RESEARCH ARTICLE

CO_2 assimilation in the chemocline of Lake Cadagno is dominated by a few types of phototrophic purple sulfur bacteria

Nicola Storelli[1,2], Sandro Peduzzi[3], Maged M. Saad[1], Niels-Ulrik Frigaard[4], Xavier Perret[1] & Mauro Tonolla[1,2,3]

[1]Department of Botany and Plant Biology, Microbiology Unit, University of Geneva, Sciences III, Geneva, Switzerland; [2]Institute of Microbiology, Bellinzona, Switzerland; [3]Alpine Biology Center (ABC), Quinto, Switzerland; and [4]Section for Marine Biology, Department of Biology, University of Copenhagen, Helsingør, Denmark

Correspondence: Mauro Tonolla, Via Mirasole 22a, CH-6500 Bellinzona, Switzerland. Tel.: + 4191 814 60 74; fax: +4191 814 60 19; e-mail: mauro.tonolla@ti.ch

Received 6 June 2012; revised 9 January 2013; accepted 10 January 2013. Final version published online 11 February 2013.

DOI: 10.1111/1574-6941.12074

Editor: Gary King

Keywords
primary production; RuBisCO; *cbbL* and *cbbM* mRNA; *Candidatus* 'Thiodictyon syntrophicum' sp. nov. strain Cad16^T.

Abstract

Lake Cadagno is characterized by a compact chemocline that harbors high concentrations of various phototrophic sulfur bacteria. Four strains representing the numerically most abundant populations in the chemocline were tested in dialysis bags *in situ* for their ability to fix CO_2. The purple sulfur bacterium *Candidatus* 'Thiodictyon syntrophicum' strain Cad16^T had the highest CO_2 assimilation rate in the light of the four strains tested and had a high CO_2 assimilation rate even in the dark. The CO_2 assimilation of the population represented by strain Cad16^T was estimated to be up to 25% of the total primary production in the chemocline. Pure cultures of strain Cad16^T exposed to cycles of 12 h of light and 12 h of darkness exhibited the highest CO_2 assimilation during the first 4 h of light. The draft genome sequence of Cad16^T showed the presence of *cbbL* and *cbbM* genes, which encode form I and form II of RuBisCO, respectively. Transcription analyses confirmed that, whereas *cbbM* remained poorly expressed throughout light and dark exposure, *cbbL* expression varied during the light–dark cycle and was affected by the available carbon sources. Interestingly, the peaks in *cbbL* expression did not correlate with the peaks in CO_2 assimilation.

Introduction

Lake Cadagno (Switzerland) is a crenogenic meromictic lake characterized by a narrow chemocline with high concentrations of sulfates; steep gradients of oxygen, sulfide, and light; and a turbidity maximum that correlates with large concentrations of bacteria of up to 10^7 cells mL^{-1} (Tonolla *et al.*, 1999, 2004; Lüthy *et al.*, 2000). Studies in other similarly stratified lakes have confirmed the importance of phototrophic sulfur bacteria to the carbon and sulfur cycles (Pimenov *et al.*, 2003; García-Cantizano *et al.*, 2005; Dimitriu *et al.*, 2008; Casamayor *et al.*, 2012). In Lake Cadagno, the chemocline is typically found at a depth of about 12 m and is characterized by the presence of a dense community of anaerobic phototrophic sulfur bacteria, including purple sulfur bacteria (PSB) of the genera *Chromatium, Lamprocystis, Thiocystis* and *Thiodictyon* and green sulfur bacteria (GSB) of the genus *Chlorobium* (Tonolla *et al.*, 1999, 2004, 2005). From 1998 to 2004 the total concentration of phototrophic sulfur bacteria increased from 4.9 (± 0.2) to 14 (± 1.4) 10^6 cells mL^{-1}, primarily due to a remarkable rise in the population of the GSB *Chlorobium clathratiforme* (Decristophoris *et al.*, 2009; Gregersen *et al.*, 2009). Previous reports have shown that despite its small volume (approximately 10% of the lake), the chemocline of Lake Cadagno is responsible for up to 40% of the total inorganic carbon photo-assimilation (Camacho *et al.*, 2001). Interestingly, significant rates of CO_2 assimilation were also found to occur during the night, indicating that primary production also relies on mechanisms other than photosynthesis. Chemoautotrophic bacteria such as those belonging to the *Thiobacillus* genus are often responsible for CO_2 fixation in the absence of light (Martinez *et al.*, 1983) but they have not been detected in Lake Cadagno (Tonolla *et al.*, 1999, 2003; Bosshard *et al.*,

2000; Gregersen *et al.*, 2009). In general, GSB are considered to be obligate photoautotrophic (Parkin & Brock, 1981), whereas PSB are capable of both photoautotrophy and photoheterotrophy; in addition, some PSB strains are capable of growing chemotrophically in the dark and under microaerophilic conditions (Kämpf & Pfennig, 1986; de Wit & van Gemerden, 1987). Previous nanoscale secondary-ion mass spectrometry (nanoSIMS) has shown that the inorganic carbon uptake is highly variable among three populations of phototrophic sulfur bacteria in the Lake Cadagno chemocline (Musat *et al.*, 2008).

Green sulfur bacteria assimilate CO_2 via the reverse tricarboxylic acid (rTCA) cycle, whereas PSB assimilate CO_2 via the Calvin-Benson-Bassham cycle in which ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO; EC 4.1.1.39) is a key enzyme. RuBisCO is found in most autotrophic organisms, as well as in eukaryotic algae and plants (Ellis, 1979; Tabita, 2005; Mulkidjanian *et al.*, 2006). There are four major forms of RuBisCO (I–IV) known in nature, distinguishable by their primary amino acid sequences (Badger & Bek, 2008; Tabita *et al.*, 2008). Forms I, II and III of RuBisCO contain catalytically active amino acid residues that are necessary for carboxylation and oxygenation. In contrast, form IV of RuBisCO (also known as the RuBisCO-like protein), which is found in several non-phototrophic bacteria (e.g. *Bacillus subtilis*) and GSB, is thought to be incapable of CO_2 fixation because it lacks several conserved residues considered essential for RuBisCO carboxylase activity (Saito *et al.*, 2009). Form I, the most abundant form of RuBisCO, is found in many eukaryotes and bacteria and is a multimeric enzyme comprising eight CbbL subunits of 50–55 kDa that are associated with eight CbbS subunits of 12–16 kDa (Schneider *et al.*, 1992). Form II is found in certain *Proteobacteria* and consists of 2–8 CbbM subunits (each of 50–55 kDa) depending on the microorganism. Form III is found only in some *Archaea* and comprises large subunit homomers in arrangements of either 2 (L_2) or 5 (L_5) subunits (Tabita *et al.*, 2008).

The aim of this study was to determine the CO_2 assimilation of the various populations of phototrophic sulfur bacteria that are found in the chemocline of Lake Cadagno. To do this, we compared the CO_2 assimilation rates in pure cultures of strains isolated from Lake Cadagno and representing the four numerically most abundant phototrophic sulfur bacteria populations. The pure cultures were cultivated, and subsequently pulsed with $NaH^{14}CO_3$, in dialysis bags positioned at a depth of 12 m (described as *in situ* conditions). In addition, the expression of the RuBisCO *cbbL* (Form I) and *cbbM* (Form II) genes in pure cultures of the strain *Candidatus* 'Thiodictyon syntrophicum' Cad16^T were determined over time during light–dark cycles under laboratory conditions (described as *in vitro* conditions). Strain Cad16^T was chosen as the model organism because it has been shown to represent one of the most prominent CO_2-assimilating populations in the lake both in the light and in the dark.

Materials and methods

Media and growth conditions

The major characteristics of the strains used in this study are described in Table 1. PSB were grown in Pfennig's medium I (Eichler & Pfennig, 1988), and GSB were grown in Pfennig's medium II (Biebl & Pfennig, 1979), both of which contain 0.25 g of KH_2PO_4 L^{-1}, 0.34 g of NH_4Cl L^{-1}, 0.5 g of $MgSO_4{\cdot}7H_2O$ L^{-1}, 0.25 g of $CaCl_2{\cdot}2H_2O$ L^{-1}, 0.34 g of KCl L^{-1}, 1.5 g of $NaHCO_3$ L^{-1}, 0.02 mg of vitamin B_{12} L^{-1} and 0.5 mL of trace element solution SL12 L^{-1} for PSB and SL10 for GSB (see Supporting Information, Table S1). The media were prepared in 2-L bottles using a flushing gas composition of 80% N_2 and 20% CO_2 according to Widdel &

Table 1. Major characteristics of strains used in this study

Name	Characteristic physiology	Reference
Candidatus 'Thiodictyon syntrophicum' strain Cad16^T	Purple sulfur bacterium; cells are ovoid-shaped, 1.4–2.4 μm in size, Gram-negative; anaerobic or microaerophilic growth; CO_2 fixation by the Calvin-Benson-Bassham (CBB) cycle	Peduzzi *et al.* (2012)
Thiocystis chemoclinalis strain CadH11^T	Purple sulfur bacterium; cells are ovoid-shaped, 2.3–3.6 μm in size, Gram-negative; anaerobic or microaerophilic growth; CO_2 fixation by the Calvin-Benson-Bassham (CBB) cycle	Peduzzi *et al.* (2011)
Lamprocystis purpurea strain CadA31	Purple sulfur bacterium; cells are ovoid-shaped, 1.9–2.3 μm in size, Gram-negative; anaerobic or microaerophilic growth; CO_2 fixation by the Calvin-Benson-Bassham (CBB) cycle	Eichler & Pfennig, (1988)
Chlorobium clathratiforme strain Cad4DE	Green sulfur bacterium; cells are rod-shaped, 0.3–1.2 μm in size, Gram-negative; strictly anaerobic growth; CO_2 fixation by the reverse tricarboxylic acid (rTCA) cycle	Tonolla *et al.* (2004)

Bak (1992) and were reduced by the addition of 1.10 mM $Na_2S.9H_2O$ and adjusted to a pH of approximately 7.0. All cultures were incubated at room temperature (20–23 °C) and subjected to a light/dark photoperiod of 12 h with a light intensity of approximately 6 μE m^{-2} s^{-1} that was generated with incandescent 60 W bulbs. All media promoted autotrophic growth except for the mixotrophic medium used for the *cbbL/cbbM* mRNA expression analysis, to which 2 mM of sodium acetate was added. Concentrations of sulfide in the cultures were measured daily and adjusted to about 1 mM throughout the experiments. The growth was followed by measuring the optical density of culture aliquots at a wavelength of 650 nm (OD_{650}) using a UV/VIS Spectrometer Lambda 2S (Perkin-Elmer Inc, Waltham, MA). All biochemical analyses were performed on cells taken from exponentially growing cultures with an OD_{650} of *c.* 0.6, which corresponds to *c.* 1.0×10^7 cell mL^{-1}.

Strain identification, total cell count and viability tests

Bacterial populations in the chemocline were identified using fluorescent *in situ* hybridization (FISH) with species-specific Cy3-labeled oligonucleotides (Table 2) in 1-μL aliquots of paraformaldehyde-fixed water samples ($n = 3$) spotted onto gelatin-coated slides [0.1% gelatin, 0.01% $KCr(SO_4)_2$] (Glöckner *et al.*, 1996). The hybridizations were performed as described by Zarda *et al.* (1997). The slides were treated with Citifluor AF1 (Citifluor Ltd., London, UK) and examined by epifluorescence microscopy using filter sets F31 (AHF Analysentechnik, Tübingen, Germany; D360/40, 400DCLP, and D460/50 for DAPI) and F41 (AHF Analysentechnik; HQ535/50, Q565LP, and HQ610/75 for Cy3). The microorganisms were counted at a 1000-fold magnification in 40 fields of 0.01 mm^2 each (Fischer *et al.*, 1995).

Cell concentrations of pure bacterial cultures in dialysis bags were determined using samples fixed with 4% formaldehyde (final concentration) and stained with 0.001% (w/v) 4′,6-diamidino-2-phenylindole (DAPI) (final concentration). To count cells, 10 μL of each fixed sample was deposited onto polycarbonate filters as described in Hobbie *et al.* (1977) and observed at 100-fold magnification using an epifluorescence microscope (Axiolab, Zeiss Germany) and filter set F31 (Zeiss). Twenty fields of 0.01 mm^2 were counted, and cell densities were expressed as the mean number of cells mL^{-1} (±SE). After the incubation period of 5 weeks in the dialysis bags at a depth of 12 m, the number of viable cells was determined using the Live/Dead BacLight Bacterial Viability kit (Invitrogen – Life Technologies Europe, Zug, Switzerland).

In situ analysis

Meromictic Lake Cadagno is located in the Piora Valley at 1921 m above sea level in the southern Swiss Alps (46°33′N, 8°43′E). Physicochemical parameters of the water column were determined using a YSI 6000 profiler (Yellow Springs, Inc., Yellow Springs, OH) and included temperature (°C), conductivity (μS cm^{-1}), pH, dissolved oxygen (mg L^{-1}), redox potential (mV) and turbidity (FTU, formazine turbidity unit) (Table S1). Two LI-193SA spherical quantum sensors (LI-COR Ltd, Lincoln, NE) were used to determine the percentage of transmission down to the chemocline of photosynthetically active radiation (%PAR-light). Sulfide concentrations were measured using Cline's reagent of water samples fixed with $ZnCl_2$ (Cline, 1969).

In situ $^{14}CO_2$ assimilation analyses were performed as follows: each of the four selected strains was grown separately under laboratory conditions until the cultures reached an OD_{650} of 0.6. This initial period of growth lasted for approximately 10 days due to the relatively long generation times, ranging from 110 to 130 h of cultures. Then, 750 mL of these pure bacterial cultures were sealed in 60-cm-long dialysis bags (inflated diameter of 62.8 mm; Karl Roth GmbH Co. KG, Karlsruhe, Germany) and acclimatized for 5 weeks (from 8 August to 12 September 2007) in the chemocline at a depth of 12 m. With pores 25–30 Å in diameter, the dialysis bags allowed for the free diffusion of molecules smaller than 20 kDa while preventing contamination of incubated cultures by environmental bacteria (Simek *et al.*, 2001, 2006; Corno & Jürgens, 2006; Blom *et al.*, 2010; Lindström & Ostman, 2011). After this 5-week pre-incubation period, the cultures were transferred from the dialysis bags into independent 100-mL sealed bottles with 125 μL of $NaH^{14}CO_3$ for primary production measurements. To limit the exposure of the bacterial culture to atmospheric oxygen, the

Table 2. Cy3-labeled oligonucleotide probes used in this study for FISH counting

Probe	Target	Sequence (5′→3′) (formamide in hybridization buffer)	Reference
S453F	*Candidatus* 'Thiodictyon syntrophicum' strain Cad16^T	CCCTCATGGGTATTARCCACAAGGCG (40%)	Tonolla *et al.* (1999)
S453H	*Thiocystis chemoclinalis* strain CadH11^T	GACGGAACGGTATTAACGCCCCGCTT (10%)	Tonolla *et al.* (2005)
Apur453	*Lamprocystis purpurea* strain CadA31	TCGCCCAGGGTATTATCCCAAACGAC (40%)	Tonolla *et al.* (1999)
Chlc190	*Chlorobium clathratiforme* strain Cad4DE	GGCAGAACAACCATGCGATTGT (20%)	Tonolla *et al.* (2005)

cultures were transferred in a CO_2-saturated environment. Due to the short exposure time, neither the pH nor the dissolved inorganic carbon (DIC) in the sample were modified by the sublimation of CO_2 (Data S2).

Measurements of radioactive inorganic carbon assimilation

The inorganic carbon fixation was evaluated in parallel in one opaque (dark) and one transparent (light) sealed 100-mL bottle. The inorganic carbon assimilation activity was measured using radioactive ^{14}C isotope (NEC-086S $NaH^{14}CO_3$; 1 mCi; 8.40 mCi/mmol; 1-mL ampoules with specific activities of 20 μCi mL^{-1}; Perkin Elmer, Schwerzenbach, Switzerland). A total of 125 μL $NaH^{14}CO_3$ solution, with an activity of 0.05 μCi mL^{-1}, was added to each 100-mL bottle. All bottles were completely filled with either a bacteria-free sample from the chemocline (filtrated with 0.22-μm filter), a chemocline water sample (12 m) or with bacterial samples from the dialysis bags. Each sample was analysed in triplicate. The filled bottles were placed at a depth of 12 m in the chemocline and incubated for 4 h (from 12:00 to 16:00 h). Subsequently, the total radioactive carbon that was assimilated was measured by the method described by Gächter & Mares (1979) to ensure a total loss of unbound radioactive carbon through the bubbling method (Schindler *et al.*, 1972). At the end of the bubbling period, 10 mL of Ready Gel scintillation liquid (Ready Gel™; Beckman Coulter, Fullerton, CA) was added to each scintillation vial, and the radioactivity was measured in a Beckman LS 6000 Scintillation Counter (Beckman, WS-BECKLS6).

Draft genome sequencing

The draft genome of *Candidatus* 'Thiodictyon syntrophicum' strain $Cad16^T$ was determined by pyrosequencing in the laboratory of Dr. S. C. Schuster (Z. Liu, K. Vogl, N.-U. Frigaard, L. P. Tomsho, S. C. Schuster and D. A. Bryant, pers. commun.) at the Genomics Core Facility of Pennsylvania State University. Paired-end reads from GX-20 FLX Titanium chemistries were assembled into 1352 primary contigs. Two contigs predicted to code for *cbbL* and *cbbM* were deposited in the NCBI public database under the respective accession numbers JQ780325 and JQ780326.

RNA extraction

Cells of strain $Cad16^T$ were grown in the laboratory under previously described conditions. At specific times, 0.5 mL of the cultures (*c.* 0.5×10^7 cells) were mixed with 1.0 mL of RNAprotect Bacteria Reagent from the RNeasy Protect Mini Kit (Qiagen) and processed according to the manufacturer's instructions. Total cellular RNA was purified with the RNeasy Mini Kit (Qiagen), resuspended in RNase-free H_2O and stored at −80 °C to minimize degradation.

Reverse transcription quantitative PCR

The levels of *cbbL* and *cbbM* gene transcription were measured by reverse transcription-quantitative PCR (qRT-PCR) analysis using a Light Cycler® instrument (Roche Applied Science) and the QuantiTect™ SYBR®Green RT-PCR kit (Qiagen) according to the manufacturer's instructions. Contigs JQ780325 and JQ780326 were used as templates to design the specific primer pairs to determine the expression of *cbbL* and *cbbM* genes. The primers used were cbbL-F (5′-cttcgagttcgtgggc-3′), cbbL-R (5′-gcacgctcgtacatct-3′) and cbbM-F (5′-caggccaagattttctctgc-3′), cbbM-R (5′-tacctgcactaccatcgtgc-3′) for *cbbL* and *cbbM*, respectively. The qRT-PCR reaction mixture contained the following components for both primer sets: 10 μL of SYBR Green buffer, 0.4 μL of both primers (0.2 μM primer final concentration), 5 μL of RNA template, 0.2 μL of qRT-PCR mix, 0.1 μL of UNG and DNAse free water (Qiagen) to a total volume of 20 μL. The qRT-PCR mix was loaded onto the LightCycler® system, and the reaction conditions were set up as follows: (1) 20-min reverse transcription at 50 °C; (2) 15-min initial activation step at 95 °C; (3) 60 reaction cycles: 0 s at 81 °C, 15 s at 94 °C, 20 s at 63 °C and 10 s at 72 °C; (4) melting curve: 0 s at 95 °C, 15 c at 60 °C and 0 s at 99 °C; (5) 2 min at a 40 °C cooling step. To monitor the progress of the PCR reaction, a fluorescence signal was measured at a wavelength of 530 nm at the end of each elongation phase. A relative quantification analysis was performed using the LIGHTCYCLER® 4.1, which enabled a comparison of the ratio of the target mRNA in each sample to the reference standard curve. The standard curve was calculated using serial dilutions of *cbbL* or *cbbM* mRNA amplified from cultured bacteria with the previous sets of primers.

Results

Physicochemical and biological properties of Lake Cadagno

Sample collection and *in situ* experiments were carried out on 12 September 2007. The main physicochemical parameters of the water column on this day, including ATP values, are shown in Fig. 1. The water column was stratified into three zones: the oxic mixolimnion (from the surface to approximately 11 m), the anoxic chemocline

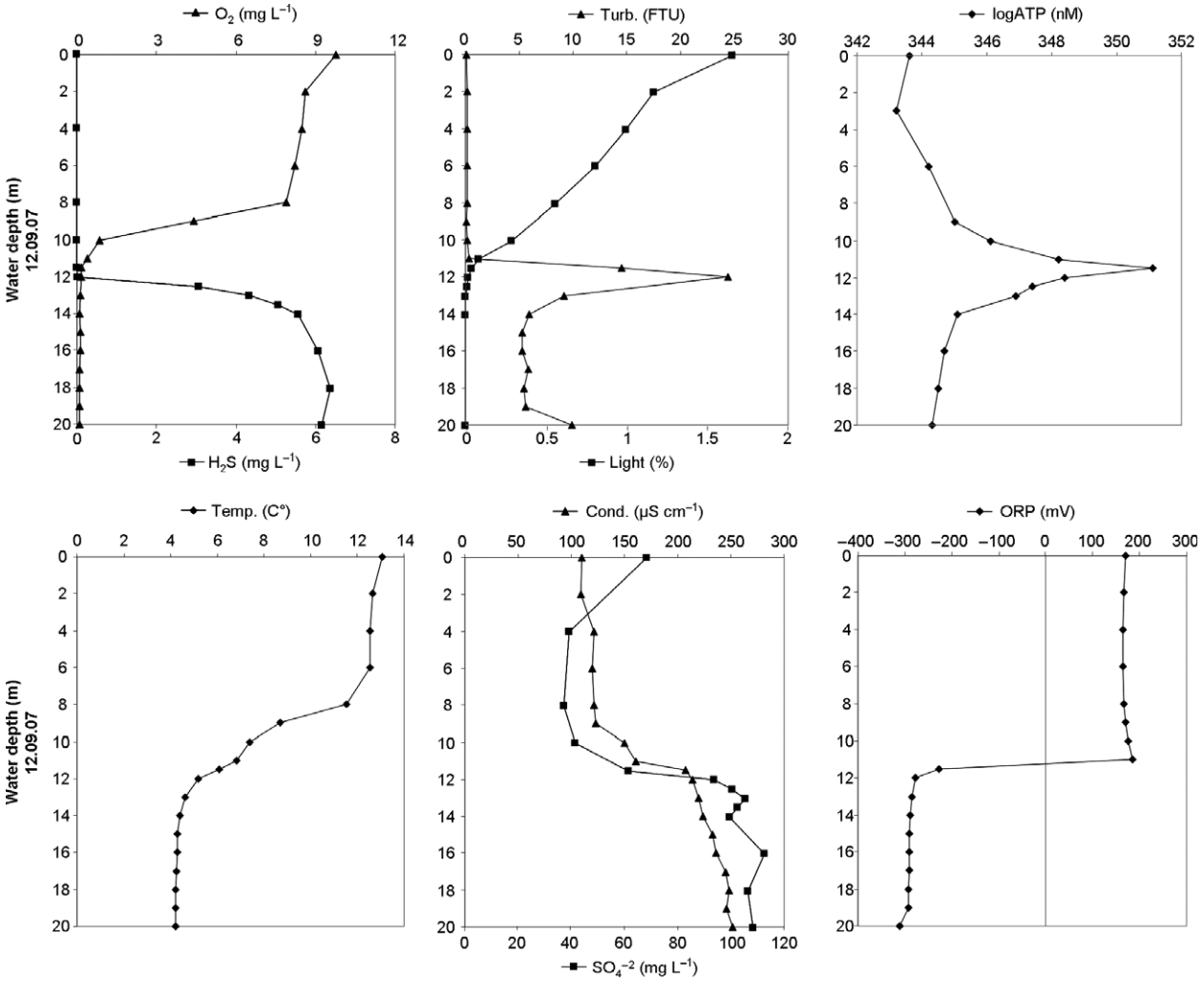

Fig. 1. Vertical distribution of oxygen, sulfide, turbidity, light, ATP, temperature, conductivity, sulfate, and oxidation reduction potential (ORP) on 12 September 2007.

(at approximately 12 m), and the anoxic, sulfide-containing monimolimnion (from approximately 13 m down to the sediment). The transition zone from the oxic to the anoxic water layer was also revealed by the redox potential (ORP) by a shift from positive to negative values at approximately 11 m. The turbidity maximum and the ATP values corresponded with a dense microbial community of *c.* 10^7 cells mL^{-1} that populated the chemocline and consisted mostly of GSB and PSB (as determined by FISH). The GSB *C. clathratiforme* was the most abundant species with 8.3×10^6 cells mL^{-1} (72.9% of the total bacterial concentration) at 12 m. The PSB *Candidatus* 'Thiodictyon syntrophicum' strain Cad16^T, *Lamprocystis purpurea* and *Thiocystis chemoclinalis* strain CadH11^T were clearly less abundant, with 1.3×10^5 cells mL^{-1} (1.2% of total), 4.9×10^4 cells mL^{-1} (0.4% of total) and 1.9×10^4 cells mL^{-1} (0.2% of total), respectively.

CO_2 assimilation in the chemocline of Lake Cadagno

To measure the inorganic carbon assimilated in the chemocline and the impact of light on this phenomenon, a water sample was collected from a depth of 12 m. After 4 h of incubation (from 12:00 to 16:00 h on a sunny day), the concentration of fixed CO_2 was determined to be 297 ($\pm$ 52) ng of ^{14}C mL^{-1} in the presence of light and 231 ($\pm$ 98) ng of ^{14}C mL^{-1} in the dark (see Fig. 2a).

In parallel, the uptake of inorganic carbon was also measured using pure cultures of the four main autochthonous strains from Lake Cadagno (Table 1), which were pre-incubated for equilibration for 5 weeks at a depth of 12 m in dialysis bags as described above. After the equilibration period, the dialysis bags were retrieved, and more than 99% of the cultures consisted of live cells.

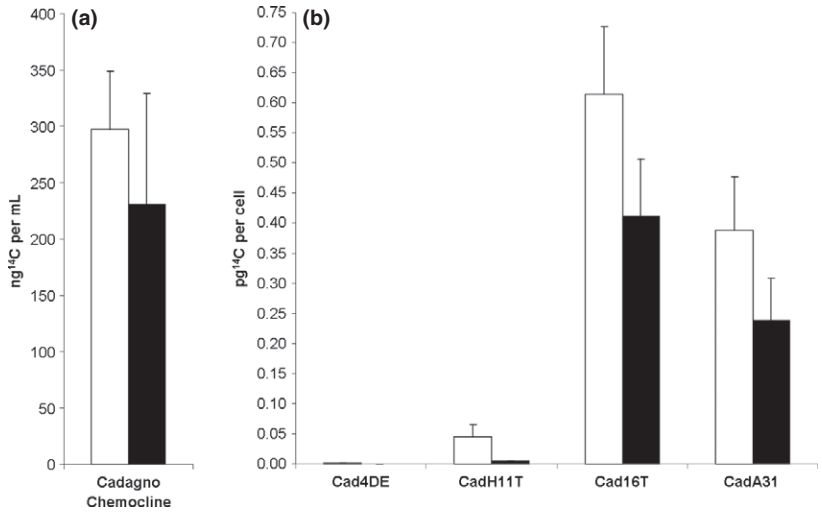

Fig. 2. Measurements of $^{14}CO_2$ assimilation by representative GSB and PSB in the chemocline of Lake Cadagno. (a) Primary production was first established using a water sample taken directly from the chemocline. (b) In parallel, CO_2 fixation by pure cultures of *Chlorobium clathratiforme* Cad4DE, *Thiocystis chemoclinalis* CadH11^T, *Candidatus* 'T. syntrophicum' Cad16^T or *Lamprocystis purpurea* CadA31 was also measured. Bacteria were incubated for 4 h at a depth of 12 m and in the presence of $NaH^{14}CO_3$, either in the presence of light (white columns) or in the dark (black columns). Activity is reported as ng of ^{14}C fixed mL^{-1} of chemocline water (Fig. 2A) or pg of ^{14}C $cell^{-1}$ within pure cultures (Fig. 2b). Each data point represents the mean of three independent measurements, with standard deviations shown as error bars.

As shown in Fig. 2b, *Candidatus* 'T. syntrophicum' Cad16^T and *L. purpurea* CadA31 were clearly the most efficient CO_2-fixing strains, with levels of 0.61 (± 0.11) and 0.39 (± 0.09) pg of ^{14}C $cell^{-1}$ in the light, respectively, whereas *C. clathratiforme* Cad4DE and *T. chemoclinalis* CadH11^T showed low to negligible activities of 0.001 (± 0.0002) and 0.04 (± 0.02) pg of ^{14}C $cell^{-1}$, respectively. Interestingly, both strains Cad16^T and CadA31 showed a strong carbon uptake of 0.41 (± 0.09) and 0.24 (± 0.07) pg of ^{14}C per cell, respectively, in opaque bottles, indicating that both strains retained the ability to fix CO_2 for at least 4 h in the dark.

As reported in Fig. 2a, a total of 528 ng (297 light + 231 dark) of CO_2 (arbitrarily set as 100%) was fixed by microorganisms found in each milliliter of the chemocline after 4 h of incubation in the presence or absence of light. Given the respective rates per cell of carbon assimilation measured for pure cultures of strains Cad4DE, CadH11^T, Cad16^T and CadA31 when incubated in the dialysis bags, we attempted to estimate the respective contributions of each population represented by the strains (in % of the total) to the global pool of carbon fixed in the lake. This estimation included the concentration of each of the populations in the chemocline as determined by FISH (see Table 3). In this model, the population represented by *Candidatus* 'T. syntrophicum' Cad16^T appeared to be the most important contributor among the four populations tested, because it assimilated as much as 25.9% of all the hypothetical carbon fixed within the chemocline (light and dark). However, the primary CO_2-assimilating organism(s) within the chemocline, responsible for 66.1% of the carbon fixation, still remain (s) unknown.

CO_2 assimilation of *Candidatus* 'T. syntrophicum' Cad16^T grown under controlled laboratory conditions

To gain an insight into the dynamics of CO_2 assimilation in the organism that was determined to be most efficient at C-fixation, a time course experiment was carried out under laboratory conditions. Cells of Cad16^T were grown in Pfennig's Medium to *c.* 0.6 OD_{650} under light/dark photoperiods of 12 h. Thereafter, $NaH^{14}CO_3$ was added every 4 h, to 50-mL aliquots that were further incubated for 4 h prior to quantification of $^{14}CO_2$. As shown in Fig. 3, CO_2 assimilation reached a maximum (0.89 ± 0.03 pg of ^{14}C per cell) during the first light period (from 07:00 to 11:00 h) and decreased rapidly once cells were exposed to the dark (0.14 ± 0.03 pg of ^{14}C per cell between 19:00 and 23:00 h), ending at a minimum assimilation activity of 0.04 ± 0.003 pg of ^{14}C $cell^{-1}$ between 23:00 and 03:00 h. The period between 11:00 and 15:00 h, with an *in vitro* assimilation of 0.53 ± 0.08 pg of ^{14}C per cell, is comparable to the *in situ* activity of 0.61 ± 0.11 pg of ^{14}C $cell^{-1}$ measured during the time frame from 12:00 to 16:00 h in the light. Similarly, the $^{14}CO_2$ assimilation of 0.41 ± 0.09 pg of ^{14}C

Table 3. Total estimated contributions to CO_2 fixation by selected groups of organisms in Lake Cadagno (phototrophic populations are represented by specific strains)

Strains	Populations in the chemocline* (10^4 cells mL^{-1})	Levels of CO_2 fixation Light + Dark† (pg C per 10^4 cells)	Total CO_2 fixed (ng C mL^{-1})	% of total
C. clathratiforme Cad4DE	831	8 + 4	11	2.0
T. chemoclinalis CadH11^T	2	450 + 40	1	0.2
Candidatus 'T. syntrophicum' Cad16^T	13	6100 + 4100	137	25.9
L. purpurea CadA31	5	3900 + 2400	31	5.8
Others	289	Not determined	Not determined	66.1
Chemocline sample‡	1140		528§	100.0

*Cells for each strain count in the chemocline (12 m) by FISH the same day of the experiment showed in Fig. 2 (12 September 2007).
†Values shown in Fig. 2B.
‡Total cell count in the chemocline (12 m) by DAPI the same day of the experiment showed in Fig. 2 (12 September 2007).
§Experimentally determined by the sum of values shown in Fig. 2A (see text).

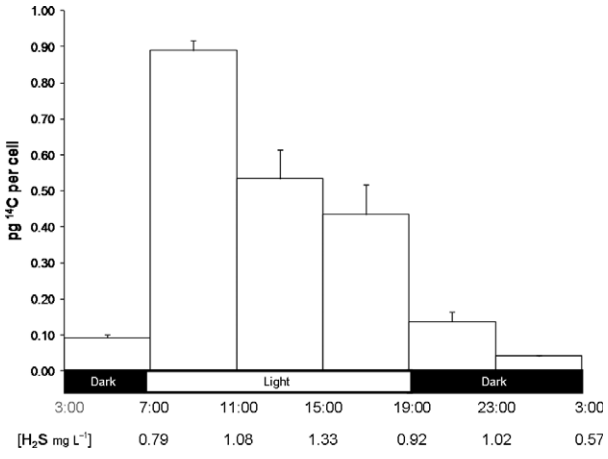

Fig. 3. Variations of $^{14}CO_2$ assimilation in cells of strain *Candidatus* 'T. syntrophicum' Cad16^T grown under laboratory conditions. Fixation of CO_2 was quantified every 4 h in pure liquid anoxic and autotrophic cultures with photoperiods of 12 h, and light/dark transitions fixed at 07:00 and 19:00 h. Assimilation is reported as pg of ^{14}C fixed per cell, with each data point representing the mean of three independent measurements. Standard deviations are shown as error bars. The concentration of H_2S was measured before every quantification of $^{14}CO_2$ assimilation.

per cell between 19:00 and 23:00 h corresponded to the *in situ* $^{14}CO_2$ assimilation in the dark. The level of sulfide was monitored during the experiment, but no correlation with other parameters was found. The maximum sulfide level was at 15:00 h after 8 h of light, and the minimum was at 3:00 h after 8 h of dark.

Expression of cbbL and cbbM genes in *Candidatus* 'T. syntrophicum' Cad16^T in the presence or absence of an organic carbon source

Two putative genes coding for the RuBisCO form I (*cbbL*) and form II (*cbbM*) were found in the genome annotation of strain Cad16^T (Data S3). To establish which form of RuBisCO is the most important for CO_2 fixation in strain Cad16^T, the transcript levels of *cbbL* and *cbbM* were determined in anoxic autotrophically or anoxic mixotrophically grown cells. When cultures reached an OD_{650} of 0.6, aliquots were collected at 2–4 h intervals, and total RNA was prepared as described in the Materials and methods section. Figure 4 shows the levels of *cbbL* and *cbbM* transcripts measured using qRT-PCR from total cellular RNA samples. In cells growing autotrophically, *cbbL* was actively transcribed, with two distinct peaks of expression at 15:00 h (12.4 ± 1.1 pg of mRNA per 10^6 cells) and 03:00 h (15.4 ± 1.1 pg of mRNA per 10^6 cells). In contrast, under mixotrophic conditions (2 mM of acetate), the expression of *cbbL* remained approximately 10-fold lower than the expression under autotrophic conditions. Although the expression was lower, under mixotrophic conditions, a high level of expression was recorded at 15:00 and 03:00 h, with 0.88 ± 0.004 and 0.69 ± 0.004 pg of mRNA per 10^6 cells, respectively. Moreover, the sampling point showing the minimal expression of *cbbL* was at 23:00 h, with 0.12 ± 0.014 pg of mRNA per 10^6 cells corresponding to the minimal expression under autotrophic conditions, with 3.21 ± 0.036 pg of mRNA per 10^6 cells.

Expression of the locus that codes for form II RuBisCO remained constant and at a low level throughout the experiment for both incubation conditions. The expression of *cbbM* was approximately 5-fold higher in autotrophic compared to mixotrophic conditions. However, the maximum level of expression, at 09:00 h (0.06 ± 0.009 pg of mRNA per 10^6 cells) in autotrophic and at 19:00 h (0.017 ± 0.002 pg of mRNA per 10^6 cells) in mixotrophic growing conditions, and the minimum level of expression, at 07:00 h (0.022 ± 0.006 pg of mRNA per 10^6 cells) in autotrophic and at 11:00 h (0.004 ± 0.001 pg of mRNA per 10^6 cells) in mixotrophic growing conditions, were independent of each other.

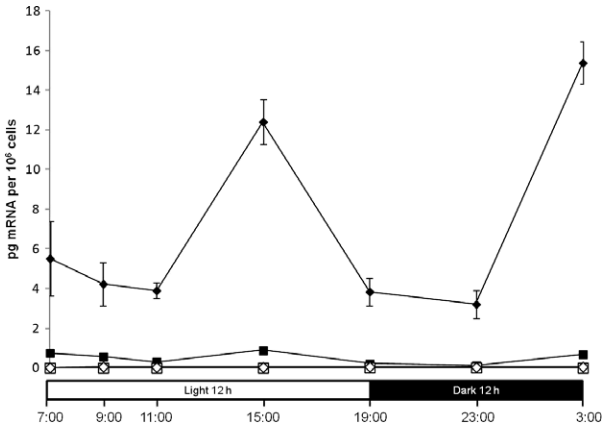

Fig. 4. Levels of *Candidatus* 'T. syntrophicum' Cad16^T *cbbL* (black) and *cbbM* (white) transcripts measured using qRT-PCR. Cells of strain Cad16^T were grown under laboratory conditions in the presence (mixotrophic; squares) or absence (autotrophic; diamonds) of acetate, using light/dark photoperiods of 12 h. The data points are reported as pg of each transcript per 10^6 cells and represent the means of three biological replicates, with standard deviations shown as vertical bars.

Discussion

The chemocline of the meromictic Lake Cadagno harbors a complex microbial ecosystem in which phototrophic sulfur bacteria play a key role in inorganic carbon fixation (Camacho *et al.*, 2001). Using dialysis bags that allowed bacterial cultures to remain pure while exposing and equilibrating them to their natural environment (Simek *et al.*, 2001, 2006; Corno & Jürgens, 2006; Blom *et al.*, 2010; Lindström & Ostman, 2011), we were able to assess their CO_2 assimilation in the chemocline at a depth of 12 m (Fig. 2). Among the strains representing the four most abundant populations of phototrophic sulfur bacteria in the chemocline, *Candidatus* 'T. syntrophicum' Cad16^T was the most effective CO_2-fixing organism, in both the presence and the absence of light. Surprisingly, the most abundant phototrophic sulfur bacterial population in the chemocline, the GSB *C. clathratiforme* constituting 73% of total bacterial cells (Tonolla *et al.*, 2005; Decristophoris *et al.*, 2009; Gregersen *et al.*, 2009) was the least efficient in terms of CO_2 assimilation per cell, with values that were approximately 100-fold lower than those measured for the *Candidatus* 'T. syntrophicum' Cad16^T. This finding confirms previous results from the Lake Cadagno chemocline phototrophic community, which showed that *C. clathratiforme* was clearly a less effective assimilator of CO_2 than were *Chromatium okenii* and *L. purpurea* (Musat *et al.*, 2008). In our study, *L. purpurea* CadA31 showed high rates of CO_2 assimilation similar to *Candidatus* 'T. syntrophicum' Cad16^T (Fig. 2b); moreover, these two species are also phylogenetically closely related (Garrity *et al.*, 2007). Musat *et al.* (2008) also showed that *C. okenii* (0.3% of the chemocline's population) contributed up to 70% of the total inorganic carbon fixed during daylight. A similar estimation is proposed by us in Table 3, taking in account both light and dark CO_2 assimilation, in which it can be seen that *Candidatus* 'T. syntrophicum' Cad16^T was the main assimilator measured, being responsible for about 25.9% of the total assimilation. However, the four most abundant populations of phototrophic sulfur bacteria under study were responsible for only 33.9% of the total CO_2 fixed in the chemocline, and the remaining 66.1% was not identified. According to Musat *et al.* (2008), we can speculate that the remaining 66.1% of CO_2 assimilation in the chemocline is carried out by *C. okenii*. Unfortunately, *C. okenii* could not be grown as a pure culture in the laboratory and therefore the CO_2 assimilation by this population could not be estimated using our approach.

The chemocline of Lake Cadagno showed high rates of carbon assimilation, not only in the presence of light of *c.* 297 ng of ^{14}C mL^{-1} but also in the dark at *c.* 231 ng of ^{14}C mL^{-1} (Fig. 2a). Similarly high CO_2 assimilation rates in the dark were also reported for other stratified lakes, such as the Spanish karstic lakes (Casamayor *et al.*, 2008, 2012; Casamayor, 2010), Lake Kinneret in Israel (Hadas *et al.*, 2001) and Big Soda Lake in Nevada (Cloern *et al.*, 1983). In Lake Cadagno, the populations represented by *Candidatus* 'T. syntrophicum' Cad16^T and *L. purpurea* CadA31 were responsible for approximately one-third (28.7%) of the dark assimilation (data not shown). Prior to the increase in the population of *C. clathratiforme* (Tonolla *et al.*, 2005; Decristophoris *et al.*, 2009; Gregersen *et al.*, 2009), analysis of the CO_2 assimilation in the chemocline showed high rates of CO_2 assimilation in the dark. At this time *C. okenii* was among the dominant populations of phototrophic sulfur bacteria (Schanz *et al.*, 1998; Camacho *et al.*, 2001). This might indicate that PSB, and in particular *C. okenii*, are the types of organisms mostly responsible for these high dark fixation rates. This speculation remains to be confirmed by further analysis. There is also the possibility that a different metabolic pathway (such as chemolithoautotrophy) in unknown organisms may contribute to the dark carbon fixation activity in this ecosystem (Yngve Borsheim *et al.*, 1985; Jorgensen *et al.*, 1991; Shively *et al.*, 1998), to the fermentation of stored glycogen (Gfeller & Gibbs, 1984; Habicht *et al.*, 2011), and to redox potential-balancing metabolisms (McKinlay & Harwood, 2010) or other unknown metabolisms (Martinez-Garcia *et al.*, 2011). To put it briefly, the chemocline of Lake Cadagno showed a high activity of dark CO_2 assimilation that is not completely understood.

Our long-term goal is to understand the microbial basis of CO_2 fixation in Lake Cadagno in the light and in the dark. For this purpose, we have established laboratory protocols to reproducibly cultivate and examine strains that we estimate represent the vast majority of the bacteria found in Lake Cadagno's chemocline – with the notable exception of *C. okenii*. The ability to maintain these bacteria as pure cultures allowed us to study the molecular mechanisms responsible for primary production. *Candidatus* 'T. syntrophicum' Cad16^T was used as a model organism to better understand the dynamics of carbon fixation in the chemocline of the Lake Cadagno. While rates of CO_2 assimilation in the light were comparable between *in vitro* (0.53 ± 0.08 pg of ^{14}C per cell) and *in situ* (0.61 ± 0.11 pg of ^{14}C per cell) conditions, this was not the case for rates in the dark. Indeed, the CO_2 assimilated *in vitro* in the first 4 h of dark, at *c.* 0.14 pg ^{14}C per cell, was approximately threefold lower than the value observed *in situ* in the dialysis bags *c.* 0.41 pg ^{14}C per cell. The absence of oxygen and organic substrates (e.g. acetate) in the autotrophic Pfennig medium I suggested two possible explanations. The absence of oxygen *in vitro* may prevent the possibility of chemolithotrophic metabolism, in a way similar to that reported for the PSB *Thiocapsa roseopersicina* (de Wit & van Gemerden, 1987; Schaub & van Gemerden, 1994; Ende *et al.*, 1996). However, the high rate of dark fixation normally combines different metabolic processes more than simple chemolithotrophy (Zopfi *et al.*, 2001). In a highly reduced environment such as the chemocline of the Lake Cadagno, the ability of cells to maintain the redox balance can be very important. Recently, it was proposed that CO_2 fixation is important not only as a primary production mechanism but also as an electron-accepting process in mixotrophic organisms (Richardson *et al.*, 1988; Hallenbeck *et al.*, 1990; Tavano *et al.*, 2005). The Calvin cycle in the purple non-sulfur bacterium *Rhodobacter palustris* was shown to re-oxidize nearly half of the reduced cofactors generated during the conversion of acetate to biomass, revealing that CO_2 fixation plays a major role in cofactor recycling (McKinlay & Harwood, 2010). The difficulty in cultivating organisms *in vitro* highlights the problems encountered in attempting to dissect a complex microbial ecosystem such as the chemocline of Lake Cadagno and underscores the need for techniques that would enable studies *in situ*, such as the dialysis bags.

High rates of CO_2 assimilation in the early hours of light exposure (Fig. 3) have also been observed in the diurnal cycles of cyanobacteria, and this often coincided with strong expression of RuBisCO-encoding genes (Pichard *et al.*, 1996; Wyman, 1999; Paul *et al.*, 2000). In the draft genome sequence of *Candidatus* 'T. syntrophicum' Cad16^T, we identified two RuBisCOs genes, a form I *cbbL* and a form II *cbbM* (Data S3). Transcription analyses confirmed that *cbbL* expression was higher at 15:00 and 03:00 h (Fig. 4). This suggests that a cyclic mechanism, possibly synchronized by light, regulates the expression of *cbbL* in autotrophic conditions. In contrast, the presence of a suitable carbon source (e.g. acetate) in the growth medium caused reduced transcription of *cbbL*. Similar observations were reported for other PSBs such as *Allochromatium vinosum* (Valle *et al.*, 1988; Kobayashi *et al.*, 1991). The absence of regulation of the transcription of *cbbM* under the tested growth conditions may suggest that this process is not regulated by environmental factors and therefore is constitutive. Differential expression of forms I and II of RuBisCO was also reported in other phototrophic bacteria, for which it was proposed that in presence of a reduced carbon source (e.g. acetate), CbbM functions primarily as a terminal electron acceptor involved in maintaining the redox balance of the cell (Wang *et al.*, 1993; Yoshizawa *et al.*, 2004; Badger & Bek, 2008; Joshi *et al.*, 2009; Laguna *et al.*, 2010).

In conclusion, our results show that of the four strains isolated from the chemocline of Lake Cadagno and for which we established *in situ* and *in vitro* growth conditions, PSB *Candidatus* 'Thiodictyon syntrophicum' strain Cad16^T exhibited the highest CO_2 assimilation activity, both in presence of light and in the dark. Laboratory experiments using pure cultures of *Candidatus* 'T. syntrophicum' Cad16^T grown in an autotrophic medium allowed us to detect that the maximal CO_2 assimilation rate occurred between 07:00 and 11:00 h, during the first 4 h of light (Fig. 2). However, this maximal activity did not correlate with the expression of any RuBisCO genes (*cbbL* and *cbbM*) (Fig. 3). In fact, RuBisCO form I *cbbL* showed two peaks of expression at 15:00 and 03:00 h, which occur more than 4 h before and 4 h the main assimilation period. The measured expression of RuBisCOs genes suggested that form I *cbbL* is dependent on environmental conditions, such as light exposure and carbon sources, whereas the form II *cbbM* gene appears to be constitutively expressed.

Acknowledgements

Financial support for this project was provided by the University of Geneva, the Institute of Microbiology and State of Ticino, and the Swiss National Science Foundation (grant no. 31003A-116591). The authors are indebted to P. Decristophoris, A. P. Caminada, N. Ruggeri and C. Strambio-De-Castillia for technical and moral support. Furthermore, we are grateful to S. Bianchi, R. Bernasconi and M. Molinari, Institute for Research in Biomedicine (IRB), in Bellinzona (Switzlerland), for their technical

support. The authors would also like to thank the FEMS and Alpine Biology Center Foundation (ABC) for their support.

References

Badger MR & Bek EJ (2008) Multiple Rubisco forms in proteobacteria: their functional significance in relation to CO_2 acquisition by the CBB cycle. *J Exp Bot* **59**: 1525–1541.

Biebl H & Pfennig N (1979) Anaerobic CO_2 uptake by phototrophic bacteria. A review. *Ergeb Limnol* **12**: 48–58.

Blom JF, Hornak K, Simek K & Pernthaler J (2010) Aggregate formation in a freshwater bacterial strain induced by growth state and conspecific chemical cues. *Environ Microbiol* **12**: 2486–2495.

Bosshard PP, Santini Y, Gruter D, Stettler R & Bachofen R (2000) Bacterial diversity and community composition in the chemocline of the meromictic alpine Lake Cadagno as revealed by 16S rDNA analysis. *FEMS Microbiol Ecol* **31**: 173–182.

Camacho A, Erez J, Chicote A, Florin M, Squires M, Lehmann C & Bachofen R (2001) Microbial microstratification, inorganic carbon photoassimilation and dark carbon fixation at the chemocline of the meromictic Lake Cadagno (Switzerland) and its relevance to the food web. *Aquat Sci* **63**: 91–106.

Casamayor EO (2010) Vertical distribution of planktonic autotrophic *thiobacilli* and dark CO_2 fixation rates in lakes with oxygen-sulfide interfaces. *Aquat Microb Ecol* **59**: 217–228.

Casamayor EO, Garcia-Cantizano J & Pedros-Alio C (2008) Carbon dioxide fixation in the dark by photosynthetic bacteria in sulfide-rich stratified lakes with oxic-anoxic interfaces. *Limnol Oceanogr* **53**: 1193–1203.

Casamayor EO, Lliros M, Picazo A, Barberan A, Borrego CM & Camacho A (2012) Contribution of deep dark fixation processes to overall CO_2 incorporation and large vertical changes of microbial populations in stratified karstic lakes. *Aquat Sci-Res Acr Bound* **74**: 61–75.

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol Oceanogr* **14**: 454–458.

Cloern JE, Cole BE & Oremland RS (1983) Autotrophic processes in meromictic Big Soda Lake, Nevada. *Limnol Oceanogr* **28**: 1049–1061.

Corno G & Jürgens K (2006) Direct and indirect effects of protist predation on population size structure of a bacterial strain with high phenotypic plasticity. *Appl Environ Microbiol* **72**: 78–86.

de Wit R & van Gemerden H (1987) Chemolithotrophic growth of the phototrophic sulfur bacterium *Thiocapsa roseopersicina*. *FEMS Microbiol Lett* **45**: 117–126.

Decristophoris PMA, Peduzzi S, Ruggeri-Bernardi N, Hahn D & Tonolla M (2009) Fine scale analysis of shifts in bacterial community structure in the chemocline of meromictic Lake Cadagno, Switzerland. *J Limnol* **68**: 16–24.

Dimitriu PA, Pinkart HC, Peyton BM & Mormile MR (2008) Spatial and temporal patterns in the microbial diversity of a meromictic soda lake in Washington State. *Appl Environ Microbiol* **74**: 4877–4888.

Eichler B & Pfennig N (1988) A new purple sulfur bacterium from stratified freshwater lakes. *Amoebobacter purpureus* sp. nov. *Arch Microbiol* **149**: 395–400.

Ellis RJ (1979) The most abundant protein in the world. *T Biochem Sci* **4**: 241–244.

Ende FP, Laverman AM & van emerden H (1996) Coexistence of aerobic chemotrophic and anaerobic phototrophic sulfur bacteria under oxygen limitation. *FEMS Microbiol Ecol* **19**: 141–151.

Fischer K, Hahn D, Daniel O, Zeyer J & Amann RI (1995) *In situ* analysis of the bacterial community in the gut of the earthworm *Lumbricus terrestris* L. by whole-cell hybridization. *Can J Microbiol* **41**: 666–673.

Gächter R & Mares A (1979) Comments on the acidification and bubbling methods for determining phytoplankton production. *Oikos* **33**: 69–73.

García-Cantizano J, Casamayor E, Gasol J, Guerrero R & Pedrós-Alió C (2005) Partitioning of CO_2 incorporation among planktonic microbial guilds and estimation of *in situ* specific growth rates. *Microb Ecol* **50**: 230–241.

Garrity GM, Lilburn TG, Cole JR, Harrison SH, Euzéby J & Tindall BJ (2007) Taxonomic outline of the Bacteria and Archaea. Part 6–Phylum 'Proteobacteria': The Deltaproteobacteria and Epsilonproteobacteria. *Taxonomic Outline of Bacteria and Archaea* Release 7.7: 148–245.

Gfeller RP & Gibbs M (1984) Fermentative metabolism of *Chlamydomonas reinhardtii* I. Analysis of fermentative products from starch in dark and light. *Plant Physiol* **75**: 212–218.

Glöckner FO, Amann R, Alfreider A, Pernthaler J, Psenner R, Trebesius K & Schleifer KH (1996) An *in situ* hybridization protocol for detection and identification of planktonic bacteria. *Syst Appl Microbiol* **19**: 403–406.

Gregersen LH, Habicht KS, Peduzzi S *et al.* (2009) Dominance of a clonal green sulfur bacterial population in a stratified lake. *FEMS Microbiol Ecol* **70**: 30–41.

Habicht KS, Miller M, Cox RP *et al.* (2011) Comparative proteomics and activity of a green sulfur bacterium through the water column of Lake Cadagno, Switzerland. *Environ Microbiol* **13**: 203–215.

Hadas O, Pinkas R & Erez J (2001) High chemoautotrophic primary production in Lake Kinneret, Israel: a neglected link in the carbon cycle of the lake. *Limnol Oceanogr* **46**: 1968–1976.

Hallenbeck PL, Lerchen R, Hessler P & Kaplan S (1990) Roles of CfxA, CfxB, and external electron acceptors in regulation of ribulose 1, 5-bisphosphate carboxylase/ oxygenase expression in *Rhodobacter sphaeroides*. *J Bacteriol* **172**: 1736–1748.

Hobbie JE, Daley RJ & Jasper S (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. *Appl Environ Microbiol* **33**: 1225–1228.

Jorgensen BB, Fossing H, Wirsen CO & Jannasch HW (1991) Sulfide oxidation in the anoxic Black Sea chemocline. *Deep Sea Res I* **38**: S1083–S1103.

Joshi GS, Romagnoli S, VerBerkmoes NC, Hettich RL, Pelletier D & Tabita FR (2009) Differential accumulation of form I RubisCO in *Rhodopseudomonas palustris* CGA010 under photoheterotrophic growth conditions with reduced carbon sources. *J Bacteriol* **191**: 4243–4250.

Kämpf C & Pfennig N (1986) Chemoautotrophic growth of *Thiocystis violacea*, *Chromatium gracile* and *C. vinosum* in the dark at various O_2-concentrations. *J Basic Microbiol* **26**: 517–531.

Kobayashi H, Viale AM, Takabe T *et al.* (1991) Sequence and expression of genes encoding the large and small subunits of ribulose 1, 5-bisphosphate carboxylase/oxygenase from *Chromatium vinosum*. *Gene* **97**: 55–62.

Laguna R, Joshi GS, Dangel AW, Luther AK & Tabita FR (2010) Integrative control of carbon, nitrogen, hydrogen, and sulfur metabolism: the central role of the Calvin-Benson-Bassham cycle. *Rec Adv Phototr Prok* **675**: 265–271.

Lindström ES & Ostman O (2011) The importance of dispersal for bacterial community composition and functioning. *PLoS ONE* **6**: e25883.

Lüthy L, Fritz M & Bachofen R (2000) *In situ* determination of sulfide turnover rates in a meromictic alpine lake. *Appl Environ Microbiol* **66**: 712.

Martinez JP, Garay E, Alcaide E & Hernandez E (1983) The genus *Thiobacillus*: physiology and industrial applications. *Acta Biotechnol* **3**: 99–124.

Martinez-Garcia M, Swan BK, Poulton NJ, Gomez ML, Masland D, Sieracki ME & Stepanauskas R (2011) High-throughput single-cell sequencing identifies photoheterotrophs and chemoautotrophs in freshwater bacterioplankton. *ISME J* **6**: 113–123.

McKinlay JB & Harwood CS (2010) Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria. *P Natl Acad Sci USA* **107**: 11669–11675.

Mulkidjanian A, Koonin E, Makarova K *et al.* (2006) The cyanobacterial genome core and the origin of photosynthesis. *P Natl Acad Sci USA* **103**: 13126–13131.

Musat N, Halm H, Winterholler B *et al.* (2008) A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. *P Natl Acad Sci USA* **105**: 17861–17866.

Parkin TB & Brock TD (1981) The role of phototrophic bacteria in the sulfur cycle of a meromictic lake. *Limnol Oceanogr* **26**: 880–890.

Paul J, Kang JB & Tabita FR (2000) Diel patterns of regulation of *rbcL* transcription in a *Cyanobacterium* and a *Prymnesiophyte*. *Mar Biotechnol* **2**: 429–436.

Peduzzi S, Welsh A, Demarta A, Decristophoris P, Peduzzi R, Hahn D & Tonolla M (2011) *Thiocystis chemoclinalis sp.* nov. and *Thiocystis cadagnonensis sp.* nov., two new motile purple sulfur bacteria isolated from the chemocline of meromictic Lake Cadagno, Switzerland. *Int J Syst Evol Microbiol* **61**: 1682–1687.

Peduzzi S, Storelli N, Welsh A, Peduzzi R, Hahn D, Perret X & Tonolla M (2012) *Candidatus* 'Thiodictyon syntrophicum', sp. nov., a new purple sulfur bacterium isolated from the chemocline of Lake Cadagno forming aggregates and specific associations with *Desulfocapsa* sp. *Syst Appl Microbiol* **35**: 139–144.

Pichard SL, Campbell L, Kang JB, Tabita FR & Paul JH (1996) Regulation of ribulose bisphosphate carboxylase gene expression in natural phytoplankton communities. I. Diel rhythms. *MEPS* **139**: 257–265.

Pimenov NV, Rusanov II, Karnachuk OV *et al.* (2003) Microbial processes of the carbon and sulfur cycles in Lake Shira (Khakasia). *Microbiology* **72**: 221–229.

Richardson DJ, King GF, Kelly DJ, McEwan AG, Ferguson SJ & Jackson JB (1988) The role of auxiliary oxidants in maintaining redox balance during phototrophic growth of *Rhodobacter capsulatus* on propionate or butyrate. *Arch Microbiol* **150**: 131–137.

Saito Y, Ashida H, Sakiyama T, de Marsac NT, Danchin A, Sekowska A & Yokota A (2009) Structural and functional similarities between a ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO)-like protein from *Bacillus subtilis* and photosynthetic RuBisCO. *J Biol Chem* **284**: 13256–13264.

Schanz F, Fischer-Romero C & Bachofen R (1998) Photosynthetic production and photoadaptation of phototrophic sulfur bacteria in Lake Cadagno (Switzerland). *Limnol Oceanogr* **43**: 1262–1269.

Schaub BEM & van Gemerden H (1994) Simultaneous phototrophic and chemotrophic growth in the purple sulfur bacterium *Thiocapsa roseopersicina* M1. *FEMS Microbiol Ecol* **13**: 185–195.

Schindler DW, Schmidt RV & Reid RA (1972) Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the 14C method. *Can J Fish Aquat Sci* **29**: 1627–1631.

Schneider G, Lindqvist Y & Branden CI (1992) RUBISCO: structure and mechanism. *Annu Rev Bioph Biom* **21**: 119–143.

Shively J, Van Keulen G & Meijer W (1998) Something from almost nothing: carbon dioxide fixation in chemoautotrophs. *Annu Rev Microbiol* **52**: 191–230.

Simek K, Pernthaler J, Weinbauer MG *et al.* (2001) Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. *Appl Environ Microbiol* **67**: 2723–2733.

Simek K, Hornak K, Jezbera J *et al.* (2006) Maximum growth rates and possible life strategies of different bacterioplankton groups in relation to phosphorus availability in a freshwater reservoir. *Environ Microbiol* **8**: 1613–1624.

Tabita F (2005) Research on carbon dioxide fixation in photosynthetic microorganisms (1971–present). *Photosynth Res* **80**: 315–332.

Tabita FR, Satagopan S, Hanson TE, Kreel NE & Scott SS (2008) Distinct form I, II, III, and IV Rubisco proteins from the three kingdoms of life provide clues about Rubisco evolution and structure/function relationships. *J Exp Bot* **59**: 1515–1524.

Tavano CL, Podevels AM & Donohue TJ (2005) Identification of genes required for recycling reducing power during photosynthetic growth. *J Bacteriol* **187**: 5249–5258.

Tonolla M, Demarta A, Peduzzi R & Hahn D (1999) *In situ* analysis of phototrophic sulfur bacteria in the chemocline of meromictic Lake Cadagno (Switzerland). *Appl Environ Microbiol* **65**: 1325–1330.

Tonolla M, Peduzzi S, Hahn D & Peduzzi R (2003) Spatio-temporal distribution of phototrophic sulfur bacteria in the chemocline of meromictic Lake Cadagno (Switzerland). *FEMS Microbiol Ecol* **43**: 89–98.

Tonolla M, Peduzzi S, Demarta A, Peduzzi R & Hahn D (2004) Phototropic sulfur and sulfate-reducing bacteria in the chemocline of meromictic Lake Cadagno, Switzerland. *J Limnol* **63**: 161–170.

Tonolla M, Peduzzi R & Hahn D (2005) Long-term population dynamics of phototrophic sulfur bacteria in the chemocline of Lake Cadagno, Switzerland. *Appl Environ Microbiol* **71**: 3544–3550.

Valle E, Kobayashi H & Akazawa T (1988) Transcriptional regulation of genes for plant-type ribulose-1, 5-bisphosphate carboxylase/oxygenase in the photosynthetic bacterium, *Chromatium vinosum*. *Eur J Biochem* **173**: 483–489.

Wang X, Falcone DL & Tabita FR (1993) Reductive pentose phosphate-independent CO_2 fixation in *Rhodobacter sphaeroides* and evidence that ribulose bisphosphate carboxylase/oxygenase activity serves to maintain the redox balance of the cell. *J Bacteriol* **175**: 3372–3379.

Widdel F & Bak F (1992) Gram-negative mesophilic sulfate-reducing bacteria. *Prokaryotes* **4**: 3352–3378.

Wyman M (1999) Diel rhythms in ribulose-1, 5-bisphosphate carboxylase/oxygenase and glutamine synthetase gene expression in a natural population of marine picoplanktonic cyanobacteria (*Synechococcus spp.*). *Appl Environ Microbiol* **65**: 3651–3659.

Yngve Borsheim K, Gijs Kuenen J, Gottschal J & Dundas I (1985) Microbial activities and chemical gradients in the chemocline of a meromictic lake in relation to the precision of the sampling procedure. *FEMS Microbiol Lett* **31**: 337–341.

Yoshizawa Y, Toyoda K, Arai H, Ishii M & Igarashi Y (2004) CO_2-responsive expression and gene organization of three ribulose-1, 5-bisphosphate carboxylase/oxygenase enzymes and carboxysomes in Hydrogenovibrio marinus strain MH-110. *J Bacteriol* **186**: 5685–5691.

Zarda B, Hahn D, Chatzinotas A, Schönhuber W, Neef A, Amann RI & Zeyer J (1997) Analysis of bacterial community structure in bulk soil by *in situ* hybridization. *Arch Microbiol* **168**: 185–192.

Zopfi J, Ferdelman TG, Jorgensen BB, Teske A & Thamdrup B (2001) Influence of water column dynamics on sulfide oxidation and other major biogeochemical processes in the chemocline of Mariager Fjord (Denmark). *Mar Chem* **74**: 29–51.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Recipe of trace elements SL10 and SL12.
Data S2. Dissolved inorganic carbon (DIC) and pH from dialysis bags.
Data S3. Genome analysis.
Table S1. Physical parameters of the Lake Cadagno of the 12 September 2007.
Fig. S1. Specific FISH counting of GSB (white) and PSB (black) compared to the total prokaryotic cells counted by DAPI (grey) at different depths of Lake Cadagno during the day of the $^{14}CO_2$ assimilation analysis from the cultures pre-incubated in dialysis bags (*in situ*, September 12, 2007).