The Impact of *Lumbricus terrestris* on Seed Germination of Four Native Herbaceous Plant Species

BIOS 35502: Practicum in Environmental Field Biology

Samantha N. Sutton

Advisor: Hannah Legatzke

Abstract

Control of invasive species is a large portion of conservation in North American forests. With the introduction of non-native earthworms to Northern hardwood forests it is adventitious to determine what impact this is having on community. In this study I investigated if the presence of *Lumbricus terrestris* has an effect on the germination rate of four species of herbaceous plants native to Gogebic County, Michigan. The four species in the study were *Mentha arvensis* (Wild Mint), *Lycopus americanus* (Water Horehound), *Oenothera biennis* (Evening Primrose), and *Calamagrotis canadensis* (Blue Joint Grass). I found no significant difference in germination between treatments with and without *L. terrestris* (p= F_{df}=0 .22$_{2}$, p=.8024). Germination rates between species were significantly different (F = 14.41; d.f.= 2; *p* =3.18e-5). The *Mentha arvensis* had very low germination and was removed from all parametric tests in order to normalize the data. The *M. arvensis* was included in the Friedman test which did result in a significant difference in the means of germination between species (p= χ^2_1=4, *p*= .0455).

Introduction

During the last period of glaciation in North America, ~10,000 years, native earthworms were expatriated from the Great Lakes region. Those existing in soils today have been introduced from mainly Europe and Asia. Spread has been intensified in areas with human settlements, agriculture, roads, and areas where fishermen dump bait worms (Holdsworth et al. 2007). The Great Lakes region is an ideal study site due to the recent introduction of earthworms. Due to the long absence of earthworms many North American plants have evolved without the soil disruption worms create. I will be testing if earthworms cause a decrease in germination rates due to prior studies suggesting that earthworms may alter plant community structure (Asshoff etal. 2010; Gundale 2002; Frelich et al. 2006). Testing and earthworm collection for this experiment occurred on property of the University of Notre Dame Environmental Research Center (UNDERC) located in the Upper Peninsula of Michigan (46 ° 13’ N, 89 ° 32’W).

Earthworms have three ecological groups: epigeic, endogeic, and anecic. In this experiment *Lumbricus terrestris* is the most commonly found anecic species in North America. Due to its large biomass and body size it is a dominant presence in areas it has colonized (Hale et al. 2005). Anecic species make deep permanent burrows into the soil. Leaf litter and other organic debris is pulled into their burrows causing a deeper transition of nutrients in the soil structure (Bouché 1972). Many studies have shown that earthworms decrease surface leaf litter and alter organic matter dynamics. (Suárez 2006; James 1991; Fragoso et al. 1993; McLean and Parkinson 1997; Burtelow et al.1998; Lavelle et al. 1998; Bohlen et al. 2004). This means that each year significant amounts of carbon are moved from soil surfaces to deeper layers. This movement of nutrients is facilitated by not only high densities of earthworms but also diversity of species. Forests with multiple earthworm ecological groups had significantly lower plant diversity than forests with fewer earthworm ecological groups (Craven et al 2016). Each earthworm ecological group occupies different strata in the soil and perform separate roles. With greater densities and diversity of non-native earthworms the soil and surrounding biotic community will be more impacted. This could be due to the estimate that earthworms have been shown to decrease forest floor weight and thickness by about 85% (Alban and Berry 1994). Some studies have concluded that leaf litter is an important component in the regeneration of various species (Facelli 1991). Less is know about the full impact these exotic invaders have on the indigenous flora in temperate hardwood forests. Previous studies have also shown that deer and earthworm densities work synergistically with one another to facilitate growth of non-native plant species (Dávalos et al 2015). If earthworms do cause loss of fitness in native species it would be advantageous to conservation to determine if germination is being affected.

It is the aim of this study to determine if the presence of earthworms can impact the germination rate of herbaceous plants. Over 80 percent of the diversity located in temperate forests is constituted by herbaceous plants (Carson unpublished data). If earthworms threaten this diversity it is important to determine the extent at which plants could be affected. If herb species are sensitive to the presence of earthworms, then a large portion of temperate forests plant diversity will be at risk. The decline of native plant species could open niches for invasive species. In this experiment I hope to test if earthworms decrease the germination rate of herbaceous plants.

Methods and Materials

Data Collection

Forty-eight 15cm pots were planted with 300 seeds per pot. I filled the pots with Miracle-Gro Potting Mix. The four species of plants tested were *Mentha arvensis* (Wild Mint), *Lycopus americanus* (Water Horehound), *Oenothera biennis* (Evening Primrose), and *Calamagrotis canadensis* (Blue Joint Grass). There were 12 pots of each species. Six pots were control and contained no earthworms and six experimental pots did contain worms. The reason for choosing these species was that all their seeds require being sown on the topsoil for germination. The pots were set up under six grow lights (65 watt) and rotated both daily and weekly to evenly distribute light among pots and species (Fig. 1 & 2). Lights were placed 30cm above the soil. All pots were given the same amount of water.

To collect earthworms for the germination experiment, I used a combination of mustard extraction and hand digging. I found mustard extraction to be more efficient means for collection. For mustard extraction, 3.78 liters of water was mixed with 40 grams of mustard powder (Mattison unpublished data). Inorganic mustard powder has been found to work much more effectively than organic mustard in earthworm extraction (Graff 2015). All of my collection took place within 100m of the shore of Tenderfoot Lake. To extract the worms, I poured half of the mustard solution into a 35 by 35cm box and waited for 5 minutes, putting worms in a collection container as they came to the surface. After the 5 minutes, I poured the other half of the mustard solution into the plot and collect the rest of the worms. This was conducted for 5 more minutes (collection lasting a total of ten minutes). All adult worms were identified to the species level and Lumbricus *terrestris* were used in the experiment. For the high worm density pots, approximately 4g of worms were added. Control pots did not contain any earthworms in order to determine the normal rate of germination. When worms were found dead at the surface of the soil they were replaced. All pots were given 150mL of water when they began to dry out. After four and a half weeks the pots were removed from the lights and each seedling was counted.

Statistical Analysis

Germination rate of each of the 48 pots was calculated by dividing the number of seeds sprouted by the total number of seeds in the pots. Only one seed out of 3,600 mint seeds sprouted so it was removed from the analysis in order to normalize the data. With the mint removed I transformed my data using an arcsin square root to make it normally distributed. I used a two-way ANOVA to compare the germination rates for each species (mint excluded) between absence of earthworms and presence of earthworms.

Results

There was no significant difference in germination rate between the pots with earthworms and those without for *Lycopus americanus, Oenothera biennis, and Calamagrotis canadensis* (2-way ANOVA, F_2=0 .22, p=.8024; Fig. 3). There was no interaction between species but each species germinated at a different rate (*p* =3.18e-5). The Tukey test between germination rate and species showed a significant difference in the means (*Lycopus americanus-Calamagrotis canadensis* p= 3.42e-5; *Oenothera biennis-Calamagrotis canadensis* p=0.0414; *Oenothera biennis-Lycopus americanus* p=0.0301; Fig. 4). The Friedman test including the the *Mentha arvensis* suggested an increase in germination with the presence of earthworms (χ^2_1=4, *p*= .0455; Fig. 5).

Discussion

As expected there was a significant difference in the mean germination rate for each species (F = 14.41; d.f.= 2; *p* =3.18e-5). This means that Lycopus *americanus*, Oenothera biennis, and Calamagrotis *canadensis* had different germination rates from one another. All species are independent of one another so it was not surprising that each had quantitatively different germination rates.

In order to normalize my data, I decided to remove Mentha *arvensis* from my data for all parametric tests. I could not run an ANOVA including *M. arvensis* due to its low germination rate causing an inability to normalize my data. After the collection of my data I wanted to determine what could have caused the low germination in my mint seeds. I found that mint can be difficult to grow from seed indoors and prefers a strong light source (Thompson and Grime 1983). To improve my experiment, I would move the lights closer the surface of the soil. Light may have been a limiting factor on germination rates overall. In order to use the data, I collected on the *M. arvensis* I ran a Friedman test to see if there was a trend in the data (Fig. 5). The Freidman test did show a significant difference between the control and the experimental pots. Due to the low germination of the *M. arvensis* and the lower power of the non-parametric test it is not a stable correlation. It is an interesting trend however. Earthworms seem to have increased germination in all species which is not what I had anticipated. The two-way ANOVA did not show a significant difference between the experimental and control (Fig. 3). In all treatments with *L. terrestris* had higher germination than in the treatments with worms absent just not to a significant degree.

Laossi et al. (2009) tested the effect of earthworms on maternal seed production and germination of those seeds. They found that *Lumbricus terrestris* increased the growth and nitrogen content of mother plants of *Poa annua, Cerastium glomeratum* and *Veronica persica*. However, *L. terrestris* decreased the germination of *V. persica* seeds through maternal effects like seed viability and seedling growth. It is possible that earthworms are beneficial to the first generation of plants but can be detrimental to plant reproduction. This could be one explanation for why there was not a decrease in germination of the seeds in my experiment. Earthworms could provide enhanced growth to plants giving non-native species a competitive edge over native plant species. A study by Brewer and Bailey (2014) found that competitive effects of non-native species on native species is greater in habitats in which high growth rates allow for a competitive advantage. It would be interesting to investigate if earthworms provide a competitive advantage by increasing growth rates of herbaceous plants. While *L. terrestris* was not found to significantly alter germination rates of the four herbaceous plants in this study it is possible that more sensitive species could face consequences from the introduction of earthworms.

Acknowledgments

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Figures

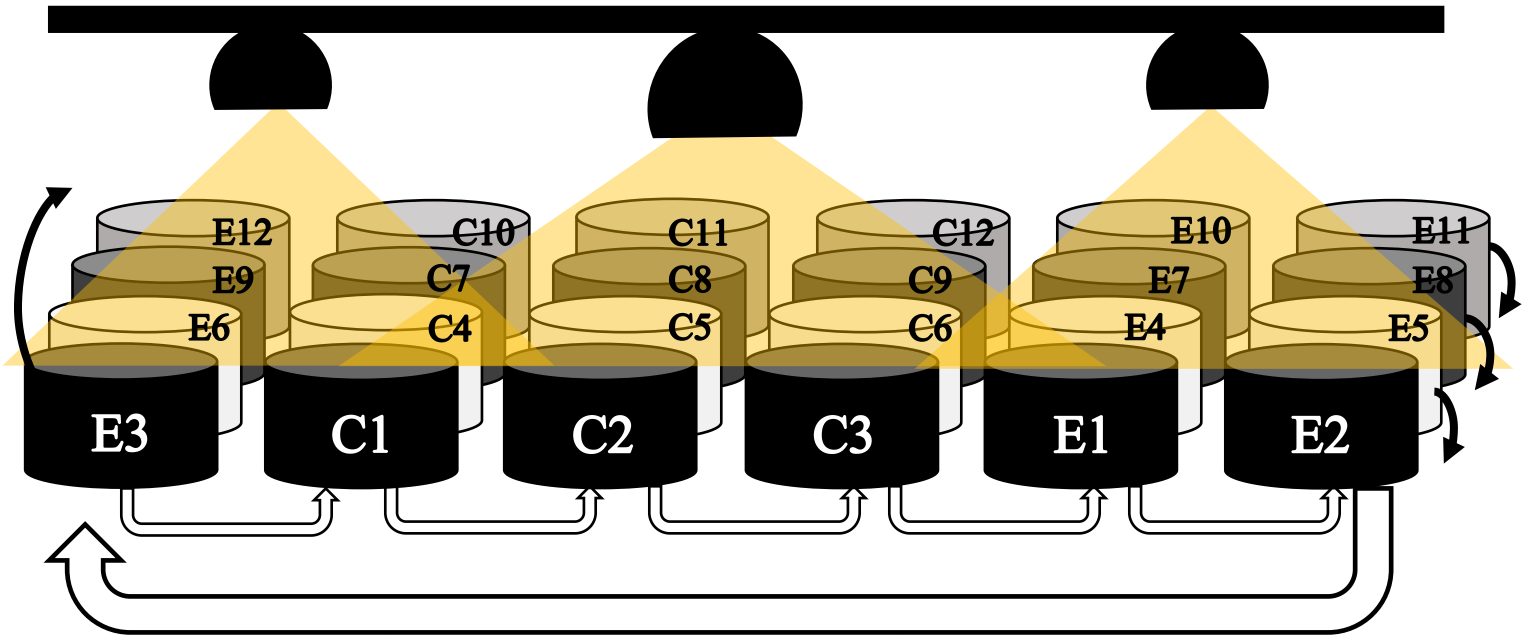

Figure 1. The above figure presents the experimental setup of germination pots. Solid arrows show the weekly rotation to move the row of species in order to minimize light variation between the plant species. The hollow arrows show the daily rotation to minimize light variation between experimental and control. The different color rows represent the four species. The E# stand for the experimental pots and the C# stand for the control pots.

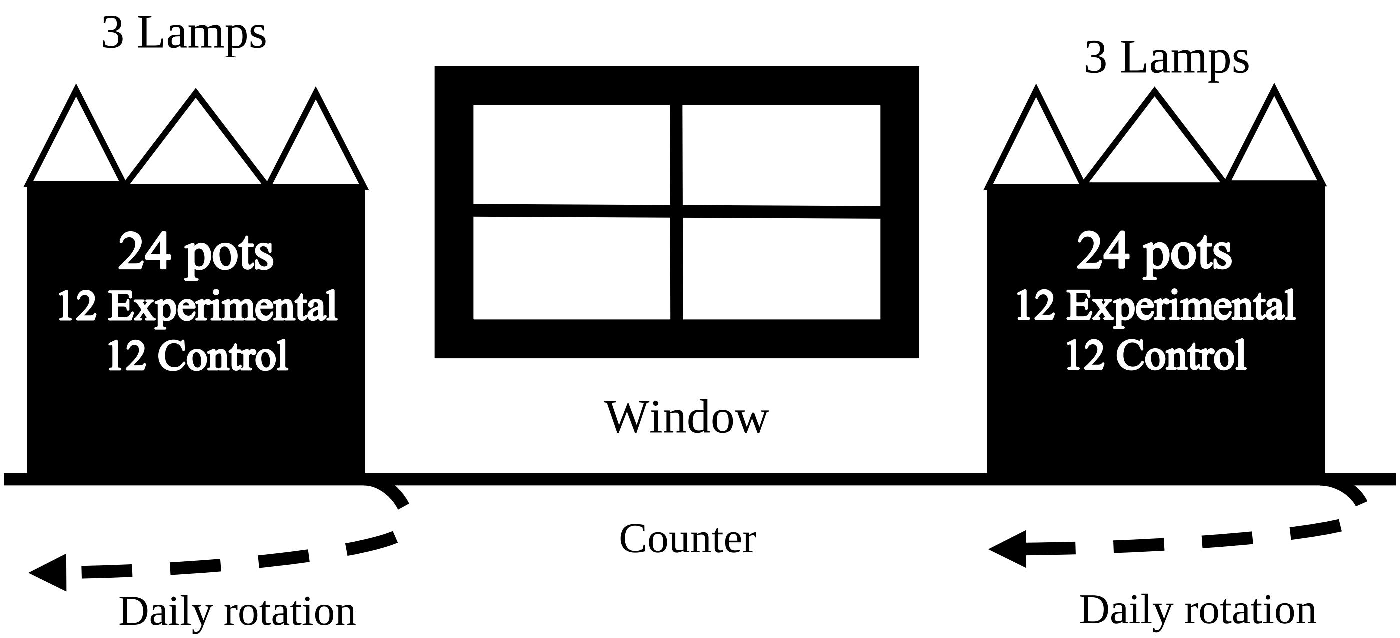

Figure 2. The figure above models the experimental setup of my experiment in the Wet Lab on the UNDERC property.

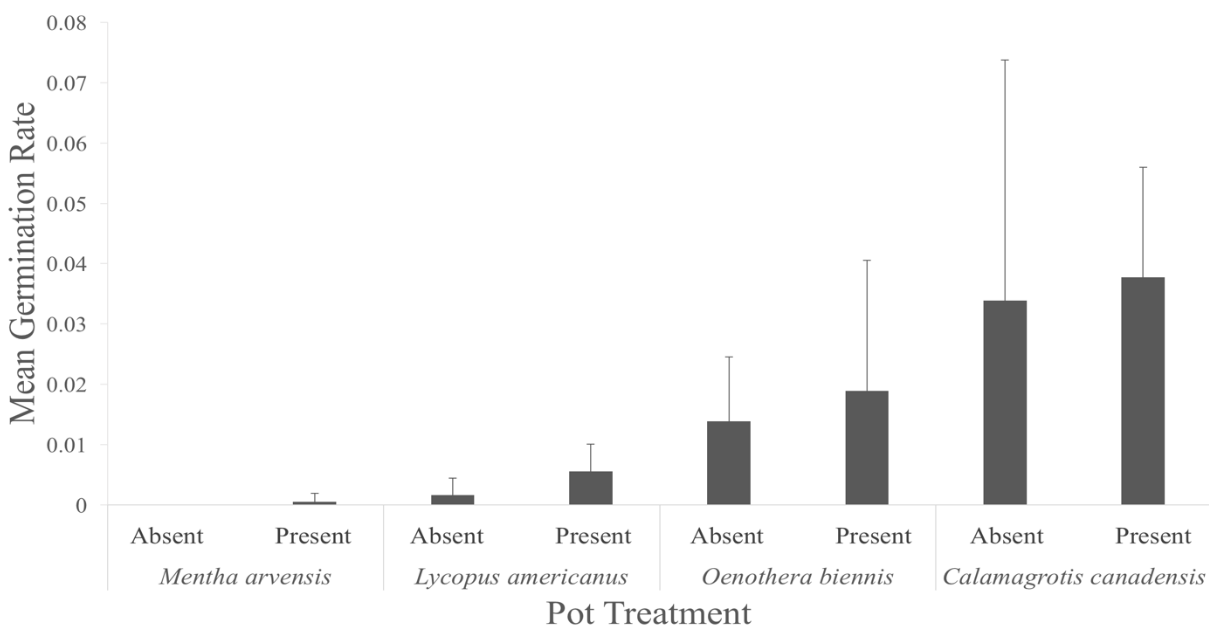

Figure 3. The mean germination rate for each treatment type, including the mint control and experimental pots. Mint was removed from the 2-way ANOVA and there was no significant difference in germination rate between the pots with earthworms and those without for *Lycopus*

americanus, Oenothera biennis, and Calamagrotis canadensis (2-way ANOVA, F_2=0 .22, *p*=.8024; Fig. 3).

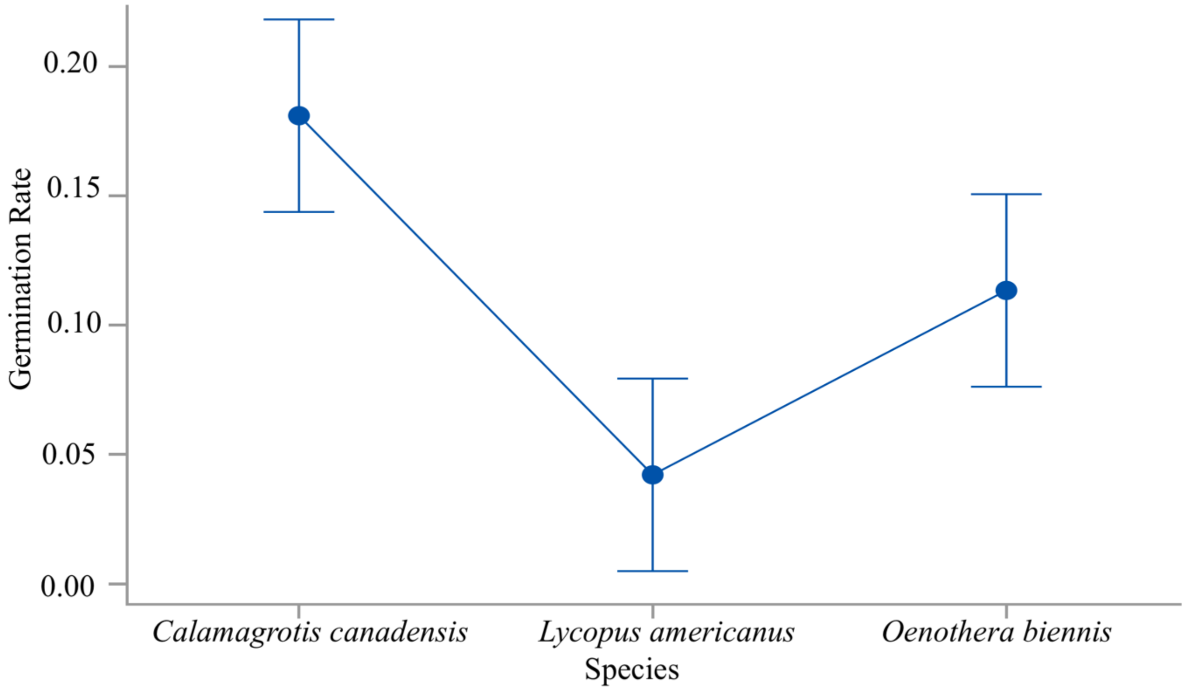

Figure 4. Germination rate compared between species. Germination rate was calculated from number of seeds sprouted out of total seeds planted. A 2-way ANOVA showed a significant difference between the means (F = 14.41; d.f.= 2; *p* =3.18e-5). The Tukey test results showed significant differences between the means of all three groups (Lycopus-Calamagrotis: *p* = 1.83e-5, Oenothera-Calamagrotis: *p* = .0358, Oenothera-Lycopus: *p* =.0250).

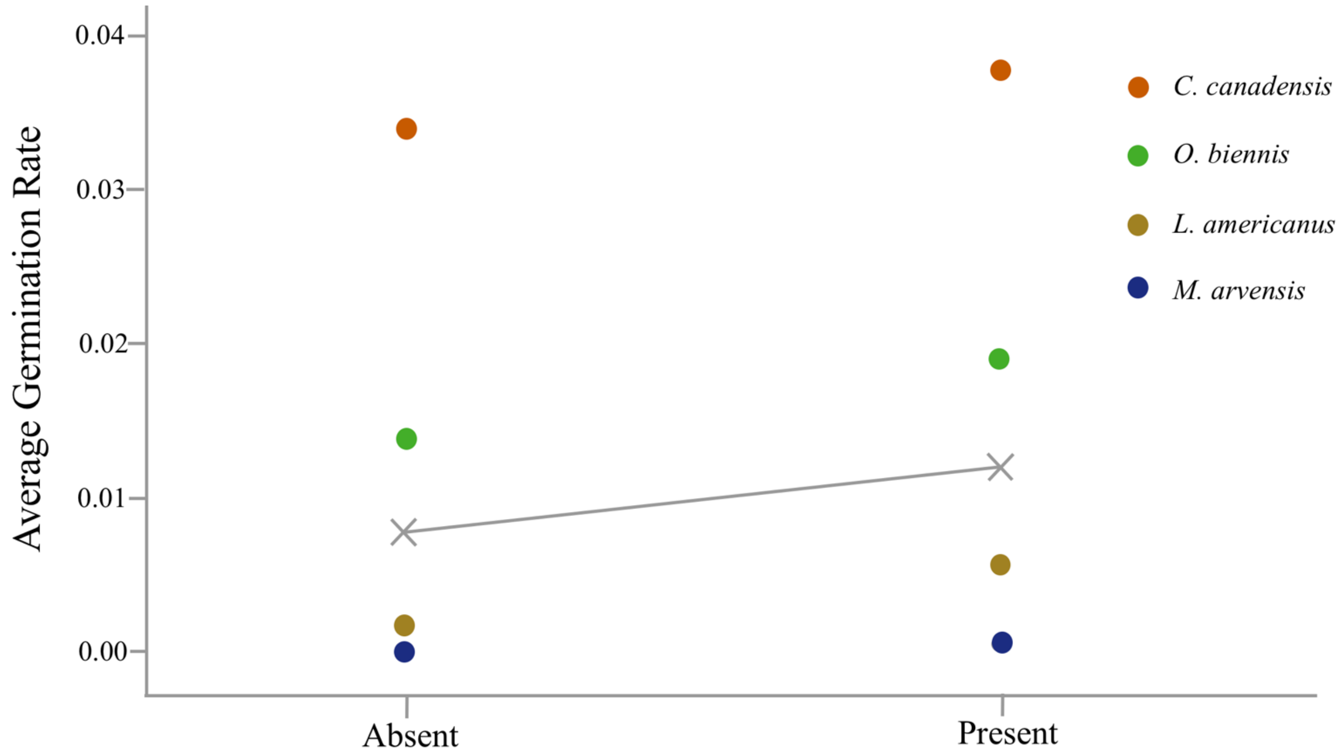

Figure 5. The Friedman test showed a significant relationship between germination rate and presence of earthworms (χ^2_1=4, *p*= .0455). The grey line shows the positive trend of germination rate with the presence of *L. terrestris*.