Functional gene abundance demonstrates clade-specific regulation of mercury methylation in boreal forest sites

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Introduction

- Permafrost thaw is one of the many ways climate change is impacting the Arctic
- It can cause elevated moisture levels in lowland boreal topsoils that can act as reservoirs of organic matter, sulfate, and inorganic mercury
- Increased anerobic microbial activity has led to changes in biogeochemical cycles leading to an increase in methylmercury (meHg) production
- *hgc*A and *hgc*B make up the biomarker gene pair associated with mercury methylation, with each gene encoding for a separate protein
- Digital polymerase chain reaction is a useful molecular tool that employs fluorescently-labeled probes to detect the presence or absence of DNA among 20,480 microchambers

Hypothesis 1: It is anticipated that in more saturated sites, results will show a higher presence of the functional genes necessary for anaerobic processes.

Hypothesis 2: The *hgc*A gene is expected to be quantified in Firmicutes, Deltaproteobacteria, and Archaea, all microbes possessing the ability to methylate mercury in anoxic conditions.

Methods

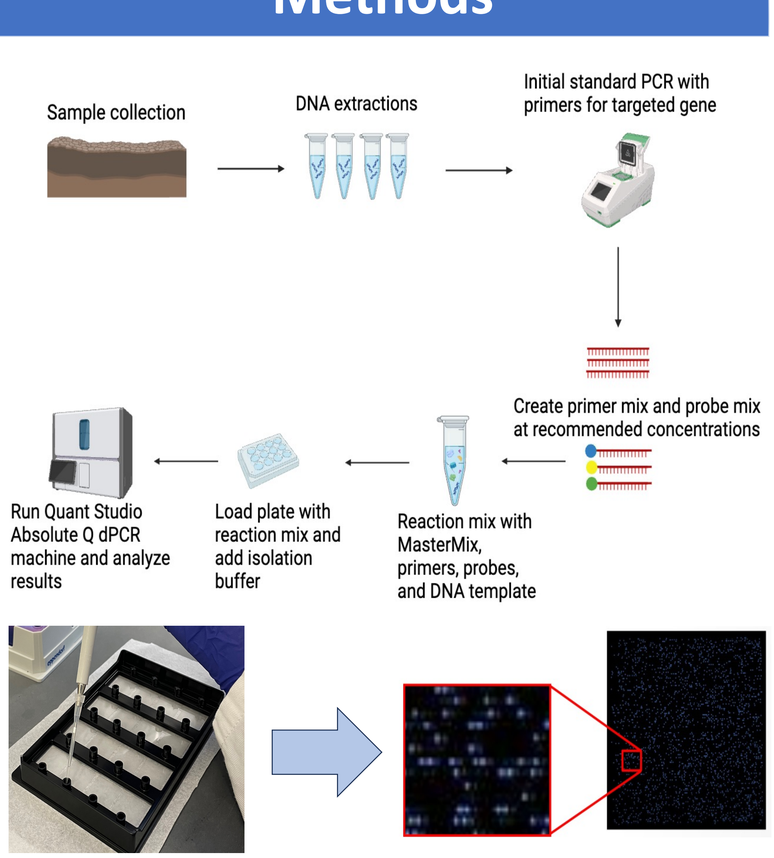

Figure 1. Image of dPCR MAP16 plate with 16 sample arrays, each comprised of 20,480 micro-chambers that are pictured to the right.

Acknowledgements

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Site Information

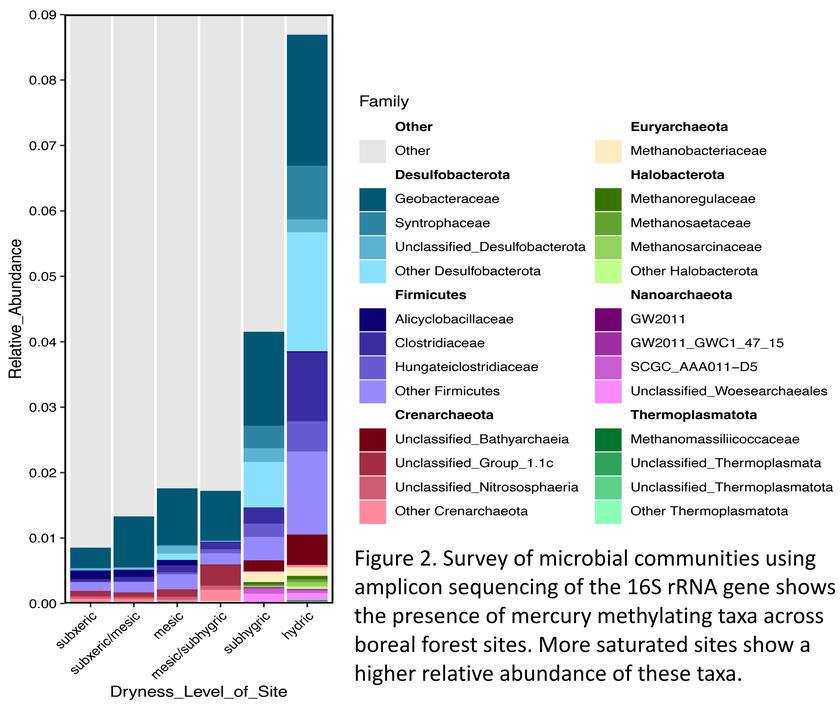
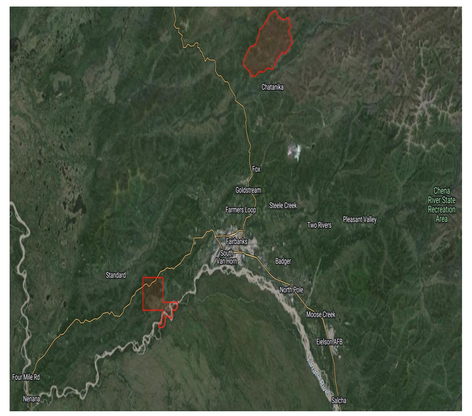


Figure 2. Survey of microbial communities using amplicon sequencing of the 16S rRNA gene shows the presence of mercury methylating taxa across boreal forest sites. More saturated sites show a higher relative abundance of these taxa.



The Caribou-Poker Creek Research Watershed (CPCRW) and Bonanza Creek Experimental Forest (BCEF). Samples were collected from water saturated sites in the upland headwater stream basins of CPCRW and floodplains and upland areas of the BCEF, pictured above.

Site	Lat	Lon	Dryness	pH
UP4A	64.7681	148.2982	Subxeric	4.93
UP4B	64.7716	148.2739	Subxeric	4.57
UP4C	65.1533	147.4908	Subhygric	5.81
BFY4	65.1498	147.4724	Subxeric/ Mesic	5
BFY7	65.1536	147.4776	Mesic/ Subhygric	4.77
BCEFM2	64.695	148.3237	Subhygric	5.4

Table 1. Characteristics of the 6 sites from which 18 samples were taken. Sites UP4A, UP4B, UP4C, BFY4, BFY7 are in the CPCRW. Temperature was averaged from three quadrants per site (subxeric = extremely dry, mesic = moderate moisture, subhygric = saturated).

Results

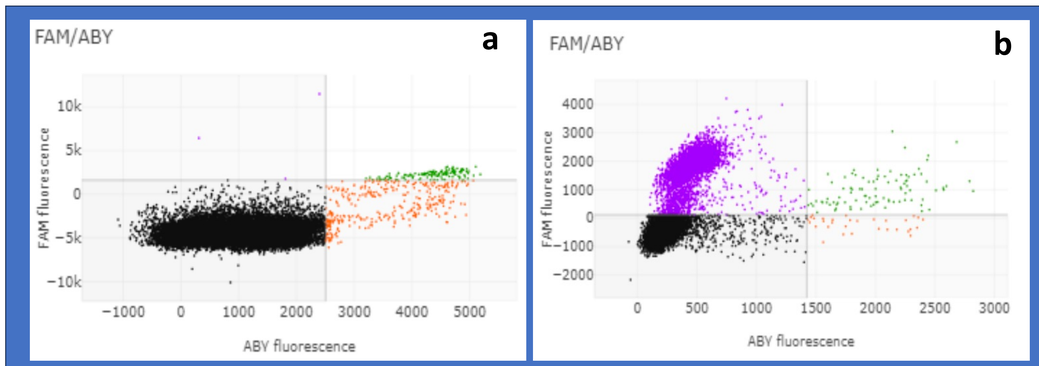


Figure 3a. Multiplex dPCR plot representing subxeric site UP4A. Positive and negative micro-chambers are shown across targets FAM and ABY, which are associated with Deltaproteobacteria and Archaea, respectively. The black dots represent microchambers that are negative for both targets.
Figure 3b. Multiplex dPCR plot representing subhygric site UP4C. Green dots represent positive chambers associated with both targets and both probes.

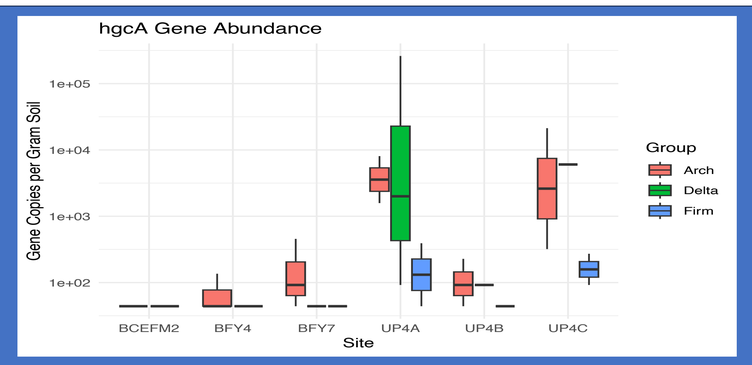


Figure 4. Plot representing the abundance of the mercury-methylating gene (*hgc*A) per gram of soil collected from each boreal forest site.

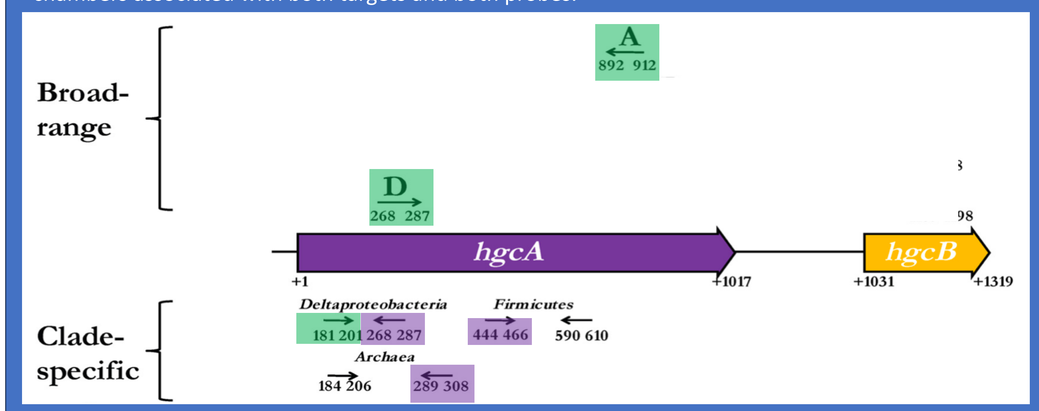


Figure 5. Schematic of broad-range and clade specific primer pairs spanning the hgcAB gene pair. The pairs highlighted green are the primer forward and reverses used for PCR. The pairs highlighted purple are the targeted sequences employed as probes, providing the necessary 3' end for DNA synthesis.

Take Away and Next Steps

Take Away:

- More copies of the *hgc*A gene were observed per gram of soil in water-saturated sites
- The 16S rRNA sequencing data and our new dPCR results suggest that Desulfobacterota, an anaerobic phylum of Deltaproteobacteria known to methylate mercury, is common in the water-saturated site, UP4C.

Next Steps:

- Acquire additional gene-specific fluorescently-labeled probes for dPCR that target methane biogeochemistry
- Quantify genes associated with methane production (*mcrA)* and oxidation (*pmo*A) using the same protocol

References:

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