Examining how the presence of invasive swallow-wort (*Vincetoxicum* sp.) affects the abundance of earthworms

A Senior Honors Thesis

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Abstract:

Swallow-wort (*Vincetoxicum* sp.), an invasive plant in Western New York, outcompetes native species such as common milkweed (*Asclepias syriaca*). Swallow-wort may release allelochemicals that change the composition of the soil to benefit its own growth. Earthworms are also an invasive species in our area. In excess, they can till the soil too much, drying it out and making it difficult for any plants to grow. While overturning the soil has its benefits, too many worms can cause the soil horizons to merge, displacing minerals and loosening up the foundation for most plants. Because no one had ever studied the relationship between these organisms, I hypothesized that neither plot type nor season should affect the presence of earthworms. In addition, I had reason to believe that swallow-wort could not only hinder earthworm abundance, but it could also promote it. During the summer and fall, I marked 30 paired forest plots in Mendon Ponds and Oatka Creek Parks where swallow-wort bordered a neighboring patch of non-swallowwort. Within these areas, vegetation was surveyed and worms were collected using the liquid extraction method. The worms were identified, measured and weighed. While non-swallowwort plots tended to have more worms, swallowwort plots had more worm biomass. However, these differences were not statistically significant for either the summer or the fall. The presence of swallow-wort does not appear to affect the presence of earthworms. In addition, the season did not seem to have an effect on the presence of earthworms. These results supported both of my hypotheses.

Introduction:

Invasive species is a term that refers to organisms that are introduced to a place where they are not native due to humans (Beck et al. 2008). The presence of humans has been shown to amplify the distribution of non-native species, whether intentionally or not (Vitousek et al. 2008). Invasive species are known present major environmental problems including decreased biodiversity, compromised ecosystem integrity, the flux rates of energy (Ehrenfeld 2010) and even some human health problems (Lee 2002).

In Western New York, one of the many introduced species that have turned invasive is swallow-wort (*Vincetoxicum* sp.). This plant is related to the native milkweed (*Asclepias syriaca*) and was introduced into North America around 135 years ago from Eastern Europe (Weston et al. 2005). Swallow-wort was first seen in Cambridge, MA in 1854 and Toronto Junction, Ontario in 1889 (Lawlor 2003). It was introduced for ornamental as well as horticultural purposes, and eventually spread south and west. Swallow-wort can survive in areas with little to dense canopy cover, in dry and moist soils. It leafs out early and reproduces rapidly, allowing it to take over extensive areas (Averill et al. 2011). Swallow-wort is a versatile vine that is hypothesized to release allelochemicals which prevent plants other than itself from growing where it establishes. However, it was observed overtaking empty niches in temperate forest undergrowth rather than actively smothering natives during this study.

In addition to swallow-wort, another invasive taxa in forests of western New York are earthworms in the order Lumbriculida. Many deciduous and coniferous forests in North America lacked these invertebrates due to the end of the Wisconsinan Glacial Episode. It was not until around the 1700s (Frelich et al. 2006) when they were reintroduced by the settlements of Europeans in North America (Callaham Jr. et al. 2006). Earthworms are central in the process of breaking down detritus and cycling nutrients. They have also been shown to assist animal species as food and shelter sources. Salamanders use the worms' burrows to survive during the winter months (Ransom 2010). However, in excess they can till the soil too much, drying it out and making it difficult for any plants to grow. While overturning the soil has its benefits, too many worms can cause all the soil horizons to merge, displacing minerals and nutrients (Hale et al. 2007) and loosening up the foundation for most plants. Furthermore, by breaking down leaf litter, earthworms can make it difficult for litter-dwelling plants to establish and take root (Hale et al. 2005). They are also known to drastically change the understory in hardwood forests (Hale et al. 2006) which includes species richness and biodiversity (Holdsworth et al. 2007). In addition to affecting plant species, earthworms have shown to negatively impact animal species as well such as ground-nesting birds (Loss and Blair 2011).

While earthworms can make it difficult for some plants to establish and grow, they are known to be correlated with the expansion of non-native plants. Research on *Rhamnus cathartica* or common buckthorn, an allelopathic species, shows that in the presence of earthworms, seedlings were significantly higher than when no earthworms were present (Roth et al. 2015). Invasive shrubs such as buckthorn and honeysuckle (*Lonicera* sp.) produce high quality leaf litter. So, their presence is positively correlated with the presence of earthworms which are essential in the process of nutrient cycling (Madritch and Lindroth 2008). It has even been determined that earthworm invasion is the driving force behind the change in plant communities rather than the invasion of non-native plants (Nuzzo and Blossey 2009).

No research exists on the interaction between earthworms and swallow-wort. So, the objective of my research was to see the relationship between them and see if there was a correlation between their presences. Though it is true that earthworms help common buckthorn establish and grow, I hypothesized that the presence of the swallow-wort would not have an effect on the abundance of worms. My rationale for this is that worms could either be inhibited by swallow-wort's potential allelopathy, or they could feed on the leaf litter contributed by the plant. I also hypothesized that season would not have an effect on the abundance of earthworms. This would be warranted by the fact that trees drop their leaves in the fall which would increase the amount of food worms can eat, but at the same time, if any allelochemicals are present in the plants, it could deter them from eating the litter.

Methods:

Two local parks, Mendon Ponds and Oatka Creek, were selected for their deciduous/coniferous forests that were known to hold swallow-wort. A total of five sites were chosen based on canopy and ground layer composition. These sites included The Plantation (43°01'52.5"N 77°33'03.0"W), Devil's Bathtub (43°01'34.5"N 77°34'30.6"W), Devil's Bathtub Pine (43°01'22.9"N 77°34'29.5"W), Wild Wings (43°01'14.4"N 77°34'46.2"W), and the Black Trail in Oatka Creek (43°00'33.1"N 77°47'53.8"W). Areas within the woods that were 20 m away from manmade paths, had near to full canopy cover and contained swallowwort were selected. In order for a plot to be considered a "swallow-wort plot," swallow-wort had to cover 75% of the quadrat. These plots were paired with non-swallowwort plots on a 15 m transect. Plots that had other vegetation or litter were considered "bare" plots. Each 1x1 m quadrat was 10 m away from the other. Worm sampling took place twice- once in the summer and once in the fall- on days when it had not rained for around 3 days. However, sampling did take place when there was snow on the ground.

Each quadrat within which worms were sampled was analyzed by identifying all plant species present, percent ground cover per type and species, canopy cover, nearest tree species and diameter at breast height (DBH). Soil moisture and temperature were measured using Vernier probes and a Data Acquisition System. In a 3x3 m perimeter around the quadrat, percent shrub cover, stem count and species were also recorded (Table 1).

Worm sampling plots were nested within the 1x1 m quadrat. In this smaller sampling square, a solution of mustard water was poured to draw out the worms. Swallow-wort was clipped before worm sampling so as not to disturb the soil. Each gallon of water was mixed with 40 g of powdered mustard (Great Lakes Worm Watch, 2018) and poured into the ground in thirds. Each third was administered every five minutes so the solution could percolate as far into the ground as possible. Worms that were collected were placed into containers with hydrogen peroxide to anesthetize them. Once brought back to the lab, they were transferred into formalin to preserve them for identification.

Worms were processed by identifying their species or group based on the Great Lakes Worm Watch Dichotomous Key (Great Lakes Worm Watch, 2018). They were also measured by length, inspected for maturity, and dry massed. The drying process happened over four days total. For the first two days, the worms were removed from the formalin and placed in the fume hood for initial drying. They were placed in a dryer for an additional two days before being massed to the nearest hundredth gram per site.

The data collected from this study were not normal. The data were attempted to be transformed, but any test for normality did not yield proper results. Because the data were not normal, nonparametric tests had were performed to compare the average number of worms and average biomass between each plot type and season. A Wilcoxon signed rank test was performed using Microsoft Excel (2015) and Minitab 18 (2017).

Results:

Wild Wings and Devil's Bathtub both had mainly maples (*Acer* sp.) as the predominant tree species, while the Plantation had red oaks (*Quercus rubra*) and Devil's Bathtub pine had pine trees almost exclusively. Oatka Creek had a canopy made mostly of hickory (*Carya* sp.), with some black cherry (*Prunus serotina*) and red oaks. These species were also present in Wild Wings and Devil's Bathtub. The only tree species that was an outlier was a flowering dogwood (*Cornus florida*) in Oatka Creek. According to the densiometer, the plantation had the lowest amount of canopy cover with about a 3% difference from the densest canopy cover which was featured in the Wild Wings area (Table 1).

These plots were also home to other vegetation. The Plantation featured garlic mustard, sensitive fern, dogwood, sedges, maple seedlings and moss. Wild Wings contained these species as well as lily (*Liliaceae)*, Virginia creeper (*Parthenocissus quinquefolia)*, maple leaf viburnum (*Viburnum acerfolium)*, buckthorn seedlings (*Rhamnus cathartica),* white snakeroot (*Ageratina altissima)*, grapevine (*Vitis* sp.), blueberry *(Vaccinium* sp.), asters (*Asteraceae)*, poison ivy (*Toxicodendron radicans)*, multiflora rose *(Rosa multiflora*), wood fern (*Dryopteris* sp.) and bedstraw (*Galium aparine)*. Devil's Bathtub had some of these species, but also had blackberry, baby aspens *(Populus tremuloides)*, false nettle (*Boehmeria* sp.), coltsfoot (*Tussilago farfara*), mint (*Lamiaceae*), and oriental bittersweet (*Celastrus orbiculatus).* The pine area featured similar vegetation as well as jumpseed (*Persicaria virginiana*). Oatka Creek's black trail held most of the species that were featured in Mendon Ponds. In addition, it also contained stinging nettle (*Urtica dioica*), baneberry (*Actaea* sp.), and grass (*Poa* sp.). Oatka Creek and the Devil's Bathtub Pine area had much more poison ivy than the other three sites. Also, Oatka Creek had more vegetation in general than most of the other bare plots (Table 1).

In terms of abiotic variables, the Plantation also contained the moistest soil with the lowest soil temperature. In contrast, Oatka Creek had the lowest soil moisture, but it did not have the highest soil temperature which was seen at Wild Wings. Denser canopy cover generally seemed to promote less soil moisture and higher temperatures (Table 1). Furthermore, while the soils in Wild Wings, Devil's Bathtub, and the Plantation were very similar, the soils in the Pine area and in Oatka Creek were very different. In the pine area, the soil was very loose and almost spongey. It is classified as Palmyra gravelly fine sandy loam (PaD) by the Soil Survey Geographic database (SSURGO). In Oatka Creek, the soil was very densely compacted. This was classified as Benson chancery loam (BcB) and was farmland of statewide importance which could explain why it was so dense.

The data were not normal mostly due to the fact that the presence of earthworms was extremely variable, resulting in an overwhelming amount of zeros in the dataset. However, in total, 419 worms were collected. The taxa that were collected included *Dendrobaena octaedra, Amynthus* sp., *Dendrodrilus rubidus, Eisenia fetida, Aporrectodea* sp., *Octolasion tyrtaeum/cyaneum, Lumbricus terrestris, Lumbricus rubellus, Aporrectodea longa, Aporrectodea trapezoides, Aporrectodea tuberculata, and Aporrectodea calignosa.* Around 45% of the total worms could be identified to species using the dichotomous key from Great Lakes Worm Watch. The rest were categorized based on which part of the soil they inhabited (Figure 1).

Between swallow-wort and non-swallowwort plots, the presence and/or absence of the invasive plant did not seem to affect the type of vegetation around or within it. However, the vine did take up to 75% or more of the ground cover. Overall, swallow-wort plots tended to have higher soil moisture and soil temperature. Before tests were run, swallow-wort plots also seemed to have less worms. However, as seen in Figure 2, the wilcoxon signed rank test revealed that plot type had no effect on the abundance of earthworms (W_{summer}= 27.0, P= 0.208; W_{fall}= 88.0, P= 0.603). In addition, Figure 3 indicates while swallow-wort plots appeared to have more earthworm biomass, plot type was not a significant effect (W_{summer}= 77.0, P= 0.481; W_{fall}= 107.0, P= 0.955).

Between seasons, summer sampling collected more worms. However, in the fall, more mature worms were collected. In the first sampling, there were a lot of juvenile worms present, but by the second there were fewer but larger worms in the soil. As seen in Figure 4, season had no significant effect on worm abundance ($W_{swallow\text{-}wort}$= 99.0, P= 0.888; W_{bare}= 144.0, P= 0.330). Furthermore, season generally had no effect on earthworm biomass (Figure 5). However, there was one indication that showed earthworms with more biomass appeared in the fall within bare plots ($W_{swallow\text{-}wort}$= 59.0, P= 0.089; W_{bare}= 61.5, P= 0.036).

Discussion:

With a threshold of 0.05 significance value, the presence of swallow-wort was shown to not have an effect on the either the abundance or the biomass of earthworms. The data showed that earthworms did not seem to prefer one sort of plot over the other, neither did they gain more sustenance from one type of plot. Generally, the season also did not seem to affect the presence of earthworms. However, it did seem to affect the biomass of worms in bare plots. This just reinforces that resampling is needed in order to solidify results, especially with the presence of earthworms being as variable as it was.

While earthworms have shown to help facilitate other allelopathic plants such as common buckthorn (Roth et al. 2014) and garlic mustard (Davalos and Dobson 2018), swallow-wort did not seem to behave in the same way. Similar vegetation was found in non-swallowwort and swallow-wort plots despite its presence and/or absence. Even buckthorn and garlic mustard were present in both types of plots. In order to figure out this sort of relationship, a similar study would have to be performed where swallow-wort, buckthorn, and garlic mustard plots are sampled within similar forest types to see if any sort of trend is present in terms of earthworms. Additionally, a greenhouse experiment where increasing amounts of worms are added to controlled pots containing germinated seeds of these three plants would help see the flip side of my study- if the presence of earthworms helps or hinders establishment and growth of invasive plant species.

Rather than focusing on the presence of swallow-wort and how it affects earthworms, it would be interesting to focus on canopy cover and forest type. One study showed that forest type and distance to agricultural clearings were two of the most important factors when determining which areas supported exotic earthworm communities (Suárez et al. 2006). Another study showed that distance to the nearest road or cabin were two of the best predictors for earthworm invasion (Holdsworth et al. 2007).

In addition, it would be beneficial to study the effects of soil type. Oatka Creek and Devil's Bathtub Pine areas yielded little to no worms. This was attributed to the soil type. Pine trees have a tendency to make the soil more acidic, reducing the understory, and containing only needle litter rather than leaf litter which may be displeasing to the earthworms. In one study, worms such as *D. octaedra* and *L. rubellus* increased the soil's acidity in a coniferous forest (Burtelow et al. 1998). Oatka Creek had tightly compacted soil which not only might have impeded the worms' movement, but the percolation of mustard water into the ground. In fact, a study done by Smith et al. showed that earthworms were in smaller numbers within an uncultivated field rather than in intensively cultivated fields. Similarly, this study reinforced the finding that the presence of conifers alters the soil such that litter quality is reduced, pH is decreased and soil structure is deteriorated, all factors which can be attributed to low abundance of earthworms (Smith et al. 2008).

While only one significant value was found in this study, based purely on observation, it was noted that more worms were present in oak and maple forests, suggesting that earthworms prefer denser canopy cover. While this observation is noted in some studies (Price and Gordon 1998), it is not expanded upon. In short, while earthworms are not the most exciting animal to study, there is so much still to learn about them, because although they are small in size, they do have a very large impact.

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Tables and Figures:

Table 1. Summary table of plots and their characteristics. The cells contain average values ± standard deviation.

Site	Plot	Most common tree species	Average distance to tree (m)	Average DBH (cm)	Average canopy cover (%)	Average Soil Moisture	Average Soil Temperature (ºC)	# species in plot	% shrub cover
PRO	SW	*Que rub*	3.78 ± 2	29.54 ± 7	94.70 ± 2	27.10 ± 3	16.00 ± 1	2	0
PRO	B	*Que rub*	4.24 ± 2	26.46 ± 7	92.15 ± 5	23.44 ± 3	15.92 ± 0	2	0
WW	SW	*Ace rub*	2.74 ± 2	35.51 ± 12	95.36 ± 1	17.52 ± 3	20.10 ± 1	4	0
WW	B	*Ace rub*	1.91 ± 1	25.64 ± 11	97.33 ± 1	15.11 ± 3	20.69 ± 1	3	0
DB	SW	*Ace sac*	4.51 ± 3	26.56 ± 13	95.94 ± 3	21.63 ± 7	19.36 ± 0	3	13
DB	B	*Ace sac*	2.53 ± 2	33.74 ± 7	98.39 ± 1	15.36 ± 5	18.80 ± 1	2	0
DBP	SW	*Pin syl*	1.20 ± 1	33.48 ± 8	95.97 ± 3	9.44 ± 2	18.98 ± 1	5	0
DBP	B	*Pin syl*	2.00 ± 1	33.93 ± 4	96.04 ± 3	11.13 ±0	18.45 ± 1	5	0
OC	SW	*Car cor*	2.72 ± 2	32.72 ± 12	95.67 ± 1	18.73 ± 4	19.12 ± 2	4	12
OC	B	*Car ova*	2.19 ± 1	26.84 ± 13	97.66 ± 1	17.13 ± 5	20.40 ± 1	7	6

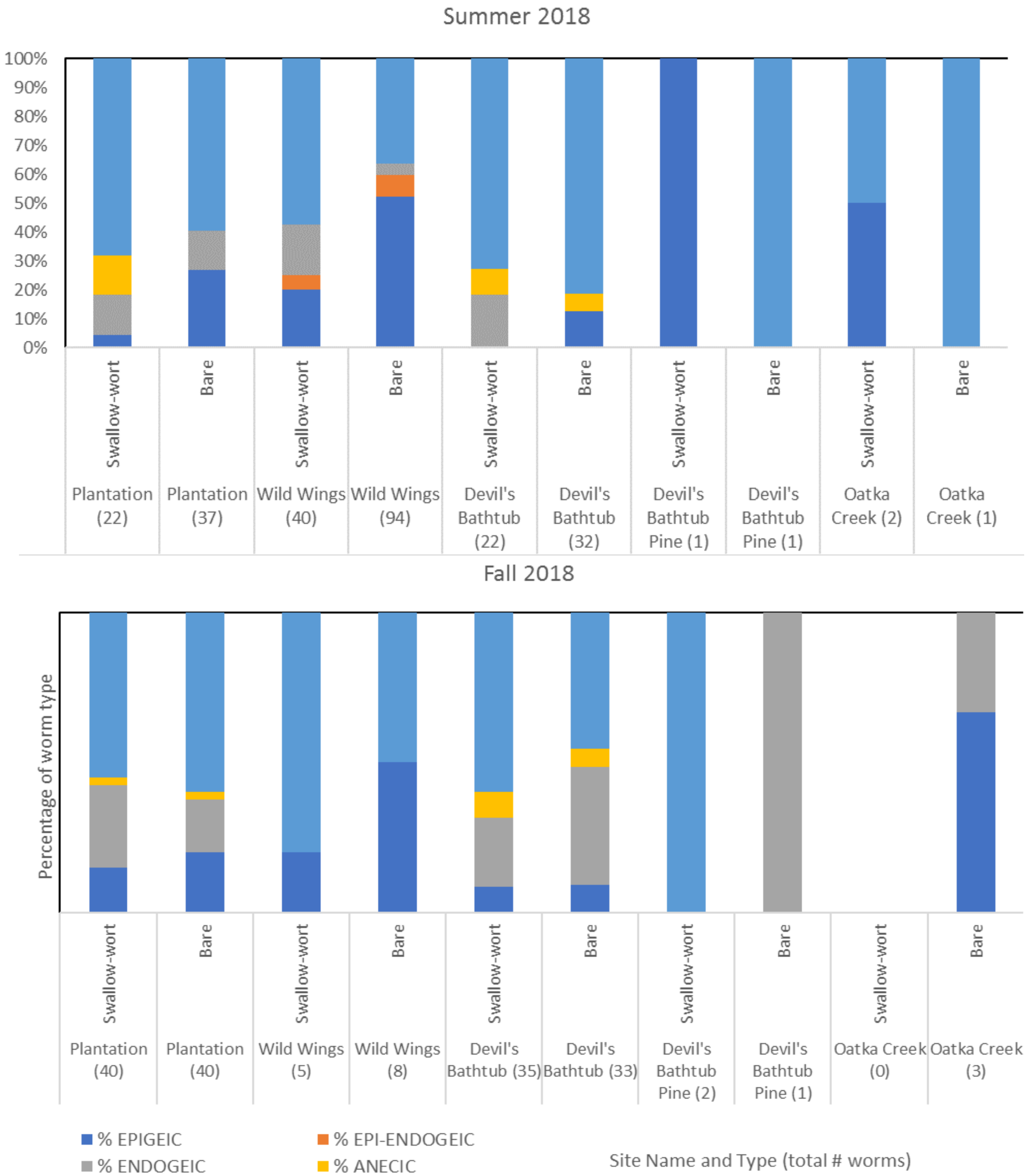

Figure 1. Each plot shows the percentage of each type of worm within a site. All bars go up to 100%. Numbers in parentheses represent how many worms were collected at each site per plot type.

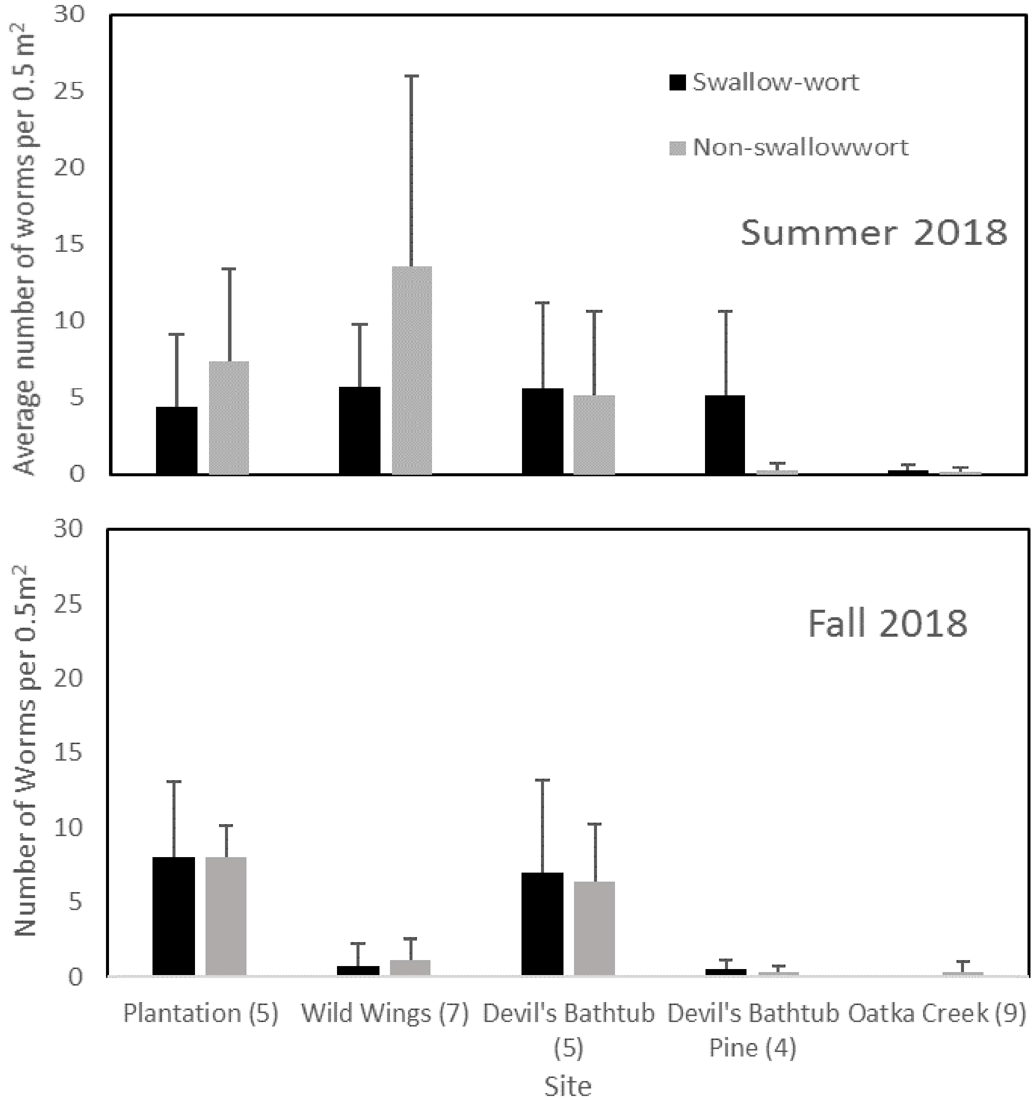

Figure 2. Each plot shows worm abundance with bars representing plot types in different sites. The first four sites were sampled in Mendon Ponds Park, while the last site was sampled in Oatka Creek Park. Numbers in parentheses indicate how many plot pairs there were. Plot type showed to have no effect on worm abundance (W_{summer}= 27.0, P= 0.208; W_{fall}= 88.0, P= 0.603).

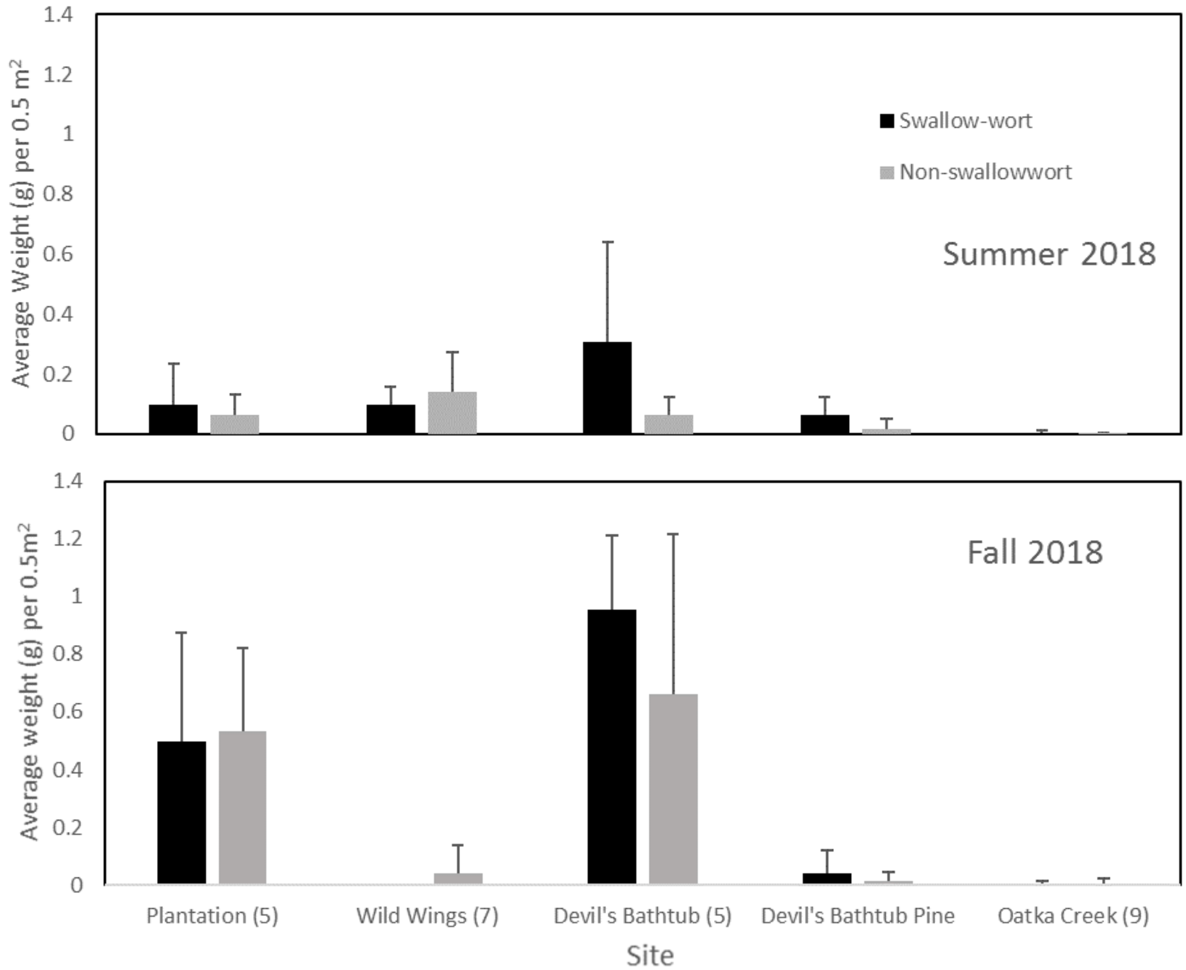

Figure 3. Each plot shows worm biomass with bars representing plot types in different sites. Numbers in parentheses indicate how many plot pairs there were. Plot type showed to have no effect on worm biomass (W_{summer}= 77.0, P= 0.481; W_{fall}= 107.0, P= 0.955).

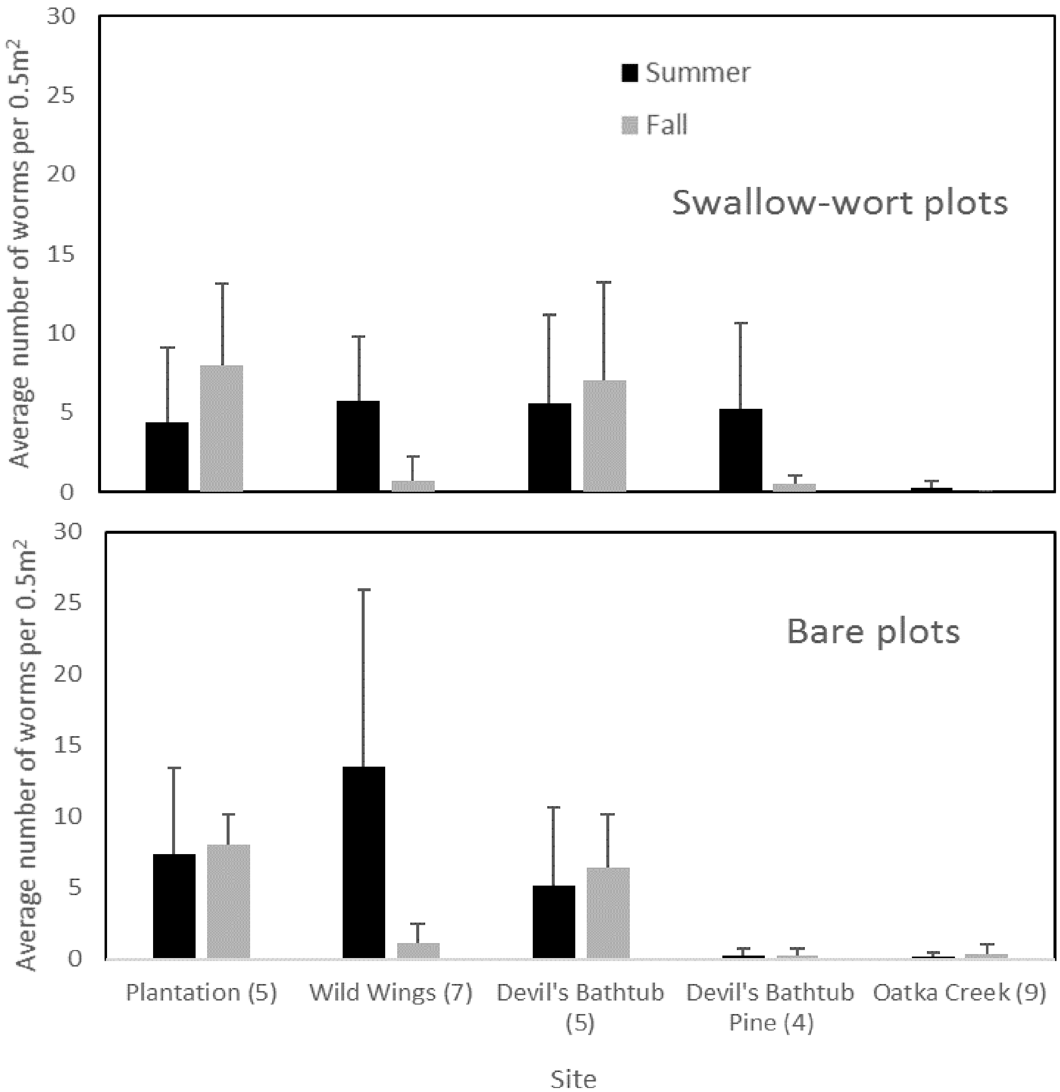

Figure 4. Each plot shows worm abundance with bars representing seasons at different sites within the same type of plots. Numbers in parentheses indicate how many plot pairs there were. Season showed to have no effect on worm abundance ($W_{swallow\text{-}wort}$= 99.0, P= 0.888; W_{bare}= 144.0, P= 0.330).

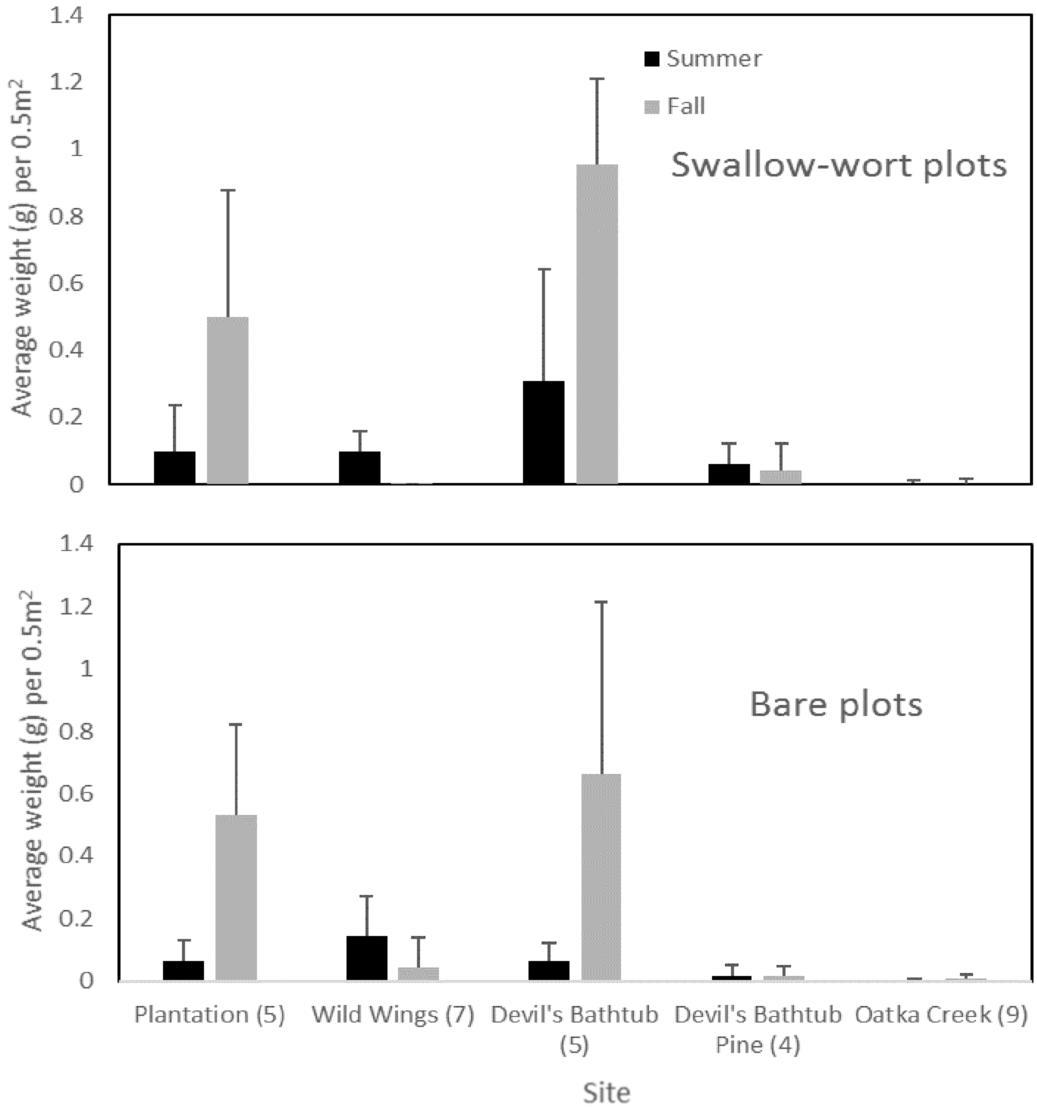

Figure 5. Each plot shows worm biomass with bars representing seasons at different sites within the same type of plot. Numbers in parentheses indicate how many plot pairs there were. Season showed to have an effect on worm biomass only in the fall ($W_{swallow\text{-}wort}$= 59.0, P= 0.089; W_{bare}= 61.5, P= 0.036).