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# Novel Metaproteomic Approaches Reveal Systematic Variations in Microbial Biogeochemical Pathways with Arctic Vegetation Types Samuel E. Miller and Jacob R. Waldbauer Department of the Geophysical Sciences, University of Chicago, Chicago, IL 60637. Contact: samuelmiller10@gmail.com

#### Introduction



- How will Arctic warming affect soil C storage?
- Greater plant growth increases C storage, but trivial in global budget • Replacement of nonvascular by vascular plants
- Accelerated microbial decomposition of massive soil C pool
- Loss of up to 9 yr worth of current anthropogenic emissions by 2100 • How does floral turnover interact with microbial activity?
- How can microbial processing of organic molecules be measured in situ?
- Do certain microbial groups perform specific heterotrophic functions?
- Metagenomes suggest functions are widely distributed among taxa
- Genomic potential may not correlate well with enzymatic activity
- Characterization of proteomes as a more direct proxy for activity
- Our recent computational advances enable soil metaproteomics
  - *Postnovo* boosts accuracy of de novo peptide sequencing by10-fold
- *ProteinExpress* greatly increases the amount of protein information

#### Sampling at Toolik Field Station, Arctic Alaska



#### Metaproteomic Workflow



Sample Peptide extract



HPLC-tandem mass spectrometry



Mass spectra Peptide ID

Protein ID

Improved by *Postnovo* (Miller et al., 2018, J. Proteome Research) Improved by ProteinExpress (Miller et al., 2018, in prep.)

## **Results: Differences between Floras**

- Relative abundances of proteins were compared between samples
- Proteins were identified from pooled meta-genomes/transcriptomes
- Proteins were classified by Functional Group (glycolysis, cellulases, ...)
- Sample Functional Group profiles naturally cluster by floral type (LDA)
  - First factor accounts for 93% of discrimination between floras • Clear separation of low and high biomass floras



Factor 1 (93.0%)

- Certain functions are more strongly expressed in more vegetated soils
  - Statistically significant pairwise differences (*t*-test, P = 0.01)
  - Sugar transporters (ribose, xylose/arabinose, sugar alcohols) • Succinoglycan (extracellular polysaccharide) biosynthesis
  - Core cellular functions such as the ribosome load strongly on Factor 1
  - Indicates greater microbial activity in more vegetated soils
- How do protein expression profiles differ between taxa?
  - Metagenomic sequence bins corresponding to 16S taxa were identified
  - Protein expression by a taxon was measured by a novel metric,  $\Phi$
- Relative abundance \* similarity between protein and bin sequences Taxa express similar suites of functions in different floras, with certain functions strongly changing in relative level within but not between taxa

16S rRNA Diversity Taxa identified in metagenomic datasets



## **Results: Taxonomic Niche Partitioning**

- Acidobacteria are the most active group, as measured by  $\Phi$
- Three natural *k*-means clusters of protein expression profiles (red boxes) . High Proteobacteria; low non-Proteobacteria
- 2. Moderate to high Proteobacteria; high non-Proteobacteria
- 3. Low Proteobacteria; high non-Proteobacteria



- Proteobacteria consume small, soluble molecules, e.g., simple sugars, CO • Rhizobiales dominate expression of sugar transporters (root exudates)
- Rhizobiales-associated functions increase most with floral biomass
- Non-Proteobacteria degrade polymers (cellulose, hemicelluloses, lignin) • Acidobacteria specialize in hemicelluloses; Actinobacteria in cellulose



- Intracellular N cycling is less skewed and more similar to overall activity • Corroboration that Proteobacteria inhabit N-depleted rhizosphere





The utility of  $\Phi$  is supported by values for certain functions • Negligible values for outer membrane functions in G+ vs. G- groups



### Discussion

• Integrate microbial niche partitioning into current model of decomposition



• A model of Arctic soil biogeochemistry and microbial ecology • Root-associated taxa/functions likely increase in a greening Arctic

