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Natural Establishment of Earthworms on Reclaimed Lignite Mine Soils

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I dedicate this work to my children, Issac, Emily and Caleb who were born soon before, during and after this project. To my darlings, I hope my work digging in the soil to search for earthworms has instilled in you a deep love for the earth that will last your lifetime.

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CHAPTER 1

Introduction to Earthworm Research

Soils are crucial to life on earth. Their ability to sustain the growing human population depends on their quality and productive capacity. As an essential natural resource, soils supply humans with food and natural fibers as well as support important wildlife communities. As the human population increases, understanding and learning to manage soils through the study of soil science has never been more important. Severely disturbed lands, such as those that have undergone surface lignite mining, with sound reclamation practices have the capacity to support healthy ecosystems. Studies that focus on soil development after severe land disturbances will aid land managers in improving land to support the growth of food, natural fiber and wildlife.

Surface lignite mining requires that the material overlying the lignite seam (overburden) be removed and either stockpiled for future use in reclamation or placed directly in a reclamation area. Removing the overburden completely

disrupts the slow actions of the five soil forming factors: climate, relief, organisms, parent material and time. The once distinct soil horizons are no longer present. It is in this medium of highly disturbed land that forests are planted and attempts to reestablish a functioning ecosystem are made.

Two widely accepted definitions for soils exist within the field of soil science, the pedologic and edaphic definitions. Pedology defines soils as a natural body, studies the properties of soil horizons and the relationships among soils within a landscape. The edaphic definition of soil focuses on soil as a habitat for living things, especially plants. These two perceptions in soil science often overlap, but occasionally contend with each other. When discussing severely disturbed land, such as at lignite mines, a pedologist would hesitate to call the reclaimed land *soils*. However, these reclaimed lands do have the ability to support plant growth and may be accepted as soils according to the edaphic definition.

Soil Organisms and Rebuilding Disturbed Land Substrates

A major factor in the development of a productive soil after a disturbance such as surface lignite mining is the return of biological diversity to the belowground ecosystem. Table 1.1 provides examples of some of the many soil organisms that help make up the belowground ecosystem. Like the aboveground ecosystem, each group of soil organisms performs unique activities that contribute to soil formation and development.

Table 1.1. Examples of some soil organisms classified by size (table adapted from Brady and Weil 2008).

Generalized grouping	Macrofauna (>2 mm)	Macroflora (>2 mm)	Mesofauna (0.1-2 mm)	Microfauna (<0.1 mm)	Microflora (<0.1 mm)
Examples	gophers, mice, earthworms, ants	feeder roots, mosses	mites, collembolans, enchytraeid worms	nematodes, rotifers, water bears	root hairs, molds, actinomycetes,

Previous research on reclaimed lignite mine sites in the south central region of the United States has investigated the status of some soil organisms after land reclamation. Research on soil microbial life revealed that soil microbes, an essential part in the mineralization of organic materials and other soil functions, continue to improve with increasing time since reclamation (Swanson 1996; Ng 2010). Research on arthropod populations has shown that population density and species composition begin to resemble that of unmined soils 20 years after reclamation (Nelson 1989).

Earthworms as Indicator Species

The belowground soil ecosystem is very complex, with organisms greatly influencing soil physical, chemical and biological properties. Earthworms, considered indicator species in the soil, not only indicate favorable soil attributes required for earthworm survival, but also indicate favorable quality of the soil as a medium for plant growth (Muys and Granval 1997; Frouz 2006; Synder and Hendrix 2008). Earthworms provide several ecosystem services that are likely to accelerate

soil restoration, improve primary production and facilitate the restoration of functional ecosystems in mined areas (Boyer and Wratten 2010). Additionally, scientists have used earthworm survival rates to evaluate the effects of soil contamination with heavy metals and pesticides (Bouche 1981; Eijackers 2010). As a measure of forest soil quality, Muys and Granval (1997) found earthworm biomass to be a suitable indicator for detecting trends in soil pH.

Due to their overwhelming influence on soil properties, earthworms have been considered by many as the most important component of the soil biota in terms of soil formation, maintenance of soil structure and soil fertility (Edwards 2004). Reproducing populations of earthworms present in the soil facilitate ecosystem processes such as stable soil aggregate formation, nutrient mineralization and soil aeration (Snyder and Hendrix 2008).

The recovery of belowground ecosystem processes, such as earthworm activity in lignite mine soils is important to long-term successful reclamation of disturbed lands. Efforts to restore ecological function to disturbed soils have traditionally focused on reestablishing vegetation and wildlife communities; however, studies on the recovery of important soil animals such as earthworms are less common (Dunger and Voigtlander 2005; Boyer and Wratten 2010).

Earthworms have been recognized throughout history as important to soil development and plant growth. Aristotle called earthworms "the Intestines of the Earth" while Gilbert White (1789) wrote:

Worms seem to be great promoters of vegetation, which would proceed but lamely without them; by boring, perforating, and loosening the soil, and rendering it pervious to rains and the fibres of plants; by drawing straws and stalks of leaves and twigs into it; and most of all, by throwing up such infinite numbers of lumps of earth called worm casts, which, being their excrement, is a fine manure for grains and grass.

Almost one hundred years later, Darwin (1881) conducted the first scientific observation on the effects of earthworms on soil structure and the development of the humic layer or, "vegetable mould." He also focused on how earthworms contribute to the pedologic evolution of soils and landscapes through their significant contribution to soil mixing. He observed that the action of bringing subsurface material to the surface played an important role in burying surface stones and even abandoned buildings. Recognizing the large influence earthworms had on soil formation was revolutionary. Prior to the publishing of Darwin's *The Formation of Vegetable Mould through the Actions of Worms with Observations on Their Habits*, earthworms were widely considered garden pests.

Earthworms and Soils

The presence of earthworms in soils indicate favorable edaphic growing conditions for plants, except for circumstances of “earthworm invasions” such as that in the Great Lakes region of the United States (Chapter 3). Earthworms have been shown to influence soil properties such as soil formation, soil turnover, soil structure, soil aeration and drainage, organic matter decomposition and nutrient mineralization.

Burrows, Casts and the Production of Humic Material

Earthworms affect soil physical properties when they ingest and excrete soil to construct burrows as part of their feeding activities. The production of earthworm casts, or globules of soil that have been ingested then expelled by the earthworms, has been shown to increase the humic fraction of the soil. Humic substances make up about 60 to 80 % of soil organic matter and are composed of complex organic molecules that are resistant to decomposition (Brady and Weil 2008). This important part of the A horizon helps retain plant nutrients, water, and favorable soil structure. Earthworm casts also have higher levels of microbial activity, and are therefore higher in polysaccharides than the surrounding soil. It is believed that the “sticky” polysaccharides bind the material in the cast to give them the quality of being water stable.

The burrowing activity and formation of humus by earthworms have been called the prominent biological processes of soil aggregation (Butt 1996). By consuming organic matter, fragmenting it and mixing it with soil mineral particles, earthworm activities form water-stable aggregates (Edwards 2004). Aggregate formation from the mixing of mineral and organic matter improves water infiltration, percolation and moisture holding capacity of the soil. Earthworms also contribute to mixing of organic and inorganic components of the soil and to decreasing the size of these particles (Shrickhande and Pathak 1951). The movement of soil materials from the deeper soil horizons to more shallow soil strata affects horizon development. The amount moved can range from 2 to 250 Mg per hectare per year (Edwards and Bohlen 1996). The formation of burrows by earthworms influences both soil aeration and drainage.

Some species of earthworms make permanent burrows, where some move about the soil freely. Earthworm burrows help to promote soil aeration, drainage, and porosity and are usually lined with protein-based mucus that helps stabilize these channels (Edwards 2004). All earthworms burrow in generally the same way:

1. They first anchor their posterior setae, which increases the hydrostatic pressure in their coelomic cavity, then
2. Stiffen their longitudinal muscles so as to project the anterior segments forward.
3. Finally, their posterior segments are then drawn up toward the anterior part

of the body by contraction of the longitudinal muscles.

This sequence is repeated as the burrows are made. As earthworms exert this pressure, they ingest soil and eventually expel it as earthworm casts (Edwards and Bohlen 1996).

Earthworm burrows create macropores in the soil (Edwards and Lofty 1972). Macropores are large soil pores with a diameter generally greater than 0.06 mm, from which water drains readily by gravity. Macropores created by earthworm burrows often open to the soil surface, have a large diameter (>5 mm), and can act as pathways for the preferential flow of water, increasing infiltration (Edwards and Bohlen 1996). Water can penetrate the surface soil between two and ten times faster when earthworms are present than when they are not (Tisdall 1978). Even smaller burrows have been shown to conduct water effectively through the soil (Linden et al. 1994). By creating burrows, particularly in heavy soils, earthworm activity significantly contributes to soil aeration (Kretzschmar 1978).

Soil Fertility

Earthworms enhance soil fertility and productivity by altering chemical conditions in the soil, especially in the upper 15 to 35 cm. Earthworms influence organic matter and nutrient availability during transit of soil materials through their gut, in freshly deposited earthworm casts, in aging casts, and during the long-term genesis of the whole soil profile (Lavelle and Martin 1992). Earthworm casts are

higher in microbes, organic matter and available plant nutrients. Roots grow well through earthworm channels, finding rich sources of nutrients in the cast and burrow lining material (Brady and Weil 2008).

By aiding the process of nitrogen mineralization (converting organic nitrogen into plant available ammonium or nitrate) earthworms can lower the carbon: nitrogen ratio in the organic matter. Organic phosphorus (P) also may have higher mineralization rates when earthworms are present (Edwards 2004). Lavelle and Martin (1992) found more exchangeable and water-extractable inorganic P in earthworm casts than in non-ingested control soils. The concentration of exchangeable P in these fresh earthworm casts further increased after four days, a result of the abundant microbial life associated with earthworm casts.

In many ecosystems earthworms are key organisms in the decomposition of plant organic matter. Their ingestion of soil and organic matter also reduces organic matter particle size and increases the surface area of these soil particles. This increases the cation exchange capacity of soils.

The presence of earthworms in soils has been shown to influence plant production. In a greenhouse experiment using grasses, the presence of earthworms in the soil enhanced shoot and root growth, root proliferation and nitrogen (N) uptake from organic litter and soil in all plant species tested (Wurst et al. 2003). In another greenhouse experiment, Zhang et al. (2010) found earthworm activities to significantly increase the dry weights and N concentration of winter oilseed rape

(*Brassica napus*) compared with plants growing without earthworms in the soil.

Plant biodiversity

Earthworm also may influence plant biodiversity. Eisenhauer et al. (2009) found that endogeic earthworms may strongly impact the composition of the soil seed bank through ingestion and excretion of seeds. This consequently influences the plant community assembly as the earthworms move the plant seeds and often scarify the seed coats to promote germination.

Earthworm Species Diversity

Earthworms are discussed in popular literature with regard to their positive influence on the soil; however, few people are aware of the species diversity and range of behaviors that exist among earthworms. Over 5,200 earthworm species have been described worldwide, and it is thought that further surveys will reveal many more species. In the eastern United States, approximately 70 species of native earthworms have been described, with another 28 species in the Pacific region (Hendrix and Bohlen 2002). Bohlen (2002) described three categories of earthworm species based on their locomotion through the soil and their feeding habits, (although many earthworm species fall somewhere in between these categories):

1. epigeic earthworms that live in the surface litter layers and feed on decomposing plant material,
2. anecic earthworms that make permanent vertical burrows and feed on O horizon plant litter and organic matter in the mineral soil,
3. endogeic earthworms are soil feeders that make horizontal burrows in the uppermost soil

Peregrine Earthworm Species

The term 'peregrine' is used to describe earthworm species that are widely distributed. These species have the ability to overcome natural migration barriers such as seas, mountain ranges and temperatures above 35° Celsius (C) or below 0°C. Some genera and species of earthworms, particularly those belonging to the Lumbricidae family, are among the most widely distributed. Often, when these species are introduced to new areas, they become dominant over the endemic species (Edwards 2004).

Earthworms on Reclaimed Lignite Mine Lands

Lignite coal, also referred to as brown coal, is a soft brown to black combustible sedimentary rock formed from naturally compressed, partially decomposed, plant material (peat). Lignite coal mining in Texas, and throughout the United States, is an important part of the economy and a reliable local source of fuel

for electric power (Clower and Reyes 2013). The process of lignite coal mining involves removing the surface material (overburden) overlaying the lignite seams, then stock piling the soil before it is used to reclaim the mined land or, if possible, immediately placing it in an already excavated area. During this process soil organisms, including earthworms, die due to the resulting severe conditions and loss of habitat (Eijsacker 2010). Recovery of the belowground ecosystem and restoration of soil health are essential to the success of land reclamation (Stewart et al. 1988; Muys and Granval 1997; Hüttnl and Bradshaw 2001).

Earthworms Return to Reclaimed Lignite Mine Soils

During the early successional stages of reclaimed lignite mine land, soil conditions are unfavorable for colonizing soil fauna (Eijsackers 2010). The lack of organic matter, water stable aggregates, soil structure and sometimes very low pH values are a few of the obstacles that limit the return of these soil organisms (Eijackers 2010). Despite these obstacles, earthworm population densities on reclaimed lignite mine lands have been found to eventually resemble those of unmined lands. Hüttnl and Weber (2001) found flora and fauna succession in forest stands on un-mined sites to be similar to the early stages of forest ecosystem development on reclaimed mined sites. The authors also found soil organism abundance and activity at reclaimed land to reach levels typical of non-mined sites after 20–30 years. They concluded that mine land restoration may be used as an

ideal case study for forest ecosystem development at “point zero” on “*terra nova*.” In east Texas, the reclaimed lignite mine lands may represent time zero for primary succession and redevelopment of the belowground ecosystem after severe disturbances.

The recovery of belowground ecosystem processes, such as earthworm activity in reclaimed lignite mine lands, is important to long-term successful reclamation. Efforts to restore ecological function to disturbed soils have traditionally focused on reestablishing vegetation and wildlife; however, studies on the recovery of important soil animals such as earthworms are less common (Dunger and Voigtlander 2005; Boyer and Wratten 2010). The influence of earthworms on soil structure, fertility and to the maturation and development of reclaimed mineral soils in the south central region of the United States has yet to be thoroughly studied.

Earthworms Help Improve Soil Quality on Reclaimed Lands

Earthworms, keystone species in the belowground ecosystem (Muys and Granval 1997) are recognized for providing several ecosystem functions that are likely to accelerate soil restoration (Boyer and Wratten 2010). Earthworms are known to incorporate organic matter into mineral soils on reclaimed lignite mine lands (Frouz et al. 2013) as well as mineralize organic matter to increase plant

available nutrients (Brady and Weil 2008). Soil physical properties such as soil structure (Stewart et al. 1988; Eijackers 2010) and stable aggregate production (Damoff 2008; Marinissen 1994) are also improved from the actions of earthworms.

In Europe, research on reclaimed lignite mine soils has found the presence of earthworms to improve soil quality. In South Wales, UK, Scullion and Malik (2000) found earthworms to increase stable soil aggregate production and the carbon content of the clay-sized fraction of the soil. Soil microbial biomass increased near the soil surface. In Germany, Dunger (1969) found that earthworm establishment on reclaimed lignite mine lands under *Alnus glutinosa* improved litter incorporation into mineral layers. After earthworms were introduced into reclaimed lignite mine lands, the incorporation of litter was accelerated and the amount of exchangeable cations and available P was increased.

Introduction to this Research

This study is part of a broader effort to better understand the role of soil biology, especially earthworms, in reclaimed mine lands. In an attempt to quantify the abundance (i.e. earthworm density [earthworms m⁻²]) and species diversity of earthworms on reclaimed lignite mine lands in east Texas, this study used the following indicators of earthworm community characteristics: reclamation type,

forest cover type, age since reclamation and select physical and chemical soil properties.

Because reclamation schemes cannot focus on every species in an ecosystem, keystone species are usually selected based on their important role in the ecosystem. Previous research on the ability of earthworms to restore and improve the functionality of the soil after surface mining suggests earthworms as a keystone species in the restoration of the overall ecosystem of reclaimed mine lands.

The primary research objectives were:

- 1) Determine earthworm abundance under afforested pine plantation and mixed pine and hardwood forest cover types on reclaimed lignite coal mine lands on a chronosequence from age 2 to 28 years since reclamation (Chapter 2).
- 2) Determine earthworm species composition in afforested pine plantation and mixed pine and hardwood forest cover types on reclaimed lignite mine lands on a chronosequence from age 2 to 28 years since reclamation (Chapter 3).
- 3) Measure selected soil parameters that affect earthworms or are influenced by earthworms under afforested pine plantation and mixed pine and hardwood forest cover types on reclaimed lignite mine lands on a chronosequence from age 2 to 28 years since reclamation (Chapter 4).

The chronosequence approach is useful as it represents time through locations producing results that indicate the presence or lack of a linear trajectory of increasing earthworm abundance through time (Walker et al. 2010). However, the results of the chronosequence approach may be confounded by the influence of landscape variability (del Moral 2007), and chronosequence studies are generally characterized by the problem of trying to compare conditions that are in fact not fully comparable (Hüttl and Weber 2001).

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CHAPTER 2

Earthworm Abundance on Beckville and Oak Hill Lignite Coal Mine Soils

Literature Review

Texas is the United States' fourth-largest coal producer, yielding nearly 46 million Mg in 2011 (Independent Statistics and Analysis 2016). Because the lignite in Texas is surface mined, the overburden above the lignite overburden becomes severely disturbed during its removal, potentially destroying the complex belowground ecosystem. When the reclamation process begins, the land is shaped to approximate natural contours. Suitable cover crops, usually grasses and forbs, are planted to stabilize the soil and slowly the belowground ecosystem begins to re-establish. Previous research on reclaimed lignite coal mine lands note that vegetation communities, including forests, are successfully restored, but little is known of the recovery of belowground ecosystems (Christian 2013).

Despite research that supports the importance of restoring the belowground ecosystem after surface mining (Scullion and Malik 2000; Snyder and Hendrix 2008 Boyer and Wratten 2010), there is a paucity of research on the recovery of earthworm communities after surface mining lignite coal and subsequent land reclamation in the United States. The mine soils that develop after surface mining

are vastly different in physical, chemical and biological properties from the pre-mining soils. For example, soil structure is damaged or eliminated as naturally formed soil aggregates are broken up during the process of overburden removal. Reduced substrates are often exposed as the overburden is removed and returned to an excavated pit. Soil biota rarely survive as the organic matter, oxygen and water from the surface horizons are often buried during excavation. These new mine soils are particularly unfavorable for earthworms because of the lack of soil structure (Stewart et al. 1988), potential compaction, low to no organic matter content, unfavorable moisture conditions, and sometimes low soil pH (Curry 2004; Eijsackers 2010). The lack of surface organic litter layers allows for greater fluctuations in soil temperature and the impact of UV radiation is increased due to the lack of a vegetative layer (Eijsackers 2010). The success of earthworms during the early stages of reclamation depends on the ability of the species to tolerate great fluctuations in temperature and moisture levels. Soil amelioration such as liming, fertilization, and the planting of grasses and forbs to promote soil stabilization (Lee 1985) help improve soil properties and encourage reestablishment of soil fauna (Curry 1994; Curry, 2004).

Earthworm colonization of reclaimed mine lands is further complicated by the limitations of earthworm migration. Some earthworm species have been shown to actively migrate less than 10 m per year (Wanner and Dunger 2002) and therefore rely more on passive transport onto severely disturbed sites (Dunger and

Voigtländer 2005). One source that facilitates the initial invasion of species in reclaimed areas may be soil that remains attached to the roots of tree seedlings used for afforestation. Birds and mammals also may carry earthworm cocoons attached to their feet, while humans may transfer cocoons in tractor or truck tires (Marinissen and van den Bosch 1992). Earthworm cocoons also may be transported by overland flow during heavy rains (Atlavinyte 1965), or in flow of intermittent streams and ditches (Schwert 1980).

Studies on reclaimed mined soils in the Lusatian lignite coal mining district in Eastern Germany found that earthworms returned to reclaimed soils as soon as three years after land reclamation with deciduous forests (Topp et al. 2001; Dunger and Voigtländer 2005) and as late as 10 years with establishment of pine forests (Dunger and Voigtländer 2005). Earthworm densities also increased with time since reclamation (Hüttl and Weber 2001; Wanner and Dunger 2002; Dunger and Voigtländer 2005) and, under optimal conditions, earthworm species richness and abundance were similar to those of unmined lands within 20 to 25 years after land reclamation (Hüttl and Weber 2001). In England, Armstrong and Bragg (1984) found total earthworm populations similar to undisturbed adjacent control areas within 10 to 20 years after surface soil replacement.

Frouz et al. (2013) found no difference between total earthworm abundances on 10–20 year old reclaimed sites and their equivalent unmined sites in Tennessee, Indiana, and Illinois. However, in this study earthworms of the family Lumbricidae

were found to dominate in the reclaimed sites, while Acanthodrilidae (especially *Diplocardia*) dominated in unmined sites.

Through their physical structure, especially their above and belowground litter inputs, trees have large impacts on chemical, physical, and biological properties of the soil system (Hendrix and Mueller 1992). Conifer afforestation has been shown to result in reduced litter quality, decreased soil pH, and deterioration of soil structure, conditions which may reduce earthworm populations (Curry 2004).

The size and structure of post reclamation earthworm communities may depend on the species of trees planted in the reclaimed areas (Neiryneck et al. 2000; Reich et al. 2005). Fewer numbers of earthworms were found under Norway spruce (*Picea abies*) and European larch (*Larix decidua*), whereas Scots pine (*Pinus sylvestris*) and oak (*Q. petraea* and *Q. robur*) influenced earthworm communities in a positive way. A positive correlation was also found between earthworm populations and the number of tree species in an afforested area (Cesarz et al. 2007). Schwarz et al. (2015) found earthworm communities to be significantly influenced by the presence of certain tree species rather than the diversity of the forest. Therefore, plant diversity may only weakly influence earthworm abundance, while the presence of certain plant functional groups may have more influence. Earthworm communities in tree stands of less than or equal to 10 years were driven by tree species composition (evenness) rather than by the number of tree species.

They recommended assessing litter and root traits to better understand tree species effects.

The lignite mines in east Texas present a unique opportunity to study the establishment of earthworms on these severely disturbed areas. Two mines in east Texas were chosen as study sites where chronosequences were established under mixed pine and hardwood and pine plantation cover types. Each chronosequence contained three time-since-reclamation sites: young post-mining sites 2–8 years after reclamation, mid-aged post-mining sites 12–18 years after reclamation, and older post-mining sites 22–28 years after reclamation.

Study Site Descriptions

The Beckville (Latitude: 32.23 °N, Longitude: -94.73 °W) and Oak Hill (Latitude: 32.22°N, Longitude: -94.73 °W) lignite coal mines are located in the Southern Mixed Forest Province (Bailey 1995) in the northern portion of east Texas. This area includes a region commonly referred to as the Piney Woods ecoregion of east Texas, where mean annual rainfall ranges from 1016 to 1270 mm and mean annual temperature ranges from 17 to 20 °C (NRCS 2015). The pre-mining soils were classified as Hapludults (NRCS 2015). These soils were formed from clayey, shale and sandstone marine deposits, as well as sand and loamy residuum. Depth to the lignite seams ranged in depth from 3 m below the soil surface to 61 m, but was usually 15 to 18 m deep (Grimes 2015).

The Beckville lignite mine uses the reclamation method called mixed-overburden, where the overburden material is immediately placed into an existing excavated pit where the lignite coal has already been removed. The oxidized and reduce overburden are mixed in the process. The surface soil at Beckville is rarely stockpiled. The new surface is then shaped approximating the original contours and the process of vegetation reclamation begins. Lime is added if soil pH is below 5.5 (Grimes 2015).

The Oak Hill lignite mine practices a method of reclamation called haul-back. Here, the reduced overburden material contains higher concentrations of pyrite (FeS_2) that if exposed to oxygen has the potential to create acidic conditions that may prevent successful establishment of vegetation for many years (Hüttl and Weber 2001). The surface layers of oxidized overburden (approximately 1.2 m deep) are separated from the underlying reduced overburden material during excavation. After mining is completed, the oxidized surface overburden is then placed on top of the unoxidized material to a minimum depth of 1.2 m. The oxidized material is occasionally stock-piled if necessary (Lamb 2015).

Unmined Reference Sites

Unmined sites in and around the Beckville and Oak Hill mines were difficult to access because of their location on private property or the density of the

understory. Therefore, previous research conducted in east Texas on unmined mixed pine and hardwood communities (Damoff 2008) and pine plantations (Wilson 2001) served as baseline data for earthworm community populations on unmined lands. The vegetative overstory at Damoff (2008) sites included *Pinus taeda*, *P. echinata* mixed with oak species (*Quercus falcata* and *Q. stellata*). The soils were predominately Alfisols and Entisols. Wilson (2001) studied earthworm abundance under pine plantations (*P. taeda*) planted on 1.8 m x 3.0 m spacing. The soils were a combination of Ultisols and Entisols (NRCS 2015) (Table 2.1).

Table 2.1. Pre-mining soil conditions at the Beckville and Oakhill mines, and reference sites (RS).

Location	Dominant Soil Order	Dominant Soil Moisture Regime	Soil pH	Clay Content (%)	Organic Matter Content
Beckville, Oak Hill (NRCS 2015)	Ultisols	Udic	5.0-5.8	12-35	Low
Mixed Pine and Hardwood RS (Damoff 2008)	Alfisols	Udic	5.1-6.0	18-26	Low
Pine Plantation RS (Wilson 2001)	Ultisols	Udic	5.0-5.8	12-35	Low

Initial Land Reclamation

After replacement and contouring of the overburden material, the reclamation process at both mines included an initial planting of winter wheat (*Triticum aestivum*) in the fall months (October through November) to stabilize the overburden material. If additional stabilization was necessary, millet

(*Setaria italica*), a spring annual, was planted the following spring. The following fall, winter wheat was drilled into the soil and fertilized. From January through March tree seedlings were planted. At pine plantation sites, second and third generation improved loblolly seedlings (*Pinus taeda*) were planted at 625 trees ha⁻¹. At the mixed pine and hardwood sites, 15 to 30 % of the area was planted in loblolly pine and 70 to 85 % were planted with hardwood trees native to the southeastern portion of the United States. Of those hardwoods, 80 % were native oaks (*Quercus spp.*). The remaining 20 % of the native hardwoods were minor species such as *Fraxinus americana*, *Liquidambar styraciflua*, *Prunus serotina*, and others (Grimes 2015).

Average temperatures during the months June through August in East Texas range from 22 to 34 °C, but may reach as high as 38 °C (US Climate Data 2015). Because the majority of earthworm species are known to be inactive at temperatures above 25 to 35 °C (Curry 2004), sampling was not conducted during the hot summer months of June through August. Field work began during late September and ended during the month of May. At each mine, 30 sites were chosen according to age class and vegetation type (Table 2.2).

At both Beckville and Oak Hill, 15 pine plantations (pine) and 15 mixed pine and hardwood (mixed) sites were selected. Because site selection was limited to appropriate age and cover type, site selection was intentional and based on availability.

Table 2.2. Experimental design to compare reclamation type (mixed overburden vs. haul-back,) cover type (pine plantation [pine] vs. mixed pine and hardwood [mixed]) and age classes (2–8 vs. 12–18 vs. 22–28). The total number of sites sampled was 60.

Age Class	Mixed Overburden		Haul-back	
	pine	mixed	pine	mixed
Years	Number of Sites			
2–8	5	5	5	5
12–18	5	5	5	5
22–28	5	5	5	5
Total	15	15	15	15

Each site represented a replicate within each age class and cover type. At each site, ten 25 m transect lines were placed 1 m apart along a 10 m base transect line. Ten soil pit locations on the resulting grid were randomly selected using a random number generator. Each soil pit was 50 x 50 cm and 30 cm deep. Soil was excavated and placed on a polyethylene tarp. No obvious earthworm burrows were seen extending below the base of the pits. Earthworms were located using hand-sorting methods described by James (1996) and kept in a polyethylene lined soil sample bag with moist soil while transported back to the laboratory for relaxation and preservation. Within 24 hours, earthworms were relaxed using a 10 % ethyl alcohol (EtOH) solution and fixed in 10 % formalin for 48 hours. The formalin was then replaced with 90 % EtOH solution for preservation. The earthworms were examined under a 40X microscope where they were identified to species when

possible. Taxonomic keys developed by Gates (1977), James (1990), Reynolds (1972), and Schwert (1990) were used for identification. Lumbricid specimens were identified using external characteristics. Diplocardian specimens required the examination through dissection for internal features used in the taxonomic keys.

Statistical Analysis

Differences in earthworm abundance among forest cover type, reclamation type and age since reclamation were analyzed using a three factor (cover, reclamation type, age) ANOVA followed by Tukey's mean separation test at the alpha = 0.05 confidence level. Earthworm densities were exponentiated prior to the analysis to meet the assumption of ANOVA. Untransformed data are presented in all tables and figures. Statistical analysis was conducted using SAS 9.2, PROC GLM for general linear models. Proc REG was used to determine the value to exponentiate the earthworm densities and proc UNIVARIATE was used to plot histograms and normal curves.

General Linear Model (GLM):

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \phi_k + (\alpha\beta)_{ij} + (\alpha\phi)_{ik} + (\beta\phi)_{jk} + \varepsilon_{ijkl}$$

Where:

μ =grand mean, α_i =age effect ($i = 1...3$), β_j =forest type effect ($j = 1...2$), ϕ_k =reclamation method ($k=1...2$), $(\alpha\beta)_{ij}$ =age x forest type interaction, $(\alpha\phi)_{ik}$ =age x reclamation method, $(\beta\phi)_{jk}$ =forest type interaction x reclamation method ε_{ijkl} =random error ($l=1...5 = n$ replicates).

Results

Earthworm densities were calculated as a mean of the number of earthworms in the 10 soil pits excavated at each site and multiplied by a factor of four to estimate the number of earthworms per square meter. A histogram of earthworm densities ($n = 60$) (earthworm m^{-2}) was plotted to determine any violation from a typical Gaussian distribution (Fig. 2.1).

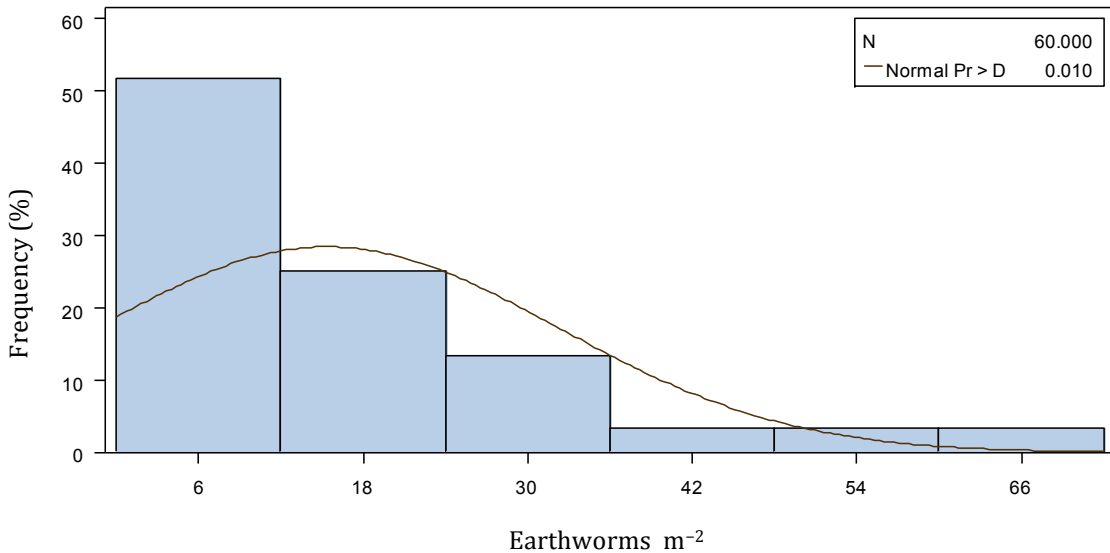


Figure 2.1. Histogram of untransformed earthworm density (earthworms m^{-2}) from the Beckville and Oak Hill mines. The height of each bar is proportional to the percentage of occurrence in each class. The mean is 15.4 earthworms m^{-2} , median is 11.4 Earthworms m^{-2} and the mode is 0 earthworms m^{-2} . Line represents a unimodal asymmetric distribution.

Figure 2.1 suggests a deviation from the assumption of normal distribution with most of the data skewed to the left. In order to achieve a normal distribution, the earthworm density values were transformed. This was done by utilizing a

relationship between the group means and group variances, where the variances are proportional to the mean to some power. The data were linearized by taking the log of the means using linear regression (PROC REG) with the following SAS code:

```
Proc sort data =mines; by reclaim cover age;
Run;
Proc mean data =mines; by reclaim cover age; output out =meanstd
(drop =_type_) mean =mean std=std; run;
Data meanstd; set meanstd; logstd =log(std); logmn =log(mean); run;
Proc reg data =meanstd;
Model logstd =logmn;
Run; quit;
```

The final equation was $\logstd = 0.73895 + 0.65762 (\logmn)$

The value of β_1 was subtracted from 1 ($1 - 0.65752 = 0.34238$) to find the value to exponentiate earthworm densities. Once the data were transformed, the histogram took on a more symmetrical normal curve (Fig. 2.2). Here, the mean = 2.0, the median = 2.3 and the mode = 0.

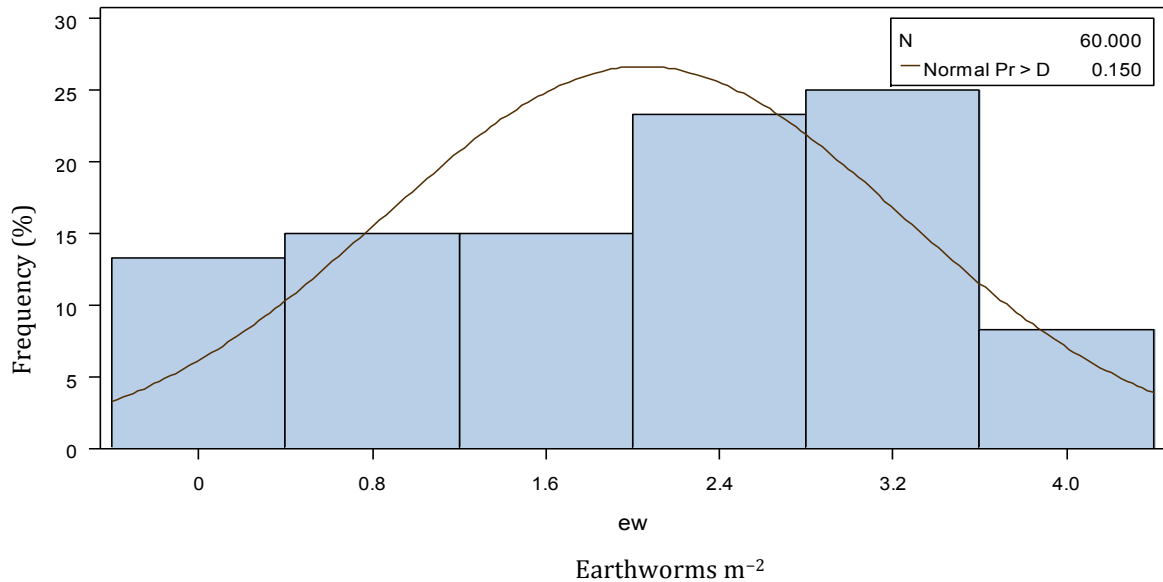


Figure 2.2. Frequency distribution of exponentiated earthworm density values (earthworms m^{-2}). All earthworms found from the Beckville and Oak Hill mines are represented. Here, the normal curve is more symmetrical than the untransformed data with both the mean (2.0 earthworms m^{-2}) and the median (2.3 earthworms m^{-2}) falling in the center of the distribution.

Table 2.3. ANOVA table for transformed earthworm density (earthworm m^{-2}). $\alpha = 0.05$.

Source of Variation	DF	SS	Mean Square	F Value	Pr > F
Reclamation Type	1	1.7367	1.7367	2.07	0.1569
Forest Cover Type	1	5.9024	5.9024	7.03	0.0108
Age Since Reclamation	2	30.1886	15.0943	17.98	<0.0001
Reclamation*Cover	1	0.8298	0.8298	0.99	0.3251
Reclamation*Age	2	3.0428	1.5214	1.81	0.1743
Age*Cover	2	0.3202	0.1601	0.19	0.8270
Reclamation*Age*Cover	2	2.2507	1.1253	1.34	0.2714

Age since reclamation ($p < 0.0001$) and forest cover type ($p = 0.0108$) were found to be significant at the 0.05 alpha level (Table 2.3). Reclamation type was not significant ($p = 0.1569$) in this analysis. At each mine, post hoc analysis found the

youngest age class (2–8 years since reclamation) to be significantly different from the 12–18 year and 22–28 years since reclamation age classes (Figure 2.3). Table 2.4 and 2.5 present the mean earthworm density values and their standard deviations at each mine. Results from unmined soils in adjacent counties are included in these tables. Damoff (2008) found 68.0 (standard deviation = 38.87) earthworms m^{-2} under unmined hardwood forests, while Wilson (2001) found 35 earthworms m^{-2} under unmined pine plantation. These values are considerably higher than earthworm densities found at the Beckville and Oak Hill reclaimed land.

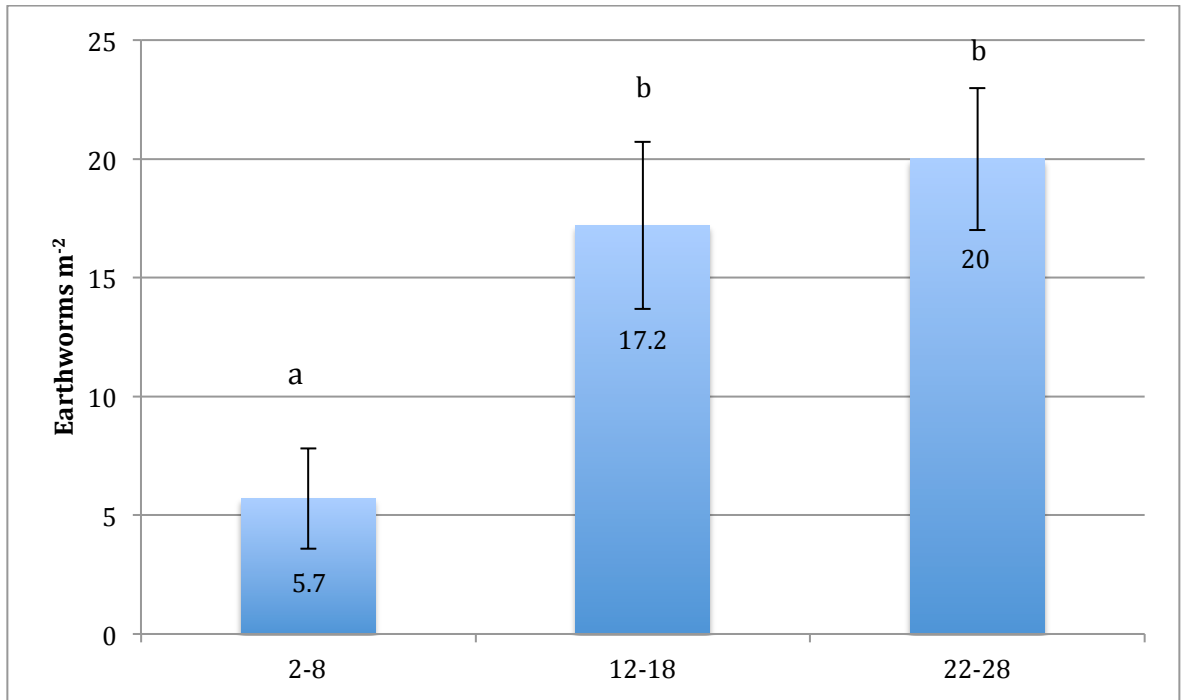


Figure 2.3. Mean earthworm abundance at each age class across cover types and reclamation type. Error bars represent standard error. Lower case letters represent Tukey mean separators. Age classes with the different letters are significantly different ($\alpha=0.05$).

Table 2.4. Earthworm densities (earthworms m⁻²) (n =50) with standard deviation in () at the Beckville mine (reclamation type =mixed overburden) for each age class since afforestation (2–8 years, 12–18 years, and 22–28 years) under both cover types (pine plantation [pine] and mixed pine and hardwood [mixed]). The letters in () represent Tukey multiple comparison analysis. Earthworm densities with different letters are significantly different ($\alpha =0.05$).

	pine	mixed	mean
Age Class (years)	Earthworms (earthworms m⁻²)		
2–8	2.1 (03.38)	6.1 (06.88)	4.1 (05.13)(<i>b</i>)
12–18	17.4 (17.19)	28.8 (20.98)	11.6 (19.09)(<i>a</i>)
22–28	4.0 (05.30)	19.0 (17.52)	16.3 (11.41)(<i>a</i>)
mean	7.8 (08.62)*	18.0 (15.13)*	
Unmined Reference Points	35.0 ¹	68.0 (38.87) ²	

*Significant at the 0.05 probability level

¹Wilson (2001) Standard deviation was not reported

²Damoff (2008)

Table 2.5. Earthworm densities (earthworms m⁻²) (n =50) with standard deviation in () at the Oak Hill (reclamation type = haul-back)mine for each age class since afforestation (2–8 years, 12–18 years, and 22–28 years) under both cover types (pine plantation [pine] and mixed pine and hardwood [mixed]). The letters in () represent Tukey multiple comparison analysis. Earthworm densities with different letters are significantly different ($\alpha =0.05$).

Age Class (years)	pine	mixed	mean
	Earthworms (earthworms m ⁻²)		
2–8	1.0 (01.93)	13.4 (25.53)	7.3 (13.73) (<i>b</i>)
12–18	18.1 (06.32)	27.4 (18.55)	22.8 (12.44) (<i>a</i>)
22–28	28.0 (23.26)	19.4 (07.43)	23.7 (15.35) (<i>a</i>)
mean	15.7 (15.76)	20.1 (17.17)	
Unmined Reference Point	35.0 ¹	68.0 (38.87) ²	

¹Wilson (2001) Standard deviation was not reported

²Damoff (2008)

At Beckville, significantly higher earthworm densities were found at the mixed pine and hardwood sites (18.0 earthworms m⁻²) when compared with the pine plantation sites (7.8 earthworms m⁻²) ($p =0.0128$). However, analysis of the earthworm densities for both cover types at Oak Hill found no significant difference ($p =0.0862$). The unmined reference points represent earthworm density means from previous research by Wilson (2001) and Damoff (2008).

Discussion

Populations of earthworms were present in these reclaimed mine soils from as early as 2–8 years since afforestation. Similarly, Frouz et al. (2013) found earthworms as soon as 2–5 years since afforestation at reclaimed mine sites across the United States. Research conducted in Germany also found earthworm recolonization to be rapid (<5 years) (Curry and Cotton 1983; Topp et al. 2001; Dunger and Voigtländer 2005). Although earthworm numbers were low at both mines (4.1 earthworms m⁻² for Beckville and 7.3 earthworms m⁻² for Oak Hill) at the 2–8 years since afforestation sites, their presence in the soil suggests the resilience and recoverability of the belowground ecosystem after severe disturbances. Snyder and Hendrix (2008) contend that presence of an earthworm community in disturbed soils indicate that habitat requirements have been fulfilled.

The 2–8 year age class was found to be significantly less than the 12–18 year and 22–28 year ages classes at both Beckville and Oak Hill lignite mines (table 2.3). This is consistent with research that shows an increase in earthworm abundance with age since afforestation (Hüttl and Weber 2001; Wanner and Dunger 2002; Dunger and Voigtländer 2005). The marked difference in earthworm numbers from aged 2–8 year to sites aged 12–18 years since reclamation may be the result of tree canopy cover establishment as well as the development of increasing soil organic matter and surface litter (Dunger and Voigtländer 2005).

Curry (2004) and Muys et al. (1992) found the response of earthworms in the soil depends on the species of trees planted and the quality and quantity of litter produced. This also is reflected in the results from the Beckville mine that found significantly higher numbers of earthworms on mixed pine and hardwood sites than on pine plantation sites. The mixed pine and hardwood sites were often planted in areas with lower elevation levels than those of pine plantation sites. These lower areas tend to remain moist after rain events compared with soils of uplands, where many of the pine plantation sites were found. Earthworms tend to prefer moist soils and therefore may have been more abundant under the mixed pine and hardwood sites.. However, there was not a significant difference between the cover types at the Oak Hill reclaimed sites. This may be the result of the surprisingly high numbers of earthworms found at the oldest pine plantation sites at Oak Hill. One site in particular was planted in a low-lying area that remained quite wet after heavy rains. This site yielded high densities of earthworms relative to other pine plantation sites.

Earthworm abundance tended to be higher, although not significant ($p = 0.1569$), at the Oak Hill mine where the haul-back method of land reclamation is practiced than at the Beckville mine, where the mixed overburden method of reclamation is used. This could be due to several factors. The oxidized layers of the overburden composed 1.2 m of the soil surface. In these soil layers, some of the microbes and organic material may have persisted through excavation. Dunger and Voigtländer (2005) suggest that soil fauna may survive the excavation process and

be responsible for helping to re-develop the new belowground ecosystem. However, the dates of soil sampling may have also played a role in earthworm abundance at the two mines. Earthworms were sampled at the Beckville mine from February 2012 through March 2013, while Oak Hill was sampled the following year. The region was recovering from an extended period of low precipitation that ended during the winter of 2011. During the drought period, a great reduction in earthworm numbers may have occurred (Curry 2004). Sampling so soon after the extended period of low precipitation may have skewed the data resulting in fewer earthworm individuals at the Beckville mine.

Earthworms have survival mechanisms they use during periods of low soil moisture. Some species survive drought in the cocoon stage while others become quiescent. Other species enter diapause in response to drought (Bouché 1977). However, prolonged droughts may greatly decrease earthworm densities. It is hypothesized that it may take as long as two years for populations to recover once soil conditions become favorable again (Edwards and Bohlen 1996). Gerard (1967) suggests that the reproductive capacity of the earthworm populations is diminished during times of drought, although some species are known to withstand dry conditions better than others. Adapted earthworm populations in areas with extremely warm summers, such as the south central region of the United States, have the capacity to survive periods of low soil moisture. However, these species of earthworms will nevertheless suffer heavy mortality, particularly among juveniles

unable to escape desiccation by moving deeper in the soil and becoming inactive (Gerard 1967).

The geology of the sites may have also influenced the data. At the 22–28 year since afforestation pine plantation sites at Beckville, earthworm abundance was lower than 12–18 year old sites and similar to 2–8 year old sites. Sites sampled from the oldest age class had a higher percentage of sand (see Chapter 4) when compared with other sites from the Beckville mine. The lower soil pH buffering capacity due to a lower cation exchange capacity, rapid drainage and lower water holding capacity of these coarser soils may have contributed to the low numbers of earthworms living in those sites.

The decrease in earthworm densities from the 12–18 year age class to the 22–28 year age class at the Beckville pine plantation sites suggests the chronosequence approach may not accurately capture earthworm succession on these reclaimed soils (Walker et al. 2010). Previous research of earthworm abundance on reclaimed mine lands show earthworm density values to increase with time since afforestation (Hüttl and Weber 2001; Wanner and Dunger 2002; Dunger and Voigtländer 2005). This study, however, showed a marked decrease, although not significant, in earthworm densities on the Beckville pine chronosequence from the 12–18 year age class to the 22–28 year age class as a result of soil characteristics unrelated to time, but to the parent material of the overburden. Chronosequences are useful in that they assume to replace a natural

linear trajectory of time, but that assumption is often violated by the natural variations that occur with differing locations (Walker et al. 2010). This study therefore suggests that a non-linear succession exists in some studies of earthworm abundance on severely disturbed sites such as surface mined soils. Proximity to older reclaimed sites or unmined sites also may influence earthworm colonization. Future studies should explore these potential edge effects as well as the relative importance of trees and understory vegetation on earthworm communities, which may change over time.

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CHAPTER 3

Earthworm Species Composition in Beckville and Oak Hill Lignite Coal Mine Soils

Literature Review

Studies in biodiversity have highlighted the intricate relationships between the flora and fauna of an ecosystem and how they work together to perform important functions (Topp et al. 2001; Postma-Blaauw et al. 2006). In the belowground ecosystem, soil biota are essential to the processes of plant nutrient dynamics and soil formation. Organisms such as earthworms can be directly related to the size of microbial populations in the soil as well as physical and chemical properties favorable to plant growth (Lavelle and Martin 1992, Hendrix 1996, Topp et al. 2001). In the belowground food web, earthworms are present on three different trophic levels, as primary consumers of plant debris, secondary consumers of decomposed organic matter and microbes and as higher-level consumers of nematodes and carnivorous microbes (Brady and Weil 2008). A diverse community of earthworm species would suggest a more complete soil food web and therefore more diverse soil biota composition. In severely disturbed reclaimed lignite coal mine soils in East Texas, the question remains as to the level of diversity that exists among earthworms inhabiting the soils.

Earthworm Systematics

Earthworm species composition is an important part of earthworm community characteristics in the belowground ecosystem. Earthworms belong to the class Oligochaeta, closely related to Polychaeta (bristle worms) and the Hirudinea (leeches). Within the class, there are 23 known families, 393 genera and over 5,000 known species (Edwards and Bohlen 1996). Native earthworms of the United States and Canada consist of members of the following families: Megascolecidae, Komarekionidae, Lumbricidae, Lutodrilidae, Sparganophilidae, Acanthodrilidae, Eudrilidae, Glossoscolecidae and Ocnerodrilidae. Pleistocene glaciations, the most recent of which ended approximately 10,000 BP (before present), are thought to have eliminated earthworm fauna from Canada and most of the northern portion of the continental USA (Gates 1977). However, glacial refugia in the southern USA and Pacific Northwest includes a number of native genera in the families Megascolecidae and Lumbricidae. Approximately 70 species of native earthworms have been described from the southern USA and many more undiscovered species are thought to exist (Hendrix 1996).

Ecological Groups

Individual earthworm species fulfill unique ecological niches in soils and therefore different ecological functions. The structure of earthworm communities and major ecological categories are based mainly on differences among burrowing and feeding activities and vertical stratification in the soil. The three major

ecological categories have broad applicability and were developed from the numerous studies of European earthworms from the Lumbricidae family. Bouché (1977) described these three major groups as evolutionary extremes with many species falling somewhere in between these groups.

- Epigeic—Earthworm species that live in the organic surface horizons or in the upper reaches of the mineral soil, have relatively high reproductive rates and grow rapidly. Species such as *Lumbricus rubellus* belong to this group.
- Anecic—Earthworm species that form permanent or semi-permanent vertical burrows in the soil. These burrows descend into the mineral horizons and open at the surface where the earthworms emerge to feed primarily on dead leaves and other decaying organic material. Examples include *Lumbricus terrestris*. Anecic earthworms are most predominant among European lumbricids.
- Endogeic—Earthworm species that inhabit the mineral soil horizons. They consume more soil than do the epigeic or anecic species. They eat more humidified organic matter, although some species will come to the surface to feed in the litter layer such as *Diplocardia caroliniana*.

These three ecological groups are frequently used in literature discussing earthworms. However, ecological comparisons across taxonomic groups should be done cautiously (Hendrix 1996).

Earthworm Diversity and Soil Development

The belowground ecosystem is comprised of a complex web of producers and consumers which all directly or indirectly influence the overall ecosystem. For example, some species of bacteria, such as nitrogen fixing bacteria, have obvious effects on plant productivity while soil meso- and macrofauna may be less directly involved. Nevertheless, these larger organisms have been shown to have indirect effects on soil processes through their interactions with other more influential organisms (Brown et al. 1999).

Earthworms and their association with microbial populations, as well as soil conditions favorable to plant growth can indicate the presence of other soil biota. Through their feeding and burrowing activities, the variety of ecological niches occupied by different species of earthworms influence soil biology, chemistry and physical properties in unique ways. Lee (1985, 1991) recommended a target earthworm community to consist of one or more anecic and epigeic species that make deep vertical burrows and then cast on the surface and bury residues; as well as one or more endogeic species that feed belowground on dead roots and organic matter and generally make horizontal burrows.

Few studies have attempted to link ecosystem function to the diversity of one soil animal such as earthworms. However, some research has observed the influence of a particular earthworm species or ecological group of earthworms on soil productivity or soil development. For example, the effects of earthworms on nitrogen (N) mineralization and plant productivity may depend on the earthworm

species present and on species interactions in the soil system. Postma-Blaauw et al. (2006) found *L. rubellus* and *L. terrestris* to enhance mineralization of applied crop residue where *Aporrectodea caliginosa* had no effect, but *A. caliginosa* and *L. rubellus* enhanced soil organic matter mineralization.

Non-native Earthworm Species

Non-native earthworm species (also commonly referred to as exotic, invasive or peregrine) were described over a century ago by Michaelsen (1890) as species that are widely distributed despite natural barriers such as mountain ranges and seas. Special characteristics of peregrine species include (Lee 1985):

- potential for hermaphroditism,
- tolerance of environmental variability,
- habitat specificity,
- opportunism in choice of food,
- ability to withstand chemical stress,
- association with cultivated soil and
- ecological plasticity.

Most earthworm species introductions have resulted from human transport of soils and plants. In addition, exotic earthworms often become established along lakes and streams because of discarded fish bait. Floods are known to transport both viable cocoons and live earthworms.

Some researchers propose that non-native earthworms have the capacity to replace existing native earthworm populations. This may happen as a result of native species intolerance for altered habitats, a loss of key biotic relationships present in intact ecosystems, competition pressures from exotic species, and the inability to

reestablish populations after disturbance (Stebbing 1962, Kalisz and Wood 1995).

The nature of earthworm species reproductive systems also may be significant in helping them survive a variety of habitats. Most earthworms are obligatory outcrossing hermaphrodites; however, some use other means of reproduction. Some species have the ability to self-fertilize and others are parthenogenetic. Parthenogenesis is a type of asexual reproduction where offspring develop from unfertilized eggs. Uniparental modes of reproduction allow for just one earthworm to begin colonizing an area. Species of the *Octolasion* and *Microscolex* are known to be parthenogenetic.

James and Hendrix (2006) state that invasions of agroecosystems by exotic earthworms are the general rule. Native species capable of tolerating the less buffered soil environment and frequent soil disturbances are less common than highly adaptive non-native invasive counterparts. In reclaimed mine lands in Texas however, the situation is different. There are not frequent disturbances such as tillage or disking after the land is reclaimed.

Exotic earthworms have changed the structure of the forest floor in temperate deciduous and mixed forests of North America that were devoid of earthworms due to glaciation. Nielsen and Hole (1964) note the conversion of podzols to a mixed mineral-humus soil in parts of Canada. The total destruction of the forest floor (O horizons) was observed in Minnesota, New Jersey, Rhode Island, Pennsylvania, and New York.

Stebbing (1962) suggested that exotic earthworm species may be out-

competing the native species on some forested sites in the central US, but Kalisz and Wood (1995) suggested that such invasions may not occur in some areas of undisturbed habitats. However, Damoff (2009) found invasive non-native earthworms on most relatively undisturbed sites in east Texas forests.

Habitat destruction or disturbance is almost always cited as a precursor to earthworm invasions. The increase in non-native species to a soil can influence soil structure, rates of organic matter decomposition, nutrient dynamics, and potentially food webs.

Earthworms Endemic to the South Central United States

Endemic earthworms are those species of native earthworms that do not spread successfully to other areas. In the southern and central United States, many species from the genus *Diplocardia* have displayed a high degree of adaptive radiation in the region's soil types and climate regimes. This genus of earthworms has only been found in North America. Species from the Lumbricid family of the *Bimastos* genus are also native to these soils.

Diplocardia spp. belong to the Megascolecidae family and are largely restricted to regions south of the Wisconsinan glaciation. The genus *Diplocardia* consists of some 50 or more species. The genus is abundantly represented in the southern states, including Texas and Oklahoma. The known distribution of the genus is from Nebraska east to Delaware, the northern limit roughly at the latitude

of Detroit, Michigan and from Nebraska south to Texas. Temperature tolerances of *Diplocardia* spp. are generally higher than those of the introduced Lumbricidae (James 1991).

Non-native invasive earthworm species are also present in the south central region of the United States. These species have spread around the world, mostly due to human transport, and have a significant impact on the soils they inhabit. Because a diversity of species exist among earthworms, this study sought to understand which earthworm species inhabit lignite mine lands after land reclamation, at what age since reclamation they survive in the soil, and if forest cover type influences earthworm species composition.

Earthworm Species Diversity

The number of species in a given earthworm community generally ranges from 1 to 15 species (Edwards and Bohlen 1996). However, Lee (1985) states that within a particular soil, less than six earthworm species are found. Most earthworm communities contain 3 to 6 species. The number of earthworm species at a given locality is most likely influenced by the characteristics of the soil, climate and organic matter resources. The history of land use is also an important factor. Communities with fewer species are often characterized by extreme soil conditions such as low pH or poor fertility, low-quality litter or a high degree of soil disturbance.

Methods

Two mines, Beckville and Oak Hill were sampled on a chronosequence under two different forest cover types (pine plantation and mixed pine and hardwood). The chronosequence had three age classes, 2 to 8 years since afforestation, 12 to 18 years since afforestation and 22 to 28 years since afforestation. Each mine practiced a different reclamation type. At Beckville the mixed overburden method was used, and the haul-back method was used at Oak Hill (see Chapter 2 for more information on reclamation type). The three variables: age class, forest cover type and reclamation type were used as factors in the analysis of species diversity and to analyze the distribution of native vs. non-native species in the reclaimed mine lands. Instead of representing earthworm species as a density such as number of species per m², this study reports earthworm species diversity as the number of species found at each age class and cover type combination.

At each mine, 30 sites were selected. Half of the 30 (15 sites) were pine plantation and the other 15 were mixed pine and hardwood. Within each cover type, five sites were 2 to 8 years since reclamation, five sites were 12 to 18 years since reclamation and five sites were 22 to 28 years since reclamation. At each site, ten 50 by 50 by 30 cm pits were dug. The soil was excavated to a polyethylene tarp where it was hand-sorted for earthworms. The earthworms were placed in a polyethylene lined soil sample bag and transported to the SFASU Forest Soils Lab.

On the same day as field collection, all earthworm specimens removed from each pit were rinsed in water and collectively relaxed in a 10 % solution of ethanol.

The concentration was incrementally increased over a 30-minute period until no response was observed when specimens were prodded with forceps. Specimens were then placed in a straight position on heavy paper saturated with 4% formalin in the bottom of a shallow plastic tub. The tub was sealed for an hour or until earthworms were in a fixed position. Earthworms were then transferred to glass vials filled with 4 % formalin and left for 48 hours. Specimens were then rinsed in water and preserved in glass vials filled with 70 % ethanol for permanent storage (Damoff 2008).

Earthworm species were identified using stereomicroscopes that ranged in magnification from 4 to 40X. Diplocardian specimens were identified using species descriptions and taxonomic keys developed by Gates (1977) and James (1990) and Lumbricid specimens were identified using the taxonomic keys developed by Reynolds (1972) and Schwert (1990). Lumbricid specimens were identified to species using external characteristics. *Diplocardia* specimens required the examination of numerous internal features through dissections.

Statistical Analysis

To determine the significance of age class, forest cover type and reclamation type of the number of species found at a site, a three-way ANOVA along with Tukey's Studentized range as a multiple comparison test was employed. All statistical analysis was performed on SAS 9.2 Proc GLM.

General Linear Model (GLM):

$$y_{ijl} = \mu + \alpha_i + \beta_j + \varphi_k + (\alpha\beta)_{ij} + (\alpha\varphi)_{ik} + (\beta\varphi)_{jk} + \varepsilon_{ij\check{k}l}$$

Where:

Y_{ijl} = mean number of earthworm species per site

μ = grand mean, α_i = age effect ($i = 1 \dots 3$), β_j = forest type effect ($j = 1 \dots 2$), φ_k = reclamation method ($k = 1 \dots 2$), $(\alpha\beta)_{ij}$ = age x forest type interaction, $(\alpha\varphi)_{ik}$ = age x reclamation method, $(\beta\varphi)_{jk}$ = forest type interaction x reclamation method $\varepsilon_{ij\check{k}l}$ = random error ($l = 1 \dots 5 = n$ replicates).

Ecological indices were calculated using standard methods (Peet, 1974).

A Shannon-Wiener Diversity Index (Ludwig and Reynolds, 1988) was calculated using the formula, $H = -\sum p_i \ln p_i$, where p_i is the relative abundance of the species ($p_i = n_i/N$; n_i is the number of individual species, N is the total number of individuals). Simpson's index using formula, $D = n_i (n_i - 1) / N(N - 1)$.

This study focused on the richness and evenness of earthworm species that are present in reclaimed lignite mine lands. Species richness reports the number of species found in a sample while evenness accounts for the number of individuals per species found in a sample. Richness reports the number of species in a community. The average number of species per sample provides the simplest, most practical measures of species richness; however, it does not provide a useful method for making inferences about the underlying community. Given the highly variable nature of the belowground ecosystem, a simple comparison of species numbers is not useful, as it is insensitive to the abundance of each species. Species diversity incorporates both the number of species in an assemblage and some measure of their relative abundances. The Shannon diversity index measures the rarity and commonness of species in a habitat. The Simpsons diversity index is

often used to quantify the biodiversity of a habitat. It takes into account the number of species present as well as the abundance.

Results

Earthworm species found are listed with the number of individuals associated with age class and cover type at the Beckville (Table 3.1) and Oak Hill (Table 3.2) reclaimed lignite mines. Eight total species were found at the Beckville mine and ten total species were found at the Oak Hill mine. Several juveniles who lacked morphological characteristics required for proper identification were classified as “unidentified.” Earthworms that were clearly from the diplocardian genus as evidenced from their lack of pigmentation and external morphological features coloration, but lacking key adult characteristics to determine the species were labeled as *Diplocardia* sp.

Table 3.1. Number of earthworm individuals by age class (years since afforestation) and species found under pine plantation (pine) and mixed pine and hardwood (mixed) at the Beckville mine (mixed overburden).

Age Class	pine			mixed			Total ¹
	2-8	12-18	22-28	2-8	12-18	22-28	
Species	Number of Individuals ¹						
<i>Aporrectodea trapezoides</i> ²	4	1	28	3	9	14	59
<i>Octolasion tyrtaeum</i> ²	-	-	1	-	-	-	1
<i>Microscolex dubius</i> ²	15	-	-	-	1	4	20
<i>Diplocardia caroliniana</i>	1	94	39	5	115	43	297
<i>Diplocardia hulberti</i>	-	41	6	4	10	62	123
<i>Diplocardia</i> species	-	1	-	-	9	2	12
<i>Diplocardia michaelsoni</i>	-	-	-	-	39	-	39
<i>Bimastos tumidus</i>	-	-	1	-	1	-	2
<i>Bimastos</i> species	-	-	-	2	-	-	2
<i>Bimastos parvus</i>	-	-	-	3	1	-	4
unidentified		3	2	3	31	-	39
Grand Total	20	140	77	20	216	125	598

¹Recorded total does not include mortality prior to preservation.

²Earthworm species not native to North America

Table 3.2. Number of earthworm individuals by age class (years since afforestation) and species found under pine plantation (Pine) and mixed pine and hardwood (Mixed) at the Oak Hill mine (haul-back).

Age Class	Pine			Mixed			Total ¹
	2–8	12–18	22–28	2–8	12–18	22–28	
Species	Number of Individuals¹						
<i>Aporrectodea trapezoides</i> ²		8	58	3	46	5	120
<i>Lumbricus rubellus</i> ²							
<i>Microscolex phosphoreus</i>	-	-	-	25	11		36
<i>Diplocardia</i> species	10	1	55		26	13	105
<i>Diplocardia caroliniana</i>	-	77	86		148	67	378
<i>Diplocardia ornata</i>	-	-	-	-	11	-	11
<i>Diplocardia fusca</i>	-	-	-	-	12	-	12
<i>Diplocardia eiseni</i>	-	-	-	-	1	-	1
<i>Diplocardia longa</i>	-	-	-	-	10	-	10
<i>Diplocardia hulberti</i>	-	-	14	-	7	-	21
<i>Diplocardia meansi</i>	-	-	-	-	12	-	12
unidentified	-	-	21	-	-	-	21
Grand Total	10	86	234	28	284	85	72

¹Recorded total does not include mortality prior to preservation.

²Earthworm species not native to North America

Total Number of Species in Each Cover Type and Age Class Combination

Native species of earthworms were represented at each age class and cover type at the Beckville mine (Table 3.3). In most cases, the number of native species was more than half of the total number of species. At the Beckville mine, there was a trend of species numbers increasing with age since reclamation at the pine plantations sites (Figure 3.1). The mixed pine and hardwood sites, however, had a high number of earthworm species at the middle age class while the youngest and oldest are around the same number of species.

Beckville

Table 3.3. Total number of earthworm species found at the Beckville mine under pine plantation (Pine) and mixed pine and hardwood (Mixed) at each age class. The numbers in parentheses represent the number of native species found at each age class and cover type.

Age Class	Pine	Mixed	Mean
Years	Number of Species (Number of Native Species)		
2–8	3.0(1.0)	4.0(3.0)	3.5(2.0)
12–18	4.0(3.0)	7.0(5.0)	5.5(4.0)
22–28	5.0(3.0)	5.0(3.0)	5.0 (3.0)
Mean	4.0(2.3)	5.3(3.7)	4.7(3.0)

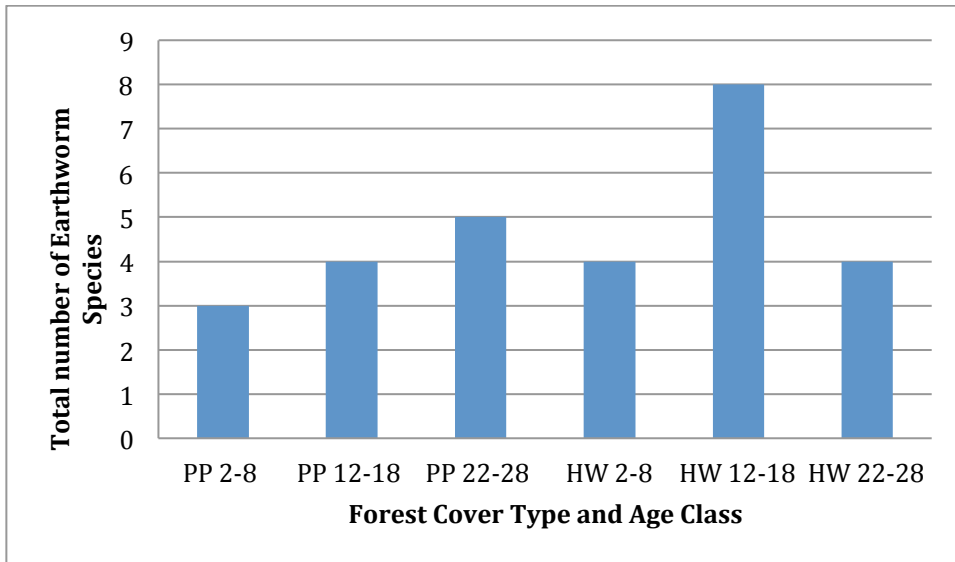


Figure 3.1. Total number of earthworm species found at Beckville under pine plantation (PP) and mixed pine and hardwood (HW) at each age class, (2–8 years since afforestation, 12–18 years since afforestation, 22–28 years since afforestation).

Native species of earthworms were represented at each class and cover type at the Beckville mine (Table 3.1). In most cases, the number of native species was more than half of the total number of species. As age since reclamation increased, species richness also increased at the Beckville mine (Figure 3.1).

Oak Hill

Native species of earthworms were well represented at the Oak Hill mine at each age class and under both cover types, save for the youngest age class under mixed pine and hardwood (Table 3.4).

Table 3.4. Total number of earthworm species found at the Oak Hill mine under pine plantation (pine) and mixed pine and hardwood (mixed) at each age class. The numbers in parentheses represent the number of native species found at each age class and cover type.

Age Class	Pine	Mixed	Means
Years	Number of Species (Number of Native Species)		
2-8	1.0(1.0)	2.0(0.0)	1.5(0.5)
12-18	3.0(1.0)	10.0(8.0)	6.5(4.5)
22-28	4.0(3.0)	3.0(2.0)	3.5(0.5)
Mean	2.7(1.7)	4.7(3.3)	3.7(2.5)

The total number of earthworm species increased with age since reclamation at the pine plantation sites. Under mixed pine and hardwood, however, the middle age class supported the highest number of earthworm species (Figure 3.2).

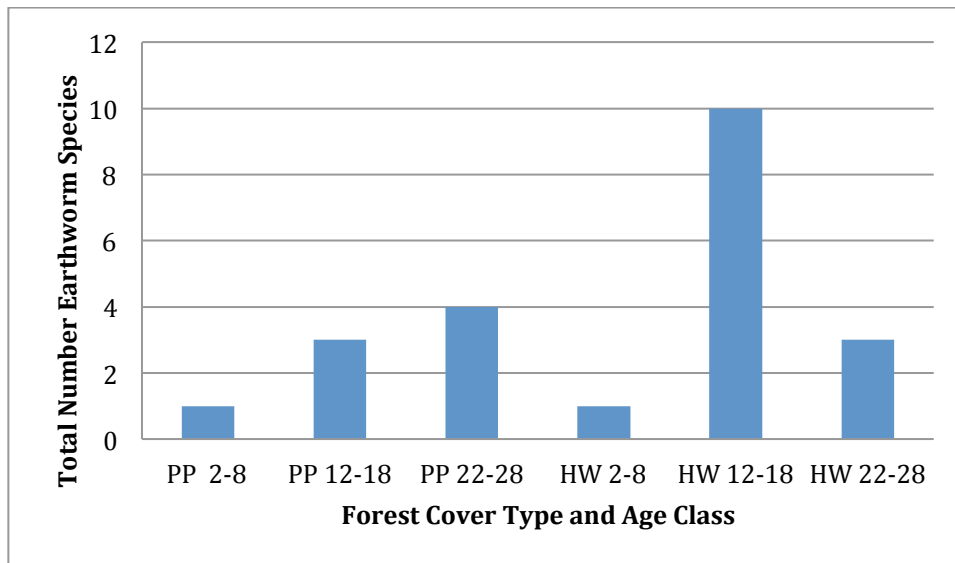


Figure 3.2. Total number of earthworm species found at Oak Hill under pine plantation (PP) and mixed pine and hardwood (HW) at each age class, (2–8 years since afforestation, 12–18 years since afforestation, 22–28 years since afforestation).

At the Oak Hill mine, mixed pine and hardwood supported a greater number of species (4.7 species) when compared with pine plantation (2.7 species). The intermediate age class (12–18 years since afforestation) supported the highest number of species (6.5 species) among the age classes. The intermediate age sites sampled under mixed pine and hardwood had the highest number of species (10 species) (Figure 3.2).

An average of 2.5 native species was found at the Oak Hill mine. Mixed pine and hardwood had the highest number of native species (3.3 species) when compared with pine plantation (1.7 species). Again, the intermediate class (12–18 years since afforestation) supported the greatest number of native species (4.5 species). The middle-aged sites under mixed pine and hardwood had the highest

number of native earthworm species (8 species). No native species were found under mixed pine and hardwood at the youngest age class (2–8 years since afforestation).

Both Mines Combined

The total number of earthworm species present in each system ranged from 1–7 at Beckville and 1–10 at Oak Hill (Figure 3.1). The number of species increased with time since afforestation at pine sites at Beckville and Oak Hill. Both mines exhibited a trend of increasing number of species to the 12–18 year age sites then decreasing at the 22–28 year age sites. At Beckville the number of species increased from 4 species at the 2–8 year sites to 8 species at the 12–18 year sites. At Oak Hill an increase occurred from 1 species at the 2–8 year sites to 10 species at the 12–18 year sites. This may be explained by the high number of diplocardian species found at the middle-aged sites.

Cover type (pine plantation or mixed pine and hardwood) ($p=0.1020$) and reclamation type (mixed overburden or haul-back) ($p=0.6360$) were not significantly correlated to the number of earthworm species found at a site. Tukey's Studentized range test found age classes to be significantly different from each other (Figure 3.3). Shannon-Wiener, Simpson and Beta diversity indices were used to describe species diversity among age classes (Tables 3.5 and 3.6).

Table 3.5. Shannon-Wiener (H) and the inverse of Simpson (D^{-1}) Diversity Index for earthworms at the Martin Lake mine complex at each age class (year since afforestation).

Years	H	D^{-1}
2–8	1.45	4.00
12–18	1.20	2.08
22–28	1.08	2.63

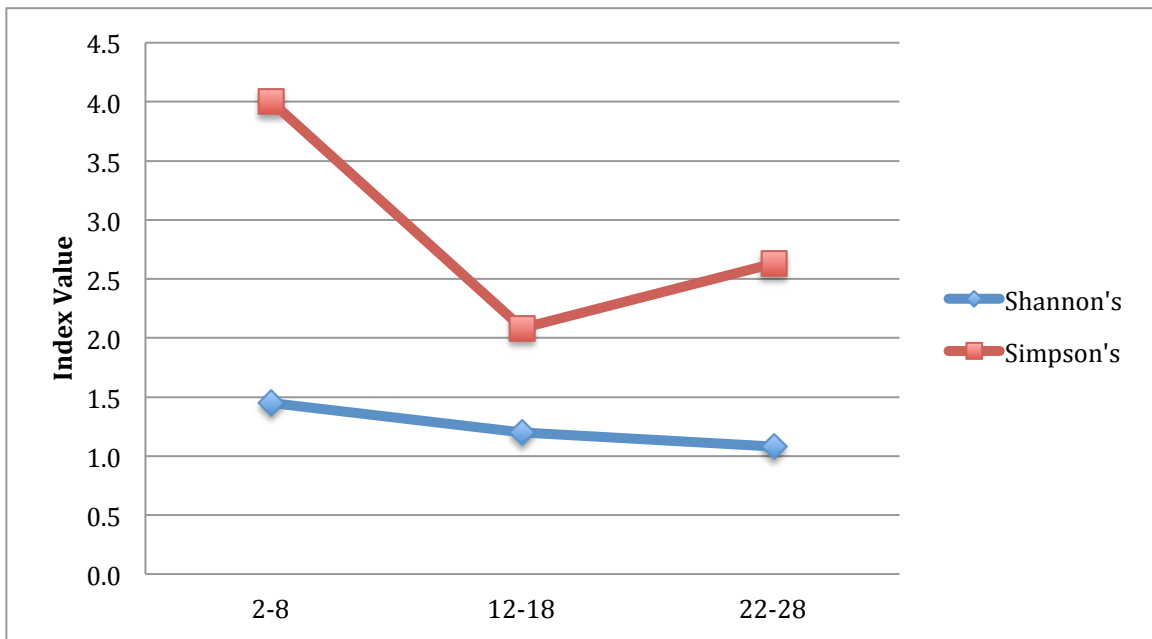


Figure 3.3. The Shannon-Wiener index and the Simpson's index values at the Martin Lake mine complex at each age class (years since reclamation).

The Shannon-Wiener index shows the youngest age class with the highest value while the oldest age class has the lowest value (Table 3.5, Figure 3.3). The inverse of the Simpson's index also shows the youngest age class with the higher

value. This may be the result of the evenness in the species found at this age group relative to the other two age groups. Shannon's is more sensitive to the addition and deletion of rare species. A species assemblage with high functional overlap among species has a lower functional diversity than an assemblage with low functional overlap. Functional diversity is those components of biodiversity that influence how an ecosystem operates or functions.

Ecological index values at each mine (Beckville-mixed overburden and Oak Hill-haul-back) are shown in Figure 3.4. The Simpson's index is higher at the Beckville mine while the Shannon's index is only slightly different between the two mines. At each cover type (pine plantation and mixed pine and hardwood) both the Shannon's and the Simpson's give higher value to the mixed pine and hardwood sites (Figure 3.5).

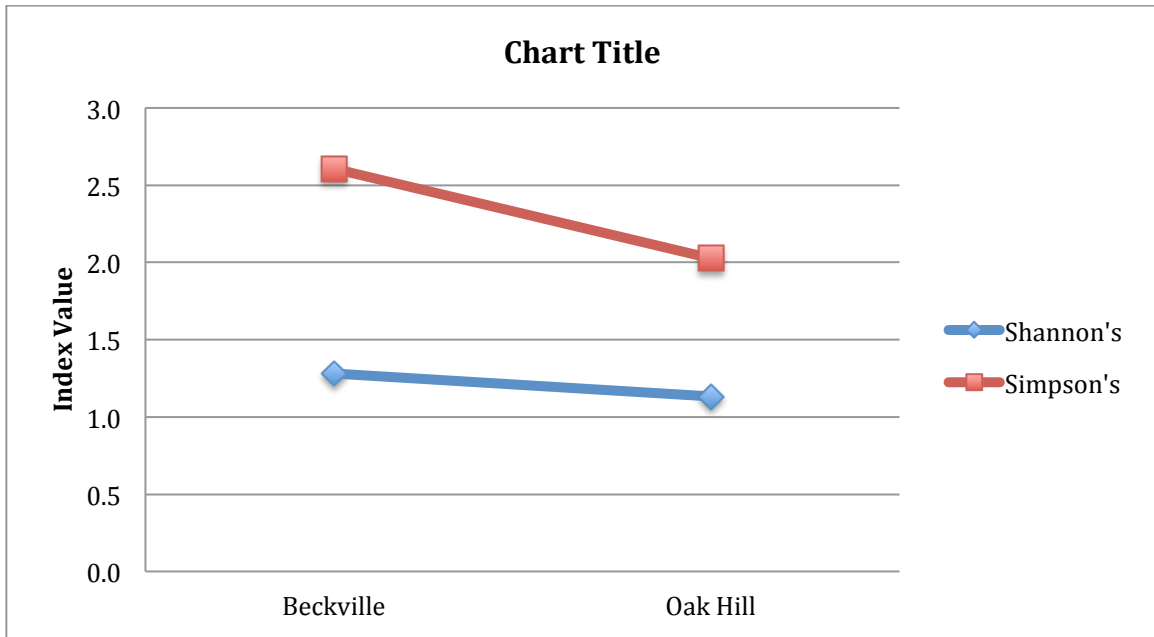


Figure 3.4. Shannon-Weiner and Simpson index values for Beckville (mixed overburden) and Oak Hill (haul-back) mines.

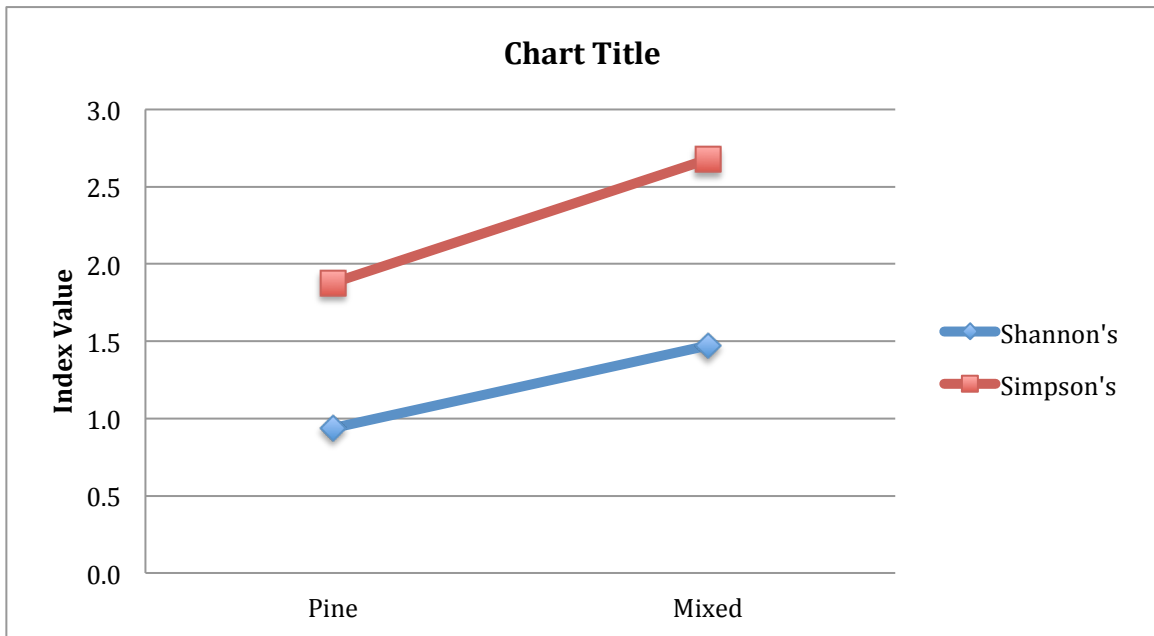


Figure 3.5. Shannon-Weiner and Simpson Index values for earthworm species diversity under pine plantation and mixed pine and hardwood sites at the Martin Lake Mine Complex.

Mean Number of Earthworms by Species

Table 3.6. Mean number of earthworm genera and species recovered from soil collected at the Beckville (BV) mine and Oak Hill (OH) mine under two forest cover types: pine plantation (pine) and mixed pine and hardwood (mixed). Values are earthworm densities (earthworms m⁻²). Values are means; n=50.

Species/Mine	Age Since Afforestation/Forest Cover					
	2–8 years		12–18 years		22–28 years	
	Pine	Mixed	Pine	Mixed	Pine	Mixed
Earthworms m ⁻²						
<i>Aporrectodea trapezoides</i>						
BV	0.3	0.2	0.1	0.7	2.2	4.7
OH	-	0.2	0.6	3.7	4.6	0.4
<i>Octolasion tyrtaeum</i>						
BV	-	-	-	-	0.1	-
OH	-	-	-	-	-	-
<i>Microscolex dubius</i>						
BV	1.2	-	-	0.1	-	0.3
OH	-	-	-	-	-	-
<i>Microscolex phosphoreus</i>						
BV	-	-	-	-	-	-
OH	-	2.0	-	0.9	-	-
<i>Lumbricus rubellus</i>						
BV	-	-	-	-	-	-
OH	-	-	-	0.9	-	-
<i>Diplocardia</i> spp. (combined)						
BV	0.1	1.4	10.9	13.8	3.6	8.6
OH	0.8	-	6.2	17.2	12.4	6.4
<i>Bimastos</i> spp. (combined)						
BV	-	0.4	-	0.2	0.1	-
OH	-	-	-	-	-	-

The widespread non-native species *Microscolex dubius* dominated the youngest age class for pine stands, while the native *Diplocardia* spp. was most

abundant in the intermediate and oldest pine stands (table 3.6). The youngest mixed pine and hardwood sites showed more species diversity than did the pine stands with two different species of *Diplocardia* and earthworms from the native *Bimastos* genera present. Of the total 559 earthworm individuals identified from the Beckville mine, only 80 individuals (14 %) were nonnative earthworm species; the rest were considered native to the south central region of the United States. Native and nonnative earthworms were found at each age class under both pine plantation and mixed pine and hardwood cover types. The largest number of native species was found at the 12–18 year age class under mixed pine and hardwood, while the least number of native species were found at the 2–8 year age class under pine plantation.

Of the 702 earthworms identified at the Oak Hill mine, 131 (19 %) of those were nonnative; therefore, 81 % of earthworms identified were considered native to the south central region of the United States. Two species of nonnative earthworms were found, (*A. trapezoides* and *L. rubellus*) while seven species within the *Diplocardia* genus were identified. Diplocardian specimens were found across each age class under both pine plantation and mixed pine and hardwood cover types. The largest number of native species was found at the 12–18 year age class under mixed pine and hardwood, while the least number of native species were found at the 2–8 year age class under mixed pine and hardwood (Figure 3.4).

Non-Native Earthworm Densities

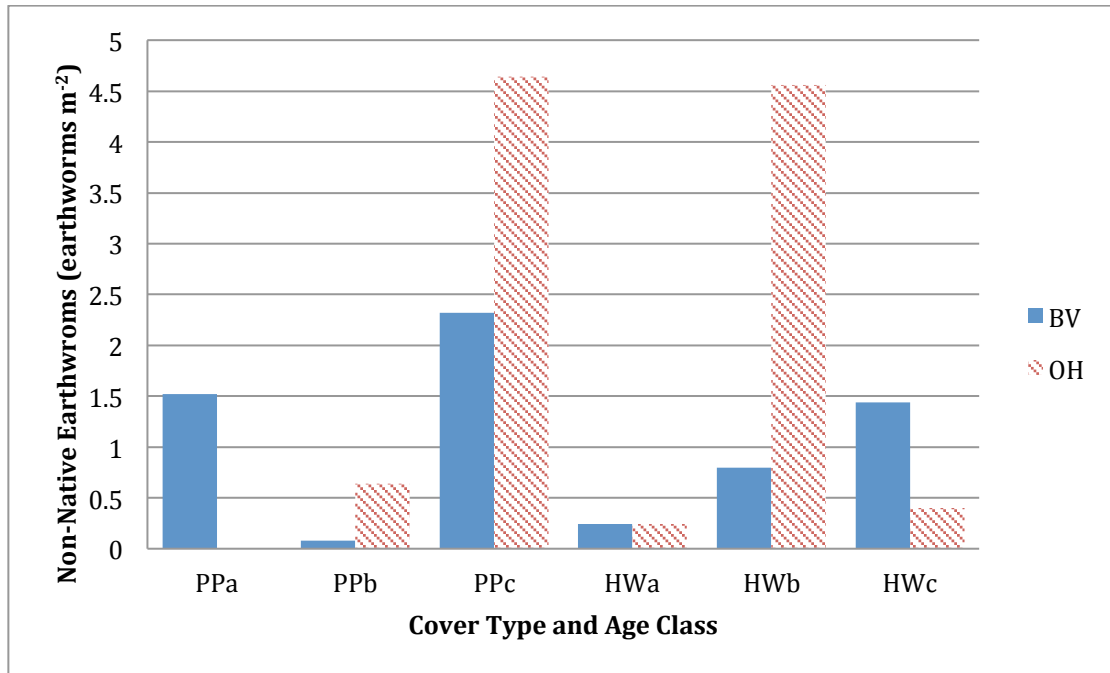


Figure 3.6. Non-native earthworms species density (earthworms m⁻²) found under each cover type (PP =pine plantation HW =mixed pine and hardwood) and age class (a =2–8 years since afforestation, b =12–18 years since afforestation, and c =22–28 years since afforestation) at the Beckville (BV) and Oak Hill (OH) reclaimed lignite mine lands.

Although there were only two non-native species found at the intermediate aged mixed pine and hardwood sites at Oak Hill, *A. trapezoides* and *M. phosphoreus*, there were a relatively large number of earthworms (4.6 earthworms m⁻²) found of those two earthworms species compared with the youngest (0.2 earthworms m⁻² *A. trapezoides* only) and oldest age class (0.4 earthworms m⁻² *A. trapezoides* only) (Table 3.5). At the pine plantation sites, earthworm densities increased with time since afforestation.

Non-native earthworms found at the Beckville mine were identified as *A. trapezoides* and *Microscolex dubius*. Pine plantations at the oldest age class (22 to 28 years since afforestation) contained the highest density of non-native earthworms (2.3 earthworms m⁻²) (Figure 3.6). The intermediate age under pine plantations contained the lowest number of non-native earthworms (0.08 earthworms m⁻²).

The greatest density of non-native earthworms were found at the Oak Hill mixed pine and hardwood sites at the 12–18 year aged sites (4.56 species m⁻²) and the Oak Hill pine plantation age 22–28 year aged sites (4.64 species m⁻²).

Native Earthworm Densities

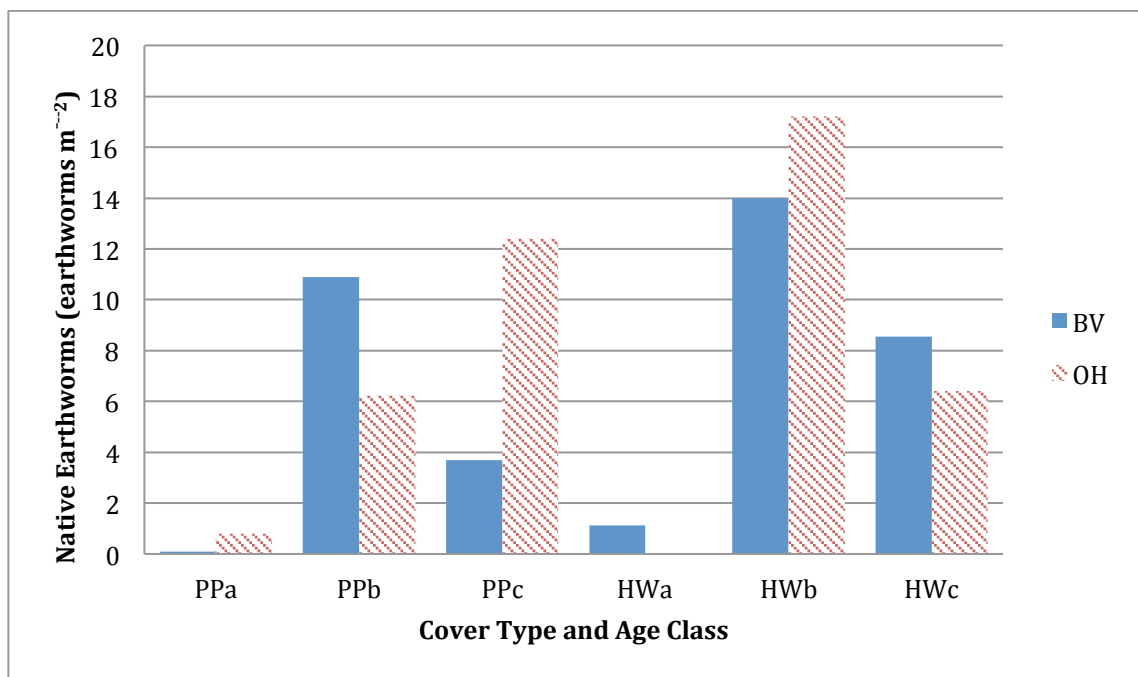


Figure 3.7. Native earthworms species density (earthworms m⁻²) found under each cover type (PP =pine plantation, HW =mixed pine and hardwood) and age class (a =2–8 years since afforestation, b =12–18 years since afforestation, and c =22–28 years since afforestation) at the Beckville (BV) and Oak Hill (OH) reclaimed lignite mine lands.

Native earthworms represented the majority of earthworms found at these reclaimed lignite mine sites. Figure 3.7 shows the abundance (earthworms m⁻²) of native earthworms found under each cover type and at each mine.

Discussion

Influence of Age, Cover and Reclamation Method on Number of Earthworm Species

The lack of organic matter, water stable aggregates, soil structure and low pH values are a few of the obstacles that limit the establishment of earthworms on reclaimed mine lands. Species tolerant of extremes in soil pH, moisture levels (drought and flooding), and compaction may be the first to colonize these reclaimed soils (Eijasckers 2010). Earthworm species found in this study are likely to tolerate adverse soil conditions such as long periods with low soil moisture and high soil temperature.

It may be that native species are better adapted to climate, soil and vegetation in a particular region and therefore may be more important to ecosystem processes than are introduced exotics. James (1991) found that native *Diplocardia* species processed larger quantities of nutrients than did introduced European lumbricid species, even though the exotics were numerically more abundant. The native earthworms apparently exploited the soil volume more effectively and were able to function over a wider range of soil temperatures (for longer periods of the

year).

For the afforested reclaimed mines in this study, earthworm species richness was most abundant at the middle age class (12–18 years since reclamation). The number of earthworm species in this study generally increased with age since afforestation. Adverse soil conditions from the onset of reclamation may be moderated by the increased ground cover, available organic matter, and improved water holding capacity as the once bare lands eventually develop into forests (see Chapter 4 for more in depth discussion of these important soil properties). Similarly, a study comparing earthworm community characteristics along an agriculture land use gradient, Smith et al. (2008) found only two species at the highest intensity land-use and up to six species at the lowest land-use intensity site. Although no significant difference between cover types were found in this study, Lee (1985) found deciduous forests to be more species rich than conifer forests because soil conditions, including pH, were more favorable. He also noted that earthworm communities in coniferous woodlands tend to be made up mainly of smaller litter-dwelling species.

Native Earthworm Species

Diplocardia spp.

Diplocardian species represented the bulk of earthworms found at the Beckville and Oak Hill mine sites. Species of this genus are recognized in the literature for their ability to withstand adverse soil conditions. Diplocardian species

are also able to enter aestivation during drought periods and are known to do well in moderately disturbed anthropogenic habitats such as lawns and gardens. Some diplocardian species commonly collected for fishing bait can survive soil temperatures of 35 degrees C in small containers (James and Hendrix 2006).

Stebbins (1962) found many diplocardian species in forest soils, while *A. trapezoides* was abundant in alluvial soils. Kalisz and Wood (1995) found the genus *Diplocardia* to be relatively insensitive to disturbance and to persist in some disturbed soils. They found *Diplocardia* to be the most common native taxon in the most fragmented natural areas of the Bluegrass regions where exotic earthworms were found to dominate, and riparian habitats supported the greatest diversity and abundance of earthworms. Kalisz and Wood (1995) also found *Diplocardia* to be resilient despite land disturbances. Perhaps because this genus typically lives in the A and E horizons and consumes humus, they may be less sensitive to many types of disturbance. Species of this genus also have the ability to aestivate during periods of extreme heat and drought. They suggest the occurrence and dominance of native earthworms may be useful as an index of ecosystem integrity.

Unlike Frouz et al. (2013), who found Lumbricidae to dominate in reclaimed sites while Acanthodrilidae (especially *Diplocardia*) dominated in unmined sites, this study found diplocardian species at all levels of the chronosequence. The presence of this native earthworm genus suggests the ability of *Diplocardia* to adapt to adverse soil conditions, even as severe as a reclaimed mine site. This includes long summer droughts when soil temperature is high and soil moisture is low.

Bimastos spp.

Many of the species in this native lumbricid genus prefer coarse woody debris as a food resource (Hendrix 1996). There are ten species in the genus *Bimastos*, two of which were found at these reclaimed mine lands. Specimens of *B. parvus* were found at the Beckville mine at the oldest age class under pine plantations. This earthworm has been called the American bark worm and may preferentially utilize coarse woody debris (Hendrix 1996). *B. tumidus* was found under mixed pine and hardwood at the youngest age class.

Non-Native Earthworm Species

Non-native earthworm species, were present at both Beckville and Oak Hill mines and at every age class and cover type combination except for the youngest Oak Hill site (2–8 year since afforestation) under pine plantation. However, non-native earthworms only represented 19 % of the total species at Oak Hill and 14 % of the species at Beckville. Kalisz and Wood (1995) found different results where non-natives were dominant in disturbed areas. However, they became less dominant with increasing distance away from such disturbed centers. For this study, non-native species did not appear to replace native species of earthworms. However, a similar study should be conducted in several years to confirm that native species are still dominant. Peregrine species may or may not negatively affect populations of endemic species. Dalby et al. (1998) found no significant

negative effects of *A. longa*, an introduced earthworm, on the survival, growth or reproduction of a native earthworm.

Aporrectodea trapezoides

Aporrectodea trapezoides dominated as the most abundant non-native species found at both mines and across age classes and cover types. *A. trapezoides* is commonly found on disturbed soils. *Aporrectodea* species, common throughout the world, have the ability to enter dormancy in response to higher temperature, low soil moisture or both (James and Hendrix 2006). Smith et al. (2008) found *Aporrectodea* to dominate the earthworm species composition in regularly tilled agricultural fields (Smith et al. 2008). This earthworm genus is relatively tolerant of agricultural activities such as disk tilling by being able to persist deeper in the subsoil than other endogeic species.

Microscolex dubius and *M. phosphoreus*

Earthworms of the genus *Microscolex* are native to the southern portion of South America, yet over the past two centuries has become widely distributed to many places in the world. *Microscolex dubius* was found at the Beckville mine under pine plantation at the youngest age class (1.2 earthworms m⁻²) and under mined pine and hardwood at the intermediate age class (0.08 m⁻²). *M. phosphoreus* was found at only one site at the Oak Hill mine under mixed pine and hardwood at the youngest age class. These earthworms may influence the development of soil as an epigeic species, thus aid in the early decomposition process of fresh litter material.

They are among the smallest adult specimens (*M. phosphoreus*) of the all species collected in this study, thus a low biomass presence. They may serve as an important prey size for small predators such as salamanders.

Lumbricus rubellus

L. rubellus were found in two of the 60 sites sampled. This species is tolerant of acidic soils (Ma et al. 1990; Sims and Gerard 1999) and also may be effective in colonizing highly disturbed soils. *L. rubellus* is known to have a short life span and high reproductive rates. They have also been linked to the early colonization of other reclaimed mine sites (Dunger et al. 2001). In this study the only occurrence of *L. rubellus* was found at sites in the 12–18 year age class under mixed pine and hardwood at the Oak Hill mine. These earthworms are mainly litter dwelling epigeic earthworms. This species was only recently reported in Texas in 2009 (Damoff and Reynolds 2009). Not too many years prior to this report it was predicted that this species would not invade the southern states of North America because of intolerance to high soil temperatures (Hendrix 1995). Based on detailed studies in the northern states, *L. rubellus* has dramatically contributed to the alteration of forest ecosystems (Hale 2004). Will the apparent establishment of *L. rubellus* in these newly reclaimed mine soils have a significant influence in shaping the above ground plant community. Also, compared to *Microscoclex* species, *L. rubellus* is several times greater in biomass, thus a greater potential to process more organic matter and to serve as a significant food source for terrestrial predators.

Octolasion tyrtaeum

Octolasion tyrtaeum was found to be the most acid tolerant of earthworms tested by Muys and Granval (1997). This European lumbricid has been reported in North America for over a century and is perhaps the most common non-native earthworm in North America (Gates 1977). However, this study yielded low densities of the earthworm (0.08 earthworms m⁻²). The only occurrence of *O. tyrtaeum* was at the intermediate age class under pine plantation at the Beckville mine. As epigeic species they likely influence how fresh litter is decomposed and serve as food source for many terrestrial predators.

Interaction between Native and Nonnative Earthworms

Earthworm communities nearly always include species that pursue different ecological strategies exploiting different food resources and habitat space (Edwards and Bohlen 1996). The interaction between introduced Lumbricidae and Megascolecidae and native earthworm species is an area of ecological concern. It has been postulated that these introduced species displace native forms (Stebbing 1962). However, *Diplocardia* species were found at most sites and often in numbers greater than any introduced Lumbricidae. James (1983) also noted that *Diplocardia* species were found in abundance in lawns that were also inhabited by Lumbricidae. The observations of these researchers along with the results of this project indicate that native and non-native earthworms can coexist. Hendrix (1996) notes that

reduced earthworm diversity may or may not strongly affect certain ecosystem processes, but more diverse assemblages may more effectively exploit soil resources and influence a wider array of processes.

Conservation of Earthworm Fauna

Public awareness is needed of biodiversity issues beyond the charismatic megafauna because of the potential impact that the loss of invertebrate species may have on ecosystem functions (Greenland 1991). It is clear that native earthworms return to these severely disturbed soils in numbers more abundant than their nonnative counterparts. However, the diversity of earthworms and the abundance of native earthworms appear to be lower than in undisturbed forests in the region. As plant communities at these reclaimed lignite mine sites continue to diversify and mature, and soil horizons develop, the potential arises for vertical dwelling earthworm species to return to these soils. Future follow-up studies should determine if non-native species that have thus far been sampled will grow in dominance over the other non-native and or native species. When this project was first being developed it was expected that the widespread and abundant non-native *Amyntas* spp. would be among the most commonly found. However, no individuals of the *Amyntas* genus were found. Research that continues to monitor these soils for earthworms will reveal more about why these earthworms are absent or if they do eventually colonize the reclaimed mine sites. If they do eventually invade, they

may overtake some or all of the other earthworm species that have pioneered the early succession of these terrestrial ecosystems.

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CHAPTER 4

Soil Parameters and Earthworm Community Characteristics at Beckville and Oak Hill Reclaimed Lignite Mines

Literature Review

Soils disturbed and moved after lignite coal mining are particularly unfavorable for earthworms. The chemical, physical and biological properties that once defined the undisturbed soils are greatly altered. Soil characteristics that developed over time are lost due to the process of extracting lignite from these surface mines. However, it has been established that earthworm populations return to these sites and with time, increase in abundance (see Chapter 2). What is less known are the links between specific soil parameters and earthworm abundance on these reclaimed lignite mine soils.

Reclamation of lignite coalmines in the east Texas region is a process involving several steps. After the overburden is removed from the lignite coal seam, it is placed in a previously excavated area. It is then contoured to approximate the previous landscape, producing landforms that function as wetlands, upland or mesic sites. Once the hydrology is established based on landscape position, vegetation reclamation begins. First native grasses, legumes and forbs are seeded into the overburden material. These plants help to stabilize the mine soil and begin to deposit some organic matter. Microbial communities begin to reestablish during

this time. The soil may be amended with fertilizer and lime to encourage plant growth and aid in the reclamation process. Once the grasses, legumes and forbs have become established, trees are planted. Luminant plants their forests as pine plantations or wildlife habitat (mixed pine and hardwood). They generally plant wildlife habitat (mixed pine and hardwood) in the lower lying areas and pine plantations in the upland areas. During the first several years after planting, the trees are monitored for survival rates and replaced if necessary, while the sites are monitored for erosion, plant cover and soil pH. If pH levels are below 5.0, the soil is ameliorated with lime to raise the pH.

The reclamation methods at Oak Hill and Beckville differ by the way in which the overburden is replaced. At Oak Hill, they use the haul-back method of reclamation. The top 1.4 m of the overburden (the oxidized overburden) is separated from the reduced overburden. If it is possible, the reduced overburden is placed immediately in an excavated pit instead of stockpiled to reduce handling costs. The mixed oxidized layer is then placed on top of the reduced overburden. This method helps prevent the oxidation of pyrite that occurs in the Oak Hill reduced overburden. Pyrite oxidation may produce acid seeps and toxically low soil and water pH levels with high solubility of metals. Pyrite occurs deep in the overburden, so land managers strive to avoid exposing it at the surface where it can become oxidized and release sulfuric acid. Pyrite oxidation releases H^+ ions, which lowers the pH of the surrounding soil and water. In addition, waters with low pH

levels can dissolve trace and semi-metal ions such as lead, arsenic, aluminum and manganese, all of which can become toxic to the flora and fauna.

At Beckville, the oxidized and reduced overburden are not separated. Using the “mixed overburden” method of reclamation, they replace the excavated soil with a heterogenous mixture of oxidized and reduced material. Pyrite does not naturally occur in high concentrations in the geology at Beckville, so land managers favor mixed overburden to the haul back because it is a much less costly approach. In addition, previous research on mixed overburden sites found soil fertility to be improved from the mixing of overburden materials (Angel 1973).

The two reclamation methods may influence soil properties that affect earthworm colonization. Earthworms may colonize the haul back site more rapidly than the mixed overburden sites. Because the A and O horizons remain near the soil surface (although mixed with the E, B and C horizons), microbial populations may still be more numerous than mixed overburden soils. These factors may contribute to earthworm colonization because the plant and microbes provide a food source for the earthworms. At the mixed overburden sites, the soil may not have as much microbial life, at least initially, because overburden from as deep as 46 m may now be at the surface. These materials may be harder for earthworms to colonize until significant plant and microbial communities have established themselves (Schröder 1986). Earthworm survival in a soil depends on several factors such as soil moisture, aeration, temperature and available food sources. In highly disturbed soils such as those in reclaimed mine lands, soil quality is often initially low,

especially in terms of organic matter and microbial communities. Based on previous research on earthworm distributions and soil properties, soil moisture, soil texture, depth, pH and organic matter content tend to be most important (Curry 2004).

Predators and parasites have a negative influence on earthworm presence in soils. Many species of birds, gophers, and other small mammals depend on earthworms as an important source of food. Parasites in the soil that can kill or debilitate earthworm reproduction can also decrease the abundance of earthworms.

Soil Moisture

Water constitutes 75-90% of earthworm body weight. Earthworms avoid water loss by moving to areas with more water or, for some species, by entering a quiescent (inactive) state in direct response to adverse conditions. Similar to this phenomenon are diapause and aestivation depending on the environment and species of earthworm. Earthworms apparently lack a mechanism to maintain constant internal water content, so that their water content is influenced greatly by the water potential of the soil (Kretzschmar and Bruchou 1991). Soil moisture content may even influence cast production (Madge 1969). Soil moisture may influence numbers and biomass of earthworms at any given location. Seasonal earthworm mortality in temperate soils has usually been attributed to moisture stress rather than to temperature extremes (Phillipson et al. 1976). Optimum soil moisture content varies for different earthworm species and ecological groups, and

within species there appears to be a considerable capacity to adapt to local conditions (Lee 1985). Nordström (1975) found a decrease in earthworm activity with decreased soil moisture content. Earthworms seem to be most active at moisture tensions approaching field capacity (\sim -10 kPa), and activity declines rapidly as the moisture tension drops to -100 kPa and ceases for most species at moisture tensions below the wilting coefficient (-1500 kPa) (Norström and Rundgren 1974; Baker et al. 1993).

Soil moisture, like soil temperature, is influenced by environmental factors as well as soil properties. Maintaining an appropriate level of soil moisture is important for supporting earthworm communities. However, some species of earthworms are capable of withstanding low soil moisture. During droughts some species of earthworms can burrow deeper into the soil where there is more moisture. They may also enter a state of quiescence until soil moisture levels become more favorable.

Earthworm species such as *Aporrectodea* spp. and *Diplocardia* spp. are active in the upper 10 cm of soil when the soil is moist, but when the soil is dry they usually migrate below 20 cm, where they quiesce—tightly coiled within spherical, mucus-lined cells (Curry 2004).

Soil pH

Most earthworm species prefer near neutral pH soils. However, some species thrive in soils with higher acidity. Soil pH also may influence earthworms to

enter quiescence (Edwards and Bohlen 1996). Earthworm castings are often more neutral than the soil in which the earthworms live. Earthworms neutralize soil acidity as it passes through their guts. Hlava and Kopecky (2013) found soil pH to influence the density and biomass of earthworms. Joschko et al. (2006) found soil pH to influence the number of earthworm species.

Earthworms are generally absent in soils with pH values less than 3.5 and are rarely found in soils with less than 4.5 pH values. Most earthworms in temperate climates tend to prefer pH values between 5.0 and 7.4 (Satchell 1967; Bouche 1972).

Soil Organic Matter

Soil organic matter may be the most important factor influencing the presence of earthworms in the soil. Earthworms feed on decomposing organic matter and the microbes associated with decomposition. Many species of earthworms actually live in the O horizon of the soil breaking down large plant debris (epigeic earthworms). Other species of earthworms feed on the more decomposed plant debris while living in the A horizon of the soil (endogeic earthworms). Other species of earthworms carry plant debris deeper into the soil profile through their vertical burrows.

Fine roots in the soil may also have a great influence on earthworm communities. Fine roots produce organic matter and carbon exudates, the rhizosphere which increases microbial populations and provides food for earthworms. The biopores (root channels) left from decomposed roots also provide

aeration pathways that promote microbial communities favorable to earthworms as sustenance.

The litter and belowground input from vegetation largely determine the nature and quality of soil organic matter. Vegetation growing on impoverished acidic soils often produces tough, unpalatable litter low in nutrients with C:N ratios higher than 60:1. These organic substrates are generally unfavorable to earthworms. However, research from Wilson (2001) found earthworm populations in pine plantations where C:N ratios were high. Litter produced from grass, herbaceous plants, and deciduous trees growing in fertile soils tend to have C:N ratios of less than 20:1. These organic substrates often support a greater abundance of earthworms. The organic matter that provides the food base for the earthworm community is vitally important in determining their distribution and abundance. Edwards and Bohlen (1996) found soil organic matter content to be a good predictor of earthworm abundance, and Hendrix et al. (1992) reported a highly significant correlation between earthworm populations and soil organic carbon content over a range of sites. Some plant litter is low in nutrients, with nitrogen (N) often in short supply. N is often considered the critical factor limiting earthworm populations in many ecosystems, both temperate (Satchell 1967) and tropical (Lee 1985).

Soil Temperature

Ambient soil temperature is the result of seasonal changes in the atmosphere that influence solar radiation reaching the soil surface, proximity of the site to the equator, the amount of shade on the site, the amount of moisture in the soil, and soil texture. Earthworms in general can tolerate a wide range of soil temperatures, from as low as 3 °C to as high as 25 °C, though most individual species have a narrower temperature tolerance. If soils become too hot or cold, earthworms will go into a state of quiescence until temperatures return to a tolerable level (Edwards and Bohlen 1996).

The range of temperatures in which most earthworms can function is narrow, with upper lethal temperature rather low (25 to 35 °C) and optimum temperature typically in the range of 10 to 20 °C for cool temperate species and 20 to 30 °C for tropical and subtropical species (Lee 1985; Edwards and Bohlen 1996). Body size for earthworms seems to be constrained in warmer soils by increased energy demands for respiration, resulting in a severely limited energy supply for tissue production from a low-energy diet. Physical, chemical and biological processes occurring in the soil are strongly influenced by temperature. Scheu (1987) found that an increase in temperature from 10 to 15 °C increased cast production by 20 %. While an increase in volumetric water content from 48 to 60 % caused a doubling of cast production.

Temperature and moisture are usually inversely related regarding earthworm abundance. Higher surface temperature and relatively dry soils are

much more limiting to earthworms than low temperature and waterlogged soils.

Earthworms are known to survive in soils below freezing through the winter.

Diplocardia sp. move down to lower soil strata at temperatures below 6° C (Dowdy 1944). Nordström (1975) found a decrease in earthworm activity when soil temperatures reaches 14–16 °C in a Swedish beechwood forest.

Soil Texture

Soil texture is a soil property that affects many other soil properties including soil temperature, soil moisture and soil pH. Specifically, many earthworms tend to prefer medium textured soils to coarse or fine textured soils (Guild 1948). Medium textured soils often maintain more favorable ratio of oxygen to moisture, have a higher pH buffering capacity than coarse textured soils and often support greater plant diversity. Hendrix et al. (1992) found that moderately and severely eroded sandy clay loam soils supported significantly higher earthworm numbers than did slightly eroded soil with higher sand content. The study also showed that silt content was most highly correlated with earthworm abundance. (Hendrix et al. 1992). Low available water capacity and organic matter of coarse textured soil may present an unfavorable habitat for earthworms (Lee 1985).

However, some earthworms from the Lumbricidae family have been shown to prefer soils with high clay content (Nordstrom and Rundgren 1974; Baker et al. 1993). Curry and Cotton (1980) found decreasing earthworm densities in coarse textured soils when compared with medium textured soils.

The objective of this portion of the study was to determine which selected soil parameters affect earthworms under afforested pine plantation and mixed pine and hardwood forest cover types on reclaimed lignite mine soils.

Methods of Soil Collection and Processing

The following soil properties were measured in the field or on the campus of Stephen F. Austin State University in the Water, Plant and Soils Lab or the Forest Soil Lab. Soil moisture was determined gravimetrically. Field capacity and wilting coefficient were measured to calculate available water capacity (AWC) using a soil ceramic pressure plate apparatus. Soil electrical conductivity (EC) and soil pH was measured using 2:1 water to soil mixture. Total carbon (TC), total nitrogen (TN) and carbon:nitrogen (C:N) were determined on the mineral portion of the soil using dry combustion on an Elementer Variomacro CN Analyzer. The concentrations of plant available nutrients P, K, and Ca were determined using Mehlich III extraction and ICP analyses. Soil bulk density (BD) was determined by the core method as described by Blake and Hartge (1986). Soil strength (ST) was determined using a cone penetrometer and methods described by Bradford (1986). Soil temperature was measured using a digital soil thermometer at 10 cm depth at each soil pit and according to methods described by Taylor and Jackson (1986). Particle size analysis (PSA) was conducted using the hydrometer method.

Statistical Analysis

Stepwise regression analysis was performed using SAS 9.1. Earthworm densities were log transformed in the model to account for the problem of non-constant variance. All tables and figures present untransformed data. Soil variables were tested for their ability to predict earthworm densities at the 0.05 alpha level using stepwise regression. The model to be tested was:

$$\begin{aligned} \text{Log earthworm density (ew m}^{-2}\text{)} = & \beta_0 + \beta_1(\text{age of site}) + \beta_3(\text{available water capacity}) \\ & + \beta_4(\text{soil temperature}) + \beta_5(\text{soil strength}) + \beta_6(\text{soil moisture}) + \beta_7(\text{sand fraction}) + \beta_8 \\ & (\text{clay fraction}) + \beta_9(\text{silt fraction}) + \beta_{10}(\text{forest cover type}) + \beta_{11}(\text{pH}) + \beta_{12}(\% \text{ nitrogen}) + \\ & \beta_{13}(\% \text{ carbon}) + \beta_{14}(\text{C:N}) + \beta_{15}(\text{mine}) + \beta_{16}(\text{phosphorus}) + \beta_{17}(\text{potassium}) + \beta_{18} \\ & (\text{calcium}) + \beta_{19}(\text{magnesium}) + \beta_{20}(\text{sodium}) \end{aligned}$$

The stepwise regression procedure first fit a simple linear regression model for each of the potential X variables. For each simple linear regression model, the F statistic was obtained. The test was set at 0.05 alpha level to help control the risk of a Type I error. The X variable with the lowest *p* value or highest F value was used first. Then another variable was added according to the same standards. This was repeated until no more variables have *p* values less than 0.05. If a variable that was already in the model has a *p* values greater than 0.05 after other variables were added, it was then dropped from the model. The final model was then validated using PROC REG in SAS 9.1.

Results

A box plot is a standardized way of displaying the distribution of data based on the minimum, first quartile, the median and the maximum values in the data set. Figure 4.5, summarizes the small variation in measured soil parameters at each mine. Although reclamation type (as represented by each mine) was not a significant variable for earthworm density prediction, a comparison of some site characteristics is useful in understanding some of the variation surrounding this project. The differences may be explained by the extended drought that occurred prior to sampling at the Beckville mine verses one year later at the Oak Hill mine. Soil temperature at the Beckville mine tended to be higher than at the Oak Hill mine (Fig. 4.5, B) and soil moisture was lower at some sites at the Beckville mine (Fig. 4.5, C). The Beckville mine also showed a larger range of available water capacity most likely as a result of some of the older sites being coarse sandy soils (Fig. 4.5, D). The C:N ratio at Oak Hill was lower than the C:N ratio at Beckville (Fig 4.5, E). This is a result of the higher concentration of total N in the Oak Hill soils (chart F). Soil pH remained within the range of 4 to 8 with median pH value at Oak Hill between 5.5 and 6 and the median at Beckville was 7.

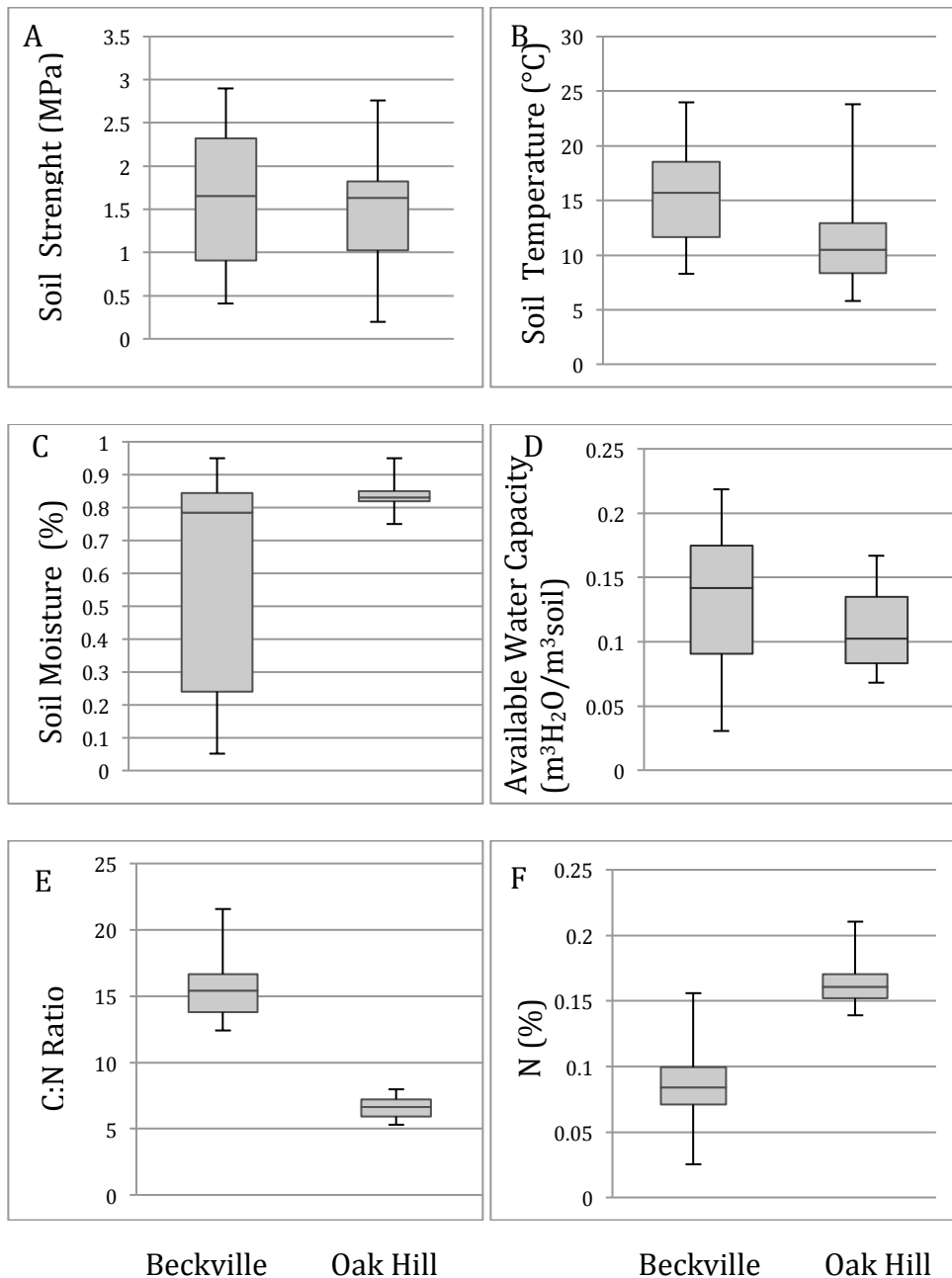


Figure 4.1. Box and whisker plots of soil strength (A), soil temperature (B), Soil moisture (C), available water capacity (D), C:N ratio (E) and concentration of total N (F) from the Beckville and Oak Hill mines. The top of each “whisker” represents the maximum value; the bottom of bottom “whisker” shows the minimum value. The top of the box represents the 1st quartile and the bottom of the box represents the 3rd quartile.

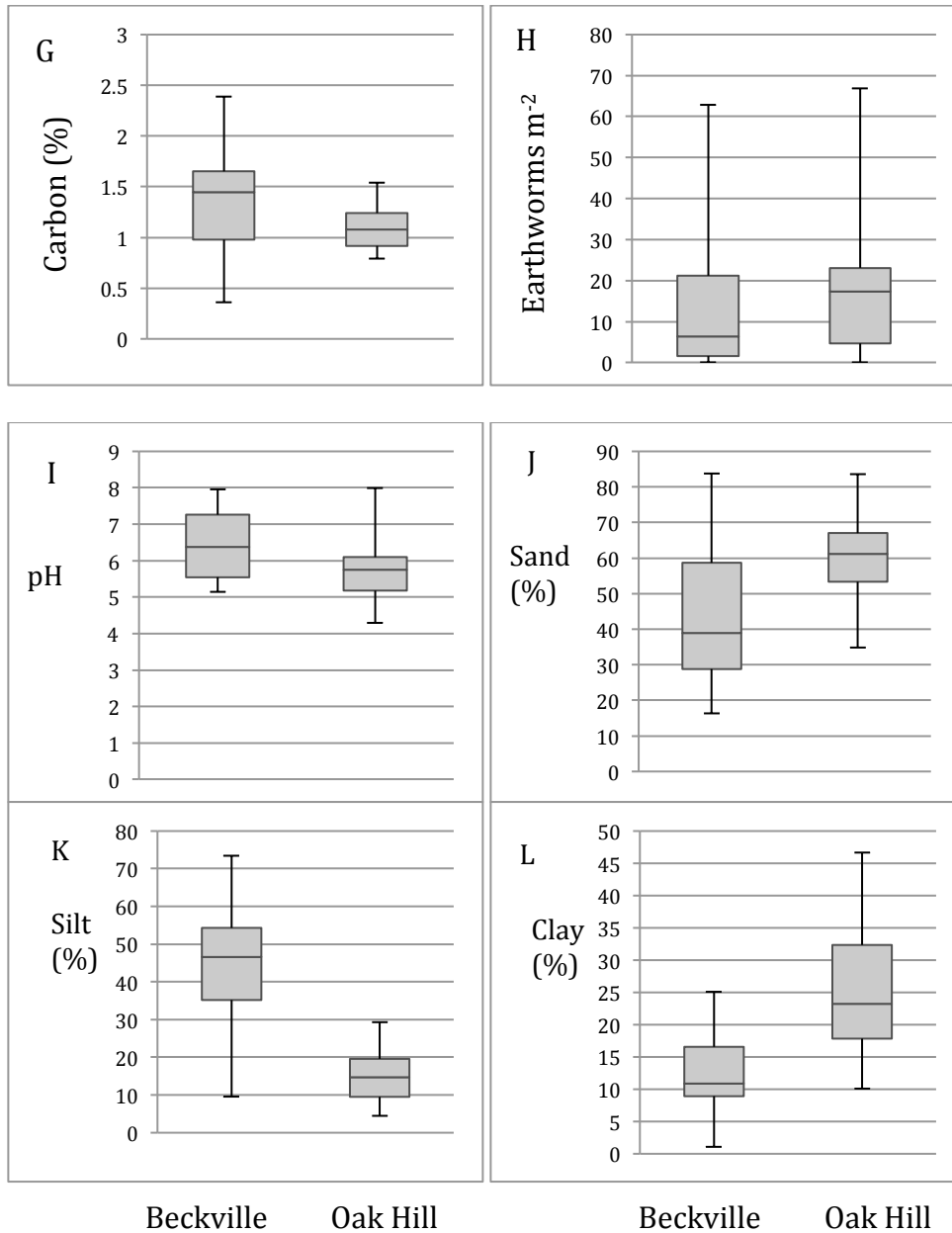


Figure 4.1 continued. Box and whisker plots of soil carbon (G), earthworm density (H), soil pH (I), sand fraction (J), silt fraction (K) and clay fraction (L) from the Beckville and Oak Hill mines. The top of each “whisker” represents the maximum value; the bottom of bottom “whisker” shows the minimum value. The top of the box represents the 1st quartile and the bottom of the box represents the 3rd quartile. The horizontal line inside of the box represents the median.

The final model was:

$$\text{Log earthworm density (ew m}^{-2}\text{)} = -13.18 + 0.26 (\text{age of site}) - 0.19 (\text{soil temperature}) + 1.41 (\text{soil pH}) + 0.06 (\text{soil sodium})$$

p < 0.001
 Adj R² = 0.411
 RMSE = 2.56

Table 4.1 presents the stepwise procedure that resulted in the final model. From the physical and chemical parameters tested during this study, soil temperature (p=0.0256), soil pH (p=0.0309) and soil sodium (p=0.0173) were included in the final regression model. Age since reclamation was also included (p=0.0006) because of its relevance to project design as a chronosequence and its strong correlation to earthworm densities.

Table 4.1. Summary of stepwise selection from the model tested.

Step	Variable Entered	Variable Removed	Partial R ²	Model R ²	C(p)	F Value	Pr > F
1	Age (years)		0.1992	0.1992	13.4246	13.44	0.0006
2	K (ppm)		0.0998	0.2990	7.2734	7.54	0.0082
3	Temp (°C)		0.0646	0.3636	3.9925	5.28	0.0256
4	pH		0.0561	0.4197	1.4123	4.93	0.0309
5		K (ppm)	0.0307	0.3891	1.9168	2.69	0.1069
6	Na (ppm)		0.0648	0.4539	-1.3815	6.06	0.0173

It was reported in Chapter 2 that the age since reclamation had a significant influence on earthworm abundances. A higher density of earthworms tended to be found on older reclaimed sites. A greater number of sites with no earthworm were observed at the youngest aged sites (between 5 and 8 years of age).

The sites with the highest number of earthworms occurred in soils with temperature that ranged from 5–15 °C; no earthworms were observed in soils with temperatures that ranged from 10–25 °C.

Discussion

The nature and properties of reclaimed mine soils are highly variable. Attempting to develop a prediction equation for earthworm densities using data obtained from reclaimed lignite mines is ambitious, and several factors challenge its accuracy. There are many variables to take into account, although it is possible to explain, with statistics, some of the interactions. This research shows that, when log transformed, earthworm densities may be predicted using the variables of soil pH, age since reclamation of the site, soil sodium and soil temperature.

Soil pH

The majority of earthworms prefer soil pH close to 7. In these reclaimed mine soils fewer earthworms were found in soils with pH levels below 6.0. The haul back sites had lower pH values even though that method specifically was implemented in an attempt to prevent acidification (Figure 4.5, I).

Soil Temperature

Soil temperature greatly affects the physical, biological and chemical processes occurring in the soil. It is known that the microbial processes and

earthworm activity are influenced markedly by soil temperature changes (Mellilo et al. 2002). In the south central region of the United States, earthworm abundances are highest during the cooler seasons of the year—late fall, winter and spring. We avoided sampling during the hot summer months of June, July, August and early September. Because soil temperature was a significant indicator of earthworms from this study, we concluded that some of the sampling times may have been too warm and this may have affected earthworm densities measured during these periods.

Soil temperature and soil moisture have been found to play a large role in the regulation of seasonal activity in earthworms, directly or indirectly by affecting, for example, food supply and microbial decomposition. Soil temperature also may be an indicator of factors that help to regulate soil temperature such as soil moisture, the depth of the O horizon, vegetative ground cover and the percent shade cast by forest growth (Nordström 1975).

Soil Sodium

Soil sodium, although at low concentrations in these soils, was found to be positively related to earthworm densities in these reclaimed mine soils is most likely the result a close association between low-lying areas and some Na accumulation in the soil. Higher levels of soil sodium tend to be associated with soils that are not leached of basic cations. Active earthworms require moist soils due to their lack of a mechanism to maintain constant internal water content so that

their water content is influenced greatly by the water content of the soil (Kretzschmar and Bruchou 1991). However, it is interesting that available water capacity and percent soil moisture were not found to be significant predictors of earthworm densities in these soils.

Confounding Factors

This project was challenged by variation in the geologic overburden materials, different management practices associated with different sites over time at the same mine (some sites were limed; some had cattle grazing previously, etc.). In addition, a very severe drought preceded the start of earthworm collection at the Beckville mine. In 2011, 97% of Texas was in extreme or exceptional drought in February of 2012, the Martin Lake mine complex was still in extreme drought (L'Oreal 2012). Field collection of earthworms at the Beckville mine began during the spring of 2012. Although certain earthworm populations, (*Diplocardia* for example) adapt to soils influenced by intense seasonal characteristics, such as low soil moisture or high temperature, and have the capacity to survive extended periods of drought, they nevertheless may have suffered significant mortality during this extreme drought, particularly among juveniles unable to escape desiccation by moving deeper into the soil and becoming inactive (Gerard 1967). Recovery of earthworm populations from the drought may take two or more years (Edwards and Bohlen 1996).

The seasonal heat common in the region also influences earthworm

community characteristics. The severity and duration of summer heat imposes constraints on the duration of earthworm activity and undoubtedly influence both the overall earthworm population density when favorable conditions exist primarily during relatively short periods in autumn, winter and spring (Curry 2004).

Although Baker (1998) found that rainfall can often explain more of the variance in earthworm numbers than any other variable in a range of agricultural soils, he found no correlation between earthworm densities and soil moisture at the time of sampling.

Soil Recovery after Lignite Mining

Soil ecosystems are resilient, and with proper management can reestablish themselves as healthy ecosystems following severe disturbances such as surface lignite mining. Earthworm communities can repopulate these disturbed soils as soon as five years since revegetation and are able to do so for a number of reasons. Earthworms have specific requirements for soil moisture, temperature and food sources. The grasses, legumes and forbs planted during the stabilization step of reclamation help regulate evaporation, soil temperature and provide a food source for soil decomposer organisms, which includes earthworms. It is because of this initial herbaceous vegetation that earthworms may become established and survive—even flourish—on reclaimed lignite mine sites in East Texas.

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CHAPTER 5

Summary

Reclamation of severely disturbed land, such as lignite mine lands, is desirable to restore ecosystem function and productivity, in addition to regulatory requirements. To better understand and manage the processes of land reclamation after a severe disturbance, knowledge of the belowground ecosystem and its functions is essential. This project investigated the abundance and species composition of an important soil organism, earthworms, as indicators of the return of overall soil function on these reclaimed lignite mine lands.

In a terrestrial ecosystem, soils have six key functions. First, soils act as a medium for plant growth. They support plant roots and supply them with water and nutrients. Second, soils control the fate of water in the hydrologic system. Soil properties such as texture, biopores and organic matter levels help determine how where and how rapidly water infiltrates and percolates through the soil profile, or if it instead runs over the surface as overland flow. Third, soils provide function in nutrient and carbon cycling. Fourth, the soil provides habitats for soil fauna and burrowing land animals. Fifth, the soils take up and release CO₂, O₂, methane and other gasses, therefore influencing the composition and physical conditions of the atmosphere. Finally, soil is an engineering medium as an ingredient for bricks, or as foundation for roads and buildings (Brady and Weil 2008). Surface mining activity

disrupts these functions and effective reclamation should set the stage for soil function to begin again.

Research that investigates the natural establishment of earthworms, because of their relationship with important soil properties, can help determine if important soil functions have returned. Understanding of soil ecosystem recovery after severe disturbances is instrumental in gauging the success of land reclamation. Soil organisms, such as earthworms, that are affected by numerous soil biological, chemical and physical properties may offer an indication of overall soil health. Less sophisticated approaches only consider soil factors in isolation and may not consider belowground from aboveground ecosystem processes together (Heneghan et al. 2008). Research on the natural establishment of earthworms on reclaimed lignite mine lands contributes to soil ecological knowledge and is therefore useful to improve understanding of successful land reclamation. Through this survey of earthworm abundance and species composition on reclaimed lignite mine soils, this project provides insight into potential reclamation practices.

Reclaimed mine soils in east Texas were surveyed for earthworm abundance and species composition to better understand the nature of belowground ecosystem recovery. Previous research on these soils addressed long-term vegetation development (Christian 2013) as well as microbial community recovery (Mott and Zuberer 1991, Ng 2014), but research that quantified a soil macro invertebrate such as earthworms was lacking.

This study was conducted at the Martin lake mine complex in east Texas at two different mines; Beckville where the mixed overburden method is practiced and Oak Hill where the haul-back method of reclamation is used. The study determined earthworm abundance and species composition on reclaimed lignite mine lands in east Texas under mixed pine and hardwood and pine plantations on a chronosequence. The chronosequence included three age classes; youngest, 2–8 years since reclamation, middle aged, 12–18 years since reclamation and oldest, 22–28 years since reclamation. Earthworms returned as soon as 2–8 years since reclamation, multiple species of earthworms were present in the soils, and species native to the area were dominate over non-native earthworm species. Soil pH and soil temperature were important indicators of earthworm abundance in these reclaimed lignite mines soils.

Earthworms were found as soon as 2–8 years after reclamation at the Martin Lake mine complex and generally increased with age of reclaimed site. The age of reclaimed sites was also significantly correlated to earthworm abundance ($p > 0.0001$), and a multiple comparison test (Tukey's) found the youngest age class to be significantly different from the middle and oldest age class. This suggests a period of natural establishment when earthworm densities remain low for several years before they gradually develop larger population densities. The lack of a significant difference between the middle and oldest age class suggest that earthworm densities remain relatively steady from 12 to 28 years since

reclamation. A study that incorporated an older reclaimed site such as a 40 or 50-year-old site, would offer insight into the size of earthworm populations over a longer period of time, and offer a better understanding of how these reclaim lignite soils recover.

Earthworms under mixed pine and hardwood forests tended to be more abundant than those under pine plantation forests ($p=0.0108$). This is most likely the result of several factors, including the diversity and palatability of leaf litter available under mixed pine and hardwood forests. However, these reclaimed lignite mine soils, mixed sites were often planted in lower lying areas, where soils retained greater moisture (Grimes 2014). Earthworms prefer moist soils and may be more abundant in areas with consistently higher moisture levels year round (Edwards and Bohlen 2008).

In studies on earthworm abundance on reclaimed lignite mines lands in Germany, earthworms did not return to soils until 10 years after reclamation in pine plantations and five years under mixed hardwood forests (Dunger et al. 2001). That earthworms were found in east Texas reclaimed mine lands under both mixed pine and hardwood and pine plantation at 2 to 8 years since reclamation speaks favorably to the effectiveness of the reclamation at these mines, the resilience of the earthworms species involved and the quality of the soils and vegetation.

Understanding the species composition of earthworms as these lands recover from surface lignite mining is also an important contribution to soil

ecological knowledge. Species richness (number of total species) at both mines exhibited a trend of increasing number of species from the youngest to the middle aged class and decreasing at the oldest age class. This may be explained by the diversity of plant species, including the ground cover that persisted while the forest canopy was still open during the middle age class. At the oldest age class, herbaceous plant species were fewer as woody tree and vine species began to increase and canopy closure occurred (Christian 2008). Earthworms benefit in the environment of fine herbaceous roots and diverse organic matter that include decomposing wood, tree leaves and herbaceous plant material (Edwards and Bohlen 2008). At the oldest sites, canopy closure most likely reduced the amount of understory herbaceous plant species.

The Simpson's species diversity index found the species composition at the youngest age class to display the most evenness. This was possibly the result of a common native earthworm species, *Diplocardia caroliniana*, which dominated the middle age class, while *D. caroliniana* and *Apporectodea trapezoides* dominated the oldest age class. The Simpson-Weiner diversity index was also used to measure earthworm species diversity. The Beckville and Oak Hill mines displayed similar diversity index values, suggesting no large differences between the two reclamation types regarding earthworm species diversity. However, the mixed pine and hardwood sites at both mines were more diverse than the pine plantation sites according to the Simpsons and Shannon's diversity indices. This is most likely the

result of the diverse plant species supporting a more diverse earthworm community, but may be confounded by the fact that the mixed pine and hardwood sites were generally located in lower landscape positions..

The majority of earthworms identified from these reclaimed lignite mine soils were native to the region. At the Beckville mine, 86 % earthworms and Oak Hill, 81 % of earthworms were native to the region. At both mines and, native species of earthworms were found as soon as 2–8 years since reclamation. The dominance of native species over non-native species is contrary to literature that reports primary succession of non-native species tends to dominate severely disturbed soils (James and Hendrix 2004).

Soil parameters were measured to see if any would correlate to earthworm abundance. Soil temperature ($p=0.0256$) and soil pH ($p=0.0309$) were found to significantly predict earthworm abundance at these reclaimed lignite mine sites. Soil temperature was inversely related to earthworm abundance. Earthworms are most abundant during the cooler months of the year in the east Texas region, January through March, while our sampling season extended from the end of September through May. The lower earthworm numbers from the warmer months was reflected in the data and in the overall low numbers of earthworms found at these sites when compared with native soils. If sampling were restricted to winter and early spring months (December through March), overall earthworm densities may have been higher. Soil pH is an important indicator of earthworm densities in

the soil (Edwards and Bohlen 2008). The mine soils displayed a wide range of soil pH values from 4.3 to 8.2. Earthworms were most abundant in soils with pH values near 7.

This research and others conducted on reclaimed lignite mine lands highlight the potential for these soils to return to a productive landscape and healthy ecosystem. In addition to the natural recovery of earthworms in this study, Christian (2013) found vegetative communities on reclaimed lignite mine lands in east Texas to resemble that of unmined reference sites within 12 to 15 years of reclamation. Ng (2013) sought to understand when a reclaimed soil returned to premined conditions by analyzing soil fertility. He found nitrogen, potassium and calcium were able to exceed premined conditions and develop native profile distribution within 10 to 15 years of reclamation. These studies and others continue to show how sound land management practices can effectively reclaim severely disturbed land.

The data produced from this study was limited by several factors; the maximum age of the reclaimed sites, the variable nature of the reclaimed soil texture and a severe drought that ended in 2011. The oldest reclaimed site sampled was 28 years since reclamation. Although the oldest sites were useful to our understanding of earthworm community development, sites as old as 40 to 50 years since reclamation might have offered a better picture of soil ecosystem recovery. Had the mine been in operation longer than 30 years prior to this project, older

reclaimed sites would have been available to sample. The variable geology of the sites also partially confounded the results. The majority of sites were classified as sandy clay loams, while the oldest sites at Beckville were sandy loams with the sand fraction representing up to 80 % of the particle size distribution. Coarse textured soil contributes to lower water holding capacity, lower cation exchange capacity, and generally higher soil temperatures than medium and fine textured soils. These sandy soils are therefore are not as favorable for earthworms. However, a study that compared earthworm abundance and species composition on reclaimed lignite mine soils to adjacent unmined soils would offer better insight into the rate of soil ecological recovery of the reclaimed sites. The adjacent unmined soils would have experienced the same severe drought conditions and thus offered a better idea of population density values and species composition that should be expected from the reclaimed soils.

Future research that informed land managers of herbaceous and woody plant species that contribute the highest quality plant litter to the organic matter fraction of the disturbed soil would be useful. Such a study would recognize the interrelationship between the above and belowground ecosystem and uses soil ecosystem knowledge to achieve the most productive and thriving ecosystem. The study would observe the potential of plant communities to contribute to the humic portion of the soil as well as organic resources for soil biota. Land managers could then make informed decisions about which herbaceous and woody species to plant

in order to improve soil ecosystem recovery.

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