Nutrient content of leaf litter and the growth of an anecic earthworm, *Lumbricus terrestris*

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Abstract

Invasive earthworm species have numerous effects on previously earthworm-free ecosystems in North America such as reducing leaf litter and speeding up nutrient cycling. This experiment examined the importance of the nutrient content of leaf litter on the growth of an aneic earthworm, *Lumbricus terrestris*, in mesocosms. Although C:N ratio could not be quantified, I provided earthworm specimens with 4 leaf treatments differing in relative C:N ratios. The treatments were (in order of decreasing expected C:N ratio) dead-leached leaves, dead leaves, fresh-leached leaves, and fresh leaves. Due to its relatively slow growth rate and large body size I predicted that *L. terrestris* would exhibit maximum growth in the leaf treatment with highest C:N ratio. As expected, the average length and mass of *L. terrestris* increased the most in the dead-leached and dead leaf treatments showing that C:N ratio may be important in determining earthworm growth. This means that earthworms will prefer habitats with large quantities of dead, leached leaves such as sugar maple stands and perhaps dried stream beds or dried vernal ponds. Interestingly, the fresh leaf treatment showed significant decreases in leaf litter mass but earthworm mass and length decreased. Perhaps, the fungus that was significantly more present on this leaf treatment decomposed the leaves and also negatively affected the earthworms. Studies of fungus-leaf and fungus-worm interactions are necessary to better understand the mechanism at work.

Introduction

In North America, there are 147 species of earthworms, of which 102 are native and 45 are exotic (Reynolds 1995). All of the native earthworm species in North America have been absent from northern temperate forests since the last glacial period (Bohlen et al. 2004). Because of this, these forests are particularly susceptible to exotic earthworm invasion through human activities, such as transport via logging roads and trucks, dumping leftover fishing bait in a terrestrial habitat, and transport of agricultural materials (Gundale et al. 2005). Currently, there are 4 exotic earthworm species on the UNDERC property – *Dendrobaena octaedra*, *Aporrectodea sp.*, *Lumbricus rubellus*, and *Lumbricus terrestris* (Lehman 2005).

Earthworms can be characterized into three different ecological groupings – epigeic, endogeic, and aneic. Epigeic species live in the soil-litter interface and feed on coarse particulate organic matter. Endogeic earthworms live and feed within the mixed mineral layer of the soil and inhabit temporary burrows. Aneic species, such as *Lumbricus terrestris*, feed on the surface organic matter, but live in deep permanent burrows (James and Hendrix 2004). Earthworm colonization usually occurs first by epigeic species such as *D. octaedra* and epi-endogenic species such as *L. rubellus* and then the colonization by endogeic and aneic species follows (Gundale et al. 2005).

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Earthworms are important organisms in forest ecosystems because they accelerate the cycling of soil nutrients by their movement, ingestion, digestion, and excretion of leaf liter and soil (Amador et al. 2003). Increased richness of the invasive earthworm community corresponds to decreases in surface liter and increases in in-soil carbon (C), nitrogen (N), and mineralizable N (Gundale et al. 2005). Despite the initial increase in soil C, the overall store of soil C in the system decreases substantially with time (Burtelow et al. 1998). Earthworms are also shown to ingest foods of a wide variety of C:N ratios and transform it to body tissue of lower C:N ratios (Syers and Springett 1984, Domínguez 2004). As earthworms feed on leaf liter, they lower the C:N ratio progressively and transform much of the organic nitrogen into the ammonium or nitrate form (Edwards 1994).

As many studies have been conducted to examine the numerous effects of earthworms on forest and agricultural ecosystems, other studies have examined the importance of the nutrient content of food sources on earthworm growth. Bohlen et al. (2004) have noted that the C:N ratio and tannin content of soil organic matter and surface plant litter affectearthworm feeding, growth, and reproduction. Nitrogen has been noted by some to be the limiting nutrient for earthworm populations with the example of a *L. terrestris* population in an English deciduous woodland that required about 100 kg ha^{-1} $year^{-1}$, greatly exceeding the amount of nitrogen actually available (Curry 2004). On the other

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hand, the opposite has been found. Binet and Trehen (1992) found that there is enough N to meet earthworm demand and earthworms excrete large amounts of N in their urine and mucus.

Since earthworms alter leaf liter and nutrient cycling and are also affected themselves by the nutrients available, it is also important to know a little about the earthworms' food sources. Studies of leaf decomposition show that leaves exposed to streams exhibit an immediate loss of dissolved organic mater (DOM) including organic carbon and nitrogen (Roberston 1988). Over the course of a year the percent nitrogen content of leaf liter on the forest floor increases as fungi and bacteria which have a low C:N ratio (4:1 to 9:1) colonize the leaf litter and retain N while C is reduced (Gosz et al. 1973). But in the short term and without microbial activity, changes in leaf liter nutrient content are much different. Over the course of 96 hours of microbial-suppressed leaching, nitrogen was shown to slowly leach from leaves while carbon was not (Sangkyu and Kang-Hyun 2003). This means if leaves are leached for a short period with little biological activity, then nitrogen should be leached out and the carbon should remain; thus, raising the C:N ratio. Also, during leaf senescence, trees have been shown to reabsorb 70% of the nitrogen from the leaves, but leave the carbon behind in the leaf's physical structure (Yasumura et al. 2006). This means that senesced, year-old leaves will have a higher C:N ratio than fresh live leaves.

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This experiment examined the importance of leaf litter nutrient content on the growth of earthworms in mesocosms. Experimental designs utilizing earthworms in mesocosms have been shown to be successful in past studies (Amador et al. 2003, Amador and Görres 2005). In this experiment, I manipulated the nutrient content of leaves by leaching them in distilled water. Although I will not be able to measure the C:N ratio of leaf treatments this summer, I can make assumptions based on previous studies of leaf decomposition. It can be assumed that dead-leached leaves will have the highest C:N ratio since nitrogen has be reabsorbed by the tree during senescence and additional nitrogen has been leached out during the distilled water leaching. The next highest C:N ratio would be dead un-leached leaves, since most of the nitrogen has been reabsorbed, but it has not been leached. The next highest C:N ratio would be the fresh leached leaves. Since they have not undergone senescence, they have not lost much nitrogen, but leaching will likely eliminate some. The fresh, live leaves would have the lowest C:N because they have neither senescenced nor leached, so the majority of nitrogen will remain in the leaf. For fast-growing organisms, phosphorous (P) is usually the most important nutrient, requiring a low N:P nutrient ratio (Elser et al.1996). Also, C:N ratios have been found to be more constrained at high than at low growth rate (Vrede et al. 2003). Organisms with larger body sizes tend to have higher C:N ratios (Sterner and Elser 2002). Compared to other earthworms, *Lumbricus terrestris*

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Methods

Earthworm Collection

I collected earthworms in a sugar maple (*Acer saccharum*) stand south of the storage building on the UNDERC property during the first research week (06/06/05 – 06/06/09). I used the "electro-shock" technique of earthworm removal to obtain young *L. terrestris* for placement in the mesocosms (Bohlen et al. 1995). In the laboratory, I identified and sorted the earthworms to species and stored them in a refrigerator (4°C) (Reynolds 1977).

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Leaf Treatments

I collected live sugar maple leaves from adult trees at the same site where I collected the earthworms. I picked leaves that were whole, green, and appeared free of disease. Half of these leaves were stored in a refrigerator (4°C) while the other half were placed in 12 L of distilled water at room temperature to leach for one week. I replaced the water with fresh distilled water daily. At the same site where I collected the fresh leaves, I also collected senesced sugar maple leaves from the ground. In the lab, I sorted through the leaves and removed any eaten or broken leaves. Once again, half of the leaves were either stored in a refrigerator (4°C) or underwent the same leaching treatment as the fresh leaves for one week. This makes four different leaf treatments: fresh, fresh leached, dead, and dead leached (Figure 1). I also used a fifth control treatment of no leaves in this experiment. I measured the total organic content for each of the treatments at the beginning of the experiment and the change in total organic content of each replicate by burning leaf samples at the end of the experiment. This was done by first drying the leaf samples for 48 hours at 60°C and then burning in a muffle furnace at 500°C for 3 hours.

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Mesocosm Setup

I used black plastic tree planters (ca. 34 cm tall; 11 cm x 11 cm square at top; with a slight taper to the base) as the earthworm mesocosms. During the first research week, I collected organic soil from the A horizon (~0-10 cm in depth) and mineral soil from the E horizon (~10-25 cm in depth) at the same sugar maple stand where the earthworms and leaves had been collected. I sifted through both soil types with a sieve to remove any earthworms, insects, large rocks, or large organic matter. I then used the sifted soil to recreate the local soil horizons in the mesocosms. Before adding any soil to the planters, I placed a 10 cm x 10 cm square of garden fabric on the bottom to stop soil and earthworms from escaping through the holes on the bottom, but allowing water to drain out. Then, the bottom 20 cm of the container were filled with mineral soil and tapped to gently pack down. I then added 10 cm of organic soil and tapped the mesocosm on the ground to gently pack the soil down.

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I randomly selected *L. terrestris* individuals and measured initial wet mass and length of the worms. In order to see optimal growth, I only used juvenile worms <65 mm long. I added one worm to each mesocosm by placing the worm on top of the soil. Later in the day, 200 ml of tap water was added to each mesocosm. After that, I randomly added one of the five treatments to each of the mesocosms. I added two grams in the case of the four leaf treatments and no leaves were added for the control. In total, I setup ten mesocosm replicates of each of the five treatments. The mesocosms received ambient lighting from the normal daily activity of the Wet Lab. Also, I added 200 ml of tap water to each mesocosm about every 10 days to maintain the soil moisture of the mesocosms.

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Growth Measurements

After 33 days, all leaf litter and earthworms were removed. I noted the depth (cm) of recovery of each earthworm and measured its wet mass and length.

Statistical Analysis

Statistical analyses were performed with SYSTAT 11 (Systat Software Inc., Point Richmond, California, USA). In order to see if there was a significant relationship between leaf treatment type and average leaf mass loss, I performed a one-way ANOVA and a post-hoc Tukey's test (dependent variable = average leaf mass loss; independent variable = leaf treatment type). To examine if there was a significant relationship between leaf treatment types and the average percent change of earthworm mass I used a one-way ANOVA and a post-hoc Tukey's test (dependent variable = average percent change of earthworm mass; independent variable = leaf treatment type). In order to determine if there was a significant relationship between leaf treatment types and the average percent change of earthworm length, I used a one-way ANOVA and a post-hoc Tukey's test (dependent variable = average percent change of earthworm length; independent variable = leaf treatment type). To determine if there was a significant relationship between leaf treatment type and the average percent change of the organic content of the leaves over the course of the 33 days I performed a one-way ANOVA and a post-hoc Tukey's test (dependent variable = average percent change of organic content; independent variable = leaf treatment type). I also

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used 2x5 contingency tables to see if there was a significant difference between leaf treatment types and either earthworm recovery or mold presence.

Results

A statistically significant relationship was found between leaf treatment type and average leaf mass loss. I found that the fresh leaf treatment had a significantly greater average leaf mass loss of 0.513 g than the three other 3 leaf treatments (Figure 2; df = 3; F = 18.858; p < 0.001). The fresh leached, dead leached, and dead leaf treatments were all statistically similar to each other.

A statistically significant relationship was found between leaf treatment type and average percent change of earthworm mass (Figure 3; df =4; F = 6.703; p = 0.001). The dead and dead-leached treatments were statistically similar and had the greatest average increase in earthworm mass of 15.26% and 48.59% respectively. The fresh and the control treatments were also significantly similar and decreased in average earthworm mass by 41.61% and 34.00% respectively. The fresh-leached treatment saw a slight increase in average earthworm mass but was not statistically different from any of the other leaf treatments.

The relationship between leaf treatment type and average percent change in earthworm length was found to be statistically significant (Figure 4; df = 4; F = 2.572; p = 0.064). The dead leached treatment which had an increase in earthworm length by 18.69% was statistically greater than the fresh leaf treatment

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which had a decrease in length by 7.93%. No other treatments were found to be statistically different from each other.

The relationship between the average percent change of the organic content of the leaves over the course of the 33 days and leaf treatment type was not found to be statistically significant (df = 3; F = 0.510; p = 0.678).

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During the course of the experiment, I observed 9 worms outside of the mesocosms in the cattle tank and they were removed from the experiment. I observed most of the escaped worms after adding water to the mesocosms. Of the 50 mesocosms set-up, 29 mesocosms had one worm present at the end of the experiment. I recovered the most number of worm replicates for the dead leaf treatment and the least number of worm replicates for the control (Table 1). Using a 2x5 contingency table, I found that there was no significant difference between the five treatment types and the number of replicates with worms at the end of the 33 days (Gotelli and Ellison 2004) (chi-square = 6.08; df = 4; p = 0.194). On the other hand, I found that there was a significant difference between the five treatment types and number of replicates that had mold present on the leaves at the end of the 33 days (Table 1, chi-square = 34.3; df = 4; p < 0.001). Two by two contingency tables showed that the fresh leaf treatment was significantly different than all other treatments. The fresh-leached treatment was also significantly different than all other treatments. All other treatments were not significantly different.

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Discussion

As my hypothesis predicted, the earthworms in mesocosms with the dead and dead-leached leaf treatments showed the greatest growth in mass and length. These two treatments with high relative C:N ratios appear to be ideal for *L. terrestris*. This is supported by the fact that earthworms can ingest food with a wide variety of C:N ratios and are able to excrete large amounts of nitrogen in their urine and mucus (Binet and Trehen 1992). Also, the earthworms in mesocosms with fresh and fresh-leached treatments, with low expected C:N ratios, exhibited small growth or even loss of mass and length. These findings support the idea that nutrient content, more specifically, the nutrient ratios, are important to the growth rate of earthworms. *L. terrestris* with its large size and slow growth rate does not require a low C:N ratio like other fast developing earthworm species may. These findings about earthworm growth in response to available nutrient ratios may have implications on the population and ecosystem scale. Without taking into consideration the effect of human-aided transport, *L. terrestris* may grow more rapidly and in larger numbers in habitats were there are large amounts of dead leaf litter available. The leaching of litter material may also come into play to determine the success of *L. terrestris.* Perhaps, sugar maple stands with lots of rain or snow melt or dried stream beds or dried vernal ponds with lots of leached leaves left behind may be good sites for earthworm inhabitation based on available food. Also, if *L. terrestris* prefer food of high C:N

Formatted: Line spacing: Double, Tabs: 37.05 pt, Left ratio, then the leaf liter that they leave behind will be low in C:N ratio (Edwards 1994). This means that what *L. terrestris* eats has an effect on the nutrients available to other organisms including other earthworm species and perhaps plant species.

Interestingly, despite the fact that earthworms in the fresh leaf treatment had a decrease in mass and length, this treatment had the greatest loss of leaf litter mass. This means that there may be some other factor besides earthworm decomposition to explain the loss of mass of the fresh leaf treatment. Combining this with the fact that leaf treatments exhibited a significant difference in presence of mold may offer an explanation. All 10 fresh leaf replicates had a white fungus present at the end of the experiment. Perhaps, this fungus is responsible for the leaf litter mass loss. Hudson (1968) describes over 40 different species of fungi or that inhabit leaf liter. At least 22 species of fungi, of which at least two have a white color, have been known to inhabit maple tree leaves (Spector 1956). Fungi have been shown to contribute to 55% of the total decomposition in a mixed-hardwood forest and even greater amounts in other forest types (Elliott et al. 1993). Not only may this fungus decompose the leaves, but it may also repel the earthworms from eating the leaves and thus explain the decrease in earthworm mass and length that was seen in the fresh leaf treatment. Studies of fungus-leaf and fungus-worm interactions are necessary to better understand the mechanism.

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Although there was no significant relationship between treatment type and successful earthworm recovery from the mesocosms, all treatments exhibited loses of earthworms. This indicates that there is room for improvement in the mesocosm experimental design. It is hard to determine the earthworms' method of disappearance. Perhaps some specimens escaped out the bottom if the garden-mesh was not held well in place over the holes in the bottom of the mesocosm. This could be solved by more firmly affixing the mesh to the mesocosm. For example, Amador et al. (2003) used fine fiberglass mesh held in place with a rubber band to plug the ends of their PVC core mesocosms. Earthworm specimens might have also escaped over the top edge of the mesocosm. The top of the soil was approximately 4 cm from the top lip of the mesocosm and with the leaves added, it is possible that earthworms climbed over the edge. This factor, along with the fact that the individual mesocosms were stored with no space between them, may account for the two mesocosms that had multiple worms recovered.

Acknowledgements

I would like to thank the Bernard J. Hank Family for their funding of this research facility and Dave Costello, Lauren Kinsman, Will Osterholz, Karen Francl, and Gary Belovsky for their assistance with this project.

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One of the most surprising results of this experiment was that the fresh leaf treatment which had the greatest average loss of mass was not the leaf treatment with the greatest average change in earthworm mass or length. In fact, the fresh leaf treatment had the lowest change in earthworm mass and length of all 5 treatments. This means that there was some other factor besides earthworm decomposition to explain the loss of mass of the fresh leaf treatment. Combining this with the fact that leaf treatments exhibited a significant difference in presence of mold may offer an explanation.

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Table 1. The number of replicates of each of the five treatment types with worms recovered and the number of replicates of each of the five treatment types with mold observed on the leaves at the end of the 33 days.

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Treatment	# of Replicates with One Worm Recovered	# of Replicates with Leaf Mold
Fresh	5	10
Fresh Leached	6	4
Dead	8	0
Dead Leached	7	1
Control	3	0

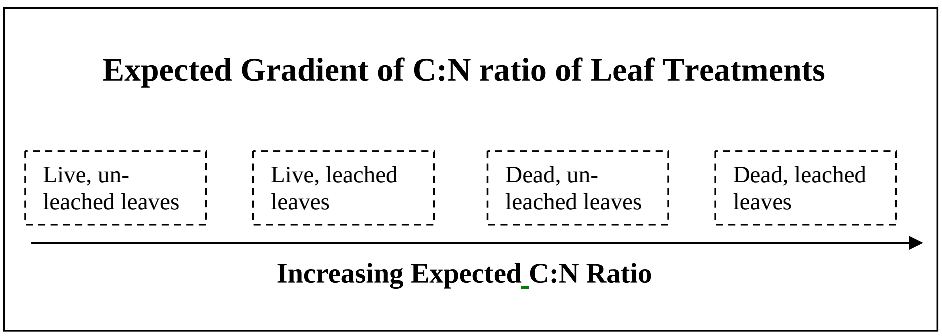

Figure 1. The expected C:N ratio of leaf treatments is lowest in live, un-leached leaves, increases in live, leached leaves, increases in dead, leached leaves, and is greatest in dead, un-leached leaves. This is based on the fact that

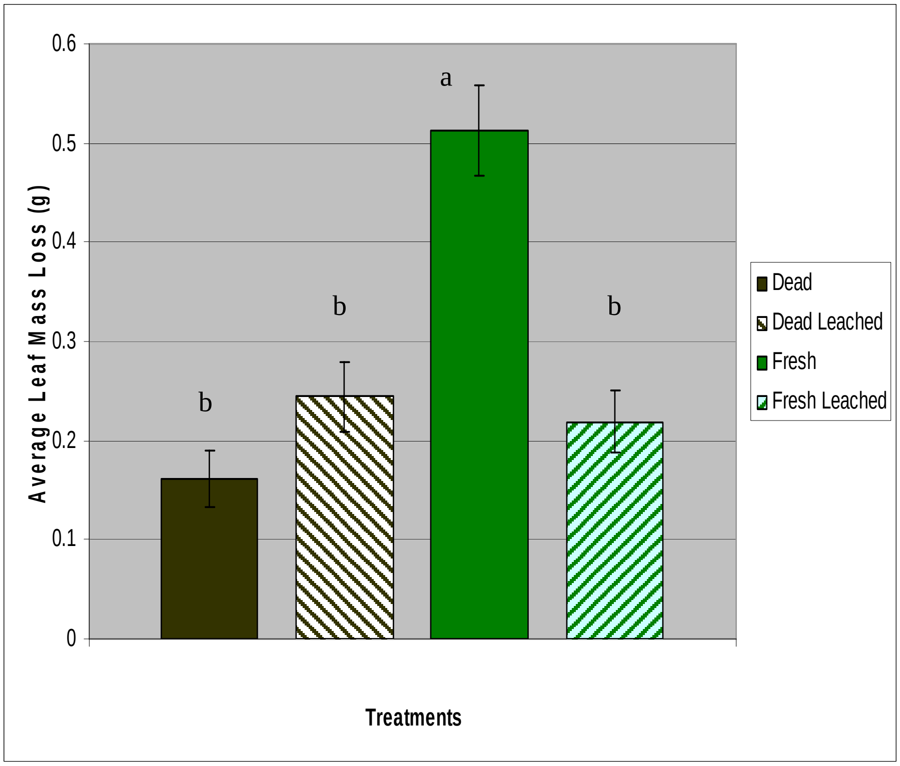

Figure 2. The average leaf mass loss (±SE) of the four leaf treatments over the course of 33 days in the mesocosms. ($df = 3$; $F = 18.858$; $p < 0.001$). The fresh leaf treatment was significant from all other leaf treatments.

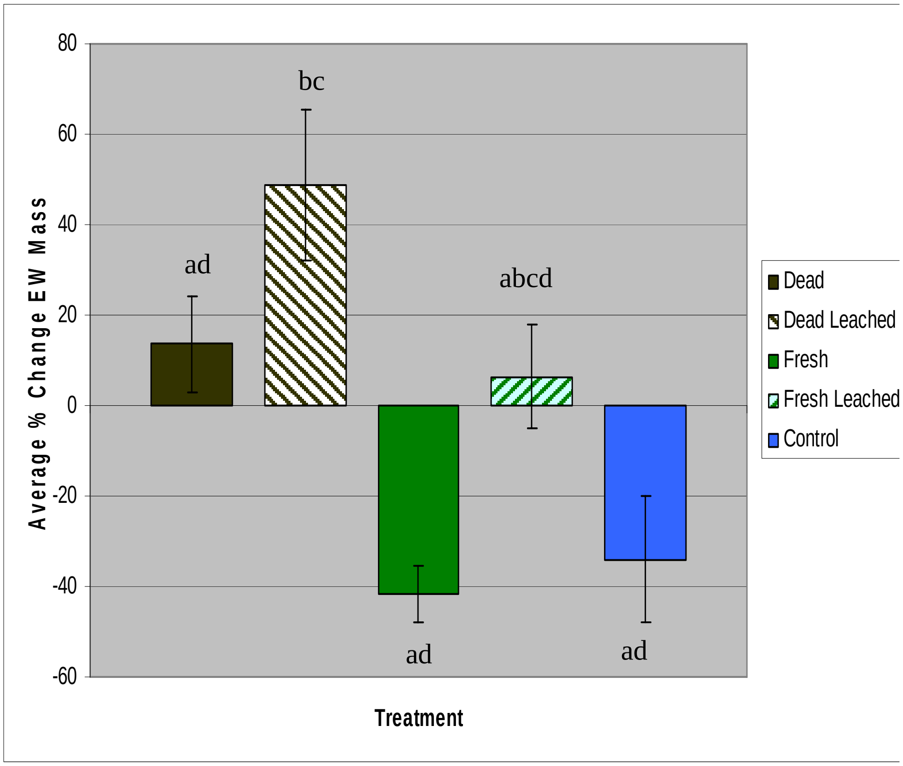

Figure 3. The average percent change (±SE) in earthworm mass of five treatments over the course of 33 days in the mesocosms ($df = 4$; $F = 6.703$; $p = 0.001$). The dead and dead leached leaf treatments are significantly different from each other. The dead leached and the fresh leaf treatments are significantly different from each other. The dead leached and the control are significantly different from each other.

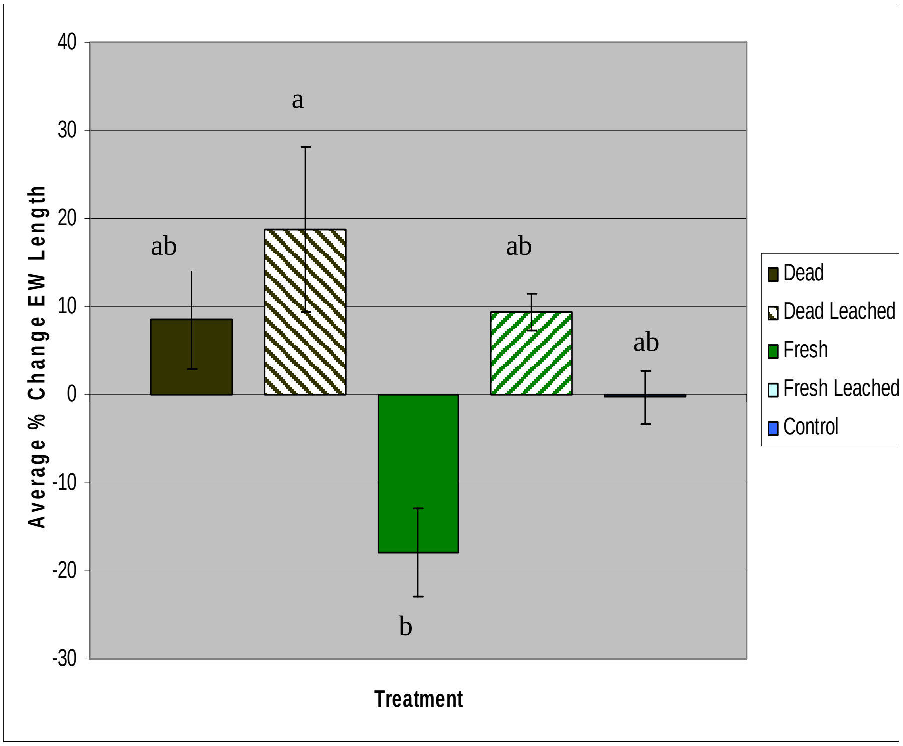

Figure 4. The average percent change (±SE) in earthworm length of five treatments over the course of 33 days in the mesocosms (df = 4; F = 2.572; p = 0.064). The dead leached and fresh leaf treatments are significantly different from each other.