The Effect of Earthworm Presence on Plant Health

INTRODUCTION

In any ecosystem, interactions occur that impact the health and behavior of species, either directly or indirectly (Thompson 1999). The relationship between plants and earthworms is an example of these type of interactions. Plants require nutrients such as nitrogen, phosphorus, and potassium for proper growth and development; for instance, enzyme activity, photosynthetic processes, and maintenance of homeostasis are all impacted by levels of these macronutrients (Shin et al. 2005). Nitrogen, phosphorus, and potassium, obtained through root systems embedded in the soil, are more accessible with the presence of earthworms (Tomati et al. 1992, Yoshitake et al. 2014). Earthworms stimulate plant growth through movement and feeding activity (Blouin et al. 2006). Furthermore, the detritivores produce castings that have more nutrients than the soil around them (Blanchart et al. 1997, Tomati et al. 1988). Earthworms also act as soil aerators, penetrating the soil while burrowing; this movement within the soil allows for plant roots to grow more compared to an environment with no worm activity (Logsdon and Linden 1992, Bertrand et al. 2015). Though research indicates that earthworms affect plant growth, some research suggests that earthworms do not impact root to shoot ratios, that is the ratio of vegetative matter to root matter of a plant (Scheu et al. 1999).

With the research that has been conducted on how earthworms impact soil nutrient availability and soil turnover, more research could be conducted to understand how earthworms impact the subsequent growth and overall health of plants. More specifically, it would be useful to understand how earthworms might impact shoot and root biomass, the ratio of shoots to roots in plants, and if there is a difference in how earthworms affect plant health across different plant families. Additionally, it may also be of interest to find if a level or population concentration of earthworms in a given area exists that provides the maximum health potential of plants or if plant health potential is directly correlated to earthworm numbers in a given area.

A genus of plants that earthworms commonly interact with is the *Brassica* genus. This genus contains an array of plants including wild-type mustard plants, cabbages, and turnips (Thieret et al. 2001). *Brassica rapa* ssp. *oleifera,* the subspecies of the wild-type Field Mustard, is related to commonly-grown produce (Terry et al. 2001). Studies have been conducted to better understand how earthworms can be used for new composting methods such as the bio-conversion process within the gut of earthworms (Manyuchi et al. 2012) or how earthworms, specifically earthworm community interactions, can serve as an indicator of crop productivity (Buckerfield 1997). Furthermore, earthworms are especially relevant to agriculture since earthworms can represent up to three-quarters of the total weight of animals on agricultural lands (Barley 1961, Logsdon and Linen 1992). Studying the impacts of a detritivore such as the European Nightcrawler can further produce insight on how earthworms impact agriculture and plant health in general.

The purpose of this research project is to better understand how earthworms are beneficial to plant health. For this study specifically, the aim is to explore how the presence of earthworms at varying densities impacts above-ground biomass and below-ground biomass. I hypothesized that earthworms will increase soil nutrient levels and aeration, leading to greater plant health. My specific predictions are that above-ground biomass, including plant height and leaf count, will increase with an increase in *E. hortensis* count, below-ground biomass will increase with an increase in *E. hortensis* count, and the shoot to root ratio will increase with an increase in *E. hortensis* count.

METHODS

Study organisms and site

The Field Mustard (*Brassica rapa)* is a weedy plant that exists in fields and disturbed areas along the east coast in states like Maryland, Virginia, and North Carolina (Thieret et al. 2001). The *Brassica* genus is a diverse group that contains agriculturally relevant plants such as broccoli and cabbage as well as wild-type plants that includes wild-type mustards and turnips (Terry et al. 2001). A subspecies, *Brassica rapa* ssp. *oleifera* (Wisconsin Fast Plant)*,* is used in classrooms and laboratories across the country for its quick maturity time (Musgrave 2000). *Brassica rapa* ssp. *oleifera* has been cultivated to have a rapid life cycle, fully maturing from a seed in about thirty days (Radu 2014), allowing for observation of plant health from seed to fruit-bearing (seed pod) stage. In addition to this rapid life cycle, *B. rapa* ssp. *oleifera* serves as a good comparison plant to agriculturally relevant plants and wild-type plants, making it an ideal study plant.

The European Nightcrawler (*Eisenia hortensis*) is wide-spread across North America and can be found in the topsoil of grassy fields and forest leaf-litter (Hale et al. 2008).

The experiment was carried out in the Virginia Tech Greenhouse on Washington St. in Section 10B. Here, the climate can be considered characteristic of spring and early summer with respect to temperature, humidity, and lighting with average temperature of 72.3ºF and average light period of 10 hours over the span of the study.

Study approach and design

This study took an experimental approach to answering the broad question of how earthworms impact plant health. To test the hypothesis, five individual *B. rapa* subsp. *oleifera* seeds were planted in two-gallon pots containing soil that were purchased to minimize unaccounted variance that might arise from microorganisms, plants, fungi, and nutrient levels found in natural soils. In eight two-gallon pots that contain five seeds per pot, different numbers of *E. hortensis* were added at day one. The following levels were added to the pots: zero worms (control), one worm, two worms, three worms, four worms, five worms, six worms, and seven worms. This process was repeated for another eight pots so that two repetitive sets of data exist in the study with 16 two-gallon pots total, 80 plants total, and 56 earthworms total. Randomized placement of containers help keep constant the amount of light and water the plants receive. Temperature and humidity in the greenhouse were held constant so that all containers experienced relatively the same conditions.

Levels of nitrogen, phosphorus and potassium were measured with a soil test kit at the beginning of the study when the macronutrient levels of the soil are the same for all containers, and at the conclusion of the study when changes are expected to have occurred in the containers due to earthworm presence. To measure plant height, measurements were taken in centimeters from the rim of the container to the shoot tip (shoot apical meristem) of the plant to account for changes in compactness of soil and variable leaf height. To measure leaf count, the number of fully unfurled leaves were counted and recorded. To measure the above-ground biomass and below-ground biomass, roots were separated from the vegetative tissue of each plant, and all parts were rinsed to remove soil particles and subsequently dried to remove excess water.

Above-ground biomass was defined as all vegetative matter and below-ground biomass was defined as roots.

Variables
Plant health was assessed in various ways including above-ground biomass, below-ground biomass, and shoot to root ratio. Plant height and leaf count were also used as assessors of plant health. Other variables being measured in this study include levels of nitrogen, phosphorus and potassium and top soil temperature.

Data analysis
In this study, the predictions were that above-ground biomass, plant height, leaf count, below-ground biomass, and the shoot to root ratio will all increase with an increase in *E. hortensis* count. A linear model was used with above-ground biomass as the response variable and earthworm count serving as the fixed effect. This model was repeated with plant height, leaf count, below-ground biomass, and the shoot to root ratio all serving as the response variable and earthworm count serving as the fixed effect as well. Nutrient levels were not measured in the data analysis as the levels of nutrients could only be measured as a coarse categorical value and not a numerical value, leaving changes in nutrient levels unspecific and, therefore, irrelevant to the study.

Running the data in version 1.2.5001 of RStudio, the following packages were used in the script: RColorBrewer, lme4, Matrix, ggplot2, dplyr, rstudio, stats, graphics, grDevices, utils, datasets, methods, and base. The Shapiro-Wilk test for normality displayed non-normal ($p < 0.05$) distributions for plant height ($p = 0.002$), leaf count ($p = 1.12e-06$), and shoot to root ratio ($p = 7.814e-07$), while above-ground biomass ($p = 0.09$) and below-ground biomass ($p = 0.07$) showed normal distribution ($p > 0.05$). However, due to the near-normal distributions of the histograms of plant height, leaf count, and shoot to root ratio, the data was analyzed using a linear model.

RESULTS

Effects of earthworm presence on above-ground biomass
Through a linear model, it was found that there was a significant relationship between earthworm count and above-ground biomass ($F = 8.733$, $p = 0.004131$). As earthworm count increased from 0 to 7 worms, average above-ground biomass also increased (Fig. 1).

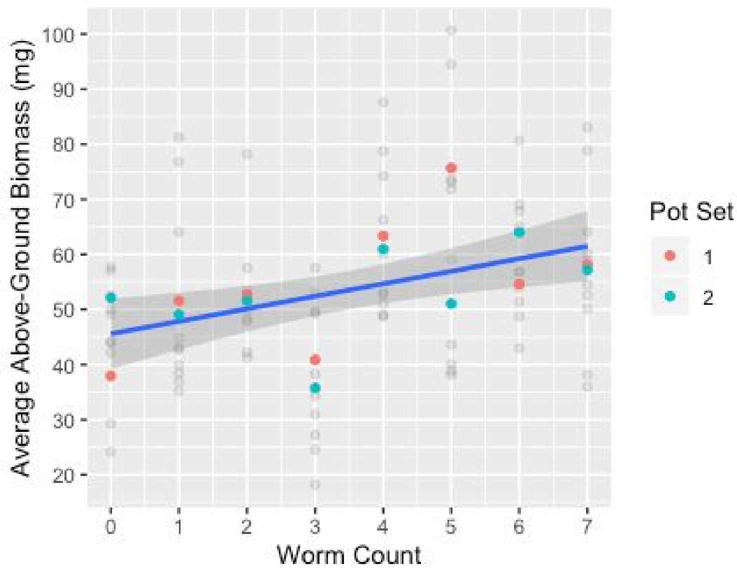

Figure 1: Effect of earthworm count on average above-ground biomass over a span of 22 days for both pot sets. Uncolored points represent individual measurements of average-above ground biomass in both pot sets.

Effects of earthworm presence on below-ground biomass

There was no significant relationship between earthworm count and below-ground biomass ($F = 0.3785$, $p = 0.5402$, Fig. 2).

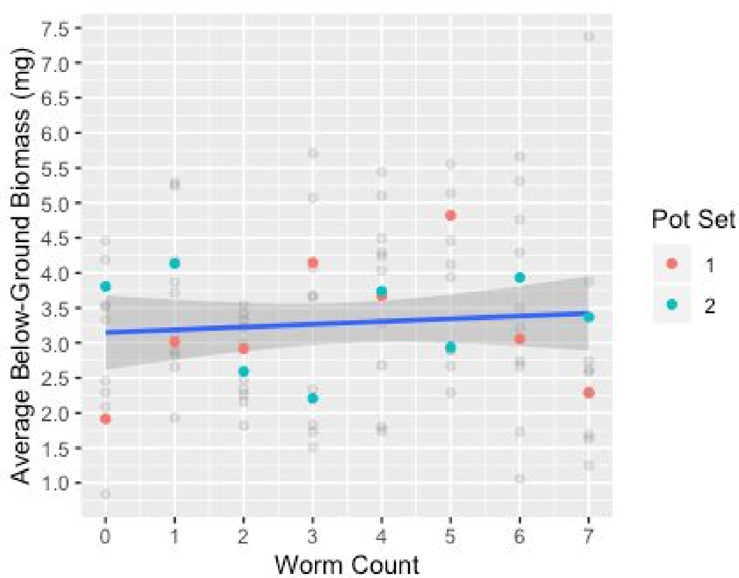

Figure 2: Effect of earthworm count on average below-ground biomass over a span of 22 days for both pot sets. Uncolored points represent individual measurements of average below-ground biomass in both pot sets.

Effects of earthworm presence on the shoot to root ratio

Through a linear model, it was found that there was a significant relationship between earthworm count and above-ground biomass ($F = 5.081$, $p = 0.02699$). As earthworm count increased from 0 to 7 worms, so did the above-ground biomass to below-ground biomass ratio, also referred to as the shoot to root ratio (Fig. 3).

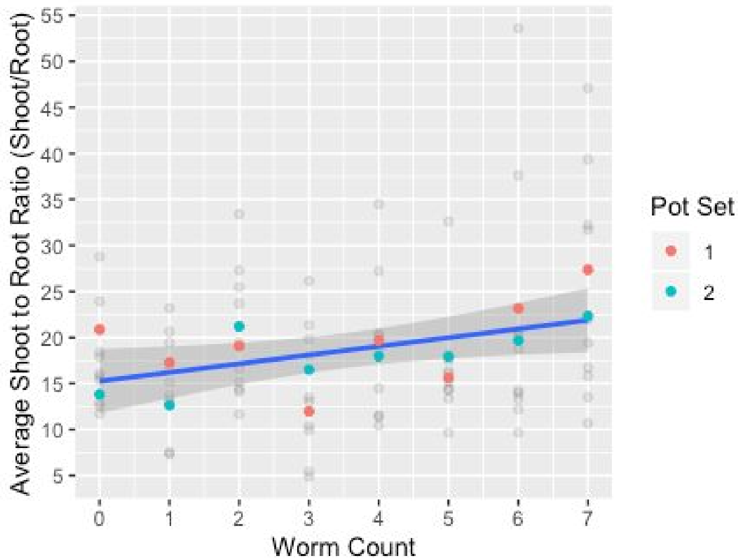

Figure 3: Effect of earthworm count on average shoot to root ratio over a span of 22 days for both pot sets. Uncolored points represent individual measurements of average shoot to root ratio in both pot sets.

Effects of earthworm presence on plant height

There was no significant relationship between earthworm count and plant height ($F = 0.1658$, $p = 0.685$; Fig. 4).

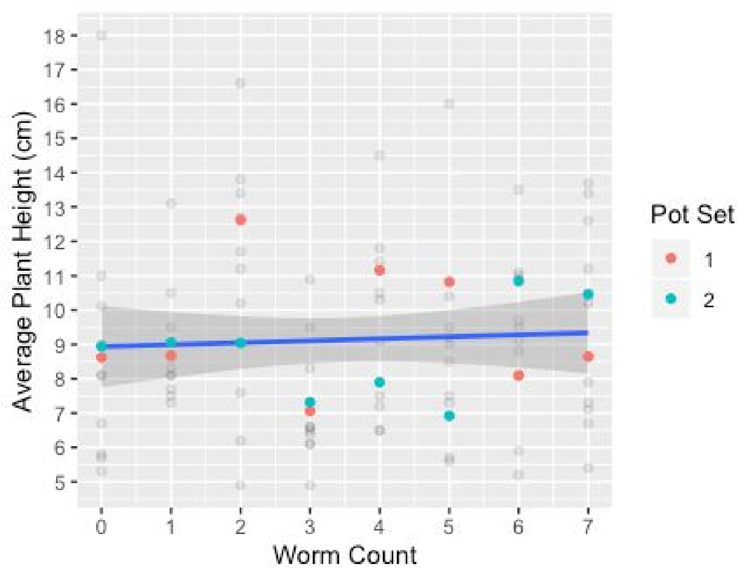

Figure 4: Effect of earthworm count on average plant height over a span of 22 days for both pot sets. Uncolored points represent individual measurements of average plant height in both pot sets.

Effects of earthworm presence on leaf count

There was no significant relationship between earthworm count and leaf count ($F = 1.101$, $p = 0.2973$, Fig. 5).

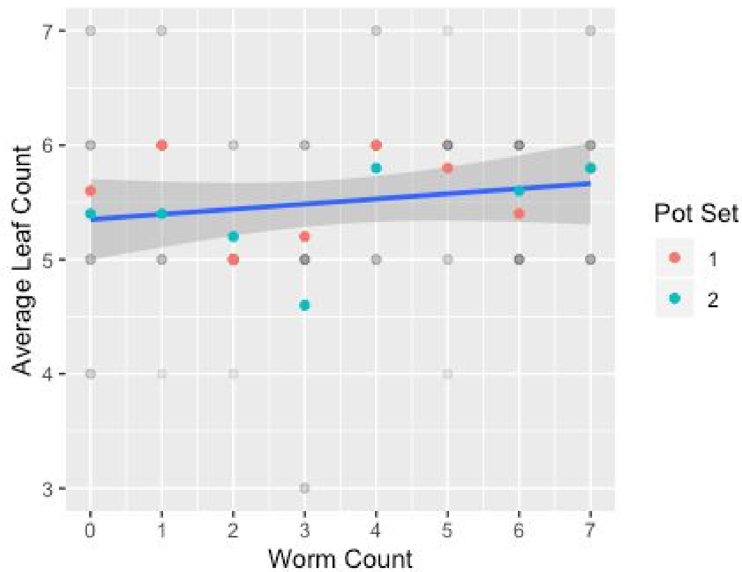

Figure 5: Effect of earthworm count on average leaf count over a span of 22 days for both pot sets. Uncolored points represent individual measurements of average leaf count in both pot sets.

DISCUSSION

The main purpose of this research project is to better understand how earthworms are beneficial to plant health. Specifically, the aim is to explore how and to what extent earthworms impact the various metrics of plant health used in this study.

The relationship between above-ground biomass and earthworm count was seen to be significant. This supports the prediction made about this relationship and validates the hypothesis that higher earthworm presence results in greater plant health (Fig. 1). A similar relationship significance and support of a prediction and the hypothesis was seen in the above-ground biomass to below-ground biomass ratio, also known as shoot to root ratio (Fig. 3). Despite the relationship between earthworm count and below-ground biomass having no significance, this doesn't necessarily deviate away from the hypothesis. This is because plants may invest in shoots and vegetative tissues with nutrients being more readily available with the earthworm activity. This investment strategy is demonstrated best in the above-ground biomass to below-ground biomass ratio (Fig. 3). Further research could give insight into whether or not the linear relationship displayed in the data collected will reach a saturation point where no greater investment can be made by plants. It may also be useful to understand how different plant genera and families may invest their metabolic energies with the presence of earthworms. Plant height and leaf count were also found to be insignificant with respect to their relationship with earthworm count but because these two properties of above-ground biomass do not reflect the total amount of vegetative tissue, the insignificance in the relationships may not entirely contradict the hypothesis.

Because the data demonstrates a relatively strong correlation between above-ground biomass and earthworm count, there is a significance to the relationship earthworms have with plants.

In terms of consistency with previous studies and reviews, the study results varied. Above-ground biomass was expected to increase with the presence of earthworms within the soil (Blouin et al. 2006, Xiao et al. 2018, Yoshitake et al. 2014). A similar trend was demonstrated in the experiment with worm count and above-ground biomass sharing a positive relationship. However, it was expected that earthworm activity and the movement through the soil would cause greater aeration and therefore would aid in the growth of root systems (Logsdon and Linden 1992). This determinant portion of plant health was not seen to have been affected by earthworm presence, leaving only above-ground biomass as a support for the hypothesis.

Other factors that could have been considered in this determination of whether or not earthworms impact plant health if resources were available can include nutrients as well as aeration. Quantifying macronutrient levels in parts per million would have better explained mechanistically how earthworms affect plant health since casting production produces nutrients from previously unbroken down organic matter (Blanchart et al. 1997, Tomati et al. 1988). However, the resources available allowed only for the categorization of nutrient levels as opposed to quantitative measurement. Furthermore, aeration could be quantified and measured in this experiment. If this was possible without disturbing root growth then it might have also given insight into how aeration of soil impacts plant health.

The significance of this work expands to understanding roles that earthworms play in ecosystems as well as roles earthworms play in agriculture. From the data collected, earthworms may have an impact on above-ground biomass. With respect to ecosystems, the presence and success of earthworms might be of importance to conservationists who hope to understand and maintain ecosystems, threatened or not, for their ability to increase above-ground biomass in plants. Conversely, it may be important to understand how an ecosystem may be impacted if a detrivore such as the earthworm were to be reduced or entirely removed and what cascade of effects would take place as a result. Some effects that could occur may include, but are not limited to, changes in soil nutrient levels, plant productivity, and soil aeration (Blouin et al. 2006, Shin et al. 2005). With regards to agriculture, the findings may be especially important because *Brassica rapa* ssp. *oleifera* belongs to the same genus as broccoli, cabbage, cauliflower, etc. (Terry et al. 2001). These genus members are a valuable source of income to farmers that make a living off of produce so understanding how earthworms increase the above-ground biomass may be of great interest in terms of increasing productivity and, consequently, revenue. Overall, the study showed some significance in areas that, if further tested on, could lead to greater understanding of how earthworms impact plant health in both natural ecosystems and agricultural ecosystems.

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