

1 **Abrupt declines in the production of marine phytoplankton driven by**
2 **warming and biodiversity loss in a microcosm experiment**

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16

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27 **Abstract:**

28 Rising sea surface temperatures are expected to lead to the loss of phytoplankton biodiversity. However,
29 we currently understand very little about the interactions between warming, loss of phytoplankton
30 diversity and its impact on oceans' primary production. We experimentally manipulated the species
31 richness of marine phytoplankton communities under a range of warming scenarios, and found that
32 ecosystem production declined more abruptly with species loss in communities exposed to higher
33 temperatures. Species contributing positively to ecosystem production in the warmed treatments were
34 those that had the highest optimal temperatures for photosynthesis, implying that the synergistic impacts
35 of warming and biodiversity loss on ecosystem functioning were mediated by thermal trait variability.
36 As species were lost, the probability of taxa remaining that could tolerate warming diminished, resulting
37 in abrupt declines in ecosystem production. Our results highlight the potential for synergistic effects of
38 warming and biodiversity loss on ocean production.

39

40 **Introduction:**

41 Experiments, mostly in grasslands, have shown strong effects of plant diversity on ecosystem
42 production (Naeem *et al.* 1994; Tilman & Downing 1994; Tilman *et al.* 1997; Loreau *et al.* 2001;
43 Hooper *et al.* 2005). Production typically increases with species richness in a saturating manner or as a
44 continuously increasing, but decelerating function (i.e. logarithmic), implying some degree of
45 functional redundancy among species (Reich *et al.* 2012). The shape of the diversity-production
46 relationship has important implications for understanding the impacts of biodiversity loss on ecosystem
47 function. If the diversity-production relationship is steep and saturates slowly, then the loss of even a
48 few species from diverse communities could have marked impacts on ecosystem function (Reich *et al.*
49 2012).

50 In spite of the fact that they contribute nearly half of global primary production (Falkowski 1994; Field
51 *et al.* 1998), the relationship between biodiversity and ecosystem functioning in marine phytoplankton
52 is poorly understood (but see (Ptacnik *et al.* 2010) for a summary of current understanding). What we
53 do know about the links between biodiversity and ecosystem functioning in marine phytoplankton
54 mostly come from observational studies. For example, global patterns of marine phytoplankton
55 biodiversity tend to show a unimodal relationship between species richness and ecosystem production
56 (Irigoien *et al.* 2004), although even this result has been challenged due to methodological concerns
57 (Cermeño *et al.* 2013). Models suggest that the unimodality could be due to stronger top-down control
58 by grazers at extremes levels of ecosystem production – the so called “kill the winner hypothesis”
59 (Vallina *et al.* 2014). Analyses have demonstrated positive associations between ecosystem production
60 and community-level diversity in cell size (Acevedo-Trejos *et al.* 2018), as well as complex interactions
61 between production and traits linked to grazing and nutrient uptake (Prowe *et al.* 2012a, b; Cermeño *et al.*
62 2016; Hodapp *et al.* 2016). In freshwater ecosystems, primary production and resource use efficiency
63 have been found to be log-linearly related to taxonomic richness (Ptacnik *et al.* 2008, Striebel *et al.*
64 2009). Nevertheless, the mechanisms underpinning patterns of phytoplankton biodiversity and
65 ecosystem production are poorly understood in both marine and freshwater ecosystems largely due to
66 dearth of controlled experiments.

67 Even less is known about the links between biodiversity and production in the face of environmental
68 change. Recent work has shown that environmental change (e.g. warming, elevated CO₂, nutrient
69 pollution, drought) can alter both diversity, ecosystem production and the relationship between diversity
70 and production though the mechanisms underlying these changes are often unclear (Reich *et al.* 2001;
71 Lewandowska *et al.* 2012, 2014; Steudel *et al.* 2012; Isbell *et al.* 2015; Striebel *et al.* 2016). The
72 insurance hypothesis and the ‘portfolio effect’ propose that biodiversity will be important for
73 maintaining ecosystem functioning in the face of rapid environmental change (Doak *et al.* 1998; Tilman
74 1999; Yachi & Loreau 1999). Because species inherently differ in their ability to tolerate abiotic change
75 (McGill *et al.* 2006), higher biodiversity provides greater insurance that some species will have traits
76 that enable them to maintain high levels of production and contribute to ecosystem functioning in
77 adverse conditions (Hooper *et al.* 2005). Thus, when environmental change exceeds the tolerance limits
78 of some species but not others, the diversity-production relationship is expected to become steeper and
79 saturate more slowly because communities with fewer species will have reduced probability of
80 including those with traits that enable them to cope with the novel environment, and ecosystem
81 production could decline rapidly with biodiversity loss. Indeed, recent work with heterotrophic bacteria
82 has shown that as temperatures depart from ambient conditions (either via warming or cooling)
83 functional redundancy rapidly declines leading to steeper, less saturating diversity-production
84 relationships (García *et al.* 2018). In phytoplankton, a recent model showed that functional diversity in
85 both thermal and nutrient traits positively affected ecosystem production, with a stronger impact of
86 diversity in thermal traits (thermal optima) than in nutrient traits (Vallina *et al.* 2017). However, to our
87 knowledge there exist no studies that have experimentally manipulated biodiversity of marine
88 phytoplankton in a climate change context.

89 Thermal tolerance curves for phytoplankton exhibit characteristic unimodality and left-skew, where
90 fitness declines more sharply above the optimum than below (Padfield *et al.* 2017; Schaum *et al.* 2017).
91 Given the large interspecific variability in thermal tolerance among phytoplankton (Boyd *et al.* 2013;
92 Barton & Yvon-Durocher 2019) and the importance of thermal tolerance for species interactions
93 (Bestion *et al.* 2018a), we hypothesised that when warming drives temperatures above the thermal

94 optimum for some species but not others, the slope of the relationship between biodiversity and
95 ecosystem functioning should become steeper because more diverse communities will have a higher
96 probability of including species that are able to tolerate warming and maintain ecosystem function as
97 temperature rises.

98 We tested this hypothesis by experimentally manipulating the species richness of marine phytoplankton
99 communities at a control temperature of 15°C, a moderate warming scenario of 25°C, and a severe level
100 of warming at 30°C, and quantifying the impact on ecosystem production in laboratory microcosms.
101 We used 16 species of marine phytoplankton encompassing most of the biogeochemically and
102 ecologically important groups (Diatoms, Dinoflagellates, Coccolithophores, Rhodophytes,
103 Chlorophytes and Prasinophytes, Table S1) and applied a random partitioning experimental design (Bell
104 *et al.* 2009) to create communities with different levels of species richness (Fig. 1). This experimental
105 design allowed quantifying the impacts of species loss on ecosystem functioning as well as evaluating
106 the relative contribution of each species to ecosystem production. To test whether changes in the
107 diversity-functioning relationship could be attributed to species-level thermal trait variance (as expected
108 under the insurance hypothesis (Doak *et al.* 1998; Tilman 1999; Yachi & Loreau 1999)) we measured
109 the thermal performance curves for photosynthesis for each species and assessed whether species'
110 relative contribution to ecosystem functioning was linked to their photosynthetic thermal tolerance (Fig.
111 1).

112

113 **Method summary**

114 **Species and culture conditions**

115 The experiment was conducted with 16 marine phytoplankton species sourced from culture collections,
116 *Amphidinium carterae*, *Bigelowiella natans*, *Chlorarachnion reptans*, *Dunaliella tertiolecta*, *Emiliana*
117 *huxleyi*, *Gephyrocapsa oceanica*, *Gymnochlora stellata*, *Micromonas pusilla*, *Nitzschia sp.*,
118 *Ostreococcus tauri*, *Porphyridium aerugineum*, *Porphyridium purpureum*, *Phaeodactylum*
119 *tricornutum*, *Rhodella maculata*, *Thoracosphaera heimii*, and *Thalassiosira pseudonana*. These strains

120 varied widely in their geographic provenance, from the North Atlantic (most strains) to the
121 Mediterranean Sea and the West and South Pacific (Table S1). Species were maintained in semi-
122 continuous culture in an Infors-HT shaking incubator (65 rpm) at 20°C on a 12:12 light-dark cycle with
123 a light intensity of 45-50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in their original culture media until the thermal performance
124 assays, and then grown on the same K+Si medium until the community experiment (see Supplementary
125 Methods).

126

127 **Thermal performance assays**

128 We characterised acute thermal performance curves for gross photosynthesis for each of the 16 species
129 (Fig. 1, see Supplementary Methods for more details). Acute thermal performance curves characterise
130 immediate responses to temperature change and quantify the impacts of temperature on the performance
131 of the photosynthetic machinery. Here we use these measurements as a proxy for the relative difference
132 between the 16 taxa in their thermal tolerance. We measured photosynthesis and respiration from 7 to
133 49°C with a Clark-type oxygen electrode as part of a Chlorolab 2 system (Hansatech Ltd, King's Lynn,
134 UK). Samples were taken during the mid-log growth phase, concentrated to yield sufficient biomass to
135 detect a sufficient signal of O₂ flux, and acclimated for 15 minutes to the assay temperature before
136 measuring photosynthesis and respiration.

137 Rates of net photosynthesis, measured as O₂ evolution, were collected across a range of light intensities
138 from 0 to 1800 $\mu\text{mol m}^2 \text{s}^{-1}$. We then used a photosynthesis-irradiance curve at each assay temperature
139 to estimate light-saturated net photosynthesis NP_{max} . Respiration (R) was measured in the dark, as
140 oxygen consumption, over a 3-minute period directly following the light response. We calculated gross
141 photosynthesis as $GP = NP_{max} + R$ and converted rates to $\mu\text{g O}_2 \text{ cell}^{-1} \text{ hour}^{-1}$.

142 We quantified the temperature-dependence of gross photosynthesis rates using the modified Sharpe-
143 Schoolfield equation (Sharpe & DeMichele 1977; Schoolfield *et al.* 1981), and calculated the thermal
144 optimum for gross photosynthesis from the equation (see Supplementary Methods).

145

146 **Biodiversity-function experiment**

147 Artificial communities for the biodiversity-functioning experiment were designed using the random
148 partition design described by Bell *et al.* (2009). We randomly divided species into communities with
149 increasing species richness levels from 1, 2, 4, 8, and 16 species, where for each species richness level,
150 the community assemblages were constructed by sampling the 16 species without replacement (Fig. 1).
151 This allowed each species to be represented an equal number of times at each richness level. This
152 process was repeated to form 5 independent partitions of the species pool, so that for each richness level
153 (R) the number of assemblages was $5 \times 16/R$. Each assemblage was then replicated 3 times. Further, all
154 replicated communities were subjected to three temperature treatments, 15, 25 and 30°C, giving for the
155 experiment as a whole a total of $3 \times 3 \times 5 \times (16 + 8 + 4 + 2 + 1) = 1395$ communities.

156 The biodiversity-function experiment was done in sixty 24 well plates filled with 2 mL of K+Si
157 medium. Each well was inoculated with 1600 cells.mL⁻¹ of each community (i.e. from 100 cells.mL⁻¹
158 per species in the case of sixteen-species communities to 1600 cells.mL⁻¹ per species for monocultures).
159 Samples were grown in three Infors-HT shaking incubators at 15, 25 and 30°C on a 12:12 light cycle.
160 Distilled water was added every 5 days to refill evaporative water loss. After 19 days, 100 µL samples
161 from each community were taken on a 96 well plate, preserved with 10 µL of 1% sorbitol and frozen at
162 -80°C after one hour of dark incubation. Cell density was determined by running flow cytometry (BD
163 Accuri C6) counting 20 µL on slow flux settings.

164

165 **Data analyses**

166 We extracted cells counts and cytometric properties from FSC files with the Bioconductor FlowCore
167 package in R v3.4.2 (R Core Team 2014). Data was filtered to remove values where either
168 $\log_{10}(\text{FSC}) < 5$, $\log_{10}(\text{SSC}) < 5$ and/or $\log_{10}(\text{FL3}) < 3.5$, which are below minimum values observed for live
169 cells of these species. We derived cell chlorophyll *a* content (pg.cell⁻¹) from FL3 values using the
170 calibration curve described in Supplementary Methods. We calculated community abundance

171 (cells.mL⁻¹) and total chlorophyll *a* content (sum across all cells per mL). These two metrics were used
172 as proxies of ecosystem production, as found in other studies (Boyd *et al.* 2013). We focus on
173 chlorophyll *a*, as it is the most widely used proxy for studying phytoplankton biomass (Field *et al.* 1998;
174 Marañón *et al.* 2014), but show that the results are largely consistent when using total community
175 abundance (Table S3-S4, S6, S8, S10, S12, Fig. S1, S3, S4, S5b, S6b, S7b).

176 The biodiversity-ecosystem functioning (B-EF) relationship was analysed using the analysis of variance
177 method described by Bell *et al.* (2009). Factors relating to temperature treatments were fitted first,
178 followed by log-transformed species richness and their interaction. The best model included the
179 temperature by richness interaction (Table 1, S3). We tested for differences in the slope of the BE-F
180 relationship between temperatures with post-hoc contrasts using the lsmeans package with tukey p-
181 value adjustment (Table S2, S4). We then extracted the residuals from relationships between ecosystem
182 functioning and species richness for each temperature treatment and fitted these residuals to the
183 presence-absence status of each of the 16 species. The species coefficients provided by this method
184 indicate the effect of each species on ecosystem production relative to an average species, where
185 positive values indicate above average contributions and negative values below average contributions
186 (Fig. S2, S3). We used linear models separating the data by climatic treatment to test whether species
187 coefficients depended on species' photosynthetic thermal tolerance (Fig. 3, S4, S5, S8, Table S5,
188 S6,S13). We also tested whether species coefficients depended on cell volume through linear models
189 separating the data by climatic treatment (Table S1, S9, S10 Fig. S7).

190 At the end of the experiment, we estimated the relative abundance of each species within the community
191 from the flow cytometry data using a randomforest analysis (see Supplementary Methods). To further
192 explore our hypothesis that variability in species' thermal tolerance plays an important role in mediating
193 the interactive effects of warming and biodiversity loss on ecosystem functioning, we used these data
194 to test whether species abundance in polyculture were linked to their respective abundance in
195 monoculture through linear models of abundance in polyculture as a function of abundance in
196 monoculture separating the data by temperature treatment (Fig. 4). We also quantified how abundance

197 in monoculture was related to thermal optima through linear models separating the data by temperature
198 treatment (Table S7-S8, Fig. 3, S4,S6).

199 Finally, we estimated net and transgressive overyielding (Table S11-S12) by comparing the mean
200 ecosystem function value of the 16-species polyculture to the mean value of all of the species grown in
201 monoculture (net overyielding) and to the mean value of the species that achieved the highest biomass
202 in monoculture (transgressive overyielding (Cardinale *et al.* 2007)).

203 **Results**

204

205 We found that ecosystem production, measured as total chlorophyll *a*, increased linearly with species
206 richness on a log-scale, implying a decelerating relationship (Fig. 2). The intercept of the richness-
207 production relationship declined sharply with warming (Fig. 2). Conversely, experimental warming
208 significantly increased the slope of the relationship between richness and ecosystem production, with
209 more than a two-fold increase (Fig. 2, Table 1, S2). The same relationship between the slope of the
210 biodiversity-ecosystem function relationship and temperature was found when using total cell
211 abundance as a proxy for ecosystem production (Fig. S1, Table S3-S4).

212 We quantified the contribution of each species present in the community to ecosystem production using
213 the linear model method from Bell *et al.* (2009), which yields a coefficient for each species, where
214 values > 0 indicate an above average effect and those < 0 are indicative of a below average contribution
215 to production (Fig. S2,S3). We found a positive correlation between a species contribution to
216 community functioning at 30°C and their thermal optimum of photosynthesis (Fig. 3a,b, Table S5),
217 while there was no correlation at 15 and 25°C (Fig. S5a, Table S5). Similar relationships were found
218 when using total cell abundance instead of chlorophyll *a* to calculate species coefficients (Fig. S4a,b,
219 S5b, Table S6). We also found that thermal optima of photosynthesis were positively linked to yield in
220 monoculture at 30°C (Fig. 3c, S4c, S6, Table S7-S8). We investigated potential links between cell
221 volume and species' relative contribution to ecosystem production and found no significant relationship
222 at any temperature (Table S9-S10, Fig. S7).

223 At the end of the experiment, we estimated the relative abundance of each species in the communities.
224 We found that the abundance of each species in polyculture was positively correlated with their
225 abundance in monoculture at all temperature levels (Fig. 4). Finally, we estimated net and transgressive
226 overyielding by comparing ecosystem production between 16-species polycultures and either the
227 average production of all monocultures (net overyielding) or the production of the best performing
228 species in monoculture (transgressive overyielding). While there was net overyielding at all
229 temperatures, we found no evidence of transgressive overyielding in the 16-species polycultures
230 compared to the monocultures for ecosystem production measured as chlorophyll *a* content (Table S11).
231 It is worth noting that while results using total cell abundance as a proxy for ecosystem production were
232 largely congruent (Table S12), with some net overyielding at all temperatures, we found some evidence
233 for transgressive overyielding at high temperatures that was not present using chlorophyll *a* content.

234 **Discussion**

235

236 By manipulating the species richness and environmental temperature of marine phytoplankton
237 communities in experimental microcosms we found that declines in ecosystem production were far
238 more pronounced under warmer temperatures – i.e. warming led to a steeper relationship between
239 biodiversity and ecosystem functioning. This key result was explicable from an understanding of
240 variability among the phytoplankton taxa in the thermal tolerance of their photosynthetic machinery,
241 with those taxa that had higher thermal tolerance also those which made the largest contributions to
242 ecosystem production in warmer environments.

243 Ecosystem production increased with species richness and was well characterised by a linear
244 relationship on a log-scale, indicating that production increased rapidly at low levels of species richness
245 but then decelerated as more species were added to the communities. The intercept of the richness-
246 production relationship, which is indicative of ecosystem production at low levels of richness, declined
247 with warming. This effect of temperature on community biomass is consistent with expectations from
248 metabolic scaling theory and is related to the exponential effects of rising temperature on metabolic

249 rates. When resource availability is fixed and independent of temperature (as was the case in these
250 microcosms), increases in temperature should result in lower equilibrium biomass because each
251 individual uses resources at a faster rate and thus the ecosystem can support fewer individuals (Savage
252 *et al.* 2004).

253 The steepness of the slope of the relationship between richness and ecosystem production provides a
254 mean to assess the importance of diversity for maintaining ecosystem functioning – where a steep slope
255 implies that species loss will have a more marked impact on ecosystem functioning (Reich *et al.* 2012).
256 Consistent with our hypothesis, we found that experimental warming significantly increased the slope
257 of the relationship between richness and production. Thus, as temperatures rose, more species were
258 required to maintain ecosystem functioning at levels comparable with the control. Indeed, only when
259 all 16 species were present were levels of production in the treatment warmed to 30°C comparable to
260 those at the control temperature. These findings are consistent with recent work on freshwater bacteria,
261 which found that as temperatures depart from ambient conditions (either via warming or cooling)
262 functional redundancy rapidly decays leading to steeper, less saturating diversity-production
263 relationships (García *et al.* 2018).

264 The steeper relationship between biodiversity and ecosystem functioning in the warmed treatments
265 implies that variance in thermal performance traits might have played an important role in shaping the
266 effects of warming and species loss on ecosystem production. To investigate this, we quantified the
267 contribution of each species present in the community to ecosystem production (Bell *et al.* 2009). We
268 then assessed whether coefficients quantifying the impact of each species in the community on
269 production under the severe warming treatment (30°C) were correlated with their optimal temperatures
270 for photosynthesis. Here we treat the thermal optimum for photosynthesis as a “trait” that is indicative
271 of variability in thermal performance among the phytoplankton species – i.e. species with higher
272 photosynthetic thermal optima are anticipated to perform better at high temperature than those with low
273 thermal optima. Our analyses do not assume a direct, causal relationship between photosynthetic
274 performance and ecosystem functioning, rather we assume that the thermal optimum for photosynthesis
275 provides useful proxy for differentiating thermal tolerance among 16 species of marine phytoplankton.

276 Indeed recent work has shown that photosynthetic performance is a key trait determining competitive
277 fitness in phytoplankton (Schaum *et al.* 2017). We found a highly significant positive correlation
278 between species' contribution to ecosystem production and their thermal optimum for gross
279 photosynthesis in the high temperature treatment, indicating that those species which contributed
280 positively to ecosystem function in under severe warming were also those with the highest thermal
281 tolerance of their photosynthetic machinery. We further found the same association between yield in
282 monoculture under severe warming and thermal tolerance. Moreover, species performance within a
283 community was positively associated with its performance in monoculture. Together, this shows that
284 the effect of species on ecosystem production is strongly dependent on the thermal tolerance of their
285 photosynthetic machinery and thus their performance in monoculture. In warmer conditions,
286 communities with low species richness have a lower probability of including those species with high
287 thermal tolerance that can contribute positively to ecosystem function.

288 Another important driver of metabolism, and consequently community structure and ecosystem
289 function in phytoplankton communities, is cell size (Marañón 2015). Cell size is a key trait for
290 understanding phytoplankton nutrient uptake (Marañón 2015), and recent work has emphasised the key
291 role of nutrient physiology traits can play in mediating phytoplankton responses to climate change
292 (Thomas *et al.* 2017; Bestion *et al.* 2018b). For instance, variability in marine phytoplankton growth
293 rate across latitudes has been shown to be strongly linked to nutrient availability (Marañón *et al.* 2014),
294 while the contribution of the smallest-sized phytoplankton cells to total phytoplankton biomass in the
295 ocean has been shown to increase with temperature (Morán *et al.* 2010). Cell volume has also been
296 found to correlate with the optimum growth temperature in marine phytoplankton with smaller cells
297 typically able to tolerate higher temperatures (Sal *et al.* 2015; Barton & Yvon-Durocher 2019). We
298 therefore investigated potential links between species' contribution to ecosystem production and cell
299 size. We found no significant association at any of the temperature treatments. This result suggests that
300 changes in the biodiversity-ecosystem relationship were not related to size dependent turnover in
301 species composition. In general, our results show that ecosystem production in the warm environment
302 was strongly dependent on the presence of species with high photosynthetic thermal optima to maintain

303 ecosystem function. Thus, when biodiversity loss removed these species and their associated traits from
304 the community, the negative impact on ecosystem functioning was marked, as evidence by the steep
305 richness-production slope in the warm treatments.

306 We estimated the net overyielding (i.e. the difference between the mean ecosystem function of the 16-
307 species polyculture and the mean ecosystem function of the monocultures) and the transgressive
308 overyielding (i.e. the difference between the mean ecosystem function of the 16-species polyculture
309 and the ecosystem function of the best functioning monoculture) (Cardinale *et al.* 2007). This allowed
310 us to tease apart overyielding due to both selection and complementarity effects from overyielding only
311 due to complementarity effects. We found that community performance was only ever as good as the
312 best species in monoculture demonstrating no evidence for transgressive overyielding in any of the 16-
313 species polycultures when using chlorophyll *a* as a proxy for biomass. This implies that selection effects
314 played an important role in mediating changes in the relationship between biodiversity and ecosystem
315 production across the temperature gradient. However, we did see some evidence for transgressive
316 overyielding at the highest temperature only when using cell abundance as a proxy. Such transgressive
317 overyielding could be driven by the coexistence of diverse size classes of algae, each with different
318 pigment characteristics related to their size, which may have led to discrepancies between calculations
319 based on total abundance and total chlorophyll *a*. Taken together, these results suggest that the loss of
320 phytoplankton species from planktonic communities might have a much more pronounced negative
321 impact on marine primary production in a warmer world.

322 It is important to consider that our findings might be impacted by the choice of phytoplankton species
323 used in this study. Because both temperate and tropical species were used in our experiments (Table
324 S1), the species pool encompassed a wide range of thermal optima. In the ocean, the regional species
325 pool for a given location might be expected to display a narrower range of thermal optima, if long-range
326 dispersal is limited. Low variance of thermal tolerance traits would be expected to lead to a less
327 pronounced impact of temperature change on the slope of the diversity-production relationship but a
328 more pronounced collapse of ecosystem function when warming exceeds the upper thermal tolerance
329 in the regional species pool. Nevertheless, recent work has demonstrated that minimum connectivity

330 times between even the most distant ocean basins are on the order of a decade (Jönsson & Watson
331 2016), which is likely to lead to mixing of temperate and tropical taxa over timescales relevant to
332 climate warming. Furthermore, planktonic microorganisms possess an enormous potential for dispersal,
333 allowing for reshuffling of communities (Finlay 2002), and the spread in thermal optima among the
334 species in our study (11.7°C) corresponds roughly to the spread of thermal optima from marine
335 phytoplankton observed within one tenth of a degree of latitude in a recent meta-analysis (10.7°C, see
336 Fig. 1 from (Thomas *et al.* 2012) and Table S14). Thus, even though the species used in our study
337 originated from diversified latitudes, it is likely that the variation in thermal optima are consistent with
338 thermal trait variation at local to regional scales in the ocean. Another important caveat is that our
339 experiments were carried out in microcosms, which might influence the broader applicability of our
340 results. Microcosm environments lack the complexity and heterogeneity of the natural environment,
341 and might not allow species to partition their niches along the full diversity of environmental axes that
342 may be possible in nature. Thus, it is likely that more complex biotic and abiotic environments could
343 lead to more niche partitioning and/or complementarity among the phytoplankton species. For instance,
344 (Burgmer & Hillebrand 2011) found that the presence of consumers modulated the effect of temperature
345 on both algal biomass and species richness, switching the impacts of warming on the species richness
346 and biomass negative to positive in the presence of grazers. Nevertheless, it is important to recognise
347 that our aim with this work was not to replicate the complexity of the natural environment, but rather
348 our principal objective with these experiments were to unpick the mechanisms that determine how
349 changes in temperature influence the relationship between phytoplankton diversity and ecosystem
350 production. Clearly, further work in more complex environmental settings are required to translate these
351 findings into natural settings.

352 Our findings highlight the potential for major synergistic negative impacts of species loss and
353 environmental warming on the production of marine phytoplankton communities. We found that the
354 slope of the relationship between species richness and ecosystem production increased significantly as
355 temperatures rose above ambient conditions. Consequently, ecosystem production declined much more
356 abruptly as species were lost from the communities in the warmer treatments and therefore a greater

357 number of species were required to maintain ecosystem functioning at levels comparable with the
358 control. This pattern was linked to variance in thermal traits in the species pool. When temperatures
359 exceeded the optimum for some species but not others, communities with low species richness had a
360 reduced probability of including taxa with thermal traits that enabled them to maintain high levels of
361 production in the warm environment and experienced dramatic declines in ecosystem functioning.
362 Overall, these results suggest that if biodiversity loss of marine phytoplankton is not correlated with
363 thermal performance traits, warming could lead to a marked negative impact of species loss on
364 ecosystem production. This could conceivably occur if other stressors which result in the loss of
365 phytoplankton species from communities – such as invasive species, nutrient limitation, pollution,
366 acidification, top-down control – are decoupled in time and/or space from ocean warming (Suchanek
367 1994; Monaco & Prouzet 2015). Indeed, major changes in food web structure due to overharvesting
368 and changes in top-down control are known to be a key driver of biodiversity loss in marine ecosystems
369 (Pauly *et al.* 1998) and are likely to be largely independent of thermal performance traits. However, if
370 biodiversity loss is directly linked to climate warming (Thomas *et al.* 2004; Bestion *et al.* 2017), it
371 should be non-random relative to thermal performance traits (Thomas *et al.* 2012) and the marked
372 negative impact on production could be buffered to some degree, because the species with lower thermal
373 tolerance that contribute least to production in the new environment (i.e. those with lower species
374 coefficients, see Fig. 3), will be the first to disappear. Overall, our results provide the first empirical
375 evidence of the critical role that species- and thermal trait diversity could play in mediating the effects
376 global warming on the primary production of marine phytoplankton.

377

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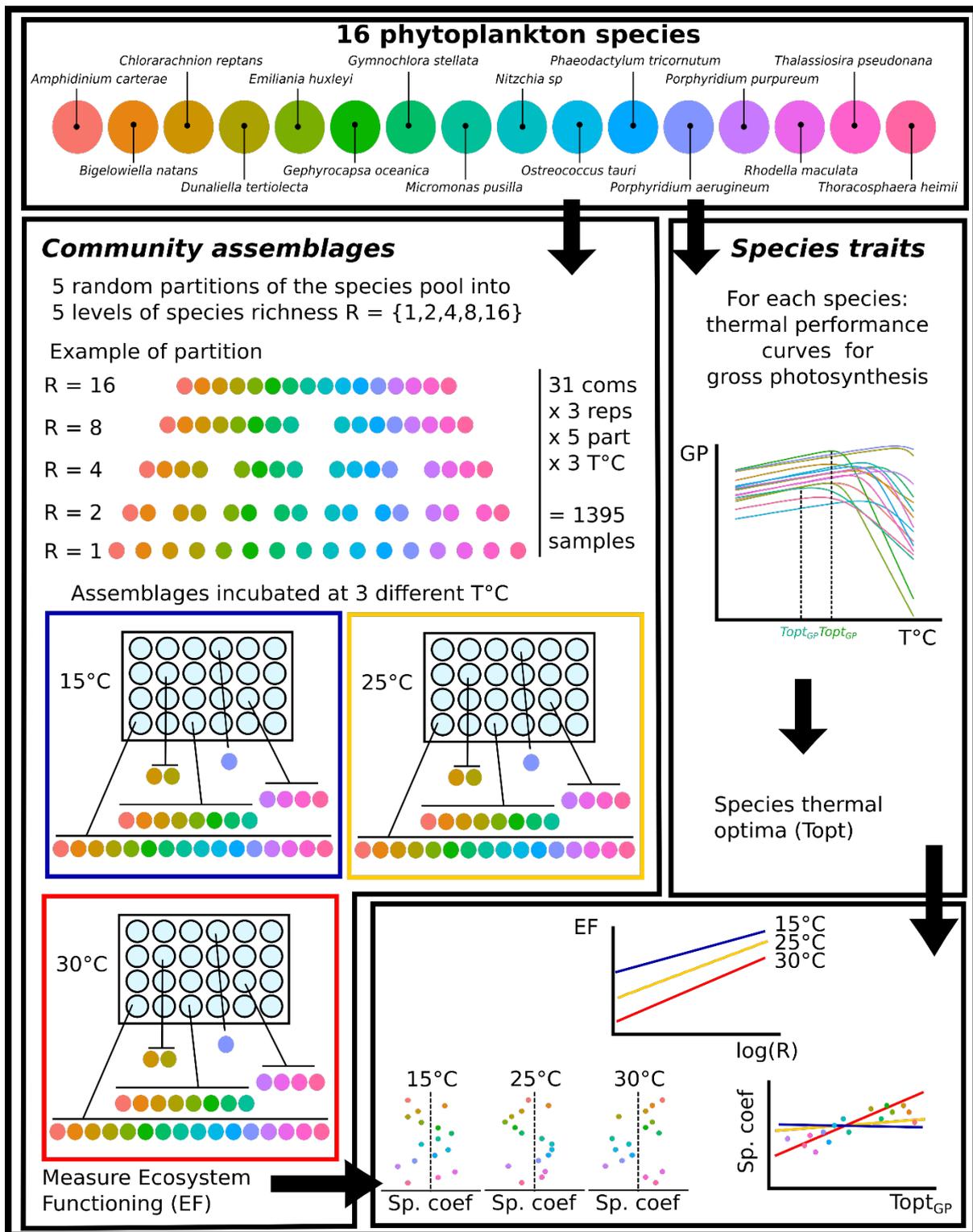
516

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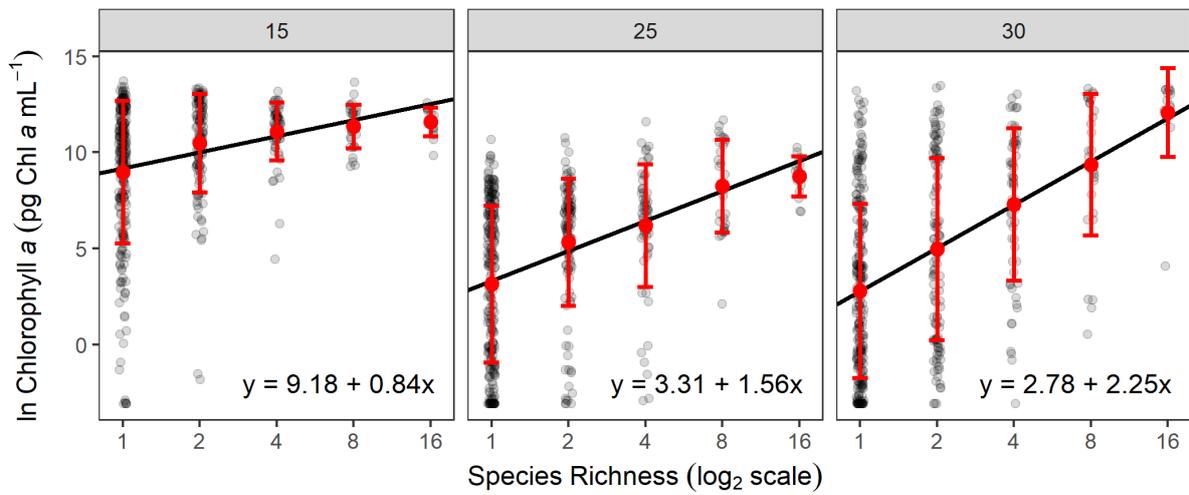
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522 **Fig. 1: Flow chart of the experimental design**



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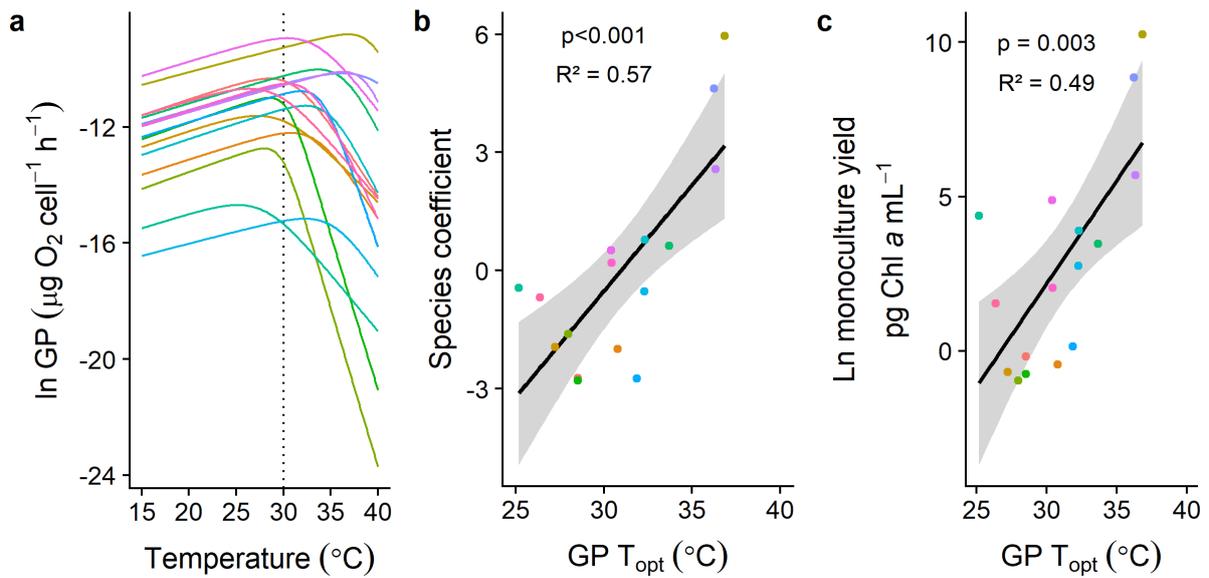


525

526 **Fig. 2. Impact of species loss and warming on ecosystem production.** Ecosystem production is
527 quantified as the total chlorophyll *a* content of the community. Grey points correspond to each of the
528 1395 replicates ($n = 465$ for each temperature treatment). Red point and bars are the mean \pm SD for
529 each level of species richness. Lines correspond to the fitted curves from the most parsimonious linear
530 model ($\ln(\ln \text{ chlorophyll } a \sim \text{ temperature } * \log_2(\text{Richness}))$), see Table 1), with the associated
531 coefficients for each temperature. Contrast analyses reveal that the slope of the richness-ecosystem
532 function relationship increased significantly with warming (Table S2), indicating that the impact of the
533 species loss on ecosystem production is more pronounced at higher temperatures.

534

535



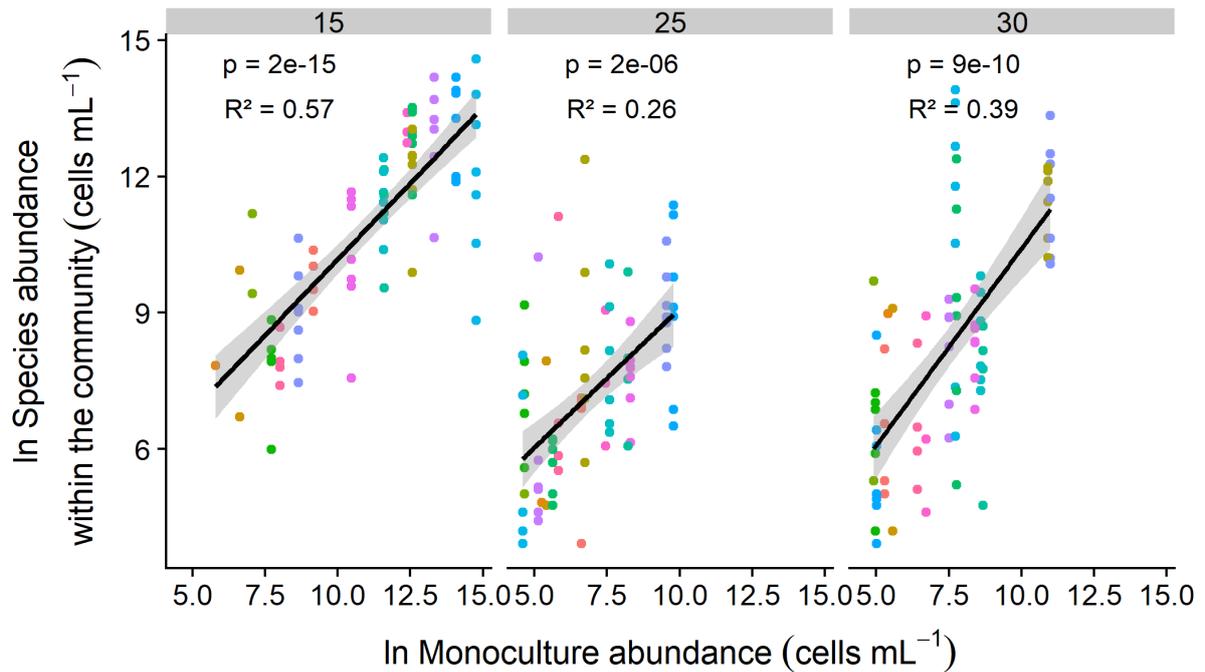
Species name

— <i>Amphidinium carterae</i>	— <i>Gymnochlora stellata</i>	— <i>Porphyridium purpureum</i>
— <i>Bigelowiella natans</i>	— <i>Micromonas pusilla</i>	— <i>Rhodella maculata</i>
— <i>Chlorarachnion reptans</i>	— <i>Nitzschia sp</i>	— <i>Thalassiosira pseudonana</i>
— <i>Dunaliella tertiolecta</i>	— <i>Ostreococcus tauri</i>	— <i>Thoracosphaera heimii</i>
— <i>Emiliana huxleyi</i>	— <i>Phaeodactylum tricornutum</i>	
— <i>Gephyrocapsa oceanica</i>	— <i>Porphyridium aerugineum</i>	

537

538 **Fig. 3. Linking thermal performance traits and species' contribution to community functioning.**

539 (a) Thermal performance curves for gross photosynthesis for each species (see Table S13 for parameters
 540 and Fig. S8 for detailed fits for each species). (b) Correlation between species coefficient at 30°C and
 541 thermal optimum for gross photosynthesis. Species coefficients represent the contribution of each
 542 individual species to the community functioning and are calculated from the residuals of the random
 543 partitions analysis of the diversity-functioning relationships for chlorophyll *a* (Fig. S2). Positive species
 544 coefficients indicate species that have a higher than average contribution to ecosystem production,
 545 negative coefficients represent lower than average contributions. (c) Correlation between mean yield in
 546 monoculture at 30°C (ln pg Chl *a* mL⁻¹) and thermal optimum for gross photosynthesis (°C). Analyses
 547 reveal that the thermal optimum for gross photosynthesis was strongly correlated with relative
 548 contribution of each species to ecosystem production at 30°C (Table S5, Fig. S5a) as well as to the yield
 549 of each species in monoculture at 30°C (Table S7, Fig. S6a).



Species name

- | | | |
|--|---|--|
| — <i>Amphidinium carterae</i> | — <i>Gymnochlora stellata</i> | — <i>Porphyridium purpureum</i> |
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| — <i>Gephyrocapsa oceanica</i> | — <i>Porphyridium aerugineum</i> | |

550

551 **Fig. 4: Relationship between focal species abundance in polyculture and its abundance in**

552 **monoculture for each temperature treatment.** Global relationship across all species. Focal species

553 abundance in polyculture is obtained with a randomforest algorithm allowing to assign each cell from

554 a polyculture to its putative species identity (see Supplementary Methods). Because the predictive

555 power of the randomforest algorithm varied with community identity, not all communities were present.

556 We calculated an average abundance of the focal species within the community as the mean of the

557 abundances for the three biological replicates, and an average abundance of the focal species in

558 monoculture as the mean of the biological replicates. There was a positive relationship between focal

559 species abundance within the community and in monoculture (linear models by temperature treatment,

560 $t = 9.95$, $df = 76$, $p = 2e^{-15}$, $R^2 = 0.57$, $t = 5.18$, $df = 76$, $p = 2e^{-06}$, $R^2 = 0.26$, and $t = 7.01$, $df = 76$, p

561 $= 9e^{-10}$, $R^2 = 0.39$, respectively for 15, 25 and 30°C).

562 **Table 1. Linear models estimating the effect of temperature, species richness and species**
563 **composition on ecosystem production.** The linear models describe the effect of temperature (T, as a
564 factor), species richness ($\log_2(R)$), and their interaction on total chlorophyll *a* content of the community
565 (index of production). At each step, terms are added to the linear model and the residual degrees of
566 freedom (res. d.f.) and sum of squares (res. SS) are re-calculated. The treatment degrees of freedom
567 (Treat. d.f), sum of squares (treat. SS) and F-statistic (F) are calculated at each step only for the term
568 that has been added to the model during that step. R^2 and AIC are calculated for each model. Lower
569 AIC values indicate an improved model. Analyses revealed that best fitting model included the
570 interaction between temperature and species richness and it explained 40 % of the variance. See Table
571 S2 for a post hoc, multiple comparisons analysis on the slope of the biodiversity-ecosystem function
572 relationship by temperature and Fig. 2 for a graphic representation of the results.

Step	Model	Res. d.f.	Res. SS	Treat. d.f.	Treat. SS	F	R^2	AIC
0	Intercept	1394	32294.1					8345.9
1	step0+T	1392	23798.3	2	8495.8	248.5	0.26	7924.1
2	step1+ $\log_2(R)$	1391	19886.6	1	3911.7	273.6	0.38	7675.6
3	step2+T* $\log_2(R)$	1389	19345.8	2	540.9	19.4	0.40	7641.1

573

574