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Final Draft

7/30/19

 Effects of annual fertilization on presence of earthworms in Northern Hardwood forests in New Hampshire’s White Mountains

***Abstract:***  Earthworms within New Hampshire’s White Mountains ecosystems have rarely been seen throughout these areas. In this experimental study we were trying to determine if earthworms were present within our research sites that include Bartlett Experimental Forest (BEF); Hubbard Brook (HB); and Jeffers Brook (JB). If they were present within any of these sites, we wanted to see which stand they would be in and if different treatment plots affected their abundance, explaining how much earthworms influence the environment and ecosystem around them. Mustard treatments will be applied to five plots within a young, mid and mature-aged stand within BEF; and a mid and mature-aged stand within Hubbard Brook HB and JB. Two treatment applications of a mustard solution were applied to each quadrat and earthworms found in different treatment applications and treatment plots were stored separately from each other, and taken to the lab to be identified. Earthworms were sparse in all stands except for the HB mid-aged stand (HBM), where they were very common. Ca showed the highest abundance of earthworms compared to all other treatment plots, while N and N+P had the lowest abundance. Most of all earthworms identified were non-native in this stand.

***Introduction:***

Earthworms have disappeared from most of northern North America 10,000 years ago during the last ice age and have been slow to re-colonize hardwood and mixed hardwood forests since (Mitchell 2019; Hendrix and Bohlen (2002). Exotic earthworms brought from European settlers from Europe and Asia have colonized many of these northern forests due to timber and petroleum activity, roads, tire, boats, and gardeners (Mitchell 2019; Hendrix et al, 2008). Due to these human activities, rural areas generally have higher abundances of earthworms near boat launches, roads and campsites where human activity is greatest (Homan et al, 2015).

 As earthworms feed on the litter of forest floors they release much of the carbon that was sitting on the forest floor into the atmosphere, altering the chemistry of the soil, soil structure, soil microflora communities, and nutrient uptake in plants (Bernard et al, 2009; Mitchell 2019). Earthworms can make the soil drier by reducing the organic matter of the forest floor replacing it with their castings, leading to the soil becoming drier and creating less favorable conditions for plant regeneration and growth (Knowles et al, 2016). From earthworms eating the organic matter on the forest floor this can lead to changes in decaying organisms; shifting fungi to bacteria, altering decomposition processes, and affecting wildlife altering the food web (Knowles et al, 2016).

 Endogeic earthworms, which are mainly found in mineral layers of soils are not tolerant of pH levels of 4.5 and below; epigeic earthworms that reside between the litter layer and soil have been found to survive in soils of a pH of 3.5 and above; anecic earthworms that burrow deep down in soil have been found to be intermediate (Potthoff et al, 2006). The soil order within the White Mountains is typically Spodosol which is established by rainfall interacting with acidic vegetative litter. The soil within the sites being tested is mostly dominated by loam and a sandy loam texture throughout many of the horizons allowing for habitable soil for earthworms (Vadeboncoeur, 2014). The acidity of the soil within the sites of the White Mountains ranges from 3.6 - 5.7 depending on the soil horizon and location, possibly explaining why earthworms have been at least locally absent (Ratliff and Fisk 2016). Differences in acidity and soil texture are due to the different parent material that is located within each site. The parent material of the BEF consists of Conway and Osceola granites; HB consists of Kinsman granodiorite; and JB consists of metamorphosed basalt (Bailey et al 2003).

 The White Mountain National Forest of New Hampshire consist of a mixed deciduous and coniferous forest, which may lack earthworms due to its acidic soils (Potthoff et al, 2006). Applications of N, P, N+P, and Ca fertilizers were added to three different sites that vary in age within the White Mountains to see how the ecosystem and environment respond to this fertilization. With these fertilizers being added we want to see if earthworms are present and what their abundance and composition is within these treated sites and the factors that could affect them. We believe that earthworms may be present within the JB because of recent sightings but further testing will be used to verify this.

***Objective:***

We want to see if earthworm species such as Bimastos rubidus, Dendrobaena octaedra, Lumbricus castaneus, Lumbricus rubellus, L. terrestris, Aporrectodea caliginosa, A. longa,

A. rosea, Allolobophora chlorotica, Eiseniella tetraedra, Eisenoides lonngbergi, and

Sparganophilus eiseni are present at early, mid and old-aged stands at BEF, JB, and HB and if so, which species are present in nitrogen, calcium, and control treated plots.

***Hypothesis:***

* At BEF and HB I expect there to be no earthworms due to a thick litter layer, its parent material being Conway and Osceola granites and Kinsman granodiorite, and the acidity of the soil being too low.
* If certain stands and plots have a high moisture level than I believe that there will be a higher abundance of earthworm’s present compared to drier stands and plots, due to more favorable conditions.
* At JB I expect there to be earthworms due to a thin litter layer and a small stream running throughout the plots possibly showing a more favorable environment for them.

***Methods***

***Site Description:***

Three sites are being studied within the White Mountains of New Hampshire: Bartlett Experimental Forest (BEF) in Bartlett, Hubbard Brook Experimental Forest (HB) in North Woodstock, and Jeffers Brook (JB), in Woodsville. These forests consist of temperate hardwood trees that have stands varying in age. BEF has a total of 9 stands, three early-aged, three mid-aged, and three old-aged (Table 1). Within BEF six stands consist of an N, P, N + P and control treated plots, while three of the stands consist of a Ca, N, P, N+ P and control treated plots. HB has two stands, one consisting of middle-aged plots, with Ca, N, P, N+P, and control treated plots and another old-aged stand, with N, P, N+P and two control plots (Table 1). JB has two stands, half are middle-aged and the others are old-aged (Table 1). Within both of these stands the plots consist of N, P, N+P, Ca, and control treated plots. Within each stand all plots consist of an area of 30 m x 30 m with a 10 m buffer, with the exception of C2 having a 7.5 m buffer. HB-mid and JB-old are also exceptions which have 20m x 20 m with a 5 m buffer. All plots have been receiving annual fertilization since May of 2011, with N as NH4 NO3 at a rate of 30 kg N/ha/yr and P as NaH2PO4 at 10 kg P/ha/yr. In October 2011, Ca in the form of CaSiO3 was added at a rate of 1150 kg Ca/ha for the first time to a fifth plot in five of the stands.

***Field Procedures:***

 A young, mid, and mature-aged stand were completed at BEF, while a mid and mature-aged stand were completed at HB and JB. Control, N, and Ca plots were tested at these stands and if earthworms were found, then all plots within the stand would be completed; If no earthworms were found then only the control, Ca, and N treatments were tested. After completing all plots within the selected stand, nearby streams, trails, and other suitable spots were hand searched for earthworms, for a total of 15 minutes, to get a better sense of the species pool in the area. If earthworms were found their coordinates were recorded.

 Upon arrival at the plot, a nearby water source was found to fill up the water jugs. Once water is found, a measurement from the outer buffer stake to the inner buffer stake will be completed to find the middle. Once in the middle, I will lay out my quadrat made of PVC pipe and cut the litter layer with my knife and peel back the Oe horizons. If any earthworms are found within this layer then they will be put into a specimen cup with a wet paper towel, labeled, and then into a cooler bag. After the litter layer is removed a pre-weighed 60g bag of dry mustard will be stirred with 6L of water in a bucket that is pre-marked with a 6L point. When the solution is mixed it will be poured slowly within the quadrat to make sure all of the solution is absorbed by the soil within the quadrat. A five-minute timer will start once all of the solution is poured and the area will be examined for earthworms. All earthworms that appear will be put into specimen cups with wet paper towels inside of them and put into a cooler, recording the abundance of earthworms per subplot, labeling each specimen cup with the date, time and subplot. After the five-minutes are up another solution of mustard water will be applied to the area within the quadrat and examined for another five minutes. After the five-minutes are up the area within the quadrat will be covered up with the removed litter. These steps will then be repeated at the other three corners of the plot. All earthworms collected will be taken back to the lab.

***Lab Procedures:***

Earthworms will be taken out of their containers, rinsed, and put into 70% isopropyl alcohol to be preserved. Keys will be used to determine species identification. After earthworms are identified they will be put into a small tube with 70% isopropyl to preserve them with the name, stand, plot, subplot, and date written on the tube to keep track of where each earthworm came from.

***Table 1:***

|  |  |  |  |
| --- | --- | --- | --- |
|  | Bartlett | Hubbard Brook | Jeffers Brook  |
| Young  | 29-39 Years | N/A | N/A |
| Mid | 44-47 Years | 48 Years | 34 Years |
| Old | 136-143 Years | 109 Years | 109 Years |

***Table 2:***

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Non-lumbricus  | Lumbricus  | Lumbricus castaneus  | Lumbricus rubellus  | Dendrobaena octaedra  | Aporrectodea caliginosa  | Aporrectodea longa  | Aporrectodea tuberculata  | Aporrectodea trapezoides  | Eiseniella tetraedra | Eisenia rosea | Unable to I.D |
| 94 | 28 | 3 | 4 | 55 | 8 | 7 | 1 | 1 | 1 | 5 | 13 |

***Graph 1:***

***Graph 2:***

***Graph 3:***



***Results:***

A total of 220 earthworms were found when testing the selected stands; Most earthworms found weren’t mature enough to have clitellum’s and were non-lumbricus dominant rather than lumbricus dominant (Table 2). The mature earthworm species that was the most abundant was the dendrobaena octaedra (Table 2) Aporrectodea trapezoides, aporrectodea tuberculate, and eiseniella tetraedra were all only found once within this area (Table 2). Lumbricus cataneus and lumbricus rubellus were only found 3-4 times, both species being non-native. Eisenia rosea, aporrectodea longa, and aporrectodea caliginosa were all found 5-8 times within the area being sparse compared to dendrobaena octaedra (Table 2). 13 species were unable to identify due to the clitellum being too small or having trouble identifying (Table 2). Ca showed the highest abundance within the JBM and HBM-aged sites, while the HB young-aged stand showed the highest abundance in N.

***Discussion:***

Earthworms in the BEF stands seem to be sparse, the one earthworm that was found in the early aged stand was non-lumbricus and is native to the area (Graph 1). The soil at this stand was moist due to there being a small stream nearby allowing for more favorable conditions for earthworms. The mid and old-aged stands at HB (HBM, HBO) were sparse of earthworms as well, the earthworms in HBM were all found in the Ca-plot. Two of the earthworms tested at HBM didn’t come out of the soil until the second application was applied and only one was found in the first application. This could be due to the thickness of the fibrous roots in the area being tested, making it difficult for the mustard to travel deep down into the soil and reach the earthworms (Osborn, 2018). Despite the earthworms coming out of the soil from different applications, all non-lumbricus and native to this area. The HBO stand was close to the HBM Ca-treatment but was lower in elevation, however no earthworms were found in any HBO plots along with the rest of the HBM plots. At Jeffers Brook mid-aged (JBM) earthworms were very abundant throughout this stand with all plots having native and non-native earthworms. They were most common in the Ca treated plot recording the highest overall abundance (Graph 6). P had the next highest abundance within the stand along with control. N and N+P both had the lowest values within this stand. This was different from what was predicted before sampling these stands. Originally N was thought to have the highest abundance of earthworms due to them taking in nitrogen, however this was not the case. Within the N and N+P plots the roots were very thick and fibrous which may have soaked up some of the mustard solution not allowing it to get deep into the soil (Osborn, 2018). At the JB old-aged stand (JBO) which is close to the JBM stand, no earthworms were found. At the JBO stand the area was very abundant with small plants, had a thick litter layer, and had lots of mature sugar maples, this may have shown proof before testing that earthworms were not in this area and in JBM. Recent studies have shown that non-native earthworms have caused sugar maples to die along with harming other species within the ecosystem (Bal et al, 2017). JBM didn’t have many sugar maples, a low abundance of plants, and a very thin litter layer throughout its stand indicating that something was eating the litter layer.

Mostly all earthworms found within JBM were all non-native, while the ones found at HBM and BEF-C2 were native. The most common earthworm species found was Dendrobaena octaedra, and Aporrectodea caliginosa this is due to the amount of litter there was on the ground and the soil conditions being favorable for these species. The least common species was Aporrectodea trapezoides and Eiseniella tetraedra, this is due to the soil conditions not being as favorable for these species rather than others. With there being so many small perennial streams allowed for more favorable conditions for earthworms throughout the JBM stand, allowing for more diversity in this stand. These invasive earthworms can alter ecosystems in many different ways increasing competition among other species (Bohlen et al, 2004). Invasive earthworms have been found to speed up nutrient cycling throughout forest communities and have been found to decrease fungal communities’ diversity, and resource availability (Bohlen et al, 2004). Due to earthworms altering soil properties and litter layers, it is found that the diversity of native plants in forests have decreased due to this (Bohlen et al, 2004). If long term earthworm invasion continues than it can lead to a major loss of native species changing the composition and structure of forest ecosystems.

 For future studies a third treatment for areas with fibrous roots could be applied to make sure that the mustard solution reaches all the way down. An alternative for fibrous roots would be cutting out the roots until the soil has been reached, however this may disrupt growth production. Testing all plots within a stand even if no earthworms are found in N, Ca, or Control, the topography of the area may change from each treatment plot which could have allowed for more favorable conditions for earthworms. Keep testing for treatment effects. Keeping track of salamanders within quadrats, it seemed that where there were no earthworms more salamanders were present and vice versa.

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