Buckthorn and Earthworm Mutualism in Southern Minnesota; Interactions between Non-Native Species

Jamie E. Mosel, Ecology, St. Olaf College, 1520 St. Olaf Ave., Northfield, MN 55057

Abstract

Earthworms were collected at three forest sites in southern Minnesota in order to examine the relationship between buckthorn (*Rhamnus cathartica*) and earthworm density and diversity. As both buckthorn and earthworms are non-native to the Great Lakes region of North America, and impact the compositions of forest ecosystems, understanding their interactions is valuable in predicting spread. Each site exhibited a varying degree of buckthorn individuals, from the absence of buckthorn at Site 1, to substantial buckthorn coverage at Site 3. Percent soil organic content and percent moisture were measured, in addition to leaf litter weight and coverage; seedling, sapling, and mature tree species were recorded. Juvenile earthworms were identified by ecological group and mature earthworms by species when possible. The greatest number of surfaced earthworms (35 individuals) per sampling subplot was found at an additional buckthorn site, Site 4; the greatest average number was at Site 1. Twice as many earthworms surfaced on average at Site 1 as compared to Sites 2 and 3. The highest mean percent moisture and percent organic content were found at Sites 1 and 4 (22.4% and 26.37%, and 10% and 8.38%). An ANOVA test produced significant p-values for soil moisture, total worms surfaced, and the anecic and endogeic ecological groups. These results suggest that, while buckthorn and earthworm densities may impact each other as seen at Sites 1 and 4, overall soil conditions and moisture levels in particular play a more decisive role in population levels.

Introduction:

Buckthorn (*Rhamnus cathartica*) and European earthworms are invasive species in the Great Lakes region, which have shown high adaptability and consequent spread throughout much of the North American continent. Buckthorn is particularly persistent in the Midwest, including Minnesota. Moreover, both organisms impact soil composition, as well as local vegetative species, thereby jeopardizing native ecosystems. European earthworms have been shown to cause significant changes in surface layer organic matter through the distribution of this matter to lower soil layers, greatly influencing the diversity of organisms that inhabit the surface layer. (Parkinson 1994) Furthermore, the drilosphere soil zone (Bouché 1975) which surrounds earthworm burrows often displays increased levels of nitrogen, phosphorus, and organic humus when contrasted to nearby soil. (Beare et al. 1994 as cited by Parkinson 1994) According to Doube and Brown (1994), this is in part due to a concentration of microbial activity in such areas. At the same time, fine roots that establish in the surface layers experience lower levels of nutrient availability, coupled with leaching of these nutrients to lower soil layers. (Frelich et. al

2006) Meanwhile, buckthorn compete with native species, and grow more vigorously in areas of earthworm activity. (Heneghan 2006, Maldritch 2009) further diminish native forest floor leaf litter, and impact nitrogen levels. (Knight 2007) A number of studies suggest that buckthorn likewise encourage heightened earthworm populations, thus providing a possible example of mutual facilitation (Madritch 2009), and a gateway for subsequent invasive species since Buckthorn and European earthworms may subsequently facilitate invasiveness (Heneghan 2006). In a study by Maldritch and Lindroth (2009), earthworms were shown to preferentially select high quality leaf litter – high quality litter often containing more nutrients, but according to Satchell (1967) as cited by Curry (1998) also distinguished from less palatable leaf litter with higher levels of carbohydrate and phenolic compounds, such as tannins – of Buckthorn over the leaf litter of native species such as *Quercus rubrum, Quercus alba*. This mutual facilitation is strengthened by Maldritch and Lindroth's study, where earthworm abundance decreased by approximately 50% over the course of 3 years in an area where buckthorn was eliminated. By sampling earthworm populations with varying levels of buckthorn individuals, I argue that there exists a similar relationship between earthworm abundance and the presence of buckthorn at four sites in southern Minnesota owned by St. Olaf College.

Methods

To determine earthworm density, three sites were initially selected. Sites 1 and 2 were both located in Norway Valley along Highway 19, a patch of restored Big Woods forest. Site 1 was level, at the top of a slope, while Site 2 was located on the edge of this south-facing slope and approximately 120 meters away. These two sites were chosen because Site 1 is absent of buckthorn, whereas Site 2 contained considerable buckthorn up until removal on September 24th, 2010. Moreover, the uprooted buckthorn were left at the site. Site 3 was located in a patch of original forest on the eastern side of the St. Olaf Natural Lands and was chosen because it exemplified considerable buckthorn presence. A fourth site was later tested, near Armstrong Road in a patch of buckthorn-dominated woods overlooking Heath Creek.

At each site, with the exception of Site 4 where only one subplot was tested, 3 33-square centimeter subplots were sampled along a 50-meter transect. The species of mature trees, of DBH greater than 10, and within a 4.5-meter radius of each subplot were recorded. Additionally, the number and species of seedlings (<0.5-meters tall), and saplings (<10-cm DBH) was recorded. Species were distinguished by leaf characteristics, when present, and by buds and bark characteristics when leaves were absent. Earthworm sampling was performed using a 1-gallon solution of 40gm mustard flour and water. The percent bare soil was observationally determined before clearing it away, and the weight of leaf litter was weighed within each subplot. Earthworms that surfaced were collected and stored in a container with soil to sustain them. Individual earthworms were recorded by ecological group – endogeic, epigeic, or anecic – as they surfaced according to presence or lack of pigmentation, as well as size. Mature individuals were identified when possible by clitella and setae pattern characteristics; juveniles, lacking clitella, could not be decisively identified. Soil cores were taken at two subplots on either end of the 50-meter transect per site. The O, A and B horizons were observed. Following the protocols of the Soil and Microclimate lab, as described in Field and Laboratory Methods for General Ecology by Brower, Zar, and Von Ende (1998), these samples were used to test soil percent moisture and soil organic content; the samples were weighed and placed in a drying oven for 48 hours at 105 degrees Celsius. The percent moisture is equivalent to the initial weight minus the weight of the dry, divided by the dry, and multiplied by 100 to achieve a percent. To determine percent soil organic content, the same samples were sieved and placed in a muffle furnace at 500 degrees Celsius for 4 hours. Percent organic content was calculated by subtracting the final weight from the initial weight, and dividing by the initial weight.

Earthworms were kept in plastic bins, separated by site, and provided soil from their respective sites. The initial weights of *Quercus rubrum, Rhamnus cathartica,* and *Acer sacarhum* leaves were recorded. These leaves placed at the top of the soil for 8 days, after which they were re-weighed, to determine the percent of leaf litter consumed. Lastly, using the statistical analysis program, R Commander, one-way ANOVAs were run between Sites 1, 2, and 3 and their mean percent soil moisture, percent soil organic, percent bare ground, leaf litter weight, total earthworms surfaced, and the proportions of anecic, endogeic, and epigeic earthworms. P-values greater than 0.5 are insignificant, and not substantial enough to warrant rejection of the null hypothesis; meanwhile, values below 0.05, or 0.01, indicate significance. Additionally, pair-wise comparisons were run for those relationships that exhibited significant p-values. A Pearson Correlation test was run to test the relationship between percent soil moisture and percent soil organic matter.

Results

The total number of earthworms surfaced, and proportions of anecic and endogeic resulted in significant p-values of 0.00094, 0.00019, and 0.0021 respectively, indicating that differences between sites had significant impacts on these characteristics. (Table 1) Furthermore, the ANOVA test between site and percent soil moisture resulted in a significant p-value of 0.00448. Pair-wise comparisons reinforce these relationships. (Table 1) The Pearson correlation test between percent soil moisture and percent soil organic matter resulted in a value of 0.056, which is close enough to be slightly significant. Graphical representation of this relationship more clearly illustrates the trend that soil organic content increases with soil moisture. (Figure 1) In comparing Sites 1, 2, and 3, greatest earthworm density was found for Site 1. Site 1 also had the largest percent soil moisture, and percent soil organic matter. Comparisons of mean leaf litter and percent bare ground were not significantly different, although values at Sites 2 and 3 were somewhat lower than at Site 1. Few mature earthworms were found at any site. (Table 3) With regards to vegetation, Site 1 was overwhelmingly *Acer saccharum*. Site 2 also had prevalent *Acer saccharum*, but also mature *Quercus rubrum*. Sites 3 and 4 had substantial buckthorn. (Table 2)

While data from Site 4 was not sufficient to be included in statistical analysis, results from a sampling performed at this site should nevertheless be taken into consideration. At a single 33-square centimeter subplot at Site 4, 35 earthworms surfaced. (Appendix 1) This is more than the site totals for both Site 2 and Site 3, and is considerably greater than the mean number of earthworms surfaced at site 1 (19). (Figure 1) Moreover, the percent soil moisture was 26.37, and thus more comparable to Site 1. Lastly, over a period of 8 days, 37% of buckthorn (*Rhamnus cathartica*) leaf litter was consumed, as compared to 22% of sugar maple (*Acer* saccharum) leaves, and 0% of red oak (*Quercus rubrum*) leaves. (Table 4)

Discussion

Based on other studies (Madritch 2009, Heneghan 2006, Knight 2007), it was anticipated that greater earthworms densities would be found at Site 3, where buckthorn were well established. The data from Sites 1, 2, and 3 of this study, however, point to the opposite conclusion.

Sampling at Site 1, which was absent of buckthorn, resulted in the greatest mean number of earthworms (19) surfaced per given subplot whereas neither Site 2 or 3 had mean earthworm numbers greater than 10. (Table 1) Even so, Site 1 was dominated by *Acer saccarhum*. While there was substantial *Acer saccarhum* present at Site 2 as well, Site 1 stands out in this regard, hosting many more seedlings, saplings, and mature trees. (Table 2) This may be explained in part by results from leaf litter consumption, which demonstrates that earthworms do appear to exhibit a preference for *Acer saccarhum*. Over 8 days, 0.30g of *Acer saccarhum* (approximately 3 leaves) were consumed -- equivalent to 22% of total *Acer saccarhum* leaf biomass offered. (Table 4) *Acer saccarhum* is particularly appealing to earthworms because it is rich in calcium. Thus, it is possible that considerable *Acer saccharum* litter supports larger populations.

More importantly, perhaps, are the values for percent soil moisture. (Holdsworth et al 2007) Site 1 exhibited the greatest earthworm density among the three sites, but also a mean percent moisture more than twice that of Site 2, and more than three times that of Site 3. Concerning soil moisture, Curry (1998) points out that earthworm distribution is often effected appreciably by moisture levels, and that earthworms prefer greater moisture (about 10 kPa).

Although it was not possible to include results from Site 4 in statistical analysis due to limited sampling, results from a single sampling at Site 4 are nevertheless important. Site 4 offers soil conditions far more similar to Site 1, and is thus better suited to comparison. While Sites 2 and 3 were both found to be dry and somewhat sandy – conditions not preferable to earthworms – sampling at Site 4 showed a percent soil moisture of 26.37% (Appendix 1). Even more revealing, more earthworms surfaced in a single subplot at Site 4 than the site totals for Sites 2 and 3. This implies pointedly that, even between buckthorn invaded sites, soil conditions are extremely important in influencing earthworm populations. Percent soil organic matter for this limited sampling was also more comparable to Site 1. This may also suggest that greater earthworm populations induce greater percent organic content within soil, perhaps by distributing the nutrient rich O horizon, resulting in less concentration by more spread. Based on the inclusion of Site 4, greater densities of buckthorn do result in larger earthworm populations.

At both Sites 1 and 4, mature *Lumbricus terrestris* and *Aporrectodea rosea* were identified based on size, pigmentation and setae arrangements. Holdsworth (2007) found that the combined presence of *Lumbricus* and *Aporrectodea* indicate heavily invaded plots. In this regard, Sites 1 and 4 constitute examples of heavy invasion. By reducing, or eliminating, the leaf litter layer, *Lumbricus* in particular may not only negatively impact understory vegetation but also the proportion of epigeic, litter dwelling worms such as *Dendrobaena*. (Holdsworth 2007) This is perhaps the case at Site 4, where significant anecic and many endogeic specimens were found, but few epigeic. (Appendix 1) To some degree, this is also true at Site 3, where no anecic specimens were found, but slightly greater proportions of epigeic specimens were present. (Table 1)

Regarding the conditions of Site 2, while Maldritch and Lindroth's (2009) study documented a substantial decrease in earthworm abundance after buckthorn removal, this was over a much greater span of time (3 years) as compared to approximately a month between removal and testing at Site 2 of this investigation. Thus, it is extremely unlikely that the earthworm population experienced reduction. In any case, Heneghan (2006) asserts that alterations in soil may remain after physical removal of the organism, and thus earthworm abundance may not be immediately affected. Still, the uprooting of buckthorn individuals did reduce earthworm access to living underground buckthorn biomass. Subsequent studies of this nature may be useful in determining whether it is buckthorn leaf litter itself, soil impacts, root biomass, or other variables that most drastically effect local earthworms.

Based on soil moisture results, overall soil conditions of an ecosystem contribute most directly in determining earthworm populations. This is evidenced in that the largest numbers of earthworms surfaced at those sites with the highest percent soil moisture (Site1 and Site 4). It is possible that the soil conditions of the sites were altered by buckthorn. Thus, in future studies, greater examination of possible variables, and greater consistency between controls is imperative to more accurately elucidate relationships between earthworms and buckthorn. Such an argument is certainly exemplified by the results of this experiment: by comparing two sites of consistent soil characteristics (Sites 1 and 4), there is a connection between greater buckthorn density and earthworm density. Yet by examining only Sites 1, 2, and 3, the opposite trend appears. Clearly, it is a combination of soil and microclimate conditions, in connection with local vegetation, which impact the size and species of an earthworm population. In turn, the compositions of these populations impart their own impacts and alterations on an ecosystem. Nevertheless, especially considering the intriguing results of studies like Maldritch and Lindroth (2009), and Heneghan (2006), further investigation of the relationship between buckthorn and earthworms is imperative, but must take a number of factors into consideration. Proper understanding of the relationship between invasive species may aid in the control of their spread, as well as effective strategies for their removal, maintenance and the successful restoration of native or sustainable ecosystems. With the growing impacts of climate change, it will be possible for many climate sensitive species, such as earthworms, to spread to areas from which they were previously limited. The spread of buckthorn and European earthworms through the Great Lakes region may detrimentally impact representative Great Lakes ecosystems, such as the Big Woods remnants, and the forests of northern Minnesota. Nevertheless, this spread offers an intriguing opportunity to examine the ways in which two non-native species – both of European origin – interact with each other and native ecosystems.

Literature Cited

Beare, M.H., Coleman, D.C., Crossley, Jr., Hendrix, P. F., and Odum, E.P. A Hierarchical Approach to Evaluating the Significance of Soil Biodiversity to Biogeochemical Cycling. *Plant and Soil*, 170: 5-22

Bouché, M. B., Action de la faune sur les états de la matière organique dans les écosystèmes. 1975.

Brower, Zar, von Ende, Field and Laboratory Methods for General Ecology, Fourth Ed. 1998.

Curry, J. P., Factors Affecting Earthworm Abundance in Soils, *Earthworm Ecology,* 1998, 37-63

Doube, B.M., Brown, G.G. Life in a Complex Community: Functional Interactions Between Earthworms, Organic Matter, Microorganisms, and Plants, *Earthworm Ecology*, 1998, 179-211

Hale, C. M.; Frelich, L. E.; Reich, P. B.; Pastor, J. Effects of European Earthworms Invasion on Soil Characteristics in Northern Hardwood Forests of Minnesota, *USA Ecosystems 2005*, 8: 911–927

Heneghan, L., J. Steffen, and K. Fagen. Interactions of an Introduced Shrub and Introduced Earthworms in an Illinois Urban Woodland: Impact on Leaf Litter Decomposition, *Pedobiologia*, 2006, 50:543–551.

Holdsworth, Andrew R., Frelich, Lee E., Reich, Peter B. Effects of Earthworm Invasion on Plant Species Richness in Northern Hardwood Forests, Conservation Biology, 2007. 21: 997-1008.

Frelich, L. E., Hale, C. M., Scheu, S., Reich, P. B., Earthworm Invasion into Previously Earthworm-free Temperate and Boreal Forests, Biological Invasions, 2006, 8: 1235-1245

Knight, K. S., Kurylo, J. S., Endress, A. G., Steward, J. R., Reich, P. B., Ecology and Ecosystem Impacts of Common Buckthorn (Rhamnus cathartica): A Review, Biological Invasions, 2007, 9: 925-937

Madritch, M. D.; Lindroth, R. L., Removal of Invasive Shrubs Reduces Exotic Earthworm Populations, *Biological Invasions*, 2009, 11, 663-671

Parkinson, D., McLean, M.A., Impacts of Earthworms on the Community Structure of Other Biota in Forest Soils, *Earthworm Ecology*, 1994, 213-226.

Satchell, J.E., Lowe, D.G., Selection of Leaf Litter by Lumbricus terrestris, *Progress in Soil Biology*, 1967, 102-119

Table 1. Mean and Stand. Deviation for Site Characteristics

	Site 1 (None)	**Site 2 (Removed)**	**Site 3 (Moderate)**	**P-value**
% soil moisture	22.24 (+2.673)	9.16 (+1.414)	4.6775 (+0.392)	0.00448
% soil organic	10 (+7.07)	3.125 (+0.884)	3.125 (+0.884)	0.30190
% bare ground	5.667 (+ 6.351)	17.333 (+28.31)	10.667 (+9.019)	0.72850
Litter (g)	23.4 (+11.27)	25.57 (+5.51)	23.4 (+5.54)	0.61610
Total worms	19.00 (+3)	6.667 (+1.528)	7.333 (+2.082)	0.00094
Anecic	2.333 (+0.577)	0 (+0)	0(+0)	0.00019
Endogeic	11.0 (+1.732)	4.333 (+0.577)	1.333 (+1.155)	0.00021
Epigeic	5.667 (+2.082)	2.333 (+2.082)	6.667 (+0.577)	0.04991

	Pairwise comparison	Pearson test (P)
% soil moisture	1 (a) 2(a) 3(b)	0.056
% soil organic	Insignificant	0.056
% bare ground	Insignificant	N/A
Litter (g)	Insignificant	N/A
Total worms	1 (a) 2(a) 3(b)	N/A
Anecic	1 (a) 2(a) 3(b)	N/A
Endogeic	1 (a) 2(a) 3(b)	N/A
Epigeic	Insignificant	N/A

Table 2. Tree Species By Site

Site	**Species**	**Seedling**	**Sapling**	**Mature**
1	*Acer saccharum*	44	35	6
	Acer negundo	0	1	0
	Fraxinus americana	1	1	0
	Fraxinus nigra	0	1	0
2	*Acer saccharum*	10	5	0
	Prunus virginiana	1	1	0
	Quercus rubrum	0	0	2
	Unknown #7 (prunus?)	0	1	0
3	*Acer saccharum*	0	0	3
	Quercus rubrum	0	0	1
	Fraxinus americana	2	0	0
	Rhamnus cathartica	7	9	0

	Ulmus americana	0	1	0
	Zanthoxylum americ.	3	4	0
4*	*Rhamnus cath.*	12	4	0
	Tilia americ.	1	0	1

Table 3. Earthworm By Species

	Juveniles	**Mature**	**Species**
Site 1	54	2	*Lumbricus terrestris*
--	--	1	*Aporrectodea rosea*
Site 2	20	0	*Octolasion?*
Site 3	22	0	*Denodrobaena oct.?*
Site 4*	30	3	*Lumbricus terrestris*
		4	*Aporrectodea rosea*

Table 4. Leaf Consumption Over 8 Days

Leaf Species	**Initial Weight(g)**	**Final Weight (g)**	**Amount Consumed**	**%Reduc.**
Rhamnus catha.	25 leaves (0.78g)	11 leaves (0.49g)	0.29g	37%
Acer saccharum	5 leaves (1.34g)	2 leaves (1.04g)	0.30g	22%
Quercus rubrum	2 leaves (1.32g)	2 leaves (1.32g)	0.0g	0.00%

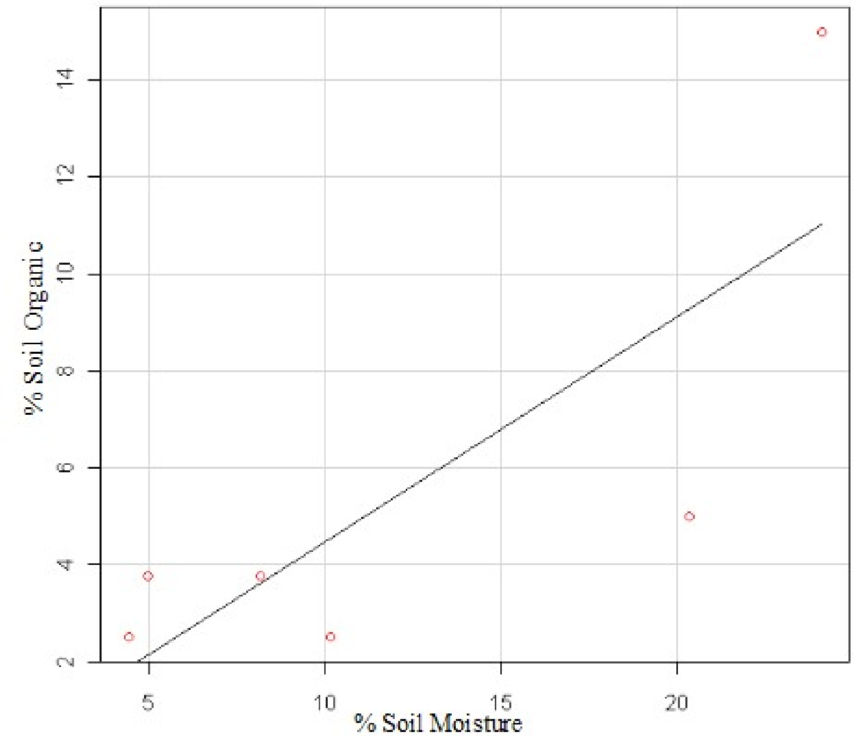

Figure 1. % Soil moisture and % Soil Organic Content Pearson Correlation Test.

Appendix 1. Soil Characteristics and Earthworm Sampling by Subplot

Site	% Moisture	% Organic	Litter (g)	% Bare
1A	20.35	5.00	22.90	2.00
1B	N/A	N/A	23.90	13.00
1C	24.13	15.00	42.90	2.00
2A	8.16	3.75	22.90	0.00
2B	N/A	N/A	21.90	50.00
2C	10.16	2.50	31.90	2.00
3A	4.96	3.75	23.40	2.00
3B	N/A	N/A	17.90	20.00
3C	4.40	2.50	28.90	10.00
4A*	26.37	8.375	28.30	5.00

Site	Earthworm Total	Anecic	Endogeic	Epigeic
1A	19	3	12	4
1B	16	2	9	5
1C	22	2	12	8

2A	7	0	4	3
2B	8	0	4	4
2C	5	0	5	0
3A	8	0	2	6
3B	5	0	0	7
3C	9	0	2	7
4A*	35	3	30	2

Appendix 2. Tree Species by Subplot

Site	Species	Seedling	Sapling
1A	*Acer sac.*	2	13
	--	--	--
1B	*Acer sac.*	6	7
	--	--	--
	Fraxinus amer	1	1
1C	*Acer sac.*	36	15
	Acer negun.	0	1
	Fraxinus nigra	0	1
2A	*Acer sac.*	10	5
	Quercus rub.	0	0
	--	--	--
	Prunus virg.	1	1
	Fraxinus nigra	1	0
	Unknown #7	0	1
3A	*Rhamnus cath.*	4	0
	Zanthoxylum a.	1	0
	Ulmus americana	0	1
	Unknowns	0	2
3B	*Rhamnus cath.*	2	6
	Zanthoxylum a.	2	4
	Quercus rub.	0	0
	Acer sac.	0	0
	Unknown	1	0
3C	*Rhamnus cath.*	1	3
	Acer sac.	0	0

	Fraxinus amer	2	0
4A	*Rhamnus cath.*	12	4
	Tilia americ.	1	0

Appendix 3. Mature Trees and DBH by Subplot

Site	**Species**	**Mature**	**DBH**
1A	*Acer sac.*	2	13.34
	--	--	17.37
1B	*Acer sac.*	2	19.40
	--	--	21.82
1C	*Acer sac.*	1	14.15
2A	*Quercus rub.*	2	18.59
3A	--	--	--
3B	*Quercus rub.*	1	15.5
	Acer sac.	2	16
	--	--	17
3C	*Acer sac.*	1 (multiple trunks)	19
4A	*Tilia americ.*	1	33.15