The following supplement accompanies the article

Green sulphur bacteria as a component of the photosynthetic plankton community in small dimictic humic lakes with an anoxic hypolimnion

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Supplement. A more detailed explanation of sample preparation, inorganic nutrient measurements and dissolved organic carbon

MATERIALS AND METHODS

Sampling procedure

Water was stored on ice in 1.0 l plastic containers and transported to the laboratory where sample processing was carried out within 6 h of sample collection. Water samples for the LH-PCR analysis were stored in 2 ml tubes at –20°C. For clone libraries 100 ml of lake water was frozen and freeze dried with an Alpha 1-4 LD plus (Christ). *In situ* profiles of water temperature, oxygen concentration, redox and pH were measured from every sampling point using a portable field meter (YSI 556 MPS, Yellow Spring Instruments). Dissolved inorganic phosphate and nitrate and ammonium concentrations were determined after the samples were filtered through 0.2 µm Millipore membrane filters. The detection limit for dissolved inorganic P was 2 mg m^{-3} and for the inorganic fractions of N was 10 mg m^{-3}. Total phosphorus and total nitrogen concentrations were determined after wet oxidation with a Lachat FIA analyser (Koroleff 1983). Water colour was determined as absorption at 420 nm with a spectrophotometer after filtration through 0.2 µm membrane or GF/C glass fibre filters (no significant difference in the filtration efficiency between the filters was found) and against Pt-Co standards. Dissolved organic carbon was determined by combustion at 900 to 950°C (Salonen 1979) or, after 2001, at 680°C with a Shimadzu TOC 5000 analyzer.

Bacterial lysates

Bacterial samples in 2 ml Eppendorf tubes were briefly thawed on ice and centrifuged (20000 × *g*, 5 min). Supernatant was removed, and 50 µl of 0.05 M NaOH/0.25% SDS solution was added to the tubes. The lysates were heated at 95°C in a water bath for 15 min and finally diluted with 950 µl of MQ-water.

Sequencing

DNA sequences were edited with ContiqExpress (Invitrogen), and the sequences (~880 bp) were compared to the GenBank database using BLAST software (Altschul et al. 1997) and the Ribosomal Database Project II programs Seqmatch and Classifier (www.rdp.cme.msu.edu). MEGA4 (Tamura et al. 2007) was used for sequence alignment and for constructing a neighbour-joining tree for selected *Chlorobium* sp. clones. The sequences are deposited in the EMBL database under Accession Numbers

HE793329 to HE793375 and HF543675 to HF543817. The sequences were divided in operational taxonomic units (OTUs) by using the CD-hit program (Huang et al. 2010), with a 97% sequence identity cut-off level.

Chlorophyll correction

For bacteriochlorophyll (BChl) determinations, 0.12 to 0.5 l sample from each water layer was filtered through a GF/C filter (45 mm diameter, Whatman) and measured as in Arvola et al. (1992). The concentration of BChl was calculated according to Takahashi & Ichimura (1970) (equation for BChl *d*), and the concentration of chlorophyll *a* (chl *a*) was calculated according to Lorenzen (1967). To correct the effect of spectral overlapping of chl *a* and BChl peaks, a model was developed from data of water layers where only one of the chlorophylls was present based on the knowledge of oxygen conditions. In these layers the relation between Abs665 and Abs654 was constant. For BChl, the absorbance correction formula was 654cor = (Abs654 – 0.53 × Abs665)/(1 – 0.51 × 0.53) (R^2 = 0.99) and for chl *a* 665cor = Abs665 – 654cor × 0.51 (R^2 = 0.96) (Fig. S1).

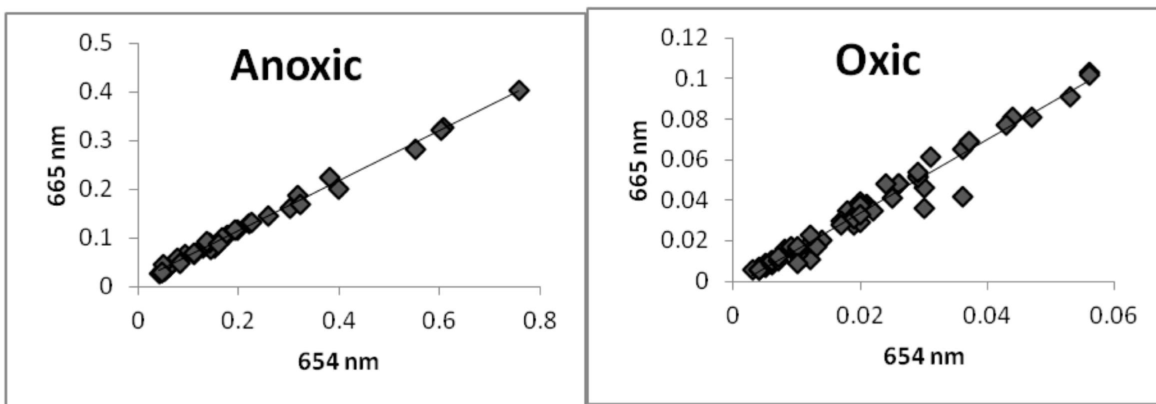

Fig. S1. Relationship between Abs_{665} and Abs_{654} in anoxic water layers, where bacteriochlorophyll forms the chlorophyll, and in oxic water layers, where chlorophyll *a* forms the chlorophyll in green sulphur bacteria-positive lakes

RESULTS

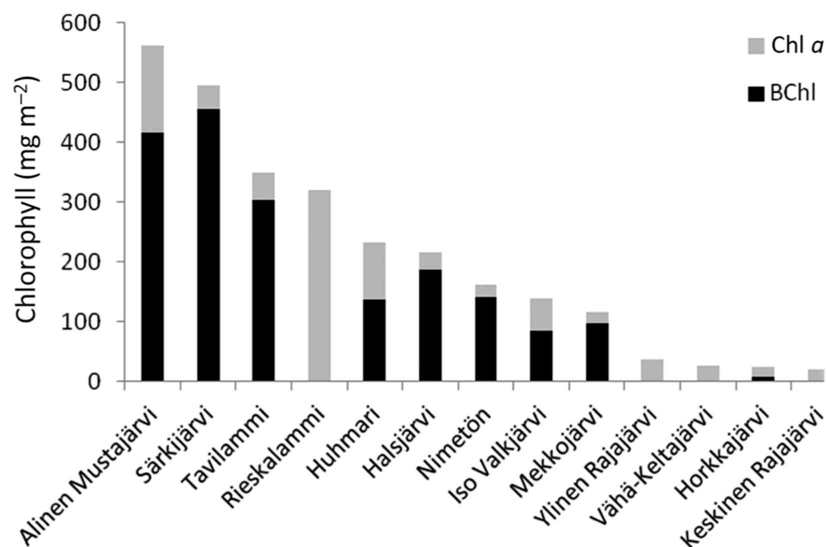

Fig. S2. Total chlorophyll amount (separated to chlorophyll *a* [chl a] and bacteriochlorophyll [BChl]) of the integrated water profiles of the study lakes

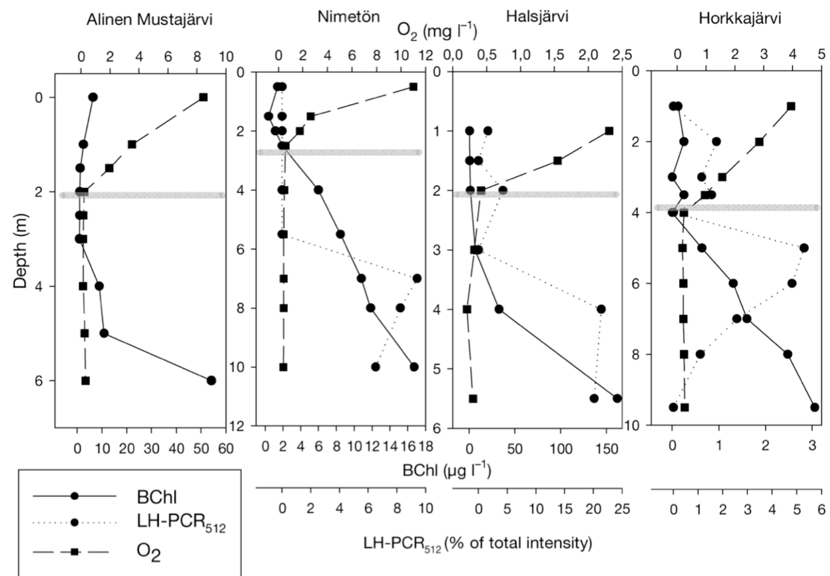

Fig. S3. Vertical winter profiles of green sulphur bacteria based on the relative proportion of LH-PCR_{512} biomarker, oxygen content and bacteriochlorophyll (BChl) concentrations. The oxic–anoxic boundary layer is indicated with a grey line

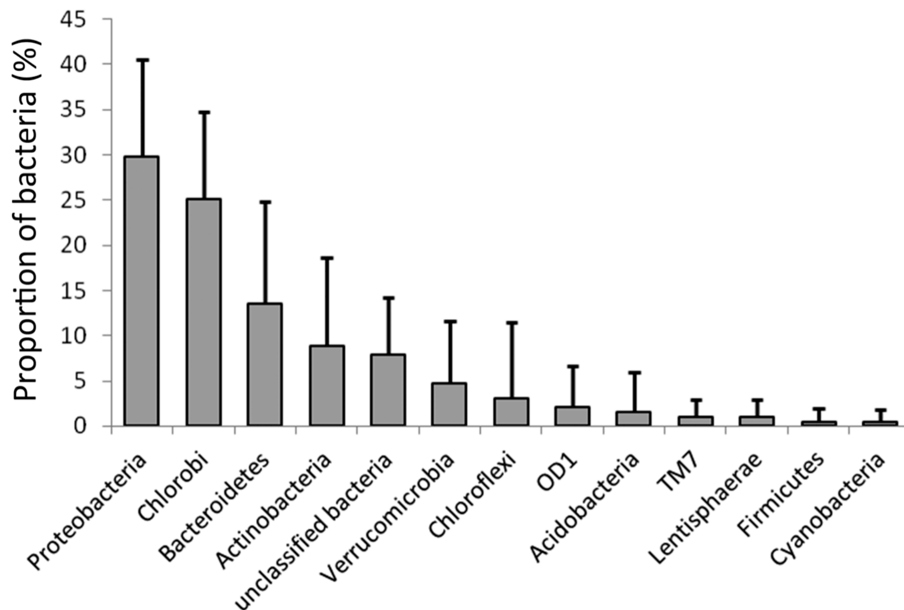

Fig. S4. Classification of the 16S rRNA gene sequences from libraries obtained from the depth of maximum bacteriochlorophyll concentration in the 8 green sulphur bacteria–positive lakes (n = 192, error bars indicate standard deviations between lakes). OD1 and TM7 are candidate phyla

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