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REINSTATEMENT OF BIOLOGICAL ACTIVITY IN SEVERELY DISTURBED
SOILS: EFFECTS OF MINING ON THE MICROBIOLOGY OF THREE MINESPOILS
AND THE MICROBIAL DEVELOPMENT IN THE MINESPOILS AFTER
AMENDATION AND PLANTING

by

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EXECUTIVE SUMMARY

Soil microorganisms and their activities are the major vectors in the decomposition of plant litter and the subsequent transformation and flow of such essential plant nutrients as nitrogen and phosphorus. The end result of their activities is the stabilization of soil organic matter upon which the development of a self-sustaining vegetative cover is based. Consequently, a project was initiated with the following objectives in mind:

1. to determine the immediate effects of coal and bitumen mining on a variety of soil microbiological factors.
2. to provide detailed information on the rates of redevelopment of biological activity when various organic and inorganic amendments are applied singly to various minespoils and subsequently planted with different herbaceous and woody plant species.

The effects of surface mining on soil microbial populations, microbial activity and decay potential were studied in a prairie grassland, a subalpine spruce-fir forest and a jack pine forest (oil sands) in Alberta. In general, mining drastically changed the composition of the bacterial and fungal communities and caused significant reductions in bacterial, actinomycete numbers, lengths of fungal hyphae, microbial respiration, microbial biomass C and ATP. However, the decomposition of cellulose filter paper placed in the field for two to three years was more rapid in the mined than unmined sites. The decrease in soil microbial activity after mining was attributed to the loss of organic matter since microbial biomass C and soil organic matter in revegetated subalpine minesoils were observed to be highly correlated.

Procedures for improving the microbial status of the prairie grassland and subalpine coal minespoils and the oil sands tailings were studied by treating each one with three different organic or inorganic amendments and then planting each with four different plant species. The grassland spoil was treated with topsoil, anaerobically-digested sewage sludge, gypsum or left untreated and planted with fall rye, crested wheatgrass, Russian

wild-rye or rambler alfalfa. The subalpine minespoil and oil sands tailings received fibrous peat, sewage sludge, mineral fertilizer or no amendment. The plant species tested on the subalpine spoil included slender wheatgrass, white spruce, alsike clover and laurel leaf willow while those on the tailings sand were slender wheatgrass, sainfoin, jack pine and bearberry. Individual plant species were studied on each amendment treatment. The response of mixtures of plant species or combinations of amendments were not examined. The effects of amendment and plant growth (fall rye on the grassland minespoil; slender wheatgrass and white spruce on the subalpine spoil; and slender wheatgrass and jack pine on the tailings sand) on soil microbial development were monitored over three to four years.

Application of sewage sludge or topsoil to the grassland spoil was highly effective in increasing microbial activity and biomass C, particularly in the upper 5 cm of the amended spoil. The development of microbial activity and biomass appeared to be linked to primary production since both parameters increased as shoot production by fall rye increased. The increased microbial biomass maintained itself for at least one year after fall rye failed, but its metabolic activity was reduced to pre-planting levels. The decay of filter papers appeared to be related to density of plant cover rather than amendment type with decomposition in the topsoil and sewage sludge treatments being faster than that in the gypsum and control treatments. No significant treatment effects on filter paper decay were measured after growth by fall rye ceased, while the decay potential in the topsoil and sludge treated spoil increased as plant cover by rambler alfalfa increased. The N_2 fixation potential of rambler alfalfa was not significantly affected by amendment, although measurements were highly variable.

The microbial status of the subalpine and oil sands spoils was most improved by the addition of peat while treatment with sewage sludge or fertilizer was less effective. The effects of amendment and planting were restricted mainly to the top 5 cm of the treated spoils. Although CO_2 efflux from the unplanted, peat amended mine-spoils increased substantially over the four year term of the study,

loss on ignition estimates indicated no significant loss of stable organic matter from this treatment. The fast growing, highly productive slender wheatgrass was more effective in stimulating the development of microbial activity and biomass C in the variously treated minespoils than the slow growing white spruce or jack pine were, again suggesting that plant productivity and soil microbial productivity are closely related. Rates of microbial development appeared to be dependent on the input of root exudates, sloughed root material and dead roots and shoots from the primary producers, which, in the case of slender wheatgrass, was particularly high in the sewage sludge treatment.

During the first two years after planting, cellulose filter paper placed in the subalpine spoil decomposed most rapidly in the sewage sludge treatment regardless of vegetation type, while those placed in the oil sands minespoil decayed fastest in the sewage sludge and fertilizer treatments planted with slender wheatgrass. Over the long term (four years), cellulose decay potential of the amended and planted subalpine spoil was not significantly altered, but the decay potential of the sludge treated sand planted with slender wheatgrass was considerably reduced. In both minespoils, the short term decomposition (one year) of slender wheatgrass leaves was faster than that of stems. Alsike clover leaves decayed more rapidly than slender wheatgrass leaves in the subalpine minespoil whilst sainfoin leaves decayed more slowly than slender wheatgrass leaves in the oil sand minespoil. Neither grass nor legume litter decomposition was significantly affected by amendment. The decay rates of filter paper could not be extrapolated to predict decay rates of plant litter. The N_2 fixation capacity of alsike clover was not significantly influenced by amendment, but sewage sludge inhibited N_2 fixation by sainfoin after the third growing season.

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1. INTRODUCTION

The chemical, physical and biological properties of a minespoil are considered to be the main factors influencing the successful establishment of a vegetative cover (Vogel, 1981). Although the chemical and physical aspects of minespoils and their influence on plant success have received a great deal of attention, the biological properties of minespoils have, until recently, been largely ignored. Both Cundell (1977) and Jurgensen (1978) stressed the importance of soil microorganisms to vegetation establishment and discussed the potentially important roles microbial processes could have in soil formation on minespoils.

The measurement of microbial activity and biomass in minespoils is considered important for the following reasons:

- i) microbial activity is one of the major factors responsible for the decomposition of plant litter and roots resulting in both the stabilization of soil organic matter and the release of nutrients for subsequent plant growth.
- ii) soil microorganisms behave as both sources and sinks of essential plant nutrients and are instrumental in the transformation and flow of C, N, and P (Ausmus et al., 1976; Paul and Voroney, 1980); also, because of their relatively rapid growth rate, they are potentially capable of quickly immobilizing nutrients which would otherwise be lost through leaching.
- iii) soil microorganisms are metabolically highly diverse and therefore have the capability to adapt to the low nutrient levels and adverse chemical and physical conditions often found in minespoils (Parkinson, 1978).
- iv) symbiotic microorganisms such as N_2 fixing bacteria and mycorrhizal fungi are considered crucial in spoils where both N and P can occur at such low levels that plant growth is adversely affected.

Research regarding microorganisms and the reclamation of minespoils has, to date, dealt mainly with the effects of mining disturbance on microbial population parameters (i.e. numbers and

types of bacteria, actinomycetes and fungi), physiological groups of microorganisms, general microbial activity, decomposition and nitrogen mineralization processes, and mycorrhizal inoculum potential. Many of these studies have included data on the microbiological characteristics of minespoils after applying amendments and/or planting.

Until a decade ago, much of the research dealing with the microbiological properties of minespoils had been carried out by Wilson and his co-workers on coal strip-mine spoils in the eastern U.S. Much of this work was summarized in Wilson (1965). One of the observations made in his investigations was that the total numbers of bacteria, fungi and actinomycetes were generally lower in unvegetated spoil compared with the revegetated spoil and nearby undisturbed areas. The root region (root surface and rhizosphere) was particularly effective in stimulating numbers of bacteria. Müller (1973) also recorded lower numbers of bacteria and actinomycetes in fresh brown-coal minespoils in Poland than in revegetated minespoils and, like Wilson (1965), concluded that microbiological activity improved as revegetation progressed. Both Müller (1973) and Wilson (1965) observed that quantitative assessments of fungi were not dramatically influenced by the presence of vegetation. However, these data must be viewed with caution because the dilution plate technique was used in both these studies - a technique which has been shown to select for fungal spores rather than the active hyphae (Warcup, 1955). More recently, Fresquez and Lindemann (1982) observed from data obtained using soil dilution plating that the undisturbed soil and soil from a reclaimed area in New Mexico, had similar bacterial, actinomycete and fungal numbers, but that microbial numbers in the nonvegetated spoil were low. However, when the spoils were amended with alfalfa or sewage sludge microbial numbers increased - a result attributed to the addition of a microbiologically available carbon source. Like Wilson (1965), Fresquez and Lindemann (1982) also recorded a stimulation of microbial numbers in the rhizosphere soil particularly in the presence of highly organic amendments.

Studies on the types of bacteria and fungi found in minespoils are limited. Müller (1973) listed the predominant fungi and bacteria isolated from minespoils in Poland and observed that no major differences existed between genera of micro-organisms in bare spoil and spoils revegetated for 2-6 yrs. Data collected by Wilson (1965) also inferred that the same types of fungi occurred in a nonvegetated spoil as in a vegetated spoil. In contrast, Lawrey (1977) and Fresquez and Lindemann (1982) reported that there were generally fewer fungal genera in minespoil than in undisturbed soil. The addition of organic amendments increased the diversity of the fungal isolates (Fresquez and Lindemann, 1982). No attempts were made to estimate the frequencies of occurrence of the fungi isolated in these studies.

The transformation of nitrogen in organic matter to ammonium and finally to nitrate is mainly a bacterial process. Since nitrogen is an essential plant nutrient and is generally lacking in mined soils, many researchers have concentrated their investigations on the effects of mining on the organisms involved in the ammonifying and nitrifying processes. Wilson (1965), Müller (1973) and Williams and Cooper (1976), all working with acidic minespoils, found that if the pH of the spoil was raised through liming and fertilization, an increase in the numbers of ammonifiers and nitrifiers resulted. Highly acidic minespoils - a consequence of the oxidation of sulphur-bearing pyrites in the spoil by Thiobacillus spp. resulting in H_2SO_4 production - therefore require the application of lime if the nitrification process is to become established. In contrast to the acidic minespoils located in the eastern U.S., Fresquez and Lindemann (1982) working with the neutral to alkaline spoils of the western U.S., stimulated ammonium oxidizers by amending the spoil with alfalfa hay and fertilizer. Although the availability of NO_3-N through nitrification is regarded as necessary to the successful establishment of a vegetative cover on minespoils, Williams and Cooper (1976) have cautioned that a too rapid nitrification of applied ammoniacal fertilizers could result in N losses through leaching, particularly in sandy soils. In fact, Lodhi

(1979) has provided evidence which suggests that as plant succession proceeds on minespoils, the vegetation inhibits the nitrification process thereby conserving N which could otherwise be lost through leaching.

Microbial activity measurements such as soil respiration ($O_2\downarrow$ and $CO_2\uparrow$), adenosine triphosphate (ATP) levels and soil enzyme (phosphatase, dehydrogenase, pectinolyase, urease, amylase) activities have been the parameters most commonly used for determining the effects of mining on microorganisms and the development of a microbial system in minespoils. In general, the results obtained from these measurements have supported the plate count data i.e. disturbance by mining results in a decrease in microbial activity, and the addition of organic matter and the establishment of vegetation leads to an increase in microbial activity. Lawrey (1977) attributed the reduced soil respiration he measured in acidic minespoils to low pH, high levels of Fe and Mn rendered more soluble by the soil acidity and low macronutrient levels. In an earlier study, Hedrick and Wilson (1956) noted that the addition of nitrogen to acidic minespoils stimulated CO_2 production and that raising the spoil pH with calcium hydroxide did not influence CO_2 efflux to the same extent. Respiration was most stimulated by the addition of a complete fertilizer, organic matter (straw) and calcium hydroxide. Recently, Stroo and Jencks (1982) compared microbial respiration and enzyme activities in undisturbed soils and in minespoils at various stages of development. As expected microbial activity was generally reduced by mining, but as organic matter and nitrogen accumulated through revegetation, activity increased. Respiration, amylase and phosphatase were significantly correlated and related to organic C and N levels. The low levels of phosphatase activity in the minespoil led the authors to suggest that P cycling in the spoil could be impaired. Soil enzyme assays (phosphatase, pectinolyase, dehydrogenase) have also been used to characterize the microbial components in neutral to alkaline coal strip mine spoils in the western U.S. (Hersman and Temple, 1979; Fresquez and Lindemann, 1982). Soil ATP levels have

been shown to be greater in native grassland soils than minespoils in Montana (Hersman and Temple, 1978) and have demonstrated a high correlation with respiratory ($\text{CO}_2\uparrow$) activity in a variety of minespoils (Hersman and Temple, 1979). Measurements of ATP, dehydrogenase, phosphatase and microbial respiration have also been used extensively for studying the effects of retorted oil shale materials on microbial activity in overlying surface soils (Hersman and Klein, 1979; Sorensen et al., 1981). The results obtained indicated that microbial activity was reduced in soils overlying processed oil shale and that a capillary barrier between the surface soil and the shale would possibly remedy the decreased microbial activity (Sorenson et al., 1981)

As mentioned previously, it is microbial activity which is responsible for much of the decomposition of incoming plant material. On minespoils, the decay process would be particularly crucial since it would be closely related to nutrient availability and the rate of soil genesis. Therefore, it seems surprising that although microbial activity in minespoils has been studied extensively, the rate of decomposition of incoming plant litter and roots produced by the the evolving plant community has received limited attention. On acid minespoils in Ohio, it was observed that strip mining had inhibitory effects during the early stages of decay, but these effects were overcome over the long term (Lawrey, 1976). The slow initial decomposition on the mine spoil was believed to be the result of low soil pH, high levels of trace metals, and a decrease in fungal diversity and soil respiration. In a subsequent study, Lawrey (1977) determined that the decay rate of a variety of litter types (placed in four areas which had been mined and subsequently revegetated) was correlated with soluble carbohydrate, ash and macronutrient components of the litter but decay rates did not differ amongst the four study sites. When investigating decomposition of tall fescue on minespoils in Missouri, Carrel et al. (1979) found that loss of dry weight was more rapid on vegetated minespoil than on bare minespoil. They also noted that litter decomposition in the vegetated minespoil was highly variable and that the initial C/N ratio of the litter was

a major factor in determining the rate of decay. Interestingly, loss of nitrogen did not differ between the bare and vegetated minespoil. Lanning and Williams (1979) also reported a relationship (negative) between C/N ratio of grass and clover shoots and roots and their decomposition rates in china clay sand wastes in Britain. Clover shoots and roots were observed to decompose faster than grass shoots and roots with net N release occurring from the clover shoots and roots. These data suggest that the use of legumes in revegetating minespoils would lead to a faster turnover of nutrients immobilized in the plant tissue, and an accumulation of organic matter and N (unless the spoil is sandy when potential losses of N through leaching would be enhanced).

Although the value of legumes for revegetating N-deficient minespoils has been generally recognized, very little research has been conducted on the conditions required by legumes to ensure their establishment (and subsequent N-fixing ability) in minespoils. Rather, revegetation efforts have leaned heavily on the use of expensive fertilizers to raise N to levels where plant (mainly grasses) growth would not be inhibited. A study reported by Skeffington and Bradshaw (1980) on N inputs into china clay wastes through leguminous plants, non-leguminous plants, free-living microorganisms and the atmosphere is one of the few to investigate the possibility of using legumes rather than fertilizer for supplying N in mined soil. They concluded that N-fixation rates by non-leguminous plants were so low that atmospheric N input was greater than input from these species. Also, N-fixation by free-living microorganisms was negligible unless an easily available carbon source was provided. However, legumes demonstrated such high N-fixation rates that they could be recommended as a means for accumulating N in this particular mine waste. In relation to the accumulation of N in legume-planted china clay wastes, it should be pointed out that Lanning and Williams (1979) measured very little N-accumulation in the same sandy china clay wastes, unless leaching rates were reduced through the addition of micaceous residues (Lanning and Williams, 1980).

Another important plant-microbial relationship essential to the successful establishment of a maintenance-free vegetative cover on minespoils is that of the endo- and ectomycorrhizal symbiosis. The role of the mycorrhizal fungi in ensuring plant success in nutrient and often moisture-stressed minespoils has been discussed by Zak et al. (1984) and Danielson et al. (1984).

With the exception of a few studies such as that reported by Stroo and Jencks (1982) it can be seen from this brief survey of the literature that much of the research dealing with the microbiological aspects of minespoils has emphasized the immediate effects of mining on various microbial parameters. Very little attention has been directed towards investigating the development of the microbial system as a vegetative cover becomes established. Also, methods of accelerating the development of a productive soil-plant system on minespoils through the incorporation of various organic and inorganic amendments have not been widely studied. As a result, the research reported was initiated with the following objectives:

1. to obtain information on the immediate effects of mining coal and bitumin on a variety of soil microbiological components (bacterial numbers and types, fungal types, hyphal length, soil respiration, microbial biomass C, asymbiotic N₂ fixing potential) and soil decomposition potential at three different minesites in Alberta.
2. to provide detailed information on the rates of redevelopment of biological activity (particularly soil respiration and microbial biomass C) when organic and inorganic amendments are applied to spoil from each of the three minesites and then planted with various herbaceous and woody plant spp.
3. to study the relationship between soil microbial activity and biomass and the decomposition of a pure cellulose substrate and incoming plant litter in each of the variously amended spoils planted with grass or legumes.
4. to assess the effects of amendment on the nodule formation and N₂ fixing ability of each of the legumes planted in each spoil.

Data regarding the effects of amendment of the three minespoils on selected soil chemical and physical characteristics, plant growth and nutrient levels in the foliage of selected plant species are presented in Visser et al. (1984), whilst Danielson et al. (1984) and Zak et al. (1984) have reported the results obtained on the ectomycorrhizal and vesicular-arbuscular mycorrhizal development of some of the plant species.

The effects of single amendments (not various combinations of amendments) on the soil respiratory activity and microbial biomass C development in the three test spoils, and how these measurements related to plant growth are the main aims of this report.

2. MATERIALS AND METHODS

The three areas chosen for the study included a shortgrass prairie site which had been mined for coal in the early 1950's and subsequently abandoned (Bow City, Alta.); a subalpine boreal forest site which is currently being mined for coal (Luscar, Alta.) and a boreal forest site which is currently being mined for oil (Suncor site, Ft. McMurray, Alta.). Short descriptions of these sites have been presented by Visser et al. (1984).

2.1 FIELD STUDY

2.1.1 Plot Design and Sampling Procedure

At each study site, five sampling plots, each 5 m x 5 m, were staked out within a 15 m x 15 m sampling frame in an area disturbed by mining and also in an adjacent undisturbed area (with the exception of the oil sands site where due to the inaccessibility of the disturbed area, plots were established only in the undisturbed jack pine woodland). Each of the five sampling plots was subsequently divided into 100 subplots, each 0.25 m x 0.25 m. These plots were used for sampling soil for general microbiological studies. Decomposition studies were performed in 5 m x 15 m plots adjacent to those used for the microbiological analysis.

The sampling procedure consisted of randomly choosing one of the subplots in each of the disturbed and undisturbed plots at each of the sites, and removing a soil core (5.5 cm dia.) and a larger 1 to 2 L sample from the middle of the subplot. In the disturbed sites, where there was no horizon development, 0-5 cm and 5-15 cm depths were sampled. In the undisturbed sites, samples were removed to a depth where organic surface and inorganic subsurface soil would be included.

Samples were removed from the following horizons in each of the undisturbed sites: the A_h and B_m in the grassland soil, the H and B_m in the subalpine soil and the FH and B in the oil sand soil. Subsamples from the soil cores were processed for various microbiological parameters while general microbial activity was measured in the larger samples. Another set of soil samples was

randomly removed from each of the sites for assessment of asymbiotic N_2 fixation. All sampling was performed in September or October. Samples were placed in plastic bags and stored at 5°C. They were sieved through a 2 mm mesh screen and processed as quickly as possible to avoid possible storage effects on the microbial components. To determine the effects of disturbance on selected soil chemical and physical parameters, subsamples from each depth or horizon were air dried, bulked and tested for sodium adsorption ratio (SAR), organic and CO_3-C , total N, P, Fe, Ca, Mg and Na. Details of the methods used are presented in Visser et al. (1984).

2.1.2 Procedures for the Determination of Microbial Parameters

2.1.2.1 Numbers of bacteria. A soil suspension was prepared by homogenizing 1 g soil in 99 ml sterile, dilute peptone (0.13%) at high speed for 3 min in a Waring blender. Serial dilutions were then prepared and 0.1 ml of the 10^{-5} , 10^{-6} , 10^{-7} dilutions spread onto pre-dried, half-strength PYE (2.5% peptone, 0.5% yeast extract) agar plates. Five replicate plates per dilution per soil sample were plated. Colonies were counted after 7-19 days incubation at 15°C.

2.1.2.2 Types of bacteria. Using a randomized grid method, 12 bacteria per replicate sample were selected and placed in pure culture resulting in 60 isolates per depth per site. The isolates were placed into broad taxonomic groups using the methods outlined by Nelson and Visser (1978).

2.1.2.3 Numbers of actinomycetes. The same homogenate, same serial dilutions and same techniques were used for quantifying the actinomycetes as those used for the bacteria, with the exception that 0.1 ml of 10^{-4} , 10^{-5} , 10^{-6} dilutions were spread onto 3 replicate, pre-dried chitin agar (Hsu and Lockwood, 1975) plates per soil sample. The plates were incubated for at least 14 days at 15°C prior to counting.

2.1.2.4 Length of fungal mycelium. 2 g and 1 g subsamples were removed from the inorganic and organic soil replicates respectively and fixed in Bouin-Hollande fixative until time of analysis. Agar films were then prepared using a modified Jones and Mollison (1948) agar film technique, i.e. soil samples were macerated in water and fixative in a Waring blender for 3 min and from the resulting suspension, soil + water agar films prepared, dried, mounted and observed using phase contrast microscopy. The line intersect method (Olson, 1950) was used to measure hyphal lengths on the films and empty (inactive) hyphae were distinguished from those with cell contents (active) by the criteria described by Frankland (1975). Five films (50 observations/film) were assessed for each soil replicate.

2.1.2.5 Types of fungi. 2.5 g and 5 g soil samples were removed from the organic and inorganic soil replicates respectively. Samples were serially washed (30, 1 min washes with sterile water) using the apparatus and methods described by Bissett and Widden (1972). Excess moisture was removed from the washed soil samples, and soil particles were then plated on 2% malt extract agar containing 100 ppm streptomycin and 50 ppm chlorotetracycline. The size of the particles plated ranged from .2-.5 mm for the organic soils and .5-1 mm for the inorganic soils. Fifteen particles (two/plate) were plated from each of the organic soil replicates and 20 particles (four/plate) were plated from each of the organic soil samples. More particles were plated from the inorganic than organic soils since organic soils are more heavily colonized by fungi than are inorganic soils. The plated soil particles were incubated at 15°C and fungi emerging from each particle isolated and identified.

2.1.2.6 Soil respiration (CO₂ efflux). Each soil sample was sieved through a 2 mm mesh sieve and as many roots as possible were removed from the sample to avoid interference from live root respiration. All roots were removed since the distinction between live and dead roots is rather tenuous. Moisture contents were

determined for each sample and samples were remoistened with distilled water if moisture levels were believed to be limiting to microbial activity. Sieved replicate samples, each equivalent to 100 g dwt, were thoroughly mixed and then placed in plastic tubes, stoppered with plastic foam plugs, and connected to an "Ultragas 3" CO₂ analyzer (Wöstoff Company, Bochum, Germany).

The CO₂ efflux was measured hourly at room temperature (22-23°C) until CO₂ evolution had stabilized. At that point (20-24 hr after beginning CO₂ measurements) respiratory activity was calculated as ml CO₂ $\uparrow$ 100 g⁻¹ dwt soil hr⁻¹.

2.1.2.7 Microbial biomass C. The same soil samples as those used for CO₂ efflux measurements were used for estimating microbial biomass C (i.e. the amount of C in the living, non-resting microbial biomass). Microbial biomass C was determined using the technique described by Anderson and Domsch (1978) which is based on the measurement of glucose stimulated respiration prior to the commencement of microbial growth. The relationship between the glucose stimulated CO₂ evolution from a soil sample and the microbial biomass in that sample was determined by Anderson and Domsch (1978) i.e. 1 ml CO₂·h⁻¹ measured at 22°C is equivalent to 40 mg microbial biomass C. Optimal glucose concentrations for maximum respiratory response were determined for representative soil samples from each soil horizon or soil depth and these glucose concentrations were then used to determine the microbial biomass C levels in the replicate samples.

2.1.2.8 Adenosine 5'-triphosphate (ATP) measurements. 1 g subsamples from each soil replicate were extracted with sodium bicarbonate and chloroform and the ATP subsequently measured using the luciferan-luciferase assay and a JRB Model II Photometer to measure the light emitted (Paul and Johnson, 1977).

2.1.2.9 N₂ (C₂H₂) fixation. The acetylene (C₂H₂) reduction assay similar to that of Hardy et al. (1968) but modified

by Paul et al. (1973) was applied to each soil sample to determine N_2 fixation potential. Sieved, remoistened soil was placed in standard sized tubes which were then sealed in 909 ml Mason jars. Ten percent of the atmosphere volume in each jar was removed with a syringe and replaced with C_2H_2 . Samples were incubated with C_2H_2 for 1 hr at $15^\circ C$ prior to making C_2H_4 measurements. The following controls were included: empty jar, empty jar containing 0.1 atm C_2H_2 and jar containing a soil core and no acetylene. The rate of ethylene (C_2H_4) production (after subtraction of any C_2H_2 detected in the controls) was used to calculate nitrogenase activity in the soil, using a theoretical ratio of 3 to 1 between C_2H_4 production and N_2 fixed (Hardy et al. 1973).

2.1.2.10 Decomposition of standard substrates. Filter paper (Whatman #1, 5.5 cm dia.) was dried to a constant weight at $35^\circ C$, bagged in 1 mm nylon mesh and tagged. The filter paper was left unamended or amended with ammonium nitrate to adjust the C/N ratio of the paper to 25/1. Since filter paper lacks nitrogen and since soil N is often limiting, particularly in soils disturbed by mining, it was hypothesized that the addition of N to the paper could potentially accelerate its decomposition.

To study the effects of disturbance on the decomposition of a recalcitrant substrate, wood (Douglas fir) dowel was also used as a test substrate. The dowel, 2 cm diameter, was cut into 5 cm segments (knots were discarded) dried to a constant wt and tagged. As for the filter paper, wood segments were left unamended or soaked in .36 M ammonium nitrate for 48 hrs prior to drying, weighing and tagging. Blocks of spruce (Picea glauca (Moench) Voss) wood (approx. 3.8 cm x 1.9 cm x 5 cm) were also used as a test substrate but only at the subalpine site.

Fifty replicates of each of the unamended or N-amended filter paper and wood segment treatments were placed on the soil surface at each of the undisturbed and disturbed sites in October. The plots designed for studying the decomposition of the two substrates (one plot for the filter paper and one for the fir dowel

at each of the disturbed and undisturbed sites) were 5 m wide x 15 m long and contained 13 rows of 4 stakes placed 1 m apart. The substrates were tied to the stakes (one amended and one unamended filter paper or wood segment/stake) and sampled regularly thereafter. At each sampling time, five filter papers and five wood segments were randomly removed from each of the disturbed and undisturbed sites. Filter papers were cleaned, dried, weighed and ashed, and the ash weight subtracted from the weight remaining prior to ashing. Wood segments were brushed clean, agitated in de-ionized water (to remove adhering soil particles) and dried to a constant weight at 35°C. Results were expressed as % dry weight remaining.

Not all the microbial parameters were investigated at all the sites. A list of the microbial parameters which were studied at each of the experimental sites is presented in Table 1. Since a disturbed plot was not established at the oil sands site, the microbial parameters measured in samples removed from the unamended control plots (pure tailings sand prior to planting) in the tank study (see pp. 6-7 of Visser et al. 1984 for a description of the tank study set-up) were compared with the microbial characteristics of the undisturbed site to assess the effects of disturbance.

2.1.2.11 Decomposition of native litter and wood residues. In addition to following the decomposition rates of standard substrates at each of the disturbed and undisturbed sites, a study was also initiated to examine the rates of decay of native litter and wood debris at the undisturbed sites only. It was felt that this information would be useful in future studies designed to compare rates of decay of plant litter in areas in various stages of reclamation to rates of decay of the same litter in an undisturbed and presumably stable system.

The methods used to examine the decomposition rates of leaf litter and wood at each of the sites were as follows:

1. prairie grassland site - Agropyron sp. standing dead grass litter was clipped approximately 1 cm above the soil surface at the grassland site in late September. Leaf blades which were still

Table 1. Microbial parameters investigated at each of the experimental sites to determine the effects of disturbance.

Parameter	Site and soil horizon or depth (cm)										
	Grassland			Subalpine			Oil Sands				
	Undisturbed	Disturbed		Undisturbed	Disturbed		Undisturbed	Disturbed			
	A _b	B _m	0-5	H	B _m	5-15	FH	B	0-5	5-15	
Number of bacteria	+	+	+	+	+	+	+	+	+	+	
Types of bacteria	+	+	+	+	+	+	+	+	+	+	
Numbers of actinomycetes	+	+	+	+	+	+	+	+	+	+	
Length of fungal mycelium	+	+	+	+	+	+					
Types of fungi	+	+	+	+	+	+	+	+	+	+	
CO ₂ efflux	+	+	+	+	+	+	+	+	+	+	
Microbial biomass C	+	+	+	+	+	+	+	+	+	+	
ATP levels	+		+	+	+	+	+	+	+	+	
N ₂ (C ₂ H ₂) fixation	+	+	+	+	+	+					
Decomposition of standard substrates (filter paper, wood dowel)	+	+		+	+		+				

green or yellow at the base (indicating it was the current year's growth) were separated from the other litter, dried to a constant weight at 35°C, weighed into approximately 5 g quantities and bagged in 1 mm nylon mesh bags (size = 13 x 15 cm). Woody branches (approx. 1 cm dia.) of Artemisia cana Pursh (sagebrush) were cut into 5 cm segments, dried to a constant weight at 35°C, weighed and tagged. Fifty litter bags and fifty branch segments were placed in the filter paper and dowel decomposition plots respectively, using the same stakes as those used for the filters and dowel segments. At each sampling time, five replicate litter bags and five branch segments were randomly sampled. Grass blades were removed from the bags and brushed clean if required, while sagebrush wood was given a 30 sec wash in distilled H₂O to remove adhering soil. The % dry weight was then determined for each substrate type.

2. subalpine site - an Abies lasiocarpa (Hook.) Nutt. (alpine fir) tree, blown over by the wind, was located near the margin of the undisturbed sampling site. Green needles and branchwood (2.5-5 cm dia.) were removed from the top 2-4 m (topside branches only) of the tree and used in decomposition studies. Green dying needles and branchwood from one tree only were used in an effort to reduce variation in decay resulting from differences in substrate quality. After drying the needles and branchwood at 35°C, they were treated in the same manner as that described for the prairie grassland Agropyron litter and sagebrush wood. At each sampling time, five replicate needle bags and five wood segments were randomly removed from each plot. The surfaces of the bag were cleaned and needles were then sprayed for 30 sec with tapwater to remove mineral material from the needle surfaces. Wood was treated in the same way as described previously. The % dry wt remaining was then determined for each substrate.

3. oil sands site - needles and branchwood of Pinus banksiana Lamb. (jack pine) were collected from a recently toppled tree as described for the subalpine site. Litter bags and branchwood segments were prepared in the same manner as described previously and placed around the stakes in the filter paper and dowel plots

respectively. Litter bags were placed underneath the lichen mat (i.e. between the sandy soil horizon (A_e) and the lichen) since the lichen would impede the movement of the bag down the soil profile. It was realized that by doing this, decomposition of the needles would be enhanced as a result of better moisture and temperature conditions under the lichen mat. Therefore, 70 pairs of pine needles were strung on each of 15 nylon threads, dried at 35°C, weighed and placed on the surface of the lichen mat. It was hoped that the weight loss of the strung needles would give some indication of the decomposition of needles as they moved through the lichen mat. Unfortunately this method can only be used to monitor weight loss of litter in the early stages of decay since weight loss is overestimated once the litter begins to fragment. Sampling and cleaning of the needles and branchwood was effected in the same manner as that described for the alpine fir substrates.

2.1.3 Effect of Revegetation on Microbial Respiration and Biomass C at the Subalpine Site

Areas which had been revegetated with grasses and legumes for 2, 6 and 7 yr and stockpiled regolith were sampled in June to determine the effects of revegetation on microbial activity and biomass C. These results were then compared to microbial activity and biomass C measured in soil from the undisturbed subalpine forest site (described previously). Five replicate soil samples (1-2 kg each) were removed randomly along a 15 m transect laid out in the revegetated areas and in the undisturbed site. Samples in the revegetated sites were removed from the 0-10 cm depth while those from the undisturbed site were removed from the FH horizon (organic soil) and the B horizon (mineral). Five samples were also randomly taken from the stockpiled regolith (organic and mineral soil and some rock which is removed from above the coal seam and stockpiled for future revegetation of mined areas). Samples were transported to the laboratory in plastic bags and processed as quickly as possible for the following parameters:

1. pH - determined electrometrically using 10 g soil in 20 ml .01 M CaCl_2 .

2. organic matter content - the Walkley-Black (Nelson and Sommers, 1982) method was used to determine organic matter in soils from the revegetated areas and regolith while loss on ignition (see method in Visser et al. 1984) was measured for soils from the undisturbed site.

3. basal respiration - samples were sieved through 2 mm sieve and CO_2 efflux was then measured using the techniques described previously (i.e. Wöstoff CO_2 analyzer).

4. microbial biomass C - the method of Anderson and Domsch (1978), described previously, was applied to all the soil samples. The optimum levels of glucose required to stimulate maximum microbial biomass were $32,000 \mu\text{g g}^{-1}$ undisturbed organic soil (FH layer), $2000 \mu\text{g g}^{-1}$ undisturbed mineral (B horizon) soil, $500 \mu\text{g g}^{-1}$ regolith soil, $2000 \mu\text{g g}^{-1}$ soil revegetated for 2 yr, $2000 \mu\text{g g}^{-1}$ soil revegetated for 6 yr and $8000 \mu\text{g g}^{-1}$ soil revegetated for 7 yr.

2.1.4 Statistical Analysis of Field Data

Many different statistical techniques were required to analyze the data collected in the field studies. Therefore, for convenience sake, the statistical tests applied to each data set have been footnoted at the end of each table of analyzed data.

2.2 SOIL TANK STUDY

It was decided that the effects of various organic and inorganic amendments on plant growth and soil microbiological characteristics in the three minespoils could be more closely monitored if the research was performed near Calgary, rather than at the more inaccessible field sites. Hence large quantities of minespoil from the grassland, subalpine and oil sand sites were transported to Calgary and each placed into separate plywood frames or soil tanks. In general, each of the three soil tanks was partitioned into 12 plots, 5 m x 7 m each and an approximately 60 cm

depth of minespoil placed in each plot. Amendments were then applied to allow three replicate plots per treatment. Anaerobically digested sewage sludge, topsoil and gypsum were applied singly to the grassland minespoil while fibrous peat, sewage sludge and mineral fertilizer were applied to the subalpine minespoil and tailings sand. Three unamended control plots were also set up for each of the three spoils.

Following amendment each plot was subdivided into four subplots with 0.5 m wide walkways between them. In the case of the grassland spoil, each amended subplot was planted with either fall rye, crested wheat, Russian wild rye or rambler alfalfa. The subalpine spoil was planted with slender wheatgrass, alsike clover, and containerized willow or white spruce seedlings, while the amended tailings sand subplots were each planted with slender wheatgrass, sainfoin and containerized bearberry or jack pine. All legume seed had been treated with inoculum of the N_2 -fixing bacteria, Rhizobium. Thus this experimental design allowed for three replicates/plant species in each of the four treatments in each spoil. A more detailed description of the experimental design, the amendments and their application, and information regarding the plant species planted in each spoil type have been presented in Visser et al. (1984).

2.2.1 Sampling Procedure

After amendment, but immediately prior to planting, two cores (5.5 cm dia.) and a larger 1-2 L sample were removed from each plot using a simple random sampling procedure. The samples were separated into 0-5 cm deep soil and 5-15 cm deep soil (except in the topsoil and peat amended pots where the 15-25 cm depth - the spoil beneath the amendment - was sampled) as they were placed in plastic bags. The cored soil samples were used for measuring various microbiological parameters and asymbiotic N_2 (C_2H_2) fixation while CO_2 efflux and microbial biomass C were measured in the larger samples.

After planting, samples were randomly removed from the plots in September/October after the first, second and third growing seasons. In the amended grassland spoil, subplots planted with fall rye were sampled. In the subalpine and oil sands spoils, samples were removed from the subplots planted with slender wheatgrass, spruce or jack pine in an attempt to assess the effects of vegetation and vegetation type on the development of microbial activity. Grass plots were sampled by removing a 10.5 cm dia. core (grassland and oil sands spoil) or a 20 cm x 20 cm block of soil (subalpine spoil) from the middle of one randomly chosen quadrat which had previously been clipped for primary production estimates. Samples taken 39 mo after planting slender wheatgrass in the subalpine and oil sands spoil were removed from beneath litterbags containing slender wheatgrass leaf blades to determine whether the microbial activity and biomass in the soil beneath the litter bag was related to the decomposition of the litter. In the spruce and pine plots, samples were extracted from an area between two randomly chosen trees. As for the pre-planting samples, cores or blocks of soils were separated into 0-5 cm and 5-15 cm (15-25 cm in the topsoil and peat-amended plots) deep soil, placed in plastic bags and stored at 5°C until processed. With the exception of the 39 mo slender wheatgrass soil samples (when one sample was removed from beneath each of two litterbags per subplot resulting in two samples per subplot) only one sample was removed from each subplot from each depth at each sampling time.

Although this study was not designed to examine the microbial development in amended but unplanted minespoil, it was decided after the first growing season that it would be useful to sample unplanted soil in an attempt to separate amendment effects on microbial development from the combined plant/amendment effects. Consequently, samples were removed from the pathways surrounding the slender wheatgrass, white spruce and jack pine plots in the subalpine and tailings sand spoils at each of the sampling times. Only pathways between the tank walls and subplots were sampled as these were seldom walked upon. Samples were removed randomly and separated

into the two depths in a similar manner to that described for the grass plots.

2.2.2 Sample Processing and Microbial Parameters Measured

Soil samples were processed in the following manner:

1. samples were placed on a 2 mm sieve and as many roots as possible removed from the soil. These roots were washed, air dried and weighed, but not returned to the soil sample for the same reasons stated earlier.

2. dead grass litter was separated from the 0-5 cm soil and categorized as leaves or stems. These were air dried, weighed, chopped into 1-2 cm segments and returned to the soil sample since this litter would eventually form part of the soil system. Green plant material was removed from the sample and discarded.

3. as much soil as possible was sieved through the 2 mm mesh; stones and woody debris were discarded. Sewage sludge aggregates present in the sludge amended samples were pressed through the sieve so they would be included in the sample.

After sieving the samples, the effects of amendment on various soil and microbiological characteristics were investigated. The parameters which were studied in each of the three spoil types are listed in Tables 2 to 4. The techniques which were used for the various measurements are as follows:

1. numbers of bacteria - as described for the field study
2. numbers of actinomycetes - as described for the field study
3. hyphal lengths - as described for the field study.
4. loss on ignition - as described in Visser et al. (1984).

5. litter input and root wts - these were estimated from the air dried litter (stems and leaves) and roots sieved out of each sample. Since the area of each core or soil block was known, litter and root wts could be converted to g m^{-2} .

6. CO_2 efflux was measured in the same manner as that described for the field study. Samples were remoistened to the

Table 2. Soil and microbial characteristics which were investigated to determine the effects of amendment on a grassland coal mine spoil (tank study).

<u>Parameter</u>	<u>Sampling times (mo after amendment)</u>	<u>Vegetation on sampled subplot</u>	<u>Sampling depth (cm)</u>	
			<u>0-5</u>	<u>5-15</u>
Bacterial numbers	0.5, 15	Unvegetated	+	+
		Fall rye	+	+
Actinomycete numbers	0.5, 15	Unvegetated	+	+
		Fall rye	+	+
Loss on ignition	0.5, 15, 27	Unvegetated,	+	+
		Fall rye		
Litter input, root wts	27	Fall rye	+	+
			(roots only)	
CO ₂ evolution	0.5, 15, 27	Unvegetated,	+	+
		Fall rye		
Microbial biomass C	0.5, 15, 27	Unvegetated,	+	+
		Fall rye		
Filter paper decomposition	12, 24	Fall rye,	+	
		alfalfa		
Wood dowel decomposition	12, 24	Fall rye,	+	
		alfalfa		
Asymbiotic N ₂ (C ₂ H ₂) fixation	0.5	Preplanting (unvegetated)	+	+
Symbiotic N ₂ (C ₂ H ₂) fixation	3, 14, 26	Alfalfa	+	

Table 3. Soil and microbial characteristics which were investigated to determine the effects of amendment on a subalpine coal spoil (tank study).

Parameter	Sampling times (mo after amendment)	Vegetation on sampled subplot(s)	Sampling depth (cm)	
			0-5	5-15
Bacterial numbers	0.5, 15	Unvegetated, grass	+	+
Actinomycete numbers	0.5, 15	Unvegetated, grass	+	+
Hyphal lengths	0.5, 15	Unvegetated, grass	+	+
Loss on ignition	0.5, 27	Unvegetated, spruce	+	+
	0.5, 15, 27	Unvegetated, grass	+	+
Litter input, root wts	27, 39	Grass, spruce (roots only)	+	+
CO ₂ efflux	0.5, 15, 27	Unvegetated, grass	+	+
		Spruce	+	+
Microbial biomass C	0.5, 15, 27	Unvegetated, grass	+	+
	0.5, 27	Spruce	+	+
Filter paper decomposition	12, 24	Grass, spruce	+	
Wood dowel decomposition	12, 14	Grass, spruce	+	
Stem and leaf decomposition	(6) ¹ , 12	Grass, clover	+	
Asymbiotic N ₂ (C ₂ H ₂) fixation	0.5	Preplanting (unvegetated)	+	+

(cont'd)

Symbiotic N ₂ (C ₂ H ₂) fixation	3, 14, 26	Clover	+
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¹Grass leaves sampled at 6 and 12 mo.

Table 4. Soil and microbial characteristics which were analyzed to determine the effects of amendment on a oil sand tailings (tank study).

Parameter	Sampling times (mo after amendment)	Vegetation on sampled subplot(s)	Sampling depth (cm)	
			0-5	5-15
Bacterial numbers	0.5, 15	Unvegetated, grass	+	+
Actinomycete numbers	0.5, 15	Unvegetated, grass	+	+
Hyphal length	0.5, 15	Unvegetated, grass	+	+
Loss on ignition	0.5, 27, 39	Unvegetated	+	+
	0.5, 15, 27, 39	Grass, jack pine	+	+
Litter input, root wt	27, 39	Grass, pine	+	+
CO ₂ efflux	0.5, 15, 27, 39	Unvegetated, grass pine	+	+
Microbial biomass C	0.5, 15, 27, 39	Unvegetated, grass pine	+	+
Filter paper decomposition	12, 24	Grass, sainfoin	+	
Wood dowel decomposition	12, 14	Grass, sainfoin	+	
Leaf and stem decomposition	(6) ¹ , 12	Grass, sainfoin	+	
Asymbiotic N ₂ (C ₂ H ₂) fixation	0.5	Preplanting (unvegetated)	+	+

(cont'd)

Symbiotic N ₂ (C ₂ H ₂) fixation	3, 14, 26	Sainfoin	+
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¹Grass leaves sampled at 6 and 12 mo.

moisture levels measured in the samples taken prior to planting (i.e. the first sample time at 0.5 mo) so that respiration measurements would be comparable amongst the various sampling times. The respiratory activity of the individual amendments was measured using a Gilson respirometer, since the liquid nature of the sewage sludge prevented this material from being processed on the CO₂ analyzer. Samples equivalent to 15 g dwt for the gypsum and 3 g dwt for the peat and sewage sludge were placed in Parkinson and Coups (1963) flasks and connected to the respirometer. Three, hourly measurements of O₂ uptake were made after samples had equilibrated for 24 h at 22°C.

7. microbial biomass was measured as described previously using the samples for which CO₂ efflux had been measured.

2.2.3 Decomposition Studies

2.2.3.1 Standard substrates (filter paper, wood dowel). The effects of amendment and vegetation types on the decomposition of cellulosic and lignified substrates were studied by following the decay of filter paper and fir wood dowel over a two year term (see Tables 2 to 4 for the vegetation types investigated). Filter papers and dowel segments were prepared in the same manner as for the field studies. One filter paper and one wood segment were placed around each of five stakes inserted in the appropriate subplots four months after planting. Two replicates of each substrate type were sampled after 12 mo incubation in the field while three replicates were sampled 24 mo after initiation of the experiment. Samples were processed as described previously and results expressed as % dry wt remaining.

In an effort to assess whether the decomposition potential of the particular vegetation types under investigation had altered since the initiation of the tank study, another set of filter papers were placed in the appropriate plots 28 mo after planting. Filters were prepared as described previously, but only two filters were placed in each plot. The % dry wt remaining was determined after 12

mo incubation in the field. Wood dowel was not used in this study since its weight loss was negligible after 24 mo in the field.

2.2.3.2 Plant residues (grass and legume litter). Litterbags (1 mm mesh and 16 cm x 8 cm) each containing either slender wheatgrass, alsike clover or sainfoin leaves or stems were placed in the subalpine and oil sand grass and legume plots, 28 mo after planting. The litter material used for this study was that clipped for standing crop estimates at the conclusion of the second growing season. Only plant material grown in the peat-treated plots was used in order to decrease variability in nutrient quality resulting from the different amendments. Plant material was dried to a constant weight at 35°C and weighed samples of stems or leaves were then placed in each bag. Leaves and stems were separated since stems tend to be more recalcitrant than leaves, therefore decomposing at a different rate than leaves do. Four bags each containing slender wheatgrass leaves, and two bags each containing grass stems were staked out in each of the grass subplots in the subalpine and oil sand tank plots. Two bags containing leaves (clover or sainfoin) and two bags containing stems (clover or sainfoin) were also placed in each of the alsike clover and sainfoin subplots. Slender wheatgrass leaves were sampled after 6 mo and 12 mo incubation in the field while all other substrate types were sampled 12 mo after placement in the field. Prior to removing the litter from the bags, samples were washed for 30 sec (15 sec on each side of bag) under cold running water in an effort to remove sand and debris adhering to the leaf and stem surfaces. Samples were then dried and % dry wt remaining was determined.

2.2.4 N₂ Fixation Studies

2.2.4.1 Asymbiotic N₂ (C₂H₂) fixation. Measurements of N₂ (C₂H₂) fixation of the variously amended spoils were conducted on samples removed immediately prior to planting the subplots. The acetylene reduction technique, as described for the field study, was used to determine N₂ fixation. Acetylene reduction was measured on

sieved samples held at field moisture levels and on soil remoistened to field capacity. It was observed that N_2 (C_2H_2) fixation was extremely low and highly variable; hence measurements of this parameter were not continued.

2.2.4.2 Symbiotic N_2 (C_2H_2) fixation. The N_2 fixation capacity of rambler alfalfa (grassland spoil), alsike clover (subalpine spoil) and sainfoin (oil sands spoil) was determined at 3, 14 and 26 mo after planting. The plants were randomly chosen and, where possible, were either flowering or filling pods, since Hardy et al. (1971) observed with soybeans and peanuts that N_2 fixation increases with fruit formation and maturation. Plants, with as much root material as possible, were collected by digging a 20 x 20 x 40 cm deep soil block around each plant, and removing the whole block in an attempt to minimize disturbance of the roots. At least three plants were removed from each plot and transported to the laboratory where roots were washed out as quickly as possible on a 1 mm mesh screen. Excess water was blotted from the roots, shoots were separated from the roots and combined roots from each subplot placed in glass sealing jars. N_2 (C_2H_2) fixation was then determined using the same techniques as described previously. Empty jars injected with acetylene and jars containing roots with no acetylene served as controls to test for residual ethylene in the acetylene gas or natural ethylene production by the roots. After measuring the amount of acetylene reduced to ethylene by the root nodules, roots were dried at 80°C and N_2 (C_2H_2) fixation calculated as nmoles N_2 fixed g^{-1} dry root hr^{-1} using a C_2H_2/N_2 conversion factor of 3. Partial correlation coefficients were computed for data collected from alsike clover and sainfoin sampled 14 mo after planting to determine if any relationships existed between the N_2 (C_2H_2) fixing capacity of the plants and root wt, shoot wt, shoot N, total soil N and soil NO_3-N .

2.2.4 Statistical Analysis

Again (as mentioned for data collected in the field

studies) many statistical techniques were required to analyze the data collected in the tank study. Therefore, to avoid confusion, the statistical tests required to analyze each data set have been footnoted on the tabulated data.

3. RESULTS

3.1. FIELD STUDY

3.1.1 Numbers of Bacteria and Actinomycetes and Hyphal Lengths

Data on the effects of surface mining on bacterial and actinomycete numbers and hyphal lengths at the three study sites are presented in Tables 5 to 7 and Fig. 1 and 2 (grassland and subalpine sites only). These results demonstrate that:

i) in the undisturbed soil at all three sites, numbers of bacteria were significantly higher in the surface organic layers of soil (i.e. soil from the A_h, H and FH horizons in the grassland, subalpine and oil sands plots respectively) than in the subsurface mineral horizons (i.e. B horizon soil at all three sites).

ii) mining disturbance resulted in an increase in bacterial numbers (particularly in the surface soil) at the grassland site. However, at the subalpine and oil sands sites, mining significantly reduced the numbers of bacteria in comparison with bacterial counts from the undisturbed surface soil horizons, but not in comparison with those from the subsurface mineral horizons.

iii) actinomycete numbers were significantly lower in the surface 0-5 cm deep soil from the disturbed plot than in the 0-15 cm undisturbed topsoil at the grassland site. Actinomycete counts in the 5-15 cm mineral soil were not affected by disturbance at this site.

iv) mining disturbance did not significantly influence actinomycete numbers at the subalpine site, while no actinomycetes were detected in soil from either the undisturbed or disturbed oil sands plots.

v) hyphal lengths, both total and those with cell contents, were significantly lower in the disturbed than undisturbed soil. At the subalpine site, mining also decreased the amount of mycelium with clamps (i.e. hyphae belonging to the basidiomycete group of fungi - a group which includes many of the ectomycorrhizal fungi). No clamped hyphae were observed in soil from the grassland

Table 5. Effect of surface mining on bacterial and actinomycete numbers and hyphal lengths at the grassland site.

Microbial Parameter	Soil and depth (cm)				MSE
	Undisturbed		Disturbed		
	Topsoil (0-15)	Mineral (15-25)	Mineral (0-5)	Mineral (5-15)	
<hr/>					
Bacteria (x 10 ⁶ g ⁻¹ dwt soil)	8.1 ^{ab2}	2.6 ^a	96.4 ^C	26.2 ^{bc}	0.79
Actinomycetes (x 10 ⁵ g ⁻¹ dwt soil)	103.1 ^{b¹}	54.8 ^{ab}	6.0 ^a	114.1 ^{ab}	
<hr/>					
Hyphal lengths (m g ⁻¹ dwt soil)					
Total	1201 ^{b²}	1250 ^b	263 ^a	216 ^a	10484
With cell contents	127 ^{b²}	166 ^b	22 ^a	18 ^a	0.23

Data was analyzed by a one-way ANOVA. Where the F-test was significant, differences were determined using the Scheffé multiple contrast procedure (P = 0.05) (Neter and Wasserman, 1974). Values in each row followed by same letter(s) do not differ significantly. MSE = mean square error.

¹Analyzed by a Kruskal-Wallis test.

²Data was ln Y transformed prior to analysis. Values presented are geometric means.

Fig. 1 Effect of surface mining on bacterial and actinomycete numbers and hyphal lengths at the grassland site.

hyphal length with cell contents.

*Bars headed by the same letter not statistically different

Table 6. Effect of surface mining on bacterial and actinomycete numbers and hyphal lengths in the subalpine soil¹.

Microbial Parameter	Soil and depth (cm)				MSE
	Undisturbed		Disturbed		
	Organic (0-10)	Mineral (10-20)	Mineral (0-5)	Mineral (5-15)	
<hr/>					
Bacteria ($\times 10^6$ g ⁻¹ dwt soil)	210 ^c	3.1 ^a	78.9 ^b	54.7 ^b	.21
Actinomycetes ($\times 10^5$ g ⁻¹ dwt soil)	5.1 ^a	0 ^{a2}	1.47 ^a	1.84 ^a	2.77
<hr/>					
Hyphal lengths (m g ⁻¹ dwt soil)					
Total	27106 ^c	2672 ^b	77 ^a	78 ^a	.10
With cell contents	4978 ^c	271 ^b	9 ^a	6 ^a	.41
With clamps	6488	106	.4	0	

¹Data was analyzed by a one-way ANOVA and Scheffé multiple contrasts. Bacteria and actinomycete data required a $\ln(y + 1)$ transformation while the hyphal length data required a $\ln Y$ transformation. Means for these parameters are geometric. Values in each row followed by the same letter do not differ significantly ($p = 0.05$). MSE = mean square error.

²No variation, therefore not included in analysis. The confidence interval calculated for the other treatments indicated this value was not significantly different from the other values.

Fig. 2

Effect of surface mining on bacterial and actinomycete numbers and hyphal lengths in the subalpine soil.

hyphal length with cell contents

*Bars headed by same letter are not statistically different

Table 7. Effect of surface mining on bacterial and actinomycete numbers in the extracted oil sands.

Microbial Parameter	Soil and depth (cm)			
	Undisturbed		Disturbed	
	Organic (0-5)	Mineral (5-15)	Mineral (0-5)	Mineral (5-15)
Bacteria ¹ (x 10 ⁶ g ⁻¹ dwt soil)	34.3 ^b (28.8)	2.5 ^a (0.6)	7.5 ^a (0.7)	8.7 ^a (1.2)
Actinomycetes	0	0	0	0

¹Data analyzed by Kruskal-Wallis test. Significant differences amongst the treatments were determined by post hoc comparisons ($p \leq 0.05$). () = standard deviation.

site. Hyphal lengths in soil from the undisturbed oil sand plots were not determined.

3.1.2 Bacteria Isolated by Dilution Plating

The most frequently isolated groups of bacteria in the undisturbed prairie grasslands topsoil were Arthrobacter, Bacillus, the coryneforms and the Gram negative nonpigmented rods while Bacillus, the coryneforms and Gram negative nonpigmented rods were the most common organisms in the subsurface mineral soil (Table 8). After mining the grasslands soil, the isolation frequency of the coryneforms increased significantly (particularly when comparing the undisturbed topsoil with the disturbed soils) while the frequencies of Bacillus and the nonpigmented Gram negative rods decreased. In general, the frequency of Gram negative bacteria decreased and the Gram positive bacteria increased after mining. The % viability (after 6 mo storage) of the bacteria isolated from the topsoil was significantly lower than the % viability of isolates from the disturbed soils (Table 8).

The groups of bacteria isolated from the undisturbed subalpine soil were dominated by coryneforms, Cytophaga, Flavobacterium (organic soil only) and nonpigmented Gram negative bacteria (Table 9). As in the prairie grasslands soil, mining caused a significant increase in the frequency of coryneforms while the nonpigmented rods tended to decrease. The number of Cytophaga isolates was also lower in the disturbed than undisturbed soil. In general, the bacterial isolation data from the subalpine soils demonstrated the same trend as that from the grasslands soil; that is, Gram negative bacteria decreased while Gram positive bacteria increased after disturbance. The viability of isolates from the undisturbed subalpine soil was significantly affected by storage in comparison with the viability of isolates from the disturbed soils (Table 9).

The coryneforms and yellow pigmented and nonpigmented Gram negative rods were the most common bacteria in the undisturbed 0-5 cm soil from the oil sands site, while the coryneforms, Cytophaga and nonpigmented Gram negative rods were very frequent in the 5-15 cm

Table 8. Effect of surface mining on the isolation frequencies of the bacterial groups in grasslands minespoil¹.

Bacterial group	Percent of total viable isolates				MSE
	Undisturbed		Disturbed		
	Topsoil (0-15)	Mineral (15-25)	Mineral (0-5)	Mineral (5-15)	
<u>Arthrobacter</u>	13.9 ^a	3.3 ^a	5.0 ^a	10.0 ^a	81.5
<u>Bacillus</u>	29.6 ^{ab}	32.7 ^b	6.7 ^{ab}	3.3 ^a	246.0
<u>Coryneforms</u> (excluding <u>Arthrobacter</u> and <u>Nocardia</u>)	26.4 ^a	40.3 ^{ab}	71.7 ^b	68.3 ^b	472.4
<u>Cytophaga</u>	3.7 (5.05)	0 (0)	0 (0)	0 (0)	
<u>Flavobacterium</u>	2.0 (4.47)	0 (0)	0 (0)	0 (0)	
<u>Nocardia</u>	1.8 (4.07)	3.5 (4.77)	0 (0)	0 (0)	
<u>Staphylococcus</u>	0 (0)	0 (0)	6.7 (14.89)	0 (0)	
Yellow pigmented Gram negative rods	2 (4.47)	0 (0)	5.0 (7.46)	3.3 (4.55)	
Nonpigmented Gram negative rods	20.6 ^b	20.1 ^b	3.3 ^{ab}	0 ^a	74.6
Gram positive cocci	0 (0)	0 (0)	0 (0)	15 (12.35)	
Total Gram negative	28.2 ^b	20.3 ^{ab}	8.3 ^a	3.3 ^a	80.6
Total Gram positive	71.8 ^a	79.7 ^{ab}	91.6 ^b	96.7 ^b	80.6
% viability after storage	86.7 ^a	98.3 ^{ab}	100 ^b	100 ^b	43.4

(cont'd)

¹Where possible, data was analyzed using a one-way ANOVA and Scheffé multiple contrasts. Values in each row followed by the same letter do not differ significantly ($p = 0.05$). MSE = mean square error. () = standard deviation.

Table 9. Effect of surface mining on the isolation frequencies of the bacterial groups from the subalpine minespoil¹.

Bacterial group	Undisturbed		Disturbed		Stockpiled regolith	MSE
	Organic (0-10)	Mineral (10-20)	Mineral (0-5 cm)	Mineral (5-15 cm)		
<u>Arthrobacter</u> ²	5.4 (7.37)	3.3 (7.47)	17.6 (12.90)	18.5 (9.15)	0 (0)	
<u>Bacillus</u>	2.5 ^a	8.7 ^a	11.6 ^a	7.6 ^a	6.4 ^a	46.0
<u>Chromobacterium</u>	0 (0)	0 (0)	0 (0)	0 (0)	3.0 (5.25)	
<u>Coryneforms</u> (excluding <u>Arthrobacter</u>)	6.0 ^a	14.4 ^{ab}	46.1 ^c	27.6 ^{abc}	19.1 ^{abc}	272.2
<u>Cytophaga</u>	16.2 (10.26)	22.9 (20.34)	0 (0)	5.6 (8.25)	3.0 (5.77)	
<u>Flavobacterium</u>	11.1 (12.10)	0 (0)	1.7 (3.71)	7.2 (7.17)	0 (0)	
<u>Flexibacter</u>	0 (0)	0 (0)	0 (0)	1.8 (4.07)	0 (0)	
<u>Nocardia</u>	0 (0)	0 (0)	5.9 (5.41)	2.0 (4.47)	3.0 (5.25)	
<u>Pseudomonas</u>	2.0 (4.47)	0 (0)	0 (0)	0 (0)	0 (0)	

<u>Staphylococcus</u>	2.0 (4.47)	2.5 (8.35)	3.3 (7.47)	4.0 (8.94)	3.6 (10.57)
Other Gram positive cocci	0 (0)	0 (0)	6.8 (7.04)	1.8 (4.07)	6.1 (17.32)
Yellow pigmented	7.0 (10.95)	3.3 (7.47)	0 (0)	5.6 (8.25)	3.3 (5.77)
Gram-negative rods	0 (0)	0 (0)	0 (0)	0 (0)	2.3 (4.79)
Orange pigmented	47.9 ^b	44.9 ^{ab}	4.3 ^a	15.0 ^a	34.7 ^{ab}
Gram-negative rods ³					0.3
Gram negative bacteria	84.2 ^c	71.1 ^{ab}	8.8 ^a	36.5 ^{ab}	55.4 ^{abc}
Gram positive bacteria	15.9 ^a	29.0 ^{ab}	91.3 ^c	61.5 ^{bc}	44.6 ^{abc}
% viability after storage	58.3 ^a	58.3 ^a	91.7 ^b	90.0 ^b	91.7 ^b
					134.5

¹Where possible, data was analyzed by a one-way ANOVA and Scheffé multiple contrasts. Values in each row followed by same letter do not differ significantly ($p = 0.05$). MSE = mean square error. () = standard deviation.

²No Arthrobacter occurred in regolith, hence this treatment was not included in the analysis.

Although the F value was significant for the other treatments, no pairwise or linear trends could be detected.

³Data was 2 arcsin $\sqrt{p}$ transformed. Means are geometric.

deep soil from the same site (Table 10). The bacterial groups present in the sand after extraction of the oil were not determined in this study.

3.1.3 Fungi Isolated by Soil Washing Technique

Tables 11 to 13 present data on the most frequently isolated fungi from the three study sites, both before and after disturbance. At the grassland site, the Chrysosporium-Pseudogymnoascus spp., and the sterile dark and sterile hyaline forms (these are organisms which will not fruit in culture; hence are termed sterile) were the most common isolates from the undisturbed soil (Table 11). Fungal isolates from the disturbed soil were dominated by Alternaria spp., Cladosporium spp., Penicillium spp., sterile dark forms and yeasts. The % of total soil particles plated which yielded fungi ranged from 70 to 90.

Mortierella spp., Mucor spp., Mycelium radicis atrovirens (MRA), Oidiodendron spp. (organic soil mainly), Penicillium spp. (organic soil mainly), Tolypocladium (mineral soil), Trichoderma polysporum and the yeasts (mineral soil mainly) formed a large proportion of the isolates from the undisturbed spruce-fir forest at the subalpine site (see Table 12). The fungal community in the stockpiled regolith consisted mainly of Acremonium spp., Chrysosporium spp., Phialophora spp. and yeasts. After the stockpiled regolith had been spread, but prior to planting, the frequency of Candida (a yeast) increased significantly. There were also significantly more isolates of Chrysosporium spp. in the disturbed soil than in the undisturbed organic soil. Therefore, the effect of mining disturbance on the fungal community of the subalpine soils was to shift it from one dominated by Mortierella, Mucor, Oidiodendron, Penicillium and Trichoderma polysporum to one dominated by the Candida and Chrysosporium spp. The % of total soil particles plated from the disturbed plot which yielded fungi was significantly lower than that from the undisturbed soils.

Of the three study sites, the effects of mining on the fungal community were most drastic at the oil sands (Table 13). The

Table 10. Isolation frequencies of the bacterial groups from the undisturbed oil sands site¹.

Bacterial group	Percent of total viable isolates			
	Organic (0-5 cm)		Mineral (5-15 cm)	
	$\bar{X}$	SD	$\bar{X}$	SD
<u>Arthrobacter</u>	1.4	3.2	6.2	9.1
<u>Bacillus</u>	2.9	3.9	0	0
<u>Chromobacterium</u>	1.4	3.2	0	0
Coryneforms				
(excluding <u>Arthrobacter</u>)	30.8 ^a	11.8	24.8 ^a	19.6
<u>Cytophaga</u>	8.5	15.5	22.8	37.6
<u>Flavobacterium</u>	2.8	3.8	3.6	8.1
Yellow pigmented				
Gram negative rods	16.9	16.4	9.9	11.3
Orange pigmented				
Gram negative rods	4.2	3.8	0	0
Nonpigmented				
Gram negative rods	31.2 ^a	20.8	32.6 ^a	31.8
Gram negative bacteria	65.0 ^a	13.5	69.0 ^a	20.1
Gram positive bacteria	35.0 ^a	13.5	31.0 ^a	20.1
% viability after storage	94.7 ^b	3.0	68.0 ^a	24.2

¹Where possible data were analyzed by Student t-test. Values in each row followed by same letter do not differ significantly ($p = 0.05$). SD = standard deviation.

²Data analyzed by Fisher's randomization t-test.

Table 11. Effect of surface mining on the per cent frequency of occurrence of the most commonly isolated fungal groups at the prairie grasslands site (i.e. having a mean frequency of occurrence >5% in any one soil replicate).

Fungal group	Soils and depth (cm)				MSE
	Undisturbed		Disturbed		
	Topsoil (0-15)	Mineral (15-25)	Mineral (0-5)	Mineral (5-15)	
<u>Alternaria</u> spp.	0 ^a	0 ^a	23.7 ^a	9.7 ^a	53.51 ³
<u>Chrysosporium</u> - <u>Pseudogymnoascus</u>	20.0 ^{ab} ¹	66.8 ^b	3.3 ^a	7.3 ^a	0.26
<u>Cladosporium</u> spp.	0.3 ^a	0 ^a	13.7 ^b	2.1 ^{ab}	0.08
<u>Fusarium</u> spp.	3.8 ^{ab} ¹	0.1 ^a	1.6 ^{ab}	5.3 ^b	0.04
<u>Mortierella</u> spp.	4.5 ^a	6.3 ^a	0.1 ^a	1.8 ^a	0.06
<u>Oidiodendron</u> spp.	0 ^a	0 ^a	5.5 ^a	9.2 ^a	21.53 ³
<u>Penicillium</u> spp.	0.6 ^a	4.9 ^{ab}	6.4 ^{ab}	23.1 ^b	0.13
<u>Trichocladium</u> sp.	0 ^a	6.0 ^a	0 ^a	0 ^a	12.4 ³
Sterile dark	24.0 ^a	4.0 ^a	17.5 ^a	12.5 ^a	121.77
Sterile hyaline	7.5 ^{ab}	15.2 ^b	1.7 ^a	5.0 ^a	23.0
Yeasts	3.5 ^{ab} ²	0.7 ^a	35.2 ^b	12.5 ^{ab}	
% of particles plated which yielded fungi	76.0	86.8	70.0	87.5	

Values in each row followed by same letter do not differ significantly ($p = 0.05$).

¹Data analyzed by a one-way ANOVA after $2 \arcsin \sqrt{p}$ transformation (sterile hyaline data did not require a transformation). Significant differences were detected by Scheffé multiple contrasts. Values are geometric means. MSE = mean square error.

²Data analyzed with a Kruskal-Wallis test.

³Data analyzed by Student t-test; except for Trichocladium. Value in MSE column is 95% confidence interval for difference.

Table 12. Effect of surface mining on the per cent frequency of occurrence of the most commonly isolated fungi at the subalpine site (i.e. those genera having a mean frequency of occurrence >5% in any one soil replicate)¹.

Fungus	Soils and depth (cm)					Stockpiled regolith	MSE
	Undisturbed		Disturbed				
	Organic (0-10)	Mineral (10-20)	Mineral (0-5)	Mineral (5-15)			
<u>Acremonium</u>	5.3 ^b	1.8 ^{ab}	0.3 ^a	0.5 ^{ab}	11.1 ^c	.031	
<u>Candida</u> ²	0	0	20.3 ^a	9.3 ^a	0		
<u>Chrysosporium</u>	3.3 ^a	8.8 ^{ab}	18.0 ^b	21.5 ^b	21.1 ^b	.089	
<u>Mortierella</u>	84.0 ^c	35.0 ^b	0.3 ^a	2.5 ^a	0	.080	
<u>Mucor</u>	14.0 ^{ab}	27.3 ^b	0.5 ^a	1.5 ^a	1.1 ^a	.177	
<u>Mycelium radicans</u>							
<u>atrovirens</u>	34.7	6.0	0	0	0		
<u>Oidiodendron</u>	20.0 ^b	4.0 ^{ab}	1.3 ^a	0.3 ^a	3.3 ^{ab}	.090	
<u>Penicillium</u>	54.7 ^b	8.5 ^a	0.8 ^a	0.5 ^a	1.1 ^a	.308	
<u>Phialophora</u>	0	9.0	0.5	0.3	30.0		
<u>Pseudogymnoascus bhattii</u>	0	5.0	0	0.5	0		
<u>Rhinocladiella</u>	11.3	3.8	2.0	0.3	0		
<u>Tolypocladium</u>	2.7	16.8	0	0	0		
<u>Trichoderma polysporum</u>	26.7	14.5	0	0	0		
<u>Verticillium</u>	7.3	0	0	0	0		

(cont'd)

Yeasts	0	19.3	2.0	11.5	43.3	
Sterile dark	2.2 ^a	9.5 ^a	5.5 ^a	3.0 ^a	8.9 ^a	.080
Sterile hyaline	0	8.3	1.3	0	6.7	
% total plated soil	100.0 ^c	98.5 ^c	45.4 ^{ab}	40.2 ^a	82.3 ^{bc}	378.1
particles yielding fungi						

1Frequency of occurrence = $\frac{\text{number of isolates per genus}}{\text{total number of plated soil particles}} \times 100\%$. Where possible, data was analyzed by a one-way ANOVA after $2 \arcsin \sqrt{p}$ transformation. Transformed data presented as geometric means. Significant differences were detected by Scheffé multiple contrasts for pairwise comparisons. Values in each row followed by the same letter do not differ significantly ($p \leq 0.05$). MSE = mean square error.

2Data analyzed by Student t-test.

Table 13. Effect of surface mining on the percent frequency of occurrence of the most commonly isolated fungal genera at the oil sands site (i.e. those having a mean frequency of occurrence >5% in any one soil replicate)¹.

Fungus	Soils and depth (cm)				MSE
	Undisturbed		Disturbed		
	Organic (0-5)	Mineral (5-15)	Mineral (0-5)	Mineral (5-15)	
<u>Acrononium²</u>	10.0 ^a	(14.5)	0	1.1 (1.9) ^a	0
<u>Beauveria</u>	0	10.0 (22.4)	0	0	0
<u>Cladosporium</u>	0	0	10.0 (8.8)	0	0
<u>Humicola</u>	0	15.3 (6.1)	0	0	0
<u>Mortierella</u>	68.5 ^b	10.0 ^a	2.2 ^a	0	122.1
<u>Mucor</u>	13.0 (7.8)	10.7 (4.3)	0	0	0
<u>Mycelium radidis atrovirens</u>	2.5 (4.3)	10.7 (10.4)	0	0	0
<u>Oidiodendron</u>	32.5 (24.2)	7.3 (3.6)	0	0	0
<u>Penicillium</u>	86.5 ^b	7.3 ^a	1.1 ^a	0	59.3
<u>Pseudogymnoascus</u>	0	16.0 (8.6)	0	0	0
<u>Thysanophora</u>	2.5 (5.6)	15.3 (13.0)	0	0	0
<u>Trichoderma</u>	44.2 (37.8)	14.7 (21.9)	0	0	0
<u>Verticillium</u>	6.0 (5.5)	0	0	0	0
<u>Yeasts</u>	0	2.0 ^a	10.0 ^a	1.1 ^a	41.4
<u>Sterile dark</u>	2.0 ^a	8.7 ^b	2.2 ^a	0	10.52

(cont'd)

Table 13. (cont'd)

Sterile hyaline ²	0	12.7 ^a	(7.2)	2.2 ^a	(1.9)	0
% total plated soil ³						
particles yielding fungi	99.7 ^c	96.4 ^c		26.5 ^b		0.4 ^a
						192.05

¹Frequency of occurrence = $\frac{\text{number of isolates per genus}}{\text{total number of plated soil particles}} \times 100\%$. Where possible, data were analyzed by a one-way ANOVA and Scheffe multiple contrasts for pairwise comparisons. (0 values were excluded from the analysis). Values in each row followed by the same letter are not significantly different ($p \leq 0.05$). MSE = mean square error.

²Data analyzed by a Student t-test. () = standard deviation.

³Data were $2 \arcsin \sqrt{p}$ transformed for analysis. Means are geometric.

fungi isolated from the undisturbed jack pine woodland were highly diverse with Mortierella, Oidiodendron, Penicillium and Trichoderma being the most common fungi in the organic soil and Humicola, Pseudogymnoascus, Thysanophora, Trichoderma and sterile hyaline forms, the most common in the subsurface mineral soil. The tailing sand, however, was very poorly colonized by fungi. Only the surface, 0-5 cm sand was colonized to any degree - the organisms being mainly Cladosporium spp. and yeasts which are both common in the atmosphere. Data on the % total soil particles plated yielding fungi provide further evidence for the sparsity of fungi in the sand after oil extraction.

3.1.4 Microbial Activity, Biomass and ATP

At the grassland and subalpine sites surface mining resulted in a significant decrease in microbial activity (CO_2 efflux), microbial biomass C and ATP levels (Tables 14 and 15, Figs. 3 and 4). Although microbial biomass C measurements were higher in the undisturbed sand than in the disturbed sand, mining did not significantly influence levels of microbial activity and ATP (Table 16, Fig. 5). Data for these two parameters, however, were exceedingly variable.

3.1.5 Asymbiotic N_2 Fixation

Measurements of asymbiotic N_2 fixation in remoistened soil from the grassland and subalpine site were extremely low in all soils tested and highly variable (Tables 17 and 18). No N_2 (C_2H_2) fixation was measured in the disturbed grassland soils and very low levels of activity were recorded for the disturbed soils and stockpiled regolith from the subalpine site. Asymbiotic N_2 fixation was not assessed for soils from the oil sands site.

3.1.6 Decay Rates of Standard Substrates (Filter Paper, Wood Dowel)

Values for the % dry wt remaining of filter paper placed in the undisturbed and disturbed grassland plots and sampled regularly

Table 14. Effect of surface mining on CO₂ efflux, microbial biomass C and ATP levels at the grassland site.

Microbial parameter	Soil type and depth (cm)			
	Undisturbed topsoil (0-15)		Disturbed mineral (0-5)	
	$\bar{X}$	SD	$\bar{X}$	SD
CO ₂ evolution (ml 100g ⁻¹ dry soil hr ⁻¹)	0.43 ^{a1}	0.05	0.16 ^b	0.07
Microbial biomass C (mg 100g ⁻¹ dry soil)	84.6 ^a	9.6	14.8 ^b	8.3
ATP (μ g 100g ⁻¹ dry soil)	32.2 ^{a2}	40.0	1.4 ^b	1.8
Soil moisture (% wet wt)	13.0 ^a	0.53	12.0 ^a	1.64

¹CO₂† data and microbial biomass data were analyzed using a Student t-test. Means ($\bar{X}$) in each row followed by the same letter do not differ significantly ($p = 0.05$). SD = standard deviation.

²The Wilcoxon test was applied to the ATP data. Values in each row followed by the same letter do not differ significantly ($p = 0.05$).

Fig. 3 Effect of surface mining on CO₂ efflux, microbial biomass C and ATP levels at the grassland site.
 *Bars headed by same letter are not statistically different

Table 15. Effect of surface mining on CO₂ efflux, microbial biomass C and ATP levels at the subalpine site¹.

Soil type and depth (cm)	Microbial Parameter					
	CO ₂ efflux (ml 100 g ⁻¹ soil hr ⁻¹)	Microbial biomass (mg C 100 g ⁻¹ soil)		ATP (µg 100 g ⁻¹ soil)	Soil H ₂ O (% wet wt)	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Undisturbed organic (0-10)	5.29 ^b	.07	978.44 ^b	.77	20.32 ^b	64.05
Disturbed mineral (0-5)	0.14 ^a	.07	24.01 ^a	1.07	1.27 ^a	.02
Undisturbed mineral (10-20)	0.55 ^b	0	66.05 ^b	1.76	2.94 ^a	.75
Disturbed mineral (5-15)	0.14 ^a	.32 ²	32.80 ^a	4.11	.24 ^a	.04
					10	.79

¹Data analyzed using a Student t-test. Undisturbed organic biomass required sq. rt. transformation and ATP data was ln Y transformed. Transformed data are presented as geometric means. Means in each column within a soil layer followed by the same letter do not differ significantly (p = 0.05). SD = standard deviation.

²Undisturbed mineral data had 0 variance, hence means were tested using the 95% confidence interval for the mineral data.

Fig. 4 Effect of surface mining on CO₂ efflux, microbial biomass and ATP levels at the subalpine site.

*Bars headed by same letter are not statistically different

Table 16. Effect of surface mining on CO₂ efflux, microbial biomass C and ATP levels at the oil sands site¹.

Soil type and depth (cm)	Microbial Parameter					
	CO ₂ efflux (ml 100 g ⁻¹ soil hr ⁻¹)	Microbial biomass (mg C 100 g ⁻¹ soil)	ATP (μg 100 g ⁻¹ soil)	Soil H ₂ O (% wet wt)		
	$\bar{X}$ $\frac{SD}{2}$	$\bar{X}$ $\frac{SD}{}$	$\bar{X}$ $\frac{SD}{}$	$\bar{X}$ $\frac{SD}{}$		
Undisturbed organic (0-5)	.10 ^a .32	21.44 ^b .36	3.16 3.98	6.3 2.7		
Disturbed mineral (0-5)	.05 ^a 0	3.61 ^a .28	0 0	5.2 1.4		
Undisturbed mineral (5-15)	.10 ^a .12	9.28 ^b 1.52	0 0	5.3 1.3		
Disturbed mineral (5-15)	.05 ^a 0	3.97 ^a .64	0 0	6.7 2.1		

¹Data analyzed by a Student t-test. Disturbed basal CO₂ † data had no variance so the mean was used as an absolute value and tested with 95% confidence interval. Values in each column within the same soil layer followed by the same letter do not differ significantly (p = 0.05).

²Required a sq. rt. transformation. Means are geometric.

Fig. 5 Effect of surface mining on CO₂ efflux, microbial biomass and ATP levels at the oil sands site.
*Bars headed by same letter are not statistically different

Table 17. Effect of surface mining on N_2 (C_2H_2) fixing potential of remoistened soil from the grassland site.

Parameter		Soil and depth (cm)			
		Undisturbed		Disturbed	
		Topsoil (0-15)	Mineral (15-25)	Mineral (0-5)	Mineral (5-15)
Moisture (% wet wt)	$\bar{x}$	33	27	32	32
	SD	1	1	1	1
N_2 (C_2H_2) fixation ¹	$\bar{x}$	.15	.21	0	0
(nmol.100g ⁻¹ dry soil hr ⁻¹)	SD	.16	.11	0	0

¹The C_2H_2/N_2 conversion factor was assumed to be 3 (Hardy et al., 1973).

Table 18. Effect of surface mining on N_2 (C_2H_2) fixing potential of remoistened soil from the subalpine site.

Parameter	Soil and depth (cm)				
	Undisturbed		Disturbed		Stockpiled regolith
	Organic (0-10)	Mineral (10-20)	Mineral (0-5)	Mineral (5-15)	
Moisture (% wet wt)	$\bar{x}$	78.6	39.5	19.1	14.4
	SD	1.4	9.8	2.6	0.9
N_2 (C_2H_2) fixation ¹	$\bar{x}$	.30	.01	.11	.05
(nmol.100 g-1 dwt soil hr-1)	SD	.28	.03	.13	.05
					.02
					.03

¹The C_2H_2/N_2 conversion factor was assumed to be 3 (Hardy et al., 1973). SD = standard deviation.

thereafter are presented in Table 19 and Fig. 6. There were no differences in wt loss of filters between the undisturbed and disturbed plots until 24 mo after the initiation of the experiment when the disturbed plot filters had lost more wt than the undisturbed plot filters. However, this difference was not significant until 44 mo after the filters were placed on the plots. At that time the wt of paper remaining on the disturbed soil was negligible while 72% of the paper remained in the undisturbed plot.

The decomposition of filter paper placed in the undisturbed and disturbed plots at the subalpine site followed a similar trend to that observed in the grassland site (Table 20, Fig. 7). Filters decayed more rapidly at the disturbed than undisturbed site, but this difference did not become obvious until 33 mo after the initiation of the experiment.

Since no long term decomposition experiment was established on a disturbed plot at the oil sands, filter paper wt loss after 1 yr incubation in the undisturbed jack pine plot was compared with the 1 yr wt loss obtained for filters placed in the control plots in the tank study. The data obtained (Table 21) demonstrated that wt loss of filter paper over the short term was not significantly different between undisturbed and disturbed plots.

Amendation of filter paper with ammonium nitrate to decrease the C/N ratio, resulted in significantly faster decay of the filters placed at the undisturbed grassland site, but had no significant effects on decay rates of filters placed in the disturbed grassland plot and both disturbed and undisturbed subalpine plots (Tables 22, 23, 24 and 25).

The wt loss of fir dowel at the grassland and subalpine sites was negligible over the 44, 45 mo term of the study (Tables 26 and 27). No differences in dowel decomposition were observed between disturbed and undisturbed plots at any of the study sites (Tables 21, 26 and 27) and amendation of the wood with ammonium nitrate did not appear to hasten the rate of decay (Tables 26 and 27). Blocks of spruce wood placed at the subalpine site also decomposed very slowly

Table 19. Effect of surface mining on the decomposition (% dry wt remaining) of cellulose filter paper at the prairie grassland site¹.

Time (mo)	Site	
	Undisturbed	Disturbed
6	99 ^d	100 ^d
12	99 ^d	97 ^d
20	99 ^d	99 ^d
24	93 ^{cd}	51 ^{bc}
32	87 ^{bcd}	26 ^{ab}
44	72 ^{bcd}	0.4 ^a

¹Data analyzed by a two-way ANOVA after $2 \arcsin \sqrt{p}$ transformation. MSE = .1710. A significant interaction was observed ($p \leq .001$). Scheffé multiple contrasts for pairwise comparisons were then calculated to detect significant differences in treatment means. Values are geometric means and where followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Fig. 6 Effect of surface mining on the decomposition of cellulose filter paper at the grassland site.

Table 20. Effect of surface mining disturbance on the decomposition of unamended filter paper at the subalpine site¹.

Time (mo)	% dry filter remaining		Time $\bar{X}$
	Undisturbed site	Disturbed site	
9	100	92	97 ^b
12	99	93	97 ^b
21	100	92	98 ^b
24	84	84	84 ^{ab}
33	86	53	69 ^{ab}
36	63	51	57 ^a
45	61	19	39 ^a
Site $\bar{X}$	90 ^b	72 ^a	

¹Data analyzed by a two-way ANOVA (MSE = 1501.18) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or site $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Fig. 7 Effect of surface mining on the decomposition of cellulose filter paper at the subalpine site.

Table 21. Effect of mining disturbance on the decomposition of filter paper and fir dowl after 1 yr on the oil sands site¹.

Substrate	Site	
	Undisturbed	Disturbed (tanks)
Filter paper	87 ^a	85 ^a
Fir dowl ²	96	99

¹Data analyzed by Student t-test. Values followed by same letter do not differ significantly ($p \leq 0.05$).

²Data not analyzed due to lack of variation.

Table 22. Decomposition (% weight remaining) of ammonium nitrate amended and unamended filter papers at the undisturbed prairie grassland plots¹.

Time (mo)	Treatment		Time $\bar{X}$
	Unamended	N-amended	
6	99	92	96 ^{dc}
12	99	95	98 ^d
20	99	90	96 ^{dc}
24	93	83	88 ^{bc}
32	87	82	85 ^b
44	72	63	67 ^a
Treatment $\bar{X}$	94 ^b	85 ^a	

¹Data analyzed by a two-way ANOVA after 2 arcsin $\sqrt{p}$ transformation (MSE = .0638) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or treatment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Values are geometric means.

Table 23. Decomposition (% dry wt remaining) of ammonium nitrate amended and unamended filter papers at the disturbed prairie grassland plots¹.

Time (mo)	Treatment		Time $\bar{X}$
	Unamended	Amended	
6	100	93	98 ^d
12	97	86	93 ^d
20	99	80	92 ^d
24	51	68	60 ^c
32	26	36	31 ^{bc}
44	0.4	2.0	1.0 ^a
Treatment $\bar{X}$	68 ^a	60 ^a	

¹Data analyzed by a two-way ANOVA after a $2 \arcsin \sqrt{p}$ transformation (MSE = .2830) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or treatment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Values are geometric means.

Table 24. Decomposition of unamended and N-amended filter papers at the undisturbed subalpine site¹.

Time (mo)	% dry wt. remaining	
	Unamended	N-amended
9	100 ^d	91abcd
12	99bcd	83abcd
21	100 ^d	88abcd
24	84 ^{abcd}	72 ^{abc}
33	86 ^{abcd}	84 ^{abcd}
36	63 ^a	67 ^{ab}
45	61 ^a	68 ^{ab}

¹Data analyzed by two-way ANOVA (MSE = 396.61). A significant interaction was observed so Scheffé multiple contrasts were applied to all values to determine treatment effects. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 25. Decomposition of unamended and N-amended filter papers at the disturbed subalpine sites¹.

Time (mo)	% dry filter paper remaining		Time $\bar{X}$
	Unamended	N-amended	
9	85	78	81 ^{ab}
12	90	83	87 ^b
21	83	65	74 ^{ab}
24	77	61	69 ^{ab}
33	53	39	46 ^{ab}
36	52	46	49 ^{ab}
45	28	32	30 ^a
Treatment $\bar{X}$	67 ^a	58 ^a	

¹Data analyzed by two-way ANOVA (MSE = 1032.0) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or treatment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 26. Decomposition (% dry wt remaining) of N-amended and unamended fir dowl at the undisturbed and disturbed prairie grassland sites¹.

Time (mo)	% dry wt remaining			
	Undisturbed site		Disturbed site	
	Unamended	N-amended	Unamended	N-amended
6	99	95	100	96
12	99	94	100	98
20	99	97	100	98
24	98	94	99	97
32	98	95	100	97
44	100	95	100	97

¹Data for the woody substrates were not analyzed due to lack of variation.

Table 27. Decomposition of unamended and N-amended fir dowl and spruce wood blocks at the undisturbed and disturbed subalpine sites¹.

Time (mo)	% dry wt remaining					
	Undisturbed site			Disturbed site		
	Unamended fir	Amended fir	Spruce	Unamended fir	Amended fir	Spruce
9	100	98	98	99	97	93
12	96	93	98	100	98	98
21	100	96	98	99	98	98
24	100	97	96	99	97	96
33	100	99	97	100	98	100
36	100	96	97	100	92	93
45	99	97	-	-	-	-

¹Data not analyzed due to lack of variation.

with no differences observed between undisturbed and disturbed plots (Table 27).

3.1.7 Decay Rates of Native Leaf Litter and Woody Residues

The rate of decay of the native woody substrates at all three sites (i.e. branchwood of Artemisia cana, Abies lasiocarpa and branches and cones of Pinus banksiana at the grassland, subalpine and oil sands sites respectively) was observed to be extremely slow with 80-90% wt remaining after 33 (oil sands site) or 45 mo incubation in the field (Tables 28-30, Figs. 8-10). The decomposition of Agropyron grass litter and fir needles was more rapid than that of the woody substrates (Tables 28 and 29) with grass litter at the grassland site decomposing faster than needles at the subalpine site. Over the first 24 mo of the study, jack pine needles placed in bags tended to lose wt more rapidly than those strung on thread (Table 30, Fig. 10). Pine and fir needle litter demonstrated short term gains in wt; the worst example being that of bagged pine needles which had significantly more wt remaining after 33 mo than 24 mo (Table 30). Filter paper decomposed at a faster rate than pine needle litter at the oil sands site (Table 30, Fig. 10). However, at the grassland site there was 72% wt remaining of the unamended filters after 44 mo in the field (Table 22) compared with 40% wt remaining of the Agropyron grass litter after the same time interval (Table 28). There was very little difference between the wt remaining of unamended filters and fir needles after 45 mo incubation at the subalpine site (Tables 24, 29).

3.1.8 Effect of Revegetation on Microbial Respiration and Biomass C at the Subalpine Site

Disturbance through mining caused a significant increase in the pH from 4.5 in the undisturbed organic soil to 5.9 in the stockpiled regolith (Table 31). As reclamation progressed the pH increased significantly to 7.3 in a site revegetated for 7 yr. Organic matter content was shown to be significantly lower in the stockpiled regolith and soil from areas in the early stages of

Table 28. Decomposition of Agropyron sp. grass litter and Artemisia cana stems at the prairie grassland undisturbed site¹.

Time (mo)	% dry wt remaining	
	Grass litter	Woody stems
6	89 ^d	95 ^a
12	61 ^c	91 ^a
20	53 ^{bc}	93 ^a
24	41 ^{ab}	90 ^a
32	45 ^{ab}	88 ^a
44	40 ^a	88 ^a
MSE	27.8	.0156

¹Data analyzed by a one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. Values in each column followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Data for woody stems required a $2 \arcsin \sqrt{p}$ transformation; means are, therefore, geometric.

Fig. 8 Decomposition of *Agropyron* sp. grass litter and *Artemisia oana* stems at the grassland undisturbed site.

Table 29. Decomposition of Abies lasiocarpa needles and branch wood at the subalpine undisturbed site.

Time (mo)	% dry wt remaining	
	Needles ¹	Branch wood
9	95 ^b	98 ^b
12	83 ^{ab}	95 ^b
21	82 ^{ab}	95 ^b
24	77 ^{ab}	93 ^b
33	78 ^{ab}	98 ^b
36	72 ^{ab}	96 ^b
45	67 ^a	84 ^a
MSE	.0353	.0438

¹Data analyzed by one-way ANOVA after 2 arcsin $\sqrt{p}$ transformation. Significant differences determined by Scheffé multiple contrasts for pairwise comparisons. For the branch wood data, the mean of the first six sample times was compared to the mean of the last sample time to determine if a difference existed. Values in each column followed by same letter(s) do not differ significantly ($p \leq 0.05$). Means are geometric. MSE = mean square error.

Fig. 9 Decomposition of *Abies lasiocarpa* needles and branch wood at the subalpine undisturbed site.

Table 30. Decomposition of various substrates at the undisturbed oil sands site¹.

Substrate	% dry wt remaining					MSE
	9 mo	12 mo	21 mo	24 mo	33 mo	
Filter paper ²	88 ^c	50 ^b	19 ^{ab}	4 ^a	6 ^a	.13
Fir dowel	98	96	99	97	97	.54
Jack pine needles (bag)	88 ^b	78 ^{ab}	77 ^{ab}	68 ^a	81 ^b	30.96
Jack pine needles (strung)	94 ^a	84 ^a	88 ^a	88 ^a	-	38.52
Jack pine branch wood ²	95 ^b	92 ^{ab}	95 ^b	88 ^{ab}	85 ^a	.046
Jack pine cones ²	99 ^b	96 ^{ab}	97 ^{ab}	94 ^a	92 ^a	.015

¹Data analyzed by one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons.

Values in each row followed by same letter(s) do not differ significantly ($p < 0.05$). MSE = mean square error.

²Data required 2 arcsin $\sqrt{p}$ transformation. Means are geometric.

Fig.10 Decomposition of various substrates at the undisturbed oil sands site.

Table 31. Microbial respiration and biomass in soil from an undisturbed subalpine forest (spruce/fir) and from adjacent areas in various stages of reclamation¹.

Soil description	pH (.01 M CaCl ₂)	Organic matter	Moisture (% wet wt)	Basal respiration (μ l CO ₂ $\uparrow$ 100g ⁻¹ dwt hr ⁻¹)	Microbial biomass C (mg C 100g ⁻¹ dwt)
Undisturbed organic (0-10 cm)	4.5 ^a	85.8 ^c	74.0 (1.7) ²	6946 ^{d3}	1151.7 ^d
Undisturbed mineral (10-20 cm)	4.7 ^a	5.9 ^a	24.3 (9.1)	86 ^a	22.4 ^{ab}
Mineral (0-10 cm) (revegetated for 7 yr)	7.3 ^d	18.8 ^b	31.3 (11.4)	1073 ^c	110.7 ^c
Mineral (0-10 cm) (revegetated for 6 yr)	7.5 ^d	6.7 ^a	15.2 (1.7)	436 ^b	45.6 ^{bc}
Mineral (0-10 cm) (revegetated for 2 yr)	6.8 ^c	2.3 ^a	12.2 (0.4)	115 ^a	25.6 ^{ab}
Stockpiled regolith (0-10 cm)	5.9 ^b	3.7 ^a	13.1 (0.5)	50 ^a	13.2 ^a
MSE	.04	14.2		6137	0.15

¹Data analyzed by one-way ANOVA and Scheffé multiple contrasts. Values in each row followed by same letter(s) do not differ significantly ($p < .05$). MSE = mean square error.

²() = standard deviation.

³Data required a ln X transformation. Values are geometric means.

revegetation than in the undisturbed organic soil. However, the organic matter level in the regolith was found to be comparable to that in the undisturbed mineral soil. The site revegetated for 7 yr had significantly more organic matter than the other disturbed and revegetated sites. Basal respiration (CO_2 efflux) and microbial biomass C were significantly greater in the undisturbed organic soil than any of the other soils. As reclamation (and soil pedogenesis) progressed, both basal respiration and microbial biomass C demonstrated an increasing trend. Interestingly, CO_2 efflux and microbial biomass C in the stockpiled regolith was not significantly different from that measured in the undisturbed mineral soil. The microbial biomass C measured in the various soils demonstrated a significant relationship to the soil organic matter content (Fig. 11).

3.2. TANK STUDY

3.2.1 Microbial Characteristics of Individual Amendments

Some of the chemical and physical characteristics of the amendments have been presented in Table 6a, Visser et al. (1984).

Data on the microbial parameters determined for each of the amendments are given in Tables 32 and 33. The bacteria were most numerous in the sewage sludge and the peat, while actinomycetes were only recorded from the peat. These results do not mean that there were no actinomycetes present in the sewage sludge, fertilizer and gypsum. It is possible that if lower soil dilutions (i.e. $< 10^{-4}$) had been plated out, actinomycetes would have been isolated from these amendments also. Hyphal length (both total and that with cell contents) was significantly greater in the sewage sludge and peat than in the fertilizer and gypsum.

The bacterial numbers and hyphal length results supported the microbial activity measured in three of the amendments, i.e. peat and sewage sludge with their high densities of bacteria and fungal hyphae were also highly active in terms of O_2 consumption (Table 34). ATP levels in the sewage sludge were also very high.

Fig. 11 Relationship between microbial biomass C and percent organic matter for subalpine soil in various stages of reclamation.

Table 32. Bacterial and actinomycete numbers in amendments prior to application to tank soils.
Values are means of 3 replicates plus standard deviations.

		Amendment			
		Sewage sludge	Fertilizer	Gypsum	Peat
Bacteria (no. $\times 10^5$ g ⁻¹ dwt soil)	$\bar{X}$	42320	1.3	0.11	6560
	SD	7240	-	.02	4860
Actinomycetes (no. $\times 10^4$ g ⁻¹ dwt soil)	$\bar{X}$	$< 10^4$	$< 10^4$	$< 10^4$	1506
	SD	-	-	-	198

¹No SD was calculated for bacteria from the fertilizer since 2 of the 3 replicates had $< 10^5$ bacteria g⁻¹ dwt fertilizer. Dilutions of $< 10^5$ were not plated out.

Table 33. Hyphal lengths in amendments prior to application to tank soils¹.

Hyphal category (m g ⁻¹ dwt amendment)	Amendment				MSE
	Sewage Sludge	Fertilizer	Gypsum	Peat	
Total hyphal length	2553 ^c	32 ^a	79 ^b	2275 ^c	0.041
Total hyphal length with cell contents	401 ^b	22 ^a	45 ^a	345 ^b	0.208

¹Data in each hyphal category were ln Y transformed and analyzed with a one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. Values in each row are geometric and where followed by the same letter do not differ significantly ($p < 0.05$). MSE = mean square error.

Table 34. Microbial activity ($O_2\downarrow$) and ATP levels in amendments prior to application to tank soils. Values are means ($n = 3$) $\pm$ SE. Fertilizer was not tested.

Amendment	$O_2\downarrow$ (μl $100g^{-1}$ dwt hr^{-1})	ATP (μg 100 g^{-1})
Gypsum	28 ± 6	ND ¹
Peat	1106 ± 293	$.10 \pm .10$
Sewage sludge	6269 ± 499	375.5 ± 137.9

¹ND = not determined.

3.2.2. Prairie Grassland Minespoil - Effects of Amendment and Plant Growth on Microbial Development

3.2.2.1 Microbial numbers. The ATP measurements made approximately 0.5 mo after amendment were highly variable with no ATP recorded from the control, untreated soil, and very high (and variable) levels in the topsoil treatment (Table 35). In general, ATP levels in the subsurface soil were so low that they were not measureable.

In the surface soil, bacterial counts were significantly higher in the sewage sludge treatment than in the gypsum and topsoil treated and the untreated minespoil (Table 36). However, in the subsurface soil (5-15 cm depth; 15-25 cm in topsoil treatment) amendment and plant growth did not significantly influence the numbers of bacteria. Overall, bacterial numbers did not differ significantly between the two sampling times at either of the soil depths in any of the treatments (Table 36). The data, particularly that from the sewage sludge treatment, was highly variable.

The actinomycete numbers recorded in the prairie minespoil plots, 0.5 and 15 mo after amendment, are presented in Table 37. These data indicate that:

i) in the surface soil, there were no immediate effects of amendment on the actinomycete counts. However, 15 mo after amendment actinomycete numbers were significantly lower in the gypsum treated spoil than in the untreated spoil.

ii) at the deeper soil depth, gypsum application resulted in fewer actinomycetes than that recorded in the untreated control spoil.

iii) in general, in the surface spoil layer, amendment had no significant influence on actinomycete numbers over the two sampling times, but in the subsurface soil actinomycetes were more numerous 15 mo after amendment than 0.5 mo after amendment.

3.2.2.2 Litter input and loss on ignition. Growing fall rye in the sewage sludge and topsoil amended spoil resulted in significantly more grass litter input than growing fall rye in the untreated and

Table 35. ATP levels in grassland spoil 0.5 mo after amendment.
Values are $\bar{X} \pm \text{SD}$.

<u>Amendment</u>	<u>Soil depth (cm)</u>	<u>ATP ($\mu\text{g } 100\text{g}^{-1}$ dry soil)</u>
Control	0-5	0
	5-15	0
Gypsum	0-5	1.1 ± 1.9
	5-15	0
Sewage sludge	0-5	0.13 ± 0.12
	5-15	0.16 ± 0.29
Topsoil	0-5	152.03 ± 162.93
	15-25	0

Table 36. Effect of amendment and time on bacterial numbers in the prairie grassland tank plots¹. Plots were planted with Fall Rye.

Amendment	Depth (cm)	No. of bacteria x 10 ⁷ g ⁻¹ dwt soil		Amendment $\bar{X}$
		Time after amendment (mo)		
		<u>0.5</u>	<u>15</u>	
Control	0-5	5.49	3.92	4.64 ^a
Gypsum		4.38	15.32	8.19 ^a
Sewage		18.64	136.65	50.47 ^b
Topsoil		11.31	5.58	7.95 ^a
Time $\bar{X}$		8.44 ^a	14.63 ^a	
Control	5-15	4.44	3.54	3.99 ^a
Gypsum		4.52	1.64	3.08 ^a
Sewage		7.46	9.34	8.40 ^a
Topsoil	15-25	4.43	2.90	3.67 ^a
Time $\bar{X}$		5.21 ^a	4.36 ^a	

¹Data analyzed by a two-way ANOVA (MSE = .845 and 11.80 for 0-5 cm and 5-15 cm depths respectively) after $\ln Y$ transformation. Values in the amendment $\bar{X}$ column or time $\bar{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All values are geometric.

Table 37. Effect of amendment and time on actinomycete numbers in the prairie grassland tank plots¹. Plots were planted with Fall Rye.

Amendment	Depth (cm)	No. of actinomycetes x 10 ⁵ g ⁻¹ dwt soil		Amendment X̄
		Time after amendment (mo)		
		<u>0.5</u>	<u>15</u>	
Control	0-5	69.22 ^{ab}	278.13 ^b	
Gypsum		58.18 ^{ab}	50.16 ^a	
Sewage		43.84 ^a	112.92 ^{ab}	
Topsoil		109.27 ^{ab}	67.26 ^{ab}	
Control	5-15	90.09	303.47	165.35 ^b
Gypsum		18.93	37.73	26.73 ^a
Sewage		105.72	95.38	100.42 ^{ab}
Topsoil	15-25	16.58	75.67	35.42 ^{ab}
Time X̄		41.58 ^a	95.35 ^b	

¹Data analyzed by a two-way ANOVA (MSE = .204 and .855 for 0-5 cm and 5-15 cm depths respectively) after $\ln Y$ transformation. A significant interaction was detected for the 0-5 cm data, hence Scheffé multiple contrasts for pairwise comparisons were applied to all values. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). For the 5-15 cm data values in the amendment $\bar{X}$ column or time $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All values are geometric.

gypsum amended spoil (Table 38). After 27 mo growth (i.e. after three growing seasons) roots in the 0-5 cm soil weighed more in the topsoil than in the other treatments; however in the deeper 5-15 cm soil root wts were greater in the sewage sludge amended spoil than in the control and gypsum treated spoil (Table 38).

Although amendment significantly influenced fall rye litter input through primary production, loss on ignition measurements in surface soil over the three sampling times were not significantly affected (Table 39). Sewage sludge appeared to significantly increase the organic matter levels of the 0-5 cm soil, but no differences in amounts of organic matter were detected in the subsurface soil in any of the treatments. In the 5-15 cm deep soil, loss on ignition values measured 15 mo after amendment were greater than those measured 27 mo after treatment. The results obtained for % loss on ignition should be viewed with caution since coal contamination of the minespoil would no doubt lead to an overestimate of the amount of "biologically available" organic carbon. Intuitively, the sewage sludge and topsoil treated spoil would have higher levels of active carbon than the gypsum treated and control spoil.

3.2.2.3 Respiratory activity and microbial biomass. The respiratory activity and microbial biomass C measured in the variously treated spoil over the term of the study are summarized in Tables 40, 41 and 42. Fig. 12 presents the microbial biomass C measurements in comparison with the shoot production by fall rye over the three growing seasons. The main features emerging from these data are as follows:

- i) CO₂ evolution from the 0-5 cm deep soil over the three sampling times demonstrated a trend with the topsoil treatment being the most active, followed by sewage sludge, gypsum and the untreated spoil (Table 40). In this surface soil, microbial activity was significantly greater in the samples removed 15 mo after amendment. At this time primary production was also at its peak (Fig. 12).

Table 38. Fall rye litter (dead leaves and stems) input and root wts for the amended grassland spoil plots¹.

Time after planting (mo)	Plant parameter	Soil depth (cm)	Litter or root wt (g dry m ⁻²)			MSE
			Control	Gypsum	Sewage sludge	
27	Litter	0-5	53.0a	59.5a	245.7b	181.1ab 2589.1
	Roots	0-5	3.8a	5.5a	7.7a	53.6b 0.3
	Roots	5-15	3.2ab	2.6a	9.1b	6.3ab 5.3
		(15-25 in topsoil)				

¹Data analyzed by one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. Values in each row followed by the same letter(s) do not differ significantly ($p \leq 0.05$). The 0-5 cm root data required a $\ln Y$ transformation, hence the means for this data are geometric.

Table 39. Effect of time on % loss on ignition of amended grassland spoil planted with fall rye^{1,2}.

Soil depth (cm)	Time after amendment (mo)	Amendment			Time $\bar{X}$
		Control	Gypsum	Sewage sludge	
0-5	0.5	6.11	5.79	8.57	7.01 ^a
	15.0	7.39	7.49	7.58	7.33 ^a
	27.0	5.67	6.08	8.55	6.64 ^a
	Amendment $\bar{X}$	6.39 ^a	6.45 ^a	8.23 ^b	6.90 ^a
5-15 (15-25 in topsoil)	0.5	6.35	7.31	7.28	6.71
	15.0	7.22	8.15	7.40	7.39 ^b
	27.0	5.62	5.44	5.80	5.69 ^a
	Amendment $\bar{X}$	6.40 ^a	6.97 ^a	6.82 ^a	6.20 ^a

¹Data analyzed by two-way ANOVA (MSE = 0.79 and 1.04 for 0-5 and 5-15 cm depths respectively). No interactions were encountered, hence Scheffé multiple contrasts for pairwise comparisons were applied to time and amendment means. Values in the time $\bar{X}$ columns or amendment $\bar{X}$ rows followed by the same letters do not differ significantly ($p < 0.05$).

² Loss on ignition values for all samples, but the topsoil (0-5 cm) are overestimates due to coal particle contamination.

Table 40. Development of microbial activity ($\mu\text{l CO}_2 \uparrow 100 \text{ g}^{-1} \text{ dwt soil hr}^{-1}$) in amended grassland spoil planted with fall rye¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			Time $\bar{X}$
		Control	Gypsum	Sewage sludge	
0-5	0.5	230	200	380	630
	15.0	430	760	800	700
	27.0	230	420	390	570
	Amendment $\bar{X}$	290 ^a	460 ^{ab}	520 ^{bc}	630 ^c
5-15 (15-25 in topsoil)	0.5	560 ^{ab}	530 ^{ab}	970 ^{ab}	820 ^{ab}
	15.0	620 ^{ab}	1090 ^b	870 ^{ab}	660 ^{ab}
	27.0	360 ^a	400 ^a	440 ^a	420 ^a

¹Data analyzed by two-way ANOVA (MSE = 15 and 25 for 0-5 and 5-15 cm depths respectively). Scheffé multiple contrasts for pairwise comparisons were applied to the time and amendment means in the 0-5 cm data set (no significant interaction was observed) and the individual means in the 5-15 cm data set (a significant interaction was observed). For the 0-5 cm data, values in the time $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$). For the 5-15 cm data, values followed by the same letters do not differ significantly ($p \leq 0.05$).

Table 41. Development of microbial biomass (mg C 100 g⁻¹ dwt soil) in amended grassland soil planted with fall rye¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			
		Control	Gypsum	Sewage sludge	Topsoil
0-5	0.5	11.72 ^a	8.11 ^a	15.72 ^{ab}	60.37 ^{bcd}
	15.0	31.89 ^{abcd}	77.61 ^{cde}	90.63 ^{cde}	128.70 ^{de}
	27.0	23.80 ^{abc}	61.23 ^{bcde}	76.64 ^{cde}	151.66 ^e
5-15 (15-25 in topsoil)	0.5	33.46 ^a	49.36 ^a	66.84 ^{ab}	61.49 ^{ab}
	15.0	61.95 ^{ab}	52.85 ^a	55.76 ^a	51.21 ^a
	27.0	45.82 ^a	51.12 ^a	51.56 ^a	121.16 ^b

¹Data analyzed by two-way ANOVA (MSE = .125 and 233.48 for 0-5 and 5-15 cm depths respectively). Significant interactions were encountered, hence Scheffé multiple contrasts for pairwise comparisons were applied to individual means. Values followed by the same letter(s) do not differ significantly ($p < 0.05$). The 0-5 cm data required a ln transformation so all means are geometric.

Table 42. The effect of sampling depth on microbial activity ($\text{CO}_2\uparrow$) and microbial biomass C in the amended grassland spoil, planted with fall rye¹.

Parameter	Time after amendment (mo)	Amendment			
		Control	Gypsum	Sewage sludge	Topsoil
Microbial activity ($\text{CO}_2\uparrow$)	0.5	**	**	**	**
	15.0	**	**	NS	NS
	27.0	NS	NS	NS	NS
Microbial biomass C	0.5	NS	**	**	NS
	15.0	NS	NS	**	*
	27.0	NS	NS	**	*

¹As a result of dependence between the 0-5 and 5-15 cm data, the effect of depth was determined by subtracting the 5-15 cm values from the 0-5 cm values and analyzing the resulting data using the model for the two-way ANOVA (i.e. microbial biomass C = $\bar{\mu} \dots +$ (amendment effect) + (time effect) + error). The null hypothesis was that each treatment mean = 0 (i.e. no difference between depths). This method allows examination of each treatment in the event that not all treatments show an effect of depth.

NS - depths not significantly different ($p < 0.05$).

* - 0-5 cm significantly greater than 5-15 cm (15-25 in topsoil) ($p < 0.05$).

** - 5-15 cm significantly greater than 0-5 cm ($p < 0.05$).

Fig.12 Primary production and the development of microbial biomass C in amended grassland spoil planted with fall rye.

ii) unlike the surface soil, amendment and plant growth had very little influence on the respiratory activity in the subsurface soil (Table 40). Respiration measured 0.5 mo after amendment was significantly higher in the subsurface than the surface soil; however, at 15 mo after amendment no significant differences were detected between CO₂ efflux from soil at either depth (Table 41).

iii) immediately after amendment, microbial biomass C in 0-5 cm soil was highest in the topsoil treatment followed by sewage sludge, gypsum and control treatments (Table 41, Fig. 12). Fifteen months after amendment, the microbial biomass had increased significantly in the gypsum and sewage sludge treated soil. The biomass in the topsoil treatment also increased, but this increase was not significant until 27 mo after amendment. The microbial biomass C demonstrated its greatest increase after the second growing season (15 mo) when fall rye was also most productive (Fig. 12).

iv) microbial biomass was less affected by amendment and subsequent plant growth in the 5-15 cm deep soil than the 0-5 cm deep soil (Table 41). However, the soil beneath the topsoil treatment (i.e. 15-25 cm deep soil) contained more microbial biomass C 27 mo after amendment than at the previous two sampling times.

v) no soil depth effects on microbial biomass C were detected in the untreated plots, while the 5-15 cm deep sewage sludge treated soil contained more microbial biomass C than the surface soil (Table 42). The biomass tended to be higher in the topsoil than in the subsurface soil material (Table 42).

Product moment correlation coefficients calculated between % loss on ignition, grass litter input, grass root wt, microbial activity and microbial biomass C demonstrated that fall rye root wts and microbial activity and biomass C were highly correlated (Table 43). Microbial activity and microbial biomass C, % loss on ignition and litter input were also strongly related. Soil respiratory activity and microbial biomass C demonstrated a positive correlation with grass litter input, but not as strong a correlation as obtained with grass root wts.

Table 43. Product moment correlation coefficients for loss on ignition, litter input, root wt, CO₂ † and microbial biomass C data collected 27 mo after amendment of the grassland soil (fall rye plots).

	% loss on ignition	Grass litter input	Grass root wt	Microbial activity (CO ₂ †)	Microbial biomass C
% loss on ignition	1	.829	-.042	.230	.398
Grass litter input		1.00	.417	.526	.607
Grass root wt			1.00	.768	.772
Microbial activity (CO ₂ †)				1.00	.873
Microbial biomass C					1.00

3.2.2.4 Decomposition of standard substrates. The decomposition potential of the grassland minespoil as measured by wt loss of cellulose filter paper after 1 yr incubation in the field was greater if the spoil had been amended with topsoil than if it had been left untreated or treated with gypsum (Table 44). This was true in amended spoil planted with either fall rye or rambler alfalfa. After 2 yrs incubation in the variously treated plots planted with fall rye there were no significant differences in wt loss of the filters amongst the four treatments. In the fall rye plots, % wt remaining in the untreated plots was significantly less after 24 mo incubation than after 12 mo incubation; this was not the case for the treated plots where no significant differences in wt loss were observed between the two sampling times. In plots planted with rambler alfalfa, there was a significant decrease in % dwt filter remaining between 12 mo and 24 mo incubation regardless of amendment (Table 44). Over the short term (12 mo) filters in the sewage sludge and topsoil treated plots tended to degrade more rapidly if the plots had been planted with fall rye rather than rambler alfalfa, but after 24 mo incubation no differences were measured between the two vegetation types.

In contrast to the filter paper, fir wood dowel demonstrated negligible wt loss over the 24 mo incubation regardless of treatment or vegetation type (Table 45).

Changes in the decomposition potential over time of the variously amended spoil were tested by measuring wt loss of filters placed in the plots 4 mo and 28 mo after planting fall rye. The results obtained are presented in Table 46 and Fig. 13 and show that the decomposition potential of the gypsum and sewage sludge treated and untreated spoil had not changed significantly over time, i.e. the growth of fall rye had not improved the decomposing ability of the spoil over the 40 mo term of the study. Filters placed in the topsoil treated plots lost less wt when placed in the plots 28 mo after planting than 4 mo after planting fall rye. Four mo after planting fall rye, the decomposition potential of the topsoil and sludge treated soil was significantly greater than that of the

Table 44. Cellulose filter paper decomposition (expressed as % wt remaining) in amended grassland spoil planted with fall rye and Rambler alfalfa¹. Filters were placed in the field 4 mo after planting.

Plant type	Time in the field (mo)	Amendment			Time $\bar{X}$
		Control	Gypsum	Sewage sludge	
Fall rye	12	89.1 ^b	87.1 ^b	53.6 ^{ab}	21.2 ^a
	24	26.6 ^a	44.1 ^{ab}	27.7 ^a	20.3 ^a
Rambler alfalfa	12	99.2	85.3	82.5	58.1
	24	73.0	34.3	28.2	13.5
	Amendment $\bar{X}$	86.1 ^c	59.8 ^b	55.4 ^{ab}	35.8 ^a
					81.3 ^a
					37.3 ^b

¹Data analyzed by two-way ANOVA (MSE = 163.9 and 160.0 for fall rye and Rambler alfalfa respectively). A significant interaction was observed for the fall rye data, hence Scheffé multiple contrasts for pairwise comparisons were applied to individual means. Values followed by the same letter(s) do not differ significantly ($p < 0.05$). Scheffé multiple contrasts for pairwise comparisons were applied to Rambler alfalfa time and amendment means since no interaction was observed for this data set. Values in the time $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p < 0.05$).

Table 45. The % wt remaining of fir wood dowel placed in amended grassland spoil plots 4 mo after planting with fall rye and Rambler alfalfa.

Plant type	Time in the field (mo)	Amendment			Time $\bar{X}$
		Control	Gypsum	Sewage sludge	
Fall rye	12	99.9	100	99.7	100
	24	100	100	99.9	99.8
	Amendment $\bar{X}$	99.95	100	99.8	99.9
Rambler alfalfa	12	99.8	100	100	100
	24	100	100	100	99.9
	Amendment $\bar{X}$	99.9	100	100	99.95

Table 46. The % wt remaining of cellulose filter paper incubated for 12 mo in amended grassland spoil plots planted with fall rye. Filters were placed in the plots 4 mo and 28 mo after planting¹.

Time after planting (mo)	Amendment			
	Control	Gypsum	Sewage sludge	Topsoil
4	89 ^C	87 ^{bc}	53 ^{ab}	21 ^a
28	89 ^C	75 ^{bc}	87 ^{bc}	79 ^{bc}

¹Data analyzed by two-way ANOVA (MSE = 266.97) and Scheffé multiple contrasts for pairwise comparisons. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Fig.13. The percent weight remaining of cellulose filter paper incubated for 12 months in amended grassland spoil plots planted with fall rye. Filters were placed in the plots 4 months and 28 months after planting.

control soil; however, 28 mo after planting, no significant differences in decomposition potential were detected amongst any of the treatments.

In contrast to the results obtained for the amended spoil planted with fall rye, decay of filter papers in plots planted with rambler alfalfa was faster 28 mo after planting than 4 mo after planting (Table 47). This was particularly true for the sewage sludge and topsoil treated plots, where filters decayed more rapidly than in the gypsum treated and untreated plots.

3.2.2.5 Symbiotic N_2 fixation. Data on the N_2 (C_2H_2) fixed by rambler alfalfa over the term of the study are presented in Table 48. Amendment did not significantly influence the amount of N_2 fixed by plants removed from the plots at each of the three sampling times, but measurements were highly variable particularly for plants from the sewage sludge and gypsum amended spoil. There did not appear to be any relationship between nodule wt and N_2 fixing capacity for alfalfa tested 14 mo after planting. At this time nodule morphology also varied amongst the treatments with roots from the topsoil treatment bearing numerous small (1 mm dia. or less) pink (indicating presence of leghaemoglobin) nodules and roots from the control, sewage sludge and gypsum treatments having large (up to 5-7 mm dia.) pink clumps of nodules. Although the effect of time on the N_2 fixing capacity of the alfalfa was not tested (since the plants may not have been in the same physiological condition after each of the three growing seasons), it appeared that N_2 fixation activity was greatest for plants collected at the conclusion of the second growing season (14 mo).

3.2.3. Subalpine Minespoil - Effects of Amendment and Plant Growth on Microbial Development

3.2.3.1 Microbial characteristics 0.5 mo and 15 mo after amendment and planting. The ATP levels in the subalpine spoil immediately after amendment were, like those in the grasslands spoil, highly

Table 47. The % dry wt remaining of cellulose filter paper after 12 mo incubation in grassland spoil plots planted with rambler alfalfa. Filters were placed in the plots at 4 mo and 28 mo after planting¹.

Time after planting (mo)	Amendment				Time $\bar{X}$
	Control	Gypsum	Sewage sludge	Topsoil	
4	100	91	87	58	88 ^b
28	95	91	50	45	74 ^a
Amendment $\bar{X}$	98 ^b	91 ^b	70 ^a	52 ^a	

¹Data analyzed by 2-way ANOVA (MSE = .258) after $2 \arcsin \sqrt{p}$ transformation. No interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to time and treatment means. Values in the time $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$). Means are geometric.

Table 48. N₂ (C₂H₂) fixation capacity (nmoles g⁻¹ dry root hr⁻¹) and nodule weight (mg dry sample⁻¹) of Rambler alfalfa planted in grassland spoil tank plots¹.

Treatment	Time after planting			
	3 mo	14 mo	26 mo	
	<u>N₂ fixed</u>	<u>Nodule wt</u>	<u>N₂ fixed</u>	<u>Nodule wt</u>
Control	37.1a	52	149.5a	9
Gypsum	155.0a	46	859.3a	19
Sewage Sludge	6.5a	30	1263.3a	5
Topsoil	103.8a	41	93.7a	39
MSE	9656.81		4.11	
				87.47

¹Data for each sampling time were analyzed using a one-way ANOVA after ln (y + 1) transformation. Values in each column followed by the same letter do not differ significantly (p < 0.05).

variable (Table 49). The sewage sludge treated spoil tended to have the highest ATP level while the untreated spoil had the lowest.

Both in the 0-5 cm and 5-15 cm deep subalpine spoil the bacteria were most numerous in plots treated with sewage sludge (Table 50). Bacterial numbers in the surface soil 0.5 and 15 mo after amendment were not significantly different; however, in the subsurface soil bacterial numbers were greater 15 mo after amendment than 0.5 mo after amendment.

Immediately after application of the amendments (0.5 mo), the number of actinomycetes in the surface soil was significantly higher in the peat treated plots than in the other treatments (Table 51). However, 15 mo after treatment and planting slender wheatgrass, actinomycetes in the 0-5 cm peat had decreased significantly, while those in the sewage sludge treated spoil had increased. Actinomycetes in the 5-15 cm spoil tended to be greater in the fertilized spoil than in the other treatments; also numbers increased from 0.5 to 15 mo after amendment at this depth (Table 51).

Total hyphal lengths at both sampling depths were also greatest in the peat amended spoil (Tables 52 and 53). No differences in total lengths were detected between the two sampling times, regardless of treatment. As for the total hyphal length, mycelium with cell contents in the 0-5 cm deep soil was longest in the peat; this was not the case in the subsurface soil where treatment did not significantly influence hyphal lengths with cell contents. Clamped hyphae were detected in all treatments at both depths indicating the presence of the basidiomycete group of fungi throughout the soil.

3.2.3.2 Microbial development in amended, unvegetated minespoil.

The % loss on ignition of samples from the amended, unvegetated pathways along the tank walls was measured 0.5 and 27 mo after amendment. As expected, the surface soil of the peat treated plots had the highest level of organic matter (Table 54). Loss on ignition in the 5-15 cm soil was not affected by treatment. Also, the organic

Table 49. ATP levels in subalpine spoil 0.5 mo after amendment.
Values are $\bar{X} \pm \text{SD}$.

Amendment	Soil depth (cm)	ATP ($\mu\text{g } 100 \text{ g}^{-1} \text{ dry soil}$)
Control	0-5	.10 $\pm$.17
	5-15	.17 $\pm$.29
Fertilizer	0-5	.17 $\pm$.29
	5-15	3.26 $\pm$ 5.66
Sewage sludge	0-5	24.40 $\pm$ 21.76
	5-15	9.17 $\pm$ 15.62
Peat	0-5	5.3 $\pm$ 9.01
	15-25	0

Table 50. Effect of amendment and time on bacterial numbers in subalpine spoil tank plots¹.
Plots were planted with slender wheatgrass.

Amendment	Depth (cm)	No. of bacteria x 10 ⁷ g ⁻¹ dwt soil		Amendment $\bar{X}$
		Time after amendment (mo)		
		0.5	15	
Control	0-5	13.77	16.19	14.93 ^a
Fertilizer		12.67	20.89	16.27 ^a
Sewage Sludge		304.87	155.28	217.58 ^c
Peat		41.15	33.91	37.36 ^b
Time $\bar{X}$		38.46 ^a	36.53 ^a	
Control	5-15	11.50	16.32	13.70 ^a
Fertilizer		9.14	18.71	13.08 ^a
Sewage Sludae		32.66	44.42	38.09 ^b
Peat	15-25	5.79	17.52	10.07 ^a
Time $\bar{X}$		11.87 ^a	22.08 ^b	

¹Data analyzed by a two-way ANOVA (MSE = .132 and .306 for 0-5 cm and 5-15 cm depths respectively) after $\ln Y$ transformation. Values in the amendment $\bar{X}$ column or time $\bar{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$). All values are geometric.

Table 51. Effect of amendment and time on actinomycete numbers in subalpine spoil tank plots¹. Plots were planted with slender wheatgrass.

Amendment	Depth (cm)	No. of actinomycetes x 10 ⁴ g ⁻¹ dwt soil		Amendment $\bar{X}$
		Time after amendment (mo)		
		0.5	15	
Control	0-5	19.86ab	175.85bc	
Fertilizer		34.17abc	102.09abc	
Sewage Sludge		13.39a	334.36c	
Peat		6113.14d	238.48c	
Control	5-15	38.66	73.73	53.39bc
Fertilizer		36.03	139.42	70.87c
Sewage Sludge		10.09	130.12	37.64ab
Peat	15-25	15.38	50.34	27.82a
Time $\bar{X}$		21.97a	90.58b	

¹Data analyzed by a two-way ANOVA (MSE = .485 and .307 for 0-5 cm and 5-15 cm depths respectively) after $\ln Y$ transformation. A significant interaction was detected for the 0-5 cm data, hence Scheffé multiple contrasts for pairwise comparisons were applied to all values. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). For the 5-15 cm data, values in the amendment $\bar{X}$ column or time $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All values are geometric.

Table 52. Hyphal lengths in 0-5 cm subalpine spoil before planting (0.5 mo) and after planting slender wheatgrass (15 mo)¹.

Hyphal category (m g ⁻¹ dwt soil)	Sampling time (mo)	Amendment			Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	
Total	0.5	216 ^a	147 ^a	179 ^a	2459 b
	15.0	159 ^a	201 ^a	235 ^a	1190 b
Total with cell contents	0.5	42 ^{ab}	13 ^a	27 ^a	348 ^c
	15.0	45 ^{ab}	59 ^{ab}	38 ^{ab}	161 ^{bc}
Total clamped	0.5	11	4	9	205 57.2 ^a
	15.0	9	4	19	7 9.6 ^a
Amendment $\bar{X}$		10 ^a	4 ^a	14 ^a	106 ^a

¹Data in each hyphal category were analyzed by a two-way ANOVA after $\ln(Y + 1)$ transformation (MSE = .065, .227 and 1.925 for total, total with contents and total clamped respectively). Scheffé multiple contrasts for pairwise comparisons were applied to all values in the first two categories (as a result of significant interactions in the ANOVA). Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). In the total clamped category, values in the yearly $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$). All values are geometric means.

Table 53. Hyphal lengths in 5-15 cm (15-25 cm in peat) subalpine spoil before planting (0.5 mo) and after planting slender wheatgrass (15 mo)¹.

Hyphal category (m g ⁻¹ dwt soil)	Sampling time (mo)	Amendment			Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat
Total	0.5	160	154	159	302
	15.0	168	197	174	193
Amendment $\bar{X}$		164 ^a	175 ^a	166 ^a	248 ^b
Total with cell contents	0.5	13	25	13	38
	15.0	45	35	35	46
Amendment $\bar{X}$		29 ^a	30 ^a	24 ^a	42 ^a
Total clamped ²	0.5	23	29	8	0
	15.0	0	6	10	8
Amendment $\bar{X}$		11 ^a	17 ^a	9 ^a	4 ^a

(cont'd)

Table 53. (cont'd)

¹Data in the total and total with cell contents categories were analyzed with a two-way ANOVA (MSE = 2.254 and 323.32 respectively) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ columns or amendment $\bar{X}$ rows followed by the same letter do not differ significantly ($p \leq 0.05$).

²Because the 0.5 mo peat values and 15 mo control values had a mean of 0 with 0 variation, the Tukey test for additivity was applied to the averages of each treatment to test for interactions. Since there was no significant interaction, the two-way ANOVA with 1 replication/treatment (i.e. mean of 3 values/treatment) was applied (MSE = 126.2). Values in the time $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$).

Table 54. Effect of time on % loss on ignition of amended, unvegetated subalpine spoil (pathways)¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	6.02 ^a	7.07 ^{ab}	7.59 ^{ab}	63.62 ^c
	27.0	6.38 ^a	6.16 ^a	10.06 ^b	68.51 ^c
5-15 (15-25 in peat)	0.5	7.38 ^a	5.93 ^a	7.40 ^a	5.35 ^a
	27.0	6.03 ^a	6.26 ^a	7.30 ^a	8.19 ^a

¹Data analyzed by two-way ANOVA (MSE = .031 and .709 for 0-5 cm and 5-15 cm depths respectively) and Scheffé multiple contrasts for pairwise comparisons. Values in each data set, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). The 0-5 cm means are geometric since the data required a $\ln Y$ transformation.

matter levels were not altered over the course of the study in any of the treatments, at either of the two depths.

Microbial activity in the 0-5 cm soil was found to be highest in the peat and sewage sludge treated spoil, particularly at 14 mo after amendment (Table 55). In the deeper 5-15 cm soil, activity was also greatest in the 14 mo samples, but amendment appeared to have little effect on the CO₂ efflux.

The microbial biomass C measurements followed a similar pattern to that observed for the microbial activity measurements with the exception that there was no significant difference between microbial biomass C at 14 mo and that at 27 mo in the 0-5 cm soil (Table 56). Immediately after amendment (0.5 mo) microbial activity and biomass C in the fertilized spoil was greater at the 5-15 cm than at the 0-5 cm depth, while in the peat treated spoil, CO₂ efflux and biomass C were always higher in the peat than in the spoil (15-25 cm depth) beneath the peat (Table 57).

3.2.3.3 Microbial development in amended spoil planted with white spruce. The weight of white spruce roots sieved from soil samples used to estimate microbial activity and biomass C levels was determined 27 mo after initiation of the study. The results are presented in Table 58 and show that root wts were greatest in the 0-5 cm peat treatment. Only the peat amendment demonstrated a significant effect of depth on root wt, with root wt in the surface soil being greater than that measured in the spoil beneath the peat (Tables 58 and 59).

The % loss on ignition measured in samples from the amended white spruce plots (Table 60) were very similar to those obtained from the unvegetated pathway samples. The addition of peat to the subalpine spoil significantly increased its organic matter content whereas fertilizer and sewage sludge amendment had no significant effect. This was true only for the 0-5 cm deep soil. Organic matter levels did not change over the 27 mo term of the study in any of the treatments. The amount of organic matter in the surface soil of the

Table 55. Development of microbial activity ($\mu\text{l CO}_2 + 100 \text{ g}^{-1} \text{ dwt soil hr}^{-1}$) in amended, unvegetated subalpine soil (pathways)¹.

Soil depth (cm)	Time after amendment (mo)	Amendment				Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	320	190	500	1460	460 ^a
	15.0	490	320	1290	4410	970 ^b
	27.0	220	200	260	2010	390 ^a
	Amendment $\bar{X}$	330 ^a	230 ^a	550 ^b	2350 ^c	
5-15 (15-25 in peat)	0.5	360	520	490	430	450 ^b
	15.0	560	480	1070	990	730 ^c
	27.0	240	260	250	360	270 ^a
	Amendment $\bar{X}$	360 ^a	400 ^{ab}	510 ^{ab}	530 ^b	

¹Data analyzed by two-way ANOVA after $\ln Y$ transformation ($\text{MSE} = 115.58$ and 771.30 for $0-5$ and $5-15$ cm depths respectively). Time and amendment means were tested by Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ columns or amendment $\bar{X}$ rows, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All values are geometric means.

Table 56. Development of microbial biomass C (mg C 100 g⁻¹ dwt soil) in amended, unvegetated subalpine spoil (pathways)¹.

Soil depth (cm)	Time after amendment (mo)	Amendment				Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	24.08	10.34	30.74	184.16	34.46 ^a
	15.0	37.84	34.32	142.17	364.08	90.55 ^b
	27.0	35.60	43.78	101.49	289.97	82.29 ^b
	Amendment $\bar{X}$	31.89 ^a	24.96 ^a	76.27 ^b	268.89 ^c	
5-15 (15-25 in peat)	0.5	30.18 ^a	41.15 ^{ab}	47.50 ^{ab}	43.73 ^{ab}	
	15.0	39.92 ^{ab}	35.16 ^{ab}	110.16 ^b	75.73 ^b	
	27.0	34.76 ^{ab}	43.38 ^{ab}	54.38 ^{ab}	30.58 ^a	

¹Data analyzed by two-way ANOVA after ln Y transformation (MSE = .1317 and .5033 for 0-5 cm and 5-15 cm depths respectively). Time and amendment means for the 0-5 cm data, and all means for the 5-15 cm data were tested with Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or amendment $\bar{X}$ row (0-5 cm data) and all values in the 5-15 cm data set, followed by the same letter do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 57. The effect of depth on microbial activity ($\text{CO}_2 \uparrow$) and microbial biomass C in amended, unvegetated subalpine spoil (pathways)¹.

Parameter	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage sludge	Peat
Microbial activity ($\text{CO}_2 \uparrow$)	0.5	NS	**	NS	*
	15.0	NS	**	NS	*
	27.0	NS	NS	NS	*
Microbial biomass C	0.5	NS	**	**	*
	15.0	NS	NS	NS	*
	27.0	NS	NS	*	*

¹See Table 42 for explanation of analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm depth significantly greater ($p \leq 0.05$) than 5-15 cm depth.

** - 5-15 cm depth significantly greater ($p \leq 0.05$) than 0-5 cm depth.

Table 58. White spruce root wts in amended subalpine spoil.

Soil depth (cm)	Time after planting (mo)	Root wt (g dwt m ⁻²)		
		Control	Fertilizer	Sewage sludge Peat
0-5	27	11.04 ^a	11.15 ^a	44.01 ^{ab} 112.37 ^b
5-15	27	54.60 ^a	63.33 ^a	86.17 ^a 26.13 ^a
(15-25 in peat)				

¹Data analyzed by one-way ANOVA (MSE = 0.78 and 1063.51 for 0-5 and 5-15 cm data respectively) and Scheffé multiple contrasts for pairwise comparisons. Values in each row, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). The 0-5 cm means are geometric since the data required a $\ln Y$ transformation.

Table 59. The effect of depth on slender wheatgrass and white spruce root distribution in amended subalpine spoil 27 mo after planting¹.

Plant type	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
Slender wheatgrass	NS	NS	NS	*
White spruce	NS	NS	NS	NS

¹Data analysis explained in Table 42.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm depth root wts significantly greater ($p \leq 0.05$) than 15-25 cm root wts in the peat.

Table 60. Effect of time on % loss on ignition of amended subalpine spoil planted with white spruce¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	6.02	7.07	7.59	63.62
	27.0	5.24	4.80	8.22	53.38
	Amendment $\bar{X}$	5.61a	5.82a	7.90a	58.28b
5-15 (15-25 in peat)	0.5	7.38ab	5.93ab	7.40ab	5.35ab
	27.0	5.09a	5.80a	6.76ab	8.51b

¹Data analyzed by two-way ANOVA (MSE = .0420 and .7710 for the 0-5 and 5-15 cm depths respectively). No interaction was observed for the 0-5 cm data, hence Scheffé multiple contrasts for pairwise comparisons were applied to time and amendment means. Values in the time mean column or amendment mean row followed by the same letter do not differ significantly ($p \leq 0.05$). For the 5-15 cm data, an interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to all means. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Geometric means are presented for the 0-5 cm data since it required a $\ln Y$ transformation.

peat amended plots was greater than that in the subsurface spoil (Table 67).

As in the unvegetated spoil, microbial activity and biomass C in the surface soil of the spruce plots were most stimulated by the addition of peat (Tables 61 and 62). Microbial activity in the 0-5 cm deep soil did not change significantly from 0.5 to 27 mo after the addition of any of the amendments (Table 61). However, the microbial biomass C in the surface soil of the peat plots increased significantly from 0.5 to 27 mo (Table 62).

In the subsurface (5-15 cm deep) soil, amendments had very little effect on either microbial activity or microbial biomass (Tables 61 and 62) at 0.5 mo following their application. Time did not significantly alter the microbial activity in any of the treatments, but did increase the level of microbial biomass C in the 5-15 cm deep sludge treated spoil. Microbial respiration and biomass C were significantly higher in the 5-15 cm deep fertilized spoil than in the 0-5 cm soil particularly at 0.5 mo after applying the fertilizer (Table 63). As expected the CO₂ efflux and microbial biomass C in the peat treated spoil were greater in the surface peat than in the spoil beneath the peat (Table 61).

3.2.3.4 Microbial development in amended spoil planted with slender wheatgrass. The quantity of slender wheatgrass litter (27 and 39 mo after planting) sieved out of the soil samples used for microbial activity and biomass measurements (and subsequently returned) was highest in the sewage sludge treated spoil samples followed by the peat, fertilizer, and control treatments (Table 64). Shoot production by slender wheatgrass was most stimulated by the application of sewage sludge (Fig. 14), therefore resulting in greater litter input in this particular treatment. The amount of dead litter deposited on the spoil surface was not significantly different between 27 and 39 mo after planting (Table 64).

The amount of slender wheatgrass root material removed from the soil samples 27 and 39 mo after planting was significantly greater in the peat treated than in the untreated spoil (Table 65),

Table 61. Development of microbial activity ($\mu\text{l CO}_2 \pm 100\text{g}^{-1}$ dwt soil hr^{-1}) in amended subalpine spoil planted with white spruce¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	320 ^{ab}	190 ^a	500 ^b	1460 ^c
	27.0	210 ^{ab}	230 ^{ab}	430 ^{ab}	3350 ^c
5-15 (15-25 in peat)	0.5	370 ^{ab}	520 ^{ab}	500 ^{ab}	450 ^{ab}
	27.0	240 ^a	360 ^{ab}	580 ^b	600 ^b

¹Data analyzed by a two-way ANOVA (MSE = 635.19 and 818.75 for the 0-5 and 5-15 cm depths respectively). Scheffé multiple contrasts for pairwise comparisons were applied to all values in each data set. Values followed by the same letter(s) do not differ significantly ($p < 0.05$). The 0-5 cm data required a $\ln Y$ transformation, hence those means are geometric.

Table 62. Development of microbial biomass (mg C 100 g⁻¹ dwt in soil) in amended subalpine soil planted with white spruce¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	25.51 ^a	11.82 ^a	33.96 ^{ab}	185.15 ^c
	27.0	32.43 ^a	42.17 ^{ab}	81.52 ^b	454.29 ^d
5-15 (15-25 in peat)	0.5	30.92 ^a	41.83 ^{ab}	48.34 ^{ab}	44.54 ^{ab}
	27.0	34.06 ^{ab}	51.42 ^{ab}	93.27 ^c	68.71 ^{bc}

¹Data analyzed by two-way ANOVA (MSE = 185.88 and 111.72 for the 0-5 and 5-15 cm depths respectively) and Scheffé multiple contrasts for pairwise comparisons. Values in each data set, followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 63. The effect of sampling depth on microbial activity ($\text{CO}_2 \uparrow$) and microbial biomass C in amended, subalpine spoil planted with white spruce¹.

Parameter	Time after amendment (mo)	Amendment		
		Control	Fertilizer	Sewage sludge Peat
Microbial activity ($\text{CO}_2 \uparrow$)	0.5	NS	**	NS *
	27.0	NS	**	NS *
Microbial biomass C	0.5	NS	**	NS *
	27.0	NS	NS	NS *

¹See Table 42 for explanation of analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm depth significantly greater ($p \leq 0.05$) than 5-15 cm depth.

** - 5-15 cm depth significantly greater ($p \leq 0.05$) than 0-5 cm depth.

Table 64. Slender wheatgrass litter (dead leaves and stems) input for amended subalpine spoil¹.

Time after planting (mo)	Litter input (g dwt m ⁻²)			Time $\bar{X}$
	Control	Fertilizer	Sewage Sludge	
27	59.60	115.87	273.90	137.03
39	108.95	105.28	264.69	147.39
Amendment $\bar{X}$	84.27 ^a	110.57 ^{ab}	269.29 ^c	142.21 ^b

¹Data analyzed by two-way ANOVA (MSE = 3149.66) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p < 0.05$).

Fig. 14 Primary production and the development of microbial biomass in amended subalpine spoil planted with slender wheatgrass.

Table 65. Slender wheatgrass root wts in amended subalpine spoil¹.

Soil depth (cm)	Time after planting (mo)	Root wt (g dwt m ⁻²)				Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	27	129.75	384.93	486.00	868.56	381.05 ^b
	39	37.04	28.65	52.11	64.71	43.50 ^a
	Amendment $\bar{X}$	69.32 ^a	105.02 ^{ab}	159.14 ^{ab}	237.09 ^b	

¹Data analyzed by two-way ANOVA after $\ln Y$ transformation ($MSE = .5295$) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or amendment $\bar{X}$ row, followed by the same letter(s) do not differ significantly ($p < 0.05$). All means are geometric.

while the weight of roots in the fertilized and sewage sludge treated spoil was intermediate. The quantity of roots in all treatments was found to be significantly greater 27 mo after planting than 39 mo after planting. The root wt data should be viewed in conjunction with the shoot production estimates presented in Fig. 14. Shoot production by slender wheatgrass peaked during the second growing season and, with the exception of the peat treatment, collapsed during the third growing season. Shoot production was not measured after the fourth (39 mo) growing season. The wt of roots measured in the slender wheatgrass plots 27 mo after planting was much greater than that recorded from the white spruce plots irrespective of treatment (Tables 58 and 65). As for the white spruce roots, the wt of grass roots was not significantly different between the two sampling depths in any of the treatments except the peat (Table 59). Here, the quantity of roots in the surface peat was greater than that in the spoil beneath the peat.

The % loss on ignition of the 0-5 cm subalpine spoil was lowest in the untreated and fertilized spoil followed by the sewage sludge amended soil (Table 66). As in the unvegetated and spruce plot samples, peat addition resulted in the highest % loss on ignition. The % organic matter in the surface soil did not vary significantly from 0.5 to 27 mo. Loss on ignition in the subsurface soil was not strongly affected by amendment or plant growth (Table 66) over the term of the study. As expected % loss on ignition in the 0-5 cm deep soil from the peat treated plots was significantly greater than that in the spoil beneath the peat (Table 67).

Microbial activity in the surface soil from the slender wheatgrass plots was, like that from the unvegetated pathways and white spruce plots, highest in the peat treatment (Table 68). CO₂ efflux from the 0-5 cm deep peat amended spoil increased significantly from 0.5 to 15 mo after treatment. Treatment of the subalpine spoil with fertilizer and sewage sludge and planting with slender wheatgrass had no significant effect on the microbial activity - one reason being the high variability demonstrated by samples from the sewage sludge treatment in particular. Microbial respiration of the

Table 66. Effect of time on % loss on ignition of amended subalpine spoil planted with slender wheatgrass¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	
0-5	0.5	6.02	7.07	7.59	63.62
	15.0	7.63	6.94	8.94	67.14
	27.0	6.83	6.48	10.98	61.10
	Amendment $\bar{X}$	6.80 ^a	6.83 ^a	9.06 ^b	63.91 ^c
5-15 (15-25 in peat)	0.5	6.38 ^{ab}	5.93 ^a	7.40 ^{ab}	5.35 ^a
	15.0	7.83 ^{ab}	6.97 ^{ab}	7.60 ^{ab}	9.63 ^b
	27.0	5.82 ^a	5.01 ^a	7.67 ^{ab}	9.95 ^b

¹Data analyzed by a two-way ANOVA (MSE = .0204 and .7454 for 0-5 cm and 5-15 cm respectively). Scheffé multiple contrasts for pairwise comparisons were applied to time and amendment means for the 0-5 cm data and to the individual means for the 5-15 cm data. For the 0-5 cm data, values in the time $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$) and for the 5-15 cm data, all values followed by the same letter(s) do not differ significantly ($p < 0.05$). The 0-5 cm data required a $\ln Y$ transformation, hence geometric means are presented.

Table 67. The effect of sampling depth on % loss on ignition in the amended subalpine spoil planted with slender wheatgrass and white spruce¹.

Plant type	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
Slender wheatgrass	0.5	NS	NS	NS	*
	15.0	NS	NS	NS	*
	27.0	NS	NS	NS	*
White spruce	0.5	NS	NS	NS	*
	27.0	NS	NS	NS	*

¹See Table 42 for explanation of analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm loss on ignition significantly greater ($p \leq 0.05$) than 5-15 cm (15-25 in the peat) loss on ignition.

Table 68. Development of microbial activity ($\mu\text{l CO}_2 \pm 100 \text{ g}^{-1} \text{ dwt soil hr}^{-1}$) in amended subalpine spoil planted with slender wheatgrass¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	320 ^{ab}	190 ^a	500 ^{ab}	1460 ^b
	15.0	290 ^a	610 ^{ab}	1320 ^{ab}	3040 ^c
	27.0	660 ^{ab}	930 ^{ab}	1440 ^b	3770 ^c
	39.0	360 ^{ab}	460 ^{ab}	800 ^{ab}	2790 ^c
5-15 (15-25 in peat)	0.5	360 ^{ab}	520 ^{ab}	490 ^{ab}	430 ^{ab}
	15.0	340 ^a	880 ^{ab}	1100 ^b	830 ^{ab}
	27.0	400 ^{ab}	350 ^{ab}	540 ^{ab}	590 ^{ab}

¹Data analyzed by a two-way ANOVA after $\ln Y$ transformation (MSE = 68.9 and 890.9 for the 0-5 and 5-15 cm depths respectively). A significant interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to individual means. Values in each data set followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Means are geometric.

surface soil from the control, fertilized and sludge treated plots did not increase significantly over the 39 mo sampling term.

Respiratory activity in the subsurface soil (5-15 cm) was significantly higher in the sewage sludge amended spoil than in the untreated spoil 15 mo after amendment (Table 68). No other treatment or time effects were detected in the deeper soil.

At 0.5 and 15 mo after amendment, CO₂ efflux in the fertilized plots was higher in the subsurface soil than in the 0-5 cm deep soil, while the 0-5 cm deep peat amended spoil was more active than the spoil beneath the peat (15-25 cm) (Table 69). Microbial respiration, 27 mo after amendment was greater in the 0-5 cm deep soil than in the 5-15 cm deep soil in all treatments.

Data on the development of microbial biomass C in the amended slender wheatgrass plots together with shoot production estimates over the first three growing seasons are presented in Table 70 and Fig. 14. The main features emerging from this data are:

i) microbial biomass C in the 0-5 cm deep soil was greatest in the peat amended spoil and remained so over the four sampling times. Over the first 27 mo of the study the microbial biomass C levels in each of the fertilizer, sewage sludge and peat treatments demonstrated an increasing trend. However, this trend did not continue into the last year of the study but instead, was maintained at the same level. Microbial biomass measurements were highest at 27 mo after amendment and planting. At this time there was a trend in biomass C levels with the largest amount occurring in the peat treatment followed by the sewage sludge, then fertilizer and untreated spoil.

ii) microbial biomass C in all treatments was highest one year after peak shoot production by slender wheatgrass (i.e. after the third growing season) (Fig. 14). Although plant production demonstrated a sharp decrease during the third growing season (27 mo), microbial biomass levels were maintained until after the fourth growing season (i.e. 39 mo after amendment).

iii) although microbial activity measurements in the deeper 5-15 cm soil did not vary over the three sampling times, microbial

Table 69. The effect of sampling depth on microbial activity ($\text{CO}_2 \uparrow$) and microbial biomass C in the amended subalpine spoil planted with slender wheatgrass¹.

Parameter	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
Microbial activity ($\text{CO}_2 \uparrow$)	0.5	NS	**	NS	*
	15.0	NS	**	NS	*
	27.0	*	*	*	*
Microbial biomass C	0.5	NS	**	**	*
	15.0	NS	NS	NS	*
	27.0	*	*	*	*

¹As a result of dependence between the 0-5 cm and 5-15 cm data, the effect of depth was determined by subtracting the 5-15 cm values from the 0-5 cm values and analyzing the resulting data using the model for the two-way ANOVA (i.e. microbial biomass C = $\bar{\mu} \dots +$ (amendment effect) + (time effect) + error). The null hypothesis was that each treatment mean = 0 (i.e. no difference between depths). This method allows examination of each treatment in the event that not all treatments show an effect due to depth.

NS - depths not significantly different ($p < 0.05$).

* - 0-5 cm depth significantly greater ($p < 0.05$) than 5-15 cm depth.

** - 5-15 cm depth significantly greater ($p < 0.05$) than 0-5 cm depth.

Table 70. Development of microbial biomass (mq C 100 g⁻¹ dwt soil) in amended subalpine soil planted with slender wheatgrass¹.

Soil depth (cm)	Time after amendment (mo)	Amendment				Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	24.83 ^{ab}	11.12 ^a	32.44 ^{ab}	184.65 ^{cde}	
	15.0	36.00 ^a	77.91 ^{abcd}	114.87 ^{bcde}	331.31 ^f	
	27.0	83.19 ^{abcde}	129.36 ^{bcde}	233.34 ^{ef}	625.85 ^g	
	39.0	66.84 ^{abc}	102.01 ^{bcde}	175.76 ^{def}	590.05 ^g	
5-15 (15-25 in peat)	0.5	30.18	41.15	47.50	43.73	40.08 ^a
	15.0	33.08	64.12	86.76	72.94	60.53 ^b
	27.0	53.14	61.42	121.07	97.15	78.72 ^c
	Amendment $\bar{X}$	37.57 ^a	54.52 ^b	79.31 ^c	67.67 ^{bc}	

¹Data analyzed by two-way ANOVA after $\sqrt{Y}$ (0-5 data) and $\ln Y$ (5-15 data) transformations (MSE = 2.183 and .0432 for 0-5 and 5-15 cm depths respectively). Significant differences were detected by applying Scheffé multiple contrasts for pairwise comparisons to the individual means (0-5 cm data) or to the time and amendment means (5-15 cm data). The 0-5 cm values followed by the same letter(s) do not differ significantly while the 5-15 cm values in the time $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p < 0.05$). All values are geometric means.

biomass C in all treatments demonstrated a significant increase over the 27 mo term of the study (Table 70). At each of the sampling times the microbial biomass in the subsurface soil was highest in the peat and sewage sludge amended soil followed by the fertilized and then the untreated soil.

iv) microbial biomass C measurements in both the surface and subsurface soil showed much stronger trends in microbial development than did the CO₂ efflux measurements.

The data presented in Table 69 show that 0.5 mo after treatment, biomass C in the fertilizer, sewage sludge and peat amended plots was significantly greater at 5-15 cm than at 0-5 cm; however, 27 mo after initiation of the experiment, biomass in all treatments was higher in the surface than subsurface soil.

Since % loss on ignition is a measure of organic matter and this, plus grass plant litter and roots, are sources of energy for the soil microorganisms, it was decided to test for significant relationships between these three parameters and microbial activity and biomass C measured in the slender wheatgrass plots 39 mo after planting. Product moment correlation coefficients were calculated (Table 71) and significant relationships were found between microbial activity and microbial biomass C and between % loss on ignition and microbial activity and biomass. Grass root wts correlated with litter input, but no significant correlations were observed between litter and root wts and microbial activity and biomass C. Undoubtedly better relationships would have resulted if:

- i) more replicates had been tested
- ii) more replicates within each amendment type had been analyzed.

3.2.3.5 Comparison of microbial development in unvegetated plots and plots planted with grass or trees. Since vegetation type, through litter production and root growth, can influence the development of organic matter, microbial activity and microbial biomass, the measurements made for these parameters in soil from the unvegetated pathways and the plots planted with white spruce and

Table 71. Product moment correlation coefficients for loss on ignition, litter input, root wt, CO₂ ↑, microbial biomass C and decomposition (% wt remaining) data collected 27 mo after amendment of the subalpine spoil (slender wheatgrass plots).

	% loss on ignition	Grass litter input	Grass root wt	Microbial activity (CO ₂ ↑)	Microbial biomass C	Decomposition (% wt remaining)
% loss on ignition	1.00	.030	.206	.952*	.974*	.077
Grass litter input		1.00	.511*	.019	.110	.267
Grass root wt			1.00	.122	.238	.011
Microbial activity (CO ₂ ↑)				1.00	.960*	.118
Microbial biomass C					1.00	.101
Decomposition (% wt remaining)						1.00

* - indicates significant correlation.

slender wheatgrass were compared. Data collected 27 mo after planting were used. The results obtained show that:

i) vegetation type had very little effect on the accumulation of organic matter in the various treatments. The amendments had a much greater effect (Table 72).

ii) in all but the peat treatment, microbial activity was highest in the slender wheatgrass plots. In the peat treated soil, vegetation type had no significant effect on the soil respiratory activity. The growth of white spruce in the control, fertilizer and sewage sludge treated spoil did not significantly influence CO_2 efflux (Table 73).

iii) as for microbial activity, slender wheatgrass stimulated the build-up of microbial biomass in all treatments except for the peat amended spoil. The microbial biomass in the white spruce plots, 27 mo after planting, was not significantly different from that recorded in the unvegetated plots (Table 74, Fig. 15). The results demonstrate that the development of microbial activity and biomass up until 27 mo after planting was more rapid if the fast growing slender wheatgrass, rather than the much slower growing white spruce, had been planted in the variously amended spoil.

3.2.3.6 Decomposition potential of the variously amended subalpine minespoil. Cellulose filter paper, placed in the slender wheatgrass plots and sampled after 12 and 24 mo incubation, decomposed fastest in the sludge treated plots followed by the fertilized, peat treated and control plots (Table 75). There was a significant wt loss of the filters between the 12 and 24 mo sample times.

Decay of the filters placed in the amended white spruce plots was also most rapid in the sewage sludge treatment (Table 75). However, there was no significant wt loss between 12 and 24 mo incubation in the field - presumably a result of the high degree of variability in the data set.

As has been the case for all the decomposition studies where fir wood dowel was used as a standard substrate, decay of the dowel, placed in the variously treated subalpine spoil planted with

Table 72. Effect of vegetation on % loss on ignition of the amended subalpine spoil, 27 mo after planting¹.

Plant type	Amendment				Plant type $\bar{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
Unvegetated	6.38	6.16	10.06	68.51	12.83 ^b
Slender wheatgrass	6.83	6.48	10.98	61.10	13.13 ^b
White spruce	5.24	4.80	8.22	53.38	10.25 ^a
Amendment $\bar{X}$	6.11 ^a	5.77 ^a	9.69 ^b	60.68 ^c	

¹Data analyzed by two way ANOVA after $\ln Y$ transformation ($MSE = .0262$) and Scheffé multiple contrasts for pairwise comparisons. Values in the plant type $\bar{X}$ column or amendment $\bar{X}$ row, followed by the same letter do not differ significantly ($p \leq 0.05$). All values are geometric means.

Table 73. Effect of vegetation on microbial activity ($\mu\text{l CO}_2 + 100 \text{ g}^{-1} \text{ dwt soil hr}^{-1}$) in the amended subalpine spoil, 27 mo after planting¹.

Plant type	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
Unvegetated	220 ^a	200 ^a	260 ^{ab}	2010 ^{de}
Slender wheatgrass	660 ^{bc}	930 ^{cd}	1440 ^{cde}	3770 ^e
White spruce	210 ^a	230 ^a	430 ^a	3350 ^e

¹Data analyzed by two-way ANOVA after $\ln Y$ transformation (MSE = 75. 0) and Scheffé multiple contrasts for pairwise comparisons. Values are geometric means where followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 74. Effect of vegetation on microbial biomass C (mg C 100 g⁻¹ dwt soil) in the amended subalpine spoil, 27 mo after planting¹.

Plant type	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
Unvegetated	35.60 ^{ab}	43.78 ^{abc}	101.49 ^{de}	289.97 ^{fgh}
Slender wheatgrass	83.12 ^{cd}	129.06 ^{def}	227.58 ^{efg}	620.40 ^h
White spruce	32.39 ^a	41.68 ^{abc}	81.27 ^{bcd}	454.60 ^{gh}

¹Data analyzed by two-way ANOVA after $\ln Y$ transformation (MSE = .0475) and Scheffé multiple contrasts for pairwise comparisons. Means are geometric and where followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Fig. 15 Effect of vegetation on microbial biomass in the amended subalpine spoil 27 months after planting.

Table 75. The % wt remaining of cellulose filter paper placed in the amended subalpine spoil, 4 mo after planting slender wheatgrass and white spruce¹.

Plant type	Time in the field (mo)	Amendment			Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat
Slender wheatgrass	12	95	74	44	97
	24	76	45	4	68
	Amendment $\bar{X}$	85.5 ^c	59.5 ^b	24 ^a	83 ^c
					77.5 ^a 48.25 ^b
White spruce	12	99	98	46	91
	24	70	48	23	65
	Amendment $\bar{X}$	84.5 ^b	73 ^b	34.5 ^a	78 ^b
					83.5 ^a 51.5 ^a

¹Data analyzed by two-way ANOVA (MSE = 58.4 and 78.7 for slender wheatgrass and white spruce respectively) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ columns or amendment $\bar{X}$ rows followed by the same letter do not differ significantly ($p \leq 0.05$).

slender wheatgrass or white spruce, was negligible over the term of the study (Table 76).

When the weight loss of filters placed in the slender wheatgrass plots at 4 mo after planting and then again at 28 mo after planting were compared after 12 mo incubation in the plots, it was observed that the decomposition potential of the soil for cellulose had not changed significantly since the tank study was initiated (Table 77).

The decay of slender wheatgrass leaves placed in the plots 28 mo after planting was equally fast in all treatments with 44-48% dry wt remaining after 12 mo decomposition (Table 78). The % dry wt remaining of the leaves decreased from 84 to 47% between the 6 and 12 mo sampling times.

A comparison of the % dry wt remaining of slender wheatgrass leaves and stems and cellulose filter paper after 12 mo in the field is presented in Table 79 and Fig. 16.

Application of the fertilizer, sewage sludge and peat to the subalpine spoil did not significantly influence the decomposition of slender wheatgrass leaves or stems over the short term. The leaves decayed significantly faster than the stems and both leaves and stems lost more weight than the filter paper over the 12 mo test period.

The correlation coefficients computed between % loss on ignition of the soil, grass litter input, grass root wt, microbial activity, microbial biomass C and the dry wt remaining of grass leaves after 12 mo decomposition suggested there were no significant relationships between any of the soil factors and short term decomposition (Table 71).

The effect of amendment on the decomposition of alsike clover leaves and stems placed in the clover plots and how the clover decomposition compared with the decay of slender wheatgrass litter was also studied. A three way ANOVA was applied to the data and the results are presented in Tables 80 and 81. There was no significant three-way interaction, but significant interactions were observed between litter type and plant type, and between plant type and

Table 76. The % wt remaining of fir wood dowel placed in amended subalpine spoil 4 mo after planting slender wheatgrass and white spruce¹.

Plant type	Time in the field (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
Slender wheatgrass	12	99	99	98	98
	24	98	98	97	98
White spruce	12	99	99	99	99
	24	99	99	98	98

¹Data not analyzed due to lack of variation.

Table 77. The decomposition of cellulose filter paper (expressed as % dwt remaining) after 12 mo incubation in amended subalpine spoil planted with slender wheatgrass. Filters were placed in the plots 4 mo and 28 mo after planting¹.

Time after planting (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
4	98.8 ^b	80.1 ^{ab}	43.6 ^a	98.6 ^b
28	97.6 ^b	96.2 ^b	86.0 ^a	91.2 ^b

¹Data analyzed by two-way ANOVA after $2 \times \arcsin \sqrt{p}$ transformation (MSE = .203). Significant differences were detected by Scheffé multiple contrasts for pairwise comparisons. Values are geometric and where followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 78. Decomposition (expressed as % dwt remaining) of slender wheatgrass leaves incubated in the amended subalpine spoil for 6 mo and 12 mo. Plots had been planted with slender wheatgrass for 28 mo prior to initiation of the experiment¹.

Incubation time (mo)	Amendment				Time $\bar{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
6	87	85	82	81	84 ^b
12	44	47	48	47	47 ^a
Amendment $\bar{X}$	66 ^a	66 ^a	65 ^a	64 ^a	

¹Data analyzed by two-way ANOVA (MSE = 15.6) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or amendment $\bar{X}$ row, followed by the same letter, do not differ significantly ($p \leq 0.05$).

Table 79. The decomposition (expressed as % dwt remaining after 12 mo in the field) of slender wheatgrass stems and leaves and cellulose filter paper placed in amended subalpine spoil 28 mo after planting slender wheatgrass¹.

Litter type	Amendment				Litter type $\bar{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
Leaves	44	47	48	47	47 ^a
Stems	77	78	73	78	77 ^b
Filter paper	97	92	84	87	90 ^c
Amendment $\bar{X}$	73 ^a	72 ^a	68 ^a	71 ^a	

¹Data analyzed by two-way ANOVA (MSE = 51.3) and Scheffé multiple contrasts for pairwise comparisons. Values in the litter type $\bar{X}$ column or amendment $\bar{X}$ row, followed by the same letter do not differ significantly ($p \leq 0.05$).

Fig. 16 The decomposition of slender wheatgrass leaves and stems and cellulose filter paper placed in amended subalpine spoil 28 months after planting slender wheatgrass.

Table 80. Three-way ANOVA table to test the effect of litter type (i.e. stems or leaves), plant type (i.e. slender wheatgrass or alsike clover) and amendment (i.e. control, fertilizer, sewage sludge and peat) on % dry litter remaining after 12 mo incubation in the subalpine soil.

Source	SS	df	MS	F	prob.1
Litter type	13680.4	1	13680.4	915.84	***
Plant type	11353.5	1	11353.5	760.06	***
Amendment	208.9	3	69.6	4.66	**
Litter type x plant type	900.4	1	900.4	60.28	***
Litter type x amendment	114.5	3	38.2	2.56	NS
Plant type x amendment	187.8	3	62.6	4.19	**
Litter type x plant type x amendment	28.9	3	9.6	.64	NS
Error	1195.0	80	14.9		
Total	27669.4	95			

1* - significant at $p \leq 0.05$.

** - significant at $p \leq 0.01$.

*** - significant at $p \leq 0.001$.

NS - not significant.

Table 81. The effect of amendment on the decomposition (expressed as % dry litter remaining after 12 mo incubation) of slender wheatgrass and alsike clover litter placed in the amended subalpine spoil, 28 mo after planting (extension of data presented in Table 80)¹.

Treatment	Plant or litter type	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
% dry wt stems or leaves remaining	Clover leaves	29	28	33	33
	Clover stems	47	45	48	54
% dry wt stems or leaves remaining	Grass leaves	44	47	47	47
	Grass stems	77	78	73	78
% dry stem and leaf wt remaining (plant type x amendment means)	Clover (leaves plus stems)	38 ^{ab}	36 ^a	40 ^{ab}	43 ^b
	Grass (leaves plus stems)	60 ^c	62 ^c	60 ^c	62 ^c
% dry clover and grass leaf or stem wt remaining (litter type x amendment means) ¹	Leaves (clover plus grass)	37 ^a	37 ^a	40 ^a	40 ^a
	Stems (clover plus grass)	62 ^b	61 ^b	60 ^b	66 ^b

(cont'd)

Table 81. (cont'd).

Treatment	Plant or litter type	% dry wt remaining (summed)	
		Leaves	Stems
% dry leaf or stem wt remaining when summed over amendments (litter type x plant type means) ¹	Clover	31 ^a	48 ^b
	Slender wheatgrass	46 ^b	76 ^c

¹Means tested by Scheffé multiple contrasts for pairwise comparisons. Values in each treatment followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

amendment (Table 78). Therefore to locate significant differences, Scheffé multiple contrasts for pairwise comparisons were applied to the individual means obtained for each amendment type where the treatments interacted. Where there was no interaction, Scheffé multiple contrasts were applied to the factor means. The results presented in Table 81 show that:

i) clover leaves and stems combined, decayed more rapidly than slender wheatgrass leaves and stems (see % dry stem and leaf wt remaining treatment). Clover litter lost more wt in the fertilized clover plots than in the peat treated plots, whereas amendment had no significant effect on the wt loss of slender wheatgrass litter.

ii) the leaves of clover and grass combined decomposed quicker than the stems (see % dry clover and grass leaf or stem wt remaining treatment). Amendment did not significantly influence the decay of the leaves or the stems.

iii) the % dry leaf or stem wt remaining of either clover or slender wheatgrass when summed over all the amendments demonstrated that leaves of either clover or grass decomposed faster than their respective stems. However clover stems and slender wheatgrass leaves did not differ in their wt loss after 12 mo incubation. Clover leaves decayed most rapidly of all the litter types tested (i.e. grass leaves and stems and clover stems).

3.2.3.7 N₂ fixation by alsike clover. The N₂ (C₂H₂) fixation capacity of alsike clover grown in the variously amended subalpine spoil was tested at the conclusion of the first three growing seasons. As was the case for the rambler alfalfa in the grasslands spoil, the results were highly variable resulting in no significant amendment effects on N₂ fixation (Table 82). Although not tested, N₂ fixation appeared to be greater after the second growing season than at the other two sample times.

Partial correlation coefficients were calculated to determine if any strong relationships existed amongst shoot wt, root wt, shoot N, total soil N, soil NO₃-N and N₂ fixation capacity

Table 82. N_2 (C_2H_2) fixation capacity (nmol g^{-1} dry root hr^{-1}) of alsike clover in subalpine spoil tank plots¹.

Treatment	Time after planting		
	3 mo	14 mo	26 mo
Control	288.2 ^a	571.4 ^a	274.1 ^a
Fertilizer	399.9 ^a	503.6 ^a	121.5 ^a
Sewage Sludge	66.0 ^a	395.2 ^a	75.0 ^a
Peat	0 ^a	625.1 ^a	44.9 ^a
MSE	73976.0	NA	20551

¹A one-way ANOVA was applied to the 3 mo and 26 mo data. Peat values in the 3 mo data set were excluded from the analysis because they had 0 variance. The hypothesis that the means of the amendments were not different from the non-random mean, 0, was tested using the confidence interval (± 526.8). Values in each column, followed by the same letter do not differ significantly ($p \leq 0.05$).

²Data analyzed with a Kruskal-Wallis non-parametric test.

after the second growing season. Except for root and shoot wt, there were no strong relationships amongst any of the parameters (Table 83). Again more replication particularly within each amendment type and the determination of N levels in soil around the root systems for which N_2 fixation was tested would have resulted in better correlations.

3.2.4. Oil Sand Tailings - Effects of Amendment and Plant Growth on Microbial Development.

3.2.4.1 Microbial characteristics 0.5 mo and 15 mo after amendment and planting. Immediately after amendment, the peat treated sand appeared to have the highest level of ATP although results were extremely variable (Table 84). In the untreated and fertilized sand, ATP occurred in such low quantities that it could not be measured.

Although the presence of microbes in the control and fertilized sand was not detected through ATP measurements, the dilution plating technique and direct observation demonstrated that the presence of bacteria, actinomycetes and fungal hyphae in these soils (Tables 85, 86, 87 and 88). Sewage sludge and peat amendment of the sand significantly increased the number of bacteria in both the 0-5 and 5-15 cm deep soil, while fertilization had no effect (Table 85). Fifteen months after amendment and planting (slender wheatgrass), bacterial counts from the 0-5 cm peat and sewage sludge treated soil had not changed significantly and were still greater than counts from the untreated sand. However, in the subsurface soil, bacteria in the peat treatment decreased significantly from 0.5 to 15 mo after application, while those in the other three treatments appeared to be unaffected by time.

The application of peat also introduced a significant number of actinomycetes, while sewage sludge and fertilizer had a negligible effect on the presence of actinomycetes in the tailing sand (Table 86). At the conclusion of the second growing season (15 mo) actinomycete counts in the 5-15 cm sewage sludge amended sand had

Table 83. Partial correlation coefficients for determining the relationships amongst shoot wt, root wt, shoot N, total soil N, soil $\text{NO}_3\text{-N}$ and N_2 (C_2H_2) fixation capacity for alsike clover after 1978 growing season.

	Shoot wt (g)	Root wt (g)	Shoot N (%)	Total Soil N (%)	Soil $\text{NO}_3\text{-N}$ ($\mu\text{g/g}$ dry soil)	N_2 (C_2H_2) fixation (nmoles/g root/hr)
Shoot wt (g)		.85	-.57	-.20	-.36	-.13
Root wt (g)			-.50	-.27	-.48	-.30
Shoot N (%)				.17	.48	.16
Total Soil N (%)					.65	-.51
Soil $\text{NO}_3\text{-N}$ ($\mu\text{g/g}$ dry soil)						
N_2 (C_2H_2) fixation (nmoles/g dry root/hr)						-.10

Table 84. ATP levels in extracted oil sand 0.5 mo after amendment.
Values are $\bar{X} \pm \text{SD}$.

Amendment	Soil depth (cm)	ATP ($\mu\text{g } 100\text{g}^{-1}$ dry soil)
Control	0-5	0
	5-15	0
Fertilizer	0-5	0
	5-15	0
Sewage Sludge	0-5	2.30 ± 3.56
	5-15	2.40 ± 4.16
Peat	0-5	27.0 ± 14.12
	15-25	0.17 ± 0.15

Table 85. Effect of amendment and time on bacterial numbers in extracted oil sands tank plots¹. Plots were planted with slender wheatgrass.

Amendment	Depth (cm)	No. of bacteria $\times 10^6$ g ⁻¹ dwt soil	
		Time after amendment (mo)	
		0.5	15
Control	0-5	7.48 ^a	8.43 ^{ab}
Fertilizer		7.86 ^{ab}	68.57 ^{bc}
Sewage Sludge		1204.31 ^d	220.27 ^{cd}
Peat		649.97 ^d	600.63 ^{cd}
Control	5-15	8.68 ^a	9.43 ^a
Fertilizer		8.78 ^a	12.59 ^{ab}
Sewage Sludge		110.07 ^c	127.70 ^c
Peat	15-25	72.29 ^{bc}	10.89 ^a

¹Data analyzed by a two-way ANOVA (MSE = .389 and .281 for 0-5 cm and 5-15 cm respectively) after a $\ln Y$ transformation. A significant interaction was detected for both data sets, hence Scheffé multiple contrasts for pairwise comparisons were applied to all values. All values are geometric and those followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 86. Effect of amendment and time on actinomycete numbers in extracted oil sands tank plots¹. Plots were planted with slender wheatgrass.

Amendment	Depth (cm)	No. of actinomycetes $\times 10^4$ g ⁻¹ dwt soil	
		Time after amendment (mo)	
		0.5	15
Control	0-5	0.62 ^a	4.21 ^{ab}
Fertilizer		0.96 ^a	0.97 ^a
Sewage Sludge		2.18 ^{ab}	20.44 ^b
Peat		3281.75 ^c	1220.40 ^c
Control	5-15	0 ^a	0.90 ^{ab}
Fertilizer		0.28 ^a	0.34 ^a
Sewage Sludge		0.63 ^a	18.31 ^b
Peat	15-25	332.99 ^d	33.91 ^{cd}

¹Data analyzed by a two-way ANOVA (MSE = .369 and .473 for 0-5 cm and 5-15 cm data respectively) after a $\ln Y$ transformation. A significant interaction was detected for both data sets, hence Scheffé multiple contrasts for pairwise comparisons were applied to all values. All values are geometric and those followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 87. Hyphal lengths in 0-5 cm oil sands tailings before planting (0.5 mo) and after planting slender wheatgrass (15 mo)¹.

Hyphal category (m g ⁻¹ dwt soil)	Sampling time (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
Total	0.5	95 ^a	37 ^a	86 ^a	1304 ^b
	15.0	41 ^a	57 ^a	66 ^a	675 ^b
Total with cell contents	0.5	77 ^{ab}	15 ^a	59 ^a	248 ^c
	15.0	27 ^a	36 ^a	24 ^a	158 ^{bc}

¹Data in each hyphal category were analyzed by a two-way ANOVA (MSE = .087 and 769.98 for total and total with cell contents respectively) and Scheffé multiple contrasts for pairwise comparisons. Values followed by the same letter(s) do not differ significantly ($p < 0.05$).

Table 88. Hyphal lengths in 5-15 cm (15-25 cm in peat) oil sands tailings (0.5 mo) and after planting slender wheatgrass (15 mo)¹.

Hyphal category (m g ⁻¹ dwt soil)	Sampling time (mo)	Amendment				Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
Total	0.5	103 ^{ab}	46 ^a	89 ^{ab}	356 ^b	
	15.0	39 ^a	26 ^a	53 ^a	34 ^a	
Total with	0.5	81	9	60	81	43
cell contents	15.0	26	20	28	15	22
Amendment $\bar{X}$		46	13	41	35	

¹Data in each hyphal category were analyzed by a two-way ANOVA (MSE = .245 and 62.02 for total and total with cell contents respectively). The total with cell contents data required a $\ln Y$ transformation. Scheffé multiple contrasts for pairwise comparisons were applied to all values in the total category (a significant interaction was detected) and to the time $\bar{X}$ and amendment $\bar{X}$ values in the total with cell contents category.

Values in the total category followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Values in the time $\bar{X}$ column or amendment $\bar{X}$ row in the total with cell contents category, followed by the same letter do not differ significantly ($p \leq 0.05$).

increased significantly compared with the 0.5 mo measurements but those in the other treatments had not changed.

Hyphal length measurements (both total and that with cell contents) were, like the bacteria and actinomycete numbers, highest in the 0-5 cm peat treated tailing sand at both sampling times (Table 87). No differences in mycelial lengths were observed between the surface sand in the control plots and that in the fertilized and sewage sludge treatments. In the deeper, subsurface soil, peat amendment did not have as significant an effect on mycelial lengths as was observed in the 0-5 cm soil (Table 88). By 15 mo after amendment and planting, total mycelial lengths in the 5-15 cm deep sand did not vary amongst the four treatments. However, hyphae having cell contents decreased significantly over the 15 mo study period (Table 88).

3.2.4.2 Microbial development in amended, unvegetated oil sand tailings. The application of peat and sewage sludge to the oil sands spoil significantly raised the organic matter level of the spoil (Table 89). The organic matter content in the four treatments did not vary 0.5 to 39.0 mo after amendment.

The microbial activity and biomass C in the unvegetated sand followed the same pattern as that observed for the % loss on ignition measurements, i.e. the peat treatment was the most active and contained the largest quantity of microbial biomass followed by the sewage sludge treated sand (Tables 90 and 91). Fertilization did not significantly affect microbial activity and biomass C. In general, CO₂ efflux and microbial biomass were highest at the 15 mo sampling time, but then decreased to levels not significant from the 0.5 mo measurements (Tables 90 and 91).

3.2.4.3 Microbial development in amended tailings sand planted with jack pine. Application of sewage sludge to the extracted oil sand stimulated the greatest amount of root production by jack pine in comparison with root wts produced in the other treatments (Table 92). This was true at both sampling depths. Root wts increased

Table 89. Effect of time on % loss on ignition of amended, unvegetated oil sands tailings (pathways)¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			Time $\bar{X}$
		Control	Fertilizer	Sewage sludge	Peat
0-5	0.5	.40	.27	1.40	26.83
	27.0	.23	.27	1.46	27.56
	39.0	.26	.20	2.10	24.41
	Amendment $\bar{X}$	.29 ^a	.24 ^a	1.65 ^b	26.24 ^c

¹Data analyzed by two-way ANOVA after $\ln Y$ transformation ($MSE = 0.24$). No interaction was determined, hence Scheffé multiple contrasts for pairwise comparisons were applied to amendment and time means. Values in the time $\bar{X}$ column or amendment $\bar{X}$ row, followed by the same letter, do not differ significantly ($p < 0.05$). All means are geometric.

Table 90. Development of microbial activity ($\mu\text{l CO}_2 \pm 100 \text{ g}^{-1} \text{ dwt hr}^{-1}$) in variously amended oil sands tailings (pathways)¹.

Soil depth (cm)	Time after amendment (mo)	Amendment				Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	0.5	70	40	200	940	140 ^a
	15.0	90	90	240	1850	250 ^b
	27.0	50	60	90	1050	130 ^a
	39.0	60	70	180	1010	160 ^a
	Amendment $\bar{X}$	60 ^a	60 ^a	170 ^b	1170 ^c	

¹Data analyzed by two-way ANOVA after $\ln Y$ transformation ($\text{MSE} = 150.0$). No interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to the amendment and time means. Values in the amendment $\bar{X}$ row or time $\bar{X}$ column, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All values are geometric.

Table 91. Development of microbial biomass (mg C 100 g⁻¹ dwt soil) in variously amended oil sands tailings (pathways)¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	3.85	1.57	17.22	90.26
	15.0	6.24	5.86	16.06	158.61
	27.0	4.13	4.13	14.04	151.26
	39.0	3.97	3.62	23.60	121.56
	Amendment $\bar{X}$	4.45 ^a	3.42 ^a	17.40 ^b	127.38 ^c
					9.84 ^a
					17.47 ^b
					13.79 ^{ab}
					14.25 ^{ab}

¹Data analyzed by a two-way ANOVA after In Y transformation (MSE = 0.22). No interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to the amendment and time means. Values in the amendment $\bar{X}$ row or time $\bar{X}$ column, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All values are geometric.

Table 92. Jack pine root wts in amended oil sands tailings¹.

Soil depth (cm)	Time after planting (mo)	Root wt (g dwt m ⁻²)				Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
0-5	27.0	0	.51	4.42	.58	0.89 ^a
	39.0	1.05	2.36	39.63	11.26	6.65 ^b
	Amendment $\bar{X}$	0.43 ^a	1.25 ^{ab}	13.84 ^c	3.41 ^b	
5-15	27.0	0	1.93	14.49	0.61	1.92 ^a
	39.0	2.34	20.12	148.23	6.59	15.81 ^b
	Amendment $\bar{X}$	0.83 ^a	6.87 ^b	47.08 ^c	2.49 ^{ab}	

¹Data analyzed by two-way MANOVA after $\ln(Y + 1)$ transformation (MSE = 11.93 and 15.52 for 0-5 cm and 5-15 cm depths respectively). In each data set values in the time $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter(s) do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All means are geometric.

significantly between 27.0 and 39.0 mo after planting in all treatments (Table 92). At 27 mo after planting, jack pine root wts did not differ between the 0-5 m and 5-15 cm deep soil but by 39 mo after planting, roots in the surface fertilized and sewage sludge treated sand were heavier than those in the subsurface soil (Table 93).

Microbial respiration and biomass C levels measured in the amended jack pine plots on four different occasions after amendment and planting are presented in Tables 94 to 97. The main features emerging from this data are:

i) with the exception of the peat amended sand, where CO_2 efflux over the term of the study was always greater than the fertilized and untreated sand, there were no obvious differences in microbial respiration in the surface soil of the various treatments (Table 94). Once the amendments had been applied, microbial activity did not change significantly over the four sampling times suggesting that the growth of jack pine did not influence the development of microbial activity in the 0-5 cm soil.

ii) in the subsurface soil CO_2 efflux 15 mo after amendment was significantly greater in the peat treated sand than in the fertilized, sewage sludge treated and untreated sand (Table 94). No amendment effects were observed at any of the other sample times. As in the surface soil, microbial activity did not change significantly over the 39 mo study period.

iii) in the 0-5 cm deep soil, microbial biomass C levels, in contrast to CO_2 efflux, demonstrated more obvious effects of amendment (Table 96). Immediately after application of the amendments (i.e. 0.5 mo) biomass in the peat treatment was significantly higher than in the sewage sludge treated sand followed by the fertilized and untreated sand. This was also the case 39 mo after amendment and planting. Like the respiration results, biomass C did not demonstrate significant increases or decreases over the 39 mo study.

Table 93. The effect of depth on jack pine root distribution in amended oil sand tailings¹.

Time after planting (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
27.0	NS	NS	NS	NS
39.0	NS	*	*	NS

¹See Table 42 for explanation of statistical analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm root wt significantly greater ($p \leq 0.05$) than 5-15 cm (15-25 in peat) root wt.

Table 94. Development of microbial activity ($\mu\text{l CO}_2 + 100 \text{ g}^{-1} \text{ dwt hr}^{-1}$) in variously amended oil sands tailings planted with jack pine¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	70 ^{ab}	40 ^a	200 ^{abc}	940 ^{cde}
	15.0	50 ^{ab}	80 ^{ab}	240 ^{abcd}	1740 ^{de}
	27.0	50 ^{ab}	60 ^{ab}	430 ^{bcde}	2150 ^e
	39.0	50 ^{ab}	80 ^{ab}	250 ^{abcd}	1340 ^{de}
5-15	0.5	60 ^{abc}	60 ^{abc}	60 ^{abc}	320 ^{cd}
	15.0	50 ^{ab}	70 ^{abc}	140 ^{bc}	440 ^d
	27.0	40 ^{ab}	50 ^{ab}	110 ^{bcd}	100 ^{bcd}
	39.0	20 ^a	40 ^{ab}	70 ^{ab}	40 ^{ab}

¹Data analyzed in the same way as that described in Table 68. MSE = 9490 and 8060 for 0-5 and 5-15 cm depths respectively. Values for each depth followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Data required a $\ln Y$ transformation, hence all means are geometric.

Table 95. The effect of sampling depth on microbial activity ($\text{CO}_2 \uparrow$) in the amended oil sands tailings planted with jack pine¹.

Time after amendation (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
0.5	NS	NS	*	*
15.0	NS	NS	NS	*
27.0	NS	NS	*	*
39.0	*	*	*	*

¹See Table 42 for explanation of statistical analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm $\text{CO}_2 \uparrow$ significantly greater ($p \leq 0.05$) than 5-15 cm (15-25 in the peat) $\text{CO}_2 \uparrow$.

Table 96. Development of microbial biomass (mg C 100 g⁻¹ dwt soil) in variously amended oil sands tailings planted with jack pine¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	3.85 ^{ab}	1.57 ^a	17.22 ^{cd}	90.26 ^{ef}
	15.0	3.24 ^{ab}	7.18 ^{bc}	20.31 ^{cde}	181.29 ^f
	27.0	3.45 ^{ab}	7.36 ^{bc}	27.82 ^{cde}	197.93 ^f
	39.0	8.57 ^{bc}	9.80 ^{bc}	39.35 ^{de}	215.53 ^f
5-15 (15-25 in peat)	0.5	4.73 ^{abc}	2.77 ^{ab}	2.77 ^{ab}	22.67 ^c
	15.0	3.14 ^{ab}	5.28 ^{abc}	7.17 ^{abc}	28.87 ^c
	27.0	2.86 ^{ab}	4.11 ^{abc}	12.03 ^{bc}	6.26 ^{abc}
	39.0	2.06 ^a	3.04 ^{ab}	10.62 ^{bc}	4.58 ^{abc}

¹Data analyzed in the same way as that described in Table 68. MSE = 5.77 and 9.02 for 0-5 cm and 5-15 cm depths respectively. Values for each depth followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Data required a $\ln Y$ transformation, hence all means are geometric.

Table 97. The effect of sampling depth on microbial biomass C in the variously amended oil sands tailings planted with jack pine.

Time after amendation (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
0.5	NS	NS	*	*
15.0	NS	NS	*	*
27.0	NS	*	*	*
39.0	*	*	*	*

¹See Table 42 for explanation of analysis.

NS - biomass C in the two depths not significantly different ($p \leq 0.05$).

* - biomass C in the 0-5 cm soil significantly greater ($p \leq 0.05$) than that in 5-15 cm (15-25 in the peat) soil.

iv) microbial biomass C in the subsurface soil of the jack pine plots was not significantly influenced by amendment or time (Table 96).

v) both microbial activity and biomass in the sludge and peat treated plots tended to be higher in the surface than subsurface soil except at 39 mo when all treatments demonstrated more CO₂ efflux and biomass in the surface soil (Tables 95 and 97).

3.2.4.4 Microbial development in amended tailings sand planted with slender wheatgrass. Litter input by slender wheatgrass grown in the fertilized, sewage sludge and peat treated sand was not significantly different amongst the three amendments with no differences recorded between the 27 and 39 mo inputs (Table 98). Data were highly variable.

Roots, however, tended to be heavier in the 0-5 cm deep peat and sludge treated soil than in the fertilized and control soil, particularly at 39 mo after planting (Table 99). In the subsurface, soil root wts in the fertilizer and sewage sludge treated plots were greater than in the untreated sand.

Twenty seven months after planting, there were more grass roots in the 0-5 cm deep fertilized and peat amended sand than in the deeper soil while sewage sludge and peat treated plots had more roots in the surface than subsurface soil at 39 mo after planting (Table 100).

Loss on ignition measured in the 0-5 cm surface soil from the slender wheatgrass plots followed a similar pattern to that obtained for the 0-5 cm unvegetated sand. Thirty nine months after the initiation of the study organic matter levels were highest in the peat and sewage sludge treated plots (Table 101). The % loss on ignition in the surface soil at 39 mo was not significantly altered from that at 0.5 mo after amendment. In the deeper soil, amendment had no well defined effects on the organic matter estimates made at any of the sampling times (Table 101). Shortly after amendment, organic matter in only the peat treated plots was greater in the surface than surface soil; however, at the conclusion of the study %

Table 98. Slender wheatgrass litter (dead leaves and stems) input for variously amended oil sands tailings¹.

Time after planting (mo)	Litter input (g dwt m ⁻²)			Time $\bar{X}$
	Control	Fertilizer	Sewage Sludge	
27	.69	126.89	113.77	80.74
39	.23	59.70	396.87	175.83
Amendment $\bar{X}$	.44 ^a	87.11 ^b	212.69 ^b	119.23 ^b
				36.75 ^a
				46.86 ^a

¹Data analyzed by two-way ANOVA after $\ln(Y + 1)$ transformation ($MSE = 0.71$). No interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to the amendment and time means. Values in the time $\bar{X}$ column or amendment $\bar{X}$ row, followed by the same letter, do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 99. Slender wheatgrass root wts in amended oil sands tailings¹.

Soil depth (cm)	Time after planting (mo)	Root wt (g dwt m ⁻²)			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	27	3.06 ^{ab}	63.43 ^c	43.82 ^{cd}	44.70 ^{cd}
	39	1.23 ^a	18.17 ^{bc}	126.47 ^d	94.63 ^d
5-15 (15-25 in peat)	27	1.90 ^a	25.03 ^{cd}	38.86 ^{cd}	5.10 ^{abc}
	39	1.82 ^a	12.55 ^{bcd}	51.42 ^d	3.22 ^{ab}

¹Data was analyzed by a two-way MANOVA after $\ln Y$ transformation (MSE = 19.78 and 15.20 for 0-5 and 5-15 cm depths respectively). Values in each data set followed by the same letter(s) do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All values are geometric.

Table 100. The effect of depth on slender wheatgrass root distribution in amended oil sands tailings¹.

Time after planting (mo)	Amendment				
	Control	Fertilizer	Sewage Sludge	Peat	
27.0	NS	*	NS	*	
39.0	NS	NS	*	*	

¹See Table 42 for explanation of statistical analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm root wt significantly greater ($p \leq 0.05$) than 5-15 cm (15-25 in peat) root wt.

Table 101. Effect of time on % loss on ignition of amended oil sands tailings planted with slender wheatgrass¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	.40 ^{ab}	.27 ^a	1.46 ^{bcd}	26.83 ^e
	15.0	.41 ^{ab}	.29 ^a	.69 ^{abc}	66.02 ^e
	27.0	.27 ^a	.41 ^{ab}	2.62 ^{cd}	40.04 ^e
	39.0	.38 ^a	.54 ^{ab}	2.54 ^d	41.68 ^e
5-15 (15-25 in peat)	0.5	.31 ^{abc}	.29 ^{ab}	.46 ^{abc}	3.06 ^d
	15.0	.33 ^{abc}	.17 ^a	.47 ^{abc}	.51 ^{abc}
	27.0	.35 ^{abc}	.33 ^{abc}	.82 ^{bcd}	1.23 ^{cd}
	39.0	.27 ^a	.30 ^{ab}	.56 ^{abc}	.44 ^{abc}

¹Data analyzed by a two-way MANOVA after ln Y transformation (MSE = 4.94 and 5.04 for 0-5 and 5-15 cm data respectively). Significant differences amongst the values in each data set were determined by Scheffé multiple contrasts for pairwise comparisons. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All means are geometric.

loss on ignition in all treatments except the control plots was higher in the 0-5 cm than 5-15 cm deep soil (Table 102).

The microbial activity in the surface soil of the amended slender wheatgrass plots followed the same trends as observed in the jack pine plots. Immediately after amendment, CO₂ efflux from the untreated and fertilized sand tended to be lower than that from the sewage sludge and peat amended plots (Table 103). Although microbial activity in the fertilized and peat treated sand appeared to increase over the first 15 mo of the study, this increase was not significant - a result of extreme variation. Time did not have a significant effect on the microbial activity so that at the end of the study period CO₂ evolution from the peat treatment was still greater than that from the sludge and fertilizer treatments (Table 103).

As in the jack pine plots, amendment had no obvious effects on microbial respiration in the subsurface soil (Table 103). CO₂ efflux from the 0-5 cm sewage sludge and peat amended sand was greater than that from the subsurface soil regardless of sampling time. At 39 mo after amendment and planting, microbial activity in the 0-5 cm deep soil from the untreated and fertilized plots was also greater than that in the subsurface soil (Table 104). Similar data were obtained for the jack pine planted soil.

In contrast to the CO₂ efflux measured for the 0-5 cm deep soil, microbial biomass C estimates for the same soils demonstrated an increasing trend over the first 27 mo of the study (Table 105, Fig. 17). This trend did not apply to the untreated sand. Also no trends in biomass development were obtained for soil from the jack pine plots suggesting that the increasing trend in microbial biomass C in the amended slender wheatgrass plots was related to the productivity of the grass (particularly in the sewage sludge and peat treatments - see Fig. 17 for slender wheatgrass primary production data). Microbial activity measurements demonstrated a lack of well-defined differences amongst the various treatments; conversely the microbial biomass C estimates obtained for each of the treatments at 39 mo after planting were all significantly

Table 102. The effect of sampling depth on % loss on ignition in the amended oil sands tailings planted with slender wheatgrass¹.

Time after amendation (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
0.5	NS	NS	NS	*
15.0	NS	NS	NS	*
27.0	NS	NS	*	*
39.0	NS	*	*	*

¹See Table 42 for explanation of statistical analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm loss on ignition significantly greater ($p \leq 0.05$) than 5-15 cm loss on ignition.

Table 103. Development of microbial activity (μ l CO₂ + 100 g⁻¹ dwt soil hr⁻¹) in variously amended oil sands tailings planted with slender wheatgrass¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	66 ^{ab}	36 ^{ab}	196 ^{bc}	936 ^{de}
	15.0	58 ^{ab}	194 ^{bc}	255 ^{bc}	2596 ^{de}
	27.0	53 ^{ab}	127 ^{abc}	495 ^{cd}	1595 ^{de}
	39.0	45 ^a	119 ^{abc}	334 ^{bc}	2951 ^e
5-15 (15-25 in peat)	0.5	60 ^{abc}	60 ^{abc}	60 ^{abc}	320 ^c
	15.0	50 ^{ab}	110 ^{abc}	80 ^{abc}	150 ^{bc}
	27.0	40 ^{ab}	50 ^{ab}	140 ^{abc}	100 ^{abc}
	39.0	40 ^{ab}	30 ^a	40 ^{ab}	60 ^{abc}

¹The 0-5 cm data was analyzed by a two-way MANOVA after ln Y transformation (MSE = 9976). Significant differences amongst means were determined by a method analogous to the Scheffé multiple contrasts for pairwise comparisons used for the ANOVA. The 5-15 cm data were analyzed by a two-way ANOVA after ln Y transformation (MSE = 138.70). Significant differences amongst the values were determined by Scheffé multiple contrasts for pairwise comparisons. Values for each depth, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 104. The effect of sampling depth on microbial activity ($\text{CO}_2 \uparrow$) in the variously amended oil sands tailings planted with slender wheatgrass¹.

Time after amendment (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
0.5	NS	NS	*	*
15.0	NS	NS	*	*
27.0	NS	*	*	*
39.0	*	*	*	*

¹See Table 42 for explanation of statistical analysis.

NS - depths not significantly different ($p \leq 0.05$).

* - 0-5 cm $\text{CO}_2 \uparrow$ significantly greater ($p \leq 0.05$) than 5-15 cm (15-25 in the peat) $\text{CO}_2 \uparrow$.

Table 105. Development of microbial biomass (mg C 100 g⁻¹ dwt soil) in variously amended oil sands tailings planted with slender wheatgrass¹.

Soil depth (cm)	Time after amendment (mo)	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
0-5	0.5	3.85 ^{abc}	1.57 ^a	17.22 ^{bcde}	90.26 ^{ef}
	15.0	3.86 ^{abc}	11.91 ^{bcd}	33.96 ^{de}	241.00 ^{fg}
	27.0	4.66 ^{abc}	14.64 ^{bcde}	96.97 ^e	267.58 ^{fg}
	39.0	3.74 ^{ab}	15.74 ^c	77.78 ^e	486.11 ^g
5-15	0.5	4.73 ^a	2.77 ^a	2.77 ^a	22.67 ^b
	15.0	3.14 ^a	5.51 ^{ab}	7.83 ^{ab}	7.48 ^{ab}
	27.0	3.62 ^a	3.94 ^a	11.68 ^{ab}	11.51 ^{ab}
	39.0	3.91 ^a	2.97 ^a	5.96 ^{ab}	4.46 ^a

¹Data analyzed by a two-way MANOVA after In Y transformation (MSE = 11.65 and 4.80 for 0-5 cm and 5-15 cm depths respectively). Significant differences amongst means for each depth were determined by a method analogous to the Scheffé multiple contrasts for pairwise comparisons used for the ANOVA. Values for each depth, followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All means are geometric.

Fig. 17 Primary production and the development of microbial biomass in amended oil sands tailings planted with slender wheatgrass.

different from each other (peat > sewage sludge > fertilizer > control).

Amendation and plant growth effects on the microbial biomass C occurred only in the 0-5 cm deep soil. There were no obvious biomass C differences recorded amongst the various treatments in the 5-15 cm deep soil (Table 105). Only the control plots and fertilized plots at 0.5 mo demonstrated no significant depth effects on microbial biomass C - the other treatments (regardless of sampling time) harbored more microbial biomass in the surface than subsurface soil (Table 106).

Although it was hypothesized that litter input and root wts in the slender wheatgrass plots were related to microbial activity and biomass, there were no significant correlations amongst these variables (Table 107). More replications within each treatment might have yielded a more significant relationship between grass productivity and microbial biomass C. Significant relationships were, however, obtained between % loss on ignition and microbial activity and biomass C.

3.2.4.5 Comparison of microbial development in unvegetated plots and plots planted with grass or pine. Organic matter estimated in soil from the amended but unvegetated pathways, in soil from the plots planted with jack pine and in soil from the plots planted with slender wheatgrass suggested that the planting of slender wheatgrass resulted in significantly more organic matter accretion than observed for the other two treatments over the 39 mo term of the study (Table 108). This observation was true for all four treatments.

Microbial biomass C, like organic matter, also tended to be higher in the 0-5 cm soil from the grass plots than soil from the unvegetated pathways and the jack pine plots (Table 109, Fig. 18). This was not the case for the control plots where primary production was negligible. Unfortunately, variation in biomass C measurements were extremely high making it difficult to further discuss the role of different vegetation types in the development of microbial activity and biomass in reclaimed minespoils. More detailed research

Table 106. The effect of sampling depth on microbial biomass C in the variously amended oil sands tailings planted with slender wheatgrass¹.

Time after amendation (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
0.5	NS	NS	*	*
15.0	NS	*	*	*
27.0	NS	*	*	*
39.0	NS	*	*	*

¹As a result of dependence between the 0-5 cm and 5-15 cm data, the effect of depth was determined by subtracting the 5-15 cm values from the 0-5 cm values and analyzing the resulting data using the model for the two-way ANOVA (i.e. microbial biomass C = $\bar{\mu}..$ + (amendment effect) + (time effect) + error). The null hypothesis was that each treatment mean = 0 (i.e. no difference between depths). This method allows examination of each treatment in the event that not all treatments show an effect due to depth. NS - depths not significantly different ($p \leq 0.05$). * - 0-5 cm biomass C significantly greater than 5-15 cm (15-25 in the peat) biomass C ($p \leq 0.05$).

Table 107. Product moment correlation coefficients for loss on ignition, litter input, root wt, CO₂ + and microbial biomass C data collected 27 and 39 mo after amendment of the oil sands tailings (slender wheatgrass plots).

	% loss on ignition	Grass litter input	Grass root wt	Microbial activity (CO ₂)	Microbial biomass C
% loss on ignition	1	.049	.329	.727	.905*
Grass litter input		1	.551	.192	.244
Grass root wt			1	.368	.476
Microbial activity (CO ₂)				1	.832*
Microbial biomass C					1

*indicates significant correlation

Table 108. Effect of vegetation on % loss on ignition of the amended oil sands, 39 mo after planting¹.

Plant type	Amendment			Plant type $\bar{X}$
	Control	Fertilizer	Sewage Sludge	Peat
Unvegetated	.26	.20	2.10	24.52
Slender wheatgrass	.38	.54	2.54	41.59
Jack pine	.27	.29	1.44	27.63
Amendment $\bar{X}$	.30 ^a	.31 ^a	1.97 ^b	30.43 ^c

¹Data analyzed by two-way ANOVA after $\ln Y$ transformation ($MSE = .200$). No significant interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to amendment and plant type means. Values in the plant type $\bar{X}$ column or amendment $\bar{X}$ row, followed by the same letter do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 109. Effect of vegetation on microbial biomass (mg C 100 g⁻¹ dwt soil) in the amended oil sands tailings 39 mo after planting¹.

Plant type	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
Unvegetated	3.97 ^{ab}	3.62 ^a	23.60 ^{cde}	121.56 ^{fgh}
Slender wheatgrass	3.74 ^a	15.74 ^{bcd}	77.78 ^{efg}	486.11 ^h
Jack pine	8.57 ^{abc}	9.80 ^{abcd}	39.35 ^{def}	215.53 ^{gh}

¹Data analyzed by two-way ANOVA after $\ln Y$ transformation (MSE = .281). Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$) as determined by Scheffé multiple contrasts for pairwise comparisons. All means are geometric.

Fig. 18 Effect of vegetation on microbial biomass in the amended oil sands tailings 39 months after planting.

with more replication is required to elucidate the relationship between primary productivity and the microbial system.

3.2.4.6 Decomposition potential of amended and planted tailings sand. Cellulose filter paper placed in the slender wheatgrass plots for 12 mo lost more wt in the sewage sludge treatment than in the other three treatments (Table 110). After spending 24 mo in the field, the % wt remaining of filters in the fertilizer and sewage sludge amended plots was significantly less than the wt remaining in the untreated and peat treated plots. Only filters in the fertilized slender wheatgrass plots lost a significant amount of wt between the two sample times.

Decomposition of filters placed in the jack pine plots was not significantly influenced by amendment and jack pine growth after 12 or 24 mo in the field (Table 110).

As in the other decomposition studies where fir wood dowel was used as a test substrate, there was very little wt loss recorded for this substrate regardless of treatment, plant type or incubation time (Table 111).

With the exception of the sewage sludge treated plots, the ability of the fertilized, peat amended and unamended sand to decompose cellulose did not change significantly over the term of the study (Table 112). In the sludge treated slender wheatgrass plots the cellulose decomposition potential of the soil decreased between 16 and 40 mo after planting. At the termination of the study (40 mo) cellulose decomposition tended to be more rapid in the fertilized plots than in the other treatments.

The decay of slender wheatgrass leaves placed in the grass plots 28 mo after planting was not significantly influenced by amendment (Table 113). A significant wt loss occurred between the 6 and 12 mo incubation times for leaves in all treatments. Interestingly, the % wt remaining of leaves placed in the oil sands plots for 12 mo (i.e. 44-46%) was very similar to that obtained for leaves placed in the subalpine plots (44-48%) for the same length of time.

Table 110. Cellulose filter paper decomposition (expressed as % wt remaining) in amended sand tailings plots planted with slender wheatgrass and jack pine. Filter paper was placed in the plots 4 mo after planting¹.

Plant type	Time in the field (mo)	Amendment				Time $\bar{X}$
		Control	Fertilizer	Sewage Sludge	Peat	
Slender wheatgrass	12	99 ^b	68 ^b	10 ^a	87 ^b	
	24	90 ^b	29 ^a	4 ^a	69 ^b	
Jack pine	12	88	91	46	67	73 ^a
	24	67	62	34	56	55 ^a
	Amendment $\bar{X}$	78 ^a	77 ^a	40 ^a	62 ^a	

¹Data analyzed by two-way ANOVA (MSE = 105 and 809 for slender wheatgrass and jack pine respectively). A significant interaction was observed for the slender wheatgrass data, hence Scheffé multiple contrasts for pairwise comparisons were applied to individual means. Values followed by the same letter do not differ significantly ($p < 0.05$).

Table 111. The % wt remaining of fir wood dowel placed in amended sand tailings plots 4 mo after planting slender wheatgrass and jack pine¹.

Plant type	Time in the field (mo)	Amendment			Time	
		Control	Fertilizer	Sewage Sludge	Peat	$\bar{X}$
Slender wheatgrass	12	99	99	98	98	98.5 ^a
	24	98	99	98	99	98.5 ^a
	Amendment $\bar{X}$	98.5 ^a	99 ^a	98 ^a	98.5 ^a	
Jack pine	12	99	99	98	98	98.5 ^a
	24	98	99	99	98	98.5 ^a
	Amendment $\bar{X}$	98.5 ^a	99 ^a	98.5 ^a	98 ^a	

¹Data analyzed by two-way ANOVA (MSE = .491 and .098 for slender wheatgrass and jack pine data respectively). Values presented in the table have been rounded off. No significant differences were observed.

Table 112. The decomposition of cellulose filter paper (expressed as % dwt remaining) after 12 mo in extracted oil sands plots planted with slender wheatgrass. Filters were placed in plots 4 mo and 28 mo after planting¹.

Time after planting (mo)	Amendment			
	Control	Fertilizer	Sewage Sludge	Peat
4	99.9 ^c	68.9 ^{bc}	6.4 ^a	90.5 ^{bc}
28	86.7 ^{bc}	36.8 ^{ab}	71.1 ^{bc}	80.5 ^{bc}

¹Data analyzed by a two-way ANOVA after $2 \times \arcsin \sqrt{p}$ transformation (MSE = 0.24). A significant interaction was observed, hence Scheffé multiple contrasts for pairwise comparisons were applied to individual means. Values followed by the same letter(s) do not differ significantly ($p \leq 0.05$). All means are geometric.

Table 113. Decomposition (expressed as % dry wt remaining) of slender wheatgrass leaves incubated in the amended oil sands tailings plots for 6 mo and 12 mo. Plots had been planted with slender wheatgrass for 28 mo prior to initiation of experiment¹.

Incubation time (mo)	Amendment				Time $\bar{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
6	88	75	78	77	79 ^b
12	46	41	46	44	44 ^a
Amendment $\bar{X}$	67 ^a	58 ^a	62 ^a	60 ^a	

¹Data analyzed by two-way ANOVA (MSE = 72.62) and Scheffé multiple contrasts for pairwise comparisons. Values in the time $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$).

A comparison of the % wt remaining for slender wheatgrass leaves, stems and filter papers after 12 mo decomposition is presented in Table 114 and Fig. 19. Neither leaf nor stem decay was affected by treatment, but leaves decayed much more rapidly than stems in all treatments. The results obtained for the filter paper were highly variable, but a significant difference in % wt remaining was observed between filters placed in the fertilized and untreated plots. Filters placed in the sewage sludge, peat and untreated plots lost approximately the same weight as the stems over the 12 mo experimental period (Fig. 19).

A multiple regression of % dry stem or leaf wt remaining = $a + b$ (% loss on ignition of soil below the respective litter bags) + c (dead litter wt above and below the litter bags at retrieval) + d (root wts in 0-5 cm deep soil below the litter bag) + e (soil respiration in 0-5 cm deep soil below the litter bag) + f (microbial biomass C in 0-5 cm soil below the respective litter bags) demonstrated no significant regression line ($p = 0.60$, $F_{6,16} = .779$). Therefore, no significant relationship could be found between any of the relevant measured parameters and the decomposition of slender wheatgrass stem and leaf litter over the short term (12 mo).

Sainfoin, the test legume species planted in the oil sands plots, was the second most productive plant over the short term after slender wheatgrass (Visser et al., 1984). Hence it was considered important to study the decomposition of its leaves and stems and compare the wts remaining with those of slender wheatgrass stems and leaves after 12 mo decay. A three-way ANOVA was applied to the data and the results are presented in Tables 115 and 116. As in the three-way ANOVA conducted on the grass and clover decomposition data from the subalpine spoil, there was no significant three way interaction, but significant interactions were observed between litter type and plant type and between litter type and amendments (Table 115). To locate significant differences data were analyzed by the same methods as described for the grass and clover decomposition data collected for the subalpine spoil.

Table 114. The decomposition (expressed as % wt remaining after 12 mo in the field) of slender wheatgrass stems and leaves and cellulose filter paper placed in extracted oil sands plots 28 mo after planting slender wheatgrass.¹

Litter type	Amendment				Litter type $\bar{X}$
	Control	Fertilizer	Sewage Sludge	Peat	
Leaves	46	50	46	44	46 ^a
Stems	81	75	66	72	73 ^b
Amendment $\bar{X}$	63 ^a	62 ^a	56 ^a	58 ^a	
Filter paper ²	84 ^b _{+13.9}	38 ^a _{+20.3}	70 ^{ab} _{+23.0}	75 ^{ab} _{+25.8}	

¹Leaf and stem data analyzed by two-way ANOVA (MSE = 157.4). Scheffé multiple contrasts for pairwise comparisons were applied to litter type means. Values in the litter type $\bar{X}$ column or amendment $\bar{X}$ row followed by the same letter do not differ significantly ($p \leq 0.05$).

²Filter paper data could not be included in the ANOVA due to considerable variations. Data was analyzed by multiple t-test based on the Behrens-Fisher t-distribution. Means followed by the same letter(s) do not differ significantly ($p \leq 0.05$). Standard deviations are also included.

Fig. 19 The decomposition of slender wheatgrass leaves and stems and cellulose filter paper placed in amended oil sands tailings 28 months after planting slender wheatgrass.

Table 115. Three-way ANOVA table to test the effect of litter type (i.e. stems or leaves), plant type (i.e. slender wheatgrass or sainfoin) and amendment (i.e. control, fertilizer, sewage sludge and peat) on % dry litter remaining after 12 mo incubation in the oil sands tailings plots.

Source	SS	df	MS	F	prob. ¹
Litter type	6864.78	1	6864.78	93.21	***
Plant type	6.93	1	6.93	0.09	NS
Amendment	369.68	3	123.23	1.67	NS
Litter type x plant type	3550.23	1	3550.23	48.21	***
Litter type x amendment	623.98	3	207.99	2.82	*
Plant type x amendment	268.03	3	89.34	1.21	NS
Litter type x plant type x amendment	167.50	3	55.83	0.76	NS
Error	589.63	80	73.65		
Total	17742.76	95			

¹ * - significant at $p \leq 0.05$

*** - significant at $p \leq 0.001$

NS - not significant.

Table 116. The effect of amendment on the decomposition (expressed as % dry litter remaining after 12 mo incubation) of slender wheatgrass and sainfoin litter placed in the extracted oil sands plots 28 mo after planting (extension of data presented in Table 115)¹.

Treatment	Plant or litter type	Amendment			
		Control	Fertilizer	Sewage Sludge	Peat
% dry leaf wt remaining (litter type x plant type x amendment)	Sainfoin	58	59	60	51
	Slender wheatgrass	46	41	46	44
% dry stem wt remaining (litter type x plant type x amendment)	Sainfoin	61	67	57	62
	Slender wheatgrass	81	75	66	72
% dry sainfoin and grass leaf or stem wt remaining (litter type x amendment means) ¹	Leaves (sainfoin plus grass)	52 ^{ab}	50 ^{ab}	53 ^{ab}	47 ^a
	Stems (sainfoin plus grass)	71 ^c	70 ^c	61 ^{bc}	67 ^c

(cont'd)

Table 116. (cont'd).

Treatment	Plant or litter type	% dry wt remaining (summed)	
		Leaves	Stems
% dry leaf or stem wt remaining when summed over amendments (litter type x plant type means) ¹	Sainfoin	57b	62b
	Slender wheatgrass	44a	73c

¹Means tested by Scheffé multiple contrasts for pairwise comparisons. Values in each category followed by the same letter(s) do not differ significantly ($p \leq 0.05$).

Table 116 presents a summary of the results. The main conclusions which can be drawn from the data are:

- i) the leaves of the grass and sainfoin combined decomposed faster than the grass and sainfoin stems in all but the sewage sludge treatment where no significant differences were recorded between the decay of leaves and stems.
- ii) when the decay of sainfoin leaves and stems together was compared with the decay of grass leaves and stems, no differences were detected between the plant types. The decomposition of sainfoin litter (i.e. stems and leaves) was equally fast amongst the four treatments - (peat, sewage sludge, fertilizer, control) - a result also obtained for slender wheatgrass.
- iii) slender wheatgrass leaves decayed more rapidly than sainfoin leaves, but sainfoin stems lost more wt than grass stems over the short term (12 mo). Sainfoin leaves and stems decayed equally fast; whereas slender wheatgrass stems were slower to decompose than the grass leaves.

3.2.4.7 N₂ fixation capacity of sainfoin. The N₂ (C₂H₂) fixation capacity of sainfoin at the conclusion of each of the first three growing seasons is presented in Table 117. After the first growing season, fixation potential was very low and no significant amendment effects were detected. N₂ (C₂H₂) fixation after 14 mo growth had increased but was highly variable so again no differences were recorded amongst the four treatments. At the conclusion of the third growing season however, sainfoin from the sludge treated tailing sand reduced significantly less C₂H₂ than sainfoin from the control, fertilized and peat amended plots.

No strong relationships were observed between N₂ fixation by sainfoin and soil NO₃-N, total soil N, shoot N, root N and shoot wt as determined by partial correlation coefficients (Table 118). Similar results were obtained for alsike clover in the subalpine spoil.

Table 117. N_2 (C_2H_2) fixation capacity (nmoles g^{-1} dry root hr^{-1}) of sainfoin in extracted oil sand tank plots¹.

Treatment	Time after planting		
	3 mo	14 mo	26 mo
Control	3.8 ^a	1032.0 ^a	151.5 ^a
Fertilizer	0.9 ^a	776.3 ^a	68.0 ^a
Sewage Sludge	1.5 ^a	4.7 ^a	2.4 ^b
Peat	5.6 ^a	233.0 ^a	125.1 ^a
MSE	32.52	9.81	3.04

¹Data was analyzed by a one-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. The 14 and 26 mo data required a $\ln(Y + 1)$ and $\ln$ transformation respectively. Values in each column followed by the same letter do not differ significantly ($p \leq 0.05$). The 14 and 26 mo means are geometric.

Table 118. Partial correlation coefficients for determining the relationships amongst shoot wt, root wt, shoot N, total soil N, soil $\text{NO}_3\text{-N}$ and N_2 (C_2H_2) fixation capacity for sainfoin after 1978 growing season.

	Shoot wt (g)	Root wt (g)	Shoot N (%)	Total Soil N (%)	Soil $\text{NO}_3\text{-N}$ ($\mu\text{g/g}$ dry soil)	N_2 (C_2H_2) fixation (nmoles/g root/hr)
Shoot wt (g)		.86	.41	.40	.18	-.29
Root wt (g)			.42	.67	.33	-.32
Shoot N (%)				.41	.38	-.39
Total soil N (%)					.86	-.17
Soil $\text{NO}_3\text{-N}$ ($\mu\text{g/g}$ dry soil)						
N_2 (C_2H_2) fixation (nmoles/g root/hr)						-.17

4. CONCLUSIONS AND DISCUSSION

4.1. FIELD STUDY

4.1.1 Microbial Numbers and Hyphal Lengths

With the exception of the grassland sites where numbers of bacteria in the 0-5 cm disturbed soil were greater than in the undisturbed topsoil and actinomycete numbers were as high in the subsurface (5-15 cm) minespoil as in the undisturbed grassland, there was a general decrease in bacterial and actinomycete counts and hyphal lengths as a result of mining at the various study sites. Many of the decreases for the individual microbial components were especially evident when results from the surface undisturbed soil were compared to those from the disturbed soil. Differences in the microbial numbers and hyphal lengths were not as obvious when the undisturbed subsurface (mineral) soil was compared with the mined soil. No doubt, the decreases in the microbial populations are a result of the removal of organic material from the soil during the mining process. The A_h horizon in the grasslands and the FH layers in the subalpine and oil sands forests are a major source of energy and nutrients for the microbes; also, many of the plant roots and their associated microorganisms are located in these layers. It therefore seems obvious that by stripping the soil of its organic constituents, a decrease in the microbial populations would automatically result. Data presented by Visser et al. (1984) show that, particularly in the two forest sites, mining resulted in the almost complete removal of the biologically available organic C.

Wilson (1965) and Müller (1973) working with acidic coal mine spoils also observed that bacterial and actinomycete numbers were lower in barren minespoils than in vegetated or undisturbed soils. However, both researchers came to the conclusion that the presence of vegetation was one of the most important factors in re-establishing the soil microflora. Input of organic matter, from the primary producers, through dead leaves and roots and root exudates, was probably the basis for stimulating microbial growth in

the revegetated minespoils they studied. Neither Wilson (1965) nor Müller (1973) measured a significant effect of mining and revegetation on the fungi. Their data, however, are difficult to interpret since soil dilution plates were used in their studies. As mentioned in the introduction, fungal plate counts tend to be nothing more than counts of inactive spores. In the present study, the effects of mining on the fungal component were determined by a direct observation technique, and it is obvious from the results obtained that mining significantly decreased the length of fungal hyphae (both total and that with cell contents) at two of the study sites.

Fresquez and Lindemann (1982), who studied the microbiological parameters in what appeared to be a similar spoil to the grassland spoil in this investigation, recorded significantly fewer bacteria in the barren spoil than in the undisturbed soil. As mentioned previously, bacterial numbers in the surface soil layers at the grassland site were significantly greater in the minespoil than undisturbed topsoil. One of the reasons for the discrepancy in results between the present study and that of Fresquez and Lindemann (1982) may be the differences in the ages of the spoils in the two studies. The spoil in this study was a result of mining in the late 1940's; the time elapsed since then may have given the bacterial populations a chance to increase. Also reclamation attempts of this spoil in the 1970's resulted in the luxurious growth of Kochia scoparia (L.) Schrad. The stimulation of bacterial growth may have occurred in the rhizosphere of this plant, thereby significantly increasing the numbers. Wilson (1965) showed that numbers of bacteria in the rhizosphere and on the surface of the plant roots in a vegetated spoil were greater than those on roots in a non-vegetated spoil.

In comparing the effects of soil disturbance on various groups of soil microorganisms quantified in this investigation, it must be remembered that different methods were used for each group (i.e. dilution plating for actinomycetes and bacteria, direct measurement for fungi). Because of this, direct comparisons amongst the groups may be misleading. Nevertheless, it appears that of the

three microbial groups studied, the bacteria were the most capable of surviving and growing under the low nutrient regimes and environmental extremes found in mined soils, while the fungi were the most sensitive.

4.1.2 Bacterial Community Composition

When the bacterial number data are viewed in conjunction with the data on the bacterial groups isolated from the grassland, and subalpine undisturbed and disturbed sites, it can be seen that mining resulted in a significant increase in the proportion of the bacterial community occupied by the coryneform-type bacteria. Prior to disturbance of the grassland, Bacillus and the unpigmented Gram negative rods were common in the soil, while the bacterial community in the undisturbed subalpine site was dominated by Cytophaga, Flavobacterium (organic soil only) and the unpigmented Gram negative bacteria. The unpigmented Gram negative bacteria were also the most frequently isolated bacteria from the unmined oil sands site, along with the coryneforms.

Nelson (1978a) found that when growing selected isolates of arctic soil bacteria in continuous culture under low temperature and limiting nutrient levels, a Bacillus sp. seemed the least adapted to these conditions while Arthrobacter (a coryneform-type bacterium) was capable of maintaining a viable population under this regime. She also observed (Nelson, 1978b) that Arthrobacter was resistant to freeze-thaw damage. The low nutrient and extreme temperature conditions often found in minespoils may give a competitive advantage to the coryneform group of bacteria over the possibly more susceptible Bacillus spp. and non-pigmented Gram-negative bacteria. The disappearance of Cytophaga as a result of mining at the subalpine site, may have been due to lack of moisture and organic matter in the minespoil. Cytophaga spp. are commonly isolated from very wet soils and are significant for their ability to degrade relatively decay resistant substrates such as cellulose and chitin (Christensen, 1977). If Cytophaga spp. in the organic mat of the undisturbed spruce-fir forest at the subalpine site were active in decomposing

the chitinous walls of the large fungal standing crop measured in that soil, the loss of this mycelial substrate through mining would undoubtedly decrease the frequency of Cytophaga. It is interesting to note that storage of the bacteria in semi-solid peptone-yeast extract agar at 5°C for six to eight months adversely affected the viability of isolates from the undisturbed soils (particularly the subalpine site) but not the viability of bacteria from the minespoils. This phenomenon may be an indication that bacteria from undisturbed systems are more specialized in terms of their nutrient, temperature and moisture requirements making them more sensitive to changing environmental conditions than are the less specialized, presumably less sensitive bacteria which survived in the disturbed soils.

4.1.3 Fungal Community Composition

Studies which have dealt with the effects of mining on the fungal genera have shown that in general the diversity of fungal taxa in barren minespoils is less than that in undisturbed soil (Lawrey, 1977; Fresquez and Lindemann, 1982). However, these investigations have demonstrated that the incorporation of organic amendments (hay, sewage sludge) or the evolution of a vegetative cover on the minespoil will increase the fungal taxa diversity, inferring that it is the lack of organic matter which limits the number of fungal genera in minespoils.

In the present study, each of the three minespoils was different in terms of its fungal generic distribution. At the grassland site, the spoil contained a large number of fungal taxa with the dominant forms being Cladosporium spp., Alternaria spp., Penicillium spp., sterile dark forms and yeasts. Cladosporium, Alternaria and yeasts are widely distributed and commonly colonize leaf litter and soil (see reviews of first two genera in Domsch et al., 1980). They are frequently isolated from the atmosphere, hence would be the first group of organisms to have the opportunity to colonize the surface of recently mined soil. Penicillium spp., a ubiquitous group of saprophytes which have the capability to utilize

a wide variety of substrates and can survive such extreme environmental conditions as low water potentials and high temperatures (see review of this taxon in Domsch et al., 1980), were the most frequently isolated fungi from the subsurface minespoil. The number of plated soil particles which yielded fungi was approximately the same for the undisturbed and disturbed sites, indicating that the grassland minespoil was well-colonized.

The diversity of fungal taxa isolated from the disturbed subalpine soil was not as great as that observed in the undisturbed spoil. Also, with the exception of Candida spp. and Chrysosporium spp. many of the fungi in the minespoil occurred with a very low frequency. Lack of organic matter, moisture and nutrient stress in the minespoil would be the major factors explaining these data. Interestingly, mining at the grassland site resulted in a significant decrease in Chrysosporium-Pseudogymnoascus, but at the subalpine site, the occurrence of Chrysosporium (mainly C. pannorum) increased significantly after mining. The increased frequency of C. pannorum in the subalpine minespoil, may be a result of the rise in soil pH after mining (pH 4.2-4.5 in undisturbed soil and 7.0-7.3 in mined soil) since this organism appears to prefer soils of a neutral pH (see review of this species in Domsch et al., 1980). Also laboratory studies of substrate utilization by this fungus have demonstrated that this organism is capable of utilizing a wide variety of substrates (starch, pectin, cellulose, chitin, tannins). This capability may give Chrysosporium pannorum a competitive advantage over other fungi, hence its frequency in the nutrient impoverished conditions of the spoil. Only one other study has recorded a Chrysosporium sp. from minespoils. Evans (1971) isolated a thermophilous Chrysosporium from coal spoil tips in England. The preference of Chrysosporium for a neutral pH would explain why this fungus has not been isolated from acidic minespoils in the eastern U.S.

In contrast to the grassland spoil, less than 50% of the subalpine minespoil particles yielded fungi. The older age of the grasslands spoil may have allowed the fungi to colonize that spoil to

a greater extent than the subalpine spoil. The subalpine spoil had been spread immediately prior to sampling. It should be noted that the fungi isolated from the stockpiled regolith were slightly different from those isolated from the disturbed plot (i.e. in addition to Chrysosporium, Phialophora sp. and Acremonium spp. were dominant forms). Also the number of soil particles yielding fungi was not substantially different from that observed for the undisturbed soils. This may infer that over the period of stockpiling, the fungal growth within the stockpiled soil decreases, while the fungal community shifts to one dominated by Chrysosporium pannorum and the yeast, Candida.

The harsh methods used to remove oil from the oil sands results in a fine-grained sand (86% of the grains fall in the 125-500 μm size range) which is devoid of organic matter and nutrients, and mycologically sterile. Fungal isolations made from the extracted sand, demonstrated that only the sand in contact with the atmosphere was colonized to any extent by fungi - these fungi consisting mainly of Cladosporium spp. and yeasts, organisms which are commonly transported through the atmosphere as mentioned previously. The poor fungal colonization of the extracted sand was further substantiated by the low number of plated particles which yielded fungi. It is obvious from these results that a highly organic amendment would be required to re-establish the fungal community in the sand tailings to a level where it would eventually attain the diversity observed in the undisturbed soils.

In general, the fungal isolation data demonstrated that the mining procedures and the age of the spoil could have a significant effect on the fungal community and its growth in the spoil. It appears that in spoils where the indigenous fungi are lacking or have been completely destroyed during mining, fungi common in the atmosphere (Cladosporium, Alternaria and yeasts) are the first colonizers, particularly in the surface minespoil.

It can be concluded from the microbial population data, that the minespoils at the three study sites exhibited a lower bacterial and actinomycete count and less fungal hyphae than that

measured in nearby undisturbed areas. One exception to this was the grassland site where numbers of bacteria were greater in the disturbed than undisturbed soil. Also mining resulted in major shifts in the structure of both the bacterial and fungal communities. After mining, the bacterial community in both the grassland and subalpine areas was dominated by coryneforms, while the fungi occurring with the greatest frequency were organisms common in the atmosphere or in the case of the subalpine spoil, Chrysosporium and Candida.

4.1.4 Soil Respiration, Microbial Biomass C and ATP

Although the microbial population data infers that mining results in reduced microbial activity, plate counts and hyphal length measurements give very little indication of the activity of the isolated organisms when in the soil. Bacterial and actinomycete plate counts can include both active, vegetative cells and inactive spores, while the presence of protoplasm in fungal hyphae at the time of sampling does not necessarily mean the mycelium is active, but rather has a potential for being active. Also, the type of media used to isolate bacteria or actinomycetes may select only a certain proportion of the community, while major difficulties are encountered in the separation of mycelium from the soil (particularly in highly organic or clayey soils). As a result, plate counts and hyphal length measurements should be coupled with soil respiration or ATP measurements in an effort to more accurately estimate the level of activity exhibited by both isolated and nonisolated organisms.

The measures of microbial activity incorporated in this investigation included soil respiration (CO_2^+), microbial biomass C and ATP. With the exception of the oil sands site, where no significant differences in CO_2 efflux were encountered between undisturbed and disturbed soil, all three parameters were significantly lower in the minespoil than in the soil from the adjacent undisturbed areas. Plate counts of bacteria and actinomycetes and fungal hyphal measurements supported these results. No doubt the decrease in microbial activity in the mined soil was

mainly due to the lack of an organically rich surface layer (with its associated plant component, more favorable nutrient levels and more active microbial system), although the high Na levels in the grassland spoil may also have contributed to inhibiting microbial activity. Malik and Azam (1980) have reported a decrease in soil microbial biomass with increasing salinity. Hedrick and Wilson (1956) also observed a lower rate of CO₂ efflux in strip-mined spoils than in undisturbed soils and attributed this mainly to a lack of sufficient N in the spoil. Lawrey (1977b) recorded lower respiratory activity in mined soils than in undisturbed soils and considered that the high levels of trace metals made available by the low soil pH contributed to the reduced microbial activity of the strip-mined soils. Recently, Stroo and Jencks (1982) concluded from their study on enzyme activity and respiration in minesoils at various stages of reclamation, that as organic matter and N accumulated in a spoil, microbial activity would slowly recover.

ATP measurements have been considered by Hersman and Temple (1979) as one of the best and most rapid methods for determining biological activity in minespoils. Their results (Hersman and Temple, 1978), like those obtained in this study, demonstrated that ATP levels in undisturbed soils were higher than those in minespoils. If one has access to the equipment required for assaying ATP, this method may be rapid, but in the present study, ATP data was highly variable. Also, ATP in the extracted oil sand was so low that it was not measureable although some microbial biomass was measured in these samples using the physiological method of Anderson and Domsch (1978). Unsuitable ATP extraction techniques and the analysis of small quantities of soil (1 g in this study) probably contributed to the variation observed.

4.1.5 Comparison of Methods for Estimating Microbial Biomass C

Because microbial biomass C, ATP, bacterial and actinomycete numbers and fungal lengths are all quantitative measurements of microbial material in a soil, it was decided to compare them via Pearson product moment correlations. Prior to

performing the correlations, all quantitative microbial estimates were converted to mg microbial biomass C 100 g⁻¹ dry soil. To achieve this, the conversion factors used by Domsch et al. (1979) in their study comparing methods for soil microbial population and biomass studies, were employed. Correlation coefficients for data from the oil sands site could not be calculated because of missing data and the presence of zeros. For the grassland disturbed and undisturbed soil, the direct observation method (which included dilution plate counts of bacteria and actinomycetes and total hyphal lengths) had correlation coefficients of -0.53 and 0.37 with the microbial biomass C (physiological technique) and ATP techniques respectively. A correlation of 0.16 was found between the ATP and microbial biomass C measurements. In contrast to the poor relationship amongst the techniques when applied to the grassland soil, correlation coefficients calculated for the subalpine soil data demonstrated a much closer relationship (direct observation method had correlation coefficients of 0.84 and 0.97 with the physiological and ATP techniques, respectively while ATP and the physiological techniques had a correlation coefficient of 0.81). Since a small number (12) of samples was used to calculate each correlation matrix only coefficients greater than 0.85 should be regarded as meaningfully significant. Some of the possible reasons for the differences in biomass C correlations between the grassland and subalpine soils and the general lack of a relationship amongst biomass values obtained by the different methods are:

1. as mentioned previously, dilution plates may be selective for a certain component of the bacterial community and therefore may underestimate the bacteria present in a particular soil. They can also overestimate the bacterial population in the vegetative phase if the bacteria are present mainly as spores. Actinomycete biomass values are probably underestimated by the dilution plate technique since they have a filamentous growth form.

2. soils high in clay, such as the grassland minespoil, may make it difficult to separate the fungal hyphae from the soil. In this study, the presence of clay in the agar films, was

responsible for a considerable amount of masking making it difficult to recognize the hyphae and therefore leading to underestimates of the fungal biomass. Masking by clay particles was not a problem in the subalpine soils, hence the hyphal lengths measured in these soils would be more accurate than those measured in the clayey, fine-textured grassland soil. The hyphae constituted approximately 90-95% of the bacterial, fungal biomass C in both the subalpine and grassland soils. Therefore an underestimate of the biomass C in the fungal component would severely affect correlations between biomass calculated from direct measurements and biomass measured by the physiological technique. On the other hand, total hyphal lengths were converted to biomass C prior to calculating the correlation coefficients. Slow decomposition rates of dead hyphae (which would be included in the total hyphae category) could result in an overestimate of the active fungal biomass calculated from direct measurements.

3. dilution plate counts of bacteria and agar film estimates of hyphae, when converted to biomass C, may be subject to major errors due to the inaccuracies in the conversion factors (e.g. cell diameter, density of cells, moisture content of cells). Van Veen and Paul (1979) pointed out some of the problems associated with the conversion factors necessary to convert bacterial counts and hyphal lengths to biomass.

4. ATP values can be underestimates as a result of inefficient extraction from clayey or highly organic soils or due to the high quench of ATP by the soils under investigation. These factors may have affected the ATP levels measured in the grassland soils and the subalpine minespoil since microbial biomass estimated from the direct observation and ATP methods were very different in these particular soils. Because the physical/chemical properties of soils can vary considerably, preliminary research to determine the most efficient extraction techniques and the ATP quenching properties of the soil extracts would be required to improve the accuracy of ATP estimates for determining microbial biomass C in different soil types. In addition, Domsch et al. (1979) emphasized that ATP assays

are difficult to convert to microbial biomass, and therefore the conversion factors are, at best, very crude. Greaves et al. (1973) in a study dealing with the relationship between microbial populations and ATP in a basin peat observed that bacteria in peat demonstrated considerable variation in their ATP contents and concluded that ATP determinations could not be used as biomass measurements. ATP determinations could, however, provide useful information on the metabolic state of microbial populations.

5. direct observation, cultural methods and ATP assays are generally performed on very small quantities of soil (usually less than 5 g). Because of the heterogeneous distribution of soil microorganisms this can lead to great variability in biomass values obtained on replicate samples by these techniques. Studies to determine optimum sample size and soil dilution would aid in decreasing this variation.

For these reasons it appears that the cultural methods, direct observations and ATP assays have major weaknesses if one wants to extrapolate the results obtained from these techniques to microbial biomass. It is the belief of the authors that the more direct physiological technique developed by Anderson and Domsch (1978) is a much more efficient and accurate method for estimating the microbial biomass C in the vastly different minespoils and soil types encountered in the present study. This method has the advantage of rapidly providing reproducible biomass values, which because of the large sample size utilized (usually 100 g/sample) exhibit much less variability than that calculated from the techniques mentioned previously. The physiological technique also allows one to measure biomass under standard temperature, moisture and substrate quality (optimum quantities of glucose are added to each soil) conditions, thereby substantially reducing the variability resulting from these three variables. This is particularly important if one is interested in comparing soils with very different chemical/physical properties. However, it should be noted that this technique is based on the conversion of 1 ml CO₂ to 40 mg microbial biomass C - a factor based primarily on biomass estimates obtained

for agricultural soils. Therefore it may not be precisely applicable to other soil types. More research is required to determine the conversion factors for soils other than agricultural soils. Nevertheless, this method has proved to be quick, efficient and sensitive enough to measure small shifts in microbial biomass and is, therefore recommended for future studies particularly with clayey soils. Microbial activity would probably be most easily and accurately determined by measuring CO₂ efflux or O₂ uptake on reasonably large soil samples. Where detailed qualitative and quantitative information is required for the individual bacterial and fungal components of the biomass, one must still resort to cultural and direct observation methods.

4.1.6 Asymbiotic N₂ Fixation

Since N is generally accepted to be lacking in minespoils, N₂ fixation by heterotrophic bacteria could be a means of increasing N levels in the spoils. Data collected in this study, however, demonstrated that the grassland minespoil exhibited no N₂ (C₂H₂) fixation potential, while the subalpine minespoil fixed negligible quantities of N under the conditions laid down in this investigation. One of the major factors controlling asymbiotic N₂ fixation is an adequate supply of C to provide energy for N₂ fixing bacteria. The lack of easily available C (in the form of organic amendments or root exudates and plant litter) in minespoils would severely limit their N₂ fixation potential. Fresquez and Lindemann (1982) recorded no Azotobacter (an asymbiotic N₂ fixing bacterium) in the coal minespoils they studied, but amendment of the minespoil with alfalfa hay, fertilizer and topsoil significantly increased Azotobacter levels particularly in the rhizosphere of their test plant, Atriplex canescens. These results emphasize the importance of an adequate C supply for the growth of the asymbiotic N₂ fixing bacteria. Also, Knowles (1977) drew attention to the fact that the rhizoplane-rhizosphere region of plants (particularly in plants which are highly productive) appears to have a high potential for N₂ fixation since many readily available C substrates (sloughed roots,

root exudates) occur there. Therefore, if the input of N through N_2 fixation is to be considered important for soils disturbed by mining, the application of a highly organic amendment and/or the planting of highly productive plant species would be a necessary requirement.

Skeffington and Bradshaw (1980) have suggested that the rates of N_2 fixation by free-living bacteria in china clay wastes are so low that their contribution to N accumulation in this particular spoil is probably negligible. One reason why this may be so is the lack of efficiency exhibited by the bacteria when fixing N. Gray and Williams (1971) presented an example where, on the basis of a requirement of 1 g of sugar to fix 5-20 mg N, Azotobacter would require 1122 kg of organic matter per year to fix 5-9 kg N/ha. This would imply a very high cell turnover rate, which according to these authors would be impossible due to the lack of available organic substrate for growth. Although Knowles (1977) has suggested that efficiencies of N_2 fixation in the soil may be 2-3 times greater than that suggested above, it would still seem that soil N accumulation by asymbiotic bacteria (particularly in minespoils) would be almost negligible.

The undisturbed soils, which contained more microbiologically available organic matter than their respective minespoils, had surprisingly low N_2 fixation potentials. This was especially true for the undisturbed grassland samples. Paul et al. (1971) in their studies on N_2 fixation in virgin grasslands in Saskatchewan, obtained similar results. Like Skeffington and Bradshaw (1980), they considered N inputs via rain and nodulated plants to be more important contributors of N. The low asymbiotic N_2 fixation by the soils they were studying was believed to be because the virgin grassland systems are in equilibrium, so N-loss mechanisms play a minor role in the system and N input requirements are, therefore, low. This theory may also apply to the grassland soil investigated in this study.

4.1.7 Decomposition Processes

The evolution of a self-maintaining vegetative cover on minespoils is ultimately dependent on the establishment of the decomposition and mineralization processes, since it is these processes which will ensure a continual nutrient supply to the primary producers. In the present investigation it was hoped that the decay rates of the standard substrates, cellulose filter paper and fir wood dowel, in both undisturbed and disturbed systems would provide comparative data of the effects of mining on the decomposition of relatively easily degradable (cellulose) and recalcitrant (wood) C resources.

4.1.7.1 Standard substrates (filter paper and wood dowel). The use of a woody substrate proved to be a poor indicator of the effects of mining on the decay of a lignified substrate, since very little weight loss occurred at either the grassland or subalpine sites over the course of the study. No differences were, therefore, detected between undisturbed and disturbed sites. Bunnell et al. (1977a, b) found that when modelling the effects of various variables on microbial respiration and substrate weight loss, the initial changes occurring in substrate weight resulted from microbial respiration rates which were 'chemical specific' and independently influenced by temperature and moisture levels. It is well recognized that woody substrates are comprised mainly of cellulose and lignin and are notorious for their high "recalcitrant C" and low N levels (the wood in this study contained .03% N). Hence, a nutrient-poor and decay resistant substrate such as this would provide very few readily available nutrients for microbial growth and therefore decompose very slowly. In an effort to remedy the high C/N ratio, wood dowel was amended with ammonium nitrate, but this did not hasten its decomposition. It is possible that much of the added N leached out of the wood before the microorganisms could utilize it or that the presence of a high level of ammonium N inhibited microbial growth. Kowalenko et al. (1978) recorded a reduction of soil microbial activity in the presence of N fertilization and attributed their

results mainly to the lowering of soil pH. Other studies (Pavlica et al. 1977; Setua and Samaddar, 1980) have revealed that ammonia can behave as a volatile inhibitor of fungal spore germination. There is also a probability that the decay resistant quality of the C in lignified substrates would control the decomposition rate of wood to a greater degree than would the levels of other nutrients. Also, the very dry conditions (total annual moisture = 335 mm) and the extremes in temperature occurring at the grassland site are not conducive to rapid microbial growth and turnover. Low soil temperatures, characteristic of soils such as those in the subalpine site, have also been regarded as a major factor in the inhibition of the decay process (Moore, 1981).

In contrast to the wood dowel, decomposition of the cellulosic filter paper proceeded at a more rapid rate. Short term differences in cellulose decomposition were not detected at any of the three sites, but over the long term (i.e. 24-33 mo) filters placed in the grassland and subalpine study areas exhibited a faster decay rate in the disturbed than undisturbed plots. A possible explanation for the results obtained at the grassland site, is that heavy spring rains (which occurred approx. 20-24 mo after the initiation of the study) resulted in increased runoff caused by the high sodium adsorption ratio of the spoil - a factor which can restrict water infiltration into the soil (Power et al., 1978). The runoff embedded and sometimes buried the filters in the spoil resulting in ameliorated microenvironmental conditions around the filters with moisture conditions being greatly improved. Once buried, the filters decayed rapidly so that 44 mo after the initiation of the study, virtually all the cellulose had been utilized.

Filters placed in the undisturbed grassland, however, were not readily incorporated into the soil matrix. The erosion observed in the disturbed plot was absent in the undisturbed plot and the grassland vegetation would occasionally elevate the filters above the soil surface. Therefore, the filters were more subject to moisture and temperature extremes resulting in reduced decomposition. Similar observations were made at the subalpine study area. Again filters

placed in the minespoil were eventually buried, while those in the undisturbed forest remained on the surface for the full term of the study. The incorporation of the filters into the forest floor appeared to be largely dependent on the growth of the feather moss (Hylocomium splendens) over the bag containing the filter. Once overgrown by the moss, the ameliorated moisture and temperature conditions would probably accelerate decay. The results obtained in this study imply that the instability of the minespoil can greatly influence the decomposition of a substrate by increasing its chances of becoming buried. Burial can protect the decomposing substrate from temperature and moisture extremes - variables which are major controlling factors affecting decay rates.

The rapid decomposition of the disturbed site filters, once buried, is interesting when viewed in conjunction with the microbial activity and biomass data discussed earlier. Although CO₂ efflux and microbial biomass C measurements were significantly lower in the disturbed than undisturbed soils from both the grassland and subalpine sites, cellulose degradation nevertheless, proceeded rapidly in the disturbed soil. One explanation for this phenomenon is that the very low available C status in the minespoils affected the microbial biomass, but once an easily available C source such as cellulose was added to such an impoverished system, the soil microorganisms (both in active and resting phases) were capable of rapidly utilizing it. Laboratory studies have confirmed that many of the fungi isolated from the disturbed soil produce cellulases (e.g. Chrysosporium-Pseudogymnoascus, Fusarium, Alternaria). Therefore, the potential for cellulose degradation was present in the grassland and subalpine minespoils. In fact, it is possible that due to the lack of available C in disturbed soils, decomposition may be more rapid than in undisturbed soils where other forms of C are available. Although both the filter paper and the minespoil generally lack N, amendment of the filters with ammonium nitrate did not accelerate their decay, presumably for the same reasons mentioned previously for the wood dowel. Also, the presence of a readily available C source such as cellulose may regulate decomposition in minespoils to a

greater degree than mineral nutrients, which were present in low quantities in the two spoil types.

The relationship between decomposition of cellulosic filter paper and that of natural litter is questionable. Natural litter and filters placed out in the undisturbed study areas demonstrated that filters decayed slowly in comparison with grass litter at the grassland site, filters decayed at the same rate as fir needles at the subalpine site, while filters placed in the undisturbed oil sands plot decomposed more rapidly than jack pine needles. Therefore, for undisturbed systems it is difficult to generalize about the relationship between the decomposition of filters and of natural litter, since the relationship appears to be site specific and is probably dependent on the litter type characteristics, climate, and the speed at which decomposing substrates are incorporated into the soil system.

Ross et al. (1978), investigating the decomposition of cellulose squares and tussock grass litter in New Zealand, made similar observations when he found that the weight losses of cellulose squares placed on the soil surface were generally correlated positively, but not significantly with total weight loss of tussock grass litter and its cellulose component. These authors also noted that variability amongst replicates of the cellulose squares was high and that microenvironment was important in determining decomposition rates. Filter paper weight loss in the present study was also highly variable, particularly in the disturbed sites. A higher degree of variability amongst weight losses of filter paper versus weight losses of native herbage was also measured by Ratliff (1980) in meadow sites in California. Lack of an adequate supply of essential nutrients in the filter substrates could possibly explain some of the variation, since microbial utilization of the cellulose would be somewhat dependent on the nutrient status of the soil under or surrounding the filter. Park (1976) emphasized the importance of mineral nutrients (particularly N) as one of the variables affecting the rate of decay of such substrates as cellulose. Natural plant litter would have a more balanced

nutritional status than pure substrates such as cellulose, making the litter more amenable as a food resource for the soil microbes.

There have been few studies to determine the decomposition potential of soils disturbed by surface mining. Lawrey (1977) investigated the relative decomposition potential of strip-mined and undisturbed areas in eastern Ohio. His results showed that decomposition of Pinus, Robinia and Acer leaf litter was initially retarded in soils affected by mining, but after six months there were no significant differences in weight loss of litter between mined and unmined areas. Decay times for litter placed on strip mines in Missouri were reported to be two to 10 times less than those for litter placed on vegetated soils (Carrel et al., 1979). For reasons discussed in the previous paragraph, the filter paper decay data collected for minespoils in this study are difficult to compare with the decomposition rates of natural litter measured by other investigators. Although filters degraded more rapidly over the long term in the mined areas at both the grassland and subalpine sites, it would be dangerous to assume that similar results would have been obtained if natural litter had been tested. Due to the high degree of variation in filter paper weight loss and the lack of relationship between filter decay and natural litter decay, it is suggested that in the future a litter rather than pure cellulose substrate be utilized to study the effects of disturbance such as mining on the general decomposition process. Grass litter produced under standard, light, moisture and nutrient conditions in a greenhouse may be a reasonable alternative.

4.1.7.2 Native plant residues (leaf litter and branchwood). It was considered important to determine the rate of disappearance of native litter and wood in the undisturbed sites, since this type of data would provide a useful baseline if and when the adjacent disturbed systems were eventually reclaimed to a level of productivity similar to that in the undisturbed ecosystems. Decay of native substrates were not followed in the minespoil, since revegetation practices have, to date, emphasized the use of fertilizers and fast growing

grass and herb species, resulting in the production of litter whose chemical quality is markedly different from that found in the undisturbed sites. Aside from the abiotic factors (i.e. temperature and moisture) chemical quality of the litter is the main variable influencing rates of decomposition (Bunnell et al., 1977a, b).

The percent dry weight remaining of wheatgrass litter decreased linearly over the first 24 mo of decomposition and then levelled off. This pattern of decay can be explained by the initial rapid loss of the easily degraded constituents of the grass such as soluble sugars, polysaccharides and cellulose followed by the much slower decay of the more recalcitrant components such as lignin. Swift et al. (1979) reviewed a number of studies which demonstrated that as the decomposition of various litter types progressed the decay resistant hemicellulose and lignin fractions tended to accumulate resulting in reduced decomposition.

The weight loss of the grass over the first 12 mo (39%) of this study was very similar to that recorded by Abouguendia and Whitman (1979) for wheatgrass placed in a mixed grass prairie in North Dakota. After 392 days of decomposition, they recorded a weight loss of 39.7%. Much of this weight loss can probably be attributed to a combination of leaching losses and the rapid microbial utilization of the water soluble constituents of the substrate. An estimate of the water soluble component of the Agropyron leaf litter was obtained from a leaching study performed under laboratory conditions. Weight losses ranged from 16.2% after 1 day of leaching to 30.8% after 10 da leaching. Therefore, the weight loss over the first year can be largely accounted for by the disappearance (through leaching or rapid microbial utilization) of the water soluble fraction of the substrate. It is doubtful that leaching would play a major role in the loss of the water soluble component, since the study area receives very little precipitation. In fact, decay of native substrates is probably severely limited by the lack of moisture characteristic of the semi-arid mixed grass prairie.

Turnover time of the mixed grasses was calculated by Abouguendia and Whitman to be 3 yr using data collected by the litter bag technique. Decay rates measured in this study suggest that the turnover time of Agropyron leaf litter is much longer. Enclosing the litter in a mesh bag may have reduced the rate at which it was incorporated into the soil once fragmentation of the leaves had occurred. It is highly likely that the presence of the mesh bag may have reduced decomposition in the same manner as that described for the filter paper.

The rate of disappearance of wood in mixed grass prairie sites has not received any attention, mainly because woody substrates form an insignificant proportion of the aboveground biomass. However, sagebrush occurs regularly on the grassland study site, and appeared to be particularly common where grazing pressure was high. Since the stems of sagebrush tend to be very woody, it was decided that a decay study of these stems would provide some indication of the potential of the grassland system to degrade a woody substrate. Weight loss from the stems was extremely slow with 88% of the stems still remaining after almost 4 yr of decomposition. Compared with the grass, the water soluble component formed a minor portion (2.6-6%) of the total substrate weight, hence the initial rapid decay observed for the grass litter would not be expected during the decomposition of the woody substrate. The faster decomposition of grass litter compared with the woody sagebrush stems can also be explained by the generally higher C/N ratio and lignin content of wood. Also, sagebrush synthesizes aromatic compounds which may behave as toxic inhibitors of microbial activity, thereby reducing decomposition. Swift et al. (1979) have termed compounds such as these, "modifiers", because they affect microbial activity by different mechanisms than those observed for C or nutrient sources. Other factors which would contribute to the decay resistance characteristic of wood, includes the physical nature of wood which because of its density is not conducive to rapid microbial growth and the lack of invertebrate activity. The ability of invertebrates to

commminute a resource and disperse microbial propagules could stimulate microbial growth; thereby accelerating decay rates.

The degradation of fir needles in the subalpine spruce-fir forest, did not proceed at a very rapid rate with the bulk of the needles (67%) still remaining after 4 yr of decomposition. Fogel and Cromack (1976) studied the decay of the similar Douglas-fir needle litter at sites in Oregon and measured approximately 65% remaining after 2 yr in the field. The faster decomposition of Douglas-fir needles compared with the decay rates of the subalpine fir needles in this study are probably the result of climatic differences with more amenable moisture and temperature conditions accelerating the disappearance of fir needles in the Oregon study. Aside from the inhibitory effects the cool temperatures characteristic of the subalpine site can have on the decay process, the other factors which would possibly account for the relatively slow disappearance of the subalpine fir needles include:

- i) a possibly higher C/N ratio and lignin content in fir needles than that in herb or grass litter,
- ii) a lower proportion of easily degraded water soluble constituents. Leaching losses from fir needles ranged from 3-7% after 1 da submersion in water to 17.6% after 10 da submersion,
- iii) the presence of a wax layer on the needles which can restrict entry of water and decomposers into the needle interior,
- iv) the presence of a high level of polyphenols in the needles.

As was mentioned previously for the filter paper placed in the undisturbed spruce-fir site, the incorporation of the decaying needles into the litter/soil system was very slow and appeared to occur only when the feather moss had overgrown the litter bag. Since measurement of the rate of decay of needle litter is still in progress, it will be interesting to see if decay accelerates once the bagged needles become part of the moss/litter layer.

The decomposition of subalpine fir branch wood was very much slower than that of the fir needles - an observation also made by Fogel and Cromack (1977) for Douglas-fir branch wood. As

discussed for the sagebrush stem decay data, the differences observed between the disappearance of litter and wood, are mainly a product of differences in resource quality, particularly in lignin content. Fogel and Cromack (1977) concluded from their study that annual weight loss of the various litter types was more influenced by lignin content than C/N ratio. They measured a lignin content of 21.8% in the needles vs. 43.4% in the branch wood which would explain why the branch wood in their study was more resistant to decay.

Weight loss of bagged jack pine needles was highly variable amongst the five sampling times. This variability was mainly a result of the inefficient removal of sand from the surfaces of the needles. Since the needles were not ashed, weight gains such as that recorded between 24 and 33 mo resulted. The adhesion of sand to the litter would also cause an underestimation of the decay rate of the needles. Litter bags containing the needles were placed under the lichen (Cladina mitis) mat since the lichen would have restricted the movement of the bag into the sand beneath. As a comparison, the decomposition of jack pine needle fascicles strung on nylon thread and placed within the lichen mat was also investigated. Over the first 24 mo of decay the strung needles decomposed more slowly than those in bags below the lichen, mainly because the strung needles were more subject to extremes in temperature and moisture. Weight loss of the strung and bagged pine needle litter was approximately 16% and 22% respectively after 12 mo compared with 30% obtained for jack pine needles by Maclean and Wein (1978) in a forest stand in New Brunswick. Higher precipitation experienced by the New Brunswick study areas would account for the faster disappearance of jack pine needles than that measured at the oil sands site. Also, the absence of a lichen mat in the New Brunswick jack pine stands could speed up the rate at which incoming litter is incorporated into the litter soil system - a factor which may enhance decay rates. The relatively slow disappearance of jack pine needles in comparison with that of wheatgrass at the grassland site is similar to that observed for fir needles at the subalpine site. It is possible that the same resource

quality factors reducing decay of the fir needles also reduce decay of the jack pine needles.

Maclean and Wein (1978) reported weight losses of 6-10% after 12 mo and 7-15% after 24 mo for jack pine branches placed in various tree stands in New Brunswick. Similar data were obtained for jack pine branchwood in this study with 8% wt loss after 12 mo and 12% after 24 mo. Interestingly the rate of decay of the jack pine branch wood over the first 24 mo of the study was approximately the same as that measured for the jack pine needles. One explanation for this observation is that moisture is the predominant variable controlling decomposition at the oil sands study area. Until moisture conditions are more conducive to microbial growth (i.e. when the needles or branches are well embedded in the lichen mat or have worked their way into the soil beneath the lichen), resource quality plays a very minor role in the decay process. More research is required to establish the importance of the moisture variable relative to the temperature and resource quality variables in the decomposition process occurring in jack pine stands at the oil sands site.

4.1.8 Organic matter accumulation and development of microbial activity and biomass. Since microorganisms require C more than any other nutrient for their growth and tissue synthesis, one would expect that as revegetation of a minespoil progresses and organic matter accumulates, microbial activity and biomass C would increase accordingly. But, with the exception of Stroo and Jencks (1982), very few studies have been conducted on the development of microbial activity in minespoils as soil genesis progresses. Stroo and Jencks observed that microbial activity in minespoils in W. Virginia increased as the age of the revegetated minespoil increased and concluded that recovery was related to organic matter and N levels. In fact, respiratory activity was highly correlated with biologically available C and mineralizable N. Also, microbial respiration in unamended sites revegetated for 20 yr approached that in the native soils. In the present study, microbial respiration, biomass C and

organic matter levels in the subalpine minesite all demonstrated increasing trends as the age of the minespoil increased. It is worthwhile noting that CO_2 evolution, biomass C and organic matter levels were not significantly different in the undisturbed mineral soil and stockpiled regolith which infers that it is the removal of the vegetation and organic matter and not the physical disturbance of the soil which controls the microbial activity in the soil after mining.

Plant growth in a minespoil results in the accumulation of organic matter as the products of primary productivity (shoots and roots and root exudates) die and are decomposed by the soil microbial component. The rate of organic matter accumulation will be determined largely by plant species, plant productivity and the rate of decay of incoming plant litter. The significant increase in organic matter between the 6 and 7 yr old sites is difficult to explain. A detailed history of each site was not available so it is possible that different fertilizer regimes and the seeding of different plant mixtures may have influenced the rates of organic matter accumulation in the 6 and 7 yr old sites. Although microbial activity and biomass C in the 7 yr old site was significantly greater than that measured in other other sites, it was still substantially less than that in the undisturbed organic mat. These observations suggest that organic matter accumulation on the subalpine minespoil is a very slow process, a conclusion also drawn by Wilson (1965) for vegetated spoils in West Virginia.

Like Stroo and Jencks (1982), a highly significant relationship was detected between microbial biomass C and percent organic matter. A regression analysis performed on biomass C and organic matter measurements from the disturbed soils only demonstrated a strong relationship between the two variables but not as strong as that detected when measurements from the undisturbed soils were included. It is considered necessary to analyze more soil samples (particularly those with organic matter ranging from 40 to 80%) before these regressions can be utilized as predictive models.

4.2. TANK STUDY

4.2.1 Rationale for the Tank Study

The evidence provided by the field study demonstrated conclusively that mining disturbance at all three study sites had an adverse effect on soil microbial activity, particularly when the surface disturbed and undisturbed soil layers were compared. Associated with the decrease in microbial activity in the minespoils was a reduction in bacterial, actinomycete numbers (with the exception of the grasslands site) and hyphal lengths and major shifts in the bacterial and fungal community structures. The loss of much of the soil organic matter during the mining process, was postulated as being the main factor affecting soil microbial activity and community dynamics since it is the organic matter which serves as the energy source for microbial growth. Also root growth tends to be concentrated in the organic horizon of the soil profile (grassland and subalpine sites in particular), hence the destruction of this horizon would eliminate the microbial activity associated with the roots (rhizoplane, rhizosphere, mycorrhizae). Therefore, if the re-establishment of microbial activity is to be considered an essential step in the successful reclamation of a minespoil, the presence of organic matter (barring the spoil has no extreme chemical/physical problems) would be an absolute necessity. The organic matter levels in a minespoil can be raised by establishing a self-maintaining vegetative cover (which would ultimately result in the accumulation of organic matter through the decomposition of plant residues) or by amelioration with an organic amendment and subsequent revegetation.

In the present study, it was hypothesized that the incorporation of an organic amendment into a minespoil would not only serve to re-establish soil microbial activity, but would ultimately result in the more rapid establishment of a stable vegetative cover. Consequently, a large quantity of minespoil from each of the three study areas was transported to Calgary, where it was amended, planted and monitored regularly for such parameters as plant success and

growth, development of microbial activity and biomass C and decomposition potential. The amendments which were tested on the grassland spoil were gypsum, sewage sludge and topsoil while those applied to the subalpine and oil sands spoil included fertilizer, sewage sludge and feather moss peat. Details of the tank design, application rates of the amendments and the plant species tested have been outlined in Visser et al. (1984).

4.2.2 Microbial Characteristics of the Individual Amendments

Of the five amendments, the anaerobically digested sewage sludge exhibited the highest microbial activity and ATP content explained mainly by the nutrient-rich composition of the sludge. The lack of biological activity in the fertilizer and gypsum was to be expected since these amendments tend to be low in moisture, highly acidic with very little biologically available carbon and potentially toxic levels of some nutrients (e.g. ammonium N in the fertilizer). Microbial respiration, numbers of bacteria and hyphal lengths in the peat and topsoil were substantially greater than that measured in the fertilizer and gypsum, but not as high as that measured in the sewage sludge presumably because the nutrient status of the sludge was better than that of the peat and topsoil (see Table 6a, Visser et al., 1984). Only the peat and topsoil harbored substantial numbers of actinomycetes. The microbial and nutrient composition of the various amendments would infer that microbial activity, bacterial numbers and hyphal lengths in the various minespoils would over the short term be most stimulated by the addition of sewage sludge and least by the gypsum and fertilizer. However, this was not the case in this study since sewage sludge was added to the minespoils at a very low rate (46 mT ha^{-1}) resulting in a dilution of the microbial inoculum and nutrients within the soil matrix. In contrast, topsoil and peat were applied as 15 cm layers resulting in the application of a heavy dose of microbial inoculum to the minespoils in the case of these two amendments.

It is worthwhile noting at this point, that the sewage sludge was mixed into the soil after it had been allowed to dry on

the soil surface. The nature of the dried sludge caused great difficulty in establishing a homogeneous sludge soil mixture. Rather the sludge was dispersed through the soil as clumps of hard carbonaceous material which represented nutrient-rich islands of intense microbial activity.

4.2.3 Microbial Development in Amended Prairie Grasslands Minespoil

4.2.3.1 Microbial numbers. The application of sewage sludge to the grasslands spoil increased the number of bacteria in the spoil surface to a greater degree than the gypsum and topsoil treatments. It is difficult to determine whether this increase was a result of the introduction of bacteria with the sewage sludge or the stimulation of reproduction by the indigenous soil bacteria resulting from the highly available nutrients present in the sludge. The stimulatory effect of the sewage sludge on the bacterial population was relatively long-lived since bacterial numbers in the sludge treated spoil were still significantly higher than in the other treatments at 15 mo after amendment. The growth of fall rye in the sludge treated plots over the first 15 mo of the study may also have contributed to the stimulation of bacterial growth particularly in the rhizosphere. Frequez and Lindemann (1982) have reported a significant increase in the number of aerobic heterotrophic bacteria in the rhizosphere of Atriplex canescens when grown in sodic coal spoil amended with sewage sludge.

The treatment of the spoil with gypsum to relieve the sodicity problem characteristic of this minespoil did not affect the numbers of bacteria, but did appear to reduce the number of actinomycetes, particularly those in the subsurface soil. The application of the highly acidic gypsum (pH 3.0) may have had an adverse effect on the actinomycetes since the streptomycetes, which can form a large proportion of the actinomycete population, are sometimes highly sensitive to acid (Kuster, 1967). Streptomyces spp. have been shown to be salt tolerant, preferring alkaline, dry, loamy,

organic soil (Kuster, 1967) which explains their abundance in the grassland topsoil. The general increase in actinomycete numbers in the 5-15 cm soil over the first 15 mo of the study can be partially explained by the development of a rhizosphere effect around the fall rye roots.

4.2.3.2 Shortcomings of ATP measurements. As was the case in the field studies, ATP determinations of the variously amended grassland minespoil was so variable that no definitive conclusions could be drawn from the data. Therefore, this technique was abandoned for any further monitoring on the effects of the amendments and plant growth on the development of microbial activity in the minespoil. Schafer et al. (1979) reported the use of ATP measurements to investigate the microbiological development which had taken place in 1 to 50 yr old stripmine spoils in Montana. Unfortunately, their data did not include any measure of the variation they encountered, making it difficult to interpret their results.

4.2.3.3 Organic matter accumulation. As mentioned previously, a productive vegetative cover on a minespoil provides the energy required by microorganisms for their growth and tissue synthesis. The rate at which the microbial component utilizes its food resource (i.e. plant litter, dead roots etc.) will eventually determine the rate at which stable organic matter accumulates. Shoot production by fall rye in the grassland minespoil was highest in the sewage sludge and topsoil treatments; therefore litter input was stimulated by these two amendments. Root production and consequently dead root input was, however, significantly greater in the topsoil treated spoil than in the sludge treatment. The high N level in the sewage sludge may have resulted in a reduction of the root system relative to the shoots (Russell, 1977). Unfortunately, primary production by fall rye ceased after the second growing season - a factor not anticipated when this study was initiated. Whether a plant species chosen to revegetate a minespoil is short or long-lived is an important factor to consider if one of the goals of reclamation is

the accretion of organic matter on the minespoil. Although significant quantities of fall rye litter and roots were deposited in the sewage sludge and topsoil-amended plots, plant growth did not influence the organic matter content in these two treatments. Organic matter accumulation is probably so slow, and the technique used to estimate organic matter (loss on ignition) so coarse that litter input over many years would be necessary before an increase in soil organic matter content could be measured. Schafer et al. (1979) estimated that 50 years would be required before organic carbon accumulation in the top 1 cm of minespoils in Montana reached equilibrium, while 200 to 400 years would be required for such equilibrium in the minespoil below 10 cm. These present data also suggest that organic matter accumulation on minespoils is a relatively slow process.

4.2.3.4 Microbial activity and biomass. Immediately after amendment both microbial activity and microbial biomass C exhibited a decreasing trend from the topsoil treatment down to the untreated minespoil. Topsoil was the most effective in improving the microbial status of the minespoil, since this amendment was characterized by high levels and a wide variety of microorganisms and was applied at a fairly heavy rate in comparison with the other two amendments. Sewage sludge with its high microbial inoculum load and nutrient rich composition was also highly effective in raising the microbial activity and biomass in the minespoil. Eiland (1981) and Macgregor and Naylor (1982) reported similar observations when they applied sewage sludge to cropland. The treatment effects occurred mainly in the top 5 cm of the minespoil, which would be expected since this is where much of the amendment was concentrated after its application.

With the exception of Wilson (1965) who reported an increase in microbial respiration of a coal minespoil after the addition of household refuse and Stroo and Jencks (1982) who used O_2 uptake to characterize minespoils varying in age and vegetation, very few studies have used soil respiration (i.e. O_2 or $CO_2\uparrow$) as a means to assess the ability of various amendments and planting

regimes to improve the microbial activity in a minespoil. This is surprising since the measurement of soil respiration is one of the most rapid and direct methods for obtaining an insight into the effects of various parameters on soil microbial activity.

The CO₂ efflux from the surface of the variously amended spoil planted with fall rye peaked at 15 mo after planting. At this point, shoot production by fall rye had also attained its peak which suggests that microbial activity in all the treatments had been stimulated by plant growth. The readily available C provided to the soil microorganisms through sloughed root material and root exudates is believed to have been the main factor stimulating microbial activity during the second growing season. It is widely recognized that plant roots release a wide variety of substances (amino acids and carbohydrates) when they are actively growing (see Russell, 1977). Little is known about the quantity of photosynthate released from plant roots through exudation and sloughing. Barber and Martin (1976) attempted to quantify root exudation and estimated that 12-18% of the photosynthetically fixed carbon of wheat and cereal plants was released by the roots. In the nutrient-poor conditions characteristic of minespoils, this amount of C could represent a major food resource for the soil microflora. The importance of root exudates as a source of C for stimulating microbial growth and activity in minespoils has been confirmed by Wilson (1965) and Fresquez and Lindemann (1982).

Plant growth did not appear to influence microbial activity in the subsurface spoil which infers that much of the root activity during the second growing season occurred in the 0-5 cm deep soil. After the third growing season, microbial activity in the top 5 cm had decreased to levels similar to those measured 0.5 mo after amendment. Interestingly, primary production by fall rye dropped sharply during the third growing season which suggests that soil respiratory activity may indeed be linked to plant growth. In the early stages of reclamation, this link may be solely dependent on levels of root activity since litter accumulation has not yet taken

place. In terms of microbial activity measurements, the variously amended grassland spoil regressed to its pre-planting stage mainly because fall rye is a short lived grass which ceased to grow after two growing seasons. Stroo and Jencks (1982) stressed the importance of vegetation to the development of microbial activity in minespoils particularly in relation to the release of root exudates. As in this study, these authors observed that when the vegetation on a minespoil failed (even after 10 years), microbial activity reverted back to levels similar to those prior to revegetation.

4.2.3.5 Microbial biomass C. As was the case for microbial activity, microbial biomass C in the 0-5 cm deep, amended minespoil also increased significantly over the first 15 mo of the study. It is assumed that the same factors were associated with the biomass stimulation as with the respiratory activity stimulation since these two parameters are often closely related, particularly when a highly available C source is present in the soil. Unlike microbial respiration, microbial biomass C did not decrease between 15 and 27 mo after amendment; instead it appeared to level out over this time period. Although microbial respiration appeared to be related to plant growth, this was not true for the microbial biomass. A possible explanation for this is that microbial biomass measurements are based on soil respiratory activity after the addition of a readily metabolizable substrate (i.e. glucose). Therefore, microbial biomass C is measured when soil nutrient conditions (i.e. C) are not limiting to the microbial populations, whereas microbial respiratory activity ($\text{CO}_2 \uparrow$ or $\text{O}_2 \downarrow$) is measured under varying C conditions. As mentioned previously, the microbial activity responsible for the oxidation of a particular resource is controlled by three main factors: moisture, temperature and the chemical composition of the resource (i.e. substrate quality). As a result, microbial respiration measured under constant temperature and moisture conditions, will vary with the amount of soil C readily available to the microorganisms. Peak CO_2 evolution after the second growing season can be explained by the elevation in the quantity of C

available to the soil microflora from root exudates or from the amendments. This source of C was negligible during the third growing season, hence microbial activity decreased. However, the microbial biomass responsible for this activity did not decrease; rather, its activity was probably limited by a lack of C. The addition of C in the form of glucose allowed the C-limited biomass to express its potential under non-limiting conditions. It appears, therefore, that the growth and turnover of microbial tissue (as evidenced by CO₂ efflux) was greater during the second growing season because of the increase in highly available (root exudate) C. The loss of this C during the third growing season resulted in less microbial growth and turnover, but there was enough C available for the biomass to maintain itself although not at the level of metabolic activity observed after the second growing season. These results suggest that the rate of microbial growth and turnover may be closely linked to plant productivity in the variously amended minespoils, whereas the microbial biomass maintains itself for some time after the cessation of plant growth. Undoubtedly, the microbial biomass would also be adversely affected if no plant growth occurred for an extended time period (i.e. when the C required to maintain the microbial biomass was no longer available). In the subsurface spoil microbial biomass, like microbial activity, changed very little over the course of the study.

Correlation coefficients calculated between grass root and litter weights and microbial activity and biomass after the third growing season implied that a reasonably close relationship existed between dead plant litter input (particularly roots) and soil microbial biomass. This relationship may be true but caution should be exercised when searching for relationships using correlation coefficients. For example, the high correlation observed between grass root weight and microbial activity and biomass may be an indirect relationship between amendment nutrient quality and microbial activity. Since both the highest root weight and microbial activity and biomass occurred in the topsoil treated spoil, followed by sewage sludge, gypsum and untreated spoil, it is possible that

amendation rather than plant growth (which had ceased during the third growing season) determined levels of microbial activity.

4.2.3.6 Decomposition process. The decomposition potential of the various treatments (as determined by the decay of filter paper) exhibited different patterns of weight loss depending on vegetation type. Over the short term (1 yr) filters placed in both the fall rye and rambler alfalfa planted plots decayed most rapidly in the topsoil treatment followed by the sewage sludge, gypsum and control treatments. However, after two years in the field, the decomposition of filters in the fall rye plots demonstrated no amendment effects, whereas the filter papers placed in the alfalfa plots followed the same pattern of decay observed during the first 12 mo of the study. These observations suggest that it was vegetation type rather than the amendment which controlled the rate of decay of the filters. If the filter decay results are viewed in conjunction with shoot production data (see Visser et al., 1982), one observes that short term decomposition in the fall rye plots was fastest in the treatments with the highest shoot production (topsoil and sewage sludge) and slowest in the treatment with poor plant growth (gypsum and control). As mentioned previously for the field sites, Ross et al. (1978) reported that decay of pure cellulose squares placed on the soil surface in tussock grasslands in New Zealand was controlled mainly by micro-environment. It is believed that variations in microclimate caused by differences in foliage density produced on the various treatments was also the main factor determining the decay of cellulose filters in this study. During the third growing season, growth of fall rye ceased, hence the microclimatic effects would presumably have been less pronounced. Although filters placed in the alfalfa plots demonstrated similar treatment effects as those placed in the fall rye plots, the differences in weight loss (particularly over the first 12 mo) between filters in the plots with greatest shoot production and those in the plots with lowest shoot production were not as pronounced as observed for the fall rye treatments. This can be explained by the longer period of time required by alfalfa to

establish itself in the topsoil treatment, and the poor germination and establishment of this plant species in the other treatments. As a result, density of the alfalfa plants was lower than that observed for fall rye and therefore the effects of microclimate on the decay of filters in the alfalfa plots was less evident and more variable. Rambler alfalfa, however, is a longer-lived species than fall rye, hence the amelioration of microclimate near the soil surface in areas planted with this species would be expected to be longer term and improve as primary production increased. This may have occurred in the topsoil treated plots over the 12-24 mo term of the study, since filters decayed rapidly in this treatment over this time period. It appears, therefore, that amendment of a minespoil may over the short term indirectly affect the potential of a spoil to degrade a pure substrate such as cellulose, by determining the germination, establishment and primary productivity (all factors which will affect plant density and microenvironmental variables) of the vegetation planted in the spoil. More research is required to discriminate between the amendment effects and the effects of plant density on the decomposition process.

The importance of vegetative cover to the decay of cellulose was substantiated when the degradation of filter paper over the short term (12 mo) was again tested 28 mo after amendment and planting fall rye and alfalfa. A comparison of the decomposition rates of filters placed in the fall rye plots 4 mo after planting and again 28 mo after planting showed that the cellulose decay potential of the two treatments which had exhibited the greatest shoot production (i.e. topsoil and sewage sludge) decreased over the 40 mo term of the study. The decay of filters placed in the control and gypsum treated plots where shoot production had been low, was much less influenced by time. These data infer that during the second growing season (when fall rye shoot production peaked) filter degradation was significantly accelerated by a dense vegetative cover. The chemical and biological properties of the amendments appeared to have very little direct effect on the decay rates,

because when growth of fall rye ceased no differences were detected in decay rates amongst the four treatments. It is, of course, highly probable that as moisture and temperature conditions at the soil surface improve with increasing plant cover and dead litter accumulation, the nutrient quality of the litter and amended minespoil would be of increasing importance in regulating cellulose decay. Obviously the link between the vegetation in a minespoil and its microbial activity and cellulose decay potential is a crucial one. When the growth of a plant species fails (as occurred in this study with fall rye), there is a danger of a minespoil regressing to its pre-planting state which it did in the topsoil treated plots.

Unlike the cellulose decomposition observed in the fall rye plots, cellulose decay potential in the Rambler alfalfa plots increased significantly with time (particularly in those plots treated with sewage sludge and topsoil). As mentioned previously Rambler alfalfa is a long-lived perennial, whose primary production increased during the term of the study. A combination of microclimatic effects caused by the vegetative cover, dead litter accumulation at the soil surface and the nutrient quality of the litter and amended minespoil surrounding the litter bag would have been the main factors governing cellulose decay in the alfalfa plots.

Wood dowel decomposition in the tank plots was as slow as that recorded in the field sites - an observation explained primarily by the decay-resistant (high lignin) quality of the wood. The very slow decay exhibited by this particular substrate makes it a rather useless material for comparing the decay potential of soils in various stages of reclamation, unless measurements are made over a very long term.

4.2.3.7 N₂ fixation by Rambler alfalfa. Since minespoils are accepted as being notoriously low in plant available N, it has been recommended (Skeffington and Bradshaw, 1980) that legumes be planted to accelerate the rate of nitrogen accumulation. In order to ensure that these legumes become well established with well nodulated roots

capable of N_2 fixation, it is important that the effects of various amendments on legume growth be recognized. The amendments used to ameliorate the grasslands spoil (i.e. sewage sludge, gypsum and topsoil) did not appear to inhibit the formation of N_2 fixing nodules during the first growing season. Unfortunately the data were highly variable at all sampling times which makes it difficult to draw any definitive conclusions on the effects of the various amendments on N_2 fixation potentials. The heterogeneous distribution of the sewage sludge and gypsum, and the difficulty in extracting complete root systems from the various treatments undoubtedly contributed to the variation. Nevertheless the results infer that even in the untreated spoil the nutrients required for successful infection, nodulation (mainly calcium) and N_2 fixation (phosphorus, sulphur and micronutrients) by Rhizobium were available in sufficient quantities. Although sewage sludge did not have a significant inhibitory effect on N_2 fixation during the first growing season, caution should be exercised when applying this amendment to legume planted minespoils. Sewage sludge contains high levels of ammonia and other forms of nitrogen which have been shown to inhibit both nodule formation and nitrogen fixation (Smittle, 1979). N_2 fixation potential of alfalfa in the sewage sludge treated minespoil increased substantially from the first to the second growing season, so if the sewage sludge contained any inhibitory substances when first applied, detoxification (perhaps through oxidation of ammonia and other N transformations) had occurred by the end of the second growing season.

Nodule weight and N_2 fixation appeared to be highest after the second growing season, particularly in the sludge and gypsum treatments. Perhaps nutrients released by these two amendments at this time stimulated plant growth, thereby causing an indirect stimulation of N_2 fixation. The difference in nodule morphology between plants from the topsoil treatment and the other three treatments is interesting. The small size of nodules on plants in the topsoil treatment may be attributed to such factors as

deficiencies of molybdenum, phosphorus or sulphur (see Munns, 1977) or high plant density (Graham and Rosas, 1978).

Skeffington and Bradshaw (1980) determined that the role of the free-living bacteria in accumulating N in derelict land was negligible when compared with N input from N₂ fixing leguminous plants. This study supports their conclusions since N₂ fixation potential of the topsoil and untreated spoil was .15 and 0 nmoles 100 g⁻¹ dry soil hr⁻¹ respectively compared with 93.7 and 149.5 nmoles g⁻¹ dry root hr⁻¹ for alfalfa grown in the topsoil and untreated spoil respectively for two growing seasons.

The extreme variability recorded in this study is problematical. Further research is required to improve the precision of the acetylene reduction technique particularly when applied to plants grown in widely contrasting soils. Trinick et al. (1976) reported that field grown plants of Lupinus luteus were highly variable in their acetylene reducing ability and that ten groups of five plants were required to reduce the coefficient of variation to 0.1. Hence, considering the highly variable nutrient conditions, particularly in the sewage sludge and gypsum treated plots, it would appear that an enormous number of plants would be required from each treatment to obtain dependable, consistent results. It is possible that if N₂ fixation potential of a legume crop was measured in the field over fairly large areas, the variation would be reduced considerably.

4.2.4 Subalpine Spoil

4.2.4.1 ATP, microbial numbers and fungal mycelial lengths.

Microbial population estimates as determined by ATP bacterial and actinomycete counts and fungal hyphal length measurements demonstrated that shortly after amendment, bacteria were most numerous in the sewage sludge and peat treatments, while the greatest number of actinomycetes and amounts of fungal hyphae occurred in the peat treatment. ATP levels were generally higher in the sewage sludge and peat treatments, but the extreme variability in the ATP

measurements made it difficult to analyze the data. As a result ATP estimates were not considered an accurate means for monitoring microbial development in the variously amended plots, and were therefore terminated. It is not surprising that the minespoil treated with sewage sludge and peat exhibited the highest levels of microbes since these two amendments introduced not only large numbers of micro-organisms, but also C and essential nutrients, such as N and P, required by the microbes for growth and maintenance. Fertilizer contained very few organisms prior to its application, and lacked the C necessary for stimulating the growth of the minespoil microflora. As in the grassland spoil, the immediate effects of the amendments on microbial numbers occurred mainly in the upper 5 cm of the treated spoil where the amendments tended to be concentrated.

Plant growth for two growing seasons did not significantly alter bacterial numbers in the 0-5 cm deep amended minespoil; however in the deeper, subsurface spoil, both bacterial and actinomycete numbers in all the treatments had increased significantly after growing slender wheatgrass for 15 mo. This increase may have been stimulated by root exudates since slender wheatgrass shoot production and presumably root production peaked during the second growing season. The potential importance of root productivity and exudation to microbial growth has been discussed previously for the grassland spoil. The lack of a response by the fungal hyphae to plant growth suggests that the bacteria and actinomycetes may respond to root exudates to a greater degree than the saprophytic soil fungi. The significant reduction in actinomycete numbers in the 0-5 cm zone of peat treated minespoil over the first 15 mo of the study is difficult to explain. Perhaps the reduction in levels of N and P in the peat (see Visser et al., 1984) adversely affected actinomycete populations. Also, the peat had been freshly excavated prior to its application to the minespoil. Changes in some of the physical and chemical qualities of the peat as a result of exposure may have caused a reduction in actinomycete numbers.

4.2.4.2 Microbial respiration and biomass in unplanted minespoil.

The higher microbial numbers measured in the top 5 cm of the peat and sewage sludge treated minespoil supported the microbial activity determinations which were also observed to be greater in the peat and sewage sludge amended spoil than in the fertilized and unamended spoil. Soil respiration in the subsurface soil was minimally affected by treatment until 15 mo after amendment when CO_2 efflux at both depths in all treatments rose significantly. This increase was most obvious in the sewage sludge and peat amended minespoil and may have been partially due to a release of nutrients from the amendments which in turn stimulated microbial activity. After this peak in microbial activity in the amended, but unvegetated minespoil, respiration in the surface soil returned to what it was shortly after amendment application, while CO_2 efflux in the 5-15 cm spoil dropped significantly lower than that recorded at the first sampling. These results suggest that any highly available nutrients, particularly C, present in the amendments had been utilized by the end of the study. However, estimates of C (measured by loss on ignition) in the variously treated unvegetated minespoil over the term of the study implied that on a very crude scale, no alteration of C levels occurred. It can, therefore, be concluded that the major portion of the C introduced with the sewage sludge and peat was of a very stable nature and not susceptible to massive microbial oxidation over the short term.

The pattern of microbial biomass development was very similar to the microbial activity pattern with the exception that biomass C (particularly in the surface soil) did not decrease after 15 mo. Interestingly, this pattern resembled that obtained for the grassland spoil planted with fall rye, suggesting that similar factors controlled microbial activity and biomass in the two spoils. As in the grassland spoil, a reduction in the quantity of highly available C in the subalpine spoil from 15 to 27 mo may have decreased microbial activity (i.e. microbial growth and turnover); however, there was enough C available for the biomass to maintain

itself at a reduced level of metabolic activity after the highly available C had disappeared.

4.2.4.3 Microbial respiration and biomass in plots planted with slender wheatgrass or white spruce. Previous investigations have suggested that vegetation is crucial to microbial activity in minespoils (Stroo and Jencks, 1982) and that the species of plant used in the revegetation program may determine the rate of development of microbial activity (Müller, 1973). Since white spruce and slender wheatgrass grow at very different rates and have distinctly different root morphologies, it was decided to follow microbial activity and biomass in the variously amended plots planted with these two plant species. The results were then compared to those obtained from amended, but unvegetated spoil.

Over the term of the study, microbial activity and biomass C estimates in spoil planted with white spruce closely resembled those measured in the unplanted spoil with the exception of the upper 5 cm soil from the peat treatment. Here, microbial respiration and biomass in peat removed from the white spruce plots at 27 mo after planting was somewhat higher than that measured in the unvegetated peat treated spoil. White spruce root weights after three growing seasons were heavier in the 0-5 cm soil in the peat treatment than in the sewage sludge, fertilizer and control treatments. Therefore, it is possible that root activity in the peat stimulated microbial activity to a greater degree than roots in the other amendments. However, it is worth noting that ectomycorrhizal infection of the white spruce roots was extremely high (Danielson et al., 1984) and that microbial stimulation resulting from root exudation may have been influenced by the presence of the mycorrhizal fungus. In the subsurface soil from the sewage sludge treated plots, microbial biomass C increased significantly between 0.5 and 27 mo after amendment, while no changes occurred at this depth in the other treatments. After three growing seasons, there was approximately twice as much root weight in the subsurface soil in the sludge amended plots than the surface soil; therefore, root activity and

nutrients released by the decomposing sludge clumps may explain this increase in microbial biomass.

Microbial activity and biomass C at the conclusion of the third growing season, were higher in the plots planted with slender wheatgrass than in the unvegetated spoil and the spoil planted with white spruce. This observation applied mainly to the 0-5 cm deep amended spoil, and was particularly true in the sewage, fertilizer and unamended plots. Peak primary production by slender wheatgrass occurred during the second growing season, resulting in the accumulation of dead litter (leaves and stems) on the spoil surface after this time. Also root weights measured in the grass plots, 27 mo after planting were substantially greater than those measured for white spruce. Sewage sludge amendment was particularly effective in enhancing grass productivity, hence the greatest amount of litter input occurred in this treatment. Root production was highest in the peat amended spoil. Although some of the amendments (sewage sludge and peat) greatly stimulated plant production, loss on ignition measurements in the grass plots did not vary over the term of the study, inferring that this method is not very sensitive for detecting small changes in organic matter, particularly in spoils contaminated with geologic forms of carbon. The soil microorganisms, however, are highly sensitive to the addition of carbon sources such as those found in litter, roots and root exudates and no doubt their growth and activity were greatly enhanced as a result of grass litter and root input during the third and fourth growing seasons. This would have been particularly true in the untreated and fertilized spoil where the supply of highly available carbon was dependent almost entirely on plant growth. These data suggest that the development of microbial activity and biomass is strongly linked with levels of primary productivity mainly because the metabolic activity of the microorganisms is regulated by the availability of an energy source. As a result, it would be expected that the development of microbial activity would be more rapid in a minespoil planted with a fast growing, highly productive grass species than a slow growing tree species, particularly if productivity was enhanced by the addition of

a nutrient rich amendment. As in this study, Müller (1973) reported a more active development of microflora in coal mine spoils planted with a grass-legume mixture than in tree vegetated spoils.

4.2.4.4 Comparison of microbial development in amended minespoil planted with grass, spruce or left unplanted. A comparison of microbial development in the unvegetated minespoil and the tree and grass vegetated minespoil demonstrated that over the first three years of this study, the presence of a grass cover was most effective in stimulating the activity and growth of the microorganisms. To increase or maintain the microbial biomass stimulated by the grass over the long term it would be essential that this vegetative cover remain productive. This was, unfortunately, not the case in this study since the growth of slender wheatgrass peaked in the second growing season and collapsed during the third growing season bringing litter and root input (i.e. future organic matter) into the soil system to a standstill. Although the microbial biomass increased and maintained itself during the third growing season (mainly as a result of litter input at this time), there was some evidence that microbial activity was in the process of declining during the fourth growing season (27-39 mo). Further sampling would be required to establish with certainty that the cessation of plant growth caused a reduction in microbial activity. Nevertheless, it appears that the choice of plant species, and the longevity of a plant species once established are important factors to consider when revegetating minespoils since this study suggests that the rate of microbial development is closely associated with the type of vegetative cover. A failure in the vegetative cover could return the soil microbial system back to its pre-planting phase. When using short-lived species such as slender wheatgrass to provide initial erosion control of a minespoil, it may be advantageous to plant longer lived woody shrubs or conifers once the grass cover has failed. The choice of plant species would of course be heavily dependent on the final land use goal.

Microbial respiration and biomass followed the same pattern in both the vegetated and unvegetated minespoil (i.e. they were

highest in the peat, followed by sewage sludge, than fertilizer and lastly control). Planting slender wheatgrass seemed to enhance the effects of the amendments more than planting white spruce presumably because of the higher productivity of the grass. It appears, therefore, that levels of plant available nutrients introduced with an amendment determines the degree of primary production (particularly of fast growing species) which in turn regulates the rate of microbial development in a minespoil. There was some evidence that fertilizer, shortly after its application, inhibited microbial activity in the upper 5 cm of the spoil. It is possible that fertilizer inhibition did occur since some investigations have demonstrated a reduction of soil microbial activity in the presence of N fertilization (Kowalenko et al., 1978) while ammonia has been shown to be a volatile inhibitor of fungal spore germination (Pavlica et al., 1977; Setua and Samaddar, 1980). Although the 0-5 cm deep soil in the slender wheatgrass plots was the "action zone" in terms of microbial development, microbial biomass measurements in the deeper 5-15 cm also demonstrated a slow but progressive increase over the first three years of the study. This increase is attributed mainly to the profuse growth of the fibrous grass roots at this depth since the microorganisms in the unamended spoil as well as in the treated spoil exhibited similar patterns of biomass development.

The microbial biomass data collected for the variously amended subalpine minespoil suggested that plant productivity and microbial development were closely related; however, correlation coefficients calculated for data collected 39 mo after planting revealed a poor relationship between grass litter/root weights and microbial activity/biomass. The lack of a relationship may be a result of the interactive influence of the chemical composition of the various amendments and plant growth on microbial activity and biomass. Plant growth and litter input in a carbon limited situation may stimulate microbial activity to a greater degree than plant growth in a minespoil where other forms of carbon have been introduced via highly organic amendments. The relationship between

plant and microbial productivity in soils containing different amounts and qualities of organic carbon warrants further study. Microbial activity and biomass, however, were highly correlated with loss on ignition. The wide gap between the organic matter levels in the untreated, fertilized and sludge amended spoil vs. the peat treated spoil partially explains this strong relationship, hence these data should be treated cautiously until soils with a wide variety of organic matter levels have been tested.

4.2.4.5 Decomposition of standard substrates (filter paper and wood dowel). The potential of the minespoil to degrade cellulosic filter paper was most enhanced by amendment with sewage sludge. Unlike the different decay rates of filters placed in the grassland minespoil planted with fall rye and alfalfa, filter decay in the subalpine spoil followed the same pattern regardless of vegetation type (i.e. the decomposition rate of the filters in each of the four treatments was approximately the same whether planted with grass or white spruce). The lower shoot production by slender wheatgrass compared with fall rye, may not have ameliorated the microclimate at the soil surface in the subalpine plots to the same extent as fall rye did in the grassland minespoil, thereby decreasing the effects of microclimate on decay. The cellulose degradation in the sewage sludge treatment appears to have been stimulated mainly by the introduction of highly available nutrients and abundant microbial inoculum in the sludge. The difficulty encountered in rototilling the sewage sludge into the rocky subalpine spoil meant that much of the sludge was concentrated in the top 5 cm of the spoil. Its effect on the decay of filters would therefore have been more pronounced in the subalpine spoil than in the grassland spoil where the sludge was incorporated to at least a 15 cm depth. It is worthwhile noting, that cellulose degradation in the peat treatment was similar to that measured in the untreated spoil, although microbial biomass in the peat was significantly greater than that in the other treatments. This suggests that factors other than microbial biomass are important in determining decay rates of substrates placed on the soil surface

(e.g. soil nutrient availability). Available P in the peat during the early stages of the study was excessively low (Visser et al., 1984) and this may have inhibited both plant growth and decomposition.

When the short term (12 mo) study of filters was again tested 28 mo after amendment and planting slender wheatgrass, it was observed that the cellulose decay potential of each of the treatments had not substantially altered since the initiation of the study (i.e. decay of filters in the sludge amended minespoil was significantly faster than in the peat, fertilizer and untreated minespoil). The lack of a change in decay potential implies that the growth of slender wheatgrass in the peat, fertilizer and untreated spoil had no significant effect on cellulose degradation over the term of the study. However, the more rapid decay in the sludge treated plots may have been a result of both the nutrient quality of the sewage sludge improving the chemical quality of the pure cellulose and the stimulated plant growth ameliorating the microclimate at the soil surface. Although slender wheatgrass productivity in the sludge treated spoil was much reduced after the second growing season, this did not appear to influence decay rate of filters placed in the plots at the conclusion of the third growing season. The thatch of undecomposed grass litter which had accumulated on the plots after the second growing season may have improved temperature and moisture conditions at the soil surface, in the same manner as foliage density of herbs and grasses has been postulated to do.

As in the previously mentioned studies, the highly lignified nature of the fir wood dowel resulted in negligible weight loss in all treatments over the first two years of the investigation.

4.2.4.6 Decomposition of grass and clover litter (leaves and stems). Slender wheatgrass leaves placed in the slender wheatgrass plots at the conclusion of the third growing season, lost approximately 16% of their weight over the winter and 50% after 1 yr in the field. It is highly likely that the initial weight loss was

due to the disappearance of the highly soluble fraction of the litter which for Agropyron litter has been estimated at approximately 16% (see field study discussion). Although the productivity of slender wheatgrass was greatly stimulated by sewage sludge and fertilizer amendment, decomposition of the incoming litter was equally fast in all treatments. The equally fast mineralization of nutrients immobilized in the litter in plant systems of low productivity vs. plant systems of high production, would result in a greater accumulation of undecomposed litter in the highly productive systems. If more nutrients become immobilized in the litter than are available for plant growth, the consequence would be poorer plant growth or even plant failure. Also a thick litter layer can become a fire hazard, or a shelter for rodents which, if they multiply extensively, can be a menace to newly planted tree seedlings. Therefore a reclamation strategy should take into account the long-term consequences of stimulating grass/legume growth via fertilization and should perhaps include measures for reducing dead litter accumulation (e.g. grazing, harvesting) if a thick litter layer develops. It should be emphasized that the decay of slender wheatgrass was followed over a very short term. It is therefore possible that once the decomposing litter is incorporated into the soil, the mineralization and cycling of the remaining nutrients would be influenced by soil amendment. Further research is required to determine long term treatment effects on mineralization of nutrients immobilized in litter which has accumulated on minespoils.

After one year in the field, the weight remaining of slender wheatgrass stems was approximately 30% greater than the amount of slender wheatgrass leaves remaining, with no significant differences being recorded between the variously amended plots. The slower decay of the stems was probably due to a difference in resource quality between the stems and leaves. The stems may have had a wider C/N ratio, a lower soluble fraction and a higher lignin content than the leaves, thereby reducing their decay. Carrel et al. (1979), utilizing the C/N ratio of grass as an index of litter quality, concluded that C/N ratio was a major factor in determining

the decay rate of litter on minespoils. Therefore in future research it may be worthwhile to follow C/N ratios and lignin contents of litter placed out on minespoils. The two different decay rates exhibited by the leaves and stems would result in two different patterns of nutrient release. This may be advantageous in soil systems which are highly leachable and where nutrient conservation is a potential problem. Conversely, the slower decay of the stems would result in slower organic matter accumulation.

Of the substrates utilized in the decomposition studies, slender wheatgrass leaves decayed most rapidly, followed by the stems and lastly the filter paper. As has been mentioned previously, natural plant litter is nutritionally much better balanced than a pure substrate such as cellulose, hence microbial growth and turnover would be expected to be more active in the litter than the filters. The comparison of the three study materials demonstrated that the decay rate of filter paper cannot be extrapolated to predict decay rates of natural litter. However, cellulose filter decomposition may be a good indicator of the levels of microbially available nutrients in minespoils which have received various amendments, since the degradation of pure cellulose is probably highly dependent on the soil nutrient conditions surrounding it.

A comparison of slender wheatgrass litter (leaves plus stems) and alsike clover litter (leaves plus stems) decay rates showed that alsike clover litter was mineralized more rapidly than grass litter. Again the difference in decay rates between the two litter types can be explained by differences in resource quality. Legume litter tends to be less "tough" than grass litter, and because legumes fix atmospheric nitrogen, the N content of clover shoots tends to be higher than that observed for grass shoots (see Table 40, Visser et al., 1984). Legume litter (particularly the leaves) may also have a larger soluble fraction than grass litter. All these factors would serve to hasten the decay of the clover litter. Since legume shoots contain more N than grass shoots, legume litter would contribute more N to the soil system when it decomposes. This would

be particularly important in minespoils where N can be the nutrient most limiting to plant growth.

Lanning and Williams (1980) compared the decomposition rates of grass and clover in china clay sand wastes and, as in this study, found that clover decomposed more rapidly than grass. They also observed that nitrogen was released only from the decaying clover, but that this nitrogen did not accumulate in the sand waste. The loss of N from the sand wastes was attributed to rapid leaching of the mineralized nitrogen, loss of shoot material through wind and rain erosion and volatilization of N as ammonia at a pH above 6.5 (Lanning and Williams, 1980). The research of Lanning and Williams emphasizes the importance of gaining insight into the ultimate fate of N released from decaying plant material on minespoils. Severe leaching losses, which are possible in the coarse textured spoil such as the subalpine spoil could eventually lead to the regression of the established plant cover. The decomposition results in this study have conclusively shown that the decay and presumably the release of N from alsike clover litter is significantly faster than that of grass litter. However, the beneficial aspects of the more rapid nutrient release from this legume (i.e. faster return of N to the primary producers) would be lost if a reclamation strategy did not take into account the potential mechanisms for nutrient loss and measures for reducing this loss if it becomes excessive.

4.2.4.7 N₂ fixation potential of alsike clover. Since legume litter exhibits a relatively high turnover rate resulting in a more rapid release of essential plant nutrients and presumably a faster accretion of stable organic matter, and since the N₂ fixing capability of legumes can stimulate the rate of N accumulation, the successful establishment of legumes within a grass/legume sward appears to be an essential requirement in the initial stages of soil genesis on a minespoil. Therefore, it is necessary that we understand the effects of various amendments on the establishment and N₂ fixing capacity of legumes planted in minespoils. In this study, no significant amendment effects on N₂ fixation capacity of

alsike clover could be detected, but, as previously mentioned for the rambler alfalfa, measurements were extremely variable.

The lack of acetylene reduction by alsike clover grown in the peat treatment for 3 mo was originally believed to be due to a deficiency of available P in the peat which according to Munns (1977) can limit N_2 fixation by limiting the growth of the host plant. However, a greenhouse pot experiment demonstrated that growth and flower production by alsike clover were indeed limited by low P levels, but nodule formation and N_2 fixation potential were not stimulated by additional P. Therefore, the lack of N_2 fixation was attributed to the high levels of NO_3-N ($450 \mu g g^{-1}$ dwt) in the peat immediately after its application. The inhibitory effects of high nitrate levels on infection, nodulation and N_2 fixation by Rhizobium have been well documented by Munns (1977). Interestingly NO_3-N levels in the peat treated minespoil dropped considerably ($97 \mu g g^{-1}$ dwt) after the first growing season, which may explain why acetylene reduction by alsike clover from the peat treatment increased substantially over the second growing season. It is also possible that high ammonia and nitrate levels in the sewage sludge reduced the N_2 fixing capacity of alsike clover, but that this inhibitory effect was obscured by high variability. As for the rambler alfalfa, N_2 fixation by alsike clover was highest after the second growing season. No significant relationships were detected between clover shoot N, total soil N, soil NO_3-N and the N_2 (C_2H_2) fixation capacity measured after the second growing season (i.e. 14 mo after planting). By growing alsike clover in spoil ranging from 1.67% N (peat treated) to .09% N (untreated), it is not surprising that no relationships existed between shoot N and N_2 fixation potential. The interaction between high levels of soil NO_3-N and the N_2 fixation process would make it extremely difficult to separate plant uptake of soil N from accumulation of N in the shoot as a result of N_2 fixation. The measurement of N_2 fixation by alsike clover grown in the subalpine minespoil mixed with different levels of a particular amendment would perhaps clarify some of the relationships which may exist between soil N, shoot N and N_2

fixation and more precisely determine the effects of amendment on the establishment of an N_2 fixing plant system. Although the data in this study are extremely variable, they do suggest that the physical and chemical characteristics (e.g. nutrient status) of a minespoil both before and after amendment requires some understanding to ensure the rapid establishment and maintenance of N_2 fixing legumes.

4.2.5 Microbial Development in Amended Oil Sands Tailings

4.2.5.1 ATP, microbial numbers and fungal mycelial lengths.

Estimates of ATP, bacteria, actinomycetes and fungal mycelium in the sand tailings shortly after amendment and 15 mo after amendment and planting slender wheatgrass followed a similar pattern to that observed for the subalpine spoil treated with the same amendments (i.e. ATP and bacterial counts were highest in the sludge treated sand while actinomycetes and fungal hyphae were most numerous in the peat treated spoil). The similar results imply that the same factors as those discussed for the subalpine spoil were involved in determining the microbial status of the tailing sand after amendment. The main difference between the extracted oil sands and the subalpine spoil appeared to be the greater effect of amendment in the subsurface soil in the oil sands minespoil. Since the sandy spoil provided no resistance to the incorporation of the sewage sludge and peat, the microbial inoculum and nutrients present in these two amendments were dispersed to a greater depth. In general, microbial counts and hyphal measurements were substantially lower in the variously amended tailing sand than in the subalpine spoil. This would be expected since the harsh procedure used to extract oil leaves the resulting sand deficient in both nutrients and microbial inoculum. Previous microbiological investigations conducted on the oil sands tailings (Rowell, 1977; Rowell, 1978) also recorded relatively low numbers of bacteria and actinomycetes in the tailing sand, but the addition of peat and overburden led to an increase in microbial numbers.

As in the case of the subalpine spoil, the short term growth (i.e. 15 mo) of slender wheatgrass had minimal effects on soil bacteria, actinomycete counts and fungal hyphae density. The lack of a plant effect through root exudation and plant litter input can probably be accounted for by the low productivity of the grass in all but the sludge treated sand. The increase in actinomycetes in the sludge amended sand, particularly at the lower depth, may have been due to the release of large quantities of root exudate resulting from the tremendous growth of the slender wheatgrass during the second growing season. It is worthwhile noting that rototilling the sludge and peat into the sand caused such large variation in microbial population estimates, that the effects of amendment and plant growth on the soil microorganisms may have been masked by this variation.

4.2.5.2 Microbial respiration and biomass in unplanted minespoil. Although microbial respiration and biomass C were generally greater in the unvegetated subalpine spoil than in the unvegetated oil sands spoil, the effects of amendment on these two parameters over the term of the study were identical in the two spoils. That is, microbial activity and biomass C were highest in the peat treated sand followed by the sewage sludge treatment and the fertilized and untreated sand (these data are supported by the bacterial, actinomycete counts and hyphal lengths). Both respiration and microbial biomass rose significantly between 0.5 and 15 mo after treatment, but whereas respiratory activity returned to levels similar to those shortly after amendment, microbial biomass C did not. Possible explanations for these results are believed to be very similar to those discussed for the subalpine spoil. The substantial increase in CO₂ efflux and microbial biomass C measured in both the subalpine and oil sands spoil, 15 mo after treatment with peat suggests that increased biological oxidation of peat C may have been taking place, but that the oxidation occurred over a very short time span (2 years). However, measurement of organic matter by the loss on ignition method, implied that much of the C introduced into the

minespoil with the sewage sludge and peat was reasonably resistant to microbial attack since no significant decreases in organic matter were detected in the 39 mo term of the study.

4.2.5.3 Microbial respiration and biomass in plots planted with slender wheatgrass or jack pine. The pattern of microbial respiration and biomass C development in the variously treated sand tailings was not significantly altered by the growth of jack pine over the four year study period (i.e. the amendments were much more important in determining microbial activity than tree growth). This is not surprising since the slow growth of a conifer such as jack pine in comparison with that of slender wheatgrass would suggest that any microbial stimulation resulting from root exudation by the pine would only occur over the long term. Also, microbial development resulting from the decay of dead plant parts would be considerably lower for pine than for the highly productive grass. Although no significant differences were detected in microbial biomass C in the sludge treatment amongst the four sampling times, there did appear to be an increasing trend in microbial biomass as time progressed. Root and shoot production by jack pine was considerably greater in the sewage sludge treatment than in the other three treatments (especially between 27 and 39 mo after planting) and therefore, may have contributed somewhat to the stimulation of microbial growth particularly during the last two years of the study. A longer term investigation would be required to determine conclusively that a relationship exists between jack pine productivity and development of soil microbial activity. Also it would be interesting to attempt to separate microbial development resulting from needle litter accumulation on the soil surface, from that stimulated by root activity.

Microbial biomass C measured 39 mo (four growing seasons) after planting tended to be higher in the 0-5 cm depth amended sand planted with slender wheatgrass than in the sand which remained unplanted or was planted with jack pine. At this time, loss on ignition was also greater in the slender wheatgrass planted plots

than in the other two treatments which implies that the input of dead grass litter and roots after the second growing season was highly effective in raising organic matter levels thereby stimulating the growth of the microbial biomass. Needless to say, the unamended sand exhibited little change in loss on ignition, microbial activity and biomass over the term of the study mainly because the growth of all test plant species was negligible in this treatment (see Fig. 4, Visser et al., 1984). With the exception of the unamended controls, the patterns of plant growth, litter input and microbial development were very similar in the oil sands and subalpine minespoils. Some microbial development occurred in the unamended subalpine spoil planted with slender wheatgrass, but the paucity of nutrients in the unamended sand tailings resulted in plant failure and no microbial development. The similarity in microbial development in the two minespoils after amendment and planting is thought to be due to the linkage between plant and microbial productivity. Factors involved in this relationship have been discussed previously (subalpine spoil) and are believed to be equally relevant to the oil sands minespoil.

Between 0.5 and 15 mo after amendment, microbial activity and biomass in the fertilized sand tailings planted with slender wheatgrass rose considerably. Since the extracted sand contained very little organic carbon, the increased microbial activity in this particular treatment can be attributed mainly to C input from root exudates and sloughed root material. This again emphasizes the importance of plant growth to the microbial system, particularly in tailing sand which has not received an organic amendment. Microbial respiration and biomass in the sludge treated 0-5 cm sand peaked at 27 mo after amendment and planting grass. Nutrient release from dead plant parts and decomposing sludge would be mainly responsible for stimulating microbial activity at this time. Although slender wheatgrass in the fertilized and sludge treated sand failed to grow after the second growing season, microbial respiratory activity and biomass were not significantly reduced. The substantial input of dead litter and roots by slender wheatgrass in these two treatments presumably provided enough energy and nutrients to maintain the

increased microbial biomass over the term of the study. The decreasing trend in CO₂ evolution from the fertilized and sludge treated sand towards the end of the study was also observed in the subalpine spoil and is presumed to have resulted from the same factors. Microbial activity and biomass in the upper 5 cm of the peat treated sand planted with slender wheatgrass was greater than that in the other three treatments and exhibited an increasing trend with time. This increasing trend is presumed to have been a result of slow biological oxidation of the peat, microbial stimulation by root exudates and plant litter/root input (which in contrast to the sewage sludge and fertilizer treatments did not cease after the second growing season but rather appeared to stabilize) or a combination of both these factors. The biological oxidation of newly excavated peat and the effects of plant growth on this oxidation warrants further research.

In contrast to the subalpine spoil, microbial development in the subsurface tailing sand did not demonstrate an increasing trend over the term of the study. Root production in the oil sands minespoil was considerably less than that in the subalpine spoil (the subalpine spoil prior to amendment was not as deficient in nutrients as the sand tailings) which may partially explain the lack of microbial development in the deeper sand. As mentioned previously, variation in the microbial data was considerable and this variation may have masked any changes in microbial activity.

Because microbial development appeared to be heavily dependent on the level of plant productivity, it was believed that the calculation of correlation coefficients between the products of grass growth (litter and roots) and microbial activity and biomass would demonstrate a strong relationship. However, as in the subalpine spoil, a significant relationship was not detected. Possible reasons for this are believed to be similar to those discussed for the subalpine spoil.

4.2.5.4 Decomposition of standard substrates (filter paper and wood dowel). As was observed for the subalpine spoil, amendment of the

tailing sand with sewage sludge hastened the short term (12 mo) decay of filter paper but only if the spoil had been planted with slender wheatgrass. The high levels of nutrients and microbial inoculum introduced with the sewage sludge plus the possible microclimatic amelioration resulting from the stimulated grass growth during the second growing season are believed to have been the main factors in accelerating the decay of filters placed on the sludge treated sand. After spending two years in the field, no significant differences were detected between % weight remaining of filters placed in the fertilized and sludge treated slender wheatgrass plots. Since primary production of the grass in these two treatments peaked during the second growing season, the resultant litter layer which accumulated above the filters during the third growing season may have stimulated the degradation of the filters by improving both microclimate around the filters and the nutrient levels within the filters (nutrients leached out of the decaying grass litter or released from the decaying sludge may have been absorbed by the filter paper).

The lack of a significant difference in % weight remaining for filters placed in the peat and untreated plots was also observed for the subalpine spoil. Since the peat harbored a wide variety of organisms and was highly active microbiologically, the slow decay of cellulose in this treatment was attributed not to a lack of microbial inoculum, but to the low levels of P which could have retarded both plant growth and the decay process.

The importance of a vegetative cover in accelerating the decomposition of a pure substrate such as cellulose is substantiated when the decay rates of filters placed in the jack pine plots are considered. Filters placed in the fertilized and sludge treated slender wheatgrass plots decomposed much more rapidly than those placed in the jack pine plots, particularly during the third growing season. Also no amendment effects on decay rates were detected for filters placed in the jack pine plots but in the case of the sewage sludge treatment (where after 24 mo decomposition 40% of the weight

remained compared with 62-78% in the other treatments), this may have been due to highly variable results.

When the 12 mo weight loss of filters was again measured 28 mo after planting slender wheatgrass, it was observed that the decomposition potential of the sludge treated sand had decreased significantly while the potential of the fertilized, peat and untreated sand had not altered significantly from that measured during the first 12 mo of the study. The reduced decomposition in the sludge treated spoil is difficult to explain since the decay potential of the sludge amended subalpine spoil did not change significantly over the 40 mo term of the study. It is possible that the development of a moss layer in the sludge treated sand planted with slender wheatgrass may have inhibited the decomposition of the filters by separating them from the nutrients being slowly released from the sludge. No such moss layer developed beneath the slender wheatgrass in the sludge treated subalpine spoil. The effects of moss layer development (particularly in recently revegetated minespoil) on the decay of incoming litter and organic matter accretion may be worthy of further research.

The highly lignified and decay resistant qualities of the fir dowl again resulted in negligible weight loss of this resource when placed in the variously amended slender wheatgrass and jack pine planted tailing sand.

4.2.5.5 Decomposition of grass and sainfoin litter (leaves and stems). Although the subalpine and oil sands minespoils were very different in terms of their physical, chemical and biological characteristics, the short term decomposition of slender wheatgrass litter was surprisingly similar in the two minespoils, regardless of treatment. Weight loss of the litter placed in the variously amended sand planted with slender wheatgrass amounted to approximately 20% over the winter (16% in the subalpine spoil) and 55% after 1 year (50% in the subalpine spoil). These data are interesting in that they imply that over the short term (i.e. 1 year of decomposition) the chemical and physical properties of a minespoil (even after

amendation) and the soil microbial biomass in the soil immediately beneath the decaying litter are insignificant in determining the initial rate of decomposition of incoming dead plant material (in fact no significant relationship was detected between % dry leaf weight remaining and soil microbial activity and biomass immediately below the slender wheatgrass litter bags). It is possible that nutrients leached from the decaying litter into the soil below it may be mineralized or recycled differently depending on minespoil treatment. As mentioned for the subalpine spoil the equally fast decay rate of grass leaves regardless of treatment may have far reaching consequences in that plant growth stimulated by nutrient additions (eg. fertilizer) may result in the accumulation of a thick mat of litter which can behave as a nutrient sink, thereby reducing plant growth and perhaps plant establishment. The decomposition rates (both short and long term) of the products of stimulated plant growth should, therefore, be taken into account when determining reclamation strategy.

The weight loss of slender wheatgrass stems after 12 mo in the variously amended tailing sand planted with slender wheatgrass was also very similar to that of stems placed in the subalpine spoil (37% weight lost in the oil sands spoil vs. 33% in the subalpine spoil). The significantly slower decay of the stems compared with the leaves can be explained by a higher C/N ratio, a smaller soluble component and a higher lignin content in the stems. The potential importance of leaves and stems decaying and releasing nutrients at two different rates on nutrient conservation and organic matter accumulation on minespoils has been discussed previously. It should be emphasized that all the slender wheatgrass decomposition studies were performed after primary production by this grass had peaked. Although the bagged litter was placed below the mat of litter which had developed on the various plots, it is possible that in a system exhibiting a highly productive vegetative cover, the rates of decay would be altered as a result of microclimatic effects.

When the decomposition rates of the filter paper and slender wheatgrass leaves and stems were compared, it was observed

that, with the exception of filters placed on the fertilized sand tailings, decay followed a similar pattern to that observed for the subalpine spoil (i.e. leaves degraded most rapidly followed by stems and lastly filter paper). The faster decomposition of the natural litter has been attributed to its better balanced nutrient content, which encourages the activity of the decomposer microorganisms. The problems of using filter paper decay rates to extrapolate to natural litter decomposition has already been discussed. The data collected in this study imply that the use of a pure substrate such as cellulose to predict the turnover of roots in variously treated tailing sand (Rowell, 1978) may result in an underestimate of root decay and nutrient mineralization from this compartment.

In contrast to the alsike clover leaves which decomposed more rapidly than slender wheatgrass leaves on the subalpine minespoil, sainfoin leaves and stems decayed more slowly than slender wheatgrass leaves on the variously amended tailings sand. Since the N content of the sainfoin leaves was, like that of alsike clover, greater than that in the slender wheatgrass leaves (2.9% in the sainfoin versus 2.3% in the grass litter) it is suggested that factors other than the C/N ratio controlled the decay of this particular legume. Perhaps sainfoin synthesizes polyphenolic compounds which inhibit decomposer organisms, thereby reducing its decay rate. The short term weight loss of the sainfoin and slender wheatgrass litter demonstrated that if a mixture of these two plant species was used to revegetate an area disturbed by mining, nutrient release from the aboveground litter could be expected to occur at three different rates (slender wheatgrass leaves would mineralize most rapidly, followed by sainfoin leaves plus stems and lastly grass stems). This type of pattern of nutrient release may be an advantage for minespoils in the early stages of soil genesis, where rapidly decaying litter would provide a continuous supply of nutrients required to maintain primary production, while the slowly decaying litter would contribute to the accumulation of stable organic matter.

The lack of an amendment effect on the decomposition of sainfoin litter is interesting, in that, contrary to the slender wheatgrass, sainfoin continued to grow after the second growing season. However, the greater density of sainfoin foliage in the sewage sludge and peat amended tailing sand did not significantly influence the rate of decay of dead sainfoin shoots, suggesting that neither the chemical and biological characteristics of the tailings sand nor foliage density were important in regulating the short term decomposition of litter. Further investigations are required to determine how amendment and vegetative cover affects long term decomposition and whether rates of decay of litter and cycling of nutrients back to the primary producers are significantly influenced by the development of a moss layer or burial of the litter by wind and water erosion.

4.2.5.6 N₂ fixation potential of sainfoin. The importance of the N₂ fixing legume in improving the N status of a minespoil (unless the minespoil is highly leachable) has been emphasized for both the grasslands and subalpine spoils. In these spoils, N₂ fixation by the legumes (i.e. rambler alfalfa and alsike clover) was not significantly affected by amendment but acetylene reduction measurements were so variable that any potential effects were presumably masked. Possible mechanisms to decrease the variability (i.e. processing more individual plants or even field measurements of acetylene reduction) were discussed. As in the other two studies, the N₂ fixation potential of the sainfoin at the conclusion of each of the first three growing seasons was highly variable with the highest N₂ fixation activity occurring after the second growing season. Acetylene reduction estimated after the first growing season was extremely low in all treatments. As for the alsike clover in the subalpine spoil, N₂ fixation by sainfoin in the fertilized, sludge and peat treated tailing sand may have been inhibited by high NO₃ (and possibly other forms of N) levels introduced with all three amendments. Because the tailing sand has less of a nutrient adsorptive capacity than the subalpine spoil, it is possible that

much of the N released from the sludge and fertilizer immediately after their application to the sand was concentrated in the soil solution, thereby enhancing the inhibitory effects of the various forms of N on N_2 fixation by sainfoin during the first growing season.

Visser et al. (1984) presented data which suggested that much of the N added with the fertilizer and not taken up by the plants had leached out of the top 15 cm of the tailing sand by the end of the first growing season. The reduced soil N levels in the fertilized plots may account for the increased N_2 fixation exhibited by sainfoin during the second growing season. However, the inhibitory effects of sewage sludge on acetylene reduction continued through the second growing season and were significant during the third growing season. Reasons for the inhibition of N_2 fixation by sewage sludge may have been due to:

(i) long term release of high levels of NO_3 from the sewage sludge aggregates as they decomposed over the term of the study. As mentioned previously, Munns (1977) in his review of mineral nutrition and the legume symbiosis, noted that various studies have demonstrated that high NO_3 levels inhibit initial nodule development, retard nodule growth and reduce rates of N_2 fixation.

(ii) high levels of heavy metals (eg. Cd, Ni, Cu) around the sewage sludge aggregates (areas where the roots tend to concentrate due to the high nutrient status of the sludge). Vesper and Weidensaul (1978) found that 1-5 ppm of Cd, Ni and Cu reduced nodule number, N_2 fixation or both. Zn also reduced nodulation but inhibited N_2 fixation only at the 5-10 ppm levels. Copper levels in the sewage sludge treated plots were significantly greater than in the other treatments (Table 9, Visser et al., 1984), a factor which combined with the high nitrogen levels may have severely reduced N_2 fixation.

Correlation coefficients calculated between shoot N, total soil N, soil NO_3 -N and N_2 (C_2H_2) fixation capacity after growing sainfoin for 14 mo revealed no significant relationships.

Identical results were obtained for the alsike clover planted in the subalpine spoil, and it is therefore believed that in the case of the sainfoin, the lack of a relationship between N fixation, and shoot and soil N status resulted from the same factors (i.e. the interactive effects of soil $\text{NO}_3\text{-N}$ levels, N fixation and shoot N levels). To separate these effects would require detailed research to determine the role of different N levels introduced via fertilizer or sewage sludge on nodule development and N_2 fixation potential.

5. SUMMARY

5.1. FIELD STUDY

The following concluding statements summarize the data collected in the field investigations.

1. With the exception of the grasslands, disturbance by mining caused a significant decrease in the number of bacteria and actinomycetes and lengths of fungal hyphae particularly when the surface soil from the disturbed and undisturbed sites were compared. At the grasslands site, bacterial numbers were greater in the mined site while actinomycete numbers tended not to be different in mined and unmined soils.

2. Fungal hyphae appeared to be more sensitive to mining disturbance than the bacteria.

3. After mining the bacterial community shifted from one dominated by Bacillus and unpigmented Gram negative rods in the grassland or Cytophaga, Flavobacterium and unpigmented Gram negative rods in the undisturbed subalpine site to one dominated by coryneform bacteria.

4. Mining also caused a major shift in the composition of the fungal communities. The most frequently isolated fungi in the grasslands and oil sands minespoil were Cladosporium spp., Alternaria spp. and yeasts - organisms common in the atmosphere. In the subalpine minespoil, Chrysosporium pannorum and Candida sp. were the dominant fungi.

5. Measurements of microbial activity (CO_2 evolution), microbial biomass C and ATP were significantly less in the minespoil than in the undisturbed soil, particularly when undisturbed and disturbed surface soils were compared.

6. The general decrease in bacterial and actinomycete numbers, fungal hyphal lengths, microbial respiration, microbial biomass C and ATP as a result of mining was attributed to reduced soil organic matter levels in the minespoils.

7. The physiological technique for estimating microbial biomass C (Anderson and Domsch, 1978) appears to be the best and most

rapid method for determining the effects of disturbance on the soil microbial biomass.

8. N_2 fixation by free-living soil bacteria was negligible or non-existent in the minespoils and very low in the undisturbed sites. Evidence from the literature suggests that N accumulation in minespoils by free-living bacteria is unimportant in comparison with N inputs from N_2 fixing legumes.

9. Decay rates of fir wood dowel were so slow that differences in the decomposition of a lignified substrate between disturbed and undisturbed sites could not be detected.

10. Decomposition rates of filter paper were similar in undisturbed soils and minespoils over the short term (i.e. 12 mo) but were more rapid in the minespoils than in the undisturbed soil over the long term (i.e. 24-33 mo). Faster incorporation of the filters into the soil matrix in the minespoil thereby improving temperature/moisture conditions is a possible explanation for these results.

11. Although microbial activity and biomass were found to be lower in the minespoils than undisturbed soil, this biomass was nevertheless highly capable of rapidly decaying a pure substrate such as cellulose.

12. The adjustment of the C/N ratios of the wood dowel and filter paper with ammonium nitrate did not accelerate the decay of either of these two substrates. The quality of the C (that in wood being more resistant to microbial attack than that in cellulose) may regulate the decay rate of a substrate to a greater degree than the level of N. It is also possible that much of the added N was lost through leaching before microbial utilization, or may have been present in such a concentrated form that it was toxic to microbial growth.

13. The relationship between filter and natural litter decomposition was found to be site specific and was postulated to be dependent on litter type characteristics (i.e. resource quality), climate and the speed at which decomposing substrates are incorporated into the soil matrix.

14. Leaf litter (wheatgrass leaves or fir needles) decayed more rapidly than the woody substrates in the undisturbed grassland and subalpine sites. The high C/N ratio and high lignin content of the wood explains its decay resistance.

15. In contrast to the grasslands and subalpine sites, jack pine needle litter decomposed at the same rate as jack pine branchwood over the short term (i.e. 24 mo) at the oil sands study area. It is postulated that moisture may be the predominant variable controlling decay at this site. Until moisture conditions are more conducive to microbial growth, resource quality may be a minor factor in controlling the decay of a particular substrate.

16. Microbial activity, microbial biomass C and organic matter increased as the age of the revegetated minespoil at the subalpine site increased. Microbial biomass C was highly correlated with soil organic matter content, suggesting that it is the accumulation of organic C resulting from the decay of the products of plant growth which is the main factor influencing the rate of re-establishment of microbial activity in minespoils.

5.2 TANK STUDY

5.2.1 Prairie Grassland Minespoil

1. Of the three amendments (i.e. gypsum, sewage sludge and topsoil), sewage sludge exhibited the greatest microbial activity with the highest bacterial numbers and largest amounts of fungal hyphae. The application of the sludge to the grassland minespoil significantly increased the number of bacteria in the upper 5 cm of the spoil compared with the other treatments. By stimulating plant growth, sewage sludge may also have increased bacterial growth in the rhizosphere of fall rye during the second growing season.

2. The highly acidic nature of gypsum may have had an adverse effect on actinomycete growth in the spoil since many actinomycetes are highly sensitive to acidity.

3. Both sewage sludge and topsoil amendment were effective in improving microbial activity and biomass in the

grassland spoil. Treatment effects were located mainly in the upper 5 cm of the spoil where the bulk of each of the amendments was concentrated.

4. The CO₂ efflux in all treatments peaked when shoot production by fall rye peaked which suggests that soil microbial activity and plant productivity are closely linked. Carbon released into the spoil in root exudates and sloughed root material was postulated to be a major source of energy for stimulating microbial growth during the early stages of reclamation, particularly in spoils lacking easily available carbon. When growth by fall rye failed during the third growing season, microbial activity returned to its pre-planting levels.

5. Microbial biomass increased with increased plant productivity, and was capable of maintaining itself at a reduced metabolic level for at least one year after plant growth failed.

6. Over the first two growing seasons, decay of cellulosic filter paper placed on the spoil surface in plots planted with fall rye or rambler alfalfa was most rapid in the topsoil treatment, followed by the sewage sludge treatment and the gypsum and untreated spoil. When the growth of fall rye ceased, the decay potential of the topsoil and sludge treatments decreased to a point where no significant effects of amendment on decomposition were detected. However, in plots planted with alfalfa (which continued to grow over the term of the study) the decay potential of the topsoil and sludge treatments increased substantially as plant cover increased. It is suggested that cellulose decay potential of the grassland minespoil in the early stages of reclamation may be related to shoot density and productivity rather than the chemical and biological characteristics of the amended spoil. Increased foliage density may ameliorate microclimatic (temperature and moisture) conditions at the soil surface, thereby accelerating cellulose decay. As moisture and temperature conditions at the soil surface improve with increasing plant cover and dead litter accumulation, the nutrient quality of the litter and amended minespoil may become increasingly important in regulating cellulose decay.

7. Rambler alfalfa, capable of fixing N_2 , was established in all treatments. No amendment effects on N_2 fixation were recorded, mainly because the data were extremely variable. N_2 (C_2H_2) fixation potential appeared to be greatest after the second growing season.

5.2.2. Subalpine Spoil

1. The immediate effects of amendment on the microbial status of the subalpine spoil was restricted mainly to the top 5 cm of the spoil since the rocky nature of the spoil made it difficult to incorporate the amendments (sewage sludge and peat) into the subsurface spoil.

2. Although sewage sludge introduced high levels of bacteria to the spoil, peat was the most effective amendment for increasing not only bacterial numbers but also actinomycete numbers and fungal hyphae.

3. The growth of slender wheatgrass over two growing seasons appeared to have a stimulating effect on bacterial and actinomycete numbers in the subsurface (5-15 cm) spoil but not in the surface 0-5 cm spoil. Root exudates and sloughed roots may have stimulated microbial growth in the deeper soil where C may have been more limiting than in the surface spoil. However, fungal hyphae were not significantly affected by plant growth which suggests that bacteria and actinomycetes may respond to root exudates to a greater degree than the saprophytic soil fungi during the initial stages of revegetation.

4. The higher microbial numbers measured in the top 5 cm of the peat and sewage sludge treated minespoil supported the microbial activity and biomass determinations which were also observed to be greater in the peat and sewage sludge treatments than in the fertilizer and control treatments. There was some evidence that release of nutrients from the sewage sludge and peat over the first 15 mo of the study stimulated microbial growth in the unplanted spoil but that these nutrients had been utilized by 27 mo after treatment. Loss on ignition measurements suggested that microbial

oxidation of stable organic matter from the sludge and peat treated spoil was negligible over the three year term of the study.

5. Over the first three growing seasons after amendment and planting microbial activity and biomass C development in the subalpine spoil were more stimulated by the growth of slender wheatgrass than by white spruce. The growth of slender wheatgrass was particularly effective in enhancing microbial activity and biomass in the upper 5 cm of the sewage sludge, fertilizer and unamended plots. Since slender wheatgrass has a more fibrous root system than white spruce and is more productive over the short term than white spruce, it has the ability to more rapidly improve the microbially available C status of an impoverished soil by introducing large quantities of dead plant parts and root exudates. This would be particularly important in the untreated and fertilized subalpine spoil where the supply of highly available carbon for stimulating microbial growth would be dependent almost entirely on plant growth. This suggests that the development of microbial productivity is strongly linked with levels of primary productivity mainly because the metabolic activity of microorganisms is regulated by the availability of an energy source. Unless alternative sources of carbon are applied to a minespoil, the energy required for microbial growth and maintenance would be solely dependent on plant productivity.

6. Since this study suggests that the rate of microbial development in an amended minespoil is closely associated with the type of vegetative cover, the choice of plant species and the longevity of a plant species are important factors to consider when determining a reclamation strategy. Short-lived species such as slender wheatgrass may stimulate microbial activity over the short term, but once primary production ceases microbial activity may rapidly return to its pre-planting level. The microbial biomass data collected for both the grassland and subalpine spoils suggest that biomass can maintain itself for some time after plant growth ceases (presumably as long as there is carbon available from dead plant parts). By planting a longer lived plant species such as woody

shrubs, deciduous trees or conifers, soon after a short-lived plant species dies, the regression of the microbial system to its pre-planting phase may be averted.

7. It appears that the levels of plant available nutrients introduced with an amendment determines the degree of primary production (particularly of fast growing species) which in turn regulates the rate of microbial development in a minespoil.

8. There was some evidence that fertilizer, shortly after its application, inhibited microbial activity in the upper 5 cm of the spoil. A decrease in pH or the inhibitory effects of ammonia resulting from the fertilizer application may have caused this decrease in activity.

9. Cellulosic filter paper decayed most rapidly in the sludge treated plots but, unlike the grassland spoil, filter decomposition was not significantly influenced by vegetation type (i.e. grass or white spruce). It was postulated that during the early stages of revegetation, microclimatic effects on decay rates resulting from the growth of slender wheatgrass were less pronounced than those observed for fall rye in the grassland spoil plots, because shoot production by slender wheatgrass was lower than that exhibited by fall rye.

10. Over the four year term of the study, the cellulose decay potential of the variously treated subalpine spoil planted with slender wheatgrass was not significantly altered and remained highest in the sewage sludge treated plots. The nutrients and microbial inoculum present in the sludge accumulated on the spoil surface may have accelerated the decay of the cellulose by improving the nutrient quality of the paper and providing microorganisms highly active in cellulose decay.

11. Although the productivity of slender wheatgrass was greatly stimulated by sewage sludge and fertilizer amendment, decomposition of the incoming litter (both stems and leaves) was equally fast in all treatments. This resulted in a greater accumulation of undecomposed litter (and greater nutrient

immobilization) in the highly productive treatments than in the treatments exhibiting low productivity.

12. Slender wheatgrass stems decomposed at a slower rate than the leaves, resulting in two different rates of nutrient release.

13. A comparison of the decay rates of slender wheatgrass stems and leaves and filter paper demonstrated that the decay rates of filters could not be extrapolated to predict decay rates of litter.

14. Alsike clover leaves and stems exhibited a faster decomposition rate than slender wheatgrass leaves and stems. The higher N content in the shoots of the N_2 -fixing alsike clover than in the slender wheatgrass shoots may have been one factor which hastened the decay of the legume litter.

15. As was the case for the Rambler alfalfa in the grassland spoil, no significant amendment effects on N_2 fixation capacity of alsike clover could be detected. Again N_2 (C_2H_2) measurements were excessively variable. During the first growing season alsike clover in the peat amended spoil demonstrated no N_2 fixation ability and this was attributed to high NO_3 levels in the peat which inhibited infection, nodulation and N_2 fixation by Rhizobium. No significant relationships were detected between clover shoot N, total soil N, soil NO_3 -N and N_2 (C_2H_2) fixation capacity measured after the second growing season.

5.2.3. Oil Sands Spoil

1. The immediate effects of amendment on the microbial status of the tailing sand was very similar to that observed for the subalpine spoil i.e. peat amendment introduced more microbial inoculum (bacteria, actinomycetes and fungal hyphae) to the sand than the other amendments.

2. The microorganisms and nutrients contained in the peat and sewage sludge amendments were incorporated to a deeper depth in the tailing sand than in the rocky subalpine spoil.

3. As in the subalpine spoil, short term growth of grass had minimal effects on microbial numbers and hyphal lengths in the surface spoil layer. Actinomycetes did increase in the 5-15 cm deep sludge-treated sand where profuse root production by slender wheatgrass may have stimulated actinomycete growth.

4. Although microbial respiration and biomass C were generally greater in the unvegetated subalpine spoil than in the unvegetated tailing sand, they were similarly affected by amendment in the two spoils over the term of the study i.e. microbial activity and biomass were greater in the peat and sewage sludge treatments than in the fertilized and unamended sand. Both the peat treated subalpine and oil sands minespoils exhibited increases in CO₂ efflux at 15 mo after amendment suggesting that the oxidation of peat C may have been stimulated at this time. However, loss on ignition measurements demonstrated no massive loss of stable organic matter over the four year study.

5. With the exception of the unamended sand where the paucity of nutrients resulted in plant failure and no soil microbial development, the patterns of plant (slender wheatgrass and jack pine) growth, litter input and microbial development in the subalpine and oil sands spoils were very similar. Jack pine, like white spruce, had negligible effects on soil microbial activity, while the growth of slender wheatgrass generally stimulated microbial growth and productivity in the 0-5 cm deep amended sand. These results support the premise that there is a strong relationship between plant and microbial productivity, particularly in C limited soils such as the fertilized tailing sand in this study where increases in microbial activity and biomass were mainly a result of the products of plant growth (i.e. root exudates, dead plant litter).

6. As in the subalpine spoil, the nutritional quality of the amendments determined the degree of primary production by slender wheatgrass which in turn appeared to regulate microbial development, particularly in the fertilizer and sewage sludge treatments. Microbial activity and biomass in the peat treated sand planted with slender wheatgrass demonstrated an increasing trend with time, but it

was difficult to determine whether this was due to the slow biological oxidation of the peat, microbial stimulation by root exudates and plant litter/root input or an elevated loss of peat C resulting from the input of root exudates (i.e. "priming effect").

7. The link between microbial development in a spoil and plant productivity emphasizes the importance of maintaining a productive plant cover on a minespoil.

8. In the first two years after amendment and planting, the decomposition of filter paper was fastest in the sewage sludge and fertilizer treated sand planted with slender wheatgrass. The higher cellulose decay potential of these two treatments was attributed to improved nutrient conditions in the sand surrounding the filters, introduction of cellulose decay microorganisms in the sewage sludge and amelioration of microclimate resulting from a greater foliage density and a thicker litter layer than that observed in the peat and control treatments.

9. Low levels of P in the peat may have reduced rates of cellulose decay in this treatment in both the oil sands and subalpine spoils.

10. Over the long term (four years) the cellulose decay potential of the sludge amended sand decreased significantly from that observed over the first two years after planting while decay rates in the other treatments were not significantly altered. The development of a moss layer in the sludge treated plots may have adversely affected decay rates of the filters.

11. The short term decay rates of slender wheatgrass litter measured three years after amendment and planting were not significantly affected by treatment and were approximately the same in the subalpine and oil sands spoils. These results imply that, during the early stages of revegetation, the chemical and physical properties of a minespoil and the microbial biomass in the soil immediately beneath the decaying litter play a minor role in determining the initial rate of decomposition of incoming dead plant material. For this reason, excess fertilization may stimulate plant growth but may not necessarily accelerate litter decay resulting in

the accumulation of litter on the spoil surface which behaves as a nutrient sink, and a protective cover for rodents.

12. As was the case for the subalpine spoil, slender wheatgrass stems lost less weight than the leaves after 12 mo in the field. Also, filter paper decomposition rates were not very comparable to those measured for grass leaves or stems.

13. Sainfoin leaves and stems decayed more slowly than slender wheatgrass leaves and stems. Also amendment did not significantly affect the decomposition rates of sainfoin litter.

14. Like the rambler alfalfa in the grasslands spoil and alsike clover in the subalpine spoil, sainfoin exhibited no significant amendment effects on N_2 fixation over the first two growing seasons. The N_2 fixation potential of sainfoin over the first growing season was extremely low in all treatments and this was attributed to inhibition by high levels of NO_3-N introduced with the fertilizer, peat and sewage sludge. Significant inhibitory effects of sewage sludge on N_2 fixation by sainfoin were recorded after the third growing season. Long term release of NO_3-N from the decomposing sewage sludge clumps and/or high levels of heavy metals (e.g. Ca, Ni, Cd) around the sludge clumps may have caused a reduction in nodule formation and N_2 fixation. No significant relationships were detected between sainfoin shoot N, total soil N, soil NO_3-N and N_2 (C_2H_2) fixation measured after the second growing season.

6. RECOMMENDATIONS FOR FURTHER RESEARCH

1. More research is required to determine the rates at which N is accumulated on minespoils via N_2 -fixing plant species since these plants provide a less expensive and more long-term alternative to maintenance fertilizer applications.

2. It is necessary to obtain information on N mineralization rates from decaying residues of N_2 -fixing species and from soil amendments under field conditions since this N supply will determine the maintenance of a specific vegetative cover. The N requirements of various plant species currently being used for reclamation purposes should also be established to determine if slow-growing species would benefit from legume nurse crops.

3. Microbial oxidation of C from amendments such as peat and the influence of plant growth (particularly fast-growing grass and herbaceous species) on this oxidation requires clarification.

4. More data are required regarding long-term decay rates of plant residues (both above- and below-ground) and the rates at which plant-available nutrients are released from these residues. Although the continual addition of nutrients may stimulate plant growth, decomposition rates of incoming plant residues are not stimulated to the same degree resulting in a thatch of litter which behaves as a slow-turnover nutrient sink. Thus effects of different management procedures such as grazing, cropping, burning and plowing in plant residues, on plant litter decay and maintenance of a vegetative cover should also be studied.

5. Data from this study suggest that spoil and amendment characteristics play a minor role in the short-term decomposition of above-ground plant residues such as leaf and stem litter. It would be profitable to define more clearly the effects of different spoil types and amendment combinations on the degradation of both above- and below-ground plant residues. It is possible root decay is much more influenced by spoil/amendment characteristics than is litter deposited on the spoil surface, particularly over the short term (12 mo).

6. This study concentrated on the effects of single amendments on individual plant species. Additional research addressing the effects of combinations of amendments (e.g. peat/fertilizer; sewage sludge/peat) on plant growth (different species) and decomposition rates of plant residues would be useful.

7. Sewage sludge, applied at low rates, has the potential for greatly enhancing plant growth over a relatively long time (5 years) on a variety of minespoils as demonstrated in this study. However, there is some indication that it may be inhibitory to such essential processes as symbiotic N_2 -fixation. Further study on the inhibitory effects of sewage sludge on plant growth, N_2 -fixation and decay rates is necessary. This research should be coupled with studies on N mineralization from the sludge since ammonium-N may be a major cause of toxicity in sludge-treated soils.

8. On minespoils, where agricultural crops are not the final land use goal, it may be advantageous to establish a short-lived, fast-growing plant species (fertilized only once) to provide erosion control and then replace this cover with long-lived, slower-growing woody shrubs and trees once the initial cover dies. It would be interesting to determine if the nutrient sink created by the fast-growing species would decay rapidly enough to provide sufficient nutrients for the establishment of the slower-growing species.

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REINSTATEMENT OF BIOLOGICAL ACTIVITY IN SEVERELY
DISTURBED SOILS: EFFECTS OF DIFFERENT AMENDMENTS TO
THREE DIFFERENT MINESPOILS ON SELECTED SOIL PHYSICAL AND
CHEMICAL PROPERTIES AND ON PLANT GROWTH

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ABSTRACT

Three different minespoils (prairie grassland, subalpine and extracted oil sands) were each treated with three different organic or inorganic amendments and then planted with four different plant species. Amendment effects on some of the soil chemical characteristics and on plant growth were then monitored over 2 and 3 years respectively.

Application of topsoil was most effective in reducing the high sodium adsorption ratio characteristic of the prairie minespoil. It also promoted the best growth by Rambler alfalfa. Fall rye and crested wheat were most productive in the sewage and topsoil treated spoil, while Russian wild rye was most stimulated by the sewage sludge treatment. Gypsum was observed to be the least effective of the amendments tested. Crested wheat, Russian wild rye and Rambler alfalfa, once established, behaved as long-term cover crops, but fall rye was found to be short-lived (2 years).

The poor fertility status of the subalpine and oil sands spoil was most improved over the long term by the addition of sewage sludge. The slender wheatgrass, Alsike clover, white spruce and willow planted in the subalpine soil, and the slender wheatgrass, sainfoin, jack pine and bearberry planted in the extracted oil sands were generally most productive in the sewage sludge treated spoils. The slow growing woody perennials (white spruce, bearberry) also performed well in the peat amended spoils, but low extractable P levels in the peat may have suppressed growth by the faster growing species. Mineral fertilizer stimulated shoot production by the grass, clover and willow in the subalpine spoil, but elicited a poor response from plants grown in the tailing sand. Much of the fertilizer applied to the sand had leached out of the rooting zone by the end of the first growing season. Slender wheatgrass followed the same growth pattern as fall rye and did not reseed readily. Heavy metals introduced with the sewage sludge were not significantly concentrated in plant tissue over the short term. The N₂-fixing legumes (clover and sainfoin) were observed to be least sensitive to low soil N levels.

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1. INTRODUCTION

Mining for energy resources such as coal and oil generally results in large tracts of land lacking vegetation. Rapid revegetation of such areas is necessary: 1) to provide erosion control, 2) to restore the land to a productive state, and 3) to improve the visual quality of the disturbed land. Vogel (1981), in his guide for revegetating coal minespoils in the eastern U.S., outlined what he considered to be the main soil characteristics influencing successful vegetation establishment. They included:

1. Chemical properties:

- i) soil pH
- ii) acid induced toxicities
- iii) nutrient deficiencies.

2. Physical properties:

- i) particle size distribution
- ii) bulk density
- iii) steepness of slope
- iv) erosion potential
- v) color of soil (dark-colored soils can result in high surface temperatures which may be lethal to seedlings and cause soils to dry out more rapidly)
- vi) aspect (whether the slope faces south, west, north or east).

3. Biological properties:

- i) decomposer microorganisms - ultimately important in recycling nutrients (for uptake by higher plants).
- ii) symbiotic microorganisms, such as N_2 fixers which improve the N status of legumes, alder etc., and mycorrhizal associations of various types which are believed to improve plant survival and growth.
- iii) soil fauna which are considered regulators of the rates of organic matter decomposition (and hence of nutrient cycling)

by comminution of plant debris, by consumption of various types of organic matter and by dispersal of microbial inoculum in the soil system.

According to Bradshaw et al. (1978) there are three main steps involved in the rehabilitation of land disturbed by mining.

1. The restoration of soil fertility and structure - aim to overcome adverse physical, nutritional and toxicity factors.

2. The establishment of plant species through natural colonization or controlled planting programs.

3. The subsequent management for ecosystem development - once vegetation has been established the system can be left untended to develop, but this is usually a very slow process. To accelerate ecosystem development on derelict land, some management (such as fertilizer additions, reseeding, planting different species of plants) related to the ultimate land use is desirable.

Many minespoils are relatively infertile and may have toxicity (e.g. acidic coal minespoils in eastern U.S.) or textural problems (e.g. sodic clay minespoils of the Northern Great Plains region of the U.S.). They are usually microbiologically impoverished lacking both decomposer and symbiotic (both mycorrhizal and nitrogen fixing) inoculum. These factors generally result in unsuccessful plant establishment and poor plant growth. To overcome the problems of soil fertility and poor soil structure, most minespoils are ameliorated with inorganic (e.g. fertilizers, lime, gypsum) or organic (e.g. sewage sludge, peat, topsoil, mulches) amendments. The spoils are contoured in such a way that the susceptibility to erosion of the site is minimized. The amendments are essential for plant establishment, survival and growth in that they can improve soil structure, provide nutrients, and also serve as a source of microbial inoculum. Ideally the type of amendment, or combination of amendments, chosen to ameliorate a minesite would have all the chemical, nutritional and biological properties required to establish and sustain a vegetative cover which would develop into a productive ecosystem with a minimum of additional management. It has become well-recognized that efficient, balanced functioning of soil

organisms (through their action in organic matter decomposition, nutrient cycling and symbiotic relationships to higher plants) is essential for ecosystem development and maintenance. As a result research was initiated which had the following objectives:

1. to study primary production of test plant species in various types of minespoil which had been previously ameliorated with organic or inorganic amendments or left unamended.

2. to provide detailed information on the rates of redevelopment of biological activity in various minespoils with and without soil amendments (using rates of increase in microbial biomass and species diversity of fungi and bacteria together with rates of increase in total soil metabolic activity as the indices of redevelopment).

3. to provide data on the types of microorganisms associated with the roots of plants grown in the test minespoils under the range of experimental treatments and to provide survey data on the speed of mycorrhiza formation and (where applicable) nodule formation on test plant roots.

4. to present conclusions and recommendations on the methods of rapid re-instatement of productive soil-plant systems on minespoils.

This report deals primarily with the results obtained on the effects of various amendments of three minespoils on some soil chemical and physical factors, plant growth, and nutrient levels in the foliage of selected plant species after one or two growing seasons.

It should be noted that, in this study, the aim was to investigate in detail the effects of single amendments and not to consider the effects of various combinations of amendments.

2. MATERIALS AND METHODS

The three areas chosen for the study were a shortgrass prairie site which had been mined for coal in the early 1950's and subsequently abandoned (Bow City, Alberta); a subalpine boreal forest site which is currently being mined for coal (Cardinal River, Alberta) and a boreal forest site which is currently being mined for oil sands (Suncor site, Fort McMurray, Alberta). The approximate locations of the three sites and the vegetation and soil types both before and after mining disturbance are presented in Table 1.

2.1. FIELD STUDY

At each study site, five sampling plots, each 5m x 5m, were laid out in a 15 x 15 m area in areas disturbed by mining and also in adjacent undisturbed areas (with the exception of the oil sands site where due to the inaccessibility of the disturbed areas, plots were established only in an undisturbed jack pine woodland). At each site, five soil cores were removed from the disturbed and undisturbed plots. The undisturbed plot cores were separated into organic and mineral layers while the disturbed cores (which showed no horizon development) were separated into 0-5 cm and 5-15 cm depths. Samples were sieved (< 2 mm) and air dried. To determine the effects of disturbance on selected soil chemical and physical parameters, subsamples from each depth from each site were bulked and tested for sodium adsorption ratio (SAR), organic and CO₃-C, total N, P, Fe, Ca, Mg, Na. The SAR was calculated from the soluble Ca, Mg and Na concentrations (me l⁻¹) in saturated soil extracts, i.e.:

$$SAR = \frac{Na}{\sqrt{1/2 (Ca + Mg)}}$$

as described by Bower and Wilcox (1965). Organic carbon was calculated by subtraction of CO₃-C from total C determined by dry combustion using an induction furnace (Allison, Bollen and Moodie, 1965; Allison and Moodie, 1965); CO₃-C was determined by a pressure-calcmeter method (Allison and Moodie, 1965). Total N was determined by a semi micro-Kjeldahl method employing a Hg catalyst (Bremner, 1965), and the total nutrients were determined by an acid digestion (HNO₃/HClO₄) procedure and atomic absorption

Table 1. Summary of vegetation and soil types at each study site.

Site	Location	Vegetation		Soil type	
		Before	After	Before	After
Prairie grassland	Bow City, Alberta	mixed grass prairie	<u>Kochia scoparia</u>	Brown	saline-sodic,
		(<u>Agropyron</u> , <u>Bouteloua</u>		chernozem	sandy clay
		<u>gracilis</u> , <u>Artemisia</u>			loam (56% sand
Subalpine Boreal	Luscar, Alberta	<u>cana</u> , <u>A. frigida</u> ,			22% clay, 22%
		<u>Mamillaria vivipara</u>			silt)
		spruce/fir forest	-	Brunisol	Regolith
		(<u>Picea engelmannii</u> ,	(seeded with		
		<u>Abies lasiocarpa</u> with	timothy, blue-		
Boreal Forest (Oil sands)	Ft. McMurray, Alberta	<u>Hylocomium splendens</u> ,	grasses, fescues,		
		<u>Empetrum nigrum</u> ,	wheatgrasses)		
		<u>Ledum groenlandicum</u>			
		understory)			
		- jack pine/lichen	-	Brunisol	Sand
Boreal Forest (Oil sands)	Ft. McMurray, Alberta	woodland (<u>Pinus</u>	(seeded with		
		<u>banksiana</u> , <u>Cladina</u>	grasses, legumes)		
		<u>mitis</u>)			
		- black spruce/tamarack			
Boreal Forest (Oil sands)	Ft. McMurray, Alberta	peatland (<u>Picea</u>			
		<u>mariana</u> , <u>Larix laricina</u>)			

spectroscopy. Many of the methods used for these analyses have been conveniently summarized by McKeague (1976). Soil pH was determined electrometrically using 10 g soil in 20 ml distilled water.

2.2. TANK STUDY

2.2.1. Set-Up

In order to monitor more closely the effects of the variously amended minespoils on plant growth and associated soil biological, chemical, and physical factors, it was decided that a major part of the research be carried out in Calgary rather than at the more inaccessible field sites. Large quantities of minespoil from the grassland, subalpine and oil sand sites were transported to Calgary and each placed into separate plywood frames (soil tanks). A plan of a soil tank and its dimensions are presented in Figure 1). Each tank was constructed with internal dividing walls to prevent mixing of amendments at the boundaries between plots. Only the first 12 plots in each tank were used in the study because not enough spoil was available to set up 16 plots. The minespoils were divided amongst the plots and levelled so that there was approximately a 60 cm deep layer of soil per plot. Amendments were then applied to allow three replicate plots per treatment (i.e. type of amendment). Three unamended control plots were also set up for each of the three spoils. The amendments tested on each of the spoil types, their origin, application rates and application procedures are presented in Table 2. Some of the chemical and physical characteristics of each amended spoil were determined using techniques described previously. Loss on ignition was measured by placing a sieved, pre-dried (105°C) soil sample in a muffle furnace, igniting it at 375°C and leaving it for 24 h. Weight loss of the sample was then determined.

After amendment but immediately prior to planting, one large (2-3 kg) soil sample was randomly removed from two depths in each of the plots to determine the immediate effects of amendment on pH, E.C., extractable cations, SAR, total N, $\text{NO}_3\text{-N}$, extractable P and DTPA extractable metals. The pH, extractable cations, SAR and total N were determined by the same methods as those described for

PLAN OF SOIL TANK

Figure 1. Plan of soil tank.

Table 2. Details of the amendments applied to each spoil type.

Spoil Type	Amendment	Application Rate	Application Procedure
Prairie Grassland	Topsoil from Bow City grass-land	15 cm layer	Spread evenly on spoil surface
	Gypsum	28.6 mT ha ⁻¹ (dry) (calculated to replace Na down to a 15 cm depth)	Spread on spoil surface; then rototilled to 15 cm depth
	Anaerobically digested sludge from Calgary, Alta. sewage lagoons.	46 mT ha ⁻¹ (dry)	Wet sludge spread on soil surface, allowed to dry, then rototilled to 15 cm depth.
Subalpine boreal forest	Feather moss peat from under spruce forest at Canmore, Alta.	15 cm layer	Evenly spread on soil surface, then rototilled into spoil.
	NPK fertilizer (23-23-0, 0-0-62)	112 kg N ha ⁻¹ 112 kg N P ₂ O ₅ ha ⁻¹	Spread by hand immediately prior to planting
	Sewage sludge (as above)	Same rate as for prairie grassland	Same procedure as for prairie grassland
Boreal Forest (Oil Sands)	Peat (as above)	Amendments applied at the same rate and in the same manner as that described above for the subalpine forest site.	
	Fertilizer (as above)		
	Sewage Sludge (as above)		

the field study samples. The electrical conductivity (E.C.) of the saturated soil extract was measured by the technique of Bower and Wilcox (1965). The depths sampled were 0-5 cm and 5-15 cm except for the peat and topsoil amended plots where 0-5 and 15-25 cm (spoil beneath amended soil) were sampled. Nitrate nitrogen was determined by extraction in .01 N CuSO_4 + .007 N Ag_2SO_4 solution and subsequent measurement by a phenol disulphonic acid colorimetric procedure (Bremner, 1965). Extractable P was measured by extraction in a .03 N H_2SO_4 + .03 N NH_4F solution and subsequent determination by an ascorbic procedure (John, 1970; Miller and Axley, 1965). DTPA extractable metals were extracted in 0.005 M DTPA, 0.01 M CaCl_2 , 0.1 M TEA, pH 7.3 and determined by atomic absorption spectroscopy (Lindsay and Norvell, 1969). Total N, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ were measured again 4 months after the application of fertilizer to the subalpine and oil sand minespoils in an effort to determine leaching losses of these nutrients after one growing season. The above-mentioned chemical and physical parameters were also assessed two growing seasons after planting fall rye in the prairie grassland minesoil and two growing seasons after planting slender wheatgrass in the subalpine and oil sand spoils.

2.2.2 Planting

After amendment each plot was subdivided into four subplots with 0.5 m walkways between them and each amended subplot was then planted with a grass, herb shrub or tree species resulting in four test plant species per plot (see Fig. 1 for the layout of one of the sewage sludge treated oil sands plots). The species planted in each of the test minespoils are listed in Table 3. Grasses and legumes were seeded by hand with each plot receiving known weights of seed. The planting rates and percentage germination of the grasses and legumes are presented in Table 4.

Spruce and pine were planted with 20 cm spacing in 14 rows of 8 seedlings each resulting in approximately 112 seedlings per subplot. Jack pine (grown by Northern Forest Research Centre, Canadian Forest Service, Edmonton) and white spruce were grown in

Table 3. Species planted in each type of minespoil.

Spoil Type	Plant Species	
	Common Name	Scientific Name
Grassland coal mine	Russian Wild Rye	<u>Elymus junceus</u> Fisch.
	Crested Wheat	<u>Agropyron cristatum</u> (L.) Gaertn.
	Fall Rye	<u>Lolium perene</u> L.
	Rambler Alfalfa	<u>Medicago sativa</u> L. var. Rambler
Subalpine coal mine	Slender Wheatgrass	<u>Agropyron trachycaulum</u> (Link) Malte
	Alsike Clover	<u>Trifolium hybridum</u> L.
	White Spruce	<u>Picea glauca</u> (Moench.) Voss
	Willow	<u>Salix</u> sp.
Extracted oil sand	Slender Wheatgrass	<u>Agropyron trachycaulum</u> (Link) Malte
	Sainfoin	<u>Onobrychis corniculatus</u> L.
	Jack pine	<u>Pinus banksiana</u> (Lamb
	Bearberry	<u>Arctostaphylos uva-ursi</u> (L.) Spreng

Table 4. Planting rates and % germination of grasses and legumes.

Spoil Type	Plant Species	Amount seed sown (g/m ²)	No. seeds/g	% germination (lab conditions)
Grassland	Russian Wild Rye	1.24	409	94
	Crested Wheat	1.79	439	61
	Fall Rye	10.39	47	98
	Rambler Alfalfa	1.10	508	85
Subalpine	Slender Wheatgrass	2.25	263	81
	Alsike Clover	0.44	1333	82
Oil Sands	Slender Wheatgrass	2.25	263	81
	Sainfoin	16.39	37	79

peat moss in 47 cm³ and 192 cm³ Spencer-Lemaire containers respectively for 20 weeks prior to planting. Willow and bearberry (purchased from the Edmonton nurseries) were planted with 25 cm spacing in 13 rows of 7 seedlings each resulting in 91 cuttings per subplot. The willow cuttings were grown in 95 cm³ cylinders of peat moss while the bearberry cuttings were rooted in pots containing 100 cm³ of a peat-sand mixture. All seeding and planting was effected in the last two weeks of June. Plots were kept free of weeds over the term of the study.

2.2.3. Primary Production Assessments

2.2.3.1. Grasses and legumes. Shoot production of the grasses and legumes was assessed at the conclusion of each of the first three growing seasons in the following way:

i) at the end of the first growing season (September), five intact grass or legume plants were removed from each of the three replicate subplots of each amendment in each spoil type. Shoots were separated from roots and dried in paper bags at 80°C for one week and weighed. Shoot production values on a m² basis were determined by multiplying the average dry shoot weight (g) per plant per amendment by the density of each plant species per subplot. The densities of each grass or legume species in each subplot had been determined four weeks after seedling emergence by counting the number of plants occurring in each of three, 25 cm x 25 cm random quadrats in each subplot.

ii) At the conclusion of the following two growing seasons (September) shoot production was assessed by means of randomly clipped quadrats. All shoots in each of three randomly placed quadrats (25 x 25 cm) in each subplot were clipped approximately 1.28 cm above the soil surface resulting in 9 replicate quadrats per treatment per sample time. Shoots were dried and weighed as described in (i) above and weights converted to a g m⁻² basis.

Much of the legume and slender wheatgrass shoot material clipped after the third growing season was dead litter (mainly stems) from the previous year. The dead litter was separated from the live

material, dried and weighed.

iii) Root production for the fall rye, slender wheatgrass and the legumes was estimated after the second growing season. Two soil cores (6 cm wide x 14 cm long) were randomly removed from each subplot and roots were washed out using 2 and 0.5 mm sieves. Roots washed out on the 2 mm sieve were picked off, dried at 80°C and weighed while the fine roots on the 0.5 mm sieve were floated out, removed from the water and dried. The dry weight of roots per soil core was then extrapolated to g dry root m⁻² to a depth of 14 cm.

2.2.3.2 Trees and Shrubs.

i) Shoot weight was determined at the conclusion (September) of each of the first three growing seasons for jack pine, bearberry and willow and four growing seasons for white spruce. At each sampling time five plants were clipped (at the soil surface) in each plot, dried (at 80°C) and weighed. Results were expressed as g dry shoot per plant.

ii) Total root weights were measured at the end of the second growing season. Willow roots were not sampled due to the inability to distinguish individual root systems. The roots of the other woody plants were sampled by digging at points midway between plants to a depth of 15 cm in the subalpine minespoil and 25 cm in the oil sands. Roots were washed out of the soil, dried at 80°C, weighed and expressed as g dry root per plant. Plant height was measured at each sample time and survival was measured for the first two years. Dieback of willow tops was determined by measuring total height and total live height of five random plants per plot. Winter injury to bearberry was evaluated by making visual estimates of five randomly selected plants per plot. Dieback of both willow and bearberry was measured at the start of the third growing season.

2.2.3.3 Foliage chemical analysis. Elemental concentrations (P, K, Ca, Mg, Mn, Cu, Zn and Fe) were determined for the foliage of slender wheatgrass, sainfoin, jack pine (needles only), and white spruce

(needles only), harvested from each plot at the conclusion of the first growing season. The methods of determination were similar to those described for nutrient analysis of the spoils after grinding the samples in a Wiley mill to pass 40 mesh screen.

Total N (Bremner, 1975) was determined for slender wheatgrass, alsike clover, willow, jack pine and sainfoin foliage collected after the second growing season since there was not enough plant sample for this analysis after the first growing season.

3. RESULTS

3.1 SOIL CHEMICAL CHARACTERISTICS

3.1.1 Field Study

The effects of mining disturbance on selected chemical characteristics of the soils under investigation are presented in Table 5. The data show that:

i) the pH of the prairie grassland soil dropped slightly after mining, whereas the pH of the subalpine and oil sands soils increased dramatically. The use of caustic soda to remove oil from the sand is presumably the main reason for the pH increase in the extracted sand tailings.

ii) the Na adsorption ratio (SAR) increased significantly at the prairie grassland site after mining. The high clay content and Na level of this minespoil explain the high SAR. The SAR and Na levels were not affected by mining at the subalpine forest site.

iii) as a result of stripping the organic mat from the subalpine site when mining, the organic C level in the resultant spoil was very low. The organic C levels measured in both the grassland and subalpine forest minespoils should be viewed with caution since these analyses were hampered by the presence of coal particles which were no doubt included in the C analyses. The extraction procedure used to remove oil from oil bearing sand results in what appears to be pure sand devoid of organic carbon and all other nutrients.

iv) the removal of the organic mat during mining at the subalpine site resulted in a decrease in total soil N; whereas the % N in the grassland site was not affected.

v) concentrations of P and Fe were greater in the minespoil than in the undisturbed soil at the subalpine site. There was virtually no P present in the oil sand tailings. Levels of Ca and Mg decreased after mining at the grassland site while mining the subalpine site resulted in higher soil Mg levels.

Therefore the main effects of mining disturbance at the three study sites were:

Table 5. Chemical characteristics of test soils before and after disturbance.

Site and Soil	pH	SAR	C(%)		N(%)	Total nutrient elements ($\mu\text{g g}^{-1}$)				
			organic-C	CO ₃ -C		P	Fe	Ca	Mg	Na
Prairie Grassland										
Undisturbed topsoil (0-15 cm)	7.5	0.2	1.8	1.0	0.2	1412	15400	26152	10154	230
Undisturbed mineral (15-25 cm)	7.3	0.2	1.7	1.3	0.2	1003	15030	37775	11005	230
Disturbed mineral (0-5 cm)	6.9	19.5	6.4	0.1	0.2	1188	17925	4990	3539	4485
Disturbed mineral (5-15 cm)	6.8	19.0	5.1	0.0	0.2	1934	16550	4990	3034	4830
Subalpine Boreal Forest										
Undisturbed organic	4.5	0.1	31.3	0	1.0	449	7140	6583	1690	230
Undisturbed mineral	4.2	0.5	4.0	0	0.2	508	9500	661	1222	460
Disturbed mineral (0-5 cm)	7.0	0.2	3.5	0	0.1	1254	30025	3127	5168	345
Disturbed mineral (5-15 cm)	7.3	0.3	10.9	0.2	0.2	845	24925	7074	4499	345
Boreal Forest - Oil Sands										
Undisturbed organic (0-5 cm)	5.1	NM	37.6 ¹	NM	NM	NM	NM	NM	NM	NM
Undisturbed mineral (5-15 cm)	6.0	NM	0.6	NM	NM	NM	NM	NM	NM	NM
Disturbed mineral (0-5cm)	8.7	NM	0.4	NM	0	1.2	2.6	NM	NM	NM
Disturbed mineral (5-15 cm)	8.7	NM	0.2	NM	0	1.0	3.2	NM	NM	NM

1 % loss on ignition determined for Oil Sands samples.

2 NM = not measured.

- i) higher SAR values (high Na and clay contents) and slightly decreased pH at the grassland site;
- ii) decreased C and N levels and increased Fe concentrations at the subalpine site; pH also increased;
- iii) increased pH and the stripping of virtually all C and other nutrients from the sandy soil at the oil sands site.

3.1.2. Tank Study

3.1.2.1 Characteristics of the amendments. The amendments applied to the various minespoils had the following chemical and physical characteristics (see Table 6a):

i) levels of organic matter, N, P were generally greater in the sewage sludge than in the other amendments. Heavy metal concentrations (particularly Zn) were also higher in the sludge than in the other amendments.

ii) gypsum (which is the mineral material resulting from the H_2SO_4 extraction of phosphate from phosphate rock) is mainly CaSO_4 and was therefore high in Ca. Phosphorus levels also tended to be high.

iii) prairie grassland topsoil when compared with the grassland minespoil (see Table 5) had higher biologically available organic matter levels and a much lower SAR.

iv) the peat used in this study was a fibrous feather moss peat high in organic matter pH and N. Peat samples from the 20-30 cm, 50-60 cm and 80-90 cm depths of the bog from which this amendment originated had $\text{NO}_3\text{-N}$ levels of 271 (104-468), 424 (270-630) and 385 (44-714) $\mu\text{g g}^{-1}$ respectively). However amounts of P (both total and extractable) were found to be limiting to plant growth during the first growing season after amendment.

In terms of nutrient organic matter addition to impoverished minespoils, it would appear that sewage sludge would offer the best possibilities. However, the high levels of ammonium N and heavy metals should be taken into consideration when applying sludge as a soil amendment.

Table 6a. Some chemical and physical characteristics of amendments.

Amendment	pH	Loss on ignition (%)	N (%)	Total nutrients ($\mu\text{g g}^{-1}$)						
				P	Ca	Fe	Cu	Zn	Pb	Cd
Sewage Sludge	8.6	50.0 (30)*	3.9	15325 (170)**	44800	11950	815	1525	788	12
Gypsum	3.0	2.4 (.1)	.01	5983 (198)	19.9 (%)	255	3	41	8	6
Topsoil	8.3	7.9 (4.3)	.18	1412 (4)	26152	15400	13	69	20	<0.5
Peat	7.2	70.0 (36)	2.5	585 (2)	51900	5700	12	36	11	1

* () = total C level.

** () = extractable P.

3.1.2.2 Immediate effects of amendment. The immediate effects of amendment on some of the physical and chemical characteristics of the three minespoils (Table 6b) were:

i) Grassland - the SAR of the minespoil after the addition of gypsum decreased in comparison with the control; also the level of ammonium acetate extractable Na increased in the gypsum treatment. The SAR and levels of extractable Na were lowest in the topsoil layer placed on the surface of the spoil. The SAR of the spoil immediately below the topsoil (i.e. 15-25 cm) was not affected by topsoil treatment. The pH of the spoil was not altered by amendment.

ii) Subalpine and Oil Sands - fertilizer decreased the pH slightly in the 0 to 5 cm depth in both these spoils. The sewage sludge treatment of the extracted oil sands also resulted in a slight drop in pH, but only at the 5-15 cm depth. As expected, extractable K and Na levels were increased by fertilization.

Tables 7, 8 and 9 present the effects of amendment on DTPA extractable metals shortly after application of the amendments, but prior to planting.

i) Grassland spoil plots - sewage sludge significantly increased the Cu, Zn and Pb levels in the 0-5 cm depth of soil compared with the levels measured in the other treatments. However, sludge-amended soil metal levels were very low in comparison with those found in the sludge prior to its addition to the soil (see Table 6a). The effects of amendment on metal levels in the deeper soil were not significant.

ii) Subalpine minespoil - as in the grassland spoil plots, sewage sludge amendment significantly increased the Cu, Zn and Pb levels over that measured in the control plots, but again only in the 0-5 cm depth of soil. The addition of peat increased the Fe and Zn levels in the surface soil. No significant amendment effects on extractable metal levels were recorded for the subsurface soil.

iii) Extracted oil sands - as for the previous two minespoils sewage sludge increased soil Cu and Zn levels, while peat amendment increased the levels of Fe and Zn in the 0-5 cm soil. However, contrary to the previous two minespoils sewage and peat

Table 6b. Some chemical and physical properties of minespoils after amendment.

Spoil Type	Treatment and Depth (cm)	pH	E.C. ¹ (mS/cm)	Extractable cations ² (me l ⁻¹)				SAR ³
				Ca	Mg	Na	K	
Grassland	Control: 0-5	7.2 ^a	4.7 ^b	4.4 ^a	1.4 ^a	47.0 ^b	0.3 ^a	27.8 ^b
	Gypsum	7.0 ^a	8.8 ^c	21.8 ^b	6.3 ^b	87.4 ^c	0.6 ^c	23.3 ^b
	Sewage Sludge	7.3 ^a	5.1 ^b	5.1 ^a	1.8 ^a	50.9 ^b	0.4 ^b	27.3 ^a
	Topsoil	7.1 ^a	1.5 ^a	6.6 ^a	2.1 ^a	8.5 ^a	0.7 ^d	4.2
	Control: 5-15	7.5 ^b	4.4 ^a	3.8 ^a	1.2 ^a	42.8 ^a	0.3 ^a	27.4 ^a
	Gypsum	7.1 ^a	8.1 ^b	15.8 ^b	4.8 ^b	79.5 ^b	0.5 ^b	24.8 ^a
	Sewage Sludge	7.5 ^b	5.7 ^{ab}	5.6 ^a	2.0 ^{ab}	55.8 ^{ab}	0.3 ^a	28.8 ^a
	Topsoil: 15-25	7.4 ^{ab}	5.3 ^a	5.8 ^a	1.8 ^{ab}	53.9 ^{ab}	0.4 ^a	29.0 ^a
Subalpine	Control: 0-5	7.1 ^b	0.5 ^a	3.9 ^a	1.1 ^a	1.0 ^a	0.1 ^a	0.45 ^a
	Fertilizer	6.7 ^a	4.2 ^b	29.9 ^b	7.3 ^b	0.9 ^b	2.0 ^c	0.27 ^a
	Sewage Sludge	6.9 ^{ab}	1.8 ^a	19.4 ^b	6.4 ^b	2.7 ^b	0.6 ^b	0.33 ^a
	Peat	7.0 ^{ab}	2.3 ^{ab}	24.9 ^b	8.1 ^b	0.8 ^a	0.3 ^b	0.58 ^a
	Control: 5-15	7.1 ^a	0.5 ^a	1.5 ^a	0.9 ^a	0.7 ^a	0.1 ^a	0.45 ^a
	Fertilizer	7.1 ^a	0.6 ^a	1.7 ^a	1.3 ^a	0.5 ^a	0.1 ^a	0.27 ^a
	Sewage Sludge	7.1 ^a	0.7 ^a	1.8 ^a	1.6 ^a	0.6 ^a	0.1 ^a	0.33 ^a
	Peat: 15-25	7.1 ^a	0.6 ^a	1.6 ^a	1.0 ^a	0.9 ^a	0.1 ^a	0.58 ^a
Oil Sands	Control: 0-5	7.3 ^b	0.5 ^a	3.3 ^a	0.7 ^a	0.8 ^a	0.1 ^a	0.53 ^{ab}
	Fertilizer	6.4 ^a	3.0 ^c	3.0 ^a	1.5 ^b	1.7 ^{bc}	5.3 ^c	1.00 ^{ab}
	Sewage Sludge	6.8 ^{ab}	2.0 ^b	8.8 ^{ab}	4.5 ^b	1.5 ^{ab}	0.8 ^a	0.60 ^a
	Peat	7.1 ^a	1.6 ^b	13.4 ^b	5.3 ^b	0.9 ^a	0.2 ^a	0.29 ^a

(cont'd)

Table 6b. (cont'd).

Spoil Type	Treatment and Depth (cm)	pH	E.C. ¹ (mS/cm)	Extractable cations ² (me 1-1)				SAR ³
				Ca	Mg	Na	K	
	Control: 5-15	7.5 ^c	0.4 ^a	2.4 ^a	0.6 ^a	0.8 ^a	0.2 ^a	.59 ^a
	Fertilizer	7.1 ^b	0.7 ^a	3.5 ^a	1.2 ^{ab}	1.6 ^b	0.5 ^{ab}	.75 ^a
	Sewage Sludge	6.4 ^a	1.8 ^b	11.6 ^b	4.6 ^c	1.5 ^b	0.6 ^b	.53 ^a
	Peat: 15-25	7.1 ^b	1.0 ^{ab}	7.6 ^{ab}	3.0 ^{bc}	0.8 ^a	0.2 ^a	.36 ^a

1 E.C. - electrical conductivity; 2 Determined by soil paste method; 3 SAR - sodium adsorption ratio.

Data analyzed by one-way ANOVA and Scheffé confidence intervals to locate significant differences.

Grassland Mg and (0-5) K, subalpine (0-5) Mg, oil sands (0-5) K data was $\ln(y + 1)$ transformed prior to analysis. Grasslands (0-5) SAR, subalpine (0-5) Ca and K and oil sands (5-15) K data analyzed by Kruskal-Wallis test.

Table 7. Effect of amendment on DTPA-extractable metals in grassland minespoil plots prior to planting.

Depth (cm)	Treatment	Metals ($\mu\text{g/g}$ dry soil)				
		Ni	Fe	Cu	Zn	Pb
0-5	Control	1.6 ^{b1}	29.6 ^a	3.2 ^b	6.5 ^b	1.0 ^a
	Gypsum	1.8 ^b	25.6 ^a	3.1 ^b	5.6 ^b	0.9 ^a
	Sewage	1.9 ^b	31.7 ^a	4.5 ^c	8.9 ^c	3.0 ^b
	Topsoil	0.7 ^a	18.5 ^a	1.4 ^a	2.3 ^a	1.3 ^a
5-15	Control	1.5 ^a	29.4 ^a	3.1 ^a	6.3 ^a	0.9 ^a
(15-25 in topsoil)	Gypsum	1.9 ^{ab}	38.9 ^a	3.3 ^a	6.6 ^a	0.9 ^a
	Sewage	1.5 ^a	29.0 ^a	3.3 ^a	6.4 ^a	1.3 ^a
	Topsoil	2.3 ^b	48.1 ^a	4.0 ^a	7.6 ^a	0.9 ^a

¹Values for each metal in each data set were subjected to a one-way ANOVA. Where the F statistic was significant, Scheffé confidence intervals were computed. Values in each column at each depth followed by the same letter, do not differ significantly ($p = 0.05$).

Table 8. Effect of amendment on DTPA-extractable metals in subalpine minespoil plots prior to planting.

Depth (cm)	Treatment	Metals ($\mu\text{g/g}$ dry soil)				
		Ni	Fe	Cu	Zn	Pb
0-5	Control	0.3 ^a ¹	16.1 ^b	1.6 ^{ab}	0.9 ^a	0.5 ^a
	Fertilizer	0.6 ^a	2.0 ^a	1.9 ^b	1.3 ^a	0.8
	Sewage	0.5 ^a	18.3 ^b	6.7 ^c	8.1 ^b	6.1 ^b
	Peat	1.1 ^a	234.0 ^c	1.2 ^a	10.3 ^b	0.9 ^a
5-15 (15-25 in peat)	Control	0.3 ^{ab}	12.4 ^a	1.6 ^a	0.6 ^a	0.7 ^a
	Fertilizer	0.5 ^b	2.7 ^a	2.0 ^a	1.1 ^a	0.9 ^a
	Sewage	0.3 ^a	16.1 ^a	1.8 ^a	0.9 ^a	0.9 ^a
	Peat	0.7 ^c	15.3 ^a	2.9 ^a	1.0 ^a	0.5 ^a

¹Values for each metal in each data set were subjected to a one-way ANOVA. Where the F statistic was significant, Scheffé confidence intervals were computed. Values in each column at each depth followed by the same letter, do not differ significantly ($p = 0.05$). Values not followed by a letter could not be included in the analysis because there was 0 variation amongst the 3 replicates within their treatments.

amendation also increased the amount of Zn measured in the deeper soil. The ease with which the sewage sludge and peat were rototilled into the sand probably accounts for the higher Zn levels in the subsurface soil of these two treatments.

3.1.2.3 Effects of time and amendation on N and P. The effects of time and amendation on extractable P, total N and $\text{NO}_3\text{-N}$ in the various minesoils are presented in Tables 10 to 18.

i) Grasslands minespoil (Tables 10-12) - in the 0-5 cm soil, gypsum and sewage sludge application resulted in significantly higher levels of extractable P than those measured in the control and topsoil treated plots. Amounts of P in the deeper soil were not significantly affected by amendation. Also time did not influence the levels of P extracted from the soil at the two sampling times (Table 10).

Sewage sludge and topsoil increased the total soil N measured in the surface soil, but not the % N assessed in the subsurface soil. Amendation did not significantly influence soil $\text{NO}_3\text{-N}$ levels in the surface 0-5 cm deep soil at either sampling time, but after the second growing season (1978) levels of $\text{NO}_3\text{-N}$ in the deeper soil were greatest in the topsoil treated plots.

ii) Subalpine minespoil (Tables 13-15) - fertilizer and sewage sludge significantly increased P levels in the 0-5 cm soil shortly after application while the P content of the deeper soil did not change. However, after two growing seasons, P levels in the 5-15 cm sewage sludge-treated plots had increased significantly. Peat significantly increased the total N of the subalpine minespoil mainly due to the high levels of $\text{NO}_3\text{-N}$ in this amendment. Fertilizer and sewage sludge also improved the $\text{NO}_3\text{-N}$ level, but only in the surface soil. It is interesting to note the significant decrease in $\text{NO}_3\text{-N}$ in the 0-5 cm soil after two growing seasons.

iii) Extracted oil sands (Tables 16-18) - extractable P levels were highest in the fertilizer and sewage sludge amended plots at both sample times. However, in the fertilized plots, amounts of P

Table 9. Effect of amendment on DTPA-extractable metals in extracted oil sands plots prior to planting.

Depth (cm)	Treatment	Metals ($\mu\text{g/g}$ dry soil)				
		Ni	Fe	Cu	Zn	Pb
0-5	Control	0.2	2.6 ^a	0.0	0.3 ^a	0.6 ^a
	Fertilizer	0.4 ^{a1}	4.3 ^{ab}	0.2 ^a	0.6 ^a	1.2 ^a
	Sewage	0.2	4.4 ^{ab}	1.1 ^b	2.6 ^b	2.8 ^a
	Peat	0.9 ^a	35.9 ^b	0.3 ^a	10.7 ^b	2.6 ^a
5-15	Control	0.2	3.2 ^a	0.1 ^a	0.3 ^a	0.3 ^a
(15-25 in peat)	Fertilizer	0.5 ^a	3.5 ^a	0.1	0.3 ^a	1.1 ^a
	Sewage	0.2	3.4 ^a	0.1 ^a	0.9 ^b	1.3 ^a
	Peat	0.5 ^a	1.9 ^a	0.2	0.9 ^b	1.3 ^a

¹Values for each metal in each data set were subjected to a one-way ANOVA. Where the F statistic was significant, Scheffé confidence intervals were computed. Values in each column at each depth followed by the same letter, do not differ significantly ($p = 0.05$). Values not followed by a letter could not be included in the analysis because there was 0 variation amongst the 3 replicates within their treatments.

Table 10. Effect of time and amendment on extractable P ($\mu\text{g/g}$ dry soil) in grasslands minespoil.

Soil depth (cm)	Amendment	Sampling date			Amendment mean	Time action	Inter- action
		1977 (prior to planting)	1978 (fall rye plots)				
0-5	Control	1.3	5.5		3.4a ²	***1	NS
	Gypsum	18.1	22.8		20.5b		
	Sewage	7.5	40.1		23.8b		
	Topsoil	3.5	1.8		2.7a		
	Yearly Mean	7.6a	17.6a				
5-15 (15-25 in topsoil)	Control	1.4	4.5		3.0b	*	NS
	Gypsum	1.2	18.3		9.8b		
	Sewage	2.8	15.5		9.2b		
	Topsoil	0.4	0.8		0.6a		
	Yearly Mean	1.45a	9.8b				

1 Each data set was analyzed using a two-way ANOVA. *** = $p < .001$; * = $.01 < p \leq .05$; NS = nonsignificant.

2 Scheffé confidence intervals were calculated for the amendment and yearly means. Values in the amendment mean column or yearly mean row followed by the same letter do not differ significantly ($p = 0.05$).

Table 11. Effect of time and amendment on total soil N (%) in grasslands minespoil.

Soil depth (cm)	Amendment	Sampling date		Amendment mean	Time action	Inter-
		1977 (prior to planting)	1978 (fall rye plots)			
0-5	Control	.11	.13	.12 ²	***1	NS
	Gypsum	.11	.13	.12 ^a		
	Sewage	.14	.20	.17 ^b		
	Topsoil	.25	.25	.25 ^c		
	Yearly Mean	.15 ^a	.18 ^b			
5-15 (15-25 in topsoil)	Control	.12	.12	.12 ^a	NS	NS
	Gypsum	.13	.14	.14 ^a		
	Sewage	.13	.15	.14 ^a		
	Topsoil	.13	.14	.14 ^a		
	Yearly Mean	.13 ^a	.14 ^a			

¹ Each data set was subjected to a two-way ANOVA. *** = $p < .001$; * = $.001 < p \leq .01$; NS = nonsignificant.

² Scheffé confidence intervals were computed for the amendment and yearly means. Values in the amendment mean column or yearly mean row do not differ significantly ($p = 0.05$).

Table 12. Effect of time and amendment on soil NO₃-N (µg/g dry soil) in grasslands minespoil.

Soil depth (cm)	Amendment	Sampling date				Inter- Time action
		1977 (prior to planting)	1978 (fall rye plots)	Amendment mean	Amendment Time	
0-5	Control	6.4	8.3	7.3a ²	NS ¹	NS
	Gypsum	15.1	3.8	9.5a		
	Sewage	25.0	17.4	21.2a		
	Topsoil	25.2	8.6	16.9a		
	Yearly Mean	17.9a	9.5a			
5-15	Control	11.1	6.3 ^{ab}	--		*
	Gypsum	8.8ab	3.3a	--		
	Sewage	36.0bc	9.7ab	--		
	Topsoil	19.0bc	63.7c	--		
	Yearly Mean	--				

¹ Each data set was subjected to a two-way ANOVA after a 1n transformation. * = .01 < p ≤ .05; NS = nonsignificant.

² Values followed by the same letter do not differ significantly (p = 0.05).

³ Scheffe confidence intervals were computed for the treatment means. Values followed by the same letter do not differ significantly (p = 0.05).

Table 13. Effect of time and amendment on extractable P ($\mu\text{g/g}$ dry soil) in subalpine minespoil.

Soil depth (cm)	Amendment	Sampling date		Amendment mean	Time action
		1977 (prior to planting)	1978 (fall rye plots)		
0-5	Control	3.4 ^b	4.7 ^{bc2}		**1
	Fertilizer	50.9 ^d	25.5 ^{cd}		
	Sewage	42.3 ^d	89.2 ^d		
	Peat	1.5 ^{ab}	0.3 ^a		
5-15 (15-25 in peat)	Control	2.9	5.0 ^{a2}		*
	Fertilizer	4.9 ^a	10.8 ^{ab}		
	Sewage	6.7 ^a	65.7 ^b		
	Peat	2.7 ^a	3.4 ^a		

1 Each data set was subjected to a two-way ANOVA after a $\ln$ transformation. ** = $.001 < p \leq .01$; * = $.01 < p \leq .05$.

2 Scheffé confidence intervals were computed for the treatment means. Values followed by the same letter in each data set do not differ significantly ($p = 0.05$).

Table 14. Effect of time and amendment on total soil N (%) in subalpine minespoil.

Soil depth (cm)	Amendment	Sampling date			Inter- Time action
		1977 (prior to planting)	1978 (fall rye plots)	Amendment mean	
0-5	Control	.09 ^a	.09a ²		***1
	Fertilizer	.13 ^a	.33 ^a		
	Sewage	.15 ^a	.18 ^a		
	Peat	1.67 ^b	.13 ^a		
5-15 (15-25 in peat)	Control	.09 ^{bc}	.09bc ²		**
	Fertilizer	.09abc	.08 ^c		
	Sewage	.09abc	.17 ^{ab}		
	Peat	.09abc	.18 ^a		

1 Data for 0-5 cm depth was $2 \arcsin \sqrt{p}$ transformed and subjected to a two-way ANOVA. Data for 5-15 cm depth was $\ln$ transformed and analyzed by a two-way ANOVA. *** = $p \leq .001$; ** = $p \leq .01$.

2 Scheffé confidence intervals were computed for the treatment means. Values at each depth followed by the same letter(s) do not differ significantly ($p = 0.05$).

Table 15. Effect of time and amendment on soil $\text{NO}_3\text{-N}$ ($\mu\text{g/g}$ dry soil) in subalpine minespoil.

Soil depth (cm)	Amendment	Sampling date		Amendment mean	Time	Inter- action
		1977 (prior to planting)	1978 (fall rye plots)			
0-5	Control	4.0a ²	3.0 ^a			***1
	Fertilizer	150.4d	4.3a			
	Sewage	25.2b	25.2b			
	Peat	448.9d	96.7cd			
5-15	Control	2.8	2.9	2.9a ³	*	NS
(15-25 in peat)	Fertilizer	13.1	2.9	8.0ab		
	Sewage	29.7	22.0	25.9b		
	Peat	5.7	25.6	15.7b		
	Yearly Mean	12.8a	13.4a			

¹ Data sets for each depth were subjected to a two-way ANOVA after a $\ln$ transformation. *** = $p \leq .001$; * = $.01 < p \leq .05$; NS = nonsignificant.

² Scheffé confidence intervals were computed for the treatment means. Values followed by the same letter do not differ significantly ($p = 0.05$).

³ Scheffé confidence intervals were computed for the amendment and yearly means. Values in the amendment mean column or yearly mean row followed by the same letter do not differ significantly ($p = 0.05$).

Table 16. Effect of time and amendment on extractable P ($\mu\text{g/g}$ dry soil) in extracted oil sands.

Soil depth (cm)	Amendment	Sampling date			Inter- action
		1977 (prior to planting)	1978 (fall rye plots)	Amendment mean	
0-5	Control	1.2 ^{ab}	1.6 ^{ab2}		***1
	Fertilizer	52.6 ^{de}	13.6 ^c		
	Sewage	26.4 ^{cd}	169.3 ^e		
	Peat	2.7 ^b	0.8 ^a		
5-15 (15-25 in peat)	Control	1.0	1.2	1.1 ^{a4}	NS NS
	Fertilizer	4.7	4.9	4.8 ^b	
	Sewage	23.2	60.3	41.8	
	Peat	1.6	0.9	1.3	
	Yearly Mean	2.4 ^a	2.3 ^a		

1 Data for the 0-5 cm depth was ln transformed and subjected to a two-way ANOVA. *** = $p < .001$; NS = nonsignificant.

2 Scheffé confidence intervals were computed for the treatment means. Values followed by the same letter do not differ significantly ($p = 0.05$).

3 The 1977 sewage data was highly variable (7.7, 5.7, 56.1 $\mu\text{g/g}$ dry soil), and inconsistent with the other data in the set. Hence the sewage values were deleted from two-way ANOVA.

4 Scheffé confidence intervals were computed for amendment and yearly means. Values in the amendment mean column or yearly mean row followed by the same letter do not differ significantly ($p = 0.05$). Sewage data was excluded from these calculations, but it can be safely assumed that the extractable P in the 1978 sewage-amended soil was significantly greater than in the other treatments. The variability in the 1977 sewage data indicates it was not significantly different from the other treatments.

Table 17. Effect of time and amendment on total soil N (%) in extracted oil sands.

Soil depth (cm)	Amendment	Sampling date		Amendment mean	Time action	Inter- action
		1977 (prior to planting)	1978 (fall rye plots)			
0-5	Control	0	.01	.005a ²	**1	NS
	Fertilizer	.01	.01	.01a		
	Sewage	.02	.05	.04a		
	Peat	.82	.68	.75b		
	Yearly Mean	.21a	.19a			
5-15 (15-25 in peat)	Control	0	.01	.005a ²	**	NS
	Fertilizer	.01	.01	.01a		
	Sewage	.01	.01	.01a		
	Peat	.06	.07	.065b		
	Yearly Mean	.02a	.025a			

¹ Because the 1977 and 1978 control treatments had a mean of 0 with 0 variation and because the 1978 peat treatment at 0-5 cm contained only 1 replicate, the Tukey test for additivity was applied to the averages of each treatment for each depth to test for interactions. Since there were no significant interactions the two-way ANOVA with 1 replication/treatment was applied to each data set at each depth. ** = .001 < p < .01; NS = nonsignificant.

² Scheffé confidence intervals were computed for the amendment and yearly means. Values in the amendment mean column or yearly mean row followed by the same letter are not significantly different (p = 0.05).

Table 18. Effect of time and amendment on soil NO₃-N ($\mu\text{g/g}$ dry soil) in extracted oil sands.

Soil depth (cm)	Amendment	Sampling date			Amendment mean	Time action	Inter-
		1977 (prior to planting)	1978 (fall rye plots)				
0-5	Control	0.1	0.8 ^{ab2}				**1
	Fertilizer	39.9 ^{cde}	2.7 ^{ab}				
	Sewage	16.2 ^{bcd}	7.0 ^{abc}				
	Peat	251.3 ^e	61.8 ^{de}				
5-15 (15-25 in peat)	Control	0.1	0.7		0.4 ^{a3}	*	NS
	Fertilizer	5.1	1.3		3.2 ^a		
	Sewage	25.5	5.1		15.3 ^b		
	Peat	24.0	8.4		16.2 ^b		
	Yearly Mean	13.7 ^a	3.9 ^b				

1 Data for 0.5 cm depth was $\ln(x + 1)$ transformed and subjected to a two-way ANOVA, while the 5-15 cm data was $\ln$ transformed prior to two-way ANOVA. *** = $p < .001$, ** = $p < .01$, * = $p < .05$; NS = nonsignificant.

2 Scheffé confidence intervals were computed for treatment means. Values followed by the same letter do not differ significantly ($p = 0.05$).

3 Scheffé confidence intervals were computed for the amendment and yearly means. Values within the amendment mean column or yearly mean row followed by the same letter do not differ significantly ($p = 0.05$).

decreased significantly between the two sampling times (presumably due to leaching and plant uptake), while P in the sludge-treated plots increased significantly (probably as a result of the decomposition and subsequent release of P from the sewage sludge aggregates).

As in the subalpine minespoil, peat amendment significantly increased total soil N at both sampling depths. All three amendments significantly increased $\text{NO}_3\text{-N}$ levels after addition to the sand with the peat amended plots showing the greatest increase. Only sewage sludge and peat increased the NO_3 levels in the subsurface soil. Levels of NO_3 in the fertilized and peat treated soil decreased significantly between the two sampling times.

3.1.2.4 Short term effect of fertilization. The short term (4 mo) effects of fertilizer application on the extractable P, $\text{NO}_3\text{-N}$ and total N measured at two depths in the subalpine and oil sand minespoils are presented in Table 19. The data show that:

i) the extractable P in the subalpine spoil did not change significantly between the two sampling times whereas there was a significant decrease in P in the fertilized oil sands over the test period. Also the P in the oil sands did not appear to accumulate in the subsurface soil indicating that in a sandy soil such as this P would move rapidly out of the rooting zone.

ii) $\text{NO}_3\text{-N}$ levels decreased significantly between the sampling times in both spoil types. It did not accumulate in the deeper 5-15 cm soil.

iii) total N had decreased significantly in the subalpine 0-5 cm soil 4 months after fertilization, but no significant decrease occurred in the 0-5 cm depth of the fertilized oil sand. In neither spoil was there any increase in % N in the 5-15 m depth. between the two sampling times whereas there was a significant decrease in P in the fertilized oil sands over the test period. Also the P in the oil sands did not appear to accumulate in the subsurface soil indicating that in a sandy soil such as this P would move rapidly out of the rooting zone.

Table 19. Changes with time of total N and extractable P and NO₃-N concentrations at two depths in a subalpine minespoil and oil sand tailings amended with mineral fertilizer.

Spoil Type	Sampling Time	Extractable P ($\mu\text{g g}^{-1}$)		NO ₃ -N ($\mu\text{g g}^{-1}$)		N (%)	
		0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm
Subalpine ¹	Immediately after fertilizer application	51 ^a	5 ^a	150 ^a	11 ^a	0.12 ^a	0.09 ^a
	Four mo. after application	46 ^a	20 ^a	40 ^b	14 ^a	0.09 ^b	0.09 ^a
Oil Sands ²	Immediately after fertilizer application	53 ^a	5 ^a	38 ^a	3 ^a	0.01 ^a	0.01 ^a
	Four mo. after application	17 ^b	7 ^a	0.5 ^b	0.5 ^a	0.01 ^a	0.01 ^a

¹Subalpine data analyzed by a t-test. Values in each column followed by same letter do not differ significantly.

²Oil Sands data analyzed by Hotelling's T² test. Values in each column followed by same letter do not differ significantly. NO₃-N data required ln (y + 1) transformation.

ii) $\text{NO}_3\text{-N}$ levels decreased significantly between the sampling times in both spoil types. It did not accumulate in the deeper 5-15 cm soil.

iii) total N had decreased significantly in the subalpine 0-5 cm soil 4 months after fertilization, but no significant decrease occurred in the 0-5 cm depth of the fertilized oil sand. In neither spoil was there any increase in % N in the 5-15 m depth.

3.2 PLANT PRODUCTION (TANK STUDY)

3.2.1. Prairie Grassland Minespoil

The effects of amendment on shoot production of crested wheat, fall rye, russian wild rye and Rambler alfalfa over the first three growing seasons are presented in Tables 20 to 23 and are summarized in Fig. 2. The data provided indicate that:

i) shoot production of all test plant species was very low until after the second growing season.

ii) sewage sludge and topsoil promoted the greatest shoot production of crested wheat compared with gypsum and control treatments. Shoot weights increased until the end of the third growing season.

iii) fall rye produced the greatest shoot weight in the sewage sludge and topsoil treated spoil. Shoot weights were highest after the second growing season, but were virtually zero after the third growing season.

iv) growth of russian wild rye was significantly greater in the sewage sludge treated soil; also, like crested wheat, shoot production by this plant species increased over the sampling time.

v) shoot weights measured for Rambler alfalfa were highest in the topsoil treatments. Only in the topsoil did shoot production increase with time. It appeared that the alfalfa had difficulty establishing itself in all but the topsoil treated minespoil. Therefore plant distribution was very patchy resulting in extreme variation.

vi) root production of fall rye and alfalfa were measured only after the second growing season. The data obtained are

Table 20. Shoot production (g dwt m⁻²) for crested wheat grown in amended grassland soil^{1,2}.

Amendment	Shoot wt (g dwt m ⁻²)			Significance Level ³		
	1977	1978	1979	Amendment	Time	Interaction
Control	4.9	167.6	305.9	159.5a	***	NS
Gypsum	12.0	219.2	310.1	180.4a		
Sewage	47.8	401.6	1399.2	616.2b		
Topsoil	168.9	360.7	1013.3	514.3b		
Yearly mean	58.4a	287.3b	757.1b			

¹Data was statistically analyzed using nonparametric tests because of significantly heterogeneous variances. Values were first analyzed for interaction effects using a test of interactions in replicated factorial design based on alignment. No interaction effects were found and significant effects of time and amendment were determined using Friedman's test for two or more observations / exp. unit.

²Values superscripted differently, differ at $p = 0.05$ as indicated by posthoc comparisons based on the Friedman's test.

³Levels of significance: *** = $p \leq 0.001$, NS = nonsignificant.

Table 21. Shoot production (g dwt m⁻²) for fall rye grown in amended grassland soil^{1,2}.

Amendment	Shoot wt (g dwt m ⁻²)			Significance Level ³		
	1977	1978	1979	Amendment	Time	Interaction
Control	62.9	233.4	2.6	99.6 ^a	***	NS
Gypsum	54.3	91.8	0.0	48.7 ^a		
Sewage	133.8	636.3	31.1	268.1 ^b		
Topsoil	233.7	857.8	3.5	365.1 ^{ab}		
Yearly mean	121.2 ^a	454.8 ^a	10.2 ^b			

¹Data was statistically analyzed using nonparametric tests because of significantly heterogeneous variances. Values were first analyzed for interaction effects using a test of interaction in replicated factorial design based on alignment. No interaction effects were found and significant effects of time and amendment were determined using Friedman's test for two or more observations / exp. unit.

²Values superscripted differently, differ at $p = 0.05$ as indicated by posthoc comparisons based on the Friedman test.

³Levels of significance: *** = $p < 0.001$, NS = nonsignificant.

Table 22. Shoot production (g dwt m⁻²) for russian wild rye grown in amended grassland soil^{1,2}.

Amendment	Shoot wt (g dwt m ⁻²)			Amendment mean	Significance Level ³		
	1977	1978	1979		Amendment	Time	Interaction
Control	7.7	179.8	290.3	159.3 ^a	***	***	NS
Gypsum	7.3	50.6	501.3	186.4 ^a			
Sewage	15.2	323.7	1049.7	462.9 ^c			
Topsoil	26.8	91.6	468.5	195.6 ^{bc}			
Yearly mean	14.3 ^a	161.4 ^b	577.5 ^c				

¹Values superscripted differently, differ at p = 0.05 based on a two-way ANOVA and Scheffé confidence intervals. Significant differences between means were obtained after ln (x + 1) transformation.

²Levels of significance: *** = p ≤ 0.001, NS = nonsignificant.

Table 23. Shoot production (g dwt m⁻²) by Rambler alfalfa grown in amended grassland soil^{1,2}.

Amendment	Shoot wt (g dwt m ⁻²)			Significance Level ³		
	1977	1978	1979	Amendment	Time	Interaction
Control	0.6	22.7	101.8	41.7a	***	NS
Gypsum	1.3	0	161.5	54.3a		
Sewage	4.2	155.6	53.6	71.1a		
Topsoil	141.4	272.9	823.2	412.5b		

¹All values were transformed [$\ln(x + 1)$] and subjected to a two-way ANOVA. *** = $p < .001$, NS = nonsignificant.

²Scheffé confidence intervals were computed for the amendment means. Values in the amendment mean column followed by the same letter do not differ significantly ($p = .05$).

Fig. 2. Shoot production in amended grassland spoil.

presented in Table 24. Generally the root weights mirrored the shoot weights, with sewage sludge and topsoil stimulating the greatest root production of fall rye and topsoil promoting the best root growth of rambler alfalfa.

3.2.2 Subalpine Minespoil

Shoot and root production of slender wheatgrass, alsike clover, white spruce and willow in the variously amended subalpine minespoil plots are presented in Tables 25 to 29. Shoot production results are summarized in Fig. 3. These data show that:

i) shoot production of slender wheatgrass (Table 25) was greatest in the sewage sludge and fertilizer amended spoil until the third growing season, when slender wheatgrass sown in the peat amended spoil demonstrated the most growth. As for the fall rye grown in the grassland spoil, shoot production of slender wheatgrass peaked after the second growing season and then decreased dramatically (especially in the sludge and fertilizer-treated spoil). During the third growing season, shoot production did not vary significantly amongst the three amendment treatments.

ii) unlike slender wheatgrass, shoot production by alsike clover (Table 26) increased significantly in all treatments over the three growing seasons. Fertilizer and sewage sludge amendment resulted in the best growth response. Although no data were collected on the reseeding success of the grass and legume species, field observations indicated that reseeding success of alsike clover was higher than that of slender wheatgrass and the two legumes tested in the grassland and oil sands minespoil.

iii) root production of slender wheatgrass (Table 27) measured after two growing seasons was significantly greater in the amended spoil compared with the control spoil. On the other hand, alsike clover root production did not appear to be affected by amendment and tended to be less than that measured for slender wheatgrass. This may have been a result of the sampling technique employed. Soil for root production estimates was sampled to a depth of 14 cm (due to the rocky nature of the subalpine spoil material, it was virtually

Table 24. Effect of amendment of prairie grassland spoil on root production over two growing seasons.

Plant Species	Root wt (g dry root m ² -1)			
	Control	Gypsum	Sewage Sludge	Topsoil
Fall Rye	53.4 ^{a1}	63.2 ^a	264.9 ^b	457.7 ^c
Rambler Alfalfa	52.1 ^{a2}	6.5 ^a	17.9 ^a	256.6 ^a

¹The nonparametric Friedman test with posthoc comparisons at $p = 0.05$ was used to determine significant effects of amendment on root wt.

²A one-way ANOVA after a $\ln(y + 1)$ transformation was applied to alfalfa data. Values in each row followed by the same letter do not differ significantly ($p = 0.05$).

Table 25. Shoot production (g dwt m⁻²) for slender wheatgrass grown in amended subalpine soil¹.

Amendment	Shoot wt (g dwt m ⁻²)				Significance Level ²	
	1977	1978	1979		Amendment	Time Interaction
Control	2.6 ^a	43.1 ^{cd}	21.0 ^{bc}		***	***
Peat	6.9 ^{ab}	111.1 ^{cde}	98.2 ^{cde}			
Fertilizer	30.0 ^{bc}	209.5 ^{de}	44.7 ^{cd}			
Sewage	53.2 ^{cd}	324.6 ^e	63.7 ^{cd}			

¹Data analysed by 2-way ANOVA after ln Y transformation. Numbers followed by the same letter are not significantly different (p = .01) as determined by Scheffé multiple contrasts.

² *** = p < .001.

Table 26. Shoot production (g dwt m⁻²) for alsike clover grown in amended subalpine spoil.

Amendment	Amendment				Time	Interaction
	1977	1978	1979	mean		
Control	2.9	42.2	202.3	82.5 ^{a2}	***	NS
Fertilizer	40.0	231.5	355.7	209.1 ^b		
Sewage	11.2	131.2	371.9	171.4 ^{ab}		
Peat	0.7	53.1	166.1	73.3 ^a		
Yearly Mean	13.7 ^a	114.5 ^b	274.0 ^c			

1 All values were transformed [$\ln(x + 1)$] and subjected to a two-way ANOVA. *** = $p < .001$, NS = not significant.

2 Scheffé confidence intervals were computed for the yearly and amendment means. Values in the amendment mean column or the yearly mean row followed by the same letter do not differ significantly ($p = .05$).

Table 27. Root production in amended subalpine minespoil after two growing seasons.

Plant Species	Root wt (g dry root m ²⁻¹)			
	Control	Fertilizer	Sewage Sludge	Peat
Slender Wheatgrass	36.0a ¹	141.5 ^b	249.5 ^b	139.4 ^b
Alsike Clover	78.2a ²	21.1 ^a	126.8 ^a	43.5 ^a

¹The nonparametric Friedman test with posthoc comparisons at $p = 0.05$ was used to analyze slender wheatgrass data.

²A one-way ANOVA was applied to the clover data. Values in each row followed by same letter do not differ significantly ($p = 0.05$).

Table 28. Effect of amendment on the growth of white spruce planted in subalpine minespoil.

Amendment	Shoot wt. (g)	Root wt. (g)	Height (cm)
Growth after one growing season			
Preplanting	.17 ^{a1}	.09 ^a	5.6 ^a
Control	.29 ^a	.37 ^b	6.3 ^a
Fertilizer	.36 ^a	.31 ^b	6.1 ^a
Sewage	.42 ^a	.35 ^b	6.3 ^a
Peat	.32 ^a	.30 ^b	5.4 ^a
Growth after two growing seasons			
Control	1.54 ^a	.88 ^a	11.0 ^a
Fertilizer	1.72 ^a	1.08 ^a	10.8 ^a
Sewage	2.26 ^a	1.31 ^a	11.6 ^a
Peat	1.27 ^a	.79 ^a	9.3 ^a
Growth after three growing seasons			
Control	6.13 ^a		16.2 ^a
Fertilizer	5.64 ^a		13.6 ^a
Sewage	9.38 ^a		17.7 ^a
Peat	6.95 ^a		16.6 ^a
Growth after four growing seasons			
Control	13.8 ^{ab}		22 ^a
Fertilizer	10.8 ^a		20 ^a
Sewage	21.2 ^b		24 ^a
Peat	21.4 ^b		26 ^a

¹Data was analyzed by 1-way ANOVA and Scheffé confidence intervals.

Values in each column in each growing season followed by the same letter do not differ significantly ($p = 0.05$). Shoot data for first and third growing seasons required a log transformation, while first growing season root data required a square root transformation.

Table 29. Effect of amendment on the growth of willow in subalpine minespoil.

Amendment	Shoot wt. (g)	Root wt. (g)	Height (cm)
Growth after one growing season			
Preplanting	.30a ¹	.10 ^a	15.9 ^a
Control	.65 ^a	.65 ^b	15.9 ^a
Fertilizer	2.30 ^c	1.46 ^c	25.8 ^b
Sewage	3.00 ^c	1.74 ^c	31.3 ^{ab}
Peat	.91 ^b	.69 ^b	16.7 ^a
Growth after two growing seasons			
Control	4.55 ^a		31.7 ^b
Fertilizer	11.06 ^b		51.3 ^b
Sewage	38.78 ^c		50.1 ^b
Peat	13.73 ^b		72.2 ^c
Growth after three growing seasons			
Control	14.40 ^a		40.1 ^a
Fertilizer	62.03 ^{ab}		69.7 ^a
Sewage	151.77 ^c		134.1 ^b
Peat	41.13 ^b		68.1 ^a

¹Data was analyzed by 1-way ANOVA and Scheffé confidence intervals.

Values in each column for each growing season followed by the same letter do not differ significantly ($\alpha = 0.05$). Height data for first two growing seasons required natural log transformation prior to analysis.

Fig. 3. Shoot production in amended sub-alpine spoil.

impossible to sample any deeper). Clover seemed to root deeper than slender wheatgrass, hence it is likely that clover root estimates would have been more comparable to slender wheatgrass root estimates if deeper samples had been processed. Also, amendment effects on clover root growth may have been found to be more pronounced if deeper samples had been taken.

iv) amendment effects on shoot production of white spruce (Table 28) were not significant until the fourth growing season, when shoot weights measured in the sewage and peat-treated plots were greater than those in the control and fertilizer treatments. The absence of significant amendment effects prior to the fourth growing season was presumably due to the slow growth of white spruce.

Root weights during the first growing season increased in all the test plots over that measured at pre-planting indicating that root growth occurred in all plots after planting (Table 28). However, no significant amendment effects were measured after either one or two growing seasons. Slow growth resulting in slow response to nutrients, and high variation may explain these results.

Height of white spruce was not affected by treatment at any of the sampling times indicating that a height measurement is not an accurate method for assessing growth response (Table 28). For example, height of white spruce after four growing seasons was not significantly different amongst any of the treatments although shoot weights in sewage sludge and peat-amended plots were double those measured in control and fertilized plots.

v) shoot production of willow (Table 29) was more stimulated by sewage sludge than by any other amendment. This was true after all three growing seasons. Growth in the fertilizer-treated soil was highly variable.

Root weights measured after the first growing season demonstrated that both sewage sludge and fertilizer were most effective in promoting root growth (Table 29). These data correlated with that obtained for shoot production.

Willow had the greatest shoot height in the fertilized minespoil after one growing season, in the peat-amended soil after

the second growing season and in the sewage sludge treated soil after the third growing season (Table 29). Again the height data is misleading because dieback of the willow shoots was significant in the peat treatment after the second growing season (Table 30) and grazing of willow by deer during the winter months was sometimes excessive.

vi) the effect of amendment type on survival of spruce and willow was measured after the first and second growing seasons (Table 30). Survival was high for both willow and spruce and was not significantly influenced by treatment. However, dieback of willow was significant in the peat-treated minespoil.

3.2.3. Oil Sands Minespoil

Data on growth of slender wheatgrass, sainfoin, jack pine and bearberry in the variously amended oil sand tailings are presented in Tables 31 to 36 and shoot production is summarized in Figure 4. These data show that:

i) estimates of shoot production of slender wheatgrass (Table 31) were highly variable making it difficult to detect any significant amendment effects on plant growth. Nevertheless, shoot production tended to be highest after the second growing season, with sewage sludge promoting the most growth followed by fertilizer and peat. It is interesting that the pattern of grass growth in the variously amended oil sand spoil was very similar to that obtained for slender wheatgrass in the subalpine spoil (i.e. a peak in plant production after the second growing season followed by very little plant growth in the third growing season, particularly in the sewage sludge and fertilizer treated sand). Very little reseeding by slender wheatgrass was observed to occur in the grass subplots, although seed germinated quite readily in the unplanted pathways.

ii) shoot weights of sainfoin (Table 32) were also highest after the second growing season. Again sewage sludge was most effective in stimulating shoot growth. Unlike slender wheatgrass, shoot weights remained relatively stable after the second growing season.

Table 30. Survival of white spruce and survival and dieback of willow grown in subalpine minespoil.

Amendment	Percent Survival				Percent dieback (willow)
	Spruce		Willow		
	1 yr	2 yr	1 yr	2 yr	
Control	93 ^{a1}	90 ^a	95 ^a	91 ^a	7 ^a
Fertilizer	93 ^a	89 ^a	97 ^a	94 ^a	12 ^a
Sewage Sludge	89 ^a	84 ^a	92 ^a	91 ^a	8 ^a
Peat	92 ^a	88 ^a	95 ^a	92 ^a	60 ^b

¹Data analyzed by one-way ANOVA after a $2 \arcsin \sqrt{p}$ transformation. Where applicable Tukey multiple comparisons were used to find differences. Values in each column followed by same letter do not differ significantly ($p = 0.05$).

Table 31. Shoot production (g dwt m⁻²) for slender wheatgrass grown in amended oil sands spoil.

Amendment	Shoot wt (g dwt m ⁻²)			Significance Level ²	
	1977	1978	1979	Amendment	Time Interaction
Control	1.6a ¹	1.8a	0.2 ^a		***
Fertilizer	45.8ab	166.6bc	17.7a		
Sewage	101.7abc	766.3bc	49.6ab		
Peat	7.6a	109.6abc	80.4ab		

¹Data was analyzed by a two-way ANOVA and Scheffé confidence intervals. Values followed by the same letter(s) do not differ significantly.

² *** = $p < .001$.

Table 32. Shoot production (g dwt m⁻²) for sainfoin grown in oil sands spoil.

Amendment	Amendment				Time	Interaction
	1977	1978	1979	mean		
Control	6.3	65.5	18.1	30.0 ^{a2}	* ¹	NS
Fertilizer	106.3	41.1	91.9	79.8		
Sewage	122.8	313.5	314.9	250.4 ^b		
Peat	46.6	219.4	190.3	152.1 ^{ab}		
Yearly Mean	58.6 ^a	199.5 ^b	174.4 ^{ab}			

¹This data could not be transformed and a non-parametric test for an interaction in a two-way design demonstrated a significant interaction. By deleting the fertilizer data, the significant interaction was removed so a Friedman's analysis could be applied to the control, sewage and peat sainfoin data. The results of this test are shown in the above table. * = 0.01 < p ≤ 0.05; NS = non-significant.

²Non-parametric posthoc confidence intervals for pairwise comparisons were computed for the yearly and amendment means. Values in the amendment mean column or the yearly mean row followed by the same letter do not differ significantly (p = .05).

Table 33. Root production in amended oil sand minespoil after two growing seasons.

Plant Species	Root wt (g dwt m ²⁻¹)			
	Control	Fertilizer	Sewage Sludge	Peat
Slender Wheatgrass	9a ¹	181.2 ^b	268.5 ^b	342.2 ^b
Sainfoin	45.2 ^a	24.4 ^a	28.7 ^a	79.6 ^a

¹Data analyzed by a one-way ANOVA after square root transformation. Values in each row followed by same letter do not differ significantly as determined by Scheffé confidence intervals ($p = 0.05$).

Table 34. Effect of amendment on the growth of jack pine in oil sands spoil.

Amendment	Shoot wt. (g dwt/plant)	Root wt. (g dwt/plant)	Height (cm)
Growth after one growing season			
Preplanting	.46 ^{a1}	.20 ^a	11.2 ^a
Control	.49 ^a	.42 ^{ab}	11.1 ^c
Fertilizer	.99 ^{ab}	.66 ^{bc}	10.7 ^a
Sewage	.99 ^b	.86 ^c	10.7 ^a
Peat	1.07 ^b	.72 ^{bc}	11.4 ^a
Growth after two growing seasons			
Control	.64 ^a	.52 ^a	14.1 ^a
Fertilizer	2.60 ^b	1.26 ^b	13.4 ^a
Sewage	10.41 ^c	4.42 ^c	17.4 ^a
Peat	2.64 ^b	1.31 ^b	14.1 ^a
Growth after three growing seasons			
Control	.65 ^a		10.6 ^a
Fertilizer	2.40 ^b		11.6 ^a
Sewage	24.47 ^d		27.9 ^b
Peat	7.71 ^c		15.8 ^a

¹All analysis was done by one-way ANOVA. Posthoc multiple comparisons were used to find differences at $p = 0.05$. Values in each column for each season followed by the same letter do not differ significantly. Shoot and root data required a natural log transformation.

Table 35. Effect of amendment on growth of bearberry planted in oil sands minespoil.

Amendment	Shoot wt. (g)	Root wt. (g)
Growth after one growing season		
Preplanting	0.99ab ¹	0.21 ^a
Control	0.81 ^a	0.38ab
Fertilizer	1.23ab	0.41ab
Sewage	1.30ab	0.45ab
Peat	1.41 ^b	0.51 ^b
Growth after two growing seasons		
Control	0.79 ^a	0.58 ^a
Fertilizer	1.16 ^a	0.70 ^a
Sewage	7.58 ^b	1.29 ^a
Peat	5.88 ^b	1.08 ^a

¹Data analyzed by one-way ANOVA and Scheffé confidence intervals. Values in each column for each season followed by same letter do not differ significantly ($p = 0.05$). Shoot wts. required natural log transformations prior to analysis.

Table 36. Survival of jack pine and survival and dieback of bearberry grown in oil sand.

Amendment	Percent Survival				Percent dieback of bearberry
	Jack pine		Bearberry		
	1 yr	2 yr	1 yr	2 yr	
Control	86 ^{a1}	54 ^a	62 ^a	40 ^{ab}	20 ^a
Fertilizer	86 ^{ab}	80 ^{ab}	31 ^b	21 ^a	19 ^a
Sewage Sludge	98 ^{ab}	97 ^b	38 ^b	30 ^{ab}	1 ^a
Peat	92 ^b	88 ^{ab}	74 ^a	58 ^b	17 ^a

¹Data was subjected to a one-way ANOVA and Tukey multiple comparisons. All but the percent dieback data was $2 \arcsin \sqrt{p}$ transformed prior to analysis. Values in each column followed by same letter do not differ significantly ($p = 0.05$).

Fig. 4. Shoot production in extracted Oil Sands.

iii) root production of slender wheatgrass (Table 33) after the second growing season was not significantly different amongst the fertilizer, sewage sludge and peat treatments, indicating that shoot/root ratios were highest in the sewage sludge amended sand. Sainfoin root production like that of alsike clover in the subalpine spoil was not affected by amendment. Again the sampling technique employed may have affected these results, since sainfoin appeared to be a very deep rooting species, particularly in the nutrient-deficient oil sand tailings used in this study.

iv) jack pine (Table 34) were most productive in the sewage sludge treated sand particularly after the second and third growing seasons. Peat was the next best amendment for promoting shoot growth followed by fertilizer. No growth occurred in the untreated sand as a result of the lack of essential nutrients required for plant production. Root growth occurred in all but the control treatments during the first growing season. After the second growing season jack pine roots sampled from the sewage sludge treatment were the heaviest - a result which mirrors the shoot weights obtained for the sludge treatment. Tree height was not influenced by amendment until the third growing season when sewage sludge treated seedlings were significantly taller than those in the other treatments. Deer, grazing on the seedlings during the winter, may have affected the height measurements.

v) bearberry (Table 35) produced the greatest shoot weights in the sewage sludge and peat treated sand particularly after the second growing season. Although root growth was best in the peat-amended spoil during the first growing season, no significant treatment effects on root production were measured after the second growing season.

vi) the survival of jack pine and bearberry after the first and second growing season are presented in Table 36. Jack pine planted in the sewage sludge amended sand demonstrated the best survival. Mortality of pine seedlings in the control treatment was severe between the first and second year after planting. Bearberry demonstrated the poorest survival record of all woody plants

investigated in this study. After the first winter, bearberry in the peat and control plots demonstrated better survival than bearberry planted in the sewage sludge-treated and fertilized plots. However, seedling losses in all treatments were high between the first and second year after planting resulting in no significant amendment effects on survival of two-year-old seedlings. The per cent dieback of bearberry was not significantly influenced by treatment.

3.3 SOIL FERTILITY AND PLANT GROWTH

In an attempt to determine whether or not shoot production was related to soil P and N status, simple correlation coefficients were calculated for the following:

i) extractable soil P, $\text{NO}_3\text{-N}$ and total N, measured in soil samples removed from the plots after amendment but prior to planting, and shoot weights of slender wheatgrass (subalpine and oil sands minespoil), alsike clover (subalpine spoil) and sainfoin (oil sands tailings) after the first growing season.

ii) extractable P, $\text{NO}_3\text{-N}$ and total N assessed for soil samples removed from slender wheatgrass subplots at the conclusion of the second growing season, and shoot production of slender wheatgrass, alsike clover and sainfoin after the second growing season.

The correlation coefficients obtained are presented in Table 37. Generally, extractable P demonstrated the best relationship with shoot weights of slender wheatgrass and first growing season shoot weights of alsike clover. No strong relationships were observed between shoot weights of both grass and legume species and $\text{NO}_3\text{-N}$ or total soil N. Small sample size (i.e. 12 values per plant species for each parameter tested) and the fact that data from all the treatments were combined into the same correlation matrix could account for the lack of relationship between plant growth and soil P and N status. Also soil samples for nutrient analysis were not removed from the same areas as those clipped for primary production estimates.

Table 37. Correlation coefficients between grass and legume shoot production (g/m^2) and selected soil nutrient levels.

Soil Type	Plant Species	Growing Season	Correlation Coefficients		
			P	$\text{NO}_3\text{-N}$	Total N
Subalpine	Slender Wheatgrass	1	.735	-.366	-.488
		2	.833	-.122	.289
	Alsike Clover	1	.823	-.078	-.353
		2	.295	-.420	.515
Oil Sands	Slender Wheatgrass	1	.434	-.356	-.334
		2	.910	-.214	-.060
	Sainfoin	1	.675	-.185	-.268
		2	.370	.096	.458

3.4 CHEMICAL ANALYSIS OF THE FOLIAGE OF SELECTED PLANT SPECIES

3.4.1. Subalpine Minespoil

Data on the effects of the various amendments on the elemental concentrations in the foliage of slender wheatgrass and needles of white spruce after one growing season are presented in Tables 38 and 39. These data show that:

i) slender wheatgrass - phosphorus was significantly greater in grass grown in the sewage sludge treated spoil, while Ca was significantly concentrated by grasses sampled from the control and peat amended plots. Levels of heavy metals (i.e. Cu, Zn, Fe) were not significantly greater in plants from the sewage sludge treatment than in those from the other treatments.

ii) white spruce - as a result of the slow growth rate of white spruce and hence its slow uptake of essential nutrients, there were no significant amendment effects on nutrient levels assessed after one growing season.

iii) total N (Table 40) measured in the foliage of slender wheatgrass, alsike clover and willow (subalpine minespoil) demonstrated that peat provided the most N for growth in comparison with the other amendments. Interestingly, treatment had no significant effect on N concentrations in the alsike clover, presumably because it can provide its own N through N fixation.

3.4.2 Oil Sand Minespoil

The elemental concentrations of slender wheatgrass, sainfoin and jack pine grown in the variously amended oil sands plots are presented in Tables 41 to 43. These data indicate that:

i) levels of P and K in slender wheatgrass plants from the amended spoil (Table 41) were significantly greater than levels measured in plants from control spoil. Phosphorus was concentrated particularly in grass grown in sewage amended sand. Zinc concentrations were significantly higher in grass from the peat plots while plants sampled from the control plots demonstrated higher Fe levels than plants in the amended plots. Amendment effects on nutrient levels in foliage of slender wheatgrass planted in the

Table 38. Elemental concentrations in foliage of slender wheatgrass after one growing season in amended subalpine spoil.

Treatment	Elements ($\mu\text{g g}^{-1}$)							
	P	K	Ca	Mg	Mn	Cu	Zn	Fe
Control	2140 ^a	14500 ^a	12500 ^b	2533 ^a	225 ^a	4 ^a	42 ^a	875
Fertilizer	3577 ^a	18558 ^a	4894 ^a	2496 ^a	400 ^a	27 ^a	40 ^a	8713 ^b
Sewage Sludge	5429 ^b	23772 ^a	5903 ^a	2441 ^a	152 ^a	36 ^a	60 ^a	1233 ^a
Peat	2757 ^a	10921 ^a	10690 ^b	2097 ^a	114 ^a	14 ^a	98 ^a	574
df	4	4	4			4	4	
T	4.43	1.11	2.49		1.11	1.47		
95% C.I.	1917	14266	1860		41	62.5		

Only one value was available for the control and peat treatments. Fertilizer and sewage data analyzed by Student t-test. Pooled sample variances were used to construct 95% confidence intervals; control or peat values outside the interval were considered different. df - degrees of freedom, T-t value; C.I. - confidence interval. Mg, Mn, and Fe data analyzed by a Wilcoxon test. Control and peat Fe values are probably different from fertilizer and sludge values. Values in each column followed by same letter do not differ significantly.

Table 39. Elemental concentrations in white spruce needles after one growing season in amended subalpine spoil.

Treatment	Elements ($\mu\text{g g}^{-1}$)						
	P	K	Ca	Mg	Mn	Cu	Zn
Control	2778 ^b	5627 ^a	12295 ^a	2175 ^a	2015 ^a	9 ^b	48 ^a
Fertilizer	2207 ^a	5130 ^a	13218 ^a	2462 ^a	1600 ^a	7 ^b	40 ^a
Sewage Sludge	2562 ^{ab}	5102 ^a	12575 ^a	2549 ^a	1966 ^a	8 ^b	50 ^a
Peat	2085 ^a	4765 ^a	13314 ^a	2374 ^a	1541 ^a	2 ^a	41 ^a
df	8	8	8	8	8	8	8
MSE	34977	424695	2876193	70386	91198	4.6	45
							.04

Data analyzed by one-way ANOVA. Significant differences located by Scheffé multiple contrasts. Fe data were In Y transformed; values are geometric means. Values in each column followed by same letter are not significantly different ($p = 0.05$). df - degrees of freedom; MSE - mean square error.

Table 40. Total N (%) measured in the foliage of selected plant species after 2 growing seasons in amended subalpine spoil.

Treatment	% N		
	Slender wheatgrass	Alsike clover	Willow
Control	1.3 ^a	2.4 ^a	1.9 ^a
Fertilizer	1.0 ^a	2.0 ^a	2.1 ^{ab}
Sewage Sludge	1.6 ^{ab}	2.9 ^a	2.7 ^{ab}
Peat	2.3 ^b	3.5 ^a	3.1 ^b
df	8	8	8
MSE	.07	.44	.14

Data analyzed by one-way ANOVA. Scheffé multiple contrasts used to locate significant differences. Values in each column followed by same letter are not significantly different ($p = 0.05$). df - degrees of freedom; MSE - mean square error.

Table 41. Elemental concentrations of foliage in slender wheatgrass after one growing season in amended oil sands.

Treatment	Elements ($\mu\text{g g}^{-1}$)							
	P	K	Ca	Mg	Mn	Cu	Zn	Fe
Control	1235 ^a	9384 ^a	20000 ^c	2432 ^b	140 ^c	10 ^{ab}	90 ^{bc}	550 ^b
Fertilizer	3664 ^b	19554 ^b	3352 ^a	1016 ^a	92 ^b	16 ^{ab}	44 ^a	336 ^a
Sewage Sludge	5818 ^c	20645 ^b	3836 ^{ab}	2115 ^b	59 ^a	33 ^b	78 ^b	256 ^a
Peat	2637 ^b	20432 ^b	7664 ^b	1463 ^a	68 ^a	0 ^a	100 ^c	279 ^a
df	6	6	6	6	6	6	6	6
MSE	175414	748914	1180502	31046	43	T=2.38	62	3379
95% C.I.						27		

Due to insufficient sample only one value was available for the control treatment elements. A one-way ANOVA and Scheffé multiple contrasts were applied to the fertilizer, sewage and peat data. The Scheffé multiple contrast was also used to determine if the control was significantly different from the other treatments. Data in each column followed by same letter are not significantly different ($p = 0.05$). df - degrees of freedom; MSE - mean square error.

Table 42. Elemental concentrations in foliage of sainfoin after one growing season in amended oil sands.

Treatment	Elements ($\mu\text{g g}^{-1}$)							
	P	K	Ca	Mg	Mn	Cu	Zn	Fe
Control	1452 ^a	7885 ^a	15667 ^a	4864 ^a	245 ^c	13 ^a	70 ^a	222 ^{ab}
Fertilizer	3158 ^{bc}	13687 ^b	12294 ^a	4033 ^a	217 ^c	13 ^a	39 ^a	267 ^b
Sewage Sludge	4077 ^c	12325 ^{ab}	13186 ^a	5092 ^a	160 ^b	13 ^a	64 ^a	211 ^{ab}
Peat	1585 ^{ab}	10890 ^b	12310 ^a	4102 ^a	61 ^a	24 ^a	49 ^a	166 ^a
df	6	6	6	6	6	6	6	6
MSE	420233	3460092	249926	197863	365	34	171	526

Due to insufficient plant material, only one value was available for the control treatment for each of the elements. Fertilizer, sewage and peat data analyzed by a one-way ANOVA and Scheffé multiple contrasts. Scheffé multiple contrast value was also used to determine if the control values were different from the other treatments. Values in each column followed by same letter do not differ significantly ($p = 0.05$).

Table 43. Elemental concentrations in foliage of jack pine after one growing season in amended oil sands.

Treatment	Elements ($\mu\text{g g}^{-1}$)					
	P	K	Ca	Mg	Mn	Zn
Control	1640 ^{ab}	6010 ^a	3966 ^a	1451 ^b	528 ^c	66 ^b
Fertilizer	2034 ^c	7065 ^a	2681 ^a	982 ^a	299 ^{ab}	37 ^a
Sewage Sludge	1985 ^{bc}	6129 ^a	3557 ^{bc}	1204 ^{ab}	350 ^b	70 ^b
Peat	1485 ^a	6021 ^a	6291 ^d	1498 ^b	265 ^a	59 ^b
df	8	8	8	8	8	8
MSE	18863	193892	161352	25073	.0083	31
						573

Data analyzed by a one-way ANOVA. Scheffé multiple contrasts were applied to locate significant differences. Values in each column followed by the same letter do not differ significantly ($p = 0.05$). df = degrees of freedom; MSE = mean square error.

¹Only 1 value was available for sewage sludge treatment.

²Mn data was $\ln Y$ transformed. Values are geometric means.

various amended oil sands plots appeared to be more pronounced than those measured for grasses from the subalpine spoil plots (see Table 38).

ii) as for slender wheatgrass, sainfoin plants also had higher levels of P and K in the amended than in the control plots (Table 42), with sewage sludge providing the most P. Amounts of Mn in plants grown in the control, fertilizer and sewage sludge treated plots were significantly greater than in plants from the peat treatment. Compared with the slender wheatgrass (Table 41) sainfoin appeared to have a greater demand for Ca and Mg.

iii) jack pine, like white spruce, demonstrated a much reduced growth rate in comparison with legumes and grasses. As a result, amendment effects on foliage chemical characteristics are difficult to interpret when analyzed after one growing season. However, jack pine did display some root growth during the first growing season, particularly in the sewage sludge amended plots (Table 34), whereas white spruce demonstrated very little root growth over a similar time period (Table 28). Therefore, there was an amendment effect on P levels in jack pine with fertilizer and sewage sludge treatments effecting higher levels of needle P than the control and peat treatments (Table 43). Trees in the peat amended spoil concentrated more Ca than those in the control.

iv) total N levels measured in the foliage of jack pine, slender wheatgrass and sainfoin after two growing seasons are given in Table 44. Shoots of jack pine and slender wheatgrass in the control treatments were too small to be analysed. The results were similar to those obtained for the plant species grown in the subalpine minespoil (Table 40). Jack pine and slender wheatgrass grown in the sewage sludge and peat treated sand had significantly higher levels of foliage N than those sampled from the control and fertilizer treatments. Sainfoin did not appear to be dependent on N introduced with the amendments since it provides its own N through N_2 fixation.

Table 44. Total N (%) measured in the foliage of selected plant species after 2 growing seasons in amended oil sand.

Treatment	% N		
	Jack pine	Slender wheatgrass	Sainfoin
Control	ND	ND	2.3 ^a
Fertilizer	1.1 ^a	0.8 ^a	1.9 ^a
Sewage Sludge	1.8 ^b	2.0 ^b	3.0 ^a
Peat	1.8 ^b	2.5 ^b	2.9 ^a
df	6	6	6
MSE	0.03	0.14	0.13

Data analyzed by one-way ANOVA. Significant differences located by Scheffé multiple contrasts. Values in each row followed by the same letter do not differ significantly ($p = 0.05$). df = degrees of freedom; MSE = mean square error; ND = not determined.

3.4.3 Correlation of Selected Soil and Foliage Chemical Characteristics

Since the nutrient levels concentrated in the foliage of the test plant species is dependent on the chemical quality of the soil in which the plants were grown, it was decided to test for a relationship between selected soil and plant chemical parameters. Simple correlation coefficients were calculated between soil and plant P, Fe, Cu and Zn levels measured during the first growing season and the results obtained are presented in Table 45. Like the soil P, N and $\text{NO}_3\text{-N}$ vs. shoot weight correlation coefficients (see Table 37), the best relationships occurred between soil P levels and P in the foliage of slender wheatgrass, sainfoin and jack pine. There was also a strong relationship between soil Cu and oil sands slender wheatgrass Cu levels. High levels of Cu in the sewage amended sand (see Table 9) may explain this result. The same relationship did not occur for the slender wheatgrass grown in the subalpine sewage treated spoil, presumably because the subalpine spoil type (more clays) in contrast to the nonadsorptive sand tailings may have rendered Cu and some of the other metals unavailable to the plants. There were no strong relationships between the other heavy metals used in the analysis and foliage metal levels indicating that over the short term (i.e. one growing season) there was no significant build-up of metals in the foliage of plants grown in the sewage sludge treated minesoils. The slow uptake of nutrients as a result of the slow growth rate of conifers such as white spruce explains the total lack of agreement between soil and needle nutrient levels. No doubt better relationships between soil and foliage chemical characteristics would have resulted if:

- i) soil samples for analysis had been removed from the vicinity in which the plants were sampled;
- ii) more replicates had been tested;
- iii) more replicates within each amendment type had been analyzed.

Table 45. Simple correlation coefficients between soil and foliage chemical characteristics ($\mu\text{g g}^{-1}$).

Spoil Type	Plant spp.	Correlation Coefficients			
		P	Fe	Cu	Zn
Subalpine	Slender Wheatgrass	.656	.525	-.070	.149
	White Spruce	-.108	-.769	.262	-.083
Oil Sands	Slender Wheatgrass	.528	-.331	.761	.413
	Sainfoin	.672	-.294	-.012	-.024
	Jack Pine	.864	-.129	.162	.007

4. DISCUSSION

4.1 PRAIRIE GRASSLAND MINESPOIL

Mining disturbance of the grassland site (Bow City) resulted in a spoil which had high levels of exchangeable Na, a high SAR, increased clay levels and a decrease in biologically available carbon (the latter being a result of the stripping of the topsoil in the mining process). Power et al. (1978) emphasized that the high exchangeable Na levels, often found in spoils from the Great Plains region, can exert a major effect on infiltration rate and water storage by dispersing the clay particles and restricting entry. This results in increased run-off and less available water for plant growth. Total N and P were not substantially different in the disturbed and undisturbed soils, but it should be emphasized that these results do not reflect the availability of N and P for plant growth. Power et al. (1978) have shown that almost all mine spoils in the Northern Great Plains are deficient in plant available P and biologically active organic N. Plant survival and growth are poor in this spoil type (see Fig. 2, control plots) mainly as a result of adverse seedbed conditions, poor root penetration (due to the impermeable nature of the minespoil), low nutrient status and poor moisture conditions (this would be particularly true at the field site where the annual precipitation is 335 mm).

The addition of gypsum (calcium sulfate) to the grassland minespoil resulted in a small decrease in the SAR coupled with an increase in EC and extractable Na as this ion was replaced by Ca. Gypsum has been widely used for sodic soil reclamation (Power et al., 1978a; Kollman, 1978; Van Rooyen and Weber, 1977; Khosla, Gupta and Abrol, 1979; Safaya and Wali, 1979). According to Power et al. (1978a) treatment of sodic soils with gypsum can reduce the exchangeable Na content 30 to 50 percent in the upper 30 cm of spoil within a few years after treatment. Because gypsum has a relatively low solubility, the length of time required to replace the exchangeable Na with Ca would be dependent on the amount of rainfall received by a site. In the semi-arid climate of the southeast portion of this province, reclamation of a sodic spoil with gypsum

would probably be long-term. Prather et al. (1978) have suggested combining gypsum with CaCl_2 or H_2SO_4 to reduce the time and leaching required to achieve reclamation. In this study the SAR was not measured after the first sample time as data on the long term effects of the gypsum on the SAR and exchangeable Na levels are not available.

A layer of 15 cm topsoil, spread on the surface of the sodic minespoil, resulted in a much reduced SAR compared with that measured in the spoil (Table 6.6). However, research conducted by Merrill et al. (1980) demonstrated that over an 8 year time span chemical diffusion resulted in significant Na migration into the topsoil spread over minespoil (SAR = 19). They concluded that this Na accumulation could potentially degrade topsoil spread over minespoil with an SAR value of 20 or more and where only 30.5 cm (12 inches) or less topsoil was available for reclamation. In this study, the SAR of the grassland spoil was measured immediately after topsoil addition. In a spoil such as this, where sodicity and salinity appear to be the major factors impeding plant growth, EC and SAR should be monitored frequently. Although sewage sludge application had no immediate effects on the SAR, it is possible that over the long term the high levels of Ca in the sludge would replace some of the Na on the exchange sites; thereby relieving some of the sodicity problems.

Primary production (both root and shoot production) by crested wheat, fall rye, russian wild rye and rambler alfalfa was most stimulated by either sewage sludge or topsoil addition. Other studies have also demonstrated that treatment of a sodic minespoil with topsoil improves plant establishment and productivity (Kollman, 1978; Power et al., 1981; Schroeder et al., 1980; Aldon, 1978) by increasing the infiltration rate, reducing run-off, improving germination (because of better seedbed conditions) and increasing plant survival as a result of better root penetration, improved soil fertility and more efficient water usage by the vegetation. Although 15 cm of topsoil was applied in the present study, recent research by Power et al. (1981) indicates that the reclamation of highly sodic

materials (SAR greater than 20), requires a minimum thickness of 90 cm of soil (20 cm topsoil plus 70 cm subsoil) to obtain maximum production of most crops in semi-arid regions. They also emphasized that if the soil material is highly erodible and upward salt migration is a problem, more than 90 cm of soil material may be required. Therefore, if maximum crop production for grazing is one of the aims in reclaiming a sodic minesite in this province, more research would be required regarding the optimum levels of topsoil to be applied taking into consideration the climatic conditions of the mined area. Needless to say, it is necessary that topsoil be stockpiled for future reclamation particularly in remote areas where other amendments are not available and the cost of transporting them to the site would be impractical.

Although sewage sludge has been used extensively for treating agricultural land, it has found only limited use for reclaiming land disturbed by mining. Sopper (1970), Stout et al. (1978), and Berry and Marx (1977) have reported results regarding the use of sewage sludge for reclaiming strip mine spoils in the eastern United States whilst Bradshaw et al. (1978) have used sewage sludge to establish plants on limestone quarry faces in England. Reasons why a nutrient-rich amendment such as sewage sludge has not been more popular for reclamation purposes may be:

i) the remoteness of many minesites resulting in high transport costs of the sludge. Agricultural land is generally within a reasonable distance of sewage sludge lagoons, i.e. near large cities where the sludge is generated.

ii) fear of heavy metal uptake by the vegetation on the minesite which could then lead to the accumulation of metals in the animals grazed on reclaimed minesites.

iii) fear of pathogens which might be present in the sludge.

iv) refusal by mine workers to handle sewage sludge due to its offensive odor, pathogen potential etc.

Nevertheless, in the present study sewage sludge applied at 46 mT ha⁻¹ was very effective in promoting the growth of crested

wheat, fall rye and russian wild rye. In contrast to the grasses, rambler alfalfa responded poorly to sewage sludge addition. This may have been a result of poor germination and establishment rather than inhibited growth due to the sludge. Also, the amount of sludge applied in this study did not improve seedbed conditions to the same extent as topsoil.

Caution must be exercised, however, when applying sewage sludge since some researchers (Wong et al., 1981; Sabey and Hart, 1975; Wollen et al., 1978; McCormick and Borden, 1973) have reported inhibition of seed germination and retarded root elongation with the application of liquid sludge or sludge extracts. Sabey and Hart (1975) observed that there was a severe inhibition of germination of sorghum sudangrass and millet when seeded shortly after liquid sludge (a mixture of 50% anaerobically digested primary and 50% aerobically digested waste activated sludges) was incorporated into the soil. However, wheat demonstrated no germination inhibition if seeded 3 mo. after sludge incorporation. They postulated that nonvolatile inorganic salts were not the causative agent of the inhibition since the inhibitory factors were destroyed by combustion at 525°C.

Wollen et al. (1978) believed that the intense biological activity which occurred when sludge was freshly incorporated into soil led to reduced oxygen tensions and the release of germination inhibitors such as ammonia and ethylene. McCormick and Borden (1973) in a study designed to measure the chemical changes of the percolate from strip minespoils treated with sewage effluent and sludge observed that many of the plants (hybrid poplar and grasses) they attempted to grow in the sludge-treated spoil died soon after planting or germination. They attributed this result to the presence of toxic concentrations of ammonia, and suggested that after applying large quantities of sewage sludge to a soil, one should wait at least 3 months before planting. As a result, if anaerobically digested sewage sludge is to be used extensively for reclaiming mine sites in Alberta, more data should be collected on the effects of different sludge applications on seed germination, plant establishment and growth. Information regarding seed germination after different

sludge application times would also be necessary in order to obtain maximum plant response from the sludge amendment.

The better growth response of the grasses and alfalfa in the topsoil and sewage-sludge treated soil in comparison with that in the gypsum-amended and control plots was also partly a result of the improved N conditions after topsoil addition and the increased quantities of N and P in the spoil after sludge amendment. The sewage sludge appeared to behave as a slow release fertilizer, releasing P and N as it decomposed. More research is required concerning the decomposition and nutrient release rates from the sludge under various soil and climatic conditions in order to predict the nutrient availability to crops over time.

As expected, sewage sludge also increased the levels of Cu, Zn and Pb in the surface soil, but levels were low in comparison with those measured in the sludge prior to application. Most metals added to soil tend to remain in the surface soil with very little movement deeper into the profile (see Williams et al., 1980 for a review of the literature regarding trace element movement in the soil). According to Williams et al. (1980), most of the metals naturally present in the soil are in forms which are relatively immobile and unavailable to plants. They concluded that after applying sewage sludge annually for 3 years to a loam soil (pH 5.2 to 5.6) at rates of 0 to 225 mT ha⁻¹yr⁻¹, the added metals like those originally present in the soil, tended to become immobilized. Only Zn and Cd were not readily chelated and were therefore likely to get into the food chain. Consequently, if only one application of sludge at a reasonably low rate (e.g. 46 mT ha⁻¹ as was used in the present study) was to be used to initiate reclamation of a minesite, accumulation of heavy metals by the vegetation would probably not be a problem.

Although the addition of gypsum to the minespoil improved the levels of P available for plant growth, plant response was generally poor. Seedbed conditions (pavement-like and impenetrable) were not significantly different from those in the control plots, so plant establishment appeared to be poor. As stated earlier, the

amelioration of a sodic spoil is a slow process dependent on precipitation input and subsequent leaching rates. So, unless gypsum is applied in combination with another amendment such as sewage sludge, one would expect revegetation to be retarded. Revegetation using gypsum could possibly be accelerated if the treated spoil was tilled and seeded every year after amelioration. In the present study, plots were seeded only at the initiation of the project.

With the exception of fall rye, shoot production of the grasses and alfalfa increased in all the treatments over the time span of the study. According to the Manual of Plant Species Suitability for Reclamation in Alberta (Watson et al., 1980), both crested wheat and russian wild rye are long-lived perennials, adapted to dry rangeland or pasture conditions because of their drought tolerance. They have high natural reseeding abilities, are suitable for alkaline soils and have moderate (crested wheat) to high (russian wild rye) tolerance to saline conditions. Crested wheat has excellent establishment qualities and is one of the best bunchgrasses for controlling erosion. In contrast to crested wheat, russian wild rye is often difficult to establish due to poor seedling vigor. In the present study, however, both crested wheat and russian wild rye did not become well established until the second growing season. The slow establishment of crested wheat may have been a result of the dry conditions after the seedlings germinated.

Rambler alfalfa is also a perennial and because of its N₂ fixing capability and drought tolerance is desirable for revegetation purposes. In this study, alfalfa established itself successfully in the topsoil treatment but not in the other treatments. The Manual of Plant Species Suitability for Reclamation in Alberta notes that alfalfa demonstrates poor tolerance to salt at the germination stage - a factor which may explain the poor shoot production results obtained for this plant species in the gypsum and sewage-treated spoil. As mentioned previously the presence of inhibitory factors in anaerobically digested sewage sludge may also have contributed to poor germination of alfalfa seed.

Of all the plant species tested on the ameliorated grassland spoil, fall rye established itself most rapidly and produced a good vegetative cover during the second growing season. Thereafter, it demonstrated very little growth and did not successfully reseed itself. Information on fall rye (perennial ryegrass) presented in the Manual of Plant Species Suitability for Reclamation in Alberta, supports the results obtained in this study - i.e. perennial ryegrass is a rapidly developing, short-lived perennial which will not last more than 3 years on the prairies. It should therefore be considered as a short-term vegetative cover planted mainly to stabilize the soil and thereby prevent surface erosion. According to the Watson et al. (1980) manual, perennial ryegrass is not particularly drought tolerant and may therefore have difficulty establishing itself in the semi-arid conditions typical of the shortgrass prairie at the minesite near Bow City, Alta.

From the plant production data collected in this study it appears that fall rye is the best choice if a rapid vegetative cover is required. However, if a long term vegetative cover is the ultimate reclamation goal crested wheat should probably be included in the seed mixture at the time of seeding. A legume, such as alfalfa should also be considered when revegetating a minesite, since it provides a readily available source of N. Jeffries et al. (1981) demonstrated that white clover growing in association with grasses on colliery spoil in England was an effective N source with N transfer from the clover to the plant becoming apparent within 22 months. after sowing. Poor establishment of alfalfa on all but the topsoil amendment should be considered when choosing legume species for reclamation of shortgrass prairie minespoil which has been rendered sodic by the mining process.

It should be mentioned that the plant species used in the present study were all introduced forage species which according to Power et al. (1978b) are generally more productive than native species but often require a higher level of management including proper seedbed preparation and seeding, good weed control, adequate fertilization and control of grazing time and intensity. Native

species in comparison have less intensive management requirements and are more resilient and adaptable under stress conditions such as prolonged drought, intense cold, short growing seasons and to limited nutrient supplies. Therefore, if proper management of forage species is not feasible on reclaimed minesites, native plants should be considered as alternatives for revegetation.

However, Curry (1980), who believes that successful reclamation of arid western lands (those where evaporation exceeds precipitation) should be defined as "restoration of the land to a state in which vegetative cover and nutrient cycles are as "closed" as they were before the man-made disturbance", fears that establishment of native plant communities may not be possible over the short-term without massive inputs of energy over a long time period. Native soils and plant communities may be the result of evolution through a sequence of climatic episodes that may not be reproducible (Curry, 1980). Nevertheless, Power et al. (1978b) have found that, although some native species require three or more years to establish good stands, some of them (some wheatgrass, needlegrass and grama species) are adapted for use on reclaimed land. The establishment of these species on mined land may be the first step in the succession toward a more stable native plant community.

4.2 SUBALPINE MINESPOIL

The major consequence of mining for coal in a subalpine area such as that found at Luscar, Alta. is the removal of the organic mat with its associated fertility, water-holding capacity and desirable seedbed conditions. In the course of excavating a pit for coal, the soil above the bedrock is removed and stockpiled for subsequent revegetation, but according to Macyk (1972) the soil to be stockpiled is sometimes so thin that the incorporation of large quantities of coarse bedrock is unavoidable. The incorporation of excessive quantities of bedrock in the "topsoil" results in revegetation problems (Macyk, 1972).

In the present study, the characteristics of the subalpine minespoil after application of "topsoil" or "regolith" (see Tables 5 and 13) but prior to planting included:

i) a neutral pH (7.0-7.3) due to the calcareous nature of the bedrock.

ii) low N (0.09%) and extractable P ($3.4 \mu\text{g g}^{-1}$) levels.

iii) elevated total Fe and Mg levels (25,000 and $4,500 \mu\text{g g}^{-1}$ respectively).

Organic matter and carbon levels of the minespoil were difficult to measure as a result of coal particle interference. However, visual observation of the spoil material indicated a significant lack of biologically available organic matter. This minespoil was characterized by large ($>2 \text{ mm}$) bedrock fragments, low levels of organic matter and plant available nutrients, and neutral pH. Therefore, the subalpine spoil was amended with peat or sewage sludge in an effort to improve the organic matter status, fertility, and water and nutrient retention capacity of the spoil. An inorganic fertilizer treatment was also included in the study since fertilization is commonly used to revegetate subalpine and alpine mine spoils (Macyk, 1972, 1974; Brown et al., 1978; Cherene, 1978).

Some of the chemical and physical characteristics of the sewage sludge included an alkaline pH (8.6), 30% C, 3.9% N and moderately high levels of P, Ca and the heavy metals. The peat used to ameliorate the subalpine spoil was also high in C (36%), and interestingly, contained high levels of N (2.5%) and large quantities of available NO_3 . However, P levels were so low in the peat (extractable P = $2 \mu\text{g g}^{-1}$ d wt soil) that they were potentially limiting to plant growth.

Immediately after applying fertilizer to the spoil, the pH of the 0-5 cm layer of soil decreased significantly as compared with the control, while the electrical conductivity and extractable cations such as Ca, Mg and K increased. According to Brady (1974), ammonium ions present in the fertilizer will tend to increase acidity when they are nitrified. Also if P is present as $\text{Ca}(\text{H}_2\text{PO}_4)_2$ in

the fertilizer granules, it will attract water from the soil resulting in a H_3PO_4 -laden solution with a pH of 1.4 moving outward from the granule (Brady, 1974). This reaction may also have contributed to a short-term drop in pH in the fertilized spoil.

If the P fertilizer contained substantial quantities of Ca, it would account for the significant increase in soil Ca levels after fertilization. Sewage sludge and peat application did not alter the pH of the minespoil, but did significantly increase the levels of extractable Ca, Mg and K in the 0-5 cm layer of the soil. The deeper soil layer (5-15 cm) was not affected by amendment, since rototilling of the amendments into the subsurface soil was severely hampered by the presence of large quantities of rock.

Primary production by slender wheatgrass (like that of crested wheat and russian wild rye in the grassland spoil) was most stimulated by the addition of sewage sludge - particularly after the second growing season. Fertilizer application was also highly effective in promoting shoot production by slender wheatgrass. The growth pattern of slender wheatgrass in all but the peat treated spoil was very similar to that demonstrated by fall rye in the grassland spoil - i.e. a peak in primary production after the second growing season followed by a substantial decrease in growth during the third growing season.

According to Watson et al. (1980), slender wheatgrass is a short-lived (3-5 years) perennial grass which becomes secondary when planted with long-lived species. The decreased growth by slender wheatgrass in the third growing season may also have been the result of nutrient immobilization in the litter and roots produced during the first two growing seasons. Due to the slow decomposition rate of this litter (46 and 76% wt. remaining after 1 year for stems and leaves respectively) plant essential nutrients such as N and P may have been in low supply during the third growing season.

The significant growth response of slender wheatgrass to the sewage sludge and fertilizer treatments appears to have been a result of the high levels of plant nutrients present in these two amendments. The data presented in Tables 13 and 15 demonstrate that

$\text{NO}_3\text{-N}$ and extractable P in the subalpine minespoil were significantly increased by the addition of sewage sludge and fertilizer. Substantial quantities of P were still present in the minespoil after the second growing season, particularly in the sewage-sludge treated plots. Although the levels of $\text{NO}_3\text{-N}$ in the sewage sludge treated soil did not alter significantly from the beginning of the first until the conclusion of the second growing season, the $\text{NO}_3\text{-N}$ in the fertilized plots had decreased to a level where it was not significantly different from that recorded for the untreated (control) spoil.

As stated previously, sewage sludge may behave as a slow release fertilizer, due to the slow decomposition of the sludge aggregates within the soil matrix. Sewage sludge aggregates sieved from the subalpine spoil two growing seasons after application contained 2.3% TKN, $94 \mu\text{g g}^{-1} \text{NH}_4\text{-N}$ and $84 \mu\text{g g}^{-1} \text{NO}_3\text{-N}$. The original levels in the sludge would have been approximately 4% TKN, 2% $\text{NH}_4\text{-N}$ and 0.01% NO_3 ; hence, substantial quantities of N were still present although the sludge had been decomposing for two years.

Conversely, the N in the fertilizer used in this study was present as ammonium nitrate - a form which makes N readily available to plants and soil organisms. If the N is not immobilized by microbes or plant roots, leaching losses of N, especially when in the $\text{NO}_3\text{-N}$ form can be severe. In this study levels of extractable P and $\text{NO}_3\text{-N}$ in unplanted, subalpine minespoil were measured 4 months after fertilization. Levels of P were not significantly altered; however, $\text{NO}_3\text{-N}$ had decreased significantly (presumably as a result of leaching) in the 0-5 cm soil and had not accumulated in the deeper (5-15 cm) soil. Therefore, after the first growing season, the N available to the slender wheatgrass may have become limiting resulting in a decrease in primary production. Macyk (1974) observed that N (100 lbs/acre) applied to a subalpine coal spoil at Grande Cache, Alberta was completely depleted after two growing seasons, and recommended additional N application at the beginning of the second growing season. From the data presented in Table 15, it appears that

amendation of a subalpine spoil with sewage sludge would not require additional N at the beginning of the second growing season; whereas, treatment with mineral fertilizer would require additional N applications in the following growing seasons.

The high levels of nutrients available to the slender wheatgrass in the sewage sludge treated plots was reflected in the elemental concentrations measured in the foliage after one growing season (Table 38). A significantly greater amount of P was present in foliage from the sludge amended plots than from the other treatments.

Interestingly, heavy metal levels (e.g. Cu and Zn) measured in plants from the sewage treated spoil treatment were not significantly greater than those measured in plants from the other treatments, although DTPA extractable Cu and Zn levels immediately after amendation were higher in the sludge treated minespoil than in most of the other treatments. As mentioned previously, Williams et al. (1980) concluded that metals added to soil through sewage sludge treatment were readily immobilized and made unavailable to plants. It should be recognized, however, that the availability of metals and their uptake by plants is dependent on such factors as chemical composition of the sludge, rate and frequency of application, soil characteristics, and plant species (Zwarich and Mills, 1979). Also, Silveira and Sommers (1977) suggested that the availability to plants of metals in sewage sludge treated soils may be elevated with time through the dissolution of metal precipitates, oxidation of metal sulfides and release of metals complexed with sludge organic matter. However, if only one application of sewage sludge at a fairly low rate is used to initiate reclamation (as was done in this study) it is doubtful that heavy metal accumulation in plants or leaching from the soil system would be a problem. More chemical analyses of the plant species grown in the sludge treated subalpine spoil would be required to analyze the effects of one application of sludge (i.e. 46 mT ha⁻¹) on the long term accumulation of metals in plant foliage.

Although application of a feather moss peat to the subalpine spoil significantly increased N levels (particularly $\text{NO}_3\text{-N}$), grass production was not stimulated to the same degree as in the sewage sludge and fertilizer treatments. The extremely low levels of extractable P in the peat ($1.5 \mu\text{g g}^{-1}$ after application) was probably one factor accounting for the low primary production in this treatment. Peat application also significantly increased levels of Fe, Zn and Ca. Both Fe and Ca can react with P to form insoluble compounds, rendering P unavailable to plants. The initial pH of the peat-amended subalpine spoil, ranged from 6.7-7.2 and according to Brady (1974) the formation of insoluble calcium salts and even more insoluble apatites occurs in this pH range. These chemical reactions may explain the low extractable P levels measured in the peat.

The high levels of $\text{NO}_3\text{-N}$ ($449 \mu\text{g g}^{-1}$ - Table 15) and Ca ($51,900 \mu\text{g g}^{-1}$ - Table 6a) account for the significantly higher concentrations (in comparison with control plots) of N and Ca immobilized in the foliage of slender wheatgrass after one and two growing seasons (see Tables 38 and 40). Although extractable P levels in the peat were exceedingly low, the P concentration in the slender wheatgrass foliage after one growing season was not significantly different from that measured for grass grown in the fertilized spoil. The role of the mycorrhizal fungi in improving the P nutrition of grass growing in a P-deficient peat such as that used in this study would probably be crucial for the survival and health of the plants (Zak *et al.*, 1984).

Alsike clover shoot production, in comparison with that of slender wheatgrass, did not peak during the second growing season, but increased over the 3 year sampling period, particularly in the sewage sludge and fertilizer treated plots. Alsike clover is a short-lived perennial (Watson *et al.*, 1980), but in this study was observed to produce large quantities of seed after the second growing season in all treatments. The seed germinated readily, and this accounted for the increased primary production in all treatments over the 3 years that it was measured. Sewage sludge and fertilizer

promoted the greatest shoot production because these amendments contained high concentrations of plant essential nutrients. Although much of the N included in the fertilizer may have leached out of the rooting zone by the end of the second growing season, this would not have affected the growth of alsike clover due to its N_2 fixing capabilities. Measurements of N fixation (using the acetylene reduction technique) demonstrated that during the second growing season, alsike clover roots in all treatments were nodulated and had the capacity to fix N_2 (Parkinson et al., 1980). The %N measured in the foliage of alsike clover after the second growing season demonstrated no significant effect of treatment on plant N levels (see Table 40), providing further evidence that even under the low N conditions found in the untreated subalpine spoil, a legume such as alsike clover is capable of fixing enough N_2 to meet its N requirements.

Application of peat to the subalpine spoil did not stimulate shoot production of alsike clover in comparison with shoot production in the control treatment. Although no data were collected regarding establishment success of alsike clover in the peat treated spoil, field observations indicated that during the first growing season clover seedlings did not establish themselves readily, and once established demonstrated very poor growth. It was postulated that the low extractable P level ($1.5 \mu\text{g g}^{-1}$) in the peat was partially responsible for the poor growth response by the clover. A greenhouse pot experiment to study the effects of added P on the growth of alsike clover in the peat treated subalpine spoil, demonstrated that additional phosphorus ($272 \mu\text{g P g}^{-1}$ peat) significantly increased plant production by alsike clover and also enhanced flower production (S. Visser, unpubl. data). No flowers were produced by plants grown in the untreated peat, indicating the significance of adequate P availability to flowering and seed formation. The lack of seed production as a result of low P levels would influence the reseeding success of alsike clover (Parkinson et al., 1980). Watson et al. (1980) also note that alsike clover requires moderate amounts of P for satisfactory growth. As mentioned

previously, a legume should be grown in association with grasses when reclaiming minespoils to provide an effective and long term N source. Under the conditions imposed in this study, alsike clover established and reseeded itself successfully when adequate levels of P were available. If it has the ability to compete with grasses, it is recommended for use on reclamation sites.

In contrast to the slender wheatgrass and alsike clover, white spruce demonstrated no significant shoot growth until the fourth growing season (see Fig. 3). Because of its slow growth rate, no effects of amendment on shoot weights were recorded until the end of the fourth year when spruce in the sewage sludge and peat treated spoil were heavier than those in the fertilized and untreated spoil. The probability that sewage sludge provided adequate levels of available nutrients to the spruce seedlings over the long term (in this instance, 4 years) would explain the better growth of spruce in the sludge-treated spoil in comparison with that measured in the control spoil. Although fertilizer treatment introduced adequate levels of available N and P to the minespoil when the trees were planted, the slow growth and uptake of nutrients by white spruce resulted in the loss of nutrients (particularly N) through leaching before the trees could immobilize them. Therefore, by the third growing season, nutrient levels in the fertilized spruce subplots were probably similar to those in the control subplots resulting in a growth response similar to that measured in the untreated minespoil. Therefore, if fertilizer is to be used to stimulate growth of slow-growing species such as white spruce, its application should be "tailored" to meet the demand of the tree i.e. continuous application over the long term.

In contrast to the results on shoot production obtained for slender wheatgrass and alsike clover grown in the peat amended spoil, shoot production by white spruce planted in the peat treatment was not significantly different from that measured in the sewage treatment at the conclusion of the fourth growing season. The different response of the grass and clover to the peat treatment compared with that observed for the spruce may be explained by:

i) the nutritional demand by the white spruce in its early stages of growth is much less than that for a fast growing grass or legume. The slender wheatgrass and clover may have taken up many of the nutrients available in the peat and immobilized them in dead plant litter and roots. On the other hand, the slow growing white spruce are incapable of rapidly immobilizing nutrients; therefore these nutrients, with the aid of mycorrhizal fungi, are available over a much longer term.

ii) it is possible that over the term of the study, some decomposition of the peat may have occurred, thereby releasing nutrients such as P which stimulated tree growth after the third growing season. Microbial respiration and microbial biomass C measurements for the peat treated subalpine spoil demonstrated a significant increase between the beginning of the first growing season and end of the second growing season, indicating that some decomposition of the peat may have occurred after its application to the spoil. More research would be required to determine whether freshly excavated peat decomposes after spreading on a spoil surface and, if so, at what rate and to what degree.

A chemical analysis of the white spruce needles after one growing season showed no real amendment effects on nutrients taken up by the tree. The slow growth and low nutrient uptake by the tree during the first growing season would explain the lack of treatment effects on needle chemical composition. Foliage analysis over the long term would be required to assess the effects of the various treatments on nutrient levels in white spruce.

The percent survival of white spruce over the first two growing seasons was high (84-90%) and was not significantly influenced by treatment.

According to Selner (1977), white spruce is considered to be the best conifer species for reclamation of coal mined areas in the eastern slopes. However, if white spruce or other woody species are planted in areas which were originally seeded with grasses, tree growth may be reduced. Research by Fales and Wakefield (1981) demonstrated that turf grasses such as perennial ryegrass, red fescue

and Kentucky bluegrass significantly reduced the growth of woody species such as flowering dogwood. They attributed the reduced growth to direct competition between the plant species for available N, and also to the production of allelopathic substances by the turf grasses. Also, if primary production of grasses has been encouraged by regular fertilizer applications, a thick mat of dead litter may develop since decomposition in subalpine areas is retarded by low temperatures. This litter layer offers protection for small rodents which multiply and can become a menace to young tree seedlings.

In comparison with the slow growing white spruce, willow demonstrated very rapid shoot growth particularly in the sewage sludge amended spoil. Response by the willow to the high levels of nutrients added to the minespoil in the form of sewage sludge and fertilizer was evident after the first growing season when shoot production in these two treatments was significantly greater than that in the control and peat treated plots. Shoot production continued to be highest in the sewage sludge amended spoil over three growing seasons, whereas shoot production in the fertilized plots was significantly less than that measured in the sludge treatment after the first growing season. As mentioned previously, loss of N through leaching from the fertilizer treated spoil during the first growing season would be one possible explanation for decreased growth during subsequent growing seasons. Additional fertilizer applications would be required to maintain the rapid growth of a shrub such as willow in the subalpine coal spoil. However, shoot production in the sewage sludge treatment was maintained at a high level over the three growing seasons - presumably as a result of nutrients being released over a long term from the slowly decomposing sludge aggregates. The lack of easily available P in the peat amended spoil may account for the low growth response by willow to this treatment.

Although percent survival of willow was high (91-97%) over the first 2 years and was not significantly affected by treatment, percent dieback after the second winter was significantly greater in the peat amended spoil than in the other treatments. This dieback

may have been a result of very dry fall weather which dried the peat creating drought conditions over the winter. Salix spp. such as Salix glauca L. are not considered to be very drought tolerant (Watson et al., 1980). However, the dieback did not appear to affect subsequent growth and survival of the plants.

Willow spp. are known as important sources of browse for moose, bighorn sheep, elk and mule deer (Watson et al., 1980). In this study evidence of browsing by deer was apparent after the second growing season, particularly in treatments where branch growth during the previous growing season had been high. The level of browsing encountered in this study did not appear to affect subsequent shoot production, although quantitative data would be required to substantiate this observation. Because shrub species such as willow are prone to browsing and dieback, height measurements to assess effects of various treatments on shoot growth can be very misleading. Shoot weight measurement after destructive sampling is a more accurate method for obtaining data on plant response to amendment.

4.3 OIL SANDS MINESPOIL

Surface mining of the Athabasca Oil Sands deposit involves the stripping of the vegetation and layers of clay, sand, gravel and boulders to expose the bitumen-bearing sand. If large quantities of peat and clay overlies the oil sand deposit, these are removed and stockpiled for future reclamation of the oil sand tailings. The oil extraction process involves the use of hot water, steam and caustic soda to remove the bitumen from the sand. This process results in the production of large volumes of an alkaline, aqueous suspension of clays which are stored in large tailings ponds. The ponds are dyked with the extracted sand. As a result of the harsh extraction process, the characteristics of the sand tailings include the following:

- i) sand content is high (96%) while silt (2.7%) and clay (1.0%) contents are low resulting in low water holding capacity (Turchenek, 1976). In the present study the sand was found to be fine-grained with 86% of the sand grains falling in the 125-500 μm

size range. The sand also appears to be hydrophobic, therefore not rewetting evenly (McCoy et al., 1976; Turchenek, 1976; Takyi et al., 1977) - a factor which leads to erosion problems particularly during heavy rainfall (Takyi et al., 1977).

ii) pH of fresh tailing sand is alkaline but tends to decrease to neutrality after one or two years of exposure (Lesko, 1974). Turchenek (1976) recorded a pH of 9.2 for tailing sand whilst Takyi et al. (1977) and McCoy et al. (1976) recorded pH values of 6.4 and 6.6 respectively. In the present study, the pH of tailing sand from the control plots was recorded as being approximately neutral (7.3-7.5) indicating that this particular sand had been exposed for some time prior to its shipment to Calgary from the Suncor site. Sodium (0.8 me l^{-1}) and E.C. (0.4 ms/cm) measurements of the sand were low - an observation also made by Takyi et al. (1977) and McCoy et al. (1976). Thus plant growth problems as a result of the NaOH used in the oil extraction were not encountered in this present study.

iii) the nutrient status is very low (Massey, 1972; Turchenek, 1976; McCoy et al., 1976; Takyi et al., 1976). Nutrient levels of the extracted oil sand used in this study were low (i.e. $\text{NO}_3\text{-N}$, $0.1 \text{ } \mu\text{g g}^{-1}$; extractable P, $1.2 \text{ } \mu\text{g g}^{-1}$). According to Turchenek (1976) the infertility of the tailing sand is due mainly to the lack of organic matter and inorganic colloids. The organic content of the sand in this study was found to be 0.2-0.4% which is similar to that obtained by Turchenek (1976). Due to the low organic matter content, the CEC, nutrient retention and water holding capacity would also be expected to be low, resulting in leaching problems if nutrients such as fertilizer are added.

Since the tailing sand has been shown to be mainly sand with associated low fertility and low water holding capacity, successful reclamation of this minespoil would require the addition of amendments to raise the nutrient status, nutrient retention and water holding capacity. Therefore, in this study, the following amendments were applied to oil sand tailings:

i) inorganic fertilizer to improve the fertility of the sand and thereby promote plant growth.

ii) sewage sludge to improve the fertility of the sand and also to increase moisture and nutrient retention.

iii) feather moss peat which, because of its high organic matter content, would significantly increase the nutrient and moisture retention, and CEC of the sand. It would also act as a source of plant nutrients.

The application of inorganic fertilizer to the sand resulted in a significant drop in pH (6.4) as compared with the control (7.3). This was also observed for the subalpine spoil, and may have been caused by the same factors as discussed for that spoil type. Levels of extractable K, extractable P and $\text{NO}_3\text{-N}$ in the sand were significantly higher after fertilizer application. However, approximately 4 months after fertilizer application, extractable P and $\text{NO}_3\text{-N}$ measurements had decreased significantly in the 0-5 cm depth of sand in the unplanted pathways (see Table 19). These nutrients had not accumulated in the 5-15 cm depth of sand, indicating the high leachability of the sand.

Addition of sewage sludge to tailing sand also improved the fertility of the sand by increasing the levels of extractable cations, extractable P and $\text{NO}_3\text{-N}$. Amounts of DTPA extractable Cu and Zn in the sand were also significantly greater after application of the sludge; particularly in the surface soil. Because of the organic nature of the sewage sludge, the CEC, moisture and nutrient retention capabilities of the sludge-amended sand were probably also improved particularly in and near the sewage sludge aggregates.

Amendation of the extracted sand with peat resulted in increased levels of DTPA extractable Fe and Zn and also $\text{NO}_3\text{-N}$. However, as was the case for the subalpine spoil, amounts of extractable P in the peat-treated sand were not significantly different from those measured in the untreated control. McCoy et al. (1976) mentioned that decomposed peat has a low extractable P content, suggesting that supplemental P would be required to promote and maintain a vegetative cover on this particular amendment.

Amendation effects on shoot production of slender wheatgrass were highly variable in all treatments. This variability may have been the result of the patchy distribution of nutrients throughout the sand particularly in the sewage sludge treated mine tailings. Since the sand is so nutrient-poor, the sewage sludge aggregates (which resulted after the liquid sludge had dried on the sand surface) probably behaved as nutrient-rich islands around which plant growth was stimulated. Also, sewage sludge may have inhibited seed germination and shoot growth during the early stages of plant development for the same reasons outlined in the grassland spoil discussion. To decrease the variability resulting from the uneven nutrient conditions, more samples should have been processed, but this was not feasible in this study due to the limited size of the plots. However, slender wheatgrass did tend to respond more favorably to the sewage sludge treatment (in comparison with the other treatments) particularly during the second growing season when primary production peaked. McCoy et al. (1976) obtained similar results when spent sand treated with sludge and NPK fertilizer; sludge significantly increased barley yield in comparison with the yield produced on spent sand and NPK fertilizer alone. The high levels of nutrients added with the sludge (particularly N and P), the relatively long term release of these nutrients and the nutrient retention capacity of the sludge (resulting in an improved CEC for the highly leachable tailing sand) are all factors which would contribute to stimulated plant growth in sludge-treated sand.

The improved fertility of the sewage-sludge amended tailing sand was evident in the foliage analysis of slender wheatgrass after one and two growing seasons i.e. concentrations of N and P were significantly greater in plants from the sludge treated sand than from the control. Levels of heavy metals (Cu and Zn) were not significantly higher in plants from the sewage sludge amended subplots than in plants from the control subplots, again demonstrating that over the short term (1 year) and with a low sludge application rate (46 mT ha^{-1}) heavy metals were not concentrated in this particular grass. The metals are probably strongly complexed

with the sludge organic matter and released over the long term as the sludge aggregates degrade.

A comparison of the foliage analysis of slender wheatgrass grown in the sewage sludge treated subalpine and extracted sand minespoils, indicated that more nutrients (particularly P) were available to the grass in the sludge treated sand than in the sludge-treated subalpine spoil. The low CEC (2.9 - Takyi et al., 1976) and associated low nutrient retentitivity of the tailings sand implies that many of the nutrients released from the sludge into the soil solution around the sand particles would be directly available to the plant roots, rather than being immobilized by clays or organic material. Since nutrient availability is greater under these conditions, elemental concentrations in the foliage of plants grown in sludge amended sand would be expected to more closely reflect nutrient availability than would plants grown in soils possessing nutrient complexing or retention properties. The high availability of nutrients and other elements in the soil solution of the tailings sand could also lead to more pronounced toxicity effects if too much sewage sludge were applied, thereby resulting in reduced plant growth.

Fertilizer and peat amendment of the extracted oil sands, also stimulated slender wheatgrass shoot production, but not to the same degree as did sewage sludge. As mentioned previously, many of the nutrients applied through the fertilizer had probably leached out of the rooting zone during the first growing season, except for those nutrients immobilized in the shoots and roots of the grass. Therefore, primary production after the first growing season may have been limited by lack of nutrients. In a growth chamber experiment, Takyi et al. (1976) observed that fertilizer applied to tailing sand improved the growth of legume-grass mixtures. They concluded that plants could be grown on tailing sand if maintained with a good fertilization program. Successful revegetation of tailings sand using fertilizer amendment would require more than one application of fertilizer (as was used in this study). Repeated fertilizer applications require careful management particularly if the

reclamation goal entails high yields of forage crops, but also if the aim is to encourage the establishment of native plants which would initiate succession toward a more stable plant community.

Peat also stimulated growth by slender wheatgrass when compared to growth in the unamended tailing sand. However, the low level of extractable P in the peat (after the second growing season, $0.8 \mu\text{g P g}^{-1}$ peat was measured in soil from the slender wheatgrass subplots) probably limited growth of the grass. As stated earlier, $\text{NO}_3\text{-N}$ levels were high in the peat and this was reflected in the amount of N measured in slender wheatgrass foliage (2.5%) after the second year. The quantity of P measured in the slender wheatgrass shoots was not significantly different from that measured in plants grown in the unamended sand. As for the slender wheatgrass grown in the peat treated subalpine spoil, this result might be explained by the presence of efficient vesicular-arbuscular mycorrhizal fungi in the grass roots. Takyi et al. (1976) observed that fertilizer applied to a tailings sand-peat mixture considerably improved the growth of a legume grass mixture, whilst McCoy et al. (1976) found that barley yields were significantly higher in a spent sand plus peat plus NPK mixture than in a spent sand plus NPK mixture. It appears, therefore, that some additional P would be required to improve plant establishment and growth in a $\text{NO}_3\text{-N}$ rich, but P deficient peat such as that used in this present study.

In agreement with Vaartnou (1974), seed of slender wheatgrass germinated readily in the untreated tailing sand in the plots. However, the low nutrient status of the sand severely limited plant growth. The pattern of shoot production by slender wheatgrass in the sewage sludge and fertilizer treated sand was similar to that observed for slender wheatgrass in the subalpine spoil - i.e. a peak during the second growing season followed by a significant decline in the third growing season. That this species is a short-lived perennial (Watson et al., 1981), and that many of the plant essential nutrients had become limiting as a result of immobilization in the slowly decomposing litter may be factors which explain this growth pattern. It should also be mentioned that the seed produced by the

slender wheatgrass (particularly in the sludge treated sand) germinated readily in the pathways, but not in the grass vegetated subplots.

Shoot production by sainfoin was, like that of the slender wheatgrass, most stimulated by sewage sludge amendment. Shoot production was consistently higher in the sludge treated sand than in the other amendments over the 3 year term of the study. Again the significantly better fertility of the sand (particularly N and P) over the long term after sludge application would account for the success of this species in this treatment.

Although sewage sludge significantly stimulated growth by sainfoin, it was observed that over the term of the study, $N_2(C_2H_2)$ fixation by this species was less than that measured for plants from the other three treatments. Measurements made during the third growing season demonstrated that reduced $N_2(C_2H_2)$ fixation by sainfoin in the sewage treatment may have been a result of high levels of nitrate or high levels of heavy metals around the sewage sludge clumps where roots tended to concentrate. Munns (1977) in his review of mineral nutrition and the legume symbiosis noted that various studies have demonstrated that high NO_3-N levels inhibit nodule development, retard nodule growth and reduce the rate of N_2 fixation. Vesper and Weidensaul (1978) found that 1-5 ppm Cd, Ni and Cu reduced nodule numbers, N_2 fixation or both. Zinc also reduced nodulation but inhibited N_2 fixation only at the 5-10 $mg\ L^{-1}$ levels. As mentioned previously Cu levels in the sewage sludge-amended tailing sand (0-5 cm depth) were significantly greater than in the other treatments. The low CEC of the sand may have resulted in a greater availability of metals such as Cu in the soil solution around the roots. The increased Cu level combined with the high N levels in the sludge treated plots may have been factors contributing to inhibited N_2 fixation in this treatment.

Fertilizer (NPK) amendment of the tailing sand also stimulated shoot production by sainfoin, but only during the first growing season. A decrease in the fertility of the sand as a consequence of fertilizer leaching or nutrient immobilization in

undecomposed shoots and roots may account for these results. Because sainfoin has the capacity to fix N_2 , loss of N from the sand should not seriously affect plant growth. However, excessive loss of P through leaching so that P becomes limiting may affect efficient nodule function. Munns (1977) mentioned that there is evidence in the literature that P fertilization improves nodulation and that nodule function may require more P than the growth of the host plant. Therefore, limiting P levels in the fertilized tailing sand after one growing season may also have indirectly affected primary production. Also excessive quantities of N in the first growing season may have delayed nodulation and associated N_2 fixation until the second growing season, when low P levels may have decreased the N_2 fixing efficiency of the nodules.

In short term pot experiments, Takyi et al. (1976) showed that slender wheatgrass-sainfoin mixtures performed considerably better in fertilized (NPK) tailing sand, but that high soil fertility gave a competitive advantage to the grass. Vaartnou (1976) also reported that productivity of various native legumes planted in tailings sand was greatly enhanced when treated with fertilizer. However, germination tended to be delayed if fertilizer was added at the time of seeding. According to Brady (1974) fertilizer salts placed directly under the seeds can result in injury to the plant as a result of the upward movement of NO_3 and K salts by capillary water. Also ammonium fertilizers may release ammonia which has been shown to inhibit seed germination (see grassland spoil discussion). As a result, fertilizer application to the highly leachable tailing sand would probably be more practical after germination of the seed when nutrient uptake by the plants can occur.

Peat treatment of the tailings sand also stimulated primary production by the sainfoin although results were highly variable. Vaartnou (1976) in a greenhouse pot experiment observed improved shoot height of three native legume species when peat was added to tailing sand. He also found that more peat mixed into the tailing sand led to greater shoot height by American milk vetch, showy locoweed and golden bean and that the addition of fertilizer had no

effect. In contrast, Takyi et al. (1976) demonstrated that peat addition to tailings sand slightly improved the yield of a slender wheatgrass-legume mixture, but that the addition of NPK fertilizer substantially increased shoot and root production. Since the peat and tailing sand used in this present study contained very low quantities of extractable P, addition of a P fertilizer (N was not required due to the high levels of $\text{NO}_3\text{-N}$ in the peat) would no doubt have resulted in improved growth and N_2 fixation capability by the sainfoin, particularly during the first growing season. As was the case for the subalpine spoil, microbial respiration and biomass of the peat amended tailing sand also demonstrated an increasing trend with time - a factor which may have resulted in the mineralization of some of the bound P in the peat, thereby stimulating growth during the second and third growing seasons.

Although germination of sainfoin seed was not inhibited in the untreated (control) tailing sand, establishment was very low. However, a few plants did survive the first growing season and were observed to grow remarkably well during the second growing season, despite the high nutrient stress in the tailing sand. Some of these plants were included in the random quadrats which were clipped for primary production estimates. In the process of sampling the roots at the conclusion of the second growing season, it was observed that sainfoin in the control subplots had rooted so deeply that they had grown out of the sand layer and into the gravel underneath the sand. The better nutrient conditions in the gravel accounted for the success of the surviving plants and explains the shoot production results obtained after the first year in the control subplots (see Table 32).

Sainfoin is a short-lived perennial which re-establishes itself mainly by seed. Sainfoin seed was observed to germinate fairly readily, but production of seed may have been severely limited by selective grazing of sainfoin flowers by deer, regardless of treatment.

The %N immobilized in the sainfoin foliage after the second growing season ranged from 1.9% in the fertilizer to 3 % in the peat

and was not significantly affected by treatment. The N_2 -fixing ability of the sainfoin or the high levels of N available in some of the treatments would account for the lack of treatment effects.

Jack pine, like slender wheatgrass and sainfoin, was most productive in the sewage sludge-treated sand tailings, presumably for the same reasons as those mentioned for the legume and the grass. Berry and Marx (1977) recorded stimulated growth by loblolly pine seedlings (Pinus taeda L.) on sewage sludge treated kaolin spoil, but that high seedling mortality occurred when the sludge application was 138 or 275 mT ha⁻¹. This mortality was attributed to toxic substances in the sludge. As noted previously, there is evidence in the literature which suggests that ammonia released from sewage sludge can severely inhibit root elongation. High ammonia levels in the heavy applications of sludge used by Berry and Marx (1977) may have been one of the toxic substances causing seedling mortality. In the present study seedling survival of jack pine was higher in the sewage sludge amended sand (97%) than in the other treatments; hence the sludge application rate (46 mT ha⁻¹) was low enough that it stimulated growth, but did not affect survival. Since shoot production (and associated response to amendment) by jack pine was minimal during the first growing season, results on chemical analyses of the needles should be treated with caution. The %N in the needles after two growing seasons was significantly greater for trees grown in the sludge treated sand than in the fertilized sand suggesting that high levels of N were available to the trees over the first two growing seasons. Estimation of heavy metal immobilization by jack pine as affected by sewage sludge treatment would require more analysis over the long term.

Fertilizer also stimulated jack pine productivity but the stimulation was considerably less than that recorded for the sewage sludge. The low growth response by jack pine during the first growing season was similar to that recorded for white spruce (subalpine spoil). The slow growth of the trees coupled with the high leachability of the tailing sand, resulted in a considerable loss in nutrients before the trees had developed a large enough root

system to take advantage of the improved fertility. The significantly lower level of N in the foliage of jack pine after two growing seasons, compared with that recorded for trees in the sludge and peat treated sand suggests that N in the fertilized plots may have become limiting after the first year. Bengston et al. (1978) found that growth of loblolly pine seedlings on an infertile, readily leached coal mine spoil was greatly increased by NP fertilizer application, but that maintenance of rapid tree growth required inputs of N on a continuing basis. Experiments conducted to study the effects of interseeding loblolly pine with a legume (Lespedeza cuneata) showed that by the sixth year, growth rate and N status of the trees planted with the legume was almost equal to trees which had received two 112 kg ha^{-1} increments of supplemental N. Perhaps sainfoin interseeded with jack pine would produce similar results. Bengston et al. (1978) also demonstrated that grasses (fescue and bermuda grass) interseeded with pine somewhat reduced pine growth and attributed this result to competition for moisture. As stated in the discussion on the subalpine site, evidence provided by Fales et al. (1981) indicated that reduced growth by woody perennials planted amongst turf grasses may involve the production of allelopathic substances by the grasses. The results obtained by these authors should be considered when planting trees and shrubs in areas already revegetated with grasses.

After sewage sludge, peat was the next most effective amendment for promoting shoot growth by jack pine, particularly during the third growing season. Interestingly, white spruce in the peat treated subalpine spoil demonstrated a very similar growth response. The nutrient retentivity properties of the peat and the high level of $\text{NO}_3\text{-N}$ in the peat coupled with a probable mineralization of bound P from the peat through microbial action may all be factors contributing to the improved growth by jack pine during the third growing season in the peat treated spoil. The large quantity of available N in the peat was reflected in the %N in the needles after two years growth when no difference was found between %N in trees grown in sludge treated sand (1.8%) and trees grown in

peat treated sand. Survival by jack pine in the peat amended sand was high (88%).

Although survival of jack pine after the first growing season was high in the untreated sand, growth was negligible due to the low fertility of the tailing sand. Presumably during the first growing season jack pine seedlings in the control subplots survived on nutrient reserves present in the peat plug in which they were grown prior to outplanting. Once these reserves were spent many of the seedlings died as was evident by their 54% survival after the second winter.

After two growing seasons, bearberry in the sewage sludge and peat amended tailing sand had produced significantly heavier shoots than bearberry in the fertilized and untreated sand. Like the conifers used in this study, bearberry is a slow growing plant (in comparison with the grass and legume) and, therefore, does not have the capacity to rapidly immobilize readily available nutrients until it becomes well established and has produced a substantial root mat. As a result, bearberry would not have benefited to the same degree as the grasses from the mineral fertilizer applied prior to planting since its root mat was negligible during the first growing season, allowing nutrients to be readily leached from the soil system. The slow release of nutrients from the sewage sludge and the nutrient retention capacity of the peat resulted in more nutrients being available in these two treatments during the second growing season when the bearberry could respond to them. It is interesting to note that all the woody perennials tested in this study (i.e. white spruce, willow, jack pine and bearberry) demonstrated very poor growth (i.e. shoot production) during the first growing season. No real growth and associated response to nutrients occurred until the second growing season when the plants had become well-established. This suggests that amendments, which provide slow release of nutrients (e.g. sewage sludge) would be more beneficial to these species over the long term than applications of highly available nutrients in the form of inorganic fertilizers. This would be particularly true when amending infertile, coarse grained minespoils

where nutrient loss through leaching can be a major problem. In contrast to the other woody species tested in this study, bearberry performed remarkably well in the P deficient peat treatments. Since bearberry is generally found on nutrient poor, coarse-textured soils (Watson et al., 1980), its demand for P may not be as great as it is for other plant species; hence it was not as sensitive to the low P levels in the peat and, as a result, successfully colonized the peat treated tailing sand.

Although bearberry, once established, appears to grow well it was very susceptible to winter kill particularly during the first winter after planting. Also, seedling mortality was found to be treatment related with seedlings planted in tailings sand treated with fertilizer or sewage sludge demonstrating the poorest survival (31% and 38% respectively). It is possible that, if bearberry is a species with low nutrient requirements, the high levels of nutrients available in the sludge treated and fertilizer sand during the first growing season may have been toxic to the seedlings as they established themselves. Once established and growing, they seem to respond favorably to high nutrient levels as demonstrated by their growth response in the sludge treated subplots during the second growing season. More research is required to determine the optimum conditions for the establishment, growth and survival of bearberry.

5. CONCLUSIONS

With the exception of the legumes (which have been introduced from Europe), all of the plant species tested in the subalpine and oil sands minespoil are native to North America. All the species, once they had established themselves, did reasonably well to very well in the variously treated minespoils. Generally, sewage sludge treatment of the minespoils promoted the best growth response from all tested plant species over the term of the study (i.e. 3-4 yrs). The high nutrient content of sewage sludge and the slow release of these nutrients over a relatively long period of time suggests that this amendment (if readily available) be strongly considered for the reclamation and revegetation of infertile, highly leachable minespoils. Even at the low application rate (once at 46 mT ha^{-1}) used in this study, sewage sludge was still stimulating growth of many of the plant species up to five years after its application.

Peat was considered the next best amendment for revegetation purposes, although the low extractable P levels in peat are believed to have inhibited the growth and establishment of some of the test plant species particularly during the first growing season. However, peat did improve the nutrient and water retention properties of the two spoil types and with an initial addition of inorganic P may be the best amendment over the very long term.

Because the minespoils (particularly oil sand tailings) are highly leachable, inorganic fertilizer treatment resulted in only very short term (one growing season) stimulation of plant growth. Over the long term, many applications of fertilizer would be required to ensure continued growth and maintenance of a vegetative cover. It would be much more practical to use fertilizer in conjunction with an organic amendment such as peat if long term revegetation by a particular plant species is the reclamation goal.

Of all the plant species tested, slender wheatgrass appeared to be the shortest lived and the least successful in reseeding itself within the grass subplots under the conditions set in this study. It is therefore considered a short term cover

species, perhaps useful for erosion control. Because it is short lived, slender wheatgrass may be a good species to plant prior to the introduction of the longer lived woody perennials such as white spruce or jack pine, particularly if a managed forest is the aim of reclamation. However, in a situation such as this conditions should be such as to allow efficient decomposition of the grass detritus with attendant nutrient recycling. Prior to planting trees in areas originally revegetated with grasses it should also be kept in mind that a grass cover (particularly a healthy grass sward) may inhibit the growth of trees through competition for water and nutrients or through the production of allelopathic substances.

Both legumes (i.e. alsike clover and sainfoin) performed very well under the climatic conditions found in the Calgary area. Through their ability to fix atmospheric N_2 , they have the capacity to improve the N status of a minespoil over the long term. Since minespoils are notoriously low in N, legumes such as alsike clover should be considered an essential component of a revegetation program and care should be taken that they become well established. If a reasonably long term shrub cover is required for revegetating and stabilizing a minespoil, willow should be considered. It grows rapidly and has the ability to withstand stresses such as heavy browsing since it can readily resprout. If the aim of a revegetation program is to initiate the early stages of a plant succession towards a more stable plant community, willow should be considered since it is a common invader of naturally disturbed areas. Because willow (along with the grasses and legumes) is fast growing it can readily immobilize nutrients in litter and roots; thereby conserving nutrients for future plant growth rather than losing them from the soil system through leaching. This process is instrumental in the initiation of soil formation - a necessary step if succession is to take place. Bearberry, once established, also grew well, but its high mortality may make it an impractical species for rapid revegetation of a mined area.

6. SUMMARY

6.1 PRAIRIE GRASSLAND MINESPOIL

i) The prairie grassland minespoil was characterized by moderately high levels of clay and SiH and exchangeable Na, a high SAR and low fertility.

ii) A layer of topsoil, 15 cm thick, spread on the surface of this minespoil was most effective in reducing the high SAR and improving seedbed and fertility conditions. Sewage sludge amendment also improved the nutrient status of the spoil, but was not effective in reducing the SAR over the short term.

iii) Fall rye and crested wheat were most productive in the sewage sludge and topsoil treated spoil; russian wild rye performed best in the sewage sludge treatment, and topsoil promoted the most growth by Rambler alfalfa. In general, gypsum was the least effective in promoting plant growth in the grassland minespoil.

iv) Plant production by crested wheat, russian wild rye and Rambler alfalfa increased steadily over the three year study term, particularly in those treatments which stimulated growth. However, the shoot weight produced by fall rye peaked in the second growing season and then decreased sharply in the third growing season, suggesting that this plant species is very short-lived, while the other three plant species can be considered long-lived perennials.

6.2. SUBALPINE MINESPOIL

i) Removal of the organic mat in the process of mining for coal in the subalpine region results in a minespoil which is dominated by large rock fragments. It is therefore characterized by low levels of organic matter and plant required nutrients and a poor water holding capacity. The pH approaches neutrality.

ii) Sewage sludge amendment of the subalpine spoil was highly effective in promoting shoot production of the test plant species, i.e. slender wheatgrass, Alsike clover, white spruce and willow, over the term of the study. Sewage sludge significantly improved the fertility of the spoil, and appeared to be releasing

nutrients over the long term. After two growing seasons, substantial quantities of N were still present in the sludge aggregates sieved from the spoil.

iii) Addition of inorganic fertilizer to the spoil stimulated shoot growth of slender wheatgrass and willow, but not to the same degree as did sewage sludge. There was some evidence that leaching losses of N from the fertilized spoil, particularly during the first growing season, may have significantly affected primary production in subsequent growing seasons. Interestingly, alsike clover performed as well in the fertilizer treatment as in the sewage sludge treatment. Since alsike clover has the capacity to fix its own N, it would be less sensitive than non N_2 fixers to leaching losses of N from the fertilized spoil.

iv) Although the peat improved seedbed conditions and introduced large quantities of NO_3-N , it was severely lacking in plant available P. Therefore, the grass, clover, and willow performed poorly in this amendment, in comparison with their growth response in the sludge and fertilizer treatments. Alsike clover was observed to be particularly sensitive to the low P levels. In a greenhouse pot experiment, clover grown in the peat fertilized with superphosphate produced significantly larger plants and more flowers than clover grown in the untreated peat.

v) White spruce performed as well in the peat amended spoil as in the sewage sludge treatment, but not until the fourth growing season. Since white spruce is extremely slow growing, it requires a long term supply of nutrients which is not readily lost through leaching. Sewage sludge appears to behave as a slow release fertilizer, while there is some evidence that over the term of the study unavailable forms of P bound in the peat may have been mineralized through microbial action.

vi) Although sewage sludge application increased levels of DTPA extractable Cu and Zn in the spoil, these metals had not concentrated in the foliage of slender wheatgrass or white spruce after one growing season.

vii) The high levels of $\text{NO}_3\text{-N}$ present in the peat amendment were reflected in the significantly higher quantities of this nutrient measured in the foliage of slender wheatgrass and willow grown in the peat-treated minespoil. Foliage N of alsike clover was not affected by treatment.

viii) Extractable P measurements in the various treatments demonstrated a strong relationship to shoot production of slender wheatgrass and alsike clover during the first growing season. However, soil extractable P levels were not highly correlated with foliage P of slender wheatgrass during the first growing season.

ix) Primary production by slender wheatgrass peaked during the second growing season, then declined sharply during the third growing season, suggesting that this grass, like fall rye, is a short-lived perennial suitable only as a short term plant cover.

x) The fast growing plant species, i.e. slender wheatgrass, alsike clover and willow can play a major role in rapidly immobilizing nutrients (which are otherwise lost through leaching), in the leaves and root mat. This process is important if succession towards a self-maintaining vegetative cover is to be initiated in the nutrient-poor conditions of a minespoil. White spruce, which is a very slow growing species, would not be very efficient in retaining nutrients within the plant-soil system during the initial stages of reclamation.

6.3 OIL SANDS MINESPOIL

i) Surface mining of the Oil Sands results in a minespoil which is almost pure sand devoid of nutrients and possessing a low CEC and poor water holding capacity. The pH of the tailing sand approaches neutrality one to two years after exposure to the elements.

ii) Amendment of the tailing sand with fertilizer significantly improved the NPK status of the sand, but many of these nutrients had leached out of the rooting zone by the end of the first growing season.

iii) The fertility status of the sand was greatly improved by the addition of sewage sludge. The fertility of the sludge amended sand appeared to be maintained over the 3 year term of the study.

iv) Application of peat to the tailing sand increased the N status of the soil, but available P was found to be potentially limiting.

v) As was observed for plants grown in the subalpine spoil, sewage sludge promoted the greatest shoot production by all four plant species (slender wheatgrass, sainfoin, jack pine and bearberry) over the term of the study.

vi) Fertilizer amendment was observed to be ineffective in stimulating plant growth presumably as a result of rapid loss of nutrients from the sand before plant uptake could occur. Slender wheatgrass performed best in the fertilized sand presumably because of its rapid growth rate and associated rapid immobilization of nutrients.

vii) The lack of extractable P in the peat is one factor which may have inhibited the primary production potential of all the test plant species. The slow growing bearberry performed best in the peat treated sand followed by jack pine and sainfoin. Some mineralization of bound P may have occurred in the peat after the first growing season.

viii) The growth pattern of slender wheatgrass was similar to that observed for slender wheatgrass grown in the subalpine spoil, suggesting this species is a short-lived perennial suitable for rapid cover of a site.

ix) Bearberry was highly susceptible to winter kill particularly in the fertilizer and sewage sludge treated tailing sand.

x) Foliage analysis of the slender wheatgrass and jack pine demonstrated that sewage sludge was the best amendment for providing N and P. Peat also supplied adequate quantities of N, but the P concentration in the jack pine needles was not significantly

different from that observed for trees in the untreated sand, suggesting P in the peat was limiting to plant growth.

xi) The %N in the foliage of sainfoin was not influenced by amendment. Nitrogen was either supplied by the amendment or by N_2 fixation.

xii) Heavy metals (Cu, Zn, Fe) were not concentrated in the foliage of slender wheatgrass, sainfoin and jack pine after one growing season in the sewage sludge treated sand in comparison with foliage metal levels of plants grown in untreated sand.

xiii) The P concentrations recorded for the jack pine needles were highly correlated with soil P levels. This relationship did not hold for the slender wheatgrass and sainfoin. Heavy metal (Fe, Cu, Zn) concentrations in the soil were not strongly correlated with metal levels recorded in the foliage of slender wheatgrass, sainfoin and jack pine, suggesting that plant build up of toxic metals introduced in the sewage sludge did not occur over the short term.

7. RECOMMENDATIONS

1. In the case of all three minespoils (grassland, subalpine and extracted oil sands) sewage sludge amendment was highly effective in improving soil fertility and thereby stimulating plant growth. Sewage sludge also appeared to behave as a slow release fertilizer since plant growth was generally stimulated over the full term of the study (i.e. 3 years). Although sewage sludge, if readily available, is recommended for the reclamation of minesites, more research is required to study:

i) the effects of different levels of sewage sludge application to a minesoil on the germination, establishment and growth by the various plant species being considered in a revegetation program. Phytotoxic levels of NH_3 produced from drying anaerobically digested sewage sludge immediately after application may require that sludge amended soil be incubated for a certain length of time before seeding or planting.

ii) the rate of metal uptake by various plant species grown in sludge amended soil to determine if concentration of toxic metals by these plants will pose a problem over the long term. It should be kept in mind that release of heavy metals from sewage sludge and their subsequent availability and uptake by plants will vary with soil type, sludge characteristics and plant spp. For example, heavy metal availability to plants may be greater in a soil with a low CEC (such as the extracted oil sands) than in a soil with a high CEC (such as the grassland spoil).

iii) the rate of decomposition and associated nutrient release from sludge aggregates since this will determine the time span over which the fertility of a sludge amended soil can be maintained.

iv) the effects of sewage sludge on mycorrhizal development since toxic factors in the sludge can suppress mycorrhizal development in plants such as slender wheatgrass (see Zak et al., 1984).

2. Because the physical nature of the grassland minespoil (i.e. high clay and high Na levels) is the major factor impeding

plant growth, it is suggested that the SAR be monitored constantly when testing the effects of amendments such as gypsum.

3. More research is required on whether upward Na migration takes place when topsoil is placed over a highly sodic minespoil, and if so, at what rate does it move. Also, the effect of different plant species on Na migration from a sodic spoil into the overlying topsoil should be studied.

4. Since peat is notoriously lacking in plant available P, it should be supplemented with inorganic fertilizer (particularly phosphate) when used as an amendment in reclamation programs. Data on the optimum levels of fertilizer to be added to a particular plot to promote establishment and growth of various plant species (ranging from grasses to legumes to trees) are required.

5. Fertilization of minespoils which are often low in CEC and water holding capacity and therefore highly leachable, may result in a heavy loss of the added nutrients from the rooting zone. Further information is required on:

i) when to apply fertilizer so that plant uptake and immobilization of the nutrients is optimized. This will depend partly on whether the plant is slow or fast growing.

ii) the levels of fertilizer required by a plant to stimulate its growth, but not inhibit the formation of symbiotic relationships such as N_2 fixing nodules or mycorrhizae.

iii) the loss of fertilizer through leaching and runoff so that fertilization programs can be made more efficient.

iv) the effect of repeated fertilizer applications (particularly to a grass/herb mixture) on plant production, litter build-up, decomposition rates of the incoming litter and rates of nutrient release from the decomposing litter. Although shoot production may be stimulated by repeated fertilization, decomposition and nutrient release from the incoming plant litter may not be accelerated, resulting in the accumulation of a thick mat of dead vegetation which can become a fire hazard or a haven for rodents.

6. There is some evidence in the literature that difficulties may arise when attempts are made to establish woody

species in an area vegetated with grasses. Grasses compete with the woody plants for moisture and nutrients and may even produce allelopathic compounds which inhibit the growth of trees or shrubs. However, grasses and legumes form an essential component of a revegetation program since their rapid growth rate provides a quick cover for erosion control. Therefore if the establishment of woody plants in an already revegetated area is one of the aims of a reclamation program, more research is required to determine the effect of various grasses on the establishment and growth of various trees and shrubs.

7. Dinitrogen fixing legumes and shrubs can be important sources of N particularly in infertile minespoils which are highly leachable. Future research should examine the optimum nutrient conditions for promoting N_2 fixation by these plants and the effects of interseeding N_2 fixing legumes or shrubs on the growth of N nutrition of woody plants such as jack pine.

8. This study has dealt with the effects of individual amendments (applied to each of three minespoils) on the establishment and growth of individual plant species. More research is required to establish the effects of various amendments (either singly or in combination with other amendments) on the growth and competition of plants placed in groups (e.g. grass/legume; legume/tree; grass/tree) or planted as a mixture.

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