

Genome-based approach for assessing microbial community dynamics and genetic potential reveals diverse methylotrophs in Slate River floodplain sediments

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The Slate River (SR), located in Colorado, USA, is a mountainous, high elevation river impacted by legacy mining leading to elevated concentrations of heavy metals in floodplain sediments. The SR floodplain experiences seasonal rise and fall in the water table related to snowmelt-induced flooding, precipitation, and evapotranspiration. These fluctuations in the water table determine the inputs of nutrients and other substances to the subsurface and oxygen penetration depth, thus strongly influencing microbial communities and biogeochemical cycling. SR subsurface floodplain sediments harbor diverse microbial communities with striking structure along depth profiles and the oxic-anoxic transition. Here, we further examine microbial metabolisms in the subsurface sediments by generating metagenome-assembled genomes (MAGs) from samples collected in June 2018 at two locations (OBJ1 and OBJ2) and depths ranging from 50 to 150 cm below ground surface. We generated 1233 MAGs (>50% completeness, <10% contamination) spanning 38 archaeal and bacterial phyla and including putative C1-cycling organisms such as methanogens and methylotrophs. While methanogens (*Methanomassiliicocales*, *Methanoregulaceae*, *Methanotrichaceae*) were limited to deeper anoxic depths, we recovered diverse and abundant genomes from putative methanotrophs across the entire depth profile, including from *Methanoperedens*, *Methylomirabilis*, *Bin18* (*Binatota*), and *Binataceae* (*Binatota*). *Methanoperedens* occur in deep, anoxic depths and reach high relative abundances in one sample (>50%). *Methanoperedens* can have significant metabolic flexibility, coupling anaerobic methane oxidation to myriad electron acceptors (including nitrate, iron, manganese, and potentially selenate, arsenate, and elemental sulfur) and can display a pleomorphic lifestyle that could allow this group to thrive in the dynamic floodplain environment. On the other hand, *Binatota* and *Methylomirabilis* occur in shallower, unsaturated depths. *Methylomirabilis* are also known for their ability to oxidize methane using nitrite (n-DAMO) and co-occur with nitrifiers in our samples. Putative methylotrophs capable of oxidizing other C1 compounds were also found in groups such as *Methyloceanibacter* and *Binatota*. Careful investigation of the recovered genomes will further elucidate connections between