**New Phytologist Supporting Information**

Trophic state alters the mechanism whereby energetic coupling between photosynthesis and respiration occurs in *Euglena gracilis*

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**Fig. S3** NPQ in *Euglena gracilis* cells grown in mixotrophy or in photoautotrophy.

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**Fig. S5** Impacts of the inhibitors of mitochondrial respiratory complexes (I, III, IV), ATP synthase, and AOX on respiratory (Rr) and photosynthetic gross oxygen evolution (Eo) rates for *Euglena gracilis* cultivated in mixotrophic and photoautotrophic conditions.

**Fig. S6** (a) End of exponential phase of batch cultures of *Euglena gracilis* cultivated in mixotrophic (TAP) and photoautotrophic (TMP) conditions, and in the presence of rotenone (Rot, 100 µM) or antimycin A (AA, 10 µM).

**Fig. S7** Transmission electron micrographs illustrating subcellular organization of *Euglena gracilis* cultured in mixotrophy, photoautotrophy and photoautotrophy + 5% CO₂.

**Fig. S8** Relationship between PSI relative electron transport rate and PSI+PSII photochemical rate, and relationship between PSII relative electron transport rate and E₀ in *Euglena gracilis* cultivated in mixotrophic and photoautotrophic conditions.

**Supplemental method 1.** Detailed procedure of the proteomic analysis
**Fig. S1** (a) Electrochromic shift (ECS) spectrum and (b) ECS linearity in *Euglena gracilis* cultivated mixotrophically and photoautotrophically. (a) Absorbance changes in the 440 to 600 nm region was induced by a single turnover flash. Measurements were done at the same chlorophyll concentration for both mixotrophic and photoautotrophic grown cells. No difference can be found between the two ECS spectra (two-way RM ANOVA; $P = 0.34$) (b) ECS signal at 510 nm was monitored after each single turnover flash of a series of 5 flashes (10 Hz). Values were normalized to one charge separation of both PSI and PSII. All measurements were performed for at least three independent culture replicates. $r^2 = 0.99$ and $P < 0.0001$ for each linear regression. Bars indicate the standard deviations.
Fig. S2 Impact of inhibitors on chlorophyll fluorescence induction curves in *Euglena gracilis* cultured in mixotrophy (TAP) or in photoautotrophy (TMP). Sodium bicarbonate (HCO$_3^-$), antimycin A (AA), potassium cyanide (KCN), salicylhydroxamic acid (SHAM), aminooxyacetic acid (AOAA), 2,5-dibromo-6-isopropyl-3-methyl-1,4-benzoquinone (DBIMB), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), and octyl gallate (OG). Measurements were performed for at least two independent culture replicates. Vertical bars indicate the standard deviation. Fm was obtained upon a 150 ms pulse of saturating light (last point of each curve). $F_0$ and Fm were normalized to 0 and 1, respectively.
**Fig. S3** NPQ after 12 minutes of illumination (100 μmol photons m⁻² s⁻¹) in *E. gracilis* cells grown in mixotrophy or in photoautotrophy. Rotenone (Rot, 100 μM), aminooxyacetic acid (AOAA, 5 mM) or sodium bicarbonate (HCO₃⁻, 10 mM) were added at the fourth minute of the light period followed by the addition (or the absence in the case of control measurement) of octyl gallate (OG, 10 μM) to inhibit the activity of plastidial terminal oxidase (PTOX). NPQ values were calculated as (Fm-Fm’)/Fm’, where Fm is the maximum fluorescence of cells before illumination and Fm’ the maximum fluorescence before light is switched off. All measurements were performed with two independent culture replicates. Vertical bars indicate the standard deviation. NPQ values were very low and identical in presence or absence of OG (two-way ANOVA; *P* = > 0.1) in mixotrophy or in photoautotrophy.
**Fig. S4** Impact of octyl gallate (OG) and salicylhydroxamic acid (SHAM) on dark respiration (Rd) in *Euglena gracilis* cultivated in mixotrophic and photoautotrophic conditions. 10^6 cells mL^−1 were adjusted in the oximeter chamber. Vertical bars indicate the standard deviation. Values were normalized to Rd values of control mixotrophic not-poisoned cells. Different letters identify significantly different means (one-way ANOVA, *P* < 0.05).
**Fig. S5** Impacts of the inhibitors of mitochondrial respiratory complexes (I, III, IV), ATP synthase, and AOX on respiratory (R_d) and photosynthetic gross oxygen evolution (E_o) rates for *Euglena gracilis* cultivated in mixotrophic (a) and photoautotrophic (b) conditions. All measurements were conducted in the presence of 10 mM NaHCO_3. 10^6 cells . mL^-1 were adjusted in the oximeter chamber. Each light step (30, 103, and 571 µmol photons m^-2 s^-1) was held for 5 min. Results are expressed as ratios with respect to the control condition. In mixotrophy, R_d value without inhibitors was 12.1 ± 2.4 fmol O_2 min^-1 cell^-1. P < 0.1, p < 0.05, or p < 0.01 of a student’s t-test are indicated by *, ** or ***, respectively). Values were compared with the E_o or R_d values of not inhibited cells (no add). Addition of KCN (blocking complex IV), and KCN+SHAM (blocking complex IV and AOX, respectively) caused equivalent reduction of E_o under moderate and high light intensities but a milder reduction of E_o at low light intensity (one-way ANOVA; P < 0.001). Addition of rotenone (blocking complex I), AA (blocking complex III) or oligomycin (blocking mitochondrial F_1F_0 ATP synthase).
synthase) caused an equal reduction of E_o at all light intensities (one-way ANOVA; P > 0.05). In photoautotrophy, addition of rotenone and AA caused a similar decline in E_o under all light intensities (one-way ANOVA; P > 0.05). Linear relationship between gross O_2 evolution (E_o) at 30 (c, r = 0.56, P = 0.03) and 103 (d, r = 0.86, P = 0.001) µmol photons m⁻² s⁻¹, and dark respiration (R_d) in presence of mitochondrial respiratory inhibitors for E. gracilis cultivated in mixotrophic and photoautotrophic conditions. Each point corresponds to the mean value of at least three independent culture replicates. All values were normalized on E_o and R_d values in absence of inhibitors. Vertical and horizontal bars indicate the standard deviations.
Fig. S6 (a) End of exponential phase of batch cultures of *Euglena gracilis* cultivated in mixotrophic (TAP) and photoautotrophic (TMP) conditions, and in the presence of rotenone (Rot, 100 µM) or antimycin A (AA, 10 µM). no add: control condition in the absence of the inhibitors. (b) Cell-specific division rate (µ) of *E. gracilis* cultured mixotrophically and photoautotrophically, and in the presence of rotenone (100 µM) and antimycin A (AA, 10 µM). All measurements were performed for at least three independent culture replicates. Vertical bars indicate the standard deviation. ***, $P < 0.01$ of a student’s t-test. Values were compared with the control cells. §, $P < 0.01$ of a student’s t-test. Values were compared with the control mixotrophic cells.
**Fig. S7** Representative examples of transmission electron micrographs illustrating subcellular organization of *Euglena gracilis* cultured in mixotrophy (a-b), photoautotrophy (c-d) and photoautotrophy + 5% CO₂ (e-f). Bars: 1 µm. (a), (c), and (e): white arrows show the mitochondrial-chloroplastic contacts; C, chloroplast; M, mitochondrion; P, pyrenoids. (b), (d), and (f): cell compartments highlighted in colors (chloroplasts in light green, pyrenoids in dark green and mitochondria in red).
**Fig. S8.** Linear relationship between PSI relative electron transport rate (rETR-PSI, µmol electrons m⁻² s⁻¹) and ECS-based photochemical rate (electron (PSI+PSII)⁻¹ s⁻¹) in *Euglena gracilis* cultivated in (a) mixotrophic (r= 0.94, P = 0.03) and (b) photoautotrophic conditions (r= 0.95, P = 0.03). Each black dot corresponds to a different light intensity: 25, 100, 345, and 615 µmol photons m⁻² s⁻¹; 3 s per light step). In a parallel experiment (white dots), 10 µM antimycin AA was added at 345 µmol photons m⁻² s⁻¹. There is no statistical difference between rETR-PSI of control and AA-poisoned cells (t-test, P = 0.34 (a), and 0.16 (b)). ECS-based photochemical rates were calculated considering the steady ECS signals of the last 500 ms of the light period minus the rate of ECS change in the first 5 milliseconds of the dark phase. These values were then normalized to the ECS signal developed by a single turnover flash (i.e. a simultaneous single charge separation in both PSI and PSII) (Bailleul *et al.*, 2010).  

(c) Linear relationship between PSII relative electron transport rate (rETR-PSII, µmol electrons m⁻² s⁻¹) and *E*. *gracilis* cultivated in mixotrophic (r= 0.99, P = 0.0003) and photoautotrophic (r= 0.98, P = 0.006) conditions. Each data value corresponds to a different light intensity: 25, 100, 345, and 615 µmol photons m⁻² s⁻¹; 2 min per light step). Photosystem I activity was monitored by recording changes in absorption of its primary electron donor (P700) due to photo-oxidation induced by increasing PPFD (25, 100, 345 and 615 µmol photons m⁻² s⁻¹) according to Roberty *et al*. (2014). Briefly, the detecting light probe of the JTS-10 spectrophotometer was filtered by a narrow band pass filter centered at 705 nm (10nm FWHM). Measurements in the dark were considered as Po, and were followed by three seconds of actinic light which gave the value of Ps. A saturating pulse (50 ms) was applied immediately, and the maximum change obtained was considered as Pm’. The maximum photo-oxidation value of P700 (Pm) was obtained after addition of DCMU (10 µM) and a three seconds of illumination followed by a saturating pulse of light. These parameters were used to calculate the relative electron transport rate through photosystem I (rETR-PSI, in µmol electron m⁻² s⁻¹) as PPFD x (Pm’-Ps) / (Ps-Po) (Roberty *et al.*, 2014). All
measurements were conducted in the presence of 10 mM NaHCO₃ and were performed for at least three independent culture replicates. Vertical bars indicate the standard deviation.

**Supplemental method 1. Detailed procedure of the proteomic analysis**

**Shotgun proteomics and protein quantification.** The relative abundance of proteins in each condition was determined using iTRAQ isobaric labelling. The iTRAQ marking was carried out according to the protocol supplied by the manufacturer (ABSciex, Foster City, USA). Samples were labeled with the 114 isobaric tag for mixotrophic cells in low light (TAP LL), 115 for mixotrophic cells in moderate light (TAP ML), 116 for photoautotrophic cells + 5 % CO₂ (TMP CO₂) and 117 tag for photoautotrophic cells (TAP). After isobaric labelling, the four samples were mixed and the mixture dried under vacuum in an SC 200 Savant Speed Vac concentrator (Thermoscientific, Waltham, USA). 16 µg of proteins for each labeled sample were then dissolved in 0.025 % trifluoroacetic acid and 5 % acetonitrile and subjected to reverse phase chromatography according to Szopinska *et al.* (2011), using an LC Ultimate nano chromatography system 3000 (Thermoscientific, Waltham, USA). The eluted peptides were analysed using an AB Sciex 4800 MALDI TOF/TOF (matrix-assisted laser desorption/ionization-time of flight) as described in Szopinska *et al.* (2011). Each sample was analysed in three repetitions.

Peak lists obtained from MS/MS spectra were identified using OMSSA version 2.1.9 (Geer *et al.*, 2004), X! Tandem version X! Tandem Vengeance (2015.12.15.2) (Craig & Beavis, 2004), MS-GF+ version Release (v2018.04.09) (Kim & Pevzner, 2014), Comet version 2018.01 rev. 3 (Eng *et al.*, 2013) and Tide version unknown (Diament & Noble, 2011). The search was carried out using SearchGUI (Vaudel *et al.*, 2011). Protein identification was conducted against a concatenated target/decoy (Elias & Gygi, 2010) version of the *E. gracilis* database (Cordoba *et al.*, unpublished) in combination with the *Euglena gracilis* reviewed sequences from UniProt (2019) complemented with a list of common contaminants found at the Global Proteome Machine 3 website (http://www.thegpm.org/crap), for a total of 50,287 (target) sequences. The decoy sequences were created by reversing the target sequences in SearchGUI. The identification settings were as follows: Trypsin, Semi-Specific, with a maximum of 1 missed cleavages, 10.0 ppm as MS1 and 0.4 Da as MS2 tolerances. Fixed modifications during refinement procedure were: iTRAQ 4-plex of K (+144.102062 Da), iTRAQ 4-plex of peptide N-term (+144.102062 Da), oxidation of M (+15.994915 Da), while
variable modifications during refinement procedure were: Acetylation of protein N-term (+42.010565 Da), Pyrolidone from E (−18.010565 Da), Pyrolidone from Q (−17.026549 Da), and Pyrolidone from carbamidomethylated C (−17.026549 Da).

Peptides and proteins were inferred from the spectrum identification results using PeptideShaker version 1.16.43 (Vaudel et al., 2015) and were validated at a 1% false discovery rate estimated using the decoy hit distribution. Spectrum counting abundance indexes were estimated using the normalized spectrum abundance factor (Powell et al., 2004) adapted for better handling of protein inference issues and peptide detectability.

A total of 544 proteins were identified. In the many analyzes that are made in MALDI, one usually reaches a number of identifications between 400-800. Labeling peptides in iTRAQ even tends to improve fragmentation and the number of identifications because the b-ion series are more complete (Gandhi et al., 2012). In the case of iTRAQ quantitative analysis, we increase the number of "laser shots (4000)" per precursor to have the signal corresponding to the reporter ions as stable as possible. By doing this, we limit the number of precursors that can be spot selected and therefore reduces the number of peptides that can participate in the identification. The quality of the quantification is therefore privileged over the number of identifications. The analyzes which make it possible to go up above the 800 identifications are carried out starting from a quantity 25 times more important of proteins (400 micrograms) by making 2D-LC by cumulating several fractions obtained by cation exchange (SCX). This greatly increases the scan time and increases the risk of a problem occurring. (Ernoult et al., 2008) The benefit of doing this kind of sequential analysis is quite minimal in terms of the number of additional identifications compared to our. We would certainly identify a little more protein but many would not be quantifiable. The capacity of the column is theoretically 1 microgram but we can inject more as we do (16 micrograms) without problem of resolution. If 400 micrograms were injected directly, resolution would be greatly lost and the proteins mainly detected would be the most abundant anyway. It is a compromise that must therefore be made and at the same time increasing the number of identifiable and quantifiable proteins is not obvious.

Finally, protein quantification was performed with Reporter software version 0.7.20 (beta). The output of Reporter corresponded to the intensity of the reporter ions aggregated and scaled to each sample. Normalized protein abundance was then log 2 transformed and significant changes (P-values lower than 0.05) between samples were computed using a linear
model framework with LIMMA empirical Bayes approach (Kammers et al., 2015; Ritchie et al., 2015).

The nucleic acid sequences of the transcripts corresponding to the proteins identified in all the iTRAQ analyzes were annotated with EggNOG-mapper v1 (Huerta-Cepas et al., 2017; Huerta-Cepas et al., 2019), by similarity using PSI-BLAST v2.2.28 searches (Camacho et al., 2009) (E-value cut-off of 0.001) against Swissprot (UniProt, 2019) and by sequence alignment to the NCBI protein database (Sayers et al., 2021) using TBLASTN v2.2.28 (Camacho et al., 2009) (E-value cut-off of 0.001).

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