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Ecological insights into hot spring microbial mats: undermat community analysis using NGS sequencing.

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Phototrophic microbial mats develop in the effluent channel of Mushroom (MS) and Octopus (OS) Springs in Yellowstone National Park (USA) over a temperature range of 45-70 °C. The upper green layer, dominated by *Cyanobacteria* and anoxygenic *Chloroflexi*, has been well studied. In contrast, the diversity and metabolic functions of the heterotrophic community in the anoxic/microoxic region of the mat are not well understood. In this study we analyzed the orange-colored undermat of the MS microbial mat using 16S rRNA gene amplicon and metagenomic analyses.16S rRNA amplicon analysis identified a highly diverse but uneven community, dominated by the anoxygenic chlorophototrophic *Roseiflexus* spp., followed by *Pseudothermotoga* sp. and members of the *Armatimonadetes, Aquificae*, and *Chloroflexi*. A high diversity of chlorophototrophic and retinalophototrophic bacteria indicate light is an important energy source even in the undermat. Metagenomic binning analysis disclosed a variety of novel organisms and members of Candidate phyla. The mat ecosystem contains nearly closed nutrient cycles. Despite the low sulfate concentration in the spring water, an active sulfur cycle is maintained in the mats and active sulfate reduction has been shown previously. Metagenomic analysis of the undermat now disclose the different key-players in the sulfur cycling. Carbon and nitrogen enter the biological cycles by cyanobacterial CO_2 and N_2 fixation. They are made available to the heterotrophic mat community in form of lactate, glyoxylate/glycolate, and amino acids. The high diversity of amino acid transporter genes in the metagenome is in accordance with isolation-based studies in our lab. After 50 years of studies of these hot spring mats, this study for the first time provides detailed information about the undermat community, and is part of a powerful combination of ？ omics- and isolation-based studies to develop a comprehensive understanding of these microbial-mat communities.