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Direct and context-dependent effects of light, temperature, and phytoplankton shape bacterial community composition

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19 **Abstract**

20 Species interactions, environmental conditions, and stochastic processes work in concert to bring
21 about changes in community structure. However, the relative importance of specific factors and
22 how their combined influence affects community composition remain largely unclear. We
23 conducted a multi-factorial experiment to 1) disentangle the direct and interaction-mediated
24 effects of environmental conditions and 2) augment our understanding of how environmental
25 context modulates species interactions. We focus on a planktonic system where interactions with
26 phytoplankton effect changes in the composition of bacterial communities, and light and
27 temperature conditions can influence bacteria directly as well as through their interactions with
28 phytoplankton. Epilimnetic bacteria from two humic lakes were combined with phytoplankton
29 assemblages from each lake (“home” or “away”) or a no-phytoplankton control and incubated
30 for 5 days under all combinations of light (surface, ~25% surface irradiance) and temperature (5
31 levels from 10°C to 25°C). Observed light effects were primarily direct while phytoplankton and
32 temperature effects on bacterial community composition were highly interdependent. The
33 influence of temperature on aquatic bacteria was consistently mediated by phytoplankton and
34 most pronounced for bacteria incubated with “away” phytoplankton treatments, likely due to the
35 availability of novel phytoplankton-derived resources. The effects of phytoplankton on bacterial
36 community composition were generally increased at higher temperatures. Incorporating
37 mechanisms underlying the observed interdependent effects of species interactions and
38 environmental conditions into modeling frameworks may improve our ability to forecast
39 ecological responses to environmental change.

40 **Keywords:** community succession, algal-bacterial interactions, context-dependence, microcosm,
41 bacterioplankton, lake

42

43 **Introduction**

44 Species interactions, environmental conditions, and stochastic processes affect the
45 abundance, diversity, and distribution of organisms in the environment. Determining the relative
46 importance of specific factors and how they interact to structure communities remains a major
47 challenge in ecology (Agrawal et al. 2007, Sutherland et al. 2012). Effects of environmental
48 conditions on a population include direct effects that can potentially be characterized by single-
49 species observations and experiments as well as indirect effects that depend on species
50 interactions and require more community-focused approaches to detect and predict (Gilman et al.
51 2010). Concurrently, the outcome (e.g., mutualism, parasitism) and strength of interspecific
52 interactions can change depending on environmental conditions as well as community
53 composition (He et al. 2013, Chamberlain et al. 2014). To predict how communities will respond
54 to seasonal environmental fluctuations and long-term changes in climate, it is necessary to
55 account for species interactions that mediate and are affected by environmental conditions.

56 The signal of changing environmental conditions can be muted, augmented, or relayed by
57 interactions with other species including mutualism, competition, parasitism, and food web
58 interactions (Gilman et al. 2010). Interaction-mediated effects of environmental change have
59 consequences for local abundances and geographic distribution as well as phenology (Miller-
60 Rushing et al. 2010, Wisz et al. 2012, HilleRisLambers et al. 2013). For example, competitive
61 interactions, predators, consumers, disease agents, or the absence of a mutualist partner may
62 counteract environmental changes expected to expand the geographic range or increase the local
63 abundance of a species (Gilman et al. 2010, HilleRisLambers et al. 2013). Additionally, shifts in
64 the phenology of one species can result in temporal mismatch and population declines in a
65 mutualist partner or consumer species (Winder and Schindler 2004, Edwards and Richardson

66 2004). In many cases, effects of environmental changes mediated by species interactions are
67 expected to be more consequential than direct effects of change (Davis et al. 1998).

68 In addition to species interactions modulating the signal of environmental change,
69 environmental conditions can alter the outcome and strength of context-dependent species
70 interactions. A change in outcome occurs when the effect of an interaction on a given species
71 (positive, negative, or neutral) depends on the environment. For example, the net costs of plants
72 associating with mycorrhizae can exceed the net benefits when nutrient availability is high or
73 light availability is low (Johnson et al. 1997). Context-dependent outcomes have been observed
74 for competition, mutualism, and predation, but are most frequently detected for mutualisms
75 (Chamberlain et al. 2014). In contrast, variation in interaction strength, or the magnitude of effect
76 sizes, across contexts is relatively consistent among interaction types (Chamberlain et al. 2014).
77 Population-level interactions can cause shifts in community structure, making it important to
78 investigate the consequences of context-dependence at the community level.

79 Planktonic communities from temperate lakes are well suited for investigating
80 interactions between biotic and environmental factors. These communities undergo annually
81 repeated seasonal succession driven both by species interactions and environmental conditions,
82 and respond rapidly to experimental treatments (Kent et al. 2006, 2007, Sommer et al. 2012,
83 Weisse et al. 2016). Moreover, lakes have been described as sentinels of climate change, in part,
84 because they respond rapidly to changes in the environment (Adrian et al. 2009). Light
85 availability and temperature are two ecologically important factors that respond to environmental
86 change in lakes. Increases in dissolved organic carbon concentration, as have been observed in
87 lakes across parts of North America and Europe (Monteith et al. 2007), decrease light availability
88 due to more rapid light attenuation and shift the vertical distribution of heat towards the surface

89 (Bukaveckas and Robbins Forbes 2000, Read and Rose 2013). Global mean lake surface
90 temperature has been increasing 0.34°C per decade in response to climate forcing (O'Reilly et al.
91 2015). Long-term climate changes are overlaid on a dynamic system where light availability and
92 temperature change with depth and over the course of a year, especially in lakes that stratify in
93 the summer and mix in the fall and spring.

94 Light and temperature can influence bacterial community composition directly as well as
95 through bacterial interactions with phytoplankton. Wavelength-specific light attenuation in the
96 water column creates a spectrum of niches for phototrophs (Stomp et al. 2007), including
97 photosynthetic organisms as well as photoheterotrophs (Martínez-García et al. 2011, Evans et al.
98 2015). Additionally, bacterial growth is affected in a strain-specific manner by light in the
99 ultraviolet range (Agogue et al. 2005, Hörtnagl et al. 2010). Bacteria have diverse optimal
100 growth temperatures and ranges, such that temperature can determine outcomes of competition
101 between bacterial populations (Upton et al. 1990, Hall et al. 2008). Phytoplankton interact with
102 bacterioplankton through mechanisms that include selective grazing by mixotrophic
103 phytoplankton (Flynn et al. 2012), serving as a habitat for bacterial epiphytes (Jasti et al. 2005),
104 and providing species-specific resources as detritus (Van Hannen et al. 1999) or labile exudates
105 released by living cells (Teeling et al. 2012, Sarmiento and Gasol 2012). As temperature
106 increases, mixotrophic phytoplankton are theorized to become more heterotrophic; this has been
107 experimentally demonstrated with the chrysophyte *Ochromonas* sp. (Wilken et al. 2012).
108 Additionally, the concentration and composition of extracellular organic carbon excreted by
109 phytoplankton depend on light and temperature conditions (Zlotnik and Dubinsky 1989, Parker
110 and Armbrust 2005).

111 Our objective was to characterize the combined effects of phytoplankton, light, and
112 temperature on bacterial community composition from two humic lakes in Northern Wisconsin
113 where interactions with phytoplankton are partially responsible for orchestrating changes in
114 bacterial composition through time (Kent et al. 2007, Paver et al. 2013). Change in light and
115 temperature with depth is especially pronounced in darkly stained humic lakes (Huovinen et al.
116 2003). We specifically aimed to determine 1) whether the influence of light and temperature on
117 bacterial communities is mediated by phytoplankton and 2) whether the effects of interactions
118 with phytoplankton depend on light and temperature conditions (Fig. S1). If light and
119 temperature affect bacteria through interactions with phytoplankton, then it is expected that the
120 variation in bacterial community composition explained by light and temperature treatment will
121 be greater in microcosms where phytoplankton are present relative to those where phytoplankton
122 are absent. If phytoplankton effects depend on the light and temperature context, the variation in
123 bacterial community composition explained by phytoplankton will change under different light
124 and temperature conditions.

125

126 **Materials and Methods**

127 *Study sites*

128 South Sparkling Bog (SSB; 46°00'13.6"N, 89°42'19.9"W) and Trout Bog (TB;
129 46°02'27.5"N, 89°41'09.6"W) are two north temperate humic lakes in Vilas County, Wisconsin
130 that have been studied as part of the North Temperate Lakes Microbial Observatory. SSB and TB
131 were selected for their similarity in maximum depth (~8m) and differences in phytoplankton
132 community composition (Paver et al. 2013). These lakes are characterized by acidic pH and high
133 levels of dissolved organic carbon (Paver et al. 2013).

134 *Experimental design*

135 We conducted a multi-factorial microcosm experiment to determine the direct and
136 interactive effects of phytoplankton presence and composition, temperature, and light on
137 bacterial community composition. On 6 July 2011, microorganisms were collected from SSB and
138 TB integrated epilimnion samples (0-1m). Filtration through a 1 μ m Polycap AS cartridge filter
139 (Whatman, Piscataway, NJ, USA) was used to separate bacteria from larger organisms.
140 Phytoplankton assemblages were collected by filtering lake water through a 100 μ m nylon mesh
141 (Spectrum Laboratories, Rancho Dominguez, CA, USA) to remove zooplankton and collecting,
142 then rinsing phytoplankton cells captured on a 20 μ m nylon mesh with SSB water filter-sterilized
143 through a 0.2 μ m Polycap AS cartridge filter (Spectrum Laboratories), which allowed smaller
144 organisms such as heterotrophic nanoflagellates and bacteria to pass through. Phytoplankton
145 collected on 20 μ m mesh were resuspended in 0.2 μ m filter-sterilized water from SSB,
146 concentrating phytoplankton from 40L of lake water to 2.5L of sterilized water. All
147 combinations of bacteria from each lake (5L of 1 μ m filtered water) were combined with 0.25L
148 of concentrated phytoplankton from one of the two lakes lake or a no-phytoplankton control
149 (0.25L of 0.2 μ m filter-sterilized SSB water) in triplicate 10L LDPE cubitainers (I-Chem,
150 Rockwood, TN, USA). Combined bacteria and phytoplankton were gently inverted to mix and
151 then partitioned into 500ml clear glass bottles (Wheaton, Millville, NJ, USA) in a predetermined,
152 randomized order (33 bottles/ treatment).

153 For each bacteria-phytoplankton combination, three bottles were used to characterize the
154 initial community composition and three bottles were incubated for five days under each of five
155 temperatures and two light levels (Fig. 1). Temperature treatments were established and
156 maintained by continuously pumping defined proportions of high temperature (\sim 25 $^{\circ}$ C) surface

157 water and low temperature (~5°C) subsurface water (1:0, 3:1, 1:1, 1:3, 0:1) into floating plastic
158 container incubators (0.73m x 0.53m x 0.46m, The Container Store, Coppell, TX, USA) (Fig.
159 S2). High light and low light treatments were established by incubating bottles at the surface and
160 bottom (~25% of surface irradiance) of each floating container incubator. Light and temperature
161 conditions were monitored throughout the incubation using HOBO light and temperature
162 pendant data loggers (Onset, Pocasset, MA, USA) with three loggers placed in each container:
163 two at opposite corners at the surface and one on the bottom.

164 *Microbial community analysis*

165 Microorganisms from initial samples and from each bottle microcosm following
166 incubation were concentrated onto 0.22µm filters (Supor-200; Pall Gelman, East Hills, NY) and
167 frozen at -20°C. DNA was extracted using FastDNA purification kits (MP Biomedicals, Solon,
168 OH, USA). Bacterial community composition was characterized using automated ribosomal
169 intergenic spacer analysis (ARISA) (Fisher and Triplett 1999) as described by Paver et al.
170 (2013). Fluorescently labeled ARISA PCR amplicons were combined with a custom 100 – 1250
171 bp Rhodamine X-labeled internal size standard (Bioventures, Murfreesboro, TN) and analyzed
172 by the Keck Center for Functional Genomics at the University of Illinois via denaturing capillary
173 electrophoresis using an ABI 3730XL Genetic Analyzer (Applied Biosystems Inc., Carlsbad,
174 California, USA). Electropherograms from each sample were aligned and peaks greater than 500
175 fluorescence units were sized and grouped into bins of operational taxonomic units using
176 GeneMarker version 1.95 (SoftGenetics, State College, PA, USA). ARISA fragments known to
177 correspond to chloroplasts were removed from the analysis. The signal strength of each peak was
178 normalized to account for run-to-run variations in signal detection by dividing the area of
179 individual peaks by the total fluorescence (area) detected in each profile.

180 *Statistical approach*

181 Pairwise Bray-Curtis similarities were calculated for every combination of samples using
182 Hellinger-transformed ARISA data and visualized using non-metric multidimensional scaling in
183 PRIMER version 6 (PRIMER-E Ltd, Plymouth Marine Laboratory, UK) (Clarke and Warwick,
184 2001). Permutational multivariate analysis of variance (PERMANOVA) was used to test: 1) the
185 effects of light and temperature on bacterial community composition following incubation for
186 each combination of bacteria and phytoplankton and 2) the effect of each phytoplankton
187 treatment (compared to the no phytoplankton control) on bacterial community composition at
188 each light and temperature level, stratified by the bacterial community source lake.
189 PERMANOVA is a non-parametric multivariate analysis of variance that generates p-values
190 using permutations (McArdle and Anderson 2001, Anderson 2001). PERMANOVA tests were
191 run using the `adonis` function from the `vegan` package (Oksanen et al. 2011) in the R statistical
192 environment (R Core Development Team, 2010).

193

194 **Results**

195 *Direct and phytoplankton-mediated light and temperature effects*

196 Over the five-day incubation, phytoplankton presence and composition, light availability,
197 and temperature affected bacterial community composition (Fig. 2). We assessed direct effects of
198 light and temperature on bacterial communities by analyzing changes in bacterial community
199 composition in no-phytoplankton control treatments. Light had a significant direct effect on the
200 composition of bacterial communities from both lakes (Fig. 3). In contrast to light, temperature
201 had a significant direct effect on the community composition of bacteria from SSB, but not
202 bacteria from TB (Fig. 3).

203 The effects of light and temperature on bacteria incubated with phytoplankton depended
204 on the specific combination of phytoplankton and bacteria (Fig. 3). When TB bacteria were
205 incubated with their “home” phytoplankton, there was a significant interaction between light and
206 temperature. Light explained small but significant variation in bacterial community composition
207 when SSB bacteria were combined with their “home” phytoplankton. Significant light effects
208 were not detected when bacteria were combined with phytoplankton from the “away” lake. In
209 contrast, temperature had a consistently significant effect on the composition of bacterial
210 communities when phytoplankton were present. Notably, the variation in bacterial community
211 composition explained by temperature was higher for bacteria incubated with phytoplankton
212 from the “away” lake than for bacteria incubated with phytoplankton from their “home” lake.

213 *Context-dependence of phytoplankton interactions*

214 We used percent variation in bacterial community composition explained by
215 phytoplankton treatment to evaluate the strength of phytoplankton effects (Fig. 4). Prior to
216 incubation, 22% of the variation in bacterial community composition in SSB phytoplankton and
217 corresponding control treatments was explained by phytoplankton treatment. Following
218 incubation, variation explained by SSB phytoplankton was only greater than the initial explained
219 variation in microcosms incubated at the highest temperature (45% variation explained). In
220 contrast, variation in bacterial community composition in TB phytoplankton and corresponding
221 control treatments due to phytoplankton treatment was not significant prior to incubation
222 ($p>0.05$). At the coldest two temperatures, 13% and 19% more variation in bacteria community
223 composition was explained by TB phytoplankton in low light compared to high light treatments.
224 As temperature increased, the variation explained by TB phytoplankton generally increased until
225 peaking at the second warmest temperature.

226 **Discussion**

227 It is well established that species interactions and environmental conditions act in concert
228 to affect community composition, but how these factors combine to determine community
229 composition is largely undefined. In this study we selected light, temperature, and
230 phytoplankton-bacterial interactions to investigate the interplay among biotic and environmental
231 factors at the community level. In general, observed treatment effects were highly similar across
232 replicates, suggesting that deterministic processes controlled the development of bacterial
233 communities over the course of the five-day experimental incubation. We observed direct light
234 effects, phytoplankton-mediated temperature effects, and temperature- and light-dependent
235 phytoplankton effects, each of which is explored below.

236 *Direct light effects*

237 Light had a consistent, direct effect on bacterial community composition in experimental
238 microcosms. One potential explanation for the direct effect of light on bacterial composition is
239 selection for phototrophic bacteria generally, or specific types of phototrophic bacteria.
240 Organisms that harvest light energy are adapted to use specific wavelengths of light and, as a
241 result, phototroph distribution within the water column reflects the available light spectrum (Vila
242 and Abella 2001, Haverkamp et al. 2008). As our study lakes have high humic content, light
243 spectra are enriched in long wavelength photons (>600nm) and light attenuates rapidly with
244 increased depth, creating specific spectral niches across modest changes in depth (e.g., 0.5m)
245 (Vila et al. 1998). Photoheterotrophic bacterial cells can comprise a sizable portion of lake
246 bacterial communities, and change in abundance with depth (Mašín et al. 2008, Martínez-García
247 et al. 2011, Lew et al. 2016). Alternatively, or perhaps additionally, direct effects of light may
248 have been caused by ultraviolet radiation. Based on the transparency properties of borosilicate

249 glass, plankton were exposed to a fraction of long wave ultraviolet A radiation, but none of the
250 ultraviolet B and shorter wavelengths of light, penetrating to their respective incubation depths
251 (Döhning et al. 1996). Effects of ultraviolet radiation can decrease the growth efficiency of
252 certain bacterial strains while having no effect or increasing the growth efficiency of other strains
253 (Hörtnagl et al. 2010). It is additionally possible that products of dissolved organic matter
254 photolysis, including low molecular weight dissolved organic matter and reactive oxygen species
255 induced changes bacterial community composition (Glaeser et al. 2010, Paul et al. 2011).

256 *Phytoplankton-mediated temperature effects*

257 In contrast to the effects of light, observed effects of temperature were primarily
258 mediated through interactions with phytoplankton. Temperature had significant direct effects on
259 bacterial community composition for SSB bacteria, but only marginally significant direct effects
260 for TB bacteria. Lack of significant direct effects of temperatures spanning approximately 15°C
261 on TB bacteria was surprising and may have been due to resource limitation. Alternatively,
262 differences in the chemistry of the lake water added along with the bacterial treatment or the
263 distribution of bacterial traits (e.g., ability to use light energy or breakdown available dissolved
264 organic matter) may explain observed lake-specific differences in temperature response.
265 Concentrations of dissolved organic carbon, total phosphorus, and total nitrogen tend to be
266 higher in TB compared to SSB (Paver et al. 2013). Temperature effects were consistently
267 significant in treatments with added phytoplankton, and enhanced in “away” phytoplankton
268 treatments, potentially due to production of organic matter novel to the bacterial community
269 (Fogg 1983, Sarmiento and Gasol 2012). Bacterial community composition is continuously
270 shaped by interactions with phytoplankton from their “home” lake (Paver et al. 2013), so initial
271 bacterial assemblages were acclimated to “home” phytoplankton resources. Our results provide

272 further evidence that bacteria rely on phytoplankton-derived dissolved organic carbon in darkly
273 stained humic lakes with high background concentrations of dissolved organic carbon (Kritzberg
274 et al. 2006, Kent et al. 2007).

275 Observed phytoplankton-dependent temperature effects along with previously published
276 findings support a signal transduction hypothesis where changes in the environment are relayed
277 to bacterial populations through their interactions with phytoplankton. In a suite of north
278 temperate humic lakes (including SSB and TB), changes in the composition of phytoplankton
279 assemblages were largely explained by environmental (e.g., water temperature, nutrients) and
280 meteorological (e.g., photosynthetically active radiation, precipitation) factors (Kent et al. 2007).
281 In contrast, changes in bacterial community composition were primarily explained by changes in
282 phytoplankton population abundances and covariation between phytoplankton populations and
283 the environment (Kent et al. 2007). Results from the current study provide experimental
284 evidence that the signal of increased temperature in these lakes is largely relayed to bacteria by
285 their interactions with phytoplankton. Experimental observations of Baltic Sea bacterial
286 communities yielded a complementary result that phytoplankton bloom stage was a more
287 important factor structuring bacterial communities than a 6°C change in temperature (Scheibner
288 et al. 2013). Previous work on bacterial growth and activity provide additional support for
289 environmental signal transduction via phytoplankton. Multiple regression and hierarchical
290 partitioning analysis of data from 300 field studies indicated that temperature has a positive
291 relationship with phytoplankton primary production, but not bacterial production (Faithfull et al.
292 2011). Despite positive correlations between temperature and bacterial production, bacterial
293 production was primarily explained by a combination of total phosphorus and primary
294 productivity (Faithfull et al. 2011). At low temperatures, bacterial growth and activity are

295 frequently temperature limited (Simon and Wünsch 1998, Vrede 2005, Adams et al. 2010).
296 When temperature is not limiting, bacterial growth in temperate lakes is commonly limited by
297 phosphorus, dissolved organic carbon, or the two in combination (Vrede 2005). Phytoplankton
298 clearly support bacterial growth and activity and have the potential to relay environmental
299 signals to bacteria with which they interact.

300 *Temperature- and light- dependent of phytoplankton effects*

301 Effects of phytoplankton on bacterioplankton were temperature, and to a lesser extent,
302 light dependent. Observed interdependence of temperature and phytoplankton effects is
303 consistent with the framework that phytoplankton provide organic matter resources to bacteria
304 (Cole 1982, Sarmento and Gasol 2012) and temperature regulates the flow of carbon from
305 phytoplankton to bacteria (Overmann 2013, Scheibner et al. 2013). Notably, when bacteria from
306 TB were incubated with “home” phytoplankton, there was a significant interaction between light
307 and temperature (Fig. 3). In contrast to high light assemblages that became increasingly different
308 from their initial composition along a somewhat linear trajectory in ordination space as
309 temperature increased, low light assemblages exhibited a curved response (Fig. 2). Bacterial
310 composition in the coldest low light treatment was uncharacteristically different from the initial
311 assemblage relative to other assemblages incubated at that temperature. These observations may
312 be explained by changes in the concentration and composition of phytoplankton exudates under
313 different temperature and light conditions. Combined effects of light and temperature have been
314 shown to affect the dominant metabolic pathways used to process carbon, thereby controlling
315 exudate release (Parker and Armbrust 2005). Alternatively, the dominant mechanism of bacteria-
316 phytoplankton interactions may change depending on light and temperature. For example, low

317 light conditions can increase the rate of bacterial consumption by mixotrophic phytoplankton
318 under certain conditions (e.g., low nutrient availability; [McKie-Krisberg and Sanders 2014]).

319 *Consequences for planktonic microbial communities*

320 Observations that light has primarily direct effects on bacterioplankton, while
321 temperature effects are mediated through interactions with phytoplankton, have implications for
322 interpreting seasonal changes in planktonic communities and forecasting future changes. The
323 pronounced, direct effect of light on bacterial community composition emphasizes the
324 importance of collecting high-resolution samples over depth and incorporating mechanisms
325 structuring bacterial communities into a depth-specific framework. Temperature is frequently
326 correlated with the succession of aquatic bacterial communities (Crump and Hobbie 2005,
327 Fuhrman et al. 2006, Shade et al. 2007). Our results demonstrate that, in some systems, much of
328 the bacterial community response to temperature is fueled by their interactions with
329 phytoplankton. Thus, if the objective is to forecast how bacterial community structure and
330 function will change in response to environmental changes, it is necessary to incorporate the
331 predicted response of phytoplankton and context-dependence of interactions linking
332 phytoplankton and bacterial assemblages. For example, elevated temperature in mesocosms
333 during the spring phytoplankton bloom in Kiel Bight accelerated the onset of the phytoplankton
334 bloom, decreased the intensity of maximum chlorophyll a and particulate organic carbon by
335 approximately 20%, and caused dissolved organic carbon concentrations to increase more
336 rapidly than under ambient temperature conditions (Biermann et al. 2014).

337 Environmental context is critical for understanding how planktonic communities will
338 change over time and in response to environmental change. Many of our observations were
339 highly dependent on the biotic or environmental context – responses seen in one lake were not

340 replicated in the other and, for TB bacteria combined with TB phytoplankton, response to
341 temperature depended on light availability. Context-dependence has also been described for
342 bacterial production in high mountain lakes where bacterial response to solar radiation treatments
343 depended on the presence of phytoplankton and whether bacteria were phosphorus-limited
344 (Medina Sánchez et al. 2002). The prevalence of non-additive interaction effects among
345 environmental factors emphasizes the importance of multi-factorial experimental investigations
346 into drivers of microbial community composition and activity. It additionally underscores a need
347 to identify mechanisms underlying context-dependent microbial responses and build these
348 mechanisms into frameworks describing aquatic microbial community dynamics.

349 *Effects of interacting factors on community composition*

350 Phytoplankton, light availability, and temperature act in concert to bring about changes in
351 bacterial community composition over time. Light availability directly affects bacterial
352 community composition while interactions with phytoplankton amplify or relay the signal of
353 increasing temperature to bacteria. The strength of phytoplankton interactions with bacteria,
354 inferred through comparisons of bacterial community composition across temperature and light
355 treatments relative to no-phytoplankton controls, depends on temperature. For certain
356 combinations of phytoplankton and bacteria, the outcome of phytoplankton interactions appears
357 to additionally be light dependent. The enhanced effect of “away” phytoplankton relative to
358 “home” phytoplankton and the lack of consistent temperature effects in treatments without
359 phytoplankton provide strong support for phytoplankton resources shaping bacterial
360 communities and their response to environmental conditions. These findings emphasize the
361 importance of observing population and community responses to multiple ecological drivers
362 simultaneously and under a range of environmentally relevant conditions. Studies aimed at

363 understanding the effects of climate change frequently compare ambient temperature conditions
364 to an elevated temperature treatment. Our results suggest that there are inflection points in
365 community responses to temperature that would be overlooked in ambient vs. elevated
366 temperature comparisons. The idea that under-sampling the range of potential temperatures
367 constrains our ability to make general inferences about temperature effects is reinforced by
368 studies that have investigated the effect of temperature on bacterial production and observed two
369 temperature optima (Simon and Wünsch 1998, Adams et al. 2010). The problem of under-
370 sampling treatment levels is potentially problematic for other factors as well, including light
371 availability (Gu and Wyatt 2016). Movement away from context-specific observations towards
372 generalizable, theoretical advances and identifying parameters that can be incorporated into
373 predictive frameworks will depend on investigations such as this into the mechanisms driving
374 observed changes in community composition.

375

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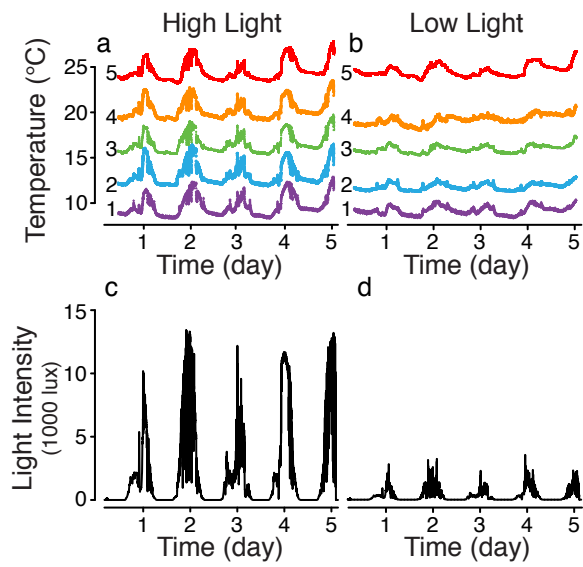
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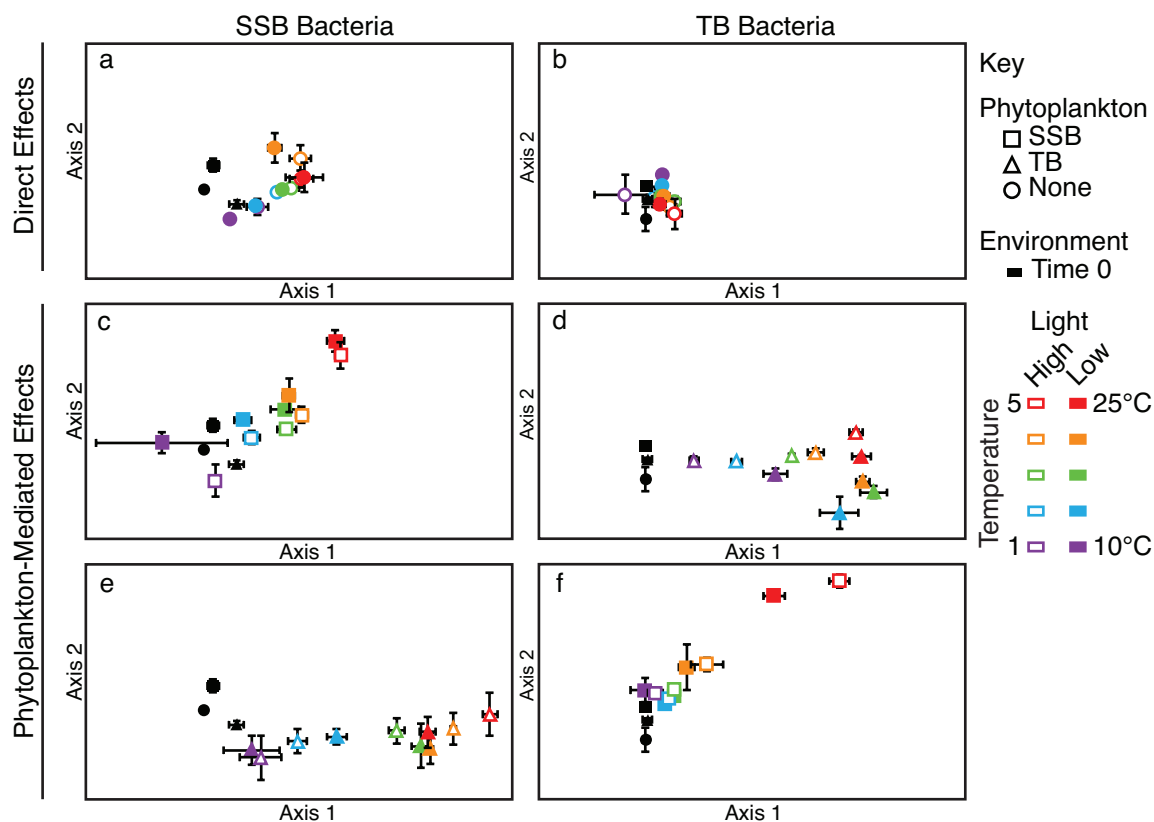
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549 **Figures**



550

551 Figure 1. Temperature (a, b) and average light intensity (c, d) at the top (high light; a, c) and bottom (low
552 light; b, d) of each temperature incubator over the five-day incubation. Temperature treatments are
553 labeled 1 (coldest) to 5 (warmest).



554

555 Figure 2. Non-metric multidimensional scaling ordination of bacterial community composition in -

556 microcosms (average \pm standard error) with SSB bacteria (a,c,e; stress value=0.08) and TB bacteria (b,d,f;

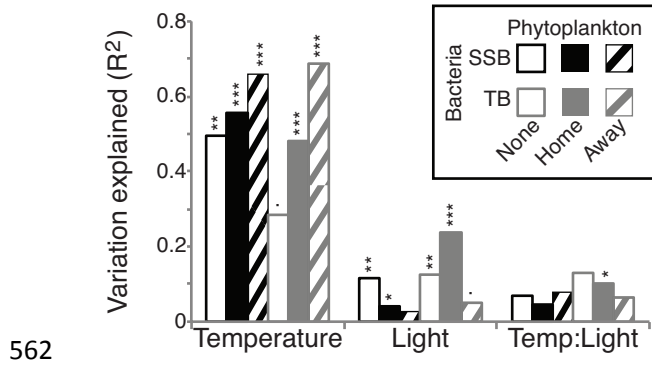
557 stress value=0.14) before and after incubation. To simplify depiction of overlapping treatments,

558 community composition in no-phytoplankton control microcosms following incubation is shown in plots

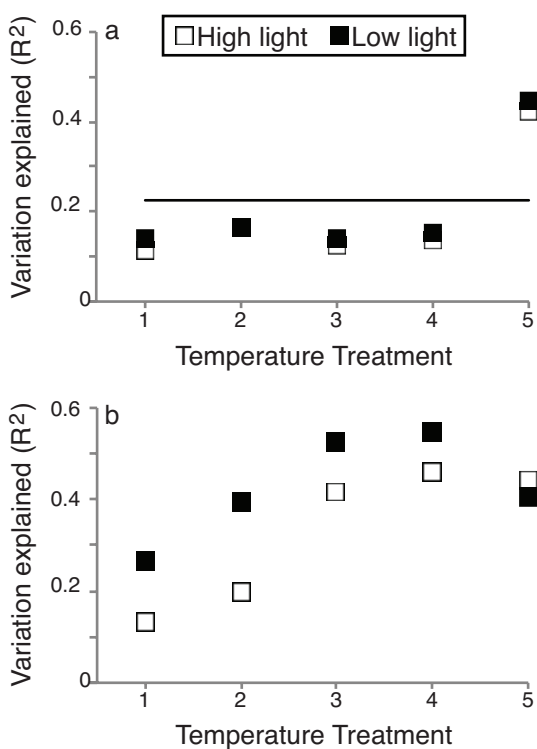
559 a and b, community composition in “home” phytoplankton treatments is shown in plots c and d, and

560 community composition in “away” phytoplankton treatments is shown in plots e and f. Community

561 composition before incubation (Time 0) is included in all plots for reference.



563 Figure 3. Variation in bacterial community composition explained by temperature, light, and the
564 interaction between temperature and light for each combination of phytoplankton and bacteria. *P*
565 values below 0.01 are indicated by symbols (*** <0.001, ** <0.01, * < 0.05, . <0.1).



566

567 Figure 4. Variation in bacterial community composition explained by SSB phytoplankton
568 treatment (a) and TB phytoplankton treatment (b) across all temperature and light conditions. A
569 horizontal line indicates variation in bacterial community composition explained by SSB
570 phytoplankton prior to incubation. TB phytoplankton did not explain significant variation in
571 bacterial community composition before incubation.