DISSERTATION

ASSESSMENT OF SOIL DEVELOPMENT FOLLOWING A SUB-ALPINE MINE RECLAMATION

Submitted by

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In partial fulfillment of the requirements

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We hereby recommend that the dissertation prepared under our supervision by Lawra A. Vanderhoof entitled *Assessment of Soil Development Following a Sub-Alpine Mine Reclamation* be accepted as fulfilling in part requirements for the degree of Doctor of Philosophy.

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ABSTRACT OF DISSERTATION

Assessment of Soil Development Following a Sub-Alpine Mine Reclamation

The Summitville Mine is located at 3,500 m in the San Juan Mountain Range, Rio Grande County, Colorado. Open-pit mining for gold created 223 hectares of waste rock and drastically disturbed land. The waste rock had acidic pH and high metal concentrations, so reclamation plans were enacted to accelerate soil formation and establish a self-sustaining plant community. Treatments were applied to the waste rock to develop a soil with adequate pH, nutrients, organic matter, and a functioning microbial community. The waste rock treatments were mushroom compost and lime (t-M), limed stockpiled topsoil (t-SM), and non-stockpiled topsoil (t-NM). Six years after reclamation, the objectives of this study were to assess soil development in the treated waste rock by measuring microbial community characteristics in relation to physicochemical properties and plant community measures and comparing these soil characteristics to native reference sites. The native reference sites were a sub-alpine meadow, forest, and a clear-cut forest. Physicochemical properties included soil pH, organic matter, nitrogen, and total metals. Plant characteristics were cover and species richness. Microbial properties included active (viable) biomass and community structure (Phospholipid Fatty Acid analysis), fungal and bacterial total biomass (Microscopy), community function measured as carbon-substrate utilization and richness (Biolog analysis), and litter decomposition. The combination of soil microbial community,

physicochemical, and plant characteristics indicated soil development in the waste rock treatments. Both t-NM and t-M were the most effective in reclaiming the waste rock. These waste rock treatments had plant cover, soil organic and inorganic N, pH, and total metals that were similar to the native reference sites. Either t-M, t-NM, or t-SM provided a functioning microbial community and litter decomposition similar to the native reference sites. However, the waste rock treatments had lower microbial active and total biomass than the native reference sites. Only t-NM was similar to the sub-alpine meadow in microbial community richness, which included bacteria, fungi, and actinomycetes. From the various soil characteristics studied, substrate utilization richness, bacterial total biomass, and soil organic matter had many significant correlations with other soil characteristics, which included inorganic N and total metal concentrations, fungal total biomass, microbial community richness, and plant species richness. Future projects could use one of these soil characteristics with their associated correlations to assess soil development after reclamation.

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CHAPTER I

Introduction

Mountainous regions of the western United States have extensively been mined for their mineral resources. The land formations in these areas provide access to numerous minerals. In 1873, gold along with silver and copper were discovered in the San Juan Mountain Range of the Colorado Rocky Mountains. An underground system of adits and shafts, known as the Summitville Mine, was created to retrieve the metals. The mine was located in the southwest corner of Rio Grande County, Colorado, approximately 29 km southwest of Del Norte, Colorado (Figure 1.1; Dodson and Benevento 2001; Winter 2000). For approximately 70 years, the Summitville Mine was regularly used to extract metallic resources.

In 1984, the Summitville Mine was reopened by a division of Galactica Resources, called the Summitville Consolidated Mining Corporation Inc. (SCMCI; Dodson and Benevento 2001; Winter *et al.* 2000). SCMCI had obtained a mining permit for 567 hectares to extract metals from Summitville's South Mountain (Dodson and Benevento 2001). Metal production by underground mining had declined by this time, so SCMCI chose a surface mining operation, called open pit heap-leach mining, to extract the metals. Surface mining has been characterized as one of the most drastic human disturbances to the land because it results in total destruction of the surface ecosystem as

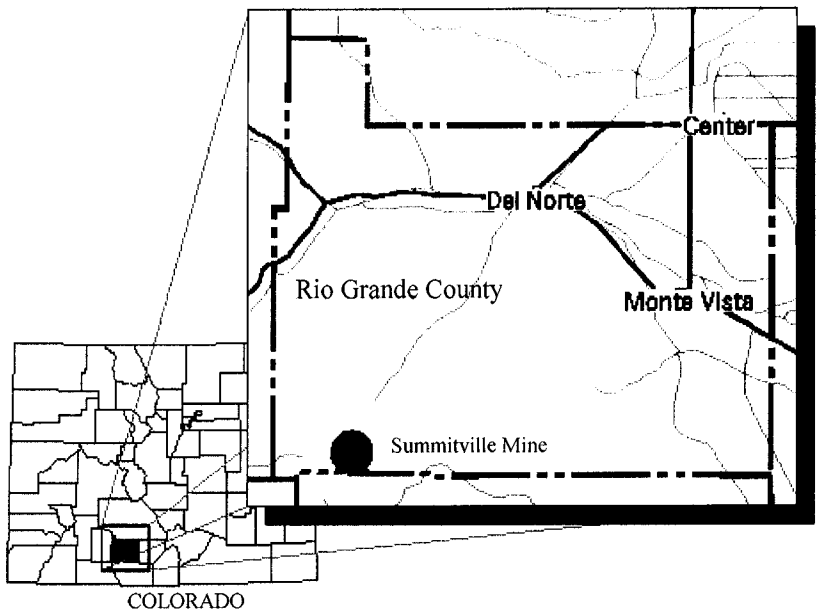

Figure 1.1. Location of the Summitville Mine in southwestern Colorado.

soil layers are removed to expose the mineral bearing ore (Box 1978; Brown *et al.* 1978). Extraction of the minerals from the ore has been described as follows:

> Gold and silver bearing ore are crushed and placed into a constructed clay and synthetic lined Heap Leach Pad. A dilute sodium cyanide solution is applied to the ore to leach out the gold and silver. After percolating through the crushed ore, the "pregnant" solution is pumped from a series of recovery sumps completed in the lowermost portions of the Heap Leach Pad. The pregnant solution is pumped to a metals recovery plant where gold and silver are removed from the solution with activated carbon. The "barren" solution has its target cyanide level restored and its pH adjusted, and then it is recycled through the Heap Leach Pad. Gold and silver are stripped from the carbon, precipitated from the stripping solution, smelted, and sold (Dodson and Benevento 2001).

Approximately 10 million tons of gold and silver bearing ore were mined from South Mountain (Dodson and Benevento 2001). A total of 223 hectares of waste rock and drastically disturbed land was created by SCMCI with the open pit mining operation (Dodson and Benevento 2001).

Together, the waste rock and exposed subsoil from South Mountain generated extremely hazardous environmental conditions. The waste rock materials contained a natural iron-sulfide mineral, called pyrite (FeS_2). When exposed to air, the pyrite minerals become oxygenated. A series of hydrous iron sulfates are biochemically formed, and sulfuric acid is produced (Caruccio *et al.* 1988). Sulfuric acid has a very low pH, commonly about 2.3, which lowered the pH of the waste rock materials (Bradshaw 1997; Caruccio *et al.* 1988). The acidic pH then increased the concentrations of available heavy metals in the waste rock. Hydrogen ions (H^+) from the sulfuric acid (H_2SO_4) displace metal cations from cation exchange sites and place them into interstitial solution (Brady and Weil 1996). The heavy metals of concern at the mine were manganese (Mn), copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd) (Redente and Richard 1998; Dodson and Benevento 2001). Acidic pH and high heavy metal concentrations are not conducive to soil formation and vegetation establishment (Pierzynski *et al.* 1994). Therefore, the waste rock remained unvegetated and exposed to wind and water erosion. Precipitation and groundwater leached the sulfuric acid and heavy metals from the waste rock, forming acid mine drainage. The acid mine drainage collected in local streams, the Cropsy Creek and Wrightman Fork, which transported the contaminants off site and into the Alamosa River. Increased acid and heavy metal loadings into the river were of particular concern due to the extensive use of the Alamosa River water for livestock, agriculture, and wildlife habitat (Plumlee and Edelmann 1995). The hazardous environmental conditions, which originated at the Summitville Mine, became a regional problem.

In December 1992, SCMCI declared bankruptcy and abandoned the Summitville Mine. The mine was left with its exposed acidic waste rock, acid mine drainage, and

high cyanide concentration in the Heap Leach Pad (Dodson and Benevento 2001). These extreme, environmental conditions warranted the immediate control of the mine by the Environmental Protection Agency's (EPA) Emergency Response Branch. The Summitville Mine was declared a Superfund Site by the EPA in May 1994.

Plans were immediately enacted to remediate the Summitville Mine and decrease the quantity of acid mine drainage leaving the site. A major part of the plan was the revegetation of the barren, acidic waste rock at the mine. Revegetation has proven to be the most economical way to reduce leaching and erosion from mined hillsides (Bellitto *et al.* 1999). Revegetating the waste rock was a challenge because of its extremely poor growth capacity and the severe climate. The waste rock was high in metal concentrations and low in pH, organic matter, and nutrients (Table 1.1). Located in the sub-alpine zone at 3,500 m, the Summitville Mine was subjected to high winds, large amounts of snow, temperature extremes, and a short growing season. Temperatures range from -9 to 13°C, where the low temperatures can retard photosynthesis and plant-uptake of nutrients and water (Appendix Table 1; Lyle 1987). Average annual precipitation is 115 cm while average annual snowfall is 8.7 m (Appendix Table 1). Snow covers the mining site most of the year except for a short growing season from June through September (Dodson and Benevento 2001). The combination of these waste rock properties and climatic conditions prevented the natural re-establishment of vegetation at the mine. Reclamation procedures were needed to accelerate soil formation and plant successional processes to provide vegetative cover at the Summitville Mine (Lyle 1987; Brown and Johnston 1980).

Table 1.1. Average chemical properties for 2000 and 2001 of the Summitville Mine waste rock compared to native soil from a local, undisturbed meadow.

Properties	Native Meadow Soil	Waste Rock
pH	5.0	2.8
Soil Organic Matter (%)	11	1
$NH_4^+ + NO_3^-$ (mg/kg)	15 + 16	12 + 0
Total Cd (mg/kg)*	4	10
Total Cu (mg/kg)	24	195
Total Pb (mg/kg)	29	147
Total Zn (mg/kg)	105	175

Note: According to Kabata-Pendias and Pendias (1984), total metal concentrations toxic to plants are Cd (3-8 mg/kg), Cu (60-125 mg/kg), Pb (100-400 mg/kg), and Zn (70-400 mg/kg).

Colorado State University (CSU) accepted the challenging task of assisting in the development of a reclamation plan for the acidic waste rock at the Summitville Mine. Initially, a greenhouse study was conducted and the results were used to design a field study on the mine's North Waste Dump (Appendix Figure 1; Redente and Richards 1998). Test plots were constructed and 8 separate treatment combinations were applied to the waste rock in 1995 (Chapter 2, Revegetation Test Plot Design). The general goal of the field study was the creation of a suitable growth medium from the waste rock treatments that would facilitate plant growth (Winter 2000). It has been reported that development of a suitable plant growth medium and establishment of vegetative cover are two important elements of mine reclamation (Bell 1999). Plant growth in the test plots was monitored annually beginning in 1996. In 1999, it was concluded that waste rock treatments of lime and mushroom compost with and without the addition of topsoil

supported the greatest plant biomass and cover compared to other treatment combinations (Winter-Sydnor and Redente 2002). In 1999, these waste rock treatments also had total plant cover comparable to that of local reference sites (Winter *et al.* 2000). The waste rock treatments were then recommended as the site-wide reclamation plan for the un-treated waste rock at the Summitville Mine.

Site-wide reclamation of the Summitville Mine proposed the establishment of a self-sustaining plant community to control erosion and groundwater contamination (Winter 2000). It has been stated that establishment of plant cover to provide surface protection, stability, and erosion control is the short-term goal of revegetation while the long-term goal is the establishment of a self-sustaining plant community (Brown and Johnston 1980; Hobbs and Norton 1996; Johnson *et al.* 1994; and Brown *et al.* 1996; Visser 1985). In order for the vegetation in the waste rock treatments to be sustainable, a soil must be developed from the reclamation process. Soil has been defined as a dynamic, natural body of inorganic mineral matter, organic matter, water, gases, and living organisms (Doran and Parkin 1994; Brady and Weil 1996). Soil provides the nutrients, water, micro-organisms and physical support for terrestrial plant life. The acidic waste rock at the mine, however, had poor physical structure and water retention, low nutrient content, high metal concentrations, and was presumed to be lacking in microbial activity. Therefore, amendments were applied to convert the waste rock into a productive and self-sustaining soil medium (Prentice *et al.* 1999; Brown and Johnston 1980; McRae *et al.* 2000). Lime was applied to increase soil pH and decrease the availability of metal concentrations by decreasing the activity of the H^+ ion in the rock (Caruccio *et al.* 1988; Brady and Weil 1996). Organic matter was applied as mushroom

compost to provide several beneficial soil characteristics. Organic matter increases the supply of nutrients and increases the cation exchange capacity of the soil, which decreases metal availability and increases nutrient retention (Sposito 1989; McRae *et al.* 2000; Bradshaw 1997; Johnson *et al.* 1994; Brown and Johnston 1980; Lyle 1987). The composition of organic matter improves the physical structure of the soil by providing a smaller surface area with more micro-pores (Johnson *et al.* 1994; Lyle 1987; McRae *et al.* 2000). This allows greater water retention in the soil (McRae *et al.* 2000; Johnson *et al.* 1994; Brown and Johnston 1980; Lyle 1987). Topsoil was applied to supply soil fertility and most importantly act as a local, natural source of microorganisms (Hargis and Redente 1984; Brown and Grant 2000; Munshower 1994; Fresquez and Lindemann 1982). Micro-organisms are essential for the development of soil. They maintain soil fertility and the nutrient cycle by converting organic matter into inorganic nutrients through decomposition (Turco *et al.* 1994; Alexander 1980; Pennanen *et al.* 1999; Lyle 1987). Microbial by-products and biomass, especially fungi, increase soil aggregation, which improves the soil's physical structure (Molope *et al.* 1987; Kumar *et al.* 1999; Eash *et al.* 1994; Turco *et al.* 1994; Alexander 1980; Lyle 1987). The combination of these amendments should enable the development of a soil to sustain plant growth over the long term.

Six years after reclamation of the waste rock, soil development in the waste rock treatments can be measured by a combination of physical, chemical, and biological soil properties (Doran and Parkin 1994). Physical properties include soil texture and water content (Doran and Parkin 1994). Chemical properties include a wide range of characteristics, such as soil nutrients (nitrogen), soil organic matter, pH, and metal

concentration (Doran and Parkin 1994). Chemical and physical soil properties have been regularly used in reclamation research to indicate soil quality (Janke and Papendick 1994; Visser 1985). It has been suggested that microbial characteristics should be used to represent soil biological properties and to provide more complete information about soil quality (Turco *et al.* 1994). Microbial biomass and community structure are sensitive to changes in soil chemical and physical properties, so these microbial characteristics can act as early indicators of soil improvement (McGrath 1996; Turco *et al.* 1994). Microbial function can indicate the recovery of microbial processes that are essential to the soil ecosystem, such as the decomposition of organic matter. Several authors conclude that microbial biomass and community structure together with microbial function are useful measurements in demonstrating reclamation development (Kelly and Tate 1998; Whitford and Elkins 1986; Turco *et al.* 1994; Moynahan *et al.* 2002). The integration of physical, chemical, and microbial properties can determine the development of soil from the waste rock treatment combinations.

A comparative approach can be used to determine if the waste rock treatments have similar soil characteristics as natural soils with sustainable plant communities (Larson and Pierce 1994; Ward 2000). A combination of the physical, chemical, and microbial soil properties described previously are used to compare the reclaimed soil to local, reference sites. Brown *et al.* (1996) state that soil properties are among the strongest indicators of successional convergence between severe disturbances and native communities. Reference sites represent natural, undisturbed communities with ecological sustainability and provide a goal for the restoration effort (Brown and Johnston 1980; Aronson *et al.* 1995). Several reference sites near the Summitville Mine property are

used in my assessment to provide a range of natural variability (Hobbs and Norton 1996; Fischer 1986). The native reference sites include a sub-alpine meadow, a sub-alpine forest, and a sub-alpine clear-cut forest. Hobbs and Norton (1996) suggest creating a "scorecard" for the comparison, which includes the current conditions of the reclaimed soil relative to the ranges of natural variability in the native reference sites. The difference in magnitude of these physical, chemical, and microbial properties between the waste rock treatments and native reference sites determines the relative development of soil in the treatments (Larson and Pierce 1994). Waste rock treatments with soil properties that closely match the native reference sites should support a long-term, self-sustaining plant community.

Few comparative studies have measured soil development in a sub-alpine ecosystem after reclamation of acidic mine spoils (Fyles *et al.* 1985; Chambers *et al.* 1987). Information is lacking on a more complete description of soil development which includes microbial structure and function in relation to soil physicochemical and plant characteristics after treatment of acidic waste rock in a sub-alpine ecosystem. The objectives of this research were to 1) describe the soil microbial community structure and function in waste rock treatments at the Summitville Mine and native reference sites of the nearby sub-alpine ecosystem in relation to soil physicochemical and plant properties six years after reclamation; 2) determine which reclaimed waste rock treatment had the most similar soil characteristics as the native reference sites by comparing soil microbial community characteristics, soil physicochemical properties, and plant community measures. The hypotheses tested in this study are as follows:

Microbial Community Structure:

H1: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have greater richness in microbial community structure (measured by the Phospholipid Fatty Acid analysis) six years after treatment than the mushroom compost amended treatment (t-M).

H2: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have similar richness in microbial community structure (measured by the Phospholipid Fatty Acid analysis) six years after treatment compared to the sub-alpine meadow (SM) but less richness compared to the sub-alpine forest (SF) and clear-cut sub-alpine forest (CC) reference sites.

H3: The mushroom compost amended treatment (t-M) will have less richness in microbial community structure (measured by the Phospholipid Fatty Acid analysis) six years after treatment than each of the three reference sites (SM, SF, and CC).

Microbial Biomass:

H4: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have greater active microbial biomass (measured by the Phospholipid Fatty Acid analysis) six years after treatment than the mushroom compost amended treatment (t-M).

H5: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have greater total bacterial biomass (measured by the microscopy analysis) six years after treatment than the mushroom compost amended treatment (t-M).

H6: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have greater total fungal biomass (measured by the Phospholipid Fatty Acid analysis) six years after treatment than the mushroom compost amended treatment (t-M).

H7: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have similar active microbial biomass (measured by the Phospholipid Fatty Acid analysis) and total bacterial and fungal biomass (measured by the microscopy analysis) six years after treatment compared to the sub-alpine meadow (SM) but less active, bacterial and fungal biomass compared to the sub-alpine forest (SF) and clear-cut sub-alpine forest (CC) reference sites.

H8: The mushroom compost amended treatment (t-M) will have less active microbial biomass (measured by the Phospholipid Fatty Acid analysis) and total bacterial and fungal biomass (measured by the microscopy analysis) six years after treatment than each of the three reference sites (SM, SF, and CC).

Microbial Community Carbon-Substrate Utilization:

H9: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have greater total carbon-substrate utilization by their microbial communities (measured by the Biolog analysis) six years after treatment than the mushroom compost amended treatment (t-M).

H10: Stockpiled and non-stockpiled topsoil treatments (t-SM and t-NM) will have greater richness in substrate utilization by their microbial communities (measured by the Biolog analysis) six years after treatment than the mushroom compost amended treatment (t-M).

H11: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have similar total carbon-substrate utilization by their microbial communities (measured by the Biolog analysis) six years after treatment compared to the sub-alpine meadow (SM) but less total carbon-substrate utilization compared to the sub-alpine forest (SF) and clear-cut sub-alpine forest (SF) reference sites.

H12: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have similar richness in substrate utilization by their microbial communities (measured by the Biolog analysis) six years after treatment compared to the sub-alpine meadow (SM) but less richness in substrate utilization compared to the sub-alpine forest (SF) and clear-cut sub-alpine forest (SF) reference sites.

H13: The mushroom compost amended treatment (t-M) will have less total carbon-substrate utilization by its microbial community (measured by the Biolog analysis) six years after treatment than each of the three reference sites (SM, SF, and CC).

H14: The mushroom compost amended treatment (t-M) will have less richness in substrate utilization by its microbial community (measured by the Biolog analysis) six years after treatment than each of the three reference sites (SM, SF, and CC).

Litter Decomposition:

H15: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have greater alfalfa and cotton decomposition six years after treatment than the mushroom compost amended treatment (t-M).

H16: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have similar alfalfa and cotton decomposition six years after treatment compared to the sub-alpine meadow (SM) but less alfalfa and cotton decomposition compared to the sub-alpine forest (SF) and clear-cut sub-alpine forest (CC) reference sites.

H17: The mushroom compost amended treatment (t-M) will have less alfalfa and cotton decomposition six years after treatment than each of the three native reference sites (SM, SF, and CC).

Physicochemical Properties:

H18: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have greater soil organic matter content and organic N six years after treatment compared to the mushroom compost treatment (t-M).

H19: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have greater soil inorganic N six years after treatment compared to the mushroom compost treatment (t-M).

H20: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have higher soil pH six years after treatment compared to the mushroom compost amended treatment (t-M).

H21: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have lower total metal concentrations six years after treatment compared to the mushroom compost amended treatment (t-M).

H22: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have similar soil organic matter content and organic N six years after treatment compared to the sub-alpine meadow (SM) but less soil organic matter and N compared to the sub-alpine forest (SF) and clear-cut sub-alpine forest (CC) reference sites.

H23: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have similar soil inorganic N six years after treatment compared to the sub-alpine meadow (SM) but less soil organic matter and N compared to the sub-alpine forest (SF) and clear-cut sub-alpine forest (CC) reference sites.

H24: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have similar soil pH six years after treatment compared to each of the three reference sites (SM, SF, and CC).

H25: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have similar total metal concentrations six years after treatment compared to each of the three reference sites (SM, SF, and CC).

H26: The mushroom compost amended treatment (t-M) will have less soil organic matter content and organic N six years after treatment than each of the three reference sites (SM, SF, and CC).

H27: The mushroom compost amended treatment (t-M) will have less soil inorganic N six years after treatment than each of the three reference sites (SM, SF, and CC).

H28: The mushroom compost amended treatment (t-M) will have lower soil pH six years after treatment than each of the three reference sites (SM, SF, and CC).

H29: The mushroom compost amended treatment (t-M) will have higher total metal concentrations six years after treatment than each of the three reference sites (SM, SF, and CC).

Plant Properties:

H30: The mushroom compost amended treatment (t-M) will have similar plant cover and species richness six years after treatment compared to the stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM).

H31: Stockpiled and non-stockpiled topsoil treatments (t-NM and t-SM) will have similar plant cover and species richness six years after treatment compared to the sub-alpine meadow (SM) but less plant cover and species richness compared to the sub-alpine forest (SF) and clear-cut sub-alpine forest (CC) reference sites.

H32: The mushroom compost amended treatment (t-M) will have similar plant cover and species richness six years after treatment compared to the sub-alpine meadow (SM) but less plant cover and species richness compared to the sub-alpine forest (SF) and clear-cut sub-alpine forest (CC) reference sites.

CHAPTER II

Materials and Methods

Revegetation Test Plot Design

The Summitville Mine test plots were established in 1995 on the North Waste Dump (NWD; Appendix Figure 1). The NWD is located in the northwest section of the mine and consisted of waste rock and pit overburden, which contained metallic sulfides (Dodson and Benevento 2001). The test plots are located on the north-facing slope of the NWD in a completely randomized block design (Figure 2.1). The design consisted of eight waste-rock treatment combinations replicated four times each for a total of 32 plots. Each plot is 10 m x 20 m with a 2 m buffer between plots and a 5 m buffer between rows of plots.

↑ North

Block 1	SM	M	NM	SB	B	SMP	NS	NSC
Block 2	NM	NS	NSC	SM	B	M	SB	SMP
Block 3	NS	SMP	SM	SB	B	NM	NSC	M
Block 4	SMP	NM	SM	M	SB	B	NSC	NS

Figure 2.1. Randomized block design for the revegetation test plots. Treatment codes: M=mushroom compost, B=biosolids, S=stockpiled topsoil, N=nonstockpiled topsoil, P=ProMac, C=capillary barrier. *Source*: Winter *et al.* 2000; Redente and Richard 1998.

The eight waste rock treatments and their amendment combinations are depicted in Table 2.1. Lime was agricultural grade limestone. Mushroom compost (MC) was a mixture of straw, dry poultry waste, cotton-seed hulls, and urea to which was added gypsum, cotton-seed mill, mycelium inoculated barley seeds, supplement (usually crushed soybeans), and a 2 inch thick casing layer consisting of peat moss, sugar beat lime, and grit lime (Personal communication with Rakhra Mushroom Farm, Alamosa, CO). Biosolids were Grade A quality from the City of Alamosa (Winter-Sydnor and Redente 2002). ProMac® was a combination of liquids and controlled release pellets formulated to inhibit iron-oxidizing bacteria (Winter *et al.* 2000). The stockpiled topsoil originated from Topsoil Stockpile #7 at the mine, and it was limed at the rate of 8.3 Mg lime/1000 Mg soil. The non-stockpiled topsoil was native live-haul soil removed during construction of the Summitville Dam Impoundment. The inert rock material consisted of 2.5 to 15.2 cm diameter rocks, which were washed of fine particles. For t-SM, t-SMP,

Table 2.1. Eight waste rock treatment combinations.

Waste Rock Treat-ments	Lime (Mg/plot)	MC [b] (Mg/plot)	Biosolids (Mg/plot)	ProMac (kg/plot)	Limed, Stockpiled Topsoil (cm)	Non-stockpiled Topsoil (cm)	Inert Rock Material (cm)
t-SM	2.04	2.36			15		
t-SMP	2.04	2.36		28.35	15		
t-NM	2.04	2.36				15	
t-M	2.04	2.36					
t-SB	2.04		2.13		15		
t-B	2.04		3.18				
t-NS	2.04				15[a]	15	
t-NSC					15[a]	15	30.5

Note: [a] Stockpiled topsoil (S) was limed because of its acidity (average pH 2.8) in t-SMP, t-SM, and t-SB while it was not limed in t-NS and t-NSC to determine the potential of material over un-amended waste rock to provide upward acidification (Winter-Sydnor and Redente 2002). [b] MC=mushroom compost

t-NM, t-SB, and t-NS, the limestone, organic matter (mushroom compost or biosolids), and/or ProMac were disked into the upper 30 cm of un-treated waste rock. Plots receiving t-NSC were excavated to 30 cm and then filled with the inert rock material (capillary barrier). For t-B and t-M, 15 cm of additional un-treated waste rock were added to their plots before the organic matter and limestone were disked into the upper 30 cm of waste rock. The stockpiled and non-stockpiled topsoils were finally applied over the amended waste rock for the appropriate treatment combinations.

After individual waste rock treatments were applied, the surface of the plots were fertilized and seeded. Fertilizer rates for t-SMP, t-SM, t-NM, t-M, t-SB, and t-B were 280 kg/ha of ammonium nitrate (33-0-0), 420 kg/ha of triple super phosphate (0-44-0), and 280 kg/ha of potassium chloride (0-0-60; Winter 2000). The fertilizer rates were higher for t-M and t-B with 420 kg/ha of ammonium nitrate (33-0-0), 840 kg/ha of triple super phosphate (0-44-0), and 420 kg/ha of potassium chloride (0-0-60; Winter 2000). Since 1997, all test plots have been fertilized annually at the beginning of each growing season with 56 kg/ha ammonium nitrate. The test plots were broadcast seeded by hand and covered with crimped certified weed-free straw (6.7 Mg/ha). The seeded plant species and their seeding rates are listed in Appendix Table 2.

Reference Sites Plot Design

Four reference sites were chosen because they contain similar physiographic characteristics (aspect, topographic position, and geologic substrate) as the reclaimed test plots and are located on or near the Summitville Mine property. Each reference site

contains four plots with the same dimensions as the test plots, 10 m x 20 m separated by 2 m buffer.

The first reference site is located west of the North Waste Dump and south of Park Creek Road off mine property (Appendix Figure 1). It is at an elevation of 3,566 m on a north-facing slope. The site consists of a sub-alpine clear-cut forest (CC), which is now a late-seral herbaceous community with sparse tree (*Picea engelmannii*) and shrub (*Ribes montigenum*) vegetation. The herbaceous community consists of graminoids (*Carex ebenea*, *Deschampsia cespitosa*, and *Agrostis scabra*) and forbs (*Mertensia ciliata*, *Ligularia amplectens*, and *Cardamine cordifolia*).

The second reference site is also located west of the North Waste Dump and south of Park Creek Road off mine property (Appendix Figure 1). It is 25 m above the CC reference site at 3,589 m on a north-facing slope. This site consists of a sub-alpine forest (SF) dominated by trees (*Picea engelmannii* and *Abies lasiocarpa*) and a shrub understory (*Vaccinium myrtillus*) with low herbaceous cover (*Arnica cordifolia*, *Erigeron coulteri*, and *Sibbaldia procumbens*).

The third reference site is located south of the Cropsy Waste Pile Footprint and Cropsy Diversion Ditch on the north-facing slope of the Cropsy Mountain at 3,596 m (Appendix Figure 1). This site consists of a sub-alpine meadow (SM) dominated by graminoids (*Deschampsia cespitosa*, *Elymus trachycaulus*, and *Carex ebenea*) and forbs (*Ligularia bigelovii*, *Erigeron coulteri*, and *Polygonium vivparium*).

The fourth reference site is located south of the Summitville Dam Impoundment on the north-facing slope of the Beaver Mud Dump Footprint at 3,429 m (Appendix

Figure 1). The soil consists of un-treated waste rock from the mine, which is void of vegetation and low in pH. This site represents the severely disturbed soil (D) of the mine.

Data Collection

Plant Cover

Plant canopy cover was measured in August 2000 and 2001 for the waste rock treatments and reference sites. Cover was estimated using the point intercept method for plant species, rock, litter, and bare ground. Two diagonal transects (20m each) were placed in each plot, and cover was observed and recorded for 80 points at 0.5-m intervals along the transects. Tree cover also was estimated. The distance along the two transects that was covered by tree canopy was recorded as tree cover. Percent cover was then determined by dividing total distance covered by trees by total distance of transects (40 m).

Plant Biomass

Aboveground plant biomass (g) was measured in the waste rock treatments in August 2000 and 2001. Four quadrats, 0.5 m x 1.0 m, were randomly placed in each plot, 10 m x 20 m. Vegetation within each quadrat was clipped to ground level and separated by species. The biomass from the four quadrats per plot was composited per species. All biomass was then dried to a constant weight at 60°C. The oven dried weight (g) of each species per plot was recorded.

Soil Samples

Soil samples were collected in August and September 2000 and 2001. Ten soil cores were randomly taken to a depth of 10 cm in each plot of the reference sites (D, CC, SM, and SF) and in each plot of three waste rock treatments (t-SM, t-NM, and t-M). Metal spades were used to collect the soil samples. Between each plot, the spades were brushed to remove soil particles and sterilized with alcohol to minimize microbial cross-contamination. Each soil core was placed in separate, sterile plastic bags, labeled, and placed in coolers. The coolers were filled with dry ice to keep the 2000 soil samples near -20°C as they were transported to the laboratory for the Phospholipid Fatty Acid analysis. The coolers were filled with freezer packs to keep the 2001 soil samples cold during transport to the laboratory for the chemical, physical, and remaining microbial analyses. Once in the lab, each set of ten cores was combined to provide one composite sample per plot. The composite sample was sieved through a 2-mm mesh screen to remove larger organic residues and soil particles (Klein *et al.* 1998). The sieves were also brushed to remove soil particles and sterilized with alcohol to minimize microbial cross-contamination. The sieved soil was returned to labeled, sterile plastic bags. The 2000 samples were stored in -80°C freezers until analysis while the 2001 samples were stored in a refrigerator until analysis. All analyses were begun within 48 hours of sample storage.

Data Analyses

Plant Richness

Plant richness was determined by the total number of species recorded per plot from the plant cover data.

Soil Percent Moisture

The composited soil samples collected in August 2001 were used to determine soil percent moisture (Gardner 1986). Ten grams of fresh soil were weighed in a glass beaker and placed in an Imperial II Radiant Heat Oven (Lab-Line Instruments, Inc.) for 48 hours at 105°C. The dried soil was re-weighed, and the wet and dry soil weights were used to determine percent moisture:

$$\% \text{ Moisture} = [(WW - DW)/DW] \times 100$$

where:

WW = soil wet weight (grams)

DW = soil dry weight (grams)

Soil pH

The composited soil samples collected in August 2000 and 2001 were used to determine soil pH in 0.01M $CaCl_2$ (McLean 1982). The soil was first air dried, and then 10 grams of the dried soil were combined with 20-mL 0.01M $CaCl_2$ solution to provide a 2:1 ratio. The mixture was stirred with a magnetic stir bar for 1 minute on a Corning Stirrer/Hot Plate after 0, 15, and 30 minutes. The stir bar was removed, and the mixture was allowed to settle for 30 minutes. An electronic Accumet Model 50

pH/ion/conductivity meter (Fisher Scientific) equipped with an electrode and Automatic Temperature Compensation probe was calibrated with pH 4.0 and 7.0 calibration buffers immediately prior and immediately after samples were measured. The measured pH values for the buffers were not allowed to be in excess of ±0.05 pH units of the expected values (4.0 and 7.0). The pH of the soil samples were measured with the pH meter and recorded.

Soil Total Carbon and Nitrogen

The composited soil samples collected in August 2001 were used to determine total carbon (C) and nitrogen (N). The soil was first air dried and then ground with a ball mill (Mulvaney 1996). Approximately 0.20 grams of the ground, dry soil were weighed onto a piece of aluminum foil. The foil was twisted to contain the soil, and the sample was placed into a LECO CNH 1000 Model high temperature induction furnace (Colorado State University Soil, Water, and Plant Testing Laboratory). The soil samples were dry combusted inside the instrument to determine total C and N (grams C or N/grams dry soil), which were expressed as total %C or total %N. Before samples were run, the LECO furnace was calibrated with 4 blanks to set the instrument to zero and 4 soil standards with a known amount of total C and N (2.276%C and 0.245%N).

Soil Inorganic Nitrogen

The composited soil samples collected in August 2001 were used to determine inorganic nitrogen (N). The extraction of exchangeable inorganic N (ammonium and nitrate) from soils followed the procedure in Mulvaney (1996). The soil was first air

dried, and then 10 grams of dry soil were added to a 250-mL beaker. A 2M KCl solution was prepared by mixing 1.5-kg reagent grade KCl in 8-L deionized water and the solution diluted to 10 L. One hundred milliliters of 2M KCl was added to the beaker, which was placed onto a shaker for 1 hour. The soil solution was allowed to settle for 15 minutes. The supernatant was filtered through a funnel, lined with filter paper, and collected into a 200-mL Nalgene plastic bottle. Before the supernant samples were measured for inorganic N concentrations, a 550 Conductivity Detector (Timberline) was calibrated with standards and a baseline solution. The standards consisted of high grade potassium nitrate and ammonium sulfate mixed in 2M KCl to provide the following 3 sets of serial dilutions (mg NH_4^+-N/L:mg KNO_3^-/L): 0:0, 0.1:0.1, 0.3:0.3, 0.6:0.6, 1:1, 2:2, 4:4, and 6:6; 0.05:0.5, 0.125:1.25, 0.25:2.5, 0.5:5, 0.75:7.5, and 1.25:12.5; 1:0, 2.5:0, 5:0, 10:0, and 15:0. The baseline solution consisted of 2M KCl that was used to zero out the instrument. After the baseline solution and sets of standards were run, the filtered supernatant was measured for inorganic N (NH_4^+ and NO_3^- mg/L) with the Conductivity Detector. The supernant samples were first measured by the Conductivity Detector to obtain total inorganic N concentrations (mg/L). The supernant samples were then measured a second time to obtain only ammonium (NH_4^+) concentrations (mg/L). The nitrate (NO_3^-) concentrations (mg/L) of the supernant samples were calculated from the differences between total inorganic N and NH_4^+ concentrations. The concentrations of soil inorganic N (mg/kg) were then determined by the following calculation:

$$N_I = (Nc \times V)/DW$$

where:

N_I = soil inorganic N (mg/kg)

Nc = NH_4^+ or NO_3^- concentration (mg/L)

V = volume of 2M KCl (L)

DW = soil dry weight (kg)

Soil Organic Nitrogen

Organic nitrogen (N) was determined for the composited soil samples, collected in August 2001, by the following calculation:

OrgN = Nt - Ni

where:

OrgN = soil organic N (mg/kg)

Nt = soil total N (mg/kg)

Ni = soil inorganic N (mg/kg)

Soil Inorganic Carbon

The composited soil samples collected in August 2001 were used to determine inorganic carbon (C) by the modified pressure-calcimeter method (Sherrod *et al.* 2002; Colorado State University Soil, Water, and Plant Testing Laboratory). The soil was first air dried, and then 1 g of dry soil was added to a 20-mL amber glass bottle. A half dram vial containing 2 mL of acid reagent (6N HCl + 3% ferrous chloride-$FeCl_2$) was carefully placed inside the amber glass bottle. The $FeCl_2$ was used to eliminate the release of CO_2 from the soil organic matter. The HCl was used to release CO_2 from the soil carbonate. The amber bottle was capped with a two pronged, butyl septum and crimped closed with an aluminum seal. The amber bottles were shook for 5 minutes or until the acid reagent

was released from the half dram vial. The amber bottles were allowed to sit for 2 hours to complete the reaction and CO_2 evolution. Three blanks were prepared as described above with no dry soil added to the amber bottle. A modified calcimeter apparatus was used to monitor the voltage output from the amber bottles, and the apparatus consisted of a pressure transducer, a digital voltmeter, and a hypodermic needle (Sherrod *et al.* 2002). The voltage outputs for the soil samples and blanks were measured with the calcimeter apparatus by inserting the needle into the septum of the amber bottles and recording the values on the voltmeter. The three blank voltmeter values were averaged to provide a mean blank value, which was then subtracted from each soil sample voltmeter value. Before the soil samples were run, a calibration curve was developed from $CaCO_3$ soil standards (0.25, 0.5, 1.0, 2.0, 3.0, 5.0, 10 g/kg) and their respective voltmeter values measured by the calcimeter apparatus. The linear regression equation from the calibration curve was then used to determine the percent of soil inorganic carbon (%C) in the soil samples from their voltmeter values and dry soil weights (Personal communication with Colorado State University Soil, Water, and Plant Testing Lab).

Soil Organic Matter

Percent soil organic matter was determined for the composited soil samples, collected in August 2001, by the following calculation (Nelson and Sommers 1996; Munshower 1994):

$$\%SOM = (\%Ct - \%Ci) \times 2.0$$

where:

%SOM = percent soil organic matter

%Ct = percent soil total carbon

%Ci = percent soil inorganic carbon

2.0 = universal ratio of organic matter/organic C in surface soils

Soil Total Heavy Metal Concentrations

The composited soil samples collected in August 2000 and 2001 were used to determine total heavy metal concentrations. Fresh soil samples were sent to the Soil, Water, and Plant Testing Laboratory at Colorado State University for analysis. Total concentrations (mg/kg) of cadmium (Cd), copper (Cu), zinc (Zn), and lead (Pb) were extracted from the soil by a nitric-perchloric-hydrofluoric acid digest (Self and Rodriguez 1999) and analyzed by an Inductively Coupled Plasma (ICP) spectrometer. These four metals were chosen for analysis because they had previously been identified at the Summitville Mine as chemicals of concern (Dodson and Benevento 2001).

Soil Texture

The composited soil samples collected in August 2000 and 2001 were used to determine texture by the particle size analysis (Gee and Bauder 1986; Ashworth *et al.* 2001). The soil was first dried at 45°C until the mass was stable. Fifty grams of dried soil were placed into a 250-mL plastic bottle. A sodium-hexametaphosphate (HMP) solution was prepared by adding 50 g of sodium-hexametaphosphate to 950-mL deionized water. One hundred milliliters of HMP solution was added to the bottle, which was filled with deionized water. The bottle was shaken by hand for a few seconds and then placed onto a reciprocating shaker over-night. The soil solution was transferred

from the bottle to a 1-L glass cylinder. The cylinder was brought to volume with deionized water. A blank was prepared as described above with no dried soil added to the plastic bottle or glass cylinder. A metal plunger was used to mix the soil and blank solutions in the cylinders for 20 seconds. A hydrometer (Fisherbrand) was lowered into the solutions. After 40 seconds from the time mixing ceased, a hydrometer reading was recorded for each solution. The solutions were allowed to sit for 2 hours at which time another hydrometer reading was recorded for each solution without mixing the solutions. The temperature of the blank and soil solutions were recorded after 40 seconds and 2 hours. The temperature did not drift more than 1°C between the blank and the soil solutions during the course of the day. The following equations were used to calculate percent clay and sand:

$$\% \text{ clay} = (100/DW)(R_{2h}-R_{Bl})$$

where:

DW = dry weight of soil (50 grams)

R_{2h} = hyrometer reading at 2 hours

R_{Bl} = hyrometer reading of blank at 2 hours

$$\% \text{ sand} = 100 - [(100/DW)(R_{40s} - R_{Bl})]$$

where:

DW = dry weight of soil (50 grams)

R_{40s} = hyrometer reading at 40 seconds

R_{Bl} = hyrometer reading of blank at 40 seconds

The textural classes of the soil samples were determined by using the percentages of clay and sand in the textural triangle (Brady and Weil 1996; Gerakis and Baer 1999).

Litter Decomposition

In each plot of the reference sites (CC, SM, SF, and D) and waste rock treatments (t-SM, t-NM, and t-M), litterbags were placed in the soil to measure decomposition by mass loss. Approximately 10 grams of cotton or dried alfalfa were weighed and separately placed into a 10-cm x 15-cm litterbag of 1-mm nylon mesh (Harmon *et al.* 1999). The cotton was cosmetic cotton balls, and the alfalfa consisted of leaves and stems of *Medicago sativa*. Four litterbags of each litter type were randomly placed in the waste rock treatment and reference plots in September 2000. A total of 96 litterbags were placed in the waste rock treatments and 128 litterbags in the reference sites. All 224 litterbags were placed to a soil depth of 5 cm. The litterbags were labeled with aluminum tags and identified by orange connecting cords and orange spray-painted stakes at the litterbag locations. The litterbags were retrieved in August 2001 and brought to the lab. Litterbags were not retrieved from the disturbed reference site (D) because heavy equipment operation occurred over the plots. Dried soil on the bags and roots were removed before the litterbags were dried at 55°C for one week (Harmon *et al.* 1999). The dried litter from each bag was separately weighed and then placed into 15-mL porcelain crucibles. The weight of the dried litter in each crucible was recorded before placing the crucibles into an Isotemp Muffle Furnace (Fisher Scientific). The litter was ashed in the furnace for 4 hours at 550°C (Harmon *et al.* 1999). Once the furnace was cool, the crucibles were re-weighed to record the ashed litter weight. Any remaining ashed

particles in the crucibles represented materials and minerals that accumulated in the litterbags over the year (Harmon *et al.* 1999). The mass loss of the cotton or alfalfa per waste rock treatment and reference site was expressed by the following equation:

DLML = PreLW – [PostLW – (AshLW - AshStd)]

where:

DLML = decomposed litter mass loss (grams)

PreLW = pre-litter weight (grams)

PostLW = post-litter weight (grams)

AshLW = ashed litter weight (grams)

AshStd = ashed cotton or alfalfa standard weight (grams)

Microbial Community Function

Microbial community function was determined as the total carbon-substrate utilization and substrate utilization richness of the soil microbial community by the Biolog analysis (Garland and Mills 1991; Haack *et al.* 1995). Gram Positive and Gram Negative Biolog micro-plates (Biolog, Inc., Hayward, CA) were used, which together provided a total of 128 different carbon-substrate containing wells plus control wells without carbon-substrates. Biolog micro-plate procedures were followed from Meyer *et al.* (1998), Garland and Mills (1991), and Haack *et al.* (1995). Six grams of fresh composited soil sample, collected in August 2001, were weighed in a sterile conical tube. Thirty milliliters of autoclaved buffer (6.804-g KH_2PO_4 mixed with 1-L distilled water) were added to the soil. The soil solutions were agitated for 15 minutes and then the solutions were kept still until the supernatant was clear (approximately 30 minutes). The

supernatant was then decanted into sterile plastic dishes. A 100-μL Biolog micro-pipettor was used to place the supernatant into the wells of the Gram Negative and Gram Positive Biolog micro-plates. The micro-plates were placed into plastic-covered containers and incubated at room temperature (25°C) for 72 hours. Utilization of a carbon-substrate by the soil microorganisms is detected by a purple color formation in the micro-plate wells. The purple color forms during microbial cellular respiration as the active microbes oxidize the carbon-substrate and reduce a tetrazolium dye to purple formazan (Bochner 1989; Garland 1996). Every 12 hours during the incubation time, the micro-plates were analyzed with a Biolog Microplate Reader (Biolog, Inc., Hayward, CA). The Biolog Reader was self-calibrating and had a 590 nm filter. The absorbance readings per well for each micro-plate were recorded by the Biolog Reader. Hour 36 was chosen as the appropriate observation time of the micro-plates for analysis. At this time, the Biolog absorbance readings indicated utilization of the carbon-substrates and no color change had occurred in the control well (Zak *et al.* 1994). A graph of the Biolog absorbance readings verses time revealed a Biolog substrate oxidation response similar to a bacterial growth curve (Haack *et al.* 1995). At 36 hours, the graphs indicated change from the lag phase into the exponential phase for microbial substrate oxidation. This earlier reading (at 36 hours) provides a closer representation of the microbial carbon-substrate utilization in the soil at the time of sampling rather than a later reading, which may reflect the microbial metabolic potential as they exhibit their exponential and stationary phases of substrate oxidation (Willig *et al.* 1996; Haack *et al.* 1995).

Total carbon-substrate utilization was determined for each reference site (CC, SM, SF, and D) and waste rock treatment (t-SM, t-NM, and t-M). Total substrate utilization

was expressed as average well color development (AWCD) for the 128 carbon-substrate wells to adjust for inoculum cell density (Garland and Mills 1991):

$$AWCD = [\Sigma(R-C)]/128$$

where:

AWCD = average well color development

R = Biolog absorbance reading of each carbon-substrate well

C = Biolog absorbance reading of control well

Each reference site and waste rock treatment had four replicates that were averaged to provide the mean AWCD for total substrate utilization. The 128 carbon-substrates were then grouped into 11 categories (Appendix Table 3 and 7). The AWCD of each category was determined as in the above equation with the denominator equaling the total number of substrates per category (Appendix Table 3). For example, the AWCD for the carbohydrate category would be calculated as follows: $AWCD = [\Sigma(R-C)]/44$. The mean AWCD for each category was determined with four replicates per reference site or waste rock treatment.

Substrate utilization richness was determined for each reference site (CC, SM, SF, and D) and waste rock treatment (t-SM, t-NM, and t-M). Richness was the total number of substrates used by the microbial community out of the 128 possible carbon-substrates.

Microbial Community Structure and Active Biomass

The Phospholipid Fatty Acid analysis (PLFA) was used to indicate the total active (viable) biomass and composition of the active, soil microbial community (White 1993;

Frostegard *et al.* 1993). Phospholipid fatty acids are found in cell membranes, and they consist of a glycerol molecule plus a phosphate group as the hydrophilic "head" and two chains of fatty acids as the hydrophobic "tail" (Brock *et al.* 1994; Appendix Figure 2). Each subset or group of microbes has its own signature phospholipid fatty acid (White 1993; Frostegard and Baath 1996, Zak *et al.* 1996). Therefore, fatty acids can provide a relative abundance of various microbial groups in the soil (Zak *et al.* 1996). During this analysis, fatty acids are quantitatively extracted and identified from collected soil samples.

Procedures for the PLFA analysis were followed from White (1992) and Allen (2000). Soil samples were collected from the waste rock treatments (t-SM, t-NM, t-M) and reference sites (CC, SF, SM, D) in August 2000. The samples were kept in coolers with dry ice to keep their temperatures near -20°C as they were transported to the laboratory. Within 24 hours, the samples were sieved, composited, and placed into -80°C freezers until analysis.

Day 1 of the PLFA analysis involved the preparation of the glassware and the initial extraction of lipids. All glassware was cleaned with phosphate-free detergent and sterilized in an oven set at 450°C for 4 hours. Twenty grams of composited soil sample were separately weighed into sterile Teflon bottles. Forty milliliters of methanol, 20-mL chloroform, and 16-mL 50mM phosphate buffer were added to each bottle. The bottles were shaken and vented several times before being placed into a sonicating bath for 1.5 minutes. The bottles were kept at room temperature on the lab countertop for 8 hours (or over-night) to extract the lipids from the soil into the organic solvents. At the end of 8 hours, each bottle's supernatant was poured into a beaker and covered with aluminum

foil. Twenty milliliters of chloroform were added to the bottles, which were shaken and vented several times, and then kept still for 15 minutes. Each bottle's supernatant was again poured into its respective beaker, and 20-mL distilled water was added to each beaker. The beakers were re-covered with aluminum foil and kept over-night on the lab countertop. The soil remaining in the bottles was removed and placed onto separate aluminum dishes, which sat under a laboratory hood for 48 hours to air dry the soil. A dried soil weight per sample was recorded.

Day 2 of the PLFA analysis completed the extraction of the lipids and involved the fractionation of those lipids. Glass pipettes were used to transfer the bottom, organic fraction in the beakers, which sat overnight, to Rapid Vap™ (RV) bottles. The RV bottles were placed into a Rapid Vap (Labconco) to remove the solvents from the organic fraction to a liquid level of 1.5mL. The Rapid Vap was set for 60% speed, 37°C, 10 minutes, and equipped with flowing nitrogen gas. Glass pipettes were used to transfer the 1.5mL organic fraction in the RV bottles to separate 10-mL test tubes. The test tubes were placed into a TekBath (Scientific Products), which was set at 37°C and equipped with flowing nitrogen gas inside the test tubes. The test tubes remained in the TekBath until all solvents were completely dried. The dried, organic fraction in the test tubes contained the extracted lipids.

While the solvents were drying in the TekBath, a silicic acid slurry (SAS) was made by combining 0.5g silicic acid powder (dehydrated at 100°C for 1 hour) with 5-mL 20mM ammonium acetate (in methanol) in a 10-mL test tube. The SAS was mixed thoroughly and quickly transferred by pipette to a disposo-pipette (pipette packed with glass wool plugs) until a 1.5 inch SAS bed was made. Five milliliters of acetone and then

5-mL chloroform were added to the disposo-pipette to make a silicic acid column (SAC). For each dried, organic fraction, a separate SAC was made.

Then, 0.2-mL chloroform was added by syringe to each 10-mL test tube to dissolve the dried, organic fraction. Each dissolved organic fraction was transferred by pipette to its respective column (SAC). Chloroform was added to the test tubes and the organic fraction transferred to the SAC two more times. Solvents were then added to each SAC one at a time from least to most polar (chloroform<acetone<methanol). Five milliliters of chloroform was added to the SAC to remove the neutral lipids. Five milliliters of acetone was added to the SAC to remove the glycolipids. Ten milliliters of methanol was added to the SAC and caught in a 10-mL test tube after leaving the column to collect the phospholipids. The 10-mL test tubes, which contained the phospholipids, were placed into the TekBath (37°C and nitrogen gas) to remove the methanol and completely dry the phospholipid fractions. The 10-mL test tubes were tightly capped to contain the nitrogen gas and stored in a freezer until the next day.

Day 3 of the PLFA analysis involved the formation and purification of the fatty acid methyl esters. The test tubes with the dry phospholipid fractions were removed from the freezer and allowed to sit for 10 minutes at room temperature. One milliliter of a methanol:chloroform mixture (5-mL methanol:5-mL chloroform) was added by syringe to the dry, phospholipid fractions in each test tube. One milliliter of 0.2N KOH (one pellet KOH plus 8-mL methanol) was also added to each test tube to remove any water. The tubes were vortexed for 20 seconds at touch speed (5-6) and then placed into a pre-heated water bath (60°C) for 30 minutes. Methanol was used in this step to cleave the fatty acid chains from the "heads" (phosphate plus glycerol) of the extracted, phospholipids.

Methyl groups ($-CH_3$) from the methanol bind to the cleaved fatty acid chains, creating fatty acid methyl esters (FAMEs).

The test tubes were removed from the water bath and allowed to cool to room temperature. Two milliliters of hexane were added to each tube and swirled by hand. A 1N acetic acid mixture (570-μL glacial acetic acid:9.4-mL distilled water) was prepared, and 200 μL were added to each test tube to neutralize the solution. The pH of the test tubes' solution was checked by litmus paper for pH 6-7. Two milliliters of distilled water were added to each test tube to break the liquid phases, and the test tubes were vortexed for 30 seconds. After a few minutes, two liquid phases settled out of the solutions. Pipettes were used to transfer the upper organic phases, containing the FAMEs, to new 10-mL test tubes. Two milliliters of hexane were added to the original test tubes, which contained the lower aqueous phases. The original test tubes were vortexed for 30 seconds and then allowed to sit. The phases settled out, and again the upper organic phases were transferred to their respective new test tubes. This step, involving the hexane, was repeated once more. The new test tubes, which contained the FAMEs, were placed into the TekBath (37°C and nitrogen gas) to remove all solvents.

Once the FAMEs were dried, 200 μL of an internal injection standard, 2,2,4-trimethylpentane (50 pmol/μL), were added to each test tube to dissolve the dried FAMEs. Glass pipettes were used to transfer the FAME solutions to separate Gas Chromatograph (GC) glass vials with glass tube inserts. The GC vials were sealed and placed into a freezer until analysis.

Day 4 of the PLFA analysis involved the quantification and identification of the FAMEs. The GC vials were removed from the freezer and placed onto the auto sampler

of a 5890 Series II Gas Chromatograph (Hewlett Packard). A computerized standard auto tune was run before each set of samples to self-calibrate the Gas Chromatograph. The dissolved FAMEs, contained in the GC vials, were separated and quantified by the Gas Chromatograph with flame ionization detection. A 5971A Mass Selective Detector/Spectrometer (Hewlett Packard) was used to determine the structure and preliminary identification of the FAMEs. A spectrum of peaks was produced for each sample, and the FAME peaks in the spectrum were identified by a computerized FAME library (Colorado State University, Chemical Engineering Department). Each identified FAME peak was given a fatty acid formula, such as 'A:BωC' (Zak *et al.* 1996, White 1992, and Frostegard and Baath 1996). 'A' is the total number of carbon atoms in the fatty acid molecule. 'B' is the number of double bonds, and 'C' is the location of the double bond from the methyl end ($-CH_3$) of the molecule. Suffixes 'c' and 't' in a formula stand for *-cis* and *–trans* geometric isomers, respectively. Prefixes 'i' and 'a' in a formula stand for methyl branches in the *iso-* (1^{st} C) and *anteiso-* (2^{nd} C) positions, respectively. Other methyl branches were given formulas, such as 10Me18:0, where the methyl branch is on the tenth carbon from the carboxyl end of the 18 carbon fatty acid molecule. The prefix 'cy' represents a cyclopropane fatty acid molecule. These FAME formulas were used to identify the groups of microbes present in the soil samples (Appendix Table 4). The microbial groups consisted of bacteria, fungi, and actinomycetes. Bacterial FAMEs included i14:0, i15:0, a15:0, 15:0, i16:0, 16:1w7c, 16:1w7t, i17:0, a17:0, cy17:0, 17:0, 18:1w7c, 18:1w7t, cy19:0, and 10Me16:0 (Appendix Table 4). The FAME, 18:2w6,9, represented fungi (Pennanen *et al.* 1998), and the FAME, 10Me17:0, represented actinomycetes (Frostegard *et al.* 1993; Lechevalier 1977;

Appendix Table 4). Individual FAMEs were 14:0, 16:0, 18:0, 20:0, 22:0, 24:0, and 12Me16:0 (Appendix Table 4). The microbial groups and individual FAMEs formed the microbial community structure of the soil samples.

The quantity of individual FAMEs in each sample was based on a comparison to the internal injection standard (FAME C19:0). First, FAME peak areas were recorded for each sample's spectrum. Second, the molar amount (pmol) of each FAME was determined by the following calculation:

$$C_X = (A_X/A_{istd}) \times C_{istd} \times D$$

where:

C_X = molar amount of FAME-X (pmol)

A_X = Area under FAME peak X

A_{istd} = Area under internal injection standard peak (C19:0)

C_{istd} = Concentration of internal injection standard (50 pmol/μL)

D = Dilution factor of internal injection standard (200 μL)

The concentrations of FAMEs per sample were determined by the following calculation:

$$C_{FAME\text{-}X} = C_x / DW$$

where:

$C_{FAME\text{-}X}$ = Concentration of FAME-X (pmol/g)

C_x = molar amount of FAME-X (pmol)

DW = soil sample dry weight (grams)

The FAMEs and their concentrations then represent the type and amount of PLFAs in the soil. The PLFAs provide an estimate of the active microbial biomass and microbial community structure of the soil samples. The total PLFA content (pmol/g) was used to determine the active microbial biomass (Zak *et al.* 1996). The mole fraction (mol%) of each PLFA was used to indicate the relative proportion of bacteria, fungi, actinomycetes, and individual PLFAs in the microbial community structure (Zak *et al.* 1996). Individual FAMEs were included in the analysis if their average mol fractions (%) were equal to or greater than 0.05%.

Total Fungal and Bacterial Biomass

The microscopy analysis was used to provide total fungal and bacterial biomass estimates in composited soil samples, collected in August 2001 (Paul and Clark 1996; Klein *et al.* 1998). According to Paul and Clark (1996), direct microscopy procedures of suspended soil are common for counting soil microbes. Fresh composited soil samples were sent to Soil Foodweb, Inc. in Corvallis, Oregon, for the microscopy analysis.

Statistical Analysis

Before statistical analyses were run:

1. the Biolog absorbance readings were subjected to square root transformations to normalize the data distribution;
2. the 0.05 alpha level ($\alpha = 0.05$) was used to determine significant differences or relationships for all statistical analyses.

The One-Way Analysis of Variance (1-Way ANOVA) on SPSS 11.0 computer software (SPSS Inc., Chicago, IL) was used to determine if the means of the waste rock treatments (t-NM, t-SM, t-M) and reference sites (SM, CC, SF, D) were different in the following measurements: total plant cover, plant species richness, soil pH, total metal concentrations, soil organic matter, N concentrations, soil moisture, litter decomposition, total substrate utilization, substrate utilization richness, active microbial biomass, active microbial richness, fungal and bacterial total biomass. A Post Hoc Multiple Comparison (PHMC) test was run if the 1-Way ANOVA resulted in a significance level equal to or less than 0.05. The Least Significant Difference (LSD) test was chosen as the PHMC test to pinpoint which means were significantly different from each other (Norusis 1997).

The Bivariate Correlation procedure on SPSS 11.0 computer software (SPSS Inc., Chicago, IL) was used to measure how the soil microbial, physicochemical, and plant properties of the waste rock treatments and reference sites were related. The Spearman's rho and significance level were computed for the soil properties and presented in a correlation matrix.

CHAPTER III

Results and Discussion

The waste rock treatments and reference sites were sampled to a soil depth of 10 cm. For the results and discussion, the waste rock treatments and reference sites are defined as follows: Treatment-SM (t-SM) is 30 cm of limed stockpiled topsoil placed over waste rock amended with lime and mushroom compost. Treatment-NM (t-NM) is 30 cm of non-stockpiled topsoil placed over waste rock amended with lime and mushroom compost. Treatment-M (t-M) is waste rock amended with lime and mushroom compost. The native reference sites consist of a sub-alpine meadow (SM), a sub-alpine forest (SF), and a sub-alpine clear-cut forest (CC). A disturbed reference site (D) is used to represent the un-treated waste rock of the Summitville Mine.

Plant Properties

Plant Cover and Species Richness

The waste rock treatments ranged in total plant cover from 76 to 79% in 2001 (Figure 3.1; Appendix Table 5). Grasses accounted for 66 to 85% of the total cover with *Alopecurus pratensis* being the most dominant grass in the waste rock treatments (Appendix Table 5). Forbs had a lower total plant cover (15 to 34%) than the grasses

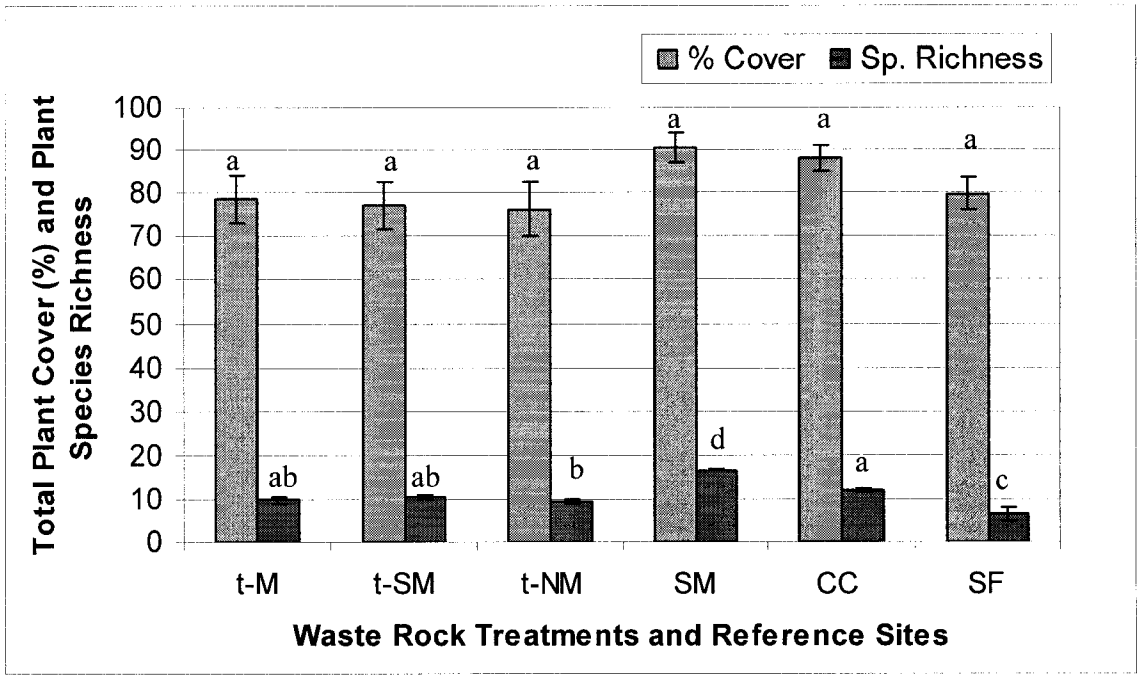

Figure 3.1. Average, total plant cover (%) and plant species richness for waste rock treatments (t-NM, t-SM, t-M) and native reference sites (SM, CC, SF) in 2001. Sample size is 4 for each treatment and reference site. Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest.

with *Achillea millefolium* being the most dominant forb in the waste rock treatments (Appendix Table 5). The waste rock treatments had greater grass biomass (62% of total biomass) than forb biomass (28% of total biomass) in 2001 (Vanderhoof *et al.* 2002). Previous revegetation studies have reported increased cover and biomass of grasses with higher nutrient availability in the soil, especially N (Meyer 2000; Doerr *et al.* 1983). Brown *et al.* (1996) stated that grasses utilize high nutrient inputs and may suppress the development of plant diversity. Topsoil and mushroom compost provided N and other essential nutrients to the plants in the waste rock treatments. Nitrogen fertilizer (ammonium nitrate) was applied annually to the waste rock treatments since 1997. These

nutrient additions may have contributed to greater grass than forb cover and biomass in the waste rock treatments.

The mushroom compost, lime, and topsoil amendments resulted in all three waste rock treatments having similar total plant cover (76 to 79%) as the native reference sites in 2001 (Figure 3.1; Appendix Table 5). The sub-alpine meadow reference site had the highest plant species richness, and the sub-alpine forest reference site had the lowest richness due to the dominance of two tree species (*Picea engelmannii* and *Abies lasiocarpa*) in 2001 (Figure 3.1; Appendix Table 5). The mushroom compost treatment (t-M) and the stockpiled topsoil treatment (t-SM) had similar plant species richness as the sub-alpine clear-cut forest reference site in 2001 (Figure 3.1). The non-stockpiled topsoil treatment (t-NM) had a plant species richness between that of the sub-alpine forest and sub-alpine clear-cut forest reference sites in 2001 (Figure 3.1). Life forms in the native reference sites included grasses, forbs, shrubs, and trees while only grasses and forbs were found in the waste rock treatments.

Physicochemical Properties

Soil pH

The waste rock treatments had greater soil pH than the extremely acidic conditions (pH 2.8 ± 0.1) of the un-treated waste rock (Table 3.1; Figure 3.2). The average soil pH in 2000 and 2001 in the waste rock treatments ranged from 4.9 to 6.0 (Figure 3.2). The lime amendment applied to the mushroom compost treatment (t-M) and the stockpiled topsoil treatment (t-SM) increased the pH of these waste rock treatments. Lime reacts with the hydrogen ion (H^+) to form calcium cations, water, and carbon

dioxide, which decreases the H^+ ion activity and increases the percentage base saturation in the soil (Caruccio *et al.* 1988; Brady and Weil 1996). The mushroom compost treatment (t-M) had the highest pH (6.0 ± 0.4) most likely due to the mushroom compost and lime amendments providing more basic cations and cation exchange sites to bind the H^+ ion (Munshower 1994; Brady and Weil 1996). The mushroom compost treatment (t-M) had a higher pH than the native reference sites (Figure 3.2). The stockpiled topsoil treatment (t-SM) had a similar pH as the sub-alpine meadow reference site (Figure 3.2). The non-stockpiled topsoil treatment (t-NM) had a pH of 4.9 (0.4) because it was not originally limed and was acidic (pH 5.2) before its application in 1995 (Redente and Richard 1998; Table 3.2). The non-stockpiled topsoil treatment (t-NM) had a similar pH as all three native reference sites (Figure 3.2).

Table 3.1. Description of soil pH ranges.

Rank	*pH Range*
Extremely acidic	<4.5
Very strongly acidic	4.5-5.0
Strongly acidic	5.1-5.5
Moderately acidic	5.6-6.0
Slightly acidic	6.1-6.5
Neutral	6.6-7.3

Source: Munshower 1994

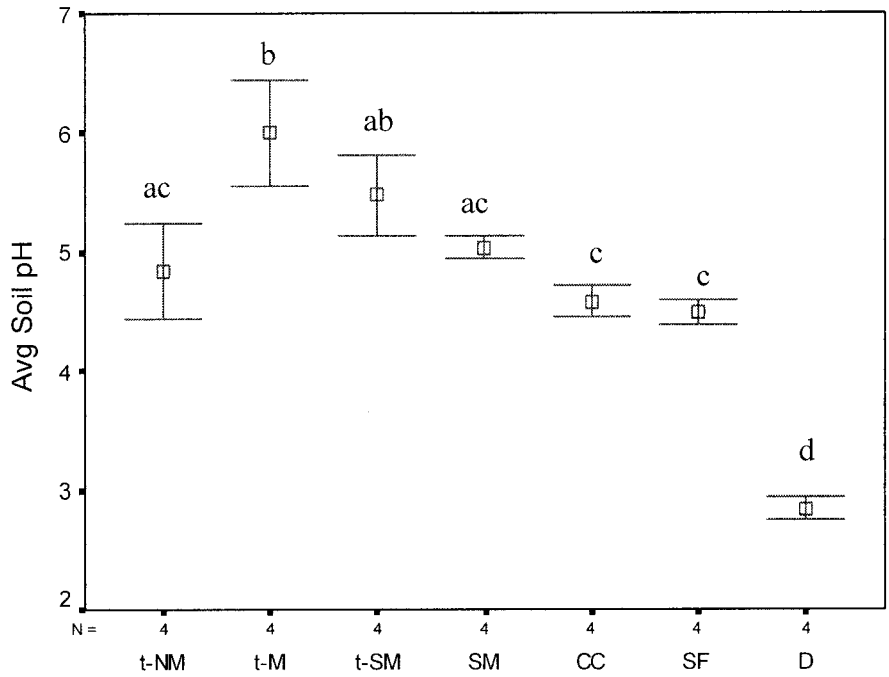

Figure 3.2. Average soil pH from 2000 and 2001 for waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D). Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

Soil Organic Matter

The waste rock treatments had greater soil organic matter (SOM) than the very low SOM (1.0 ± 0.6 %) of the un-treated waste rock in 2001 (Table 3.2 and Figure 3.3). The waste rock treatments ranged in SOM from 2.7 to 7.9% in 2001 (Figure 3.3). According to Johnson *et al.* (1994), the goal of organic matter application is to achieve 3 to 6% SOM, which the waste rock treatments attained. The mushroom compost

treatment (t-M) had the highest SOM (7.9 ± 1.9%) of the waste rock treatments (Figure 3.3). This was to be expected since the mushroom compost had 22.2% organic matter when applied in 1995 (Winter-Sydnor and Redente 2002). The mushroom compost treatment (t-M) had a similar SOM level as the sub-alpine meadow and sub-alpine forest reference sites (Figure 3.3). The non-stockpiled topsoil treatment (t-NM) had a similar SOM level (5.0 ± 0.1 %) as t-M, however, it was lower in SOM than the native reference sites (Figure 3.3). The stockpiled topsoil treatment (t-SM) had 2.7% SOM (0.2), which was similar to t-NM, but also lower than the native reference sites (Figure 3.3). The SOM level of t-SM may be the result of a decline in organic matter during storage of the stockpiled topsoil. Other studies have reported a significant decrease in organic carbon of stockpiled topsoil compared to undisturbed soils (Kundu and Ghose 1997; Schwenke *et al.* 1999). Schwenke et al. (1999) explained that lower organic C in stockpiled topsoil is attributed to continued microbial decomposition and lack of organic residue inputs to balance the losses.

Table 3.2. Description of soil organic matter (SOM) ranges

Soil Organic Matter Levels	**SOM (%)**
Very low	<2.0
Low	2.1-3.5
Medium	3.6-5.0
High	5.1-10.0
Very High	>10.0

Source: Munshower 1994

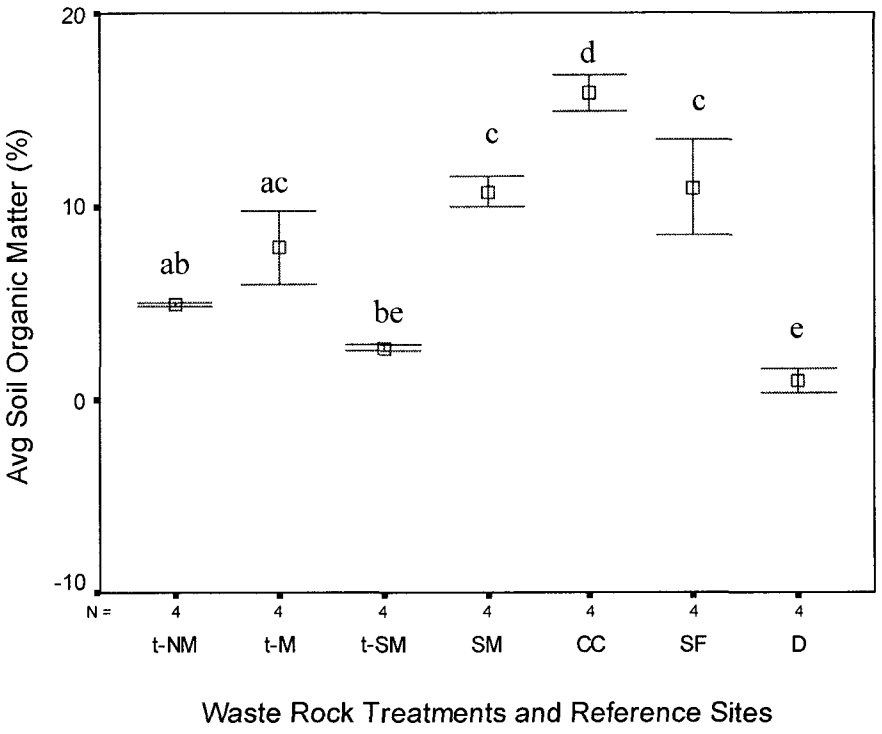

Figure 3.3. Average soil organic matter (%) for waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D) in 2001. Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

Soil Nitrogen

Nitrogen (N) is an essential nutrient needed in the soil to sustain plant growth and development (Hopkins 1999; Munshower 1994; Bradshaw 1997). Plants take up inorganic N from the soil in the form of ammonium (NH_4^+) and nitrate (NO_3^-). The main supply of inorganic N to the soil is through microbial decomposition of organic materials (Alexander 1980; Alexander 1998; Lyle 1987; Bradshaw 1997). Therefore, it is

important to measure the organic N in soil to determine the potential source of N to plants and to measure the inorganic N ($NH_4^+$and NO_3^-) as the current source of N.

The waste rock treatments had higher organic N concentrations than the un-treated waste rock in 2001 (Table 3.3). This was to be expected since the waste rock treatments had greater percentages of soil organic matter than the un-treated waste rock (Figure 3.3). The mushroom compost treatment (t-M) had the greatest organic N concentration of the waste rock treatments and also had the greatest soil organic matter level (Table 3.3; Figure 3.3). The mushroom compost treatment (t-M) was similar to all three native

Table 3.3. Average organic N and inorganic N (ammonium and nitrate) concentrations (mg/kg) for waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D) in 2001. Standard error of the means is represented in ( ). Different letters per column represent significant mean differences (α=0.05). N codes: NH_4^+=ammonium, NO_3^-=nitrate. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

Waste Rock Treatments and Reference Sites	Sample Size	Organic N (mg/kg)	Total Inorganic N (mg/kg)	NH_4^+ (mg/kg)	NO_3^- (mg/kg)
t-M	4	4,160 ^{ab} (1,048)	7.7 ^{a} (1.1)	5.4 ^{a} (0.2)	2.2 ^{a} (0.9)
t-SM	4	913 ^{d} (115)	9.4 ^{a} (1.6)	8.5 ^{ac} (1.6)	0.9 ^{a} (0.6)
t-NM	4	2,034 ^{cd} (58)	10.9 ^{a} (2.7)	10.4 ^{abc} (2.9)	0.5 ^{a} (0.5)
SM	4	5,629 ^{a} (283)	31.0 ^{b} (1.5)	15.2 ^{b} (2.7)	15.7 ^{b} (3.4)
CC	4	5,355 ^{a} (200)	22.3 ^{c} (2.8)	13.9 ^{bc} (0.8)	8.4 ^{c} (2.1)
SF	4	3,149 ^{bc} (644)	8.5 ^{a} (2.4)	8.5 ^{ac} (2.4)	0.0 ^{a} (0.0)
D	4	409 ^{d} (284)	12.4 ^{a} (1.9)	12.4 ^{bc} (1.9)	0.0 ^{a} (0.0)

reference sites in organic N concentration (Table 3.3). The non-stockpiled topsoil treatment (t-NM) had a lower organic N concentration than t-M and a similar organic N concentration as the sub-alpine forest reference site (Table 3.3). The stockpiled topsoil treatment (t-SM) had an organic N concentration similar to t-NM, however, it was lower in organic N than the native reference sites (Table 3.3).

The waste rock treatments had total inorganic N concentrations similar to the un-treated waste rock and the sub-alpine forest reference site in 2001 (Table 3.3). The sub-alpine meadow reference site had the highest total inorganic N concentration in 2001 (Table 3.3). The mushroom compost, lime, stockpiled and non-stockpiled topsoil amendments resulted in similar NH_4^+ and NO_3^- concentrations among the waste rock treatments. None of the waste rock treatments had NO_3^- concentrations similar to the sub-alpine clear-cut forest or sub-alpine meadow reference sites (Table 3.3). The non-stockpiled topsoil treatment (t-NM) had a similar NH_4^+ concentration as all three native reference sites (SM, CC, and SF; Table 3.3). The stockpiled topsoil treatment (t-SM) and the mushroom compost treatment (t-M) were similar to the sub-alpine forest reference site in NH_4^+ concentrations with t-SM also having a similar concentration as the sub-alpine clear-cut forest reference site (Table 3.3).

In general, the NH_4^+ concentrations were higher than the NO_3^- concentrations for all soils in 2001, except the sub-alpine meadow reference site (Table 3.3). Some possible explanations for the soil inorganic N trend are described as follows. It should be noted that other possible processes may be occurring. One explanation may be the fact that microorganisms transform organic N into NH_4^+ and NO_3^-. This study found both NH_4^+ and NO_3^- concentrations to be positively correlated to bacterial total biomass ($R = 0.559$

and 0.529, respectively; Appendix Table 6). The NH_4^+ ion can then accumulate in soil by binding to cation exchange sites of soil particles and organic matter (Lyle 1987; Dancer 1975). The NH_4^+ ion can also be transformed further into NO_3^- by bacteria in the soil during the process of nitrification (Myrold 1998). The negative charge on NO_3^- would keep this N ion in soil solution where it could be easily taken up by plants or leached from the soil (Munshower 1994; Dancer 1975; Brady and Weil 1996; Hopkins 1999). Both plant up-take and the leaching process could lower the NO_3^- concentration relative to NH_4^+. Erosion and leaching of the bare un-treated waste rock may have resulted in no measurable concentration of NO_3^- at reference site D (Table 3.3). The unmeasurable NO_3^- concentration at the sub-alpine forest reference site could have been a combination of nitrification and constant plant up-take of NO_3^- by the tree species.

Soil Total Metal Concentrations

Total metal concentrations of the waste-rock treatments and reference sites were measured as the sum of cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) concentrations. These four metals were identified as metals of concern at the Summitville Mine because of their high concentrations in the waste rock (Dodson and Benevento 2001). At relatively high concentrations, these metals are known to cause toxicity in plants and microorganisms (Hopkins 1999; Kabata-Pendias and Pendias 1984; Baath 1989). Damage to organisms by metals can occur through deficiencies of other essential nutrients, blocked biological functional groups (transport systems), denatured enzymes, and disrupted cell and organelle membranes (Hopkins 1999; Ross and Kaye 1994). The results of metal toxicity in plants are abnormal, stunted shoot and root growth

and disordered metabolic cycles, such as photosynthesis and respiration (Kabata-Pendias and Pendias 1984). Metal toxicity in microorganisms results in reduced microbial biomass and activity (Anderson and Domsch 1993; Baath 1989; Knight *et al.* 1997; Pennanen *et al.* 1996; Konopka *et al.* 1999; Chander and Brookes 1993; Pierzynski *et al.* 1994). Average total metal concentrations from 2000 and 2001 in the waste rock treatments and reference sites are shown in Figure 3.4.

The waste rock treatments had lower total metal concentrations than the un-treated waste rock (528 ± 69 mg/kg; Figure 3.4). The mushroom compost lowered the total metal concentration of the mushroom compost treatment (t-M) by increasing the bulk material of the waste rock and thereby diluting its total metal concentration. This study found a negative correlation between total metal concentrations and soil organic matter (R = -0.387; Appendix Table 6). The mushroom compost treatment (t-M) had a total metal concentration similar to the sub-alpine clear-cut forest reference site (Figure 3.4). The stockpiled topsoil added to t-SM provided the lowest total metal concentration of the waste rock treatments and a similar total metal concentration as the sub-alpine meadow and sub-alpine forest reference sites (Figure 3.4). The non-stockpiled topsoil treatment (t-NM) had a greater total metal concentration than t-SM, but was similar in total metal concentration to t-M and the sub-alpine clear-cut forest reference site (Figure 3.4). The non-stockpiled topsoil treatment (t-NM) remained significantly similar to the un-treated waste rock in total metal concentration due to its Cu and Pb concentrations (Figure 3.4; Table 3.4). According to Kabata- Pendias and Pendias (1984), total Pb concentrations of 100-400 mg/kg and total Cu concentrations of 60-125 mg/kg could be phytotoxic. The results from Winter-Sydnor and Redente (2002) indicate otherwise.

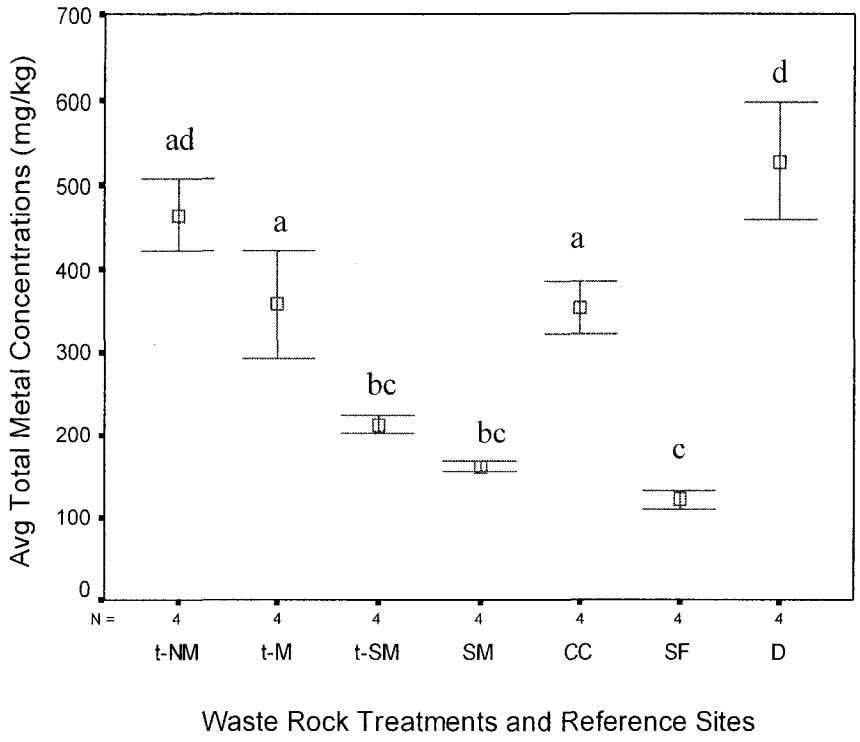

Figure 3.4. Average, total metal concentrations (mg/kg) from 2000 and 2001 of waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D). Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

They state that the non-stockpiled topsoil treatment (t-NM) had 477 ± 55 mg Pb/kg and 152 ± 22 mg Cu/kg in 1999. No visual signs of phytotoxicity were observed, and plant tissue analysis in 1999 indicated "normal" levels of Cu and Pb (Winter-Sydnor and Redente 2002). Paschke and Redente (2002) reported an EC50-plant (concentration of metal that reduced seedling biomass by 50% after 60 days) of 266 mg Cu/L for slender wheatgrass and 328 mg Cu/L for tufted hairgrass. Both grasses are dominant species in the waste rock treatments (Appendix Table 5). The non-stockpiled topsoil treatment

Table 3.4. Average, total metal concentrations (mg/kg) from 2000 and 2001 of cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) for waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D). Standard error of the means is represented in ( ). Different letters per row represent significant mean differences (α=0.05). Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

Waste Rock Treatments and Reference Sites	Sample Size	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
t-M	4	2.1 [b] (0.3)	122 [b] (39)	135 [b] (17)	99 [b] (17)
t-SM	4	4.2 [a] (0.6)	44 [a] (3)	60 [c] (5)	105 [b] (5)
t-NM	4	4.6 [a] (0.2)	100 [bc] (4)	243 [d] (35)	117 [b] (6)
SM	4	3.5 [ac] (0.1)	24 [a] (1)	29 [ac] (4)	105 [b] (9)
CC	4	6.5 [d] (0.3)	60 [ac] (5)	47 [ac] (16)	240 [a] (11)
SF	4	2.9 [bc] (0.5)	25 [a] (4)	10 [a] (1)	83 [b] (9)
D	4	10.2 [e] (0.8)	196 [d] (29)	147 [b] (13)	175 [c] (29)

(t-NM) had total Cu concentrations less than 266 mg/L. It is predicted that the Cu and Pb concentrations of t-NM were not causing plant toxicity or hindering plant growth.

Higher concentrations of Cu and Pb can cause toxicity in microorganisms. Knight *et al.* (1997) reported Cu concentrations of 143 mg Cu/kg at soil pH 4.5 to significantly decrease microbial biomass and function, measured as carbon-substrate utilization. Chander and Brookes (1993) found microbial biomass to decrease by 12% in soil contaminated with Cu at concentrations of 197 mg Cu/kg. The same authors reported that a combination of Cu (191 mg/kg) and Zn (367 mg/kg) in soil caused a larger decrease in microbial biomass (29%) compared to either metal at higher concentrations. A higher concentration of Pb than Cu is needed to harm soil microorganisms. Konopka *et al.*

(1999) found active microbial biomass to decrease as Pb content in soil increased to 809 mg Pb/kg. All three waste rock treatments had Cu and Pb total concentrations less than the toxic concentrations reported by these studies.

Microbial Properties

Active Microbial Biomass and Microbial Community Structure

Active microbial biomass and microbial community structure were determined by the extraction, identification, and quantification of microbial phospholipid fatty acids (PLFA) in soil samples of the waste rock treatments and reference sites (Appendix Table 4). The un-treated waste rock had an active microbial biomass of 4,700 pmol/g (1,000) in 2000 (Figure 3.5). Other studies have reported low microbial abundance in mine waste material (Moynahan *et al.* 2002; Visser 1985; Noyd *et al.* 1995; Fresquez and Lindemann 1982; Miller and Cameron 1978). The waste rock treatments ranged in active microbial biomass from 13,500 to 17,800 pmol/g in 2000 (Figure 3.5). The mushroom compost, lime, stockpiled and non-stockpiled topsoil amendments provided similar active microbial biomass among the waste rock treatments (Figure 3.5). The use of non-stockpiled topsoil plus plant growth raised the active microbial biomass of t-NM from that of site D. Topsoil served as a native source of micro-organisms to inoculate the treatments (DePuit and Redente 1988; Hargis and Redente 1984; Munshower 1994; Fresquez and Lindemann 1982). Topsoil and mushroom compost acted as a source of nutrients, especially carbon and nitrogen, which can be used to build microbial biomass

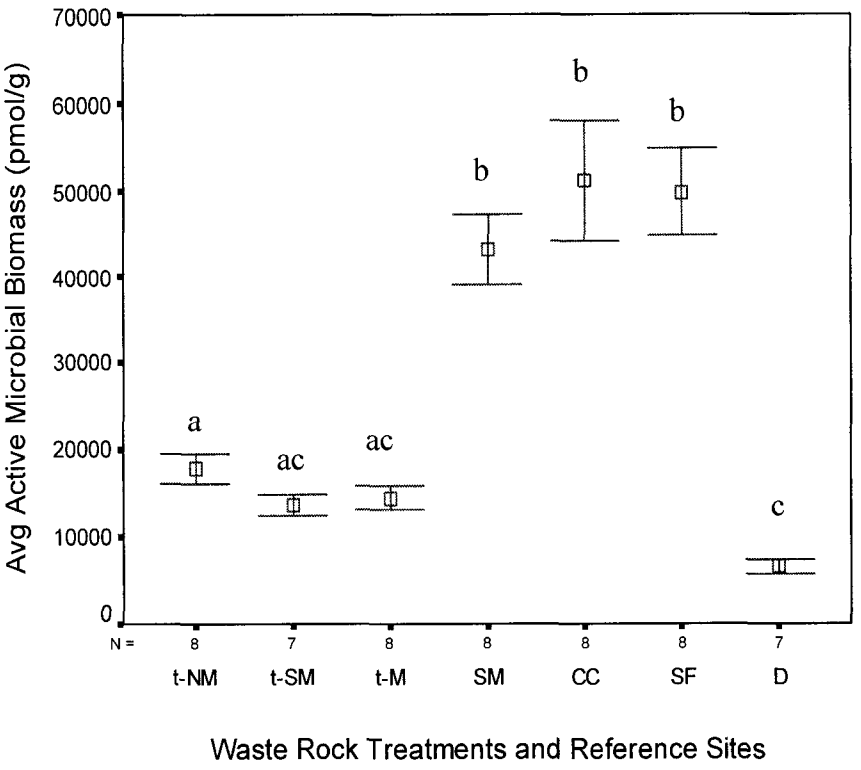

Figure 3.5. Average, soil active microbial biomass (pmol/g) for waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D) in 2000. Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

and provide energy for reproduction (Wagner and Wolf 1998). Zak *et al.* (1996) and Ohtonen *et al.* (1999) found that plants increased the active microbial biomass (measured by the PLFA analysis) in soil. Other studies have shown active microbial biomass to be dependent on soil pH where lower pH resulted in less microbial biomass (Babich and Stotzky 1978; Baath *et al.* 1980; Pennanen *et al.* 1998; Anderson and Domsch 1993; Blagodatskaya and Anderson 1998). This study shows a positive correlation between

active microbial biomass and soil pH (R=0.491; Appendix Table 6). All three waste rock treatments have greater soil pH than the un-treated waste rock (Figure 3.2). Six years after reclamation, the waste rock treatments did not have similar active microbial biomass as the native reference sites in 2000 (Figure 3.5).

The waste rock treatments changed the structure of the microbial community. The waste rock treatments had a greater richness in microbial community structure than the un-treated waste rock (Table 3.5). MacNaughton *et al.* (1999) reported that microbial communities in contaminated ecosystems are typically less diverse than those in non-stressed systems. Moynahan *et al.* (2002) found mine tailings with acidic pH to have low microbial counts and diversity relative to lime and topsoil amended plots with higher soil pH. This study found richness in microbial community structure to be positively correlated with soil pH (R = 0.549; Appendix Table 6). The non-stockpiled topsoil treatment (t-NM) had the highest richness in microbial community structure of the waste rock treatments and a similar richness as the sub-alpine meadow reference site (Table 3.5). The stockpiled topsoil treatment (t-SM) and the mushroom compost treatment (t-M) had lower richness in microbial community structure than t-NM and the native reference sites (Table 3.5).

The major differences in the PLFA profiles were that the un-treated waste rock had no recorded active biomass for actinomycetes and PLFAs 20:0, 22:0, 24:0, and 12Me16:0 (Table 3.5). Actinomycetes were present in the waste rock treatments and native reference sites (except the sub-alpine forest), which had higher soil pH and soil organic matter than the un-treated waste rock. Actinomycetes are sensitive to acidic soil pH and may be absent or markedly reduced in soil with a pH below 5.0 (Alexander 1980;

Table 3.5. The microbial community structures of the waste rock treatments and reference sites in 2000. The community structures are represented by phospholipid fatty acids (PLFA), which are expressed as mol% of the total PLFAs. Average PLFA richness of the community structure is listed for the waste rock treatments and reference sites. Standard error of the means is represented in ( ). Different letters represent significantly different means (α=0.05). Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=clear-cut sub-alpine forest, SF=sub-alpine forest, D=un-treated waste rock.

		Waste Rock Treatments			Reference Sites			
PLFA - mol%		**t-M**	**t-NM**	**t-SM**	**SM**	**CC**	**SF**	**D**
Individual:	14:0	1.3 (0.07)	1.9 (0.08)	1.5 (0.1)	0.7 (0.07)	1.0 (0.06)	1.3 (0.09)	1.1 (0.2)
	16:0	11.8 (0.3)	13.6 (0.4)	15.4 (3.1)	9.7 (0.2)	10.8 (0.2)	10.7 (0.3)	24.3 (0.9)
	18:0	2.0 (0.07)	2.0 (0.1)	1.7 (0.1)	1.8 (0.05)	2.2 (0.08)	2.2 (0.05)	2.1 (0.2)
	20:0	0	0.5 (0.1)	0.1 (0.1)	0.2 (0.06)	1.5 (0.1)	1.5 (0.07)	0
	22:0	0	1.1 (0.1)	0.2 (0.1)	0.6 (0.1)	2.2 (0.1)	2.0 (0.3)	0
	23:0	0	0	0.03 (0.03)	0	0.1 (0.04)	0.3 (0.05)	0
	24:0	0	1.0 (0.2)	0.2 (0.1)	0.7 (0.2)	0.9 (0.1)	1.0 (0.05)	0
	12Me16:0	0	0.3 (0.1)	0	0.7 (0.1)	0.7 (0.06)	0.4 (0.1)	0
Bacteria:	i14:0	1.4 (0.1)	0.8 (0.07)	0.9 (0.1)	0.6 (0.08)	0.3 (0.04)	0.4 (0.05)	0
	i15:0	6.6 (0.3)	6.9 (0.3)	5.4 (0.4)	5.3 (0.2)	5.7 (0.2)	4.6 (0.2)	6.4 (0.6)
	a15:0	7.7 (0.2)	6.6 (0.3)	6.2 (0.4)	5.9 (0.2)	4.0 (0.1)	3.1 (0.5)	2.6 (0.4)
	15:0	0	0	0	0.1 (0.07)	0.2 (0.05)	0.5 (0.09)	0
	i16:0	4.7 (0.1)	2.3 (0.08)	2.0 (0.03)	1.9 (0.05)	1.4 (0.08)	1.1 (0.08)	4.2 (0.3)
	16:1w7c	12.3 (0.4)	9.1 (0.4)	12.8 (2.3)	8.0 (0.3)	9.8 (0.3)	8.0 (0.3)	13.6 (0.6)
	16:1w7t	3.7 (0.2)	1.9 (0.1)	2.9 (0.5)	4.3 (0.2)	3.3 (0.1)	2.7 (0.3)	1.4 (0.1)
	i17:0	2.1 (0.1)	1.1 (0.04)	0.9 (0.1)	1.3 (0.07)	1.0 (0.04)	0.7 (0.04)	0.1 (0.1)
	a17:0	2.3 (0.1)	1.2 (0.03)	1.1 (0.05)	1.2 (0.09)	1.3 (0.09)	1.3 (0.2)	0.3 (0.1)
	cy17:0	3.6 (0.1)	3.7 (0.5)	9.2 (1.8)	5.1 (0.2)	5.2 (0.2)	4.9 (0.1)	9.7 (0.2)
	17:0	0.2 (0.1)	0.2 (0.05)	0.2 (0.08)	0.4 (0.1)	0.3 (0.08)	0.4 (0.08)	0
	18:1w7c	8.8 (0.4)	12.7 (0.3)	9.3 (0.7)	10.6 (0.2)	9.9 (0.2)	10.8 (0.1)	5.3 (0.4)
	18:1w7t	10.2 (0.2)	9.8 (0.3)	11.2 (0.3)	21.4 (0.3)	20.8 (0.4)	16.8 (0.6)	4.5 (0.3)
	cy19:0	10.5 (0.4)	7.4 (0.2)	6.4 (0.7)	8.9 (0.5)	8.4 (0.4)	7.1 (0.2)	10.2 (0.5)
	10Me16:0	2.7 (0.3)	2.5 (0.4)	2.8 (0.4)	4.0 (0.4)	4.5 (0.2)	3.6 (0.3)	5.0 (0.6)
Fungi:	18:2w6,9	7.3 (0.5)	11.8 (0.8)	8.2 (0.8)	5.3 (0.4)	3.2 (0.2)	13.6 (1.1)	8.5 (1.2)
Actinomycetes:	10Me17:0	0.03 (0.03)	0.5 (0.1)	0.1 (0.1)	0.3 (0.1)	0.04 (0.04)	0	0
Average PLFA Richness		17.6 (0.3)a	21.4 (0.4)b	18.6 (0.9)a	21.9 (0.5)bd	23.3 (0.6)d	23.0 (0.6)d	14.4 (0.3)c

Alexander 1998). The un-treated waste rock had an extremely acidic pH of 2.8 (0.1). Other researchers found populations of bacteria and actinomycetes to increase with greater amounts of organic matter (Noyd *et al.* 1995; Kelly and Tate 1998; Visser 1985). Moynahan *et al.* (2002) reported lime and topsoil treated mine tailings to have significantly more actinomycetes and 1-2 orders of magnitude more bacteria than the disturbed acidic tailings. Segal and Mancinelli (1987) reported significantly more viable numbers of bacteria and actinomycetes in local topsoil compared to spent oil shale. PLFAs 20:0, 22:0, 24:0, and 12Me16:0 were also present in the native reference sites and the non-stockpiled and stockpiled topsoil treatments (t-NM and t-SM, respectively), except 12Me16:0 in t-SM (Table 3.5). However, all four PLFAs remained absent from the mushroom compost treatment (t-M; Table 3.5). PLFA 20:0 has generally been identified with eukaryotes while the other PLFAs have not currently been identified (McNaughton *et al.* 1999; Appendix Table 4). The presence of these PLFAs in the topsoil treatments (t-NM and t-SM) and the native reference sites indicate that t-NM and t-SM received indigenous microorganisms from the topsoil of the local mining area. The indigenous microorganisms have not colonized the mushroom compost treatment (t-M). Moynahan *et al.* (2002) found that topsoil placed over acidic mine tailings provided indigenous soil microbiota and increased the microbial community to a greater degree than non-topsoiled treatments. The same study found that topsoil treatments had similar microbial diversity as the undisturbed, native soil.

The waste rock treatments and reference sites contained four bacterial PLFAs that represent sulfur-oxidizing bacteria. These PLFAs include 16:1ω7c, cy17:0, 18:1ω7c, and cy19:0 (Appendix Table 4). Sulfur-oxidizing bacteria (SOxB) are important in this study

because of their prominent role in the oxidation of pyrite and the development of acid mine drainage (Germida 1998). The un-treated waste rock had the greatest proportion of SOxB at 61 mol% (1) of bacterial PLFAs (Figure 3.6). This was to be expected since SOxB are acidophiles, and the waste rock had a pH of 2.8 (0.1). According to Alexander (1998), SOxB are not only tolerant of the acidic conditions that they produce, but are actually unable to grow at soil pH greater than 4.0. This study found a negative correlation between soil pH and the proportion of SOxB ($R = -0.646$; Appendix Table 6). The waste rock treatments decreased the proportion of SOxB from that of the un-treated waste rock. The mushroom compost treatment (t-M) had the lowest proportion of SOxB of the waste rock treatments at 46 mol% (2) and the highest soil pH (6.0 ± 0.4; Figure3.6). The mushroom compost treatment (t-M) successfully reached a similar proportion of SOxB as the sub-alpine clear-cut forest and sub-alpine forest reference sites (Figure 3.6). The stockpiled and non-stockpiled topsoil treatments (t-SM and t-NM, respectively) had 53 mol% SOxB (1) and 50 mol% SOxB (1), respectively (Figure 3.6). These topsoil treatments (t-NM and t-SM) did not have similar proportions of SOxB as the native reference sites (Figure 3.6).

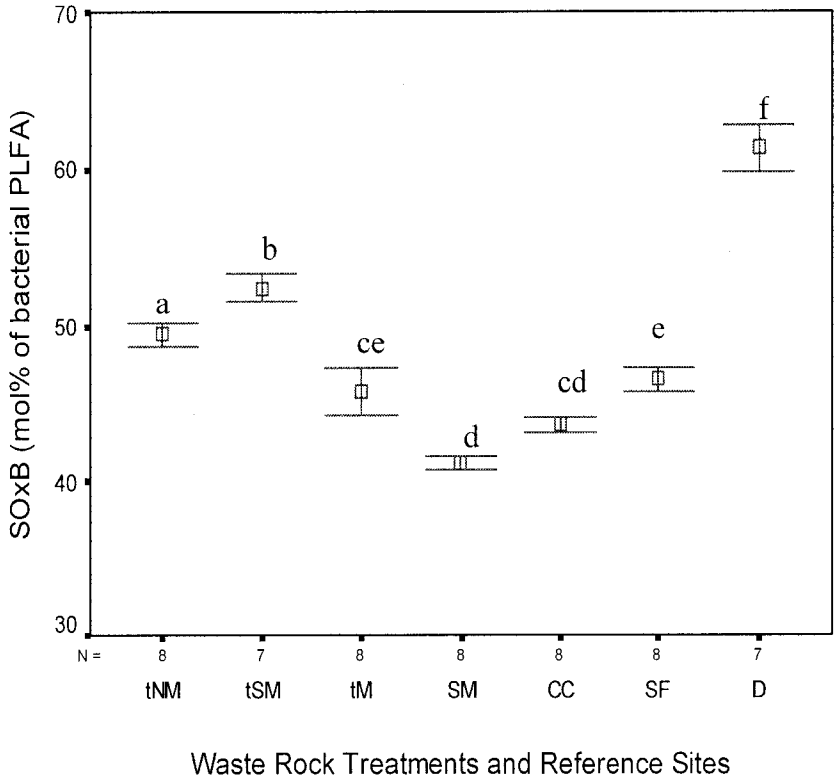

Figure 3.6. Average amount of sulfur-oxidizing bacteria (SOxB), expressed as mol% of bacterial phospholipid fatty acids (PLFA), of waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D) in 2000. Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

Total Microbial Biomass

The total bacterial and fungal biomass of the waste rock treatments and reference sites was measured by the microscopy analysis. The waste rock treatments had greater bacterial total biomass than the un-treated waste rock (site D) in 2001, however, the waste rock treatments were significantly similar to site D in fungal total biomass in 2001

(Figure 3.7 and 3.8). The mushroom compost treatment (t-M) had a greater bacterial total biomass than the stockpiled topsoil treatment (t-SM) while the non-stockpiled topsoil treatment (t-NM) had a bacterial total biomass in between and significantly similar to t-M and t-SM (Figure 3.7). This study found bacterial total biomass to be positively correlated with soil organic matter (R = 0.716) where t-M had the highest soil organic matter level of the waste rock treatments (Figure 3.3; Appendix Table 6). The mushroom

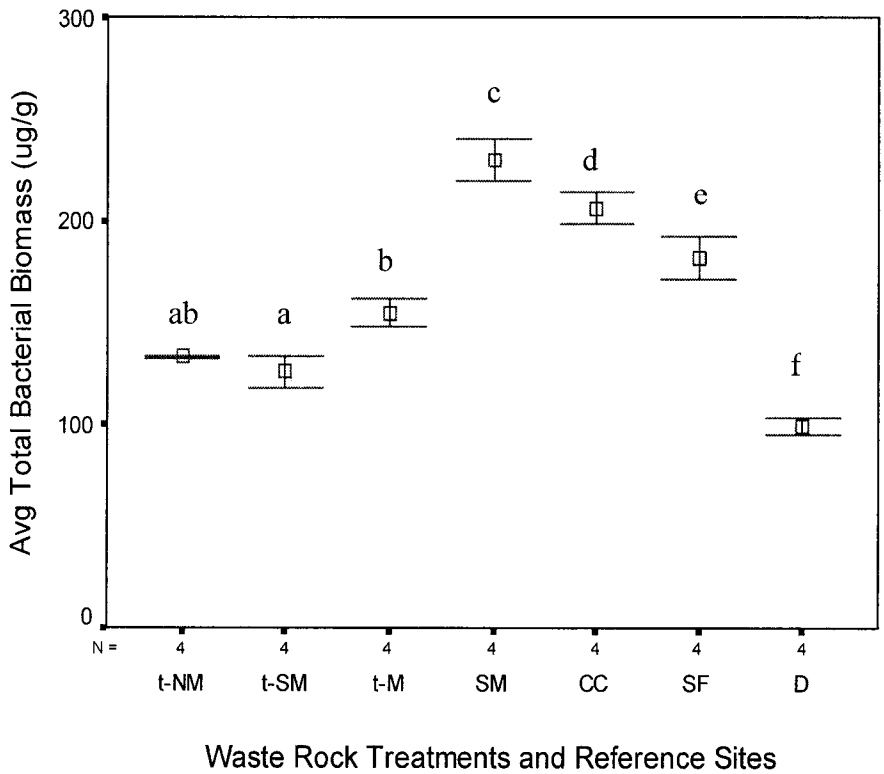

Figure 3.7. Average, soil bacterial total biomass for waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D) in 2001. Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

compost, lime, stockpiled and non-stockpiled topsoil amendments provided similar fungal total biomass among the waste rock treatments (Figure 3.8). In general, it was found that bacterial and fungal total biomass were negatively correlated with total metal concentrations (R= -0.599 and –0.560, respectively; Appendix Table 6). The waste rock treatments had lower total metal concentrations than the un-treated waste rock (Figure 3.4).

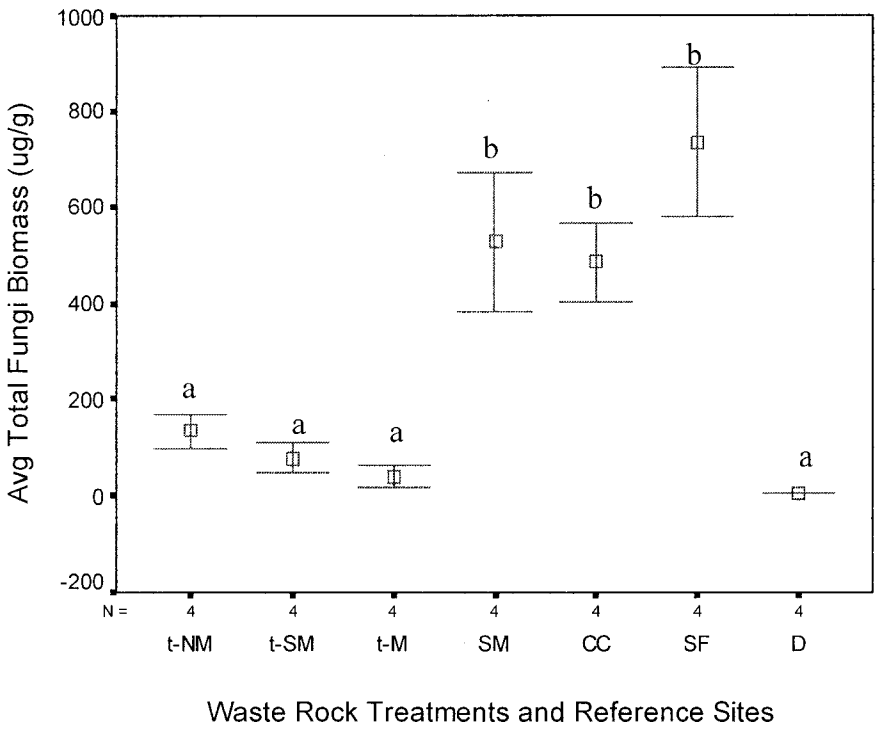

Figure 3.8. Average, soil fungal total biomass for waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D) in 2001. Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

The waste rock treatments did not have similar bacterial and fungal total biomass compared to the native reference sites in 2001 (Figure 3.7 and 3.8). The differences in magnitude between the waste rock treatments and native reference sites were less for bacterial total biomass than fungal total biomass. Allen *et al.* (1999) noted that bacteria are generally favored rather than fungi after a soil disturbance. The native reference sites had more fungal total biomass than the waste rock treatments possibly due to the successional stages of the sites. A study by Ohtonen *et al.* (1999) at the Lyman Glacier (1800 m) in Washington found that it took over 60 years of natural succession for the microbial community to shift from a bacterial-dominated to a fungal-dominated community. Successional studies by Klein *et al.* (1995 and 1998) in a shortgrass steppe community found the proportion of fungal biomass to increase with time after cultivation was abandoned, and total fungal hyphal lengths to have higher values in late-seral and uncultivated sites than early-seral sites. Sylvia (1998), Kumar *et al.* (1999), and Carroll and Wicklow (1992) concluded that soil ecosystems in later stages of succession are dominated by fungi due to their associations with plants. This study found fungal total biomass to be positively correlated to total plant cover (R=0.489) and soil organic matter (R = 0.803; Appendix Table 6). The waste rock treatments will continue to increase in bacterial and fungal total biomass as succession proceeds in the treatment plots and organic matter accumulates.

Microbial Community Function

Microbial community function of the waste rock treatments and reference sites was determined by microbial utilization of 128 carbon-substrates during the Biolog

analysis. Several studies have used the Biolog analysis to determine the functional capabilities of the soil microbial community (Garland and Mills 1991; Zak *et al.* 1994; Knight *et al.* 1997; Moynahan *et al.* 2002; Pennanen *et al.* 1998; Meyer *et al.* 1998). Moynahan *et al.* (2002) concluded that a microbial community that can utilize a variety of carbon-substrates in the Biolog analysis will be more able to decompose a diverse amount of plant litter in the field. Microbial decomposition of many litter types is an important aspect of soil development. Restored decomposition by the microbial community ensures the maintenance of soil fertility and the nutrient cycle.

The waste rock treatments increased the extent of total substrate utilization by the microbial community relative to the un-treated waste rock in 2001 (Figure 3.9). Several authors have concluded that elevated metal concentrations and acidic soil pH can reduce the ability of the microbial community to utilize carbon substrates (Kelly and Tate 1998; Knight *et al.* 1997; Moynahan *et al.* 2000; Alexander 1980). The un-treated waste rock had an extremely acidic pH and high metal concentrations, especially cadmium and copper. This study found a negative correlation between total metal concentration and total substrate utilization by the microbial community ($R = -0.427$; Appendix Table 6). The waste rock treatments ranged in total substrate utilization from 3.6 to 5.9 mean AWCD (Figure 3.9). The mushroom compost, lime, stockpiled and non-stockpiled topsoil amendments resulted in similar total substrate utilization by the microbial communities of the waste rock treatments. This study found bacterial and fungal total biomass to be positively correlated with total substrate utilization ($R = 0.564$ and 0.441, respectively) where bacterial biomass had the stronger correlation (Appendix Table 6). Soil organic matter was also found to have a positive correlation to total substrate

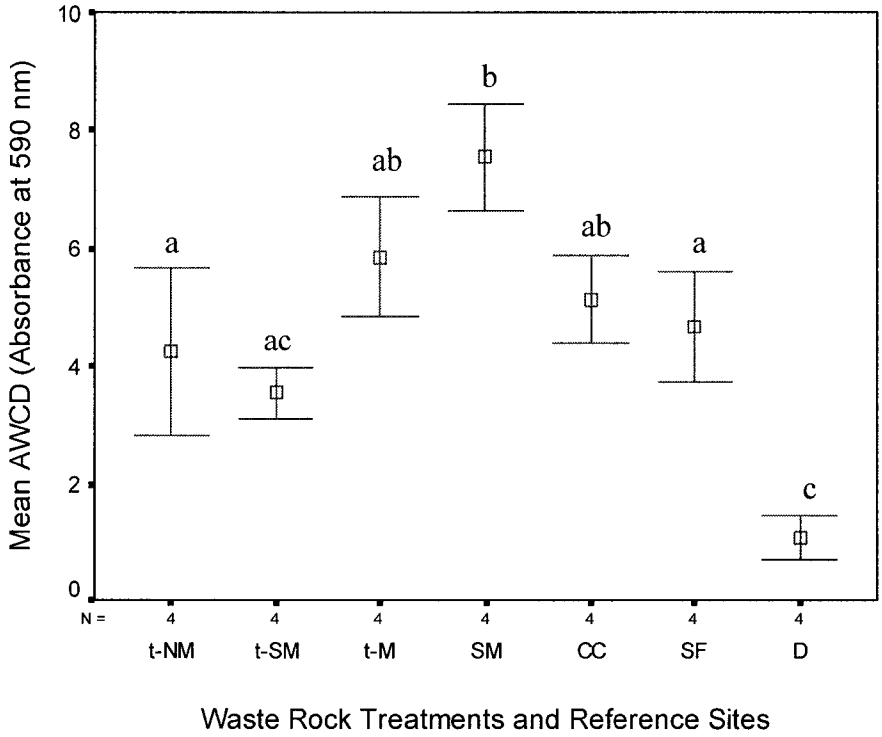

Figure 3.9. Total substrate utilization, expressed as the mean AWCD (average well color development), by the soil microbial community for waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D) in 2001. Well-color absorbance readings were subjected to square root transformations before analysis. Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

utilization by the microbial community (R = 0.571; Appendix Table 6). For both soil organic matter and bacterial total biomass, the mushroom compost treatment (t-M) had more than the stockpiled topsoil treatment (t-SM).

The waste rock treatments have similar total substrate utilizations by their microbial communities as the native reference sites in 2001 (Figure 3.9). The mushroom

compost treatment (t-M) had a similar total substrate utilization as all three native reference sites (Figure 3.9). The stockpiled and non-stockpiled topsoil treatments (t-SM and t-NM, respectively) were similar to the sub-alpine clear-cut forest and sub-alpine forest reference sites in total substrate utilization (Figure 3.9). Other research found similar results in total substrate utilization by microbial communities after reclamation of disturbed soil. Moynahan *et al.* (2002) showed mine tailings treated with topsoil and an undisturbed reference site to have higher substrate utilization levels by the microbial community than un-treated mine tailings, which had the lowest substrate utilization level. A mixture of sewage sludge and fly-ash applied to metal-contaminated soil from a Zn smelter significantly improved the substrate utilization levels of the microbial community (Kelly and Tate 1998).

The waste rock treatments have microbial communities that can utilize a greater number of carbon-substrates relative to the un-treated waste rock in 2001 (Figure 3.10). Appendix Table 7 lists 11 carbon-substrate categories and their utilization levels by the microbial communities of the waste rock treatments and reference sites. Knight *et al.* (1997) and Meyer *et al.* (1998) reported that the number of substrates used by the soil microbial community decreased with the increase in heavy metals and soil acidity. This study found a negative correlation between substrate utilization richness and total metal concentrations ($R = -0.597$; Appendix Table 6). After four years of soil acidification, Kytoviita *et al.* (1990) found reductions in bacterial utilization of a variety of carbon substrates, such as starch, protein, pectin, and cellulose. The waste rock treatments ranged in substrate utilization richness from 81 to 103 substrates out of the 128 total substrates (Figure 3.10). The mushroom compost, lime, stockpiled and non-stockpiled

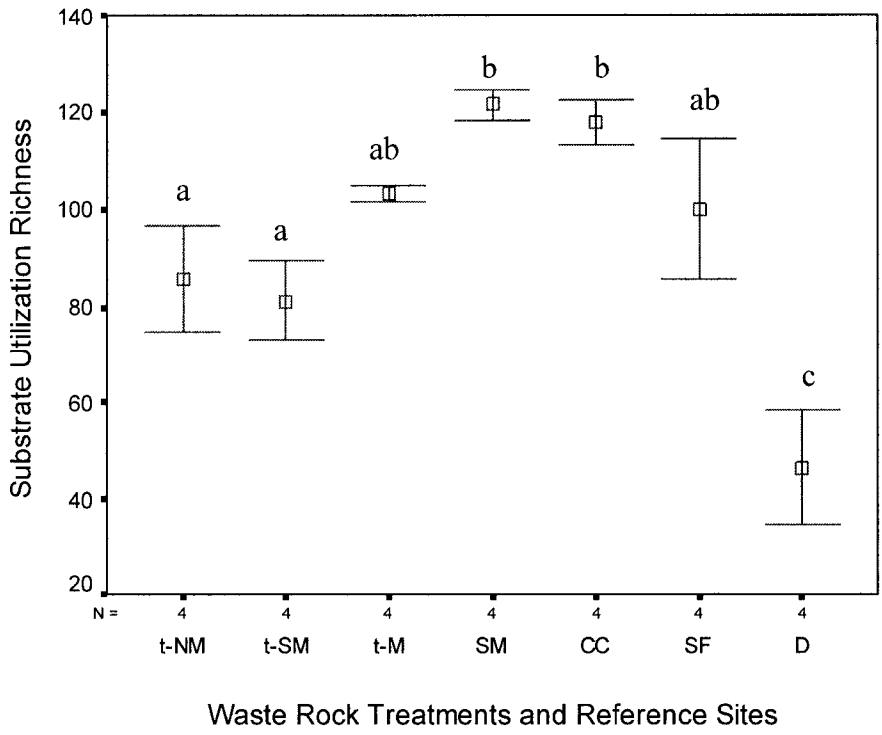

Figure 3.10. The number of carbon-substrates utilized by the microbial community of the waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D) in 2001. Gram positive and negative Biolog micro-plates were used to provide a total of 128 carbon-substrates. Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

topsoil amendments of the waste rock treatments resulted in similar richness in substrate utilization by the microbial communities (Figure 3.10). Substrate utilization richness was found to be positively correlated with total substrate utilization (R = 0.625; Appendix Table 6). Both Biolog analyses provided similar results for microbial community function. The amount of soil organic matter and total bacterial and fungal biomass were positively correlated to substrate utilization richness (R = 0.672, 0.779, and 0.785,

respectively; Appendix Table 6). A weak correlation was found between substrate utilization richness and active microbial richness (R = 0.395) with a stronger correlation between substrate utilization richness and plant species richness (R = 0.532) and total plant cover (R = 0.408; Appendix Table 6).

The waste rock treatments had similar substrate utilization richness by their microbial communities as the native reference sites in 2001 (Figure 3.10). The mushroom compost treatment (t-M) had a similar substrate utilization richness by its microbial community as all three native reference sites (Figure 3.10). The stockpiled and non-stockpiled topsoil treatments (t-SM and t-NM, respectively) were similar to the sub-alpine forest reference site in substrate utilization richness (Figure 3.10).

Microbial community function was determined in the field by cotton and alfalfa litter decomposition after one year. Microbial decomposition of organic matter depends on the ability of the microbial community to metabolize the carbon source and the limitations imposed by the physicochemical conditions in the field (Tate 1985). Decomposition of organic matter can also be affected by other factors that were not controlled for in this study. Microorganisms initially present on the cotton and alfalfa may assist the soil microorganisms in the decomposition of these organic materials, since neither the cotton nor alfalfa were sterilized before their application in the field. Other soil organisms, such as the mesofauna (mites and collembolan) and microfauna (nematodes), could have also contributed to the cotton and alfalfa decomposition in the field (Brady and Weil 1996). The decomposition of alfalfa and cotton was not determined in the un-treated waste rock because no litterbags could be collected from site D due to construction over the site.

The waste rock treatments ranged in alfalfa decomposition from 2.2 to 2.5g in 2001 (Figure 3.11). The mushroom compost, lime, stockpiled and non-stockpiled topsoil amendments provided similar alfalfa decomposition in the waste rock treatments (Figure 3.11). All three waste rock treatments had similar alfalfa decomposition as the native reference sites in 2001 (Figure 3.11). Alfalfa was easily decomposed by all microbial communities because of its nutritional content. Alfalfa is a legume with a relatively high

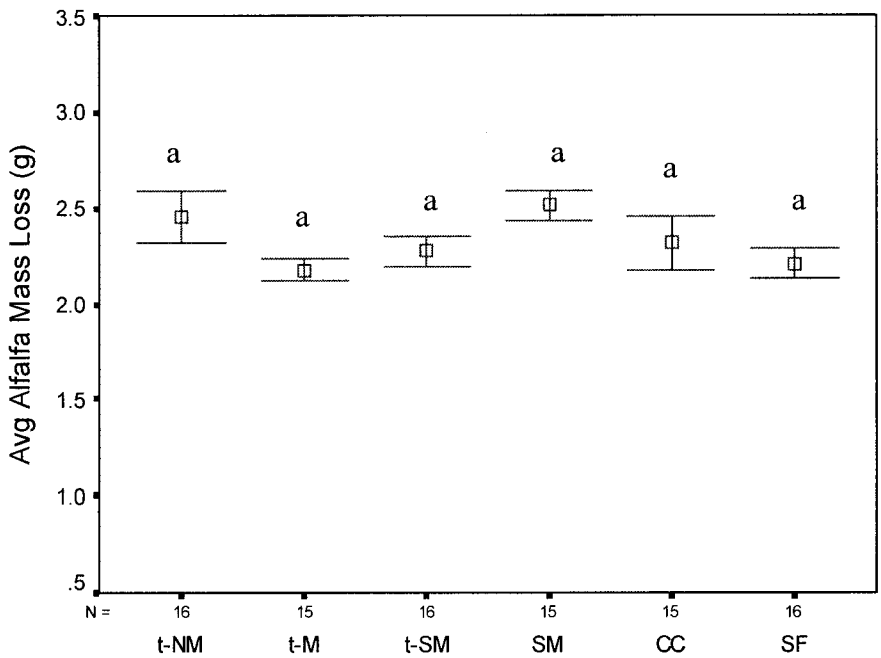

Figure 3.11. Average alfalfa decomposition, expressed as mass loss (g), for waste rock treatments (t-NM, t-SM, t-M) and native reference sites (SM, CC, SF) after one year (2000-2001). Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

proportion of protein (2-4 %) in its biomass compared to other herbaceous plants (Munshower 1994). Simple proteins along with sugars and starches are easily decomposed and rapidly oxidized by the soil microbial community as a source of energy (McRae *et al.* 2000). Heneghan *et al.* (1998) reported that litter decomposition after 250 days is positively related to the initial %N of the litter (r^2=0.997). Another possible reason for the universal alfalfa decomposition may be due to experimental procedures. Some of the dried alfalfa could have broken into smaller pieces and fallen through the mesh holes of the litterbags contributing to the mass loss of alfalfa.

The waste rock treatments ranged in cotton decomposition from 0.7 to 2.3g in 2001 (Figure 3.12). The non-stockpiled topsoil treatment (t-NM) had the highest cotton decomposition of the waste rock treatments (Figure 3.12). The stockpiled topsoil treatment (t-SM) had lower cotton decomposition than t-NM with the mushroom compost treatment (t-M) having the lowest cotton decomposition of the waste rock treatments (Figure 3.12). The non-stockpiled and stockpiled topsoil treatments (t-NM and t-SM, respectively) had similar cotton decomposition as native reference sites. The non-stockpiled topsoil treatment (t-NM) was similar to the sub-alpine meadow and sub-alpine forest reference sites in cotton decomposition (Figure 3.12). The stockpiled topsoil treatment (t-SM) only had similar cotton decomposition as the sub-alpine forest reference site (Figure 3.12). The mushroom compost treatment (t-M) was not similar to any of the native reference sites in cotton decomposition as reported for alfalfa decomposition. Cotton decomposition varied more among the waste rock treatments than alfalfa because of the composition of cotton. Cotton is made of 91% cellulose and 0.4% waxes and fatty substances (Cotton Website 1997). Cellulose and waxes are more complex compounds

and harder for the microbial community to decompose than simple proteins, sugars, and starches (McRae *et al.* 2000; Brady and Weil 1996). Alexander (1998) stated that decomposition of complex constituents of organic matter typically requires functional cooperation from a diverse microbial community. This study found a positive correlation between the microbial community function (measured by substrate utilization richness) and the richness in microbial community structure (R = 0.395; Appendix Table 6). The

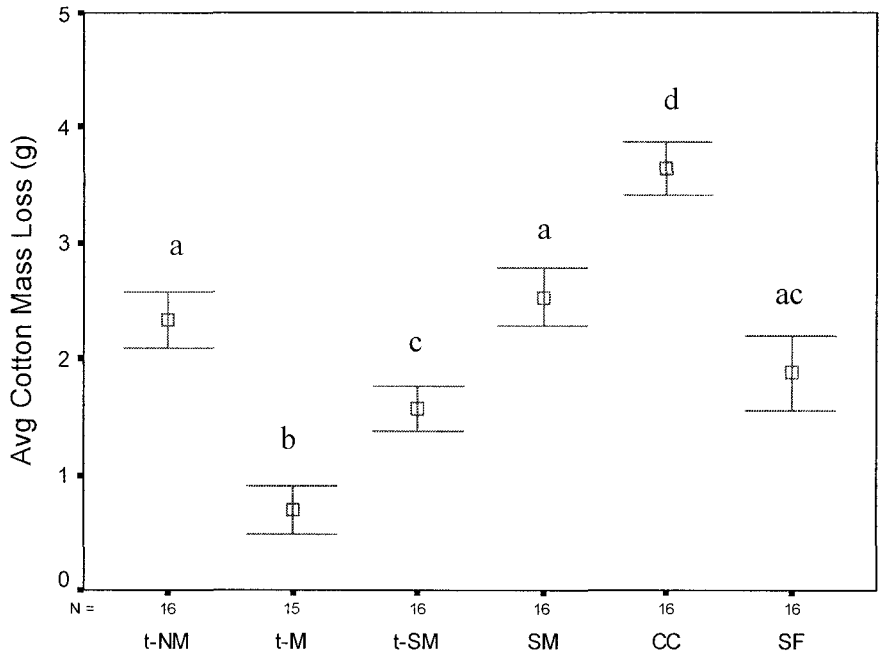

Figure 3.12. Average cotton decomposition, expressed as mass loss (g), for waste rock treatments (t-NM, t-SM, t-M) and native reference sites (SM, CC, SF) after one year (2000-2001). Different letters represent significant mean differences (α=0.05). T-bars represent ± standard error of the means. Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

non-stockpiled topsoil treatment (t-NM) had the greatest richness in microbial community structure of the waste rock treatments (Table 3.5). Fungi and actinomycetes are the primary decomposers of cellulose and waxes in organic matter (Morton 1998; Alexander 1998). The non-stockpiled topsoil treatment (t-NM) also had the greatest proportion (mol%) of active fungi and actinomycetes in its microbial community structure (Table 3.5).

Summary of Results

The following is a summary of the soil microbial community, physicochemical, and plant community characteristics measured in 2000 and 2001. These results are shown in Tables 3.6 and 3.7 and represent soil and plant conditions six years following reclamation at the Summitville Mine.

Plant Properties

The mushroom compost and lime amendments of t-M, the non-stockpiled topsoil of t-NM, and the limed stockpiled topsoil of t-SM all had similar plant cover and species richness (Table 3.6). From these results, I accept hypothesis H30. The plant cover of t-M, t-NM, and t-SM was similar to the three native reference sites (SM, CC, and SF; Table 3.7). The plant species richness of t-M and t-SM was similar to the sub-alpine clear-cut forest while t-NM had plant species richness between that of the sub-alpine clear-cut forest and the sub-alpine forest (Table 3.7). Based on these results, I reject hypotheses H31 and H32.

Table 3.6. Summary of the plant community measures, physicochemical properties, and microbial community characteristics of the waste rock treatments (t-M, t-SM, t-NM) in 2000 and 2001. Standard error of the means is represented in ( ). Different letters per row represent significantly different means (α=0.05). Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock.

	Waste Rock Treatments		
Properties	**t-M**	**t-SM**	**t-NM**
Plant cover (%)	78.8 (5.5) **A**	77.2 (5.3) **A**	76.3 (6.2) **A**
Plant species richness	9.8 (0.6) **A**	10.5 (0.6) **A**	9.5 (0.6) **A**
pH	6.0 (0.4) **A**	5.5 (0.3) **AB**	4.9 (0.4) **B**
Soil organic matter (%)	7.9 (1.9) **A**	2.7 (0.2) **B**	5.0 (0.1) **AB**
Organic N (mg/kg)	4,160 (1,048) **A**	913 (115) **B**	2,034 (58) **B**
Inorganic N - NH_4^+ and NO_3^- (mg/kg)	7.7 (1.1) **A**	9.4 (1.6) **A**	10.9 (2.7) **A**
Total metals (mg/kg)	358 (65) **A**	213 (12) **B**	464 (43) **A**
Active microbial biomass (pmol/g)	14,500 (1,400) **A**	13,700 (1,200) **A**	17,900 (1,700) **A**
Bacterial total biomass (ug/g)	155 (7) **A**	126 (8) **B**	134 (1) **AB**
Fungal total biomass (ug/g)	40 (21) **A**	77 (32) **A**	134 (35) **A**
Active microbial richness	17.6 (0.3) **A**	18.6 (0.9) **A**	21.4 (0.4) **B**
Sulfur-oxidizing bacteria (mol%)	46 (2) **A**	53 (1) **B**	49 (1) **C**
Total substrate utilization (mean AWCD)	5.9 (1.0) **A**	3.6 (0.4) **A**	4.2 (1.4) **A**
Substrate utilization richness	103 (2) **A**	81 (8) **A**	86 (11) **A**
Alfalfa decomposition (g)	2.2 (0.1) **A**	2.3 (0.1) **A**	2.5 (0.1) **A**
Cotton decomposition (g)	0.7 (0.2) **A**	1.6 (0.2) **B**	2.3 (0.2) **C**

Table 3.7. Summary of the plant community measures, physicochemical properties, and microbial community characteristics of the native reference sites (SM, CC, SF) compared to the waste rock treatments (t-M, t-SM, t-NM) in 2000 and 2001. Native reference site codes (SM, CC, SF) per row represent significantly similar means (α=0.05) between the waste rock treatment and native reference site. The abbreviation, NS, indicates the means are not significantly similar (α=0.05) between the waste rock treatment and native reference sites. Standard error of the means is represented in ( ). Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest.

	Waste Rock Treatments			**Native Reference Sites**		
Properties	**t-M**	**t-SM**	**t-NM**	**SM**	**CC**	**SF**
Plant cover (%)	**SM, CC, SF**	**SM, CC, SF**	**SM, CC, SF**	90.6 (3.3)	88.0 (3.0)	79.8 (3.7)
Plant species richness	**CC**	**CC**	NS	16.5 (0.3)	12.0 (0.4)	6.5 (1.6)
pH	NS	**SM**	**SM, CC, SF**	5.0 (0.1)	4.6 (0.1)	4.5 (0.1)
Soil organic matter (%)	**SM, SF**	NS	NS	10.7 (0.8)	15.8 (0.9)	11.0 (2.4)
Organic N (mg/kg)	**SM, CC, SF**	NS	**SF**	5,629 (283)	5,355 (200)	3,149 (644)
Inorganic N-NH_4^+, NO_3^- (mg/kg)	**SF**	**SF**	**SF**	31.0 (1.5)	22.3 (2.8)	8.5 (2.4)
Total metal (mg/kg)	**CC**	**SM, SF**	**CC**	162 (6)	353 (31)	121 (11)
Active microbial biomass (pmol/g)	NS	NS	NS	43,200 (4,100)	51,200 (6,900)	49,900 (5,100)
Bacterial total biomass (ug/g)	NS	NS	NS	230 (10)	206 (8)	182 (10)
Fungal total biomass (ug/g)	NS	NS	NS	526 (145)	484 (83)	734 (157)
Active microbial richness	NS	NS	**SM**	21.9 (0.5)	23.3 (0.6)	23.0 (0.6)
Sulfur-oxidizing bacteria (mol%)	**CC, SF**	NS	NS	41 (0.4)	44 (0.4)	47 (1)
Total substrate utilization (mean AWCD)	**SM, CC, SF**	**CC, SF**	**CC, SF**	7.5 (0.9)	5.1 (0.8)	4.7 (0.9)
Substrate utilization richness	**SM, CC, SF**	**SF**	**SF**	122 (3)	118 (5)	100 (14)
Alfalfa decomposition (g)	**SM, CC, SF**	**SM, CC, SF**	**SM, CC, SF**	2.5 (0.1)	2.3 (0.1)	2.2 (0.1)
Cotton decomposition (g)	NS	**SF**	**SM, SF**	2.5 (0.3)	3.6 (0.2)	1.9 (0.3)

Physicochemical Properties

The mushroom compost and lime amendments of t-M resulted in a higher soil pH than the non-stockpiled topsoil of t-NM (Table 3.6). The limed stockpiled topsoil of t-SM was similar in pH to both t-M and t-NM (Table 3.6). From these results, I reject hypothesis H20. The soil pH of t-M was higher than all three native reference sites (SM, CC, and SF; Table 3.7). Therefore, I reject hypothesis H28. The soil pH of t-NM was similar to all three native reference sites while t-SM was only similar in pH to the sub-alpine meadow (Table 3.7). From these results, I reject hypothesis H24.

The mushroom compost and lime amendments of t-M provided greater soil organic matter than the limed stockpiled topsoil of t-SM (Table 3.6). The non-stockpiled topsoil of t-NM was similar in soil organic matter to both t-M and t-NM (Table 3.6). The soil organic N of t-M was the greatest of the waste rock treatments with both topsoil treatments (t-SM and t-NM) having similar organic N concentrations (Table 3.6). Based on these results, I reject hypothesis H18. The soil organic matter of t-M was similar to the sub-alpine meadow and sub-alpine forest while t-NM and t-SM were lower than all three native reference sites (SM, CC, and SF) in soil organic matter (Table 3.7). The soil organic N of t-M was similar to all three native reference sites (SM, CC, and SF; Table 3.7). The organic N of t-NM was similar to the sub-alpine forest while t-SM had lower organic N than the native reference sites (Table 3.7). From these results, I reject hypotheses H22 and H26.

The mushroom compost and lime amendments of t-M, the non-stockpiled topsoil of t-NM, and the limed stockpiled topsoil of t-SM all had similar soil inorganic N, measured as ammonium and nitrate (Table 3.6). The soil inorganic N of t-M, t-NM, and

t-SM was similar to the sub-alpine forest (Table 3.7). From these results, I reject hypotheses H19, H23, and H27.

The limed stockpiled topsoil of t-SM had the lowest total metal concentration of the waste rock treatments with both the non-stockpiled topsoil treatment (t-NM) and the mushroom compost treatment (t-M) having similar total metal concentrations (Table 3.6). From these results, I reject hypothesis H21. The total metal concentrations of t-NM and t-M were similar to the sub-alpine clear-cut forest (Table 3.7). The total metal concentration of t-SM was similar to the sub-alpine meadow and sub-alpine forest (Table 3.7). Based on these results, I reject both hypotheses H25 and H29.

Microbial Properties

The mushroom compost and lime amendments of t-M, the non-stockpiled topsoil of t-NM, and the limed stockpiled topsoil of t-SM all resulted in similar active microbial biomass (Table 3.6). The bacterial total biomass of t-M was greater than t-SM with t-NM having a similar bacterial total biomass as both t-M and t-SM (Table 3.6). The fungal total biomass of t-M, t-NM, and t-SM was similar (Table 3.6). Based on these results, I reject hypotheses H4, H5, and H6. The active microbial biomass, bacterial total biomass, and fungal total biomass of t-M, t-NM, and t-SM were all lower than the three native reference sites (SM, CC, and SF; Table 3.7). Therefore, I reject hypothesis H7 and accept hypothesis H8.

The non-stockpiled topsoil of t-NM had the greatest richness in microbial community structure of the waste rock treatments with both the limed stockpiled topsoil treatment (t-SM) and the mushroom compost treatment (t-M) having similar richness in

microbial community structure (Table 3.6). From these results, I reject hypothesis H1. The microbial community richness of t-NM was similar to the sub-alpine meadow and lower than the other native reference sites (CC and SF; Table 3.7). The microbial community richness of t-SM and t-M was lower than the three native reference sites (SM, CC, and SF; Table 3.7). Based on these results, I reject hypothesis H2 and accept my hypothesis H3.

The mushroom compost and lime amendments of t-M decreased the proportion of sulfur-oxidizing bacteria in the soil more than the non-stockpiled topsoil of t-NM and the limed stockpiled topsoil of t-SM (Table 3.6). The proportions of sulfur-oxidizing bacteria of t-NM and t-SM were higher than the native reference sites (SM, CC, and SF; Table 3.7). The proportion of sulfur-oxidizing bacteria of t-M was similar to the sub-alpine clear-cut forest and the sub-alpine forest (Table 3.7).

The mushroom compost and lime amendments of t-M, the non-stockpiled topsoil of t-NM, and the limed stockpiled topsoil of t-SM all resulted in similar total substrate utilization by their microbial communities (Table 3.6). Therefore, I reject hypothesis H9. The total substrate utilization by the microbial community of t-M was similar to all three native reference sites (SM, CC, and SF; Table 3.7). The total substrate utilizations of t-NM and t-SM were similar to the sub-alpine clear-cut forest and the sub-alpine forest (Table 3.7). From these results, I reject hypotheses H11 and H13.

The mushroom compost and lime amendments of t-M, the non-stockpiled topsoil of t-NM, and the limed stockpiled topsoil of t-SM all resulted in similar substrate utilization richness by their microbial communities (Table 3.6). Therefore, I reject hypothesis H10. The substrate utilization richness by the microbial community of t-M

was similar to all three native reference sites (SM, CC, and SF; Table 3.7). The substrate utilization richness of t-NM and t-SM was similar to the sub-alpine forest (Table 3.7). Based on these results, I reject hypotheses H12 and H14.

The mushroom compost and lime amendments of t-M, the non-stockpiled topsoil of t-NM, and the limed stockpiled topsoil of t-SM all provided similar alfalfa decomposition (Table 3.6). The greatest cotton decomposition occurred in t-NM while t-M had the lowest cotton decomposition of the waste rock treatments (Table 3.6). The cotton decomposition of t-SM was in between that of t-NM and t-M (Table 3.6). Based on these results, I reject hypothesis H15. The alfalfa decomposition of t-M, t-NM, and t-SM was similar to all three native reference sites (SM, CC, and SF; Table 3.7). The cotton decomposition of t-NM was similar to the sub-alpine meadow and sub-alpine forest while the cotton decomposition of t-SM was only similar to the sub-alpine forest (Table 3.7). The cotton decomposition of t-M was lower than the native reference sites (SM, CC, and SF; Table 3.7). From these results, I reject both hypotheses H16 and H17.

CHAPTER IV

Conclusion

Six years after the test plots were established at the Summitville Mine, the combination of soil microbial community characteristics with physicochemical properties and plant community measures indicated soil development in the waste rock treatments. The waste rock treatments had acceptable soil pH and total metal concentrations, soil organic and inorganic nutrients, and soil microbial community structure and function to support plant life. This research found non-stockpiled topsoil (t-NM) or the combination of mushroom compost and lime amendments (t-M) to be the most effective treatments for the reclamation of acidic waste rock in a sub-alpine ecosystem. Both t-NM and t-M had more soil characteristics that were similar to the native reference sites than the limed stockpiled topsoil of t-SM. The non-stockpiled topsoil treatment (t-NM) and the mushroom compost treatment (t-M) had plant cover and microbial community function that were similar to the surrounding native reference sites. These waste rock treatments (t-NM and t-M) also resulted in similar soil organic N, inorganic N (ammonium and nitrate), and total metal concentrations as the native reference sites.

The waste rock treatments developed soil microbial community characteristics similar to the sub-alpine native reference sites six years after reclamation. This study found that the application of mushroom compost and lime amendments (t-M), non-stockpiled topsoil (t-NM), or limed stockpiled topsoil (t-SM) resulted in microbial

community function similar to the native reference sites. Microbial community function was measured as carbon-substrate utilization, the richness of utilized carbon-substrates, and litter decomposition. The waste rock treatments had functioning microbial communities, however, this study reveled that the waste rock treatments did not have similar microbial biomass as the native reference sites. Active microbial biomass and total bacterial and fungal biomass were lower in all three waste rock treatments (t-NM, t-SM, and t-M) than the native reference sites. Only the non-stockpiled topsoil treatment (t-NM) was similar to the sub-alpine meadow reference site in microbial community richness, which included bacteria, fungi, and actinomycetes. It is important that the waste rock treatments have a functioning microbial community to carry out essential soil processes, such as decomposition and nutrient cycling, which will help maintain the plant community. The microbial active and total biomass of the waste rock treatments is predicted to increase as the plant community and soil continue to develop over time.

One of the unique aspects of this research was the use of many different analyses to measure a range of soil microbial community, physicochemical, and plant characteristics. This study found three soil characteristics and their respective analyses to have significant correlations with many other soil characteristics.

1). Substrate utilization richness (Biolog micro-plates):
- Plant cover (R = .408)
- Plant species richness (R =.532)
- Soil organic matter (R = .672)
- Bacterial total biomass (R = .779)
- Fungal total biomass (R = .785)
- Total metal concentration (R = -.597)
- Microbial community richness/structure (R = .395)

2). Bacterial total biomass (Microscopy):
- Soil organic matter (R = .716)

Total metal concentration (R = -.599)
Ammonium-NH_4^+ (R = .559)
Nitrate-NO_3^- (R = .529)
Fungal total biomass (R = .780)
Substrate utilization richness (R = .779)
Microbial community richness/structure (R = .569)

3). Soil organic matter (Total and inorganic carbon):
Total metal concentration (R = -.387)
Nitrate-NO_3^- (R = .562)
Bacterial total biomass (R = .716)
Fungal total biomass (R = .803)
Substrate utilization richness (R = .672)

The relevance of these correlations is that one of these soil characteristics could be used to assess soil development after reclamation without having to run each individual soil analysis. Soil organic matter is the easiest and least expensive characteristic to measure. However, substrate utilization richness and bacterial total biomass provide more significant correlations than soil organic matter. The soil characteristic and the analysis chosen will depend on specific objectives and any economic constraints that may exist.

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APPENDIX

Appendix Table 1. Summitville Mine climate data for January 1998 through December 2001. Temperature and precipitation data were recorded from Wolf Creek Pass weather station (ID number = 59181 and elevation = 3,243 m). Temperature (T) is represented as an average, while precipitation (ppt) is a total for each given month. NA = data not available.

	Avg. T (°C)	Total ppt (cm)		Avg. T (°C)	Total ppt (cm)
Jan-98	-8.0	6.7	Jan-99	-7.2	8.5
Feb-98	-9.0	8.5	Feb-99	-6.7	7.7
Mar-98	-6.0	18.1	Mar-99	-2.2	3.4
Apr-98	-4.0	9.1	Apr-99	-3.3	21.2
May-98	4.0	2.0	May-99	-1.1	10.3
Jun-98	8.0	0.8	Jun-99	7.2	3.3
Jul-98	13.0	12.2	Jul-99	11.1	19.9
Aug-98	11.0	7.9	Aug-99	10.0	22.6
Sep-98	9.0	3.7	Sep-99	6.1	19.6
Oct-98	1.0	27.3	Oct-99	3.9	1.6
Nov-98	-4.0	15.0	Nov-99	0.6	1.6
Dec-98	-7.0	7.4	Dec-99	-7.8	2.1
Total-98:		118.7	Total-99:		121.8

	Avg. T (°C)	Total ppt (cm)		Avg. T (°C)	Total ppt (cm)
Jan-00	-7.4	6.6	Jan-01	-9.3	13.8
Feb-00	-6.6	14.0	Feb-01	-8.0	16.0
Mar-00	-5.4	17.8	Mar-01	-5.3	10.1
Apr-00	0.7	3.1	Apr-01	-0.9	20.0
May-00	6.0	3.8	May-01	5.5	4.2
Jun-00	10.5	5.9	Jun-01	10.0	2.5
Jul-00	12.5	6.0	Jul-01	12.4	13.3
Aug-00	11.7	8.8	Aug-01	10.6	13.9
Sep-00	8.7	6.7	Sep-01	8.2	2.3
Oct-00	1.7	20.2	Oct-01	3.0	4.1
Nov-00	-8.5	10.1	Nov-01	-3.0	9.6
Dec-00	-7.1	6.2	Dec-01	NA	NA
Total-00:		109.2	Total-01:		109.8

Appendix Table 2. Seed mixture and seeding rate used on revegetation test plots. Environmental factors influencing plant growth, including a rating of acid tolerance (AT) and average required precipitation (ppt), are shown. N = native; I = introduced; P = Perennial.

	Common Name	Scientific Name	Environmental Factors	Seeding Rate (lb PLS/ac)
	Grasses:			
N/P	Slender wheatgrass	*Agropyron trachycaulum*	16" ppt; AT = 3*	3.0
I/P	Redtop	*Agrostis alba*	20" ppt; AT = 3	1.0
N/P	Bentgrass	*Agrostis scabra*	no information	1.0
I/P	Meadow foxtail	*Alopecurus pratensis*	25" ppt; AT = 2	1.0
N/P	Mountain Brome var. Bromar	*Bromus marginatus*	16" ppt; AT = 0	3.0
I/P	Orchardgrass var. Latar	*Dactylis glomerata*	18" ppt; AT = 2	2.0
N/P	Tufted hairgrass	*Deschampsia caespitosa*	20" ppt; AT = 2	1.0
I/P	Sheep fescue var. Durar	*Festuca ovina*	10-12" ppt; AT = 1	1.0
N/P	Alpine Timothy	*Phleum alpinum*	no information	1.0
N/P	Alpine bluegrass	*Poa alpina*	20" ppt; AT = 1	1.0
N/P	Canada bluegrass var. Reubens	*Poa compressa*	18" ppt; AT = 2	1.0
	Forbs:			
N/P	Western yarrow	*Achillea millefolium*	AT = 1	1.0
N/P	Engleman aster	*Aster englemannii*	AT = 1	0.5
I/P	Cicer milk vetch var. Lutana	*Astragulus cicer*	12-18" ppt; AT = 1	1.0
I/P	Sainfoin var. Eski	*Onobrychis viciaefolia*	15-18" ppt; AT = 1	3.0
N/P	Rocky Mt. Penstemon	*Penstemon strictus*	AT = 0	3.0
I/P	Inoculated alsike clover	*Trifolium hybridum*	35" ppt; AT = 2	2.0
I/P	Inoculated red clover	*Trifolium pratense*	35" ppt; AT = 1	2.0

Note: (*) Indicates plant adaptation to acidic conditions: 0 = not adapted; 1= marginal; 2 = average; 3 = best.

Source: Winter *et al.* 2000; Redente and Richard 1998

Appendix Table 3. The 128 carbon-substrates of the Gram Positive and Gram Negative Biolog micro-plates grouped into 12 categories (Garland and Mills 1991; Zak *et al.* 1994)

Carbohydrates	*Carboxylic Acids*	*Amino Acids*
α-D-glucose	α-hydroxybutyric acid	D-alanine
α-D-lactose	α-keto butyric acid	D-serine
α-methyl-D-galactoside	α-keto glutaric acid	D-L-carnitine
α-methyl-D-glucoside	α-keto valeric acid	γ-amino butyric acid
α-methyl-D-mannoside	acetic acid	glycyl-L-aspartic acid
adonitol	β-hydroxybutyric acid	glycyl-L-glutamic acid
arbutin	*cis*-aconitic acid	hydroxy L-proline
β-methyl-D-galactoside	citric acid	L-alanine
β-methyl-D-glucoside	D-galactonic acid lactone	L-alanyl-glycine
cellobiose	D-galacturonic acid	L-asparagine
D-arabitol	D-gluconic acid	L-aspartic acid
D-fructose	D-glucosaminic acid	L-glutamic acid
D-galactose	D-glucuronic acid	L-histidine
D-mannitol	D-malic acid	L-leucine
D-mannose	D-saccharic acid	L-ornithine
D-melezitose	D-L-lactic acid	L-phenylalanine
D-melibiose	formic acid	L-proline
D-psicose	γ-hydroxybutyric acid	L-pyroglutamic acid
D-raffinose	itaconic acid	L-serine
D-ribose	L-lactic acid	L-threonine
D-sorbitol	L-malic acid	
D-tagatose	malonic acid	*Amines*
D-trehalose	N-acetyl-L-glutamic acid	phenyl ethylamine
D-xylose	ρ-hydroxyphenylacetic acid	putrescine
gentiobiose	propionic acid	2-amino ethanol
i-erythritol	pyruvic acid	
L-arabinose	quinic acid	*Esters*
L-fucose	sebacic acid	mono-methyl succinate
L-rhamnose	succinic acid	methyl pyruvate
lactulose		
m-inositol	*Polymers*	*Aromatic Chemicals*
maltose	α-cyclodextrin	inosine
maltotriose	β-cyclodextrin	thymidine
mannan	dextrin	uridine
N-acetyl-D-galactosamine	glycogen	urocanic acid
N-acetyl-D-glucosamine	inulin	*Alcohol*
N-acetyl-D-mannosamine	tween 40	glycerol
palatinose	tween 80	
sedoheptulosan		*Miscellaneous*
stachyose	*Amides*	adenosine-5-monophosphate
sucrose	alaninamide	adenosine
turanose	glucuronamide	amygdalin
xylitol	lactamide	D-lactic acid methyl ester
3-methyl glucose	succinamic acid	fructose-6-phosphate
		salicin
Phosphorylated Chemicals	*Brominated Chemicals*	thymidine-5-monophosphate
D-L-alpha-glycerol phosphate	bromo-succinic acid	uridine-5-monophosphate
glucose-1-phosphate		2-deoxy adenosine
glucose-6-phosphate		2,3-butanediol

Appendix Table 4. Fatty Acid Methyl Esters (FAMEs) and their respective microbial groups and genera identified by the Phospholipid Fatty Acid analysis for the waste rock treatments and reference sites. NA=not available.

FAME (A:BωC)	*Name of FAME*	*Microbial Group or Genera*
Saturated, Straight Chain		
14:0	Tetradecanoic acid	All Organisms [a]
15:0	Pentadecanoic acid	Bacteria[1,2,3,5]
16:0	Hexadecanoic acid	All Organisms [a]
17:0	Heptadecanoic acid	Bacteria[1,2,3,5]
18:0	Octadecanoic acid	All Organisms [a]
20:0	Icosanoic acid	Eukaryote[7]
22:0	Docosanoic acid	NA
23:0	NA	NA
24:0	Tetracosanoic acid	NA
Saturated, iso or anteiso Methyl Branched Chain		
i14:0	iso-tetradecanoic acid	Gram (+) Bacteria[3]
i15:0	iso-pentadecanoic acid	Gram (+) Bacteria[1,2,3,5] *Bacillus* or *Arthrobacter* [4]
a15:0	anteiso-pentadecanoic acid	Gram (+) Bacteria[3] *Bacillus* or *Arthrobacter* [4]
i16:0	iso-hexadecanoic acid	Gram (+) Bacteria[1,2,3,5]
i17:0	iso-heptadecanoic acid	Gram (+) Bacteria[3] *Bacillus* or *Arthrobacter* [4]
a17:0	anteiso-heptadeconoic acid	Gram (+) Bacteria[3] *Bacillus* or *Arthrobacter* [4]
Saturated, Methyl Branched Chain		
10Me16:0	10-Methylhexadecanoic acid	Gram (+) Bacteria[1,5] *Desulfobacter* [4]
10Me17:0	10-Methylheptadecanoic acid	Actinomycetes[3,14]
12Me16:0	12-Methylhexadecanoic acid	NA
Cyclopropane Fatty Acid		
cy17:0	cis-9,10-Methylenehexadecanoic acid	Gram (-) Bacteria[1,2,3,5] *Thiobacillus*[8,13]
cy19:0	cis-9,10-Methyleneoctadecanoic acid	Gram (-) Bacteria[1,2,5,7] *Thiobacillus*[8,9,13]

Appendix Table 4. (continued)

FAME (A:BωC)	*Name of FAME*	*Microbial Group or Genera*
Unsaturated, *cis* or *trans* Branched Chain 16:1ω7c	cis-Hexadec-7-enoic acid	Gram (-) Bacteria[6] *Thiobacillus*[8,9,11,12,13] or *Beggiatoa*[10]
16:1ω7t	trans-Hexadec-7-enoic acid	Gram (-) Bacteria[5,6]
18:1ω7c	cis-Octadec-9-enoic acid	Gram (-) Bacteria[6] *Thiobacillus*[8,10,12]
18:1ω7t	trans-Octadec-9-enoic acid	Gram (-) Bacteria[6]
18:2ω6,9	Octadeca-6,9-dienoic acid	Fungi [5]

Source: [1]Zak *et al.* 1996; [2]Frostegard and Baath 1996; [3]Frostegard *et al.* 1993; [4]White 1992 and Allen 2000; [5]Pennanen *et al.* 1998; [6]White *et al.* 1998; [7]McNaughton *et al.* 1999; and [8]Katayama-Fujimura *et al.* 1982; [9]Wakao *et al.* 1991; [10]Jacq *et al.* 1989; [11]Ghosh and Mishra 1985; [12]Fullarton *et al.* 1995, [13]Chang *et al.* 1997; [14]Lechevalier 1977.

Note: [a] These FAMEs are present in a diversity of microbes and are therefore not considered biomarkers for a particular microbial group (Findlay and Dobbs 1993; Cavigelli *et al.* 1995).

Appendix Table 5. Average plant cover (%), separated by species, for the waste rock treatments (t-SM, t-NM, t-M) and native reference sites (CC, SM, SF) in 2001. Percent cover was measured by the point intercept method. Standard error (stderr) is provided for totals. Different letters among waste rock treatments and native reference sites represent significant mean differences (α=0.05). Treatment codes: S=stockpiled topsoil, N=nonstockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: CC=sub-alpine clear-cut forest, SM=sub-alpine meadow, SF=sub-alpine forest.

Plant Species	CC (%)	SM (%)	SF (%)	t-SM (%)	t-NM (%)	t-M (%)
Agropyron trachycaulum	0.0	14.1	0.0	8.4	6.9	6.5
Agrostis scabra	13.1	0.0	0.0	0.0	0.0	0.0
Agrostis stolonifera	0.0	0.0	0.0	0.6	1.0	0.7
Alopercurus pratensis	0.0	1.6	0.0	18.4	13.3	12.7
Bromus inermis	0.0	0.0	0.0	0.9	0.0	0.3
Bromus marginatus	0.3	0.0	0.0	0.3	0.0	0.3
Carex ebenea	4.4	15.4	0.0	0.0	0.3	0.0
Carex sp.	0.6	0.0	1.7	0.3	0.0	0.0
Dactylis glomerata	0.0	0.0	0.0	14.4	2.5	15.1
Deschampsia ceaspitosa	16.3	13.8	0.0	8.8	12.3	6.2
Festuca ovina	0.3	0.6	0.7	1.6	5.5	1.7
Festuca sp.	0.0	0.0	0.3	0.0	0.0	0.0
Phleum pratense	0.0	0.0	0.0	9.7	6.3	8.0
Phleum alpinum	1.3	0.0	0.0	0.0	0.0	0.0
Poa alpina	0.0	0.0	0.3	0.6	1.3	1.3
Poa glauca	0.0	0.3	0.0	1.3	0.7	2.9
Poa sp.	0.3	0.0	0.0	0.0	0.0	0.0
Trisetum spicatum	0.0	0.6	0.0	0.0	0.0	0.0
Other grass species	0.9	0.3	0.0	0.0	0.0	0.0
Total Graminoids (%)	**37.5**	**46.7**	**3.0**	**65.3**	**50.1**	**55.7**
stderr	15.5	0.9	1.9	4.6	1.3	4.7
	a	ab	c	b	ab	ab
Ribes montigenum	0.6	0.0	0.0	0.0	0.0	0.0
Total Shrub (%)	**0.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Abies lasiocarpa	0.0	0.0	1.8	0.0	0.0	0.0
Picea engelmannii	0.0	0.0	49.1	0.0	0.0	0.0
Total Trees (%)	**0.0**	**0.0**	**50.9**	**0.0**	**0.0**	**0.0**
Achillea millefolium	0.0	1.6	0.0	10.9	25.4	21.7
Agoseris glauca	0.0	0.6	0.0	0.0	0.0	0.0
Allium geyeri	0.0	0.3	0.0	0.0	0.0	0.0
Arnica cordifolia	1.0	0.3	2.9	0.0	0.0	0.0
Cardamine cordifolia	3.8	0.0	0.0	0.0	0.0	0.0
Castilleja sulphurea	0.0	0.6	0.0	0.0	0.0	0.0
Cerastium arvense	0.0	1.6	0.0	0.0	0.0	0.0
Cirsium coloradense	0.0	1.3	0.0	0.0	0.0	0.0
Cirsium tweedyi	0.0	1.9	0.0	0.0	0.0	0.0
Conioselinum scopulorum	0.3	1.3	0.3	0.0	0.0	0.0
Descurainia pinnata	0.3	0.0	0.0	0.0	0.0	0.0

Appendix Table 5. (continued)

Plant Species	CC (%)	SM (%)	SF (%)	t-SM (%)	t-NM (%)	t-M (%)
Epilobium sp.	1.6	0.0	0.0	0.0	0.0	0.0
Erigeron coulteri	0.6	10.4	1.6	0.0	0.0	0.0
Erigeron melanocephalus	0.0	0.3	0.0	0.0	0.0	0.0
Gentiana parryi	0.0	0.6	0.0	0.0	0.0	0.0
Geum rossii	0.0	1.9	0.0	0.0	0.0	0.0
Juncus drummondii	0.3	0.9	0.0	0.0	0.0	0.0
Ligularia amplectens	3.8	0.0	0.6	0.0	0.0	0.0
Ligularia bigelovii	0.0	4.4	0.0	0.0	0.0	0.0
Luzula parviflora	0.6	0.0	0.0	0.0	0.0	0.0
Mertensia ciliata	34.7	0.0	0.0	0.0	0.0	0.0
Pedicularis racemosa	0.0	0.3	0.0	0.0	0.0	0.0
Penstemon strictus	0.0	0.3	0.0	0.3	0.7	0.0
Polemonium pulcherrimum	0.0	0.0	0.3	0.0	0.0	0.0
Polemonium sp.	0.6	0.0	0.0	0.0	0.0	0.0
Polenomium viscosum	0.0	0.3	0.0	0.0	0.0	0.0
Polygonium viviparum	0.0	2.2	0.0	0.0	0.0	0.0
Potentilla diversifolia	0.0	5.0	0.0	0.0	0.0	0.0
Potentilla gracilis	0.0	0.0	0.0	0.0	0.0	0.0
Saxifraga odontoloma	0.6	0.0	0.0	0.0	0.0	0.0
Sedum sp.	0.6	0.0	0.0	0.0	0.0	0.0
Senecio amplectens	0.0	0.0	0.0	0.0	0.0	0.3
Senecio crassulus	0.0	0.3	0.0	0.0	0.0	0.0
Sibbaldia procumbens	0.0	0.0	1.6	0.0	0.0	0.0
Solidago sp.	0.0	2.5	0.0	0.0	0.0	0.0
Taraxicum sp.	0.0	1.6	0.0	0.3	0.0	1.3
Trifolium pratense	0.0	0.6	0.0	0.0	0.0	0.0
Trifolium sp.	0.0	1.9	0.0	0.0	0.0	0.0
Vaccinium myrtillis	0.0	0.0	11.8	0.0	0.0	0.0
Viola sp.	0.9	0.0	0.0	0.0	0.0	0.0
Moss	0.0	0.0	1.3	0.0	0.0	0.0
Mushroom	0.0	0.0	0.3	0.0	0.0	0.0
Other forb species	0.0	1.0	5.0	0.3	0.0	0.0
Total Forbs (%)	**49.7**	**44.0**	**25.7**	**11.8**	**26.1**	**23.3**
stderr	13.8	3.0	6.5	0.8	6.4	1.1
	a	ab	bc	c	bc	c

Total Cover:	CC (%)	SM (%)	SF (%)	t-SM (%)	t-NM (%)	t-M (%)
Bare Ground	3.2	6.0	5.6	9.1	6.7	12.7
Rock	0.0	1.0	0.3	3.1	3.2	1.3
Litter	8.9	2.5	65.3	10.6	13.8	7.3
Plant	**87.8**	**90.7**	**79.7**	**77.1**	**76.2**	**79.0**
stderr	3.0	3.3	3.7	5.3	6.2	5.5
	a	a	a	a	a	a

Appendix Table 6. Bivariate correlation matrix of soil microbial, physicochemical, and plant characteristics measured in waste rock treatments and reference sites in 2000 and 2001. Each correlation has a Spearman's rho correlation coefficient (-1 to 1), significance level (α=0.05), and number (N) of samples. Bold type and asterisks represent significant correlations with (*) being between 0 and ±0.500 and (**) being greater than ±0.500.

Spearman's rho Nonparametric Correlation		Total Plant Cover (%)	Plant Species Richness	pH	SOM (%)	Organic N (mg/kg)
Total Plant Cover (%)	Correlation Coefficient	1.000	.335	.166	.325	-.374
	Sig. (2-tailed)	.	.110	.437	.121	.072
	N	24	24	24	24	24
Plant Species Richness		.335	1.000	-.245	.034	-.283
		.110	.	.249	.875	.181
		24	24	24	24	24
Soil pH		.166	-.245	1.000	.166	.204
		.437	.249	.	.399	.318
		24	24	28	28	26
Soil Organic Matter-SOM (%)		.325	.034	.166	1.000	**-.389***
		.121	.875	.399	.	**.049**
		24	24	28	28	**26**
Soil Organic N (mg/kg)		-.374	-.283	.204	**-.389***	1.000
		.072	.181	.318	**.049**	.
		24	24	26	**26**	26
Soil Ammonium-N (mg/kg)		.043	-.097	-.086	.334	-.316
		.843	.652	.677	.095	.116
		24	24	26	26	26
Soil Nitrate-N (mg/kg)		.082	-.259	.215	**.562****	-.153
		.702	.221	.291	**.003**	.455
		24	24	26	**26**	26
Total Metal Concentration (mg/kg)		**-.536****	**-.676****	-.305	**-.387***	.316
		.007	**.000**	.114	**.042**	.116
		24	**24**	28	**28**	26
Soil Moisture (%)		.295	.079	.097	**.953****	**-.449***
		.162	.712	.623	**.000**	**.021**
		24	24	28	**28**	**26**
Active Microbial Biomass (pmol/g)		-.276	-.272	**.491***	.111	.383
		.192	.199	**.008**	.574	.053
		24	24	**28**	28	26
Active Microbial Richness		-.268	-.095	**.549****	.208	.188
		.205	.657	**.002**	.289	.359
		24	24	**28**	28	26
Sulfur Oxidizing Bacteria (mol% bacterial PLFA)		.184	.204	**-.646****	-.131	-.247
		.388	.340	**.000**	.507	.223
		24	24	**28**	28	26
Total Bacterial Biomass (ug/g)		.305	.117	.162	**.716****	-.374
		.148	.585	.411	**.000**	.060
		24	24	28	**28**	26
Total Fungal Biomass (ug/g)		**.489***	.335	.039	**.803****	**-.510****
		.015	.109	.844	**.000**	**.008**
		24	24	28	**28**	**26**
Total Substrate Utilization (mean AWCD)		.274	.276	.367	**.571****	-.296
		.195	.193	.055	**.001**	.141
		24	24	28	**28**	26
Substrate Utilization Richness		**.408***	**.532****	.080	**.672****	-.282
		.048	**.007**	.684	**.000**	.163
		24	**24**	28	**28**	26
Cotton Decomposition (g)		-2.17	-.172	**.390***	-.052	**.427***
		.309	.420	**.040**	.793	**.030**
		24	24	**28**	28	**26**
Alfalfa Decomposition (g)		-.244	-.309	.066	.138	-.070
		.251	.142	.739	.485	.734
		24	24	28	28	26

Appendix Table 6. (continued)

Spearman's rho Nonparametric Correlation		NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Total Metal Concentration (mg/kg)	Moisture (%)	Active Microbial Biomass (pmol/g)
Total Plant Cover (%)	Correlation Coefficient	.043	.082	**-.536****	.295	-.276
	Sig. (2-tailed)	.843	.702	**.007**	.162	.192
	N	24	24	**24**	24	24
Plant Species Richness		-.097	-.259	**-.676****	.079	-.272
		.652	.221	**.000**	.712	.199
		24	24	**24**	24	24
Soil pH		-.086	.215	-.305	.097	**.491***
		.677	.291	.114	.623	**.008**
		26	26	28	28	**28**
Soil Organic Matter-SOM (%)		.334	**.562****	**-.387***	**.953****	.111
		.095	**.003**	**.042**	**.000**	.574
		26	**26**	**28**	**28**	28
Soil Organic N (mg/kg)		-.316	-.153	.316	**-.449***	.383
		.116	.455	.116	**.021**	.053
		26	26	26	**26**	26
Soil Ammonium (NH_4^+-N) (mg/kg)		1.000	.285	-.172	.352	.020
		.	.159	.401	.078	.922
		26	26	26	26	26
Soil Nitrate (NO_3^--N) (mg/kg)		.285	1.000	.087	**.608****	.147
		.159	.	.673	**.001**	.472
		26	26	26	**26**	26
Total Metal Concentration (mg/kg)		-.172	.087	1.000	-.360	-.130
		.401	.673	.	.060	.511
		26	26	28	28	28
Soil Moisture (%)		.352	**.608****	-.360	1.000	.115
		.078	**.001**	.060	.	.560
		26	**26**	28	28	28
Active Microbial Biomass (pmol/g)		.020	.147	-.130	.115	1.000
		.922	.472	.511	.560	.
		26	26	28	28	54
Active Microbial Richness		.369	.303	-.357	.242	**.831****
		.064	.132	.062	.215	**.000**
		26	26	28	28	**54**
Sulfur Oxidizing Bacteria (mol% bacterial PLFA)		.026	-.150	-.015	-.111	**-.679****
		.898	.465	.941	.574	**.000**
		26	26	28	28	**54**
Total Bacterial Biomass (ug/g)		**.559****	**.529****	**-.599****	**.756****	.238
		.003	**.005**	**.001**	**.000**	.222
		26	**26**	**28**	**28**	28
Total Fungal Biomass (ug/g)		.255	.333	**-.560****	**.831****	-.010
		.209	.096	**.002**	**.000**	.960
		26	26	**28**	**28**	28
Total Substrate Utilization (mean AWCD)		.132	**.470***	**-.427***	**.506****	.344
		.519	**.015**	**.023**	**.006**	.073
		26	**26**	**28**	**28**	28
Substrate Utilization Richness		.297	.379	**-.597****	**.703****	.096
		.141	.056	**.001**	**.000**	.629
		26	26	**28**	**28**	28
Cotton Decomposition (g)		-.057	.179	.070	-.163	.198
		.783	.382	.723	.407	.152
		26	26	28	28	54
Alfalfa Decomposition (g)		-.282	.217	**.389***	.132	.074
		.164	.288	**.041**	.502	.594
		26	26	**28**	28	54

Appendix Table 6. (continued)

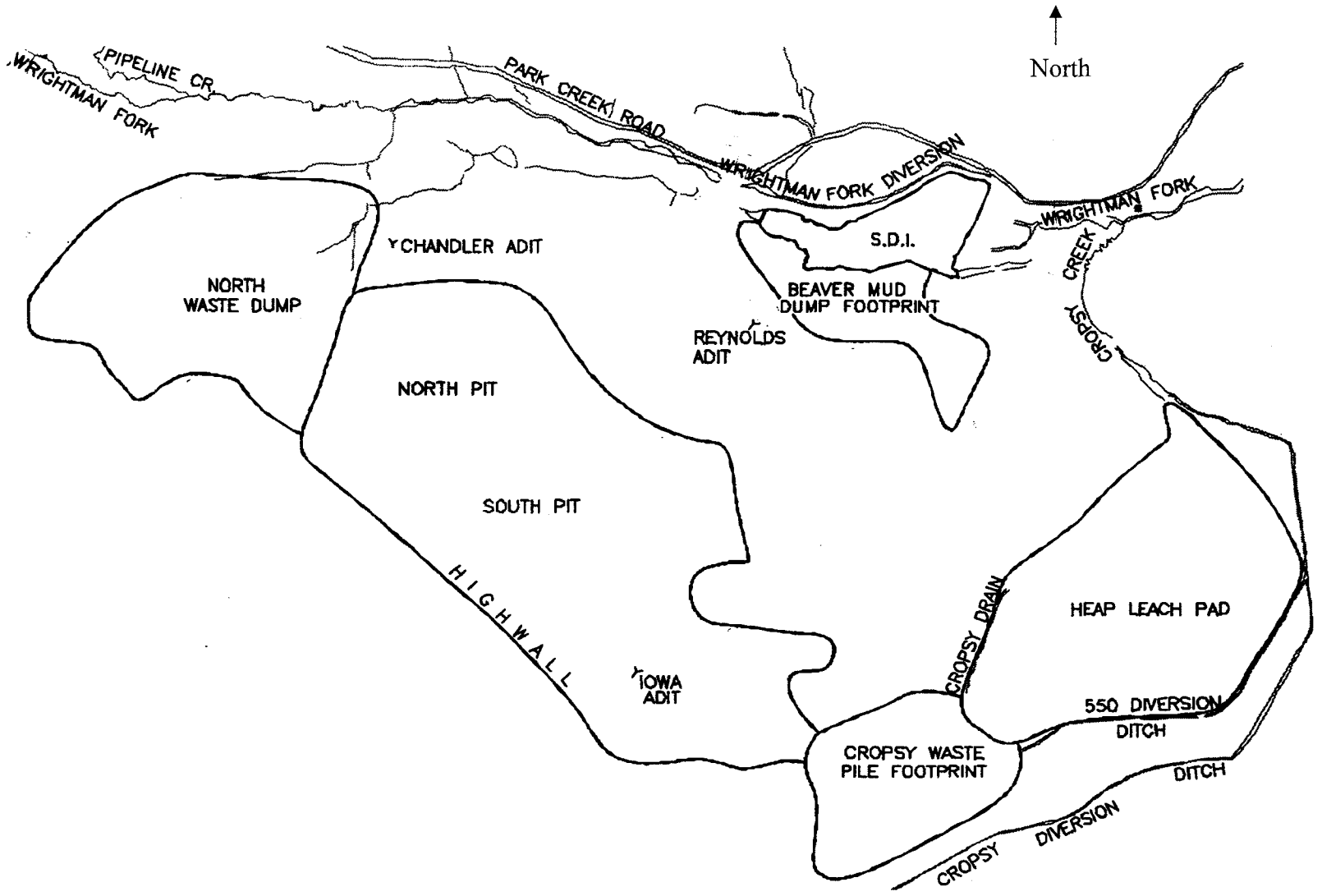
Spearman's rho Nonparametric Correlation		Active Microbial Richness	Sulfur Oxidizing Bacteria (mol%)	Total Bacterial Biomass (ug/g)	Total Fungal Biomass (ug/g)	Total Substrate Utilization (mean AWCD)
Total Plant Cover (%)	Correlation Coefficient	-2.68	.184	.305	**.489***	.274
	Sig. (2-tailed)	.205	.388	.148	**.015**	.195
	N	24	24	24	**24**	24
Plant Species Richness		-.095	.204	.117	.335	.276
		.657	.340	.585	.109	.193
		24	24	24	24	24
Soil pH		**.549****	**-.646****	.162	.039	.367
		.002	**.000**	.411	.844	.055
		28	**28**	28	28	28
Soil Organic Matter-SOM (%)		.208	-.131	**.716****	**.803****	**.571****
		.289	.507	**.000**	**.000**	**.001**
		28	28	**28**	**28**	**28**
Soil Organic N (mg/kg)		.188	-.247	-.374	**-.510****	-.296
		.359	.223	.060	**.008**	.141
		26	26	26	**26**	26
Soil Ammonium (NH_4^+-N) (mg/kg)		.369	.026	**.559****	.255	.132
		.064	.898	**.003**	.209	.519
		26	26	**26**	26	26
Soil Nitrate (NO_3^--N) (mg/kg)		.303	-.150	**.529****	.333	**.470***
		.132	.465	**.005**	.096	**.015**
		26	26	**26**	26	**26**
Total Metal Concentration (mg/kg)		-.357	-.015	**-.599****	**-.560****	**-.427***
		.062	.941	**.001**	**.002**	**.023**
		28	28	**28**	**28**	**28**
Soil Moisture (%)		.242	-.111	**.756****	**.831****	**.506****
		.215	.574	**.000**	**.000**	**.006**
		28	28	**28**	**28**	**28**
Active Microbial Biomass (pmol/g)		**.831****	**-.679****	.238	-.010	.344
		.000	**.000**	.222	.960	.073
		54	**54**	28	28	28
Active Microbial Richness		1.000	**-.588****	**.569****	.199	**.436***
		.	**.000**	**.002**	.310	**.020**
		54	**54**	**28**	28	**28**
Sulfur Oxidizing Bacteria (mol% bacterial PLFA)		**-.588****	1.000	-.105	-.073	-.357
		.000	.	.594	.713	.062
		54	54	28	28	28
Total Bacterial Biomass (ug/g)		**.569****	-.105	1.000	**.780****	**.564****
		.002	.594	.	**.000**	**.002**
		28	28	28	**28**	**28**
Total Fungal Biomass (ug/g)		.199	-.073	**.780****	1.000	**.441***
		.310	.713	**.000**	.	**.019**
		28	28	**28**	28	**28**
Total Substrate Utilization (mean AWCD)		**.436***	-.357	**.564****	**.441***	1.000
		.020	.062	**.002**	**.019**	.
		28	28	**28**	**28**	28
Substrate Utilization Richness		**.395***	-.134	**.779****	**.785****	**.625****
		.037	.496	**.000**	**.000**	**.000**
		28	28	**28**	**28**	**28**
Cotton Decomposition (g)		.199	**-.414***	-.030	-.184	**.402***
		.148	**.002**	.878	.349	**.034**
		54	**54**	28	28	**28**
Alfalfa Decomposition (g)		.075	-.229	-.227	.077	.092
		.590	.096	.245	.696	.643
		54	54	28	28	28

Appendix Table 7. The patterns of carbon-substrate utilization by the soil microbial community, expressed as the mean AWCD (average well color development), of the waste rock treatments (t-M, t-SM, t-NM), native reference sites (SM, CC, SF), and a disturbed reference site (D) in 2001. Well-color absorbance readings were subjected to square root transformations before analysis. Standard error of the means is represented in ( ). Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock. Br=Brominated, Phos=Phosphorylated.

Waste Rock Treatments and Reference Sites	Alcohol (AWCD)	Amide (AWCD)	Amine (AWCD)	Amino Acid (AWCD)	Aromatic Chemical (AWCD)	Br Chemical (AWCD)	Carbohydrate (AWCD)	Carboxylic Acid (AWCD)	Ester (AWCD)	Phos. Chemical (AWCD)	Polymer (AWCD)
t-M	6.0 (1.7)	5.2 (1.3)	3.6 (0.5)	7.9 (1.3)	4.4 (0.8)	11.9 (0.5)	3.9 (1.0)	9.3 (1.2)	6.3 (1.1)	4.5 (1.3)	5.1 (1.3)
t-SM	2.6 (0.9)	2.2 (0.5)	2.2 (0.4)	4.7 (0.5)	2.4 (0.3)	10.8 (1.3)	1.9 (0.3)	6.5 (0.7)	3.1 (0.8)	2.8 (0.5)	3.3 (0.4)
t-NM	5.7 (3.2)	4.1 (1.4)	3.2 (1.6)	5.4 (1.6)	4.2 (1.4)	8.8 (1.0)	3.5 (1.8)	6.9 (1.5)	4.8 (2.7)	5.5 (2.8)	5.5 (2.4)
SM	8.8 (0.9)	6.3 (1.0)	5.5 (0.3)	8.4 (0.8)	6.9 (0.9)	11.0 (0.4)	6.5 (0.9)	9.9 (1.0)	6.3 (1.5)	6.9 (1.3)	7.3 (0.7)
CC	4.8 (1.0)	3.8 (0.6)	5.9 (1.1)	6.3 (1.0)	4.4 (0.9)	7.4 (1.2)	4.0 (0.8)	6.9 (0.9)	3.9 (0.3)	4.2 (1.0)	4.9 (0.5)
SF	4.4 (1.7)	4.5 (0.2)	3.5 (1.8)	4.5 (1.3)	4.0 (0.7)	8.6 (0.9)	3.8 (0.8)	6.6 (1.3)	3.6 (1.0)	3.0 (1.2)	5.1 (0.9)
D	1.4 (0.8)	1.0 (0.5)	1.4 (0.6)	1.0 (0.5)	0.5 (0.4)	0 (0)	1.0 (0.3)	1.5 (0.5)	0 (0)	0.6 (0.4)	1.8 (0.4)

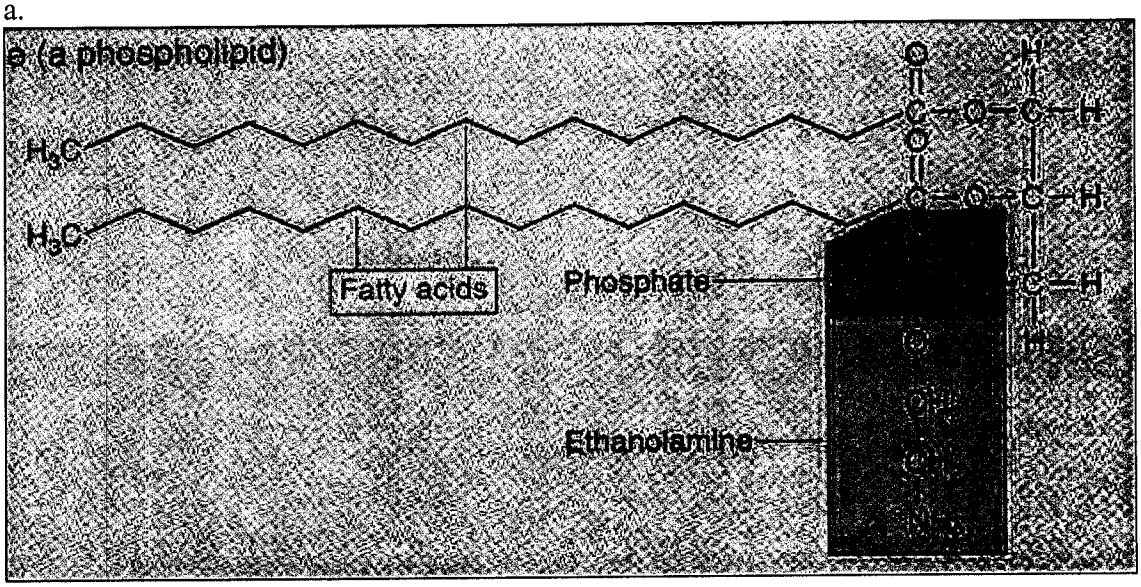
Appendix Table 8. Average soil texture and moisture (%) of waste rock treatments (t-NM, t-SM, t-M), native reference sites (SM, CC, SF), and disturbed reference site (D) in 2001. Different letters represent significant mean differences (α=0.05). Standard error of the means is represented in ( ). Treatment codes: N=non-stockpiled topsoil, S=stockpiled topsoil, M=mushroom compost and lime amended waste rock. Reference codes: SM=sub-alpine meadow, CC=sub-alpine clear-cut forest, SF=sub-alpine forest, D=un-treated waste rock.

Waste Rock Treatments and Reference Sites	**Sample Size**	**Texture**	**Moisture (%)**
t-NM	4	Sandy Clay Loam	25.9 [a] (1.9)
t-SM	4	Sandy Clay Loam	20.1 [a] (0.4)
t-M	4	Sandy Loam	27.9 [a] (3.4)
SM	4	Sandy Loam	43.5 [b] (1.6)
CC	4	Loam	58.8 [c] (3.8)
SF	4	Loam	39.0 [b] (5.8)
D	4	Sandy Loam	19.5 [a] (0.6)

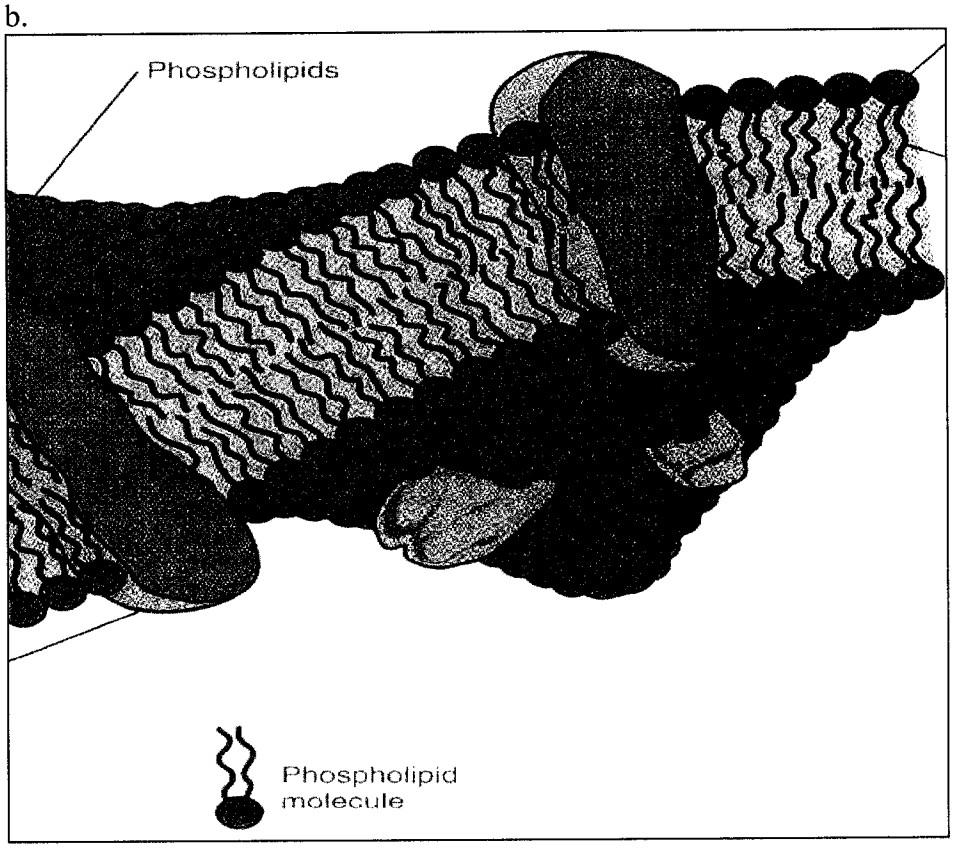


Appendix Figure 1. Map of Summitville Mine. S.D.I.=Summitville Dam Impoundment *Source*: Revised from Dodson and Benevento 2001.

a.



b.



Appendix Figure 2. a). Molecular structure of a phospholipid. b). Phospholipid bilayer of a cell membrane. *Source*: Brock *et al.* 1994.