



Methane oxidation in the anoxic hypolimnion of a deep south-alpine lake

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Atmospheric methane is one of the most important greenhouse gases in Earth's atmosphere. Its main source is biogenic, particularly in anoxic aquatic habitats. Methane efflux from aquatic environments to the atmosphere is controlled by microbial methane oxidation, which can be mediated under aerobic or anaerobic conditions. In marine habitats, where sulphate is an abundant electron acceptor, the anaerobic oxidation of methane (AOM) is coupled to sulphate reduction. In fresh-water systems, in contrast, where sulphate concentrations are 2-3 orders of magnitude lower, the main microbial pathway of methane elimination is its aerobic oxidation. However, recent evidence has shown that in fresh-water ecosystems AOM can be coupled to denitrification. In this study biogeochemical, lipid biomarker, stable carbon isotope and molecular ecological techniques were combined in order to investigate the modes of methane oxidation in Lake Lugano. The northern basin of Lake Lugano is meromictic, with a permanent anoxic hypolimnion below 135 m water depth. Reducing conditions were evidenced by increasing concentrations of H_2S and NH_4^+ . Methane concentrations decreased from $50 \mu\text{M}$ in the bottom water to about 20-40 nM at the oxycline, but the depth distribution of methane suggests its consumption well below the oxycline, at 160 to 200 m water depth. Concomitant with decreasing methane concentrations, we observed an increase in the $\delta^{13}\text{C}$ -values of the residual methane, corresponding to an apparent C-isotope effect (ϵ) of about -7‰ within the anoxic zone. A negative shift in the $\delta^{13}\text{C}$ -values of monoenoic C16 fatty acids in association with the observed methane gradients provides evidence for the incorporation of ^{13}C -depleted, methane-derived carbon into bacterial biomass. Turnover rate measurements indicate two methane oxidation maxima, one located at the oxycline and a second one at about 35 m below the oxycline. At the same time, sulphate reduction rate measurements show that sulphate reduction cannot account for the observed methane consumption. Alternative electron acceptors such as NO_2^- , Fe^{3+} and Mn^{4+} are currently under investigation. Analysis of functional genes has revealed the presence of particulate methane monooxygenase (pmoA) – a gene associated with the aerobic pathway of methane oxidation – in the anoxic hypolimnion. Quantitative analysis of pmoA will provide information on the spatial distribution of methanotrophs within the anoxic water column.