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Soil science is an essential part of ecosystem and agriculture health but is unfortunately not a priority in most non-science students' education. To counter this, the goal of this study was to determine the best protocol for a general education science lab that would use soil microcosms to explore nutrient cycling from leaf litter decomposition. The experimental procedure was designed to test different combinations of soil organism type, organism density, and leaf density in simple Tupperware microcosms. The best combination was defined as a combination that met the following traits: significant decrease in leaf litter mass and high nutrient flow from leaf litter to the soil. Statistical testing revealed that high densities (10-11 individuals) of pillbugs (*Armadillidium*) combined with medium (2 g) or low (1 g) leaf litter densities produced the largest change in leaf litter mass and nutrient flow into the soil. The lab protocol can be used in any general science course to teach non-science students the importance of soil health, and the relationship between soil, nutrients, and soil fauna.

Introduction

Nearly every terrestrial ecosystem requires soil as a basis of primary productivity. Soils allow for the plant growth that makes up the "critical base of food chains in nearly all ecosystems" (MSU, 2017). Plants are necessary to provide food, nutrients, and shelter for other organisms in the ecosystems. Without healthy soils, ecosystems would not be able to exist, as soils hold nutrients and water that plants need to grow, as well as providing habitats for various animals (IDNR, 2017). Soils are also necessary for retaining water and moisture for the plants (USFS, 2016). Healthy soil is also a necessity for successful agriculture. Crops need soil in order to grow, and soil contains billions of microorganisms, such as bacteria, that decompose dead organic matter. Decomposition in turn releases nutrients that are essential for plant growth (Wallace, 2001).

Soil is primarily composed of various minerals, but also consists of water, organic matter, gases, and microorganisms (DeGomez, et al, 2015). The texture of soil depends on the amount of clay, sand, and/or silt the soil contains. Sand particles are the largest, while clay particles are the smallest: sandy soils are looser and do not retain water, while clay soils are very tightly-packed (DeGomez, et al, 2015). The texture of a plot of soil will depend on its location and the ecosystem it is a part of. Figure 1 below is an image created by U.S. Department of Agriculture's Natural Resources Conservation Service that demonstrates various soil textures. Notice that it is possible to determine soil type based on the different proportions of the three soil particle types; this is accomplished by following the crisscrossing lines in the center of the pyramid. For example, if a soil has 50% sand, 30% clay, and 20% silt, the soil type would be sandy clay loam, because that is the spot on the pyramid where the lines from all three particle types cross. The key to productive soil is a balance of the soil particle sizes known as loam.

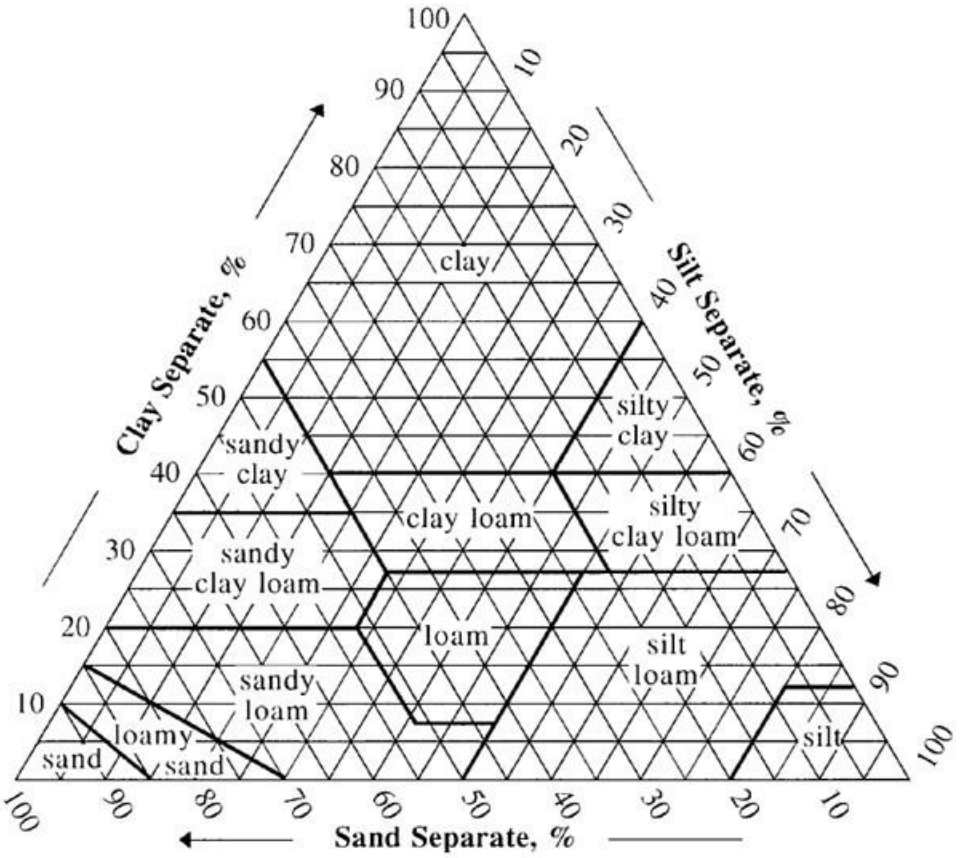

Figure 1. Soil texture pyramid produced by the U.S. Department of Agriculture's Natural Resources Conservation Service (USDA, 1979).

Soil porosity is another character of the soil and is based on pore space. Pore space is simply the amount of soil volume that is not made up of solid material: these are the gaps in the soil particles (Nimmo, 2013). Pores are necessary to allow movement and storage of water, air, and chemicals, and are also the habitats of many soil fauna. Soil porosity depends on the types of soil particles present and how tightly compacted the soil is (Naghdi et al. 2018). Generally, too few pores prevent water from permeating the soil, while too many pores prevent the soil from retaining the moisture. Soils with enough porosity to hold water but still maintain air flow are the healthiest (Ball, 2001).

Soils are an important stock of the nitrogen and phosphorus: the nutrients are stored in the soil, which are then taken up by plants, which are taken up by animals that consume the

plants (Withgott and Laposata, 2014). Such nutrients are essential for the survival and growth of plants, animals, and especially humans. Nitrogen is a necessary component of proteins, amino acids, amino sugars, and other compounds (Allison, 1957). Phosphorus, on the other hand, is used to transfer energy between cells of living organisms and helps stimulate plant growth (Pagliari et al. 2017). Nitrate is a negatively-charged ionic form of nitrogen and is more soluble and available to plants (Lamb et al. 2014). Phosphate is also an ionic form of phosphorus and is water-soluble and generally available to plant matter in the soil as well (Pagliari et al. 2017). Nitrate and phosphate are much easier to test for in soil and indicate the amount of available nitrogen and phosphorus respectively in each sample.

Soil organisms, also referred to as soil fauna, are an important component of the soil and are the reason that soil can be so productive and healthy. Microorganisms help break down debris such as leaf litter and transfer nutrients back into the soil (The Environmental Literacy Council, 2015). Leaf litter refers to the fallen, decomposing leaves that land on soil ecosystems. Said nutrients that are released from the process of decomposition are absolutely necessary for proper plant growth (The Environmental Literacy Council, 2015; U.S. Forest Service, 2016). Larger organisms such as mites, arthropods, and insects, consume, mix, and transport soil materials. Soil fauna burrow through the soil and assist with aerating the soil to further assist decomposition, and any tunnels left behind help water penetrate into the soil to become stored and later absorbed by plants (Janet, 2001). Two important soil organisms are earthworms (Phylum Annelid) and pillbugs (Family Armadillidiidae). Both are considered to be soil macrofauna. Macrofauna are the largest forms of soil organisms.

Earthworms are well-known soil organisms that provide benefits to the physical, biological, and chemical aspects of soil. Earthworms increase plant growth as well, by

fragmenting and breaking down detritus into usable nutrients (Atiyeh et al, 2002). They are semi-aquatic, meaning they need a sufficiently moist environment to survive. They are also nocturnal and have a harder time thriving in colder temperatures (Darwin, 1881). They enrich topsoil and improve the permeability and aeration of the soil by burrowing through the soil, increasing pore space, ingesting soil, and plant debris, and excrete nutrient-rich casts (Werner and Bugg, 1990). Studies found that the presence of earthworms increase the amount of nitrogen found in the soil and show that earthworms assist in nutrient flow in the soil (Bugg, 1994, Römbke et al 2005).

Pillbugs are terrestrial crustaceans (Order Isopoda) that prefer habitats that are relatively moist, without being too damp. They are typically found under fallen leaves and rotting wood. Pillbugs have the ability to roll up into a ball for protection and are unique among terrestrial isopods due to this characteristic (Smigel and Gibbs, 2008). They prefer to live in large-particle soil, as opposed to finer soils, as the former provides a habitat for easier burrowing and more water retention (Holland, 2014). While they are usually detritivores, breaking down decomposing organic matter and returning nutrients to the soil, they have been shown to be herbivores as well as carnivores; they are adaptable organisms that can feed upon whatever food source is available (Holland, 2014, Rushton and Hassall, 1983).

Soil Education

Despite the importance of soils to ecosystem and agricultural health, very little education is dedicated to teaching students about this topic, especially at more advanced levels. Soil ignorance is common in American society. Although an interest in the soil is common for young children, most formal soil education ends in childhood and is not pursued in adulthood. Any soil education that is taught usually presents soil as simply part of a larger system (Harrison, 2012). In addition, education, specifically in secondary schools and at the high school level, about soils

usually does not place any emphasis on soil quality and how organic matter affects soil health (Zuazagoitia and Villarroel, 2016). Many standardized soil lesson plans do not delve deep enough into soil information. Thus, there has been a call for more scientific education on soils for all ages, especially education funded by various American governmental departments (Glasener, 2013).

It is this lack of proper soil education, especially in a college setting, that leads to this Honors Research project. The question that this project strives to answer is "Is there a feasible way to incorporate a soil lab into a general education science class?" In order to answer this question, an experiment had to be run to determine the protocol for the lab in question. The project will be used to create a soil lab to be implemented into the Earth Systems Science course at Illinois Wesleyan University (IWU).

Methods

Experimental design

The overall goal of this experiment is to test feasibility of such a lab protocol. The pedagogical goal of the lab would be to demonstrate nutrient flow from leaf litter into the soil, and how the presence of soil macrofauna influences such flow. The point of this research, then, is to determine if such nutrient flow can actually be observed. Thus, the goal of the experimental design is to determine the best treatment that will be used in a class setting to demonstrate nutrient flow. The two macrofauna used in this experiment are earthworms (Genus *Lumbricus*) and pillbugs (Genus *Armadillidium*), since these are two essential soil organisms that are easy and inexpensive to acquire, and also fairly easy to work with and study.

Different organism densities and leaf litter densities were tested in this experiment. Hättenschwiler & Gasser (2005) determined that organism type and organism density influenced litter decomposition rates of leaf litter. Various combinations of soil fauna and leaf litter densities had to be tested in our experiment in order to determine which treatment best demonstrates nutrient flow. Personal experience has demonstrated the importance of leaf litter density in small microcosms. During a previous freshwater microcosm project in Earth System Science, my lab group added leaves to observe their effects on the ecosystem. The leaves ended up disrupting the ecosystem, taking up all the oxygen in the water as they decomposed, and killed nearly all of the freshwater organisms in each microcosm (Bergquist, unpublished data). Adding too many leaves has proven to be destructive to the microcosm ecosystem, so it was essential to test which leaf litter densities had a positive effect on the microcosm.

The experiment was carried out by utilizing a total of 24 containers that contained different treatments: 9 treatments per each organism, and 6 control samples. There were three different leaf litter treatments that were used: high leaf litter density, medium leaf litter density, and low leaf litter density. There were also three different organism treatments: high organism density, medium organism density, and low organism density. The containers always contained either earthworms or pillbugs; the two species were kept separate. 18 of the containers held leaves and one of the types of organisms, and 6 were used as a control and contained only leaves.

The 18 containers each had a different level of leaf and organism density. 9 of the containers contained pillbugs and the other 9 contained earthworms. The containers had one level of leaf density and one level of organism density. Table 1 demonstrates how the containers were laid out. The purpose of this was to determine what combination of leaf density, organism type, and organism density demonstrated the strongest increase in nutrient flow.

Table 1. A 3x3 table to demonstrate different combinations of leaf and organism densities in the containers.

	High organism density	***Medium organism density***	***Low organism density***
High leaf density	High leaf density, high organism density	High leaf density, medium organism density	High leaf density, low organism density
Medium leaf density	Medium leaf density, high organism density	Medium leaf density, medium organism density	Medium leaf density, low organism density
Low leaf density	Low leaf density, high organism density	Low leaf density, medium organism density	Low leaf density, low organism density

Course Justification

Earth Systems Science is a course offered every spring and taught by Dr. Aaron Wilson. It is a course worth 1.25 credits as it includes a weekly lab and occasional field studies. It is a core course for the Environmental Studies (ES) major at IWU. As it is a 100-level introductory course, it is often taken by non-science majors who need to fulfill a lab credit. In fact, the majority of students taking the course have been non-Environmental Studies (ES) majors (WA Wilson, personal communication). In the past four semesters of the course, the percentage of ES majors within a class ranged from 7.4% to 30.7%, with a mean of 20.4%. Students in the business/finance field, on the other hand, ranged from 19.2% to 60.9% of the course population with a mean of 20.4%. Overall, it has been observed that most students that take the course are not science or ES majors. The goal of this project is to develop a soil lab into a general education science course, and Earth Systems Science is the perfect course for the job. This course provides a lab structure and a soil lab would demonstrate many of the concepts that are taught in lecture, which will be touched on later. There are not many soil-based courses at Wesleyan: this course might be the only opportunity for non-science majors to learn about the importance of soil.

Experimental Procedure

The experimental design was based on an experiment by Hättenschwiler and Gasser, 2005. The authors studied the effects of different levels of soil macrofauna diversity and density on leaf litter from the forest floor. The study in question took place over the span of six months, utilizing soil collected directly from the forest floor. Unfortunately, it would not have be feasible for this honors research to directly replicate the work of Hättenschwiler and Gasser: it would have not been possible to spend six months on the project and collecting soil from forested areas requires special permits. Due to budget and time constraints, this study had to make many changes.

The experiment involved the creation of 24 soil microcosms. A microcosm (in biology) is a miniature ecological community that replicates the natural environment. In this case, the containers filled with soil, leaves, and macrofauna would replicate a normal temperate soil ecosystem. The topsoil used for this experiment was store-bought Timberline soil purchased from Oldcastle Lawn & Gardens, Inc. Various twigs and pebbles were removed and discarded from the bag before the soil was tested for nitrate and phosphate levels with a color disc test kit (Hach). The 24 containers were 24-ounce Tupperware. All of the lids were perforated with approximately 8 air holes each, drilled with a cordless drill. The lids were also all labeled with labeling tape. Each container was filled with soil to the 12-oz mark. This was to approximately standardize the amount of soil per container: at the time the soil was being added, a scale that could handle such weight was not available, so it was not possible to fill the containers with the same mass of soil.

Before the leaves and organisms were added to the containers, the soil was sufficiently and thoroughly wetted, because both organisms require a moist environment and the presence of

water would accelerate the decomposition process. The soil was also mixed to ensure it was thoroughly wetted. Then, all the leaves and organisms were added. All relevant observations of soil texture, quality, organism behavior, etc. were recorded.

The leaves for the experiment were collected from a corner of the quad on the Illinois Wesleyan Campus. The leaves were collected during a warm spell in the mid-fall, so they were dried and had not started decomposing. The leaf density levels were as follows: low (1 gram of leaves), medium (2 grams of leaves), and high (4 grams of leaves). Since there was limited space in the containers, more than 4 grams of leaves would not fit into the containers, while less than 1 gram would barely cover the top of the soil. The leaves that were not used for the experiment were disposed of back on the quad.

The organisms were purchased from Carolina Biological. Due to the number of organisms in each package, the organism densities were not equal between the earthworms and pillbugs. The earthworm density levels were as follows: high (15 earthworms), medium (8 earthworms), and low (4 earthworms). The pillbug levels were as follows: high (10-11 pillbugs), medium (5 pillbugs), and low (2 pillbugs). Pillbugs were more expensive and fewer could be added per container. Leftover earthworms were disposed of in compliance to federal law to prevent the spread of invasive organisms. More earthworms could not be added to the containers due to the limited space and resources in the containers: OECD recommends 100 worms per 1 kilogram, which comes out to 1 worm per 10 grams. While it was not possible to weigh the soil containers (especially since the containers did not all contain the exact same amount of soil), it is safe to approximate that adding too many earthworms to the very small containers would have been damaging to the specimens. There were 6 control containers that only contained leaves: 2 high leaf densities, 2 medium leaf densities, 2 low leaf densities.

The containers were stored in an environmental chamber kept at 20° C (+/- 2 degrees), with an alternating pattern of 12 hours of light and 12 hours of darkness. Earthworms need such a diurnal cycle to function properly: this technique is recommended by the OECD guidelines for testing soil toxicology using earthworms (OECD, 2003). The containers were stored for nine weeks and were watered every week with deionized water. The amount of water each container received was approximated. Water was added to the soil until its mass matched the mass from the previous week. Both earthworms and pillbugs need a moist environment, so weekly watering's were essential.

At the end of the nine weeks, the leaves were removed from the containers and weighed, with the results recorded. The number of living adults and the number of offspring found in each container was recorded. All of the organisms in each container were counted with the same procedure. One container at a time was upturned into a shallow bin. Any soil clumps were broken up, and living organisms were gently pulled from the soil and put into the now-empty Tupperware containers. Once all the organisms were separated from the soil, the adults and juveniles were counted. Unfortunately, since the juveniles of both species were very small, it is possible that some were missed or not properly counted. All organisms were placed in containers that were put in a freezer, because they were not suited to be released into the environment as they could be considered invasive organisms.

Once the leaves and organisms were removed, select soils were tested for nitrate and phosphate. It was not feasible to test every soil sample for nutrients due to time constraints: each test takes several minutes and had to be run one at a time. Thus, samples with the most noticeable change in leaf mass, a few with the least noticeable change in leaf mass, and some control soils were tested.

Statistical analysis

Due to resource constraints, it was not possible to make replications of the treatments. Instead, pseudo-replication was utilized. The treatments will be considered as "paired" when they are combined across the 3x3 setup rows or columns (Table 1). In order to determine if there was a significant difference in leaf mass between treatments, the remaining leaf mass for every sample was subtracted from the initial leaf mass. The differences were then averaged for each organism density level (high pillbug, high earthworm, medium pillbug, medium earthworm, low pillbug, low earthworm) and plotted in a bar graph. The differences were also averaged for different densities of organisms (high leaf densities for the pillbugs, high leaf densities for the earthworms, medium leaf densities for the pillbugs, medium leaf densities for the earthworms, low leaf densities for the pillbugs, and low leaf densities for the earthworms). The differences in leaf mass in the control groups were also calculated and averaged.

A paired t-test was conducted in Excel in order to determine if there was a significant difference in leaf mass between treatment groups. The original average weight of the leaves per treatment group (for example, the average weight of the high leaf density was 4 grams) were compared to the average and standard deviation of the leaf masses after the treatments. This was done for all levels of leaf densities by treatment (pillbugs, earthworms, and control).

The differences in the level of nitrate and phosphate in certain samples were calculated and compared. The replications were too few to compare statistically. A regression was also calculated between pillbug densities, including the control group, and decrease in leaf litter. Due to the low level of replication in this study, and the fact that the goal was to determine if this type of project would be possible in a classroom setting, we are using $\alpha = 0.10$ to determine significance.

Results

Change in Leaf Mass

The only treatments that produced any decrease in leaf mass were the pillbug treatment groups. In the earthworm samples and the control, the leaf mass actually increased, as shown in figures 2 and 3 below. The decreases in leaf mass were negative in the earthworm and control treatments while the differences were positive for the pillbug treatment.

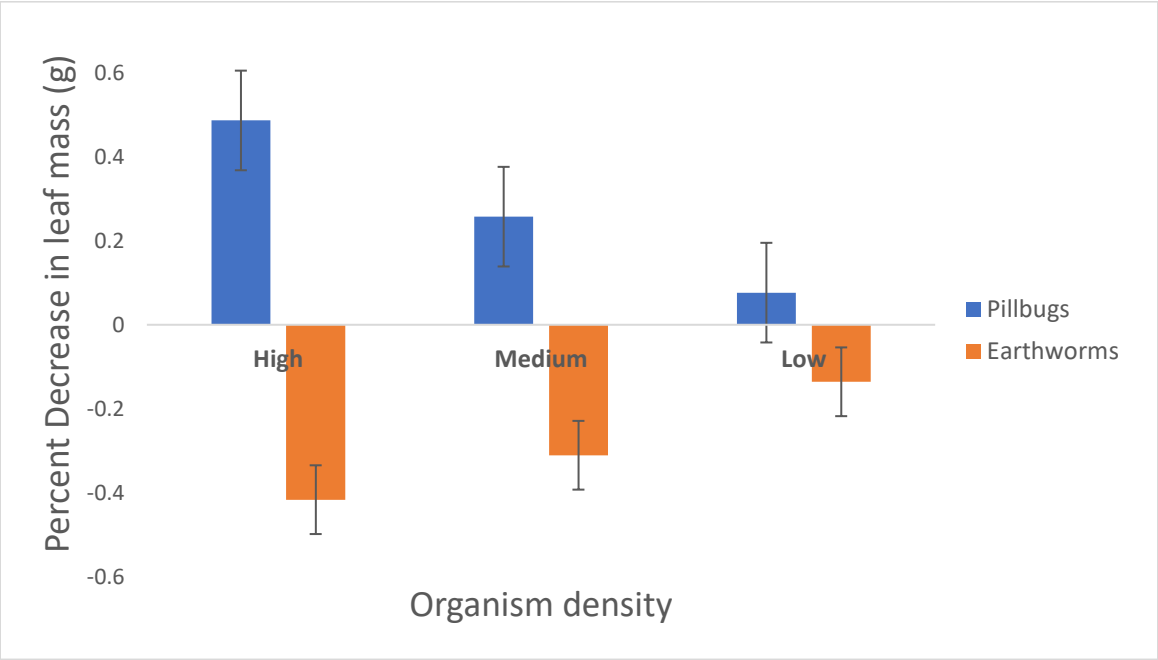

Figure 2. Percent decreases in overall leaf mass (as compared to starting values), averaged by leaf densities, by organism densities in both pillbugs and earthworms. Error bars demonstrate standard error (SE).

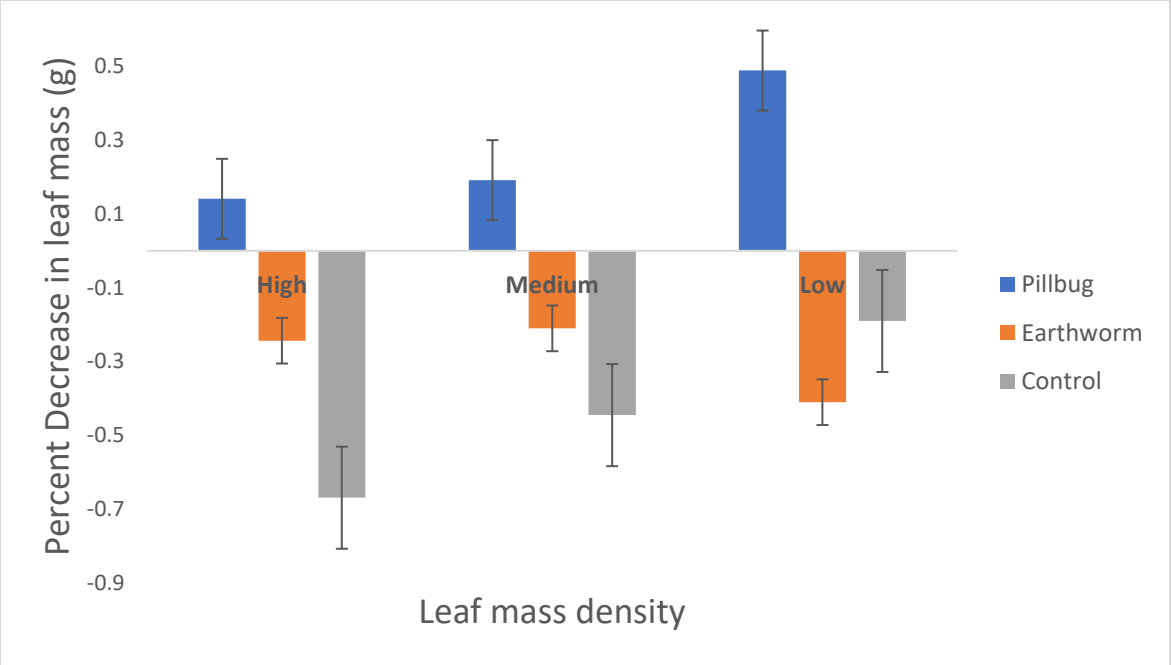

Figure 3. The percent decreases in leaf mass by the leaf mass densities in the pillbugs, earthworms and control treatments. Error bars represent standard error (SE).

There was a significant decrease in leaf mass, compared to starting values, in the medium leaf density pillbug treatment ($p = 0.0641$), the low leaf density pillbug treatment ($p = 0.0859$), the medium leaf density earthworm treatment ($p = 0.045$), the low leaf density earthworm treatment ($p = 0.081$), and the medium leaf density control treatment ($p = 0.03$).

The regression showed a significant relationship between pillbug density and decrease of leaf litter mass. The goal of a regression analysis is to understand which independent variables are related to the dependent variable and their relationship. In this case, there was a highly significant relationship between overall pillbug density and change in leaf litter mass ($p= 0.002$). Figure 4 below shows the relationship between high (10 pillbugs), medium (5 pillbugs), low (2 pillbugs), and control (0 pillbugs) densities of pillbugs. As the number of pillbugs increase, the negative change (decrease) in leaf mass also increases.

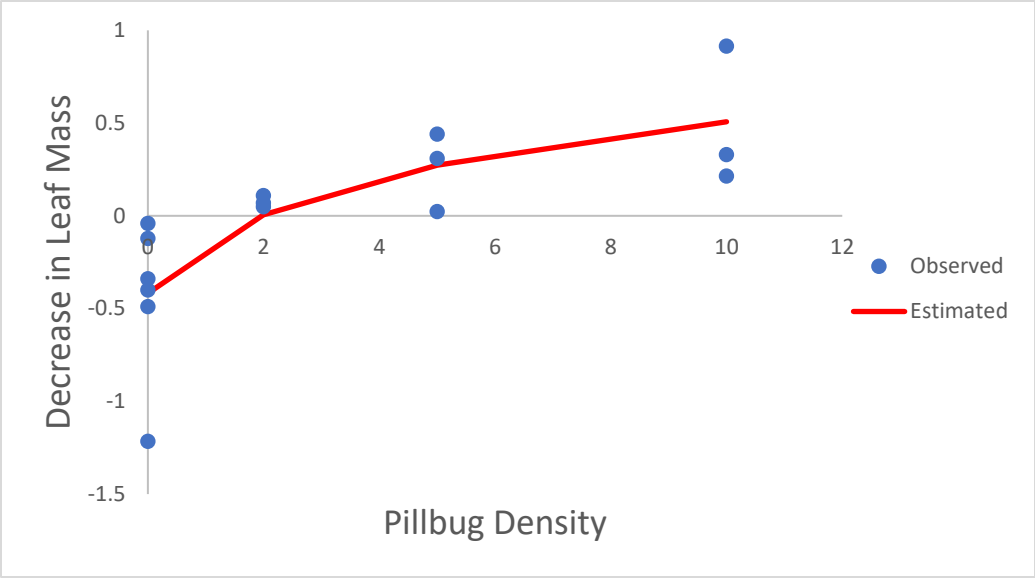

Figure 4. A regression analysis demonstrating the relationship between pillbug density and the decrease in leaf mass density.

Chemical analysis

Nine total soil samples were tested for nitrate and phosphate levels: the soil from the bag before any treatments were made, the high leaf density-high pillbug density, low leaf density-high pillbug density, low leaf density-medium pillbug density, high leaf density-high earthworm density, medium leaf density-medium earthworm density, low leaf density-low earthworm density, the first high leaf density control, and the second low leaf density control. The starting nitrate and phosphate levels for the soil were 2.02 mg/L and 1.5 mg/L, respectively. In every soil sample tested for nitrate and phosphate, there was a decrease in phosphate level. In many samples, there was not a detectable concentration of phosphate. However, in almost every case there was a dramatic increase in nitrate level. The only case in which that was not true was in the one high leaf density control group, which had no change in nitrate level. Overall, the largest increases occurred in the pillbug treatments, while the lowest increase was in the second low-leaf density control treatment. Table 2 below shows the soils tested and the starting and ending nutrient levels.

Table 2. All of the soil treatments tested for nutrients, the starting nutrient levels, and the ending nutrient levels. "BD" refers to nutrient levels "below detection".

Organism type	Organism Density	Leaf Density	Starting (P) mg/L	Ending (P)	Staring (N) mg/L	Ending (N)
Pillbug	High	High	1.5	BD	2.02	28.28
		Low	1.5	BD	2.02	32.32
	Medium	Low	1.5	0.5	2.02	30.30
Earthworm	High	High	1.5	1.0	2.02	26.26
	Medium	Medium	1.5	0.3	2.02	20.20
	Low	Low	1.5	BD	2.02	20.20
Control	N/A	High 1	1.5	BD	2.02	2.02
	N/A	Low 2	1.5	BD	2.02	18.18

Organism Survival and Reproduction

Overall, more pillbugs survived than the earthworms. There were 16 adult earthworms found and 62 juveniles, while there were 29 adult pillbugs and 126 juveniles, compared to the original 81 adult earthworms and 53 adult pillbugs. Despite the fact that more earthworms were initially used in the experiment, a lower percentage survived than did the pillbugs. Figure 5 below shows the percent decrease in number of adult organisms for both species.

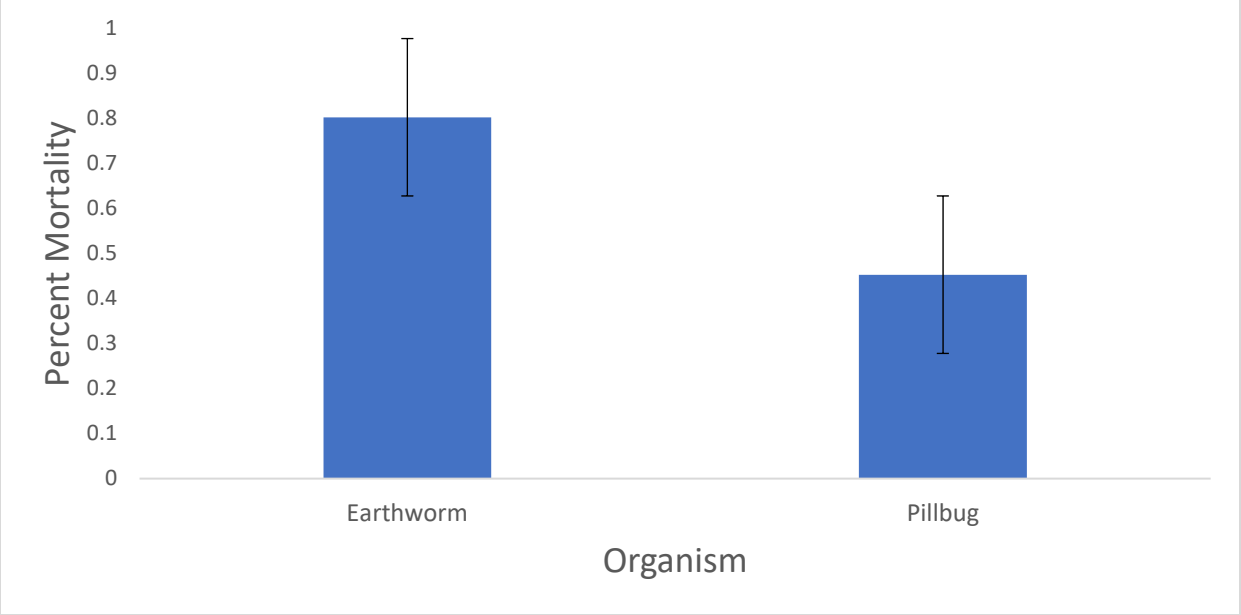

Figure 5. Percent mortality in number of adult organisms for both earthworms and pillbugs. Error bars represent standard error (SE).

Discussion

The leaf mass decreased in the pillbug treatment, likely due to the fact that pillbugs, with their strong mandibles, are able to chew on the solid leaves and break them down efficiently (Schmitz, 1986). Earthworms, on the other hand, do not have such chewing appendages. Instead, they prefer to consume leaves that are already decaying and slowly digest the organic matter in their guts, which, while maximizing the amount of nutrients returned to the soil, is a much slower process (Curry and Schmidt, 2007). Since the leaves were not fully consumed by the earthworms, they likely increased in mass due to the colonization of various microorganism and fungus growing on the leaves. In addition, the leaves in the earthworm treatment microcosms were extremely damp and weighed down with water due to the weekly watering. The leaves had had time to dry out before weighing but were not completely dry. Due to time constraints, it was not possible to leave them to dry fully before weighing. These results contrast with Hättenschwiler and Gasser. In their study, all of the leaf litters that contained earthworms had lower leaf masses when compared to leaf litter that had no soil fauna. This difference is likely

explained by the difference in duration of these experiments: this project lasted 9 weeks, while Hättenschwiler and Gasser studied their organisms for nearly 7 months.

Although the phosphate level in the soil decreased, the nitrate level did increase dramatically, showing that there is nutrient flow occurring when leaf litter and soil fauna are added to the soil. Again, easily visible results are desirable in a classroom experiment.

The fact that more pillbugs than earthworms survived at the end of the experiment is likely due to the fact that, again, pillbugs are hardier and can survive on leaves that have not already been broken down. Pillbugs are also better adapted to cope with a lack of moisture. Smigel and Gibbs (2008) discovered that when pillbugs engaged in conglobation, the rolling-up behavior, water loss significantly decreased. If moisture was running out, the pillbugs would be able to curl up and retain more water. It is also not surprising that more juvenile pillbugs were found: female pillbugs carry eggs under their thoraxes for weeks until they hatch and grow, and females can produce up to 600 juveniles (Holland, 2014). It is entirely possible that the females shipped had mated with a male already and were carrying eggs on their thorax when they were added to the microcosms. Earthworms also reproduce relatively fast, but it is possible that due to higher mortality rates and more difficult habitats, they might have been unable to devote any resources to reproduction.

There might have been an issue with overcrowding in the earthworm treatment, which would explain the dramatic mortality rate. As stated before, the recommended rate of earthworms per gram of soil is 1 worm per 10 grams of soil. The high and medium earthworm densities were definitely overcrowded. Earthworms were much cheaper, and thus more earthworms were used per container. In the high earthworm density treatment, only one living juvenile worm was found at the end of the experiment time. This probably happened because of

a lack space and moisture in the containers: the increased number of earthworms meant more respiration taking place, meaning that the soil dried out, despite the weekly watering. Evers et al. (2010) found that in their treatment groups, earthworm mortality was lowest in low-density treatments (10 earthworms per 4kg of soil); it is a noticeable pattern that earthworms survive longer if kept in smaller populations.

The differences in adult mortality, reproduction rate, and change in leaf mass demonstrate that pillbugs would be the ideal organism to use in a classroom lab version of this experiment. It would be more effective to have treatments of high pillbug density, since there was the most noticeable difference in leaf mass due to high pillbug density, and the high-density treatment had the highest proportion of adult surviving pillbugs and the highest number of juveniles. The most effective leaf treatment would be either medium or high density of leaves. The low-leaf density caused the leaves to be eaten away to nearly nothing and left the pillbugs with little food by the end of the experiment. However, this would likely not be an issue in a classroom setting, as the lab would run for four weeks at most, giving the pillbugs plenty of food. The higher levels of leaves showed the highest increase in nitrate levels. More leaves also trap water inside the containers, which help keep the soil moister than do low levels of leaves. A medium level of leaves would likely be the most effective as they are easier to handle. 4 grams of leaves nearly spill out of the small Tupperware containers and they are very easy to lose track of. 2 or 3 grams of leaves would fit better in the containers and still provide food to the pillbugs and nutrients to the soil.

Earthworms would not be the best soil fauna to use in the experiment because they are less likely to survive in more difficult environments and require more work. As stated before, earthworms consume organic matter that is already decomposing. The leaves that were collected

for the experiment were thoroughly dried and had not had time to start breaking down. This meant that the leaf litter was not suitable for earthworm consumption. Hättenschwiler & Gasser worked around this issue by allowing their leaf litter to decompose on its own for over three months before the organisms were added.

The earthworms also needed a 12-hour night and day cycle and quickly dried out and died when there was not enough soil moisture. They also typically reside in the bottom layers of the soil, in contrast to the pillbugs, which were often found on the surface of the soil and even within the leaf litter. It is harder to keep track of and view the earthworms, which makes it more difficult to determine if they are surviving and able to break down the leaf litter.

Changes would have to be made in the experiment to be suited for a lab experience. There was the issue during testing that the soil dried out very quickly. This is likely because when the water was first added to the soil, all of the clay particles absorbed the water and tightened up, resulting in very dense soil that did not retain water well. Clay soils have a very high water holding capacity: due to the small particle size, there are fewer pores in clay, which allows a low amount of water to flow out (Ball, 2001). However, once clay begins to dry (as it did in the experiment between watering's), it tightens up and shrinks (Zamek, 2003). To fix this, sand should be mixed in to the topsoil before the experiment begins. The ideal type of soil contains a mixture of sand, clay, and silt, and is commonly referred to as loam (Cornell University, 2017). Mixing sand into the topsoil will also mean that the starting nutrients will be lowered, since sand is nutrient-poor, and thus the results from adding the leaf litter and organisms will be more noticeable. In addition, pillbugs prefer soil with larger particles for ease of burrowing. Once the water is added for the first time, the soil should be mixed thoroughly to ensure that it does not become too dense to retain moisture.

This experiment was designed to create a baseline protocol, as described in Appendix A, that could be used for any soil science or earth science course. The benefit of a microcosm is that students will be able to make their own changes to the experiment. The control of the lab would be the standard topsoil/sand mixture with a set leaf density and organism density. The lab groups would then be able to make changes as they see fit in order to test if such changes have an influence on nutrient flow.

Conclusion

As the study shows, it is absolutely feasible to determine a soil lap protocol into a general education science class. It is possible that by adding a few grams of leaf litter and less than a dozen pillbugs to microcosms, one can design a lab protocol to be used for a general lab class. Unfortunately, with the time constraints, this project will not be able to test whether or not such a lab protocol actually increases student investment or interest. In the future, the protocol would be used to conduct an experiment that tests for knowledge and interest in soil quality.

Appendix A

Sample protocol. The protocol below demonstrates how to test the soil samples for Nitrate and Phosphate levels. This is an important part of the soil experiment, but not the full protocol for the setup of this lab.

Nitrate and Phosphate Testing Protocol

Background

Both nitrogen and phosphorous are essential elements to soil communities. Nitrate is a form of nitrogen that is water-soluble and readily taken up by organisms. Phosphate is similar, but it is an available form of the element phosphorus. Nitrogen is a building block of proteins, nucleic acids and other cellular constituents that makes it essential for plant growth. Phosphorus is used as a catalysis for photosynthesis, is a vital component of DNA and ATP (the energy unit of plants), and overall necessary for the health and life of plants. With the right balance of these nutrients in the soil, there can be flowering, diverse plant growth. The plants in turn provide resources and shelter to other organisms in an ecosystem, prevent soil erosion, and regulate the climate. Nutrient-rich soil is required for successful ecosystems.

Instructions

1. Wear gloves, goggles, and proper lab attire for this lab.

2. Select either a nitrate or phosphate kit.

3. Measure out three grams of soil and put them in a 50-mL tube.

4. Measure 15 mL of DI in a graduated cylinder and pour it into the tube. Mix for one minute and let sit until the soil particles.

5. Every time the sample is poured into a color-viewing tube, add more water to the soil in the 50-mL tube and shake again. This is to ensure that the mixture poured into the color-viewing tubes is mostly liquid and not dark mud that would not easily show color change.

Nitrate test kit

1. Fill one of the color-viewing tubes to the mark with demineralized water (can use squirt bottles on the bench). Stopper the tube and shake vigorously. Empty and repeat—this process rinses the tube out.

2. Add the soil sample to the 5-mL line on the tube.

3. Add contents of one NitraVer 6 Nitrate Reagent Powder Pillow to the tube. Stopper the tube and shake for 3 minutes. Then allow the sample to stand undisturbed for an additional 30 seconds. You *may* notice some particles of cadmium (from the powder pillow) drifting to the

bottom of the tube. **NOTE:** *Cadmium is a toxic metal regulated under RICRA. Handle carefully and dispose of the cadmium in a waste container.*

4. Pour the prepared sample into a second color-viewing tube **carefully** so that any cadmium particles or soil particles remain in the first tube. *Rinse the first tube into the waste container.*

5. Add the contents of one NitriVer 3 Nitrite Reagent Powder Pillow to the sample. Stopper the tube and shake for 30 seconds. A red color will develop if nitrate is present. Allow at least 10 minutes to stand but **no more** than 20 minutes.

6. Insert the tube containing the sample into the right top opening of the color comparator.

7. Rinse out the tube used in step 4, *washing the cadmium into a waste container*. Fill the tube to the mark with the original, untreated sample. Place in the left top opening of the comparator.

8. Hold the comparator up to a light source such as the sky, window or lamp and view through the openings in front. Rotate the disc to obtain a color match. Read the mg/L nitrate---nitrogen present in the sample and **multiply by 40.4** to get the concentration of nitrate.

9. **Record** your value **BUT DO NOT** tell the other members what you came up with. **Gently** spin the disc and give the comparator to a group member. **At least 3** group members need to take a reading.

10. Once you have all made your comparison, **average** your values and **record** that value on your Report Sheet.

Phosphate Test Kit

1. Fill two tubes to the first line (5 mL) with the soil sample. Put one tube into the left opening of the color comparator box.

2. Add one PhosVer 3 Phosphate Reagent Powder Pillow to the second tube. Swirl to mix. A blue color develops. **Wait** 1 minute but **no more** than 4 minutes to read results.

3. Put the second tube in the color comparator box. Hold the comparator up to a light source such as the sky, window or lamp and view through the openings in front. Rotate the disc to obtain a color match. Read the mg/L nitrate nitrogen present in the sample and **divide by 10.**

4. **Record** your value **BUT DO NOT** tell the other members what you came up with. **Gently** spin the disc and give the comparator to a group member. **At least 3** group members need to take a reading. 6. Once you have all made your comparison, **average** your values and **record** that value on your Report Sheet.

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