Microbe-substrate interactions following simulated microbial inoculation to thawed yedoma permafrost in anaerobic environments

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The relative roles of ancient versus modern microbial communities in yedoma permafrost carbon decomposition and subsequent greenhouse gas (GHG) production are poorly understood. We anaerobically incubated sediments collected from a 12-m yedoma profile in Interior Alaska to examine: (1) interaction between thawed substrate and microbial community composition (16S RNA) and function (metagenomics); (2) how mixing modern CH4-producing communities with microbial communities present in frozen permafrost affects community composition and function following thaw; and (3) subsequent effects on CO2 versus CH4 production. Inoculation with modern CH4-producing communities from surface sediment collected from an adjacent thermokarst lake (Methanobacteriales, Methanomicrobiales, and Methanosarcinales) altered both microbial community development and organic matter utilization. For most depths, the inoculation increased CH4 (7.6 - 390x) and CO2 (1.0 - 2.7x) production and decreased CO2:CH4 ratios (36 – 99 % decrease) compared to controls. Combined data from our metagenomic functional pathway and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) analyses show this increase in anaerobic GHG production following inoculation is the result of enhanced intermediate organic matter (OM) degradation (carbohydrate-active enzyme classes) breaking down more recalcitrant OM classes (carbohydrate-like compounds and lignin-like compounds) compared to the controls. Yedoma sediments with the highest initial substrate potentials (high relative abundance of aliphatic- and peptide-like compounds) that had not thawed since their formation experienced the strongest effects from inoculation, supporting previous suggestions that GHG production in thawed yedoma is microbially-limited. Changes in microbial community composition (R2 = 0.90 and 0.51 for CO2 and CH4, respectively) and organic matter characterization (R2 = 0.68 and 0.33) provided better fits for estimating anaerobic GHG production potentials than initial microbial community composition (R2 = 0.32 and 0.41) and organic matter characterization (R2 = 0.49 and 0.29). This suggests predicting the evolution of microbial communities following thaw in conjunction with substrate potential will yield more accurate estimates of GHG production potentials compared to characterizing initial communities alone.

Investigating microbial dormancy within the permafrost microbiome

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Arctic ecosystems are experiencing warming at twice the rate of the global average and this is causing rapid permafrost thaw. During thaw, previously unavailable soil carbon and nutrients serve as inputs for microbial decomposition which can release greenhouse gases into the atmosphere and further accelerate climate change. Microbial communities in permafrost soils contain a diverse array of active, dead, and dormant microbes that experience widespread environmental and ecological change during thaw. An important metabolic strategy employed by dormant bacteria to survive stressful environments is to form endospores. Bacterial endospores represent a pool of latent metabolic capacity whereby they are metabolically inactive under frozen conditions and could be metabolically active under thawed conditions. Favorable abiotic conditions (i.e. elevated temperature, pH, and available labile soil carbon) following thaw could possibly resuscitate large proportions of the endospore-forming community. However, which members of the permafrost microbiome are dormant and which will be resuscitated upon thaw is unknown. We conducted a 1-month permafrost incubation experiment to assess changes to bacterial dormancy over the course of permafrost thaw. Our samples include permafrost replicates collected from above the CRREL Permafrost Tunnel and Farmer's Loop sites near Fairbanks, AK and Utqiaġvik, AK. Throughout the incubation, we monitored the relative abundance and identity of endospore-formers present in permafrost immediately before and after thaw via isolation of endospores and subsequent 16S rRNA gene sequencing. This work provides insight into the effect of thaw on permafrost microbial diversity, which will advance understanding of microbial decomposition and the release of greenhouse gases in thawing permafrost landscapes.

Permafrost microbial communities are structured by latitudinal and soil chemical gradients

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The pan-arctic biogeography of permafrost microbial communities (PMCs) and their functional potential is an important area of research, because PMCs may reveal information about permafrost history, biogeochemistry, and response to thaw. Permafrost soils vary by climate zone (both historic and current), history of vegetation and disturbance, parent material, and age, all likely affecting PMCs. We asked whether PMCs clustered into unique functional groups that are reflective of the biogeochemistry of the associated soils. We analyzed metagenomes from 133 samples from across North America, Europe, and Asia. The phylogenetic composition of communities did not cluster into components based upon environmental factors such as carbon content or pH, or permafrost characteristic such as permafrost age or depth, though a unimodal relationship between microbial diversity and soil pH was observed. In contrast, the functional composition of PMCs, based on largest variation in functional gene distribution, clustered into six types differentiated by latitude, pH, and soil depth. PMC clusters each had a dominant functional profile that included exclusively or in unique combination the following attributes: 1) fermentation and methanogenesis 2) toxin/antitoxin defense mechanisms, 3) osmoprotectants, 4) nitrate and nitrite reduction 5) the use of 'alternate' substrates (e.g., waxes, fatty acids, acetate, alkanes) that are metabolized exclusively through acetyl-CoA rather than pyruvate and 6) iron uptake and utilization. At the panarctic scale, significant variation in the functional potential of PMCs was observed that could be explained by environmental data. However, marker genes were not predicted by environmental data, despite significant variation among sites. These patterns may help to explain variation in microbial processes observed across permafrost soils such as methane and nitrous oxide production, iron reduction, and amino acid cycling, and these data could help to identify locations with high potential for climate feedbacks following thaw.

Changes in Permafrost Microbial Community Composition after Thaw

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Arctic and Subarctic permafrost is thawing at an unprecedented rate, significantly altering the ecosystem. Microbial blooms triggered by permafrost thaw accelerate global warming, change permafrost structure and impact the vegetation. Where and when these blooms will occur is poorly understood. Our study examined the microbial communities in permafrost over a controlled thaw regime. Samples consisted of five different permafrost soils collected in interior and northern Alaska. Both abiotic and biotic factors were examined in to investigate drivers of the microbiome and associated processes. We seek to determine which bacteria represent the core microbiome of Alaskan permafrost soils and how these groups change through thaw.

Investigating the Preservation Process of DNA in the Cold and Arid Paleoshores of the Antarctic Untersee Oasis

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The perennially frozen Lake Untersee and its paleoshores make up the Untersee Oasis, located in Eastern Antarctica, where the mean annual temperature is -10˚C [1]. The lake itself contains actively growing, modern microbial mats, and the cold, arid paleoshores of Lake Untersee are home to dry paleomats, which are remnants of microbial mats that lived within the lake hundreds to thousands of years ago. The combination of the modern and desiccated paleomats provide a unique sample set for the study of biosignature preservation in cold, dry conditions. Recent studies of biomarker preservation have been focused on lipids and other fatty acids which are well preserved in these cold and dry conditions [2]. However, in this study, we focus on the preservation of DNA, a much more information rich molecule. I have analyzed and sequenced DNA extracted from both paleomats found in the shores and modern mats within the lake. Using metagenomics, I study the changes in community structure and composition through time. I study the chemical changes that DNA undergoes through the early stages of diagenesis during which most degradation occurs. Previous studies found the chemical changes to include strand break and deamination damage to bases such as cytosine, adenine and guanine [3]. Here, I assess the DNA quality in the mats by studying the changes in strand length through time. I use Uracil N-Glycosylase treatments to study deamination in the samples.

[1] Andersen, T., Journal of Applied Meteorology and Climatology, 2015; [2] Wilhelm, M. B., Organic geochemistry, 2017; [3] Shapiro, B., Science, 2014

Microbial response to a long-term anoxic batch scenario of permafrost-affected soil

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Permafrost-affected soils are widespread in the Arctic and store about half the global soil organic carbon. This large carbon pool becomes vulnerable to microbial decomposition through permafrost warming and deepening of the seasonal thaw layer (active layer). Here we combined greenhouse gas (GHG) production rate measurements with a metagenome-based assessment of the microbial taxonomic and metabolic potential before and after five years of incubation under anoxic conditions at a constant temperature of 4°C in the active layer, permafrost transition layer and intact permafrost. Warming led to a rapid initial release of CO2 and, to a lesser extent, CH4 in all layers. After the initial pulse, especially in CO2 production, GHG production rates declined and conditions became more methanogenic. Functional gene-based analyses indicated a decrease in carbon- and nitrogen-cycling genes, and a community shift to the degradation of less labile organic matter. This study reveals a decrease in the relative abundance of major metabolic pathway genes and an increase in carbohydrate-active enzyme classes in long-term batch scenarios of thawed permafrost soils and suggests that when labile carbon is steadily depleted, the less labile carbon fractions are increasingly utilized maintaining low but constant microbial GHG production.

Life in the freeze: Microbial community growth and greenhouse gas production across a Holocene to Pleistocene permafrost chronosequence revealed by Stable Isotope Probing

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Permafrost is an extreme habitat which hosts a community of microorganisms that may survive and reproduce for millennia despite acute limitations in water and substrate availability. Many studies focus on DNA-based methods for determining microbial community composition, however these do not distinguish active and in-active cells or preserved DNA. In order to better understand microbial growth and transformation of permafrost C to greenhouse gas, we combined DNA-stable isotope probing (SIP) with process measurements data to reveal activities of microbial communities across a permafrost chronosequence from Holocene (5 ka) to Pleistocene (19 ka, 33 ka). Soils were collected, stored, and incubated at in situ temperatures (-3 °C) with either 18O-water, to target all active microbes, or 13C-glucose, to target actively growing microorganisms which may derive C from glucose. During the incubation period, we collected headspace gas measurements at 3-week intervals and destructively harvested samples for molecular analyses at 6, 12, and 18 months. We found evidence that microbial populations are actively growing and respiring across the entire Holocene to Pleistocene age permafrost gradient. The 5 ka soils yielded the most glucose-derived CO2, though this represented less than 0.05 % of the total 13C added. The 19 ka and 33 ka samples also produced 13CO2, with rates decreasing with increases in permafrost age. The 19 ka samples produced an order of magnitude more CH4 than either the 5 ka or 33 ka permafrost. Actively growing bacteria and archaea were found in all of the samples, although the relative abundance of the microbial population decreased with increasing soil age. DNA-SIP facilitated characterization of the metabolic pathways in growing organisms that likely drove differences in flux of CO2 and CH4. Results indicate the microorganisms are actively growing in intact permafrost and changes in C availability due to slight shifts in temperature may lead to substantial shifts in community composition and the potential release of GHG.

Mercury, methylmercury, and microbial communities in a degrading palsa of the Hudson Bay Lowlands, Far North Ontario

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The Hudson Bay Lowlands (HBL) is the second largest northern peatland in the world, hosting North America's lowest latitude continuous permafrost. The mobilisation of inorganic mercury from thawing permafrost may contribute mercury for methylation and subsequent uptake for biota and human exposure. Based on published circumpolar estimates, 81-150 mg/m2 of mercury may be stored in the top 100 cm of HBL peats; values amongst the highest in permafrost zones of the Northern Hemisphere, although this has not been directly quantified. Moreover, estimates of the inorganic or total mercury pool does not provide any insights into the amounts of methylmercury found there, which is the form of mercury that bioaccumulates, biomagnifies. This paper assesses shifts in microbial community diversity and composition, inorganic mercury, and methylmercury concentrations in surface peat in degrading palsa fields of the HBL in northern Ontario to test the hypothesis that the transition from palsas to thermokarst fens via permafrost degradation creates biogeochemical conditions that enhance methylmercury production. We measured rates of permafrost degradation, and collected cores of permafrost, active layer, and thermokarst from a palsa in a degrading palsa field. One set of core subsamples were freeze-dried, and solid peat was analysed for total and methylmercury using certified ultra-trace methods. Microbial community composition was examined in another set of core subsamples via DNA extractions and high-throughput sequencing of the 16s rRNA gene. In the study region, we found that palsas lost ~0.83% of their area per year as they collapsed into adjacent thermokarst fens. As a result of shifting biophysical conditions, microbial diversity was significantly higher (<0.001) in samples from thermokarst than intact palsas. Through relating surficial permafrost features to microbial community and mercury data, we make important links between the potential release of inorganic mercury stores in permafrost, and methylmercury production in thermokarst as palsas degrade.

Seasonal variation in microbial community depth profiles: implications for understanding nutrient movements

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Climate warming is resulting in permafrost thaw and the deepening of the seasonally-thawed soil active layer. These changes are likely to be accompanied by changes to soil biogeochemistry and nutrient cycling, mediated by shifts in the permafrost microbiome, with implications including the potential for feedback to climate change. To date, however, research has largely focused on changes to the permafrost microbiome from spring snowmelt through summer, when cold regions are most accessible. In this study, we explore active layer, transition zone and permafrost bacterial and fungal communities from summer through fall, using 16S rRNA gene and ITS amplicon sequencing of samples collected from Imnavait Creek, Alaska in June, August and October 2019. Our preliminary sampling suggests changes in the depth profile of the microbial communities between these time points, potentially reflecting processes such as movement of pore water, or microbial dispersal. Our findings suggest that summer sampling alone may not generate a complete picture of the linkages between microbial communities and nutrient movements through groundwater, highlighting the importance of capturing seasonal variation by sampling throughout the year.

Climate change effects on microbial activity in Arctic permafrost and considerations for modeling this system in transition

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Permafrost is thawing at unprecedented rates, significantly altering the landscape through changing subsurface conditions, soil properties, and vegetation characteristics. Permafrost microbes activate as permafrost soils warm and thaw. The contribution of temperature or starting inoculum on the microbial trajectory and potential function after thaw remains a research gap. We hypothesize that the initial soil microbial composition rather than the temperature dictates the end-state microbial community along a short transect of permafrost. We sampled permafrost from five discrete locations representing different ages of deposition in the Cold Regions Research and Engineering Laboratory Permafrost Tunnel in Alaska. In a laboratory incubation study, we gradually warmed the permafrost samples from -3˚C to 6˚C and continuously measured heterotrophic respiration. DNA was extracted and metagenomes were analyzed. Under frozen conditions, microbial respiration rates from different PT locations were similar to one another, ranging from 2 to 12 mg C-CO2 kg-1 d-1. Respiration increased during thaw for three of the permafrost locations, but remained stable for two of the permafrost locations. There was an average of 21.9 million reads per sample. Analysis of the shotgun metagenomes revealed that the trajectory of dominant taxa and their potential function during thaw in a given permafrost location was more influenced by starting inoculum than by incubation temperature. Location was found to be a significant ($p = 0.001$) factor in differentiating taxonomy and potential functional profiles. Furthermore, the five most abundant classes in the permafrost samples include Alphaproteobacteria, Actinobacteria, Clostridia, Betaproteobacteria, and Bacilli. The differential response of microbiome from different locations has important implications for modeling soil biochemical processes across a dynamic landscape. What remains unknown are the effects of long-term thaw and in turn microbial composition on soil function.

Mycorrhizal species characterization of tundra plant roots

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North American Arctic plant communities are shifting from graminoid-dominated to shrub-dominated in a process known as shrubification, a change that is associated with shifts in belowground microbial ecology such as mycorrhizal colonization and rhizosphere community composition, and biogeochemical functions such as soil carbon sequestration and decomposition. The variation of mycorrhizal species colonization across different tundra plant types remains a knowledge gap. Likewise, it is unclear the extent to which mycorrhizal colonization can affect the community composition of root associated bacteria and fungi. Since rhizosphere microorganisms can have substantial impacts on carbon fluxes between soil organic matter and the atmosphere, we wanted to examine if mycorrhizal fungi influence the rhizosphere community composition of various plants throughout the tundra. It has been shown that different tundra plant species will harbor different compositions of mycorrhizal fungi and rhizosphere communities; however, less is known about mycorrhizal and rhizosphere variation within a plant species. Since most tundra plants are generalists and can harbor many different species of mycorrhizal symbiotes, it was expected that within plant species variation of mycorrhizal composition would be high. To that end, we hypothesized that the mycorrhizal fungi composition would be an explanatory variable of rhizosphere community composition. Our sampling was conducted August of 2021 near Toolik Lake Field Station, Alaska. Roots and rhizosphere soil of various tundra plants were collected and sequenced using amplicon sequencing with general bacterial, fungal and mycorrhizae-specific primers to determine the rhizosphere and mycorrhizal species composition. The primary purpose of this study was to look at variation within and between the mycorrhizal and rhizosphere community compositions across different tundra plant species. We hope this work will serve as foundational information for other researchers involved in tundra biogeochemistry and mycorrhizal fungi.

Assembly of microbial communities in thawing permafrost

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Background: Arctic permafrost soils store one third of the world's soil carbon and amplified Arctic warming is thawing permafrost. Microorganisms in thawing permafrost can access previously unavailable soil organic matter and release greenhouse gases through decomposition. The rate and type of gases released depends on thaw-induced changes in microbial community structure and functional capacity. However, predicting these changes and subsequent carbon release is challenging since the community shifts – collectively known as assembly – are the result of a combination of eco-evolutionary and environmental processes, some of which can be directly predicted from species identity ("deterministic" forces) and others which are random with respect to species ("stochastic" forces). Due to compounding perturbations and the potential for hysteresis during assembly, predicting multi-year effects of thaw on communities is particularly challenging without first understanding the deterministic and stochastic forces in play. Yet, this mechanistic understanding of community assembly is vital to design more accurate predictive models of permafrost carbon dynamics. Objective: To better understand how the ecological processes structuring these microbial communities and their functions over time, we used shotgun metagenomics to investigate the changes in active layer soil microbial communities over six years (2011-2017) along a permafrost thaw gradient in Stordalen Mire, near Abisko Sweden. We then used ecological models to identify and quantify assembly processes. Results: We found that soil communities transitioned from homogenizing to heterogeneous selection as permafrost thawed, and that habitat-specific selection increased across years. This suggests a switch from abiotic pressure to competition during thaw which increased from 2011 to 2017. We also identified genes and organisms most associated with selective forces and therefore most likely to be affected in thawing permafrost. These results give us a better idea of the ecological forces structuring post-thaw microbial communities, and will help us better predict carbon dynamics in thawing permafrost ecosystems.

The Transition From Stochastic to Deterministic Bacterial Community Assembly During Permafrost Thaw Succession

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Climate warming has resulted in permafrost thaw across the Northern high latitudes over the past several decades. Thaw-induced environmental changes lead to shifts in microbial communities and their associated functions, however the ecological processes that shape the final composition of microbial communities are not well understood. Understanding how the processes that structure the identity and abundance (i.e. assembly) of pre- and post-thaw microbial communities may inform our ability to predict the functional outcomes of permafrost thaw. Deterministic processes are driven by abiotic and biotic selection pressures, and stochastic processes are associated with more uncertainty. The objective of this study was to determine the relative contribution of stochastic and deterministic assembly processes in active layer and permafrost soils before and after thaw. Specifically, we aimed to characterize the effect of time since thaw on assembly processes and evaluate the impact of increased temperature on assembly dynamics. We characterized microbial community assembly during permafrost thaw using in situ observation and a laboratory incubation encompassing active layer, transition zone, and permafrost soils from the Storflaket Mire in Abisko, Sweden where permafrost thaw has occurred over the past decade. We found microbial community assembly was driven by stochastic processes immediately after thaw. As post-thaw succession progressed, deterministic processes became increasingly important in structuring microbial communities. Furthermore, laboratory-induced thaw was reflective of assembly dynamics in situ immediately after thaw, however, short-term lab studies might not capture the long-term effects of permafrost thaw on microbial community dynamics. Our results suggest predictions of microbial community dynamics in the weeks to years after thaw confounded by the high amount of stochasticity structuring the communities.

Altered microbial structure and function after thermokarst formation

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Permafrost thaw could induce substantial carbon (C) emissions to the atmosphere, and thus trigger a positive feedback to climate warming. As the engine of biogeochemical cycling, soil microorganisms exert a critical role in mediating the direction and strength of permafrost C-climate feedback. However, our understanding about the impacts of thermokarst (abrupt permafrost thaw) on microbial structure and function remains limited. Here we employed metagenomic sequencing to analyze changes in topsoil (0-15 cm) microbial communities and functional genes along a permafrost thaw sequence (1, 10, and 16 years since permafrost collapse) on the Tibetan Plateau. By combining laboratory incubation and a two-pool model, we then explored changes in labile and stable soil C decomposition along the thaw sequence. Our results showed that topsoil microbial α-diversity decreased, while the community structure and functional gene abundance did not exhibit any significant change at the early stage of collapse (1 year since collapse) relative to non-collapsed control. However, as the time since the collapse increased, both the topsoil microbial community structure and functional genes differed from the control. Abundances of functional genes involved in labile C degradation decreased while those for stable C degradation increased at the late stage of collapse (16 years since collapse), largely driven by changes in substrate properties along the thaw sequence. Accordingly, faster stable C decomposition occurred at the late stage of collapse compared to the control, which was associated with the increase in relative abundance of functional genes for stable C degradation. These results suggest that upland thermokarst alters microbial structure and function, particularly enhances stable C decomposition by modulating microbial functional genes, which could reinforce a warmer climate over the decadal timescale.