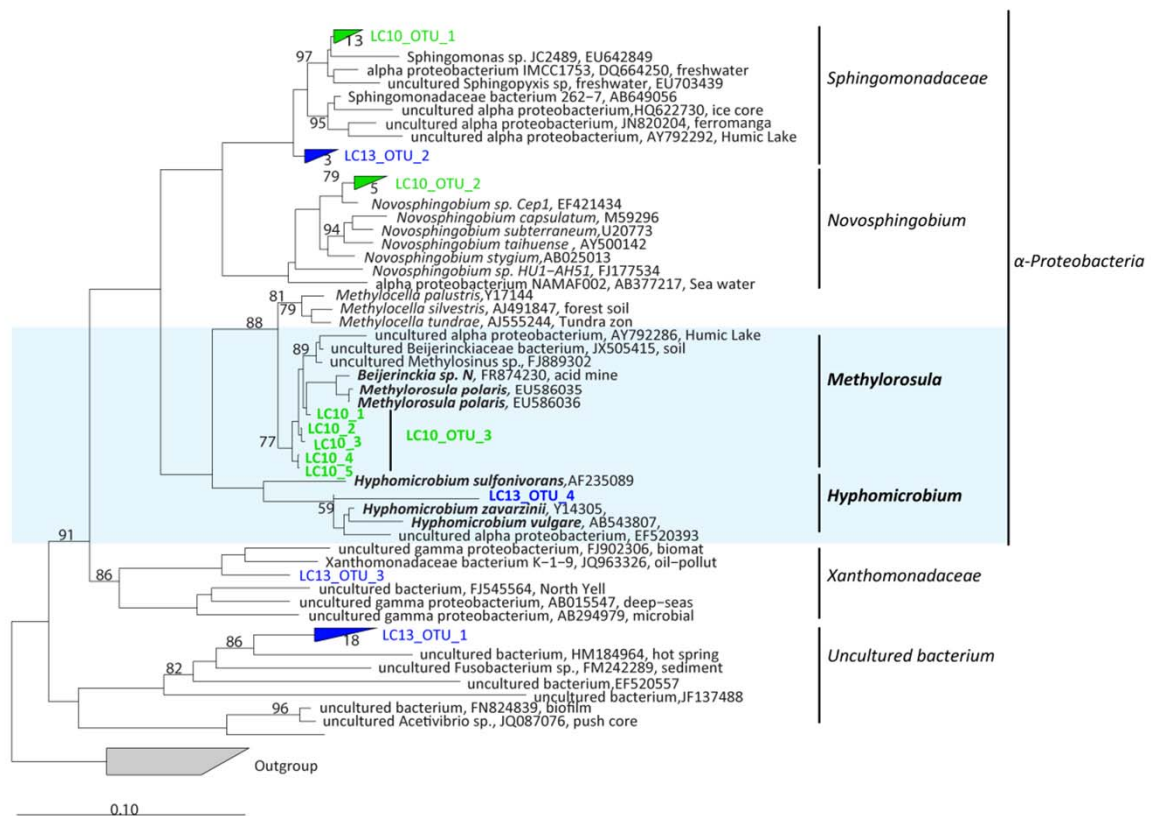
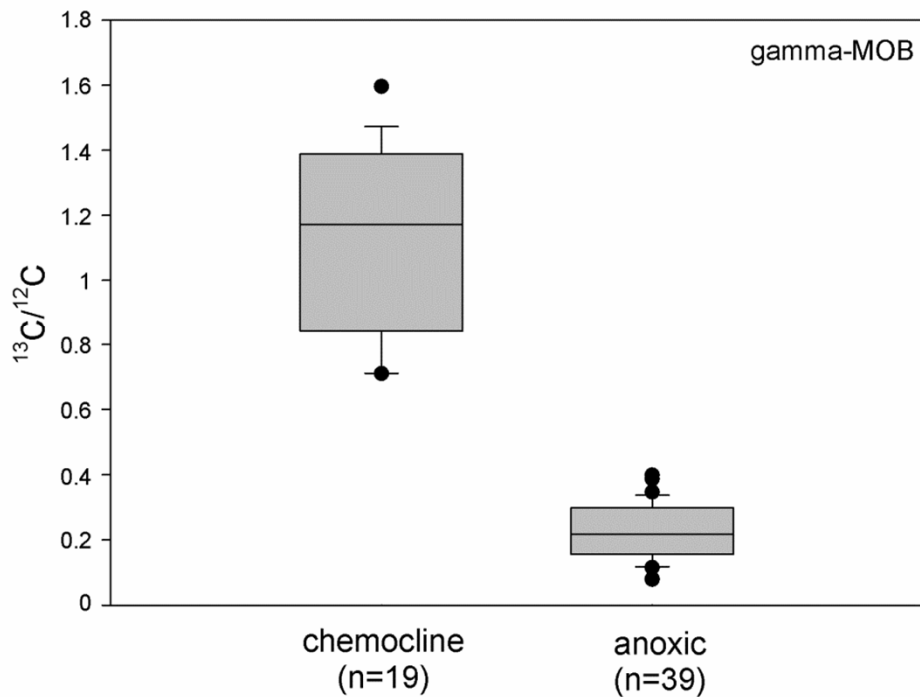


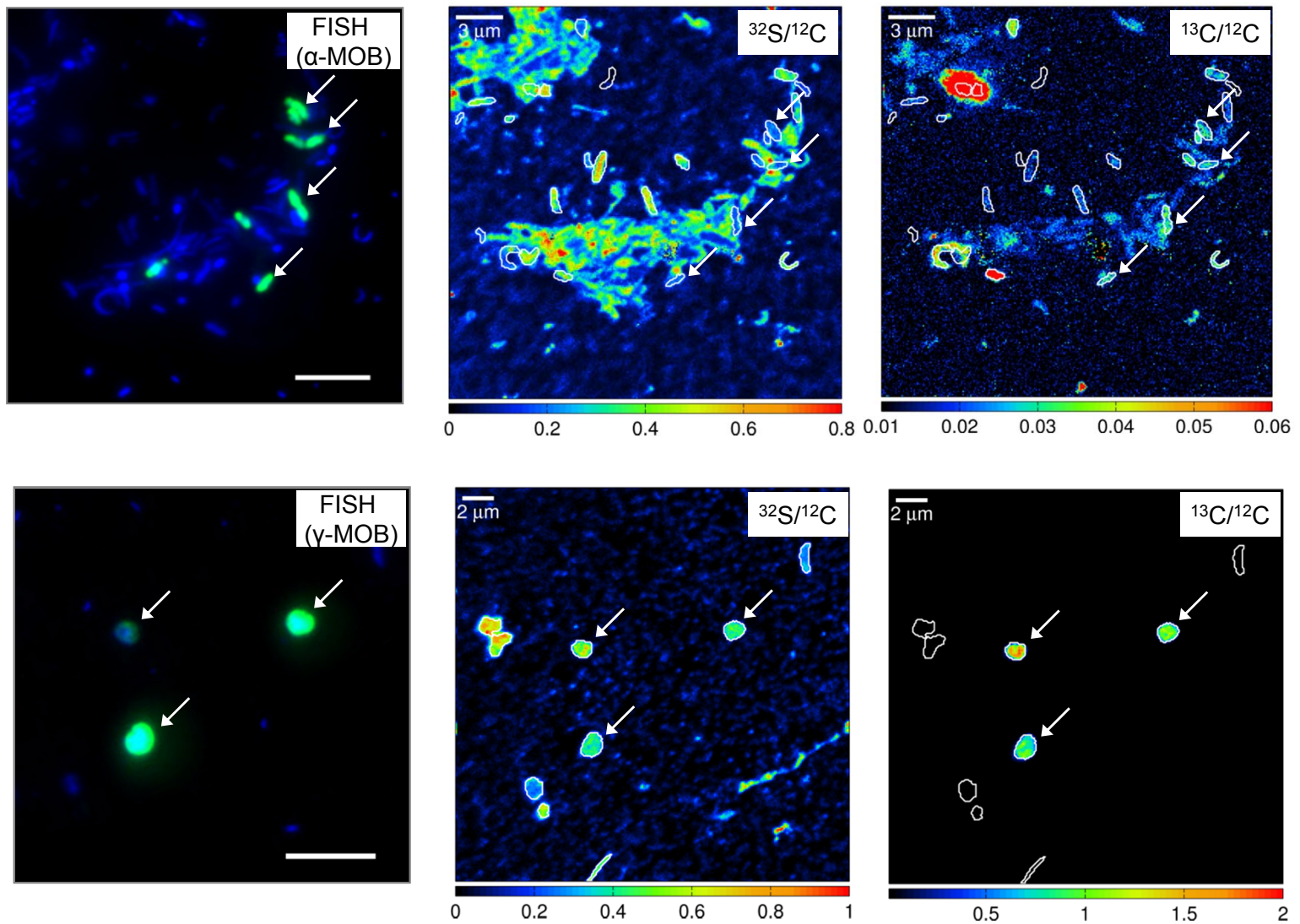
Supplementary Figure 1. Water column parameters from August 2011. Chemical profiles of oxygen (full line), sulfide (empty squares), ammonium (grey circles), methane (filled circles) and sulfate (filled triangles) concentrations. Zones of methane and sulfide oxidation are highlighted in light and dark grey, respectively. Note that the plotted sulfate concentrations are 10-fold lower than in reality.



Supplementary Figure 2. Phylogenetic tree of type II MOB 16S rRNA gene clone using a Maximum Likelihood method based on the Jukes-Cantor model. The partial 16S rRNA gene sequences were aligned to the SILVA reference database (SSURef 115, release data: 20-07-13). Clustering of operational taxonomic units (OTUs) of LC10 and LC13 indicate sequences from chemocline (10m, green) and anoxic (13m, blue) water of Lago di Cadagno with similarities of >97%. Bootstrap values higher than 70% are indicated at branch nodes. The scale bar represents the number of changes per nucleotide position.

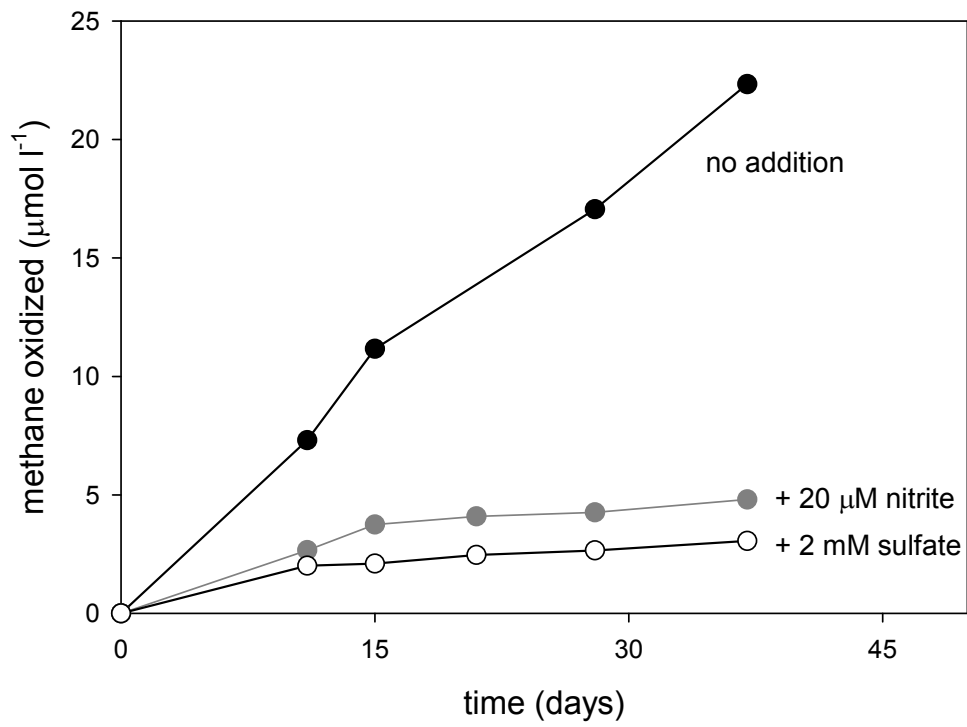


Supplementary Figure 3. The ^{13}C -enrichment of individual gamma-MOB due to $^{13}\text{CH}_4$ assimilation in samples from the chemocline and the anoxic water of Lago di Cadagno. All single cell measurements were performed by nanoSIMS. Box plots show the median enrichment, standard error and outliers for each depth sample. Note that all cells show a strong ^{13}C -enrichment ($^{13}\text{C}/^{12}\text{C}$ ratio >0.1) relative to natural abundance ($^{13}\text{C}/^{12}\text{C}$ ratio ~ 0.01) indicating substantial incorporation of $^{13}\text{CH}_4$.

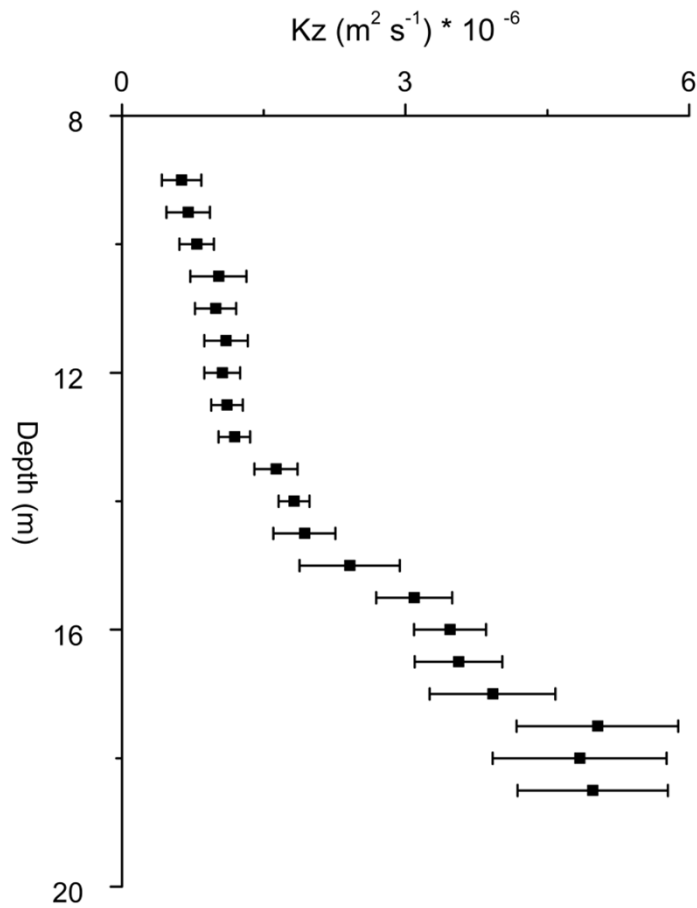


Supplementary Figure 4. Methane carbon assimilation by single cells of alpha (upper panel) and gamma (bottom panel) MOB from 11 m depth.

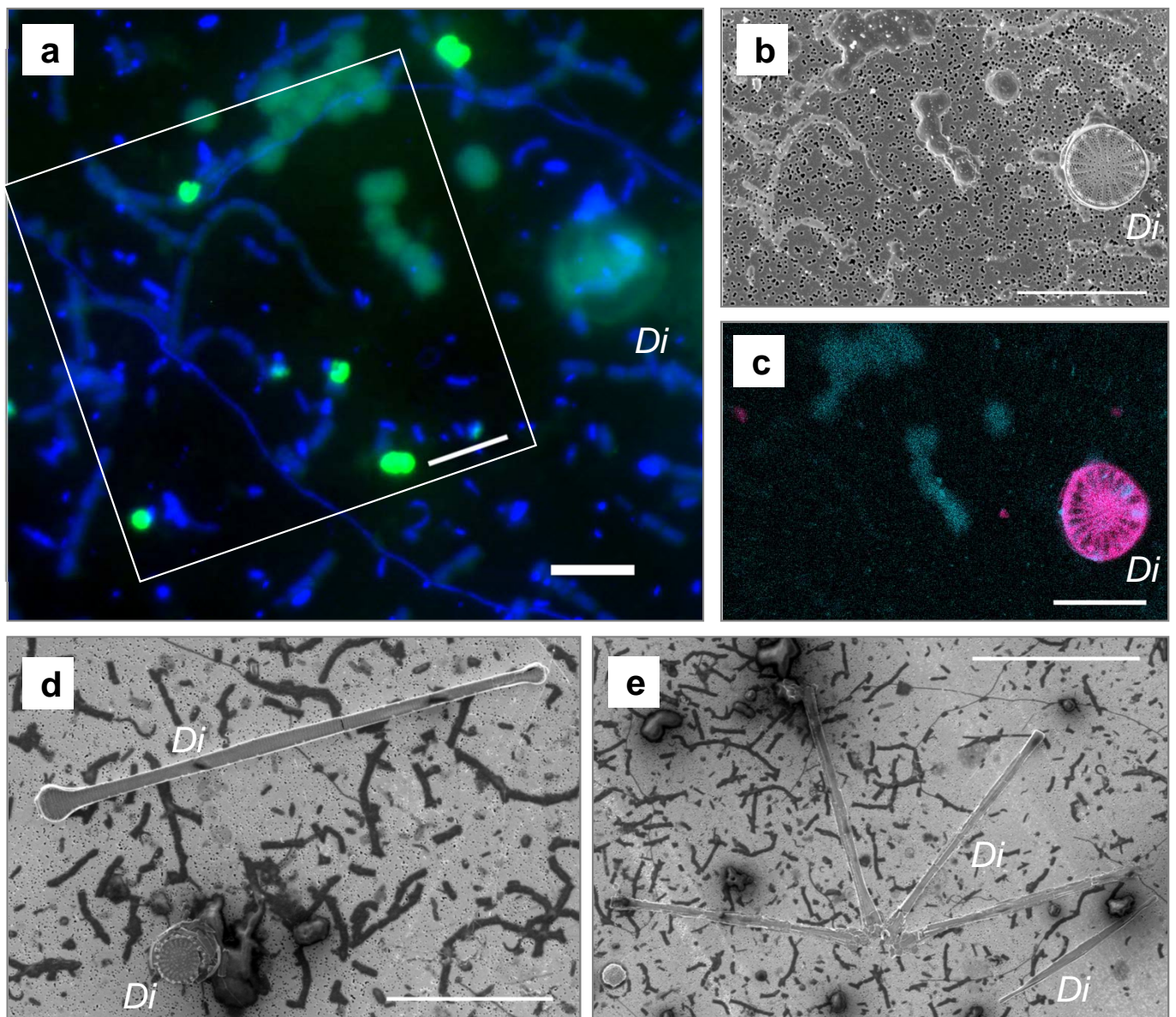
Arrows indicate hybridized MOB. Corresponding $^{32}\text{S}/^{12}\text{C}$ and $^{13}\text{C}/^{12}\text{C}$ nanoSIMS images show distribution of cell biomass and assimilation of ^{13}C -labeled methane, respectively. Note different scales of nanoSIMS ratios. Scale bar on fluorescent images represents 5 μm . Scale bar on nanoSIMS images represents 3 μm in the upper panels and 2 μm in the lower panels.



Supplementary Figure 5. *In vitro* methane oxidation rates in batch incubations from the chemocline with added nitrite or sulfate. The additions of nitrite or sulfate did not stimulate methane oxidation.



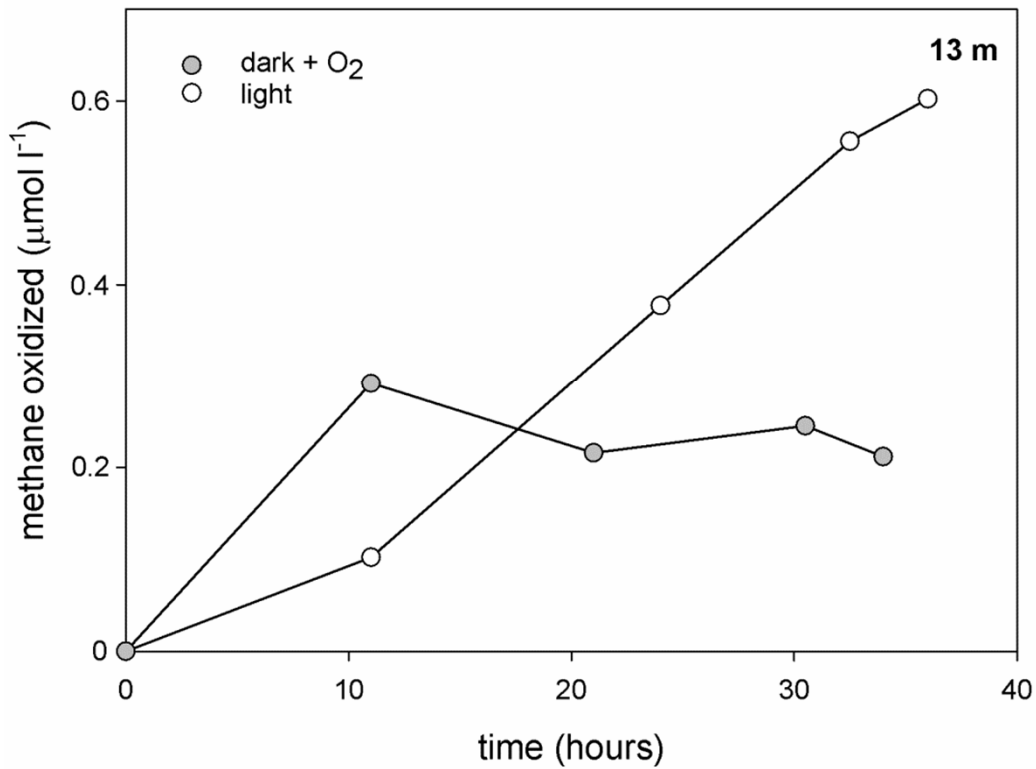
Supplementary Figure 6. Graph showing the Eddy Diffusivity/Turbulent diffusion coefficient (K_z) values across the chemocline of Lago di Cadagno according to Uhde 1992. Average values were determined from the temperature and by using the SF6 methods. The standard deviation varies between 33% in the uppermost part of the anoxic zone to between 9 and 18% in the chemocline at 12-14 m.



Supplementary Figure 7. Centric and pennate diatoms found at 13 m depth in Lago di Cadagno.

(a) Larger field of view of a fluorescent micrograph from Figure 4. Note green autofluorescence of the centric diatom (*Di*). White inset corresponds to the CARD-FISH image from Figure 4 showing gamma MOB in bright green. (b) Scanning electron micrograph and (c) energy-dispersive X-ray spectroscopic mapping of sodium (in turquoise) and silica (in magenta) of the same field of view. (d, e.) Up to 200 centric and pennate diatoms were counted per millilitre of water from 13 m depth. Water from 10 m depth contained *ca.* 2.5-times more diatoms.

Scale bar represents 5 μm in (a), 7 μm in (b, c), 20 μm in (d) and 40 μm in (e).



Supplementary Figure 8. Methane oxidation in the light and with addition of oxygen in the anoxic water from 13 m depth. Note that the addition of oxygen resulted in higher initial methane-oxidation rates which ceased during the course of the incubation whereas light treatment resulted in lower but steady linear rates.