The Impacts of Extreme Precipitation on different Fungal Communities at the University of Michigan Biological Station

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Abstract

The increase in extreme precipitation events due to anthropogenic climate change has been reported extensively. This study explores the affects of one such extreme event on the enzymatic activity of saprotrophic fungi in three different environments. Saprotrophic fungi are crucial members of the decomposition process, and understanding how changes in precipitation patterns affects them will have significant impacts on our understanding of carbon and nutrient cycling. We found that the treatment resulted in a marginally significant decrease in fungal activity in two of the sites, and that fungal activity did not vary significantly between our three sites. The decrease in fungal activity offers us insights into the relationship fungus has with water.

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Introduction:

As anthropogenic climate change progresses, the frequency of extreme precipitation is expected to increase in the state of Michigan. This has already begun, as the Midwest has seen a 37% increase in the amount of rain that falls in the most intense 1% of precipitation from 1958 through 2012 (Janowiak et al, 2014). The precipitation regime is therefore predicted to become more episodic, and high-intensity events are representing a larger proportion of the total annual and seasonal rainfall (Handler, 2014). This change has had and will continue to have impacts on ecosystem processes such as erosion, precipitation and growth (Knapp et al, 2008) . Various soils that are less able to hold water will especially be impacted by the increase in proportion of rain falling in extreme events, as they will not be able to hold any of the water that comes down in those events, and so may experience drought-like conditions, even if the total rainfall has not

changed (Handler, 2014). As rainfall begins to follow a pattern of intense rainfall followed by drought, communities living in the soil are going to be impacted (Reichstein et al, 2013). These communities are responsible for most of the decomposition in an ecosystem, and so are key to nutrient and energy cycles. One member of this community is saptrotrophic fungi.

Saprotrophic fungi are a diverse and important type of decomposers. They are effective in this role— their hyphae allow them to penetrate most materials while extracellular enzymes metabolize the dead organic matter (Talbot et al, 2012). The wide range of enzymes produced by saprotrophic fungi make them particularly adept at decomposing the recalcitrant lignocellulose, making them key members of the decomposer community (Kjoller, et al., 1982).

One group of such enzymes are phenol oxidases, which are used in plants to produce lignin, a compound rich in phenols. Phenol oxidases break down lignin and humus, releasing fixed carbon and energy for fungal growth (Sinsabaugh , 2010). One study found that fungus was unable to break down lignin when it lacked the ability to produce phenol oxidases, evidence of its importance to fungal communities (Ander et al., 1976). Though the enzyme is associated with fungi, bacteria and archaea, the largest class of the enzyme found in the soil, is traditionally associated primarily with fungi, which makes it a good proxy for overall enzymatic activity (Sinsabaugh , 2010). This ability to produce enzymes that break down the tougher organic matter ensures the reliance of the ecosystem on fungus's contribution to the cycling of nutrients. Thus, anything that alters the fungal activity will also alter the cycles of nutrients in an ecosystem.

Ergosterol is a component of the fungal cell membranes, at it can act as a proxy for fungal presence and abundance in diverse site (Martin 1990).

We tested the activity of phenol oxidases on three contrasting ecosystems: A dune, moraine and wetland, all found near on University of Michigan Biological Station (UMBS) land

near Pellston. The dune site was formed initially by glacial outwash selectively depositing sand (Karowe, 2018). This was later shaped into dune on the shore of the glacial lake Algonquin, around 11,000 years ago (Farrand, 1988). The high porosity inhibits retention of water and nutrients in the site, so the dune is categorized by its well-drained soil with low nutrients (Spurr, 1964). The wetlands parent material was also formed by glacial outwash, but it was close to a creek that seasonally flooded the site, making it more poorly drained with higher nutrients. Our last site was an interlobate moraine, a hill of unsorted till of various sizes that collected between two lobes of the glacier and then deposited during its retreat (Karowe, 2018). Because of the variety in particle sizes, moraines usually retain water and nutrients more than a dune, but not as well as the wetland. The differences in nutrient and water retention at our three sites should affect the fungal communities and their response to extreme precipitation events. The differences in amount of accumulated carbon acros the three sites should also have an impact on the pre-existing enzymatic activity.

There have been a number of studies exploring the effects of moisture on fungal communities. One study, conducted in the wet Northwestern forests of Oregon, found that respiration logs sheltered from rain had higher respiration rates than those unsheltered (Prograr et al., 2000). Another study found that fungal communities were more abundant and diverse during drought than during rain, and suggested that fungal aabundance only increases in response to rainfall in dry soils where rain is a limiting resource (Hawkes, 2011). This is supported by a study conducted in a dry moisture-limited woodland, which found that fungal abundance increased during the monsoon seasons as opposed to the dry seasons (Cregger et al., 2012).

No studies were found to directly explore the effects of extreme precipitation on fungal communities, and none were conducted in the biome found on University of Michigan Biological

Station (UMBS) land. The purpose of this study is to investigate the effects of an extreme precipitation event on the activity of saprotrophic fungi living in the soil of three distinct environments in Michigan. We hypothesize that the change in fungal activity in response to an extreme precipitation event is due to water availability in an ecosystem. If water is a limiting resource, then extreme precipitation will increase fungal activity. If water is not a limiting resource, then fungal activity will decrease in response to an extreme precipitation event. Due to the different water retention capabilities of our three sites, we predicted that the dune would experience an increase in activity, as water is a limiting resource in that environment, the moraine would experience a decrease in activity, as water is not a limiting resource, and would not change in the wetland, as it is already saturated with water and fungal activity would not change in response to added water.

We predict that phenol concentration will be positively correlated to ergosterol and fungal activity. In sites with higher phenol concentration, we expect higher presence and activity of carbon, due to the fact that fungus are the only organisms that can break phenol. We predicted that Phenolic concentration would be highest in the dune site, due to the high input of phenolic-rich detritus. We predicted that fungal presence, when taking available carbon into account, would be the highest in the dune, due to its higher phenol concentration. We also predict that fungal activity, not taking amount of carbon available into account, would be highest in the moraine site, as it is most productive site. However, we predicted that fungal activity per percent carbon would be higher in the dune, again due to its high phenolic content. We predicted that percent carbon would be highest in the wetland site, as it had a high accumulation of carbon, and that pH would be highest in the wetland due to its ability to buffer, and would be lowest in the dune, due to the high input of acidic

Materials and methods

We selected our sites using the UMBS GIS map. Our dune and wetland site were along Hogsback road, and our moraine site off Riggsville road. All three sites were on UMBS property near Pellston. At each site, we constructed three 1 m^2 plots—two experimental and one control.

In order to calculate the amount of water falling that would classify as an extreme rain event in the ecosystem and climate at UMBS, we examined hourly precipitation data for Gaylord county for the years 2005 through 2011. The highest rainfall in those 6 years was 1.37 inches, and the second highest rainfall was .97 inches (NOAA, 2018). We chose to use the rate 1 inch per hour to simulate extreme precipitation, both for ease of use and because it would have been the second highest extreme weather even occurring in 6 years at UMBS.

In order to simulate a 1 inch per hour rate we constructed a sprinkler system by attaching a small Nalgene bottle with punched holes to a carboy with a plastic tube. We calculated a rate of 4 inches an hour, and so to approximate the desired rate, we would turn on the carboys for a minute, and then turn them off for three hours, for a total of a half hour. We did not rain for a full hour because we did not want to overwater and cause runoff in our plots. For our simulated rain, we used deionized water.

At each plot and at each time we took five 10 cm cores in order to better capture the fungal diversity of each plot. We passed them throw a 2mm sieve and maintained them on ice prior to analysis. At two plots, plot 1 and plot 3, in the wetland site more than five cores were taken at each time due to the high density of roots in the plot preventing the soil corer from going down to 10 cm. The amount of cores obtained at those plots was based on an estimation of obtaining a similar amount of soil material as the other plots.

Following simulated extreme rainfall we again took soil cores at time 0, time 12, time 24 and time 72 to see how fungal activity changed as time elapsed. We placed all soil cores from one plot in one bag and put it on ice. We then weighted out 20 grams into tins and placed them in drying ovens for at least 24 hours. We then re-weighed the 20 gram samples to obtain soil mosture content and used the soil to perform hydrometer analysis to obtain soil texture of the three sites.

To verify fungal presence, we tested for ergesterol concentration at all three plots prior to water addition. We performed Carbon and Carbon:Nitrogen tests to ascertain the amount of carbon in each of our sites, and examined phenols to more specifically examine carbon available to fungus, also all on just our time 0 soil samples. We then ran an enzyme assay using l-dopamine to investigate the amount of phenol oxidase activity in the soil samples for all our time periods.

To verify significance, we ran ANOVAs on our percent carbon, pH, Phenols per percent carbon, phenols per percent carbon, ergosteral per percent carbon, and initial phenol oxidase activity. In the case of phenols per percent carbon, we also ran a two-sided t-test for the moraine and dune site. We ran a two-factor ANOVA on our phenol oxidase activity data to see if the temporal trends were statistically significant.

Results

The three sites were varied in their litter. The dune's litter was made up of leaves from *Quercus rubra, Pinus strobus, Populus grandidentata* and *Pinus strobus*. The wetland's litter contained *Pinus strobus, Thuja occidentalis, Pinus resinosa, Quercus rubra, Populus grandidentata* and *Acer rubrum* leaves. The moraine site contained leaf litter from *Fagus*

grandifolia, Populus grandidentata, and *Acer rubrum.* The soil texture at the wetland was loamy sand, while the soil texture at both the dune and moraine was sand, though the moraine did have a small percentage of clay present. The pH varied significantly between the two sites, the dune site was the most acidic and the wetland site was the least acidic (figure 5, F= 13.10, P-value= .0065). Specifically, the dune varied significantly from both the moraine and the wetland site (P-value= .0048, .0491).

Our three sites varied in their percent carbon. Wetland had the highest percent carbon, followed by Moraine, and finally dune. These results were significant (Figure 1, F-value= 86.78, P-value= .00013). Post-hoc analysis revealed that the wetland was significantly different from the dune and moraine (P-value= .0013, .0033). The concentration of phenols were not found to be statistically different. The dune had the highest ergosteral per percent carbon, followed by wetland and then dune. These results were statistically significant (figure 3, F-value= 83.71, P-value= .000143). The wetland had the highest ergosterol per phenol concentration, followed by moraine and then dune. These results were statistically significant (figure 4, F-value= 17.9258, P-value= .0052). Post hoc analysis revealed that it was the differences between the wetland and moraine and wetland and dune that were statistically significant (P-value= .02, .0209). Initial enzymatic activity seemed to be higher in the moraine site than in the other two sites, but the results were not statistically significant (Figure 6).

The addition of water seemed to have an effect on the moraine and dune site, but did not on the wetland site, though our results were only marginally significant (Figure 7, F-value 3.41, p-value= .1074). Phenol oxidase activity decreased initially in the moraine and dune treatment plots, where it initially increased in the control plots (Figure 8). Phenol oxidase activity in both

the dune and the moraine had an inverse relationship with soil moisture, at least initially. As the soil moisture increased, the phenol oxidase activity decreased.

Discussion

Our hypothesis was correct in some ways and incorrect in others. In our wetland site, the soil moisture content did not follow any trend, so we can conclude that our treatment was ineffective and that any changes in phenol oxidase activity were not due to our treatment. This is similar to what we predicted—that the site was already so wet that our treatment would have no affect on enzymatic activity. In the dune and moraine site, the simulated extreme precipitation event did seem to have an affect on soil moisture content. It is important to note that the moraine control and treatment plots started at similar soil moisture levels, but the dune treatment sites began with higher soil moisture content than the control site. However, in both cases, our addition of moisture seemed to increase the soil moisture content in the treatment sites, though they followed similar temporal trends as the control sites. Because our treatment did seem to affect soil moisture content in the treatment plots of our moraine and dune sites, we can make some conclusions about the affect of our treatment on the two sites.

In our treatment plots, the increase in moisture led to a decrease in phenol oxidase activity, and as the moisture content began to decrease the enzymatic activity began to increase. We expected this in our moraine plot, where water was not a limiting factor, but this relationship was not expected in the dune plot. There are two possible explanations for this. Either water was not limited in the dune plot, or fungal activity only increases in response to heavy rainfall when water is extremely limited, in deserts or in severe droughts. Though the soil texture at the dune

was sand, it had just rained a week before our experiment, which may have explained our results that water did not increase fungal activity in the dune site (Vogel, 2018).

Though we did observe some temporary effects of our treatment on enzymatic activity, but we also observed longer effects on fungal presence and activity in our different sites. The pH of the three sites varied, specifically the wetland was significantly higher in pH than the dune and moraine, which may be a component of the basic qualities of a wetland that allow it to better buffer the acidic litter inputs of some of the more phenolic leaf litter. Our wetland had the highest percent carbon, which can be explained by the buildup of carbon that occurs in most wetlands.

However, the quality in litter across the three sites were not statistically different. This is interesting considering the differences in leaf litter—the dune had more phenol-rich litter sources like conifers, while the moraine had litter sources that were less rich in phenols like most broadleaf trees. This is different that the expected high phenolic content in the dune site and lower phenolic content in the moraine. It is possible that higher density of trees, and therefor the higher deposition of litter, can account for the similarities in phenol content between the moraine and the dune. It is also possible that phenols are higher in the dune but we the variance in our data did not allow us to conclude that significantly.

Though phenol concentration did not vary across the three sites, ergosterol did vary. Ergosterol per percent carbon was highest in the dune site, indicating that there was a higher concentration of fungus relative to overall carbon in the dune site. However, ergosterol per phenols were not significantly different between the three sites, though the dune did have the highest ergosterol per phenols. This difference in results may indicate that phenols do vary between the three sites, and are in fact higher in the dune, or they may indicate that ergosterol is

not significantly related to amount of phenols in a site. If this is true, then fungal presence cannot be predicted by how high phenol concentration is in the site.

Initial enzyme activity did not vary significantly between our two sites. This may indicate that the factors we were using to predict fungal activity: Ergosterol, phenol and total carbon, are not in fact correlated with differences in enzymatic activity. It is also possible that differences are subtle enough that we failed to detect them in our small sample size.

Further research opportunities include a more in-depth look at differences in fungal activity between three different sites and the indicators we use to examine those differences. We would want to verify that there was, in fact, no difference between phenols and phenol oxidase activity between our three sites by using a larger sample size. We would also explore other enzyme activity's as proxies for fungal activity, to see if they are more strongly correlated with fungal presence or phenol concentration.

Though we did see an interesting temporary decrease in fungal activity in response to the addition of water in our moraine and dune site, in order to draw any conclusions about the overall affects of the increase in episodic rainfall on fungal communities and their ecosystem, we would need to perform a longer study examining both the effects of extreme rainfall, and the droughts that follow them, on fungal communities.

Appendix

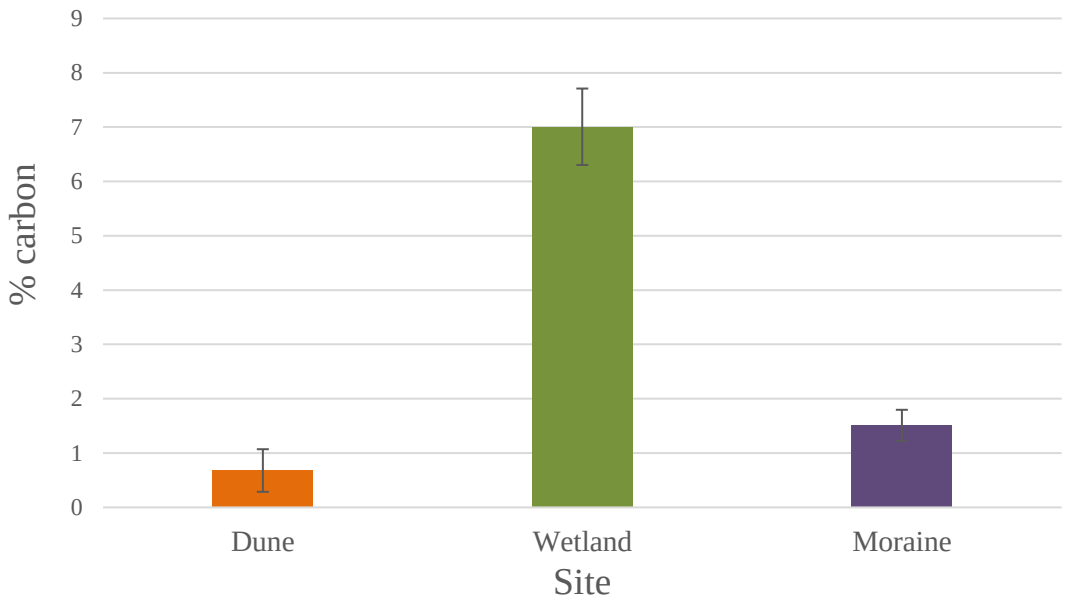


Figure 1. Average percent soil carbon at the dune, wetland and moraine site at UMBS. Wetland significantly different than dune and moraine, dune and moraine not statistically significant.

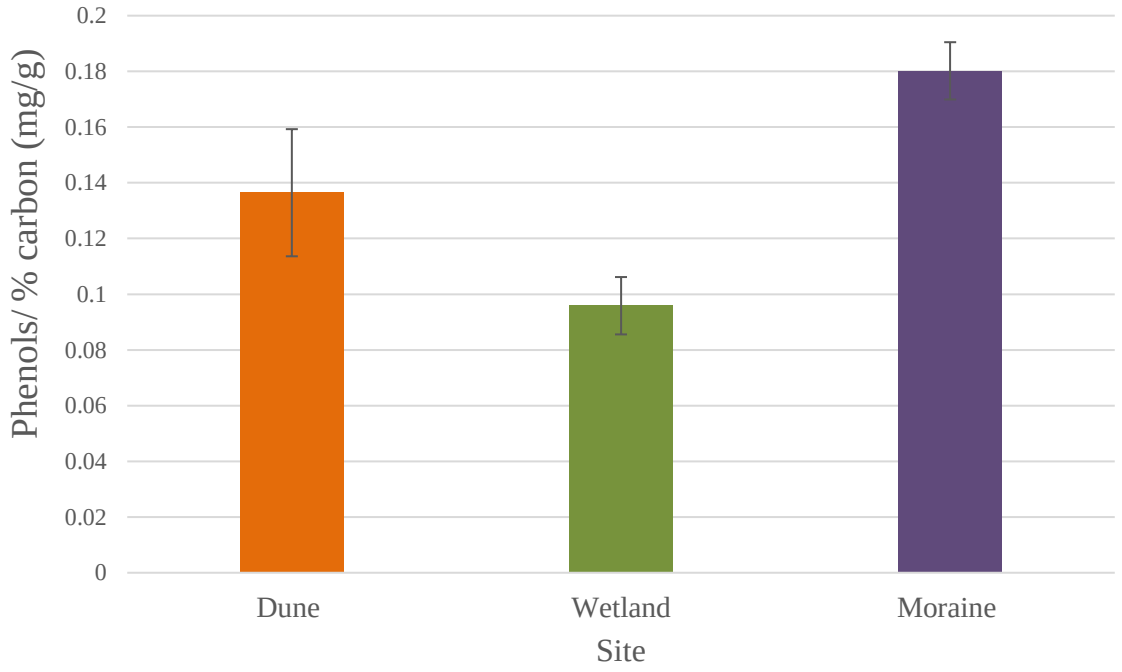


Figure 2. Phenols (mg/g) per percent carbon for Dune, Wetland and Moraine.

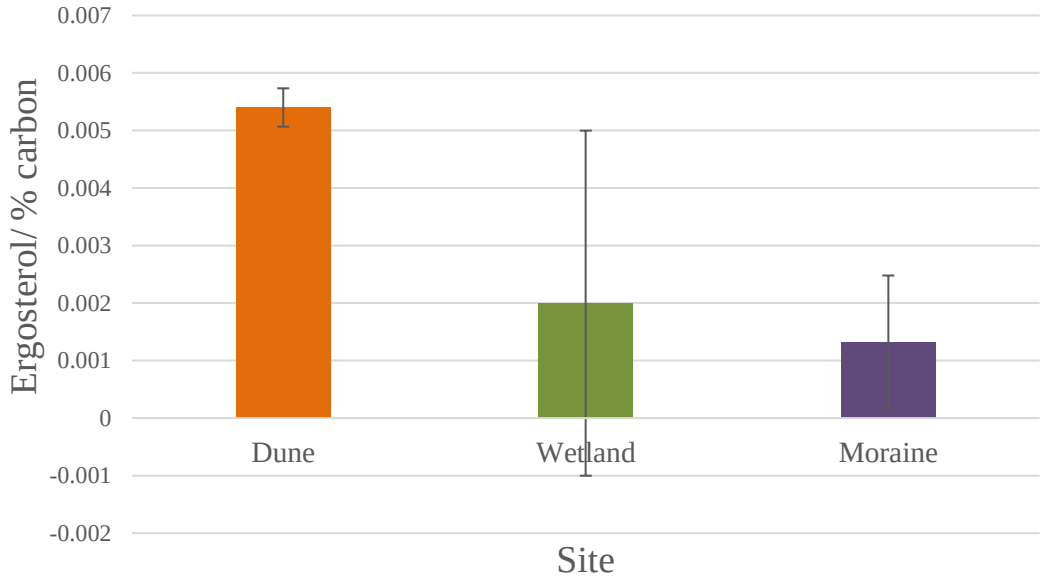


Figure 3. Ergosterol per percent carbon for dune, wetland and moraine.

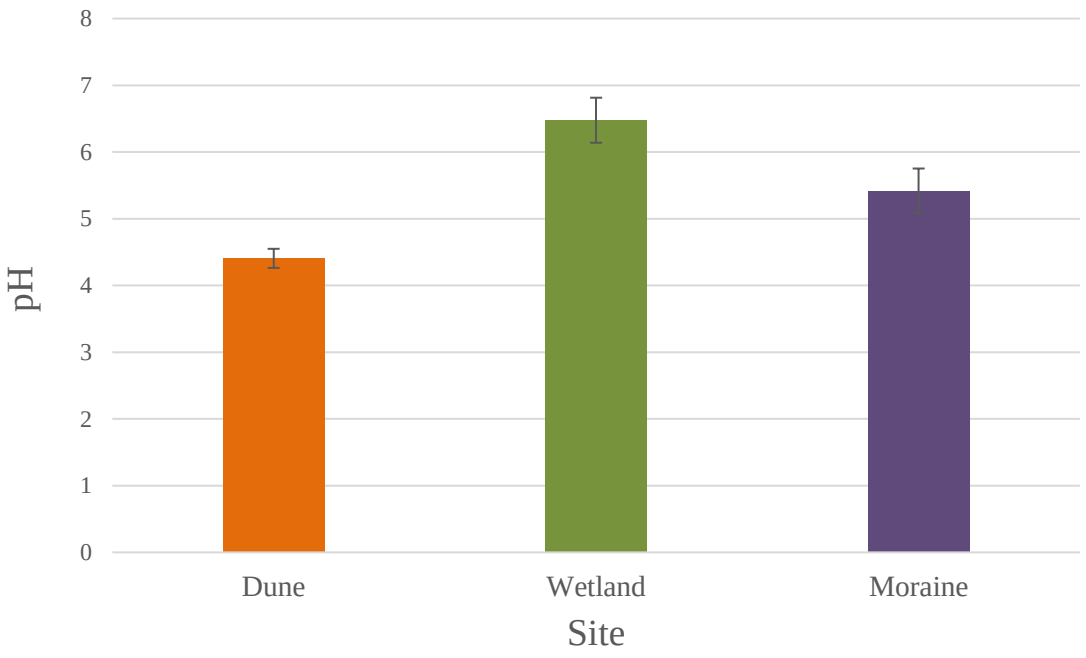


Figure 4. Average pH of dune, wetland and moraine.

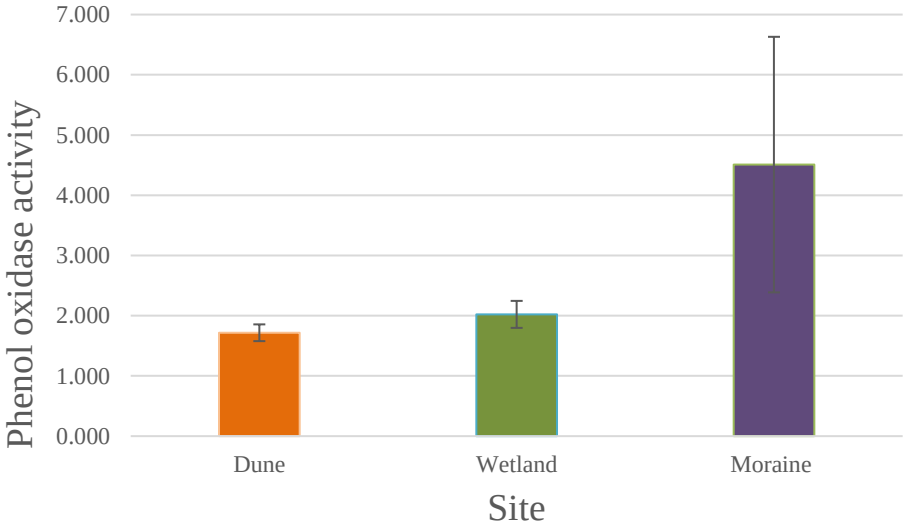
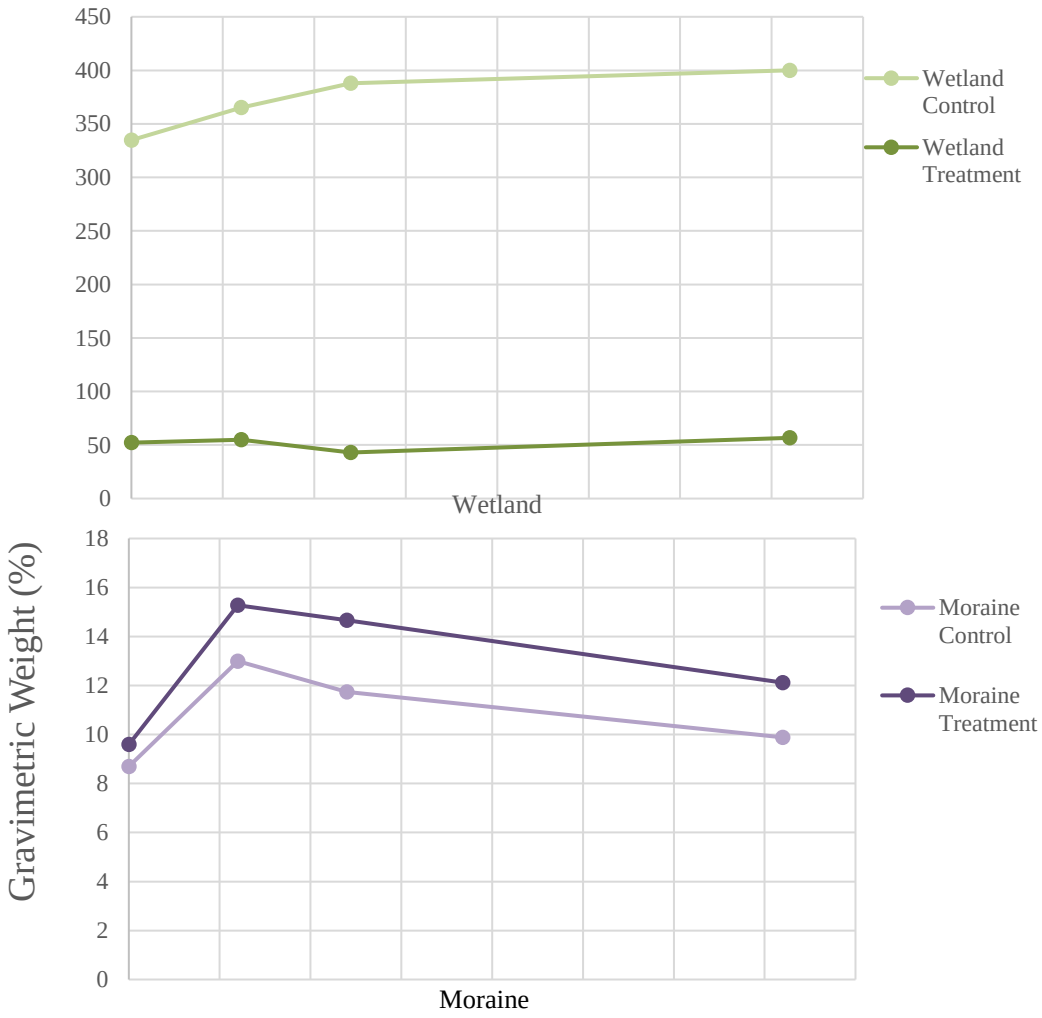
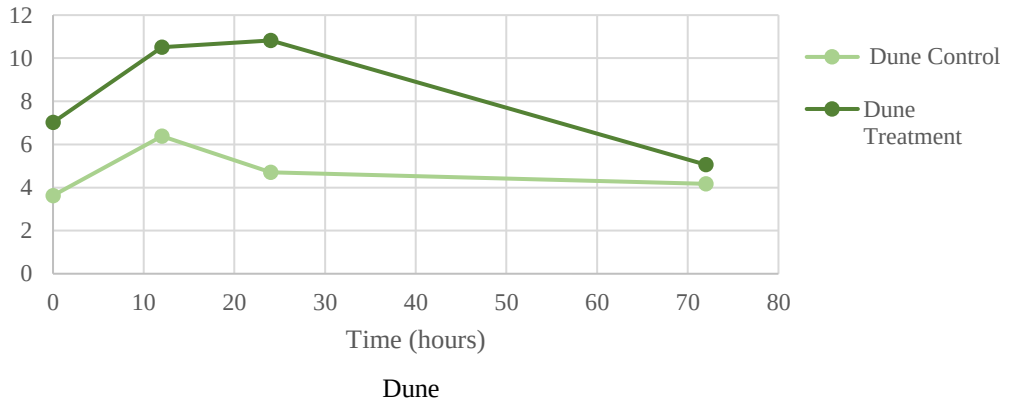


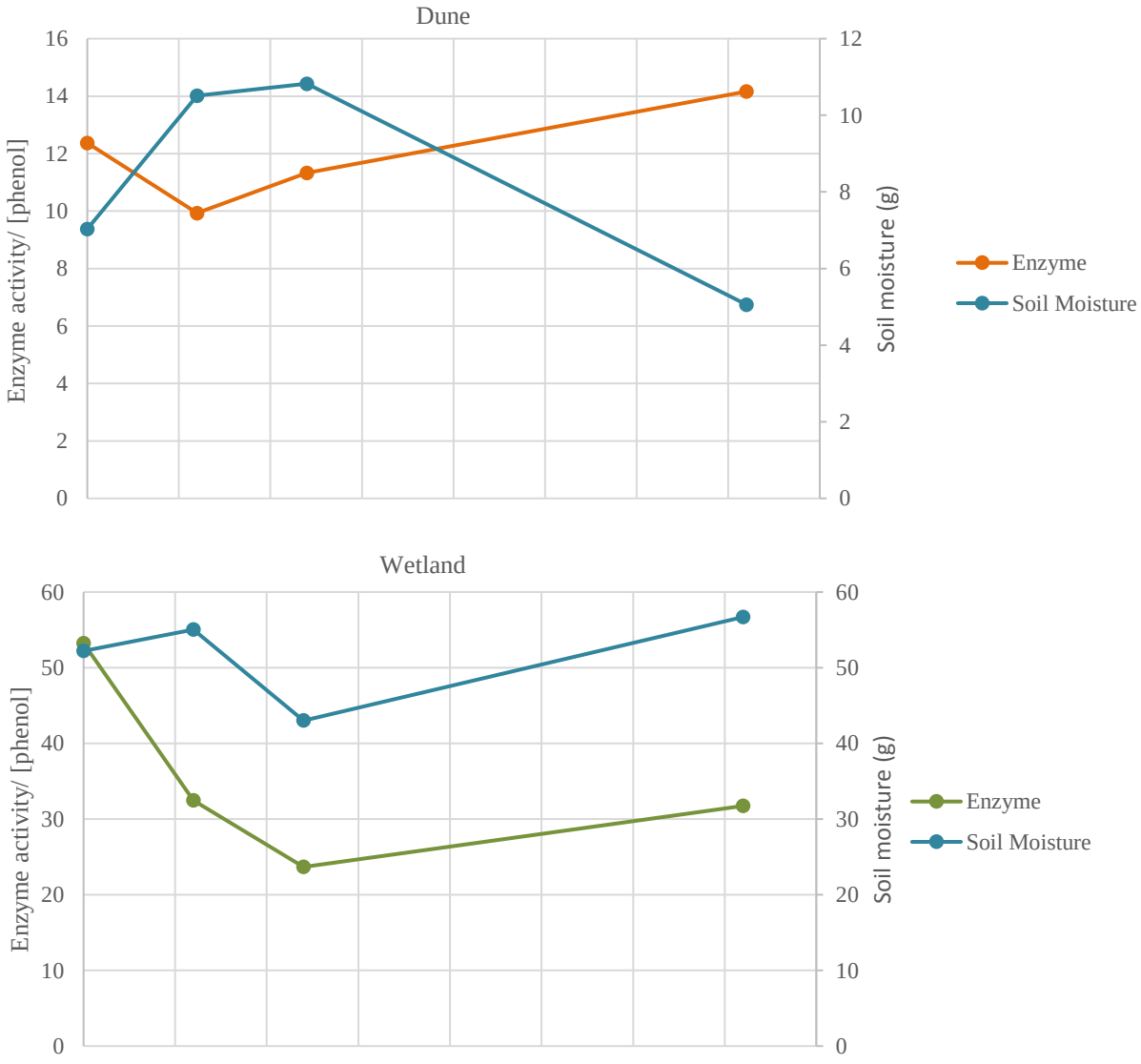
Figure 5. Initial phenol oxidase activity at time 0 for dune, wetland and moraine.


Gravimetric Weight (%)
450
400
350
300
250
200
150
100
50
0
Wetland
Wetland Control
Wetland Treatment
18
16
14
12
10
8
6
4
2
0
Moraine
Moraine Control
Moraine Treatment



Dune

Figure 6. Gravimetric weight of our soil at Time 0, 12, 24 and 72 for the dune, wetland and moraine



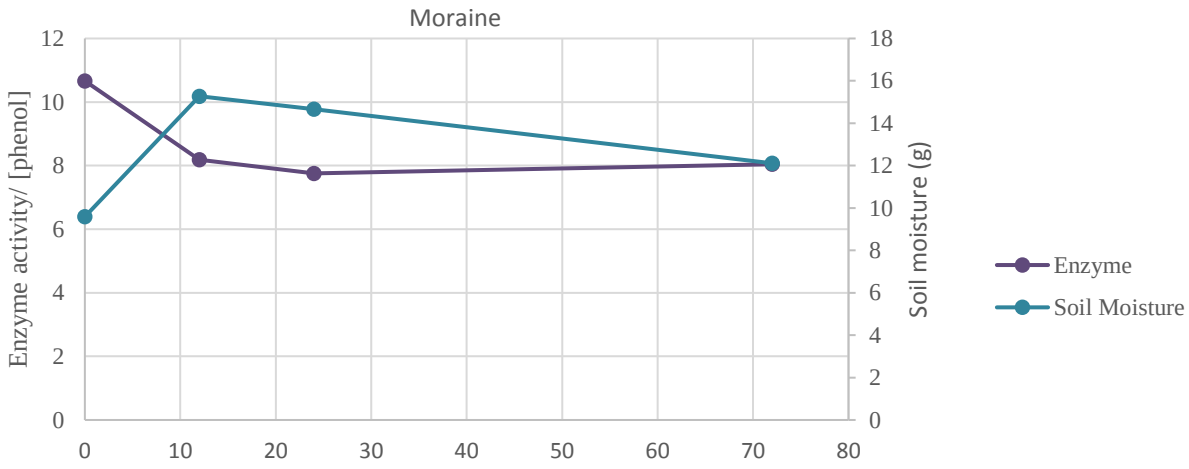


Figure 7. Soil moisture (g) and enxyme activity per phenol concentration for dune, wetland and moraine

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