**Figure S1** Organic Lake expedition. A) Schematic of the Vestfold Hills showing the location of Organic Lake, adapted from Gibson (Gibson, 1999). The Vestfold Hills is approximately 400 km2 in area and contains a remarkable diversity of more than 300 lakes which range in salinity from fresh to hypersaline (Gibson, 1999). Most of the saline lakes were originally pockets of seawater and retain a marine-derived biota (Gibson, 1999). B) Aerial photograph of Organic Lake (inset), and surrounds including Ace Lake, Long Fjord and the open Southern Ocean. C) Sampling site at Organic Lake showing mobile work shelters (MWSs) tethered by ropes and ice screws for protection against strong winds (up to 140 km/h in 2008/09), and sampling equipment, thermometer and YSI probe. The hole in the floor of the MWS enabled direct access to lake water below the surface ice. D) Surveying the water level of Organic Lake and taking lake bathymetry measurements (see Figure S2).

**Figure S2** Vertical profiles of physical and chemical parameters of Organic Lake taken *in situ* at the deepest point in the lake on 9 November 2008. σT (1000−density) was calculated from temperature and conductivity.

**Figure S3** Bathymetry of Organic Lake 9 November 2008.

**Figure S4** Epifluorescence microscopy images of Organic Lake microbiota (<20 µm) filtered onto 0.01 µm polycarbonate membrane and stained with SYBR Gold. (**A**) 1.7 m, (**B**) 4.2 m, (**C**) 5.7 m, (**D**) 6.5 m, (**E**) 6.7 m. Scale bar = 20 µm.

**Figure S5** PCA analysis of physico-chemical parameters and cell/VLP counts of Organic Lake profile. Data points are the sampling depths 1.7, 4.2, 5.7, 6.5 and 6.7 m. The overlaid vector diagram shows the relative contribution of the variables to explaining the difference between samples. PC1 explained 74.3% and PC2 14.7% of the variation between samples. Abbreviations: cond, conductivity; temp, temperature; turb, turbidity.

**Figure S6** Phylogenetic tree of rhodopsin homologs including proteorhodopsin, bacteriorhodopsin, actinorhodopsin and xanthorhodopsin. *Halobacterim salinarum* R1 halorhodopsin was used as an out-group. The tree was computed from a 78 amino acid region spanning the motif involved in ‘spectral tuning’ using the neighbor-joining algorithm. Organic Lake sequences from this study are shown in red and marked with an asterisk (\*). Numbers in parentheses are counts of sequences which clustered with the Organic Lake homolog shown in the tree with 90% amino acid identity. Sequences with confirmed activity are shown in bold. Accession numbers from top to bottom are: EAZ99241, EDP63929, EGF32634, ZP\_09955974, AEG32267, EDY76405, EDY88259, YP\_445623, ACN42850, EIC91904, ZP\_02194911, AAZ21446, AAT38609, AEE49633, EAS71907, sequence from John Bowman (personal communication), EAQ40507, EAQ40925, EAR12394, EHQ04368, EAZ94876, EIA08356, AEE20201, EEG43331, ZP\_09501337 and YP\_001689404.

**Figure S7** Genomic maps of Organic Lake scaffolds containing the OL-R1 rhodopsin homolog. All genes surrounding OL-R1 had best BLAST matches to *Octadecabacter* sequences. The scale below shows the number of base pairs. The sample depth and filter from which the scaffold was assembled is shown in parentheses beside the scaffold ID.

**Figure S8** Phylogenetic tree of DddD DMSP lyase homologs. *E. coli* carnitine coenzyme A transferase was used as an out-group. *Dinoroseobacteria shibae* DFL 12 and *Ruegeria pomeroyi* DSS-3 homologs are non-functional outgroup (Todd *et al.*, 2011). The tree was computed from a 75 amino acid region within the conserved amino-terminal class III coenzyme A domain (CaiB) using the neighbor-joining algorithm. Organic Lake sequences from this study are shown in red and marked with an asterisk (\*).Numbers in parentheses are counts of sequences which clustered with the Organic Lake homolog shown in the tree with 90% amino acid identity. Sequences with confirmed DMSP lyase activity are shown in bold. Accession numbers from top to bottom are: EBA01716, AEV37420, ACY01992, ADZ91595, EAQ63474, ABR72937, ACV84065, ACY02894, ABI89851, YP\_002822700, EEE36156, ABV95365, AAV94987 and EGB36199.

**Figure S9** Genomic maps of Organic Lake scaffolds containing the OL-dddD homolog. DddT and choline dehydrogenase had best BLAST matches to *Halomonas* sp. HTNK1(*Gammaproteobacteria*) and *Hoeflea phototrophica* DFL-43 (*Alphaproteobacteria*), respectively. The numbers represent base pairs. The sample depth and filter from which the scaffold was assembled is shown in parentheses beside the scaffold ID.

**Figure S10** Phylogenetic tree of DddL DMSP lyase homologs from Organic Lake and public databases. The tree was computed from an 84 amino acid N-terminal region using the neighbor-joining algorithm. Organic Lake sequences from this study are shown in red and marked with an asterisk (\*). Numbers in parentheses are counts of sequences which clustered with the Organic Lake homolog shown in the tree with 90% amino acid identity. Sequences with confirmed DMSP lyase activity are shown in bold. Accession numbers from top to bottom are: EEB86351, ADK55772, EAQ07081, EEE47811, EAV43167, EAU41122, EAQ10619, ABV95046, EAQ04071, ABA77574 and EHJ04839.

**Figure S11** Phylogenetic tree of DddP DMSP lyase homologs from Organic Lake and public databases. The tree was computed from a 129 amino acid C-terminal region including the predicted catalytic sites using the neighbor-joining algorithm. Organic Lake sequences from this study are shown in red and marked with an asterisk (\*). Numbers in parentheses are counts of sequences which clustered with the Organic Lake homolog shown in the tree with 90% amino acid identity. Sequences with confirmed DMSP lyase activity are shown in bold. Accession numbers from top to bottom are: ZP\_01755203, YP\_167522, YP\_613011, YP\_682809, EAP77700, ZP\_01741265, ZP\_01036399, ZP\_01881042, ZP\_05063825, AFO91571, YP\_509721, ZP\_01448542, AEQ39103, AEQ39091, XP\_001823911, XP\_389272 and ACF19795.

**Figure S12** Phylogenetic tree of DmdA DMSP demethylase homologs from Organic Lake and public databases. The tree was computed from a 128 amino acid region using the neighbor-joining algorithm. Organic Lake sequences from this study are shown in red and marked with an asterisk (\*). Numbers in parentheses are counts of sequences which clustered with the Organic Lake homolog shown in the tree with 90% amino acid identity. Sequences with confirmed DMSP lyase activity are shown in bold. Accession numbers from top to bottom are: EDZ60447, YP\_265671, EDZ61098, EAU51039, YP\_003550401, EDP61332, EAQ26389, ABV94056, AAV94935, AAV95190, EDY79173, EDY89914, EAW42451, AAV94935 and AAV97197.

**Table S1** Summary of metagenomic data for Organic Lake samples.

**Table S2** Full list of KEGG Orthologs (KO) involved in carbon, nitrogen and sulfur conversions that were searched for in the Organic Lake metagenome. Abbreviations: rTCA, tricarboxylic acid cycle; WL, Wood-Ljungdahl pathway; AAnP, aerobic anoxygenic phototrophy; DNRA, dissimilatory nitrate reduction to ammonia; anaerobic ammonia oxidation; ASR, assimilatory sulfate reduction; DSR, dissimilatory sulfate reduction; SRB, sulfate-reducing bacteria.

**Table S3** Sequences used in this study as BLAST queries for retrieving homologs in the Organic Lake metagenomes. (%ID) is the minimum amino acid sequence identity for matches to be considered a homolog.

**Table S4** Microbial taxa detected in the Organic Lake water column profile. Analysis of SSU gene sequences shown in phylum, class and genus ranks as defined by the SILVA taxonomy except RF3 which is placed with the *Firmicutes* according to Tajima *et al.* (1999).SSU gene sequences were classified to the genus level or to the lowest rank with bootstap confidence >85% (see materials and methods). The best BLAST matches to environmental SSU clone sequences are shown for the abundant candidate divisions RF3 and OD1.

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