
Biodiversity and thermal ecological function: The influence of freshwater algal diversity on local thermal environments - Submission to ECOLOGY & EVOLUTION

Anouch Missirian^{1,*}, Eyal G. Frank², Jess T. Gersony³, Jason C. Y. Wong¹, Shahid Naeem⁴

1 School of International and Public Affairs, Columbia University, New York, NY, USA

2 Harris School of Public Policy, University of Chicago, Chicago, IL, USA

3 Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA

4 Department of Ecology, Evolution, and Environmental Biology, Columbia University, New York, NY, USA

* Corresponding author, am4215@columbia.edu

Abstract

The influence of temperature on diversity and ecosystem functioning is well studied; the converse however, i.e. how biodiversity influences temperature, much less so. We manipulated freshwater algal species diversity in microbial microcosms to uncover how diversity influenced primary production, which is well documented in biodiversity research. We then also explored how visible-spectrum absorbance and the local thermal environment responded to biodiversity change. Variations in the local thermal environment, that is, in the temperature of the immediate surroundings of a community, are known to matter not only for the rate of ecosystem processes, but also for persistence of species assemblages and the very relationship between biodiversity and ecosystem functioning. In our microcosm experiment, we found a significant positive association between algal species richness and primary production, a negative association between primary production and visible-spectrum absorbance, and a positive association between visible-spectrum absorbance and the response of the local thermal environment (i.e., change in thermal infrared emittance over a unit time). These findings support an indirect effect of algal diversity on the local thermal environment pointing to a hitherto unrecognized biodiversity effect in which diversity has a predictable influence on local thermal environments.

Keywords

biodiversity, thermal ecology, ecosystem function, thermography, global change, temperature.

Short title

Biodiversity and thermal ecological function

1 Introduction

2 The varied influences of biodiversity on ecosystem functions and properties, and the abiotic components of these
3 systems, are well studied (3, 20, 29). The local thermal environment – most frequently measured as air, water, or
4 soil temperature –, which can be considered an ecosystem-level property, however, has largely been treated as the
5 result of extrinsic or abiotic factors such as climate, special attention being devoted to the impacts of changing
6 temperature on biodiversity and ecosystem properties in the face of recent, unprecedented changes in climate (12).
7 Temperature's effect on ecosystem functioning and biodiversity has been investigated: most notably, the effect of

8 temperature change on individuals (2), community diversity (18, 26), ecosystem functions (21), but also on many
9 other facets of the ecosystem such as pest dynamics, niche shift, and community turnover, in terrestrial and
10 marine systems alike, as well as the very relationship between biodiversity and ecosystem functioning, e.g.,
11 (1, 5, 9, 15, 32, 33)). The influence of biological diversity on temperature, however, is less well studied, despite
12 temperature being an environmental parameter of fundamental ecological importance.

13 It is important to note that the influence of vegetation type on albedo (e.g., when boreal forest replaces
14 grassland – see for instance (7, 8)) is well studied. However, whether the change in plant species richness shows
15 predictable impacts on albedo is unknown. Our focus, then, is on whether a change in the *diversity* of a given
16 community can affect its thermal properties. Back to the albedo example for instance, whether the change in
17 plant species richness shows predictable impacts on albedo is unknown. So is the broader influence of biodiversity
18 on local thermal environments.

19 Given the roles biodiversity can play in primary productivity (27) and other ecosystem properties (e.g.,
20 stability (10, 17, 28), efficiency (22)), biodiversity effects could translate into a change in the local thermal
21 properties of the system, though the direction and magnitude are difficult to predict. Indeed, in terrestrial
22 systems for example, if more diverse communities had higher albedo or greater evapotranspiration associated with
23 greater production, the local temperature could decrease. On the other hand, local temperature could just as well
24 decrease in more diverse communities if increasing diversity led to increasing dominance by darker plants, hence
25 to increased absorbance, leading to visible spectrum radiation being re-emitted as thermal radiation. Germanely,
26 diversity could have either or both of these countervailing effects in aquatic systems: increasing temperature by
27 increasing productivity, decreasing temperature by increasing efficiency, on top of community albedo effects.

28 To explore this issue, we manipulated algal biodiversity in freshwater microcosms to test for diversity effects on
29 local thermal environments; microcosm refers to the closed system in its entirety, i.e. culture vessels with their
30 culture medium and phytoplankton community. Algal species are key members of aquatic communities that are
31 concentrated in upper surfaces of the water column where light is abundant. They play a key role in aquatic
32 environments as primary producers, and in global biogeochemical cycles; yet little is known of their patterns of
33 diversity (see (13)) and how they relate to primary production (see (30)). Because planktonic algal species contain
34 a variety of pigments (4, 11), they absorb visible spectrum light (0.40 – 0.90 μm), some of which is used for
35 photosynthesis, but a large portion of the remainder is re-emitted as thermal infrared (7.5 – 13.0 μm), which
36 produces sensible heat that warms the water around them. Global changes are affecting freshwater and marine
37 communities and their diversity (24), and therefore make algal communities of additional interest from an
38 environmental perspective. While the impacts of temperature change on algal communities, or indeed any
39 biological community, are important as climate change increases, the role biological communities play in their
40 changing thermal environments is unknown and could be important for understanding more clearly the two-way
41 interaction between temperature and ecosystems.

42 Materials and Methods

43 We used a microcosm setup for maximal control over the variables of interest, our objective being to observe
44 whether or not community diversity, more precisely here, species richness, has an effect on the local thermal
45 properties of ecosystems. Thus our focus is not on productivity (or its proxies, such as Chlorophyll a or greenness):
46 the relationship between producer diversity and production has been well studied. Rather, we focus on the effect
47 of species richness on the radiation of thermal infrared (sensible heat) resulting from the absorption of visible light.
48 We chose species richness as a measure of diversity to minimize the numbers of degrees of freedom and the
49 magnitude of this novel experiment, and also to conform to the long tradition of experiments in biodiversity and
50 ecosystem functioning (19, 27) and thus make ours comparable to that rich body of literature. We measured
51 sensible heat using thermography. Thermography quantifies thermal infrared radiation, in particular that emitted
52 by the focal organism(s) (e.g., mammals, molluscs (14, 25), or here, phytoplankton); it is distinct from greenness,
53 which concerns reflected light (most often from chlorophyll in relatively transparent freshwater algal species).

54 We used algae as a model group. The algae communities consisted of 0 (control), 1, 2, 4, or 8 species drawn
55 from a pool of eight species. These were: *Ankistrodesmus falcatus*, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*,
56 *Cosmarium turpinii*, *Eudorina elegans*, *Haematococcus droebakensis*, *Selenastrum capricornutum*, *Staurastrum*
57 *gracile*. All species are freshwater algae that are commonly found in lakes and other water bodies under temperate

58 climates, with standard nutrient and growth medium requirements (e.g., none uses silicon, and all grow at
 59 ambient temperature). We chose species that are unicellular (i.e., none were colonial, though some formed cell
 60 aggregates), and as morphologically diverse as was possible so as to maximize functional complementarity and
 61 facilitate enumeration (similar to (31)).

62 **Experimental Design** Given 8 species, it is possible to form $2^8 = 256$ species combinations of any size, and in
 63 particular 107 combinations of size 1, 2, 4, and 8. We explored the majority of possible combinations opting to
 64 maximize coverage of diversity rather than replication of individual combinations (see Table S1).

65 Specifically, we assembled 169 communities of 0, 1, 2, 4, or 8 species in transparent sterilized plastic culture
 66 flasks (15 mL, optically clear virgin polystyrene); each was labeled and filled with 1,000 cells (except the controls)
 67 and algal growth medium to total 15 mL (Alga-Gro® Freshwater Medium, from Carolina Biological Supply
 68 Company, Burlington, NC, USA; the algal cultures themselves were also all obtained from Carolina Biological
 69 Supply Company). Microcosms were prepared in three batches of equal size, the first one prepared one week
 70 before the two others but in otherwise similar conditions, in order to facilitate sampling. The inoculation species
 71 densities of 1,000, 500, 250, 125, for 1-, 2-, 4-, 8-species communities, respectively, were prepared from
 72 monocultures of known densities. Finally, the microcosms were established under white, full-spectrum lamps for
 73 13 days (corresponding to about 13 generations) at ambient temperature (22°C); this corresponds approximately
 74 to their optimal temperature. The microcosms' position under the lamps was randomized daily to minimize effects
 75 of possible heterogeneity in the light environment. A conceptual diagram of the experiment is presented in Fig. 1.

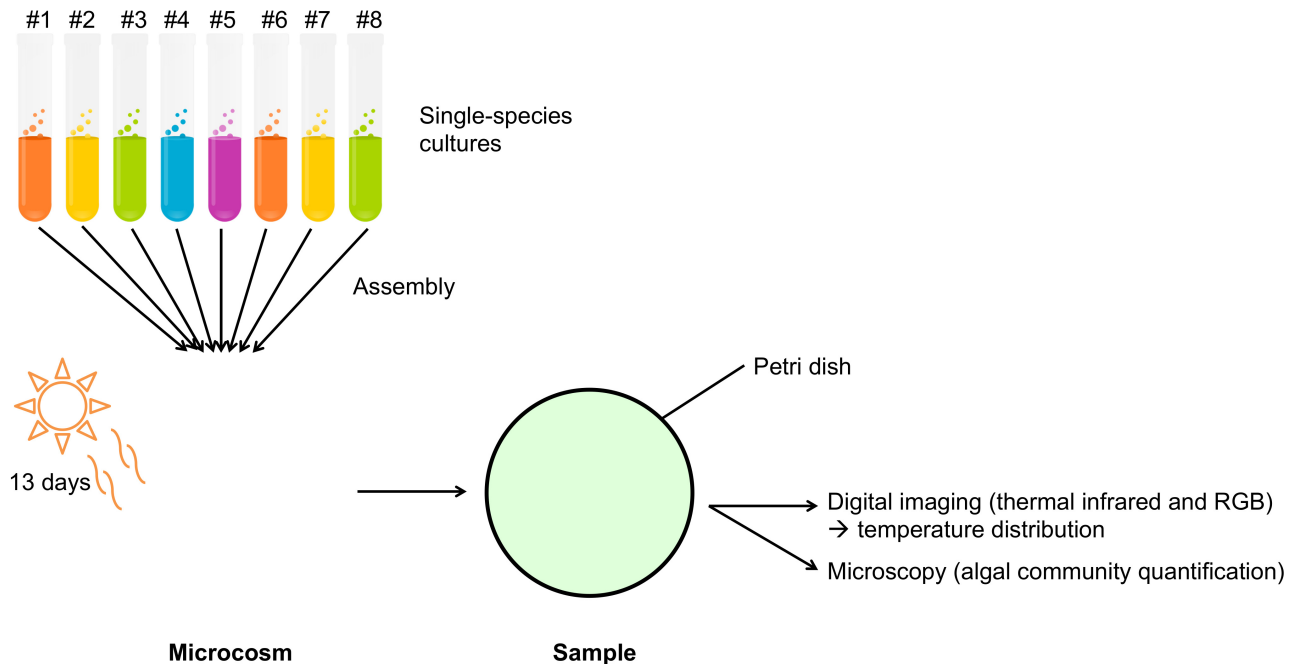


Figure 1. Experimental design: conceptual diagram

RGB refers to red, green, and blue sensors in visible spectrum camera.

76 **Measurement** After 13 days of incubation in a nutrient-rich environment and under constant exposure to light,
 77 the flasks were vortexed and 1 mL was used for counting (10 photographs of each slide were taken with an inverted
 78 microscope at magnification 40x for future counting) and the remaining 14 mL were used to perform thermal
 79 imagery. For thermography, the 14 mL samples were individually poured into a Petri dish, promptly covered, and
 80 exposed to fiber-optic, low temperature white light (Lumina, Chiu Technical Corporation, Kings Park, NY, USA,
 81 150 W) for 60 seconds (other durations were tested and yielded similar results) to allow algae to absorb light. We
 82 removed the lid and took an infrared image with a FLIR T650SC (FLIR Systems, Nashua, NH, USA), as well as a

83 photograph in the visible spectrum, thus measuring both temperature and visible light (RGB) reflectance of the
84 culture (the color and opacity possibly depending on the density, health and composition of the communities). For
85 the second and third batches (processed together), we also took a thermal image before we heated the culture
86 (which required removing the lid for approximately 5 seconds), inserting our controls at regular intervals between
87 the samples to control for possible warming over the time it took to make measurements. This enabled us to
88 compute ΔT , the temperature change before/after exposure to light ($N = 109$). The control flasks serve as a
89 baseline for the visible and thermal imagery measurements.

90 **Data processing** The images obtained by optical microscopy were counted manually. Because of similar
91 morphologies in spite of our efforts to pick dissimilar species (compare Figures S2-S3), we were unable, in many
92 instances, to discriminate among four species when they were in polyculture; these were *Chlamydomonas*,
93 *Chlorella*, *Eudorina* and *Haematococcus*. Where necessary (e.g. in the calculation of complementarity and
94 selection effects), we therefore decided to aggregate the counts of these four species (hereafter referred to as the
95 “isomorphic group” or IG), i.e. in all measures of biovolume and cell count; because of their similar shape, size,
96 chloroplast density, they may share some important functional and ecological features, and obviously have a
97 similar cell volume for purposes of biovolume estimation. From now on, “group” refers to either of the four other
98 species or the isomorphic group (hence five groups). While removing the isomorphic group from the analysis is
99 technically feasible, it accounts for half of the species present. Therefore many species assemblages comprise at
100 least one of those species (87% of our samples), and removing them would reduce the number of (non-control)
101 samples on which to perform the analyses to 20 (down from 164 initially), most of which monocultures. We
102 therefore do not exclude them from our analyses. Nonetheless, and anticipating on the Results section, we note
103 here that we reanalyzed the data where separating the isomorphic group was feasible, e.g. that presented in
104 Tables 1–2, Figures 2–3, since we are using information on the initial composition or on biovolume. The
105 complementarity and selection effects, however, are impossible to compute without lumping together the four
106 species). This did not alter the results. For consistency, we prefer presenting the results for the four species plus
107 isomorphic group throughout the paper.

108 We estimated biovolume for each group based on the optical microscope images (available in the Supporting
109 Information, Table S2), as data available from different sources on our species’ unitary biovolume (the volume of a
110 single cell) seemed not to converge.

111 Selection and complementarity effects were measured following (16). The selection effect refers to the fact that,
112 given a set of species, a random draw from that pool may select a species with a level of function above average;
113 and thus by increasing diversity (i.e. here, the number of species), one increases the likelihood of picking those
114 high-function species. The complementarity effect, on the other hand, refers to the fact that species may occupy
115 different ecological niches, thus improving resource use efficiency, and may in addition interact in a synergistic (or
116 an antagonistic) way. Overall, these interactions yield a level of function different from what might have been
117 expected by extrapolating function from the monocultures’.

118 The temperature and RGB (visible spectrum) profiles were extracted from each infrared image using FLIR
119 ExaminIR and ImageJ (Rasband 1997 - 2014) software, and operations on data were conducted in Python with
120 the Python Data Analysis Library (pandas, <https://pandas.pydata.org/>); the regressions and other statistical
121 tests were run in Stata.

122 **Data analysis** The effect of community composition and richness on the RGB profile was assessed, using the
123 mean RGB value of each culture or its standardized value (minus sample average, divided by standard deviation).
124 We examined the effect of several covariates on the maximum and minimum points of the temperature profiles,
125 the (average) temperature after exposition to light, and the amplitude of the change before/after the light
126 treatment – the linear regressions (ordinary least squares, OLS) are described below, and their results are detailed
127 in the next section.

128 The aforementioned covariates include time trends and a measure of the selection and complementarity effects
129 as defined in (16). The time trends were meant to control for a possible warming over time of the flasks in the
130 measurement room. To learn about the relationship between biovolume and RGB, we estimated the specification

131 given in Equation (1):

$$\text{RGB}_k = \alpha_0 + \alpha_1(\text{Biovolume})_k + \sum_{f=1}^F \alpha_f(\text{Functional Group})_{kf} + \varepsilon_k \quad (1)$$

132 where $(\text{Biovolume})_k$ is the biovolume measured in flask k , $(\text{Functional Group})_{kf}$ is a dummy for the (initial)
133 presence/absence of the functional group f in flask k ; α_0 is the intercept, and ε_k is the error term. The results are
134 presented in Table 2 of the Results section.

To investigate the effect of each hypothesized causal mechanism of influence of biodiversity on temperature (namely: albedo, activity, other unknown channels), we regressed each of our temperature variables on each of the suspected causes, as specified in Equation 2 in its most generic form and fullest specification:

$$\begin{aligned} (\text{Temp})_k = \alpha_0 + \sum_{f=1}^F \alpha_f(\text{Functional Group})_{kf} + \lambda_1 \text{Time}_k \times (\text{1st Batch})_k + \lambda_2 \text{Time}_k \times (\text{2nd Batch})_k \\ + \gamma_1 \text{RGB}_k + \gamma_2 (\text{RGB}_k)^2 + \beta_1 (\text{Complementarity Effect})_k + \beta_2 (\text{Selection Effect})_k + \varepsilon_k \end{aligned} \quad (2)$$

135

where $Temp$ stands for: ΔT , T_a (the temperature after), T_{min} or T_{max} .

136 In Equation 2, $(\text{RGB})_k$ is the mean RGB value for flask k , $(\text{Temp})_k$ stands for either T_a (the average temperature
137 of the content of flask k measured *after* exposure to the light source) or ΔT (the temperature *change* before/after
138 exposure) or T_{min} or T_{max} (extreme values measured in the microcosm). $(\text{Functional Group})_{kf}$ is a dummy
139 variable that receives a value of 1 if functional group f is present in flask k for all functional groups. Time_k is a
140 linear time trend for the time at which the flask was analyzed (to account for heating of the room).
141 $(\text{First Batch})_k$ and $(\text{Second Batch})_k$ are dummy variables that receive a value of 1 if the flask belongs to the first
142 or second batch analyzed, respectively. Finally, ε_k is the error term, and α_0 is the regression constant.

143 The results are discussed in the Results section.

144 Results

145 Biodiversity significantly affects productivity

146 In this study, productivity is measured as the biovolume of the community after 13 days of growth with abundant
147 light and nutrients. As shown on Table 1 and on Fig. S5 (see Supporting Information), the biovolume increases
148 with the richness of the microcosm, but no single species has a significant effect on total biovolume (Table 1).

149 It should be noted (see Fig. 2) that individual species behaviors are idiosyncratic: for instance, in
150 *Ankistrodesmus* and *Selenastrum* biovolume increases as the number of species increases, but *Cosmarium* did
151 much better in monoculture than in co-culture (in *Ankistrodesmus*, from a median biovolume of about $10^5 \mu\text{m}^3$ in
152 monoculture to about $3.10^5 \mu\text{m}^3$ in the company of the seven other species, as opposed to *Cosmarium* starting in
153 monoculture with a median biovolume of $3.10^5 \mu\text{m}^3$ and a fat upper tail, lower values at $n = 2$ and $n = 4$ and back
154 to about $3.10^5 \mu\text{m}^3$ with all 8 species). Interestingly, *Cosmarium* is the species with the highest unitary biovolume.

155 Our results are overall consistent with the widely observed positive saturating relationship between plant
156 species richness and primary production (3, 29).

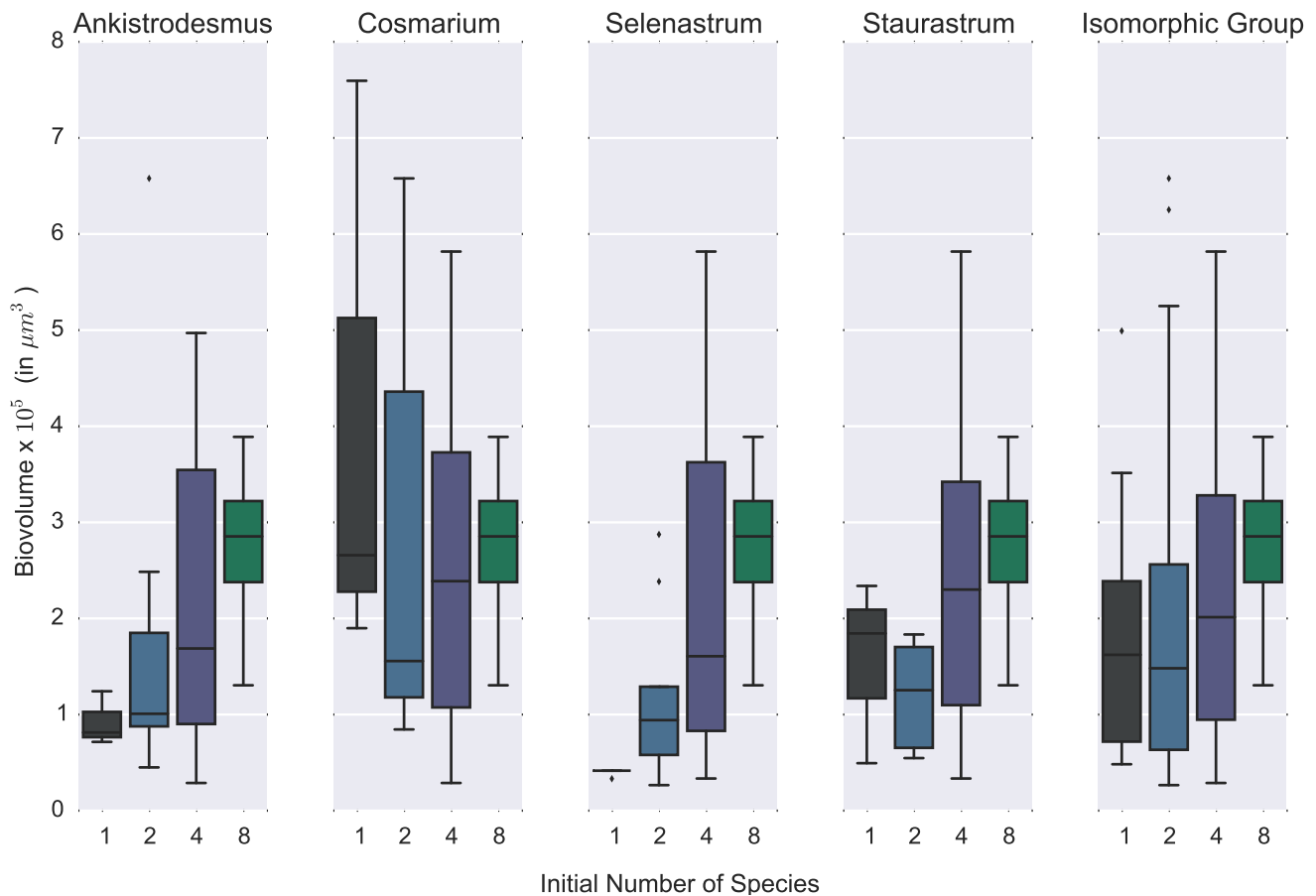


Figure 2. Flask biovolume per species

Each column presents the biovolumes of the flasks containing a particular species, be it as a monoculture or in an assemblage of 2, 4, 8 species. The horizontal line corresponds to the median, the box shows the quartiles, the whiskers describe the rest of the distribution, and the points beyond the whiskers are outliers.

157 **Biovolume significantly affects light absorption**

158 Although it is likely that higher biovolumes would lead to greater visible-spectrum absorbance, there is no reason
159 *a priori* to assume that species-specific volumes and pigment content are correlated. We tested this by estimating
160 the regression described by Equation (1), where mean RGB is regressed against biovolume; the results are
161 summarized in Table 2. Further illustration of this relationship is provided by Fig. S6 of the Supporting
162 Information. The negative relationship between biovolume and RGB is tenuous yet visible on Fig. S6a, reflecting
163 the negative coefficient obtained in column (1) of Table 2. Fig. S6b shows that this relationship persists even
164 when the effect of individual species' presence is controlled for; according to column (2) of Table 2, accounting
165 species identity even strengthens and makes this negative effect become statistically significant at the 10 % level.
166 We note that while 0.1 is not frequently used as a significance level in Ecology, (34) notes that “there is nothing
167 sacred about the value of .05” and that biological significance, rather than statistical significance (while necessary)
168 should be emphasized.

169 Table 2 also points to the importance of some individual species: the presence of *Selenastrum* seems to
170 increase reflectance – which is consistent with the fact that *Selenastrum* tended to thrive in any combination of
171 species, and therefore produced a lot of biovolume and opacity (apparently not at the expense of the other species)
172 – and the presence of *Cosmarium* seems to decrease light absorption – which is consistent with our observation
173 that *Cosmarium* did, at best, reproduce less than the other genera (and that competition with other genera was
174 in general detrimental to it), thus making the microcosm not as opaque as it could have been.

175 Adding the initial richness of the microcosm to the initial specification explained more of the variance
176 (Table S3, Fig. 3) than when solely considering the effect of biovolume; the importance of individual species is still
177 supported (see Table S3, though *Cosmarium*'s influence is not significant anymore), but the negative effect of
178 biodiversity (through higher productivity) on the RGB mean is no more. A positive slope is found with the linear
179 specification (column (1) in Table S3 and green line on Fig. 3), and a quadratic specification provides a better fit
180 (column (2) in Table S3 and blue line on Fig. 3), i.e. RGB mean is high in monocultures, decreases in
181 low-diversity mixes, and increases in high-diversity mixes.

182 Based on these results, the first channel (albedo) of influence of diversity on thermal properties seems valid
183 (though somewhat complex), and appears to be mediated by the system's increased productivity (higher
184 biovolume).

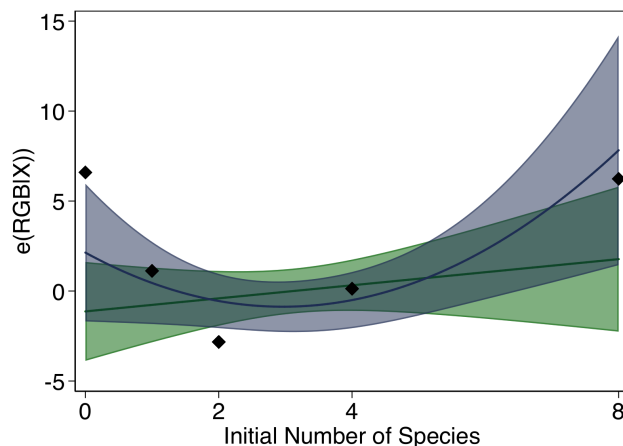


Figure 3. Influence of species richness on visible light reflectance.

Dots represents the mean residual of RGB regressed on the dummies for the functional groups. Solid lines represent a linear fit and quadratic fit, in green and blue, respectively. Shaded areas represent 95% confidence intervals.

185 Local temperature is not directly influenced by biodiversity

186 We now turn to the effect of each hypothesized causal mechanism of influence of biodiversity on temperature, and
187 estimated the model described in Equation 2. The temperature variables we considered were T_a (the average
188 temperature of the content of flask k measured *after* exposure to the light source), ΔT (the temperature *change*
189 before/after exposure), T_{min} , and T_{max} (extreme values measured in the microcosm).

190 We focus here on the results for ΔT , reported in Table 3. The regression tables for the other temperature
191 variables and specifications are consigned in Supporting Information, in tables S4, S5, S6, S7 (T_a , ΔT , T_{max} ,
192 T_{min} , respectively, no RGB), S8, S9, S10, S11 (idem, but linear in RGB), S12, S13, S14 (T_a , T_{max} , T_{min} ,
193 respectively, quadratic in RGB (full specification)). The distribution of the dependent variables T_a , T_{max} , T_{min} is
194 also available in Supporting Information, Fig. S4. Briefly, Tables S4, S8, S12 show that under the specification
195 used, only the time trends and the presence of *Selenastrum* have a robust and significant effect on the
196 temperature of the microcosms after exposition to light (T_a , $N = 169$). As regards the extreme values of the
197 temperature distribution within the microcosm (T_{min} and T_{max} , $N = 169$), while potentially of ecological
198 significance, they do not seem to be affected in a robust manner by anything other than the time trend. These
199 results are presented in Tables S6-S7, S10-S11, S13-S14. While mean RGB and some genera (those with the largest
200 contribution to biovolume) appear to have a significant effect under some specifications, these effects all disappear
201 when the time trend is taken into account (compare columns (3) and (6) of Tables S10, S11, S13, S14), or when
202 the selection and complementarity effects are included, which makes the reality of these effects doubtful.

203 If we restrict our analysis to the data of the second batch ($N = 109$), we can compute the temperature
204 difference ΔT (before/after exposition to the source of light), which is more relevant a variable, and proceed to
205 similar regressions (specification following Equation 2 with ΔT as the dependent variable), whose results are
206 reported in Table 3.

207 Temperature difference is not, unlike the other temperature variables, affected by the warming of the room
208 (the time trend). Rather, as can be seen in Table 3, the reflectance of the suspension, as well as the presence of
209 the isomorphic functional group (IG), are the main drivers of the change in temperature due to exposition to light.
210 The effect of the functional group disappears when selection and complementarity effects are included, but this
211 may be caused by the loss of 20 samples (the monocultures and the controls, for which these variables cannot be
212 computed), thus decreasing statistical power and possibly blurring the picture as a result (lower R^2). We note
213 that the exclusion of 20 samples is likely to have reduced statistical power, so we are cautious in our interpretation
214 of the results. The presence of elements of the IG functional group in the microcosm decreases the temperature
215 change, and this effect seems robust to addition/deletion of controls, see also Table S5). The RGB mean ('albedo')
216 is also an important driver of the magnitude of the temperature change. However, none of the biodiversity effects
217 is significant, in any regression specification we tried.

218 This absence of a distinct, one-sided, biodiversity effect is also visible in Fig. 4: no clear pattern pertaining to
219 the number of species emerges but for the fact that monoculture extremes (encountered for instance with
220 *Ankistrodesmus*, *Selenastrum* and *Staurastrum*) are tempered by the addition of other species. This seems not to
221 be a dilution effect, judging by the differences between $n = 1$ and $n = 2$ for those species. Therefore, if anything,
222 biodiversity, in our microcosm experiment, dampens the thermal properties of the community.

223 Fig. 5 summarizes our findings. An increase in species richness increases biovolume (with a constant number of
224 cells at time $t=0$); an increase in biovolume decreases the mean RGB value; and a decrease in RGB is associated
225 with a decrease in temperature (or possibly an increase in temperature change). However, no empirical evidence
226 supports any effect of biovolume on temperature (change), nor of biodiversity on temperature (change) directly.

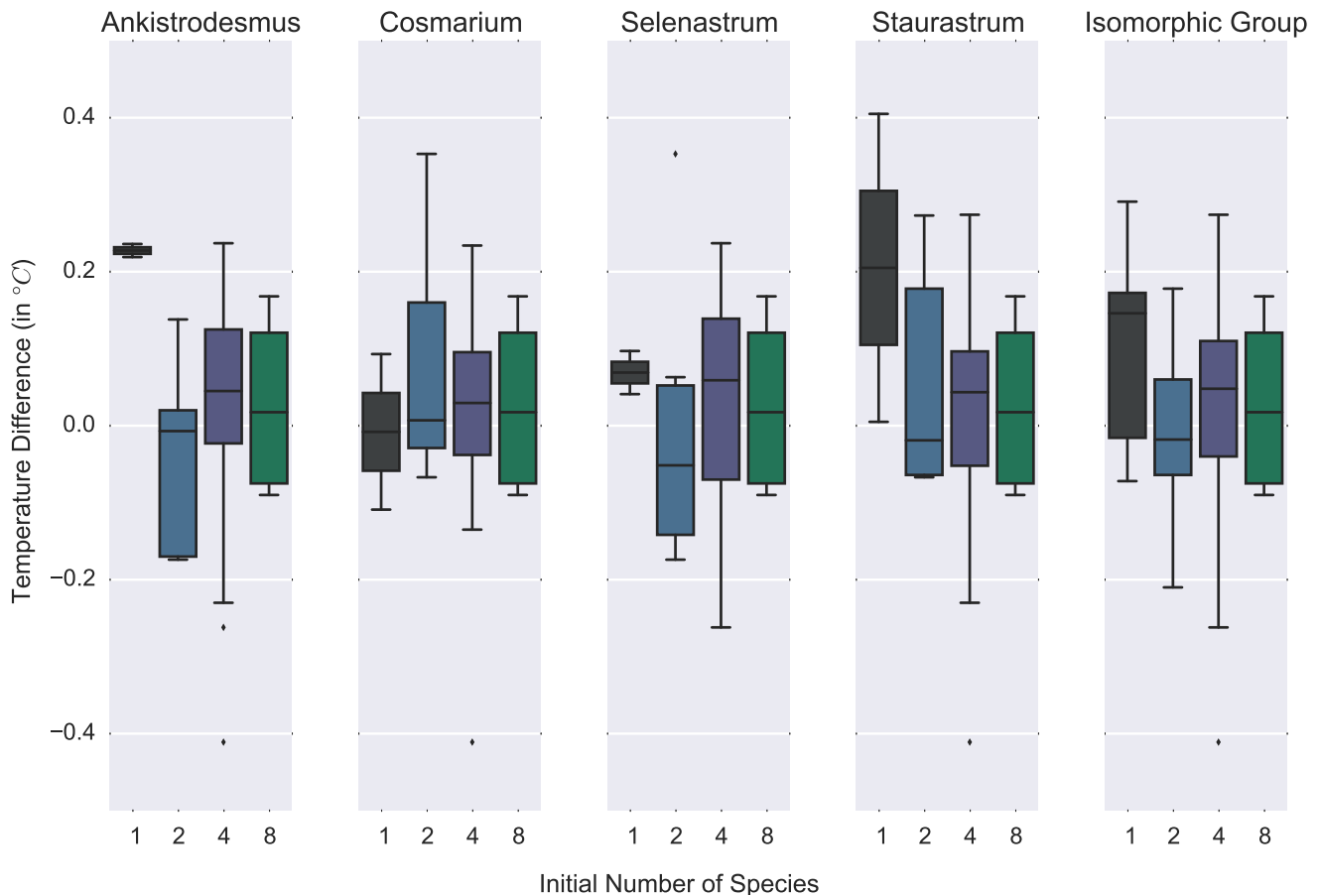


Figure 4. Flask change in temperature, per species

Each column presents the change in temperature (before/after exposition to light) of the flasks containing a particular species, be it as a monoculture or in an assemblage of 2, 4, 8 species. The horizontal line corresponds to the median, the box shows the quartiles, the whiskers describe the rest of the distribution, and the points beyond the whiskers are outliers.

227 Discussion

228 Algal species richness in this microcosm study exhibited the positive relationship with primary production
 229 observed in many BEF experiments, but showed no direct relationship with the local thermal environmental
 230 properties, assessed in this case as the change in temperature, measured by thermography, that occurred after a
 231 60-second exposure to light. Primary production, or algal community biovolume, also did not show a positive
 232 relationship with local thermal environmental properties. Instead, the likely causal chain of the influence of
 233 diversity over the local thermal environment is through its impact on biovolume and RGB reflectance (color).
 234 Fig. 5 summarizes these relationships.

235 Our study focuses on these issues and illustrates both the approach and complications one may encounter in
 236 attempting to identify biodiversity effects that may be subtle or otherwise difficult to detect. We were able to
 237 generate a diversity effect on production, as many BEF experiments have found in the past; this change in
 238 production had an effect on visible-spectrum absorbance (or, its inverse, reflection, which we measured through
 239 RGB imagery). The diversity-induced change in absorbance did impact local temperature, but the effects were
 240 weak (low R^2 s) and ultimately did not provide a statistically significant link between biodiversity, and the local
 241 thermal environment (Fig. 5). The fact that we were unable to find any effect of biodiversity and biovolume on
 242 temperature (despite the other relationships found) could indicate that there is indeed no effect, or that our
 243 sample size was too small and our experimental protocols too imprecise (in particular, the fact that we were
 244 unable to discriminate between genera of the 'IG' functional group). In addition, it should be kept in mind that,

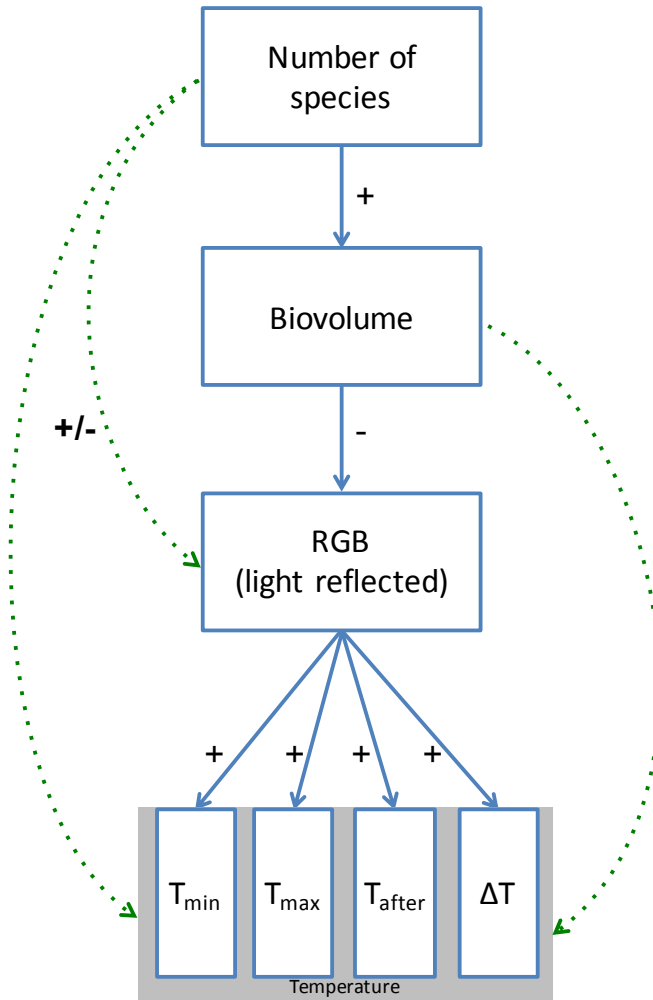


Figure 5. Summary schematic of collective findings and conclusions.

Solid lines represent the statistically significant and unambiguous results, with a plus or minus sign representing the type of the relationship. Dotted lines represent the non-significant relationships. In our microcosms, increased species richness led to an increased biovolume, which in turn led to higher mean RGB (reflectance) values, and higher mean RGB values were significantly associated with higher thermal outcomes: higher ΔT (the temperature difference before/after exposition to light), higher T_a (the temperature difference after exposition to light), higher T_{min} and T_{max} (local extrema).

245 as any typical BEF experiment, this protocol does not enable to distinguish between “noise” variation
 246 (measurement error, etc.) and variation caused by community composition, the latter of which is at play in
 247 communities made up of 1 to 4 species, but not 8 (all species).

248 Given the challenges of measuring potentially subtle effects in algal communities, if one considers that small
 249 changes in temperature affect numerous microbial processes in phytoplankton and their associated microbial
 250 communities, our findings potentially touch upon important possibilities for the impacts of changing biodiversity
 251 on ecosystem functions and properties. It has also been recently noted that microcosm experiments manipulating
 252 biodiversity tended to underestimate outcomes occurring in the wild (in terms of community production and
 253 stability) (6). Given the vast surface area of freshwater and marine systems and the clarity of the mechanism we
 254 identified, even though the direct linkage between diversity and temperature was difficult to detect, the
 255 implications are clear. Our results should encourage an alteration in the way albedo is modeled, and go beyond
 256 the uniform and time-invariant value attributed to bodies of water, and more generally, to biomes. Such diversity
 257 effects could translate to important temperature-mediated biogeochemical consequences at large scales in our
 258 world where changes in climate and biodiversity are co-occurring.

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262 Author contributions

263 All authors contributed equally to this work.

264 Competing interests

265 The authors declare no competing interests.

266 Data Accessibility

267 Experimental data necessary to reproduce the analyses are made available on Dryad doi:10.5061/dryad.59p3vv5.

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Tables

Table 1. Influence of microcosm composition on biovolume. Each column corresponds to a separate regression: the independent variables are, in column (1), species richness (linear), in column (2), a quadratic in species richness, in column (3), species richness (linear) and dummies (indicator functions) indicating the presence/absence of each group in the initial species mixture, in column (4), a quadratic in species richness and the group dummies.

Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. $N = 162$. Robust standard errors are indicated in parentheses.

Dependent Variable: Biovolume				
	(1)	(2)	(3)	(4)
N_{Species}	19704.682*** (6556.075)	34765.725* (17619.573)	24697.360* (12963.029)	43228.318* (24767.124)
N_{Species}^2		-2141.077 (1982.076)		-2179.164 (2244.786)
<i>Ankistrodesmus</i>			-39205.166 (26992.927)	-42324.962 (26806.441)
<i>Cosmarium</i>			49323.755 (33073.750)	46096.612 (33004.238)
<i>Selenastrum</i>			-38465.483 (27172.388)	-41133.504 (27390.329)
<i>Staurastrum</i>			-21112.737 (27379.180)	-23953.073 (27880.794)
Isomorphic Group			12150.509 (44893.394)	-3248.527 (50141.158)
Constant	139032.475*** (25403.689)	118424.819*** (35827.400)	131024.894*** (36880.235)	117830.291*** (38599.427)
R^2	0.046	0.050	0.107	0.111

Table 2. Regression: species presence and biovolume effect on standardized RGB Each column corresponds to a separate regression on standardized RGB: the independent variables are, in column (1), standardized biovolume, in column (2), standardized biovolume and group dummies (signalling the initial presence/absence of each group in the microcosm).

Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

Dependent Variable: Standardized RGB		
	(1)	(2)
Standardized Biovolume	-0.107 (0.077)	-0.140* (0.079)
<i>Ankistrodesmus</i>		-0.253 (0.167)
<i>Cosmarium</i>		0.292* (0.160)
<i>Selenastrum</i>		-0.392** (0.155)
<i>Staurastrum</i>		0.084 (0.165)
Isomorphic Group		-0.292 (0.246)
Constant	0.005 (0.079)	0.353 (0.250)
R^2	0.011	0.099
N	162	162

Table 3. Regression results: Influence of microcosm composition and greenness on ΔT . Each column corresponds to a separate regression on ΔT : the independent variables are, in column (1), a quadratic in RGB mean, in column (2), a quadratic in RGB mean and group dummies, in column (3), a quadratic in RGB mean, group dummies and a time trend, in column (4), selection and complementarity effects only, in column (5), a quadratic in RGB mean, group dummies, selection and complementarity effects, in column (6), a quadratic in RGB mean, group dummies, a time trend, selection and complementarity effects.

Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

Dependent Variable: Temperature Difference (°C)						
	(1)	(2)	(3)	(4)	(5)	(6)
(RGB Mean)	0.068 (0.066)	0.202*** (0.071)	0.200*** (0.071)		0.180** (0.089)	0.184** (0.089)
(RGB Mean) ²	-0.000 (0.000)	-0.001*** (0.000)	-0.001*** (0.000)		-0.001** (0.000)	-0.001** (0.000)
<i>Ankistrodesmus</i>		0.007 (0.025)	0.008 (0.025)		-0.002 (0.031)	-0.001 (0.031)
<i>Cosmarium</i>		-0.027 (0.025)	-0.024 (0.026)		-0.023 (0.032)	-0.016 (0.034)
<i>Selenastrum</i>		-0.008 (0.028)	-0.003 (0.031)		0.003 (0.031)	0.011 (0.035)
<i>Staurastrum</i>		-0.006 (0.027)	-0.001 (0.028)		-0.007 (0.030)	-0.000 (0.031)
Isomorphic Group		-0.128*** (0.043)	-0.121*** (0.045)		-0.097 (0.073)	-0.083 (0.072)
Time Trend Second Batch			-0.000 (0.000)			-0.000 (0.000)
Selection Effect				0.000 (0.000)	-0.000 (0.000)	-0.000 (0.000)
Complementarity Effect				0.000 (0.000)	-0.000 (0.000)	-0.000 (0.000)
Constant	-4.868 (4.620)	-14.029*** (4.968)	-13.845*** (4.952)	0.015 (0.022)	-12.566** (6.168)	-12.849** (6.213)
R^2	0.019	0.121	0.123	0.006	0.078	0.081
N	109	109	109	84	84	84

Figure Legends

Figure 1. Experimental design: conceptual diagram RGB refers to red, green, and blue sensors in visible spectrum camera.

Figure 2. Flask biovolume per species Each column presents the biovolumes of the flasks containing a particular species, be it as a monoculture or in an assemblage of 2, 4, 8 species. The horizontal line corresponds to the median, the box shows the quartiles, the whiskers describe the rest of the distribution, and the points beyond the whiskers are outliers.

Figure 3. Influence of species richness on visible light reflectance Dots represents the mean residual of RGB regressed on the dummies for the functional groups. Solid lines represent a linear fit and quadratic fit, in green and blue, respectively. Shaded areas represent 95% confidence intervals.

Figure 4. Flask change in temperature, per species Each column presents the change in temperature (before/after exposition to light) of the flasks containing a particular species, be it as a monoculture or in an assemblage of 2, 4, 8 species. The horizontal line corresponds to the median, the box shows the quartiles, the whiskers describe the rest of the distribution, and the points beyond the whiskers are outliers.

Figure 5. Summary schematic of collective findings and conclusions Solid lines represent the statistically significant and unambiguous results, with a plus or minus sign representing the type of the relationship. Dotted lines represent the non-significant relationships. In our microcosms, increased species richness led to an increased biovolume, which in turn led to higher mean RGB (reflectance) values, and higher mean RGB values were significantly associated with higher thermal outcomes: higher ΔT (the temperature difference before/after exposition to light), higher T_a (the temperature difference after exposition to light), higher T_{min} and T_{max} (local extrema).

Supporting Information

Additional experimental details

Timing of the experiment The first batch of microcosms was assembled on November 26th, 2014, and the last series of microcosms were imaged on December 15th, 2014, allowing for a 13 day incubation period for all the microcosms.

Sampling schedule and processing The first batch of microcosms was prepared on November 26th, 2014, and sacrificed 13 days later (i.e. December 9th): thermal and visible imagery was conducted, and samples were subsequently disposed of. The second and third batches were prepared in the same fashion together on December 2nd, 2014, and imaged together on December 15th, 13 days after assembly as well. Thirteen days was the duration required to ascertain reasonable (observable) growth was taking place; it is consistent with the duration specified on growth medium for “most algae [to] show substantial growth”. Before assembly, the pure algal strands were stored at room temperature under a 24 W T5 fluorescent lamp. After assembly, the microcosms were stored at room temperature and were shuffled daily to homogenize exposition to light.

S1 Table

Table S1. Community combinatorics.

	C_n^k	replicates	nb. microcosms
0 species (control)	1		5
1 species	8	8 triplicates	24
2 species	28	12 duplicates, 16 singles	70
4 species	70	24 duplicates, 46 singles	54
8 species	1	1 sextuplicate	6
Total			169

Species information

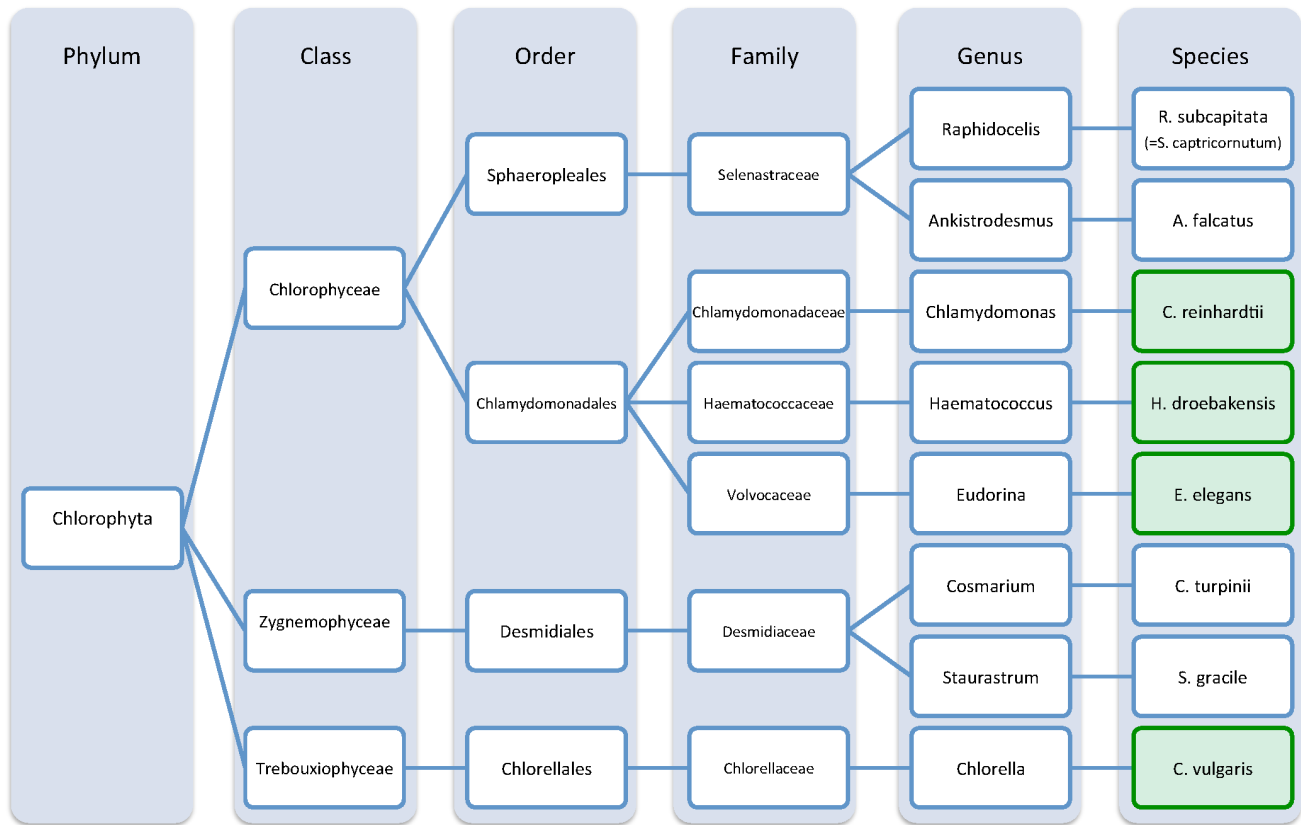
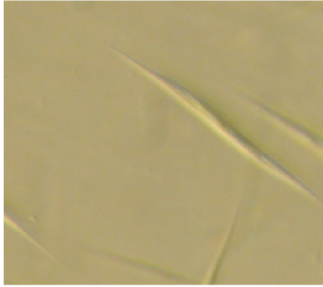


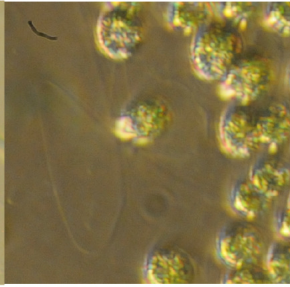
Figure S1. Phylogenetic information.

Phylogenetic relationships of the eight species used. Shaded in green: the four isomorphic species.

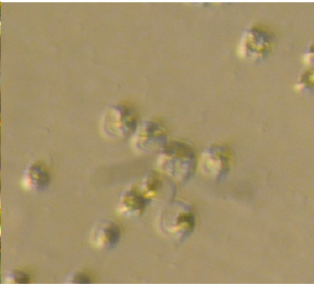
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falcatus*



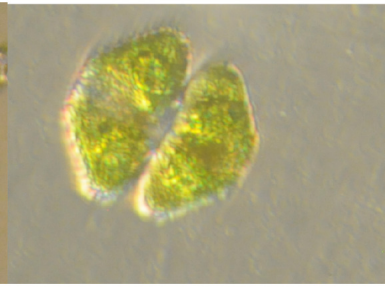
*Chlamydomonas
reinhardi*



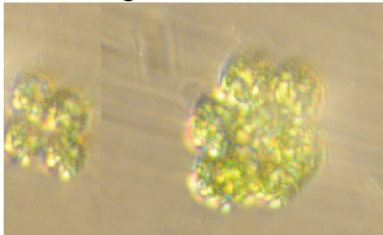
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vulgaris*



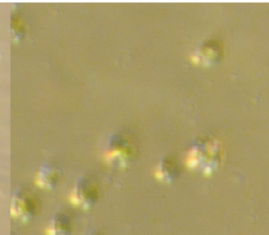
*Cosmarium
turpinii*



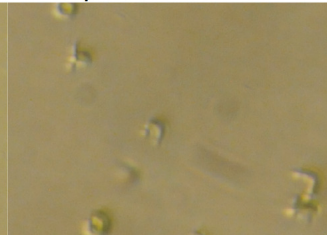
*Eudorina
elegans*



*Haematococcus
droebakensis*



*Selenastrum
capricornutum*



*Staurastrum
gracile*

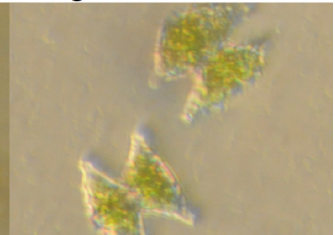


Figure S2. Algal morphology.

Plate used for species identification.

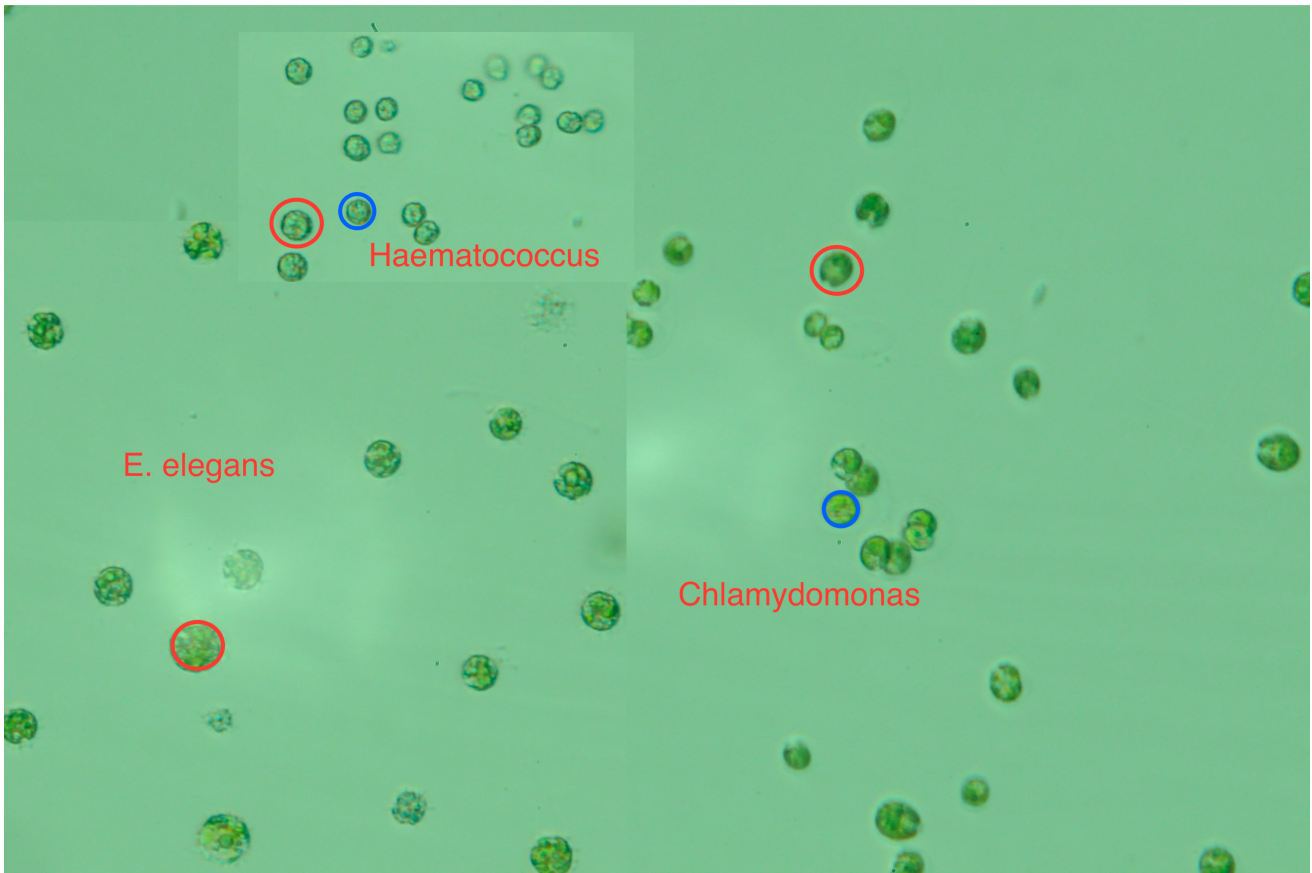


Figure S3. Algal morphology, experimental conditions.

Mosaic of photographs of slides under inverted microscope at magnification 40x, used for enumeration. Annotated to highlight morphological closeness between *Chlamydomonas*, *E. elegans*, *Haematococcus*.

Empirical biovolumes

Table S2. Biovolumes

Species	Biovolume (μm^3)	replicates	Std. err.
<i>Ankistrodesmus falcatus</i>	116	75	9.9
<i>Chlamydomonas reinhardtii</i>	313	75	21.7
<i>Chlorella vulgaris</i>	95	75	8.1
<i>Cosmarium turpinii</i>	33 226	20	2 355.8
<i>Eudorina elegans</i>	456	75	46.2
<i>Haematococcus droebakensis</i>	154	75	10.2
<i>Selenastrum capricornutum</i>	45	75	2.9
<i>Staurastrum gracile</i>	2 882	20	196.5

Temperature distributions in the sample

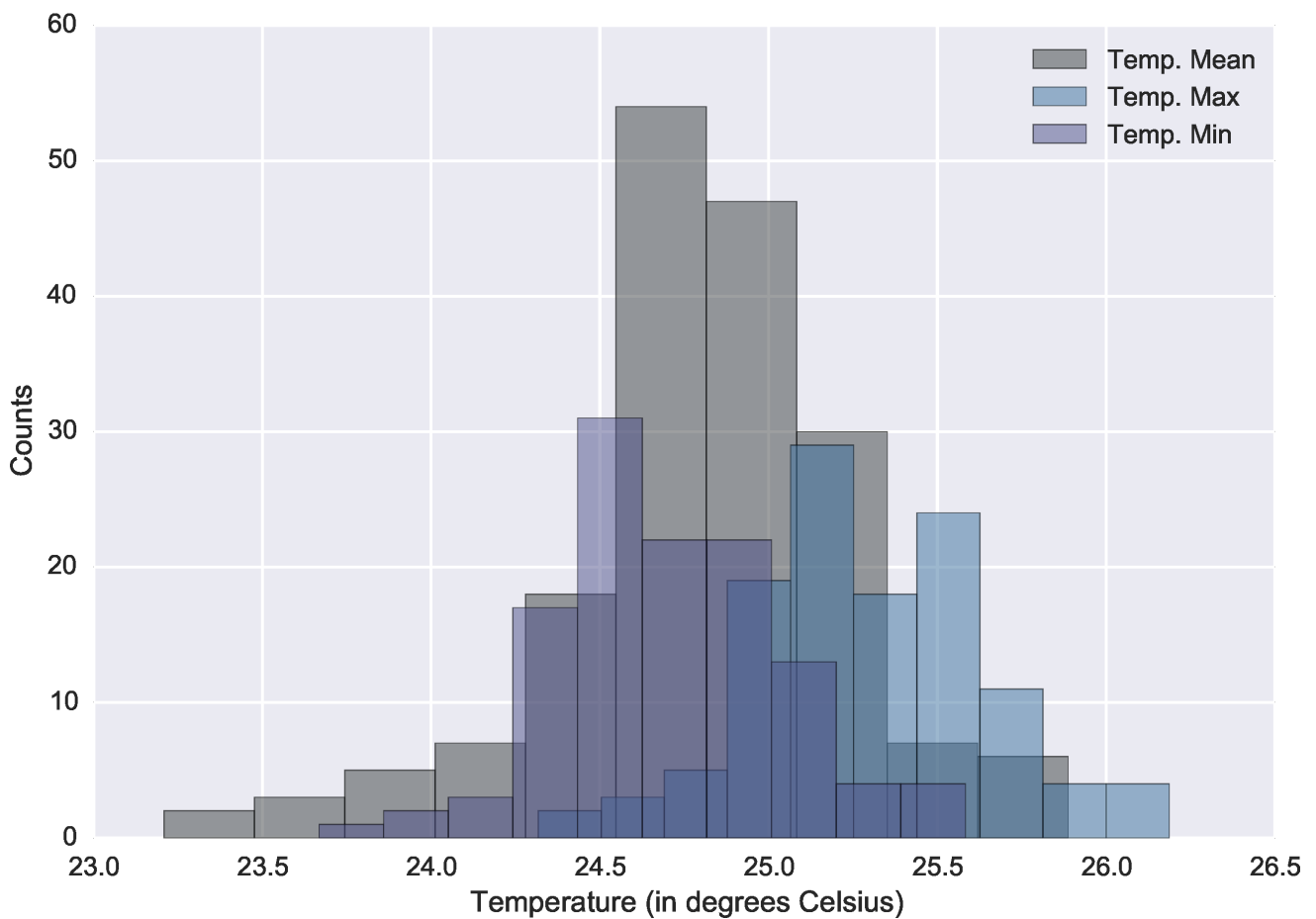


Figure S4. Distribution of temperature (min, max, average) in the sample.

Species richness is positively correlated to biovolume

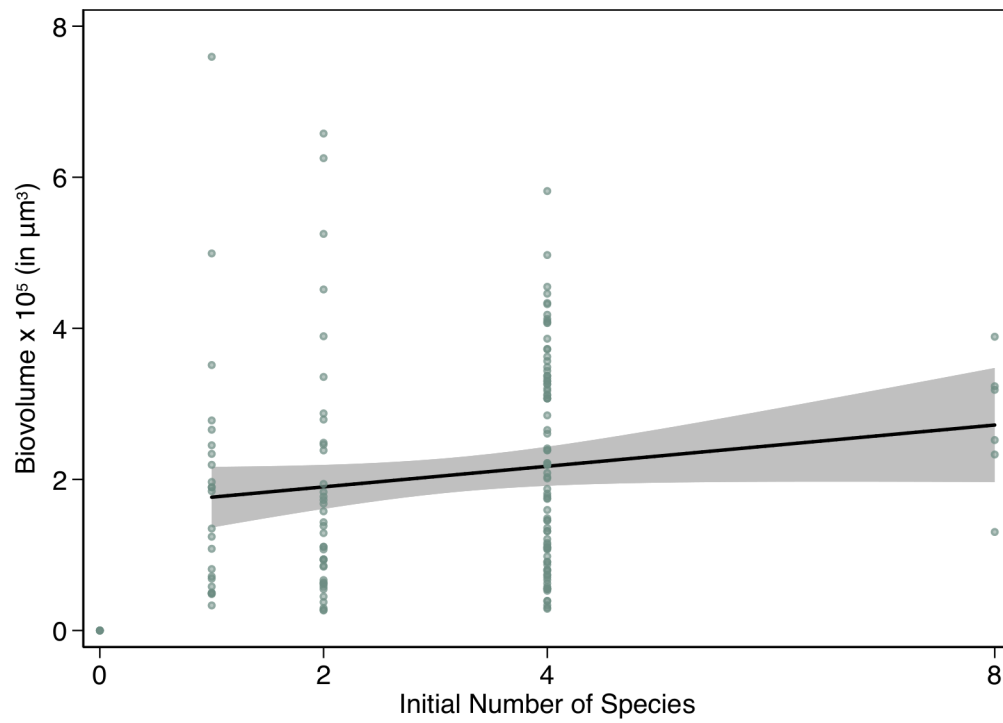


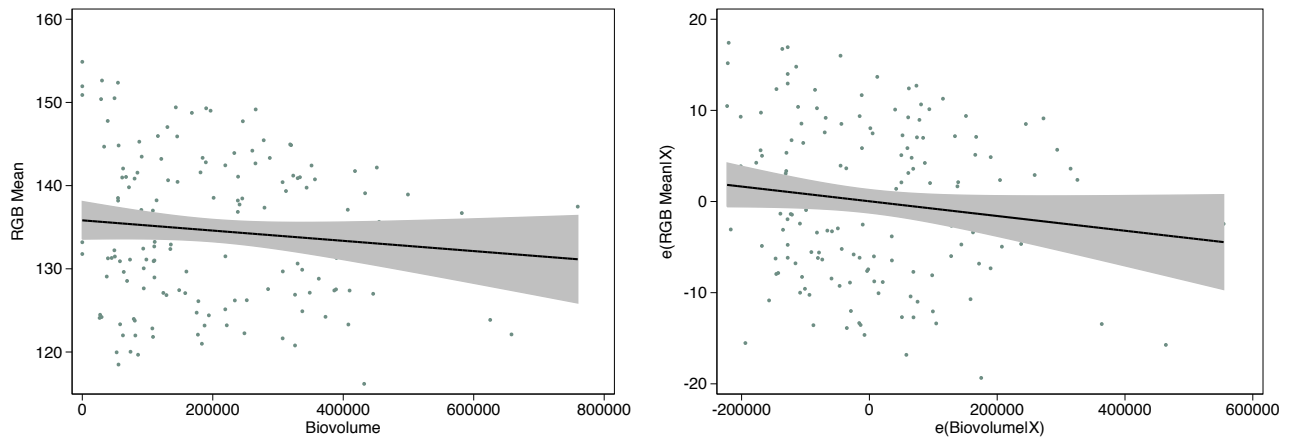
Figure S5. Microcosm biovolume and initial species richness

Solid line represents the linear best fit of the measured biovolume as a function of the number of initial species. Shaded area represents 95% confidence intervals.

Biovolume is negatively correlated to RGB

In both regressions presented in Table 2, biovolume is negatively associated with RGB values (albeit insignificantly so in Column (1)). Column (2) shows that this relationship persists, and is strengthened (i.e. becomes significant at the 10% level), when the effect of individual species' presence is controlled for. Note that Column (2) also points to the importance of some individual species: the presence of *Selenastrum* seems to increase “greenness” – which is consistent with the fact that *Selenastrum* tended to thrive in any combination of species, and therefore produced a lot of biovolume and opacity (apparently not at the expense of the other species) – and the presence of *Cosmarium* seems to decrease light absorption – which is consistent with our observation that *Cosmarium* did, at best, reproduce less than the other genera (and that competition with other genera was in general detrimental to it), thus making the microcosm not as opaque as it could have been.

Fig. S6a shows the RGB values versus the biovolume (analogous to Column (1) of Table 2), and hints at a negative but weak relationship between biovolume and RGB; on Fig. S6b are plotted the residuals from the regression of RGB on the functional group dummies, against the residuals from biovolume on the functional group dummies – i.e. a partial regression plot – which shows that the negative slope persists even when the effect of individual species' presence is subtracted from both biovolume and RGB (mirroring the findings of Column (2) in Table 2).



(a) Scatter Plot

(b) Partial Regression Plot

Figure S6. Influence of biovolume on RGB Mean

Scatter plot and the partial regression plot showing the negative linear relationship between the mean RGB and biovolume. Solid line shows the linear best fit. Shaded area shows the 95% confidence intervals.

There is a quadratic relationship between species richness and RGB

Table S3. Regression results: Microcosm composition and RGB mean value.

Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$

Dependent Variable: RGB Mean		
	(1)	(2)
N_{Species}	1.487** (0.750)	-1.315 (1.431)
N_{Species}^2		0.330** (0.162)
<i>Ankistrodesmus</i>	-3.682** (1.521)	-3.170** (1.502)
<i>Cosmarium</i>	0.297 (1.511)	0.846 (1.497)
<i>Selenastrum</i>	-4.827*** (1.358)	-4.402*** (1.366)
<i>Staurastrum</i>	-1.310 (1.644)	-0.862 (1.641)
Isomorphic Group	-5.310** (2.474)	-3.069 (2.469)
Constant	138.099*** (2.097)	140.139*** (2.312)
R^2	0.100	0.123
N	169	169

Other regressions

Table S4. Regression results: Microcosm composition and T_a .

Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

	Dependent Variable: Temperature After (°C)				
	(1)	(2)	(3)	(4)	(5)
<i>Ankistrodesmus</i>	-0.085 (0.070)	-0.104* (0.053)		-0.082 (0.087)	-0.093 (0.071)
<i>Cosmarium</i>	-0.004 (0.071)	-0.047 (0.059)		0.023 (0.082)	-0.045 (0.072)
<i>Selenastrum</i>	0.102 (0.070)	0.120** (0.059)		0.115 (0.079)	0.137* (0.072)
<i>Staurastrum</i>	0.088 (0.069)	0.079 (0.062)		0.115 (0.085)	0.090 (0.077)
Isomorphic Group	-0.020 (0.060)	-0.001 (0.070)		0.000 (0.114)	-0.009 (0.120)
Time Trend First Batch		-0.001*** (0.000)			-0.001** (0.000)
Time Trend Second Batch		0.000*** (0.000)			0.001*** (0.000)
Selection Effect			-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Complementarity Effect			-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Constant	24.779*** (0.058)	24.726*** (0.066)	24.846*** (0.074)	24.771*** (0.153)	24.629*** (0.156)
R^2	0.030	0.396	0.009	0.048	0.429
N	169	169	129	129	129

Table S5. Regression results: Microcosm composition and ΔT .Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

	Dependent Variable: Temperature Difference (°C)				
	(1)	(2)	(3)	(4)	(5)
<i>Ankistrodesmus</i>	-0.006 (0.027)	-0.005 (0.027)		-0.011 (0.031)	-0.011 (0.031)
<i>Cosmarium</i>	-0.015 (0.025)	-0.012 (0.027)		-0.002 (0.032)	0.000 (0.034)
<i>Selenastrum</i>	-0.010 (0.027)	-0.007 (0.028)		0.002 (0.029)	0.003 (0.032)
<i>Staurastrum</i>	0.002 (0.027)	0.006 (0.030)		0.001 (0.029)	0.003 (0.032)
Isomorphic Group	-0.081* (0.043)	-0.076* (0.043)		-0.052 (0.080)	-0.048 (0.080)
Time Trend Second Batch		-0.000 (0.000)			-0.000 (0.000)
Selection Effect			0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Complementarity Effect			0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Constant	0.116*** (0.043)	0.124** (0.049)	0.015 (0.022)	0.066 (0.091)	0.068 (0.094)
R^2	0.057	0.058	0.006	0.019	0.020
N	109	109	84	84	84

Table S6. Regression results: Microcosm composition and T_{max} .Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

	Dependent Variable: Temperature Maximum (°C)				
	(1)	(2)	(3)	(4)	(5)
<i>Ankistrodesmus</i>	-0.094 (0.072)	-0.114** (0.053)		-0.092 (0.089)	-0.105 (0.072)
<i>Cosmarium</i>	-0.001 (0.073)	-0.053 (0.059)		0.029 (0.084)	-0.055 (0.071)
<i>Selenastrum</i>	0.093 (0.072)	0.106* (0.058)		0.100 (0.081)	0.113 (0.072)
<i>Staurastrum</i>	0.080 (0.071)	0.063 (0.062)		0.105 (0.088)	0.067 (0.078)
Isomorphic Group	-0.020 (0.062)	-0.007 (0.067)		0.016 (0.123)	-0.018 (0.131)
Time Trend First Batch		-0.001*** (0.000)			-0.001** (0.000)
Time Trend Second Batch		0.001*** (0.000)			0.001*** (0.000)
Selection Effect			-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Complementarity Effect			-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Constant	25.098*** (0.060)	25.033*** (0.064)	25.162*** (0.076)	25.089*** (0.162)	24.931*** (0.163)
R^2	0.026	0.427	0.010	0.044	0.448
N	169	169	129	129	129

Table S7. Regression results: Microcosm composition and T_{min} .Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

	Dependent Variable: Temperature Minimum (°C)				
	(1)	(2)	(3)	(4)	(5)
<i>Ankistrodesmus</i>	-0.069 (0.066)	-0.085 (0.052)		-0.065 (0.082)	-0.075 (0.069)
<i>Cosmarium</i>	-0.024 (0.068)	-0.059 (0.058)		-0.001 (0.078)	-0.065 (0.070)
<i>Selenastrum</i>	0.086 (0.066)	0.105* (0.058)		0.096 (0.074)	0.114 (0.072)
<i>Staurastrum</i>	0.079 (0.066)	0.075 (0.061)		0.104 (0.081)	0.079 (0.076)
Isomorphic Group	-0.026 (0.057)	-0.005 (0.068)		-0.013 (0.105)	-0.026 (0.114)
Time Trend First Batch		-0.001*** (0.000)			-0.001** (0.000)
Time Trend Second Batch		0.000*** (0.000)			0.001*** (0.000)
Selection Effect			-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Complementarity Effect			-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Constant	24.546*** (0.055)	24.505*** (0.064)	24.585*** (0.071)	24.535*** (0.141)	24.406*** (0.146)
R^2	0.025	0.346	0.008	0.040	0.382
N	169	169	129	129	129

Table S8. Regression results: mean RGB and T_a (linear).Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

	Dependent Variable: Temperature After (°C)					
	(1)	(2)	(3)	(4)	(5)	(6)
(RGB Mean)	0.020*** (0.004)	0.022*** (0.004)	-0.001 (0.005)		0.027*** (0.005)	-0.001 (0.006)
<i>Ankistrodesmus</i>		-0.038 (0.063)	-0.108* (0.056)		-0.066 (0.079)	-0.093 (0.072)
<i>Cosmarium</i>		-0.049 (0.065)	-0.048 (0.059)		-0.074 (0.073)	-0.044 (0.072)
<i>Selenastrum</i>		0.176*** (0.066)	0.115* (0.064)		0.173** (0.075)	0.135* (0.077)
<i>Staurastrum</i>		0.081 (0.061)	0.077 (0.063)		0.083 (0.073)	0.089 (0.078)
Isomorphic Group		0.039 (0.073)	-0.006 (0.070)		-0.051 (0.173)	-0.009 (0.119)
Time Trend First Batch			-0.001*** (0.000)			-0.001** (0.000)
Time Trend Second Batch			0.000*** (0.000)			0.001** (0.000)
Selection Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Complementarity Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Constant	22.084*** (0.489)	21.689*** (0.550)	24.918*** (0.635)	24.846*** (0.074)	21.166*** (0.659)	24.727*** (0.787)
R^2	0.157	0.207	0.396	0.009	0.269	0.429
N	169	169	169	129	129	129

Table S9. Regression results: mean RGB and ΔT (linear).Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

Dependent Variable: Temperature Difference (°C)						
	(1)	(2)	(3)	(4)	(5)	(6)
(RGB Mean)	0.002 (0.002)	0.002 (0.002)	0.002 (0.002)		0.003 (0.003)	0.003 (0.003)
<i>Ankistrodesmus</i>		-0.004 (0.026)	-0.002 (0.026)		-0.012 (0.031)	-0.011 (0.031)
<i>Cosmarium</i>		-0.019 (0.026)	-0.014 (0.027)		-0.013 (0.033)	-0.007 (0.034)
<i>Selenastrum</i>		-0.003 (0.028)	0.005 (0.031)		0.010 (0.030)	0.016 (0.035)
<i>Staurastrum</i>		-0.001 (0.028)	0.006 (0.030)		-0.006 (0.030)	-0.000 (0.032)
Isomorphic Group		-0.077* (0.044)	-0.067 (0.045)		-0.058 (0.078)	-0.046 (0.078)
Time Trend Second Batch			-0.000 (0.000)			-0.000 (0.000)
Selection Effect				0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Complementarity Effect				0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Constant	-0.242 (0.274)	-0.106 (0.304)	-0.170 (0.324)	0.015 (0.022)	-0.343 (0.355)	-0.371 (0.374)
R^2	0.010	0.062	0.066	0.006	0.036	0.038
N	109	109	109	84	84	84

Table S10. Regression results: mean RGB and T_{max} (linear).Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

	Dependent Variable: Temperature Maximum (°C)					
	(1)	(2)	(3)	(4)	(5)	(6)
(RGB Mean)	0.022*** (0.004)	0.024*** (0.004)	-0.001 (0.005)		0.029*** (0.005)	-0.001 (0.006)
<i>Ankistrodesmus</i>		-0.043 (0.063)	-0.117** (0.055)		-0.076 (0.080)	-0.105 (0.072)
<i>Cosmarium</i>		-0.049 (0.066)	-0.053 (0.059)		-0.075 (0.075)	-0.054 (0.071)
<i>Selenastrum</i>		0.173** (0.067)	0.102 (0.064)		0.162** (0.076)	0.111 (0.077)
<i>Staurastrum</i>		0.073 (0.063)	0.062 (0.063)		0.070 (0.075)	0.066 (0.079)
Isomorphic Group		0.044 (0.074)	-0.011 (0.067)		-0.039 (0.178)	-0.018 (0.130)
Time Trend First Batch			-0.001*** (0.000)			-0.001** (0.000)
Time Trend Second Batch			0.001*** (0.000)			0.001*** (0.000)
Selection Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Complementarity Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Constant	22.123*** (0.496)	21.737*** (0.555)	25.179*** (0.634)	25.162*** (0.076)	21.254*** (0.670)	25.030*** (0.788)
R^2	0.181	0.227	0.427	0.010	0.281	0.448
N	169	169	169	129	129	129

Table S11. Regression results: mean RGB and T_{min} (linear).Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

Dependent Variable: Temperature Minimum (°C)						
	(1)	(2)	(3)	(4)	(5)	(6)
(RGB Mean)	0.018*** (0.004)	0.020*** (0.004)	-0.001 (0.005)		0.025*** (0.005)	0.000 (0.006)
<i>Ankistrodesmus</i>		-0.028 (0.060)	-0.087 (0.055)		-0.051 (0.075)	-0.075 (0.070)
<i>Cosmarium</i>		-0.064 (0.062)	-0.059 (0.058)		-0.090 (0.070)	-0.065 (0.071)
<i>Selenastrum</i>		0.151** (0.063)	0.103 (0.064)		0.149** (0.071)	0.114 (0.076)
<i>Staurastrum</i>		0.073 (0.059)	0.074 (0.062)		0.075 (0.070)	0.079 (0.077)
Isomorphic Group		0.026 (0.071)	-0.007 (0.069)		-0.059 (0.160)	-0.026 (0.115)
Time Trend First Batch			-0.001*** (0.000)			-0.001** (0.000)
Time Trend Second Batch			0.000** (0.000)			0.001** (0.000)
Selection Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Complementarity Effect				-0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Constant	22.139*** (0.476)	21.793*** (0.530)	24.603*** (0.646)	24.585*** (0.071)	21.273*** (0.622)	24.403*** (0.804)
R^2	0.138	0.183	0.346	0.008	0.244	0.382
N	169	169	169	129	129	129

Table S12. Regression results: mean RGB and T_a (quadratic).Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

	Dependent Variable: Temperature After (°C)					
	(1)	(2)	(3)	(4)	(5)	(6)
(RGB Mean)	0.338*** (0.094)	0.325*** (0.101)	0.098 (0.098)		0.398*** (0.137)	0.114 (0.124)
(RGB Mean) ²	-0.001*** (0.000)	-0.001*** (0.000)	-0.000 (0.000)		-0.001*** (0.001)	-0.000 (0.000)
<i>Ankistrodesmus</i>		-0.019 (0.063)	-0.098* (0.056)		-0.047 (0.077)	-0.086 (0.072)
<i>Cosmarium</i>		-0.042 (0.063)	-0.047 (0.059)		-0.053 (0.073)	-0.039 (0.071)
<i>Selenastrum</i>		0.148** (0.065)	0.107* (0.065)		0.136* (0.074)	0.126 (0.077)
<i>Staurastrum</i>		0.105* (0.060)	0.083 (0.063)		0.109 (0.073)	0.098 (0.080)
Isomorphic Group		-0.020 (0.070)	-0.025 (0.072)		-0.096 (0.153)	-0.023 (0.120)
Time Trend First Batch			-0.001*** (0.000)			-0.001** (0.000)
Time Trend Second Batch			0.000*** (0.000)			0.001** (0.000)
Selection Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Complementarity Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Constant	0.679 (6.390)	1.370 (6.834)	18.163*** (6.666)	24.846*** (0.074)	-3.518 (9.190)	16.972** (8.320)
R^2	0.204	0.246	0.400	0.009	0.312	0.432
N	169	169	169	129	129	129

Table S13. Regression results: mean RGB and T_{max} (quadratic).Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

	Dependent Variable: Temperature Maximum (°C)					
	(1)	(2)	(3)	(4)	(5)	(6)
(RGB Mean)	0.342*** (0.095)	0.327*** (0.102)	0.087 (0.096)		0.407*** (0.139)	0.105 (0.123)
(RGB Mean) ²	-0.001*** (0.000)	-0.001*** (0.000)	-0.000 (0.000)		-0.001*** (0.001)	-0.000 (0.000)
<i>Ankistrodesmus</i>		-0.024 (0.063)	-0.109* (0.056)		-0.057 (0.079)	-0.098 (0.072)
<i>Cosmarium</i>		-0.042 (0.064)	-0.052 (0.059)		-0.053 (0.075)	-0.049 (0.071)
<i>Selenastrum</i>		0.145** (0.066)	0.095 (0.064)		0.124* (0.074)	0.103 (0.077)
<i>Staurastrum</i>		0.096 (0.061)	0.067 (0.063)		0.096 (0.075)	0.074 (0.080)
Isomorphic Group		-0.016 (0.072)	-0.028 (0.069)		-0.084 (0.160)	-0.032 (0.133)
Time Trend First Batch			-0.001*** (0.000)			-0.001** (0.000)
Time Trend Second Batch			0.001*** (0.000)			0.001*** (0.000)
Selection Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Complementarity Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Constant	0.566 (6.437)	1.444 (6.930)	19.194*** (6.513)	25.162*** (0.076)	-3.894 (9.294)	17.871** (8.286)
R^2	0.227	0.264	0.430	0.010	0.324	0.451
N	169	169	169	129	129	129

Table S14. Regression results: mean RGB and T_{min} (quadratic).Notes: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$. Robust standard errors in parentheses.

	Dependent Variable: Temperature Minimum (°C)					
	(1)	(2)	(3)	(4)	(5)	(6)
(RGB Mean)	0.295*** (0.090)	0.287*** (0.097)	0.085 (0.097)		0.331** (0.135)	0.078 (0.124)
(RGB Mean) ²	-0.001*** (0.000)	-0.001*** (0.000)	-0.000 (0.000)		-0.001** (0.001)	-0.000 (0.000)
<i>Ankistrodesmus</i>		-0.011 (0.060)	-0.079 (0.055)		-0.036 (0.074)	-0.070 (0.070)
<i>Cosmarium</i>		-0.057 (0.061)	-0.058 (0.058)		-0.072 (0.071)	-0.061 (0.070)
<i>Selenastrum</i>		0.127** (0.063)	0.096 (0.064)		0.119* (0.070)	0.109 (0.076)
<i>Staurastrum</i>		0.094 (0.059)	0.079 (0.062)		0.096 (0.071)	0.085 (0.078)
Isomorphic Group		-0.026 (0.068)	-0.024 (0.072)		-0.096 (0.145)	-0.035 (0.116)
Time Trend First Batch			-0.001*** (0.000)			-0.001* (0.000)
Time Trend Second Batch			0.000** (0.000)			0.000** (0.000)
Selection Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Complementarity Effect				-0.000 (0.000)	-0.000 (0.000)	0.000 (0.000)
Constant	3.506 (6.089)	3.914 (6.546)	18.766*** (6.591)	24.585*** (0.071)	0.909 (9.024)	19.177** (8.332)
R^2	0.178	0.216	0.349	0.008	0.277	0.384
N	169	169	169	129	129	129