Classifying the Functional Microbial Diversity in Relation to pH within a North Canton Bog

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Abstract

Bogs are rare naturally occurring wetlands that formed from the movement of glaciers about 10,000 years ago. Bogs are supplied by their own underground water source, which creates a unique ecosystem. In this study, I examined the functional microbial communities of a local North Canton bog in relation to the water's pH, total nitrogen, and total phosphorus. Microbial growth is dependent on the condition of the environment, which results in some bacteria being better adapted to live in more acidic environments. I hypothesized that microbial diversity and pH would be positively correlated because fewer species can grow in acidic environments, so as the pH increases more species will colonize the area leading to an increase in diversity. To test this hypothesis, samples were collected from three different sites around the bog and inoculated on Biolog Ecoplates. Ecoplates use 31 different carbon sources and provide a general classification of the microbial community present in each site of the bog. Using the Ecoplates, I measured the microbial functional diversity and species evenness and compared those values to variation in pH, total nitrogen, and total phosphorus. There was no relationship between diversity and pH, but diversity positively correlated with total nitrogen and total phosphorus. Additionally, species evenness, which is a component of diversity, increased with pH. Bogs are important carbon sinks and support a wide variety of flora and fauna. Their microbial diversity, however, has rarely been explored. This is one of the first studies to estimate the functional diversity of a bog's microbial community. Microorganisms are important in nutrient cycling and understanding what impacts microbial diversity can have implications for the entire ecosystem.

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Introduction

Wetlands provide a wide range of ecological services as they mitigate flooding, purify water, store carbon, and provide habitat for many flora and fauna (Keddy et al., 2009). Types of wetlands include swamps, marshes, fens, and bogs. Despite their diversity and important ecological services, wetlands only cover about 8.5% of the earth's land (Zedler and Kercher, 2005). While all types provide important ecological services, swamps and marshes seem to be the primary focus of conservation efforts and research (Coppenolle and Temmerman, 2020). Bogs, likewise, have also been well studied, but with a primary focus on their role as carbon sinks (Loisel et al., 2021; Gorham 1991).

Bogs perpetuate a very unique ecosystem largely because of how they are formed. Bogs in North America, for example, were formed from glaciers that spanned from Canada down to the southern United States about 10,000 years ago (EPA, 2021). As the glaciers advanced, they brought with them rocks, seeds, and microbes into the area. As the climate began to warm, the glaciers receded northward, leaving fragments of melted ice that became the bogs in the midwestern United States (ODNR, 2021). Over thousands of years, decaying organic material called peat was deposited in these bogs and contributed to bogs' current role as carbon sinks (EPA, 2021). Due to the lack of this knowledge or care, farmers and other settlers in the area used these bogs as dumping grounds for wood and trash (Andreas and Knoop, 1992). Now with the knowledge of their natural history and their ecological significance, preserving and protecting bogs should be a priority in conservation efforts of the United States midwest.

The term "bog" is used colloquially to describe different types of wetlands, but it should be properly defined to distinguish its unique characteristics. A bog is a wetland

that cannot drain its water, and bogs have a build-up of peat which makes the water that does accumulate following rain appear brown (Andreas, 1985). Little water circulation and the build-up of peat both cause a bog to have a nutrient-poor and acidic environment (EPA, n.d.). The nutrient-poor environment allows for the ground cover around the bog to be dominated by sphagnum moss (EPA, n.d.). The acidic environment, with pH ranging from 3.5-5.5, is an important distinction for bogs when comparing them to other wetlands (Andreas, 1985). Not only does pH help define the physical characteristics of the bog, but also helps define what organisms can live in this type of environment.

Low pH values can cause physiological stress on organisms, which will limit the organism's zone of habitation (Hamilton, 2012). Species are limited by the range of pH they can survive, but not all species have the same ranges. For example, carnivorous plants like sundews and shrubby vegetation in the families Ericaceae and Cyperaceae have adapted to live in acidic environments and can be found in some bogs (EPA, n.d.; Andreas, 1985). Similarly, some bacteria, like those in the phylum Acidobacteria, a group of bacteria commonly found in bogs (Dedysh et al., 2006), are uniquely adapted to living in highly acidic environments (Kielak et al., 2016). Because of this adaptation, these bacteria are classified as acidophiles and their response to pH has been well-studied.

Since the early 1900s, scientists have identified many species of bacteria that can survive moderate to extreme acidic conditions ($pH < 5$) (Johnson and Quatrini, 2020). pH, which is a measurement of protons in a solution, plays an important role in the physiology of bacteria (Jin and Kirk, 2018). For example, when the pH of the environment is lower than the pH of bacteria, protons will move across the cell membrane from the environment into the cell (Baker-Austin and Dopsin, 2007). This

influx of excess protons interferes with the chemical reactions of DNA transcription, protein synthesis, and enzyme activities (Baker-Austin and Dopsin, 2007). Even if proton concentration does not directly affect cellular processes, pH will change the charge of essential nutrients, like phosphate and nitrogen, which prevents them from being stable enough to be taken up by the cell (Kirchman, 2018). If a cell loses its ability to perform any of these tasks, it cannot survive long. Because of this, some bacteria have developed physiological adaptations that minimize these harmful effects. Some acidophiles, for example, have evolved highly impermeable membranes, smaller membrane pores, and active proton pumps to diminish the number of protons present in the cell (Baker-Austin and Dopsin, 2007). With these adaptations or lack thereof, species will distribute themselves based on their tolerance to low pH environments.

Although much is known about how pH affects individual species of bacteria (Baker-Austin and Dopsin, 2007), much less is known about how environmental pH affects microbial communities, particularly in aquatic environments. While studying the microbial diversity of freshwater lakes of different trophic levels, Dickerson and Williams (2014) found that pH and microbial diversity were different across the lakes, but since it was not the focus of their study, they suggested that further investigation would be needed to understand pH's influence on microbial differences in aquatic microbial communities. Conversely, Percent et al., (2008), in a study on the microbial community in other freshwater lakes, found that bacterial community richness and diversity both positively correlated with pH. Not every group of bacteria, however, correlated with pH, which further supports the idea that how bacteria are affected by pH may differ depending on their genetics and physiology.

Methods of genetic sequencing and physiological classification are commonly used to identify the presence of bacteria. However, using this method to describe large communities of bacteria can be impractical and not even necessary to understand the ecological diversity present among microbial communities. Biolog EcoPlates®, for example, are designed to categorize microbial organisms, not on a species level, but on a community level based on their utilization of different carbon sources (Garland, 1997). This method of community-level physiological profiling (CLPP) is commonly used when studying microbial species in the environment. EcoPlates involve the use of different carbon sources and a redox dye (tetrazolium violet) to estimate the functional diversity of microorganisms in a sample.

More specifically, EcoPlates consist of 96 wells, broken down into three equal sets of 31 different carbon sources and 1 control well. The carbon source substrates selected are comparable to the sources found in natural environments to observe functional differences in the bacterial assemblages present (Dickerson and Williams, 2014). These bacterial assemblages are categorized into groups called functional guilds based on the carbon source they utilize (Table 1). These functional guilds are used to distinguish bacteria based on their functionality in the ecosystem, rather than by species. The utilization of a carbon source can be determined by the color change of the well because the tetrazolium violet dye is reduced as a side effect of metabolism (Freeberg et al., 2019). The utilization of each carbon source can be quantified by the average well color development (AWCD) which is measured through light absorbance by a spectrophotometer (Dickerson and Williams, 2014). By applying these concepts, EcoPlates have been used to study microbial functional diversity in wetlands (Sura et al.,

2012), rivers (Wang et al., 2010), and lakes (Dickerson and Williams, 2014). For example, Dickerson and Williams (2014) used EcoPlates to measure diversity (through the Shannon diversity index), substrate richness, substrate evenness, and metabolic activity in three freshwater lakes of different trophic levels.

Table 1. Carbon Substrates in Biolog EcoPlates (from Dickerson and Williams, 2014; Christian and Lind, 2006)

Amino Acids	**Carbohydrates**	**Carboxylic acids**	**Amines**	**Polymers**
L-Arginine	β-Methyl-D-Glucoside	Pyruvic Acid Methyl Ester	Phenylethyl-amine	Tween 40
L-Asparagine	*D-Galactonic Acid γ-Lactone*	D-Galacturonic Acid	Putrescine	Tween 80
L-Phenylalanine	D-Xylose	D-Glucosaminic Acid		α-Cyclodextrin
L-Serine	*i-Erythritol*	Itaconic Acid		Glycogen
L-Threonine	D-Mannitol	α-Keto Butyric Acid		
Glycyl-L-Glutamic Acid	N-Acetyl-D-Glucosamine	D-Malic Acid		
γ-Amino Butyric Acid	D-Cellobiose	2-Hydroxy Benzoic Acid		
	Glucose-1-Phosphate	4-Hydroxy Benzoic Acid		
	α-D-Lactose			

In freshwater systems, microorganisms play an important role in biogeochemical cycling and being a source of nutrients for higher trophic levels (Dickerson and Williams, 2014). Therefore, measuring the functional diversity of a microbial community can give insight into the health of the broader biological community and ecosystem as a whole. To study how bacteria may be used as indicators of poor water quality, Wang et al. (2010) researched the functional bacterial distribution of four rivers in China based on their nutrient levels. They discovered that the functional bacteria present did reflect the differences in the trophic levels. Samples from richer nutrient levels cultured more bacteria colonies, indicating higher bacterial density but lower bacterial diversity. Even though changes in the bacterial community occur through natural factors, such as

seasonality, these changes can also occur from algal blooms and anthropogenic activities (Dikerson and Williams, 2014). To understand the effects herbicide has on microbial communities in wetlands, Sura et al. (2012) tested biofilm growth between one side of a wetland that was given herbicide and one side that was left untreated. Biofilms in particular are sensitive to changes in their ecosystem, so they can be used as indicators of toxic effects occurring in ecosystems (Sura et al., 2012). So, by studying the functional diversity of a microbial community, we can gain insight into the health of the whole ecosystem.

As previously stated, bogs host a unique ecosystem because of their low pH, however, the relationship between low pH and microbial composition is seldom studied. Dedysh et al. (2006) conducted a study in an acidic bog to understand the overall bacterial community composition through genetic sequencing. A different study conducted by Bragina et al. (2015) also used genetic sequencing to study the functional bacterial diversity in an Alpine bog. I aim to research the connection between functional microbial diversity and pH by cultivating bacteria from water samples with differing pH in a bog in North Canton, Ohio. I expect these differences in pH to arise naturally within the bog, as different areas will be influenced by the flora, fauna, and human development of the surrounding landscape.

Here, I hypothesized that a higher pH environment is more conducive to higher bacterial diversity than a lower pH environment because lower pH environments have more protons, and protons interfere with microbial metabolic processes (Jin et al., 2018). Only certain groups of bacteria have evolved adaptations to highly acidic environments, and I further hypothesized that these groups will competitively exclude a wide diversity

of microorganisms. Therefore, if my hypothesis is correct, then the areas of the bog with higher pH would have greater microbial diversity and evenness than the areas of the bog with a lower pH.

Methods

Study Site

Microbes were sampled from an unclassified bog located in North Canton, Lake Township, Stark County, Ohio (40.921138, -81.389952) (Figure 1). This wetland is approximately 60 meters long and 40 meters wide. Even though unclassified, this wetland is likely to be a bog due to the brown appearance of the water, the little water circulation, and the presence of sphagnum moss throughout (Andreas, 1985). Because of these characteristics, the bog water is most likely to have a relatively low pH between 3.5 and 5.5 during most of the year (Andreas, 1985). Additionally, with the influence of wildlife and human activity, there is likely to be variation in pH between different areas throughout the bog. For example, a road is approximately 25 meters from the west side of the bog. Proximity to a road has been correlated with high pH (Radziemska and Fronczyk, 2015; Lee et al., 2012). To identify variations in pH, three sites were chosen given their location towards or away from the road and distance from each other. Site 1 had an average pH of 5.70 ± 0.06, Site 2 had an average of 5.98 ± 0.06, and Site 3 had an average of 5.76 ± 0.16. By attempting to take samples from a variety of sites, I sought to better describe the role pH has in affecting the functional diversity of microbes.

Figure 1. Map showing the study site located at North Canton, Lake Township, Stark County, Ohio (40.921138, -81.389952). Site 1: (40.921273, -8.3898311), Site 2: (40.9211666, -81.3902135), Site 3: (40.9208704, -81,3900427). Map data from ©2022 Imagery©2022 Maxar Technologies, State of Ohio/OSIP, U.S. Geological Survey, USDA Farm Service Agency.

Microbial Sampling

For each round of sampling, water samples were collected from different sites of the bog on the same day to accurately account for any potential weather differences. For the first two rounds of sampling (February 16th, 2022 and February 21st, 2022, respectively), the bog water was covered by snow and ice, so a shovel was used to break open the ice, and samples were taken from the liquid water underneath. For the last round of samples (February 28th, 2022), the ice had mostly melted, so a sampling rod (a

50-milliliter centrifuge tube attached to a meter-long stick) was used to collect samples. Along with the collection of samples, pH and temperature were measured using a pH/Temperature meter (HI991300, Hanna Instruments, Inc.). To compare nutrient levels across the sites, total nitrogen and total phosphorus levels were measured using a DR/890 Hach Portable Datalogging Colorimeter. The pH and temperature were measured in the field, while the water samples for microbial and chemistry testing were stored on ice and transported to Malone University for laboratory testing.

Analysis of Samples

To measure microbial functional diversity, each water sample was assigned to its own EcoPlate. Due to the 96-well design of the EcoPlates, samples are repeated in triplicates in a single plate, so each sample has three data points per carbon source (Garland, 1997). Each well received 130 μL of the sample for the designated plate (Dickerson and Williams, 2014). Plates were incubated at 26 ± 2° C for the duration of the study (Dickerson and Williams, 2014). Because three sampling rounds were taken, each site was sampled three times for a total of nine replicates per site.

To measure the growth of microbes within the plates, the well absorbance levels were measured using a Spectro Star spectrophotometer set at 590 nm (Dickerson and Williams, 2014). To standardize samples, I estimated when the bacteria are in their exponential growth phase by analyzing the samples every 12 hours for about 120 hours (5 days) after incubation (Dickerson and Williams, 2014; Tiquia, 2010). The growth phase of the bacteria can be determined by measuring the average well color development (AWCD). The AWCD is a function of substrate utilization because of the color change of the tetrazolium violet dye. The AWCD was calculated by averaging the absorbance of

each well on a single EcoPlate (Garland, 1997). The absorbances were corrected for each plate by subtracting the absorbance of each carbon source from the absorbance of the water-control well (Feigl et al., 2017). By graphing AWCD over time, I identified 36 hours to be where the AWCD was increasing quickly in a linear phase, indicating exponential growth by the microbes (Tiquia, 2010; Figure 2).

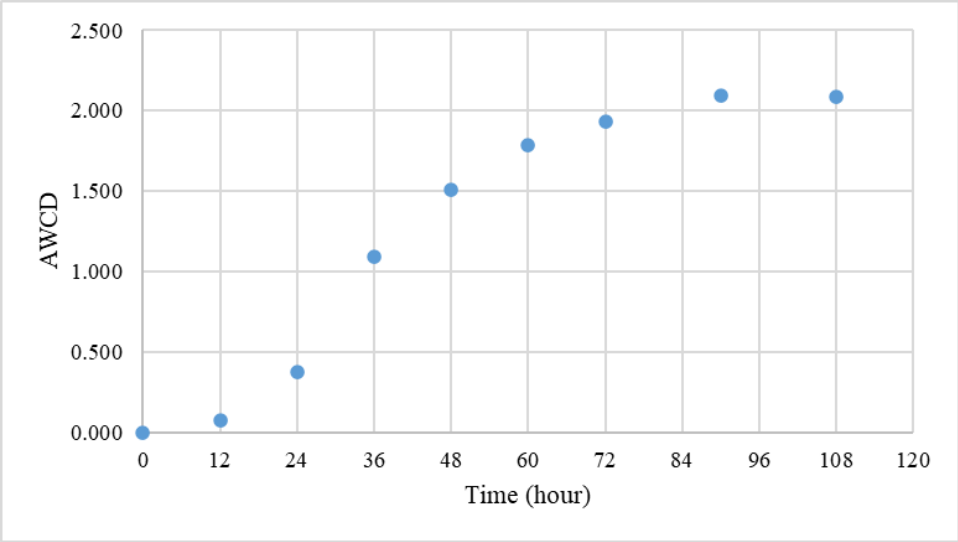

Figure 2. Average Well Color Development absorbance at 590 nm (AWCD) plotted against time taken from Site 3 on February 16th, 2022. The inflection point on the curve at or near 36 hr indicates the bacteria are likely still in the log-growth phase.

To estimate aspects of functional diversity, I calculated the Shannon-Weiner Diversity Index (H) and Species Evenness (E). The diversity index was calculated via the following equation (Tribedi et al., 2015; Tribedi and Sil, 2013).

$$H = - \sum p_i * ln(p_i) \qquad \text{(Equation 1)}$$

Where p_i equals the activity of each substrate (absorbance) divided by the sum of activities of all substrates (∑absorbances). An absorbance of 0.25 at 590 nm is considered the threshold for a positive response, so only absorbance values greater than 0.25 were used (Garland, 1997).

Species Evenness (E) was calculated via the equation below.

$$E = \frac{H}{\ln S} \quad \text{(Equation 2)}$$

Where H is substrate diversity, and S is the number of wells with an absorbance greater than 0.25 (Rana et al., 2021).

Data analysis

To compare diversity and evenness between the different sampling sites, I used a one-way ANOVA test followed by a Tukey's post hoc analysis. Carbon sources were categorized into carbon guilds (Table 1) according to Christian and Lind (2006) and Dickerson and Williams (2014) and analyzed via a two-way ANOVA test followed by a Tukey's post hoc analysis to identify more specific functional differences between the sites. All tests were carried out in R (R Core Team, 2022) and differences were considered significant if $P < 0.05$.

To compare the relationships between diversity and pH, diversity and total nitrogen, diversity and total phosphorus, and evenness and pH, a regression analysis was performed in Excel for each individual comparison.

Results

Regression Analyses

There was no relationship between the Shannon Diversity Index and pH (Figure 3, Table 2). However, Site 3 and the Combined-Sites data are trending towards a negative relationship, while Site 2 is trending towards a positive relationship.

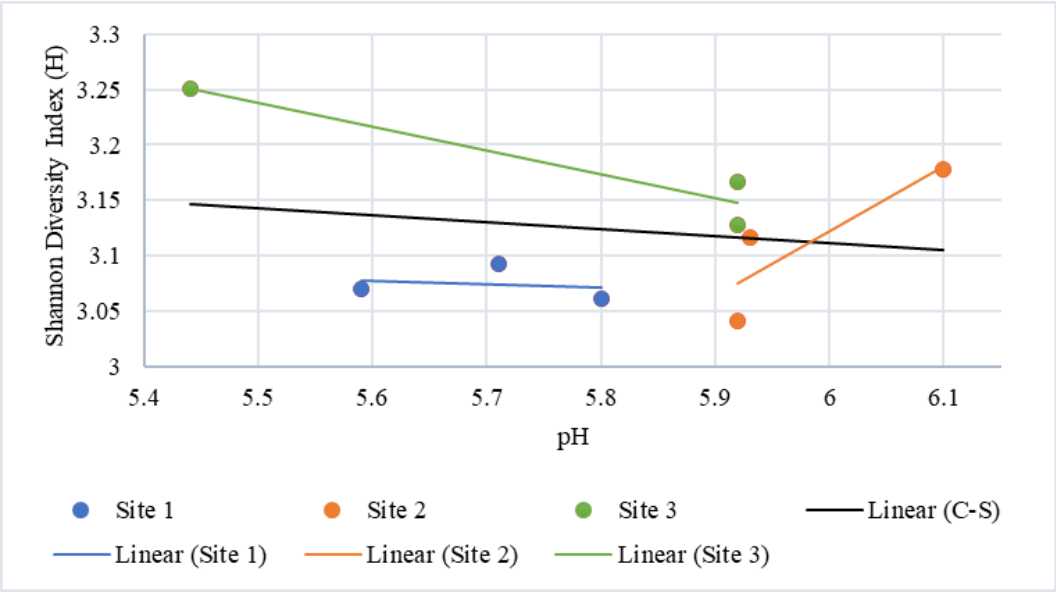

Figure 3. Regression analysis for the Shannon Diversity Index and pH. N = 3 for each site. "C-S" means Combined Sites. See Table 2 for regression coefficients.

There was a positive relationship between the Shannon Diversity Index and the total nitrogen of the Combined-Sites data (Figure 4, Table 2). All three sites alone did not indicate a relationship, however, each is trending towards a positive relationship. Notably, Site 3 has only two data points because the total nitrogen from February 28th was beyond the limit that the DR/890 Hach Portable Datalogging Colorimeter could read.

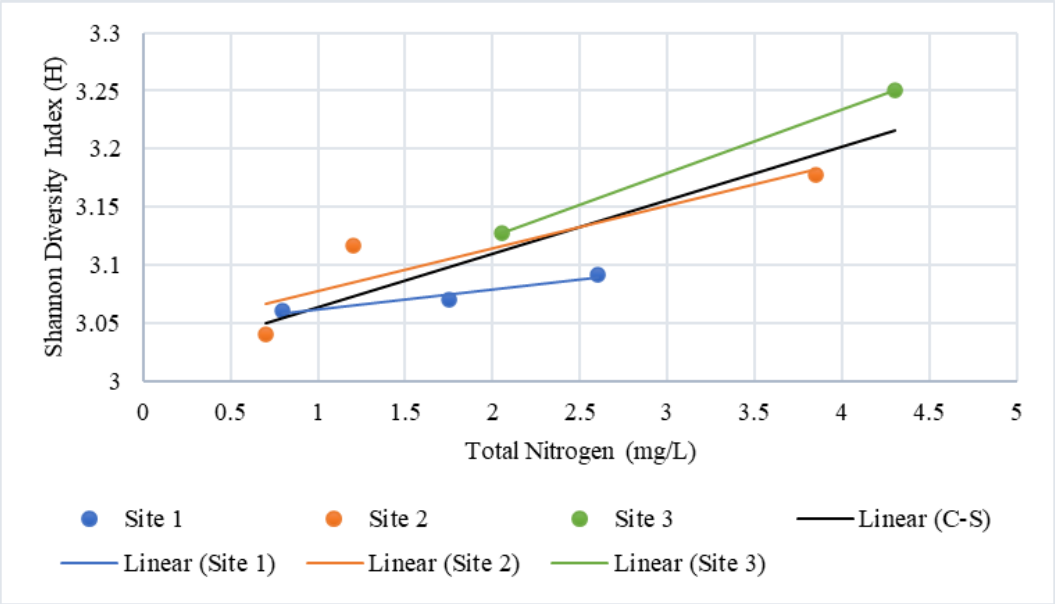

Figure 4. Regression analysis for the Shannon Diversity Index and total Nitrogen (mg/L). N =3 for sites 1 and 2, and N = 2 for site 3. "C-S" means Combined Sites. See Table 2 for regression coefficients.

There was a positive relationship between the Shannon Diversity Index and the total phosphorus of the Combined-Site data (Figure 5, Table 2). All three sites alone did not indicate a relationship, however Sites 2 and 3 are trending towards a positive relationship.

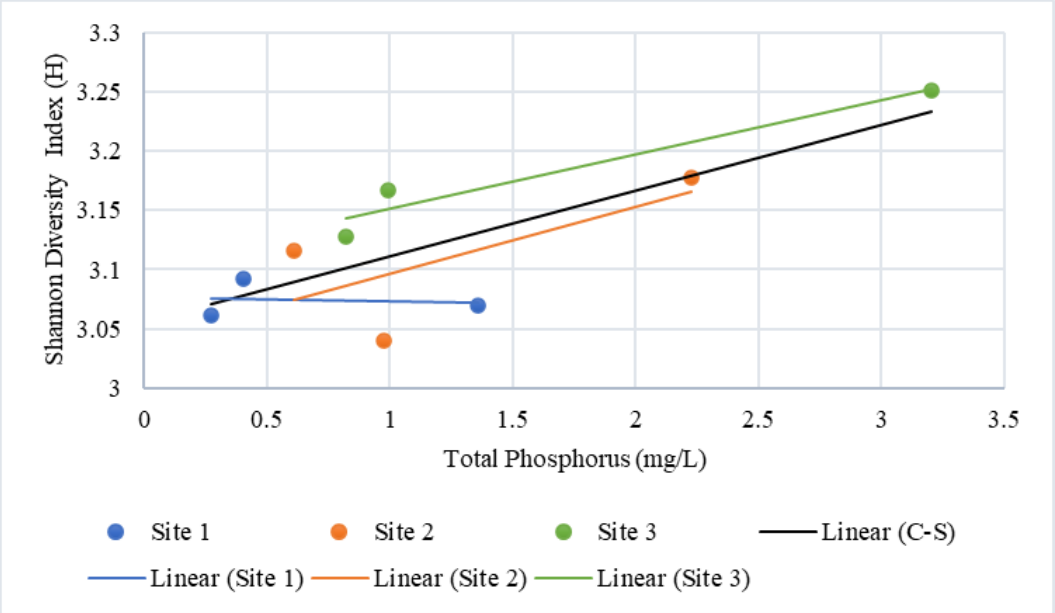

Figure 5. Regression analysis for the Shannon Diversity Index and total phosphorus (mg/L). N = 3 for all sites. "C-S" means Combined Sites. See Table 2 for regression coefficients.

There was a positive relationship between evenness and pH in Site 3 and the Combined-Sites data (Table 2; Figure 6). There was no relationship between evenness and pH at Sites 1 and 2, however, both sites are trending towards a positive relationship.

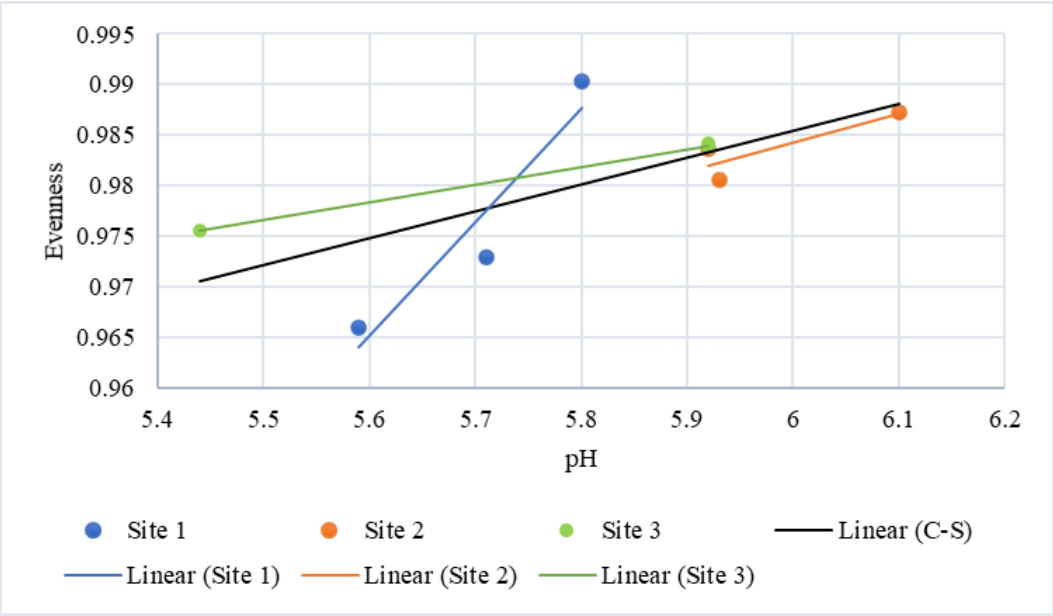

Figure 6. Regression analysis for pH and evenness. N = 3 for all sites. “C-S” means Combined Sites. See Table 2 for regression coefficients.

Table 2. Regression Analysis for Diversity vs pH, Diversity vs Total Nitrogen, Diversity vs Total Phosphorus, and pH vs Evenness. “N” represents the sample size. “b” represents the y-intercept and “m” represents the slope of the regression line.

	N	b	95% CI	p	m	95% CI	p	R^2
Diversity vs pH								
Site 1	3	3.245	(-7.488, 13.978)	0.162	-0.030	(-1.913, 1.853)	0.873	-0.922
Site 2	3	0.393	(-26.71, 25.924)	0.881	0.586	(-3.812, 4.984)	0.340	0.482
Site 3	3	4.425	(-0.689, 9.538)	0.058	-0.2158	(-1.103, 0.671)	0.199	0.811
Combined-Sites	9	3.484	(1.806, 5.163)	0.002	-0.062	(-0.351, 0.226)	0.626	-0.102
Diversity vs Nitrogen								
Site 1	3	3.045	(2.932, 3.159)	0.002	0.017	(-0.044, 0.078)	0.174	0.853
Site 2	3	3.041	(2.527, 3.555)	0.008	0.037	(-0.181, 0.255)	0.277	0.645
Site 3	2	3.016	(3.016, 3.016)	N/A	0.055	(0.055, 0.055)	N/A	1
Combined-Sites	8	3.018	(2.961, 3.075)	<0.001	0.046	(0.023, 0.069)	0.003	0.769
Diversity vs Phosphorus								
Site 1	3	3.077	(2.794, 3.359)	0.005	-0.004	(-0.342, 0.335)	0.916	-0.965
Site 2	3	3.040	(1.968, 4.111)	0.018	0.057	(-0.684, 0.798)	0.510	-0.030
Site 3	3	3.105	(2.810, 3.400)	0.005	0.046	(-0.102, 0.194)	0.158	0.879
Combined-Sites	9	3.056	(2.997, 3.114)	<0.001	0.056	(0.016, 0.095)	0.012	0.562
Evenness vs pH								
Site 1	3	0.334	(-2.418, 3.086)	0.366	0.113	(-0.370, 0.595)	0.207	0.796
Site 2	3	0.812	(-0.455, 2.079)	0.078	0.029	(-0.183, 0.240)	0.335	0.495
Site 3	3	0.880	(0.820, 0.941)	0.003	0.018	(0.007, 0.028)	0.030	0.996
Combined-Sites	9	0.826	(0.687, 0.965)	<0.001	0.027	(0.003, 0.050)	0.034	0.425

Diversity

The Shannon Diversity Index (H) varied across the sites (Figure 7, $F_{2,24} = 8.739$, $P = 0.001$). More specifically, Site 1 (3.02 ± 0.03) had less diversity than Site 3 (3.17 ± 0.02), but Site 2 (3.08 ± 0.02) was not different from either site (Figure 7).

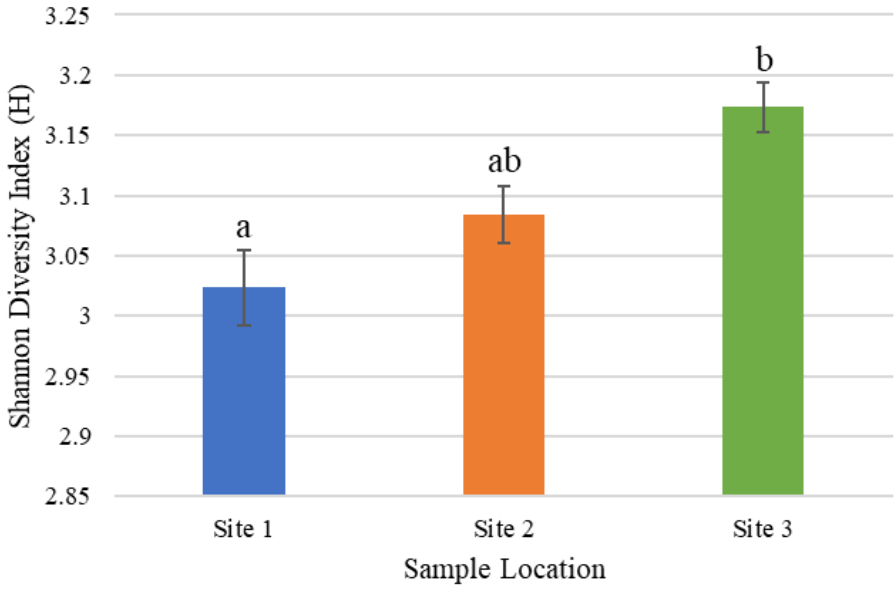

Figure 7. Average diversity across sites. The standard error of the mean is indicated by the error bars. Mean values with the same letter are not significantly different. N = 9 for each site.

Average Well Color Development (AWCD)

The AWCD varied across the sites (Figure 8, $F_{2,24} = 3.794$, $P = 0.037$). Specifically, Site 1 (0.94 ± 0.03) had lower AWCD than Site 2 (1.14 ± 0.06), but Site 3 (1.06 ± 0.06) was not different from either site.

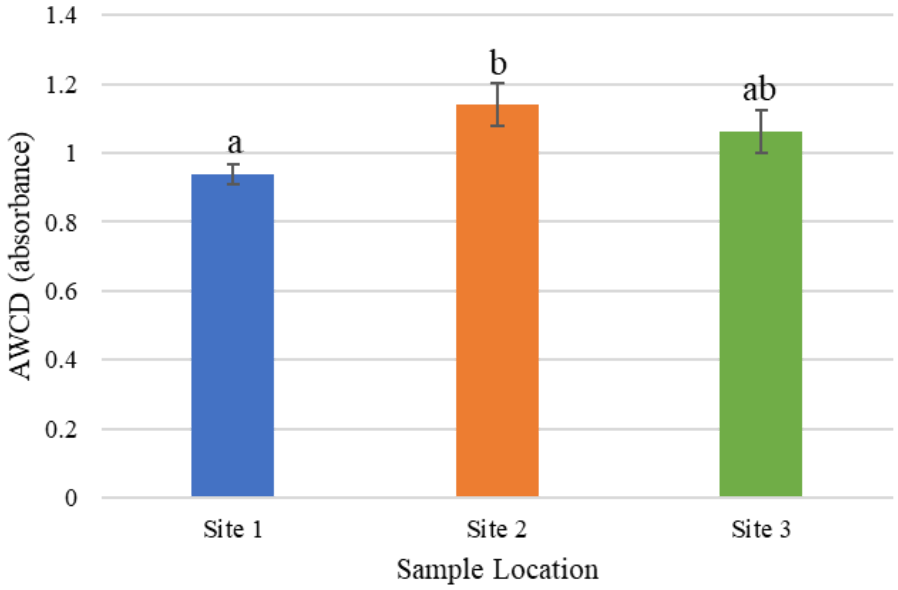

Figure 8. Average Well Color Development (AWCD) across sites. The standard error of the mean is indicated by the error bars. Mean values with the same letter are not significantly different. N = 9 for each site.

Carbon Guilds

Both sample location ($F_2 = 5.044$, $P = 0.008$) and carbon guild ($F_4 = 24.312$, $P < 0.001$) had an impact on absorbance and there was an interaction between the two ($F_8 = 2.182$, $P = 0.033$). Not many differences were found between guilds overall, however, amino acids were higher for all three sites than polymers for all three sites (Figure 10). In looking across Site 1, amino acids were also higher than carbohydrates, and in Site 2, amino acids were higher than amines.

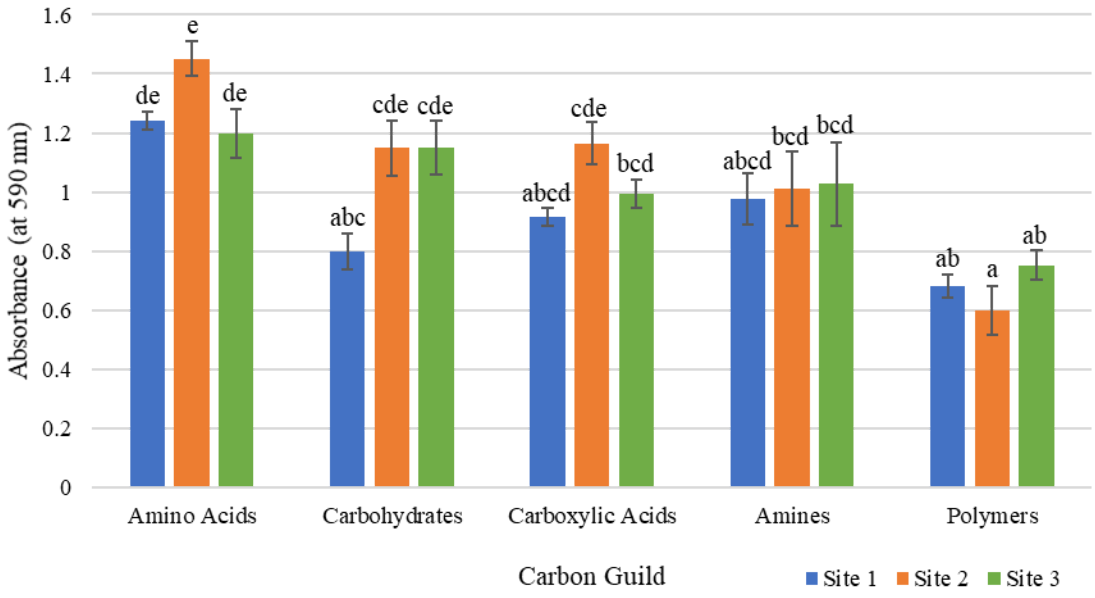

Figure 10. Carbon Guild Analysis. The standard error of the mean is indicated by the error bars. Mean values with the same letter are not significant. N = 9 for each site within each guild.

Discussion

Trends in Diversity

My hypothesis, that diversity would positively correlate with pH, was not supported, because there was no significant relationship between pH and diversity. There was, however, a slight positive relationship between pH and evenness for the Combined-Sites data. Because evenness is a component of diversity, a significant relationship in evenness could mean a significant relationship with diversity (Moore, 2013). This leads me to conclude that there may be a relationship between pH and diversity, but this was not evident in the results because of the conditions and limitations of the study.

A few different factors may explain this. A larger sample size would provide more data for statistical analysis and help identify any outliers. For example, the left-most data point from Site 3 could be an outlier but it is hard to say with such a small sample

size (See Figure 3). Along with a larger sample size, a larger variation of pH would be informative as well.

The variation of pH during the study period (5.44-6.1) may not have been wide enough to properly describe the relationship between diversity and pH. The pH range observed is also surprising since research indicates bogs have a pH from 3.5-5.5 (Andreas, 1985). This could indicate that the wetland in this study is in fact not a bog, and further research would be needed to classify it as a fen. Alternatively, other factors may have impacted the sites' pH. Because sampling occurred in February, the weather varied across days and sampling dates were chosen based on warmer weather for better access to the bog water. This meant that sample sites were exposed to the incoming snow that would have melted into the sites and then froze again between sampling dates. Snow typically has a pH from 5 to 6, (Mesner and Geiger, 2005) so it is likely that the melting snow would have raised the local pH of each sampling site.

Effects of Nitrogen and Phosphorus

Diversity positively correlated with total nitrogen and total phosphorus. This relationship may be better understood due to the range of variation between samples. Total nitrogen had a range of 0.7 to 4.3 mg/L and total phosphorus had a range of 0.28 to 3.2 mg/L.

It is not surprising that higher levels of nitrogen would correlate to higher levels of microbial diversity, based on what is known about nitrogen's role in the environment. Nitrogen is important for plant growth and microbes play a role in nitrogen cycling, and additionally, nitrogen is found in many different forms (Isobe et al 2020). Total nitrogen, the measurement used in this study, is a measure of multiple nitrogen species (ammonia,

organic nitrogen, nitrate, nitrite, etc) (Scott, 2012). However certain bacteria (like ammonifiers and nitrifiers) play specific roles in nitrogen cycling (Isobe et al., 2020). This means multiple forms of nitrogen could promote diversity through different types of microbes.

Contrary to an increase in total nitrogen, higher phosphorus has been linked to a decrease in microbial diversity. In a study by Zheng et al. (2019), different sources of phosphate were studied in activated sludge to identify the effects they had on the chemical and microbial composition of a sample. When phosphorus was the sole source added, microbial diversity decreased, and they inferred that this could be due to the competitive advantage that species that utilize sole phosphorus have. However, in the case of my study, it can be assumed that phosphorus was not present in just one form, and we know from the total nitrogen data that phosphorus was not the only chemical present. So we can infer that like nitrogen, when more phosphorus, especially in its multiple forms, is present, it is able to be utilized by multiple microbial guilds, hence increasing microbial diversity.

Trends in Diversity and Carbon Guilds Across Sites

When comparing across sites, diversity was greatest at Site 3 with Sites 1 and 2 being statistically the same. Because Site 3 is on the southern side of the bog, it will most likely get more sun compared to the other sites (Figure 1). Getting more sunlight would then allow more growth of photosynthetic microbes but could also be inhibitory for other microbes (Lindell et al., 1996). Depending on the different functional groups those bacteria are classified in, diversity could be a function of increased exposure to sunlight.

To understand the variation in diversity, I also measured the AWCD. In contrast to diversity, AWCD at Site 2 was significantly higher than at Site 1, but neither site was different from Site 3. AWCD is measured by taking the average of the total absorbance across the EcoPlate and describes the microbial metabolic activity of the sample (Tribedi and Sil, 2013). Similarities across the site, therefore, simply mean the metabolic activity of all microbes is similar across the sites. To better understand why diversity may vary across the sites, one can explore the specific carbon guilds.

One of the reasons the Biolog EcoPlates were used in this study is for their ability to measure differences in microbial functional groups across samples via the use of different carbon substrates (Dickerson and Williams, 2014). By grouping the carbon substrates into broader guilds (Table 1), differences in substrate utilization then indicate the different phylogeny and ecological roles of the microbes present (Dickerson and Williams, 2014). All three sites, with considerable variation, showed the greatest substrate utilization in the amino acid guild and the least substrate utilization in the polymers guild.

The amount of amino acids present in the bog is a reflection of nitrogen available in the composition of the soil and peat (Scarpelli, 2016). Differences in the amount of amino acid utilizing microbes could be an indication that soil composition is not the same throughout the bog. The plants present on the different sides are having an effect on the microbial community in the water as dissolved organic matter typically comes from the soil and leaf litter (Mattsson et al., 2008). As mentioned previously, plants also depend on the nitrogen and pH of their soil, so the differences in plants around the bog could be a further reflection of those differences. Given the sampling time period took place in

winter, it was difficult to identify the local vegetation to determine differences in plant species around the different sites.

The polymer carbon guild of the EcoPlate is notably smaller in both the number of carbon sources and in the comparable utilization. This difference could be due to the fact that polymers are not as commonly found in nature as the other carbon guilds. Glycogen is most commonly found in animals and microorganisms (Rocha Leão, 2003), which may still be found in the bog, but probably not as much as the plant matter. Tween-80 and Tween-40 are two of the other polymers used in the EcoPlates, however, these are more commonly found in human manufactured products like cosmetics and pharmaceuticals (Nielson et al 2016; UL Prospector, n.d.). Since this bog is located on private property, it remains fairly untouched by human pollution, and so it is not surprising that microbes of the polymer guild were not found in abundance.

Study Limitations

Sampling for this study was conducted during the month of February in 2022. Throughout the month, temperatures ranged from about 10°C to 60°C (WeatherSpark, 2022). During sampling, the measured water temperature ranged between 0.4°C and 2.4°C. Additionally, the extent of ice on the bog significantly decreased between sampling periods, especially between February 21st and February 28th (personal observation). Therefore, combining the data based on site may not be appropriate given the variation in temperature and ice around the bog across the weeks.

Previous research has shown that decreases in temperature can alter microbes' affinity for substrates, limiting their growth (Nedwell, 1999). This limitation of substrate utilization may be due to the lipids in the cell membrane becoming more solid, thus

limiting the transport of nutrients across the cell (Nedwell, 1999). In contrast, during higher temperatures, molecules inside and outside of the cell can move faster, optimizing metabolism for microbes (Blamire, 2000). This could mean that the microbial communities were changing in response to temperature variation throughout the sampling period.

Additionally, Butler et al. (2019) found a significant decrease in bacterial abundance during ice cover, but an increase in phylogenetic diversity during ice cover. Therefore, despite this study being conducted on a much finer spatial and temporal scale, the amount of ice cover in the bog may also have influenced the microbial community.

Conclusion and Future Study

Microbial diversity and the elements that influence it have been studied in controlled environments, however, much less is known about microbial diversity in natural environments. While most studies focus on the physical characteristics of bogs and their role in important ecological services, this research was one of the first studies to explore microbial diversity in a bog. However, because of severe weather throughout the sampling period, sampling was limited to warmer days of which there were few. Few sampling dates and only sampling on warm days likely biased the data and introduced confounding variables that were difficult to separate. Nevertheless, the results and methods described here provide a framework that other researchers can use to better explore how the environment influences microbial diversity.

The goal of this study was to understand how pH specifically affected microbial diversity in a local bog. Further research is required, however, to account for the dynamically changing environment of a bog, especially in the middle of the changing

seasons of Ohio. Future studies should explore how the microbial diversity changes across warmer seasons when temperatures are more stable. Additionally, because of the surface ice conditions, samples were taken at the surface of the water, which is more prone to fluctuations in temperature (Kiselev et al., 2018) and potentially pH (Bergsma, 2009). Collecting samples deeper in the bog may provide a more stable abiotic environment which can help give a clearer insight into the differences in microbial diversity. This study focused on a single bog in Northeast Ohio. Sampling from other bogs and wetlands in the area would provide even greater understanding of the microbial community and its interactions with its environment.

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