

Interactions between nitrogen fixation and methane cycling in boreal peat bogs

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Microbial N₂ fixation supplies an important nitrogen input to boreal peatlands, extremely oligotrophic ecosystems dominated by *Sphagnum* mosses. In this study, we coupled major and trace nutrient analyses, potential rate measurements, gene amplicon and omics sequencing to characterize interactions between N₂ fixation and CH₄ cycling at the S1 peat bog in Marcell Experimental Forest (Minnesota, USA). Total dissolved nitrogen (NO₃⁻+NO₂⁻+NH₄⁺) and phosphate were both consistently < 2 μM in surface peat, suggesting severe nutrient limitation. While dissolved Fe was 18-35 μM, Mo, V and Cu were extremely scarce (2-40 nM), suggesting that alternative metalloenzymes containing Fe in place of other metals may be expressed. However, few alternative nitrogenase transcripts were recovered with both primer and omics approaches, suggesting that Mo is likely still required for the nitrogenase cofactor despite its scarcity in peat bogs. Diazotrophy measured by both ¹⁵N₂ incorporation and the acetylene reduction assay was 7-fold higher in anoxic than oxic incubations. No significant difference in N₂ fixation rates measured by either method was observed with or without 1% CH₄. Anoxic ¹⁵N₂ incorporation was 3-4x higher in treatments lacking C₂H₂, suggesting that the acetylene reduction assay underestimates N₂ fixation by inhibiting diazotrophs sensitive to C₂H₂. Aerobic methanotrophy was also inhibited by 1% C₂H₂. Comparison of the phylogenetic composition of *nifH* DNA and cDNA amplicon sequences and *nifH* genes in metagenomes and metatranscriptomes from fresh peat sampled at the S1 bog revealed notable differences between primer- vs. omics-based approaches, as well as DNA vs. cDNA sequences. Metatranscriptomes from surface peat were dominated by archaeal *nifH* sequences most similar to *Methanosarcinales*, whereas metagenomes were dominated by nitrogenase paralogs. *In silico* analysis of *nifH* primers previously used in peatland studies revealed multiple mismatches to methanogenic archaeal *nifH* genes. This study suggests that methanogenic archaea may be important soil diazotrophs that have gone undetected due to a combination of C₂H₂ inhibition during ARA measurements and lack of amplification by common *nifH* primers.