Investigating the Impacts of Acid Mine Drainage on Ecosystem Functioning Using a Leaf Litter Decomposition Analysis

Undergraduate Thesis

Presented in fulfillment towards the requirements for a Bachelor of Science Degree with Honors Research Distinction from the School of Environment and Natural Resources within the College of Food, Agricultural, and Environmental Sciences at The Ohio State University

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[Abstract]

Acid mine drainage (AMD), a significant source of anthropogenic stress throughout the world, has relevant effects on carbon cycling. There are 1,300 miles of streams affected by AMD in Ohio alone. To investigate the impact of AMD on ecosystem function, multiple streams were surveyed at The Wilds Conservation Center in Cumberland, Ohio. The primary goal of this study is to quantify how AMD affects ecosystem process and function in benthic communities. Leaf litter bags were deployed across three separate stream sections. Two sites were chosen along Watson Road: one lower section with neutral pH (~7) and one upper section with low pH (~4). A third comparison site was selected at Miller Creek. Sixteen leaf litter bags were deployed at all sites in sets of three and subsequently collected once a month from October 2019 – February 2020. Water quality parameters (pH, temperature, dissolved oxygen (DO), conductivity) were recorded at the time of deployment. The leaf litter bags were retrieved and transported back to the lab and the percent of ash-free dry mass remaining after each collection day was determined. Leaf litter decomposition rates were slower in the Watson Upper site, which had more severe AMD pollution as compared to Lower Watson and Miller Creek. Other published studies suggest that marked decreases in decomposition rates are a result of AMD's effect on water chemistry. Subsequently, this has adverse effects on the biodiversity of both macroinvertebrate and microbial communities in those areas. Benthic communities are critical to the processing of organic carbon, an important component of the carbon cycle and global carbon budget. Results from this study will provide knowledge that is to be used in future remediation efforts by The Wilds. Knowing which rates of organic matter decomposition correspond to desired community functioning can help create benchmark levels for the successful management of AMD remediation sites.

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Introduction

Anthropogenic stressors lead to a loss of biodiversity which subsequently affects carbon cycling in aquatic systems (Burrows et al., 2017). Until recently, the critical role that catchment headwaters play in calculations of the global carbon budget has been overlooked. Catchment headwaters, which makeup approximately 65-90% of the global drainage-basin area, in addition to other inland waters, are critical links in the cycling of carbon between atmospheric and terrestrial pools (Looman et al., 2016). The structure and function of many inland waters are being rapidly altered by modern anthropogenic stressors and climate change (Looman et al., 2016). The biological assemblages making up these environments typically face multiple stressors if they have been anthropogenically altered. The specific responses that species have to combinations of these different stressors often differ from what is predicted to result when only one stressor is considered (Tolkkinen et al., 2015). For example, in naturally acidic streams, there can be a high diversity of organisms, and a decrease in consumer diversity does not always imply an impairment in ecosystem function. However, the ability of organisms which live in naturally stringent freshwater environments to cope with novel anthropogenic pressures remains largely unstudied (Tolkkinen et al., 2015).

Global carbon cycling is influenced by organic matter decomposition, a fundamental ecosystem process. Organic matter decomposition is controlled by a collection of hierarchically-organized and networked factors that regulate the flux of local carbon stocks, which in turn influence the concentrations of atmospheric CO_2 and CH_4 (Burdon et al., 2020). The primary source of energy and carbon in small woodland streams is the autumnal input of riparian leaf litter. Shredding invertebrates and microbes are key players in the decomposition of this leaf material (Tolkkinen et al., 2015). The process of organic matter decomposition also affects food web dynamics and other aspects of ecosystem function in headwater streams and a wide range of other aquatic systems. As a consequence, anthropogenic stressors that affect critical food web compartments often influence breakdown rates, which makes decomposition assays a dynamic functional indicator in efforts to quantify changes in ecosystem functioning (Burdon et al., 2020).

Numerous studies have shown that water acidity and heavy-metal content affect on organic matter decomposition rates (Tolkkinen et al., 2015). Two factors, high acidity (a pH of 1.5-4.5) and high mineralization, in addition to complex salt composites, the presence of sulfuric acid, and high conductivity, are all characteristics of acid mine drainage (AMD) (Plaul, 2014). AMD is the most widely documented human-induced consequence of mining worldwide (Hogsden & Harding, 2012). AMD inputs into streams degrade habitats and exert chemical and physical stress on stream biota. Many studies have investigated the effects of AMD on benthic communities; however, few have illustrated how AMD affects ecosystem processes (Hogsden & Harding, 2010), a primary interest of this study. This effect was investigated using a leaf litter decomposition analysis. This type of analysis has been used frequently to study the functional integrity of streams. Understanding the rate at which leaf litter is broken down in an ecosystem can help to elucidate how energy flows through that system (Hauer & Lambert, 2017).

In Ohio alone, 1,300 miles of streams are affected by AMD (ODNR, 2017). The primary goal of this study was to quantify how AMD affects ecosystem functioning across a series of ponds in southeastern Ohio. It was hypothesized that higher AMD inputs will have more significant impairments on ecosystem functioning. More specifically, the objectives of this study were:

1) Quantify the rate of leaf litter decomposition across different AMD loads,
2) Compare decomposition rates resulting from different AMD loads, and

3) Understand how AMD is affecting larger ecosystem processes in AMD affected environments.

Methods

Site description

The study site is located near Cumberland, OH at The Wilds Conservation Center [39.8295° N, 81.7330° W]. Surface mining of coal took place in the Cumberland area for more than 40 years until the Federal Reclamation Act was passed in 1971. This Act required replacement of topsoil and plantings for erosion control erosion control. As a result, the mining operation here was shut down and 40km^2 of land was gifted by the Central Ohio Coal Company to The Wilds. Today, The Wilds is a surface mine reclamation site and a state-of-the-art conservation and research center. A chain of seven ponds located at The Wilds that are with different AMD loads were used in this investigation. Figure 1 provides an aerial view of the positions of the ponds along Watson Rd. An additional site, Miller Creek, which has undergone AMD remediation, serves as a reference site and is also located at The Wilds.

Study design

A leaf litter decomposition study was designed to analyze rates of decomposition of leaf biomass and differences in functional composition of macroinvertebrate communities across three different sections of the chain of ponds. Three sites were compared that differed in the pH of the water. The first site, Watson 1-2 (along Watson Road), had an average pH of 4. The second site, Watson 6-7, had an average pH of 7. The third site, Miller 1, also had an average pH of 7. Sixteen leaf litter bags were deployed at each site in sets of four. An additional six leaf litter bags were used to calculate handling loss; two bags were placed in each site. Each leaf litter bag, including the handling loss bags, was approximately 1.9m^3 (25.4cm x 25.4cm x 3cm) and constructed from green chicken netting and plastic ties. Leave from a cottonwood tree [*Populus sect. Aigeiros*] were used and 3.54g - 4.26 g (mean = 4.04 g) dry mass of leaves were placed in bags. The leaves were collected across the street, directly west of the football stadium on the Ohio State University campus. The leaves had fallen recently and only dry leaves that appeared to not have been colonized by microbes or to have begun to breakdown were chosen. The leaves were then dried in the lab for at least 48 hours at 55°C. The holes in the chicken netting were 3.5cm in diameter, big enough to allow a variety of differently sized macroinvertebrates to enter while not allowing any leaves to fall out.

The leaf litter bags were deployed on 20 October, 2019 and pH, temperature, dissolved oxygen (DO), and conductivity of each collection site were measured and recorded on the first day of exposure (Table 1). The leaf litter bags were positioned in sets of four in the ponds and secured to metal stakes. Handling loss bags were also placed in the ponds with the other bags and secured for a brief time but then subsequently removed on the same day and transported back to the lab for analysis. One set of four bags was collected from each of the locations monthly between October 2019 and February 2020. The exact collection dates were: 30 October, 2019, 20 November, 2019, 2 January, 2020, and 4 February, 2020. Only three of the four leaf litter bags collected from each site the first collection day were used in the decomposition analysis as one bag of these bags been designated for use in another study.

Leaf litter bags secured in Miller 1

The leaf litter bags were transported back to the lab after each collection. The leaves from each pack were gently removed and rinsed to remove any silt or debris. The cleaned leaves were then dried in an oven at 50-60°C for at least 24 hours. After drying, the leaves were weighed and their dry mass (DM) was recorded.

Mineral deposits, which can cause errors in final DM, were expected to be encountered in the study. To overcome this, the DM was converted to ash-free dry mass (AFDM). To determine AFDM, 7.5 cm diameter aluminum weighing pans were used. The aluminum pans were initially heated in a muffle furnace for 30 minutes at 550°C so that an accurate tare weight could be recorded. The dried leaves were then milled and 250mg of the milled leaves from each sample were placed on a tared pan. Each pan was then placed back in the muffle furnace at 550°C for another forty minutes and stirred briefly with a dissecting needle after twenty minutes.

When removed from the oven and cooled, the remaining weight of the milled leaves was recorded. The percent of organic matter was calculated as follows:

% organic matter = [(sample dry mass – sample ash mass)/(sample dry mass)] x 100

Leaf pack dry mass (LPDM) was then converted to AFDM:

AFDM = (LPDM) x (% organic matter)

Finally, AFDM for each sample was converted to %AFDM remaining:

%AFDM remaining = (Final AFDM/ Initial AFDM) x 100

This process was repeated twice for each leaf packet and each handling loss pack. The natural log (ln) of the mean %AFDM remaining for each collection time was plotted on the y-axis against days of exposure on the x-axis. The AFDM of the handling loss leaf packs was assumed to be equivalent to 100% remaining on Day 0. The processing coefficient (-k) is the slope of the regression line that results from the plotting of these points for each site. A 2-way ANOVA and post-hoc Tukey’s HSD statistical analysis was performed on the resulting data using RStudio to determine whether or not any significant differences in leaf-litter processing were present.

Results

Water temperature and atmospheric pressure were consistent across all sites at approximately 12°C and 740 mmHg respectively (Table 1). The conductivity of each site was above 2000 specific conductivity (SPC) µS/cm which is unusually high (healthy freshwater conductivity ranges from 0-1,500 SPC-µS/cm). The pH for Watson 1-2 was approximately 4.8, which indicates a much higher concentration of AMD present. This site also exhibited visibly higher levels of environmental degradation. There was little to no water flow, very little vegetation, there appeared to be a biofilm on top of the water, and there was an orange substance coating the leaves retrieved from this site. I speculate the orange substance was iron deposits, but it could have also been produced by a particular microbial species, however, it was not tested to be determined for sure.

The pH for both Watson 6-7 and Miller 1 were more circumneutral at approximately 7 and both of these sites, especially Miller 1, visibly appeared to be healthier. The water was more clear and there was more flow at these two sites. There also appeared to be significantly more vegetation and there was no orange substance coating the leaves retrieved from these sites.

The leaf litter decomposition rates for each site are illustrated in Figures 2 - 4. The processing coefficient (-k), which is the negative slope of the line of the days of exposure vs. %AFDM remaining, was 0.0016 for Watson 1-2, and 0.0047 for Watson 6-7, and 0.0053 for Miller 1. The mean %AFDM remaining for each site were compared with each other in Figure 5, and the processing coefficient slopes were compared against each other in Figure 6.

A 2-way ANOVA test indicated that there is a significant difference between the % AFDM remaining in each of the leaf litter bags and the days of exposure (p-value = 0.025) and there was a significant difference between sites and their corresponding %AFDM remaining over time (p-value < 0.004) [Table]). A subsequent Tukey's HSD test indicated there was only a significant difference between Miller 1 and Watson 1-2 (p-value < 0.00061), but there were no significant differences (p-value > 0.099) between Miller 1 and Watson 6-7 or between Watson 1-2 and Watson 6-7 (Table 3).

Discussion

The observed data supported the general hypothesis that AMD affects ecosystem function. The lower pH environments found in waters polluted by high levels of AMD appear to impede decomposition rates (Kittle et al., 1995). The processing coefficient (-k) was found to increase gradually downstream as the pH increased and the AMD load decreased.

There was a negative relationship between rates of leaf litter decomposition and AMD pollution loads. The results demonstrate a significant difference in the leaf litter decomposition rates between Watson 1-2 and Miller 1. While the difference between Watson 1-2 and Watson 6-7, and between Watson 6-7 and Miller 1, is not statistically significant, it is clear from the visual comparison of data between sites (Figures 5 & 6) that there appears to be a difference in the interactions taking place in the respective sites.

Results from other experimental field studies suggest that the strongest predictor of stream biota and subsequent ecosystem processing is the chemistry of the water (Hogsden & Harding, 2012). This finding could explain why the leaf litter break down rate at Watson 6-7 did not differ significantly from the other two sites but is more similar to the rate of Miller 1. While conductivity was high at all sites, pH was circumneutral around 7 at Watson 6-7 and Miller 1. It is certainly possible that the difference in the decomposition rates of the two streams could be attributed to the remediation of Miller 1. Remediation of AMD-affected waters has been shown to result in increased pH levels and significantly higher levels of local macroinvertebrate taxa richness, biomass, diversity, and density, all of which would contribute to higher rates of processing (Williams & Turner, 2015). Watson 6-7 has not undergone remediation. However, the pH of this site is closer to neutral and approximately the same as that of Miller 1, which appears to have positively affected the macroinvertebrate community and thus the rate of decomposition to some degree. While the general agreement is that water chemistry tends to be the primary determinant of biological health in AMD-affected sites, biological health can also be highly variable and contingent on a variety of other factors that have synergistic effects (Williams & Turner, 2015).

Additionally, it is important to note that the water quality of sites that are remediated typically improves more quickly than the structure and function of the biological community at unremediated sites (Williams & Turner, 2015). This is because macroinvertebrates only reproduce and travel upstream to different locations at particular times of the year. Therefore, if the water chemistry improves at a different time from when macroinvertebrates would colonize an area, one would not see greater diversity and richness of macroinvertebrate species for some time (Williams & Turner, 2015). It is unknown if Watson 6-7 previously had a lower pH or how long its pH has been at the same level as Miller 1. However, it is probable that, without other factors in play, the water chemistry has not been this way for a sufficient time to have been colonized by macroinvertebrates, critical to leaf litter decomposition, but intolerant of the water quality previously characteristic of the site.

Furthermore, decomposition of leaf litter is a process that is affected by several different variables. These variables include different elements that make up the stream ecosystem, such as leaf species, microbial activity, invertebrates, and the physical and chemical features of the stream (Hauer & Lamberti, 2017). Many studies investigating AMD have examined effects on benthic invertebrate communities. Macroinvertebrate and microbial species richness, abundance, and diversity are frequently reduced compared to an unaffected stream in streams affected by AMD. Sensitive invertebrate species, such as mayflies [*Ephemeroptera*], caddisflies [*Trichopter*a], and mollusks [*Mollusca*] , are typically discounted from AMD environments, while more tolerant species, such as chironomids [*Chironomidae*], beetles [*Coleoptera*], and true bugs [*Hemiptera*], which are not directly involved in organic matter decomposition, are capable of enduring the harsh conditions (Hogsden & Harding, 2012). Data on the composition of microbial communities in AMD-affected waters are less readily available and there is non-consensus within the scientific community as to the typical composition of these communities in these environments, other than that biodiversity levels are lower in AMD-affected systems (Hogsden & Harding, 2012). The predominant conclusion from previous studies is that sites affected by AMD have lower decomposition rats due to lower macroinvertebrate and microbial diversity and richness and significantly fewer numbers of those species that are less tolerant of AMD environments (Hogsden & Harding, 2012). One small sample of macroinvertebrates was taken from each environment (Table 4). There were much fewer different taxa and a much lower number of total macroinvertebrates identified at the Watson 1-2 site. Interestingly, there were a higher number of different taxa identified at Watson 6-7 but a lower number of total macroinvertebrates compared to Miller 1, from the one sample. This sample is not large enough for any conclusions to be drawn, however, it gives an idea of what could have been seen at these sites if more sampling had been done.

Moreover, macroinvertebrate communities, through their foraging activities on the nutrient supplies in the streams, such as leaf litter, are essential mediators of particulate organic carbon processing (Burrows et al., 2017). The processing of carbon is an essential ecosystem function as it supports the transfer of energy throughout the entire biosphere. Inland waters, which include the ponds in this study, are of particular importance to this process as they fix both inorganic and organic carbon through photosynthesis and mineralization (Hanson et al., 2015). While inland waters only cover less than 1% of the Earth's surface, they transport minerals and bury similar amounts of carbon as that of the entire terrestrial sink for anthropogenic emissions (Burrows et al., 2017). Since inland waters process, store, and export a disproportionately high rate of carbon compared to other mediating factors, they have significant influence on the carbon balance of the watersheds they drain. Therefore, inland waters and the macroinvertebrate and microbial communities that inhabit them and facilitate organic matter processing are dynamic components of the Earth's carbon cycle (Burrows et al., 2017). Carbon is cycled through these affected sites at a slower rate than usual when there is a decrease in the rate of organic matter decomposition as a result of AMD. A change in the rate of carbon cycling disturbs the natural balance of that cycle which needs to be maintained in order for the ecosystem to be able to continue to support the same levels of biodiversity.

As shown in Figure 6, it is clear that the processing rate of Watson 1-2, the site with the highest AMD load, is significantly slower than that of Miller 1, and, though non-significant, also differs from that of Watson 6-7. The Watson 1-2 site, as a result of its impeded leaf litter breakdown, is cycling carbon at a significantly slower rate than the other two sites in the area. Inhibition of leaf decomposition is most likely a result of the water chemistry's negative effect on the biodiversity of the macroinvertebrate community, which plays an essential role in this process. This finding is also concerning, given that the carbon cycling of inland waters influences the surrounding landscape. However, knowledge of these interactions is still largely incomplete (Hanson et al., 2015). There is also concern with data from recent studies which suggest that changes in rates of carbon cycling coupled with the climate will have a net positive effect on global climate warming in the twenty-first century (Dorrepaal, 2007 & Burrows et al., 2017). This is thought to be the case because, while local factors, such as taxonomic composition of the benthic community, typically control leaf litter decomposition on a smaller scale (e.g. a single pond), the contemporary climate is considered to be the controlling factor affecting leaf litter decomposition over larger areas (Strickland et al., 2015).

Thus, the interaction of global climate warming and other anthropogenic stressors, and more specifically AMD, could prove to have detrimental effects on ecosystem biodiversity and critical ecosystem processes.

While further testing is needed, it is likely that the landscape surrounding the Watson 1-2 site is also being affected by AMD and that Watson 1-2 itself is being influenced by regional climate changes. An investigation that involves a more extended period of exposure and a larger number of leaf litter bag replicates, as well as further documentation of site-specific statistics and species composition of the benthic communities of the respective sites, would be illuminating. These additional measures may show a significant difference between all three of the sites and better illustrate the specific interactions taking place and the structure of the ecosystems being affected by varying loads of AMD.

Conclusion

The inland waters affected by AMD are more likely to exhibit decreased rates of leaf litter decomposition. This slowing is due to the negative effects of AMD on site-specific water chemistry which in turn affects macroinvertebrate and microbial communities that are critical to the process of organic carbon decomposition. There are thousands of miles of streams across Ohio and North America that are affected by AMD. Therefore, this has implications on the global carbon budget due to the disproportionate amount of carbon that inland waters store and export and the importance of organic carbon decomposition in the process of carbon cycling.

Acknowledgments

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Literature Cited

Armstrong, Alona., Waldron, Susan., Ostle, Nicholas J., Richardson, Harriett., Whitaker, Jeanette. (2015). Biotic and Abiotic Factors Interact to Regulate Northern Peatland Carbon Cycling. *Ecosystems*, 18, 1395-1409.

Burdon, F.J., et al. (2020). Stream microbial communities and ecosystem functioning show complex responses to multiple stressors in wastewater. *Global Change Biology*, 26, 6363-6382.

Burrows, R.M., et al. (2017). High rates of organic carbon processing in the hyporheic zone of intermittent streams. *Scientific Reports*, 7, 13198.

Dorrepaal, E. (2007). Are plant growth-form-based classifications useful in predicting northern ecosystem carbon cycling feedbacks to climate change? *Journal of Ecology*, 95, 1167-1180.

Edwards, K. J., Gihring, T. M., and Banfield, J. F. (1999). Seasonal Variation in Microbial Populations and Environmental Conditions in an Extreme Acid Mine Drainage Environment. *Applied and Environmental Microbiology*, 65, 3627-32.

Frank, Paul. (1983). Acid Mine Drainage. *Environment*, 25, 40-42.

Hanson, P.C., et al. (2015). Integrating Landscape Carbon Cycling: Research Needs for Resolving Organic Carbon Budgets of Lakes. *Ecosystems*, 18, 363-375.

Hauer, F.R. & Lamberti, G.A. (2017). Methods in Stream Ecology. Volume 2*: Ecosystem Function, Third Edition*. Academic Press.

Hogsden, K.L., & Harding, J.S. (2012). Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. *Freshwater Science*, 31(1), 108-120.

Kittle, D.L., McGraw, J.B., & Garbutt, K. (1995). Plant Litter Decomposition in Wetlands Receiving Acid Mine Drainage. *Journal of Environmental Quality*, 24(2), 301-306.

Looman, A., et al. (2016). Carbon cycling and exports over diel and flood-recovery timescales in a subtropical rainforest headwater stream. *Science of the Total Environment,* 550, 645-657.

Ohio Division of Natural Resources. (2017). Acid Mine Drainage Abatement Program.

Plaul, P., Shulenina, Z. M. (2014) Acid Mine Drainage. *Value Inquiry Book Series*, 276, 3-5.

Simate, Geoffrey S., Ddlovu, Sehliselo. (2014). Acid mine drainage: Challenges and opportunities. Journal of Environmental Chemical Engineering, 2, 1785-1803.

Strickland, M.S., et al. (2015). Climate history shapes contemporary leaf litter decomposition. *Biogeochemistry*, 122, 165-174.

Ramanathan, B., Boddicker, A. M., Roane, T. M., Mosier, A. C. (2017). Nitrifer Gene Abundance and Diversity in Sediments Impacted by Acid Mine Drainage. *Frontiers in Microbiology,* 8, 2136.

Talukdar, B.K., et al. (2016). Evaluation of genetic toxicity caused by acid mine drainage of coal mines on fish fauna of Simsang River, Garohills, Meghalaya, India. *Ecotoxicology and Environmental Safety*, 131, 65-71.

Tolkkinen, M., et al. (2015). Multi-Stressor impacts on fungal diversity and ecosystem functions in streams: natural vs. anthropogenic stress. *Ecology*, 96(3), 672-683.

Wei, X., Zhang, S., Han, Y., Wolfe, A. (2017). Mine Drainage: Research and Development. *Water Environment Research*, 89, 1384-1402.

Weiping, Hu., Jorgensen, Sven Erik., Fabing, Zhang., Yonggen, Chen., Longyan, Yang. (2011). A model on the carbon cycling in Lake Taihu, China. *Ecological Modeling,* 222, 2973-2991.

Wildman, Alissa. (2017). Native grasses look positive on strip-mine land. The Columbus Dispatch, https://www.dispatch.com/news/20170923/native-grasses-look-promising-on-strip-mine-land

Williams, K.M., & Turner, A.M. (2015). Acid mine drainage and stream recovery: Effect of restoration on water quality, macroinvertebrates, and fish. *Knowledge & Management of Aquatic Ecosystems*, 416, https://doi.org/10.1051/kmae/2015014

Figures & Tables

Figure 1.
Aerial image of Watson Road at The Wilds Conservation Center in Cumberland, OH [39.8295° N, 81.7330° W]. The seven ponds located along this road are polluted by varying loads of AMD. Two sites were chosen along this road to take place in a leaf litter decomposition analysis. An upper site (most northern ponds in outlined in yellow and red) was named Watson 1-2 and had an average pH of 4. A lower site (most southern ponds outlined in white and orange) was named Watson 6-7 and had an average pH of 7. A third comparison site, Miller Creek, was chosen. It is also located in The Wilds, it had an average pH of 7, and it has undergone remediation treatment for acid mine drainage (AMD).

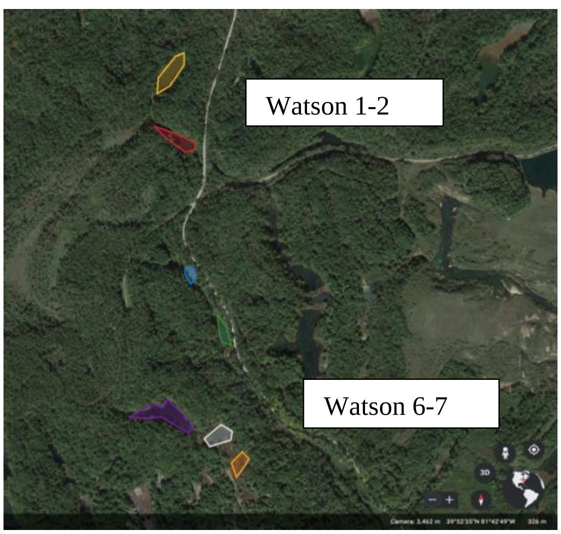

Figure 2: Leaf litter decomposition between 20 October, 2019, and 4 February, 2020 at Watson 1-2, one of the sites chosen to take place in a leaf litter decomposition analysis. The pH of the site when the leaf litter bags were deployed was 4.84. One set of 4 leaf litter bags was collected once a month between October 2019 and February 2020 and the remaining percentage of ash-free dry mass (AFDM) was determined. AFDM was plotted on the y-axis against days of exposure on the x-axis. The slope of the regression line was equal to the processing coefficient (-k) of the site. The processing coefficient for Watson 1-2 was 0.0016.

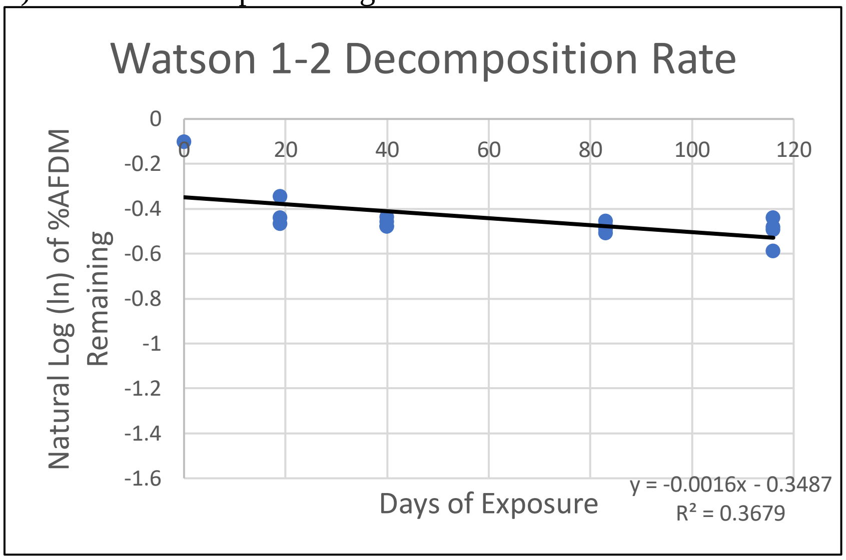

Figure 3: Leaf litter decomposition between 20 October, 2019, and 4 February, 2020 at Watson 6-7, one of the sites chosen to take place in a leaf litter decomposition analysis at The Wilds in Cumberland, OH [.39.8295° N, 81.7330° W]. The pH of the site when the leaf litter bags were deployed was 7.88. One set of 4 leaf litter bags was collected once a month between October 2019 and February 2020 and the remaining percentage of ash-free dry mass (AFDM) was determined. AFDM was plotted on the y-axis against days of exposure on the x-axis. The slope of the regression line was equal to the processing coefficient (-k) of the site. The processing coefficient for Watson 6-7 was 0.0047.

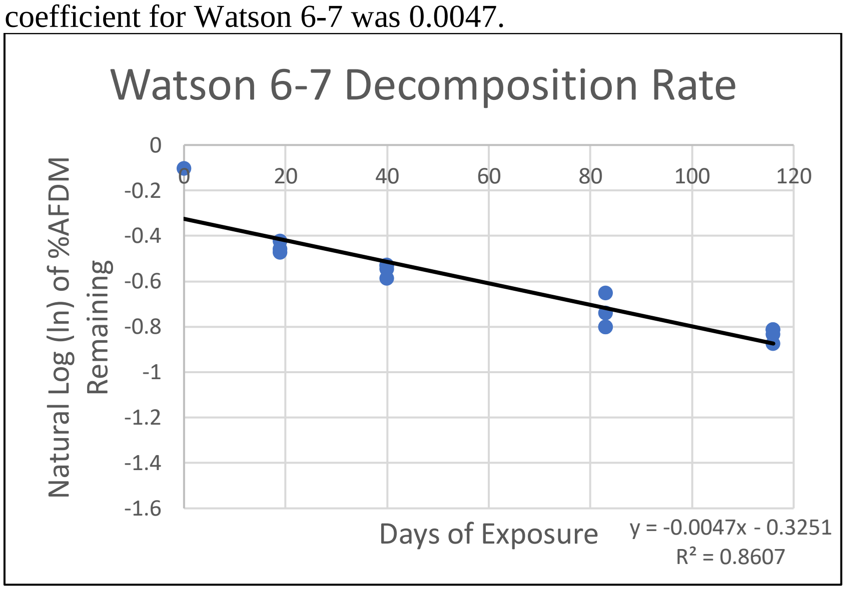

Figure 4: Leaf litter decomposition between 20 October, 2019, and 4 February, 2020 at Miller 1, one of the sites chosen to take place in a leaf litter decomposition analysis at The Wilds in Cumberland, OH [.39.8295° N, 81.7330° W]. The pH of the site when the leaf litter bags were deployed was 7.84 and this site has undergone remediation treatment for acid mine drainage (AMD). One set of 4 leaf litter bags was collected once a month between October 2019 and February 2020 and the remaining percentage of ash-free dry mass (AFDM) was determined. AFDM was plotted on the y-axis against days of exposure on the x-axis. The slope of the regression line was equal to the processing coefficient (-k) of the site. The processing coefficient for Miller 1 was 0.0053.

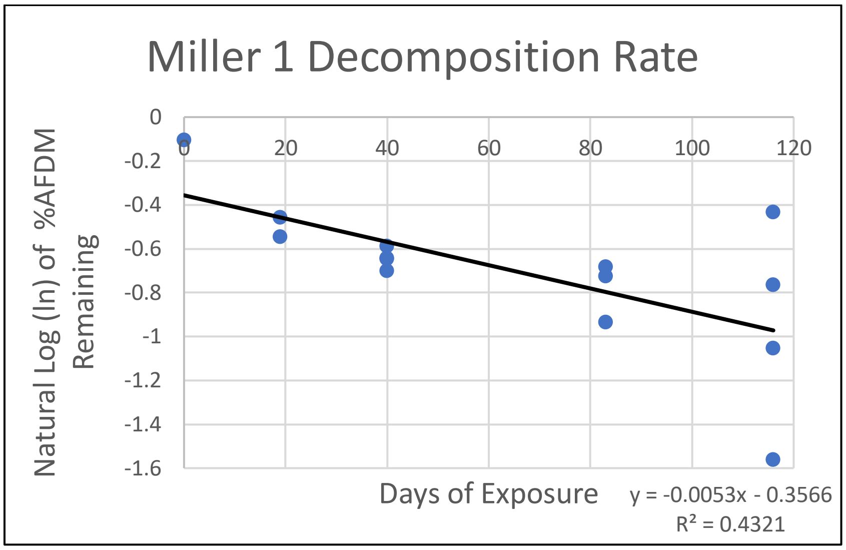

Figure 5: Leaf litter decomposition between 20 October, 2019 and 4 February, 2020 in three locations at The Wilds in Cumberland, OH [39.8295° N, 81.7330° W]. The three locations used in the study were Watson 1-2, Watson 6-7, and Miller 1, and they had a pH of 4.84, 7.88, and 7.84 respectively on the day the leaf litter bags were deployed. Watson 1-2 and Watson 6-7 are affected by acid mine drainage (AMD) and Miller 1 has undergone remediation treatment for AMD. One set of 4 leaf litter bags was collected from each site once a month between October 2019 and February 2020 and the remaining percentage of ash-free dry mass (AFDM) was determined The mean percent of ash-free dry mass remaining for each site was calculated and plotted against days of exposure on the x-axis.

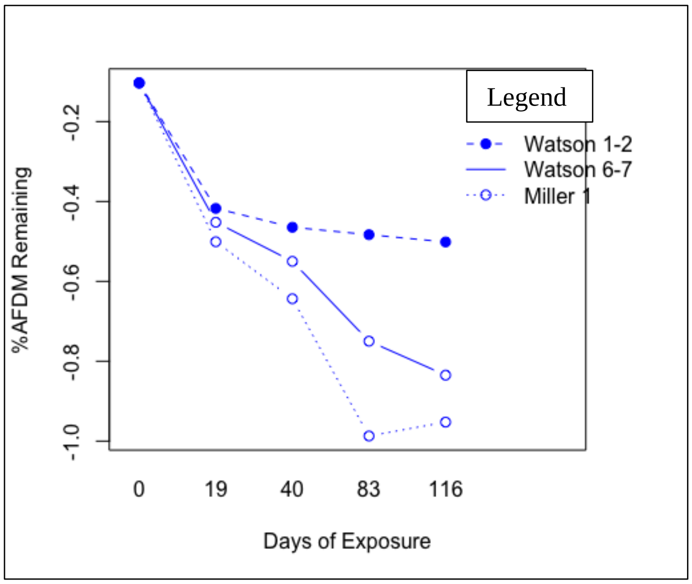

Figure 6: Leaf litter decomposition processing coefficients between 20 October, 2019 and 4 February, 2020 from three locations at The Wilds in Cumberland, OH [39.8295° N, 81.7330° W]. The three locations used in the study were Watson 1-2, Watson 6-7, and Miller 1, and they had a pH of 4.84, 7.88, and 7.84 respectively on the day the leaf litter bags were deployed. Watson 1-2 and Watson 6-7 are affected by acid mine drainage (AMD) and Miller 1 has undergone remediation treatment for AMD. One set of 4 leaf litter bags was collected from each site once a month between October 2019 and February 2020 and the remaining percentage of ash-free dry mass (AFDM) was determined The mean percent of ash-free dry mass remaining for each site was calculated and plotted against days of exposure on the x-axis and the processing coefficient (-k) was equal to the slope of the regression line.

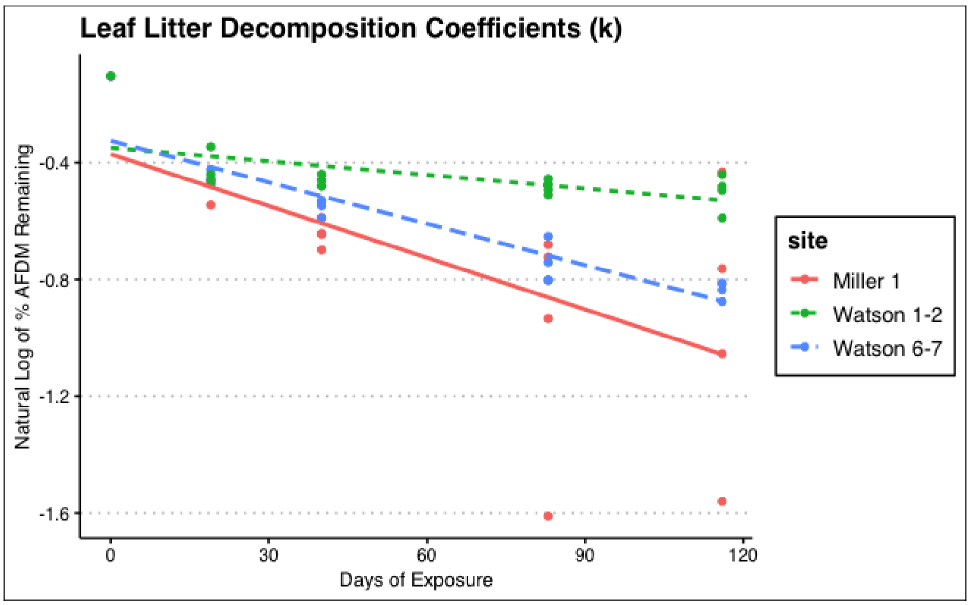

Table 1: Measurements of the conditions of the three sites being used in a leaf litter decomposition analysis at The Wilds in Cumberland, OH [39.8295° N, 81.7330° W] The measurements were taken on deployment day, 20 October, 2019, using a Yellow Springs Instrument (YSI). The measurements were only taken on the same day, but not at the same time of day. Watson 1-2 and Watson 6-7 are affected by acid mine drainage (AMD) and Miller 1 has undergone remediation treatment for AMD.

Site	Temperature ºC	mmHg	DO%	DO mg/L	SPC-µS/cm	pH
Watson 1-2	12.8	739.3	76.8	8.08	2458	4.84
Watson 6-7	11.6	741.7	93.8	10.13	2364	7.88
Miller 1	11.7	743.8	84.1	9.06	2035	7.84

Table 2: A 2-way ANOVA test of the data collected from a leaf litter decomposition analysis at The Wilds in Cumberland, OH [39.8295° N, 81.7330° W]. 16 leaf litter bags were deployed across three different sites. The three locations used in the study were Watson 1-2, Watson 6-7, and Miller 1, and they had a pH of 4.84, 7.88, and 7.84 respectively. Watson 1-2 and Watson 6-7 are affected by acid mine drainage (AMD) and Miller 1 has undergone remediation treatment for AMD. There was a significant difference (alpha = 0.05) in leaf litter breakdown found between at least two of the collection days and at least between two of the sites.

	Degrees of freedom	Sum Squares	Mean Square	F value	P (>F)
days of exposure	1	0.8377	0.8377	12.471	0.001019**
site	2	1.1061	0.5531	0.5531	0.000962***
days of exposure: site	2	0.1322	0.0661	0.0661	
residuals	42	2.8211	0.0672	0.0672	

Table 3: Post hoc Tukey's honestly significantly different (HSD) test for a 2 way ANOVA to find which sites from a leaf litter decomposition analysis at The Wilds in Cumberland, OH [39.8295° N, 81.7330° W] presented significant differences from one another. The three locations used in the study were Watson 1-2, Watson 6-7, and Miller 1, and they had a pH of 4.84, 7.88, and 7.84 respectively. Watson 1-2 and Watson 6-7 are affected by acid mine drainage (AMD) and Miller 1 has undergone remediation treatment for AMD. Diff = the mean difference between the two groups. Lwr = lower end point of the interval. Upr = upper endpoint of the interval. P adj = gives p-value after adjustments for multiple comparisons. There was only a significant difference found between Watson 1-2 and Miller 1.

	diff	lwr	upr	p adj
Watson 1-2-Miller 1	0.3717252	0.14910953	0.59434097	0.00006082**
Watson 6-7-Miller 1	0.193886	-0.02873974	0.4165017	0.09873771
Watson 6-7-Watson 1-2	-0.1778393	-0.40045498	0.04477645	0.1399178

Table 4: This table shows preliminary data from one sample taken at three different sites at The Wilds in Cumberland, OH [39.8295° N, 81.7330° W]. The three locations used in the study were Watson 1-2, Watson 6-7, and Miller 1, and they had a pH of 4.84, 7.88, and 7.84 respectively. Watson 1-2 and Watson 6-7 are affected by acid mine drainage (AMD) and Miller 1 has undergone remediation treatment for AMD. Macroinvertebrates were identified down to the family they belonged and the total number of invertebrates found in each sample were also counted.

Site	**Total number of Different Taxa**	**Total Number of Macroinvertebrates**
Watson1-2	2	4
Watson6-7	7	13
Miller1	3	41