Non-native Earthworm Taxis and Facilitation Involving Invasive Species in North America

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Abstract

Non-native earthworms exhibit a positive taxis towards Amur Honeysuckle (*Lonicera maackii*), and possibly other invasive plant species, compared to native North American plant species. Following previously shown information about the ecological impacts of Non-native earthworms in North America, we set up an experiment involving choice chambers of native Red Maple (*Acer rubrum*) and Non-native Honeysuckle and Callery Pear (*Lonicera maackii* and *Pyrus calleryana*) leaf litter, counting earthworms in each side to measure their taxis. Though no significant taxis was reported for *P. calleryana*, a significant positive taxis was found for *L. maackii* and found to be caused by the chemical composition of the plant's leaf litter.

Introduction

Earthworms are known to impact ecological systems, particularly plant communities, and the impact varies greatly by the system in which they are inhabiting. Generally, earthworms have been considered great for agriculture and research has suggested numerous benefits arising from their presence. Rombke et al. (2005) found that earth worms have a distinct ability to engineer their own habitats; they provide nutrients to plants, relocating seeds, and improve the activity of the microbial community. The overall effect found was an improvement in soil structure, water filtration, and agricultural yield. Rombke et al. also stated that while some earthworms, such as *Eisenia Fetida*, can improve erosion by creating water-stable aggregates, they can also have detrimental effects on the soil, causing erosion by removing surface litter. Noted by Frelich et al. (2019), the impact that earthworms have on their environment has been found to be far more complicated than it may initially seem; non-native earthworms accelerate leaf litter decomposition and upper-layer soil mixing, destroying the litter layer, and ultimately cause cascading effects that may even facilitate non-native plant species.

Evidence exists of a positive feedback cycle between non-earthworms and non-native plant invasion, where non-earthworms will in turn benefit from the presence of the non-native plants they have facilitated (Frelich et al., 2019). This idea is further supported by Stinson et al., (2018) who had concluded that the invasive plant Garlic Mustard (*Alliaria petiolata*) facilitates the arrival of non-native earthworms, suggesting broader effects in regard to invasive species as a whole.

Invasive plants have had numerous detrimental effects in North America, two of note being Amur Honeysuckle (*Lonicera maackii)* and Callery Pear (*Pyrus calleryana*). *L. maackii* is native to Northern China and surrounding areas, it was imported into the United States to aid with soil erosion issues. The plant suppresses local plant species survivorship, growth, and reproduction; and enhances decomposition and nutrient turnover processes (McNeish and McEwan, 2016). *P. calleryana* was initially an ornamental plant native to China and Vietnam. The species exhibits rapid growth and abundant flowering, along with a very wide environmental tolerance; such factors can potentially allow it to impede the establishment of other, particularly native, plants (Culley and Hardiman, 2007). Invasive plants such as *L. maackii* and *P. calleryana* alter nutrient cycling rates because of the rate at which their leaves decompose; the litter quality of *L. maackii* differs far more from native trees such as *Acer rubrum* and has high nitrogen and mass loss, whereas *P. calleryana* appears similar to native trees with the exception of lignin content (Boyce, 2022).

Further research into the relationship between non-earthworms and invasive plants was seen as necessary and provoked an inquiry into whether non-earthworms would favor the leaf litter of invasive over native plants. If a relationship between the two were to be found to be more concrete, steps could be taken to reduce the prevalence of invasive species by methods previously not considered, such as those involving the management of non-earthworm populations. We addressed this issue by comparing the leaf litter preference of the non-North-American-native *Eisenia Fetida* earthworms in choice chambers which held *L. maackii* or *P. calleryana* leaf litter and *Acer rubrum* as the control, hypothesizing that the earthworms would favor the non-native species. Equal numbers of earthworms were added to each side of the choice chamber, and then the numbers of earthworms were counted for each side of the chamber. If our hypothesis were correct, we predicted that there would be a significantly greater number of earthworms in the invasive leaf litter side of the chamber.

Materials and Methods

Experimental Design

The overall design for our study was to compare the number of earthworms on each side of a container ("Choice Chamber") divided between a non-native plant species' leaf litter and the leaf litter of a native plant. We placed a hardware cloth divider inside of a plastic container to create separate sides for the two types of leaf litter in each choice chamber. The non-native species we used were Amur Honeysuckle (*Lonicera maackii*) and Callery Pear (*Pyrus calleryana*). The native plant species was Red Maple (*Acer rubrum*). Leaves from each species were shredded and mixed with shredded newspaper, then dechlorinated water was added to each side of the choice chamber. Non-native earthworms of the species *Eisenia Fetida* were added to each side of the choice chambers. The containers were kept in a dark cabinet for one week before we counted the number of earthworms on each side.

Choice Chamber Setup

Thirty-one 725ml plastic containers were divided into two sides, each by a hardware cloth divider that had 40.3mm^2 openings. The lids of the containers had 1.6mm diameter holes to provide openings for air.

Experimental Procedure

Each choice chamber was separated into a native and invasive species side. Each table of students created one chamber with *P. calleryana* and one with *L. maackii*, both with a side containing Red Maple; roughly half of the choice chambers had *P. calleryana* and the other *L. maackii*. 10g of leaves of a non-native species and 10g of a native species were fragmented by hand over 30-second periods for each choice chamber. Each 10g of leaves were mixed with 15g of shredded paper before being placed into respective labeled sides of the choice chambers. 50ml of dechlorinated water was added to each side of the choice chambers, and then 10 earthworms of the species *Eisenia Fetida* were also added. Each choice chamber was placed into a dark cabinet for one week. After that time had passed, we recorded the number of earthworms on each side of the chamber.

Statistical Analysis

A chi-square test was used to test for statistical significance. We used 1 degree of freedom due to the testing of two categories; invasive and native. The *L. maackii* chamber calculated to a 9.522 chi-square, or a p-value between 0.01-0.001; *P. calleryana* came out to be a chi-square of 0.242, a p-value of 0.50-0.70.

Results

The choice chamber experiment, which had control for native species and experimental groups of non-native species, yielded extensive results. The main goal of the experiment was to gather data on whether there would be a significantly greater number of earthworms in the invasive leaf litter side of the chamber, with replication. Our results encompass every course section.

We counted the earthworms across 30 chambers with Honeysuckle (*Lonicera maackii*) and Red Maple (*Acer rubrum*) leaf litter, and 29 chambers with Callery Pear (*Pyrus calleryana*) and Red Maple. We measure the scaled values, which were collected by taking each recorded value divided by the sum of the invasive and native chamber sides, and then multiplying by 20. The standard error for the *L. maackii* chamber type was 0.5 for both the native and invasive halves. The *P. calleryana* chamber had 0.8 for the standard error of both the invasive and native halves. According to the scaled values and the data displayed on figure 1, *L. maackii* had a greater taxis for the earthworms with 13.8 more earthworms on the invasive side. *P. calleryana* had a somewhat lower taxi with 2.2 more earthworms on the invasive side.

We conducted a chi-square test with the expected frequency of 10 for each chamber side. The *L. maackii* chamber yielded a 0.001-0.001 p-value from a chi-square of 9.522; a significant value. *P. calleryana* yielded a p-value of 0.50-0.70 from a chi-square of 0.242; the observed taxis towards *P. calleryana* was not supported by statistical significance.

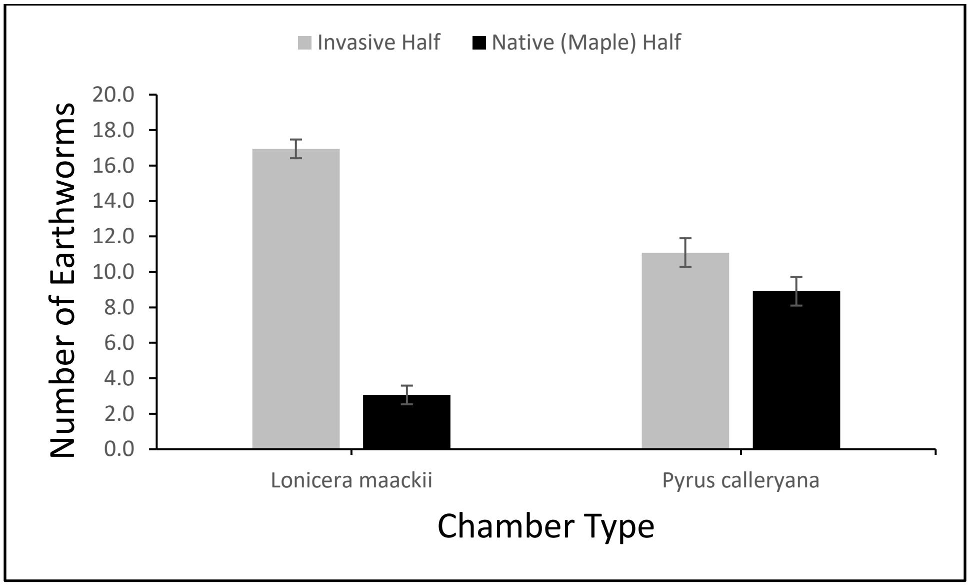

Fig 1. Earthworm count by chamber type. Each type of choice chamber by non-native species under study, Amur Honeysuckle (*L. maackii)* and Callery Pear (*P. calleryana)*, with their respective scaled earthworm counts after a week of isolation. Measured in a mean over all chamber instances. *L. maackii* had a mean of 16.9 to the 3.1 earthworms of *A. rubrum*. *P. calleryana* had a mean of 11.1 for *A. rubrum*'s 8.9 earthworms.

Discussion

In order to better understand the ecological impact of earthworms, an inquiry into whether earthworms preferred non-native over native plants was conducted. Specifically, we aimed to measure earthworm taxis for leaf-litter of two categories. The studied non-earthworms, *Eisenia Fetida*, exhibited a positive taxis towards Amur Honeysuckle (*L. maackii*) leaf-litter over Red Maple (*A. rubrum*) with statistical significance, though the observed inclination towards Callery Pear (*P. calleryana*) was insignificant. Though the results differ greatly for both chamber types, and there are possible reasons for such a result, we cannot conclude that the data supports the hypothesis of earthworms preferring non-native species; however, the data contributes to the broader concept of the facilitation cycle between invasive plants and earthworms.

Earthworms have been recorded to prefer certain litter types over others with a preference for nitrogen-rich material (Curry and Schmidt, 2006). It is also known that *L. maacki* leaf litter consists of greater nitrogen than *P. calleryana* in terms of proportion to carbon, and *P. calleryana* was found to be similar to *Acer rubrum* in decomposition such that it would not disturb native ecosystems in eastern North America (Boyce, 2022). It is reasonable to then conclude that some non-native species are not preferred by earthworms, explaining the discrepancy between *L. maacki* and *P. calleryana* taxis. Moreover, some invasive species could create far more desirable environments for earthworms, specifically non-native earthworms.

Previous research in non-native earthworm facilitation has shown the spread of invasive plant species to be associated with a rise in earthworm presence (Stinson et al, 2018). Furthermore, the removal of invasive plants is accompanied by a significant drop in non-native earthworm population size (Madritch and Lindroth, 2009). Earthworms are significantly associated with a positive taxis towards non-native plants, though our study shows a slight limitation regarding chemical composition.

Further research into the subject matter may be necessary to discover whether other non-native plant species of North America follow similar taxis patterns for non-native earthworms. To further extrapolate such findings, broader data could be collected, such as individual leaf litter quality and chemical composition, along with the rate of decomposition. An approach with more variety in plant species may be beneficial, as well.

Our data becomes particularly interested when these previous findings are contrasted with those of Frelich et al. (2019), non-native earthworms facilitate invasive species; conversely, invasive species facilitate non-native earthworms. However, we have found that not all non-native plant species participate in this facilitation cycle, possibly determined by chemical composition. Regarding the experiment itself, we found a positive taxis towards *L. maacki* specifically, though the hypothesis of both non-native plant species provoking a positive taxis for *Eisenia Fetida* could not be wholly supported. This result suggests a more complex picture of the non-native earthworm and invasive plant species facilitation cycle in North America could be more complex than previously known.

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