

Changes in Bacterial Communities in Coastal Plain Streams: The Influence of Beaver Wetlands

Kaylan Gee, Kirsten Ansorge, *Jennifer Edmonds, Ph.D.

*Faculty Sponsor

Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487, USA

Beaver dams create wetlands that are fundamentally different from other wetlands. Their geomorphic position in the landscape, short persistence at a particular location, and close association with human management make them dynamic, but understudied as a component of low-gradient stream networks in the southeastern United States. In these ecosystems, beavers create a mosaic of patches that vary in time with dam failure and conversion of the wetland back to a stream configuration. This work relates beaver dam demise in a Coastal Plain stream (Payne Creek) to bacterial community composition in benthic sediments. We used a molecular "fingerprint" analysis (T-RFLP) to test the prediction that the influence of beaver-created wetlands on sediment bacterial communities is evident as long as two decades after a wetland drained. Significant differences in samples from 3 stream reaches, collected 6 times from January-October 2009, suggest this wetland legacy is present, likely mediated through differences in organic matter abundance and bioavailability. We also found temporal changes in bacterial community composition possibly linked to shorter time frame phenomena such as leaf fall, flooding, or dramatic changes in temperature.

Introduction

A wetland is a transitional area of land where the soil is saturated with moisture, either seasonally or permanently. Wetlands, often called the "kidneys" of the environment, play a major role in environmental regulation of water and waste. If there are areas of urbanization or agriculture in the watershed, pesticides, fertilizers, and other pollutants may enter the wetland system. The pollutants are taken up into the biomass of vegetation and bacteria in the wetland. Bacteria can also chemically alter the pollutants and, in many cases, reduce their toxicity [6]. Recently, there has been a movement to protect wetlands against pollution and to prevent them from being directly destroyed by urban and agricultural development [12]. Common types of wetlands include swamps, marshes, floodplains, and bogs. Wetlands can have saltwater, freshwater, or brackish water. We examined freshwater beaver wetlands in the coastal plain physiographic province of the southeastern United States. These wetland ecosystems have been highly understudied by researchers.

Beavers are considered one of the world's few species of "ecosystem engineers," or animals that significantly alter their environment and, thus, increase biodiversity in the area. When a beaver

builds a dam on a stream, a wetland develops upstream. This new wetland provides a unique habitat for various species of organisms [14]. Human impact on beaver populations through alteration or elimination of their habitat can have a major impact on the overall landscape and wetland development [1]. We focused on how the demise of beaver dams influences biodiversity and ecosystem function in relation to bacterial species. Seventeen years of observations by UA faculty in local stream networks with embedded beaver wetlands has allowed us to develop a strong conceptual framework for how ecosystem processes might change in response to beaver activity. Observations tell us that, as a wetland transitions back into a stream after the failure of a beaver dam, the sediment and organic build-up that had been concentrated around the impoundment remains in the stream, significantly impacting microbial growth in the water and sediment. The soils outside the stream that were once water-saturated drain and eventually become a beaver meadow, which is gradually reforested. This transformation creates a slow change in the intensity and character of terrestrial-aquatic linkages, particularly with regards to the bioavailability of organic matter available for decomposition in the

sediments. Therefore, the study of beaver wetlands is essential to understand how these changes influence carbon cycling in streams.

Beaver wetlands create sudden change in an ecosystem, whereas other types of wetlands have a much more gradual impact on the environment. For instance, local vegetation is significantly altered when beavers dam a part of a stream. New types of aquatic or semi-aquatic vegetation grow in place of non-aquatic species. Forests are turned into bogs as beaver dams convert them into aquatic ecosystems. After beavers abandon the area, forestation gradually returns [5]. Beaver wetlands also influence the production of greenhouse gases. Beaver dams generate conditions that are favorable for methanogenesis, leading to higher production of methane in the area. These higher levels of methane have an impact on overall methane concentrations across the northern hemisphere [10]. Our conceptual model considered beaver-created wetlands as nodes on the landscape that are extremely dynamic. Beaver ponds may be created in a week and may remain embedded in the stream network for as little as 4 years or for as long as 4 decades. Age of a beaver wetland depends on size, climatic conditions, and human interference. The transition from beaver wetland back to stream ecosystem is a frequent occurrence in the Gulf Coastal Plains, based on our personal observation, but has been relatively understudied.

Although most research relating to extant beaver wetlands is concentrated in northern North America, there have been few investigations into southeastern beaver-created wetlands. The beaver populations in the Southeast have varied significantly over the last half-century, decreasing after 1983 due to changes in trapping policies [11]. Southeastern beaver wetlands tend to be restricted to watershed areas of about 2500 hectares that show no discrimination or preference for streams crossed by roads [4]. Hydrological research in the southeast has shown that the water upstream of beaver dams tends to be of a higher pH and has higher concentrations of dissolved organic carbon (DOC) than does the water downstream of the dams [9]. DOC is a significant chemical component of wetland waters. The source of DOC is plant matter, which is produced in the aquatic ecosystem or transported by runoff coming from

terrestrial soils [8]. DOC influences the removal of aquatic pollutants, particularly hydrophobic organic contaminants, like some pesticides, in one of two ways. One way is by stimulating larger populations of heterotrophic bacteria that chemically transform the pollutant during growth on the DOC. The second is by the direct absorption of the pollutant to DOC compounds. The larger the bacterial population, the more pollutants can possibly be removed from the water [6]. Levels of DOC in wetlands vary, and levels tend to fluctuate most dramatically during the summer and fall when there is more bacterial activity breaking down DOC [7].

We specifically focused on the relationship between DOC sources to coastal plain streams and the bacterial communities living in the sediments. It is believed that bacterial community structure is similar to community structure formed by macro organisms [3]. Communities of bacteria will often self-organize so that different species with similar characteristics cluster together to fill the same niche in their environment, which is known as functional redundancy. Functional redundancy in bacterial communities dampens the variability in a particular bacterial process as different species thrive under different environmental conditions, maintaining the level of each function. For example, a community of bacteria living in an area of varying temperature consists of different species of bacteria, each with a different temperature tolerance. The functions of the community such as photosynthetic activity or decomposition of DOC may be preserved even with variations in temperature levels. However, if environmental conditions (temperature, water chemistry, light levels) change dramatically, a new community structure will develop [13]. Here, we discuss our year-long investigation into the differences in bacterial diversity in wetland versus non-wetland environments in Payne Creek in the Talladega National Forest. We focused on how bacterial communities differed in stream sediments that previously were covered by wetlands, versus areas that have never been known to contain beaver wetlands.

TWE Site Description

Our study was conducted at Payne Creek in the Talladega National Forest (Figure 1). It is a

second-order Coastal Plain stream located in west-central Alabama (32.9206° N, 87.4405° W). Payne Creek is a nutrient-poor stream with low alkalinity and specific conductance. The stream length of the study was approximately 1000 meters long and composed of unconsolidated sand and silt, sometimes over 2 meters deep. Measurements of stream discharge water temperature and water chemistry have been intermittently recorded since 1990 and constantly recorded since 2001. Occasionally, excessive rainwater and runoff flood Payne Creek.

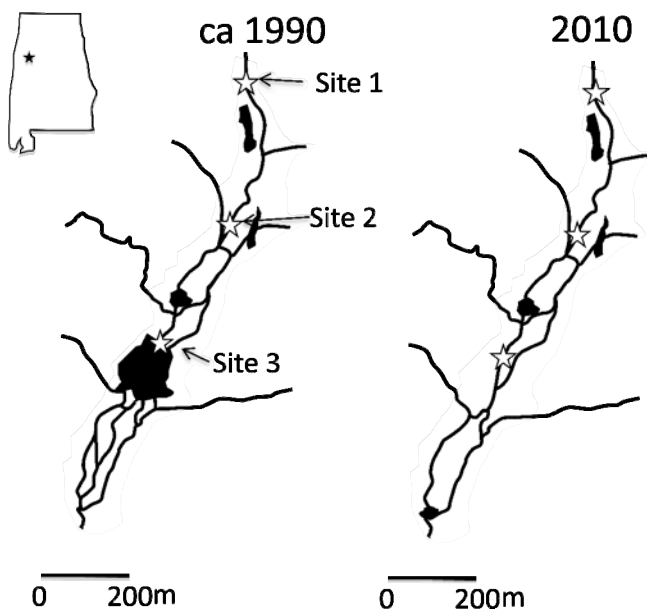


Figure 1. The star in the state of Alabama identifies the location of the Talladega National Forest. The two stream networks are Payne Creek in its current configuration versus when there was a large beaver pond and associated wetland 20 years ago. Black lines and shapes represent the ponds and streams of Payne Creek. The outlined white stars demonstrate the exact locations of sampling for this project.

Approach

We compared the bacterial communities in stream sediments, which were once a part of a large beaver wetland that drained 13 years ago. Our goal was to determine if the legacy of this wetland could be found in the composition and function of the bacterial community. An experimental design known as "space for time substitution" was used to infer the influence of the wetland. By knowing the location (or space) of the wetland in the past, sediment samples

from inside and outside of its former location could be compared. This allowed identification of the differences in bacterial community composition that might be due to the wetland's previous existence.

The bacterial gene chosen for comparison was the 16S rRNA gene. This gene codes for the smaller subunit of the ribosome, which is necessary for protein creation, and is a well-known phylogenetic marker used for identifying bacterial species. We performed a terminal restriction fragment length polymorphism (T-RFLP) analysis to generate a community "fingerprint" for each sample. Then, we statistically evaluated these fingerprints to look for the legacy of beaver wetlands in the stream sediments.

Methods

Continuous (15 minutes intervals) monitoring of water stage and temperature was completed using a Model 107 T probe in association with a Campbell Scientific Inc. data logger (Logan, UT) permanently mounted in the field. Stage-discharge relationships were established by making instantaneous measurements of discharge using a flow meter and channel width and depth measurements. Sediments were sampled at each site to a depth of 2 centimeters after removing the top 0.2 centimeters of sediment that would be the lighted benthic sediment. A sterile syringe was used, and sediment samples were stored in a cryovial and flash frozen at -70° C in the field. Once back in the lab, they were stored at -80° C. Water samples were taken in acid washed polypropylene containers, stored on ice until returning to the laboratory, and then filtered through a pre-combusted Whatman GF/F filter with a nominal pore size of 0.7 microns. DOC was measured on a Shimadzu 5000 TOC analyzer; nitrate, phosphate, and ammonium were measured on a Lachett autoanalyzer using standard colorimetric methods.

DNA was extracted from 1-5 grams of frozen soil using MoBio soil DNA extraction kits. Extracted nucleic acids were precipitated and resuspended in the final kit solution containing ethylenediaminetetraacetic acid (EDTA). The extracts were then stored at -20° C. Bacterial 16S rRNA genes were amplified with the primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492Reverse (5'-AAGGAGGTGATCCANCCRCA-3') [2]. These primers

are meant for use with microorganisms with the Bacteria domain. The forward primer was labeled with the fluorescent molecule, fluorescein (FAM). All PCR amplifications were carried out with Ready-To-Go PCR beads. Each PCR had a total reaction volume of 25 μ L, with 3 μ L of DNA template. Final concentrations of FAM-27F and 1492Reverse were 400 nM. Thermal cycling conditions for this amplification were as follows: (1) 3 minutes at 95° C; (2) 30 cycles of 30 seconds at 95°C, 45 seconds at 56° C, and 30 seconds at 72° C; (3) an additional elongation step at 72° C for three minutes.

Gel electrophoresis was used to confirm the 16S rRNA amplicon at a length of approximately 1500 base pairs. After confirming the amplicon, it was purified from a fluorescently-stained 1% agarose gel using a Qiagen gel extraction kit. Restriction enzyme digestion of the PCR product was carried out with the enzyme *HaeIII*, using the manufacturer's recommended instructions. This step cuts each PCR amplicon at a specific location and creates fragments of different sizes, depending on the 16S rRNA gene sequence of each species amplified. Digested samples were further purified by ethanol precipitation and resuspended in 12 μ L of deionized formamide. One μ L of DNA fragment length standard Gene-Scan-500 TAMRA (tetramethylrhodamine) was added to the sample, and all samples were denatured at 95° C. The terminal restriction fragment lengths were separated by capillary electrophoresis on an ABI Prism 310 genetic analyser. Fragments were quantified using GENESCAN analysis software, and each fragment appears as a peak on the electropherogram (Supplementary Figure S1).

Fragments smaller than 50 base pairs were excluded from the analysis in order to avoid the detection of primers. The T-RFLP data were analyzed using a Visual Basic program that reconciled minor shifts in fragment length between successive electropherograms. Peaks of less than 3% of total electropherogram area were not considered in the analysis. The relative abundance of fragments within samples was determined by calculating the area under each peak as a percentage of the total area. The fragment lengths present in each sample and the percent contribution to the total community were used to characterize the individual bacterial communities. Since particular peaks in this analysis

may represent more than one bacterial taxon, the number of peaks was not considered an indication of species richness. Rather, the peaks were used for comparative purposes.

Sample fingerprints were compared using the software package PRIMER 5 (Plymouth Marine Laboratory, Plymouth, UK). To assess differences in community compositions, we used a nonparametric analysis of similarity (ANOSIM). We generated one Bray-Curtis similarity matrix using the fragment composition of the bacterial communities. This matrix was characterized in two ways, based upon sample location (Site 1, 2, or 3) or sampling date (February, March, April, May, July, or October). Differences among these groups were visualized using nonmetric multidimensional scaling (MDS) plots of the similarity matrix to produce a two-dimensional ordination figure. ANOSIM was used to test whether there were significant differences in bacterial community composition between sample sites and dates.

Results and Discussion

During 2009, we sampled on six different dates from three sites and generated T-RFLPs for each sample (Table 1). Site 1 was located farthest upstream where no beaver dam had been known to exist. This site contained sandy soil that was frequently removed and then replenished due to flooding. Site 2 was between sites 1 and 3 and may have previously been a small beaver wetland, as indicated by the vegetation structure when our collaborators first started sampling there. Site 3 was the farthest downstream, where it is known to have been a large beaver wetland that was intensively studied by UA researchers from 1990-1996. Fragment lengths found in the T-RFLP data ranged from 51–328 base pairs for all the samples, and the number of fragments per sample ranged from 8–15 fragments. Bray-Curtis similarity values were graphically represented in a 2 dimensional MDS (Figures 2 and 3). The stress value was interpreted as the difficulty in representing the difference between all the samples in graphing space, with a 3-dimensional representation usually being more accurate (lower stress) than a 2-dimensional graph. Although our stress value was higher than desired, we maintained

Table 1. Number of replicate samples at each site for each sampling date.

Sampling Date	Site 1	Site 2	Site 3	Total
23 February 2009	4	2	2	8
23 March 2009	1	2	2	5
27 April 2009	1	1	1	3
28 May 2009	1	-	2	3
28 July 2009	2	-	2	4
22 October 2009	3	-	4	7

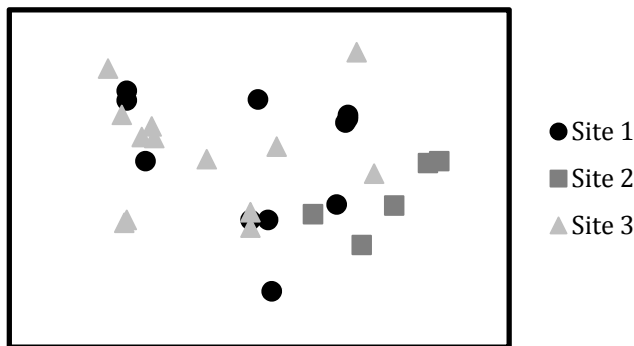
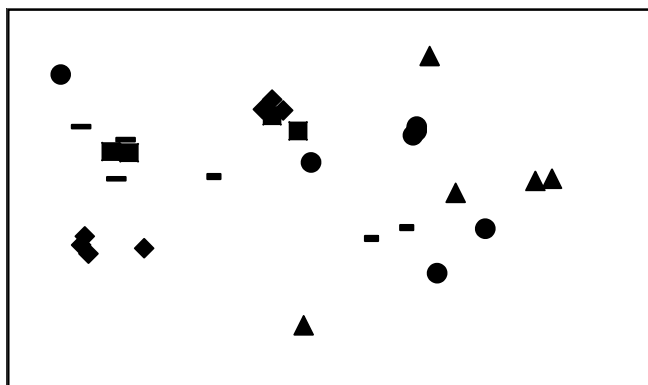


Figure 2. MDS of Bray-Curtis similarity values, based on sampling site, generated using T-RFLP data from soil samples collected in 2009.



February	●	May	—
March	▲	July	■
April	-	October	●

Figure 3. MDS of Bray-Curtis similarity values, based on sampling date, generated using T-RFLP data from soil samples collected in 2009.

the 2-dimensional graphs for simplicity of presentation. ANOSIM analysis of the Bray-Curtis matrix showed significant differences in bacterial community diversity between 2 of the 3 sampling sites when grouping across all dates. There was a strong significant difference between sites 1 and 3, with a significance level of 0.001 (Table 2). Sites 1 and 2 were also significantly different at a level of 0.022. Sites 2 and 3 were not statistically different from each other. The samples collected in February and October (regardless of site) also varied significantly from the samples collected in other months. The significance level of February versus March, April, July, and August ranged from 0.002-0.022. The difference between February and May was not significant, but only 3 samples were analyzed from May, which makes our statistical power low. The significance level between October and the other months ranged from 0.05 to 0.017, indicating a significant difference in community composition in October compared to all other sampling dates (Table 2).

Table 2. Statistical output from two-way crossed ANOSIM.

	R Statistic	Significance Level
<i>Global test (by site)</i>	0.975	0.001
Pairwise Test (by site)		
Site 1 vs. Site 2	1.0	0.022
Site 1 vs. Site 3	1.0	0.001
Site 2 vs. Site 3	0.75	0.111*
<i>Global test (by date)</i>	0.953	0.001
Pairwise tests (by date, only significant tests reported)		
Feb vs. Mar	1.0	0.022
Feb vs. April	1.0	0.022
Feb vs. May	1.0	0.067*
Feb vs. Jul	1.0	0.022
Feb vs. Oct	1.0	0.002
Oct vs. Mar	1.0	0.017
Oct vs. April	1.0	0.05
Oct vs. May	1.0	0.017
Oct vs. Jul	1.0	0.007

* Test not significant but data are reported for completeness of comparisons.

The significant difference between sites 2 and 3, as compared to the site at the top of the reach, supported our prediction that the beaver wetland left a legacy that could be quantified based on bacterial diversity. Water chemistry data indicated that site 3 contained approximately twice as much DOC as site 1. This suggests that the presence of a beaver wetland impacts the amount and type of organic matter in an area, resulting in larger pools of organic carbon, nitrogen, and phosphorus associated with the sediments. Site 1 had never been known to contain a beaver wetland and had lower levels of stream water DOC. Site 2 had DOC levels between those of sites 1 and 3, indicating the possibility that it may have once been a beaver wetland. Also, when researchers first began studying Payne Creek in 1973, they noticed alder vegetation at site 2, which was also indicative of a past beaver wetland.

We detected differences in community structure throughout the year, indicating that changes in environmental conditions may be influencing bacterial community dynamics. The difference between samples in February and those in other months was likely due to lower temperatures (Supplementary Table S1). Low temperatures possibly selected for species in the sediments that are adapted to colder temperatures, as the total number of fragments in the February samples were not different than the number found in other months. The differences found in October may have been created by an increase in fallen leaves or due to a recent large flood (Supplementary Table S1). The average discharge of Payne Creek in October was 3-15 times higher than any other sampling date. More organic matter was passing through Payne Creek during this time, much of which originated from the soils the runoff recently passed through. The DOC in the stream in October would likely have been of a different quality or bioavailability than that found during base flow periods, whether it came from freshly fallen leaves or floodwaters, stimulating different bacterial communities to grow. There is also a possibility that the reworking of the sediments during the flood prior to our October sampling brought new bacterial species, or that bacteria derived from soil water were a greater portion of the community due to the recent flood. We will be completing a large-scale sequencing effort to further

evaluate changes in community structure and the functional gene capabilities of these bacterial communities in light of the results presented here.

Conclusion

Based on our findings, we conclude that the presence of a beaver wetland leaves a lasting impact on the function and composition of bacterial communities in coastal plain stream ecosystems even after the wetland has drained. In the future, we will generate additional T-RFLPs for the various sites and dates to create a more robust and even data set. Sediment samples will continue to be collected from the sites to see if temporal patterns in bacterial communities we found in 2009 are consistent across years. The aim of this study is to understand differences in the way that carbon, nitrogen, and phosphorous are cycled through the ecosystem as a result of changes in the composition of bacterial communities.

References

- [1] Butler D & Malanson G. (2005). The geomorphic influences of beaver dams and failures of beaver dams. *Geomorphology*, 71:48-60.
- [2] Edmonds JW. (2009). Microbial Community Response to Seawater Amendment in Low-Salinity Tidal Sediments. *Microbial Ecology*, 58:558-568.
- [3] Horner-Devine MC & Bohanna BJM. (2006). Phylogenetic clustering and Overdispersion in Bacterial Communities. *Ecology*, 87:S100-S108.
- [4] Jakes AF, Snodgrass JW & Burger J. (2007). Castor Canadensis (Beaver) Impoundment Association with Geomorphology of Southeastern Streams. *Southeastern Naturalist*, 6:271-282.
- [5] Johnston CA & Naiman RJ. (1990). The use of geographic information system to analyze long term landscape alteration by beaver. *Landscape Ecology*, 4:5-19.
- [6] Luo J, Ma M, Liu C, Zha J & Wang Z. (2009). Impacts of particulate organic carbon and dissolved organic carbon on removal of polycyclic aromatic hydrocarbons, organochlorine pesticides, and

- nonylphenols in a wetland. *Journal of Soils and Sediments*, 9:180-187.
- [7] Mann C & Wetzel R. (1995). Dissolved organic carbon and its utilization in a riverine wetland ecosystem. *Biogeochemistry*, 31:99-120.
- [8] Mann C & Wetzel R. (1996). Loading and utilization of dissolved organic carbon from emergent macrophytes. *Aquatic Botany*, 53:61-72.
- [9] Margolis B, Castro M & Raesly R. (2001). The impact of beaver impoundments on the water chemistry of two Appalachian streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 58:2271-2283
- [10] Naiman RJ, Manning T & Johnston CA. (1991). Beaver population fluctuations and tropospheric methane emissions in boreal wetlands. *Biogeochemistry*, 12:1-15.
- [11] Snodgrass JW. (1997). Temporal and spatial dynamics of beaver-created patches as influenced by management practices in a south-eastern North American landscape. *Journal of Applied Ecology*, 34: 1043-1056.
- [12] Tockner K & Stanford J. (2002). Riverine Flood Plains: present state and future trends. *Environmental Conservation*, 29:308-330.
- [13] Ward DM. (2006). Microbial diversity in natural environments: focusing on fundamental questions. *Antonie van Leeuwenhoek*, 90:309-324.

Kaylan Gee is a sophomore from Irmo, South Carolina, majoring in microbiology and Spanish with minors in Computer-Based Honors and the Blount Undergraduate Initiative. She conducted her research for this paper in the lab of Dr. Jennifer Edmonds on microbial ecology. She has been honored as the recipient of a Computer-Based Honors Fellowship. Kaylan is the Vice President of Administration for her sorority, Kappa Alpha Theta, a Save First volunteer, and a Justice on the SGA Judicial Board.

Kirsten Ansorge is a sophomore from Hoover, Alabama, majoring in microbiology with minors in Computer-Based Honors and chemistry. She conducted her research for this paper with Dr. Jennifer Edmonds. She also works with Dr. Matthew Jenny of the Department of Biological Sciences using similar DNA analysis methods. Kirsten is a National Merit Finalist, Presidential Scholar, and a member of the Honors Program Student Association.