



## Anaerobic nitrite-dependent methane-oxidizing bacteria – novel participants in methane cycling of drained peatlands ecosystems

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Northern peatlands are one of the key sources of atmospheric methane. Process-based studies of methane dynamic are based on the hypothesis of the balance between microbial methane production and oxidation, but this doesn't explain all variations in and constraints on peatland CH<sub>4</sub> emissions. One of the reasons for this discrepancy could be anaerobic methane oxidation (AOM) - the process which is still poorly studied and remained controversial.

Very little is known about AOM in peatlands, where it could work as an important "internal" sink for CH<sub>4</sub>. This lack of knowledge primarily originated from researchers who generally consider AOM quantitatively insignificant or even non-existent in northern peatland ecosystems. But not far ago, Smemo and Yavitt (2007) presented evidence for AOM in freshwater peatlands used indirect techniques including isotope dilution assays and selective methanogenic inhibitors. Nitrite-dependent anaerobic methane oxidation NC10 group bacteria (n-damo) were detected in a minerotrophic peatland in the Netherlands that is infiltrated by nitrate-rich ground water (Zhu et al., 2012). Present study represents the first, to our knowledge, characterization of AOM in human disturbed peatlands, including hydrological elements of artificial drainage network.

The experiments were conducted with samples of peat from drained peatlands, as well as of water and bottom sediments of ditches from drained Dubnensky mire massif, Moscow region (Chistotin et al., 2006; Sirin et al., 2012). This is the key testing area of our research group in European part of Russia for the long-term greenhouse gases fluxes measurements supported by testing physicochemical parameters, intensity and genomic diversity of CH<sub>4</sub>-cycling microbial communities. Only in sediments of drainage ditches the transition anaerobic zone was found, where methane and nitrate occurred, suggested the possible ecological niche for n-damo bacteria. The NC10 group methanotrophs were analyzed by PCR amplification of 16S rRNA (Ettwig et al. 2009) and *pmoA* (Luesken et al. 2011) genes followed by construction of clone libraries. Phylogenetic analysis revealed only one n-damo bacterium distantly related to uncultured anaerobic methanotrophs found *in situ*. It may represent a new cluster of NC10 bacteria with an identity of less than 96 and 86% to the 16S rRNA and *pmoA* genes of "*Ca. Methylomirabilis oxyfera*," respectively. An enrichment of nitrite-reducing methanotrophic NC10 bacteria was successfully obtained from this sample in a static anaerobic culture with methane and nitrite at an *in situ* pH of 6.3. The bacterial abundance in enrichment was estimated using quantitative PCR and FISH (DBACT-0193-a-A probe) analysis and was found to increase up to 10 times for 120 days. The results of this study expand our knowledge of the diversity and distribution of NC10 bacteria in the environment and their potential contribution to nitrogen and methane cycles in northern peatland ecosystems. We think that AOM may be more active in anthropogenic disturbed peatlands with greater supply of elements that could potentially serve as electron acceptors. In spite of generally low concentration, seasonal increases in nitrate content in drained peatlands may work as an important control of CH<sub>4</sub> fluxes.

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