

Review of: "Multiple groups of methanotrophic bacteria mediate methane oxidation in anoxic lake sediments"

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Much attention is presently concentrated at anaerobic methane oxidation (AOM) in freshwater basins, where sulfate concentration is below 10 mg/L and classical AOM carried out by methanotrophic archaea (ANME) and sulfate-reducing bacteria is inefficient. Methane profiles in the water column and sediments of freshwater lakes indicate, however, active microbial processes in the anoxic zone of such basins. AOM coupling with denitrification and reduction of variable-valency metals (primarily iron and manganese), was recently established. A number of publications report the AOM process carried out by methanotrophic archaea and bacteria in anoxic zones under sulfate limitation. In some cases, however, in the anoxic zones of water and sediments, where methane oxidation was revealed by physicochemical, gas geochemical, and radiotracer studies, no known ANME archaea of the family Methanoperedenaceae and bacteria oxidizing methane with nitrite (Cand. *Methylomirabilis oxyfera*) were found, while microorganisms phylogenetically related to aerobic methanotrophic bacteria were revealed. The reviewed article represents an attempt to reveal the microbial agents and the mechanisms of anaerobic methane oxidation in reduced lake sediments using radioisotopic, stable isotopic, and molecular techniques.

The authors have carried out extensive work and confirmed that none of the typical electron acceptors (NO_2^- , NO_3^- , SO_4^{2-} , Fe^{3+} , and Mn^{4+}) could stimulate AOM in the studied lake sediments. Molecular techniques revealed type I and type II methanotrophs in the sediments. A drastic increase of methane oxidation rate was observed during sediment incubation under anoxic conditions. In spite of the absence of expressed novelty, the work involves a broad spectrum of methodical approaches and provides deep analysis of the data, using abundant literature data. It will certainly be of use to researchers working on anaerobic methane oxidation in sulfate-free environments by the microorganisms known as classical aerobic methanotrophs.

Some minor remarks are listed below.

1. Analysis of the methanotrophic bacterial community of the studied lake revealed the presence of gamma-proteobacterial Methylococcaceae, alpha-proteobacterial Methylocystaceae (in lower numbers), and high occurrence of filamentous methanotrophic *Crenothrix*. A very similar ratio of methanotrophs has been reported for the anoxic zone of Arctic lakes (in the water column, rather than in the sediments). In our opinion, the discussion would have gained from the relevant reference (Cabrol et al, doi.org/10.1016/j.scitotenv.2020.139588).

2. Discussion of the possible role of methanogenic archaea should have been more detailed. As early as 1980, Zehder and Brock showed their ability to carry out “reverse methanogenesis,” especially at high methane concentrations. Methane content in the studied sediments was relatively high, exceeding its solubility in pore water. Moreover, molecular analysis revealed relatively high abundance of Euryarchaeota in the studied sediments.