



Methane production in the different types of West Siberian boreal wetlands: inhibition experiments, isotopic signatures, microbial communities

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Wetlands are the largest natural source of methane, the important greenhouse gas. Wetlands emit methane as a result of methanogenesis, the final stage of organic matter decomposition in anaerobic conditions. Rates, pathways and other characteristics of methanogenesis are regulated by a number of factors that are still poorly known in the boreal peatlands. So the goal of our study was to investigate special features of methanogenesis at the different scales: 1) within one wetland at different depths (at 20 and 50 cm below the water table level – further WTL), 2) in the different types of wetlands of the same climatic zone (ombrotrophic bog, poor fen and minerotrophic fen – further OB, PF and MF, respectively), 3) during the different seasons (mid-July and mid-September), and 4) under the different climatic conditions (middle and southern taiga zones). All studied types of wetlands were chosen due to their high contribution to the regional methane emission.

During the study, wetland peat was sampled for the incubation experiments and 16S rRNA amplicon sequencing of total extracted DNA. Incubation experiments included: 1) the control incubation without any substrate addition, 2) the incubation with the fluoromethane as an acetoclastic methanogenesis inhibitor, 3) the incubation with 2-bromoethanesulfonate (BES) as an inhibitor of entire methanogenesis, and 4) the incubation with an additional substrate (H₂/CO₂). During the incubation experiments, the concentration dynamic of methane, carbon dioxide (with their isotopic signatures), hydrogen and low-molecular fatty acids were monitored.

We found that methane production in the control experiments significantly increased in a row OB < PF < MF and decreased with a depth (more sharply in OB than in PF and MF). Acetate concentration varied strongly between the ecosystems: it was about 0.2 mM in all OB samples, while in PF and MF samples it was 5-10 times higher. During the control experiments, the acetate concentration remained relatively constant. Hydrogen concentrations were above 10 ppm for all wetlands except the south taiga OB.

All incubation experiments indicated the dominance of acetoclastic methanogenesis at the depth of 20 cm below WTL in all types of wetlands. The contribution of acetoclastic pathway to the total methanogenesis was 65-90%. The lowest fraction of acetoclastic methanogenesis was found in the south taiga OB samples. It was consistent with a relatively low hydrogen concentration in these samples. In contrast, hydrogenotrophic methanogenesis dominated in peat sampled at the depth of 50 cm below WTL.

Experiments with a substrate addition showed that homoacetogenesis (microbial conversion of hydrogen to acetate) was active in all samples. Methane production, however, did not increase in OB with an increase in acetate concentration, while in PF and MF it increased strongly. This may be explained by the fact that high-affinity Methanosae-taceae were dominant among acetoclastic methanogens in OB, while in PF and MF low-affinity acetate-using Methanosarcinaceae were much more abundant. It was confirmed by preliminary results of 16S rRNA amplicon sequencing.

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