



## **Methanogenesis limitations in degraded peatlands after their hydrological restoration**

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Peatlands are ecosystems that can have a high degree of carbon sequestration due to CO<sub>2</sub> fixation and low decomposition rates, but on the other hand, they are a source of CH<sub>4</sub>. Past drainage or mining can disturb these natural functions with rewetting being the main method used to bring back their original ecosystem properties. Methanogenic community composition and its activity seems to be very sensitive to environmental changes and therefore its limited activity after restoration can reflect the not fully restored functioning of the microbial community and its processes in the rewetted peatlands.

To find the cause for this methanogenesis limitation we determined the abundance and composition of the methanogenic community and methane potential production in pristine, long-term drained and rewetted bogs and spruce swamp forests (SSF) in the Šumava Mountains (Czech Republic), using high-throughput barcoded sequencing, qPCR and anaerobic incubation of peat samples in relation to peat biochemical properties. Long-term drainage led to a strongly reduced diversity, abundance and activity of the methanogenic community in both peatland types. In restored sites, methanogenic abundance and community composition reached a pristine like state, however their activity measured as CH<sub>4</sub> production remained as low as in drained sites. Substrate limitation was expected; therefore we further added different substrates during anaerobic incubation of the peat samples. In addition to glucose and ethanol, we added natural complex substrates from peatland plants (sedges, Sphagnum) to simulate the effect of the spreading of peatland species and their litter on methanogenic activity. The results unambiguously confirmed the limitation of methanogens by substrate availability due to the previous long-term drainage and strongly decomposed peat. The addition of natural substrates led to an increase in CH<sub>4</sub> production, which was close to values in pristine sites. The limited CH<sub>4</sub> production reflected a not fully restored vegetation structure with sufficient litter production. Thus, the re-establishment of peatland vegetation structure should lead to regeneration of methanogenic activity and microbial processes in restored peatlands. Moreover, the results showed that there is no risk of increased CH<sub>4</sub> emissions in the case of nutrient poor bogs and SSF after re-wetting. For management, the methanogenic community composition, abundance and activity could be used as indicators of restoration success.