# EVALUATION OF TOPSOIL SUBSTITUTES FOR RESTORATION OF APPALACHIAN HARDWOODS ON STRIP MINED LAND

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# Evaluation of Topsoil Substitutes for Restoration of Appalachian Hardwoods on Strip Mined Land

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**(ABSTRACT)**

Current surface mine reclamation in Appalachia involves returning the land to approximate original contour by grading the surface and planting grasses and early-successional trees. This results in a greatly altered ecosystem compared to the native forest that was there prior to mining. The reclaimed land is usually degraded economically and environmentally because mine soils are usually less productive than the native soils, and because the mined sites do not provide the same level of ecosystem services. This research addressed constraints to the return of the native ecosystem by assessing how mine spoil properties and treatments affect native tree species and soil microorganisms. A 4x2x3 factorial greenhouse experiment was used to examine the growth of one-year-old *Fraxinus americana*, *Quercus rubra,* and *Liriodendron tulipifera* as well as herbaceous plant occurrence and microbial biomass and activity. Three mine spoils, brown, weathered sandstone (BWS), white, unweathered sandstone (WUS), and gray, unweathered shale (GUH) were compared with undisturbed forest topsoil (UFT) to determine their suitability for tree growth. Half of each of the four media was inoculated with a 2.5-cm layer of topsoil. BWS was the optimal spoil material for the growth of *F. americana, Q. rubra* and microbial populations. Foliar nutrient analysis indicated that *L. tulipifera* was highly dependent on nutrient levels and was unable to grow well on any of the spoil types due to deficiencies. Inoculation with topsoil increased tree growth on the GUH spoil, and increased microbial activity and presence of herbaceous plants across all growth media.

The field study was used to determine what spoil properties most influenced three-year-old *Quercus alba* growth. This information was used to test a mine quality classification model. Northeast facing sites with sandy spoils high in nutrients, moderate in pH, and high in microbial populations were optimal for tree growth. These variables explained 52% of the variation in tree growth. Tree growth was also highly correlated with tree foliar nutrient levels, further suggesting that tree growth was influenced by spoil nutrients. Microbial biomass and dehydrogenase production were also regressed against soil properties and were dependant on a moderate pH, high nitrogen levels, and low salt content. These variables explained 53% of the variability in microbial biomass and 50% of the variability in enzyme production. These studies suggest that tree growth and soil microbial populations are closely linked, and both are affected by mine spoil properties. During mined land reclamation, mine spoils conducive to tree growth should be selected if return of the native ecosystem is the reclamation goal.

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# INTRODUCTION

Nearly 500,000 ha of native Appalachian forests and soils have been eliminated by surface mining since the implementation of the Surface Mining Control and Reclamation Act (SMCRA) in 1977. Since this time few forests have been successfully restored due to conversions to other land uses or because of inadequate reclamation or reforestation programs. Although the law requires replacement of topsoil, this regulation is nearly always waived in the Appalachian region, and mine spoils are placed on the surface as the plant growth medium. Native hardwoods usually survive and grow poorly due to a number of physical, chemical, and biological mine spoil properties that exist at excessive or inadequate levels. The development of a soil ecosystem is needed for healthy plant growth and good forest productivity. The natural progression of restoring disturbed land involves a succession of soil organisms and plant life over long periods of time, decades or centuries. To meet the requirements of reclamation laws, strip-mine reclamation is an attempt to reduce this lengthy process, creating a healthy soil and successful plant community in a small fraction of what it would take if left to nature. Using topsoil that already has a community of soil biota might speed this process considerably (Miller 1998).

Restoring land to its original use and level of productivity is important because coal is a limited resource and after it has been mined, the people of the Appalachian region must look to other industries. Forest resources have been an economic mainstay for the region for a century or more. Current reclamation practices often replace native hardwood stands with grasses and legumes or unmanaged forests consisting of early-successional trees and shrubs. If left unmanaged, this ecosystem is much less valuable to

landowners and people of the region than a planted forest made up of commercially-valuable mid to late successional hardwood species. The replacement of hardwood forests would create an economically viable system, as the land could then be used for timber, wildlife habitat, hunting and recreation. It is therefore vital to determine an effective, efficient, and low cost way to restore native forests.

Restoring the native Appalachian hardwood forest for all its products and ecosystem services is the goal of many landowners and surrounding communities. Accordingly, my research objective was to test the hypotheses that certain mine spoils are better for hardwood reforestation, and that native tree survival and growth is enhanced by inoculating mine spoils during the reclamation process with topsoil containing a variety of symbiotic organisms that might increase the success of these forests.

My specific objectives were:

1. To determine the relative suitability of three different mine spoil types for native hardwood tree species and soil microorganisms.
2. To determine if topsoil inoculation improves tree growth and increases microbial populations.
3. To determine the relationship between mine soil properties and white oak growth and nutrition.
4. To test the accuracy of a site quality classification model for reclaimed mined sites in the Appalachian region.

# LITERATURE REVIEW

### *The Mixed Mesophytic Forest*

The native hardwood forest of Appalachia consists of a rich collection of vegetation that plays an essential role in the environmental health, biodiversity, economy, esthetics, and culture of the Appalachian Region. Many of the late successional hardwoods are valuable timber species, playing an important role in the economy of the area, while other tree and herbaceous species have cultural and environmental importance. Many of the understory plants are gathered for medicine or food, such as ginseng and ramps (Duke, 1997; Jones and Lynch, 2002). Basket weaving and doll making are also uses of nontimber forest products (Alexander et al., 2002). The diverse wildflower populations are important esthetically and environmentally. From the overstory come sourwood and basswood honey, which are valued for their unique specialty flavors (Hill, 1998). Large mast trees such as oaks and hickories are important timber species and supply food for wildlife. Many of the state flowers and trees of the seven states in the Appalachian region are symbols of the diversity and beauty of the native hardwood forest in this region. They include flowering dogwood (*Cornus florida*), tulip poplar (*Liriodendron tulipifera*), hemlock (*Tsuga canadensis*), rhododendron (*Rhododendron maximum*) and mountain laurel (*Kalmia latifolia*).

This mixed mesophytic forest is an extremely diverse collection of woody and herbaceous species. It is dominated by beech (*Fagus grandifolia*), basswood (*Tilia americana*), sugar maple (*Acer saccharum*), sweet buckeye (*Aesculus octandra*), red oak (*Quercus rubra*), white oak (*Quercus alba*), and hemlock (*Tsuga canadensis*). Birch

(*Betula lenta*)), black cherry (*Prunus serotina)*, cucumber tree (*Magnolia acuminate*), white ash (*Fraxinus americana*) and red maple (*Acer rubrum*) also comprise a large portion of the forest, with black gum (*Nyssa sylvatica*), black walnut (*Juglans nigra*) and hickories (*Caria*) present, but not abundant (Braun, 1950). In addition to these 15 are another 22 species that occur in different regions (Braun, 1950). This forest is unique in its diversity and an invaluable asset to the people of the region.

The unique characteristics of these diverse forests are currently being eliminated over large areas by surface mining. Over 500,000 ha have been affected by strip mining in the Appalachian region since the implementation of SMCRA in 1978 (OSM, 1999). Traditionally, these areas are graded and hydroseeded with non-native herbaceous vegetation, reclaiming them to grassland. However, there has been a recent shift towards reforestation of reclaimed mined land to unmanaged forest land. These reforestation efforts, though a step closer to the return of the native forest, usually involve planting monocultures of early successional species. Black locust and a variety of other early-successional trees are able to survive and grow on these mined sites (Vogel and Berg, 1973; Filcheva et al., 2000), but this leads to forest stands with little or no diversity or value. Because of the competitive herbaceous vegetation and the physical and chemical properties of the mine spoils, many of the native species of the mixed mesophytic forest are unable to establish themselves where they once occurred in abundance. Although early successional species such as black locust, autumn olive, and Virginia pine may return some forest cover, the value of the forest and its future potential, economically, environmentally, culturally, and esthetically, is largely degraded.

### *The Coal Industry*

Fifty-one percent of U.S. electrical energy is derived from coal, most of which is mined within the borders of the country (Energy Information Administration, 2003). Large areas of the U.S. are underlain with coal, including the Appalachian region and areas in the west. In 1981, there was still over 4 million ha of strippable coal in the United States (National Research Council, 1981). This large resource has been exploited by a large mining industry. Since the implementation of SMCRA in 1977, 22.2 billion metric tons of coal have been mined (OSM, 2004), and there are still an estimated 249.5 billion metric tons of extractable coal reserves (Energy Information Administration, 2003). In 2001 alone, 334.9 million metric tons of coal were mined from the Appalachian region and 992.7 from the country as a whole (U.S. Department of Labor, 2003). This has greatly increased from the 625 million metric tons mined in the U.S. in 1977 (OSM, 2004). With our country's great dependency on this energy source, it is apparent that coal mining will continue far into the future. It is therefore necessary to understand and control the reclamation process so that the land is returned to productive uses for future generations.

Coal was once extracted mostly by deep mining. However, in many areas it has become more economically feasible to surface mine. Large machinery has increased the amount of coal that can be extracted and decreased the number of workers needed (Ripley et al., 1996). Now 67% of coal is surface mined (Energy Information Administration, 2003). In 2002, 334,173 ha of land in the U.S. were under permit to be mined (OSM, 2002a). Surface mining involves the removal of overlying material to expose a seam of coal. The coal is then removed and the material replaced.

The main types of surface mining in the Appalachian region are contour mining and mountaintop removal. Contour mining involves the removal of soil and rock overlying a coal seam around the contour of a mountain (Fig. 1). The spoil from such sites is taken to previously mined areas to be used as fill. The temporary highwalls that this mining creates are filled in with these spoils. As spoils increase in volume by 25-30%, compared to intact rock, the excess is dumped in an adjacent valley.

The operation continues to move inwards on the mountain until the soil and rock above the coal seam is too deep to remove economically (OSM, 2002b). If the entire mountain can be removed because the coal seam is thick enough, or the equipment is more capable, the operation is called mountain top removal (Fig. 1).

This alteration of large areas of land by mining has had a major environmental impact on the Appalachian region. The natural vegetation and soil that once stood on these areas has been completely eliminated. Early strip mining often did not include reclamation of the mine land. The land was left as it was after the coal was removed. This often consisted of a highwall, a bench where the coal was removed, and an outslope of spoil material that was often unstable (OSM, 2002b). These unreclaimed sites often had high erosion rates and toxic spoils were often left on the surface. Re-growth and development of vegetation was often poor due to poor soils and lack of replanting. As the mining industry grew, and these problems became more apparent, action was taken by the government to implement reclamation practices on strip mine sites though the Surface Mining Control and Reclamation Act of 1977, SMCRA.

Figure 1. Examples of contour mining (left) and mountaintop removal (right) at Pritchard Mine WV, 2003.

### *Mined Land Reclamation Procedures since Implementation of SMCRA*

In 1977, nearly 34,000 ha of land in West Virginia had been abandoned after strip mining (U.S.D.A.-S.C., 1979). The unstable slopes, poor water quality and poor soil conditions on these abandoned lands, and in other states throughout the country, caused the US Congress to enact Public Law 95-87, otherwise known as SMCRA. Reclamation regulations based on this act include the return of topsoil, return of the approximate original contour of the land, and a 90% growing rate on the strip mined land compared with rates previous to mining. Companies wishing to strip mine an area are also required to submit a proposal explaining their procedure for reclaiming the land (SMCRA, 1977).

This law addressed many of the environmental problems caused by strip mining. Highwalls were no longer abandoned, and erosion was reduced with the establishment of ground cover. In the Midwest, the mollic epipedon was saved and replaced on the surface after mining. Toxic spoils that would have contaminated water were deeply buried. However, the implementation of these regulations left some problems unaddressed and created new ones. Replacement of forested areas is not a requirement, and native forests are often replaced by less productive herbaceous and woody species. Returning the approximate contour of the land led to compactive grading while the requirement of ground cover led to the use of aggressive grass species. Another issue is that some regulations of SMCRA were considered too difficult to implement on the steep slopes of the Appalachians and are often waived, such as the replacement of topsoil. This review will consider these issues as they pertain to reforestation of mined land with native hardwood species.

Replacement of topsoil

One requirement of the SMCRA that is not currently being followed by most coal operations in the Appalachians is the replacement of topsoil during reclamation. This rule requires removal of topsoil separate from the rest of the mine spoils and its replacement on top of the reclaimed land after strip mining. Topsoil, in this context, includes the A and E horizons although some researchers recommend that topsoil for forestry post-mining use include the O and A horizons, as well as the B, BC and some Cr horizons (Burger and Zipper, 2002) (Fig. 2).

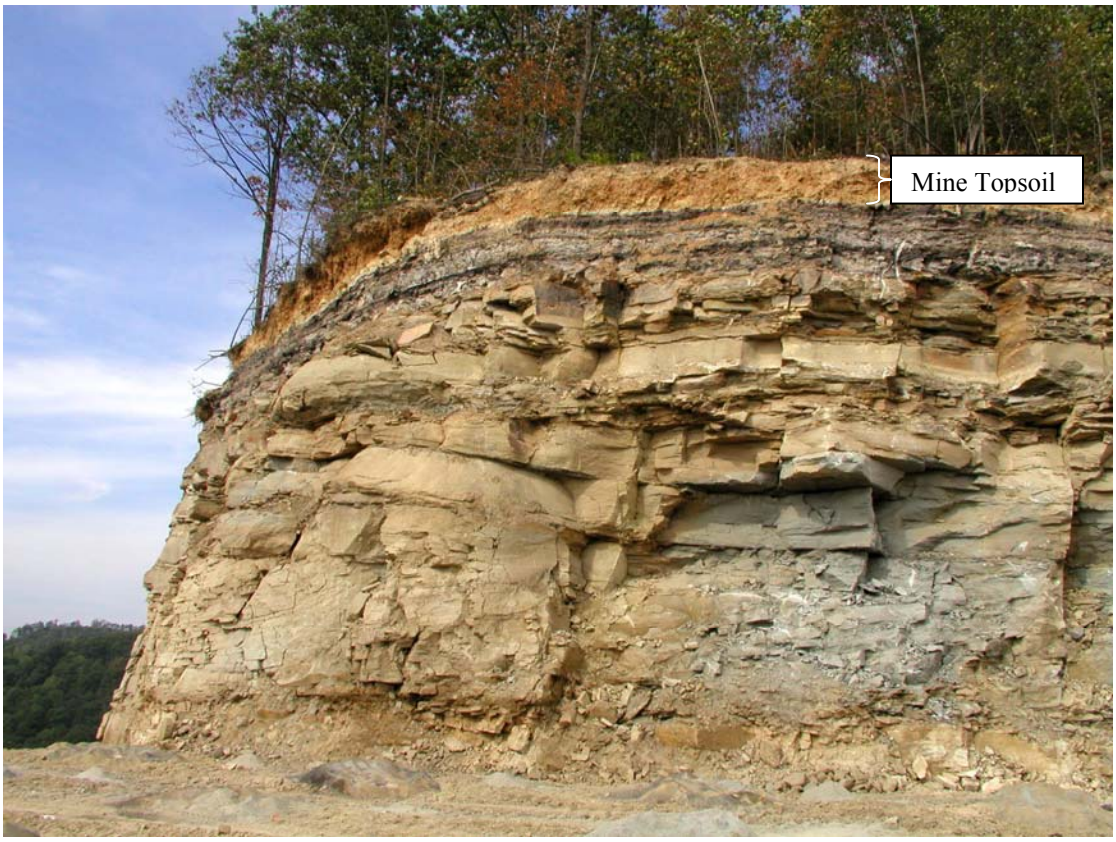


**Figure 2**. Highwall depicting "topsoil" including the O, A, B, BC and Cr layers. Beneath the mine topsoil are lower rock horizons which are often placed on the surface during reclamation.

Topsoil is often not replaced because the slope is considered too steep and the topsoil too shallow. Instead, the topsoil gets buried and mine spoils are placed on the surface as topsoil substitutes. The law states that spoils may be used if they are an equivalent or better growth medium than the original topsoil. Many are now making the argument that because these spoils are more alkaline, they create a better growth medium. However, there is much debate about this because the native forest grows on a fairly acidic soil.

Ground cover

The focus on immediately establishing a ground cover in order to comply with SMCRA regulations is another issue complicating reforestation. Since the implementation of SMCRA, many studies have tested the success of various ground

covers that would alleviate erosion problems (Barnhisel, 1977; Asay, 1979; Hinchman, 1979).

However, many of these early experiments did not involve the testing of native plants. Instead, metal-tolerant species that could survive harsh conditions were used. Planting these non-native, hardy plants has become common due to the fact that they grow quickly in unfavorable environments. These characteristics result in vegetation that can cover the inhospitable substrate of mine spoils quickly, resulting in land covered in vegetation for erosion control, but by grasses and weedy species that are usually too competitive for tree establishment and growth. Some tree species, such as black locust, are able to compete with ground cover (Vogel and Berg, 1973). However, rapid forest succession cannot occur with these aggressive herbaceous species, and the return of native hardwood stands is arrested if not eliminated entirely (Chaney et al., 1995).

More recently, tree-compatible ground cover mixes which allow the growth of native hardwoods have been developed (Torbert and Burger, 1990). Other studies have examined the use of herbicides immediately around trees and broadcast spraying to allow tree growth (Burger et al., 2005). However, these practices have not yet become common reclamation techniques.

Grading

Another issue that has arisen after the implementation of SMCRA is grading. SMCRA requires the replacement of the approximate contour of the land, which involves grading the minespoil. This greatly compacts the spoil, creating a poor medium for plant growth. It can severely limit tree success, resulting in high mortality rates and slow growth (Ashby, 1990).

### *Biological Characteristics Needed for Succession of Plant Species*

When attempts have been made to plant a more diverse array of native hardwoods, including the oaks, sugar maple, and black cherry, on reclaimed mine sites, their survival and growth is often poor. Mined sites have many of the raw characteristics typical of primary successional sites. Mine spoils often have highly variable physical and chemical properties, ranging from very acid pyritic materials to alkaline shale. Compared to native soils, mine spoils can be high in rock fragments, have low moisture content, low porosity, poor structure or high bulk density (Bussler et al., 1984). High pH and soluble salts or low nutrient levels can also adversely affect tree growth (Torbert et al., 1990). This combination of factors often leads to a material fit only for primary succession of pioneer species.

The two most important factors dictating plant establishment in these primary succession spoils are the ability to seed into the area and the subsequent ability of the plant to germinate, emerge, and grow in the given harsh conditions (Chapin, 1993). Some early successional plant species that are wind and bird disseminated, and have an opportunistic growth habit, are better adapted for reclaimed strip mines. However, many of the native species that occur in the mixed mesophytic forest are less likely to become established on mined sites, and, when they do, they cannot tolerate mine spoil conditions.

One example of an important native genera that may have difficulty is *Quercus*. In Appalachia, where fire and other disturbances are common, oaks represent a mature successional stage in forest development (Johnson et al., 2002). They are an essential component of the native hardwood forest and their replacement on these sites is an important step toward the return of these forests. Due to their large tap root they are

drought tolerant, suggesting that once established they could compete on these harsh sites. However, the chemical and physical properties of the soil medium play an important role in tree development. A section of this study addresses the question of how these different spoil properties may affect oak growth.

In order to restore the late successional native hardwood stand as quickly as possible, we must attempt to skip the first few stages of succession. With current strip mine practice of hydroseeding with grass, or even planting with early successional trees, the native forest may take extended periods to return. However, the goal is to try to reduce this timescale by creating a hospitable environment conducive to the establishment of a diverse group of species including later successional tree and herbaceous plants.

One requirement for plant success during primary succession is the ability to grow in inhospitable soil conditions. Again, many native species are unable to accomplish this. Mine spoils are highly variable, depending on which geologic strata they come from. Some of these spoils are better media than others for late-successional plant growth. Attempting to approximate the native forest soils of the area may aid in plant establishment.

The soils of the Appalachian Mountains are mostly Ultisols and Inceptisols (Hicks, 1998). Although the A horizon may be thin in places, it is well weathered soil that supplies the needed conditions for the given ecosystem. Other soils may appear to be better media because of their high pH or high CEC, but these soils would probably lead to the development of a different ecosystem. Just as the limestone valleys and the sandstone ridges of the Ridge and Valley area have different soils and different

ecosystems, the vastly different spoil media on many reclaimed strip mines would also harbor a different collection of herbaceous and tree species.

In order to regain the collection of species that grew on these mountains prior to mining, it may be important to select and treat spoils so that they best approximate the native soils of the area. Little research has addressed this issue, but selection of spoils similar to native topsoil would imply choosing sandstone spoils with low rock content, a slightly acid pH, high organic matter content, and the ability to rapidly develop good soil structure. Many of the spoils placed on the surface are blasted rock that weather slowly. These materials have very high rock content, high pH, disproportionately high or low micro nutrients, low macronutrients, no organic matter, and poor structure. However, reclamation practices have begun to alter these conditions by different selection and treatment of the mine spoils. The following is a critique of the current practices, how they are, or are not, conducive to the growth of native hardwoods, and how they could be improved.

### *Current Reclamation Techniques for Restoring Native Hardwoods*

Since the implementation of SMCRA, the majority of land has been planted with herbaceous ground cover and little effort has been devoted to the establishment of hardwoods. However, due to recent changes in the implementation of the law in West Virginia, companies are now required to plant with commercially valuable trees if they do not develop the land to a higher economic use or replace the original contour. Many of these species are high-value native hardwoods and efforts are now being made to insure the success of these trees. In order to successfully grow these species, the trees

must be handled carefully, the herbaceous ground cover kept to a minimum, and the spoil medium must have physical, chemical and biological properties conducive to growth.

Stocking and planting

Trees must be of very high quality and must be treated with care in order to survive on these sites. A professional tree planter is often hired in order to insure proper planting. Trees must be planted to a sufficient depth, which is often difficult on these rocky slopes. Similar to any other tree planting operation, trees should not be allowed to dry out. Even when proper guidelines are followed, the conditions of the soil can limit the growth of even the healthiest and most well planted tree. High bulk densities, high rock or salt content, along with a myriad of other poor conditions, can be detrimental to tree growth (Rodrigue and Burger, 2004).

Soil preparation

Due to current reclamation techniques, the physical properties of mine spoils are often inadequate for the growth of native hardwoods. Current practice involves grading mine spoils to return the approximate contour of the slope, decrease erosion and create a more stable landform (Harwod and Thames, 1988). This involves running heavy equipment over the soil which can compact it, leading to even higher bulk densities, a break down of structure, and a decrease in infiltration, resulting in an increase in erosion (National Research Council, 1981). This compaction also creates a medium that is impenetrable to plant roots (Jansen and Melsted, 1988), making re-vegetation of the area more difficult, especially with more sensitive tree species.

When compaction is severe from grading, ripping can be used to loosen the soil. This is a type of deep plowing that can decrease the bulk density of the spoil. An

alternative to grading is loose tipping. Instead of packing the material down with heavy equipment, it is tipped by a dump truck and leveled with an excavator to the correct depth. This procedure prevents heavy machinery from driving over the surface of the soil. This minimizes compaction and results in much higher soil quality (Moffat and Bending, 2000), decreasing the bulk density and allowing root penetration and growth where it may have been restricted if the soils were tracked in.

Hydroseeding ground covers and fertilizer

Because mine spoils are low in some available nutrients, the addition of fertilizer can greatly improve plant growth. After fertilizer is added, growth and survival may then be limited by other factors such as high bulk density or low water holding capacity. Hardy species, such as black locust that can survive these adverse physical conditions can increase growth by three to four times from fertilizer amendments (Vogel and Berg, 1973). Growth of pines was also found to increase significantly in southwestern Virginia with the addition of a slow-release fertilizer (Klemp et al., 1986). However, some tree species are often unable to survive the harsh conditions of reclaimed sites even with the addition of fertilizer. Thus this treatment is an easy and effective amendment to the soil but is often inadequate by itself.

Species selection and combinations

One technique for restoring native hardwoods is to under-plant a stand of previously planted nurse trees such as black locust. Black locust is very hardy and can grow on most mine spoils. Over a period of time it can greatly improve soil conditions, both by adding organic matter and fixing nitrogen. A variety of hardwood species can be successfully under-planted several years after the black locust have established. In a 30-year study, 16 tree species were under-planted in locust and pine stands, and were very successful (Ashby and Kolar, 1977). This technique, however, takes a great deal more time and energy.

Species selection can be geared toward soil type. Some species are better at growing on more acid sites while others can tolerate alkaline sites. Figure three shows species site specificity for commercial hardwood growth in the Appalachians depending on site quality, pH, aspect and slope.

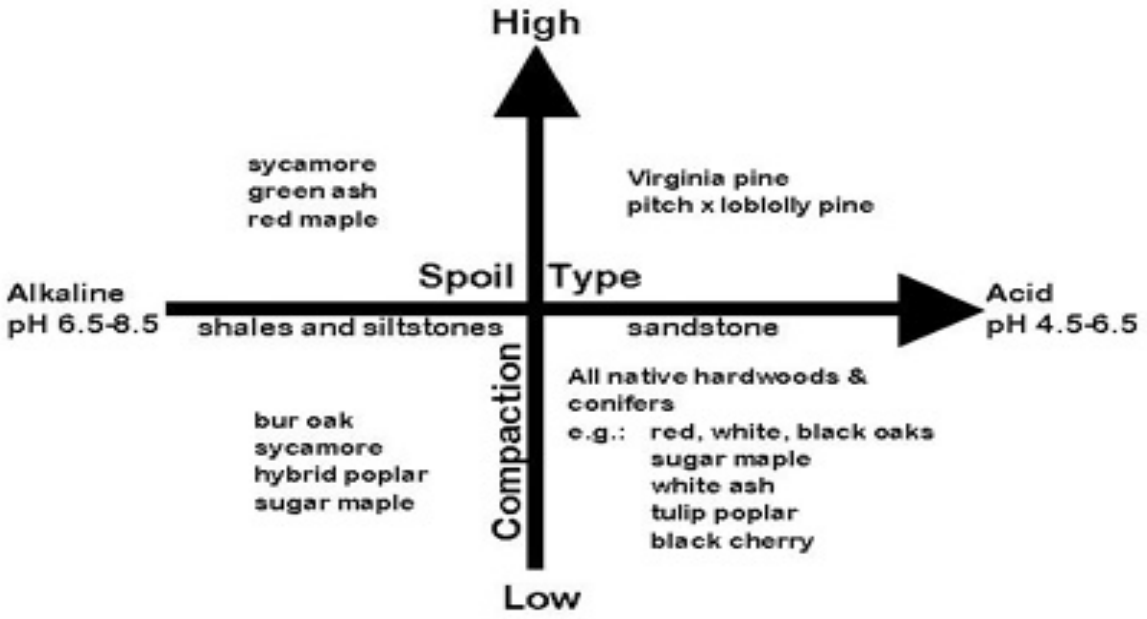


**Figure 3.** Tree species recommended for different soil types and levels of compaction (Burger and Zipper, 2002).

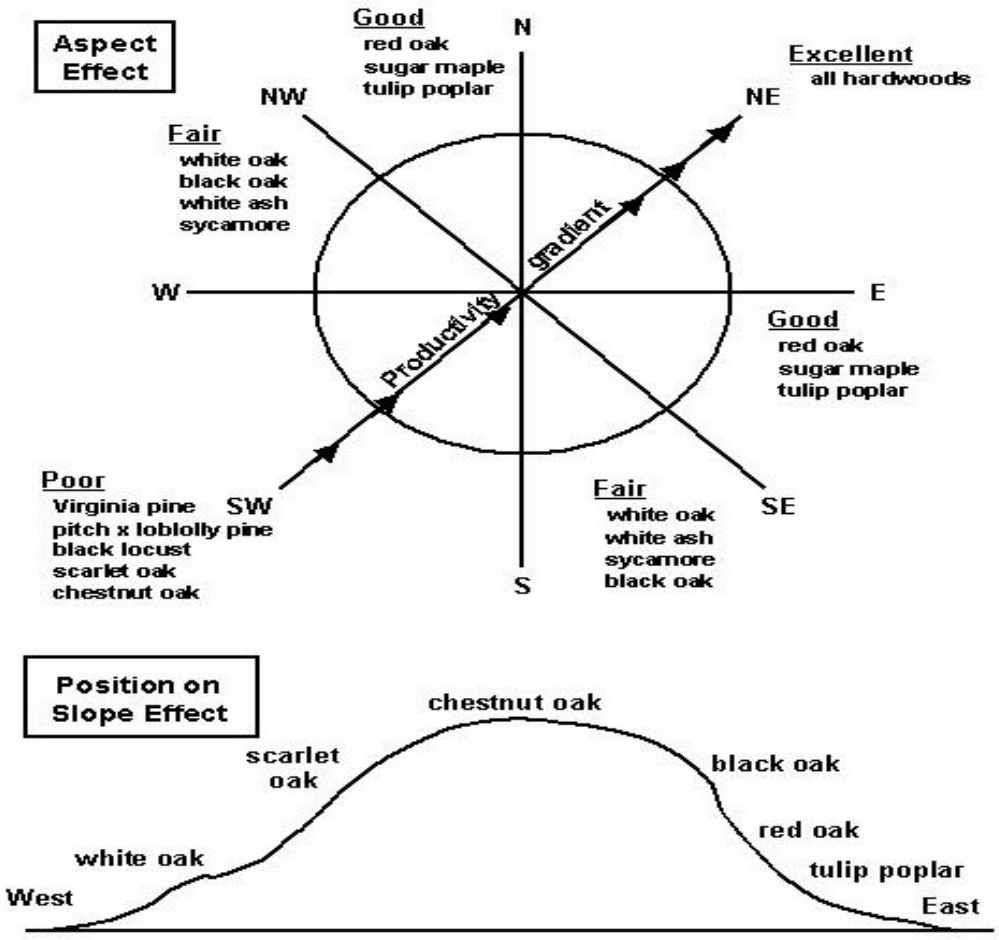


**Figure 4**. Tree species recommended for different slopes and aspects (Burger and Zipper, 2002).

Soil amendments have also been found to greatly increase the success of native trees. The addition of fertilizer has become common practice for strip mined sites, and the addition of sewage sludge and compost have been investigated and found to have positive effects on tree growth (Daniels and Zipper, 1999).

Sewage sludge/Compost

The addition of sewage sludge/compost mixes adds to the nutrients in the soil and increases the overall organic matter content. This increase in organic matter can help alleviate some poor physical conditions of mine spoil. Organic matter decreases bulk

density and increases water holding capacity (Daniels and Haering, 1990). Sludge also supplies a source of slow release nutrients which can further aid the long term success of trees. It can also lead to an increase in soil biota over time (Sopper and Seaker ,1988).

The addition of organic matter to the soil through composting was found to improve immediate growth and establishment of plants. One study demonstrated an increase in biomass of five to seven times that of non-amended soils (Noyd et al., 1997). This suggests that organic matter is an important factor for initial soil quality. Replacement of topsoil along with residual stumps and surface organic matter would lead to an initial input of organic matter along with an introduction of soil organisms that are vital for the mixing and incorporation of organic matter into the soil. This would imply that replacement of topsoil would have similar positive effects. However, a long term study of organic amendments and topsoil replacement demonstrated an equilibration of organic matter and site productivity on amended and non-amended plots after 16 years (Bendfeldt et al., 2001). This indicates that soil amendments play a less important role in long term soil quality. Initially, however, these organic amendments, or top soil replacements, could help the establishment of native trees. However, this material must be hauled in and applied, which raises costs and reduces feasibility. Although current studies suggest that risk of ground water contamination is low and that other environmental risks are nominal, these aspects must also continue to be monitored.

Topsoil

Topsoil has similar properties to the above amendments. It is higher in organic matter than mine spoil and it also adds N and P when used as an amendment. Although

the amount of nutrients added by the replacement of topsoil is fairly low, when applied in conjunction with fertilizer, levels can be increased considerably.

Replacement of topsoil increased height and diameter of loblolly pines compared to sites where topsoil was not replaced. This experiment was carried out on alkaline Pottsville shale and on sites where topsoil was replaced (Cross et al., 1987). The topsoil and shale in this experiment are similar to those found in the Appalachian region, suggesting that tree growth may be effected similarly.

Topsoil also contains soil microorganisms. These organisms can greatly add to the quality of the soil by actively mixing and breaking down organic mater over a period of time. This accelerates the rate at which soil structure is formed and allows for greater incorporation of organic matter. Nutrient availability and uptake by roots also increases due to enhanced breakdown of organic matter and increased uptake by mycorrhizae.

Another advantage of topsoil is that it is already on site. Instead of having to truck in sewage sludge or compost, topsoil can simply be segregated, stockpiled and replaced after mining. However, stockpiling may affect the soil biota. Instead of being spread thinly over a forest floor the soil is piled and is often left for years. Aeration is greatly reduced and oxygen cannot get into the center of the pile. This can greatly diminish microbial activity and number of soil macro-invertebrates. Rives and collogues (1980) found that mycorrhizae were substantially reduced in topsoil that had been stockpiled for three years.

New practices involve the cycling of topsoil, removing it from one area that is about to be mined and taking it to another area where mining has been completed. This would eliminate the need to stockpile for long periods of time.

### *Topsoil Seed Bank*

Another potential benefit of replacing native topsoil is the seed bank contained within it. One of the great difficulties associated with re-establishment of native species on reclaimed sites is the inability of many native species to seed in and establish. In order to seed into a large barren area, a species must have a light seed that can travel great distances. Primary successional species are well adapted to this strategy with small winged or cottony seeds. They are thus able to seed into the center of even large mines. However late-successional species often have large heavy seeds that must be carried by animals and are subject to predation. Oaks and hickories have seeds that rarely travel more than the circumference of the canopy unless carried by animals. Other species such as maple and poplar have seeds that travel 61-122 m. However, this is not nearly far enough to establish on large strip mines. Understory forest vegetation also has difficulty seeding into these primary successional areas. Most of these tree and herbaceous species would be out-competed by primary successional species with light seeds. On mine sites, this problem is partly alleviated by the planting of trees and hydro seeding of grasses. The use of non-native grass monocultures to stabilize mine spoils can create problems with succession of native woody plant species. These aggressive grasses are used as an initial ground cover to reduce erosion. However, native tree species are often unable to compete with these monocultures, leading to arrested succession, low species diversity and low productivity (Carter and Ungar, 2001). In a seven-year study of reclaimed mine land in Indiana, seedling survival of black walnut and red oak were only 4% and 1%. Chemical control of competing herbaceous species increased survival rates to 66% and 48% (Andersen et al., 1989).

Another study demonstrated very slow growth of red oak and black walnut. After 12 years, neither species was successful enough to fulfill SMCRA requirements unless ground cover was initially controlled. Poor growth was a result of shoot dieback and mortality that resulted from competition with ground cover grasses (Chaney et al., 1995). Another study showed significant positive effects of chemical weed control on the growth of pines in Virginia (Klemp et al., 1986). These studies show that ground cover can have an important negative impact on seedling survival of native trees.

Instead of multiple chemical applications, the use of native soils could be a viable alternative for reduction of groundcover competition without increasing erosion. After only one growing season, seed banks have been shown to produce $1.9x10^6$ shoots $ha^{-1}$, with a diversity of 134 taxa (Farmer et al., 1982). This suggests that the seed bank in native soils may lead to enough plant establishment initially that seeding a monoculture for erosion control is unnecessary. Use of the native seed bank could increase the establishment of natural herbaceous species leading to faster development of the native ecosystem.

The growth of woody plants from the seed bank would also speed up recovery of the native forest. In the above mentioned seed bank experiment, 16 of the species were trees and shrubs, making up 11.64% of the total plants. Several of these species, *Liriodendron tulipifera* and *Rubus allegheniensis*, occurred on all of the plots (Farmer et al., 1982). This suggests that the seed bank of native topsoil is a significant source of native woody plant regeneration.

Although only a fraction of the seeds found in a seed bank can be found in the standing vegetation, many of the others are from slightly earlier or slightly later

successional seres (Pickett and McDonnell, 1989). Many seeds of later successional species do not last long in the seed bank and are often non-viable after only one growing season. Others, however, can last extended periods in the forest floor and have been found to be viable after 40 years (Pickett and McDonnell, 1989).

The seeds in the bank may not lead to the target late successional forest, but they consist of species from a later sere and are have a greater proportion of native species than the primary successional seeds that are brought with the wind. Seed banks may thus lead to a much higher seedling survival rate and faster development of a native ecosystem.

**Figure 5.** In the foreground is vegetation derived from a native seedbank, while the background is a conventionally hydroseeded bank at Pritchard Mine, WV, 2003.

### *Mine Spoil Properties and Their Influence on Tree Growth*

Mine spoils that are currently being used as topsoil substitutes have very different physicochemical properties from original surface soils. Spoils are taken from very deep horizons that consist mainly of rock. They have not undergone the same physical, chemical, and biological weathering processes that surface soils have, nor have they had the opportunity to accumulate organic matter and nitrogen. They are very different in physical and chemical properties and can be inhospitable for re-establishment of most tree species (Brenner, 1979; Chaney et al., 1995).

Mine land reforestation with native hardwoods is not commonly practiced in the Appalachian region due to the lack of success of these trees. The differences in these mine spoils from the original surface soil are enough to create a medium inadequate for forest growth and development (Bussler et al., 1984). Early studies found that a variety of vegetation types had poor success on mine spoils. This was thought to be a result of low fertility, high acidity, and low water holding capacity (Tyner and Smith, 1945). In a later study, factors such as water storage capacity, salt content, and toxicity from heavy metals were directly correlated with plant growth (McFee et al., 1981). Brenner and collogues (1979; 1984) found that organic matter and soil moisture were the primary factors limiting tree and shrub establishment. Temperature and soil moisture also influence germination and seed establishment rates (Bell and Ungar, 1981). This study dealt with a survey of 30 different tree and shrub species which showed over all low success when planted in strip mine spoils. Another key factor is soil depth, having a strong direct correlation with growth of eastern white pine (Andrews et al., 1998).

Black locust and, in some studies, a variety of other trees, are able to survive and grow well in these conditions (Vogel and Berg, 1973; Filcheva et al., 2000). However, many of these trees have low production values and do not approximate the native forest. As a result these forests are of little economic value for land owners and do not provide the same level of ecosystem services.

Physical properties

Compared to native topsoils, mine spoils are usually high in rock fragments, have low moisture content, low porosity, poor structure and high bulk density (Bussler et al., 1984). All of these factors play an important role in tree growth. High % rock fragments leave less fine earth for tree rooting, leading to less nutrient and water uptake. Low moisture content can make arid regions or even dry years in wet climates extremely inhospitable to tree growth. Low porosity and poor structure decrease the amount of air and water that a soil can hold. High bulk density, one of the most important factors, can create a physical barrier to root growth and development along with water flow. These factors pose lesser or greater difficulties depending on the mine spoil and the manner in which it was reclaimed. In some cases these factors may not be a problem and tree growth may be limited by chemical or biological properties. Other times, these factors may be so extreme that native tree survival is severely limited without some modification of the substrate.

*Bulk Density*

Mine spoils are void of organic matter or soil structure and can be easily compacted, which greatly increases soil bulk density. This could be partly alleviated with the replacement of topsoil. Bulk density of sites where topsoil was replaced verses

bulk density on sites that used mine spoil as a topsoil substitute was 15% lower (Chong et al., 1986). Bulk density of the overburden layers studied by Anderson and coworkers (1989) were 1.69 – 1.77g $cm^{-3}$ compared to 1.53g $cm^{-3}$ in replaced topsoil and 1.29 – 1.70g $cm^{-3}$ at the reference sites. Root development is limiting at bulk densities above 1.7g $cm^{-3}$, suggesting that mine spoils are usually too compacted for proper plant growth.

The use of heavy machinery to grade reclaimed sites can also greatly increase the bulk density by compacting the soil and creating a medium that is impenetrable to plant roots (Jansen and Melsted, 1988) making re-vegetation of the area more difficult. Even when topsoil is replaced, grading can compact the soil to such an extent that an inhospitable medium is created. Bulk density on reclaimed sites in Indiana where topsoil was replaced ranged from 1.54 to 1.89g $cm^{-3}$, much higher than on comparable undisturbed sites (1.29-1.70g $cm^{-3}$) (Bussler et al., 1984). On these sites the topsoil layer had the lowest bulk density, 1.54g $m^{-3}$. Soil porosity, another vital factor for development of root systems, was considerably lower (37.3-28.6%) than undisturbed soils (41.6-28.2%) (Bussler et al., 1984). Once again, it was the topsoil of the mined site that had the greatest porosity, 37.3%, despite being lower than the undisturbed soil.

The replacement of topsoil is not always an improvement, however, as it can be greatly compacted by grading. When topsoil is compacted it can be an inferior growth medium to mine spoil. Vogel and Gray (1987) found that tree survival was only 36% on mine spoil but was even lower, 4%, on topsoiled sites. This was thought to be a direct result of low water holding capacity due to compaction from grading. Ashby (1998) also found that hardwoods survived poorly on graded topsoil. Ungraded and graded mine spoil plots had higher survival and growth rates than the graded topsoil sites.

Torbert and Burger (1990) found that these higher bulk densities due to grading can limit tree growth. Sycamore trees had a 70% survival rate on moderately graded and ripped sites while survival was only 50% on intensely graded sites. Growth was also much higher on the ripped and less intensively graded sites. In a later study they found a tree survival of 42% on graded sites compared with 70% on un-graded sites (Torbert and Burger, 1996). This was thought to be a result of low water holding capacity and the restriction of root growth due to compaction from grading. Often machinery must make several passes over the soil in order to spread it uniformly. These repeated passes may be even more detrimental to the quality of the soil than some amount of non-uniformity that might occur with one pass (Doll, 1987).

Current procedures of stockpiling topsoil before it is replaced may also severely compact the soil (Holland and Phelps, 1986). This is a present concern that not only destroys soil structure, making a poorer plant growth medium, but also may limit growth and survival of soil organisms due to poor aeration (Harris and Birch, 1989).

*Water availability*

Water availability can also be much different on mine spoils, leading to droughty or waterlogged conditions that can cause tree mortality. Rodrigue and Burger (2004), in a study of 14 mined sites across seven states, found it the third most important factor for tree growth on a range of older reclaimed sites. However, they found that on many reclaimed sites, available water holding capacity can be higher than on adjacent native soils due to a deeper soil profile. Thurman and Sencindiver (1986) also found high water availability on reclaimed sites compared to undisturbed sites in West Virginia.

On the other end of the spectrum, soils on reclaimed sites can be waterlogged due to poor hydraulic conductivity. Mine soils have poor structure with few macropores. As a result, water does not flow well and often saturates the soils or even puddles on the surface.

*Coarse fragment content*

Coarse fragment content is also an important factor, with tree growth inversely proportional to rock content. Rodrigue and Burger (2004) found that it was the second most important factor affecting tree growth across a range of reclaimed sites. They found that coarse fragment content can be very high, ranging from 14-83%. Rock fragments decrease the amount of fine earth available to roots, which, in turn, reduces the water holding capacity, fertility and volume available to root penetration (Torbert et al., 1990; Childs and Flint, 1990; Thurman and Sencindiver, 1986; Rodrigue and Burger, 2004).

*Organic matter*

Brenner and colleagues (1979; 1984) found that organic matter and soil moisture were the primary factors limiting tree and shrub establishment. Temperature and soil moisture also influence germination and seed establishment rates (Bell and Ungar, 1981). The study by Bell and Ungar (1981) dealt with a survey of 30 different tree and shrub species which showed overall low success when planted in strip mine spoils. Another key factor is soil depth, having a strong direct correlation with growth of eastern white pine (Andrews et al., 1998).

*Lasting effects*

Poor physical soil quality is not just a matter of present concern but has been shown to persist for extended periods. After 31 years, diversity of a planted tree stand

on coal spoil in southeastern Ohio decreased (Carter and Ungar, 2001). Of the species planted, some died and there was very little invasion by other species, resulting in a very low diversity. Factors believed to contribute to the lack of establishment included high soil temperature and low moisture availability (Carter and Ungar, 2001).

Chemical properties

Chemical properties also vary among spoil types. Mine spoils are commonly very low in fertility, high in salts, contain toxic levels of trace metals and can have extremely high or low pH. These problems are a function of spoil type and have greater or lesser impact depending on vegetation type.

*pH*

The pH of mine spoils also differs from that of topsoil. Often it is more acidic, although some mine spoils are more alkaline than the native topsoil. This difference in pH can influence microbial communities, preventing growth of native mycorrhizae or bacteria while promoting growth of other organisms not found on undisturbed sites. This change can have an important impact on native tree growth.

One of the greatest limiting factors in much of the Appalachian area to re-vegetation is low pH due to the oxidation of pyrite (Bell and Ungar, 1981; Johnson and Skousen, 1995). pH can greatly restrict plant growth by increasing aluminum toxicity. pH also limits microorganisms. Earthworms only occur in soils above pH 3.5. Other important microorganisms are influenced by pH, and community composition shifts greatly depending on the pH of the soil (Wei- Chun Ma 1989).

Acidity is not a problem with spoil materials consisting of limestone low in pyretic materials. In fact, mine soils can be too alkaline for native trees. In Alabama,

Cross and colleagues (1988) found that grasses grew well on alkaline mine spoils but that native trees were slow to establish, with only patches of Virginia pines and winged sumac after 12 years. In Ohio, Kost and colleagues (1998) found that replacement of more acidic topsoil on calcareous overburden increased survival and growth of black pine, and green ash at age nine. Other species did not survive well on any of the plots. The success of these two species was attributed to the topsoil's lower pH, higher water holding capacity, and lower level of salt compared to the alkaline rocky mine spoils.

Thomas and colleagues (2001) examined the chemical properties of several sites in the same region of southern West Virginia and found that pH in mine spoils of ages two, seven, and eleven were all higher than in native soils. Only the 23-year-old mine spoil had a pH of 4.6 compared to 4.7 on the native soil. He also found that N and P levels were higher, though this was probably a result of high levels of carbolithic material, which is not available to vegetation or microbial communities. There is very little literature on alkaline mine spoils and their effects on native tree growth. This is currently a point of contention in the mining community, as some view this alkaline medium as a superior growth medium while others do not.

*Fertility*

Mine spoils often have an inadequate supply of available nitrogen and phosphorous causing nutrient deficiencies in trees. Furthermore, they are initially devoid of organic matter or microbes. Because the majority of nutrients are held in the organic matter and microbial pool, even added fertilization is quickly lost from the system. However, as plants establish and a microbial community develops, nutrients also

accumulate. Reeder (1977) found very low levels of N mineralization in fresh mine spoils and shales compared with older spoils and soil.

Many mine spoils are often high in geogenic organic matter in the form of coal, but the N and C in most of these materials cannot be directly used by vegetation (Waschkies and Huttl, 1999). Other nutrients may be found in these mine spoils, but they are often contained in rocks and cannot be accessed by roots or microbes.

Mine reclamation operations have used fertilizers along with the addition of sewage sludge and other soil amendments to increase available nutrients. However, these additions are often a temporary solution and an overall decline in available nutrients usually occurs after several years (Daniels and Zipper, 1999).

*Soluble salts*

Alkaline spoils are also often high in salts. Although salt levels must be very high to effect crops, they only need to be slightly elevated to effect tree growth (Torbert et al., 1988). Many mine spoils have salt levels higher than this threshold, adversely effecting tree growth.

Salinity also inhibits the establishment of microorganisms. Earthworms were inhibited with salt concentrations above 7mmho/cm and collembolan were inhibited above 8mmho/cm (Wei-Chun Ma, 1989).

### *Quantifying Success of Trees and Soil Interactions on Reclaimed Sites*

It is clear that the physical and chemical properties of mine spoils have an important impact on tree growth. In order to understand how different strip mine spoil materials and reclamation practices may affect the success rates of native hardwoods, one

must quantify the effects of site and mine spoil properties on tree growth. Characterization of site properties and physical, chemical and biological soil properties can be used as the independent variables and tree growth as a dependant variable in a regression analysis. This works well if a wide variety of mine spoil types are being examined.

Another option for examining the suitability of mine soils for trees is to examine foliar nutrient levels. One of the most important aspects of proper tree growth is adequate nutrition. Mine spoils are void of organic matter, the main source of nitrogen within a forest system. Thus all nutrients must come from the spoil parent material, dry and wet deposition, nitrogen fixation or fertilization. Although nutrient availability can be found by analyzing soil, in order to understand how much of these nutrients are actually being utilized by the tree, one can perform a foliar analysis.

The Diagnosis and Recommendation Integrated System (DRIS) was developed in order to better quantify nutrient imbalances and deficiencies in plant foliage (Beaufils, 1973). This analysis involves finding levels of different nutrients in leaf tissue of an array of trees growing on different soils. The levels and ratios of the nutrients in the leaves of the trees that are most successful are considered the optimum. All other nutrient levels are lacking in some way, decreasing the success of the other trees.

By using a combination of these techniques, a better understanding can be gleaned of what spoil properties are essential to tree growth. By quantifying this information, the potential value of spoil selection, topsoil replacement and site treatment can be evaluated.

### *Soil Biota and Soil Physical and Chemical Interactions*

In addition to the diverse collection of native hardwoods, another essential part of the Appalachian ecosystem is the soil organism community. These microorganisms have an integral connection with the chemical and physical properties of the soil and thus the growth of vegetation.

Through looking at primary succession on sites with extreme conditions, the critical impact of microorganisms can be seen. These extreme examples are simple, slow moving systems with few species allowing us to better tease apart the role that microbes play in primary succession (Wynn-Williams, 1993). In extremely arid, cold, or nutrient poor soils, bacteria are the first and often the only colonizers for a number of years. These sites have much too extreme conditions to support any other life and even the bacterial populations are small. These microorganisms help weather and free nutrients from the soil and are also important in the formation of soil aggregates (Wynn-Williams, 1993). It is only after the soils have weathered to a certain extent and enough nutrients and soil stability are present that other organisms can establish themselves.

There are a range of organisms that live and interact in topsoil. They can be divided into three categories: microfauna (< 200 μm length), such as nematodes and bacteria, mesofauna (200 μm – 2 mm), such as Collembola, mites and fly larvae; and macrofauna (2 – 20 mm), such as earthworms, ants and termites (Abbott, 1989).

Soil biota and the physical and chemical properties of soil are integrally connected. With a decrease in soil quality, the number and diversity of soil microorganisms decreases (Hutson, 1980a; Hutson, 1980b; Moore and Luxton, 1986; Blair et al., 1996; Topp et al., 2001; Mummey et al., 2002a). At the same time,

microorganisms have been shown to increase soil quality (Blair et al., 1996; Boyle, 1996; Moldenke et al., 1996; Topp et al., 2001).

This information suggests that microbes also play an invaluable part in primary succession of more complex systems such as reclaimed strip mines. Appalachian strip mines, although not as extreme as the arctic or arid desert, are low in several plant-essential elements and have poor soil structure. As microbial populations improve the soil, their presence will lead to better plant success.

Importance of soil biota to soil and plant health

Soil physical and chemical properties are manipulated and conditioned by soil organisms in a variety of ways. Decomposers play an essential role in nutrient cycling by breaking up plant litter and other detritus and returning it to a mineral form that can be utilized by plants (Hutson, 1980b). They are also important to the formation of good soil structure by mixing, aerating and creating more stable soil aggregates (Abbott, 1989). Mycorrhizae form essential symbioses with plant roots, increasing root area and making nutrients and water more available. Nitrogen fixers also play a vital role by increasing levels of nitrogen in the system.

Microarthropods such as Collembola have a varied diet that includes detritus and leaf litter. They shred and break up these materials into a form available to other organisms. They also eat bacteria, fungal hyphae and other living organisms. This releases the nutrients that these organisms tie up and maintains the balance of different biota in the microcosm. The material consumed by Collembola is converted to feces, which is a more available form of nutrients for smaller organisms such as bacteria and fungi. These organisms are then, in turn, consumed by nematodes and protozoa (Hutson,

1980a). This cycling has the end result of breaking up litter into its mineral constituents as well as regulating the extent to which nutrients get tied up in any one organism.

Mineralogical nutrients are then available to the large surface area of the mycorrhizal fungi which transport them to plant roots (Moldenke et al., 1996). Mycorrhizal fungi can greatly improve tree success through nutrient uptake (Hutson, 1980b). This complex system of micro-, meso- and macrofauna are essential for nutrient availability and uptake needed for a healthy plant community (Boyle, 1996).

Soil organisms, such as earthworms, also improve soil structure through burrowing and integrating organic mater into the soil (Moldenke et al., 1996). Earthworm species that cast at the surface also increase soil porosity. Burrows are the proper size to allow fast drainage (1-10 mm diameter), and are beneficial for decreasing erosion. Finer pores are created by earthworm casts and are important for increasing water holding capacity. Ants and termites also increase porosity in the soil from tunneling to form mounds. Soil perturbation by ants, termites, and earthworms also helps to incorporate organic matter decreasing bulk density (Abbott, 1989).

Earthworm casts are also an important soil aggregate as they are covered in a mucilaginous material produced by bacteria in the gut of the worm that helps to hold the soil together. This increase in soil aggregation and porosity and decrease in bulk density caused by these macrofauna improve soil quality and subsequent root growth (Abbott, 1989). Earthworms also play an important role in movement of microfauna, and in breaking up litter for the release of nutrients.

Diversity of these soil organisms is also important to soil quality. In an experiment examining the disappearance of litter and bio-perturbation by the earthworm

*Lumbricus rubellus* and the isopod *Porcellio scaber* on strip mined sites, combinations of the two organisms led to an increase in litter disappearance by 271% (Topp et al., 2001). This implies that different organisms may occupy separate niches involved in the breakdown and incorporation of organic matter in mine soils. Thus, greater diversity leads to faster and more thorough development of mine soils.

There is still some debate about whether some forms of native soil biota, such as mycorrhizal fungi, have a significant effect on plant growth on mine spoils. In several earlier studies both trees and herbaceous species inoculated with specific types of mycorrhizae were found to grow significantly better (Marx, 1975; Cordell et al., 1987). A variety of these well adapted species of fungi were found by Kiernan and colleagues (1983) on abandoned older sites and she confirmed their potential importance in reclamation. However, more recent studies have shown that inoculation with native mycorrhizae do not result in a significant increase in plant growth (Andersen et al., 1989; Noyd et al., 1997; Schonholtz et al., 1987). This could be due to the adaptation of non-native species to growth in harsh environments such as mine spoils. The inoculation with native mycorrhizae on the other hand may have little effect due to the harsh environment of mine spoils. However, the reaction of these different types of mycorrhizae may be significantly different on sites where topsoil is replaced. Because the native mycorrhizae would be in more natural soil conditions, they may have a much greater impact. Mycorrhizae is also only one aspect of soil biota and, in combination with the rest of the native soil ecosystem, their effect may be more apparent.

Some organisms that could be reintroduced through the replacement of topsoil may be detrimental to tree growth. The various herbivores that feed on the native trees of

the area may have an advantage on these new sites. Trees would be more susceptible to attack because of the less than optimal growing conditions. This has been seen in black locust which had a greater number of attacks by wood boring beetles on reclamation sites compared to adjacent undisturbed sites (Urbanek, 1989).

Microfauna also play an invaluable role in the soil. They allow for the final breakdown of organic matter into a mineralized form that is available to plants. Almost all nitrogen that becomes available to plants comes from the bodies of bacteria and other microorganisms. These bacteria use nitrogen as an energy source through the process of nitrification. *Nitrosomonas* converts ammonium to nitrite which is then converted to nitrate by *Nitrobacter* (Fisher and Binkley, 2000). This nitrate can then be absorbed by higher plants.

Nematodes occur in large numbers in the soil and are serious pests of some agricultural crops, but little is known of their affect on forest vegetation. Different species occupy a variety of different niches, consuming both living roots and detritus. The ratio of detrimental effects to positive effects of this group of animals is poorly understood, though it is clear that they are an important part of the soil ecosystem. Their populations can be over $10^6/m^2$ and their interactions with other soil biota, plants and the soil itself is very complex (Fisher and Binkley, 2000).

The belowground ecosystem is very complex and poorly understood. Measuring the system is usually done by measuring certain processes such as measuring overall respiration or ATP levels. This provides a measure of the soil biota as a whole, but shifting interactions and changes in species composition are hard to quantify. Organisms such as nematodes and fungi are difficult to identify or isolate from the soil medium.

Thus, although general roles of these organisms are known, an intimate understanding of the complexities of the system is still unknown. As a result, much of the work studying the changes of soil biota on disturbed sites is general, with increases or decreases in biota as a whole. More specific information is being learned dealing with the larger animals such as Collembola, mites and earthworms, but often the smaller organisms are still lumped together as soil biota. However, even with this poor understanding, it is becoming apparent that these groups are invaluable for the proper function of the soil and thus success of vegetation.

### *Most Important Groups of Soil Biota and How to Quantify Them*

The range of types of soil organisms and the activities they carry out in the soil is immense. Predators, herbivores, detritovores, parasites and photosynthetic organisms create a very complex system that interact in countless ways, with vegetation and with the physicochemical properties of the soil. For example, soil organisms affect nutrient availability in three major ways, organic matter decomposition, mycorrhizal symbiosis, and N-fixation. Decomposition is key to the cycling of nutrients while N-fixation adds N to the system. Mycorrhizae are vital for increasing uptake and the availability of these mineralized nutrients. Some of the organisms that carry out these functions, the resulting affects on the soil, and the proper assays to accurately measure these biota are explained below.

Decomposers

As mentioned above, a variety of macro and micro organisms contribute to the decomposition of soil organic matter. These materials are made up of sugars, proteins and carbohydrates that are more or less easily broken down. Over time, different components are broken down by soil fauna and flora, slowly mineralizing nutrients and making them available for plant uptake. This process is essential to the cycling of nutrients through the system.

The group of organisms that contributes to the breakdown of organic matter is extremely varied. They range from fungi to earthworms to microarthropods to bacteria. As a result, their roles are difficult to quantify. Any sort of general microbial assay would also incorporate predators, herbivores, symbiointes and other organisms that may not directly contribute to decomposition. However, these organisms play a critical role in soil ecology, and many of the other organisms would not be there without their presence. Thus general assays may still be a significant way of enumerating these decomposers.

Most assays give total biomass of organisms in the soil. Chloroform fumigation-extraction is a commonly used technique but it is only able to quantify about 30% of the organisms (Kunc, 1994; Tate, 2000) (Appendix I). It also gives no specifics of what type of organisms there are, but it does give a general estimation of total biomass. Other techniques such as ergosterol, muramic acid, and glucosamine biomarkers can measure a specific type of organism (Bentham et al., 1992; Kunc, 1994), such as bacteria or fungi, but a whole array of different tests must be conducted to get a complete picture of the soil biota. The fatty acid methyl ester (FAME) analysis is able to tease apart gram positive and gram negative bacteria, fungi, protozoa, and eukaryotes (Mummey et al., 2002a;

Mummey et al., 2002b). This would allow at least some general characterization of the organisms in the soil, although the species composition would still be unknown. However, this procedure is still very time consuming and can only reasonably be done for small groups of samples. ATP analysis can be performed to estimate the total activity of soil organisms (Kunc, 1994; Tate, 2000) (Appendix I). This activity has often been found using respiration analysis. However, respiration measurements should not be performed in this situation due to the alkalinity of the mine spoils. High soil calcium carbonate levels can skew results by overestimating activity levels. ATP analysis gives a similar measure of activity (Kunc, 1994; Tate, 2000). Dehydrogenase can also be measured to get an estimate of enzyme activity important to mineralization of nutrients needed for tree growth (Tabatabai, 1982; Dick et al., 1996) (Appendix I). Because these enzymes are only produced by decomposers, a better estimate of decomposer populations can be made.

With the use of these three techniques, triangulation can be used to pinpoint the proportion of decomposers and the total biomass as well as the total activity of soil organisms in a system. Microbial populations can then be correlated to tree growth and possible relationships between the two can be examined.

Mychorrhizal fungi

As mentioned above these organisms increase the absorptive surface of roots. There are two types of mycorrhizae, endomychorrizae and ectomychorizae. There is a large energy trade off on the part of the host plant for supporting these fungi. However, the benefits they bring through the increase in nutrient influx outweigh the costs. Due to

the small diameter of mycelia, they take much less energy to cover a large area than would even fine roots, which have a much greater diameter (Aber and Melillo, 1991).

Due to their importance for tree growth, it is essential to be able to detect their presence and extent. Ectomychorrizae can be seen by the naked eye, and thus their presence or absence can be determined. Endomychorrizae, however, cannot. Their presence or absence can be determined using a staining method. Estimates of the levels of these organisms can also be gained by the analyses used for quantifying decomposers. The total amount of fungi in the soil could be known from the FAME analysis. This would include decomposer fungi but would give a rough estimate of these organisms. It is very difficult to distinguish between mycorrhizal fungi and other types of fungi.

N-fixation

N-fixation is a vital function of microorganisms. These organisms are often introduced to reclamation sites through the use of legumes or other symbiotic plants. This genus of bacteria is *Rhizobium;* it is an obligate symboint protected by plant roots in nodules that shield them from oxygen and acidity (Aber and Melillo, 1991). However, some N-fixing organisms are free living. Some of these N-fixers are photosynthetic cyanobacteria. Others use large amounts of energy from the decomposition of organic matter, such as *Clostridium* and *Azobacter* (Fisher and Binkley, 2000). They often live in the rhizosphere and live off carbon produced by roots. In the soil at large, they may not fix large amounts of nitrogen, with rates of <0.01 to 1.4 kg $ha^{-1}$ nitrogen annually (Fisher and Binkley, 2000). However, in a severely deficient system such as mine spoil that has

little or no nitrogen and very little organic matter, any source of nitrogen input is extremely valuable to the success of native hardwoods.

N-fixing bacteria are difficult to quantify. All current techniques tend to have high error rates. The most accurate technique for measuring small amounts is the acetylene reduction assay (Weaver et al., 1994). This assay may need to be performed over a longer period due to the trace amounts of organisms in new mine soils. It tends to become less accurate the longer it is run; however, relative amounts between different soils can still be estimated.

***Soil Biota in Mine Spoils verses Topsoil***

Mine spoils alone have very low levels of organisms both in terms of diversity and number. Very early on, Visser found that fungal hyphae were much lower in disturbed mine spoil. Bacterial numbers were also much lower than in the undisturbed A horizon of the native soils. These bacteria were also different in numbers creating a different composition in the mine spoils . Hutson (1980b) found significantly greater levels of degradation of oak leaves on a control site verses an industrial reclamation site. This lack of soil organisms in mine spoils may result in poor soil development and thus lower tree survival and growth.

The replacement of topsoil increases diversity and number of soil biota on surface mined sites (Moore and Luxton, 1986). It also reestablishes biota common to developed ecosystems without the need of the complete succession of pioneering species. Topsoil holds many of the organisms found in the undisturbed forest soil prior to strip mining as well as providing an environment conducive to reestablishment of these organisms (Wanner and Dunger, 2002). In his discussion of techniques for reclaiming with native

hardwoods, Miller (1998) states that the development of a healthy and diverse soil microorganism population through replacement of topsoil is essential to the establishment of native trees.

There has been some controversy as to whether topsoil is the best way to increase numbers and diversity of soil organisms. In New Mexico, Elkins and colleagues (1984) found that topsoil had little effect compared to amendments with bark. This, however, may be due to the arid conditions and low amount of organic matter in topsoil of the area. Other studies have demonstrated the qualities of amending mine spoil with sewage sludge for the increase in soil biota.

Some microorganisms are able to endure harsh environments and reestablish in an area quickly (within a year) (Moore and Luxton, 1986). However, even organisms that can establish on the inhospitable conditions of mine spoils, such as Collembola and trombidiid mites, colonize areas with higher organic matter much more quickly (Wanner and Dunger, 2002). Giving these organisms a head start allows them to play a major role in litter breakdown and increase in nutrient availability for vegetation in the critical time period when trees are first planted. Also, this higher level of diversity leads to a healthier and more stable ecosystem which could in turn lead to greater productivity.

A greater diversity of Collembola has been found in reclaimed sites using topsoil verses shale (Moore and Luxton, 1986). It is essential to encourage the establishment of these organisms on reclaimed land. In an experiment in England, most litter breakdown on strip mined sites was thought to result from these pioneering microarthopods. Different sized mesh bags containing oak leaves were buried and mesh sizes that allowed Collembola and larger organisms in had the greatest litter breakdown. Mesh sizes that let

in only smaller organisms had lower levels of breakdown. These reclamation sites did not contain earthworms, and litter breakdown was much slower than on reference sites with earthworms (Hutson, 1980a).

Comparison studies of undisturbed soils and reclamation sites where topsoil has been replaced has shown that even after extended periods, in this case 20 years, microbial biomass on mined sites averaged only 20% of undisturbed sites (Mummey et al., 2002a). Although this is low, it is much higher than on sites where the topsoil has not been replaced and may be due to other factors such as soil compaction.

Another comparison study between microbes on sites with topsoil, sites without, and undisturbed reference sites demonstrated that sites with topsoil had comparable biota to undisturbed sites. Enzyme activity after three months was also equivalent to the undisturbed soil. Non-topsoiled sites, on the other hand, had much lower levels of microbial populations (Fresquez et al., 1986).

Microbial respiration should decrease with increasing stabilization of the system. Native forests have very low levels due to the equilibrium that is reached. Stephens and colleagues (2001) found an inverse correlation between mine soil age and microbial respiration. With an increase in mine soil age there was a decrease in respiration, except for the very young site (two years). Although these lower levels of respiration are ideal, higher levels are needed initially in order to attain the needed balance.

One problem with the reestablishment of some soil biota is motility. While aerial colonizing organisms established quickly, organisms moving from the adjacent substrate took much longer (Wanner and Dunger, 2002). As topsoil would have many of these organisms in it initially, substrate-moving organisms such as *Testae amoebae* would be

able to establish quickly as they may already be present in the topsoil. In reclaimed areas without topsoil, these organisms would have to move in from adjacent areas. This is also true of earthworms. A site that had good conditions for earthworm establishment had none because they had not been introduced. Once artificially introduced, the earthworms established quickly. Similarly, earthworms might not establish on strip mined lands, even under ideal conditions. If, however, they were introduced with the replacement of the topsoil, they have a better chance of re-establishing (Abbott, 1989).

Even if some organisms are killed in the movement of the topsoil, replacement of this material is also an important factor for creating a conducive environment for the immigration and establishment of organisms. Immigration of *testae amoebae* was correlated to amount of organic matter in the substrate (Wanner and Dunger, 2002). Topsoil has more organic matter than mine spoils and thus suggests a favorable environment for establishment.

Grading can also result in an alteration in the success of soil fauna. Grading results in a very flat surface on which soil fauna must establish and survive. A study in the Rhineland demonstrated that surface patterns greatly affect the success of soil fauna. On landscapes consisting of crests and troughs, troughs were found to have much higher diversity and number of soil fauna. This is thought to be a result of a sheltered microclimate (Topp et al., 2001). On graded land, these microclimates do not exist. Instead the land is completely flat and exposed. If one pass strike off is used, the soil is not compacted down and flattened and these small microclimates would remain allowing for a better chance of invasion and establishment by soil biota.

Grading and other site prep can also alter basic physicochemical properties of the soil that are vital to the survival of soil biota. Water is one of the main factors affecting the number and type of soil biota. Water is an essential medium for biota to survive and proliferate, and conditions that are too dry can decrease activity substantially. As was already mentioned, mine spoils opposed to topsoil as well as grading opposed to not grading can alter the levels of water and thus hinder microbial populations (Fisher and Binkley, 2000).

Soil pH can also have an extreme impact on the soil biota. Restrictions have been well noted for acidic mine spoils, but basic soils may also limit some organisms. Actinomycetes, cannot live in soils above pH 5 (Fisher and Binkley, 2000). These organisms are not well understood, but are known to be important in decomposition of organic matter. Shifts in species composition may be substantial with a change in pH. Fungi are often dominant in acidic soils while some types of bacteria cannot survive at a low pH. The change in species composition due to a change in pH could have serious repercussions for other aspects of the environment that interact with the soil biota such as the vegetation and the soil itself.

As explained above, these soil properties are vital for native tree growth. Soil organisms are an essential manipulator of these physiochemical properties for the development of a suitable medium for native tree growth and survival.

Over all, there is enough evidence for the ability of topsoil to increase soil biota to make it an important and valuable means of reclamation. It has been demonstrated that topsoil initially introduces some organisms to the environment as well as creating an environment conducive to their colonization.

However, it is difficult to tell how much soil is needed to improve the reclamation medium enough to significantly increase soil biota. Lindemann and Fresquez (1984) found that topsoil inoculations of 14.5 Mg/ha did not have a significant impact on biological activity. Topsoil spread at a depth of 30cm, however, significantly increased mycorrhizal infection, along with other microbial numbers and activity. They also tested the effects of adding sewage sludge or alfalfa and concluded that the addition of carbon was more important than the inoculation with microbes (Fresquez and Lindemann, 1982). The topsoil they used had been stockpiled, which can significantly reduce microbial activity. Topsoil that is not stockpiled may have higher levels of organisms and may thus be more effective for inoculation of mine spoils.

On the other hand, Thorne and colleagues (1998) found that topsoil proved to be an important source of mycorrhizal inoculum, as it was very effective at increasing levels of nodules on roots compared to mine spoils without topsoil additions.

As noted, many essential decomposers have difficulty colonizing mine spoils. Without their presence, the breakdown of organic matter is greatly slowed. This could be the reason for the increase in litter layer on many mine sites compared with the adjacent mine sites. Organic matter in its raw form has nutrients that are unavailable for plant uptake. Without facilitation by soil organisms it decomposes extremely slowly. Without decomposers, organic matter is not incorporated into the soil where it could aid in the development of physical properties.

Absence of mycorrhizal fungi can lead to a substantial decrease in a plant's ability to absorb water and nutrients. In a nutrient poor environment, the lack of mycorrhizae could be the difference between stunted/dead trees or successful tree growth.

Although there has been little study on free living nitrogen fixers, they are known to add small amounts of nitrogen to the system. In a system that has no labile nitrogen pool and few other nitrogen inputs, this small source of nitrogen could have disproportionate value. Their presence could add that essential N to a starved system that would allow better establishment.

### *Combining All Factors of the System*

Current strip mine reclamation practices are not conducive to the return of the native ecosystem that was there prior to mining. Many of the native hardwood species cannot thrive in the harsh conditions of strip mine spoils. Other native herbaceous and woody species are unable to establish and microbial and macro-invertebrate populations are diminished in number and diversity. This is a result of the poor physicochemical properties of these spoils. These materials are taken from deep in the geologic profile and are often very different from the native soil of the area. This leads to the slow return of the native ecosystem to these areas.

It is clear that in order to improve spoil selection and treatment for the successful reestablishment of the mixed mesophytic forest, interactions of tree, herbaceous, and microbial growth with different spoil physicochemical properties must be further examined. It is clear that soil properties have an important impact on the return of the ecosystem, but how all of the different soil properties, the vegetation and the soil organism populations interrelate is not well understood. This is especially true of the native hardwood systems in the Appalachian region. By teasing apart these factors, a better understanding of what factors are critical could lead to an improvement in

reclamation practices. This may result in the speeding up of the return of the native ecosystem leading to improved ecosystem and economic benefits.

Also, because soil biota can have such a great impact on the creation of soil properties, severe disturbances caused by strip mining may be greatly reduced over a period of time with the presence of these organisms. However, the severe disturbance itself may destroy these organisms. It is thus imperative that researchers take all three factors, soil biota, soil physicochemical properties and the growth of vegetation, into consideration. There is no direct cause and effect relationship. Instead each part is affected and, in turn, influences the system as a whole.

In conclusion most mine spoil physiochemical properties are inadequate to support a full complement of native hardwoods and must be amended. Of the various soil amendments, topsoil has the unique property of containing native soil organisms. Investigation of these organisms show that they are vital to the development of a healthy soil and proper growing conditions for native trees.

# CHAPTER III.

# GROWTH OF THREE APPALACHIAN HARDWOODS IN DIFFERENT MINE SPOIL TYPES WITH AND WITHOUT TOPSOIL INOCULATION


## Abstract

The goal of many landowners who own reclaimed mined land is to restore the diverse mixed mesophytic forest for environmental, economic, and cultural reasons. However, native hardwoods tend to grow poorly on mined sites due to their physical, chemical and biological mine spoil properties. A 4x2x3 factorial greenhouse experiment was conducted with one-year-old seedlings. We examined the suitability of four growth media: undisturbed forest topsoil (UFT), brown, weathered sandstone (BWS), white, unweathered sandstone (WUS), and gray, unweathered shale (GUH), as well as the effects of inoculation with topsoil (none verses inoculated), on the growth of three native hardwood species: *F. americana, Q. rubra,* and *L tulipifera*. Tree growth, foliar nutrients, and soil properties were measured and characterized. The BWS was the mine spoil material most conducive to growth for *F. americana* and *Q. rubra.* *L. tulipifera* did not respond to any treatments. Foliar nutrient analysis indicated that adequate nutrition of *Q. rubra* was independent of spoil type, *F. americana* was somewhat dependant on spoil type for nutrient uptake, and *L. tulipifera* was highly dependent, ($p=0.49$, $p=<0.0001$ and $p<0.0001$, respectively). Topsoil inoculation significantly increased growth on the GUH spoil type, but not the WUS or BWS spoil types. Topsoil inoculation significantly increased the number of herbaceous plants growing in the pots and improved foliar nutrient indices in *F. americana* and *L. tulipifera*. Many properties, such as pH, microbial activity, and water availability of the BWS more closely approximated the

control soil than the white sandstone or shale. The results of this study show that trees are sensitive to spoil type and that certain spoil types should be selected during the reclamation process. Topsoil inoculation should also be considered as it may increase tree growth on some spoil materials, improve tree nutrition and help return the diverse native plant population that was present prior to mining.

## Introduction

The native hardwood forest of Appalachia consists of a rich collection of vegetation that plays an essential role in the economy, esthetics, environmental biodiversity, and culture of the Appalachian Mountains. Many of the late successional hardwoods are valuable timber species, playing an important role in the timber economy of the area, while other tree and herbaceous species have cultural and environmental importance. Many of the understory plants are gathered for medicine or food, such as ginseng and ramps (Duke, 1997; Jones and Lynch, 2002). Basket weaving and doll making are also examples of nontimber forest products in Appalachia (Alexander et al., 2002). The diverse wildflower populations are important esthetically and environmentally. From the overstory come sourwood and basswood honey, which are valued for their unique specialty flavors (Hill, 1998). Large mast trees such as oaks and hickories are not only important timber species but supply food for wildlife. Many of the state flowers and trees of the seven states in the Appalachian region are symbols of the diversity and beauty of the native hardwood forest in this region. They include flowering dogwood (*Cornus florida*), tulip poplar (*Liriodendron tulipifera*), hemlock (*Tsuga*

*canadensis*), rhododendron (*Rhododendron maximum*) and mountain laurel (*Kalmia latifolia*).

This mixed mesophytic forest is dominated by beech (*Fagus grandifolia*), basswood (*Tilia americana*), sugar maple (*Acer saccharum*), sweet buckeye (*Aesculus octandra*), red oak (*Quercus rubra*), white oak (*Quercus alba*), and hemlock (*Tsuga canadensis*). Birch (*Betula lenta*)), black cherry (*Prunus serotina)*, cucumber tree (*Magnolia acuminate*), white ash (*Fraxinus americana*) and red maple (*Acer rubrum*) also comprise a large portion of the forest, with black gum (*Nyssa sylvatica*), black walnut (*Juglans nigra*) and hickories (*Caria*) present, but not abundant (Braun, 1950). In addition to these 15 are another 22 species that occur in different regions (Braun, 1950). This forest is unique in its diversity and an invaluable asset to the people of the region.

The mixed mesophytic forests are currently being eliminated over large areas by surface mining. Over 500,000 ha have been affected by strip mining in the Appalachian region since the implementation of SMCRA in 1978 (OSM, 1999). Traditionally, these areas are graded and hydroseeded with non-native herbaceous vegetation, reclaiming them to grassland. However, there has been a recent shift towards reforestation of reclaimed mined land (OSM, 1999). These reforestation efforts, although a step closer to the return of the native forest, usually involve planting monocultures of early successional species. For example, black locust is able to survive and grow (Vogel and Berg, 1973; Filcheva et al., 2000), but planting this species leads to forest stands with little or no diversity. Because of the competitive herbaceous vegetation and the physical and chemical properties of the mine spoils, many of the native species of the mixed mesophytic forest are unable to establish themselves where they once occurred in

abundance. Although early successional species such as black locust, autumn olive, and Virginia pine may return some forest cover, the value of the forest and its future potential, economically, environmentally, culturally, and esthetically, is largely degraded.

When attempts have been made to plant a more diverse array of native hardwoods, including the oaks, sugar maple, and black cherry on reclaimed mined sites, their survival and growth is often poor. Mined sites have many of the characteristics typical of primary successional sites. The two most important factors dictating plant establishment in primary succession is the ability to seed into the area and the subsequent ability of the plant to germinate, emerge, and grow in the given harsh conditions (Chapin, 1993). Some early successional plant species that are wind and bird disseminated, and have an opportunistic growth habit, are better adapted for reclaimed strip mines. However, many of the native species that occur in the mixed mesophytic forest are less likely to become established on mined sites, and, when they do, they do not grow well because they cannot tolerate mine spoil conditions.

In order to seed into a large barren area, a species must have a light seed that can travel great distances. Primary successional species are well adapted to this with small winged or cottony seeds. They are thus able to seed into the center of even large mines. However, late successional species often have large heavy seeds that do not travel the distances needed to seed into these areas.

An alternative seed source is the seed bank of the native forest topsoil. This may be an invaluable source for the return of these late successional native species. After only one growing season, seed banks from native topsoils placed on mine spoils have been shown to produce $1.9 \times 10^6$ shoots $ha^{-1}$, with a diversity of 134 taxa (Farmer et al., 1982).

Seed banks may not contain all the target late successional forest species, but they are of a later sere and are higher in native species than the primary successional seeds that are brought with the wind. Seed banks may thus lead to a much higher seedling survival rate and faster development of a native ecosystem.

The other aspect of establishment on these sites is the ability of plants to survive and grow in the spoil medium. There are a variety of spoils placed on the surface during reclamation and few are selected to maximize tree growth. Mine spoils have highly variable physical and chemical properties, ranging from very acid pyritic materials to alkaline shale. Compared to native soils, mine spoils can have poor chemical properties such as extreme pH, high soluble salts, and low levels of nutrients (Torbert et al., 1990). They can also have poor physical properties such as low moisture content or porosity, poor structure or high bulk density and high levels of rock fragments (Bussler et al., 1984). Within this array of spoil types some are probably more suited to the growth of native forest species.

With current reclamation practices consisting of hydroseeding with grasses and legumes or planting with early successional trees, the return of late-successional native forests may take several hundred years. In order to quickly restore the late successional forest, it may be possible to skip the first few stages of succession. Our overall goal is to reduce this timescale by creating a hospitable environment for reforestation and natural forest processes. This may require a spoil medium conducive to the reestablishment and growth of later successional species, returning the soil seed bank, and inoculating the spoil with forest soil organisms.

Therefore, as part of our work toward this broad goal, the objectives of this study were: 1) to determine the relative suitability of three different mine spoil types for hardwood growth, 2) to determine if topsoil inoculation improves tree growth, 3) to evaluate the establishment of native herbaceous understory plants from spoils inoculated with topsoil.

## Methods and Procedures

### Soil and Treatment Characterization

Mine spoils for this study were collected with the help of the Pritchard Mining Co., whose mine is located south of Charleston, West Virginia. This surface mine uses a combination of contour and mountain top removal mining. The area was forested with the Appalachian oak forest type (Braun, 1950) previous to mining. Reclamation practices at this mine involve returning a variety of rock types to the surface, grading, planting with a non-native grass and legume species mix and planting a variety of tree species. No effort is made to select specific topsoil substitutes for trees, which is typical throughout the Appalachian coal fields.

Three different mine spoils and undisturbed native topsoil were collected for this greenhouse experiment in March 2004. The three spoil types, brown, weathered sandstone (BWS), white, unweathered sandstone (WUS), and gray, unweathered shale (GUH), were taken from various levels of the Kanawha geologic formation and all are used during reclamation as topsoil substitutes depending on their presence during mining. The undisturbed forest topsoil (UFT) was used as the control and was collected from the upper 30 cm of soil of the adjacent forest stand.

The Kanawha formation is 210 m thick at its north end and becomes progressively thicker, reaching 600m at its southern reach in West Virginia (Blake et al., 1994). The sampled area is on the shallower end of this spectrum in Boone county West Virginia. In West Virginia this formation runs through Kanawha, Boone, Fayette, Raleigh, Logan, Wyoming, Mingo, and McDowell counties in the southwestern part of the state. It is comprised of sandstones, siltstones, shale and coal. Because of its numerous coal seams, it is intensively mined.

The native soils on the site are typical of the area. The site is located in an upland region of Clymer-Dekalb-Gilpin soil types, which is strongly sloping to very steep, well drained, and acid, (Soil Survey, 1981). The majority of the slopes were very steep Clymer- Dekalb complexes before mining occurred. Clymer is a fine-loamy, mixed, mesic Typic Hapludult, while Dekalb is a loamy-skeletal, mixed, mesic, Typic Dystrochrept (Soil Survey, 1981). Forest site index for red oak ranges from 65 to 75. The original soil is rarely used as the final growth medium during reclamation. Soils are usually lost deep within the mine when spoils are returned during reclamation.

BWS is the bedrock located directly beneath the soil solum. This weathered rock is the parent material of these forest soils and is normally exploited by deep rooted trees. It has a pH comparable to the native topsoil, is easily weathered when placed on the surface, and occasional has trace amounts of native forest topsoil mixed with it when it is used on the surface as a topsoil substitute.

WUS is also used as a topsoil substitute during reclamation. It contains limestone concretions that are common in shale siltstone and sandstone marine deposits in the area

(Blake et al., 1994). Limestone would suggest that the pH of this soil is much higher than the topsoil. Calcareous sandstone also occurs at the base of these marine layers.

GUH is common in the geologic profile, occurring in several shale members located above the coal seams. Similar to the WUS, GUH is a marine deposit and may have many similar characteristics (Blake et al., 1994). It is, however, much finer grained, creating silty and clayey mine soil material.

Greenhouse Methods

Each of three tree species, *Fraxinus americana* L., *Liriodendron tulipifera* L., and *Quercus rubra* L., were planted in three mine spoil types, BWS (brown weathered sandstone), WUS (white, unweathered sandstone), and GUH (gray unweathered shale), along with the control, UFT, (undisturbed forest topsoil) (Table 1). Half of the 7.57 L pots were inoculated with 2.5 cm of native topsoil, which was spread on the surface (creating a 1:214 topsoil to spoil ratio). This 4x2x3 factorial design was replicated 10 times for a total of 240 pots.

**Table 1**. Greenhouse experiment layout with a 4x2x3 factorial design across UFT (undisturbed forest topsoil), BWS (brown weathered sandstone), WUS (white unweathered sandstone), and GUH (gray unweathered shale) that were inoculated or not inoculated and planted to three different tree species.

| | UFT | BWS | WUS | GUH |
|---|---|---|---|---|
| Not Inoculated | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* |
| Inoculated with Native Topsoil | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* |

The surface of all pots was covered with a hydroseed paper mulch to simulate field conditions and to decrease potential contamination from pot to pot. A watering

treatment of 1.5L was applied to each pot once a week. Trees were planted in May, 2004 and were harvested in October, 2004.

Laboratory Methods

In order to determine which properties of the different spoil types most influenced tree growth, their physical, chemical and biological properties were characterized. Soil samples were collected in October when trees were harvested. The ten reps of each treatment combination were divided into four groups of two or three pots per group. Soil from pots of each group was combined creating four composite samples per treatment combination. Mulch was removed from all pots before combining, but topsoil on the surface of the inoculated pots was mixed into the composite samples. Sub-samples from each composite sample were sieved through a 2mm sieve, kept at 4°C, and processed within 2.5 weeks of collection. An adenosine triphosphate (ATP) procedure was used to estimate microbial activity(ATPlite, 2002). The procedure was modified for the analysis of soils. Soil was placed in a saline solution and shaken for an hour to put microbial populations into solution. The samples were then centrifuged at 1000 rpm to remove particulate matter while allowing microbes to remain in solution. This solution was then used in the standard ATP procedure (ATPlite, 2002). Dehydrogenase concentration was found through a 2,3,5-Triphenyltetrazolium chloride (TTC) indicator (Tabatabai, 1982). Microbial biomass was measured by chloroform fumigation (Anderson and Domsch, 1978; Gregorich et al., 1990; Jenkinson and Powlson, 1976).

The remainder of the composite samples was dried and sieved through a 2 mm sieve and an array of physical and chemical properties was measured. Bulk density was found by weighing the contents of the pot and determining the volume of the soil; coarse

fragment content was determined by weight. Soil particle size was determined using the hydrometer method (Bouyoucos, 1936). Total soluble salts was measured with an electrical conductivity meter (Bower and Wilcox, 1965), and pH was measured in a 2:1 water:soil suspension using a pH glass electrode. Nitrogen availability was measured via aerobic incubation (Bremmer 1965a) and inorganic nitrogen was measured using a KCl extraction method (Bremmer, 1965b). Total nitrogen and carbon were found using a carbon nitrogen analyzer (Vario MAX, 2000 Elementar Americas, Inc., Hanau, Germany). Exchangeable cations were extracted using the ammonium acetate method and determined on an ICP spectrophotometer (SpectroFlame Modula Tabletop ICP, 1997, Spectroanalytical instruments, Germany; Thomas, 1982).

Leaves were harvested from each tree in mid August from the upper portion of the crown, dried at 65°C for 7 days, and ground to pass a 1-mm sieve. Foliar nitrogen content was found using a C-N analyzer (Vario MAX, 2000 Elementar Americas, Inc., Hanau, Germany). Potassium, calcium, magnesium, manganese and phosphorus were determined by dry ashing the samples, extracting using a 6 N HCl solution, and analyzing the extracts with an ICP spectrophotometer referenced above (Jones and Steyn, 1973).

Tree height was measured at the beginning and end of the growing season to determine incremental stem growth. At the end of the growing season, stems and roots were harvested, dried at 65°C for 1 week, and weighed to determine dry incremental stem biomass and total root biomass.

Data Analysis

This experiment was a completely randomized 4x2x3 factorial design that was statistically examined using an analysis of variance (SAS, 2004). Tree growth and spoil

physical, chemical and biological properties were compared among spoil types, between inoculated and un-inoculated pots, and among species.

Foliar samples were analyzed for nutrient sufficiency and balance using the Diagnosis and Recommendation Integrated System (DRIS) (Beaufils, 1973; Walworth and Sumner, 1987). This analysis involves comparing the variance in foliar nutrient levels and nutrient ratios between the subpopulation of trees that are most successful and the rest of the population. Mean nutrient ratios from a collection of different studies were used as a standard by which imbalances were assessed. Nutrient levels and ratios of trees that were growing well in each of these studies were used (Brockway et al. 1979; Burg, 1979; Costea et al., 1984; Farmer et al., 1970; Fiedler et al., 1973; Furlan et al., 1983; Gouin and Walker, 1977; Henry, 1973; Heinsdorf, 1975; Heinsdorf and Krauss, 1974; Höhne, 1978; Johnson et al. 1982; McClenahen and Dochinger, 1981; Messanger, 1975; Mitchell and Chandler, 1939; Schomaker and Rudolph, 1964; Trillmilch and Uebel, 1982; Wood et al., 1973). The high and low of the good population from each study were averaged to get an overall mean across studies. These levels and ratios were used as 'norms' for comparison with nutrient levels and nutrient ratios of trees in this experiment.

A nutrient index of sufficiency or balance for a given foliar nutrient level 'A' is developed using the following equation:

$$\text{Nutrient 'A' index} = \frac{[f(A/B) + f(A/C) + \ldots. + f(A/N)]}{z}$$

where

$f(A/B) = ((A/B)/(a/b) - 1) \times (1000/CV)$, when $A/B \geq a/b$,

or,

$f(A/B) = (1 - (a/b)/(A/B)) \times (1000/CV)$, when $A/B < a/b$,

where A through N are the levels of foliar nutrients of interest, A/B is the nutrient ratio of the target population, a/b is the nutrient ratio of nutrient A to nutrient B for the 'norm' from the literature, z is the number of functions in the equation, and CV is the coefficient of variation of the norm (Walworth and Sumner, 1987). The closer the nutrient index is to zero, the closer it is to the level of the good population or the optimum. If it is negative, the nutrient is deficient, and if it is positive, the nutrient is in excess. The sum of the absolute values of the nutrient indices were added to obtain a nutrient balance index (NBI). The higher the number, the greater the nutrient imbalance, and the poorer the tree growth. If the NBI is close to zero, foliar nutrient concentrations are close to optimal.

In this study we used foliar nutrient norms compiled from the literature to determine nutrient deficiencies and imbalances within single populations of young *F. americana, Q. rubra,* and *L. tulipifera* . This analysis was done to find relative levels of nutrient imbalances among trees within the study. It was used to distinguish between the nutrient levels of trees that were growing relatively well on certain treatments and trees that were growing poorly, as well as comparing these levels to those reported in the literature.

## Results

### Tree Growth Response

Spoil type had a significant effect on tree height ($p=0.0013$), incremental biomass ($p<0.0001$), and root biomass ($p<0.0001$) (Table 2). Species was also significant for all dependant variables ($p<0.0001$). The main effect of inoculation was not significant for

any dependent variable, although the interaction of spoil and inoculation was significant for incremental height ($p=0.0167$), and the interaction of spoil and species was significant for incremental biomass ($p<0.0001$), and root biomass ($p<0.0001$).

**Table 2.** Significance of main effects and interactions of spoil type, topsoil inoculation and species on tree growth.

| Treatment | Incremental Height (cm) | Incremental Biomass (g) | Root biomass (g) |
|---|---|---|---|
| Spoil | 0.0013*** | <0.0001*** | <0.0001*** |
| Species | <0.0001*** | <0.0001*** | <0.0001*** |
| Inoculation | 0.2592 | 0.3311 | 0.9782 |
| Spoil x Inoculation | 0.0167** | 0.6691 | 0.6822 |
| Spoil x Species | 0.2254 | <0.0001*** | <0.0001*** |
| Inoculation x Species | 0.5505 | 0.6549 | 0.4006 |
| Spoil x Inoculation x Species | 0.7964 | 0.9056 | 0.6955 |

Significance at the 0.1, 0.05, and 0.01 level is marked by a '*', '**', or '***' respectively.

Incremental tree height of the $GUH_{non\text{-}I}$ (non-inoculated gray, unweathered shale) was about two-thirds that of the $UFT_{non\text{-}I}$ (Fig. 1). Inoculation of the $GUH_I$ increased the incremental height by about 50%. Incremental height of the $WUS_I$ treatment was also less than the UFT treatments. The heights of the BWS treatments were no different from the UFT treatments.

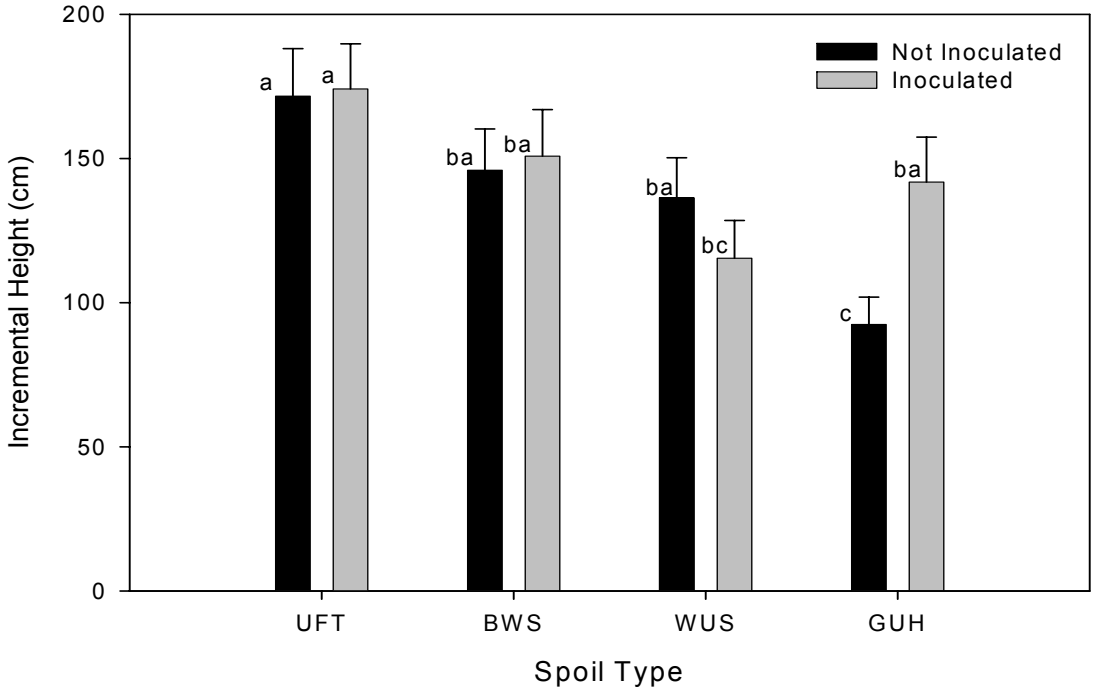


**Figure 1.** Effect of mine spoil type and topsoil inoculation on incremental height of three native hardwood species. Different letters signify significantly different means using Fisher's LSD (0.05).

There was a significant interaction between spoil type and species for both shoot and root biomass (Table 4). Shoot and root biomass of *F. americana* was different among nearly all treatments. Shoot biomass responded in the order UFT>BWS>WUS=GUH (Fig. 2). Root biomass responded to spoil type treatments in the order UFT>BWS>WUS>GUH.

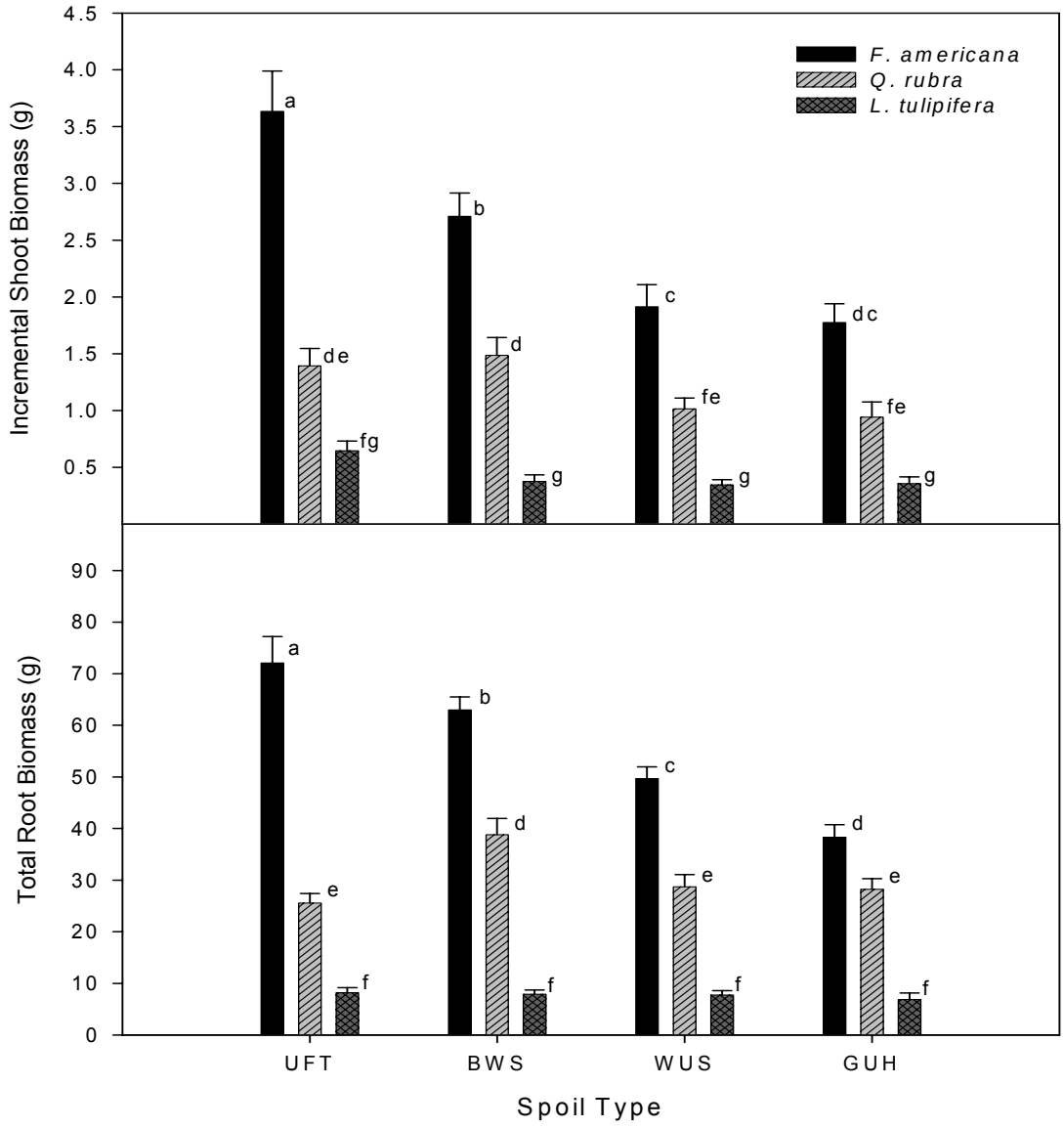


**Figure 2.** Interaction between spoil and topsoil inoculation on means and standard errors of shoot and root biomass. Different letter signify significantly different means using Fisher's LSD.

Overall, *Q. rubra* grew at half the rate of *F. americana* and was less sensitive to spoil type. Shoot growth of *Q. rubra* responded to treatments in the order UFT=BWS, but BWS>WUS=GUH. For root growth, the order was BWS>UFT=WUS=GUH. Shoot and root growth of *L. tulipifera* was considerably less than the other two species and was similar among spoil type treatments.

There was a significant interaction (p=0.003) between spoil type and the number of native, volunteer, herbaceous plants per pot. There was nearly an average of four volunteer, herbaceous plants in the UFT pots, one plant in every other BWS pot and an occasional plant in the WUS and GUH spoil types (Fig 3). Topsoil inoculation greatly increased herbaceous plants in the BWS, WUS, and GUH pots to two to three per pot on average.

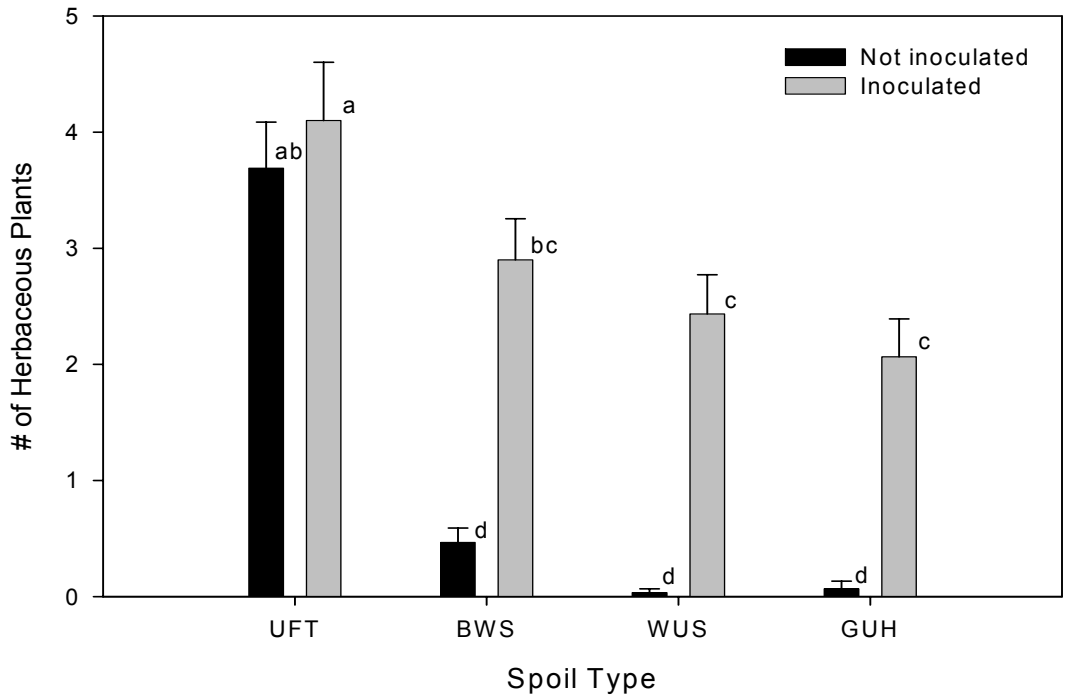


**Figure 3.** Number of understory plants found in three spoil types and a native control soil that were either inoculated or not inoculated with topsoil. Different letters signify significantly different means using Fisher's LSD.

Soil Characterization

Almost all spoil physical, chemical and biological properties tested were different across spoil types (Tables 3, 4, and 5). Physical properties were characterized prior to setting up the experiment. The BWS had the highest fine earth fraction (57%), followed by the UFT (41%), WUS (38%) and GUH (32%). The UFT had the highest silt plus clay content (48%) followed by the GUH (45%). Silt plus clay of the WUS (21%) was half that of the UFT and the GUH while the BWS was intermediate at 33%. The wilting point and $H_2O$ availability of the GUH (4.8% and 5.06%) were half that of the UFT (9.51% and 10.20%). The BWS had an even lower wilting point (3.66%), but higher water availability (7.6%) than the GUH. The WUS had the lowest wilting point and water availability (1.3% and 4.86%).

**Table 3.** Mean values of physical soil properties for three mine spoils and a control and for inoculated verses not inoculated spoils. Different letters signify significantly different values based on Fisher's LSD.

| Measurement | UFT | BWS | WUS | GUH |
|---|---|---|---|---|
| % Fines | 41 [b] | 57 [a] | 38 [b] | 32 [b] |
| % Silt + Clay [†‡] | 47.82 [a] | 33.15 [c] | 21.04 [d] | 44.84 [b] |
| Wilt pt (% by wt)[†‡] | 9.51 [a] | 3.66 [c] | 1.30 [d] | 4.80 [b] |
| $H_2O$ Retention (% by wt)[†‡§] | 10.20 [a] | 7.60 [ba] | 4.86 [b] | 5.06 [b] |

† represents significant correlation of a soil property with incremental stem height. ‡ represents significant correlation of a soil property with incremental stem biomass. § represents significant correlation of a soil property with total root biomass

Mine soil pH ranged from 5.19 to 8.86 across treatments. The pH of the BWS (5.56) was only slightly higher than the UFT (5.21), while the WUS and GUH were very alkaline (8.86 and 8.39, respectively). Topsoil inoculation neutralized the alkaline spoils slightly to pH 8.17 for the WUS and 7.58 for the GUH.

Soluble salt content ranged from 0.127 dS $m^{-1}$ to 0.562 dS $m^{-1}$. The EC of the GUH (0.616 dS $m^{-1}$) was almost three times as high as the UFT (0.236 dS $m^{-1}$). The

WUS (0.317 dS $m^{-1}$) was comparable in salinity to the UFT (0.236 dS $m^{-1}$) while the BWS (0.127 dS $m^{-1}$) was slightly lower.

Exchangeable acidity ranged from 0 to 0.64 $cmol^{+}$ $kg^{-1}$. Neither the GUH nor the WUS had any exchangeable acidity due to their high level of alkalinity. The UFT had 0.64 $cmol^{+}$ $kg^{-1}$ of charge due to exchangeable acidity, while BWS had only half that amount (0.28 $cmol^{+}$ $kg^{-1}$). Topsoil inoculation did not affect exchangeable acidity on any of the spoil types.

The CEC ranged from 1.60 $cmol^{+}$ $kg^{-1}$ to half that amount (0.71 $cmol^{+}$ $kg^{-1}$) across treatments. The GUH (1.60 $cmol^{+}$ $kg^{-1}$) was similar to the UFT (1.50 $cmol^{+}$ $kg^{-1}$), while the BWS was slightly lower (1.14 $cmol^{+}$ $kg^{-1}$) and the WUS was less than half (0.71 $cmol^{+}$ $kg^{-1}$). Topsoil inoculation increased the CEC of the BWS and WUS slightly to 1.216 and 0.87 $cmol^{+}$ $kg^{-1}$, respectively.

Salt-extractable available nitrogen levels ranged widely across spoil types from 9.93 to 27.72 mg $kg^{-1}$. Compared to the UFT (23.91 mg $kg^{-1}$), available N was about half that amount in the WUS (12.91 mg $kg^{-1}$) and GUH (11.2 mg $kg^{-1}$), while the BWS was intermediate (17.80 mg $kg^{-1}$) and not different from the other treatments. Topsoil inoculation had no affect on N levels for any of the soil types.

**Table 4.** Mean values of chemical soil properties for three mine spoils and a native control soil and for inoculated verses not inoculated spoils. Values are on a whole soil basis. Different letters signify significantly different values based on Fisher's LSD test.

| Soil Property | | UFT | BWS | WUS | GUH | Mean |
|---|---|---|---|---|---|---|
| pH†‡§ | Not Inoc. | 5.23 [e] | 5.53 [d] | 8.86 [a] | 8.39 [b] | **7.00** |
| | Inoc. | 5.19 [e] | 5.59 [d] | 8.17 [b] | 7.58 [c] | **6.63** |
| | Mean | **5.21** | **5.56** | **8.515** | **7.985** | |
| EC (dS $m^{-1}$) | Not Inoc. | 0.229 | 0.123 | 0.303 | 0.616 | **0.318 [a]** |
| | Inoc. | 0.243 | 0.131 | 0.331 | 0.508 | **0.303 [a]** |
| | Mean | **0.236[b]** | **0.127[c]** | **0.317[b]** | **0.562[a]** | |
| Exchangeable Acidity ($cmol^{+}$ $kg^{-1}$) †‡§ | Not Inoc. | 0.62 | 0.32 | 0.00 | 0.00 | **0.23 [a]** |
| | Inoc. | 0.66 | 0.24 | 0.00 | 0.00 | **0.23 [a]** |
| | Mean | **0.64** | **0.28** | **0.00** | **0.00** | |
| CEC ($cmol^{+}$ $kg^{-1}$) | Not Inoc. | 1.50 [b] | 1.14 [d] | 0.71 [f] | 1.60 [a] | **1.23** |
| | Inoc. | 1.51 [b] | 1.22 [c] | 0.87 [e] | 1.56 [a] | **1.29** |
| | Mean | **1.51** | **1.18** | **0.79** | **1.58** | |
| KCl Extractable Inorganic N (mg $kg^{-1}$) | Not Inoc. | 27.72 | 19.32 | 10.05 | 9.93 | **16.76 [a]** |
| | Inoc. | 20.10 | 16.28 | 15.77 | 12.46 | **16.15 [a]** |
| | Mean | **23.91[a]** | **17.80[ab]** | **12.91[b]** | **11.20[b]** | |

† represents a significant correlation of a soil property with incremental stem height. ‡ represents a significant correlation of a soil property with incremental stem biomass. § represents a significant correlation of a soil property with total root biomass.

Microbial biomass, dehydrogenase level and ATP level were highest on the UFT (Table 5). Microbial biomass ranged from 1.31 to 0.19 mg $kg^{-1}$ across treatments. The BWS, WUS and GUH all had very low levels, 0.35, 0.19 and 0.19 mg $kg^{-1}$, respectively, which were only a fraction of the level on the UFT (1.31 mg $kg^{-1}$). Inoculation did not affect microbial biomass. Dehydrogenase and ATP levels also ranged an order of magnitude from 90.17mg $kg^{-1}$ and 1536.55 to 0.41mg $kg^{-1}$ and 214.82, respectively. Levels of ATP and dehydrogenase were highest on the UFT (90.17 mg $kg^{-1}$ and 1536.55), followed by the BWS (2.17 mg $kg^{-1}$ and 249.66), which had levels that were only a fraction of the UFT. GUH levels were slightly lower (0.79 mg $kg^{-1}$ and 214.82), and WUS had very low levels (0.41mg $kg^{-1}$ and 156.96). Inoculation increased dehydrogenase levels over five times on the BWS from 2.17 to 11.58mg $kg^{-1}$ and on the GUH from 0.79 to 6.42 mg $kg^{-1}$ but did not effect the WUS. ATP levels doubled due to

inoculation on the BWS from 249.66 to 427.99, and increased on the GUH from 214.82 to 444.52; they remained unchanged on the WUS.

**Table 5.** Mean values of biological soil properties for three mine spoils and a control and for inoculated verses not inoculated spoils. Values are on a whole soil basis. Different letters signify significantly different values based on Fisher's LSD.

| Measurement | | UFT | BWS | WUS | GUH | Mean |
|---|---|---|---|---|---|---|
| Microbial Biomass (chloroform fumigation mg $kg^{-1}$) †‡ | Not Inoc. | 1.17 | 0.20 | 0.10 | 0.10 | **0.39** |
| | Inoc. | 1.45 | 0.50 | 0.28 | 0.28 | **0.63** |
| | Mean | **1.31** [a] | **0.35** [b] | **0.19** [b] | **0.19** [b] | |
| Dehydrogenase (mg TPF $kg^{-1}$)†‡ | Not Inoc. | 90.10[a] | 2.17[d] | 0.41[f] | 0.79[e] | **23.37** |
| | Inoc. | 90.17[a] | 11.58[b] | 0.72[e] | 6.42[c] | **27.22** |
| | Mean | **90.14** | **6.875** | **0.565** | **3.605** | |
| ATP(light response)†‡§ | Not Inoc. | 1536.55 [a] | 249.66 [c] | 156.96 [d] | 214.82 [dc] | **539.50** |
| | Inoc. | 1461.45[a] | 427.99[b] | 208.81[d] | 444.52[b] | **635.69** |
| | Mean | **1499** | **338.83** | **182.89** | **329.67** | |

† represents significant correlation of a soil property with incremental stem height. ‡ represents significant correlation of a soil property with incremental stem biomass. § represents significant correlation of a soil property with total root biomass.

Foliar Nutrient Levels

Foliar nutrient levels of trees growing on the different spoil types and the control soil varied considerably (Table 6). These levels were often different from foliar nutrient concentrations found in the literature and were also different among species.

Foliar N and K concentrations of *F. americana* norms were generally similar to tree foliar concentrations on the various treatments. Ca and Mg foliar concentrations of trees growing on spoils were much higher than the norms, while P was comparable to the norm. *Q. rubra* and *L. tulipifera* nutrient levels were generally lower in N, P, and K concentration on spoil treatments compared to their norms.

Foliar N concentration of *F. americana* growing on the UFT (17.17 mg $kg^{-1}$), BWS (16.21 mg $kg^{-1}$) and GUH (16.42 mg $kg^{-1}$), were all close to the norms (16.0 mg $kg^{-1}$) (Table 7). Trees on the WUS, however, had a lower N level of 14.35 mg $kg^{-1}$. The native foliar P concentration for *F. americana* (2.3 mg $kg^{-1}$) was about twice as high as

any of the treatments. Trees on the UFT had the lowest levels (0.90 mg $kg^{-1}$) followed by WUS (0.96 mg $kg^{-1}$), GUH (1.08 mg $kg^{-1}$) and finally BWS (1.12 mg $kg^{-1}$). The K native foliar level (7.0 mg $kg^{-1}$) was similar to the UFT (6.69 mg $kg^{-1}$), BWS (7.17 mg $kg^{-1}$) and WUS (6.84 mg $kg^{-1}$), while the GUH was several units higher (10.20 mg $kg^{-1}$). Both Ca and the Mg were found in much higher foliar concentrations on all of the treatments compared to their respective norms (7.0 and 3.1 mg $kg^{-1}$). Ca levels decreased in the order UFT (12.80 mg $kg^{-1}$)>GUH (11.03 mg $kg^{-1}$)=WUS (10.8 mg $kg^{-1}$)>BWS (8.24 mg $kg^{-1}$). Mg concentrations of trees on the WUS (4.31 mg $kg^{-1}$) and the GUH (4.46 mg $kg^{-1}$) were higher than the UFT (3.41 mg $kg^{-1}$), while trees on the BWS were not different (3.99 mg $kg^{-1}$).

Foliar N concentration of *Q. rubra* was lower for trees on all treatments compared to the norm (25.2 mg $kg^{-1}$). Concentrations for trees on the BWS (15.64 mg $kg^{-1}$), WUS (16.33 mg $kg^{-1}$), and GUH (16.71 mg $kg^{-1}$) were all about three mg $kg^{-1}$ lower than the UFT (19.40 mg $kg^{-1}$), which was almost six mg $kg^{-1}$ lower than the norm. The P norm (1.4 mg $kg^{-1}$) was also greater than the UFT (1.23 mg $kg^{-1}$) which was equivalent to the GUH (1.05 mg $kg^{-1}$), but greater than the BWS (0.93 mg $kg^{-1}$) or the WUS (0.93 mg $kg^{-1}$). The K concentration for trees on the spoil treatments were lower than the norm but did not different from each other. Similar to *F. americana* foliar levels, Ca and Mg concentrations for *Q. rubra* were higher on the treatments than the native forest norms. The UFT had lower concentrations of Ca (7.55 mg $kg^{-1}$) than the GUH (9.70 mg $kg^{-1}$) which did not vary from the BWS (8.29 mg $kg^{-1}$) or the WUS (9.10 mg $kg^{-1}$). The concentrations of Mg for the trees on the GUH (4.94 mg $kg^{-1}$), WUS (4.7 mg $kg^{-1}$) and GUH (4.89 mg $kg^{-1}$) were also higher than on the UFT (3.54 mg $kg^{-1}$).

*L. tulipifera* showed little variability in nutrient concentrations among treatments. In general, N, P, and K concentrations on the treatments were lower than the native forest norms, while Ca and Mg were higher than the norms.

**Table 6.** Mean foliar levels of three species (mg $kg^{-1}$) for literature-derived norms, and the same species growing in a control soil, and three different mine spoil types . Different letters signify significantly different values based on Fisher's LSD.

| Tree species | Nutrient | Normal Foliar Nutrient Conc.†‡§ | UFT | BWS | WUS | GUH |
|---|---|---|---|---|---|---|
| *F. americana* | N | 22.14 | 17.17[a] | 16.21[a] | 14.35[b] | 16.42[a] |
| | P | 2.53 | 0.90[c] | 1.12[a] | 0.96[bc] | 1.08[ba] |
| | K | 11.49 | 6.69[b] | 7.17[b] | 6.84[b] | 10.20[a] |
| | Ca | 12.72 | 12.80[a] | 8.24[c] | 10.8[b] | 11.03[b] |
| | Mg | 2.63 | 3.41[b] | 3.99[ba] | 4.31[a] | 4.46[a] |
| *Q. rubra* | N | 22.50 | 19.40[a] | 15.64[b] | 16.33[b] | 16.71[b] |
| | P | 1.81 | 1.23[a] | 0.93[b] | 0.93[b] | 1.05[ba] |
| | K | 7.99 | 6.85[a] | 6.44[a] | 5.90[a] | 6.40[a] |
| | Ca | 7.52 | 7.55[b] | 8.29[ba] | 9.11[ba] | 9.70[a] |
| | Mg | 2.24 | 3.54[b] | 4.94[a] | 4.70[a] | 4.89[a] |
| *L. tulipifera* | N | 20.67 | 16.06[a] | 12.52[a] | 9.97[a] | 12.99[a] |
| | P | 1.78 | 0.85[a] | 0.98[a] | 0.68[a] | 0.84[a] |
| | K | 12.13 | 7.57[a] | 5.81[bc] | 4.40[c] | 6.29[ba] |
| | Ca | 18.32 | 12.79[a] | 11.45[a] | 18.51[a] | 17.79[a] |
| | Mg | 3.16 | 3.56[a] | 4.97[a] | 7.13[a] | 7.13[a] |

† Native nutrient norms for *F. americana* are from the mean of a group of good growth populations from the literature (Brockway et al. 1979; Furlan et al., 1983; McClenahen and Dochinger, 1981; Mitchell and Chandler, 1939; Ricklefs and Matthew, 1982). ‡ Norms for *Q. rubra* are from a group of good growth populations from the literature (Auchmoody and Hammock, 1975; Brockway et al. 1979; Burg, 1979; Costea et al., 1984; Fiedler et al., 1973; Henry, 1973; Heinsdorf, 1975; Heinsdorf and Krauss, 1974; Höhne, 1978; Johnson et al. 1982; McClenahen and Dochinger, 1981; Messanger, 1975; Trillmilch and Uebel, 1982; Wood et al., 1973). § Norms for *L tulipifera* are from a group of good growth populations from the literature (Auchmoody and Smith, 1977; Brockway et al., 1979; Farmer et al., 1970; Gouin and Walker, 1977; Schomaker and Rudolph, 1964).

Almost all mean foliar nutrient concentrations and ratios across treatments for all three species were different than the norms found in the literature (Table 7).

**Table 7.** Mean foliar nutrient levels from well nourished trees reported in the literature compared to mean foliar nutrient levels from trees in this greenhouse experiment.

| Tree | Nutrient | Literature Norm[†‡§] | | | Mean study value | | | T-test for mean diff | p-value for mean diff |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | CV | Mean | SD | CV | | |
| *F. americana* | N | 22.14 | 3.8 | 17.16 | 16.04 | 1.75 | 10.91 | 4.15 | 0.005*** |
| | P | 2.53 | 0.58 | 22.92 | 1.02 | 0.14 | 13.73 | 7.37 | 0.0001*** |
| | K | 11.49 | 4.5 | 39.16 | 7.72 | 1.93 | 25.00 | 2.17 | 0.0699* |
| | Ca | 12.72 | 4.4 | 34.59 | 10.72 | 2.24 | 20.90 | 1.00 | 0.3712 |
| | Mg | 2.63 | 0.67 | 25.48 | 4.04 | 0.77 | 19.06 | 4.94 | 0.0006*** |
| | N/P | 9.39 | 1.71 | 18.21 | 16.02 | 2.55 | 15.92 | 7.97 | <0.0001*** |
| | N/K | 2.13 | 0.93 | 43.66 | 2.17 | 0.48 | 22.12 | 0.10 | 0.9244 |
| | N/Ca | 2.04 | 0.21 | 10.29 | 1.56 | 0.4 | 25.64 | 2.02 | 0.0517* |
| | N/Mg | 8.69 | 3.23 | 37.17 | 4.13 | 1.02 | 24.70 | 3.13 | 0.0388** |
| | P/K | 0.25 | 0.08 | 32.00 | 0.14 | 0.03 | 21.43 | 3.34 | 0.0192** |
| | P/Ca | 0.21 | 0.07 | 33.33 | 0.1 | 0.03 | 30.00 | 3.85 | 0.0164** |
| | P/Mg | 0.9 | 0.19 | 21.11 | 0.26 | 0.05 | 19.23 | 8.01 | 0.0004*** |
| | K/Ca | 0.79 | 0.2 | 25.32 | 0.76 | 0.26 | 34.21 | 0.27 | 0.7908 |
| | K/Mg | 4.93 | 2.96 | 60.04 | 1.97 | 0.55 | 27.92 | 2.64 | 0.0381** |
| | Ca/Mg | 4.37 | 1.78 | 40.73 | 2.72 | 0.7 | 25.74 | 2.05 | 0.107 |
| *Q. rubra* | N | 22.5 | 3.99 | 17.73 | 16.09 | 2.58 | 16.03 | 5.96 | <0.0001*** |
| | P | 1.81 | 0.55 | 30.39 | 1.03 | 0.22 | 21.36 | 6.88 | <0.0001*** |
| | K | 7.99 | 2.03 | 25.41 | 6.4 | 0.86 | 13.44 | 3.79 | 0.0006*** |
| | Ca | 7.52 | 2.95 | 39.23 | 8.04 | 1.74 | 21.64 | 0.04 | 0.9702 |
| | Mg | 2.24 | 0.96 | 42.86 | 4.52 | 0.77 | 17.04 | 9.91 | <0.0001*** |
| | N/P | 13.27 | 3.97 | 29.92 | 16.75 | 2.26 | 13.49 | 3.92 | 0.0004*** |
| | N/K | 3.04 | 0.9 | 29.61 | 2.7 | 0.48 | 17.78 | 1.71 | 0.0959* |
| | N/Ca | 3.04 | 1.43 | 47.04 | 2.05 | 0.59 | 28.78 | 3.25 | 0.0028*** |
| | N/Mg | 13.21 | 6.79 | 51.40 | 3.92 | 1.12 | 28.57 | 6.5 | <0.0001*** |
| | P/K | 0.24 | 0.1 | 41.67 | 0.16 | 0.02 | 12.50 | 4.05 | 0.0004*** |
| | P/Ca | 0.23 | 0.08 | 34.78 | 0.13 | 0.06 | 46.15 | 5.1 | <0.0001*** |
| | P/Mg | 0.94 | 0.38 | 40.43 | 0.24 | 0.08 | 33.33 | 8.98 | <0.0001*** |
| | K/Ca | 1.01 | 0.41 | 40.59 | 0.78 | 0.28 | 35.90 | 2.51 | 0.0158** |
| | K/Mg | 4.26 | 2.36 | 55.40 | 1.47 | 0.37 | 25.17 | 5.86 | <0.0001*** |
| | Ca/Mg | 4.33 | 1.77 | 40.88 | 1.94 | 0.39 | 20.10 | 6.59 | <0.0001*** |
| *L. tulipifera* | N | 20.67 | 4.76 | 23.03 | 11.82 | 2.94 | 24.87 | 6.85 | <0.0001*** |
| | P | 1.78 | 0.57 | 32.02 | 0.84 | 0.25 | 29.76 | 4.81 | 0.0010*** |
| | K | 12.13 | 5.71 | 47.07 | 6.02 | 1.96 | 32.56 | 3.16 | 0.0123** |
| | Ca | 18.32 | 10.26 | 56.00 | 15.13 | 3.9 | 25.78 | 0.91 | 0.3854 |
| | Mg | 3.16 | 1.86 | 58.86 | 5.7 | 1.87 | 32.81 | 3.6 | 0.0009*** |
| | N/P | 14.51 | 4.11 | 28.33 | 16.34 | 5.17 | 31.64 | 0.98 | 0.3334 |
| | N/K | 2.34 | 1.14 | 48.72 | 2.3 | 0.78 | 33.91 | 0.1 | 0.9184 |
| | N/Ca | 1.88 | 1.28 | 68.09 | 0.93 | 0.37 | 39.78 | 2.21 | 0.0566* |
| | N/Mg | 10.39 | 6.5 | 62.56 | 2.7 | 1.95 | 72.22 | 3.53 | 0.0074 |
| | P/K | 0.16 | 0.05 | 31.25 | 0.15 | 0.04 | 26.67 | 0.69 | 0.4954 |
| | P/Ca | 0.14 | 0.1 | 71.43 | 0.06 | 0.02 | 33.33 | 2.27 | 0.0517* |
| | P/Mg | 0.75 | 0.46 | 61.33 | 0.17 | 0.09 | 52.94 | 3.77 | 0.0052*** |
| | K/Ca | 0.88 | 0.6 | 68.18 | 0.43 | 0.18 | 41.86 | 2.23 | 0.0545* |
| | K/Mg | 5.3 | 3.9 | 73.58 | 1.24 | 0.74 | 59.68 | 3.11 | 0.0141** |
| | Ca/Mg | 7.02 | 3.91 | 55.70 | 2.82 | 0.73 | 25.89 | 3.2 | 0.0122** |

[†] Native norm concentration for *F. americana* are from the mean of a group of good growth populations from the literature (Brockway et al. 1979; Furlan et al., 1983; McClenahen and Dochinger, 1981; Mitchell and Chandler, 1939; Ricklefs and Matthew, 1982). [‡] Native norm concentrations for *Q. rubra* are from a group of good growth populations from the literature (Auchmoody and Hammock, 1975; Brockway et al. 1979; Burg, 1979; Costea et al., 1984; Fiedler et al., 1973; Henry, 1973; Heinsdorf, 1975; Heinsdorf and Krauss, 1974; Höhne, 1978; Johnson et al. 1982; McClenahen and Dochinger, 1981; Messanger, 1975; Trillmilch and Uebel, 1982; Wood et al., 1973). [§] Native norms for *L tulipifera* are from a group of good growth populations from the literature (Auchmoody and Smith, 1977; Brockway et al., 1979; Farmer et al., 1970; Gouin and Walker, 1977; Schomaker and Rudolph, 1964).

Using foliar norms from well-nourished trees for comparison, spoil types had a significant influence on N (p=0.0118), K (p=0.0234), Ca (p<0.0001), and Mg (p=0.0169) foliar indexes for *F. americana*, while inoculation influenced K (0.0617), and Ca (0.0807) levels (Table 8). *Q. rubra* foliar levels were influenced by spoil type in the case of P (p=0.0078) and Ca (p=0.02), but not K or NBI. Foliar levels of *L. tulipifera* were influenced by all spoil types for all nutrients, N (p<0.0001), P (p=0.0009), K (0.0001), Ca (p<0.0001) and Mg (p<0.0001), and the NBI (nutrient balance index) (p<0.0001). Inoculation significantly affected K, Mg and NBI levels (p=0.0098, p=0.0014 and p=0.0009, respectively). There was a significant interaction between spoil type and inoculation for N (p=0.0889), K (p=0.0566), Ca (p=0.0025), Mg (p=0.0151), and NBI (p=0.0057) for *L. tulipifera.*

**Table 8.** Significance of foliar nutrient indexes for three native tree species on three mine spoils and a control soil that have or have not been inoculated with native topsoil.

| Tree | Treatment | N | P | K | Ca | Mg | NBI |
|---|---|---|---|---|---|---|---|
| | Spoil | 0.0185* | 0.0017* | 0.0105* | <0.0001* | 0.0742* | 0.1021 |
| *F. americana* | Inoc | 0.2793 | 0.8773 | 0.0454* | 0.0668* | 0.2985 | 0.3863 |
| | SpoilxInoc | 0.9812 | 0.3281 | 0.5818 | 0.8188 | 0.9900 | 0.8789 |
| | Spoil | 0.0061* | <0.0001* | 0.1295 | 0.0982* | <0.0001* | <0.0001* |
| *Q. rubra* | Inoc | 0.6797 | 0.0272* | 0.3105 | 0.2406 | 0.2900 | 0.3696 |
| | SpoilxInoc | 0.6339 | 0.6903 | 0.3598 | 0.6238 | 0.1734 | 0.0454* |
| | Spoil | 0.0001* | 0.0009* | 0.0001* | <0.0001* | <0.0001* | <0.0001* |
| *L. tulipifera* | Inoc | 0.2090 | 0.6294 | 0.0098* | 0.7996 | 0.0014* | 0.0009* |
| | SpoilxInoc | 0.0889* | 0.1476 | 0.0566* | 0.0025* | 0.0151* | 0.0057* |

'*' depicts significance at the 0.10 level.

The overall *F. americana* nitrogen foliar concentrations for the study soils were about 25% less than the norms suggesting that trees growing in the mine soils as well as the control soil were nitrogen deficient. The nutrient indices and NBI for *F. americana* on the UFT were better balanced than the other treatments (Table 9). The nitrogen index on the BWS (-0.84) was closer to the optimum and did not differ from the UFT (-1.89),

while the levels for trees growing on the WUS and the GUH were more deficient (-16.42 and -11.28, respectively). Inoculation did not change the nitrogen index.

Like nitrogen, the overall phosphorous foliar levels for the study trees were about 30% less than the norms showing that all trees were P deficient regardless of growth medium. Phosphorous was equally deficient on the UFT, WUS, and GUH (-65.25, -61.19 and -61.31, respectively) and slightly less deficient on the BWS (-42.27). Overall potassium foliar levels for the study trees were variable compared to the norms, with higher levels for the *F. americana* and *Q. rubra* but lower levels for the *L. tulipifera.* The potassium index of WUS (-17.70) and the BWS (-12.31) did not differ from the UFT (-15.18). Trees on the GUH (2.31) were sufficient with an index close to zero. Inoculation doubled the deficiency of potassium from -6.34 to -15.10 across spoil types.

Calcium foliar levels were similar to norms. Within treatments, Ca was most deficient on the BWS (-2.68) compared to the UFT which had the highest excess (33.63). Trees on the GUH were in slight excess (12.43), and the WUS was also in excess (23.07). Topsoil inoculation did not change foliar Ca levels.

Foliar magnesium levels were about twice as high as norms. Mg was in greater excess on the WUS (72.24) compared to the UFT (48.69), while the BWS (58.09) and GUH (57.85) did not differ from the UFT. Inoculation did not affect Mg indices for *F. americana*. NBI was lower for trees on the BWS (58.09) compared to the WUS (72.24) but did not differ from the UFT (167.86) or the GUH (156.09). The UFT and GUH did not differ from the WUS. Topsoil inoculation had no affect on NBI.

*Q. rubra* nutrient indices were highly variable within treatments, leading to few significant differences between spoil types. Inoculation had no affect on any nutrient

levels for *Q. rubra.* N was less deficient on the UFT (-6.70) compared to the BWS (-19.10), WUS (-16.18) or GUH (-18.22). P was also more deficient for trees on the WUS (-35.64), BWS (-35.40) and the GUH (-30.38) compared to the UFT (-16.04). Levels of foliar K did not differ across treatments. Foliar Ca levels were in excess on the GUH (5.00) and WUS (5.61) compared to deficiencies on the UFT (-4.73). BWS was closest to sufficient (-4.73) and did not differ from any other treatments. Similar to P and K, Ca levels were highly variable for trees within treatments. Mg levels were in greater excess on the WUS (72.24) than the UFT (48.69). BWS (58.09) and GUH (57.85) were intermediate in excess and did not differ from the other treatments. NBI was closer to balanced on the BWS (133.49) than the WUS (190.62) but did not differ from the UFT (167.86) or the GUH (156.09). The WUS also did not differ from the UFT or the GUH.

In general, nutrient indices and the NBI of *L. tulipifera* on the UFT were close to balanced compared to indices for trees on the spoil materials. Nitrogen was close to balanced in trees on the UFT (2.46) while it was deficient on the BWS (-10.21) and GUH (-14.79) and even more deficient on the WUS (-21.46). P was slightly deficient on the BWS (-6.67) while it was more deficient on the UFT (-16.25). Phosphorous was the most deficient for trees on the GUH (-23.80) and the WUS (-25.74). Inoculation did not have an effect on the phosphorous index. Potassium was deficient for trees on the GUH (-11.92) and the WUS (-25.50) compared to the UFT (-0.420), while trees on the BWS were similar (-9.38). Inoculation decreased potassium deficiency to half the level of the non-inoculated mean, from -16.28 to -7.33.

Calcium was sufficient for trees on the GUH (1.67), which was similar to trees on the UFT (-2.15). Trees on the WUS, were in excess (8.99) while trees on the BWS were

deficient (-7.26). Inoculation had no effect on Ca levels for *L. tulipifera*. The Mg levels in trees on the UFT were in slight excess, while the BWS, GUH and WUS were higher in increasing order (33.52, 48.84, and 63.70, respectively). Inoculation with topsoil decreased Mg levels in trees on the BWS and WUS from an excess of 48.81 and 76.16 to 18.23 and 51.23, respectively. NBI was lowest on the UFT (48.10) followed in increasing order by the BWS (69.33), GUH (106.44) and WUS (145.33).

**Table 9.** Mean values of foliar nutrient indexes for three native tree species on three mine spoils, and a control that have or have not been inoculated with native topsoil. Different letters among values depicts a significant difference based on Fisher's LSD 0.5.

| *F. americana* | | | UFT | BWS | WUS | GUH | Mean |
|---|---|---|---|---|---|---|---|
| | N | Mean | **-1.89**$^{a}$ | **-0.84**$^{a}$ | **-16.42**$^{b}$ | **-11.28**$^{ba}$ | |
| | P | Mean | **-65.25**$^{b}$ | **-42.27**$^{a}$ | **-61.19**$^{b}$ | **-61.31**$^{b}$ | |
| | | Not inoc | -13.56 | -5.45 | -16.38 | 10.04 | **-6.34**$^{a}$ |
| | K | Inoc | -16.79 | -19.16 | -19.01 | -5.42 | **-15.10**$^{b}$ |
| Nutrient Index | | Mean | **-15.18**$^{b}$ | **-12.31**$^{b}$ | **-17.70**$^{b}$ | **2.31**$^{a}$ | |
| | | Not inoc | 29.64 | -7.93 | 22.50 | 7.97 | **13.05**$^{a}$ |
| | Ca | Inoc | 37.61 | 2.57 | 23.65 | 16.88 | **20.18**$^{a}$ |
| | | Mean | **33.63**$^{a}$ | **-2.68**$^{c}$ | **23.07**$^{ba}$ | **12.43**$^{b}$ | |
| | Mg | Mean | **48.69**$^{b}$ | **58.09**$^{ba}$ | **72.24**$^{a}$ | **57.85**$^{ba}$ | |
| NBI | | Mean | **167.86**$^{ba}$ | **133.89**$^{b}$ | **190.62**$^{a}$ | **156.09**$^{ba}$ | |
| *Q. rubra* | | | | | | | |
| | N | Mean | **-6.70**$^{a}$ | **-19.10**$^{b}$ | **-16.18**$^{b}$ | **-18.22**$^{b}$ | |
| | P | Mean | **-16.04**$^{a}$ | **-35.40**$^{b}$ | **-35.64**$^{b}$ | **-30.38**$^{b}$ | |
| Nutrient Index | K | Mean | **-3.02** | **-6.16** | **-9.70** | **-9.15** | |
| | Ca | Mean | **-4.73**$^{b}$ | **-0.44**$^{ba}$ | **5.61**$^{a}$ | **5.00**$^{a}$ | |
| | Mg | Mean | **30.49**$^{b}$ | **61.11**$^{a}$ | **55.91**$^{a}$ | **52.74**$^{a}$ | |
| NBI | | Mean | **68.68**$^{b}$ | **129.78**$^{a}$ | **124.45**$^{a}$ | **115.88**$^{a}$ | |
| *L. tulipifera* | | | | | | | |
| | | Not inoc | 4.73$^{a}$ | -18.06$^{c}$ | -25.38$^{c}$ | -13.21$^{bc}$ | **-12.98** |
| | N | Inoc | 0.19$^{a}$ | -2.36$^{ba}$ | -17.53$^{c}$ | -16.38$^{c}$ | **-9.02** |
| | | Mean | **2.46**$^{a}$ | **-10.21**$^{b}$ | **-21.46**$^{c}$ | **-14.79**$^{cb}$ | |
| | P | Mean | **-16.25**$^{b}$ | **-6.67**$^{a}$ | **-25.74**$^{c}$ | **-23.80**$^{cb}$ | |
| | | Not inoc | -2.77$^{ba}$ | -9.39$^{ba}$ | -37.85$^{c}$ | -15.11$^{b}$ | **-16.28**$^{b}$ |
| | K | Inoc | 1.93$^{a}$ | -9.37$^{ba}$ | -13.15$^{b}$ | -8.73b$^{a}$ | **-7.33**$^{a}$ |
| Nutrient Index | | Mean | **-0.420**$^{a}$ | **-9.38**$^{ba}$ | **-25.50**$^{c}$ | **-11.92**$^{b}$ | |
| | | Not inoc | -1.96$^{cd}$ | -9.41$^{e}$ | 14.91$^{a}$ | -1.45$^{cbd}$ | **0.52** |
| | Ca | Inoc | -2.34$^{cd}$ | -5.10$^{ed}$ | 3.08$^{cb}$ | 4.80$^{b}$ | **0.11** |
| | | Mean | **-2.15**$^{b}$ | **-7.26**$^{c}$ | **8.99**$^{a}$ | **1.67**$^{b}$ | |
| | | Not inoc | 17.03$^{c}$ | 48.81$^{b}$ | 76.16$^{a}$ | 48.45$^{b}$ | **47.61**$^{a}$ |
| | Mg | Inoc | 15.70$^{c}$ | 18.23$^{c}$ | 51.23$^{b}$ | 49.22$^{b}$ | **33.59**$^{b}$ |
| | | Mean | **16.36**$^{d}$ | **33.52**$^{c}$ | **63.70**$^{a}$ | **48.84**$^{b}$ | |
| | | Not inoc | 53.99$^{c}$ | 98.24$^{b}$ | 182.13$^{a}$ | 100.10$^{b}$ | **108.64**$^{a}$ |
| NBI | | Inoc | 42.21$^{c}$ | 40.42$^{c}$ | 109.51$^{b}$ | 112.67$^{b}$ | **76.20**$^{b}$ |
| | | Mean | **48.10**$^{c}$ | **69.33**$^{c}$ | **145.82**$^{a}$ | **106.44**$^{b}$ | |

Correlations to tree growth

Many physical, chemical and biological soil properties were significantly correlated with tree growth across all three species. However the relative importance of different properties differed somewhat among species.

For *F. Americana,* the physical properties, wilting point and water retention, were positively correlated with incremental stem height and biomass as well as total root biomass (Table 10). Total root biomass was also positively correlated with percent coarse fragment content, while incremental stem biomass was also correlated with percent silt plus clay.

All chemical variables except CEC were correlated with growth of *F. amercana.* pH, and EC, were negatively correlated with incremental stem growth, biomass and total root biomass. Exchangeable acidity and extractable N were positively correlated with incremental stem growth, incremental stem biomass and total root biomass of *F. americana*. Dehydrogenase and ATP levels were positively correlated with incremental stem height, biomass, and total root biomass.

Foliar nutrient concentrations and DRIS indices were not as well correlated to *F americana* growth, and were often negatively correlated. Foliar P concentration was negatively correlated to incremental stem height and total root biomass, while foliar K was negatively correlated to incremental stem biomass and total root biomass. Foliar Mg was negatively correlated with all three, incremental stem height, incremental stem biomass, total root biomass. Foliar indices were also poorly correlated with *F. americana* growth. N was positively correlated with incremental stem biomass and total root

biomass, while Ca was positively correlated with incremental stem height and biomass. K indices were negatively correlated with incremental stem height and total root biomass.

**Table 10.** Relationship of mine soil properties and foliar nutrient concentrations and indices to growth of *F. americana*.

| *F. Americana* | | | | | | | |
|---|---|---|---|---|---|---|---|
| Property | | Incremental Stem Height (cm) | | Incremental Stem Biomass (g) | | Total Root Biomass (g) | |
| | | Corr. Coeff. | p value | Corr. Coeff. | p value | Corr. Coeff. | P value |
| % Fines | | 0.1831 | 0.3158 | 0.2773 | 0.1245 | 0.4411 | 0.0115** |
| % Silt + Clay | | 0.2538 | 0.1611 | 0.3854 | 0.0294** | 0.2214 | 0.2232 |
| Wilt pt (% by wt) | | 0.4992 | 0.0036*** | 0.6629 | <0.0001*** | 0.5580 | 0.0009*** |
| $H_2O$ Retention (% by wt) | | 0.6085 | 0.0002*** | 0.7951 | <0.0001*** | 0.8086 | <0.0001*** |
| pH | | -0.4994 | 0.0042*** | -0.6852 | <0.0001*** | -0.7462 | <0.0001*** |
| EC (dS $m^{-1}$) | | -0.3442 | 0.0580* | -0.4458 | 0.0120** | -0.6438 | <0.0001*** |
| Ex Acidity (cmol+ $kg^{-1}$) | | 0.6169 | 0.0002*** | 0.7982 | <0.0001*** | 0.7990 | <0.0001*** |
| CEC ($cmol^{+}$ $kg^{-1}$) | | 0.0792 | 0.6719 | 0.1804 | 0.3314 | 0.0082 | 0.9650 |
| Available N (mg $kg^{-1}$) | | 0.4196 | 0.0168** | 0.3996 | 0.0235** | 0.4271 | 0.0148** |
| Dehydrogenase (mg TPF $kg^{-1}$ $24h^{-1}$) | | 0.6902 | <0.0001*** | 0.8323 | <0.0001*** | 0.7783 | <0.0001*** |
| ATP (light level) | | 0.6121 | 0.0003*** | 0.7725 | <0.0001*** | 0.7316 | <0.0001*** |
| Foliar Nutrient Levels (mg kg-1) | N | 0.0444 | 0.8092 | 0.2818 | 0.1181 | 0.1928 | 0.2904 |
| | P | -0.4034 | 0.0220** | -0.2522 | 0.1638 | -0.3069 | 0.0876* |
| | K | -0.2760 | 0.1262 | -0.4267 | 0.0149** | -0.5867 | 0.0004*** |
| | Ca | 0.0822 | 0.6547 | 0.2173 | 0.2322 | 0.0883 | 0.6308 |
| | Mg | -0.3908 | 0.0270** | -0.4029 | 0.0222** | -0.449 | 0.0100*** |
| Foliar Nutrient Indices | N | 0.2293 | 0.2069 | 0.3569 | 0.0452** | 0.3776 | 0.0331** |
| | P | -0.0553 | 0.7636 | -0.1220 | 0.5061 | -0.0034 | 0.9853 |
| | K | -0.3318 | 0.0636* | -0.2539 | 0.1609 | -0.3382 | 0.0583* |
| | Ca | 0.3393 | 0.0574* | 0.3136 | 0.0805* | 0.2362 | 0.1931 |
| | Mg | -0.1705 | 0.3509 | -0.2395 | 0.1868 | -0.2051 | 0.2600 |
| | NBI | 0.0582 | 0.9748 | -0.0025 | 0.9891 | -0.016 | 0.9308 |

*, **, *** represent significance at the 0.10, 0.05, and 0.01 levels, respectively.

*Q. rubra* growth was also well correlated with soil properties (Table 11). The physical property percent fines was positively correlated with all three growth variables, incremental stem height, incremental stem biomass and total root biomass, while incremental stem height and biomass were also positively correlated with water retention

and total root biomass was negatively correlated with wilting point. Similar to *F. americana*, stem height and biomass of *Q. rubra* was negatively correlated with pH and EC and positively correlated with exchangeable acidity. Incremental stem height and biomass were also correlated with ATP level, but not dehydrogenase activity.

Foliar nutrient concentrations and indices were not well correlated with the growth of *Q. rubra*. N was negatively correlated with incremental stem height, biomass, and total root biomass, while K was positively correlated with incremental stem height. Ca was also negatively correlated with incremental stem height and biomass. There were no correlations of nutrient indices with tree growth.

**Table 11.** Relationship of mine soil properties and foliar nutrient concentrations and indices to growth of *Q. rubra*.

| *Q. rubra* | | | | | | | |
|---|---|---|---|---|---|---|---|
| Property | | Incremental Stem Height (cm) | | Incremental Stem Biomass (g) | | Total Root Biomass (g) | |
| | | Corr. Coeff. | p value | Corr. Coeff. | p value | Corr. Coeff. | p value |
| % Fines | | 0.4248 | 0.0154** | 0.4875 | 0.0047*** | 0.3946 | 0.0254** |
| % Silt + Clay | | 0.0863 | 0.6387 | 0.0431 | 0.8148 | -0.2807 | 0.1196 |
| Wilt pt (% by wt) | | 0.2051 | 0.2601 | 0.2024 | 0.2665 | -0.2980 | 0.0976* |
| $H_2O$ Retention (% by wt) | | 0.3994 | 0.0236** | 0.4444 | 0.0108** | -0.0916 | 0.6180 |
| pH | | -0.4984 | 0.0037*** | -0.5267 | 0.0020*** | -0.1005 | 0.5843 |
| EC (dS $m^{-1}$) | | -0.4447 | 0.0108** | -0.4545 | 0.0090*** | -0.1288 | 0.4822 |
| Ex Acidity ($cmol^+ kg^{-1}$) | | 0.3648 | 0.0401** | 0.4024 | 0.0224** | -0.1599 | 0.3820 |
| CEC ($cmol^+ kg^{-1}$) | | 0.0806 | 0.6612 | 0.0729 | 0.6918 | -0.1863 | 0.3074 |
| Available N (mg $kg^{-1}$) | | 0.1865 | 0.3069 | -0.0365 | 0.8426 | -0.2240 | 0.2179 |
| Dehydrogenase (mg TPF $kg^{-1}$ $24h^{-1}$) | | 0.2607 | 0.1496 | 0.2440 | 0.1784 | -0.2530 | 0.1624 |
| ATP (light level) | | 0.3045 | 0.0902* | 0.3164 | 0.0777* | -0.2765 | 0.1255 |
| Foliar Nutrient Levels (mg $kg^{-1}$) | N | -0.3650 | 0.0400** | -2.993 | 0.0961* | -0.4652 | 0.0073*** |
| | P | 0.0568 | 0.7574 | -0.0278 | 0.8800 | -0.3633 | 0.0410** |
| | K | 0.3054 | 0.0892* | 0.1574 | 0.3895 | -0.2164 | 0.2342 |
| | Ca | -0.3232 | 0.0712* | -0.4572 | 0.0085*** | -0.1987 | 0.2756 |
| | Mg | -0.0126 | 0.9457 | -0.1614 | 0.3776 | 0.1522 | 0.4056 |
| DRIS Nutrient Indices | N | 0.1862 | 0.3075 | 0.1559 | 0.3943 | -0.1967 | 0.2805 |
| | P | 0.2302 | 0.2052 | 0.1147 | 0.5319 | -0.1935 | 0.2886 |
| | K | 0.1810 | 0.3214 | 0.2553 | 0.1584 | 0.1290 | 0.4816 |
| | Ca | -0.2338 | 0.1979 | -0.1998 | 0.2730 | 0.0302 | 0.8699 |
| | Mg | -0.1998 | 0.2730 | -0.1518 | 0.4070 | 0.1753 | 0.3372 |
| | NBI | -0.1548 | 0.3976 | -0.1226 | 0.5039 | 0.2123 | 0.2434 |

*, **, *** represent significance at the 0.10, 0.05, and 0.01 levels, respectively.

Although growth differences of *L. tulipifera* were slight, they were well correlated with soil properties (Table 12). Percent silt plus clay, wilting point and water retention were all positively correlated with incremental stem height and incremental stem biomass. pH was negatively correlated with both incremental stem height and incremental stem weight, but not root biomass. Exchangeable acidity was positively correlated with incremental stem height and biomass, while CEC was positively correlated with

incremental stem height only. All three microbial indicators were positively correlated with incremental stem height and incremental stem biomass.

Similar to *F. americana* and *Q rubra*, growth of *L. tulipifera* was not well correlated with nutrient concentrations or indices. N concentration was positively correlated with incremental stem height and biomass. Ca and Mg were negatively correlated with incremental stem height and incremental stem biomass while K was negatively correlated with total root biomass. K indices were positively correlated with incremental stem height and biomass. No other indices were correlated with tree growth.

**Table 12.** Relationship of mine soil properties and foliar nutrient concentrations and indices to growth of *L. tulipifera*.

| *L. tulipifera* | | | | | | | |
|---|---|---|---|---|---|---|---|
| Property | | Incremental Stem Height (cm) | | Incremental Stem Biomass (g) | | Total Root Biomass (g) | |
| | | Corr. Coeff. | p value | Corr. Coeff. | p value | Corr. Coeff. | p value |
| % Fines | | -0.1060 | 0.5636 | 0.0191 | 0.9175 | 0.1393 | 0.4470 |
| % Silt + Clay | | 0.4096 | 0.0199** | 0.3394 | 0.0574* | -0.0904 | 0.6226 |
| Wilt pt (% by wt) | | 0.5268 | 0.0020*** | 0.5173 | 0.0024** | 0.0872 | 0.6352 |
| $H_2O$ Retention (% by wt) | | 0.4453 | 0.0107** | 0.5207 | 0.0022*** | 0.2228 | 0.2203 |
| pH | | -0.3279 | 0.0669** | -0.4071 | 0.0208** | -0.1448 | 0.4290 |
| EC (dS $m^{-1}$) | | 0.0308 | 0.8673 | -0.1114 | 0.5438 | -0.1964 | 0.2813 |
| Ex Acidity ($cmol^+ kg^{-1}$) | | 0.4743 | 0.0061*** | 0.5396 | 0.0014*** | 0.2278 | 0.2106 |
| CEC ($cmol^+ kg^{-1}$) | | 0.3159 | 0.0782* | 0.2309 | 0.2036 | -0.1828 | 0.3168 |
| Available N (mg $kg^{-1}$) | | -0.0037 | 0.9840 | 0.0384 | 0.8345 | 0.1700 | 0.3522 |
| Microbial Biomass (chloroform fumigation mg $kg^{-1}$) | | 0.5578 | 0.0009*** | 0.4869 | 0.0047*** | 0.1546 | 0.3981 |
| Dehydrogenase (mg TPF $kg^{-1}$ $24h^{-1}$) | | 0.4372 | 0.0124** | 0.4720 | 0.0064*** | 0.0990 | 0.5900 |
| ATP (light level) | | 0.5126 | 0.0027*** | 0.5238 | 0.0021*** | 0.0943 | 0.6078 |
| Foliar Nutrient Levels (mg $kg^{-1}$) | N | 0.4206 | 0.0165* | 0.3761 | 0.0339* | -0.0263 | 0.8864 |
| | P | -0.1426 | 0.4363 | -0.2363 | 0.1929 | -0.1429 | 0.4352 |
| | K | 0.0746 | 0.6849 | -0.0367 | 0.8419 | -0.3364 | 0.0598* |
| | Ca | -0.3047 | 0.0899* | -0.4175 | 0.0174* | -0.1555 | 0.3954 |
| | Mg | -0.3046 | 0.0901* | -0.3519 | 0.0482* | -0.0384 | 0.8345 |
| DRIS Nutrient Indices | N | 0.2731 | 0.1304 | 0.2821 | 0.1178 | 0.0518 | 0.7785 |
| | P | -0.0886 | 0.6295 | -0.1397 | 0.4457 | -0.0055 | 0.9760 |
| | K | 0.3598 | 0.0431* | 0.3640 | 0.0406* | -0.0730 | 0.6913 |
| | Ca | -0.0979 | 0.5940 | -0.2547 | 0.1596 | -0.1156 | 0.5286 |
| | Mg | -0.2907 | 0.1065 | -0.2165 | 0.2340 | 0.0595 | 0.7463 |
| | NBI | -0.2037 | 0.2635 | -0.1636 | 0.3710 | 0.1173 | 0.5228 |

*, **, *** represent significance at the 0.10, 0.05, and 0.01 levels, respectively.

## Discussion

Effect of Spoil Type

This study showed that the spoil growth medium plays a critical role in tree growth and success of native hardwoods on reclaimed strip mine sites. Based on the growth response of *F. americana* and *Q. rubra*, BWS was a superior medium for tree growth over WUS or GUH. Both tree species had greater stem and root biomass on the BWS than on the other spoils. *L. tulipifera* did not show a growth response to any treatment. This may be because *L. tulipifera* is very sensitive to site quality and is very site specific even on undisturbed soils.

The better growth response of *F. americana* and *Q. rubra* on the BWS may be due to a number of soil physical, chemical, and biological properties that differentiate the growth media. The higher % fines and higher water retention more closely approximated the UFT creating a physical environment with better water/air balance, which is more conducive to tree growth. The lower pH, higher microbial activity, and, to some extent, more balanced nutrient availability also created an environment more conducive to tree growth (Tables 3, 4, and 5). Many of these soil properties were highly correlated with incremental stem height and biomass as well as total root biomass of some or all tree species, further suggesting that these properties were influencing tree growth (Tables 11, 12 and 13).

The low % coarse fragments of the BWS compared to the WUS, GUH or even the UFT created a larger rooting medium (Table 3). Root growth of *F. americana* and all growth parameters of *Q. rubra* were positively correlated with % fines, but *L. tulipifera* was not, suggesting that other factors were having a greater influence on the growth of

this species (Tables 11, 12 and 13). The higher water retention of the BWS and the UFT, kept trees well watered between watering events. The WUS and the GUH, on the other hand, may have become droughty due to their lower water retention. Water retention did correlate well with all growth parameters of *F. americana* and to incremental tree height and incremental biomass of both *Q. rubra* and *L. tulipifera*, further supporting its importance to tree growth.

Rodrigue and Burger (2002) found water/air balance an important factor affecting a variety of eastern hardwood species growing on mined land in a seven-state region. Coarse fragment content and available water holding capacity were the second and third most important variables in their regression analysis of tree growth on mined sites. High amounts of rock fragments lead to a decrease in fine earth available for exploitation by tree roots, which leads to a reduction in nutrient and water availability (Torbert et al., 1985; Childs and Flint, 1990; Thurman and Sencindiver, 1986; Rodrigue and Burger, 2004).

Silt plus clay and wilting point may also be important factors due to their association with soil water/air balance. These physical properties also correlated well with growth of *F. americana*, and *L. tulipifera,* but not as well to growth of *Q. rubra* in this study. Both silt-plus-clay % and wilting point were highest on the UFT, which may have led to the measured improved tree growth. However, these properties were not distinguishing factors of the BWS from the other spoils, and were most likely not the determining soil factors differentiating tree growth on the BWS, WUS and GUH.

Chemical properties that may have played a role in growth of trees in this experiment include pH, as well as phosphorous and nitrogen levels (Table 4). pH of the

BWS was much closer to that of the UFT then the pH of the WUS or the GUH. The three native species in this study grow naturally on soils with pH around 5, and may have adaptations that favor this pH over more alkaline materials.

Soil reaction is widely documented as playing an important role in nutrient availability and toxicity in forest soils (Fisher and Binkley, 2000). In southwest Virginia, Showalter and colleagues (2005) found a negative correlation between white oak (*Quercus alba*) growth and increasing pH level across a variety of mine spoil types that ranged in pH from 3.2 to 7.8.

On a site in the Virginian Appalachians, Torbert and colleagues (1990) found that pitch x loblolly hybrid pine had much higher growth on acidic sandstone (pH= 5.7 compared to an alkaline siltstone (pH=7.1). Pines can usually tolerate more acidic soils than hardwoods (Fisher and Binkley, 2000). However, the soils of Appalachian hardwood forests are largely acidic (Hicks, 1998), showing that hardwoods are also adapted to moderately acidic soils.

In central Ohio, Kost and colleagues (1998) found that black pine (*Pinus nigra* Arnold) survived better after nine years on a two-layered soil including topsoil with a pH of 6.8 and low coarse fragment content (26%) covering a high pH overburden (7.4) with a higher coarse fragment content (51%). Tree growth was compared to that on overburden alone (Survival was 60% compared to 37%). Green ash (*Fraxinus pennsylvanica* Marsh), black pine, and silver maple (*Acer saccharinum* L.) had better growth rates on the topsoil (136, 165, and 57 cm) compared to the overburden alone (102, 116, and 41 cm). The success of these species could be attributed to either the lower coarse fragment content or lower pH, or a combination of the two. These studies

corroborate the tree mine soil property relationships found in our experiment; that is, native hardwoods appear to prefer soils with a moderately low pH similar to native soils of the area.

Indirect factors may have also played a role in the growth differences measured in this study, The lower pH of the BWS (5.56) may have been better for the growth of native soil organisms. Wei-Chun Ma (1989) found that earthworm populations in fly ash-amended mine soils in the Netherlands were negatively impacted by high pH's. Four- to five-year-old sites amended with fly ash with a pH of 8.2 had 49 worms $m^{-2}$; 15-18 year old sites with a pH of 7.9 had 265 worms $m^{-2}$ ; and control sites had a pH of 7.6 and 333 worms $m^{-2}$. Other soil fauna such as millipedes and isopods were also negatively impacted by increasing amounts of fly ash in soil mixtures. Microorganisms may also be influenced by pH and can shift in composition based on the acidity or alkalinity of the medium.

pH was also negatively correlated with growth of all three species in this study, further showing the importance of a moderately acid pH in the range of 4.8 to 6.5 for optimum growth. It is clear from the results of this study that alkaline soil reaction above pH 7.5 is detrimental to tree growth.

Because there is not an accurate soil analysis to determine P, K, Ca or Mg availability across materials with a wide range in pH and parent material, foliar nutrient levels of N, P, K, Ca and Mg were used in conjunction with soil KCl extractable inorganic N as indicators of soil nutrient availability. Nutrient analyses helped clarify how well available nutrients were being taken up and utilized by *F. americana, Q. rubra,*

and *L. tulipifera* on the different materials and whether these nutrients were affecting growth.

In general, foliar nutrient levels on trees growing on the UFT more closely approximated norms for trees growing naturally compared to the other spoil materials, suggesting that the UFT was most representative of a good growth medium (Table 6). Foliar nutrient levels on all materials were higher or lower than the norms for a given nutrient or nutrient ratio depending on the material and the species. Trees on the GUH and the WUS were generally high in Ca and Mg, low in P and N and variable in K, depending on species, suggesting that P and N were deficient in these materials. N and P are the primary limiting nutrients to tree growth (Fisher and Binkley 2000) both in the natural forest and when planted on a variety of soil types.

Soil KCl extractable inorganic N and foliar N concentrations were lower across all treatments compared to norms compiled from the literature. This suggests that trees growing in all treatments were nitrogen deficient. However, there were few data that differentiated among treatments, suggesting that N deficiencies were uniform across treatments and that physical properties were more important in differentiating the spoils from each other. However, this uniform deficiency suggests that N is an important factor limiting tree growth on these sites and that efforts should be taken to increase N regardless of the material used for reclamation.

Available soil nitrogen was highest on the UFT (27.72 mg $kg^{-1}$), which did not differ from the BWS (19.32 mg $kg^{-1}$), but was higher than on the WUS (12.91 mg $kg^{-1}$) and the GUH (11.20 mg $kg^{-1}$). The BWS did not differ from the WUS or the GUH (Table 4).

Based on the DRIS analysis, the WUS had greater N deficiencies in the foliage of *F. americana* than on the other treatments (Table 9). The foliar N index was lower on all spoils compared to the UFT for *Q. rubra. L. tulipifera* also showed the highest N index for UFT followed by BWS, GUH and WUS.

The DRIS foliar nutrient indices generally correlated with the soil analysis data showing that trees were able to extract more nitrogen from the UFT compared to the other materials. Although the evidence was not as strong, the BWS was a close second in nitrogen levels. This may have been due to the higher levels of microbial biomass and activity on the UFT followed by the BWS compared to the other materials. Decomposers mineralize N while other microbes are N-fixing, increasing N levels in the soil and making it more available (Hutson 1980).

Similar to the N indexes, foliar N concentrations of *F. americana* were lower on the WUS compared to the other treatments. Also similar to the N indexes , *Q. rubra* had higher N concentrations on the UFT compared to the spoil materials. The foliar N concentrations of *L. tulipifera*, on the other hand, did not differ across treatments.

Growth of *Q. rubra* and *L. tulipifera* correlated with foliar N concentrations but not soil N levels or DRIS N indices. Growth of *F. americana,* on the other hand, correlated with soil N levels and DRIS N indices but not foliar N concentrations.

Across all treatments, the foliar N concentrations were far lower than the N norms, suggesting that N was a limiting nutrient on these materials. Growth of *F. americana* correlated with differential adsorption based on treatment, having lower levels on the WUS compared to the other materials. *Q. rubra* was more able to extract N from the UFT, but showed no differences among the spoil materials. The highly variable foliar N

levels of *L. tulipifera* suggested that this species did not differ in N uptake across treatments and was equally deficient on all materials. The combined results suggest that the UFT had the highest available N, while the WUS had the lowest available N. But levels were low across all treatments, potentially having a negative impact on the growth of all trees.

Mineralizable N is essential for successful tree growth (Fisher and Binkley, 2000) and is often very low on fresh spoil materials (1.52 mg $kg^{-1}$) (Schoenholtz et al., 1992). Reeder (1977) also found very low levels of N mineralization in five mine spoils (0 to 11 mg $kg^{-1}$) from Colorado and Wyoming compared with the native topsoil (57 mg $kg^{-1}$). In Virginia, Schoenholtz and colleagues (1992) found that replacement of topsoil on mine sites increased mineralizable soil N levels from 45.76 mg $kg^{-1}$ to 66.95 mg $kg^{-1}$ but did not lead to improved growth of pitch x loblolly hybrid pine (*Pinus rigida* L. x *P. tadea* L.) or herbaceous vegetation. Growth appeared to be more a function of water availability than of nitrogen, with woodchip applications improving tree growth and increasing water availability. This suggests that other soil properties may have been more important on this study than nitrogen levels. However, even though the effects of nitrogen may have been masked by the more influential effects of soil physical properties, it is clear that nitrogen levels were deficient. The correlation of spoil type with foliar N levels in *F. americana* show that trees growing on the WUS may have a greater defficiency than the other materials, which could lead to decreased growth over longer periods of time.

Foliar P concentrations on all treatments were deficient for all three tree species compared to norms compiled from the literature (Table 6). However the level of foliar P deficiency differed across treatments depending on species. The P level of *F. americana*

was most sufficient on the BWS followed by the GUH, but the level in *Q. rubra* was most sufficient on the UFT followed by the GUH. *L. tulipifera* did not differ across treatments but was low on all materials. WUS was poor for all species, but foliar P concentrations of trees on the UFT, BWS, and GUH were more species dependent.

The DRIS analysis suggested that P levels of *F. americana* were most sufficient on the BWS, while P levels of *Q. rubra* were highest on the UFT, and P levels of *L. tulipifera* was most sufficient on the BWS followed by the UFT (Table 9).

The similar results of the foliar P concentrations and the DRIS P indices suggest that *F. americana* was most able to extract P from the BWS, while *Q. rubra* was more able to extract P on the UFT. *L.* tulipifera showed less conclusive results, but may have taken up the most P on the BWS.

However, these foliar concentrations and nutrient indices showed varying levels of correlation with tree growth depending on species. *L. tulipifera* showed no correlation, while the only *Q. rubra* growth parameter that correlated with foliar P was root biomass . Nutrient concentration was correlated with stem height and root biomass of *F. americana* suggesting a stronger relationship for this species.

P availability was not a strong differentiating factor for growth of *L. tulipifera* across treatments. *Q. rubra* showed slight correlation, suggesting that the higher availability of P on the UFT may have improved growth over the other treatments for this species. The stronger correlations of *F. americana* suggest that the higher P availability on the BWS may have played a more important role in the differential growth of this species across treatments.

Even though there was little correlation of *L. tulipifera* and *Q. rubra* it is clear that all materials were deficient in available P, which was having a negative impact on growth of all species across all treatments. The importance of sufficient P for proper tree growth is well established (Fisher and Binkley, 2000). This suggests that P fertilization is an important reclamation practice for all materials that are to be used for a native tree growth medium. However, it is also apparent that the BWS was a superior medium due to higher levels of available P for the growth of *F. americana*.

Trees growing on the BWS were generally low in foliar Ca and Mg concentrations. Compared with norms compiled from the literature, Ca levels were more deficient in foliage of *F. americana* growing on the BWS compared to the UFT, WUS or GUH (Table 12). Growth of *F. americana,* however, did not correlate with Ca concentrations, and tree growth of *Q. rubra* and *L. tulipifera* were negatively correlated with Ca concentrations. This suggests that other factors that parallel Ca concentrations, such as pH, may play a more important role in tree growth.

On the other hand, growth of *F. americana* was positively correlated with DRIS Ca indices, suggesting that Ca nutrient balance may have played a role in growth (Table 8). Indices were much lower on the BWS compared to the UFT, WUS or GUH, suggesting that Ca deficiencies on the BWS may have been detrimental to tree growth. Growth of *Q. rubra* and *L. tulipifera* were not correlated to Ca DRIS indices, suggesting that Ca nutrient balance was not important to growth. However, any Ca deficiencies occurring in the BWS were less important to growth than other factors, as is shown by the superior growth on the BWS. Mg concentrations and DRIS values were similar to Ca.

Potassium was also deficient for all species across all treatments compared to the norms established from the literature. The different levels of K in *F. americana, Q. rubra,* and *L. tulipifera* on different treatments suggests that these species are taking up potassium in very different ways. While *F. americana* had highest concentrations on the GUH, *Q. rubra* was undifferentiated across treatments and *L. tulipifera* had highest levels on the UFT followed by the GUH. *F. americana* growth correlated with K concentration suggesting that the higher levels on the GUH may have improved growth for this species. The correlations between growth of *Q. rubra* as well as *L. tulipifera* to K concentrations were weak.

*F. americana* growth did correlate with K DRIS indices, with the lowest defficiences on the GUH. *Q. rubra* growth did not correlate to K DRIS indices, but *L. tulipifera* did with the lowest deficiencies on the UFT followed by the BWS. The foliar concentrations and DRIS indices of K indicate that *F. armericana* was most able to take up K on the GUH, which positively affected growth, while *Q. rubra* did not differentiate. *L. tulipifera* was most able to take up K on the UFT followed by the BWS.

Overall balanced nutrition may not be an important factor to the growth of these trees as shown by the lack of correlation of tree growth with NBI. This may be because that while the WUS and GUH were more imbalanced in N and P, the BWS was imbalanced in Ca, creating equivalent NBI levels. It is apparent that these materials supply different nutrition dependant on the nutrient and the tree species.

Microbial populations were also higher on the BWS over the WUS or the GUH. Microbes are a crucial component for nitrogen and phosphorous availability on newly reclaimed sites. Decomposers play an essential role in nutrient cycling by breaking up

plant litter and other detritus and returning it to a mineral form that can be utilized by plants (Hutson, 1980).

Levels of dehydrogenase and ATP on the BWS were lower than on the UFT but were much higher than on the WUS or the GUH and positively correlated to tree growth (Table 7). Microbes are responsible for the decomposition of soil organic matter and mineralizing nutrients for root uptake. They also fix N and form important symbioses with plant roots. BWS had the most microbial biomass and activity of the three spoil materials, which were both correlated with tree growth. This may have been due to a combination of its lower pH and higher water retention, which is more conducive to microbial growth and activity.

Bussler and colleagues (1984) found on reclaimed forestland in southwestern Indiana that, while chemical properties were similar or better on mine spoils compared to adjacent native soils, water holding capacity and rock fragments were much different. Rock fragments on the topsoil were around 1.53 g $cm^{-3}$ while they were 1.77 g $cm^{-3}$ in the overburden. Also, water-holding capacity of the topsoil was 16.5% compared to spoil (10.8 to 11.7%). This suggests that although chemical properties may be playing a role, it may be that they are auto-correlated to soil physical properties, which are more important to tree growth on strip mined sites. However, the similarities in chemical properties may also have resulted from the replacement of topsoil on these sites.

Overall, this study follows the literature, suggesting that physical properties are more crucial to native hardwood growth than are chemical properties. Although pH and N and perhaps Ca are playing a role, water retention and coarse fragment content are more important to tree growth.

Effect of Topsoil Inoculation

Topsoil inoculation is more important for tree growth on some materials than others. While it had no effect on tree growth on the WUS or the BWS, it did have a positive impact on tree growth on the GUH. Topsoil inoculation had an effect on many of the spoil properties, increasing or decreasing their levels to closer approximate the UFT. Inoculation decreased the very high pH of the GUH, which increased foliar concentrations of some nutrients, while decreasing some that may have been in excess. These changes may have also improved the soil environment for the growth of a native soil fauna population (Wei-Chun Ma, 1989). The amendment may not have been as important on the BWS due to the fact that it already had a conducive environment for soil fauna and microbial growth. The WUS may have had such low nitrogen and water retention and high coarse fragments that even though the addition of topsoil decreased pH and improved the material somewhat, other factors were limiting. The GUH, on the other hand, may have been a poor material for tree growth by itself, but had the potential for good growth due to high nutrient content, and high moisture retention, both of which would create a better environment for microbial growth.

In a study conducted in Alabama, Cross and colleagues (1987) found that after seven years replacement of topsoil on an alkaline Pottsville shale *Pinus taeda* increased in height (4.68 m) and in diameter (7.34 cm) compared to sites where topsoil was not replaced (4.04 m and 6.30 cm, respectively). This shale was higher in pH (6.25) and coarse fragments (56.6%) than the topsoil (5.0 and 31.6%, respectively).

Although there was only an increase in growth on the GUH, inoculation caused a positive change in foliar K, and Mg indices and NBI's of *L. tulipifera* across spoil types, suggesting that this addition could have important value to tree nutrition for more sensitive species (Table 12). As mentioned above, Schoenholtz and colleagues (1992) found that topsoil addition increased total and mineralizable N levels by 23 and 46% over the control after three years. Vegetation productivity, however, did not increase. Plants were more affected by water availability than N availability on these plots. On this study, physical properties may also have been more important, though N levels may affect tree nutrition in the long run.

In southwest Virginia, Moss and colleagues (1989) found that addition of 30 cm of mixed A, E, B and C horizons on top of spoil did little to change soil properties and had no effect on survival or growth of planted pitch x loblolly pines (*Pinus rigida* Mill. x *P. taeda* L.). This manner of topsoil addition that includes the B and C horizons is much more realistic in the Appalachians because the A horizon can be thin in places. This suggests that the nutritional value of topsoil in the Appalachian region is still debatable. However, the spoil material that was used for Moss's study was already a 2:1 sandstone/siltstone mix, which had physical and chemical properties that were similar to the topsoil, and may have been less impacted by topsoil addition compared to spoils such as WUS and GUS that were very different from native topsoil.

Growth of understory vegetation was significantly higher on the topsoil-inoculated pots than the pots that were not inoculated. Although the difference among spoils was not significant, the trend suggests that it is not only the topsoil inoculation that is important but also the material with which it is mixed. Native plants did better overall

on the BWS compared to the GUH or WUS, showing that it is more hospitable overall to the establishment of understory vegetation. Farmer and colleagues (1982) found that native forest topsoil placed on mine spoils in southern Appalachia can produce substantial native vegetation. Native seed banks produced $1.9x10^6$ shoots $ha^{-1}$, with a diversity of 134 taxa after one growing season, supporting the potential growth of topsoil seedbanks. Not only is this important in light of sprouting seeds from the topsoil inoculation, but also for the establishment of seeds that may be transported to the area by wind or animals. Furthermore, inoculated pots had significantly higher microbial levels, further adding to the return of the native ecosystem. The return of microbial populations can lead to improvements in the establishment of the native forest by mineralization of nutrients, nitrogen fixation and symbiotic relationships with tree roots.

Although inoculation only improved growth on the GUH, the added benefit of improved understory vegetation growth from the native seed bank and increased microbial growth suggests that inoculation with the native topsoil may be an important step in the reclamation process regardless of spoil type. Inoculation improves the potential for the return of a diverse native hardwood ecosystem including the understory vegetation and microbial populations.

Species Differences

Not only was overall tree growth influenced by spoil type but the three species displayed different growth responses to each spoil, creating a statistical interaction. *L. tulipifera* did not react well to any of the spoils and grew about the same on all treatments. Zeleznik and Skousen (1996) also found that *L. tulipifera* survival and growth was much poorer on strip mined sites compared to other trees planted, with survival rates ranging

from 3 to 21%. Planted *L. tulipifera* is highly sensitive to site quality, growing well on good sites and poorly on poor sites (Beck, 1990). All of the spoil materials would be considered poor soils comparatively due to their low organic matter, low nutrient levels and poor soil structure. The control soil came from a side slope, which is typically a poorer soil than that found on toeslopes or cove positions. *L. tulipifera* grows best on high quality cove positions. Both nutrient levels and soil physical properties such as water availability and aeration have been correlated with growth of *L. tulipifera* and are important to its survival and growth (Beck, 1990). Beck (1990) reported that in the first few years of life, water availability and drainage are critical for the survival and good growth of *L. tulipifera.* Also, this tree is commonly propagated from stump sprouts or seeds in the forest floor, and is not often planted as seedlings (Beck, 1990).

*F. americana* and *Q. rubra* showed clear but somewhat different responses to spoil type. *F. americana* had the greatest growth response and displayed different growth rates on each spoil. Next to the UFT, the BWS was the best medium for *F. americana* growth, based on both root and stem biomass. *Q. rubra* was more successful on the BWS than any other medium including the UFT. This may have been due to the higher % fines on the BWS compared to the UFT.

Among species, *F. americana* was the most successful on all spoils. This suggests that it was most able to adapt to the highly variable properties of these different materials. In eastern Ohio, Zeleznik and Skousen (1996) found that *F. americana* was the most successful species planted compared to *L. tulipifera* and *Pinus strobus*. After 46 years *F. americana* had a survival rate of 43% and was doing equally well on spoils that ranged in physical properties. In central Ohio, Kost and colleagues (1998) found that

among six species *F. pennsylvanica* had the best survival rate (95%), with the next highest being *P. nigra* at 60%, on alkaline spoils covered with acid topsoil after nine years. This supports our findings that *Fraxinus* is able to survive and grow well on spoils when other species are not. Whether this is due to its ability to tap nutrient pools or if it has greater tolerance to other limiting spoil properties is still debatable.

Torbert and colleagues (1985) found on a reclaimed site in Virginia that *F. americana* had a survival rate of 91% and a growth rate of 24 cm on herbicide sprayed plots after 3 years. In contrast, *L. tulipifera* had survival rates of 54% and height growth of only 11 cm. Interestingly, *Q. rubra* did not fair well on their experiment, with a survival rate of 43% and growth of only 6 cm. In this study, *Q. rubra* growth was intermediate, not as vigorous as the *F. americana*, but growing adequately on all spoil types.

## Conclusions

The return of the diverse mixed mesophytic forest of the Appalachian Mountains on reclaimed strip mine sites may be greatly influenced by spoil type. This research shows that the growth of two of the three native hardwood species used in this study are affected by the physical, chemical, and biological properties of mine spoils. Spoils that better approximate the native soils of the region, such as BWS (brown weathered sandstone), were more conducive to native hardwood growth than the WUS (white unweathered sandstone) or GUH (grey unweathered shale).

The inoculation with native topsoil improved tree growth on the GUH, added a native seed pool, and increased microbial populations. In addition, the added topsoil

created an environment that was more conducive to establishment of volunteer plants . All of these factors suggest that adding native topsoil to spoils could speed up the return of the native mixed mesophytic forest by creating an environment rich in native microbes and seeds. In addition, inoculation shifted the properties of all three spoils toward those of the UFT (undisturbed forest topsoil), which should increase the likelihood of native plant establishment.

Our study shows that some species are much more adept at growing on reclaimed sites and that the timely return of the diverse forest that was there prior to mining requires spoil selection and treatment. While *F. americana* may have grown well, species that are more sensitive to site quality such as *L. tulipifera* may need good quality sites in order to establish and grow well.

This study showed that spoil selection, return of topsoil, and species selection to match soil characteristics are all important factors to consider during the reclamation process. In order to best establish the native mixed mesophytic forest, brown weathered sandstone was found to be the best topsoil substitute tested in this study. The benefits of topsoil replacement were biological as native plants were introduced in the seed pool and soil microbial biomass and activity were greater; however, it has little direct effect on spoil fertility. Many of the mid- to late-successional native hardwoods with life histories similar to ash and red oak can be expected to grow well on BWS spoil amended with topsoil.

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# CHAPTER IV.

# PHYSICAL, CHEMICAL, AND BIOLOGICAL MINE SOIL PROPERTIES INFLUENCE WHITE OAK SEEDLING GROWTH


## Abstract

Landowners in Appalachia are becoming increasingly interested in restoring the native hardwood forest on reclaimed mined land. Trees are usually planted in topsoil substitutes consisting of blasted rock strata, and reforestation attempts using native hardwoods are often unsuccessful due to the variable nature of these mine spoils. The purpose of this study was to determine which mine soil properties most influence white oak (*Quercus alba*) seedling growth, and to test whether these properties are adequately reflected in a preliminary mine soil classification model. Seventy-two 3-yr-old white oaks were randomly selected across a reclaimed site in southwestern Virginia that varied greatly in spoil/site properties. Tree height was measured and soil samples adjacent to each tree were analyzed for physical, chemical and biological properties. Our original mined land classification model used rock type, compaction and slope aspect as mapping criteria; however, mapping units were not well correlated with differences in tree height. Tree height, ranging from 15.2 to 125.0 cm, was regressed against mine soil and site properties. Microbial biomass, pH, exchangeable potassium, extractable inorganic nitrogen, texture, aspect, and extractable phosphorous accounted for 52% of the variability in tree growth. The regression model shows that white oaks were most successful on northeast-facing aspects, in slightly-acidic, sandy loam, fertile mine soils that are conducive to microbial activity. Based on the regression model, nutrient availability was highly influential on tree growth and not adequately represented in the

classification model. This suggests that pH should be included as a classification criterion as it was correlated with all nutrient variables in the regression model.

Key words: Site index, native hardwoods, mine soil classification

## Introduction

The eastern deciduous hardwood forest of Appalachia is one of the most valuable, productive, and diverse temperate forests in the world. It is important, both environmentally and economically to the seven states in the Appalachian region. However, large areas of this forest are being eliminated due to surface mining, and very few reclaimed mines are restored to native forest.

Over 500,000 ha of land have been surface mined in the eastern United States since the implementation of SMCRA in 1978 (OSM, 1999). Federal and state regulations based on SMCRA have helped improve water and environmental quality as well as safety of active and reclaimed sites, but current reclamation procedures are not conducive to reforestation (Burger and Zipper 2002). Mine soils are usually too compacted, salty, and infertile for native forest plant communities. These large areas of land have mostly been reclaimed to grassland or wildlife habitat (grassland with wildlife shrubs). Reclamation usually involves the replacement of the landscape to approximate original contour and planting with an herbaceous ground cover. Many reclaimed grassland sites have low productivity and are often abandoned due to their poor quality and remote location. If conditions were conducive to native forest plants, their return would create a valuable economic resource as well as play an important role in watershed control, water quality, carbon sequestration, and wildlife habitat.

Due to the steep slopes of the Appalachian Mountains, returning land to approximate original contour is often unfeasible or expensive. In West Virginia, commercial forestry is an acceptable land use on mines for which a waiver of the approximate original contour requirement has been obtained. Reforestation is an attractive alternative under these circumstances, but, in order for coal operators to get bond release, tree growth must be successful, with a site index comparable to adjacent native forest.

Black locust (*Robinia pseudoacacia*) and a variety of other early-successional trees are able to survive and grow on these mined sites (Vogel and Berg 1973; Filcheva et al. 2000). However, these species have little commercial value and do not provide the same level of ecosystem services as the native mixed mesophytic hardwoods that are usually present prior to mining.

In this area where fire and other disturbances are common, oaks (*Quercus*) represent a mature successional stage in forest development (Johnson et al, 2002). They are an essential component of the native hardwood forest and their replacement on these sites would be a positive step toward the return of these forests. Due to their large tap root, and high root to shoot ratio, they are drought tolerant suggesting that once established they could compete on these harsh sites. However, the chemical and physical properties of the soil medium will play an important role in their development.

Mine spoils have highly variable physical and chemical properties, ranging from very acid pyritic materials to alkaline shales. Compared to native soils, mine spoils can be high in rock fragments, have low moisture content, low porosity, poor structure or

high bulk density (Bussler et al., 1984). Chemical properties such as high pH and soluble salts and low nutrient levels can also adversely affect tree growth (Torbert et al., 1990).

Understanding how the combinations of different properties affect tree growth would aid in classifying and mapping mined land, and in developing successful forestry practices. Sites could then be mapped and tree species selected based on site and soil types.

Therefore, objectives of this study were to: 1) test the accuracy of a site quality classification model (Burger et al. 2002) using a reforested mined site containing a broad gradient of spoil types and potential site quality classes; and 2) to understand the relationship between mine soil properties and white oak growth in order to improve the usefulness of the model.

## Methods and Procedures

### Site Description and Classification

A previous study involving 10 reclaimed sites across 3 states was used to select preliminary mine soil quality classification criteria that included rock type, compaction, and aspect (Fig. 1) (Burger et al., 2002). These criteria were based on a conceptual model of mine soil quality that includes soil toxicity, soil strength, air/water balance, and nutrient level. Listed from most to least important, these soil properties are the determining factors for native hardwood growth on reclaimed mine sites. The mapping criterion rock type represents all four factors to some extent. The level of soil compaction determines soil strength and air/water balance, while aspect is an important criterion for estimating air/water balance.

These three criteria were measured and regressed against tree growth on all plots. The slope of the regression line for each criterion was used to develop a weighting factor (WF) for each criterion to reflect its relative importance. Sites were classified by assigning a value of 1 to 5 for each criterion, which was then multiplied by their respective WF and added to obtain a soil/site quality class ranging from I to V. This study was used to test the site classification model and determine if the criteria used adequately represented the factors that influenced white oak growth.

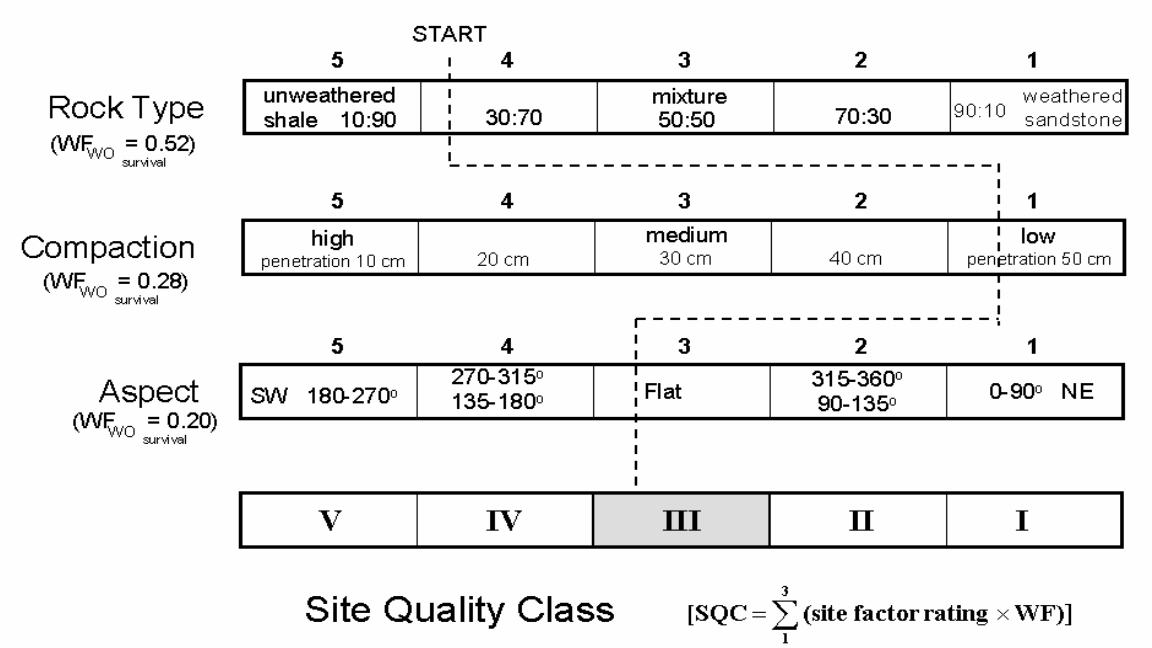



**Figure 1.** Site factor gradients used to determine overall mined site quality class (SQC) (Burger et al., 2002).

This study was conducted during the summer of 2004. The study area is located in southwestern Virginia on a reclaimed coal mine owned by Rapoca Energy Co. (Fig. 2). This area is in the Kanawha geologic formation and many different rock strata above the coal seam were placed on the surface during reclamation. As a result, the area consists of a wide variety of mine soil types, varying in both physical and chemical properties.

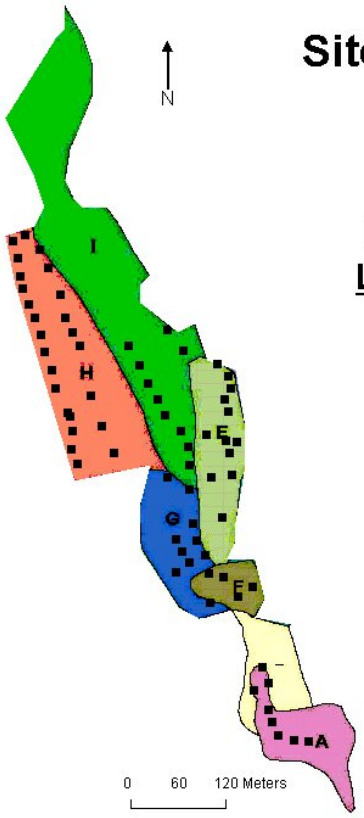
**Figure 2.** The Rapoca reclamation site in southwest Virginia immediately after reclamation, 2002.

In 2001, immediately after final grading, reclaimed soils were mapped and areas of the site with similar mine-soil properties were delineated as soil mapping units. For each mapping unit, site quality class was determined using the model described above (Fig. 1). The site was planted in 2002 with native hardwoods at a density of 1482 trees $ha^{-1}$. using species mixes appropriate to each mapping unit's soil properties and site quality.

Field and Laboratory Methods

Tree performance was used to judge the accuracy of the site class assignments. Seventy-two three-year-old white oak trees were randomly chosen across a range of spoil types, which included 6 mapping units (Fig. 3). White oak was chosen because it was a component of all species mixes and is a valuable timber species of the late successional

native forest of the Appalachians. Glyphosphate was sprayed in a 1m diameter circle around each tree at the beginning and in the middle of the growing season to reduce herbaceous competition. Tree heights were measured at the end of the growing season, and averages were calculated for each mapping unit.




**Site classification of six mined land map units.**

| Unit Label | Rock Type | Compac-tion | Aspect | Site Quality | Site Class |
|---|---|---|---|---|---|
| A | 2 | 2 | 3 | 2.2 | II |
| E | 2 | 4 | 3 | 2.8 | III |
| F | 3 | 2 | 4 | 2.9 | III |
| G | 4 | 4 | 5 | 4.2 | IV |
| H | 5 | 3 | 4 | 4.2 | IV |
| I | 1 | 1 | 5 | 1.8 | II |

**Figure 3**. Soil/site quality classification and mapping of the Rapoca reclamation site in southwestern Virginia. The data points on the map indicate randomly selected white oaks that were used to test the classification criteria. Values are based on the site quality class scheme in Figure 1.

Soil samples were taken to a 40 cm depth within a 50 cm radius from the base of each tree. Samples were dried and sieved through a 2 mm sieve and an array of physical and chemical properties were measured. Bulk density was found using volume displacement and coarse fragment content was found by mass fractionation Soil particle size was determined using the hydrometer method (Bouyoucos, 1936). Total soluble

salts was measured with an electrical conductivity meter (Bower and Wilcox, 1965) and pH was determined in a 2:1 water-soil suspension using a pH meter. Nitrogen availability was measured using aerobic incubation (Bremmer, 1965a) and inorganic nitrogen was determined using a KCl extraction method (Bremmer, 1965b). Total nitrogen and carbon were found using a carbon nitrogen analyzer (Vario MAX, 2000. Elementar Americas, Inc., Hanau, Germany). Exchangeable cations were extracted using the ammonium acetate method and analyzed using an ICP spectrophotometer (SpectroFlame Modula Tabletop ICP, 1997, Spectroanalytical instruments, Germany; Thomas, 1982). Available nutrients were found using the Mehlich I test and available P was measured using the sodium bicarbonate method (Olsen and Sommers, 1982).

The site factors aspect, slope, and distance from native forest were also determined for each tree. Aspect was scaled by assigning numerical values ranging from 1 for a northeast aspect and decreasing in value in either direction around the compass to 0 for a southwest aspect.

At the end of the growing season, the trees were excavated and soil adjacent to tree roots was used for the characterization of soil microbial properties (Table 1). Soil was kept at 4°C and was processed within 2.5 weeks of collection. Dehydrogenase concentration was found through a 2,3,5-Triphenyltetrazolium chloride (TTC) indicator (Tabatabai, 1982). Microbial biomass was measured by chloroform fumigation (Anderson and Domsch, 1978; Gregorich et al., 1990; Jenkinson and Powlson, 1976).

Leaves were harvested in mid August from the upper portion of the crown, dried at 65°C for 7 days, and ground to pass through a 1mm sieve. Nitrogen was found using the C-N analyzer referenced above. Potassium, calcium, magnesium, manganese and

phosphorus were determined by dry ashing the samples, extracting the nutrient elements using a 6 N HCl solution, and analyzing the extracts with the ICP spectrophotometer referenced above (Jones and Steyn, 1973).

Data Analysis

Tree height was regressed with site quality class to determine if the classification criteria represented factors influencing tree growth (SAS, 2004). Tree height was also regressed with soil and site properties. The independent variables were transformed based on established relationships with tree growth (Henderson et al., 1990; Kiniry et al., 1983, Fisher and Binkley, 2000). Potassium and available nitrogen were transformed using the square root function and microbial biomass and pH were transformed using a natural log function. An arc sine function was used to transform aspect and silt plus clay percent in order to achieve a normal distribution (Little and Hills, 1978). Backward Cp and R-squared selection were used to eliminate variables based on multi-collinearity and biological significance. Two experimental trees were eliminated based on differences in growth pattern. One had a double stem while the other was much larger than any of the other trees. Two additional outliers were eliminated based on soil properties. Both had phosphorous levels over ten times higher than any of the other samples. This was most likely due to a lab error.

Foliar samples were analyzed for nutrient sufficiency and balance using the Diagnosis and Recommendation Integrated System (DRIS) (Beaufils 1973). This analysis involves comparing the variance in foliar nutrient levels and nutrient ratios between the subpopulation of trees that are most successful and the rest of the population. Ratios of the good population were used as a standard by which imbalances in the

remaining trees were assessed. A nutrient index for a given foliar nutrient level 'A' is developed using the following equation:

$$\text{Nutrient 'A' index} = \frac{[f(A/B) + f(A/C) + \ldots. + f(A/N)]}{z}$$

where

$$f(A/B) = ((A/B)/(a/b) - 1) \times (1000/CV) \text{ when } A/B \geq a/b,$$

or,

$$f(A/B) = (1-(a/b)/(A/B)) \times (1000/CV) \text{ when } A/B < a/b,$$

where A through N are the levels of foliar nutrients of interest, A/B is the nutrient ratio of the target population, a/b is the nutrient ratio of nutrient A to nutrient B for the good subpopulation, z is the number of functions in the equation and CV is the coefficient of variation of the good population (Walworth and Sumner, 1987). The closer the nutrient index is to zero, the closer it is to the level of the good population or the optimum. If it is negative, the nutrient is deficient, and if it is positive, the nutrient is in excess. The sum of the absolute values of the nutrient indices were added to obtain a nutrient balance index (NBI). The higher the number, the greater the nutrient imbalance and the poorer the tree growth. If the NBI is close to zero, foliar nutrient concentrations are close to optimal.

This analysis can be used to develop nutrient norms for a given plant species. Populations that are fertilized at various levels are compared and the nutrient levels in plants that are most successful are used as norms. In this study we used foliar nutrient levels to compare relative nutrient deficiencies and imbalances within a single population of young white oak trees. Nutrient means of the upper quartile of the population based

on tree height were used as the standard against which foliar levels were compared. This analysis was done to find relative levels of nutrient imbalances among trees on the study site. It was simply used to distinguish between the nutrient levels of trees that are growing relatively well on certain sites and trees that were growing poorly.

## Results and Discussion

Average tree growth for each mapping unit was plotted as a function of site quality class (Fig. 4). We hypothesized that tree growth would increase as site quality increased, where Class I is highest quality and Class V is lowest. However, the relationship between tree growth and designated site class for the mapping units was not significant ($p>0.1$) Measured tree growth showed that site class was incorrectly assigned to several of the mapping units. This suggests that the classification criteria do not represent all the soil and site factors influencing tree growth, and that additional classification criteria are needed to adequately estimate site quality class.

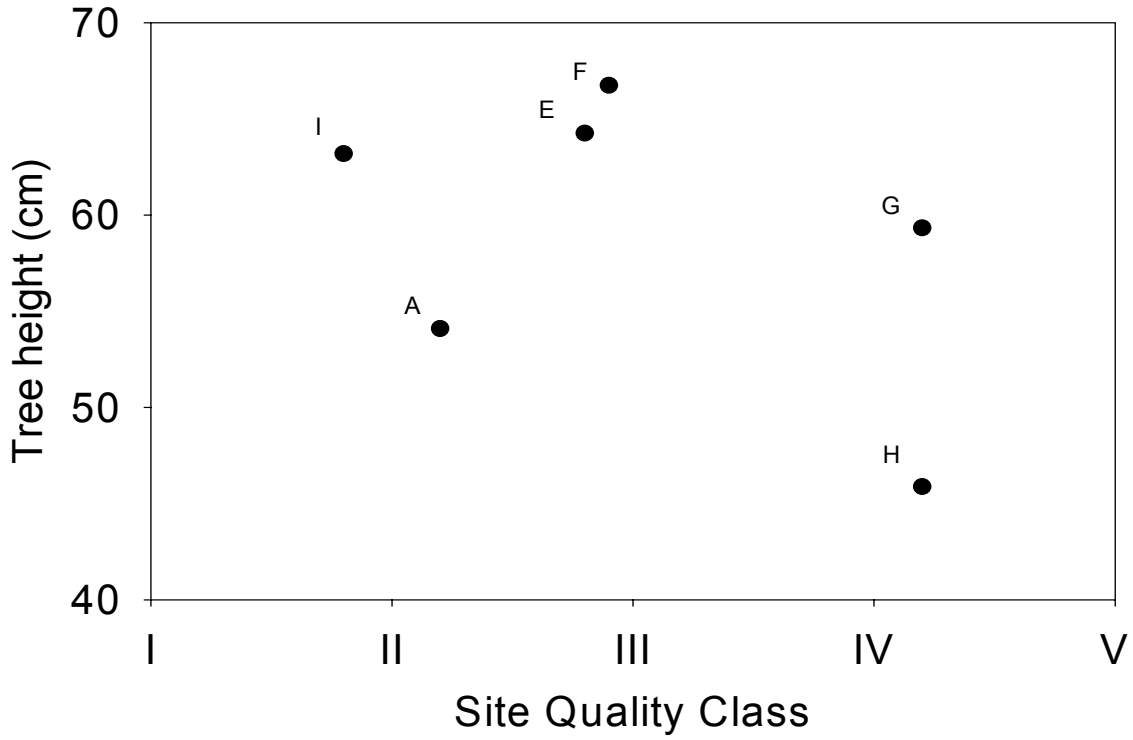



**Figure 4.** Mean tree height verses soil/site quality for mapping units on the Rapoca reclamation site in southwestern Virginia, 2004.

A spectrum of site and soil physical, chemical and biological properties, hypothesized to influence tree growth, were measured and are presented in table 1. In nearly all cases, values ranged considerably among the sampled points. For example, slope steepness ranged from 0 to 55 and slope aspect covered the spectrum from northeast to southwest. Bulk density of the fine earth fraction ranged from 0.61 to 1.39 g $cm^{-1}$ and silt plus clay ranged from 17 to 64%. Chemical properties were also highly variable with soil exchangeable nutrients ranging by an order of magnitude. pH ranged from 3.24 to 7.79 and EC ranged from 0.14 to 4.72 dS $m^{-1}$. Microbial activity was virtually absent on some sites and quite prevalent on others. As a result of this broad range in soil and site properties, tree height ranged from 15 to 125 cm.

**Table 1.** Mean values and ranges for tree height and soil/site properties associated with each tree on the Rapoca Mine Site, VA, 2004.

| Variable | | Mean | Min | Max | St Dev | CV |
|---|---|---|---|---|---|---|
| Tree Height (cm) | | 56.4 | 15.2 | 125.0 | 22.5 | 40.0 |
| Site Properties | | | | | | |
| Aspect † | | 0.27 | 0.0 | 1.0 | 0.29 | 107.44 |
| % Slope | | 24 | 0 | 55 | 16 | 69 |
| Dist from Native Forest (m) | | 69 | 6 | 162 | 41 | 59 |
| Physical Properties | | | | | | |
| % Silt + Clay | | 45 | 17 | 64 | 9 | 20 |
| % rock fragments | | 35 | 21 | 51 | 6 | 16 |
| Bulk Density (fine earth) | | 0.96 | 0.61 | 1.39 | 0.18 | 18.27 |
| Chemical Properties | | | | | | |
| EC (dS $m^{-1}$) | | 0.92 | 0.14 | 4.72 | 1.03 | 112.29 |
| pH | | 5.2 | 3.2 | 7.8 | 1.5 | 27.8 |
| Anaerobic N (mg $kg^{-1}$) | | 9.96 | 0.49 | 62.95 | 10.12 | 101.66 |
| Available N (mg $kg^{-1}$) | | 5.20 | 1.59 | 21.55 | 3.52 | 67.78 |
| P (mg $kg^{-1}$) | | 7.24 | 0.92 | 34.86 | 5.75 | 79.44 |
| Melich I Available Nutrients (mg $kg^{-1}$) | K | 33.5 | 8.7 | 72.3 | 13.2 | 39.5 |
| | Ca | 542.3 | 54.0 | 1426.4 | 368.3 | 67.9 |
| | Mg | 142.0 | 24.9 | 267.2 | 62.6 | 44.1 |
| | Zn | 2.2 | 0.8 | 5.9 | 1.1 | 50.6 |
| | Mn | 19.8 | 6.1 | 52.2 | 11.1 | 56.0 |
| | Cu | 1.1 | 0.4 | 1.8 | 0.3 | 25.9 |
| | Fe | 41.9 | 12.0 | 138.9 | 24.3 | 58.0 |
| | B | 0.09 | 0.05 | 0.20 | 0.04 | 47.67 |
| C-N analyzer (mg $kg^{-1}$) | N | 0.05 | 0.03 | 0.15 | 0.02 | 41.95 |
| | C | 1.08 | 0.30 | 4.39 | 0.76 | 70.53 |
| Exchangeable cations (mg $kg^{-1}$) | Ca | 252.5 | 44.5 | 539.9 | 117.1 | 46.4 |
| | Mg | 100.5 | 27.9 | 147.0 | 23.2 | 23.1 |
| | K | 161.5 | 28.4 | 343.6 | 73.2 | 45.3 |
| | Na | 65.1 | 16.0 | 107.2 | 17.0 | 26.1 |
| CEC (cmol $kg^{-1}$) | | 3.01 | 0.99 | 5.65 | 1.11 | 37.02 |
| Biological Properties | | | | | | |
| Dehydrogenase (mg $kg^{-1}$) | | 11.42 | 0.25 | 80.83 | 15.47 | 135.46 |
| Microbial biomass (mg $kg^{-1}$) (dissolved organic carbon) | | 38.1 | 3.3 | 201.8 | 35.8 | 93.9 |

† aspect was calculated using a continuum where southwest=0 and northeast=1.

To better understand which soil and site factors were influencing white oak seedling growth, and to determine additional classification criteria to improve the model (Fig. 1), tree height was regressed against soil and site properties. From greatest to least importance in the regression model, microbial biomass, pH, extractable soil potassium,

total extractable inorganic nitrogen, % silt plus clay, aspect, and extractable soil phosphorous were found to be the most important factors influencing white oak growth on this site (Table 2). Over 52% of the variation in tree height was described by the seven soil and site factors:

$$\textbf{\textit{Tree ht}} = 33.10 + 9.19\ln(\textbf{\textit{bio}}) - 29.01\ln\textbf{\textit{pH}} + 6.61\sqrt{(\textbf{\textit{K}})} + 10.05\sqrt{(\textbf{\textit{N}})} - 61.15\arcsin(\textbf{\textit{siltclay}}) + 11.33\arcsin(\textbf{\textit{as}}) + 0.02(\textbf{\textit{P}})^2$$

$$\mathbf{R^2 = 0.5205}$$

**Table 2.** Variables and standardized coefficients for the above regression model describing 52% of the variation in tree growth.

| Variable | Standardized coefficient | p value |
|---|---|---|
| ***Bio*** = biomass of microbes (mg $L^{-1}$) | 0.40622 | 0.004 |
| ***pH*** = pH | -0.40334 | 0.0068 |
| ***K*** = potassium (mg $kg^{-1}$) | 0.38567 | 0.0019 |
| ***N*** = total extracted nitrogen (mg $kg^{-1}$) | 0.35289 | 0.0048 |
| ***Siltclay***= % silt + clay | -0.26324 | 0.0345 |
| ***as*** = aspect | 0.24696 | 0.0318 |
| ***P*** = phosphorus (mg $kg^{-1}$) | 0.19187 | 0.0796 |

Examined on a mapping unit basis, the means of the significant soil properties varied widely (Table 3). These areas are easily distinguished by their vastly different physical, chemical and biological properties, suggesting that their map unit delineation was warranted. However, these properties also varied widely within mapping units.

**Table 3.** Mean values and ranges for height and soil/site properties that were included in the regression model for each mapping unit on the Rapoca Mine Site, VA, 2004.

| Variable | Mapping Unit | | | | | |
|---|---|---|---|---|---|---|
| | F | E | I | G | A | H |
| Height (cm) | 66.74 | 64.26 | 63.19 | 59.33 | 54.10 | 45.89 |
| Microbial biomass (mg $kg^{-1}$) (chloroform fumigation) | 84.15 | 32.51 | 38.96 | 64.38 | 66.95 | 16.59 |
| pH | 6.57 | 4.64 | 4.75 | 6.22 | 6.19 | 4.96 |
| Melich I Available K (mg $kg^{-1}$) | 33.22 | 36.04 | 39.47 | 30.17 | 45.71 | 29.09 |
| Available N (mg $kg^{-1}$) | 11.19 | 4.02 | 4.99 | 8.04 | 6.47 | 3.43 |
| % Silt + Clay | 42.93 | 50.39 | 49.34 | 43.39 | 47.50 | 39.84 |
| Aspect † | 0.13 | 0.73 | 0.19 | 0.00 | 0.00 | 0.17 |
| P (mg $kg^{-1}$) | 7.12 | 6.77 | 9.31 | 6.52 | 8.88 | 6.63 |

† aspect was calculated using a continuum where southwest=0 and northeast=1.

Microbial biomass was represented with a mean of 37.36 mg/kg of dissolved organic carbon; it was skewed toward lower levels with many sites having virtually no microbes, and only a few sites having levels above 100 mg/kg (Fig. 5). Microbial biomass was positively correlated with tree growth. Since correlation does not necessarily imply causation, it is unknown whether microbial populations have a significant effect on tree growth or whether microbial biomass responded to the site factors that influenced tree growth. The fact that microbial biomass was independent of other regressors in the model suggests that it is a causative factor. Symbiotic mycorrhizal colonization of the tree root systems would be an example of causation. In any case, microbial activity is a good indicator of soil quality (Miller, 1998) as microbes play important roles in decomposition of organic matter, nitrogen mineralization and fixation, and symbiosis. Hutson (1980) found that low population densities of organisms on industrial reclamation sites led to significantly less degradation of oak leaves than on control sites. Microbes are the main mechanism for the release of plant available nutrients from organic matter (Brady and Well, 1996). In his discussion of techniques for

reclaiming with native hardwoods, Miller (1998) stated that the development of a healthy and diverse soil microbial population through replacement of topsoil is essential to the establishment of native trees.

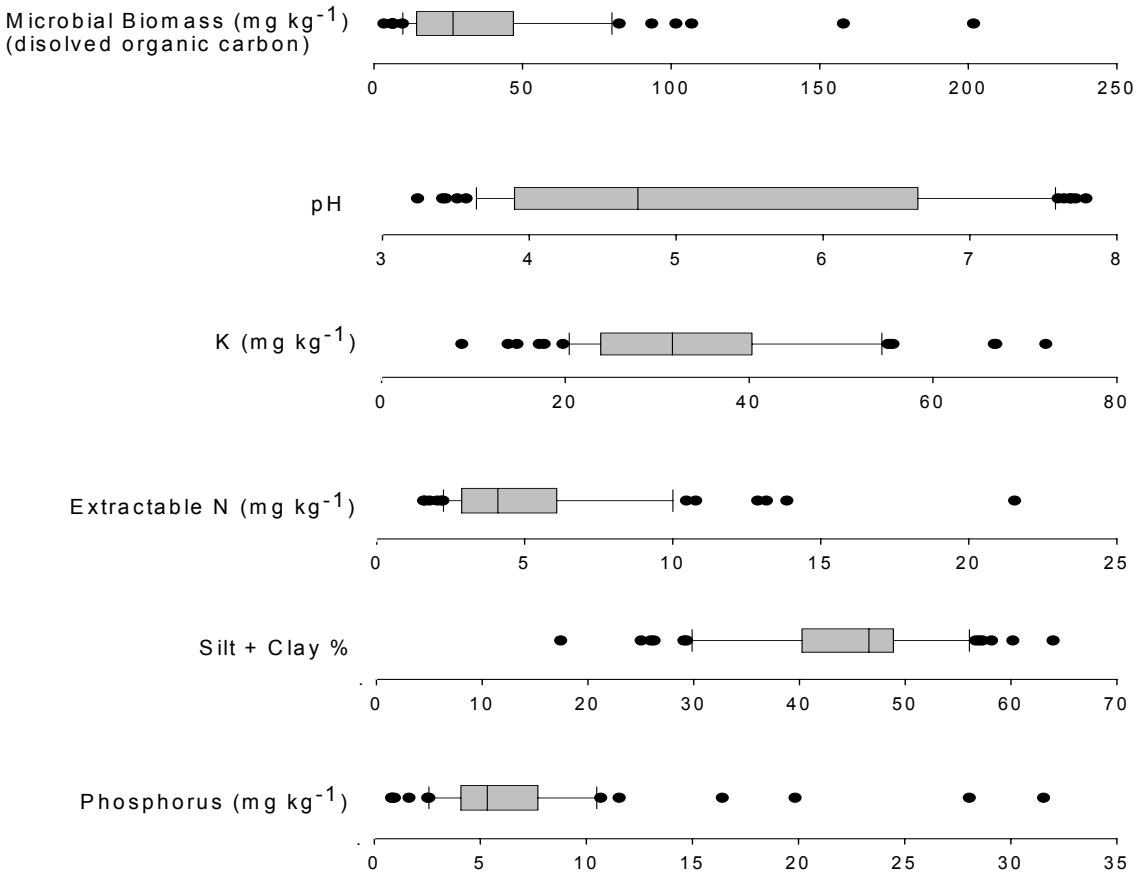



**Figure 5**. Boxplots for variables in the regression model, listed from most to least important to tree growth.

pH ranged from 3.24 to 7.79 with a mean of 5.20 (Fig. 5). It had a significant negative correlation with tree growth and was the second most important variable in the regression model. pH is related to many other soil chemical and biological properties. It is closely related to spoil type and also dictates nutrient availability.

The average level of nitrogen measured across the study area was 5.02 mg/kg (Fig. 5). This level is considered deficient (Fisher and Binkley, 2000), suggesting that the higher levels found on some sites would have a positive impact on tree growth. Levels of potassium and phosphorous ranged widely with phosphorous skewed toward smaller amounts, while potassium was more evenly distributed. These nutrients, P, K, and N, were positively correlated with tree growth and were found to be statistically significant components of the regression model. Tree growth was a function of the square root of the level of N and K and the level of P squared, which is a typical response relationship. Nutrients that were collinear with potassium such as calcium and magnesium were removed from the regression analysis; therefore, K may be seen as representing a group of cations important to tree growth.

Soil types ranged from loamy sands to sandy clay loams with 17 to 65% silt-plus-clay (Fig. 5). Percent silt-plus-clay was negatively correlated with tree growth. This negative correlation suggests that oak trees grow relatively better in sandier mine soils. This may be caused by low hydraulic conductivity and poor drainage in the more finely-textured mine soils, or it may be because silt-plus-clay content correlates with chemical factors that influence tree growth. Torbert and colleagues (1990) also found a strong positive correlation between percent sand and tree growth.

Aspect ranged from northeast to southwest. Tree height was positively correlated to aspect increasing from southwest to northeast. Northeast aspects are more mesic and cooler during the growing season, making them more conducive to tree growth.

Because soil fertility factors played a dominant role in the model of tree growth as a function of soil and site properties, (Equ. 2), foliar nutrient concentrations were

analyzed to better understand the role of soil fertility on tree growth. DRIS nutrient analyses showed that nitrogen, potassium, and calcium were deficient or in excess and that ratios of N/K, K/Ca, and K/Mg were out of balance. This suggests that levels of N, K, P, Mg, and Ca are all crucial nutrients affecting the growth of these trees on these sites. The nutrient indices suggested that all mapping units were deficient in N and P and over abundant in Ca and Mg (Table 5). K was either sufficient or slightly deficient depending on the mapping unit.

The nutrient levels of the better growing population were much closer to levels found in foliage from white oaks growing in the native Appalachian hardwood forest. Auchmoody and Hammock (1975) found levels of 25.6 mg/kg for N, 1.5 mg/kg for P, 7.3 mg/kg for K, 4.7 mg/kg for Ca, 0.9 mg/kg for Mg and 1.0 mg/kg for Mn (Table 4). These trees were located on a mixed oak stand in the Fernow Experimental Forest in Parsons, West Virginia. The upland oak site index was 66 and the stand was 60 years old. Foliage samples were taken from the upper part of the crown in late August. A comparison of nutrient levels with those occurring on the study site, shows that levels of N are insufficient on the mine site, while P and K are sufficient and Ca, and Mg are in excess compared to these native forest levels.

When these nutrient indices are added (absolute values) using the DRIS technique to create a nutrient balance index (NBI), the values show that there is a clear relationship between the extent of imbalance and the amount of tree growth ($p=0.0001$) (Fig. 6). Mapping unit H had the lowest tree height growth levels, the lowest predicted site quality and the highest nutrient imbalances. This further supports the importance of nutrient levels in the designation of spoil quality and the ability of trees to grow in them. These

DRIS results strongly support the inclusion of soil available N, P, and K in the regression model.

**Table 4.** DRIS foliar nutrient ratios for good and poor populations of three-year-old white oaks on a mine site in Virginia.

| Nutrient | | Native Unfertilized White Oak Nutrient Levels[†] | High Height Growth[‡] | | Low Height Growth | | F-test for Variance Ratio | p value |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | CV | Mean | CV | Sl2/SH2 | |
| N | 21.90 | 25.60 | 22.75 | 12.62 | 17.99 | 28.82 | 3.26 | 0.0104* |
| P | 1.45 | 1.50 | 1.74 | 21.97 | 1.30 | 40.37 | 1.88 | 0.1575 |
| K | 6.4 | 7.30 | 7.48 | 19.52 | 8.03 | 30.03 | 2.73 | 0.0272* |
| Ca | 6.70 | 4.70 | 8.73 | 29.87 | 10.93 | 46.77 | 3.84 | 0.0039* |
| Mg | | 0.90 | 1.75 | 43.61 | 2.56 | 42.75 | 2.07 | 0.1045 |
| Mn | | 1.00 | 2.09 | 67.47 | 1.41 | 112.0 | 1.25 | 0.6329 |
| N/P | | 17.07 | 13.54 | 19.78 | 15.02 | 35.96 | 4.07 | 0.0028* |
| N/K | | 3.51 | 3.12 | 17.10 | 2.53 | 45.93 | 4.74 | 0.0010* |
| N/Ca | | 5.45 | 2.81 | 27.97 | 2.02 | 53.66 | 1.91 | 0.1491 |
| N/Mg | | 28.44 | 15.53 | 43.70 | 8.77 | 60.79 | 0.62 | 0.1994 |
| N/Mn | | 25.60 | 35.43 | 166.7 | 50.04 | 121.3 | 1.05 | 0.9450 |
| P/K | | 0.21 | 0.24 | 31.82 | 0.17 | 46.24 | 1.08 | 0.9226 |
| P/Ca | | 0.32 | 0.21 | 30.27 | 0.15 | 57.43 | 1.76 | 0.2063 |
| P/Mg | | 1.67 | 1.20 | 51.00 | 0.65 | 69.86 | 0.54 | 0.1017 |
| P/Mn | | 1.50 | 2.37 | 160.6 | 3.22 | 112.0 | 1.12 | 0.7340 |
| K/Ca | | 1.55 | 0.95 | 39.14 | 0.92 | 65.36 | 2.62 | 0.0332* |
| K/Mg | | 8.11 | 5.42 | 60.08 | 3.79 | 56.48 | 0.43 | 0.0245* |
| K/Mn | | 7.30 | 3.59 | 0.29 | 5.72 | 0.27 | 1.13 | 0.7203 |
| Ca/Mg | | 5.22 | 5.55 | 32.37 | 4.56 | 35.81 | 0.83 | 0.5907 |
| Ca/Mn | | 4.70 | 4.19 | 0.44 | 7.78 | 0.42 | 1.04 | 0.9934 |
| Mg/Mn | | 0.90 | 0.84 | 0.65 | 1.82 | 0.38 | 0.70 | 0.3268 |

[†] From Auchmoody and Hammock, 1975. [‡] Upper quartile of the population. Significance on a 0.1 level is denoted by a '*'.

**Table 5.** Mean nutrient indices for three-year-old white oaks located across six mapping units.

| Map Unit | N Index | P Index | K Index | Ca Index | Mg Index | NBI |
|---|---|---|---|---|---|---|
| H | -37.95 | -30.66 | 70.35 | 18.86 | 32.16 | 168.60 |
| A | -4.08 | -13.04 | -38.72 | 0.47 | 26.34 | 81.41 |
| E | -3.97 | -3.22 | -16.71 | 5.68 | 5.69 | 38.94 |
| I | -10.51 | -14.17 | 25.02 | 10.49 | 7.93 | 63.09 |
| G | -4.36 | -16.31 | -8.34 | 7.87 | 14.88 | 79.26 |
| F | -6.40 | -23.05 | -29.82 | 10.62 | 26.29 | 89.31 |

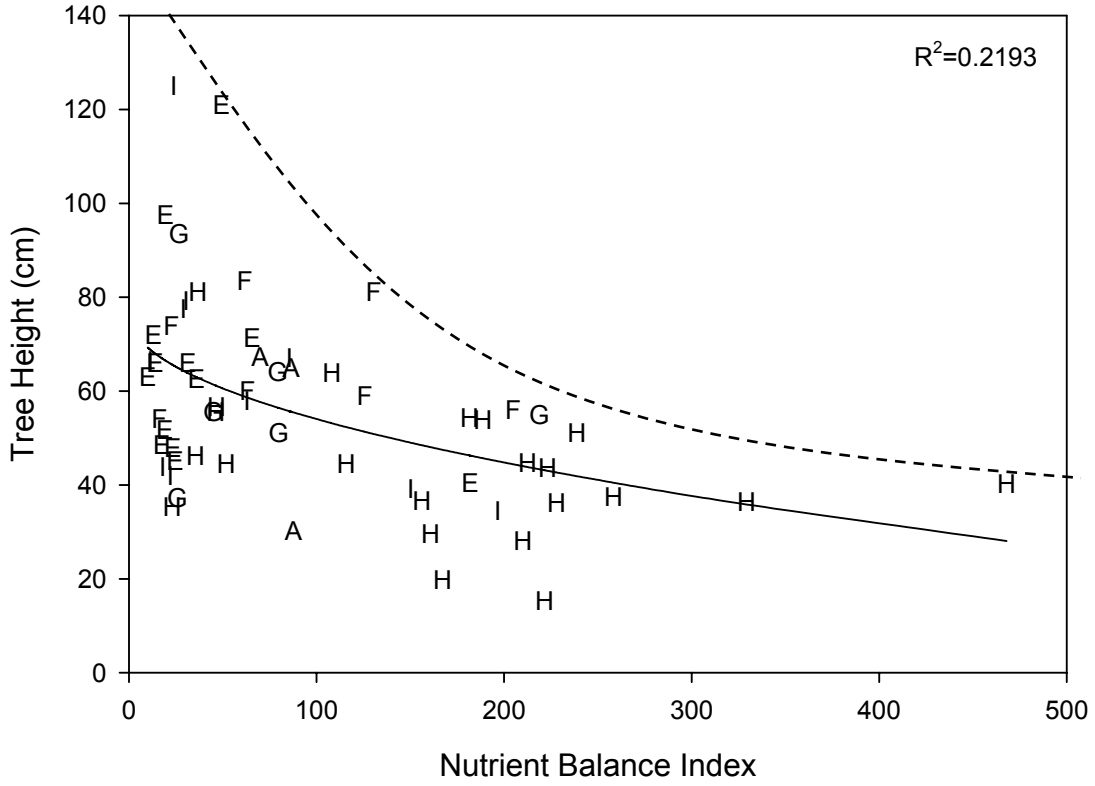



**Figure 6.** Tree height as a function of nutrient balance for 65 three-year-old white oak seedlings across six mapping units. The dashed boundary line represents a tree height limit for a given NBI. The solid curve is the regression line of tree height as a function of the square root of nutrient balance index.

The regression model and nutrient analysis show that the four factors in the conceptual model for mine soil quality (toxicity, soil strength, air/water balance, and nutrient levels) were not all important on this site. There were no variables in the final regression model indicating that soil toxicity or density was a problem, suggesting that these mine soils were nontoxic and uncompacted. Air/water balance was represented in the regression model through the variables silt plus clay and aspect, suggesting that water availability and aeration influenced white oak growth on this site. Nutrient level was also an essential soil attribute on this site. It appeared in the regression model through soil variables characterizing N, P, and K concentrations. The importance of available

nutrients was further supported by the DRIS analysis, where NBI levels paralleled tree growth.

Of the two factors important to white oak growth on this site, air/water balance was adequately addressed by the combination of the three mapping criteria. Nutrient level, however, was not adequately represented by the preliminary mapping criteria. Although rock type partially addresses this, it alone does not represent variability of nutrient levels across sites. An added criterion that represents nutrient levels is thus needed in the classification model.

Nutrient levels, although established as important criteria of soil quality, are not easily measured in the field and thus may not be useful additions to the field mapping criteria. However, the essential soil nutrients influencing white oak growth on this site, according to both the regression model and the DRIS analysis, were correlated with pH (Table 6).

**Table 6**. Correlation coefficients of pH with soil nutrient levels and other soil properties on the Rapoca Mine site in southwest Virginia.

| | Correlation Coefficient | Significance |
|---|---|---|
| Total Extracted N (mg $kg^{-1}$) | 0.42067 | 0.0002 |
| K (mg $kg^{-1}$) | 0.38222 | 0.0009 |
| P (mg $kg^{-1}$) | -0.23450 | 0.0507 |
| Ca (mg $kg^{-1}$) | 0.77269 | <0.0001 |
| Mg (mg $kg^{-1}$) | 0.86313 | <0.0001 |
| % Silt + Clay | -0.24561 | 0.0376 |
| Microbial Biomass (mg $kg^{-1}$) | 0.54324 | <0.0001 |

The positive correlation with nutrients and negative relationship to tree growth suggest that an intermediate pH may be optimal. Soil reaction has been used in the past to assess mine soil quality for agronomic crops, but it is often modeled as a positive linear relationship with productivity for all situations, where a higher pH indicates a

better soil. Although pH levels up to 6.5 can lead to an increase in phosphorus availability and CEC, pH above this level can decrease the availability of some micronutrients and shift microbial composition, especially mycorrhizal relationships on which native trees depend. High pH can also complicate tree growth by increasing herbaceous competition. At high pH, grasses have a distinct advantage and often out-compete trees (Burger and Zipper, 2002). Furthermore, pH was not well correlated to rock type on this site, varying widely within each mapping unit. This suggests that pH should be judged as tree and site specific and should be added as an additional criterion for soil classification and mapping.

## Conclusions

Tree growth was somewhat correlated with a mine soil classification criteria that included rock type, compaction, and aspect (Burger et al., 2002), but additional criteria were needed for adequate characterization of mine site quality for trees. Our regression model of tree height as a function of site and soil properties showed that sandy loam soils with a northeast aspect, high nutrient levels, and high microbial populations were the most conducive to growth of young white oaks on this reclaimed mine site. Of these properties, soil particle size and aspect are represented in the existing classification model and account for the range in water/air balance (Burger et al., 2002). Soil fertility was also a growth determining factor. It is controlled to a large extent by soil pH, which is an easily measured property that could be included as a classification criterion.

Including pH with aspect, rock type, and bulk density may improve forest site quality classification and mapping. Soil quality maps should account for known

interactions between site and soil factors and late successional tree growth. With soil quality maps, native trees can be selected and planted accordingly, leading to increased survival and growth,  a better chance of timely bond release for miners, and a better chance of restoring a healthy native hardwood stands.

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# CHAPTER V:

# INFLUENCE OF PHYSICAL AND CHEMICAL MINE SOIL PROPERTIES ON MICROBIAL POPULATIONS


## Abstract

The return of the native forest ecosystem in the Appalachian region after strip mining is important to landowners and local communities for economic and environmental reasons. An essential part of the reestablishment of this ecosystem is the return of native soil microbial populations. When a site is reclaimed, blasted rock is usually placed on the surface and microbes must reinvade and reestablish on these barren sites. The purpose of this study was to determine the size and activity of microbial populations on mine spoils with a range of physical and chemical properties. We hypothesized that mine spoils with properties most similar to native soils would have the highest microbial biomass and activity. This investigation was carried out in two parts, a greenhouse study and a field study. The greenhouse study measured microbial biomass and activity on an undisturbed forest topsoil (UFT), and three mine spoil types: brown weathered sandstone (BWS), white unweathered sandstone (WUS), and gray, unweathered shale (GUH). These growth media were placed in pots, planted with three tree species, and half the pots were inoculated with 2.5 cm of topsoil. On a reclaimed mined site, the influence of spoil physical and chemical properties on microbial biomass and enzyme production across a range of spoil types was determined. The greenhouse study showed that microbial populations, activity and enzyme production were highest on the UFT followed by the BWS, and were higher on topsoil-inoculated spoils compared to non-inoculated spoils. The field study showed that pH, salt content and nitrogen

accounted for 53% of the variation in microbial biomass and 50% of the variation in enzyme activity. These properties were also found conducive to soil organisms in the BWS spoil in the greenhouse experiment compared to the GUH and WUS spoil types. This suggests that topsoil-inoculated BWS is most favorable for the return of microbial populations on reclaimed sites due to its moderate acidity, high nitrogen level and low salt content. Applying native topsoil and BWS to the surfaces of reclaimed mines may increase the speed of recovery of below and aboveground ecosystems.

## Introduction

The eastern deciduous hardwood forest of Appalachia is a diverse and unique ecosystem. An essential part of this ecosystem is the soil and the organisms that live within. Microorganisms are influenced by the physical and chemical properties of the soil, and, in turn, the growth of vegetation is influenced by soil microorganisms. With a decrease in soil quality, the number and diversity of soil microorganisms decreases (Hutson 1980a; Hutson 1980b; Moore and Luxton, 1986; Blair et al., 1996; Topp et al. 2001; Mummey et al., 2002a). At the same time, microorganisms increase soil quality, (Blair et al., 1996; Boyle, 1996; Moldenke et al., 1996; Topp et al. 2001), which can increase productivity and diversity of vegetation. These ecosystem components and processes interact in complex ways that allows the ecosystem to function productively.

When viewed as a part of the greater forest ecosystem, many microorganisms function as vital facilitators of plant growth. Decomposers play an essential role in nutrient cycling by breaking up plant litter and other detritus and returning it to a mineral form that can be utilized by plants (Hutson, 1980b). They are also important to the formation of good soil structure by mixing, aerating and creating more stable soil aggregates (Abbott, 1989). Mycorrhizae form essential symbioses with plant roots, increasing root area and making nutrients and water more available. Nitrogen fixers also play an important role by increasing levels of nitrogen in the system. With the modification or elimination of these microbes, other aspects of the forest ecosystem may change or be eliminated. The health of microbial populations is an essential consideration when the return of the native ecosystem is the goal during reclamation.

Strip mine reclamation practices often involve placing blasted rock from a deep geologic horizon on the surface. These materials are vastly different physically and chemically and contain few, if any, of the microbial populations found in the native forest soils. Even after time, microbial populations that develop are often lower in number and less diverse than on adjacent undisturbed soils (Visser, 1979; Hutson, 1980a, Mummey et al., 2002b). The native microbial populations that were present prior to mining are eliminated, and those that develop may be less healthy and have very different composition from populations in the original forest soil.

The reestablishment of microorganisms on reclaimed surface mines is influenced by several soil and site factors. The return of topsoil inoculates the spoil with microorganisms and can greatly increase microbial numbers due to the organic matter and nutrients that are added to the spoil (Fesquez and Lindemann, 1982; Moore and Luxton, 1986). Topsoil addition can also create an environment more conducive to later establishment of microorganisms (Wanner and Dunger, 2002).

The physical and chemical properties of the spoil also play an important role. Many microbes are affected by pH, nutrient levels, and water levels (Fisher and Binkley, 2000). Distance from native forest may determine the speed and extent of reintroduction on disturbed sites (Wanner and Dunger, 2002). Although these factors have been examined individually, their interaction and relative importance are not well known.

The objectives of this study were to 1) to determine the effects of different mine spoil materials on microbial populations, 2) to examine the impact of topsoil inoculation and its interaction with mine spoil type on microbial populations, and 3) to determine

which site and mine spoil physical and chemical properties are most important to the development of healthy microbial populations.

## Methods and Procedures

This investigation was done in two parts: a greenhouse study using spoils taken from a mined site in West Virginia operated by the Pritchard Mining Co., and a field study that is located in southwestern Virginia on land owned by Rapoca Coal Co. (Fig. 2) .

### *Greenhouse Study Methods*

Soil and Treatment Characterization

The Pritchard Mine which is a surface mine that uses a combination of contour mining and mountain top removal. The area was forested with Appalachian oak types previous to mining. Reclamation practices at this mine involve returning a variety of rock types to the surface, grading, hydroseeding a grass and legume species mix and planting a variety of tree species.

Three different mine spoils and an undisturbed topsoil were collected for this greenhouse experiment in March 2004. The three spoil types, brown, weathered sandstone (BWS), white, unweathered sandstone (WUS), and gray, unweathered shale (GUH), were taken from various levels of the Kanawha geologic formation; all are used during reclamation as topsoil substitutes depending on their presence during mining. The undisturbed forest topsoil (UFT) was used as the control and was collected from the upper 30cm of soil on the adjacent forest stand.

The Kanawha formation is 210 m thick at its north end and becomes progressively thicker, reaching 600m at its southern reach in West Virginia (Blake et al., 1994). The sampled area is on the shallower end of this spectrum in Boone county West Virginia. In West Virginia this formation runs through Kanawha, Boone, Fayette, Raleigh, Logan, Wyoming, Mingo, and McDowell counties in the southwestern part of the state. It is comprised of sandstones, siltstones, shale and coal. Because of its numerous coal seams, it is intensively mined.

The native soils on the site are typical of the area. The site is located in an upland region of Clymer-Dekalb-Gilpin soil types, which is strongly sloping to very steep, well drained, and acid (Soil Survey, 1981). The majority of the slopes were very steep Clymer- Dekalb complexes before mining occurred. Clymer is a fine-loamy, mixed, mesic Typic Hapludult, while Dekalb is a loamy-skeletal, mixed mesic Typic Dystrochrept (Soil Survey, 1981). The original soil is rarely used as the final growth medium during reclamation. Soils are usually lost deep within the mine when spoils are returned during reclamation. One or more of the three spoil types chosen for this study, BWS (brown, weathered sandstone), WUS (white, unweathered shale) or GUH (gray, unweathered shale), is used as a topsoil substitute.

BWS is the bedrock located directly beneath the soil solum. This weathered rock is the parent material of these forest soils and is often exploited by deep-rooted native trees. It has a pH comparable to the native topsoil, is easily weathered when placed on the surface, and often has trace amounts of native forest topsoil mixed with it.

WUS is also placed on the surface during reclamation. It contains limestone concretions that are found in shale siltstone and sandstone marine deposits in the area

(Blake et al., 1994). Limestone would suggest that the pH of this soil is much higher than the topsoil. Calcareous sandstone also occurs at the base of these marine layers.

GUH is common in the geologic profile occurring in several shale members located above the coal seams. Similar to the WUS, GUH is a marine deposit and may have many similar characteristics (Blake et al., 1994). It is, however, much finer grained, creating silty and clayey mine soil material.

Greenhouse Methods

Each of three tree species, *Fraxinus americana, Liriodendron tulipifera,* and *Quercus rubra*, were planted in three mine spoil types and the topsoil (Table 1). Half of the 7.57 L pots were inoculated with 2.5 cm of native topsoil, which was spread on the surface. This 4x2x3 factorial design was replicated 10 times for a total of 240 pots.

**Table 1**. Greenhouse experiment layout with a 4x2x3 factorial design across three growth media, half of which were inoculated with topsoil and planted with three different tree species.

| | UFT | BWS | WUS | GUH |
|---|---|---|---|---|
| Not Inoculated | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* |
| Inoculated with Native Topsoil | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* | *L. tulipifera*<br>*Q. rubra*<br>*F. Americana* |

The surfaces of all pots were covered with a paper mulch to simulate field conditions and to decrease potential contamination from pot to pot. A watering treatment of 1.5L was applied to each pot once each week.

Laboratory Methods

In order to determine which properties of the different spoil types were most important, the physical, chemical and biological properties were characterized. Soil was

combined creating four composite samples per treatment combination. Sub-samples from each composite sample were sieved through a 2 mm sieve, kept at 4 °C and processed within 2.5-wks of collection. An adenosine triphosphate (ATP) procedure was used to estimate microbial activity. The procedure was modified for the analysis of soils. Soil was placed in a saline solution and shaken for an hour to put microbial populations into solution. The samples were then centrifuged at 1000 rpm to remove particulate matter while allowing microbes to remain in solution. This solution was then used in the standard ATP procedure (ATPlite, 2002). Dehydrogenase activity was found using a 2,3,5-Triphenyltetrazolium chloride (TTC) indicator (Tabatabai, 1982). Microbial biomass was measured by chloroform fumigation (Anderson and Domsch, 1978; Gregorich et al., 1990; Jenkinson and Powlson, 1976).

These three procedures were used in combination. ATP analysis determines the level of activity in the soil. Chloroform fumigation is an indicator of total biomass, and DHA is an indicator of the level of decomposers in the system. Through the combination of these factors, several different aspects of soil quality can be examined. Total biomass shows how much overall living material is in the soil, while ATP depicts the overall activity of the microbial populations. The DHA assay estimates the decomposer population.

The remainder of the composite samples was dried and sieved through a 2 mm sieve and an array of physical and chemical properties were measured. Bulk density was found by weighing the mass of the pot contents and determining its volume. Total coarse fragments (>2mm) were determined by sieving and weighing. Spoil particle size was analyzed using the hydrometer method (Bouyoucos, 1936). Total soluble salts were

measured with an electrical conductivity meter (Bower and Wilcox, 1965), and pH was measured in a 2:1 water:soil suspension using a pH glass electrode. Nitrogen availability was measured via aerobic incubation (Bremmer, 1965a) and inorganic nitrogen was measured using a KCl extraction method (Bremmer, 1965b). Total nitrogen and carbon were found using a carbon nitrogen analyzer (Vario MAX, 2000. Elementar Americas, Inc., Hanau, Germany). Exchangeable cations were extracted using the ammonium acetate method and analyzed on an ICP spectrophotometer (SpectroFlame Modula Tabletop ICP, 1997, Spectroanalytical instruments, Germany; Thomas, 1982). Available nutrients were estimated using a Mehlich I extraction with subsequent analysis using the ICP referenced above.

Data Analysis

This experiment was a completely randomized 4x2x3 factorial design with 10 replications; it was statistically analyzed using an analysis of variance (SAS, 2004). Microbial biomass, dehydrogenase activity, and ATP level as well as spoil physical, and chemical properties were compared among spoil types and inoculated and un-inoculated soil. Tree species was used as a blocking factor.

***Field Study Methods***

The field study is also located in the Kanawha geologic formation. Many different rock strata above the coal seam were placed on the surface during reclamation, and, as a result, the area consisted of a wide variety of mine soil types, varying in both physical and chemical properties (Fig. 1).

**Figure 1.** The Rapoca Coal Co. reclamation site in southwest Virginia immediately after reclamation, in 2002.

In 2001, the area was reclaimed using conventional practices that included returning the surface to approximate original contour, and grading and hydroseeding with a grass seed/fertilizer mix. The site was planted in 2002 with a native hardwood mix at a density of 1482 trees per ha. In 2004, seventy-two three-year-old white oak trees were randomly chosen across a range of spoil types.

Soil samples were taken to a 40 cm depth within a 50 cm radius at the base of each tree. Soil samples were prepared and analyzed using the same methods and procedures described above for the greenhouse study.

At the end of the growing season, the trees were excavated and soil adjacent to the tree was used for the characterization of soil microbial properties (Table 1). Soil samples were prepared and analyzed using the methods described above for the greenhouse study.

## Results

### *Greenhouse Study*

Soil Microbial Response

Spoil type and inoculation had a significant effect on dehydrogenase activity ($p<0.0001$), ATP level ($p<0.0001$) and total microbial biomass ($p<0.0001$ and $p=0.0584$, respectively) (Table 2). The interaction between spoil type and inoculation was also significant for dehydrogenase activity and ATP level ($p<0.0001$ and $p=0.0034$, respectively).

**Table 2.** P values for the effect of spoil, topsoil inoculation and their interaction on dehydrogenase, ATP and microbial biomass.

| Treatment | Natural log of dehydrogenase activity (mg TPF $kg^{-1}$ 24 $h^{-1}$) | ATP (light level) | Microbial Biomass (DOC mg $kg^{-1}$) |
|---|---|---|---|
| Spoil | <0.0001*** | <0.0001*** | <0.0001*** |
| Inoculation | <0.0001*** | <0.0001*** | 0.0584* |
| Spoil x Inoculation | <0.0001*** | 0.0034*** | 0.9789 |

*,**, and *** represent significance at the 0.1, 0.05, and 0.001 level respectively.

Dehydrogenase activity in the UFT was 10 times greater than that in the BWS (brown weathered sandstone) and 100 times greater than that in the WUS (white unweathered sandstone). The GUH (grey unweathered shale) also had low levels of dehydrogenase, with levels falling between those in the BWS and the WUS. Topsoil

inoculation increased dehydrogenase activity substantially on both the BWS and the GUH, but did not effect the WUS.

Similarly, ATP levels were much lower on the WUS compared to the UFT, while both the BWS and GUH were intermediate. Again, topsoil inoculation increased ATP level on both the BWS and GUH, but not the WUS.

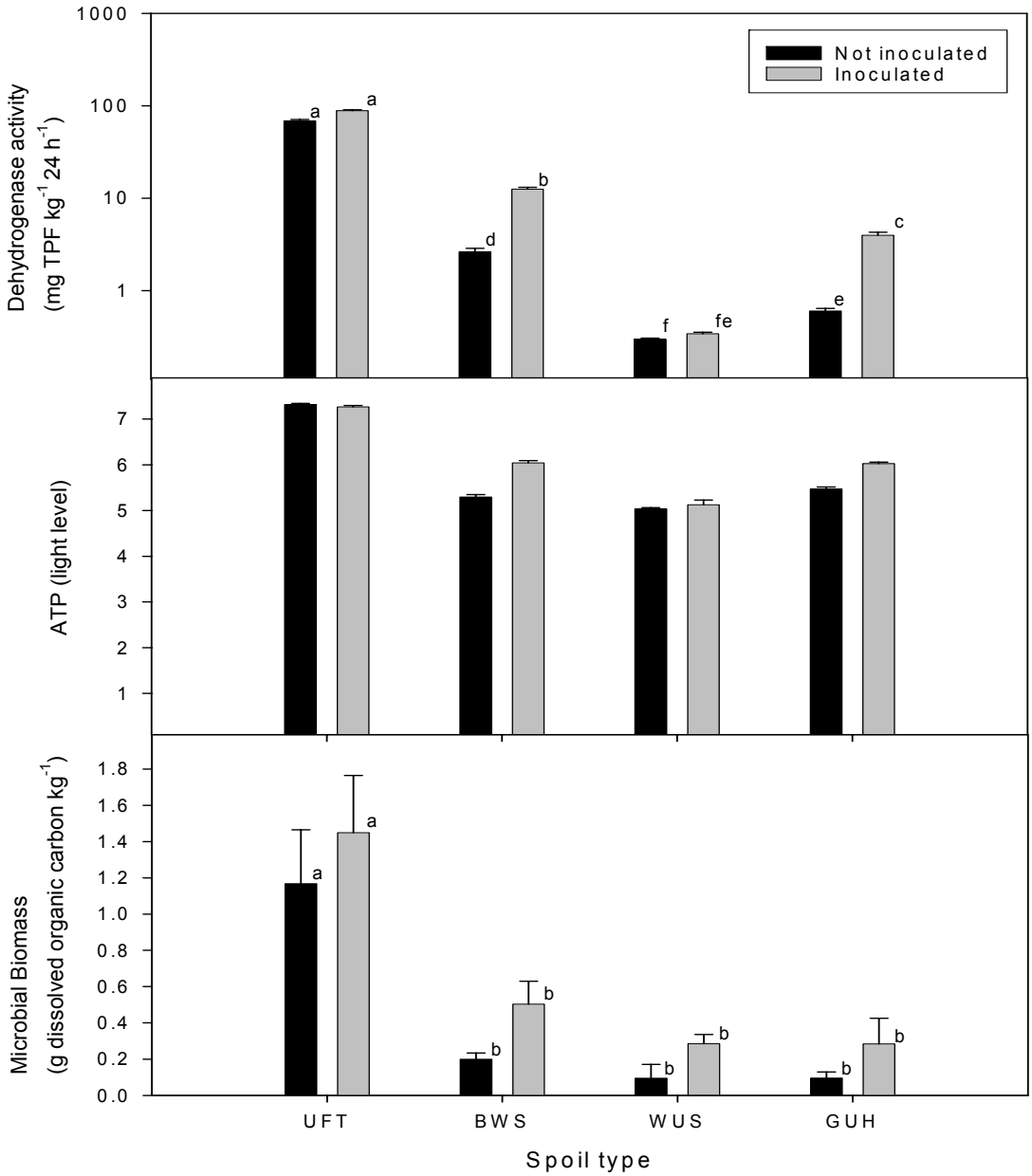


**Figure 2.** Effect of topsoil inoculation and spoil type on dehydrogenase activity, ATP, and biomass levels of soil microbial populations. Different letters signify significantly different values by Fisher's LSD (0.05).

Microbial biomass was also greatest on the UFT compared to the spoil materials. There was no difference in microbial biomass among the spoils. Topsoil inoculation did not significantly affect microbial biomass, but had a consistent trend of increasing it.

Soil Characterization

Almost all spoil physical and chemical properties tested were different across spoil types (Tables 3 and 4). Physical properties were characterized using composite samples prior to setting up the experiment. The UFT had the highest silt plus clay content (48%) followed by the GUH (45%). Silt plus clay of the WUS (21%) was half that of the UFT and the GUH while the BWS was intermediate at 33%. The wilting point and $H_2O$ availability of the GUH (4.8% and 5.06%) were half that of the UFT (9.51% and 10.20%). The BWS had an even lower wilting point (3.66%), but a higher water availability (7.6%) than the GUH. The WUS had the lowest wilting point and water availability (1.3% and 4.86%).

**Table 3.** Mean values of physical soil properties for three mine spoils and a control and for inoculated verses not inoculated spoils. Different letters signify significantly different values based on Fisher's LSD (0.05).

| Measurement | UFT | BWS | WUS | GUH |
|---|---|---|---|---|
| % Silt + Clay †‡§ | 47.82 [a] | 33.15 [c] | 21.04 [d] | 44.84 [b] |
| Wilt pt (% by wt) †‡§ | 9.51 [a] | 3.66 [c] | 1.3 [d] | 4.80 [b] |
| $H_2O$ Avail. (% by wt) †‡§ | 10.20 [a] | 7.60 [ba] | 4.86 [b] | 5.06 [b] |

† represents significant correlation of a soil property with incremental stem height. ‡ represents significant correlation of a soil property with incremental stem biomass. § represents significant correlation of a soil property with total root biomass

Mine soil pH ranged from 5.19 to 8.86 across treatments (Table 4). The pH of the BWS (5.56) was only slightly higher than the UFT (5.21), while the WUS and GUH were very alkaline (8.86 and 8.39, respectively). Topsoil inoculation acidified the alkaline spoils slightly to pH 8.17 for the WUS and 7.58 for the GUH.

Soluble salt content ranged from 0.127 dS $m^{-1}$ to 0.562 dS $m^{-1}$. The EC of the GUH (0.616 dS $m^{-1}$) was almost three times as high as the UFT (0.236 dS $m^{-1}$). The WUS (0.317 dS $m^{-1}$) was comparable in salinity to the UFT (0.236 dS $m^{-1}$) while the BWS (0.127 dS $m^{-1}$) was slightly lower.

Exchangeable acidity ranged from 0 to 0.64 $cmol^{+}\ kg^{-1}$. Neither the GUH nor the WUS had any exchangeable acidity due to their high level of alkalinity. The UFT had 0.64 $cmol^{+}\ kg^{-1}$ of charge due to exchangeable acidity, while BWS had only half that amount (0.28 $cmol^{+}\ kg^{-1}$). Topsoil inoculation did not affect exchangeable acidity on any of the spoil types.

The CEC ranged from 1.59 $cmol^{+}\ kg^{-1}$ to half that amount (0.70 $cmol^{+}\ kg^{-1}$) across treatments. The GUH (1.60 $cmol^{+}\ kg^{-1}$) was similar to the UFT (1.50 $cmol^{+}\ kg^{-1}$), while the BWS was slightly lower (1.13 $cmol^{+}\ kg^{-1}$) and the WUS was less than half (0.706 $cmol^{+}\ kg^{-1}$). Topsoil inoculation increased the CEC of the BWS and WUS slightly to 1.21 and 0.86 $cmol^{+}\ kg^{-1}$ , respectively.

Salt-extractable available nitrogen levels ranged widely across spoil types from 9.93 to 27.72 $mg\ kg^{-1}$. Compared to the UFT (23.91 $mg\ kg^{-1}$), available N was about half that found in the WUS (12.91 $mg\ kg^{-1}$) or GUH (11.2 $mg\ kg^{-1}$), while the BWS was intermediate (17.80 $mg\ kg^{-1}$) and was not different from the other treatments. Topsoil inoculation had no affect on N levels for any of the soil types.

The Melich I extractable nutrients, P, Ca, Mn, Cu, Fe, and Zn displayed an interaction between spoil type and topsoil inoculation (Table 5). P and Fe ranged in concentration over an order of magnitude from 0.82 to 12.33 $mg\ kg^{-1}$ for P and from 6.95 to 234.75 $mg\ kg^{-1}$ for Fe. Levels were over 10 times more concentrated on the $GUH_{non\text{-}I}$ (12.33 and 60.9 $mg\ kg^{-1}$) and $WUS_{non\text{-}I}$ (12.03 and 234.75 $mg\ kg^{-1}$) compared to the UFT, (0.82 and 7.79 $mg\ kg^{-1}$), while the $BWS_{non\text{-}I}$ was only slightly higher than the UFT in P concentration (1.62 $mg\ kg^{-1}$) and was comparable in Fe concentration (6.95 $mg\ kg^{-1}$).

Topsoil inoculation decreased P and Fe levels to 9.79 and 203.73 mg $kg^{-1}$ on the $WUS_I$ and 9.41 and 39.48 mg $kg^{-1}$ on the $GUS_I$, respectively, but had no effect on the BWS.

Ca ranged from 76.19 to 544.95 mg $kg^{-1}$. The BWS treatment had a lower concentration (76.19) than the UFT (122 mg $kg^{-1}$), while Ca in the GUH and WUS were over three times as concentrated as the UFT (350 and 545 mg $kg^{-1}$ ,respectively). Similar to P and Fe, Ca levels were lowered by topsoil inoculation to 313 mg $kg^{-1}$ on the $GUH_I$ and 531 mg $kg^{-1}$ on the $WUS_I$. In contrast, levels were raised by inoculation on the BWS to 97.71 mg $kg^{-1}$.

Mg, Cu, Zn and Mn also varied across spoil types from 27.12, 0.105, 0.27 and 2.64 to 122.64, 0.847, 1.38, and 27.77, respectively. Cu concentration was much higher on all of the spoils compared to the control, and concentrations generally decreased with topsoil inoculation. The highest level of Cu was on the $GUH_{non\text{-}I}$ and decreased in the order $GUH_{non\text{-}I} > GUH_I > BWS_{non\text{-}I} = BWS_I > WUS_{non\text{-}I} = UFT_I = UFT_{non\text{-}I}$, and $UFT_{non\text{-}I} = WUS_I$, but $WUS_I < UFT_I = WUS_{non\text{-}I}$. Zn was higher in concentration on the GUH compared to the UFT but lower on the BWS and WUS. Topsoil inoculation slightly increased levels on the BWS and slightly decreased levels on the GUS while not affecting WUS concentrations. Concentrations decreased in the order $GUH_{non\text{-}I} > GUH_I > UFT_I = UFT_{non\text{-}I} > WUS_{non\text{-}I} = WUS_I > BWS_I > BWS_{non\text{-}I}$. Mn concentration was higher on WUS and the GUH compared to the UFT, while BWS was lower in concentration. Topsoil inoculation substantially increased Mn concentration on all spoil types. Concentrations decreased in the order $WUS_I$ followed by $GUH_I > GUH_{non\text{-}I} > WUS_{non\text{-}I} > UFT_I = UFT_{non\text{-}I} > BWS_I > BWS_{non\text{-}I}$.

**Table 4.** Mean values of chemical soil properties for three mine spoils and a control and for inoculated verses not inoculated spoils. Different letters signify significantly different values based on Fisher's LSD (0.05).

| Measurement | | | UFT | BWS | WUS | GUH | Mean |
|---|---|---|---|---|---|---|---|
| pH†‡§ | | Not Inoc. | 5.23 [e] | 5.53 [d] | 8.86 [a] | 8.39 [b] | **7.00** |
| | | Inoc. | 5.19 [e] | 5.59 [d] | 8.17 [b] | 7.58 [c] | **6.63** |
| | | Mean | **5.21** | **5.56** | **8.515** | **7.985** | |
| EC (dS $m^{-1}$) †‡ | | Not Inoc. | 0.229 | 0.123 | 0.303 | 0.616 | **0.318 [a]** |
| | | Inoc. | 0.243 | 0.131 | 0.331 | 0.508 | **0.303 [a]** |
| | | Mean | **0.236[b]** | **0.127[c]** | **0.317[b]** | **0.562[a]** | |
| Exchangeable Acidity ($cmol^{+}$ $kg^{-1}$) †‡§ | | Not Inoc. | 0.616 | 0.318 | 0.000 | 0.000 | **0.234 [a]** |
| | | Inoc. | 0.663 | 0.237 | 0.000 | 0.000 | **0.225 [a]** |
| | | Mean | **0.640** | **0.278** | **0.000** | **0.000** | |
| CEC ($cmol^{+}$ $kg^{-1}$) †‡ | | Not Inoc. | 1.500 [b] | 1.136 [d] | 0.706 [f] | 1.598 [a] | **1.227** |
| | | Inoc. | 1.510[b] | 1.216 [c] | 0.867 [e] | 1.556 [a] | **1.287** |
| | | Mean | **1.505** | **1.176** | **0.786** | **1.576** | |
| KCl Extractable Inorganic N (mg $kg^{-1}$) †‡ | | Not Inoc. | 27.72 | 19.32 | 10.05 | 9.93 | **16.76 [a]** |
| | | Inoc. | 20.10 | 16.28 | 15.77 | 12.46 | **16.15 [a]** |
| | | Mean | **23.91[a]** | **17.80[ab]** | **12.91[b]** | **11.20[b]** | |
| Melich I Extractable Nutrients (mg $kg^{-1}$) | P †‡§ | Not Inoc. | 0.82 [d] | 1.62 [c] | 12.03 [a] | 12.33 [a] | **5.55** |
| | | Inoc. | 0.82 [d] | 1.33 [c] | 9.79 [b] | 9.41 [b] | **4.36** |
| | | Mean | **0.82** | **1.47** | **10.91** | **10.81** | |
| | K‡ | Not Inoc. | 14.05 | 20.36 | 6.79 | 23.75 | **16.24[a]** |
| | | Inoc. | 13.74 | 19.17 | 7.89 | 23.42 | **16.06 [a]** |
| | | Mean | **13.89[c]** | **19.77[b]** | **7.34[d]** | **23.58 [a]** | |
| | Ca †‡§ | Not Inoc. | 122.08 [d] | 76.19 [f] | 544.95 [a] | 349.94 [b] | **271.66** |
| | | Inoc. | 124.37 [d] | 97.71 [e] | 530.77 [a] | 313.31 [c] | **266.54** |
| | | Mean | **123.22** | **86.95** | **537.86** | **330.82** | |
| | Mg†‡§ | Not Inoc. | 27.30 | 60.05 | 121.72 | 94.66 | **75.93 [a]** |
| | | Inoc. | 27.12 | 60.61 | 122.64 | 88.30 | **74.67 [a]** |
| | | Mean | **27.21[d]** | **60.33[c]** | **122.18[a]** | **91.48[b]** | |
| | Fe†‡§ | Not Inoc. | 7.28 [e] | 6.95 [e] | 234.75 [a] | 60.90 [c] | **77.82** |
| | | Inoc. | 7.79 [e] | 8.19 [e] | 203.73 [b] | 39.48 [d] | **64.80** |
| | | Mean | **7.53** | **7.57** | **219.24** | **49.72** | |
| | Cu†‡§ | Not Inoc. | 0.157 [ed] | 0.271 [c] | 0.174 [d] | 0.847 [a] | **0.352** |
| | | Inoc. | 0.174 [d] | 0.261 [c] | 0.105 [e] | 0.608 [b] | **0.287** |
| | | Mean | **0.166** | **0.266** | **0.139** | **0.722** | |
| | Zn† | Not Inoc. | 1.09 [c] | 0.27 [f] | 0.98 [d] | 1.38 [a] | **0.92** |
| | | Inoc. | 1.14 [c] | 0.38 [e] | 0.96 [d] | 1.27 [b] | **0.94** |
| | | Mean | **1.12** | **0.33** | **0.97** | **1.32** | |
| | Mn†‡ | Not Inoc. | 10.01 [e] | 2.64 [g] | 15.28 [d] | 17.32 [c] | **11.19** |
| | | Inoc. | 10.54 [e] | 4.23 [f] | 27.77 [a] | 22.94 [b] | **16.37** |
| | | Mean | **10.28** | **3.44** | **21.52** | **20.25** | |
| | B†‡§ | Not Inoc. | 0.112 | 0.062 | 0.042 | 0.075 | **0.073 [a]** |
| | | Inoc. | 0.107 | 0.062 | 0.045 | 0.075 | **0.072 [a]** |
| | | Mean | **0.110[a]** | **0.062[c]** | **0.044[d]** | **0.075[b]** | |

† represents significant correlation of a soil property with ATP level. ‡ represents significant correlation of a soil property with dehydrogenase. § represents significant correlation of a soil property with microbial biomass.

There was a significant relationship between microbial properties and almost all soil and site factors (Table 5). Microbial biomass, ATP and DHA were positively correlated with all physical properties. They were negatively correlated with pH, EC (electrical conductivity), and almost all Mehlich I available nutrients, and positively correlated with CEC, exchangeable acidity and available inorganic nitrogen.

**Table 5.** Correlation coefficients of microbial properties to physical and chemical mine soil properties.

| Property | | ATP (light level) | | Dehydrogenase activity (mg TPF $kg^{-1}$ 24 $h^{-1}$) | | Microbial Biomass (g dissolved organic carbon $kg^{-1}$) | |
|---|---|---|---|---|---|---|---|
| | | Corr. Coeff. | Sig | Corr. Coeff. | Sig | Corr. Coeff. | Sig |
| % Silt + Clay | | 0.6455 | <0.0001*** | 0.7021 | <0.0001*** | 0.49506 | 0.004* |
| Wilt pt (% by wt) | | 0.8825 | <0.0001*** | 0.8744 | <0.0001*** | 0.74846 | <0.0001* |
| $H_2O$ Avail. (% by wt) | | 0.8413 | <0.0001*** | 0.8796 | <0.0001*** | 0.76498 | <0.0001* |
| CEC | | 0.5184 | <0.0001*** | 0.6135 | <0.0001*** | 0.1786 | 0.3281 |
| pH | | -0.6618 | <0.0001*** | -0.8266 | <0.0001*** | -0.6091 | 0.0002*** |
| Mehlich I Avail Nut mg $kg^{-1}$ | P | -0.6386 | <0.0001*** | -0.7968 | <0.0001*** | -0.6131 | 0.0002*** |
| | K | -0.2255 | 0.028** | -0.0776 | 0.4549 | -0.2175 | 0.2319 |
| | Ca | -0.5389 | <0.0001*** | -0.7378 | <0.0001*** | -0.4699 | 0.0067*** |
| | Mg | -0.8195 | <0.0001*** | -0.9039 | <0.0001*** | -0.6855 | <0.0001*** |
| | Fe | -0.5126 | <0.0001*** | -0.7188 | <0.0001*** | -0.4228 | 0.0159** |
| | Cu | -0.2868 | 0.0048*** | -0.2630 | 0.01*** | -0.3793 | 0.0323** |
| | Zn | 0.2028 | 0.0488** | 0.0274 | 0.7923 | 0.0788 | 0.6678 |
| | Mn | -0.2773 | 0.0065*** | -0.4143 | <0.0001*** | -0.2668 | 0.1400 |
| | B | 0.7624 | <0.0001*** | 0.8129 | <0.0001*** | 0.6677 | <0.0001*** |
| EC (ds $m^{-1}$) | | -0.2234 | 0.0295** | -0.2915 | 0.0041*** | -0.2329 | 0.1995 |
| Ex Acidity (cmol $kg^{-1}$) | | 0.8866 | <0.0001*** | 0.8849 | <0.0001*** | 0.8240 | <0.0001*** |
| KCl Extractable Inorganic N (mg $kg^{-1}$) | | 0.2351 | 0.0218** | 0.1989 | 0.0533* | 0.1131 | 0.5378 |

Field Study

A field spectrum of site and soil physical and chemical properties hypothesized to influence soil microbes were analyzed and are presented in table 6. In nearly all cases, values ranged considerably among the sampled points. For example, bulk density of the fine earth fraction ranged from 0.61 to 1.39 g $cm^{-1}$ and silt plus clay ranged from 17 to

64%. Chemical properties were also highly variable with soil exchangeable nutrients ranging by an order of magnitude. pH ranged from 3.24 to 7.79 and EC ranged from 0.14 to 4.72 ds $m^{-1}$. As a result of this broad range in soil and site properties, microbial biomass and activity was virtually absent on some sites and quite prevalent on others.

**Table 6.** Mean values and ranges for microbial and soil/site properties associated with each site on the Rapoca Mine Site, VA,2004.

| Variable | | Mean | Low | High | St Dev | CV |
|---|---|---|---|---|---|---|
| Microbial Properties | | | | | | |
| Dehydrogenase activity (mg TPF $kg^{-1}$ 24 $h^{-1}$) | | 11.42 | 0.25 | 80.83 | 15.47 | 135.46 |
| Microbial biomass (mg dissolved organic carbon $kg^{-1}$) | | 38.1 | 3.3 | 201.8 | 35.8 | 93.9 |
| Site Properties | | | | | | |
| Dist from Native Forest (m) | | 69 | 6 | 162 | 41 | 59 |
| Physical Properties | | | | | | |
| % Silt + Clay | | 45 | 17 | 64 | 9 | 20 |
| Bulk Density (fine earth) | | 0.96 | 0.61 | 1.39 | 0.18 | 18.27 |
| Chemical Properties | | | | | | |
| EC (dS $m^{-1}$) | | 0.92 | 0.14 | 4.72 | 1.03 | 112.29 |
| pH | | 5.2 | 3.2 | 7.8 | 1.5 | 27.8 |
| Anaerobic N (mg $kg^{-1}$) | | 9.96 | 0.49 | 62.95 | 10.12 | 101.66 |
| KCL Extractable Inorganic N (mg $kg^{-1}$) | | 5.20 | 1.59 | 21.55 | 3.52 | 67.78 |
| P (mg $kg^{-1}$) | | 7.24 | 0.92 | 34.86 | 5.75 | 79.44 |
| Melich I Available Nutrients (mg $kg^{-1}$) | K | 33.5 | 8.7 | 72.3 | 13.2 | 39.5 |
| | Ca | 542.3 | 54.0 | 1426.4 | 368.3 | 67.9 |
| | Mg | 142.0 | 24.9 | 267.2 | 62.6 | 44.1 |
| | Zn | 2.2 | 0.8 | 5.9 | 1.1 | 50.6 |
| | Mn | 19.8 | 6.1 | 52.2 | 11.1 | 56.0 |
| | Cu | 1.1 | 0.4 | 1.8 | 0.3 | 25.9 |
| | Fe | 41.9 | 12.0 | 138.9 | 24.3 | 58.0 |
| | B | 0.09 | 0.05 | 0.20 | 0.04 | 47.67 |
| C-N analyzer (mg $kg^{-1}$) | N | 0.05 | 0.03 | 0.15 | 0.02 | 41.95 |
| | C | 1.08 | 0.30 | 4.39 | 0.76 | 70.53 |
| Exchangeable cations (mg $kg^{-1}$) | Ca | 252.5 | 44.5 | 539.9 | 117.1 | 46.4 |
| | Mg | 100.5 | 27.9 | 147.0 | 23.2 | 23.1 |
| | K | 161.5 | 28.4 | 343.6 | 73.2 | 45.3 |
| | Na | 65.1 | 16.0 | 107.2 | 17.0 | 26.1 |
| CEC ($cmol^{+}$ $kg^{-1}$) | | 3.01 | 0.99 | 5.65 | 1.11 | 37.02 |

† aspect was calculated using a continuum where southwest=0 and northeast=1.

To better understand the influence of site and soil physical and chemical properties on microbial populations, the variables microbial biomass and dehydrogenase

activity was regressed against site and soil properties of the field study. From greatest to least importance in both regression models, pH, EC (electrical conductivity), and available inorganic nitrogen, were the most important factors influencing microbial biomass and dehydrogenase level. Almost 53% of the variability in microbial biomass was explained by these three soil chemical properties:

$$\textbf{\textit{Biomass}} = -2.69 + 4.86(\textbf{\textit{pH}}-5)^2 - 9.81\ln\textbf{\textit{EC}} + 9.48\sqrt{\textbf{\textit{N}}}$$

$$\mathbf{R^2 = 0.5255}$$

**Table 7.** Variables, standardized coefficients, and significance level for the above regression model describing 53% of the variation in microbial biomass.

| Variable | Standardized coefficient | p value |
|---|---|---|
| ***pH***=pH | 0.45727 | <0.0001 |
| ***EC***=Electrical Conductivity (dS $m^{-1}$) | -0.36763 | 0.0004 |
| ***N***=KCl extractable inorganic N (mg $kg^{-1}$) | 0.26640 | 0.0122 |

pH, EC and total extractable inorganic nitrogen also accounted for almost 50% of the variability in dehydrogenase activity on these sites:

$$\textbf{\textit{Dehyd}} = -5.16 + 2.27(\textbf{\textit{pH}}-5)^2 - 3.41\ln\textbf{\textit{EC}} + 3.70\sqrt{\textbf{\textit{N}}}$$

$$\mathbf{R^2 = 0.4970}$$

**Table 8.** Variables, standardized coefficients, and significance for the above regression model describing 50% of the variation in dehydrogenase.

| Variable | Standardized coefficient | p value |
|---|---|---|
| ***pH***=pH | 0.50074 | <0.0001 |
| ***EC***=Electrical Conductivity (dS $m^{-1}$) | -0.29901 | 0.0039 |
| ***N***=KCl Extractable Inorganic N (mg $kg^{-1}$) | 0.24432 | 0.0234 |

pH ranged from 3.2 to 7.8 across sites (Fig. 3), and was the most important variable in the regression model for both microbial biomass and dehydrogenase level.

Optimal microbial growth and enzyme production occurred at a pH of 5 and decreased at higher and lower pH's.

EC ranged from 0.14 to 4.72 dS $m^{-1}$ with fairly low levels on most sites, giving a low skewed mean of 0.92 dS $m^{-1}$. Microbial activity was highest at low salt concentrations, and decreased with an increase in salinity.

Total extractable inorganic nitrogen had values that ranged from 1.59 to 21.55 mg $kg^{-1}$ and was also a variable in the regression model. The average level of nitrogen measured across the study area was 5.02 mg $kg^{-1}$. This level is considered deficient in forest soils (Fisher and Binkley, 2000).

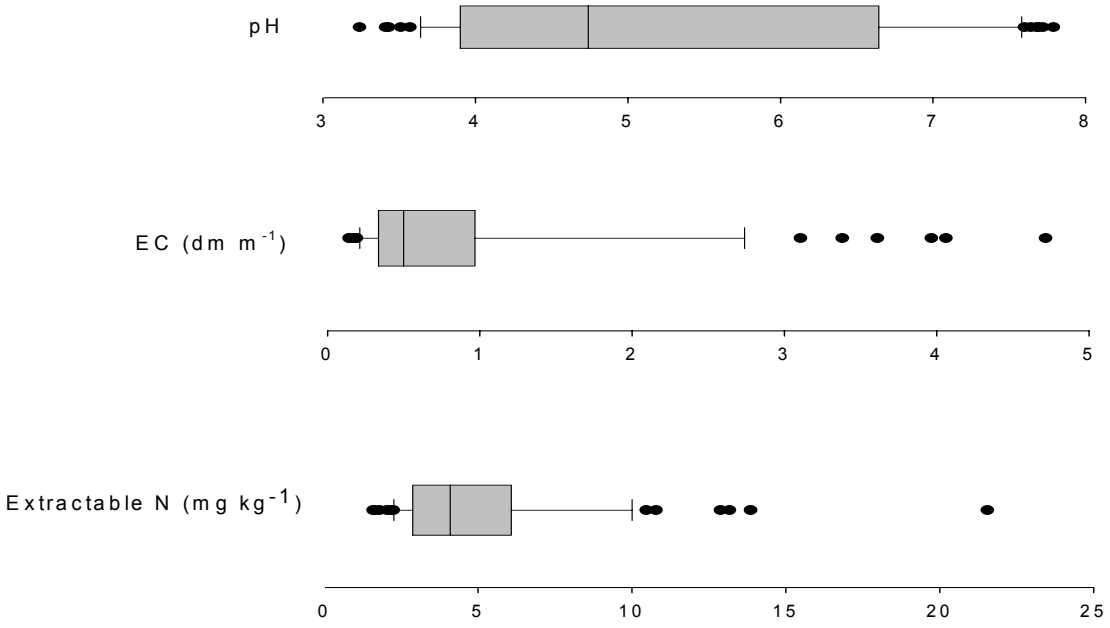


**Figure 3**. Boxplots for variables in the regression model, listed from most to least important to microbial biomass and dehydrogenase level in the regression models.

For the field study, the relationship between microbial biomass and distance from native forest was determined (Fig. 4). With an increase in distance from native forest, both microbial biomass (p=0.0049) and dehydrogenase activity (p=0.0014) decreased.

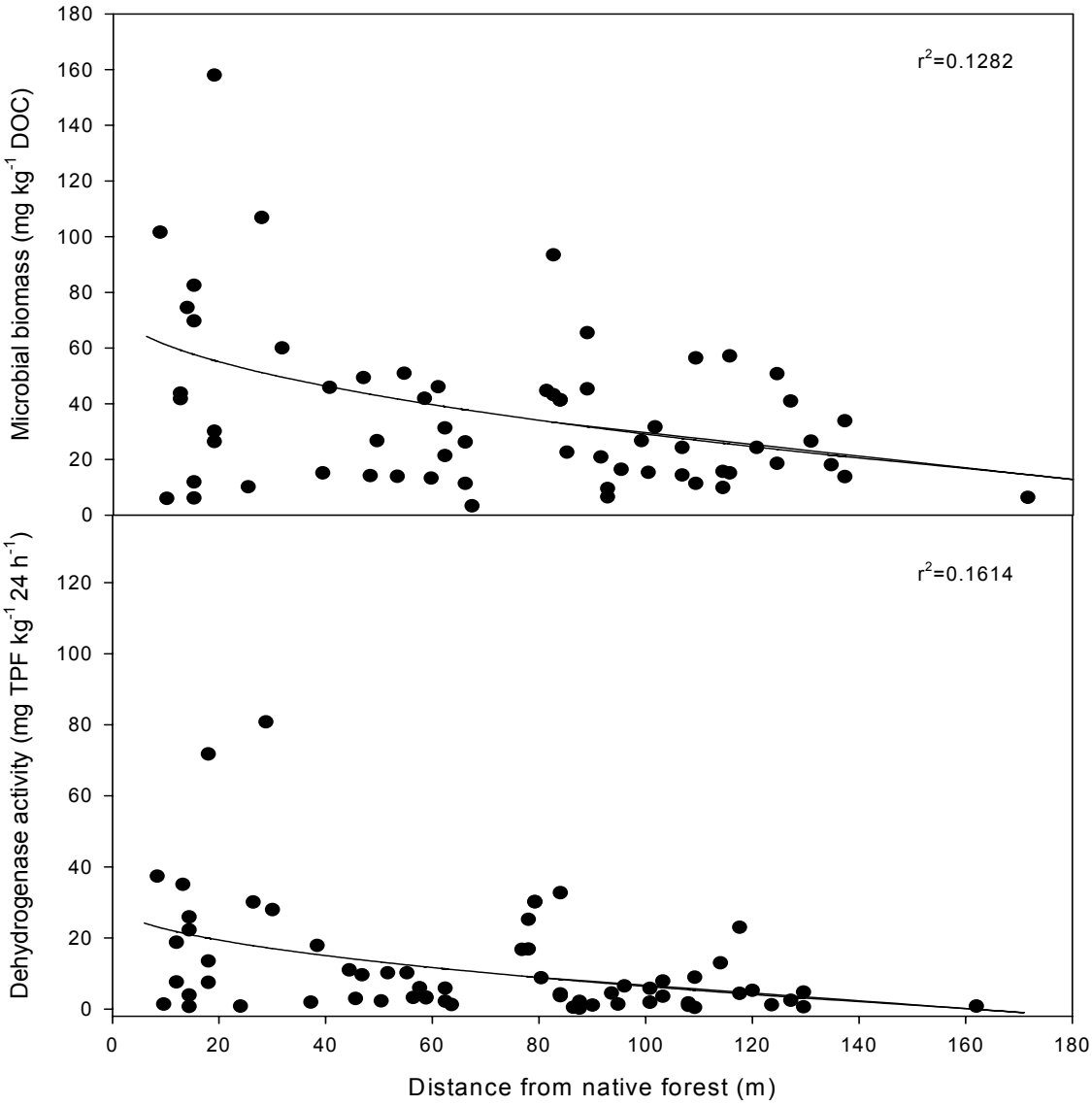


**Figure 4.** Microbial biomass and dehydrogenase activity as a function of distance from native forest on the Rapoca Coal Co. mine site in southwestern Virginia. Regressions lines are microbial biomass and dehydrogenase activity as a function of the square root of distance from native forest.

## Discussion

### Greenhouse Study

The greenhouse study shows that both spoil type and topsoil inoculation influenced microbial biomass and activity. The UFT (undisturbed forest topsoil) had the greatest microbial populations based on all three microbial variables. Based on ATP and dehydrogenase activity, BWS (brown weathered sandstone) was the best spoil material, closely followed by GUH (gray unweathered shale), while WUS (white unweathered sandstone) was the least conducive to soil microbial growth. Microbial biomass was not different among spoil types.

Topsoil inoculation also had an important positive impact on microbial populations. Almost all spoil chemical and physical properties were significantly correlated with microbial variables, and some of these properties were vital to the development of active microbial populations. The physical properties, silt plus clay, wilting point, and water retention were positively correlated with ATP, dehydrogenase activity, and microbial biomass (Table 7). These were highest on the UFT, closely followed by the BWS and the GUH, all of which had a greater water retention which is more conducive to the growth of microbes (Table 3). The WUS, on the other hand, had a much lower water retention, wilting point and % silt plus clay, and may have desiccated between watering, killing off microbial populations. Water is an essential medium for biota to survive and proliferate, and conditions that are too dry, such as those that commonly occurred on the WUS, can decrease activity substantially (Fisher and Binkley, 2000). In the United Kingdom, Harris and Birch (1989) found much higher levels of microbial activity on control soils compared to identical restored soils. Restored soils

had dehydrogenase levels of 10 to 220 ug $g^{-1}$ while undisturbed soils had much higher levels (140 to 580 ug $g^{-1}$) These soils are removed and stockpiled for two years and then replaced. All physical and chemical properties were similar except for water holding capacity which was 0.66 g water $g^{-1}$ soil on undisturbed sites compared to an average of about 0.47 g water $g^{-1}$ soil. Water-holding capacity had a significant positive correlation with nitrifying potential.

Chemical properties also influenced the presence and activity of microbes. Microbial properties were negatively correlated with pH, electrical conductivity (EC), and almost all Mehlich I available nutrients. In general, the lowest levels of these soil properties were found on the UFT followed by the BWS, while the GUH and the WUS were quite high. Many of these soil properties could be auto-correlated, none the less, some or all are having a negative impact on soil microbes. pH and EC are indicators of nutrient availability and content; pH dictates their availability, while EC is an indicator of total concentrations. These two variables can thus be viewed as "master variables" affecting microbial populations. They may have a direct toxicity effect due to excessive levels of $H^+$ or soluble salts, or they may be affecting the soil chemistry and nutrient availability.

Other studies have found that a higher salinity than those found on this site are needed to inhibit the establishment of soil organisms. Earthworms were inhibited with salt concentrations above 7dS $m^{-1}$ and collembolan were inhibited above 8dS $m^{-1}$ (Wei-Chun Ma, 1989). Although salinity was not that high on this site, microorganisms such as bacteria and amoebae may be more sensitive. Also, the soil as a whole may not have very high levels, but pockets within the soil may be very high.

Research has shown that soil pH can have an adverse impact on soil biota. Limitations have been well reported for acidic mine spoils, but alkaline soils may also limit some organisms. Actinomycetes, cannot live in soils above pH 5 (Fisher and Binkley, 2000). These organisms are not well understood, but are known to be important in decomposition of organic matter. Shifts in species composition may be substantial with a change in pH. Fungi are often dominant in acidic soils while some types of bacteria cannot survive at a low pH. The change in species composition due to a change in pH could have serious repercussions for other aspects of the environment that interact with the soil biota such as the vegetation and the soil itself. Microbial properties were positively correlated to available nitrogen, exchangeable acidity, and CEC (Table 5). N is an essential nutrient that is usually in short supply in mine soil. CEC is a measure of the availability of a soil to retain cations. Exchangeable acidity is a measure of soil reaction. All of these properties could directly influence microbial biomass and activity. However, these properties are auto-correlated with the soil physical property silt plus clay, which may have been the more important soil property affecting microbes.

Available nitrogen was highest on the UFT followed by the BWS, while the WUS and the GUH were lower. Microbial properties were positively correlated to soil N. Soil N levels in soil are enhanced by microbes and, in turn, essential to their development and growth. In West Virginia, Stroo and Jencks (1982) found lower levels on mineralizable N across 11 mine spoils (mean 25.45 mg kg-1) ranging from acidic to neutral, 3 to 20 years old and either barren, grass-legume covered or locust covered compared to native soils (mean 82.1 mg kg-1). N mineralization was positively correlated to microbial respiration, amylase, and phosphatase levels across sites. Also, with an increase in age of

the sites there was an increase in N, P, and microbial indicators. Higher nitrogen levels lead to the development of microbial populations, while the presence of microbes increases nitrogen levels. Cause and effect cannot be determined because of the close relationship between these two variables. Whatever the case, N is closely linked to microbial populations and should be viewed as an essential soil property to their development.

Topsoil inoculation had a positive effect on ATP and dehydrogenase levels, and showed a trend for increasing microbial biomass. Topsoil addition shifted the physical and chemical properties of the spoils and inoculated the spoils with microbial populations. pH was lower on the WUS and GUH as a result of topsoil addition, as were Mehlich I available nutrient levels.

Topsoil holds many of the organisms found in the undisturbed forest soil prior to strip mining as well as providing an environment conducive to reestablishment of these organisms (Wanner and Dunger, 2002). Inoculation of spoils with topsoil increased microbial activity in this study. In England, Moore and Luxton (1986) found that topsoil increased the diversity and number of soil fauna on reclaimed mined sites.

Fresquez and colleagues (1986) did a comparison of microbial populations on sites with topsoil, sites without, and on undisturbed reference sites. They demonstrated that sites with topsoil had comparable biota to undisturbed sites. Enzyme activity after three months was also equivalent to the undisturbed soil. Non-topsoiled sites, on the other hand, had much lower microbial populations.

It is difficult to know how much soil is needed to improve the reclamation medium enough to significantly increase soil biota. In New Mexico, Lindemann and

colleagues (1984) found that topsoil inoculations of 14.5 Mg/ha did not have a significant impact on biological activity. Topsoil spread at a depth of 30 cm, however, significantly increased mycorrhizal infection with *streptomyces* numbers on the spoil at $0.1x10^6$ $g^{-1}$ compared to 1.3x106 $g^{-1}$ on the 30 cm of topsoil. Dehydrogenase activity was 0.08 mg $g^{-1}$ on the spoil compared to 1.03 mg $g^{-1}$ on the topsoil. Topsoil inoculation alone did cause an increase in number of fungal genera from 6 to 8, while 30 cm of topsoil had even more types (11). They also tested the effects of adding sewage sludge or alfalfa and got similar increases in fungi and dehydrogenase activity and concluded that the addition of carbon was more important than the inoculation with microbes. The topsoil they used had been stockpiled, which can significantly reduce microbial activity. Topsoil that is not stockpiled has higher numbers of organisms and may be more effective for inoculation of mine spoils.

This greenhouse study showed that even very low amounts of topsoil, 2.5 cm on the surface, can greatly increase microbial activity. However, the effectiveness of topsoil addition was found to be spoil dependent. It appears that the BWS and GUH had much greater increases in microbial activity and enzyme production while the addition of topsoil to WUS had little effect. The WUS may have been too droughty with a high pH and EC, despite the addition of topsoil.

Field Study

In the field, the regression analysis showed that pH, available inorganic nitrogen and salinity were the most important variables affecting the presence of large and active microbial populations. Spoils with a pH of 5 supported the highest levels of dehydrogenase and total microbial biomass suggesting that this is the optimal pH for microbial populations in this area. Soil nitrogen was positively correlated with microbial biomass, dehydrogenase and ATP levels. Soil nitrogen and soil microbial populations are closely linked. It has been established that higher levels of nitrogen lead to larger microbial populations, but that the presence of microbes can also increase nitrogen levels. When an increase in soluble salt levels, microbial populations declined. This may have been a toxicity effect or simply a change in osmotic pressure that is not conducive to microbial growth.

Distance from the native forest influenced the presence and activity of microbial populations. On average, microbes occurred in greater abundance and were producing more enzymes the closer they were to the native forest. Although soil factors were also important limiting factors, it is apparent, that location played a critical role in microbial establishment.

In eastern Germany on plots with substrate barriers, Wanner and Dunger (2002) found that aerially colonizing organisms such as protests and spiders established after only a few months, while microarthopods took longer, establishing by the end of the 616 day study period. Macofauna such as earthworms and other insects were more dependent on substrate locomotion and did not establish. Fauna moving through the substrate may already be present in topsoil used for spoil inoculation. In reclaimed areas without

topsoil and distant from native forest stands, these organisms would have to move in from adjacent areas. This suggests that large strip mine areas that have no native forest in the vicinity may take much longer to develop normal soil fauna populations.

Overall Implications

Spoil properties had an important effect on microbial populations for both the greenhouse study and the field study. Both studies demonstrated that spoil types with a pH around 5, low salt content, and high available inorganic nitrogen were most conducive to healthy microbial populations.

The growth of these microbes correlated or even contributed to the successful growth of vegetation (Showalter et al. 2005, Chapter III). This is apparent in the parallel of good tree growth on the greenhouse site as well as the inclusion of microbial populations as the primary factor in the regression model describing successful tree growth in the field.

The positive relationship of microbial factors to tree growth observed in the greenhouse study suggests that the same physical and chemical factors that are important to tree growth may also effect microbial populations. Secondly, microbial populations may play an important role in the creation of some physical and chemical characteristics that lead to successful tree growth. It is difficult to tease apart these two factors. One can only conclude that a healthy microbial population is a good indicator of healthy soil conditions for tree growth. How much the successful microbial populations are due to good soil conditions, and how much of the good soil condition is created by microbial processes cannot be determined.

This relationship of microbial populations to tree growth is further supported by the field study. Microbes were the most important variable in the regression equation. Again, whether this is a cause and effect relationship or a simple correlation cannot be ascertained, but it is clear that tree growth and microbial growth are closely linked.

The relationship of microbes to soil physicochemical properties is important both to the return of native plant populations as well as the return of the rest of the ecosystem other soil organisms as well as larger animals.

## Conclusions

Microbial activity, enzyme production and biomass were affected by spoil type and treatment. The greenhouse experiment showed that brown weathered sandstone was most suitable for microbial growth, while the field study reaffirmed that the characteristics of this material, high nitrogen levels, low salt content and moderate pH, were best for microbial growth on reclaimed strip mined sites. In comparison to the shale and the sandstone, the BWS had the most moderate pH, the lowest EC and the highest nitrogen levels, thus supporting the findings from the field.

Furthermore, topsoil inoculation plays an important positive role in the development of microbial populations. This was corroborated in the field study by the higher microbial activity and populations found in close proximity to the native forest. This suggests that native soil may be an important source for the establishment of new populations on microbially-devoid fresh spoil material.

These conclusions suggest that selection of spoil materials and return of topsoil may be important if the return of the native ecosystem is the reclamation goal.

**Acknowledgments**

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# CHAPTER VI:

# SUMMARY AND RECOMMENDATIONS

Restoring the native mixed mesophytic hardwood forest on reclaimed mined land is important to landowners and Appalachian communities because of the products and services these forests provide. The economy and culture of this area have been dependent on these forests for more than two centuries. However, current reclamation practices are often not conducive to the return of the native forest. My research shows that such reclamation practices must be modified in order to create an environment that restores land capability for native forests. Current reclamation practice is often not specific for forestry post-mining land uses. The surfaces of reclaimed land are commonly composed of overburden spoils with physical and chemical properties that limit survival and normal growth of most native tree species. However this study shows that with proper spoil selection and treatment, reforestation with native, commercially-valuable species is possible.

Spoil type and its treatment had a large influence on the growth of native hardwoods, herbaceous vegetation and microbial populations. The greenhouse experiment showed that BWS (brown, weathered sandstone) that occurs just below the forest topsoil, or spoils that approximate this material, are more conducive to successful tree and microbial growth. Spoil characterization and foliar nutrient analyses showed the BWS had a high fine earth fraction and water availability, a moderately acidic pH, and a high nitrogen content compared to other spoils used in this experiment. These soil properties created an environment that more closely approximated the undisturbed forest

topsoil and were more conducive to the growth of *F. americana* and *Q. rubra* compared to the other spoils. *L. tulipifera* did not grow well on any of the mine spoil treatments, but its foliage was higher in nitrogen and had a better balance of nutrient levels on the BWS. The presence and activity of soil micro-organisms were also much higher in the BWS with higher ATP and dehydrogenase levels. By comparison, the other spoils tested in the greenhouse experiment were not as conducive to tree or microbial growth. Soil characterization and foliar nutrient analysis showed that the WUS (white, unweathered sandstone) and GUH (gray unweathered shale) had higher coarse fragment contents, lower water holding capacities, they were more alkaline, lower in N, and had higher levels of other macro- and micronutrients, levels that excessive in some cases. These materials were not conducive to growth of *F. americana, Q. rubra,* or *L. tulipifera.* Compared to the UFT and BWS, microbial populations had lower ATP and dehydrogenase levels in the GUH and very low levels in the WUS.

Adding native topsoil to spoils during reclamation could improve their quality for reforestation. The addition of topsoil did not have an impact on tree growth on the BWS or the WUS, but it did improve growth on the GUH. In addition, it shifted levels of soil properties of all three spoils to more closely approximate the UFT (undisturbed forest topsoil) of the area. Topsoil inoculation also improved foliar nutrient levels of *L. tulipifera* across all three spoils. It also greatly increased microbial populations and it provided a native seed bank which introduced other herbaceous and woody plants.

The findings of the greenhouse study dealing with the effects of spoil type and soil properties on tree growth were re-enforced by the field study of three-year-old *Q. alba* and soil microbial growth on a spectrum of mine spoil types. Our regression model

of tree height as a function of site and soil properties showed that sandy loam soils with a northeast aspect, high nutrient levels, and high microbial populations were the most conducive to tree growth. In addition, regression models of both microbial biomass and dehydrogenase level as a function of soil properties showed that soils with a moderately acidic pH, low electrical conductivity and high nitrogen levels were conducive to the growth and activity of microbial populations.

The field study also showed that microbial populations were correlated to distance from native forest. This suggests that introduction and establishment of microbes is enhanced by the proximity of native forest soils. This finding is further supported by the improved soil microbial growth on topsoil-inoculated spoils on the greenhouse experiment.

*Q. alba* growth on the field study was used to re-evaluate mapping classification criteria that used rock type, compaction and aspect to map and determine site quality of reclaimed mine spoils for forests. Tree growth and site quality did not correlate well, showing that these mapping criteria were inadequate for a good estimate of soil/site quality In an attempt to improve the classification model, a regression analysis of tree height as a function of mine site and soil properties was done. The properties silt plus clay and aspect in the regression model were well represented by the rock type and aspect mapping criteria. However, the regression analysis also showed that nutrient levels and pH were important factors influencing tree growth. Foliar nutrient analyses further supported the importance of soil nutrient supply to tree growth. pH correlated well to soil nutrient levels, it was a variable in the regression analysis, and it is easily measured in the field. We recommend that pH be added as a soil mapping criterion. The addition of pH

as a criterion of the classification model should improve its use for mapping forest site quality on mined land.

Currently there is little differentiation of topsoil substitutes for placement on the surface, leading to highly variable spoil conditions that may or may not be conducive to native ecosystem recovery. This study shows that a brown, weathered sandstone/topsoil mix is best suited for native trees and other components of the Appalachian forest system. Use of this material as a preferred topsoil substitute should be recommended when forestry is the designated post-mining land use.

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# APPENDIX I
# DETAILS OF LAB PROCEDURES

## MICROBIAL BIOMASS CARBON BY CHLOROFORM FUMIGATION-EXTRACTION

**Remarks:**

The chloroform fumigation-extraction (CFE) method is useful for determining an estimate of microbial biomass carbon, nitrogen, and other elements. The procedure, although seemingly complicated at first, is simple, rapid, and therefore useful in studies where large numbers of samples are required. The CFE method was adapted from the "standard" method of chloroform fumigation-incubation (CFI) and is gaining widespread acceptance. The CFI method is much more complex and less rapid.

The CFE method works by lysing the microbial cells with chloroform and then extracting the carbon with a dilute salt solution. The extractable carbon is compared with non-fumigated samples that serve as a control. The chloroform does not kill all cells, and all carbon is not extracted, but studies have determined that for most soils and most conditions, approximately 35% of the microbial biomass carbon is recovered.

Note: While this procedure is fairly easy, any release of chloroform outside the hood is dangerous and potentially deadly. If you feel lightheaded or get a headache, close the fume hood door and leave the lab for a few minutes.

**Equipment:**

50 mL beakers
vacuum desiccator (no desiccant)
vacuum pump

**Reagents:**

ethanol-free chloroform ($CHCl_3$)
0.5 M potassium sulfate ($K_2SO_4$)

**Procedure:**

| Procedure | Remarks |
|---|---|
| | |
| 1. Weigh 25 g field-moist soil into 50 mL beakers. Prepare 4 replicate samples (2 fumigated and 2 non-fumigated) for each soil. Skip steps 2 – 8 for non-fumigated samples. | If soil has been air-dried, add DIW to bring to field-moist condition and allow soil to equilibriate for 24 hrs. |
| 2. Place into a vacuum desiccator containing 250 mL of DIW to maintain moisture content. | Remember to remove the desiccant from the base of the desiccator |
| 3. IN THE HOOD, place a 100-mL beaker containing | Wear protective clothing and never do alone in the lab. Chloroform is |

| | |
|---|---|
| 25 mL of chloroform ($CHCL_3$) into the desiccator. | very dangerous. |
| 4. Turn on the vacuum pump for 3-5 minutes until the $CHCL_3$ boils or splatters on the sides of the desiccator. | Make sure you have a strong vacuum pump so that the $CHCL_3$ will boil. |
| 5. Close vacuum desiccator valve and then turn off vacuum. | |
| 6. Remove vacuum tube and CAREFULLY open valve. | |
| 7. Repeat steps 5 and 6 four more times or until $CHCL_3$ has evaporated. | The $CHCL_3$ may stop boiling and sometimes not all of it will evaporate. |
| 8. Leave desiccator under vacuum in a closed and darkened fume hood for 24 hours. | |
| 9. Transfer soil to 150 ml erlenmeyer flasks and add 100 mL 0.5 M $K_2SO_4$. Cover flasks with parafilm. | |
| 10. Shake flasks on low for 2 hours and allow to settle (4-5 hrs). | Make sure flasks are stable on the shaker. They have a tendency to fall and spill. |
| 11. Filter extract through Whatman #2 filter paper into 150 ml beakers. Pour subsample into a scintillation vial. | |
| 12. Filter 30 mL of pure $K_2SO_4$ solution for each blank (do at least 2); filter paper adds carbon. Pour filtrate into a scintillation vial. | |
| 13. Take samples to TOC analyzer. Lab results reported in mg/L Carbon. Convert mg/L to mg/kg soil and use calculation below to determine MBC. | |

Microbial biomass carbon is calculated using the following equation and adjusted for soil moisture content.

$$MBC = \frac{[\text{extractable C (fumigated)} - \text{extractable C (unfumigated)}]}{k_{ec}}$$

where $k_{ec} = 0.35$ (depends on soil characteristics)

**References: (Anderson, and Domsch, 1978; Gregorich et al. 1990; Jenkinson and Powlson, 1976)**

## SOIL ENZYMES: DEHYDROGENASE ASSAY

### Introduction

All biochemical processes in soils are mediated by enzymes. Enzymes are catalysts that increase the rates of chemical reactions by decreasing the activation energy required for specific reactions. Because enzymes increase the rate of chemical reactions, they play a significant role in nutrient cycling processes in soils. Every species of enzyme has a lock and key relationship with specific reactions. This lock and key relationship between enzyme and reaction allows us to isolate the enzymes involved in some very important reactions in forest soils. There are techniques available for assaying a large number of soil enzymes; however, standard methods only exist for assaying enzymes involved in C, N, P, and S cycling. Development of standard methods has been problematic because rates of enzyme reactions depend on (i) the concentration of enzyme and substrate, (ii) temperature, (iii) pH, (iv) necessity of coenzymes, (v) presence of natural inhibitors, and (vi) ionic strength of soil.

Of the soil enzyme assays, the dehydrogenase assay is perhaps the most significant in forest soils. Essential macronutrients such as nitrogen are made available to plants through biological oxidation of organic matter. Biological oxidation of organic matter is primarily carried out through dehydrogenation. As part of biological oxidation, dehydrogenase enzymes transfer hydrogen from organic compounds to hydrogen acceptors. Studies have shown that dehydrogenase activity is highly correlated with soil respiration and nitrification potential, and as such should be a good indicator of microbial activity and nutrient availability. The general reaction for dehydrogenation is:

$$XH_2 + A \rightarrow X\,AH_2$$

where $XH_2$ is an organic compound donating hydrogen to a hydrogen acceptor, A

**Equipment:**

spectrophotometer (wavelength 485 nm)
test tubes (16 x 150 mm) w/stoppers
#2 filter paper
leaching rack
pipettes
37$^{o}$C incubator
funnels
100 ml volumetric flasks (or 50 ml volumetric flasks if microbial levels are low such as in B horizons or mine spoils)
glass mixing rods
cuvettes

**Reagents:**

1. Calcium carbonate ($CaCO_3$), reagent grade
2. 2,3,5-Triphenyltetrazolium chloride (TTC), 3%
   - Dissolve 3 g TTC in approximately 80 ml deionized water, and adjust the volume to 100 ml with deionized water.
3. Methanol (CH3OH), analytical reagent grade
4. Triphenyl formazan (TPF) standard solution (i.e., for calibration)
   - Dissolve 100 mg TPF in approximately 80 ml methanol, and adjust the volume to 100 ml with methanol. Mix thoroughly.

| Procedure | Remarks |
|---|---|
| **I. CALIBRATION CURVE** | |
| 1. In a volumetric flask, dilute 10 ml of TPF standard solution to 100 ml with methanol. | |
| 2. Pipette 5, 10, 15, and 20 ml aliquots of the dilute TPF solution into separate 100-ml volumetric flasks, and bring up to volume with additional methanol. Mix thoroughly. | 2. Corresponds to 500, 100, 1500, and 2000 µg TPF/100 ml. |
| 3. Decant samples from each volumetric flask into separate cuvettes, and measure the intensity of red color using the spectrophotometer set at 485 nm. | |
| 4. Plot the absorbance readings against the amount of TPF in each standard solution. | |
| **II. DEHYDROGENASE ASSAY** | |
| 1. Add 0.2 g CaCO3 to 20 g of sieved (<2 mm), air-dried, soil. Mix thoroughly. | |
| 2. Place 6 g of the mixture into each of 3 test tubes, and add 1 ml TTC reagent and 2.5 ml deionized water to each test tube. Include one blank. | |
| 3. Mix the contents of each test tube using a glass rod, stopper each tube, and incubate at 37$^{o}$C for 24 hrs. | |
| 4. Add 10 ml methanol to each test tube, stopper each tube, and shake for 1 min. | |
| 5. Place funnels with #2 filter paper on leaching rack, and position 100-ml volumetric flasks under each funnel. | If microbial levels are low, 50-ml volumetric flasks should be used instead to decrease dilution. |
| 6. Quantitatively transfer the contents of each test tube into the funnels using 10-ml increments of methanol. Continue adding 10-ml increments of methanol to each funnel until the reddish color has disappeared | |

| | |
|---|---|
| from the filter paper. Bring the flask up to volume with additional methanol. | |
| 7. Decant a sample from the volumetric flask into a cuvette, set the spectrophotometer to 485 nm, and measure the intensity of the reddish color. Use the same cuvette for all samples to decrease error. Rinse out the cuvette with the sample before filling and measuring g it. Read an additional blank cuvette filled with methanol only. | |
| 8. Determine the amount of TPF produced using the calibration curve. Remember to correct for the dilution of the solution. | |

**Reference: (Tabatabi, 1982)**

## SOIL ACTIVITY: ATP ANALYSIS

### Introduction

Adenosine TriPhosphate (ATP) is the basic unit of energy in all living organisms. When free energy is created by light in plants or by the breakdown of food in other organisms, it is captured and stored in the chemical bonds of ATP. It can then be transported and used elsewhere in the organism. The free energy released by the breaking of high energy phosphate bonds in this molecule can then be used for carrying out other reactions that would otherwise be energetically unfavorable. ATP has 3 phosphate groups but only two of them are high energy. After the loss of one group it becomes adenosine diphosphate (ADP). With the loss of both groups it becomes adenosine monophosphate (AMP).

ATP is a good measure of biological activity in the soil, because it is only produced by living cells undergoing metabolic activity and is broken down very rapidly outside of these cells.

ATPlite, the system you will be using, lyses these cells and stabilizes the ATP while eliminating enzymes that break it down (ATPases). The ATP then reacts with firefly luciferase and produces light. The amount of light produced is proportionate to the amount of ATP. The reaction that takes place is:

$$ATP + \text{D-Luciferin} + O_2 \rightarrow \text{Oxyluciferin} + AMP + PP_i + CO_2 + \text{light}$$

ATP is a measure of total activity in the soil. It can differ greatly from other assays such as dehydrogenase or chloroform fumigation. Dehydrogenase measures a specific enzyme activity that is not produced by all cells. ATP measures activity of all cells including mycorrhizae and bacteria that do not produce this enzyme. On the other hand, chloroform measures total biomass in the soil. This includes inactive cells. ATP measures energy of the system independent of how many cells there are. This is important because bacteria may be dormant or have low activity but have a high biomass.

**Equipment:**

TopCount machine (This must be turned on and allowed to warm up for several hours before it can be used)
micropipette
pipette tips
syringe
microplate
microplate cover stickers
centrifuge tubes
centrifuge
shaker
Light proof container for dark adapting samples

**Reagents:**

1. Saline solution (8g NaCl/L $H_2O$)
2. ATP kit

Note: ATP is everywhere. Be very careful about contamination with this experiment.

Note: Light affects this experiment. If possible, do as much of the experiment as possible in low lighting.

| Procedure | Remarks |
|---|---|
| **I. CALIBRATION CURVE** | |
| 1. Using a micropipette, add 960ul of DI water to a vial of lyophilized ATP standard solution. Swirl the vial for 1 minute to dissolve the ATP. | |
| 2. Prepare a dilution series from $1x10^{-5}$ mM down to blank. Pipette 50, 40, 30, 20, 10 and 0 µl of ATP solution into separate 5ml beakers. Then pipette 0, 10, 20, 30, 40 and 50 µl of DI water into the same beakers making different dilutions. | |
| 3. Pipette 100 µL of saline solution 18 wells of the plate. | |
| 4. Add 50 µL of mammalian cell lysis solution to each well and cover the plate with a clear microplate sticker. Shake on fast for 5 minutes. | |
| 5. Remove and discard the sticker. Add 10 µL of the ATP dilution series to each well. Do three reps for each dilution. | |
| 6. Cover with a new microplate sticker. Place in a light proof box. Shake on fast for 5 minutes. | |
| 7. Dark adapt (protect from light) plate for 10 more minutes | |
| 8. Place plate in front loading chamber and place black | Ask Dave Mitchem or Julia |

| | |
|---|---|
| metal plate on top. | Showalter for more specifics on how to use the TopCount machine |
| 9. Click on assay wizard and set up an ATP white run. | |
| 10. Click start and run assay. | |
| 11. Plot the TopCount light readings against the amount of ATP in each standard solution. | |
| **II. ATP ASSAY** | |
| 1. In centrifuge tubes, weight out 10 g of soil. Add 50ml of saline solution with a tipping pipette. Do two blanks. | |
| 2. Put caps on tubes and shake on low for 1 hour. | |
| 3. While samples are shaking take out ATPlite kit and allow need reagents to warm to room temperature | |
| 4. Using a syringe, reconstitute a vial of lyophilized substrate solution by adding 5ml of substrate buffer solution. Agitate gently until the solution is homogeneous. | |
| 5. Put tubes in the centrifuge and spin at 1500rpm for 10 minutes. | 1500rpm is about 1/3 the way around the dial on the centrifuge. |
| 6. Using a micropipette, put 100µl aliquots of sample in each well on a microplate. | Leave the first 4 wells empty as blanks.<br>Do at least 3 reps per sample.<br>Change the micropipette tip between each sample to avoid contamination |
| 7. Put 50µl of mammalian cell lysis in each well. | |
| 8. Cover plate with a clear microplate sticker. | |
| 9. Shake on fast for 5 minutes | |
| 10. Remove the sticker and discard. | |
| 11. Put 50µl of ATP luciferase in each well | |
| 12. Cover with a new sticker. Cover and protect plate from light from this point on | |
| 13. Shake on fast for 5 minutes. | |
| 14. Dark adapt (protect from light) plate for 10 more minutes | |
| 15. Place plate in front loading chamber and place black metal plate on top. | Ask Dave Mitchem or Julia Showalter for more specifics on how to use the TopCount machine |
| 16. Click on assay wizard and set up an ATP white run. | |

| | |
|---|---|
| 17. Click start and run assay. | |
| 18. Determine the amount of ATP produced using the calibration curve. | |

**References: (ATPLite, 2004)**

# APPENDIX II
# BACKGROUND INFORMATION OF FIELD STUDY

## Hardwood Reforestation Field Trials on Mined Land

**Goals:**

The purpose of the field trial program is to aid efforts by mining firms to change reclamation practices, so as to reforest with hardwoods, on a trial basis. Despite widespread awareness of mine reforestation benefits, some mining firms have expressed reluctance to change reclamation practices currently in use.

Powell River Project is willing to work with mining operators to establish mine reclamation – hardwood reforestation field trials, in close communication with Virginia DMLR. The hope is that, by establishing a field trial, operating firm personnel can gain experience in conducting reclamation that is

- cost effective,
- consistent with current regulations, and
- creates a productive hardwood forest.

Mining firms establishing successful field trials will gain the capability to reclaim for quality hardwoods where they find such reclamation to be desirable. Such firms will improve their capability to identify situations where hardwood reforestation can be accomplished routinely, without excessive cost.

**Process:**

Operators choosing to establish field trials will designate an area. The amount of acreage is up to the operator and the landowner. Areas of 10 to 50 acres would work well, although larger or smaller areas would also be suitable.

Operators choosing to establish a field trial should review their permit and inform their inspector. Depending on the post-mining land use and reclamation plan, it may be possible to establish a field trial on an existing permit with no permit change or with only a minor modification. Virginia DMLR personnel can be consulted to determine whether or not modification of an existing permit would be required.

Virginia Tech personnel, working through Powell River Project, are available to consult with operators on site, speak with the inspector or other DMLR personnel, or do whatever else may be necessary to help assure successful reforestation.

PRP personnel would like to document reclamation procedures on both the field-trial area and adjacent areas being reclaimed using conventional methods. We would also like to return to the site periodically, so as to document reclamation success. However, by agreeing to establish a field trial, operators are not making a commitment to allow future site access.

### *Field Trials Address Operator Concerns:*

*Costs:* By becoming more familiar with the procedures to establish productive hardwood forest through reclamation on a mining site, operators will be able to make decisions about when and where to

implement hardwood reforestation, considering costs and other issues. Operators are encouraged to select a field-trial site where near-surface brown-sandstone retrieval and surface placement can be accomplished through routine operation.

Successful hardwood reforestation creates an increase in timber value that more than offsets any increased seedling, planting, or maintenance costs required by hardwoods, relative to pines (see Virginia Cooperative Extension pub. 460-138).

*Past Failures:* Hardwood seedlings tend to grow slowly above the ground during the first two years after planting, while roots grow rapidly. Major factors associated with past hardwood failures are compacted soils (which prevent planters from opening holes deep enough for hardwood seedlings), soils with chemical properties unsuited to trees (neutral-to-alkaline pH and/or soluble salts depress survival and early growth), aggressive non-tree-compatible groundcover (which outcompetes hardwood seedlings for light and nutrients), improper care of seedlings, and improper planting practices.

PRP personnel are willing to work closely with operators to help prevent the problems cited above and assure successful reforestation.

*Operational Change:* PRP personnel are available to speak with employees whose efforts are necessary for successful reforestation in offices, group meetings, or the field.

*Regulatory Concerns:* DMLR communications with operators (see references), as well as our communications with DMLR personnel, indicate that PRP-recommended reforestation procedures, intended for use in the field-trial program, are consistent with the DMLR regulations. If the operator requests, PRP personnel are willing to inform DMLR administrative personnel of the cooperative agreement to conduct a field trial, and to meet with company and DMLR personnel.

The best insurance against regulatory problems is effective reclamation procedures in the field coupled with regular and consistent communication with the regulatory agency. PRP personnel are willing to work with operators to assure success in both of these areas, if requested.

**Contacts:**

Operators willing to establish field trials, with Powell River Project assistance may contact Carl Zipper (540-231-9782; czip@vt.edu), Jim Burger (540-231-7680; jaburger@vt.edu), or Jon Rockett (540-328-0162; jrockett@vt.edu)

## References

Virginia Department of Mines, Minerals, and Energy, Division of Mined Land Reclamation. 1996. Guidelines for Husbandry and Reclamation Practices Appropriate to Forestry Post-Mining Land Uses. Memorandum to Operators 3-96 (Available from http://www.mme.state.va.us/Dmlr/default.htm and http://www.mcrcc.osmre.gov/tree/state_guidelines.htm). 7/9/96.

Virginia Department of Mines, Minerals, and Energy, Division of Mined Land Reclamation. 2001. Reforestation Reclamation Practices. Guidance Memorandum No. 2-01. 5/29/01.

**Table 1.** Composition of the hydroseed mix used on the Rapoca mine site.

| Species of Grasses and Legumes: | Pounds per Acre |
|---|---|
| rye | 30 |
| red top | 2 |
| weeping lovegrass | 2 |
| perennial ryegrass | 5 |
| orchard grass | 15 |
| birdsfoot trefoil | 5 |
| Fertilizer Analysis: | Pounds per Acre |
| 10-20-20 | 300 |
| Type of Mulch | Pounds, Bales per Acre |
| wood cellulose fiber | 1500 |

**Seedling Species Mix Planted on the Rapoca Mine Site**
**Rapoca Energy Co. Commercial Forestry Reclamation Project**

April 6, 2002 Jim Burger

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**Species on Hand:**

Crop Trees
- White ash 15000
- Red maple 1750
- Sycamore 3700
- Sugar maple 6200
- Bur oak 1750
- Chestnut oak 3000
- White oak 11200
- Red oak 6200

Wildlife Trees
- Dogwood 1800
- Bristle locust 4000
- White pine 3,400

---

**Crop Tree Mixes:** 600 crop trees/acre (Plantable acres: 70 to 75)

***Species Mix #1:***
Acid, moist sites
28 acres
Plant 150 each/acre
- White oak
- Sugar maple
- White ash
- N. Red oak

***Species Mix #2:***
Acid, dry sites
15 acres
Plant 200 each/acre
- Chestnut oak
- White ash
- White oak

***Species Mix #3:***
Alkaline sites
14 acres
Plant 120 each/acre
- White ash
- Sycamore
- Red maple
- Bur oak
- White oak

***Species Mix #4:***
Flat, compacted sites
12 acres
Plant mixture of all remaining trees

---

**Wildlife Tree Mix:** Plant across all acres:
- Bristle locust 50/acre
- White pine 50/acre
- Dogwood 25/acre

**See attached map for site specific assignment of Species Mixes**
**Soil and Site Factor Experiment:**

- A soil and site map of the Rapoca site has been constructed. 18 different site types have been identified.

- Soil samples have been collected from each site type for chemical and physical analysis.

- *Dave Mitchem will mark the corners of 10 one acre plots on different site types.*

- *Rick Williams will plant a systematic mixture of all species in each of the one acre plots. Each tree will be marked by a pin flag.*

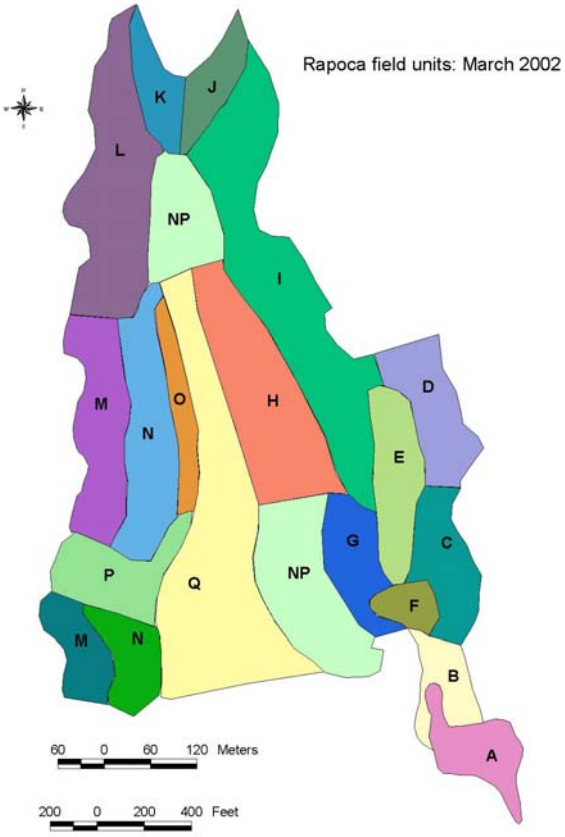
- Dave Mitchem will add additional species at a later date.

- The survival and growth of each species as a function of soil and site type will be measured and monitored over time.


**Figure 1**. Location of tree seedling mix plantings on the Rapoca mine site.

**Table 2.** Rapoca field units based on rough estimates (GPS points and 1999 aerial photo).

| Unit label | Acres | Rock Type | Compaction | Aspect | SQC | pH | EC |
|---|---|---|---|---|---|---|---|
| A | 2.4 | 2 | 2 | 3 | | | |
| B | 1.8 | 4 | 3 | 3 | | | |
| C | 3.0 | 3 | 1 | 2 | | 5.5 | |
| D | 3.3 | 4 | 2 | 2 | | | |
| E | 3.3 | 2 | 4 | 3 | | | |
| F | 1.1 | 3 | 2 | 4 | | | |
| G | 2.7 | 4 | 4 | 5 | | 7.8 | 0.17 |
| H | 7.0 | 5 | 3 | 4 | | 7.9 | 0.23 |
| I | 10.9 | 1 | 1 | 5 | | | |
| J | 1.8 | 1 | 1 | 2 | | | |
| K | 1.8 | 1 | 1 | 5 | | | |
| L | 7.2 | 3 | 1 | 2 | | 7.1 | |
| M (north) | 4.4 | 2 | 1 | 1 | | | |
| M (south) | 2.0 | 2 | 1 | 1 | | | |
| N (north) | 4.9 | 2 | 1 | 1 | | | |
| N (south) | 2.1 | 2 | 1 | 1 | | | |
| O | 1.7 | 4 | 3 | 2 | | | |
| P | 3.2 | 4 | 2 | 1 | | | |
| Q | 11.5 | 2 | 5 | 3 | | | |
| NP (north) | 3.1 | | | | | | |
| NP (south) | 5.3 | | | | | | |
| Total acreage (estimated) | 84.3 | | | | | | |




**Rapoca field units. Based on GPS points and 1999 aerial photo.**

| Unit Label | Acres | Rock Type | Compac-tion | Aspect | Site Quality | Site Class |
|---|---|---|---|---|---|---|
| A | 2.4 | 2 | 2 | 3 | 2.2 | II |
| B | 1.8 | 4 | 3 | 3 | 3.5 | IV |
| C | 3.0 | 3 | 1 | 2 | 2.2 | II |
| D | 3.3 | 4 | 2 | 2 | 3 | III |
| E | 3.3 | 2 | 4 | 3 | 2.8 | III |
| F | 1.1 | 3 | 2 | 4 | 2.9 | III |
| G | 2.7 | 4 | 4 | 5 | 4.2 | IV |
| H | 7.0 | 5 | 3 | 4 | 4.2 | IV |
| I | 10.9 | 1 | 1 | 5 | 1.8 | II |
| J | 1.8 | 1 | 1 | 2 | 1.2 | I |
| K | 1.8 | 1 | 1 | 5 | 1.8 | II |
| L | 7.2 | 3 | 1 | 2 | 2.2 | II |
| M (north) | 4.4 | 2 | 1 | 1 | 1.5 | II |
| M (south) | 2.0 | 2 | 1 | 1 | 1.5 | II |
| N (north) | 4.9 | 2 | 1 | 1 | 1.5 | II |
| N (south) | 2.1 | 2 | 1 | 1 | 1.5 | II |
| O | 1.7 | 4 | 3 | 2 | 3.3 | III |
| P | 3.2 | 4 | 2 | 1 | 2.8 | III |
| Q | 11.5 | 2 | 5 | 3 | 3 | III |

**Influence of mine site quality on commercial forest value**

| **Site quality class** | V | VI | III | II | I |
|---|---|---|---|---|---|
| Oak site index ($ft_{50}$) | 40 | 50 | 60 | 70 | 80 |
| Commercial use | None | Firewood | Ties | Sawtimber | Veneer |
| % Return on Investment (Probert 1999) | -5 | 0 | 3 | 7 | 10 |

**Figure 2.** Mine soil/site quality mapping scheme for the Rapoca mine site.

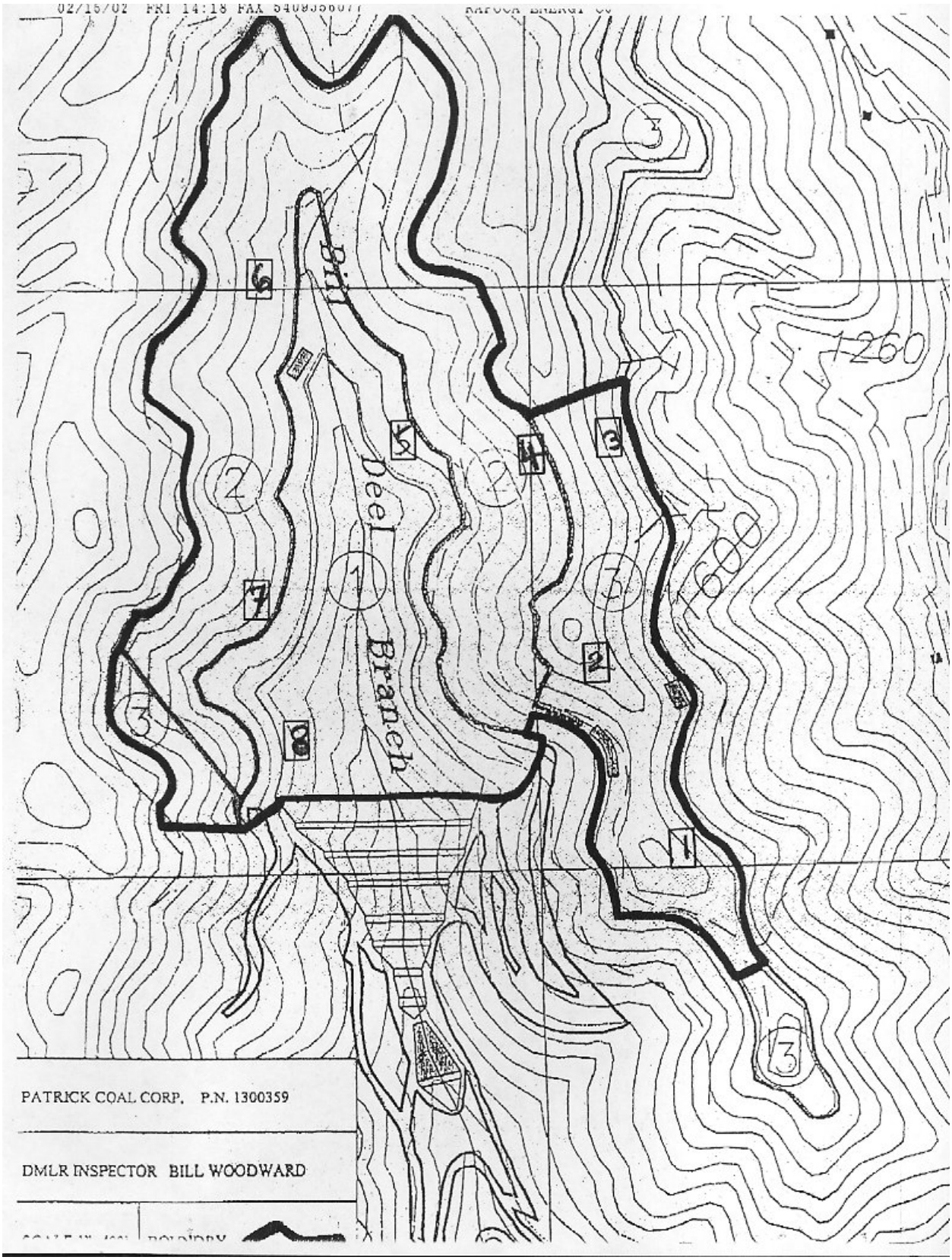



**Figure 3.** Location of plots on the Rapoca mine site.

# APPENDIX III
# SUMMARY TABLES

## Rapoca Field Data

**Table 1.** Height diameter and weight of 73 three-year-old white oaks located on the Rapoca mine, southwestern VA, 2004.

| Tree # | Old ht (cm) | New ht (cm) | ht ch (cm) | Diameter (mm) | New wt (g) | Old wt (g) | Total wt (g) |
|---|---|---|---|---|---|---|---|
| 1 | 22.6 | 30 | 7.4 | 6.2 | 0.48 | 3.29 | 3.77 |
| 2 | 49.1 | 53.9 | 4.8 | 6.2 | 1.22 | 9.16 | 10.38 |
| 3 | 34.8 | 44.6 | 9.8 | 6.8 | 0.67 | 6.23 | 6.90 |
| 4 | 31.3 | 54.2 | 22.9 | 6.8 | 1.87 | 5.32 | 7.19 |
| 5 | 48 | 51 | 3 | 8.9 | 0.85 | 14.54 | 15.39 |
| 6 | 30.5 | 36.5 | 6 | 6.6 | 0.43 | 5.18 | 5.61 |
| 7 | 42.2 | 44.4 | 2.2 | 8.2 | 0.86 | 8.27 | 9.13 |
| 8 | 26.4 | 63.9 | 37.5 | 6.9 | 7.32 | 13.95 | 21.27 |
| 9 | 29.6 | 55.5 | 25.9 | 8.5 | 2.81 | 13.68 | 16.49 |
| 10 | 38.1 | 56.6 | 18.5 | 9.2 | 6.40 | 17.40 | 23.80 |
| 11 | 31.7 | 44.4 | 12.7 | 9.2 | 3.12 | 10.54 | 13.66 |
| 12 | 22.8 | 29.5 | 6.7 | 3.4 | 0.10 | 1.50 | 1.60 |
| 13 | 11.1 | 19.8 | 8.7 | 5.9 | 0.58 | 2.67 | 3.25 |
| 14 | 27.5 | 36.4 | 8.9 | 5.4 | 0.14 | 2.53 | 2.67 |
| 15 | 13.2 | 28 | 14.8 | 3.7 | 1.34 | 2.55 | 3.89 |
| 16 | 61.5 | 124.2 | 62.7 | 9.6 | 26.03 | 60.55 | 86.58 |
| 17 | 36.1 | 46.1 | 10 | 6 | 1.22 | 7.47 | 8.69 |
| 18 | 38.4 | 39.5 | 1.1 | 5.5 | 0.06 | 2.21 | 2.27 |
| 19 | 54 | 81.1 | 27.1 | 7.7 | 3.48 | 14.13 | 17.61 |
| 20 | 23.6 | 37.5 | 13.9 | 10.1 | 2.43 | 16.91 | 19.34 |
| 21 | 12.8 | 15.2 | 2.4 | 4.4 | 0.37 | 1.84 | 2.21 |
| 22 | 33.2 | 35.2 | 2 | 4.5 | 1.55 | 3.74 | 5.29 |
| 23 | 38 | 40.1 | 2.1 | 7.8 | 1.44 | 7.85 | 9.29 |
| 24 | 41.9 | 43.5 | 1.6 | 7.3 | 0.16 | 8.76 | 8.92 |
| 25 | 31.2 | 36.1 | 4.9 | 4.8 | 0.62 | 5.01 | 5.63 |
| 26 | 34.2 | 34.6 | 0.4 | 9.2 | 0.39 | 5.75 | 6.14 |
| 27 | 59.2 | 67.1 | 7.9 | 7.4 | 4.47 | 24.05 | 28.52 |
| 28 | 9 | 30.2 | 21.2 | 1.9 | 1.48 | 2.62 | 4.10 |
| 29 | 38.1 | 65 | 26.9 | 6.7 | 3.89 | 21.15 | 25.04 |
| 30 | 45.9 | 48.2 | 2.3 | 5.6 | 0.86 | 8.01 | 8.87 |
| 31 | 13.9 | 19.5 | 5.6 | 3.8 | 0.10 | 1.19 | 1.29 |
| 32 | 10.8 | 16.5 | 5.7 | 2.8 | 0.01 | 0.23 | 0.24 |
| 33 | 27.1 | 29 | 1.9 | 6.2 | 0.12 | 5.5 | 5.62 |
| 34 | 38.6 | 41 | 2.4 | 3 | 0.45 | 3.36 | 3.81 |
| 35 | 28.9 | 34.6 | 5.7 | 4.9 | 0.34 | 3.17 | 3.51 |
| 36 | 47.9 | 62.5 | 14.6 | 7.4 | 2.42 | 21.02 | 23.44 |
| 37 | 23.8 | 66 | 42.2 | 5.1 | 3.57 | 9.22 | 12.79 |
| 38 | 36.4 | 47.8 | 11.4 | 7.8 | 4.00 | 15.70 | 19.70 |
| 39 | 46.8 | 47.2 | 0.4 | 5.6 | 0.65 | 4.92 | 5.57 |
| 40 | 45.3 | 71.2 | 25.9 | 10 | 14.96 | 37.82 | 52.78 |
| 41 | 42.7 | 45 | 2.3 | 7.8 | 1.44 | 4.86 | 6.30 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 42 | 40.9 | 72 | 31.1 | 9.9 | 11.62 | | 41.64 | 53.26 |
| 43 | 13.5 | 63 | 49.5 | 5.4 | 3.73 | | 10.44 | 14.17 |
| 44 | 70.4 | 97.5 | 27.1 | 10.1 | 10.46 | | 44.79 | 55.25 |
| 45 | 36.4 | 66 | 29.6 | 6.9 | 2.75 | | 8.94 | 11.69 |
| 46 | 45.3 | 51.6 | 6.3 | 9 | 5.27 | | 23.20 | 28.47 |
| 47 | 68.2 | 121 | 52.8 | 8.2 | 16.90 | | 40.99 | 57.89 |
| 48 | 36.2 | 48.4 | 12.2 | 5.9 | 1.87 | | 9.67 | 11.54 |
| 49 | 37.3 | 40.4 | 3.1 | 5.9 | 0.23 | | 5.25 | 5.48 |
| 50 | 24.1 | 41.9 | 17.8 | 7.5 | 3.50 | | 10.48 | 13.98 |
| 51 | 32.6 | 67 | 34.4 | 7.5 | 3.02 | | 12.37 | 15.39 |
| 52 | 37.2 | 39 | 1.8 | 5.7 | 0.62 | | 9.96 | 10.58 |
| 53 | 31.4 | 34.5 | 3.1 | 5.8 | 0.12 | | 4.13 | 4.25 |
| 54 | 43.9 | 79.2 | 35.3 | 8.8 | 15.78 | | 32.27 | 48.05 |
| 55 | 97.7 | 125 | 27.3 | 3.7 | 40.60 | | 149.43 | 190.03 |
| 56 | 32.5 | 43.7 | 11.2 | 4.6 | 0.86 | | 4.13 | 4.99 |
| 57 | 25 | 57.8 | 32.8 | 7.4 | 4.03 | | 8.13 | 12.16 |
| 58 | 42.2 | 66.3 | 24.1 | 8.8 | 4.96 | | 24.23 | 29.19 |
| 59 | 52.5 | 77.5 | 25 | 9.2 | 3.99 | | 26.38 | 30.37 |
| 60 | 38.1 | 51 | 12.9 | 4.8 | . | . | . | |
| 61 | 54.4 | 93.5 | 39.1 | 8.6 | 18.15 | | 57.94 | 76.09 |
| 62 | 52.8 | 54.9 | 2.1 | 6.5 | 1.49 | | 15.07 | 16.56 |
| 63 | 46.5 | 55.5 | 9 | 10.4 | 0.90 | | 19.48 | 20.38 |
| 65 | 32 | 37.1 | 5.1 | 8.6 | 0.23 | | 7.85 | 8.08 |
| 66 | 45.1 | 64 | 18.9 | 7 | 4.32 | | 15.94 | 20.26 |
| 67 | 49.5 | 56 | 6.5 | 6.3 | 0.48 | | 10.63 | 11.11 |
| 68 | 19.4 | 60 | 40.6 | 5.3 | 4.14 | | 7.48 | 11.62 |
| 69 | 70 | 83.4 | 13.4 | 12 | 6.99 | | 42.87 | 49.86 |
| 70 | 55.2 | 81 | 25.8 | 8.4 | 6.61 | | 25.76 | 32.37 |
| 71 | 48.7 | 58.9 | 10.2 | 7.4 | 1.19 | | 13.68 | 14.87 |
| 72 | 40.9 | 54 | 13.1 | 5.6 | 5.45 | | 13.60 | 19.05 |
| 73 | 40.5 | 73.9 | 33.4 | 8.2 | 12.60 | | 20.94 | 33.54 |

**Table 2.** Physical soil properties of mine spoils located adjacent to 73 3-year-old white oaks located on the Rapoca mine, southwestern VA, 2004.

| tree # | siltclay | aspect | x3 val | slope | Slope aspect | BD | Rock % | watera vl | meters |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 45.10 | 0.88 | 0.97 | 40 | 190.27 | 0.88 | 36.53 | 0.07 | 18.0 |
| 2 | 17.42 | 0.88 | 0.97 | 32 | 190.70 | 0.93 | 39.56 | 0.05 | 37.2 |
| 3 | 39.33 | 0.88 | 0.97 | 48 | 189.85 | 1.38 | 45.65 | 0.05 | 50.4 |
| 4 | 34.67 | 0.88 | 0.97 | 41 | 190.22 | 0.77 | 37.90 | 0.05 | 62.4 |
| 5 | 42.03 | 0.88 | 0.97 | 29 | 190.85 | 0.75 | 34.75 | 0.07 | 80.4 |
| 6 | 40.37 | 0.88 | 0.97 | 24 | 191.12 | 0.97 | 20.71 | 0.08 | 109.2 |
| 7 | 52.27 | 0.88 | 0.97 | 42 | 190.17 | 0.89 | 29.30 | 0.08 | 162.0 |
| 8 | 37.40 | 0.88 | 0.97 | 29 | 190.85 | 0.89 | 32.18 | 0.07 | 162.0 |
| 9 | 26.26 | 0.88 | 0.97 | 30 | 190.80 | 1.17 | 34.44 | 0.06 | 127.2 |
| 10 | 25.03 | 0.88 | 0.97 | 18 | 191.43 | 1.18 | 40.09 | 0.06 | 87.6 |
| 11 | 25.94 | 0.75 | 0.99 | 19 | 191.36 | 0.92 | 39.20 | 0.05 | 63.6 |
| 12 | 47.87 | 0.75 | 0.99 | 22 | 191.20 | 0.87 | 38.54 | 0.07 | 56.4 |
| 13 | 48.70 | 0.75 | 0.99 | 34 | 190.56 | 0.99 | 28.79 | 0.08 | 45.6 |
| 14 | 46.74 | 0.75 | 0.99 | 32 | 190.66 | 1.29 | 34.02 | 0.07 | 14.4 |
| 15 | 30.19 | 0.75 | 0.99 | 27 | 190.93 | 1.09 | 34.73 | 0.06 | 9.6 |
| 16 | 55.65 | 0.75 | 0.99 | 38 | 190.34 | 0.84 | 37.11 | 0.07 | 6.0 |
| 17 | 48.39 | 0.75 | 0.99 | 47 | 189.46 | 1.03 | 37.20 | 0.07 | 14.4 |
| 18 | 49.06 | 0.75 | 0.99 | 43 | 189.67 | 1.11 | 41.53 | 0.06 | 24.0 |
| 19 | 29.07 | 0.75 | 0.99 | 44 | 189.62 | 0.91 | 37.87 | 0.06 | 55.2 |
| 20 | 47.41 | 0.88 | 0.97 | 50 | 189.34 | 1.08 | 35.96 | 0.07 | 90.0 |
| 21 | 52.75 | 0.88 | 0.97 | 51 | 189.29 | 0.99 | 31.52 | 0.07 | 108.0 |
| 22 | 47.68 | 0.88 | 0.97 | 50 | 189.34 | 1.19 | 34.69 | 0.07 | 123.6 |
| 23 | 45.55 | 0.88 | 0.97 | 36 | 190.08 | 0.92 | 27.15 | 0.08 | 62.4 |
| 24 | 31.71 | 0.88 | 0.97 | 37 | 190.03 | 0.94 | 46.94 | 0.05 | 46.8 |
| 25 | 29.33 | 0.88 | 0.97 | 41 | 189.82 | 0.89 | 42.61 | 0.05 | 18.0 |
| 26 | 48.36 | 0.25 | 0.16 | 8 | 184.90 | 1.16 | 29.62 | 0.08 | 20.4 |
| 27 | 46.99 | 1.00 | 0.81 | 4 | 184.91 | 1.01 | 42.53 | 0.06 | 12.0 |
| 28 | 48.16 | 1.00 | 0.81 | 5 | 184.87 | 0.64 | 43.54 | 0.07 | 14.4 |
| 29 | 47.36 | 1.00 | 0.81 | 5 | 184.87 | 0.99 | 38.69 | 0.07 | 13.2 |
| 30 | 50.61 | 0.75 | 0.16 | 6 | 184.95 | 1.03 | 32.05 | 0.08 | 19.2 |
| 31 | 47.78 | 1.00 | 0.81 | 9 | 184.69 | 1.07 | 28.86 | 0.08 | 22.8 |
| 32 | 47.50 | 1.00 | 0.81 | 7 | 184.78 | 1.19 | 32.69 | 0.07 | 9.6 |
| 33 | 47.84 | 1.00 | 0.81 | 9 | 184.69 | 1.30 | 35.22 | 0.07 | 16.8 |
| 34 | 51.13 | 0.75 | 0.99 | 2 | 184.98 | 1.37 | 46.55 | 0.06 | 31.2 |
| 35 | 62.95 | 0.75 | 0.99 | 2 | 184.98 | 1.26 | 27.10 | 0.08 | 34.8 |
| 36 | 47.91 | 0.25 | 0.99 | 43 | 183.19 | 0.92 | 32.00 | 0.07 | 44.4 |
| 37 | 49.05 | 0.25 | 0.99 | 28 | 183.99 | 1.13 | 37.38 | 0.07 | 57.6 |
| 38 | 48.68 | 0.25 | 0.99 | 11 | 184.90 | 0.72 | 27.10 | 0.07 | 86.4 |
| 39 | 57.28 | 0.00 | 0.81 | 23 | 184.49 | 1.28 | 26.49 | 0.09 | 94.8 |
| 40 | 44.37 | 0.25 | 0.99 | 14 | 184.74 | 0.82 | 31.56 | 0.07 | 100.8 |
| 41 | 56.67 | 0.50 | 0.16 | 2 | 185.45 | 0.86 | 30.58 | 0.07 | 100.8 |
| 42 | 60.18 | 0.25 | 0.99 | 12 | 184.85 | 1.16 | 30.82 | 0.07 | 109.2 |
| 43 | 49.28 | 0.25 | 0.99 | 6 | 185.17 | 1.02 | 28.45 | 0.07 | 114.0 |
| 44 | 44.81 | 0.25 | 0.99 | 5 | 185.22 | 0.74 | 33.16 | 0.07 | 117.6 |
| 45 | 58.16 | 0.25 | 0.99 | 10 | 184.96 | 1.03 | 25.91 | 0.07 | 117.6 |
| 46 | 49.81 | 0.50 | 0.59 | 4 | 185.36 | 1.10 | 34.21 | 0.07 | 120.0 |
| 47 | 42.19 | 0.25 | 0.99 | 15 | 184.69 | 0.84 | 36.20 | 0.06 | 129.6 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 48 | 56.98 | 0.25 | 0.99 | 10 | 184.96 | 1.06 | 31.82 | 0.06 | 129.6 |
| 49 | 40.13 | 0.25 | 0.99 | 7 | 185.12 | 1.09 | 50.82 | 0.05 | 84.0 |
| 50 | 37.21 | 1.00 | 0.81 | 17 | 184.75 | 0.89 | 23.68 | 0.06 | 78.0 |
| 51 | 46.51 | 1.00 | 0.81 | 21 | 184.57 | 1.03 | 29.22 | 0.07 | 78.0 |
| 52 | 44.22 | 0.75 | 0.16 | 10 | 185.26 | 0.94 | 35.68 | 0.07 | 84.0 |
| 53 | 48.24 | 1.00 | 0.81 | 12 | 184.97 | 0.68 | 43.31 | 0.06 | 87.6 |
| 54 | 55.65 | 1.00 | 0.81 | 40 | 183.74 | 1.06 | 33.31 | 0.07 | 93.6 |
| 55 | 47.29 | 0.88 | 0.97 | 46 | 183.07 | 0.87 | 31.31 | 0.06 | 103.2 |
| 56 | 55.11 | 0.88 | 0.97 | 55 | 182.60 | 0.63 | 35.42 | 0.07 | 108.0 |
| 57 | 39.74 | 0.88 | 0.97 | 54 | 182.65 | 0.91 | 40.31 | 0.05 | 103.2 |
| 58 | 55.46 | 0.75 | 0.99 | 3 | 185.33 | 1.06 | 32.52 | 0.07 | 96.0 |
| 59 | 63.98 | 0.00 | 0.81 | 0 | 185.49 | 1.17 | 32.70 | 0.07 | 84.0 |
| 60 | 46.56 | 1.00 | 0.81 | 11 | 185.01 | 0.99 | 33.99 | 0.06 | 58.8 |
| 61 | 48.64 | 1.00 | 0.81 | 54 | 183.13 | 0.79 | 33.09 | 0.07 | 58.8 |
| 62 | 42.72 | 1.00 | 0.81 | 5 | 185.27 | 1.04 | 34.96 | 0.07 | 38.4 |
| 63 | 44.24 | 1.00 | 0.81 | 15 | 184.84 | 0.62 | 41.14 | 0.07 | 30.0 |
| 65 | 41.51 | 1.00 | 0.81 | 3 | 185.36 | 0.76 | 36.41 | 0.07 | 18.0 |
| 66 | 36.64 | 1.00 | 0.81 | 4 | 185.32 | 0.72 | 48.50 | 0.05 | 14.4 |
| 67 | 41.97 | 0.88 | 0.52 | 12 | 185.12 | 0.89 | 38.99 | 0.07 | 51.6 |
| 68 | 41.71 | 0.88 | 0.52 | 8 | 185.24 | 1.05 | 40.71 | 0.07 | 26.4 |
| 69 | 48.07 | 0.88 | 0.52 | 15 | 185.02 | 0.99 | 36.81 | 0.07 | 28.8 |
| 70 | 37.43 | 0.88 | 0.52 | 19 | 184.90 | 0.61 | 33.34 | 0.06 | 12.0 |
| 71 | 41.36 | 0.88 | 0.52 | 12 | 185.12 | 1.39 | 41.16 | 0.06 | 8.4 |
| 72 | 48.45 | 0.88 | 0.52 | 17 | 184.96 | 1.07 | 32.39 | 0.06 | 76.8 |
| 73 | 41.52 | 0.88 | 0.97 | 20 | 184.44 | 0.99 | 38.77 | 0.06 | 79.2 |

**Table 3.** Soil nutrients of mine spoils located adjacent to 73 3-year-old white oaks located on the Rapoca mine, southwestern VA, 2004.

| Tree # | P mg $kg^{-1}$ | An $NH_4$ mg $kg^{-1}$ | KCl N mg $kg^{-1}$ | Mehlich-1 Extractable nutrients (mg $kg^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | K | Ca | Mg | Zn | Mn | Cu | Fe | B |
| 1 | 3.45 | 8.60 | 3.12 | 43.16 | 1336 | 267 | 4.63 | 49.19 | 1.21 | 42.27 | 0.19 |
| 2 | 31.54 | 2.02 | 2.86 | 22.97 | 1136 | 175 | 4.11 | 29.43 | 0.91 | 69.93 | 0.06 |
| 3 | 3.16 | 8.89 | 2.46 | 44.03 | 1198 | 201 | 4.40 | 43.05 | 1.03 | 39.14 | 0.16 |
| 4 | 2.69 | 3.07 | 2.85 | 31.67 | 1426 | 204 | 5.90 | 47.32 | 1.24 | 58.68 | 0.12 |
| 5 | 4.78 | 4.29 | 3.66 | 36.54 | 1336 | 207 | 5.74 | 41.30 | 1.50 | 58.20 | 0.13 |
| 6 | 8.64 | 2.75 | 4.82 | 25.37 | 846 | 136 | 2.85 | 20.54 | 1.43 | 93.80 | 0.08 |
| 7 | 8.59 | 2.08 | 4.35 | 24.04 | 314 | 76 | 2.33 | 14.00 | 1.41 | 75.93 | 0.07 |
| 8 | 5.38 | 1.95 | 3.88 | 23.74 | 214 | 66 | 1.90 | 13.09 | 1.36 | 68.63 | 0.07 |
| 9 | 6.31 | 1.51 | 3.67 | 17.70 | 222 | 71 | 1.57 | 11.47 | 1.05 | 74.47 | 0.07 |
| 10 | 3.63 | 2.06 | 2.88 | 24.56 | 479 | 126 | 2.16 | 14.32 | 1.26 | 26.12 | 0.06 |
| 11 | 4.39 | 0.80 | 3.29 | 22.50 | 421 | 109 | 2.07 | 13.92 | 1.16 | 25.96 | 0.06 |
| 12 | 4.54 | 10.28 | 2.93 | 38.11 | 543 | 139 | 2.27 | 14.87 | 1.41 | 26.49 | 0.06 |
| 13 | 5.03 | 9.26 | 5.17 | 38.45 | 649 | 176 | 2.14 | 17.95 | 1.28 | 27.63 | 0.07 |
| 14 | 2.64 | 4.91 | 3.97 | 35.63 | 694 | 151 | 3.30 | 21.77 | 1.32 | 31.61 | 0.07 |
| 15 | 5.04 | 2.24 | 4.80 | 20.89 | 316 | 76 | 2.28 | 16.97 | 0.85 | 64.94 | 0.07 |
| 16 | 6.85 | 7.01 | 3.79 | 35.22 | 400 | 111 | 1.70 | 15.66 | 0.75 | 40.18 | 0.06 |
| 17 | 6.47 | 3.81 | 4.39 | 27.01 | 334 | 87 | 2.70 | 18.72 | 1.13 | 52.06 | 0.06 |
| 18 | 6.07 | 1.45 | 3.82 | 23.97 | 401 | 106 | 2.86 | 19.70 | 0.99 | 41.34 | 0.06 |
| 19 | 2.57 | 3.41 | 4.58 | 23.61 | 478 | 139 | 1.93 | 13.11 | 1.24 | 23.42 | 0.06 |
| 20 | 6.78 | 0.73 | 1.63 | 23.05 | 430 | 93 | 1.73 | 13.83 | 0.96 | 59.24 | 0.06 |
| 21 | 6.76 | 0.49 | 2.05 | 23.28 | 361 | 87 | 1.92 | 15.48 | 1.10 | 57.87 | 0.07 |
| 22 | 5.53 | 1.74 | 2.70 | 26.78 | 214 | 69 | 2.02 | 9.08 | 0.98 | 67.01 | 0.07 |
| 23 | 9.66 | 2.66 | 4.70 | 8.74 | 503 | 114 | 2.40 | 15.95 | 1.17 | 138.92 | 0.07 |
| 24 | 1.64 | 1.21 | 1.59 | 33.96 | 1070 | 168 | 4.46 | 35.76 | 1.11 | 41.81 | 0.11 |
| 25 | 2.58 | 14.62 | 1.79 | 52.23 | 1323 | 204 | 5.28 | 52.17 | 1.32 | 49.07 | 0.17 |
| 26 | 8.28 | 6.69 | 5.88 | 6.33 | 150 | 53 | 1.34 | 10.35 | 1.13 | 129.63 | 0.07 |
| 27 | 16.41 | 13.52 | 8.02 | 66.67 | 471 | 196 | 1.44 | 12.01 | 0.86 | 27.30 | 0.06 |
| 28 | 6.75 | 20.31 | 3.48 | 36.13 | 583 | 245 | 0.96 | 14.40 | 0.56 | 16.20 | 0.11 |
| 29 | 4.42 | 12.39 | 7.90 | 34.33 | 663 | 254 | 0.92 | 12.57 | 0.67 | 15.39 | 0.12 |
| 30 | 12.63 | 25.04 | 4.32 | 43.49 | 381 | 129 | 1.77 | 33.16 | 0.82 | 31.80 | 0.07 |
| 31 | 14.17 | 11.97 | 4.60 | 22.05 | 139 | 48 | 0.78 | 9.11 | 0.85 | 62.10 | 0.07 |
| 32 | 9.28 | 6.90 | 2.49 | 20.87 | 282 | 85 | 1.68 | 12.25 | 1.21 | 80.70 | 0.07 |
| 33 | 8.84 | 3.16 | 4.85 | 14.25 | 356 | 69 | 1.43 | 15.35 | 0.84 | 92.25 | 0.06 |
| 34 | 16.59 | 1.56 | 1.88 | 22.98 | 317 | 94 | 1.71 | 17.32 | 0.91 | 42.98 | 0.05 |
| 35 | 4.06 | . | 2.47 | 25.52 | 228 | 114 | 1.90 | 30.98 | 1.02 | 35.50 | 0.07 |
| 36 | 0.84 | 11.76 | 4.57 | 42.16 | 471 | 246 | 2.11 | 12.72 | 1.02 | 15.09 | 0.07 |
| 37 | 4.33 | 14.43 | 6.36 | 55.11 | 509 | 150 | 1.32 | 10.33 | 0.88 | 18.35 | 0.06 |
| 38 | 10.36 | 8.71 | 2.72 | 31.35 | 149 | 81 | 2.92 | 32.30 | 1.09 | 69.26 | 0.07 |
| 39 | 8.38 | 4.63 | 2.44 | 37.49 | 297 | 138 | 3.38 | 19.85 | 1.76 | 36.61 | 0.07 |
| 40 | 5.32 | 4.09 | 2.24 | 23.95 | 140 | 58 | 1.57 | 13.89 | 1.37 | 57.49 | 0.07 |
| 41 | 7.08 | 2.53 | 2.27 | 27.07 | 205 | 96 | 2.22 | 12.50 | 1.46 | 39.15 | 0.07 |
| 42 | 3.94 | 7.47 | 3.49 | 29.06 | 140 | 87 | 1.25 | 10.38 | 1.04 | 34.80 | 0.07 |
| 43 | 5.35 | 20.34 | 6.47 | 72.27 | 525 | 152 | 1.93 | 14.02 | 1.14 | 21.61 | 0.14 |
| 44 | 8.75 | 23.60 | 4.09 | 66.84 | 343 | 189 | 1.67 | 10.76 | 1.27 | 16.51 | 0.07 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 45 | 8.77 | 12.09 | 4.06 | 37.79 | 273 | 193 | 1.33 | 10.00 | 0.96 | 15.56 | 0.07 |
| 46 | 0.81 | 2.50 | 3.15 | 19.74 | 54 | 57 | 1.78 | 14.74 | 1.84 | 17.70 | 0.07 |
| 47 | 6.66 | 17.71 | 4.46 | 26.16 | 84 | 25 | 1.15 | 6.06 | 1.08 | 92.32 | 0.06 |
| 48 | 4.75 | 6.18 | 6.14 | 21.82 | 132 | 89 | 1.57 | 12.82 | 1.09 | 32.86 | 0.07 |
| 49 | 4.44 | 4.85 | 3.80 | 13.77 | 337 | 74 | 3.54 | 21.54 | 1.08 | 51.74 | 0.05 |
| 50 | 4.47 | 12.78 | 5.35 | 42.74 | 112 | 137 | 1.37 | 14.12 | 0.69 | 18.78 | 0.08 |
| 51 | 19.84 | 26.81 | 5.88 | 46.01 | 194 | 96 | 1.34 | 12.74 | 0.71 | 29.09 | 0.07 |
| 52 | 4.12 | 16.01 | 5.07 | 32.80 | 1201 | 255 | 2.32 | 46.37 | 1.09 | 37.05 | 0.19 |
| 53 | 5.05 | 7.18 | 4.79 | 14.74 | 512 | 48 | 1.76 | 11.28 | 1.25 | 76.20 | 0.06 |
| 54 | 2.52 | 2.31 | 2.74 | 25.34 | 222 | 59 | 2.13 | 11.14 | 1.13 | 50.75 | 0.07 |
| 55 | 28.05 | 20.19 | 10.46 | 55.64 | 350 | 104 | 1.92 | 14.29 | 1.10 | 41.90 | 0.07 |
| 56 | 10.67 | 14.71 | 7.66 | 51.67 | 386 | 94 | 2.13 | 18.21 | 1.29 | 56.38 | 0.06 |
| 57 | 5.14 | 4.58 | 3.19 | 44.17 | 381 | 176 | 1.19 | 14.32 | 0.72 | 19.16 | 0.06 |
| 58 | 6.95 | 2.11 | 2.18 | 55.33 | 299 | 234 | 0.81 | 8.16 | 0.74 | 12.01 | 0.07 |
| 59 | 0.95 | 1.80 | 2.61 | 26.25 | 106 | 159 | 3.23 | 12.32 | 0.87 | 16.42 | 0.07 |
| 60 | 5.12 | 1.95 | 2.54 | 17.16 | 180 | 61 | 1.19 | 7.92 | 1.25 | 57.42 | 0.07 |
| 61 | 11.55 | 30.60 | 8.69 | 46.84 | 213 | 66 | 1.54 | 10.24 | 1.20 | 64.57 | 0.07 |
| 62 | 4.94 | 19.76 | 12.87 | 29.92 | 862 | 201 | 1.95 | 24.65 | 0.91 | 26.47 | 0.13 |
| 63 | 6.09 | 25.92 | 13.16 | 31.78 | 789 | 174 | 1.29 | 19.13 | 0.59 | 19.01 | 0.18 |
| 65 | 4.06 | 12.42 | 6.02 | 22.89 | 817 | 225 | 1.27 | 18.57 | 0.70 | 21.43 | 0.13 |
| 66 | 6.27 | 23.57 | 4.93 | 32.45 | 956 | 204 | 1.18 | 30.90 | 0.41 | 17.36 | 0.15 |
| 67 | 5.07 | 11.44 | 8.87 | 32.95 | 1100 | 213 | 1.71 | 33.92 | 0.67 | 23.55 | 0.18 |
| 68 | 3.81 | 15.31 | 10.78 | 29.05 | 1048 | 204 | 2.19 | 39.13 | 1.07 | 28.94 | 0.18 |
| 69 | 7.72 | 17.50 | 13.85 | 32.86 | 935 | 221 | 1.58 | 26.22 | 0.88 | 23.76 | 0.19 |
| 70 | 9.69 | 62.95 | 21.55 | 54.00 | 837 | 178 | 1.93 | 25.53 | 0.87 | 22.47 | 0.20 |
| 71 | 3.77 | 6.65 | 9.70 | 22.95 | 853 | 200 | 1.41 | 21.71 | 0.88 | 23.00 | 0.12 |
| 72 | 7.70 | 14.35 | 8.95 | 33.80 | 268 | 112 | 2.30 | 20.15 | 1.42 | 34.82 | 0.07 |
| 73 | 9.53 | 7.32 | 4.60 | 26.94 | 609 | 116 | 2.33 | 24.13 | 1.35 | 69.87 | 0.06 |

**Table 4.** Chemical soil properties of mine spoils located adjacent to 73 3-year-old white oaks located on the Rapoca mine, southwestern VA, 2004.

| Tree # | pH | EC | C-N analizer | | Ammonium Acetate Ex Cations (mg $kg^{-1}$) | | | | CEC |
|---|---|---|---|---|---|---|---|---|---|
| | | | N mg $kg^{-1}$ | C mg $kg^{-1}$ | Ca | Mg | K | Na | |
| 1 | 7.57 | 0.96 | 0.05 | 1.59 | 316 | 87 | 200 | 55 | 3.52 |
| 2 | 4.63 | 2.50 | 0.04 | 0.69 | 239 | 78 | 145 | 47 | 2.67 |
| 3 | 7.41 | 1.27 | 0.04 | 1.01 | 275 | 64 | 149 | 35 | 2.76 |
| 4 | 6.38 | 3.38 | 0.04 | 0.90 | 181 | 96 | 112 | 59 | 2.25 |
| 5 | 6.16 | 4.72 | 0.06 | 1.71 | 297 | 78 | 194 | 51 | 3.34 |
| 6 | 3.51 | 3.97 | 0.07 | 2.40 | 215 | 104 | 171 | 83 | 3.02 |
| 7 | 3.64 | 1.21 | 0.05 | 1.24 | 130 | 88 | 92 | 62 | 1.86 |
| 8 | 3.64 | 0.93 | 0.08 | 3.03 | 107 | 89 | 73 | 60 | 1.59 |
| 9 | 3.43 | 1.33 | 0.04 | 0.58 | 99 | 100 | 65 | 66 | 1.55 |
| 10 | 5.13 | 0.50 | 0.04 | 0.88 | 273 | 76 | 163 | 45 | 2.95 |
| 11 | 4.55 | 0.43 | 0.04 | 0.82 | 236 | 82 | 143 | 50 | 2.66 |
| 12 | 5.72 | 0.38 | 0.07 | 1.75 | 302 | 76 | 186 | 47 | 3.27 |
| 13 | 6.07 | 0.39 | 0.05 | 1.34 | 319 | 65 | 227 | 46 | 3.65 |
| 14 | 5.92 | 0.41 | 0.05 | 1.19 | 284 | 85 | 188 | 56 | 3.28 |
| 15 | 3.71 | 0.96 | 0.03 | 0.53 | 90 | 73 | 59 | 48 | 1.30 |
| 16 | 4.30 | 0.75 | 0.03 | 0.56 | 241 | 96 | 151 | 60 | 2.84 |
| 17 | 3.99 | 0.73 | 0.03 | 0.52 | 175 | 89 | 110 | 56 | 2.17 |
| 18 | 4.39 | 0.58 | 0.03 | 0.57 | 224 | 90 | 131 | 52 | 2.55 |
| 19 | 5.56 | 0.56 | 0.04 | 0.90 | 311 | 87 | 193 | 54 | 3.43 |
| 20 | 3.66 | 1.34 | 0.04 | 0.64 | 181 | 69 | 116 | 44 | 2.14 |
| 21 | 3.87 | 0.87 | 0.03 | 0.59 | 154 | 82 | 106 | 56 | 2.03 |
| 22 | 3.76 | 0.93 | 0.04 | 0.78 | 122 | 81 | 80 | 53 | 1.66 |
| 23 | 3.24 | 3.61 | 0.05 | 1.51 | 191 | 99 | 139 | 72 | 2.58 |
| 24 | 6.81 | 1.52 | 0.03 | 0.75 | 258 | 82 | 137 | 43 | 2.68 |
| 25 | 6.93 | 1.33 | 0.05 | 0.97 | 257 | 79 | 148 | 45 | 2.76 |
| 26 | 3.20 | 2.32 | 0.10 | 3.27 | 80 | 89 | 56 | 62 | 1.35 |
| 27 | 5.10 | 0.31 | 0.05 | 0.93 | 492 | 28 | 283 | 16 | 4.61 |
| 28 | 6.57 | 0.23 | 0.05 | 0.73 | 540 | 101 | 305 | 57 | 5.40 |
| 29 | 6.90 | 0.31 | 0.04 | 0.56 | 505 | 89 | 309 | 54 | 5.24 |
| 30 | 4.53 | 0.47 | 0.10 | 3.07 | 275 | 94 | 187 | 64 | 3.29 |
| 31 | 3.92 | 0.50 | 0.17 | 6.57 | 81 | 96 | 58 | 68 | 1.41 |
| 32 | 3.54 | 1.64 | 0.08 | 2.76 | 164 | 76 | 111 | 51 | 2.07 |
| 33 | 3.33 | 2.21 | 0.07 | 2.37 | 122 | 101 | 79 | 65 | 1.77 |
| 34 | 4.02 | 0.88 | 0.05 | 1.72 | 212 | 103 | 113 | 55 | 2.39 |
| 35 | 3.94 | 0.78 | 0.05 | 1.32 | 190 | 111 | 139 | 81 | 2.65 |
| 36 | 6.72 | 0.15 | 0.04 | 0.65 | 501 | 112 | 341 | 76 | 5.65 |
| 37 | 5.69 | 0.30 | 0.06 | 1.39 | 291 | 74 | 183 | 46 | 3.19 |
| 38 | 3.74 | 0.98 | 0.07 | 1.71 | 123 | 147 | 90 | 107 | 2.19 |
| 39 | 4.58 | 0.29 | 0.04 | 0.90 | 241 | 117 | 177 | 86 | 3.24 |
| 40 | 3.93 | 0.46 | 0.04 | 0.78 | 102 | 115 | 70 | 79 | 1.70 |
| 41 | 3.83 | 0.97 | 0.04 | 0.59 | 168 | 101 | 117 | 70 | 2.30 |
| 42 | 4.10 | 0.49 | 0.04 | 0.45 | 157 | 86 | 109 | 59 | 2.09 |
| 43 | 5.58 | 0.41 | 0.08 | 1.68 | 289 | 95 | 207 | 68 | 3.54 |
| 44 | 5.45 | 0.15 | 0.06 | 0.95 | 373 | 125 | 249 | 83 | 4.41 |
| 45 | 4.95 | 0.15 | 0.06 | 0.86 | 356 | 88 | 264 | 65 | 4.27 |
| 46 | 4.47 | 0.18 | 0.04 | 0.74 | 95 | 121 | 62 | 80 | 1.64 |
| 47 | 3.57 | 0.69 | 0.06 | 0.83 | 44 | 98 | 28 | 62 | 0.99 |
| 48 | 4.15 | 0.50 | 0.05 | 0.75 | 165 | 118 | 113 | 81 | 2.35 |
| 49 | 4.22 | 0.87 | 0.03 | 0.77 | 146 | 131 | 72 | 64 | 1.88 |
| 50 | 4.77 | 0.14 | 0.06 | 1.30 | 223 | 116 | 170 | 89 | 3.11 |
| 51 | 4.74 | 0.19 | 0.08 | 2.31 | 198 | 135 | 140 | 95 | 2.84 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 52 | 7.72 | 0.52 | 0.04 | 0.72 | 336 | 142 | 216 | 91 | 4.06 |
| 53 | 3.41 | 4.06 | 0.05 | 0.88 | 106 | 102 | 60 | 58 | 1.51 |
| 54 | 3.79 | 1.13 | 0.04 | 0.67 | 114 | 135 | 76 | 90 | 1.91 |
| 55 | 4.62 | 0.48 | 0.08 | 1.46 | 247 | 117 | 170 | 80 | 3.17 |
| 56 | 4.31 | 0.97 | 0.08 | 1.72 | 205 | 107 | 132 | 69 | 2.60 |
| 57 | 4.80 | 0.24 | 0.04 | 0.47 | 420 | 127 | 251 | 76 | 4.60 |
| 58 | 4.96 | 0.30 | 0.03 | 0.37 | 509 | 93 | 344 | 62 | 5.59 |
| 59 | 4.35 | 0.36 | 0.03 | 0.30 | 308 | 137 | 207 | 92 | 3.85 |
| 60 | 3.74 | 0.75 | 0.12 | 4.09 | 112 | 95 | 74 | 62 | 1.65 |
| 61 | 4.02 | 0.88 | 0.15 | 4.39 | 133 | 91 | 89 | 61 | 1.85 |
| 62 | 7.38 | 0.38 | 0.04 | 0.69 | 345 | 141 | 224 | 92 | 4.16 |
| 63 | 7.05 | 0.30 | 0.06 | 1.02 | 387 | 126 | 228 | 74 | 4.25 |
| 65 | 7.43 | 0.24 | 0.03 | 0.55 | 411 | 142 | 261 | 90 | 4.75 |
| 66 | 7.68 | 0.40 | 0.05 | 0.85 | 272 | 106 | 140 | 54 | 2.88 |
| 67 | 7.69 | 0.37 | 0.05 | 0.79 | 352 | 122 | 215 | 74 | 3.98 |
| 68 | 7.64 | 0.41 | 0.05 | 0.90 | 315 | 94 | 187 | 56 | 3.43 |
| 69 | 7.60 | 0.37 | 0.05 | 0.89 | 389 | 118 | 246 | 75 | 4.39 |
| 70 | 6.98 | 0.45 | 0.09 | 1.40 | 278 | 132 | 185 | 88 | 3.51 |
| 71 | 7.79 | 0.29 | 0.03 | 0.55 | 319 | 108 | 187 | 63 | 3.52 |
| 72 | 4.52 | 0.38 | 0.04 | 0.77 | 224 | 119 | 152 | 80 | 2.93 |
| 73 | 3.78 | 3.11 | 0.04 | 0.56 | 141 | 91 | 86 | 56 | 1.84 |

**Table 5.** Biological properties of soils collected from the rhizoshpere of 73 three-year=old white oak trees at the Rapoca Mine Stie, VA, 2004. ATP values may not be accurate due to lab technique.

| Tree # | ATP (light level) | Dehydrogenase (mg/kg) | Chloroform (mg/kg dissolved organic carbon) |
|---|---|---|---|
| 1 | 1061.272 | 13.52384897 | 26.40244 |
| 2 | 191.3038 | 1.967788974 | 15.13331 |
| 3 | 311.1728 | 2.306978111 | 13.91983 |
| 4 | 56.16683 | 5.859107914 | 11.40708 |
| 5 | 735.7031 | 8.812192318 | 22.62193 |
| 6 | 102.6762 | 0.484978202 | 15.16798 |
| 7 | . | . | . |
| 8 | 393.4756 | 0.844923174 | 6.346947 |
| 9 | 64.44323 | 2.478219518 | 18.06498 |
| 10 | 136.0463 | 2.184621065 | 9.556115 |
| 11 | 15.69802 | 1.267412642 | 3.304391 |
| 12 | 146.2695 | 3.276791432 | 13.31012 |
| 13 | 40.56144 | 2.977737452 | 14.16568 |
| 14 | 53.53874 | 3.938600656 | 11.9071 |
| 15 | 56.17831 | 1.409497711 | 5.989593 |
| 16 | . | . | . |
| 17 | 43.79137 | 0.719104037 | 6.129665 |
| 18 | 24.54409 | 0.822828601 | 10.16102 |
| 19 | 517.6672 | 10.23357222 | 41.92749 |
| 20 | 119.3183 | 1.135041576 | 16.42499 |
| 21 | 789.309 | 1.728718485 | 9.90337 |
| 22 | 184.5925 | 1.192121402 | 26.56991 |
| 23 | 140.1335 | 2.21951738 | 26.27803 |
| 24 | 374.6008 | 9.656486391 | 26.73242 |
| 25 | 450.0399 | 7.515881335 | 30.1006 |
| 26 | 99.77706 | 3.229691601 | . |
| 27 | 152.3117 | 7.650803149 | 43.79864 |
| 28 | 1461.505 | 25.93388419 | 82.50779 |
| 29 | 164.9915 | 35.07717481 | 74.55062 |
| 30 | 228.3536 | 26.86642132 | 92.54574 |
| 31 | 143.2831 | 0.214217117 | 23.61329 |
| 32 | 42.4509 | 0.709601605 | 6.742818 |
| 33 | 102.2454 | 2.192843957 | 15.74179 |
| 34 | 184.7143 | 1.215638827 | 21.59725 |
| 35 | 51.97923 | 2.058984917 | 16.41032 |
| 36 | 998.5638 | 11.01957419 | 49.40072 |
| 37 | 97.43596 | 5.985771842 | 46.08637 |
| 38 | 110.1827 | 0.515170128 | 20.8379 |
| 39 | 21.302 | 1.397146468 | 15.37611 |
| 40 | 596.402 | 5.841938619 | 14.39793 |
| 41 | 275.705 | 1.943282733 | 24.28183 |
| 42 | 1273.873 | 8.96095508 | 57.18278 |
| 43 | 331.6454 | 13.00305035 | 24.30449 |
| 44 | 1654.461 | 23.02318747 | 50.76907 |
| 45 | 783.7943 | 4.423183579 | 18.58477 |
| 46 | 1516.468 | 5.279247185 | 40.96285 |

| | | | |
|---|---|---|---|
| 47 | 206.6004 | 0.641555016 | 13.78549 |
| 48 | 183.1449 | 4.799173295 | 33.8772 |
| 49 | 166.3587 | 4.255680586 | 45.30983 |
| 50 | 494.2269 | 16.90072127 | 43.20188 |
| 51 | 1347.59 | 25.24425093 | 93.47211 |
| 52 | 3084.561 | 32.73871108 | 65.51188 |
| 53 | 25.3336 | 0.245380019 | 6.518083 |
| 54 | 239.3588 | 4.488819516 | 26.72143 |
| 55 | 281.5266 | 3.603794073 | 56.46417 |
| 56 | 76.71327 | 1.044095224 | 15.65795 |
| 57 | 1348.776 | 7.884216403 | 11.37894 |
| 58 | 196.401 | 6.538911984 | 31.6788 |
| 59 | 342.6529 | 3.73866838 | . |
| 60 | 161.1062 | 3.386554063 | 21.35305 |
| 61 | 585.115 | 3.125961731 | 31.31365 |
| 62 | 514.8223 | 17.91425719 | 45.82796 |
| 63 | 169.5967 | 27.9831352 | 60.03857 |
| 65 | 2319.254 | 71.7658874 | 157.9932 |
| 66 | 1588.347 | 22.26953529 | 69.77187 |
| 67 | 200.023 | 10.23785486 | 50.92734 |
| 68 | 377.8449 | 30.11237536 | 106.9157 |
| 69 | 1436.253 | 80.82718787 | 201.7955 |
| 70 | 321.3761 | 18.80176674 | 41.77731 |
| 71 | 5292.04 | 37.42048009 | 101.5849 |
| 72 | 413.5557 | 16.77700244 | 44.70443 |
| 73 | 1456.924 | 30.22383303 | 41.37296 |

**Table 6.** Foliar nutrient levels for 73 three-year-old white oaks located on the Rapoca mine, southwestern VA, 2004.

| | Foliar Nutrient Level (mg $kg^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| Tree | C | N | K | Mn | P | Ca | Mg |
| 1 | . | . | 8.23 | 0.278 | 0.616 | 6.78 | 3.02 |
| 2 | 529.8 | 22.27 | 5.29 | 0.145 | 0.976 | 18.83 | 4.56 |
| 3 | 525.1 | 17.39 | 4.88 | 0.133 | 0.959 | 18.48 | 4.43 |
| 4 | 548.9 | 9.99 | 6.89 | 0.128 | 0.709 | 8.78 | 2.66 |
| 5 | 524.3 | 9.81 | 9.69 | 0.271 | 0.762 | 15.75 | 2.37 |
| 6 | 530.9 | 13.18 | 7.94 | 1.254 | 1.210 | 5.21 | 2.71 |
| 7 | 509.2 | 19.34 | 7.36 | 1.856 | 2.113 | 8.41 | 2.20 |
| 8 | 527.7 | 12.19 | 14.58 | 1.303 | 1.475 | 5.30 | 1.61 |
| 9 | 502.7 | 18.26 | 7.65 | 3.061 | 2.051 | 10.07 | 2.08 |
| 10 | 521.3 | 20.46 | 4.53 | 1.060 | 0.860 | 6.20 | 1.12 |
| 11 | 535.3 | 11.9 | 7.44 | 0.150 | 1.701 | 9.65 | 2.77 |
| 12 | 516.2 | 11.83 | 8.91 | 0.180 | 1.159 | 14.06 | 2.91 |
| 13 | 522.5 | 15.67 | 8.32 | 0.548 | 0.755 | 11.74 | 3.10 |
| 14 | 498 | 9.8 | 9.30 | 0.560 | 0.792 | 21.11 | 3.69 |
| 15 | 532.9 | 8.9 | 8.77 | 0.324 | 0.718 | 10.12 | 2.58 |
| 16 | 538.9 | 23.6 | 10.43 | 2.380 | 1.908 | 6.16 | 0.75 |
| 17 | 527.6 | 20.26 | 9.28 | 3.518 | 1.686 | 6.81 | 0.85 |
| 18 | . | . | 12.55 | 1.657 | 1.201 | 16.32 | 2.16 |
| 19 | 545.4 | 25.76 | 7.47 | 0.337 | 1.494 | 7.03 | 1.70 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 20 | 512.3 | 8.4 | 8.32 | 3.192 | 0.650 | 10.00 | 3.09 |
| 21 | 515.5 | 9.74 | 7.51 | 3.056 | 0.609 | 7.18 | 3.01 |
| 22 | 519.9 | 19.73 | 8.12 | 1.541 | 1.762 | 5.90 | 1.08 |
| 23 | 508.1 | 19.3 | 14.50 | 2.408 | 1.394 | 4.12 | 5.96 |
| 24 | 537.1 | 9.84 | 11.82 | 0.196 | 0.533 | 10.13 | 2.01 |
| 25 | 516.2 | 14.96 | 6.16 | 0.206 | 1.056 | 22.64 | 4.07 |
| 26 | . | . | . | . | . | . | . |
| 27 | 535.3 | 23.16 | 8.07 | 0.860 | 1.190 | 7.10 | 2.26 |
| 28 | 532.1 | 22.91 | 6.70 | 0.202 | 1.533 | 7.62 | 3.03 |
| 29 | 517.8 | 24.6 | 6.04 | 0.881 | 1.906 | 14.70 | 4.02 |
| 30 | . | . | . | . | . | . | . |
| 31 | . | . | . | . | . | . | . |
| 32 | . | . | . | . | . | . | . |
| 33 | . | . | . | . | . | . | . |
| 34 | . | . | . | . | . | . | . |
| 35 | . | . | . | . | . | . | . |
| 36 | 537.7 | 21.29 | 6.43 | 0.488 | 1.493 | 11.01 | 1.92 |
| 37 | 525.6 | 20.58 | 8.06 | 2.385 | 1.404 | 6.89 | 1.07 |
| 38 | 516.9 | 24.67 | 8.68 | 2.642 | 2.401 | 11.41 | 1.95 |
| 39 | 483.8 | 17.22 | 7.04 | 6.690 | 4.783 | 21.32 | 2.29 |
| 40 | 533 | 21.49 | 6.53 | 0.833 | 1.675 | 15.29 | 1.90 |
| 41 | 495 | 19.7 | 6.74 | 3.591 | 1.954 | 9.03 | 1.46 |
| 42 | 531.3 | 27.28 | 7.88 | 5.003 | 2.322 | 8.58 | 1.65 |
| 43 | 536.8 | 26.76 | 8.66 | 0.666 | 1.685 | 8.74 | 1.54 |
| 44 | 540.6 | 26.33 | 7.26 | 2.078 | 1.987 | 8.73 | 2.01 |
| 45 | 534.7 | 26 | 7.03 | 2.631 | 1.956 | 11.42 | 2.52 |
| 46 | 516.1 | 24.47 | 5.54 | 5.592 | 2.246 | 9.28 | 1.72 |
| 47 | 526.7 | 23.73 | 8.24 | 3.608 | 2.648 | 7.95 | 1.05 |
| 48 | 533.9 | 26.31 | 7.74 | 3.384 | 2.229 | 8.11 | 1.68 |
| 49 | 531.1 | 17.31 | 6.89 | 0.738 | 0.736 | 11.38 | 3.68 |
| 50 | 522.7 | 20.98 | 5.50 | 2.995 | 1.377 | 8.62 | 1.42 |
| 51 | 517.9 | 16.73 | 4.44 | 4.655 | 2.139 | 14.11 | 2.17 |
| 52 | 523.6 | 19.46 | 6.55 | 0.400 | 1.124 | 19.46 | 3.46 |
| 53 | 533 | 12.44 | 10.90 | 0.558 | 0.479 | 9.14 | 2.02 |
| 54 | 526.7 | 20.18 | 8.83 | 2.824 | 1.546 | 5.66 | 0.79 |
| 55 | 519.9 | 21.93 | 7.89 | 1.485 | 1.536 | 7.84 | 1.05 |
| 56 | 509.3 | 23.07 | 7.63 | 2.760 | 1.479 | 8.24 | 1.69 |
| 57 | 530.7 | 15.17 | 10.74 | 0.948 | 1.358 | 6.59 | 1.70 |
| 58 | 533.3 | 21.63 | 7.05 | 2.063 | 1.783 | 8.46 | 1.60 |
| 59 | 537 | 25.61 | 8.53 | 2.852 | 1.852 | 6.42 | 1.53 |
| 60 | 525.7 | 21.4 | 3.84 | 1.073 | 0.936 | 4.08 | 1.17 |
| 61 | 525.6 | 24.35 | 8.65 | 1.706 | 1.576 | 9.04 | 1.24 |
| 62 | 521.3 | 19.22 | 5.99 | 0.202 | 1.016 | 22.90 | 4.55 |
| 63 | 540.4 | 21.47 | 7.95 | 0.164 | 1.456 | 8.22 | 2.19 |
| 65 | 530.1 | 19.99 | 11.89 | 0.391 | 1.866 | 8.06 | 1.34 |
| 66 | 541.2 | 20.57 | 6.60 | 0.215 | 1.313 | 6.08 | 2.32 |
| 67 | 550.7 | 20.8 | 4.73 | 0.086 | 0.596 | 11.14 | 3.42 |
| 68 | 538.6 | 21.11 | 10.72 | 0.082 | 1.457 | 7.07 | 2.10 |
| 69 | 547.3 | 21.15 | 6.68 | 0.110 | 1.385 | 9.25 | 2.62 |
| 70 | 556.1 | 18.29 | 4.24 | 0.096 | 1.198 | 10.09 | 3.81 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 71 | 534.5 | 21.22 | 6.45 | 0.146 | 1.317 | 18.05 | 3.66 |
| 72 | 527.1 | 24.14 | 9.48 | 4.099 | 1.933 | 9.80 | 1.88 |
| 73 | 524.9 | 21.7 | 7.45 | 1.655 | 1.700 | 7.18 | 1.69 |

## Greenhouse Layout

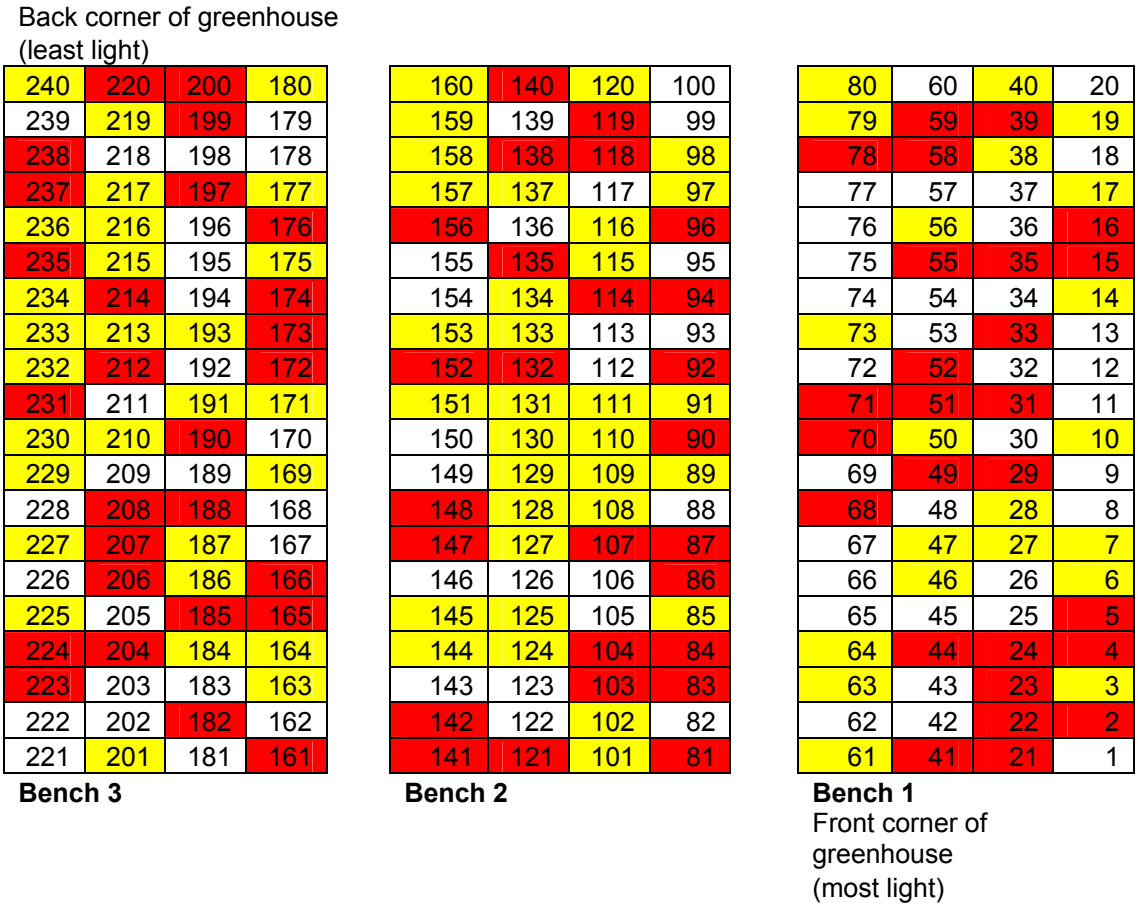



**Figure 1.** Layout of pots by number in the greenhouse. Red represents *Q. rubra*, white *F. americana* and yellow *L. tulipifera*.

## Greenhouse Data

**Table 7.** Growth of three tree species grown on three mine spoils and a control, which were either inoculated or not inoculated with topsoil. A represents *F. americana*, O is *Q. alba*, and P is *L. tulipifera.* C is control, t is BWS, ss is white unweathered sandstone and sh is grey unweathered shale. M is inoculated with topsoil and u is not inoculated.

| Tree # | treat-ment | Original tree parameters | | | Incremental growth | | | Root |
|---|---|---|---|---|---|---|---|---|
| | | Height (cm) | Basal diam (cm) | Stem wt (g) | Height (cm) | Basal diam (cm) | Stem wt (g) | |
| 1 | a t m | 667 | 1.142 | 28.729 | 850 | 1.29 | 4.0476 | 82.6 |
| 2 | o ss m | 492 | 0.701 | 19.355 | 580 | 0.89 | 0.5937 | 33.9 |
| 3 | p c u | 553 | 0.493 | 4.397 | 620 | 0.61 | 0.4949 | 5.37 |
| 4 | o c u | 541 | 0.73 | . | 600 | 0.71 | . | . |
| 5 | o c m | 586 | 0.67 | 9.3257 | 690 | 0.75 | 0.6856 | 27.2 |
| 6 | p c u | 462 | 0.449 | 4.9353 | 670 | 0.64 | 0.9365 | 7.23 |
| 7 | p sh m | 442 | 0.549 | 5.776 | 685 | 0.78 | 1.1739 | 5 |
| 8 | a sh u | 574 | 0.822 | 15.113 | 671 | 1.04 | 0.7268 | 21.8 |
| 9 | a ss m | 585 | 1.352 | 32.199 | 779 | 1.48 | 4.0407 | 62.3 |
| 10 | p ss m | 452 | 0.621 | 4.3907 | 525 | 0.62 | 0.2664 | 6.55 |
| 11 | a ss m | 615 | 0.88 | 18.173 | 780 | 1.05 | 1.8377 | 58.3 |
| 12 | a sh u | 536 | 1.113 | 28.661 | 559 | 1.21 | 2.2003 | 44.1 |
| 13 | a sh u | 492 | 0.659 | 11.3 | 621 | 0.66 | 1.232 | 32.1 |
| 14 | p c m | 571 | 0.592 | 3.6108 | 613 | 0.57 | 0.0764 | 6.54 |
| 15 | o sh m | 501 | 0.817 | 9.3971 | 629 | 0.74 | 0.289 | 10.1 |
| 16 | o ss m | 566 | 0.498 | 9.2122 | 660 | 0.7 | 0.6102 | 23.2 |
| 17 | p c m | 413 | 0.524 | 4.9822 | 669 | 0.62 | 1.0039 | 6.52 |
| 18 | a t u | 590 | 1.012 | 13.367 | 724 | 1.28 | 1.877 | 52.4 |
| 19 | p t u | 613 | 0.648 | 5.6133 | 629 | 0.69 | 0.0137 | 1.45 |
| 20 | a ss m | 547 | 0.945 | 17.504 | 682 | 1.04 | 1.8092 | 49.6 |
| 21 | o t m | 546 | 0.702 | 13.719 | 850 | 0.81 | 2.3097 | 48.1 |
| 22 | o sh m | 469 | 0.779 | 15.108 | 646 | 0.91 | 1.4645 | 34.3 |
| 23 | o t u | 590 | 0.574 | 8.6256 | 811 | 0.72 | 0.8171 | 26.2 |
| 24 | o t u | 412 | 1.085 | 18.558 | 690 | 1.04 | 1.9403 | 57.6 |
| 25 | a t u | 522 | 0.949 | 20.478 | 674 | 1.32 | 2.6746 | 60 |
| 26 | a c u | 513 | 0.649 | 8.5979 | 744 | 0.93 | 1.8112 | 72.2 |
| 27 | p ss u | 468 | 0.501 | 4.756 | 561 | 0.55 | 0.2889 | 4.71 |
| 28 | p c m | 449 | 0.481 | 4.0933 | 545 | 0.6 | 0.3286 | 6.23 |
| 29 | o ss u | 483 | 0.631 | 10.617 | 592 | 0.69 | 1.1858 | 33.2 |
| 30 | a ss u | 553 | 0.905 | 17.244 | 811 | 1.3 | 2.9203 | 64.2 |
| 31 | o c m | 581 | 0.52 | 6.88 | 727 | 0.7 | 0.9468 | 20 |
| 32 | a ss u | 448 | 0.914 | 15.794 | 550 | 1.12 | 3.5444 | 64.8 |
| 33 | o t m | 431 | 1.04 | 12.276 | 600 | 1.06 | 1.0309 | 33.8 |
| 34 | a t u | 542 | 1.32 | 21.613 | 765 | 1.55 | 3.7103 | 69 |
| 35 | o sh u | 518 | 0.529 | 8.1895 | 591 | 0.65 | 0.442 | 29 |
| 36 | a sh m | 570 | 1.089 | 14.23 | 749 | 1.2 | 1.3532 | 38.4 |
| 37 | a t u | 602 | 1.087 | 20.65 | 762 | 1.35 | 1.3768 | 49.1 |
| 38 | p sh m | 512 | 0.579 | 4.0407 | 538 | 0.59 | 0.01 | 1.16 |
| 39 | o c u | 471 | 0.775 | 8.1488 | 616 | 0.92 | 1.0807 | 29.8 |
| 40 | p c m | 622 | 0.464 | 2.8838 | 201 | 0.42 | 0.7565 | 2.02 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 41 | o t u | 571 | 0.915 | 16.798 | 762 | 0.99 | 1.204 | 53.8 |
| 42 | a sh u | 595 | 0.981 | 15.328 | 775 | 1.26 | 1.5411 | 30.6 |
| 43 | a c m | 572 | 1.115 | 19.743 | 725 | 1.48 | 3.1983 | 81.2 |
| 44 | o sh u | 574 | 0.585 | 11.407 | 714 | 0.89 | 0.7923 | 27.7 |
| 45 | a sh m | 616 | 1.178 | 25.921 | 790 | 1.65 | 2.3327 | 63.5 |
| 46 | p sh m | 504 | 0.442 | 4.2058 | 580 | 0.54 | 0.2053 | 3.34 |
| 47 | p ss u | 665 | 0.63 | 6.4059 | 739 | 0.82 | 0.3801 | 7.74 |
| 48 | a sh u | 567 | 1.082 | 22.476 | 738 | 1.61 | 2.6185 | 47.3 |
| 49 | o ss u | 513 | 0.733 | 10.133 | 581 | 0.84 | 0.2836 | 20 |
| 50 | p sh u | 475 | 0.527 | 3.7502 | 529 | 0.63 | 0.1646 | 5.74 |
| 51 | o sh m | 505 | 0.79 | 10.643 | 710 | 0.75 | 0.56 | 24.9 |
| 52 | o ss u | 650 | 0.771 | 17.079 | 831 | 1 | 1.3635 | 32.3 |
| 53 | a t m | 545 | 0.899 | 24.222 | 743 | 1.16 | 3.2184 | 65.5 |
| 54 | a t u | 554 | 1.367 | 23.966 | 831 | 1.75 | 2.3636 | 62.6 |
| 55 | o t m | 526 | 0.599 | 7.306 | 602 | 0.62 | 0.3569 | 20.3 |
| 56 | p sh u | 510 | 0.759 | 8.7969 | 606 | 0.96 | 0.5635 | 13.9 |
| 57 | a ss u | 512 | 0.782 | 12.586 | 603 | 1.07 | 1.4329 | 42.8 |
| 58 | o t m | 630 | 0.785 | 20.472 | 891 | 0.9 | 1.7826 | 35.6 |
| 59 | o ss u | 467 | 0.379 | 7.1265 | 576 | 0.76 | 0.5874 | 17.3 |
| 60 | a sh u | 517 | 0.674 | 10.134 | 583 | 1.14 | 0.4262 | 21.1 |
| 61 | p t m | 531 | 0.639 | 6.319 | 628 | 0.9 | 0.4905 | 9.72 |
| 62 | a c u | 636 | 0.898 | 16.102 | 812 | 1.4 | 3.2726 | 58.6 |
| 63 | p c u | 475 | 0.52 | 4.4657 | 615 | 0.81 | 0.556 | 8.43 |
| 64 | p ss m | 475 | 0.455 | 3.6309 | 494 | 0.52 | 0.1918 | 3.48 |
| 65 | a c m | 730 | 1.34 | 38.431 | 1010 | 1.42 | 4.4928 | 97.6 |
| 66 | a c u | 503 | 0.976 | 19.864 | 775 | 1.3 | 4.8899 | 85.4 |
| 67 | a ss m | 576 | 0.791 | 13.877 | 690 | 0.92 | 1.0915 | 49.7 |
| 68 | o ss u | 546 | 0.582 | 9.346 | 883 | 0.84 | 1.2 | 24.1 |
| 69 | a c m | 541 | 0.689 | 13.767 | 611 | 1.07 | 1.228 | 37.9 |
| 70 | o t m | 535 | 0.749 | 11.563 | 773 | 0.92 | 1.2939 | 40.4 |
| 71 | o sh m | 485 | 0.646 | 7.6884 | 574 | 0.69 | 0.6424 | 27.3 |
| 72 | a c m | 556 | 0.865 | 14.25 | 770 | 1.36 | 2.8638 | 54.9 |
| 73 | p c u | 547 | 0.75 | 7.6707 | 690 | 0.9 | 1.0289 | 7.09 |
| 74 | a t m | 562 | 0.918 | 24.186 | 715 | 1.24 | 2.7208 | 51.3 |
| 75 | a ss u | 585 | 0.889 | 16.836 | 704 | 1.02 | 1.5204 | 42.2 |
| 76 | a t u | 680 | 1.082 | 20.872 | 820 | 1.65 | 2.8864 | 69 |
| 77 | a ss u | 566 | 0.819 | 16.079 | 881 | 1 | 2.9736 | 41.7 |
| 78 | o t u | 606 | 0.846 | 19.789 | 804 | 1.04 | 2.2228 | 45.7 |
| 79 | p ss u | 519 | 0.424 | 3.0656 | 583 | 0.52 | 0.182 | 3.02 |
| 80 | p sh u | 509 | 0.571 | 5.1383 | 564 | 0.73 | 0.2157 | 5.46 |
| 81 | o sh m | 514 | 0.601 | 7.3887 | 606 | 0.55 | 0.8762 | 25.2 |
| 82 | a ss m | 471 | 0.88 | 12.878 | 686 | 1.12 | 2.0901 | 56.1 |
| 83 | o sh m | 555 | 0.905 | 16.677 | 900 | 0.92 | 2.3552 | 39.1 |
| 84 | o c u | 442 | 0.638 | 9.5103 | 716 | 0.86 | 2.9612 | 26.1 |
| 85 | p ss u | 571 | 0.672 | 5.9918 | 594 | 0.76 | 0.1736 | 7.99 |
| 86 | o ss m | 594 | 0.621 | 12.222 | 741 | 0.71 | 1.5706 | 35 |
| 87 | o t m | 595 | 0.857 | 19.701 | 802 | 0.94 | 2.0812 | 46.1 |
| 88 | a t m | 770 | 0.981 | 27.519 | 971 | 1.19 | 4.3599 | 66.1 |
| 89 | p ss m | 431 | 0.619 | 5.913 | 454 | 0.71 | 0.4501 | 10.5 |
| 90 | o ss m | 481 | 0.521 | 8.6059 | 624 | 0.63 | 0.6836 | 18.1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 91 | p t m | 615 | 0.511 | 4.5566 | 621 | 0.71 | 0.148 | 4.17 |
| 92 | o ss m | 671 | 0.689 | 14.271 | 713 | 0.84 | 1.162 | 24.5 |
| 93 | a sh u | 672 | 1.152 | 34.07 | 850 | 1.37 | 3.3133 | 45.6 |
| 94 | o sh u | 571 | 0.561 | 8.4799 | 676 | 0.6 | 0.9173 | 17 |
| 95 | a t m | 645 | 0.8 | 12.638 | 700 | 1.12 | 0.9928 | 34.3 |
| 96 | o t u | 606 | 0.822 | 15.398 | 861 | 0.94 | 1.6567 | 36.7 |
| 97 | p ss m | 475 | 0.451 | 3.4955 | 482 | 0.47 | 0 | 2.85 |
| 98 | p ss u | 562 | 0.617 | 5.7781 | 640 | 0.82 | 0.3431 | 10.2 |
| 99 | a t m | 626 | 1.09 | 23.68 | 803 | 1.44 | 2.5544 | 78 |
| 100 | a ss m | 450 | 0.65 | 8.781 | 560 | 0.76 | 0.986 | 36.7 |
| 101 | p ss m | 440 | 0.533 | 4.0418 | 535 | 0.73 | 0.418 | 7.21 |
| 102 | p c u | 511 | 0.506 | 4.1033 | 561 | 0.52 | 0.2117 | 5.11 |
| 103 | o t m | 534 | 0.632 | 10.014 | 663 | 0.74 | 1.0926 | 27.7 |
| 104 | o ss m | 502 | 0.64 | 11.636 | 671 | 0.78 | 0.9729 | 27.6 |
| 105 | a t u | 506 | 0.78 | 11.668 | 601 | 1.2 | 1.5143 | 58.4 |
| 106 | a sh u | 521 | 0.736 | 11.379 | 659 | 1.3 | 1.6827 | 34.5 |
| 107 | o c u | 616 | 0.472 | 6.6961 | 750 | 0.64 | 1.0224 | 23.4 |
| 108 | p c m | 661 | 0.559 | 6.788 | 842 | 0.64 | 0.7055 | 11.3 |
| 109 | p ss m | 602 | 0.602 | 6.864 | 751 | 0.71 | 0.6713 | 5.45 |
| 110 | p t u | 686 | 0.523 | 5.9442 | 755 | 0.72 | 0.2374 | 8.5 |
| 111 | p t u | 615 | 0.617 | 8.4 | 721 | 0.84 | 0.7418 | 14.6 |
| 112 | a t u | 693 | 0.432 | 35.413 | 910 | 1.94 | 3.8302 | 74.4 |
| 113 | a ss u | 496 | 0.913 | 16.853 | 645 | 0.95 | 1.7071 | 50.3 |
| 114 | o c m | 506 | 0.702 | 9.457 | 764 | 0.72 | 1.7743 | 16.5 |
| 115 | p t m | 536 | 0.611 | 5.6054 | 582 | 0.69 | 0.2074 | 5.74 |
| 116 | p ss m | 475 | 0.574 | 3.8568 | 509 | 0.56 | 0.1637 | 4.32 |
| 117 | a ss m | 532 | 0.93 | . | 724 | 1.11 | 3.3079 | 60.4 |
| 118 | o sh m | 480 | 0.801 | 9.9698 | 648 | 0.83 | 1.0708 | 37.3 |
| 119 | o ss m | 472 | 0.537 | 7.8845 | 773 | 0.62 | 1.2533 | 27.5 |
| 120 | p c m | 586 | 0.625 | 5.2838 | 670 | 0.7 | 0.3501 | 6.74 |
| 121 | o t u | 603 | 0.53 | 7.4802 | 695 | 0.64 | 0.6215 | 24.4 |
| 122 | a sh u | 406 | 0.651 | 6.8528 | 490 | 0.81 | 0.7203 | 28.3 |
| 123 | a ss u | 483 | 0.647 | 9.5907 | 621 | 0.88 | 1.0287 | 31.5 |
| 124 | p sh m | 555 | 0.509 | 5.9812 | 641 | 0.63 | 0.2418 | 7.35 |
| 125 | p sh m | 392 | 0.536 | 5.6071 | 509 | 0.62 | 0.545 | 7.9 |
| 126 | a t m | 623 | 0.99 | 17.652 | 819 | 1.36 | 2.872 | 73.9 |
| 127 | p ss m | 423 | 0.552 | 5.6062 | 591 | 0.67 | 0.8045 | 12 |
| 128 | p sh u | 484 | 0.463 | 3.3026 | 536 | 0.6 | 0.1828 | 4.22 |
| 129 | p sh m | 437 | 0.507 | 4.6475 | 592 | 0.7 | 0.5922 | 6.89 |
| 130 | p ss u | 498 | 0.652 | 7.4288 | 565 | 0.65 | 0.4034 | 11.7 |
| 131 | p ss u | 488 | 0.548 | 6.6207 | 668 | 0.65 | 0.3504 | 8.99 |
| 132 | o c m | 557 | 0.712 | 12.126 | 728 | 0.84 | 0.9532 | 26.8 |
| 133 | p t u | 511 | 0.815 | 7.0473 | 534 | 1.04 | 0.9457 | 10.3 |
| 134 | p sh m | 433 | 0.511 | 4.0615 | 486 | 0.57 | 0.1556 | 3.85 |
| 135 | o sh m | 540 | 0.517 | 8.398 | 673 | 0.66 | 0.724 | 19.8 |
| 136 | a sh u | 468 | 0.784 | 14.087 | 591 | 1.18 | 1.6286 | 38.5 |
| 137 | p ss u | 571 | 0.684 | 9.0175 | 658 | 0.95 | 0.538 | 14.5 |
| 138 | o ss u | 551 | 0.761 | 12.466 | 754 | 1 | 1.1549 | 27.2 |
| 139 | a t m | 642 | 0.744 | 17.955 | 786 | 1.02 | 2.581 | 54.3 |
| 140 | o c m | 582 | 0.698 | 10.834 | 740 | 0.82 | 1.6112 | 41.8 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 141 | o sh u | 620 | 0.546 | 8.6645 | 774 | 0.68 | 0.6304 | 20.9 |
| 142 | o ss u | 562 | 0.69 | 12.591 | 740 | 0.88 | 1.0767 | 42 |
| 143 | a c m | 490 | 0.879 | 14.116 | 581 | 1.09 | 1.9807 | 45.8 |
| 144 | p ss u | 414 | 0.443 | 3.6684 | 474 | 0.65 | 0.2139 | 6.03 |
| 145 | p ss m | 466 | 0.456 | 4.2163 | 507 | 0.54 | 0.2017 | 4.6 |
| 146 | a t m | 501 | 0.982 | 21.904 | 688 | 1.19 | 3.0856 | 59.6 |
| 147 | o c u | 563 | 0.701 | 12.32 | 805 | 0.76 | 1.7033 | 22.3 |
| 148 | o ss m | 582 | 0.648 | 13.498 | 774 | 0.94 | 1.8357 | 53.4 |
| 149 | a c m | 628 | 0.889 | 18.523 | 739 | 1.25 | 2.8524 | 66.6 |
| 150 | a sh m | 449 | 0.65 | 11.397 | 614 | 0.97 | 1.9035 | 28.3 |
| 151 | a sh u | 606 | 0.825 | 17.153 | 659 | 1.3 | 0.4419 | 28.3 |
| 152 | o sh m | 607 | 0.468 | 6.0092 | 715 | 0.55 | 0.8398 | 24.7 |
| 153 | p c m | 433 | 0.497 | 5.6709 | 595 | 0.68 | 0.8846 | 12.8 |
| 154 | a c u | 741 | 1.301 | 33.558 | 1025 | 1.37 | 5.5676 | 93.7 |
| 155 | a t m | 395 | 1.151 | 27.375 | 764 | 0.39 | 2.9907 | 65.8 |
| 156 | o sh u | 526 | 0.673 | 8.1702 | 495 | 0.71 | 0.4059 | 24.4 |
| 157 | p c m | 524 | 0.593 | 6.837 | 620 | 0.7 | 0.5408 | 12.1 |
| 158 | p t u | 602 | 0.672 | 7.9513 | 660 | 0.79 | 0.305 | 7.44 |
| 159 | p c u | 666 | 0.514 | 5.8725 | 733 | 0.65 | 0.2836 | 5.9 |
| 160 | p t u | 608 | 0.52 | 3.76 | 660 | 0.76 | 0.1325 | 2.61 |
| 161 | o c u | 691 | 0.826 | 15.728 | 814 | 1.05 | 1.4132 | . |
| 162 | a sh m | 546 | 0.813 | 13.289 | 714 | 1.03 | 1.5798 | 31.7 |
| 163 | p t u | 473 | 0.551 | 4.5309 | 616 | 0.71 | 0.5121 | 10.2 |
| 164 | p sh u | 526 | 0.611 | 5.2403 | 579 | 0.65 | 0.1357 | 4.89 |
| 165 | o c m | 429 | 0.751 | 10.817 | 691 | 0.83 | 1.734 | 26.4 |
| 166 | o t u | 541 | 0.548 | 5.4679 | 631 | 0.64 | 0.503 | 18.3 |
| 167 | a sh m | 511 | 0.682 | 11.218 | 721 | 0.91 | 1.9113 | 39.4 |
| 168 | a c m | 747 | 0.416 | 36.033 | 1119 | 1.42 | 7.4663 | 112 |
| 169 | p sh m | 459 | 0.566 | 4.1254 | 526 | 0.59 | 0.2482 | 3.63 |
| 170 | a ss u | 564 | 0.819 | 17.585 | 698 | 1.1 | 1.963 | 49.8 |
| 171 | p t m | 529 | 0.543 | 4.7956 | 570 | 0.72 | 0.2091 | 6.21 |
| 172 | o sh u | 504 | 0.826 | 13.303 | 671 | 0.9 | 1.7044 | 43.8 |
| 173 | o ss u | 484 | 0.812 | 19.312 | 715 | 0.96 | 1.4766 | 36.3 |
| 174 | o c u | 519 | 0.634 | 9.1937 | 609 | 0.89 | 0.6027 | 24 |
| 175 | p ss m | 524 | 0.68 | 4.9046 | 578 | 0.61 | 0.2068 | 7.82 |
| 176 | o c m | 559 | 0.511 | 10.58 | 754 | 0.71 | 1.0271 | 34.6 |
| 177 | p t m | 451 | 0.595 | 19.773 | 582 | 0.84 | 0.6631 | 12 |
| 178 | a c u | 550 | 0.877 | 17.48 | 818 | 1.19 | 3.886 | 55.9 |
| 179 | a sh m | 556 | 1.094 | 16.627 | 612 | 1.28 | 1.9196 | 47.3 |
| 180 | p c u | 434 | 0.615 | 7.241 | 694 | 0.92 | 1.7563 | 21.3 |
| 181 | a sh m | 528 | 0.69 | 9.2265 | 691 | 0.84 | 1.4449 | 29 |
| 182 | o t u | 661 | 0.8 | 13.699 | 724 | 0.8 | 0.9308 | 33.6 |
| 183 | a ss u | 568 | 0.898 | 15.926 | 685 | 1.08 | 1.6441 | 44.9 |
| 184 | p t m | 567 | 0.627 | 6.2337 | 636 | 0.72 | 0.3155 | 8.99 |
| 185 | o t m | 490 | 0.685 | 13.42 | 733 | 0.89 | 2.1094 | 26.2 |
| 186 | p c u | 516 | 0.599 | 5.2788 | 671 | 0.6 | 0.6331 | 5.54 |
| 187 | p ss u | 456 | 0.7 | 9.925 | 580 | 0.84 | 0.6753 | 15.7 |
| 188 | o sh m | 516 | 0.719 | 12.907 | 894 | 0.9 | 2.3393 | 48.7 |
| 189 | a c m | 706 | 1.302 | 25.139 | 966 | 1.23 | 4.346 | 103 |
| 190 | o ss m | 551 | 0.671 | 9.7295 | 615 | 0.64 | 0.9196 | 45.1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 191 | p t u | 587 | 0.69 | 7.0258 | 756 | 0.73 | 0.8572 | 10.7 |
| 192 | a c u | 561 | 1.18 | 18.468 | 705 | 1.3 | 3.4798 | 51.8 |
| 193 | p sh u | 609 | 0.58 | 5.583 | 702 | 0.66 | 0.3118 | 7.99 |
| 194 | a t u | 561 | 1.049 | 22.133 | 770 | 1.4 | 3.0971 | 66.5 |
| 195 | a c u | 482 | 1.115 | 24.534 | 844 | 1.63 | 6.0627 | 116 |
| 196 | a c m | 566 | 0.821 | 19.002 | 756 | 1.33 | 3.3239 | 66.7 |
| 197 | o c m | 448 | 0.781 | 7.7156 | 635 | 0.8 | 1.1988 | 20.7 |
| 198 | a sh m | 445 | 0.791 | 14.001 | 556 | 1.14 | 1.5713 | 46.2 |
| 199 | o sh u | 481 | 0.635 | 8.9731 | 591 | 0.78 | 1.1358 | 26.4 |
| 200 | o c m | 580 | 0.514 | 9.724 | 859 | 0.8 | 1.9956 | 31.3 |
| 201 | p sh u | 445 | 0.529 | 4.3321 | 446 | 0.6 | 0.2616 | 3.69 |
| 202 | a ss m | 481 | 0.803 | 14.478 | 660 | 1 | 1.8201 | 40.2 |
| 203 | a ss u | 596 | 0.805 | 21.354 | 782 | 1.19 | 1.7936 | 64.9 |
| 204 | o sh u | 665 | 0.621 | 8.75 | 695 | 0.79 | 0.3771 | 29.1 |
| 205 | a t u | 674 | 0.765 | 19.839 | 770 | 1.03 | 1.4548 | 67.5 |
| 206 | o sh u | 576 | 0.675 | 12.453 | 621 | 0.86 | 0.6515 | 34.5 |
| 207 | o t m | 535 | 0.609 | 11.197 | 731 | 0.77 | 1.3643 | 26.7 |
| 208 | o c u | 598 | 0.718 | 11.19 | 809 | 0.87 | 0.4623 | 16 |
| 209 | a c u | 618 | 0.985 | 20.988 | 726 | 1.2 | 3.1126 | 64.5 |
| 210 | p c u | 567 | 0.848 | 0.6605 | 669 | 0.8 | 7.1943 | 11.8 |
| 211 | a c u | 543 | 1.06 | 24.643 | 912 | 1.33 | 4.4894 | 66.2 |
| 212 | o ss m | 533 | 0.81 | 9.2309 | 523 | 0.72 |  | 8.82 |
| 213 | p c m | 442 | 0.483 | 5.8701 | 582 | 0.66 | 0.7501 | 9.79 |
| 214 | o ss u | 590 | 0.586 | 15.453 | 710 | 0.95 | 1.1238 | 26.3 |
| 215 | p t m | 506 | 0.413 | 3.8848 | 550 | 0.57 | 0.1204 | 4.46 |
| 216 | p t m | 440 | 0.65 | 5.0265 | 479 | 0.74 | 0.3381 | 6.85 |
| 217 | p sh m | 542 | 0.647 | 7.2092 | 631 | 0.98 | 0.4542 | 9.76 |
| 218 | a sh m | 614 | 0.949 | 16.249 | 764 | 1.1 | 2.3684 | 52.7 |
| 219 | p sh u | 553 | 0.55 | 6.7415 | 607 | 0.75 | 0.4657 | 6.26 |
| 220 | o t u | 526 | 0.649 | 11.94 | 709 | 0.89 | 1.1335 | 47.9 |
| 221 | a ss m | 551 | 0.811 | 15.517 | 689 | 0.93 | 0.9908 | 53.1 |
| 222 | a sh m | 519 | 0.992 | 19.6 | 785 | 1.05 | 3.0424 | 46.1 |
| 223 | o c m | 454 | 0.87 | 16.306 | 739 | 1.05 | 2.7528 | 8.18 |
| 224 | o ss u | 524 | 0.475 | 8.7957 | 565 | 0.76 | 0.2577 | 18.6 |
| 225 | p c u | 582 | 0.507 | 4.6062 | 690 | 0.6 | 0.4643 | 4.26 |
| 226 | a ss u | 574 | 0.748 | 14.966 | 744 | 1.07 | 1.1806 | 41.1 |
| 227 | p t m | 472 | 0.576 | 4.5947 | 539 | 0.64 | 0.255 | 4.49 |
| 228 | a c m | 583 | 1.1 | 25.095 | 845 | 1.52 | 3.2232 | 69.5 |
| 229 | p t u | 537 | 0.623 | 4.8597 | 589 | 0.87 | 0.1861 | 5.34 |
| 230 | p sh m | 477 | 0.495 | 4.438 | 514 | 0.62 | 0.0984 | 1.84 |
| 231 | o t m | 536 | 0.809 | 16.636 | 763 | 1 | 2.6657 | 57.5 |
| 232 | p t m | 575 | 0.648 | 9.4208 | 646 | 0.78 | 0.4385 | 13.4 |
| 233 | p c m | 551 | 0.412 | 5.9215 | 669 | 0.62 | 0.5253 | . |
| 234 | p sh u | 415 | 0.8 | 7.1506 | 556 | 0.81 | 0.7643 | 8.56 |
| 235 | o sh m | 641 | 0.785 | 11.927 | 670 | 0.8 | 0.6424 | 20.8 |
| 236 | p t u | 618 | 0.512 | 6.5768 | 739 | 0.69 | 0.4355 | 10.9 |
| 237 | o c u | 612 | 0.787 | 13.777 | 736 | 0.9 | 1.1584 | 39.4 |
| 238 | o t u | 606 | 0.783 | 16.585 | 900 | 1 | 2.6147 | 70 |
| 239 | a c u | 570 | 0.837 | 14.2 | 632 | 1.15 | 1.1272 | 42.3 |
| 240 | p sh m | 420 | 0.532 | 4.4596 | 480 | 0.62 | 0.2691 | 4.37 |

**Table 8.** Foliar nutrient levels of three tree species grown on 3 mine spoils and a control, which were either inoculated or not inoculated with topsoil. A represents *F. americana*, O is *Q. alba*, and P is *L. tulipifera.* C is control, t is BWS, ss is white sandstone and sh is shale. M is inoculated with topsoil and u is not inoculated.

| | | Foliar Nutrient Levels (mg $kg^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| Treatment | rep | N | P | K | Ca | Mg |
| acm | 1 | 16.82 | 0.751 | 5.54 | 13.14 | 3.52 |
| acm | 2 | 19.09 | 1.014 | 7.37 | 13.67 | 3.94 |
| acm | 3 | 18.19 | 0.992 | 6.69 | 16.72 | 4.38 |
| acm | 4 | 15.79 | 0.794 | 7.39 | 10.71 | 2.65 |
| acu | 1 | 16.6 | 0.813 | 5.69 | 12.79 | 3.49 |
| acu | 2 | 15.38 | 0.887 | 7.82 | 11.10 | 2.67 |
| acu | 3 | 18.81 | 1.012 | 6.86 | 11.42 | 3.46 |
| acu | 4 | 16.71 | 0.978 | 6.13 | 12.86 | 3.14 |
| ashm | 1 | 15.23 | 0.990 | 7.74 | 12.33 | 5.02 |
| ashm | 2 | 16.29 | 1.038 | 8.14 | 10.88 | 4.84 |
| ashm | 3 | 14.96 | 1.092 | 10.89 | 11.88 | 3.98 |
| ashm | 4 | 17.49 | 1.222 | 9.21 | 10.21 | 3.97 |
| ashu | 1 | 16.92 | 1.220 | 13.22 | 14.48 | 6.09 |
| ashu | 2 | 16.92 | 1.192 | 10.48 | 10.48 | 3.84 |
| ashu | 3 | 17.78 | 0.819 | 9.14 | 8.35 | 3.85 |
| ashu | 4 | 15.83 | 1.030 | 12.78 | 9.62 | 4.11 |
| assm | 1 | 12.64 | 0.939 | 6.52 | 11.10 | 4.55 |
| assm | 2 | 14.14 | 0.927 | 7.18 | 11.21 | 4.10 |
| assm | 3 | 13.96 | 0.972 | 6.53 | 11.02 | 4.99 |
| assm | 4 | 13.94 | 0.936 | 6.00 | 9.14 | 3.69 |
| assu | 1 | 12.33 | 0.872 | 8.43 | 10.48 | 4.17 |
| assu | 2 | 16.21 | 1.157 | 7.27 | 10.98 | 3.68 |
| assu | 3 | 17.26 | 1.055 | 7.27 | 10.21 | 4.38 |
| assu | 4 | 14.33 | 0.851 | 5.50 | 12.30 | 4.93 |
| atm | 1 | 17.65 | 1.181 | 6.87 | 7.88 | 3.28 |
| atm | 2 | 15.39 | 1.082 | 7.59 | 7.77 | 3.84 |
| atm | 3 | 15.7 | 1.107 | 5.84 | 9.41 | 4.42 |
| atm | 4 | 17.18 | 1.337 | 6.53 | 11.14 | 5.30 |
| atu | 1 | 15.06 | 1.009 | 8.38 | 6.65 | 4.46 |
| atu | 2 | 13.27 | 1.088 | 6.10 | 8.80 | 4.38 |
| atu | 3 | 16.79 | 1.053 | 6.75 | 7.65 | 3.25 |
| atu | 4 | 18.67 | 1.102 | 9.26 | 6.63 | 2.97 |
| ocm | 1 | 19.35 | 1.071 | 6.16 | 7.82 | 3.50 |
| ocm | 2 | 17.59 | 1.270 | 6.65 | 6.20 | 3.87 |
| ocm | 3 | 18.56 | 1.962 | 9.18 | 4.44 | 3.68 |
| ocm | 4 | 17.72 | 1.042 | 6.24 | 10.09 | 3.85 |
| ocu | 1 | 17.4 | 0.972 | 5.35 | 7.80 | 3.05 |
| ocu | 2 | 19.53 | 0.986 | 7.05 | 7.22 | 2.93 |
| ocu | 3 | 20.65 | 1.192 | 7.38 | 8.80 | 3.52 |
| ocu | 4 | 24.37 | 1.342 | 6.82 | 8.05 | 3.90 |
| oshm | 1 | 17.74 | 1.046 | 7.20 | 8.63 | 4.79 |
| oshm | 2 | 15.95 | 1.257 | 6.35 | 8.89 | 4.78 |
| oshm | 3 | 15.05 | 1.070 | 6.75 | 11.41 | 4.46 |
| oshm | 4 | 20.44 | 1.165 | 7.41 | 9.94 | 4.91 |
| oshu | 1 | 16.63 | 0.928 | 5.38 | 7.89 | 4.67 |

| | | | | | | |
|---|---|---|---|---|---|---|
| oshu | 2 | 18.69 | 1.045 | 6.06 | 10.52 | 4.79 |
| oshu | 3 | 14.18 | 0.940 | 5.68 | 8.89 | 4.53 |
| oshu | 4 | 14.97 | 0.967 | 6.34 | 11.43 | 6.21 |
| ossm | 1 | 16.98 | 0.919 | 5.62 | 8.09 | 4.24 |
| ossm | 2 | 18.39 | 1.091 | 6.47 | 7.87 | 5.05 |
| ossm | 3 | 11.89 | 0.744 | 6.22 | 8.94 | 4.20 |
| ossm | 4 | 17.98 | 1.163 | 6.33 | 9.25 | 4.69 |
| ossu | 1 | 13.6 | 0.794 | 6.16 | 9.82 | 5.61 |
| ossu | 2 | 17.49 | 0.968 | 5.65 | 11.26 | 4.96 |
| ossu | 3 | 16.01 | 0.809 | 4.95 | 8.13 | 3.89 |
| ossu | 4 | 18.33 | 0.924 | 5.82 | 9.56 | 5.00 |
| otm | 1 | 14.06 | 0.789 | 5.57 | 6.70 | 4.88 |
| otm | 2 | 15.71 | 0.956 | 6.45 | 7.37 | 5.15 |
| otm | 3 | 19.96 | 1.048 | 5.40 | 13.16 | 5.18 |
| otm | 4 | 13.74 | 0.972 | 7.50 | 7.49 | 4.58 |
| otu | 1 | 16.73 | 0.932 | 5.66 | 7.93 | 5.61 |
| otu | 2 | 13.02 | 0.927 | 6.71 | 9.11 | 5.81 |
| otu | 3 | 15.52 | 0.880 | 7.78 | 8.01 | 4.22 |
| otu | 4 | 16.37 | 0.898 | 6.43 | 6.57 | 4.11 |
| pcm | 1 | 16.58 | 1.705 | 11.68 | 15.45 | 3.38 |
| pcm | 2 | 16.24 | 0.623 | 7.28 | 11.71 | 3.86 |
| pcm | 3 | 13.94 | 0.643 | 6.42 | 11.71 | 4.08 |
| pcm | 4 | 14.89 | 0.710 | 6.40 | 13.03 | 2.88 |
| pcu | 1 | 17.23 | 0.752 | 8.03 | 12.23 | 3.18 |
| pcu | 2 | 15.85 | 0.672 | 7.99 | 10.46 | 3.41 |
| pcu | 3 | 15.99 | 0.752 | 3.26 | 14.08 | 3.86 |
| pcu | 4 | 17.73 | 0.901 | 9.49 | 13.61 | 3.86 |
| pshm | 1 | 15.94 | 0.505 | 4.49 | 13.68 | 5.09 |
| pshm | 2 | 9.88 | 1.225 | 7.21 | 20.29 | 8.03 |
| pshm | 3 | 11.93 | 0.684 | 7.43 | 19.50 | 7.24 |
| pshm | 4 | 10.54 | 0.624 | 6.49 | 22.07 | 7.38 |
| pshu | 1 | 12.75 | 0.755 | 4.14 | 19.98 | 8.43 |
| pshu | 2 | 17.67 | 0.990 | 7.08 | 12.46 | 5.27 |
| pshu | 3 | 11.42 | 1.160 | 7.49 | 17.89 | 7.98 |
| pshu | 4 | 13.79 | 0.769 | 5.97 | 16.49 | 7.59 |
| pssm | 1 | 10.36 | 0.645 | 4.82 | 16.23 | 6.37 |
| pssm | 2 | 11.08 | 0.719 | 5.65 | 15.19 | 5.71 |
| pssm | 3 | 11.26 | 0.696 | 5.05 | 20.34 | 6.97 |
| pssm | 4 | 10.82 | 0.829 | 6.37 | 16.54 | 7.91 |
| pssu | 1 | 9.32 | 0.579 | 3.28 | 21.20 | 7.44 |
| pssu | 2 | 9.83 | 0.685 | 2.96 | 21.81 | 8.74 |
| pssu | 3 | 8.66 | 0.666 | 4.03 | 19.70 | 7.31 |
| pssu | 4 | 8.41 | 0.597 | 3.05 | 17.08 | 6.61 |
| ptm | 1 | 16.62 | 1.050 | 4.11 | 9.55 | 3.20 |
| ptm | 2 | 12.17 | 0.902 | 5.11 | 9.58 | 4.03 |
| ptm | 3 | 13.61 | 1.075 | 6.59 | 13.73 | 3.36 |
| ptm | 4 | 14.93 | 1.226 | 6.46 | 12.25 | 4.19 |
| ptu | 1 | 12.48 | 1.007 | 8.45 | 12.33 | 5.20 |
| ptu | 2 | 9.59 | 1.043 | 5.38 | 14.42 | 5.67 |
| ptu | 3 | 12.35 | 0.846 | 5.50 | 10.07 | 7.06 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ptu | 4 | 8.42 | 0.700 | 4.88 | 9.64 | 7.01 |

**Table 9.** Physical properties of three mine spoils and a control, inoculated or not inoculated with topsoil across three tree species. A represents *F. americana*, O is *Q. alba*, and P is *L. tulipifera.* C is control, t is BWS, ss is white sandstone and sh is shale. M is inoculated with topsoil and u is not inoculated.

| Trt-ment | rep | % Fines | % Sand | % Silt | % Clay | % Silt + clay | Wilting pt |
|---|---|---|---|---|---|---|---|
| acm | 1 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| acm | 2 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| acm | 3 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| acm | 4 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| acu | 1 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| acu | 2 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| acu | 3 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| acu | 4 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| ashm | 1 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| ashm | 2 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| ashm | 3 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| ashm | 4 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| ashu | 1 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| ashu | 2 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| ashu | 3 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| ashu | 4 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| assm | 1 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| assm | 2 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| assm | 3 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| assm | 4 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| assu | 1 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| assu | 2 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| assu | 3 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| assu | 4 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| atm | 1 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| atm | 2 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| atm | 3 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| atm | 4 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| atu | 1 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| atu | 2 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| atu | 3 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| atu | 4 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| ocm | 1 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| ocm | 2 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| ocm | 3 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| ocm | 4 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| ocu | 1 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| ocu | 2 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| ocu | 3 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| ocu | 4 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| oshm | 1 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| oshm | 2 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| oshm | 3 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| oshm | 4 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| oshu | 1 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| oshu | 2 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| oshu | 3 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| oshu | 4 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| ossm | 1 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| ossm | 2 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| ossm | 3 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| ossm | 4 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| ossu | 1 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| ossu | 2 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| ossu | 3 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| ossu | 4 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| otm | 1 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| otm | 2 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| otm | 3 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| otm | 4 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| otu | 1 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| otu | 2 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| otu | 3 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| otu | 4 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| pcm | 1 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| pcm | 2 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| pcm | 3 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| pcm | 4 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| pcu | 1 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| pcu | 2 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| pcu | 3 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| pcu | 4 | 0.54 | 52.18 | 38.17 | 9.65 | 47.82 | 9.51 |
| pshm | 1 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| pshm | 2 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| pshm | 3 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| pshm | 4 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| pshu | 1 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| pshu | 2 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| pshu | 3 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| pshu | 4 | 0.37 | 55.16 | 22.91 | 21.92 | 44.84 | 4.80 |
| pssm | 1 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| pssm | 2 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| pssm | 3 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| pssm | 4 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| pssu | 1 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| pssu | 2 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| pssu | 3 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| pssu | 4 | 0.42 | 78.96 | 13.04 | 8.00 | 21.04 | 1.30 |
| ptm | 1 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| ptm | 2 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| ptm | 3 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| ptm | 4 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| ptu | 1 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ptu | 2 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| ptu | 3 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |
| ptu | 4 | 0.62 | 66.85 | 17.93 | 15.23 | 33.15 | 3.66 |

**Table 10.** Chemical properties of three mine spoils and a control, inoculated or not inoculated with topsoil across three tree species. A represents *F. americana*, O is *Q. alba*, and P is *L. tulipifera*. C is control, t is BWS, ss is white sandstone and sh is shale. M is inoculated with topsoil and u is not inoculated.

| Trt-ment | Rep | ph | EC | Melich I extractable nutrients (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | P | K | Ca | Mg | Zn | Mn | Cu | Fe | B |
| acm | 1 | 4.96 | 158.25 | 1.07 | 9.10 | 166.99 | 25.69 | 1.39 | 13.27 | 0.27 | 14.24 | 0.11 |
| acm | 2 | 5.20 | 189.34 | 1.07 | 10.70 | 152.00 | 25.15 | 1.39 | 12.90 | 0.16 | 9.42 | 0.11 |
| acm | 3 | 4.83 | 163.28 | 1.07 | 10.17 | 139.69 | 23.01 | 1.50 | 13.92 | 0.16 | 9.53 | 0.11 |
| acm | 4 | 5.20 | 345.84 | 1.07 | 10.70 | 176.08 | 27.30 | 1.98 | 15.90 | 0.37 | 9.10 | 0.11 |
| acu | 1 | 5.09 | 160.95 | 1.07 | 11.77 | 152.54 | 24.62 | 1.39 | 13.22 | 0.16 | 9.21 | 0.11 |
| acu | 2 | 5.22 | 207.01 | 1.07 | 9.63 | 144.51 | 22.48 | 1.34 | 11.45 | 0.21 | 8.99 | 0.11 |
| acu | 3 | 5.22 | 135.82 | 1.07 | 8.03 | 132.73 | 20.87 | 1.23 | 11.56 | 0.16 | 9.10 | 0.11 |
| acu | 4 | 5.11 | 239.27 | 1.07 | 9.63 | 149.32 | 24.08 | 1.28 | 12.95 | 0.21 | 9.47 | 0.11 |
| ashm | 1 | 7.43 | 576.27 | 10.82 | 20.90 | 342.61 | 84.72 | 1.31 | 26.80 | 0.67 | 44.08 | 0.07 |
| ashm | 2 | 7.52 | 591.13 | 11.20 | 20.15 | 343.73 | 85.09 | 1.31 | 25.64 | 0.71 | 42.06 | 0.07 |
| ashm | 3 | 7.57 | 699.38 | 11.57 | 25.75 | 370.60 | 95.54 | 2.05 | 30.60 | 0.75 | 53.41 | 0.07 |
| ashm | 4 | 7.49 | 735.37 | 11.57 | 24.63 | 374.71 | 94.05 | 1.46 | 27.62 | 0.75 | 48.33 | 0.07 |
| ashu | 1 | . | . | . | . | . | . | . | . | . | . | . |
| ashu | 2 | 8.33 | 648.02 | 14.18 | 22.77 | 400.08 | 94.80 | 1.46 | 19.78 | 0.97 | 66.25 | 0.07 |
| ashu | 3 | 8.32 | 695.36 | 14.93 | 23.14 | 399.34 | 98.53 | 1.87 | 21.12 | 1.08 | 70.16 | 0.07 |
| ashu | 4 | 7.99 | 1017.83 | 13.81 | 21.27 | 397.85 | 91.81 | 1.49 | 20.86 | 0.93 | 73.90 | 0.07 |
| assm | 1 | 7.83 | 374.18 | 10.18 | 7.64 | 616.19 | 127.74 | 1.06 | 33.10 | 0.08 | 234.81 | 0.04 |
| assm | 2 | 7.80 | 239.07 | 10.61 | 6.79 | 602.61 | 123.49 | 1.10 | 30.98 | 0.08 | 233.96 | 0.04 |
| assm | 3 | 8.19 | 374.19 | 10.61 | 6.79 | 592.43 | 120.52 | 1.02 | 32.72 | 0.13 | 227.38 | 0.04 |
| assm | 4 | 7.96 | 313.37 | 10.61 | 7.21 | 627.65 | 128.59 | 1.10 | 32.80 | 0.13 | 245.71 | 0.04 |
| assu | 1 | 8.81 | 418.80 | 12.73 | 4.67 | 586.48 | 117.55 | 1.02 | 16.47 | 0.17 | 258.57 | 0.04 |
| assu | 2 | 8.67 | 364.50 | 12.73 | 5.52 | 638.26 | 128.59 | 1.06 | 17.70 | 0.17 | 277.16 | 0.04 |
| assu | 3 | 8.81 | 299.43 | 12.73 | 5.94 | 586.06 | 117.55 | 1.02 | 16.00 | 0.21 | 257.17 | 0.04 |
| assu | 4 | 8.69 | 291.96 | 13.16 | 5.52 | 626.38 | 130.71 | 1.06 | 17.44 | 0.17 | 272.66 | 0.04 |
| atm | 1 | 5.39 | 109.90 | 1.86 | 19.84 | 99.84 | 65.73 | 0.43 | 4.40 | 0.25 | 8.74 | 0.06 |
| atm | 2 | 5.46 | 129.07 | 1.86 | 17.98 | 112.24 | 63.25 | 0.43 | 4.40 | 0.19 | 8.81 | 0.06 |
| atm | 3 | 5.33 | 82.10 | 1.86 | 11.78 | 93.01 | 42.17 | 0.43 | 4.59 | 0.37 | 8.99 | 0.06 |
| atm | 4 | 5.53 | 129.84 | 1.24 | 16.74 | 104.80 | 61.39 | 0.37 | 4.03 | 0.19 | 8.00 | 0.06 |
| atu | 1 | 5.45 | 109.36 | 1.86 | 16.74 | 77.51 | 54.57 | 0.25 | 2.36 | 0.31 | 6.51 | 0.06 |
| atu | 2 | 5.53 | 256.15 | 1.24 | 16.12 | 81.85 | 55.19 | 0.25 | 2.85 | 0.31 | 7.44 | 0.06 |
| atu | 3 | 5.37 | 112.04 | 1.86 | 20.46 | 86.81 | 65.73 | 0.37 | 3.10 | 0.81 | 7.81 | 0.06 |
| atu | 4 | 5.49 | 158.06 | 1.86 | 17.98 | 86.81 | 60.15 | 0.31 | 2.73 | 0.25 | 7.38 | 0.06 |
| ocm | 1 | 5.24 | 220.72 | 1.07 | 14.45 | 165.92 | 29.44 | 1.50 | 14.18 | 0.21 | 10.22 | 0.11 |
| ocm | 2 | 5.21 | 431.11 | 1.07 | 14.99 | 150.39 | 25.69 | 1.39 | 13.27 | 0.21 | 9.69 | 0.11 |
| ocm | 3 | 5.28 | 194.68 | 1.07 | 17.13 | 160.03 | 28.90 | 1.55 | 14.08 | 0.27 | 9.79 | 0.11 |
| ocm | 4 | 5.14 | 280.14 | 1.07 | 19.80 | 167.52 | 29.44 | 1.61 | 16.48 | 0.21 | 10.76 | 0.11 |
| ocu | 1 | 5.27 | 265.42 | 1.07 | 17.13 | 171.27 | 29.44 | 1.55 | 13.81 | 0.21 | 9.37 | 0.11 |
| ocu | 2 | 5.17 | 310.65 | 1.07 | 20.34 | 170.73 | 30.51 | 1.61 | 14.99 | 0.21 | 10.86 | 0.11 |
| ocu | 3 | 5.21 | 235.56 | 1.07 | 19.27 | 162.70 | 30.51 | 1.55 | 13.92 | 0.21 | 9.58 | 0.11 |
| ocu | 4 | 5.26 | 336.11 | 1.07 | 16.06 | 188.39 | 34.79 | 1.71 | 15.04 | 0.21 | 9.26 | 0.16 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| oshm | 1 | 6.22 | 245.51 | 6.72 | 21.27 | 290.36 | 67.92 | 1.27 | 21.76 | 0.45 | 27.99 | 0.07 |
| oshm | 2 | 7.67 | 448.97 | 11.57 | 25.75 | 400.83 | 93.68 | 1.60 | 29.75 | 0.78 | 52.59 | 0.07 |
| oshm | 3 | 7.54 | 556.66 | 11.20 | 26.50 | 387.40 | 94.42 | 1.57 | 29.30 | 0.78 | 51.84 | 0.07 |
| oshm | 4 | 7.84 | 516.36 | 11.57 | 24.26 | 386.28 | 91.44 | 1.49 | 28.18 | 0.75 | 51.80 | 0.07 |
| oshu | 1 | 8.35 | 359.46 | 13.81 | 25.01 | 385.53 | 95.54 | 1.60 | 19.52 | 0.97 | 74.16 | 0.07 |
| oshu | 2 | 8.38 | 616.65 | 14.18 | 23.89 | 409.04 | 93.68 | 1.57 | 19.56 | 0.97 | 70.28 | 0.07 |
| oshu | 3 | 8.57 | 626.24 | 14.56 | 24.26 | 398.97 | 92.18 | 1.75 | 19.82 | 0.97 | 71.66 | 0.07 |
| oshu | 4 | 8.38 | 583.93 | 14.93 | 26.12 | 419.49 | 98.53 | 1.57 | 20.56 | 1.01 | 74.75 | 0.07 |
| ossm | 1 | 7.99 | 758.86 | 10.61 | 8.06 | 568.24 | 117.98 | 1.06 | 31.06 | 0.08 | 216.22 | 0.04 |
| ossm | 2 | 8.35 | 212.71 | 11.03 | 8.91 | 597.94 | 128.16 | 1.15 | 30.51 | 0.13 | 231.88 | 0.08 |
| ossm | 3 | 8.32 | 316.51 | 11.46 | 7.64 | 564.42 | 116.70 | 1.10 | 29.62 | 0.13 | 217.92 | 0.04 |
| ossm | 4 | 8.36 | 226.85 | 10.61 | 7.64 | 585.21 | 118.82 | 1.10 | 31.15 | 0.08 | 225.26 | 0.04 |
| ossu | 1 | 8.76 | 233.62 | 13.58 | 7.64 | 593.27 | 122.22 | 1.10 | 17.61 | 0.17 | 256.58 | 0.04 |
| ossu | 2 | 9.01 | 302.46 | 13.58 | 7.64 | 624.68 | 124.77 | 1.15 | 17.23 | 0.17 | 273.51 | 0.04 |
| ossu | 3 | 8.89 | 255.28 | 14.00 | 7.64 | 609.83 | 119.25 | 1.02 | 17.53 | 0.25 | 264.94 | 0.04 |
| ossu | 4 | 8.96 | 238.16 | 13.58 | 7.64 | 639.11 | 129.43 | 1.15 | 17.70 | 0.17 | 277.12 | 0.04 |
| otm | 1 | 5.66 | 255.91 | 1.24 | 21.08 | 97.36 | 63.25 | 0.37 | 3.72 | 0.31 | 7.26 | 0.06 |
| otm | 2 | 5.57 | 104.27 | 1.24 | 22.32 | 106.66 | 71.31 | 0.43 | 4.34 | 0.37 | 8.31 | 0.06 |
| otm | 3 | 5.64 | 99.75 | 1.24 | 22.32 | 91.77 | 70.69 | 0.37 | 3.72 | 0.37 | 7.88 | 0.06 |
| otm | 4 | 5.69 | 149.58 | 1.24 | 23.56 | 102.32 | 73.17 | 0.37 | 4.22 | 0.31 | 8.50 | 0.06 |
| otu | 1 | 5.33 | 95.52 | 1.86 | 23.56 | 87.43 | 73.79 | 0.31 | 3.84 | 0.12 | 8.43 | 0.06 |
| otu | 2 | 5.68 | 88.46 | 1.86 | 22.94 | 83.71 | 62.01 | 0.31 | 4.34 | 0.25 | 6.95 | 0.06 |
| otu | 3 | 5.51 | 110.11 | 1.86 | 24.80 | 89.91 | 71.31 | 0.31 | 3.10 | 0.25 | 9.55 | 0.06 |
| otu | 4 | 5.74 | 124.03 | 1.24 | 22.94 | 86.81 | 68.83 | 0.31 | 2.73 | 0.25 | 7.01 | 0.06 |
| pcm | 1 | 5.34 | 109.41 | 1.07 | 13.38 | 170.20 | 28.37 | 1.28 | 12.42 | 0.21 | 10.76 | 0.11 |
| pcm | 2 | 5.23 | 203.57 | 1.07 | 15.52 | 169.13 | 28.37 | 1.50 | 13.06 | 0.21 | 9.58 | 0.11 |
| pcm | 3 | 5.37 | 382.60 | 1.07 | 14.45 | 166.45 | 27.30 | 1.34 | 12.15 | 0.21 | 9.05 | 0.11 |
| pcm | 4 | 5.26 | 238.77 | 1.07 | 14.45 | 163.77 | 26.76 | 1.45 | 13.49 | 0.21 | 9.85 | 0.11 |
| pcu | 1 | 5.31 | 199.90 | 1.07 | 12.85 | 165.92 | 28.37 | 1.34 | 12.95 | 0.21 | 10.76 | 0.11 |
| pcu | 2 | 5.31 | 203.85 | 1.07 | 12.31 | 161.63 | 28.37 | 1.34 | 11.94 | 0.21 | 8.78 | 0.11 |
| pcu | 3 | 5.33 | 234.08 | 1.07 | 13.92 | 153.61 | 26.23 | 1.34 | 11.72 | 0.16 | 9.05 | 0.11 |
| pcu | 4 | 5.27 | 216.28 | 1.07 | 17.66 | 158.96 | 27.30 | 1.39 | 13.27 | 0.27 | 9.63 | 0.11 |
| pshm | 1 | 7.94 | 616.34 | 12.69 | 23.89 | 372.47 | 92.56 | 1.53 | 26.24 | 0.82 | 55.94 | 0.07 |
| pshm | 2 | 7.90 | 381.36 | 10.82 | 22.39 | 368.73 | 83.97 | 1.38 | 24.52 | 0.67 | 39.60 | 0.07 |
| pshm | 3 | 8.00 | 373.40 | 10.82 | 22.77 | 365.00 | 87.71 | 1.42 | 24.89 | 0.67 | 41.76 | 0.07 |
| pshm | 4 | 7.86 | 354.77 | 11.20 | 22.77 | 382.17 | 88.45 | 1.42 | 25.75 | 0.71 | 43.07 | 0.07 |
| pshu | 1 | 8.32 | 827.69 | 14.56 | 23.89 | 419.12 | 97.41 | 1.64 | 21.46 | 1.01 | 71.02 | 0.07 |
| pshu | 2 | 8.47 | 516.61 | 14.18 | 24.63 | 448.98 | 94.42 | 1.57 | 20.30 | 1.01 | 69.16 | 0.07 |
| pshu | 3 | 8.59 | 514.48 | 14.93 | 24.26 | 409.42 | 96.29 | 1.64 | 20.15 | 1.04 | 74.23 | 0.07 |
| pshu | 4 | 8.55 | 373.43 | 14.18 | 22.02 | 401.58 | 88.08 | 1.49 | 19.07 | 0.90 | 65.69 | 0.07 |
| pssm | 1 | 8.17 | 384.53 | 11.03 | 9.34 | 589.46 | 123.07 | 0.98 | 29.66 | 0.13 | 222.37 | 0.04 |
| pssm | 2 | 8.38 | 264.74 | 11.46 | 8.49 | 567.39 | 117.55 | 1.02 | 29.54 | 0.13 | 213.29 | 0.04 |
| pssm | 3 | 8.30 | 231.91 | 11.46 | 7.64 | 578.85 | 117.98 | 1.06 | 29.66 | 0.13 | 223.77 | 0.04 |
| pssm | 4 | 8.39 | 272.77 | 11.46 | 8.49 | 622.56 | 131.13 | 1.15 | 31.32 | 0.17 | 237.73 | 0.04 |
| pssu | 1 | 8.89 | 264.51 | 13.58 | 7.21 | 530.04 | 103.12 | 0.93 | 14.85 | 0.13 | 223.48 | 0.04 |
| pssu | 2 | 9.06 | 288.76 | 14.00 | 8.49 | 650.99 | 127.74 | 1.40 | 18.04 | 0.34 | 273.59 | 0.04 |
| pssu | 3 | 8.96 | 406.82 | 14.00 | 6.37 | 614.07 | 119.67 | 1.06 | 17.19 | 0.17 | 256.32 | 0.04 |
| pssu | 4 | 8.85 | 271.05 | 13.58 | 7.21 | 603.88 | 120.10 | 1.10 | 17.02 | 0.21 | 254.79 | 0.04 |
| ptm | 1 | 5.93 | 84.96 | 1.24 | 17.98 | 145.72 | 53.33 | 0.56 | 8.93 | 0.25 | 14.39 | 0.06 |
| ptm | 2 | 5.58 | 89.03 | 1.86 | 17.36 | 107.28 | 50.23 | 0.43 | 4.03 | 0.25 | 8.00 | 0.06 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ptm | 3 | 5.66 | 174.41 | 1.24 | 21.70 | 117.82 | 62.01 | 0.43 | 5.02 | 0.25 | 9.74 | 0.06 |
| ptm | 4 | 5.63 | 162.94 | 1.24 | 17.36 | 96.74 | 50.85 | 0.37 | 3.84 | 0.31 | 8.31 | 0.06 |
| ptu | 1 | 5.46 | 79.51 | 1.86 | 22.32 | 81.23 | 58.91 | 0.25 | 2.79 | 0.31 | 8.81 | 0.06 |
| ptu | 2 | 5.60 | 99.91 | 1.86 | 21.08 | 78.13 | 53.33 | 0.25 | 2.11 | 0.25 | 6.70 | 0.06 |
| ptu | 3 | 5.47 | 100.59 | 1.86 | 16.74 | 76.27 | 45.89 | 0.31 | 2.36 | 0.19 | 7.50 | 0.06 |
| ptu | 4 | 5.68 | 136.20 | 1.86 | 18.60 | 78.13 | 50.85 | 0.31 | 2.17 | 0.25 | 6.64 | 0.06 |

**Table 11.** Chemical properties of three mine spoils and a control, inoculated or not inoculated with topsoil across three tree species. A represents *F. americana*, O is *Q. alba*, and P is *L. tulipifera.* C is control, t is BWS, ss is white sandstone and sh is shale. M is inoculated with topsoil and u is not inoculated.

| Trt-ment | rep | NH4 | Ex acidity | CEC | Exchangable cations (cmol/kg) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Ca | Mg | K | Na | Al | Mn |
| acm | 1 | 25.15 | 86.53 | 87.60 | 0.65 | 0.19 | 0.0483 | 0.1765 | 0.0060 | 0.0003 |
| acm | 2 | 23.14 | 86.53 | 87.60 | 0.65 | 0.19 | 0.0483 | 0.1765 | 0.0060 | 0.0003 |
| acm | 3 | 13.59 | 86.53 | 87.60 | 0.65 | 0.19 | 0.0483 | 0.1765 | 0.0060 | 0.0003 |
| acm | 4 | 33.46 | 86.53 | 87.60 | 0.65 | 0.19 | 0.0483 | 0.1765 | 0.0060 | 0.0003 |
| acu | 1 | 55.56 | 86.53 | 87.70 | 0.70 | 0.21 | 0.0449 | 0.2044 | 0.0074 | 0.0003 |
| acu | 2 | 22.42 | 86.53 | 87.70 | 0.70 | 0.21 | 0.0449 | 0.2044 | 0.0074 | 0.0003 |
| acu | 3 | 23.93 | 86.53 | 87.70 | 0.70 | 0.21 | 0.0449 | 0.2044 | 0.0074 | 0.0003 |
| acu | 4 | 29.23 | 86.53 | 87.70 | 0.70 | 0.21 | 0.0449 | 0.2044 | 0.0074 | 0.0003 |
| ashm | 1 | 10.22 | 0.00 | 1.92 | 1.15 | 0.62 | 0.0767 | 0.0742 | 0.0000 | 0.0000 |
| ashm | 2 | 8.55 | 0.00 | 1.92 | 1.15 | 0.62 | 0.0767 | 0.0742 | 0.0000 | 0.0000 |
| ashm | 3 | 31.42 | 0.00 | 1.92 | 1.15 | 0.62 | 0.0767 | 0.0742 | 0.0000 | 0.0000 |
| ashm | 4 | 9.22 | 0.00 | 1.92 | 1.15 | 0.62 | 0.0767 | 0.0742 | 0.0000 | 0.0000 |
| ashu | 1 | 0.00 | 0.00 | 2.02 | 1.19 | 0.63 | 0.1144 | 0.0860 | 0.0000 | 0.0001 |
| ashu | 2 | 7.85 | 0.00 | 2.02 | 1.19 | 0.63 | 0.1144 | 0.0860 | 0.0000 | 0.0001 |
| ashu | 3 | 25.02 | 0.00 | 2.02 | 1.19 | 0.63 | 0.1144 | 0.0860 | 0.0000 | 0.0001 |
| ashu | 4 | 8.36 | 0.00 | . | . | . | . | . | . | . |
| assm | 1 | 8.51 | 0.00 | 1.00 | 0.67 | 0.27 | 0.0138 | 0.0464 | 0.0000 | 0.0015 |
| assm | 2 | 8.79 | 0.00 | 1.00 | 0.67 | 0.27 | 0.0138 | 0.0464 | 0.0000 | 0.0015 |
| assm | 3 | 9.41 | 0.00 | 1.00 | 0.67 | 0.27 | 0.0138 | 0.0464 | 0.0000 | 0.0015 |
| assm | 4 | 1.71 | 0.00 | 1.00 | 0.67 | 0.27 | 0.0138 | 0.0464 | 0.0000 | 0.0015 |
| assu | 1 | 9.49 | 0.00 | 0.78 | 0.53 | 0.21 | 0.0066 | 0.0312 | 0.0000 | 0.0023 |
| assu | 2 | 7.41 | 0.00 | 0.78 | 0.53 | 0.21 | 0.0066 | 0.0312 | 0.0000 | 0.0023 |
| assu | 3 | 7.66 | 0.00 | 0.78 | 0.53 | 0.21 | 0.0066 | 0.0312 | 0.0000 | 0.0023 |
| assu | 4 | 6.57 | 0.00 | 0.78 | 0.53 | 0.21 | 0.0066 | 0.0312 | 0.0000 | 0.0023 |
| atm | 1 | 9.85 | 25.78 | 26.77 | 0.39 | 0.44 | 0.0567 | 0.0949 | 0.0000 | 0.0002 |
| atm | 2 | 16.08 | 25.78 | 26.77 | 0.39 | 0.44 | 0.0567 | 0.0949 | 0.0000 | 0.0002 |
| atm | 3 | 19.05 | 25.78 | 26.77 | 0.39 | 0.44 | 0.0567 | 0.0949 | 0.0000 | 0.0002 |
| atm | 4 | 15.33 | 25.78 | 26.77 | 0.39 | 0.44 | 0.0567 | 0.0949 | 0.0000 | 0.0002 |
| atu | 1 | 12.22 | 23.75 | 24.76 | 0.34 | 0.49 | 0.0606 | 0.1089 | 0.0000 | 0.0002 |
| atu | 2 | 40.57 | 23.75 | 24.76 | 0.34 | 0.49 | 0.0606 | 0.1089 | 0.0000 | 0.0002 |
| atu | 3 | 7.54 | 23.75 | 24.76 | 0.34 | 0.49 | 0.0606 | 0.1089 | 0.0000 | 0.0002 |
| atu | 4 | 13.55 | 23.75 | 24.76 | 0.34 | 0.49 | 0.0606 | 0.1089 | 0.0000 | 0.0002 |
| ocm | 1 | 20.03 | 86.53 | 87.63 | 0.62 | 0.21 | 0.1081 | 0.1551 | 0.0046 | 0.0004 |
| ocm | 2 | 28.37 | 86.53 | 87.63 | 0.62 | 0.21 | 0.1081 | 0.1551 | 0.0046 | 0.0004 |
| ocm | 3 | 21.71 | 86.53 | 87.63 | 0.62 | 0.21 | 0.1081 | 0.1551 | 0.0046 | 0.0004 |
| ocm | 4 | 10.88 | 86.53 | 87.63 | 0.62 | 0.21 | 0.1081 | 0.1551 | 0.0046 | 0.0004 |
| ocu | 1 | 15.00 | 86.53 | 87.71 | 0.70 | 0.23 | 0.0777 | 0.1627 | 0.0035 | 0.0004 |
| ocu | 2 | 24.67 | 86.53 | 87.71 | 0.70 | 0.23 | 0.0777 | 0.1627 | 0.0035 | 0.0004 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ocu | 3 | 11.45 | 86.53 | 87.71 | 0.70 | 0.23 | 0.0777 | 0.1627 | 0.0035 | 0.0004 |
| ocu | 4 | 48.21 | 86.53 | 87.71 | 0.70 | 0.23 | 0.0777 | 0.1627 | 0.0035 | 0.0004 |
| oshm | 1 | 17.21 | 0.00 | 1.75 | 1.04 | 0.55 | 0.0796 | 0.0844 | 0.0013 | 0.0001 |
| oshm | 2 | 7.04 | 0.00 | 1.75 | 1.04 | 0.55 | 0.0796 | 0.0844 | 0.0013 | 0.0001 |
| oshm | 3 | 8.79 | 0.00 | 1.75 | 1.04 | 0.55 | 0.0796 | 0.0844 | 0.0013 | 0.0001 |
| oshm | 4 | 17.57 | 0.00 | 1.75 | 1.04 | 0.55 | 0.0796 | 0.0844 | 0.0013 | 0.0001 |
| oshu | 1 | 6.31 | 0.00 | 1.79 | 1.08 | 0.59 | 0.0663 | 0.0593 | 0.0000 | 0.0001 |
| oshu | 2 | 7.02 | 0.00 | 1.79 | 1.08 | 0.59 | 0.0663 | 0.0593 | 0.0000 | 0.0001 |
| oshu | 3 | 6.05 | 0.00 | 1.79 | 1.08 | 0.59 | 0.0663 | 0.0593 | 0.0000 | 0.0001 |
| oshu | 4 | 26.10 | 0.00 | 1.79 | 1.08 | 0.59 | 0.0663 | 0.0593 | 0.0000 | 0.0001 |
| ossm | 1 | 8.21 | 0.00 | 0.96 | 0.64 | 0.25 | 0.0203 | 0.0458 | 0.0000 | 0.0011 |
| ossm | 2 | 7.64 | 0.00 | 0.96 | 0.64 | 0.25 | 0.0203 | 0.0458 | 0.0000 | 0.0011 |
| ossm | 3 | 61.92 | 0.00 | 0.96 | 0.64 | 0.25 | 0.0203 | 0.0458 | 0.0000 | 0.0011 |
| ossm | 4 | 10.87 | 0.00 | 0.96 | 0.64 | 0.25 | 0.0203 | 0.0458 | 0.0000 | 0.0011 |
| ossu | 1 | 7.62 | 0.00 | 0.81 | 0.55 | 0.21 | 0.0138 | 0.0238 | 0.0000 | 0.0027 |
| ossu | 2 | 7.56 | 0.00 | 0.81 | 0.55 | 0.21 | 0.0138 | 0.0238 | 0.0000 | 0.0027 |
| ossu | 3 | 6.36 | 0.00 | 0.81 | 0.55 | 0.21 | 0.0138 | 0.0238 | 0.0000 | 0.0027 |
| ossu | 4 | 6.32 | 0.00 | 0.81 | 0.55 | 0.21 | 0.0138 | 0.0238 | 0.0000 | 0.0027 |
| otm | 1 | 19.32 | 25.78 | 26.91 | 0.38 | 0.55 | 0.0753 | 0.1267 | 0.0000 | 0.0002 |
| otm | 2 | 6.90 | 25.78 | 26.91 | 0.38 | 0.55 | 0.0753 | 0.1267 | 0.0000 | 0.0002 |
| otm | 3 | 39.30 | 25.78 | 26.91 | 0.38 | 0.55 | 0.0753 | 0.1267 | 0.0000 | 0.0002 |
| otm | 4 | 12.18 | 25.78 | 26.91 | 0.38 | 0.55 | 0.0753 | 0.1267 | 0.0000 | 0.0002 |
| otu | 1 | 11.53 | 23.75 | 24.84 | 0.32 | 0.55 | 0.0838 | 0.1292 | 0.0000 | 0.0003 |
| otu | 2 | 9.33 | 23.75 | 24.84 | 0.32 | 0.55 | 0.0838 | 0.1292 | 0.0000 | 0.0003 |
| otu | 3 | 17.02 | 23.75 | 24.84 | 0.32 | 0.55 | 0.0838 | 0.1292 | 0.0000 | 0.0003 |
| otu | 4 | 13.85 | 23.75 | 24.84 | 0.32 | 0.55 | 0.0838 | 0.1292 | 0.0000 | 0.0003 |
| pcm | 1 | 18.83 | 86.53 | 87.68 | 0.66 | 0.21 | 0.1001 | 0.1800 | 0.0045 | 0.0003 |
| pcm | 2 | 19.85 | 86.53 | 87.68 | 0.66 | 0.21 | 0.1001 | 0.1800 | 0.0045 | 0.0003 |
| pcm | 3 | 11.82 | 86.53 | 87.68 | 0.66 | 0.21 | 0.1001 | 0.1800 | 0.0045 | 0.0003 |
| pcm | 4 | 14.34 | 86.53 | 87.68 | 0.66 | 0.21 | 0.1001 | 0.1800 | 0.0045 | 0.0003 |
| pcu | 1 | 19.81 | 86.53 | 87.65 | 0.64 | 0.21 | 0.0591 | 0.2085 | 0.0040 | 0.0002 |
| pcu | 2 | 21.29 | 86.53 | 87.65 | 0.64 | 0.21 | 0.0591 | 0.2085 | 0.0040 | 0.0002 |
| pcu | 3 | 39.90 | 86.53 | 87.65 | 0.64 | 0.21 | 0.0591 | 0.2085 | 0.0040 | 0.0002 |
| pcu | 4 | 21.21 | 86.53 | 87.65 | 0.64 | 0.21 | 0.0591 | 0.2085 | 0.0040 | 0.0002 |
| pshm | 1 | 8.58 | 0.00 | 1.77 | 1.07 | 0.57 | 0.0718 | 0.0552 | 0.0018 | 0.0002 |
| pshm | 2 | 14.60 | 0.00 | 1.77 | 1.07 | 0.57 | 0.0718 | 0.0552 | 0.0018 | 0.0002 |
| pshm | 3 | 9.62 | 0.00 | 1.77 | 1.07 | 0.57 | 0.0718 | 0.0552 | 0.0018 | 0.0002 |
| pshm | 4 | 6.73 | 0.00 | 1.77 | 1.07 | 0.57 | 0.0718 | 0.0552 | 0.0018 | 0.0002 |
| pshu | 1 | 9.43 | 0.00 | 1.82 | 1.09 | 0.59 | 0.0705 | 0.0709 | 0.0000 | 0.0001 |
| pshu | 2 | 8.10 | 0.00 | 1.82 | 1.09 | 0.59 | 0.0705 | 0.0709 | 0.0000 | 0.0001 |
| pshu | 3 | 7.16 | 0.00 | 1.82 | 1.09 | 0.59 | 0.0705 | 0.0709 | 0.0000 | 0.0001 |
| pshu | 4 | 7.79 | 0.00 | 1.82 | 1.09 | 0.59 | 0.0705 | 0.0709 | 0.0000 | 0.0001 |
| pssm | 1 | 7.87 | 0.00 | 0.95 | 0.64 | 0.26 | 0.0221 | 0.0227 | 0.0017 | 0.0011 |
| pssm | 2 | 6.74 | 0.00 | 0.95 | 0.64 | 0.26 | 0.0221 | 0.0227 | 0.0017 | 0.0011 |
| pssm | 3 | 53.75 | 0.00 | 0.95 | 0.64 | 0.26 | 0.0221 | 0.0227 | 0.0017 | 0.0011 |
| pssm | 4 | 3.84 | 0.00 | 0.95 | 0.64 | 0.26 | 0.0221 | 0.0227 | 0.0017 | 0.0011 |
| pssu | 1 | 27.40 | 0.00 | 0.78 | 0.55 | 0.20 | 0.0101 | 0.0254 | 0.0000 | 0.0017 |
| pssu | 2 | 5.33 | 0.00 | 0.78 | 0.55 | 0.20 | 0.0101 | 0.0254 | 0.0000 | 0.0017 |
| pssu | 3 | 8.96 | 0.00 | 0.78 | 0.55 | 0.20 | 0.0101 | 0.0254 | 0.0000 | 0.0017 |
| pssu | 4 | 19.97 | 0.00 | 0.78 | 0.55 | 0.20 | 0.0101 | 0.0254 | 0.0000 | 0.0017 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ptm | 1 | 14.84 | 25.78 | 26.85 | 0.45 | 0.43 | 0.0695 | 0.1193 | 0.0000 | 0.0001 |
| ptm | 2 | 19.98 | 25.78 | 26.85 | 0.45 | 0.43 | 0.0695 | 0.1193 | 0.0000 | 0.0001 |
| ptm | 3 | 14.71 | 25.78 | 26.85 | 0.45 | 0.43 | 0.0695 | 0.1193 | 0.0000 | 0.0001 |
| ptm | 4 | 7.75 | 25.78 | 26.85 | 0.45 | 0.43 | 0.0695 | 0.1193 | 0.0000 | 0.0001 |
| ptu | 1 | 18.23 | 23.75 | 24.66 | 0.31 | 0.43 | 0.0686 | 0.1014 | 0.0015 | 0.0003 |
| ptu | 2 | 66.52 | 23.75 | 24.66 | 0.31 | 0.43 | 0.0686 | 0.1014 | 0.0015 | 0.0003 |
| ptu | 3 | 11.33 | 23.75 | 24.66 | 0.31 | 0.43 | 0.0686 | 0.1014 | 0.0015 | 0.0003 |
| ptu | 4 | 10.15 | 23.75 | 24.66 | 0.31 | 0.43 | 0.0686 | 0.1014 | 0.0015 | 0.0003 |

**Table 12.** Microbial properties of greenhouse pots across three spoil types and a control and inoculated and un-inoculated with topsoil.

| Treatment | rep | Dehydrogenase (mg/kg) | Microbial Biomass (dissolved organic carbon mg/kg) | ATP (light level) |
|---|---|---|---|---|
| acm | 1 | 57.64 | . | 1804.21 |
| acm | 2 | 77.02 | . | 1410.84 |
| acm | 3 | 61.46 | .. | 1228.03 |
| acm | 4 | 72.89 | . | 1907.69 |
| acu | 1 | 61.44 | . | 1409.36 |
| acu | 2 | 75.51 | . | 2003.09 |
| acu | 3 | 120.13 | . | 2025.40 |
| acu | 4 | 68.42 | . | 1992.85 |
| ashm | 1 | 5.99 | . | 581.79 |
| ashm | 2 | 5.77 | . | 383.33 |
| ashm | 3 | 3.90 | . | 380.30 |
| ashm | 4 | 6.70 | . | 414.59 |
| ashu | 1 | . | . | . |
| ashu | 2 | 0.42 | . | 142.38 |
| ashu | 3 | 0.92 | . | 108.20 |
| ashu | 4 | 1.51 | . | 242.86 |
| assm | 1 | 0.70 | . | 25.63 |
| assm | 2 | 0.93 | . | 41.79 |
| assm | 3 | 0.72 | . | 364.11 |
| assm | 4 | 0.91 | . | 251.31 |
| assu | 1 | 0.48 | . | 145.80 |
| assu | 2 | 0.58 | . | 173.26 |
| assu | 3 | 0.80 | . | 190.51 |
| assu | 4 | 0.36 | . | 192.16 |
| atm | 1 | 8.28 | . | 332.32 |
| atm | 2 | 13.42 | . | 405.41 |
| atm | 3 | 12.57 | . | 546.22 |
| atm | 4 | 11.54 | . | 251.75 |
| atu | 1 | 1.63 | . | 297.77 |
| atu | 2 | 1.46 | . | 268.10 |
| atu | 3 | 0.83 | . | 332.15 |
| atu | 4 | 2.78 | . | 270.35 |
| ocm | 1 | 43.48 | . | 1506.66 |
| ocm | 2 | 82.76 | . | 1540.45 |
| ocm | 3 | 95.86 | . | 1060.27 |
| ocm | 4 | 128.52 | . | 1763.70 |

| | | | | |
|---|---|---|---|---|
| ocu | 1 | 81.03 | . | 1207.74 |
| ocu | 2 | 111.13 | . | 1285.94 |
| ocu | 3 | 105.95 | . | 1029.51 |
| ocu | 4 | 100.11 | . | 1078.28 |
| oshm | 1 | 16.98 | . | 492.41 |
| oshm | 2 | 5.42 | . | 386.79 |
| oshm | 3 | 10.19 | . | 340.75 |
| oshm | 4 | 3.66 | . | 159.51 |
| oshu | 1 | 0.91 | . | 151.64 |
| oshu | 2 | 0.99 | . | 320.84 |
| oshu | 3 | 0.62 | . | 188.06 |
| oshu | 4 | 0.49 | . | 402.55 |
| ossm | 1 | 1.12 | . | 210.43 |
| ossm | 2 | 0.58 | . | 202.53 |
| ossm | 3 | 0.61 | . | 276.37 |
| ossm | 4 | 1.57 | . | 170.75 |
| ossu | 1 | 0.34 | . | 201.06 |
| ossu | 2 | 0.20 | . | 164.54 |
| ossu | 3 | 0.44 | . | 114.59 |
| ossu | 4 | 0.40 | . | 180.95 |
| otm | 1 | 11.12 | . | 436.57 |
| otm | 2 | 13.38 | . | 309.82 |
| otm | 3 | 7.42 | . | 353.30 |
| otm | 4 | 6.72 | . | 596.11 |
| otu | 1 | 3.57 | . | 91.97 |
| otu | 2 | 1.52 | . | 172.74 |
| otu | 3 | 1.13 | . | 212.73 |
| otu | 4 | 1.70 | . | 270.36 |
| pcm | 1 | 85.35 | | 821.42 |
| pcm | 2 | 119.24 | | 1430.87 |
| pcm | 3 | 132.17 | | 1613.39 |
| pcm | 4 | 125.67 | | 1449.88 |
| pcu | 1 | 90.05 | | 1779.37 |
| pcu | 2 | 116.93 | | 1557.27 |
| pcu | 3 | 55.64 | | 1370.23 |
| pcu | 4 | 94.87 | | 1699.56 |
| pshm | 1 | 3.38 | | 471.75 |
| pshm | 2 | 8.08 | | 724.42 |
| pshm | 3 | 5.53 | | 489.67 |
| pshm | 4 | 1.47 | | 508.97 |
| pshu | 1 | 0.75 | | 218.99 |
| pshu | 2 | 1.21 | | 241.04 |
| pshu | 3 | 0.32 | | 122.29 |
| pshu | 4 | 0.52 | | 224.20 |
| pssm | 1 | 0.48 | | 194.31 |
| pssm | 2 | 0.46 | | 163.68 |
| pssm | 3 | 0.37 | | 197.96 |
| pssm | 4 | 0.22 | | 406.86 |
| pssu | 1 | 0.39 | | 123.48 |
| pssu | 2 | 0.22 | | 154.24 |

| | | | |
|---|---|---|---|
| pssu | 3 | 0.39 | 98.53 |
| pssu | 4 | 0.33 | 144.44 |
| ptm | 1 | 19.96 | 614.12 |
| ptm | 2 | 14.77 | 500.12 |
| ptm | 3 | 8.54 | 343.21 |
| ptm | 4 | 11.24 | 446.97 |
| ptu | 1 | 3.12 | 170.72 |
| ptu | 2 | 0.83 | 355.15 |
| ptu | 3 | 1.95 | 325.13 |
| ptu | 4 | 5.53 | 228.76 |

# VITA

Julia Showalter was born in Morgantown, West Virginia, and received her Bachelor of Science degree in Biology from West Virginia University in May of 2002. After completing her Master of Science degree in Forestry at Virginia Tech in the summer of 2005, her career objectives are to provide technical services through the Peace Corps. Upon her return she wishes to pursue a Ph.D. degree in Forestry and attain an academic teaching and research position.