Landscape topography structures the soil microbiome in arctic polygonal tundra

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In the Arctic, environmental factors governing microbial degradation of soil carbon (C) in active layer and permafrost are poorly understood. Here we determined the functional potential of soil microbiomes horizontally and vertically across a cryoperturbed polygonal landscape in Alaska. With comparative metagenomics, genome binning of novel microbes, and gas flux measurements we show that microbial greenhouse gas (GHG) production is strongly correlated to landscape topography. Active layer and permafrost harbor contrasting microbiomes, with increasing amounts of Actinobacteria correlating with decreasing soil C in permafrost. While microbial functions such as fermentation and methanogenesis were dominant in wetter polygons, in drier polygons genes for C mineralization and CH4 oxidation were abundant. The active layer microbiome was poised to assimilate N and not to release N2O, reflecting low N2O flux measurements. These results provide mechanistic links of microbial metabolism to GHG fluxes that are needed for the refinement of model predictions.

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Ice wedge polygons are important topographical and hydrological features in otherwise low relief coastal arctic tundra. Ice wedge development begins at the ground surface as frost cracks and forms a polygonal pattern in the top soil as ice wedges enlarge over time. Approximately 20% of the Arctic Coastal Plain of northern Alaska contains polygonal grounds and thaw lakes that develop in ice-rich permafrost. Depending on their growth or degradation state, ice wedges can create various sizes (~5–20 m) and types of polygons. Development of this microtopography regulates water distribution over large areas and affects soil properties that are important for C cycling, such as soil moisture, other biogeochemical cycles, and freeze-thaw dynamics. With a predicted increase in surface air temperature of 5–6 °C by the end of this century4 an accelerated soil warming in the polygonal tundra will lead to a deepening of the seasonally-thawed active layer, enlarge permafrost thaw area and changes in the distribution of water across this landscape. Therefore, the estimated 1035 Pg C stored in arctic ecosystems could undergo rapid microbial decomposition, resulting in a significant positive feedback on the climate in the form of greenhouse gas (GHG) emissions.

A major challenge in linking microbial functions to GHG emissions in arctic soils is the identification of environmental factors that govern the distribution of microbial functions and C fluxes. Besides the complexity introduced by the high microbial diversity in these soils, our understanding of environmental controls and spatial variation on microbial C metabolism, with respect to hydrological patterns, is limited. To resolve the microbial metabolism leading to GHG emissions across the polygonal tundra in the Arctic, we used multiple DNA sequencing approaches to determine the soil microbial community composition and metabolic potential. We focused our studies in the Barrow Environmental Observatory (BEO) located in Barrow, Alaska. Polygonal grounds constitute 65% of the surface in the Barrow Peninsula. The landscape of the BEO includes a mosaic of drained thaw lakes and interstitial polygons where different polygon types—high-centered (HC), flat-centered (FC) and low-centered (LC)—represent different states of permafrost degradation and moisture distribution (Fig. 1). Variance in landscape features (e.g., rim, center, and trough) have been hypothesized to result in distinct microbial habitats that might respond differently to warming. We collected 29 active layer soil cores along a 500 m transect that extended laterally across HC, FC, and LC polygons in the BEO in September 2011. The top 0–50 cm, including surface organic (0–10 cm) and deeper mineral (20–50 cm) layers of the active layer, was collected from centers, rims, and troughs of the polygons along the transect (Fig. 1). Additionally, two deep cores, 1 and 2.65 m in depth, were taken from a FC polygon within the transect in April 2012 to access the vertical distribution of the microbial communities in the permafrost. The soil microbiomes compositions in the samples were determined by sequencing of 16S rRNA genes (16S) and the functional potential was inferred by sequencing of total metagenomic DNA. The sequence data were correlated to in situ GHG flux measurements and geophysical and geochemical soil characteristics. Here we show that soil microbiomes and their functional potentials in arctic polygonal grounds are governed by the landscape microtopography.

Results

Microbial community composition is linked to polygon types.

We found a shift in microbial community composition along the polygon transect, with different polygon types and soil horizons having distinct microbiomes (Fig. 1, Supplementary Fig. 1). Although the soil microbiomes primarily clustered by polygon type (ANOSIM R = 0.432, p < 0.001) (Supplementary Fig. 1), the two soil horizons were populated with phylogenetically different microbial populations (organic and mineral; ANOSIM R = 0.117, p = 0.044). We detected a weak spatial autocorrelation between different polygon types (Moran’s I: 0.144, SD: 0.018, p = 1.964E−05) but not between soil horizons (Moran’s I: −0.035, SD: 0.079, p = 0.983). Additionally, there were significant correlations between organic C content (Adonis analysis: F1,25 = 2.02, r² = 0.057, p = 0.032) and total nitrogen (N) content (F1,28 = 2.33, r² = 0.065, p = 0.019) of the samples and the observed microbial community clustering patterns (Supplementary Fig. 1). These results are in alignment with parallel measurements of the soils’ geophysical and geochemical properties, which demonstrated differences among polygon types. The phylogenetic alpha diversity (Faith’s PD) was also significantly different among the different polygons (ANOVA F2,27 = 18.64, p < 0.001) and soil horizons (ANOVA F1,28 = 14.34, p < 0.001) (Supplementary Table 3, Supplementary Fig. 2). However, within each individual polygon type no significant differences were observed between the alpha diversity of organic and mineral horizons. In general, the HC polygons (both horizons) had the highest alpha diversity (Supplementary Fig. 2).

Proteobacteria (HC 40 ± 10%, FC 35 ± 14%, LC 28 ± 22%) and Actinobacteria (HC 22 ± 5%, FC 34 ± 10%, LC 35 ± 15%) were the two most abundant microbial phyla across the transect (Fig. 1). Using canonical correspondence analysis, we found that 55% of the observed variation in microbial community composition at the OTU level could be explained by a combination of environmental features (Supplementary Fig. 3), including polygon type, soil horizon, pH, total C, and organic C content. HC and FC polygons were dominated by Alphaproteobacteria (ANOVA F2,27 = 3.64, p = 0.015) and Acidobacteria (ANOVA F2,27 = 6.31, p = 0.016) whereas the wetter LC polygons contained significantly more Bacteroidetes (ANOVA F2,27 = 5.15, p = 0.031) and Verrucomicrobia (F2,27 = 7.22, p = 0.012) (Fig. 1, Supplementary Fig. 3). In addition, the deeper mineral soil horizons had higher relative levels of Actinobacteria (ANOVA F1,28 = 6.60, p = 0.021) (HC 24 ± 5%, FC 37 ± 9%, LC 47 ± 11%) than the surface organic soil horizons (Fig. 1).

Several functional traits were derived for representative members of the soil microbiomes across the polygon transect based on the similarities of 16S sequences to known species with characterized physiologies. Representatives of methanogenic Euryarchaeota were more abundant in the LC polygons (max. 4.6% relative abundance), with high levels of Methanothermobacterium and Methanosetaea in the organic horizons and Methanosetaea and Methanosarcina in the mineral horizons (Supplementary Fig. 4a). Many different representatives of potential methane oxidizing Bacteria were also detected in all of the polygons, including Verrucomicrobia (0.02–0.37%), Alpha- (0.04–0.92%, most abundant genus: Methyllobacterium), Betaproteobacteria (0.12–1.00%, most abundant genus: Methyllobium) and Gammaproteobacteria (0.01–0.28%, most abundant genus: Crenothrix) (Supplementary Fig. 4b). The organic horizons of the LC polygons had a higher relative abundance of methylotrophic bacteria (max. 2.5% relative abundance) in comparison to mineral layers. On the other hand, representative ammonia-oxidizers were more prominent in the HC polygons, including Candidatus Nitrososphaera, Nitrosipina, and Nitrobacter with relative abundances varying between 0.08–0.15% (Supplementary Fig. 4c).

In addition to sampling along the lateral transect, we also sampled two deep cores (1 and 2.65 m depth) from a FC polygon to determine the microbial community distribution with depth into the permafrost (Fig. 2). We analyzed the geochemistry and the soil microbiome in 5 cm intervals through these cores (Supplementary Table 1). While organic and mineral layers were
acids, soil pH increased up to 8.5 with depth (Fig. 2, Supplementary Table 1). Soil C content on the other hand, decreased with depth. NO$_3^-$, NO$_2^-$, and total Fe concentrations also decreased with depth whereas SO$_4^{2-}$ concentrations increased in the deep permafrost layers (Fig. 2). Actinobacteria was the most abundant phylum throughout the soil profiles, accounting up to 68% of the total microbiome. Within this phylum, Actinomycetales and Solirubrobacterales were the most abundant orders at 42% and 30% relative abundance, respectively (Supplementary Fig. 5). Although Actinomycetales were abundant along the whole depth profile, the relative abundance of Solirubrobacterales increased in deeper permafrost layers (1–2.64 m). Most of the Actinobacterial orders, except Acidimicrobiales, were negatively correlated to high C, N, total Fe, NO$_3^-$, and NO$_2^-$ content in the organic active layer soils. However, below the active layer, correlations between Actinobacterial orders and sample chemistry were marginal. The permafrost layers also had high relative amounts of Bacteroidetes (max. 29%) and candidate phylum OP9 (max. 23%). Additionally, more Euryarchaeota were detected in the deeper permafrost samples compared to the active layer (max. 13% and max. 3% relative abundance, respectively).

The microbial diversity of the active layer was significantly different from that of the permafrost (Supplementary Fig. 6). Alpha diversity (Faith’s PD and Shannon H$’$) generally decreased with depth, but some depths had transient spikes in diversity (Supplementary Fig. 5). We also found that the diversity decreased in the transitional layers between the active layer and permafrost, at ~50 cm depth. At this depth we detected higher pH and C content in comparison to the overlying acidic active layer.
We hypothesize that the abrupt shift in soil chemistry resulted in the decreased diversity at this location. The microbiomes also differed in composition with depth (Fig. 2). The observed clustering was strongly and significantly influenced by pH, depth and changes in material density (calculated from CT-scanning images; Fig. 2, Table 1). Network analyses of the ratio of shared total operational taxonomic units (OTUs), revealed differences in microbiome clustering patterns with depth (Supplementary Fig. 7). Interestingly, no clusters were observed across the polygon transect (ANOVA F 2,14 = 0.004) and transporters (ANOVA F 2,14 = 4.018, p = 0.030). Genes for nitrification, NO3− reduction and assimilation, stress response and pyrimidine metabolism pathways were more abundant in the HC polygons (Fig. 3a), whereas genes for H2 production, ABC transporters, valine, leucine, and isoleucine degradation and biosynthesis pathways were more abundant in the FC polygons. The LC polygons had higher amounts of genes for fermentation, N-fixation, osmotic stress response, glycosidase hydrolase, and glutamine/glutamate metabolism.

Previously, significant seasonal differences were observed across the polygon transect, such as higher CH4 flux from the LC polygons in early summer compared to late summer10. Here we identified subunits of a key enzyme responsible for CH4 production (methyl coenzyme M reductase, mcrABG) that was significantly higher in the LC polygons compared to the other polygon types (Fig. 3b), corresponding to higher abundances of methanogen 16S rRNA genes in the LC polygons (Fig. 3b). Active layer thickness (ALT) was also significantly deeper (ANOVA F2,27 = 8.14, p = 0.004) in this polygon type due to the more extensive thaw. Subsequent sampling of the site revealed that the LC polygons were consistent

**Microbial genomic potential for GHG production.** The metagenome data revealed both phylogenetic and functional variations across the polygon transect (ANOVA F2,14 = 1.79, p = 0.047) (Fig. 3). The functional gene repertoires were based on functional assignments of the metagenomes using the FOAM9 database. FOAM enables screening of metagenomes for environmentally relevant genes, including global C and N cycles, derived from KEGG Orthologs (KOs). The functional gene compositions of the metagenomes from HC polygons were highly similar across the organic and mineral layers. By contrast there were differences in functional gene compositions between the layers for the FC and LC polygons. Specific high-level FOAM9 categories (Supplementary Fig. 8) that differed significantly across the polygon transect included methanogenesis (ANOVA F2,14 = 5.34, p = 0.012) and transporters (ANOVA F2,14 = 4.018, p = 0.030). Genes for nitrification, NO3− reduction and assimilation, stress response and pyrimidine metabolism pathways were more abundant in the HC polygons (Fig. 3a), whereas genes for H2 production, ABC transporters, valine, leucine, and isoleucine degradation and biosynthesis pathways were more abundant in the FC polygons. The LC polygons had higher amounts of genes for fermentation, N-fixation, osmotic stress response, glycosidase hydrolase, and glutamine/glutamate metabolism.
sources of CH₄ (Supplementary Fig. 9, Supplementary Fig. 10). The in situ CH₄ flux was significantly higher (Akaike Information Criterion (AIC): 301.04, F₂,4₁ = 11.17, p = 0.00013) in centers of LC polygons compared to the other polygon types (Fig. 3b), although CH₄ fluxes were sporadically detected at lower rates in wetter areas, such as troughs, along the transect (Supplementary Fig. 9, Supplementary Fig. 10). Both phylogenetic marker gene and functional gene distributions in the LC polygons suggest that multiple Archaeal species and pathways were involved in the CH₄ production, including genes from hydrogenotrophic (CO₂-reducing), acetoelastic, and methylotrophic methanogens, with no significant differences in their relative distributions (Supplementary Fig. 11) in organic and mineral layers. LC polygons had 2-fold higher relative abundance of methanogenesis genes in comparison to HC and FC polygons (Supplementary Fig. 11). By contrast, the potential for CH₄ oxidation was detected along the potential for CH₄ oxidation was detected along the BEO were also observed (Supplementary Fig. 13). By contrast, genes encoding enzymes for metabolism of oligosaccharides and other simple carbohydrates were more predominant in the LC polygons, including alpha-mannosidase (EC:3.2.1.24) (ANOVA F₂,₁₄ = 3.2, p = 0.049), sugar utilization via hexose degradation (ANOVA F₂,₁₄ = 4.18, p = 0.038), starch degradation (Supplementary Fig. 14, Supplementary Fig. 15) and ABC transporters involved in sugar transport (Supplementary Fig. 23). The LC polygons also had a significantly higher proportion of genes involved in mixed acid fermentation pathways (Spearman’s rho = 0.44, p = 0.041) compared to the other polygon types. However, there were no significant differences in many other central metabolic pathways among polygons, including pyruvate fermentation (Supplementary Fig. 16), homoacetogenesis, fatty acid oxidation, or hydrocarbon degradation (Supplementary Fig. 17, Supplementary Fig. 18, Supplementary Fig. 19).

Several genes encoding anaerobic terminal electron acceptor pathways were represented in the metagenomes. The LC polygons had up to 2-fold higher levels of decalene cytochromes (mtr) (Supplementary Fig. 20), suggesting that there is a significantly higher potential for Fe reduction in this polygon type. Previous, lab-scale incubations using Barrow LC polygon soils showed that Fe reduction and methanogenesis occur concurrently in the organic soils, supporting our findings. Our results suggest that Fe reduction capacity is not equally distributed across the polygonal landscape and, similar to methanogenesis, might be limited to the wetter LC polygons. By contrast, genes involved in the sulfur cycle were found across all polygon types and soil horizons in relatively similar abundances (Supplementary Fig. 21).

The relative abundances of genes involved in N cycling varied among polygon types and soil horizons. N-fixation genes were significantly higher in abundance (p < 0.01) in LC polygons and mineral soils (Fig. 3c), whereas the HC and FC polygons had higher abundances (up to 0.1% of total gene content) of genes involved in nitrification; for the HC polygons this corresponds to the relative high abundance of putative nitrifiers that were detected (Supplementary Fig. 4c). All polygons had low genomic potential for denitrification and N₂O production. Genes for N- assimilation (nirB, nrf) from NO₂⁻ to NH₄ were found in all polygons in similarly high abundance, as well as genes involved in conversion of organic N to NH₄ (for example, gdh and uredC). Our metagenome data are supported by gas flux measurements that found negligible amounts of N₂O production (0.130–0.742 nmol N₂O m⁻² s⁻¹) at only 5 of 153 points across the polygon site at Barrow, with no apparent link to soil moisture.

**Novel microbial genomes across the polygons.** Thirty three nearly complete (>89%) and low contamination (<15%) draft microbial genomes were binned from the assembled metagenomic sequences (Fig. 4, Supplementary Table 5). The genome bin sizes were between 2.1 and 7.6 Mb with a variable GC content ranging from 36–69%. Most of the genomes constituted, on average, 1% (min. 0.25%, max. 5.96%) of the total reads in their respective metagenomes; in total 10–14% of the metagenome
reads could be mapped back to genome bins (Supplementary Table 5). We assembled full-length 16S sequences in half of the genome bins, ranging between 82 and 95% similarity to 16S sequences from previously sequenced genomes (Supplementary Table 6). **Proteobacteria**, **Bacteroidetes**, **Acidobacteria**, and **Actinobacteria** were the four main phyla that the genome bins were assigned to. The majority of the low GC genomes were identified as **Bacteroidetes**, whereas most of the high GC genomes were represented in **Actinobacteria** (Supplementary Table 6, Supplementary Table 7).
Across different polygon types, genome bins were from genetically different but functionally similar microbial species. The bins showed low similarity to publicly available genomes (Fig. 4b) suggesting that they represent novel species. All of the genome bins contained a variety of genes encoding degradation of cellulose, hemicellulose, and polysaccharides. Almost all of the genomes contained glucosidases for degradation of oligosaccharides, but genes encoding the initial steps in cellulose breakdown were found in just some of the genomes (Fig. 4). The majority of the chitinases were located in Bacteroidetes and Actinobacteria genome bins. Hemicellulose and cellulose degradation potential were more common than chitinases across different phyla. The lower occurrence of complete cellulose breakdown pathways in Barrow genomes relative to those for utilization of cellobiose (Fig. 4) is consistent with previous research showing that cellobiose utilization outnumbered cellulase-metabolic pathways in currently known genomes.

Genomic analyses indicated that most of the genomes represented facultative anaerobes, with pathways for fermentation of glucose, lactate, and pyruvate (Figs. 4a and 5). Several of the genomes had multiple copies of alcohol and aldehyde dehydrogenases. Formate and CO-dehydrogenases were mainly detected in genomes binned from the HC and FC polygon metagenomes. Three genomes—one in each polygon type—also contained putative ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) genes for CO₂ assimilation. Since molecular hydrogen is an important metabolic intermediate in saturated environments (i.e., wetlands) we investigated the presence of hydrogenases in Barrow genomes. Fe,Fe-hydrogenase complexes, which are found in fermenters that produce high molar ratios of H₂, were not detected in any of the genomes. Ni,Fe-hydrogenases that are commonly found in organisms using H₂ as a donor for respiratory metabolism were mainly present in FC polygon genomes.

The genomes expanded upon our findings from the metagenomes regarding the potential for cycling of N. All of the genomes contained genes required for N- assimilation, and many also had genes encoding transporters for NH₃ and NO₃⁻/NO₂⁻ (Fig. 5, Supplementary Table 7). Denitrification pathways were sparse and mostly incomplete across all polygon types. These functions were mainly found in FC polygons where four out of eight genomes contained incomplete denitrification pathways. In addition, one Bacteroidetes bin from a LC polygon contained an incomplete denitrification pathway while the remaining LC genomes did not comprise any known denitrification genes. Additionally, we did not detect genes for nitrification, N-fixture, or aerobic CH₄ oxidation in any of the genomes.

Data mining of the binned genomes also supported the metagenome data with respect to the potential for methanogenesis in the LC polygons. We assembled one partially complete methanogen (completeness 41.06%, contamination: 9.1%) from a LC polygon (Fig. 5). This new candidate Archaeal strain E.001 had 87% 16S similarity to a previously described hydrogenotrophic methanogen, Methanolinea tarda strain NOB1-17, and its mcrA gene had 76% identity (blastp e-value: 6E⁻⁵⁸) to Methanocella paludicola, another hydrogenotrophic methanogen isolated from a rice paddy (Rice Cluster 1). Additionally, the genome bin contained a mtd (F420-dependent methylene tetrahydromethanopterin tetrahydromethanopterin dehydrogenase; EC 1.5.99.9) gene which exhibits 56% identity (blastp e-value: 2E⁻¹⁰⁴) to Methanolinea tarda. This draft genome also contained the complete pathway for hydrogenotrophic methanogenesis (Fig. 5).

Additional genes identified in the genome bins included those encoding cold and heat shock proteins, alkaline phosphatases and sulfate adenylyltransferases (Figs. 4a and 5). A large number of cold-shock proteins have previously been identified in psychrophiles and cold-adapted microorganisms. Therefore, phylum-wide availability of these genes was anticipated. Unlike the cold-shock response, heat shock can be triggered by nonspecific stress factors which could further support the survival of arctic microorganisms under changing environmental conditions.

Four out of eight Actinobacteria genome bins had genes for spore formation (Fig. 4a). Seven genomes had genes for flagella suggesting the potential for motility.

**Discussion**

The majority of arctic microbiology research to date has focused on high soil moisture locations such as LC polygons, as potential hot-spots for CH₄ production. A recent IPCC report indicates a stronger climate forcing by CH₄ emissions (equivalent to 34 g of CO₂ per g of CH₄) than previously anticipated. This comparison highlights the importance of CH₄ emissions from permanently inundated areas. However, as water is heterogeneously distributed spatially and temporally in the tundra, oxygen availability would also be expected to be unevenly distributed. As a result aerobic and anaerobic processes alike are of importance to understanding GHG production from polygonal landscapes. Disturbances in polygonal grounds, such as caused by thermo-erosion, can occur on a decadal scale and result in severe and immediate impacts on the terrain hydrology, ALT, and vegetation distribution. Thus, understanding the distribution of the microbial and environmental controls over the polygonal landscape that regulates the emission of GHG is essential.

Here we demonstrated that microbial diversity and composition changed both horizontally across the polygonal landscape and with depth into the permafrost layer. A key feature of the permafrost community was an increasing dominance of Actinobacteria with depth, corresponding with a decline in carbon and nutrients (Fig. 2). In particular, Solirubrobacterales increased in abundance with depth (Supplementary Fig. 8a); presumably due to their ability to survive cold, nutrient limited conditions, such as the Dry Valleys of Antarctica. Actinomycetales were otherwise the dominant order in the permafrost, and their co-occurrence with representatives of Solirubrobacterales may be due to niche partitioning, as survival strategy to utilize limited resources.
Our novel findings go beyond generalizations to illustrate which specific C decomposition pathways (e.g., genes for cellulose and chitin degradation; Fig. 4a) are present in the soil microbiome and the metabolic capacity of specific populations (genomes) from the site and how those data are correlated to gas flux measurements. In particular, there were contrasting carbon metabolic pathways in the HC and LC polygons, with a higher relative abundance of genes for degradation of more complex organic compounds in the HC polygons and for metabolism of simple carbohydrates and fermentation in the LC polygons. We also found significant differences in genes involved in the nitrogen cycle. For example, higher levels of genes for nitrogen fixation were detected in LC polygons and higher levels of genes for nitrification in HC polygons. Another novel finding was the link between low levels of genes for NO2 production across the polygons that corresponded to low measured NO2 flux at the Barrow site.

The balance between methane generation and oxidation was a key differentiator across the polygon transect. The key gene for methanogenesis was only observed in the LC polygons, corresponding to the significantly higher measured CH4 flux at this location (Fig. 3b), as we previously reported for bog samples at a different site in Alaska. Other genes involved in methanogenesis were found in all polygon types, but they had ~100x higher abundance in the LC polygons, compared to the HC and FC polygons (Supplementary Fig. 9). We hypothesize that methanogenesis is favored in the wetter regions of the the LC polygons, not only because they retain water, but also because they have warmer season temperatures that delay freezing of top soils and result in accumulation of organic matter in the polygon centers. High amounts of organic material favor methanogenesis both by provision of substrates for fermentation and for maintaining low oxygen levels. CH4 was previously measured in pore water samples collected below 20 cm depth from both HC and FC polygons at Barrow. However, we found that the amount of CH4 flux from wetter areas in HC and FC polygons was much lower than that measured from the LC polygons. We hypothesize that the accumulated CH4 was oxidized in the upper soil layers in the HC and FC polygons before it reached the surface. Our metagenome data suggest that methane oxidizers were more abundant in those polygon types, thus supporting this hypothesis. In the extreme case, in some mineral arctic soils, CH4 flux can be entirely suppressed by CH4 oxidation thus becoming a sink for atmospheric CH4.

One hypothesis is that hydrological conditions (i.e., lateral movement and drainage) and soil structure allow the presence of both micro-aerophilic and anaerobic conditions in the LC polygons, thus supporting both CH4 production and oxidation. However, in the mineral soils of the LC polygons there was a significantly lower relative abundance of pmo genes compared to the rest of the samples. These samples also had the lowest abundance of Methylibium (Supplementary Fig. 2b) which was otherwise the most abundant CH4 oxidizing bacteria in HC and FC polygons. The reduced potential for CH4 oxidation (based on pmo gene abundance) could be one of the underlying mechanisms leading to high CH4 fluxes from this polygon type.

In inundated areas such as LC polygons 75–95% of the subsurface CH4 has been reported to be lost to the atmosphere due to the combined upward transport of mediated by plants and ebullition. During this transport only 0.8–8.7% of subsurface CH4 is available for microbial oxidation. The dominant vegetation type at our location was Carex aquatilis, a sedge with aerenchyma known to transport CH4. Thus despite the high potential for CH4 production in the wetter LC polygons, vegetative transport and removal from the system may be factors that limit CH4 oxidation by the resident microbes.

By targeting genes involved in cycling of N we provide the first metagenomic evidence that the biomes of arctic polygonal grounds are enriched in genes for N-assimilation. This finding suggests that the microbial communities are poised to take up N as it becomes available in this N poor environment. Previously, 815N measurements identified snow melt and nitrification potential in HC polygons as the main sources of NO3- in Barrow polygons. Although Lipson et al. showed that denitrification
potential was present at the Barrow site; our metagenome findings suggest that NO$_3^-$ was utilized as a N source, but not lost through denitrification. Also observed negligible amounts of N$_2$O flux from this landscape with no apparent links to polygon types, soil moisture or other environmental conditions (Fig. 3b).

The metagenome data suggest that the wetter LC polygons are favorable for both methanogenesis and Fe reduction processes (Supplementary Fig. 20). Previously, lab-scale incubation studies from Barrow LC polygons showed that Fe reduction and methanogenesis occur concurrently$^{12}$ in the organic soils, supporting our findings. Microbes involved in both processes are potential consumers of available organic acids and fermentation products$^{35}$ and we found that the LC polygons were enriched with genes involved in degradation of simpler carbohydrates (i.e., sugars) and mixed acid fermentation pathways (Supplementary Fig. 15, Supplementary Fig. 16) that could be linked to these processes. A combination of anaerobic conditions and availability of organic acids from root exudates from sedges$^{36,37}$ are likely facilitators of microbial potential for fermentation in the wet areas.

Increased CH$_4$ emissions with permafrost thaw is a common observation in arctic tundra$^{38-40}$. However, the influence of increasing soil temperatures on GHGs in polygonal grounds depends strongly on the interactions between temperature, soil moisture and drainage potential$^{10}$. At BEO, CH$_4$ flux was predominantly observed from wetter, LC polygons (Fig. 3b). However, despite the differences in soil moisture distribution (Supplementary Table 8), we did not observe significant differences among CO$_2$ fluxes in FC and LC polygons; although the HC polygons had intermittently higher fluxes in the late summer months (Supplementary Fig. 9, Supplementary Fig. 10)$^{10}$. These findings could be due to differences in soil organic matter deposition, decomposition and root respiration rates over the season. In the HC polygons there were several genes encoding enzymes for degradation of C polymers that were significantly (~10×) more abundant when compared to the other polygon types. These included genes encoding: xylan lyase (Supplementary Table 9, Supplementary Table 10), chitinase (Supplementary Table 11, Supplementary Table 12) (Supplementary Fig. 11). This enrichment of hydrolytic enzymes suggests that complex plant polymers are available as microbial growth substrates in HC polygons. In contrast, the wetter LC polygons were enriched with genes for anaerobic processes, such as sugar and mixed acid fermentation, iron reduction, and methanogenesis (Supplementary Table 8, Supplementary Table 9, Supplementary Table 10, Supplementary Table 11, Supplementary Table 12, Supplementary Table 13, Supplementary Table 14, Supplementary Table 15, Supplementary Table 16, Supplementary Table 17, Supplementary Table 18, Supplementary Table 19, Supplementary Table 20, some of which resulted in anaerobic CO$_2$ fluxes. As polygonal landscapes transition into a drier and more high-centered state$^{36,41,42}$, we hypothesize that the corresponding decrease in soil moisture that leads to death of vascular plants will at least transitionally provide plant residues that serve as a substrate for the resident soil microbes.

We binned several novel draft genomes from the metagenomes (Figs. 4 and 5). These bins were both taxonomically and...
functionally diverse and contained multiple genes involved in breakdown and utilization of diverse structural polysaccharides (Figs. 4 and 5). This wide range of substrate utilization capacity has been observed in several microbial isolates from the Arctic35 and hypothesized to be related to improved resilience to fluctuating temperatures and nutrient-deficient conditions in tundra soils. Our results, moreover, point towards functional heterogeneity as a mechanism to cope with changing moisture conditions because facultative metabolism was found across the genome bins. This potential was accompanied by presence of cellulose-degrading enzymes and alcohol dehydrogenases both in organic and mineral soil layers (Figs. 4a and 5). In arctic peat soils gradients of aerobic to anaerobic conditions have been hypothesized to structure microbial metabolic potential35,36. Based on our findings we also hypothesize that soil structure and drainage create dry or wet micro pockets within the same soil horizon that favor the growth and survival of taxonomically diverse microorganisms with facultative metabolism.

We assembled one partially complete Archaeal bin that based on 16S and mcrA gene identification showed low similarity to previously described hydrogenotrophic methanogens37,38. This methanogen was co-localized with fermentative bacteria (Fig. 4a) that can produce H2 thus potentially supporting hydrogenotrophic methanogenesis. However, the metagenome results showed that multiple Archaeal species and pathways were potentially involved in production of CH4 within LC polygons (Supplementary Fig. 11). In fact, in seasonal succession of methanogenesis, the acetoclastic pathway was found to be dominant when active layer soils were thawed35 and hydrogenotrophic methanogenesis to be dominant at the end of summer.33. This methanogen represents one of many Archaeal genomes that were present in these samples.

We also screened the genome bins for genes involved in stress survival. Spore forming was not widely detected in Barrow genome bins (Fig. 4a); supporting the previous assessment44 that spores are not the best survival strategy for freezing conditions. We hypothesize that instead of dormancy and spore formation that the microorganisms at Barrow use a combination of cold and heat shock proteins, cryoprotectants (including sugars originating from soil organic matter45) and low activity—enabling critical cellular functions such as DNA repair—to survive harsh winter conditions.

In summary, the structure and functions of soil microbial communities in polygonal grounds are usually shaped by the landscape topography. Our research demonstrates that a metagenomic approach can resolve metabolic roles for uncultivated soil microorganisms and provide the framework needed to predict their metabolic response when the landscape evolves. Our results demonstrate that distribution of genes involved in soil organic C degradation is significantly related to the landscape topography. This knowledge is important because it provides evidence towards predicting the microbial metabolic response to increased temperatures in arctic soils that can potentially contribute to a positive climate feedback39,46,47. Our results advances previous findings of genes and pathways involved in soil organic C degradation in other arctic soil metagenomes21,37,48–51. Specifically, the knowledge gained provides more mechanistic detail about the metabolic potential of the soil microbiome and can help to define the metabolic routes for GHG production for refinement of model predictions. The combination of detailed hydrological, biogeochemical, vegetation, and microbial data obtained from the BEO site10,52,53 suggest that under a warmer climate, microbial contributions to GHG emissions from these ecosystems will ultimately depend on how soil moisture (i.e., wetter vs drier) is distributed across the landscape.

Methods

Sampling location and characteristics. Site selection, sample collection, and sample characterization were previously reported41. Soil and permafrost samples were collected from the BEO which is located approximately 6 km east of Barrow, AK (71.3° N, 156.5° W). This Arctic Coastal Plain research site contains thaw lakes, drained thaw lake basins, and interstitial ice-wedge polygons that cover more than half of the land surface42. Active layer soils in the BEO are classified as Gelisols and cryosere based on an organic-rich mineral layer underlain by a horizon of mineral soil and a frozen organic-rich mineral layer. In total 31 soil samples from identified organic and mineral layers of HC, FC, and LC polygons were collected prior to seasonal freeze on 24 September 2011. During the sampling period, peak plant growth and active layer thaw depths were also measured. The LC polygons at this location are characterized by narrow, saturated, troughs, relatively high and dry topographic ridges, and depressed year-round saturated centers. By comparison, the HC polygons have seasonally saturated troughs, dry ridges and dome shaped high and dry centers. FC polygons contain seasonally saturated troughs that border the polygon with elevated but flat centers. Mosses and liverworts are widely found in all polygon types. Carex aquatilis (sedge), Eriophorum (sedge), Petasites frigidus (perennial herb), Luzula nivalis (perennial herb), and Dupontia fisheri (sedge) are among most commonly observed plants at this location53. Among sedges Carex aquatilis was shown to be more abundant in LC polygons whereas Eriophorum and Dupontia fisheri were more abundant in FC polygons54. The mean canopy height across different polygon features was lower in HC polygons (1.9–10.8 cm) in comparison to FC (4.1–11.3 cm) and LC (7.9–15.5 cm) polygons.

Upon collection, the samples were kept frozen at −20 °C. Thaw depth measurements were obtained using a steel probe with centimeter graduations starting at the bottom of the probe. Since the measurements were collected at the end of the thawing season, the thaw depth measurements are considered to be equivalent to the ALT. Average active layer depth along this transect is 0.37 m. Samples were analyzed to determine density, C and N content, pH, electrical conductivity (μS), and moisture. Additionally, major and trace cations, including Na+, Mg2+, Fe3+, Al3+, Si4+, K+, Mn2+, Cu2+, and Zn2+ were measured from extracted pore water4. During sampling, soil temperatures were between 1.2 and 1.8 °C at all sampling locations. Active layer (0.00–0.53 m depth) soil temperatures fluctuate between −22 °C and 5 °C over the course of a year52. HC polygons were shown to contain a higher amount of C (23.2 ± 5.4% relative to other polygons (FC: 12.9 ± 5.1% and LC: 15.4 ± 8.4%)). FC polygons were cooler than the other polygons. FC polygons had the lowest average ALT (HC: 33.5 ± 11.9 cm, FC: 34.5 ± 5 cm, and LC: 45.7 ± 7 cm) and the highest Fe3+ concentrations (HC: 3.1 ± 1.9 μg L−1, FC: 3.1 ± 1.9 μg L−1 and LC: 3.4 ± 2.9 μg L−1). The soil samples were freeze-dried over a 7 day period until no further weight loss was observed. The gravimetric moisture content was calculated from the weight difference before and after the freeze-drying process. Note that soils in the BEO are highly porous and have a high water holding capacity4. The average gravimetric soil moisture (g water gr−1) was highest in the LC polygons; values for all sampling locations were: HC Organic: 0.71 ± 0.10, Mineral: 0.59 ± 0.05; FC Organic: 0.76 ± 0.07, Mineral: 0.41 ± 0.12; LC Organic: 0.83 ± 0.05, Mineral: 0.50 ± 0.03 (Supplementary Table 8). When observed, water levels in troughs were measured from the bottom of the active layer. Dissolved oxygen concentrations measured in nearby locations showed 86–92% O2 in surface and at 31–46% O2 in mineral soil depths51. Permafrost at the site is continuous, ice-rich, and is present to depths greater than 350 m.52

Duplicate permafrost cores were obtained via drilling with a 3 inch core barrel (SPRE core). The centers of a FC polygon (the south end of the transect) and a LC polygon (the center of a FC polygon) were collected in April of 2012. The total lengths of the cores are 1.00 and 2.65 m. This is the deepest and most intact core pair that was obtained during this sampling campaign. The permafrost temperature range at this location is between −17°C and −1°C at 0.5 m to −12°C and −5°C at 2.65 m. Cores were stored frozen at −20°C and scanned using a modified Siemens Somatom HiQ third-generation medical computed tomography (CT) scanner53. X-ray CT scanning is a nondestructive method that provides the three-dimensional density (g cm−3) distribution of the scanned samples in order to differentiate ice content from higher density materials such as organics or clays54. After scanning, cores were sub-sampled while frozen under aseptic conditions with a 400 μm diameter mini-corer in 5 cm depth intervals. Each depth interval was divided into four sections, homogenized and analyzed independently. The rest of the core was subsequently sliced with a chop saw into disks for chemical analysis (water chemistry, C and N content, Supplementary Table 1). Anions and cations were measured in pore water samples with rhizon samplers and analyzed with IC and ICP-MS system as described previously41.

DNA extraction and 16S analysis. Total DNA was extracted from 2 g material as described earlier55. Extractions were purified using an AllPrep DNA/RNA kit (Qiagen, Valencia, CA, USA) and were quantified using Quant-iT dsDNA HS assay kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s manual. PCR reactions were performed as described earlier55. Active layer samples were sequenced on Illumina GA-Ix platform (at the Lawrence Berkeley National Lab, CA, USA) and permafrost samples at Illumina MiSeq platform (at JGI, CA, USA) resulting in ~95 bp single-end reads and permafrost samples were sequenced on Illumina MiSeq platform (at JGI, CA, USA), resulting in ~250 bp paired end reads.
The paired-end sequences were overlapped and merged using FLASH. Quality filtering and demultiplexing were performed as described previously. Sequences were grouped into operational taxonomic units (OTUs) based on 97% sequence identity, and chimeric sequences were removed, using UPARSE. OTUs were given taxonomic assignments in QIIME version 1.7.0 using RDP classifier and Greengenes release 13.5. Phylogenetic trees were created using FastTree under QIIME’s default parameters. The tree was used for the calculation of alpha- (Shannon’s H), Chao1 and Faith’s PD) and beta-diversity (weighted UniFrac distance) metrics. Variable sequencing depth was normalized across samples, by performing a single rarefaction at a depth of 7500 sequences per active layer soil or 22,400 sequences per permafrost core. The weighted UniFrac distance matrices were used to visualize the community composition.

Metagenome sequencing and analysis. Sixteen metagenome shotgun sequencing libraries were prepared to represent three polygon features (rim, trough, and polygon)10. Fluxes of CO2 and CH4 were measured on 10 years, 2012 and 2013. Point locations for CO2 and CH4 measurements were described in Vaughn et al. (2016)10. Briefly, a chamber (25 cm diameter) was placed on a similar PVC base. CO2 and CH4 fluxes were measured using a static opaque chamber (25 cm diameter) placed on a PVC base (installed approximately 15 cm deep) for 4–8 in unvegetated plots, a floating chamber whose base extended 4 cm below the water surface was used. Fluxes were calculated from the slope of the linear section of the LGR plot of GHG concentration versus time. CO2 and CH4 fluxes were collected in two consecutive years, 2012 and 2013. Point locations for CO2 and CH4 flux measurements are represented in Fig. 1b. In 2012, fluxes were measured from four locations per polygon (n = 4); three replicate measurements from the polygon centers and one measurement from polygon troughs in HC and FC polygons and a polygon rim in LC polygons as a single time point measurement on 12 August 2012. In 2013, 1 x 1 m plots were established in center, rim, and trough of each polygon (n = 3 per polygon)16. Fluxes of CO2 and CH4 were measured on 10–12 July, 7–16 August, 4–7 September, and 2–4 October 201310. N2O fluxes were measured using a static opaque chamber (25 cm diameter) placed on a similar PVC base. N2O fluxes were collected from adjacent polygons to the ones studied here. In 1–3 July, 5–11 August, and 12 September 2012 chamber measurements were collected from center, rim, and trough (n = 3 per polygon) of 16 polygons. Samples of headspace gas were collected via syringe through sepa on the chamber at 10 min intervals for 40 min, and analyzed within 24 h using a Shimadzu GC-2014 electron capture detector.

Statistical analysis. All statistical tests were performed by using ade4 and vegan packages in the R statistical environment (http://www.r-project.org). Results are defined to be significant at p < 0.05. Ordination of the whole community detected by 16S amplicon sequencing was created using UniFrac matrix calculated by QIIME software and presented in a principal coordinates analysis plot. Analysis of similarity (ANOSIM) was used to test differences in community structure within the samples (1000 Monte Carlo permutations). Permutational multivariate analysis of variance (Adonis) tests were performed on weighted UniFrac values to determine the amount of variation explained by soil chemistry. Pearson (r) correlation and Spearman (rho) coefficients were determined between microbial abundances, geographical factors, and soil chemistry to test the alternative hypothesis that relative abundance of each prokaryotic phylum or gene is positively associated with geographical factors and soil chemistry. Moran’s I, which calculates the degree of correlation between observations as a function of the spatial distance separating them, was used as implemented in ncf package to determine the presence of spatial autocorrelation in the distribution of microbial communities across different polygon features. Microbial network analyses were performed in R using the phyclone package70. The network is based on the Bray-Curtis distance measure, and only connected nodes with a distance < 0.4 were shown. In metagenomes, to test differences between relative abundances of different genes amongst polygons one-way ANOVA followed by the Tukey honestly significant difference test was used. The differences among CO2 and among CH4 flux measurements were tested based on Akaike information criterion (AIC) values and Type III Sums of Squares using a linear package to accommodate unbalanced data structure.

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Author contributions
N.T., S.S.H. and J.K.J conceived the study and designed the experiments; N.T. prepared samples for 16S rRNA gene and metagenome sequencing, N.T. and E.P. performed data analysis, N.T., S.W. and Y.W. sterile sub-sectioned permafrost samples and performed DNA extraction, C.U. and S.S.H. collected active layer soil and permafrost core samples, T.K. provided CT-scanning and analysis of the permafrost core, S.G.T. facilitated 16S rRNA gene sequencing of permafrost samples, M.S.T. provided in situ gas-flux measurements, N.T. and J.K.J wrote the manuscript with feedback from all authors.

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