

The permafrost/active layer microbial community in the Eravna Bazin (Buryatia, Russia)

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Motivation and Aim: Soil microbial communities play key role in biogeochemical cycles and greenhouse gas formation during the decomposition of the released organic matter in the thawing permafrost. The Eravna Basin, bounded by the Vitim Plateau in the northeast and the Selenga Middle Mountains in the south, is a model system for microbial ecology studies in various soil-ecological niches. The object of our studies was the Darkhituy–Khaimisan transect soils (the Eravna Basin, Buryatia). The soils of the transect polygon were well-characterized earlier [1, 2]. The active layer, which is a seasonally thawing surface and subsurface soil layer, suffers from repeated environmental disturbances due to frozen–thaw cycles [3, 4]. The active layer thickness differs across the sampling sites and was a significant factor determining bacterial and archaeal diversity. Active layer soils are exposed to multiple freeze thaw cycles during the change in seasons [4]. Environmental conditions of studied sites representing distinct habitats (larch forest, birch forest, meadow steppe and thermokarst lake) in the Eravna Basin characterized different amplitudes of freeze-thaw conditions over seasons and had a strong impact on taxonomical changes in the microbial communities present in the active layer during the initial period of soil thawing [5]. The aim of our research was to assess the taxonomic prokaryotic diversity at the seasonally thawing permafrost soils across Darkhituy–Khaimisan transect. We investigated changes in the microbial communities present in permafrost and the active layer of three sites representing distinct habitats. We explore the relationship between the biogeochemical differences among habitats and the permafrost/active layer microbial community via a spatial (across habitats, and with depth through the active layer) community survey using high-throughput Illumina sequencing.

Methods and Algorithms: We collected the seasonally thawed soil core and permafrost from the Darkhituy slope (1,015 m a.s.l.) and an adjacent thermokarst dried-up lake Khaimisan from the Eravna Basin (52° 38' N, 111° 24' E) during initial thawing phase in May, 2021, as well as the maximum thawing in September, 2022. Soil moisture, temperature, pH, soil organic carbon, soil nitrate and ammonia nitrogen were determined as previously described [5]. To isolate DNA from soil samples, was used a reagent kit (MACHEREY-NAGEL NucleoSpin Soil) from MACHEREY-NAGEL (Germany) according to the manufacturer's instructions. Library preparation and sequencing were carried out in accordance with the manufacturer's recommendations for operation on the Illumina MiSeq instrument (Illumina, USA) using the MiSeq® ReagentKit v3 (600 cycle) in a paired-end run (2*300 n). The research was done using equipment of the Core Centrum “GenomicTechnologies, Proteomics and Cell Biology” in ARRIAM (Saint-Petersburg, Russia). For the presentation of taxonomic analysis data, the tools of the QIIME software package [6] and SILVA software [7] were used. Statistical and

mathematical processing of multidimensional data of environmental parameters of the studied soils was carried out as previously described [5].

Results: The most common phyla across all samples of distinct permafrost thaw stages included *Proteobacteria* (34 %), *Actinobacteriota* (32 %), *Bacteroidota* (7 %), *Crenarchaeota* (7 %), *Firmicutes* (6 %), *Verrucomicrobiota* (4 %), *Acidobacteriota* (4 %), *Halobacterota* (1 %) and *Planctomycetota* (1 %). The relative abundance of dominant phyla *Crenarchaeota*, *Halobacterota*, *Verrucomicrobiota* and *Planctomycetota* as well as co-dominant taxa such as *Gemmatimonadota* and *Desulfobacterota* varied significantly ($p < 0.05$) between sample sites, with *Crenarchaeota*, *Verrucomicrobiota* and *Gemmatimonadota* abundant in the samples of the steppe meadows and the larch-birch forest and rare in lake Khaimisan soil samples, whereas Khaimisan soil samples were dominated by *Halobacterota*. Furthermore, the relative abundance of some of the dominant phyla varied significantly ($p < 0.05$) between Darkhitui soil horizons, with *Crenarchaeota*, *Acidobacteria*, *Planctomycetota*, *Verrucomicrobiota* and *Gemmatimonadota*, being more abundant in top-soil and subsurface soil horizon, whereas deep frozen horizons were dominated by *Actinobacteriota* and γ -*proteobacteria*. Specifically in Khaimisan permafrost samples, *Actinobacteriota*, with the families *Micrococcaceae*, *Microbacteriaceae* and *Nocardioidaceae* accounting for up to 37.4 % of reads in the permafrost samples and *Gammaproteobacteria* with family *Comamonadaceae* (11 %) and *Hydrogenophilaceae* (6.9 %) increased with depth. *Methanoperedenaceae*, *Devosiaceae*, *Hyphomicrobiaceae*, *Pyrinomonadaceae* and *Xanthomonadaceae* dominated during initial thawing in spring. *Beijerinckiaceae*, *Geodermatophilaceae*, *Intrasporangiaceae* and *Micromonosporaceae* were abundant during maximal thawing in autumn. Significant differences ($p < 0.05$) of mean relative abundance at family level between initial and maximal thawing stages were found in Darkhituy meadow steppe. Thus, bacteria *Chthoniobacteraceae*, *Gemmatimonadaceae*, *Microbacteriaceae*, *Propionibacteriaceae*, *Pyrinomonadaceae*, unclassified_ *Rhizobiales*, *Xanthobacteraceae* and archaea *Nitrososphaeraceae* prevailed during initial thawing. Principal Coordinate Analysis (PCoA) of the beta diversity of soil microbial community revealed that microbial community structure on family level remained similar in the active layer depth regardless of the thawing stage. Top-soil horizons in May and in September were characterized by similar microbial community. While, microbial community structure in permafrost under birch forest varied significantly with domination of *Nocardioidaceae*, *Rhizobiaceae*, *Streptomycetaceae*. These three families accounted for more than 40 % of total microbial diversity. Similarly, analogous composition of microbial community was identified in the active layer under meadow steppe at different thawing stages. Microbial community structure varied significantly in permafrost layer at this sample site with high relative abundance of *Flavobacteriaceae*, *Oxalobacteraceae*, *Pseudomonadaceae*, *Sphingobacteriaceae*, *Comamonadaceae*, *Caulobacteraceae*, and *Methylophilaceae*. In the permafrost of the Khaimisan soil core, the abundance of gammaproteobacteria *Comamonadaceae* increased sharply (up to 23.9 %). The most abundant ASV demonstrates the greatest homology with strains of the psychrophilic, aerobic, chemoorganotrophic genera *Polaromonas* and *Variovorax*. Abundance of *Actinobacterota* increased toward permafrost depths under birch forest, reaching almost 51 % of the total abundance, mainly governed by the families *Nocardioidaceae*, *Streptomycetaceae*, *Micrococcaceae* and *Nocardiaceae*. Members of the genus *Streptomyces* make up almost 12 % of the permafrost community. The

increased abundance of *Bacteroidota* was found at permafrost under steppe. The taxa that dominated this 250-cm layer in our study included both the copiotrophic *Bacteroidia* and *Gammaproteobacteria*. Although *Bacteroidia* were found as one of the clearest thaw zone indicator taxa, we found its dominance (up to 36.6 %) in permafrost. *Flavobacterium*, *Pseudomonas*, *Massilia*, unclassified_ *Comamonadaceae* and *Pedobacter* were the five most abundant genera, which together account for 54 % of all sequences in permafrost.

Conclusion: A comprehensive analysis of the microbial distribution at the seasonally thawing permafrost soils of the Vitim Plateau revealed a site- and horizon-specific patterns of the microbial distribution at the seasonally thawing permafrost soils. High level of microbial diversity in all soil horizons of active layer during the maximum thawing and in intact permafrost was found. *Actinobacteriota* have been identified as one of the most obvious indicator taxa of permafrost horizons. *Proteobacteria* increased toward permafrost depths is mainly due to members of the copiotrophic family *Comamonadaceae*, which grow rapidly when supplied with fresh, labile nutrients.

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