Dynamics of phototrophic sulfur oxidizing bacteria inhabiting Trunk River's "Lemonade"

A Bioinformatic Approach

Microbial Diversity Course Report
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Abstract:

The shallow brackish pond Trunk River in Falmouth, MA develops yellow sulfur-rich pools (nicknamed Lemonade) days after a physical disturbance event. The microbial community composition dynamics associated with the Lemonade were examined using bioinformatic techniques. The dominant phyla in the Lemonade appear to be the Proteobacteria and the Chlorobi. At the class level, Chlorobia tended to dominate the community in deeper in the water column while the Gammaproteobacteria were found in greater abundances at shallower depths. This phenomenon is likely due to differential sensitivity to oxygen among these classes, however the appropriate statistics were not performed to support this assertion.

Introduction:

Biogeochemistry is the study of the elemental composition of the planet and how biology, chemistry, and geology work to alter this composition. The biogeochemical cycle of sulfur is of particular interest in this study due to the element's relatively large abundance on earth as well as its diversity of potential redox states. In oxic waters, sulfate, with oxidation state of +6, is the most abundant sulfur species while reduced compounds, such as hydrogen sulfide (oxidation state -2), occur in greater concentrations as the O_2 levels decrease[1]. Bacteria and Archaea can take advantage of the electrons exchanged during dissimilatory sulfur redox chemistry and conserve energy to fuel both metabolism as well as autotrophic carbon fixation pathways[2].

Dissimilatory sulfur oxidation (DSO) can occur in metabolically and phylogenetically diverse groups of microbes. While DSO is limited to the chemolithotrophic order Sulfolobales in the Archaeal domain, this process can be observed among chemolithotrophic and photolithotrophic bacteria from phyla such as the Proteobacteria and the Chlorobi[1,3]. Chemolithotrophic sulfide oxidizing proteobacteria include members of the genera *Thiothrix*, *Beggiatoa*, and *Thiomargarita*. Organisms of the genus *Chromatium* are photolithotrophic proteobacteria and are known as the 'purple sulfur bacteria'[4]. The Chlorobi phylum contains of photolithotrophic sulfur oxidizers known as the 'green sulfur bacteria'. The *Chlorobium* genus is exemplar of this group of bacteria[5].

This miniature project was designed to examine the dynamics the photolithotrophic sulfur oxidizing bacteria Proteobacteria and Chlorobi from Trunk River, a shallow brackish pond located in Falmouth, MA from a bioinformatic frame of reference. Physical disturbance of Trunk River sediment results in the formation of yellow pools (nicknamed Lemonade) likely composed of elemental sulfur and other compounds released during sulfur redox reactions mediated by sulfur bacteria. Samples were collected during summers of 2014 and 2015 for chemical, molecular, and bioinformatic analyses in order to better understand the biogeochemical dynamics associated with the disturbance of Trunk River and the resultant Lemonade.

Materials and Methods:

Sample collection. All samples were taken from Trunk River, a brackish pond in Falmouth, MA (41.°32'07"N, 70.°38'28.2"W) during the summers of 2014 and 2015 by affiliates of the Microbial Diversity Summer Course at the Marine Biological Laboratories in Woods Hole, MA. In 2014, two samples were taken of the Lemonade for subsequent DNA extraction and 16S sequencing. In 2015, a systematic approach was taken to sample for both chemical and biological profiling. Four holes (labeled, A, B, E, K) were dug in the sediment order to initiate the formation of the Lemonade. Samples were taken at 4 depths below the water-air interface (5 cm, 10 cm, 25 cm and 35 cm) on 8 different days. Several other Lemonade samples collected that year outside of this organized system were included to make further enrich the total Lemonade data set.

Chemical analyses. Oxygen, pH, and temperature. Chemical measurements taken at the time of collection using a YSI Professional Series Model Pro multiparameter probe equipped with a quarto probe that allowed measurements of pH, ORP, ISE, and temperature. Calibration for pH was done with pH 4, 7, and 10 buffers. Calibration for dissolved oxygen was done in oxygen saturated water and in an anoxic solution of sodium ascorbate and NaOH. After sample collection at each site, the probe was lowered to each sample collection depth and allowed to stabilize before taking measurements. Sulfur Species: Samples were transferred back to lab and dissolved hydrogen sulfide was measured using the colorimetric assay developed by Cline in 1969[6]. Sulfate was measured via Ion Exchange Chromatography using a ThermoFisher/Dionex ICS2100 equipped with a AS18 column using a 13 minute, 33 mM NaOH isocratic program.

DNA Extraction and Sequencing. DNA was extracted and was subjected to PCR to amplify 16S rRNA genes as previously described[7]. Positive amplicons were subjected to Illumina MiSeq sequencing for downstream informatics analysis.

Informatics analysis. Illumina reads were assembled and concatenated into a single FASTA file for easier manipulation. The QIIME pipeline[8] was used to process and analyze the compiled 16S data following a tutorial written by Jeff Werner of SUNY Cortland (http://www.wernerlab.org/teaching/qiime/overview). Briefly, MacQIIME (version 1.9.1 20150604 OS10.7) was used to trim barcodes from the individual sequences and OTUs were picked using the uclust script. Representatives from each OTU were taken and compiled to create a smaller and easier to work with data set. This representative set was taxonomically classified using the GreenGenes reference database. An OTU table was generated and summarized in order to gain insight into these microbial communities.

Results and Discussion:

A bioinformatic approach was taken in order to gain insight into the microbial community dynamics associated with the Trunk River Lemonade. The data collected from the systematic survey in 2015 were labeled using a 3 character alphanumeric code as follows. The first character is a number that refers to the day at which the sample was collected and can vary from 1 through 8. The second character is a letter that corresponds to which site the sample was taken from (A, B, E, K). The last character is a number that refers to the depth at which the sample was taken. (1 – 5 cm, 2 – 10 cm, 3 – 25 cm, 4 – 35 cm).

A taxonomy area plot was generated using macQIIME that highlighted the taxonomic diversity within all collected samples at the phyla level (Figure 1). The dominant phyla across all samples collected appear to be the Proteobacteria and the Chlorobi, which is consistent with the knowledge that these phyla are respectively home to photolithotrophic the purple and green sulfur bacteria, which are likely the biological sources for the elemental sulfur that gives the Lemonade its distinct yellow color[1,3].

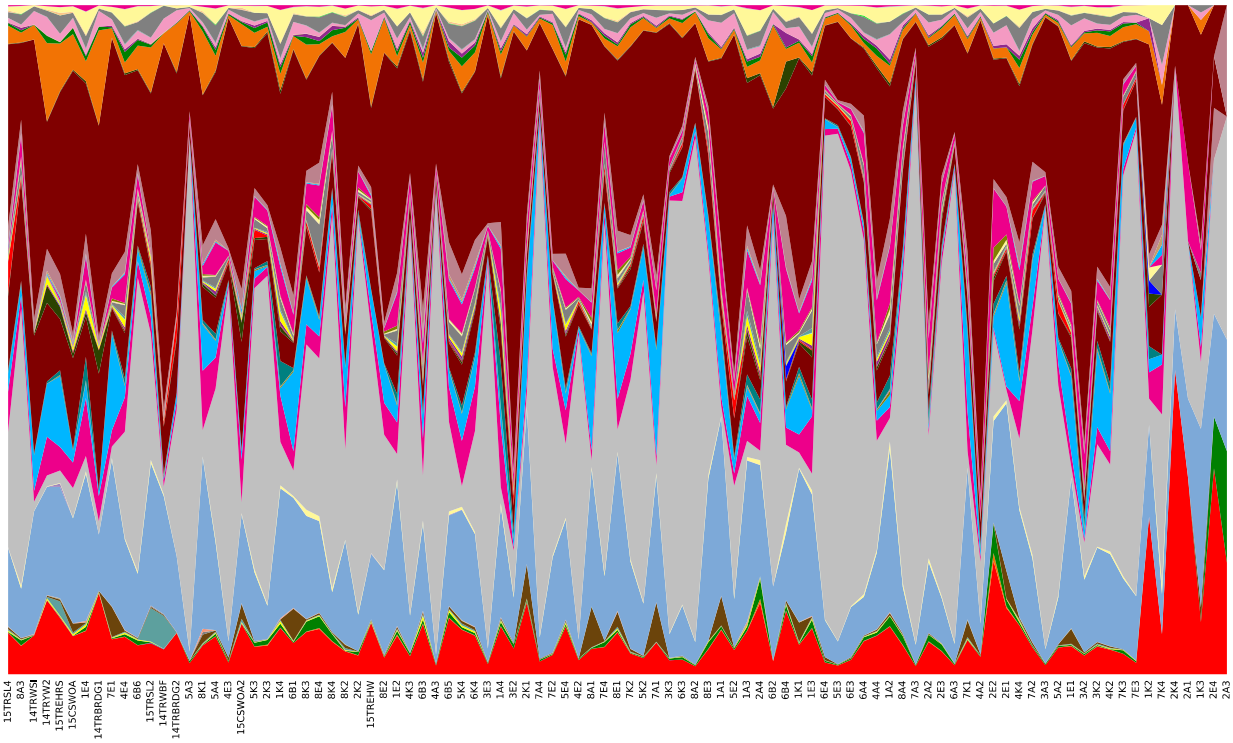

Figure 1. Taxonomic area plot depicting the phyla present in samples collected from Trunk River Lemonade. All samples used in this study are found on the x- axis. The dominant phyla in all many samples appear to be the Chlorobi (gray area) and Proteobacteria (maroon area).

The total OTU table was manipulated in Microsoft Excel such that all phyla aside from the Proteobacteria and Chlorobi would be removed. A new taxonomy area plot was constructed using only Proteobacteria and Chlorobi data to further show the dynamics between these two groups (Figure 2). In all samples, there seemed to be a negative association between these phyla; whenever the Chlorobi become abundant, the Proteobacterial abundance is diminished with the reverse also being true.

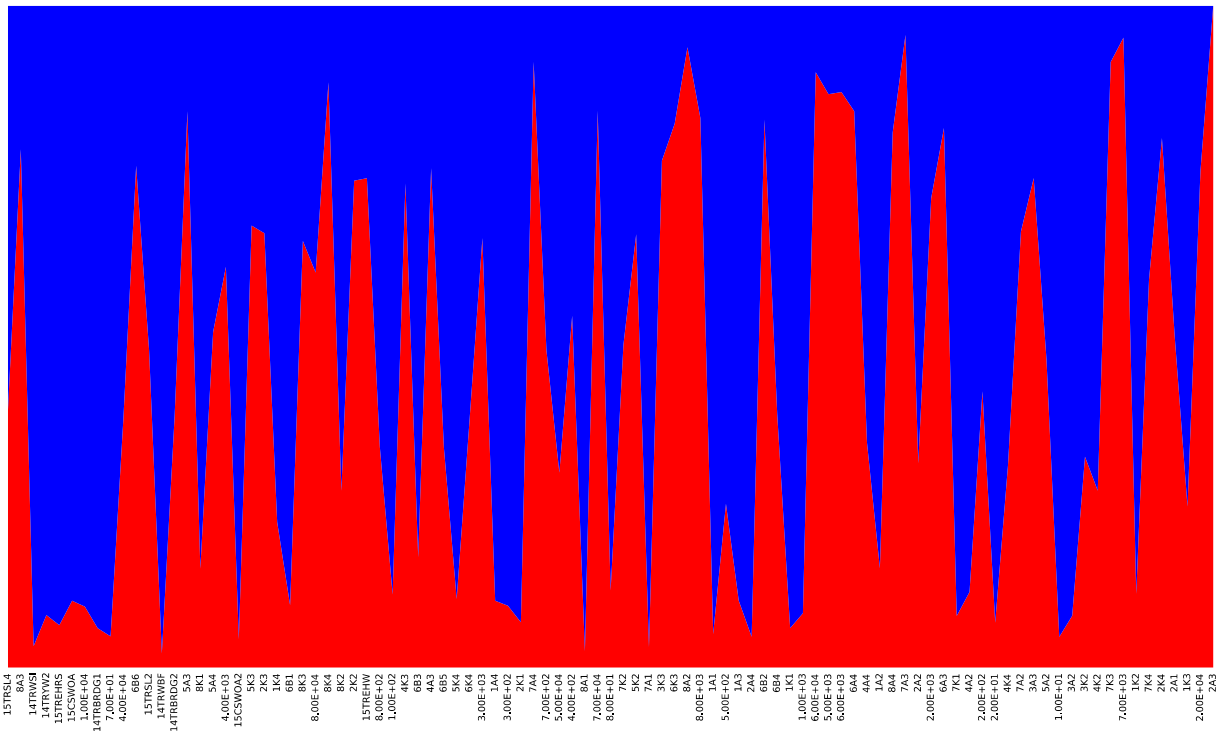

Figure 2. Taxonomic area plot depicting the dynamics between the two dominant phyla. Sample designations are found on the X-axis. The Proteobacteria (blue) appear to dominate in samples were the abundance of Chlorobi (red) are diminished.

The data from Figures 1 and 2 are slightly misleading as the samples are arranged along the X-axis in a random order, giving the appearance of a cyclic turnover in Chlorobi and Proteobacteria abundances. The OTU table was further manipulated to allow for more accurate representation and interpretation of the collected data. In addition, the OTU's were further resolved to the Class taxonomic level to gain even further insight into the dynamics of these data. Figure 3 shows the 16S amplicon data separated by sampling site and were arranged by increasing depth from left to right. Figure 3A describes the data collected from the A-Hole site. Higher abundances of organisms from the class Chlorobia are found towards the right of the figure, suggesting these organisms prefer to deeper waters. Gammaproteobacteria (phylum containing the purple sulfur bacteria) is the major class observed when Chlorobia abundances are down. Figure 3B shows data collected from the B-Hole site. At this depth, Chlorobia dominate in both shallow and deep waters, with Deltaproteobacteria becoming more prominent at intermediate depths. Many sulfate reducing bacteria are found within the deltaproteobacteria clade, suggesting that a difference in local chemistry at B-Hole could be selecting for the presence of sulfate-reducers as opposed to sulfur oxidizers. E-Hole data is shown in Figure 3C, and a clearer trend of Chlorobia becoming increasingly abundant as depth increases can be observed. Proteobacteria abundances tended to decrease with depth. Figure 3D shows data collected from the K-Hole, where a similar trend of Chlorobia dominating with depth is observed again. The Deltaproteobacteria make a greater appearance with depth as well.

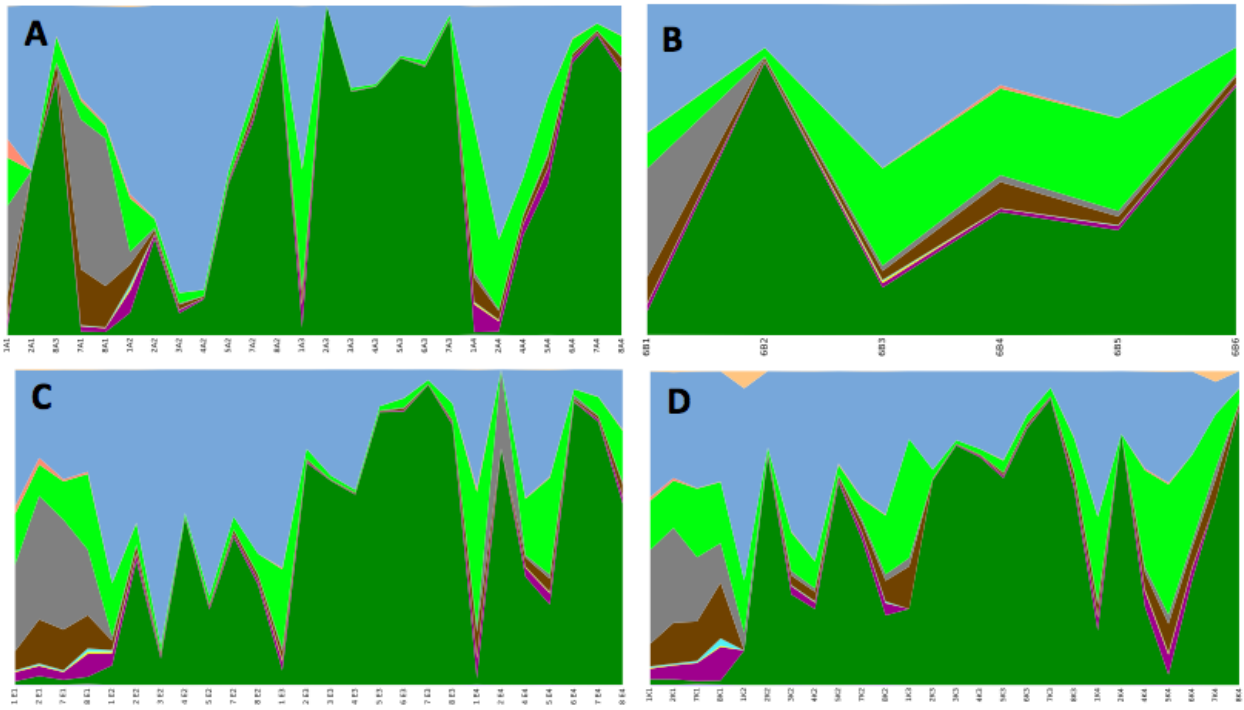

Figure 3. Class level taxonomic area plots of samples collected and separated by sampling site. Depth increases from left to right in all images. Dark green: Chlorobia, Neon green: Deltaproteobacteria, Gray: Betaproteobacteria, Blue: Gammaproteobacteria. **A**: Samples from the A-Hole Site. **B**: Samples from the B-Hole site. **C**: Samples from the E-Hole site. **D**: Samples from the K-Hole site.

A set of trends could be observed in Figure 3 that hinted at Chlorobia preferring to live in deeper waters. In order to visualize this trend from a different point of view, the OTU Table was manipulated again to organize the samples by depth with time increasing from left to right (Figure 4). Figure 4A shows the data collected from all sample sites at 5 cm depth. The proteobacteria appear to be the dominant phyla at this depth, with the Gammaproteobacteria and the Betaproteobacteria making up the largest portions of the community at the class level. Sulfur oxidizers can also be found within the Betaproteobacteria, with an example coming from the genera *Thiobacillus*[9]. Figure 4B shows the data collected at the 10 cm depth. Here, the Chlorobia begin to have higher abundances with the Gammaproteobacteria making up most of the rest of the community. Samples from the 25 cm depth is shown in Figure 4C, which shows that the Chlorobia have become the major class in this community, with the Gammaproteobacteria making up the majority of the rest of the community at this depth. Figure 4D shows the data collected at 35 cm depth. Here, the Chlorobia still make up a large portion of the total communites but do esn't seem to be as abundant as at 25 cm. The Deltaproteobacteria make a more pronounced appearance at this depth, with the Gammaproteobacteria filling in most of the rest of the community.

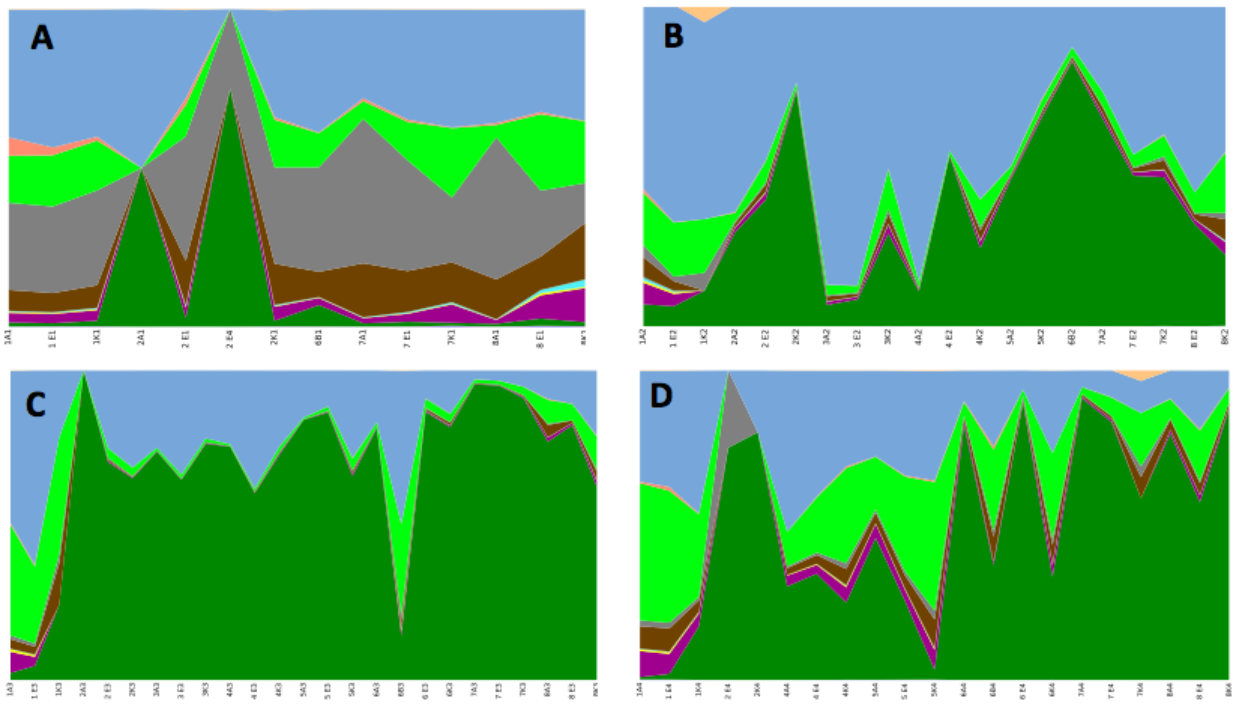

Figure 4. Class level taxonomic area plots of samples collected and separated by depth. Time increases from left to right in all images. Dark green: Chlorobia, Neon green: Deltaproteobacteria, Gray: Betaproteobacteria, Blue: Gammaproteobacteria. **A**: Samples from the 5 cm depth. **B**: Samples from the 10 cm depth. **C**: Samples from the 25 cm depth. **D**: Samples from the 35 cm depth.

The major trend that reoccurred throughout this study was the observation that the organisms of the Chlorobia class tended to be found deeper in the water column at these Lemonade sites. Perhaps this phenomenom was due to the physicochemistry of the environment changing as a function of depth. As shown by Microbial Diversity affiliates from 2015, dissolved oxygen decreased as you traveled further down in the water column (Supplemental Figure 1). Though both the purple and green sulfur bacteria are classified as anaerobic anoxygenic phototrophs, could the Chlorobi be more sensitive to oxygen than the Gammaproteobacteria? The data presented in this study cannot specifically answer that question but is important to note that this study was only resolved to the class taxonomic level. Most, if not all, Chlorobi are anaerobic while that is not the case with all Gammaproteobacteria. It is possible that many of the Gammaproteobacteria in our sample were actually oxygen tolerant and would be happier in the upper portions of the water column, where oxygen is present. Further analysis at deeper taxonomic levels would need to be performed in order to support this claim.

It is difficult to observe and interpret trends in datasets without performing the appropriate statistical measures to back any claims made. The 2015 Microbial Diversity Affiliates were able to take a lot of chemical measurements which are found in the mapping file used to construct the data for this report. QIIME can be used to correlate the appearance of a group of organisms with a specific parameter using a metadata correlation script. This this test should be performed to better quantify the data set in order to be more confidant in asserting at the Chlorobia don't tolerate oxygen as well as the Gammaproteobacteria.

In addition, statistical analyses also need to be performed on the data in order to quantify the diversity seen within individual sample. Over 9 million reads were processed to generate this data with drastic variation in the number of reads per sample (from as low as 5 to as high as 600,000). Alpha diversity and rarefaction analysis would be welcomed as it would aide in describing the diversity observed within each individual sample. If the majority of the samples are not representative of the environments from whence they came, how can we make any claims at all about trends we've observed? In addition, beta diversity and other distance metric tests should be run on this data to better quantity the differences in the community structure between samples. If the sampling sites and holes end up not being significantly different from each other, then do any of the trends observed have any tangible meaning? It's hard to say without numbers fall back on.

Conclusive Remarks:

Trunk River Lemonade is an interesting and unique environment that definitely warrants further study. Any work on either the biological or chemical nature of this site should be backed up with appropriate statistics in order to have concrete evidence to help guide even more in depth study of this location.

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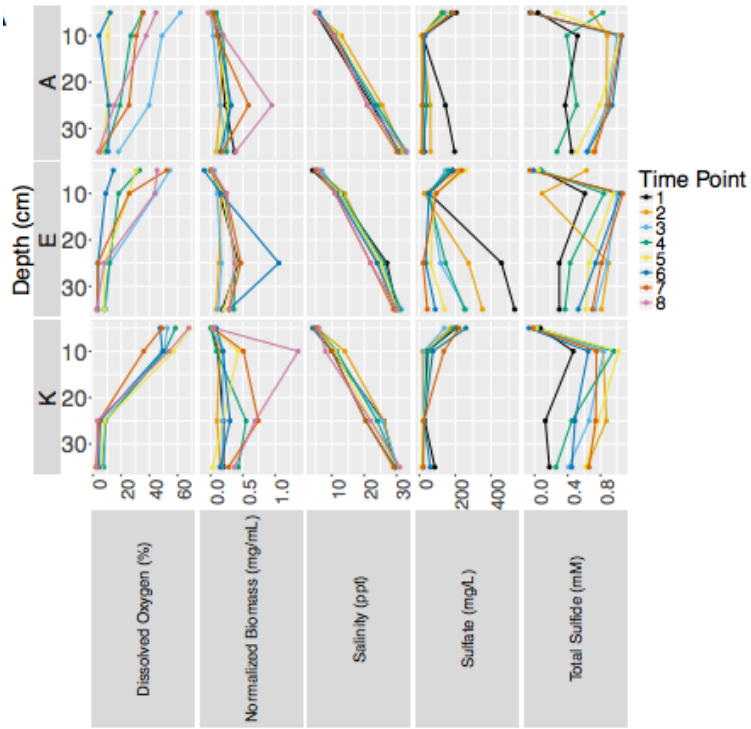
Supplemental Data:



Supplemental Figure 1. Physicochemical data collected in 2015 by Microbial Diversity affiliates. As observed, Dissolved oxygen decreases with depth and there are dynamics between sulfate and sulfide in these samples[7].