IMPACTS OF INVASIVE EARTHWORMS ON CARBON STORAGE IN SOUTHERN BOREAL HARDWOOD FORESTS

By

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Thesis

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ABSTRACT

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Keywords: Biomixing; Boreal forest; Carbon; Climate change; Detritivore; Earthworms; Invasive species; *Lumbricidae*; Organic matter; Soil carbon; Thunder Bay

The introduction of invasive, non-native earthworms into forest ecosystems has increased as recreational, industrial, and commercial activities increase in northern Ontario. The presence of these ecosystem engineers in previously uninvaded ecosystems has resulted in significant changes in soil structure, vegetation communities and soil organic carbon. In this study, field activities gathered samples of earthworms and soil from five different sites in the Thunder Bay district. Lab analysis was then completed to determine the relationships between ash-free dry mass of earthworms, total organic carbon, soil texture, pH and understory species richness. Results indicated that the presence of earthworms in northern boreal hardwood forest ecosystems has led to significant decreases in carbon storage of approximately 7%. This reduction in soil carbon storage may result in an increased release of carbon emissions and the reduction of the efficacy of the boreal forest as a critical global carbon sink.

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1.0 INTRODUCTION

The focus of this study was to determine and quantify the influence of invasive earthworms on soil carbon stocks in boreal hardwood forests. Soil carbon is a major component of the global carbon cycle, and the boreal forest one of the world's largest global carbon sinks. Low decomposition rates in the boreal forest results in the accumulation of organic matter, increasing soil carbon stocks and minimizing the carbon emissions (Frelich et al. 2006). Earthworm invasions in boreal ecosystems have the potential to alter important soil processes and threaten the sustainability of the boreal soil carbon stocks.

Earthworms of the family *Lumbricidae* were introduced to human settlement areas from Europe hundreds of years ago through the deliberate movement of soils as a result of the beneficial effects on soil mixing (biomixing), nutrient availability, aeration, and water infiltration seen on farms and in urban areas in Europe (Hendrix and Bohlen 2002). Since their initial introduction, earthworms have become a driving force behind changes in soil structure and carbon storage in the areas in which they have been introduced. Changes in soil carbon storage are primarily caused by decreases in the ability of soil structures to retain carbon, which in turn leads to an increase in the release of carbon dioxide into the atmosphere. These changes are the result of soil organic matter mineralization and increased microbial respiration caused by earthworms in the soil (Lemtiri et al. 2014). This release of carbon dioxide is a major consequence of structural changes caused by invasive earthworms and may accelerate climate change.

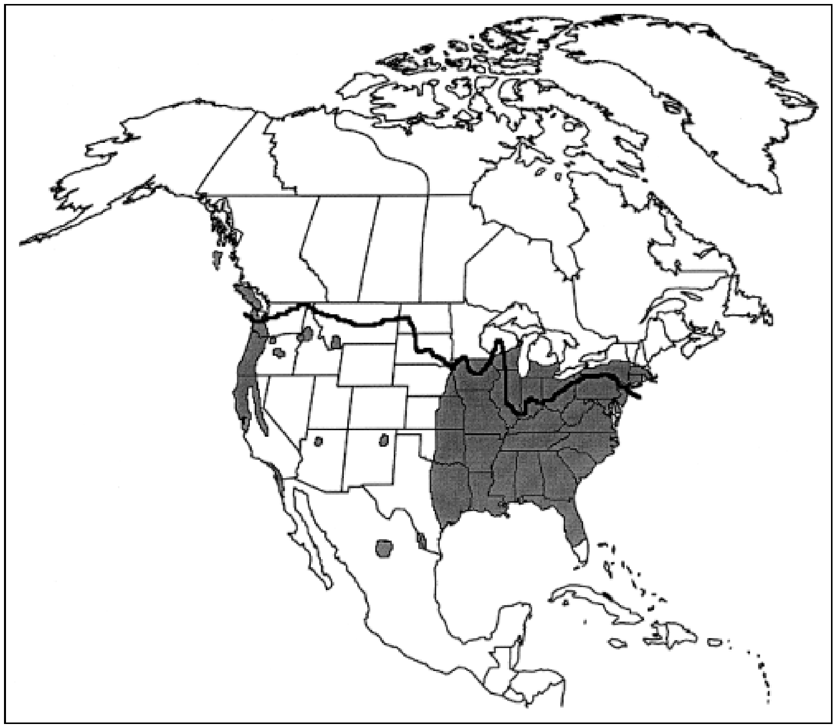
Understanding the mechanisms driving these changes, as well as their impacts on forest ecosystems is essential to be able to understand and manage boreal carbon stocks.

1.1 Literature Review

1.1.1 Earthworm Biology, Behaviour and Distribution

Earthworms are a large group of invertebrate detritivores with a nearly global distribution. All earthworms are classified under the phylum Annelida, class Clitellata, subclass Ogliochaeta and order Opisthophora (Hendrix and Bohlen 2002). There are 16 known families of earthworms and approximately 3500 species (Hendrix and Bohlen 2002).

In North America, native earthworms are limited to the region south of the southern limit of the Wisconsin ice sheet, which receded roughly 10,000 years ago and runs from east to west across the northern United States (Figure 1; Callaham et al. 2006; Hendrix and Bohlen 2002). Currently there are no native earthworm species found in Canada east of the Pacific Northwest region (Hendrix and Bohlen 2002), whereas approximately 100 native earthworm species occur across the United States (U.S., Hendrix and Bohlen 2002). The recolonization of native earthworms from the US to Canada following the retreat of the glaciers has been very slow, estimated at only five metres per year (Holdsworth et al. 2007). The distribution of native earthworm species in their present range is complex with many communities overlapping (simplified in Figure 1 as a single grey shaded area). This occurs because many different species of worms are able to coexist at a single location through partitioning of resources and the utilization of different feeding strategies.



Source: Hendrix and Bohlen 2002

Figure 1. Distribution of roughly 100 native North American earthworms (shaded area) in relation to the southern boundary of the Wisconsin ice sheet.

All earthworms, regardless of species, are hermaphrodites, possessing both eggs and sperm, allowing individual worms to reproduce without the need for mating (Williamson n.d.). However, earthworms generally mate sexually rather than through self-fertilization (Williamson n.d.). According to Williamson (n.d.), eggs are laid in early spring in capsules containing three to six eggs each. Earthworms mature in in four to five months and are sexually active by late June.

Mature earthworms have two major requirements for survival: adequate soil moisture, to prevent drying of skin and to maintain body temperature, and adequate food, in the form of deciduous leaf litter and organic matter (Williamson n.d.). For this reason, earthworms are often less abundant in sandy soils as the soil has a higher likelihood of causing skin abrasions and does not possess the necessary food and moisture (Williamson n.d.). Additionally, when soil is drier worms are found deeper in the soil profile where moisture is greater (Williamson n.d.).

Earthworms can be broadly classified into three major groupings based on where they are found in the soil profile and their effect on biomixing (the combination of soil organic matter with mineral soil layers) processes (Bohlen et al. 2004a). Bohlen et al. (2004a) identified these three groupings as:

1. Epigeic: species residing in the upper layer of organic matter, promoting mixing of the organic layer with the upper mineral soil;
2. Endogeic: species residing in the mineral soil which further enhance the mixing of organic matter and mineral soil; and
3. Anecic: species found deep in the mineral soil, 1-2 metres below the surface, leading to a unique form of mixing in which mineral soil is brought to the surface and organic matter is brought deeper into the soil.

These groups of worms may live at separate sites or may all exist at one site, leading to increased biomixing within the entire soil profile (Bohlen et al. 2004a).

1.1.2 Introduction and Patterns of Invasion

European and Asian earthworms have been described by Bohlen et al. (2004a) as a “peregrine species,” which readily colonize new habitats and tolerate a wide range of environmental conditions. Nevertheless, recolonization by native earthworm species following the Wisconsin Glaciation has been slow (Callaham et al. 2006). The natural migration rate of earthworms has been estimated to be approximately five metres per year, indicating a total northward migration of 50 kilometres since glacial retreat

(Holdsworth, Frelich and Reich 2007). Rather than through the re-establishment of natural populations, earthworm populations in North America have been restored to pre-glaciation levels as a result of human-induced introduction of non-native earthworm species.

According to Callahan et al. (2006) the introduction and invasion of non-native species, including earthworms, is characterised by four general stages. These stages and the primary barrier to the success of each stage are detailed in Table 1 (below).

Table 1. Stages of invasion of non-native species and the primary barrier for each stage.

Stage of Invasion	Barrier to Success
Transportation	Geography
Colonization	Abiotic Conditions
Establishment	Biotic Interactions
Landscape Spread	Landscape Factors

Source: Hellmann et al. 2008

The introduction of non-native species to North America began approximately 400 years ago, with the onset of European colonization (Callaham et al. 2006). In the early stages of introduction, earthworms were brought over from Europe accidentally in soil and organic matter on ships. This human-assisted migration allowed species of European earthworms, of the family *Lumbricidae,* to cross the Atlantic Ocean, a major ecological barrier (Hendrix and Bohlen 2002). Present day earthworm populations are now well beyond the establishment stage and are heading towards the landscape spread stage of invasion (Hellmann et al. 2008).

The ability of earthworms to overcome landscape factors that have the potential to limit their spread is further aided by intentional and accidental human-assisted migration, including mechanisms such as:

1. Construction of logging roads: dirt in the tire treads and wheels may help to transport small epigeic species;
2. Direct release of unused fishing bait: primarily anecic species (the primary issue in the Boreal forest);
3. Relocation of till or horticultural materials: may carry all groups of earthworms;
4. International commerce (especially agriculture and logging): soil materials may contain species of all groups; and
5. Vermicomposting: deliberate introductions of species of endogeic and anecic earthworms (Bohlen et al. 2004; Callaham et al. 2006).

Presently, there are 45 known species of earthworms that have been intentionally and unintentionally introduced to North America (Hendrix and Bohlen 2002). The largest group has come from Europe, containing 25 species, most of which are in the family *Lumbricidae* (Hendrix and Bohlen 2002). The second largest group has been introduced from Asia, containing 14 species (Hendrix and Bohlen 2002). The remainder of the species were introduced from Africa and South America, with 3 species introduced from each (Hendrix and Bohlen 2002).

1.1.3 Ecological Impacts of Invasive European Earthworms

European earthworms have become keystone detritivores in North American temperate forests by causing physical transformation of soils and altering resources for other organisms (Frelich et al. 2006; Lavelle et al. 2006). The impacts of earthworms on soil structure, chemistry and texture, as well as on vegetation community structure can be viewed as both beneficial and detrimental, depending upon the ecosystem in which they are found.

Earthworms can be extremely beneficial in agricultural systems, increasing litter decomposition, nutrient transformation, plant nutrient uptake, soil aggregation, soil porosity, and enhancing water infiltration and solute transport (Hendrix and Bohlen 2002). These effects help to improve soil quality and growth of agricultural crops. In reference to earthworms and their influence on agricultural systems, Charles Darwin wrote, “It may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organised creatures” (Feller et al. 2003). Even though earthworms have beneficial impacts in agricultural systems, the impacts they have on natural forest soils may be far from beneficial.

Soil invertebrates, such as earthworms, are key mediators of soil function in natural ecosystems (Lavelle et al. 2006). Their impacts include, but are not limited to, the incorporation of litter into the mineral soil, alteration of porosity and aggregation, microbial communities, pest and disease control, and acceleration of plant succession (Lavelle et al. 2006).

In general, earthworm impacts can be described as causing a shift in soil dynamics from a slow-cycling, fungus-dominated system to a fast-cycling, bacteria-dominated ecosystem (Bohlen et al. 2004a). Beginning at the surface level, at what is called the “invasion front”, visible changes on the forest floor include decreasing diversity and abundance of herbaceous plants, decreasing abundance and density of tree seedlings and a reduction in thickness of the surface litter layer (Hale et al. 2005;

Hendrix and Bohlen 2002). The most critical impact of earthworms on soils is the reduction of the surface organic layer (LFH) as a result of consumption of leaf litter and organic matter and incorporation into mineral soil layers (Frelich et al. 2006; Holdsworth, Frelich and Reich 2008; Ross et al. 2015). Biomixing and the secretion of mucus by the earthworms leads to increased (and larger) aggregation of soil particles, increasing porosity and drainage (Lemtiri et al. 2014; Ross et al. 2015). The resulting increase in water infiltration causes an increase in nutrient leaching, especially nitrogen, and solute transport in the soil (Hendrix and Bohlen 2002).

In some cases, the loss of the surface organic layer and the production of casts (earthworm feces) stimulates a process called “surface sealing” (Hendrix and Bohlen 2002). Unlike the other changes in soil structure, this process results in a limitation of water infiltration, an increase in surface run-off and an increase in erosion. The alteration of infiltration, whether an increase or decrease, and alteration of soil structure often leads to altered soil temperature, soil moisture and nutrient availability.

Earthworms are described as regulators of nutrient cycling, often affecting cycling at many different spatial and temporal scales (Lavelle et al. 2006). As a result of increased leaching and decomposition of surface organic matter, reductions in the carbon to nitrogen ratios, a critical consideration for plant growth and productivity, have often been recorded (Holdsworth, Frelich and Reich 2008; Holdsworth, Frelich and Reich 2007). In some cases, the increased compaction caused by earthworm burrowing, nesting and the production of casts can also lead to decreased nutrient availability. Changes in nutrient availability and the loss of a thick LFH layer has significant negative consequences for the native vegetation communities. The decrease in vegetation community abundance and diversity seen as a result of earthworm invasion is largely a result of the compounding processes outlined in Table 2 (below). The severity of these impacts depends more upon the diversity of earthworm species present than on the abundance of all earthworms (Hale et al. 2005). The combination of differing feeding strategies employed by each species increases the amount of biomixing and therefore causes greater reduction in the surface organic matter layer.

Table 2. Impacts of earthworms on native vegetation communities.

Impact or Action	Resulting Influence on Vegetation
Deep burial of seeds	Reduction in regeneration
Loss of surface organic matter	Reduction in regeneration as a result of increased grazing by deer (increased deer to plant ratio) and environmental exposure
Increased leaching of nitrogen and phosphorous	Decreased availability of nitrogen and phosphorous leading to decreased productivity
Feeding on roots	Decreased growth and diversity
Feeding on fungal hyphae	Reduction and alteration in soil fungal communities causing decreased mycorrhizal activity
Passage of seeds through earthworm gut	Decreased viability of seeds

Source: Hale et al. 2005; Frelich et al. 2006

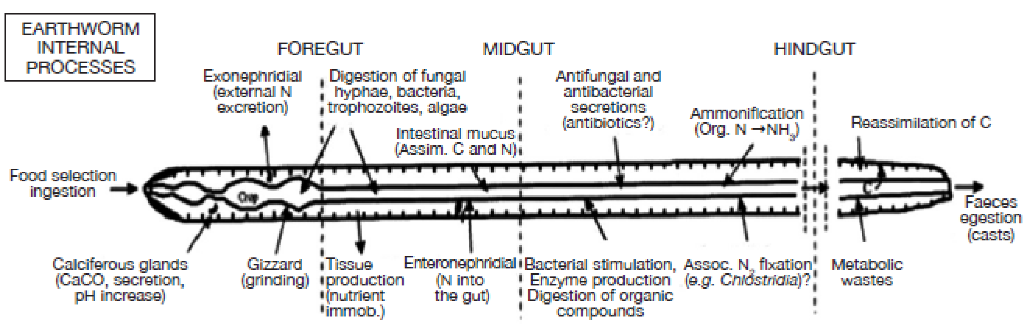
Together, all of these impacts can lead to what is known as “forest decline syndrome”, when the lush, diverse understory plant community is replaced by a sparse community with low diversity (Frelich et al. 2006). Additionally, the reduction in diversity of native plant species may increase susceptibility of the site to invasion by non-native species; a phenomenon known as an “invasional meltdown” (Hendrix and Bohlen 2002).

1.1.4 Carbon Storage and Climate Change

The boreal forests found in northern Ontario and around the world are some of the most globally important carbon sinks (Bohlen et al. 2004a). Boreal forests contain 338-471 billion tons of carbon, which is approximately 247-344 tons of carbon per hectare of land (Lal 2004). Terrestrial ecosystems, such as the boreal forest, and the soil they contain, play a very important role in the global carbon budget (Lal 2004). The soil organic carbon pool is four times the size of the biotic organic carbon pool and roughly three times the size of the atmospheric organic carbon pool (Lal 2004). Between 35 and60% of the total organic carbon in ecosystems is contained in soils, with an additional 20% contained within the litter layer (Seely, Welham and Kimmins 2002). Additionally, stands dominated by deciduous species, such as *Populus* (poplar), have been found to have greater soil carbon storage than stands dominated by coniferous species (Seely, Welham and Kimmins 2002; Shipitalo and Bayon 2004). The annual carbon budget in mature northern hardwood forests has previously been shown to be at, or near, equilibrium (Barr et al. 2002). The activities of introduced earthworm species in these forests threaten the long-term stability and equilibrium of the critical boreal carbon pool.

Earthworm activities such as feeding, biomixing and the production of casts are the primary drivers behind the loss of carbon in natural forest ecosystems. Studies finding carbon loss indicate that the reduction of the surface organic layer causes the movement of carbon lower into the mineral soil layers where decomposition is accelerated (Lemtiri et al. 2014). Decomposition of the litter layer is a major component of the global carbon budget, representing up to 70% of the annual carbon flux at natural rates (Aerts 2006). Acceleration of decomposition of this layer would serve to increase the annual carbon flux increasing climate change. It is estimated that approximately 20% of the annual global carbon dioxide emissions come from soil (Lubbers et al. 2013).

The stimulation of microbial activity, and therefore microbial respiration, in the gut of earthworms (Figure 2) causes increased mineralization and release of carbon (Bohlen et al. 2004a). Carbon losses in the upper soil layers of up to 28%, as a result of earthworm activity, have been recorded (Bohlen et al. 2004b). This reduction is comparable to those caused by logging activities, which have been estimated to reduce carbon by approximately 17-71% (Bohlen et al. 2004b). Carbon losses in the form of carbon dioxide emissions have been shown to increase by up to 33%, increasing the net global warming potential of soils by up to 16% as a result of earthworm presence (Lubbers et al. 2013). A study by Zhang et al. (2013) indicates potential average losses of upwards of 600 kilograms of carbon per hectare per year over 14 years.



Source: Lemtiri et al. 2014

Figure 2. Internal process within the earthworm gut ("drilosphere") from ingestion to excretion.

Zhang et al. (2013) and Bohlen et al. (2004a), have suggested that reductions in carbon storage due to earthworm activity in soils represent only a short-term change.

After the initial decrease, the level of carbon storage within the soil stabilizes at a new equilibrium level, leading to long term carbon stabilization. This paradox is described as the “earthworm dilemma”, a situation in which earthworm presence causes a short-term increase in carbon dioxide emissions, but a long-term stabilization of organic carbon at a new lower level (Lubbers et al. 2013).

1.1.5 Implications for Management

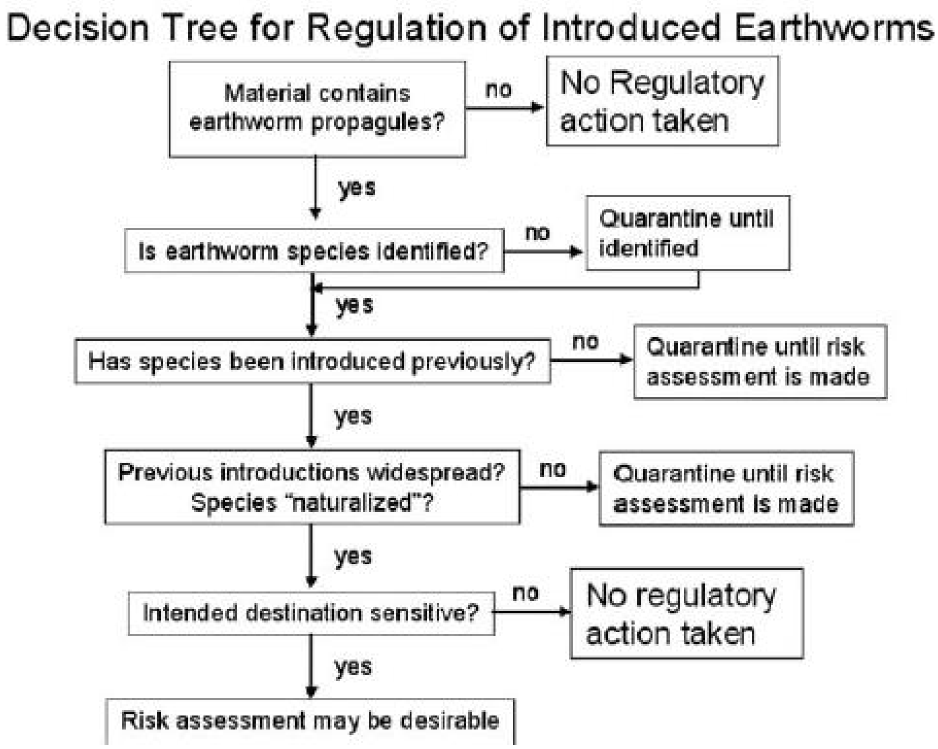
Policy options for the management and regulation of invasive earthworm populations in North America have not been widely discussed nor tested and are highly varied (Callaham 2006). However, primary suggestions include a dating system to identify the time of invasion; a classification system, to be used when deciding which species of non-native worms should or should not be approved for import and transport; rapid response frameworks for controlling invasions when they occur; and education programs to increase awareness of the impacts of invasive earthworms (Frelich et al. 2006; Bellard et al. 2013; Bohlen et al. 2004a).

The most widely agreed upon option to control current and future invasions is to develop a classification system to determine the risk associated with each species of earthworm. This should be used to determine which species may be transported within a region, which can be transported outside of a region, and which should not be transported at all. Classification should be based upon three primary factors:

1. The potential impacts of a species on soil processes and environmental quality;
2. The potential impacts of a species on beneficial or desirable microbial, animal, and plant communities; and

3. The potential for the species to be vectors for the introduction and dispersal of pathogens (Hendrix and Bohlen 2002).

Additionally, the size of the present population and the invasive success of the species in other regions should also be considered (Hendrix and Bohlen 2002). Callaham et al. (2006) suggest classification for regulation using the decision tree described in Figure 3 below.



Source: Callaham et al. 2006

Figure 3. Proposed decision tree for the regulation of earthworms and earthworm-containing materials.

This model could be used to determine whether species should be permitted entry or whether further precautions should be taken. If coupled with an effective classification system, this could provide a strong regulatory framework for the development of policies regarding the management and control of non-native, and potentially invasive, earthworm populations.

When developing policies, there are four possible scenarios. The first is the "do nothing" or "no action" approach (Hendrix and Bohlen 2002). This approach requires no direct regulations and instead considers all species to be safe until they are proven otherwise. The second option is to impose selective restrictions on earthworm species, allowing only those which are approved, based on a selective low-risk assessment, to be transported (Hendrix and Bohlen 2002). The third option is similar to the second, but places more severe restrictions on species, requiring a strict approval protocol and quarantine before species are permitted entry (Hendrix and Bohlen 2002). The final option is a complete ban on the import and transport of all earthworm species (Hendrix and Bohlen 2002). This fourth and final option is the most severe and has the potential to cause many economic issues, especially for industries such as the vermicomposting and scientific research industries (Callaham et al. 2006). It is necessary to consider a range of policy options and regulations in order to determine one that is able to mitigate most (if not all) earthworm associated environmental damage and still maintain the success of earthworm-driven industries.

1.2 Significance of Research

Introduced European earthworms have a significant impact on northern forest ecosystems. By altering the structure and productivity of forest soils earthworms act as keystone detritivores. The effects caused by earthworm activity are the driving force behind the increase in carbon emissions and the loss of organic carbon from the soils in the boreal forest region of northwestern Ontario. The significance of the boreal forest as a major global carbon pool and component of the global carbon budget, makes understanding and mitigating these changes critical to the future survival and sustainability of these ecosystems. Management strategies must be able to identify, quantify and minimize the potential for future spread and attempt to control the present populations of earthworms in order to protect the boreal forest and the world from the global warming-causing carbon losses.

1.3 Objective

The objective of this study was to determine the impact of invasive non-native earthworms on soil organic carbon storage in boreal hardwood forests. The effects of earthworms on soil carbon were considered with respect to the known biological mechanisms leading to loss of carbon storage in boreal soils and the influence this may have on the sustainability of the boreal carbon stocks.

1.4 Hypothesis

The null hypothesis states that invasive earthworms do not have significant impacts on the storage of soil organic carbon. Comparatively, the alternative hypothesis states that invasive earthworms will lead to a significant decrease in the storage of soil organic carbon. An additional possibility is the potential for earthworms to influence an increase in the storage of soil organic carbon, as suggested by some researchers in the United States.

2.0 MATERIALS AND METHODS

2.1 Study Area

Five sites were chosen in the Thunder Bay District, Ontario, based upon proximity to potential sources of introduction and the presence of a stand dominated by poplar species (*Populus* sp.). These sites can be viewed on the map in Figure 4. Two sites were selected south of the city of Thunder Bay, Fallingsnow (Site B, green icon) and Silver Mountain (Site C, purple icon). The site selected in town was located on the Lakehead University Campus, adjacent to the Bora Laskin Building (Site D, blue icon). Two additional sites were chosen north of the city along Highway 527; the first near No Name Lake (Site A, red icon) and the second down a forest road approximately 20 kilometres north on Highway 527 (Site E, yellow icon). The final site, on the forest road north of Thunder Bay, was found to be absent of earthworms and was analyzed as the natural level of soil organic carbon in boreal hardwood forests. These five sites were chosen in order to provide a statistically viable number of samples, and to provide representation of the general area around Thunder Bay. All sites supported similar overstory and understory species, and soil conditions; ensuring consistent soil and litter compositions between sites.

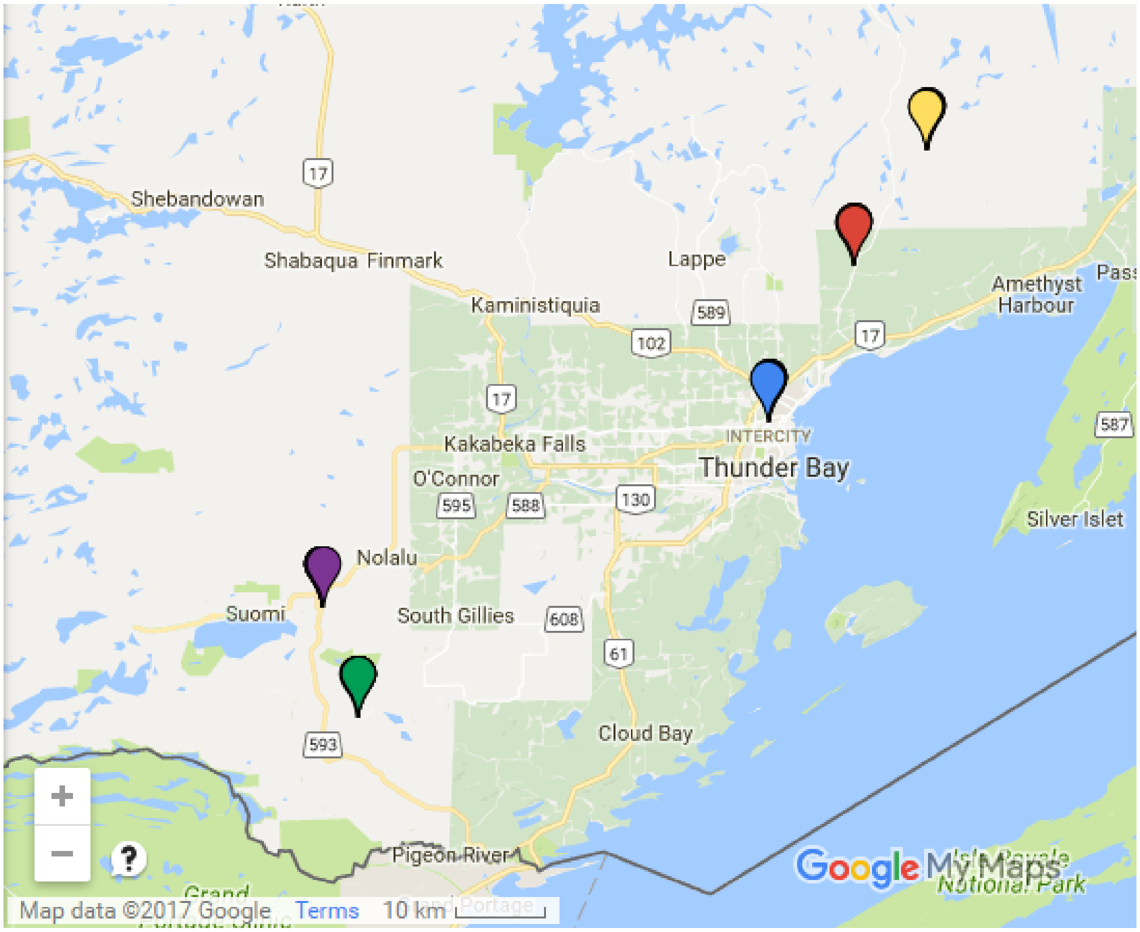
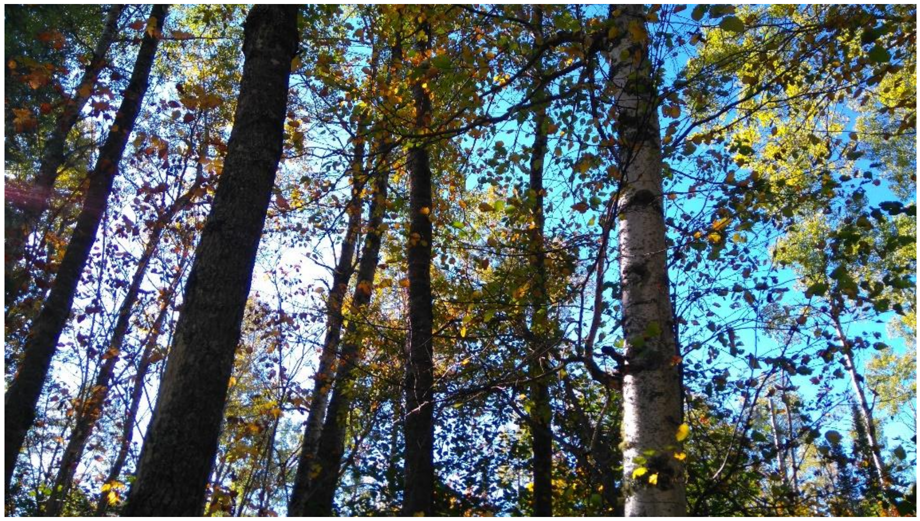


Figure 4. Location of sampling sites in the area surrounding the City of Thunder Bay, Ontario.

Overstory conditions at each site were characteristic of boreal hardwood forests. Dominant overstory species at each site were trembling aspen (*Populus tremuloides* Michx.), balsam fir (*Abies balsamifera* (L.) Mill.), white spruce (*Picea glauca* (Moench) Voss), and paper birch (*Betula papyrifera* Marshall; Figure 5). Other species with incidental presence were jack pine (*Pinus banksiana* Lamb.) and eastern white cedar (*Thuja occidentalis* L.).



Source: Nick Beals

Figure 5. Typical overstory species composition at selected sites.

Ground vegetation communities were also similar across all sites. However, not all species were present at all sites with the abundance and richness varying somewhat between sites. The primary species found at almost every site included bracken fern (*Pteridium* Gleditsch ex Scop.**)**, sedges (*Carex* sp.), and large-leaved aster (*Eurybia macrophylla* (L.) Cass.; Figure 6). Additionally, sarsasparilla (*Aralia hispida* Vent.), rasspberry (*Rubus* sp.), red osier dogwood (*Cornus stolonifera* Michx.), bluebead lily (*Clitonia borealis* (Aiton) Raff.), and fragrant bedstraw (*Galium triflorum* Michx.) were also found at many of the sites.

Source: Nick Beals

Figure 6. Typical ground vegetation composition at selected sites.

Soils at all sites were classified as moist silty-sand to sandy-loam soils composed of primarily of coarse sand material. The surface of the soil was covered by a mixture of broadleaf litter, woody debris, and some conifer litter. The proportion of conifer litter was dependent upon the proportion of conifer trees in the overstory, with most sites possessing only minor conifer litter content.

Dominant overstory species and the soil types were used to classify sites according to the Provincial Ecological Land Classification System for Ontario. Under this classification system, all five sites were determined to be “Moist-Coarse: Aspen – Birch Hardwood, B070Tt/Tl (Banton et al. 2009). These sites are characterised by a hardwood canopy consisting mostly of trembling aspen and/or birch at greater than 50% of the total canopy (Banton et al. 2009). Mixed with the dominant hardwoods, balsam fir, white spruce, black spruce and jack pine are often found (Banton et al. 2009). The understory generally consists of the same hardwood and conifer species with ground cover typically shrub and herb rich (Banton et al. 2009). Soils on these sites range from sand to coarse loam and are usually greater than 15 cm deep with a moisture regime of four or five (moist), although soil conditions are variable (Banton et al. 2009). A similar result was determined when the sites were classified according to the Canadian guide to Terrestrial and Wetland Ecosites of Northwestern Ontario. According to the national system, each of the sites was classified as ecosite 16 (Racey et al. 1996). This ecosite is described as a hardwood-fir-spruce mixedwood stand on sandy soil and possessed similar overstory, understory and soil conditions ass those described by the provincial classification system.

2.2 Field Sampling

At each site, a set of two transects were completed, each measuring 150 metres in length. Along each transect, three plots were located 50 metres apart. Beginning at the potential point of introduction, in most cases a road, a point of commencement (POC) was marked with the 150m transect run from the POC into the stand and each plot marked with an additional GPS waypoint.

Sampling at each plot consisted of a visual estimation of percent cover of ground vegetation species, a visual observation of overstory species, soil sampling of the Ah horizon, and sampling of earthworm populations (Figure 7). The Ah horizon is classified as the mineral soil layer found below the organic layer which shows signs of biomixing and organic matter incorporation, usually the upper 15 centimetres of the soil.

Source: Nick Beals

Figure 7. Hand sampling of the Ah layer at a sample plot.

Sampling of worm populations was completed using a a liquid mustard extraction. This technique involves a solution of 1:100 ratio of ground mustard powder and water. Each plot required two doses of this solution. In order to begin the sampling, a wooden frame measuring 35 centimetres by 40 centimetres was placed on the ground in an area cleared of any visible organic matter and duff layer. After photographing the plot and the surrounding vegetation, one litre of the mustard solution was poured on the soil (Figure 8). During the next five minutes, any worms that came to the surface were collected, rinsed and then placed in a labelled sample bag. After those five minutes, the second litre of mustard solution was poured on the soil and the collection process repeated. This technique is environmentally friendly, non-harmful (the mustard only acts as an irritant) and is relatively simple to employ. Despite the effort required to transport the necessary water onto the site, the technique was successful, with large numbers of worms sampled.

Source: Nick Beals

Figure 8. Soil plot with liquid mustard solution applied for earthworm extraction.

2.3 Lab Analysis

2.3.1 Sample Preparation

Samples were prepared according to the Forest Resources and Soils Testing Laboratory (FoReST Lab) standardized testing techniques. Each sample was first emptied onto a plastic tray covered in a layer of paper towel and allowed to air dry for at least 24 hours (Figure 9). Each sample was then ground through a two-millimetre standard metal sieve to ensure equal particle size for testing (Figure 9). Once ground, any excess material remaining in the sieve was discarded and the prepared sample placed back into the labelled sample bag. This preparation ensured a consistent particle size for testing and removed larger pieces of wood, leaves or other organic material which would have skewed the lab results.

Figure 9. Air drying of soil samples (Left) and fully prepared soil sample (Right).

2.3.2 Direct Estimation of Organic Matter (Loss-On-Ignition)

In order to estimate the total organic carbon in each of the soil samples, a loss-on-ignition test was performed. This test relies on the complete combustion of soil samples in a muffle furnace. Soil samples of 5 grams (g; +/- 0.005 g) were weighed, into individual desiccator-dried porcelain crucibles (Figure 10) using a Denver Instruments APX-203 scale (accurate to 0.001g; Figure 10). Weight was recorded as the air-dry weight. In addition to the 30 field samples, a quality control (QC) sample was also weighed into a crucible. The QC sample ensured that the equipment was calibrated correctly by providing a known carbon content value that should have been obtained after the combustion process.

Figure 10. Soil sample being weighed using the APX-203 3-point scale (Left) and porcelain crucibles in the desiccator (Right).

Filled crucibles were placed into a Fisher Scientific Isotemperature Programmable Muffle Furnace (Figure 11) for two hours at 105 degrees Celsius (°C; Figure 12) for two hours. After drying, crucibles were removed from the muffle furnace and placed in a desiccator for 15 minutes to cool. After cooling, crucibles were removed from the desiccator and weighed to determine oven-dry weight. After weighing all samples, the crucibles were placed back into the muffle furnace for combustion.

Figure 11. Fisher Scientific Isotemperature Programmable Muffle Furnace.

Figure 12. Soil samples inside of the muffle furnace.

The muffle furnace was programmed according to the standard operating procedures from the FoReST Lab. The set combustion period lasted for five hours at 360°C, long enough for complete combustion of soil organic matter to occur (Figure 13). Once the combustion period was completed, the muffle furnace was stabilized at 105°C until samples were removed the following day (approximately seven hours later, for a total of 12 hours). Upon removal, all samples were again placed in the desiccator for a 15-minute cooling period before being weighed. Final weights were recorded as the ignited weight.

Figure 13. Fully combusted soil samples.

Percent total organic carbon (%TOC) was calculated based upon the assumption that combusted organic matter was approximately 58% carbon (Pluske, Murphy and Sheppard 2017).

2.3.3 pH Analysis

The pH of each sample was also measured using a calcium chloride ($CaCl_2$) solution at a 1:2 ratio. This method was chosen over the alternative pH test in distilled water because it provides a more accurate result, regardless of the natural seasonal variation. PH tests in water tend to vary according to the season in which the soil was gathered, whereas pH measured in $CaCl_2$ remains relatively constant.

To begin, ten grams (g) of each soil sample were weighed into individual 100 millilitre (mL) glass beakers. For those soil samples which contained more organic matter, only 5g of soil was weighed into the beaker. As with the %TOC test, a quality control sample was also used to provide a baseline to ensure the pH meter was correctly calibrated. Using a plastic bottle with a measuring cup attachment, 20mL of 0.01 molar (M) $CaCl_2$ was then added to each beaker (Figure 14). If a soil sample absorbed all of the solution, additional $CaCl_2$ was added until a layer of liquid was visible on top of the soil solution. Any changes in the ratio of soil to the $CaCl_2$ solution was noted. This layer of liquid was critical for ensuring an accurate measurement of the soils' pH.

Figure 14. Soil samples mixed with $CaCl_2$ solution.

Once the solution was added to the correct level, each beaker was stirred for approximately 10 seconds (s). Over the next 30 minutes, each sample was stirred between four and five times, approximately six minutes apart. Once all samples had been stirred, the soil was allowed to settle out of suspension (approximately 30 minutes).

Using an Acumet Research AR20 pH Conductivity Meter (Figure 15), the pH of each solution was measured, being careful to only measure the solution and not the settled soil material.

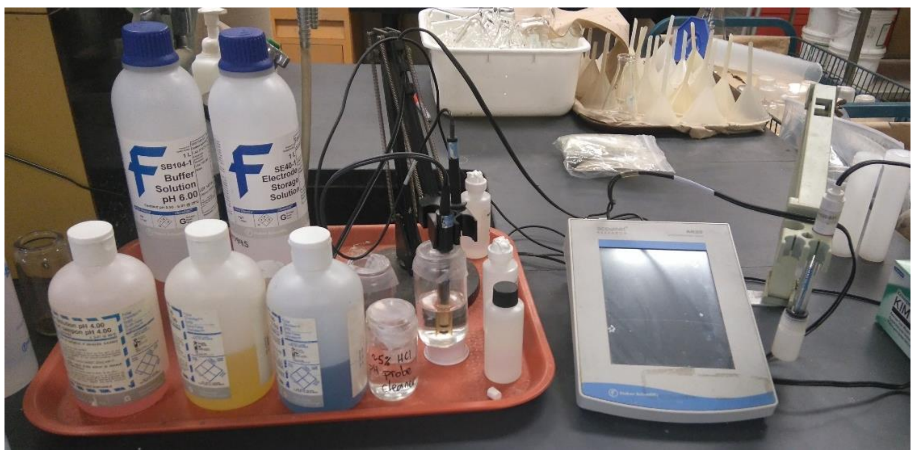


Figure 15. Acument AR20 pH Conductivity Meter.

2.3.4 Texture Classification (Bouyoucos Hydrometer)

Soil texture was measured using the Bouyoucos Hydrometer method; a hydrotexture method for determining the proportion of sand, silt and clay in a soil sample. This method relies on the suspension of soil in a mixture of water and calgon (sodium metaphosphate) that settles out over a period of 12 hours, providing a more accurate result than texture test completed by touch alone. Additionally, this test allows determination of precise percentages of each soil particle types in the sample (sand, silt, and clay) as compared to a broad soil type classification. The hydrometer measures the relative density of the solution in the cylinder, a value that changes over time as lighter soil particles settle out of solution. Sand particles settle out first, followed by silt and, finally, clay (Figure 16). The rate at which the relative density changes indicates the proportion of each of these particles in the soil sample.

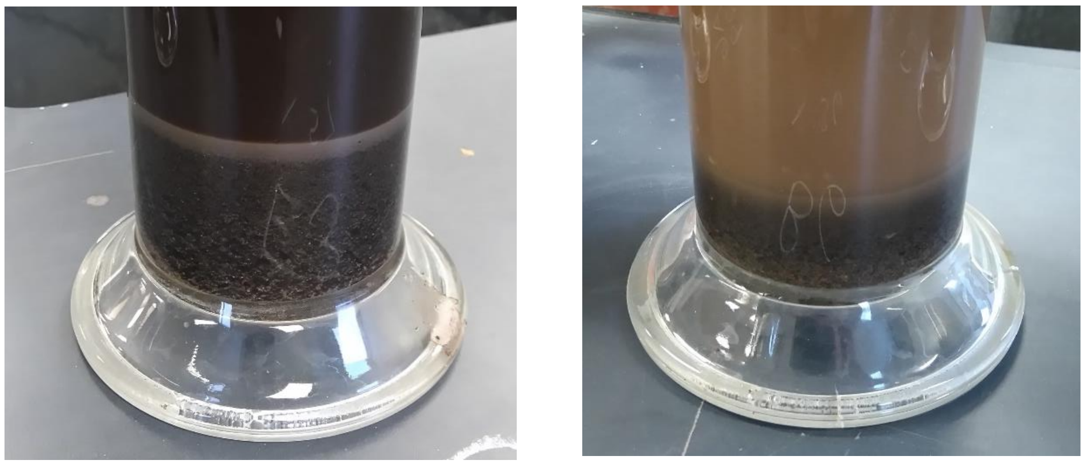


Figure 16. Soil samples with different particle compositions and rates of precipitation.

A hydrotexture test is composed of two steps: preparation and analysis. Soils were prepared by measuring 50g of each soil sample (one sample selected from each of the five sites) into a dispersion cup (Figure 17). To each cup, 400mL of water was then added, followed by 50mL of calgon (Figure 17). Each cup was then placed on a milkshake mixing machine for 15 minutes to fully mix the solution (Figure 18). After mixing, each sample was left to settle for 48 hours.

After the settling period, each sample was poured into a 1000mL glass sedimentation cylinder with water added to bring the total volume up to 1000mL (Figure 19). Before adding additional clean water, water was used to wash out the dispersion cup and ensure that all soil was in the sedimentation cylinder. In addition to the five soil samples, a blank was also prepared composed of 950mL of water and 50mL of calgon.

Figure 17. Soil samples in the dispersion cups (Left) and Calgon (Sodium metaphosphate).

Figure 18. Dispersion cup on the milkshake mixing machine.

Figure 19. Soil samples in the sedimentation cylinders.

Beginning with the blank, a rubber stopper was placed in the top of the cylinder and then gently inverted 25 times to ensure that the solution was fully mixed. Without thoroughly mixing the solution, the results would not be accurate as the sedimentation rates would be altered. After mixing, the stopper was immediately removed and rinsed, and a hydrometer (Figure 20) placed gently into the cylinder. A reading was then recorded at 30s, 45s, 1 minute, 2 minutes and 5 minutes. After completing the first five readings, the same process was completed with the first soil sample, returning to take a reading of the blank at the 10-minute mark. This process was repeated with each of the samples with additional readings taken at 1 hour, 2 hours, 4 hours, 8 hours and 12 hours. The results from the blank readings were used to correct the hydrometer readings from the soil samples to ensure accurate calculations.

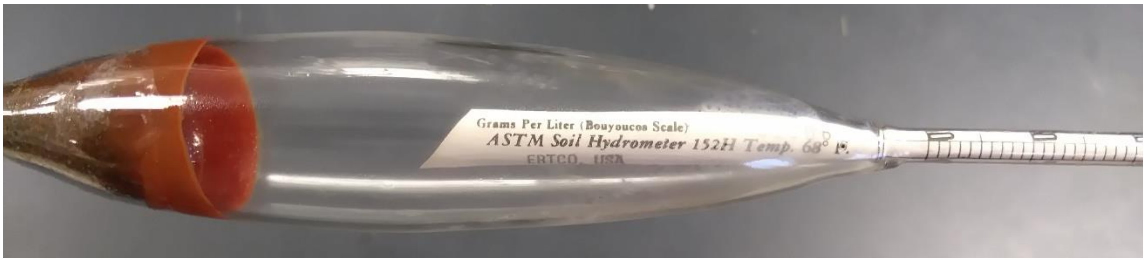


Figure 20. ASTM Soil Hydrometer.

2.3.5 Ash-Free Dry Mass

Total worm biomass at each site was determined by oven drying worms to determine their Ash-Free Dry Mass (AFDM). Worms from each site were placed in weighed and labelled aluminum trays (each site in a separate tray). Trays were then oven dried for five hours at 75 degrees Celsius (°C). Once all samples were completely dry, the weight of the tray and dried worms was weighed to determine weight of dried worms.

2.4 Statistical Analysis

2.4.1 Pearson Correlation

In order to determine the correlation between the biomass of earthworms (measured using Ash-Free Dry Mass) and percent total organic carbon (%TOC), a Pearson Correlation Coefficient was calculated using a Pearson Correlation test. This test indicated whether the number of earthworms present at a site affected the carbon present in the soil.

The Pearson Correlation test was run using the International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS). SPSS uses an input spreadsheet containing the desired variable values to produce an output table which describes the Pearson Correlation Coefficient (r) as well as the significance level (whether there is evidence that a linear correlation is present in the sample). The correlation coefficient is determined from a scatterplot and a line of best fit. The r-value determined by the test indicates the strength and direction of the correlation. An r-value that is equal to 1 represents a perfect positive correlation, whereas an r-value that is equal to -1 represents a perfect negative correlation (Owen et al. n.d.). The closer the r-value is to 1 or -1, the stronger the correlation. If the r-value was close to or equal to 0 there is a weak or absent correlation. The significance level used for this test was equal to 0.05 or a 95% confidence level.

A Pearson Correlation was run using both the original data set and a data set in which the %TOC was transformed using a natural logarithmic transformation suggested by Fletcher, Mackenzie and Villouta (2005) for dealing with ecological data which has a high proportion of zeroes and is positively skewed. This transformation allowed for the incorporation of the null values in the correlation and regression analyses to provide a more accurate representation of the data set. The transformation applied the following mathematical operation to each of the %TOC values, in which ln is the natural logarithm, y is the %TOC value and c is a constant, in this case 1:

$$\ln(y + c)$$

2.4.2 Regression

As a further indicator of the relationship between the presence of earthworms and the total soil organic carbon, a regression was completed in both SPSS and in Microsoft Excel. In comparison to the Pearson Correlation, the regression was completed using only plots from the original data set with earthworms present to allow for the data to be plotted on a logarithmic scale, which normally does not allow for the incorporation of zero values. When the regression was run using the transformed data, the transformation allowed the incorporation of all plots, not only those with a presence of earthworms. In SPSS the program used the same inputs as the Pearson Correlation but instead produced an r and r^2 value. These values represent the direction and strength of the correlation. The r value is the same as the Pearson Correlation Coefficient produced in the previous test. In Excel, a scatterplot was produced and a line of best fit from which the r^2 value and an equation of best fit were determined. The difference between the values determined in SPSS and Excel was slight, with SPSS relying on a linear relationship and Excel instead using an exponential relationship to better fit the data. The results of both regressions were plotted on a logarithmic scale to minimize the skewedness of the data.

In order to test for possible background correlations, a regression was completed using Excel comparing the pH and hydrogen ion concentration of the soil samples to the corresponding %TOC and AFDM.

2.4.3 Comparison of Means

As a comparison of the overall differences between those sites with earthworms and those sites with no earthworms, a two-way Analysis of Variance (ANOVA) was completed in SPSS and a bubble chart indicating group means and standard deviation was created in Excel. The ANOVA in SPSS utilized the same input as the Pearson Correlation and the regression, but produced an output table that described the significance of the variation in percent total organic carbon (%TOC) between the sites with earthworms and the sites without. This test used the same level of significance as the other tests, 0.05.

In addition to a comparison of the means for sites with and without earthworms, the means for other relationships were also examined using an ANOVA test and a bubble graph. These relationships included:

1. The relationship between soil texture class and total ash-free dry mass at each plot;
2. The relationship between soil texture class and percent total organic carbon at each plot; and
3. The relationship between total ash-free dry mass and species richness of the understory at each plot.

3.0 RESULTS

The results of this study supported the alternative hypothesis that earthworms cause significant impacts on soil structure and a decrease in overall carbon storage in the soil of Boreal hardwood-dominated forests.

3.1 Summary of Results

Table 3. Summary of observed and calculated results

Site	A	B	C	D	E
Location	No Name Lake	Fallingsnow	Silver Mountain	Lakehead U Campus	Resolute Forest Road
Dominant Overstory	Poplar White birch Balsam fir	Poplar White birch Balsam fir White spruce	Poplar White birch Balsam fir	Poplar White birch Balsam fir White spruce	Poplar White birch White spruce Mountain maple
Dominant Understory	Large leaf aster Raspberry Bracken fern Blue bead lily Bunchberry Sarsasparilla Grass	Raspberry Sarsasparilla Grass Fragrant bedstraw	Large leaf aster Bracken fern Grass	Raspberry Grass Horsetail	Bunchberry Sarsaparilla Grass Horsetail
Soil Texture (Particle Composition)	Silty sand (72% Sand, 25% Silt, 3% Clay)	Sandy loam (65% Sand, 26% Silt, 9% Clay)	Silty sand (55% Sand, 39% Silt, 6% Clay)	Loamy sand (78% Sand, 19% Silt, 3% Clay)	Silty Sand (70% Sand, 26% Silt, 4% Clay)
Average Percent Total Organic Carbon (%)	10.95	24.02	6.58	4.38	23.55
Average Ash-Free Dry Mass (g)	0.48	0.01	0.69	0.29	0.00
Average pH	4.36	5.12	5.14	4.95	3.67

3.2 Pearson Correlation

The calculated Pearson Correlation Coefficient for the original data set was -0.402 with a significance level (p-value) of 0.028 for all 30 values. These results indicated a moderate, negative correlation that was significant at the 0.05 level (Appendix).

The Pearson Correlation Coefficient for the transformed data set was -0.441 with a significance level (p-value) of 0.026 for all 30 values. These results indicated a moderate, negative correlation that was significant at the 0.05 level. The results from the analysis of the transformed data set were slightly more significant than the results calculated using the original data set.

3.3 Regression

The results of the regression indicated the same moderate, negative relationship that was indicated by the Pearson Correlation (Appendix). In addition to the -0.402 r value, an r^2 value of 0.162 was also determined for the original data set. The relationship between the total biomass and percent total organic carbon (%TOC) can be seen in Figure 21 and is modeled by the equation $y = 11.168e^{-0.818x}$. This graph shows a relatively constant decrease in %TOC as earthworm biomass increases to a certain point and then a stabilization of %TOC at a new lower level.

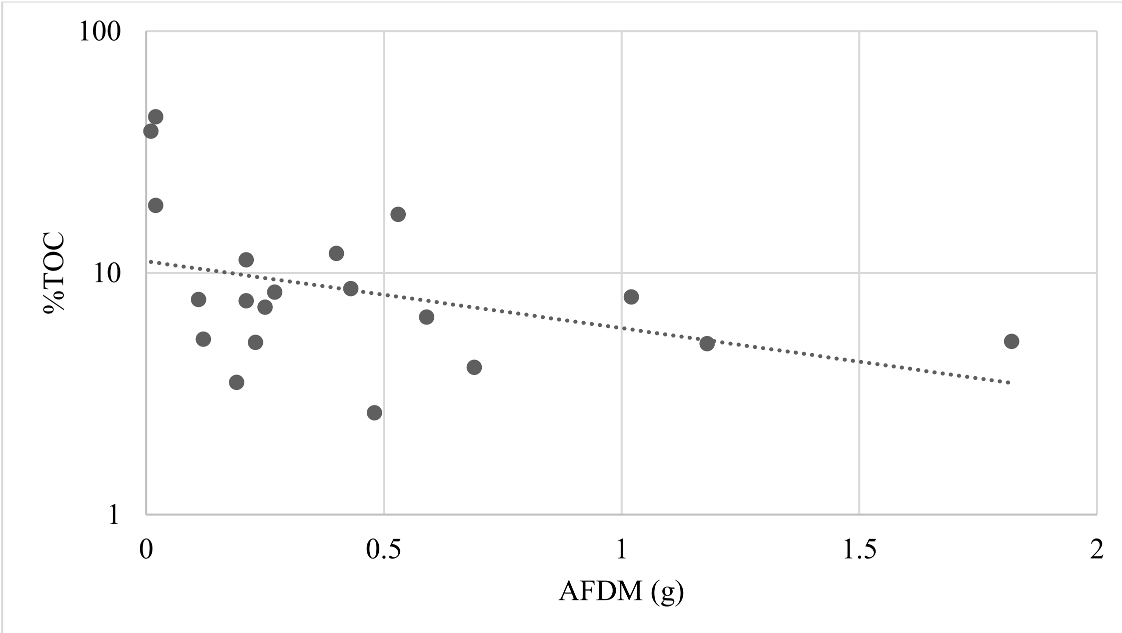


Figure 21. Regression of percent total organic carbon (%TOC) versus ash-free dry mass of earthworms (AFDM) for each site.

The results of the regression of the transformed data indicated a similar moderate, negative relationship. In addition to the -0.441 r value, an r^2 value of 0.194 was also determined. The relationship between the total biomass and percent total organic carbon (%TOC) can be seen in Figure 22 and is modeled by the equation $y = 2.5688e^{-0.225x}$. This graph shows a relatively constant decrease in %TOC as earthworm biomass increases, with less visible stabilization of %TOC levels.

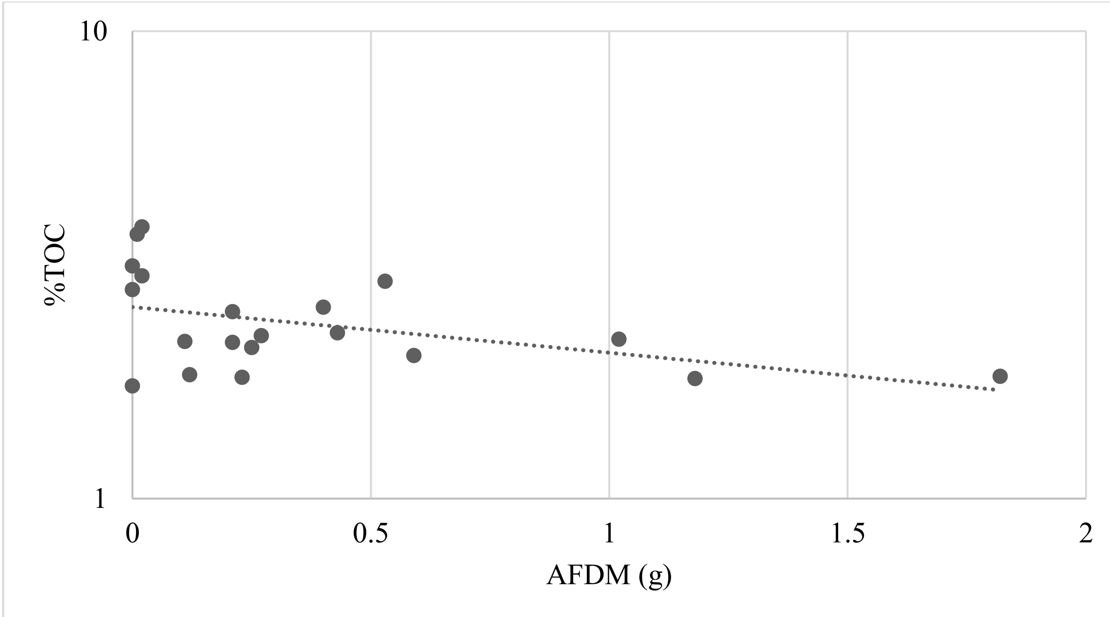


Figure 22. Regression of transformed percent total organic carbon (%TOC) versus ash-free dry mass of earthworms (AFDM) for each site.

In the case of the regressions comparing pH to %TOC and AFDM, no relationship was determined, therefore no r or r^2 value could be found. This suggests that the pH of the soil did not affect the carbon content or presence of earthworms in the soil, as was predicted.

3.4 Comparison of Means

The results of the ANOVA indicated a significant difference in %TOC between sites with and without earthworms. At a confidence level of 0.05, the ANOVA test returned a significance value of $p < 0.001$ (Appendix). Differences in %TOC are shown in Figure 23. The sites without earthworms were shown to have an average %TOC more than twice as high as the sites with earthworms present. This indicated a significant change in carbon storage as a result of the presence of earthworms in the soil. The size of the circles on the graph indicated a similar standard deviation for both site groupings.

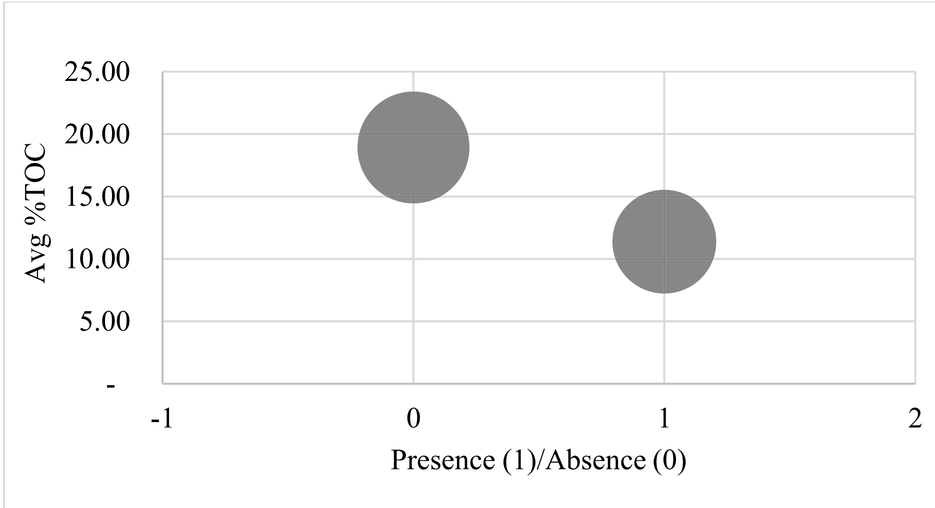


Figure 23. Comparison of average percent organic carbon content between sites with and without earthworms present, determined by two-tailed ANOVA.

The comparison of the herb layer richness and average ash-free dry mass (AFDM) indicated no relationship between the two variables. This suggests that the amount of earthworms present at a site did not significantly affect the richness of the herbaceous species in the ground cover at each of the sites. A similar result was found when texture was compared to earthworm abundance (AFDM) at each plot. In this case, the texture of the soil at each of the plots did not have a significant impact on the AFDM of earthworms. However, when the texture of the soil was compared to the percent total organic carbon at each of the plots, a significant difference was found. The ANOVA run in SPSS returned a significance value of 0.013 at a confidence level of 0.05. The soils which were classified at sandy loams showed the highest level of carbon, whereas the soil classified as loamy sands were shown to have the lowest level of carbon (Figure 24).

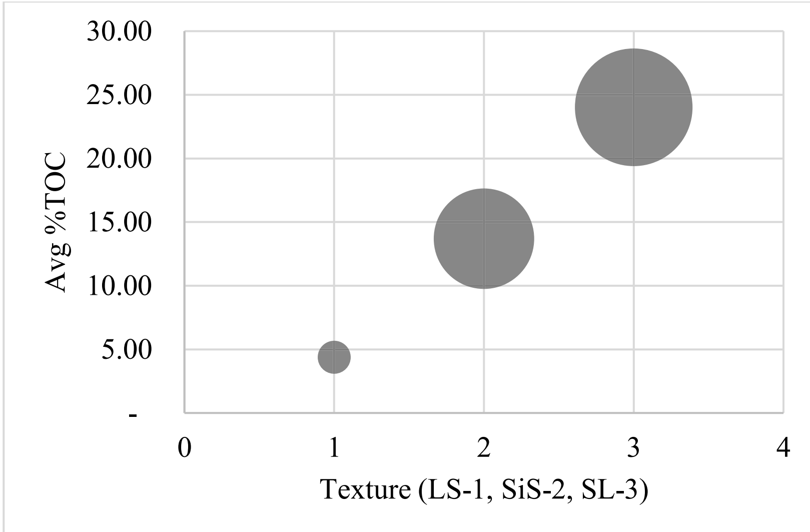


Figure 24. Comparison of texture class and average percent total organic carbon content, determined by a two-tailed ANOVA.

4.0 DISCUSSION

The results of this research suggest a strong negative impact on total organic carbon storage in northern boreal hardwood forests caused by the presence of earthworms in the soil. The results of the Pearson Correlation and the regression suggest that those sites which possessed a greater mass of earthworms did not have a significantly greater %TOC than those sites with a lower mass of earthworms. This is consistent with Hale et al. (2005) who found diversity of earthworm species has a greater impact than total earthworm biomass. This is likely a result of niche partitioning which allows for a greater decomposition of organic matter and increased biomixing. This factor was not considered in this research study, but the lack of a significant relationship between increasing earthworm biomass and decreasing soil carbon suggests that there may be other, more complex factors at play.

Soil texture also affects soil carbon storage, as was indicated by the ANOVA between soil texture and total organic carbon content. Sandy loam soils had the greatest carbon content, while loamy sand soils had the lowest carbon content. This is because soils with a higher proportion of finer soil particles, such as the loamy sand, have been found to have lower levels of organic matter present and therefore naturally lower levels of carbon prior to earthworm invasion (Najmuldeen and Mohammad 2010). Comparatively, soils with a higher proportion of sand particles have been shown to have lower levels of earthworm biomass and therefore lower levels of decomposition and biomixing, and higher levels of organic carbon. This is proposed to occur as a result of greater irritation to the earthworms' skin caused by the higher proportion of large abrasive particles (Williamson n.d.).

Ross et al. (2015) and Zhang et al. (2013), reported a reduction in soil carbon in forest ecosystems within a couple years of earthworm invasion, with soil carbon levels stabilizing at a lower level. This trend was consistent with the results of this study which indicated that the reduction in percent total organic carbon was greater as earthworm biomass increased from 0 grams to approximately 0.25 grams, after which soil carbon levels appeared to remain stable, regardless of increasing earthworm biomass. While there was a significant relationship between the presence or absence of earthworms, beyond a certain point, an increase in the total biomass did not cause a greater decrease in soil organic carbon storage. The stabilization of carbon at a new lower level is likely a result of the conversion of the soil from a digestible mor to an indigestible mull humus type (Ponge 2003). Mor soils are characterised by undecomposed, raw humus with little micro or macrofaunal activity, resulting in immobilization of nutrients (Ponge 2003). As micro and macrofaunal activity becomes greater, the humus develops into a mull, characterised by rapid disappearance of litter and rapid nutrient cycling (Ponger 2003). This transition to a mull means that there is less food for earthworms, resulting in a decrease in their activity and therefore a decrease in the further loss of carbon.

The potential decrease in carbon storage that resulted simply from the presence of earthworms indicated by the results of this research was an average of about 7%. Given the present average mass of carbon per hectare in the boreal forest of approximately 300 tonnes, a decrease of 7% carbon could mean a loss of 21 tonnes of carbon per hectare, or a total of 29 picograms (Pg: billion tonnes) of carbon across the entire boreal forest. More accurate estimations of carbon losses could have been predicted had bulk density also been measured for each of the soil samples. These estimates are based upon estimates from soil carbon concentrations rather than actual masses. Despite this shortcoming, the significance of the relationship between the presence of earthworms in the soil and the resulting storage of organic carbon cannot be understated. The influence of earthworms on the storage of carbon in boreal soils is an important component of the annual global carbon flux. The boreal forest is the largest carbon sink of all forest biomes, meaning that changes in carbon storage in this ecosystem have the potential to influence global climate change. This is also an important consideration for Canada's ability to meet promised targets under the Paris agreement (2016). At present, Canada will be unable to meet their 2020 and 2030 goals; an increase in carbon emissions resulting from the invasion of earthworms in northern boreal forests may decrease Canada's ability to meet targets even further (CATP 2017).

Earthworm introductions have been found to lead to a decrease in the floristic diversity and alter species regeneration (Frelich et al. 2006). While the results of this research did not indicate any significant changes, many previous studies provided strong proof for the changes in herbaceous species caused by earthworm presence. These changes are likely to be more noticeable in forest regions that have a naturally higher diversity of herbaceous species. The boreal forest is generally low in herb layer diversity and therefore the loss of or decrease in abundance of some herbaceous species would not necessarily be noticeable at a local scale, compared to the loss of many species in more floristically diverse regions. In regions with naturally low diversity, however, the loss of a single species can mean a significant change in the function and productivity of ecosystems. Furthermore, the loss of herbaceous species cover is likely to lead to increased soil erosion, causing nutrient losses and leaching that may alter the capacity of the soil to sustain a stable population of plant species.

The further spread of earthworms, through natural and assisted migration, is an issue for consideration in the short and long-term. Sources of assisted migration, including fishing, vermiculture, horticulture and logging, are becoming ever more prevalent and popular, increasing the likelihood of introducing earthworms into previously uninvaded regions (Bohlen et al. 2004a).

Earthworms are very hardy organisms capable of living in a variety of ecosystems. Despite their presumed dislike of sandy soils, this study found that earthworms can live in areas previously thought to be uninhabitable. If given the opportunity, it is likely that in the next few hundred years, earthworms will be common-place in almost every ecosystem on Earth. Earthworms' natural habitat plasticity, the variety of sources of introduction and spread, and the significance of the impacts they have on natural ecosystems present a strong case for the classification of earthworms as a globally invasive species. Without any such classification, their spread is not likely to be reduced or controlled in any way. Additionally, education of citizens and companies who regularly use earthworms for various purposes is important for helping to mitigate the impacts of earthworms on natural systems, especially the boreal forest.

5.0 CONCLUSION

Until recently, earthworms were thought to be an important part of soil communities, and a critical to maintaining soil fertility and the growth and survival of plant species. Upon further consideration, it was discovered that they in fact have many negative, and in some cases significant, impacts on natural ecosystems, especially forests. Earthworms are considered to be keystone detritivores, causing structural and functional changes in the ecosystems in which they occur. This study showed that the presence of earthworms in the soil in southern boreal hardwood ecosystems caused significant reductions in soil carbon. The presence of earthworms in the soil was found to result in a rapid reduction of soil carbon, eventually stabilizing at a new lower level of soil carbon. These impacts have the potential to significantly destabilize the boreal forest carbon pool, leading to increased carbon emissions and therefore increased global climate change. The potential impacts of widespread invasion of earthworms into previously uninvaded ecosystems necessitates the development of robust management strategies for controlling migration of current populations and preventing the introduction of future populations.

LITERATURE CITED

Aerts, R. 2006. The freezer defrosting: global warming and litter decomposition rates in cold biomes. Journal of Ecology 94:713-724 (online).

Banton, E., J. Johnson, H. Lee, G. Racey, P. Uhlig, and M. Wester. 2009. Ecological Land Classification Operational Draft. Queen's Printer for Ontario. 532pp (online).

Barr, A.G., T.J. Griffis, T.A. Black, X. Lee, R.M. Staebler, J.D. Fuentes, Z. Chen, and K. Morgenstern. 2002. Comparing the carbon budgets of boreal and temperate deciduous forest stands. Canadian Journal of Forest Research 32:813-822 (online).

Bellard, C. W. Thuiller, B. Leroy, P. Genovesi, M. Bakkenes, and F. Courchamp. 2013. Will climate change promote future invasions?. Global Change Biology 19(12):3740-3748 (online).

Bohlen, P.J., S. Scheu, C.M. Hale, M.A. McLean, S. Migge, P.M. Groffman, and D. Parkinson. 2004a. Non-native invasive earthworms as agents of change in northern temperate forests. Frontiers in Ecology and the Environment 2(8):427-435 (online).

Bohlen, P.J., D.M. Pelletier, P.M. Groffman, T.J. Fahey, and M.C. Fisk. 2004b. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. Ecosystems 7:13-27 (online).

Callaham Jr., M.A., G. González, C.M. Hale, L. Heneghan, S.L. Lachnicht, X. Zou. 2006. Policy and management responses to earthworm invasions in North America. Biological Invasions 8:1317-1329 (online).

[CATP] Climate Action Tracker Partners. 2017. Climate Action Tracker: Canada. Climate Analytics, Ecofys and NewClimate Institute. http://climateactiontracker.org/countries/canada.html. Feb. 21, 2017.

Dukes, J.S., J. Pontius, D. Orwig, J.R. Garnas, V.L. Rodgers, N. Brazee, B. Cooke, K.A. Theoharides, E.E. Stange, R. Harrington, J. Ehranfeld, J. Gurevitch, M. Ledrau, K. Stinson, R. Wick, and M. Ayres. 2009. Responses of insect pests, pathogens and invasive plant species to climate change in the forests of northeastern North America: What can we predict?. Canadian Journal for Forest Research 39:231-248 (online).

Feller, C., G.G. Brown, E. Blanchart, P. Deleporte, S.S. Chernyanskii. 2003. Charles Darwin, earthworms and the natural sciences: various lessons from past to future. Agriculture, Ecosystems and Environment 99:29–49 (online).

Fletcher, D., D. Mackenzie, and E. Villouta. 2005. Modelling skewed data with many zeros: A simple approach combining ordinary and logistic regression. Environmental and Ecological Statistics 12:45-54 (online).

[FoReST] Forest Resources and Soils Testing Laboratory. 2014. Direct Estimation of Organic Matter. SOP # FS012-B. Lakehead University Centre for Analytical Services. 10pp.

Frelich, L.E., C.M. Hale, S. Scheu, A.R. Holdsworth, L. Heneghan, P.J. Bohlen, and P.B. Reich. 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biological Invasions 8:1235-1245 (online).

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 (online).

Hendrix, P.F., P.J. Bohlen. 2002. Exotic earthworm invasions in North America: ecological and policy implications. BioScience 52(9):801-811 (online).

Holdsworth, A.R., L.E. Frelich, and P.B. Reich. 2007. Regional extent of an ecosystem engineer: earthworm invasion in northern hardwood forests. Ecological Applications 17(6):1666-1677 (online).

Holdsworth, A.R., L.E. Frelich, P.B. Reich. 2008. Litter decomposition in earthworm-invaded northern hardwood forests: role of invasion degree and litter chemistry. Ecoscience 15(4):536-544 (online).

Kalra, Y.P. and D.G. Maynard. Methods manual for forest soil and plant analysis. Report: NOR-X-319. Canadian Forest Service. 116pp.

Lal, R. 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123:1-22 (online).

Lavelle, P., T. Decaëns, M. Aubert, S. Barot, M. Blouin, F. Bureau, P. Margerie, P. Mora, and J.P. Rossi. 2006. Soil invertebrates and ecosystem services. European Journal of Soil Biology 42:S3-S15 (online).

Lemtiri, A., G. Colinet, T. Alabi, D. Cluzeau, L. Zirbes, É. Haubruge, F. Francis. 2014. Impacts of earthworms on soil components and dynamics: a review. Biotechnology Agronomy Society and Environment 18(1):121-133 (online).

Loss, S.R., R.M. Hueffmeier , C.M. Hale , G.E. Host , G. Sjerven , L.E. Frelich. 2013. Earthworm invasions in northern hardwood forests: a rapid assessment method. Natural Areas Journal 33(1):21-30 (online).

Lubbers, I.M., K.J. van Groenigen, S.J. Fonte, J. Six, L. Brussaard, and J.W. van Groenigen. 2013. Greenhouse-gas emissions from soils increased by earthworms. Nature Climate Change 3:187-194 (online).

Najmuldeen, H. and A. Mohammad. 2010. Effects of soil texture on chemical compositions, microbial populations and carbon mineralization in soil. Egyptian Journal of Experimental Biology 6(1):59-64 (online).

Owen, A., E. Marshall, M. Petrie, J. Matthews, D. Green, T. Croft and S. Davis. N.d. Pearson's Correlation. Loughborough University and Coventry University. 8pp (online).

Pluske, W., D. Murphy. J. Sheppard. 2016. Total Organic Carbon. Government of Australia. http://www.soilquality.org.au/factsheets/organic-carbon. Dec. 11, 2016.

Ponge, J. 2003. Humus forms in terrestrial ecosystems: a framework to biodiversity. Soil Biology and Biochemistry 35(7):935-945 (online).

Racey, G.D., A.G. Harris, J.K. Jeglum, R.F. Foster and G.M. Wickware. 1996. Terrestrial and Wetland Ecosites of Northwestern Ontario. Queen's Printer for Ontario. 110pp (online).

Ross, D.S., J. Gorres, C. Danks, and S. Wilmot. 2015. Earthworm and land-use legacy effects on belowground carbon in the managed northern forest. University of Vermont. 24pp (online).

Seely, B., C. Welham, H. Kimmins. 2002. Carbon sequestration in a boreal forest ecosystem: results from the ecosystem simulation model, FORECAST. Forest Ecology and Management 169:123-135 (online).

Shipitalo, M.J. and R. Le Bayon. 2004. Quantifying the effects of earthworms of soil aggregation and porosity. Earthworm Ecology 2:183-200 (online).

Williamson, R.C. n.d. Effective Management of Earthworm Castings. University of Wisconsin. 43pp (online).

Zhang, W., P.F. Hendrix, L.E. Dame, R.A. Burke, J. Wu, D.A. Neher, J. Li, Y. Shao, and S. Fu. 2013. Earthworms facilitate carbon sequestration through unequal amplification of carbon stabilization compared with mineralization. Nature Communications 4(2576):1-9 (online).

APPENDIX

Pearson Correlation all values – original data set

		AFDM of worms (g)*	%TOC
AFDM of worms (g)*	Pearson Correlation	1	-.402[*]
	Sig. (2-tailed)		.028
	N	30	30
%TOC	Pearson Correlation	-.402[*]	1
	Sig. (2-tailed)	.028	
	N	30	30

*. Correlation is significant at the 0.05 level (2-tailed).

Regression all values – original data set

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.402[a]	.162	.132	11.26814

a. Predictors: (Constant), AFDM of worms (g)*

*significant at the 0.05 level

ANOVA (presence/absence) all values – original data set

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1653.233	1	1653.233	17.894	.000
Within Groups	2586.915	28	92.390		
Total	4240.147	29			

ANOVA (AFDM by Texture) – original data set

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.656	2	.328	1.951	.162
Within Groups	4.540	27	.168		
Total	5.196	29			

ANOVA (%TOC by Texture) – original data set

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1158.827	2	579.413	5.077	.013
Within Groups	3081.321	27	114.123		
Total	4240.147	29			

ANOVA (Species richness by AFDM) – original data set

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	48.467	18	2.693	1.039	.490
Within Groups	28.500	11	2.591		
Total	76.967	29			

Pearson correlation (all values) – transformed data set

		AFDM (g)	Trans. %TOC
AFDM (g)	Pearson Correlation	1	-.441*
	Sig. (1-tailed)		.026
	N	20	20
Trans. %TOC	Pearson Correlation	-.441*	1
	Sig. (1-tailed)	.026	
	N	20	20

*. Correlation is significant at the 0.05 level (1-tailed).

Regression (all values) – transformed data set

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.441[a]	.194	.149	.566779

a. Predictors: (Constant), AFDM (g)