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

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Abstract

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

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

Introduction

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

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

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

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

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

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

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

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

Materials and Methods

Study sites

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

Experimental design

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

For each bacteria-phytoplankton combination, three bottles were used to characterize the initial community composition and three bottles were incubated for five days under each of five temperatures and two light levels (Fig. 1). Temperature treatments were established and maintained by continuously pumping defined proportions of high temperature (~25°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.

Statistical approach

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

Results

Direct and phytoplankton-mediated light and temperature effects

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

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

Context-dependence of phytoplankton interactions

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

Discussion

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

Direct light effects

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

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

Consequences for planktonic microbial communities

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

Environmental context is critical for understanding how planktonic communities will change over time and in response to environmental change. Many of our observations were 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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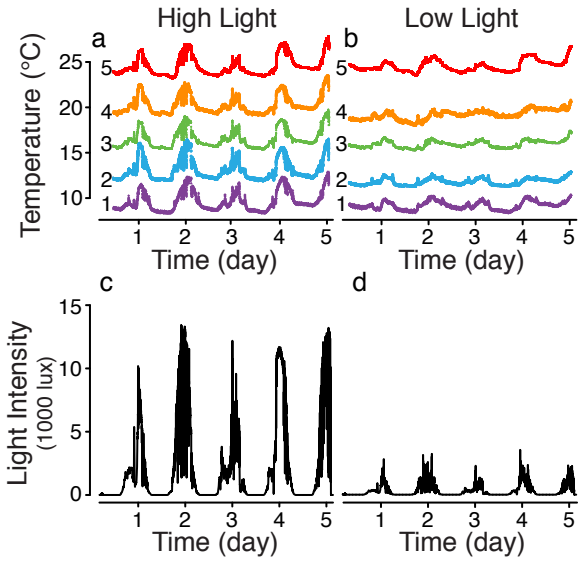
549 **Figures**



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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).

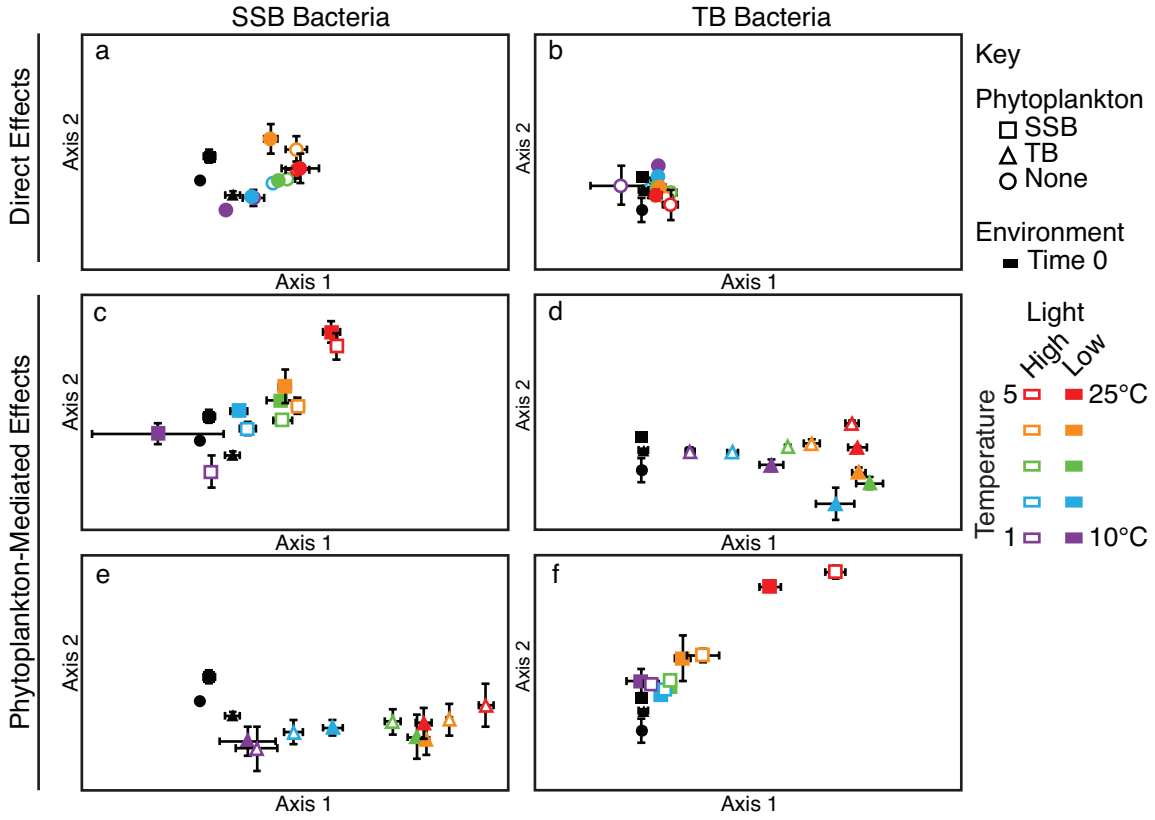

Figure 2. Non-metric multidimensional scaling ordination of bacterial community composition in microcosms (average ± standard error) with SSB bacteria (a,c,e; stress value=0.08) and TB bacteria (b,d,f; stress value=0.14) before and after incubation. To simplify depiction of overlapping treatments, community composition in no-phytoplankton control microcosms following incubation is shown in plots a and b, community composition in "home" phytoplankton treatments is shown in plots c and d, and community composition in "away" phytoplankton treatments is shown in plots e and f. Community composition before incubation (Time 0) is included in all plots for reference.

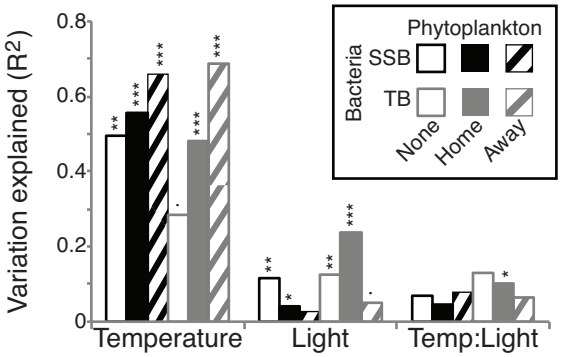

Figure 3. Variation in bacterial community composition explained by temperature, light, and the interaction between temperature and light for each combination of phytoplankton and bacteria. *P* values below 0.01 are indicated by symbols (*** <0.001, ** <0.01, * < 0.05, . <0.01).

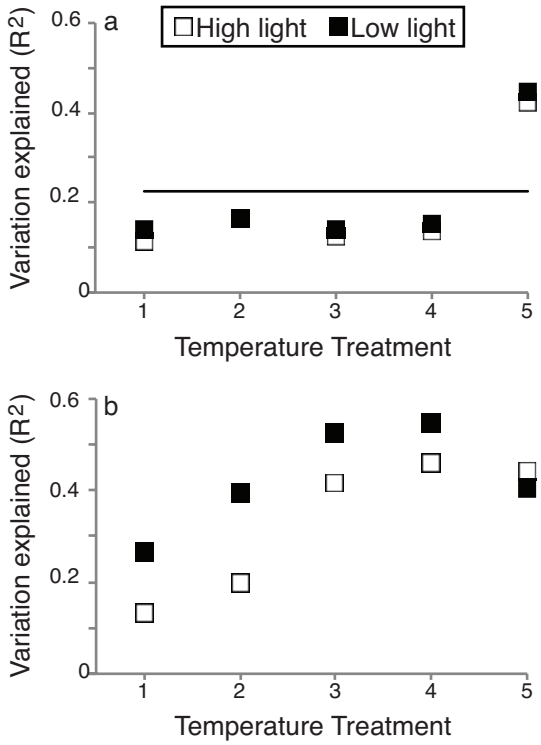

Figure 4. Variation in bacterial community composition explained by SSB phytoplankton treatment (a) and TB phytoplankton treatment (b) across all temperature and light conditions. A horizontal line indicates variation in bacterial community composition explained by SSB phytoplankton prior to incubation.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