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By Jessica H. Johnston

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Hardwood Reforestation on Post-Mined Land under Varying Soil Replacement Strategies in the Eastern Interior Region

For the degree of Master of Science

Is approved by the final examining committee:

Phillip E. Pope
Chair

Arthur P. Schwab

Eileen J. Kladivko

Douglass F. Jacobs

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HARDWOOD REFORESTATION ON POST-MINED LAND UNDER VARYING SOIL REPLACEMENT STRATEGIES IN THE EASTERN INTERIOR REGION

A Thesis
Submitted to the Faculty
of
Purdue University
by
Jessica Hannah Johnston

In Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

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ABSTRACT

Johnston, Jessica H. M.S., Purdue University, August 2012. Hardwood Reforestation on Post-Mined Land Under Varying Soil Replacement Strategies in the Eastern Interior Region. Major Professors: Prof. Phillip E. Pope and Prof. Arthur P. Schwab.

Approximately two million hectares of land in the U.S. have been impacted by surface coal mining and subsequent reclamation. Soil replacement strategies follow standards set by state and federal regulations; however, permits allow for new techniques, such as the soil replacement method studied in this project, in order to improve the quality of the land. A new strategy, which involves dumping soil in overlapping piles with minimal equipment compaction, has been studied in the Appalachian coal region on a range of site conditions. However, this approach has not been evaluated in the Eastern Interior region, which includes Indiana. This study assessed the relationships of soil physiochemical and biological conditions on plant survival, growth, and root development by investigating growth responses of seedlings planted on standard graded (GR) plots and those planted on loosely dumped (LD) soil. The study, located on a reclaimed mine site in southern Indiana, used a split plot design. A total of 3200 seedlings of four hardwood species, northern red oak (*Quercus rubra* L.*)*, white oak (*Quercus bicolor* W.*)*, Shumard oak (*Quercus shumardii* B.), and American chestnut (*Castanea dentate* B.), were used.

Soil physical properties of the LD and GR soils showed significant treatment effects. Bulk density, moisture retention and porosity of the LD soil were favorable for plant growth. The GR soil had a significantly higher bulk density (1.74 g/cm^3) compared to the LD soil (1.54 g/cm^3) resulting in root impairment. The chemical properties of the LD and GR soils showed little variation and had low fertility status, organic carbon contents, and cation exchange capacities consistent with that of a subsurface soil. The presence and activity of soil microorganisms measured through microbial biomass (MB) and fluorescein diacetate (FDA) hydrolysis were low and reflected the poor soil quality with slight treatment differences.

Seedling survival was significantly higher in the LD treatment compared to the GR treatment at the end of the second growing season. Above ground growth of the four hardwood species did not favor one soil replacement method over the other as few treatment effects were observed. A few exceptions to this generalization were observed which favored the LD treatment: average plant biomass significantly increased and plant water potential significantly decreased (less water stressed) in the LD treatment. The similarities in above ground growth among treatments is attributed, in part, to the short duration of the study (17 months) which may not be a sufficient amount of time for differences in above ground growth to manifest themselves.

In contrast, the below-ground growth parameters indicate that the method of soil replacement influenced root morphology and architecture due to the difference in soil properties. Increased lateral and tap root dry weights, root volume and projected root area (measured by WinRHIZO) were observed in seedlings grown on the LD treatment. It is likely that bulk density was the main root-restricting factor.

American chestnut seedlings ranked highest in root and shoot parameters, as well as plant water potential; yet this species ranked lowest in survival and had the greatest amount of dieback. In contrast, Shumard oak seedlings generally ranked second or third in root and shoot parameters; and yet had the highest survival rate and largest increase in lateral root dry weight compared to all other species.

Overall, this study showed that seedling survival and growth was favorable on the LD treatment. The below ground root responses measured with traditional or WinRHIZO analyses were clear indicators that the seedlings favored the LD treatment. In general, above-ground growth parameters were not sensitive indicators of growth over the 17-month study period, whereas below-ground root parameters proved to be sensitive indicators of seedling growth.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Coal is the most abundant fossil fuel produced in the United States. The US is the global leader in both coal reserves and production (Institute for Energy Research 2012). US coal production reached 1.04 billion metric tons in 2007, and is projected to increase by 25 percent to 1.32 billion metric tons by 2030 (Institute for Energy Research 2012). This compares to an estimated annual world consumption of 7.16 billion metric tons (2010) and represents approximately 26 percent of the global energy consumption. In the US, over 90 percent of the coal consumed is used to generate electricity which provides approximately one half of the total electricity generated.

Coal represents an important component of the overall energy budget for the US, and it is generally accepted that coal has allowed the US to have greater degree of energy independence. However, surface coal mining operations in the US often compromise certain aspects of soil health, which later result in negative impacts on plant growth and the environment (Rokich et al. 2001). Since the 1950's, surface mining has become the predominant method for extracting coal to avoid the hazards and costs associated with subsurface mining, particularly in mountainous terrain (Auch et al. 2005). Currently, it is the predominant approach in the Eastern Interior region, which includes the southern border of Illinois, western border of Indiana, and western tip of Kentucky. In 1984, surface coal mining had disturbed 930,000 hectares (ha) in the US, and it was estimated with reasonable certainty that four million ha of strippable reserves remained (MacMahon 1987). In Indiana alone, surface coal mining exceeds 2000 ha per year (Chaney et al. 1995).

Despite protection performance standards set by the Surface Mining Control and Reclamation Act (SMCRA) of 1977, there is general consensus that the soil disturbed during surface coal mining and subsequent soil replacement is detrimental for forest land use (Rodrigue & Burger 2004;Groninger et al. 2006). Under SMCRA, mine operators are required to grade and recontour the post-mined land surface with the excavated material in order to restore the land to its' approximate original contour (Code of Federal Regulations 2012). They are also required to use topsoil, or the best available soil material, to support revegetation. In some cases, the original topsoil from a pre-disturbed mine site consisting of A and B horizon materials, which provide improved soil structure and increased nutrients for plant growth, is set aside for future soil replacement. At many mine sites, however, low fertility subsurface C horizon and 'spoil' materials are used resulting in poor soil physiochemical conditions (Rodrigue & Burger 2004;Chatterjee et al. 2009). Even when surface horizon soil materials are used, plant growth can be negatively impacted due to intensive grading and the resulting compaction that impedes root development (Bussler et al. 1984;Rokich et al. 2001).

Current reclamation strategies often prohibit the return of native forests (Auch et al. 2005). Ecosystem and forest reconstruction following land disturbance from coal mining has slowly improved due to considerable research in the past four decades (Bowen et al. 2005). In Indiana, for example, reintroduction of fine hardwood species on post-mined lands is gaining acceptance (Groninger et al. 2006). The research in this project is focused on soil replacement methods on post-mined land and their effects on hardwood seedling growth and survival.

1.2 Reforesting Reclaimed Surface Coal Mining Sites

In the Eastern Interior region, mining companies are required to develop a successful vegetative cover on reclamation sites for a minimum of five years before their bonds are released. Bonds are used by regulatory agencies to hold money from mining companies and return all funds upon fulfillment of reclamation plans. Thus, bonds are posted to offer monetary inducement to reclaim the land according to SMCRA. If companies do not fulfill reclamation plans, the money posted for bond will become available to the regulatory agency to complete reclamation (Greb et al. 2006). Forests have oftentimes been reclaimed to grasslands, wildlife habitat (grasslands with a mix of woody wildlife food plants), or unmanaged forest (ground cover grasses with a mix of black locust, pine species, and woody shrubs) as these land uses provide a more predictable bond release (Skousen et al. 2009;Rathfon et al. 2004). Hayland-pasture land uses predominated by grass cover are utilized to decrease erosion and improve watersheds in compliance with SMCRA (1977) but often increase the time to return the land to its previous native land use (Hall et al. 2010). Pre-mined sites that were originally forested and later converted to grassland after mining neglect the following standard that requires the mining operations at a minimum to—*restore the land affected to a condition capable of supporting the uses which it was capable of supporting prior to any mining* (Legal Information Institute 2011;Rodrigue & Burger 2004;Groninger et al. 2006). Between 1996 and 2002, 12 percent of reclaimed Indiana mine land was released to forest (Figure 1.1). This was surpassed by pastureland (19 %), fish and wildlife habitat (25%), and cropland (32%) (Briggeman et al. 2007). The benefits of reforestation include restoring the ecosystem of the land by providing a forest habitat, increasing carbon sequestration, and having a renewable timber resource (Zipper et al. 2011;Groninger et al. 2006).

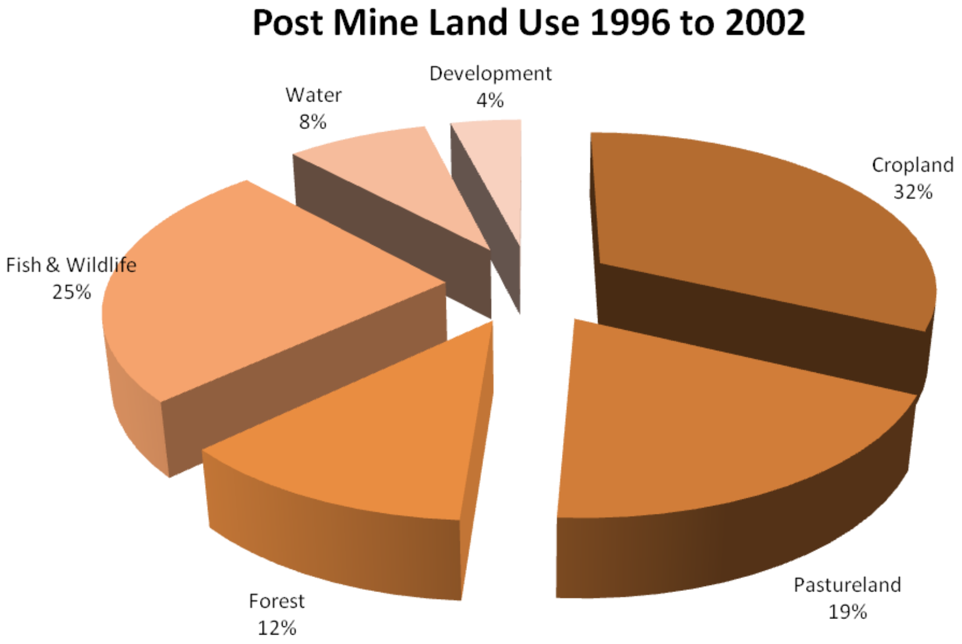


Figure 1.1. Distribution of post mine land uses in Indiana from 1996 to 2002. Data from Briggeman et al. 2007.

The cost of planting trees often exceeds the cost of other revegetation efforts, sometimes up to 50 percent more (Conrad 1999). However, the potential economic benefit of reforestation that results in a quality hardwood forest could be significant. In 2002, the estimated rotation-age stumpage values on a mixture of hardwood species planted on mined sites ranged from $3,064 to $19,528 per hectare. Stumpage value depends on species and stem quality, which translate into the likely products including pulpwood, firewood, and lumber. On several sites, the stumpage values of forests on reclaimed mine sites were higher than on non-mined reference sites (Rodrigue et al. 2002). However, harvesting timber on reclaimed mine land may not be a viable option for all reclaimed sites. Groninger et al. (2006) reported that many post-SMCRA sites in Indiana had stocking levels below the minimum levels found in published guides and models for timber products-based management. Despite this finding, the lower yielding sites provided good forest habitat for wildlife (Groninger et al. 2006).

(Torbert et al. 2000) examined reforestation of three pine species over an 11-year period on sites reclaimed via pre- and post-SMCRA standards in southwest Virginia. The three species of pine had increased growth on the pre-SMCRA site due to favorable soil conditions including increased aeration and water drainage. However, long term tree growth on many pre-SMCRA sites was limited by bedrock due to shallow topsoil cover. This study concluded that these three pine species would have likely grown better on most of the post-SMCRA sites had the soil physiochemical properties improved. When high soil quality was used to reclaim post-mined land, minimal differences were found in site productivity between reclaimed sites and their non-mined counterparts in the Eastern Interior region (Rodrigue & Burger 2004). This study also found that when comparing pre- and post-SMCRA mine reclamation sites, forest productivity was more influenced by soil physiochemical properties than whether the site was reclaimed via pre- versus post-SMCRA regulations. The main physiochemical properties influencing site establishment included base saturation, available water holding capacity, total porosity, and soluble salts. However, as a result of the regulations within SMCRA, many post-SMCRA sites have poor site productivity due to improper selection of soil replacement material, lack of soil biota, and compaction of the land due to grading requirements (Bussler et al. 1984;Rodrigue & Burger 2004).

1.3 Soil Replacement Strategies

Bradshaw (1987) addressed the problems involved with the reconstruction process on mine sites and stated:

> “The starting point must be the soil, or at least the substrate into which plants must establish and root, for although soil can exist without plants, there are few plants that can exist without soil.”

Bradshaw states that there are four simple soil requirements for optimal plant growth: (1) a suitable medium for the physical development of roots, (2) an adequate water supply, (3) a sufficient nutrient supply, and (4) lack of toxicity. In reality, the state of soil quality in the early developing years (<25 years) on many reclaimed mine sites rarely meets all four of these characteristics due to the physiochemical nature associated with soil used for reclamation (Bussler et al. 1984;Moffat & Bending 2000;Jacinthe & Lal 2007). One approach to improve soil quality on mine sites is to conserve and replace surface soils (Bradshaw 1987;Jacinthe & Lal 2007). Soil microbial colonization is generally more advanced in the surface soil horizon compared to sub surface material; thus, surface soil replacement on mine land often leads to more rapid ecosystem development (Bradshaw 1987). In a 15 year study comparing topsoil versus no topsoil application on reclaimed mine land in Ohio, a significant effect of topsoil on tree growth was observed. Growth parameters including height, diameter, and biomass were several times higher with topsoil application than without (Jacinthe & Lal 2007).

It is critical for seedling development, whether using topsoil or the best available soil material, to use an adequate depth of soil above the graded cast overburden on mine sites (Torbert et al. 1988). Long term effects of four topsoil replacement depths (0, 20, 40, and 60 cm) have been shown to influence mine reclamation success (Bowen et al. 2005). As the depth of topsoil replacement increased, total aboveground biomass and litter cover significantly increased. Litter cover at the 0 cm topsoil depth treatment was 17% compared to 43% cover in the 60 cm topsoil depth treatment. In addition, soil organic C and total N were significantly higher in the 40 and 60 cm topsoil treatments compared to the 0 and 20 cm treatments, which was likely due to the increased biomass and litter cover. Plant biomass accumulation was higher in the deeper topsoil treatments due to increased rooting media and available organic matter. Cumulative water infiltration also significantly increased for the 40 and 60 cm topsoil depth treatments compared to the 0 cm topsoil depth treatment. However, this was due, in part, to higher clay content and restricted drainage associated with the spoil material located beneath the topsoil.

In the past 10-15 years, reforestation practices for mine reclamation have improved dramatically by continually upgrading methods of soil handling, adapting equipment, and decreasing ground traffic and resulting compaction. Conventional grading practices involve repeated travel of heavy equipment to grade both the underlying burden and topsoil (or best available material). Methods designed to counteract the problems that arise with the heavy equipment trafficking include soil ripping, loose tipping, and loose grading. According to Skousen et al. (2009), soil ripping involves placement of topsoil or the best available material on the surface of graded overburden, and then using equipment (e.g. single blade ripper attached to a bulldozer) to rip and disk the mine soil to a specific depth (e.g. 1 m). Moffat & Bending (2000) discuss a variety of techniques to create the ripping effect. (Casselman et al. 2006) also verifies the positive benefits that ripping the soil can provide, some of which include lowering bulk density and increasing soil aeration. Loose tipping involves placement of topsoil (or the best available material) on the surface of ripped overburden prior to spreading and leveling the 'topsoil' to a minimum depth of 250 mm through the use of an excavator (Moffat & Bending 2000). Loose grading, similar to loose tipping, uses non-rubber tracked equipment to dump the topsoil (or best available material) in adjacent piles that are a minimum of 1.3 m deep and then leveling the piles with the lightest available equipment (Skousen et al. 2011). In the present study, mine trucks unloaded B and C horizon soil into adjacent piles ranging from 1.5 to 4 m deep with 30-50% overlap. Several attributes of this approach were derived from the forestry reclamation approach (FRA) described by Zipper et al. (2011). The technique in the present study was termed the 'loosely dumped' method, as there was no grading of the soil piles involved.

In a recently published study, soil ripping prior to seedling planting improved seedling survival and growth on reclaimed mine land (Skousen et al. 2009). Topsoil (30 cm) was placed and graded above the mine overburden on four 400 m^2 plots at each of the four sites. Half of each plot (200 m^2) was ripped using a single blade ripper attached to a bulldozer, which ripped the mine soil to a depth of 1 m; while the remaining half of the plot was left compacted. Species including black cherry, red oak, and yellow poplar were planted as seedlings on both ripped and non-ripped treatments. After seven years, survival was significantly greater for seedlings planted on the ripped treatment. This was due, in part, because the ripped treatment provided a more favorable soil media that was conducive for root proliferation and had greater accessibility to water. In the first year at one site, red oak seedling survival was at 96% in the ripped treatment. By the seventh year, red oak survival declined to 47% in the ripped treatment compared to 12% in the non-ripped treatment.

In a study comparing sites that used soil ripping (in which topsoil was placed prior to ripping) to soil loose tipping, it was found that survival was consistently higher and growth was greater on the sites that used the loose tipping approach (Moffat & Bending 2000). Soil penetrometer measurements found no differences in soil resistance between the two methods in the soil materials above the 0.6 m depth. However, soil ripping was unable to relieve compaction below the 0.6 m depth due to the limitations of ripping and disking. It was concluded that loose tipping, on the other hand, provided a non-compacted, deeper rooting zone.

The loose grading approach, similar to the loose tipping approach, is accomplished by grading the overburden using standard practices and then transporting and dumping topsoil in adjacent piles during dry conditions. The piles are lightly graded using an excavator or the lightest available equipment. This approach was recommended for use as part of the Forestry Reclamation Approach (FRA) that was created in 2005. Reclamation scientists developed the FRA after studying how forests develop under varying soil replacement strategies on both older and newer mine sites (Zipper et al. 2011). Preliminary studies have shown soil erosion may occur during the first two years using this approach. However, the loose Appalachian soil surface, containing organic debris and forest floor rocks, was found to enhance water infiltration. This approach may be a more sustainable option than the standard graded approach for long-term runoff and surface erosion control in appropriate landscapes (Skousen et al. 2011). While loose grading as a top soil replacement strategy has been studied extensively on Appalachian mine sites (Zipper et al. 2011), this approach has not been evaluated in the Eastern Interior region.

1.4 Soil Physiochemical Properties on Reclaimed Mine Sites

Soil physiochemical properties refer to both the physical and chemical states of the soil. Physiochemical properties of reclaimed mine soil vary widely by geographic location and depend upon the method of soil replacement, prior land use, nature of reclaimed soil (surface versus subsurface material), and age of the site (Jacinthe & Lal 2007). A useful tool in understanding the repercussions of mining on soil properties is to compare results with non-mined reference soils. One study evaluated soil pH, organic C content, and nutrient levels of reclaimed mine land and non-mined reference sites after 0, 5, 10, 20, and 25 years of site establishment (Graham & Haynes 2004). At time zero, organic C content was only 24% of that present under non-mined native forest land (reference site). After 25 years, the soil C content increased to 93% of that present under non-mined native forest land. During the 25-year period, exchangeable Ca and Mg increased from 53.6 to 73.4 mmol kg^{-1} and 8.2 to 13.8 mmol kg^{-1}, respectively (T_0 - T_{25}); while soil pH and Olsen P decreased from 7.7 to 6.3 pH_{water} and 35 to 10 mg kg^{-1}, respectively. In a separate study, Kuznetsova et al. (2011) assessed changes in soil nutrient levels over time on a reforested mine site and found similar trends. Over the 7-year study, soil N concentration increased, while pH and P concentration decreased ($p<0.001$). It was thought that the P availability decreased in the soil due to the uptake and assimilation of P in plants. The older tree species had 26 – 34% more P in leaves compared to younger seedlings (Kuznetsova et al. 2011).

In addition to studying soil chemical properties, another experiment assessed the changes in soil physical properties between soil depths on a reclaimed mine site and its non-mined reference site in Indiana (Bussler et al. 1984). The mine land was reclaimed with topsoil consisting of A and B soil horizon material: C1 (0-29 cm), and underlying graded cast material layers: C2 (29-147 cm) and C3 (147-152 cm). Soil chemical properties were similar or more favorable in the reclaimed sites compared to the soil of the non-mined reference sites in the rooting zone. In contrast, soil physical properties in the reclaimed sites were less favorable in the rooting zone. Mean available water holding capacity was 16.5% in the topsoil depth (C1) and ranged from 10.8% to 11.7% in underlying graded cast layers (C2 and C3). The bulk densities were 1.53 (C1) and 1.77 (C2) g cm^{-3}. In the non-mined reference sites, bulk densities ranged from 1.29 to 1.51 g cm^{-3} in the 0-66 cm depth. Another study found similar bulk density trends in the surface (0-10 cm) of mined (1.87 g cm^{-3}) and non-mined reference sites (1.18 g cm^{-3}) (Acton et al. 2011).

One study examined soil bulk density values in standard (ST) graded topsoil, graded overburden (OV) with no topsoil, and ripped topsoil (RT) and found values ranged from 1.3 to 1.7 g cm^{-3} yet were not significantly affected by topsoil application (ST, RT, or OV) after 15 years (Jacinthe & Lal 2007). Compaction did not vary among topsoil treatments in either the surface or subsurface soil layers, which was opposite of previous findings (Rokich et al. 2001;Bussler et al. 1984). It is likely that bulk density values were greater within the first few years; however, this study's measurements were taken after 15 years and therefore did not show significance. Acton et al. (2011) showed that bulk density values measured in the surface soil of reclaimed sites decreased over time. Bulk densities decreased from 1.87 to 1.51 g cm^{-3} between the 2nd and 10th year of their study. However, the bulk density in the subsurface soil (10 – 50 cm) did not significantly change throughout the course of the 14-year study.

The choice of topsoil application technique has been shown to affect soil C storage in minesoils, as does the choice of tree species. A 15-year study by Jacinthe et al. (2007) examined the effects of ripping topsoil in relation to the partition of C storage between soil and above ground biomass. Recent C storage in the soil (last 15 years), derived from decomposed litter and roots, was measured by subtracting fossil C from the total organic carbon. The topsoil application techniques consisted of 1) the standard (ST) graded topsoil, 2) ripped topsoil (RT), and 3) overburden (OV) in which no topsoil was used. In this study, topsoil consisted of a mixture of A, B, and C horizon materials and the RT treatment plowed the soil to 80 cm after topsoil was graded. The ST and RT treatments represented post-SMCRA sites and used only 30 cm of topsoil; whereas the OV treatment represented pre-SMCRA sites. The RT treatment showed decreased bulk density and overall less compaction than the ST treatment. This study found recent soil

C storage in the Austrian pine (*Pinus nigra*) plots averaged 44.1, 49.5, and 19.1 Mg C ha^{-1} under ST, RT, and OV treatments, respectively. In contrast, soil C storage on the slower growing green ash plots yielded lower averages for the RT treatment compared to the ST treatment. Thus, there is some evidence to suggest that species may influence the effectiveness of the soil replacement treatment in terms of soil C storage. Other important considerations for the lower soil C storage on the green ash RT treatment include the destruction of soil aggregates and increased soil aeration.

1.5 Soil Biological Properties

In addition to favorable soil physiochemical properties, reforestation success on reclaimed mine land also relies on adequate population density and diversity of soil microorganisms. Soil is a living entity with an ecosystem of its own. The microbial communities in the rhizosphere, the region of soil surrounding plant roots, benefit both plant growth and soil quality. In addition to enhancing nutrient availability, some microbial communities are able to produce root growth promoting hormones such as auxins (Marschner et al. 2004;Khalid et al. 2004). On harsh sites, such as reclaimed coal mining sites, rhizosphere interactions between roots and soil microorganisms are especially important (Lohmus et al. 2006;Fresquez & Lindemann 1982). History shows us that natural colonization in soil with inadequate nutrient levels and organic matter is slow. Re-establishment of soil colonization in derelict materials, such as glacial moraine or kaolin wastes, may take 30-70 years (Bradshaw 1987). Minimizing disturbances when excavating and re-spreading topsoil is difficult to achieve and therefore often overlooked. However, there are positive plant benefits on reclaimed sites when stumps, roots, and woody debris are mixed into the replacement soil due to the preservation of microbial communities (Skousen et al. 2011).

The population size and activity of soil microorganisms are generally more indicative of a functioning soil than the measurement of species diversity of microbial organisms within the soil (Graham & Haynes 2004). One study compared microbial numbers and dehydrogenase activity among forest reclaimed mine sites, reclaimed mine sites without vegetation, non-mined forest reference sites, and coal mine topsoil spoil piles (Fresquez & Lindemann 1982). They found that microbial numbers and activity were greater on the undisturbed soil and forest reclaimed sites than the stockpiled topsoil or non-vegetated spoil. The non-vegetated spoil had the lowest microbial counts and activity. They also found that dehydrogenase activity did not correlate well with microbial population counts. Other studies have suggested that in early developing soil ecosystems, there is less competition for energy and therefore decreased rates of enzyme activity (Graham & Haynes 2004).

Another study measured soil microbial parameters on alder plantations growing on three land types: forest, abandoned agricultural land, and oil-shale reclaimed mining area (Lohmus et al. 2006). Microbial parameters included the measurement of: substrate-induced respiration (SIR) which measures active microbial biomass, basal respiration (BAS) which measures microbial respiration activity, and metabolic quotient (Q=BAS/SIR) on bulk soil samples. The alder stands on the reclaimed mine area and abandoned agricultural land had SIR values that were 2.3 to 10.8 times lower than the forest stand. When BAS and SIR were repeatedly measured, the SIR values were more stable than BAS which may have been due to changes in weather. This study concluded that the interactions between soil microbial biomass/activity and root parameters exist; however, further investigations are needed to fully understand these relationships.

1.6 Root Morphology

The impact of soil disturbance due to mining is particularly distressing on root morphology and architecture. In a study investigating root development and architecture of tree seedlings of various species which naturally vary in rooting patterns, Rokich et al. (2001) observed root systems of native *Banksia spp.* seedlings on reclaimed sites with varying topsoil media application as well as an undisturbed woodland reference site. Significant differences in root growth and architecture were observed for all species growing on the reclaimed sites compared to the woodland site, as well those growing on the ripped compared to the non-ripped treatments. In general, the roots obtained from the woodland and disturbed ripped sites had longer primary (tap) roots and less lateral root production compared to the roots on the reclaimed non-ripped sites, which had short tap roots and numerous lateral roots. In contrast to these findings, Kormanik (1986) observed lateral root morphology on sweetgum (*Liquidambar styraciflua* L.) during early stages of seedling growth and development and found seedlings with few initial lateral roots were experiencing greater first-year transplant stress than those with many lateral roots. Rokich et al. (2001) also found that root mass significantly declined with depths > 20 cm after one year on the non-ripped reclaimed sites. In some areas, root development was completely inhibited as a result of bulk density values exceeding 1.7 g cm^{-3}. The maximum depth of penetration was similar across the woodland reference site and the disturbed sites where ripping was applied. Ripping was shown to increase root development and architecture in most of the species investigated after one year.

(Simmons & Pope 1987) examined the influence of soil compaction on yellow poplar and sweet gum seedling root growth. Seedlings were transplanted into greenhouse pots containing soil compacted to bulk densities of 1.25, 1.40 and 1.55 g cm^{-3}. For yellow poplar, significant increases in root biomass were observed when bulk density decreased from 1.55 to 1.25 g cm^{-3}. These findings were consistent with (Rokich et al. 2001). Simmons & Pope (1987) also reported significant increases in total root length for yellow poplar when bulk density decreased from 1.55 to 1.40 g cm^{-3}. Sweetgum showed a similar trend that was slightly more sensitive to the degree of soil compaction. The total root length, biomass, and fibrosity of sweetgum seedlings significantly increased with each incremental decrease in bulk density.

Young tree seedlings growing in unfavorable conditions will often adapt their root systems to survive (Rosenvald et al. 2011;Rokich et al. 2001;Lohmus et al. 2006). One study examined the effects of fine-root morphological adaptations in a chronosequence of silver birch stands on a reclaimed mining site (Rosenvald et al. 2011). This study used WinRHIZO, a root imaging and analysis software, to measure first and second order root parameters including length, projected area, and diameter. Young birch trees had the highest fine-root specific root length (SRL) (m g^{-1}) indicating exploration through stony alkaline soil. As stand age and soil improvement increased, fine-root SRL decreased logarithmically due to less root tips per unit mass and thicker root development. The authors concluded that finer short-roots, higher SRL, and higher root tip frequency per mass unit are often found in young seedlings growing in unfavorable conditions. The rate of change of short-root morphology was greatest during the first 5 years of growth.

Another study utilized WinRHIZO to measure fine-root parameters, in addition to soil parameters, of alder plantations growing on a forest, an abandoned agricultural land, and an oil-shale reclaimed mining area (Lohmus et al. 2006). Similar to the findings of Rosenvald et al. (2011), Lohmus et al. (2006) reported specific root length (SRL) and specific root area (SRA) decreased with stand development. In addition, the SRL and SRA were found to be significantly different among study areas; however, they were similar in the same type of soil (e.g. in Mollic Gleysol). Furthermore, the authors concluded fine-root growth parameters should be included in root-soil microbial studies based on the finding of a positive correlation between SRA and a community-level physiological profile of culturable bacteria.

1.7 High value hardwoods on reclaimed mine land

Due to sometimes extreme and often growth limiting soil conditions associated with reclaimed mine land, species selection for reforestation can be challenging. In hardwood forests in the Midwestern region of the US, sassafras (*Sassafra albidum*) and sugar maple (Acer *saccharum*) are often pioneer communities prior to oak establishment (Ashby 1987). Generally, reforestation of mine lands has attempted to skip the pioneer stage with the objective of successfully planting late-successional hardwoods on initially bare land surface mined for coal. The suitability of sites to support late-successional forests is becoming an important consideration. Late-successional species including red oak (*Quercus rubra*), white oak (*Q. alba*), and bur oak (*Q. macrocarpa*) often fail on reclaimed mine land due to the uncertainty of soil conditions (Ashby 1987); however, there are exceptions. In W. Clark Ashby's article

(Ashby 1987), he probes the questions, “Why do certain species succeed on open sites while others almost invariably fail? And why are some communities rapidly invaded while others seem to be extremely stable?” These questions arose as a response to his findings of black locust plantings, in which low survival was observed over time due to invasion of a diversity of species.

W. Clark Ashby’s observations were tested in a recent survey of forest productivity of 7-14 year old stands on post-SMCRA reclaimed mine sites in Indiana; Groninger et al. (2006) found black locust (*Robinia psedoacacia*) and green ash (*Fraxinus pennsylvanica*) to be the predominantly occurring species. Black locust comprised 46% of all tallied stems, followed by green ash, which comprised 14% of all stems. Groninger et al. (2006) corroborated Ashby’s (1987) findings in reporting that black locust was subject to early decline, despite its ability to grow on harsh sites due to its initial hardy survival rate. Northern red oak and white oak comprised the third and fourth highest amounts of basal area across the 22 selected study sites (Groninger et al. 2006).

Late-successional hardwood species, such as oaks, are often used as trial species on reclaimed mine land due to their large economic and aesthetic benefits (Auch et al. 2005). On hardwood sites where tree density does not reach the strict tree survival standard of 1235 trees/ha to reach bond release (Sullivan & Amacher 2010), these sites still support mast-producing trees and habitat for a large range of wildlife species (Groninger et al. 2006).

Remedial silvicultural treatments including vegetation management, tillage, and fertilization may ameliorate harsh conditions associated with reclaimed land (Casselman et al. 2006;Chaney et al. 1995). In a study assessing the effects of varying levels of silvicultural treatments on the survival and growth of native hardwood species, white pine, and hybrid poplar plantings on reclaimed mine sites in the Appalachian region, hardwood stands had the highest mean survival on all sites ranging from 50% (Ohio) to 85% (Virginia) (Casselman et al. 2006). The silvicultural treatments included weed control (WC) only, weed control plus tillage (WC + T), and weed control, tillage, and fertilization (WC + T + F). Tillage was used to reduce the overall soil compaction and increase aeration. In the first year, hardwood seedlings had negative net growth due to dieback. Hardwood seedling growth was slow; which was also found by Chaney et al. (2006), who reported red oak trees on their study site grew in height at approximately 10 cm year^{-1} on reclaimed mine land where weed control was used. Casselman et al. (2006) found that hardwoods had overall increased height growth in the WC + T + F treatment compared to the WC treatment. In general, all species had increased survival and growth on the WC + T compared to the WC treatment as the tillage decreased soil impedance and increased root proliferation.

Fewer studies have used American chestnut (*Castenea dentata*) in their species selection due to the outbreak of chestnut blight fungal pathogen *Cryphonectria parasitica*, which was accidentally introduced approximately a century ago and has rapidly spread and killed off American chestnut trees (Saielli et al. 2012). The American Chestnut Foundation (TACF) has been working toward reestablishing this species in North America for the past 30 years (Simmons 2009). TACF research involves crossing the American chestnut and Chinese chestnut (*Castenea mollissima*) to form an F1 hybrid, which is then back- crossed three times with the American chestnut leading to a BC3F1 (Jacobs 2007). Selected blight-resistant BC3F1 trees are intercrossed twice to the final cross for reintroduction, BC3F3. American chestnut grows well in acidic soil conditions and is drought resistant. In a study comparing American chestnut and oak seedling physiological responses to drought, pre-dawn leaf water potential was generally higher for the American chestnut seedlings compared to *Quercus* species; however, the lack of (significant) differences among the species pre-dawn leaf measurements suggests the seedlings had similar rooting depths (Abrams et al. 1990). Based on these findings, as well as the many hardwood studies on mine sites, the species selected for this thesis project should be comparable and appropriate for mine land use. The species include: northern red oak (*Quercus rubra* L.*),* white oak (*Quercus bicolor* W.*)*, Shumard oak (*Quercus shumardii* B.), and American chestnut (*Castanea dentate* B.).

1.8 Project Objectives

The overall goal of this research project was to evaluate whether selected hardwood species would survive and become established on a reclaimed mine site using a modified soil replacement method. The specific objectives were to evaluate the effects of the traditional standard graded (GR) soil replacement and the newly proposed ‘loosely dumped’ (LD) soil replacement techniques on seedling survival and growth. Four representative hardwood species were used consisting of northern red oak, white oak, Shumard oak, and American chestnut. This study was designed to determine the effects that the GR and LD soil replacement treatments have on the soil physiochemical and biological properties. Lastly, this study aimed to assess the relationship of soil physiochemical properties to the growth and root architecture of the four species.

In this study, three null hypotheses (assertions that could be proven false) were proposed. First, it was hypothesized that the method of soil replacement would have no effect on the properties of the rooting media (soil physiochemical and biological properties). The second null hypothesis was that any differences in soil physiochemical properties resulting from the two soil replacement methods would not impact growth and root architecture of the four hardwood species. Lastly, the third null hypothesis was that soil replacement treatments would not affect seedling survival and growth.

CHAPTER 2. ASSESSMENT OF SOIL PHYSIOCHEMICAL AND BIOLOGICAL PROPERTIES UNDER TWO SOIL REPLACEMENT STRATEGIES ON RECLAIMED MINE LAND

2.1 Introduction

Soil quality on reclaimed mine sites varies widely in its suitability for plant growth. Overall soil quality depends on the soil nutrients and amount of organic matter of the replacement soil, and the impact of the method of replacement on soil physical properties; all of which are among the critical factors that influence tree seedling development and survival. Understanding and optimizing the soil chemical, physical, and biological processes will lead to increased seedling survival and growth. It is common for mine operators to transfer B and C soil horizon material from the mining area to the reclaimed mine site. Thus, soil quality with respect to nutrient and organic matter levels is often marginal and sometimes insufficient (Torbert et al. 1988;Rodrigue & Burger 2004). While nutrient availability is often one of the main growth-limitations for many forests (Lohmus et al. 2006), additional growth-limiting factors that impact survival and growth of hardwood seedlings result from competing vegetation (Chaney et al. 1995), soil compaction (Casselman et al. 2006;Rokich et al. 2001), availability of water (Rokich et al. 2001), and lack of soil microorganisms (Lohmus et al. 2006).

Several studies have observed negative changes in root systems of seedlings grown on compacted soil (Conlin & van den Driessche 1996;Simmons & Pope 1987;Rokich et al. 2001). One study showed a dramatic decrease in root biomass beyond the 20 cm depth due to an impenetrable subsoil layer with a high bulk density (Rokich et al. 2001). Of all the soil physical and chemical properties measured in this present study, the difference in bulk density between the two soil replacement treatments was hypothesized to be the greatest growth-limiting factor, based on prior mine reclamation studies (Bussler et al. 1984;Rokich et al. 2001). This chapter presents a detailed comparison of the soil physical, chemical and biological properties of two soil replacement methods at a reclaimed mine site in Southwestern Indiana. This data provides the baseline soil characterization data that will be needed to properly understand the survival and growth of the selected hardwood species described in Chapter 3.

The overall goal of the present study was to evaluate survival, growth and establishment of selected hardwood species on a reclaimed mine site using a modified soil replacement method for forestry reclamation. In order to understand limitations of plant growth, soil chemical, physical, and biological properties were evaluated. This study aimed to assess the relationship of soil physiochemical and biological properties to the survival and growth of selected hardwood species.

2.2 Site Establishment and Experimental Design

The study site consisted of reclaimed land on Peabody Energy’s Somerville Mine located between Evansville and Oakland City, Indiana. This mine area is typical of many of the recently reclaimed sites within southwestern Indiana and southeastern Illinois since inception of the Surface Mining Control and Reclamation Act (SMCRA) in 1977. Following mining operations in the summer of 2009, soil material (B and C horizon) from a nearby pasture site was deposited over graded overburden between fall 2009 and spring 2010. Soil replacement occurred in one of two ways: (1) the standard graded (GR) approach, or (2) the loosely dumped (LD) approach. In the LD technique, haul trucks unloaded B and C horizon soil in overlapping piles ranging from 1.5 to 4 m deep. Piles overlapped by 30-50%. This approach was termed in this study as the ‘loosely dumped’ method, as there was no grading of the soil piles involved. Eight one-acre plots were laid out side by side with 10 m buffer strips between the plots. Seedlings were planted in May 2010. Details on seedling establishment are described in Chapter 3.

The basic experimental design was a split plot, with four blocked replicates and two soil replacement treatment plots in each block (Figure 2.1). Within each plot, four tree species were planted, with approximately 100 plants per species completely randomized in each plot. The specific analysis of variance model depended on the frequency and nature of measurements taken during the timeframe of the experiment.

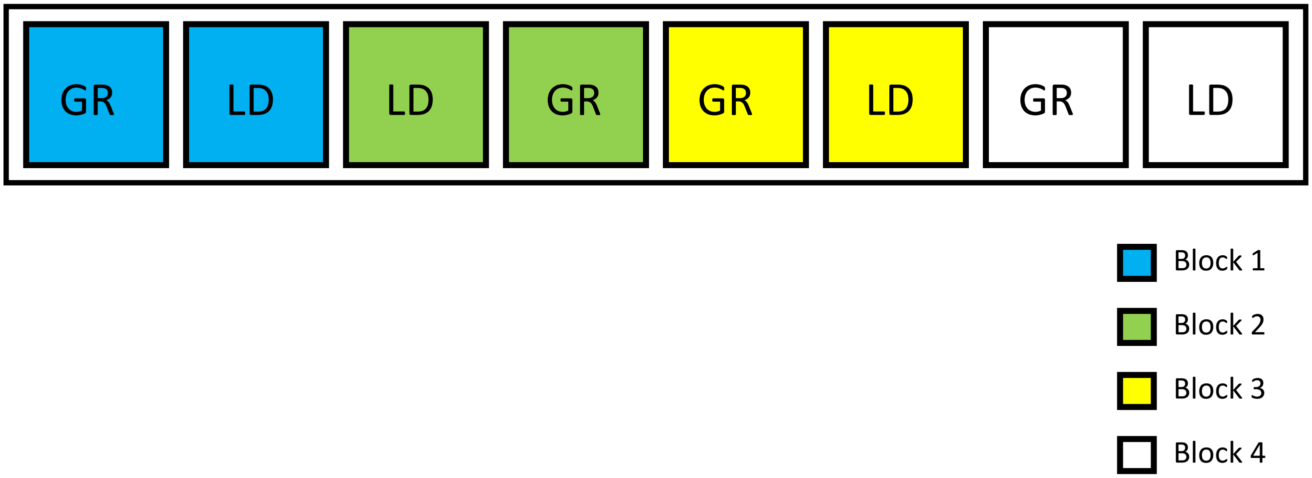


Figure 2.1. Experimental design was a split plot with four blocked replicates and two soil replacement treatments: graded (GR) and loosely dumped (LD).

2.3 Materials and Methods

2.3.1 Sample Collection for Soil Physiochemical Properties

In June of 2010 and 2011, soil was sampled at the 0-20 and 20-40 cm depths in three randomly selected locations on each plot. Soil was sampled using a split core metal sampler (4.8 cm in diameter). Bulk and undisturbed core samples were obtained simultaneously at each sampling location. One continuous core was sampled and the undisturbed soil cores were obtained from the 10 and 30 cm depths. Each undisturbed core was 2.54 cm thick and 4.8 cm in diameter corresponding to a volume of 46 cm^3. Bulk samples of approximately 135 cm^3 were dried and ground in the laboratory at Purdue University (West Lafayette, IN) and then shipped to AgSource Laboratories (Lincoln, NE) for analysis of pH, buffer pH, soluble salts, CEC, Bray 1 P, percent total N, percent organic matter, and extractable K, Mg, Ca, and Na. In 2011, only the 0-20 cm bulk samples were sent for analysis. Undisturbed soil cores were analyzed at Purdue for gravimetric and volumetric water holding capacity, soil strength (needle penetrometer resistance), bulk density, and total porosity. On-site soil erosion was also measured at several intervals in the spring, summer, and fall during the first two growing seasons.

2.3.2 Soil Chemical Properties

Soil pH was measured by AgSource labs using a Lignin pH probe and Windmill© box pH meter. A 1:1 (w:v) ratio of water to soil was used. Buffer pH was measured using the difference between the original pH and the ending pH after adding Sikora buffer solution (Brown 1980). Sikora buffer was modified from the SMP buffer and was shown to produce the same buffer pH values as SMP in addition to having greater stability than SMP (Pagani et al. 2009). Soluble salts were measured using electrical conductivity in 1:1 soil:water slurry, and values were reported in mmhos/cm (Brown 1980). Organic matter was measured by AgSource labs by loss of ignition. Soil was ground to pass a 0.2 mm sieve, and 2 g soil samples were ignited to 400°C for 4 hours (Brown 1980). Values were recorded in percent organic matter.

The Bray I method was used by AgSource labs to determine available soil phosphorus (P). Using an automatic dispenser, 10 mL Bray I extracting solution containing DI water, 0.03 M ammonium fluoride, and 0.025 M HCl was added to 1 g soil samples. Samples were poured through Ahlstrom 642 filter paper and transferred to test tubes. Sample extracts were analyzed by flow injection analysis (FIA) with a method blank and a minimum of one reference sample at the start of each 30 sample run. Calibration standards of P ranged from 0 to 300 ppm. The raw FIA data included the 1:10 dilution factor (1 g soil to 10 mL extracting solution) and therefore no further calculations were needed (Knudsen & Beegle 1988).

The modified Kjeldahl digestion followed by FIA was used by AgSource labs to determine total nitrogen. Soil samples were weighed to approximately 500 mg and then digested with catalyst tablets (1.5 g K_2SO_4 and 0.125 g $CuSO_4$) and concentrated sulfuric acid (H_2SO_4, 96%). Calibration standards for percent N ranged from 0.00 to 2.00%. Soil concentration was determined as, for example (Bremmer 1986;Egan 2002):

$$\text{Soil \%} = \frac{200\text{ mg N-NH}_3}{\text{L}} \times \frac{0.05\text{ L}}{500\text{ mg soil}} \times 100 = 2.00\%$$

Modified ammonium acetate extraction was used by AgSource labs to determine cation concentrations of K, Mg, Na, and Ca. An extracting solution adjusted to pH 7.3 contained 1 M ammonium acetate, 0.005 M DPTA, and 0.2 M sorbitol. Analyte concentrations were determined on 3 g soil samples using inductively coupled plasma optical emission spectrometry (ICP-OES). The sample extracts were analyzed on an ICP-OES spectrometer with a method blank and a minimum of one reference sample at the start of each run. Calibration standards for K, Mg, and Na ranged from 250 to 1000 ppm; while Ca ranged from 2550 to 14000 ppm. The ICP measurement was recorded in mg/L and all calibration standards were volume based. Cation exchange capacity was also measured using the ammonium acetate method (Brown 1988).

2.3.3 Soil Physical Properties

2.3.3.1 Textural Analysis

AgSource labs determined particle size analysis using the hydrometer method. Soil was weighed and mixed with sodium pyrophosphate and water prior to being transferred to the hydrometer. Hydrometer readings took place 40 seconds and 6 hours + 30 minutes after the soil solution was transferred to the hydrometer and thoroughly mixed. Temperature was recorded at the time of each measurement. Method detection limits did not apply; however, reading errors in the hydrometer translated to +/- 2%. Calculations on 50 g samples were as follows (Bouyoucos 1951;Bouyoucos 1962):

$\%\ \text{Sand} = 100 – [2 \times (R_{40} + T_c)]$

$\%\ \text{Clay} = 2 \times (R_6 + T_c)$

$\%\ \text{Silt} = 100 – (\%\ \text{Sand} + \%\ \text{Clay})$

Where: R_{40} = 40 second reading with blank subtracted

R_6 = 6 h reading with blank subtracted

T_c = temperature correction

2.3.3.2 Water Holding Capacity

The remaining soil physical properties were conducted at Purdue University. Gravimetric and volumetric water holding capacities were measured on undisturbed soil cores at saturation and at -0.05 and -0.1 bar (1 bar = 1000 kpa) using a sand tension table. Prior to any soil work, the sand table was checked for adequate sand-silt packing material, a flat contour on the bed, and 2.5 cm of water sitting above the packing material. Soil cores were weighed and wrapped in a cheese cloth held together with a rubber band and then placed in 2 cm of water for 48 hours or until the weight of the saturated cores equilibrated. A control core with no soil was treated equally to the other soil cores throughout the experiment. Saturated weights were recorded. Cores were transported to the sand tension table with a Whatman circle 70 mm filter paper between the core and packing material to increase soil connection to the packing material. A 50 mL beaker of water was placed in the sand table prior to covering the table with a lid in order to minimize evaporation. A flask connected to the sand table via tubing was fastened 100 cm directly beneath the sand table (See figure 2.2). Water was released from the table as the tubing was opened in order to equilibrate the soil cores to 100 cm tension (-0.1 bar). The distance from the middle of the soil core to the outlet of the flask was 100 cm.

In 2011, soil cores were also equilibrated to 50 cm tension (-0.05 bar). Gravimetric and volumetric water holding capacities were calculated as (Klute 1986) (Vomocil 1965):

Gravimetric water holding capacity at -0.1 bar:

$$\frac{\text{Control Adjusted Mass of Soil + Water} - \text{Control Adjusted Oven Dry Soil}}{\text{Control Adjusted Oven Dry Soil}}$$

Volumetric water holding capacity at -0.1 bar:

$$\frac{\text{Control Adjusted Mass of Soil + Water} - \text{Control Adjusted Oven Dry Soil}}{\text{Core Ring Volume } (46\ \text{cm}^3)}$$

Where oven dry soil values were reported after samples had oven dried at 105 °C and equilibrated in weight. Control adjusted values were calculated by subtracting the weight of the cheese cloth on the control core from the original measured values. Core ring volume was calculated as:

$$V = \pi \cdot \text{inner core radius}^2 \cdot \text{height of core ring}$$

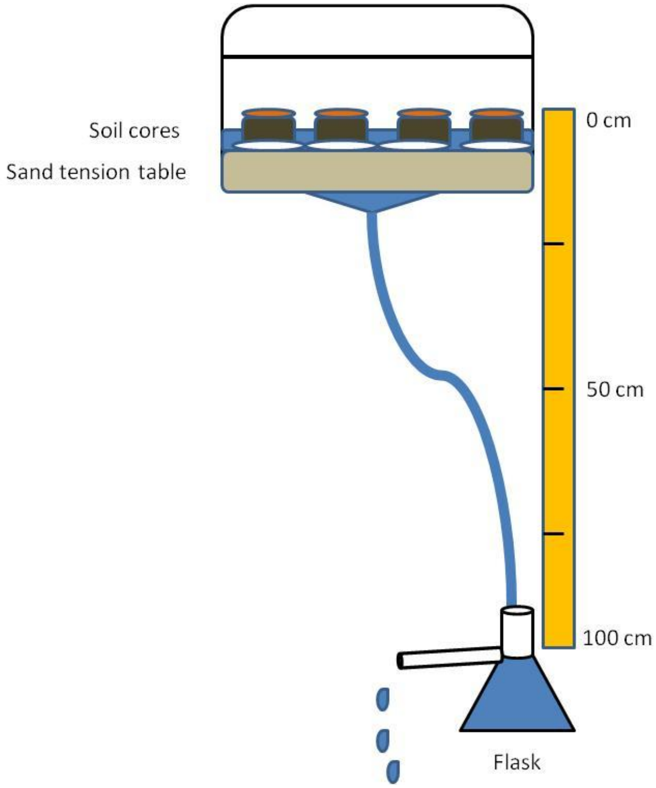


Figure 2.2. Assembly of sand tension table with flask located either 50 or 100 cm below the middle of the soil cores in order to reach -0.05 or -0.1 bar respectively. Figure shows cores equilibrating at -0.1 bar matric potential.

2.3.3.3 Soil Mechanical Resistance

A laboratory Precision penetrometer 1/10 mm division (Precision Scientific Co., Chicago) was used on all soil cores collected in June 2010 and July of 2011 to determine mechanical resistance of the soil to penetration by a metal probe. This measurement was taken when soil cores were equilibrated to -0.1 bar (Davidson 1965). See figure 2.3.

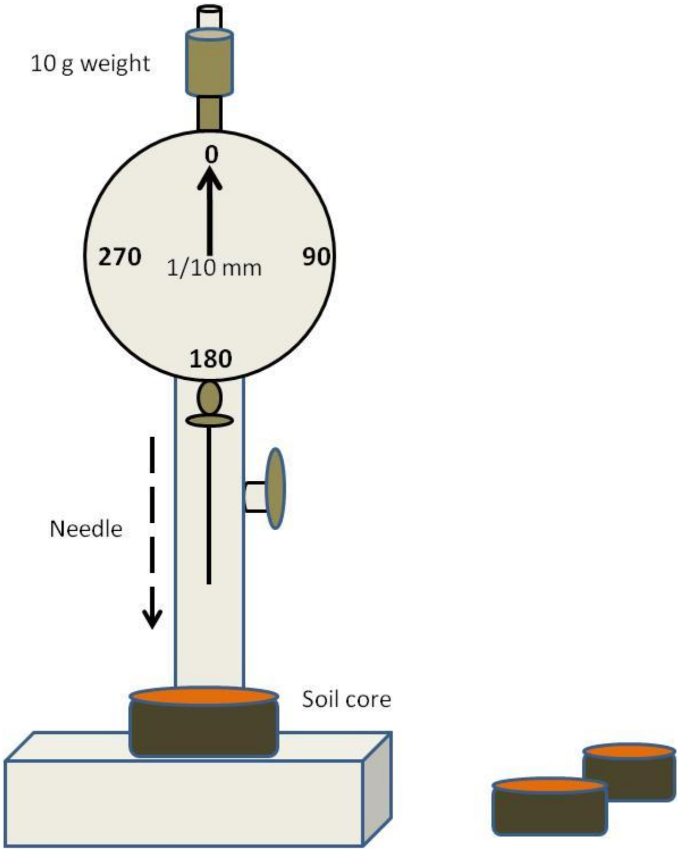


Figure 2.3. Soil penetrometer had a 10 g weight above needle; needle was dropped and the depth of needle penetration was recorded based on 1/10 mm increments.

2.3.3.4 Bulk Density and Total Porosity

Bulk density values were determined on the soil samples using the core method. Soil cores were dried to 105° C and weighed once they equilibrated in weight. Bulk density was calculated (Blake & Hartge 1986) as:

$$\frac{\text{Mass of Oven Dry Soil}}{\text{Soil Volume}}$$

Total porosity (p_t) was calculated (Danielson & Sutherland 1986) as:

$$p_t = 1 - \frac{p_b}{p_s} = 1 - \frac{V_s}{V_t}$$

Where p_t is given as a decimal fraction, p_b is the dry bulk density, p_s is the solid phase density, V_s is the soil particle volume, and V_t is the total volume of the sample. Values of p_b were calculated from the cores in the lab; while p_s values used 2.65 g cm^{-3}, as this value represents the approximate particle density of a standard mineral soil.

2.3.4 Soil Erosion and Settling

Soil erosion (or settling) and accumulation were measured through soil changes on stakes placed on two of the eight treatment plots (one GR and one LD). Stakes were driven approximately 60 cm into the ground with 30 cm remaining above soil. On the GR plot, 24 stakes were placed in a grid to cover the entire plot. On the LD plot, 4 randomly selected soil mounds had line transects of 6 stakes placed descending the mounds (Figure 2.4). Mounds were selected so that each faced a different cardinal direction (e.g. east-facing mound). Stake heights were recorded in July and September 2010, and again in June 2011. Measures comparing across time and stakes in an individual treatment were analyzed using a T-test. Significance was tested at α=0.05.

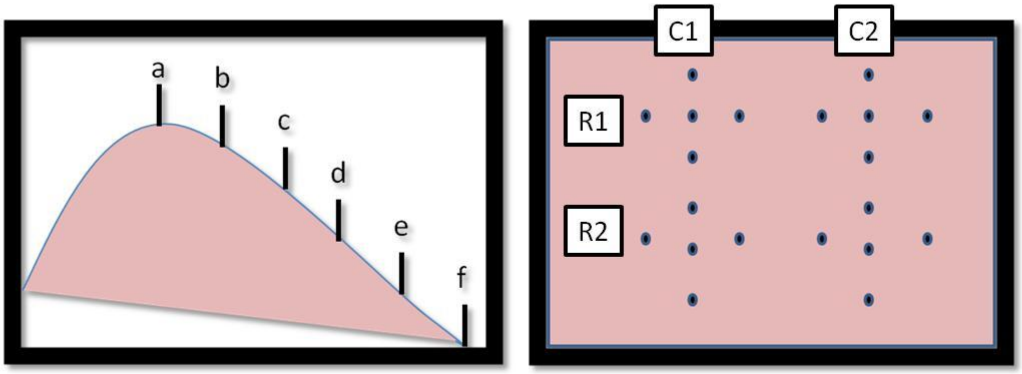


Figure 2.4. Left image shows a line transect of stakes descending down a soil mound (a-f) in the LD plot, while the right image is an aerial view of the columns (C1 and C2) and rows (R1 and R2) grid-placement of stakes on the GR plot.

2.3.5 Sample Collection for Soil Microbiological Properties

A pilot study was conducted on the rhizosphere soil obtained from American chestnut seedlings using three different microbial assessments. Indole acetic acid (IAA) analysis was used to determine the presence of plant-growth promoting bacteria. Fluorescein diacetate (FDA) hydrolysis was used to estimate the enzyme activity of microorganisms in the soil. In addition, CO_2 incubation methods were used for microbial biomass (MB) analysis. In June of 2011, soil was sampled at the 0-20 cm and 20-40 cm depths within 15 cm of the rhizosphere of ten randomly selected American chestnut seedlings across all of the plots for a total of 20 samples. The American chestnut species was desirable for this pilot study due to the genetic similarities as all seedlings of this species were selected BC3F2 seedlings. For a control, soil was sampled at an abandoned pasture site adjacent to the area where the replacement soil was obtained consisting of three surface horizon (0-20 cm) and three sub-surface (20-40 cm) samples. Soil was sampled using a split core metal sampler (4.8 cm in diameter). Bulk and core samples were obtained simultaneously at each sampling location. One continuous core was sampled and the undisturbed soil cores were obtained from the 10 and 30 cm depths. Each undisturbed core was 2.54 cm thick and 4.8 cm in diameter corresponding to a volume of 46 cm^3. Soil samples were kept in coolers and stored at 4 °C within 24 hours of collection. Twenty six undisturbed soil cores were used for IAA analysis, and 26 bulk samples were used for the FDA hydrolysis and MB analyses.

2.3.6 Soil Microbiology Assessment

2.3.6.1 Indole-3-acetic Acid (IAA) Analysis

Twenty six undisturbed soil cores were placed into individual jars. One hundred mL of MeOH was added to each jar and the jars were sealed. The soil and MeOH mixture was shaken for approximately 30 seconds. Soil samples were stored in a dark cooler held at 2 °C for 3 days. The soil and supernatant separated, and the supernatants were collected and filtered through a Whatman No. 2 filter paper. The soil was discarded. The supernatants (20 mL each) were prepared for a chloroform extraction by adding a phosphate-citrate buffer (0.5 M $NaPO_4$ and 0.1 M citrate) and 50 mL methanol to each sample. Samples were held at 2°C for 22 days. The supernatants were extracted with a chloroform extraction solution. The inorganic aqueous phase was discarded using a separation flask. The extracts were evaporated to dryness by removing the seals and keeping the samples at 2°C. The extracts were dissolved in 500 µL and kept at 2°C.

Samples were analyzed using reverse-phase HPLC separation. This was performed with a Waters 2695 Separations Module (Milford, MA) on a Nucleodur C18 Isis column (C18; 150 mm x 3.0 mm I.D.; particle size, 3µm). The mobile phases (A and B) contained polar solvents as mobile phase A was comprised of 2% methanol and 0.2% trifluoroacetic acid, while mobile phase B was comprised of 98% methanol and 0.2% trifluoroacetic acid. The polar mobile phases moved through the column (the stationary phase) to allow the sample to interact with the stationary phase and become separated. Samples were run under the following elution profile at a volume rate of 0.4 ml/min: 0-5 min: solvent A 76% / solvent B 24%; 5-20 min: solvent A 76% / solvent B 24% → solvent A 0% / solvent B 100%, convex gradient curve; 20-25 min: solvent A 0% / solvent B 100%; 25-30 min: return to initial mobile phase conditions, concave gradient curve (Figure 2.5). Sample injections of 25 µL were monitored by absorbance using a Waters 996 Photodiode Array Detector on wavelengths from 200-500 nm. The IAA standard was run in sync with a ferulic acid standard due to multiple projects being run on this instrument. The IAA standard concentration used was 0.02 mg/ml dissolved in 2% acetic acid to water (V/V) and peaked at 21 minutes.

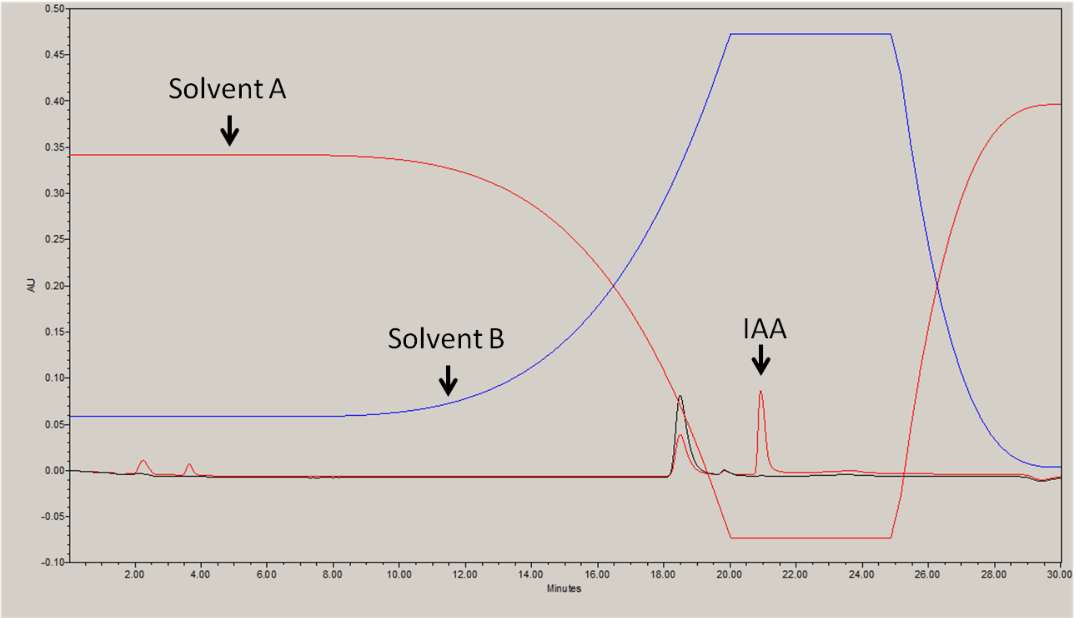


Figure 2.5. Mobile phases: A (red line) and B (blue line) are shown with the chromatogram of IAA and ferulic acid standards. IAA standard of 0.02 mg/ml dissolved in 2 percent acetic acid to water (V/V) peaked at 21 minutes. Amino acids (found in standards by default) peaked around 3 minutes and ferulic acid standard peaked at 18.5 minutes.

2.3.6.2 Soil Analysis for Fluorescein diacetate (FDA) Hydrolysis

Enzyme activity of microbial populations was measured using the fluorescein diacetate hydrolysis method. Fluorescein accumulates in cells that possess intact membranes or active metabolism. Four sub-samples measured to 1 g were taken from each of the 26 bulk soil samples, totaling 104 samples (soil was set aside in biomass procedure, as previously mentioned). Samples were placed into 50 mL Erlenmeyer flasks (A, B, C, and D). All flasks had 50 mL 0.1*M* tris(hydroxymethyl)aminomethane (THAM) buffer added, while flasks A, B, and C had 0.5 mL fluorescein diacetate lipase (FDA) substrate solution added. Flasks labeled D represented the controls and had 0.50 mL acetone added. Flasks were swirled for 15 seconds and placed in a pre-heated incubator held at 37 °C for 3 hours. After 3 hours, samples were removed from the incubator and 2 mL of acetone was added to each flask to terminate FDA hydrolysis. A 30 mL soil suspension was removed from each solution, transferred to a 50 mL centrifuge tube, and centrifuged at 8,000 rev min^{-1} for 5 minutes in a Beckman J2-HS centrifuge. The filtrate was transferred to a disposable cuvette and the absorbance was measured on a Genesys™ 10 UV spectrophotometer set at a wavelength of 490 nm. Results were reported as mg fluorescein released per kg soil per 3 hours (mg kg^{-1} 3 h^{-1}). A complete description of this method can be found in the USDA's Standard Operating Procedures for FDA Hydrolysis (Soil Survey Laboratory Methods Manual 2004).

2.3.6.3 Soil Microbial Biomass

Total microbial respiration was measured using the microbial biomass fumigation incubation procedure to determine biomass of soil microorganisms (Soil Survey Laboratory Methods Manual 2004). Twenty-six bulk soil samples were put through a 2 mm sieve. Field moist water content was determined on each of the samples by drying approximately 10 g soil at 110°C overnight and recording the water loss. A sub-sample of 4 g were removed from each sample and set aside in a dark cooler held at 4° C for the fluorescein diacetate (FDA) procedure described in Section 2.3.5.3. Six sub-samples of 17.5 g each were collected from the 26 bulk soil samples, totaling 156 samples, and placed into 40 mL beakers (**A, B, C**; D, E, F representing **fumigated** and non-fumigated respectively, as half of the samples underwent fumigation). Soil density was equilibrated by tapping the beaker against the counter until the volume reach a pre-measured bulk density line (17.5 cm^3).

Calculations based on recorded water loss from each of the 26 bulk soil samples were made to determine how much water was needed to add to the soil samples in order to bring samples to 60 percent water-filled pore space and were as follows:

Gravimetric water content required for soil to be at 60% water-filled pore space:

$$\text{Gravimetric } H_2O = \frac{0.60 \times \{1 - (D_b / 2.65)\}}{D_b}$$

Where D_b: bulk density.

Gravimetric water needed for soil to reach 60% water-filled pore space in air dried soils:

$$H_2O_{add}\ (g\ g^{-1}) = [\ (H_2O_{0.60)}\ -\ (H_2O_f)\ ]\ x\ [\ W\ /\ (1 + H_2O_f)\ ]$$

Where H_2O_{add} is the amount of water needed to reach 60 % water filled pore space and H_2O_f is the amount of water already in the soil.

W: soil dry weight (g)

Deionized water was slowly and meticulously added with a syringe to bring each of the samples to 60 percent water-filled pore space without disrupting sieved particles (to avoid clumping). Samples were covered with parafilm and stored overnight at 4°C to equilibrate. Fumigant samples labeled A, B, C were placed in a desiccant cabinet in the hood with a vacuum pump. A 50 mL beaker with 40 mL chloroform and 8-10 boiling stones was placed on each of the three shelves. The cabinet vacuum pressure was maintained at 30 psi for 3 minutes. The vacuum pressure was brought back down to 0 psi and fumigation continued for 24 hours. The chloroform was evacuated using the vacuum hose at a pressure of 30 psi for 15 minutes. The chloroform beakers were removed.

Fumigated and non-fumigated samples were incubated in Mason jars with a base trap consisting of a scint vial containing 5 mL of 2 N KOH. Five mL of deionized water was added to the bottom of each Mason jar to prevent desiccation (See figure 2.6). Six controls consisting of only a basetrap and no soil sample were also incubated. Lids were airtight as the samples incubated in order to trap all CO_2 production. After 10 days of incubation, the base trap KOH scint vials were removed from the mason jars and immediately capped. The jars were aerated, replaced with a new KOH trap (5 mL of 2 N KOH), and sealed for the following 10-20 day incubation period. After 20 days, the second basetrap was removed and immediately capped. In order to prepare samples for the gas chromatograph (GC), a 250 µL aliquot from each basetrap was transferred into a GC vial and capped. GC vials with 50 µL $NaCO_3$ and 250 µL KOH were made for quality control samples. Prior to analysis, all GC vials were injected with 1 mL of 4 N HCl in order to release the trapped CO_2. Analysis was completed on the Varian CP-3800 gas chromatograph.

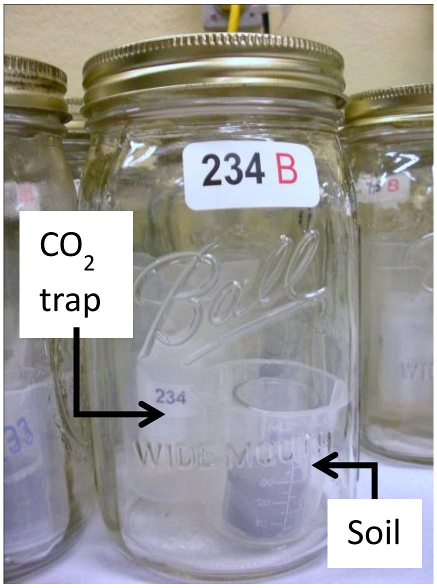


Figure 2.6. Microbial biomass fumigation incubation mason jar holding soil sample and scint vial containing KOH trap.

Calibration curves and retention times for gas under analysis were established by analyzing the certified standard gas mixture (1% CO_2) by the procedure used for analysis of the samples. The flow rate was 30 mL min^{-1}. For each incubation period, CO_2-C production was determined by subtracting the CO_2 produced in the non-fumigated samples from the CO_2 produced in the fumigated samples, since the fumigated samples had a larger carbon source for food as supplied from dead microorganisms. Data was reported as mg C kg^{-1} soil. A more complete description of the microbial biomass protocol can be found in the USDA's Soil Survey Laboratory Methods for Microbial Biomass Fumigation Incubation Procedure (Soil Survey Laboratory Methods Manual 2004).

2.3.7 Statistical Analysis for Soil Properties

Soil chemical properties were collected in 2010 at 2 depths, and at 1 depth only in 2011. The analysis of variance model in 2010 was a split block with treatments and depth as fixed effects and blocks random. In 2011, the experimental design was a randomized complete block at one depth only. For one depth only, an analysis of variance was performed comparing treatments and year as a split plot experimental design.

Soil physical properties were collected at one depth in 2010, and at two depths for each treatment in 2011. The 2010 data were analyzed as a randomized complete block experimental design. The 2011 data were analyzed as a split block experimental design with treatment and depth as fixed effects in the model. For one depth only, an analysis of variance was performed comparing treatments and year as a split plot experimental design.

The preliminary study of carbon and microbial activity on treated plots and an adjacent reference sample area compared the three sample sites using t tests. The effect of treatment on soil erosion was also determined by a t test.

All statistical analysis was performed using SAS software (SAS Institute Inc. 2009). The GLM procedure was used to finalize the model, with error variances pooled where possible at $P>0.25$. Where more than one error variance remained in the model, the final analysis of variance and LS-Mean separation tests were performed using PROC MIXED. Tests of fixed effects were declared significant at $P<0.05$.

2.4 Results and Discussion

2.4.1 Site characterization

The location of the site and general description of plot layout are given in Section 2.1. The soil physical and chemical properties reflect the overall composition of the pre-disturbed soil. The original (or pre-disturbed) soil was obtained from a nearby abandoned pasture site. The surface horizons (the A and the upper portion of the B horizon) of this soil were removed and used as top soil on a nearby agricultural reclamation site and not included in this study. The subsurface material containing the B and C horizon material was excavated and transported to the experimental site for this study. The predominant soil series of the excavation site was a Stendal silt loam (Figure 2.7)(Schulze 2012). The Stendal soil series was formed from loess and is somewhat poorly drained and found in bottom land positions which experience frequent flooding events during the winter and spring. The Bg subsoil of the Stendal series is described as mottled yellowish brown and light gray, friable silt loam about 68 cm thick, while the Cg horizon is 152 cm of light brownish gray, mottled silt loam. The Bg and Cg horizons have a hue of 10YR or 2.5Y, value of 5 to 7, and chroma of 1 or 2 (McWilliams 1989). In the current study, soil bulk soil samples of the 0-20 cm and 20-40 cm depths were dried and characterized by hue, value, and chroma using the Munsell color system. The soil characteristics of these samples were consistent with those reported for the Stendal soil series (McWilliams 1989) with a hue of 10YR, value of 5 to 6, and a chroma of 4 to 6. Peabody personnel communicated that the replacement soil also contained soil from the Hosmer soil series. Contrary to the Stendal silt loam, the Hosmer silt loam includes a fragipan (McWilliams 1989).

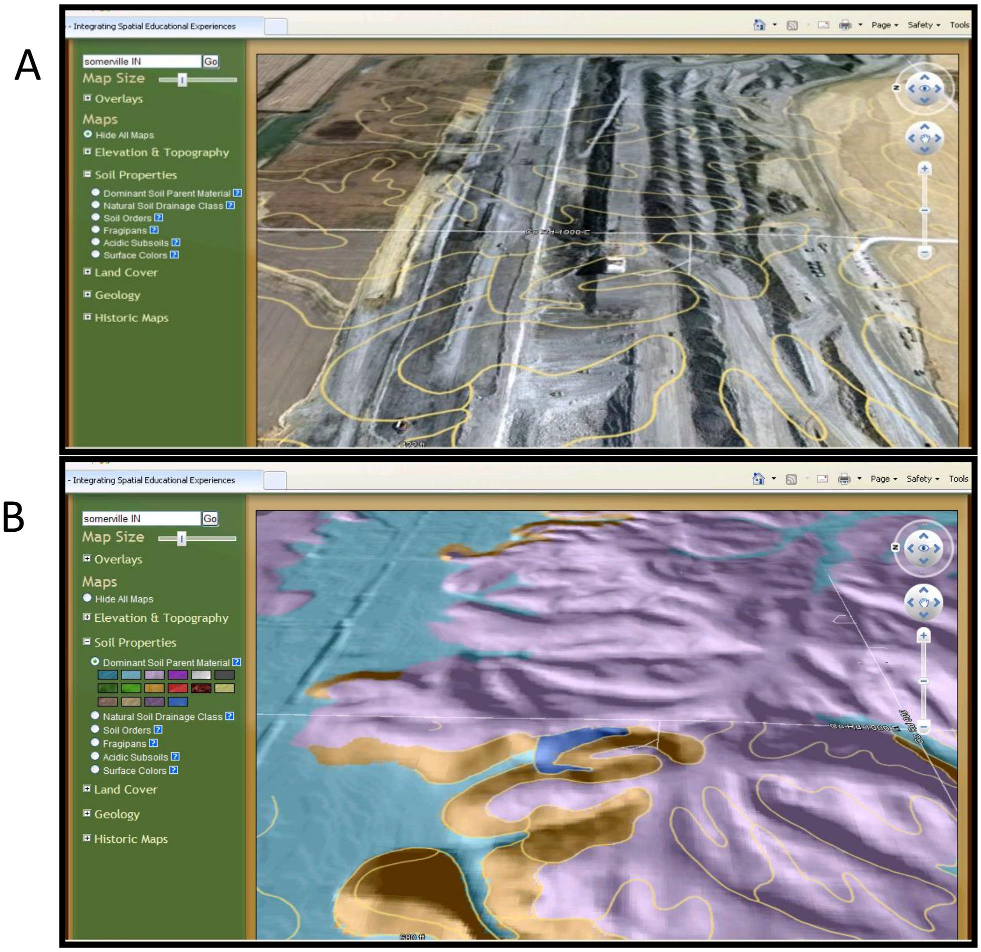


Figure 2.7. Comparison of images obtained from (Schulze 2012) identifying the excavation area (A) with the overlying dominant soil parent material map (B). The predominant soil series of the excavation site was found to be a Stendal silt loam (light purple color).

2.4.2 Soil Physical Properties

2.4.2.1 Soil Particle Size Analysis

Table 2.1 shows a comparison of soil particle size distribution (PSD) of the two soil replacement treatments for 2010 and 2011 for the surface horizon (0-20 cm). Significant differences were observed between the two time periods for percent clay and percent silt (Table 2.1). There were no significant changes in soil PSD between treatments. Soil PSD was also determined in 2010 for two depths (0-20 cm, and 20-40 cm), shown in Appendix A (Table A.1).

A Stendal soil pedon (ID: 1979-IN055-032) obtained from Greene, IN was analyzed at Purdue University (National Cooperative Soil Survey 2012). The properties of this soil pedon were compared to selected properties of the soils investigated in this study. This soil pedon showed similar soil PSD in the 74-152 cm depth compared to the 0-20 cm depth of the disturbed soil at the experimental site (National Cooperative Soil Survey 2012). The percent clay, silt, and sand of the Stendal soil averaged 19, 62, and 19 percent respectively. PSD was not significantly different between treatments at the experimental site. The percent clay, silt, and sand averaged across treatments in 2011 were 20, 59, and 22 percent respectively. Both treatments were classified as silt loams (Hillel 1988). The percent clay decreased slightly from 23 to 20 percent with a corresponding increase in percent silt between 2010 and 2011 (Table 2.1). Changes were more prevalent in the LD treatment. These changes across both treatments may have been caused from disruption and dispersion of finer materials in soil aggregates and siltstone materials present in the B and C horizon, leading to the accumulation of finer particles further down in the profile. (Jacinthe & Lal 2007) observed a high percentage of clay-size particles on an old overburden treatment on reclaimed mine land. The author reported that the high clay content was likely due to rapid weathering and disaggregation of clay-stones present in the overburden that took place over the 15 year period. In terms of particle size and qualitative soil characteristics, the replacement soil matched those of the lower B and C horizons associated with the Stendal soil series.

Table 2.1. Comparison of soil particle size changes in the 0-20 cm depth over two years (2010 and 2011), for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Soil particle size changes between 2010 and 2011 significant at α=0.05 are indicated with *.

	Percent Clay				Percent Silt				Percent Sand		
Treatment	2010		2011	Mean	2010		2011	Mean	2010	2011	Mean
GR	22		20	21	57		59	58	21	21	21
LD	24		19	22	53		58	56	23	23	23
Mean	23	*	20		55	*	59		22	22	

2.4.2.2 Water holding capacity

Gravimetric water contents (Θ_g) were obtained from soil cores at -0.1 bar matric potential and saturation in 2010 and 2011. In 2011, gravimetric water contents at -0.05 bar matric potential were also obtained. Table 2.2 shows a comparison of gravimetric water contents at -0.1 matric potential and saturation corresponding to the LD and GR soil replacement treatments for 2010, 2011 and the means for two years. Treatment differences (α=0.05) were observed in 2010 and for the means of the two years as indicated in Table 2.2. Differences were also observed between the two time periods for the LD treatment and overall.

The gravimetric water contents at -0.1 bar matric potential and saturation were significantly higher for cores collected from the LD treatment in 2010 and overall (Table 2.2). In 2010, gravimetric water content at -0.1 bar matric potential was 0.25 g H_2O/g soil in cores obtained from the LD treatment which was significantly higher than the GR treatment average of 0.21 g H_2O/g soil. Gravimetric water content at -0.1 bar matric potential decreased by 2 percent between 2010 and 2011 for the LD treatment; whereas gravimetric water content at saturation decreased by 6 percent between these two years for the LD treatment. Both gravimetric and volumetric water contents were obtained in this study and showed similar results with the gravimetric water contents showing slightly greater significance. A comparison of gravimetric and volumetric water contents between the two treatments and two depths in 2011 is shown in Table A.2. Volumetric water contents in the surface horizon (10 cm) recorded for the two treatments in 2010 and 2011 are shown with the gravimetric water content data in Table A.3.

In the surface horizon (10 cm), the LD treatment retained more water than the GR treatment soil samples (Table A.3). This is attributed to greater porosity and proportion of macropores in the LD soil. Moisture retention did not vary with depth in this study. This behavior is consistent with prior work where Bussler et al. (1984) showed that the moisture retention curves of mined soil had little variation with depth from the surface to a depth of 130 cm; unlike the typical variation observed in undisturbed soils. Bussler et al. (1984) showed that the moisture retention curves of the undisturbed soil in their study retained less moisture in the A2 horizon and greater moisture in the B2 horizon.

The moisture retention behavior of the LD and GR soils showed greater water holding capacity at field capacity (-0.1 bar) in the LD treatment in 2010. This result and similar soil physical property comparisons (e.g., bulk density) indicate that the method of soil replacement directly influences water holding capabilities and soil structure. The lack of variation of moisture retention with depth (to a depth of 40 cm) is indicative of a homogeneous constructed soil with little spatial order (Indorante & Jansen 1981).

In 2011, moisture retention at all three water potentials showed minimal differences across each treatment (Table A.2). Moisture retention was analogous at saturation and -0.1 bar in 2010 for the GR treatment as well (Table 2.2). These findings are likely due to the nature of post-mine land soil structure. The transportation and replacement of B and C horizon material creates a new soil structure that lacks typical soil aggregate arrangement, including macropore distribution. It was likely that the tension at -0.1 bar did not reach an adequate threshold to extract water from the micropores; and therefore, no change in moisture retention was observed.

Table 2.2. Comparison of gravimetric water contents at -0.1 bar matric potential and saturation for soil cores obtained at 10 cm depth over two years, for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Treatments having different letters are significant at α=0.05 where a=largest mean and b=lesser mean. Changes between 2010 and 2011 significant at α=0.05 are indicated with *.

	Ɵg -0.1 bar				Ɵg Saturation			
Treatment	2010		2011	Mean	2010		2011	Mean
GR	0.21 b		0.22	0.21 b	0.21 b		0.22	0.22 b
LD	0.25 a	*	0.23	0.24 a	0.30 a	*	0.24	0.27 a
Mean	0.23		0.23		0.26	*	0.23	

2.4.2.3 Depth of Needle Penetration (Soil Resistance)

Depth of needle penetration in soil cores collected at the 10 cm depth in the two treatments for 2010, 2011 and the means for two years is shown in Table 2.3. Measurements of soil needle penetration resistance were taken after soil cores were equilibrated to -0.1 bars using a sand tension table to ensure treatment differences were due to the effect of treatment rather than moisture. The depth of needle penetration is inversely related to soil resistance. A large difference was observed between the two soil replacement treatments in 2010. The lack of differences in 2011 was likely due, in part, to the settling that occurred during the first year. Another contributing factor to the changes observed in 2011 was the root development of grass on the site which decreased bulk density. The depth of needle penetration in the cores corresponding to the LD treatment averaged 11.9 mm (low resistance) compared to the GR average of 3.0 mm (high resistance) in 2010. Moffat & Bending (2000) measured soil resistance using a Bush recording penetrometer and observed significantly less resistance for the mound treatment (similar to LD) compared to the ripped and disked treatment with time. This was likely a result of fewer equipment passes (less impact) associated with the mound treatment. In this study, the mean penetration resistance at the 12 cm depth was 0.4 MPa for the mound treatment and 0.7 MPa for the disked treatment after five growing seasons (Moffat & Bending 2000). Despite initially breaking up the soil through ripping and disking, Moffat & Bending (2000) reported that the ripped treatment may have re-compacted after 5 growing seasons due to a collapse of the macropores formed during the initial ripping operation. This study also reported that penetration resistance increased with depth. In the current study, the depth of needle penetration (2011) was slightly greater at the 30 cm depth; however, this was not significant (Table A.4).

Table 2.3. Depth of needle penetration of soil cores obtained at 10 cm depth in 2010, 2011, and the means for the two years for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Treatments having different letters are significant at α=0.05.

Soil Property	Depth of Needle Penetration (mm)			
Treatment	2010		2011	Mean
GR	3.0	b	6.0	4.4
LD	11.9	a	9.1	10.4
Mean	7.4		7.5	

2.4.2.4 Bulk Density and Total Porosity

Soil physical properties including bulk density and total porosity of soil cores collected at the 10 cm depth in the two treatments for 2010 and 2011 are shown in Table 2.4. Significant differences were observed between the two soil replacement treatments in 2010 and for the means of the two years. These measured properties did not change between 2010 and 2011. Table 2.5 shows bulk density and total porosity in 2011 on cores obtained at the 10 and 30 cm depths. Soil bulk density values increased slightly with depth in the LD treatment (non-significant), and decreased with depth in the GR treatment (significant at α=0.05). Significant differences in total porosity were observed in comparing the two soil replacement treatments at the 10 cm and 30 cm depths; however, both treatments showed negligible changes in total porosity with increasing depth.

The Stendal soil pedon (ID: 1979-IN055-032) had a bulk density range of 1.45 to 1.65 g cm^{-3} in the 25-152 cm depth which was reflected in the cores measured from the LD treatment (National Cooperative Soil Survey 2012). In 2010, bulk density was 1.54 g

cm^{-3} for cores obtained from the LD treatment and 1.74 g cm^{-3} for cores obtained from the GR treatment (Table 2.4). Bulk densities greater than 1.6 g cm^{-3} have been reported to inhibit root growth on reclamation sites (Moffat & Bending 2000). Thus, the threshold for root impairment fell between the mean bulk densities of the LD and GR treatments.

In 2010, soil cores from the LD treatment averaged a significantly greater total porosity of 0.42 cm^3 cm^{-3} compared to the GR treatment mean of 0.34 cm^3 cm^{-3} (Table 2.4). Mine reclamation methods that include intensive grading cause some parts of the reclaimed soil to compact to the degree in which there is no structure, and the mineral particles become bound together (McSweeney & Jansen 1984). Soil in this state has been termed by McSweeney & Jansen (1984) as having a 'massive' structure. Reclaimed mine soils generally have mix of massive and fritted (loosely compressed aggregates) structures. In the current study, the massive soil structure contributed to the low total porosity and high bulk densities in the GR treatment, while the LD treatment likely was more composed of loosely compressed aggregates, or rather, a fritted soil structure. In 2011, bulk density and total porosity had no significant treatment differences (Table 2.4); however, trends were similar to the means reported in 2010.

Table 2.5 shows bulk densities of cores collected from the GR treatment decreased ($\alpha=0.05$) from 1.73 to 1.70 g cm^{-3} with depth. Total porosity varied among treatments at the 10 cm depth as the GR treatment averaged 0.34 cm^3 cm^{-3} and the LD treatment averaged 0.40 cm^3 cm^{-3}. Similar treatment differences were also seen in cores obtained at the 30 cm depth. However, porosity was not significantly influenced by depth for either treatment. Previous mine studies have shown varying degrees of compaction with depth depending upon the use of equipment and moisture content of the soil material during the time of operation (Bussler et al. 1984;McSweeney & Jansen 1984). Oftentimes, the degree of compaction changes when soil is sampled above and below the junction of the topsoil and subsoil (McSweeney & Jansen 1984). In the case of this current study, both treatments had 1 – 1.5 m of B and C horizon material used as 'topsoil' over the graded cast overburden. Therefore, compaction changes around the topsoil and subsoil junction were not assessed.

Of all the soil physical and chemical properties measured in this study, the difference in bulk density between the LD and GR soils was perhaps the most influential on plant growth and survival. Consistent with prior studies, the traditional GR soil replacement treatment resulted in high soil bulk density that was detrimental to plant growth (Moffat & Bending 2000;Bussler et al. 1984).

Table 2.4. Comparison of bulk density and total porosity of soil cores obtained at the 10 cm depth for 2010, 2011, and the means of the two years for the graded (GR) and loosely dumped (LD) soil replacement treatments. Treatments having different letters are significant at α=0.05 where a=largest mean and b=lesser mean.

	Bulk Density (g/cm^3)			Total Porosity (cm^3/cm^3)		
Treatment	2010	2011	Mean	2010	2011	Mean
GR	1.74 a	1.73	1.74 a	0.34 b	0.34	0.34 b
LD	1.54 b	1.58	1.56 b	0.42 a	0.40	0.41 a
Mean	1.64	1.66		0.38	0.37	

Table 2.5. Comparison of bulk density and total porosity of the soils corresponding to the loosely dumped (LD) and standard graded (GR) soil replacement treatments for 2011 for the 10 and 30 cm depths. Changes between 10 and 30 cm depths significant at α=0.05 are indicated with *.

	Bulk Density (g/cm^3)			Total Porosity (cm^3/cm^3)		
Treatment	10 cm	30 cm	Mean	10 cm	30 cm	Mean
GR	1.73 *	1.70	1.72 a	0.34 b	0.36 b	0.35 b
LD	1.58	1.61	1.59 b	0.40 a	0.40 a	0.40 a
Mean	1.66	1.65		0.37	0.38	

2.4.2.5 Soil Erosion and Settling

Soil replacement on the study site occurred between fall 2009 and spring 2010. In summer 2010, stakes were installed in plots 1 and 2 representing the GR and LD treatments, respectively. Changes in soil depth due to erosion (or settling) or accumulation were monitored at the 2-month- (September 2010) and 11-month-interval

(June 2011). In June 2011, settling and erosion was evident in the top and middle portions of the soil mounds as shown in Table 2.6 and Figure 2.8. The lower portions of the mounds were not monitored due to the formation of ponds (up to 1 m deep) that formed at the base of the mounds. The ponds formed between neighboring soil mounds, where there was no natural drainage due to the flat topography and the impermeable cast layer beneath the 'top soil'. Over the two year period, soil from the top and mid portions of the mounds was redistributed to the base of the mounds due to settling and erosion. The loss of soil varied significantly among mounds and was greatest for the east and west-facing aspects of mounds as the average soil losses (in the mid and upper portions of the mound) were 7.7 and 8.2 cm, respectively. This may have been due to the construction of the site and the direction that the equipment unloaded soil to create the overlapping piles.

There was less erosion in the GR treatment as the rows and columns averaged between 1.1 and 3.0 cm soil loss in June 2011. There were no differences in soil loss between the measured rows and columns for either time period. The east and south-facing aspects of mounds had significant increases in erosion between the 2- and 11-mo monitoring periods. Overall, the LD treatment had greater soil loss in the mid and upper portions of the soil mounds compared to the GR treatment in 2011 (Table 2.6). Soil was not monitored off-site for either of the treatments; and therefore, quantitative measurements were not analyzed. However, off-site soil loss on the GR treatment was presumably greater due to observation of large erosion channels in comparison to the LD treatment which primarily lost soil on the mounds bordering the perimeter of the plot. Contrary to the GR treatment, the LD treatment had greater redistribution of soil within the plots due to settling and (on-site) erosion. Reclamation sites where slopes are prevalent can experience large soil losses. In a study done on controlled and non-controlled vegetation sites in Germany with an inclination slope of 8°, erosion channels up to 1.5 m in depth were evident on sites where no vegetation efforts were installed. In contrast, sites where mulch and hay were transferred and vegetation was quickly set in place, erosion channels were observed to be < 5 cm (Baasch et al. 2012). In the present study, erosion channels were a greater issue for the GR treatment.

Table 2.6. Comparison of 2-mo and 11-mo soil changes in the graded (GR) and loosely dumped (LD) treatments. Positive values are indicative of erosion (or settling), while negative values represent soil accumulation. IDs, described in Figure 2.4, having different letters are significant at α=0.05 using a T-test where a=largest mean, etc. Changes between 2-mo and 11-mo significant at α=0.05 are indicated with *.

Treatment	ID	2-Mo. Soil Change (cm)		11-Mo. Soil Change (cm)	
GR	C1	-0.1		1.1	
GR	C2	-0.1		2.4	
GR	R1	-0.1		1.3	
GR	R2	-0.1		3.0	
LD	East	-0.1	*	7.7	a
LD	North	1.2		4.9	ab
LD	South	-0.7	*	1.9	b
LD	West	-0.4		8.2	a
GR	Mean	-0.1		2.0	b
LD	Mean	0.0		5.7	a

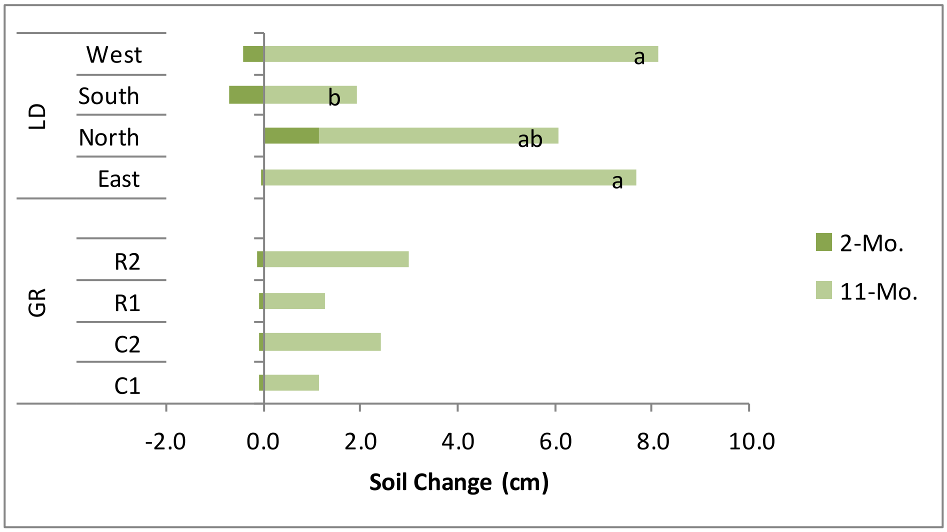


Figure 2.8. Comparison of 2-mo and 11-mo soil changes (cm) in the graded (GR) and loosely dumped (LD) treatments. Positive values are indicative of erosion (or settling), while negative values represent soil accumulation. Soil mounds in the LD treatment having different letters are significant at α=0.05 using a T-test .

2.4.3 Soil Chemical Properties

Selected chemical properties of the soils corresponding to the LD and GR soil replacement treatments for 2010 and 2011 for the surface horizon (0-20 cm) are shown in Table 2.7. The site was fertilized with 224 kg/ha 18-46-0 (N-P-K) in April 2010 prior to collecting soil samples. Fertilizer application varied among treatments as the fertilizer was lightly rotor-tilled 10 to 15 cm into the ground of the GR treatment, while fertilizer and seed were added into a slurry mix and hydroseeded in the LD treatment. Significant differences were observed between the two treatments for pH, soluble salt, total N, Mg^{2+} and Na^{+} (Table 2.7). There were no significant changes in soil chemical properties observed from 2010 to 2011. Soil chemical properties were also measured in 2010 for two depths (0-20 cm, and 20-40 cm) which showed significant differences among depths for total N and P (Table A.5).

Soil chemical measurements from the study site were compared to those reported for the Stendal soil pedon (25-172 cm), and a typical temperate deciduous forest floor soil, as shown in Table 2.7. Chemical properties of the underlying C horizon material (50-150 cm) of the Stendal soil series is described to have a pH range of 4.9-5.3 and contain 0.2-0.9 percent organic matter (National Cooperative Soil Survey 2012). On the experimental site, pH was significantly higher in the soil corresponding to the LD treatment as shown in Table 2.7. Soil pH averages in the LD and GR treatments were 6.1 and 5.5 respectively. Soil pH was moderately acidic across both treatments and showed negligible buffering capacity, which is ideal for oak species as they tend to grow best on moderately acidic to neutral soils (Rodrigue & Burger 2004). Soil corresponding to the GR treatment showed slightly higher soluble salt levels averaging 0.33 mmhos/cm compared to the LD treatment average 0.28 mmhos/cm, although these changes were significant. Soil from both GR and LD treatments contained soluble salt levels < 2 mmhos/cm which was indicative of little to no salinity (Binkley 1986). Cation exchange capacity was <14 $cmol_c$/kg for both treatments in both years and was not different among treatments. The percent organic matter averaged 1.1 percent for both treatments. Total N averaged 0.046 percent in the GR treatment which was slightly higher than the LD treatment average of 0.034 percent. Overall P levels were low as each treatment averaged 4 mg/kg compared to a typical temperate deciduous forest floor range of 9 to 44 mg/kg (Brady & Weil 2002). K slightly increased by 20 mg/kg across both treatment between 2010 and 2011. Exchangeable Mg^{2+} and Na^+ were slightly higher among soil corresponding to the LD treatment. Both treatments had exchangeable Mg^{2+} and Na^+ levels that were higher compared to the Stendal soil pedon.

Overall, the soil chemical properties of both the LD and GR soils were consistent with a homogeneous subsurface replacement soil. Few significant differences were found in the soil chemical properties among treatments. (Indorante & Jansen 1981) reported pH and exchangeable Mg^{2+} were 1.5 to 2 times more variable on average on the disturbed sites in comparison to undisturbed sites, which was consistent with the soil variation findings from this current study. More importantly, the soils had low fertility status, low organic carbon contents, and low cation exchange capacities. Bussler et al. found similar nutrient status on mine land reclaimed with topsoil compared to this current study. The authors reported low levels of available P (8.3 kg ha^{-1}) and exchangeable K (0.13 cmol kg^{-1}) and Ca (6.2 cmol kg^{-1}) in the rooting zone (0-29 cm). This study also found marginal CEC levels (16.5 cmol^{+} kg^{-1}) and organic matter (1.08%) in the rooting zone. In the current study, the pH fell into the range of 4.5-7.0, which was suitable for the selected hardwood species (Binkley 1986). Although pH was favorable, the low fertility, organic matter and CEC of both the LD and GR soils resulted in soils with little ability to retain critical nutrients for growth. This may explain the fact that although the soil was fertilized with N and P prior to the study and soil collection, little N and P were detected in the soil analysis suggesting that the added N and P was leached prior to soil sampling. For these soils, soil organic matter may be the most limiting soil constituent that governs nutrient retention.

Table 2.7 Comparison of chemical properties of the soils corresponding to the loosely dumped (LD) and standard graded (GR) soil replacement treatments for 2010 and 2011 for the surface horizon (0-20 cm). Horizontal comparisons having different letters are significant at α=0.05 where a=largest mean and b=lesser mean.

^{A} National Cooperative Soil Survey 2012

^{B} Brady & Weil 2002

		Treatment								Stendal Soil Series (25-172 cm)	Typical Range for Forest Floor (O horizon) in Temperate Deciduous Region
		Graded				Loosely Dumped					
Soil Chemical Properties	Units	2010	2011	Mean		2010	2011	Mean			
pH		5.6	5.5	5.5	b	6.2	6.1	6.1	a	4.9 - 5.9	5.6 - 6.0*
Buffer pH		7.0	6.9	6.9	b	7.1	7.0	7.1	a		
Soluble Salts	mmhos/cm	0.34	0.32	0.33	a	0.3	0.27	0.28	b		
CEC	cmol$_c$/kg	11	13	12		13	13	13			12 - 18*
OM	%	1.1	1.2	1.1		1.1	1.1	1.1		0.2 - 0.9	1-6
Total N	%	0.039	0.053	0.046		0.028	0.039	0.034			0.16
Bray 1 P	mg/kg	3.8	4.5	4.2		4.4	3.3	3.8			9-44
K	mg/kg	37	61	49		37	61	49			22-67
Mg	mg/kg	515	550	532	b	641	649	645	a		
Na	mg/kg	84	78	81	b	120	99	110	a		
Ca	mg/kg	849	977	913		982	935	958			
K	%	0.9	1.2	1.1		0.8	1.2	1.0		0.8 - 0.9	
Mg	%	38.8	35.6	37.2		43.2	41.8	42.5		6.0 - 7.1	
Na	%	3.3	2.6	3.0		4.1	3.4	3.8		0.8 - 0.9	
Ca	%	38.6	38.4	38.5		38.8	36.0	37.4		22.3 - 23.1	
H	%	18.5	22.2	20.4		13.1	17.6	15.3		68.8 - 69.2	

2.4.4 Soil Microbiological Properties

2.4.4.1 Indole acetic acid (IAA) analysis

Indole acetic acid (IAA) analysis was used to determine the presence of plant-growth promoting bacteria. Studies have shown that 80% of root associated microorganisms are able to synthesize IAA (Barazani & Friedman 1999). Representative HPLC results for indole acetic acid (IAA) analysis are summarized in Figure 2.9. IAA was not detected in either the original pre-disturbed soil or in the rhizosphere soil. The IAA standard had a retention time of 21 min (Figure 2.9D). No peaks were observed in the soil samples in the range of 18 to 22 min, indicating that IAA was not present. Features that were observed in the HPLC chromatograms were amino acids eluting around 3 min and a complex set of features in the 23 to 30 min that were not identified. IAA has typically been studied *in vitro*. Khalid et a. (2003) found IAA microbial products in the soil; however, isolates were taken to the laboratory to study the effects on root systems (elongation and biomass). This study concluded that certain isolates resulted in increased root elongation and weight, as well as increased shoot biomass. Auxins produced by these isolates averaged 7.0 mg IAA-equivalents liter^{-1} *in vitro.*

In poor nutrient soils, such as those found in mine studies, the interaction between roots and rhizobacteria is especially important (Lohmus et al. 2006). In the current study, the presence of IAA microbial products was not present in soil from either the reclamation site or the undisturbed pasture site near where soil was taken for the study. Seedlings growing in soil conditions where microorganisms are scarce may face greater limitations for nutrient availability and root growth stimulation. However, these results may be due, in part, to the sensitivity of this method. The concentration of the IAA standard was 20 mg IAA-equivalents liter^{-1}, meaning soil samples with effective IAA producers were at the low end of the detection range. Methods to overcome this include larger samples and more sensitive detectors (e.g. electrochemical detection, mass spectrometry, etc.). Despite detection limitations, IAA was likely not present due to the low organic matter and overall lack of microbial biomass. Levels of quality assurance were also lacking from this study including 1) adding a known concentration of IAA prior to extraction and 2) adding a known concentration of IAA to the extract immediately before analysis. Future studies may wish to study the effects of soil inoculation with IAA isolates, provided the soils would have a sufficient level of organic matter to support plant growth promoting bacteria.

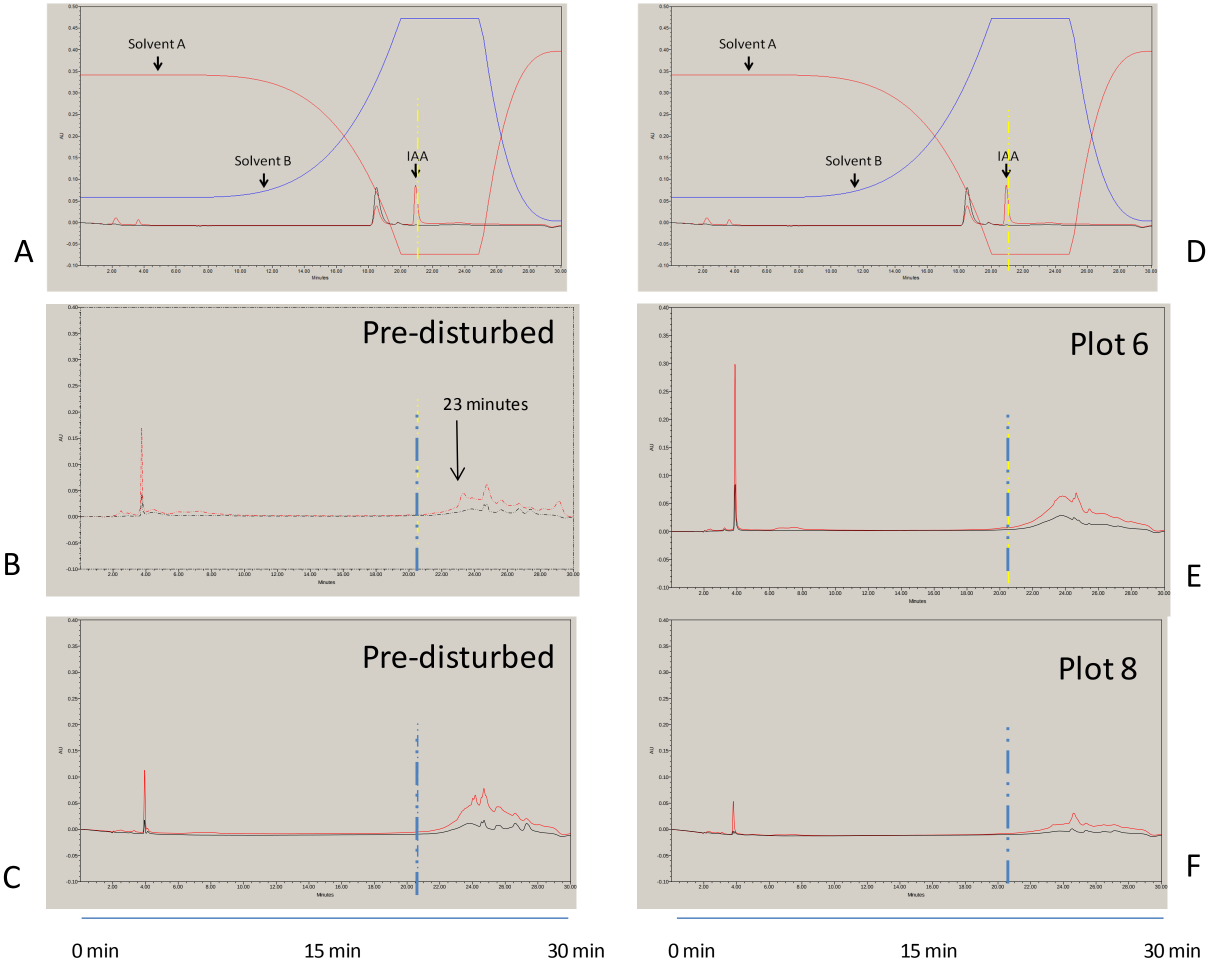


Figure 2.9. (A and D) Chromatograms of IAA standards of 0.02 mg/ml dissolved in 2 percent acetic acid to water (V/V) peaked at 21 minutes (A and D same image). Chromatograms B, C, E, and F of soil extract samples taken from the pre-disturbed pasture site (B and C) and the experimental site (E and F). HPLC analysis did not confirm the presence of IAA as dashed line (21 minutes) indicates where IAA peak would have occurred based on the standard.

2.4.4.2 Fluorescein Diacetate (FDA) Hydrolysis and Soil Microbial Biomass (MB)

Enzyme activity of microbial populations was measured using the fluorescein diacetate (FDA) hydrolysis method. Soil microbial enzyme production levels were significantly greater in the pre-disturbed soil obtained from the pasture site compared to both treatments on the experimental site (Table 2.8). In the pre-disturbed site, 445.9 mg fluorescein were released per kg of soil per hour compared to the GR and LD mean values of 177.5 and 109.7 mg fluorescein released per kg of soil per hour. These values were the mean of the 10 and 30 cm depth samples, as there was no significant change with depth.

In addition, microbial biomass measured through a CO_2 incubation method was also analyzed. Microbial populations were significantly greater in the pre-disturbed soil obtained from the pasture site compared to the LD treatment on the experimental site (Table 2.8). The GR treatment and the pre-disturbed soil showed no significant difference in microbial biomass despite their difference in means. Microbial biomass measured through CO_2 incubation was 50.8 mg C per kg soil in the pre-disturbed soil compared to the GR and LD treatment mean values of 12.5 and 7.5 mg C per kg soil, respectively. The GR and LD treatment were not statistically different. These values were the mean of the 10 and 30 cm depth samples, as there was no significant change with depth.

It is generally agreed that the enzyme production and biomass of soil microorganisms are more indicative of a functioning soil than the measurement of species diversity of microbial organisms within the soil (Graham & Haynes 2004). Both FDA and biomass results in the present study showed greater microbial enzyme production and biomass associated with the pre-disturbed site. In a study comparing microbial biomass and enzyme activity among forest reclaimed mine sites and non-mined forest reference sites, the presence of soil microorganisms was greater in the undisturbed soil (Fresquez & Lindemann 1982). In the present study, trends also showed greater establishment of microbial communities in the GR treatment compared to the LD treatment. It was predicted that the LD treatment would have shown greater enzyme activity of microbial populations and overall microbial biomass as microbial communities are known to be influenced by soil chemical and physical properties (Marschner et al. 2004). Marschner et al. (2004) indicated that there is a strong relationship between microbial populations and plant roots. In the present study, the dense grass vegetation and associated root system in the GR treatment may have, in part, stimulated microbial growth and activity.

Table 2.8. Comparison of soil microbial enzyme activity and overall biomass among the two soil replacement treatments, as wells an undisturbed site adjacent to where soil was removed for soil replacement. Treatments having different letters are significant at α=0.05 using a T-test where a=largest mean, etc.

	Soil Microbial Enzyme Activity		Soil Microbial Population Count	
Treatment	mg fluorscein released kg soil^{-1} 3h^{-1}		Soil Biomass Flush mg C kg^{-1} soil	
Graded	177.5	b	12.5	ab
Loosely Dumped	109.7	b	7.5	b
Undisturbed	445.9	a	50.8	a

2.5 Conclusions

Overall, the soil physical properties of the LD and GR soils showed significant treatment effects. In particular, the bulk density, moisture retention and porosity of the LD soil were more favorable for plant growth associated with minimal soil compaction. In contrast, the GR soil had a significantly higher bulk density (1.74 g/cm^3) compared to the LD soil (1.54 g/cm^3) resulting in a soil where root impairment is to be expected. The GR also had lower water holding capacity at field capacity (-0.1 bar) and lower porosity than the LD soil especially during the first year. Prior studies have shown that bulk density values greater than 1.6 g/cm^3 (Moffat & Bending 2000) or 1.7 g cm^{-3}

(Rokich et al. 2001) may cause root restriction. Although the LD soil had favorable physical properties, it was found to be more susceptible to erosion and soil redistribution within the site. The chemical properties of the LD and GR soils were consistent with those of a homogeneous soil constructed with subsurface material. Based on soil color, texture and related properties, soil from the experimental site resembled the lower B and C horizons of the Stendal soil series.

Unlike the treatment differences observed in soil physical properties, soil chemical properties showed negligible changes among treatments. The soil chemical properties reflected those of a subsurface replacement soil originating from the Stendal soil series. The soils had low fertility status, low organic carbon contents, and low cation exchange capacities consistent with that of a subsurface soil. These soil properties, in particular, determine the ability of a soil to retain nutrients and support soil microbial communities. This may explain, in part, why the fertility status of these soils is low despite being fertilized prior to the beginning of this study. For these soils, soil organic matter may be the most limiting soil constituent that governs nutrient retention and support of microbial communities.

The presence and activity of soil microorganisms measured through microbial biomass (MB) and fluorescein diacetate (FDA) hydrolysis were low in comparison to the undisturbed pasture site. This was reflective of poor soil quality associated with the B and C horizon material. Soil microbial counts and activity were slightly higher in the GR treatment which may have been due to the dense grass vegetation and associated root system in the GR treatment. The overall lack of soil microorganisms and enzyme activity added further stress on the seedlings and contributed to transplant shock.

CHAPTER 3. SEEDLING SURVIVAL AND GROWTH UNDER VARYING SOIL REPLACEMENT STRATEGIES: AN EMPASIS ON ROOT STRATEGIES

3.1 Introduction

After the passing of the Surface Mining Control and Reclamation Act (SMCRA), added concern was raised about the suitability of sites to support tree growth (Rodrigue & Burger 2004). Using the conventional graded approach, mine operators were required to grade the land to restore the approximate original contour (Legal Information Institute 2011). This standard graded soil replacement method was well suited for the reestablishment of herbaceous plants that could survive a shallow rooting zone and general lack of soil nutrients (Zipper et al. 2011). However, research in the area of reforestation of mined land has led to the implementation of a forest re-establishment process called the Forestry Reclamation Approach (FRA) (Skousen et al. 2009). The FRA was set up to provide awareness on how to create a suitable rooting medium to reclaim mine sites with native tree species. To meet this standard, mine operators need to avoid unnecessary soil compaction and maintain adequate soil fertility through selection of the replacement soil and use of compatible ground covers. Prior studies have shown reforestation to be successful on uncompacted, loose soil material (Skousen et al. 2009) with adequate soil depth (Torbert et al. 1988). The traditional graded soil replacement method can result in soil compaction and high bulk density values, which are known to limit water infiltration and root development (Simmons & Pope 1987).

Although seedling survival and growth have improved using the FRA standard, some negative effects can occur, such as increased soil erosion. At the same time, the loose soil method may enhance water infiltration. Thus, the FRA may become a more sustainable option than the standard graded approach for long-term runoff and surface erosion control in landscapes where site drainage occurs(Skousen et al. 2011). While loose grading as a top soil replacement strategy has been studied extensively on Appalachian mine sites, this approach has not been evaluated in the Eastern Interior region (including Indiana) and is one of the goals of this study.

In the present study, the overall goal was to evaluate survival and growth of selected hardwood species on a reclaimed mine site using a modified forestry reclamation approach soil replacement method. The specific objectives were to evaluate the effects of the traditional standard graded (GR) soil replacement and the newly proposed 'loosely dumped' (LD) soil replacement techniques on seedling survival and growth. Northern red oak (*Quercus rubra),* white oak (*Quercus bicolor)*, Shumard oak (*Quercus shumardii*), and American chestnut (*Castanea dentata*) were used. Seedling survival and growth (root collar diameter, shoot length, dieback, and biomass) were measured. In addition, below ground growth of the seedlings was quantified by measuring the overall root morphology and architecture of the four hardwood species grown on the two types of soil. The study interpreted seedling growth responses, including root development, based on soil physiochemical and biological properties.

3.2 Seedling Establishment

In April 2010, nursery grown (1 + 0) seedlings of northern red oak (*Quercus rubra),* white oak (*Quercus bicolor)*, and Shumard oak (*Quercus shumardii*) were obtained from the Vallonia State Nursery in southern Indiana located near Vallonia in Jackson County. Operational practices were used in the production of seedlings grown in the nursery, and seed source for all species was collected from trees within relative proximity of the nursery. Four orchard mixes of (1+0) blight resistant BC3F2 open-pollinated American chestnut (*Castanea dentata*) seedlings were also used in this study. The American Chestnut Foundation has been selectively breeding the American chestnut and Chinese chestnut for the past 30 years to develop the BC3F2 hybrid. (Jacobs 2007;Simmons 2009). These seedlings were crossed between American chestnut and Chinese chestnut twice (F2) and then back crossed three times with the American chestnut (BC3) in order to create a blight resistant hybrid BC3F2. Approximately 800 out of the 2000 oak seedlings obtained from each oak species were hand selected based on morphological characteristics including height and root collar diameter. Fewer chestnuts were selected (approximately 600 out of the 1000 obtained) as these seedlings were from orchard mixes. To minimize variability within species, height and root collar diameter were sorted and ranged from 50-85 cm, and 6-10 mm respectively, for American chestnut, 80-120 cm and 7-11 mm for red oak, 60-100 cm and 8-11 mm for shumard oak, and 15-40 cm and 4-7 mm for white oak seedlings. Root systems were not characterized in the initial pre-sorting. Seedlings were stored in bags in a cooler at 2 °C for about 4 weeks prior to outplanting.

3.3 Ground Preparation and Outplanting

The site was fertilized with 224 kg/ha 18-46-0 (N-P-K) and seeded with 11.2 kg/ha perennial ryegrass, 5.6 kg/ha annual ryegrass, and 2.2 kg/ha ladino white clover in early to mid-April 2010. The timing and method of application varied among soil replacement treatments. A hydro-seeder applied the fertilizer and seed to the loosely dumped soil replacement treatments. The seed was dumped into the fertilizer and water mixture. The hydro-seeder has an agitator to keep the fertilizer and seed in suspension during the application process. The pump and hose nozzle tip was sized to handle the mixture. A rainfall event (2.7 cm) took place within 5 days after the fertilizer and seed was applied. Standard graded plots were lightly disked followed by application of granular fertilizer and seed, which was pressed in with Brillion roller one week following the loosely dumped treatment application.

On May 5, 2010, a planting crew hired by Stone Forestry hand-planted seedlings using their standard practices which included severing the tap roots at 20 cm length and using a hoe-dad planting tool. Approximately 76 American chestnuts and 108 seedlings of each oak species were randomly planted in a 3 x 3 m grid per soil replacement treatment per block, totaling 3200 seedlings. In order to keep track of the species designation, the site was flagged with color-coded flags representing the four species. The exterior rows of each plot were also planted with seedlings to serve as buffer rows.

Weed control was conducted in the spring of 2011 after seedlings had been planted for approximately one year. The herbicide consisting of 4.68 L/ha glyphosate and 0.59 L/ha Plateau® (post-emergence herbicide) was mixed with water and surfactant on the site and sprayed using a backpack sprayer with a hand-held nozzle attachment. The herbicide was applied approximately 1 m diameter around each seedling. Seedlings were shielded with a cardboard poster in order to avoid herbicide damage to the leaves.

3.4 Materials and Methods

3.4.1 Baseline Plant Growth Measurements

A sub-sample of 100 sorted and selected seedlings (25 seedlings of each species) from Vallonia Nursery were measured in a laboratory at Purdue University for shoot height, root collar diameter, and lateral and tap root volume by water displacement (Bohm 1979a). Twenty five seedlings of each species were sorted based on similar height and root collar diameter measurements that were selected for planting. Seedling tap roots were cut to 20 cm in length to roughly mimic the size of the root systems planted on the site. Tap root dry weights obtained from seedlings excavated in July 2010 compared favorably to those of the seedlings measured in the subsample (i.e. were within 0.4 g of the corresponding species). Individual seedling components (i.e. shoot, root) were dried at 70 °C for a minimum of 72 hours or until weight of dry matter no longer fluctuated. After dry weights were recorded, samples were sent to A&L laboratory (Fort Wayne, IN) for nutrient analysis of N, P, K, Ca, Mg, S, Zn, Mn, Fe, Cu, B, Al, and Na as described in the plant nutrient analysis section.

3.4.2 Plant Growth

In May 2010, all seedlings planted within treatments (excluding those planted on buffer rows) were measured for height to live bud and root collar diameter. In April and

September of 2011, all seedlings on each plot were measured for height to new (live) bud, total height, and root collar diameter, and recorded for browse, damage, and survival. Seedlings that were excavated were not considered to be dead in the survival data. Therefore, survival included the excavated seedlings in the baseline number, as it was assumed that those seedlings would still have been living had they not been harvested. The length of dieback was measured on seedlings that had dieback in April and September of 2011. On seedlings that had dieback, this was calculated by subtracting the height to live bud from the total height of the seedlings (Figure 3.1).

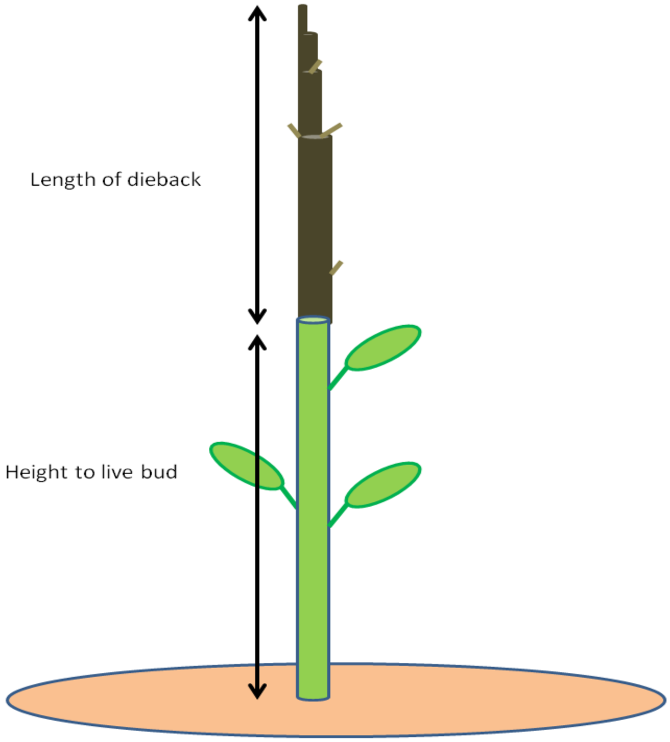


Figure 3.1. Dieback was calculated by subtracting the height to live bud from the total height of the seedlings.

3.4.3 Sampling Measurements

Seedlings were excavated in July 2010 and September 2011. Five seedlings per species in each plot (80 seedlings per treatment) were excavated with shovels and destructively harvested in July 2010. In September 2011, this number was modified to three seedlings per species at each plot (48 seedlings per treatment). Seedlings were wrapped in bags to prevent root desiccation and transported in coolers. Upon arrival to

Purdue, seedlings were measured for height and root collar diameter. Leaves were removed and stored separately from shoots. Roots were washed free of soil and tap root length was measured followed by removal and storage of lateral roots. Roots were stored in Ziploc bags and kept in a refrigerator at 3 °C. In 2010, tap and lateral root volumes were measured using water displacement (Bohm 1979b) prior to removal and storage of lateral roots. Root growth and architecture of lateral roots were analyzed using WinRHIZO as described in the root growth section. In 2010, tap roots were also separately analyzed for root parameters using WinRHIZO. Individual seedling components (i.e. shoot, leaf, tap root, and lateral roots) were dried at 70 °C for a minimum of 72 hours or until weight of dry matter no longer fluctuated. After dry weights were recorded, leaf material was ground in a Wiley mill with mesh size of 40 mm. Lateral roots, shoots, ground leaves, and 1 representative tap root from each species were sent to A&L laboratories (Fort Wayne, IN) and analyzed for N, P, K, Ca, Mg, S, Zn, Mn, Fe, Cu, B, Al, and Na as described in the plant nutrient analysis section.

3.4.4 Root Growth

Roots were washed free of soil and refrigerated at 3 °C until images were acquired using a flatbed scanner. Root images were taken in a plastic tray filled one-third full of water in order to minimize root overlap. Images were imported into Adobe Photoshop (v. 9.0.2) where they were cropped to remove dust and other debris from the image (See figure 3.2). Images were analyzed using WinRHIZO software (v. 4.1, Regent Instruments Inc., Quebec, Canada, 2001) for root length, volume, surface area (SA), projected area (PA), and number of tips within each 0.0 – 0.5 mm diameter class up to 4.5 – 5.0 mm. Projected area was calculated from surface area by transforming SA data to compute a 2-D PA variable.

Figure 3.2. Root image scanned for the WinRHIZO analysis.

3.4.5 Plant Nutrient Analysis

Nutrient concentrations in plants were analyzed by A&L laboratories using an emission spectrograph. Stock standard solutions were prepared using the concentrations as shown in Table 3.1. Standard concentrations (g/L) were based on 1 g sample taken up in 5 mL buffer solution containing 50 g Li_2CO_3, 200 mL HNO_3, and 1 L H_2O.

Table 3.1. Stock solution concentrations used for standards in emission spectrograph (A&L laboratories).

Element	Element g/L	Salt g/L	Solvent
P	10	31.64	H_2O
K	125	238.36	H_2O
Ca	40	99.89	1 N HNO_3
Mg	20	33.16	1 N HNO_3
Zn	1	1.00	1 N HNO_3
Mn	10	1.29	1 N HNO_3
Fe	10	1.00	1 N HNO_3
Cu	1	1.00	1 N HNO_3
B	1	5.72	H_2O
Al	10	1.00	1 N HNO_3
Na	10	25.42	H_2O

Plant material was dried and ground in a Wiley mill with No. 20 steel sieve. Samples were weighed to 1.0 g and placed in crucibles. Samples were held at 500 °C for a minimum of 4 hours, cooled, and then 5.0 mL buffer solution (same solution used in standards) was added. On the spectrograph, electrodes were spaced 4 mm apart in holders. Aliquots of each sample were placed in porcelain boats and immersed in solution while the emission spectrograph sparked 10 seconds to condition electrodes and photomultiplier tubes, and then an additional 30 seconds for integration. Mixed element standard solutions and known tissue standards were used in order to calibrate the spectrograph.

In order to determine sulfur content on plant samples, A&L laboratories weighed samples to 2.0 grams and placed in a crucible with Na_2CO_3. Crucibles were held over a sulfur-free flame until contents fused. Contents were then placed in a 600 mL beaker where 100 mL H_2O was added. Solution was diluted to volume and filtered. Aliquot of prepared solution were diluted to 200 mL with H_2O and HCl until 0.5 mL free acid was present. Samples were heated to boiling point and 10 mL 10% $BaCl_2$ solution was added. Samples were filtered through ashless paper, and 15 mL boiling H_2O was added to precipitate. Precipitate was dried, filtered, ignited and weighed as $BaSO_4$. The following calculation was used to determine sulfur concentration: Precipitate wt (g) x 0.1374 = S (Jones 1984).

Total nitrogen was determined by A&L laboratories using the Kjeldahl method. Samples were weighed to 1-2 g in digestion flasks and a volume of H_2SO_4 was added to each flask (volume corresponded to weight of sample; 35 mL for 1 g). Samples were shaken thoroughly and then let to stand for a minimum of 30 minutes with occasional shaking. Five grams of $Na_2S_2O_3 \cdot 5H_2O$ was added to each flask. Flasks were shaken and heated to a boil until foaming ceased. Samples were heated to a boil for the second time after the addition of 0.7 g HgO and 15 g K_2SO_4. Samples were cooled and 200 mL H_2O and a few Zn granules were added to the solution. Flasks were tilted and 50 mL NaOH-thiosulfate solution was added to each flask. Flasks were connected to distillation bulbs and heated until a minimum of 150 mL distillate was collected per sample. Blank detections on reagents were corrected (Jones 1984).

Root N content (lateral and tap) was calculated using the formula below. Due to the limited N data on tap roots in 2011, total root N (tap + lateral) values were calculated for each species based upon corresponding species' N tap root concentration from that year.

$$\begin{array}{llcl} & \text{\% Total N (lateral)} & \text{x} & \text{Lateral DW} \\ + & \text{\% Total N (rep species tap)} & \text{x} & \text{Tap DW} \\ \hline & \text{Root Total N (g) (Lateral and Tap)} & & \end{array}$$

$$\frac{\text{Root Total N (g)}}{\text{Root DW (g)}} = \text{Root Total N (g/g)}$$

3.4.6 Plant Moisture Stress

August 19th, 2010 predawn plant water potentials (ψ) were measured using a pressure bomb to assess plant moisture stress. The timing of these measurements coincided with droughty conditions. Specifically, the study area received approximately 0.86 cm of rain in the two weeks prior to measurements. In addition, the 0.86 cm of precipitation fell prior to a 9-day drought period that led up to measuring ψ. These measurements were taken between 11 PM EDT and 5 AM EDT on the 20th of August. To conduct measurements, one fully developed leaf was collected near the terminus of each stem of five randomly selected seedlings per species per treatment replicate. The bottom of the petiole was then sliced with a razor and placed into the pressure bomb gasket and tightened. These measurements were repeated again in mid-September of 2011. Nine days prior to the September measurements, the study site received 0.05 cm of rain. However, an additional rainfall of 1.19 cm occurred prior to measuring plots 7 and 8.

3.4.7 Statistical Analysis for Seedling Measurements

Plant growth was measured for each tree in each year. The experimental design was a split plot, split block with surface treatment as the whole unit, species as the subunit, and time as a split block treatment.

Root growth characteristics were measured in each year, with 5 and 3 trees sampled for each tree species and plot in 2010 and 2011, respectively. Plant moisture stress was also measured in each year, on five randomly selected trees per species and plot. Analysis of variance was performed using the means of the trees measured for each species and plot. The experimental design was a split-split plot, with surface treatment as the whole unit, species as the subunit, and time as the sub-sub unit.

Percent seedling survival was determined at the beginning and end of the second year, and analyzed within each sampling time. Plant nutrient information was measured at the end of the experiment for three trees per species and plot. The analysis of variance was performed on the means of three samples per species and plot. The experiment was a split plot experimental design, with surface treatment as the whole unit and species as the subunit treatment. Within tree species, surface treatment means were compared to the mean of baseline data as a constant using a t test.

Percent survival was arcsine transformed and the other measurements were log or square root transformed prior to analysis of variance, as determined by the Box-Cox regression procedure (Box et al. 1978). Results are presented in back-transformed units. All statistical analysis was performed using SAS software(SAS Institute Inc. 2009). The GLM procedure was used to finalize the model, with error variances pooled where possible at $P>0.25$. Where more than one error variance remained in the model, the final analysis of variance and LS-Mean separation tests were performed using PROC MIXED. Tests of fixed effects were declared significant at $P<0.05$.

All root and shoot measurements obtained from the 96 excavated seedlings in September 2011 were run using Pearson product-moment correlation. Pearson correlation analyzes the relationship between two variables and provides information about how well the linear model fits the relationship between the two variables. Variables tested in this study were from individual seedlings, i.e. the root volume and shoot dry weight of an individual seedling from a treatment plot, with $n=96$. Statistical analysis was performed using Origin 8.6 (OriginLab Corporation 2011).

3.5 Results and Discussion

3.5.1 Seedling Survival and Growth

Seedling survival was determined for two growing seasons (2010-2011). Seedling ‘field’ height and root collar diameter (RCD) were measured for live seedlings. The term ‘field’ height simply refers to heights taken in the field, whereas ‘excavated’ height and RCD will be used for the harvested seedlings. The bareroot nursery seedlings (1+0) were planted in May of 2010 and seedling data was collected within two weeks of post-planting. Seedling survival was measured after the first growing season (April of 2011) and at the end of the second growing season (September of 2011) and is presented in Figure 3.3. Excavated seedlings were included in the survival data and the baseline seedling number was not adjusted; as it was assumed that the excavated seedlings would have been alive had they not been harvested. Seedling survival was 80% and nearly identical across both treatments after the first year. At the end of the second growing season (September 2011), seedling survival was significantly higher in the LD compared to the GR treatment. Survival was 53% for the LD treatment compared to 42% for the GR.

A similar study comparing seedling survival on graded and ripped (less compacted) treatments of 16 tree and shrub species observed > 75 percent survival after two years, with the exception of three species (*Pyrus* spp., A. *saccharinum*, and L. *styraciflua*) whose survival ranged from 18-68 % (Ashby 1997). The low survival of these three species was attributed to animal browse and herbicide damage. Seventy-one percent survival was observed on the control treatment (no rip/no herbicide) compared to 85% (no rip/herbicide), 80% (rip, no herbicide), and 80% (rip/herbicide) treatments. The current study had much lower survival after one year, which may have been caused by the planting method and rapid weed growth that occurred in the second growing season despite weed control measures (see Confounding Factors, section 3.5.7). Chaney et al. (1995) reported that survival decreased rapidly during the first 4 years following planting. After 4 years, red oak seedlings planted on reclaimed mine land had 56% survival on treatments that had weed control, and 10% on treatments with no vegetation control. After 11 years, red oak survival was 43% with weed control, and nearly 0% on the treatment with no weed control (Chaney et al. 1995).

Seedling survival among species is shown in Figure 3.4. American chestnut seedlings had the lowest second-year survival compared to all other species. In September 2011, survival was 26% (AMC), 50% (REO), 59% (SHO), and 57% (WHO). Shumard oak seedlings had the highest survival rate.

A non-mining study (Jacobs et al. 2005) assessed red oak and white oak seedling survival on a field planting site in southern IN. Seedlings were obtained from Vallonia nursery, which is where the current study obtained seedlings. At the end of the second growing season, seedling survival of red and white oak ranged from 94 to 97% due to effective weed control and elimination of deer browsing. As this current study shows, seedlings on reclaimed mine land had lower survival rates due to unfavorable vegetation competition, soil compaction, and soil moisture availability. Several mine studies have shown negative relationships between soil density and tree survival due to the water limitation and root impediment associated with denser soils (Zipper et al. 2011;Skousen et al. 2009). Skousen et al. (2009) suggested slightly higher survival rates on the ripped mined land treatment compared to the graded treatment were due to increased root access to water. In this current study, seedling survival was only slightly affected by soil density as treatment differences were minimal. It is more likely that vegetation competition and poor planting attributed to the low survival rates across both treatments as described in the Confounding Factors section 3.5.7.

Seedling field height and RCD measurements showed significant differences among species within each time period (Figure 3.5 A-B and Table 3.2 A-C). First-year measurements were taken within two weeks after planting and were heavily influenced by nursery conditions. A subset of 100 seedlings of each species was sorted and selected based on height and RCD prior to planting for more in depth analysis at Purdue University. Among the four species, the field height and RCD means of the 3200 seedlings were within 21 cm and 3.1 mm, respectively, of the selected subset seedlings (Table B.1). The subset of nursery seedlings was slightly larger in height and RCD than the planted seedlings.

Since the seedlings were planted in May of 2010, no dieback was observed for this measurement period (Figure 3.5 A). At the beginning (April 2011) and end of the second growing season (September 2011), length of dieback was observed and measured for all four species. Measurement of dieback is discussed more fully in the plant analysis methods section. In Figure 3.5 A, the mean total field height for each species is shown which is comprised of the height of the seedling to live bud (living portion of the seedling; shaded region in Figure 3.5 A) and dieback (the non-living portion of the stem above the living stem that contributes to the overall height; non-shaded region in Figure 3.5 A). American chestnut and red oak seedlings had significantly greater first- and second-year field height and root collar diameter compared to Shumard and white oak (Figure 3.5 B). Red oak seedlings had slightly greater field height at the beginning of the second growing season in April 2011. This was due in part to the 66 cm dieback mean that occurred on the American chestnut seedlings. Length of dieback varied among species within the two soil replacement treatments (Table 3.2 C and Figure B.1). In the GR treatment, dieback averaged 47 cm (AMC), 37 cm (REO), 43 cm (SHO), and 16 cm (WHO); whereas in the LD treatment, dieback averaged 65 cm (AMC), 29 cm (REO), 34 cm (SHO), and 10 cm (WHO).

Second-year field RCD increased from the time of planting by 0.9 mm for red oak, 1.5 mm for white oak and American chestnut, and 2.2 mm for Shumard oak (Table 3.2 B). For all species expect American chestnut, seedling field height was greatest at the time of planting. In contrast, root collar diameter was greatest at the end of the second growing season for all species (Table 3.2 A). In other words, seedling field height (to the live bud) decreased over the two growing seasons due to dieback; in contrast to field RCD which increased for all species over this same time interval. Casselman et al. (2006) reported the average height growth of hardwood species planted on reclaimed mine land was -2.3 cm in the first year due to dieback. Skousen et al. (2009) found negative height growth due to both deer browse and dieback; in contrast to an increase in RCD after 7 years. In this current study, 3.1 meter high deer fencing was installed shortly after site establishment, ensuring seedling protection from deer browsing.

Seedling height and RCD trends in the excavated seedlings showed similar differences among species for both years (Table B.2). Overall height and RCD measurements among species excavated in 2011 were slightly larger than seedling recorded in the field (3 days prior to excavations) in 2011 (Tables 3.2 and B.3). No treatment differences were observed among height and RCD measurements of the excavated seedlings in 2010 and 2011; however, height and RCD significantly increased among both treatments within this time interval (Table B.3). Table 3.3 A-B shows that shoot dry weight changes were significantly different among treatments when averaging 2010 and 2011 measurements. Seedlings from the LD treatment increased in shoot dry weight by 12.4 g compared to the GR treatment gain of 9.2 g between July 2010 and September 2011 (Table 3.3 A). When averaging seedlings from both treatments, average shoot weight significantly increased from 7.96 to 18.64 g between the two growing seasons. Another study found that varying spoil types significantly affected hardwood shoot and root development (Showalter et al. 2010). Under favorable site conditions (weathered sandstone spoil), the shoot and root biomass of *F. americana* seedlings increased by 2 and 32 g, respectively, compared to unfavorable sites.

Red oak, Shumard oak, and American chestnut seedlings had significantly greater shoot dry weight compared to the white oak seedlings in 2010 and 2011 (Table 3.3 B). White oak has a reputation for being a slow growing species. It was chosen for this study because it has been known to thrive on low quality sites (Kormanik et al. 2002). All species significantly increased in shoot dry weight between the two growing seasons. The largest increase was observed in the American chestnut species as this species averaged a 15.3 g increase in shoot dry weight between the two growing seasons. Despite the low survival rate and large amount of dieback observed in this study, the American chestnut seedlings that initially survived were able to show rapid growth on the soil conditions of the reclaimed mine land.

Leaf dry weight of American chestnut, red oak, and Shumard oak seedlings was greater than white oak seedlings in 2010 and 2011 (Table B.4). Leaf dry weight averaged across treatments increased by 7.5 g from 2010 to 2011; however, there were no treatment differences (Table 3.4 and Figure 3.6 A-B). There were no leaf comparisons between field seedlings and the pre-sorted and selected nursery seedlings as the nursery seedlings were dormant and had not developed leaves at the time of planting.

No treatment differences or interactions were observed for height and root collar diameter within the first two growing seasons (Table 3.2). In summary, the gross seedling morphology measurements (height and RCD) were not found to be sensitive to the method of soil replacement after two growing seasons. Skousen et al. (2009) found varying soil treatments significantly influenced the growth of five tree species growing on reclaimed mine land. They reported that seedlings had significantly greater growth on ripped sites compared to the compact, non-ripped, sites. Another study observed that regardless of topsoil application, if the site was severely compacted, growth limitations on seedlings would prevail and result in poor growth (Casselman et al. 2006).

Above ground growth and survival of the four hardwood species observed in this study were comparable or slightly less than those reported in prior mine reclamation studies. Seedling survival was greater on the LD replacement soil with the exception of red oak. Root collar diameter increased for both treatments; however, overall height did not increase due to dieback. Shoot biomass was significantly greater overall in the LD treatment. In addition, seedlings growing on LD replacement soil had less dieback with the exception of American chestnut. In general, few treatment effects were observed in above ground growth parameters and survival. This is attributed, in part, to the fact that this study measured seedlings over a 16 month time period and this time period may not have provided a sufficient amount of time to observe above ground growth sensitivity to treatments.

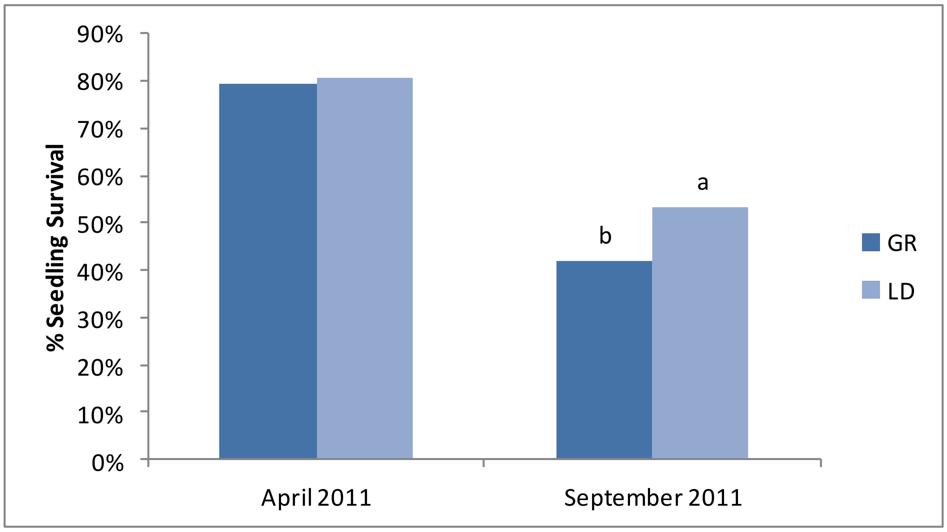


Figure 3.3 Seedling survival at the beginning (April) and end (September) of the second growing season across the graded (GR) and loosely dumped (LD) treatments. Treatments having different letters are significant at α=0.05 where a=largest mean, etc.

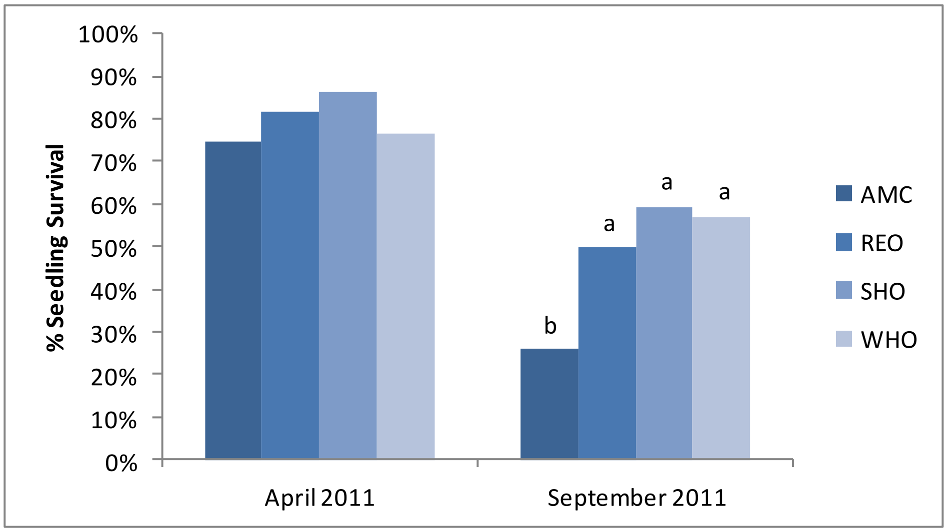


Figure 3.4 Seedling survival of American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) at the beginning (April) and end of the second growing season (September). Species having different letters are significant at α=0.05 where a=largest mean, etc.

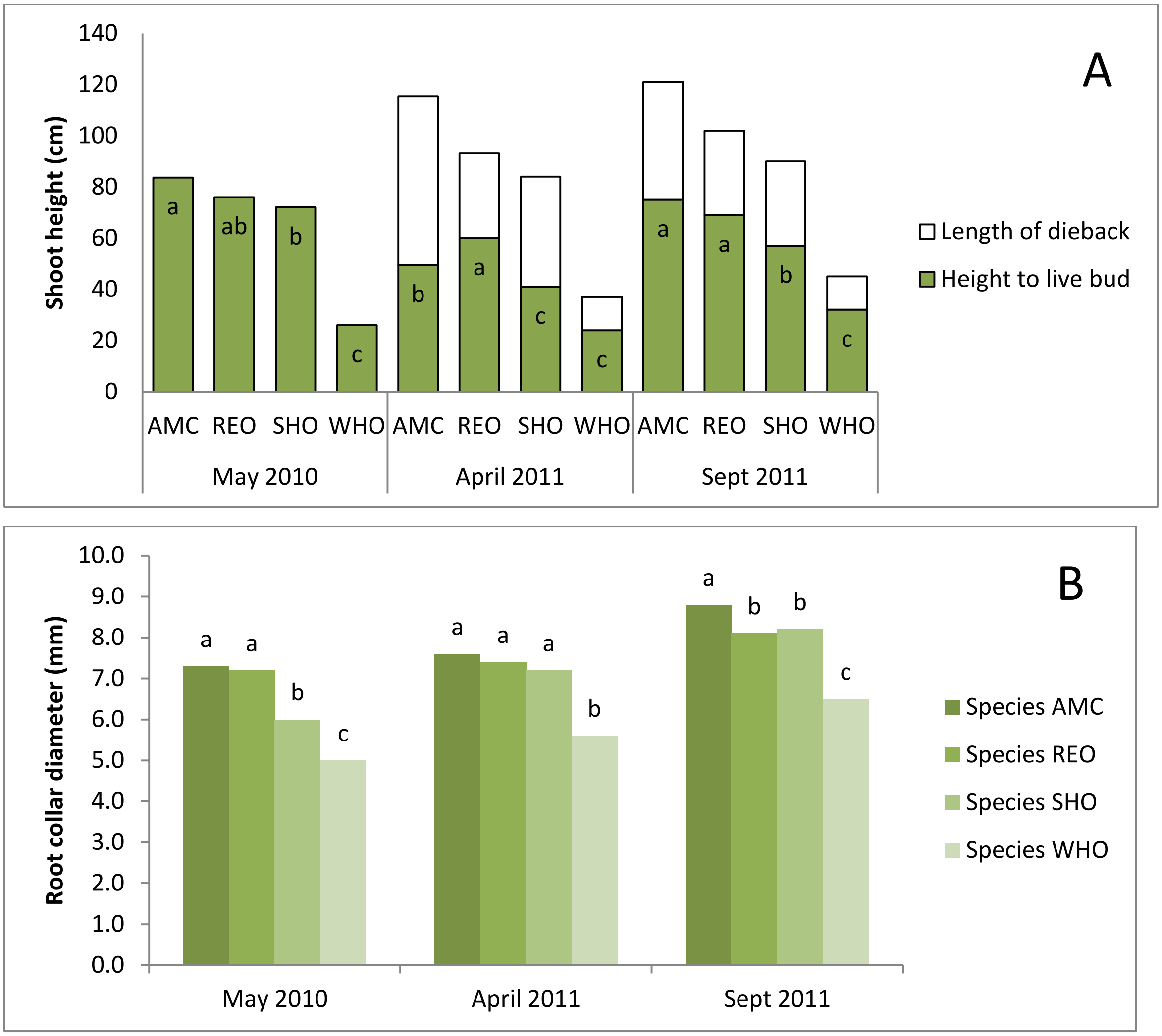


Figure 3.5 A-B. Shoot height to live bud, length of dieback (A), and root collar diameter (B) of American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) for the graded (GR) and loosely dumped (LD) treatments. Species having different letters within each sampling period are significant at α=0.05 where a=largest mean, etc. Dieback was calculated during each measurement period by subtracting height to new bud from the total height of the seedling.

Table 3.2 A, B, C. Seedling field height (A), root collar diameter (RCD) (B), and length of dieback (C) of American chestnut (AMC), red oak (REO), shumard oak (SHO), and white oak (WHO) over two growing seasons (2010 and 2011), for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Means followed by the same letter are not significantly different. Capital letters are used for row comparison of dates or treatments; lowercase letters are used for species comparison.

A

	Treatment		Date			
Spp.	GR	LD	May 2010	April 2011	Sept 2011	Spp. Mean
			Seedling Height (cm)			
AMC	68	71	84 A a	50 B b	75 AB a	70 a
REO	67	69	76 A ab	60 B a	69 B a	68 a
SHO	54	60	72 A b	41 B c	57 C b	57 b
WHO	26	28	26 c	24 c	32 c	27 c
Mean	54	57	64 A	44 B	59 AB	

B

	Treatment		Date			
Spp.	GR	LD	May 2010	April 2011	Sept 2011	Spp. Mean
			Root Collar Diameter (mm)			
AMC	8.1	7.6	7.3 AB a	7.6 B a	8.8 A a	7.9 a
REO	7.8	7.3	7.2 AB a	7.4 B a	8.1 A b	7.6 b
SHO	7.4	7.2	6.0 C b	7.2 B a	8.2 A b	7.3 b
WHO	5.8	5.6	5.0 C c	5.6 B b	6.5 A c	5.7 c
Mean	7.2	6.9	6.5 BC	6.9 B	7.8 A	

C

	Treatment		Date		
Spp.	GR	LD	April 2011	Sept 2011	Spp. Mean
			Length of Dieback (cm)		
AMC	47 a	65 a	66	46	55 a
REO	37 a	29 b	33	33	33 b
SHO	43 a	34 b	43	33	38 b
WHO	16 b	10 c	13	13	13 c
Mean	33	28	33	29	

Table 3.3. A-B. Comparison of shoot dry weight among treatments (A) and species (B) including American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) over two growing seasons (2010 and 2011) and for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Means followed by the same letter are not significantly different. Changes between 2010 and 2011 significant at α=0.05 are indicated with *.

A

Treatment	2010		2011	Mean	
	Shoot Dry Weight (g)				
GR	7.35		16.51	11.01	b
LD	8.62		21.04	13.47	a
Mean	7.96	*	18.64		

B

	Treatment		Year				Species	
Species	GR	LD	2010		2011		Mean	
	Shoot Dry Weight (g)							
AMC	17.68	19.47	12.41	*	27.72	a	18.55	a
REO	15.92	16.78	12.71	*	21.02	a	16.35	a
SHO	12.40	18.02	10.31	*	21.66	a	14.94	a
WHO	4.22	5.60	2.48	*	9.56	b	4.86	b

Table 3.4. Leaf dry weights of excavated seedlings over two growing seasons (2010 and 2011) for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Changes between 2010 and 2011 significant at α=0.05 are indicated with *.

Treatment	2010		2011	Mean
	Leaf Dry Weight (g)			
GR	2.62		8.91	4.83
LD	2.50		11.44	5.35
Mean	2.56	*	10.10	

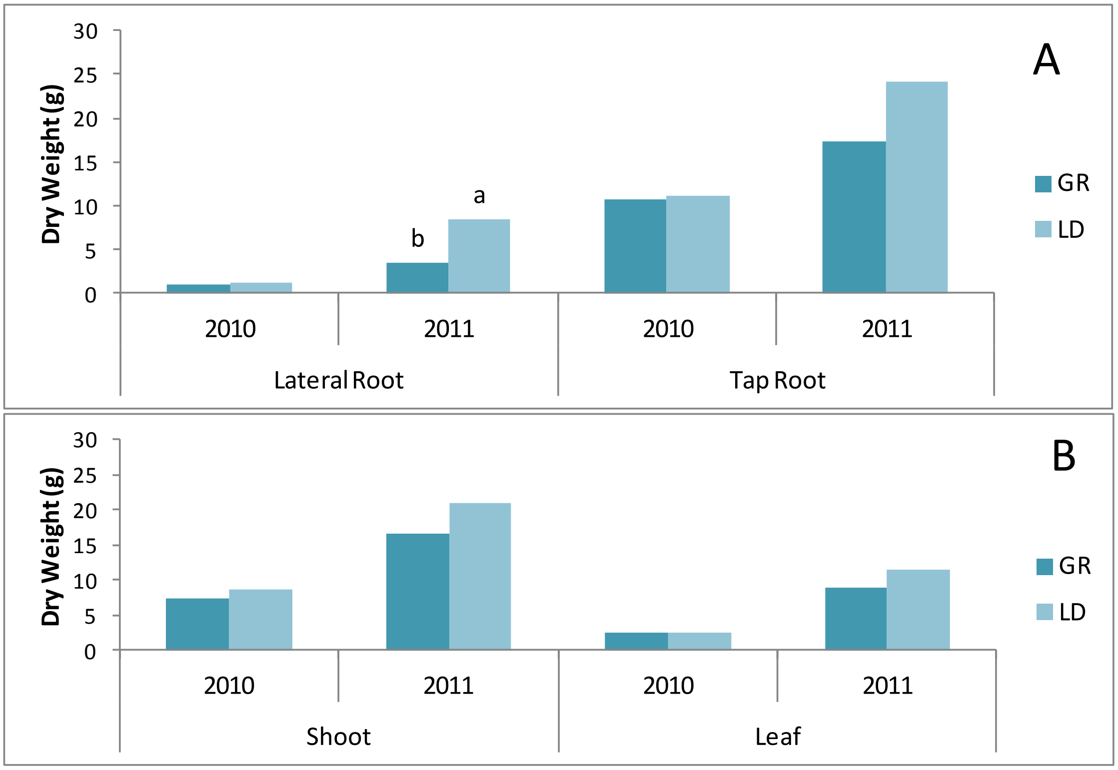


Figure 3.6 A-B. Lateral and tap root, shoot, and leaf dry weight means of excavated seedlings from the graded (GR) and loosely dumped (LD) treatments. Species having different letters within each treatment are significant at α=0.05 where a=largest mean, etc.

3.5.2 Root Morphology and Architecture

Root morphology and architecture measurements were obtained from seedlings excavated in July of 2010 and September of 2011. Tap root length slightly increased between 2010 and 2011 in seedlings planted in the LD treatment, and was significantly greater in the LD compared to the GR treatment in 2011 (Table 3.5). Lateral root dry weights were significantly greater (α=0.05) in the LD compared to the GR treatment in 2011 (Figure 3.6 A and Table 3.5). Leaf, shoot, and tap root dry weights had larger mean values in the LD treatment in 2010 and 2011, but were not significant (Tables 3.3A, 3.4, and 3.5).

Lateral and tap root dry weights of the four species over the two growing seasons are shown in Tables 3.6 and B.6. Lateral root dry weights for Shumard oak showed the greatest increase between 2010 and 2011 compared to the other species.

The average lateral root dry weight for the Shumard oak seedlings significantly increased from 0.76 - 6.55 g between the two growing seasons which equates to a 5.79 g increase. The other species significantly increased in lateral root dry weight by 4.45 g for American chestnut, 5.04 g for red oak, and 2.51 g for white oak (Table 3.6). In July 2010, excavated seedlings increased in lateral root dry weight compared to the corresponding nursery seedling measurements representative of the seedlings at the time of planting (May 5, 2010) (Table 3.7). American chestnut and white oak seedlings planted in the LD treatment significantly increased in lateral and tap root dry weight compared to the corresponding nursery seedlings over the 2.5 month period (May 2010 to July 2010). All species planted in the LD treatment, as well as most species in the GR treatment, significantly increased in lateral and tap root dry weight between May 2010 (nursery subset) and September 2011 (Table 3.7).

Further details on lateral root morphology and architecture of the excavated seedlings are presented in the Root Architecture section below. Interpretation of the tap root data (Tables 3.5, 3.7, and B. 6; Figure 3.6A) was confounded by the trimming of the tap root to a length of ~ 20 cm by the planting crew (see Confounding Factors, section 3.5.7). In 2011, tap root length was 6 cm larger in the LD treatment compared to the GR treatment (Table 3.5). Tap root dry weights averaged across both treatments increased by 10 g in the second year.

Of all the seedling growth parameters measured in this study, root morphology was perhaps the most sensitive to the soil replacement methods. Consistent with other laboratory and growth chamber compaction studies, the traditional GR soil replacement treatment resulted in decreased root volume compared to the LD treatment due to the associated high soil bulk density (Conlin & van den Driessche 1996;Simmons & Pope 1987). Simmons & Pope (1987) reported significant decreases in root biomass, total root length, and fibrosity occurred for sweetgum seedlings with each incremental increase in bulk density (1.25 to 1.40 g cm^{-3}, and 1.40 to 1.55 g cm^{-3}. In the present study, bulk density averaged 1.54 g cm^{-3} for cores obtained from the LD treatment which corresponded to an average lateral root dry weight increase of 7.2 g between the two growing seasons. In contrast, bulk density averaged 1.74 g cm^{-3} for cores obtained from the GR treatment, and lateral root dry weight only increased by 2.5 g from 2010 to 2011 (Tables 2.4 and 3.5).

Studies have also shown similar relationships between root biomass and depth in highly compacted soils (Conlin & van den Driessche 1996;Rokich et al. 2001). In a growth chamber study by Conlin & van den Driessche (1996), root weight in the 10-20 cm depth section increased with increasing compaction, while the 30-40 cm section decreased with increasing soil compaction. However, overall root biomass decreased with increasing soil compaction. Rokich et al. (2001) found that root mass significantly declined with depths > 20 cm after one year on standard graded soil reclamation sites. In some areas, root development was completely inhibited as a result of bulk density values exceeding 1.7 g cm^{-3}. Negative root responses were observed in the present study in seedlings measured from the GR treatment. This result and the root architecture results presented in section 3.5.3 indicate that the method of soil replacement directly influences root morphology. It is likely that bulk density was the main root-restricting factor as the GR treatment in the present study exceeded the bulk density threshold (1.7 g cm^{-3}) reported by Rokich et al. (2001).

Table 3.5. Comparison of lateral and tap root dry weights, as well as tap root length over two growing seasons (2010 and 2011) for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Treatments having different letters are significant at α=0.05 where a=largest mean and b=lesser mean. Changes between 2010 and 2011 significant at α=0.05 are indicated with *.

	2010		2011	Mean	2010		2011	Mean	2010		2011	Mean
	Lateral Root Dry Weight (g)				Tap Root Dry Weight (g)				Tap Root Length (cm)			
GR	0.92	*	3.41 b	1.77 b	10.66		17.45	13.64 b	19		20 b	19 b
LD	1.18	*	8.37 a	3.14 a	11.24		24.10	16.46 a	19	*	26 a	22 a
Mean	1.04	*	5.35		10.95	*	20.51		19	*	23	

Table 3.6. Comparison of lateral root dry weights of excavated American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) over two growing seasons (2010 and 2011), for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Species having different letters are significant at α=0.05 where a=largest mean, etc. Changes between 2010 and 2011 significant at α=0.05 are indicated with *.

	Treatment		Year	
Species	GR	LD	2010	2011
	Lateral Root Dry Weight (g)			
AMC	2.23	3.58	1.38 *	5.83
REO	2.43	3.25	1.25 *	6.29
SHO	1.58	3.15	0.76 *	6.55
WHO	1.41	2.66	0.89 *	3.40

Table 3.7. Lateral and tap root dry weights comparison of pre-planted nursery seedlings and excavated American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Changes between pre-planted measurements and field measurements significant at α=0.05 are indicated with *.

Lateral Root Dry Weight (g)					
		AMC	REO	SHO	WHO
May 2010	Pre-planting	0.84	1.06	0.52	0.40
July 2010	GR	1.20	1.32	0.84	0.66 *
	LD	1.74 *	1.32	0.82	1.34 *
Sept 2011	GR	4.44 *	4.78 *	3.82 *	2.07 *
	LD	7.98 *	13.35 *	13.74 *	6.27 *

Tap Root Dry Weight (g)					
		AMC	REO	SHO	WHO
May 2010	Pre-planting	6.27	13.22	13.31	7.17
July 2010	GR	8.62	14.37	12.51	8.68
	LD	9.89 *	13.48	14.32	10.07 *
Sept 2011	GR	15.62 *	21.12 *	19.58	16.80 *
	LD	15.70 *	28.05 *	36.57 *	25.80 *

3.5.3 Root Architecture

Images of lateral root systems taken from seedlings excavated in July 2010 and September 2011 were analyzed using the WinRHIZO root image analysis software (Figure 3.7). The image analysis software provided a measure of lateral root volume, surface area, and projected root area across ten diameter classes from 0 to > 4.5 mm in 0.5 mm increments. The tap root was not included in this analysis.

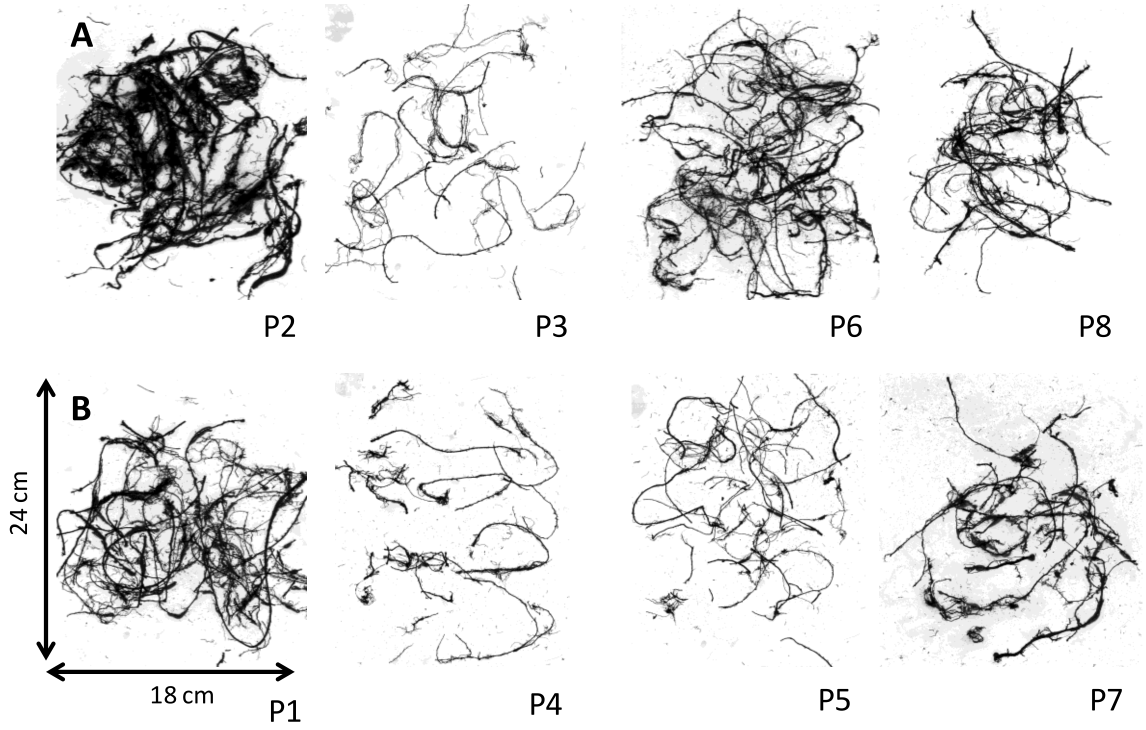


Figure 3.7. Lateral root images (area: 24 x 18 cm) of excavated White oak seedlings obtained in July 2010 from the loosely dumped plots (Row A) and the graded treatment plots (Row B) analyzed by WinRHIZO for root architecture parameters including projected root area, volume, and number of tips.

3.5.3.1 Volume

WinRHIZO estimated lateral root volume on the excavated seedlings in July 2010 and September 2011. In addition, the water displacement method was used to estimate lateral root volume on selected nursery seedlings used for baseline estimates in May

2010 and on the excavated seedlings in July 2010 (Table 3.8) (Bohm 1979b). Red oak seedlings had significantly greater lateral root volume compared to white oak seedlings in May and July of 2010, while American chestnut and Shumard oak were not significantly different from the other species. Correlations were made between the two methods used to estimate root volume and are shown in Table 3.9. The estimated values obtained using the two methods were highly correlated for all species except Shumard oak. The r^2 values ranged from 0.72 to 0.94 for the three hardwood species excluding Shumard oak. These highly correlated values demonstrate that the WinRHIZO root analysis method provides an accurate assessment of below ground growth. A similar experiment studying the correlation between the water displacement and WinRHIZO root scanning methods for measuring root volume on Bermuda grass found a highly significant linear regression relationship in two trials where r^2 was > 0.996 and $P < 0.0001$ (Pang et al. 2011).

The results obtained in the present study for Shumard oak are consistent with the findings of the Pang et al. (2011) study which showed that samples with very small volumes (<0.1 cm^3) had larger errors in the water displacement method compared to the root scanning method. Shumard oak seedlings had the least lateral root dry weight in 2010, averaging 0.76 g, compared to other species. It is likely that the error in measurement increased for Shumard oak seedlings using the water displacement method, which may explain why the correlation between water displacement and WinRHIZO was not significant.

Based on the strong correlations shown in Table 3.9, lateral root volume was solely measured using WinRHIZO for the excavated seedlings in September 2011. Comparisons in root volumes across the ten diameter classes measured by WinRHIZO for the July 2010 and September 2011 excavations are shown in Tables 3.10 and B.7 and Figure 3.8A-B. In 2011, lateral root volumes ranged from 0.06 to 1.53 cm^3 for the GR treatment and 0.07 to 5.49 cm^3 for the LD treatment when looking across all diameter classes. Lateral root volume increased between 2010 and 2011 for all classes of roots > 2 mm in diameter (Tables 3.10 and B.7). In 2011, lateral root volume was significantly greater in the LD treatment for all diameter classes > 3 mm in diameter (Figure 3.8 B). No treatment differences were observed in 2010 reflecting the fact that the measurements were obtained only three months after planting (Figure 3.8 A). However, trends in root volume were already developing which favored the LD treatment. Lateral root volume significantly increased in the LD treatment between 2010 and 2011 in diameter classes > 3.0 mm (Table 3.10 and B.7). There were no significant volume differences in the diameter classes observed among species (Table B.7).

These results provide greater detail with the respect to root architecture and are consistent with the root morphology measurements presented in section 3.5.2. The volume displacement and WinRHIZO analyses showed highly correlated values which demonstrated that the WinRHIZO root analysis method provides an accurate assessment of below ground growth. Given soil chemical properties were similar between the two treatments, it is likely that root volume, especially in diameter classes > 3.0 mm, was significantly influenced by the soil physical properties related to the soil replacement method. In highly compact soils, roots must generate a force greater than the frictional resistance of soil in order to develop (Gebauer et al. 2011;Gregory 2006). In order to do so, roots respond to compaction by decreasing root elongation and increasing the root diameter near the root tip (Gregory 2006). In the present study, root volume significantly decreased in the GR treatment compared to the LD treatment for diameter classes > 3.0 mm. Despite the lack of diameter data in the present study, seedlings in the GR treatment likely had less elongation based upon root volume data. When trees cease to elongate their roots, their conductive pathway between roots and leaves becomes shortened. Thus, a more favorable water potential gradient is achieved and the trees are defensively set up to survive water stress (Gebauer et al. 2011). However, the study by Gebauer et al. (2011) also noted the importance in studying beyond the morphological parameters of roots. The authors suggested assessing the histology (study of tissue) of root cell walls in order to fully evaluate root functions.

Table 3.8. Lateral root volumes measured by water displacement of selected nursery seedlings sampled in May 2010 (100 seedlings) and excavated seedlings obtained in July 2010 (160 seedlings). Species having different letters are significant at α=0.05 where a=largest mean, etc. No significant changes between dates were observed.

	Lateral Root Volume (cm^3)	
	May 2010	July 2010
American Chestnut	3.4 ab	4.7 ab
Red Oak	3.8 a	5.2 a
Shumard Oak	2.8 ab	4.9 ab
White Oak	2.1 b	3.2 b

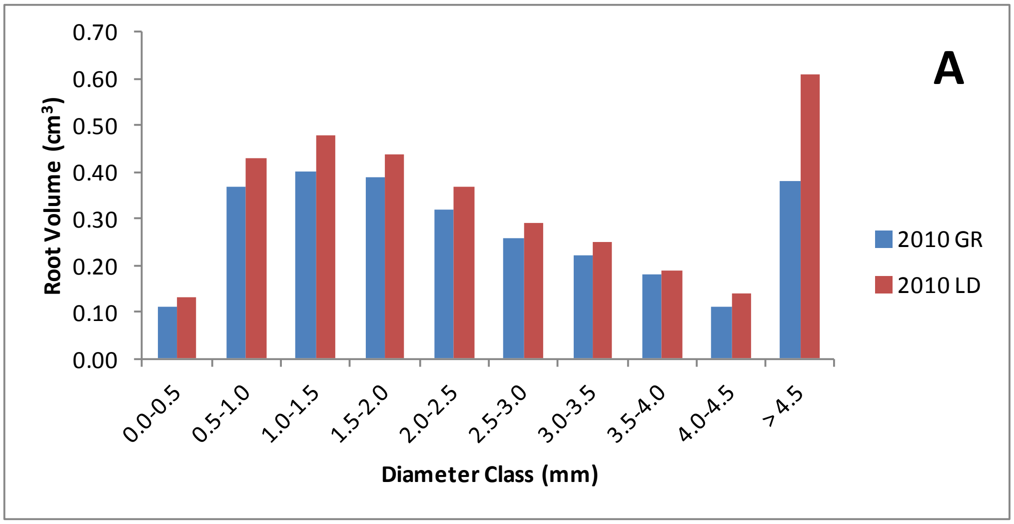
Table 3.9. Correlations (r^2) of the two methods used to measure lateral root volume: water displacement and WinRHIZO on the excavated seedlings obtained in July 2010 (160 seedlings). Species having significant correlations at α=0.05 are indicated with *.

Species	Water Displacement and WinRHIZO Correlation (r^2)
American Chestnut	0.94 *
Red Oak	0.72 *
Shumard Oak	0.16
White Oak	0.87 *

Table 3.10. Comparison of lateral root volume across diameter classes over two years (2010 and 2011), for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Treatments having different letters are significant at α=0.05 where a=largest mean and b=lesser mean. Root volume changes between 2010 and 2011 significant at α=0.05 are indicated with *.

Diameter Class (mm)	0-0.5			0.5-1.0			1.0-1.5			1.5-2.0			2.0-2.5		
Treatment	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
GR	0.11	0.06	0.09	0.37	0.40	0.38	0.40	0.49	0.45	0.39	0.44	0.42	0.32	0.48	0.40
LD	0.13	0.07	0.10	0.43	0.48	0.40	0.48	0.54	0.51	0.44	0.61	0.52	0.37	0.73	0.54
Mean	0.12 *	0.07		0.40	0.39		0.44	0.52		0.41	0.52		0.35 *	0.60	

Diameter Class (mm)	2.5-3.0			3.0-3.5			3.5-4.0			4.0-4.5			>4.5		
Treatment	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
GR	0.26	0.45	0.35	0.22	0.32 b	0.27 b	0.18	0.32 b	0.25 b	0.11	0.28 b	0.19 b	0.38 *	1.53 b	0.86 b
LD	0.29	0.68	0.47	0.25 *	0.81 a	0.49 a	0.19 *	0.87 a	0.47 a	0.14 *	0.78 a	0.40 a	0.61 *	5.49 a	2.44 a
Mean	0.28 *	0.56		0.23 *	0.53		0.19 *	0.56		0.13 *	0.50		0.49 *	3.20	



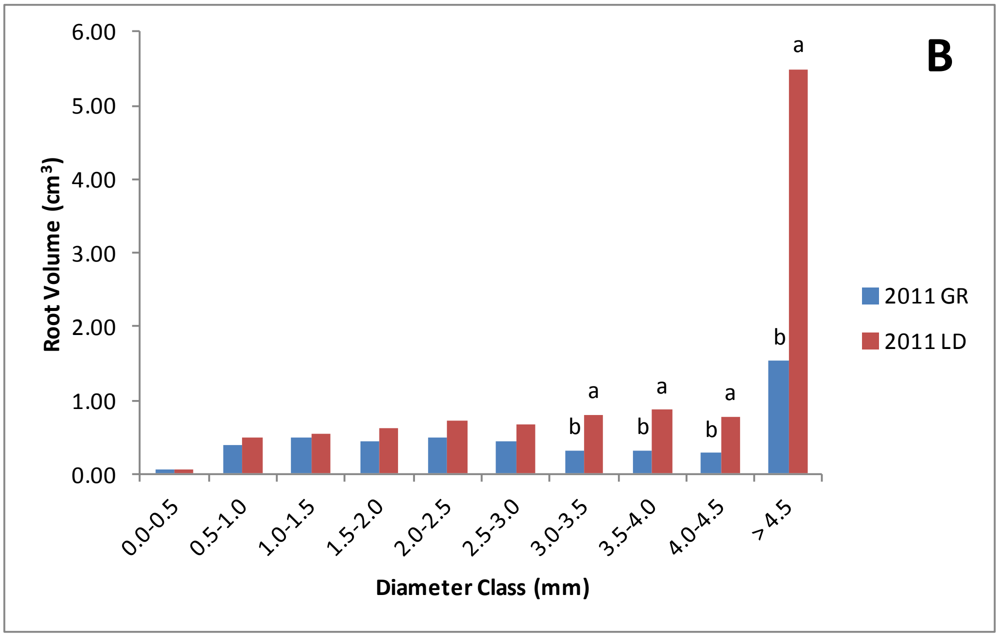


Figure 3.8. A-B. Comparison of lateral root volume across diameter classes for the graded (GR) and loosely dumped (LD) treatments in 2010 (A) and 2011 (B). Treatments having different letters are significant at α=0.05 where a=largest mean and b=lesser mean.

3.5.3.2 Projected Root Area

Estimated projected area (PA) of the lateral roots varies slightly from surface area estimates since PA is a 2-D measurement based on pixel area occupied by the root. In contrast, surface area was calculated from projected area values assuming a circle cross section of roots. PA was measured on the excavated seedlings in 2010 and 2011 (Tables 3.11 and B.9). Lateral root PA increased between 2010 and 2011 for all classes of roots > 2 mm in diameter (Table 3.11). In addition, PA increased in the LD treatment in 2011 for all classes of roots > 3 mm in diameter. There were no significant PA differences in the diameter classes observed among species (Table B.8). Projected area, similar to root volume, was significantly influenced by the soil physical properties related to the soil replacement method. A study by Moffat & Bending (2000) compared soil replacement applications on mine land. When comparing ripped topsoil to the loosely tipped soil replacement method (see section 1.3 for details on methods), the rooting area of the trees in the loosely tipped soil replacement treatment doubled that of the ripped treatment. This was due, in part, to the fact that the loosely tipped ground had lower bulk density values at all depths compared to the ripped, or plowed, ground. The loosely tipped treatment was similar to this present study's LD treatment.

Table 3.11. Comparison of projected root area across diameter classes over two years (2010 and 2011), for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Treatments having different letters are significant at α=0.05 where a=largest mean and b=lesser mean. Projected root area changes between 2010 and 2011 significant at α=0.05 are indicated with *.

Diameter Class (mm)	0-0.5			0.5-1.0			1.0-1.5			1.5-2.0			2.0-2.5		
Treatment	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
GR	5.40	2.65	3.90	6.54	7.13	6.83	4.24	5.12	4.67	2.90	3.26	3.08	1.86	2.75	2.28
LD	6.03	3.18	4.49	7.68	6.73	7.20	5.04	5.59	5.31	3.26	3.23	3.82	2.13	4.17	3.07
Mean	5.71 *	2.91		7.10	6.93		4.63	5.35		3.06	3.84		1.99 *	3.42	

Diameter Class (mm)	2.5-3.0			3.0-3.5			3.5-4.0			4.0-4.5			>4.5		
Treatment	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
GR	1.20	2.09	1.62	0.86 *	1.26 b	1.05 b	0.61 *	1.09 b	0.84 b	0.33 *	0.84 b	0.55 b	0.82 *	3.14 b	1.79 b
LD	1.36	3.19	2.18	0.97 *	3.15 a	1.91 a	0.66 *	2.94 a	1.59 a	0.43 *	2.33 a	1.19 a	1.09 *	10.97 a	4.74 a
Mean	1.28 *	2.61		0.91 *	2.10		0.64 *	1.90		0.38 *	1.49		0.95 *	6.46	

3.5.3.3 Lateral Root Tips

In addition to volume and PA, WinRHIZO estimated the number of lateral root tips. Lateral root tips increased between 2010 and 2011 in the diameter classes 3.5-4.0 mm and 4.5-5.0 mm (Table 3.12). In contrast, lateral root tips decreased for the 0.0-0.5 mm diameter class between the two growing seasons. Root tips are important as one of their key roles is to facilitate penetration into the soil (Gregory 2006). Roots will change direction and growth rate depending upon gravity, light, nutrients, and water (Zeleznik et al. 2006;Gregory 2006). In a study looking at ozone fumigation on roots, the number of root tips was significantly affected by light and ozone fumigation (Zeleznik et al. 2006). In unfavorable light conditions and ozone levels, the number of root tips significantly decreased, especially in the diameter classes 0-1 and 1-2 mm. The present study found that majority of all root tips were found in the diameter classes that ranged from 0-1.5 mm. However, only the 0-0.5 mm class was negatively affected with time. This diameter class is the most likely to regenerate with time as previous studies have reported that fine root systems are able to turnover their entire mass in less than one year (Zeleznik et al. 2006).

In the 3.0-3.5 lateral root diameter class, the number of tips were significantly different among species as they averaged 1.7 tips for red oak, 1.5 tips for American chestnut, 1.3 tips for Shumard oak, and 1.2 tips for white oak (Figure 3.9 and Table B.9). This trend was also observed in estimated tip counts of diameter classes 1.5-2.0, 2.5-3.0, and 3.5-4.0 mm (Table B.9). The projected root area showed similar rank in species for several upper diameter classes as well. In April 2011, seedling field height also showed the same rank in species. Whether these were significantly correlated to each other are shown in the Regression section 3.5.4.

Table 3.12. Comparison of root tip counts across diameter classes over two years (2010 and 2011), for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Root tip changes between 2010 and 2011 significant at α=0.05 are indicated with *.

Diameter Class (mm)	0-0.5			0.5-1.0			1.0-1.5			1.5-2.0			2.0-2.5		
Treatment	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
GR	1617.7	300.0	696.7	42.3	43.5	42.9	10.1	8.9	9.5	4.4	3.5	3.9	2.2	2.2	2.2
LD	1720.8	409.3	839.3	38.9	45.2	41.9	9.7	8.1	8.9	4.2	3.7	3.9	2.2	2.6	2.4
Mean	1668.4	* 350.5		40.5	44.3		9.9	8.5		4.3	3.6		2.2	2.4	

Diameter Class (mm)	2.5-3.0			3.0-3.5			3.5-4.0			4.0-4.5			> 4.5		
Treatment	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
GR	1.7	1.5	1.6	1.4	1.4	1.4	1.1	1.3	1.2	1.1	1.1	1.1	1.2	1.5	1.3
LD	1.7	1.9	1.8	1.3	1.6	1.4	1.2	1.4	1.3	1.2	1.3	1.2	1.2	1.9	1.5
Mean	1.7	1.7		1.3	1.5		1.2 *	1.4		1.1	1.2		1.2 *	1.7	

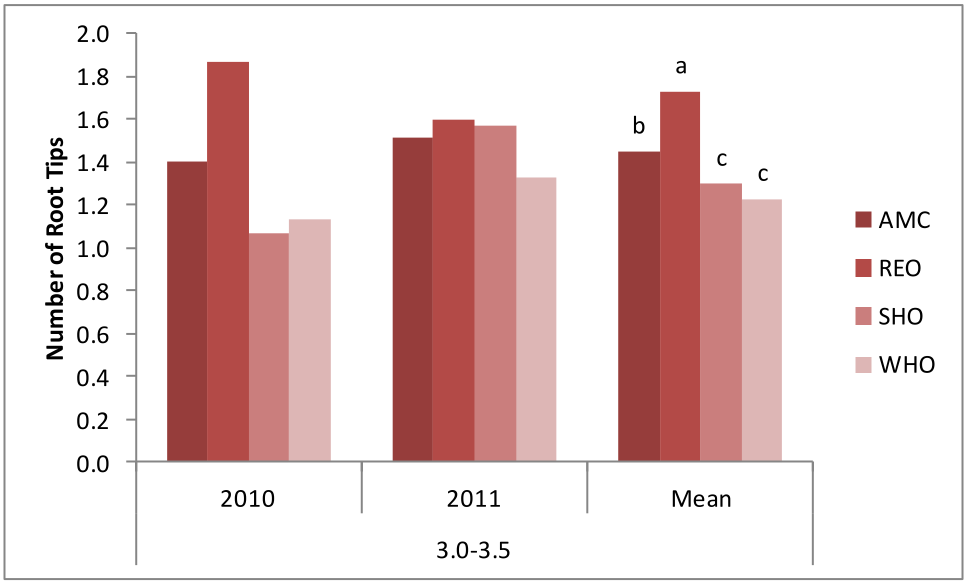


Figure 3.9. Comparison of the number of lateral root tips in the 3.0-3.5 mm diameter class over two years (2010 and 2011), for American chestnut (AMC), red oak (REO), shumard oak (SHO), and white oak (WHO). Species having different letters are significant at α=0.05 where a=largest mean, etc.

3.5.4 Seedling Growth Relationship

All root and shoot measurements obtained from 96 excavated red oak, Shumard oak, white oak, and American chestnut seedlings in September 2011 were run using Pearson correlations. Pearson correlation analyzes the relationship between two variables and provides information about how well the linear model fits the relationship between the two variables. Variables tested in this study were from individual seedlings, i.e., the root volume and shoot dry weight of an individual seedling from a treatment plot, with n=96. Tables 3.13 – 3.16 show the individual seedling relationships (for 96 seedlings) between height, RCD, shoot dry weight (DW), leaf DW, and lateral root DW to corresponding seedling WinRHIZO measurements including number of tips and volume for the ten individual diameter classes. In general, red oak and American chestnut seedlings showed the strongest relationship between the traditional above ground growth parameters (height, RCD) and the WinRHIZO below ground root morphology measurements). White oak seedlings showed the weakest relationship.

All of the species excluding white oak showed a relationship between root volume and seedling height. However, the root volume diameter classes that correlated with height were different for each species. For American chestnut and red oak seedlings, the diameter classes >4.0 mm were more strongly correlated (α=0.01) to height than other diameter classes. Root volume in the 0-0.5 mm diameter class of red oak seedlings also showed a strong relationship to height. Red oak and American chestnut seedlings were the only species that showed a relationship between root volume and number of tips to RCD. However, the diameter classes where this relationship occurred were species dependent.

The majority of the WinRHIZO parameters in larger diameter classes (> 5.0 mm) correlated strongly to lateral root dry weight, even for White oak. As an example, the relationship of root volume in the 4.0-4.5 mm class to lateral root dry weight is shown in Figure 3.10. This figure demonstrates the sensitivity of WinRHIZO and how it compares to conventional morphological measurements such as biomass. Prior studies support the generalization that seedlings with greater root systems yield higher survival and above ground growth, yet few studies have quantified root parameters to determine how much root development is sufficient to support a high-functioning seedling (Thompson 1985). One of the main objectives of this current project was to understand the relationship between root growth (explained by soil properties) and above ground growth and survival. The current study has also shown the importance of the relationship between root morphology derived parameters from WinRHIZO to seedling height and root collar diameter. In a 10 year study measuring field survival and growth of white spruce, survival was not linearly related to initial height (Mullin and Svaton 1972). Instead, there was a maximum after which height increase no longer corresponded to increased survival. After the maximum was reached, seedling survival was likely more dependent upon the root system size and architecture, as well as drought conditions on site.

Table 3.13. Relationship of traditional growth parameters to WinRHIZO root morphology measurements (V: volume and T: root tips) in 10 diameter classes (Class 1: 0-0.5 mm, class 2: 0.5-1.0 mm, etc.) for Shumard oak seedlings. Bolded and bordered regions are significant at α=0.01.

		Height	RCD	Shoot DW	Leaf DW	Lat DW
T= Root Tips	TD1	0.41	-0.02	0.23	0.30	0.15
	TD2	0.24	0.01	0.11	0.05	0.03
	TD3	0.26	-0.22	0.24	0.05	-0.02
	TD4	0.27	0.24	0.30	0.21	0.19
	TD5	-0.01	-0.40	0.05	0.03	0.23
	TD6	0.09	0.04	-0.01	0.12	0.51
	TD7	0.30	-0.09	0.00	0.14	-0.10
	TD8	0.04	0.32	0.31	0.23	0.07
	TD9	0.25	0.30	0.33	0.33	0.33
	TD10	0.01	0.09	-0.08	0.07	**0.63**
V= Root Volume	VD1	0.46	0.19	0.31	0.33	0.02
	VD2	0.32	0.14	0.29	0.17	-0.15
	VD3	**0.61**	0.10	0.31	0.23	0.04
	VD4	**0.58**	-0.09	0.18	0.18	0.37
	VD5	0.48	-0.19	0.18	0.24	0.41
	VD6	**0.60**	0.05	0.49	0.52	**0.57**
	VD7	**0.63**	0.30	**0.71**	**0.70**	0.43
	VD8	0.45	0.23	**0.57**	**0.63**	**0.56**
	VD9	0.22	0.05	0.16	0.29	**0.65**
	VD10	0.18	0.32	0.17	0.32	**0.97**
	Height		0.19	**0.65**	**0.61**	0.31
	RCD	0.19		0.40	0.49	0.32
	Shoot DW	**0.65**	0.40		**0.89**	0.25
	Leaf DW	**0.61**	0.49	**0.89**		0.39
	Lat DW	0.31	0.32	0.25	0.39	

Table 3.14. Relationship of traditional growth parameters to WinRHIZO root morphology measurements (V: volume and T: root tips) in 10 diameter classes (Class 1: 0-0.5 mm, class 2: 0.5-1.0 mm, etc.) for white oak seedlings. Bolded and bordered regions are significant at α=0.01.

		Ht	RCD	Shoot DW	Leaf DW	Lat DW
T= Root Tips	TD1	0.15	0.52	0.33	0.14	**0.64**
	TD2	0.18	0.53	0.40	0.29	0.42
	TD3	0.28	0.30	0.30	0.26	0.36
	TD4	0.49	0.47	**0.64**	**0.60**	0.27
	TD5	0.37	0.35	0.31	0.11	0.53
	TD6	0.32	0.13	0.14	-0.09	**0.59**
	TD7	0.25	0.31	0.31	0.13	**0.70**
	TD8	-0.33	-0.18	-0.21	-0.22	-0.02
	TD9	0.09	0.15	0.05	0.06	0.01
	TD10	0.00	0.42	0.27	0.21	0.43
V= Root Volume	VD1	0.03	0.28	0.05	-0.05	0.52
	VD2	0.28	0.47	0.40	0.39	0.26
	VD3	0.42	0.49	0.55	0.35	**0.62**
	VD4	0.31	0.39	0.38	0.15	**0.78**
	VD5	0.35	0.46	0.46	0.22	**0.81**
	VD6	0.37	0.55	0.47	0.26	**0.73**
	VD7	0.09	0.24	0.22	0.01	**0.78**
	VD8	0.09	0.21	0.21	0.07	**0.72**
	VD9	-0.11	0.22	0.20	0.10	**0.79**
	VD10	0.17	0.53	0.43	0.31	**0.85**
	Height		0.53	**0.77**	**0.71**	0.15
	RCD	0.53		**0.80**	**0.74**	0.38
	Shoot DW	**0.77**	**0.80**		**0.94**	0.32
	Leaf DW	**0.71**	**0.74**	**0.94**		0.15
	Lat DW	0.15	0.38	0.32	0.15	

Table 3.15. Relationship of traditional growth parameters to WinRHIZO root morphology measurements (V: volume and T: root tips) in 10 diameter classes (Class 1: 0-0.5 mm, class 2: 0.5-1.0 mm, etc.) for red oak seedlings. Bolded and bordered regions are significant at α=0.01.

		Height	RCD	Shoot DW	Leaf DW	Lat DW
T= Root Tips	TD1	0.44	0.46	0.47	0.46	0.48
	TD2	0.29	0.33	0.34	0.33	0.41
	TD3	**0.62**	0.45	**0.61**	**0.58**	**0.57**
	TD4	0.32	0.44	**0.60**	**0.73**	**0.72**
	TD5	0.40	0.39	0.49	0.42	0.46
	TD6	-0.16	0.01	0.11	0.01	-0.01
	TD7	0.20	-0.10	-0.01	-0.01	0.06
	TD8	**0.60**	**0.67**	**0.67**	0.54	0.54
	TD9	0.54	0.47	**0.64**	**0.74**	**0.77**
	TD10	0.50	**0.60**	**0.67**	**0.67**	**0.71**
V= Root Volume	VD1	**0.57**	0.49	0.51	0.39	0.45
	VD2	0.45	0.38	0.36	0.26	0.29
	VD3	0.42	0.37	0.42	0.44	0.47
	VD4	0.42	0.39	0.46	0.45	0.52
	VD5	0.44	0.45	0.51	0.43	0.55
	VD6	0.52	0.41	**0.56**	0.54	**0.69**
	VD7	**0.62**	0.45	**0.68**	**0.69**	**0.80**
	VD8	**0.65**	**0.56**	**0.79**	**0.77**	**0.93**
	VD9	**0.59**	0.49	**0.78**	**0.87**	**0.91**
	VD10	0.52	0.54	**0.74**	**0.82**	**0.97**
	Height		**0.65**	**0.71**	**0.65**	**0.59**
	RCD	**0.65**		**0.74**	**0.61**	**0.58**
	Shoot DW	**0.71**	**0.74**		**0.84**	**0.79**
	Leaf DW	**0.65**	**0.61**	**0.84**		**0.84**
	Lat DW	**0.59**	**0.58**	**0.79**	**0.84**	

Table 3.16. Relationship of traditional growth parameters to WinRHIZO root morphology measurements (V: volume and T: root tips) in 10 diameter classes (Class 1: 0-0.5 mm, class 2: 0.5-1.0 mm, etc.) for American chestnut seedlings. Bolded and bordered regions are significant at α=0.01.

		Height	RCD	Shoot DW	Leaf DW	Lat DW
T= Root Tips	TD1	0.39	0.4	0.36	0.32	**0.67**
	TD2	**0.54**	0.49	0.47	0.41	**0.76**
	TD3	0.22	**0.71**	**0.54**	0.45	0.33
	TD4	**0.61**	**0.65**	**0.53**	0.46	**0.61**
	TD5	0.12	0.11	0.13	0.22	0.24
	TD6	0.18	0.42	0.15	0.25	0.25
	TD7	0.26	0.09	0.11	0.02	0.37
	TD8	0.14	0.31	0.18	0.33	0.17
	TD9	0.32	0.36	0.4	0.22	0.42
	TD10	0.44	**0.6**	**0.65**	0.51	**0.73**
V= Root Volume	VD1	0.44	0.34	0.32	0.21	**0.65**
	VD2	0.44	0.5	0.38	0.29	**0.59**
	VD3	0.48	**0.62**	**0.61**	0.5	**0.61**
	VD4	**0.55**	0.39	0.42	0.32	**0.6**
	VD5	0.49	0.37	0.37	0.33	**0.7**
	VD6	0.42	0.4	0.29	0.28	**0.64**
	VD7	**0.53**	0.48	0.44	0.38	**0.85**
	VD8	**0.59**	0.5	**0.58**	0.5	**0.91**
	VD9	**0.63**	**0.62**	**0.75**	**0.69**	**0.98**
	VD10	**0.52**	**0.7**	**0.78**	**0.75**	**0.91**
	Height		**0.54**	**0.66**	0.49	**0.61**
	RCD	**0.54**		**0.85**	**0.8**	**0.68**
	Shoot DW	**0.66**	**0.85**		**0.91**	**0.74**
	Leaf DW	0.49	**0.8**	**0.91**		**0.71**
	Lat DW	**0.61**	**0.68**	**0.74**	**0.71**	

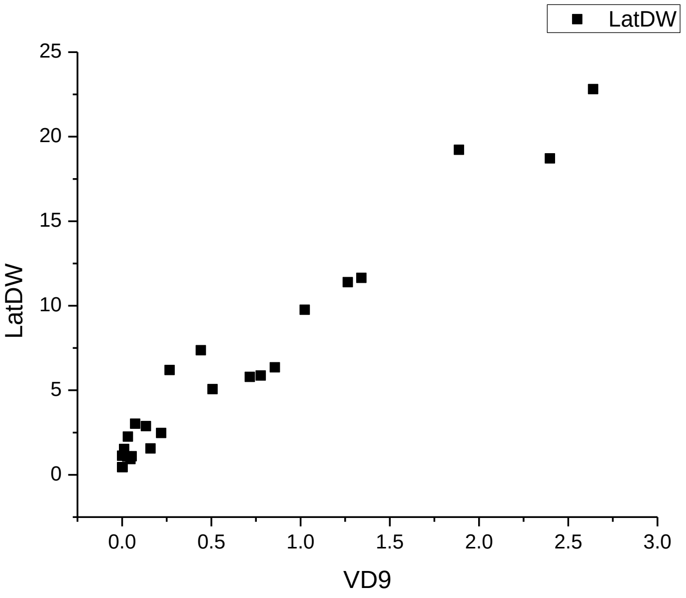


Figure 3.10. A plot of 24 American chestnut seedlings showing the relationship of root volume in the 4.0-4.5 diameter class to lateral root dry weight, n=96.

3.5.5 Plant Moisture Stress

Pre-dawn plant water potentials (ψ) were measured on the four species for the two soil replacement treatments during droughty conditions in 2010 and 2011 (Tables 3.17 and 3.18). The microclimate of the site around the time of measurement is further explained in the Materials and Methods section (3.3.8). Seedlings planted in the LD treatment had significantly higher ψ than those planted in the GR treatment (Table 3.17 and Figure B.2A). The ψ averaged -19.5 bars for the GR and -16.4 bars for the LD treatment. Comparing treatment means, the lowest ψ were observed in 2010. Despite droughty conditions, water-stressed American chestnut seedlings maintained significantly higher ψ than the oak species (Table 3.18 and Figure B.2B). Averaging 2010 and 2011 data, the ψ for American chestnut seedlings averaged -12.7 bars, while the oak seedlings ranged from -16.8 bars (Shumard oak) to -22.8 bars (white oak). In a study comparing water treatments on 3 Mediterranean oak species, pre-dawn ψ differences were observed among species (Siam et al. 2009). This study found that in mid-June, when drought conditions were beginning to progress (the month of June received < 15 mm rainfall); water-stressed *Q. ithaburensis* seedlings had significantly higher pre-dawn ψ than *Q. pubescens* and *Q. frainetto* seedlings. The pre-dawn ψ were -4 bars for *Q. ithaburensis*, -15 bars for *Q. pubescens*, and -10 bars for *Q. frainetto.*

In the present study, pre-dawn water potentials were affected by the soil replacement treatments. Seedlings planted in the LD treatment were less water stressed than those in the GR treatment during the drought conditions. This may be explained, in part, by the soil moisture conditions of the two treatments. The LD treatment had a greater water holding capacity at field capacity (-0.1 bar) and higher total porosity compared to the GR treatment.

Table 3.17. Comparison of pre-dawn ψ (bars) over two growing seasons (2010 and 2011) for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Treatments having different letters are significant at α=0.05 where a=largest mean and b=lesser mean.

Treatment	2010	2011	Mean	
	Pre-dawn ψ (bars)			
GR	-19.9	-19.0	-19.5	b
LD	-17.3	-15.6	-16.4	a
Mean	-18.6	-17.3		

Table 3.18. Comparison of pre-dawn ψ (bars) of American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) over two growing seasons (2010 and 2011), and for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Species having different letters are significant at α=0.05 where a=largest mean, etc.

	Treatment		Year			
Species	GR	LD	2010	2011	Species Mean	
			Pre-dawn ψ (bars)			
AMC	-14.2	-11.1	-12.0	-13.4	-12.7	a
REO	-20.6	-18.4	-21.0	-18.1	-19.5	bc
SHO	-18.5	-15.1	-16.4	-17.2	-16.8	b
WHO	-24.5	-21.1	-25.0	-20.6	-22.8	c

3.5.6 Plant Nutrition

Plant tissue was analyzed for macro- and micro-nutrients among seedlings in the selected baseline nursery group, as well as for the 96 excavated seedlings at the end of the second growing season. The analyzed nutrients included N, P, K, Ca, Mg, Mn, Fe, Cu, B, Al, and Zn. The nursery group is represented in Tables 3.19 and B.10 by the 2010 measurements and includes shoot and root tissues. The excavated seedlings are indicated by the 2011 measurements and include shoot, leaf, and lateral root tissues. For every nutrient, at least one plant tissue type (shoot, root, etc.) for either the 2010 or 2011 group was found to vary significantly by species (Tables 3.19 and B.10). The most major differences were observed in percent total N for the 2010 measurements. American chestnut and red oak had significantly greater % total N in root tissue (Table 3.19). In the excavated group (2011), K levels were larger for the white oak species for shoot, leaf, and lateral root tissues compared to the other species (Table 3.19). Results from the nursery subset were heavily influenced by fertilizer treatments provided through the nursery. A general trend of reduced nutrient concentrations was observed in plant tissues from 2010 to 2011. This was likely due to the low nutrient status of the replacement soil. Plant tissues are often examined to asses forest soil nutrition (Binkley 1986). They also are used to assess physiological properties such as photosynthesis as studies have confirmed the relationship between leaf % N and net photosynthesis. One study showed that photosynthesis reached a maximum at foliar concentrations of 1.7% N (Binkley 1986). In this current study, foliar % N ranged from 1.45 to 1.59%.

Treatment effects were observed in some cases. Table 3.20 shows percent total N was greater in seedling tissues, especially lateral root tissue, from the GR treatment; while P had slightly greater quantities in the LD seedlings. This may be due to the higher N available in the soil on the GR treatment. Other studies have shown conflicting findings as soil compaction caused a decrease in concentration of N and K in shoot, while P was not affected (Gregory 2006). Table 3.20 shows Ca concentration in the leaf and lateral root tissues was greater in the LD seedlings. Table B.11 show the remaining treatment comparisons among nutrients. There was slight favoritism for the GR treatment with respect to Fe and Zn. Soil chemical results showed slightly greater N levels in the soil of the GR treatment, which may explain the elevated N levels observed in all tissues of the GR treatment. Soil nutrition is often a good indicator of plant nutrition (Binkley 1986); however, it is a limited predictor of seedling performance when water availability is low (Landis et al. 2005).

Table 3.19. N, P, and K nutrient levels in plant tissue in the nursery subset (2010) and the excavated seedlings (2011) for American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO).

	% Total N					P (percent)				
Species	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011
AMC	0.86 a	0.55	1.39 a	1.45	0.65	0.10 ab	0.05	0.17 a	0.13	0.10 a
REO	0.87 a	0.51	1.34 a	1.59	0.61	0.08 b	0.05	0.16 a	0.11	0.08 b
SHO	0.70 b	0.52	0.87 b	1.56	0.56	0.07 b	0.05	0.11 b	0.11	0.06 c
WHO	0.84 a	0.54	0.99 b	1.50	0.54	0.12 a	0.05	0.17 a	0.10	0.06 bc

	K (percent)				
Species	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011
AMC	0.26	0.20 c	0.66 a	0.34 c	0.31 b
REO	0.24	0.22 bc	0.43 bc	0.49 b	0.29 b
SHO	0.28	0.24 b	0.31 c	0.55 a	0.33 b
WHO	0.23	0.29 a	0.54 ab	0.50 ab	0.42 a

Table 3.20. N, P, Ca, and S nutrient levels of the excavated seedlings (2011) for the graded (GR) and loosely dumped (LD) soil replacement treatments.

	% Total N				P (percent)		
Treatment	Shoot	Leaf	Lateral Root	Root	Shoot	Leaf	Lateral Root
GR	0.57 a	1.58 a	0.71 a	0.55 a	0.05	0.10 b	0.07
LD	0.49 b	1.47 b	0.46 b	0.47 b	0.05	0.12 a	0.08

	Ca (percent)			S (percent)		
Treatment	Shoot	Leaf	Lateral Root	Shoot	Leaf	Lateral Root
GR	0.75	1.00 b	0.42 b	0.04	0.13	0.05 a
LD	0.82	1.00 a	0.52 a	0.04	0.12	0.04 b

3.5.7 Confounding Factors

There were several anthropogenic factors, as well as uncontrolled natural factors, that influenced the results in this study. The anthropogenic factors were due in part to inadvertent mistakes by the mining company personnel and the tree planting crew. These decisions led to both direct and indirect influences on seedling growth and survival. The planting crew was accustomed to severing tap roots at 20 cm length in order to alleviate planting difficulties. However, on a study where one of the main objectives is to relate root development to soil replacement treatments, severing the root system hinders the potential for normal root development. This factor therefore warrants reader discretion when interpreting seedling survival and growth results.

In addition to severing the tap roots, the planting crew used hoedad tools to plant the seedlings. In general, hoedad tools are used to plant pine seedlings or those characterized by smaller root systems. In contrast, spades or augers are used to plant seedlings with longer tap roots, such as hardwoods. Ideally, the planters would have used a tool designed to match the size and shape of the seedlings being planted, instead of vice-versa, in which the planters altered the size and shape of the seedlings to match the planting tool. A large portion of seedlings were not planted properly in the GR treatment. Air pockets were observed near the root collar diameter, which allowed the root system to desiccate, killing 92+ seedlings in the GR treatment (Table B.12). An additional 133 seedlings in the GR treatment were damaged due to the improper planting technique.

The vegetation at the site, especially on the GR treatment, was thick and reached >2 m in height. The species of the grass species was not identified; however, it was not in the original ground cover mix that was seeded prior to planting seedlings in May 2010. Despite vegetation control that took place in May 2011, the vegetation competed heavily against the seedlings for available nutrients, water, and sunlight. Seedlings in the GR treatment experienced greater shade due to the towering vegetation in 2011; whereas those growing on the LD treatment generally had full to moderate sunlight.

In the LD treatment, ponds quickly formed at the base of the mounds during the rainy season in the summer. Due to the graded cast overburden and flat topography of the experimental site, a perched water table remained on the LD treatment for the duration of the study. In other words, the water in the ponds never infiltrated into the ground or was able to runoff the LD plots. A large percent of the mortality that occurred in the LD treatment was due to trees that were planted in a location that later filled up with water (the ponds had not filled at the time of planting). At least 92 seedlings died in the LD treatment due to lack of O_2 in the soil from being planted in a location that later filled with water (Table B.12). An additional 113 seedlings were damaged from being near the vicinity or directly in a pond. In placement of the trees, the ponds allowed several hydrophytic vegetative species, such as cattail, to survive on the LD plots. The ponds were also a source of habitat for tadpoles, frogs, and ducks.

In August 2011 which was towards the end of the second growing season, the deer fencing was knocked down by Peabody Energy personnel in three locations in order to mow plots 1 and 7. However, the rows that were mowed in these plots killed a moderate portion of the seedlings which contributed to the high mortality that occurred in the second year. In addition, erosion was not able to be monitored at the end of the second growing season due to the mowing, as well as rabbit browse on the stakes.

3.6 Conclusions

Above ground growth and survival of the four hardwood species observed in this study were comparable or slightly less than those reported in prior mine reclamation studies. Survival was significantly higher in the LD treatment compared to the GR treatment at the end of the second growing season. Survival rates of the four hardwood species averaged 10 percent higher on the LD treatment. In general, above ground growth parameters did not favor one soil replacement method over the other as few treatment effects were observed. A few exceptions to this generalization were observed in shoot biomass and plant water potential, which favored the LD treatment. Average plant biomass significantly increased and plant water potential significantly decreased (less water stressed) in the LD treatment. The similarities in above ground growth across treatments is attributed, in part, to the fact that this study measured seedlings over a 17 month time period and this time period may not have provided a sufficient amount of time to observe above ground growth sensitivity to treatments.

In contrast, the below-ground growth parameters indicate that the method of soil replacement influenced root morphology and architecture due to the difference in soil properties. Positive root responses including lateral and tap root dry weights, root volume and projected root area were observed in seedlings grown on the LD treatment. It is likely that bulk density was the main root-restricting factor as the GR treatment in the present study exceeded the bulk density threshold (1.7 g cm^{-3}) reported by Rokich et al. (2001).

Prior studies support the generalization that seedlings with greater root systems yield higher survival and above ground growth, yet few studies have quantified root parameters to determine how much root development is sufficient to support a high-functioning seedling (Thompson 1985). In the present study, American chestnut seedlings ranked highest in root and shoot parameters, as well as plant water potential; and yet this species ranked lowest in survival and had the greatest amount of dieback. In contrast, Shumard oak seedlings generally ranked second or third in root and shoot parameters; and yet had the highest survival rate and greatest increase in lateral root dry weight compared to all other species.

Seedlings excavated from the LD treatment in 2011 exhibited greater lateral root development compared to those from the GR treatment. Shumard oak seedlings in the LD treatment, for example, had a lateral root dry weight of 3.15 g compared to the GR treatment average of 1.58 g. As Kormanik (1986) found in a non-mining study, lateral root morphology may be the primary indicator of seedling quality. In order to conclude whether or not the selected species were suitable for either soil replacement treatment, the root and shoot parameters as well as the survival rates should be examined.

CHAPTER 4. FINAL CONCLUSION AND RECOMMENDATIONS

This project evaluated the plantation establishment success of American chestnut (*Castanea dentata*) and high quality oak (*Quercus* spp.) seedlings on a reclaimed mine site through application of new, cost effective reforestation technologies. The effects of the standard graded (GR) and loose dumping (LD) soil replacement strategies were assessed on a Peabody Energy mine site in southwestern Indiana. This study examined the relationship of soil physiochemical and biological properties to plant survival and growth. Quantitative assessment of seedling growth was accomplished by measurement of above-ground (root collar diameter, height, leaf dry weight) and below-ground (root biomass, volume, projected area) parameters. In addition, changes in root architecture were evaluated as a means of studying the impact of the two soil replacement methods. Short term (2 years) effects of the two soil replacement strategies were shown to influence seedling survival and growth.

In this study, three null hypotheses (assertions that could be proven false) were proposed. The first was that the method of soil replacement would have no effect on the properties of the rooting media (soil physiochemical and biological properties). This null hypothesis is clearly false as soil physical properties of the LD and GR soils showed significant treatment effects. Bulk density, moisture retention and porosity of the LD soil were favorable for plant growth. In contrast, the GR soil had a significantly higher bulk density (1.74 g/cm^3) compared to the LD soil (1.54 g/cm^3) resulting in root impairment. The moisture retention behavior of the LD and GR soils showed greater water holding capacity at field capacity (-0.1 bar) in the LD treatment in 2010. These findings (and those described more fully in Chapter 2) indicate that the method of soil replacement directly influences water holding capabilities and soil structure. Of all the soil physiochemical and biological properties measured, the difference in bulk density between the LD and GR treatments had the greatest impact on seedling growth and survival.

The amount of soil transported off-site from the LD or GR treatments was not measured; therefore, a quantitative measure of soil erosion could not be determined. However, off-site soil loss on the GR treatment may have been greater due to observation of large erosion channels in comparison to the LD treatment which primarily lost soil on the mounds bordering the perimeter of the plot. Contrary to the GR treatment, the LD treatment had significantly greater redistribution of soil from the upper to lower portions of the soil mounds within the plots due to settling and (on-site) erosion. An unintended consequence of the LD treatment was that the soil redistribution of fines (silt and clay particles) from the upper portion of the mounds to the valleys between mounds may have also contributed to the poor drainage and ponding conditions in the LD plots.

The replacement soil used for the LD and GR treatments had low fertility status, low organic carbon contents, and low cation exchange capacity (CEC) (detailed in Chapter 2), consistent with those of the original subsurface soil. Over the course of the two year study, soil chemical properties were not significantly different among treatments. Although the site was fertilized with 224 kg/ha 18-46-0 (N-P-K) in April of 2010 prior to planting in order to improve the fertility status of the soil, soil analysis after fertilization indicated that the fertility status of the soil was low which impacted the overall project. The lack of nutrients retained by the soil was attributed to the lack of soil organic matter and low CEC of replacement soil. This was also ultimately reflected in the low nutrient concentrations measured in seedling root and shoot components (Chapter 3). Competing vegetation was dense across both treatments and likely consumed a large fraction of the total nitrogen and phosphorus applied. A general finding across both treatments was that the concentration of soil nutrients was less than optimum for seedling growth. As a means of improving soil nutrient retention, incorporation of the organic-matter-rich surface horizon (A horizon) of the replacement soil would have been helpful.

The presence and activity of soil microorganisms measured through microbial biomass (MB) and fluorescein diacetate (FDA) hydrolysis were low, consistent with the chemical properties (low soil organic matter and low fertility status) of the replacement soil, and reflected poor soil quality. Soil microbial enzyme production levels were significantly greater in the pre-disturbed soil obtained from the pasture site compared to both treatments on the experimental site. Comparing treatments, soil in the GR treatment had slightly greater microbial populations and enzyme production. The denser grass vegetation and associated root system in the GR treatment may have, in part, stimulated microbial growth and activity.

The second null hypothesis was that any differences in soil physiochemical properties resulting from the two soil replacement methods would not impact growth and root architecture of the four hardwood species. Prior studies have shown that bulk density values > 1.6 to 1.7 g/cm^3 (Moffat & Bending 2000;Rokich et al. 2001) may cause root restriction. In 2010, the bulk density average in the LD soil (1.54 g cm^{-3}) replacement treatment fell below this mean, while the GR treatment average (1.74 g cm^{-3}) exceeded these reported thresholds. In fact, negative root responses were clearly observed in the present study in seedlings measured from the GR treatment (Chapter 3). After three months, trends in root volume were already developing which favored the LD treatment. In 2011, lateral root volume was significantly greater in the LD treatment for all root diameter classes > 3 mm. Root volume, especially in diameter classes > 3.0 mm, was most impacted by the treatment-induced difference in soil physical properties.

In addition to bulk density, soil water holding capacity is often a major growth limiting factor. The method of soil replacement influenced water holding capabilities and soil structure. The GR had lower water holding capacity at field capacity (-0.1 bar) and lower porosity than the LD soil especially during the first year which may have directly influenced pre-dawn plant water potential (ψ). Seedlings planted in the LD treatment (-16.4 bars) had significantly higher ψ than those planted in the GR treatment (-19.5 bars).

This study found that treatment-induced differences in soil physical properties (e.g., compaction of the GR soil) influenced overall root proliferation especially in regard to lateral root volume. Although prior studies support the generalization that seedlings with greater root systems yield higher survival and above ground growth, few studies have provided a quantitative assessment of root development/architecture (Thompson 1985). This study showed that root volume was highly/strongly correlated with above-ground growth parameters (e.g., leaf dry weight). In addition, a number of root morphology derived parameters were also strongly correlated with above-ground growth parameters of the seedlings.

Lastly, the third null hypothesis was that soil replacement treatments would not affect seedling survival and growth. At the end of the second growing season, seedling survival was significantly higher in the LD (53%) compared to the GR treatment (42%). Above ground growth of the four hardwood species did not favor one soil replacement method over the other as few treatment effects were observed. The similarities in above ground growth among treatments is attributed, in part, to the fact that this study measured seedlings over a 17 month time period and this time period may not have provided a sufficient amount of time to observe above ground growth sensitivity to treatments. Although the ‘traditional’ above-ground measurements did not show treatment effects, changes were observed in root growth and morphology. Positive root responses including lateral and tap root dry weights, root volume and projected root area were observed in seedlings grown on the LD treatment.

Overall, the LD treatment was a favorable alternative for reforestation in comparison to the GR treatment. This was attributed to the lower bulk density of the LD soil as it was able to provide a more open soil structure favorable for root growth. The conventional GR treatment, on the other hand, faced a number of negative factors including soil compaction, limited water availability, and lower total porosity. There were several factors (both anthropogenic and natural) that both treatment faced (detailed in Confounding Factors section 3.5.7), and many of which could have been avoided. The most important confounding factors being (i) the tap roots were severed prior to planting, (ii) the seedlings were poorly planted, (iii) competing vegetation, and (iv) development of ponds on the LD treatment. In addition, the soil fertility status was low and sub-optimal for seedling growth for both the LD and GR treatments.

Although the LD plots had favorable soil physiochemical properties, ponding in the lower portions between mounds resulted in lower seedling survival. In general, the LD treatment could have been improved if soil piles were created closer together, or some natural drainage could have been implemented allowing for water to drain off site. The accumulating water in the ponds may have been avoided on a different landscape, i.e., land with a slope gradient. A major improvement for this project would have occurred had the seedlings been properly planted. Hand planting is a critical step in reforesting seedlings on the LD treatment and deserves proper care. Use of tools for hardwoods, such as spades or augers, would have been better suited for planting seedlings with long tap roots. In addition, planters should avoid pruning roots as this adds significantly to transplant stress.

The LD treatment may have benefitted more if the site had been establishing during a dryer period. The use of equipment during wet conditions likely compacted the ground beneath the piles and consequentially contributed to the ponding. Weed control was also a major issue that should have been prevented with greater measure of weed control throughout the growing season. However, the LD treatment was not able to be mowed due to limited site access, so hand spraying would be the most viable option. Lastly, incorporation of A horizon soil would have greatly benefited the site by providing more nutrients, organic matter, and soil microbial biota.

This study provided contributions to surface mine reclamation efforts. The LD treatment was found to be a favorable alternative for reforestation in comparison to the GR treatment in terms of its ability to facilitate/support seedling growth and survival. However, it is recommended that this treatment be used on appropriate landscapes where natural drainage would be implemented. Future studies examining the LD treatment will be able to apply recommendations from this study and increase the potential use of this method. The results from this study have application to improve post-mined land with productive, healthy hardwood forests.

REFERENCES

REFERENCES

Abrams,M.D., Schultz,J.C. & Kleiner,K.W. (1990) Ecophysiological Responses in Mesic Versus Xeric Hardwood Species to An Early-Season Drought in Central Pennsylvania. *Forest Science*, **36**, 970-981.

Acton,P.M., Fox,J.F., Campbell,J.E., Jones,A.L., Rowe,H., Martin,D. & Bryson,S. (2011) Role of Soil Health in Maintaining Environmental Sustainability of Surface Coal Mining. *Environmental Science & Technology*, **45**, 10265-10272.

Ashby,W.C. (1987) Forests. *Restoration Ecology: A synthetic approach to ecological research* (eds W. R. Jordan, M. E. Gilpin & J. D. Aber), pp. 89-108. Press Syndicate of the University of Cambridge.

Ashby,W.C. (1997) Soil ripping and herbicides enhance tree and shrub restoration on stripmines. *Restoration Ecology*, **5**, 169-177.

Auch, T., Burger, J., and Mitchem, D. O. Hardwood Stocking After Five Years on Reclaimed Mined Land in Central Appalachia: A Preliminary Analysis. National Meeting of the American Society of Mining and Reclamation. 2005. 6-19-2005.
Ref Type: Conference Proceeding

Baasch,A., Kirmer,A. & Tischew,S. (2012) Nine years of vegetation development in a postmining site: effects of spontaneous and assisted site recovery. *Journal of Applied Ecology*, **49**, 251-260.

Barazani,O. & Friedman,J. (1999) Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? *Journal of Chemical Ecology*, **25**, 2397-2406.

Binkley,D. (1986) *Forest Nutrition Management*. A Wiley-Interscience Publication, New York.

Blake,G.R. & Hartge,K.H. (1986) Bulk Density. *Methods of Soil Analysis Part 1-Physical and Mineralogical Methods* (ed A. Klute), pp. 363-375. Soil Science Society of America, Inc., Madison.

Bohm,W. (1979a) *Methods of Studying Root Systems*.

Bohm,W. (1979b) *Methods of Studying Root Systems*. Springer-Verlag, New York.

Bouyoucos,G.J. (1951) A Recalibration of the Hydrometer Method of Making Mechanical Analysis of Soils. *Journal of Agronomy*, **43**, 434-438.

Bouyoucos,G.J. (1962) Hydrometer Method Improved for Making Particle Size Analysis of Soils. *Journal of Agronomy*, **54**, 464-465.

Bowen,C.K., Schuman,G.E., Olson,R.A. & Ingram,J. (2005) Influence of Topsoil Depth on Plant and Soil Attributes of 24-Year Old Reclaimed Mined Lands. *Arid Land Research & Management*, **19**, 267-284.

Box,G.E.P., Hunter,W.G. & Hunter,J.S. (1978) Simplification and Increased Senstitivity from Transformation. *Statistics for Experimenters: An introduction to design, data analysis, and model building* J. Wiley, New York.

Bradshaw,A.D. (1987) The reclamation of derelict land and the ecology of ecosystems. *Restoration Ecology: A synthetic approach to ecological research* (eds W. R. Jordan, M. E. Gilpin & J. D. Aber), pp. 53-74. Press Syndicate of the University of Cambridge.

Brady,N.C. & Weil,R.R. (2002) *The Nature and Properties of Soils*. Prentice Hall, Upper Saddle River.

Bremmer,J.M. (1986) *Methods of Soil Analysis* (ed A. Klute), pp. 1085-1121. Soil Science Society of America, Inc., Madison.

Briggeman, R., Horton, K., Mayes, B., and Schieferstein, J. Assessment of Reforestation on Reclaimed Indiana Surface Coal Mine Sites. 2007. Indiana Department of Natural Resources, Office of Surface Mining.
Ref Type: Online Source

Brown, J. R. Recommended chemical soil test procedures. 449, pp. 5-8. 1980. North Dakota Agricultural Experiment Station.
Ref Type: Report

Brown, J. R. Recommended cation tests and measures of cation exchange capacity. 221, pp. 15-16. 1988. North Central Region Publication.
Ref Type: Report

Bussler,B.H., Byrnes,W.R., Pope,P.E. & Chaney,W.R. (1984) Properties of Minesoil Reclaimed for Forest Land-Use. *Soil Science Society of America Journal*, **48**, 178-184.

Casselman,C.N., Fox,T.R., Burger,J.A., Jones,A.T. & Galbraith,J.M. (2006) Effects of silvicultural treatments on survival and growth of trees planted on reclaimed mine lands in the Appalachians. *Forest Ecology and Management*, **223**, 403-414.

Chaney,W.R., Pope,P.E. & Byrnes,W.R. (1995) Tree Survival and Growth on Land Reclaimed in Accord with Public-Law-95-87. *Journal of Environmental Quality*, **24**, 630-634.

Chatterjee,A., Lal,R., Shrestha,R.K. & Ussiri,D.A.N. (2009) Soil Carbon Pools of Reclaimed Minesoils Under Grass and Forest Landuses. *Land Degradation & Development*, **20**, 300-307.

Code of Federal Regulations. Mineral Resources. Title 30, pp. Part 700-1290. 2012.
Ref Type: Bill/Resolution

Conlin,T.S.S. & van den Driessche,R. (1996) Short-term effects of soil compaction on growth of Pinus contorta seedlings. *Canadian Journal of Forest Research*, **26**, 727-739.

Conrad, G. Enhancement of reforestation at surface coal mines. 1999. OSM. Summary Report of State Reforestation and Tree Planting Statistics: Technical Interactive Forum.
Ref Type: Report

Danielson,R.E. & Sutherland,P.L. (1986) Porosity. *Methods of Soil Analysis Part 1 - Physical and Mineralogical Methods* (ed A. Klute), pp. 443-461. Soil Science Society of America, Inc., Madison.

Davidson,D.T. (1965) Penetrometer measurements. *Methods of Soil Analysis, Part I* pp. 472-484. American Society of Agronomy, Madison.

Egan, L. Determination of total Kjeldahl nitrogen in plant digests by flow injection analysis colorimetry. 13-107-06-2-G. 2002. QuikChem.
Ref Type: Report

Fresquez,P.R. & Lindemann,W.C. (1982) Soil and Rhizosphere Microorganisms in Amended Coal-Mine Spoils. *Soil Science Society of America Journal*, **46**, 751-755.

Gebauer,R., Volarik,D. & Martinkova,M. (2011) Impact of soil pressure and compaction on tracheids in Norway spruce seedlings. *New Forests*, **41**, 75-88.

Graham,M.H. & Haynes,R.J. (2004) Organic matter status and the size, activity and metabolic diversity of the soil microflora as indicators of the success of rehabilitation of mined sand dunes. *Biology and Fertility of Soils*, **39**, 429-437.

Greb, S. F., Eble, C. F., Peters, D. C., and Papp, A. R. Coal and the Environment. 10. 2006. American Geological Awareness Series.
Ref Type: Report

Gregory,P. (2006) *Plant Roots*. Blackwell, Ames.

Groninger,J.W., Fillmore,S.D. & Rathfon,R.A. (2006) Stand characteristics and productivity potential of Indiana surface mines reclaimed under SMCRA. *Northern Journal of Applied Forestry*, **23**, 94-99.

Hall,S.L., Barton,C.D. & Baskin,C.C. (2010) Topsoil Seed Bank of an Oak-Hickory Forest in Eastern Kentucky as a Restoration Tool on Surface Mines. *Restoration Ecology*, **18**, 834-842.

Hillel,D. (1988) *Environmental Soil Physics*. Academic Press, San Diego.

Indorante,S.J. & Jansen,I.J. (1981) Soil Variability on Surface-Mined and Undisturbed Land in Southern Illinois. *Soil Science Society of America Journal*, **45**, 568.

Institute for Energy Research. Energy Overview: Coal. 2012. Washington DC.
Ref Type: Online Source

Jacinthe,P.A. & Lal,R. (2007) Carbon storage and minesoil properties in relation to topsoil applications techniques. *Soil Science Society of America Journal*, **71**, 1788-1795.

Jacobs,D.F. (2007) Toward development of silvical strategies for forest restoration of American chestnut (Castanea dentata) using blight-resistant hybrids. *Biological Conservation*, **137**, 497-506.

Jacobs,D.F., Salifu,K.F. & Seifert,J.R. (2005) Relative contribution of initial root and shoot morphology in predicting field performance of hardwood seedlings. *New Forests*, **30**, 235-251.

Jones,J.B. (1984) Plants. *AOAC Official Methods of Analysis* (ed J. B. Jones), pp. 39-61. Association of Official Analytical Chemists, Arlington.

Khalid,A., Arshad,M. & Zahir,Z.A. (2004) Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, **96**, 473-480.

Klute,A. (1986) Water Retention: Laboratory Methods. *Methods of Soil Analysis Part 1-Physical and Mineralogical Methods* (ed A. Klute), pp. 635-662. Soil Science Society of America, Inc., Madison.

Knudsen, D. and Beegle, D. Recommended phosphorous tests. 221. 1988. Fargo, North Dakota, North Central Regional Publication.
Ref Type: Report

Kormanik,P.P. (1986) Lateral Root Morphology As An Expression of Sweetgum Seedling Quality. *Forest Science*, 595-604.

Kormanik, P. P., Sung, S. S., Kormanik, T. L., Tibbs, T., and Zarnoch, S. J. Nutrition and Irrigation Regime Affect Size and Early Growth of White Oak Seedlings. 13th Central Hardwood Forest conference. 2002. 4-1-2001.
Ref Type: Conference Proceeding

Kuznetsova,T., Lukjanova,A., Mandre,M. & L+¦hmus,K. (2011) Aboveground biomass and nutrient accumulation dynamics in young black alder, silver birch and Scots pine plantations on reclaimed oil shale mining areas in Estonia. *Forest Ecology and Management*, **262**, 56-64.

Landis, T. D., Haase, D. L., and Dumroese, R. K. Plant Nutrient Testing and Analysis in Forest and Conservation Nurseries. USDA Forest Service Proceedings RMRS-P-35. 2005.
Ref Type: Conference Proceeding

Legal Information Institute. U.S. Code: Environmental protection performance standards: Title 30: Ch 25: Sub Ch V: 1265. Cornell University Law School . 2011.
Ref Type: Online Source

Lohmus,K., Truu,M., Truu,J., Ostonen,I., Kaar,E., Vares,A., Uri,V., Alama,S. & Kanal,A. (2006) Functional diversity of culturable bacterial communities in the rhizosphere in relation to fine-root and soil parameters in alder stands on forest, abandoned agricultural, and oil-shale mining areas. *Plant and Soil*, **283**, 1-10.

MacMahon,J.A. (1987) Disturbed lands and ecological theory: an essay about a mutualistic association. *Restoration Ecology: A synthetic approach to ecological research* (eds W. R. Jordan, M. E. Gilpin & J. D. Aber), Press Syndicate of the University of Cambridge.

Marschner,P., Crowley,D. & Yang,C.H. (2004) Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. *Plant and Soil*, **261**, 199-208.

McSweeney,K. & Jansen,I.J. (1984) Soil Structure and Associated Rooting Behavior in Minesoils. *Soil Science Society of America Journal*, **48**, 607-612.

McWilliams, K. M. Soil Survey of Gibson County, Indiana. 1989. National Cooperative Soil Survey.
Ref Type: Generic

Moffat,A.J. & Bending,N.A.D. (2000) Replacement of soil and soil-forming materials by loose tipping in reclamation to woodland. *Soil Use and Management*, **16**, 75-81.

National Cooperative Soil Survey. National Cooperative Soil Characterization Database. Pedon ID: 1979-IN055-032 . 2012. 2-22-0012.

Ref Type: Online Source

OriginLab Corporation. Origin 8.6. 2011. North Hampton, MA, OriginLab Corporation.
Ref Type: Computer Program

Pagani, A., Mallarino, A. P., and Sawyer, J. E. Soil pH and lime management for corn and soybean. North Central Extension-Industry Soil Fertility Conference 25. 2009. Des Moines, IA.
Ref Type: Conference Proceeding

Pang,W., Crow,W.T., Luc,J.E., McSorley,R. & Giblin-Davis,R.M. (2011) Comparison of Water Displacement and WinRHIZO Software for Plant Root Parameter Assessment. *Plant Disease*, **95**.

Rathfon, R. A., Fillmore, S. D., and Groninger, J. W. Status of reforested mine sites in southwestern Indiana reclaimed under the Indiana Mining regulatory program. FNR-251, pp. 1-15. 2004. Purdue Univ. Coop. Ext. Pub.
Ref Type: Report

Rodrigue,J.A. & Burger,J. (2004) Forest Soil Productivity of Mined Land in the Midwestern and Eastern Coalfield Regions. *Soil Science Society of America Journal*, **68**, 833.

Rodrigue,J.A., Burger,J.A. & Oderwald,R.G. (2002) Forest productivity and commercial value of pre-law reclaimed mined land in the eastern United States. *Northern Journal of Applied Forestry*, **19**, 106-114.

Rokich,D.P., Meney,K.A., Dixon,K.W. & Sivasithamparam,K. (2001) The impact of soil disturbance on root development in woodland communities in Western Australia. *Australian Journal of Botany*, **49**, 169-183.

Rosenvald,K., Kuznetsova,T., Ostonen,I., Truu,M., Truu,J., Uri,V. & Lohmus,K. (2011) Rhizosphere effect and fine-root morphological adaptations in a chronosequence of silver birch stands on reclaimed oil shale post-mining areas. *Ecological Engineering*, **37**, 1027-1034.

Saielli,T.M., Schaberg,P.G., Hawley,G.J., Halman,J.M. & Gurney,K.M. (2012) Nut cold hardiness as a factor influencing the restoration of American chestnut in northern latitudes and high elevations. *Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere*, **42**, 849-857.

SAS Institute Inc. SAS/STAT 9.2 User's Guide. Second. 2009. Cary, NC, SAS Institute Inc.
Ref Type: Computer Program

Schulze, D. G. Integrating Spatial Educational Experiences. 2012.
Ref Type: Online Source

Showalter,J.M., Burger,J.A. & Zipper,C.E. (2010) Hardwood Seedling Growth on Different Mine Spoil Types with and without Topsoil Amendment. *Journal of Environmental Quality*, **39**, 483-491.

Siam,A.M.J., Radoglou,K.M., Noitsakis,B. & Smiris,P. (2009) Differences in ecophysiological responses to summer drought between seedlings of three deciduous oak species. *Forest Ecology and Management*, **258**, 35-42.

Simmons,G.L. & Pope,P.E. (1987) Influence of soil compaction and vesicular-arbuscular mycorrhizae on root growth of yellow poplar and sweet gum seedlings. *Canadian Journal of Forest Research*, **17**, 970-975.

Simmons, M. American chestnut ready to reign again. 2009. American Chestnut Foundation.
Ref Type: Online Source

Skousen,J., Gorman,J., Pena-Yewtukhiw,E., King,J., Stewart,J., Emerson,P. & DeLong,C. (2009) Hardwood Tree Survival in Heavy Ground Cover on Reclaimed Land in West Virginia: Mowing and Ripping Effects. *Journal of Environmental Quality*, **38**, 1400-1409.

Skousen, J., Zipper, Carl E., Burger, J., Barton, C. D., and Angel, Patrick N. Selecting materials for mine soil constructions when establishing forests on Appalachian mine sites. 8. 2011. The Appalacian Regional Reforestation Initiative (ARRI). Forest Reclamation Advisory.
Ref Type: Report

Soil Survey Laboratory Methods Manual. Microbial Biomass Fumigation Incubation. USDA Burt, R. 42, pp. 388-391. 2004.
Ref Type: Report

Sullivan,J. & Amacher,G.S. (2010) Private and Social Costs of Surface Mine Reforestation Performance Criteria. *Environmental Management*, **45**, 311-319.

Thompson, B. E. Seedling Morphological Evaluation --What You Can Tell By Looking. Evaluating Seedling Quality: Principles, Procedures, and Predictive Abilities of Major Tests. 1985. Oregon State University. 10-18-1985.
Ref Type: Conference Proceeding

Torbert,J., Burger,J., Schoenholtz,S. & Kreh,R. (2000) Growth of three pine species after eleven years on reclaimed minesoils in Virginia. *Northern Journal of Applied Forestry*, **17**, 95-99.

Torbert,J.L., Tuladhar,A.R., Burger,J. & Bell,J.C. (1988) Minesoil Property Effects on the Height of Ten-Year-Old White Pine. *Journal of Environmental Quality*, **17**, 189-192.

Vomocil,J.A. (1965) Porosity. *Methods of soil analysis, part 1* (ed C. A. Black), pp. 299-314.

Zeleznik,P., Hrenko,M., Then,C., Koch,N., Grebenc,T., Levanic,T. & Kraigher,H. (2006) CASIROZ: Root Parameters and Types of Ectomycorrhiza of Young Beech Plants Exposed to Different Ozone and Light Regimes. *Plant Biology*, **9**, 298-308.

Zipper,C.E., Burger,J.A., Skousen,J.G., Angel,P.N., Barton,C.D., Davis,V. & Franklin,J.A. (2011) Restoring Forests and Associated Ecosystem Services on Appalachian Coal Surface Mines. *Environmental Management*, **47**, 751-765.

APPENDICES

Appendix A

Table A. 1. Comparison of soil particle size changes in the 0-20 cm and 20-40 cm depths in 2010, for the two soil replacement treatments: graded (GR) and loosely dumped (LD).

Treatment	Depth (cm)	% Clay	% Silt	% Sand
GR	0-20	22	57	21
LD	0-20	24	53	23
GR	20-40	24	59	18
LD	20-40	24	54	22

Table A. 2. Comparison of gravimetric and volumetric water contents at -0.1 and -0.05 bar matric potential and saturation of soil cores taken from 10 and 30 cm depths within the loosely dumped (LD) and standard graded (GR) soil replacement treatments for 2011.

	ϴg -0.1 bar			ϴg -0.05 bar			ϴg Saturation			ϴv -0.1 bar			ϴv -0.05 bar			ϴv Saturation		
Treatment	10 cm	30 cm	Mean	10 cm	30 cm	Mean	10 cm	30 cm	Mean	10 cm	30 cm	Mean	10 cm	30 cm	Mean	10 cm	30 cm	Mean
GR	0.22	0.22	0.22	0.22	0.23	0.22	0.22	0.22	0.22	0.37	0.37	0.37	0.39	0.38	0.38	0.39	0.38	0.38
LD	0.23	0.23	0.23	0.24	0.23	0.24	0.24	0.23	0.24	0.37	0.36	0.37	0.38	0.37	0.38	0.39	0.37	0.38
Mean	0.23	0.22		0.23	0.23		0.23	0.23		0.37	0.37		0.38	0.38		0.39	0.38	

Table A. 3. Comparison of gravimetric and volumetric water contents at -0.1 bar matric potential and saturation of soil cores obtained at the 10 cm depth for 2010, 2011 and the means for two years for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Treatments having different letters are significant at α=0.05 where a=largest mean and b=lesser mean. Changes between 2010 and 2011 significant at α=0.05 are indicated with *.

	ϴg -0.1 bar				ϴg Saturation				ϴv -0.1 bar			ϴv Saturation			
Treatment	2010		2011	Mean	2010		2011	Mean	2010	2011	Mean	2010		2011	Mean
GR	0.21 b		0.22	0.21 b	0.21 b		0.22	0.22 b	0.35	0.37	0.36	0.36 b	*	0.39	0.37 b
LD	0.25 a	*	0.23	0.24 a	0.30 a	*	0.24	0.27 a	0.37	0.37	0.37	0.44 a	*	0.39	0.41 a
Mean	0.23		0.23		0.26	*	0.23		0.36	0.37		0.40		0.39	

Table A. 4. Depth of needle penetration of soil cores obtained at 10 and 30 cm depths in 2011 for the two soil replacement treatments: graded (GR) and loosely dumped (LD).

Soil Property	Depth of Needle Penetration (mm)		
Treatment	10 cm	30 cm	Mean
GR	6.0	5.6	5.8
LD	9.1	8.7	8.9
Mean	7.5	7.1	

Table A. 5. Comparison of soil chemical properties among the 0-20 cm and 20-40 cm depths in 2010 averaged across both treatments. Properties having different letters are significant at α=0.05 where a=largest mean and b=lesser mean.

Soil Chemical Properties	Units	Depth (cm)		
pH	-log[H+]	0-20	5.9	
		20-40	5.9	
Soluble Salts	mmhos/cm	0-20	0.33	
		20-40	0.30	
Total N	%	0-20	0.034	a
		20-40	0.031	b
Bray 1 P	mg/kg	0-20	4	a
		20-40	3	b
Mg	mg/kg	0-20	579	
		20-40	589	
Na	mg/kg	0-20	102	
		20-40	114	

Appendix B

Table B. 1. Comparison of nursery and field seedling measurements.

	Shoot Height (cm)			
	AMC	REO	SHO	WHO
May 2010 (nursery subset)	71	97	86	28
May 2010 (field)	84	76	72	26
	RCD (mm)			
	AMC	REO	SHO	WHO
May 2010 (nursery subset)	8.2	9.2	9.1	5.6
May 2010 (field)	7.3	7.2	6.0	5.0

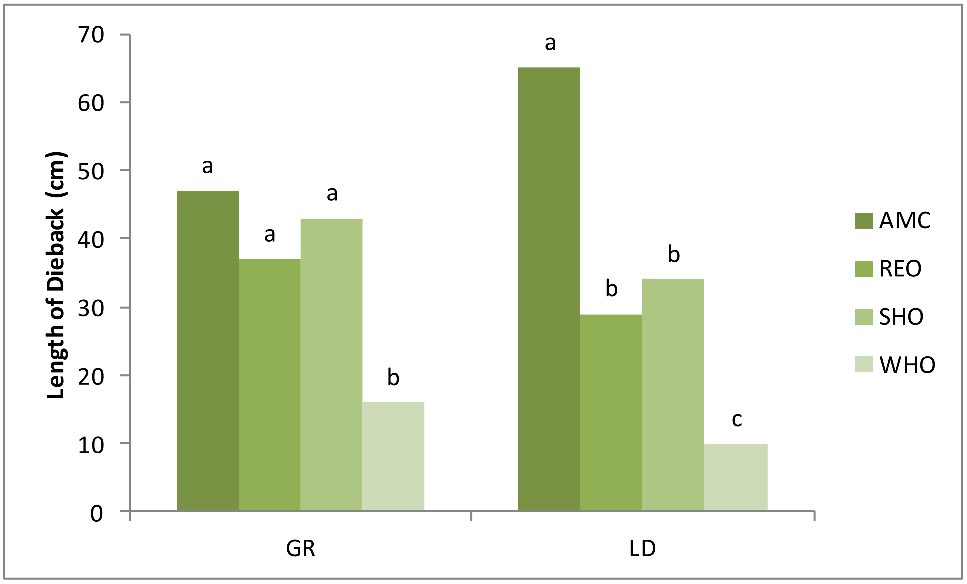


Figure B. 1. Length of dieback of American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) for the graded (GR) and loosely dumped (LD) treatments. Species having different letters within each treatment are significant at α=0.05 where a=largest mean, etc.

Table B. 2. Comparison of excavated seedling height and root collar diameter (RCD) of American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) over two growing seasons (2010 and 2011), for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Species having different letters are significant at α=0.05 where a=largest mean, etc.

	Treatment		Year		Treatment		Year	
Species	GR	LD	2010	2011	GR	LD	2010	2011
	Height (cm)				Root Collar Diameter (mm)			
AMC	97	100	94	104	9.9	9.9	7.9	11.9
REO	81	88	83	85	10.0	9.6	8.2	11.5
SHO	74	78	74	77	9.5	9.5	7.2	11.8
WHO	37	40	33	44	7.6	8.0	5.8	9.8

Table B. 3. Comparison of excavated seedling height and root collar diameter over two growing seasons (2010 and 2011) for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Changes between 2010 and 2011 significant at α=0.05 are indicated with *.

	Treatment		Year		Treatment		Year	
Species	GR	LD	2010	2011	GR	LD	2010	2011
	Height (cm)				Root Collar Diameter (mm)			
AMC	97	100	94	104	9.9	9.9	7.9	11.9
REO	81	88	83	85	10.0	9.6	8.2	11.5
SHO	74	78	74	77	9.5	9.5	7.2	11.8
WHO	37	40	33	44	7.6	8.0	5.8	9.8

Table B. 4. Leaf dry weights of excavated American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) over two growing seasons (2010 and 2011), for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Species having different letters are significant at α=0.05 where a=largest mean, etc.

	Treatment		Year	
Species	GR	LD	2010	2011
	Leaf Dry Weight (g)			
AMC	6.26	6.01	3.71	10.15
REO	4.72	5.40	2.70	9.43
SHO	4.98	6.58	2.70	12.07
WHO	3.69	3.83	1.57	8.99

Table B. 5. Comparison of tap root length and dry weight values of American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) over two growing seasons (2010 and 2011) and for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Species having different letters are significant at α=0.05 where a=largest mean, etc.

	Treatment		Year		Treatment		Year	
Species	GR	LD	2010	2011	GR	LD	2010	2011
	Tap Root Length (cm)				Tap Root Dry Weight (g)			
AMC	20	19	18	21	11.6	11.8	8.91	15.38
REO	19	23	20	23	17.12	18.21	13.40	23.25
SHO	19	26	19	26	15.01	22.35	13.30	25.19
WHO	19	21	18	22	11.6	15.3	9.04	19.64

Table B. 6. Comparison of lateral root volume across diameter classes over two years (2010 and 2011), for the two soil replacement treatments: graded (GR) and loosely dumped (LD). Treatments or times having different letters are significant at α=0.05 where a=largest mean and b=lesser mean.

Time	Treatment	0.0-0.5	0.5-1.0	1.0-1.5	1.5-2.0	2.0-2.5	2.5-3.0	3.0-3.5	3.5-4.0	4.0-4.5	> 4.5
2010	GR	0.11	0.37	0.40	0.39	0.32	0.26	0.22	0.18	0.11	0.38
2010	LD	0.13	0.43	0.48	0.44	0.37	0.29	0.25	0.19	0.14	0.61
2011	GR	0.06	0.40	0.49	0.44	0.48	0.45	**0.32 b**	**0.32 b**	**0.28 b**	**1.53 b**
2011	LD	0.07	0.48	0.54	0.61	0.73	0.68	**0.81 a**	**0.87 a**	**0.78 a**	**5.49 a**
2010	Mean	**0.12 a**	0.40	0.44	0.41	**0.35 b**	**0.28 b**	**0.23 b**	**0.19 b**	**0.13 b**	**0.49 b**
2011	Mean	**0.07 b**	0.39	0.52	0.52	**0.60 a**	**0.56 a**	**0.53 a**	**0.56 a**	**0.50 a**	**3.20 a**
Mean	GR	0.09	0.38	0.45	0.42	0.40	0.35	**0.27 b**	**0.25 b**	**0.19 b**	**0.86 b**
Mean	LD	0.10	0.40	0.51	0.52	0.54	0.47	**0.49 a**	**0.47 a**	**0.40 a**	**2.44 a**

Table B. 7. Comparison of lateral root volume across diameter classes over two years (2010 and 2011), for American chestnut (AMC), red oak (REO), shumard oak (SHO), and white oak (WHO).

Diameter Class (mm)	0-0.5			0.5-1.0			1.0-1.5			1.5-2.0			2.0-2.5		
Species	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
AMC	0.11	0.08	0.10	0.37	0.45	0.41	0.45	0.66	0.55	0.43	0.69	0.55	0.34	0.85	0.57
REO	0.12	0.06	0.09	0.40	0.32	0.36	0.50	0.46	0.48	0.50	0.49	0.49	0.43	0.62	0.52
SHO	0.11	0.06	0.09	0.34	0.35	0.34	0.33	0.50	0.41	0.29	0.56	0.41	0.25	0.59	0.4
WHO	0.14	0.07	0.10	0.50	0.45	0.48	0.49	0.46	0.47	0.45	0.38	0.42	0.38	0.40	0.39

Diameter Class (mm)	2.5-3.0			3.0-3.5			3.5-4.0			4.0-4.5			4.5-5.0		
Species	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
AMC	0.28	0.73	0.48	0.23	0.63	0.41	0.20	0.71	0.42	0.15	0.58	0.33	0.57	2.56	1.39
REO	0.36	0.58	0.47	0.35	0.60	0.47	0.24	0.64	0.41	0.19	0.56	0.35	0.50	4.20	1.9
SHO	0.16	0.59	0.34	0.10	0.61	0.3	0.08	0.60	0.28	0.03	0.58	0.22	0.04	4.27	1.28
WHO	0.32	0.37	0.34	0.29	0.34	0.31	0.26	0.34	0.3	0.18	0.31	0.24	1.28	2.09	1.66

Table B. 8. Comparison of projected root area across diameter classes over two years (2010 and 2011), for American chestnut (AMC), red oak (REO), shumard oak (SHO), and white oak (WHO). Root volume changes between 2010 and 2011 significant at α=0.05 are indicated with *.

Diameter Class (mm)	0-0.5			0.5-1.0			1.0-1.5			1.5-2.0			2.0-2.5		
Species	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
AMC	5.15	3.48	4.27	6.57	7.88	7.21	4.74	6.87	5.75	3.18	5.05	4.06	1.95	4.86	3.24
REO	5.82	2.42	3.94	7.05	5.65	6.33	5.24	4.77	5	3.68	3.56	3.62	2.47	3.50	2.97
SHO	5.26	2.71	3.88	6.01	6.22	6.11	3.48	5.15	4.28	2.13	4.09	3.03	1.46	3.33	2.3
WHO	6.67	3.08	4.71	8.94	8.13	8.53	5.18	4.76	4.97	3.39	2.83	3.1	2.16	2.24	2.2

Diameter Class (mm)	2.5-3.0			3.0-3.5			3.5-4.0			4.0-4.5			4.5-5.0		
Species	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
AMC	1.32	3.42	2.25	0.90	2.47	1.59	0.68	2.39	1.4	0.43	1.74	0.98	1.20	5.35	2.9
REO	1.69	2.72	2.18	1.37	2.33	1.82	0.81	2.17	1.4	0.57	1.65	1.04	1.11	8.16	3.82
SHO	0.74	2.73	1.58	0.40	2.39	1.18	0.28	2.04	0.96	0.09	1.73	0.65	0.09	8.53	2.58
WHO	1.48	1.72	1.6	1.15	1.33	1.23	0.88	1.15	1.01	0.55	0.92	0.72	2.11	4.33	3.12

Table B. 9. Comparison of the number of lateral root tips across diameter classes over two years (2010 and 2011), for American chestnut (AMC), red oak (REO), shumard oak (SHO), and white oak (WHO). Species having different letters are significant at α=0.05 where a=largest mean, etc.

Diameter Class (mm)	0-0.5			0.5-1.0			1.0-1.5			1.5-2.0			2.0-2.5		
Species	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
AMC	1468.0	413.0	778.6	41.0	54.0	47.1	9.0	10.0	9.3	4.0	4.0	4.1	3.0	3.0	2.6
REO	1574.0	295.0	682.4	38.0	38.0	38.2	11.0	8.0	9.3	5.0	3.0	4.2	2.0	3.0	2.4
SHO	1340.0	319.0	654.2	32.0	36.0	34.4	9.0	7.0	8.0	4.0	4.0	3.9	2.0	2.0	1.9
WHO	2499.0	387.0	983.5	53.0	51.0	52.1	12.0	9.0	10.2	4.0	3.0	3.5	3.0	2.0	2.3

Diameter Class (mm)	2.5-3.0			3.0-3.5				3.5-4.0			4.0-4.5			4.5-5.0		
Species	2010	2011	Mean	2010	2011	Mean		2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
AMC	1.7	2.0	1.9	1.4	1.5	1.5	b	1.2	1.3	1.3	1.2	1.3	1.2	1.3	1.5	1.4
REO	2.2	1.7	1.9	1.9	1.6	1.7	a	1.3	1.7	1.5	1.1	1.3	1.2	1.1	2.0	1.5
SHO	1.5	1.7	1.6	1.1	1.6	1.3	c	1.0	1.4	1.2	1.1	1.3	1.2	1.0	1.8	1.3
WHO	1.5	1.4	1.5	1.1	1.3	1.2	c	1.2	1.1	1.2	1.2	1.0	1.1	1.4	1.5	1.4

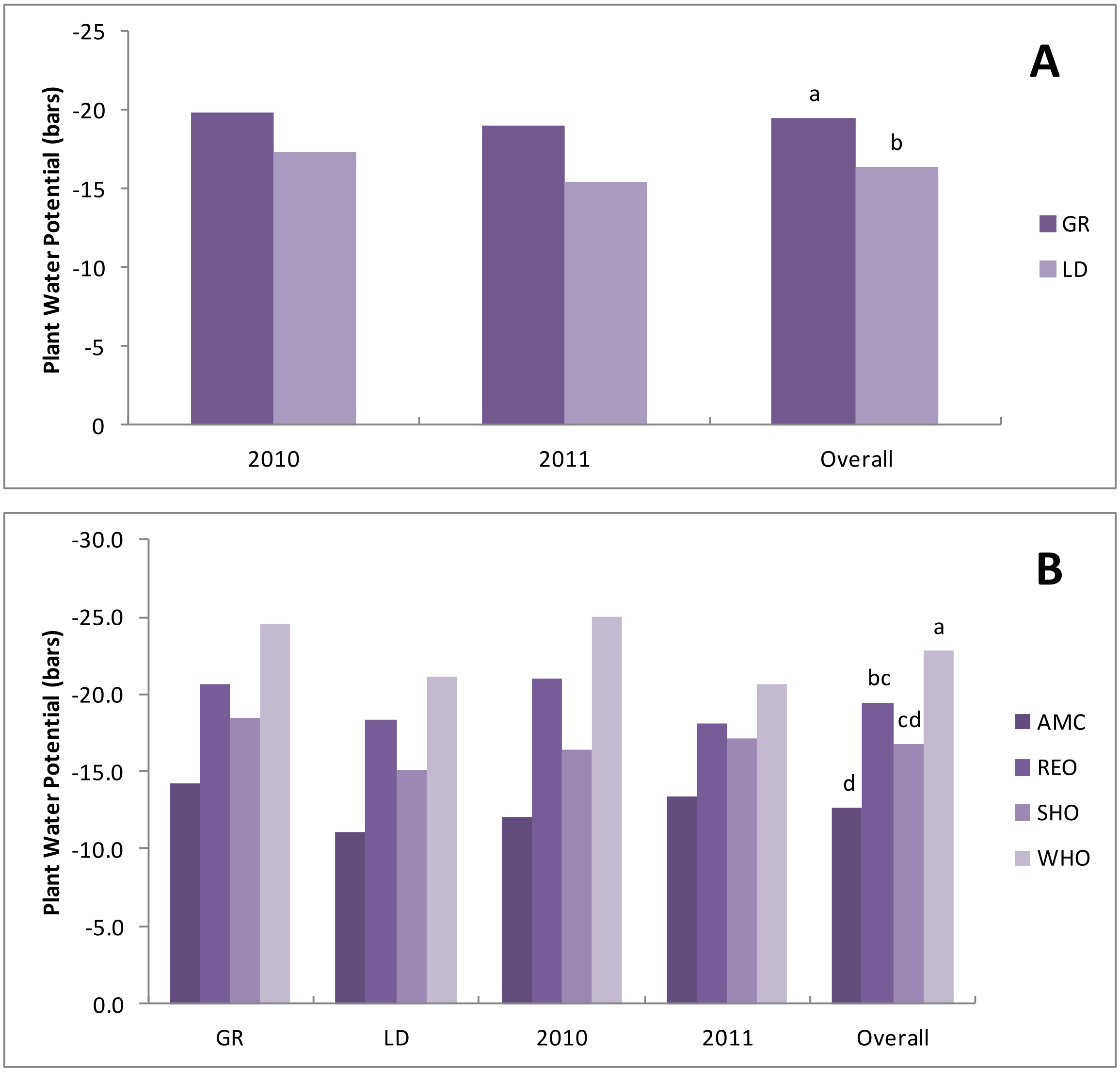


Figure B. 2 A-B. Pre-dawn ψ measured in bars averaged across all four species in the loosely dumped (LD) and graded (GR) soil replacement treatments (A); as well as averaged within species (B): American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO) for the 2 treatments and 2 time periods (2010 and 2011). Treatments or species having different letters are significant at α=0.05 where a=largest mean, etc.

Table B. 10 Nutrient levels including Al and B (**A**), Cu and Fe (**B**), Mn and Mg (**C**), Na and Ca (**D**), and Zn and S (**E**) in plant tissue in the nursery subset (2010) and the excavated seedlings (2011) for American chestnut (AMC), red oak (REO), Shumard oak (SHO), and white oak (WHO).

	Al (ppm)					B (ppm)				
Species	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011
AMC	285 a	127 a	214	196	597 a	14 a	12	10 ab	37 a	9 a
REO	25 b	46 b	231	165	501 ab	8 c	11	8 bc	26 b	7 ab
SHO	44 b	50 b	165	172	435 b	7 c	9	6 c	18 b	6 b
WHO	51 b	55 b	143	209	449 b	12 b	9	11 a	23 b	6 b

	Cu (ppm)					Fe (ppm)				
Species	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011
AMC	14 a	4 a	7 a	4	6	110 a	71	232 a	227	666 a
REO	3 bc	3 b	4 b	4	5	66 b	64	230 a	215	531 ab
SHO	2 c	3 b	3 c	4	4	82 b	74	139 b	244	414 b
WHO	4 b	4 a	4 bc	4	6	106 a	74	144 b	290	502 ab

	Mn (ppm)					Mg (percent)				
Species	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011
AMC	416 a	735 c	125 b	2195 b	336	0.20 a	0.25 a	0.22 a	0.65 a	0.30 a
REO	589 a	1122 a	222 a	3408 a	360	0.11 c	0.21 b	0.14 b	0.41 c	0.19 b
SHO	217 b	796 bc	118 b	2396 b	283	0.12 c	0.21 b	0.15 b	0.52 b	0.17 b
WHO	484 a	1026 ab	90 b	2443 b	320	0.15 b	0.18 b	0.14 b	0.31 d	0.18 b

	Na (percent)					Ca (percent)				
Species	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011
AMC	0.02	0.01 a	0.02	0.01	0.03	0.83 b	0.76 b	0.68 a	1.09	0.54 a
REO	0.01	0.01 ab	0.03	0.01	0.04	0.47 c	0.70 b	0.38 b	1.10	0.51 a
SHO	0.01	0.01 b	0.02	0.01	0.04	0.54 c	0.73 b	0.28 b	1.04	0.37 b
WHO	0.01	0.01 b	0.01	0.01	0.04	1.18 a	0.97 a	0.78 a	1.10	0.47 ab

	Zn (ppm)					S (percent)				
Zn (ppm) Species	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 2011	Shoot 2010	Shoot 2011	Root 2010	Leaf 2011	Lateral Root 201
AMC	14 b	10 b	13	17 b	10 a	0.06	0.04 b	0.09 a	0.14 a	0.0
REO	14 b	10 b	9	24 a	7 b	0.07	0.04 b	0.08 b	0.12 b	0.0
SHO	10 b	8 b	9	19 b	6 c	0.06	0.04 b	0.07 c	0.12 b	0.0
WHO	22 a	14 a	14	13 c	8 b	0.07	0.04 a	0.08 bc	0.11 b	0.04 a

Table B. 11. Nutrient levels including Al, B, Cu, Fe, K, Mg, Mn, Na, and Zn in plant tissue of the excavated seedlings (2011) for the graded (GR) and loosely dumped (LD) soil replacement treatments.

	Al (ppm)			B (ppm)		
Treatment	Shoot	Leaf	Lateral Root	Shoot	Leaf	Lateral Root
GR	63	179	510	10	23	7
LD	76	192	480	11	29	7

	Cu (ppm)			Fe (ppm)		
Treatment	Shoot	Leaf	Lateral Root	Shoot	Leaf	Lateral Root
GR	3	4	6 a	78 a	250	559
LD	3	4	5 b	64 b	238	497

	K (percent)			Mg (ppm)			Mn (ppm)		
Treatment	Shoot	Leaf	Lateral Root	Shoot	Leaf	Lateral Root	Shoot	Leaf	Lateral Root
GR	0.24	0.47	0.34	0.21	0.49	0.20	871	2386	314
LD	0.23	0.47	0.33	0.21	0.46	0.22	968	2836	335

	Na (percent)			Zn (ppm)		
Treatment	Shoot	Leaf	Lateral Root	Shoot	Leaf	Lateral Root
GR	0.01	0.01	0.04	11	20 a	8 a
LD	0.01	0.01	0.04	10	17 b	7 b

Table B. 12. The final account of type of damage, type of animal browse, and cause of mortality was recorded for all seedlings planted on the graded (GR) and loosely dumped (LD) experimental plots.

Tree Notes		GR	LD	Total
Damage	Broken Top	53	49	102
	Poor Planting	133	4	137
	Pond	0	113	113
Animal Browse	Rodent/Vole	78	14	92
	Rabbit	50	63	113
Mortality	Excavated	128	128	256
	Natural/Unknown	786	671	1457
	Animal Browse	9	7	16
	Poor Planting	92	2	94
	Pond	0	38	38
Percent Damaged		12%	10%	11%
Percent Browsed		8%	5%	6%
Percent Survival		45%	55%	50%