

Title: An Investigation of the Role of Methoxylated Aromatic Compounds in Methanogenesis from Peat and an Update on Ongoing SPRUCE Metagenomic Analyses

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Project Abstract: Boreal peatlands are simultaneously important ecosystems for terrestrial carbon storage as well as large sources of the greenhouse gas methane. Methanogenic Archaea are thought to primarily use acetoclastic (acetate), hydrogenotrophic (carbon dioxide and hydrogen) or methylotrophic (C-1 compounds) pathways and substrates to produce methane. Recently, an organism using Methoxylated Aromatic Compounds (MACs) to produce methane was characterized from coal bed systems, however the prevalence, ecology and detailed mechanisms of these organisms and pathways remain unknown. As MACs are common in peat, we conducted laboratory microcosm experiments using peat from the SPRUCE site and six different methoxylated aromatic compounds to peat from various depths in the peat profile and incubated anaerobically for ~100 days. Gas headspace was monitored for CH₄ and CO₂ over time and final samples were harvested for microbial analysis using 16S rRNA gene sequencing. Experiments to date show a high heterogeneity in gas production response to MAC amendment across peat cores and depths within core profiles. Rates of methanogenesis were generally slow, but increased over the course of incubation period (up to 0.94 ug C-CH₄ / g peat per day vs. 0.26 in controls), as did the CH₄:CO₂ ratio of headspace gases (up to 12.82 vs. 0.96 in controls), and were highest with 2-methoxyphenol, 1,3,5-trimethoxybenzene, and 3,4,5-trimethoxybenzyl alcohol additions. Several MAC incubations produced significantly more methane than no-substrate controls as well as methanol used as a comparative known methylotrophic substrate. Together results to date suggest that MAC utilizers are present in peatland systems but may be in low abundance, and that high concentrations of certain methoxylated compounds may have toxic effects on some microbes. DNA analysis of microbial communities are ongoing and follow up experiments are being designed with ¹³C-labeled substrates to better distinguish MAC methanogenesis from other potential pathways. When complete these investigations should provide valuable insights the prevalence and mechanisms for MAC based methanogenesis in peatland ecosystems. In addition to these MAC experiments, an update on new metagenomic analyses of microbial community changes during the SPRUCE experimental treatments will be presented resulting from data just coming in via a collaboration with the Joint Genome Institute.