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Characterizing Bacterial Communities Across a Nutrient Gradient Over Winter Months

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ABSTRACT

When trying to understand how anthropogenic activities affect patterns in community ecology, bacterial communities are frequently neglected. These bacterial communities are essential to the overall health and stability of several different ecosystems. As nutrient pollution increases, some bacterial taxa may increase, causing an imbalance in food web dynamics and disrupting biogeochemical cycles. Next-generation sequencing coupled with water chemistry measurements has allowed for association testing between site water characteristics and bacterial taxa. By examining bacteria along a natural nutrient gradient over winter months in Overpeck Creek in northern New Jersey, this research shows that anthropogenic activities, specifically nutrient pollution, can alter bacterial diversity and taxa composition. These findings indicate that Overpeck Creek suffers from extensive nutrient pollution and that winter bacteria are altered more by nitrogen and carbon than phosphorus pollution. Extended work needs to be conducted to understand if seasonal changes in bacterial diversity patterns are moderated by nutrient pollution in Overpeck Creek.

MONTCLAIR STATE UNIVERSITY

CHARACTERIZING BACTERIAL COMMUNITIES ACROSS A NUTRIENT GRADIENT
OVER WINTER MONTHS

by

EMILY JIN SEARLES STONE

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

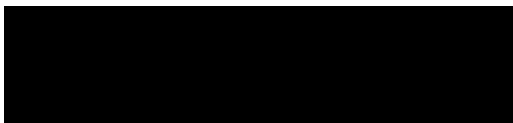
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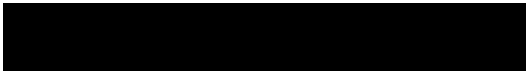
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OVER WINTER MONTHS

A THESIS

Submitted in partial fulfillment of the requirements

For the degree of Master of Science

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EMILY JIN SEARLES STONE

Montclair State University

Montclair, NJ

2021

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INTRODUCTION

Freshwater ecosystems house diverse communities and are valuable in providing ecosystem services (Kumaraswamy et al. 2020). Ecosystem services that fresh waters provide include decomposition and cycling of nutrients, carbon sequestration, local climate regulation, and natural flood protection (Wilson and Carpenter 1999). These services can determine ecosystem health, supporting productivity, and overall functionality. Productivity is the foundation of environmental functionality. Functionality, the process of any physical, chemical, or biological translocation of energy across an ecosystem, contributes to biodiversity. However, anthropogenic activity and industrialization often disrupt ecosystem functions, productivity, and ecosystem services (Znachor et al. 2020).

Water pollution is still a significant issue in the United States. For decades the United States used watersheds and wetlands as a suitable place to deposit harsh chemicals, minerals, toxic materials, and excess waste products (Griffith et al. 2010). Point and non-point sources of pollution have led to the deposition of excess nutrients in aquatic systems. Increased nutrient pollution is largely due to urbanization and anthropogenic activities such as modern agricultural practices (Kumaraswamy et al. 2020; Ponader et al. 2007). Excess nutrients can be associated with or used by disease-causing prokaryotes that enter freshwater systems through fecal pollution, increasing disease risks for humans who use these water sources (Donovan et al. 2008; Lee et al. 2014). In particular, the Musconetcong River watershed was shown to violate New Jersey water standards because of fecal contamination (David Hsu et al. 2019). Another freshwater system in northern New Jersey, the Passaic River, has been polluted for decades, causing biodiversity to diminish and harmful bacteria to increase (Donovan et al. 2008, Nie et al. 2018). The Pompton River has a high risk of being contaminated by fecal indicator bacteria, even though there is less land use

surrounding the river compared to the Passaic River (Rossi et al. 2020). Regulating nutrient pollution and maintaining freshwater ecosystems has led to better conservation, management and overall helped maintain sustainable levels of biodiversity while minimizing harmful and pathogenic bacterial populations.

Bacterial communities are the foundation for creating a healthy freshwater system. One of the primary ways bacteria contribute to ecosystems is through regulating biogeochemical cycles (Horner-Devine et al. 2003). The nitrogen cycle is a complex cycle that involves several different kinds of bacteria. Nitrogen-fixing bacteria create ammonia from atmospheric nitrogen that is either used by bacteria or further converted into nitrites by nitrifying bacteria. Nitrites can then be converted into nitrates by bacteria before denitrifying bacteria create atmospheric nitrogen to restart the cycle. Bacteria that are carrying out these functions allow for higher ecosystem functioning.

In freshwater systems, an excessive increase in nitrogen can have detrimental effects. Nutrient pollution from synthetic fertilizers has increased in freshwater systems causing eutrophic conditions to occur more frequently (Horner-Devine et al. 2003). In addition, human activities have increased the amount of available nitrogen in systems by 33-35% (Nogales et al. 2010). Nitrogen often contributes to eutrophication and over-enrichment in coastal waters, whereas phosphorous is the primary driver of eutrophic conditions in freshwater systems (Howarth et al. 2000). How plants and aquatic fauna respond to these conditions is widely understood, but there is a lack of understanding of how bacterial taxa respond; this topic may prove to be an important area to study since these organisms are necessary for biogeochemical cycling (Horner-Devine et al. 2003).

A study conducted by Horner-Devine and others (2003) aimed to understand how productivity rates of algae influenced the composition and richness of bacterial communities. Altering primary productivity by changing nitrogen and phosphorous levels in mesocosms, researchers were able to show that bacterial abundance increased with the primary productivity of the algae. The study showed that bacterial richness can correspond with fluctuations in primary productivity, similar to the algal richness, and can shift between different taxonomic groupings (Horner-Devine et al 2003). Although informative, laboratory studies have clear disadvantages. It is difficult to generalize the results to other situations. Field studies are needed to verify findings of laboratory studies and to expand previous inferences at multiple field sites. A more realistic observation of how these organisms respond to nutrient pollution should include samples taken along a naturally occurring nutrient gradient where water quality is investigated (Jiao et al. 2021). Yet, natural systems often exhibit extreme variation in environmental conditions over weeks and months, and seasons, requiring high-frequency long-term sampling and making investigations of bacterial communities difficult and cost-prohibitive.

To overcome these issues, researchers use next-generation sequencing and conduct metagenomic studies. The use of genomic sequencing allows for a more conclusive characterization of microbial communities (Brooks et al. 2015). Studies typically analyze the prokaryotic 16S ribosomal RNA gene (16S rRNA), which comprises nine different variable regions. These variable regions provide the highest possible discriminating power since the V2, V3, and V6 sites have the maximum available heterogeneity (Shah et al. 2011). As a result, 16S rRNA analyses can better describe bacterial communities than culture-dependent studies. In addition, the gene is capable of describing otherwise rarely isolated or phenotypically uncommon strains and can lead to recognizing novel pathogens and unculturable bacteria (Clarridge 2004).

Overall, genomic sequencing allows researchers to study community structures, phylogenetic composition, diversity, and metabolic capacity from the 16S gene (Shah et al. 2011).

Overpeck Creek in Bergen County, New Jersey, provides a natural nutrient gradient to study bacterial communities. The concentration of nutrients varies from north to south due to increased wastewater and fertilizer contamination farther south in the creek. To create a foundation to examine bacteria in Overpeck Creek, New Jersey, five different sites situated along the freshwater system were chosen based on the accessibility. The site directly across from the selected source of nutrient pollution Overpeck Golf Course is predicted to have high order abundance. The influx of nutrients should provide otherwise limiting resources for numerous bacterial groups. Therefore, increased nutrients will likely play a large role in determining the bacterial taxa present. In addition, the site has several free-floating particulate debris; proteobacteria are often associated with debris, adhering to the surfaces and colonizing the material (Zhang et al. 2007). Locations above Overpeck Golf Course will likely have fewer prokaryotes associated with nitrogen and phosphorous nutrient pollution but may contain bacterial communities often associated with increased salinity since the road density within the watershed is highest in the northern reaches of the stream. This study was conducted in the winter and aims to understand how bacteria communities change across pollution gradients over winter months and understand the associations between bacterial taxa, nutrients, and road salts.

METHODOLOGY

FIELD SITES

Overpeck Creek, a tributary of the Hackensack River, is a freshwater system in Bergen County, New Jersey. The creek is considered a freshwater non-trout, saline estuarine (FW2-NT/SE2) body of water. For these bodies of water, the New Jersey Department of Environmental

Protection (NJDEP) declares that the distinction between the fresh and saline waters is determined by salinity measurements, where freshwater are the sections that have less than or equal to 3.5 ppt salinity during high tide. The waters of Overpeck Creek change from freshwater to saline water headwaters to downstream. The creek is sourced by precipitation, where runoff enters Overpeck Creek and partial leaching from surrounding terrain. This creek has a watershed that begins at the northern New York-New Jersey border, runs through Tenafly, Englewood, and Bergenfield, New Jersey, and connects to the Hackensack River. Like many of the streams in northern New Jersey, Overpeck Creek has been exposed to high levels of contamination over the past two decades resulting in increased nitrogen, carbon, and phosphorus concentrations and increased salinity.

Along Overpeck Creek, there are several parks, interstate highways, and residential and municipal roads, all of which contribute to increased pollution. A road's impact on a system depends on several factors: the amount of vehicle traffic occurring on the roadway, the slope of the road, and the density of surrounding roadways (Schuler and Relyea 2018). Pollutants such as heavy metals from car parts and salts used for deicing (e.g., sodium chloride) flow off roads during rainfall or snow melt events and can enter a stream relatively quickly in urban areas. These pollutants enter surface waters such as streams instead of entering groundwater systems due to the high density of impermeable surfaces in urban areas. Heavy metal contamination and road salt influence are likely to impact the chemical properties of the water, which could impact the bacteria in the water. In Overpeck Creek, the northern parts of the stream are surrounded by roads, and there are more green areas and parks at the southern end of the stream. In the regions where there are more parks, the concentration of road contaminants is likely decreased due to the influx of freshwater from grassy and forested areas. However, the stream also runs directly through a golf course before emptying into a larger area. Runoff and leaching from golf courses contribute to

high nutrient pollution. Based on their proximity to the source of nutrient pollution and ease of access, sites were selected accordingly.

The five sites for this study are North Englewood, MacKay Ice Rink, Dinosaur Overpeck Park, South Overpeck Park, and McGown Park. **Figure 1** provides an overview of site locations, characteristics, and proximity to nearby interstate highways along Overpeck Creek. The Dinosaur site is located across the golf course, while the South Park location is 700m south. Because the dinosaur site is straight across from Overpeck Golf Course, the nutrient pollution source, it has been considered “highly impacted” by nutrient pollution. The South Park site is presumed to be under fewer nutrient influences since it is further from the nutrient pollution source. These sites are located in Overpeck Creek Park, where a large Canada goose population resides during the winter months. These geese can spread pathogenic bacteria such as *Listeria*. MacKay ice rink is north of Overpeck Golf Course, making it a site with minimal nutrient impact but having relatively higher salinity levels. MacKay Ice Rink is found in a residential area with multiple storm drains emptying into the freshwater system. Over the winter months, this area receives an adequate amount of road salt to prevent ice from accumulating on nearby roads. As snow and ice melt, any road salt that dissolved can runoff into nearby water systems (Lind et al. 2018). McGowan is the southernmost site closest to the Hackensack River and is subject to strong tidal influences and the highest salinities. Being in such proximity to the Hackensack River, tidal influence is likely to affect the bacterial communities (Campbell and Kirchman 2013; Denaro et al. 2005; Nogales et al. 2007). The northernmost site in Englewood, New Jersey, served as the control site. This site is believed to be the least impacted by nutrient pollution and likely has a lower influence from salt pollution than the more southern sites.

SAMPLE COLLECTION

Samples were collected on two different occasions, December 2020 and January 2021. 2,500mL was taken from the surface water at the field sites by partially submerging a sterilized bucket below the water surface. Taking surface water not only allows for straightforward retrieval of samples but also makes the study easily replicable. A 250mL bottle with deionized water was opened and used as a control at each site. At each field site, personal protective equipment was changed, and new sterile containers were used.

December samples were taken a day after multiple days of rainfall, averaging 0.16cm of precipitation daily. January samples had slightly higher rainfall averaging 0.66cm daily before sampling. Water temperatures were the same for sites at Overpeck Park at 0.89°C. Further north, MacKay Ice Rink had a temperature of 7.55°C, and the North Englewood site had a temperature of 7.72°C. McGowan Park, which has some tidal influence, temped at 7.0°C. Temperatures differed from the first set of samples, with the second set of samples temping at 3.0°, 4.7°, 6.7°, 6.7°, and 4.2°C.

Until the samples could be filtered, they were frozen in the lab. 500mL of water was filtered from each site and broken down into two sampling efforts, 250mL each. Under a certified sterile hood, I used sterile 0.22 um cellulose nitrate (CN) filters in sterile collection containers to collect the bacteria from each water sample. Upon completion, I placed each filter into a Qiagen® bead tube. A total of 22 bacterial samples were filtered for DNA extraction and amplification. The remaining liter of water was used to quantify the chemical aspects of Overpeck Creek using a spectrophotometer. An additional positive sample inoculated with Firmicutes was included to ensure proper DNA amplification and sequencing took place.

DNA EXTRACTION & AMPLIFICATION

DNA extraction followed the Qiagen® DNeasy PowerWater Kit protocol closely, excluding the time the filters were steeped in the lysing solution. Once the lysing solution was added, I set the bead tubes in a heated bead bath for 24hr. The CN filters left in bead tubes for an extended period potentially allowed more bacterial cells to lyse, giving a higher DNA yield.

Before being run through PCR, samples were stored at 5°C. PCR amplification followed standard protocol and was conducted by Adam Parker, the technical specialist of the Montclair State University Biology Department. Primers used were given Illumina adapter overhang sequences to create full-length primer sequences. The primers used can be found in **Table 1** below. These primers have been shown to cover a more significant percentage of Bacterial taxa than other primer sets in Klindworth's and other's 2012 study, which compared various primer sets to one another. Upon completion, samples were examined at the hypervariable V3 region. By analyzing samples at the V3 region, it is possible to get more precise taxonomic classifications (Bukin et al. 2019). The 16S rRNA marker is the standard approach when investigating microbial diversity (Bukin et al. 2019; Klindworth et al. 2012). To maximize sequence output, I used a 16s primer kit from Illumina.

Illumina MiSeq is capable of performing genomic DNA sequencing and data analyses of microbial communities. Successfully characterizing community composition is dependent upon primer selection and creating little to no bias. Primer choice is arguably the most critical step when doing culture-independent community composition studies. Using a suboptimal primer set can cause gaps in analysis, which can omit taxa or entire clades, resulting in inconclusive results (Klindworth et al. 2012). The primers chosen for this project employed Klindworth's and other's primer set from their 2012 study. The primers bind to the oligonucleotides, the sequencing binding site, where corresponding nucleotides pair with the DNA. Sequences are produced by these

matches before being overlaid to a reference genome. When completed, data analysis can take place where bioinformatic procedures are necessary.

QIIME programming was used to obtain bacteria taxa from the sequences. This bioinformatics program is designed to provide information from given 16S rRNA sequences along with statistical data. To use QIIME, I used a standardized QIIME pipeline, developed by Dean Bobo, a Research Associate in the Biology Department at Montclair State University. The 70% similarity, the default for identification, was applied for the pipeline.

WATER ANALYSIS

To understand differences in water chemistry among the sites, I used a calibrated Hach DR6000 spectrophotometer. Water characteristics have a significant impact on what bacterial taxa may be present.

To better understand the characteristics of field sites, several different chemical tests were carried out. Each test followed the given Hach standardized protocols. The tests conducted included: chloride testing using the mercuric thiocyanate method (TNT 879), heavy metal testing for nickel, fluoride, and copper, the dimethylglyoxime method (TNT 856), USEPA SPADNS method¹ (TNT 878), and porphyrin method¹, persulfate digestion method (TNT826) that calculates total nitrogen, ascorbic acid method (TNT 843) that is used to quantify phosphorous, turbidimetric method¹ (TNT 865) which looks at sulfate levels, USEPA¹ direct method (TNT 810) that examined total organic carbon, metal phthalein colorimetric method (TNT 869), that tested for water hardness and also provided magnesium and calcium ions, and heteropoly blue method for silica. The objective of measuring water chemistry is to understand how each parameter determines the bacteria present, the number of sequences detected (a proxy for bacterial abundance), and bacterial richness (number of bacterial orders). Prokaryote phyla were also considered for the study.

I used a generalized linear model (GLM) with a normal distribution to understand how each water quality parameter affected order richness. Data that did not fit the assumptions of generalized linear model tests were normalized by taking the square root of the data. Order composition (the types of orders present) is an important indicator of functional differences among sites. To determine the drivers of compositional differences among the sites in Overpeck Creek, I used the measured water chemistry values and Bray-Curtis Dissimilarity values to conduct a Permutation ANOVA (PERMANOVA). I conducted the PERMANOVA in R using the *vegan* community ecology package 2.5-7. Compositional dissimilarity was calculated using Bray-Curtis dissimilarities from the *vegan* package. The program, Tableau® 64-bit version, was used to create **Figure 2** and **Figure 3**.

RESULTS

The compositional differences among sites in Overpeck Creek were primarily explained by the concentration of Total Dissolved Organic Carbon (TDOC) ($p=0.005$). Temperature and nutrient concentration did not significantly explain prokaryotic compositional differences among sites in Overpeck Creek ($p>0.05$). Archaea were detected at very few sites. The Phyla *Euryarchaeota* and *Nanoarchaeota* characterized the Dinosaur Overpeck park site. McGowan Park had also had *Nanoarchaeota* present in the samples from December. *Methanobacterium* spp. of phylum *Euryarchaeota* was found at the highly impacted site in December. *Candidatus paearchaeota*, *Nanoarchaeota archaeon*, and *Woesearchaeales* spp., from phylum *Nanoarchaeota*, were also found in December samples. Other than these two sites, no other sites had detected any organisms from Archaea, including the North Englewood site. However, this site, along with several other sites, observed *Firmicutes. Proteobacteria* from Domain Bacteria were found across sites (**Figure 2**)

Burkholderiales of Class *Gammaproteobacteria* was the most prominent in four of the sites. In the southernmost site McGowan Park, many of the organisms from *Burkholderiales* came from *Alcaligenaceae*, a family that harbors GKS98, a freshwater prokaryote. Other families of *Burkholderiales* discovered at McGowan Park included: *Aquaspirillaceae*, *Burkholderiaceae*, *Comamonadaceae*, *Hydrogenophilaceae*, *Methylophilaceae*, *Neisseriaceae*, *Nitrosomonadaceae*, *Oxalobacteraceae*, *Rhodocyclaceae*, and *Sutterellaceae*. The orders of Class *Alphaproteobacteria* contributing the most were *Rhizobiales* and *Rhodobacterales*. Again, the highly impacted site Dinosaur Overpeck Park and McGowan Park had the highest reads among all the sites. There were not many *Cyanobacteria* present across locations.

Cyanobacteria orders *Chloroplast* and *Synechococcales* were mainly discovered at McGowan Park, followed by the Dinosaur Overpeck Site. There were only three reads from *Cyanobacteria* at the North Englewood site. *Cyanobacteria* of the *Chloroplast* order found at the highly impacted site included: *Chlorophyta* symbionts, *Chroomonas coerulea*, *Chrysochromulina* spp., *Euglena viridis*, *Neotessella volvocina*, *Trachydiscus minutus*, and *Tupiella akineta*. *Phascus applanatus*, *Planctonema lauterbornii*, *Synura peterseni*, *T. minutus*, and several uncultured *Cryptomonadaceae* characterized McGowan Park.

Other prokaryotes of interest include genera *Cloacibacterium* and *Listeria* and members of Order *Pseudomonadales*. *Cloacibacterium*, a *Flavobacteriales*, was most prominent at McGowan Park during December. During the same sampling period, *Listeria* was found at Overpeck Creek at the South Park location. *Pseudomonadales* included *Acinetobacter bohemicus*, *A. celticus*, and *A. harbinensis*, all of which were discovered at McGowan Park and the Dinosaur site. *Gracilibacteria*, a wastewater bacterium of phylum *Patescibacteria*, was found in both sampling periods for McGowan Park and in January at Dinosaur Overpeck Park.

Filtered controls found *Escherichia shigella*, a *Gammaproteobacteria* of Phylum *Proteobacteria*. The additional positive sample, along with a few site samples, also detected *E. shigella*.

The McGowan Park and Dinosaur Overpeck Park sites had the highest diversity values of all the sites (**Table 4, Figure 3**). December samples from McGowan Park had the highest abundance with 39,733 individuals recorded, followed by the highly impacted site in January.

Total dissolved nitrogen and TDOC contributed to changes in order richness across sites (**Table 2**). As total dissolved nitrogen and TDOC increased, so did the number of bacterial orders (**Figure 4**), whereas the number of bacterial orders decreased as dissolved silica concentration increased (**Figure 5**).

There was no significant association explaining diversity among sites ($P > 0.05$). Water chemistry analysis indicated that fluoride, sulfate, and nickel were under the detectable limit in most sites. Only McGowan Park had reads within range for the December sampling period. The Dinosaur site had the highest TDOC available. It also had the highest value of total dissolved nitrogen in January compared to other sites. North Englewood in December had the lowest total dissolved organic carbon available for bacteria to utilize and had very few *Burkholderiales* present. Phosphorous had the highest detectable value in January at the South Park location, 700m away from Overpeck Golf Course, the area of interest for nutrient pollution for this study. Although this point source of pollution has been the focal point of the study, several other sources of pollution may have contributed to community composition. Runoff is particularly a critical non-point pollution source that can alter bacterial communities in freshwater systems. All of the surrounding roadways in Overpeck Creek's watershed serve as a non-point pollution source, particularly during

rainfall, where stormwater contaminated with heavy metals, salts, and nutrients can enter the river (Schuler and Relyea 2018).

Little copper was found in the samples. A complete list of water characterization from all sites is summarized below (**Table 3**).

DISCUSSION

Pollution in freshwater ecosystems is a significant threat affecting biota universally (Kumaraswamy et al. 2020). Bacterial communities are arguably more sensitive to ecological changes since these organisms have faster growth rates and can respond to lower levels of pollutants quicker than other larger organisms (Denaro et al. 2005). Nitrogen and phosphorous increase in aquatic systems because of agricultural practices, discharged wastewater, animal wastes, and residential fertilizer runoff (Drury et al. 2013; Kumaraswamy et al. 2020; Liu et al. 2017; Ponader et al. 2007; Saarenheimo et al. 2017). These changes in water quality affect bacterial communities present. Increased nutrients, hydrocarbons, and sodium chloride have favored proteobacteria (Bouvier and del Giorgio 2002; Denaro et al. 2005; Pecher et 2019). Higher salinity levels often select alpha-proteobacteria more than beta-proteobacteria (Bouvier and del Giorgio 2002; Campbell and Kirchman 2013).

Analyzing community composition through sequencing 16S rRNA and creating a library for comparison is the standard approach for understanding microbial diversity (Klindworth et al. 2012). The alternative method is to use a culture-based approach. A culture-based approach was not used because a limited number of bacterial taxa can be cultivated in a laboratory setting (Horner-Devine et al. 2003). Furthermore, recreating the natural community structure under laboratory settings is challenging. Therefore, it is easier and more reliable to study community composition using genomics (Festa et al. 2017). However, there were not enough sampling efforts

to adequately produce realistic results. Additional sampling efforts are needed to characterize bacterial communities found in Overpeck Creek in its entirety. Numerous potential pathogens were found at the sites (e.g., *Listeria*, *Cloacibacterium*, *Acinetobacter* spp. of the *Pseudomonadales*, *E. shigella*) even though there were minimal sampling efforts.

Medical professionals consider *Acinetobacter* species to be core pathogens, being the root cause of a range of infections (Adewoyin and Okoh 2018). This genus has been found in several different aquatic environments such as streams, aquaculture farms, and heavily in wastewater (Adewoyin and Okoh 2018). Sewage can also contain the bacteria, *Cloacibacterium* and *Gracilibacteria*, which were found in the study. Thus, wastewater is arguably a critical component in forming the different bacterial communities found in Overpeck Creek.

A wastewater treatment plant is located along Overpeck Creek, and experiences combined sewage overflow on occasion. Two discharging pipes are close to McGowan Park, 65m and 320m away. Wastewater plants such as these contribute additional nutrients to aquatic systems and can potentially introduce new bacteria to the river (Saarenheimo et al. 2017). For example, Saarenheimo and colleagues (2017) were able to find *Bacteroidetes*, *Firmicutes*, and *Gracilibacteria* in two different discharged wastewaters. These three taxa were present in Overpeck Creek during the time this project was being carried out. All sites during the first set of sampling detected *Firmicutes*. *Gracilibacteria* and *Bacteroidetes* were only found in January for the Overpeck sites. McGowan Park contained the highest variety of sequences of *Gracilibacteria*, detecting the uncultured genus *Absconditabacteriales* (SR1). Similar studies also confirm that these groups are prominent in freshwater systems with a wastewater treatment plant nearby (Behnami et al. 2018; Drury et al. 2013; Liu et al. 2017). Therefore, continuing to monitor Overpeck Creek will provide insights into whether these taxa can persist in the environment.

Future studies should look into sampling closer to the point sources of pollution to determine if the taxa come from the combined sewage overflow.

It is unlikely that the *Pseudomonadales* originated from an animal source (Adewoyin and Okoh 2018) like *Listeria*, often in the feces of deer and birds (Lyautey et al. 2007; Weis and Seeliger 1975). Birds can distribute the pathogen across sites, yet it was only detected in one location, South Park, during the first sampling set. *Listeria* species are found in natural and urban settings and have a significant relationship between pH and seasonality (Orsi and Weidmann 2016). Linke and others (2014) point out that wastewater effluent, fertilized fields, wildlife, and watercourses contribute to *Listeria* in low-lying areas. Seeing how deer and birds are more active in the fall and winter seasons, sampling during different periods may show that *Listeria* is only present during colder months. *Listeria* may only be present due to the large Canada goose populations that use Overpeck Park during migrations. While only two of the seventeen *Listeria* species are considered pathogenic, further investigations on *Listeria* in Overpeck Creek should be observant when sampling.

It was hypothesized that the highly impacted site, the Dinosaur Overpeck site, was to have high abundance compared to other locations due to nitrogen and phosphorous inputs. However, phosphorous did not play a significant part in determining richness or abundance. Compared to nitrogen, phosphorous is not as biologically accessible. Nitrogen can be obtained through several different organic compounds and from the atmosphere, whereas phosphorous is found within rocks and sediment and is typically the more limiting nutrient in freshwater environments. This makes it difficult for organisms to use phosphorous and is a more limiting resource than nitrogen in freshwater systems. Phosphorous levels in Overpeck Creek are exceedingly higher than typical

freshwater systems and violates the threshold put forth by the New Jersey Department of Environmental Protection of 0.1mg/L.

According to the NJDEP's 7:9 water quality criteria, which was last amended in April of 2020, nutrients, excluding those inputted by natural conditions, cannot exceed concentrations that would cause changes in pH indicative of excessive photosynthetic activity, create unsuitable water quality through algal densities, form diurnal fluctuations in dissolved oxygen, produce detrimental changes to aquatic ecosystems, or cause other indicators of use impairment caused by nutrients. Because phosphorous levels are so high, this limiting resource may have shifted nutrient use by the microbes so that nitrogen became a more limiting resource. This change would explain why nitrogen has a more significant relationship in determining the order richness seen across sites.

Water hardness, dissolved silica, and TDOC contributed to the orders present across sites. Dinosaur Overpeck did, however, have high abundance as predicted. McGowan Park ultimately had the highest recorded abundance at 39,733. Even so, the highly impacted site consistently showed high abundance during both sampling periods, unlike McGowan that had a difference of 36,434 during the second sampling effort. The extreme contrast between the two sampling periods may have been caused by tidal influence. Tidal reports were taken from NOAA. With the changing tides, salinity levels also change. The first sampling effort taken in December was performed as the tide was nearly at its lowest point of 0.37m that day. The second sampling in January was taken at high tide, where the water level raised 1.64m. The high tide may have shifted the bacterial community and reduced the number of bacteria able to withstand the increased salinity level. A comprehensive list of richness and abundance can be found below (**Table 4**).

Silica concentrations were the highest at the North Englewood site. Silica explained the differences in richness across sites. Very few taxa and low abundance were detected at the North

Englewood site. Many of the genera at this site consisted of *Proteobacteria*. *Burkholderiaceae*, *Diplorickettsiaceae*, and *Enterobacteriaceae* of *Gammaproteobacteria*, and *Sphingomonadaceae* of *Alphaproteobacteria* provided the most individuals detected.

No *Cyanobacteria* were present at the MacKay ice rink, contrary to what was predicted. Like *Listeria*, a seasonality factor exists for *Cyanobacteria* (Bertos-Fortis et al. 2016). Sampling during the winter, when temperatures are lower, likely played a part in preventing optimal growing conditions.

The tidal influence was expected to influence the prokaryotes found at McGowan Park. Many of the prokaryotes found at this site were from *Burkholderiales*, a *Gammaproteobacteria* known for having high nutritional versatility, being able to use various organic compounds to source carbon (Festa et al. 2017). Because *Burkholderiales* have shown to be diverse in utilizing different sources for carbon, this most likely explains why this group was found across sites and in such high numbers. Additionally, *Burkholderiales* are more varied genetically than other clades, alluding to the ecological diversity in this group (Festa et al. 2017).

Nogales and others (2007) found that higher pollution levels from hydrocarbons favored *Gammaproteobacteria*, while the relative abundance of *Alphaproteobacteria* and *Cyanobacteria* decreased. There was a higher percentage of *Gammaproteobacteria* found compared to *Alphaproteobacteria*. 23%-28% of taxa detected in samples from McGowan consisted of *Gammaproteobacteria*, whereas *Alphaproteobacteria* only made up between 6%-10%. *Cyanobacteria* only contributed 2%-6% of the taxa.

Based on Nogales and colleagues' (2007) findings, hydrocarbons could have influenced community composition. *Burkholderiales*, the most prominent group found at the McGowan site, are known to be found in several different ecosystems due to their ability to source carbon from

various organic compounds (Festa et al. 2017). A generating station that uses kerosene, a mixture of hydrocarbons, is found 650m upstream from McGowan Park. A more thorough investigation is needed to confirm how hydrocarbons contribute to the bacterial communities found in Overpeck Creek. Several roadways also surround McGowan Park and the other sites, where heavy metals pollution can be caught up in stormwater runoff.

The surrounding roadways likely had hydrocarbons that ran off into Overpeck Creek. Oil and PAH enter the environment through poor automotive maintenance and automobile emissions (Wada et al. 2015). These pollutants accumulate on pavement and are washed into the environment creating non-point source pollution (Opher et al. 2009). Several different models allude to annual average daily traffic being the most influential factor for runoff pollution followed by rainfall events (Opher et al. 2009). Before sampling, there were periods of rainfall which most likely washed these pollutants into Overpeck Creek.

Many factors might have caused bias in this study. Several different reasons may have caused field bias: proximity to the shoreline, depth of sample collection, sampling effort, the distance between sites, the quantity of water filtered, rainfall before sampling, and sampling season all contribute to the taxa detected. Sampling close to the shoreline increases the chances of detecting soil biota. When rainfall is involved, the issue is compounded. Rossi and others (2020) argue that rain alters the density of fecal indicator bacteria present since new environmental inputs from storm runoff affect them. Between the northernmost and southernmost sites, four discharge points release a mixture of storm runoff directly onto the surface water of the river. Therefore, it is possible that the prokaryotes detected in this study presented here came from soil biota and were not present in the freshwater system before sampling.

Any prokaryotes that entered the creek from the shoreline are more likely to be at the water surface before dispersing. A sampling at different depths may prove to show a completely different community composition. For example, microbes that enter the system by a discharge point would likely be found beneath the surface water. Similarly, bacteria that reside on the substrate or vegetation can only be obtained by sampling further into the water column. By only sampling at the surface, any prokaryotes that exist elsewhere in the water column are omitted by default.

Temperature is often responsible for determining bacteria community composition. The temperature has been shown numerous times to affect prokaryotes in several studies (Bachran et al. 2018; Bouvier and del Giorgio 2002; Nogales et al. 2007; Nogales et al. 2010). Lower temperatures during the winter months likely affected community composition. Having only two sampling efforts also truncates the list of possible prokaryotes in the system. Increasing sampling efforts over an extended period may provide a more comprehensive representation of diversity, abundance, and richness. The small window for sampling illustrates the limitations of the study presented here.

Sampling during warmer seasons will likely change the taxa found in Overpeck Creek and the richness. Because temperature is a principal component in developing bacterial communities (Bouvier and de Giogrio 2002; Nogales et al. 2007), warmer months may show completely different taxa in Overpeck Creek. In addition, during these warmer months, there will likely be a substantial increase in fertilizer usage at the Overpeck Golf Course, affectively altering the bacteria present at both the Dinosaur and South Overpeck Park locations. However, these are just speculations and will need to be thoroughly investigated before any conclusive statements can be made.

Laboratory and metagenomic bias are also highly possible. Even though metagenomics is capable of discovering otherwise undetectable or unculturable prokaryotes, bias can still occur. For instance, the use of inappropriate primer pairs can lead to questionable conclusions (Klindworth et al 2012). PCR amplification, DNA extraction, and primer selection and design can all contribute to bias (Brooks et al. 2015). Brooks and others (2015) suggest that reducing how many PCR cycles occur will help avoid chimera formation; extracting DNA in triplicate and using various DNA extraction combinations will also help mitigate bias.

Correctly detecting microbes is essential when trying to characterize different communities. It is crucial to understand and evaluate how bacterial communities change over different localities and times. Findings show that more work needs to be conducted over an extended period but provides a foundation for examining bacteria in Overpeck Creek in northern New Jersey.

CONCLUSION

Monitoring changes in nutrient concentration is complex because of the periodic input from point and non-point pollution sources (Ponader et al. 2007). The only way to work around this issue is to sample and develop a reliable framework as a baseline. Conducting a prolonged study may prove to yield more significant results than the ones found here. Long-term studies are valuable as a resource; surveying an area for an extended period allows for a thorough investigation of changing environmental conditions and how these conditions shape diversity (Znachor et al. 2020).

Another focus should be on understanding how various pollutants enter freshwater systems and how these pollutants shape favorable charismatic organisms and ecologically important bacterial communities (Kumaraswamy et al. 2020). While nutrient pollution is a focal point of

this study, issues surrounding Overpeck Creek go beyond that. Seeing as numerous bacteria taxa present associated with wastewater in the limited number of samplings, fecal indicator bacteria and pathogenic microbes may be more of an issue than previously thought. However, few sampling efforts have made it difficult to state that the results truly represent bacterial taxa present confidently.

However, this study provides a foundation to build upon; continuing to sample the area over extended periods will help document changing aquatic conditions due to changes in anthropogenic activity and allow potential monitoring of pathogens found in Overpeck Creek.

FIGURES

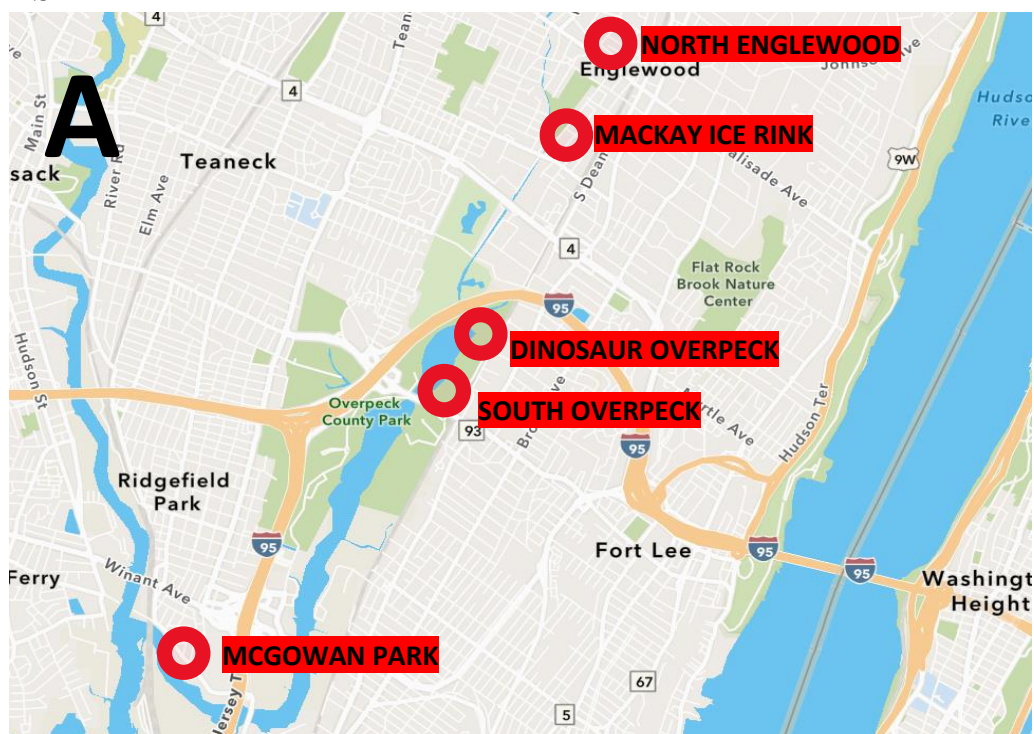


FIGURE 1: Site locations with proximity to major interstate highways. North Englewood: Site with minimal salt and nutrient pollution. MacKay Ice Rink: Surrounded by residential roadways and a parking lot, several storm drains discharge into the river. Dinosaur Overpeck Park: Highly impacted site from nutrient pollution, large particulate matter. South Overpeck Park: 700m south from the highly impacted site, presumed to be under lower nutrient influence. McGowan Park: Southern-most site experience tidal influence from the Hackensack River

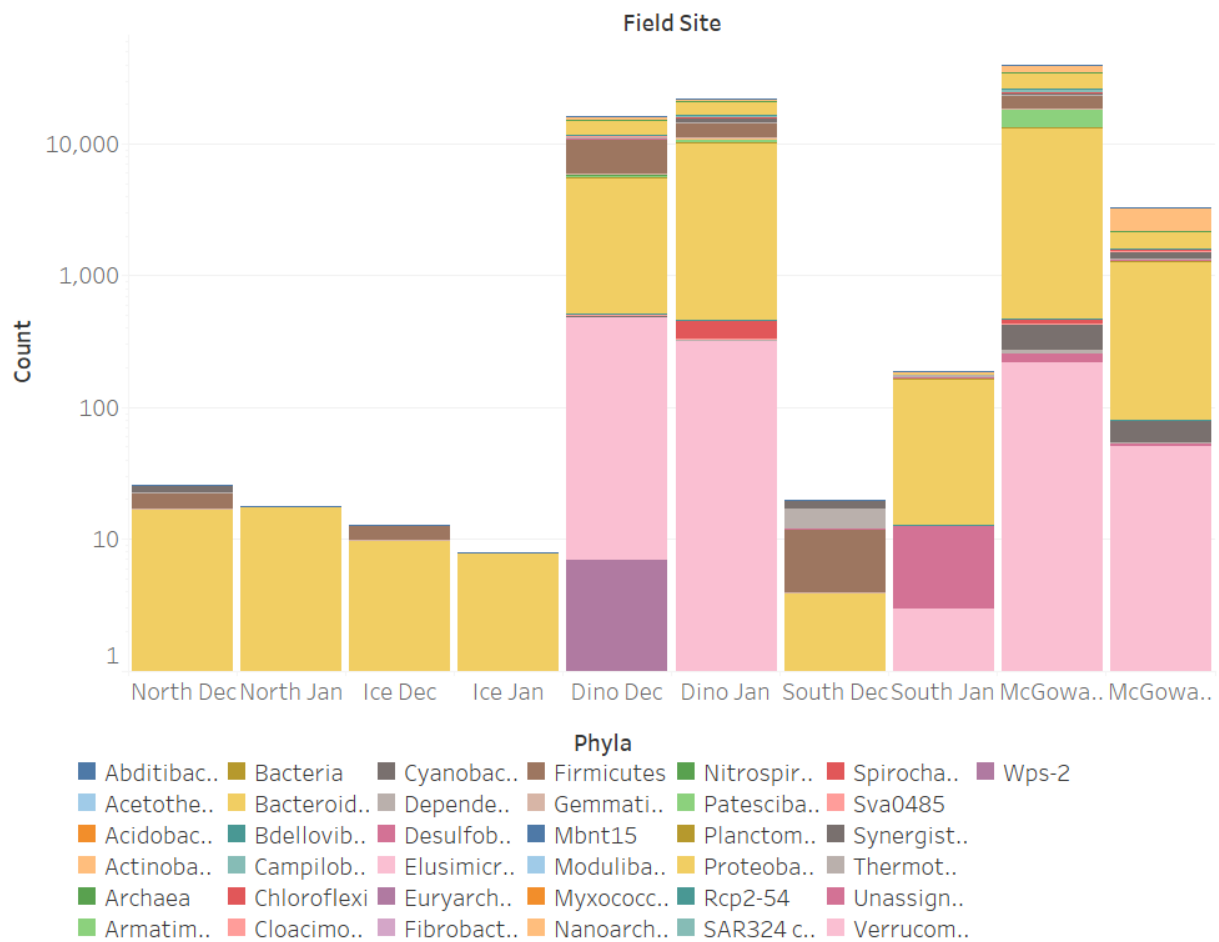


FIGURE 2: Different phyla found at each of the field sites on a logarithmic scale.

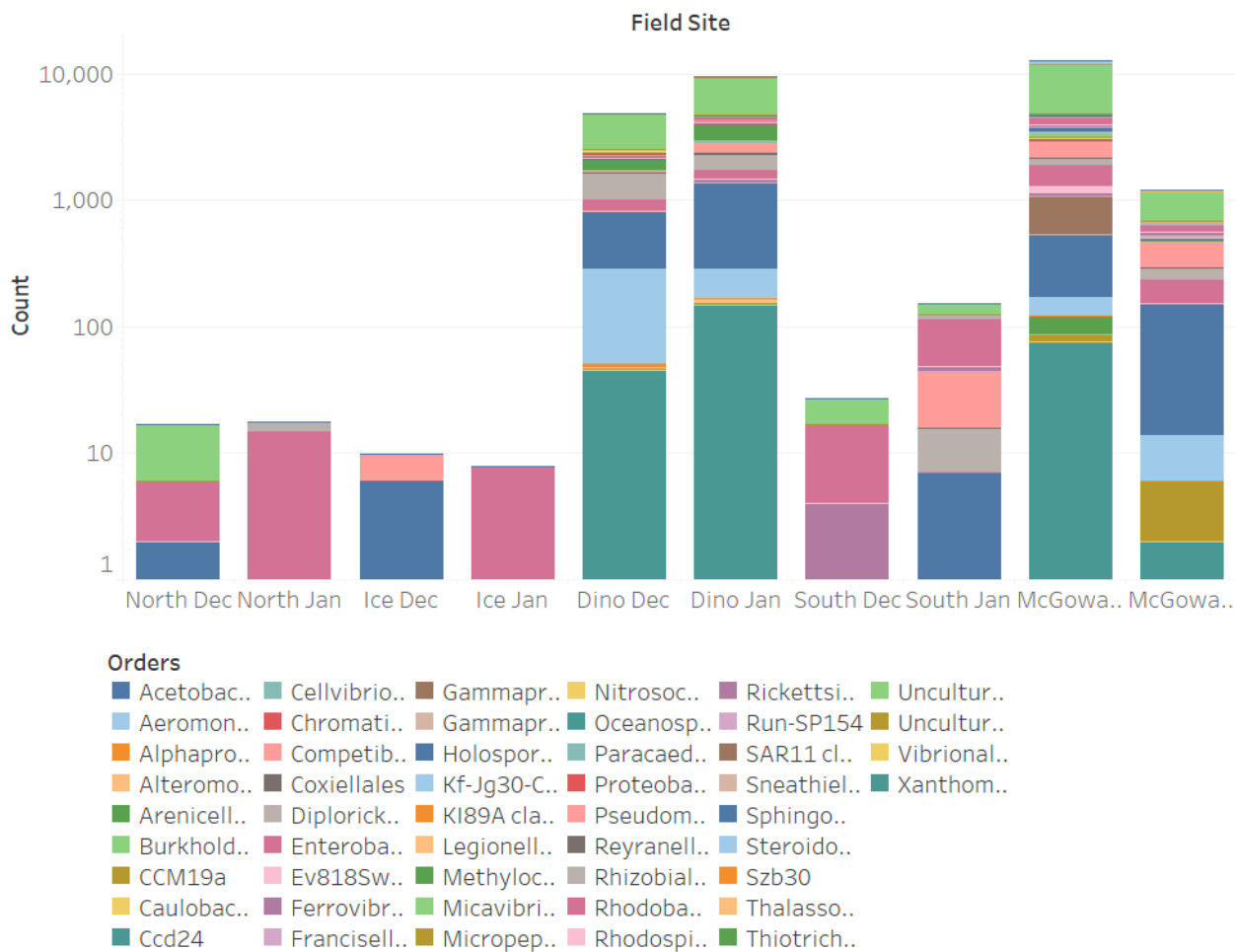


FIGURE 3: Illustration of Proteobacteria orders found in 20 samples on a logarithmic scale.

FIGURE 4: POSITIVE EXPLANATORY FACTORS OF BACTERIAL ORDERS IN OVERPECK CREEK.

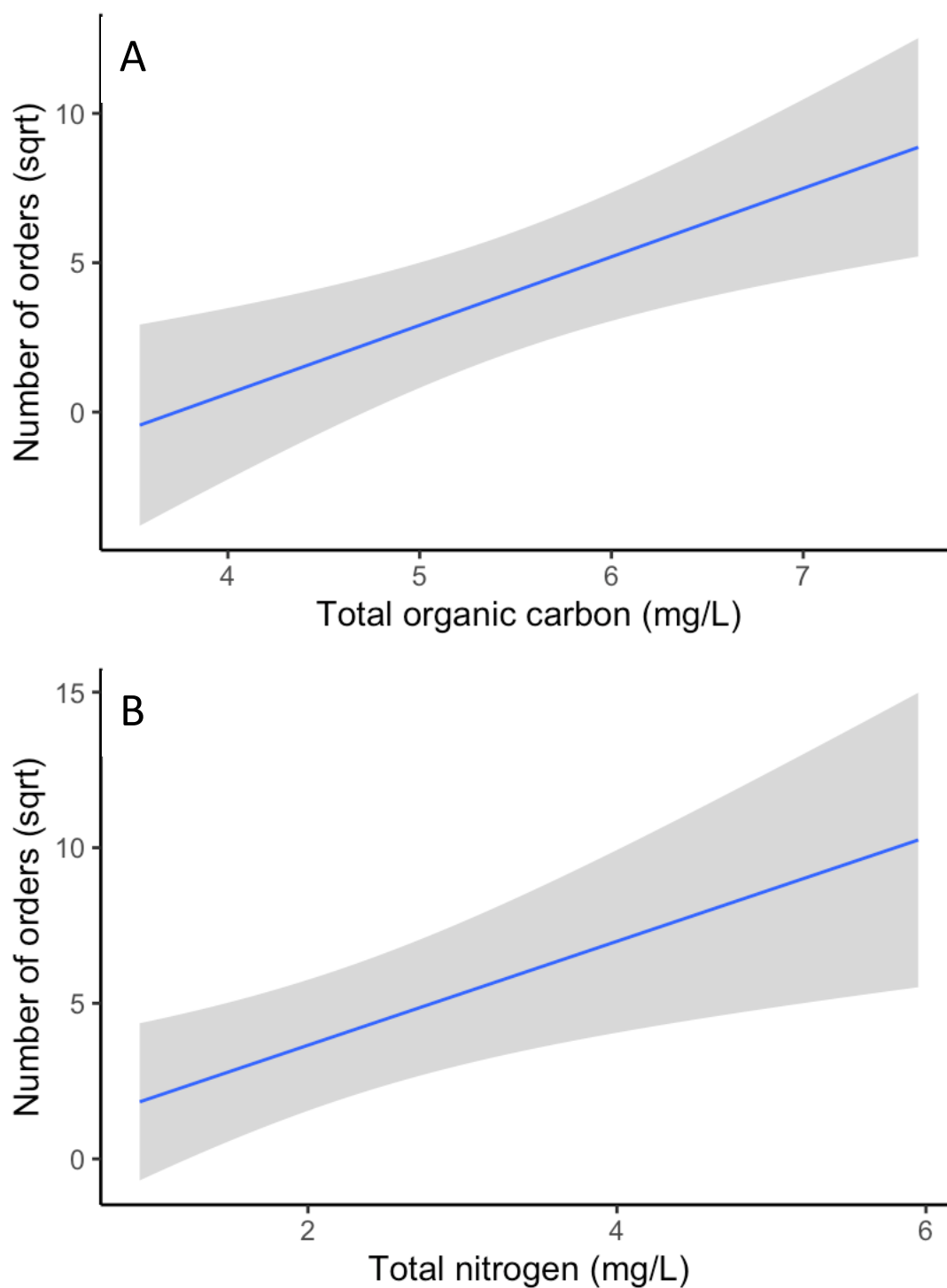


Figure 4: **A.** A positive relationship between total dissolved organic carbon and the number of bacterial orders among the sites in Overpeck Creek. **B.** A positive relationship between total dissolved nitrogen and the number of bacterial orders among the sites in Overpeck Creek.

FIGURE 5: NEGATIVE EXPLANATORY FACTORS OF BACTERIAL ORDERS IN OVERPECK CREEK.

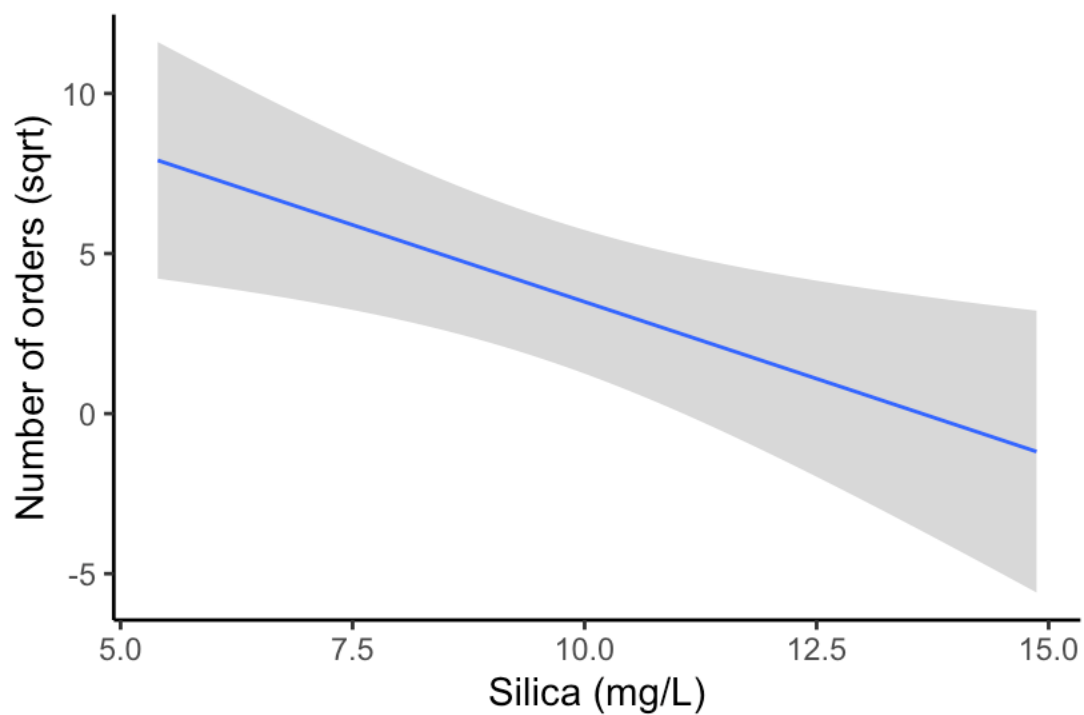


Figure 5: A negative relationship between dissolved silica and the number of bacterial orders in Overpeck Creek.

TABLES

TABLE 1: PRIMERS

Forward Primer

5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG 3'

Reverse Primer

5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC 3'

Illumina Forward Overhang

5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG - [locus-specific sequence] 3'

Illumina Reverse Overhand

5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG - [locus-specific sequence] 3'

TABLE 2: RESULTS OF THE GENERALIZED LINEAR MODELS

Order Richness		
Linear Regression	ChiSq	P-value
Total Dissolved Nitrogen	11.788	<0.001
Total Dissolved Organic Carbon	13.801	<0.001
Total Dissolved Phosphorus	0.007	0.932
Chloride	3.186	0.074
Dissolved Silica	9.640	0.002
Hardness	2.144	0.143

Order Diversity		
Linear Regression	ChiSq	P-value
Total Dissolved Nitrogen	10.950	<0.001
Total Dissolved Organic Carbon	7.047	0.008
Total Dissolved Phosphorus	0.033	0.855
Chloride	3.431	0.063
Hardness	1.873	0.171
Dissolved Silica	12.879	<0.001

Table 2: The results of the Generalized Linear Models showing associations between order richness (top) and order diversity (bottom) and measured water quality parameters. Bold values indicate statistically significant relationships.

DECEMBER 2020

	1. North Englewood	2. Mackay Ice Rink	3. Dinosaur Overpeck Park	4. South Overpeck Park	5. McGowan Park	
Total Dissolved Nitrogen	1-16 mg/L	1.4 g/L	0.9 mg/L	1.2 mg/L	5.8 mg/L	
	1-210 ug/L	1.0 ug/L	2.0 ug/L	0.0 ug/L	2.0 ug/L	
Copper	1.5-30 mg/L	3.5 mg/L	6.51g/L	7.3 mg/L	4.1 mg/L	6.6 mg/L
TDOC	1-100 mg/L	14.9 mg/L	11.5 mg/L	8.9 mg/L	8.9 mg/L	5.6 mg/L
Dissolved Silica	3-50 mg/L	16.5 mg/L	8.8 mg/L	9.4 mg/L	8.7 mg/L	>DL
	5-100 mg/L	47.6 mg/L	39.1 mg/L	39.0 mg/L	35.2 mg/L	46.7 mg/L
CA ²⁺	20-350 mg/L	187 mg/L	134.0 mg/L	136.0 mg/L	124.0 mg/L	>DL
Nickle	0.1-6.0 mg/L	<DL	<DL	<DL	<DL	0.1 mg/L
Sulfate	150-900 mg/L	<DL	<DL	<DL	<DL	170.0 mg/L
Total Dissolved Phosphorous	0.15-4.5 mg/L	0.4 mg/L	0.3 mg/L	0.3 mg/L	0.2 mg/L	0.7 mg/L
	0.1-2.5 mg/L	<DL	0.1 mg/L	<DL	<DL	0.2 mg/L
Fluoride	70-1000 mg/L	108.0 mg/L	137.0 mg/L	115.0 mg/L	132.0 mg/L	1086.0 mg/L
Chloride						

TABLE 3: WATER CHARACTERIZATION

Table 3: Complete water characterization from various chemical tests conducted for selected sites. Values below the detectable limit of HACH dr6000 are represented as <DL, those that are above are shown as >DL.

JANUARY 2021

Total Dissolved Nitrogen	1-16 mg/L	1.1 mg/L	1.5 mg/L	5.9 mg/L	1.0 mg/L	1.5 mg/L
	1-210 ug/L	1.0 ug/L	1.0 ug/L	0.0 ug/L	2.0 ug/L	1.0 ug/L
Copper	1.5-30 mg/L	4.2 mg/L	4.2 mg/L	7.6 mg/L	4.7 mg/L	5.7 mg/L
TDOC	1-100 mg/L	13.4 mg/L	11.9 mg/L	5.4 mg/L	6.5 mg/L	8.5 mg/L
Dissolved Silica	3-50 mg/L	9.2 mg/L	8.8 mg/L	10.8 mg/L	4.2 mg/L	>DL
	5-100 mg/L	41.8 mg/L	48.5 mg/L	39.5 mg/L	28.4 mg/L	34.6 mg/L
CA ²⁺	20-350 mg/L	142.0 mg/L	157.0 mg/L	143.0 mg/L	87.9 mg/L	147.0 mg/L
Nickle	0.1-6.0 mg/L	<DL	<DL	<DL	<DL	<DL
Sulfate	150-900 mg/L	<DL	<DL	<DL	<DL	<DL
Total Dissolved Phosphorous	0.15-4.5 mg/L	0.2 mg/L	0.7 mg/L	0.2 mg/L	0.7 mg/L	0.4 mg/L
	0.1-2.5 mg/L	<DL	<DL	0.1 mg/L	<DL	<DL
Fluoride	70-1000 mg/L	99.3 mg/L	114.0 mg/L	127.0 mg/L	73.4 mg/L	232.0 mg/L
<i>Detectable Range</i>		6. <i>North Englewood</i>	7. <i>MackKay Ice Rink</i>	8. <i>Dinosaur Overpeck Park</i>	9. <i>South Overpeck Park</i>	10. <i>McGowan Park</i>

TABLE 4: SITE RICHNESS & ABUNDANCE

Table 4: Specific site richness, abundance, and diversity index.

SITE	MONTH	ABUNDANCE	RICHNESS	DIVERSITY INDEX
1. <i>North Englewood</i>	DECEMBER	26	5	3.634
2. <i>MacKay Ice Rink</i>	DECEMBER	13	3	2.770
3. <i>Dinosaur Overpeck Park</i>	DECEMBER	15,961	124	8.107
4. <i>South Overpeck Park</i>	DECEMBER	51	8	6.237
5. <i>McGowan Park</i>	DECEMBER	39,733	135	14.447
6. <i>North Englewood</i>	JANUARY	18	2	1.384
7. <i>MacKay Ice Rink</i>	JANUARY	8	48	1.000
8. <i>Dinosaur Overpeck Park</i>	JANUARY	22,059	171	15.147
9. <i>South Overpeck Park</i>	JANUARY	174	12	4.647
10. <i>McGowan Park</i>	JANUARY	3,339	48	12.752

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