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# The Effects of Seasonality and Land Use on Microbe Populations in Three Ecological Areas

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# THE EFFECTS OF SEASONALITY AND LAND USE ON MICROBE POPULATIONS IN THREE ECOLOGICAL AREAS

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## ABSTRACT

The Huron River watershed is comprised of three primary land uses: agricultural, natural, and urban. Land usage affects the biological and chemical conditions within the watershed, therefore affecting water quality. Recent studies have provided evidence that water quality can be assessed by examining the composition of the microbial communities found in biofilms. In this study we analyzed and compared samples collected from a natural site and urban site over the course of three seasons; fall (August), winter (December), and spring (April). Microbial diversity was determined by isolating, amplifying, and genotyping the 16S rRNA gene. The results show that microbial community structure varies with seasons, material composition, and site location.

## INTRODUCTION

Microbes have become an increasingly popular area of study. This is understandable, since microorganisms drive biogeochemical cycles, such as nutrient mineralization and the transformations of carbon into organic matter that control the functioning of ecosystems (Kucera & Dick, 2008).

Biofilms form when bacteria adhere to surfaces in aqueous environments and begin to excrete a slimy, glue-like substance that can anchor the biofilms to many surfaces (in this case, rock, sediment, and a plastic bobber). A biofilm can be formed by a lone bacterial species, but it is more likely that natural biofilms consist of various species of bacteria, algae, debris, protozoa, and fungi. Once they are a part of a surface, biofilm microorganisms bring about a variety of disadvantageous or favorable reactions, depending on the immediate environmental conditions. Biofilm microorganisms arbitrate many processes in aquatic ecosystems, including nitrification and denitrification. These processes are important to understand in freshwater ecosystems. Metabolically vigorous organisms participate in many critical roles in many environmental processes.

Assessment of microbial diversity and dynamics in biofilms will lead to a better understanding of how land usage and seasonality affects biogeochemical conditions in the Huron River watershed. This information can be used to detect particular areas in the Huron River watershed with superior or meager biogeochemical circumstances. This watershed, because of presence in both urban and unmanaged areas, influences the biogeochemical cycling in many regions. Watershed studies usually focus on characterization of microbial (usually bacterial) populations or communities (Gutknecht, 2006). The specific areas the biofilms will be collected from in the larger study include agricultural areas impacted by nearby farm animals and chemicals used for crops, urban areas impacted by human interaction, and natural areas that are the least impacted by any outside influences. These three ecological areas will be sampled over the course of a year to see not only how the three types of areas vary in their microbial populations, but also to see how these populations vary when light and foliage conditions change over time. There have been previous studies, such as that of Moss et al. (2006) that showed an increased microbial diversity in the summer months. It is important to consider seasonality in order to assess whether certain seasons provide more nutrients for the microbes, thus resulting in a higher amount of eutrophication of the watershed in those areas. This study will focus on two sites, one urban and one natural.

It is not known whether there is a general trend in how microbial populations behave in response to certain seasonal, land use, and water level conditions. As stated by Rocha (2008):

> It is clear that the microbial diversity of sands, as well as the different metabolic pathways possible in this environment, have only very recently begun to be studied, and a strong effort must be made in this area. It is crucial that further information on microbial diversity and community structure present in sands and their link to biogeochemical function is obtained. (p. 126).

It is especially important to understand the impact of microbial populations on regional water quality. A study performed by Jones et al. (2008) found that

> seasonal variations in temperature, sunlight, and tributary inflow will result in variations in most, if not all, water quality parameters. This is particularly true in enriched systems where the activity

> of primary producers during the growing season can have major effects on water quality parameters, ranging from orthophosphorous levels to dissolved oxygen to BOD. (Jones et al., 2008).

Another important aspect of the overall study is to identify how algal populations are linked to the microbial diversity found in biofilms in the Huron River watershed. The relationship between microbes and algae has already been identified by various previous studies, including Haynes et al., who find "algal-bacterial coupling in estuarine sediments is likely to involve particular bacterial taxa, rather than a response from the entire bacterial community" (Haynes et al., 2007). The completed study will help to expand on previous studies by identifying the particular microbial species that correspond with increased or decreased algal populations and how land usage and seasonality affect these relationships.

Microbial diversity and dynamics will be examined in this study. Four sampling dates per site will be used over the course of a year. This article focuses on two sites and three sampling dates. The full project will encompass all sites, all sampling dates, and also examine the influence of varying light, water levels, and nutrients. These variables will then be compared with the microbial populations in order to find some correlation between them.

## MATERIALS AND METHODS

### Sample Collection

Samples were recovered from a total of twelve sites over the course of a year. There were four sites for each of the three ecological areas. At each site, biofilm samples were removed from three different substrata: natural rocks, surficial sediments, and an artificial element (plastic bobbers) which were set in the waterway for a period of two to four weeks and then recovered. The biofilms that adhered to each material were examined. This article discusses two sites: sites 32 (natural) and 56 (urban), which reside at latitude 42.33 N and longitude -84.04 W and latitude 42.24 N and longitude -83.75 W, respectively.

### T-RFLP

The total DNA of the sample was separated from any debris in that sample (sand, rock, etc.) using a Powersoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA). Polymerase Chain Reaction (PCR) was then performed using laboratory PCR protocol, which

includes Mastermix, a fluorescent forward primer (FAM8F) and a 1525R reverse primer. These primers amplified the 16S rRNA gene and attached a fluorescent marker to that gene which was what was needed to identify the different microbes in the samples. The 1525R primer was used because the sequence recognized by 1525R (5′-AAGGAGGTGATCCAGCC-3′) is a universal bacterial primer (Lane, 1991). The samples were placed in a thermocycler to complete the PCR reaction at 94°C for 10 minutes then cycled 35 times between 94°C for 1 minute, 46.5°C for 1 minute, 72.0°C for 2 minutes, and ended with 72.0°C for 10 minutes. Once the thermocycler was finished, it remained at 4°C until the sample plate was retrieved and stored at -10°C.

The products from this process were then cleaned-up using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI) to make sure there were no lingering debris and extracted DNA fragments of 100bp to 10kb. DNA concentrations were determined spectrophotometrically at 260 nm. $A_{260}$PCR products were further analyzed after gel electrophoresis and staining with gel red. Samples were then restriction digested using MspI Restriction Enzyme, Buffer 2, and Nuclease Free Water. Once the ingredients were added, the samples were placed in a 37°C water bath for no longer than 16 hours. They were then cleaned up once again using the Wizard® SV Gel and PCR Clean-Up System. Samples were then sent to the University of Michigan DNA sequencing center (SeqCore) for fragment analyses. ROX1000 (Applied Biosystems, Foster City, CA) was used as the molecular weight standard.

Sequenced samples were then analyzed using Terminal Restriction Fragment Length Polymorphism. T-RFLP is a molecular fingerprinting technique that allowed us to see the changes in the microbial population over time between all the samples at the two different sites. This is a lower resolution technique.

> The technique is dependent on automated sequencing using thermocycler conditions and gene mapping systems that make use of fluorescent dye chemistry for fragment detection. By using a 5' fluorescently tagged primer in the PCR amplification step (FAM8F), followed by restriction with an endonuclease and size selection on automated systems, only the restriction fragment that includes the primer, i.e., the terminal fragment, is detected by the fluorescent sensor. (p. 323).

T-RFLP fragments were analyzed using a DNA sequencer (Peak

Scanner), which generated a fingerprint that is unique to each particular sample site. The fingerprint was in the form of a chromatogram that provided different sized peaks that told us that there were different types of microbes in the sample. Each individual peak represented a particular microbe.

After all of the microbes were identified, we could determine which microbes were present during the different times of the year and how light, water levels, and nutrient availability affected this. For this particular study we were only looking at how the seasonal variations, substratum, and site location/land use affected microbial community structure and diversity. Depending on the correlations that are discovered we can see how each ecological area is affecting the microbial populations of the Huron River watershed, whether negatively or positively.

## Analysis

The genotyped samples from the University of Michigan's SeqCore were then converted to .txt files by ***Peak Scanner™*** developed by Applied Biosystems. The .txt files provided a dendrogram that showed the relatedness of the samples by analysis from K9 and R console (http://www.r-project.org). K9 and R console use a statistical database to determine which samples are more closely related to each other based on the peak locations identified by Peak Scanner.

# RESULTS

Two sites out of a total of twelve were analyzed. Using K9 and R console, dendrograms were produced using several different parameters, and seasonal and material variations. The dendrograms produced are phylogenetic trees based on the 16S rRNA bacterial gene taken from both collection sites. In Figure 1, sites 32 and 56 were analyzed and compared. The purpose of this dendrogram is to show how the different seasons (fall, winter, and spring) and different substrata (sediment, rock, and bobber) relate to each other in microbial community structure.

By combining both samples into one dedrogram we can see how the two sites relate to each other in community structure as well as how the seasons and material of the samples contribute to relatedness. The Bray-Curtis distance is the measure of relatedness; the closer the branches containing the samples are to 0.0, the more related the microbial communities of the samples are to each other.

There are a total of 60 samples. The samples abbreviations are based on whether the samples are from the winter, spring, or fall collec-

tions; the site number; whether the samples are from rock, sediment, or bobber; the label of 1 or 2 because each rock, sediment, and bobber had two collections; and finally the samples were analyzed in duplicates so the samples with two digits at the end, the second digit represents the duplicate and will always be a 2. An example in Figure 1 of this abbreviation system is "SP56R12". The "SP" means the sample is from the spring collection. The "56" shows that the sample was collected from site 56. The "R" means this sample was collected from rock. Then the "1" means it is the first rock sample collected from that site. The "2" at the end means that this is the duplicate. Figure 2. represents a dendrogram produced when only site 32 is examined. The parameter of different site locations is removed,

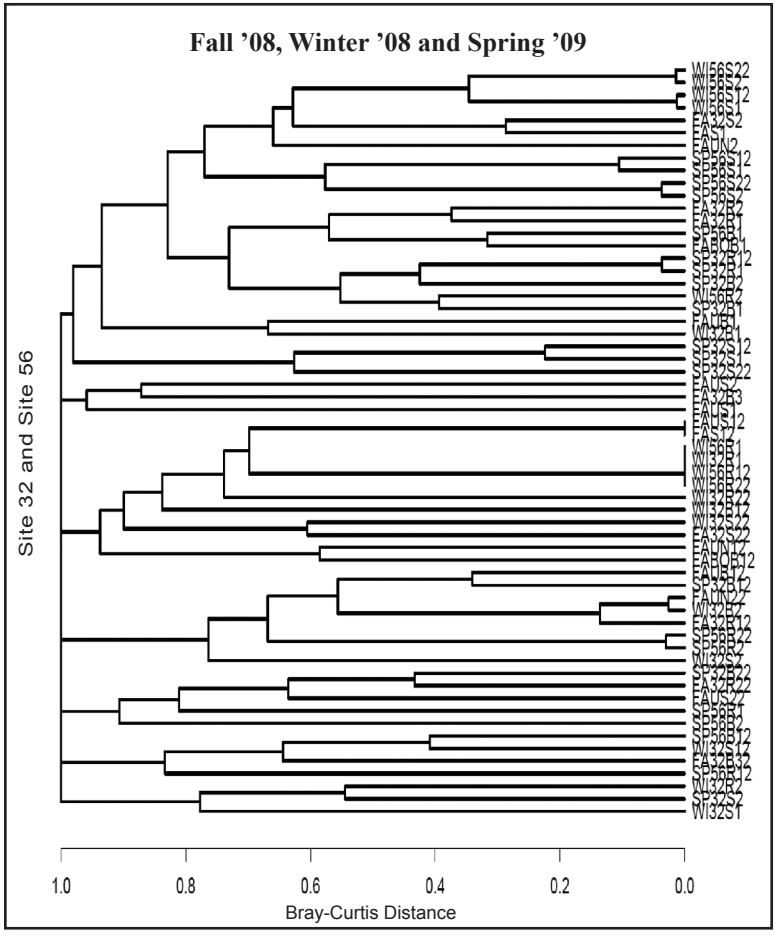


**Figure 1.** Dendrogram representing both sites, 32 (natural) and 56 (urban).

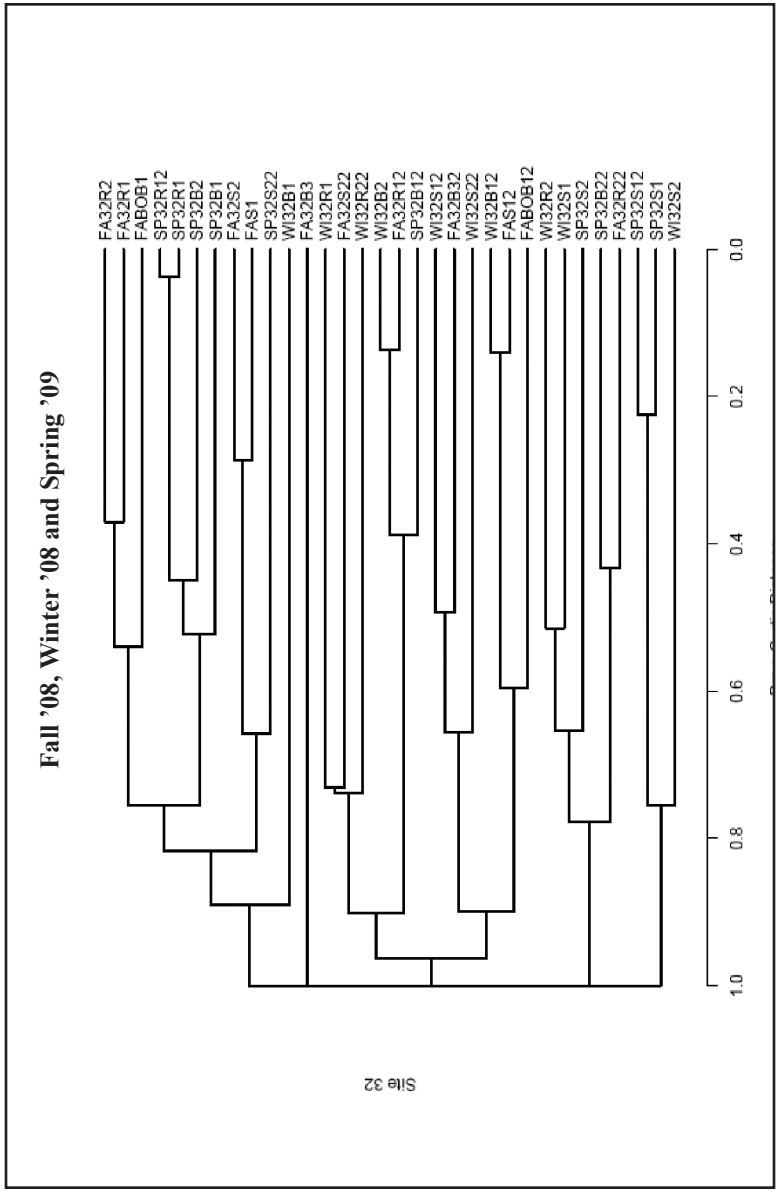


**Figure 2:** Dendrogram of natural area site 32. This dendrogram incorporates all of the different samples collected from each season as well as each material collected (rock, sediment, and bobber). All samples were analyzed in duplicates to account for analytical error. Figure 2 was produced using the same method as Figure 1. There are a total of 32 samples.

having removed site 56 results. The impact of seasons and materials on the microbial population dynamics and site 32 can now be assessed alone.

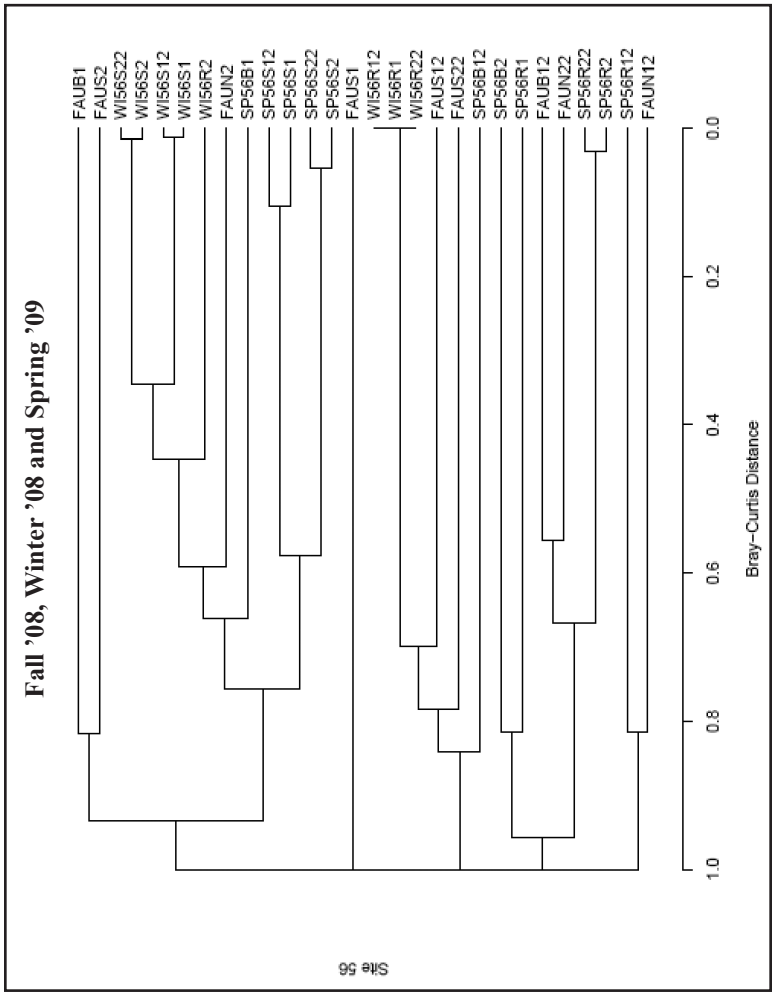


**Figure 3:** Dendrogram of only site 56. The purpose of this dendrogram is to show how the samples relate in microbial community structure when examining the parameters of seasonal and material variations. The abbreviations for the plastic bobbers in this dendrogram switch between using "B", "UB", and "UN". Other than this variation from the previous figures (Figs. 1 and 2), the abbreviation system explained above is exactly the same.

Figure 3 was produced in order to examine how the different seasons and materials used affected the microbial diversity of site 56 when examined alone.

## CONCLUSION

The T-RFLP analysis demonstrated the annual variation in microbial population dynamics. Because of time limitations, this article does not include comparisons of microbial data to environmental conditions (e.g., water levels, nutrients, light, etc.), and focuses on the impact of different seasons—in this case fall (August), winter (December), and spring (April)—on the microbial diversity of natural site 32 and urban site 56 along the Huron River watershed.

Dendrograms from Buesing et al. (2009) comparing freshwater marsh microhabitats from January, April, July, and October at three sites determined that there was little to no impact of seasonality on microbial diversity. Our results contradict those of Buesing et al. When considering the dendrograms from Figures 2 and 3, we can see distinct groupings between seasons. There are a few outliers, but the majority group by seasons. These outliers may be the result of cross-contamination when preparing samples for genotyping. The findings by Buesing et al. (2009) are also contradicted by a parallel study performed by Moss et al. (2006). In Moss's study, they found that microbial diversity increased during summer months. While Moss's study encompassed estuarine sediment habitats of East Sabine Bay, Florida, it is still comparable to this study.

Another parameter that was examined, substratum, was also found to group microbial communities. There are major groupings between sediment samples, rock samples, and bobber samples as indicated by dendrograms in Figures 1, 2, and 3. Seasonal variability and material could not be separated in order to examine those results independently of each other. Because of this, the results show the interaction of seasonal variability with substratum composition.

The last parameter examined by Figure 1, site location, also exhibits grouping. There are substantial groupings between the samples from site 32 independent from the sample groupings of site 56. This is a reasonable result as site 32 samples are from a natural area while site 56 samples are from an urban area. These distinct groupings support the assumption that because of the different ecological impacts of the surrounding areas, there should be significant differences between the natural site and urban site.

Nutrient availability was not included in this paper, and so the

effects of this, along with the community structure, cannot be related to fluctuations in algal populations of the Huron River watershed at this time. This study is ongoing and will produce a more complete report in the future. When these results are incorporated, we will be able to examine how microbial diversity and community structure affect biogeochemical cycling of the Huron River watershed.

Clone libraries will also be produced for the larger, complete study so as to connect the general relatedness represented by the dendrograms (Figures 1, 2, and 3) from the T-RFLP analysis to specific microbial taxa. This analysis will help us to identify particular microbes that may be more abundant in some areas than others. This result can then be related to the other data parameters to strengthen what we know about how biogeochemical cycling is affected by microbial diversity and community structure.

## REFERENCES

Buesing, N., Filippini, M., Burgmann, H., & Gessner, M. O. (2009). Microbial communities in contrasting freshwater marsh microhabitats. *FEMS Microbiol Ecol., 69* (1), 84–97.

Gutknecht, J. L. M., Goodman, R. M., & Balser, T. C. (2006). Linking soil process and microbial ecology in freshwater wetland ecosystems. *Plant and Soil, 289*, 17–34.

Haynes, K., Hofman, T. A., Smith, C. J., Ball, A. S., & Underwood, G. J. C. (2007). Diatom-derived carbohydrates as factors affecting bacterial community composition in estuarine sediments. *Applied and Environmental Microbiology, 73*, 6112–6124.

Jones, C. R., Kelso, D. P., & Schaeffer, E. (2008). Spatial and seasonal patterns in water quality in an embayment-mainstream reach of the tidal freshwater Potomac River, USA: A multiyear study. *Environ. Monit. Assess, 147*, 351–375.

Kucera, J. M. and Dick, R. P. (2008). PLFA profiling of microbial community structure and seasonal shifts of a Douglas-fir chronosequence. *Microbial Ecology*, *55*, 500–511.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In E. Stackebrandt & M. Goodfellow (Eds.), *Nucleic acid techniques in bacterial systematics*. New York: John Wiley and Sons.

Marsh, T. L. (2005). Culture-independent microbial community analysis with terminal restriction fragment length polymorphism. *Methods in Enzymology*, *397*, 309–326.

Moss, J. A., Nocker, A., Lepo, J. E., & Snyder, R. A. (2006). Stability and change in estuarine biofilm bacterial community diversity. *Applied and Environmental Microbiology*, *72*, 5679–5688.

Rocha, C. (2008). Sandy sediments as active biogeochemical reactors: Compound cycling in the fast lane. *Aquatic Microbial Ecology, 53*, 119–127.