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# Anoxygenic Photosynthesis and Dark Carbon Metabolism under micro-oxic conditions in the Purple Sulfur Bacterium *“Thiodictyon syntrophicum”* nov. strain Cad16<sup>T</sup>

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28 **1. ABSTRACT**

29

30 The microbial ecosystem of the meromictic Lake Cadagno (Ticino, Swiss Alps) has been  
31 studied intensively to understand metabolic functions driven by the highly abundant  
32 anoxygenic phototrophic sulfur bacteria of the families Chromatiaceae and Chlorobiaceae. It  
33 was found that the sequenced isolate “*Thiodictyon syntrophicum*” nov. sp. str. Cad16<sup>T</sup>,  
34 belonging to the Chromatiaceae, may fix 26% of all bulk inorganic carbon in the chemocline  
35 at day and night. With this study, we elucidated the mode of dark carbon fixation of str.  
36 Cad16<sup>T</sup> with a combination of long-term monitoring of key physicochemical parameters with  
37 CTD, <sup>14</sup>C-incorporation experiments and quantitative proteomics of *in situ* dialysis bag  
38 incubations of pure cultures. Regular vertical CTD profiling during the study period in  
39 summer 2017 revealed that the chemocline sank from 12 to 14 m which was accompanied by  
40 a bloom of cyanobacteria and the subsequent oxygenation of the deeper water column.  
41 Sampling was performed both day and night in September. While CO<sub>2</sub> assimilation rates were  
42 higher during the light period, the relative change in the proteome (663 quantified proteins)  
43 was only 1% of all CDS encoded in str. Cad16<sup>T</sup>. Oxidative respiration was thereby  
44 upregulated at light, whereas stress-related mechanisms prevailed during the night. These  
45 results indicate that the low light availability due to high cell concentrations and the  
46 oxygenation of the chemocline induced a mixotrophic growth in str. Cad16<sup>T</sup>.  
47 The complete proteome data have been deposited to the ProteomeXchange with identifier  
48 PXD010641.

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## 61 2. INTRODUCTION

62

63 Inorganic carbon, nitrogen and sulfur is cycled in diverse microbial metabolism networks in  
64 paired redox reactions and organic compounds are thereby produced in the scale of  $10^9$  t year<sup>-1</sup>  
65 worldwide [1]. Light has been used as a source of energy in phototrophic anoxygenic bacteria  
66 since at least 3.85 Ga (billion years before the present) [2]. During carbon fixation, the  
67 transfer of electrons along a gradient of carrier molecules with sequentially lower potential  
68 allows the generation of energy bound to phosphate (ATP) and reductants [e.g., NAD(P)H  
69 and reduced ferredoxin]. The electrons required to replenish the oxidized electron acceptor  
70 pool can be derived from the oxidation of reduced sulfur species such as sulfide, sulfite and  
71 thiosulfate, or H<sub>2</sub> and Fe (II) [3].

72 The anoxygenic photosynthetic purple sulfur bacteria (PSB) of the family Chromatiaceae are  
73 found widespread in aquatic, sulfidic oxygen minimum-zones where light is still available [4].  
74 As an adaption to low light availability around 0.1–20  $\mu\text{mol m}^2 \text{s}^{-1}$  and only limited  
75 wavelength (450–600 nm, and infrared above 750 nm), PSB contain pigments of the  
76 carotenoid and bacteriochlorophyll *a* and *b* (BChl) classes [5], as well as multiple copies of  
77 antenna peptides (LHC), to subtly modulate charge separation within the membrane bound  
78 type II reaction center (RC) [6, 7]. Carbon is typically fixed through the Calvin-Benson-  
79 Bassham (CBB) cycle [8]. In order to store both, reduction-equivalents and oxidized carbon,  
80 PSB intracellularly concentrate elemental sulfur-chains (S-S<sub>n</sub><sup>0</sup>) in protein covered globules  
81 (SGBs) [9] and glycogen and polyhydroxybutyrate (PHB) [10], respectively. These  
82 assimilates may subsequently allow for chemotrophic growth in the dark [11, 12].

83 The possibility of chemolithoautotrophic growth of PSB under microaerophilic conditions in  
84 the dark has been proposed by van Niel [13] and has been described first for *Thiocapsa*  
85 *roseopersicina* BBS [14]. The PSB *T. roseopersicina* inhabiting shallow tidal flats is  
86 especially adapted to the daily changes of oxygen concentrations and competes with  
87 chemotrophic non purple sulfur bacteria (*Thiobacillus* spp.) and *Beggiatoaceae* spp. [15].  
88 Chemoheterotrophic, chemoautotrophic and mixotrophic growth has since been shown for  
89 different PSB spp. [16–21]. Several strains of *Allochromatium vinosum* and *T. roseopersicina*  
90 have shown mixotrophic growth under a 5% oxygen atmosphere and acetate, and different  
91 reduced sulfur compounds [18].

92 The ecological significance and the impact on biogeochemistry of PSB were extensively  
93 studied in permanently stratified lakes [22–27]. In Lake Cadagno (Piora valley, Swiss Alps),  
94 underwater springs in gypsum rich dolomite provide a steady inflow of solute-rich water. In  
95 combination with solute-poor surface water, a stable and steep gradient in redox potential,  
96 salinity, sulfide and oxygen concentrations at around 12 m depth is formed [28]. Within this  
97 chemocline, a dense population of phototrophic sulfur oxidizing bacteria (PSOB) of the  
98 family Chromatiaceae and Chlorobiaceae (GSB; green sulfur bacteria) thrive to dense  
99 populations with up to  $10^7$  cells  $\text{ml}^{-1}$  between June and October [29]. *In situ* chemocline  
100 incubation experiments with  $^{14}\text{C}$ -uptake in Lake Cadagno [30, 31] and other lakes [32], as  
101 well as with nanoSIMS  $^{13}\text{C}$ -stable-isotope labelling [33] revealed both light-driven and dark  
102 carbon fixation of PSB. Thereby it was found, that the population of PSB isolate “*Thiodictyon*  
103 *syntrophicum*” sp. nov. str. Cad16<sup>T</sup> (str. Cad16<sup>T</sup>, thereafter) [34] assimilated 26% of the total  
104 carbon the chemocline during dark and light incubations [31]. Alternatively, the relative  
105 contribution of different PSB and GSB spp. to total carbon assimilation normalized to  
106 biomass during daytime was estimated with stable isotope analysis for Lake Cadagno.  
107 Thereby, str. Cad16<sup>T</sup> only photosynthetically fixed 1.3 to 2 % of the carbon, as estimated from  
108 the daily bulk  $\delta^{13}\text{C}$ -mass balance [35].

109 Additional insight came from an *in vitro* quantitative proteomics study with str. Cad16<sup>T</sup>  
110 growing anaerobically under light and dark with 1 mM  $\text{H}_2\text{S}$  [36]. Photosynthesis-driven  
111 growth of str. Cad16<sup>T</sup> resulted in the relative  $>1.5\times$  expression of 22 proteins. Most notably,  
112 the poly(R)-hydroxyalkanoic acid synthase subunit PhaE and the phasin (PhaP) involved in  
113 the synthesis of PHB were found. In contrast, among the 17 proteins overexpressed under  
114 dark conditions, three enzymes of the dicarboxylate/4-hydroxybutyrate (DC/HB) cycle were  
115 detected, indicating dark carbon fixation through this typically *archeal* pathway.

116 The complete genome of str. Cad16<sup>T</sup> gave evidence of the biological functions encoded [37].  
117 Similar to *Allochromatium* spp. or *Lamprocystis* spp., str. Cad16<sup>T</sup> expresses a type II (quinone  
118 type) RC, the membrane-bound protein cascade of cyclic electron transport to generate ATP  
119 and reverse electron transport to produce NAD(P)H, and also contains a *cbb3* type  
120 cytochrome *c*. The latter may enable microaerobic growth and Fe(III) oxidation of str. Cad16<sup>T</sup>  
121 [38]. However, no genetic evidence for the possible syntrophic relationship within aggregates  
122 of *Desulfocapsa* sp., and also only incomplete Sox and no thiosulfate dehydrogenase Tsd  
123 proteins, responsible for  $\text{SO}_3^{2-}$  oxidation, as previously described for str. Cad16<sup>T</sup> by Peduzzi  
124 and colleagues [34], were found.

125 With this study, we aimed at elucidating the metabolic pathways of PSB str. Cad16<sup>T</sup> in more  
126 detail, to better understand the key metabolic mechanisms involved. The main objectives of  
127 this study were: (i) to study differences between day and night in the metabolism of PSB str.  
128 Cad16<sup>T</sup> *in situ* at the chemocline of Lake Cadagno and (ii) to monitor the environmental  
129 factors longitudinally that determine the metabolic activity of the chemocline community. We  
130 used a combination of CO<sub>2</sub> assimilation analysis using <sup>14</sup>C-scintillation and proteomics using  
131 label-free quantitation tandem mass spectrometry (LFQ-MS<sup>2</sup>) to quantify metabolic activity  
132 and the pathways involved in str. Cad16<sup>T</sup>. Relative light intensity and temperature at the depth  
133 of the chemocline were measured constantly and several CTD profiles were taken during the  
134 incubation. The carbon assimilation rates and protein profiles obtained, thereby revealed a  
135 mixotrophic metabolism of str. Cad16<sup>T</sup> influenced by the unique microaerobic *in situ*  
136 conditions encountered in summer 2017.

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### 139 **3. MATERIALS AND METHODS**

#### 140 **3.1. Study Site and Field Measurements**

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142 The *in situ* incubations were performed in Lake Cadagno between 13 July 2017 to 23  
143 September 2017 with dialysis bags attached to a mooring (46°33',05,1" N/8°42',43,0" E,  
144 max. depth 18 m) (**suppl. Figure S 1b and c**). From 13 July to 13 September 2017 different physical  
145 and chemical parameters were measured alongside the incubations in order to monitor the *in*  
146 *situ* chemocline conditions and adjust the incubation depth, if necessary. A CTD  
147 (Conductivity, Temperature, Depth) 115 probe (Sea & Sun Technology GmbH, Germany)  
148 equipped with temperature, salinity, oxygen, redox potential, chlorophyll *a* (Chl *a*) and  
149 turbidity sensors was used to measure physicochemical profiles. Profiles from 13 July 2017  
150 were taken as an estimate of chemocline depth (**suppl. Figure S2** HOBO UA-002-64 Pendant data  
151 loggers (Onset Computer Corporation, MA, USA) measured relative light (Lux; 180–1'200  
152 nm) and temperature at 60 min intervals. Two sensors were placed near the surface (0.05 m  
153 depth) and other pairs were positioned 0.4 m apart at the upper and lower part of the rig,  
154 respectively (**suppl. Figure S 1c**). An empirical conversion factor of Lux = 0.018 PAR and 0.016

155 PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was used for the surface and below the water, respectively as in ref. [39].  
156 The passive HOBO logger values were analyzed after retrieval at the end of the experiment.  
157 We additionally had access to CTD data from a parallel project from Dr. Oscar Sepúlveda  
158 Steiner and colleagues from EAWAG (Dübendorf, Switzerland) where two CTD profiles  
159 were taken daily.

160

## 161 3.2. Flow Cytometry for Cell Counting

162

163 Flow-cytometry based cell counting was performed as in Danza and colleagues [40]. In short,  
164 phototrophic bacteria were identified using  $50 \mu\text{l}$  sub-samples in triplicates with a BD Accuri  
165 C6 cytometer (Becton Dickinson, San José, CA, USA). A forward scatter threshold of FSC-H  
166  $10'000$  was applied to exclude abiotic particles. A second red fluorescent (FL3-A) threshold  
167 above  $1'100$  was applied to select for cells emitting autofluorescence due to Chl and BChl.  
168 The flow rate was set to  $66 \mu\text{l min}^{-1}$ .

169

## 170 3.3. Estimates of Biovolume and Biomass of Bacterial Cells

171

172 The biovolume was calculated for strain Cad16<sup>T</sup> assuming a median diameter of  $2 \mu\text{m}$  (range;  
173  $1.4\text{--}2.4 \mu\text{m}$ ) for a spherical cell. Biomass was estimated using the conversion factor  
174 determined for Lake Cadagno PSB strains  $4.5 \text{ fmol C m}^{-3}$  [33].

175

## 176 3.4. Estimates of Carbon Uptake Rates

177

178 We took the estimate from Camacho *et al.* [30] that half of the carbon is fixed through  
179 oxygenic and anoxygenic photosynthesis, respectively. Musat and colleagues estimated  
180 anoxygenic carbon assimilation for PSB *C. okenii* and GSB *C. clathratiforme* to be 70% and  
181 15% of the total daily  $\text{CO}_2$  assimilation, respectively [33]. We therefore calculated the uptake  
182 rates for the three populations as follows (Eq. 1) where  $A_{\text{day}}$  is the total uptake rate per cell of  
183 the phototrophic community and  $F$  the fraction as estimated in [33]:

184

$$185 \quad A_{\text{cyano}} = A_{\text{day}} \times 0.5 \times F \quad (1)$$

### 187 3.5. Bacterial Pure Cultures and Media

188

189 Str. Cad16<sup>T</sup> was isolated in 2003 [41] and was subsequently grown in pure culture on  
190 Autotrophic Pfennig's medium II [42] at the Laboratory of Applied Microbiology in  
191 Bellinzona, Switzerland (LMA) (Figure S 1a). The medium contained 0.25 g of KH<sub>2</sub>PO<sub>4</sub> l<sup>-1</sup>, 0.34  
192 g of NH<sub>4</sub>Cl l<sup>-1</sup>, 0.5 g of MgSO<sub>4</sub>·7H<sub>2</sub>O l<sup>-1</sup>, 0.25 g of CaCl<sub>2</sub>·H<sub>2</sub>O l<sup>-1</sup>, 0.34 g of KCl l<sup>-1</sup>, 1.5 g of  
193 NaHCO<sub>3</sub> l<sup>-1</sup>, 0.02 mg of vitamin B12 l<sup>-1</sup> and 0.5 ml of trace element solution SL12 l<sup>-1</sup>. The  
194 medium was autoclaved under a 80% N<sub>2</sub>/ 20% CO<sub>2</sub> atmosphere [43] and 1.1 mM Na<sub>2</sub>S·9H<sub>2</sub>O  
195 was added aseptically. The pH was adjusted to 7.0. Cultures were grown in 500 ml glass  
196 bottles at ambient temperature and under a 12/12 h light/dark-regime with a 60 W  
197 incandescent lamp (6 μmol quanta m<sup>-1</sup> s<sup>-1</sup>). Cells were grown up to a concentration of around  
198 3 × 10<sup>6</sup> cells ml<sup>-1</sup>. Cell concentrations repeatedly were measured by flow cytometry.

199

200 Cellulose dialysis bags with a 14 kDa cutoff (D9777-100FT, Sigma-Aldrich, Buchs, CH)  
201 were rinsed for 1.5 h in Na<sub>2</sub>CO<sub>3</sub> (40 g l<sup>-1</sup>) and 0.01 M EDTA at 60 °C while stirring. The bags  
202 were cleaned with ddH<sub>2</sub>O, cut into 0.6 m long pieces, closed by a knot on one end and  
203 autoclaved for 20 min at 121 °C. On site, about 80 ml of bacteria culture were filled randomly  
204 in each bag, which was closed, attached to a rig and installed in the chemocline within 30 min  
205 (suppl. Figure S 1b). In total 18 dialysis bags were placed at 12 m depth from 13 July to 23  
206 August 2017 and then lowered to 14 m for the remaining campaign.

207

### 208 3.6. <sup>14</sup>C-Incubations

209

210 The scintillation experiment was performed as in Storelli *et al.* [31]. In short, subsamples  
211 from three dialysis bags were pooled together randomly, 7 ml NaH<sup>14</sup>CO<sub>3</sub> (NaH<sup>14</sup>CO<sub>3</sub>; 1.0  
212 mCi; 8.40 mCi mmol<sup>-1</sup>, 20 μCi ml<sup>-1</sup>; Cat. No. NEC-086S Perkin-Elmer, Zurich, Switzerland)  
213 were added and incubated in cleaned and autoclaved 50 ml translucent Duran glass bottles  
214 (SCHOTT AG, Mainz, Germany). Six replicates of str. Cad16<sup>T</sup> cultures were incubated for 4  
215 hours during day (1:00–4:00 pm) and night (9:00 pm–12:00 am), respectively. Chemocline  
216 background fixation rates were determined in 50 ml chemocline samples. Filtered chemocline  
217 lake water (0.45 μm) was used as negative control. Upon retrieval, the amount of β-activity

218 (<sup>14</sup>C) assimilated by microbes during the incubation time was measured in the laboratory  
219 following standard method that included acidification and bubbling of the samples [44].

220

221 The inorganic dissolved carbon concentration was determined with the CaCO<sub>3</sub> *Merck*  
222 *Spectroquant* kit No. 1.01758.0001 and the *Merck spectroquant Pharo 100* photospectrometer  
223 (Merck & Cie, Schaffhausen, Switzerland). Samples were taken at 14 m depth, filtered with  
224 0.45 μm filters, pH was tested with indicator paper (MColorpHast, Merck KGaA, Germany)  
225 to lie within 6.8–7.0 and triplicate samples were measured.

226

227 Scintillation was done on a *Guardian 1414* liquid scintillation counter (Perkin Elmer Wallac,  
228 MA, USA) running with the WinSpectral v.1.40 software. Raw data was statistically analyzed  
229 using *t*-tests in Excel (Microsoft Office 2010, v-.14.0.7168.5000).

230

### 231 3.7. Protein Extraction and Digest

232

233 Subsamples were transferred to 50 ml tubes upon retrieval and stored at 4 °C in the dark.  
234 They were then brought to the lab within 30 min and centrifuged 10'000 *g* at 4 °C for 10 min.  
235 The supernatant was discarded and the pellets were re-suspended in 1× PBS pH 7.0 and 1%  
236 EDTA-free Protease Inhibitor Cocktail (*v/v*; Thermo Fisher Scientific, Rheinach, Switzerland)  
237 and frozen at -20 °C until further processing.

238

239 The cells were thawed, lysed in 5% SDC (w/w) in 100 mM ammonium-bicarbonate buffer  
240 containing 1% EDTA-free Protease Inhibitor Cocktail (*v/v*; Thermo Fisher Scientific,  
241 Rheinach, Switzerland) and sonicated for 15 min at 200 W at 10 °C with a Bioruptor  
242 ultrasonicator (Diagenode SA, Belgium). Samples were then shipped to the Functional  
243 Genomic Center Zurich (FGCZ) on dry ice for further processing. Protein concentration was  
244 estimated using the Qubit Protein Assay Kit (Thermo Fisher Scientific, Rheinach,  
245 Switzerland). The samples were then prepared using a commercial iST Kit (PreOmics,  
246 Germany [45]) with an updated version of the protocol. Briefly, 50 μg of protein were  
247 solubilized in 'lyse' buffer, boiled at 95 °C for 10 min and processed with High Intensity  
248 Focused Ultrasound (HIFU) for 30 s setting the ultrasonic amplitude to 85%. Then the  
249 samples were transferred to a cartridge and digested by adding 50 μl of the 'Digest' solution.  
250 After incubation (60 min, 37 °C) the digestion was stopped with 100 μl of Stop solution. The



251 solutions in the cartridge were removed by centrifugation at 3'800 g, while the peptides were  
252 retained by the iST-filter. Finally the peptides were washed, eluted, dried and re-solubilized in  
253 'LC-Load' buffer for Tandem Mass spectrometry (MS<sup>2</sup>)-analysis.

254

### 255 3.8. Liquid Chromatography and MS<sup>2</sup>-Analysis

256

257 MS<sup>2</sup> analysis was performed on a *QExactive* mass spectrometer coupled to a nano *EasyLC*  
258 *1000* HPLC (Thermo Fisher Scientific, Rheinach, Switzerland). Initial solvent composition  
259 was 0.1% formic acid for channel A and 0.1% formic acid, 99.9% acetonitrile for channel B,  
260 respectively. For each sample 4  $\mu$ L of peptides were loaded on a commercial *Acclaim*  
261 *PepMap* Trap Column (75  $\mu$ m  $\times$  20 mm; Thermo Fisher Scientific, Rheinach, Switzerland)  
262 followed by a *PepMap* RSLC C18 Snail Column (75  $\mu$ m  $\times$  500 mm; Thermo Fisher Scientific,  
263 Rheinach, Switzerland). The peptides were eluted at a flow rate of 300 nl min<sup>-1</sup> by a gradient  
264 from 5 to 22% B in 79 min, 32% B in 11 min and 95% B in 10 min. Samples were acquired in  
265 a randomized order. The mass spectrometer was operated in data-dependent mode (DDA),  
266 acquiring a full-scan MS spectra (300–1'700  $m/z$ ) at a resolution of 70'000 at 200  $m/z$  after  
267 accumulation to a target value of 3'000'000, followed by HCD (higher-energy collision  
268 dissociation) fragmentation on the twelve most intense signals per cycle. HCD spectra were  
269 acquired at a resolution of 35'000, using a normalized collision energy of 25 a. u. and a  
270 maximum injection time of 120 ms. The automatic gain control (AGC) was set to 50'000  
271 ions. Charge state screening was enabled and singly and unassigned charge states were  
272 rejected. Only precursors with intensity above 8'300 were selected for MS<sup>2</sup> (2% underfill  
273 ratio). Precursor masses previously selected for MS<sup>2</sup> measurement were excluded from further  
274 selection for 30 s, and the exclusion window was set at 10 ppm. The samples were acquired  
275 using internal lock mass calibration on  $m/z$  371.101 and 445.120.

276

### 277 3.9. Protein Identification and Label Free Protein Quantification

278

279 The acquired raw MS<sup>2</sup> data were processed by MaxQuant v.1.4.1.2, followed by protein  
280 identification using the integrated Andromeda search engine. Each file was kept separate in  
281 the experimental design to obtain individual quantitative values. Spectra were searched  
282 against a forward str. *Cad16*<sup>T</sup> database (6'237 coding genes), concatenated to a reversed

283 decoyed *fasta* database and common protein contaminants (NCBI Assembly No.  
284 ASM281377v1; release date: 2017/12/07). Carbamidomethylation of cysteine was set as fixed  
285 modification, while methionine oxidation and N-terminal protein acetylation were set as  
286 variable. Enzyme specificity was set to trypsin/P allowing a minimal peptide length of 7  
287 amino acids and a maximum of two missed-cleavages. Precursor and fragment tolerance was  
288 set to 10 ppm and 0.05 Da for the initial search, respectively. The maximum false discovery  
289 rate (FDR) was set to 0.01 for peptides and 0.05 for proteins. Label free quantification was  
290 enabled and a 2-min window for match between runs was applied. The re-quantify option was  
291 selected. For protein abundance, the intensity (Intensity) as expressed in the protein groups  
292 file was used, corresponding to the sum of the precursor intensities of all identified peptides  
293 for the respective protein group. Only quantifiable proteins (defined as protein groups  
294 showing two or more razor peptides) were considered for subsequent analyses. Protein  
295 expression data were transformed (hyperbolic arcsine transformation) and missing values  
296 (zeros) were imputed using the *missForest* R-package v.1.4 [46] The protein intensities were  
297 normalized by scaling the median protein intensity in each sample to the same values.

298

299 Scaffold v.4.8.4 (Proteome Software Inc., Portland, OR) was used to validate MS<sup>2</sup> based  
300 peptide and protein identifications. Peptide identifications were accepted if they could be  
301 established at greater than 42.0% probability to achieve an FDR less than 0.1% by the Peptide  
302 Prophet algorithm with *Scaffold* [47] delta-mass correction. Protein identifications were  
303 accepted if they could be established at greater than 54.0% probability to achieve an FDR less  
304 than 1.0% and contained at least two identified peptides. Protein probabilities were assigned  
305 by the *Prophet* algorithm [48]. Proteins that contained similar peptides and could not be  
306 differentiated based on MS<sup>2</sup> analysis alone were grouped to satisfy the principles of  
307 parsimony. Proteins sharing significant peptide evidence were grouped into clusters. For the  
308 two-group analysis the statistical testing was performed using (paired) *t*-test on transformed  
309 protein intensities (hyperbolic arcsine transformation). Proteins were called significantly  
310 differentially expressed if linear fold-change exceeded 2-fold and the *q*-value from the *t*-test  
311 was below 0.01.

312 As an alternative method to find differentially expressed proteins, we used the correlation  
313 adjusted *t*-Score algorithm provided by the R-package *sda* v.1.3.7 [49] to further analyze the  
314 dataset of 1'333 proteins identified with MaxQuant.

315

### 316 3.10. Protein Functional Annotation

317

318 BlastKOALA v.2.1 [50] and eggNOG v.4.5.1 [51] were used to classify the proteins into  
319 functional categories. The complete KEGG-dataset for str. Cad16<sup>T</sup> can be found under Ref.  
320 [52].

321

### 322 3.11. Genomic Data Availability

323

324 The complete genome of str. Cad16<sup>T</sup> [52] is available under the GenBank assembly  
325 GCA\_002813775.1.

326

### 327 3.12. Proteomic Data Availability

328

329 The complete proteomic data of str. Cad16<sup>T</sup> have been deposited to the ProteomeXchange  
330 Consortium [53] via the PRIDE partner repository [54] under the accession PXD010641 and  
331 project DOI 10.6019/PXD010641

332

## 333 4. RESULTS

334

### 335 4.1. Physicochemical Parameters from July to August 2017

336

337 Fluctuations in light intensity were positively correlated with the predicted surface radiation  
338 and negatively associated with cloud cover, respectively (**suppl. Figure S3 a**). Daily relative  
339 average surface light intensity at 12:00 pm was  $1'448.9 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  (4.3–3'769.6)  
340 (**suppl. Figure S3 b**). The surface temperature was stable at an average of 15 °C. High temperatures  
341 up to 40 °C were measured, due the solar heating of the logger casing (**suppl. Figure S3 b**). The  
342 accumulated sunlight were above average for the weeks observed from (**suppl. Figure S4**). From  
343 13 July to 23 August 2017 at 12 m depth, an average of  $3.7 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  (0.2–26.2)

344 was measured. At 12.4 m depth, a mean of  $1.3 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  (0.04–7.8) was registered  
345 (suppl. Figure S3 c and d). Within this period, changes of turbidity, oxygen and Chl-*a* profiles in the  
346 daily CTD profiles indicated that the chemocline had been sinking from 12 to 13–14.5 m  
347 depth (suppl. Figure S5). To ensure chemocline conditions for the incubations, we adjusted the  
348 depth of the rig from 12 to 14 m depth. This resulted in an unexpected reduced relative light  
349 intensity at the position of the rig at 14 m for the days 23 August to 13 September 2017. Only  
350 an average of  $0.4 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  (0.2–4.0) was measured at 14 m depth from 24 August  
351 to 13 September 2017, and no light was measured at 14.4 m depth after 23 August 2017.  
352 Temperature was stable around  $5 \text{ }^\circ\text{C}$  (4.6–6.1) at both incubation depths with a positive trend  
353 over the months (suppl. Figure S3 c and d).

354

## 355 4.2. Chemical and Physical Analysis of Lake Cadagno at Sampling Date

356

357 Physicochemical measurements and endpoint-sampling was done on 12 and 13 September  
358 2017. The weather was cloudless with weak wind. Two CTD profiles at 1:30 pm and 9:00 pm  
359 showed a comparable situation for temperature, dissolved oxygen and conductivity (Figure 1).  
360 Water temperature was stable at  $12 \text{ }^\circ\text{C}$  from the surface down to the thermocline at 9.5 m,  
361 whereas it dropped to  $5 \text{ }^\circ\text{C}$  at 16 m depth at both time points. Dissolved oxygen (DO) was  
362 measured at  $8.5 \text{ mg l}^{-1}$  ( $265.6 \mu\text{M}$ ) throughout the mixolimnion. From 7 m to 9.5 m depth,  
363 DO-concentrations steadily declined to  $6 \text{ mg l}^{-1}$ , and then more rapidly to  $2 \text{ mg l}^{-1}$  at 10.5 m  
364 depth, at both time points measured. At 14 m depth,  $0.4 \text{ mg l}^{-1}$  ( $12.5 \mu\text{M}$ ) and  $0.3 \text{ mg l}^{-1}$  ( $9.4$   
365  $\mu\text{M}$ ) were measured during day and night, respectively. Conductivity increased along the  
366 profile from 0.13 in the mixolimnion to  $0.22 \text{ mS cm}^{-1}$  hypolimnion, both at day and night. In  
367 contrast, a pronounced turbidity peak (30 FTU) was observed at a depth of 13 m at 1:30 pm  
368 whereas a broader distribution of the FTU values (6–16 FTU) from 13 to 14 m depth was  
369 observed at 9:00 pm. Water samples taken at 1:30 pm from 14 m depth showed a milky and  
370 pink coloration, characteristic for a concentrated PSB community. The total inorganic  
371 dissolved carbon concentrations measured at 14 m depth were  $1.26 \text{ mM}$  at 2:00 pm and  $1.46$   
372  $\text{mM}$  9:00 pm.

373

374

### 375 4.3. Microbial Counts and Evaluation

376

377 Initial str. Cad16<sup>T</sup> cell concentrations were on average  $3.1 \times 10^6$  cells ml<sup>-1</sup> in July, as measured  
378 by flow cytometry (FCM). The rigged cultures were checked on 23 August 2017 and all  
379 dialysis bags were found intact and cells were judged healthy due to the turbid-pinkish  
380 appearance. When retrieved for sampling on 12 September 2017, all dialysis bags were still  
381 intact, the population was uniformly distributed within the dialysis bags and the cells grew to  
382 a mean concentration of  $9.3 \times 10^6$  cells ml<sup>-1</sup>. No significant difference in cell concentration  
383 and internal complexity was measured between the two sampling groups ( $P = 0.74$ ). In total,  
384 the str. Cad16<sup>T</sup> cultures grew 3-fold from July to September.

385 The average cell concentration in the lake sample taken at 14 m was  $4.23 \times 10^6$  cells ml<sup>-1</sup> at  
386 1:30 pm, whereas it was  $1.69 \times 10^5$  cells ml<sup>-1</sup> at 9:00 pm. However, the later value has to be  
387 questioned as the FCM count was below the values obtained for the 0.45  $\mu$ m filtered lake  
388 water of the <sup>14</sup>C background control despite that the measured turbidity values were similar  
389 (4–5 FTU) at the two time points at 14 m sampling depth (**Figure 1**). FCM revealed that the  
390 phototrophic microbial community at 1:30 pm ( $4.23 \times 10^6$  cells ml<sup>-1</sup>) consists mainly of *C.*  
391 *okenii*, *C. clathratiforme* and cyanobacteria spp. with  $1.48 \times 10^6$  cells ml<sup>-1</sup>,  $7.45 \times 10^5$  cells ml<sup>-1</sup>  
392 and  $1.49 \times 10^6$  cells ml<sup>-1</sup>, representing 35, 35 and 17% of the total phototrophic population,  
393 respectively.

394

395

### 396 4.4. *In situ* Carbon Fixation Rates

397

398 Absolute carbon fixation rates at the chemocline were comparable between day and night  
399 when tested, with medians of 757 nM h<sup>-1</sup> and 587 nM h<sup>-1</sup>, respectively (**Figure 2**). Cad16<sup>T</sup> fixed  
400 carbon in both conditions of light or dark (**Figure 3**). For str. Cad16<sup>T</sup> <sup>14</sup>C-bicarbonate median  
401 uptake rates normalized per cell were significantly different between the conditions with  
402  $1'074$  amol C cell<sup>-1</sup> h<sup>-1</sup> (range: 937–1'585) during the day, and 834 amol C cell<sup>-1</sup> h<sup>-1</sup> (range;  
403 650–969) during the night (**Figure 3**). When the uptake rates were normalized to average  
404 biovolume ( $3.6 \mu\text{m}^3$  cell<sup>-1</sup>), 316 amol C  $\mu\text{m}^{-3}$  h<sup>-1</sup> in light and 230 amol C  $\mu\text{m}^{-3}$  h<sup>-1</sup> in the dark  
405 were obtained, and a carbon-based doubling time range of 5 to 23 h was calculated for str.  
406 Cad16<sup>T</sup>. For the dominant phototrophic populations of the chemocline we estimated uptake

407 rates in amol C cell<sup>-1</sup> h<sup>-1</sup> only at day because of uncertain FCM values at night. Thereby PSB  
408 *C. okenii*, GSB *Chlorobium* spp. and cyanobacteria assimilated in average 173.35, 73.70 and  
409 245.68 amol cell<sup>-1</sup> h<sup>-1</sup>, respectively.

410

#### 411 4.5.Total Proteins identified with LC-MS<sup>2</sup>-LFQ

412

413 A total of 11 samples, five samples for category ‘light’ and six samples for category ‘dark’,  
414 were processed and protein quantification was performed using the MaxQuant package. We  
415 used corrected *t*-test based statistics in order to identify and quantify proteins. The samples  
416 Cad16T\_dia\_7 (‘light’) and Cad16T\_dia\_12 (‘dark’) were identified as outliers in cluster  
417 analysis and were excluded from further data analysis. Therefore, the data analysis was made  
418 with four samples of the category ‘light’ and five samples of the category ‘dark’. Overall a  
419 total of 1’333 proteins (21% of the total coding CDS) with at least two peptides were  
420 identified.

421

#### 422 4.6. Proteins Quantified with LC-MS<sup>2</sup>-LFQ

423

424 Peptide identifications were accepted if they could be established at >42.0% probability to  
425 achieve an FDR less than 0.1% with Scaffold delta-mass correction, resulting in 12’576  
426 spectra included. Protein identifications were accepted if they could be established at >54.0%  
427 probability to achieve an FDR less than 1.0% and contained at least two identified peptides.  
428 The number of quantified proteins per condition was similar, with an average of 374 for “day”  
429 and 354 for “night”, respectively. Between 102 and 663 proteins were quantified in each  
430 biological replicate (7.2–37% of all IDs). Consequently, 684 proteins were quantified over all  
431 samples. Thereof, 21 contaminants were excluded. The remaining 663 CDS were classified  
432 with blastKOALA and EggNOG, with 627 annotated CDS for (99%) COG and 460 annotated  
433 CDS (69.4%) for blastKOALA, respectively.

434

435 As expected, many of the proteins with unchanged abundance belonged to the functional  
436 categories energy conversion, genetic information processing, carbohydrate and amino  
437 metabolism and protein modification (Table 1). Among the most abundant proteins detected  
438 were F<sub>0</sub>F<sub>1</sub> ATPase subunits (AUB84561.1, AUB81565.1, AUB81565.1 and AUB84563.1)

439 and chaperons (GroEL; AUB81575.1, AUB80010.1, AUB84066.1, DnaK; AUB84026.1).  
440 Since cell growth depends on protein synthesis, we found 36 ribosomal subunits as well as  
441 elongation factor Tu (AUB80476.1) to be equally abundant.

442 The cells always contained the established components of the dissimilatory sulfate reduction  
443 pathway such as ATP-sulfurylase Sat (AUB82369.1), the adenylylsulfate reductase AprAB  
444 (AUB82371.1, AUB82370.1) and the sulfite reductase Dsr complex (AUB83448.1–  
445 AUB83455.1). A Sqr sulfide:quinone reductase and a glutathione amide reductase GarA  
446 homolog to *A. vinosum* putatively involved in intracellular sulfur shuttling [55] were also  
447 present. In PSB, sulfur oxidation provides the electrons for cyclic electron transport driven by  
448 light. Consequently, in str. Cad16<sup>T</sup> PufMCL (AUB85378.1–AUB85380.1) and PuhA  
449 (AUB85431.1) subunits forming the RC II and six different PufAB antenna proteins  
450 (AUB85355.1–AUB85357.1, AUB85363.1, AUB85361.1, AUB85710.1) were expressed.

451 Additionally we found enzymes for BChl synthesis, terpenoid backbone biosynthesis and  
452 carotenoid synthesis. Noteworthy, elements of the reduction pathways driven by  
453 photosynthesis are shared with oxidative phosphorylation in PSB. We found in total 23  
454 protein subunits involved in substrate respiration including the NADH dehydrogenase  
455 subunits NuoCDEFG an HoxFU, the cytochrome reductase CytB and Cyt1, seven F-type  
456 ATPase subunits and two *cbb3* cytochrome *c* oxidase subunits. PSB use the ATP and  
457 NAD(P)H derived from photosynthesis to fix CO<sub>2</sub> through the CBB cycle. For str. Cad16<sup>T</sup> a  
458 complete CBB cycle with the key enzymes CbbM/RbcL RuBisCO form II (AUB81831.1) and  
459 phosphoribulokinase PrkB (AUB79979.1) were present. The fixed carbon enters the central  
460 carbon metabolism as 3-phospho-D-glycerate. In both growth conditions, str. Cad16<sup>T</sup> contains  
461 enzymes for glycolysis and gluconeogenesis, as well as pyruvate oxidation, the glyoxylate  
462 cycle and the citrate cycle (TCA cycle) in unvaried abundance. Additionally, the presence of  
463 malic enzyme (MaeB; AUB82893.1) may allow the entry of malate into the central carbon  
464 pathway via pyruvate, is shown for *A. vinosum*. In both conditions examined, the PHB  
465 synthase subunits PhaC and PhaE are expressed (AUB80707.1 and AUB84676.1). Also  
466 enzymes necessary for amino acid biosynthesis and Co-factor and vitamin synthesis were  
467 expressed under both groups analyzed.

468 In a second step, we analyzed the proteome data for significant changes between growth  
469 conditions. However, we found a large within-group variation and no proteins were  
470 significantly differently expressed between the two growth conditions using (paired) corrected  
471 *t*-test on transformed protein intensities (q.mod <0.01).

472

## 473 4.7. Proteins Differentially Expressed

474

475 The expression dataset was alternatively analyzed using correlation-adjusted *t*-scores (CAT  
476 scores) in order to additionally address the correlative structure of the dataset as only 4.5% of  
477 the proteins are differentially expressed. Thereby, 60 proteins were found differentially  
478 expressed (1% of all coding CDS) at a local false discovery rate of  $l\text{fdr} < 0.05$ . (Table 3)

479 During the ‘day’ period 21 CDS were found relatively more abundant for str. Cad16<sup>T</sup>.  
480 Thereof, all CDS were annotated with eggNOG (Table 2). Growing in the light, str. Cad16<sup>T</sup>  
481 over-expressed four proteins involved in oxidative phosphorylation that were subunits of  
482 NADH:quinone oxidoreductase, cytochrome *bc1* complex respiratory unit and AtpC subunit  
483 of F-type ATPase. Two enzyme involved in the central carbon pathway were abundant, the  
484 glycogen synthase GlgA and the glucose-6-phosphate isomerase GPI involved in glycolysis.

485 The enzyme acetolactate synthase I/III small subunit associated with thiamine synthesis and  
486 the A 7,8-dihydroneopterin aldolase FolB involved in tetrahydrofolate biosynthesis were  
487 additionally found. Also membrane transport systems were found, including the ABC-2 type  
488 transport system and Twin-arginine translocation (Tat). Additionally, chaperone-type proteins  
489 DnaJ and proteolytic ClpX were also over-expressed.

490 Under the condition ‘night’ 39 proteins were shown to be more abundant and thereof 35  
491 entries (89%) were COG annotated (Table 3). Analysis of this data suggested that the NAD(P)H  
492 isocitrate dehydrogenase Idh1 responsible first carbon oxidation from oxaloacetate to 2-  
493 oxoglutarate in the TCA cycle as well as a FabH 3-oxoacyl-[acyl-carrier-protein] synthase III  
494 involved in fatty acid biosynthesis initiation and elongation were abundant. The  
495 adenylylsulfate reductase subunit alpha was found, and AprA is responsible for sulfite  
496 oxidation to 5'-adenylyl sulfate [56]. Cad16<sup>T</sup> further expressed proteins associated to cell  
497 division (FtsZ), cell wall formation CpxP, Lysine and branched amino acid synthesis and  
498 nucleotide metabolism (Ppx-GppA; exopolyphosphatase and RutE; 3-hydroxypropanoate  
499 dehydrogenase). Elements of two secretion pathways were identified, a putative polar amino  
500 acid transport system and type II general secretion system. Additional three proteins detected  
501 to be more abundant in the dark are involved in stress response. The BolA-family  
502 transcriptional regulators, is a general stress-responsive regulator. Rubrerythrin may provide  
503 oxidative stress protection via catalytic reduction of intracellular hydrogen peroxide and a  
504 ATP-dependent serine protease mediates the degradation of proteins and transitory regulatory  
505 proteins, and thereby ensures cell homeostasis



## 506 **5. DISCUSSION**

507

508 We compared light to dark carbon fixation metabolism of PSB str. Cad16<sup>T</sup> through a  
509 combination longitudinal monitoring of physicochemical condition of the Lake  
510 Cadagno chemocline, scintillation and quantitative proteomics of cultures incubated *in*  
511 *situ*.

512

### 513 **5.1. Monitoring Data**

514

515 During the incubation experiment from July to September 2017 the chemocline was  
516 actively monitored with CTD and light and temperature profiles were measured  
517 passively. This detailed record allowed us to understand the prevailing physicochemical  
518 conditions experienced by the str. Cad16<sup>T</sup> cultures. Thereby, the average light  
519 availability measured was 10× lower than previously recorded at the chemocline,  
520 whereas the available DIC concentration was comparable to 2013 [31]. CTD  
521 measurements (this study) and FCM counts of the chemocline throughout the summer  
522 2017 [57] revealed dense PSB and GSB populations (10<sup>6</sup> cells ml<sup>-1</sup>) and an additional  
523 cyanobacterial bloom down to the monimolimnion from July 2017 on [40] that probably  
524 resulted in increased self-shading below a depth of 13 to 14 m [57]. As a consequence,  
525 the maximal peak of turbidity related to the PSOB sunk from around 12 to around 14 m  
526 depth. For this reason, we decided to hang the dialysis bags lower from 12 to 14 m at  
527 the 23 August 2017. The resulting low light conditions at 14 m depth possibly reduced  
528 net photosynthesis and subsequent carbon storage capacity and growth for str. Cad16<sup>T</sup>  
529 as previously observed for PSB *Chromatium* spp. in the Lakes Cisó and Vilar [58, 59].

530 Whereas the monimolimnion has been typically described as anoxic [28], recurrent  
531 blooms of oxygenic plankton [30, 57] and optode-based measurements of dissolved  
532 oxygen has revealed micro-oxic conditions below 20 nM at the chemocline [60]. Our  
533 study helps to understand the consequences of these conditions for the PSOB.

534

## 535 5.2. Carbon Uptake Rates

536

537 Measured chemocline CO<sub>2</sub>-fixation rates at both conditions were within the ranges  
538 previously obtained for Lake Cadagno that were between 85 to 8'000 nM h<sup>-1</sup> with light,  
539 and between 27 to 7'608 nM h<sup>-1</sup> in dark conditions, respectively (**suppl.** Fehler! Verweisquelle  
540 konnte nicht gefunden werden.) [30, 33, 61, 31]. Comparable CO<sub>2</sub>-assimilation rates have been  
541 also measured in other stratified sulfureta as in Spanish karstic lakes with up to 3'000  
542 nM h<sup>-1</sup> both in light or dark conditions [62]. Importantly, it was estimated that only half  
543 of the bulk carbon is fixed by anoxygenic photosynthesis in the Lake Cadagno  
544 chemocline [30]. Thereof, PSB *C. okenii* is responsible for 70% of the total anoxygenic  
545 phototrophic CO<sub>2</sub> assimilation [33] and the concentration was 10<sup>6</sup> cells ml<sup>-1</sup>—equal to  
546 35% of the total phototrophic microbes— at 14 m depth in this study. Taken together, *C*  
547 *okenii* may have accounted for an average 173.35 amol C cell<sup>-1</sup> h<sup>-1</sup> in light conditions in  
548 our study, which is 60-fold lower than previously observed [33]. The average GSB  
549 assimilation rate with 73.7 amol C cell<sup>-1</sup> h<sup>-1</sup> was higher than observed (1–30), but within  
550 the same magnitude [33]. Oxygenic photosynthesis contributed 245.68 amol C cell<sup>-1</sup> h<sup>-1</sup>  
551 to the C-fixation that is 10–100 less than observed in the Baltic sea [63].

552

553 For str. Cad16<sup>T</sup>, the median uptake rate of 1'073.9 amol C cell<sup>-1</sup> h<sup>-1</sup> during the day was  
554 within the range measured for other PSB (100–30'000 amol C cell<sup>-1</sup> h<sup>-1</sup>) [33, 31],  
555 however 10× lower compared to a previous *in situ* <sup>14</sup>C-assimilation study with strain  
556 Cad16<sup>T</sup> (around 12'000 amol C cell<sup>-1</sup> h<sup>-1</sup>) [31]. In the former experiment, autotrophic  
557 CO<sub>2</sub> assimilation rates of str. Cad16<sup>T</sup> were additionally measured *in vitro*, with values  
558 between 8'541.7–18'541.7 amol C cell<sup>-1</sup> h<sup>-1</sup> with light, and around 2'916.7 amol C cell<sup>-1</sup>  
559 h<sup>-1</sup> in the dark, respectively. For C-fixation at night, highly variable C-assimilation rates  
560 in chemocline bulk samples were measured, ranging from 7–45% of the rates measured  
561 with light [24]. Schanz and colleagues thereby found a positive correlation between  
562 photosynthetically driven increase in overall biomass and dark fixation rates [24]. In our  
563 study, the median dark fixation rate of 834.4 amol C cell<sup>-1</sup> h<sup>-1</sup> was significantly lower  
564 than during the day, and again around 10× lower than measured by Storelli and  
565 colleagues [31]. This overall discrepancy may be explained by the 10-fold lower light  
566 availability that possibly reduced photosynthetic carbon assimilation and carbon storage  
567 (see above). Furthermore the sampling times in our study were adapted to the natural

568 light-dark hours. This was in order to account for possible circadian effects, whereas in  
569 the former study incubations were performed in parallel at daytime, using clear and  
570 opaque sample bottles.

571 The differences between the study results may further be explained with the varied cell  
572 counting methodologies. Fluorescent *in situ*-hybridization (FISH) was used to count  
573 cells in the study of Storelli *et al.* [31]. Furthermore, we did not control for dead cells in  
574 FCM which might have led to a relative overestimation of the str. Cad16<sup>T</sup> cell  
575 concentration. Moreover, str. Cad16<sup>T</sup> forms aggregates, and some stuck to the dialysis  
576 bags visible as a pinkish residue, possibly reducing the number of planktonic cells for  
577 FCM counting.

578

579 When normalized to biomass, the carbon uptake values at light were higher as  
580 previously measured for PSB *C. okenii* and *Lamprocystis* sp. [33]. Uptake rates  
581 normalized to biovolume depend on the cell volume estimation (i.e. volume depends on  
582  $r^3$ ) and the conversion factors chosen. We used values previously used in literature (see  
583 methods). For str. Cad16<sup>T</sup>, the estimated carbon-based doubling time was between 4 and  
584 23 h, which is in contrast to previous estimates of 121 h *in vitro* [34], and a median of  
585 333.6 h (25<sup>th</sup> percentile; 196.8 h, 75<sup>th</sup> percentile; 652.8 h) for the bulk biomass in the  
586 chemocline [24]. In contrast, the average doubling time obtained by FCM counting was  
587 948.0 h (39.5 d) which is 40× longer than our <sup>14</sup>C-uptake based calculations, and 8×  
588 longer than *in vitro* [34]. We did not control for the presence and metabolic activity of  
589 the syntrophic *Desulfocapsa* sp. nov. str. Cad626 [69] as it was observed that the str.  
590 Cad16<sup>T</sup> *in vitro* cultures we used lost the initially co-occurring str. Cad626 after several  
591 years under laboratory growth conditions.

592 The significantly higher inorganic C-uptake rate during the day compared to night-time  
593 rates suggests active photosynthesis of str. Cad16<sup>T</sup> at low light intensities, as it was  
594 described for other PSB [24, 64]. Noteworthy, the presence of up to  $3 \times 10^5$  ml<sup>-1</sup>  
595 oxygenic phototrophic microbes down to 16 m depth [40] resulted in a partly  
596 oxygenated chemocline with around 0.6 mg O<sub>2</sub> l<sup>-1</sup> (19 μM), as measured with CTD.  
597 Consequently, the chemocline waters retained some of the produced oxygen through the  
598 night (**Figure 1**). Taken together, str. Cad16<sup>T</sup> may have used the O<sub>2</sub> present as electron  
599 acceptor during mixotrophic growth under both conditions. As a consequence, some of  
600 the CO<sub>2</sub> assimilated might have been constantly respired with thiosulfate as electron  
601 donor as found for *T. roseopersicina* str. M1 [15]. In accordance, str. Cad16<sup>T</sup> grew in

602 the dark with thiosulfate and 5% O<sub>2</sub> *in vitro* [41]. Interestingly however, we found only  
603 SoxXY and SoxB and no TsdA thiosulfate-oxidizing enzyme homologues encoded in  
604 the str. Cad16<sup>T</sup> genome [52]. As the complete Sox-complex is essential for the complete  
605 thiosulfate oxidation to sulfate in *A. vinosum* DSM 180<sup>T</sup> [65], str. Cad16<sup>T</sup> possibly uses  
606 an alternative mechanism. One alternative may be thiosulfate uptake through CysTWA  
607 (AUB80378.1, AUB80379.1 and AUB80380.1) and CysP (AUB80377.1) and oxidation  
608 to sulfite via the intermediate S-sulfocysteine by cysteine synthase B CysM  
609 (AUB82938.1) and possibly monothiol glutaredoxin of the Grx4 family (AUB83488.1)  
610 as suggested by Dahl [66]. However, with the applied methods we could not determine  
611 the relative contributions of photosynthetic or chemotrophic activity to the total increase  
612 in biomass. To conclude, <sup>14</sup>C-fixation rates and FCM counting indicated an actively  
613 growing str. Cad16<sup>T</sup> population during the incubation. The findings on carbon  
614 assimilation rates are further reflected in the proteome (discussed below).

615

### 616 5.3. Stable Fraction of the Strain Cad16<sup>T</sup> Proteome

617

618 In order to understand differences in metabolism, the str. Cad16<sup>T</sup> light/dark proteome  
619 has been studied extensively. Previously, Storelli and colleagues [36] performed a  
620 comparable proteomics study on light/dark metabolism, str. Cad16<sup>T</sup> *in vitro* with 2D-  
621 difference gel electrophoresis (2D-DiGE), where they identified around 1'400 proteins,  
622 quantified 56 and, thereof, found 37 differentially expressed proteins. With six  
623 biological replicates instead of three, and the increased resolution and high-throughput  
624 capacity of LC-MS<sup>2+</sup> compared to 2D-DiGE [67], we expected to substantially increase  
625 protein identification and quantitation in comparison. Overall, the number of identified  
626 proteins was comparable in both studies, with 1'400 in Ref [36] to 1'333 in our study.  
627 Noteworthy, LC-MS<sup>2</sup> analysis returns sequence information on all proteins identified,  
628 whereas 2D-DiGE is limited to a chosen subset, based on the relative intensity and size  
629 of the protein spots on the gel. Therefore, we could quantify as much as 663 proteins  
630 that are 12× more than in the former study. Nevertheless, the number of unique proteins  
631 with different abundance between the two growth conditions was comparable, with 62  
632 in our study versus 37 in the former, respectively. Interestingly, we retrieved only 28 of  
633 these proteins in the constantly expressed fraction of the proteome.

634 In addition to technical differences, the former study was performed *in vitro* under an  
635 anaerobic atmosphere, with higher light intensities ( $6 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and at  $20 \text{ }^\circ\text{C}$ , which  
636 may have an influence on metabolic rates and relative activity. In conclusion, the results  
637 of the present study can only be compared to some extent with previous findings.

638

639 The photosynthesis apparatus was abundant in both growth conditions, as several LHC  
640 proteins were detected. In accordance, also the enzymatic pathway for BChla and  
641 carotenoid synthesis was expressed. In *T. roseopersicina* BChla was absent at  
642 prolonged exposure to  $60 \mu\text{M O}_2$  in darkness [68]. In contrast, for str. Cad16<sup>T</sup> we did  
643 not observe a loss of pigmentation. This might suggest that BChla was not regulated on  
644 expression level and/or the micro-oxic conditions did not influence BChla biosynthesis.

645 The central role of dissimilatory sulfur oxidation during photosynthesis is well  
646 established for PSB [69] and proteins involved were found expressed in str. Cad16<sup>T</sup>.

647 The proteins Sqr, Dsr and Sat were present and SGBs were also observed  
648 microscopically in both conditions. The CBB cycle is central in purple bacteria, not  
649 only for autotrophic carbon fixation, but also to regenerate the pool of reduced co-  
650 factors NAD[P]H<sub>2</sub> [70]. Strain Cad16<sup>T</sup> contains two forms of RuBisCo, RbcAB form I  
651 and RbcSL form II. In a previous study Storelli and colleagues [31] detected  
652 constitutive transcription of the *rbcL* gene under autotrophic condition *in vitro* under a  
653 12/12 h dark/light regime, whereas the form I *rbcA* was induced by light. In contrast  
654 both *rbc* genes were transcribed equally under heterotrophic conditions with acetate,  
655 with and without light, respectively [31]. In our study we detected RbcL to be equally  
656 abundant in both conditions and no other RuBisCo subunits were found. Therefore, the  
657 sole presence of the dimeric form II RuBisCo may underline the importance of CO<sub>2</sub>  
658 fixation mediated by the CBB cycle in maintaining the redox-balance under chemo or  
659 mixotrophic growth at low light and dark conditions, as described for purple bacteria  
660 [71].

661

662 In str. Cad16<sup>T</sup>, the CsrA (AUB84364.1) seems to be the main carbon storage regulator  
663 where it was detected under both conditions. Glycolysis under mixotrophic conditions  
664 might thereby be regulated through mRNA transcription and stability as in *A. vinosum*  
665 [56]. We additionally found enzymes involved in the central carbon pathways TCA,  
666 EMP and glyoxylate cycle in unvaried abundance. Noteworthy, isocitrate lyase was  
667 found expressed, that is involved in the glyoxylate cycle, that prevents loss of CO<sub>2</sub> and

668 ensures production of NAD[P]H<sub>2</sub> otherwise occurring through the isocitrate  
669 dehydrogenase and 2-oxoglutarate dehydrogenase in the TCA [72]. Further, the malic  
670 enzyme was abundant, that generates oxaloacetate via malate through anaplerotic  
671 reactions without ATP [73]. The *ccb3* cytochrome *c* oxidase found in both conditions is  
672 used in aerobic respiration and additionally used for FeS oxidation and it was speculated  
673 that str. Cad16<sup>T</sup> is also involved in both aerobic [38] and anaerobic cryptic iron cycling,  
674 as found for *Thiodictyon* str. F4 [74]. Taken together, the metabolic fate of the carbon  
675 fixed is only partly elucidated by proteome analysis and metabolic carbon pathways  
676 may be active under the conditions examined.

677

#### 678 5.4. Differentially Expressed Proteome

679

680 In light conditions, members of the oxidative respiration pathway were upregulated,  
681 indicating an active substrate respiration with light as in *T. roseopersicina* [20].  
682 However, the cytochrome *bc* and the NADH-dehydrogenase complex and redox carrier  
683 molecules are also used in cyclic electron transport during photosynthesis in PSB.  
684 During light conditions, both chemotrophic and phototrophic metabolism compete for  
685 electrons in str. Cad16<sup>T</sup>. We also found evidence for glycolysis / gluconeogenesis since  
686 GPI was overexpressed. Interestingly, the glycogen synthase GlgA was additionally  
687 found abundant in the light. Glycogen synthesis and sulfur oxidation was found to be  
688 ineffectively regulated in *A. vinosum* [75] and we might speculate that in str. Cad16<sup>T</sup>  
689 both processes are highly active even at slow growth, allowing for both, intracellular  
690 sulfur and glycogen accumulation. Altogether, these results indicate an active  
691 phototrophic and chemotrophic metabolism competing for electrons in the light.

692

693 In the dark, the micro-oxic condition may have led to the production of reactive oxygen  
694 species in str. Cad16<sup>T</sup> that would explain the upregulated proteins involved in stress  
695 induced damage-control.

696 Interestingly, AprA was more abundant in the night period, indicating a relative higher  
697 activity of sulfite oxidation possibly coupled to chemotrophy. SGBs are consumed  
698 under dark autotrophic conditions in PSB *C. okenii* and *C. minus* observed *in vitro* [12],  
699 however the side scatter values measured did not vary between the two sample groups  
700 in this study when monitored with FCM. This may be due to glycogen and PHB storage

701 inclusions, structures that add up additional structural complexity and de-  
702 polymerization kinetics may be different to those of SGB. Supporting evidence is given  
703 by the unchanged presence of enzymes involved in of glycogen and PSB synthesis.

704

705 In summary, the 60 proteins found differentially expressed represent only about 1% of  
706 all protein coding CDS and about 5% of the identified proteins. Therefore, their impact  
707 on metabolic pathways is unclear and has to be further examined

## 708 **6. CONCLUSION AND OUTLOOK**

709

710 PSB str. Cad 16<sup>T</sup> is metabolically flexible and grows phototrophically as well as  
711 chemotrophically in the light as shown in this study. In dark conditions, low levels of  
712 oxygen may enable respiration of different small organic molecules. In order to  
713 understand the dark carbon metabolism, uptake experiments with labelled acetate and or  
714 pyruvate should be therefore included in the future. The long-term observation of the  
715 chemocline revealed the relative importance of oxygenic phototrophs on the  
716 oxygenation of the chemocline. However, it is not clear if O<sub>2</sub> or reduced sulfur is used  
717 as terminal electron acceptor in str. Cad16<sup>T</sup>. Furthermore, the oxidation of Fe(II) to fix  
718 CO<sub>2</sub> should be tested for str. Cad16<sup>T</sup> *in vitro*, both under microaerobic and anaerobic  
719 conditions as found in Lake Cadagno in 2017. To better estimate the relative metabolic  
720 contributions of the different phototrophic energy metabolism at microaerobic  
721 conditions oxygenic photosynthesis-inhibitors [76] should be included in future  
722 experiments as a control. The contribution of phytoplankton to dark carbon uptake [77]  
723 has also not yet been elucidated in Lake Cadagno and therefore a sequential size  
724 dependent fractionation of sub-samples would be interesting. In order to further  
725 understand transcriptional control over the light to dark metabolism also mRNA  
726 sequencing experiments would be needed. To complete the understanding metabolomic  
727 studies would give insight into the relative amount of metabolic intermediates produced  
728 under different regimes. We further observed a large variability in the C-uptake rates  
729 between different studies that cannot be readily explained by differences in the  
730 chemocline community composition and/or technical variances. Therefore, NanoSIMS  
731 experiments [78] on Cad16<sup>T</sup> may help to determine the within-population heterogeneity  
732 in inorganic C-assimilation under variable growth regimes.

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## 950 **8. AUTHOR CONTRIBUTIONS**

951 SML, NS, FD, JFP, AB and MT conceived the study. SML, NS, FD and SR installed the  
952 mooring and performed field measurement and sampling. NS and FD prepared scintillation  
953 samples. FD did flow cytometry cell enumeration. SML extracted total protein and performed  
954 scintillation measurements. SML, AB, MW and JFP and MT analyzed physicochemical,  
955 proteomic and scintillation data. SML, NS; FD and MT prepared the manuscript. All authors  
956 contributed to writing and agreed on the manuscript before review.

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984 **10. TABLES AND FIGURES**

985 **10.1. Tables**

986 **Table 1 | Functional categories of “*Thyodictyon syntrophicum*” str. Cad16<sup>T</sup> proteins quantified using EggNOG v.4.5.1 [50] and**  
 987 **blastKOALA v.2.1 [50] was used to classify proteins according to COG and KEGG classification, respectively.**

<b>COG Code</b>	<b>Description</b>	<b>No. Entries</b>
<a href="#">J</a>	Translation, ribosomal structure and biogenesis	64
<a href="#">K</a>	Transcription	15
<a href="#">L</a>	Replication, recombination and repair	7
<a href="#">D</a>	Cell cycle control, Cell division, chromosome partitioning	5
<a href="#">V</a>	Defense mechanisms	3
<a href="#">T</a>	Signal transduction mechanisms	19
<a href="#">M</a>	Cell wall/membrane biogenesis	28
<a href="#">N</a>	Cell motility	1
<a href="#">U</a>	Intracellular trafficking and secretion	10
<a href="#">O</a>	Posttranslational modification, protein turnover, chaperones	50
<a href="#">C</a>	Energy production and conversion	93
<a href="#">G</a>	Carbohydrate transport and metabolism	35
<a href="#">E</a>	Amino acid transport and metabolism	52
<a href="#">F</a>	Nucleotide transport and metabolism	12
<a href="#">H</a>	Coenzyme transport and metabolism	27
<a href="#">I</a>	Lipid transport and metabolism	18
<a href="#">P</a>	Inorganic ion transport and metabolism	37
<a href="#">Q</a>	Secondary metabolites biosynthesis, transport and catabolism	6
<b>KEGG Functional Category</b>		
	Genetic information processing	78
	Carbohydrate metabolism	78
	Energy metabolism	41
	Metabolism of cofactors and vitamins	29
	Amino acid metabolism	28
	Environmental information processing	27
	Nucleotide metabolism	17
	Metabolism of terpenoids and polyketides	11
	Lipid metabolism	9
	Metabolism of other amino acids	5

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**Table 2 | List of “*Thiodictyon syntrophicum*” str. Cad16<sup>T</sup> proteins identified as more abundant in the light period.** Lfdr: local false discovery rate, cat: *t*-score for each group and feature the cat score of the centroid versus the pooled mean [79].

Accession	Name	COG category	cat score	lfdr
AUB81546.1	cytochrome c1	C	5.44	9.53E-11
AUB83791.1	NADH-quinone oxidoreductase subunit I	C	4.35	8.32E-05
AUB84560.1	F0F1 ATP synthase subunit epsilon	C	3.51	2.26E-04
AUB83997.1	acetolactate synthase small subunit	E	2.88	1.23E-02
AUB81260.1	glucose-6-phosphate isomerase	G	3.63	2.26E-04
AUB83261.1	starch synthase	G	4.48	2.53E-06
AUB79823.1	thiazole synthase	H	2.74	2.93E-02
AUB81699.1	dihydroneopterin aldolase	H	3.41	1.29E-03
AUB83078.1	DNA topoisomerase I	L	2.98	3.81E-03
AUB84659.1	outer membrane lipoprotein carrier protein LolA	M	5.43	2.03E-10
AUB82245.1	HflK protein	O	6.34	1.24E-13
AUB82609.1	ATP-dependent protease ATP-binding subunit ClpX	O	3.38	1.29E-03
AUB84025.1	molecular chaperone DnaJ	O	6.17	1.24E-13
AUB79510.1	hypothetical protein	S	2.84	1.23E-02
AUB81403.1	hypothetical protein	S	3.53	2.26E-04
AUB82197.1	hypothetical protein	S	3.54	2.26E-04
AUB82687.1	hypothetical protein	S	3.96	1.21E-04
AUB83592.1	histidine kinase	S	3.51	1.29E-03
AUB84052.1	host attachment protein	S	3.51	2.26E-04
AUB84690.1	hypothetical protein	U	3.02	3.81E-03
AUB80231.1	multidrug ABC transporter ATP-binding protein	V	3.83	1.21E-04

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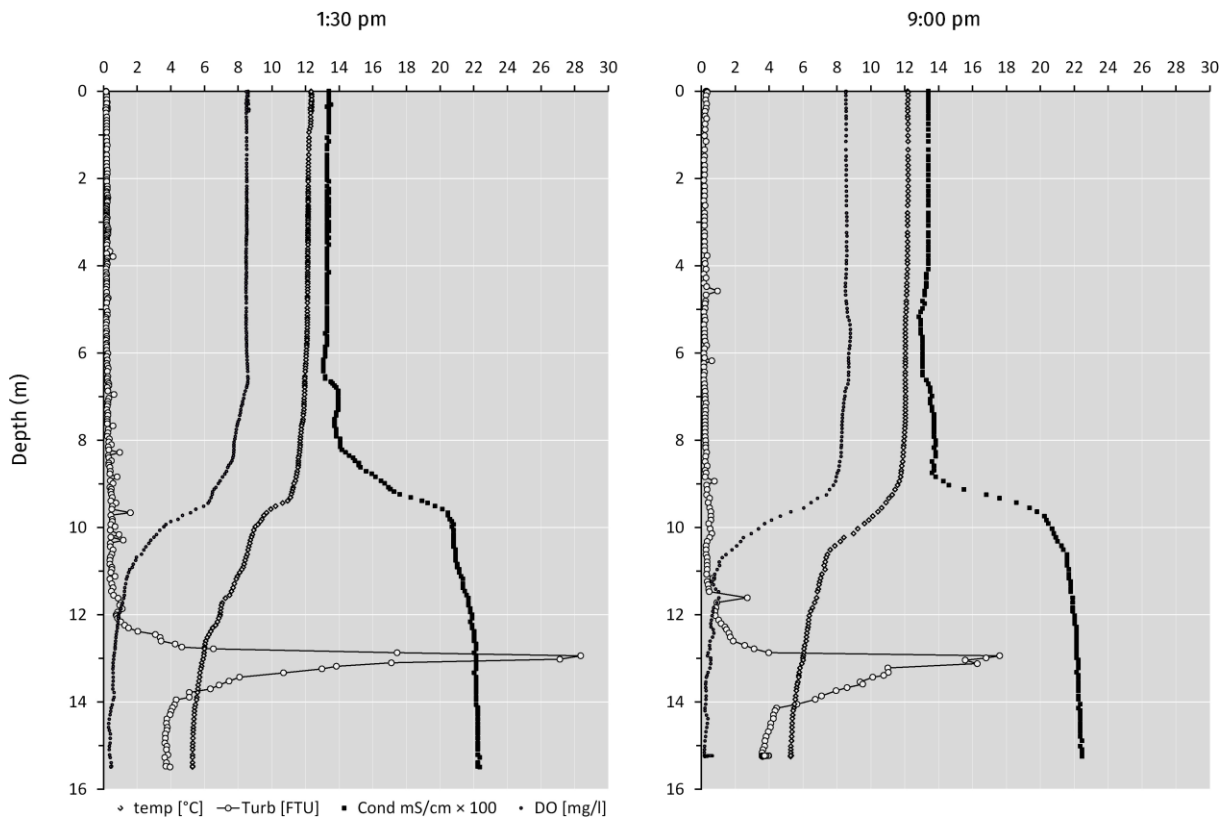
**Table 3 | List of “*Thyodictyon syntrophicum*” str. Cad16<sup>+</sup> proteins identified as more abundant in the dark period.** Lfdr: local false discovery rate, cat: *t*-score for each group and feature the cat score of the centroid versus the pooled mean [79].

Accession	Name	COG		
		category	cat score	lfdr
AUB80187.1	isocitrate dehydrogenase (NADP)	C	2.94	3.81E-03
AUB81294.1	oxidoreductase	C	3.54	2.26E-04
AUB82371.1	adenylylsulfate reductase alpha subunit	C	3.03	3.81E-03
AUB83156.1	NADH dehydrogenase NAD(P)H nitroreductase	C	3.03	3.81E-03
AUB84003.1	rubrerythrin	C	2.54	4.44E-02
AUB80125.1	cell division protein FtsZ	D	2.67	2.93E-02
AUB79575.1	extracellular solute-binding protein, family 3	E	2.62	4.44E-02
AUB81750.1	decarboxylase	E	2.63	2.93E-02
AUB83785.1	4-hydroxy-tetrahydrodipicolinate synthase	E	2.94	4.18E-03
AUB84954.1	acetolactate synthase	E	2.76	1.95E-02
AUB84325.1	proline iminopeptidase	E	2.91	4.18E-03
AUB82337.1	adenylosuccinate synthase	F	2.54	4.44E-02
AUB79762.1	ubiquinone biosynthesis regulatory protein kinase UbiB	H	2.86	1.23E-02
AUB80927.1	uBA THIF-type NAD FAD binding	H	2.64	2.93E-02
AUB83525.1	synthase	I	5.36	6.66E-10
AUB81024.1	lysyl-tRNA synthetase	J	3.31	1.29E-03
AUB79657.1	OmpA MotB	M	5.26	9.24E-09
AUB80121.1	cell wall formation (By similarity)	M	3.04	3.81E-03
AUB83127.1	choloylglycine hydrolase	M	2.98	3.81E-03
AUB83783.1	conserved repeat domain protein	M	2.58	4.44E-02
AUB83066.1	twitching motility protein	N, U	3.14	3.81E-03
AUB79946.1	heat-shock protein	O	2.65	2.93E-02
AUB82608.1	endopeptidase La	O	5.02	1.01E-07
AUB85620.1	DnaK-related protein	O	2.78	1.23E-02
AUB81090.1	protein of unknown function (DUF971)	S	3.55	2.26E-04
AUB82842.1	dinitrogenase iron-molybdenum cofactor biosynthesis protein	S	3.30	3.81E-03
AUB83253.1	nitrogen fixation protein	S	2.96	3.81E-03
AUB83860.1	tellurite resistance protein TehB	S	2.76	1.23E-02
AUB83943.1	short-chain dehydrogenase reductase SDR	S	2.91	4.18E-03
AUB84318.1	transposase	S	2.99	3.81E-03
AUB84331.1	dienelactone hydrolase	S	2.91	4.18E-03
AUB85354.1	protein of unknown function (DUF2868)	S	3.78	2.26E-04
AUB82296.1	Ppx GppA phosphatase	T	2.79	1.23E-02
AUB82910.1	YciI-like protein	T	2.63	2.93E-02
AUB79943.1	general secretion pathway protein G	U	2.96	3.81E-03
AUB80110.1	hypothetical protein	-	3.21	3.81E-03
AUB81753.1	ATPase	-	4.76	1.20E-06
AUB82624.1	hypothetical protein	-	2.81	1.23E-02
AUB82926.1	hypothetical protein	-	2.99	3.81E-03

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1003 10.2. Figures



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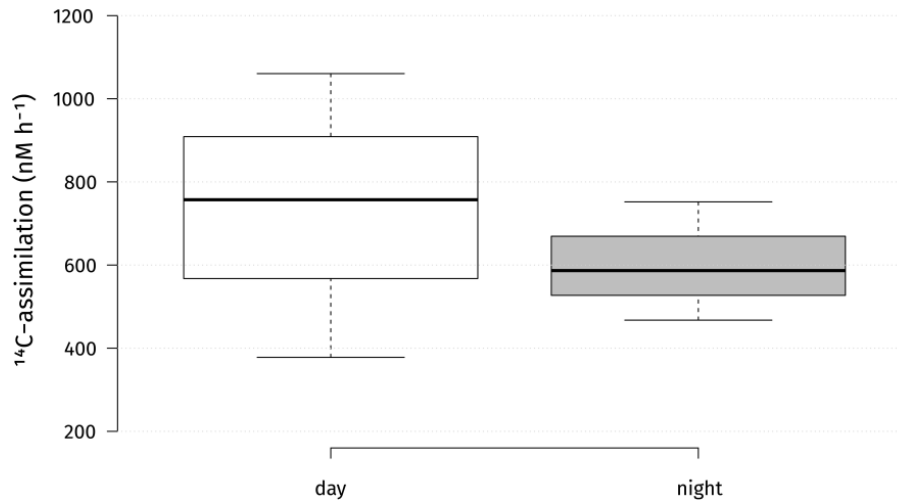
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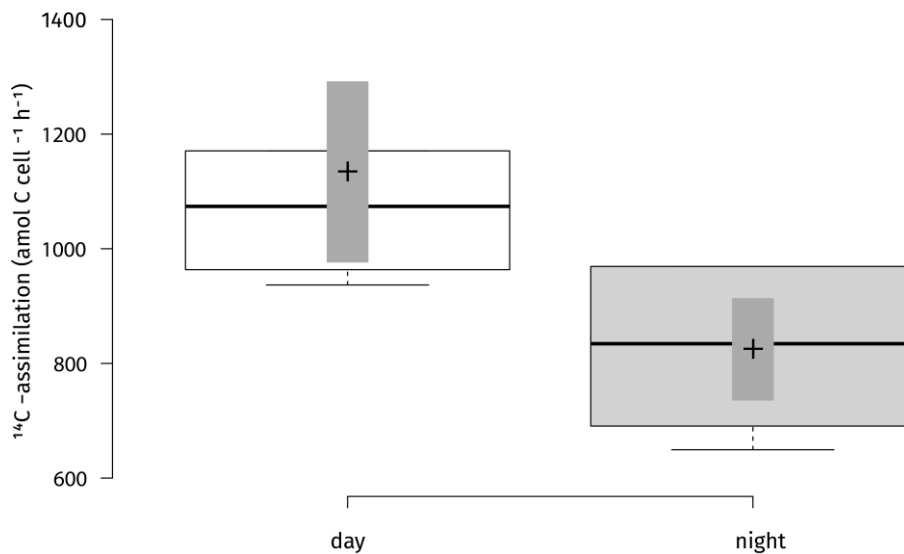
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**Figure 1 | Conductivity, temperature, depth (CTD) profiles from Lake Cadagno on September 12 (1:30 pm) and (9:00 pm) 2017.** Measurements were taken from the platform. Probe was equilibrated for 5 min at 0.5 m depth before measuring. Temperature ( $\diamond$ ), FTU: Formazin Turbidity Unit ( $\odot$ ), Cond: Conductivity ( $\blacksquare$ ), DO: Dissolved Oxygen ( $\bullet$ ).



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**Figure 2 | Absolute microbial  $^{14}\text{C}$ -uptake rates in the chemocline of Lake Cadagno during day and night.** Center lines show the medians; box limits indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25<sup>th</sup> and 75<sup>th</sup> percentiles, outliers are represented by dots.  $n = 3$  sample points.



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**Figure 3 | Carbon uptake rates per cell for strain Cad16<sup>T</sup> cultures for two conditions (day / night) during 4 h incubation *in situ*.**  $^{14}\text{C}$ -scintillation experiments were performed on six biological replicates. Two side  $t$ -test statistics was applied. The difference in uptake rates between the two conditions was statistically significant at  $p < 0.05$  ( $p = 0.02$ ). Center lines show the medians; box limits indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25<sup>th</sup> and 75<sup>th</sup> percentiles, outliers are represented by dots; crosses represent sample means; bars indicate 83% confidence intervals of the means; data points are plotted as open circles.  $n = 6$  sample points.

1027 **11. APPENDIX**

1028 **11.1. Supplementary Tables**

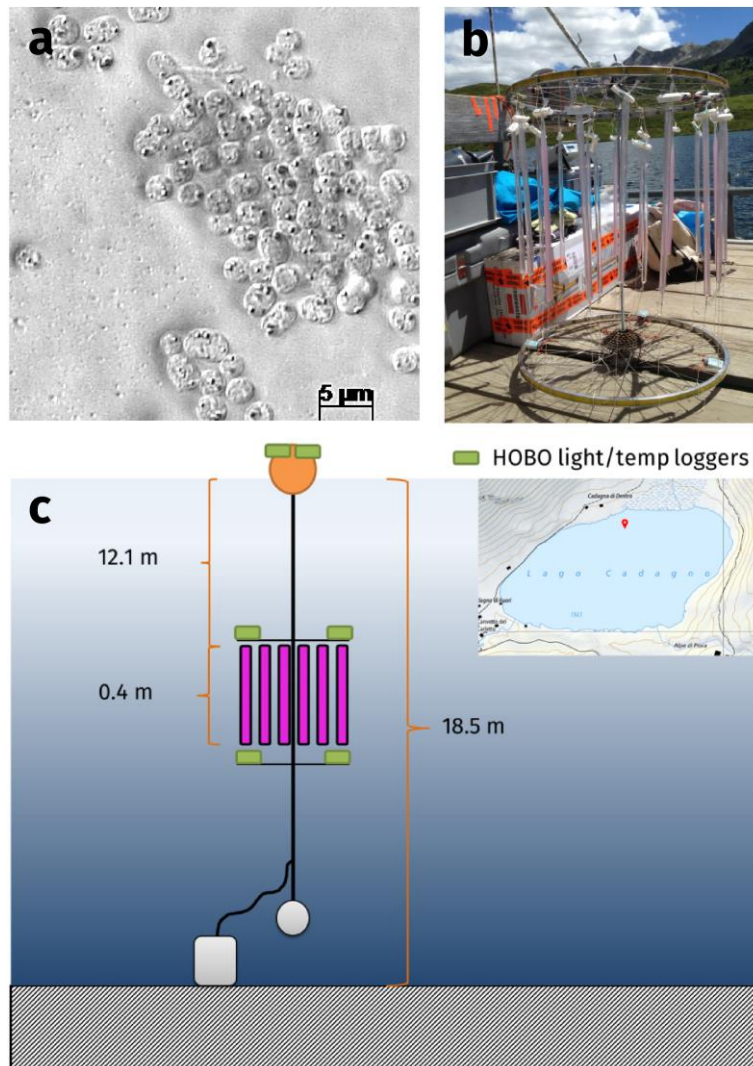
1029 **Table S 1| Absolute carbon uptake rates of the microbial community in the Lake Cadagno chemocline.**

Photosynthesis	Conditions			depth [m]	Reference
	light		dark		
	oxygenic	anoxygenic			
Carbon Uptake Rates [nM h <sup>-1</sup> ]	3'825	4'175	7'608	11.5	Camacho <i>et al.</i> 2001 [30]
		1'200	-	11.5	Musat <i>et al.</i> 2008 [33]
		85	25	12.5	Halm <i>et al.</i> 2009 [61]
		6'187	4'812	12.0	Storelli <i>et al.</i> 2013 [31]
		987	-	11.4	Berg <i>et al.</i> 2016 [38]
		757	587	14.5	This study

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1037 11.2. Supplementary Figures



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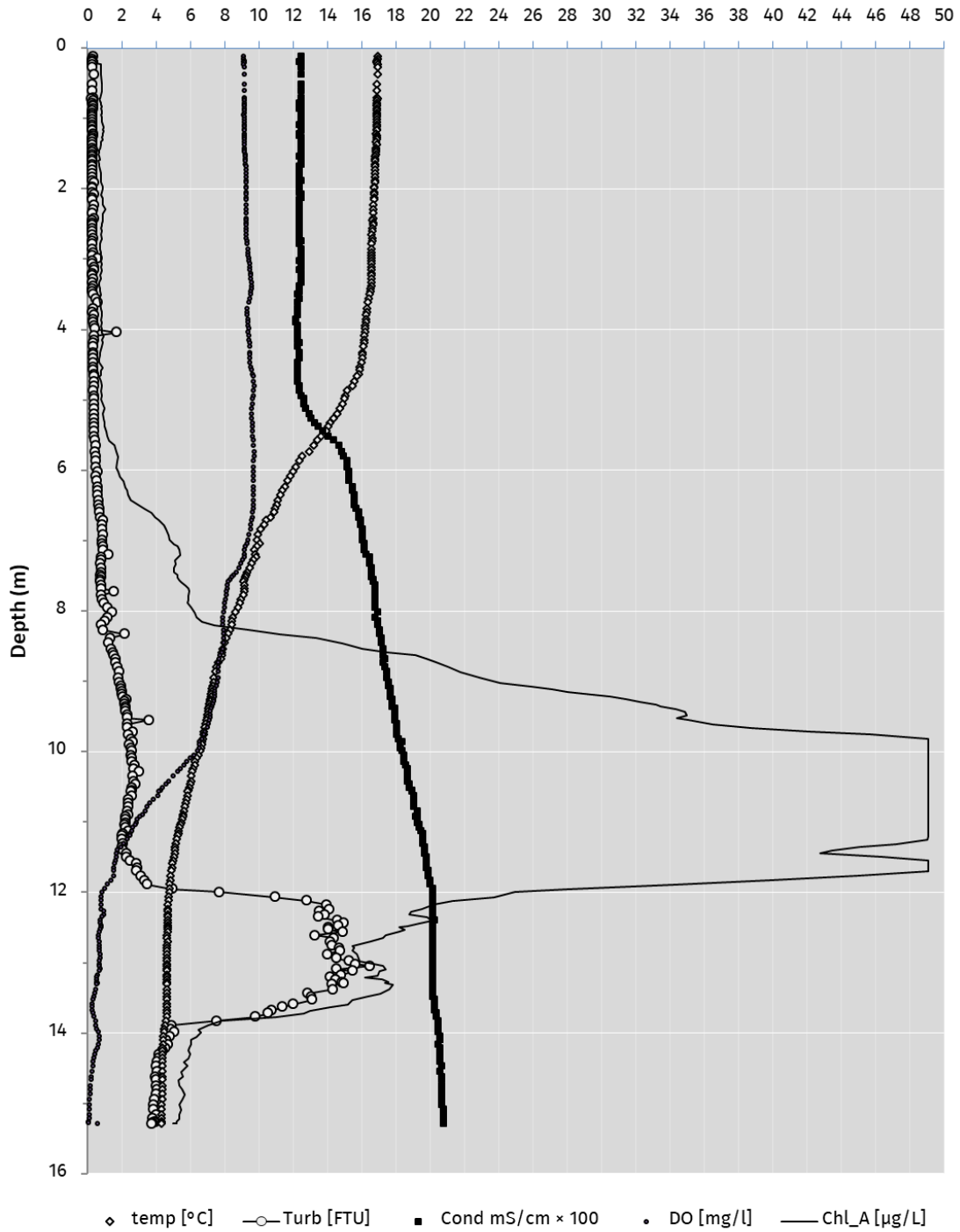
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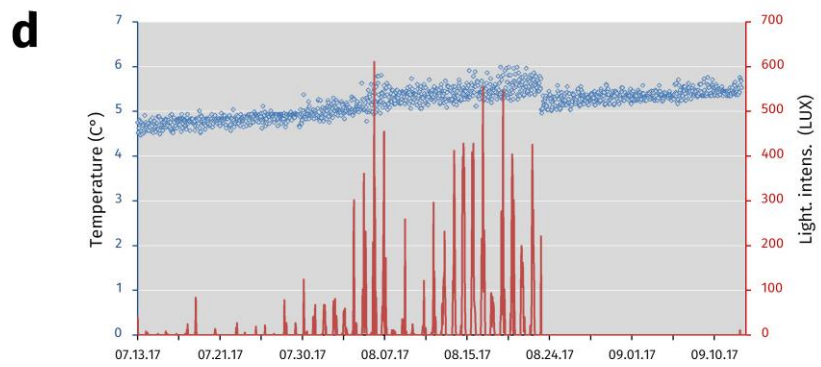
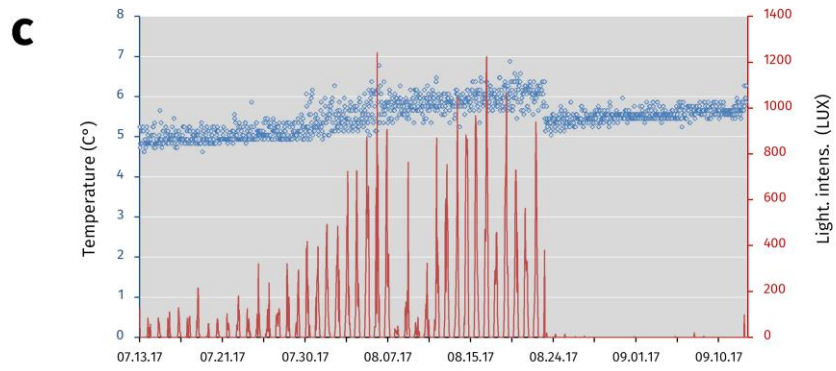
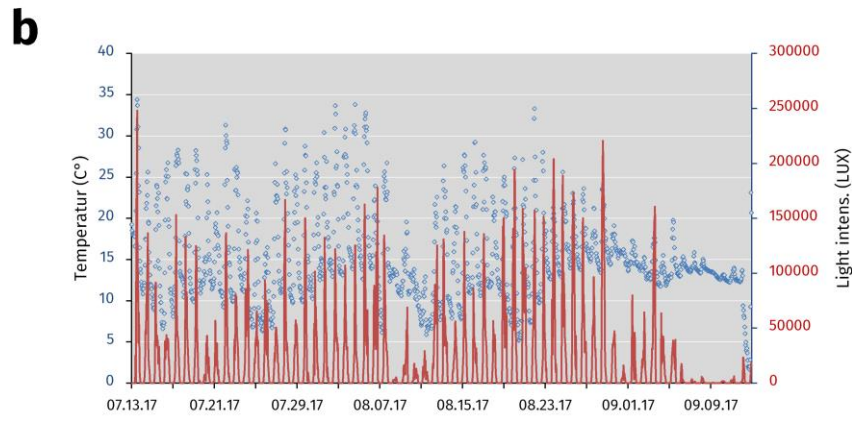
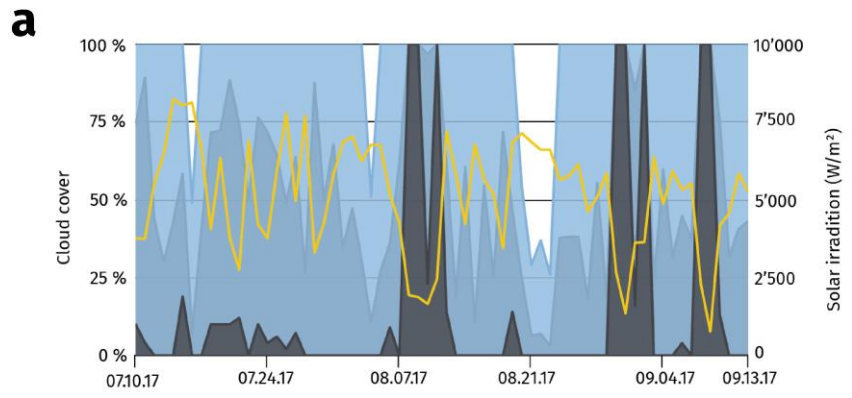
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**Figure S 1 | Depiction of strain “*Thiodictyon syntrophicum*” sp. nov. strain Cad16<sup>T</sup> cells, sampling site and the experimental setup. a)** Phase-contrast microscopic image of pure cultures of str. Cad16<sup>T</sup> with sulfur inclusions visible as highly refractive particles. **b)** Set-up of the strain Cad16<sup>T</sup> cultures in dialysis tubes attached to a support grid. **c)** Mooring scheme and location of the incubation experiment (initial depth, July to August) on Lake Cadagno. In pink indicated the dialysis tubes, in green the HOBO temperature and light sensors.



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**Figure S2 | Conductivity, temperature, depth (CTD) profile on July 13 2017 at 2:44 pm above the deepest point of Lake Cadagno.** Chlorophyll a (—), Temperature (◊), FTU: Formazin Turbidity Unit (○), Cond: Conductivity (■), DO: Dissolved Oxygen (•),

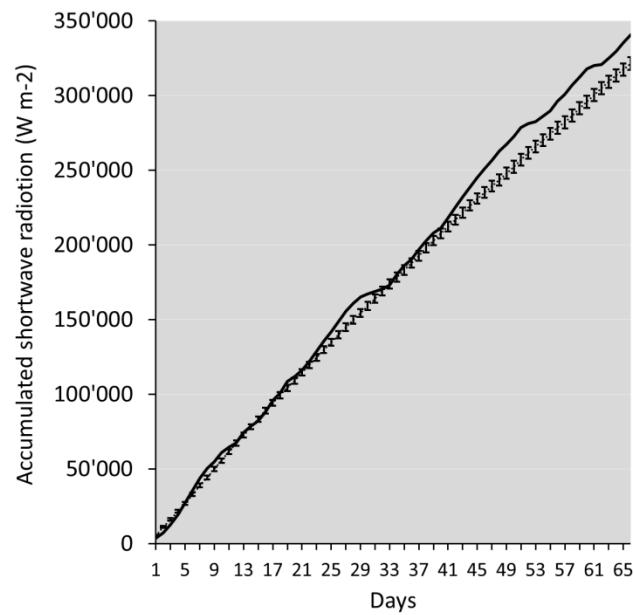


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1052 **Figure S3 | Meteorological data for the Piora valley and temperature and relative light availability at different depths of the**  
 1053 **mooring in Lake Cadagno from July 13 to September 13 2017. a)** Sun light and cloud cover graph for the Piora valley from July  
 1054 13 to September 13 2017. Data from meteoblue.com (yellow; sum of the daily shortwave radiation in  $W m^{-2}$ , blue; maximal  
 1055 daily cloud cover in%, light blue; mean daily cloud cover in%, grey; minimal daily cloud cover in%) **b)** Temperature (black  
 1056 ovals) and average light available (red line) at the surface buoy of Lake Cadagno. The data logger was partly immersed in  
 1057 water, dampening the light and temperature readings (see values in August) **c)** A steady increase in average temperature  
 1058 and light availability from July to August is visible. The increase in the available light can be explained through the  
 1059 downward movement of photosynthetic bacteria over the season (see suppl. Fig. S5 ) Re-positioning of the rig on the August  
 1060 23 2017 results in both, a drop in temperature, and light availability. Available light was reduced to an average of  $0.4 \mu mol m^{-2} s^{-1}$ . **d)** Low light availability and temperature are characteristic for the depth of around 12.4 m. As in c), temperature and  
 1061 available light values are steadily rising from July to August. No light was detected during daytime at 14.4 m depth after  
 1062 repositioning in August. Data was logged in hourly intervals for b)-d).

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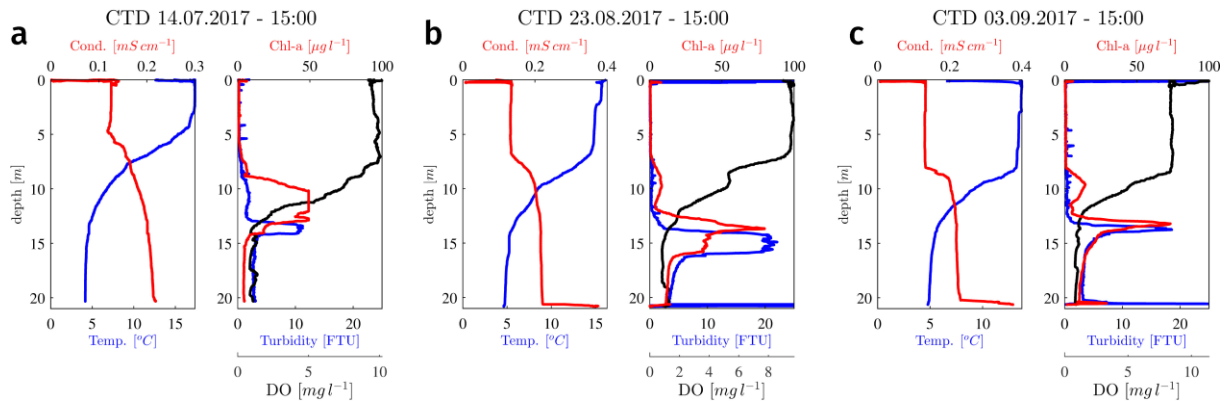
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1067 **Figure S4 | Accumulated surface shortwave radiation for 66 days from July 10 to September 12 2017 at Piora valley.** The  
 1068 values for 2017 show above-average values (solid line). The mean values from 1985 to 2017 for the same time period are  
 1069 represented by the dotted line. Error bars indicate standard-error. Global radiation (diffuse and direct) on a horizontal  
 1070 plane given in Watt per square meter. Values derived from simulation data from meteoblue.com.

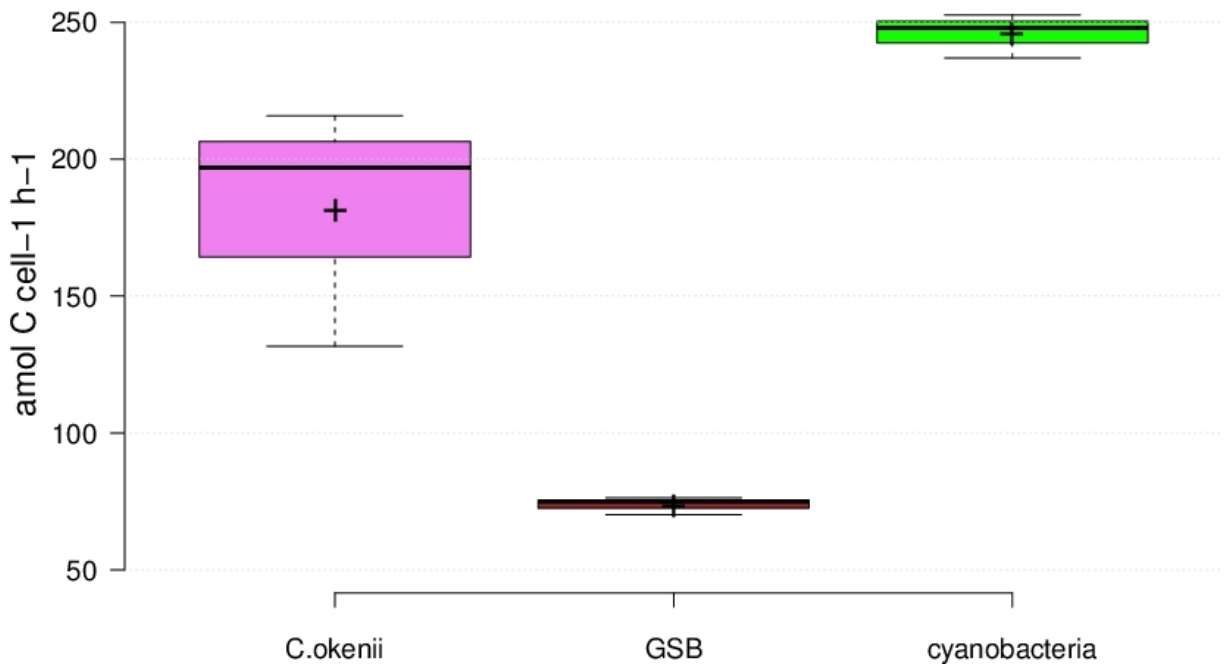
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**Figure S5 | Vertical conductivity, temperature, depth (CTD) profiles from Lake Cadagno from July to September 2017 at the deepest point of the lake.** Temperature , conductivity, dissolved oxygen , chlorophyll a and turbidity are displayed for: **a)** July 14 2017 **b)** August 23 2017 **c)** September 03 2017. Formazin Turbidity Unit (FTU). The data is courtesy of Dr. Oscar Sepúlveda Steiner, Dr. Damien Bouffard and Prof. Johnny Wüest. Plots courtesy of Dr. O. Sepúlveda Steiner, APHYS Laboratory, EPFL, Switzerland.



**Figure S6 | Average inorganic uptake rates at the chemocline of the three populations counted with FCM at 1:30 pm.** *Chromatium okenii* (pink), green sulfur bacteria (GSB; brown) and cyanobacteria (green). Center lines show the medians; box limits indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25<sup>th</sup> and 75<sup>th</sup> percentiles, outliers are represented by dots; crosses represent sample means.  $n = 3$  sample points.