

1 **Main Manuscript for:**

2 **Cyclic and pseudo-cyclic electron pathways play antagonistic roles**
3 **during nitrogen deficiency in *Chlamydomonas reinhardtii***

4
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16 **Author's Contributions:**

17 Y.L.-B., G.P., A.B., and O.D. conceived the study. O.D. performed most of the experiments. P.A. and
18 O.D. carried out biochemical experiments. M.H. and O.D. performed starch and lipid analysis. G.P.
19 supervised the MIMS experiments. O.D. drafted the manuscript with contributions from Y.L.-B., A.B.
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44 **Abstract (250 words)**

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46 Nitrogen (N) deficiency is a frequently encountered situation that constrains global biomass
47 productivity. In response to N deficiency, cell division stops and photosynthetic electron transfer are
48 downregulated, while carbon storage is enhanced. However, the molecular mechanism
49 downregulating photosynthesis during N deficiency and its relationship with carbon storage are not
50 fully understood. The Proton Gradient Regulator-like 1 (PGRL1)-involved in cyclic electron flow (CEF)
51 and Flavodiiron proteins involved in pseudo-(CEF) are major players in the acclimation of
52 photosynthesis. To determine the role of PGRL1 or FLV in photosynthesis under N deficiency, we
53 measured photosynthetic electron transfer, oxygen gas exchange and carbon storage in the knockout
54 of *Chlamydomonas pgrl1* and *flvB* mutants. Under N deficiency, *pgrl1* maintains higher net
55 photosynthesis and O₂ photoreduction rates, while *flvB* shows similar responses compared to control
56 strains. The amount of cytochrome *b₆f* was maintained at a higher level in *pgrl1*. The photosynthetic
57 activity of *pgrl1 flvB* double mutants decreases in response to N deficiency similar to the control
58 strains. Furthermore, the triacylglycerol content of *pgrl1* was twice higher than the controls under N
59 deficiency. Taken together, our results suggest that in the absence of PGRL1, FLV-mediated O₂
60 photoreduction through PCEF maintains net photosynthesis at a high level, resulting in increased
61 triacylglycerol biosynthesis. This study reveals that PGRL1 and FLV play antagonistic roles during N
62 deficiency. It further illustrates how nutrient status can affect the regulation of photosynthetic energy
63 production in relation to carbon storage and provides new strategies for improving lipid productivity in
64 algae.

65

66 **Significance statement**

67 Nitrogen (N) deficiency, an often-encountered phenomenon in nature, triggers growth arrest and
68 massive lipid accumulation in microalgae. The downregulation of photosynthesis is necessary to
69 ensure cell viability. We demonstrate that a well-conserved protein in chlorophytes, the Proton
70 Gradient Regulator-like 1 (PGRL1) is a key (down) regulator of photosynthesis. In its absence, cells
71 exhibited sustained photosynthesis and over-accumulated lipids thanks to the Flavodiiron (FLV)
72 protein. We propose that both PGRL1 and FLV, by having antagonistic roles in N deficiency, manage
73 the redox landscape, carbon storage and biomass production. Our work revolves around the current
74 paradigm of photosynthesis regulation during N deficiency and provides a new framework for
75 improving lipid accumulation in microalgae for biotechnological purposes.

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88 Introduction

89 Nitrogen (N) deficiency is one of the most harsh environmental situation that constrains global primary
90 biomass productivity in all ecosystems (1–3). Under N deficiency, cell division and photosynthetic CO₂
91 assimilation are downregulated, the carbon and energy are used to synthesize starch and
92 triacylglycerols (TAGs) (4–7). The downregulation of electron transfer reactions, together with a re-
93 routing of the excess of reducing power towards carbon storage, prevents over-production of reactive
94 oxygen species, thus ensuring cell fitness (8–12).

95 Due to the variability in their natural habitat, microalgae must constantly adjust the production
96 of energy by photosynthesis to match the metabolic demand and have therefore developed a set of
97 regulatory mechanisms to fine-tune electron transfer reactions. During photosynthesis, energy in the
98 form of ATP and NADPH is mostly produced by the linear electron flow (LEF) (13). The balance of
99 ATP and NADPH is essential for optimal CO₂ capture and metabolism to which cyclic electron flow
100 (CEF) and pseudo-cyclic electron flow (PCEF) play a critical role (14–17). Two pathways of CEF
101 around PSI have been described in the green microalga *Chlamydomonas reinhardtii* (*Chlamydomonas*
102 hereafter), one involving the type II NADPH dehydrogenase (NDA2) (18), and the other the proton
103 gradient regulator 5 (PGR5)/PGR-like 1 (PGRL1) proteins (19). Both CEF pathways reduce the
104 plastoquinone (PQ) pool by using either NADPH or ferredoxin (Fd) as electron donor respectively (18,
105 20). CEF has been shown to take part in the acidification of thylakoid lumen through generating an
106 extra proton motive force (*pmf*) in addition to the one produced by LEF (18, 19, 21, 22). PCEF
107 mediated by Flavodiiron proteins (FLVs), by transferring electrons from Fd toward O₂, also contributes
108 to the establishment of the *pmf* and therefore to the lumen acidification (23–25). The *pmf* is used to
109 either (1) produce ATP (26), (2) trigger light energy quenching via a low luminal pH (27–29) or (3)
110 repress electron transfer at the level the cytochrome *b₆f* complex through the photosynthetic control
111 triggered by the low luminal pH (21, 30–32). Both CEF and PCEF have been shown to be critical
112 under various conditions of light, CO₂ availability or sulfur deficiency, where both mechanisms have
113 been found to play a synergistic role (14, 24, 25, 33, 34).

114 Despite the importance of CEF and PCEF in response to dynamic environments, little is
115 known about their role during N deficiency. The role of NDA2-involved CEF have been recently
116 addressed during CO₂-limiting photoautotrophic N deficiency and the lack of NDA2 was shown to
117 impair the establishment of non-photochemical quenching (22). The role of PGRL1-PGR5 pathway
118 has been investigated under mixotrophic N deficiency and the lack of PGRL1 was shown to decrease
119 the rate of CEF and consequently TAG production (35). However, the role of PGRL1 during
120 photoautotrophic N deficiency and the possible existence of interactions between carbon/energy sinks
121 (TAG and starch) and (P)CEF pathways has not been explored so far. The latter appears particularly
122 important considering that massive carbon reallocation occur under N deficiency (4). Fully
123 understanding the role of these regulatory pathways and how they affect reserve formation is also
124 needed towards engineering photosynthesis and improving lipid production in conditions of nutrient
125 deficiency.

126 Here, we evaluated the contribution of PGRL1-mediated CEF in the regulation of
127 photosynthesis during N deficiency and its interaction with carbon reserve accumulation in
128 *Chlamydomonas* cells grown in photoautotrophic conditions in non-limiting CO₂ concentrations (using
129 CO₂-enriched air), conditions which favor the accumulation of carbon reserves (7, 36, 37). By
130 monitoring photosynthetic activity based on chlorophyll fluorescence and O₂ exchange rates
131 measurements, we observed a continuous and high net and gross photosynthetic activity, elevated O₂
132 uptake and hyper-accumulation of TAGs in a PGRL1-deficient strain under N deficiency compared to
133 the control strains. By characterizing mutants deficient in either PGRL1, FLVs or both we showed that
134 FLVs, by maintaining a strong PCEF in the *pgrl1* mutant, allows maintaining high photosynthetic rates
135 and high TAG accumulation. Finally, we discuss how modulating photosynthetic electron flow could
136 constitute an efficient strategy to boost TAG production under nutrient deficiency.

138 Results

139 A PGRL1-deficient mutant showed sustained photosynthetic activity under N deficiency

140 To first assess the role of PGRL1 in the regulation of photosynthesis during photoautotrophic N
141 deficiency, we simultaneously monitored chlorophyll fluorescence and O₂ exchange following a dark-
142 light-dark transition in N-replete and N-deprived cells. We compared the photosynthesis efficiency of
143 the PGRL1-deficient strain (*pgrl1* hereafter) with its control wild-type strain (137AH) and two
144 complemented lines (19) (**Fig. 1 and Supplemental Fig. S1 and S2**). Under N replete condition, both
145 chlorophyll fluorescence and net O₂ evolution patterns were mostly similar in *pgrl1* and control cells
146 (**Fig. 1 A, B, E and F**). *pgrl1* exhibited higher O₂ uptake and gross O₂ production rates in the light as
147 compared to control lines (**Fig. 1B and F**), as previously reported (14, 25). After 2 days of N

148 deficiency, the PSII operating yield measured in the light decreased by about 75% in the WT and only
149 by 35% in *pgrl1* (**Fig. 1 C, D, E and F; Supplemental Fig. S1C**). As previously reported (5, 38) the
150 dark respiration rate was stimulated in the WT under N deficiency, this effect being reduced in *pgrl1*
151 (**Supplemental Fig. S2 and S4**). Conversely, *pgrl1* showed twice higher net O₂ evolution and light-
152 dependent O₂ uptake as compared to the WT (**Fig. 1 D and F**). By using *pgrl1* complemented lines
153 (19) we observed full recovery in the *pgrl1::PGRL1-2* and a partial recovery in *pgrl1::PGRL1-1*
154 (**Supplemental Fig. S1 and S2**). The preservation of photosynthetic activity in *pgrl1* suggests that
155 CEF contributes to repressing the activity of photosynthetic electron transfer under photoautotrophic
156 N-deficiency.

157
158 **Cyt *b_{6f}*, PsaD and FLVs accumulate to higher levels in *pgrl1* as compared to its control under N**
159 **deficiency**

160 The amount of photosynthetic proteins drops when Chlamydomonas cells undergo N deficiency under
161 mixotrophic conditions (5, 39). However, this effect strongly depends on growth conditions, as the
162 amount of photosynthetic proteins is maintained at high level when cells are grown under CO₂-limiting
163 photoautotrophic conditions (22). By loading similar protein amounts, we observed detectable amount
164 of PGRL1, NDA2, FLVA and FLVB proteins up to 48h of N deficiency although FLVs were found at
165 lower amount compared to N-replete conditions (**Fig. 2 A and B**). A strong reduction in the amounts of
166 Cyt *f*, PsaD, PsaC and PsaD subunits were observed when N deficiency was performed in non-limiting
167 CO₂ photoautotrophic, thus indicating a reduction in the amounts of Cyt *b_{6f}*, PSII and PSI complexes
168 (**Fig. 2 A and B**). We conclude from these analyses that N-limitation induces a strong decrease in
169 most proteins involved in photosynthetic electron transport with the exception of the CEF proteins
170 PGRL1 and NDA2.

171 To gain insights into the mechanisms behind the high photosynthetic activity in *pgrl1* during N
172 deficiency, we compared the relative abundance of major proteins involved in photosynthetic
173 complexes and in CEF and PCEF in the *pgrl1* and the WT during N deficiency (**Fig. 2 C and D**).
174 Interestingly, we observed a higher accumulation of the PSI subunit PsaD and reduced degradation of
175 Cyt *f* in *pgrl1* compared to the WT (**Fig. 2 C and D**). FLVA amount was found more abundant after 2 d
176 of N deficiency despite a reduced level in N-replete in *pgrl1* compared to the WT (**Fig. 2 C and D**). It is
177 worth noting that the hallmark of autophagy i.e. ATG8 was barely detectable in the mutant whereas it
178 was highly induced in the WT under N deficiency (**Fig. 2 C and D**). All the other proteins tested
179 accumulated to a similar amount in *pgrl1* and WT (**Fig. 2 C and D**). We conclude from this experiment
180 that *pgrl1* mutant keeps a higher photosynthetic activity during N deficiency than the WT thanks to a
181 lower decrease of the Cyt *b_{6f}* amount and higher accumulation of PSI and FLVs proteins.

182
183 **FLV-mediated O₂ photoreduction drives photosynthesis in *pgrl1* under N deficiency**

184 The above data (**Fig. 1 and 2**) suggest that PGRL1 is involved in the downregulation of PET, and that
185 a light-dependent O₂ uptake mechanism is activated in *pgrl1* during N deficiency. We then questioned
186 the nature of molecular actors involved in the increased O₂ uptake. As FLVs have been reported to be
187 the main O₂ uptake mechanism in the light (24, 25, 40), we evaluated steady-state O₂ exchange rate
188 in *pgrl1*, *flvB* as well as in the *pgrl1 flvB* double mutants (**Fig. 3**). In N-deficiency, the light-dependent
189 O₂ uptake, was highly increased in *pgrl1* but strongly impaired in *flvB* and *pgrl1 flvB* mutants
190 (**supplemental Fig. S3 and S4B**) which mirrors what has been reported for N-replete conditions (24,
191 25). Interestingly, while both O₂ uptake in the light and net O₂ production measured during steady-
192 state photosynthesis remained high in *pgrl1*, no marked difference in gross or net O₂ exchange were
193 detected between *flvB*, *pgrl1 flvB* and their respective control lines (**Figure 3 A-C and supplemental**
194 **Fig. S2 and S3**). Immunoblot analyses showed similar accumulation of NDA2, Cyt *f*, PsaD, and ATG8
195 proteins in the *pgrl1 flvB* compared to controls under N replete and deficiency (**Fig. 3D**). Taken
196 together, these data show that the absence of FLV-mediated PCEF in the *pgrl1* background restores
197 the effect of N limitation observed in control strains. We conclude that FLV-mediated PCEF by
198 maintaining photosynthetic electron transfer reactions in *pgrl1* allows net photosynthesis to be
199 maintained at a high level during N deficiency.

200
201 ***pgrl1* over accumulates TAGs under N deficiency**

202 Emerging literature suggests that alterations of energy management pathways affect not only biomass
203 production but also its composition (16, 17, 36, 41). Starch and TAGs are major forms of carbon
204 storage in Chlamydomonas during N deficiency. Since *pgrl1* mutant shows stronger net photosynthetic
205 rate, we measured its ability to accumulate storage compounds. Starch accumulation was similar in
206 *pgrl1* and control lines under both N replete and deficiency (**Fig. 4A; Supplemental Fig. S5, S7 A and**
207 **B**). In contrast, thin-layer chromatography (TLC) revealed twice more TAG accumulation in *pgrl1*

208 compared to the control lines under N deficiency whereas no difference was observed under N-replete
209 condition (**Fig. 4B; Supplemental Fig. S5 and S7 C and D**). Lipid droplet imaging confirmed the
210 observation of TAG quantification by TLC (**Fig. 4C**). To determine whether the increase in TAG is due
211 to membrane lipid remodeling or to an improved *de novo* fatty acid biosynthesis in the chloroplast, we
212 quantified total fatty acids by gas chromatography coupled to a mass spectrometer (GC-MS). We
213 observed a net increase in total fatty acids in *pgrl1*, suggesting an increase in *de novo* fatty acid
214 biosynthesis (**Supplemental Fig. S6**).

215 We then evaluated whether the changes in photosynthesis and TAG amount affect biomass
216 accumulation, we followed changes in cell volume for 6 d during N deficiency. Cell volume is used
217 here as a proxy for biomass accumulation. The cell volume increased twice faster in the *pgrl1*
218 compared to the control lines (**Supplemental Fig. 5A**). We conclude from above experiments that the
219 higher photosynthetic activity in *pgrl1* results in increased biomass and TAG productivity under N
220 deficiency.

221 We then analyzed starch and TAG amount in *flvB* as well as *pgrl1 flvB* double mutants before
222 and under N deficiency (**Supplemental Fig. S7**). Similar to the observations on photosynthetic activity,
223 the *pgrl1 flvB* double mutant accumulated similar amounts of TAG and starch as compared to control
224 strains either in N replete or N limited conditions (**Supplemental Fig. S7I-L**). Intriguingly, under
225 optimal growth but not under N deficiency, the *flvB* single mutants accumulated much higher amount
226 of starch than WT (**Supplemental Fig. S7E**) and accumulated lower amounts of TAG under N
227 deficiency (**Supplemental Fig. S7H**). These data indicate that modifying regulatory mechanisms of
228 photosynthetic electron transfer reactions strongly affects carbon storage in both quality and quantity
229 (**Fig. 1 and 2**).

230 We conclude that during phototrophic N-deficiency, photosynthetic electron flow mediated by
231 FLVs allows to drive photosynthetic TAG accumulation while PGRL1 tends to constrain it, likely via an
232 activation of photosynthetic control. We propose that both PGRL1 and FLVs, by having an
233 antagonistic role in N-deprivation, manage the redox landscape, carbon storage and biomass
234 production.

235

236 Discussion

237 The ability of microalgae to coordinate their energy production to meet the metabolic demand is crucial
238 for their survival in a constantly fluctuating environment. Mechanisms involved in photosynthesis
239 regulations have been abundantly studied in response to light or CO₂ levels, but not much is known
240 during nutrient deficiency when a massive re-orientation of metabolic pathways occur. In the absence
241 of N, the major cellular energy sinks (cell division, protein biosynthesis and photosynthetic CO₂
242 fixation) are restricted whereas the carbon and energy are stored as TAGs or starch. Moreover, the
243 photosynthetic electron transfer (PET) is downregulated to match the demand and maintain cell
244 viability. In this study, we demonstrated that PGRL1 is involved in the downregulation of PET during N
245 deficiency. This down-regulation likely operates via the photosynthetic control mechanism (see below
246 for details). Lack of PGRL1 resulted in sustained efficient photosynthetic activity after 2 d of N
247 deficiency. We have further shown that the release of photosynthetic control in *pgrl1* mutants resulted
248 in higher FLVs-mediated PCEF. By channeling excess electrons toward O₂, PCEF prevents PSI
249 acceptor side-limitation, allows maintaining PET at high level and favors high TAG accumulation. A
250 schematic diagram explaining the role of PGRL1 in the downregulation of photosynthesis and
251 metabolic consequences of its absence during N deficiency is presented in **Fig. 5**.

252 PGRL1-involved CEF contributes to photosynthetic control during N deficiency

253 Unlike in higher plants (21, 42–44), the photosynthetic control mechanism has been poorly studied in
254 microalgae. The term 'photosynthetic control' refers to mechanisms that restrict the reactions of PET
255 during the steady-state photosynthesis mostly occur in response to environmental fluctuations (31,
256 44). The Cyt *b₆f* is considered as the central hub for controlling PET between PSII to PSI and prevents
257 the over reduction of P700 (44, 45). This is achieved either by slowing down the turnover rate
258 (conductance) of the Cyt *b₆f* triggered by the acidification of the thylakoid lumen (44) followed (or not)
259 by the selective autophagy of Cyt *b₆f* (39). In addition to Cyt *b₆f*, the PGRL1-PGR5-dependent CEF
260 was shown to contribute to the photosynthetic control in higher plants (21, 43, 46) and lately in
261 microalgae (19, 47–50). However, a recent work in *Chlamydomonas* reported that PGRL1 only slightly
262 contribute to the luminal proton generation and that the *pgrl1* displayed similar proton gradient to the
263 WT during photoautotrophic N deficiency under CO₂ limiting conditions (22). Our work shows that
264 impairing the accumulation of the PGRL1 removes a bottleneck of photosynthetic electron flow during
265 N deficiency under non-limiting CO₂ conditions (**Fig. 1 and 3; Supplemental Fig. S1 and S2**). Similar
266 observations were reported under sulphur deprivation where deletion of PGRL1 or PGR5 resulted in

267 sustained linear electron flow toward H₂ production in *Chlamydomonas* (19, 49). The contribution of
268 PGRL1 in the photosynthetic control is likely through the acidification of lumen that will exert a
269 backpressure on Cyt *b₆f* conductance, slowing down the electron transfer toward plastocyanin.
270 However, further works will be required to shed light on the exact participation of PGR5/PGRL1 in the
271 photosynthetic control. Recently, PGR5 (which interacts with PGRL1 during the CEF (43) was shown
272 to be required for efficient Q cycle through the reduction of the heme-*c_i* in the *b₆f* using reduced
273 ferredoxin (50). The operation of the Q cycle is crucial for lumen acidification to trigger the onset of
274 photosynthetic control (51–53). By doing so, PGRL1-PGR5 connects the stromal redox poise to the
275 *b₆f*-dependent photosynthetic control.
276

277 **FLVs become predominant in the absence of PGRL1-mediated cyclic electron flow**

278 So far, a few studies reported a compensation mechanism between PGRL1 and FLVs during algal
279 adaptation to high light or low-CO₂ conditions (14, 25). Under high light or low-CO₂ conditions, the
280 increased activity of FLVs in the PGRL1-deficient strain had no significant improvement on the net
281 photosynthesis but rather FLVs serve either as a safety valve of excess electrons or to balance
282 ATP/NAD(P)H by generating more *pmf* (14, 25). Similar compensation has also been observed in
283 higher plants where orthologous expression of FLVs rescues the *pgr5* mutants phenotype without
284 further improvement photosynthesis (46, 54) although some level of increased biomass were
285 measured in WT *Arabidopsis* expressing FLVs under light fluctuations thanks the protective role of
286 FLVs in these conditions (55). In contrast, our results suggest that under N deficiency, FLVs and
287 PGRL1 have an antagonistic role. Indeed, the increased activity of FLVs in the PGRL1-deficient strain
288 resulted in higher net photosynthesis and TAG accumulation than in the WT (**Fig. 3D and 4B**), which
289 shows that rather than compensating each other, PGRL1 is indirectly controlling the activity of FLVs by
290 limiting the electron flow. Our observation of the sustained accumulation of Cyt *b₆f* and increased
291 accumulation of PSI in the PGRL1-deficient strain under N deficiency (**Fig. 2C**) could be explained by
292 the strong activity of FLVs draining electrons from the photosynthetic chain thereby releasing the over-
293 reduction pressure and preventing PSI from damage. However, we were expecting that removal of
294 both PGRL1 and FLVs should have more severe consequences on cell physiology and metabolism.
295 Instead, we observed similar photosynthetic activity and TAG biosynthesis in the *pgr1 flvB* double
296 mutants as their control strains (**Fig. 3 and supplemental Fig. S7**), i.e. the additional removal of FLVs
297 suppressed the phenotype of PGRL1-deficient strain. The WT-like phenotype of *pgr1 flvB* double
298 mutants further supports our conclusion that PGRL1 and FLV are antagonist during N deficiency. A
299 third pathway taking over could explain this WT-like phenotype of the double mutant *pgr1 flvB*. The
300 NDA2 pathway or chloroplast-mitochondria electron flow (CMEF) could be good candidates. NDA2
301 protein level was shown to increase during air photoautotrophic N deficiency and also the CEF rate in
302 *Chlamydomonas* was decreased by 50% in *nda2* mutants (22). However, we observed a similar
303 protein level of NDA2 in the *pgr1 flvB* as their control lines (**Fig. 3C and Supplemental Fig. S4D**).
304 Nevertheless, the protein level might not always correlate with the activity and other regulatory
305 mechanisms (e.g., phosphorylation or redox regulation) can modulate enzyme activity. However, the
306 high rates of respiration in the dark in N-deprived WT and *pgr1 flvB* double mutants (**Fig. 1 and**
307 **Supplemental Fig. S3 and S4A**) is a good indication that mitochondria is strongly active and likely
308 reflects a strong CMEF, which would take over CEF and PCEF in N-deprived conditions. Altogether,
309 we conclude that FLVs act as a major driving force leading to the increased net photosynthesis in
310 *pgr1* under N deficiency (**Fig. 3**).
311

312 **Relationship between cellular redox landscape and carbon storage**

313 A major biotechnological challenge in algal domestication for biofuel is the low lipid productivity. In
314 *Chlamydomonas* and many other microalgae, starch and TAG massively accumulate but mostly under
315 stress conditions in particular N deficiency when cell division stops and productivity is impaired.
316 Considerable efforts have focused on the study of the molecular mechanisms behind the onset of
317 reserve accumulation by monitoring omics responses to a stress (5, 56–59), or focused on specific
318 steps of fatty acid and TAG biosynthesis, which have resulted in some limited improvement in
319 productivity (60, 61). Improving productivity requires a better understanding of the crosstalk between
320 photosynthetic carbon fixation, environmental signals and the redox balance, which all govern reserve
321 accumulation (62–64). Here by studying mutants affected in CEF and PCEF, we explored the
322 relationships between the cellular redox status and carbon storage. The increased accumulation of
323 TAG but not starch observed in the PGRL1-deficient strain under N deficiency (**Fig. 4 A and B**) is due
324 to an increase in *de novo* biosynthesis in the chloroplast as evidenced by the observed increase in
325 total fatty acids (**Supplemental Fig. S6**). The increase in TAG amount is a consequence of the
326 continuous production of NADPH and ATP through PCEF in the PGRL1-deficient strain under nitrogen

327 deficiency. By alleviating the limitation of the acceptor side of PSI, PCEF released the over-reduction
328 pressure of the photosynthetic chain, thereby sustaining the PET reactions. In line with our finding, the
329 *Chlamydomonas pgd1* mutants (e.g. *Plastid galactoglycerolipid degradation 1*) with reduced LEF rate
330 are shown to produce less TAG under N starvation (11, 65). The report that the *pgr1* mutant made
331 less TAG than WT under mixotrophic conditions (66) is not surprising. It is well known that the
332 bioenergetics of *Chlamydomonas* under photoautotrophic conditions differ from mixotrophic conditions
333 (67–69). The presence of acetate can drastically affect cellular bioenergetics level i.e. its uptake
334 consumes ATP and its metabolism produces NADH, therefore further favoring oil synthesis (67, 68,
335 70, 71).
336 Moreover, in addition to higher TAG amount, we also observed higher biomass (cell volume) in the
337 PGRL1-deficient strain during N deficiency (**Supplemental Fig. 5A**). This high biomass can be
338 interpreted by the combined increased of photosynthetic activity (**Fig. 1**), TAG biosynthesis (**Fig. 4**)
339 and reduced autophagy as indicated by the amount of ATG8 (**Fig. 2**).
340 Here we show that removing the photosynthetic control mediated by PGRL1 allows the FLVs-
341 mediated PCEF to massively take part in the energization of the *de novo* fatty acid biosynthesis
342 pathway resulting in improved TAG biosynthesis and double TAG productivity. Taken together, our
343 data highlight the importance of energetic balance on carbon reserve formation for biomass production
344 and for altering biomass composition. Rewiring the alternative photosynthetic electron flows opens
345 new possibilities in fine tuning algal productivity and the organic compounds they are producing.

346 **Materials and methods**

347 **Growth conditions and strains**

348 The *pgr1* from the background 137AH, its two complementing strains, *flvB* mutants from the
349 background CC-4533 and the double mutants *pgr1 flvB* were previously described (19, 24, 25). Cells
350 were routinely cultivated in an incubation shaker (INFORS Multitron pro) maintained at 25°C, with 120
351 rpm shaking and constant illumination at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Fluorescent tubes delivering white light
352 enriched in red wavelength supplied lightings in the INFORS. All experiments were performed under
353 photoautotrophic condition with minimum medium with or without nitrogen source (MM and MM-N)
354 buffered with 20 μM MOPS at pH 7.2 in air enriched with 1% CO_2 . Due to cell aggregation (*pgr1*) that
355 prevent accurate cell counting, total cellular volume was measured using a Multisizer 4 Coulter
356 counter (Beckman Coulter) and the different strains were diluted to reach a similar cellular
357 concentration before N deficiency experiments.

358 **Measurement of chlorophyll fluorescence using a Pulse Amplitude Modulation (PAM)**

359 Chlorophyll fluorescence was measured using a PAM fluorimeter (Dual-PAM 100, Walz GmbH,
360 Effeltrich, Germany) on the MIMS chamber as described in (40) using green actinic light (1250 μmol
361 $\text{photon m}^{-2} \text{s}^{-1}$, green LEDs). Red saturating flashes (8,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 600 ms) were
362 delivered to measure the maximum fluorescence (FM) every 30 s (before and upon actinic light
363 exposure). The maximum PSII quantum yield was calculated as $Fv/Fm = (Fm - F_0)/Fm$ where F_0 is the
364 basal fluorescence obtained with the measuring light and Fm the fluorescence emitted after saturating
365 pulse (72). PSII operating yield (Φ_{PSII}) was calculated as $\Phi_{\text{PSII}} = (F_M' - F_s)/F_M'$ with F_M' the
366 fluorescence value after saturating pulse, F_s the stationary fluorescence during actinic light exposure.

367 **O₂ exchange measurement using Membrane Inlet Mass Spectrometry (MIMS)**

368 O_2 exchanges were measured in the presence of [^{18}O]-enriched O_2 using a water-jacketed,
369 thermoregulated (25°C) reaction vessel coupled to a mass spectrometer (model Prima ΔB ; Thermo
370 Electronics) through a membrane inlet system (73). The cell suspension (1.5 mL) was placed in the
371 reaction vessel and bicarbonate (10 mM final concentration) was added to reach a saturating CO_2
372 concentration. One hundred microliters of [^{18}O]-enriched O_2 (99% $^{18}\text{O}_2$ isotope content; Euriso-Top)
373 was bubbled at the top of the suspension just before vessel closure and gas exchange
374 measurements. O_2 exchanges were measured during a 3-min period in the dark, then the suspension
375 was illuminated at 1250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 5 min using green LEDs followed by 3-min in the
376 dark. Isotopic O_2 species [$^{18}\text{O}^{18}\text{O}$] ($m/e = 36$), [$^{18}\text{O}^{16}\text{O}$] ($m/e = 34$), and [$^{16}\text{O}^{16}\text{O}$] ($m/e = 32$) were
377 monitored, and O_2 exchange rates were determined (73). Argon gas was used to correct O_2 exchange
378 measured by the spectrometer as described in (73).

379 All others methods are described in **SI Materials and Methods**.

380 **Acknowledgments**

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390 assistance in performing immunoblot. We also acknowledge the ZoOM microscopy facility.

391

392 **Figures**

393 **Figure 1. *pgrl1* showed sustained photosynthesis after 2 d of N deficiency.**

394 **A-C**, Chlorophyll fluorescence was measured using a Dual-PAM during the dark-light-dark transition
395 from N-replete (**A**) and N-deficient (**B**) conditions.

396 **D-F**, O₂ exchange rates were measured using a MIMS in the presence of [¹⁸O]-enriched O₂. Net O₂
397 evolution (green) was calculated as gross O₂ production (blue) – gross O₂ uptake (red).

398 **E**, PSII quantum yield before and after 2 d of N deficiency. PSII (Y) was measured using green actinic
399 light (1250 μmol photon m⁻² s⁻¹, green LEDs) and calculated as ΦPSII= (F_M'-F_s)/ F_M' with F_M' the
400 fluorescence value after saturating pulse, F_s the stationary fluorescence during actinic light exposure.
401 Asterisks represent statistically significant differences compared to the control 137AH (* *p*<0.05, **
402 *p*<0.01 and *** *p*<0.001) using one-way ANOVA.

403 **F**, The Net O₂ production before and after 2 d of N deficiency in the wild-type 137AH, *pgrl1* and the
404 complemented lines *pgrl1::PGRL1-1* and *pgrl1::PGRL1-2*.

405 **G**, Immunoblot analysis of PGRL1 accumulation in the wild-type 137AH, *pgrl1* and the complemented
406 lines. L30 antibody was used as a positive control. Coomassie blue staining was used as the loading
407 control. Protein samples were obtained from 2 d N-starved cells.

408 Wild type 137AH, *pgrl1*, *pgrl1::PGRL1-1* and *pgrl1::PGRL1-2* N-replete cells cultivated
409 photoautotrophically with 1% CO₂ in air under continuous light (50 μmol photons m⁻² s⁻¹) were
410 transferred into N-free media for 2 d prior to measurements. Shown are an average of at least three
411 biological replicates ± SD.

412

413 **Figure 2. *pgrl1* accumulated more cytochrome Cyt *b₆f* and PSI during N deficiency.**

414 **A**, Immunodetection of photosynthetic proteins in the wild-type 137AH during N deficiency. Cells were
415 harvested at 0, 16, 24, 40 and 48 of N deficiency. Total proteins were extracted from the same cell
416 number (20 million of cells) to quantitatively follow changes in specific protein amounts. Shown are
417 representative images of three biological replicates. We validated our quantitative analysis by showing
418 unchanged accumulation of the housekeeping α-tubulin protein and the timely induction of ATG8
419 autophagy protein despite the gradual degradation of total cellular proteins as shown by Coomassie
420 staining during N deficiency.

421 **B**, Heatmap showing the abundance of proteins during N deficiency in the WT cells. The time 0 (N
422 replete) was used for normalization. The color scale is shown in the right. Data are average of at least
423 two biological replicates.

424 **C**, Immunodetection of photosynthetic proteins in the wild-type 137AH and *pgrl1* during N deficiency.
425 Total proteins were extracted from cells harvested at 0, 8, 22, 30 and 50 h of N deficiency. Samples
426 were loaded at equal total protein amounts as shown by Coomassie blue staining. Shown are
427 representative images of three biological replicates.

428 **D**, Heatmap showing the proteins abundance during N deficiency in the *pgrl1* cells. The data from
429 different time points were normalized to the WT protein amount. The color scale is shown in the right.
430 Data are average of at least two biological replicates.

431

432 **Figure 3. The photosynthesis in *pgrl1* is driven by FLV-mediated O₂ photoreduction under N 433 deficiency.**

434 **A**, Gross O₂ uptake measured between 5-7min as shown by the supplemental figure 3B.

435 **B**, Gross O₂ production measured between 5-7min as shown by the supplemental figure 3B

436 **C**, Net O₂ production measured between 5-7min as shown by the supplemental figure 3B

437 **D**, Immunodetection of photosynthetic protein in *pgrl1 flvB* and their control lines.

438 O₂ exchange rates were measured using a MIMS in the presence of [¹⁸O]-enriched O₂. Net O₂
439 evolution (green) was calculated as gross O₂ production (blue) – gross O₂ uptake (red). Total proteins
440 were extracted from N-replete and 2 days N-deprived cells and samples were loaded at equal total
441 proteins amounts as shown on Coomassie blue staining. Cells cultivated photoautotrophically with 1%
442 CO₂ in air under continuous light of 50 μmol photons m⁻² s⁻¹ were transferred into N-free media for 2
443 days prior to measurements or sampling for immunoblot. Shown are an average of at least three
444 biological replicates ± SD. Asterisks represent statistically significant difference compared to the WTs
445 (* *p*<0.05, ** *p*<0.01, *** *p*<0.001 and **** *p*<0.0001) using one-way ANOVA.

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Figure 4. *pgrl1* over accumulated TAGs under N deficiency

A, Starch amount.

B, TAG amount.

C, representative images of confocal microscopy observation of lipid droplet stained with Bodipy dye. Pseudo-colors were used: BODIPY-stained LDs in yellow, chlorophyll autofluorescence in red. DIC: differential interference contrast. Scale bar = 5 μm .

Cells cultivated photoautotrophically with 1% CO_2 in the air under continuous light of 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ were transferred into N-free media for 2 d prior to sampling for starch and TAG or confocal imaging. Asterisks represent statistically significant difference compared to the control 137AH (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$) using one-way ANOVA.

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Figure 5. Schematic diagram on the role of PGRL1 in the downregulation of photosynthesis and metabolic consequences of its absence during N deficiency

We proposed that PGRL1-mediated CEF is involved in the downregulation of photosynthesis during N deficiency through the Cyt *b6f*-dependent photosynthetic control (black arrow). In the absence of PGRL1, FLVs-mediated PCEF is strongly induced to drive the photosynthetic electron transfer. As a result, excess photosynthetic electrons are diverted toward fatty acid and triacylglycerol biosynthesis (* out of the chloroplast). The red arrows show the pathways upregulated in the PGRL1-deficient strain compared to the WT (gray arrows).

Reference

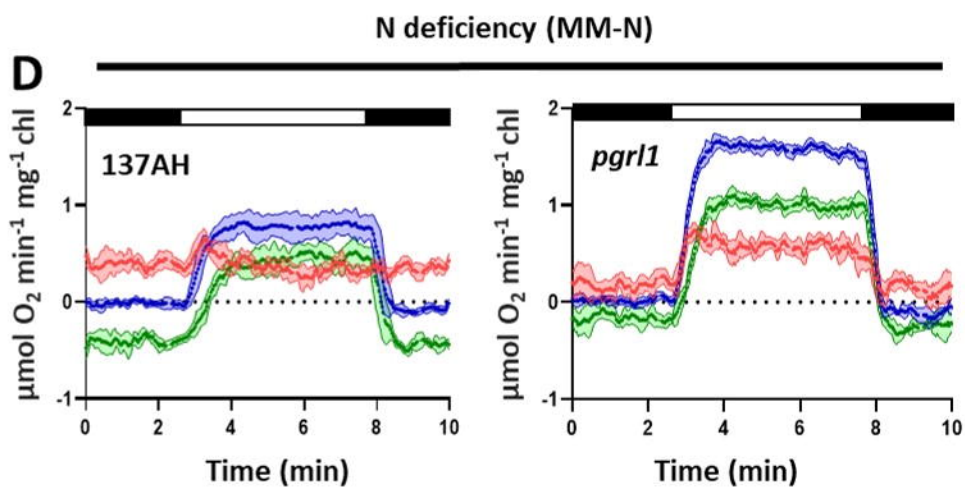
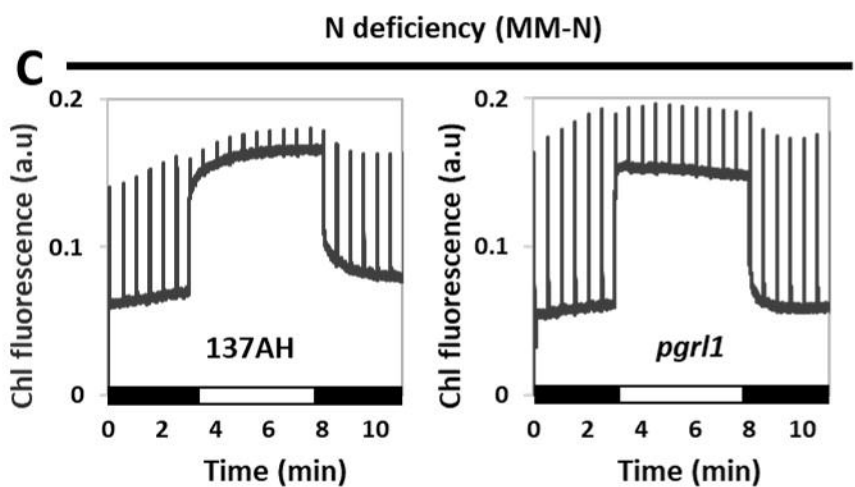
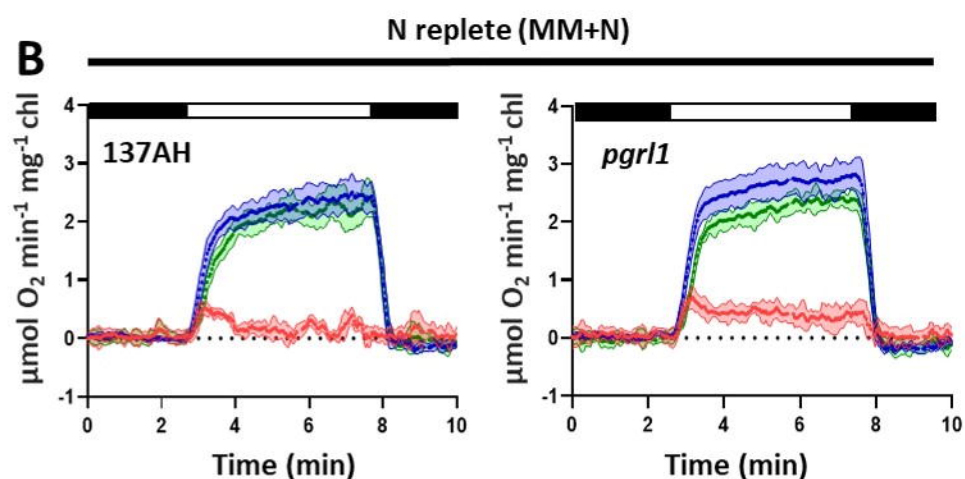
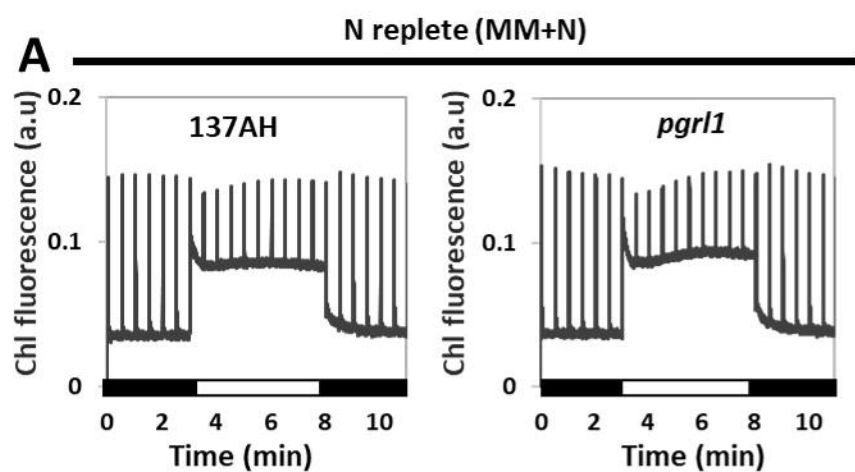
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498

1. P. M. Vitousek, R. W. Howarth, Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* **13**, 87–115 (1991).
2. D. S. LeBauer, K. K. Treseder, Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**, 371–379 (2008).
3. E. Du, *et al.*, Global patterns of terrestrial nitrogen and phosphorus limitation. *Nat. Geosci.* **13**, 221–226 (2020).
4. M. Siaut, *et al.*, Oil accumulation in the model green alga *Chlamydomonas reinhardtii*: characterization, variability between common laboratory strains and relationship with starch reserves. *BMC Biotechnology* **11**, 7 (2011).
5. S. Schmollinger, *et al.*, Nitrogen-Sparing Mechanisms in *Chlamydomonas* Affect the Transcriptome, the Proteome, and Photosynthetic Metabolism. *The Plant Cell* **26**, 1410–1435 (2014).
6. M. T. Juergens, *et al.*, The Regulation of Photosynthetic Structure and Function during Nitrogen Deprivation in *Chlamydomonas reinhardtii*. *Plant Physiology* **167**, 558–573 (2015).
7. M. Schulz-Raffelt, *et al.*, Hyper-accumulation of starch and oil in a *Chlamydomonas* mutant affected in a plant-specific DYRK kinase. *Biotechnology for Biofuels* **9**, 55 (2016).
8. Y.-M. Zhang, H. Chen, C.-L. He, Q. Wang, Nitrogen Starvation Induced Oxidative Stress in an Oil-Producing Green Alga *Chlorella sorokiniana* C3. *PLOS ONE* **8**, e69225 (2013).
9. J.-J. Park, *et al.*, The response of *Chlamydomonas reinhardtii* to nitrogen deprivation: a systems biology analysis. *The Plant Journal* **81**, 611–624 (2015).
10. M. Gargouri, P. D. Bates, J.-J. Park, H. Kirchhoff, D. R. Gang, Functional photosystem I maintains proper energy balance during nitrogen depletion in *Chlamydomonas reinhardtii*, promoting triacylglycerol accumulation. *Biotechnology for Biofuels* **10**, 89 (2017).
11. Z.-Y. Du, *et al.*, Galactoglycerolipid Lipase PGD1 Is Involved in Thylakoid Membrane Remodeling in Response to Adverse Environmental Conditions in *Chlamydomonas*. *The Plant Cell* **30**, 447–465 (2018).
12. Q.-G. Tran, *et al.*, Impairment of starch biosynthesis results in elevated oxidative stress and autophagy activity in *Chlamydomonas reinhardtii*. *Sci Rep* **9**, 9856 (2019).
13. J. F. Allen, Cyclic, pseudocyclic and noncyclic photophosphorylation: new links in the chain. *Trends in Plant Science* **8**, 15–19 (2003).

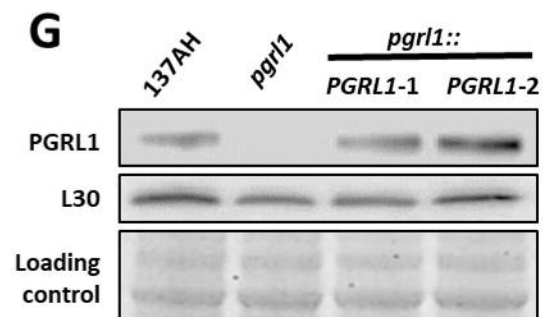
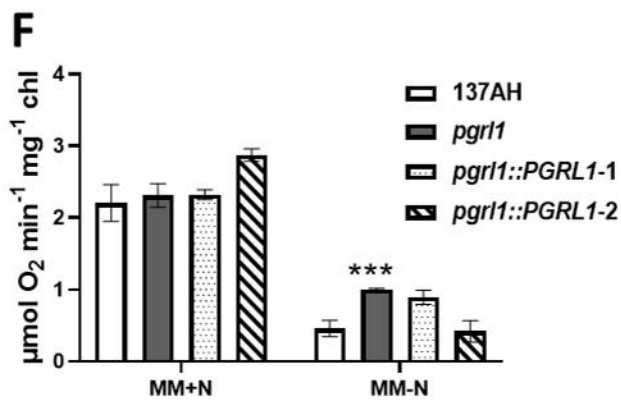
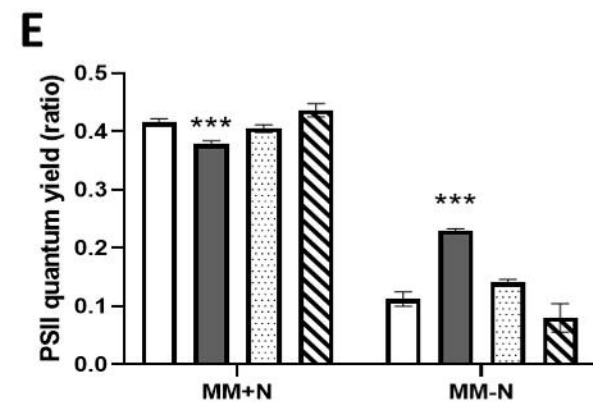
- 499 14. K.-V. Dang, *et al.*, Combined Increases in Mitochondrial Cooperation and Oxygen
500 Photoreduction Compensate for Deficiency in Cyclic Electron Flow in *Chlamydomonas*
501 *reinhardtii*. *The Plant Cell* **26**, 3036–3050 (2014).
- 502 15. B. Bailleul, *et al.*, Energetic coupling between plastids and mitochondria drives CO₂ assimilation
503 in diatoms. *Nature* **524**, 366–369 (2015).
- 504 16. A. Burlacot, G. Peltier, Y. Li-Beisson, Subcellular Energetics and Carbon Storage in
505 *Chlamydomonas*. *Cells* **8**, 1154 (2019).
- 506 17. S. Saroussi, *et al.*, Alternative outlets for sustaining photosynthetic electron transport during
507 dark-to-light transitions. *Proc Natl Acad Sci USA* **116**, 11518–11527 (2019).
- 508 18. C. Desplats, *et al.*, Characterization of Nda2, a Plastoquinone-reducing Type II NAD(P)H
509 Dehydrogenase in *Chlamydomonas* Chloroplasts. *J. Biol. Chem.* **284**, 4148–4157 (2009).
- 510 19. D. Tolleter, *et al.*, Control of Hydrogen Photoproduction by the Proton Gradient Generated by
511 Cyclic Electron Flow in *Chlamydomonas reinhardtii*[W]. *Plant Cell* **23**, 2619–2630 (2011).
- 512 20. A. P. Hertle, *et al.*, PGRL1 Is the Elusive Ferredoxin-Plastoquinone Reductase in Photosynthetic
513 Cyclic Electron Flow. *Molecular Cell* **49**, 511–523 (2013).
- 514 21. Y. Munekage, *et al.*, PGR5 Is Involved in Cyclic Electron Flow around Photosystem I and Is
515 Essential for Photoprotection in *Arabidopsis*. *Cell* **110**, 361–371 (2002).
- 516 22. S. I. Saroussi, T. M. Wittkopp, A. R. Grossman, The Type II NADPH Dehydrogenase Facilitates
517 Cyclic Electron Flow, Energy-Dependent Quenching, and Chlororespiratory Metabolism during
518 Acclimation of *Chlamydomonas reinhardtii* to Nitrogen Deprivation. *Plant Physiology* **170**,
519 1975–1988 (2016).
- 520 23. C. Gerotto, *et al.*, Flavodiiron proteins act as safety valve for electrons in *Physcomitrella patens*.
521 *PNAS* **113**, 12322–12327 (2016).
- 522 24. F. Chaux, *et al.*, Flavodiiron Proteins Promote Fast and Transient O₂ Photoreduction in
523 *Chlamydomonas*. *Plant Physiology* **174**, 1825–1836 (2017).
- 524 25. A. Burlacot, *et al.*, Alternative photosynthesis pathways drive the algal CO₂-concentrating
525 mechanism. *Nature*, 1–6 (2022).
- 526 26. J. F. Allen, Photosynthesis of ATP—Electrons, Proton Pumps, Rotors, and Poise. *Cell* **110**, 273–
527 276 (2002).
- 528 27. G. Peers, *et al.*, An ancient light-harvesting protein is critical for the regulation of algal
529 photosynthesis. *Nature* **462**, 518–521 (2009).
- 530 28. G. Bonente, *et al.*, Analysis of LhcSR3, a Protein Essential for Feedback De-Excitation in the
531 Green Alga *Chlamydomonas reinhardtii*. *PLOS Biology* **9**, e1000577 (2011).
- 532 29. E. Erickson, S. Wakao, K. K. Niyogi, Light stress and photoprotection in *Chlamydomonas*
533 *reinhardtii*. *The Plant Journal* **82**, 449–465 (2015).
- 534 30. H. H. Stiehl, H. T. Witt, Quantitative treatment of the function of plastoquinone in
535 photosynthesis. *Z Naturforsch B* **24**, 1588–1598 (1969).
- 536 31. C. Foyer, R. Furbank, J. Harbinson, P. Horton, The mechanisms contributing to photosynthetic
537 control of electron transport by carbon assimilation in leaves. *Photosynth Res* **25**, 83–100
538 (1990).
- 539 32. Y. Munekage, *et al.*, Cytochrome b6f mutation specifically affects thermal dissipation of
540 absorbed light energy in *Arabidopsis*. *The Plant Journal* **28**, 351–359 (2001).
- 541 33. M. Jokel, *et al.*, *Chlamydomonas* Flavodiiron Proteins Facilitate Acclimation to Anoxia During
542 Sulfur Deprivation. *Plant and Cell Physiology* **56**, 1598–1607 (2015).
- 543 34. M. Jokel, X. Johnson, G. Peltier, E.-M. Aro, Y. Allahverdiyeva, Hunting the main player enabling
544 *Chlamydomonas reinhardtii* growth under fluctuating light. *The Plant Journal* **94**, 822–835
545 (2018).
- 546 35. H. Chen, *et al.*, Ca²⁺-regulated cyclic electron flow supplies ATP for nitrogen starvation-induced
547 lipid biosynthesis in green alga. *Sci Rep* **5**, 15117 (2015).
- 548 36. F. Kong, *et al.*, Interorganelle Communication: Peroxisomal MALATE DEHYDROGENASE2
549 Connects Lipid Catabolism to Photosynthesis through Redox Coupling in *Chlamydomonas*. *The*
550 *Plant Cell* **30**, 1824–1847 (2018).

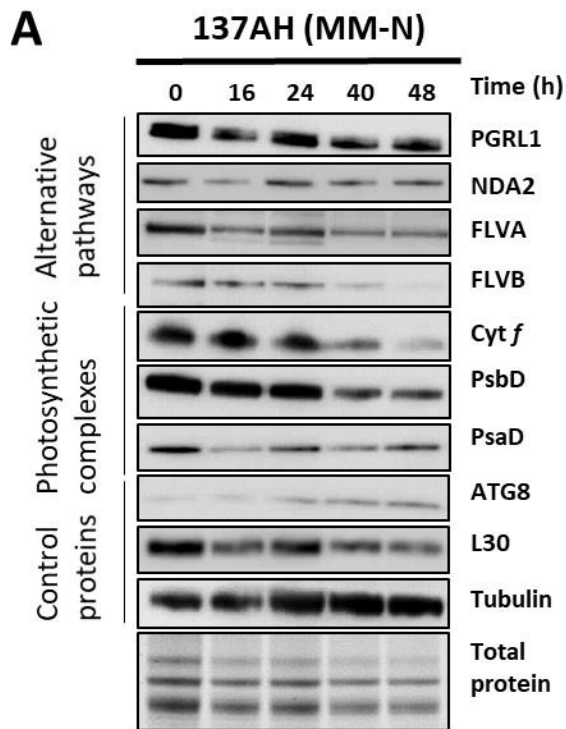
- 551 37. S. Wu, *et al.*, Elevated CO₂ improves both lipid accumulation and growth rate in the glucose-6-
552 phosphate dehydrogenase engineered *Phaeodactylum tricornutum*. *Microb Cell Fact* **18** (2019).
- 553 38. G. Peltier, G. W. Schmidt, Chlororespiration: an adaptation to nitrogen deficiency in
554 *Chlamydomonas reinhardtii*. *Proceedings of the National Academy of Sciences* **88**, 4791–4795
555 (1991).
- 556 39. L. Bulté, F.-A. Wollman, Evidence for a selective destabilization of an integral membrane
557 protein, the cytochrome b₆/f complex, during gametogenesis in *Chlamydomonas reinhardtii*.
558 *European Journal of Biochemistry* **204**, 327–336 (1992).
- 559 40. A. Burlacot, *et al.*, Flavodiiron-Mediated O₂ Photoreduction Links H₂ Production with CO₂
560 Fixation during the Anaerobic Induction of Photosynthesis. *Plant Physiol.* **177**, 1639–1649
561 (2018).
- 562 41. S. Saroussi, E. Sanz-Luque, R. G. Kim, A. R. Grossman, Nutrient scavenging and energy
563 management: acclimation responses in nitrogen and sulfur deprived *Chlamydomonas*. *Current*
564 *Opinion in Plant Biology* **39**, 114–122 (2017).
- 565 42. K. Sonoike, I. Terashima, Mechanism of photosystem-I photoinhibition in leaves of *Cucumis*
566 *sativus* L. *Planta* **194**, 287–293 (1994).
- 567 43. G. DalCorso, *et al.*, A complex containing PGRL1 and PGR5 is involved in the switch between
568 linear and cyclic electron flow in *Arabidopsis*. *Cell* **132**, 273–285 (2008).
- 569 44. J. E. Johnson, J. A. Berry, The role of Cytochrome b₆f in the control of steady-state
570 photosynthesis: a conceptual and quantitative model. *Photosynth Res* **148**, 101–136 (2021).
- 571 45. S. Saroussi, *et al.*, Reversible restriction of electron flow across cytochrome b₆f in dark
572 acclimated cells limited for downstream electron sinks. 2022.10.04.507358 (2022).
- 573 46. H. Yamamoto, T. Shikanai, PGR5-Dependent Cyclic Electron Flow Protects Photosystem I under
574 Fluctuating Light at Donor and Acceptor Sides. *Plant Physiology* **179**, 588–600 (2019).
- 575 47. D. Petroustos, *et al.*, PGRL1 Participates in Iron-induced Remodeling of the Photosynthetic
576 Apparatus and in Energy Metabolism in *Chlamydomonas reinhardtii**. *Journal of Biological*
577 *Chemistry* **284**, 32770–32781 (2009).
- 578 48. X. Johnson, *et al.*, Proton Gradient Regulation 5-Mediated Cyclic Electron Flow under ATP- or
579 Redox-Limited Conditions: A Study of Δ ATPase *pgr5* and Δ rbcl *pgr5* Mutants in the Green Alga
580 *Chlamydomonas reinhardtii*1[C][W]. *Plant Physiol* **165**, 438–452 (2014).
- 581 49. J. Steinbeck, *et al.*, Deletion of Proton Gradient Regulation 5 (PGR5) and PGR5-Like 1 (PGRL1)
582 proteins promote sustainable light-driven hydrogen production in *Chlamydomonas reinhardtii*
583 due to increased PSII activity under sulfur deprivation. *Front Plant Sci* **6**, 892 (2015).
- 584 50. F. Buchert, L. Mosebach, P. Gäbelein, M. Hippler, PGR5 is required for efficient Q cycle in the
585 cytochrome b₆f complex during cyclic electron flow. *Biochemical Journal* **477**, 1631–1650
586 (2020).
- 587 51. C. A. Sacksteder, A. Kanazawa, M. E. Jacoby, D. M. Kramer, The proton to electron
588 stoichiometry of steady-state photosynthesis in living plants: A proton-pumping Q cycle is
589 continuously engaged. *Proc Natl Acad Sci U S A* **97**, 14283–14288 (2000).
- 590 52. W. A. Cramer, S. S. Hasan, E. Yamashita, The Q Cycle of Cytochrome bc Complexes: a Structure
591 Perspective. *Biochim Biophys Acta* **1807**, 788–802 (2011).
- 592 53. G. Finazzi, J. Minagawa, G. N. Johnson, “The Cytochrome b₆f Complex: A Regulatory Hub
593 Controlling Electron Flow and the Dynamics of Photosynthesis?” in *Cytochrome Complexes:*
594 *Evolution, Structures, Energy Transduction, and Signaling*, Advances in Photosynthesis and
595 Respiration., W. A. Cramer, T. Kallas, Eds. (Springer Netherlands, 2016), pp. 437–452.
- 596 54. S. Wada, *et al.*, Flavodiiron Protein Substitutes for Cyclic Electron Flow without Competing CO₂
597 Assimilation in Rice. *Plant Physiology* **176**, 1509–1518 (2018).
- 598 55. L. Basso, K. Sakoda, R. Kobayashi, W. Yamori, T. Shikanai, Flavodiiron proteins enhance the rate
599 of CO₂ assimilation in *Arabidopsis* under fluctuating light intensity. *Plant Physiology*, kiac064
600 (2022).

- 601 56. R. Miller, *et al.*, Changes in Transcript Abundance in *Chlamydomonas reinhardtii* following
602 Nitrogen Deprivation Predict Diversion of Metabolism1[W][OA]. *Plant Physiol* **154**, 1737–1752
603 (2010).
- 604 57. I. K. Blaby, *et al.*, Systems-level analysis of nitrogen starvation-induced modifications of carbon
605 metabolism in a *Chlamydomonas reinhardtii* starchless mutant. *Plant Cell* **25**, 4305–4323
606 (2013).
- 607 58. K. Zienkiewicz, Z.-Y. Du, W. Ma, K. Vollheyde, C. Benning, Stress-induced neutral lipid
608 biosynthesis in microalgae — Molecular, cellular and physiological insights. *Biochimica et*
609 *Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1861**, 1269–1281 (2016).
- 610 59. T. Takeuchi, C. Benning, Nitrogen-dependent coordination of cell cycle, quiescence and TAG
611 accumulation in *Chlamydomonas*. *Biotechnology for Biofuels* **12**, 292 (2019).
- 612 60. F. Kong, Y. Yamaoka, T. Ohama, Y. Lee, Y. Li-Beisson, Molecular Genetic Tools and Emerging
613 Synthetic Biology Strategies to Increase Cellular Oil Content in *Chlamydomonas reinhardtii*.
614 *Plant Cell Physiol* **60**, 1184–1196 (2019).
- 615 61. Y. Li-Beisson, J. J. Thelen, E. Fedosejevs, J. L. Harwood, The lipid biochemistry of eukaryotic
616 algae. *Progress in Lipid Research* **74**, 31–68 (2019).
- 617 62. P. Geigenberger, A. Kolbe, A. Tiessen, Redox regulation of carbon storage and partitioning in
618 response to light and sugars. *Journal of Experimental Botany* **56**, 1469–1479 (2005).
- 619 63. L. Michelet, *et al.*, Redox regulation of the Calvin–Benson cycle: something old, something new.
620 *Front Plant Sci* **4** (2013).
- 621 64. P. Geigenberger, A. R. Fernie, Metabolic Control of Redox and Redox Control of Metabolism in
622 Plants. *Antioxid Redox Signal* **21**, 1389–1421 (2014).
- 623 65. X. Li, *et al.*, A Galactoglycerolipid Lipase Is Required for Triacylglycerol Accumulation and
624 Survival Following Nitrogen Deprivation in *Chlamydomonas reinhardtii*. *The Plant Cell* **24**, 4670–
625 4686 (2012).
- 626 66. H. Chen, *et al.*, Ca²⁺-regulated cyclic electron flow supplies ATP for nitrogen starvation-
627 induced lipid biosynthesis in green alga. *Sci Rep* **5**, 15117 (2015).
- 628 67. X. Johnson, J. Alric, Interaction between Starch Breakdown, Acetate Assimilation, and
629 Photosynthetic Cyclic Electron Flow in *Chlamydomonas reinhardtii*. *J. Biol. Chem.* **287**, 26445–
630 26452 (2012).
- 631 68. X. Johnson, J. Alric, Central Carbon Metabolism and Electron Transport in *Chlamydomonas*
632 *reinhardtii*: Metabolic Constraints for Carbon Partitioning between Oil and Starch. *Eukaryot Cell*
633 **12**, 776–793 (2013).
- 634 69. M. Saint-Sorny, P. Brzezowski, S. Arrivault, J. Alric, X. Johnson, Interactions Between Carbon
635 Metabolism and Photosynthetic Electron Transport in a *Chlamydomonas reinhardtii* Mutant
636 Without CO₂ Fixation by RuBisCO. *Frontiers in Plant Science* **13** (2022).
- 637 70. C. Goodson, R. Roth, Z. T. Wang, U. Goodenough, Structural correlates of cytoplasmic and
638 chloroplast lipid body synthesis in *Chlamydomonas reinhardtii* and stimulation of lipid body
639 production with acetate boost. *Eukaryot Cell* **10**, 1592–1606 (2011).
- 640 71. U. Goodenough, *et al.*, The path to triacylglyceride obesity in the sta6 strain of *Chlamydomonas*
641 *reinhardtii*. *Eukaryot Cell* **13**, 591–613 (2014).
- 642 72. K. Maxwell, G. N. Johnson, Chlorophyll fluorescence—a practical guide. *J Exp Bot* **51**, 659–668
643 (2000).
- 644 73. A. Burlacot, F. Burlacot, Y. Li-Beisson, G. Peltier, Membrane Inlet Mass Spectrometry: A
645 Powerful Tool for Algal Research. *Front Plant Sci* **11**, 1302 (2020).

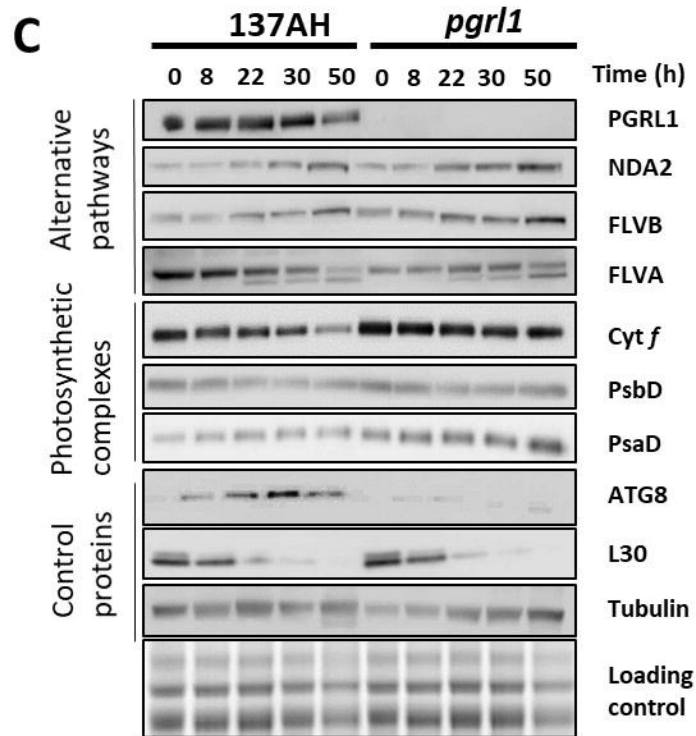
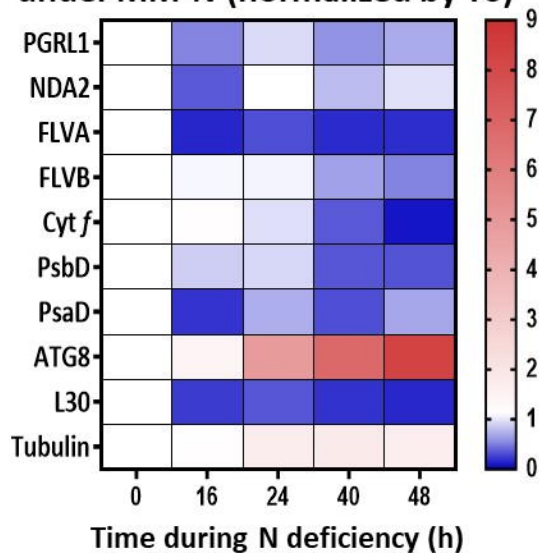


■ Gross O₂ uptake ■ Gross O₂ production ■ Net O₂ production





B **Proteins abundance in 137AH under MM-N (normalized by T0)**



D **Proteins relative abundance in *pgrl1* (*pgrl1*/WT)**

