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Effects of inorganic electron acceptors on methanogenesis and methanotrophy and on the community structure of bacteria and archaea in sediments of a boreal lake

Antti J. Rissanen (1,3), Anu Karvinen (2), Hannu Nykänen (3), Sari Peura (3,4), Marja Tiirola (3), Anita Mäki (3), and Paula Kankaala (2)

(1) Tampere University of Technology, Department of Chemistry and Bioengineering, Tampere, Finland (antti.rissanen@tut.fi), (2) University of Eastern Finland, Department of Biology, Joensuu, Finland, (3) University of Jyväskylä, Department of Biological and Environmental Science, Jyväskylä, Finland, (4) Uppsala University, Department of Ecology and Genetics, Uppsala, Sweden

Lake sediments are globally significant sources of CH_4 to the atmosphere, but the factors controlling the production and consumption of CH_4 in these systems are understudied. Increasing availability of electron acceptors (EA) (other than CO_2) in sediments can decrease or even suppress CH_4 production by diverting the electron flow (from H_2 and organic substances) from methanogenic to other anaerobic respiration pathways. However, whether these changes in microbial function extend down to changes in the structure of microbial communities is not known. Also anaerobic oxidation of methane (AOM) could be enhanced by increased availability of EAs (SO_4^{2-}, NO_3^-, Fe^{3+} and Mn^{4+}), but information on the role of this process in lake sediments is scarce. We studied the effects of inorganic EAs on the potential for CH_4 production and consumption and on the structure of microbial communities in sediments of a boreal lake.

Anoxic slurries of sediment samples collected from two depths (0 – 10 cm; 10 – 30 cm) of the profundal zone of a boreal, mesotrophic Lake Ätäskö, were amended with 1) CH_4 or with CH_4 and either 2) 10 mM Mn^{4+}, 3) 10 mM Fe^{3+}, 4) O_2 or 5) CH_2F_2 (inhibitor of aerobic methane oxidation) and incubated at +10˚C for up to 4 months. Furthermore, slurries from the 10 – 30 cm layer were amended with CH_4 and either 6) 2 mM NO_3^- or 7) 2 mM SO_4^{2-} and incubated at +4 ˚C for up to 14 months. The processes were measured using ^{13}C-labelling and by concentration measurements of CH_4 and CO_2. Effects of treatments 1-3 on microbial communities were also analysed by next-generation sequencing of 16S rRNA, as well as methyl coenzyme-M reductase gene amplicons and mRNA transcripts.

CH_4 production (max. 83 nmol $g_{dw}^{-1}d^{-1}$) took place in the anaerobic treatments but was generally decreased by the addition of NO_3^-, SO_4^{2-}, Fe^{3+} and Mn^{4+}. Although the structure of sediment archaeal community was resistant to Fe^{3+}/Mn^{4+} - additions, slight changes in the structure of bacterial community occurred. Besides decreasing the availability of methanogenic substrates, the Mn^{4+}/Fe^{3+} - induced changes in the bacterial community also probably decreased the H_2:acetate – ratio in the substrate pool. This led to increase in the relative activity (mRNA level) of some operational taxonomic units assigned to aceticlastic *Methanosaetaceae* and decrease in the relative activity of hydrogenotrophic *Methanoregulaceae* in the sediment. CH_4 oxidation (0.02 - 0.30 nmol $g_{dw}^{-1}d^{-1}$ in anaerobic and 18 - 73 nmol $g_{dw}^{-1}d^{-1}$in aerobic treatments) took place without EA additions and was enhanced only by O_2. This suggests decoupling of the process from the reduction of other inorganic EAs. The results also indicate that Fe^{3+}/Mn^{4+} - reduction did not increase CH_4 oxidation via increased availability of SO_4^{2-} by cryptic sulfur cycle or via increased availability of organic EAs. Furthermore, ANME – archaea were only $\leq$ 3% of sediment archaeal community and their relative activity was decreased during incubations. Thus, EA driving CH_4 oxidation in the anoxic sediments of the lake remains unknown or the process was methanogen-driven via trace methane oxidation.