

Effects of Leaf Litter and Soil Moisture and pH on Macroinvertebrate Abundance

Alana Brauns

Introduction

Despite the importance of old-growth forests environment for invertebrates, only around 100,00 hectares of old-growth remains in Appalachia (White et al. 2018). Macroinvertebrates are a vast group of organisms that can take on numerous roles within an ecosystem, and can act as predators, prey, or as pollinators (Greenberg and Forrest 2003). Many macroinvertebrates are classified as detritivores, animals that feed on decaying plant matter on the forest floor. Forests, then, are often considered an ideal habitat for a host of macroinvertebrate populations (Bogyó et al. 2015). Their populations contribute to soil biodiversity, which has been shown to have a large impact on maintaining ecosystem productivity and multifunctionality (Delgado-Baquerizo et al. 2020). Macroinvertebrates are often important to forest ecosystems due to their role in decomposition of organic and plant materials and upkeep of soils (Kalisz and Powell 2000). Since there is a close connection between macroinvertebrates and organic materials on forest floors, old-growth forests prove to be a suitable habitat to proliferate due to their stable environmental conditions (Schowalter 2017). While macroinvertebrates are known to be incredibly diverse in old-growth forests, it is also important to note the micro-scale factors at play that may affect their abundance in these habitats.

Soil environment, specifically pH and soil moisture, plays an important role in interaction with soil invertebrates in forest habitats. Soil is the building block for forest ecosystems, and acts as the main habitat for many macroinvertebrates (Kula and Lazorík 2016). A study from 2024 found that, under certain conditions, shifts in soil pH could contribute to a change in community composition (Hu et al. 2024). Likewise, soil moisture often has a large impact on invertebrate composition within a given habitat (Wenk et al. 2016). Soil remains an important part of the environment for detritivores, but leaf litter also has an important and crucial role in how detritivores and other invertebrates interact with their environment (Suriel et al. 2025). Detritivores and macroinvertebrates benefit from not only a higher percent of leaf litter cover in habitats, but a thicker cover of leaf litter, as well (Bogyó et al. 2015). Despite the prevalence of leaf litter and its importance to these populations, more articles seemed to focus on soil properties while the effect of leaf litter on populations was more of a sidenote.

The main goal of this study was to investigate the importance of soil pH and moisture versus leaf litter pH and moisture for shaping macroinvertebrate communities living in leaf litter within an old-growth forest habitat. Leaf litter and soil samples were taken from Stadium Woods, an old-growth forest, over the course of multiple days and were measured for both pH and soil moisture. Invertebrates were collected and counted at each site before being released. In this study, I hypothesized that leaf litter pH and moisture will be a more important driver of macroinvertebrate abundance than those same soil properties due to the invertebrates' reliance on leaf litter as a food source and as a habitat.

Methods

2.1 Study Area

Stadium Woods is an old-growth forest surrounded by the Virginia Tech campus. The forest covers about 11.5 acres of campus, and is on the east side of Lane Stadium. White oak trees that can be found in the forest have been estimated to be over 300 years old. Research done of the woods has “shown the old-growth urban forest to have a balanced, uneven-aged structure.” (“Old Growth Forest” n.d.)

2.2 Sampling Design

Sites were chosen in Stadium Woods, an old growth forest on the Virginia Tech campus (37.222456, -80.416532). Three transects were sampled using a 50 meter measuring tape and sampling took place approximately every 10 meters (Bogyó et al. 2015, Suriel et al. 2025). If debris or other objects were in the way of a sampling plot, the next nearest meter was chosen. A one meter squared sampling area was arranged using meter sticks to create a square in which all sampling of invertebrates, leaf litter, and soil would take place. A total of 13 plots were sampled over three transects.

2.3 Soil and Leaf Litter Sampling

Both the tubes and the plastic bags used to collect leaf litter and soil were weighed before being taken out to the field. Soil samples were taken using a soil sample probe. Soil was taken from each of the four corners of the sampling square and mixed together in a 50 ml tube. Leaf litter was also collected at each of the four corners of the sampling square. Leaf litter was put into a quart sized plastic bag and mixed together for uniform sampling of the site. Leaf litter and soil samples containers were labelled with the date and the plot ID. Depth of leaf litter was taken using a ruler and measured in mm. Recent weather, anything unique about the sampling site, date, and coordinates were also noted. Upon leaving the study area, soil samples were sifted through a 3mm sieve to rid samples of larger organic matter.

Soil and leaf litter samples were brought into the lab for analysis. To determine soil and leaf litter moisture, samples were weighed fresh, freeze-dried for at least one day, then reweighed to determine the percentage of water in the sample. The samples were first frozen overnight before being dried in a freeze drier for at least a day. The gravimetric water content equation was used to determine the percentage of water in the soil and leaf litter. To test for pH, leaf litter samples were ground with an electric processor to create uniformity in the sample. Soil did not need to be ground. 10 ml of ground leaf litter was mixed with 20 ml of milli-Q water and tested for pH using a pH meter. 5 ml of soil was mixed with 10 ml of milli-Q water and tested for pH using the same method.

2.4 Invertebrate Identification

Invertebrate sampling took place within a plot. A timer was set for four minutes to search for and collect macroinvertebrates. One helper was present to help with invertebrate collection. Any invertebrates found were placed in plastic bags to be photographed, identified, and recorded at the end of the four minute period. Invertebrates found were photographed with a plot ID label. iNaturalist was used to identify species down to the most specific taxonomic rank possible. Pictures were then cross referenced with other pictures taken within Blacksburg and Stadium Woods that had been uploaded to iNaturalist to verify the accuracy of suggested identifications for the invertebrate.

2.4 Data Analysis

To test how soil and leaf litter parameters affect macroinvertebrate abundance, I used a linear mixed model with transect ID as a random effect. Four predictor variables were used in the same model, with soil pH, soil moisture, leaf litter pH, and leaf litter moisture content as fixed effects and abundance as the response variable. To test the relationship between soil moisture and leaf litter moisture, another linear mixed model with transect ID as a random effect was used. Soil moisture was used as the fixed effect and leaf litter moisture was the response variable. Soil pH and leaf litter pH was the same as the moisture model, with soil pH being used as the fixed effect and leaf litter pH as the response variable. Type II Wald chi-square tests were used to assess statistical support for the fixed effects .

Results

Across 13 plots, we identified 45 total macroinvertebrates. Most plots (11 of 13) contained at least one individual. There were 14 different macroinvertebrate families identified across all plots. Formicidae was the most abundant family with 16 individuals identified.

Table 1. List of macroinvertebrate families and their abundance

Family	Abundance
Anyphaenidae	1
Arionidae	4
Carabidae	3
Cleidogonodidae	1
Formicidae	16
Geophilidae	2
Geoplanidae	1
Icheumonid	1
Lumbridae	1
Lycosoidea	2
Raphidophoridae	2
Trachelipodidae	1
Unknown Family 1	3
Unknown Family 2	1

To test the effects of leaf litter and soil properties on macroinvertebrate abundance, I used a Type II Wald chi-square test with soil pH, leaf litter pH, soil moisture, and leaf litter moisture as predictors and abundance as the response variable. There was no significant effect of leaf litter moisture (figure 1a, $X^2 = 2.1$, $p = 0.15$), leaf litter pH (figure 1b, $X^2 = 0.01$, $p = 0.91$), soil moisture (figure 1c, $X^2 = 1.4$, $p = 0.25$), or soil pH (figure 1d, $X^2 = 0.41$, $p = 0.52$). Data shown on the plots of figure 1 are scattered. However, all plots at a given transect have similar pH for most tested factors.

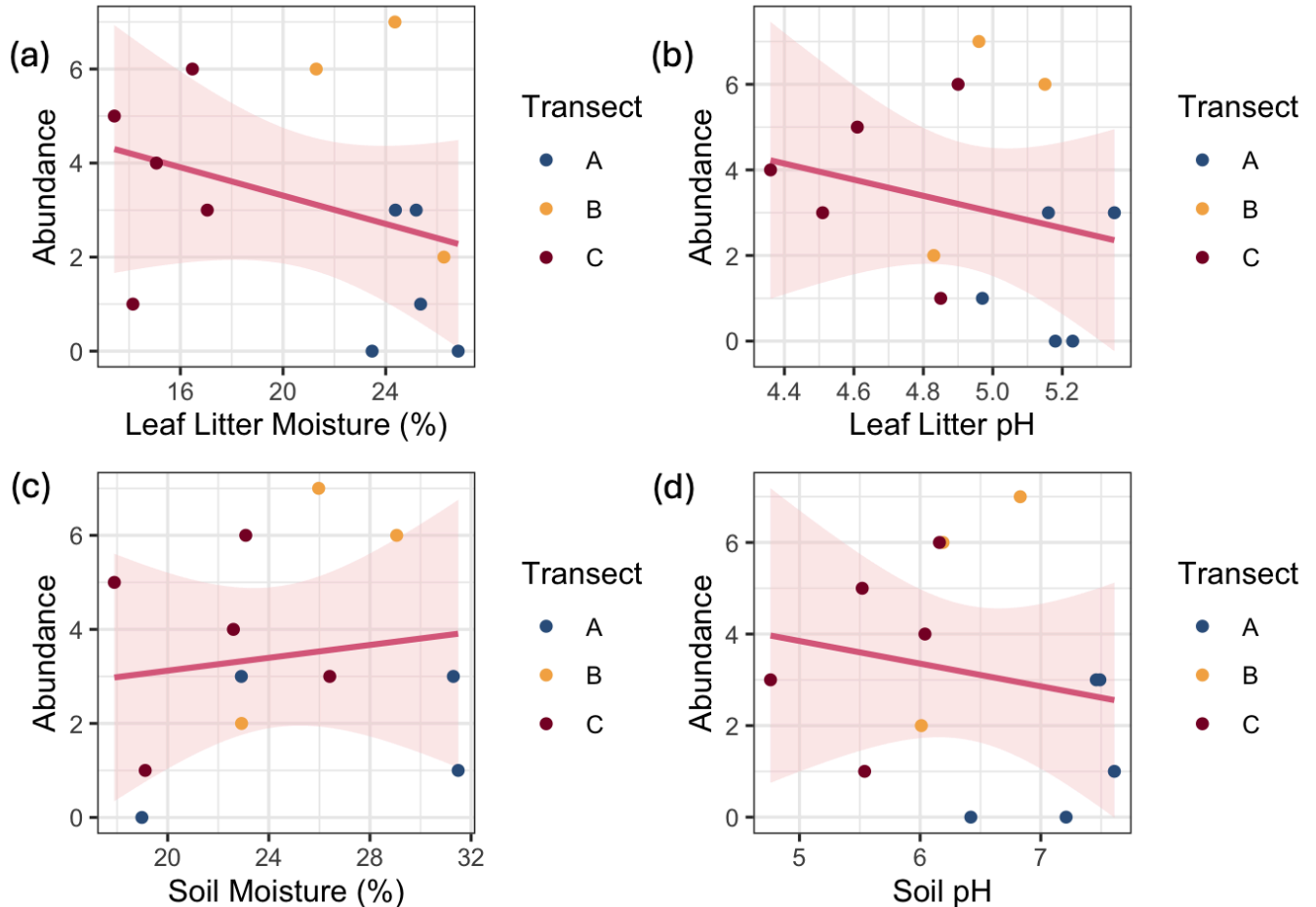


Figure 1: Effects of leaf litter moisture, soil moisture, leaf litter pH, and soil pH on macroinvertebrate abundance in Stadium Woods. Each point represents a single 1m² plot. Abundance is the total abundance of individuals across the families.

When examining the relationship between soil moisture and leaf litter moisture, again using a Type II Wald chi-square test, it was also found that there was no significant relationship between the two variables (Figure 2a, $X^2 = 1.9$, $p = 0.17$). There was no significant relationship between leaf litter and soil pH, either (Figure 2b, $X^2 = 0.75$, $p = 0.39$). However, soil and leaf litter moisture values appear to be more scattered compared to soil and leaf litter pH values.

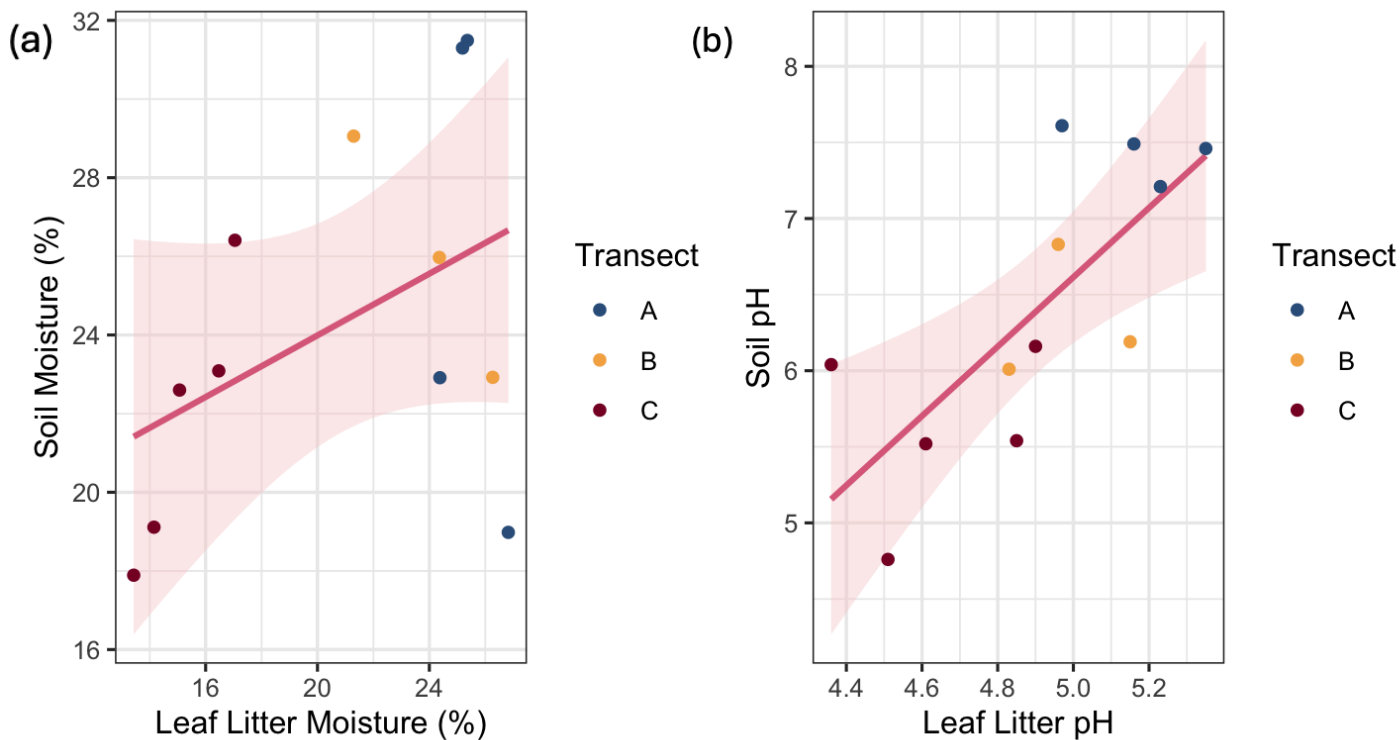


Figure 2: Relationship between soil moisture and leaf litter moisture percent and soil pH to leaf litter pH. Each point represents a single 1m² plot.

Discussion

Macroinvertebrate abundance is often determined by soil conditions such as pH and moisture, as shown by many scientific articles. However, less is known about the impact of leaf litter parameters such as pH and moisture of leaf litter. While the initial hypothesis, that leaf litter has a more significant effect than soil on macroinvertebrate abundance, was not supported by the data, there is still interesting information that can be extracted from the data collected.

Soil pH (figure 1d) and pH of leaf litter (figure 1c) in Stadium Woods had levels similar to those found in other old growth forests in the Appalachia region (Kalisz and Powell 2000). One reason why there may not have been a significant relationship between soil and leaf litter pH and macroinvertebrate abundance is due to the low number of macroinvertebrates identified during the study period. While there is no strong correlation shown in the data, there is a trend shown on both graphs that abundance may decrease as pH becomes more neutral. It is hard to say, however, if these trends would be present if more macroinvertebrates had been collected. Previous studies have found that an increase in pH has the opposite effect on some macroinvertebrate species, such as the earthworm, and abundance will increase with increasing pH. However, unlike the earthworm, there did not appear to be a correlation between chemical measures for soil quality and abundance of other macroinvertebrate species (Kalisz and Powell 2000). There is not enough data to support the prediction that leaf litter pH may have a greater effect on these invertebrates than the soil, but more testing would be required with a larger

sampling size to fully determine whether the hypothesis is true or not. It is also worth noting that family Formicidae, ants, were the most abundant organism found over the course of the sampling period. Many expectations were made with the thought that detritivores would be the most dominant macroinvertebrate found within leaf litter. However, while ants can act as detritivores, they can also act as predators and scavengers. There is a possibility that the abundance of the ants may not have been driven by moisture or pH, but rather by prey or food distribution.

Though not considered while formulating the hypothesis for this study, another reason that no clear effect may have been seen may have been due to the area of Stadium Woods. Stadium woods is in the middle of both living spaces, a football field, and the rest of the Virginia Tech campus. Though considered an old-growth forest, the woods take up a small portion of the land on campus, only about 11.5 acres. Forest edges and forest interiors have been shown to have different rates of macroinvertebrate abundance and diversity. Studies done show the abundance of certain macroinvertebrates increases with edge effect and decreases as an individual moves to the forest interior (Bogyó et al. 2015). Because Stadium Woods is such a small area, it would be hard to determine how much the edge effect may influence abundance within Stadium Woods, or how much of the woods could be considered the interior of a forest. Stadium Woods would also be relatively affected by human disturbances, which would in turn affect macroinvertebrate abundance, diversity, richness, and the soil and leaf litter properties of the woods. It is often hard to determine what human disturbances have occurred in old-growth forests across the nation, and would therefore make it more difficult to quantify the changes done to these forests (White et al. 2018).

Unlike the trend with soil and leaf litter pH, soil moisture (figure 1b) and leaf litter moisture (figure 1a) had opposite trends when it came to abundance. While still not significant, leaf litter moisture seemed to continue the trend of having a negative correlation with macroinvertebrate abundance, but soil moisture had a positive correlation with abundance. There also seemed to be a stronger trend between soil and leaf litter pH (figure 2b) than in soil and leaf litter moisture (figure 2a). Centipede abundance has been correlated with a higher moisture content in soils, although there is no mention if that same correlation exists for leaf litter and centipede abundance (Kula and Lazorík 2016). There is still not significant enough evidence or enough sample data to support the idea of leaf litter properties having a greater effect on abundance than soil properties.

A longer testing period and more samples are needed for a more thorough examination into the effects of leaf litter on macroinvertebrate abundance and how leaf litter properties compared to soil properties when it comes to effects on macroinvertebrates. More transects across Stadium Woods, or in another old growth forest, would be needed earlier in the season before the weather became too cold to gain more samples.

While no significant relationships came out of this study, there are still basic trends that are shown throughout the data that provide useful context to the abundance of macroinvertebrates in old growth forests. Understanding macroinvertebrate abundance in old-growth forests such as Stadium Woods is not only important because macroinvertebrate populations are crucial to the environment, but also because of the decline of old-growth forests in Appalachia and the vast amounts of knowledge needed to protect any remaining forests. Preserving our natural landscapes has always been and will continue to be important to maintaining biodiversity and

abundance of any number of organisms. Without furthering our understanding of the connection between the chemical and physical properties of forests and other habitats and their connection to life living within them, there would be a much larger struggle to protect the natural landscapes we still have.

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