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Developing methods to assess the role of *Lumbricus terrestris* on seed predation in prairie restoration

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DEVELOPING METHODS TO ASSESS THE ROLE OF *LUMBRICUS TERRESTRIS* ON SEED PREDATION IN PRAIRIE RESTORATION

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ABSTRACT

Tallgrass prairie used to cover Iowa and much of the midwest, but now only a small percentage of it remains. Many prairie restoration efforts are in place to revitalize Iowa's former prairie landscape. Native prairie seed that is genetically diverse and regionally appropriate is expensive, and only 5-10% of the seeds planted germinate into seedlings. Ungerminated seed loss can occur many ways, one being due to seed predation. The common earthworm, *Lumbricus terrestris*, is a seed predator that has been observed to be present at prairie restoration sites by its numerous middens. The goal was to develop methods that would allow future researchers to study how significant of a seed predator *L. terrestris* is in prairie restoration sites. A series of trials were run in the laboratory to test if the methods used were successful tools to study earthworm granivory. The species of seeds offered and the density of seeds offered were only variables altered. The results confirmed that earthworms will eat native prairie seed. The native seeds they consumed the most included *Solidago rigida* and *Monarda fistulosa*. The results from the density trials indicated that as the density increased, the number of seeds the earthworms consumed also increased. The next step is to develop a model system where earthworm seed predation can be measured in soil, with the focus being on developing methods for tracking/recovering seeds.

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BACKGROUND & INTRODUCTION

About 10,000 years ago, the climate warmed causing glacial ice sheets to retreat from the midwest, altering the landscape (U.S. Department of the Interior, 2022). This change created a suitable environment for a thriving prairie ecosystem. Prairie used to cover a third of North America's 3.7 million square miles, and was located predominantly in the midwest (U.S. Department of the Interior, 2022). Prairies were, and are, incredibly resilient thanks to their deep and complex root systems which allowed them to successfully adjust to the hot/humid summers and cold/bitter winters characteristic of the area. Their deep root system also allowed them to survive when wildfires repeatedly tore through the grasslands . The fires killed any brush and trees that were not native to the prairie, allowing deep rooted native prairie plants to regrow without competition from faster growing and shallow rooted plants. The fires also helped return rich nutrients to the soil and burn away dead material resting above ground. Over thousands of years, this cycle created very rich and fertile soil (Iowa Prairie Network, 2022).

When European settlers moved westward across the United States in the 19th century and discovered the tallgrass prairies in Iowa, they also discovered that Iowa had superior soil and very bountiful lands ideal for agriculture– but they were not the first. The lands were already occupied and being utilized by local Native American tribes, but not to the scale that the Europeans intended to take it to. The Native Americans were very knowledgeable about how to live off of the land and knew it was bountiful/fertile. Indigenous peoples used the land in ways that did not permanently alter or destroy biodiversity (VOA, 2012). They traded and shared some of their knowledge with the Europeans, with one of the most significant trades being corn seed and the knowledge of how to mass grow it (VOA, 2012). The Europeans utilized this information and plowed the prairies anywhere that was suitable for domestic crops, like wheat and corn. While Iowa has become an agricultural powerhouse, the tallgrass prairies have suffered immensely, with only a small percentage of them remaining (Smith, 1998).

Today, only 0.1% of Iowa's native tallgrass prairie is still around, making a once very ecologically rich landscape one of the most endangered ecosystems in the world (Smith, 1998; Gruchow, 1995). Many prairie restoration efforts to revitalize prairie lands are in place by different entities across the country. The Tallgrass Prairie Center, located in Cedar Falls, Iowa, is one of those entities that is working to return Iowa to its former glory through research, education, and action (Figure 1).

When restoring prairies, a lot of factors have to be considered. Using a genetically diverse population (to make the prairie a more stable and weed resistant system) and regionally appropriate seed (that is biologically adapted for a specific area's climate) is essential. But the type of soil that predominates in the location, the light requirements of the species, the geological distribution of the region, and the phenology and life span of the plants are just some factors that go into selecting seeds for a prairie seed mix (Tallgrass Prairie Center, 2022). One more significant factor that influences the types and amount of seed that is used in prairie restoration is the cost of the seed. Native prairie seed prices are extremely high and can cost more than a thousand dollars per pound (Tallgrass Prairie Center, 2022).

In addition to native prairie seed being very expensive, seedling establishment rates of native prairie seed is low with only 5-10% of planted seeds actually germinating into seedlings (Williams et al., 2007). There are many events that can occur to prevent a seed from germinating into a seedling, including improper planting, seed dormancy, removal from the site (due to erosion or weather), and soil pathogens. Another event that can play a major role in seed loss is seed predation (or granivory). Seed predation is when seeds are used as a primary food source for animals. It is known that granivores, such as small mammals, birds and insects, are very prevalent in prairie fields and consume significant amounts of native prairie seed (Meier et al., 2020; Riebkes et al. 2018). In order to hopefully increase the seedling establishment rate, previous research has assessed how significant seed predators are to the removal of native prairie seed, and if there are ways to deter them until seedlings have emerged (Pellish et al., 2017; Riebkes et al., 2018).

It is common to witness granivores such as mice, deer, squirrels, insects and birds foraging for seeds or nuts. Most people are not aware that earthworms are granivores as well. In order to get a better understanding of where the ungerminated prairie seeds are going, the seed predator, *Lumbricus terrestris* (also known as the common earthworm), was the focus for this research. Earthworm middens have been widely observed at prairie restoration sites, so it is known that they are present and active. Methods for studying how significant a seed predator *L. terrestris* is in prairie restoration have not been fully developed by the scientific community. The goal of this research was to develop methods that would allow future researchers to eventually answer the big question: How significant of a seed predator is *L. terrestris* is in prairie restoration sites?

Figure 1: Irvine Prairie near Dysart Iowa, a eastern Iowa prairie restoration site established and managed by the Tallgrass Prairie Center. This was the location for previous seed predation research (Meier 2020).

LITERATURE REVIEW

In 2020, I conducted a precursor to this study which centered around how sacrificial seed (inexpensive seed used as a "sacrifice" to satisfy granivores in hopes of decreasing native prairie seed loss) impacted native seedling establishment/germination in prairie restoration. Part of that research included observing seed loss from seed cards in a buffet-style experiment. Seed cards were an experimental tool we used to assess granivory; they were 11 cm x 14 cm cards with consistently twenty seeds adhered to them. The seed cards were secured to the ground for a period of time in order to be accessible by nearly all seed predators. After the experimental period was over, the seeds were counted to observe and quantify the loss. Control cages were built to exclude seed predators but still allowed exposure to the weather. Our first set of control cages did not exclude insects, but our second set of control cages excluded all predators. In this study, one of our tentative observations was that roughly 30% of seeds were removed by small mammals, birds, and possibly earthworms; roughly 55% of seeds were removed by insects; and roughly 3% of seeds were removed by weather (Meier et al., 2020). This data showed that seed predation is widespread, many species participate, and more research needs to be gathered on specific seed predators (Betzer, 2020). During this study we saw many earthworm middens over the experimental area (a freshly planted prairie restoration area on retired farm ground). The density of the earthworm middens led us to wonder about how much of a role they played in the prairie seed loss.

In an article published in 1973, McRill and Sagar discuss an experiment done with earthworms and seeds. *L. terrestris* was placed in a petri dish with damp filter paper and a variety of seeds (14 species and sizes). The earthworm was left in a dark, room temperature environment for 18 hours before it was removed and placed into a new petri dish. Any remaining seeds were counted in the first dish; the earthworm's casts were recovered in the second dish and any seed found in casts were counted and tested for viability. This experiment demonstrated that earthworms are granivores, they will consume a variety of seed types, and some seeds are still viable after passing through *L. terrestris’* gut.

In the 2006 experiment by Perreault and Whalen titled “Earthworm burrowing in laboratory microcosms as influenced by soil temperature and moisture”, some important methodology questions were answered. In their study, earthworms were held in large (37 L) plastic containers filled with soil from their field site. Litter, including ground soybean stems, leaves, and composted cattle manure, was added to the soil surface as a food source for the earthworms. The experimental chambers were placed in incubators that controlled temperature ranging 5ºC to 20ºC. These methods may be useful to assess earthworm seed predation in the laboratory, but it does not offer insight on how to track/recover the seeds *L. terrestris* eats.

Part of Perreault and Whalen (2006) experiment tracked earthworms’ weight gain over a period of time in specific conditions. So, before being introduced to their experimental habitat “earthworms were placed in plastic jars on damp paper towels to void their guts for 24 [hours], then patted dry and weighed” to obtain their initial empty gut weight (Perreault & Whalen, 2006). They tested a range of soil temperatures and soil moisture to determine the ideal conditions for earthworms to thrive, measured by the amount of burrows, surface castings, activity, and eventual weight gain. At the end of the 1 week experimental period, earthworms were removed from their chambers, voided their guts for 24 hours in a plastic jar, and were weighed to obtain their final empty gut weight. It was determined that "the greatest weight gain by *L. terrestri*s was recorded at 20ºC [68ºF] in soil moistened to 5 kPa".

This study offered great insight into laboratory methods to observe earthworms and the ideal conditions for earthworm activity. Conducting laboratory experiments at 20ºC would likely yield the best results.

In a 1990 doctoral thesis by Daniel, one of the topics discussed the life cycle of the earthworm, *L. terrestris*. He collected juvenile earthworms and soil from his experimental site to use in the study. Earthworms were placed in experimental chambers where their conditions could be controlled. Multiple experiments were conducted, each running for 1 week, with one variable (soil temperature, soil moisture, food availability) being adjusted each time.

The results of one of his experiments showed that earthworm "food consumption increased with rising temperature" until the temperature reached 21ºC, where food consumption decreased. No earthworm activity or consumption occurs lower than 0ºC or higher than 28ºC. The upper lethal temperature limit for *L. terrestris* is about 28.5ºC. This is very similar to the results found by Perreault and Whalen (2006) later on.

Daniel also found evidence that the behavior of *L. terrestris* is age specific; juvenile earthworms are more likely to be epigean, close to the soil surface or on top of the soil surface; and adult earthworms are more hypogean, living in deep burrows. Daniel's (1990) study provided environmental parameters for my study, and useful information on the best life stage for studying granivory of *L. terrestris*.

A very recent (2022) study by Regnier et al. discussed how non-native earthworms shifted seed predation dynamics of a native weed, giant ragweed. In a 2 year study, these researchers analyzed "relative rates of seed removal by earthworms and mice" of seeds dispersed at various times and in varied vegetative cover. They set up plots in 10 sites; half in low plant cover habitats and half being in high cover habitats. Seasonal patterns, temperatures, and weather were recorded and taken into account. Earthworm and mice population densities were also taken into consideration. (Mice population density was assumed to be insignificantly changing, earthworm population density was calculated using earthworm middens.)

Seed removal was monitored by attaching 40 cm long sewing threads to giant ragweed seeds and dispersing them along transects in the plots. Stakes marked the opposite end of each string. Additional free giant ragweed seeds were dispersed to simulate natural seed distribution. Tagged seeds were checked on frequently and categorized as intact, buried by earthworms (thread drug into burrow opening), or predated on by mice (damaged, detached, teeth marked).

Results of the study showed that earthworms were the predominant seed forager under warmer and wetter conditions in low plant cover habitats; while mice obtained "more seeds under colder, drier conditions and in high cover habitats". Environmental factors (temperature, weather, etc.) influenced which seed predator got to the seeds first, and the first one there usually became the predominant forager.

The study proposed that *L. terrestris* may behave mutualistically with giant ragweed– earthworms buried seeds at depths that were out of reach for mice, but "still conducive to seedling emergence" in all experimental conditions. These earthworm caches of seeds reduce seed availability for rodents and act as seed dispersal mutualists with giant ragweed. "The ability of *L. terrestris* to compete with rodents for seeds in all test environments suggests *L.*

terrestris may affect seed predation dynamics of other large-seeded species in other plant communities."

This study correlates very nicely with the present study– it discussed methods for comparing seed predation between earthworms and other granivores. It confirms that earthworms do forage for seed in crop fields and grass pasture environments. It also proposes a new idea of earthworms being mutualistic to a specific kind of plant. This opens up the possibility that earthworm seed foraging could be either beneficially impactful (keeping viable seeds away from other seed predators) or disadvantageous (consuming seeds as much as or more so than other seed predators). If replicated, their methods could be useful for tracking/recovering seeds in outdoor earthworm seed predation experiments.

As mentioned, a precursor to this study was conducted at a prairie restoration site on retired farm ground. While there, we observed many earthworm middens over the course of the entire area designated to the prairie. With only a small percentage of prairie seed actually germinating into seedlings, this suggested to us that earthworms may play a role in the removal of native prairie seed in prairie restoration sites. The literature reviewed contained many helpful starting points, but methods to study this phenomenon are not fully developed. With the overarching goal to someday improve prairie restoration successes, once methods can be established to test earthworm seed predation, we will be one step closer.

METHODS

To develop methodology that would allow me to study how significant a seed predator L. terrestris is, I started in an indoor controlled environment with the intention to expand from there. Studying *L. terrestris* in its natural environment is ideal, but outside there are so many other variables– such as other seed predators, unpredictable weather, and more– that do not have to be accounted for in a laboratory. Methods that needed to be developed prior to moving on to further outdoor phases included, but were not limited to, knowing: what seeds *L. terrestris* was most likely going to consume, how much does *L. terrestris* consume in a given time period, how long should the intervals between data collection be, the effect of seed density on consumption rates, and the suitability of methods used for other seed predators.

Beginning in a laboratory and running straightforward experiments that would have clear results was the starting point. With each laboratory phase, the goal was to more closely replicate the natural environment of *L. terrestris* until the methodology was refined enough to be able to take it outside to the prairie.

Many of the methods used in this study were based on the literature reviewed. None of the methods described in the literature perfectly fit the research's goal, but the design ideas from each source were helpful starting points when developing the methodology; adapting some of their methods so their purposes were suitable for studying earthworms as seed predators.

For the experiments conducted during this research, *L. terrestris* live specimens were obtained from a local earthworm producer, Hank's Live Bait & Tackle, and/or caught from the soil local to the target prairie restoration research sites. The same group of three dozen earthworms were used for two to three weeks before a new batch of three dozen earthworms were bought. Caught earthworms were used to intermix with the bought earthworms. The most active earthworms were the ones picked for each trial.

PHASE ONE:

Questions I attempted to address with Phase One methodology: Does *L. terrestris* eat seeds? What seeds will *L. terrestris* consume? How does the density of seeds affect how many seeds are consumed by *L. terrestris*?

A series of experiments were run in succession with a single variable being altered for each trial (Table 1). For all trials, adult earthworms (10 cm – 15 cm in length when extended) were removed from a refrigerator (~3ºC) and placed individually in 12 plastic containers (measuring roughly 12 cm x 12 cm x 5 cm; Figure 2). Each plastic container had small perforations in the lid to allow for gas exchange. A damp paper towel was placed at the bottom of each plastic container to keep *L. terrestris* moist and comfortable during the trial period (McRill & Sagar, 1973). Each trial was run in a dark, temperature-controlled (~20ºC) room for 24 hours (Perreault et al., 2006). The amount and/or type of seed placed in the container was variable trial to trial. After each trial, the seeds remaining in each container were counted and recorded. Earthworms were placed back at ~3ºC until they were needed again.

These methods restricted *L. terrestris* to only having access to the food source provided and made it possible to accurately quantitate the food source remaining.

To begin, I wanted to get an idea of what types of seeds *L. terrestris* was inclined to consume. The first trial began on April 6, 2022. It consisted of three types of native prairie seeds (*Eryngium yuccifolium, Heliopsis helianthoides, and Asclepias syriaca*) in four containers each. Each container contained ten seeds. These species were chosen because they were native, varied in size, were plentifully available, and helped us learn what *L. terrestris* would be inclined to consume.

Since *L. terrestris* did not consume many seeds in trial one, I replaced the types of seeds offered. The second trial began April 13, 2022. It consisted of one type of native prairie seed (*H. helianthoides*) and two other non-native model seeds (*Sesamum indicum* and *Salvia hispanica*) in four containers each. Each container contained ten seeds. These species were chosen because *L. terrestris* only showed interest in one of the native seed species from trial one, so two model species were offered in order to confirm that the environment this method used was suitable. *S. indicum* and *S. hispanica* were good options because they are high in fats which I hypothesized would make them more desirable.

At this point, I had an idea of the seed size and composition that the earthworms were preferring. The third trial began on April 21, 2022. It consisted of two types of native prairie seeds (*Solidago rigida* and *Monarda fistulosa*) and one other non-native model seed (*S. indicum*) in four containers each. Each container contained ten seeds. The two native species were chosen because they resembled the oily non-native model seed *S. indicum*, which the earthworms were consuming well.

After establishing that *L. terrestris* would consume seeds well with these methods, I changed the quantity of seeds offered and kept the seed type the same for the next two trials. The fourth trial began on April 23, 2022. It consisted of two quantities of one type of non-native model seed (*S. indicum*) in six containers each. The first set of six containers contained ten seeds each, the second set contained twenty-five seeds each. This species was chosen because it was cheap, I had a plentiful amount of it, and it was being consumed well by the earthworms (which was the most important because the density was the variable being altered for this trial).

The fifth trial began on May 2, 2022. It consisted of two quantities of one type of non-native model seed (*S. indicum*) in six containers each. The first set of six containers contained fifty seeds each; the second set contained one hundred seeds each. This was a continuation of density alteration observations that began in trial four with a model seed species.

Since the quantity of seeds offered used a model seed species in trials four and five, the next two trials used native seed species to see if the same results would be achieved. The sixth trial began May 3, 2022. It consisted of two types of native prairie seeds (*S. rigida* and *M. fistulosa*) in six containers each. Each container contained ten seeds. These species were chosen because they were consumed well by *L. terrestris* in trial three and they were a native prairie species. This was the first trial observing the effects of seed density with native seed.

The seventh trial began May 17, 2022. It consisted of two types of native prairie seeds (*S. rigida* and *M. fistulosa*) in six containers each. Each container contained twenty-five seeds. This was a continuation of density alteration observations that began in trial six with native prairie seed species.

Figure 2: Earthworms shown in plastic containers on damp paper towel squares with seeds scattered on the surface. The lids are removed in these photos.

Trial	Species of Seed	Native or Non-Native	# of Identical Containers	# of Seeds per Container
I	*Eryngium yuccifolium, Heliopsis helianthoides, Asclepias syriaca*	Native	4	10
II	*Heliopsis helianthoides*	Native	4	10
	Sesamum indicum, Salvia hispanica	Non-Native	4	10
III	*Solidago rigida, Monarda fistulosa*	Native	4	10
	Sesamum indicum	Non-Native	4	10
IV	*Sesamum indicum*	Non-Native	6	10, 25
V	*Sesamum indicum*	Non-Native	6	50, 100
VI	*Solidago rigida, Monarda fistulosa*	Native	6	10
VII	*Solidago rigida, Monarda fistulosa*	Native	6	25

Table 1: Summation of the seven trials ran during Phase One

PHASE TWO:

Questions attempting to address with Phase Two methodology: Are seed cards– a tool used to assess granivory in prairie restoration sites– a useful method to assess seed predation by *L. terrestris* (Meier et al., 2020)? What other methods can be used to monitor seed removal by *L. terrestris*?

Mini seed cards were made using 5.5 cm x 7 cm coarse rectangular segments of sandpaper (3M Paper Sheet 346U, 36 grit) with ten seeds randomly spaced and glued to the surface using a generous base layer and top coat of adhesive aerosol (3M Super 77

Multipurpose Adhesive Aerosol) (Riebkes et al. 2018, Meier et al., 2020). The procedure followed the procedure outlined in Phase One with one exception: seeds were only offered via seed cards inside of the plastic containers (Figure 3). Trial eight began on May 19, 2022 and ran for 24 hours. *S. rigida* seeds were chosen for this experiment because they are native prairie seeds and they were consumed well by the earthworms in phase one.

Figure 3: *L. terrestris* shown in a plastic container on a damp paper towel square with a seed card.

RESULTS

PHASE ONE:

The experiments in Phase One demonstrated that *L. terrestris* will eat seeds, and prairie seeds can be a part of an earthworm's diet (Figure 4). The experiments in Phase One also confirmed that the methods used were an effective design to test earthworm granivory in a controlled laboratory environment (Table 2). In this study, earthworms consumed only relatively small seeds, as they did not consume any of the larger *A. syriaca* (140 seeds/g) or *E. yuccifolium* (260 seeds/g). The native prairie seed they consumed the most was *S. rigida* (1500 seeds/g) and *M. fistulosa* (2500 seeds/g). They also consumed the model seed *S. indicum* (460 seeds/g) well.

Figure 4: *Lumbricus terrestris*

LUMBRICUS TERRESTRIS CONSUMPTION OF NATIVE PRAIRIE SEED

			10 seeds offered	**25 seeds offered**
		Seed Species	% seeds consumed	% seeds consumed
a.		*Asclepias syriaca*	0.00% ± 0.00% (N=4)	
b.		*Eryngium yuccifolium*	0.00% ± 0.00% (N=4)	
c.		*Heliopsis helianthoides*	7.50% ± 2.89% (N=4)	
d.		*Solidago rigida*	43.33% ± 11.89% (N=6)	35.33% ± 14.37% (N=6)
e.		*Monarda fistulosa*	43.33% ± 14.61% (N=6)	36.67% ± 13.74% (N=6)

Table 2: *Lumbricus terrestris* consumption of native prairie seed. Individual *L. terrestris* were offered a specific quantity (10 seeds or 25 seeds) of a variety of native prairie seed in 12 cm x 12 cm x 5 cm plastic containers containing only the seeds and a damp paper towel. Native prairie seed offered include *Asclepias syriaca,* 140 seeds/g (a), *Eryngium yuccifolium,* 260 seeds/g (b), *Heliopsis helianthoides,* 220 seeds/g (c), *Solidago rigida,* 1500 seeds/g (d), and *Monarda fistulosa,* 2500 seeds/g (e). Percentage of seeds consumed is shown as the average of the replicate experiments. Number of replicate experiments is indicated by N.

When the density of the model seed species was altered, two trends were observed. The raw number of seeds consumed by an individual *L. terrestris* in 24 hours increased as the seed density (number of seeds offered in the same area) increased, but not at the same rate (Figure 5). The percentages of seeds consumed decreased as the seed density increased.

SESAMUM INDICUM **SEED DENSITY VS.** ***LUMBRICUS TERRESTRIS*** **CONSUMPTION**

a.

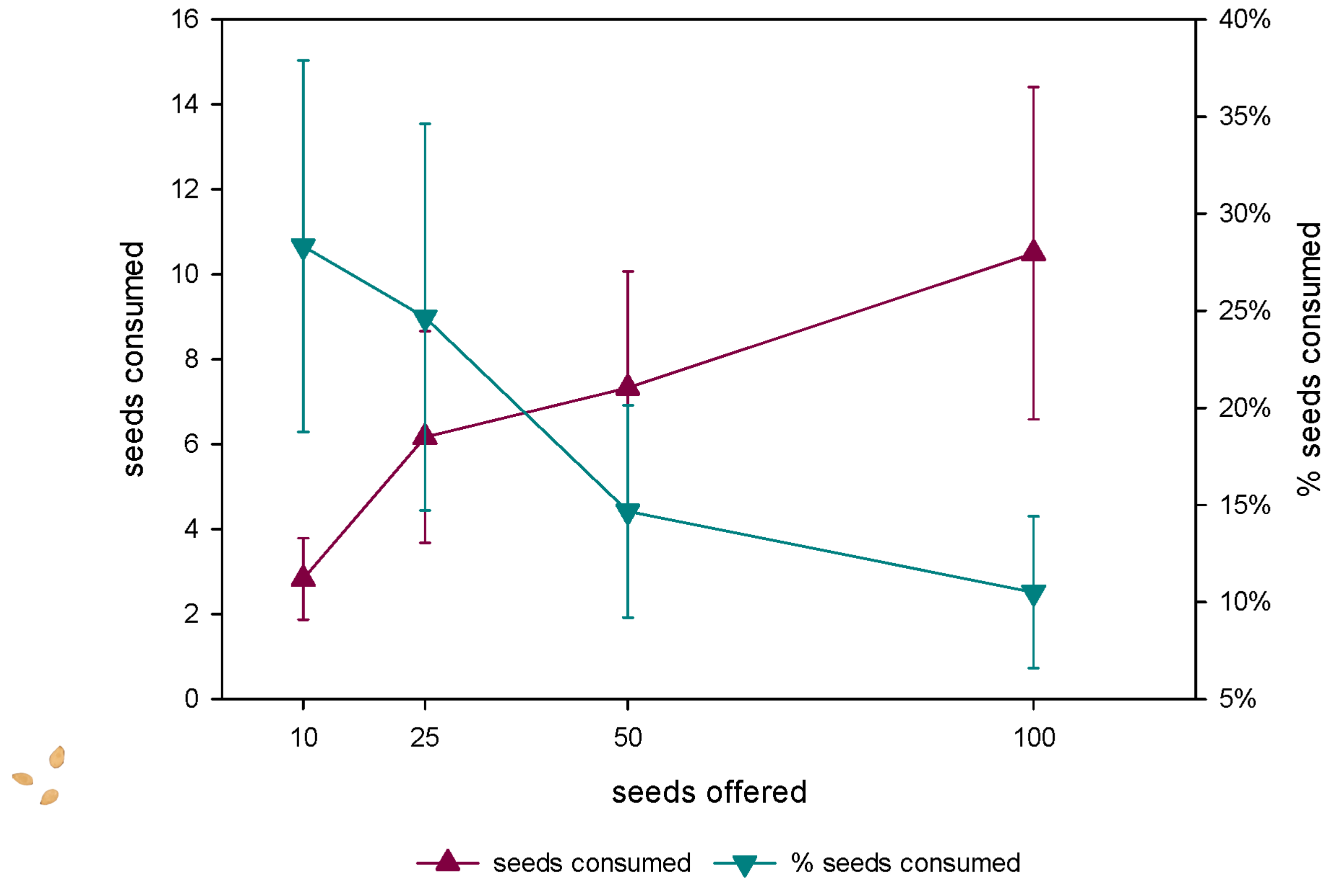

Figure 5: *Sesamum indicum*, 460 seeds/g (a) seed density compared to *Lumbricus terrestris* consumption. Individual *L. terrestris* were offered a specific quantity of *S. indicum* in 12 cm x 12 cm x 5 cm plastic containers containing only the seeds and a damp paper towel. Each quantity (10 seeds, 25 seeds, 50 seeds, and 100 seeds) was replicated 6 times (with 6 earthworms). Number of seeds consumed and percentage of seeds consumed are shown as the average of the 6 replicate experiments.

PHASE TWO:

No *S. rigida* seeds were removed from any of the seed cards in the twelve replicate containers in trial eight. The experiments in Phase Two confirmed that *L. terrestris* will not consume *S. rigida* seeds off of seed cards. A different set of methods will need to be developed in order to assess earthworm granivory in prairie restoration sites.

DISCUSSION & CONCLUSION

As previously discussed, many prairie restoration efforts are in place to revitalize Iowa's former tallgrass prairie landscape. Native prairie seed that is genetically diverse and regionally appropriate is expensive, and only 5-10% of the seeds planted actually establish mature plants. Ungerminated seed loss can occur many ways, one being due to seed predation. There are many types of granivores (birds, rodents, insects, deer, etc.) known, but the one seed predator this research focused on was the common earthworm, *L. terrestris*.

The goal for this study was to develop methods that would allow me and future researchers to study how significant of a seed predator *L. terrestris* is in prairie restoration sites. Two preliminary phases were completed in pursuit of this main question.

The results from the trials in phase one confirmed that earthworms will eat native prairie seed. The native seeds they consumed well included *S. rigida* and *M. fistulosa*, which are both relatively small seeds. Since a large variety of seeds were not tested, *L. terrestris'* specific seed size, texture, and composition preferences cannot be determined. The results from the density trials may indicate that the more seed that is spread/planted– the more seed that is offered to the earthworms– the more *L. terrestris* will try to consume. There may come a point where *L. terrestris* has reached its limit and an increase is no longer observed, but that needs further testing.

The next step is to develop a model system where earthworm seed predation can be measured in soil– first in a lab, and then in the field. The focus needs to be on selecting the right species of seeds and developing methods for tracking/recovering seeds. Questions that still need to be answered include: how can seeds be tracked or recovered once placed on soil (and tracked in a way that does not prevent the earthworm from predating upon it), how can you guarantee seed loss is from *L. terrestris* and not another factor, and does the quantity of *L. terrestris* in the soil matter and/or how can that be monitored.

Creating artificial earthworm biomes in the lab using large plastic containers and soil collected from local target sites would be an ideal way to test methods in a controlled way that could later be moved outdoors to *L. terrestris’* natural environment. Possible methods that could be used to track and recover seeds include: dyeing seeds with fluorescent dye or paint, placing them on soil surface, and using black light to help track remaining seeds; or attaching threads to seeds at one end, and a stake at the other end, and tracking where they get pulled to (Regnier et al., 2022). A preliminary trial of the thread tracking method was unsuccessful, but methods were not sufficiently well developed to present results here).

During the trials, even though every earthworm experienced the same environment before, during, and after the trials, some were much more active and/or hungry than others. Also, due to the nature of how my earthworms were obtained, age was unknown. This aspect needs to be more closely monitored in future experiments.

In the end, the overall goal is to have more successful, effective, and efficient prairie restoration. Researching ways to increase the amount of native seeds that germinate into seedlings by finding techniques to temporarily deter seed predators would be a huge advancement.

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