Earthworms in the Urban Environment:
Can Population Augmentation Improve Urban Soil Properties?

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in
partial fulfillment of the requirements for the degree of

Master of Science
in
Forestry

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July 17, 2009
Blacksburg, Virginia

Keywords: urban forest, urban forestry, urbanization, earthworms, earthworm population
augmentation, *Lumbricus terrestris*, disturbed soil, soil ecology, soil compaction

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(Abstract)

Urban forests perform essential ecological functions, and their performance is dependent on soil quality, which is often degraded by human activity. Because earthworms play a key role in soil health, augmenting earthworm populations in urban soils may improve tree performance. However, we know very little about earthworm ecology in highly urbanized soils. The objectives of our study were: (1) to assess earthworm demographics across a range of urban land uses and (2) to evaluate earthworm augmentation techniques for rehabilitating disturbed soils and improving tree growth.

1. We conducted an observational study across three landuse types to assess earthworm abundance and diversity as well as associated soil properties. Earthworm abundance and biomass in were affected by land use type, disturbance time frame, and seasonality. Earthworm abundance and biomass were affected by a suite of complex soil and temporal variables, and soil temperature and moisture seemed to be the most influential properties.

2. We conducted an earthworm inoculation experiment on a compacted cut-fill field soil with a very low existing earthworm population. In 2008, three soil treatments (control, compost, and compost + earthworm) were applied to 2 m^2 plots into which two common urban tree species with contrasting soil tolerances were planted (*Acer rubrum* and *Cornus florida*). We measured

soil physical and chemical properties along with earthworm survival and soil respiration. Earthworm inoculation did not succeed in improving bulk density or increasing soil organic matter, yet it was successful in increasing soil CEC, Fe, and Mn relative to compost only effects.

Dedication

This master's thesis is dedicated to my mom and dad, who have always encouraged me to follow my heart and pursue my dreams, and in memory of my sister, who will always be there in my heart and when I fulfill those dreams.

Acknowledgements

I'd like to thank the following people for all of their hard work on this project: John Peterson, general go-to guy; Dan Henry, Urban Forester for the City of Roanoke; my worm wranglers, lab helpers, and ditch digging crew, Jeannette Hoffman, Mason Patterson, Taylor Duke, Chris Jones, Velva Groover, Sarah Dickinson, Dawn Park, Julia Bartens, Tyler Wright, Cindy Green, Stephanie Worthington, Joe Hoffman, and Jake Carter; Tal Roberts, equipment provider and rescue team; the CSES Soils lab for analyzing our multiple soil samples; the Forestry Soils lab for allowing us to borrow supplies; the Laboratory for Interdisciplinary Statistical Analysis, specifically Ciro Velasco and Nels Johnson, for helping with statistical analysis; and Dr. Erik Ervin and Frederick 'Dickie' Shepherd at the Turfgrass Research Center for showing us around the site and letting us plant trees in the middle of lawns.

A special thank you is extended to my committee: Dr. Susan D. Day, Dr. W. Lee Daniels, and, chairperson, Dr. P. Eric Wiseman.

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Chapter 1: Introduction

1.1   Background

Urbanization is an increasingly important environmental issue that affects the general health of trees and forests world-wide. As cities expand and populations grow, the natural landscape changes drastically. Trees are removed to create space for buildings, parking lots, and roads; as a result, greenscapes are decreased and impervious surfaces increased. Urban trees and forests are essential for clean air and water, carbon storage, stormwater runoff reduction, air temperature moderation, energy conservation, and an improved human aesthetic experience (Nowak and Walton 2005). In most cases, the value of benefits reaped from urban trees far exceeds the cost to manage them, making urban forests a wise investment for improving city environments (McPherson et al. 2005). However, the poor soils in cities present a challenge to the growth of healthy, sustainable urban forests.

Earthworms have been shown to improve soil chemical and physical properties as well as fertility and general soil health (Card et al. 2002; Edwards and Bohlen 1996; Hidalgo et al. 2006; Lee 1985). Although widely studied in agricultural (Bohlen et al. 1995; Eriksen-Hamel and Whalen 2007) and natural forest settings (Bohlen et al. 2004c; Burtelow et al. 1998; Groffman et al. 2004), there is little information about earthworm ecology in urban ecosystems. Additionally, researchers have noted the positive effects of earthworm presence on plant growth in the greenhouse and garden (Hidalgo et al. 2006; Scheu 2003), but earthworm applications in arboriculture have rarely been explored (Edwards and Bohlen 1996).

1.2   Justification and Significance

The disturbance caused by human activities has had a detrimental impact on the function of urban soils, which has indirectly affected the health and growth of urban trees. These soils are altered through road and building construction, human waste and trash disposal, and atmospheric deposition (Scheyer and Hipple 2005). Protection and remediation of the soil is imperative to the growth and survival of city trees. Due to the positive impacts earthworms have on soil, restoring earthworm populations and function in this setting may be a possible mechanism for improving urban forest performance.

1.3   Objectives and Hypotheses

Two studies were conducted to explore the impact of earthworm presence on urban soils. The first was an observational study used to assess earthworm abundance and diversity as well as associated soil properties. The second was a designed inoculation experiment used to evaluate earthworm population augmentation strategies to rehabilitate disturbed urban soils and improve tree growth.

1.3.1   Observational Study

Objectives

1. To assess earthworm population trends across three urban landscape settings and two soil disturbance timeframes within the urban forest and assess soil habitat characteristics that can explain those differences.

2. To characterize earthworm abundance in urban landscapes to provide insight on the merits of earthworm inoculation to improve soil health

Research Hypotheses

A. Earthworm populations are more diverse and numerous in landuse types with the least amount of artificiality and disturbance (forested fragments and lawns vs. parking lot islands).

B. Earthworm population is a function of the amount of time since soil disturbance (older sites will support larger and more diverse populations).

C. Earthworm populations are more diverse and earthworm density increases in landuse types with lower bulk density, higher soil organic matter and moisture, soil pH close to 7.0, and soil temperatures between 15-25°C (forested fragments and lawns vs. parking lot islands).

1.3.2   Inoculation Experiment

<u>Objective</u>

To evaluate earthworm augmentation strategies for improving soil conditions for urban tree growth.

<u>Research Hypotheses</u>

A. Earthworms can help rehabilitate urban soil and improve tree health.

B. Earthworm inoculation will decrease bulk density, increase porosity, and improve soil aggregation.

C. Earthworm inoculation will increase soil cation exchange capacity and organic carbon.

D. Earthworm inoculation will improve soil health (as described above) thereby increasing tree growth.

E. Earthworm inoculation will result in long-term and sustainable population augmentation.

Chapter 2: Literature Review

2.1   Healthy, Sustainable Urban Forest Management

2.1.1   Urban Forest Features

Urban forests are "ecosystems characterized by the presence of trees and other vegetation in association with people and their developments" (Nowak et al. 2005). The Society of American Foresters defines the term urban forestry as "[t]he art, science, and technology of managing trees and forest resources in and around urban community ecosystems for the physiological, sociological, economic, and aesthetic benefits trees provide society" (Society of American Foresters 2007). A term originating from the University of Toronto, "urban forestry" was officially recognized by the U.S. federal government in 1968 and funding was given to the USDA Forest Service in 1972 to help state and local governments develop urban forestry programs (Koch 2000).

Although relatively new in the U.S., deliberate incorporation of trees into human settlements has a long history in human civilization (Koch 2000). In ancient times, trees were sometimes worshipped directly and were commonly used for aesthetic improvement. As cities developed throughout history, so did the desire to use trees for anything from garden boundaries to lining transportation routes, such as streets, pathways, and canals. Early American city plans often included tree plantings, but it was not until the late 18th century that trees were actually planted as instructed by the plans. Cities such as Philadelphia, PA and Washington, D.C.,

boasted tree-lined streets and, eventually, cities such as Detroit passed laws requiring trees in boulevards and public squares (Koch 2000). In the wake of the U.S. industrial revolution, cities were overcrowded and polluted, prompting city beautification efforts. Although a successful endeavor to make cities more habitable, city parks did not keep people within the city boundaries, leading to a population boom in suburban areas, which also required tree maintenance. It has recently been suggested that congestion in suburban areas has been encouraging people to move back to the cities, which is creating new-found demand for sustainable urban forests (Koch 2000). Today, urban areas (i.e. densely populated lands) are where 75% of Americans reside (McPherson 2003) and urban forests are vital to a healthy quality of life in the U.S.

In the United States, there are roughly 3.8 billion urban trees, with 2.8% of the country's tree cover in its urban areas and an average of 27.1% tree cover in those urban areas (Nowak et al. 2001). Comparatively, the Commonwealth of Virginia has 156.5 million urban trees, putting it within the top ten states in the country for high urban tree populations. About 5% of the state is covered with urban trees and 35.3% of urban areas are covered with trees. Urban trees can be found on the following land use types, as described by Grey (1996): city parks, public squares, grounds of city buildings, monuments and cemeteries, boulevard medians, streetsides, parking lots, and riparian zones, all of which are traditionally maintained by the city government. Urban forests are also found on utility easements, public lands (federal and state), and private lands, such as residential yards, commercial properties, educational grounds, and undeveloped lands.

2.1.2   Urban Forest Benefits

Trees and associated vegetation in the urban environment provide many environmental, social, and economic benefits. Many authors attribute improved environmental quality, enhanced human health and well-being, and increased real estate value and economic vitality to the presence of an urban forest (McPherson 2003; McPherson et al. 2005; Nowak and Dwyer 2007; Nowak et al. 2001). In addition to the benefits of urban forests are the associated economic costs of maintaining those trees (e.g. annual pruning and planting) and the social costs of living with those trees (e.g. human allergies) (Lohr et al. 2004; McPherson et al. 2005; Nowak and Dwyer 2007).

Nowak and Dwyer (2007) explore the benefits and costs associated with the urban forest, and McPherson et al. (2005) provide empirical evidence of selected benefits and costs in five cities in the U.S., including benefits of decreased air temperature, the removal of air pollutants, energy conservation, and increased real estate value, and the costs of allergies, injuries from branch or tree failures, and annual maintenance. Lohr et al. (2004) studied the public's perception of the urban forest and concluded that, in general, people ranked the social, environmental, and practical benefits of trees very highly and indicated that the problems created by urban trees were not so bad as to warrant their removal or reduced use.

2.1.3   Urban Forest Issues

Increasing urbanization world-wide is severely affecting the natural forest resource available. In the U.S., urban land is predicted to increase from 3.1% in 2000 to 8.1% in 2050 (Nowak and Walton 2005). During this time frame, over 5% of existing forestland will be subsumed by urban growth, resulting in forest management and tree health concerns, including new forest edge, increased fire risk in the wildland-urban interface, exposure of trees to urban stress, reduced timber harvesting, increased pressure for recreational activities, increased probability of pest introduction, and increased forest fragmentation, which alters or degrades wildlife habitat (Nowak and Walton 2005; Nowak et al. 2005). In Virginia, almost 50% of urban growth occurred in forested land cover types between the years 1990 and 2000 (Nowak et al. 2005), presenting an even more intense critical need for management of urban forested areas in this state.

Due to the positive impacts of trees in the urban environment, more trees need to be incorporated in cities. American Forests (2002) recommends an overall urban tree canopy cover of 40%, yet urban areas in the U.S. have an average of 27% urban tree canopy cover (Nowak et al. 2001). In Virginia, urban areas have an average of 35% tree canopy cover. Unfortunately, major tree health challenges[1] are faced by city tree managers, making it difficult to increase canopy cover.

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[1] See review of Duryea, M.L., and M.M. Malavase. 2003. How Trees Grow in the Urban Environment. Forest Resources and Conservation Department, F.C.E. Service, I.o.F.a.A. Sciences, and U.o. Florida (eds.), Gainesville, FL.

2.2   Urban Soils

Soil is essential for human survival and serves as the foundation for human activity on this planet. It provides a surface to walk on, to build on, and to live on. It supplies a medium for plants to grow, which are then used as a food source, for building materials, and in many other necessary materials. Additionally, it acts as a filter for water and a transporter of many important nutrients (Craul 1999; Scheyer and Hipple 2005; Singer and Munns 2002). Unfortunately, urban soils are often disrupted and are unable to provide the necessary space, water movement, and nutrient availability needed for basic tree health (Craul 1999; Scheyer and Hipple 2005).

2.2.1   Human Impact on Soil Development

Soil is formed through five major formation factors: parent material, climate, organic activity (plants and animals), topography (relief), and time (Singer and Munns 2002), and consists of both biotic and abiotic components. Due to the increasingly widespread impact humans have had through population increase and urban development, a sixth factor has been identified: human activities (Dudal 2004). This sixth factor has been the impetus for characterizing and classifying soils previously described as man-made or anthropogenic. These include those affected by human induced changes of soil class, new parent materials, deep soil disturbance, change of landform, and change of topsoil. In 1998, the World Reference Base for Soil Resources proposed a thirteenth soil order called Anthrosols. Soils within this order have been greatly altered by human activity and are often those impacted by long term applications of

organic matter or of wetland cultivation (Dudal 2004). Urban soils, therefore, would largely fall within the Anthrosol order.

2.2.2   *Urban Soil*

Urban soils have a man-made surface layer "produced by mixing, filling, or by contamination of land surface in urban and suburban areas" (Bockheim 1974). Comparatively, forested soils are soils that have developed under the influence of a forest cover and continues to be influenced by that forest cover and cultivated soils are soils that are developed under agricultural activities (Fisher and Binkley 2000).

Urban soils differ from natural soils because of the enormous human influence associated with urban areas (Dudal 2004; Scheyer and Hipple 2005). As soil properties change, so does the capacity of the soil to properly function. Many human activities affect the function and health of urban soils, often necessitating restoration following disturbance. Site preparation and manipulation disturb soils, thus making them react much differently to environmental influences (e.g. precipitation and pollution) common to urban areas (Scheyer and Hipple 2005). Table 2.1 summarizes many of the physical, chemical, and biological impacts urbanization has on urban soils.

Table 2. 1. List of human impact on urban soil function and health. Summarized from Scheyer and Hipple (2005) and Harris (1991)^.

Physical
Ability to support structures
Human-constructed soil layers change water movement in soils
Less water infiltration and higher evaporation due to impervious surfaces and compacted soils
More water runoff brings sediments and pollutants to lakes, streams, and ground water
Higher soil temperature due to large areas of pavement and large buildings that absorb heat
Increased wind erosion and dust from plant removal and pulverized soil aggregates

Chemical
Little or no addition of organic matter
Elevated salt content
Interrupted nutrient cycling
Increases chemical reaction rates from higher soil temperatures
Higher pH (addition of cement, plaster and road salts) reducing available nutrients for plant use
Metals deposited from past land use, automobile emissions, building materials, poorly managed dumps and landfills, city fires

Biological
Heat stress in plants
Modified micro-organism activity
Change in size and composition of soil organism community (diversity)^
Reduction in soil macrofauna (e.g. earthworms) population, biomass, and activity^
Sequestration and transport of heavy metals in macrofauna and throughout the food chain^
Toxic effects on soil organisms^

2.3   Biology and Ecology of Earthworms

"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."
Charles Darwin, 1881

2.3.1   Earthworm Ecology

Earthworms are segmented worms placed in the phylum Annelida and within the class Chaetopoda. Although the vast majority of species are terrestrial, there are a few species of earthworm that, like their close relations, bristle worms and leeches, are aquatic. The most common worms in North America and Europe are further classified into the family Lumbricidae (Martin et al. 2008)[2].

Earthworms, are placed into three functional groups based on location in the soil profile, burrowing and feeding behaviors, size, and color (Edwards 1998; Lee 1985). **Epigeic** species reside in the upper organic layer and contribute to a limited mixing of mineral and organic soil, which makes nutrients more rapidly available to plants. They tend to be the smallest earthworms, are usually red in color, and create temporary burrows, if at all.

The **endogeic** species create continuous, semi-permanent, horizontal burrows, which helps aerate the soil. These worms are unpigmented, small to medium in size, and live mostly in the O horizon, feeding mostly on dead roots and further contributing to the mixing of organic matter with mineral soil. **Anecic** worms build permanent, vertical burrows that are up to two meters in depth and open to the soil surface. They feed at the surface and pull that litter into their burrows, which incorporates organic material with the mineral soil in deeper layers but also

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[2] There is much discussion about the proper taxonomic classification of terrestrial earthworms. Martin et al. (2008) describes the most recent. For more detailed history, refer to Edwards and Bohlen (1996).

brings mineral components to the surface. These species are the largest and longest lived worms and are brownish in color.

Earthworms are hermaphroditic, containing male and female sex organs on each individual. Eggs are produced for the majority of the year and are stored inside a cocoon, which is deposited either near the soil surface, if conditions are moist enough, or deeper down, during times of drought. The number of cocoons laid depends on the climate and weather of that year and can be used as a measure of environmental stress. Most cocoons are laid in the spring when temperature, soil moisture, and food supplies are optimal (Edwards and Bohlen 1996; Lee 1985).

2.3.2   *Earthworm Distribution and Dispersal*

Edwards and Bohlen (1996) characterized the geographical distribution of earthworms as **peregrine** – species that are widely distributed, migrate easily, or are spread by man, or **endemic** – species that are not well adapted to migration (Edwards and Bohlen 1996). Most peregrine species are within the Megascoledicae or the Lumbricidae families; the Lumbricids are among the most adaptable and aggressively pervasive species of earthworms. Over the past century, these particular worms have been noted for their ability to live in environments that are constantly disturbed by human activities and have often been associated with the disappearance of endemic worm populations (Stephenson 1930).

Gates (1970) further distinguished peregrine earthworm species using the term **anthropochorous** to describe species that can thrive in human-disturbed soils, and the term **allochthonous** to describe species that are not constrained to "man-modified" habitats. Human activities have contributed to the presence of anthropochorous, exotic earthworms both by eradicating native worm habitat, through forest harvesting and land conversion, and by accidental introduction (soil movement, landscaping, bait used for fishing, etc.).

In "Ecological Risk Assessment of Non-indigenous Earthworm Species", Proulx (2003) explains earthworm presence and distribution in North America by invoking the "Post Quaternary Introduction Theory" (Reynolds et al. 1974). This theory indicates that glaciation during the most recent ice age destroyed native earthworm habitat and, therefore, native populations above the Quaternary glacial boundary were eradicated. Because native earthworms have a preference for fine textured soils (Fender 1995), they have not repopulated previously glaciated regions of the continent, leaving a conglomeration of niches open for exotic colonization. Still, native species are present and often dominate pristine, undisturbed tracts of land south of the glacial boundary (Kalisz and Dotson 1989). Based on ideas presented by both Proulx (2003)and Kalisz and Dotson (1989), it could be concluded that issues related to exotic earthworm presence arise from previous landuse as well as landscape history (previously glaciated or not) and the functional group and species of earthworm.

2.3.3 Role of Earthworms in Soil Function

Earthworms are widely regarded as fauna that are critical to the health and function of soils. Earthworms improve soil function by modifying several key physical and chemical soil properties, including soil structure, water-holding capacity, fertility, and nutrient cycling.

Effects of Earthworms on Soil Chemical Properties

Earthworms also modify soil chemical properties. They mix organic matter from the surface with mineral soil in lower horizons through ingestion and fragmentation. Casts, or excrement, which are frequently deposited at the surface, also contain nutrients in plant-available forms and play a key role in nutrient cycling within the soil (Card et al. 2002; Edwards and Bohlen 1996).

Nutrient cycles, such as carbon and nitrogen, are highly influenced by earthworm activity. According to studies summarized by Edwards and Bohlen (1996), earthworms can either increase organic carbon content by promoting plant productivity or decrease soil carbon content by enhancing mineralization, thus making carbon unavailable for plant uptake. Lee (1985) describes studies that explored the relationship between earthworms and the nitrogen cycle. One concluded that earthworms have also been found to increase the rate of nitrogen cycling, making nitrogen more rapidly available for plant uptake. Another deduced that the nitrogen released when an earthworm dies is readily mineralized and microbes in earthworm guts

transform inorganic nitrogen to organic forms. Finally, studies described by both Edwards and Bohlen (1996) and Lee (1985) have shown that earthworm casts have a lower C:N ratio and more available phosphorus than surrounding soil, making them a rich source of plant nutrients.

Although earthworms play a part in most nutrient cycling, they assimilate carbon rather inefficiently. For example, in a study summarized by Edwards and Bohlen (1996) they consume a large amount of organic carbon, yet contribute very little to overall CO_2 output, unless populations are very large (e.g. 1000 individuals m^{-2}). In an experiment where earthworms were inoculated in soil columns, CO_2 emission rates were significantly higher in inoculated columns during the first month, but then declined significantly over the next three months of monitoring to a rate lower than columns without earthworms (Borken et al. 2000). Overall, earthworms did not contribute much to CO_2 flux over the course of this study. The initial peak in CO_2 was due to the creation of burrows and incorporation of organic matter into the mineral soil. This activity most likely stabilized the organic matter, preventing subsequent microbial breakdown, which caused the rate to steadily decline for the rest of the study. Conversely, Bohlen and Edwards (1995) described a study where an increase in soil respiration with the addition of earthworms and various nutrient sources was reported. The researchers attributed the increase to earthworm respiration as well as the stimulation of microbial respiration from earthworm activities. Additionally, the rate of CO_2 efflux varied in intensity depending on the nutrient source.

Earthworm Effects on Soil Physical Properties

Earthworms increase soil macroporosity and aeration, aggregate formation, and water-holding capacity. Edwards and Bohlen (1996) and Lee (1985) both describe studies where researchers determined that earthworm burrows are partially stabilized by mucus secreted by the earthworm and are a main component of vertical macroporosity in the soil. They frequently intersect with one another, which provides a means for water percolation below surface, thereby increasing water drainage and decreasing surface run-off. Additionally, studies summarized also concluded that earthworm burrows created pores for root growth and enhances root aeration and hydration (Card et al. 2002; Edwards and Bohlen 1996).

Soils rich in colloidal particles (e.g. clay and organic matter) typically have higher water-holding capacities. Earthworms can enhance the water-holding capacity of a soil by incorporating organic matter into mineral horizons, constructing burrows (which increases porosity), and promoting aggregate formation with the aid of the mucus secreted by the earthworm's skin (Card et al. 2002). Earthworm casts are also important in the formation of soil aggregates due to the earthworm's preferential ingestion of organic matter and fine soil particles. In a study summarized by Edwards and Bohlen (1996), researchers found that these aggregates have higher proportions of clay and silt fractions than the surrounding soil and can be more stable than those in soils without earthworms. Additional studies concluded that the stability of the casts depends on the species of earthworm, intestinal secretions, ingestion of plant fibers and fungal hyphae, presence of bacterial gums, wet-dry cycling, and age of cast(Edwards and Bohlen 1996).

<u>Earthworm Effects on Soil Biological Properties</u>

Earthworms have been shown to have an effect on soil microbial populations, which are typically comprised of fungi, yeast, and bacteria. At least two studies have reported increased microbial populations in earthworm casts compared to the surrounding soil, attributing this phenomenon to several factors, including original microbial populations in the earthworm gut, high amounts of ammonia and partially digested organic matter (summarized by Edwards and Bohlen 1996), and higher moisture content (summarized by Lee 1985), all of which create a more favorable environment for micro-organisms. However, it has been reported that microbial populations are highest in freshly deposited casts and then decline as the cast becomes older, indicating that this phenomenon is short-lived (Edwards and Bohlen 1996).

2.3.4   Earthworm Influence on Plant Growth and Response

<u>Effect of Earthworm Inoculation on Plant Growth</u>

Earthworm-induced changes to soil structure and fertility, as discussed above, have been observed to enhance leaf and fruit production, root:shoot ratio, and stem and height growth (Derouard et al. 1997; Gutiérrez-Miceli et al. 2007; Hidalgo et al. 2006; Scheu 2003). In a potting substrate study, earthworm castings alone had greater nutrient content (especially N) and the highest pH and EC compared to soils without a casting amendment (Hidalgo et al. 2006). It was found that the castings increased stem diameter, root growth, dry weight, and flower number

in potted marigolds grown in a greenhouse. Rounder castings produced by larger earthworms were also found to improve plant growth while casts produced by smaller earthworms were thought to disaggregate the soil too much, providing limit benefit for plant growth (Derouard et al. 1997). This study also suggested that using multiple earthworm functional groups at once should produce better plant growth results.

Gutierrez-Miceli et al. (2007) looked at the effects of vermicomposting on tomato plants in a greenhouse. The researchers grew tomato seedlings in pots with various ratios of castings to soil medium. Ratios of the vermicomposted material increased plant heights and stem diameters and improved fruit quality and potential juice production more than the control, which was a generic soil mixture without vermicompost, and lower ratios of vermicompost to soil mix.

In studies summarized by Baker et al. (2006), researchers also found that pasture root growth improved as a result of increased water infiltration and aeration, root contents were higher in inoculation sites than in control sites due to increased carbohydrate levels in organic matter content, and that the increased number of larger pores as a result of earthworm activities enhanced tree seedling growth. However, other researchers found positive and negative yields in the same study with no clear soil effects on plant growth.

<u>Response of Differing Plant Species to Earthworm Inoculation</u>

Plant species can differ in their response to earthworm inoculation (Eriksen-Hamel and Whalen 2007; Pashanasi et al. 1992). Pashanasi et al. (1992) grew three tree species (*Bixa orellana*, *Eugenia stipitata*, and *Bactris gasipae*) in pots filled with potting media composed of three parts soil: one part sawdust and inoculated with four concentrations of earthworms. Each tree species responded differently to the treatments; two of the species (*Bixa* and *Eugenia*) experienced significant increases in biomass accumulation in reaction to worm inoculation while the third species (*Bactris*) actually showed lower biomass accumulation compared to the control treatment over time. Apparently, the rooting systems and abundance of roots were related to these responses: the tree showing the greatest growth increase (*Bixa*) had long and fine roots while the tree with the poorest growth (*Bactris*) had thicker, shorter roots. These physiological differences affected where the roots found nutrients and the latter tree could not take advantage of the improved conditions due to its limited ability to explore the soil.

Another study involving soybean and maize agroecosystems also observed a relationship between rooting structure and ability to benefit from improved soil conditions (Eriksen-Hamel and Whalen 2007). Here the researchers found that soybeans, which have finer roots mostly in the top 0-10 cm of soil, showed a positive growth relationship when earthworms were present compared to maize, which has a deeper rooting system. This is most likely due to the fact that an endogeic species was used for inoculation, which would impact the top 0-10 cm of soil, precisely where the soybeans roots resided. The deeper roots of the maize would grow past this improved zone, limiting this plant's ability to profit from improved conditions.

Baker et al. (2006) reviewed a study reporting increased wheat and soybean grain yields and black oat dry mass, which were attributed to earthworm castings that increased water infiltration and plant nutrient availability. However, another study found no difference in soil properties as a result of earthworm inoculation and, therefore, no differences between wheat and black bean yields.

2.3.5   Earthworm Limitations and Response to Adverse Conditions

As previously discussed, urban soils are characterized by severe compaction, restricted aeration, modified pH, and soil pollution and contamination (Scheyer and Hipple 2005) and are managed in various ways (irrigation and fertilization) depending on the land owner. All of these factors can affect earthworm population dynamics and limit the earthworm's ability to function and survive in an urban environment, which may have implications for landscape trees and other vegetation.

Effect of Water Supply on Earthworms

Water is the most limiting requirement for earthworm survival (Lee 1985). Earthworms depend on appropriate water levels and have no anatomical adaptations to cope with water loss. Most of the water they acquire is consumed with their food although they do drink water stored in soil pores. They also respire through their skin, which must be kept moist, and acquire oxygen from the air or from oxygenated water in the soil. Suitable soil moisture is species-specific, but

in general, they will survive in soils that are between permanent plant wilting point and near field capacity (Lee 1985).

Effect of Soil Physical Properties on Earthworms

Physical soil limitations arise from compaction, varying soil texture, soil temperature, and light exposure. Highly compacted soils will limit movement and ultimately survival if earthworms are not mobile enough to reach their food and water sources. Specific limits in bulk density and porosity have received little attention in the literature. Stovold et al. (2004), however, studied the effects of compaction on earthworms and concluded that earthworms prefer to burrow in looser soil (packed using 96 kPa of pressure) and tend to avoid more compacted soil (386 kPa), and can burrow at a faster rate in looser soil compared to more compacted soil. Additionally, earthworms exhibited behavior such that they were referred to as crevice burrowers, preferring to use cracks in the soil rather than create their own burrows.

Earthworm skin is very thin and permeable and, therefore, soil texture has a strong influence on species composition and population abundance. Coarsely textured soils (high sand content) contain larger, more abrasive particles, which are damaging to earthworm skin, and tend to be excessively well-drained. Soils with high clay content can retain too much water leading to anaerobic conditions. Klok et al. (2007) found that as the amount of clay increased, individual earthworm biomass decreased and overall population growth slowly decreased indicating that these soils inhibited earthworm fitness.

Soil temperature affects earthworm metabolic rates and available soil water. Most earthworms in Europe have been reported to survive in optimum soil temperatures between 15°C and 20°C. *Lumbricus rubellus* (an endogeic species) prefer temperatures ranging between 15°C and 20°C and *Eisenia fetida* (an epigeic species) prefer 25°C (Lee 1985).

Earthworms are highly sensitive to light and respond with a "withdrawal reflex" where they immediately retract any portion of their bodies exposed to light. The ultraviolet light intensity of sunlight is lethal to earthworms; however, depending on the degree of pigmentation of some species, they can withstand limited sunlight exposure (Edwards and Bohlen 1996; Lee 1985).

Effect of Soil Chemical Properties on Earthworms

Earthworms are sensitive to soil C:N ratio, pH, and certain applied chemicals. The majority of the earthworm's diet consists of dead plant material, especially that found in the litter layer, which provides a source of organic carbon and nitrogen. Suitable C:N ratios are species-specific and tend to limit populations(Edwards and Bohlen 1996; Lee 1985). For example, in studies reported by Edwards and Bohlen (1996) and Lee (1985), *L. rubellus* seems to prefer a C:N ratio between 8.5:1 and 21:1 and *E. fetida* prefers C:N ratios above 13:1. C:N ratios are highly influenced by seasonal litter layer removal, a common practice in urban areas that reduces the litter deposited by deciduous tree species, which have higher C:N ratios and are more palatable for detritivores.

Highly acidic soil limits earthworm populations usually becoming lethal below a pH of 4.0. Klok et al. (2007) found that earthworm survival was limited in soils below a pH of 3.4, but acidic limits varied by species.

Earthworms are sensitive to certain chemicals and pesticides commonly used on lawns and turf as well as high amounts of ammonium nitrate and salts, most likely originating from winter road salting. Populations can be reduced using the insecticides Carbaryl (1-naphthyl methylcarbamate) and Diazinon (O,O-diethyl-O-(2-isopropyl-6-methyl-pyrimidine-4-yl)phosphorothioate), and, to a lesser extent, the herbicide 2,4-D (2,4-Dichlorophenoxyacetic acid) (Card et al. 2002) and fungicides, such as the carbendazim-based benomyl, can be toxic to earthworms (Edwards and Lofty 1980).

Earthworm Response to Adverse Soil Conditions

To cope with adverse conditions in their environment, earthworms exhibit several behaviors that increase their survivorship. When the soil surface becomes too dry or cold, they migrate to deeper layers. If avoiding the situation is not possible, they may become inactive until conditions improve. In response to low soil temperatures and periods of drought, earthworms will enter a state of hibernation referred to as aestivation (Edwards and Bohlen 1996; Lee 1985). Finally, because egg cocoons can tolerate desiccation and extreme temperatures better than the worms, cocoons are deposited at the onset of adverse conditions, remaining in the soil to hatch when conditions improve, thereby increasing reproductive capabilities (Edwards and Bohlen 1996).

Urban Soil Limitations on Earthworm Growth and Survival

Adverse soil conditions for earthworm survival are common in urban areas. As described above, urban soils tend to be compacted with varying textures and parent materials, are often highly alkaline, and have poor aggregation. In addition, tree canopies are frequently not connected enough to protect surface dwelling species from severe light exposure, especially in parking lot islands and street tree cutouts. Islands and cutouts also tend to be severely compacted in the bottom and isolated from larger bodies of soil, possibly limiting earthworm migration. In addition, earthworms have been known to ingest and retain heavy metals and other pollutants, which results in the redistribution of these elements and chemicals within the ecosystem (Edwards and Bohlen 1996; Kennette et al. 2002; Lee 1985; Lukkari et al. 2004; Pizl and Josens 1995). Finally, these systems tend to have variable amounts of organic matter and leaf litter because people seasonally remove leaves and other yard debris (Scheyer and Hipple 2005), ultimately removing the earthworms' food source.

Impediments to Earthworm Inoculation in Urban Soil

For earthworm inoculation to be an effective environmental management technique, the above mentioned limitations should be mitigated prior to inoculation. First, it is recommended to till the soil and ensure adequate moisture levels as well as to protect the soil surface from sunlight and predators shortly after inoculation (Eriksen-Hamel and Whalen 2007). In addition, soil pH should be modified relatively close to neutral and sufficient amounts of palatable organic

material should be provided. Finally, it is important to prevent or limit pesticide use near inoculation sites.

Edwards and Bohlen (1996) make the following suggestions to ensure the survival of introduced earthworms for the purpose of environmental management:

- Introduce the most suitable species of earthworm when ameliorating the soil.
- Identify the soil's population limit. An overabundance of earthworms will not produce positive effects on soil health unless there are enough resources for survival.
- Add casts to improve soil structure and fertility. Casts have a higher pH and can contribute to stronger aggregates.
- Add lime to acid soils before inoculation. This favors population growth in areas with these conditions.

2.4   Earthworms in Natural and Disturbed Systems

2.4.1   Earthworms in Forested Ecosystems

The majority of literature available discussing earthworms in the forested setting relate to exotic earthworm invasions, almost all of which were researched in the northern states of the U.S. (e.g. Minnesota, Wisconsin, New York) where glaciation eradicated native earthworm populations and exotic invasions are drastically affecting the natural forest floor. Some studies have been directed at determining the natural ecology of earthworms in forested ecosystems,

most of which were conducted outside of the U.S. (Cesarz et al. 2007; Räty and Huhta 2004; Szlávecz and Csuzdi 2007).

Earthworm Ecology in Forested Ecosystems

In the forest, earthworm populations are driven by both biotic soil properties (presence of other species, diversity of the forest, and quantity and quality of litter) (Cesarz et al. 2007; Räty and Huhta 2004) and abiotic soil properties (moisture, pH, texture) (Cesarz et al. 2007; Räty and Huhta 2004), as well as human activities (accidental transport and previous landuse) (Räty and Huhta 2004; Szlávecz and Csuzdi 2007).

Cesarz et al. (2007) compared earthworm communities in relation to tree diversity in a deciduous forest in Germany. They determined that differences in litter quality and quantity played a stronger role in earthworm density than small differences in soil moisture content. Additionally, higher earthworm densities were found in the most diverse stands in May but by February, these stands contained the lowest density. The authors attributed overall diversity to the fact that a diversity of tree species created a diversity of microhabitats and the seasonal decrease in diversity to the seasonal decrease in litter amount.

Räty and Huhta (2004) compared earthworm communities of anthropogenous birch stands of different origins (birch after spruce, birch after field) with those of natural deciduous forests in Finland. They found that earthworm populations were driven more by previous land

use than anticipated. Earthworms were able to survive and reproduce in birch stands established after field use because they had invaded during cultivation. Furthermore, it was determined that afforestation didn't seem to affect earthworm populations as much as previous land use, a conclusion drawn from the fact that cultivated soils preceding the birch stands tended to have smaller particle sizes (more clayey) and a higher pH than the soils originally occupied by spruce.

Similarly, Szlavecz and Csuzdi (2007) compared earthworm species composition and abundance in physically adjacent forest stands to various stages of old field succession in eastern Maryland, USA. There was a distinct earthworm abundance gradient, with successional forests showing higher abundance, mature forests showing lower abundance, and clearcuts possessing no population, and there was a decrease in the proportion of anecic earthworms accompanied by an increase in epigeic species in mature stands. The observed patterns in abundance seemed to be driven by a combination of land use change and a shift in plant composition over time. They concluded that as forests enter into later stages of succession, they can no longer support a large population of litter feeding earthworms.

Effects of the Invasion of Earthworms to Forested Ecosystems

In the U.S., earthworm invasion is a major issue in northern temperate forests and affected natural resource agencies have devoted much research effort to determining how exotic earthworms have changed these forests. The Minnesota Department of Natural Resources has produced an ecological risk assessment of exotic earthworms (Proulx 2003) in which the author

describes the issues surrounding exotic earthworms, the threats they pose, and management

recommendations.

The issue of exotic earthworms in the temperate north inspired an entire special issue in

the journal *Ecosystems* in which five papers were published about a large research project

conducted in New York (Bohlen et al. 2004a; Bohlen et al. 2004b; Fisk et al. 2004; Groffman et

al. 2004; Suárez et al. 2004). Results from these papers are presented in Table 2.2.

Bohlen et al. (2004c) found that earthworms alter nutrient storage and availability, affect

populations and communities of other flora and fauna, and alter the soil food web, which

includes microbes, fungi, other invertebrates, trees, and plants. Burtelow et al. (1998) observed

higher soil pH and microbial biomass in areas with exotic worm populations. Additional studies

Table 2. 2. Summary of five studies describing the effects of earthworm invasion on various soil and plant properties in New York state.

Author	Soil Properties Studied	Effects of Earthworm Invasion
(Bohlen et al. 2004a)	Entire ecosystem	Physical effects (increased soil porosity, altered soil hydrology, soil aggregate stability) Geochemical effects (redistribution of soil, altered nutrient availability) Biological effects (consumption of detritus and forest floor)
(Fisk et al. 2004)	Soil respiration Root distribution Litter fall flux	No influence on soil respiration Earthworms do not seem to be affecting net C emission from soil Fine root mass in top 12 cm of mineral soil and forest floor was higher in non-worm plots Earthworms altered the vertical distribution of fine roots in the surface 12 cm
(Bohlen et al. 2004b)	Soil carbon and nitrogen	Removed forest floor thereby decreasing soil carbon storage and reducing C:N ratios
(Groffman et al. 2004)	Microbial biomass	Reduced pools of microbial biomass in the forest floor and increased pools in mineral soil Increase in respiration in mineral soil
(Suárez et al. 2004)	Soil phosphorus cycling	Increased amounts of unavailable inorganic P Increased amounts of readily exchangeable P, increasing its loss in leaching water

have related exotic earthworm invasion to the near extermination of the rare fern species, *Botrychium mormo*, which requires a thick litter layer to survive (Gundale 2002).

It has been suggested that earthworm populations will likely increase with increased human activity and global climate change (Bohlen et al. 2004c). This idea is supported by the work of Kalisz and Dotson (1989) in which land use history in the mountains of eastern Kentucky was studied in relation to the occurrence of exotic earthworms. They concluded that native populations of earthworms were dominant on tracts of land that had not been disturbed by human practices (e.g. timber harvesting and construction of roads and other infrastructure) and that exotic earthworms were only present in areas that had been disturbed.

2.4.2   Earthworms in Cultivated Systems

Earthworms are commonly studied in agricultural settings due to the numerous positive impacts they have on soil quality. Many researchers have focused on studying the relationship between earthworm communities and land use type and management intensity (Didden 2001; Johnson-Maynard et al. 2007; Smith et al. 2008; Topoliantz et al. 2000), while others have focused on the effects that earthworm inoculation have on soil properties and plant growth (Baker et al. 2006; Desjardins et al. 2003; Edwards and Lofty 1980; Eriksen-Hamel and Whalen 2007; Schindler Wessells et al. 1997; Shuster et al. 2001).

Earthworm Ecology in Agricultural Systems

Land use type and land use intensity are strong drivers of earthworm abundance and community composition in agricultural systems. In the Netherlands, populations seemed to be more robust (higher densities, higher biomass, and higher species richness) in grasslands when compared to horticultural farms (Didden 2001). This difference was attributed to the physical and chemical makeup of the soil and to the intensity of various management practices employed, such as tilling, crop harvesting, and crop protection measures, which break up soil aggregates, result in less soil organic matter with lower quality, or contaminate the soil with pesticides.

Management intensity has an effect on earthworm populations, often reducing numbers due to tillage, fertilizing, harvesting, and crop protection (herbicide) practices (Didden 2001; Smith et al. 2008). Smith et al. (2008) researched diversity and abundance across a land use intensity gradient and found that population abundance seemed to follow land use intensity. The more intense the practice (high fertilizer input and intensively managed row-crop agriculture to old-field and old-growth forest ecosystems), the lower the worm population measured. This is supported by the work of Johnson-Maynard et al. (2007), who found that earthworm density was higher in non-till plots than in tilled plots. Conversely, in examining the influence of different tillage and chemical input practices, Topoliantz et al. (2000) found that earthworm densities were lowest under reduced tillage and highest under deep plowing. They attributed this counter-intuitive result to the use of disking in reduced tilling, which was more disruptive to the soil structure than the relatively gentle turning over associated with their deep plowing.

Inoculation and Use of Earthworms in Agricultural Systems

In addition to studies related to how earthworms function under various land use
practices and types, much research has been invested into understanding how earthworm
population manipulation (inoculation and reduction) can be used to enhance agricultural soil and,
therefore, improve crop growth and production (Desjardins et al. 2003; Edwards and Lofty 1980;
Schindler Wessells et al. 1997; Shuster et al. 2001).

Desjardins et al. (2003) evaluated the impact of epigeic earthworms on soil organic
matter dynamics and found that inoculation decreased the carbon levels in the topsoil at a higher
rate than without inoculation. Conversely, Shuster et al. (2001) discovered that inoculation with
deep-burrowing, anecic worms increased average soil organic carbon and changed its spatial
distribution from uniform to patchy. Edwards and Lofty (1980) also studied anecic species and
found that straw debris from previous harvests was incorporated into the topsoil in plots
inoculated with earthworms. Their results showed that inoculation improved barley production
with increased populations and weight, deeper roots, and taller height and larger amount of
foliage. They attributed these differences to the plant available nutrients that line earthworm
burrows. The influence of different earthworm population densities on soil respiration was
investigated by Schindler Wessells et al. (1997). They found that the inoculation of earthworms
did influence respiration rates seasonally and that the addition of organic nutrients yielded higher
respiration rates. However, it was determined that respiration was either increased by the
earthworms themselves or from an increase in microbial respiration stimulated by earthworm
activity.

2.5   Earthworms in Urban Systems

There is a clear lack of attention in the literature to earthworm populations and community dynamics in the urban setting, as well as the use of earthworms to remediate urban soils. Urban areas are growing at a high rate, replacing forested and agricultural lands. As discussed earlier, urban soil function is often disrupted, requiring remediation to restore health and sustain vegetation. Due to the extensive study of earthworms in natural and agricultural settings and the widespread use of earthworms in agricultural soils, it seems plausible that the same treatments could be applied to the urban environment.

2.5.1   Earthworm Ecology in Urban Settings

Urban earthworm studies either compare earthworm populations along a gradient of urban to rural soils (Steinberg et al. 1997; Szlavecz et al. 2006) or along a gradient of urbanization within the urban area (Pizl and Josens 1995; Smetak et al. 2007). The gradient of urbanization has been determined through various methods: distance from city center (Pizl and Josens 1995; Steinberg et al. 1997), distance and type of development surrounding the stands (Szlavecz et al. 2006), or site age (Smetak et al. 2007). Steinberg et al. (1997) and Szlavecz et al. (2006) both found a higher abundance of earthworms and biomass at sites ranked as the most urban along the gradient. Steinberg et al. speculated that earthworm density was higher in urban areas because the exotic species found most likely originated from New York City's seaports.

Conversely, Pizl and Josens (1995) and Smetak et al. (2007) found the opposite pattern, extracting more earthworms and higher earthworm biomass from sites farther from the city center or considered on the more rural end of the urban gradient. This result parallels the conclusion drawn by Harris (1991) that urban soils are associated with a reduced number of organisms and biomass as well as a reduction in species diversity.

Upon further investigation into related soil properties, Pizl and Josens (1995) found higher bulk density, higher pH, higher concentrations of heavy metals, and higher amounts of organic matter in the urban core than the farthest sample site. Although, harsher soil conditions were present closer to the city center, there seemed to be no detrimental effect of urbanization on earthworm species richness or community structure. Similarly, Smetak et al. (2007) measured higher bulk density, lower soil moisture, and lower amounts of C and N in sites considered to be the more urban and disturbed, also showing the harsh conditions present at these sites. Soils that have had the highest amount of disturbance, such as urban soils, usually have a poorly developed catalogue of soil biota (Harris 1991).

2.5.2   *Use of Earthworms in the Urban Setting*

Although earthworm biology in the urban environment has received some attention, the application of earthworm technology for use in urban soils has been relatively ignored. In the urban setting, earthworms have been used in a bioassay approach for monitoring the status of soil ecosystems at potentially contaminated urban sites (Hankard et al. 2005) or in waste and

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environmental management (Edwards and Bater 1992; Edwards and Bohlen 1996; Lee 1985).

Related research has been conducted using earthworms on such sites as reclaimed mines and

former industrial lands. These experiments have seen relative inoculation success and soil

rehabilitation, much like similar experiments conducted in cultivated systems (Baker et al. 2006;

Curry 1988; Haimi 2000; Pospiech and Skalski 2006). In addition to the chemical, physical, and

biological responses seen, earthworms have also shown an ability to remove and relocate the

heavy metals associated with industrial and mining pollution (Zorn et al. 2005).

2.6   Earthworm Sampling and Inoculation Methods

2.6.1   *Earthworm Sampling*

Earthworms have been collected and studied since the time of Darwin. The most effective

method for sampling populations is a combination of physical and chemical methods,

specifically hand-sorting and application of a chemical repellant (Edwards 1991; Edwards and

Bohlen 1996; Lee 1985). Until recently, no standardized earthworm sampling methodology had

been developed and, as a result, many techniques have been used. The high variation between

studies has caused many researchers to wonder what constitutes the most appropriate method for

earthworm population assessment.

Physical Earthworm Extraction

Hand-sorting is the process by which a known volume of soil is separated from the landscape and carefully sorted to capture adult and juvenile worms as well as eggs and worms in the diapause state. It is laborious and time consuming, but accurate for sampling endogeic and epigeic species (Edwards 1991; Edwards and Bohlen 1996; Lee 1985). Samples need to be handled carefully when hand-sorting due to the risk of cutting earthworms with the soil sampling tool. (Jiménez et al. 2006). Hand-sorting was found to be a superior method for sampling during times of the year when there are large amounts of smaller, juvenile worms (Callaham and Hendrix 1997).

Chemical Earthworm Extraction

Chemical extraction involves the application of a chemical repellant that irritates the earthworm's skin causing individuals to come to the soil surface. In this way, earthworms are easily collected and the soil structure is not compromised. This method is ideal for collecting anecic or deep-burrowing earthworms (Callaham and Hendrix 1997), but its effectiveness depends on high activity rates, which is dictated by seasonal weather variation (Edwards 1991). In addition, some chemical repellants are extremely toxic and their use is not permitted in fragile soils or soils where other soil inhabitants need to be conserved. Formaldehyde (Raw 1959) is the most commonly used repellant and is recommended in ISO 23611-1 - the only known

standardized earthworm collection methodology (Rombke et al. 2006), but it is a known carcinogen.

Recently, mustard solutions (Gundale 2002; Heneghan et al. 2007) have been used for circumstances that require minimal disruption of the soil structure and are restricted to non-toxic extraction. Mustard extraction depends on the glucosinolate breakdown product, allyl isothiacyanate, which produces the same irritated reaction earthworms have to formalin solutions. Extraction using mustard solution is comparable to formalin extraction and has been known to yield similar abundances, biomass, and functional groups (Zaborski 2003). However, Bartlett et al. (2006) cautioned using mustard extraction as the sole sampling method due to a bias towards capturing large, sexually mature anecic worms.

Other Earthworm Extraction Techniques

Other methods are either expensive or time consuming, which have made their use uncommon. The electric-octet method involves inserting electrodes into the earth at fixed distances and shocking the soil. This method is effective at targeting earthworm species while limiting disruption of soil microbe and arthropod communities as well as impacts on plants (Coja et al. 2007). However, the apparatus is expensive to construct and this method is extremely sensitive to soil moisture. Funnels and flotation are additional methods but are time consuming and ineffective when large amounts of organic matter are present (Edwards 1991).

<u>Earthworm Extraction Comparisons</u>

Several studies have been conducted that compare these various methods to one another to determine which are the most appropriate and effective (Callaham and Hendrix 1997; Chan and Munro 2001; Edwards 1991; Högger 1993; Lawrence and Bowers 2002). Edwards (1991) summarized extraction techniques to assess soil invertebrate populations which included hand-sorting and formalin extraction. His conclusion was that choosing the appropriate method depended on the invertebrate being measured and the time and goals of the project. Techniques such as flotation and funnel methods are not appropriate to sample earthworms and hand-sorting and chemical extraction are preferred.

Callaham and Hendrix (1997) compared hand-sorting and formalin extraction for sampling in the southern Appalachian Piedmont. They concluded that formalin was not as effective at estimating populations from the months of November to January because of the high number of juveniles present in the soil during that time. Other than that limitation, formalin was just as reliable as hand-sorting and even more effective at collecting anecic species. Regressions were developed describing the significant, positive relationship between hand-sorting and formalin for various species found.

In a comparison among five sampling methods, Coja et al. (2007) found that hand-sorting yielded more earthworms than chemical extraction and the electric octet methods, but also cautioned that this method required destroying the intact soil profile and needed to be done in a timely fashion to ensure live earthworm identification.

A study performed in Clarke County, VA compared "hot" mustard extraction and hand-sorting (Lawrence and Bowers 2002). They found that using mustard provided a "good and consistent index" of earthworm populations. Like other researchers, Lawrence and Bowers suggest that chemical extraction is more efficient for sampling large, anecic worms, but in this study, the difference between mustard and hand-sorting was not significant. Most importantly, this study found that mustard was effective at assessing populations across land use types, which supports its use in the current investigation.

Chemical extraction alone seems to be just as effective for assessing earthworm populations as hand-sorting, and several studies have compared various chemical extraction techniques. Högger (1993) compared the use of mustard flour to that of formalin and concluded that no significant difference emerged between a 0.33% mustard flour solution and a 0.25% formalin solution. It is suggested that different mustard pastes can give different extraction efficiencies, which indicates that mustard extraction has not been standardized.

Chan and Munro (2001) also evaluated the use of formalin versus mustard, and the efficacy of mustard for collecting an anecic species versus an endogeic species. Their results indicated that mustard extraction was more effective than formalin for the anecic worms and that hand-sorting underestimated the populations because worms were able to escape to deeper layers before the soil was pulled out of the ground. To create the most optimal mustard concentration, the researchers shook 106 g of dry mustard powder in 1 l of 5% acetic acid overnight and then diluted that extract in water with a ratio of 15 ml to 1 l. The endogeic sampling produced contrary results because these worms seemed to be adversely affected by the mustard and stayed

within the top 10 cm of soil. Overall, the researchers concluded that mustard extraction was a more efficient and environmentally friendly chemical to use for earthworm population assessment than formalin.

Other researchers have chosen to combine hand-sorting with chemical extraction to better capture the various functional groups. Cesarz et al. (2007) hand-sorted soil from a pit 0.11 m^2, 20 cm deep, and then used a 0.33% mustard solution to extract anecic worms from the dug hole. In Finland, Raty and Huhta (2004) chose to use modified wet funnels and then formalin extraction to extract the deeper burrowing species. Finally, the ISO has proposed using a combined hand-sorting/chemical extraction technique as the standardized method of collecting earthworm samples for population analysis (Rombke et al. 2006) (Figure 2.3).

2.6.2   *Earthworm Inoculation*

Inoculation techniques have been developed for soil amelioration and land reclamation, including the earthworm inoculation unit (EIU) (Butt et al. 1995; Butt et al. 1997), transport of worm-rich topsoil (Butt et al. 1995; Nuutinen et al. 2006), and broad surface scattering often used to study the effects of earthworm activities on agricultural fields (Blair et al. 1997; Bohlen et al. 1995; Butt et al. 1995).

Butt et al. (1997) developed the earthworm inoculation unit (EIU) for use in land reclamation citing the importance of using a more controlled inoculation population (appropriate species, good physical health, and good physiological condition) to ensure successful

establishment and survival. This technique has been used successfully in England with the addition protective netting to prevent avian predation. Nuutinen et al. (2006) found unsuccessful results using the EIU, but attribute this to harsh soil conditions that were not conducive to earthworm survival.

Transporting worm-rich top soil and turf plugs has also been used as an inoculation technique (Butt et al. 1995; Nuutinen et al. 2006). It is appropriate to use when inoculating a smaller, surface or litter-dwelling earthworm species and has been known to be successful because of the transportation of a protective environment and the transportation of all earthworm life stages (Butt et al. 1995).

Broadcast scattering of earthworms is commonly used for application in cultivated fields (Blair et al. 1997; Bohlen et al. 1995), and takes advantage of larger, deep-burrowing worms. It usually entails acquiring live adult earthworms from a bulk producer of worms, such as a bait wholesaler. Earthworms are scattered across the surface of the soil and allowed to burrow on their own (Nuutinen et al. 2006) and are often covered with soil (Eriksen-Hamel and Whalen 2007). Screening or topdressing with hay or mulch can be used to protect the earthworms from sunlight exposure and predators (Blair et al. 1997; Bohlen et al. 1995).

Chapter 3: Soil Characteristics and Earthworm Population Dynamics in Roanoke, VA: An Observational Study

3.0   Introduction

An observational study was conducted in Roanoke, VA to assess earthworm population trends across three urban land use types and two soil disturbance time frames within the urban forest and to assess soil habitat characteristics that help explain population trends. Of major interest for this study was to characterize earthworm abundance in urban landscapes to provide insight on the merits of earthworm inoculation to improve soil health: are earthworm populations low enough that it might reasonably be feasible to increase them through inoculation? Earthworms were sampled at 36 sites throughout the city in the spring and fall of 2008 to determine earthworm abundance, biomass, and diversity, and the effect that season had on each.

3.1   Materials and Methods

3.1.1   Study Area Description

The observational study was conducted in Roanoke, Virginia, which is Virginia's fourth largest metropolitan area and is considered the cultural and commercial center of the southwestern region of the state. It covers approximately 111 km^2, with a population density of 8.5 people ha^{-1} (U.S. Census Bureau 2000). Roanoke has a temperate climate with average daily

temperatures ranging from 7.4°C to 19.3°C and an average yearly precipitation of 102.3 cm. The growing season is usually 171 days, given temperatures above freezing (Ealy 1997).

Physiography, Geology, and Native Soils

Roanoke is located within two major land resource areas: the Blue Ridge physiographic province (35%) to the east and the Southern Appalachian Ridges and Valley physiographic province (65%) to the west (Ealy 1997). The study sites in this city were located across five of the eleven general soil map units recorded for the area. Frederick-Urban land-Chilhowie soils originate from limestone and calcareous shale, are gently sloping to very steep, well drained, and have a clayey subsoil. They are mainly used for urban development such as parking lots, buildings, roads, and other impervious surfaces. The Chiswell-Litz-Urban land unit originates from shale, siltstone, sandstone, and interbedded limestone, has gently sloping to very steep terrain, is well drained, and has a loamy subsoil. Woodland or urban development are often found in this unit. In the Blue Ridge, Hayesville-Evard-Urban land soils are made up of gneiss, granite, schist, sandstone, quartzite, and phyllite. They are gently sloping to very steep, well drained, and have clayey and loamy subsoils. Like the Chiswell-Litz-Urban land soils, this unit is primarily woodland or urban development. The Edgemont unit is also composed of gneiss, granite, schist, sandstone, quartzite, and phyllite and are strongly sloping to very steep, well drained, and have loamy subsoil. Due to the limiting slope, these soils are mainly used for woodland and sites for homes. Finally, the Speedwell-Urban land-Derroc soil unit originates

from alluvial material, is nearly level and well drained, and has a loamy subsoil. It is used for pasture, hay, urban development, and some cultivated crops (Ealy 1997).

Land Use and Native and Urban Ecology

Historically, farming was the main land use type in the Roanoke Valley. During the mid-1970's, urban expansion began replacing this land with housing developments, industry, and shopping centers. Major industrial activities include railroad, trucking, and furniture, rubber goods, construction equipment, and small electrical appliance manufacture (Ealy 1997). For the purpose of this study, the city was categorized as forested, residential, or commercial.

As of 2002, the City of Roanoke exhibited a 32% canopy cover; more specifically, commercial areas in the city had 16% canopy cover and residential had 39% (American Forests 2002). Observed urban trees in the City of Roanoke include, but are not limited to, *Acer* x*freemanii*, *Acer rubrum*, *Acer saccharum*, *Amelanchier arborea*, *Betula nigra*, *Cercidiphyllum japonicum*, *Cercis Canadensis*, *Chamaecyparis thyoides*, *Cornus florida*, *Ginkgo biloba*, *Gleditsia triacanthos*, *Lagerstroemia indica*, *Liriodendron tulipifera*, *Nyssa sylvatica*, *Quercus alba*, *Pinus strobus*, *Platanus occidentalis*, *Prunus* spp, *Pyrus* spp, *Robinia pseudoacacia*, *Thuja* spp.*Tilia cordata*, *Ulmus Americana*, *Ulmus pumila*, and *Zelkova serrata*.

3.1.2   Landscape Classification and Study Sites

All study sites were located either on Roanoke City or Roanoke School District property and were chosen to reflect a range of urban land uses and timeframes since soil disturbance. More specifically, we investigated three land use types (naturalized areas, residential areas, and commercial areas) in combination with two soil disturbance timeframes (less than 10 years ago and greater than 10 years ago), resulting in six landscape classifications. These classifications were termed Forested–Old (FO), Forested–New (FN), Residential–Old (RO), Residential–New (RN), Commercial–Old (CO), and Commercial–New (CN).

Study sites were chosen through field reconnaissance and consultation with the Roanoke City urban forester. The unifying criterion across landscape classes was that substantial tree cover had to be present at each study site. Once study sites were identified, sampling plots were established within the root zones of randomly selected trees. No preference was given to species or age of the tree, but the tree had to be representative of the prevailing tree cover composition for that landscape class. Below are a general description of each landscape classification and a listing of the Roanoke City facilities where study sites were established for each class.

Forested–Old (FO)

These sites were located within relatively undisturbed greenways and forest fragments. The selected trees had not experienced substantial soil disruption within their root zones in the past decade. Groundcover typically consisted of tree litter and unmanaged herbaceous vegetation.

Lick Run Greenway (I)

Lick Run Greenway (II)

Mill Mountain Greenway

Murray Run Greenway

Smith Park

Tinker Creek Greenway

Forested–New (FN)

These sites were located in outlying areas of recreational parks where vegetation management had been largely abandoned and the adjacent forest was encroaching on maintained areas. The selected trees had experienced soil disruption near the roots within the past decade. Groundcover typically consisted of tree litter and irregularly maintained herbaceous vegetation.

Booker T. Washington Park

Fallon Park

Highland Park

Kennedy Park

Maher Fields

Wasena Park

Residential–Old (RO)

These sites were located in residential neighborhoods where there had been limited soil
disturbance within the root zones of established trees in the past decade. The selected trees were
either located within narrow planting strips between the curb and sidewalk or in lawns adjacent
to the sidewalk. Groundcover typically consisted of mulch around the tree trunk surrounded by
turf that was mowed on a regular basis.

Hamilton Terrace

Lafayette Boulevard

Lakewood Park

Linwood Road

Montrose Avenue

Terrace Road

<u>Residential–New (RN)</u>

These sites were primarily located in parks in residential neighborhoods where the landscape had been substantially modified in the past decade. In most instances, sampling was conducted within the root zone (but not in the original root ball) of a recently transplanted landscape tree. The selected trees were typically located within a narrow planting strip, in a parking lot, or in an adjacent lawn. Groundcover typically consisted of mulch around the tree trunk surrounded by turf that was mowed on a regular basis.

Eureka Park

Fallon Park

Fishburn Park

Maher Fields

Robertson–Brown Park

Wasena Park

<u>Commercial–Old (CO)</u>

These sites were primarily located at municipal buildings and public school campuses. The selected trees had not experienced substantial soil disruption within their root zones in the past decade. Trees were typically located within parking lot islands or parking lot border plantings.

Groundcover typically consisted of mulch in parking lot islands and occasionally turf within the border plantings.

Fishburn Park Elementary School

Grandin Village Center

Hotel Roanoke

Roanoke City Jail

Roanoke Civic Center

Virginia Western Community College

Commercial–New (CN)

These sites were primarily located at municipal buildings and public school campuses where the landscape had been substantially modified in the past decade. In most instances, sampling was conducted within the root zone (but not in the original root ball) of a recently transplanted landscape tree. The selected trees were typically located within parking lot islands or parking lot border plantings. Groundcover consisted of mulch only.

Crystal Spring Village

Jefferson Center

Patrick Henry High School

Roanoke Civic Center

Roanoke Higher Education Center

Roanoke Valley Visitor's Center

3.1.3 Statistical Design

The observational study was conducted using a randomized complete block statistical design. The experimental unit was a study site[3] characterized as one of the six landscape classes described above. Each landscape class was replicated across six blocks[4] for a total of 36 experimental units. Within each experimental unit, there were three observational units, which were the root zones of individual landscape trees. In total, there were 108 sampling plots in the observational study (6 blocks × 6 landscape classes × 3 subsamples). Blocking was used to control experimental error due to geographical location and time of sampling.

3.1.4 Earthworm and Soil Sampling Procedures

To assess seasonal variation in earthworm abundance earthworm sampling was conducted at two seasonal intervals in 2008 – spring (May 1–15) and fall (October 10–24).

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[3] Usually a neighborhood block, city park, or single parking lot.
[4] One block contained each of the six landscape classes, all of which were close enough to each other to be sampled in one single day.

Within each experimental unit, three suitable landscape trees were selected, and a soil pit (25 cm wide × 25 cm wide × 10 cm deep) was excavated in the root zone of each tree. Earthworms were then collected from the pits using a combination of physical and chemical extraction methods (Cesarz et al. 2007). Physical methods are preferred for sampling epigeic and endogeic species (Edwards 1991, 1998; Edwards and Bohlen 1996) whereas chemical methods are effective for deep burrowing anecic species (Edwards 1991).

First, soil excavated from each pit was spread on a tarp and earthworms were handpicked from the soil (Smetak et al. 2007). Next, a solution of 50 g hot mustard powder (Spice World, Glen Head, NY) combined with 3.78 L of water was poured evenly over the bottom of the excavated soil pit (1.89 L per pit). Earthworms that emerged within the time it took for the solution to percolate were collected (20 minutes maximum if soil was poorly drained), rinsed with water, and placed in plastic containers with damp potting soil. Plastic containers with both physically and chemically extracted worms were then placed in a cooler for transport to the lab for counting and further analysis.

During earthworm collection, soil temperature and moisture were measured in undisturbed soil at three points around the perimeter of each pit at 15 cm depth using a temperature probe (Cole-Parmer Traceable Calibration; Control Company; Friendwood, TX) and moisture meter (Field Scout TDR 100; Spectrum Technologies, Inc.; Plainfield, IL) (Manufacturer, Location). During the spring sampling session only, soil samples were collected from the 0–10 cm depth of undisturbed soil adjacent to each pit for chemical analysis. At the

same time, three soil cores (5 cm diameter × 5 cm length) were collected from the 2.5 – 7.6 cm depth for bulk density measurement.

3.1.5 Laboratory Analysis

Earthworms were immediately processed upon arrival at the lab. They were first sorted into three age categories (juvenile, adolescent, and adult) based on morphological characteristics. Juveniles were worms that had no indication of clitellum development, adolescents showed slight swelling or lightening where the clitellum should develop, and adults had full clitellum development. Once sorted, earthworms were counted and total weight of each age class was recorded. For the fall sampling, earthworms were further sorted into groups based on similar morphotype for use as an indicator of species diversity.

Soil samples were sent to the Virginia Tech Soil Testing Lab to measure pH (H_2O); cation exchange capacity (CEC); organic matter content (loss on ignition); soluble salt content; and acid extractable soil nutrients (phosphorus, potassium, calcium, magnesium, zinc, manganese, copper, iron, boron) (Mullins and Heckendorn 2009). A portion of each soil sample was also subjected to particle size analysis to determine clay, silt, and sand content using the hydrometer method developed by Day (1965). Soil cores were dried at 105°C to constant mass and bulk density was calculated based on core mass and volume.

3.1.6   Statistical Analysis

All data sets were evaluated for data normality and homogeneity of variance. Analysis of variation for treatment factor effects on independent variables was conducted using the GLM procedure in SAS version 9.2 (SAS Institute; Cary, NC). Where main effects of independent variables were found significant, multiple comparisons of independent variables were conducted using Student's *t*-test. Where interactions of independent variables were found significant, the simple effects of these variables on the response were investigated using the slice procedure within GLM in SAS. All statistical tests were conducted at the α=0.05 level.

3.2   Results

3.2.1   Soil Observations

Soil Temperature and Moisture

Soil temperature and moisture were measured concomitantly with earthworm sampling in both spring and fall 2008. In spring, soil temperature was significantly affected by both land use type and disturbance time frame (Table 3.1). Soil temperature was higher in commercial sites than in residential sites, which was, in turn, higher than in forested sites. In addition, soil temperature was higher in recently disturbed sites than in older sites. A significant interaction between land use and disturbance time frame was observed for fall soil temperature (Figure 3.1).

Analysis of the simple effect of land use type within each disturbance time frame revealed that soil temperature in recent residential sites was higher than in recent commercial sites, which was, in turn, higher than recent forested sites. Conversely, soil temperature in older commercial sites was higher than in older residential sites, which was higher than older forested sites. Analysis of the simple effect of disturbance time frame within each land use type revealed that soil temperature was higher in recent residential sites than older residential sites. Disturbance time frame had no effect on soil temperature in forested or commercial sites.

Volumetric soil moisture was significantly affected by land use, but not by disturbance time frame, in both spring and fall 2008 (Figure 3.2). In addition, a significant interaction between the landscape variables was observed for soil moisture in both seasons. In both spring and fall, analysis of the simple effect of land use type within each disturbance time frame revealed that recent forested sites were wetter than both recent residential and recent commercial sites, which did not differ. Patterns of soil moisture were much more complex among older landscapes. In both spring and fall, residential sites were wetter than commercial sites; forested sites were intermediate to the other site types, but were not significantly different from either. Analysis of the simple effect of disturbance time frame within each land use type revealed that recent forested sites were wetter than older forested sites in both spring and fall. Conversely, recently disturbed residential sites were drier than older residential sites in both spring and fall. Disturbance time frame had no effect on soil moisture in commercial sites.

Table 3. 1. Soil temperature and moisture observed across three land use types and two disturbance time frames in Roanoke, Virginia during May 2008. Measurements were made in 0–10 cm soil depth adjacent to earthworm sampling pits (n=36 for land use; n=54 for disturbance time frame). Standard error of mean shown in parentheses.

	Spring 2008		Fall 2008	
	Temperature	Moisture	Temperature	Moisture
	°C	% v/v	°C	% v/v
Land Use				
Forested	15.7 (0.3) c[z]	25.3 (1.7) a	15.9 (0.3) b	14.3 (1.7) a
Residential	17.9 (0.2) b	22.3 (1.7) a	18.0 (0.3) a	10.5 (1.5) b
Commercial	19.1 (0.3) a	17.1 (1.1) b	18.1 (0.3) a	8.7 (1.1) b
Disturbance Time Frame				
Recent (< 10 yrs.)	18.2 (0.3) a	21.1 (1.5) a	17.7 (0.3) a	10.6 (1.1) a
Not Recent (> 10 yrs.)	16.9 (0.3) b	22.0 (1.2) a	17.0 (0.2) b	11.7 (1.3) a
ANOVA[y]				
Land Use (LU)	<0.0001	<0.0001	<0.0001	<0.0001
Disturbance Time Frame (DT)	<0.0001	0.3823	<0.0001	0.2195
LU × DT	0.8392	<0.0001	<0.0001	0.0003

[z]For each categorical variable, means within a column followed by different letters are significantly different using Student's t-test (α=0.05).

[y]Analysis of variance for main effects (LU, DT) and interaction (LU × DT) of categorical variables (p-values shown).

Figure 3. 1. Soil temperature observed across three land use types and two disturbance time frames in Roanoke, VA during October 2008. Measurements were made in 0–10 cm soil depth adjacent to earthworm sampling pits. Uppercase Latin and lowercase Greek letters denote statistical significance of land use simple effect within each level of disturbance time frame. Lowercase Latin letters denote statistical significance of disturbance time frame simple effect within each level of land use. Columns not connected by same letter are significantly different (α=0.05).

Figure 3. 2. Soil moisture observed across three land use types and two disturbance time frames in Roanoke, VA during May and October 2008. Measurements were made in 0–10 cm soil depth adjacent to earthworm sampling pits. Uppercase Latin and lowercase Greek letters denote statistical significance of land use simple effect within each level of disturbance time frame. Lowercase Latin letters denote statistical significance of disturbance time frame simple effect within each level of land use. Columns not connected by same letter are significantly different (α=0.05).

Soil physical and chemical properties were also measured during earthworm sampling in spring 2008. Both land use and disturbance time frame had a significant effect on bulk density (Table 3.2). Residential sites had high bulk density than both forested sites and commercial sites, which did not differ. Additionally, recently disturbed sites were more compact than older sites. Neither land use nor disturbance time frame had a significant effect on sand content or silt content. Likewise, land use had no effect on clay content; however, clay content was significantly higher in recently disturbed sites than in older sites.

A significant interaction between land use and disturbance time frame was observed for both soil pH and CEC (Table 3.3). Analysis of the simple effect of land use type within each disturbance time frame revealed that soil pH was lower in old forested sites than in old residential sites, which was, in turn, lower than in old commercial sites (Figure 3.3). Additionally, soil pH was lower in recent forested sites than both recent residential and recent commercial sites, which did not differ. Analysis of the simple effect of disturbance time frame within each land use type revealed that soil pH was lower in old forested sites than in new forested sites. Disturbance time frame had no effect on soil pH in the residential or commercial sites.

Analysis of the simple effect of land use type within each disturbance time frame revealed that soil CEC was lower in older forested sites than in older residential or older commercial sites, which did not differ (Figure 3.4). There was no land use type effect on recently

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disturbed sites. Analysis of the simple effect of disturbance time frame within land use type revealed that soil CEC was lower in older forested sites than recently disturbed forested sites. Disturbance time frame had no effect on soil CEC in the residential or commercial sites. Soluble salt content and organic matter content were unaffected by land use and disturbance time frame.

A significant interaction between land use and disturbance time frame was observed for soil calcium, magnesium, and iron (Table 3.4). Analysis of the simple effect of land use type within each disturbance time frame revealed that soil calcium was lower in old forested sites than in both old residential sites and old commercial sites, which did not differ (Figure 3.5). Land use type had no effect on recently disturbed sites. Analysis of the simple effect of disturbance time frame within each land use type revealed that soil calcium was lower in old forested sites than in new forested sites. Disturbance time frame had no effect on soil calcium in the residential or commercial sites.

Analysis of the simple effect of land use type within each disturbance time frame revealed that soil magnesium was lower in old forested sites than in both old residential sites and old commercial sites, which did not differ (Figure 3.6). Land use type had no effect on recently disturbed sites. Analysis of the simple effect of disturbance time frame within each land use type revealed that soil magnesium was lower in old forested sites than in new forested sites. Additionally, soil magnesium was higher in old commercial sites than in recently disturbed commercial sites. Disturbance time frame had no effect on soil magnesium in the residential sites.

Analysis of the simple effect of land use type within each disturbance time frame revealed that soil iron was lower in recently disturbed forested sites than in recently disturbed residential sites; recently disturbed commercial sites were intermediate (Figure 3.7). Conversely, soil iron was higher in old forested sites than both old residential sites and old commercial sites, which did not differ. Analysis of the simple effect of disturbance time frame within each land use type revealed that soil iron was lower recently disturbed forested sites than in older forested sites. Conversely, soil iron was higher in both recently disturbed residential and recently disturbed commercial sites than in older residential and older commercial sites, respectively.

Soil phosphorous was not significantly affected by either land use type or disturbance time frame. Potassium was higher in recently disturbed sites than in older sites, but did not differ across land use types. Manganese was also higher in recently disturbed sites; in addition, manganese differed across land use types. Manganese was highest is commercial sites, followed by residential and forested sites, which also differed from one another.

Table 3. 2. Soil physical properties observed across three land use types and two disturbance time frames in Roanoke, Virginia during May 2008. Measurements were made in 0–10 cm soil depth adjacent to earthworm sampling pits (n=36 for land use; n=54 for disturbance time frame). Standard error of mean shown in parentheses.

	Bulk Density	Sand Content	Silt Content	Clay Content
	g cm^{-1}	%	%	%
Land Use				
Forested	1.12 (0.03) b^z	46 (2) a	37 (2) a	17 (1) a
Residential	1.18 (0.02) a	45 (2) a	38 (2) a	17 (1) a
Commercial	1.10 (0.04) b	46 (2) a	39 (2) a	16 (1) a
Disturbance Time Frame				
Recent (< 10 yrs.)	1.16 (0.02) a	44 (2) a	38 (1) a	18 (1) a
Not Recent (> 10 yrs.)	1.11 (0.04) b	47 (2) a	38 (2) a	15 (1) b
ANOVA^y				
Land Use (LU)	0.0067	0.8123	0.8200	0.4759
Disturbance Time Frame (DT)	0.0224	0.1400	0.9260	0.0273
LU × DT	0.3758	0.1989	0.5385	0.1146

[z]For each categorical variable, means within a column followed by different letters are significantly different using Student's t-test (α=0.05).

[y]Analysis of variance for main effects (LU, DT) and interaction (LU × DT) of categorical variables (p-values shown).

Table 3. 3. Soil chemical properties observed across three land use types and two disturbance time frames in Roanoke, Virginia during May 2008. Measurements were made in 0-10 cm soil depth adjacent to earthworm sampling pits (n=36 for land use; n=54 for disturbance time frame). Standard error of mean shown in parentheses.

	pH	Cation Exchange Capacity	Soluble Salts	Organic Matter
		meq 100 g^{-1}	ppm	%
Land Use				
Forested	7.0 (0.2) b[z]	12.8 (0.9) b	116.2 (6.7) a	7.1 (0.6) a
Residential	7.6 (0.1) a	14.8 (0.9) a	106.6 (6.8) a	6.5 (0.7) a
Commercial	7.7 (0.1) a	15.2 (0.9) a	111.8 (8.3) a	7.6 (0.9) a
Disturbance Time Frame				
Recent (< 10 yrs.)	7.6 (0.1) a	14.6 (0.8) a	113.1 (6.5) a	6.4 (0.6) b
Not Recent (> 10 yrs.)	7.2 (0.1) b	13.8 (0.7) a	110.0 (5.4) a	7.7 (0.6) a
ANOVA[y]				
Land Use (LU)	<0.0001	0.0012	0.5433	0.3784
Disturbance Time Frame (DT)	<0.0001	0.1522	0.6579	0.0476
LU × DT	<0.0001	<0.0001	0.1762	0.1734

[z]For each categorical variable, means within a column followed by different letters are significantly different using Student's t-test (α=0.05).

[y]Analysis of variance for main effects (LU, DT) and interaction (LU × DT) of categorical variables (p-values shown).
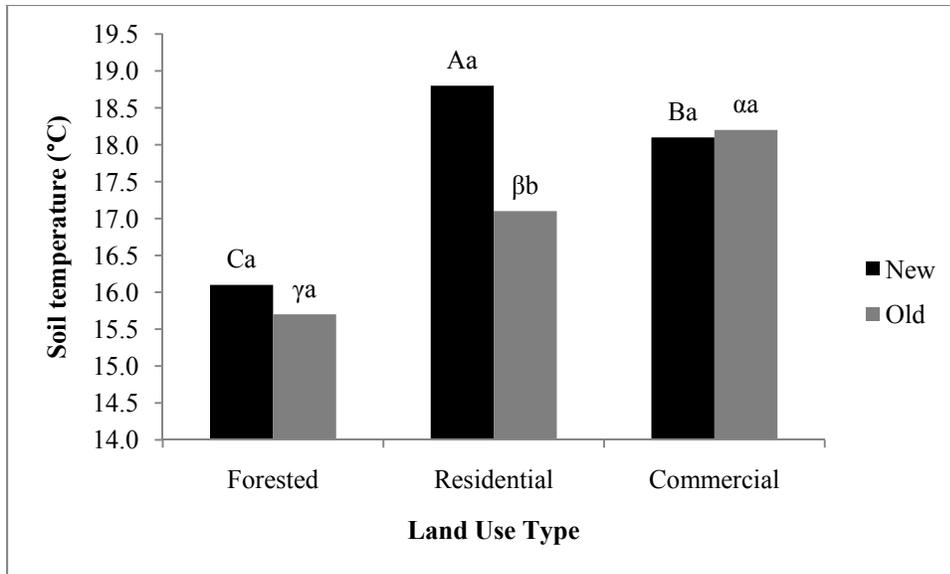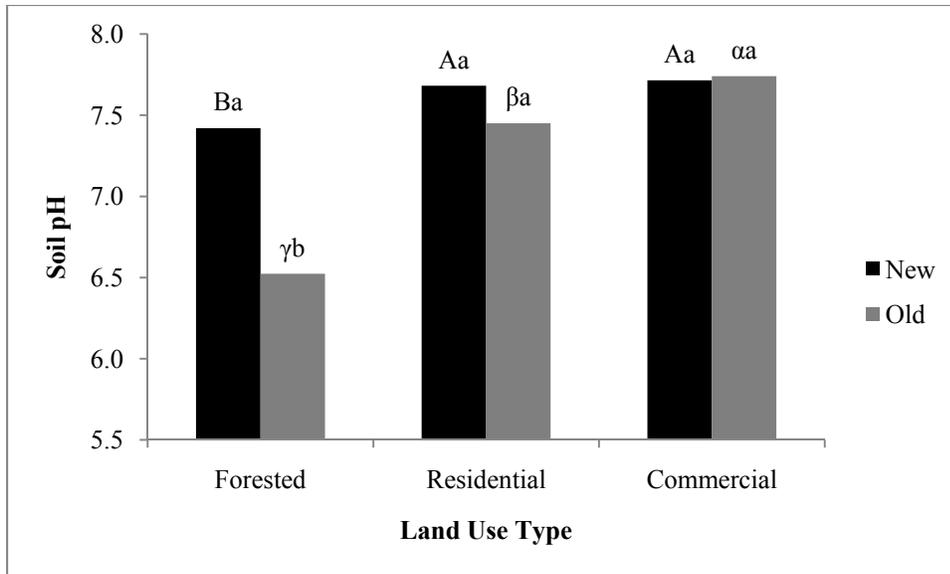
Figure 3. 3. Soil pH observed across three land use types and two disturbance time frames in Roanoke, VA during May 2008. Measurements were made in 0–10 cm soil depth adjacent to earthworm sampling pits. Uppercase Latin and lowercase Greek letters denote statistical significance of land use simple effect within each level of disturbance time frame. Lowercase Latin letters denote statistical significance of disturbance time frame simple effect within each level of land use. Columns not connected by same letter are significantly different (α=0.05).
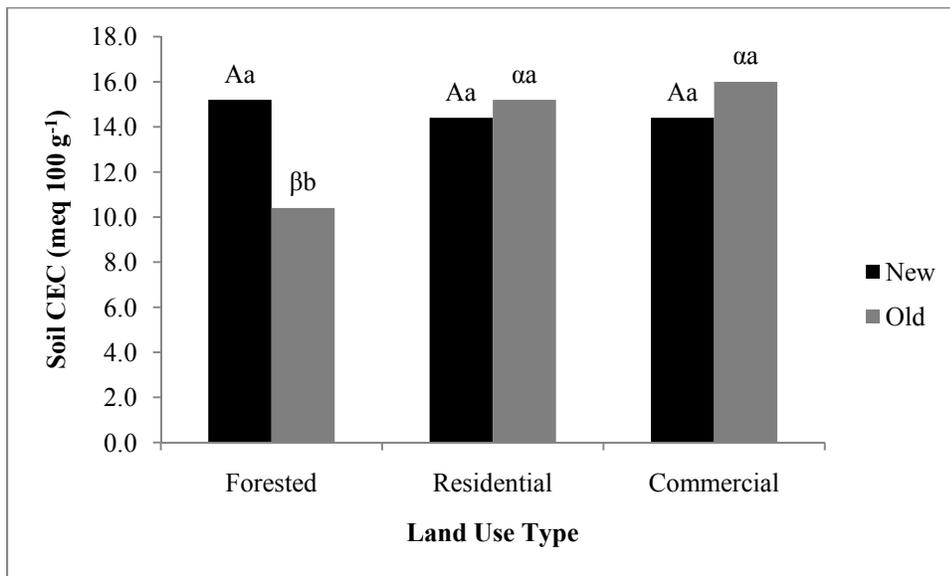
Figure 3. 4. Soil CEC observed across three land use types and two disturbance time frames in Roanoke, VA during May 2008. Measurements were made in 0–10 cm soil depth adjacent to earthworm sampling pits. Uppercase Latin and lowercase Greek letters denote statistical significance of land use simple effect within each level of disturbance time frame. Lowercase Latin letters denote statistical significance of disturbance time frame simple effect within each level of land use. Columns not connected by same letter are significantly different (α=0.05).
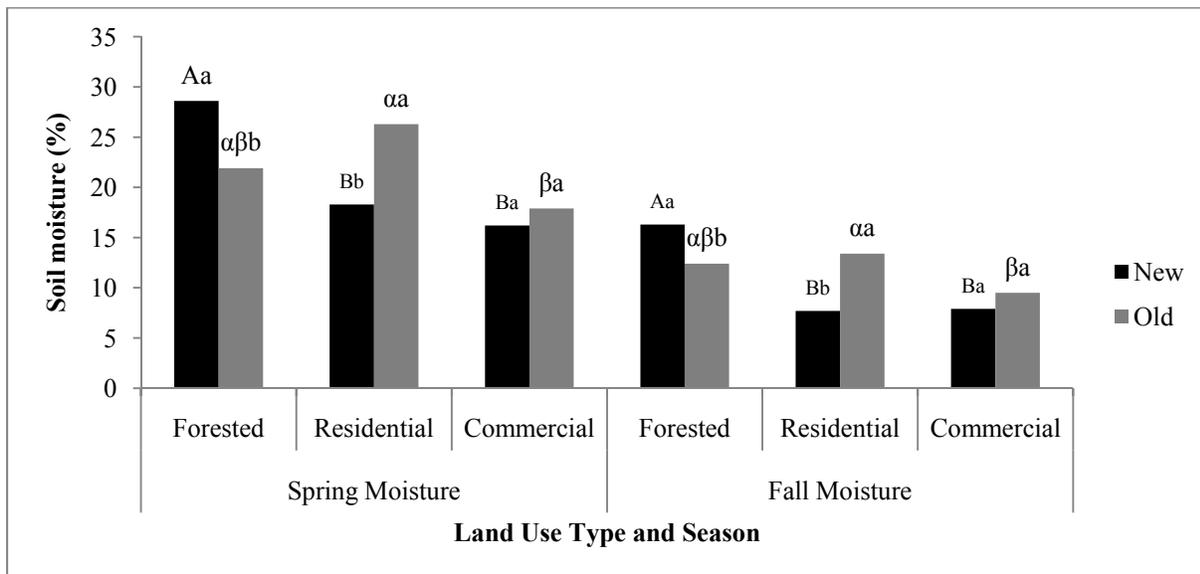
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Table 3. 4. Soil nutrient content observed across three land use types and two disturbance time frames in Roanoke, Virginia during May 2008. Measurements were made in 0–10 cm soil depth adjacent to earthworm sampling pits (n=36 for land use; n=54 for disturbance time frame). Standard error of mean shown in parentheses.

	P	K	Ca	Mg	Fe	Mn
			mg kg^{-1}			
Land Use						
Forested	24 (6) a[z]	148 (13) a	1864 (165) b	304 (22) b	9.0 (1.7) a	19.4 (1.4) c
Residential	28 (6) a	169 (13) a	2160 (150) a	434 (35) a	8.9 (1.5) a	25.2 (1.8) b
Commercial	17 (2) a	149 (8) a	2255 (155) a	416 (40) a	7.2 (0.7) a	32.3 (2.0) a
Disturbance Time Frame						
Recent (< 10 yrs.)	22 (4) a	172 (10) a	2220 (142) a	367 (18) a	9.6 (1.2) a	27.6 (1.8) a
Not Recent (> 10 yrs.)	24 (4) a	138 (10) b	1965 (114) b	402 (36) a	7.1 (1.0) b	23.7 (1.3) b
ANOVA[y]						
Land Use (LU)	0.1955	0.2555	0.0011	0.0002	0.1137	<0.0001
Disturbance Time Frame (DT)	0.7409	0.0041	0.0041	0.1885	0.0018	0.0123
LU × DT	0.0008	0.4382	<0.0001	<0.0001	<0.0001	0.3263

[z]For each categorical variable, means within a column followed by different letters are signficantly different using Student's t-test ($\alpha=0.05$).

[y]Analysis of variance for main effects (LU, DT) and interaction (LU × DT) of categorical variables (p-values shown).
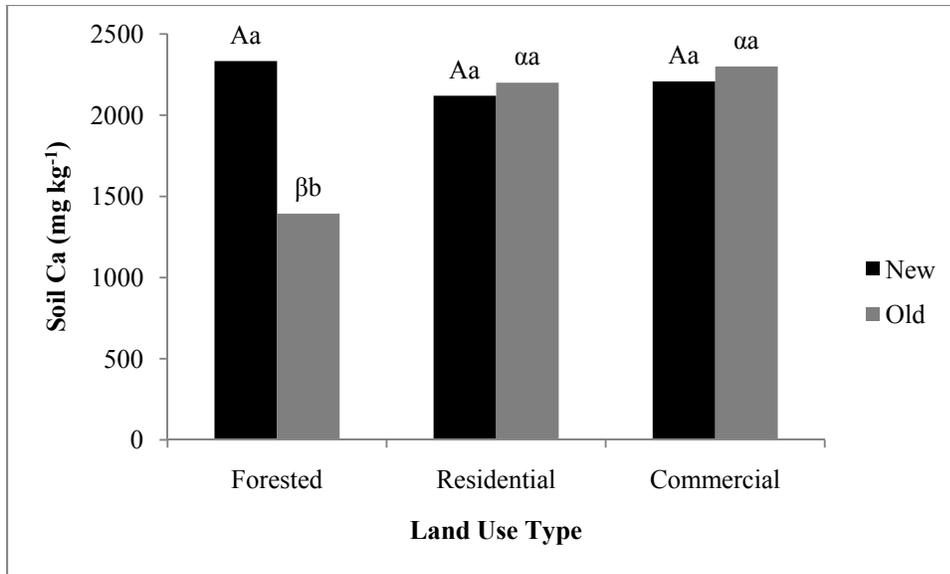
Figure 3. 5. Soil Ca observed across three land use types and two disturbance time frames in Roanoke, VA during May 2008. Measurements were made in 0–10 cm soil depth adjacent to earthworm sampling pits. Uppercase Latin and lowercase Greek letters denote statistical significance of land use simple effect within each level of disturbance time frame. Lowercase Latin letters denote statistical significance of disturbance time frame simple effect within each level of land use. Columns not connected by same letter are significantly different (α=0.05).
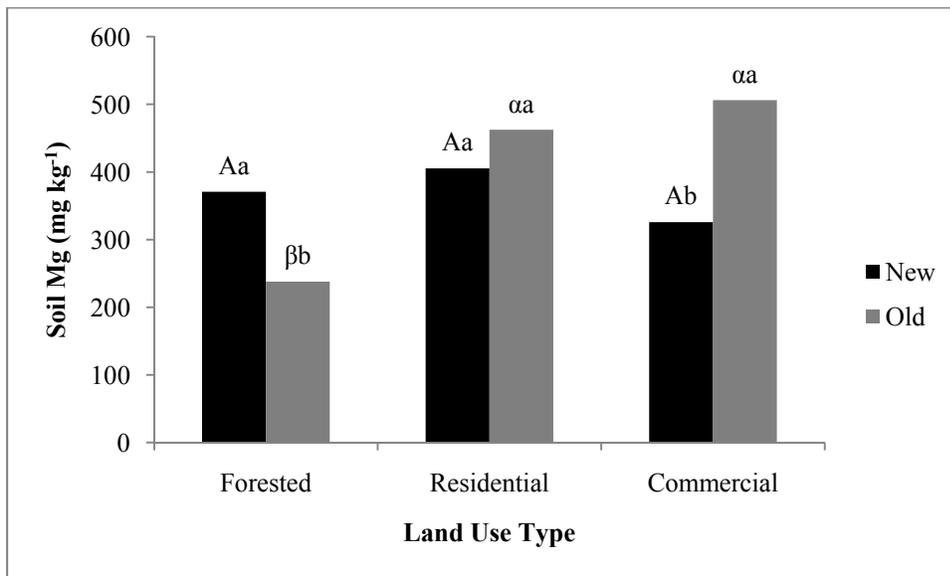
Figure 3. 6. Soil Mg observed across three land use types and two disturbance time frames in Roanoke, VA during May 2008. Measurements were made in 0–10 cm soil depth adjacent to earthworm sampling pits. Uppercase Latin and lowercase Greek letters denote statistical significance of land use simple effect within each level of disturbance time frame. Lowercase Latin letters denote statistical significance of disturbance time frame simple effect within each level of land use. Columns not connected by same letter are significantly different (α=0.05).

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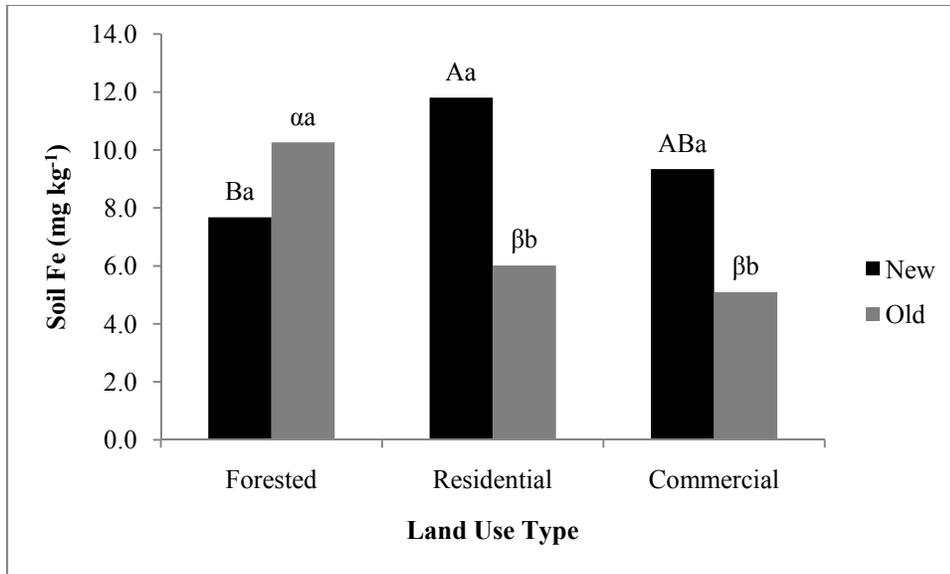
Figure 3. 7. Soil Fe observed across three land use types and two disturbance time frames in Roanoke, VA during May 2008. Measurements were made in 0–10 cm soil depth adjacent to earthworm sampling pits. Uppercase Latin and lowercase Greek letters denote statistical significance of land use simple effect within each level of disturbance time frame. Lowercase Latin letters denote statistical significance of disturbance time frame simple effect within each level of land use. Columns not connected by same letter are significantly different (α=0.05).

3.2.2 Earthworm Observations

Abundance

In spring 2008, land use type significantly affected total earthworm abundance (Table 3.5). More earthworms were found in forested sites than in commercial sites. Earthworm abundance in residential sites was intermediate to forested and commercial sites, but was not significantly different from either. Disturbance time frame had no significant effect on total earthworm abundance.

Similar patterns in spring earthworm abundance were found when age classes of worms were examined individually (Table 3.5). For the adult age class, a significant interaction between land use and disturbance time frame was observed. Analysis of the simple effect of land use type within each disturbance time frame revealed that there were more adult worms in recent forested sites than in either recent residential or recent commercial sites, which did not differ from one another (Figure 3.8). There were no significant differences in adult abundance among land use types that had not been recently disturbed. Analysis of the simple effect of disturbance time frame within each land use type revealed that there were significantly more adult worms in recent forested sites than in older forested sites. Disturbance time frame had no effect on adult abundance in the residential or commercial sites.

Analysis of adolescent earthworm abundance showed a significant effect of land use and a non-significant effect of disturbance time frame (Table 3.5). As with the total population, adolescent worms were more abundant in forested sites than commercial sites; residential sites were intermediate to the other sites and did not statistically differ from either. Neither land use nor disturbance time frame significantly affected juvenile earthworm abundance.

Table 3. 5. Earthworm abundance observed across three land use types and two disturbance time frames in Roanoke, Virginia during May 2008. Each value is the mean earthworm count sampled from a 1 m^2 × 0.1 m deep soil pit (n=36 for land use; n=54 for disturbance time frame). Standard error of mean shown in parentheses.

	Total[z]	Adult	Adolescent	Juvenile
	Earthworm Count			
Land Use				
Forested	109 (19) a[y]	16 (3) a	30 (6) a	63 (14) a
Residential	66 (22) ab	2 (1) b	15 (8) ab	49 (16) a
Commercial	45 (15) b	2 (1) b	8 (3) b	34 (12) a
Disturbance Time Frame				
Recent (< 10 yrs.)	84 (18) a	9 (2) a	21 (6) a	53 (12) a
Not Recent (> 10 yrs.)	63 (13) a	4 (1) a	14 (3) a	44 (11) a
ANOVA[x]				
Land Use (LU)	0.0402	<0.0001	0.0271	0.3335
Disturbance Time Frame (DT)	0.3182	0.0545	0.2907	0.5758
LU × DT	0.3173	0.0029	0.6227	0.4139

[z]Sum of adult, adolescent, and juvenile eartworms observed in each plot.

[y]For each categorical variable, means within a column followed by different letters are significantly different using Student's *t* -test (α=0.05).

[x]Analysis of variance for main effects (LU, DT) and interaction (LU × DT) of categorical variables (*p* -values listed).
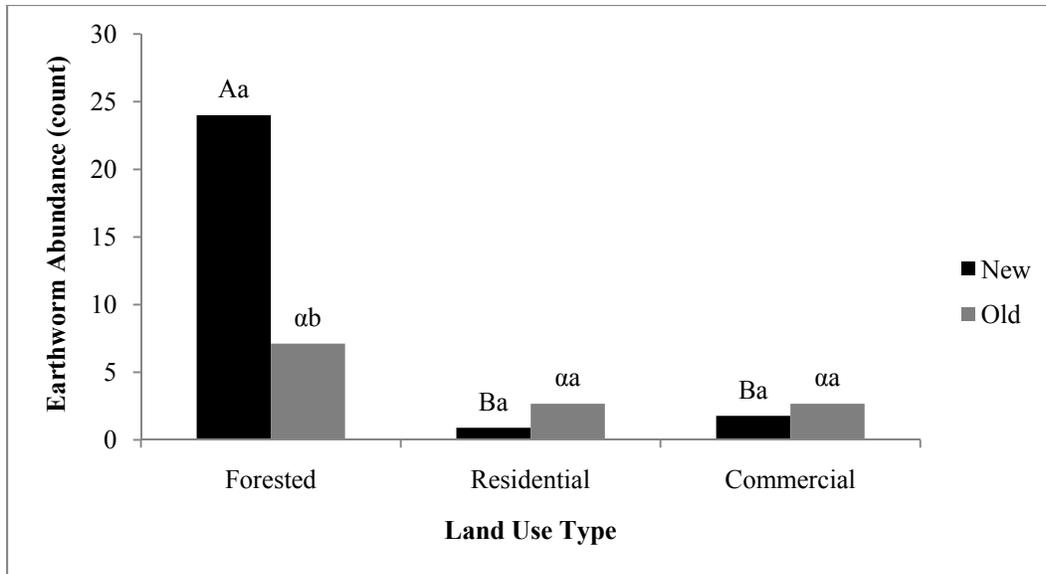
Figure 3. 8. Adult earthworm abundance observed across three land use types and two disturbance time frames in Roanoke, VA during May 2008. Each value is the mean earthworm count m^{-2} (n=18 for each treatment combination). Uppercase Latin and lowercase Greek letters denote statistical significance of land use simple effect within each level of disturbance time frame. Lowercase Latin letters denote statistical significance of disturbance time frame simple effect within each level of land use. Columns not connected by same letter are significantly different (α=0.05).

As in the spring, land use significantly affected total earthworm abundance in fall 2008 (Table 3.6). More total earthworms were observed in forested sites than in both residential and commercial sites, which did not differ. The same land use effect was observed for the adult, adolescent, and juvenile age classes. Disturbance time frame had no effect on total or age-class-specific worm abundance. However, a significant interaction between land use and disturbance time frame was observed for the juvenile age class. Analysis of the simple effect of land use type within each disturbance time frame revealed that there were more juvenile worms in new forested sites than in either new residential or new commercial sites, which did not differ. There were no significant differences in juvenile abundance among land use types that had not been recently disturbed (Table 3.6). Analysis of the simple effect of disturbance time frame within each land use type revealed that there were more juvenile worms in recent forested sites than in

old forested sites (Figure 3.9). Disturbance time frame had no effect on juvenile abundance in the

residential or commercial sites.

Table 3. 6. Earthworm abundance observed across three land use types and two disturbance time frames in Roanoke, Virginia during October 2008. Each value is the mean earthworm count sampled from a 1 m^2 × 0.1 cm deep soil pit (n=36 for land use; n=54 for disturbance time frame). Standard error of mean shown in parentheses.

	Total[z]		Adult		Adolescent		Juvenile	
	Earthworm Count							
Land Use								
Forested	108	(16) a[y]	7	(3) a	45	(8) a	56	(12) a
Residential	37	(12) b	0	(1) b	22	(9) b	15	(4) b
Commercial	32	(10) b	1	(1) b	19	(6) b	12	(6) b
Disturbance Time Frame								
Recent (< 10 yrs.)	63	(12) a	2	(1) a	26	(5) a	35	(9) a
Not Recent (> 10 yrs.)	56	(10) a	4	(2) a	32	(8) a	20	(4) a
ANOVA[x]								
Land Use (LU)	<0.0001		0.0040		0.0385		0.0001	
Disturbance Time Frame (DT)	0.6404		0.3065		0.5049		0.1076	
LU × DT	0.1287		0.7684		0.2741		0.0280	

[z]Sum of adult, adolescent, and juvenile earthworms observed in each plot.

[y]For each categorical variable, means within a column followed by different letters are significantly different using Student's t-test (α=0.05).

[x]Analysis of variance for main effects (LU, DT) and interaction (LU × DT) of categorical variables (p-values listed).
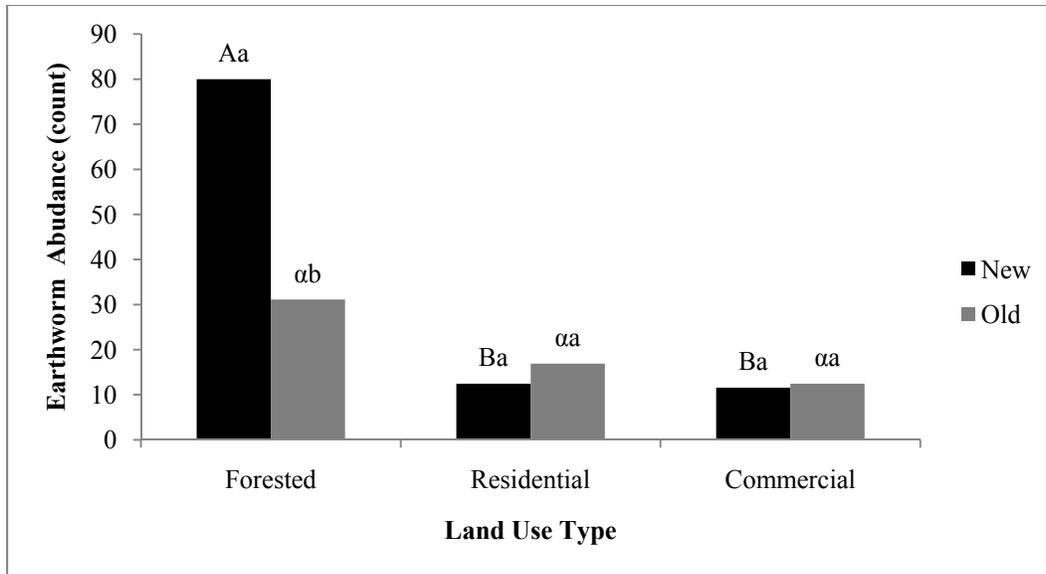
Figure 3. 9. Juvenile earthworm abundance observed across three land use types and two disturbance time frames in Roanoke, VA during October 2008. Each value is the mean earthworm count m^{-2} (n=18 for each treatment combination). Uppercase Latin and lowercase Greek letters denote statistical significance of land use simple effect within each level of disturbance time frame. Lowercase Latin letters denote statistical significance of disturbance time frame simple effect within each level of land use. Columns not connected by same letter are significantly different (α=0.05).

<u>Biomass</u>

In spring 2008, land use significantly affected total, adult, and adolescent earthworm biomass, but not juvenile biomass (Table 3.7). More total, adult, and adolescent earthworm biomass was found in forested sites than in both residential and commercial sites, which did not differ. Disturbance time frame had no effect on total or age-class-specific worm biomass.

A significant interaction between land use and disturbance time frame was observed for both total and adult worm biomass. Analysis of the simple effect of land use type within each disturbance time frame revealed that there was more total worm biomass in recent forested sites than in either recent residential or recent commercial sites, which did not differ (Figure 10).

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There were no significant differences in total worm biomass among land use types that had not been recently disturbed. Analysis of the simple effect of disturbance time frame within each land use type revealed that there was more total worm biomass in recent forested sites than older forested sites. Disturbance time frame had no effect on total worm biomass in the residential or commercial sites. Analysis of the simple effects of land use type and disturbance time frame on adult worm biomass revealed the same statistical outcomes as reported for total worm biomass.

Land use and disturbance time frame affected fall 2008 earthworm biomass similarly to spring 2008 (Table 3.8). More total as well as age-class-specific worm biomass was found in forested sites than in both residential and commercial sites, which did not differ. Again, disturbance time frame had no effect on total or age-class-specific worm biomass. However, unlike spring 2008, no significant interactions were observed between the landscape classification variables for fall 2008 worm biomass.

Table 3. 7. Earthworm biomass observed across three land use types and two disturbance time frames in Roanoke, Virginia during May 2008. Each value is the mean earthworm count sampled from a 1 m^2 × 0.1 cm deep soil pit (n=36 for land use; n=54 for disturbance time frame). Standard error of mean shown in parentheses.

	Total[z]			Adult			Adolescent			Juvenile		
						Grams						
Land Use												
Forested	47	(8)	a[y]	18	(5)	a	14	(3)	a	15	(4)	a
Residential	17	(7)	b	5	(4)	b	5	(2)	b	7	(2)	a
Commercial	9	(5)	b	1	(1)	b	3	(1)	b	5	(4)	a
Disturbance Time Frame												
Recent (< 10 yrs.)	27	(6)	a	10	(3)	a	8	(2)	a	9	(9)	a
Not Recent (> 10 yrs.)	22	(6)	a	6	(3)	a	7	(2)	a	10	(3)	a
ANOVA[x]												
Land Use (LU)	0.0001			0.0025			0.0069			0.1164		
Disturbance Time Frame (DT)	0.5403			0.2862			0.8307			0.8791		
LU × DT	0.0188			0.0103			0.8566			0.1910		

[z]Sum of adult, adolescent, and juvenile earthworms observed in each plot.

[y]For each categorical variable, means within a column followed by different letters are significantly different using Student's t-test (α=0.05).

[x]Analysis of variance for main effects (LU, DT) and interaction (LU × DT) of categorical variables (p-values listed).
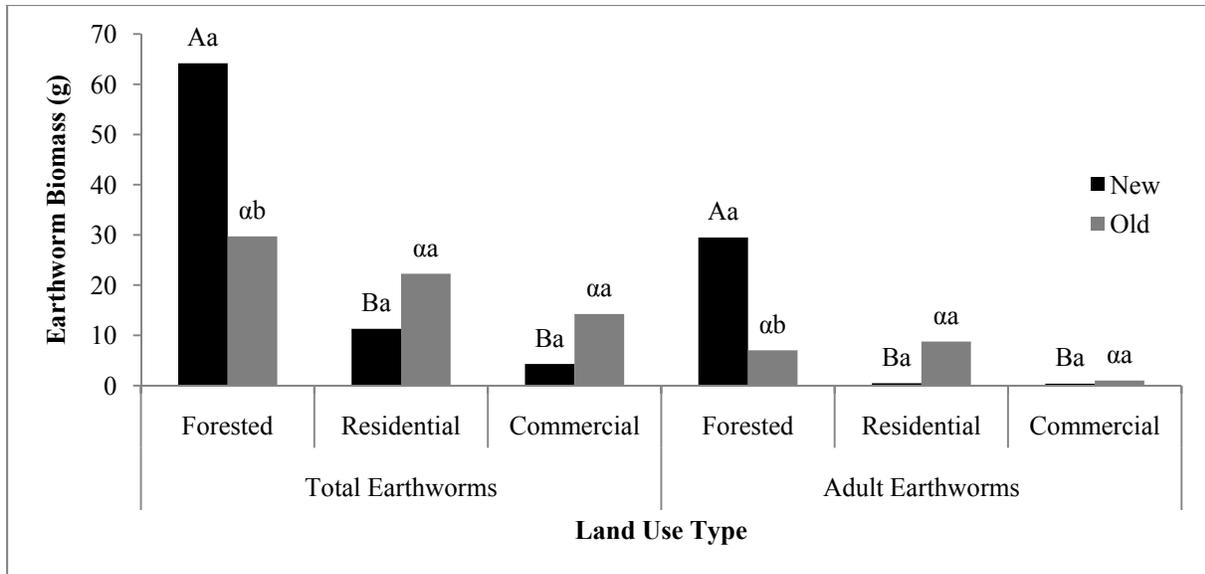
Figure 3. 10. Total and adult earthworm biomass observed across three land use types and two disturbance time frames in Roanoke, VA during May 2008. Each value is the mean earthworm count m^{-2} (n=18 for each treatment combination). Uppercase Latin and lowercase Greek letters denote statistical significance of land use simple effect within each level of disturbance time frame. Lowercase Latin letters denote statistical significance of disturbance time frame simple effect within each level of land use. Columns not connected by same letter are significantly different (α=0.05).

Table 3. 8. Earthworm biomass observed across three land use types and two disturbance time frames in Roanoke, Virginia during October 2008. Each value is the mean earthworm biomass sampled from a 1 m^2 × 0.1 cm deep soil pit (n=36 for land use; n=54 for disturbance time frame). Standard error of mean shown in parentheses.

	Total[z]			Adult			Adolescent			Juvenile		
	Grams											
Land Use												
Forested	35	(6)	a[y]	7	(3)	a	22	(4)	a	7	(1)	a
Residential	12	(5)	b	1	(1)	b	8	(4)	b	2	(1)	b
Commercial	11	(4)	b	2	(1)	b	7	(3)	b	1	(1)	b
Disturbance Time Frame												
Recent (< 10 yrs.)	19	(4)	a	3	(2)	a	11	(2)	a	4	(1)	a
Not Recent (> 10 yrs.)	20	(5)	a	3	(1)	a	14	(4)	a	2	(1)	a
ANOVA[x]												
Land Use (LU)	0.0004			0.0332			0.0090			0.0007		
Disturbance Time Frame (DT)	0.8561			0.8950			0.5173			0.1106		
LU × DT	0.3608			0.4827			0.4099			0.1259		

[z]Sum of adult, adolescent, and juvenile earthworms observed in each plot.

[y]For each categorical variable, means within a column followed by different letters are significantly different using Student's *t* -test (α=0.05).

[x]Analysis of variance for main effects (LU, DT) and interaction (LU × DT) of categorical variables (*p* -values listed).

Diversity

Land use had a significant effect on the diversity of earthworms observed in fall 2008.

More morphotypes were found in forested sites than in residential and commercial sites, which

did not differ (Table 3.9). However, disturbance time frame had no significant effect on

earthworm diversity.

Table 3. 9. Earthworm diversity observed across three land use types and two disturbance time frames in Roanoke, Virginia during fall 2008. Each value is the mean number of earthworm types sampled from a 625 cm^2 × 10 cm deep soil pit (n=36 for land use; n=54 for disturbance time frame). Standard error of mean shown in parentheses.

	Type Count
Land Use	
Forested	2.5 (0.3) a
Residential	1.2 (0.2) b
Commercial	0.7 (0.2) b
Disturbance Time Frame	
Recent (< 10 yrs.)	1.5 (0.2) a
Not Recent (> 10 yrs.)	1.4 (0.2) a
ANOVA[y]	
Land Use (LU)	<0.0001
Disturbance Time Frame (DT)	0.5168
LU × DT	0.1802

[z]For each categorical variable, means within a column
followed by different letters are significantly different using
Student's t-test (α=0.05).

[y]Analysis of variance for main effects (LU, DT) and
interaction (LU × DT) of categorical variables (p-values
listed).

<u>Seasonality</u>

Neither total earthworm abundance nor total biomass significantly differed between spring and fall 2008 (Figure 3.11). However, there were seasonal differences in both abundance and biomass for specific age classes. More adult and juvenile worms were observed in spring than in fall, whereas adolescent worms were more prevalent in the fall than in spring. Similarly, more juvenile biomass was observed in spring than in fall, whereas more adolescent biomass was observed in fall than in spring. Seasonal differences in adult biomass were marginal. Significant differences in worm abundance were also observed between age classes within each season (lowercase letters). In the spring, more juveniles were observed than adults and adolescents, which did not differ in their abundance. In the fall, both juveniles and adolescents were more abundant than adults. In terms of seasonal biomass, no significant differences were found between age classes in the spring sampling; however, in the fall, more adolescent biomass was observed than that of both adult and juvenile biomass, which did not differ.
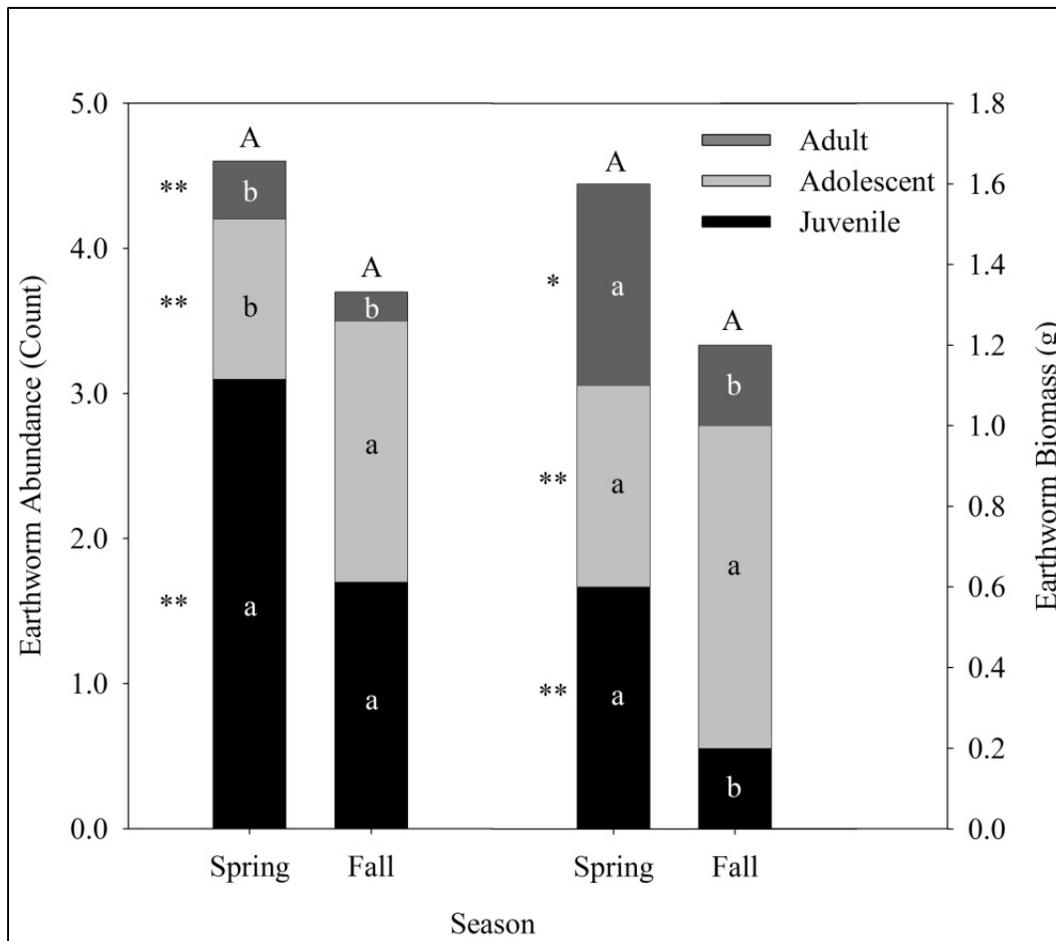
Figure 3. 11. Seasonal earthworm abundance (left) and biomass (right) observed at urbanized sites in Roanoke, Virginia during May and October 2008. Values are based on earthworms sampled from 625 cm^2 × 10 cm deep soil pits (n=108 for each season). Uppercase letters denote between-season comparisons of total earthworm abundance and biomass (Student's paired *t*-test; α=0.05). Lowercase letters denote within-season comparisons of all earthworm age classes for abundance and biomass (Fisher's LSD; α=0.05). Asterisks denote between-season comparisons of specific earthworm age classes for abundance and biomass (Student's paired *t*-test; **P<0.05; *P<0.1).

3.3   Discussion

3.3.1   Soil Observations

<u>Physical Properties</u>

Soil temperature was higher in commercial sites than residential and then forested sites in both spring and fall. This was most likely due to lower urban tree canopy cover in residential and commercial sites, which would lead to higher soil temperatures. Additionally, commercial sites could also have been affected by the heat island effect, increasing soil temperature simply because of the higher amount of infrastructure (buildings, roads, etc.) surrounding street trees and trees planted in parking lot islands. Anecdotal field observation suggested that residential sites had fewer trees than forested sites and that the trees tended to be larger and have a more extensive canopy than commercial plantings, possibly due to a larger soil volume. This could explain why residential sites had intermediate soil temperatures. Furthermore, newer sites also had higher soil temperatures than sites that had been undisturbed for more than ten years. Newer plantings tended to have younger trees that were smaller and, therefore, had smaller canopies, which could explain why the soil was warmer at these sites.

Soil moisture in Roanoke's urbanized areas was lower than observed in other cities (Pizl and Josens 1995; Smetak et al. 2007; Steinberg et al. 1997; Szlavecz et al. 2006), most likely due to a very dry year, and seemed to follow the pattern of disturbance. Sampling occurred in the late

spring and late fall of 2008, and the spring and summer of 2008 were relatively dry in southwest Virginia. Total precipitation in Roanoke in 2008 was 84.6 cm, an 11.5 cm departure from the 30 year norm. Rainfall monthly deficits averaged 3.6 cm from September through November 2008. Earthworms are extremely sensitive to dry soil conditions because they have high water content in their bodies and breathe through their permeable skin, requiring moisture for this process (Lee 1985). Furthermore, drier conditions make it difficult to sample earthworms because they are mostly active when soil conditions are advantageous - appropriate moisture and temperature. Chemical extraction does not work as well under dry conditions because earthworms aestivate when conditions are not favorable. All of these factors could have impacted worm extraction.

Moisture content was higher in recently forested sites, followed by older residential, older forested, older commercial, and then newer residential and commercial sites. Smetak et al. (2007) noted slightly higher moisture in urban parks that were more than 75 years old and lower moisture in younger residential sites. Similarly, Szlavecz et al. (2006) recorded slightly higher moisture in rural sites than in urban sites, most likely due to their distance from the city center, resulting in decreased soil temperatures and increased interception due to lower heat-island effects and lower amounts of impervious surfaces. In Roanoke, forested sites had lower temperatures than in residential and commercial sites, and anecdotal observations suggested that forested sites had thicker litter layers, both of which would contribute to soil moisture conservation.

Soil bulk density was higher in residential sites than in both commercial and forested sites. Additionally, it was higher in recently disturbed sites than in older sites. Often, sampling

plots for the residential land use were located in recreational parks, and bulk density may have

been higher due to heavier foot traffic and human use than commercial sites, which were located

in turf strips and parking lot islands – areas little used for human travel. Recently disturbed sites

most likely had higher bulk densities because bulk density decreased following disturbance, a

phenomenon also found by Smetak et al. (2007). Regardless of differences in land use and

disturbance time frame, measured bulk densities were very low and would not be root-limiting

for tree roots, however, sampling only came from the 0-10 cm depth. During land development,

the subsoil is often compacted and less dense topsoil is laid down during the final grading

process (Scheyer and Hipple 2005). It is likely that the subsoil was much denser, and, therefore,

possibly root-limiting below the depth of sampling. Although Pizl and Josens (1995) reported

similar bulk densities in Brussels, Smetak et al. (2007) listed higher bulk density measurements

across all of their sites in Moscow, ID, particularly in young residential sites, which would be

considered root-limiting (and earthworm limiting) depending on the soil texture at these sites

(Natural Resources Conservation Service 2000).

Particle size analysis revealed that the typical surface soil texture across land use types

and disturbance time frames in Roanoke was loam. Although, silt and sand content did not differ

across land use and time, clay content was higher in recently disturbed sites, indicating that fill

material used in these sites may have had higher clay content than fill used in older sites. Klok et

al. (2007) described a relationship between percent clay in the soil and the growth rates of *L.*

rubellus, an epigeic earthworm species. In their experiment, population growth rates and

individual earthworm weight decreased as soil clay content increased. However, soil texture did

not vary by land use in Roanoke, nor could it be directly related to earthworm abundance. This

may be due to the limitation of sampling only the top 10 cm of soil and not sampling deeper soil layers where the larger, deep-burrowing earthworms live. Additionally, because earthworms were not identified to the species, and, therefore, to the functional group, it would be difficult to draw any relationship between earthworms and soil texture in this study.

Chemical Properties

On average, soil pH reported across urbanized sites in the city of Roanoke was very close to neutral and fell within appropriate ranges for tree planting and crop rearing (Craul 1999; Maguire and Heckendorn 2009). Pizl and Josens (1995) reported soil pH ranging from 5.93 to 7.03 in Brussels – the lower pH values coming from the farthest distances from the city center – and Szlavecz et al. (2006) reported a range of 4.2 – 5.3 in Baltimore, MD (with no obvious land use influence). Neither study correlated soil pH directly to earthworm abundance, but both mentioned the relationship between soil pH and other soil characteristics – specifically metal toxicity and nutrient cycling – which could affect earthworm populations. Earthworms can survive in acidic soils as low as 4.0, but generally prefer a range closer to 7.0 (Card et al. 2002) Urban soils tend to be more alkaline due to a mixture of deeper, less weathered soil horizons and the common use of building materials that leach calcium into the soil. Kennette et al. (2002) reported that *L. terrestris* showed no negative survival effect from exposure to high amounts of heavy metals, attributing this phenomenon to the high pH of the soil (between 7 and 8), which could have reduced dissolved concentrations of the metals. Earthworm survival in alkaline soils (above 7.0) has not been widely reported and should be investigated further.

CEC was significantly lower in old forested sites than any other land use type and disturbance time frame combination, most likely due to a suite of factors: weathering, pH, and organic matter content. In older sites, soils would be more weathered, which would lower CEC, and pH was lower, which also would lower CEC. Organic matter content did not vary across land use types, but was higher in older sites, which may have contributed to a higher CEC. There is limited literature regarding earthworm population dynamics and the soil CEC.

Soluble salts concentrations were not significantly affected by land use type or disturbance time frame. Virginia Cooperative Extension states that at levels lower than 844 ppm, soluble salts will not cause salt injury to crops (Maguire and Heckendorn 2009). Our average reported levels were all lower than 125 ppm, falling within an appropriate range. Additionally, although earthworms are extremely sensitive to salts (Card et al. 2002), salt content in Roanoke did not seem to be population-limiting due to the fact that forested new sites were on the upper end of the soluble salt range, and more earthworms were extracted from these sites.

Land use type did not significantly affect soil organic matter; however, organic matter content was higher in older sites than in recently disturbed sites. Because earthworms are detritivores, they require a source of decomposing, organic matter; thus, low levels of organic matter could limit earthworm populations. Reported organic matter content was extremely high because loss on ignition was used and this process measures everything that is burnable in the soil sample, not just organic matter, overestimating the actual content. Regardless, total earthworm abundance was not affected by disturbance time frame, indicating that differences in organic matter between time frames could not be associated with earthworm populations.

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3.3.2   Earthworm Observations

Earthworm abundance and biomass were generally higher in forested sites than in residential and commercial sites, a pattern that emerged both in spring and fall. Soil temperature, moisture, and pH were the most variable soil attributes across these land use types, and may help explain observed differences in earthworm abundance. Lower observed soil temperature in forested sites was most likely a function of greater tree canopy cover, which would reduce the amount of ultraviolet light that reaches the ground. Earthworms are extremely sensitive to direct sunlight and would tend to flourish in sites with more protection from the sun's harmful rays (Edwards and Bohlen 1996; Lee 1985). High soil temperature also influences soil moisture by increasing the rate of evaporation. Forested sites tended to be wetter than residential and commercial sites, which should favor earthworm activity.

Soil pH and calcium content were lower in older forested sites, presumably due to acidifying organic matter inputs and the absence of urban infrastructure, which can leach calcium. Earthworms can survive in slightly acidic soil and have been known to increase soil pH to above neutral if living in more acidic soil for a given amount of time (Card et al. 2002; Lee 1985). Forest soil tends to be more acidic due to litter decomposition. It would be expected that forested sites had more organic matter than both residential and commercial sites simply because litter is often removed from human-occupied sites; however, our analyses revealed no differences in organic matter content near the soil surface. It is possible that the organic matter in forested sites would be higher in the absence of earthworms, but is being reduced by the higher earthworm populations present (Bohlen et al. 2004a; Bohlen et al. 2004b; Gundale 2002).

Although soil bulk density was lower in forested sites than residential sites, the difference was biologically irrelevant. However, it is known that dense soil can impede earthworm movement through the soil (Joschko et al. 1989), and the slight differences in bulk density measured at the soil surface in this study might be indicative of more pronounced differences at greater soil depth.

Similar population and biomass distribution patterns by land use type have occurred in other studies. Out of five comparable urban earthworm studies (Pizl and Josens 1995; Smetak et al. 2007; Steinberg et al. 1997; Szlávecz and Csuzdi 2007; Szlavecz et al. 2006), four reported fewer earthworms in the highly disturbed end of the urban-suburban continuum than in the less disturbed end. This pattern was often attributed to the fact that highly disturbed, urbanized soil cannot provide an appropriate habitat for earthworms to survive and grow. However, Pizl and Josens (1995) found a more complex relationship and could not connect higher abundance to higher biomass. The authors ascribed the recorded variations to different species compositions rather than to age structure. Only Steinberg et al. (1997) reported more earthworms and higher biomass in their highly urbanized study sites than in their rural sites, attributing the higher abundance to the New York City seaport – a potential point of earthworm introduction – and the lower abundance in the rural areas to past farming practices, which included tilling, a practice that is known to reduce earthworm populations (Didden 2001; Johnson-Maynard et al. 2007).

Further exploration of earthworm abundance and biomass within age classes revealed some interesting patterns, which often varied by season. Adult abundance and biomass were affected by land use type, soil disturbance time frame, and season, showing that more adults and

more adult biomass were extracted from forested new sites in spring. In spring, forested sites were significantly cooler than other land use types and forested new sites were significantly wetter than forested old sites. Forested new sites benefit from limited daily traffic and disruption combined with close proximity to managed areas that may receive more moisture and nutrients. These sites were usually in abandoned areas with little direct management, yet they often bordered managed turf that may have been irrigated and fertilized, such as soccer fields. This junction may have provided habitat that was more conducive for earthworm survival and growth than more isolated sites could provide.

Exploration of adolescent earthworm data revealed that abundance and biomass were also consistently higher in forested sites than commercial sites, and differed across seasons. Abundance and biomass were both higher in spring than in fall, most likely because juveniles from the spring had grown into the adolescent phase in fall. Additionally, in fall, adolescents were more prevalent than adults and contributed more biomass than either adults or juveniles, probably because there were so many more adolescents than adults and because adolescents are larger than juveniles.

In spring, land use type did not have a significant effect on juvenile abundance nor biomass; however, both abundance and biomass were affected by land use type in fall. Furthermore, the majority of earthworms extracted were juveniles, a pattern found in Moscow, ID (Smetak et al. 2007) and Brussels, Belgium (Pizl and Josens 1995). Earthworms produce more cocoons when conditions are most hospitable (Edwards and Bohlen 1996; Lee 1985), and although earthworms are capable of reproducing year round and have been known to produce

cocoons throughout the year, reproduction is more so a function of soil conditions rather than season. Temperature and moisture data across all land use types suggest that the soil habitat was more conducive to reproduction in the spring. This would explain the lack of difference between juvenile abundance and biomass between land use types in spring. In fall, higher juvenile abundance and biomass were recorded in forested sites, a phenomenon also reported in Baltimore, MD (Szlavecz et al. 2006). Higher juvenile abundance is to be expected throughout the year since most earthworm species (*r*-selected strategists) produce many offspring per adult. Most hatchlings die before reaching reproductive age; therefore earthworm fecundity is very high to ensure adequate survivorship to adulthood. Additionally, a significant interaction between land use type and disturbance time frame did affect abundance. Like adult abundance in spring, in fall, more juveniles were extracted from forested new sites than any other site. The high amount of juveniles in these sites is probably a relic of the high amount of adults in these sites in the previous season, reproducing high amounts of juveniles.

Although earthworms sampled in Roanoke were not identified to the species level, morphotyping revealed higher earthworm diversity in the forested sites than in the commercial and residential sites. This coincides with what Szlavecz and Csuzdi (2007) reported in Baltimore, MD and Smetak et al. (2007) reported in Moscow, ID. It seems that the least disturbed sites supported a higher diversity of earthworm species. This could be because sites that are less disturbed have soils that are more hospitable for earthworm establishment, survival, and reproduction, and a higher abundance of earthworms may lend itself to being more diverse. Additionally, forested soils are likely more heterogeneous in the litter layer and A horizon than residential and commercial sites, which would create more niches for different species to inhabit.

It would be interesting to explore the species composition in Roanoke to determine how many of these various earthworms were considered exotic, which would further explain the diversity of worms within each land use type and disturbance time frame combination.

3.4   Conclusion

In urbanized landscapes of Roanoke, Virginia, earthworm abundance and biomass were affected by land use type, disturbance time frame, and seasonality. In spring, higher abundance and biomass were extracted from recently disturbed, forested sites. Similarly, in fall, higher earthworm abundance, biomass, and diversity were observed across both forested sites. Soil properties associated with forested sites included higher soil moisture and lower soil temperatures, bulk density, pH, and CEC, whereas recently disturbed sites were associated with higher temperature, bulk density, clay content, pH, potassium, calcium, iron, and manganese, and lower organic matter. Clearly, earthworm abundance and biomass are affected by a suite of complex soil and temporal variables. In Roanoke, it seems that soil temperature and moisture were the most influential soil properties.

Future studies should include measuring tree properties along with earthworm abundance over a longer period of time. This would allow the researcher to determine whether the trees were benefitting from earthworm presence rather than making an indirect connection between earthworms and trees using soil as a proxy. Additional studies would also benefit from species identification to determine both functional group and the presence or absence of natives and exotics. A more detailed analysis of soil organic matter might also contribute to a better understanding of earthworm abundance. C:N ratios help describe the palatability of soil organic matter for detritivores and soil microbes but these ratios cannot be determined from general organic matter content values because they do not give specific carbon and nitrogen content. Organic matter palatability could help explain earthworm abundance in certain sites. Further

research also should be done exploring soil properties in the subsoil – deeper than 10 cm, which would allow researchers to make inferences about deeper burrowing, anecic worms. Finally, the use of a designed experiment would permit causative inferences regarding earthworm population dynamics and soil properties, rather than an observational study which only allows for associative inferences.

Chapter 4: The Influence of Earthworm Inoculation on a Simulated Urban Soil

4.0   Introduction

An inoculation experiment was conducted at the Turfgrass Research Center on the Virginia Tech campus in Blacksburg, VA to evaluate earthworm augmentation strategies for improving soil conditions for urban tree growth. Of major interest for this experiment was to determine the efficacy of using earthworms in addition to the widely accepted rehabilitation technique of pneumatic decompaction and organic matter incorporation used to improve urban soil and, eventually, tree health. Sixty plots were installed in spring 2008, treated with tilling only or with tilling and earthworm inoculation in summer 2008, and eventually planted with either *Acer rubrum* or *Cornus florida* saplings in fall 2008. Earthworm presence was assessed and soil conditions characterized to determine the effectiveness of earthworm inoculation as a soil compaction and nutrient remediation treatment.

4.1 Materials and Methods

4.1.1 Site Description

The inoculation experiment was performed at Virginia Tech's Turfgrass Research Center (TRC) in Blacksburg, Virginia. Much like Roanoke, VA, Blacksburg has a temperate climate with an annual average of 10.6°C and average daily temperatures ranging from 4.0°C to 17.3°C, and an average annual precipitation of 108 cm.

<u>Land Use and Soils</u>

Historically, this area was used for farming, but approximately 60 years ago, the site was cut to the clay subsoil during runway construction for an airport that was never completed.

The study site falls within the Groseclose-Urban land complex with 2-7% slopes (Creggar et al. 1985). The Groseclose soils are characterized by a loam surface layer and a sticky, plastic clay subsoil and substratum with clay loam beneath. Groseclose soils have slow permeability, moderate available water capacity, high subsoil shrink-swell potential, low natural fertility and organic matter content and extremely to strong acidic surface and subsoil layers if unlimed, however, all of these characteristics are variable in disturbed soil.

4.1.2   Treatments

Three treatments were evaluated for their effect on soil properties and tree development: (1) control, (2) soil tilling plus compost amendment, and (3) soil tilling plus compost amendment and earthworm inoculation. The control treatment (Con) consisted of only applying ground hardwood bark mulch to the surface of prepared plots (plot preparation described below). The soil tilling plus compost amendment treatment (T/C) involved incorporating compost into the soil using both a mechanical tiller and a pneumatic excavation tool followed by surface mulching. Finally, the soil tilling plus compost amendment and earthworm inoculation treatment (T/C+W) used the same soil preparation as the till only treatment followed by scattering live adult earthworms on the soil surface prior to applying mulch.

4.1.3   Statistical Design

The earthworm inoculation experiment was conducted using a generalized randomized block statistical design. Experimental treatments were evaluated on two common landscape tree species of contrasting soil quality tolerance – red maple (*Acer rubrum* L. 'Red Sunset') and flowering dogwood (*Cornus florida* L.'Cherokee Brave'). Red maple is noted for its tolerance of poor soils whereas flowering dogwood is generally considered intolerant (Dirr 1998). The experimental site was divided into five blocks within which two replicates of each species × treatment combination were randomly assigned to a treatment plot, totaling 60 experimental units (2 species × 3 treatments × 5 blocks × 2 replicates).

4.1.4   Plot Construction and Treatment Installation

In March 2008, a skid-steer excavator (Bobcat® S185, Bobcat Co., Gwinner, ND) was used to scrape the sod and surface soil (approximately 20 cm deep) from the field site to replicate the disturbance of urban land development and diminish existing earthworm populations. Next, a pedestrian trencher (Ditch Witch ® 1030, Charles Machine Works Inc., Perry, OK) was used to cut 10 cm wide × 45 cm deep trenches in a grid pattern across the plot, creating sixty 2 m × 2 m treatment plots. The plots were arranged into six 10-plot rows with a 2 m buffer row between each treatment plot row. These plots were then enclosed with 6-milimeter plastic sheeting supported by 60 cm wooden stakes to a soil depth of 45 cm and extending 15 cm above ground line to minimize earthworm migration between plots. Finally, trenches were backfilled to grade with the excavated soil.

Treatments were applied shortly after plot construction in late May 2008. Due to the high clay content and extreme compaction of the soil on the site, T/C and T/C+W treatment plots underwent a three-step treatment process: (1) manual loosening of the soil with a shovel to 20 cm depth, (2) mechanical loosening of the soil with a garden tiller to 20 cm depth, and (3) compost incorporation to 20 cm depth with a pneumatic excavator (Supersonic Air Knife, Easy Use Air Tools Inc., Allison Park, PA). Compost used for the T/C and T/C+W treatments was pre-packaged, retail-grade cow manure (0.5-0.5-0.5 Composted Manure and Humus; Garden Pro; Dagsboro, DE) devoid of earthworms. About 18 kg of compost was spread over each plot to a depth of 5 cm and then worked into the soil using the Air Knife.

Two weeks after compost amendment, the T/C+W treatment plots were inoculated with earthworms (*Lumbricus terrestris* L.) acquired from a mail-order vendor (Knutson's Recreational Sales Inc., Brooklyn, MI). For each plot, 48 live adult worms were sprinkled onto the soil surface. Immediately after earthworm inoculation, locally acquired ground hardwood bark mulch was applied to the surface of the 60 treatment plots to a 10 cm depth. Plots were maintained weed free through the 2008 growing season by hand weeding and spot treatment with Glyphosate (Glyphomate 41, PBI/Gordon Corp., Kansas City, MO).

In late October 2008, trees were transplanted into the treatment plots. Locally acquired nursery stock grown in 26-liter containers of soilless media was used for the experiment to ensure that no earthworms were introduced with the root balls. After carefully raking back the mulch from the soil surface, a planting hole was excavated in the center of each treatment plot using a 30 cm power auger. Trees were randomly assigned to the treatment plots, placed in the planting holes, and backfilled with excavated soil. Finally, trees were secured to trellises spanning the plot rows and irrigated with 10 L of water. One supplemental irrigation of 10 L of water was provided one week after planting. Total tree height and trunk diameter 15 cm above ground line were measured and recorded immediately after planting.

4.1.5 Soil and Earthworm Sampling

Soil samples were collected from each treatment plot at the $0 - 10$ cm depth two months before treatment, one day after treatment, one day before earthworm inoculation, four months after earthworm inoculation, and nine months after earthworm inoculation, and analyzed as

described in the observational study. Soil bulk density cores were collected from each treatment plot at the 2.5 – 7.6 cm depth two months before treatment, three weeks after earthworm inoculation, and nine months after earthworm inoculation.

The contribution of biomass and nutrients of earthworm carcasses was also determined. Six dozen adult *L. terrestris* were procured from Blacksburg Feed and Seed (Blacksburg, VA), weighed, euthanized, dried to a constant mass (65°C), and then weighed again. Their average weight dozen^{-1} contributed roughly 28 g of mass to each 2-m^2 inoculated plot.

Earthworm survival assessments were performed one, four, and nine months after inoculation. Extraction, handling, and characterization were conducted as described for the Observational Study. In addition, visual inspection of earthworm casts at the soil surface and surface burrow openings at the bottom of the dug pits were performed at the time of each earthworm survival assessment to assess current earthworm activity.

Monthly measurements of soil CO_2 efflux rate were commenced six months after earthworm inoculation to assess treatment effects on soil respiration in plots containing red maples. A permanent efflux measurement site was established in each treatment plot by inserting a 10 cm diameter × 17 cm long PVC ring into the soil to a 10 cm depth about 30 cm from the tree root ball. Soil CO_2 efflux rate was measured using an LI-6250 infrared gas analyzer (Li-Cor Environmental Science Division, Lincoln, NE) linked to an LI-6200 portable photosynthesis console. On a clear, calm day each month, a dynamic closed cuvette chamber was affixed to the PVC ring and used to capture CO_2 diffusing from the soil surface and circulate the gas to and

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from the analyzer (Janssens et al. 2000). When the CO_2 concentration was observed to be rising steadily, a 30 s sampling period was initiated and the analyzer calculated efflux rate to the nearest 0.01 $\mu mol/m^2/s$. Soil temperature and moisture were simultaneously measured to a depth of 10 cm using a temperature probe (Cole-Parmer Traceable Calibration Thermometer; Control Company; Friendwood, TX) and moisture meter (Field Scout TDR 100; Spectrum Technologies, Inc.; Plainfield, IL).

4.1.6 Laboratory Analysis

Earthworms collected during survival assessments of the experimental treatments were subjected to the same protocol described for the Observational Study. Soil chemical properties (pH, nutrient content, organic matter content, and soluble salt content) as well as physical properties (bulk density and particle size analyses) were also analyzed using the procedures of the observational study.

4.1.7 Statistical Analysis

All data sets were evaluated for data normality and homogeneity of variance. Analysis of variation for treatment factor effects on independent variables was conducted using the GLM procedure in SAS version 9.2 (SAS Institute; Cary, NC). Where main effects of independent variables were found significant, multiple comparisons of independent variables were conducted using Student's *t*-test. Where interactions of independent variables were found significant, the

simple effects of these variables on the response were investigated using the slice procedure within GLM in SAS. All statistical tests were conducted at the α=0.05 level.

Repeated measures analysis of variation for treatment factor effects on soil respiration was conducted using the mixed procedure repeated measured analysis using Huynh-Feldt covariance structure in SAS version 9.2.

4.2   Results

To evaluate the efficacy of earthworm inoculation for improving urban soil properties and landscape tree performance, a series of measurements were taken before, during, and after application of soil treatments. Here, results of the analysis of treatment effects on earthworm presence, soil physical and chemical properties, and soil respiration are presented for the first ten months following experiment installation.

4.2.1   Earthworm Presence Assessment

Earthworms were extracted from T/C+W plots at every post-inoculation assessment interval, but were not extracted from T/C plots until the five and ten month intervals (Table 4.1). Two weeks after inoculation, no worms were detected in T/C plots, but worms were detected in 15% of the T/C+W plots, albeit at very low abundance. Five months post-inoculation, earthworms were found in 20% of both T/C and T/C+W plots; in plots where worms were found,

both treatments yielded the same number of worms per sampling pit on average. Ten months post-inoculation, 35% of both T/C and T/C+W plots harbored earthworms when averaged across tree species. Examination of earthworm abundance in plots where worms were detected showed no significant difference between soil treatments or between tree species. Earthworm abundance in T/C+W plots at ten months post-inoculation was similar to earthworm abundance observed in residential and commercial landscapes in Roanoke, Virginia during 2008.

Table 4. 1. Earthworm presence in field plots at three time intervals following soil rehabilitation. T/C plots were rehabilitated by a combination of mechanical and pneumatic soil loosening, compost incorporation, and organic mulch top-dressing. T/C+W plots were rehabilitated in the same manner, but were inoculated with *Lumbricus terrestris* prior to mulching. For earthworm extractions, each value is the mean count sampled from a 625 cm^2 × 10 cm deep soil pit. Standard error of mean shown in parentheses.

| | Worm-Positive Plots | | Worms Extracted From | |
| | (% of total plots) | | Worm-Positive Plots (count) | |
Post-Inoculation Interval	T/C	T/C+W	T/C	T/C+W
Two Weeks[z]				
Unplanted Plots	0	15	0.0 (0.0) a	1.0 (0.1) a
Five Months[y]				
A. rubrum	20	20	1.5 (0.5) a	1.5 (0.5) a
Ten Months[x]				
A. rubrum	20	30	1.0 (0.1) Aa	2.7 (0.9) Aa
C. florida	50	40	1.2 (0.2) Aa	2.5 (0.9) Aa

[z]For each soil treatment, total plots equal 20. Values followed by different letters are significantly different using Student's t-test ($\alpha=0.05$).

[y]For each soil treatment, total plots equal 10. Values followed by different letters are significantly different using Student's t-test ($\alpha=0.05$).

[x]For each soil treatment, total plots equal 10 per species. Uppercase letters denote statistical significance ($\alpha=0.05$) of soil treatment simple effect within each species. Lowercase letters denote statistical significance ($\alpha=0.05$) of species simple effect within each soil treatment.

4.2.2. Physical and Chemical Soil Properties

Treated plots were significantly different ($p < 0.0001$) than Con plots throughout the progression of pre-treatment to five and then to ten months after treatment (Table 4.2, Table 4.3, Table 4.4, Table 4.5) . With the exception of bulk density, which decreased, soil treatment increased the value of each soil property discussed below. By five months after treatment, T/C+W plots were significantly different than T/C plots for five out of the ten soil properties measured (Table 4.4, Table 4.5). By ten months, T/C+W plots were significantly different from T/C plots for five out of the eleven soil properties measured (Table 4.4, Table 4.5). In addition, there was a significant tree species effect on two out of eleven soil properties at ten months after planting (Table 4.5).

Pre-Inoculation

Disturbance at the experimental site during preparation for treatment installation significantly affected both physical and chemical soil properties (Table 4.2, Table 4.3). Bulk density increased, and both cation exchange capacity and organic matter content decreased after disturbance. Phosphorus, potassium, calcium, and magnesium content also decreased. However, soil pH and soluble salts were unchanged.

Soil properties in treated plots after tilling and compost incorporation (but prior to earthworm inoculation) were all significantly different than in Con plots (Table 4.2, Table 4.3).

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In all cases, T/C and T/C+W plots did not differ (which was the objective). The rehabilitation treatments decreased bulk density and increased soil pH. Organic matter content and CEC doubled, and soluble salts increased seven-fold. Soil content of all measured macro and micronutrients increased.

Table 4. 2. Soil properties measured at 0–10 cm depth during three installation stages of soil rehabilitation experiment. Before disturbance denotes the unaltered plot. After disturbance denotes the plot after excavation and grading. Pre-inoculation denotes after soil cultivation and organic matter amendment, but before inoculation with *Lumbricus terrestris*. Standard error of mean shown in parentheses.

	Bulk Density	pH	Cation Exchange Capacity	Soluble Salts	Organic Matter
	$g\,cm^{-1}$		$meq\,100\,g^{-1}$	ppm	%
Before Disturbance[z]	1.16 (0.03) b[w]	5.8 (0.1) a	6.9 (0.3) a	59.7 (11.5) a	5.0 (0.7) a
After Disturbance[y]	1.41 (0.03) a	5.6 (0.1) a	5.3 (0.2) b	35.0 (7.2) a	1.5 (0.1) b
Pre-Inoculation[x]					
Con	1.42 (0.03) a[v]	5.7 (0.1) b	5.4 (0.2) b	39.3 (11.6) b	1.4 (0.1) b
T/C	1.11 (0.03) b	6.7 (0.1) a	12.2 (0.2) a	280.9 (13.9) a	2.9 (0.1) a
T/C+W	1.12 (0.03) b	6.8 (0.1) a	12.1 (0.2) a	294.4 (24.1) a	2.9 (0.1) a

[z] For bulk density, n=10. For all other properties, n=7.

[y] For all properties, n=20.

[x] For all properties, n=20.

[w] For comparisons before and after disturbance, means within a column followed by different letters are significantly different using Student's t-test (α=0.05).

[v] For pre-inoculation stage, means within a column followed by different letters are significantly different using Student's t-test (α=0.05).

Table 4. 3. Soil nutrient content measured at 0–10 cm depth during three installation stages of soil rehabilitation experiment. Before disturbance denotes the unaltered plot. After disturbance denotes the plot after excavation and grading. Pre-inoculation denotes after soil cultivation and organic matter amendment, but before inoculation with *Lumbricus terrestris*. Standard error of mean shown in parentheses.

	P	K	Ca	Mg	Fe	Mn
	mg kg^{-1}					
Before Disturbance[z]	3 (1) a[w]	77 (10) a	665 (61) a	248 (12) a	22.1 (0.3) a	6.1 (0.2) a
After Disturbance[y]	2 (0) b	31 (2) b	439 (30) b	199 (14) b	14.4 (2.6) a	7.3 (1.1) a
Pre-Inoculation[x]						
Con	2 (0) b[v]	27 (1) b	422 (32) b	196 (15) b	11.0 (0.9) b	7.5 (1.1) b
T/C	10 (1) a	85 (3) a	2013 (65) a	220 (8) ab	121.4 (3.8) a	32.1 (0.9) a
T/C+W	10 (1) a	87 (4) a	1994 (96) a	232 (7) a	116.0 (5.0) a	32.3 (1.2) a

[z]For all properties, n=7.

[y]For all properties, n=20.

[x]For all properties, n=20.

[w]For comparisons before and after disturbance, means within a column followed by different letters are significantly different using Student's *t*-test (α=0.05).

[v]For pre-inoculation stage, means within a column followed by different letters are significantly different using Student's *t*-test (α=0.05).

Post-Inoculation

Soil treatment effects on soil properties persisted at both five and ten months after earthworm inoculation (Table 4.4, Table 4.5). At the ten month interval (five months after tree planting), a significant species effect was observed for soil pH and manganese content. Tree species did not affect any other soil properties at the ten month interval.

Bulk density was not measured five months after inoculation, but remained lower in treated plots than in Con plots after ten months. After five months, soil pH was significantly higher in T/C+W plots than T/C plots which was higher in Con plots; pH remained higher in rehabilitated plots after ten months, but the difference in the two treatments subsided.

Additionally, pH and manganese content were higher in plots planted with *A. rubrum* than with *C. florida* ten months after inoculation.

Significant differences in CEC as well as calcium, iron, and manganese content persisted among the three soil treatments through ten months post-inoculation. In all cases, values were highest in T/C+W, followed by T/C plots, and then Con plots. Although magnesium content in T/C plots had subsided to Con levels by ten months, elevated magnesium content persisted in T/C+W plots. Phosphorus and potassium content remained higher in rehabilitation treatments than the Con after ten months, but the two treatments did not differ. Elevated soluble salt content in rehabilitated plots had subsided by ten months after inoculation.

Soil organic matter content showed interesting responses to soil rehabilitation treatments (Figure 4.1). A marginally significant interaction (p=0.0640) between soil treatment and post-inoculation interval was observed for soil organic matter. Analysis of the simple effect of soil treatment within each post-inoculation interval revealed that soil organic matter was lower in Con plots five months post-inoculation than in both T/C and T/C+W plots, which did not differ. Subsequently, soil organic matter was higher in T/C plots ten months post-inoculation than in T/C+W plots, which was, in turn, higher than in Con plots. Analysis of the simple effect of post-inoculation interval within each soil treatment revealed that soil organic matter was higher ten months post-inoculation than five months post-inoculation for each soil treatment.

Table 4. 4. Soil properties measured at 0–10 cm depth in field plots at two time intervals following soil rehabilitation. T/C plots were rehabilitated by a combination of mechanical and pneumatic soil loosening, compost incorporation, and organic mulch top-dressing. T/C+W plots were rehabilitated in the same manner, but were inoculated with *Lumbricus terrestris* prior to mulching (n=30 for species; n=20 for soil treatment). Standard error of mean shown in parentheses.

	Bulk Density	pH	Cation Exchange Capacity	Soluble Salts	Organic Matter
	$g\ cm^{-1}$		$meq\ 100\ g^{-1}$	ppm	%
Post-Inoculation (5 Months)					
Species					
A. rubrum	—[z]	6.5 (0.2) a[y]	8.6 (0.4) a	105.8 (10.6) a	3.5 (0.3) a
C. florida	—	6.3 (0.2) a	8.3 (0.3) a	100.2 (7.4) a	3.3 (0.3) a
Soil Treatment					
Con	—	5.5 (0.1) c	6.5 (0.3) c	49.8 (4.2) b	1.9 (0.1) b
T/C	—	6.6 (0.2) b	8.9 (0.3) b	131.2 (7.6) a	4.4 (0.4) a
T/C+W	—	7.1 (0.2) a	10.0 (0.3) a	128.0 (9.2) a	3.9 (0.2) a
ANOVA					
Species (SP)	—	0.1305	0.3411	0.4919	0.3127
Soil Treatment (ST)	—	<.0001	<.0001	<.0001	<.0001
SP × ST	—	0.6230	0.3377	0.4408	0.5942
Post-Inoculation (10 Months)					
Species					
A. rubrum	1.28 (0.03) a	6.6 (0.2) a	6.6 (0.2) a	56.0 (4.1) a	5.5 (0.5) a
C. florida	1.25 (0.04) a	6.3 (0.2) b	6.5 (0.2) a	73.1 (8.2) a	5.1 (0.5) a
Soil Treatment					
Con	1.43 (0.03) a	5.6 (0.1) b	5.5 (0.1) c	53.9 (11.5) a	2.9 (0.2) c
T/C	1.16 (0.04) b	6.7 (0.2) a	6.6 (0.2) b	73.5 (2.3) a	7.3 (0.7) a
T/C+W	1.19 (0.03) b	7.0 (0.2) a	7.7 (0.3) a	74.6 (3.4) a	5.8 (0.4) b
ANOVA[x]					
Species (SP)	0.4897	0.0303	0.6642	0.3104	0.3653
Soil Treatment (ST)	<.0001	<.0001	<.0001	0.4263	<.0001
SP × ST	0.6748	0.2959	0.3082	0.3536	0.0601

[z]Bulk density not measured at the five month interval.

[y]For each categorical variable within a time frame, means within a column followed by different letters are significantly different using Student's *t*-test ($\alpha=0.05$).

[x]Analysis of variance for main effects (SP, ST) and interaction (SP × ST) of categorical variables (*p*-values listed).

Table 4. 5. Soil nutrient content measured at 0–10 cm depth in field plots at two time intervals following soil rehabilitation. T/C plots were rehabilitated by a combination of mechanical and pneumatic soil loosening, compost incorporation, and organic mulch top-dressing. T/C+W plots were rehabilitated in the same manner, but were inoculated with *Lumbricus terrestris* prior to mulching (n=30 for species; n=20 for soil treatment). Standard error of mean shown in parentheses. One outlier was removed from K data.

	P	K	Ca	Mg	Fe	Mn
			mg kg^{-1}			
Post-Inoculation (5 Months)						
Species						
A. rubrum	5 (1) a[z]	87 (8) a	1115 (92) a	245 (11) a	39.7 (4.8) a	21.1 (1.7) a
C. florida	5 (1) a	81 (6) a	1032 (83) a	250 (9) a	36.4 (4.5) a	18.9 (1.4) a
Soil Treatment						
Con	3 (1) b	43 (3) b	487 (22) c	217 (12) b	7.8 (0.3) c	10.9 (0.7) c
T/C	5 (1) a	109 (8) a	1266 (55) b	256 (12) a	46.8 (3.8) b	22.8 (1.6) b
T/C+W	6 (1) a	100 (5) b	1469 (59) a	270 (10) a	59.6 (2.9) a	26.3 (1.1) a
ANOVA[y]						
Species (SP)	0.5147	0.2345	0.1537	0.6424	0.3557	0.0993
Soil Treatment (ST)	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001
SP × ST	0.0805	0.0463	0.1349	0.7158	0.3243	0.0390
Post-Inoculation (10 Months)						
Species						
A. rubrum	6 (1) a	124 (5) a	854 (66) a	179 (8) a	39.8 (5.3) a	20.1 (1.4) a
C. florida	6 (1) a	116 (6) a	788 (63) a	177 (7) a	35.1 (4.2) a	18.2 (1.0) b
Soil Treatment						
Con	3 (1) b	99 (5) b	454 (41) c	172 (11) b	9.6 (0.5) c	12.8 (0.6) c
T/C	7 (1) a	130 (7) a	902 (51) b	167 (6) b	42.9 (3.8) b	20.9 (0.9) b
T/C+W	8 (1) a	130 (6) a	1107 (57) a	196 (9) a	59.9 (4.7) a	23.8 (1.5) a
ANOVA						
Species (SP)	0.3277	0.1712	0.1906	0.7635	0.1949	0.0389
Soil Treatment (ST)	<.0001	<.0001	<.0001	0.0181	<.0001	<.0001
SP × ST	0.0556	0.6228	0.0932	0.4506	0.3161	0.8320

[z]For each categorical variable within a time frame, means within a column followed by different letters are significantly different using Student's *t*-test (α=0.05).

[y]Analysis of variance for main effects (SP, ST) and interaction (SP × ST) of categorical variables (*p*-values listed).
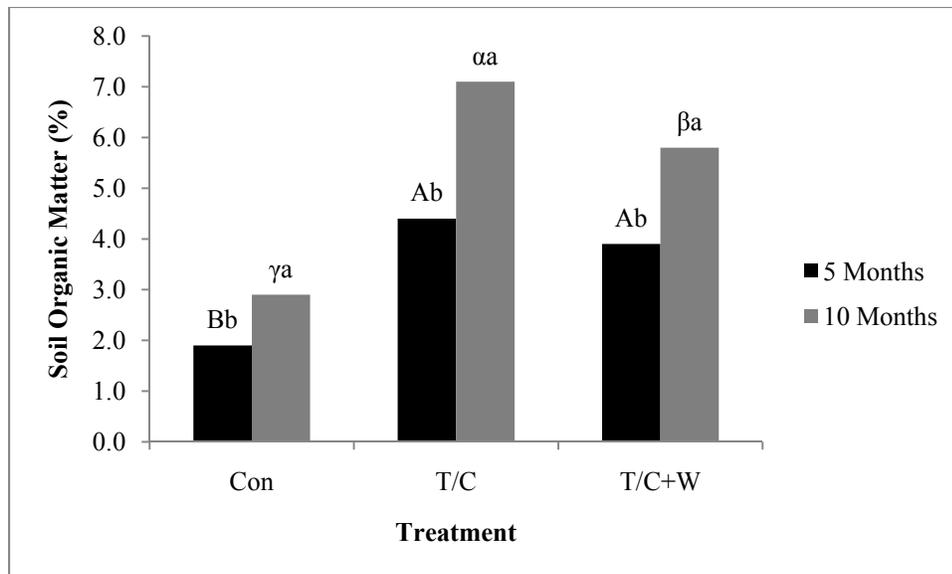
Figure 4. 1. Soil organic matter measured at 0–10 cm depth in field plots at two time intervals following soil rehabilitation. T/C plots were rehabilitated by a combination of mechanical and pneumatic soil loosening, compost incorporation, and organic mulch top-dressing. T/C+W plots were rehabilitated in the same manner, but were inoculated with *Lumbricus terrestris* prior to mulching (n=20 for each treatment–interval combination). Uppercase Latin and lowercase Greek letters denote statistical significance of soil treatment simple effect within each level of time since inoculation. Lowercase letters denote statistical significance of time since inoculation simple effect within each level of soil treatment. Columns not connected by same letter are significantly different.

4.2.3 Soil Respiration

Date of measurement had a significant effect on soil respiration, while soil treatment had a marginal effect (Table 4.6). There was no date by soil treatment interaction. During the course of measurement, CO_2 efflux was highest in April, ten months after inoculation, followed by March, February, and December, which did not differ from one another, and lowest in January (Figure 4.2). On average, across all dates, soil respiration in Con plots was significantly different from T/C+W plots and marginally significantly different from T/C plots. Additionally, the two soil treatments did not differ from one another.

Table 4. 6. Repeated measures analysis of soil CO$_2$ efflux measured in field plots at monthly intervals following soil rehabilitation. T/C plots were rehabilitated by a combination of mechanical and pneumatic soil loosening, compost incorporation, and organic mulch top-dressing. T/C+W plots were rehabilitated in the same manner, but were inoculated with *Lumbricus terrestris* prior to mulching.

Model Effects	Type 3 Tests of Fixed Effects		
	Num DF	Den DF	Pr > F
Block	4	8	0.0209
Soil Treatment (ST)	2	8	0.0649
Date (D)	4	47	<0.0001
ST × D	8	47	0.1765

Soil Treatments	Least Squares Means		
	Mean	SE	Pr > $|t|$
Con	1.120	0.152	<0.0001
T/C	1.546	0.151	<0.0001
T/C+W	1.699	0.151	<0.0001

Comparisons	Differences of Least Squares Means		
	Estimate	SE	Pr > $|t|$
Con v. T/C	-0.426	0.214	0.0818
Con v. T/C+W	-0.579	0.214	0.0269
T/C v. T/C+W	-0.153	0.214	0.4946
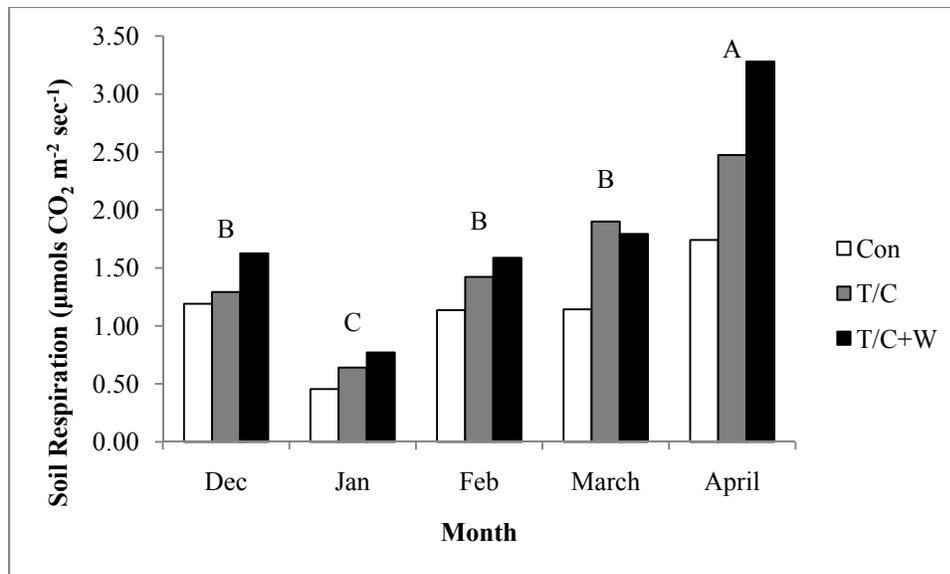
Figure 4. 2. Soil respiration measured using a LiCor-6200 with 3.5" head space in collars at field site in Blacksburg, Virginia 5 through 10 months after application of soil treatments. Only plots planted with *Acre rubrum* were measured across all treatments (n=30). Columns not connected by same letter are significantly different.

4.3   Discussion

4.3.1   Earthworm Presence Assessment

Earthworm presence assessments suggested that worm mortality was high in the experimentally inoculated plots; however, many soil properties showed a significant effect of inoculation treatment, indicating that inoculation had some short term benefits. There were anecdotal observations of middens – casts brought to the surface by anecic worms – and burrow holes in the plots during each assessment event, proving that earthworms were active during each assessment. Furthermore, juvenile worms were common ten months post-inoculation, suggesting that inoculated earthworms were not only surviving, but were reproducing.

Earthworm population establishment may not have been successful due to mortality, escape, or sampling limitations. Soil treatment attempted to create a more hospitable environment for worms, but could not change the texture of the soil. The Groseclose soil series found at the site is described as being sticky, plastic clay in the subsoil, and because the site had been graded, filled, and then scraped over the years, this subsoil was just below the turf. This soil has slow permeability and a high water holding capacity, and these characteristics may have made the site too wet for earthworms to survive. However, the dry mass of the inoculated earthworms was so minute that changes in soil properties in inoculated plots could not have been due to the mere presence of worm carcasses. At the time of inoculation, the earthworm barriers were intact and strong, but after several months, the barriers tended to fail aboveground allowing for possible ingress and egress from plot to plot and from outside the plots. This could help explain the presence of earthworms in T/C plots, specifically in edge plots located close to the surrounding turf, and the seeming absence of worms in some T/C+W plots. Finally, sampling techniques may not have been successful in extracting a representative sample of earthworm survival. The sampling pits only excavated 1/64th of the surface area of each plot, leaving much area not sampled. Additionally, because of the clayey nature of the soil, the chemical technique needed to extract the deep-burrowing worms used in inoculation, may not have been effective due to an inability of the mustard solution to permeate into the soil.

Although, it may seem that earthworm inoculation was unsuccessful, there was a slight (non-significant) increase in the number of inoculated plots that yielded worms during sampling and a slight (non-significant) increase in the number of worms extracted, over the course of the study. Bohlen et al. (1995) also found an increase in abundance and biomass after a year, in

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addition to an inoculation effect on nutrient cycling and soil aggregate stability, attributed mostly to *L. terrestris*. Samplings over the coming years are needed to draw definitive conclusions regarding inoculation success.

4.3.2   Soil Properties

Soil physical and chemical properties were altered by plot installation disturbance, soil treatment, and, in some cases, earthworm inoculation. Examination of earthworm dry mass revealed that 26.8 ± 2.5 g of worms were applied to each inoculated plot; it seems unlikely that merely earthworm carcasses could have affected soil properties – the worms must have been active in the plots for some duration. Changes in chemical properties were mostly driven by the addition of compost. Because pneumatic soil decompaction and organic incorporation is becoming a widely accepted soil rehabilitation technique for degraded landscape tree soil, the effect of earthworm inoculation in addition to these practices is of particular interest.

Bulk Density

Bulk density significantly increased and soil organic matter significantly decreased after initial soil disturbance, indicating that the site preparation succeeded in replicating a degraded urban soil. The measured bulk density was low enough to allow for root penetration and eventual earthworm burrowing. High bulk density can limit root growth and deter earthworm movement within the soil. Earthworms prefer to move within looser soils and avoid more compacted soils

(Stovold et al. 2004), so soils with higher bulk densities would encourage egress from the exact soils that need their ameliorative benefits. The pre-inoculation treatment of tilling and compost incorporation successfully decreased bulk density and increased organic matter content, which should have created a more habitable setting for earthworm survival.

Ten months after soil treatment, bulk density was still significantly lower in treated plots than Con plots, a relic of initial tilling and compost addition during soil treatment. However, there appeared to be no earthworm effect on soil density over the course of this study. Baker et al. (2006) summarized two studies that looked at the effect of inoculation on bulk density. Under the more controlled setting of a greenhouse, researchers found that earthworm inoculation did have an effect on bulk density; however, other researchers found that field inoculation did not change bulk density. In experiments exploring the relationship between earthworms and soil physical properties under different tilling intensities, Johnson-Maynard et al. (2007) also failed to demonstrate an earthworm effect on bulk density. The researchers surmised that either the earthworm population was too low to make a difference, the earthworms had limited activity due to harsh environmental conditions, or a discrepancy in scale between the differences caused by earthworms and the scale of measurements. They also proposed future researchers take samples over a longer time period to detect effects on bulk density. Bulk density measurements taken in the current experiment may have been incomplete due to the fact that samples were only taken twice, making it difficult to monitor initial changes in soil compaction. Additionally, these measurements may have been affected by soil settling after being tilled. It would be interesting to determine how bulk density changes on a month to month or every three month basis following inoculation.

pH

Soil pH at the site remained stable following soil disturbance, but significantly increased following pre-inoculation soil treatment. The pH observed at that time was appropriate for tree planting (an ideal range would be between 5.0 and 7.5) (Craul 1999) as well as earthworm survival. Although earthworm tolerance of soil pH is species-specific, a general range runs from as low as 4.0 to as high as 7.0, with optimal conditions closer to 7.0 (Card et al. 2002; Edwards and Bohlen 1996; Lee 1985), further suggesting that the rehabilitated soil conditions were suitable for earthworm survival.

Five months after treatment, soil pH was highest in plots inoculated with earthworms, followed by T/C plots, with Con plots being the lowest. Despite only modest changes in pH, the two soil treatments no longer differed ten months after treatment; however, soil pH was still significantly higher than in Con plots. Throughout the experiment, pH in Con plots remained stable, indicating that mulching did not affect soil pH and that the treatment practices employed (e.g. tilling and compost amendment) drove the increase in pH. Elevated pH five months post-inoculation in earthworm plots may have been attributable to increased earthworm activity. A considerable number of earthworm castings were observed in inoculated plots in November 2008. Many case studies presented by Lee (1985) and Edwards and Bohlen (1996) observed that earthworm casts often have a higher pH than the soil surrounding them. Diminished earthworm activity over the winter months may explain the lack of significant difference between treated plots in April 2008.

An additional note should be made regarding the relationship between soil pH and tree species. Ten months following inoculation, soil pH was significantly higher in plots planted with *A. rubrum* than *C. florida*. Although, there seemed to be a significant effect of tree species on soil pH, there was no tree species x soil treatment interaction, indicating that this phenomenon was not related to earthworm inoculation and may have been due to differences in root turnover or root exudates between the two tree species.

Cation Exchange Capacity

CEC at the site decreased with disturbance and increased with soil treatment, and remaining higher in treated plots than Con plots. However, the CEC was considered low for a clay/clay loam soil such as that found at the Turfgrass Research Center - Havlin et al. (1998) note that 20–50 meq 100 g^{-1} is typical CEC for clays and clay loams.

CEC is dependent on clay mineralogy and organic matter content, both of which have negative surface charge and are considered colloidal particles with high surface area to volume ratio. A CEC that is high provides a greater capacity to hold nutrients available for plant uptake, and a CEC that is low can allow important ions to leach out of the soil with the soil solution before plants can use them (Singer and Munns 2002). CEC is also very dependent on soil pH, where lower CEC is associated with a lower pH. Although CEC impacts on earthworm survival are not well understood, an understanding of the influence of clay, organic matter, and pH can lead to an explanation of worm survival under certain conditions. A soil with higher pH, clay

content, and organic matter will have higher CEC. Because earthworm populations do well under higher pH and organic matter conditions, it could be hypothesized that earthworms would do well in soils with higher CEC.

Five and ten months following inoculation, CEC was highest in T/C+W plots than T/C plots, which were higher than Con plots, however, throughout the experiment, CEC did tend to decrease with time across all treatments. Regardless of this decline, worm inoculation does have an effect on cation exchange capacity, making it higher than similarly treated plots without worms, most likely due to a reduction in organic matter in the top 10 cm of soil.

Soluble Salts

Soil disturbance associated with site preparation did not affect soluble salt content, but pre-inoculation soil treatment increased content in the treated plots due to the addition of compost. High levels of soluble salts can inhibit plant growth and development, often manifesting itself through leaf scorching or stunted growth. When salts are high, the osmotic water potential is lowered, making it harder for plants to take up water and necessary nutrients in the soil solution (Singer and Munns 2002). Just as plants are affected by high soluble salt content, earthworms are sensitive to high amounts of salts (Card et al. 2002) because they are mostly water and require water to function and breathe. They are particularly susceptible to high salt content because they acquire water from the foods they eat and from water stored in soil pores and have no natural defense against water loss (Lee 1985). However, soluble salt content at

this site was never recorded above 294 ppm, and lethal levels have been reported at 5,000 ppm (Lee 1985).

Soluble salt content in treated plots declined by more than half after five months' time, yet remained higher than in Con plots. After ten months, salt content decreased by half of the five month measurements and was no longer significantly different from levels in the Con plots. Interestingly, salt content gradually increased (not statistically analyzed) from pre-inoculation to five months and to ten months post-inoculation in Con plots. This pattern could be a result of salt leaching out of the top layer of soil throughout the year. Soluble salts were clearly higher in pre-inoculation, tilled plots due to the addition of compost. Con plots did not have compost incorporated in the soil, but did have mulch applied as a top dressing, which could be the reason why salt content tended to increase over time.

In studies exploring the vermicomposting of sewage sludge, researchers described high levels of soluble salts in earthworm casts leading to the suggestion that casts be treated to leach out the salts prior to use in planting (Lee 1985).

Organic Matter

Site preparation reduced organic matter from 5.0% to 1.5%, however, these results are most likely overestimates of actual organic matter present due to the use of loss on ignition as the test for soil organic matter content. Low organic matter can support tree planting yet soils with

low organic matter content cannot support large earthworm populations (Edwards and Bohlen 1996), thus, to ensure organic matter was not a limiting factor to inoculation success, compost was added as part of the pre-inoculation treatment. After pre-inoculation soil treatment, organic matter was increased significantly, presumably creating a better habitat for earthworm survival. Case studies reported by Edwards and Bohlen (1996) reported larger numbers of earthworms in fields augmented with much less manure per hectare than our application, signifying that our organic matter incorporation was more than sufficient for the inoculation size.

Five months post-inoculation, organic matter was higher in treated plots than Con plots. However, ten months following inoculation, T/C plots had significantly higher organic matter content than T/C+W plots, which were higher than Con plots. Desjardins et al. (2003) also found lower organic matter in plots inoculated with epigeic worms, however, (Shuster et al. 2001) found that inoculation with anecic worms actually increased average soil carbon. Deep-burrowing worms feed at the soil surface and pull organic matter into their burrows, further incorporating it with the mineral soil in lower soil horizons (Edwards and Bohlen 1996; Lee 1985). Lower organic matter in the top 10 cm of soil in the T/C+W plots may indicate that earthworm activity had moved the organic matter below 10 cm, which was below our sampling area. This is consistent with the behavior of the anecic worms used for inoculation. Additionally, organic matter was higher ten months after inoculation in every treatment. This is most likely due to decomposition of mulch and further incorporation by decomposers into the top 10 cm of soil.

<u>Soil Nutrients</u>

Macronutrients (P, K, Ca, and Mg)

      The addition of compost to the soils during pre-inoculation soil treatment increased several soil nutrients, specifically the macronutrients phosphorous, potassium, calcium, and magnesium, and the micronutrients iron and manganese. Maguire and Heckendorn (2009) make recommendations for the macronutrients measured at our site, all of which were increased with the compost addition. Phosphorous was considered to be low prior to pre-inoculation soil treatment and was increased to a moderate level after treatment. Potassium was decreased to a low level through disturbance, but also increased to a moderate level with compost addition. Calcium was at a moderate level during the disturbance phase of site preparation, but was significantly increased to a very high level by pre-inoculation soil treatment. Finally, magnesium was consistently at very high levels, regardless of the significant decrease after disturbance.

      As discussed previously, earthworm casts have higher levels of exchangeable P, K, Ca, and Mg than the surrounding soil (Edwards and Bohlen 1996; Lee 1985). The magnitude of higher levels is likely species-specific, with even higher levels related to litter–feeding species than deeper burrowing species, which consume more soil than plant material. Edwards and Bohlen (1996) discuss the work of Basker et al. (1994) in which conflicting results were found. Earthworms actually decreased potassium levels in one laboratory soil and, in others, the researchers could find not consistent trends in earthworm influence on exchangeable cations,

leading to the conclusion that higher levels in casts could be due to the preferential ingestion of certain materials high in those cations, not because of earthworm gut processes.

Five and ten months post-inoculation, soil phosphorous was higher in treated plots than Con plots. After earthworm inoculation, we would expect higher levels of P in T/C+W plots because earthworms are known to increase available P in soils (Edwards and Bohlen 1996). Earthworm casts tend to have more available P than the surrounding soil and some researchers have even found increases in casts $5 - 10$ times greater than the surface soil (Lee 1985). Additionally, earthworm activities have been found to increase P plant uptake from plant litter (Edwards and Bohlen 1996). P levels in Con plots remained relatively stable throughout the experiment, indicating that the amended compost contained higher amounts of P. Additionally, there is an emerging trend of slightly higher P in T/C+W plots than T/C plots and a trend of increasing P from five months post-inoculation to ten months post-inoculation. Further exploration in the coming months might yield a significant worm effect.

Higher levels of potassium would also be expected in T/C+W plots post-inoculation. As discussed previously, earthworm casts tend to have higher levels of exchangeable cations than the surrounding, undigested soil. Treated plots were consistently higher than Con plots, indicating that the compost amendment was a source of K. Interestingly enough, soil K continuously increased over the term of the experiment, most likely due to leaching from the mulch application and a pooling in the top 10 cm of the soil.

Soil calcium was significantly higher in T/C+W plots than in T/C plots, which was higher than in Con plots. The higher level of Ca in T/C+W plots is to be expected because concentrations of available Ca are usually significantly greater in casts than the surrounding soil (Edwards and Bohlen 1996), an effect that can only occur with earthworm inoculation. This effect appeared after five months and remained after ten months. Throughout the term of the experiment calcium decreased across both tilled treatments after pre-inoculation treatment, indicating that the compost was also a source of Ca.

A higher concentration of magnesium in T/C+W plots was expected for the same reasons as Ca. However, this effect appeared after ten months, indicating that the influence of earthworm activity on Mg is more delayed than Ca. Higher levels of Mg were consistently measured in tilled plots, specifically T/C+W plots, which were significantly higher than Con plots pre-inoculation and significantly higher than both T/C and Con plots ten months post-inoculation. Much like Ca, Mg levels increased in Con plots after five months and then decreased after ten months.

Micronutrients

The micronutrients measured remained stable through site preparation, and then increased with pre-inoculation soil treatment. Iron and manganese are closely related within the soil solution; often Fe deficiencies can be caused by high levels of Mn and, conversely, high Fe levels can cause Mn deficiencies (Vitosh et al. 1994). Compost amendment during the pre-

inoculation soil treatment succeeded in increasing Fe levels in treated plots. Five and ten months post-inoculation, iron was higher in T/C+W plots than in T/C plots, which was higher than in Con plots, indicating earthworm activity effected Fe soil content. Soil Fe content remained stable after five and ten months post-inoculation across treatments.

After five and ten months, soil Mn was significantly higher in T/C+W plots than in T/C plots, which was higher than in Con plots. Like Fe, Mn stayed stable after five and then ten months post-inoculation across treatments. Clearly, compost amendment increased Mn, which then decreased and settled in treated plots. In addition, a tree species effect appeared after ten months. Much like soil pH, Mn levels were higher in plots planted with *A. rubrum* than in plots planted with *C. florida*. Again, this might be attributed to differing root turnover or root exudates.

4.3.3 *Respiration (CO$_2$ Efflux)*

Soil respiration was highly affected by measurement date and marginally affected by soil treatment. Higher respiration rates occurred in April 2009, followed by December 2008, February 2009, and March 2009. Lowest respiration rates occurred in January 2009. Microbial respiration closely followed seasonal changes in temperature, which explains this seasonal pattern (Fisk et al. 2004; Schindler Wessells et al. 1997). Averaged across measurement dates, respiration was marginally higher in treated plots than Con plots. The incorporation of organic

matter into the top soil layer of treated plots probably provided ideal conditions for decomposing microbes to proliferate, contributing to higher respiration rates.

Schindler Wessells et al. (1997) found similar patterns while exploring the relationship between soil respiration and earthworm population augmentation and diminishment. Soil respiration at their research sites varied by season, which the authors attributed to increased biological activity in the soil and temporal differences in resource availability. Additionally, earthworms have been shown to directly affect respiration, contributing 5-6% to total soil respiration (Edwards and Bohlen 1996). Earthworms are also attributed with indirectly affecting respiration by stimulating microbial respiration (Lee 1985). These interactions would contribute to an overall increase in soil respiration.

Soil respiration should be taken throughout the next year to determine additional temporal effects and possible inoculation effects. Additionally, respiration measurements should have begun directly following inoculation. Borken et al. (2000) described an initial increase in CO_2 efflux one month after inoculation and then a steady decline for the rest of the study and attributed this peak and fall to the creation of burrows and incorporation of organic matter into the mineral soil during the first month. The authors explain the subsequent decrease by hypothesizing that this activity stabilized the organic matter, which prevented further microbial breakdown and associated microbial respiration.

4.4   Conclusion

Earthworm inoculation did not succeed in improving bulk density or increasing soil organic matter, however, inoculation was successful in increasing soil CEC, Fe, and Mn relative to compost only effects. Soil treatment did improve many soil characteristics, most of which had lasting improvements during the course of the study. Additionally, inoculation was successful in establishing a population of earthworms that could be extracted ten months after inoculation and showed signs of activity and reproduction throughout the course of the study.

Trees will need to be measured for growth over the course of several more years to determine the effect earthworm inoculation has had on improving soils enough to effect tree growth. Additionally, earthworm populations should be assessed for abundance, biomass, diversity, if earthworm ingress has occurred due to the breakdown of the earthworm barriers, and identification of age classes, all of which will help determine if inoculation has resulted in a long-term and sustainable population. Finally, soil samples, especially bulk density, and soil respiration should be taken on a regular basis to assess long-term inoculation effects.

Overall Summary

Comparisons of earthworm abundance and soil properties between the inoculation experiment and the observational study revealed similarities in earthworm abundance but few soil property similarities appeared. In urbanized landscapes of Roanoke, Virginia, higher abundance and biomass were extracted from forested sites. Soil properties associated with forested sites included higher soil moisture and lower soil temperatures, bulk density, pH, and CEC. Earthworm inoculation was successful in establishing a population of earthworms that could be extracted ten months after inoculation and showed signs of activity and reproduction throughout the course of the study. Inoculation was successful in increasing soil cation exchange capacity, iron, and manganese, but not successful in improving bulk density or increasing soil organic matter.

Earthworm extraction was much lower five months after inoculation than abundance in Roanoke measured at the same time of year – fall. However, extraction ten months after inoculation revealed similar numbers to those found in residential and commercial sites in the spring. Prior to inoculation, soil bulk density was similar to commercial and forested sites and was higher ten months after inoculation and similar to residential sites. Soil pH and organic matter content were both much higher prior to inoculation than any site found in Roanoke and remained lower ten months after inoculation. Soil CEC was also lower than any Roanoke land use type ten months after inoculation. In general, soil nutrients were consistently lower during the course of the experiment than any site in Roanoke, with the exception of iron, which was consistently much higher than any land use type.

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