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An Honors Thesis presented to the Faculty of the
Department of Biology at Colby College
in partial fulfillment of the requirements for the
Degree of Bachelor of Arts with Honors

By
Ella Maddi

Waterville, ME
May 20, 2019

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Abstract

Asian Jumping worms (Megascolecid spp) were introduced to North America in the early 1900s and have been altering soils in forest ecosystems as they spread throughout the continent. The worms differ from other introduced earthworms in their ability to consume large densities of organic material and potentially alter nutrient cycling in the ecosystems that they invade (Greiner et al. 2012). This study examined if and how Asian jumping worms alter turfgrass systems by changing the biological or physical properties of soil. To do this we conducted a mesocosm experiment and observational field study. For the mesocosm study we inoculated boxes that replicated a backyard environment with Asian jumping worms, and we evaluated how microbial extracellular enzyme activity, soil chemistry, biomass, and organic matter changed over time in boxes with and without worms. For the field study we collected soil samples from Coastal Maine Botanic Gardens and analyzed enzyme activity and ammonium concentration for sites with and without worms. The mesocosm study did not produce evidence of worms altering any of the biological or physical metrics that we studied. However, in the field study, we found that sites with high worm presence had increased chitinase and peptidase activity. Overall, the results of the study are inconclusive but support previous findings that worms effect grassy ecosystems differently than forest ecosystems (Qui and Turner 2017). Further studies are needed to determine how Asian Jumping worms effect turf grass systems and how those effects differ from what has been found in forests.

Introduction

Earthworms are important ecosystem engineers that have transformed North American forest ecosystems since their introduction into previously glaciated regions. Nonnative earthworms consume leaf litter and through their movement mix soil layers, disperse seeds, and disrupt mycorrhizae. This activity leads to a rapid disruption in decomposition rates and nutrient fluxes unfamiliar to North American forest ecosystems (Eldor 2015). In the 17th and 18th centuries European settlers introduced *Lumbricus* species into northeastern hardwood forests through trade of horticultural products (Greiner et al. 2012). In the early 1900s, a new invasive group of Megascolecidae worms were introduced to North American forests. In the past few decades these worms have posed new threats to hardwood ecosystems (Greiner et al. 2012). Although it is unknown how and when the worms were introduced to North America, it is suspected that in the early 20th century they were brought from their native environment in eastern Asia to North America in potting soils (Chang et al. 2016). The Megascolecidae family consists of three species that concern ecologists due to their destructive potential: *Amyntas agrestis*, *Amyntas tokioensis*, and *Metaphire hilgendorfi*. These species are characterized by their distinct smooth, white clitellum and erratic behavior when disturbed, leading to their common name of the Asian jumping worms (Chang et al. 2016).

Asian jumping worms have a life history distinct from European *Lumbricus* species. Asian jumping worms are epigeic earthworms which means that live and consume nearly exclusively the leaf litter and top organic soil layer. They have an annual life cycle in which the juveniles emerge from an overwintering cocoon in the spring, grow quickly, and reproduce then die in the fall (Greiner et al. 2012). Megascolecidae worms have more dietary flexibility, larger body sizes, and a higher growth rate than *Lumbricus* species (Qiu and Turner 2017). Overall, this

means that these worms are consuming large amounts of organic matter over short time periods. Unlike European worms they do not have the agricultural benefit of mixing soil layers or aerating compacted soils. The unique life history traits of Megascolecidae therefore combine to make a species of conservation concern with the potential to cause irreversible damage to hardwood forests (Qiu and Turner 2017).

Asian jumping worms accelerate organic material decomposition and as a result leave behind dense aggregates of castings. The castings can promote erosion and facilitate invasive seed dispersal (Eldor 2015). Furthermore, the castings are microbial hotspots that further accelerate nutrient cycling. Previous experiments conducted in forests found that Megascolecidae invasion led to reduced surface litter, increased total carbon, increased nitrogen, and increased phosphorous (Qiu and Turner 2017). These findings support concerns that the worms may increase nutrient transformation into more liable compounds through mineralization. Although mineralization is generally beneficial to plant growth, when mineralization occurs too quickly plants cannot take up all available nutrients and the soluble compounds are lost during leaching (Qiu and Turner 2017). However, as the case with most earthworm species, the extent of the impact of Asian jumping worms is greatly dependent on the ecosystem composition.

Despite the growing concern surrounding Megascolecidae worms, most research is focused on forest ecosystems leaving a critical knowledge gap around the effects of these species on turf and garden systems. The importance of this gap is underscored by the fact that Asian jumping worms are moving into urban environments occupying residential gardens. Anecdotes from homeowners include observations of worms “eating” their lawns (Patterson 2017). Although worms do not eat grass, these reports do raise questions about the role of these invasive worm species in and potential impact on urban ecology specifically within turfgrass systems.

Turfgrass describes a uniform stand of grass that is maintained at a relatively low height often used for recreational or aesthetic purposes (Potter and Braman 1991). The turfgrass ecosystem is composed of the roots, stems, leaves of individual grasses, stolons, and the organic debris called thatch (Potter and Braman 1991). Turfgrass occupies more square kilometers than any other irrigated crop in America (Wu and Bauer 2012) and supports a \$25 billion industry. Turfgrass is critical for urban landscaping as it is used for backyards, golf courses, athletic fields, cemeteries, road sides, and parks (Potter and Braman 1991). In addition to its aesthetic value, turfgrass supplies oxygen, filters dust, moderates surface temperature, and reduces erosion, runoff, glare, and noise pollution (Potter and Braman 1991). Turfgrass systems are a fundamental and ubiquitous component in urban and suburban ecosystems. However, turfgrass in urban environments is especially susceptible to non-native earthworm invasions because well managed turfgrass has high soil moisture content that can facilitate worm movement and survival. Furthermore, strategies for pest management are complicated in urban environments due to concerns regarding pesticide use and human health in densely populated area.

The importance of turfgrass as a crop and the evident, but unknown impacts of an Asian jumping worm invasion motivated me to investigate how Asian jumping worms are affecting turfgrass ecosystems. There are multiple ways that worms can alter the soil of turfgrass ecosystems. The presence of worms can alter soil community composition by altering the abundance and diversity of microarthropods and microbes. Earthworms can change the microbial community by creating microbial hotspots where the worms deposit their castings. Worms consume organic matter and that organic matter is colonized by microbes in the earthworm gut (Eldor 2015). Once the organic matter is defecated as casts, microbes continue to colonize and break down the material until all the easily decomposable compounds are either mineralized or

immobilized (Eldor 2015). If the compounds are mineralized then the decomposed material is turned into nutrients that is available for plant uptake. High mineralization rates caused by worm activity can lead to increased plant root growth and increased soil biota activity (Eldor 2015). However, should the earthworms convert the organic matter layer into castings faster than the organic matter layer accumulates, over all nutrient availability may decrease and the microbes will immobilize rather than mineralize soluble compounds. When microbes immobilize compounds they are not turning organic material into plant available forms, but keeping the nutrients for themselves to benefit their own cellular growth. As a result, plant productivity decreases (Lou et al. 2017).

Investigating how Asian jumping worms change microbial activity and abundance provide information about changing nutrient pools, especially nitrogen. The fungi and bacteria that compose the microbial community are the foundation of soil composition and aboveground biodiversity (Luo et al. 2017). In a broad sense, the productive capacity of an ecosystem can be measured by the rate at which microbes transform nitrogen into soluble compounds (Eldor 2015). Microbes break down simple molecules such as sugars and starches when nutrients are readily available, but turn to more indigestible polyphenols such as tannins and lignin when resources are scarce. Microbes use their extracellular enzymes to break down specific amino acids. Therefore, by looking at which extracellular enzymes are present in the soil we can make inferences about resource availability and demand in the ecosystem (Luo et al. 2017).

For this study, I quantified the activity of the extra cellular enzymes β -N-acetyl-glucosaminidase (NAG), Leucine- amino peptidase (LAP), peroxidase, and phenol oxidase in soils with and without Asian jumping worms. NAG is a chitinase that catalyzes cellulose degradation (Luo et al. 2017). Previous studies have shown a correlation between nitrogen

mineralization rates and NAG activity in cropping systems (Luo et al. 2017). LAP cleaves the terminal nitrogen peptide bonds in proteins (Sinsabaugh 1994). Peroxidase and phenol oxidase are oxidative enzymes that catalyze the decomposition of recalcitrant compounds such as lignin (Lou et al. 2017). NAG, LAP, peroxidase, and phenol oxidase are all common enzymes with known substrates and established protocols for assessing enzyme activity. Furthermore, they target compounds that are closely associated with nitrogen and carbon cycling

Initially I sought to examine whether the Asian jumping worms will even occupy a turfgrass environment when presented with the option of living in an organically rich mulched bed or an adjacent patch of turfgrass. Once I determined that the worms do indeed freely migrate from infested mulch patches to adjacent turfgrass, the next task was to determine to what extent do the worms use turfgrass as a resource and what are the subsequent impacts. Should the worms be causing damage to the turfgrass ecosystem, what is the mechanism of damage? I predicted that the worms could be changing the turfgrass physically or biologically. Potential physical alterations include decreasing the aggregate stability making the soil unsuitable for turf growth. Potential biological changes include the worms shifting nutrient availability, leading to inadequate resources for the turfgrass.

This study is composed of two components: a mesocosm study conducted in Geneva, NY and a field study conducted in Boothbay, ME. Asian jumping worms have been identified in 13 counties throughout New York state (Reynolds 2014). New York has classified the worms as a prohibited species. Although the Asian jumping worms are not thought to be widely prevalent in Maine they have been identified in both Boothbay and Augusta. No studies have been done to quantify abundance or range of the worms in Maine, so the extent of their invasion outside of managed gardens is unknown. The mesocosm study aimed to investigate the effect of worms on

turfgrass over a 124 day study period while the field study aimed to assess the effect of an established population of worms on turfgrass at one time point. Using the extracellular enzymes I formerly mentioned to assess microbial response, I hypothesized that in the mesocosm study, initially after worm invasion nitrogen mineralization, available carbon, and overall turf health would increase leading to an increase in NAG, LAP, ammonium, shoot biomass and root biomass. However, following the initial increase in nutrient availability and turfgrass health, I suspected that nitrogen and carbon sources would be exhausted leading to nitrogen immobilization and a decrease in overall turfgrass health leading to an increase in peroxidase and phenol oxidase and a decrease in thatch biomass and organic matter. For the field study I hypothesized that the soils with high worm activity would have lower levels of NAG, LAP, ammonium, and organic matter than the soils with low worm activity.







Methods

Mesocosm study

To test the effect of Asian jumping worms on turfgrass ecosystems I conducted a mesocosm study with six different treatments. One hundred eight 35.6 cm x 20.3 cm x 12.4 cm plastic boxes were transformed into mesocosms replicating a common garden layout with one of three amendments abutting a patch of turf. The mesocosms were randomly assigned an amendment of either hardwood mulch, compost mulch or no amendment. All boxes received a patch of Kentucky blue grass (*Poa pratensis*) and Tall Fescue (*Festuca arundinacea*) blend and 0.64 cm of the assigned amendment. Under the amendment and turfgrass, the boxes were filled with a sandy divot mix made for golf course fairway repairs. The mesocosms were kept in a greenhouse at 24° C in the Cornell AgriTech greenhouses (Geneva, NY).

Asian jumping worms (Megascolecidae spp.) were collected from the Cornell Botanical Gardens. Over 200 Megascolecidae worms were identified using a key described in Chang et al. (2016). I inoculated 54 out of 108 mesocosm boxes with three worms. The mesocosms were watered as needed when the turfgrass exhibited characteristics of dryness, and the turfgrass was clipped every three days to a height of 5cm. Clippings were removed as to reduce additional nutrient input sources. Twenty-eight days later boxes in which worms had died were reinoculated with fresh worms and all dead worms were removed. Boxes were collected at three time points for analysis over a five-month period. Thirty-three days after the initial inoculation thirty-six boxes were randomly selected and taken apart for biological analysis. Seventy-two days after the initial inoculation thirty-six more boxes were randomly selected and taken apart. Finally, 124 days after the initial inoculation the last thirty-six boxes were collected for analysis.

Table 1. Shows the experimental design of the mesocosm study. Six treatments were used to assess the effect of worm and amendment, worm interaction on turfgrass

Amendment	No worm	worm
Hardwood mulch		
compost		
control		

Physical Analysis

In order to characterize the effect of Asian jumping worms on the physical characteristics of turfgrass, I looked at percent greenness of the turfgrass, presence of castings, thatch mass, clipping mass, and root biomass. To measure greenness we used the application Canopeo (Canopeo 2019), which calculates percentage of greenness based on photographs we took of each patch of turfgrass. To assess worm activity, presence of castings was quantified on a binary scale of presence versus absence depending on observations. Both percent greenness and casting presence were measured weekly.

Thatch mass, clippings mass, and root biomass were measured only after the boxes were destructively harvested. The patch of turfgrass was gently removed from the mesocosm and all soil was washed from the roots. Thatch mass was determined by randomly choosing a 2.5 x2.5

cm square within the turfgrass and scraping out all the deceased and decomposing blades of turf. All removed detritus was dried and measured. The patch of turfgrass was then uniformly cut to a height of 2.5cm. All the clippings were dried and weighed to quantify clipping mass. All roots grown beyond the thatch layer were clipped, dried, and weighed to find root biomass. I determined the location of each worm as either occupying the mulch or turfgrass patches. I sifted through mulch and soil under the mulch and any worms present were removed. All patches of turfgrass were submerged in a mustard powder solution to flush any remaining worms out of the turfgrass. All worms were removed and their location recorded.

Finally, I quantified organic matter content of the soil by using a loss on ignition protocol (Wright et al 2012). Five grams of each soil sample were measured into tin weigh boats and placed in an oven at 105° C for 24 hours. The samples were then weighed again to get an initial dry mass and promptly put into a muffle furnace. The samples were burned at 700° C for 8 h and then left to cool for 12 h. The soils are then put back into the oven for 24 h and weighed once more. The quantity of organic matter was calculated by taking the difference between the initial and final masses.

Biological Analysis

During the destructive harvest, the amendment and patch of turfgrass were removed and all remaining soils were collected and separated into two categories: 1) soils under the mulch and 2) soils under the turf. Both soils under the mulch and soils under the turf were analyzed for extracellular enzyme activity specifically β -N-acetyl-glucosaminidase (NAG), Leucine- amino peptidase (LAP), phenol oxidase, and peroxidase via fluorometric and spectrophotometric analyses. For both the fluorometric and spectrophotometric assays, 1g of each soil sample was blended with a sodium acetate buffer to create a soil slurry and 200 μ l of the soil slurry was

added 3 columns of a 96 well plate. Then, for the fluorometric assay 50 μ l of fluorescing agent was added to the first column. The fluorescing agent for NAG is 4- methylumbelliferone (MUB) and for LAP is 7- amino- 4- methylcoumarine (MC). Additionally, 50 μ l of substrate was added to the second two columns. The substrate for NAG is 4-MUB-N-actetyl-B-D-glucosaminide and for LAP is L-Leucine-7-amino-4-methylcoumarine. Then, the plates are incubated for 5 hours. Once the incubation is complete 10 μ l of NaOH is added to every well and the plate is read at an excitation of 360 nm and emission of 460nm.

For the spectrophotometric assay, 50 μ l of the sodium acetate buffer is added to the first column of wells, and 50 μ l of substrate is added to the second and third columns. The substrate for phenol oxidase is L-3, 4-dihydroxyphenylalanine (L-Dopa) and for peroxidase is L-Dopa and hydrogen peroxide. Then, the plates are incubated for 24 hours. Once incubation is complete pipette out 150 μ l of supernatant into clean 96 well plates and read the plates at 650nm. The enzyme assays were conducted in accordance with German et al. (2011) to estimate nmol per hour per gram dry soil for NAG and LAP and μ mol per hour per gram dry soil for peroxidase and phenol oxidase.

Additionally, the soils under the turfgrass were tested for ammonium content (μ g/ g dry soil) via standard colorimetric analysis (Kandeler and Gerber 1988). To do this, 5 g of soil was extracted in 25 mL of potassium sulfate. Extracts were made by shaking soil and potassium sulfate solution on a shake plate for 1 hour at 170 rpm. Then supernatants were poured through a filter paper into scintillation vials. A standard curve was made from diluted ammonium sulfate with the following concentrations: 1.0 ppm, 0.5 ppm, 0.2 ppm, 0.1ppm, 0.05 ppm, and 0.01ppm. Each standard and sample extract was loaded into a 96-well plate. Then 40 μ l of ammonia salicylate was added to all wells, and 3 min later 40 μ l of ammonia cyanurate was added to all

wells. The plate was incubated for 20 min and read on an absorbance plate reader at 650nm. All biological analysis of the mesocosm soils were conducted at Cornell AgriTech at the New York Agricultural Experiment Station.

Field Study

To look at the effects of an established Asian jumping worms population of turfgrass I collected soil samples from areas with high and low worm activity from the Coastal Maine Botanic Garden. Coastal Maine Botanical Garden is composed of 295 acres and has been a botanical garden since 2007. Soil samples were collected in early October (average temperature = 49°F (9°C)). I characterized sites as high worm activity sites based on the presence of Asian jumping worms or the presence of abundant castings and as low worm activity sites based on the absence of earthworms and castings. I sampled eight mulch sites with high worm activity and I sampled eight neighboring patches of turfgrass. Then, I sampled six mulch sites with low worm activity and sampled six neighboring patches of turfgrass for a total of twenty-eight sample sites. At each sample site five 5cm soil cores were taken and then mixed together to get a soil sample representative on the entire mulch or turf patch. The sites I sampled varied in soil type and it turfgrass management. According to the USDA soil surveys the site consisted of three soil types: Lyman-Brayton variant rock outcrop (3-8% slope), Lyman-Brayton Tunbridge outcrop (8-15% slope), and Lyman-Brayton Tunbridge outcrop (15-45% slope). Additionally, some of the sampled turf was harvested sod while other patches were a variety of species grown from a seeded mixture.

Biological Analysis

All of the field samples were filtered through a 2 mm sieve and picked through to remove rocks and large pieces of organic material such as roots and blades of grass. All samples

remained frozen at -20 °C degrees from the time they were collected until time of analysis. As in the mesocosm samples, the field samples were analyzed for NAG and LAP activity using the same protocol previously mentioned. Furthermore, the field soils were tested for ammonium and nitrate content using the same protocol described for the mesocosm samples. However, due to experimental error (standard range – sample range mismatch), the results for the nitrate assays were not included in our statistical analysis. Finally, a loss on ignition method was used calculate the organic matter of the field soil samples. All biological analyses of the field soils were conducted at Colby College.

Statistical Analysis

For all metrics I analyzed the distribution of values and standardized the data as follows to eliminate biologically inappropriate negative values. All enzyme values reported as negative were assigned a quantity of 0. In the fluorometric analyses the quench values return as negative if the control wells are no different than the experimental. Therefore, it is biologically appropriate to assign negative values as 0. Additionally, any value that was outside two standard deviations for the distribution of that metric was considered an outlier due to experimental error and removed from the data set. Then, each metric was plotted against every other metric and I calculated Pearson correlation values. For the mesocosm experiment, I used a linear regression model to assess the effect of worm, amendment, time, and worm x time on each metric. For the field study, I used both a linear regression and multiple linear regression models to assess the effect of worm and amendment on each metric. All analyses were done using R. (R Core Team 2017)

Results

Mesocosm

For the mesocosm study I looked at microbial enzyme activity, specifically NAG, LAP, peroxidase, and phenol oxidase, and ammonium concentrations. Additionally, I analyzed the physical properties root biomass, shoot biomass, thatch biomass, and organic matter. We quantified the effect of worm, amendment, and time on each of these metrics. In order to accomplish this we set up a greenhouse study with 108 mesocosms with six treatments: worm, hardwood mulch; worm, compost mulch; worm no amendment; no worm, hardwood mulch; no worm, compost mulch; no worm no amendment. Descriptive statistics can be found in the supplementary data.

In the mesocosm experiments, there were no significant differences in means between mesocosms with and without worms for any of the biological or physical properties of the turfgrass (Table 3). For biological characteristics, neither NAG, LAP, peroxidase, phenol oxidase, nor ammonium exhibited different mean values in experimental versus control mesocosms (Table 2). For physical characteristics neither root, shoot, thatch, or organic matter showed differing means for the experimental and control mesocosms.

Although there was not an effect of worm presence on overall means of NAG, LAP, peroxidase, phenol oxidase, ammonium, root biomass, shoot biomass, thatch, or organic matter, we did observe difference in means of LAP, peroxidase, and phenol oxidase at specific destructive harvests (Table 3). Soil properties were sampled after 33 (July harvest), 72 (August harvest), and 124 (October harvest) days after mesocosms were inoculated with worms. In mesocosms with worms, there was increased mean LAP activity in July ($t = -2.54$, $n = 18$, $p = 0.02$) and August ($t = -2.76$, $n = 18$, $p = 0.01$) (Figure 1). In mesocosms with worms, there was decreased

mean peroxidase and phenol oxidase activity in August ($t = -2.54$, $n = 18$, $p = 0.02$; $t = -2.54$, $n = 18$, $p = 0.02$).

Additionally, almost all of the biological and physical characteristics changed over time (Table 3). From the first harvest in July to the final harvest in October, net NAG, LAP, peroxidase, phenol oxidase, ammonium decreased ($F = 4.51$, $n = 36$, $p = 0.013$; $F = 72.32$, $n = 36$, $p < 0.001$; $F = 39.52$, $n = 36$, $p < 0.001$; $F = 179.2$, $n = 36$, $p < 0.001$; $F = 48.73$, $n = 36$, $p < 0.001$) (Figure 1). Net root biomass and thatch biomass also decreased from July to October ($F = 4.51$, $n = 36$, $p = 0.01$; $F = 31.36$, $n = 36$, $p < 0.001$). Finally, there was no difference in means of any of the biological or physical properties in the hardwood mulch, compost, or control amendments (Table 2).

Table 2 .Results of comparison of means testing in the mesocosm study. p-values for the effect of worm (t-test) and amendment (ANOVA) on biological and physical properties of turfgrass for mesocosm study.

	Worm (n= 54)		Amendment (n=36)	
	p-value	t- value	p- value	F- value
NAG	0.73	-0.35	0.18	1.79
LAP	0.26	-1.13	0.42	0.66
Phenol oxidase	0.41	-8.30	0.83	0.05
Peroxidase	0.36	-0.93	0.88	0.02
Ammonium	0.24	-1.18	0.82	0.05
Root	0.26	-1.14	0.44	0.61
Shoot	0.76	-0.31	0.90	0.02
Thatch	0.56	0.58	0.26	1.28
Organic matter	0.82	0.23	0.88	0.02

Table 3. comparison of mean values of all metrics measured in mesocosms with worms, without worms (control), with no amendment, hardwood mulch, compost mulch, and for the July, August, and October destructive harvest.

Metric	Worm	No worm	No amendment	Hardwood	Compost	July	August	October
NAG (nmol/h/g)	0.83	0.92	1.13	0.78	0.71	0.84	1.35	0.43
Peroxidase (μmol/h/g)	1.05	1.18	1.15	1.07	1.13	1.94	0.67	0.63
Phenol oxidase (μmol/h/g)	1.13	1.22	1.25	1.02	1.23	1.59	0.67	1.27
Root (g)	0.20	0.29	0.27	0.28	0.19	0.60	0.10	0.02
Shoot (g)	1.70	1.74	1.79	1.58	1.78	1.71	1.66	1.73
Thatch(g)	0.42	0.39	0.43	0.41	0.37	0.58	0.27	0.33
Organic matter (g)	1.05	1.04	1.03	1.05	1.07	1.03	1.03	1.07
LAP (nmol/h/g)	11.22	14.35	16.01	9.40	13.23	26.44	1.41	9.23
Ammonium (μg/g-dry soil)	1.98	2.21	2.19	1.99	2.15	3.02	2.01	1.31

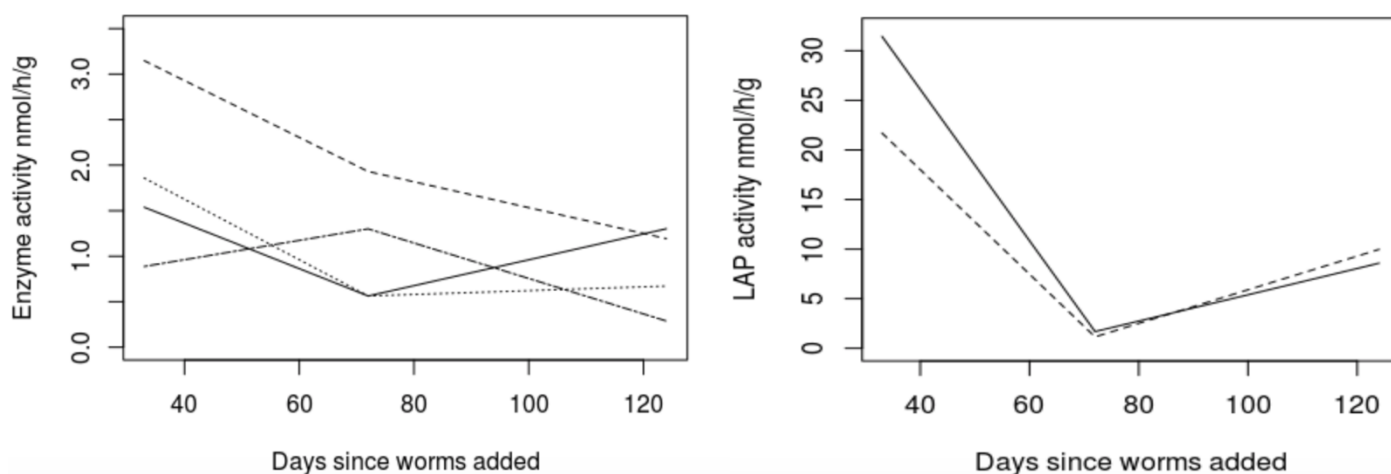


Figure 1. A) Enzyme activity of NAG (nmol/h/g) (dot dashes), peroxidase (μmol/h/g) (dots), phenol oxidase (μmol/h/g) (solid), and ammonium concentrations (μg/g-dry soil) (dashes) over time. B) LAP activity (nmol/h/g) in control group (solid) and experimental (dashes) over time.

Field Study

For the field study I analyzed 28 soil samples from the Coastal Maine Botanical Garden. Six samples were low worm presence in mulch beds, 6 were low worm presence in turfgrass, 8 were greater worm presence in mulch beds, and 8 were greater worm presence in turfgrass. Each of these samples were tested for NAG, LAP, peroxidase, phenol oxidase, and for ammonium and nitrate concentrations. We quantified the effect of worm presence, ground cover, and time on each of these metrics. Exploratory statistics can be found in the supplementary data.

In the field study experiments, I found that sites with higher worm presence had an increased mean value of NAG and LAP activity ($t=3.03$, $n=14$, $p=0.01$; $t=2.10$, $n=14$, $p=0.05$) (Figure 2). There was no difference in means of ammonium concentration or organic matter between sites with high and low worm presence ($t=-0.39$, $n=14$, $p=0.70$; $t=0.86$, $n=14$, $p=0.41$) (Figure 2). However, mean ammonium concentrations were lower in mulch sites than in turf sites ($t=3.64$, $n=14$, $p=0.001$) (Figure 3). Additionally, mean organic matter biomass was higher in mulch sites than in turf sites ($t=-2.14$, $n=14$, $p=0.04$) (Figure 3). Furthermore, I found no interaction effect between worm and ground cover on any of the properties that we tested. Finally, although I tested for nitrate content, I did not include the results because of experimental error (mismatch between standard range and sample range). Generally, nitrate concentration ranges from 0 to 111.61 $\mu\text{g/g-dry soil}$ with a mean of 6.58 $\mu\text{g/g-dry soil}$.

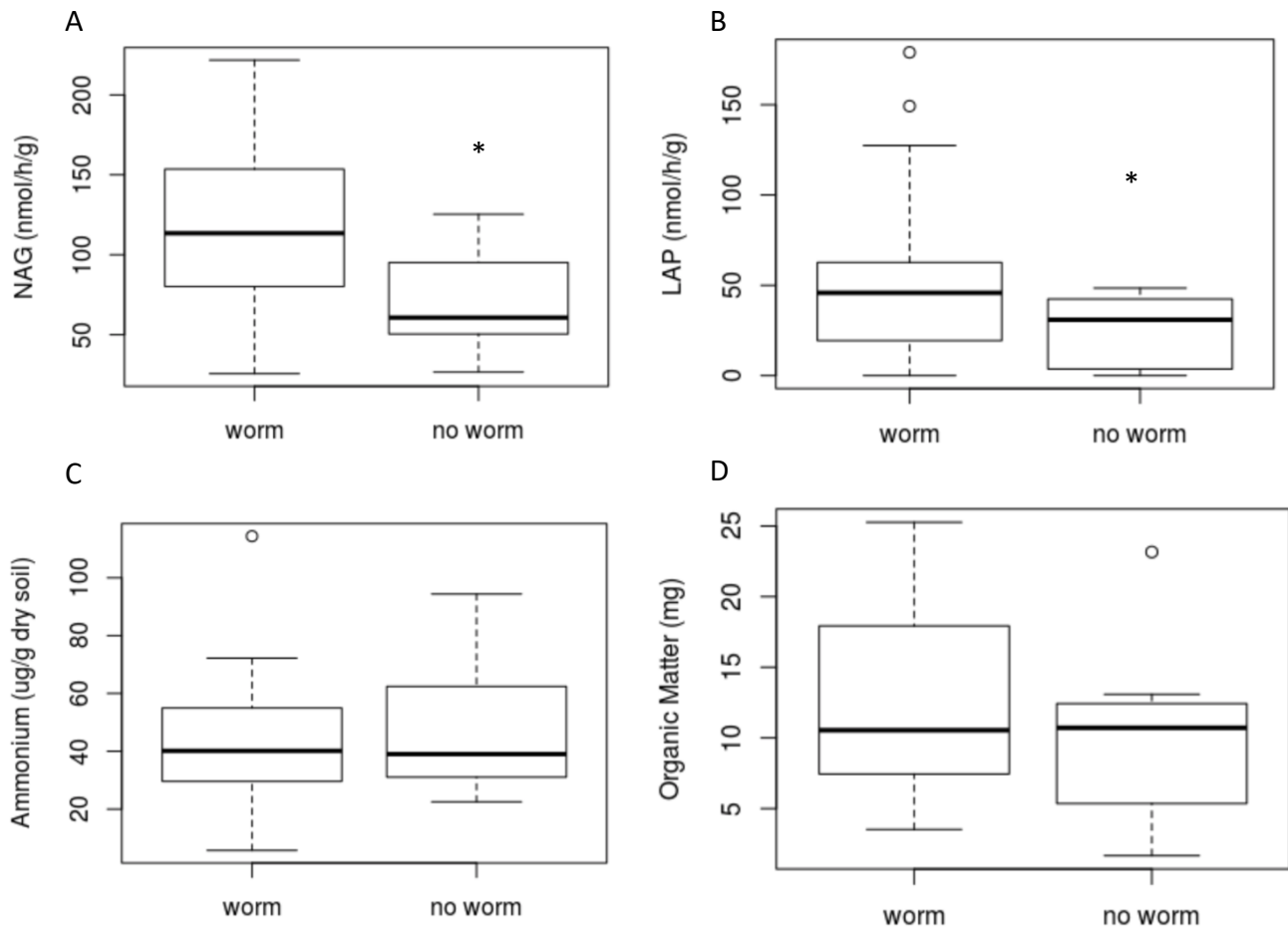


Figure 2. Effect of worm on A) NAG activity ($p=0.006$); B) LAP activity ($p=0.046$); C) Ammonium concentrations ($p=0.704$); D) Organic matter content ($p=.402$) in the field study. Significance denoted by *

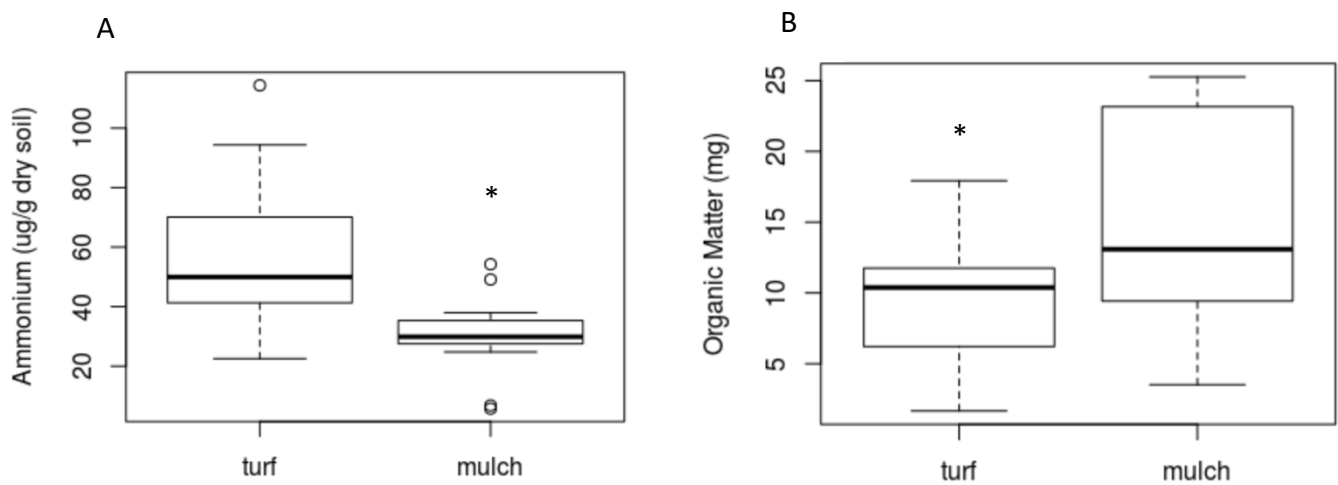


Figure 3. Effect of amendment on A) Ammonium concentration ($\mu\text{g/g dry soil}$) ($p=0.001$) and B) Organic matter (mg) ($p=0.044$)

Discussion

The mesocosm and field studies exhibited conflicting and inconclusive results. The mesocosm study showed no significant effect of worm presence on any biological or physical properties of turfgrass, but the worms did decrease LAP activity in July and August and phenol oxidase and peroxidase activity in August. The field study showed that the worms increased NAG and LAP activity but had no effect on ammonium or organic matter. The observed trends do not support my hypothesis that the worms initially increase nutrient availability and plant health but then subsequently deplete available nutrients leading to decreased plant health. Furthermore, these findings indicate that Asian jumping worms may effect turfgrass ecosystems differently than they effect forest ecosystems. This underscores the need for continued research to discover how Asian jumping worms might alter turfgrass.

The analysis of the biological properties of the mesocosms showed that the worms only changed LAP, peroxidase, and phenol oxidase at specific time points. Although time has an effect on all the biological characteristics this significance only indicates that enzyme activity changes throughout the season which is expected as the microbial community responds to environmental changes. LAP is a peptidase that cleaves the terminal nitrogen peptide bonds in proteins. It is generally associated with nitrogen acquisition (Jian et al. 2016). It is widely hypothesized that microbes only excrete their extracellular enzymes when there are available substrates to break down because extra cellular enzymes are energetically expensive to produce. Therefore, the decrease of LAP activity seen in the presence of worms shows an unexpected trend. Since worms breakdown large particles of organic matter into more decomposable inorganic compounds, we expect that the worms would increase nitrogen acquisition and thereby LAP activity. Should the decrease in LAP activity indicate a decrease in nitrogen availability we

would expect to see a significant decrease in ammonium concentrations. However, this change was not observed. This discrepancy may suggest there may be more dynamics at play considering the complex relationship between LAP and nitrogen availability.

Both peroxidase and phenol oxidase are oxidases that breakdown recalcitrant nutrients locked up in more difficult material to decompose. Phenol oxidase and peroxidase are associated with nutrient-poor conditions, where all easily digestible compounds have been immobilized. Therefore, nutrients left are occupied in lignin and tannins and take more energy to breakdown less nutritious substrates. The lower levels of peroxidase and phenol oxidase indicate that the worm may be increasing digestible nutrient availability. This trend is especially evident in August. Many of the biological and physical metrics measured showed either dips or peaks during the August destructive harvest. This may be due to weather conditions being hot and dry. Despite the experiment taking place in the greenhouse, the mesocosms were still affected by fluctuations in the outside environment, especially humidity. Furthermore, the mesocosms were poorly drained, did not dry out between watering, and fungal growth was observed on the surface of mesocosm soils. The impacts of these non-ideal conditions are unknown. Since the greatest difference between the control and experimental groups for peroxidase and phenol oxidase was in August, it is possible that in the short term the worms can continue to increase nutrient availability in stressful environmental conditions that lead to nutrient scarcity. Potentially, in times of drought where the control group runs low on nutrient availability the worms continue to mineralize macronutrients that the enzyme community cannot, thereby keeping nutrient availability high. Additional, more targeted studies would be needed to further support this claim.

Although the microbial enzymes did not initially increase then decrease in the way I hypothesized, they did follow expected trends with respect to one another. NAG and the oxidases had opposite patterns of activity. This is logical given that NAG decomposes easily available compounds while the oxidases target more recalcitrant nutrients. Additionally, ammonium concentrations generally decreased with NAG activity. This is expected as well because NAG activity is associated with increased mineralization rates, so when NAG activity decreases mineralization rates between inorganic N and ammonium should decrease, decreasing the overall ammonium concentrations. Analyzing these trends allows us to gain a greater ecological understanding of how the Asian jumping worms change their microenvironment over time, and how these changes can manifest as physical changes to the turfgrass ecosystem.

Finally, there was no effect of amendment on enzyme activity. This indicates that the worms do not exhibit a preference for hardwood or compost over no amendment. Additionally, no amendment is more conducive to worm activity than any other. The implications of this finding are that Asian jumping worms can live in a variety of environments and use various types of amendment as vectors for invasion, especially in home gardens, parks, and other mulched landscapes.

Similarly to biological characteristics, the mesocosm study showed there was no effect of worm on any of the turfgrass's physical properties. Neither root, shoot, thatch (layer of organic matter between shoot and soils), nor organic matter biomass significantly changed in the presence of worms. Given that none of the metrics produced a significant difference, I can conclude that within the time frame of our experiment the worms do not change the physical characteristics of the turfgrass ecosystem. The worms may be slightly altering turfgrass, but generally these alterations are not large enough to manifest themselves as visible physical

changes. This indicates either that our mesocosm study was not long enough to capture such effects or that the damage to lawns done by worms as reported by homeowners is being caused by synergistic interactions that were not represented in our mesocosms. For example, perhaps soil type, density of worms, or environmental factors could lead to exaggerated changes in turfgrass quality. Finally, the worms could be changing turfgrass quality through changing aggregate stability by replacing soils with castings or the facilitation of invasive species by seed dispersal.

Through the mesocosm study I was able to look at the effect of Asian jumping worms on turfgrass over a five month time frame. Through the field study I could determine the potential effects of a successfully established Asian jumping worm population in an uncontrolled turfgrass ecosystem. The field study allowed me to investigate how the worm interacted in a more natural environment and observe the long term effects of invasion not detectable within my 124 day mesocosm experiment. The field study showed that worms increased NAG and LAP activity. NAG and LAP are positively correlated with nitrogen mineralization rates. However, similarly to the mesocosm study, the observed changes in ammonium concentrations do not support the claim that the worms increased mineralization rates. Additionally, I used a lower standard in the nitrate assay than for the ammonium assay, meaning that in general ammonium concentrations were higher than nitrate concentrations. Five potential explanations exist for these conflicting trends. First, the nitrogen released through mineralization is immediately immobilized by another class of microbes. This means that there is a general shortage of nitrogen, so rather than releasing nitrogen for plant use, the nitrogen is kept within the microbial community. Secondly, the enzymes could be mineralizing the nitrogen at a fast rate because the worms are producing an excess of decomposed organic material. As a result, nitrification is also occurring quickly, but

since nitrate is easily lost we are seeing low nitrate values due to leaching. Although possible, this explanation remains unlikely since it would be unexpected to observe different leaching rates between the two treatments. Next, it is possible that the different soil types had chemical differences that could account for differentiation in cation exchange altering observed ammonium values. Additionally, it is important to note that given the trends with a greater sample size the ammonium concentration values could become increasingly significant. Finally, there may be other factors or interactions that are changing nutrient dynamics such as fertilizer inputs. An increase in fertilizers could impact the turfgrass similarly to worms making it difficult to differentiate between the effects of one versus another.

In an attempt to control for effects of fertilization along and attempt to map the potential routes of invasion, I compared high and low worm activity in both turfgrass and neighboring mulch patches. I found that ammonium concentrations were higher in turf patches than in mulch. Mulch is generally not fertilized, so this shows that there was most likely some effect of fertilization on the turf samples. Ground cover also had an effect on organic matter biomass. Organic matter was significantly higher ($p=0.045$) in mulch patches than in turf. This result is expected because the mulched beds at the botanical garden have been layered with mulch for many years meaning that most of the soil at this point is organic matter. Since other enzymes were not affected by ground cover we can conclude that worms can flow freely between mulch and turfgrass patches. This means if worms are introduced through mulch amendments they are not confined to the area which they are introduced and can subsequently invade neighboring landscapes. This could potentially have more adverse implications for turfgrass environments that border forests.

I hypothesized that the worms would initially benefit plant growth by increasing decomposition of organic matter creating hotspots for microbes which would increase mineralization rates. However, by looking at trends of biological and physical properties it does not appear the worms effect the turfgrass ecosystem in this way. There appear to be multiple interactions occurring in the ecosystem that were not accounted for in the metrics that I chose to analyze at the time scale of the experiment. I was able to confirm that worms can move freely between mulched patches and adjacent turfgrass. This was not only evident through the observed changes in the mulch versus turfgrass but also the abundance of castings showing that the worms were consuming material in both environments. Additionally, I observed various trends for how the worms can impact biological characteristics of turfgrass. The trend of greatest concern is the increase of NAG and LAP in the areas of established worm populations. A short-term increase of these enzymes may benefit ecosystem function, but too much activity can lead to excessive nitrification and leaching. However, due to the limited strength of the statistical tests due to my small sample sizes it appears as if the overall effect of worms on biological characteristics is neutral. Similarly, effect of the worm on physical characteristics appears to be generally neutral. The changes in nutrient cycling were not great enough to have a substantial impact on turfgrass health. There appears to be potential for the worms to benefit plant health during times of environmental stress. However, this relationship would take further experimentation to determine.

Since Asian jumping worms have never been studied in the context of turfgrass, my hypothesis was formulated based on the effects Asian jumping worms have on forest ecosystems. Previous studies have shown that in temperate deciduous forests with Asian jumping worms there is increased soil carbon and nitrogen (Greiner et al. 2012 and Burtlelow et al. 1998).

Furthermore, multiple studies have found that the worms can alter the microbial community. Chang et al. (2017) found that over the course of two years, forest plots with Asian jumping worms increased in microbial biomass compared to plots without the worms. Additionally, Burtelow et al. (1998) found plots with an arboretum with the worms had higher carbon and nitrogen microbial biomasses. They also found an increase in carbon mineralization rates and an increase in denitrification potential (Burtelow et al. 1998). This finding suggests that the worms may be associated with an acceleration of the nitrogen cycle. This suggestion was additionally supported by their finding that in forest areas with worms there was an increase in nitrate concentrations, but a decrease in ammonium concentrations. The differences between my findings and those of previous studies may conclude that Asian jumping worms have different effects on turfgrass and forests that cause differencing degrees of damage in each ecosystem.

The only other study that has previously looked at the effect of Megascolecidae species on grassy ecosystems was a study done by Qui and Turner (2017) looking the effects of *A. agrestis* and *A. tokioensis* invasion on temperate forests and prairie soil in the Midwest. Their experiment included both a mesocosm and field component similar to my experimental design. Qui and Turner (2017) found that areas occupied by worms had lower total percentage of carbon, carbon to nitrogen ratio, and total nitrogen in the soil, and that organic matter increased 16%. This finding is consistent with results from the field study where areas occupied by worms had 19% more organic matter. Qui and Turner (2017) also reported a significant decrease in nitrate availability, and a slight decrease in ammonium concentration, but no difference in availability of inorganic nitrogen in areas occupied by worms compared to control areas. These findings are consistent with results for both the mesocosm and field studies. In the mesocosm, ammonium concentrations were 7.13% lower in mesocosm samples with worms, and 8.23% lower in the

field study. Finally, when comparing their forest results to the prairie results, Qui and Turner (2017) concluded that the worm invasion has a greater impact on forest ecosystems than on prairie ecosystems.

Qui and Turner (2017) attributed the differences between worm effects on forest and prairie differences to the inherent environmental difference in leaf litter and soil moisture. Forests ecosystems have more leaf litter than grassy ecosystems, and leaf litter is preferential food for the worms. Leaf litter is also a nutrient input that can have significant impacts on nutrient availability in the soil. Leaf litter also helps to retain soil moisture which may facilitate microbial enzyme activity. Overall, the authors justified these mixed results by concluding, “given high densities of *Amythas*, the incorporation of litter nutrients into soils exceeds earthworm induced nutrient losses, leading to a transient enrichment of nutrient pools in top soils” (Qui and Turner 2017). Ultimately, they claim that the worms are altering their environment but in a way that is concealed in short term investigations.

The discrepancies between observed and expected values in the current study may be explained by flaws in experimental design. My mesocosm experiment had much lower enzyme values relative to field soils. This may have been due to a decision to use sandy and low nutrient divot mix as the soils. The divot mix did not retain water well, creating a low moisture environment that may have discouraged extracellular enzyme activity. This issue was likely exacerbated by the dry and hot weather over the growing season. Many of the turfgrass boxes dried out before the end of the experiment. As a result, I had to eliminate greenness of the turf as a metric because so many of the mesocosms turned brown. In the field experiment, some of my plots had drastically different intensities of grooming. In the most intensively managed plots the turfgrass is removed and replaced with new sod every two years and the mulch is constantly

being replenished. In the least managed plots, the turfgrass was grown from a seeded mixture and mulch was added a couple times a year. Since the worms have invaded the mulch supply at the botanical gardens, higher worm activity is correlated with more frequently mulched areas and therefore more heavily managed and groomed turfgrass. This bias is difficult to control for and my sample size may not have been large enough to account for the differential management practices between the worm and control samples.

The difficulty in isolating effect of Asian jumping worms on their environment and the lack of conclusive literature regarding the topic emphasize the need for further research on Megascolecids and their pattern of invasion. Once patterns and implications of invasion are better understood we can start to develop strategies to mitigate any damage caused by the worm. Turf is one of the most abundant crops in America and is extremely important for health of urban ecosystems. Turfgrass supplies oxygen reduces erosion, runoff, glare, noise pollution, filters dust, and moderates surface temperature (Potter and Braman 1991). Should the Asian jumping worm become a major turfgrass pest throughout the US, an entire \$25 billion industry could be at risk. Understanding the specific effects of the worm on turfgrass is important not only because of the relevance of turfgrass in urban ecosystems but because potential management strategies would be very different from those used in forest ecosystems. To many turfgrass consumers the aesthetic value of the turf is more important than the ecological function. Therefore, if the worm is capable of accelerating nutrient leaching, such effects can be compromised by adding more nutrient inputs such as fertilizers. However, the use of fertilizers is not a sustainable long-term solution as the excessive nitrogen can affect drinking water supplies especially in urban environments. Additionally, studies have shown that Asian jumping worms have a greater detrimental effect on forest ecosystems than on grasses. Here we confirm that worms can use

turfgrass ecosystems as suitable habitats (Qui and Turner 2017). This renders the forests that abut turfgrass plots vulnerable to invasion. Overall, invasive species cause billions of dollars' worth of damage, and compromise the environment's ability to provide ecosystem services. It is crucial to recognize the effect of Asian jumping worms early in their invasion to prevent further spread and conserve the valuable ecosystems they invade.

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Supplemental data

Mesocosm

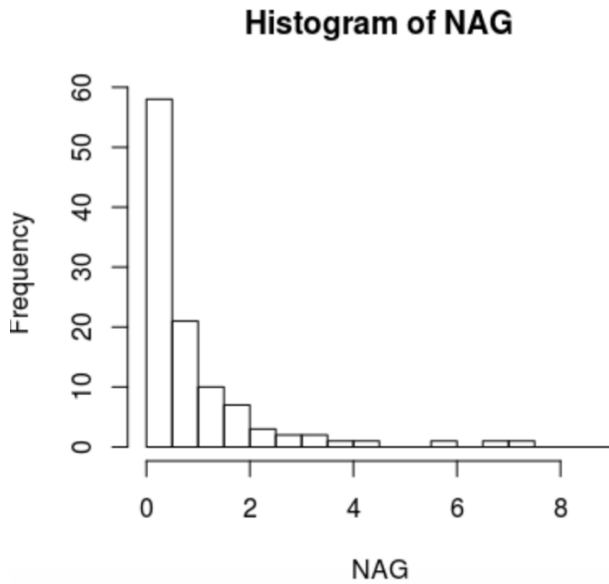


Figure 4. Histogram of NAG activity values (nmol/h/g) for the mesocosm study

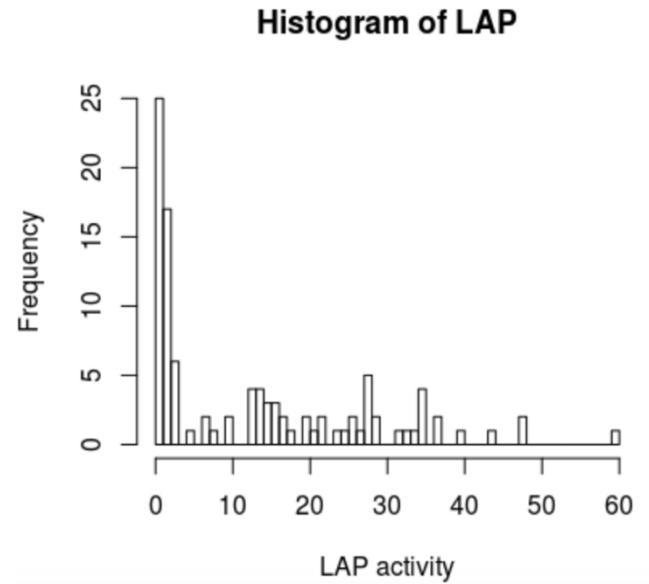


Figure 5. Histogram of LAP activity values (nmol/h/g) for the mesocosm study

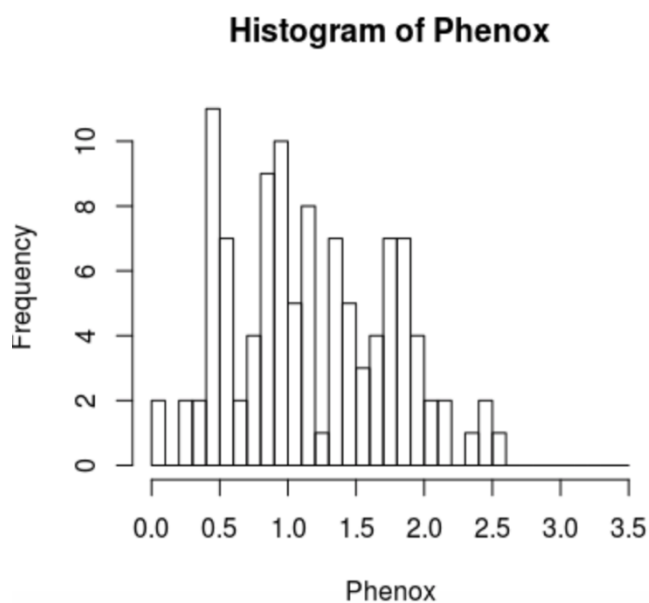


Figure 6. Histogram of phenol oxidase activity values ($\mu\text{mol/h/g}$) for the mesocosm study

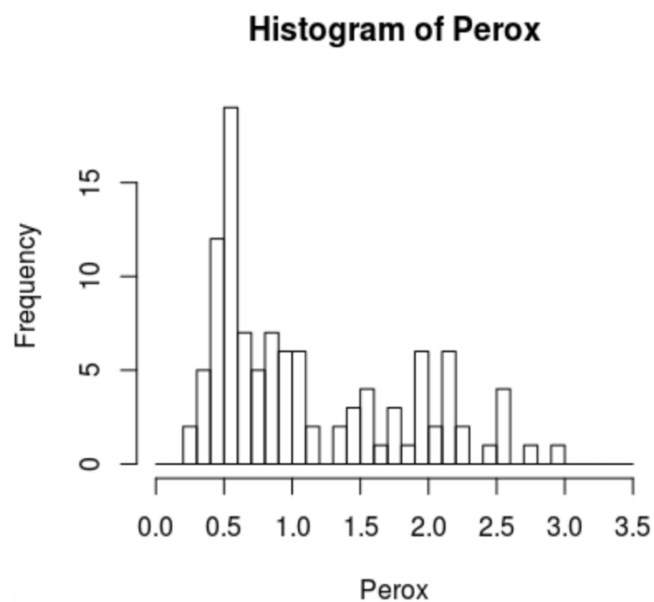


Figure 7. Histogram of peroxidase activity values ($\mu\text{mol/h/g}$) for the mesocosm study

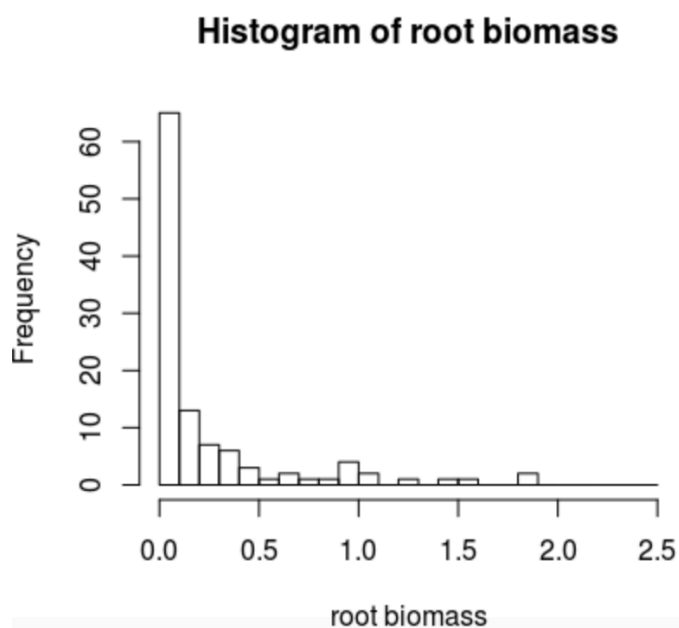


Figure 7. Histogram of root biomass values (g) for the mesocosm study

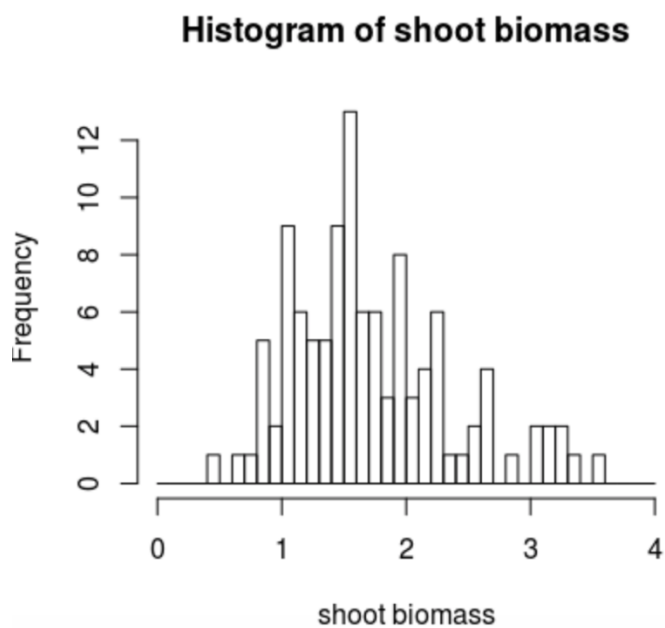


Figure 8. Histogram of shoot biomass values (g) for the mesocosm study

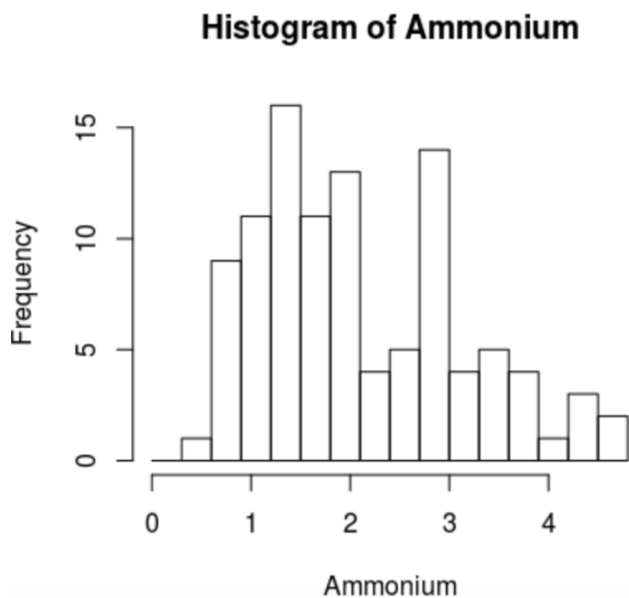


Figure 11. Histogram of ammonium concentration values ($\mu\text{mol/g}$) for the mesocosm study

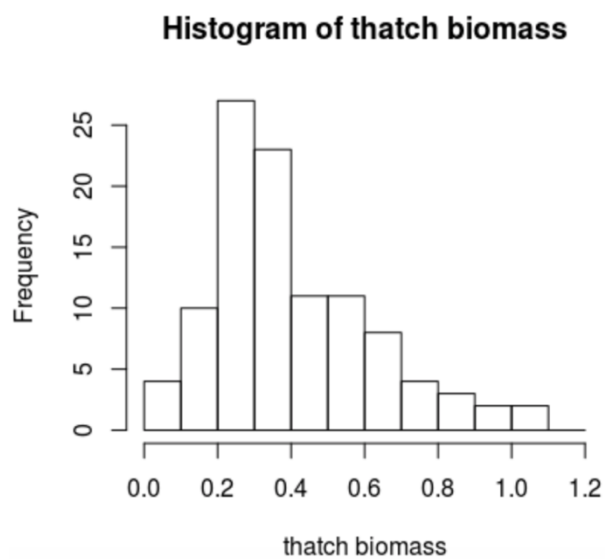


Figure 9. Histogram of thatch biomass values (g) for the mesocosm study

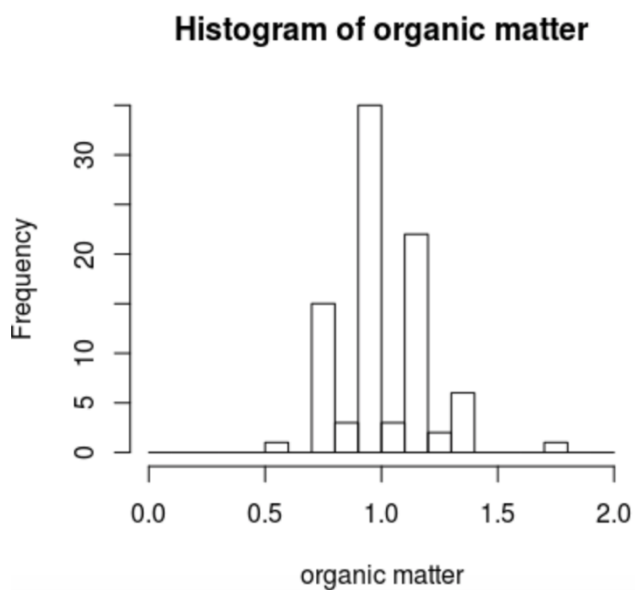


Figure 10. Histogram of root biomass values (g) for the mesocosm study

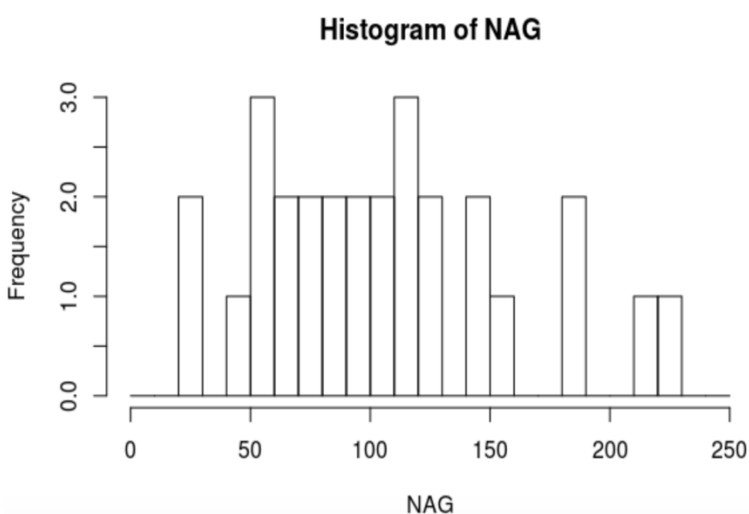


Figure 12. Histogram of NAG activity values (nmol/h/g) for the field study

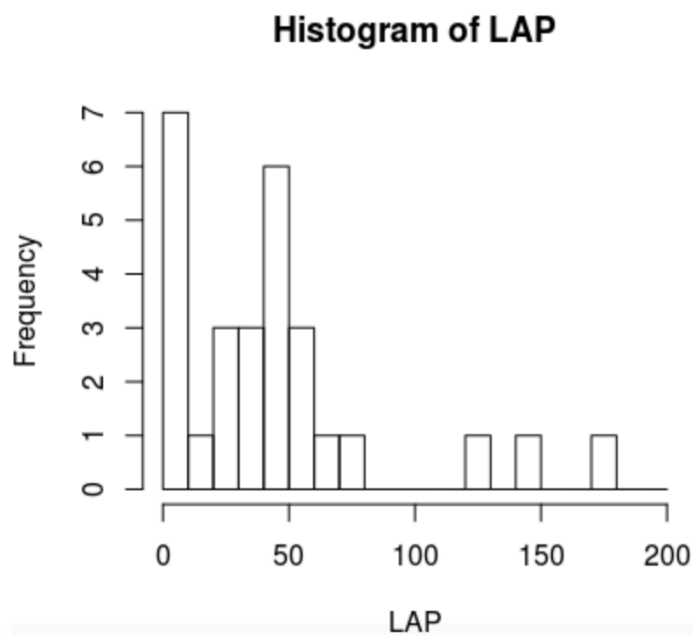


Figure 13. Histogram of LAP activity values (nmol/h/g) for the field study

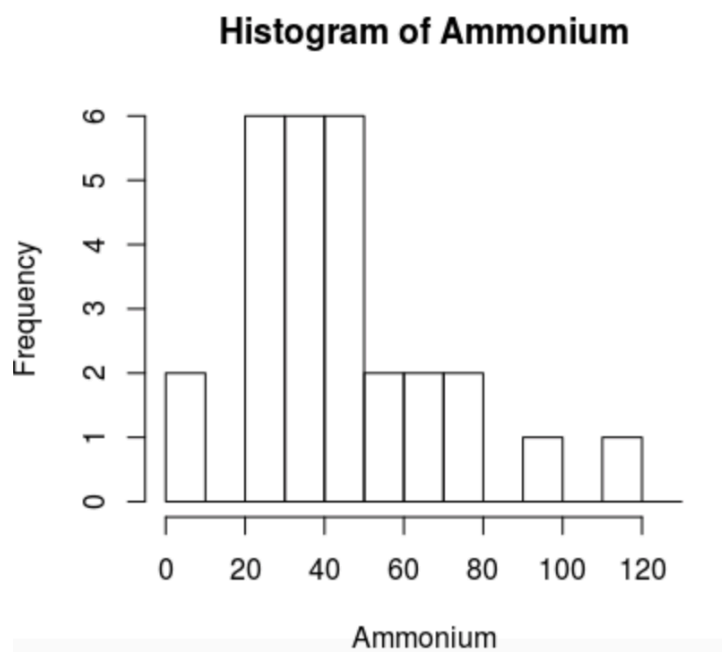


Figure 14. Histogram of ammonium concentration values ($\mu\text{mol/g}$) for the field study

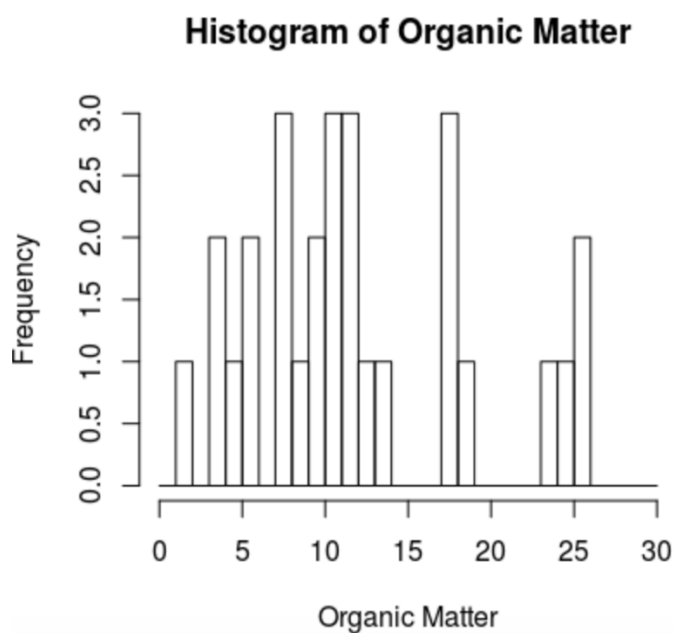


Figure 15. Histogram of organic matter values (mg) for the field study