Contributions of Microbial Community Metabolic Expression to Biogeochemical Cycling in a Highly Stratified Meromictic Pond over a Diel Cycle

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Abstract:
Earth’s biogeochemical cycle results largely from a complex interaction of individual microscopic organisms among growth, competition and cooperation. Meromictic lakes represent unique ecosystems for investigating microbial biogeochemistry due to the steep chemical gradients that support distinct resident microbial communities that interact within and across these layers. Here, we investigated microbial communities and biogeochemistry in Siders Pond, a meromictic pond on Cape Cod, MA, by sampling 8 depths spanning aerobic to anaerobic habitats over a 24-hour diel cycle. Together with environmental characterization of the Siders Pond water column, we examined the gene content and transcription patterns of microbial communities and specific populations to understand the taxonomy and metabolism both through the water column and over time. Members of the Cyanobacteria, Proteobacteria, Actinobacteria, and Bacteroidetes dominated the upper layers of Siders Pond while below the chemilne the Archaea, e.g. Woesearchaeota, were more abundant. The surface samples had the highest transcription levels followed by the samples collected at the transition between the oxic and anoxic conditions. Genes expressed in the upper layers were associated with oxygenic photosynthesis. High levels of phosphate and phosphonate transporter genes suggested phosphate limitation. Below the chemilne genes for anoxic resynthesis, sulfate reduction, methanogenesis, and motility were among the highest expressed. Overall gene expression was highest during daylight. Changes in gene expression over time were detected for oxic and anoxic photosynthesis, carbon fixation, and motility. Other functions, e.g. sulfur reductase were expressed at high levels during the whole period. Expression trends and networks related to the 24 hour cycle will be presented and compared to a thermodynamic model developed for Siders that determines optimal metabolic pathway expressions based on the principle of maximum entropy production. Together, results facilitate our understanding of how microbial communities organize and coordinate microbial biogeochemistry in aquatic ecosystems.

Methods
Siders Pond was sampled over a 24-hour period by collecting water from eight depths over 7 casts (Fig. 1B). Chemistry and microbial cell density were measured (Figs 1C, 1D). rRNA and DNA were collected on 0.2 mm Sterivex filters and immediately frozen. DNA was extracted with phenol chloroform while RNA was extracted, purified, and converted to cDNA. Metagenomic and metatranscriptomic libraries were prepared and sequenced on an Illumina MiSeq. Pair-end reads were quality filtered, assembled, and annotated at JGI. Ribosomal RNA was removed from the metatranscriptome.

EMIRGE was used to reconstruct full length 16S rRNA genes from the metagenomes and the genes classified with Mothur. Relative abundances of the 16S rRNA genes were determined from the NonPrir EMIRGE output. The metagenomes were merged and the metatranscriptome was mapped against the meta genome CDSs using Kallisto. Genes were grouped by their KEGG Orthology (KO) assignments and the transcripts per million (TPM) recalculated and normalized using means clustering, edger, and the TMM method. Genes with the highest counts were extracted.

To compare the metatranscriptome profiles the vegan library in R was used to calculate a Jaccard dissimilarity index followed by an NMDS analysis with a binary distance (Fig. 3A). Clustering (Fig. 3B) of both genes (left) and samples (top) and samples associated with metabolites was done using Euclidean distance. Transcription of carbon, nitrogen, or sulfur metabolism were determined by summarizing the TPMs of the respective genes and calculating their percentage distribution with depth.

To search for genes showing periodicity in their expression patterns, a RATH (Rhythmicity Analysis Incorporating Nonparametric methods) analysis was done for genes associated with metabolism. Genes with significant periodicity (p<0.05) were extracted and analyzed for the functions.

Conclusions
The stratification of Siders Pond is reflected in the microbial community compositions and in the gene expression patterns. Distinct clusters suggests a depth-specific taxonomic and functional diversity. i.e. an abundance of Chlorobi and Sulfur and Nitrogen metabolism genes at 8m depth. Genes identified as having periodic signals may shed light on metabolisms that are linked to day-night cycling. Their pathways can help explain how microbial communities shift and reorganize over short temporal scales, as well as inform the modeling effort.

References

Acknowledgements
Thank you Leslie Matyshak, Emily Redding, and Suzanne Thomas for preparing sequence libraries and making chemistry measurements. Thank you Carolto for help with data analysis and images.

Funding support: NSF-GEO 1451356.

Fig. 1A

Siders Pond
- Coastal pond in Falmouth, MA.
- Area: 4 hectares
- Maximum depth: 15m
- Meromictic: permanently stratified with no mixing of layers.
- Upper oxygenated layer, misoxygenation, is separated from lower anaerobic layer.
- monoximolimnion, by a chemilne with increased salinity, phosphate, and nitrogen.

Fig. 2A

Abundance of microbes and gene transcripts.

Table 2B

A. Taxonomy of full length 16S rRNA sequences show distinct communities according to depth. Between 96 (surface) and 116 (10m) ribosomal sequences were reconstructed from each of the metagenomes. Three major community structures were determined corresponding to the misoxygenation (Proteobacteria, Actinobacteria, Cyanobacteria), chemilne (Chlorobi) and monoximolimnion (Archaea, Chloroxygenic, Proteobacteria, Planctomycetes) of Siders. B. The most abundant gene transcripts from the Siders metatranscriptomes is listed according to the sample depths.

Periodicity was detected for genes expressed at all depths. Periodicity was observed mainly for genes of energy and carbon metabolism. Only 3 genes between expressed with periodicity at both upper (surface, 3m, 6m) and bottom (8m and 12m) layers. Two genes showed periodicity in more than one upper layer and six genes in both bottom layers.

Fig. 4B

Table 4A

Periodicity was detected for genes expressed at all depths. Periodicity was observed mainly for genes of energy and carbon metabolism. Only 3 genes were expressed with periodicity at both upper (surface, 3m, 6m) and bottom (8m and 12m) layers. Two genes showed periodicity in more than one upper layer and six genes in both bottom layers.

Fig. 4C

Modeling
A Maximum Entropy Production based thermodynamic model developed for Siders Pond is used to predict optimal pathway usage for the system. The model consists of 28 reactions, and it is outlined in Fig. 4A. Gene expression profiles over time and space extracted from the metatranscriptome datasets are shown in Fig. 4B. Profiles of metabolic genes are compared to the expression levels predicted in the model predicted to inform and refine the model (Fig. 4C).