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The effects of warming on carbon and microbial community wetland dynamics at Turnbull National Wildlife Refuge, Washington

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THE EFFECTS OF WARMING ON CARBON AND MICROBIAL COMMUNITY WETLAND DYNAMICS AT TURNBULL NATIONAL WILDLIFE REFUGE, WASHINGTON

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ABSTRACT

THE EFFECTS OF WARMING ON CARBON AND MICROBIAL COMMUNITY WETLAND DYNAMICS AT TURNBULL NATIONAL WILDLIFE REFUGE, WASHINGTON

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Wetlands are biodiverse ecosystems that play a key role in the biogeochemical cycling of carbon. In the face of global warming, wetland hydroperiods could shift causing changes in their functionality. My field experiment surveyed 3 plots within 12 wetlands of each hydroperiod class (i.e. 12 permanent, 12 semi-permanent, 12 ephemeral). This survey was paired with a warming experiment by placing open top warming chambers on half of each wetland type. In chapter one, I compared carbon dynamics across hydroperiods and treatment by measuring soil organic carbon (in Summer 2018) and effluxes of carbon dioxide and methane (in Summer 2018, Fall 2018, and Spring 2019). I found no differences across wetland type or warming treatment in soil organic carbon. Results also showed that when comparing wetland fluxes within each season, there were no differences between wetland types or warming treatments. CO_2 fluxes were consistently higher than CH_4 fluxes within and across all seasons. The seasonality of CO_2 and CH_4 fluxes differed, which lead to a significant interaction between gas and season.

In chapter two, I report on differences in both wetland soil microbial abundance and diversity between treatments. Total abundance was measured by qPCR to quantify

16S rRNA gene copy numbers. Soil microbial diversity, composition, and relative abundance was determined using Illumina sequencing protocol for the amplification of the V4-V5 region of the 16S rRNA gene. Results showed that both abundance and diversity decreased with warming and depth within permanent wetlands, although no variation in species composition was found. Abundance also decreased with warming in ephemeral wetlands, but diversity did not. Although Chapter 1 highlights a general stability in carbon dynamics with warming, Chapter 2 illustrates that the microbial communities are changing with warming and that they might not be as stable over time. As global warming progresses, it is important to continue wetland ecosystem research in longer term studies due to its high potential for climate change mitigation.

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Introduction

Functional Importance of Wetlands

Wetlands are biodiverse systems known for providing critical habitat, improving water quality (Halabisky et al. 2017; Shiau et al. 2016), and mitigating floods (Evers et al. 2017). These habitats are important for wildlife and are a refuge during droughts. Water quality in particular is improved in wetlands by trapping sediment and soils, filtering out nutrients and removing contaminants in the water (Hayes et al. 2017). Wetlands are unique because they sequester carbon; anaerobic conditions in the soil (Larsen et al. 2015; Sutfin et al. 2016) make decomposition of organic material slower than in other soils allowing accumulation of organic material (Jiang et al. 2016). Decomposition is so slow that it creates deep, highly fertile soil that could potentially hold decade to millennia old carbon (Larsen et al. 2015). Wetlands with organic rich soils are net carbon sinks and are important in the global cycling of carbon dioxide and other gases (Jahangir et al. 2016; Kayranli et al. 2010).

Rich in organic matter, wetland soils known as histosols, allow highly productive plant communities and rival tropical rainforests in overall productivity (Kayranli et al. 2010). Although known for being carbon sinks, wetlands may also act as greenhouse gas sources due to the natural release of methane and carbon dioxide from microbial oxidation-reduction reactions (Bridgham et al. 2013; Shiau et al. 2016). Examining carbon release from wetlands is of critical importance because as the input of greenhouse gases into the atmosphere increases, the positive feedback of warming with climate change and greenhouse gas release will continue (Turetsky et al. 2014). Methane, in

particular, is known to be 25-30 times more efficient than carbon dioxide at trapping heat (Shiau et al. 2016). Understanding how environmental factors affect carbon fluxes will be important in understanding climate change mitigation.

Anthropogenic Impacts on Wetlands

Land use changes affect roles of wetlands in the global carbon cycle. Human alteration of wetlands and climate change have shifted the balance of carbon and methane movement between wetlands and the atmosphere (Evers et al. 2017; Shiau et al. 2016). With the increase in human population, there has been a major increase in farming, infrastructure, and roads without effective environmental mitigation. Wetland drainage for agricultural usage, in particular, has been a major source of wetland losses causing soil organic carbon that had accumulated slowly over centuries to be lost in a matter of days (Cao et al. 2017; Mitra et al. 2005).

Historically during urban development, wetlands were drained to create crop lands and new housing developments. Currently as populations continue to increase, there is an increasing demand on urban development. This means drainage of wetlands for agricultural use and continued urban sprawl will lead to large carbon dioxide and methane release into the atmosphere (Maucieri et al. 2017). Destruction of these wetlands can also cause increased water pollution due to removal of natural filtration systems in place, as well as diminished nutrient availability with lower water levels (Kayranli et al. 2010). This will continue to cause positive feedback reactions with warming and greenhouse gas emissions. These land use changes with climate change could test the adaptability of wetlands to new changes in temperature and precipitation regimes (Mitra et al. 2005).

Current climate change models have predicted that we can expect increased precipitation levels and flash floods during wet seasons followed by longer periods of drought during the dry season (Crowther et al. 2016; Wang et al. 2017). This will affect wetlands in two fundamental ways: the number of functioning wetlands will decline, and the geographic location of wetlands will shift (Day et al. 2008). This is largely due to the effect temperature and precipitation has on the hydrology of wetlands (Kayranli et al. 2010; Shanley et al. 2015). The hydrology of each wetland is the major factor determining how the soil develops, and it is therefore critical to its overall function (US 2008). Wetlands are classified based on their hydroperiod (Evers et al. 2017), and each class will react differently to warming and drought (Ma et al. 2017).

Wetland Classification

Some wetlands are permanently flooded, while others only seasonally (Woodward et al. 2014). There are three major types of wetlands based on differences in hydroperiod (Correa-Araneda et al. 2017), including permanent, semi-permanent and ephemeral wetlands (Halabisky et al. 2017; Woodward et al. 2014). Permanent wetlands are inundated with water year-round, while semi-permanent wetlands hold water most of the year but dry out by the end of the fall season. Ephemeral wetlands temporarily hold water, usually seasonally in the spring and early summer, but dry out by late summer (Correa-Araneda et al. 2017; Halabisky et al. 2017). Each of these wetland types support high levels of biodiversity and provide specific ecosystem functions including its impact on biogeochemical cycles. It is important to note that geographic location and anthropogenic changes may determine how the inputs and outputs of carbon dioxide and

methane from wetlands affect habitat and water quality functions (Hardy et al. 2003; Moreno-Mateos et al. 2012; Schmidt et al. 2015).

Current Changes in Carbon and Methane Fluxes

Preliminary studies have found that increased temperatures stimulate carbon dioxide and methane emissions, while increasing water levels decrease carbon dioxide and increase methane emissions, reducing total carbon stocks (Fellman et al. 2017). Drying of wetlands reduced or eliminated carbon sinks, converting some wetlands into net carbon sources (Maucieri et al. 2017). As dry periods and atmospheric temperatures continue to increase, more carbon will be released into the atmosphere due to wetland drought. Since carbon storage is enhanced under anaerobic conditions, permanent wetlands provide optimal conditions for the accumulation of organic matter (Crowther et al. 2016). Therefore, we can expect a higher loss of organic carbon as more permanent wetlands continue to dry out. Similarly, drainage of wetlands has caused changes in soil carbon emission rate with decreasing moisture rates (Maucieri et al. 2017). This also shows that carbon dioxide fluxes and dissolved organic carbon production are significantly affected by soil temperature (Oertel et al. 2016; Romero-Olivares et al. 2017) and moisture (Manzoni et al. 2012; Shiau et al. 2016).

Methane fluxes are also likely to respond to increased temperatures. Processes such as denitrification and methane production are dependent on the oxygen status of soil and sediment (Romero-Olivares et al. 2017). Anaerobic soils and sediments produce methane, while in well-drained soils methane oxidation prevents the release of methane. The water level of wetlands not only influences the amount of methane emitted to the atmosphere, but also the retention of carbon in that system (Kayranli et al. 2010). In fact,

maximum methane fluxes occurred under warmer, wetter conditions (Turetsky et al. 2014). This is because warming accelerates metabolic processes, and as less oxygen is available under wetter conditions more methane production will occur (Bardgett et al. 2008; Fierer 2017).

Current Limitations

Wetland drainage and climate change can cause major decline in the number of functioning wetlands at a global scale. Historically, as much as 221 million acres of wetlands covered land in the United States alone. Currently, over half of these wetlands have been lost. In fact, there is now less than 50% of the worlds functioning wetlands left and this is predicted to decrease over the next century. Even with mitigation and creation of new wetlands, many will have to become well established for over 100 years to be considered carbon sinks. This makes wetland conservation an important effort at a global scale (Davidson 2014).

Currently, there is a need for determining how wetlands differ in their stability and function. How soil organic carbon stocks, carbon dioxide, and methane fluxes are changing in soil with warming has been the main area of focus for most wetland studies (Fellman et al. 2017). Many studies have been done in greenhouse or laboratory settings, under controlled environments. Relating these findings to what can happen under natural conditions with other environmental factors is a major limitation on current wetland research. In fact, studies that have done field experiments limit their study to areas that exhibit little to no environmental fluctuations during sampling.

Purpose and Objectives

The purpose of this project is to measure the effects of experimental warming on wetland ecosystem functioning. Specifically, the study will examine how climate change is likely to impact carbon storage and fluxes. Our main objectives were to compare controlled vs. warmed plots in permanent, semi-permanent, and ephemeral wetlands and determine differences in soil organic carbon, methane fluxes, and carbon dioxide fluxes.

Hypotheses

Hypothesis 1: Permanent wetlands will store the most soil organic carbon due to inundated conditions.

Hypothesis 2: Permanent wetlands will emit the most methane and least carbon dioxide.

Hypothesis 3: Experimental warming will shift permanent wetlands to more ephemeral conditions, triggering higher carbon emissions.

Methods

Experimental Design

We used aerial image data depicting wetland hydroperiods and predicted changes in hydroperiod over the next decade (Halabisky et al. 2017) to classify wetlands at Turnbull National Wildlife Refuge (Figure 1.1). We classified wetlands based on their hydroperiod as either permanent, semi- permanent, or ephemeral, selected 12 wetlands of each type, and randomly selected 3 plots within each wetland. All plots were at least 4.6 m apart (Table 1.1, Figure 1.2).

Soil Organic Carbon and Carbon Emissions

Soil cores were collected from each plot from depths of 0-10cm, and 10-20 cm layers. A dry or wet soil core sampler was used depending on the water level of each wetland. If water levels within the plot exceeded 1 meter, a wet soil core sampler was

used. Replicates in each wetland were composited into singular homogenous samples, running them through 10mm sieve for soil organic carbon analysis (Tan 2005). Samples from each wetland were collected once during the Summer 2018 field season, specifically June 10-July20. Soil moisture was calculated by comparing wet weight of the soil to the dry weight. Soil organic matter was quantified by using a drying oven (50°C) and then a muffle furnace (450°C) to compare dry weight to ash weight. We then estimated the amount of soil organic carbon (SOC) present by assuming 58% of the dry organic matter was carbon (Pribyl 2010).

Methane and carbon dioxide emission levels were collected at half of the permanent and ephemeral wetlands using a static chamber to collect gas samples (using methods described by Shiau et al. 2016). We used either a floating or stationary static chamber depending on the water level of each wetland at each sampling season (Figure 1.3). If water levels at the plot were greater than or equal to 0.3 meters high, a floating chamber was used. Chambers were connected to an external pneumatic valve which when opened, was pumped to deliver gas into a specialized gas collection bag. Chambers were flushed out prior to collecting gas at both 5min and 15min intervals. These samples were then sent out for gas chromatography analysis at Isotech lab, Champaign, Illinois. Carbon dioxide and methane gas samples were collected in Summer 2018, Fall 2018, and Spring 2019 to account for changes in seasonal flux variability. Measurements were taken at the same 2-4-hour time period each day to control for diurnal fluctuations.

Experimental warming

A warming study was performed by using passive open- top chambers at one plot within 18 of the 36 wetlands (6 of each type) to mimic warming due to accelerated

climate change (using methods described by Johnson et al. 2013). Warming chambers were modified by adding flexibility and drainage to allow regular movement and flow of water in and out of the chamber during waterlogged states (Figure 1.4). Chambers warmed plots an average of 3 °C and ranged between 2-5 °C warming capabilities depending on the season. Soil organic carbon, carbon dioxide, and methane samples were collected as mentioned above, and compared to non- warmed (control) plots.

Statistical Analyses

A statistical analysis of variance (ANOVA) type II was used to compare total soil organic carbon, carbon dioxide fluxes, and methane fluxes between warmed and controlled permanent, semi-permanent, and ephemeral wetlands using R (version 3.5.3). Pairwise comparisons were analyzed using *emmeans* functionality in R studio, which allowed us to examine interactions or differences between specific treatments and wetland types as well as any interactions between treatments.

Results

Soil Moisture

Soil moisture between ephemeral, semi-permanent, and permanent wetland types were not significantly different ($p=0.072$), although a slight difference was seen between permanent and ephemeral wetlands specifically ($p=0.058$) (Figure 1.5). Warming treatments did not affect total soil moisture between wetland types ($p=0.24$).

Soil Organic Carbon

No differences were seen between wetland type in total soil organic carbon (SOC) ($p=0.59$) (Figure 1.6). Although an increased trend in SOC can be seen in ephemeral

wetlands in comparison to permanent and semi-permanent types. Warming treatments did not affect total organic carbon between wetland type (p=0.40).

Carbon Fluxes

In general, carbon dioxide fluxes were higher than methane fluxes across all seasons (p=0.0071) (Figure 1.7). There was also a significant interaction between season and gas (p=0.034). Pairwise statistics show that CO_2 fluxes in summer ephemeral control wetlands were significantly higher than CH_4 fluxes (p=0.0022). Similarly, CO_2 fluxes were higher than CH_4 fluxes in fall permanent control (p=0.0002) and fall permanent warmed wetlands (p=0.045). Fall ephemeral wetland CO_2 fluxes were also higher in control plots compared to warmed plots (p=0.0028). Comparing seasons, ephemeral wetland CO_2 fluxes were higher in the summer than in the fall (p=0.052) and in the spring (p=0.015). In contrast, permanent wetland CO_2 fluxes were higher in the fall than in the spring (p=0.0052).

When comparing carbon dioxide and methane fluxes across season, there were differences in carbon dioxide fluxes between seasons (p=0.040), although not specifically between wetland type (p=0.30) and warming treatment (p=0.34) (Figure 1.8, Figure 1.9). Pairwise tests showed permanent control CO_2 fluxes were higher in the fall than in the spring (p=0.050). Between control and warmed treatments, fall ephemeral wetlands showed higher CO_2 fluxes in the control than in the warmed plots (p=0.021). There were also CO_2 flux changes between ephemeral and permanent warmed wetlands in the fall season, showing ephemeral wetlands had higher CO_2 fluxes (p=0.035). In contrast, no differences in methane fluxes were seen across season (p=0.67), wetland type (p=0.31) or warming treatment (p=0.27). Although, a slight difference can be seen between summer

vs. fall permanent warmed wetlands ($p=0.068$) and summer vs. spring permanent warmed wetlands ($p=0.077$) that show a slightly higher CH_4 flux in summer in both instances.

Discussion

Overall, wetland carbon dynamics showed to be relatively stable within these systems during the duration of this research. Not only were there no differences in total organic carbon, soil moisture, and carbon fluxes across wetland types, but we also didn't see any differences across our warming treatments. This could possibly be due to a variety of factors that give wetlands their specificity and uniqueness across hydroperiods within the same region.

Carbon Storage and Wetlands

Soil organic carbon (SOC) did not differ between ephemeral, semi-permanent, and permanent wetlands. This is an unusual finding, because permanent wetland soils are in anaerobic conditions longer, creating higher carbon retention capacity. Many other studies have shown anaerobic conditions promote higher carbon storage (Hayes et al. 2017; Kayranli et al. 2010; Sutfin et al. 2016), but Fellman et al. (2017) results were similar to ours in that organic carbon did not differ between wetland types. They explained that this is most likely due to the complexity and diversity of soil organic matter, and the higher likelihood that temperature dependence of microbial decomposition of soil carbon compounds of differing chemical composition and substrate vary (Bardgett et al. 2008). Alternatively, another possibility is that ephemeral wetlands at TNWR may accumulate more SOC than expected because of their high collection of litter on the soil surface, especially during early fall. Organic materials from this surface

litter may be incorporated into the soil organic matter after winter snow pack and spring rains.

Soil moisture means were not different across wetland types, which was surprising since wetland types are defined by differences in hydroperiod, and were sampled in the summer, when differences are likely to be largest. In fact, previous studies that also used gravimetric soil content to calculate total soil moisture have shown that there are usually differences between wetland type (Fellman et al. 2017; Mitra et al. 2005). In our study specifically, large variation in soil moisture within each wetland type caused by differences in elevation, gradient (slope), and vegetation cover (Natural Resources Conservation Service) may have made it difficult to detect the differences between wetland types. In addition, ephemeral and semi-permanent microbiomes can have higher tolerance and retain soil moisture in drought seasons when soils were collected (Manzoni et al. 2012; Toth et al. 2017). Few studies differentiate wetland types and their soil moisture differences, but those that do illustrate that soils in ephemeral or semi-permanent wetlands retain their hydric soils through drought, which can most likely be attributed to soil microbial communities (Don et al. 2017; Graaff et al. 2015; Serna-Chavez et al. 2013).

Wetland Carbon Fluxes

Carbon dioxide fluxes were consistently ~30x higher and experienced more variability than methane fluxes in each season and overall. A similar finding has been seen across different wetland ecosystem studies. It seems that even when anaerobic properties are at their highest potential, the amount of methane fluxes still don't exceed those of carbon dioxide fluxes (Hernandez et al. 2018; Mitra et al. 2005). This suggests

that of all the carbon leaving the wetland system, a higher proportion comes from aerobic processes. There was also a clear interaction between season and gas. Mainly, these interactions were due to differences in CO_2 fluxes, as no changes were seen across or within each season in CH_4 fluxes in either wetland type, or warming treatment. Additionally, differences between carbon dioxide and methane were smaller in the spring compared to fall and summer seasons, when spring fluxes were lower. This might be attributed to the fact that aerobic microbes are transforming methane into carbon dioxide and water before it gets released through the water surface (Mitra et al. 2005). Our results were different from other studies specifically in that they didn't show significant seasonal variation with methane flux. Previous studies have shown that methane fluxes increase with high water levels due to increased anaerobic conditions (Altor & Mitch et al. 2008, Hernandez et al. 2018). Although, Hernandez et al. (2018) specifically showed that even though they found the highest fluxes in the months of heavy rain, there were no differences seen across wetland type. Since we also didn't find differences across wetland type, this might suggest that hydroperiod, along with moisture level, don't affect the differences seen in carbon fluxes.

Across wetland type within the fall season, ephemeral wetlands experienced less CO_2 fluxes than permanent wetlands. This is not surprising, due to the fact that ephemeral wetlands at this season have very low to no surface water compared to permanent flooded wetlands, lowering their anaerobic capabilities and therefore providing more suitable conditions for aerobic processes (Bridgham et al. 2013; Jahangir et al. 2016; Kayranli et al. 2010). Ephemeral wetland carbon dioxide fluxes increased in the summer compared to the fall and the spring. In contrast, permanent wetlands had higher carbon dioxide fluxes

in fall compared to spring. Previous studies show that as temperatures increase, we can expect higher carbon dioxide and methane fluxes within medium wetland water levels (Altor & Mitsch 2008; Hernandez et al. 2018). This would make the most sense when looking at summer ephemeral fluxes, since they would be in optimal mid to low surface water levels and high temperatures conditions for high carbon dioxide flux (Cao et al. 2017). A similar observation could be said for the permanent wetlands. Experiencing highest fluxes in the fall compared to spring would make sense due to the same pattern seen in Cao et al. (2017), where anything higher than mid-level water levels would start reducing carbon dioxide fluxes due to higher anaerobic capacities. Permanent wetlands already hold their waterlogged state year-round, and during spring months this water level is often exaggerated, limiting the potential for aerobic processes.

Effects of Warming on Wetland Carbon Storage and Fluxes

Not surprisingly, there were no changes seen in SOC with warming treatment. Incorporation of organic material to the soil organic carbon pool is a very slow process, and the four-month time point when we collected our soils simply wasn't enough time for warming treatments to create an impact (Kayranli et al. 2010; Trumbore et al. 2000). Another reason we might not have seen differences across wetland type is that ephemeral wetlands are wetter than we realized, especially considering that we saw no differences in soil moisture between warmed and control treatments. Various factors including groundwater level, temperature, substrate availability, nutrient level and microbial population affect decomposition rate and therefore affect carbon sequestration (Mitra et al. 2005). Larger amounts of methane are produced from the lower anaerobic levels, while the upper levels produce carbon dioxide and oxidize methane released from lower

levels. Similar to other studies, our results show that there is high variation within a single wetland, including changes in slope and gradient within the landscape and spatial diversities (Mitra et al. 2005).

When comparing fluxes in fall warming treatments, control plots in ephemeral wetlands showed higher carbon dioxide fluxes than warmed plots. This was more plausible due to the fact that plots in the warmed treatment no longer had stagnant water. Cao et al. (2017) showed that whenever water levels are too high or too low, CO_2 fluxes will decrease due to optimal fluxes occurring at "medium" water levels. Therefore, although our ephemeral control plots showed higher fluxes than the warmed plots it might just be because the surface of the plot has lost all water due to high evapotranspiration in the warming chamber.

Based on these results we can see that within a year study there is very few to no changes in carbon storage or carbon fluxes with warming. This is an unusual finding in wetland carbon flux studies, although most of those have been greenhouse warming studies (e.g. Kayranli et al. 2010; Yang et al. 2014). It is important to note that differences seen here incorporate the dynamic nature of a field study, where previous findings may have been skewed due to the general limitations you may find in a regular greenhouse study. Although, Mitra et al. (2005) did find similar results to ours in that they described wetlands should be relatively small sources of greenhouse gases if kept in healthy conditions, meaning that the soil remains undisturbed and the native plant communities are allowed to thrive. Methane production in our study was specifically low compared to other studies (Shiau et al. 2016; Turetsky et al. 2014), which might mean that wetland methane production in our region isn't as much of a concern compared to

other regions. Although, this doesn't mean that we won't see these changes over time if we were to continue this study. As mentioned previously with soil organic carbon results, carbon sequestration is a very slow process. For this reason, wetlands are crucial in that they act as a carbon sink and therefore hold decade to millennia old carbon. Even though we saw generally stable systems across all wetland hydroperiod types and warming treatments, this might not be the case over time. This is especially true when considering the potential these studies have for climate change mitigation efforts. If we were to ignore the future impacts of temperature and precipitation regime changes to wetlands, these might no longer be the stable systems we see today. Future directions of this research should include more dynamic mechanisms to measure soil carbon stock changes with warming. For example, a more detailed comparison of labile vs. recalcitrant carbon sources, as well as an overall carbon pool measurement might be useful in distinguishing how carbon sequestration might differ across wetland types and warming treatments. More importantly, it would be of great interest to study the wetland microbial communities that play a big part in the biogeochemical cycling of carbon in these systems (Chapter 2).

References

Altor, A. and Mitch, A. (2008). Pulsing hydrology, methane emissions and carbon dioxide fluxes in created marshes: a 2-year ecosystem study. Wetlands 28, 423- 438.

Bardgett, R. D., Freeman, C. and Ostle, N. J. (2008). Microbial contributions to climate change through carbon cycle feedbacks. Isme Journal 2, 805-814.

Bridgham, S.D., Cadillo-Quiroz, H., Keller, J.K., and Zhuang, Q.L. (2013). Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales. Global Change Biology 19, 1325-1346.

Cao, R., Xi, X.Q., Yang, Y.H.S., Wei, X., Wu, X.W., and Sun, S.C. (2017). The effect of water table decline on soil CO_2 emission of Zoige peatland on eastern Tibetan Plateau: A four year in situ experimental drainage. Applied Soil Ecology 120, 55-61.

Correa-Araneda, F., De Los Rios-Escalante, P., Figueroa, R., and Parra-Coloma, L. (2017). Temporal Distribution of Crustaceans in Forested Freshwater Wetlands: Responses to Changes in the Hydroperiod. Crustaceana 90, 721-734.

Crowther, T. W., Todd-Brown, K. E. O., Rowe, C. W., Wieder, W. R., Carey, J. C., Machmuller, M. B., Snoek, B. L., Fang, S., Zhou, G., Allison, S. D., Blair, J. M., Bridgham, S. D., Burton, A. J., Carrillo, Y., Reich, P. B., Clark, J. S., Classen, A.T., Dijkstra, F. A., Elberling, B., Emmett, B. A., Estiarte, M., Frey, S. D., Guo, J., Harte, J., Jiang, L., Johnson, B. R., Kroel-Dulay, G., Larsen, K. S., Laudon, H., Lavallee, J. M. et al. (2016). Quantifying global soil carbon losses in response to warming. Nature 540, 104-108.

Davidson, N. (2014). How much wetland has the world lost? Long-term and recent trends in global wetland area. Marine and freshwater research 65, 934-941.

Day, J.W., Christian, R.R., Boesch, D.M., Yanez-Arancibia, A., Morris, J., Twilley, R.R., Naylor, L., Schaffner, L., Stevenson, C. (2008). Consequences of climate change on the ecogeomorphology of coastal wetlands. Estuaries and coasts 31, 477-491.

Don, A. Bohme, I.H., Dohrmann, A.B., Poeplau, C., and Tebbe, C.C. (2017). Microbial community composition affects soil organic carbon turnover in mineral soils. Biology and Fertility of Soils 53, 445-456.

Evers, S., Yule, C.M., Padfield, R., O'Reilly, P., and Varkkey, H. (2017). Keep wetlands wet: the myth of sustainable development of tropical peatlands - implications for policies and management. Global Change Biology 23, 534-549.

Fay, P. A., Guntenspergen, G. R., Olker, J. H., and Johnson, W. C. (2016). Climate change impacts on freshwater wetland hydrology and vegetation cover cycling along a regional aridity gradient. Ecosphere 7(10), 1-12.

Fellman, J. B., D'Amore, D. V., Hood, E., and Cunningham, P. (2017). Vulnerability of wetland soil carbon stocks to climate warming in the perhumid coastal temperate rainforest. Biogeochemistry 133, 165-179.

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15, 579-590.

Graaff, de M.A., Adkins, J., Kardol, P., and Throop, H.L. (2015). A meta-analysis of soil biodiversity impacts on the carbon cycle. Soil 1, 257-271.

Halabisky, M., Lee, S.Y., Hall, S.A., and Rule, M. (2017). Mapping wetland hydrology across an ecoregion and developing climate adaptation recommendations. University of Washington, Remote sensing and geospatial lab, University of Washington climate impacts group, SAH Ecologia LLC, US Fish and Wildlife Service Turnbull Lakes National Wildlife Refuge 1-38.

Hardy, J. (2003). Climate change causes, effects, and solutions. John Wiley& Sons 1-44.

Hayes, M.A., Jesse, A., Hawke, B. Baldock, J., Tabet, B., Lockington, D., and Lovelock, C.E. (2017). Dynamics of sediment carbon stocks across intertidal wetland habitats of Moreton Bay, Australia. Global Change Biology 23, 4222-4234.

Hernández, M.E. and Moreno-Casasola Barceló, P. (2018). Carbon fluxes and stocks in freshwater wetlands in Mexico. Madera y Bosques 24, 1-12.

Jahangir, M.M.R., Richards, K.G., Healy, M.G., Gill, L., Muller, C., Johnston, P., and Fenton, O. (2016). Carbon and nitrogen dynamics and greenhouse gas emissions in constructed wetlands treating wastewater: A review. Hydrology and Earth System Sciences 20, 109- 123.

Jiang, Y., Zhuang, Q., and Stitch, S. (2016). Importance of soil thermal regime in terrestrial ecosystem carbon dynamics in the circumpolar north. Global and planetary change 142, 28-40.

Johnson, C.P., Pypker, T.G., Hribljan, J.A., and Chimner, R.A. (2013). Open Top Chambers and Infrared Lamps: A Comparison of Heating Efficacy and CO_2/CH_4 Dynamics in a Northern Michigan Peatland. Ecosystems 16: 736-748.

Kayranli, B., Scholz, M., Mustafa, A., and Hedmark, A. (2010). Carbon Storage and Fluxes within Freshwater Wetlands: A Critical Review. Wetlands 30, 111-124.

Larsen, S. and Alp, M. (2015). Ecological thresholds and riparian wetlands: an overview for environmental managers. Limnology 16, 1-9.

Ma, M. J., Baskin, C. C., Yu, K. L., Ma Z., and Du, G. Z. (2017). Wetland drying indirectly influences plant community and seed bank diversity through soil pH. Ecological Indicators 80, 186-195.

Manzoni, S., Schimel, J. P., and Porporato, A. (2012). Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930-938.

Maucieri, C., Barbera, A. C., Vymazal, J., and Borin, M. (2017). A review on the main affecting factors of greenhouse gases emission in constructed wetlands. Agricultural and Forest Meteorology 236, 175-193.

Mitra, S., Wassmann, R., and Vlek, P.L.G. (2005). An appraisal of global wetland area and its organic carbon stock. Current Science 88 (1), 25-35.

Moreno-Mateos, D., Power, M. E., Comin, F. A., and Yockteng, R. (2012). Structural and Functional Loss in Restored Wetland Ecosystems. PLOS Biology, 10.

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., and Erasmi, S. (2016). Greenhouse gas emissions from soils A review. Chemie Der Erde-Geochemistry 76, 327-352.

Pribyl, D.W. (2010). A critical review of the conventional SOC to SOM conversion factor. Geoderma 156, 75-83.

Romero-Olivares, A. L., Allison, S. D., and Treseder, K. K. (2017). Soil microbes and their response to experimental warming over time: A meta-analysis of field studies. Soil Biology & Biochemistry 107, 32-40.

Schmidt, J., Schulz, E., Michalzik, B., Buscot, F., and Gutknecht, J. L. M. (2015). Carbon input and crop-related changes in microbial biomarker levels strongly affect the turnover and composition of soil organic carbon. Soil Biology & Biochemistry 85, 39-50.

Shanley, C. S., Pyare, S., Goldstein, M.I., Alaback, P.B., Albert, D.M., Beier, C.M., Brinkman, T.J., Edwards, R.T., Hood, E., MacKinnon, A., McPhee, M.V.,

Patterson, T.M., Suring, L.H., Tallmon, D.A., and Wipfli, M.S. (2015). Climate change implications in the northern coastal temperate rainforest of North America. Climatic Change 130, 155-170.

Serna-Chavez, H. M., Fierer, N., and van Bodegom, P. M. (2013). Global drivers and patterns of microbial abundance in soil. Global Ecology and Biogeography 22, 1162-1172.

Shiau, Y.J., Burchell, M.R., Krauss, K.W., Birgand, F., and Broome, S.W. (2016). Greenhouse Gas Emissions from a Created Brackish Marsh in Eastern North Carolina. Wetlands 36, 1009-1024.

Sutfin, N.A., Wohl, E.E., and Dwire, K.A. (2016). Banking carbon: a review of organic carbon storage and physical factors influencing retention in floodplains and riparian ecosystems. Earth Surface Processes and Landforms 41, 38-60.

Tan, K. (2005). Soil sampling, preparation, and analysis. Taylor & Francis. Second Edition, 1-62.

Toth, Z., Tancsics, A., Kriszt, B., Kroel-Dulay, G., Onodi, G., and Hornung, E. (2017). Extreme effects of drought on composition of the soil bacterial community and decomposition of plant tissue. European Journal of Soil Science 68, 504-513.

Trumbore, S. (2000). Age of soil organic matter and soil respiration: radiocarbon constraints on belowground dynamics. Ecological Applications 10(2), 399-411.

Turetsky, M.R., Kotowska, A., Bubier, J., Dise, N.B., Crill, P. Hornibrook, E.R.C., Minkkinen, K., Moore, T.R., Byers-Smith, I.H., Nykanen, H., Olefeldt, D., Rinne, J., Saarnio, S., Shurpali, N., Tuittila, E.S., Waddington, J.M., White, J.R., Wickland, K.P. and Wilmking, M. (2014). A synthesis of methane emissions from

71 northern, temperate, and subtropical wetlands. Global Change Biology 20, 2183-2197.

US Environmental Protection Agency. (2008). Methods for evaluating wetland condition, Wetland Hydrology. Retrieved from: <https://www.epa.gov/sites/production/files/documents/wetlands_20hydrology.pdf>.

Wang, B., Lerdau, M., and He, Y. (2017). Widespread production of nonmicrobial greenhouse gases in soils. Global Change Biology 23, 4472-4482.

Woodward, C., Shulmeister, J., Larsen, J., Jacobsen, G. E., and Zawadzki, A. (2014). The hydrological legacy of deforestation on global wetlands. Science 346, 844-847.

Chapter 2: The effects of warming on wetland microbial community abundance and diversity

Introduction

Soil microbial communities play critical roles within wetland ecosystems. Wetlands have high overall microbial abundances and also high microbial diversity (He et al. 2015). Higher microbial diversity is associated with greater ecosystem stability and productivity (Maron et al. 2018). Microbes are involved in wetland ecosystem services that impact soil fertility and nutrient cycling (i.e. plant communities) and water quality (i.e. wildlife communities) (Maron et al. 2018).

Soil microbes regulate biogeochemical cycles that influence global warming (Oertel et al. 2016; Romero-Olivares et al. 2017). Wetland hydroperiods can affect how soils respond to warming, specifically how microbial community structure and biodiversity respond (Toth et al. 2017; Wiedenbeck 2011). Microbial activity is a predictor of decomposition rates, which can decrease with low moisture and drought (Bardgett et al. 2008). Drought restructures soil bacterial communities and causes a decrease in overall microbial functioning (Cheng et al. 2017). Soil warming can also increase microbial respiration rates (i.e. CO_2 emissions) due to an increase in metabolized carbon pools (Manzoni et al. 2012). Other studies have shown that respiration rate decreases with low water availability in wetlands, due to high environmental stress (Don et al. 2017; Neilson et al. 2011). In fact, in long term warming studies soil respiration steadily decreases over time (Romero-Olivares et al. 2017) across different biomes (Graaff et al. 2015), resulting in levels more similar to control temperatures. Soil diversity has a significant correlation with ecosystem function based on soil respiration

(Cheng et al. 2017). Specifically, a decrease in soil biodiversity reduces soil carbon respiration and decomposition, impacting carbon cycling processes (Don et al. 2017). Manipulation of the microbial community structure affects soil organic carbon turnover in soils (Cheng et al. 2017). Warming significantly enhances soil CO_2 fluxes and reduces soil carbon contents, increasing decomposition of decade and millennia old soil organic matter decomposition (Cheng et al. 2017; Graaff et al. 2015; Serna-Chavez et al. 2013).

Methane cycling in soils are also controlled by microbial processes. Methane production in soils occur when organic matter is broken down anaerobically through the process of methanogenesis. Microbial decomposers degrade organic material, allowing them to take up needed energy (Freitag et al. 2010). Anaerobic degradation of this organic material is done by methanogens, which are Archaea that produce methane as the metabolic byproduct in anaerobic environments (Xie et al. 2017). The five major genera within Methanogens include *Methanobacterium, Methanocella, Methanosaeta, Methanosarcina,* and *Methanomassiliicoccus* (Hanson and Hanson 1996). In contrast, methanotrophs are mainly bacteria that metabolize methane as a source of carbon and energy in aerobic environments. Some methanotrophs, methane-oxidizing archaea and sulfate-reducing bacteria, can metabolize methane in anaerobic environments. Some key genera of Methanotrophs include *Methylomonas, Methylobacter, Methylococcus, Methylocystis, Methylosinus, and Methylomicrobium* (Whiting and Chanton 2001).

As warming increases temperature and drought, microbial community structures might change between methane producing and consuming processes (Cheng et al. 2017). Since methane emission depends on the balance of methanogenesis and methanotrophy, examining the soil microbiome can allow us to better understand how methane emissions

are influenced. Current limitations in wetland ecology include a lack of insight into how microbial community abundance, diversity, stability, and functionality may change with future climate change.

Purpose and Objectives

The purpose of this study was to measure the effects of experimental warming on wetland microbial communities. Specifically, our main objectives were to compare controlled vs. warmed plots in permanent, semi-permanent, and ephemeral wetlands and determine differences in microbial abundances and diversities.

Hypotheses

Hypothesis 1: Permanent wetlands will have a higher total abundance of microbes. Warming will decrease microbial abundance across all wetland types.

Hypothesis 2: Permanent wetlands will have lower microbial diversity due to specialized anaerobic requirements. Warming will decrease microbial diversity across all wetland types.

Hypothesis 3: Wetland types with have different microbial compositions. Warming will shift microbial composition.

Methods

Soil Collection and DNA extraction

Soil samples were collected in Summer 2018 (June 10-July 20) using a soil core sampler to remove a core of 20 cm in length. A wet core sampler was used if water exceeded 1 m. Otherwise a dry core sampler was used. The top 10 cm and bottom 10 cm were cut apart from each soil core. A total of three replicates (three soil cores) were taken from each plot and combined into a single sample for each wetland. Each consolidated

soil core sample was passed through a 2mm sieve to homogenize the sample, stored on ice in transport to EWU, and then stored in -20° C until DNA extraction. To prevent cross contamination across samples, each soil core and sieve were handled with disposable gloves sterilized using 2% bleach solution followed by a sterile water wash that was also used to sterilize the equipment itself. Equipment was allowed to air dry before the next sample was taken. Soil cores were separated at the 10cm mark to separate the 0-10cm and 10-20cm soil depths using sterile gloves. To make sure this decontamination method worked, a swab was taken from equipment after sterilization process and DNA was extracted and run through PCR to confirm negative control. A Qiagen PowerSoil DNA extraction kit was used to extract DNA from each sample and stored in -60° C until processed for qPCR and PCR for Illumina Sequencing analysis (Walke et al. 2015).

Soil Microbial Abundance: qPCR

To compare differences in total soil microbial abundance between samples (Table 2.1), we used a quantitative PCR (qPCR) assay to quantify 16S rRNA gene copy numbers and therefore estimate absolute microbial abundance in each treatment (Fierer et al. 2005). A universal 16S primer set (Eub338F/Eub518R) was used, including plasmid DNA as a standard. The plasmid consisted of a 16S rRNA gene fragment inserted into the pCR®2.1-TOPO® vector (Invitrogen, Carlsbad, CA, USA). A standard curve was run in triplicate reactions of 10-fold dilutions of plasmid DNA. Samples were run in duplicate, and no template controls were run in triplicate. The gene copy numbers in each sample were then calculated from the standard curve, by averaging the replicate values. The assays were run in Bio-Rad 96-well plates (cat# HSP9601) on the Real-Time PCR

Detection System (Bio-Rad CFX Connect Real-Time System). Each 15 µl reaction contained 2.75 µl PCR water (MO BIO Laboratories, Inc., Carlsbad, CA, USA), 0.15 µl BSA (10 µg/µL final concentration), 7.5 µl SSoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA), 0.75µl of each primer (10µM stock), and 3µl template DNA. PCR conditions were 10 min at 95°C, and 40 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec (Fierer et al. 2005; Fierer 2017).

Soil Microbial Community Diversity: Illumina Sequencing

A subset of permanent and ephemeral wetlands (3 of each) were used to analyze microbial diversity in top 10 cm and bottom 10-20 cm control plots as well as top 10cm warmed plots (Table 2.2). The V4-V5 region of the 16S rRNA gene was amplified using barcoded 515F (Parada et al. 2016) and 926R (Quince et al. 2011; Parada et al. 2016) primers. Each primer sequence consisted of appropriate adapters complementary to the oligonucleotides on the Illumina flow cell and specific primer for the V4-V5 region, including appropriate barcode on the forward primer. The DNA sequences from each sample had a unique barcode sequence, allowing for samples to be multiplexed and run on a single Illumina flow cell. All samples were amplified in triplicate reactions, each containing a total reaction volume of 25 µl including 11.75 µl ultra-clean PCR grade water, 0.25µl BSA (10µg/µL stock?), 10 µL 5PRIME Hot Master Mix (cat# 10847-706, supplier # 2200400-QuantaBio), 0.5 µl Forward Primer IL515F + barcode, 0.5 µl Reverse Primer IL926R, and 2 µl DNA. PCR conditions were as follows: initial denaturation at 94C for 3 minutes, followed by 35 cycles of denaturation at 94C for 45 seconds, annealing at 50C for 1 minute, elongation at 72C for 1.5 minutes, and a final elongation

step at 72C for 10 minutes. PCR products and negative controls were analyzed on 1.5% agarose gel to confirm proper amplification. DNA concentrations were then measured using Qubit 4.0 Fluorometer and the 1X dsDNA High Sensitivity assay kit. Based on concentration determined by the fluorometer for each sample, equal amounts of DNA per sample were combined into a single pooled sample. The pooled sample was then purified using the Qiagen QIAquick PCR purification kit, quantified with Qubit as above, and sent to Dana Farber Cancer Institute at Harvard University for DNA sequencing using a 250bp paired-end approach on the Illumina MiSeq platform to characterize the diversity and composition of the soil microbiome (Caporaso et al. 2012; Freitag et al. 2010; Parada et al. 2016).

Experimental warming

A warming study was set up by using passive open- top chambers at one plot within 18 of the 36 wetlands (6 of each type) to mimic warming due to accelerated climate change (using the methods of Johnson et al. 2013). Warming chambers were modified by adding flexibility and drainage to allow regular movement and flow of water in and out of the chamber during waterlogged states (Chapter 1: Figure 3). Soil microbial abundance and diversity samples were analyzed as mentioned above and compared to non-warmed (control) plots.

Statistical Analyses

A type II analysis of variance (ANOVA) was used to compare soil microbial abundance (16S rRNA gene copy number) between warmed and controlled permanent, semi-permanent, and ephemeral wetlands using R (version 3.5.3). Pairwise comparisons were analyzed using *emmeans* functionality in R studio, which allowed us to examine

interactions or differences between specific treatments and wetland types as well as any interactions between treatments. The bioinformatics pipeline QIIME 2, Quantitative Insights into Microbial Ecology (© 2016-2019, QIIME 2 development team, Bolyen et al. 2018), was used to analyze DNA sequence data and determine alpha and beta diversity as well as overall soil microbial community composition. Data was rarefied to 37,946 sequences/sample, for all other normalization method samples with fewer than 37,946 sequences/sample were removed from the raw data. DADA2 (Callahan et al. 2016) was used to cluster sequences into operational taxonomic unit (OTU) features, using 100% sequence similarity, and GreenGenes database (version 13_8, 2013) to assign taxonomy. Alpha diversity was measured using Faith's Phylogenetic Diversity, Observed OTUs (i.e. OTU richness), and Shannon Diversity. Differences in alpha diversity metrics across wetland type, warming, and depth were determined using Kruskal-Wallis tests, as well as pairwise interactions. Beta Diversity was measured using Weighted UniFrac (phylogenetics-based) matrices, visualized by principle coordinate analysis (PCoA). Differences in community structure across groups were tested using PERMANOVA. Relative abundances were measured through Analysis of Composition of Microbes (ANCOM) statistical test.

Results

Soil Microbial Abundance: qPCR

Total microbial abundance differed between depth ($p=0.00071$) and treatment ($p=0.045$), but not by wetland type ($p=0.082$) (Figure 2.1). In pairwise comparisons, no specific combinations of wetland type, treatment, and depth were different from one another. In ephemeral wetlands, microbial abundance generally decreases with warming

as well as with soil depth. Microbial abundance also decreases with warming in permanent wetlands, but the effect of depth varies between warmed and control treatments. Finally, microbial abundance within semi-permanent wetlands seems to increase with warming but not generally between depths.

Soil Microbial Community Diversity: Illumina Sequencing

The total number of sequences found in our study was 1,036,455. Average sequence count per sample was 60,967, ranging from 37,946 to 81,119. There was a total of 16,686 features (total number of OTUs) obtained in our soil microbiome. The average features per sample were 62.12, ranging from 1 to 11,634.

No overall differences were seen in Faith phylogenetic alpha diversity between wetland type ($p=0.77$), warming treatment ($p= 0.34$), or depth ($p=0.058$) (Figure 2.2). However, pairwise comparisons showed that warming increased diversity in the top 10cm of ephemeral wetlands ($p=0.0495$) and decreased diversity in the top 10 cm of permanent wetlands ($p=0.050$). Control permanent wetlands had higher diversity than control ephemeral diversity, and diversity declined with depth in permanent wetlands ($p=0.050$).

For Shannon diversity, no differences were seen between wetland type ($p=0.92$), warming treatment ($p= 0.63$), or depth ($p=0.058$). Again, there were also individual pairwise comparisons that showed specific differences between warming treatment, wetland type, and depth. For example, ephemeral warmed top 10 cm wetlands showed higher diversity than permanent warmed top 10 cm wetlands ($p=0.050$). In permanent wetlands specifically, microbial diversity decreased with warming ($p=0.050$), and with depth ($p=0.050$).

Lastly, there were no differences in Observed OTUs (i.e. OTU richness) between wetland type (p=0.29) or warming treatment (p= 0.34), but we did see a decrease in diversity with depth (p= 0.038). Again, certain pairwise comparisons did show significance differences in diversity with warming, wetland type, and depth. Ephemeral warmed top 10cm wetlands exhibited higher diversity than permanent warmed top 10cm and permanent control 10-20cm wetlands and (p=0.050). Warming in permanent wetlands significantly decreased diversity (p=0.050), and also decreased diversity at lower depths (p=0.050).

There were no significant differences in microbial community composition across depth, treatment, or wetland type for beta diversity (Figure 2.3). Specifically, weighted UniFrac showed no statistical differences across depth (p=0.086, pseudo-F= 1.73), treatment (p=0.77, pseudo-F= 0.59) or wetland type (p=0.46, pseudo-F= 0.90). Although, there was a high trend seen in difference between depths (Figure 2.3).

When comparing specific taxonomic differences through relative abundance measures, there were no differences across wetland type, depth, or warming treatment. Meaning that there were no OTUs found in one wetland that wasn't present in another. The most dominant groups found in our soil microbiome included Acidobacteria (85-100% relative abundance), Betaproteobacteria (75-95%), and Deltaproteobacteria (68-90%). PCoA shows an ordination based on a distance or dissimilarity matrix to show that although no specific differences were found, there were specific trends seen within treatments (Figure 2.3). These show that although no taxonomic differences in relative abundance were found, there was visible grouping of communities by wetland type and more specifically by permanent wetlands. Relative abundances are shown in Figure 2.4,

and it is worthy to note here that both methanogen (methane producing Methanobacteria and Methanomicrobia) and methanotroph (methane oxidizing Methylacidiphilae) bacteria and archaea are present across all wetlands.

Discussion

Overall, our findings showed that with warming, microbial abundances are reduced in both ephemeral and permanent wetland types. Additionally, that a reduction in microbial diversity occurs specifically in permanent wetlands. These are critical findings that could illustrate the potential impacts of climate change on microbial communities, especially how they might relate to the carbon measurements we found in Chapter 1.

Soil Microbial Abundance: qPCR

Microbial abundances significantly differed between depth and treatment and showed a high trend of differing between wetland type. The general pattern shows that ephemeral control wetlands had the highest total microbial abundance. Within ephemeral wetlands, warming decreased microbial abundance. This pattern has been seen in previous studies where increase in drought and temperatures create more moisture limiting conditions and therefore lower microbial abundances while increasing microbial decomposition and respiration rates (Bardgett et al. 2008; Cheng et al. 2017; Romero-Olivares et al. 2017). This is especially true in our data, where although moisture level (wetland hydroperiod type) didn't have a large effect on microbial communities, warming temperature did. Relating this data to Chapter 1, it is important to note that although previous studies as mentioned show an increase in activity (measured by CO_2 and CH_4) with warming, we did not see this in our carbon data. It seems that our findings are showing that both abundance and activity of microbes are decreasing with warming in

wetlands. Our study also showed that microbial abundances decrease with depth. This has been a common finding in phospholipid-derived fatty acids (PLFA) studies, a chemotaxonomic marker method, that find that bacterial and fungal PLFA's decrease with depth (Balasooriya et al. 2008; Zhang et al. 2016).

In contrast, permanent wetlands show a trend of increased abundance in deeper soils within the control plots but then a decrease within warmed plots. Again, the decrease of abundance with warming is a common finding in other studies that have tested drought and warming effects on soil microbes (Manzoni et al. 2012; Romero-Olivares et al. 2017; Toth et al. 2017; Weidenbeck et al. 2011). The increase in abundance of deeper soils could be due to the fact that permanent wetlands might have a more specialized and diverse anaerobic community compared to ephemeral wetlands. Although this has not been a common finding in the past, these results might be explained further by looking at differences in soil microbial diversity (Don et al. 2017; Weidenbeck et al. 2011; Zogg et al. 1997). Finally, microbial abundance within semi-permanent wetlands seem to increase with warming but not generally between depths. This is a very unusual finding in that warming actually increased microbial abundances (Baben et al. 2014; Manzoni et al. 2012; Yun et al. 2013). One possible explanation could be that because of the high variability in water level and water moisture in semi-permanent wetlands year-round, the microbial community could be better adapted to warming temperatures and therefore have specific mechanisms in place to keep a stable microbial community. For example, a recent study by Kueneman et al. (2019) showed that in amphibian skin microbiomes more variable environments, including variable temperatures, had more microbial diversity. This was largely driven by the fact that more

microbes were capable of dormancy under more variable conditions. A similar study by Valter de Oliveria and Margis 2015 showed that the microbial seed bank in riverine systems remain stable across seasonal shifts in river temperatures, although shifts in diversity might occur across season. To better understand why these differences might be occurring in our wetlands, looking into changes in microbial diversity might illustrate the potential for more dormancy genes under warming conditions, specific wetland types, or depth.

Soil Microbial Community Diversity: Illumina Sequencing

Warming and depth affected microbial alpha diversity in Faith (richness and phylogeny), Shannon (evenness and richness) and Observed OTUs (species richness), meaning that diversity was different within the local species pool. The main pattern seen here showed that alpha diversity is consistently lowered within permanent wetlands when warmed. In addition, within both ephemeral and permanent wetlands, alpha diversity decreased with depth. In contrast, there were no differences seen in beta diversity with wetland type, depth, and warming treatment. Meaning that there are no shifts in species composition across sites. Although, we can see high trends in depth specifically where microbial communities in permanent wetlands are clustered together showing higher similarity to each other compared to other treatments. Here the data suggests that paired with our qPCR analysis, both warming and depth treatments have a significant impact to the wetland microbial abundance and diversity. Our data is similar to previous studies that have shown that warming and stimulated drought conditions decrease microbial diversity (Cheng et al. 2017; Graaff et al. 2017; Toth et al. 2017). One study done by Graaff et al. (2017) specifically showed that as a result of reduction in soil microbial

diversity, decomposition and soil carbon respiration also decrease. Comparing this to Chapter 1, where we didn't see changes in carbon fluxes (CO_2 or CH_4) across warming treatment, illustrates that warming in wetland systems might not affect respiration like studies have shown in other systems. Although, this doesn't mean decomposition won't be affected, especially as warming progresses in time. This gives us an insight into direct effects on the wetland carbon cycle. As warming continues to decrease soil microbial diversity, we might see shifts in carbon pools and plant communities as a result of the reduction in decomposition (Baben et al. 2014; Crowther et al. 2016; Kayranli et al. 2010).

When comparing specific taxonomic differences in the wetland microbiome, there were no differences across wetland type, depth, or warming treatment. Meaning that there we could not identify taxa characteristic of any one particular treatment. The most abundant classes in our study included Acidobacteria that include major groups of decomposing bacteria, known to use both inorganic and organic nitrogen as their N sources, Betaproteobacteria that are known for nitrogen fixation, and Deltaproteobacteria that reduce sulfate or elemental sulfur (Gupta 2000; Kielak et al. 2016). These are known across soil studies to be really ubiquitous groups across soils around the world. Generally, methanogens including the Archaea Methanomicrobia and Methanobacteria, as well as methane oxidizers such as Methylacidiphilae were present across all wetlands, although at low relative abundances (2-15%). In other words, the bacteria and archaea responsible for both production and oxidation of methane were present across all treatments. Other notable bacteria present in our soil microbiome include decomposers,

photosynthesizers, and nitrifying bacteria such as Rubrobacteria, Synechococcophycideae, and Nitrospira respectively.

Based on these data, the wetland soil microbiome is a very complex system made up of a diverse soil microbial community. These soil microbial communities are much more sensitive in determining changes in wetland type, warming treatment and depth compared to carbon storage and carbon flux measurements. It could be, as mentioned in Chapter 1, that carbon measurements become more accurate and sensitive over a longer experimental time frame and that could be what caused such low significance across our carbon measurements. Alternatively, our carbon data might be indicating that there are no effects of wetland type or warming treatment on carbon storage or carbon fluxes. Based on our microbial data, I would argue that these systems are not as stable and resistant as we thought in Chapter 1. This is clearly seen when looking at changes in microbial abundance within semi-permanent wetlands, where warming actually stimulated abundances instead of decreased them as we saw in the permanent and ephemeral wetlands. As alpha diversity decreased with warming, we might expect changes in microbial communities over time. Although semi-permanent wetland DNA was not sequenced, I predict that warming would also decrease diversity in these wetlands even after exhibiting higher total abundances with warming. Mainly due to the fact that we didn't find any differences across wetland type specifically, diversity could decrease in semi-permanent wetlands leaving room for remaining species to thrive, resulting in higher total abundances. I would argue that although we didn't see significant shifts in beta diversity (species composition) or relative abundance, the changes we did see in species richness may influence species composition given enough time. Especially

considering that we are already seeing high trends in grouping of permanent wetlands and communities in the 0-10cm soil layers illustrated in our PCoA ordination.

Overall, gathering information on the wetland microbiome seems to give a better picture of the wetland ecosystem dynamics. Therefore, as we continue to move forward in wetland ecosystem studies, I would urge the importance of sampling using both molecular and ecological techniques as it gives us the potential to better predict the health status of these systems in the future, especially in the face of climate change.

References

Balasooriya, W.K., Denef, K., Peters, J., Verhoest, N.E.C., and Boeckx, P. (2008). Vegetation composition and soil microbial community structural changes along a wetland hydrological gradient. Hydrology and Earth System Sciences 12, 277 291.

Baben, D., Vogel, C., Zuhlke, S., Schloter, M., Pronk, G.J., Heister, K., Spiteller, M., Kogel-Knabner, I., and Smalla, K. (2014). Soil Mineral Composition Matters: Response of Microbial Communities to Phenanthrene and Plant Litter Addition in Long Term Matured Artificial Soils. PLOS ONE 9(9), 1-12.

Bardgett, R. D., Freeman, C., and Ostle, N. J. (2008). Microbial contributions to climate change through carbon cycle feedbacks. Isme Journal 2, 805-814.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E., Da Silva, R., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki,

M., Fouquier, J., Gauglitz, J.M. et al. (2018). QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. Retrieved from: <https://doi.org/10.7287/peerj.preprints.27295v2>.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016). "DADA2: High-resolution sample inference from Illumina amplicon data." Nature Methods 13, 581-583.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., and Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. Isme Journal 6, 1621-1624.

Cheng, L., Zhang, N. F., Yuan, M. T., Xiao, J., Qin, Y. J., Deng, Y., Tu, Q. C., Xue, K., Van Nostrand, J. D., Wu, L. Y., He, Z. L., Zhou, X. H., Leigh, M.B., Konstantinidis, K. T., Schuur, E. A. G., Luo, Y. Q., Tiedje, J. M., and Zhou, J. Z. (2017). Warming enhances old organic carbon decomposition through altering functional microbial communities. Isme Journal, 11, 1825-1835.

Don, A. Bohme, I.H., Dohrmann, A.B., Poeplau, C., and Tebbe, C.C. (2017). Microbial community composition affects soil organic carbon turnover in mineral soils. Biology and Fertility of Soils 53, 445-456.

Fazli, P., Che Man, H. Shah, U.K.M., and Idris, A. (2013). Characteristics of Methanogens and Methanotrophs in Rice Fields: A Review. Asia-Pacific Journal of Molecular Biology and Biotechnology 21, 3-17.

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15, 579-590.

Fierer, N., Jackson, J.A., Vilgalys, R., and Jackson, R.B. (2005). Assessment of Soil Microbial Community Structure by Use of Taxon-Specific Quantitative PCR Assays. Applied and Environmental Microbiology 71, 4117-4120.

Freitag, T.E., Toet, S., Ineson, P., and Prosser, J. (2010). Links between methane flux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog. FEMS microbiology ecology 73, 157-165.

Graaff, de M.A., Adkins, J., and Throop, H.L. (2015). A meta-analysis of soil biodiversity impacts on the carbon cycle. Soil 1, 257-271.

Gupta, R.S. (2000). The phylogeny of proteobacteria: Relationships to other eubacterial phyla and eukaryotes. FEMS Microbiology Reviews 24, 367- 402.

Hansen, R.S. and Hanson, T.E. (1996). Methanotrophic bacteria. Microbiological reviews 1, 439-471.

He, S., Hu, W., Ma, D., Lan, H., Yang, Y., and Gao, Y. (2015). Patterns in Wetland Microbial Community Composition and Functional Gene Repertoire Associated with Methane Emissions. mBio 6, 1-15.

Johnson, C.P., Pypker, T.G., Hribljan, J.A., and Chimner, R.A. (2013). Open Top Chambers and Infrared Lamps: A Comparison of Heating Efficacy and CO_2/CH_4 Dynamics in a Northern Michigan Peatland. Ecosystems 16: 736-748.

Kielak, A.M., Barreto, C.C., Kowalchuck, G.A., van Veen, J.A., and Kuramae, E.E. (2016). The Ecology of Acidobacteria: Moving beyond Genes and Genomes. Fronteirs in Microbiology 7, 744.

Kueneman, J.G., Bletz, M.C., McKenzie, V.J., Becker, C.G., Joseph, M.B., Abarca, J.G., Archer, H., Arellano, A.L., Bataille, A., Becker, M., Belden, L.K., Crottini, W.M.,

Hughey, M., Jarek, M., Kearns, P.J., Kerby, J.L., Kielgast, J., Kurabayashi, A., Longo, A.V., Loudon, A., Medina, D., Nunez, J.J., Perl, R.G.B., Pinto-Tomas, A., Rabemananjara, F.C.E., Rebollar, E.A., Rodriguez, A., Rollins-Smith, L., Stevenson, R., Tebbe, C.C. et al. (2019). Community richness of amphibian skin bacteria correlates with bioclimate at the global scale. Nature Ecology & Evolution 3, 381–389.

Manzoni, S., Schimel, J. P., and Porporato, A. (2012). Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930-938.

Maron, P.A., Sarr, A., Kaisermann, A., Leveque, J., Mathieu, O., Guigue, J., Karimi, B., Bernard, L., Dequiedt, S., Terrat, S., Chabbi, A., and Ranjard, L. (2018). High Microbial Diversity Promotes Soil Ecosystem Functioning. Applied and Environmental Microbiology 84: 1-13.

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., and Erasmi, S. (2016). Greenhouse gas emissions from soils A review. Chemie Der Erde-Geochemistry 76, 327-352.

Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series andglobal field samples. Environmental Microbiology 18(5), 1403-1414.

Pichler, M., Coskun, O.K., Ortega-Arbulu, A.S., Conci, N., Worheide, G., Vargas, S., and Orsi, W.D. (2017). A 16S rRNA gene sequencing and analysis protocol for the Illumina MiniSeq platform. MicrobiologyOpen 7, 1-9.

Quince, C., Lanzen, A., Davenport, R.J., and Turnbaugh, P.J. (2011). Removing noise from pyrosequenced amplicons. BMC Bioinformatics 12, 38.

Romero-Olivares, A. L., Allison, S. D., and Treseder, K. K. (2017). Soil microbes and their response to experimental warming over time: A meta-analysis of field studies. Soil Biology & Biochemistry 107, 32-40.

Serna-Chavez, H. M., Fierer, N., and van Bodegom, P. M. (2013). Global drivers and patterns of microbial abundance in soil. Global Ecology and Biogeography 22, 1162-1172.

Toth, Z., Tancsics, A., Kriszt, B., Kroel-Dulay, G., Onodi, G., and Hornung, E. (2017). Extreme effects of drought on composition of the soil bacterial community and decomposition of plant tissue. European Journal of Soil Science 68, 504-513.

Valter de Oliveira, L.F., and Margis, R. (2015). The Source of the River as a Nursery for Microbial Diversity. PLOS ONE 10, 1-11.

Walke, J. B., Becker, M.H., Hughey, M.C., Swartwout, M.C., Jensen, R.V., and Belden, L.K. (2015). Most of the Dominant Members of Amphibian Skin Bacterial Communities Can Be Readily Cultured. Applied Environmental Microbiology 81, 6589-6600.

Whiting, G.J. and Chanton, J.P. (2001). Greenhouse carbon balance of wetlands: methane emission versus carbon sequestration. Tellus series B-chemical and physical meteorology 53, 521-528.

Wiedenbeck, J. and Cohan, F.M. (2011). Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. FEMS Microbiology Reviews 35, 957-976.

Xie, W., Jiao, N., Ma, C.L, Fang, S., Phelps, T., Zhu, R.X., and Zhang, C.L. (2017). The response of archaeal species to seasonal variables in a subtropical aerated soil: insight into the low abundant methanogens. Applied microbiology and biotechnology 101, 6505-6515.

Yun, J., Yu, Z., Li, K., and Zhang, H. (2013). Diversity, abundance and vertical distribution of methane-oxidizing bacteria (methanotrophs) in the sediments of the Xianghai wetland, Songnen Plain, northeast China. Journal of Soil Sediments 13, 242- 252.

Zhang, Q., Wu, J., Yang, F., Lei, Y., Zhang, Q., and Cheng, X. (2016). Alterations in soil microbial community composition and biomass following agricultural land use change. Nature 10, 1-10.

Table 1.1. Experimental Design

Wetland Type	**Wetland Number**	**Control Plot**	**Warming Plot (In 18 of the 36 wetlands)**	**Total Plots**
Permanent	12	3	1	42
Semi-Permanent	12	3	1	42
Ephemeral	12	3	1	42
				126

Table. 1.2. Methane and carbon dioxide sampling

Wetland Type	**Wetland Number**	**Control Plot**	**Warmed Plot**	**Seasons**	**Total Samples**
Permanent	6	2	2	3	72
Ephemeral	6	2	2	3	72
					144

Table 2.1. qPCR Samples

Wetland Type	**Control 0-10 cm**	**Control 10-20 cm**	**Warmed 0-10 cm**	**Warmed 10-20 cm**	**Total Samples**
Permanent	12	12	6	6	36
Semi-Permanent	12	12	6	6	36
Ephemeral	12	12	6	6	36
					108

Table 2.2. Illumina Sequencing Samples

Wetland Type	**Control 0-10 cm**	**Control 10-20 cm**	**Warmed 0-10 cm**	**Total Samples**
Permanent	3	3	3	9
Ephemeral	3	3	3	9
				18

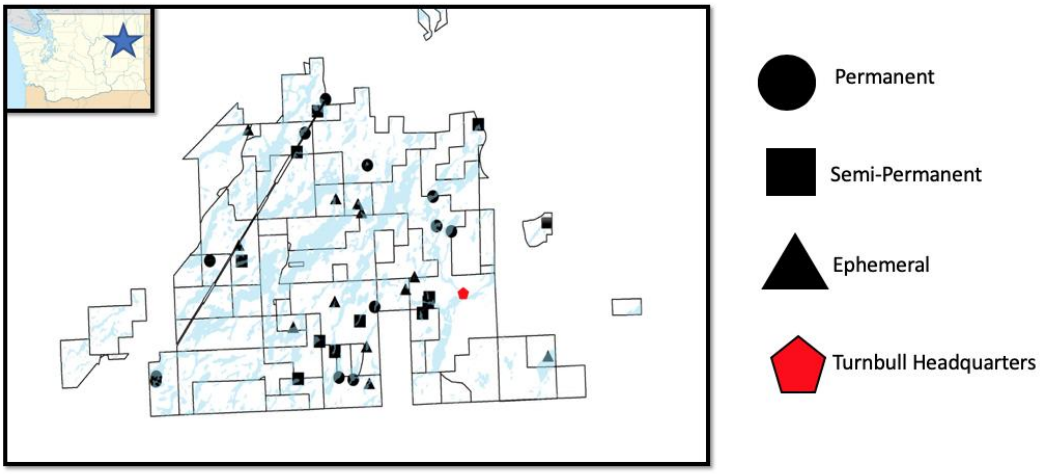

Figure 1.1. Map of Turnbull National Wildlife Refuge, Spokane County, WA, USA.

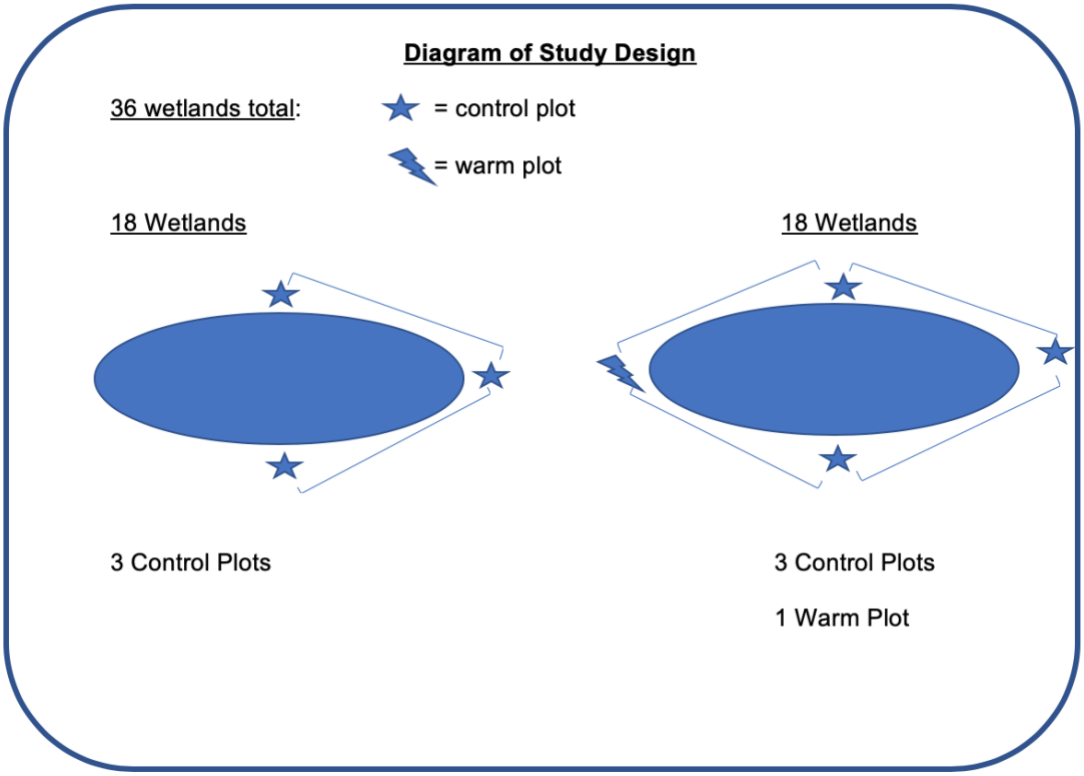

Figure 1.2. Illustration of Study Design. Note that each bracket represents that each plot was at least 4.6 m away from other plots.

Figure 1.3. Gas static floating (top left two) and stationary chambers (top right two).

Figure 1.4. Open-top warming chamber design. Made of 16.5 cm wide by 61 cm tall 0.16 cm thick polycarbonate panels positioned in a regular hexagon that is 61 cm diameter.

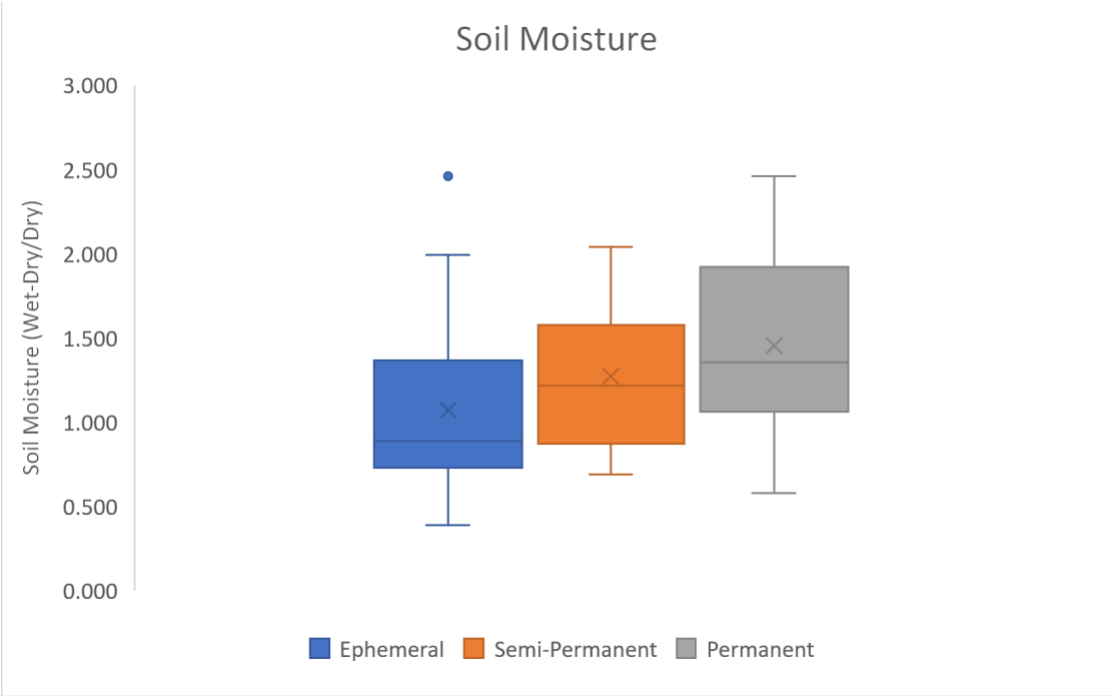

Figure 1.5. Soil moisture as a percentage of dried soil between wetland types. Median represented by X and mean represented by line. Error bars represent +- S.E.

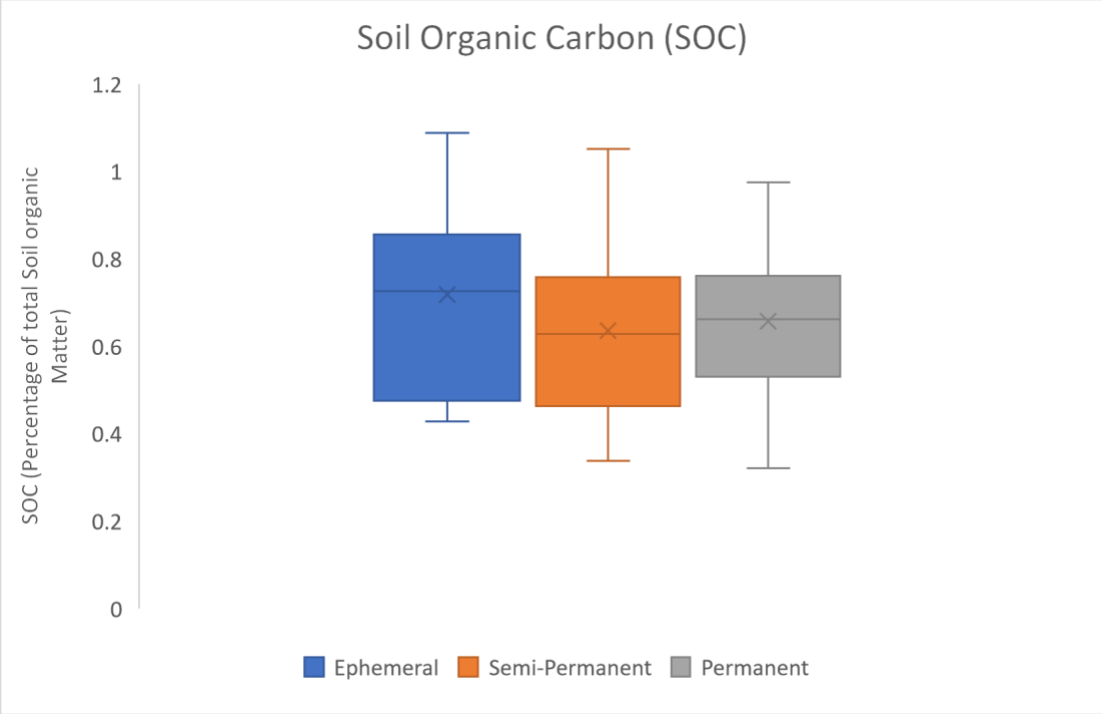

Figure 1.6. Soil organic carbon as a percentage of total organic matter between wetland type. Median represented by X and mean represented by line. Error bars represent +- S.E.

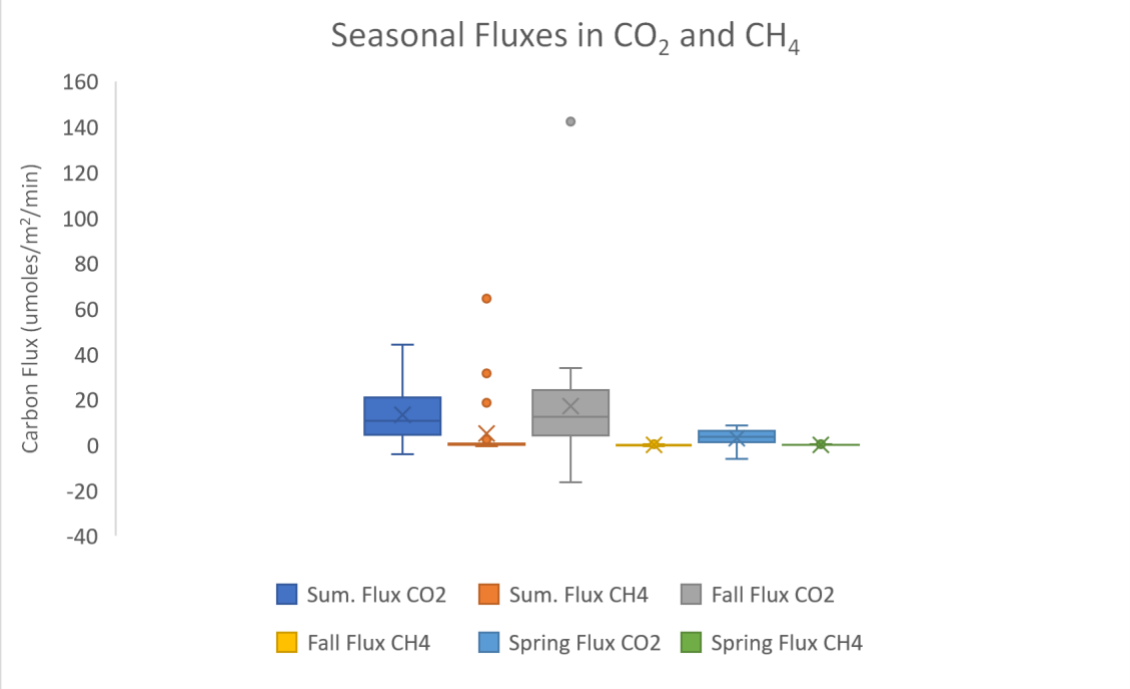

Figure 1.7. Carbon fluxes (CO_2 and CH_4) per season. Median represented by X and mean represented by line. Error bars represent +- S.E.

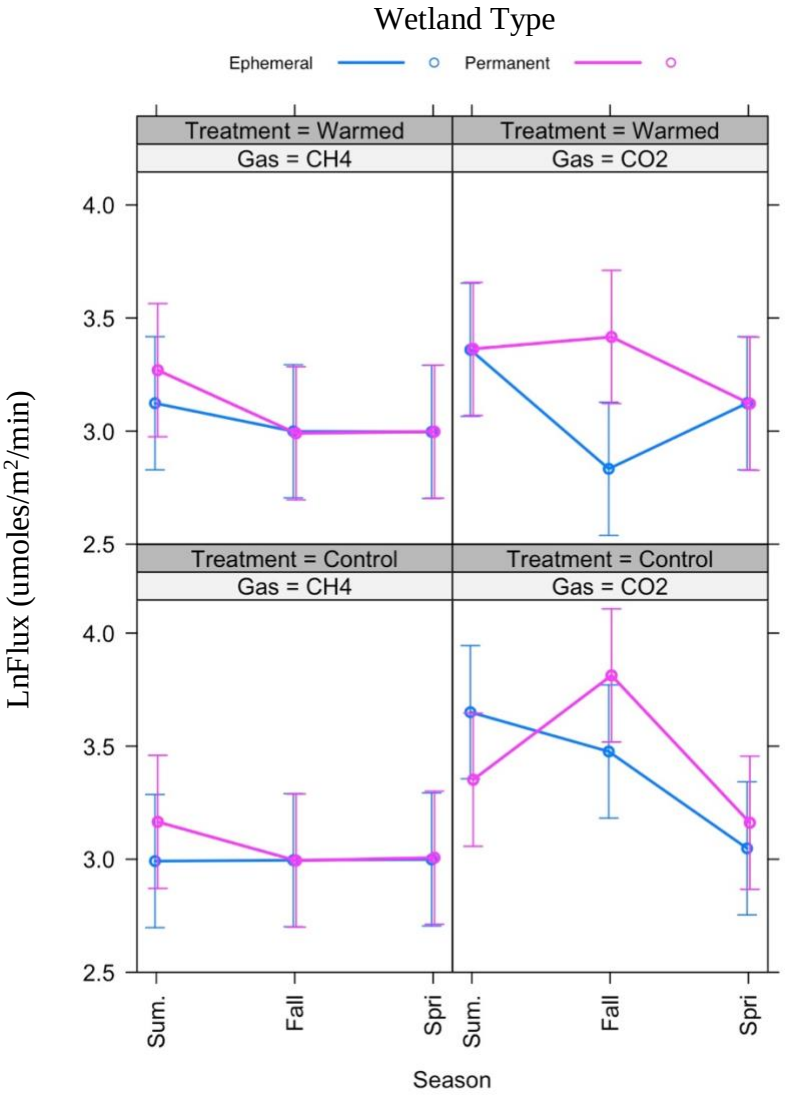

Figure 1.8. Methane and carbon dioxide fluxes within and across season, wetland type, and treatment. Blue line represents ephemeral wetlands and pink line represents permanent wetlands. Error bars represent +- S.E.

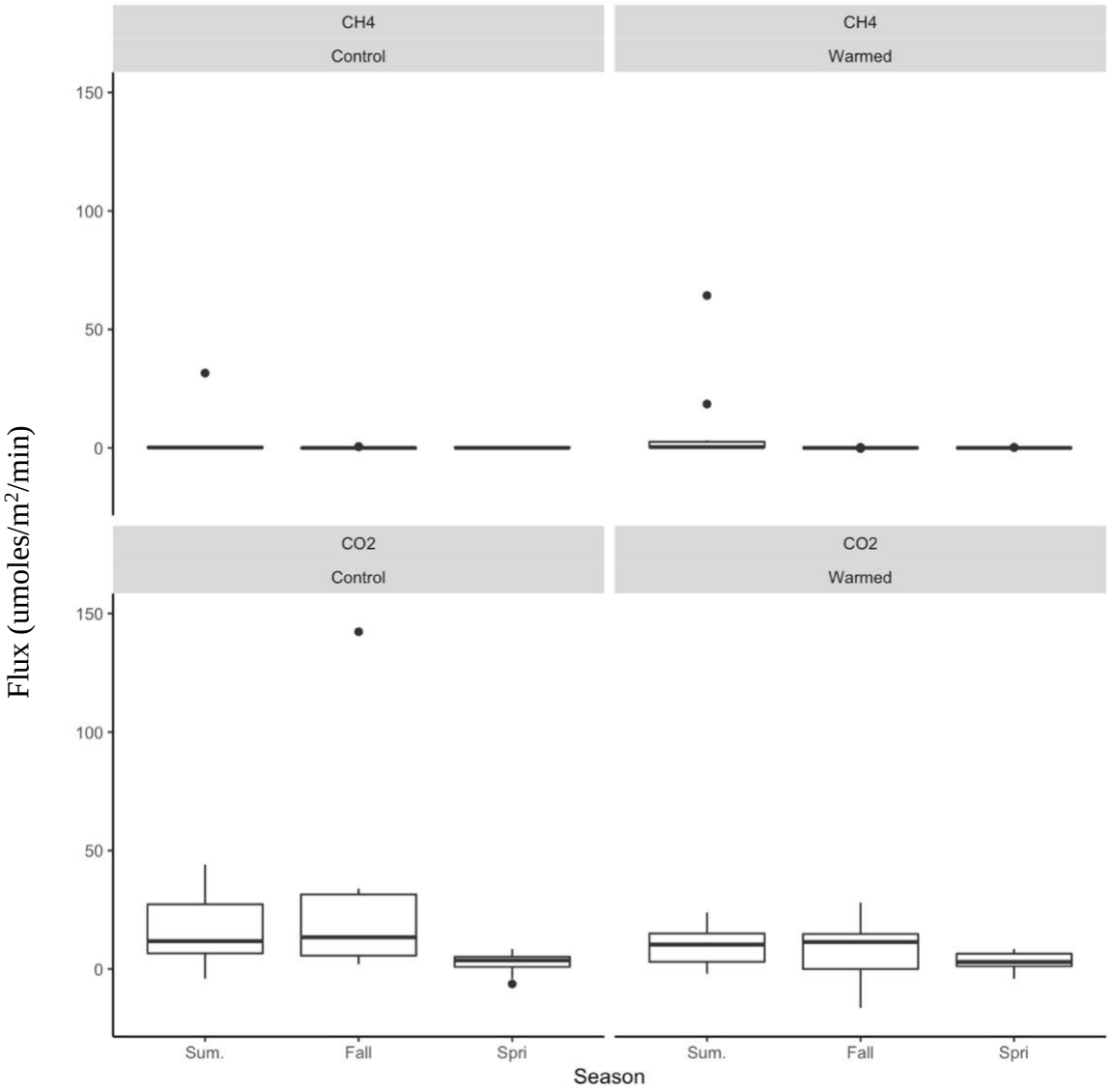

Figure 1.9. Methane and carbon dioxide fluxes within and across season, wetland type, and treatment. Error bars represent +- S.E.

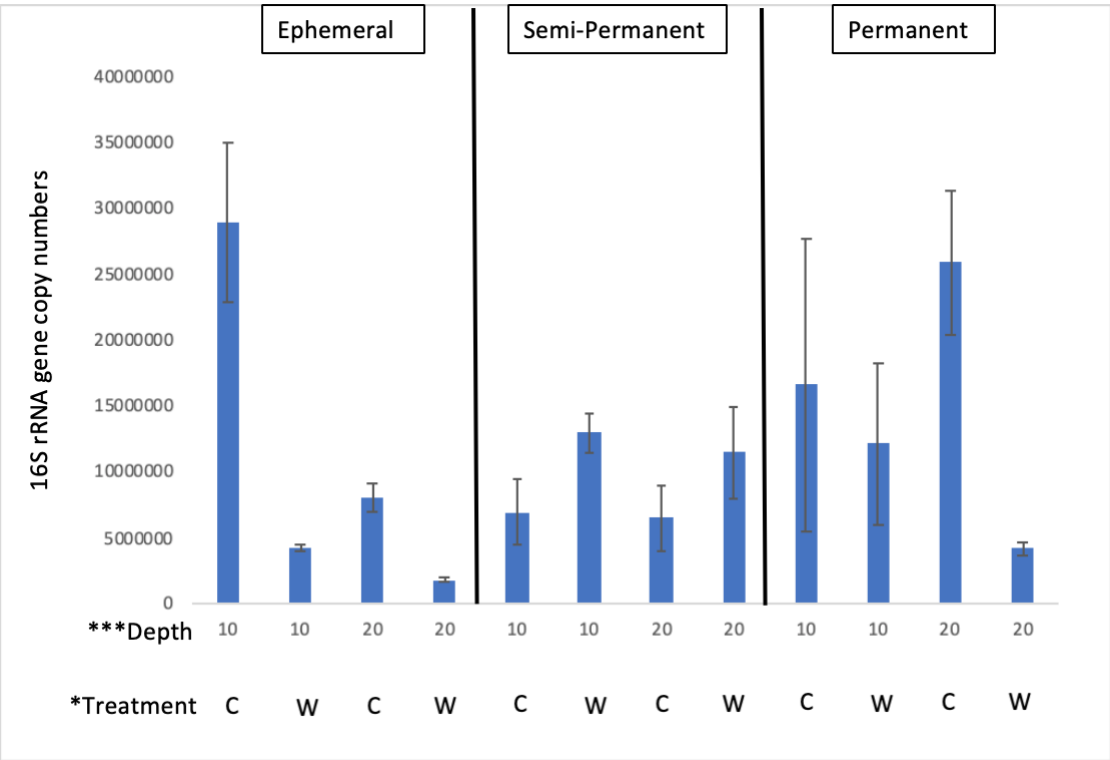

Figure 2.1. Microbial abundance by Wetland Type, Depth, and Treatment. Bars are shown by Wetland Type, Depth ***p<0.001, and Treatment *p<0.05, mean values +/- SE for each group, where C refers to control and W refers to warmed treatments.

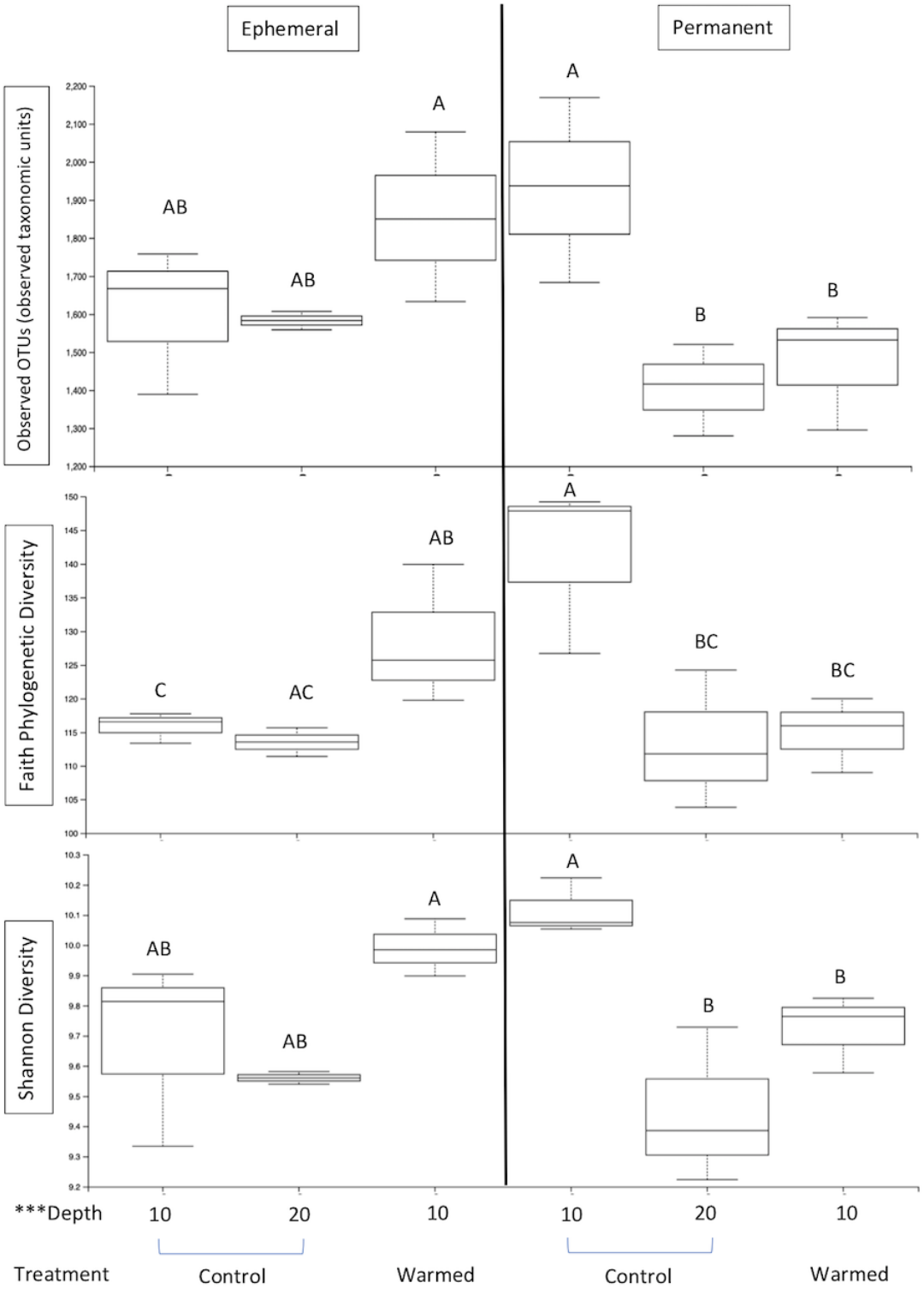

Figure 2.2. Observed number of OTUs, Faith's phylogenetic diversity, and Shannon diversity characterized by Wetland Type, Depth, and Treatment. Where ***p<0.001, and *p<0.05. Error bars represent +- S.E.

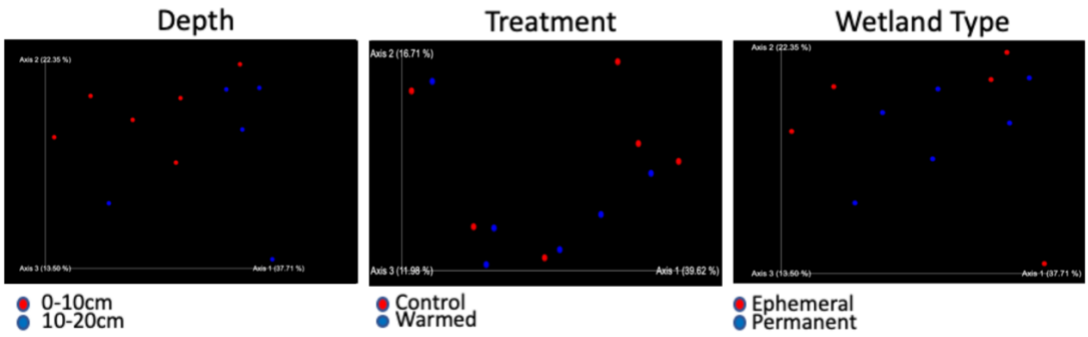

Figure 2.3. Principal coordinate analysis (PCoA) of the weighted UniFrac distance matrix characterized by Depth, Treatment, and Wetland Type.

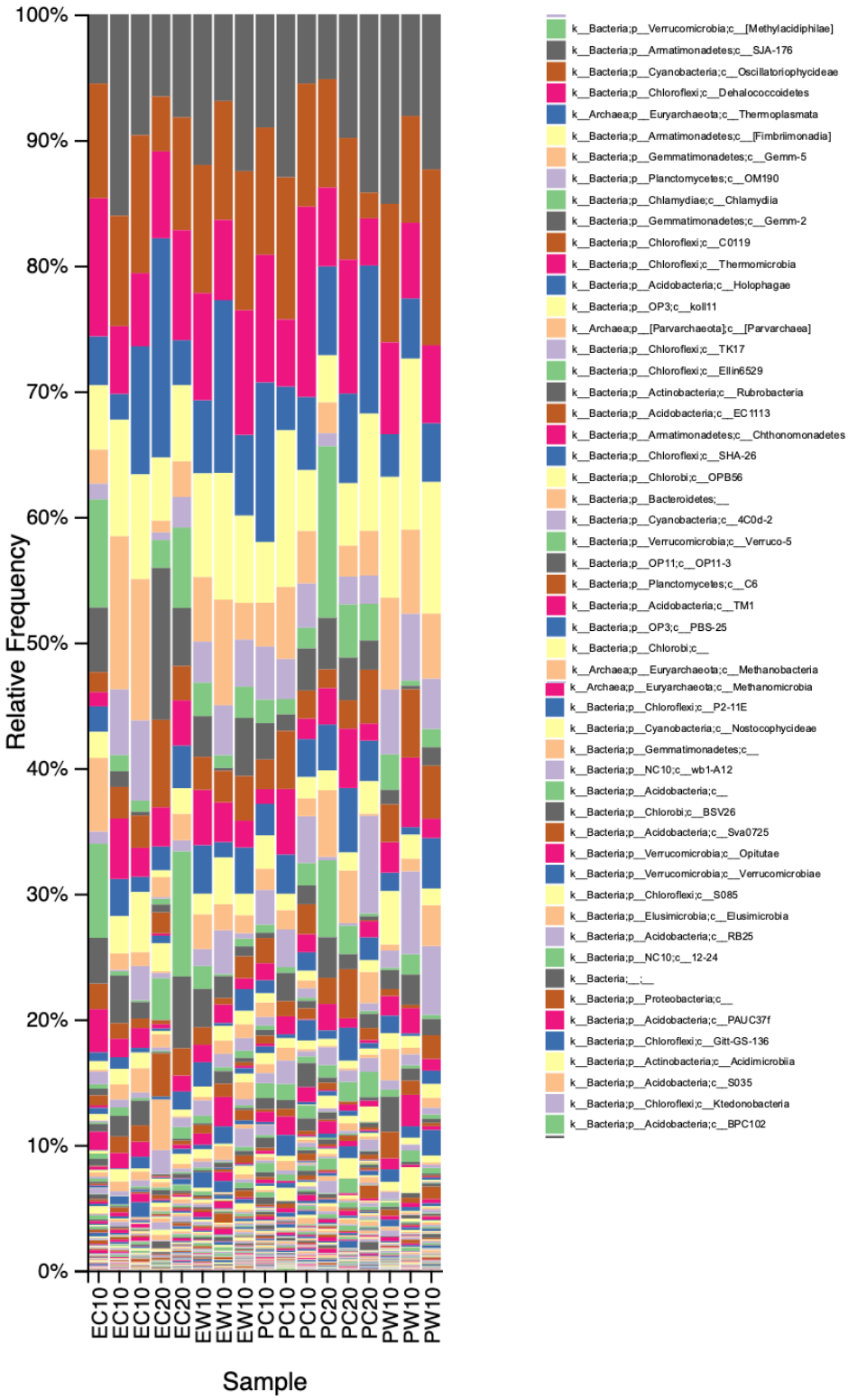

Figure 2.4. Relative abundances of microbial classes across wetlands. Where on the x axis, E refers to ephemeral and P to permanent wetlands. C is control and W is warmed. Finally, 10 is 0-10cm soil depth and 20 is 10-20cm soil depth.

Appendix

Soil Moisture Original Data

	Df	Sum Sq	Mean Sq	F value	P value
Wetland.Type	2	1.355	0.6775	2.77	0.0721
Residuals	51	12.472	0.2446		

Soil Moisture Difference (Warming)

	Df	Sum Sq	Mean Sq	F value	P value
Wetland.Type	2	0.957	0.4783	1.59	0.236
Residuals	15	4.512	0.3008		

SOC original data

	Df	Sum Sq	Mean Sq	F value	P value
Wetland.Type	2	0.00191	0.0009533	0.542	0.585
Residuals	51	0.08968	0.0017585		

SOC Difference (Warming)

	Df	Sum Sq	Mean Sq	F value	P value
Wetland.Type	2	0.002848	0.001424	0.985	0.396
Residuals	15	0.021684	0.001446		

CH_4 vs. CO_2across all seasons and all treatments
Response: LnFlux

	Df	F value	P value
Intercept	1	1.4964e+05	< 2.2e-16***
Season	2	3.9920e-01	0.671716
Wetland.Type	1	1.0340e+00	0.311258
Gas	1	7.5070e+00	0.007084 **
Treatment	1	1.2579e+00	0.264290
Season: Wetland.Type	2	8.4060e-01	0.433963
Season: Gas	2	3.4663e+00	0.034393 *
Wetland.Type: Gas	1	1.9969e+00	0.160212
Season: Treatment	2	8.6120e-01	0.425249
Wetland.Type: Treatment	1	6.9000e-03	0.933823
Gas: Treatment	1	1.6998e+00	0.194815

Season: Wetland.Type: Gas	2	1.6295e+00	0.200334
Season: Wetland.Type: Treatment	2	2.3000e-03	0.997683
Season: Gas: Treatment	2	1.6377e+00	0.198734
Wetland.Type: Gas: Treatment	1	4.6880e-01	0.494845
Season: Wetland.Type: Gas: Treatment	2	4.7400e-01	0.623672
Residuals	120		

Pairwise~Gas|Wetland.Type*Treatment*Season
Wetland.Type = Ephemeral, Treatment = Control, Season = Fall

	Contrast estimate	SE	df	t.ratio	P value
CH_4-CO_2	-0.4809	0.21	120	-2.287	0.0240

Wetland.Type = Permanent, Treatment = Control, Season = Fall

	Contrast estimate	SE	df	t.ratio	P value
CH_4-CO_2	-0.8189	0.21	120	-3.894	0.0002

Wetland.Type = Permanent, Treatment = Warmed, Season = Fall

	Contrast estimate	SE	df	t.ratio	P value
CH_4-CO_2	-0.4261	0.21	120	-2.026	0.0450

Pairwise~Season | Wetland.Type*Season*Gas
Wetland.Type = Ephemeral, Gas = CO_2

	Contrast estimate	SE	df	t.ratio	P value
Sum-Fall	0.350235	0.149	120	2.355	0.0522
Sum-Spri	0.419309	0.149	120	2.820	0.0154

Wetland.Type = Permanent, Gas = CO_2

	Contrast estimate	SE	df	t.ratio	P value
Sum-Fall	-0.257142	0.149	120	-1.729	0.1985
Sum-Spri	0.216218	0.149	120	1.454	0.3168
Fall-Spri	.473360	0.149	120	3.183	0.0052

Pairwise~Treatment | Wetland.Type*Season*Gas
Wetland.Type = Ephemeral, Season = Fall, Gas = CO_2

	Contrast estimate	SE	df	t.ratio	P value
Control-Warmed	0.64281	0.21	120	3.057	0.0028

Carbon Dioxide Flux
Response: LnFlux

	Df	F value	P value
Intercept	1	230.8503	< 2e-16 ***
Season	2	3.4017	0.03986 *
Wetland.Type	1	1.0808	0.30268
Treatment	1	0.9255	0.33990
Season: Wetland.Type	2	1.1557	0.32174
Season: Treatment	2	1.2926	0.28208
Wetland.Type: Treatment	1	0.7406	0.39290
Season: Wetland.Type: Treatment	2	0.6389	0.53141
Residuals	60		

Pairwise~Season|Wetland.Type*Treatment
Wetland.Type = Permanent, Treatment = Control

	Contrast estimate	SE	df	t.ratio	P value
Fall-Spri	0.6516	0.271	60	2.406	0.0497

Pairwise~Season | Wetland.Type*Season*Gas
Wetland.Type = Ephemeral, Season = Fall

	Contrast estimate	SE	df	t.ratio	P value
Control-Warmed	0.6428	0.271	60	2.373	0.0208

Pairwise~Wetland.Type|Season*Treatment
Season = Fall, Treatment = Warmed

	Contrast estimate	SE	df	t.ratio	P value
Ephemeral-Permanent	-0.58317	0.271	60	-2.153	0.0353

Methane Flux
Response: LnFlux

	Df	F value	P value
Intercept	1	1.4964e+05	<2e-16 ***
Season	2	3.9920e-01	0.6726
Wetland.Type	1	1.0340e+00	0.3133
Treatment	1	1.2579e+00	0.2665
Season: Wetland.Type	2	8.4060e-01	0.4365
Season: Treatment	2	8.6120e-01	0.4278
Wetland.Type: Treatment	1	6.9000e-03	0.9340
Season: Wetland.Type: Treatment	2	2.3000e-03	0.9977
Residuals	60		

Total Abundance
Analysis of Deviance Table (Type II tests)
Response: LogSQMean

	Df	F value	P value
Depth	1	12.2491	0.0007077***
Treatment	1	4.1290	0.0449173 *
Wetland.Type	2	2.5624	0.0823865
Depth: Treatment	1	0.1377	0.7114163
Depth: Wetland.Type	2	0.0395	0.9612610
Season: Treatment	2	2.3986	0.0962664
Depth:Treatment: Wetland.Type	2	0.3273	0.7216523
Residuals	96		

Alpha diversity: Faith Phylogenetic
Kruskal-Wallis (all groups)

	Result
H	10.673202614379079
p-value	0.058258533165010576

Kruskal-Wallis (pairwise)

		H	p-value	q-value
Group 1	**Group 2**			
EC10 (n=3)	**EC20 (n=2)**	1.333333	0.248213	0.372320
	EW10 (n=3)	3.857143	0.049535	0.185755
	PC10 (n=3)	3.857143	0.049535	0.185755
	PC20 (n=3)	0.428571	0.512691	0.699124
	PW10 (n=3)	0.047619	0.827259	0.886349
EC20 (n=2)	**EW10 (n=3)**	3.000000	0.083265	0.208161
	PC10 (n=3)	3.000000	0.083265	0.208161
	PC20 (n=3)	0.000000	1.000000	1.000000
	PW10 (n=3)	0.333333	0.563703	0.704629
EW10 (n=3)	**PC10 (n=3)**	2.333333	0.126630	0.211051
	PC20 (n=3)	2.333333	0.126630	0.211051
	PW10 (n=3)	2.333333	0.126630	0.211051

PC10 (n=3)	**PC20 (n=3)**	3.857143	0.049535	0.185755
	PW10 (n=3)	3.857143	0.049535	0.185755
PC20 (n=3)	**PW10 (n=3)**	0.047619	0.827259	0.886349

Alpha Diversity: Observed OTUs

Kruskal-Wallis (all groups)

	Result
H	11.424836601307192
p-value	0.04357785875506338

Kruskal-Wallis (pairwise)

		H	**p-value**	**q-value**
Group 1	**Group 2**			
EC10 (n=3)	**EC20 (n=2)**	0.333333	0.563703	0.563703
	EW10 (n=3)	1.190476	0.275234	0.317577
	PC10 (n=3)	2.333333	0.126630	0.237432
	PC20 (n=3)	1.190476	0.275234	0.317577

	PW10 (n=3)	1.190476	0.275234	0.317577
EC20 (n=2)	**EW10 (n=3)**	3.000000	0.083265	0.178424
	PC10 (n=3)	3.000000	0.083265	0.178424
	PC20 (n=3)	3.000000	0.083265	0.178424
	PW10 (n=3)	1.333333	0.248213	0.317577
EW10 (n=3)	**PC10 (n=3)**	0.428571	0.512691	0.549312
	PC20 (n=3)	3.857143	0.049535	0.178424
	PW10 (n=3)	3.857143	0.049535	0.178424
PC10 (n=3)	**PC20 (n=3)**	3.857143	0.049535	0.178424
	PW10 (n=3)	3.857143	0.049535	0.178424
PC20 (n=3)	**PW10 (n=3)**	1.190476	0.275234	0.317577

Alpha Diversity: Shannon Diversity

Kruskal-Wallis (all groups)

	Result
H	11.947712418300654
p-value	0.03551130796052839

Kruskal-Wallis (pairwise)

		H	p-value	q-value
Group 1	**Group 2**			
EC10 (n=3)	**EC20 (n=2)**	0.333333	0.563703	0.603967
	EW10 (n=3)	2.333333	0.126630	0.211051
	PC10 (n=3)	3.857143	0.049535	0.148604
	PC20 (n=3)	1.190476	0.275234	0.344042
	PW10 (n=3)	0.047619	0.827259	0.827259
EC20 (n=2)	**EW10 (n=3)**	3.000000	0.083265	0.178424
	PC10 (n=3)	3.000000	0.083265	0.178424
	PC20 (n=3)	0.333333	0.563703	0.603967
	PW10 (n=3)	1.333333	0.248213	0.344042
EW10 (n=3)	**PC10 (n=3)**	1.190476	0.275234	0.344042
	PC20 (n=3)	3.857143	0.049535	0.148604
	PW10 (n=3)	3.857143	0.049535	0.148604

PC10 (n=3)	**PC20 (n=3)**	3.857143	0.049535	0.148604
	PW10 (n=3)	3.857143	0.049535	0.148604
PC20 (n=3)	**PW10 (n=3)**	2.333333	0.126630	0.211051

Beta Diversity:
Wetland Type-Beta Weighted Unifrac

PERMANOVA results	
Method	PERMANOVA
Test statistic name	Pseudo-F
Sample size	17
Number of groups	2
Test statistic	0.898109
p-value	0.458
Permutations	999

Depth-No Warmed Beta Weighted Unifrac

PERMANOVA results	
Method	PERMANOVA
Test statistic name	Pseudo-F
Sample size	11

Number of groups	2
Test statistic	1.73
p-value	0.086
Permutations	999

Warming Treatment: No 20 Depth Beta Weighted Unifrac

PERMANOVA results	
method name	PERMANOVA
test statistic name	pseudo-F
sample size	12
number of groups	2
test statistic	0.585009
p-value	0.775
number of permutations	999

Vita

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