Impact of water level on methane emission in wetlands

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II. ABSTRACT

Methane has become of increasing importance in climate change over recent years, as it is one of the main contributors to global warming. Wetlands are the main natural source of methane emissions into the atmosphere and are responsible for roughly 30% of global methane emissions (EPA, 2021). Several factors contribute to the amount of methane being released from wetlands such as temperature, vegetation, microbial activity, and water level. It is unclear how large of a role water level plays in the emission of methane from wetlands. The hypothesis being tested is wetlands with lower water levels will emit more methane than wetlands with higher water levels. This is expected because higher water levels cause dilution and reduce the supply of organic substrate for methanogens and the productivity of aquatic vegetation. Three methane chambers were installed in the inlet and outlet of a wetland located at the University of Maryland Golf Course. Water levels and temperature conditions were monitored at the sites by using a ruler and a thermometer, these observations were compared with data from depth sensors and temperature loggers installed at the site. A bi-weekly sampling of the gas emitted from the wetland sites occurred over several months, mostly during the cold seasons of the year. The methane concentration of these samples is analyzed by the Gas Chromatograph (GC) to see the methane accumulation rate in 30-minute increments at the sites. The GC results test the hypothesis, as the methane concentrations is compared with the corresponding water levels at the time of the sample. The inlet and outlet sites have been sampled seven times, while this is not a significant amount of data collected, these results aided in correcting experimental design and finding potential relationships between variables. From our current research there appears to be no prominent relationship between temperature and methane emission. However, our results do show a variation in the effects of low and high-water levels on methane emissions. The current data points towards higher water level producing larger methane accumulation rates compared to lower water levels. This experiment would benefit from a collection of more samples during different seasons and an environment that experiences larger ranges of water level and temperature conditions.

III. INTRODUCTION AND BACKGROUND

Methane is approximately 25% more efficient at trapping greenhouse gases than carbon dioxide and therefore is greatly contributing to climate change (USEPA, 2021). Methane emissions are becoming an increasingly important topic as methane concentration in the atmosphere in 1988 was increasing at a rate of 1.1% per year over two decades (Singh et al., 2000). The annual increase in atmospheric methane in 2020 was 14.7 ppb (to 1891.9 ppb total), which is a record high (NOAA, 2021) (Figure 1). Methane can be released into the atmosphere through anthropogenic sources and natural sources. Wetlands are the main contributor of methane in the atmosphere by natural sources and are responsible for roughly 15% to 45% of global methane emissions (USEPA, 2021). According to Segers (1998) these uncertainties in estimation are due to the different environmental variables in wetlands such as seasonal and climate changes. Figure 2 from Saunois et al. (2016) shows wetlands were emitting more methane daily than fossil fuels, agriculture and waste, and biomass and biofuel burning from 2003 to 2012. The emission of methane gas by wetlands is becoming a subject of interest by environmental scientists interested in the fight against global climate change. Wetlands have a large role in climate change as they can produce, store, and emit greenhouse gases (Salimi et al.,

2021). Methane is produced and consumed in wetlands by microbial communities. Methanogens are anaerobic bacteria that carry out methanogenesis by taking in dissolved organic material or carbonates and producing methane (Fenchel, et al., 2012). Methanogenesis occurs in highly reduced, anaerobic conditions (Sowers, 2009; Fenchel, et al., 2012). Methanogens are found in a variety of anaerobic environments, including freshwater, and can grow in cold and warm conditions (Sowers, 2009). However, they tend to thrive in higher temperatures, as warm water contains less dissolved oxygen than colder water environments (USGS, 2019). Barbera et al. (2019) discusses that methane produced in these conditions is released into the environment through bubbling, direct diffusion, or by aerenchyma of vascular plants. In contrast, methanotrophs consume and oxidize the methane produced by methanogenic bacteria reducing the release of methane in the atmosphere from the wetlands (Murrel and Jetton, 2009). Methanotrophs exist in both aerobic and anaerobic environments. Aerobic methanotrophs take in methane and oxygen to oxidize, and anaerobic methanotrophs take in methane and something other than oxygen, such as sulfate or iron to oxidize (Fenchel, et al., 2012). Methane emissions in wetlands are equal to the difference of methane produced by methanogenesis minus the methane consumed by methanotrophs.

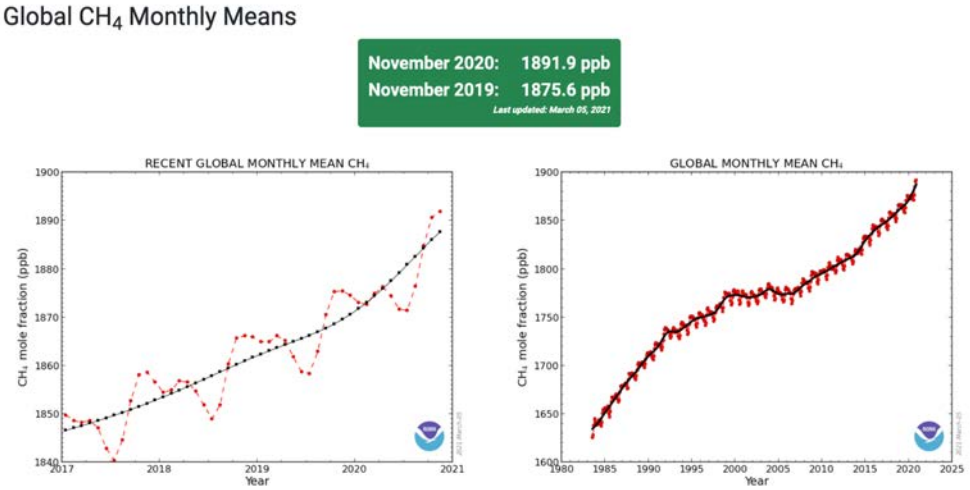


Figure 1 Recent NOAA Global Methane Monthly Means data (2020).

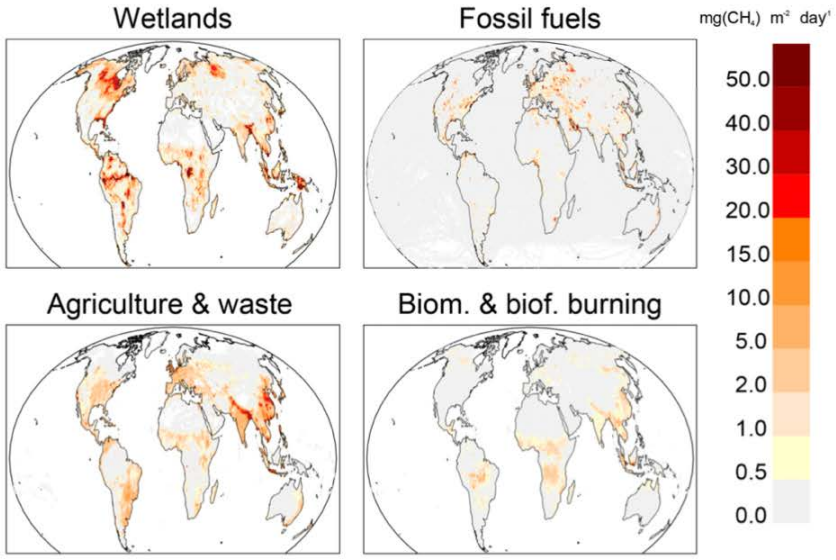


Figure 2 Comparison of Methane emission ($mg(CH_4)m^{-2}d^{1}$) in wetlands, fossil fuels, agriculture & waste, and Biomass & Biofuel Burning from 2000-2012. From Saunois et al. (2016).

Wetlands exist globally, in different types of climates and cover around 5% to 8% of the land surface (Salimi et al., 2021). There are two main types of wetlands: boreal and tropical wetlands. Boreal wetlands are in northern areas of high latitudes and at relatively low temperatures. Tropical wetlands are in lower latitudes with relatively high temperatures. Both types of wetlands are currently being affected by climate change. In tropical wetlands, increasing temperatures are causing an increase in precipitation which is then promoting methanogenesis by affecting surface inundation, water table depth, and soil moisture (Zhang et al., 2017). In boreal wetlands, increase in temperature is causing the thawing of permafrost and increasing the rates of soil microbial activity which leads to greater methane production (Zhang et al., 2017; Walter et al., 2007). By using the representative concentration pathway, Zhang et al. (2017) calculated that if there are no climate mitigations, methane emissions in boreal wetlands are predicted to increase by 18.05 Tg (to 41.69 Tg) at the end of the 21st century, and methane emissions in tropical wetlands are predicted to increase by 48.36 Tg (to 87.37 Tg). However, the budget of atmospheric methane has limited accuracy in climate change projections, as factors such as bubbling in boreal wetlands are not incorporated in the global methane budget (Walter et al., 2007).

Methane emission in wetlands is extremely sensitive to environmental changes, so it is important to consider how methane emission is changing with changing climate. Temperature plays the main role in the methane emission occurring in wetlands; it has a positive feedback relationship with methane production (Whiting and Chanton, 1993; McCalley et al., 2014). Rising temperatures are affecting methane production in wetlands through thawing permafrost, the oxygen concentration in water, vegetation growth, microbial activity, and finally water level (Whiting and Chanton, 1993; McCalley et al., 2014).

The variable of interest for this proposed research is water level, as many scientific studies have collectively found an inconclusive answer to how exactly water level is affecting methane emission in wetlands (Harrison et al. 2017; Hondula, et al., 2021; Singh et al., 2000).

A paper by Harrison et al. (2017) discusses an experiment that was performed in a reservoir environment that experiences pronounced summer drawdowns in water level. Harrison et al. (2017) found in their research that there is a relationship occurring between methane flux and water level. The paper found that methane consumption and the rate of bubbling are being influenced by water level, which is overall impacting the methane emissions at the site. The results showed that average pre-drawdown rates of methane flux were not very high, but during the drawdowns, rates of methane flux were higher than 90% of the other reported reservoir methane flux rates. However, the research described in the paper is dealing with a much more controlled site than the wetland of focus for this project, as Harrison et al. (2017) could easily study the drawdowns in a reservoir. Harrison et al. (2017) discusses that there is a lot of uncertainty in their research and indicates that more investigation needs to be done on the subject to further understand the relationship between changing water levels and methane flux. Harrison et al. (2017) has overall inconclusive findings that appear to be a common theme in different studies focusing on changing variables, such as water level, and methane emission. Similarly, Hondula et al. (2021) performed a study on small freshwater ecosystems in which they focused on several different factors in inundated and non-inundated soils. This study suggests that methane fluxes were seen in rising and falling water levels, however they concluded that inundated duration was more impactful that water depth. They found that falling water levels appeared to be producing a larger methane flux than rising ones. They discuss that differences in water level appear to be a better indicator of methane fluxes, as there appears to be a lagged relationship between water level and methane emission (Hondula, et al., 2021). Tangen, B. A., and Bansal, S. (2019) also found fluxes of methane to be strongly affected by hydrological states and produce a lag effect of several weeks from transitions of wet to dry states.

Singh et al. (2000) discusses implications of seasonal changes and methane emission in wetlands. This article goes in-depth about how different seasonal factors affect methane production, which relates to this proposed research. The paper infers low emissions of methane are caused by lower water depth and intermittent drying up of lakes, which drastically reduces the methane formation in oxic conditions. Singh et al. (2000) suggests based on their findings that there are more methane emissions in summer seasons, in which water levels tend to be higher. However, similar to other papers, Singh et al. (2000) concluded that the correlation between water level and methane emissions is difficult to find as there are many other contributing factors occurring. Singh et al (2000) infers seasonal methane fluctuations in wetlands are caused by vegetation, water depth, temperature, organic carbon, and soil pH. The paper suggests that vegetation plays a big role in methane production in wetlands: in the research they found that less vegetated areas produced less methane compared to unvegetated areas. Vegetation is dependent on water level, so the amount of vegetation is considered over the course of this experiment. Soil pH is seen as a stable characteristic and does not change with the season in Singh et al. (2000) and does not need to be taken into consideration. In much of the literature, there are yet many uncertainties about methane emissions in wetlands.

IV. OBJECTIVES OF RESEARCH

The focus of this project is to investigate how water levels are influencing the methane emission of wetlands. Temperature has an unavoidable impact on water level, and it appears hard to separate the two variables if they are correlated with each other in the field. Therefore, two hypotheses were included in this thesis. The water level alternative hypothesis states, “wetlands

with lower water levels/depth are expected to emit more methane than higher ones." This hypothesis is reasoned by the knowledge that higher water levels dilute the supply of organic substrate for methanogens and the productivity of aquatic vegetation. The temperature alternative hypothesis states, "wetlands with higher temperatures are expected to emit more methane than lower temperature wetlands." This hypothesis is reasoned by the knowledge that methanogens tend to thrive in warmer environments, because there is less dissolved oxygen in water with higher temperatures (USGS, 2019; Sowers, 2009). The water level null hypothesis states, "there is no relationship between methane emissions at wetlands with high water levels/depths or low water levels/depths." The temperature null hypothesis states, "there is no relationship between methane emissions at wetlands with high temperatures or low temperatures."

V. METHODS OF ANALYSIS

1. Sampling Methods

Field research was conducted to view the impact of water level on methane emission in wetlands. Methane was sampled from two outlet sites and one inlet site (Figure 3) in a wetland located at the University Golf Course in College Park, MD (Figure 4). Various personnel were at the site to assist me in collecting the samples, such as Dr. Haghnegahdar, Jiayang Sun, Dr. Evans, Samantha Volz, or Dr. Prestegaard. The sites were sampled bi-weekly from early November to April, mainly during the winter and early spring season. Vegetation was a variable that needed to be considered based on studies on vegetation playing a role in methane production in wetlands (Singh et al., 2000). The outlet site has less vegetation than the inlet of the wetland, so these two sites were selected to aid in isolating vegetation as a factor. The sites were sampled at the same time daily (9 am - 12 pm) to maintain similar temperature conditions throughout the seasons. Methane samples were collected by syringing gas from methane chambers installed at the sites. Before sampling the lids from the gas chambers were removed for several minutes to air out any gas that had accumulated in the chamber. Then three to four measurements of water depth were recorded from inside each chamber using a meter stick and then averaged. The ground at the wetland was very uneven therefore several measurements of the water depth needed to be taken to account for the uncertainty. Additionally, water and air temperatures were taken with a thermometer prior to and after sampling, these temperatures were then also averaged. After the chamber lid was placed back on, the gas chambers needed to be sealed to prevent any gas from escaping during sampling. To create the seal, water was poured into the area between the lid of the chamber and its base. When the chamber is completely sealed it is difficult to remove the lid as the water creates a suction effect. At each site, a gas sample was collected using a 20 mL syringe at 0 minutes, 30 minutes, 60 minutes, 90 minutes, and 120 minutes. The sample collection was time sensitive, as it is important to make sure samples are collected at the exact same time increments. By collecting samples at thirty-minute increments we were able to view estimates of methane accumulation rates at the different sites. To get a clear reading of the methane in the chamber, any possible residual gas in the syringe and tube was pushed out before each sample collection. After each collection the syringes and vials must be sealed correctly, so no gas is lost from the sample.

Figure 3 A photo from Google Earth of the University of Maryland Golf Course. The blue arrows point to the two different sites sampled at the Wetland. The Outlet Site (upper arrow) and the Inlet Site (lower arrow).

Figure 4 Photos of the three sites at the University of Maryland Golf Course that were monitored and sampled throughout this experiment. (From left to right; Inlet Site (GI), Outlet Site (GO), Outlet Pond Site (GOP).

2. Gas Chromatographic Determination of Methane Concentration

After collecting samples from the field, the concentration of methane was measured in the lab using the Flame Ionization Detector Gas Chromatograph (GC). This instrument determines the concentrations of methane in the collected samples from the wetland. The GC burns off the other gases in the sample, and the concentration of oxygen and methane is recorded in the results. Two cubic centimeters (cc) of the collected samples were injected into the instrument. To check for precision and reduce human error each sample was analyzed by the GC twice. The GC produces a methane area curve (Figure 4), that we then compare to the concentration of methane in the air of the lab room, which is used as the standard. The area curve is converted to find the methane concentration of the sample in parts per billion (ppb). The concentration of methane is converted from the area curve by the equation: $\frac{(area\ of\ methane\ curve\ of\ sample * 2000\ ppb)}{area\ of\ methane\ curve\ in\ lab\ air}$ (2000 ppb represents the atmospheric average methane concentration). The methane production rates for each sample are then able to be compared with the varying water levels and temperature conditions.

Figure 5 An example of the results produced by the Gas Chromatograph. The first two odd-looking curves before time .5 represent the previous gas in the GC being removed. The area curve at around time

1.317 is the oxygen concentration, and the last area curve at time 2.203 represents the methane concentration of the sample.

VI. PRESENTATION OF DATA, AND ANALYSIS OF UNCERTAINTY

1. Sampling Uncertainty

This is an overall new technique and a learning process; therefore, experimental, and human errors were expected to occur over the course of this thesis. Throughout this thesis my skills using the different instruments and techniques have improved. This improvement can be seen as errors in sampling data were reduced over time. The error source in technique and equipment (e.g., the seals of the gas chamber and syringes) was monitored by the investigation of the methane accumulation results. The methane concentration in the samples is expected to increase with time as methane accumulates in the chamber, therefore it is possible to spot a collection error in the data if the concentrations do not follow this trend or have extreme outliers. After the samples containing methane gas are processed by the GC, the data from Figure (6) was condensed into individual graphs for each chamber. Datum that was clearly an inaccurate representation of the sampling period were not analyzed in final figures. For example, on day one of sampling the valve of GO and GOP was left open from time zero to time thirty minutes, this error was shown on the graph as there was a clear drop in methane concentration for the second measurement at both these sites (Figure 6). The data containing sampling errors are highlighted in red on Figure (7), these data show R^2 values lower than .5 which represent large variance from the linear model that further establishes these data as sampling errors.

Throughout the process of sampling the starting methane concentrations in the chambers at time zero were abnormally higher than the expected atmospheric methane concentration of 2,000 ppb. This anomaly could be explained by (1) higher average methane concentration surrounding these sites, (2) disruption causing additional methane bubbles from the ground to fill the chamber, (3) or from additional possible stored gas in the chambers. Air samples were collected at the site to gain more information of the methane concentration in the surrounding air at the wetland. The samples were taken at the surface near the chamber, at a meter distance from the chamber, and at over a meter distance from the chamber. The air samples gave a mixed reading of results varying from 2,262 ppb to 15,111 ppb (Figure 8). The variability in the data could potentially be due to environmental factors such as wind. More research is necessary to find the expected starting point of methane concentration at this site, but these results confirmed the methane concentration collected at time zero from the chamber is much higher than the methane concentration in the surrounding air. The initial samples from the chambers are between 30,000 ppb to 260,000 ppb (Figure 6). The high starting methane concentration could be caused by disruption around the site when setting up the chambers and collecting the samples. If you simply poke a stick into the wetland, methane bubbles will rise to surface, therefore it is reasonable to think walking around the chambers could cause additional bubbles of methane to be released from the ground. To reduce this effect longer tubes were attached to the chambers so samples could be collected farther away. However, this modification did not reduce the effect of disruption that occurs while preparing the chambers. The initial gas in the chamber is much higher than it should be according to Figure (8) results and further thinking and redesign of this aspect of the sampling procedure should be explored to reduce this uncertainty. Fortunately, this high starting concentration, however, should not impact the methane accumulation rate of the chambers.

Samples are left out for several days to a week before being run through the GC, during this time there could be possible gas leakage from the syringes. To see if gas was lost from the samples over time, the samples were retested a week later to see if the methane concentration decreased. There appeared to be no significant loss in methane concentration in the sample over the course of a week. A sample measured on 11/10/21 had a recorded methane concentration of 34,129 ppb, the same sample retested over two weeks later on11/19/21 had a methane concentration of 35,691.1 ppb. The syringes appear to be sufficient in staying sealed over the course of at least two weeks.

Lastly, there were several environmental uncertainties to be aware of during sampling. Sampling at the same time (9 am to 12 pm) every other week allowed for the potential to collect samples when water levels are different but have similar temperature conditions for sequential weeks within the same cold and warm seasons. This thesis took place from early November to April, and therefore data was only collected from winter and early spring seasons. This limited period of sampling did not include data from the warmer seasons. In winter and early spring, when the water temperature is lower the dissolved oxygen concentration in the water is high, and in the summer and fall the opposite relationship occurs (USGS, 2019). Since methanogens tend to thrive in more anaerobic conditions, we were unable to collect samples when methanogenesis was more active (Sowers, 2009). This additional data could have potentially given more insight on the effects of temperature on methane emissions. Furthermore, the temperature was measured with a thermometer before and after sampling, and these two measurements were averaged to reduce uncertainty in the changing temperature over the two hours. These temperature measurements were then compared with the temperature data collected by loggers installed at the site by Dr. Prestegaard. Water level measurements taken at the site also had uncertainty associated with them. The water depth was measured using a ruler; however, the sediment was unlevel in the wetland and it was difficult to get an accurate reading. A +/- 5 cm error is assigned to these measurements to limit the uncertainty. The three to four recorded measurements of water depth usually varied by an average of 2-5 cm, therefore an uncertainty of +/- 5 cm was applied.

2. Analytical Uncertainty

Additionally, there is a potential instrument error while using the GC. The minimum gas required to flush the GC of previous samples is 2 cc, and we injected only 2 cc of sampled gas into the GC. There is potential error in not injecting enough gas into the instrument to completely clear the GC. In addition to this, it is difficult to consistently inject the exact same amount of gas into the GC for each sample. To reduce this error, the samples were analyzed twice and averaged. The standard deviation (STD) for the samples processed by the GC was calculated by finding the variance associated with the two GC readings of each sample (Figure 6). The STD for the samples was relatively small compared to the large methane concentrations and appear relative to other sources of uncertainty (See Figure 6).

To further investigate the two hypotheses additional graphs were created to compare the relationships between water depth and methane accumulation rate, and between temperature and methane accumulation rate (Figure 9a and 9b). These graphs aid in looking for a trend between the two variables and methane emission. A linear regression model was fit to the data to test the null hypothesis. A linear regression model was chosen to view the relationship between the independent and dependent variables. The hypotheses predicted a linear relationship between the variables e.g. “wetlands with lower water levels are expected to emit more methane than higher

ones" and "wetlands with higher temperatures are expected to emit more methane than lower temperature wetlands." A relationship other than linear was not explored in this thesis, but possibilities of a non-linear relationship could be investigated in future papers. Furthermore, a three-dimensional graph was created to compare the relationship of the two variables of temperature and water level (Figure 10). Figure 10 allows us to see if temperature and water level are related or in fact independent of each other when effecting the methane accumulation rate. There is some uncertainty associated with these graphs as methanogens and methanotrophs tend to have a 2-to-3-week incubation period. This would require a trailing 14-to-21-day average to further excess the hypothesis. This is of importance because previous papers (Hondula et al., 2021); Tangen, B. A., & Bansal, S., 2019) discuss an apparent lagged relationship between water level and temperature and methane emission. The significance of this will be explored further in the discussion section.

11/5/21						
Time (min)	GO Methane Conc. (ppb)	GO STD of GC (ppb)	GOP Methane Conc. (ppb)	GOP STD of GC (ppb)	GI Methane Conc. (ppb)	GI STD of GC (ppb)
0	34129.00	45.67	3598376.97	3785.78		
30	5454.41	39.11	1188562.03	1261.53		
60	193578.35	39.11	3329138.74	234.90		
90	247822.94	106.60	3690368.87	307.63		
120	271158.38	604.06	3522242.74	577.11		
11/17/21						
Time (min)	GO Methane Conc. (ppb)	GO STD of GC (ppb)	GOP Methane Conc. (ppb)	GOP STD of GC (ppb)	GI Methane Conc. (ppb)	GI STD of GC (ppb)
0	84621.60	58.78	180287.82	234.02	87081.01	2356.84
30	67739.00	44355.13	342634.30	1363.08	22308.84	19.96
60	113834.99	1.11	385630.93	5257.13	30279.98	532.37
90	45470.94	73.20	380250.96	168.58	93365.23	11978.28
120	51410.87	1266.59	420950.51	7306.75	89322.41	1618.18
12/3/21						
Time (min)	GO Methane Conc. (ppb)	GO STD of GC (ppb)	GOP Methane Conc. (ppb)	GOP STD of GC (ppb)	GI Methane Conc. (ppb)	GI STD of GC (ppb)
0	102344.01	133.64	542918.78	166.67	995448.95	1364.91
30	156976.82	666.69	1598291.28	2198.27	1113598.30	2231.31
60	152083.17	590.11	1527400.81	373.89	1146635.99	4219.37
90	169807.11	717.74	1406501.50	2312.39	1133129.36	1458.01
120	176031.14	609.63	1503818.09	2099.17	1138097.33	1030.07
2/9/22						
Time (min)	GO Methane Conc. (ppb)	GO STD of GC (ppb)	GOP Methane Conc. (ppb)	GOP STD of GC (ppb)	GI Methane Conc. (ppb)	GI STD of GC (ppb)
0	265004.48	167.13	362681.88	10.32	293455.96	218.94
30	383358.55	153.60	629933.53	1076.37	332697.57	274.08
60	378474.33	338.61	925873.83	1085.54	327793.74	405.90
90	385153.84	284.17	939625.64	127.24	335414.94	533.03
120	382039.54	266.97	939688.05	135.26	326231.40	7513.95
2/23/22						
Time (min)	GO Methane Conc. (ppb)	GO STD of GC (ppb)	GOP Methane Conc. (ppb)	GOP STD of GC (ppb)	GI Methane Conc. (ppb)	GI STD of GC (ppb)
0	131758.78	5410.97547	91017.13	1442.07435	8883.25	158.13
30	141181.70	4401.417847	116099.71	7470.210249	21694.75	690.60
60	149610.39	4135.127375	368295.14	n/a	41704.20	1887.14
90	167097.01	5555.265021	315050.72	1568.650305	59528.43	2231.56
120	183306.62	7230.314207	295610.22	1162.645317	83387.67	3290.74
3/7/22						
Time (min)	GO Methane Conc. (ppb)	GO STD of GC (ppb)	GOP Methane Conc. (ppb)	GOP STD of GC (ppb)	GI Methane Conc. (ppb)	GI STD of GC (ppb)
0	172970.59	2647.38758	367144.00	5260.798668	59074.81	1465.28
30	213185.23	2836.594955	368975.53	8043.208067	103401.40	2627.05
60	241903.60	4397.240038	430654.60	13240.22187	168612.86	4438.92
90	258929.03	5068.938852	472971.03	14284.14641	181593.15	4348.74
120	280073.36	5379.653234	736635.22	19634.24389	189944.12	4225.84
4/11/22						
Time (min)	GO Methane Conc. (ppb)	GO STD of GC (ppb)	GOP Methane Conc. (ppb)	GOP STD of GC (ppb)	GI Methane Conc. (ppb)	GI STD of GC (ppb)
0	70515.24	541.19	345762.35	5435.17	8789.27	1014.13
30	315687.44	122.65	634883.81	7734.77	11634.40	194.60
60	327762.27	322.05	1110903.73	1178.58	18726.86	28.78
90	345808.86	433.06	870101.94	2646.32	28071.40	21.93
120	343060.64	1480.08	955749.37	7159.19	34689.03	6.85

Figure 6 Table showing raw data of the methane concentration in ppb of every sample collected at each site in 30-minute increments. The dates of the sampling period are highlighted in yellow.

Data for site GI was not collected on 11/5/21 because this site was not added until 11/17/21. The standard deviation (STD) of the GC for the methane concentrations of samples is included in the table. The STD appears to be relatively small compared to the large methane concentrations, therefore the GC produces a small degree of variation.

Site: GO	Date	Air T (C°)	Water T (C°)	Water Depth (cm)	Methane Accumulation Rate (ppb/min)	Regression Estimate	R^2
	11/5/21	14.18	n/a	5.1	2388.10	y=2388.1x + 7123.2	0.85
	11/17/21	12.3	9.72	5.4	-295.63	y=-295.63x + 90353	0.26
	12/3/21	9.82	6.22	3.8	534.02	y=534.02x + 119408	0.76
	2/9/22	5.4	0.64	4.5	786.22	y = 786.22x + 311633	0.50
	2/23/22	8.28	13.75	5	430.04	y=430.04x + 128789	0.98
	3/7/22	10.46	13.7	6.3	866.50	y = 866.5x + 181422	0.97
	4/11/22	11.2	7.45	6.8	1917.40	y = 1917.4x + 165524	0.59
Site: GOP	Date	Air T (C°)	Water T (C°)	Water Depth (cm)	Methane Accumulation Rate (ppb/min)	Regression Estimate	R^2
	11/5/21	14.18	n/a	5.5	7831.80	y=7831.8x + 25958	0.12
	11/17/21	12.3	9.11	8.1	1729.80	y=1729.8x + 238162	0.75
	12/3/21	9.82	6.11	6.3	5766.70	y=5766.7x + 969784	0.39
	2/9/22	5.4	1.25	8	4879.00	y = 4879x + 466820	0.80
	2/23/22	8.28	13.5	8.7	2027.10	y = 2027.1x + 115587	0.59
	3/7/22	10.46	14.92	8.3	2809.90	y = 2809.9x + 306680	0.76
	4/11/22	11.2	7.55	7.5	4850.60	y = 4850.6x + 192442	0.59
Site: GI	Date	Air T (C°)	Water T (C°)	Water Depth (cm)	Methane Accumulation Rate (ppb/min)	Regression Estimate	R^2
	11/5/21	n/a	n/a	n/a	n/a	n/a	n/a
	11/17/21	12.3	10.72	1.9	251.80	y=251.8x + 49364	0.12
	12/3/21	9.87	9.8	0	1016.10	y=1016.1x + 1.0444e6	0.59
	2/9/22	9.07	6.78	6.5	227.56	y = 227.56x + 309465	0.40
	2/23/22	8.48	12.39	4.6	622.81	y = 622.81x + 5671.2	0.99
	3/7/22	10.65	13.56	3.8	1133.10	y = 1133.1x + 72539	0.89
	4/11/22	13.4	10.55	5.5	227.46	y = 227.46x + 6734.9	0.98

Figure 7 Table showing: Air T(degC), Water T(degC), Water Depth (cm), Methane Accum. Rate (ppb/min), Regression Estimate, and Regression Line Fit (R^2) for each sampling period at the three sits. Top to bottom: GO (Golf course Outlet Site), GOP (Golf course Outlet Pond Site), GI (Golf course Inlet Site). The rows highlighted in red signify the data that was thrown out due to a R^2 values smaller than .5, caused by data with large variance from the regression model. Data from sites are expected to accumulate at a consistent rate as the chamber fills with gas, these data with small R^2 values represent errors in sampling.

Site	Methane Conc. at surface	Methane Conc. at .5 to 1 m	Methane Conc. at + 1 m
GI	10279.40	2505.84	2432.24
GO	2624.62	2262.73	2277.73
GOP	2273.62	5584.88	15111.19

Figure 8 Table showing the measured methane concentrations in the air surrounding the three different chambers (GI, GO, GOP) at the wetland. Data shows the methane concentration from the air at the surface of the chambers, at .5 to 1 m distance from the chamber, and at over 1 m distance from the chamber. These air samples were collected to see the expected methane concentration at time zero at these sites and how it compares to the atmospheric average concentration of 2,000 ppb. These data show a variety of different methane concentrations. Mainly we see a methane concentration around 2,200 ppb to 2,500 ppb which is consistent with the atmospheric average. However, at the GI and GOP sites there appears to be a unusually high concentration of methane at varying distances. These anomalies could be caused by environmental factors such as wind, but this remains uncertain. Data confirms the initial (T0) samples collected from the chambers are much higher than the methane concentration of surrounding air at the sites.

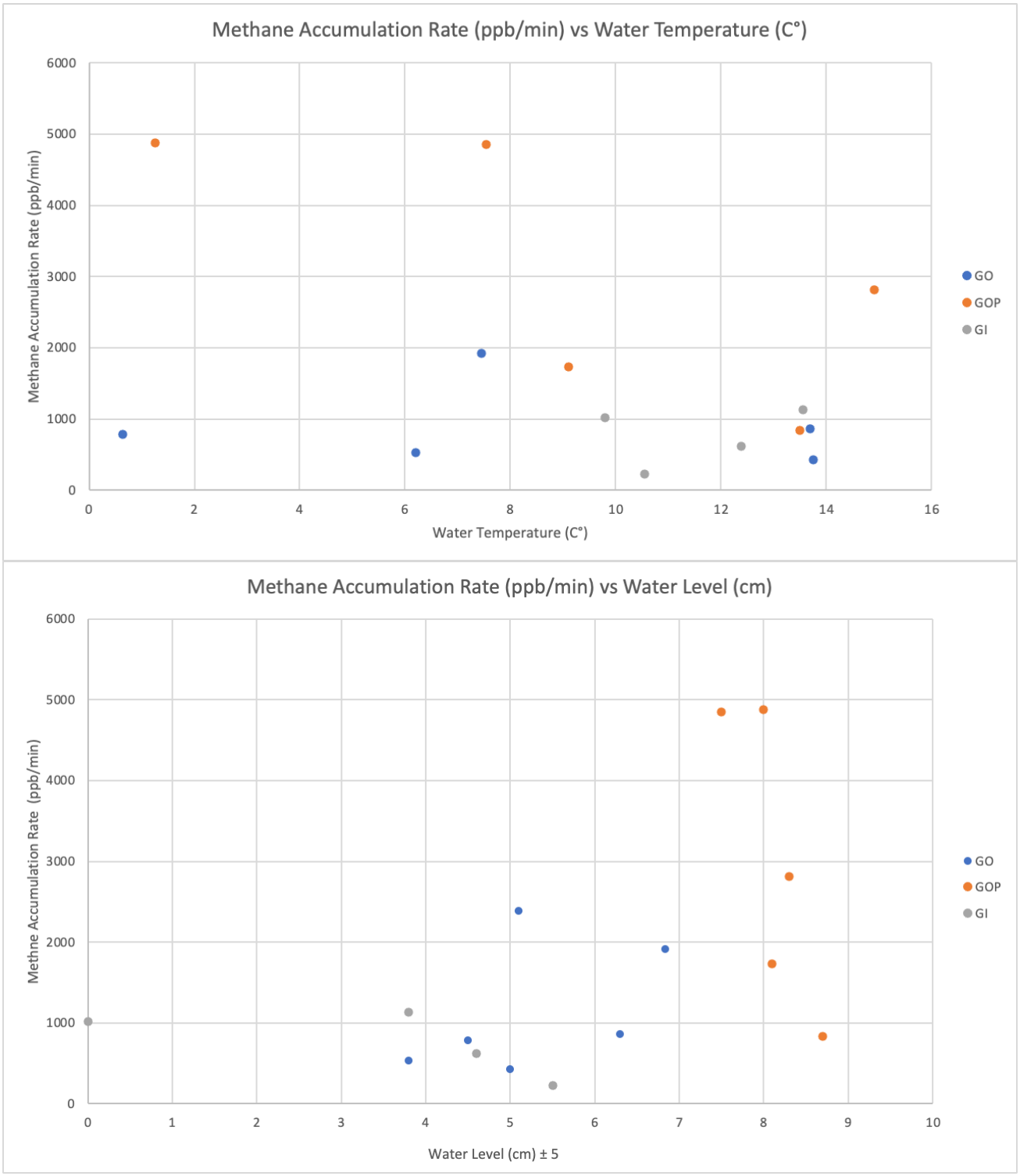


Figure 9 (9a.) Graph showing Methane Accumulation Rate (ppb/min) against Water Temperature (C°) of all three sites (Top). **(9b.)** Graph showing Methane Accumulation Rate (ppb/min) against Water level (cm) of all three sites (Bottom).

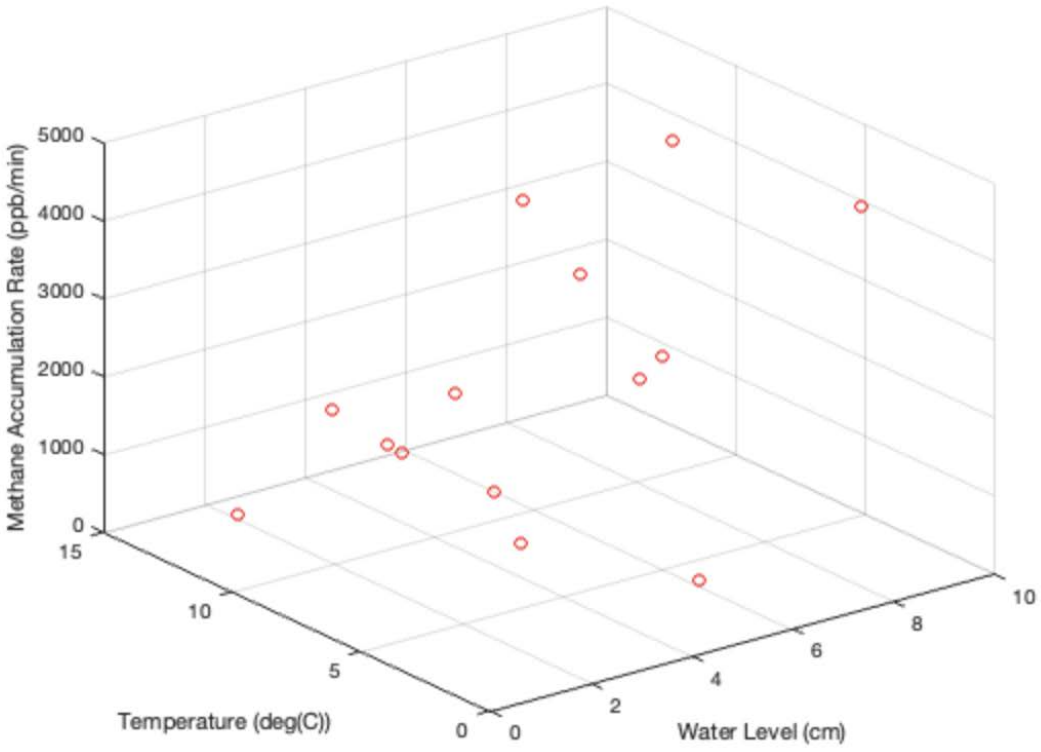
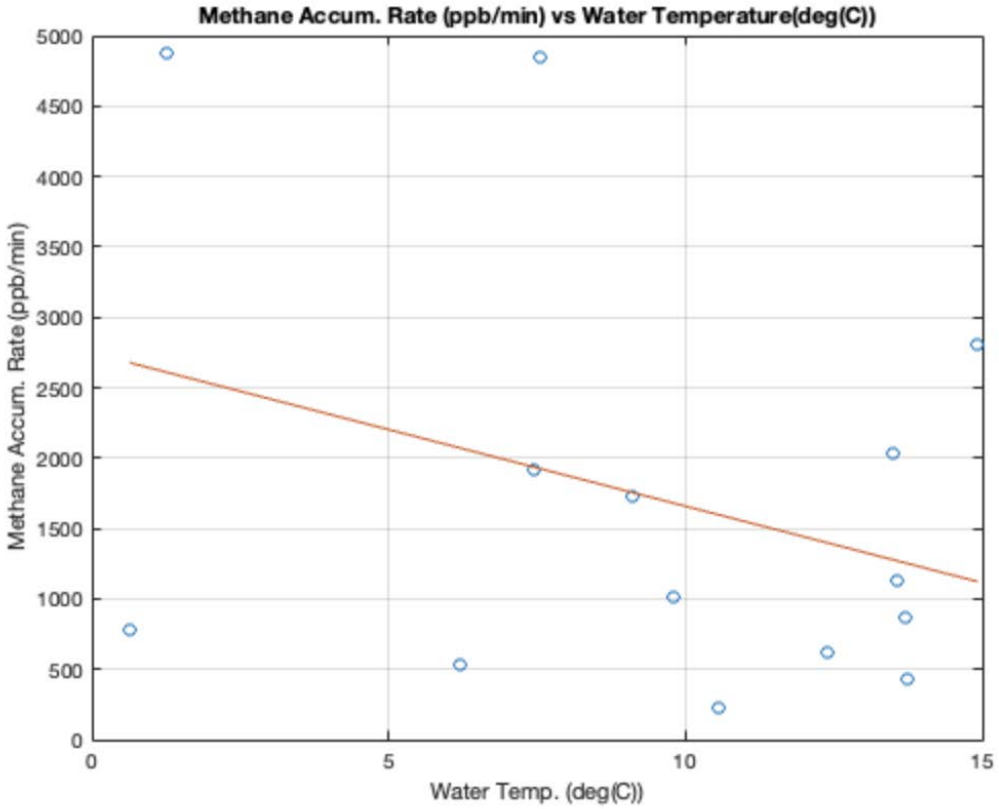


Figure 10 Three-dimensional graph plot of Methane Accumulation Rate (ppb/min) vs. Water Temperature (C°) vs. Water Level (cm). This plot allows a look at the two variables, water level and temperature, at the same time.


Methane Accum. Rate (ppb/min) vs Water Temperature(deg(C))
Methane Accum. Rate (ppb/min)
Water Temp. (deg(C))
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
0
5
10
15

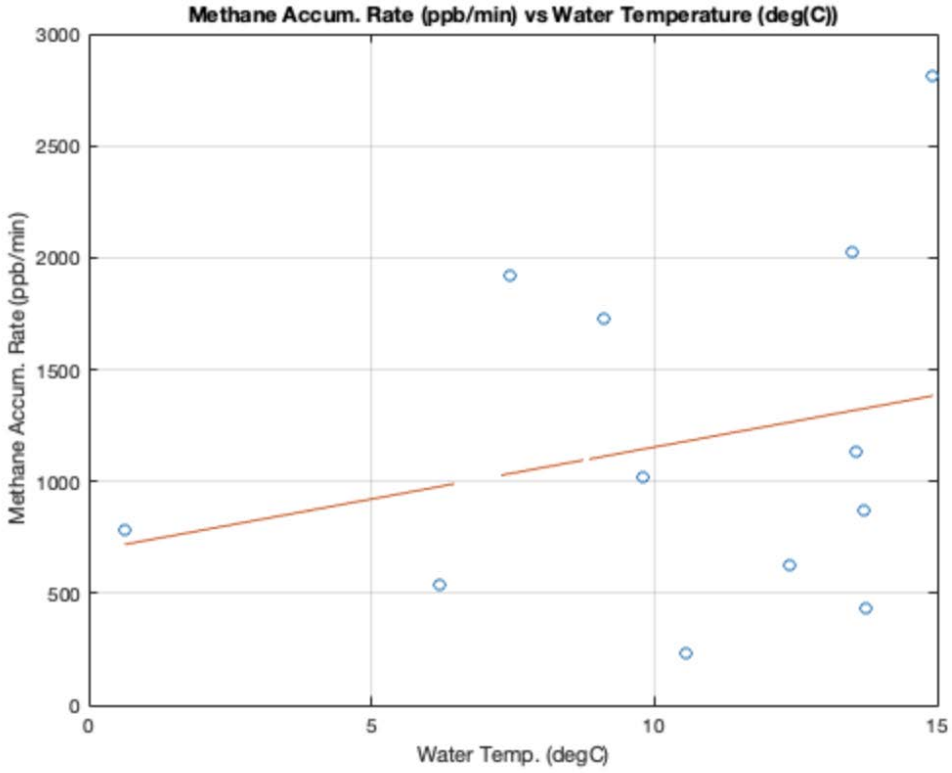

Methane Accum. Rate (ppb/min) vs Water Temperature (deg(C))
Methane Accum. Rate (ppb/min)
Water Temp. (degC)
0
500
1000
1500
2000
2500
3000
0
5
10
15

Figure 11(a,b) Figure (**11a.**)(top) shows Methane Concentration Rate (ppb) vs Water Temperature (deg(C)) of all samples fit to a linear regression model. Figure (**11b.**) (bottom) shows Methane Concentration Rate (ppb) vs Water Temperature (deg(C)) of samples, without the two outliers seen in **(11a.)**, fit to a linear regression model.

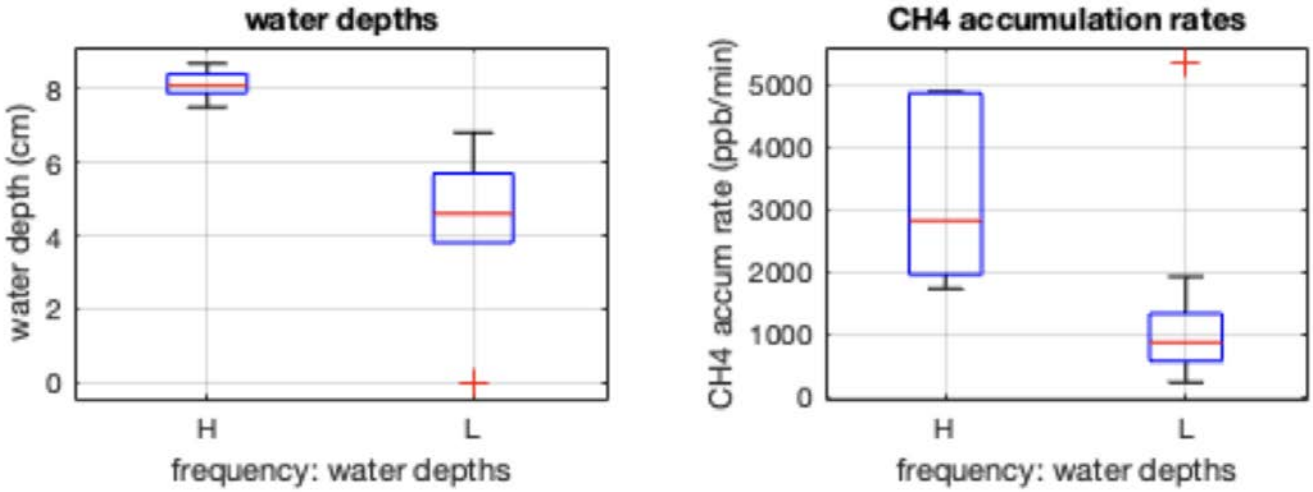


Figure 12 Boxplot **12a** (left) shows the frequency of the various sampled water levels for low and high-water depths. Boxplot **12b** (right) shows the frequency of different methane accumulation rates occurring at low and high-water levels. H is representative of high-water levels; L is representative of low water levels.

Sampling date	11/17/21	12/3/21	2/9/22	2/23/22	3/7/22	4/11/22
Outlet Site WL (m)	0.87	0.86	0.894	0.88	0.88	n/a
OutletSite T(deg(C))	12.26	9.91	5.82	5.97	6.79	n/a
Inlet Site WL (m)	n/a	0.41	0.11	0.11	0.12	n/a
Inlet Site T(deg(C))	n/a	9.83	4.77	8.73	11.17	n/a
3 Weeks prior to sampling	10/27/21	11/12/21	1/19/22	2/2/22	2/14/22	3/21/22
Outlet Site WL (m)	0.88	0.93	0.9	0.89	0.89	0.87
OutletSite T(deg(C))	15.93	13.02	6.95	5.91	6.06	8.26
Inlet Site WL (m)	n/a	n/a	0.46	0.41	0.1	0.15
Inlet Site T(deg(C))	n/a	n/a	6.4	4.7	4.36	10.82

Figure 13 Table showing the temperature and water depth conditions data recorded by temperature loggers and depth sensors at the wetland. The table is separated by data from days of sampling and data from days 3 weeks prior to sampling to show the lag conditions for the microbes.

VII. DISCUSSION OF RESULTS

Figure (10) includes a three-dimensional graph of the relationship of the methane accumulation rate for each chamber versus water level and water temperature. The 3-D plot was used to see if there was a relationship occurring between temperature and water level, and if temperature had an impact on water level's effect on methane emission. Figure 10 shows high and low temperatures correlated with both high and low water levels, therefore representing a lack of correlation between the two variables. This figure rules out the possibility of the temperature being responsible for the methane emissions associated with water level, and therefore allows us to look at the two variables independent of each other.

Figure (9a) shows the relationship between methane accumulation and temperature. The data appears sporadic, but except for two data points there could be a potential tendency of higher methane accumulation rates in higher temperatures. To further access a relationship between the temperature and methane emissions a linear regression model was used. The linear regression model for methane accumulation rate vs. water temperature had a slope of -108.8293 ppb/min*deg(C) with a standard error of 90.5598 ppb/min*deg(C) (Figure 11a). The null hypothesis was accepted as the t-observed for slope (-1.2017) was inside the t critical ($\alpha = .05$) value of +/- 1.782. This means the slope between methane accumulation rate and temperature is zero and there is no predictive relationship is occurring. There appeared to be a tendency toward higher methane accumulation rate with increasing T except for two values. One of those values (2/9/22, GOP, T: 1.25 deg(C), Meth. Accum. Rate: 4879 ppb/min - See Figure 7) was sampled when there were ice sheets in the wetland. The other value (4/11/22, GOP, T: 7.55 deg(C), Meth. Accum. Rate: 4850.6 ppb/min - See Figure 7) was sampled during an unseasonably cold day after a large rainstorm. The ice and rainstorms may have impacted the actual temperature conditions of the water. As discussed, prior methane emissions may rely on a two-to-three-week lag time in relation to water level and temperature. Temperature conditions from three weeks earlier appear to have been warmer. Three weeks before 2/9/22 temperatures were five degrees warmer (6.96 deg(C)) than the water temperature and three weeks before 4/11/22 temperatures were ten degrees warmer (17.23 deg(C)) (Prestegaard et al., 2022). It appeared reasonable to remove this data and run the linear regression again due to these samples being possibly unrepresentative of a response to the current conditions at the time of sampling. However, this is a comparison of water temperature and air temperature, since the water temperatures for these sites are unavailable, therefore removing the samples isn't completely justified. If the two samples are removed the linear regression model has a slope of 46.4714 ppb/min*deg(C) with a standard error of 57.6115 (Figure 11b). The exclusion of these points reduced the standard error and shifted the slope from negative to positive. However, even with the exclusion of these points the null hypothesis was accepted and a strong relationship between methane accumulation rate (ppb/min) and temperature (deg(C)) was not found, as the t observed value for slope (.806) was within the t-critical value for $\alpha = .05$ (+/-1.82). The three-week lag time of the microbial communities reacting to changing variables provides an area of uncertainty in the results. The

consideration of this lag altered the regression slope from negative to positive, and therefore may have an impact on revealing a relationship between the independent and dependent variables.

Similarly, in Figure (9b) there appeared to be a potential trend favoring higher methane accumulation rates with high water levels. The GOP site had significantly higher water levels than the other two sites (GI and GO). These high and low water level sites were separated to test for the difference between means for high and low water depth samples of methane accumulation rates using the two-sample t-test of difference between means. Boxplots were created to qualitatively evaluate the high and low water level data (Figure 12). By comparing the mean methane accumulation rates for high and low water levels it appeared there is a difference between the two. The two-sample t-test rejected the null hypothesis as the t-observed (2.207) was outside the t-critical value for $\alpha = .05$ (+/- 2.179). The standard error of means associated with this test was 855.6277. In Figure (b) there is an outlier at a water depth of 3.8 and its associated methane accumulation rate, 5334.02 ppb/min. This outlier was interesting and was investigated further to see if it was influenced by prior water level conditions. According to Dr. Prestegaard's water depth sensors the water level at the time of the sample was around .86 m, three weeks prior the water level was around .93 m. In terms of cm, which the water levels are usually measured in, the difference in water depth appears to be significant. To speculate further, the two-sample t-test was performed again with the absence of the outlier. The results greatly favored the difference between means in the methane accumulation rate of high and low water depths, as the null hypothesis was rejected. The new t-observed (3.7054) was further outside the t-critical value for $\alpha = .05$ (+/- 2.179). The standard error of means associated with this test was lower at 746.6687. However, to exclude this point from the data it is necessary to discuss whether all the other water depths were not likely influenced by big changes in water depth three few weeks prior. Examining the temperature loggers and water depth sensors at the site helped determine the rational of removing the outlier. The other water depths only varied by approximately .01 m, whereas the outlier decreased by .07 m from 11/17/21 to 12/3/21(Figure 13). Since the water depth of the outlier varied by .06 m more than any of the other samples it is reasonable to exclude this from the data for being inconsistent with the current environmental conditions at the time. Overall, it appears low and high-water levels have different impacts on methane emission in wetlands. However, it is unclear in which ways water level impacts methane emissions, but based on Figure (9b) the data appears to be in favor of rejecting the alternative hypothesis for water level. Higher water levels appear to be producing more methane than lower ones. The higher water levels could potentially be producing more methane due to higher water depths reducing the amount of dissolved oxygen in the water (USGS, 2019).

Future experiments could aid in further investigating the effect of water level and temperature on methane emissions. Additional collection of data could help make it clearer whether the null hypotheses should be rejected or accepted. More data will allow for a more in-depth ANOVA analysis to assess the contributions of temperature and water depth. If sampling occurred over longer periods of time the different seasons could provide a larger range of temperature and water depth data. It would also be useful to perform this experiment in an environment where water level can be more controlled like Harrison et al. (2017) studying drawdowns in reservoirs. An environment with a larger range of water levels would also be useful as there was a small variation in water depth at the Golf course wetland, however, to sample gas in higher water depths a taller gas chamber will be needed. There were several occasions gas was unable to be sampled due to extremely high-water levels flooding the

chambers. Overall, this problem should not yet be dismissed, and more research and design modifications in the future will aid in finding more conclusive answers.

VIII. CONCLUSIONS AND BROADER IMPLICATIONS

This research project was expected to provide an answer to the question of how water level is impacting methane emissions in wetlands. This thesis investigated methane emissions over the course of several months in different areas of a wetland located at the University of Maryland Golf Course, as the sites experienced different temperature conditions and fluctuating water levels. Samples were collected at the sites by syringing gas from methane chambers in 30-minute increments. The selected sites at the outlet and inlet of the wetland were sampled bi-weekly and the concentration of methane collected was compared to water level and temperature data. Currently, it appears the impact of temperature on methane emissions is limited, as the null hypothesis was upheld in linear regression models of the data. However, the data shows there is a difference in the effect of low and high-water levels on methane emissions in wetlands. The alterative water level hypothesis was not rejected nor upheld, but with additional research using this experimental design the effect of water levels on methane emission can be explored further. This experiment uses new instruments and techniques that have not been frequently used in the past to study methane in wetlands and can provide others with knowledge and procedural skills to use when testing this problem or similar problems in the future. There has been a lot of trial and error over the course of the on-site sampling that have helped the experimental design become more reliable and efficient. It would be very interesting to see this experiment being carried out over longer time periods in which the wetland sites can be sampled more frequently in all seasons. The potential key to finding a relationship between water level and methane emission could simply be in a larger sample size. This project will allow others to continue to search for answers regarding the impact of methane, how it varies with environmental effects, and the importance of its different sources. The broader implications of this research are to provide more information about methane emissions in wetlands to help further understand the growing climate crisis. Hopefully, in the future a more in-depth investigation using the mass spectrometer in the Panorama Lab could provide isotopic signatures from the purified methane samples, which could give a better understanding of the workings of methanogens and methanotrophs in wetland sites with varying water levels. This future work could allow for more insight into the mystery of methane and provide information on how and where all this methane in the atmosphere is coming from.

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