



Aerobic methanotrophs drive the formation of a seasonal anoxic benthic nepheloid layer in monomictic Lake Lugano

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In the southern basin of Lake Lugano, thermal stratification of the water column during summer and autumn leads to a lack of exchange between surface and deep water masses, and consequently to seasonal bottom water anoxia, associated with high methane concentrations. With the onset of bottom water anoxia, a dense layer of high particulate matter concentration – a so-called benthic nepheloid layer (BNL) – develops in the bottom waters. A sharp redox gradient marks the upper boundary of the BNL. At its maximum, the BNL extends 15 – 30 m from the sediment into the water column. We investigated the identity of the BNL and key environmental factors controlling its formation in the framework of a seasonal study. Compound specific C-isotope measurements and Fluorescence In Situ Hybridisation (FISH) of suspended particulate organic matter, radioactive tracer based measurements of methane oxidation, as well as investigation of geochemical water column parameters were performed in spring and autumn. Our analyses revealed that the microbial biomass within the BNL is dominated by methanotrophic bacteria. Aerobic methane oxidation (MOx) was restricted to a narrow zone at the top of the BNL, reaching maximum rates of up to 1.8 $\mu\text{M}/\text{day}$. The rates of MOx activity effectively consumed most (>99%) of the uprising methane, leading to the formation of a sharp CH_4 concentration gradient and a strongly suppressed kinetic isotope effect ($\varepsilon = -2.8\text{‰}$). CH_4 oxidation was limited by the diffusive supply of O_2 from the upper hypolimnion, implying that methanotrophy is the primary driver of the seasonal expansion of the anoxic bottom water volume, and explaining the vertical migration of the BNL in response to its own O_2 consumption. The bulk organic matter extracted from the BNL was strongly depleted in ^{13}C ($\delta^{13}\text{C} < -60\text{‰}$), providing evidence for the incorporation of CH_4 -derived carbon into the biomass, suggesting that the BNL was composed of MOx-communities. This was further evidenced by four dominant fatty acid biomarkers ($\text{C}_{16:1\omega5}$, $\text{C}_{16:1\omega6}$, $\text{C}_{16:1\omega7}$, and $\text{C}_{16:1\omega8}$), which were strongly C-isotopically depleted, with $\delta^{13}\text{C}$ values between -62‰ ($\omega6$) and -80‰ ($\omega7$). The fingerprint of isotopically depleted FAs indicates a dominance of Type I MOx bacteria in the BNL, which we could confirm with FISH using specific probes. Isotope mixing considerations suggest that 77 – 96 % of fatty acid carbon in the BNL is CH_4 -derived. FISH revealed that up to 30% of microbial cells in the BNL are methanotrophic. The cell size of methanotrophs was significantly larger than of other microbial cells, and an independent approach to quantify the contribution of methanotroph-carbon to the BNL biomass, based on methanotrophic cell size, confirmed our C-isotope-based estimate.