A Study on the Effects of Extreme Rain Events on Fungal Activity

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Abstract

Saprotrophic fungi play a key role in the carbon cycle by breaking down organic carbon in the form of leaf litter or downed wood into inorganic forms with which they perform humification, mineralization, or export as dissolved organic carbon (Boddy, 2008). Human activity has altered Earth's natural carbon cycle, and one outcome of this in an increase in severity and frequency of extreme precipitation events (O'Neill, 2018). Our study investigates how sensitive fungal communities in varying ecosystems are to an extreme precipitation event in terms of enzymatic activity. We induced an extreme precipitation event on three different landscape ecosystems on the University of Michigan Biological Station property an outwash plain dune, wetland, and moraine. At each site, we analyzed both the conditions of their present fungal community and the community's enzymatic response to an imposed rain event over time. We found that when comparing the concentrations of phenol oxidase, an enzyme fungus uses to break down phenols, to soil moisture content, the two were inversely correlated in the dune and moraine sites, but positively correlated in the wetland site. From this we derived that in the dune and moraine sites, water is neither limiting nor excessive, therefore additional water will decrease fungal activity by disinsentivising cooperative linking or fungal communities brought about by a common need for a limited resource. However, in the wetland site where water already exists in excess, the addition of water will have no effect on fungal communities.

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Introduction:

Climate Change

Humans continue to impose pressures on the Earth's natural carbon cycle by shifting the amount of carbon naturally held in geological and terrestrial reservoirs, as fossil fuels and forests respectively, to our atmosphere and oceans. This disrupts the naturally cyclic flow between these reservoirs which has brought about many unanticipated consequences such as changes in precipitation patterns (O'Neill, 2018). Our study focuses specifically on the increased abundance and severity of extreme precipitation events. In the 20th century, the frequency of rainfalls greater than 4 inches in a day has already increased by 50% in the Midwest. This trend is projected to continue increasing over the coming century, however the dispersal or rain events is also expected to become more highly targeted to the wet seasons. Therefore, the increase in extreme precipitation events will be paired with an increase in extreme drought conditions during the dry seasons. These rapid and variable changes in climatic conditions in northern Michigan are expected to impose immense pressures on all the living organisms that inhabit this region (Handler, 2013).

Saprotrophic Fungi Function

Saprotrophic fungi are an essential component to an ecosystem; inhabiting the soil they perform the critical task of breaking down organic carbon in the form of leaf litter or downed wood into inorganic forms available for plant uptake. The tool with which fungi are able to break down plant matter is their hyphae which secretes enzymes for extracellular digestion. The litter they break down varies from small to large plant fragments such as buds, leaves, and coarse woody debris. Because the abundance of these litter types varies by location within a forest and between seasons, fungi must be able to break down a wide range of organic matter (Boddy, 2008). An important enzyme that fungus employs is phenol oxidase which degrades phenolic compounds, a carbon ring structure that is very difficult to break down (Sinsabaugh, 2010). One such molecule characterized by its high phenolic content is lignin, found in the cell wall of plants (Hakkila, 2012). Phenol oxidase works to degrade compounds including lignin, and with the broken-down carbon, fungi perform humification, mineralization, or export it as a dissolved organic carbon which contributes to soil respiration (Boddy, 2008).

Previous Literature

A study titled "Fungal Community Responses to Precipitation" was conducted in northern California for the purpose of predicting the functional responses of fungi to climate changes, and what consequence this might have for the soil carbon cycle. Looking in response to short term ambient rainfall, they found fungus to be less abundant, diverse, and more variable during periods of rain than periods of drought. They interpreted these results to indicate that species with a wider range of tolerances are better able to coexist during droughts because this stress moderates their ability to compete with one another. They observed no change in carbon storage during the four-year period of their study but did measure an increase of carbon dioxide flux into the atmosphere with increased rain (Hawkes, 2011).

Another manipulative study titled "Diversity and Co-Occurrence Network of Soil Fungi are More Responsive Than Those of Bacteria to Shifts in Precipitation Seasonality in a Subtropical Forest" reduced rainfall during the dry season and increased rainfall during the wet season in the subtropical forest of China while keeping the total annual rainfall constant; this study was meant to mimic anticipated climate change for the region. Their study revealed that increasing the severity of both the wet and dry seasons reduced fungal abundance, richness, and activity over time. However, they also observed that during periods of drought, the interactions among fungal communities intensified (He, 2017).

Lastly there was a study titled "Drought Consistently Alters the Composition of Soil Fungal and Bacterial communities in Grasslands from two Continents" explored how imposed drought affected abundance, richness, and function of fungal communities. They found that mesic ecosystems were much more sensitive to drought than already xeric ecosystems. In the mesic locations, there was a significant increase in abundance, richness, and enzymatic activity of fungal communities during imposed drought. They hypothesized that this was due to plants having a much lower tolerance for drought than fungal communities in mesic areas, the increased deaths of fine roots and shedding of foliage drove more fungal activity (Ochoa-Hueso, 2018).

Landscape Ecosystems

The University of Michigan Biological Station is located on the edge of where ancient glacial landforms resided. Therefore, the soil characterizing the landscape ecosystems on the property have been established by either moraines or outwash plains. Moraines are formed at the far end of receding glaciers where unsorted material, called till, is deposited. Soils in these locations consist of a mixture of sand, silt, and clay particles. This variation reduces water drainage and nutrient leaching. Outwash plains are formed where glacial runoff carried and deposited large sand particles from the moraine. These soils, which are composed primarily of sand, allow water to drain more easily and have a higher leaching rate than till. Following the receding of the glaciers came the formation of glacial lake Algonquin which extended partially into the University of Michigan Biological Station property. This led to the formation of dunes and swales which are still visible in the topography today. This unique history has given rise to a great diversity of landscape ecosystems on the University of Michigan Biological Station property supporting an array of different soil conditions which would react differently to changes in precipitation patterns (Sommers, 1984).

Hypothesis

In our experiment, we plan to explore specifically extreme precipitation events in northern Michigan and its effect on fungal activity. We hypothesize that in ecosystems that are limited by water, the rain event will drive fungal decomposition by providing a resource that allows fungi to thrive and reproduce (Boddy, 2008). In ecosystems where water is not limiting we hypothesize that the rain will decrease fungal activity by reducing the incentive for fungal communities to cooperatively link to one another to take in soil resources more efficiently (He, 2017). Lastly, in ecosystems where water is excessive, we hypothesize that the addition of water will have no effect on fungal activity because there is already water in the soil that is not being taken up for decomposition which has led to its buildup. We hypothesize that our dune site will be an ecosystem limited by water due to excessive drainage, and that it will therefore have suppressed fungal community abundance. Our moraine site will not be limited in water having a moderate drainage rate and therefore a high fungal community abundance. The wetland site will have excessive water due to a low drainage rate, we also expect the fungal community to be suppressed here due to anaerobic conditions.

Materials & Methods:

To test this hypothesis, we simulated an extreme precipitation event on the University of Michigan Biological Station property and took measurements of enzyme concentration and abundance. We conducted the experiment at three different landscape ecosystems to capture the wide range of communities existing on this property, and to quantify how preexisting environmental conditions influence the impact of an extreme rain event on fungal activity in terms of scale and direction.

Sampling Sites

To determine which sites to sample, we used GIS data for the University of Michigan Biological Station property (Tallant, 2018). We identified three major landscape ecosystems to represent the diverse distribution of ecosystems on the property; these were a high-level outwash plain that was previously a dune, a low-level outwash plain that is currently a wetland, and a moraine. At each location, we delineated three meter squared plots, two of the three plots were designated as treatment plots to receive an extreme precipitation event, and the third plot was kept constant as a control. The plots were spaced about ten meters apart from one another on relatively level ground to assure that water poured on one plot could not seep into neighboring plots.

Rainfall

Using data from NOAA, National Atmospheric and Oceanic Association, we found that the most extreme rain event between the years 2005 and 2011 was 1.37 inches in an hour which was much higher than the average rate of rainfall during that time period at .15 inches an hour (PRECIP, 2018). With this context, we decided on the rainfall rate of one inch an hour for a duration of 30 minutes. In total, half an inch of water rained on each meter squared plot, amounting to 10 liters per plot. The water used for the rain was deionized to eliminate potential confounding variables from contaminants. To simulate rain, we attached a hose with a sprinkler head to a five-liter carboy. The rate of water flow from this system was four times the desired rate at four inches an hour; therefore, while conducting the precipitation event we rained for one minute, and stopped for three minutes, continuing this pattern for the entire 30-minute period.

Soil Sampling

On the first day of sampling we conducted a number of one time measurements to gain an understanding of existing environmental and fungal conditions at each site. We qualified leaf litter by recording the leaf species observed and measuring the depth of the organic layer at each site. Following these measurements, we took five initial soil cores from each plot (2 cm wide by 10 cm deep) homogenized these cores, and immediately stored them on ice. Next, we simultaneously conducted the precipitation event for 30 minutes on the two treatment plots. After completing this process at each site, we sifted the soils from each plot through a two-millimeter sieve. 20 g of this sifted sample was separated to dry in the 60-degree oven to take soil moisture measurements and the remaining sample was stored in the 40 degree refrigerator for chemical analyses. This soil sampling process was repeated 12, 24, and 72 hours following the rain event to analyze fungal activity over time.

Chemical Analyses

In the lab, we began by quantifying environmental comparisons between the landscape ecosystems. This involved conducting a pH test, a carbon to nitrogen ratio test for productivity, a hydrometer analysis for soil texture, and moisture content measurements for each plot at each time interval for analyzing drainage rates. To understand initial environmental conditions pertinent to fungal communities, we additionally conducted a test for phenolics to determine the carbon content targeted for fungal breakdown, and a test for ergosterol, a chemical found in the cell walls of fungi, to evaluate preexisting abundance of fungal communities. Lastly, to analyze fungal activity at each time interval, we tested for the phenol oxidase enzyme using L-Dopa which phenol oxidase breaks down into melanin. Excluding the phenol oxidase concentration and moisture content, all tests were conducted only on the time 0 samples.

Statistics

To determine whether static data taken at time 0 was statistically different between the sites we conducted ANOVA analyses. The variables for which we conducted these analyses were carbon to nitrogen ratio, percent carbon, percent phenolics, ergosterol, and pH. To compare amount of phenol oxidase between the two sites over time we conducted a two factor ANOVA analysis.

Results:

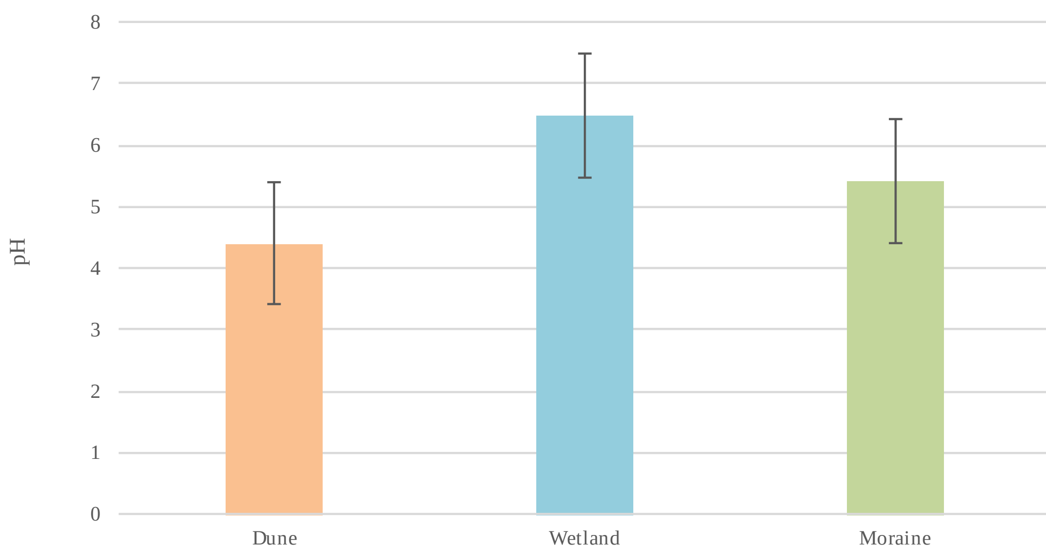

Figure 1: Average pH values for dune, wetland, and moraine sites at the University of Michigan Biological Station. (p-value .00647194)

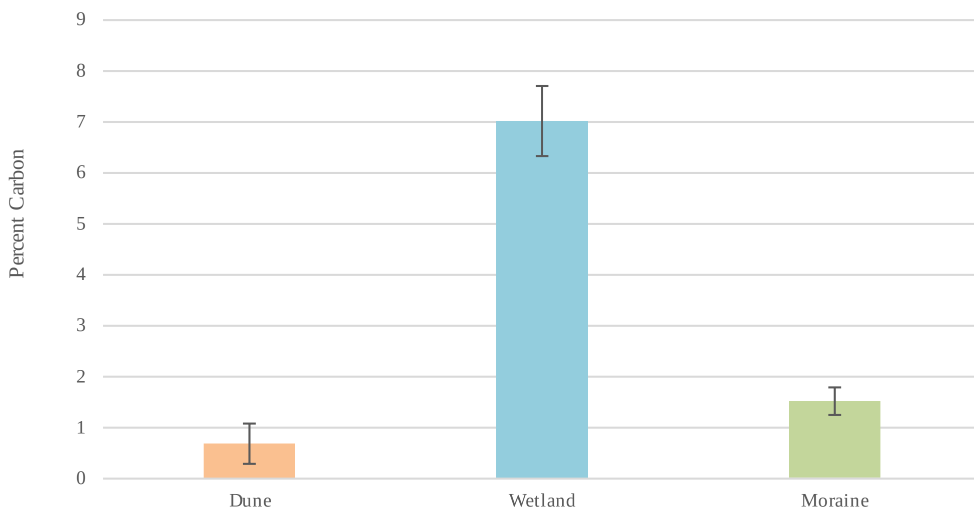

Figure 2: Percent carbon in soil for dune, wetland, and moraine sites at the University of Michigan Biological Station. (p-value 0.000131224)

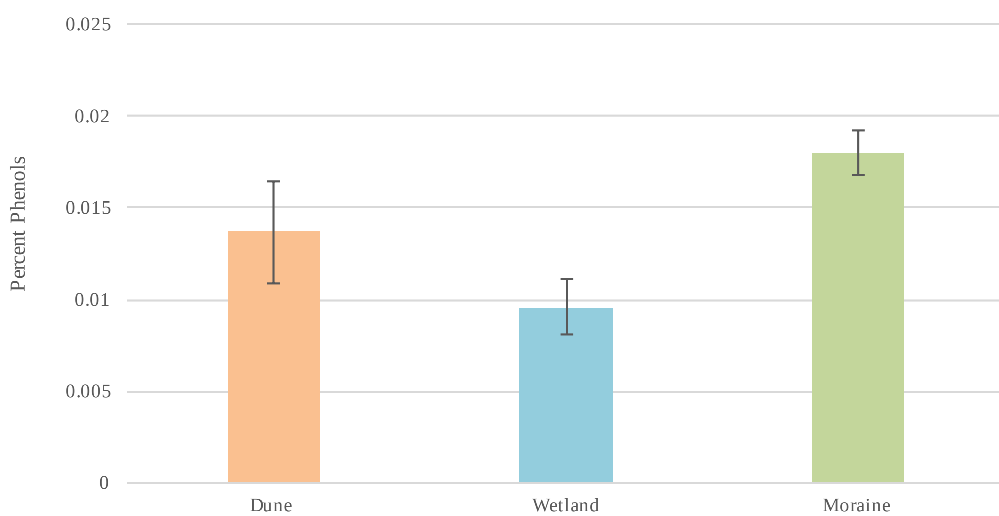

Figure 3: Percent phenols in total carbon for dune, wetland, and moraine sites at the University of Michigan Biological Station. (p-value .10721507)

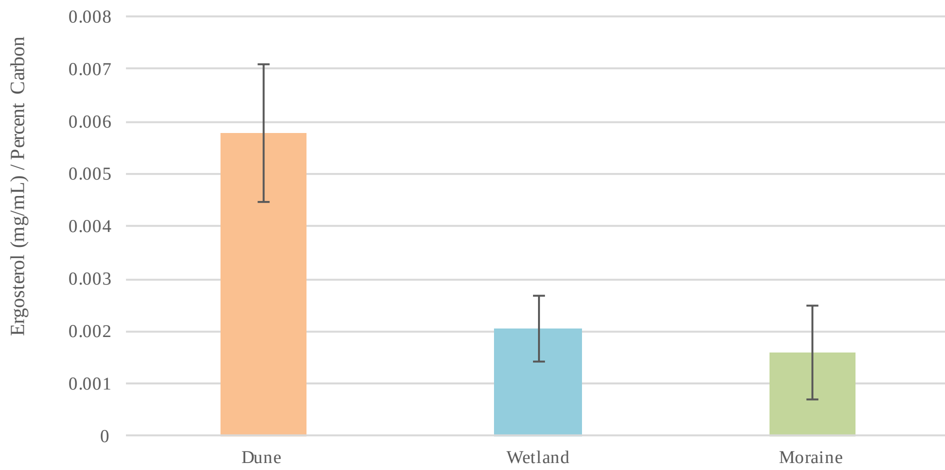

Figure 4: Amount of ergosterol proportional to percent carbon for dune, wetland, and moraine site samples at the University of Michigan Biological Station. (p-value 0.071169514)

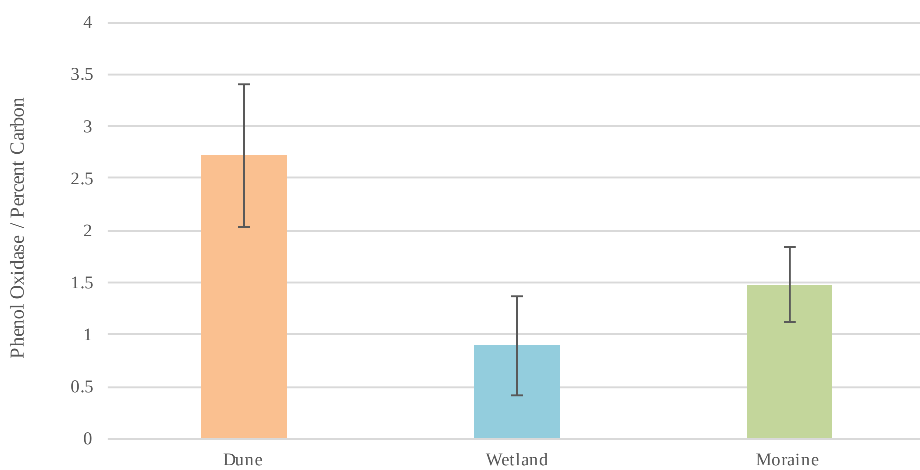

Figure 5: Initial phenol oxidase concentrations proportional to percent carbon in dune, wetland, and moraine sites at the University of Michigan Biological Station. (p-value 0.152939859)

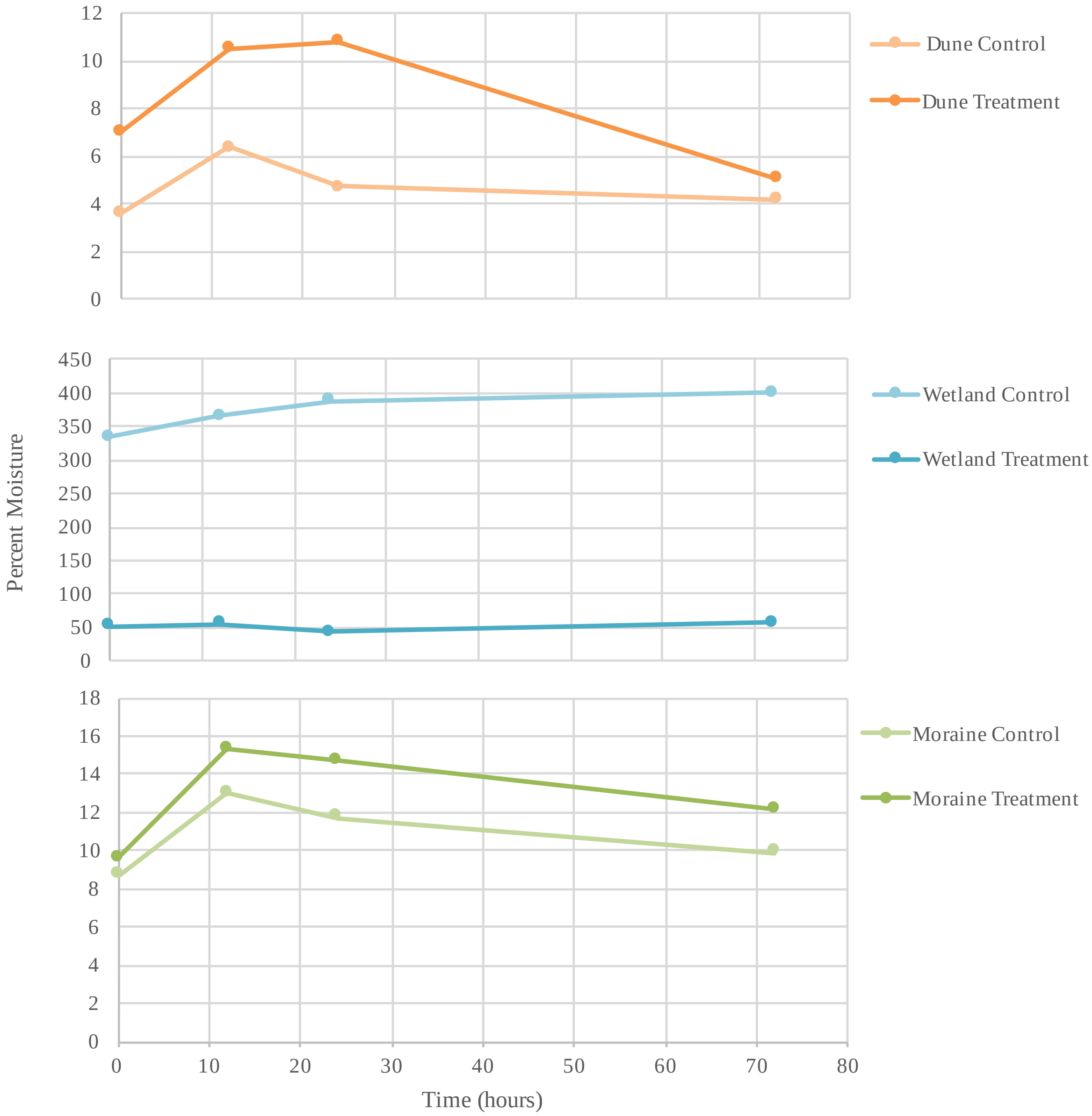

Figure 6: Soil moisture content comparison between control and treatment plots for dune, wetland, and moraine sites at the University of Michigan Biological Station.

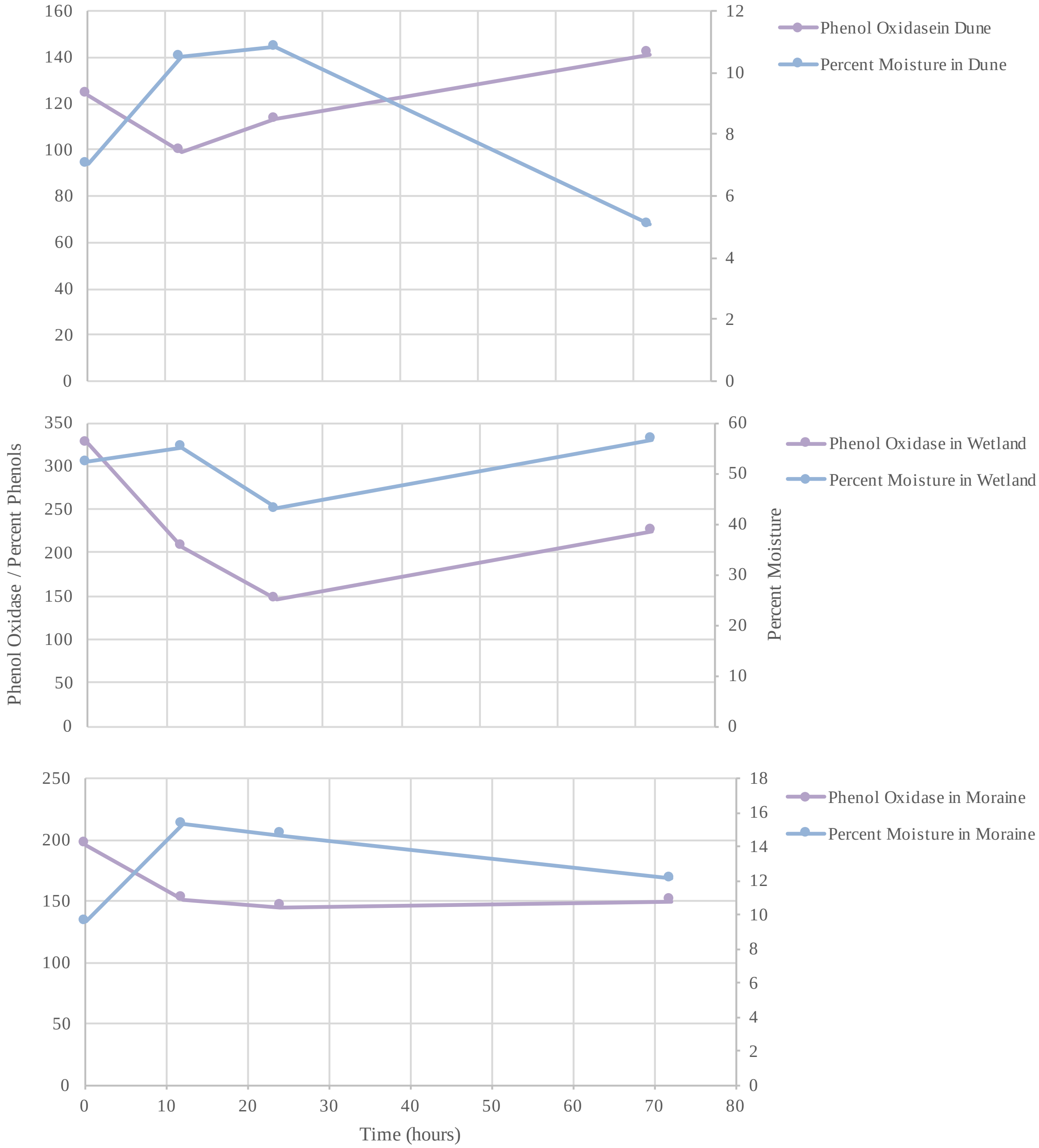

Figure 8: Comparison between soil moisture content and phenol oxidase concentration for each site, dune, wetland, and moraine at the University of Michigan Biological Station.

The leaf litter present in the dune site included *Populus grandidentata, Pinus resinosa, Pinus strobus, and Quercus rubra.* The depth of the O horizon was about one centimeter, the soil texture was sand, and the average pH was acidic at 4.41 (Figure 1). At the wetland site the leaf litter was comprised of *Pinus resinosa, Pinus strobus, Quercus rubra, and Tsuga occidentalis.* The depth of the O horizon was greater than ten centimeters, the soil texture was loamy sand for what was sampled beneath the organic layer, and the average pH was slightly basic at 6.477 (Figure 1). The moraine site had leaf litter including tree species: *Fagus grandifolia, Populus grandidentata, and Quercus rubra.* The depth of the O horizon at this location was two to three centimeters, the soil texture was also sand, and the average pH of soil samples was slightly acidic at 5.420 (Figure 1).

The percent carbon in the soil in the dune was .679% followed by the moraine at 1.510%, and then the wetland which was very high relative to the other sites at 7.007% (Figure 2). The ANOVA analysis indicated that the difference in mean percent carbon between these sites was significant. Following this with a Post Hoc analysis that showed that there was a significant difference between the dune and wetland (p-value .00133) and the moraine and wetland (p-value .00334), but not between the dune and moraine. The percent phenols of this carbon for the dune, wetland, and moraine sites were 1.368%, .959%, and 1.800%, respectively (Figure 3). An ANOVA analysis revealed that there was not a significant difference between the mean percent phenols of carbon in these sites.

The initial concentration of ergosteral per percent carbon at the dune site was the highest at .00576, followed by the wetland at .00206, and the moraine at .00160. An ANOVA analysis revealed that the mean ergosteral per percent phenols between the sites was only marginally significant (p-value .0712). The initial concentration of phenol oxidase per percent carbon was the highest at the dune site at 2.726, followed by the moraine at 1.475, and lastly the wetland at .898. An ANOVA analysis on this data showed that this data was also not significantly different between sites.

Figure 6 shows that the control plot at the dune site began with a lower moisture content, but that the imposed precipitation event did result in a consistently higher moisture content in the treatment plots throughout the 72- hour period of the study. In the wetland site, the control plot was measured to have a much higher moisture content than the control plots throughout the study. In the moraine site, the treatment and control plots began at about the same moisture level and our imposed precipitation event caused the treatment plots to have a consistently higher moisture content that the control plot throughout the study.

Figure 8 shows that in the dune site, soil moisture and phenol oxidase content was inversely correlated. This same inverse trend was found in the moraine site, however, the wetland site showed a positive correlation.

The two factor ANOVA analysis on the phenol oxidase concentration in the control plots as compared to the treatment plots over time revealed that the difference was only marginally significant (p-value .1074).

Discussion:

Based on our comparative observational analysis of the wetland, moraine, and dune, we found that these three landscape ecosystems exhibit highly different soil properties and plant communities, which determines the amount of water and carbon available to fungal communities. The dune, characterized by sandy, acidic soils drains quickly and has minimal organic carbon content due to a thin O horizon. This community supported a mixture of hardwoods and pines. The wetland on the other hand, consisted of almost entirely organic carbon in the top ten centimeters of the soil, and is nearly saturated due to periodic flooding. The leaf litter at this site was most heavily concentrated with needles from pines and cedars. The moraine had an intermediate value for carbon content at an O horizon depth of 2-3cm, however, these characteristics were much more closely proportional to the dune site. The tree species at this site did not include pines, but did include hardwoods, needles have a higher lignin content. Despite these vast variances in litter and soil quality, the percent phenols of the total carbon were not found to be different between the three sites. This indicates that the quality of the organic carbon at each site, characterized in terms of phenol content, were consistent between the sites despite having varying carbon inputs from different tree species. Contrary to our hypothesis, the three sites did not reveal a significant difference in fungal abundance proportional to percent carbon.

The soil moisture content over time data revealed both preexisting differences between treatment and control plots, a differing relative drainage of moisture over time between sites, and varying severity in effect of our extreme precipitation event between sites. For the months preceding our experiment, this region was experiencing a period of uncommonly high temperatures and drought. However, a week prior to our experiment there was a moderate natural rain event. This led to variability in soil moisture between areas within each site which can be observed in the initial differences between moisture content of control and treatment plots before beginning the imposed precipitation event, specifically in the dune site. Our precipitation event did appear to have an effect on the soil in the dune and moraine sites as their moisture level was consistently higher throughout the study. By comparing the two sites, it is evident that the dune site drained more quickly, as hypothesized. The wetland site in general did not appear to react to our imposed rain event as the soil moisture content in the treatment plots was erratic throughout the study. Additionally, the control plot at the wetland site was an extreme outlier to the data because the soil there consisted exclusively of saturated organic matter and therefore could not be used as a reference for further analysis.

Comparing the trends of soil moisture content and the phenol oxidase concentration showed that both the dune and the moraine exhibited an inverse relationship between the two variables while the wetland revealed a positive correlation. According to previous literature, fungal activity increase in response to introduced water only in cases where water is extremely limited. Because this trend was not observed we can conclude that water was not limiting in either the dune or moraine, and instead, the addition of water may have disincentivized fungal communities from linking to interact cooperatively (He, 2017). Because moisture content did not strongly correlate with the methods of our study in the wetland site, we concluded that the addition of water had no effect on the fungal communities there. It is likely that this ecosystem already has an overabundance of water and organic carbon, therefore the fungal communities simply continued reacting to natural variations in flooding.

Although our variation in phenol oxidase concentration as compared to the control plots was only marginally significant, when scaled to the duration of anticipated drought and heavy precipitation events in the future, these trends could amount to a significant difference and severe impact on ecosystems. Fungi are incredibly flexible communities that can tolerate a relatively wide range of environmental strains. However, as oscillations between wet and dry seasons become increasingly more intense and frequent, the seasonal changes between fungal abundance and activity will have a severe impact on their ability to consistently regenerate (He, 2017). At this scale, the plant community’s response to drought and heavy rain, such as the deaths of roots and shedding of foliage, would also amplify the effect of climatic changes on fungal activity. To make conclusions about how fungal interactions vary during long periods of extreme weather in a way that either enhances or reduces activity would require a long term observational study involving multiple precipitation events.

Fungi play a central role in the carbon cycle by taking in organic carbon from forest litter and exporting carbon in the form of humification, mineralization, and dissolved organic carbon (Boddy, 2008). In a balanced ecosystem, the rate at which fungi convert carbon into these form correlates with the respiration rate of above ground communities (O'Neill, 2018). As this balance is offset and the decomposition process either increases or decreases in rate, the forest has the potential to either become a more severe carbon source or sink. This could thus induce a positive feedback loop, to either counter or further the unnatural shifts of carbon between natural reservoirs.

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