



Large shifts in riverine microbiomes exposed to mountaintop coal mining activities in Central Appalachia: regional and longitudinal assessments

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Appalachia – a biodiversity hot spot – is currently threatened by mountaintop mining of coal. Mountaintop coal mining alters river water chemistry, leading to increased specific conductivity associated with the alkaline mine drainage (elevated salts dominated by Ca^+ , Mg^+ , SO_4^{2-} , and HCO_3^-), in turn impairing aquatic life. We performed a regional assessment of the impact of this major land cover change on river microbiomes (amplicon sequencing of 16S rRNA and 18S rRNA genes) sampled from 93 rivers across Central Appalachia (West Virginia, USA) spanning a gradient of exposures to mountaintop coal mining. We identified the sensitive and tolerant microbial taxa to this gradient of mountaintop mining exposure, and we calculated the thresholds to stream conductivity where large synchronous changes in diversity were observed using the Threshold Indicator Taxa Analysis (TITAN). We found that rivers exposed to mountaintop coal mining showed decreased microbial diversity and large shifts in community composition. Nearly 25% of the bacterial taxa were sensitive to the increase in stream conductivity. Large synchronous declines in the bacterial community were identified starting at low levels of mining impacts (stream conductivity thresholds of $150 \mu\text{S}/\text{cm}$), which are markedly lower than the current EPA aquatic life benchmark ($300 \mu\text{S}/\text{cm}$) for Central Appalachian rivers. We complemented this survey with a longitudinal sampling on a single river network spanning the largest mountaintop mining complex in West Virginia (Hobet Mine Complex), where we studied the influence of multiple confluences between reference and impacted rivers (i.e. very contrasted chemical signatures) on microbiome structure. We investigated the factors driving the assembly mechanisms of the aquatic microbial communities exposed to these multiple coalescence events by analyzing mixing ratios and the chemical or microbiome similarity at each confluence. This study demonstrates the high sensitivity of river microbiomes to very low levels of mountaintop mining activity and leverages the distribution of microorganisms along a strong environmental gradient to resolve the natural history of thousands of freshwater microorganisms. We also present new insights on the outcome of sequential coalescence events in situ in a river network.