

22 **Abstract**

23 Marine phytoplankton are responsible for about half of the photosynthesis on Earth. Many are
24 mixotrophs, combining photosynthesis with heterotrophic assimilation of organic carbon but the
25 relative contribution of these two carbon sources is not well quantified. Here, single-cell
26 measurements reveal that *Prochlorococcus* at the base of the photic zone in the Eastern
27 Mediterranean Sea are obtaining only ~20% of carbon required for growth by photosynthesis.
28 Consistently, laboratory-calibrated evaluations of *Prochlorococcus* photosynthesis indicate that
29 carbon fixation is systematically too low to support published *in situ* growth rates in the deep
30 photic layer of the Pacific Ocean. Furthermore, agent-based model simulations show that
31 mixotrophic cells maintain realistic growth rates and populations 10s of meters deeper than
32 obligate photo-autotrophs, deepening the nutricline and Deep Chlorophyll Maximum by ~20 m.
33 Time-series of *Prochlorococcus* ecotype-abundance from the subtropical North Atlantic and North
34 Pacific suggest that up to 30% of the *Prochlorococcus* cells live where light intensity is not enough
35 to sustain obligate photo-autotrophic populations during warm, stratified periods. Together,
36 these data and models suggest that mixotrophy underpins the ecological success of a large
37 fraction of the global *Prochlorococcus* population and its collective genetic diversity.

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40 Photosynthesis by phytoplankton provides most of the energy and fixed carbon that support
41 marine food webs and carbon reservoirs¹. However, few phytoplankton are strictly photo-
42 autotrophic². Many phytoplankton also utilize dissolved organic matter, taking up particulate
43 detrital organic matter or preying upon other living cells and even harvesting organelles².
44 Mixotrophic lifestyles, in which cells both fix carbon and use exogenously available organic
45 carbon, may enhance fitness when the relative availability of inorganic resources differs from
46 physiological demands³. This may occur, for example, where light intensity is low but inorganic
47 nutrients are abundant. Despite the potential importance of mixotrophy to phytoplankton life
48 history, the contribution of heterotrophic carbon assimilation to phytoplankton growth is not well
49 quantified⁴. Simulations suggest that mixotrophy may be a globally significant carbon source for
50 phytoplankton⁵ but this prediction is currently difficult to quantitatively test with empirical data.
51 One reason is that dissolved organic carbon (DOC) in the oceans constitutes an extremely complex
52 mixture of compounds^{6,7}, most of which are uncharacterized. This means that uptake
53 measurements using specific organic carbon sources (e.g. glucose, amino acids)^{8,9} do not
54 represent the entirely available DOC pool and may underestimate the actual DOC uptake rates,
55 and hence mixotrophy of major phytoplankton species¹⁰.

56 *Prochlorococcus* are the most abundant phototrophic cells on Earth, actively growing at depths
57 ranging from the ocean surface down to the base of the photic zone (~160 m)¹¹. Across these
58 depths, photosynthetically available radiation (PAR) varies over 3-4 orders of magnitude, a
59 challenge which the diverse *Prochlorococcus* lineage faces using a variety of adaptations in their
60 photosynthetic apparatus^{11,12}. These adaptations have led to the diversification of
61 *Prochlorococcus* into high-light and low-light adapted clades^{11,12}. In addition, *Prochlorococcus* are
62 mixotrophs, able to uptake dissolved organic compounds such as glucose⁸, pyruvate¹³, amino
63 acids⁹, nucleotides¹⁰ and perhaps DMSP^{14,15}. Yet, to what extent DOC uptake can supplement or
64 replace photosynthetically fixed carbon for respiration and/or growth in this globally-abundant
65 lineage is still unknown¹⁰. Available evidence suggests that while mixotrophy helps
66 *Prochlorococcus* survive limited periods of darkness, axenic cells die after ~1 week if not exposed
67 to light^{13,16} indicating that light harvesting, and possibly photosynthesis, are likely obligate.

68 Here, we take a multi-faceted approach to evaluate the contribution of heterotrophic carbon
69 assimilation to *Prochlorococcus* in the oceans. We first use isotopic measurements to quantify
70 photosynthesis and N uptakes in wild *Prochlorococcus* populations at the base of the photic zone

71 in the Mediterranean Sea. Then we compare observed growth rates from the Pacific Ocean with
72 purely photo-autotrophic growth rates simulated by a laboratory-calibrated photo-physiological
73 model. We also use an individual-based model to illustrate how mixotrophy provides a fitness
74 advantage and deepens the nutricline. Finally, we use time-series observations of vertical profiles
75 of *Prochlorococcus* ecotypes in subtropical gyres to show that several clades rely extensively on
76 mixotrophic carbon assimilation. Overall, these results suggest that up to a quarter of depth
77 integrated carbon assimilation by *Prochlorococcus* originates from DOC, with implications for
78 global C cycles, and that mixotrophy is essential to support a significant fraction of
79 *Prochlorococcus* diversity.

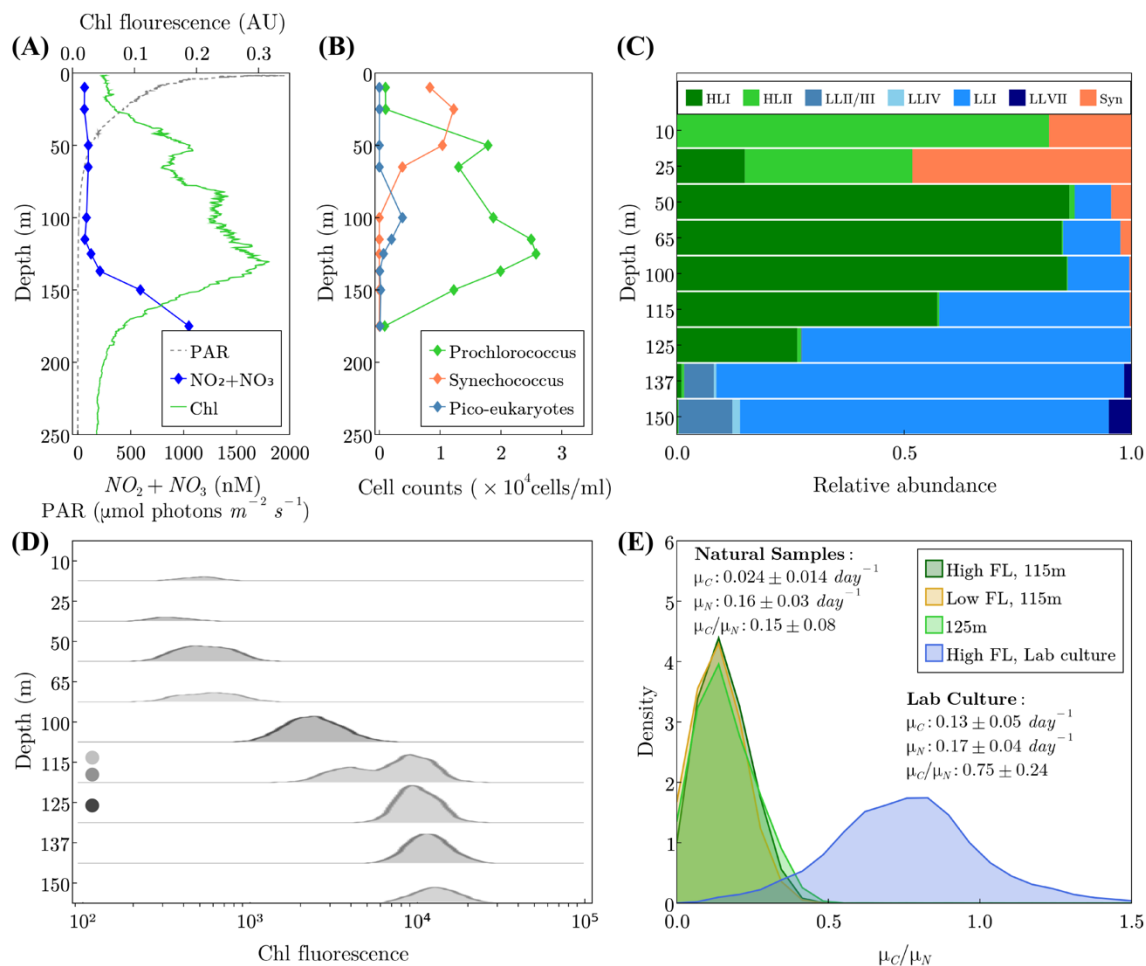
80

81 **Results and discussion**

82 *Carbon and nitrogen uptake in wild samples from the base of the photic zone.*

83 To evaluate the relative contributions of photosynthesis and heterotrophic carbon uptake in a
84 natural *Prochlorococcus* population from the base of the photic zone, where light may be limiting,
85 we assess the *Prochlorococcus* population structure and per-cell activity during late summer in
86 the ultra-oligotrophic Eastern Mediterranean Sea¹⁷. At the time of sampling, the water column
87 was highly stratified, nutrients were depleted down to around 140 m, and a prominent Deep
88 Chlorophyll Maximum (DCM) was observed at depth of ~115 m (Figure 1A). *Prochlorococcus* were
89 the numerically dominant phytoplankton below the surface (Fig 1B), and could be divided into
90 two populations based on the per-cell fluorescence – a low fluorescence population from the
91 surface to 115 m and a high fluorescence population from 115-150 m, with an overlap at 115 m
92 (Figure 1C, D). The shift in the per-cell chlorophyll fluorescence in *Prochlorococcus* with depth is
93 commonly observed^{18–20}, and is usually attributed to a change in the genetic composition of the
94 population, from High-Light adapted cells (HL, low fluorescence) to Low-Light adapted (LL, high
95 fluorescence) ones¹⁹. However, phenotypic heterogeneity (acclimation) can also contribute to this
96 phenomenon²¹, and indeed amplicon sequencing of the Internal Transcribed Spacer between the
97 16S and 23S genes (ITS)^{21,22} revealed a gradual transition from HL to LL clades around the DCM,
98 suggesting both genotypic and phenotypic shifts with depth (Figure 1C). The flow cytometry and
99 genetic data are both consistent with previous studies^{21,23}, and suggest that the water column had
100 been relatively stable for at least 3-4 days prior to sampling²⁰. Notably, the light intensity at the

101 DCM ($\sim 3\text{-}5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ during the afternoon, Figure 1A) is potentially enough under
 102 laboratory conditions to support the growth of some LL strains but not sufficient for active growth
 103 of most HL strains²⁴. Since HL cells comprise >50% of the *Prochlorococcus* population at 115 m
 104 and about 25% at 125 m, this suggests that a significant fraction of the *Prochlorococcus* cells in
 105 these samples are living under conditions where photosynthesis cannot support growth (Figure
 106 1C).



107

108 **Figure 1: Nutrient uptake of naturally occurring *Prochlorococcus* populations at the Eastern**
 109 **Mediterranean Sea.** (A) Depth profiles of Photosynthetically Available Radiation (PAR), $\text{NO}_2 + \text{NO}_3$
 110 and Chlorophyll. (B) Phytoplankton cell counts using flow cytometry. (C) Relative abundance of
 111 different *Prochlorococcus* clades across the water column, determined by ITS sequencing. Note
 112 the change in Chl fluorescence without a concomitant change in population structure between 60
 113 to 100 m, as well as the presence of LL clades above 115 m and HL clades at 125 m. (D) Density

114 plots of *Prochlorococcus* per-cell chlorophyll fluorescence. Note the double population at 115 m.
115 The circles represent the populations sorted and analyzed by nanoSIMS. (E) Density plots of the
116 ratios of C-specific C uptake rate (μ_C) to N-specific N uptake rate (μ_N) from NanoSIMS analysis of
117 each sorted sub-population from 115 m, the single population from 125 m, and lab cultures. The
118 numbers of cells measured in each population are 45 (LL 115m), 49 (HL 115m), 55 (125m), and
119 489 (lab culture).

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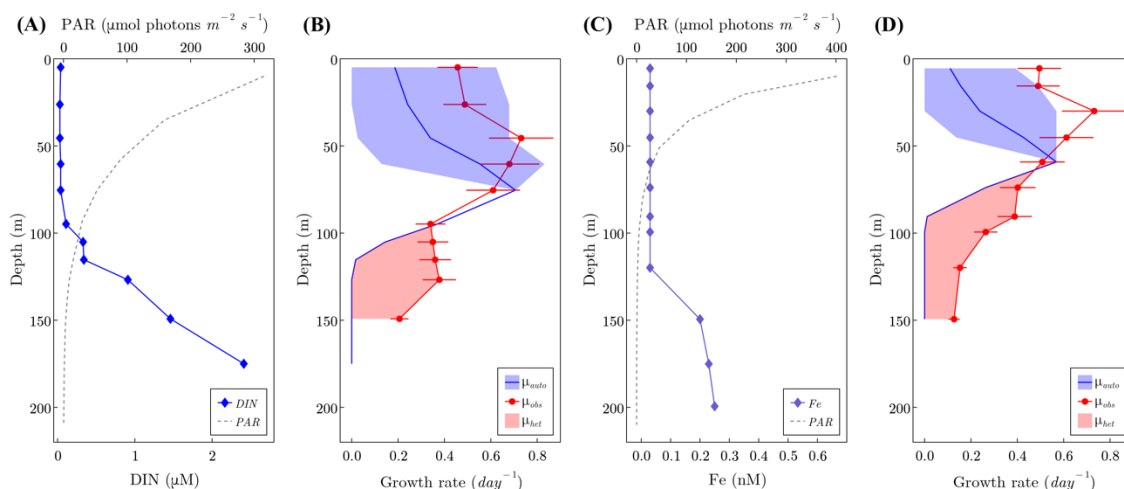
121 We next measured the uptake of ^{13}C -labelled bicarbonate (representing C-fixation through
122 photosynthesis) and of ^{15}N -labeled ammonium (representing nitrogen uptake) in single
123 *Prochlorococcus* cells from the DCM, using Nanoscale Secondary Ion Mass Spectrometry
124 (NanoSIMS). Essentially all of the *Prochlorococcus* cells at 115 and 125 m depth were active
125 (photosynthesized and took up NH_4). The observation that essentially all of the *Prochlorococcus*
126 cells in natural samples are active is consistent with a similar study in the North Pacific²⁵, and
127 suggests that dead or chlorotic cells observed in laboratory cultures^{13,26} may be relatively rare in
128 nature, at least during midday at the DCM. Nevertheless, the per-cell photosynthesis rates at
129 these depths were not sufficient to support the growth rates indicated by the nitrogen-specific
130 nitrogen uptake rates, even though the uptake experiments were performed when light intensity
131 was maximal (Figure 1E). Previous studies from multiple oceanic regions based on cell cycle
132 analysis and on ^{14}C incorporation into divinyl-chlorophyll indicate that *Prochlorococcus* cells at
133 depths of 100-150 m replicate every 4-7 days (a growth rate of $0.14\text{-}0.25 \text{ day}^{-1}$)²⁷⁻³⁰. However,
134 the observed C-specific C uptake rate (μ_C) was only $\sim 0.024 \text{ day}^{-1}$, too low to support these
135 expected growth rates, while the observed N-specific N uptake rate (μ_N) was $\sim 0.16 \text{ day}^{-1}$
136 indicating a doubling time of ~ 6 days. Furthermore, μ_C/μ_N was only ~ 0.15 in the field, much lower
137 than normal cells which are expected to be ~ 1 ($\mu_C \approx \mu_N$). Indeed, μ_C/μ_N in lab cultured
138 *Prochlorococcus* was ~ 0.75 (Figure 1E). Taken together, these quantitative observations suggest
139 that $>80\%$ of the C required for the expected growth rate of these *Prochlorococcus* cells at the
140 DCM must come from non-photosynthetic sources.

141

142 *Evaluation of potential growth rate profiles.*

143 Our Mediterranean samples suggest that a large fraction of carbon assimilated by *Prochlorococcus*
144 in the deeper reaches of the photic zone is of organic origin. By comparing measured profiles of
145 growth rates and modeling photosynthetic carbon fixation from sites in the Pacific, we ask if this
146 is consistent in other regions and infers the water column integrated contribution of
147 heterotrophy. Vaultot et al.³¹ and Liu et al.³² reported vertical profiles of *Prochlorococcus* division
148 rates based on cell-cycle analysis in the Equatorial Pacific (EqPac, 0°N, 140°W) and North Pacific
149 Subtropical Gyre (HOT, 22°45'N, 158°W; Station ALOHA), respectively. These data were obtained
150 in the context of an extensive biogeochemical survey (JGOFS EqPac)³³ and time-series station
151 (HOT)³⁴ and are associated with rich contextual data sets including observations of cell counts,
152 photon fluxes and nutrient concentrations (Figure 2A, C). Calibrated by observed, noon-time PAR
153 profiles, we simulated the daily cycle of photosynthesis and the vertical profiles of
154 *Prochlorococcus*' carbon-specific, net photosynthesis rate (day^{-1}). We simulated both HL and LL
155 ecotypes, using laboratory calibrations of the photosynthesis-irradiance relationship from Moore
156 and Chisholm²⁴. Similarly, using allometric scaling for fixed-nitrogen, phosphate and dissolved iron
157 uptake rates^{35,36}, along with observed environmental concentrations, we evaluated the nutrient-
158 specific uptake rates (day^{-1}). Full details are presented in Materials and Methods. The estimated,
159 purely autotrophic growth rates were determined by the most limiting resource at each depth
160 (Figure 2B, D). Light and carbon fixation strongly limited the simulated autotrophic growth in the
161 deeper region of the photic layer, while fixed nitrogen (HOT), iron (EqPac) and carbon fixation,
162 due to photo-inhibition, were important near the surface (Figure 2). While the observed growth
163 rates at the surface were mostly within the ranges predicted from the photophysiological
164 parameters of HL and LL strains (blue shade in Figure 2B and D), the model failed to resolve the
165 observed growth rates below ~75-100 m at both stations. Rather, the model unequivocally
166 suggests that photosynthesis alone cannot account for the observed division rates at depth. We
167 interpret the differences between the modeled autotrophic and observed actual growth rates at
168 depth (red shading) to infer the minimal rate of organic carbon assimilation of *Prochlorococcus*.
169 The two stations represent very different physical and biogeochemical regimes, yet show similar
170 qualitative structure. Mixotrophy appears to become significant at different depths at the two
171 stations (95 m at HOT and 60 m at EqPac) but at similar level of PAR ($\sim 15 \mu mol photons m^{-2} s^{-1}$,
172 $\sim 5\%$ of surface PAR). Using observed cell densities^{31,32} and assumed cellular carbon quotas³⁷ we
173 estimated the vertically integrated autotrophic net primary production for *Prochlorococcus* to be
174 $\sim 0.35 gC m^{-2} day^{-1}$ at HOT and $\sim 0.20 gC m^{-2} day^{-1}$ at EqPac, with vertically integrated

175 heterotrophic contributions (based on the red shading in Figures 2B and D) of ~ 0.075
 176 $gC\ m^{-2}\ day^{-1}$ at HOT and $\sim 0.069\ gC\ m^{-2}\ day^{-1}$ at EqPac. In other words, assimilation of
 177 organic carbon is inferred to support $\sim 18\%$ of total *Prochlorococcus* biomass production at HOT
 178 and $\sim 25\%$ at EqPac. Furthermore, organic carbon uptake contributes $\sim 80\%$ at HOT and 54% at
 179 EqPac of the total production below the depth where the contribution of mixotrophy is greater
 180 than photosynthesis, broadly consistent with the isotopic inference from the deep photic zone in
 181 the Mediterranean. We note that this model does not take into account exudation of organic
 182 carbon by *Prochlorococcus* which is not well constrained experimentally and would likely reduce
 183 the inferred growth rates at the surface^{38–41}. Indeed, mixotrophy (uptake of glucose and amino
 184 acids) has been observed in surface *Prochlorococcus*^{9,10}, suggesting that our estimate provides a
 185 lower bound of the contribution of mixotrophy to integrated *Prochlorococcus* production.



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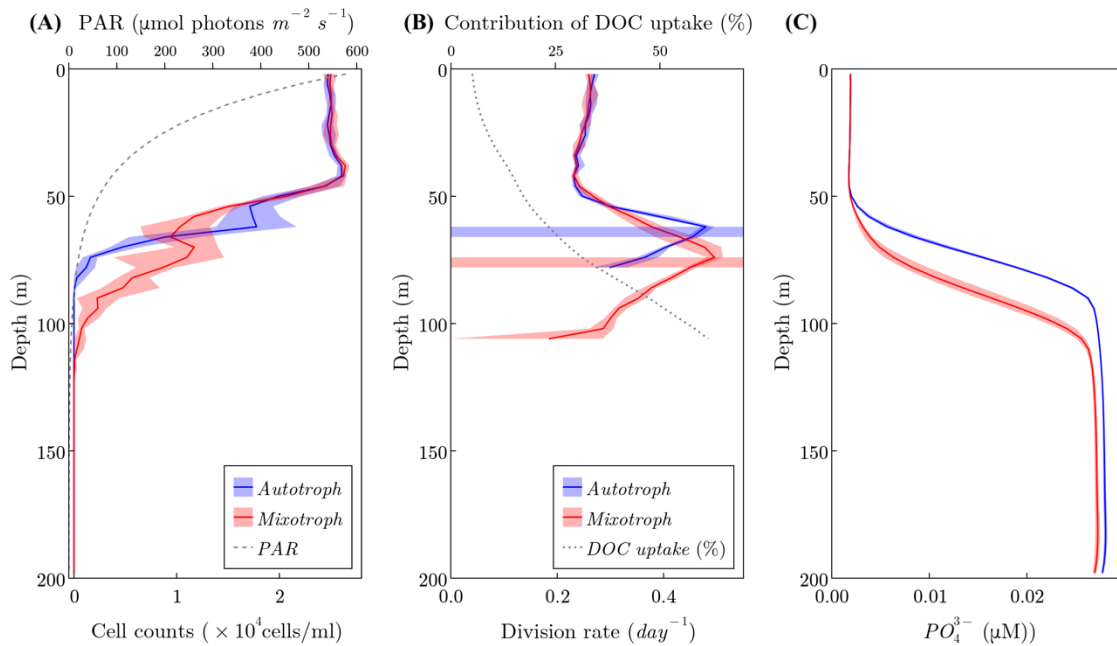
187 **Figure 2. Simulated growth rates at HOT and EqPac.** (A), (C) Observations of PAR and dissolved
 188 inorganic nitrogen (DIN) at the Hawaii Ocean Time-series (HOT, panel A) and of PAR and dissolved
 189 iron (Fe) at the equatorial Pacific (EqPac, panel C). (B), (D) Simulated autotrophic growth rate
 190 (blue line) and observed growth rates (red line with dots, data from⁴²) at HOT (B) and EqPac (D).
 191 The blue shade represents the difference between simulated HL and LL ecotypes, and the red
 192 shade represents the inferred heterotrophic growth rate. A 19% error of observed growth rate is
 193 included both at HOT and EqPac according to Vaultot et al.³¹.

194

195 *Simulations in a dynamic water column.*

196 To investigate the implications of mixotrophy on biogeochemical dynamics, we employed an
197 individual-based modeling approach (see Method for details), simulating trajectories of individual
198 *Prochlorococcus* cells (or super-agents representing many cells) through light and nutrient
199 environments in a two-dimensional, highly resolved turbulent fluid flow (see supporting movie).
200 Inorganic nutrients and a DOC-like tracer are represented by density-based equations. Briefly,
201 individuals fix carbon by photosynthesis and take up inorganic nitrogen and phosphorus. Two
202 idealized types of individuals are simulated separately, one with a strict photo-autotrophic
203 lifestyle and the other which is mixotrophic and able to assimilate carbon from the DOC-like
204 substance. The mixotrophic individual cannot live strictly heterotrophically, as suggested by Coe
205 et al.¹³, which we parameterize as requiring 1% of the incorporated C to come from
206 photosynthesis. In Figure 3A we illustrate horizontally-averaged profiles of cell density from the
207 purely autotrophic and mixotrophic simulations, illustrating how mixotrophy supports a
208 population of *Prochlorococcus* below ~75 m. The simulated daily division rate of $\sim 0.2 \text{ day}^{-1}$ at
209 depth (Figure 3B) is consistent with the published cell-cycle profiles from the subtropical and the
210 Equatorial Pacific^{31,32} and is a bit higher than the aforementioned inferred division rate in the
211 Mediterranean based on NH_4 uptake. Mixotrophs and autotrophs share the same division rate
212 ($\sim 0.3 \text{ day}^{-1}$) in the mixed layer (surface 50 m) where the inorganic nutrient is the limiting factor
213 in the simulations. The autotrophs then reach a maximum daily division rate of $\sim 0.5 \text{ day}^{-1}$ at 60
214 m depth where the transition of N to C limitation happens, and then decrease rapidly to zero at
215 90 m depth due to light limitation. In contrast, the mixotrophs have a deeper maximum growth
216 rate of $\sim 0.5 \text{ day}^{-1}$ at 80 m depth where the transition of N to C limitation occurs and gradually
217 decrease to $\sim 0.2 \text{ day}^{-1}$ at 125 m depth (Figure 3B). The deeper maximum division depth of the
218 mixotrophs and their ability to maintain a population at depths where photosynthesis is not
219 sufficient are supported by the DOC utilization, which is presented as a black line in Figure 3B. In
220 the mixotrophic simulation, the contribution of DOC uptake to the vertically integrated total
221 production is ~12%; ~43% when light becomes the limiting factor, below the red stripe in Figure
222 3B. The contribution of DOC and the maximal depth at which *Prochlorococcus* can grow are
223 broadly consistent with the division rate profile model and are sensitive to parameter values
224 which control the nutritional value of the DOC-like substance (and which cannot be *a priori*
225 constrained by empirical data at this point; see Materials and Methods). Notably, the horizontal
226 stripes in Figure 3B indicate the depth at which limitation shifted from nutrients to C in the two

227 ensembles of simulations. This horizon is deeper when the cells are mixotrophic and leads to a
228 significantly deeper nutricline in the simulation with mixotrophic cells (Figure 3C).



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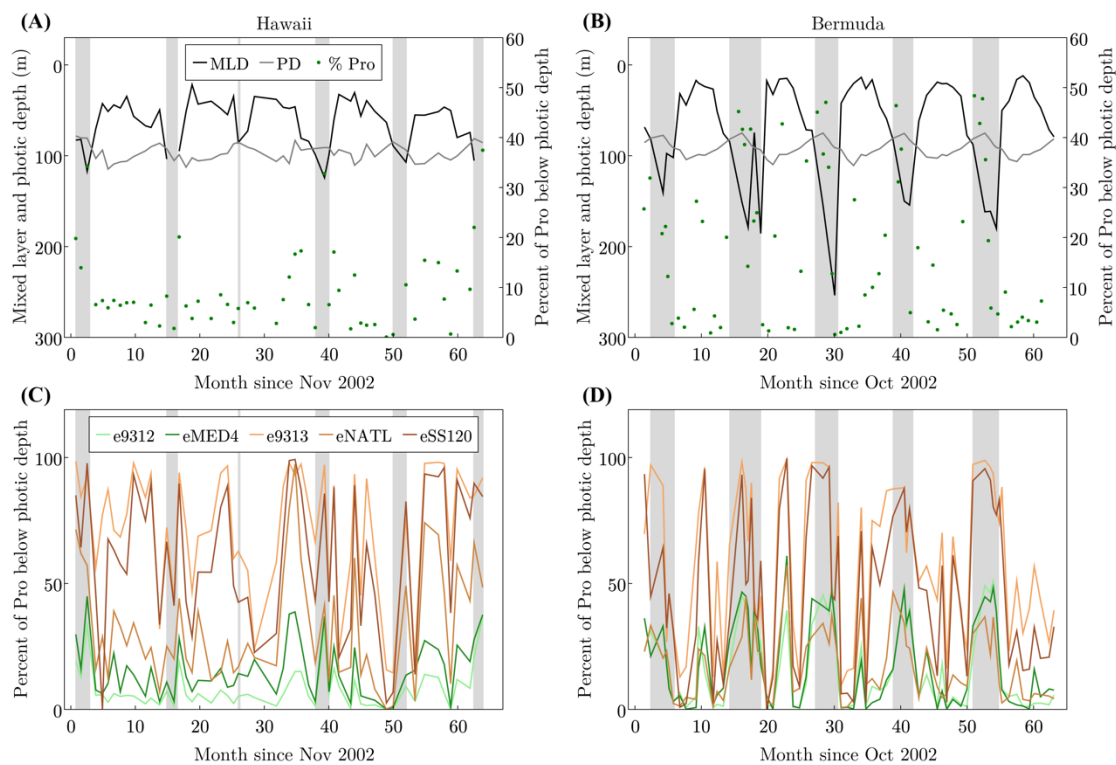
230 **Figure 3. Vertical profiles of simulated autotrophs and mixotrophs in the individual-based**
231 **model.** The red and blue shades in all panels indicate the differences between an ensemble of 10
232 model runs. (A) Vertical profiles of cell density of simulated autotrophs (blue) and mixotrophs
233 (red). The vertical profile of PAR is represented as the gray dashed line. (B) Vertical profiles of cell
234 division rate of autotrophs (blue) and mixotrophs (red). The blue and red stripes indicate the
235 transition point from nutrient limitation to carbon limitation of phytoplankton growth. The black
236 dotted line represents the contributions of DOC uptake to total carbon acquisition in mixotrophs.
237 (C) Vertical profiles of phosphate in simulations of autotroph (blue) and mixotroph (red).

238

239 *Interpretation of vertical distributions of Prochlorococcus ecotype at time-series stations.*

240 To what extent does mixotrophy supports natural, genetically-diverse, populations of
241 *Prochlorococcus*? To answer this question, we calculated the fraction of the *Prochlorococcus* cells
242 and of individual ecotypes living below the depth where they can be supported by photosynthesis
243 alone over a 5-year time series in the north Atlantic and Pacific gyres (Hawaii and Bermuda time
244 series study sites, respectively²³). We consider only the time of the year when the water column

245 is stratified (white regions in Figure 4), defined here as a mixed layer depth that is shallower than
246 the photic depth (light intensity is $>10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for high-light strains or > 2.8
247 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for low-light strains, experimentally-determined minimal light
248 requirement for active growth of high-light and low-light adapted strains during a 14:10 day-night
249 cycle²⁴). This is because at other times cells below the photic depth but still within the upper mixed
250 layer could be transferred closer to the surface and therefore receive increased light. An average
251 of $\sim 8\text{-}10\%$ of the *Prochlorococcus* cells during these stratified periods are likely to be light-starved
252 (Figure 4 A, B), including the vast majority of LL adapted ecotypes (Figure 4C, D).



253

254 **Figure 4: Estimating the number of *Prochlorococcus* cells and of specific ecotypes found below**
255 **their photic depth at Hawaii and Bermuda.** (A), (B) The percent of total *Prochlorococcus* cells
256 (Pro) found below their photic zone at Hawaii (A) and Bermuda (B), defined as the integrated
257 illumination level supporting the growth of representative strains in laboratory cultures²⁴ (grey
258 line shows this depth for HL strains). The black line shows the mixed layer depth (MLD), the grey
259 line shows the photic depth (PD), the green dots represent the percentages of *Prochlorococcus*
260 below photic depth, the grey areas are non-stratified conditions where cells may be mixed from

261 depth to the surface. (C), (D) The percentage of each *Prochlorococcus* ecotype below its photic
262 depth. The data are taken from Malmstrom et al.²³.

263

264 **Conclusions**

265 We have presented several lines of evidence illustrating the importance of mixotrophic carbon
266 assimilation by *Prochlorococcus*. The uptake of isotopically labelled nitrogen uptake in samples
267 from the Mediterranean Sea indicate doubling times at the DCM of about a week, consistent with
268 cell-cycle based observations from the Equatorial and Subtropical Pacific^{27,29-32}. The associated
269 uptake of labelled carbon suggests that this growth rate is only viable if more than three-quarters
270 of assimilated carbon is sourced from organic matter. Using a laboratory-calibrated model of
271 carbon-specific photosynthesis rates and local environmental data, we compared carbon-limited
272 growth rates with observed cell-cycle observations at the Pacific locations. We estimated that
273 18-25% of depth integrated, net carbon assimilation by *Prochlorococcus* is heterotrophic at those
274 sites, with as much as 80% heterotrophic carbon supply at the DCM. We note that while this shifts
275 perception of the photo-autotrophic nature of primary producers, products such as remote-
276 sensing based estimates of global-scale primary production are typically calibrated with data from
277 isotopically labeled inorganic carbon studies and hence, other sources of error notwithstanding,
278 are appropriately estimating photosynthesis and not growth rates. We explored the wider
279 consequences of the phenomenon in simulations with an individual-based model that resolves a
280 DOC-like substance. These simulations suggest that such extensive mixotrophy in the deeper
281 photic layer will significantly deepen the nutricline. This is significant for carbon cycle simulations,
282 most of which do not currently resolve mixotrophy and may predict, or inappropriately tune, a
283 too-shallow nutricline. Finally, investigation of the ecotypic, vertical biogeography in the
284 subtropical North Pacific and North Atlantic²³ indicates that low-light adapted *Prochlorococcus*
285 spend 50-100% of their time, depending on season, below the deepest horizon for photo-
286 autotrophically viable maintenance of the population. We propose that reliance on mixotrophy,
287 rather than on photosynthesis, underpins the ecological success of a large fraction of the global
288 *Prochlorococcus* population and its collective genetic diversity.

289

290 **Materials and Methods**

291 *Isotope labelling and phylogenetic analysis of a natural marine bacterioplankton population at sea*

292 Mediterranean seawater was collected during August 2017 (station N1200, 32.45°N, 34.37°E)
293 from 11 depths by Niskin bottles and divided into triplicate 250 ml polycarbonate bottles. Two
294 bottles from each depth were labeled with 1mM Sodium bicarbonate-¹³C and 1mM Ammonium-
295 ¹⁵N chloride (Sigma-Aldrich, USA) and all 3 bottles (2 labelled and 1 control) were incubated at the
296 original depth and station at sea for 3.5 hours around mid-day. The short incubation time was
297 chosen to minimize isotope dilution and potential recycling and transfer of ¹³C and ¹⁵N between
298 community members²⁵. After incubation, bottles were brought back on board and the incubations
299 were stopped by fixing with 2X EM grade glutaraldehyde (2.5% final concentration) and stored at
300 4 °C until sorting analysis. Cell sorting, NanoSIMS analyses and the calculation of uptake rates
301 were performed as described in Roth-Rosenberg et al.²⁶.

302

303 *DNA collection and extraction from seawater*

304 Samples for DNA were collected on 0.22 µm Sterivex filters (Millipore). Excess water was removed
305 using a syringe, 1 ml Lysis buffer (40 mM EDTA, 50 mM Tris pH 8.3, 0.75 M sucrose) was added
306 and both ends of the filter were closed with parafilm. Samples were kept at -80°C until extraction.
307 DNA was extracted by using a semi-automated protocol including manual chemical cell lysis
308 before the automated steps. The manual protocol began with thawing the samples, then the
309 storage buffer was removed using a syringe and 170 µl lysis buffer added to the filters. 30 µl of
310 Lysozyme (20 mg/ml) were added to the filters and incubated at 37°C for 30 min. After incubation,
311 20 µl proteinase K and 200 µl buffer AL were added to the tube for 1 hour at 56°C (with agitation).
312 The supernatant was transferred to a new tube and DNA was extracted using the QIAcube
313 automated system and QIAamp DNA Mini Protocol: DNA Purification from Blood or Body Fluids
314 (Spin Protocol, starting from step 6, at the BioRap unit, Faculty of Medicine, Technion). All DNA
315 samples were eluted in 100 µl DNA free distilled-water.

316

317 *ITS PCR amplification*

318 PCR amplification of the ITS was carried out with specific primers for *Prochlorococcus*
319 CS1_16S_1247F (5'-ACACTGACGACATGGTCTACACGTACTACAATGCTACGG) and Cs2 ITS_Ar (5'-
320 TACGGTAGCAGAGACTTGGTCTGGACCTCACCTTATCAGGG)^{21,22}. The first PCR was performed in
321 triplicate in a total volume of 25 µl containing 0.5 ng of template, 12.5 µl of MyTaq Red Mix

322 (Bioline) and 0.5 μ l of 10 μ M of each primer. The amplification conditions comprised steps at 95°C
323 for 5 min, 28/25 (16S/ITS) cycles at 95°C for 30 sec, 50°C for 30 sec and 72°C for 1 min followed
324 by one step of 5 min at 72°C. All PCR products were validated on a 1% agarose gel and triplicates
325 were pooled. Subsequently, a second PCR amplification was performed to prepare libraries.
326 These were pooled and after a quality control sequenced (2x250 paired-end reads) using an
327 Illumina MiSeq sequencer. Library preparation and pooling were performed at the DNA Services
328 (DNAS) facility, Research Resources Center (RRC), University of Illinois at Chicago (UIC). MiSeq
329 sequencing was performed at the W.M. Keck Center for Comparative and Functional Genomics at
330 the University of Illinois at Urbana-Champaign (UIUC).

331

332 *ITS Sequence processing*

333 Paired-end reads were analyzed using the Dada2 pipeline⁴³. The quality of the sequences per
334 sample was examined using the Dada2 'plotQualityProfile' command. Quality filtering was
335 performed using the Dada2 'filterAndTrim' command with parameters for quality filtering
336 `truncLen=c(290,260)`, `maxN=0`, `maxEE=c(2,2)`, `truncQ=2`, `rm.phix=TRUE`, `trimLeft=c(20,20)`.
337 Following error estimation and dereplication, the Dada2 algorithm was used to correct sequences.
338 Merging of the forward and reverse reads was done with minimum overlap of 4 bp. Detection and
339 removal of suspected chimeras was done with command 'removeBimeraDenovo'. In total,
340 388,417 sequences in 484 amplicon sequence variants (ASVs) were counted. The ASVs were
341 aligned in MEGA6⁴⁴ and the first ~295 nucleotides, corresponding to the 16S gene, were trimmed.
342 The ITS sequences were then classified using BLAST against a custom database of ITS sequences
343 from cultured *Prochlorococcus* and *Synechococcus* strains as well as from uncultured HL and LL
344 clades.

345

346 *Individual-based Model*

347 PlanktonIndividuals.jl (v0.1.9) was used to run the individual-based simulations. A full
348 documentation is available at <https://juliaocean.github.io/PlanktonIndividuals.jl/dev/>. Briefly, the
349 cells fix inorganic carbon through photosynthesis and nitrogen, phosphorus and DOC from the
350 water column and grow until division or grazing. Cell division is modeled as a probabilistic function
351 of cell size. Grazing is represented by a quadratic probabilistic function of cell population. Cells
352 consume nutrient resources which are represented as Eulerian, density-based tracers. We set up

353 two separate simulations, each of them either has a population of an obligate photo-autotroph
354 or a mixotroph which also consumes DOC. The initial conditions and parameters are the same for
355 the two simulations except the ability of mixotrophy. The simulations were run with a time step
356 of 1 minute for 360 simulated days to achieve a steady state. We run the two simulations for
357 multiple times in order to get the range of the stochastic processes. The code of this configuration
358 is available at https://github.com/zhenwu0728/Prochlorococcus_Mixotrophy.

359

360 *Evaluation of autotrophic growth rates.*

361 We evaluated the carbon-specific, daily-averaged carbon fixation rate, \mathbb{P} as a function of light
362 intensity (I , μE) as follows:

$$363 \quad \mathbb{P} = \frac{1}{\Delta t} \int_0^{\Delta t} \frac{q_{Chl}}{q_C} P_S^{Chl} \left(1 - e^{-\alpha_{Chl} I / P_S^{Chl}} \right) e^{-\beta_{Chl} I / P_S^{Chl}} dt .$$

364 Here, following Platt et al.⁴⁵: P_S^{Chl} is an empirically constrained coefficient representing the
365 Chlorophyll-a specific carbon fixation rate ($mol C \cdot (mol Chl)^{-1} \cdot s^{-1}$) and $\frac{q_{Chl}}{q_C}$ is the molar
366 Chlorophyll-a to carbon ratio. α_{Chl} and β_{Chl} are empirically determined coefficients representing
367 the initial slope of the photosynthesis-light relationship and photo-inhibition effects at high
368 photon fluxes, respectively. Here we impose empirically determined values for α_{Chl} and β_{Chl} and
369 P_S^{Chl} from the published study of Moore and Chisholm²⁴. To find the maximum estimate for
370 *Prochlorococcus* photosynthesis at different light intensities we use photo-physiological
371 parameters for a High-Light adapted ecotype (MIT9215), acclimated at $70 \mu mol photons \cdot m^{-2} \cdot$
372 s^{-1} and a Low-Light adapted ecotype (MIT9211), acclimated $9 \mu E$. $\Delta t = 24$ hours. I is the hourly
373 PAR, estimated by scaling the observed noon value at each depth with a diurnal variation
374 evaluated from astronomical formulae based on geographic location and time of year^{33,34}. The
375 Chlorophyll to Carbon ratio, $\frac{q_{Chl}}{q_C}$, is estimated as a function of growth rate and light intensity using
376 the model of Inomura et al.⁴⁶ which was calibrated by laboratory data from Healey⁴⁷.

377 The Chlorophyll to carbon ratio, $\frac{q_{Chl}}{q_C}$, can be modeled as a function of growth rate and light
378 intensity^{46,48}. Here we use the Inomura⁴⁶ model (equation 17 therein) where parameters were
379 calibrated with laboratory data from Healey⁴⁷. An initial guess of the growth rate and the

380 empirically informed light intensity are used to estimate $\frac{q_{chl}}{q_c}$, which is then used to evaluate the
381 light-limited, photoautotrophic growth rate

$$382 \quad \mathbb{V}_C^{auto} = \frac{P}{q_c}$$

383 from which the Chlorophyll to carbon ratio is again updated. The light-limited growth rate is used
384 to re-evaluate the Chlorophyll to carbon ratio. Repeating this sequence until the values converge,
385 \mathbb{V}_C^{auto} and $\frac{q_{chl}}{q_c}$ are solved iteratively.

386 The nitrogen-specific uptake rate of fixed nitrogen (day^{-1}) is modeled as

$$387 \quad \mathbb{V}_N = \mathbb{V}_N^{max} \frac{1}{Q_N} \frac{N}{N + K_N}$$

388 where values of the maximum uptake rate, \mathbb{V}_N^{max} and half-saturation, K_N , are determined from
389 empirical allometric scalings³⁵, along with a nitrogen cell quota Q_N from Bertilsson et al.³⁷ (0.77
390 $fmol N cell^{-1}$).

391 The P-limited growth rate, or the phosphorus-specific uptake rate of phosphate (day^{-1}), is
392 modeled as

$$393 \quad \mathbb{V}_P = \mathbb{V}_P^{max} \frac{1}{Q_P} \frac{PO_4^{3-}}{PO_4^{3-} + K_P}$$

394 where values of the maximum uptake rate, \mathbb{V}_P^{max} and half-saturation, K_P , are determined from
395 empirical allometric scalings³⁵, along with a nitrogen cell quota Q_P from Bertilsson et al.³⁷ (0.048
396 $fmol P cell^{-1}$).

397

398 Iron uptake is modeled as a linear function of cell surface area (SA), with rate constant (k_{Fe}^{SA})
399 following Shaked et al.³⁶.

$$400 \quad \mathbb{V}_{Fe} = k_{Fe}^{SA} \cdot SA \frac{1}{Q_{Fe}}$$

401 The potential light-, nitrogen-, phosphorus- and iron-limited growth rates ($\mathbb{V}_C, \mathbb{V}_N, \mathbb{V}_P, \mathbb{V}_{Fe}$) were
402 evaluated at each depth in the water column and the minimum is the local modeled photo-

403 autotrophic growth rate estimate, absent of mixotrophy (blue lines, Figure 2B, D). The the model
404 is available at https://github.com/zhenwu0728/Prochlorococcus_Mixotrophy.

405

406 A significant premise of this study is that heterotrophy is providing for the shortfall in carbon
407 under very low light conditions, but not nitrogen. It is known that *Prochlorococcus* can assimilate
408 amino acids⁹ and therefore the stoichiometry of the heterotrophic contribution might alter the
409 interpretations. However, it is also known that *Prochlorococcus* can exude amino acids³⁸ which
410 might cancel out the effects on the stoichiometry of *Prochlorococcus*.

411 For the estimates of photo-trophic growth rate from local environmental conditions (Figure 2) we
412 employed photo-physiological parameters from laboratory cultures of *Prochlorococcus*²⁴. For the
413 purposes of this study, we have assumed that the photosynthetic rates predicted are Net Primary
414 Production which means that autotrophic respiration has been accounted for in the
415 measurement. However, the incubations in that study were of relatively short timescale (45 min),
416 which might suggest they are perhaps more representative of Gross Primary Production. If this is
417 the case, our estimates of photo-autotrophic would be even lower after accounting for
418 autotrophic respiration, and thus would demand a higher contribution from heterotrophic carbon
419 uptake. In this regard, our estimates might be considered a lower bound for organic carbon
420 assimilation.

421

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435

436 **Author contributions**

437 DA, DRR, TLK, AV, MV and DS designed experiments, DRR, DA, TLK, LZ and DS performed
438 experiments and field analyses, DRR, DA, TLK, AV, and FE performed NanoSIMS analyses, DA, DRR,
439 TLK, AV, LZ, FE, HPG, MV and DS analyzed experimental results. WZ, MJF, OW and DS designed
440 and executed the growth rate simulations. WZ designed and executed the individual-based model
441 simulations. WZ, DA, DRR, TLK, MJF and DS wrote the manuscript with contributions from all
442 authors.

443

444 **Competing interests**

445 The authors declare no competing interests.

446

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