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Solange Duhamel, France van Wambeke, Dominique Lefèvre, Mar Benavides, Sophie Bonnet

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6 **Mixotrophic metabolism by natural communities of unicellular cyanobacteria in the**
7 **western tropical South Pacific Ocean**
8

9 ^{1,*}Solange Duhamel, ²France Van Wambeke, ²Dominique Lefevre, ^{2,3,4}Mar Benavides, ^{2,3}Sophie
10 Bonnet
11

12 ¹Lamont Doherty Earth Observatory, Columbia University, Palisades, NY 10964, USA

13 ²Aix Marseille Université, Université de Toulon, CNRS, IRD, Mediterranean Institute of
14 Oceanography (MIO) UM 110, 13288, Marseille, France

15 ³Aix Marseille Université, Université de Toulon, CNRS, IRD, Mediterranean Institute of
16 Oceanography (MIO) UM 110, 98848, Noumea, New Caledonia

17 ⁴Now at: Marine Biological Section, Department of Biology, University of Copenhagen,
18 Strandpromenaden 5, 3000 Helsingør, Denmark
19

20 **Corresponding author: S Duhamel, Lamont-Doherty Earth Observatory of Columbia*
21 *University, 61 Route 9W, Palisades, NY 10964-8000, USA*

22 *E-mail: sduhamel@ldeo.columbia.edu; Phone: 845-365-8774; Fax: 845-365-8150*
23

24 *Running title: Mixotrophy in natural marine cyanobacteria*
25

26 *Originality-Significance Statement:* Marine unicellular cyanobacteria of the genus
27 *Prochlorococcus* and *Synechococcus* are major contributors to primary production and carbon
28 (C) export in the open ocean. These picocyanobacteria are considered to be photoautotrophic, but
29 recent evidence suggests they may also benefit from assimilation of organic compounds
30 (mixotrophy). Most studies investigating the light-dependent organic C uptake potential of
31 marine cyanobacteria have been performed with cultures, while only one field study has
32 demonstrated glucose uptake by *Prochlorococcus* in the Atlantic Ocean. Hence, in situ data is
33 lacking to assess the potential mixotrophic nutrition of these globally relevant marine
34 cyanobacteria, how it compares to their autotrophic nutrition mode (CO₂ fixation), and its
35 environmental controls (nutrients, light levels, etc.).

36 To fill this gap, we used a combination of radiolabeled organic molecules in incubation
37 experiments where both light availability and photosynthetic electron transport were controlled,
38 followed by flow cytometry cell sorting to separate picoplankton groups in natural samples along
39 an oligotrophic to ultraoligotrophic transect in the Western Tropical South Pacific (WTSP)
40 Ocean. Our results demonstrate that natural *Prochlorococcus* and *Synechococcus* can incorporate
41 organic molecules, including glucose, and that rates are reduced in the dark or when
42 photosynthesis is inhibited. This mixotrophic metabolism by unicellular cyanobacteria was
43 widespread in the tested trophic gradient in the WTSP Ocean. In comparison, the low-DNA-
44 content bacteria, a group dominated by photoheterotrophic bacteria from the SAR11 group, also
45 presented large (~35%) light-enhanced cell-specific glucose uptake, but generally lower than for
46 *Prochlorococcus* and *Synechococcus* (~50%). Nevertheless, parallel group specific C uptake
47 from ¹⁴C-sodium bicarbonate suggest that the most abundant marine phytoplankton,
48 *Prochlorococcus* and *Synechococcus* remain primarily autotrophic. Our results also indicate that

49 mixotrophy by marine picocyanobacteria is more likely to be an adaptation to low inorganic
50 nutrient availability as cell specific uptake rates of organic molecules containing nutrients were
51 much higher (35.5 ± 16.5 time for leucine uptake by *Prochlorococcus*) than for glucose.

52 These findings are a significant contribution for developing a mechanistic understanding
53 of the diverse cellular physiologies of different bacterioplankton groups, and to improve our
54 understanding of microbial adaptations to light and nutrient availability, but also our knowledge
55 of marine dissolved organic matter cycling, and the role of light in bacterioplankton
56 biogeochemical functions in the ocean.

57

58 *Keywords:* cyanobacteria; mixotrophy; photoheterotrophy; carbon cycling; organic molecules

59 *Conflict of Interest:* The authors declare no conflict of interest

60 **Summary**

61

62 Cyanobacteria are major contributors to ocean biogeochemical cycling. However,
63 mixotrophic metabolism and the relative importance of inorganic and organic carbon
64 assimilation within the most abundant cyanobacteria are still poorly understood. We
65 explore the ability of *Prochlorococcus* and *Synechococcus* to assimilate organic
66 molecules with variable C:N:P composition and its modulation by light availability and
67 photosynthetic impairment. We used a combination of radiolabeled molecules
68 incubations with flow cytometry cell sorting to separate picoplankton groups from the
69 western tropical south Pacific Ocean. *Prochlorococcus* and *Synechococcus* assimilated
70 glucose, leucine, and ATP at all stations, but cell-specific assimilation rates of N and P
71 containing molecules were significantly higher than glucose. Incubations in the dark or
72 with an inhibitor of photosystem II resulted in reduced assimilation rates. Light-enhanced
73 cell-specific glucose uptake was generally higher for cyanobacteria (~50%) than for the
74 low nucleic acid fraction of bacterioplankton (LNA, ~35%). Our results confirm previous
75 findings, based mainly on cultures and genomic potentials, showing that *Prochlorococcus*
76 and *Synechococcus* have a flexible mixotrophic metabolism, but demonstrate that natural
77 populations remain primarily photoautotrophs. Our findings indicate that mixotrophy by
78 marine cyanobacteria is more likely to be an adaptation to low inorganic nutrient
79 availability rather than a facultative pathway for carbon acquisition.

80 **Introduction**

81 Unicellular marine cyanobacteria (e.g., *Prochlorococcus* and *Synechococcus*) are
82 major contributors to primary production and carbon (C) export in the open ocean (Li et
83 al., 1983, Richardson & Jackson 2007, Johnson & Lin 2009, Martiny et al., 2009).
84 Cyanobacteria are aerobic oxygenic prokaryotes that use a chlorophyll-based light-
85 harvesting complex and CO₂ as a C source. Therefore, marine representatives have been
86 classically considered photoautotrophs (Karl 2007), and *Prochlorococcus* and
87 *Synechococcus* have long been considered to be unable to use organic C sources such as
88 glucose (Waterbury et al., 1986, Bèjà & Suzuki 2008). However, the simple picture that
89 marine cyanobacteria are purely photoautotrophic is actually much more complex.
90 Indeed, recent molecular evidences indicate that organic compound uptake genes are
91 ubiquitous within marine picocyanobacteria (Yelton et al., 2016), suggesting that these
92 photosynthetic microorganisms may present mixotrophic metabolism. *Prochlorococcus*
93 and *Synechococcus* have the genetic capacity to use not only organic molecules with key
94 limiting chemical elements such as nitrogen (N) and phosphorus (P), but also molecules
95 devoid of such elements, such as glucose (Gao and Xu 2012, Gomez-Baena et al., 2008,
96 Muñoz-Marín et al., 2013, 2017, Yelton et al., 2016). Unicellular marine cyanobacteria
97 are thus potentially capable of degrading and assimilating a wide range of organic
98 molecules, but only a handful of studies have measured assimilation rates of some
99 selected organic molecules such as amino acids, adenosine-5'-triphosphate (ATP) or
100 dimethylsulfoniopropionate (Michelou et al., 2007, Mary et al., 2008a,b Duhamel et al.,
101 2012, Ruiz-Gonzalez et al., 2012b, Björkman et al., 2015, Evans et al., 2015). Results
102 suggest that marine picocyanobacteria may obtain nutrients mixotrophically via the

103 uptake of N and/or P-containing organic molecules when facing nutrient limitation
104 (Yelton et al., 2016).

105 Direct evidences that marine picocyanobacteria use organic molecules to obtain
106 energy or C are scarce, particularly under *in situ* conditions. To date, only one study by
107 Muñoz-Marín et al. (2013) demonstrated that *Prochlorococcus* in the Atlantic Ocean can
108 take up organic C lacking other essential nutrients at nanomolar concentrations in the
109 light (using radiolabeled glucose). A limited number of studies have attempted to
110 quantify the relative contribution of mixotrophy compared to photoautotrophy in C
111 assimilation by natural communities of marine cyanobacteria. First attempts to quantify
112 the contribution of glucose uptake to total C assimilation in *Prochlorococcus* indicated
113 that it may be very small (<1%; Muñoz-Marín et al. 2013). However, measurements of C
114 assimilation from inorganic and organic substrates were done separately (in the same
115 sampling area but on separate cruises and by different users) and only one taxon was
116 considered, making comparison between fluxes and taxonomic groups difficult (Paoli et
117 al., 2008, Muñoz-Marín et al., 2013, Benavides et al., 2017). Thus, it is still unclear if
118 organic C assimilation by marine unicellular cyanobacteria is ubiquitous, and how it
119 contributes to total C uptake to different groups of picocyanobacteria (e.g. high-light and
120 low-light adapted *Prochlorococcus* and *Synechococcus*, Partensky & Garzarek, 2010) in
121 comparison to CO₂ fixation.

122 Additional critical gaps in our understanding of picocyanobacteria mixotrophic
123 metabolism concern regulating factors. In particular, we still don't know how organic C
124 assimilation by marine unicellular cyanobacteria depends upon light availability and
125 photosynthetic electron transport in natural settings (Moore, 2013). Recent findings

126 showed that light enhances picocyanobacteria uptake rates of amino acids, ATP or
127 dimethylsulfoniopropionate (Michelou et al., 2007, Mary et al., 2008a, Duhamel et al.,
128 2012, Ruiz-Gonzalez et al., 2012b, Björkman et al., 2015, Evans et al., 2015).
129 Interestingly, organic nutrient assimilation by the low nucleic acid bacterioplankton
130 (LNA), which has been shown to be numerically dominated by the photoheterotrophs
131 SAR11 (Mary et al., 2006, Hill et al., 2010, Gómez-Pereira et al., 2013, Zubkov et al.,
132 2015), was enhanced to a comparable extent to that by *Prochlorococcus* (Gómez-Pereira
133 et al., 2013). However, the mechanisms of this light enhancement are not well
134 understood. Recently, Muñoz-Marín et al. (2017) explored the potential role of
135 photosynthetic electron transport in the regulation of glucose uptake by *Prochlorococcus*
136 SS120 in laboratory cultures. Using different photosynthesis inhibitors, they showed that
137 glucose uptake was significantly reduced or even inhibited. This remains untested with
138 natural populations.

139 To improve our understanding of marine unicellular cyanobacteria utilization of
140 organic molecules and answer pressing questions about their mixotrophic metabolism in
141 the wild, we conducted a set of experiments in the WTSP during the OUTPACE cruise
142 (Oligotrophy to ULtra-oligotrophy PACific Experiment). Based on the ubiquity of
143 picocyanobacterial organic compound uptake genes (Yelton et al. 2016), but low glucose
144 uptake rates by *Prochlorococcus* in the Atlantic Ocean (Muñoz-Marín et al. 2013), we
145 hypothesize that mixotrophy by *Prochlorococcus* and *Synechococcus* is an adaptation to
146 nutrient limitation rather than a facultative pathway for carbon acquisition. To test this
147 hypothesis, we measured group-specific assimilation rates of organic molecules
148 containing C only, C and N, or C, N and P (glucose, leucine, and ATP, respectively) in

149 *Prochlorococcus* and *Synechococcus*. We then compared C assimilation from glucose
150 and sodium bicarbonate to test how much C is assimilated via autotrophic or mixotrophic
151 pathways. We also compared the light enhancement of leucine, ATP, and for the first
152 time, glucose, by natural *Prochlorococcus*, *Synechococcus* and LNA, and tested the
153 effect of the photosystem II inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).
154 We hypothesize that as for organic nutrient assimilation, organic C uptake is enhanced in
155 the light, and that it is partially tied to photosynthetic electron transport.

156

157 **Results**

158 *Description of the study area*

159 Three incubation experiments were carried out in the WTSP at the long duration (LD, 72
160 h) stations A, B and C (thereafter LDA, LDB, LDC, respectively) chosen for their
161 contrasted biogeochemical conditions (Table 1, Moutin et al. 2017). At LDA and LDB,
162 seawater was sampled within the well-lit top mixed layer (ML at 54% PAR, 7 and 9 m
163 depths, respectively) and at the deep chlorophyll maximum (DCM, 0.3% PAR, 70 and 90
164 m, respectively), while LDC was sampled at 60 m depth (10% PAR), to compare results
165 between microbial communities adapted to different light levels and nutrient conditions.
166 The ML was 14, 21, and 34 m deep, at LDA, LDB, and LDC, respectively (Moutin et al.,
167 2018).

168 Station LDA was relatively oligotrophic (Table 1), with moderately high
169 chlorophyll *a* concentrations compared to typical open ocean regional values (0.36 ± 0.05
170 $\mu\text{g l}^{-1}$, Benavides et al., 2017). Station LDB was sampled in an elevated chlorophyll *a*
171 patch, about twofold the concentration at LDA in the ML ($0.83 \pm 0.07 \mu\text{g l}^{-1}$, Benavides et

172 al., 2017; de Verneuil et al., 2018). Compared to LDA, LDB exhibited similar low
173 nutrient concentrations in the ML but depleted inorganic nutrients at the DCM (Table 1).
174 *Prochlorococcus*, *Synechococcus* and LNA abundances were 2.3, 1.4 and 3.6-fold higher
175 in the ML of LDB than at LDA; and respectively were 2.3–6.1, 13.1–31.8 and 1.5–4.5
176 fold higher in the ML than at the DCM (Table 1). LDC presented characteristics of the
177 oligotrophic south Pacific gyre (lower surface chlorophyll concentrations and deeper
178 DCM, Claustre et al., 2008, Moutin et al. 2017). Glucose concentrations were on average
179 2.6 to 4.3-fold higher at LDA than at LDB and LDC. Leucine and ATP concentrations
180 were on average 1.4 to 4.5-fold higher at LDA than at LDB and LDC (Table 1).

181

182 *Assimilation rates of organic molecules in light incubations*

183 The assimilation of radiolabeled organic molecules with C alone, or C with N or P
184 (glucose, leucine and ATP, respectively) was measured in separate incubations. Flow
185 sorting of radiolabeled cells after incubation in the light showed that *Prochlorococcus*,
186 *Synechococcus* and LNA assimilated glucose, leucine and ATP at all sampled stations
187 and depths (Fig.1). Group-specific assimilation rates of glucose followed trophic
188 gradients and were generally higher at LDB>LDA>LDC, and higher in the ML than at
189 the DCM (Fig. 1 a, b). Cell specific rates of glucose assimilation by LNA were 4.8 and
190 1.7-fold higher than *Prochlorococcus* in the ML at LDA and LDB, respectively, and 2.3-
191 fold higher at LDA-DCM, but *Prochlorococcus* presented higher cell specific rates than
192 LNA at LDB-DCM and LDC-60m (4.1 and 1.3 times, respectively, Fig. 1a).
193 *Synechococcus* presented 1.4±0.2-fold higher cell specific assimilation rates than
194 *Prochlorococcus* at LDB (p<0.05) and LDC, but about half the rate by *Prochlorococcus*

195 at LDA, although differences were not significant at LDA and LDC. Because
196 *Synechococcus* was much less abundant than *Prochlorococcus* and LNA (on average
197 *Prochlorococcus*, *Synechococcus* and LNA represented 46 ± 5 , 2 ± 2 and $52\pm 7\%$ of the
198 cumulated abundance (*Prochlorococcus*+*Synechococcus*+LNA), respectively; Table 1),
199 the contribution of the *Synechococcus* group to glucose assimilation rates was at least an
200 order of magnitude lower than that of *Prochlorococcus* and LNA groups (0.90 ± 1.12 ,
201 0.08 ± 0.11 and 1.95 ± 2.52 pmol Glc l⁻¹ h⁻¹ on average, for *Prochlorococcus*,
202 *Synechococcus* and LNA groups, respectively, Fig. 1b). Consequently, *Synechococcus*
203 accounted for < 3% of the total glucose assimilation by the three combined sorted groups
204 (*Prochlorococcus*+*Synechococcus*+LNA), while LNA accounted for > 65% at LDA and
205 LDB-ML, and *Prochlorococcus* contributed to 70 and 54% at LDB-DCM and LDC.
206 Thus, *Prochlorococcus* can be a large contributor to glucose assimilation rates in
207 comparison to SAR11-like photoheterotrophic groups. But compared to the total
208 microbial community glucose assimilation (i.e. bulk rates), *Prochlorococcus* accounted
209 for only $4.9\pm 3.3\%$, similar to results obtained in the Atlantic Ocean (Muñoz-Marín et al.,
210 2013).

211 Group-specific assimilation rates of leucine and ATP were also higher at LDB
212 and LDA than at LDC, and were higher in the ML than at the DCM ($p < 0.05$, Fig. 1). On
213 average, *Prochlorococcus* and LNA groups accounted for 50 ± 23 and $21\pm 7\%$ of leucine
214 assimilation by the total microbial community, respectively; and 17 ± 12 and $40\pm 17\%$ of
215 ATP assimilation by the total microbial community, respectively. The *Synechococcus*
216 group contributed to < 1% of the leucine and ATP assimilation by the total microbial
217 community. Cell specific leucine and ATP assimilation rates by *Synechococcus* were

218 roughly an order of magnitude lower than by *Prochlorococcus*, except for ATP at LDB
219 and LDC, where assimilation rates by *Synechococcus* were similar to those by
220 *Prochlorococcus*.

221

222 *Light-enhanced uptake of organic molecules and relationship to photosynthesis*

223 Flow sorting of labeled cells incubated in light or dark bottles showed that light
224 enhanced the cell specific uptake of all radiolabeled organic molecules tested here,
225 including glucose, and in most cases to a larger extent in *Prochlorococcus* and
226 *Synechococcus* as compared to LNA (Fig. 2). On average, incubations in the light
227 represented an enhancement of 44 ± 18 , 57 ± 30 and $35\pm 11\%$ of dark glucose uptake,
228 73 ± 23 , 57 ± 30 and $35\pm 11\%$ of dark leucine uptake and 56 ± 25 , 35 ± 24 and $43\pm 30\%$ of
229 dark ATP uptake by *Prochlorococcus*, *Synechococcus* and LNA, respectively. In
230 comparison, incubations in the light represented an enhancement of 92 ± 11 and $99\pm 2\%$ of
231 dark ^{14}C -sodium bicarbonate uptake by *Prochlorococcus* and *Synechococcus*,
232 respectively; where dark uptake was negligible (Fig. 2g). Interestingly, the light
233 enhancement of organic molecule uptake rates was higher at the DCM than in the ML for
234 *Prochlorococcus* and *Synechococcus* (light to dark ratios of glucose, leucine and ATP
235 uptake were 1.3–2.1 (ML) vs. 2.2–5.5 (DCM), 1.1–4.1 (ML) vs. 2.2–11.4 (DCM), and
236 1.9–10.5 (ML) vs. 1.5–1.7 (DCM), respectively; Fig. 3, 4, 5). In comparison, light
237 enhancement of organic molecule uptake by LNA was mostly similar between ML and
238 DCM. In SYBR stained samples, high-DNA-content bacteria (HNA) could be properly
239 distinguished from *Prochlorococcus* only in samples from the DCM and differences
240 between light and dark incubations were either not significant or higher in the dark (*t*-test,

241 $p < 0.05$, Fig. 2 b, d, f), and thus this group is not further discussed here. However, these
242 results indicate that the light enhancement of organic molecule assimilation by
243 *Prochlorococcus* could not be a result of by-sorting HNA bacteria overlapping in size
244 with *Prochlorococcus*.

245 Additions of the photosystem II inhibitor DCMU resulted in reduced
246 *Prochlorococcus*, *Synechococcus* and LNA glucose uptake to a level not statistically
247 different from rates in the dark, except for *Prochlorococcus* and LNA at LDB-ML where
248 uptake was higher in DCMU compared to the dark (Fig. 3). Cell specific leucine uptake
249 was lower when DCMU was added, significantly for *Prochlorococcus* and LNA at all
250 stations/depths but only in the ML for *Synechococcus* (Fig. 4). For *Prochlorococcus*,
251 DCMU resulted in higher or equal rates than in the dark while for LNA, DCMU resulted
252 in lower rates than in the dark. In most cases, DCMU resulted in decreased cell specific
253 ATP uptake for the three groups compared to light samples, but differences between dark
254 and DCMU were not significant (except for *Synechococcus* at LDC, Fig. 5).

255

256 *Bacterial production*

257 Because bacterial production is commonly measured using leucine or thymidine
258 assays, the effect of light and DCMU on the incorporation rates of leucine and thymidine
259 into trichloroacetic acid (TCA) insoluble material (Leu_{inc} and Tdr_{inc} , respectively) was
260 estimated in separate incubations. Light affected Leu_{inc} to a larger extent than Tdr_{inc} (Fig.
261 6a, b, e). Leu_{inc} and Tdr_{inc} rates were 12–57% ($40 \pm 21\%$) and 2–27 % ($11 \pm 14\%$) lower in
262 the dark than in the light, respectively (Fig 6e). The addition of DCMU resulted in an
263 average decrease of $68 \pm 10\%$ and $49 \pm 23\%$ in Leu_{inc} and Tdr_{inc} rates in the light,

264 respectively (Fig. 6a, b). The Leu_{inc} to Tdr_{inc} ratio was on average 1.6±0.5 times higher in
265 the light than in the dark, 1.5±0.3 times higher in the light than with DCMU (Fig.6c).

266

267 **Discussion**

268 *Characterization of Prochlorococcus and Synechococcus mixotrophic metabolism.*

269 Owing to their capability to utilize sunlight and atmospheric CO₂ for growth,
270 *Prochlorococcus* and *Synechococcus* are commonly considered photoautotrophs. Yet,
271 recent evidence has shown that the uptake of organic N- (leucine, amino acids) and P-
272 (ATP) molecules by *Prochlorococcus* and *Synechococcus* is enhanced in the light
273 (Michelou et al., 2007, Mary et al., 2008b, Duhamel et al., 2012, Gomez-Pereira et al.,
274 2013). Therefore previous studies (e.g. Björkman et al., 2015, Gomez-Pereira et al., 2012,
275 Michelou et al., 2007, Moore 2013, Muñoz-Marín et al., 2013, 2017, Zubkov 2009,
276 Zubkov et al., 2003) since the early work of Rippka (1972) have commonly defined this
277 nutritional plasticity in marine cyanobacteria as photoheterotrophy, although *sensus*
278 *stricto* this term defines organisms that use light for energy, but cannot use CO₂ as their
279 sole C source. However, these studies demonstrating light-enhancement of N or P-
280 containing organic molecules uptake did not directly verify if marine cyanobacteria could
281 also use organic molecules containing only C (e.g. glucose) and if light also enhances
282 organic C assimilation. To the best of our knowledge, only Muñoz-Marín et al. (2013)
283 demonstrated that *Prochlorococcus* could assimilate glucose, a molecule devoid of
284 heteroatoms (N or P), in natural seawater. Our results geographically expand these
285 findings from the Atlantic Ocean and demonstrate that not only *Prochlorococcus* but also
286 *Synechococcus* assimilate glucose in biogeochemically distinct marine environments of

287 the WTSP. Interestingly, cell- and group- specific assimilation rates of glucose appeared
288 to follow trophic gradients, similar to organic C uptake by *Trichodesmium* (Benavides et
289 al., 2017). Further, we demonstrate that light enhanced cell specific glucose uptake by
290 nearly 50% for *Prochlorococcus* and *Synechococcus*, suggesting that variability in light
291 availability (e.g. changes in light intensity due to euphotic layer PAR gradient, diel
292 sunlight rhythm, cloud coverage) could largely impact organic C assimilation by these
293 cyanobacteria. Similar results were also found in cultures of the low-light
294 *Prochlorococcus* SS120 strain in which 24h incubation in the dark induced a 40%
295 decrease in glucose uptake (Gomez-Baena et al., 2008). Thus, marine cyanobacteria may
296 be a significant competitor of heterotrophic bacteria for this labile molecule, especially
297 during the day, potentially creating temporal patterns between strictly heterotrophic and
298 mixotrophic bacterioplankton. Expanding this finding to all labile C molecules, such
299 temporal patterns could affect dissolved organic matter (DOM) remineralization and C
300 sequestration via the microbial C pump.

301 We also explored the participation of photosynthetic electron transport in the
302 regulation of organic molecules assimilation using the photosynthesis inhibitor DCMU
303 (Rippka, 1972; Stanier, 1973; Neilson & Lewin, 1974; Paerl, 1991; Moore, 2013). In
304 chlorophyll and bacteriochlorophyll containing organisms, DCMU blocks electron flow
305 between photosystem II and plastoquinone, resulting in no O₂ and NADPH production,
306 but allowing ATP synthesis through cyclic electron flow around photosystem I. Our
307 results show that DCMU completely inhibited CO₂ fixation by *Prochlorococcus* and
308 *Synechococcus* but only partially inhibited their assimilation of glucose, leucine and
309 ATP. For most samples and molecules examined, the assimilation rates were significantly

310 higher or not statistically different in the light with DCMU compared to the dark,
311 suggesting that organic molecules incorporation is partially tied to photosynthetic
312 production of energy in the light. The light harvested by *Prochlorococcus* and
313 *Synechococcus* photosynthetic apparatus may thus transfer energy into ATP that can be
314 used in the active transport of organic molecules. Similarly, Muñoz-Marín et al. (2017)
315 found different inhibitory effects on *Prochlorococcus* SS120 glucose uptake using the
316 quinone analogue inhibitor of cytochrome b6f complex in photosystem I –DBMIB
317 (~100%), or the inhibitor of photosystem II –DCMU (~50%), and argued that the ATP
318 generated by photosystem I could maintain up to 50% of the glucose uptake. Seawater
319 used in our experiments was sampled in the early morning and thus cells were likely light
320 energy depleted, assuring that continuing uptake in our dark incubations was not a result
321 of stored energy during the light phase. Therefore, our results demonstrate that natural
322 populations of *Prochlorococcus* and *Synechococcus* are nutritionally and metabolically
323 flexible. We confirm that *in situ*, picocyanobacteria can assimilate selected organic
324 molecules including glucose, a molecule devoid of heteroatoms, and show that
325 assimilation rates are reduced but continue even in the dark or when photosynthesis is
326 impaired. This mixotrophic metabolism could explain the recent findings of the
327 substantial presence of *Prochlorococcus* in the aphotic ocean (Jiao et al., 2014) and the
328 survival of specific strains of *Prochlorococcus* in extended darkness (Coe et al., 2016).

329

330 *Relative importance of mixotrophy for picocyanobacterial C assimilation.*

331 Cell-specific glucose uptake in natural *Prochlorococcus* was on average
332 0.00021 ± 0.00011 fg C cell⁻¹ h⁻¹, similar to results by Muñoz-Marín et al. (2013) in the

333 Atlantic Ocean (0.00010 ± 0.00008 fg C cell⁻¹ h⁻¹, where added and ambient glucose
334 concentrations were comparable to those in our study). *Synechococcus* had higher cell
335 specific glucose assimilation rates than *Prochlorococcus*, but using an average biovolume
336 of 0.17 and 0.33 μm^3 for *Prochlorococcus* and *Synechococcus*, respectively (Grob et al.,
337 2007), we calculated that glucose uptake by *Prochlorococcus* and *Synechococcus* was
338 similar (0.0013 ± 0.0007 and 0.0009 ± 0.0005 fg C μm^{-3} h⁻¹, respectively). Compared to
339 carbon uptake from ¹⁴C-sodium bicarbonate (4.4 ± 1.7 and 44 ± 36 fg C cell⁻¹ d⁻¹ for
340 *Prochlorococcus* and *Synechococcus*, respectively), glucose uptake represented a small
341 fraction (<1%) of total (inorganic + organic) C uptake, similar to the values calculated
342 using results in Muñoz-Marín et al. (2013). This implies that mixotrophy may represent a
343 marginal fraction of *Prochlorococcus* and *Synechococcus* C uptake in the Atlantic and
344 Pacific Oceans, and confirms culture-based studies where *Prochlorococcus* actively takes
345 up glucose when available, but remains primarily autotrophic (Muñoz-Marín et al.,
346 2017). Yet considering that glucose is only one of the greatly diverse dissolved organic C
347 molecules present in the ocean (Moran et al., 2016), the low assimilation rates may be the
348 result of glucose uptake competition with other sugars. Thus, other organic C compounds
349 need to be tested as substrates for mixotrophic growth by marine cyanobacteria.
350 However, mixotrophy by marine picocyanobacteria may represent a greater advantage in
351 response to inorganic nutrient limitation than to access an alternative form of C. Indeed,
352 we found that cell specific uptake of leucine and ATP by *Prochlorococcus* were on
353 average 35.5 ± 16.5 and 3.4 ± 2.3 times greater than cell specific uptake rates of glucose
354 (mol:mol). Still, Muñoz-Marín et al. (2013) argued that the bioenergetic advantage of

355 glucose uptake vs. glucose synthesis de novo may save *Prochlorococcus* significant
356 energy for other metabolic uses.

357

358 *Photoheterotrophy by LNA bacteria.*

359 The LNA group has been previously characterized using molecular tools and
360 results consistently show that it is largely dominated by SAR11 (Mary et al., 2006,
361 2008b, Gomez-Pereira et al., 2013, Morán et al., 2015), a highly abundant group in the
362 subtropical Pacific (West et al., 2016). SAR11 is a clade of proteorhodopsin-based
363 photoheterotrophic bacteria, which are characterized by light-controlled growth and
364 proteorhodopsin expression (Lami et al., 2009). Like in other studies (Mary et al., 2008b,
365 Zubkov 2009, Gomez-Pereira et al., 2013, Evans et al., 2015) we showed that light
366 enhances the assimilation of leucine and ATP by the LNA group. In our experiments,
367 Tdr_{inc} was an excellent proxy of strict heterotrophic bacterial production as the Tdr_{inc}
368 rates were similar in light and dark incubations, unlike Leu_{inc} rates (Fig. 6). This confirms
369 that increased Leu_{inc} rates in the light were mostly due to photoheterotrophy and
370 mixotrophic capacities of *Prochlorococcus*, but not to an indirect effect related to
371 enhanced phytoplankton excretion/exudation in the light. Light-enhanced cell-specific
372 glucose uptake by LNA bacteria was large (~35%), albeit lower than for
373 *Prochlorococcus* and *Synechococcus*, implying that photons can supply a significant part
374 of the energy demand during daytime and the requirement for organic molecules as
375 energy sources is significantly decreased. Surprisingly, the addition of DCMU reduced
376 organic molecules incorporation by LNA. DCMU has been widely used to study
377 phytoplankton metabolism (Jeanjean 1976, Lewis et al., 1984, Garrigue et al., 1992,

378 Ikeya et al., 1997, DeLorenzo et al., 2001, Laurent et al., 2013, Halsey et al., 2014),
379 including photoheterotrophy (Estep & Hoering 1981, Paerl 1991, Johnson & Alric 2012,
380 Knoop et al., 2013, You et al., 2015, Oren et al., 2016, Muñoz-Marín et al., 2017). These
381 studies indicate that DMCU is not lethal, that its inhibitory effect is reversible and that it
382 does not affect heterotrophic processes, even in autotroph-heterotroph symbiotic
383 associations (Vandermeulen et al., 1972, Mühlbauer & Eichacker 1998, Francoeur et al.,
384 2007). Combined with good efficiency against algal photosynthesis (Fig. 6d), and
385 because it was also chosen to study photoheterotrophic metabolism in culture isolates of
386 *Prochlorococcus* (Muñoz-Marín et al., 2017), we used DCMU as selective inhibitor of
387 the photosystem II in cyanobacteria. However, we observed a reduction in organic
388 molecules incorporation in LNA which suggests that DCMU affected LNA directly or
389 indirectly. A direct effect could be either toxicity on heterotrophic metabolism of strict
390 heterotrophs or an action on the light-driven proton pump, proteorhodopsin. Yet, at the
391 end of incubation, LNA cell abundances were similar between treatments, indicating that
392 DCMU may not be lethal to LNA. Moreover, DCMU did not inhibit the light-driven
393 proton pump of the cyanobacteria *Gloeobacter violaceus* which has two types of light-
394 driven proton pumps, chlorophyll-based photosystems and rhodopsin (Choi et al., 2014).
395 Alternatively, the reduction of activity by LNA in DCMU samples may be indirect,
396 resulting from the inhibition of photosynthate production by phytoplankton
397 (photosynthesis was inhibited, Fig. 6d) on which bacteria rely greatly as a source of labile
398 organic substrates for growth (Church et al., 2004, Ruiz-Gonzalez et al., 2012a, 2013).
399

400 *Implication of picocyanobacterial uptake of leucine for bacterial production*
401 *measurements in the ocean.*

402 Despite being important for our understanding of biological productivity in the
403 ocean, light enhanced bacterial production and uptake of leucine by the most abundant
404 marine microbes, *Prochlorococcus*, *Synechococcus* and LNA, have been the subject of a
405 limited number of studies (Church et al., 2004, Michelou et al., 2007, Mary et al., 2008b,
406 Ruiz-Gonzalez et al., 2013, Björkman et al., 2015). In incubations with saturating
407 concentrations of leucine (20 nM), previous reports found that *Prochlorococcus*
408 contributes significantly to bacterial production estimates in the North Pacific (Björkman
409 et al., 2015), North Atlantic (Michelou et al., 2007) and Mediterranean Sea (Talarmin et
410 al., 2011a), both in dark or in the light. Here we show that light enhanced bulk Leu_{inc}
411 rates (incorporation rates of leucine into TCA insoluble material), as well as cell-specific
412 leucine uptake by *Prochlorococcus*, *Synechococcus* and LNA in the tropical southwest
413 Pacific Ocean. However, light significantly enhanced bulk Tdr_{inc} rates to a much lesser
414 extent (dark to light ratio was 73–100 %, mean 89%). This may be due to thymidine
415 being preferentially used by heterotrophic bacteria than leucine which can be used by
416 mixotrophic phytoplankton (Michelou et al., 2007, Björkman et al., 2015). Indeed, in our
417 samples, over a third of the total leucine uptake was attributable to the *Prochlorococcus*
418 group. Unfortunately, due to low thymidine specific activity, we were not able to measure
419 its group-specific uptake. This should be verified in future studies as cyanobacteria tested
420 so far do not incorporate Tdr in culture, which was related to a probable lack of
421 thymidine kinase (Pollard & Moriarty 1984). The contribution of picocyanobacteria to
422 bacterial production estimates, particularly using ^3H -leucine, should thus be considered

423 when measuring bacterial production in marine environments, even in dark incubations
424 (Talarmin et al., 2011a, Björkman et al., 2015). Longnecker et al. (2006) found higher
425 $\text{Leu}_{\text{inc}}/\text{Tdr}_{\text{inc}}$ ratio in dark incubated HNA than in the LNA group, and also higher in
426 surface samples than below, particularly in the open-sea station. Besides the general
427 hypothesis of higher rates of protein synthesis relative to DNA synthesis in larger cells,
428 this result could also be partially due to the difficulty to separate *Prochlorococcus* from
429 HNA during cell sorting after SYBR green DNA staining, particularly in sub-surface
430 waters. As seen from seawater cultures, the $\text{Leu}_{\text{inc}}/\text{Tdr}_{\text{inc}}$ ratio can be representative of
431 decoupling between cell division and biomass production (Chin-Leo & Kirchman 1990).
432 In situ, Leu_{inc} rates has been shown also to vary more than Tdr_{inc} rates along diel cycles
433 (Riemann & Bell 1990). Consequently, the use of $\text{Leu}_{\text{inc}}/\text{Tdr}_{\text{inc}}$ ratio as a proxy of
434 unbalanced growth should be misleading when samples are incubated in the light as we
435 demonstrated that it was also affected by photoheterotrophic processes (higher in the light
436 than in the dark or with DCMU).

437

438 **Conclusion**

439 We present several lines of evidence that natural *Prochlorococcus* and
440 *Synechococcus* can assimilate organic molecules with variable C:N:P composition, as
441 well as organic molecules devoid of heteroatoms (i.e. glucose). *Prochlorococcus* and
442 *Synechococcus* assimilated organic molecules in the light but also in the dark or when
443 photosynthesis was altered by DCMU, albeit at significantly reduced rates, verifying
444 previous findings in culture indicating that cyanobacteria are nutritionally versatile. Yet
445 *Prochlorococcus* and *Synechococcus* C uptake from glucose was small compared to CO_2

446 uptake, indicating that they obtain carbon primarily through an autotrophic metabolism.
447 Nevertheless, mixotrophy by these unicellular cyanobacteria was widespread in
448 biogeochemically distinct regions of the WTSP Ocean and cell and group-specific
449 assimilation rates were generally higher in surface than at the DCM. However, cell-
450 specific assimilation rates of the N- and P- containing molecules (leucine and ATP) were
451 significantly higher than that of glucose. Thus, mixotrophy by marine cyanobacteria is
452 more likely to be an adaptation to low inorganic nutrient availability. Many details of
453 marine cyanobacteria mixotrophic metabolism remain to be elucidated. In particular,
454 additional experiments will be necessary to evaluate the global importance of organic vs.
455 inorganic C uptake by marine cyanobacteria and assess the diel variability in these
456 processes, in order to improve C fluxes models (Gasol et al., 2008, Zubkov 2009).
457 Further study of mixotrophic metabolism is likely to contribute not only to our
458 understanding of microbial adaptations to light and nutrient availability but also to our
459 knowledge of marine DOM cycling, and the role of light in modulating bacteria and
460 cyanobacteria biogeochemical functions in the ocean.

461

462 **Experimental Procedures**

463 *Field sampling*

464 This study was conducted in the WTSP along trophic gradients during the
465 OUTPACE cruise (DOI: <http://dx.doi.org/10.17600/15000900>, RV *L'Atalante*, February–
466 April 2015) between New Caledonia and Tahiti (Moutin et al., 2017). Three incubation
467 experiments were carried out at the long duration stations LDA, LDB, and LDC, selected
468 for their contrasted biogeochemical conditions (Table 1). At LDA and LDB, seawater

469 was sampled within the well-lit top mixed layer (ML at 54% PAR, 7 and 9 m depths,
470 respectively) and at the DCM (0.3% PAR, 70 and 90 m, respectively). LDC was sampled
471 at 60 m depth (10% PAR).

472

473 *Bacterioplankton enumeration*

474 Bacterioplankton groups were enumerated from untreated samples using a BD
475 Influx flow cytometer (BD Biosciences, San Jose, CA, USA). *Prochlorococcus* and
476 *Synechococcus* were enumerated in unstained samples while the low-DNA-content
477 (LNA) and high-DNA-content (HNA) bacteria groups were discriminated in a sample
478 aliquot stained with SYBR Green I DNA dye (0.01% final), following published
479 protocols (Gasol et al., 1999, Duhamel et al., 2014). Using a forward scatter detector with
480 small particle option and focusing a 488 plus a 457 nm (200 and 300 mW solid state,
481 respectively) laser into the same pinhole greatly improved the resolution of dim surface
482 *Prochlorococcus* population from background noise in unstained samples. However, in
483 stained samples from the ML, *Prochlorococcus* overlapped with HNA bacteria and HNA
484 abundances were calculated by subtracting *Prochlorococcus* enumerated from unstained
485 samples. Calibration and alignment were done using 1- μ m yellow-green microspheres
486 (Polysciences, USA).

487

488 *Incubation experiments*

489 Seawater collected at the LD stations was distributed into acid-washed and
490 sample rinsed transparent polycarbonate bottles for separate incubations with different
491 radioactive-labeled molecules and under different treatment conditions. For each

492 radioactive molecule tested, a killed control was prepared by adding paraformaldehyde
493 (0.5 % final w/v) for 30 min before adding the radioisotope. Bottles were incubated in
494 on-deck blue-shielded incubators to mimic the amount of transmitted light at the
495 corresponding sampled depth and cooled with surface seawater. Samples were treated
496 with or without addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, at 20 μ M
497 final, for 15 min before adding the radioisotope). For dark incubations, the bottles were
498 masked using multi-layers of matte black aluminum foil (Rosco Matte Black Cinefoil).

499 For each treatment, D-[6- 3 H(N)]-glucose (45.7 Ci mmol^{-1}), L-[3,4,5- 3 H(N)]-
500 Leucine (112 Ci mmol^{-1}) and [2,5',8- 3 H]-Adenosine-5'-triphosphate (52 Ci mmol^{-1}),
501 were used in separate incubations to measure assimilation rates of organic C alone
502 (glucose, Glc), and N- (leucine, Leu) and P- (Adenosine-5'-triphosphate, ATP) –enriched
503 organic compounds, respectively (Perkin Elmer, Waltham, MA, USA). Isotope additions
504 were kept as low as possible considering the specific activity and sensitivity of the cell
505 sorting procedure described below: 3 H-glucose, 3 H-leucine and 3 H-ATP were added at a
506 final concentration of 2, 7 and 1 nmol l^{-1} , respectively. Samples were incubated for 4 to 6
507 h and uptake linearity was checked before each experiment. Additional incubations were
508 done using [14 C]-sodium bicarbonate (43.3 mCi mmol^{-1}) at 3.3 $\mu\text{Ci ml}^{-1}$ final
509 concentration from dawn to dusk (~8 h). At the end of incubation, samples were fixed
510 with paraformaldehyde (0.5 % final, for 15-min in the dark), 20 μl were sampled to
511 measure total activity (dpm l^{-1} , with β -phenylethylamine for ^{14}C samples) and determine
512 the concentration of added molecules (S^* , nmol l^{-1}), 4-ml were filtered onto 0.2- μm
513 polycarbonate membranes to monitor incorporation by the total microbial community
514 (total activity –dpm l^{-1} , and total microbial assimilation rate – $\text{nmol l}^{-1} \text{h}^{-1}$) and 30 to 50 ml

515 was gently concentrated to 4 ml and preserved at -80°C for flow cytometry cell sorting.
516 Radioactivity was measured using scintillation cocktail with low background and high ^3H
517 counting efficiency (Ultima Gold LLT, Perkin Elmer) and a Packard Tri-Carb 3110 TR
518 liquid scintillation counter with ultra-low-level option kit. The turnover time (h) was
519 calculated by dividing the total activity (dpm l^{-1}) by the activity on the 0.2- μm filter (dpm
520 $\text{l}^{-1} \text{h}^{-1}$). The total microbial assimilation rate ($\text{nmol l}^{-1} \text{h}^{-1}$) was calculated by dividing the
521 substrate concentration (ambient concentration (S_a) plus S^*) by the turnover time (h). The
522 ambient concentrations of glucose, leucine and ATP were estimated using a concentration
523 series bioassay of untreated live samples as described by Wright and Hobbie (1966) and
524 modified by Zubkov and Tarran (2005), which represents an upper estimate of ambient
525 concentrations. Thus, calculated assimilation rates represent an upper estimate of
526 assimilation rates at in situ concentrations.

527

528 *Flow cytometry cell sorting*

529 Bacterioplankton groups were characterized as described above. Note that
530 because of the overlap in *Prochlorococcus* with HNA in stained ML samples, we did not
531 systematically sort the HNA population for which results were biased by the contribution
532 of *Prochlorococcus* activity. The Influx flow cytometer was set at the highest sorting
533 purity (1.0 drop single mode) and potential attached cells were discarded using a pulse
534 width vs. forward scatter plot. The drop delay was calibrated using Accudrop Beads (BD
535 Biosciences, USA) and sorting efficiency was verified manually by sorting a specified
536 number of 1- μm yellow-green microspheres (Polysciences, USA) onto a glass slide and
537 counting the beads under an epifluorescence microscope. Using 1.0 drop single mode we

538 systematically recovered 100% of the targeted beads. Performance was validated by
539 sorting *Prochlorococcus* and *Synechococcus* from natural samples and reanalysing the
540 sorted cells flow cytometrically to confirm sort purity, which exceeded 96 %, a result
541 similar to Baer et al. (2017). Increasing numbers of cells from the same incubation
542 sample were sorted (20,000–600,000 LNA and 10,000–300,000 *Prochlorococcus* and
543 *Synechococcus*). Sorted cells were assessed by liquid scintillation following Talarmin et
544 al. (2011b). The ^{14}C -labeled samples were acidified with 0.5 ml of 1N HCl for 24 h to
545 remove any unincorporated ^{14}C -sodium bicarbonate. For each group, at least three
546 samples were sorted and regression analysis between the number of cells sorted and the
547 radioactivity taken up by the sorted cells was used to calculate the per cell activity (dpm
548 cell^{-1}). Radioactivity in the killed control sorted samples (dpm cell^{-1}) was deduced from
549 radioactivity in the respective sorted samples. The cell-specific assimilation rate (nmol
550 $\text{cell}^{-1} \text{h}^{-1}$) was calculated by dividing the radioactivity per cell (dpm cell^{-1}) by the total
551 microbial activity (dpm l^{-1}) measured in the same treatment, and then multiplied by the
552 total microbial assimilation rate at ambient plus added organic substrate concentration
553 (S_a+S^* , nmol $\text{l}^{-1} \text{h}^{-1}$). Statistical tests (one-way ANOVA, Tukey's multiple comparisons
554 test) were carried out to assess significant differences between treatments ($P < 0.05$)
555 using Prism 6 (GraphPad software, La Jolla, CA, USA).

556

557 *Bulk bacterial production*

558 Samples were incubated with [methyl- ^3H]-thymidine (Tdr, 20 nM, 48.8 Ci mmol $^{-1}$)
559 1) or with L-[3,4,5- ^3H (N)]- leucine (Leu, 6 nM, 112 Ci mmol $^{-1}$) for 5 to 6 h. Killed
560 controls and incubation terminations were fixed with formalin 1% final concentration.

561 Tdr samples were treated by the filtration technique (Bell, 1993, ice cold- trichloroacetic
562 acid (TCA) extract) and Leu samples by the centrifuge technique (Smith & Azam, 1992).
563 In both methods, an ethanol rinse was included. Note that bulk bacterial production
564 experiments could not be done at LDA but were done at the short duration station 8
565 instead (hereafter SD8, in the ML, 12m). We occasionally checked that we were working
566 at saturating concentration of Tdr and Leu by testing activities using a range of
567 concentrations of Tdr varying from 5 to 60 nM and of Leu varying from 2 to 45 nM. We
568 confirmed that the Tdr concentration was saturating at all stations and that Leu
569 concentration was saturating at all stations, excepted at SD8 where measured rates were
570 about half the maximum velocities.

571

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585

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826 **Table and Figure Legends:**

827 Table 1. Ancillary data Characteristics of the seawater samples collected at stations LDA, LDB and LDC used in experiments.

828 Latitude (Lat.), longitude (Long.), fraction of surface photosynthetically active radiation (PAR, %) received at the corresponding

829 depth (m), temperature (T, °C), *Prochlorococcus* (Pro), *Synechococcus* (Syn), and LNA cell abundances (10^3 cell ml⁻¹), phosphate and

830 nitrate concentrations (PO_4^{3-} and NO_3^- nmol l⁻¹), and ambient concentrations of glucose (Glc), leucine (Leu) and ATP (nmol l⁻¹).

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Name	Depth m	Coordinates		PAR %	T °C	10 ³ cell ml ⁻¹			nmol l ⁻¹				
		Long.	Lat.			Pro	Syn	LNA	PO ₄ ³⁻	NO ₃ ⁻	Glc	Leu	ATP
LDA-ML	9	164°41E	19°12S	54	29.4	255	28.6	195	40	< 20	1.93	1.78	1.14
LDA-DCM	90			0.3	25.2	112	0.9	128	240	820	1.43	1.80	1.12
LDB-ML	7	170°51W	18°14S	54	29.9	598	39.2	699	30	< 20	0.56	0.52	0.65
LDB-DCM	70			0.3	25.3	98	3.0	156	90	< 20	0.45	0.40	0.81
LDC	60	165°45W	18°40S	10	26.4	145	1.2	156	110	20	0.52	0.56	0.48

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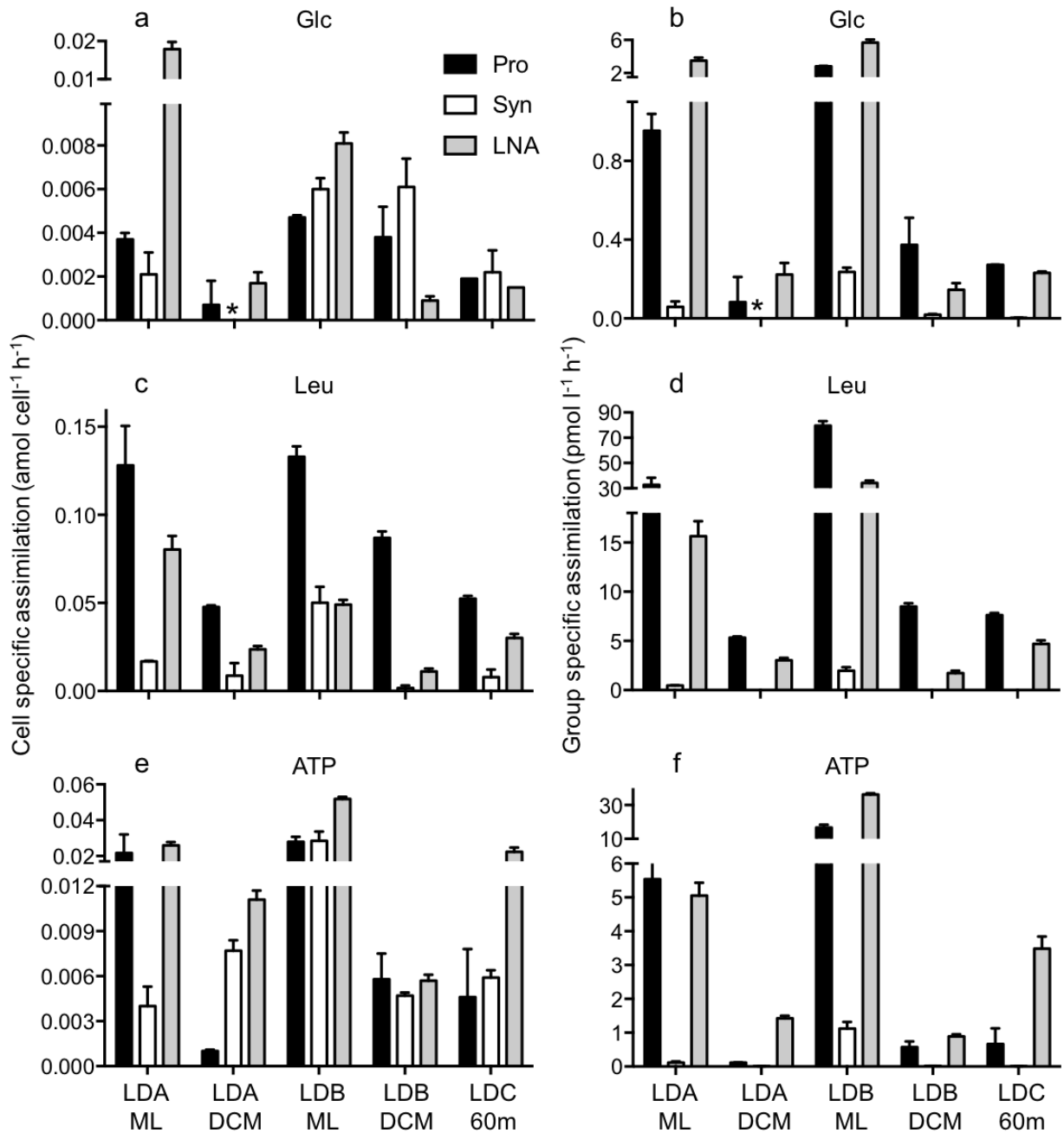
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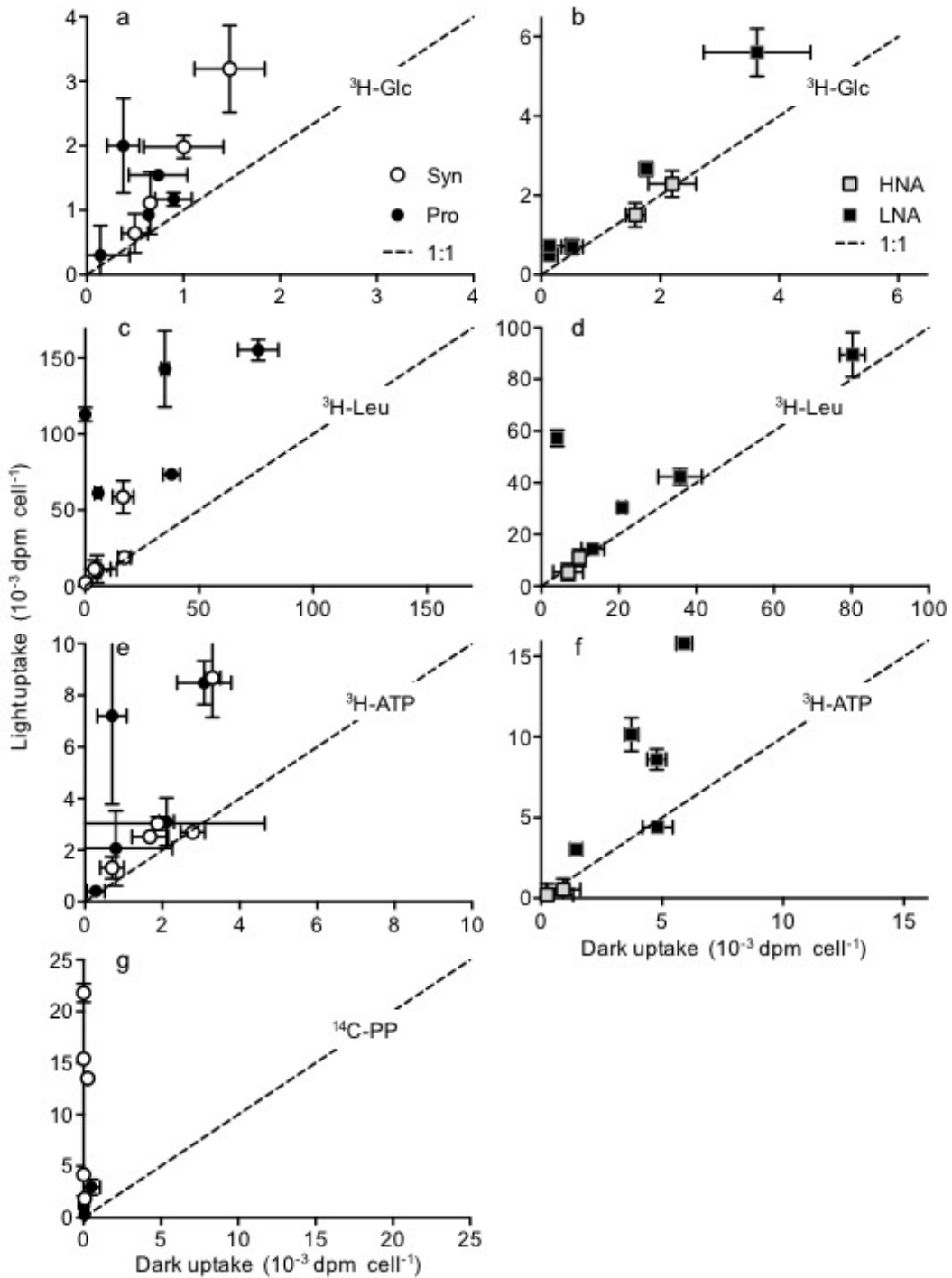
838 Fig. 1: Cell specific (a, c, e, $\text{amol cell}^{-1} \text{h}^{-1}$) and group specific (b, d, f, $\text{pmol l}^{-1} \text{h}^{-1}$)
 839 assimilation rates of glucose (a, b), leucine (c, d) and ATP (e, f) by *Prochlorococcus*
 840 (Pro, black bars), *Synechococcus* (Syn, white bars) and LNA bacteria (LNA, grey bars) in
 841 incubations in the light. Error bars represent standard deviation on triplicate samples. *
 842 indicate non-measurable rates (killed control corrected rates \leq killed control).
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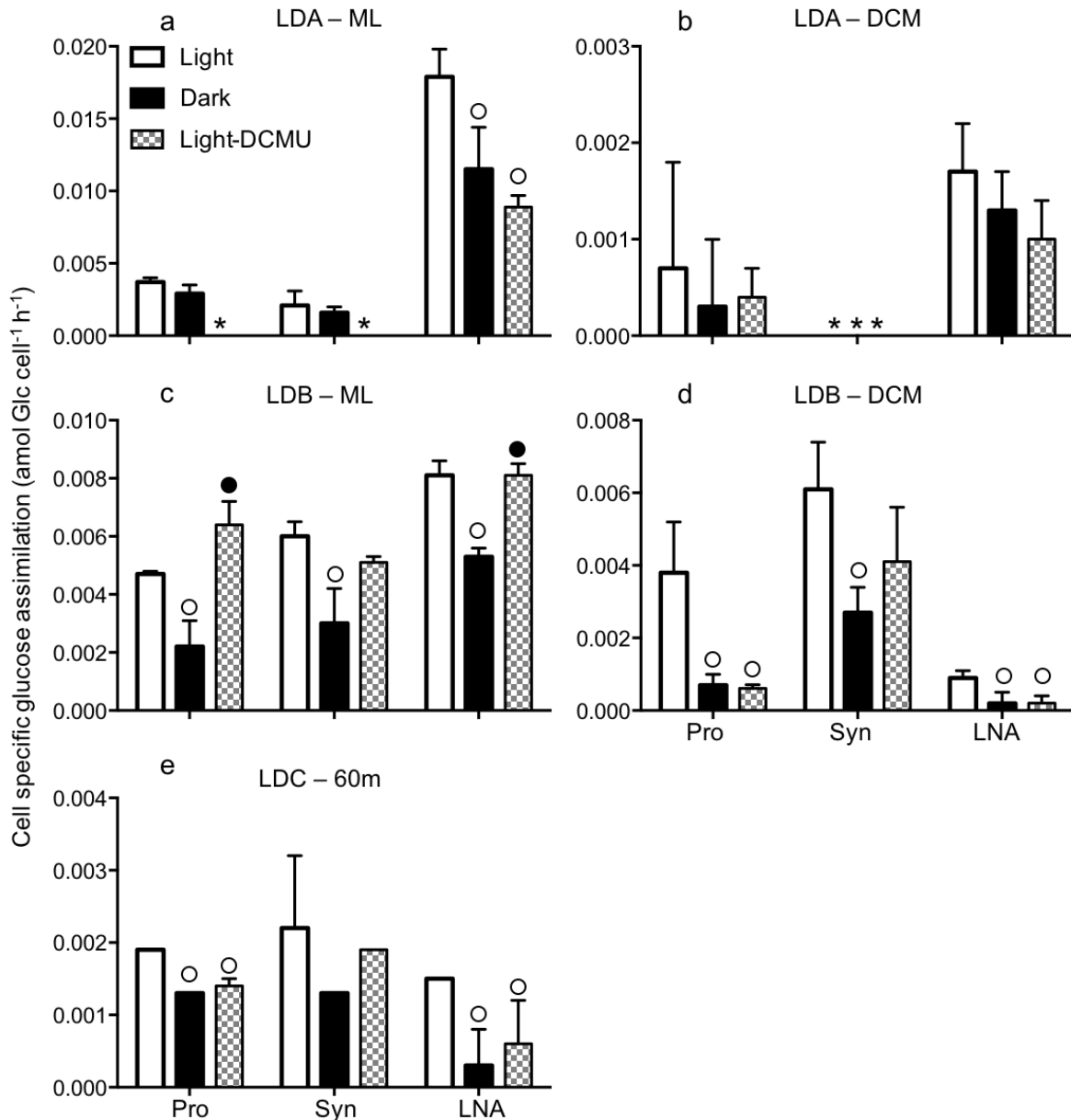
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846 Fig. 2: Scatter plots comparing cell specific uptake (10^{-3} dpm cell $^{-1}$) in the light
 847 (ordinate) and in the dark (abscissa) by picocyanobacteria (Pro: black filled circles, Syn:
 848 white filled circles; a, c, e, g) and bacteria (LNA: black filled squares, HNA: grey filled
 849 squares; b, d, f), for ^3H radiolabeled glucose (^3H -Glc, a, b), leucine (^3H -Leu, c, d) and
 850 ATP (^3H -ATP, e, f), and for ^{14}C radiolabeled sodium bicarbonate (^{14}C -PP, g). Error bars
 851 represent standard deviation on triplicate samples. The dotted lines represent the 1:1 ratio.
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854 Fig. 3: Cell specific glucose assimilation ($\text{amol Glc cell}^{-1} \text{h}^{-1}$) at LDA – ML (a), LDA –
 855 DCM (b), LDB – ML (c), LDB – DCM (d) and LDC – 60m (e), in incubations in the
 856 light (white bars), in the dark (black bars), and in the light with DCMU (checker board
 857 pattern) for *Prochlorococcus* (Pro), *Synechococcus* (Syn) and LNA bacteria (LNA). Error
 858 bars represent standard deviation on triplicate samples. One-way ANOVA multiple
 859 treatment comparison results are represented by white or black circles when values are
 860 significantly ($P < 0.05$) different from the light or the dark treatments, respectively. *
 861 indicate non-measurable rates (killed control corrected rates \leq killed control).
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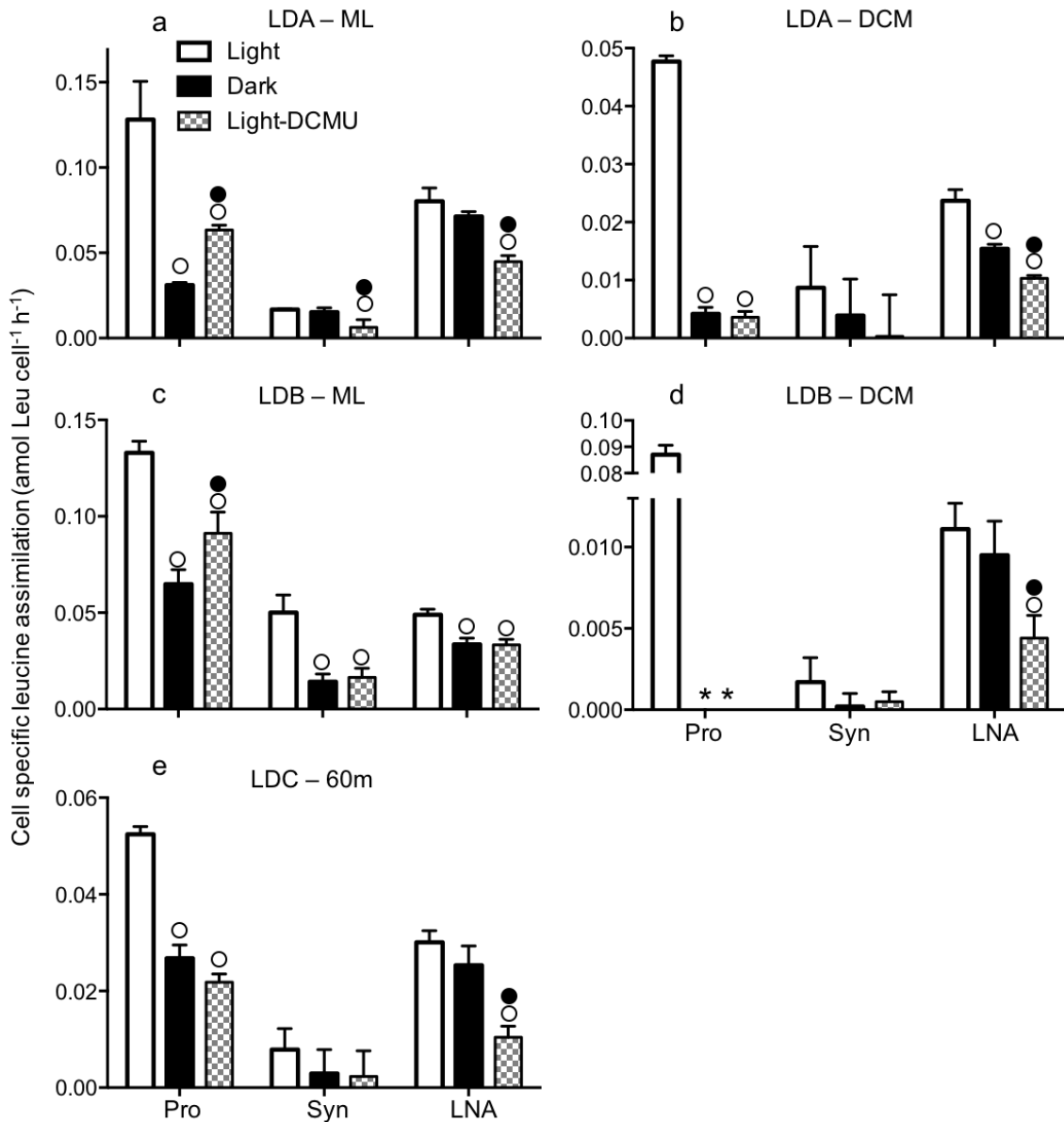


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866 Fig. 4: Cell specific leucine assimilation rate (amol Leu cell⁻¹ h⁻¹) at LDA – ML (a),
 867 LDA – DCM (b), LDB – ML (c), LDB – DCM (d) and LDC – 60m (e), in incubations in
 868 the light (white bars), in the dark (black bars), and in the light with DCMU (checker
 869 board pattern) for *Prochlorococcus* (Pro), *Synechococcus* (Syn) and LNA bacteria
 870 (LNA). Error bars represent standard deviation on triplicate samples. One-way ANOVA
 871 multiple treatment comparison results are represented by white or black circles when
 872 values are significantly (P < 0.05) different from the light or the dark treatments,
 873 respectively. * indicate non-measurable rates (killed control corrected rates ≤ killed
 874 control).
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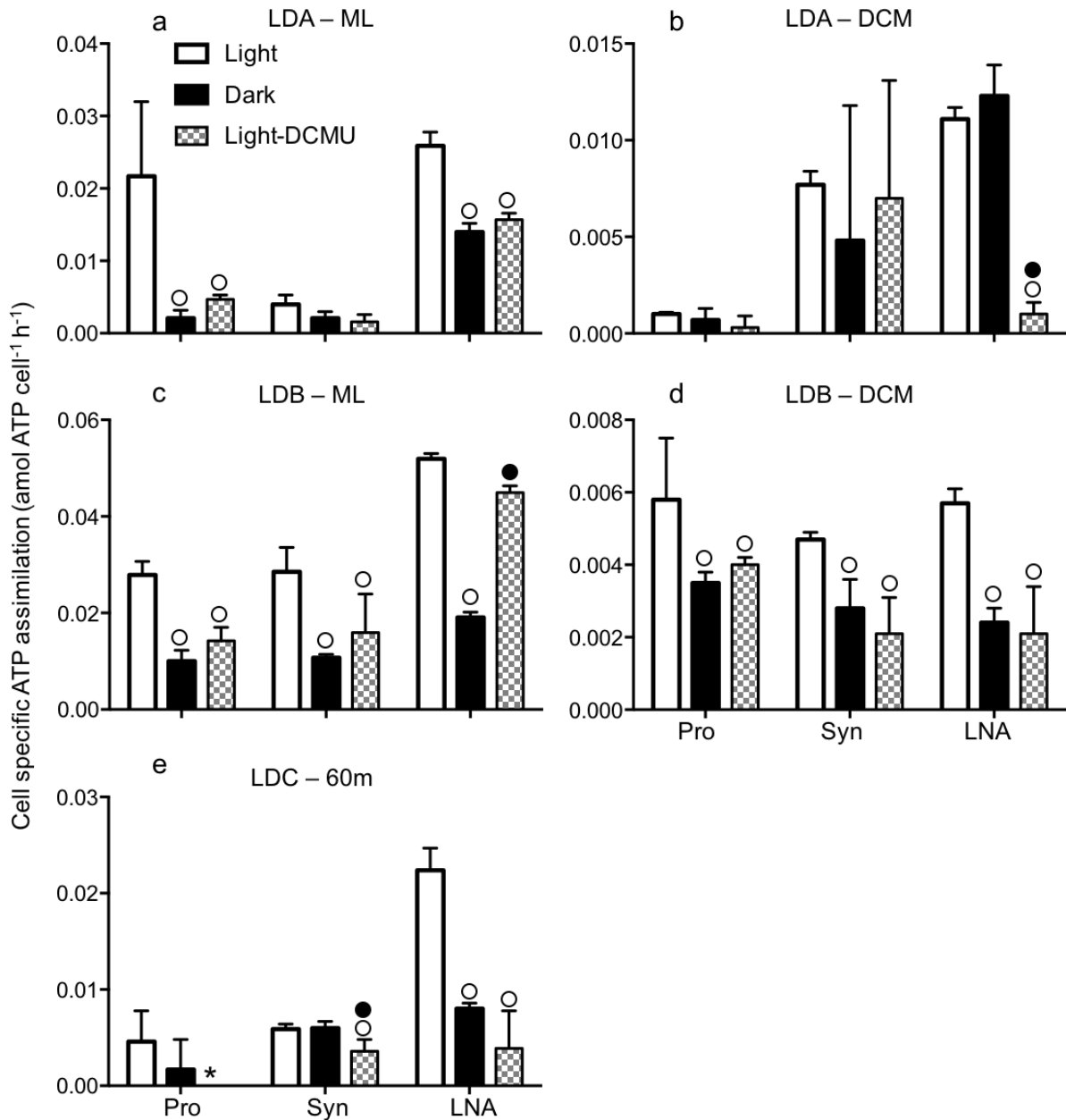


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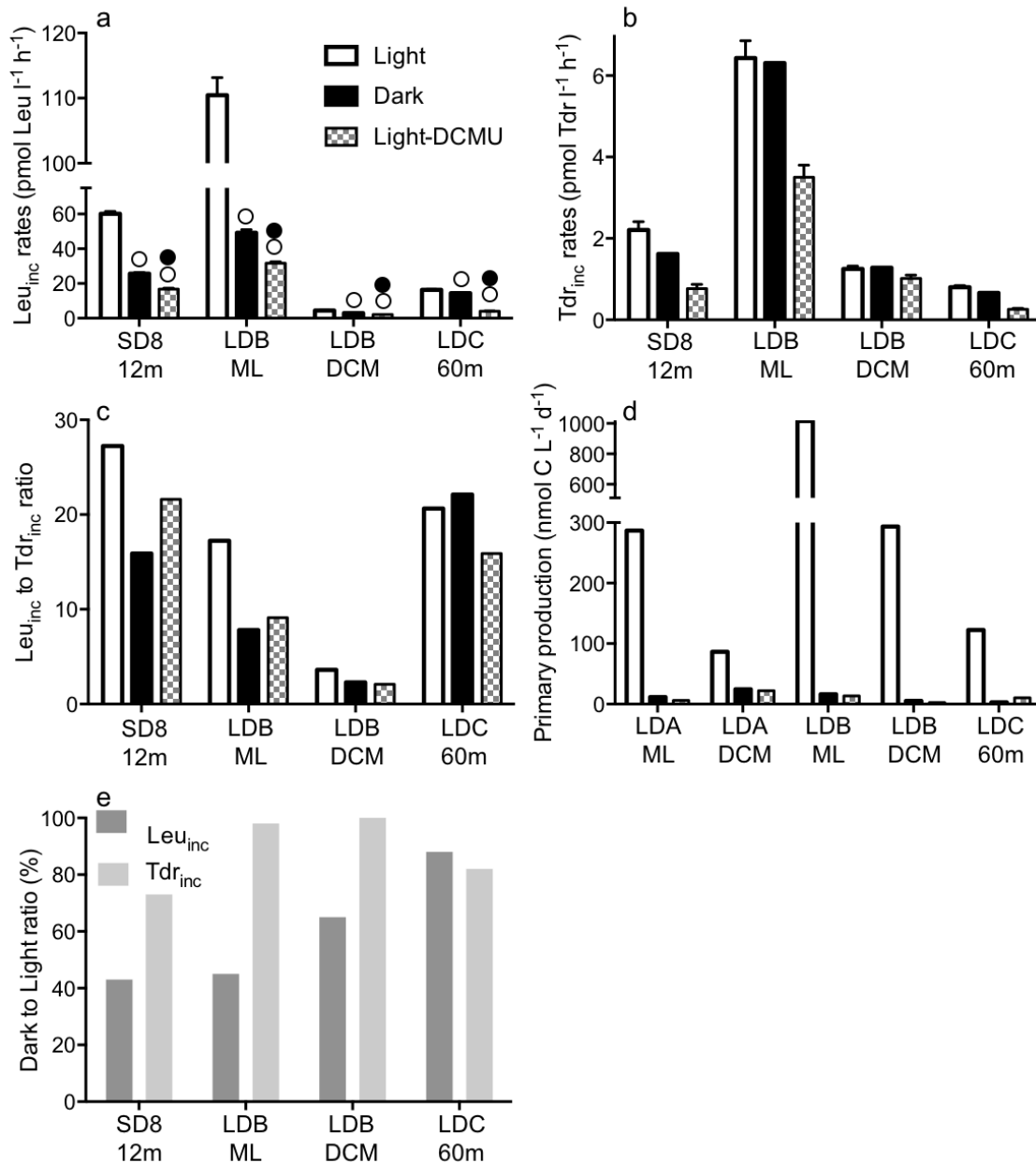
879 Fig. 5: Cell specific ATP assimilation rate ($\text{amol ATP cell}^{-1} \text{h}^{-1}$) at LDA – ML (a), LDA
 880 – DCM (b), LDB – ML (c), LDB – DCM (d) and LDC – 60m (e), in incubations in the
 881 light (white bars), in the dark (black bars), and in the light with DCMU (checker board
 882 pattern) for *Prochlorococcus* (Pro), *Synechococcus* (Syn) and LNA bacteria (LNA). Error
 883 bars represent standard deviation on triplicate samples. One-way ANOVA multiple
 884 treatment comparison results are represented by white or black circles when values are
 885 significantly ($P < 0.05$) different from the light or the dark treatments, respectively. *
 886 indicate non-measurable rates (killed control corrected rates \leq killed control).
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890 Figure 6. Bacterial production rates measured using leucine (a, Leu_{inc} , $\text{pmol Leu l}^{-1} \text{h}^{-1}$)
 891 or thymidine (b, Tdr_{inc} , $\text{pmol Tdr l}^{-1} \text{h}^{-1}$) incorporation into TCA insoluble material;
 892 leucine to thymidine incorporation ratio (c, Leu_{inc} to Tdr_{inc} ratio); primary production
 893 rates (d, $\text{nmol C l}^{-1} \text{d}^{-1}$) in incubations in the light (white bars), in the dark (black bars),
 894 and in the light with DCMU (white and grey checker board pattern); dark to light ratio (e,
 895 %) for Leu_{inc} (grey bars, Leu) and Tdr_{inc} (light grey bars, Tdr). Error bars represent
 896 standard deviation on triplicate samples (a) or absolute difference between duplicate
 897 samples (b, thymidine in the light and light with DCMU). One-way ANOVA multiple
 898 treatment comparison results are represented by white or black circles when values are
 899 significantly ($P < 0.05$) different from the light or the dark treatments, respectively.
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