

## Seasonal variations of contaminant removal and composition of the (active) microbial communities in Northern peatlands treating mining-affected waters

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Mining-affected waters are a huge challenge to natural ecosystems. At a mine site in Finnish Lapland, mining-affected waters are purified in two treatment peatlands (TPs) before they are released into downstream waters. The TPs experience long winters and are snow- and partly ice-covered from October to May. Contaminants in inflow waters include nitrogen compounds, sulfate, metals and metalloids (e.g. arsenic, antimony, nickel). The TPs were intensively monitored from 2015 to 2018 (water quality, air and peat temperatures, snow- and ice-cover, groundfrost depth). Snow- and ice-cover thickness as well as ground-frost depth were assessed throughout the winter, and soil temperatures were recorded continuously in different peat layers year-round. Maximal ground-frost depth of up to 30 cm were observed in March and April. Lower peat layers, however, remained unfrozen throughout the winter with temperatures ranging from 1 to 5°C.

Removal of nitrogen and sulfate was higher in summer months while removal of arsenic, antimony and nickel was similar throughout the year. Potential process rates for aerobic respiration, arsenic/antimony turnover and sulfate reduction were assessed at different incubation temperatures in microcosm studies with peat soil. Potential process rates were generally higher at higher incubation temperatures and decreased with decreasing temperatures, but significant rates were also observed at temperatures close to 0°C. Thus, microbes are potentially active in the deeper layers of the TPs even in cold winter months.

The composition of the (active) microbial community was compared in soil samples taken in triplicate at 4-6 weeks intervals from 2 depths (10 cm, 70 cm) from both TPs in winter 2015/2016. The community composition of the samples was compared on DNA and cDNA level by length-heterogeneity PCR (LH-PCR) of the bacterial 16S rRNA gene and samples were selected for additional next-generation sequencing of 16S rRNA gene amplicons based on the LH-PCR results. LH-PCR revealed differences in microbial community composition between the two TPs, the two layers in the TPs and the months while amplicon sequencing identified the active microbial players. Triplicate samples generally clustered closely together and were separated from samples taken at different timepoints, indicating seasonal variations in the (active) microbial community. Based on amplicon sequencing of cDNA the most active taxa included Proteobacteria, Chloroflexi and Acidobacteria, and the relative abundance of individual taxa varied throughout the year.

The collective results showed that (i) microorganisms in the studies TPs are subjected to cold temperatures in winter time, (ii) contaminant removal in TPs shows seasonal variations, (iii) peat microorganisms have the potential to catalyze removal-related processes even in the winter time, and (iv) there are seasonal differences in the active microbial communities in TPs.