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Microbial Responses of Deep Catotelm Peat to *In Situ* Experimental Warming and *Ex Situ* Amendments of Temperature, pH, Nitrogen and Phosphorus

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The Spruce and Peatland Responses Under Climatic and Environmental Change (SPRUCE) experiment is an ecosystem-level manipulation examining how peatland systems respond to increased temperature and CO₂ levels. This experimental manipulations are expected to lead to various changes in ecosystem processes, including microbially mediated biogeochemical cycles that may ultimately alter the overall C balance of these ecosystems. The initial phase of this experiment began over the summer of 2014 by heating deep subsurface peat to +2.25, +4.5, +6.75 and +9.0 °C above ambient plots with a target heating zone of 1.5-2 meters depth. This deep peat heating phase lasted just over 1 year. The response of microbial communities to *in-situ* warming is assessed with qPCR and 16S rRNA amplicon sequencing at eleven discrete depths across the peat profile to a depth of 200 cm. Additionally, metagenomic sequencing was used to characterize microbial metabolic and functional potential on four depths per profile. After one year of deep peat warming, microbial community structure and abundance of bacterial, archaeal, fungal, and methanogenic populations showed strong vertical stratification across the peat depth profile yet no clear response to the temperature treatments. In an effort to identify factors that may be limiting decomposition and microbial community change in deep peat, we conducted a microcosm incubation of deep peat (150-200 cm depth) at 6 and 15 °C to mimic ambient and +9 °C SPRUCE conditions. Additional treatments included elevated pH and the addition of N and P. Microcosms were monitored for CO₂ and CH₄ production, and microbial community dynamics were assessed using qPCR and amplicon sequencing. Increasing temperature resulted in both greater CO₂ and CH₄ production, while elevated pH only resulted in greater CH₄ production. The effects of elevating temperature and pH in combination with N, P, or N+P additions were more variable. Although temperature had little effect on the overall microbial community structure from amplicon 16S rRNA gene sequencing results, there was a shift in the size of bacterial and archaeal populations evident from qPCR assessments. In contrast, N addition (alone and in combination with other variables) as well as pH amendments seemed to have the largest influence on community structure. Collectively both our *in situ* and *ex situ* results suggest that microbial responses in the deep catotelm peat is likely limited by factors other than temperature.