Snow microbiome functional analyses reveal novel aspects of microbial metabolism of complex organic compounds

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INTRODUCTION

Abiotic parameters, such as temperature, pH, and pressure, create stress on microorganisms, especially in extreme environments (Rothschild & Mancinelli, 2001). The cryosphere, an extreme cold environment, covers a large portion of Earth's surface. Over 14% of the world's biosphere is located at the planetary poles, while 90% by volume of the ocean is colder than 5°C (Price & Sowers, 2004). Taxonomic surveys based on 16S rRNA gene sequencing have described significant microbial diversity in glacial ice (Cameron et al., 2016; Christner, Mosley-Thompson, Thompson, & Reeve, 2001; Christner et al., 2000), cryoconite (Uetake et al., 2016).
community shifted from early spring cooperation to late spring
and network analysis, the study revealed that the snow microbial
Vogel, & Larose, 2019). Using a combined method of marker genes
2011) in Svalbard, Norway (Bergk-Pinto, Maccario, Dommergue,
prevalence in permafrost soils; for review, see Nikrad, Kerkhof, and
One pioneering metagenomic study correlated microbiome
functionality with chemical parameters, such as mercury concentra-
tion in the Arctic spring snow samples (Maccario, Vogel, & Larose,
Another notes that biological activity in the snow is a poorly
studies have been carried out in a wide va-
ety of environments including the human gut (Gevers et al., 2014; Qin et al., 2012; Zhu et al., 2018), groundwater (Hemme et al., 2015),
acid mine drainage (Chen et al., 2014), beach sand (Rodriguez-R et al., 2015), etc., and identified potential diagnostic, therapeutic,
bioremediation targets. With ample data, comparative analysis of
metagenomes/metatranscriptomes under different conditions high-
lights the key microbial members and their molecular functions that
result from and/or contribute to niche differences (Zhu, Delmont, Vogel, & Bromberg, 2015; Zhu, Mahlich, Miller, & Bromberg, 2018).
While such analyses have not yet been widely applied to cold envi-
ronment samples, they could help elucidate microbial mechanisms of
survival and adaptation at low temperatures.

Bergk-Pinto et al. studied the microbial ecology in 20 snow sam-
10°C, as they were completely melted. Filters were stored in Eppendorf
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have equally impacted late and early spring samples, suggesting that their differences are still a reliable source of functional evidence. Details on sampling conditions, sample site, and chemical analyses can also be found in Bergk Pinto et al. (2019). Sequencing data were quality filtered using Mothur (Schloß et al., 2009) with settings described in Schloss, Gevers, and Westcott (2011). Base over-representation was controlled using FastQC (Andrews, 2010). Usearch (Edgar, 2010) was used to identify and remove remaining adaptors.

### 2.2 | Analysis

The post-quality-control reads were submitted to mi-faser web service (Miller, Zhu, & Bromberg, 2017; Zhu et al., 2018) for annotation. For each sample, mi-faser returns a read abundance table of enzyme functionality detected in the sample, that is, the EC profile (EC stands for Enzyme Commission (1992)). For all further analysis, read abundance was standardized by the total number of reads in each sample. To create the pathway profile of a sample, for each known KEGG functional pathway (Kanehisa, Sato, Kawashima, Furumichi, & Tanabe, 2016), we divided the sum of the reads mapping to all enzymes in this pathway by the total number of enzymes in this pathway. The NMDS diagrams were generated with the (enzyme and pathway) profiles of samples assigned to four groups, early_DNA (early spring metagenomes), early_RNA (early spring metatranscriptomes), late_DNA (late spring metagenomes), and late_RNA (late spring metatranscriptomes). The Euclidean distances between the same-sample DNA and RNA NMDS points were calculated and compared across the four groups. The significance of differences in distance distributions was evaluated using a two-tailed t test at 0.05 threshold. Organic acid levels were standardized across all samples to the sum total of their abundances in all samples. The Spearman correlation coefficients, as well as the significance of correlations, were calculated by the R function cor.test with algorithm AS89 (Best & Roberts, 1975).

### 3 | RESULTS AND DISCUSSION

#### 3.1 | Early to late spring dissimilarity and metagenome-to-metatranscriptome divergence highlight community activity in late spring samples

While the metagenome reflects the potential function of a microbial community, metatranscriptomic analyses reflect genes that are transcribed, highlighting the implicitly active fraction of these functions. In analyzing the metagenomes and metatranscriptomes of early and late spring polar snow samples, we observed that (a) the early spring samples were more diverse than the late spring samples in both potential and active microbial functionality (measured as the Euclidean distance between entries on the NMDS plot; Materials and Methods; EC profile sample distance: early spring = 4.8 ± 2.3, late spring = 0.4 ± 0.3, Figure 1a, Figure A1a,b; pathway profile sample distance: early spring = 1.4 ± 0.9, late spring = 0.1 ± 0.1, Figure 1b, Figure A1c,d) and that (b) metagenome-to-metatranscriptome similarity of the same sample was significantly lower in early than in late

![FIGURE 1](image-url) NMDS suggests higher microbial functional beta-diversity in early spring samples than in late spring ones. The average Euclidean inter-sample distance between (a) sample EC profiles is 4.8 ± 2.3 for early spring samples, and 0.4 ± 0.3 for late spring samples and (b) sample pathway profiles is 1.4 ± 0.9 for early spring samples and 0.1 ± 0.1 for late spring samples. Intuitively, observe that early spring samples are widely distributed in both panels, while late spring samples tend to concentrate
spring (in both comparisons of the EC profiles, $t$ test $p$-value < 0.001, Figure 2a and the pathway profiles, $t$ test $p$-value = 0.025, Figure 2b). Note that for all comparisons, ~29% ECs (195 of 683) in our data could not be mapped to known KEGG pathways (Appendix B at https://doi.org/10.6084/m9.figshare.12290711).

The discrepancy in functional annotation of metagenomes (DNA) and metatranscriptomes (cDNA) of the same samples has previously been observed in environments such as the human gut (Franzosa et al., 2014) and open ocean (Shi, Tyson, Eppley, & DeLong, 2010). The genes observed in the metagenomes represent potential functions that may or may not be expressed in the environment at the time of sampling and could belong to inactive community members. The metatranscriptome-specific functions, on the other hand, belong to active members of the community at the time of sampling (Yu & Zhang, 2012). The exceedingly low metagenome-to-metatranscriptome similarity (high distance/dissimilarity) in the early spring samples (Figure 2) suggests that the active members (organisms and molecular functions) in early spring occur at such low abundance that metagenomic sequencing fails to detect them. We speculated that the potential functional diversity in the early spring metagenome samples (Figure 1; Figure A1; DNA datasets) might come from the DNA of dead or inactive cells preserved in the snow. Interestingly, many microorganisms identified in snow and ice via 16S rRNA gene surveys are non-psychrophiles (Cowan & Tow, 2004) and their membership in the community needs further investigation. Meanwhile, the diversity of active microbial functionality in the early spring metatranscriptomes (Figure 1; Figure A1; RNA datasets) reflected diverse microbial activities (238 enzymatic functions involved in 84 metabolic pathways including cell size reduction, changes in fatty acid and phospholipid membrane composition, and decrease in the fractional volume of cellular water). This observation is in line with the known variety of survival strategies employed by microbes at low temperature (Nikrad et al., 2016; Price & Sowers, 2004).

With the warming in the late spring, the active community made up a larger fraction of the sequenced reads and, thus, manifested in more homogeneity. Previous 16S rRNA-based taxonomic analysis on the same dataset also observed a shift in the community from early to late spring (Bergk Pinto et al., 2019). While the early spring samples contained a core community of 59 OTUs, there were only 29 OTUs in the late spring samples, with 42 early spring core OTUs disappearing from the core community of late spring samples (and 12 late spring-specific OTUs appearing) (Bergk Pinto et al., 2019). The early spring community thus contained a higher diversity of organisms of which only a small fraction was likely active; the inactive community members could no longer be detected in the late spring samples. As a result, we observed a decrease in functional diversity (Figure 1; Figure A1) and an increase in the metagenome-to-metatranscriptome similarity (Figure 2). Also, our result suggests that despite the taxonomic diversity in the late spring samples, their functional potential and activity were highly similar (Figure 1; Figure A1), highlighting the advantages of functional analyses to the 16S rRNA gene surveys.

### 3.2 | Microbial use of complex organic compounds in the snow

Snow provides a medium and nutrients for microbial growth and associated physicochemical processes (Domine & Shepson, 2002); growth implies the utilization of nutrients. In glacial ice metagenomes, numerous genes related to xenobiotics, biopolymers, and other carbon sources were detected, suggesting that ice microorganisms have the potential to degrade a wide range of substrates (Stibal, Šabacká, & Žárský, 2012). The levels of all three organic acids (oxalate, acetate, and formate) measured in our study remained in low concentration in the early spring samples (Appendix C: https://doi.org/10.6084/m9.figshare.12290720). They increased in the late spring (Figure A2), possibly concomitant with increased microbial activity. Increased activity of microbial community members in the late spring snow might thus be related to the changes in organic acid levels in the samples.

Microbial preferences for different carbon classes were studied in Antarctic snow, showing a higher rate of carbon uptake when snow microcosms were amended with a combination of simple and complex carbon sources (Antony et al., 2012). The appearance of organic acids in the snow may have both abiotic (e.g., aerial deposition) and biotic (e.g., microbial activity) origins. In our study, the clear correlation (co-interia (DolÉDec & Chessel, 1994)) of organic acid concentrations with microbial activity levels, captured by metatranscriptomes, strongly indicated active metabolism in the late spring samples (Table 1; Figures A3-A7). Note that both mi-faser and EggNog Mapper (Huerta-Cepas et al., 2017) functional profiles recognized this correlation (Table A1), albeit mi-faser reached a higher level of significance.
TABLE 1  The metagenomic/metatranscriptomic pathways significantly correlate (Spearman’s ρ; p-value <0.05) with the levels of organic acids in the late spring samples

| Pathway                      | Oxalate | Acetate | Formate |
|------------------------------|---------|---------|---------|
|                              | ρ       | p-value | ρ       | p-value | ρ       | p-value |
| Fatty acid biosynthesis      | 0.63    | 0.001   | 0.42    | 0.040   | 0.56    | 0.004   |
| Biosynthesis of unsaturated fatty acids | 0.61    | 0.002   | 0.66    | 0.004   | 0.55    | 0.005   |
| Fatty acid elongation        | 0.46    | 0.025   | 0.55    | 0.005   | 0.34    | 0.102   |
| Geraniol degradation         | 0.46    | 0.025   | 0.55    | 0.005   | 0.34    | 0.102   |
| Styrene degradation          | −0.33   | 0.116   | −0.42   | 0.041   | −0.33   | 0.110   |

Bold values indicate p < .05.

Among the enzymes that were not mapped to known KEGG pathways, two tRNA-methyltransferases (2.1.1.61 and 2.1.1.217; p-value <0.05, Materials and Methods) showed a significant correlation with organic acid levels. tRNA methylation regulates important steps in protein synthesis and is essential for microbial growth in high temperature (Hori, 2014). Our results suggest that it could be also important in low-temperature conditions.

To summarize, we identified five pathways in our metagenomes/metatranscriptomes that significantly correlated with organic acid levels in the late spring samples (p-value <0.05 highlighted in bold in Table 1; Figures A3-A7; Materials and Methods): fatty acid biosynthesis, biosynthesis of unsaturated fatty acids, fatty acid elongation, geraniol degradation, and styrene degradation. The top three pathways were related to fatty acid synthesis and elongation. Fatty acids are essential due to their role in membrane synthesis and critical in low temperatures that affect membrane fluidity (Cronan & Thomas, 2009). The following degradation pathways were also important. Geraniol is a terpene produced by a variety of plants for its antibacterial activities (Friedman, Henika, & Mandrell, 2002). Terpenes are released from plants (Marmulla & Harder, 2014) and deposited in arctic snowpacks like other volatile organic compounds (Kos, Kanthasami, Adebchina, & Ariya, 2014). Geraniol degradation allows some bacteria, for example Pseudomonas putida, to utilize geraniol as their sole carbon and energy source (Vandenbergh & Wright, 1983). Pseudomonas putida is also known to degrade styrene (O’Connor, Duetz, Wind, & Dobson, 1996) and polystyrene (Ward, Goff, Donner, Kaminsky, & O’Connor, 2006). Therefore, the organic acid level correlation (with geraniol degradation) and anticorrelation (with styrene degradation) may suggest a change of nutrient availability in the environment. Pseudomonas putida is known to possess diverse metabolic capabilities to degrade a variety of organic solvents. Most of its strains are mesophilic, but one (KT2440) has been reported as psychrotolerant (optimal growth at 30°C but can proliferate at 4°C) (Fonseca, Moreno, & Rojo, 2011). To the best of our knowledge, no microbial metabolism of geraniol and styrene has been reported at low temperatures. Our functional omics study thus provides new evidence suggestive of active microbial degradation of complex organic compounds at subzero temperatures.

4 | CONCLUSIONS

We defined microbial activity at low temperatures as the gene abundance level in metagenomic and metatranscriptomic datasets from snow in early and late spring. Our results highlight the novel microbial activity of complex organic compound degradation at low temperatures. A further in-depth exploration of the functionality of the cryosphere inhabitants can contribute to our understanding of microbial metabolism at low temperatures and aid in the discovery of novel enzymes with potential industrial and bioremediation value.

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CONFLICTS OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Chengsheng Zhu: Conceptualization (lead); formal analysis (lead); methodology (lead); visualization (lead); writing – original draft (lead); writing – review & editing (lead). Maximilian Miller: Software (lead). Nicholas Lusskin: Software (supporting). Benoît Bergk Pinto: Data curation (equal); writing – review & editing (supporting). Lorrie Maccario: Data curation (equal); writing – review & editing (supporting). Max Häggblom: Writing – review & editing (supporting). Catherine Larose: Writing – review & editing (supporting). Yana Bromberg: Conceptualization (equal); resources (lead); supervision (lead); writing – review & editing (lead).

ETHICS STATEMENT

None required.
DATA AVAILABILITY STATEMENT
The associated data and materials are accessible via figshare: metagenomic and metatranscriptomic data: https://doi.org/10.6084/m9.figshare.12300560; correlation code: https://doi.org/10.6084/m9.figshare.12290771; pathway profile: https://doi.org/10.6084/m9.figshare.12290750; Appendix B (ECs that were not mapped to KEGG pathways): https://doi.org/10.6084/m9.figshare.12290711; Appendix C (Organic acid levels in early and late spring samples): https://doi.org/10.6084/m9.figshare.12290720.

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### APPENDIX A

#### TABLE A1  Co-inertia results comparing the Eggnog mapper vs. mi-faser

| Mapper       | Annotation  | Metagenomes Co-inertia | p-value | Metatranscriptomes Co-inertia | p-value |
|--------------|-------------|-------------------------|---------|-------------------------------|---------|
| Eggnog mapper | Genes id    | 0.44                    | 0.033*  | 0.43                          | 0.072   |
|              | GO terms    | 0.45                    | 0.003*  | 0.44                          | 0.023*  |
|              | KEGG pathways | 0.59                  | 0.0002***| 0.37                          | 0.064   |
| mi-faser     | EC          | 0.51                    | 0.0009***| 0.5                           | 0.001** |
|              | KEGG pathways | 0.48               | 0.0003***| 0.49                          | 0.0004***|

Note: Co-inertia of organic acid levels with the Eggnog mapper or mi-faser abundances. The co-inertia analysis measures the co-variance of the organic acid levels and the functional profile abundances determined by Eggnog mapper and mi-faser. The significance of each co-inertia was tested using a permutation test (10,000 permutations).

* \( p < .05. \)

** \( p < .01. \)

*** \( p < .001. \)
**FIGURE A1** NMDS plots of (a) EC profiles of early spring samples, (b) EC profiles of late spring samples, (c) pathway profiles of early spring samples, and (d) pathway profiles of late spring samples
**Figure A2**  Increased levels of (a) oxalate, (b) acetate, and (c) formate in the late spring samples. X- and Y-axis represent the NMDS dimensions; Z-axis represents the organic acid level in nmol/kg.

**Figure A3**  Fatty acid biosynthesis functional abundance correlates with organic acid levels. The pathway abundance is in standardized read-count units, that is, the sum of the reads mapped by mi-faser to all enzymes in this pathway standardized and rescaled by (1) the pathway size, that is, the total number of enzymes in this pathway and (2) the sample size, that is, the total number of reads in the sequencing sample. The dashed lines are simply visual aids of the data correlation.
**FIGURE A4** Biosynthesis of unsaturated fatty acids functional abundance correlates with organic acid levels. The pathway abundance is in standardized read-count units, that is, the sum of the reads mapped by mi-faser to all enzymes in this pathway standardized and rescaled by (1) the pathway size, that is, the total number of enzymes in this pathway and (2) the sample size, that is, the total number of reads in the sequencing sample. The dashed lines are simply visual aids of the data correlation.

**FIGURE A5** Fatty acid elongation functional abundance correlates with organic acid levels. The pathway abundance is in standardized read-count units, that is, the sum of the reads mapped by mi-faser to all enzymes in this pathway standardized and rescaled by (1) the pathway size, that is, the total number of enzymes in this pathway and (2) the sample size, that is, the total number of reads in the sequencing sample. The dashed lines are simply visual aids of the data correlation.
FIGURE A6 Geraniol degradation functional abundance correlates with organic acid levels. The pathway abundance is in standardized read-count units, that is, the sum of the reads mapped by mi-faser to all enzymes in this pathway standardized and rescaled by (1) the pathway size, that is, the total number of enzymes in this pathway and (2) the sample size, that is, the total number of reads in the sequencing sample. The dashed lines are simply visual aids of the data correlation.

FIGURE A7 Styrene degradation functional abundance correlates with organic acid levels. The pathway abundance is in standardized read-count units, that is, the sum of the reads mapped by mi-faser to all enzymes in this pathway standardized and rescaled by (1) the pathway size, that is, the total number of enzymes in this pathway and (2) the sample size, that is, the total number of reads in the sequencing sample. The dashed lines are simply visual aids of the data correlation.