St. Olaf College

Local Ecology Research Papers

Comparing Soil Composition and Earthworm Population Densities in a Chronosequence of Restored Tallgrass Prairie Ecosystems

YiWynn Chan

2021

Comparing Soil Composition and Earthworm Population Densities in a Chronosequence of Restored Tallgrass Prairie Ecosystems

YiWynn Chan
Bio 371 Field Ecology
Professor Kathleen Shea

December 13th, 2021

Abstract

Minnesota was once made up of 50% diverse prairie land. However, the European settlement destroyed 99% of prairies for agriculture purposes (Sampson et al. 1994). The settlers also introduced non-native earthworms to Northern America, which alter the physical, chemical and biological properties of soil (Frelich et al. 2019). As St. Olaf has been restoring plots of prairie lands (St. Olaf 2021), it is important to understand the relationship between earthworm populations, soil composition and surface litter. Therefore, this study set out to compare soil moisture, bulk density, organic matter and earthworm population densities across the chronosequence of St. Olaf's restored prairie lands. I found that the number of years post restoration did not affect the soil composition and litter weight. However, there was a notable difference in the oldest prairie site restored in 1989. Furthermore, I found that earthworm population densities were significantly different across all sites, and that litter weight and type of earthworms were correlated. Based on the differences seen in the 1989 site, I hypothesize that 1989 may have had a different land use history and the prairies will remain fairly similar in the near future; or that the prairie ecosystem begins changing 27-32 years post restoration. This study provides preliminary information useful for conducting further research to evaluate the success of St. Olaf Natural Land's prairie restoration efforts.

Keywords: prairie, soil composition, invasive earthworms, restoration, chronosequence

Introduction

Natural prairies are highly diverse ecosystems. According to St. Olaf (2021), natural prairies hold up to 250 species of grasses and wildflowers within a square mile. Before European settlement in Northern America, prairies comprised much of the geographic midsection of the vegetative ecosystems in North America. However, such vegetative systems have since declined by as much as 99.9% (Sampson et al. 1994). This decline is mainly due to the lack of knowledge and understanding towards the diversity of these grasslands (CEQ 1991 as cited in Okerman 1997). Since prairies leave behind a lot of organic matter which creates fertile soil, the lands were cleared by settler farmers for agricultural use. Thus, hectares of land which once held intraspecific relationships between unique animals, plants and birds have now been lost (CEQ 1991 as cited in Okerman 1997).

In recent years, ecologists have studied the benefits of prairie lands. According to Sampson et al. (1994), apart from having a very diverse ecosystem, prairies function as massive carbon sinks which reduces the presence of greenhouse gasses. Thus, agencies such as The Nature Conservancy and US Fish and Wildlife Service have been active in efforts of prairie restoration (Haakenson, 2003). As prairie lands become more established over time, plant roots and bodies eventually decay and add significant amounts of organic matter to the soil (Packard & Mutel 1997 as cited in Okermann 1997).

According to Varvel et al. (2006), soil characteristics take a substantial amount of time before any change can be measured. Since St. Olaf's prairies were restored in different years ranging from 1989 to 2005, it would be interesting to investigate how the soil composition in these lands have changed in relation to the number of years the land has been restored. This chronosequence approach would highlight the changes in soil and vegetation properties over temporal and spatial scales (Kalinina et al. 2015; Boecker et al. 2015 as cited in Bai et al. 2018).

Earthworms are good indicators of an ecosystem's health. They are known for altering the physical, chemical and biological properties of soil in the upper mineral soil horizons and forest floor (Hale et al. 2005, 2006, 2008 as cited in Bal et al. 2017). They also increase soil organic carbon turnover rates, which changes soil carbon and nitrogen fluxes along with its nutrient contents (Bohlen et al. 2004 as cited in O'keefe and McCulloh 2020). However, as earthworms are invasive species introduced to North America by European settlers (Frelich et al. 2019), they unwelcomely speed up the rate of litter reduction in Northern American ecosystems (Bohlen et al. 2004 as cited in O'keefe and McCulloh 2020). This is because north-eastern Northern American forests developed in the absence of earthworms as they formed after the last glacial maximum approximately 22,000 years ago (Hendrix and Bohlen 2002). Thus, the native plants have evolved and adapted to nutrient depleted conditions to maintain optimal stoichiometry regardless of soil fertility (Kery and Gregg 2004 as cited in Dobson et al. 2017).

Earthworm activity has been found to intermix soils, thus exposing soils to periods of warmer and dryer or more freezing temperatures (Hale et al. 2006; Hale et al. 2008 as cited in Bal et al. 2017). Furthermore, invasive earthworms are known to alter Ca, Mg, K and P components in soil, affecting the soil's nutrient availability (Li et al. 2002 as cited in Bal et al. 2017). As these alterations are hypothesized to be functions of time and space (Bal et al. 2017), studying earthworm densities in prairie sites restored in different years would provide preliminary information on the effects of earthworms on soil composition and vegetation. Studies relating to the population density of earthworms in St. Olaf's prairies have yet to be conducted. Therefore, the findings of this experiment could serve as insightful information for St. Olaf's Natural Lands stewards as to whether the years since restoration changes soil composition and earthworm densities.

This study set out to compare soil quality in terms of moisture, bulk density and organic matter as well as earthworm population densities in restored prairie lands. To help us understand the chronosequence in soil composition of the prairies, I compared soil samples from four sites which were restored in different years. To better understand the possible effects of earthworms on soil and vegetation, I investigated the earthworm populations in relation to litter weight in the different prairies. Thus, the objectives of this study were:

1) To compare the soil percent moisture dry weight, bulk density and percent organic matter of prairies restored in different years
2) To compare the earthworm population density of prairies restored in different years
3) To determine if there is a correlation between the amount of litter weight, earthworm density and different earthworm species

<u>Methods</u>

Site Description

I conducted this study in the St. Olaf Natural Lands prairie site, which is located in Northfield, Minnesota. St. Olaf College has been conducting extensive restoration on former farmland since 1989. The Biology and Environmental Studies departments have planted seedlings to restore ~40ha of Big Woods (maple-basswood) and ~60ha of native tall grass prairies (2018). Such efforts are necessary and beneficial to the local land as they support diversity of plants and small animals native to forest and prairie environments. To date, St. Olaf's restored prairies have 10 native grass species and 25-40 species of native wildflowers (St. Olaf 2018). The prairie land has an average latitude of 44.46667 degrees and a longitude of -93.19260 degrees. I conducted this study on the prairies restored in the years 1989, 1994, 1998 and 2004 as seen in Figure 1.

Soil Composition
I collected 3 soil samples from each prairie site, totaling 12 samples. Each plot was at least 50m away from any trails and forest edges, and 30m apart from each other. At each plot, I collected soil samples using a soil corer as described by Shea et al. (2012) in "Methods for Soil Analysis at St. Olaf". Observations on the soil's color and texture were made before placing the soil samples into their respective ziplock bags.

The soil samples were collected for soil bulk density, soil moisture and soil organic content. In the laboratory, I weighed the samples before placing them for 48 hours at 105°C and weighed them again after to calculate the percent moisture dry weight and bulk density of the soil samples. To determine the percent organic matter, I further heated soil samples (<2mm) at 500°C for four hours.

Earthworm Population
I collected earthworms from 2 plots at each prairie site. The earthworms were collected 2 weeks apart, on September 25th, 2021 and October 8th, 2021. The former collection date was conducted in the late morning whereas the latter was conducted in the early afternoon. It should be noted that while the time of the day was relatively different and the tallgrass were not as damp in the latter date, both dates yielded a similar number of earthworms at each site.

I collected the earthworms using plot squares of 0.109cm^2. Prior to collecting the earthworms, I cleared and collected plants and litter on top of the intended earthworm collection area. I then poured 1.9L of mustard solution onto the square plot twice with an interval of 4 minutes, picking up the earthworms as they surfaced (Field Ecology 371 2019).

In the laboratory, I identified the earthworms into three categories of epigeic, endogeic and anecic as described in Hale (2013), while making careful observations. As described in Field Ecology 371 (2019), I euthanized each earthworm in 10% isopropanol for 1-2 minutes, before transferring them into 10% buffered formalin for tissue fixation. After two days, the earthworms were rinsed and preserved in 70% isopropanol. I also measured the length of the straightened earthworm to estimate its biomass. Furthermore, I left the plant litter to air dry for a week before placing them in the dry oven at 65°C for 48 hours to measure the litter dry mass.

Data Analyses

I compiled the data on soil composition and calculated the percent moisture dry weight, bulk density and percent organic matter using the the following equations found in Brower et al. (1998):

$$Percent\ Moisture\ Dry\ Weight = \frac{Wet\ weight - Dry\ weight}{Dry\ Weight} \times 100$$

$$Bulk\ Density = \frac{Soil\ Dry\ Weight}{Volume\ of\ Sample\ Collected}$$

$$Percent\ Organic\ Matter = \frac{Weight\ after\ 105°C - Weight\ after\ 500°C}{Weight\ after\ 105°C} \times 100$$

Furthermore, I measured the length of the earthworms, calculated the density of the earthworms per sampled plot, and identified the earthworms into their respective categories of anecic, endogeic and epigeic. I also measured the litter dry weight of each plot.

Using RStudio version 3.6.0 (2021), I constructed box plots and ran an Analysis of Variance (ANOVA) to determine whether percent moisture dry weight, bulk density and percent organic matter varied by the different prairie sites. I also conducted ANOVA to investigate if earthworm density and litter density varied by the different prairie restoration years. Furthermore, I constructed contingency tables and ran a chi-square test to determine whether earthworm species were independent of prairie sites. I also conducted a correlation test to investigate if weight litter is correlated with the percentage of endogeic and percentage of anecic earthworms present.

Results

The four prairie sites in this study were dense with tall grass and prairie plants. 1989 was easy to walk through, however 1994 had thick litter and thorny plants. 2004 had different vegetation, with more flowering tall grass. 1989 and 1998 had relatively softer soil texture.

There were a total of 35 earthworms collected from all 8 plots, with one plot from 1989 not having any earthworms and the most earthworms in one plot from 1994, having 10 earthworms. Across all the plots, there were a total of 10 anecic earthworms, 22 endogeic earthworms and 3 epigeic earthworms. The length of the earthworms ranged from 1.9cm to 7.2cm. The density of earthworms in the measured plots ranged from 9.18 earthworms per square meter to 91.83 earthworms per square meter. Of the 7 plots with earthworms, the quickest time it took for at least one earthworm to emerge was ~1 minute, and the longest time taken was ~7 minutes.

Soil Composition

There was no evident relationship between the chronosequence of restored prairie and the measured soil composition. The mean for percent moisture dry weight, bulk density and percent organic matter were all not statistically significant with p-values of 0.081 (Table 1), 0.093 (Table 2) and 0.165 (Table 3) respectively. This indicates that there was not enough of a difference between the means to show that years since prairie restoration affect the soil composition.

While the results were statistically insignificant, the 1989 site had relatively different means for percent moisture dry weight (Figure 2), bulk density (Figure 3), and percent organic matter (Figure 4), compared to the other sites. According to the ANOVA test, for percent moisture dry weight, 1989 had a low mean of 17.7% with a standard deviation of 0.854, while the other sites had a mean which ranged from 27.5% - 31.0% (Table 1). For bulk density, 1989 had a high mean of 1.35 g/cm^3, with a standard deviation of 0.082, while the other sites had a mean which ranged from 1.08g/cm^3 -1.16 g/cm^3 (Table 2). As for percent organic matter, 1989 had a low mean of 4.75%, with a standard deviation of 0.775, whereas the other sites had a mean of 6.49%-7.86% (Table 3).

Earthworm Population and Litter

The earthworm populations were significantly different (p-value = 0.006) in each site. As seen in Table 4, 1989 had the lowest mean density of 4.59 earthworms per square meter (n=1), with a standard deviation of 6.49. 1994 had the highest mean density of 82.6 earthworms per square meter (n=18), with a standard deviation of 13.0. While the earthworm densities varied from site to site, the type of earthworms were not influenced by the different sites (p-value = 0.096). As seen in Table 5, the most popularly found earthworm categories are anecic and endogeic. 1989 had 100% anecic (n=1); 1994 had 77.78% endogeic (n=14); 1998 had 75% endogeic (n=6); 2004 had 50% anecic (n=4). Epigeic earthworms were only found on the 1994 and 2004 sites, with 5.56% (n=1) and 25% (n=2) respectively.

According to the ANOVA test, there was no apparent significant difference (p-value=0.194) between the mean of litter weight among the different prairie sites. As seen in Table 6, 1989 had the lowest dry litter weight of 97.7g with a standard deviation of 18.5. Apart from 1989, there was however an increasing trend in litter weight from newest restored prairie to oldest restored prairie. 1994 had a mean of 232g with a standard deviation of 8.17, 1998 and 2004 each had a mean of 198g and 188g with a standard deviation of 87.4 and 47.3 respectively. As with the soil composition, 1989 had earthworm density and dry litter weight which was relatively different from that of the other sites.

There was a strong positive correlation between the weight of litter and density of earthworms (Figure 5). As the litter weight increased, the density of earthworms also increased. This correlation was strong (R=0.76) and statistically significant (p-value=0.028). In looking at the specific species, there was a strong positive correlation between the weight of litter and percentage of endogeic earthworms (Figure 6). As the litter weight increased, the percentage of endogeic earthworms also increased. This correlation was strong (R=0.8) and the results were statistically significant, with a p-value of 0.032. On the other hand, there was a negative correlation between litter weight and percent anecic earthworm (Figure 7). As the litter weight increased, the percentage of anecic earthworms decreased. This correlation was strong and statistically significant, with a R value of -0.88 and p-value of 0.009.

Discussion

While some of the findings of this study were expected, certain aspects are in opposition to the existing literature. This study found that the number of years of prairie restoration did not affect the soil composition and litter weight. However, there was a notable difference seen in the oldest prairie site which was restored in 1989.

As there was no significant difference in soil composition and litter weight across the prairie sites, it was not expected that there would be a difference in earthworm population densities across the sites. The results of this study however found that earthworm population densities were significantly different across all sites. This suggests that there could be other factors influencing the earthworm population densities in these prairie sites which were not investigated in this study.

Soil Composition

The soil composition was fairly similar across the chronosequence of restored prairie sites. As seen in Figures 2, 3 and 4, the sites 1994, 1998 and 2004 all had similar means for percent moisture dry weight, bulk density and percent organic matter. Since the difference in restoration periods of the studied prairie sites are relatively short (15 years) and were restored not too long ago (32 years), it is expected that the physical soil composition of all sites remain relatively similar. As Jones (2006) cited Varvel et al. (2006), soil characteristics change at a fairly slow rate, and thus a period of 27 years since restoration might not portray an observed difference in the sites.

Although the results were not significant, the prairie site restored in 1989 had relatively different means for all three soil composition aspects. Its percent moisture dry weight and percent organic matter were relatively lower than that of the other three sites while its bulk density was relatively higher than that of the other sites. This suggests that the soil characteristics may have begun changing some time between 27 to 32 years after restoration. The higher percent organic matter in 1989 may be indicative that the restoration is successful as Okermann (1997) mentioned that more established prairie lands have more organic matter in the soil.

As vegetation restoration can alter litter input, as well as the physical, chemical and biological properties of the soil (Ren et al. 2017; Zhao et al. 2017 as cited in Bai et al. 2018), future studies should be conducted to monitor how 1989 site continues to change over time. Furthermore, as there was an evident change in soil composition from 1989 to 1994, more studies should be conducted on St. Olaf's prairie lands that were restored in 1993 and 1994. A yearly or twice-yearly study could be conducted to track the changes in the land from 27 to 32 years, therefore, determining whether there is a tipping point at which the land's soil composition changes.

In looking at the soil composition differences between 1989 and the other prairie sites, it should be noted that 1989 is at a relatively different location (refer to Figure 1). 1989 is comparatively smaller than the other prairies, located further away from the other prairie sites and mainly bordered by existing or restored forests. According to Zhang et al. (2015), the different land use and management history of restored lands would cause soil composition and vegetation to alter at different rates. As St. Olaf has been slowly acquiring different plots of land since 1989 (St. Olaf 2021), the 1989 site could have had a different land use history from that of the other studied sites.

Earthworm Population and Litter

As with soil composition, the earthworm population density in the 1989 site was evidently different from that of the other sites (Table 4). According to Hensley et al. (2021), earthworm densities decrease after a certain amount of time since restoration. While Hensley et al. found that younger restoration sites had more earthworms, Wodika et al.'s (2014) study found that earthworm abundance increased across the chronosequence of restored prairie sites, which is synonymous with this study. I found that the older restoration site (1994) had a higher earthworm population density as compared to 1998 and 2004. This highlights the possibility that earthworm populations respond differently in different restored prairie sites.

Another point to consider would be the time at which the prairie sites were last burnt. According to Thomason et al (2017) as cited in Hensley et al. (2021), prairie burns have been reported to increase earthworm densities. The 1994 site had the second most recent prairie burn (spring 2018), behind the 1989 site (spring 2019), which could explain the high earthworm density in the 1994 site (Shea 2022). However, the 1989 site was yet again an anomaly as it had the lowest earthworm density despite being the site which was most recently burnt.

In considering the litter weight found in the prairie sites, apart from 1989, there was an increasing trend in litter weight with longer established prairie sites (Table 6). Camill et al. (2004) found that litter mass increased as restored prairies became more established. Camill et al. also mentioned that prairie burns would also significantly reduce surface litter, which explains 1989's relatively lower litter weight as it is the most recent site in this study to have undergone a prairie burn in spring of 2019 (Shea 2022). However, if prairie burns are taken into consideration, the high earthworm density in 1994 (Table 4) and low litter weight in 1989 (Table 6) are contradicting as the burnt site should have high earthworm density and low litter weight.

The positive correlation between litter weight and earthworm density (Figure 5) indicates that areas with lots of litter had many earthworms. However, numerous studies have shown that areas with high earthworm densities would reduce the litter layer greatly (Alban and Berry 1994; Ashton et al. 2005 as cited in McCay and Scull 2018; Ista 2001) and could in turn affect the vegetation density on the prairie (Ista 2001). According to Wallace (1988), depending on the effects of vegetation on the soil, different organisms would inhabit different soil areas. Comparing these two studies, it seems as though the vegetation density and earthworm population density might depend on whether earthworms were prevalent on these sites prior to restoration. A possible hypothesis on the positive correlation trend observed is that the earthworm population (until 1994) might still be increasing due to the high abundance of litter, and will eventually reach a point when the population is greater than the litter available. The resulting lack of litter would thus not be able to support high earthworm densities, which could explain the low litter and low earthworm density seen in 1989.

To further investigate the effects of litter weight on earthworms, I looked at the correlation of litter weight on specific earthworm types. The most populous earthworm was endogeic earthworms, which increased in density as the litter weight increased (Figure 6). On the other hand, the second most populous was the anecic earthworms, which decreased as litter weight increased (Figure 7). While the correlation for endogeic earthworms is expected as they feed on both surface and upper mineral soil layers (Hale 2007 as cited in Henshue et al. 2016), an opposite (positive) correlation for anecic earthworms was expected as they only feed on surface litter (Reynolds 1977 as cited in Henshue et al. 2016). There should be an increase in anecic earthworms with an increase in litter. As anecic earthworms live in deep burrows (Reynolds 1977 as cited in Henshue et al. 2016), there is a possibility that a wait duration of 8 minutes may not have been long enough for all the anecic earthworms to surface.

Future of St. Olaf's Prairie Sites

Based on the findings of this study, it is unclear whether the soil composition, earthworm densities and litter weight differences in 1989 are due to the fact that the restored prairie site begins to change between 27-32 years after restoration or its further location. If it is the former which caused the soil and its organisms to change significantly, a similar change in the other restored prairies in the near future could be expected. However, there is also a possibility that the difference in 1989 could be due to its location or land use history instead, in which we would expect all the prairie sites to remain fairly similar in the near future.

Conclusion

The findings of this study suggest that the chronosequence of St. Olaf restored prairie sites were not noticeably different, with the exception of 1989. This lack of distinction in soil composition and litter weight between the sites was most likely due to the short 32 year period since the oldest site (1989) was restored (Varvel et al. 2006 as cited in Jones 2006). The difference seen in the 1989 plot was likely due to its location and land use history, or that 27-32 years post restoration is when the prairie ecosystem begins to change. To determine if this period is when the prairie ecosystem begins to change, twice-yearly studies should be conducted on 1993 and 1994 sites in the next 4-5 years to determine if a similar pattern as seen in 1989 would surface.

As no significant differences in soil composition and litter was found, the differences seen in the earthworm population was likely due to other factors such as soil chemistry or the type of vegetation which was not included in this study. Since this is the first study on earthworms in the St. Olaf prairies, future studies should be conducted to analyze the chemical composition of the soil in relation to earthworm densities. Furthermore, the earthworm population across the chronosequence of prairies should be investigated more thoroughly, including identifying the specific species. These studies should also take into account when the prairie sites were last burnt and its land use history as this could shine light on the anomalies seen in the results.

Future studies on the type of vegetation currently found on St. Olaf prairies should also be conducted to better understand how different vegetation and litter might affect the earthworm population density, or whether it is an inverse relationship that is seen. In order to better predict the recruitment and abundance of native plant species, it is important to better understand the complex interactions between soil composition, earthworm densities, litter and burns. All this information would be useful in evaluating the success of St. Olaf Natural Land's prairie restoration (Wodika, Klopf, & Baer, 2014 as cited in Hensley et al. 2021), and would provide necessary information on whether management intervention is needed to ensure the prairies remain healthy.

Acknowledgements

I would like to thank Professor Kathy Shea for her guidance throughout the execution of this project, for her help in brainstorming a research topic, guidance in conducting field work, data analysis and writing this report. I would also like to express my gratitude to Jack Haselhorst and Rachel Ellis for their help and companionship in collecting samples from the prairie sites. A huge thank you goes out to the entire Field Ecology 2021 class for their camaraderie, support and guidance throughout the entire 2021 Fall semester.

Works Cited

Alban, D. H., and E. C. Berry. 1994. Effects of earthworm invasion on morphology, carbon, and nitrogen of a forest soil. Applied Soil Ecology 1:243–249.

Ashton, I. W., L. A. Hyatt, K. M. Howe, J. Gurevitch, and M. T. Lerdau. 2005. Invasive species accelerate decomposition and litter nitrogen loss in a mixed deciduous forest. Ecological Applications 15:1263–1272.

Bai, Y., X. Zha, and S. Chen. 2019. Effects of the vegetation restoration years on soil microbial community composition and biomass in degraded lands in Changting County, China. Journal of Forestry Research 31:1295–1308.

Bal, T. L., A. J. Storer, and M. F. Jurgensen. 2017. Evidence of damage from exotic invasive earthworm activity was highly correlated to sugar maple dieback in the upper Great Lakes Region. Biological Invasions 20:151–164.

Boecker, D., C. Centeri, G. Welp, and B. M. Möseler. 2015. Parallels of secondary grassland succession and soil regeneration in a chronosequence of central-hungarian old fields. Folia Geobotanica 50:91–106.

Bohlen, P. J., P. M. Groffman, T. J. Fahey, M. C. Fisk, E. Suarez, D. M. Pelletier, and R. T. Fahey. 2004. Ecosystem consequences of exotic earthworm invasion of north temperate forests. Ecosystems 7:1–12.

Bouché, M. B. 1977. Strategies lombriciennes. Ecol Bull 25:122–132.

Brower, J., J. Zar and C. vonEnde. 1998. Field and laboratory methods for general ecology. 4th ed. WCB/McGraw-Hill, Dubuque, Iowa.

Camill, P., M. J. McKone, S. T. Sturges, W. J. Severud, E. Ellis, J. Limmer, C. B. Martin, R. T. Navratil, A. J. Purdie, B. S. Sandel, S. Talukder, and A. Trout. 2004. Community- and ecosystem-level changes in a species-rich tallgrass prairie restoration. Ecological Applications 14:1680–1694.

Council on Environmental Quality (CEQ). 1991. Environmental qualityTwenty-first annual report. Council on Environmental Quality, Washington, D.C.

Dobson, A. M., B. Blossey, and J. B. Richardson. 2017. Invasive earthworms change nutrient availability and uptake by forest understory plants. Plant and Soil 421:175–190.

Eisenhauer, N., and S. Scheu. 2008. Invasibility of experimental grassland communities: The role of earthworms, plant functional group identity and seed size. Oikos 117:1026–1036.

Field Ecology 371 Lab Manual. 2019. European earthworm distribution and diversity. Unpublished.

Frelich, L. E., B. Blossey, E. K. Cameron, A. Dávalos, N. Eisenhauer, T. Fahey, O. Ferlian, P. M. Groffman, E. Larson, S. R. Loss, J. C. Maerz, V. Nuzzo, K. Yoo, and P. B. Reich. 2019. Sideswiped: Ecological cascades emanating from earthworm invasions. Frontiers in Ecology and the Environment 17:502–510.

Haakensen, K. A. 2003. Assessing the Progress of Prairie Restoration on the campus of St. Olaf College. Unpublished. https://wp.stolaf.edu/naturallands/files/2016/02/merged_Haakenson_PDF.pdf.

Hale, C. 2013. Earthworms of the Great Lakes. Kollath+Stensaas Pub. Duluth, Minnesota.

Hale, C. M., L. E. Frelich, and P. B. Reich. 2006. Changes in hardwood forest understory plant communities in response to European earthworm invasions. Ecology 87:1637–1649.

Hale, C. M., L. E. Frelich, P. B. Reich, and J. Pastor. 2005. Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. Ecosystems 8:911–927.

Hale, C. M., L. E. Frelich, P. B. Reich, and J. Pastor. 2007. Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: A mesocosm study. Oecologia 155:509–518.

Hendrix, P. F., and P. J. Bohlen. 2002. Exotic Earthworm Invasions in North America: Ecological and policy implications. BioScience 52:801.

Henshue, N., C. Mordhorst, and L. Perkins. 2017. Invasive earthworms in a northern Great Plains Prairie fragment. Biological Invasions 20:29–32.

Hensley, C., K. R. Brye, M. C. Savin, E. E. Gbur, and L. S. Wood. 2021. Earthworm density differences among tallgrass prairies over time in the Ozark highlands. Agrosystems, Geosciences & Environment 4.

Ista, A. 2001. Impacts of European earthworms on maple-basswood forests. Unpublished. https://wp.stolaf.edu/naturallands/files/2016/01/Ista_2001_merged.pdf.

Jones, A. M. 2006. Impact of soil quality by long-term no-till practices and artificial drainage systems. Unpublished. https://wp.stolaf.edu/naturallands/files/2016/02/Jones_2006_merged_AG.pdf.

Kalinina, O., A. N. Barmin, O. Chertov, A. V. Dolgikh, S. V. Goryachkin, D. I. Lyuri, and L. Giani. 2015. Self-restoration of post-agrogenic soils of Calcisol–Solonetz Complex: Soil development, carbon stock dynamics of Carbon Pools. Geoderma 237-238:117–128.

Kéry, M., and K. B. Gregg. 2004. Demographic analysis of dormancy and survival in the terrestrial orchid Cypripedium Reginae. Journal of Ecology 92:686–695.

Li, X. 2002. Influence of earthworm invasion on soil microbial biomass and activity in a Northern Hardwood Forest. Soil Biology and Biochemistry 34:1929–1937.

McCay, T. S., and P. Scull. 2019. Invasive lumbricid earthworms in northeastern North American forests and consequences for leaf-litter fauna. Biological Invasions 21:2081–2093.

Okerman, A. K. 1997. Comparison of two prairie restoration sites to examine density, maturity and species diversity. Unpublished. https://wp.stolaf.edu/naturallands/files/2016/02/merged_Okerman_PDF.pdf.

O'Keefe, K., and K. A. McCulloh. 2020. Do invasive jumping worms impact sugar maple (Acer saccharum) water-use dynamics in a central hardwoods forest? Biological Invasions 23:129–141.

Ren, C., J. Chen, J. Deng, F. Zhao, X. Han, G. Yang, X. Tong, Y. Feng, S. Shelton, and G. Ren. 2017. Response of microbial diversity to C:N:P stoichiometry in fine root and microbial biomass following afforestation. Biology and Fertility of Soils 53:457–468.

Reynolds, J. W. 1977. The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Royal Ontario Museum, Toronto.

RStudio Team (2021). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Samson, F., and F. Knopf. 1994. Prairie conservation in North America. BioScience 44:418–421.

Shea, K. 2012. Methods for Soil Analysis at St. Olaf College. Unpublished. https://erenweb.org/wp-content/uploads/2012/08/EREN-PFPP_Appendix-II_SoilMethods_StOlaf_30July2012.pdf.

Shea, K. 2022. Personal Communication.

St. Olaf College. (2019). St. Olaf College Natural Lands. St. Olaf College. https://wp.stolaf.edu/naturallands/files/2019/02/Natural-Lands-Version-2019b_use.pdf.

St. Olaf College. 2018, March 15. Prairies. St. Olaf College. https://wp.stolaf.edu/naturallands/prairies/.

St. Olaf College. 2021, September 15. Natural lands. St. Olaf College. https://wp.stolaf.edu/naturallands/.

Thomason, J. E., M. C. Savin, K. R. Brye, and E. E. Gbur. 2017. Native earthworm population dominance after seven years of tillage, burning, and residue level management in a wheat-soybean, double-crop system. Applied Soil Ecology 120:211–218.

Varvel, G., W. Riedell, E. Deibert, B. McConkey, D. Tanaka, M. Vigil, and R. Schwartz. 2006. Great Plains Cropping System Studies for Soil Quality Assessment. Renewable Agriculture and Food Systems 21:3–14.

Wallace, A. 1988. Comparison of chemical characteristics and soil invertebrate diversities in coniferous and deciduous forest soils. Unpublished. https://wp.stolaf.edu/naturallands/files/2016/02/Wallace_1988_merged.pdf.

Wodika, B. R., R. P. Klopf, and S. G. Baer. 2014. Colonization and recovery of Invertebrate Ecosystem Engineers during prairie restoration. Restoration Ecology 22:456–464.

Zhao, D., M. Xu, G. Liu, L. Ma, S. Zhang, T. Xiao, and G. Peng. 2017. Effect of vegetation type on microstructure of soil aggregates on the Loess Plateau, China. Agriculture, Ecosystems & Environment 242:1–8.

Appendix

St. Olaf College Natural Lands

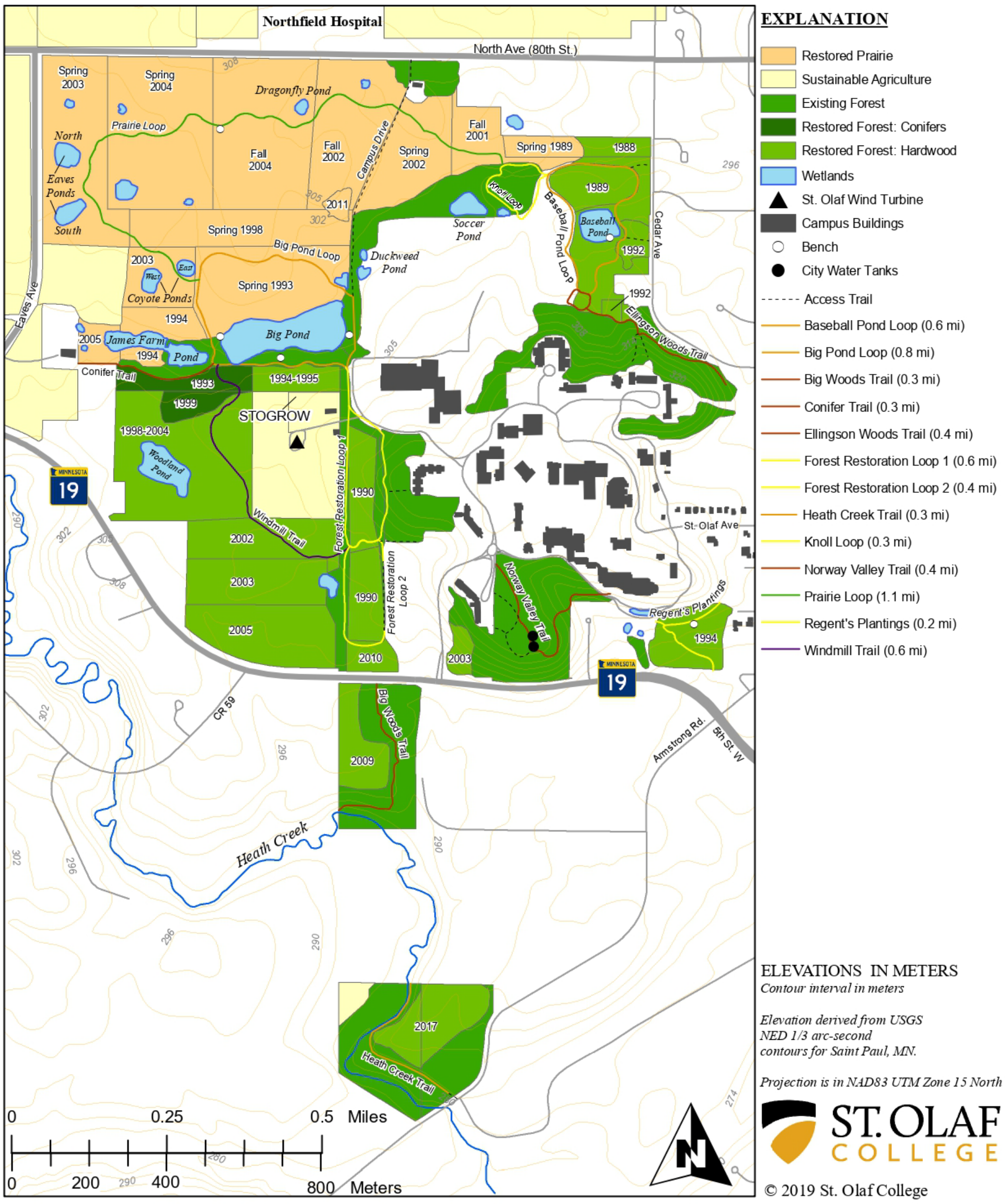

Figure 1. A map of St. Olaf's Natural Lands highlighting the restored prairie sites and its respective years restored (St. Olaf 2019).

Table 1. The analysis of variance showing the mean, standard deviation, count and p-value of soil percent moisture dry weight in relation to the different prairie sites.

Prairie Site	% Moisture Dry Weight			P-Value: 0.081 DFn: 3 DFd: 8 F-value: 3.244
	Mean	**SD**	**Count**	
1989	17.7	0.854	3	
1994	29.9	6.07	3	
1998	27.5	1.32	3	
2004	31.0	9.79	3	

Table 2. The analysis of variance showing the mean, standard deviation, count and p-value of soil bulk density in relation to the different prairie sites.

Prairie Site	Bulk Density			P-Value: 0.093 DFn: 3 DFd: 8 F-value: 3.035
	Mean	**SD**	**Count**	
1989	1.35	0.082	3	
1994	1.08	0.173	3	
1998	1.16	0.054	3	
2004	1.16	0.106	3	

Table 3. The analysis of variance showing the mean, standard deviation, count and p-value of soil percent organic matter in relation to the different prairie sites.

Prairie Site	% Organic Matter			P-Value: 0.165 DFn: 3 DFd: 8
	Mean	**SD**	**Count**	
1989	4.75	0.775	3	
1994	7.86	2.67	3	

1998	6.94	0.939	3	**F-value: 2.207**
2004	6.49	0.788	3	

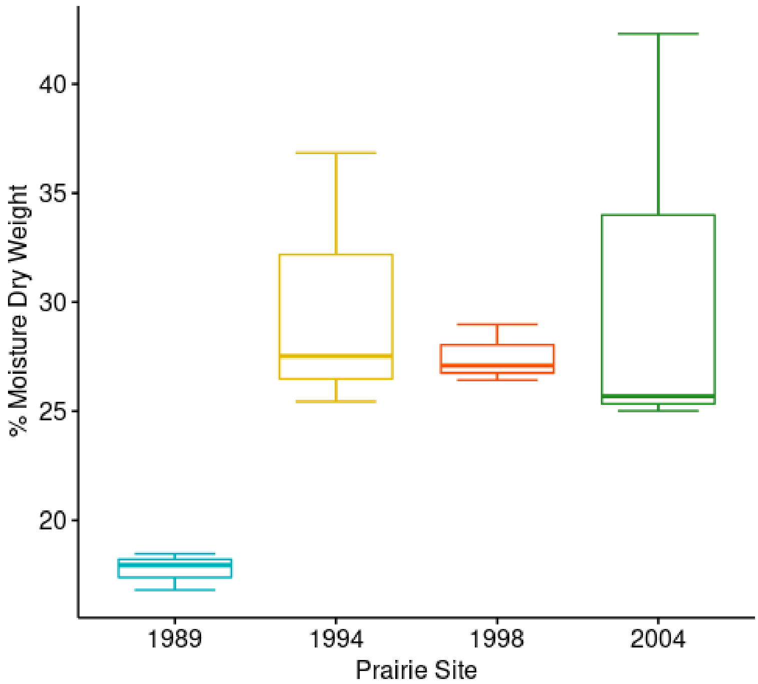

Figure 2. A box plot illustrating the soil percent moisture dry weight of each prairie site. Means were not significantly different (p-value = 0.081).

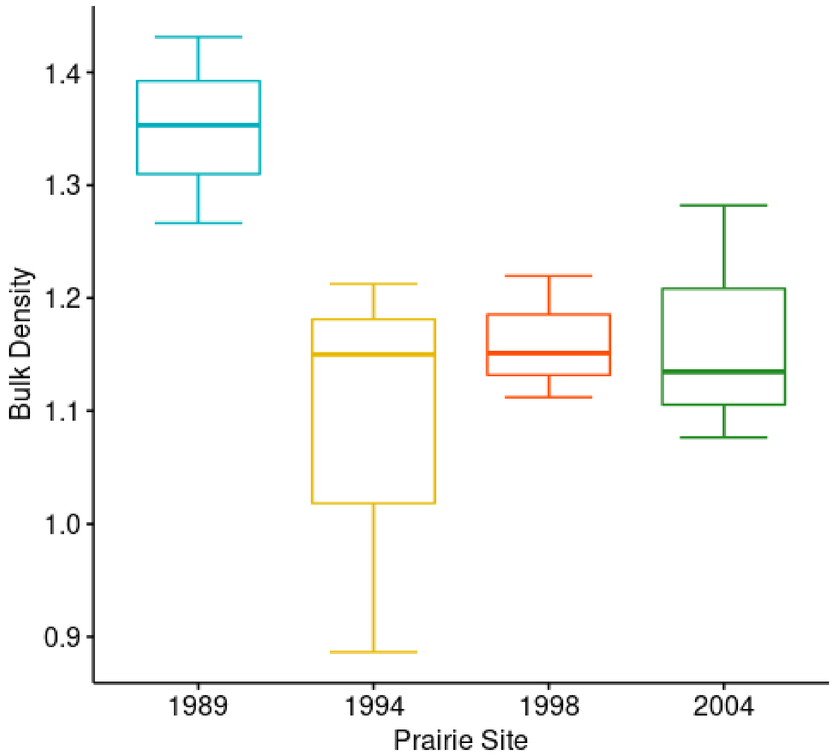

Figure 3. A box plot illustrating the soil bulk density of each prairie site. Means were not significantly different (p-value = 0.093).

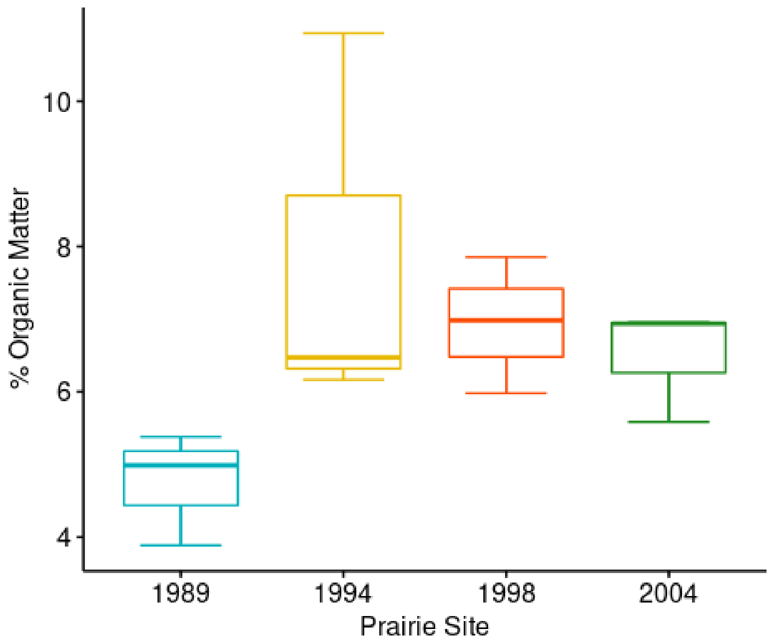

Figure 4. A box plot illustrating the soil percent organic matter of each prairie site. Means were not significantly different (p-value = 0.165).

Table 4. The analysis of variance showing the mean, standard deviation, count and p-value of earthworm density in relation to the different prairie sites.

Prairie Site	Earthworm Density			P-Value: 0.006 DFn: 3 DFd: 4 F-value: 21.741
	Mean	SD	Count	
1989	4.59	6.49	2	
1994	82.6	13.0	2	
1998	36.7	0	2	
2004	36.7	13.0	2	

Table 5. Contingency table depicting the independence of earthworm types relative to the respective prairie sites. The chi-square test yielded a chi-square value of 10.759, with a degree of freedom of 6 and p-value of 0.096.

Sites	Anecic	% Anecic	Endogeic	% Endogeic	Epigeic	% Epigeic
1989	1	100%	0	0%	0	0%
1994	3	16.67%	14	77.78%	1	5.56%
1998	2	25%	6	75%	0	0%
2004	4	50%	2	25%	2	25%

Table 6. The analysis of variance showing the mean, standard deviation, count and p-value of square plot litter dry weight (cleared for earthworm extraction) in relation to the different prairie sites.

Prairie Site	Weight of Dry Litter			P-Value: 0.194 DFn: 3 DFd: 4 F-value: 2.552
	Mean	SD	Count	
1989	97.7	18.5	2	
1994	232	8.17	2	
1998	198	87.4	2	
2004	188	47.3	2	

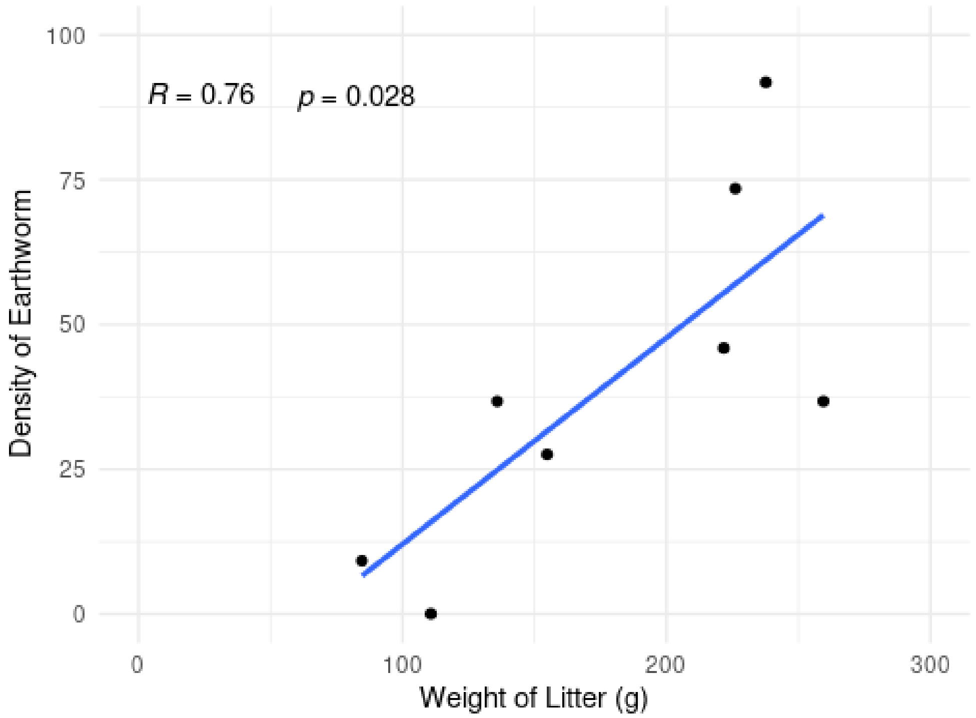

Figure 5. A scatterplot illustrating the correlation between the dry weight of litter taken from the square plot clearings and the density of earthworms.

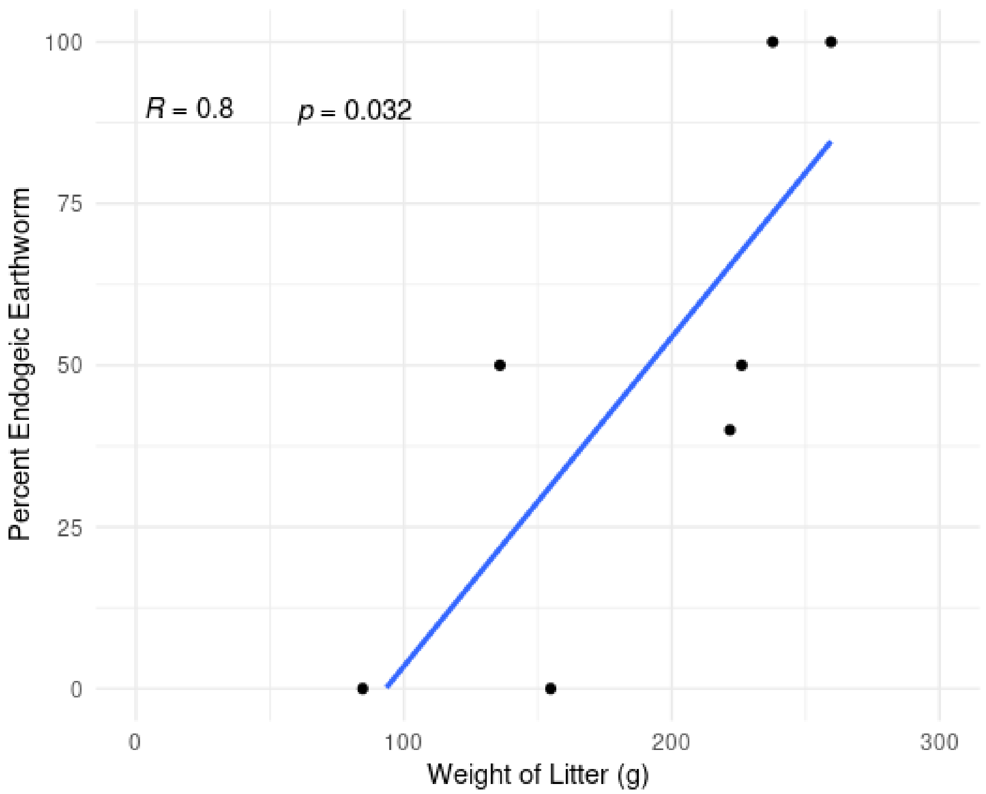

Figure 6. A scatterplot illustrating the correlation between the dry weight of litter taken from the square plot clearings and the percentage of endogeic earthworms.

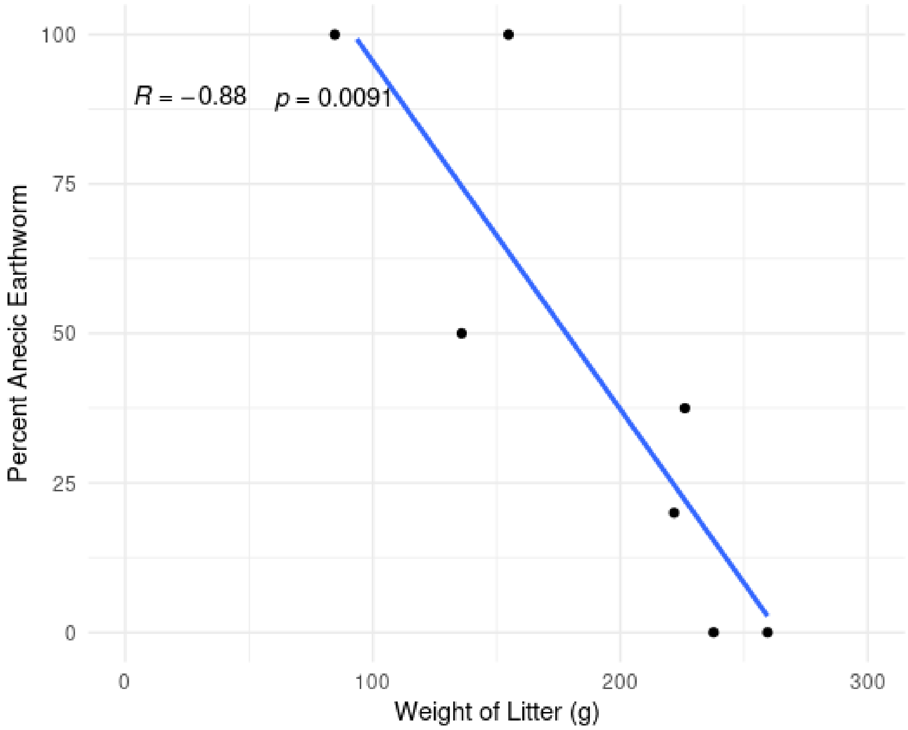

Figure 7. A scatterplot illustrating the correlation between the dry weight of litter taken from the square plot clearings and the percentage of anecic earthworms.