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A Survey of Earthworm Communities Across Areas of Varying Land Management in Northwest Arkansas

An Honors Thesis submitted in partial fulfillment of the requirements of Honors Studies in
Environmental, Soil, and Water Science

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Abstract

Earthworms are an integral component of the soil ecosystem, impacting factors including soil formation, maintenance of soil structure, and nutrient recycling. Earthworm abundances and distributions are related to abiotic soil properties and can influence the community structure. The goal of this study was to survey earthworm populations across areas of different land management histories and soil characteristics to determine if a relationship between earthworm density and land management characteristics was present. Earthworm and soil samples were collected at irregular temporal intervals from September 2017 to March 2018. In total, five sites were sampled, three at the Botanical Garden of the Ozarks in Fayetteville, AR (garden, restored riparian streambank, and field) and two sites (intermound and mound areas) at Chesney Prairie in Siloam Springs, AR. Samples were collected from 30 cm wide x 30 cm long x 20 cm deep monoliths extracted with a garden spade. Subsamples of the soil were collected, and earthworms were boiled and preserved in 90% ethanol. The earthworm community density per m^2 was examined across the five sampling sites. Abiotic characteristics of the soil including moisture, temperature, carbon-to-nitrogen ratio, and texture were examined across three sites from September 2017 to November 2017. Native and exotic genera of earthworms were morphologically identified. *Bimastos* is a native genus that was observed in the garden sampling site, the most managed site where exotics are often expected to outcompete the native species. More exotic species were observed at all sampling sites where adults were detected, which may be due to migration of species or through the transport of soil or plants in a botanical garden setting. If exotic species continue to outcompete the natives, soil processes may be altered due to different rates of activities in the soil, which may ultimately affect the other organisms and plants in an undisturbed ecosystem compared to a frequently disturbed system.

Introduction

The beneficial role of earthworms in breaking down plant material and incorporating the organic material into the soil was first acknowledged by Darwin in 1881 (Darwin, 1881). Earthworms are keystone species and ecological engineers, significantly influencing various soil properties and processes essential for the functioning of terrestrial ecosystems (Hendrix et al., 2006). Soil processes affected by earthworm activity include soil aggregation, soil porosity, nutrient cycling and microbial activity (Lavelle and Pashanasi, 1989; Lee, 1985). As earthworms burrow through the soil, casts are created which bind soil particles together, potentially increasing or decreasing soil aggregation, depending on the species present. The burrows create macropore space in the soil and alter pore space dynamics such as increasing water infiltration. Earthworms are also decomposers and breakdown detritus physically and chemically. The breakdown of material leads to an increase in microbial activity in casts compared to the surrounding soil (Lavelle et al., 1989; Hendrix et al., 2006; Lavelle and Pashanasi, 1989). Field-collected casts have been reported to have greater populations of fungi, actinomycetes, and bacteria and more enzyme activity compared to the surrounding soil (Edwards and Bohlen, 1996).

Earthworm community distribution is generally heterogeneous, where abundances are related to abiotic soil factors (Guild, 1952). Environmental properties including soil moisture, temperature, texture, and nutrient content can influence earthworm populations (Edwards and Bohlen, 1996). Earthworms collected from the field typically have a water content 65% to 75% of body weight (Lee, 1985). While earthworms can survive some loss of body water, most earthworms have limited physiological defenses against the water loss. Increased temperatures are often associated with moisture shortages, which produce seasonal earthworm mortality in temperate soils due to moisture stress rather than temperature extremes (Lee, 1985). When the soil moisture begins to decrease towards a critical value, earthworms burrow deeper into the soil to find moisture, enter quiescence (dormancy), or die (Wood and Samuel, 1993). Soil temperature influences activity, metabolism, growth, respiration and reproduction of earthworms (Edwards and Bohlen, 1996). Optimum temperatures vary by species but typically range from 10 to 25 °C (Edwards and Bohlen, 1996). Temperatures that are outside survivable limits may kill earthworms.

Earthworms are considered semi-continuous or continuous breeders, as they breed several times during their lifespan and release cocoons. Although juveniles emerge from these cocoons at any time during the year, emergence can vary by season due to temperature and moisture variations (Lee, 1985). Mortality due to water stress mainly affects juveniles because juveniles are unable to escape desiccation by moving deeper into the soil (Curry, 1994). Since earthworms are soft-bodied organisms, soil texture can influence earthworm species' composition and abundance and density of populations. Earthworms avoid coarse textured soils because of the abrasiveness and susceptibility to drought. Clay content of soils also affects earthworm activity because the soil may become anaerobic with an elevated water content and reduced gas exchange (Lee, 1985).

The carbon (C) and nitrogen (N) content of the soil can also influence earthworm activity. In 1972, Bouché observed that the minimum C:N ratio in the soil for seven lumbricid species, including *Aporrectodea caliginosa*, *Lumbricus rubellus*, and *Lumbricus terrestris*, ranged from seven to nine. An exotic family, Lumbricidae, were able to tolerate a wide range of C:N ratios. The availability of nitrogen is one of the most important factors that limits earthworm populations and distributions (Lee, 1985). All earthworm species have specific soil property optima, which influence community composition within soils.

Relatively few terrestrial systems modified by human activity are free of introduced exotic earthworms and, in the southern United States, both native and exotic species have been recorded (James and Hendrix, 1994; Kalisz and Wood, 1995). After the introduction of exotic earthworms into an area previously inhabited by native species, the exotics may become prominent, fail to persist, or co-inhabit the area with native species (Hendrix et al., 2006). When competing with native species, exotic earthworms often acquire and conserve resources more efficiently, colonize new habitats, and spread rapidly due to a greater tolerance of variable environmental conditions (Kalisz and Wood, 1995; Lavelle, 1988; Hendrix et al., 2006). Once introduced into an ecosystem, the exotic species can alter pre-existing soil properties including porosity and nutrient dynamics, which can affect ecological dynamics in the ecosystem.

Understanding factors influencing exotic and native earthworm population distributions will become increasingly important since exotics have the ability to disrupt native ecosystems, ultimately affecting other species' ecology and diversity. Data revealing how community composition of exotic and native earthworm species is related to abiotic soil factors will further our understanding of earthworm ecology and, consequently soil functions in managed systems.

The objectives of this study were to (1) examine changes in earthworm developmental stage structure across ecosystems of varying management intensity, (2) observe the effect of land management and soil abiotic factors including soil moisture, temperature, texture and nutrient content (total nitrogen and total carbon) on total earthworm population densities, and (3) survey earthworm community composition at each sampling site using morphological identifications.

Materials and Methods

Study Sites

Five study sites within Northwest Arkansas were sampled based on differing land management histories and soil characteristics. Three sites were located at the Botanical Garden of the Ozarks (BGO) (36°08'12.3"N 94°07'10.6"W) in Washington Country, AR. The BGO sites included (1) a heavily managed garden bed located at the edge of an irrigated flower garden that received compost and mulching, (2) the toe slope of a restored riparian streambank located downslope from the garden beds that was restored in April 2016, and (3) a mowed grass field adjacent to the restored bank. The fourth and fifth sampling sites were at Chesney Prairie in Benton County, AR (36°13'05.1"N 94°28'58.9"W), a remnant tallgrass prairie with mound topography, which received biennial prescribed burning. Both mound and intermound topography were sampled at Chesney Prairie. Earthworm samples were collected at irregular intervals from September 2017 to March 2018 (Tables 1 and 2). At least two replicates were collected at each site for every sampling date to ensure replication within sites.

Abiotic Soil Property Analysis

Soil temperature and moisture were recorded in-situ with a thermometer at a depth of 10 cm and a theta probe at a depth of 7 cm, respectively. Soil subsamples were collected from the sorted and mixed monolith from each replication during fall collections (September-December). Samples of collected soil were sieved through a 2-mm sieve and air-dried for three days before analysis. Particle sizes were determined using a 2-hour hydrometer method with 50 mL of 1 *M* sodium hexametaphosphate and 1 L of deionized water and particle sizes were used to determine soil texture for each sampling site. Soil samples (40.0 ± 0.1 g) were not treated with hydrogen peroxide to remove organic matter before analysis. Total organic carbon and total nitrogen were analyzed by dry combustion using a Vario Max CN combustion analyzer (Elementar Americas INC., Mt. Laurel, NJ).

Earthworm Collection and Identification

The earthworms were collected as part of a service-learning project during the fall semester (2017) course ENSC 3103 Plants and Environmental Restoration. A monolith of soil 30 cm wide x 30 cm long x 20 cm deep from the soil surface was excavated as one replicate sample. If the soil surface was covered by mulch, the material was removed before excavation. The monolith was hand sorted and collected earthworms were boiled to kill, and were fixed and preserved on site in vials containing 95% ethanol. Adult earthworms were morphologically identified to the genus level using a dichotomous key (Dindal, 1990) using external physical features including the location of the clitellum, arrangement of setae, appearance of the prostomium, and earthworm pigmentation. Earthworms were recorded as adults if mature features including the clitellum and tubercula pubertatis were present. Juvenile earthworms lack identifiable physical characteristics present in adults and, therefore, no juveniles were identified morphologically. Juveniles were included in density calculations.

Data Analysis

The average density of earthworms was calculated based on a per m^2 basis across site and sampling date. Average density was calculated as a logarithmic transformation to account for variability within the samples and reported values are back-transformed. Any partial earthworms collected were discarded and did not contribute to the final dataset. Average soil moisture and temperature for each sampling site and date was plotted with standard deviation error bars. A two-way ANOVA was used to determine interaction effects between date and sampling site for number of earthworms collected, soil moisture, soil temperature, and C:N. If there was a significant interaction, a Tukey HSD was used to determine the significant differences between the groups. A linear regression, with a 95% confidence interval, was performed on a scatter plot of soil moisture and temperature against total number of earthworms collected per 0.09 m^2.

Results and Discussion

Earthworm Densities over Time and across Locations

The five sampling sites of this study were sampled irregularly with the garden and the field being sampled eight times from September 2017 to March 2018 (Table 1). The streambank was sampled six times from September to November 2017, and the prairie (intermound and mound) was sampled once on September 28, 2017. At Chesney Prairie on September 28, 2017, no earthworms were detected at the mound sampling sites and only juveniles were collected from the intermound sites.

For September 21, 2017, the most earthworms (32 per m^2) were collected at the field site compared to the number of earthworms collected at the garden and streambank sites (Table 2). When comparing the densities of the prairie (intermound) with the garden, streambank and field September sampling, the prairie (intermound) was significantly different with 100 earthworms per m^2. The number of earthworms collected decreased from October 5th to October19th in the garden, streambank and field sampling sites. During November 2017 the most earthworms were collected in the field at 250 per m^2 on November 16. In February and March of 2018, the density of earthworms in the field decreased to 226 per m^2 in February and increased to 273 per m^2 in

March, which differed from the garden sampling site where 56 and 55 per m^2 were found in February and March, respectively.

From September to November 2017, there was a significant difference between the number of earthworms collected on each sampling date (*P* = 0.04) and there was a significant difference among the different sampling sites (*P* = 0.047). November 16, 2017 was the only date that significantly differed to two other dates, October 19 and November 2, 2017 (*P* = 0.05). After early November, the number of earthworms collected at the garden and field sampling sites were significantly different where the most earthworms were collected at the field sampling site. The field sampling site was significantly different than the garden, streambank, and prairie sampling sites (*P* = 0.003).

The trend in percentage of juveniles decreased as management intensity increased with the garden having 73%, the streambank 75%, field at 86%, and the intermound had 100% juveniles (Table 1), but there were no significant differences between the percentages of juvenile-to-total earthworms collected on each sampling date (*P* = 0.49) or between the sampling locations (*P* = 0.20). Furthermore, there was no significant correlation between the percentage of juveniles-to-total earthworms with moisture or temperature (data not shown). There was a significant difference in the number of adult earthworms collected by sampling date (*P* = 0.01), but not by sampling location (*P* = 0.83). On November 16, 2017, there were significantly more adult earthworms collected compared to the other sampling dates (*P* = 0.03).

The calculated earthworm densities ranged from 1 to 273 per m^2 for total earthworms, 0 to 40 per m^2 for adult earthworms, and 1 to 231 per m^2 for juvenile earthworms. The total earthworm and juvenile density of this study fell within the range of the study in agricultural ecosystems in eastern Arkansas where earthworm densities ranged from 271 to 508 per m^2 for total earthworms, and 176 to 371 per m^2 for juvenile earthworms (Thomason et al., 2017). The adult densities of this study, 1 to 40 per m^2, did not align with the study in eastern Arkansas where the researchers observed 71 to 185 per m^2. The difference may be due to environmental differences, such as nutrient content.

Although adult densities did not correspond directly, the proportion of juvenile earthworms observed in the garden fell within the percentage range recorded in a highly managed agricultural ecosystem in the Lower Mississippi River Valley region of eastern Arkansas (Thomason et al., 2017). The garden at the BGO is the most heavily managed site out of the sampled locations since the garden received applications of compost and fertilizers as well as human disturbance through planting. The nutrient differences between the garden and the field sites may have influenced the metabolism and reproduction of earthworms compared to the field. If the collection period was longer, a change in earthworm densities may have been observed. An increased density of juvenile earthworms may occur in the spring because of favorable environmental conditions for reproduction and hatching from cocoons (Decaens et al., 2008).

Relationship of Soil Characteristics and Earthworm Populations

For soil textural analysis, the garden was a loam, the streambank was a sandy loam, the field was a silt loam, and the prairie, both mound and intermound, were silt loams (Table 3). Since the textures of the sampling sites were either sandy loam, silt loam, or a loam, densities may not have been influenced by soil texture. Earthworms are soft-bodied organisms that come in direct contact with the soil and large particles such as sand may deter earthworms from inhabiting the area because of potential abrasion (Lavelle, 1988). Furthermore, coarse texture soils are drought-prone and in turn, affect earthworm abundances (Lee, 1985). Earthworms are also infrequently found in soils with a high clay content because increased moisture can create anaerobic conditions (Edwards and Bohlen, 1996).

Average soil moisture varied between sites on specific dates. In September 2017, the field sampling site had a significantly drier moisture compared to the streambank and garden sites (Figure 1). Furthermore, the two prairie sampling sites were significantly different from each other. The prairie sites were also significantly different from the garden, and streambank sites, but not the field at the BGO. In November, the field moisture was significantly less than the garden and streambank. In February 2018, the field had a significantly greater moisture than the garden, but there was no difference between the field and the garden in March (Figure 1). For soil temperature, there were no differences between the garden, streambank, and field during September 2017, but the prairie sampling sites, both mound and intermound, were significantly different than the BGO sampling sites; although, there was not a significant difference between the prairie sampling locations in September (Figure 2). On October 19, 2017, there was a significant difference between the garden and streambank temperatures. The temperature of November 2, 2017 varied significantly between sampling sites, where the field had an increased temperature, garden was in the middle, and the streambank had the lower temperature (Figure 2). Furthermore, on November 9, 2017, temperatures were significantly different between the sampling sites at the BGO. The field had the highest temperature, then the garden, and the streambank had the lowest temperature.

Total earthworms collected (per 0.09 m^2) were independently regressed against temperature and moisture. Earthworm density per unit area was related positively to volumetric moisture (%) for all samples collected from September 2017 to March 2018 (n = 64, $P = 0.0001$, Figure 3). Boyle (2018) observed a similar trend with earthworms and soil moisture in turfgrass systems in Northwest Arkansas. Although the *P*-value was significant, the R^2 value was low, indicating that there was large variability among samples. On a gravimetric basis, optimal moisture conditions for earthworms range from 20 to 30% (Grant, 1955). The volumetric moistures in this study ranged from 5.6 to 40%. Suboptimal conditions were present during the study, especially at the prairie sampling sites, and may have affected the number of earthworms collected.

Similar to moisture, the total earthworms collected (per 0.09 m^2) and temperature had a significant regression with a *P*-value of 0.001 (Figure 4). A significant negative trend was observed where earthworm density decreased as temperature increased. Similar to moisture, the *P*-value was significant and the R^2 value was low, indicating that there was large variability among samples. Evans and Guild (1948) determined that the number of earthworms present significantly correlated to the temperature of a soil in a grass field. Temperatures within this study for most sampling locations and times were within the optimal temperatures observed for earthworms by Edwards and Bohlen in 1996, which range between 10 and 25°C. Since temperatures within this study were generally in the range of optimal temperatures, moisture conditions could have significantly influenced the number of earthworms collected.

There was no significant linear relationship between the total number of earthworms plotted against total soil C:N ($P > 0.05$) (Figure 5). The total soil C:N values in this study ranged from 8.6 to 20.2, which were higher than the minimum values observed by Bouché in 1972, which ranged from seven to nine (Lavelle, 1988). Finally, for C:N there was a significant difference between the garden and the other four sampling sites (Figure 6). The difference may be due to input from gardening activities such as mulching with materials that contain a wide range of C:N, which would increase the carbon input into the soil leading to an increased C:N.

The earthworm community diversity at a specific location is dependent on soil characteristics, climate, organic matter composition and quality, and historical and current site management (Hackenberger and Hackenberger, 2014). Moisture and temperature are related soil properties and influence not only earthworm metabolism and growth, but community structure as well.

Earthworm Identifications

A total of four genera were identified, including *Amynthas, Aporrectodea, Bimastos,* and *Lumbricus,* in the garden bed, streambank, and field sampling sites sampling sites (Table 4). The number of estimated species ranged from four in the field, to five at the streambank site, to six in the garden. The observed species richness in the study coincides with the general expectation that species richness within a favorable environment that does experience drought or frost commonly ranges between three to six and rarely exceeds eight to ten species (Hackenberger and Hackenberger, 2014; Lavelle, 1988). The three genera that were identified at the garden, streambank, and field samplings sites were all exotic and the lack of differences (minus one individual identified as a native species) indicates that the sites are not different from one another in terms of community composition.

Two previous studies completed in Arkansas observed that in highly managed ecosystems, native species outnumbered exotic species of earthworms. In a study in Eastern Arkansas along the Lower Mississippi River Delta, earthworms were collected and identified from a wheat-soybean double-crop system that received seven years of various treatments including, tillage managements, fertilizer inputs, and residue treatment through burning (Thomason et al., 2017). Out of the earthworms collected, around 65% of adult earthworms collected were native *Diplocardia sylvicola* earthworms. An exotic species, *Apporectodea trapezoides*, was present, but overall, there was little difference in diversities among sampled communities. The second study was conducted on golf course turfgrass systems in the transition zone of Arkansas and Oklahoma between November 2015 and December 2017 (Boyle, 2018). Morphological identifications indicated the presence of exotic genera *Apporectodea* and *Amynthas* spp. and a native genus, *Diplocardia* spp. A decline in *Diplocarida* spp. was reported after invasion by the non-native *Apporectodea caliginosa* and *Octalasion cyaneum* in a prairie study (James, 1991). *Apporectodea* spp. were identified in all sampling locations where adults were present (James, 1991). Native species may have a competitive disadvantage in some conditions compared to exotic species due to size, but the study performed by Boyle (2018) suggests that this may not always be the case.

This current study did not coincide with the two previously mentioned studies where native earthworms were in greater densities than exotic species. Instead, more exotic genera were identified compared to natives. *Bimastos* spp. was the only native genus of earthworm and was collected on 2 November 2017 at the most managed sampling site, the garden. *Apporectodea, Amynthas, and Lumbricus* spp. are exotic genera that were observed across the BGO sampling locations. When native and exotic earthworms inhabit the same ecosystem, the earthworms compete for nutrients. Habitat disturbance, such as fertilizer amendments, increase the availability of the resources to the exotic earthworms, enhancing their ability to invade the disturbed sites (Fragoso et al, 1999). Exotic earthworms typically dominate in disturbed systems, but in lower diversities compared to undisturbed systems (Hendrix et al., 2006).

Dispersive activities may have influenced the earthworm densities at the BGO sampling sites. Exotic species can be dispersed actively or passively. Active dispersal describes earthworms’ movement through a soil and involves transporting themselves to a new location. Passive dispersal is when earthworms are transported by another agent such as a heavy rain or human activity (Lee, 1985). Since the BGO is a managed garden site, passive activity through human interaction by importing soil and plants from various locations potentially influenced the earthworm community composition.

Reynolds (2014) presented earthworms identified from a survey of specimens collected across 50 out of 75 counties in Arkansas and identified earthworms from the following families: *Acanthodrilidae*, *Lumbricidae*, *Megascolecidae*, and *Sparganophilidae*. The garden, streambank, and field site are within Washington County, AR, one of the counties included in Reynolds (2014). Reynolds identified genera including *Amynthas, Apporectodea, Bimastos, Dendrobaena, Diplocardia, and Octolasion.* Three genera, *Amynthas*, *Apporectodea*, and *Bimastos*, identified in this study were previously recorded by Reynolds in the county in 2014. *Lumbricus* is a new genus identified for Washington County. This genus of earthworm was previously observed in the Ozarks Highland Region in Missouri and Oklahoma, but not Arkansas (Reynolds, 2014). Therefore, *Lumbricus* may have migrated passively through human interaction by being released as fishing bait into the environment or through the transport of soil and plants containing cocoons or earthworms (Reynolds, 1977; Casson et al., 2002).

Sources of Error

Earthworm populations in this study may not be represented correctly due to irregular sampling of the sites. Each site was not sampled the same number of times or dates, e.g. the prairie sampling site. If sites such as the streambank and Chesney Prairie were sampled more often, the result may allow for a more well-rounded comparison of seasonal earthworm age differences. Furthermore, it should be noted that in this study, multiple people participated in sampling earthworms, which produced a human error in density calculations.

Conclusion and Future Work

The influence of five land management intensities and histories on earthworm densities and populations and relationships of densities with abiotic soil properties was investigated in Northwest Arkansas during September 2017 to March 2018. There was a significant difference in earthworm densities when comparing the prairie (intermound) sampling site to the sites at the BGO September 2017. There was a significant difference in earthworms densities collected from the field sampling site compared to the garden, streambank, and prairie (intermound) sampling sites ($P = 0.003$). The most earthworms were collected from the field site during the last sampling of November 2017 and in February and March of 2018, possibly due to reduced management compared to the garden sampling site. Temperature and moisture did have a significant effect on the number of earthworms collected, but the total number of earthworms collected per unit area varied greatly among sampling dates. The number of juveniles collected influenced the ability to identify the earthworms since few adult earthworms were collected. There were no differences in the earthworm communities at the garden, streambank, and field sampling sites and three genera were present in common across the sites, *Amynthas, Apporectodea*, and *Lumbricus.* One only native genus was identified in this study, which was collected from the garden, the most managed site. Future studies should survey populations of earthworms in prairie systems to better understand seasonal dynamics. If sites such as Chesney Prairie were sampled more frequently, more representative adult earthworm collections may allow for the identification of genera and species present. Future studies should include the molecular identification of earthworms to ensure species identification of specimens collected in Northwest Arkansas. Based on the adult earthworms identified, the exotic species of earthworms were present at greater densities than native species and may result in changes in soil processes.

Potential changes include differences in nutrient cycling and modification of the soil structure, which would in turn affect other organisms and plants in the ecosystem.

Tables and Figures

Table 1. The sampling sites, number of sampling dates, date range, the total numbers of adult and juvenile earthworms collected and percentage of juveniles out of the total amount of earthworms collected across all sampling times at each sampling site from September 2017 to March 2018.

Site Management	Number of Sampling Dates	Sampling Date Range	Adult Earthworms Collected	Juvenile Earthworms Collected	Juvenile / Total (%)
Garden	8	9/21/17- 3/30/18	15	41	73
Riverbank	6	9/21/17- 11/9/17	16	46	75
Field	8	9/21/17- 3/30/18	20	119	86
Prairie (intermound)	1	9/28/2017	0	49	100
Prairie (mound)*	1	9/28/2017	ND	ND	ND

*Not detected, i.e. no earthworms were collected at time of sampling

Table 2. The density of earthworms (back-transformed value of the average of the log-transformed adult plus juvenile densities) collected per m^2. A dash indicates that the sampling site was not sampled on that date.

	Sampling Site			
Sampling Date	Garden	Streambank	Field	Prairie (intermound)
9/21/2017	18	27	32	-
9/28/2017	-	-	-	100
10/5/2017	73	72	59	-
10/19/2017	22	19	20	-
11/2/2017	25	22	19	-
11/9/2017	54	99	17	-
11/16/2017	26	102	250	-
2/26/2018	56	-	226	-
3/30/2018	55	-	273	-

The prairie (mound) sampling site was not included in the ANOVA. There were significant differences with the total amount of earthworms collected at each date (P = 0.04), and a significant difference for the total amount of earthworms collected at each site (P = 0.047). The number of earthworms collected in the field was larger than in the garden, streambank, and prairie (intermound).

Table 3. Average particle size distribution for composite soil samples (0-20 cm) and average C:N (standard deviation) collected September 2017 to November 2017.

Sampling Site Name	Sand (%)	Clay (%)	Silt (%)	C:N	Soil Texture
Garden	38.5	15.2	46.3	15.65 (2.36)	Loam
Streambank	63.9	12.2	23.9	10.82 (1.41)	Sandy Loam
Field	35.1	13.4	51.4	10.15 (0.76)	Silt Loam
Prairie (intermound)	23.9	20.9	55.2	10.53 (0.80)	Silt Loam
Prairie (mound)	24.1	13.9	62.0	10.06 (0.53)	Silt Loam

The sampling sizes for the garden was 13, streambank was 12, field was 13, prairie (intermound) was 5, and prairie (mound) was 5.

Table 4. The number of sampling dates and sampling date range with tentative earthworm genera and species richness from adult earthworms collected from every sampling site. No adults were observed in the prairie, so species or genera could not be identified and are unknown.

Site Management	Number of Sampling Dates	Sampling Date Range	Estimated Genera Richness	Tentative Genera Identifications	Estimated Species Richness
Garden	8	9/21/17-3/30/18	4	*Amynthas, Aporrectodea, Bimastos, Lumbricus*	6
Streambank	6	9/21/17-11/9/17	3	*Amynthas, Aporrectodea, Lumbricus*	5
Field	8	9/21/17-3/30/18	3	*Amynthas, Aporrectodea, Lumbricus*	4
Prairie (intermound)	1	9/28/2017	Unknown	Unknown	Unknown
Prairie (mound)*	1	9/28/2017	Unknown	Unknown	Unknown

*No earthworms detected at time of sampling

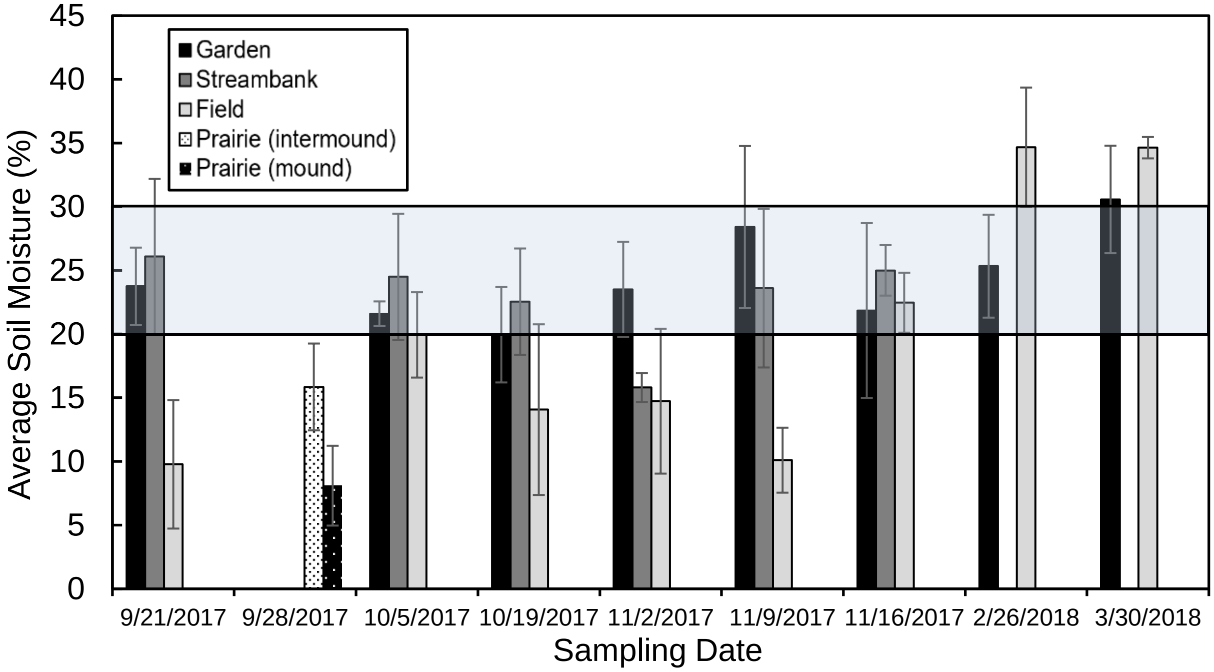

Figure 1. The average volumetric moisture as a percent for the garden, streambank, field, prairie (intermound), and prairie (mound) sampling sites on each sampling date with standard deviation. The shaded region indicates optimal soil moistures ranging from 20 to 30% (Grant, 1955).

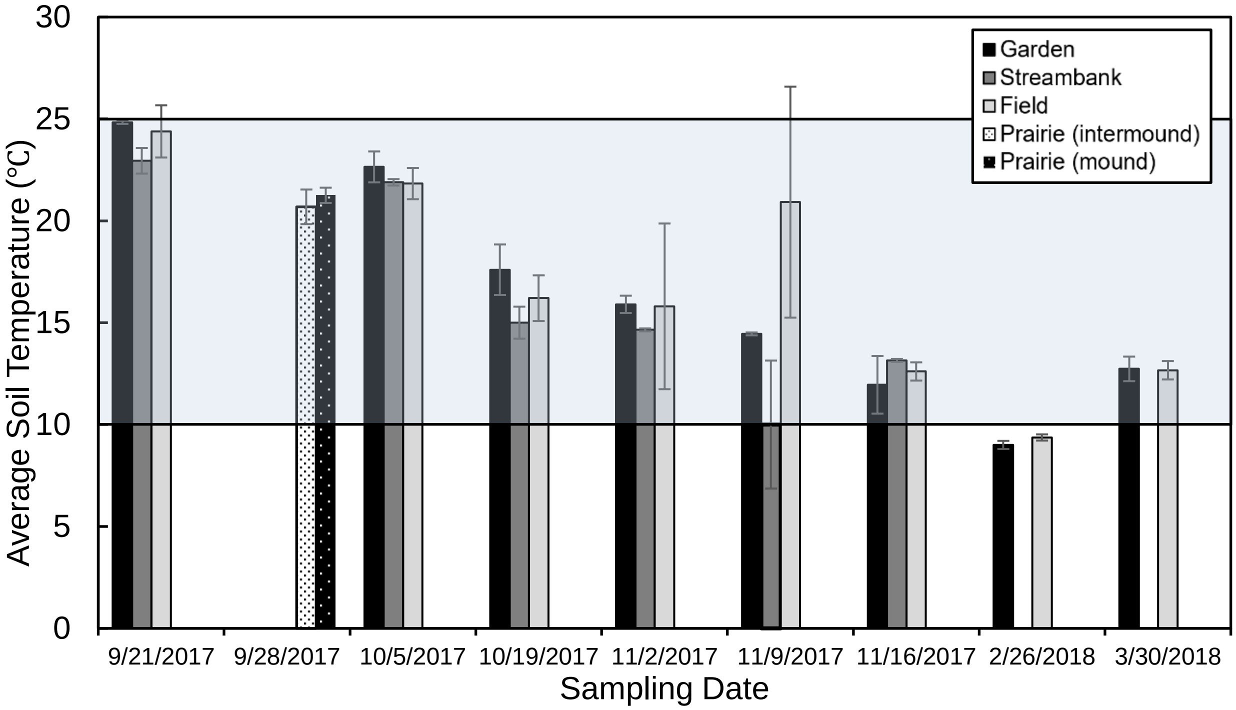

Figure 2. The average temperature in Celsius for the garden, streambank, field, prairie (intermound), and prairie (mound) sampling sites on each sampling date with standard deviation. The shaded region indicates optimal soil temperatures ranging from 10 to 25 ℃ (Edwards and Bohlen, 1996).

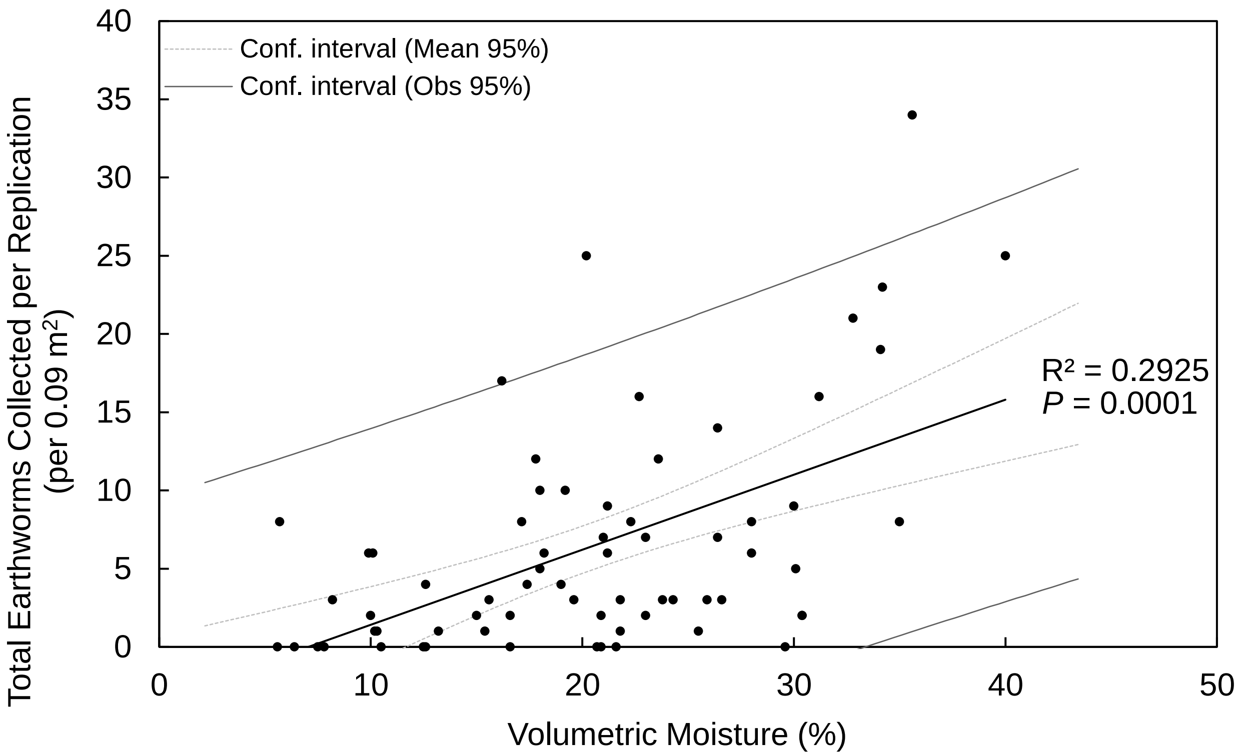

Figure 3. Total earthworms collected, September 2017 to March 2018, from the garden, streambank, field, and prairie (intermound) sampling sites against volumetric moisture (%). The Conf. interval (Mean 95%) is the confidence interval of the mean of earthworms collected given volumetric moisture and related to the R-squared value. Therefore, 29.25% of the variability with the number of earthworms collected is explained by volumetric moisture. The Conf. interval (Obs 95%) is the confidence of a single observation to be within the lines given volumetric moisture and is related to the *P*-value.

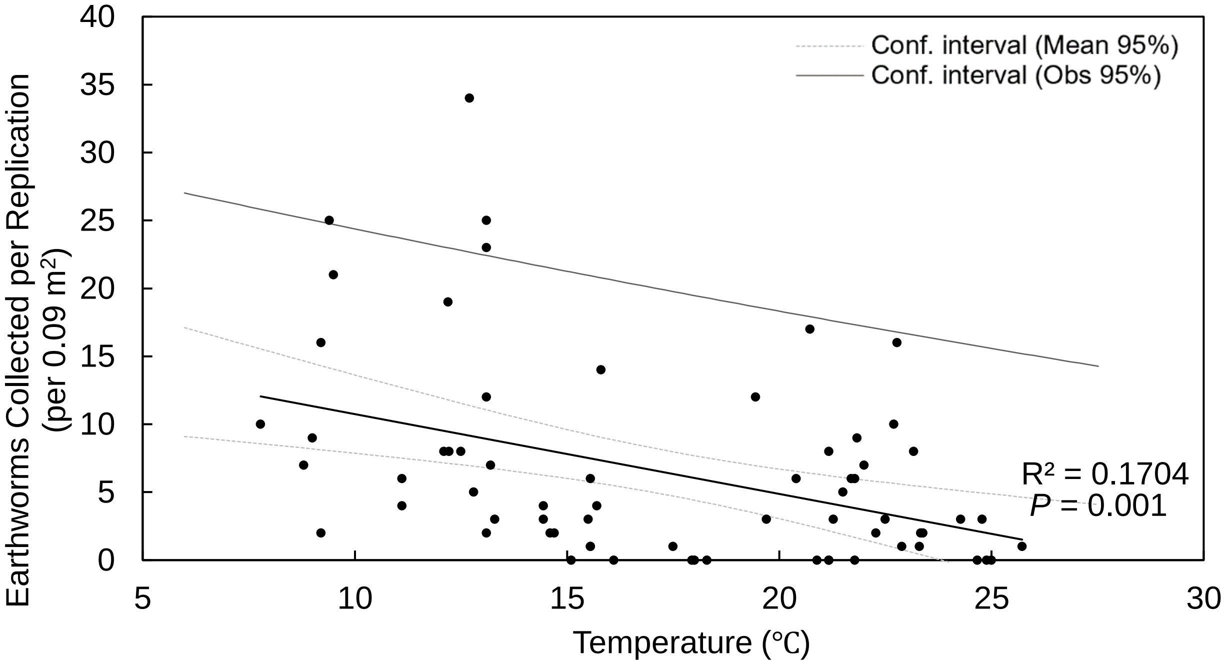

Figure 4. Total earthworms collected, September 2017 to March 2018, from the garden, streambank, field, and prairie (intermound) sampling sites against temperature (℃). The Conf. interval (Mean 95%) is the confidence interval of the mean of earthworms collected given temperature and related to the R-squared value. Therefore, 17.04% of the variability with the number of earthworms collected is explained by soil temperature. The Conf. interval (Obs 95%) is the confidence of a single observation to be within the lines given temperature and is related to the *P*-value.

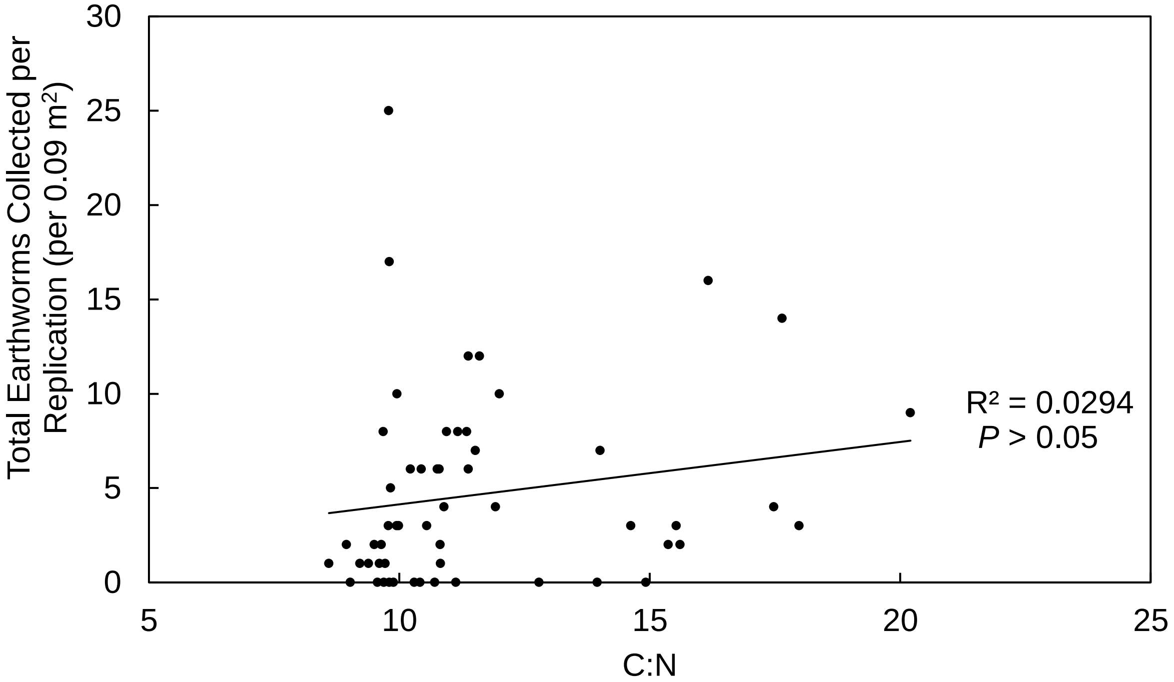

Figure 5. Total earthworms collected, September 2017 to March 2018, from the garden bed, streambank, field, and prairie (intermound) sampling sites plotted against soil C:N. Only 2.94% of the variability with the number of earthworms collected is explained by the C:N. No significant trend between total number of earthworms collected and soil C:N was observed through the linear regression ($P > 0.05$).

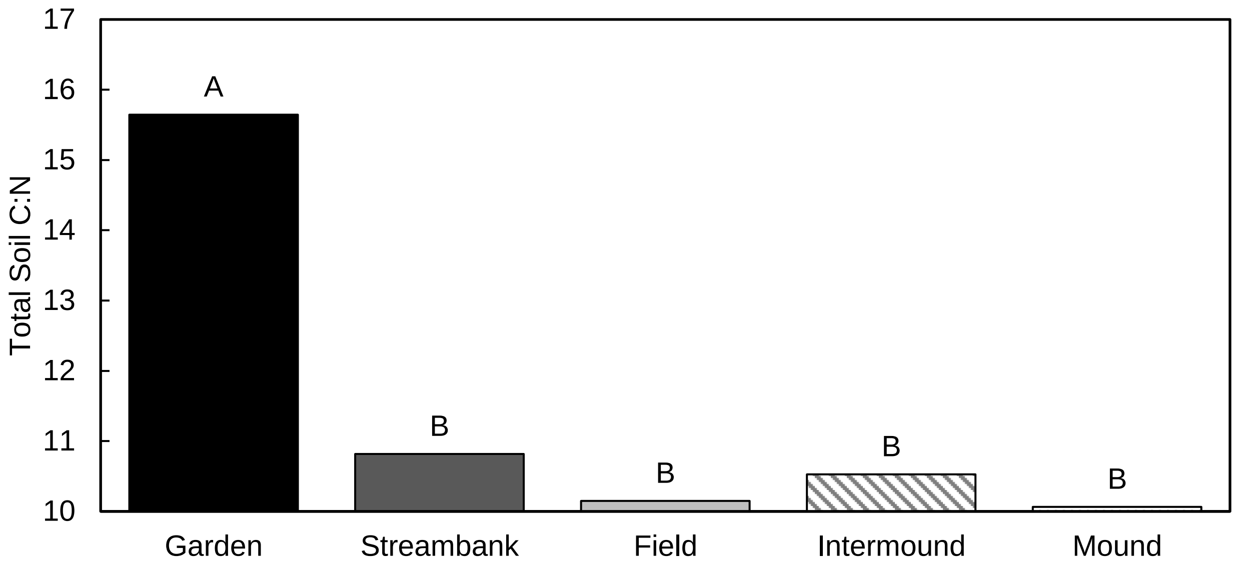

Figure 6. A comparison of average soil C:N at each sampling site (n = 19 for garden and field, 12 for streambank, and 5 for the intermound and mound) from variable number of collections occurring from September 2017 to November 2017 (P = 0.29). The garden had a significantly greater C:N than the streambank, field, prairie (intermound), and prairie (mound) sampling sites ($P < 0.0001$). Sites that do not share the same letter are significantly different.

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