Extraction of DNA from biofilm found on submerged wood in different flow regions

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Abstract

Large wood is often added to aquatic systems to improve the habitat for macrofauna, but it can also serve as a surface for colonizing bacteria. Bacterial biofilm communities form on wood surfaces in river ecosystems may be crucial in understanding their role in structural integrity of wooden structures in river systems. This project began by submerging a string of wood slices into the Mississippi River at different locations with differing flow rates. The flow rates were classified for each location as: high (1.1-1.9 m/s), medium (0.6-0.8 m/s), or low (0.0-0.1 m/s). Slices were removed from the river at five different timepoints. Layers of biofilm had formed on the surface of all the log slices. The biofilm layer was removed by scraping the surface with a razor blade in three 2 cm x 2 cm squares, for a total of 12 cm^2 for each slice. The amount of biofilm found on the wood slices generally increased over time. On average, 9.0 mg of biofilm/cm^2 was recovered from wood slices in low flow regions. Wood slices in the medium and high flow regions had an average of 9.5 and 7.9 mg/cm^2, respectively. DNA was then extracted from the biofilm samples and 7.4, 4.6, and 9.3 ng DNA/mg biofilm was found for low, medium, and high flow regions, respectively. The DNA was sent off to be sequenced and will be analyzed to look at the development of the biofilm community over time, and to compare the community composition on submerged wood in the different flow regions of the Mississippi River.

Objectives

- Extract DNA from biofilm layer that formed on wood slices submerged in the Mississippi River
- Sequence extracted DNA
- Use statistical analysis to analyze sequence data in order to identify trends in microbial community composition on the wood slices when comparing sites, timepoints, and flow regimes.

Methodology

Strings of wood slices (Figure 1) were submerged in the Mississippi River at different locations, with differing flow rates. Slices were removed from the river at up to five different timepoints. Three 2 cm x 2 cm squares of biofilm were scraped from each wood slice using sterile razor blades. DNA was extracted from the biofilm using the PowerBiofilm DNA kit, from QIAGEN (Düsseldorf, Germany). DNA was sent to Mr. DNA (Shallowater, TX) to be sequenced. Sequence data was analyzed using R, a free statistical analysis program (https://www.r-project.org). Various phylogenetic analysis packages were used within this program. Phylogenetic analysis of community composition was also done using Microsoft Excel.

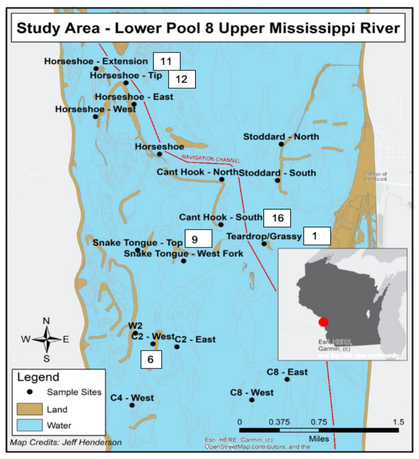

Figure 1. Wood slices prior to submersion strung to a flotation device (left). Map of the Mississippi River with sample locations marked underneath or to the right of location names (right).

Results

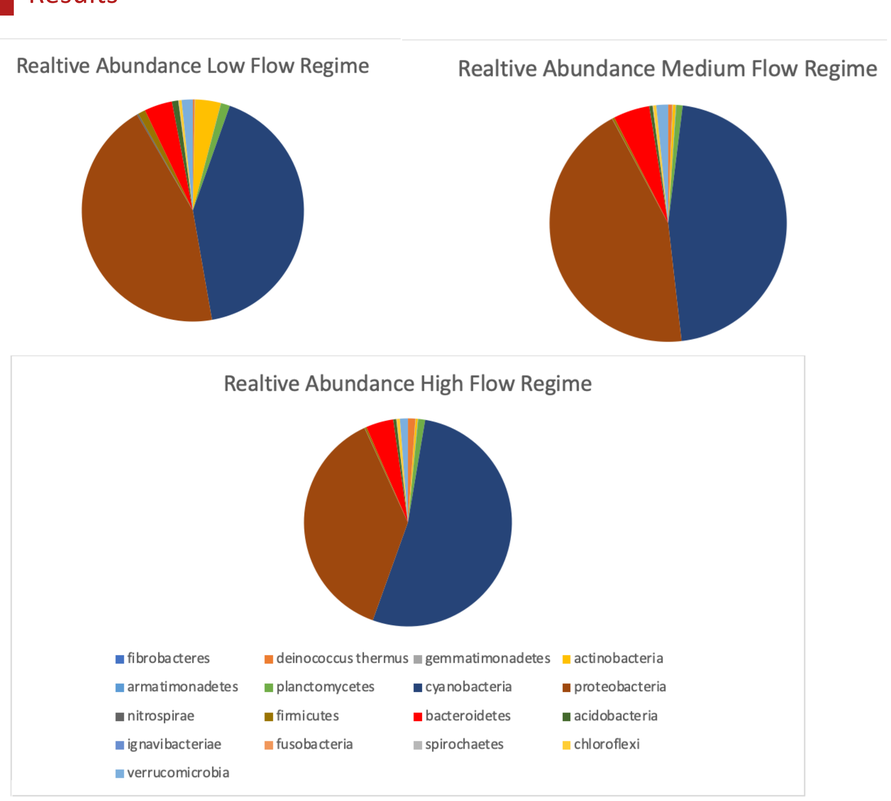

Figure 2. Pie charts representing the average relative abundance of various Bacterial phyla in three different flow regimes.

Results (continued)

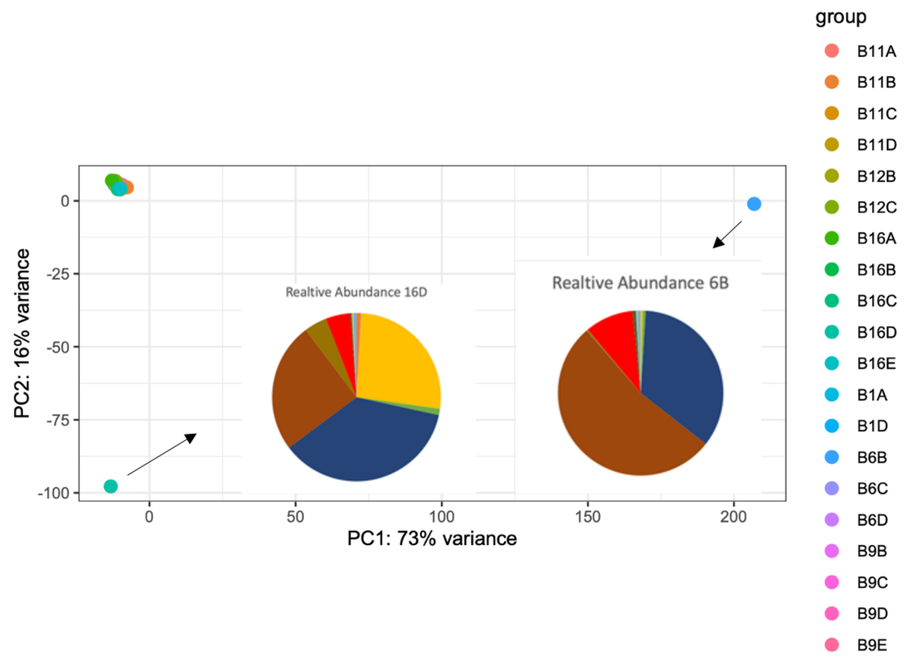

Figure 3. Principle component analysis (PCA) of Bacterial communities on the wood slices. Two major outliers were identified (6B and 16D). The pie charts for their relative abundancies have been superimposed on the PCA chart to show their community makeup. Further analysis done with heat maps (data not shown) showed that sample 16D had high numbers of a subset of Proteobacteria and Bacteroidetes OTUs that was not found in any of the other communities. The identification of these outliers led to their removal in future analyses.

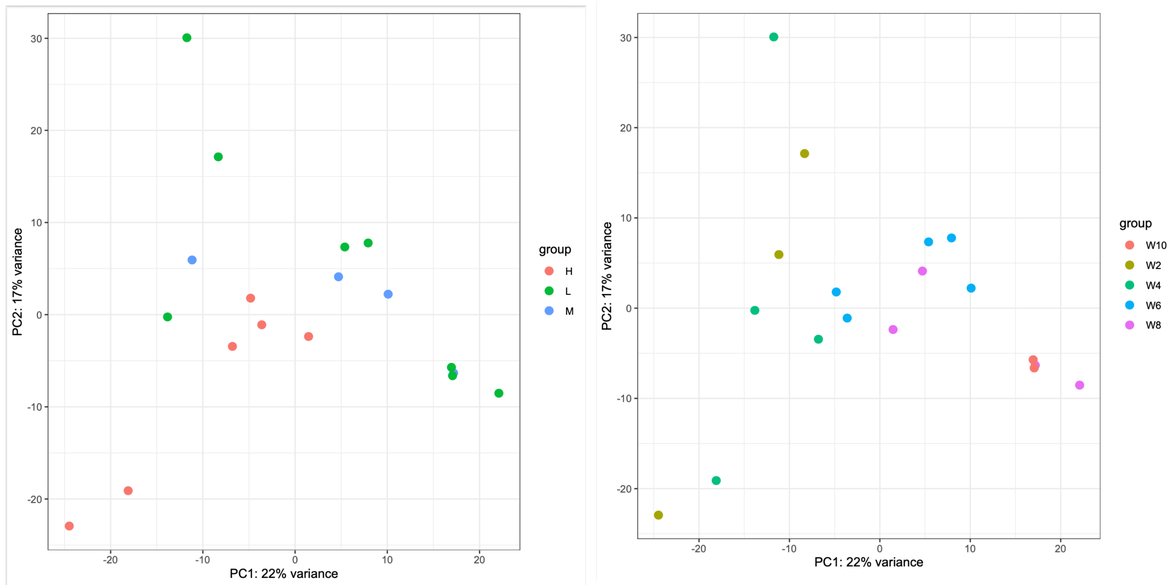

Figure 4. PCA of Bacterial communities on the wood slices (excluding the two outliers). The image on the left represents a PCA that groups samples based on the flow rate of the Mississippi River where they were submerged (H=high, M=medium, L=low). Based on this analysis, there is a moderate correlation between community composition and flow rate. The image on the right is a PCA that groups samples based on time submerged in the Mississippi river (W10=10 weeks, W8=8 weeks, and so on). This data shows that community composition has a strong correlation with time. It is evident that the microbial communities on submerged wood slices change over time. As submersion time increases, the communities become more similar to one another. No correlation was found between location and community formation (data not shown).

Conclusion

- In general, the microbial communities that formed were made up of mostly Proteobacteria, Cyanobacteria, and Bacteroidetes.
- Community formation appears to be more heavily impacted by flow rate and submersion time than location.
  - Moderate correlation between flow rate and community composition
  - The factor that influenced community composition the most was found to be submersion time.
- The communities changed over time and appeared to become more similar.

Acknowledgements

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