Amendment of Gold Mine Tailings with Modified Humic Substances to Promote Soil Development and Plant Growth

By : Carl Szczerski

A Thesis Submitted to the Faculty of Graduate Studies
in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Botany
University of Manitoba
Winnipeg, Manitoba

June, 2007

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Abstract

To promote the establishment of vegetation, modified humic substances were added to gold mine tailings in rates of 2 g C kg^{-1}, 3 g C kg^{-1} and 4 g C kg^{-1} tailings as an amendment. Growth chamber and field studies were conducted to determine the effect of modified humic substances on soil chemical and physical properties. The physiological response of *Brassica juncea, Festuca pratensis, Festuca rubra, Poa pratensis, Medicago sativa, Agropyron trachycaulum and Agropyron elongatum* was also investigated. Humic substances increased the tailings macro aggregation. Conductivity of the tailings increased from slightly saline to moderately/highly saline following amendment addition while pH of the tailings remained unchanged. Application of modified humic substances in mine tailings led to variable growth responses with only *M. sativa* exhibiting any growth stimulation. *Brassica juncea*, *A. elongatum* and *F. rubra* exhibited no effect of amendment on their growth while *A. trachycalum* and *F. pratensis* both showed an inhibitory effect.

Acknowledgements

It would be a serious folly to think that this thesis would have been completed without the people around me. No proper measure of thanks can be attributed to Dr. Renault, for her guidance, knowledge, help in the field and of course providing the opportunity to conduct this research to start with. Furthermore not enough can be said for her patience where my procrastinations and delays would have caused most people to loose hope. This work was possible due to the funding provided by NSERC and BlackEarth Humates Ltd. I also want to thank Dr. Goh for his help in getting this project off the ground and both Dr. Goh and Dr. Markham for providing significant insight and expertise. I also want to thank fellow graduate students Colin Nakata and Mason Kulbaba for their guidance in writing this thesis. In addition nothing short of an army of volunteers, Colin Nakata, Scott Green, Karen Kivinen, David Szczerski, and Pamela Ouellette, made the field work possible.

Table of Contents

List of Figures

List of Tables

1 Introduction

In Canada mining is a major sector of the economy generating up to 16% of the national export revenue and approximately 20 billion dollars (SCNR, 1996). While the economic benefits are apparent, mining contributes large amounts of processed waste material in the form of tailings. Tailings associated with sulfur bearing metals are of particular concern due to the biotic and abiotic oxidation of these sulfide minerals releasing acidity and metals into the environment (Blowes and Ptacek, 1994). This phenomena known as acid mine drainage (AMD) has been identified as the most serious environmental problem facing the global metal mining industry (Government of Canada, 1996). Prior to adequate legislation, little was required in terms of environmental reclamation or mitigation plans and many older mine sites became abandoned or orphaned with little treatment or capital invested. One such mine site is the Central Manitoba gold (Au) mine tailings, deposited in the 1920's – 1930's, which has little established natural vegetation and no active management program.

Cost effective reclamation for such sites typically involves tailings stabilization to prevent contaminated particle wind blow, exposure of new oxidizable material, and reduce or eliminate AMD caused by water and oxygen movements into and through the tailings impoundment (Ripley et al., 1996). The majority of low cost techniques focus on the establishment of vegetation in combination with or without chemical, biological, or physical treatments to aid in tailings stabilization. Selected vegetation typically consists of commercially available, native, or metal tolerant species. In addition revegetation can also be thought of as a successional process where the initial vegetation consisits of pioneer species. The primary goal of the initial stages of revegetation then is to establish vegetative cover that requires little to no maintenance in order to aid in the prevention of erosion and build up organic material in preparation for more permanent vegetation (Ripley et al., 1996).

However, successful establishment of vegetation may be limited by the physical and chemical properties associated within sulfide tailings. These properties include: potentially toxic level of heavy metals, high salinity, low pH, macronutrient deficiencies, low organic carbon content, relatively poor soil structure, and a high degree of surface compaction (Ripley et al., 1996; Tordoff et al., 2000). In order to overcome these

properties, selection of species with appropriate tolerances is desirable. Another possible solution is the addition of organic amendments which can facilitate plant growth by improving the biological quality of the tailings (Tordoff et al., 2000). Organic amendments which add humic substances, may lead to an improvement in tailings biological and physical quality as humic substances are known to increase water and nutrient holding capacity, decrease soluble metal concentrations by complexing with metals and play a role in the formation of soil structure (MacCarthy, 2001). BlackEarth modified humic substances are one possible source of humic substances and have been shown to significantly improve the carbon content and structure of mine tailings (Ibrahim and Goh, 2004).

Three separate experiments, 2 in the growth chamber and 1 in the field were conducted using BlackEarth soluble 80 modified humic substances as an organic amendment in mine tailings. The first objective of the experiments were to assess the effect of modified humic substances applied in rates of 2 g C kg^{-1}, 3 g C kg^{-1} and 4 g C kg^{-1} on tailings physical and chemical properties. The second objective was to study the growth and physiological responses of the selected species grown in tailings amended with modified humic substances. It was hypothesized that modified humic substances would promote soil development in terms of aggregation, increase the carbon content of the tailings, and stimulate overall growth of the selected species. Plant species (*Brassica juncea, Poa pratensis, Festuca pratensis, Festuca rubra, Medicago sativa, Agropyron trachycaulum and Agropyron elongatum*) were selected based on history of use in reclamation, commercial availability, and tolerances to water stress and low pH. The first growth chamber experiment focused on the short term response of the selected species in amended tailings specifically investigating emergence, growth responses, and photosynthetic pigment content. The second growth chamber experiment was conducted over a 3 month period, with investigations into tailings properties including pH and conductivity. In addition, physiological parameters were measured including: growth responses, photosynthetic pigment content, anthocyanin content, electrolyte leakage and transpiration. Two field seasons were conducted in order to determine the effect of modified humic substance amendment on tailings physical and chemical properties as well as plant physiological responses in a natural setting. Soil measurements were

conducted at various times of the year and up to 1 year following amendment application to investigate changes in pH, conductivity, organic carbon and aggregation. Physiological responses were also monitored with seed emergence and plant survival until harvest measured. The growth response, photosynthetic pigment content, and elemental content of the selected species were also investigated.

2. Literature Review

2.1 Introduction

Mining and mineral processing results in the production of a large quantity of mine waste (tailings). Mine tailings can increase the level of heavy metals in the environment though wind blow and acid mine drainage affecting the quality of the surrounding water, land and air. Various reclamation methods are available to facilitate the growth and establishment of vegetation, stabilize the tailings surface and reduce the potential for acid mine drainage in tailings management. However, the establishment of vegetation is limited by the chemical and physical properties of the mine tailings impairing long term reclamation and management.

This review will concentrate on procedures used in the reclamation and revegetation of sulfide mine tailings. A discussion on the geological processes and mineralogy within the Central Manitoba tailings that lead to specific challenges in the establishment of vegetation will also be discussed.

2.2 Mining in Canada

2.2.1 Economic Benefits and Concerns

In Canada, mining plays an important and beneficial role in the economy generating approximately 20 billion dollars and accounting for 16% of the nations export revenue (SCNR, 1996). This sector is one of the major drivers of the rural Canadian economy employing over 380,000 people in direct and spin off rated positions (SCNR, 1996). However, benefits of this industry do not come without impact on the environment. Mining affects the environment at various levels and stages including exploration, mining and milling, smelting and refining, and post operational waste management (SCNR, 1996). Ore extraction, refining and deposition of waste materials can lead to the release of heavy metals and acidity into the environment affecting the quality of the surrounding water, land and air. In Canada, the primary pollutants released from all stages of mining include lead, mercury, cadmium, arsenic, copper, nickel, antimony, and sulfur dioxide (SCNR, 1996).

2.2.2 Generation of Tailings and Environmental Risk

As of 2006, Canada possessed approximately 7 billion tonnes of tailings from metal mining spread over 41,000 hectares of land and generates approximately 650 million additional tonnes of tailings per year (Tremblay, 2006). Environmental problems associated with tailings depend on the nature of the mineral composition. Tailings can be categorized into acid producing or potentially acid producing and neutral/basic (SCNR, 1996). The category of largest concern is the acid producing wastes that contain sulfur bearing minerals. Oxidation of sulfide minerals in the presence of water and oxygen leads to acidification and release of heavy metals from the minerals into the environment (Boulet and Laroque, 1998; Tordoff et al., 2000).

Mine tailings also present a difficult substrate for revegetation, as tailings possess a range of physical (poor structure, low water retention) and chemical (low pH, low nutrient status, toxic concentration of metals) characteristics that make for an unfavorable environment for plant growth. Research is needed to prevent AMD and associated environmental challenges with mine tailings, as increasing demand for metals and development of technology to economically process lower grade ores in the future, is likely to drive further accumulation of tailings (SCNR, 1996).

2.2.3 Active, Abandoned and Orphaned Mines

Three different status levels exist for mines in general: active, abandoned, and orphaned. Active status refers to mines that are currently in production. Abandoned and orphaned status refers to mines that are no longer active. Abandoned mines have a known owner while orphaned mines have an unknown owner. As of 2006 it has been estimated that there are more than 10,000 abandoned, or orphaned mine sites in Canada (Tremblay, 2006). Owners are responsible for mine closure, clean up and reclamation efforts while in the case of orphaned sites the government assumes the responsibility.

2.3 Central Manitoba Gold Mine

2.3.1 History and Use

The Central Manitoba gold (Au) mine tailings impoundment, located 35 kilometres south east of Bissett in Nopiming Provincial Park, is one example of an abandoned acid generating mine waste site in Canada. The tailings associated with this site were generated during the mines operation from 1928 – 1937 in which approximately 347,000 tonnes of ore material was processed for 4,200 kilograms of gold (Richardson and Ostry, 1996). Five different gold bearing veins were mined producing varying grades of gold ore and tonnage. Fine grained tailings were produced during milling and deposited in a poorly constructed impoundment covering approximately 20 hectares, left untreated, and open without any surface cover.

Due to mining legislation of the 1920 – 1930's the Central Manitoba tailings site was left untreated for natural revegetation processes to occur. A pilot seeding and fertilization project was conducted on a portion of the tailings in 1971 by the Mines Branch of the Manitoba Department of Energy and Mines (Slivitzky, 1996). The project was abandoned in 1977 when the seeded and fertilized tailings were used for highway improvements. The tailings, despite this short lived reclamation effort, remain relatively open today with most of the vegetation clustered in small islands. However, despite the sites relative openness, approximately 100 plant species are able to survive within the Central Manitoba tailings (Sherriff, Punter and Punter, personal communication). The majority of the plant diversity is along the edge of the tailings or within the small islands of vegetation. However, both woody and non woody species have been able to establish directly in the tailings. Deciduous woody species include: *Populus balsamifera (*balsam poplar), *Populus tremuloides* (trembling aspen), *Betula papyrifera* (white birch*), Salix bebbiana* (beaked willow), *Salix exigua* (sandbar willow), *Salix lucida* (shiny willow), *Salix petiolaris* (basket willow) and *Larix laricina* (tamarack). Non deciduous woody species include: *Pinus banksiana* (jack pine), *Picea glauca* (white spruce), and *Picea mariana* (black spruce). Non woody species include: *Agropyron trachycaulum* (slender wheatgrass), *Aster laevis* (smooth aster), *Chenopodium rubrum* (coast-bite), *Epilobium angustifoliu* (fireweed), *Equisetum variegatu* (variegated horse-tail), *Juncus brevicaudatus* (short-tailed rush), *Melilotus alba* (white sweet-clover), *Polygonum*

aviculare (prostrate knotweed), *Polygonum persicaria* (lady's thumb), *Puccinellia nuttalliana* (nuttall's salt-meadow grass), *Rumex salicfolius* (narrow-leaved dock), *Senecio pauperculus* (balsam groundsel), *Solidago missouriensis* (low goldenrod), *Suaeda maritiama* (sea-bite), and *Taraxacum officinalis (*common dandelion) (Sherriff, Punter and Punter, personal communication).

2.3.2 Tailings Mineralogy

Minerals within tailings impoundment can be classified as primary or secondary. Primary minerals are those that made up the original ore and associated rock while secondary minerals are those which form within the tailings impoundment by chemical weathering reactions of the primary minerals.

The primary mineral composition of the Central Manitoba gold mine tailings reflects the mineralogy of the gold bearing veins which were comprised of quartz, potassium-feldspar, calcite, mica and sulfide bearing minerals; including pyrite (iron sulfide) and chalcopyrite (copper-iron sulfide) and pyrrhotite (iron sulfide) (Richardson and Ostry 1996; Salzsauler, 2001). Formation of secondary minerals in mine tailings depends primarily on the acidic (pH) and redox potential (Eh) of the system (Alpers et al. 1994). Secondary mineral composition thus may vary with depth and location within a tailings impoundment. Common secondary minerals associated with sulfide tailings typically include various iron and metallic sulfates (such as jarosite), iron oxides and oxyhydroxides (including goethite, lepidocrocite, and ferrihydrite), and various metallic carbonates. Little work on secondary mineral identification in the Central Manitoba site has been conducted but manganese and iron oxide fractions as well as copper carbonate minerals are present within the impoundment and within the salt crusts that form along the surface (Salzsauler, 2001).

2.4 Chemical Reactions in Tailings

2.4.1 Geochemical Zones

In situations where mine tailings experience wet-dry cycles, such as the Central Manitoba site, three distinctive layers may develop in the tailings. Blowes and Ptacek

(1994) describe three geochemical zones that develop in tailings due to oxidation of sulfide minerals, an upper vadose sulfide oxidation and acid generation zone (oxidation), an acid neutralization and chemical precipitation zone (hardpan), and an attenuation and dissolution zone that is water saturated (reduction).

Salzsauler (2001) identified and described these three distinctive zones in the Central Manitoba tailings. The oxidized zone occupies the upper 30 cm and tends to have a homogenous yellow brown colour. From 30 cm – 60 cm a transition zone exists between the upper oxidized zone and the lower reducing zone. The transition zone is a heterogeneous layer consisting of yellow, red, brown and blue layers. Within these layers iron, manganese, copper, nickel and cobalt concentrate marking a hardpan zone instead of a single distinctive layer. The reducing zone that exists below 60 cm of the tailings is blue grey in colour and contains metals in reduced form.

2.4.2 Oxidation Mechanisms

2.4.2.1 Abiotic Oxidation and Associated Reactions

Oxidation of the sulfide bearing primary minerals pyrite, chalcopyrite and pyrrohotite in the oxidation and transition zones of the Central Manitoba tailings, leads to the release of iron, copper and acidity. Exposed tailings subject to wet dry cycles are susceptible to increased rates of oxidation (Boulet and Laroque, 1997). Four principle acid generating reactions are associated with sulfide tailings impoundments, oxidation of sulfide minerals, oxidation of Fe^{2+} (ferrous), hydrolysis of Fe^{3+} (ferric) and precipitation of metal hydroxide and hydroxysulfate phases (Blowes and Ptacek, 1994).

Nicholson (1994) described four stages of complete pyrite and pyrrohotite oxidation summarized below using pyrite as a model. Incomplete oxidation products and other chemical transformations are also possible, such as formation of elemental sulfur or conversion of pyrrohotite to pyrite, but are not included in the oxidation mechanisms. Pyrite and pyrrohotite oxidation (1) occurs in the presence of dissolved oxygen. In this reaction the sulfur atom is oxidized while the ferrous ion is released. Under acidic pH conditions < 3.5 a significant quantity of the ferrous ion is oxidized (2) to the ferric ion and will remain in solution (Nicholson, 1994). Pyrite (3) reacts with ferric ions in a rapid reaction and under low pH conditions generates Fe^{2+} that in turn oxidizes to Fe^{3+} (2). The

cycling of iron in reactions (2) and (3) along with pyrite, generates large quantities of acidity and maintains a low pH environment (Nicholson, 1994). Under pH conditions >3.5 low concentrations of ferric ions are maintained due to precipitation of ferrihydrite or ferric hydroxide (4), reducing the rate of iron cycling (Nicholson, 1994). Chalcopyrite oxidation mechanism follows a simular system as seen in pyrite with the exception that Cu^{2+} is also released into solution (Rimstidt et al., 1994).

Pyrite Model

(1) $2\ FeS_2$ (pyrite) $+ 7\ O_2 + 2\ H_2O \rightarrow 2\ Fe^{2+} + 4\ SO_4^{2-} + 4\ H^+$ (sulfide oxidation)

(2) $4\ Fe^{2+} + O_2 + 4\ H^+ \rightarrow 4\ Fe^{3+} + 2\ H_2O$

(3) $FeS_2 + 14\ Fe^{3+} + 8\ H_2O \rightarrow 15\ Fe^{2+} + 2\ SO_4^{2-} + 16\ H^+$

(4) $Fe^{3+} + 3\ H_2O \leftrightarrow Fe(OH)_3 + 3\ H^+$

2.4.2.2 Biotic Oxidation and Associated Reactions

Mine tailings contain a diverse assemblage of bacterial species due to variation in oxidized compounds, nutrient requirements, pH optima, and oxygen requirements (Gould et al., 1994). These bacteria include iron oxidizing and reducing bacteria as well as sulfur oxidizing and sulfate reducing bacteria all of which play key roles in the cycling of iron and sulfur from one form to another (Johnson et al., 2002). Thus, these bacteria can influence the chemistry of the impoundment. The oxidation and dissolution of sulfide minerals are of particular importance as these processes are accelerated by acidophilic bacteria such as *Leptospirillum ferrooxidans* and *Thiobacillus ferrooxdans* (Johnson et al., 2002). It has been suggested that these bacteria are capable of catalyzing abiotic oxidation by a factor of 10 or 100 (Nicholson, 1994).

2.4.3 Acid Neutralization Reactions

The principle acid neutralization and buffering mechanisms in mine tailings impoundments are associated with carbonate, hydroxide, and aluminosilicate mineral dissolution. These acid consuming minerals result in a general maintenance of pore water pH preventing a decrease in pH over time (Blowes and Ptacek, 1994). Neutralization reactions are of particular importance in tailings due to their ability to

buffer the continued generation of acidity through the oxidation of sulfide minerals that can occur for periods of time up to centuries.

The most significant pH buffering reactions in tailings involve carbonate minerals (calcite, dolomite, siderite, ankerite). These minerals are effectively able to maintain a neutral pH within the tailings and facilitate the precipitation of metal oxyhydroxides or hydroxysulfates. Thus the presence of carbonates can lower the concentration of dissolved metals, especially iron, in solution and prevent AMD (Blowes and Ptacek, 1994). Metal hydroxides such as gibbsite and goethite buffer the tailings at pH 4 and 3.5 respectively. At very low pH, after all the carbonate and hydroxide minerals have been depleted, aluminosilicate minerals become the last important acid neutralization mechanism (Blowes and Ptacek, 1994).

2.4.4 Acid Mine Drainage

Acid mine drainage results from the abiotic and biotic oxidation of sulfide minerals and associated reactions that generate acidity and release heavy metals. When the buffering ability of the tailings impoundment is overcome by the generation of acid, metals are released into the acid water solution and may travel off site.

The Central Manitoba tailings impoundment on average contains 1 % by weight calcite and 0.9 % by weight sulfide minerals (Londry and Sherriff, 2005). The pH associated with the mine tailings as a result of the buffering and neutralization reactions of the calcite should be neutral (Salzsauler, 2001). The recorded pH values varied from 3.5 to 7 (Renault et al., 2002; Londry and Sherriff, 2005). It was determined that only ~20% (area) of the total tailings possessed a pH < 4 due to the exhaustion of calcite by a higher local load of sulfide minerals (Londry and Sherriff, 2005). No studies have investigated metal content and acidity in drainage waters moving off site or into the ground water. However, it is assumed that AMD is occurring at the Central Manitoba mine tailings impoundment (Slvitzky, 1996; Salszauler, 2001; Londry and Sherriff, 2005).

2.5 Tailings Properties/Characteristics

Reclamation and establishment of vegetation may be limited by physical and chemical properties associated within sulfide tailings. These properties include potentially toxic levels of heavy metals, high salinity, low pH, macronutrient deficiencies, low organic carbon, poor soil structure, and surface compaction (Ripley et al., 1996; Tordoff et al., 2000). The Central Manitoba tailings impoundment itself is a heterogeneous site with variable topography, areas with sporadic vegetation, variable degrees of exposure to wind and water drainage, variable pH and metal content all resulting in slightly different limitations based on location within the impoundment.

2.5.1 Iron and Copper

Total iron content of the Central Manitoba tailings was found to range from 18,000 to 54,000 $\mu g\ g^{-1}$ and total copper content was found to range from 800 $\mu g\ g^{-1}$ to 20,000 $\mu g\ g^{-1}$ depending on depth and location (Renault et al., 2000; Salzsauler, 2001). The phytotoxicity of these metals varies depending on their bioavailability which is determined by the soil pH, redox potential (Eh), cation exchange capacity, organic matter content, clay mineralogy, carbonate and hydrous Fe/Mn oxides content (Viets, 1962; McLaren and Crawford, 1973; Shuman 1985; Sims, 1986; Kabata-Pendias and Pendias, 1992). Average total iron content in agricultural soils is approximately 50,000 $mg\ kg^{-1}$ but soluble iron content usually is low in comparison to the total iron content except under low pH conditions (Kabata-Pendias and Pendias, 1992). At neutral and alkaline pH values iron precipitates as oxides and hydroxides decreasing the soluble iron content. Acidic soils are much higher in soluble iron resulting in potential toxicity to plants while conversely at higher pH deficiencies may be observed in soil.

Typical copper content in soils ranges from 13 - 24 $\mu g\ g^{-1}$ with soluble concentrations being low as copper readily precipitates with anions such as sulfate, carbonate and hydroxide in neutral conditions (Kabata-Pedias and Pendias, 1992). At low pH, dissolution of carbonate and hydroxide minerals occurs releasing copper into solution. Senkiw (2004) determined soluble copper content in Central Manitoba tailings at pH 3.3 and found a range from 400 - 600 $\mu g\ g^{-1}$.

Sorbtion of copper and iron ions to the charged surfaces of carbonates, oxides, hydroxides and clay particles reduces the bioavailability of these metals (McLaren and Crawford, 1973; Shuman, 1985). Low pH can also influence the bioavailability of iron and copper by reducing the surface charge available for sorption of the ions (Kabata-Pendias and Pendias, 1992). Occlusion of iron and copper ions in hydroxides, carbonates and phosphates may also occur binding both metals in a non diffusible form (Shuman, 1985). Iron and copper also bind to organic matter (including humic substances) forming both soluble and insoluble complexes (McLaren and Crawford, 1973; Shuman, 1985).

2.5.2 Metal Toxicity

Iron and copper are both essential micronutrients in plants playing a role in many enzymatic functions including photosynthesis. However, elevated levels of iron and copper in the mine tailings may lead to an accumulation of metals in toxic concentrations resulting in toxicity.

Iron is an important component in plant enzyme systems playing a role in electron transport, nitrate and sulfate reduction, nitrogen assimilation, and energy production (Jones, 1998). Typically iron ranges from 100 to 500 $\mu g\ g^{-1}$ in plant leaf tissue (Kabata-Pendias and Pendias, 1992). Toxic concentrations of iron in plant tissue vary according to species and soil type but generally occur at values higher than 500 $\mu g\ g^{-1}$ (Jones, 1998). Toxicity symptoms expressed in both the root and shoot vary greatly within species, age of plant, nutrient status, and cultivar (Foy et al., 1978). Iron toxicity in leaf tissue varies according to species but generally toxicity results in localized necrotic spots such as bronzing observed in *Oryza spp.* (rice) cultivars or freckle disease observed in *Saccharum spp.* (sugarcane) (Foy et al., 1978). Millikan (1949) observed stunted root and shoot growth along with deep green foliage in *Linum usitatissimum* (flax) grown with excess iron. Toxic concentrations of iron also affect the photosynthetic apparatus interfering with the function of photosystem II in *Pisum sativum* (Pea) (Suh et al., 2002). Suh et al. (2002) suggested that excess iron gave rise to excess cytochrome b6/f content in the thylakoid membrane resulting in inhibition of photosystem II.

Copper plays a role in the function of chloroplast protein plastocyanin, electron transport linking photosystem I and II and is part of the enzymes that reduce both atoms

of molecular oxygen (Jones, 1998). Copper also participates in protein and carbohydrate metabolism, nitrogen fixation, and in the desaturation/hydroxylation of fatty acids (Jones, 1998). Leaf tissue copper content varies according to species and soil type but ranges from 1 to 20 $\mu g\ g^{-1}$ (Kabata-Pendias and Pendias, 1992). Copper toxicity in plant tissue typically is observed around 20 to 100 $\mu g\ g^{-1}$ (Jones, 1998). Symptoms of copper toxicity include suppression of root growth including both elongation and formation of lateral roots (Savage et al., 1981). Copper is also known to induce: tissue damage and reduced elongation of root cells, alteration of membrane permeability causing root leakage of ions and solutes, peroxidation of chloroplast membrane lipids and inhibition of photosynthetic electron transport and immobilization of copper in cell walls (Kabata-Pendias and Pendias, 1992). Copper toxicity can also induce an iron deficiency which may lead to chlorosis of the leaves (Jones, 1998). Toxic concentrations of copper is also thought to inhibit Fe and Mn metabolism in the shoot tissues resulting in the impairment of the photosynthetic pathway (Maksimiec and Baszynski, 1996).

2.5.3 Nutrient Deficiency

Deficiencies of nutrients in the tailings may be a limiting factor in plant growth in the Central Manitoba tailings (Renault et al., 2002). Nutrient deficiencies of magnesium, calcium and potassium were observed in *Larix laricina* (tamarack), *Picea glauca* (white spruce), *Pinus banksiana* (jack pine), *Cornus sericea* (red-osier dogwood), and *Betula glandulosa* (bog birch) planted at the Central Manitoba site (Renault et al., 2002). Phosphorus and nitrogen levels in the Central Manitoba tailings were found to be low with 70-100 $\mu g\ g^{-1}$ and 100 $\mu g\ g^{-1}$ respectively (Green and Renault, 2007).

2.5.4 Tailings Structure and Carbon

Ibrahim and Goh (2004) found that Central Manitoba tailings possessed a poorly developed structure and low organic carbon content. Water stable macro aggregation (>0.25 mm) was found to be less than 13%. Low organic carbon (2.3 $g\ kg^{-1}$), low clay content (<3.5%) and the high sand content (48.5%) impart relatively poor water retention abilities, nutrient holding ability, and cation exchange capacity further exacerbating the nutrient limitations of the Central Manitoba tailings. The poor soil structure of the

tailings in combination with sparse vegetation leaves the tailings prone to surface erosion by wind and water resulting in a disturbed and shifting soil surface increasing, the amount of sulfide minerals available for oxidation.

2.5.5 Hydrology

Revegetation of the Central Manitoba tailings impoundment is also impaired by the hydrology of the site. Surface disruption due to water run off tends to be high as water redistributes the tailings causing a shifting unstable surface (Londry and Sherriff, 2005). The water table within the impoundment is also high, residing below the surface due to the basin like nature of the site. Groundwater entry from the north and south sides of the impoundment is sufficient along with capillary action bringing water up to the transition zone (Londry and Sherriff, 2005).

2.6 Tailings Disposal

Research over the past 20 years has been conducted into the long term management and disposal of mine tailings (Ripley et al., 1996). Modern disposal methods generally attempt to prevent and reduce the effects and risks associated with mine waste specifically AMD from sulfide tailings. However, this concern was not included in the long term management and control of AMD prior to the 1980's, resulting in tailings impoundments that have acidic effluents containing heavy metals (Ripley et al., 1996). Two basic methods exist for dealing with AMD, treatment and prevention. Treating effluents is an expensive process that removes heavy metals from the water but does not address the generation of acidity. The second option is to reduce or limit the oxidation of sulfide bearing minerals in the tailings impoundment thus preventing AMD.

Deep water placement is one promising method of preventing acid generation due to anaerobic conditions on the bottom of lakes (Pederson et al., 1994). New methods in the construction of tailings impoundments have been suggested to improve their design and operation in order to contain drainage waters (Ripley et al., 1996). Flooding tailings impoundments at the time of decommission has also been considered and has been shown in models by Romano et al. (2003) to significantly lower the rate (over 99%) and depth of oxidation.

2.7 Reclamation: Stabilization and Revegetation

Reclamation is defined as the rehabilitation or return of disturbed land to productive uses; including all activities of spoil movement, grading and seeding (Bartels, 2000). Reclamation of mine tailings involves stabilization and generally includes the use of vegetation with or without amendments. Reclamation programs thus attempts to overcome and prevent the negative chemical and physical characteristics associated with tailings that impair plant growth and establishment. Stabilization of the tailings surface can be achieved with a combination of physical, chemical, and biological treatments. These treatments are designed to prevent contaminated particle wind blow and exposure of new oxidixable materials, reduce or eliminate AMD caused by water and oxygen movements into and through the tailings impoundment (Ripley et al., 1996). Vegetation can also be used as a method of stabilization allowing for root biomass to hold and stabilize the surface. Successful establishment of vegetation also leads to an addition of organic matter to the surface and improves the visual appearance of the tailings impoundment (Tordoff et al., 2000). Tailings with low pH and high metal content may have amendments applied in order to aid in the establishment and survival of vegetation by reducing the toxic effects of metals and improve the physical and chemical characteristics of the tailings (Tordoff et al., 2000). Phytoremediation, specifically the extraction of metal by plants, is a promising method of aiding in the reclamation of mine tailings (Salt et al., 1998).

2.7.1 Tailings Stabilization: Sealants and Covers

Various methods and materials exist and many can be combined to stabilize the surface of tailings impoundments. The primary methods of tailings stabilization include the use of sealants, covers, and vegetation. These materials are applied to the surface of tailings in order to reduce the rate of sulfide oxidation by preventing the passage of water or air into the tailings and eliminate the movement of tailings by erosion.

The use of sealants such as concrete, asphalt, rubber, latex and clay materials has been studied and proven to be extremely effective in preventing AMD (Ripley et al., 1996). Chemical treatments of the tailings surface can also be conducted using materials including resins, sodium silicate, lignin sulphonate, and latex gelatin that provide a

surface crust resistant to wind and water erosion (Ripley et al., 1996). However, these sealant materials are expensive and possess high maintenance costs as they are subject to chemical and or physical breakdown (Johnson and Bradshaw, 1977).

Cover materials using capillary barriers have also been shown by Nicholson et al. (1989) to act as effective sealants of sulfide tailings. Other waste rock materials such as shale, slate quarry waste and limestone chips can also be added to the surface in substantial quantities to create a capillary break (as opposed to barrier) (Tordoff et al., 2000). These course materials prevent the upward movement of water from the tailings into any substrate containing vegetation. Other less effective methods of physical stabilization include snow fences or other general windbreaks. These structures are capable of preventing wind erosion and trapping snow for increased moisture in the spring but are not effective in preventing water erosion (Ripley et al., 1996).

Organic mulches such as hay and straw, applied as a cover directly on the tailings surface, have been found to improve conditions including water infiltration, erosion control, increased seed germination, prevention of soil crust formation, facilitating soil structure formation and nutrient supply (Lyle, 1987). Other organic mulches such as wood chips, sawdust, bark, or paper mill sludge and animal litter have also been widely used as surface stabilizers (Lyle, 1987; Sabey et al., 1990). Organic covers can also be used primarily to act as an oxygen barrier, consuming oxygen before it enters the tailings surface reducing oxygen flux. Aerobic activity of the microorganisms consuming the organic materials reduces the amount of oxygen that penetrates into the tailings surface. The ability of pulp and paper residue to act as a cover material was studied by Cabral et al. (2000) who found that a residue depth of 0.5 m was required in order to create an anaerobic environment at the tailings surface.

2.7.2 Revegetation

Revegetation can be thought of as a successional process where the initial vegetation seeded or planted are pioneer species. The primary goal of the initial stages of revegetation is to establish vegetative cover that requires little to no maintenance in order to aid in the prevention of erosion and build up organic material in preparation for more permanent vegetation (Ripley et al., 1996). Revegetation may be achieved through two

routes, the first through natural processes allowing vegetation to slowly reclaim the tailings directly. However, natural processes are inherently slow and are likely to span human lifetimes potentially disrupting and damaging surrounding ecosystems with contamination. The second option, through an active program, generally involves NPK fertilizer application, stabilization treatments, and or amendment application to the tailings in some combination with seeding or planting of seedlings (Ripley et al., 1996).

Species suitable for seeding or planting within a tailings impoundment may vary depending on the nature of the tailings, the local climate, the micro habitats within the impoundment and the reclamation program being utilized (Johnson and Bradshaw, 1977). In general the use of commercial forage species, grasses and legumes, is preferred due to seed being readily available, nutritional requirements being well known, rapid germination, and initial growth and productivity are generally high (Ripley et al., 1996).

Grasses and legumes provide a number of benefits that allow them either to survive or aid in the reclamation and stabilization of the tailings surface. Legumes that are inoculated with *Rhizobium* spp. bacteria have the ability to form nitrogen fixing associations that are beneficial in the reclamation process due to the long term additions of nitrogen to the tailings environment (Tordoff et al., 2000). However, legumes do not tend to perform well in acid tailings where only a few species such as *Trifolium repens* (red clover) can survive and grow in acidic conditions (Johnson et al., 1977). Grass species are commonly used in revegetation efforts due to the diverse selection of species available and wide range of tolerances observed (low nutrients, drought and acidity) (Baker, 1987). Grasses also posses fibrous root systems making them highly suitable for the stabilization of the tailings surface preventing erosion (Skousen and Zipper, 1996).

2.7.2.1 Direct Seeding

Vegetation can be established directly into the mine waste in cost effective process' using commercially available, native, or metal tolerant species. Commercially available plants offer the benefit of an easy to acquire and economical seed source. Grass/legume mixtures are the most common type of seeds obtained and are combined with a fertilizer to alleviate nutrient deficiencies (Ripley et al., 1996). Native species are ecologically adapted to the prevailing climate making their use favorable and in some

locations the only legal choice of vegetation (Ripley et al., 1996). However, the direct establishment of commercial and native plant species in tailings is only recommended for neutral/basic tailings or capped acidic tailings that do not have acidity or high heavy metal concentration problems (Tordoff et al., 2000). Despite obvious advantages of heavy metal tolerant species there are limited heavy metal tolerance species adapted for boreal climatic conductions. There is also a lack of heavy metal tolerant nitrogen fixing legumes and a limited number of broad range metal tolerators as species tend to be single metal specific, a quality only suitable for homogenous tailings (Bradshaw et al., 1978; Palmer 1990).

Regardless of vegetation type standard NPK fertilizers are commonly applied in all cases to alleviate nutrient deficiencies and may need to be applied on a repeated basis until the cation exchange capacity and nutrient holding capacity of the tailings has increased by natural additions of organic matter (Ripley et al., 1996).

Phytoremediation is a relatively new approach in reclamation with major scientific progress in the area of metal phytoextration. Two direct seeding approaches are used in extracting metals from the environment. The first approach, induced extraction, uses a metal chelating agent applied to a metal containing substrate in order to induce a rapid update of metals by vegetation (Salt et al., 1998). The second approach, continuous extraction, is with the use of naturally occurring hyperaccumulating plants to continuously extract metals from a metal containing substrate (Salt et al., 1998). Hyper accumulating plants include members of the Brassicaceae and Fabaceae families (Ni, Zn, and Se) as well as *Thalapsi calaminare* (Zn) and *Thalapsi caerulescens* (Zn) (Ingrouille and Smirnoff, 1986; Salt et al., 1998). Onsite treatment of mine tailings favors continuous extraction where the application of a chelating agent on the surface could lead to increased contamination of ground water or possibly increase the metal content in drainage waters (Romkens et al., 2002). However, the use of hyperaccumulators is limited due to low biomass and slow growth rates of most hyperaccumulating species. These characters limit their potential to quickly remove metals from mine tailings resulting in the slow remediation of a contaminated environment Thus phytoremediation as primary method of reclamation is limited but attempts are ongoing in improving

existing lines of phytoextrating plants and exploration for new possible high biomass hyperaccumulating plants (Salt et al., 1997).

2.7.2.2 Direct Seeding with the use of an Amendment

In mine tailings with poor physical properties that inhibit germination and growth or are too toxic for commercial or native species, amendments may be used. The addition of one or several amendments to mine tailings provides a number of direct benefits including a reduction in the toxicity of the tailings and improvement of the substrate physical properties to allow for the establishment of vegetation (Tordoff et al., 2000).

Two common amendment classes applied to sulfide tailings are organic materials and lime. A number of different organic amendments exist that can be applied into the tailings include sewage sludge, domestic refuse, peat, topsoil, and paper mill sludge (Tordoff et al., 2000). These organic materials play a number of roles if applied directly to the mine waste including: improvement of the soil structure, increased water and nutrient holding capacity (CEC), a long term nutrient store, complexation of organic compounds with metals to form insoluble complexes reducing phytotoxicity (Tordoff et al., 2000). Liming agents such as calcite, hydrated lime, dolomite and fly ash are also commonly used as an amendment, and may be used in combination with organic materials. Liming agents used as amendments raise the pH and precipitate heavy metals preventing toxicity problems for establishing vegetation (Johnson and Bradshaw, 1977).

A variety of species may be employed and with natural and heavy metal tolerant specialy typically favored. The establishment of seeded or planted vegetation is generally obtained quickly following amendment addition and standard NPK fertilizer application. However, vegetative regression, a decrease in plant health and die back, may occur with organic amendments, typically appearing a few years following seeding, requiring additional fertilizer applications (Goodman et al., 1973). Regression is also associated with the reappearance of heavy metal toxicity in tailings that tend to have upward movements of soluble metal salts. The remobilization of metals might also occur as organic material complexed with the metals begins to be decomposed by microbial action (Lucas, 1948). Other problems arise from root growth being restricted

to the amended layer effectively forming a thin zone over the tailings making it prone to erosion when a major environmental disturbance occurs (Johnson and Bradshaw, 1977; Tordoff et al., 2000).

2.7.2.3 Seeding with Sealants and Covering Materials

The establishment of vegetation following the application of a capillary barrier, break or sealant to the surface of a mine tailings impoundment is a relatively straight forward process. A suitable surface substrate is applied above the sealant, break or barrier with significant depth in order to reduce biological intrusion from root growth interacting with the sealant or barrier (Bussiere et al., 2004). The negative properties associated with the tailings are effectively removed and commercial or native vegetation can be established. Attempts to cover seals and barriers with shallow substrates have resulted in mechanical damage due to root penetration, thus limiting their beneficial effects (Ripley et al., 1996). Soils resting on impervious surfaces are also inherently unstable as saturation of the substrate above the sealant can occur and may result in mass flow of the soil. Course material layers, such as those in capillary and break covers, added above a sealant may be used to facilitate lateral drainage and increase the stability of soils (Nicholson et al., 1989).

Organic covers have many similar benefits and drawbacks as in organic amendments. A variety of commercial, native or heavy metal tolerant species may be employed. Liming agents may also be applied and repeated fertilizer applications may be required due to regression of vegetation though some organic materials such as sewage sludge and animal litter that contain significant quantities of nutrients (Johnson and Bradshaw, 1977; Tordoff et al., 2000). Under some moisture conditions in combination with thick mats of organic mulches, oxygen movement may be inhibited such that plant growth and survival may be reduced due to poor aeration (Lyle, 1987).

2.7.3.4 BlackEarth Humic Amendment

Treating soils with humic containing materials has a wide range of practical applications including remediation and reclamation of soils and tailings. Humic substances are an amorphous and complex mixture of true humic and non humic

components (Hayes and Clapp, 2001). Three main fractions are observed in humic containing materials, humic acids (true humic), fulvic acids (true humic) and humin (aggregate of true and non humic substances) (MacCarthy, 2001). Humic substances are composed of true humic materials with large surface areas possessing many functional and charged groups. This property of humic substances allows them to be active compounds in soil participating in many agronomic, environmental, and geochemical processes (Stevenson, 1982).

Commercial humic substances originate from a variety of different sources such as peat, soil and lignite (general term) a material associated with coal deposits. Humic substances from BlackEarth Humates Ltd., originate from a lignite material termed humalite. Similar to coals, humalite originates from ancient organic matter but has not undergone sufficient compaction and heating to form a high grade coal (NEB, 1984). Ayuso et al. (1997) found that humified organic matter associated with coal contains a high level of humic substances that possessed similar chemical properties to soil humified organic matter. However, humic materials associated with coal deposits are more oxidized, containing lower amounts of nitrogen and possess higher aromaticity in comparison to soil humic materials (Ayuso et al. 1997). Modification of lignites generally occurs in which the active and true humic substances converted into a water soluble form through a variety of extraction procedures. BlackEarth Ltd. commercial soluble humic substances are extracted using a proprietary process but contain fulvic and humic acid like substances along with non humic components (BlackEarth Humates Ltd., 2005).

Organic matter and humic substances play important roles in soil that are useful for reclamation purposes, including the stabilization of soil aggregates and the promotion of plant growth (Hayes and Clapp, 2001). Humic substances, also play a number of other beneficial roles in soils and can be considered beneficial for reclamation including pH buffering, binding of charged particles, increasing the soil cation exchange capacity, and serving as a reservoir for nutrients (nitrogen, phosphorus, and potassium) and retaining soil moisture (MacCarthy, 2001). Furthermore the ability of humic substances to remove metal ions from soil solution and decreasing their bioavailability makes them extremely useful for reclamation of heavy metal contaminated land (Livens, 1991). Humic

substances in soil are also widely known for their role in the formation of soil structure by binding particles together and forming stable aggregates (Stevenson, 1982). Improvements in soil structure increase aeration, water holding capacity and water permeability providing significant advantages for the growth and establishment of vegetation. Whiteley (1993) applied an ammonium lignite slurry and found it to significantly increase the aggregate stability, but suggested its use only for soils with significant clay quantities. Piccolo et al. (1997) found that humic material derived and extracted from coal was able to increase the aggregate stability of different Italian soils. Humic substance extracts from lignite have also been shown to have a positive and stimulatory effect on germination and increase initial root length in *Hordeum vulgare* (barley) and *Lepidium sativum* (watercress) (Ayuso et al., 1996). Humic substances can also stimulate root growth in *Triticum aestivum* (wheat) (Malik and Azam, 1985) and *Pelargonium hortorum* (geranium) seedlings (O'Donnell, 1973) as well as increase total fresh weight of *Letuga sativa* (lettuce) and *Lycopersicum esculentum* (tomato) seedlings (Piccolo et al., 1993).

2.8 Conclusion

Mining in Canada has many economical benefits especially in rural portions of the country. However, these benefits are contrasted by generation of heavy metal pollution and acidity into the environment. The generation of tailings and especially acid generating mine waste is one of the largest problems associated with mining in Canada. These tailings if left untreated may release heavy metals into groundwater and local ecosystems posing a significant threat to the environment. One example of this condition is the Central Manitoba mine tailings site where the tailings have been abandoned without any reclamation program for the past 70 years.

Reclamation programs for sites such as the Central Manitoba are critical if acid mine drainage is to be mitigated or prevented. However, reclamation generally suffers from significant challenges such as high salinity, localized low pH and toxic concentrations of metals coupled with poor soil structure, low nutrient status and site hydrology. These problems prevent the establishment and long term survival of vegetation in the mine waste. Thus the goals of Central Manitoba reclamation are to

facilitate the growth and establishment of vegetation, stabilize the tailings surface, and reduce the potential for acid mine drainage. Reclamation of mine waste using organic amendments is low cost and the most likely method to achieve positive results. Organic amendments offers many advantages for reclamation including an improvement in soil structure, increases in water retention, a carbon source for microbial activity and complexation with metals. Limitations in reclamation cannot be overcome completely by the use of amendments and management practices such that the selection of appropriate species is critical. Legumes and grasses have a well established record in reclamation offering the potential to fix nitrogen (legumes), tolerate drought and saline stress (grasses) and act as effective surface stabilizers with their rooting systems.

In order for the successful reclamation of acid generating mine waste a complex understanding of the geological, chemical, physical, and biological process that are occurring and have occurred is needed. With knowledge of these processes, tailings sites such as the Central Manitoba can be rehabilitated to some productive state and the risk of acid mine drainage eliminated or reduced.

3. Materials and Methods

3.1 Plant Materials

The selection of plant species was based on: 1) established use in tailings reclamation, 2) characteristics such as heavy metal tolerance, drought tolerance, tolerance to salinity, and ability to control erosion, 3) species currently growing on site or successfully used in reclamation studies at the Central Manitoba tailings impoundment and 4) potential ability to accumulate heavy metals. No more than 5 of the listed species were used in a given experiment.

- *Brasica juncea* (Brown mustard)
    - Tolerance and ability to accumulate various heavy metals such as lead, chromium, cadmium, nickel, zinc, and copper (Nanda-Kumar et al. 1995).
- *Poa pratensis* (Kentucky bluegrass)
    - Moderate drought tolerance and very high ability to control erosion (Hardy BTT Ltd., 1989).
    - Found growing in the Central Manitoba tailings impoundment (Sherriff, Punter and Punter, personal communication).
- *Festuca pratensis* (meadow fescue)
    - Good tolerance to acidic soils
- *Festuca rubra* (red fescue)
    - High tolerance to drought, saline and acidic growing conditions (Hardy BTT Ltd., 1989).
    - High ability to control erosion (Hardy BTT Ltd., 1989).
- *Medicago sativa* (alfalfa)
    - Potential to accumulate gold (Gardea-Torresdey et al. 2000)
    - Ability to fix nitrogen and found growing in the Central Manitoba tailings impoundment (Sherriff, Punter and Punter, personal communication).
- *Agropyron trachycaulum* (slender wheatgrass)
    - Medium drought and high saline tolerance and a high level of erosion control (Hardy BTT Ltd., 1989).

- Demonstrated good germination and survival in peat amended mine tailings at Central Manitoba tailings site (Renault et al. 2002) and also found growing in the impoundment (Sherriff, Punter and Punter, personal communication).

- *Agropyron elongatum* (tall wheatgrass)
  - Very high tolerance to saline and acidic growing conditions (Hardy BTT Ltd., 1989).
  - High drought tolerance and medium ability to control erosion (Hardy BTT Ltd., 1989).

Seeds of *B. juncea* (lot #14656) were purchased from Richters (Canada) and seeds of *P. pratensis* (lot #14231394079513), *F. pratensis, F. rubra, M. sativa* (lot #3098 MN), *A. trachycaulum* and *A. elongatum* were provided by Brett Young Seeds Ltd (Winnipeg, Manitoba, Canada).

3.2 Growth Chamber Experiments

3.2.1 Tailings Collection and Storage

Tailings were collected on two separate occasions from the Central Manitoba tailings impoundment, once in May 2003 and again in September 2003. Tailings in both collection times were taken from the top 15 cm of surface on the east side of Provincial highway #304, with a geographical location corresponding to 50° 54' 16" N and 95° 20' 6" W. No previous experimentation or analysis of the tailings from this area of the impoundment had been published at the time of collection. Tailings were air dried following collection, mixed thoroughly and stored in covered PVC containers in a cool dry room. Tailings collected in May 2003 were used in the short term experiment while tailings collected in September 2003 were used in the long term experiment.

3.2.2 Growth Conditions

Experiments were conducted under spring growing conditions with a photoperiod of 14 hours and a constant temperature of 20 °C. Light levels in the growth chambers were approximately 270 μmol m^{-2} s^{-1}.

3.2.3 Amendment Application

Water soluble modified humic substances from BlackEarth Humates Ltd. were added to air dried Central Manitoba mine tailings in treatment rates of 0, 7.5, and 15.0 g amendment kg^{-1} tailings. Amendment was applied in these rates in order to reach concentrations of 0, 2 and 4 g carbon kg^{-1} tailings, respectively. The amount of modified humic substances required to promote favorable chemical and physical changes in Central Manitoba tailings were suggested by Ibrahim and Goh (2004). Tailings and amendment were mixed together evenly in a dry state. Water was added to raise the moisture to approximately 35 % gravimetric water content.

3.2.4 Fertilizer Application

Plant Prod ™ soluble fertilizer 20/20/20 (N/P/K) with chelated micronutrients (iron 0.1%, manganese 0.05%, zinc 0.05%, and copper 0.05%) and unchelated micronutrients (boron 0.02% and molybdenum 0.0005%) was used in the short term and long term growth chamber experiments.

3.2.5 Short Term Growth Chamber Experiment

3.2.5.1 Experimental Setup

Five kilograms of hydrated tailings (~35% water by weight) from each treatment were placed in seeding trays 25 cm x 50 cm. Each treatment (0 g C kg^{-1}, 2 g C kg^{-1} and 4 g C kg^{-1} tailings) was replicated four times. Following application of amendment, the tailings in the seeding trays were incubated in the growth chamber for four weeks to allow for favorable structural changes to occur within the tailings as suggested by Ibrahim and Goh (2004). Following incubation, forty seeds (2 rows of 20) of *B. juncea*, *P. pratensis*, *F. pratensis*, *M. sativa* and *A. trachycaulum* were seeded in each tray and covered with 1 cm layer of moist peat. Seeding trays were initially covered with clear plastic lids for 2 weeks following seeding. Watering was conducted on a regular basis every other day following seeding. Two hundred mL (4.2 g L^{-1}) of fertilizer was added to each seeding flat at 1 week and 3 weeks post seeding.

3.2.5.2 Tailings Analysis

Tailings samples were collected prior to amendment application and at harvest. Two samples from each seeding flat were collected from random locations. Samples were air dried and stored at 5 °C until further analysis. A saturated paste extract of the tailings was used in order to determine the pH as outlined by SPAC (2000). Distilled water was added to 30 grams of air dry tailings and incubated for 1 hour. Samples were placed in Buchner funnels with Whatman #1 filter paper under suction. The filtrate was stored at 10 °C until further analysis. The pH of the filtrate was measured using a Dual Channel pH/Ion meter (Accumet AR25, Fisher Scientific, Georgia, USA).

3.2.5.3 Emergence, survival and growth

Seedling emergence and survival of planted seeds in each of the five species was measured over a 21 day period. Each row of plants were harvested after 4 weeks post seeding and washed with distilled water. Root and shoots were separated and pooled on a row basis before fresh biomass was recorded. Plants were lyophilized and dry biomass of roots and shoots recorded. Shoot height was measured in *M. sativa* and *B. juncea* from the base of the stem to the shoot apical meristem. *P. pratensis, F. pratensis* and *A. trachycaulum* shoot height was measured from the base of the stem to the tip of the tallest leaf.

3.2.5.4 Photosynthetic Pigment Content

Two plants of each species, 1 from each row, were randomly selected for pigment extraction from each treatment after 4 weeks post seeding. Three mature healthy leaves of *M. sativa* and *B. juncea* were chosen at random from the same plant and 1 disk (0.25 cm^2) taken from each leaf. Leaf tissue was sampled from leaves of *P. pratensis, F. pratensis* and *A. trachycaulum* by collecting the top 3 cm of three blades and then discarding the tip (1 cm) of each blade. The three samples taken from each plant were then pooled and the combined fresh weight recorded (F_w g) with samples then lyophilized. Chlorophyll a, chlorophyll b, and carotenoids were extracted from lyophilized leaf tissue using two 80% acetone extractions. Leaf pigments were quantified

from the two combined extracts (E_t in mL) as outlined by MacKinney (1941) and Davies (1976) on a fresh weight basis using a spectrophotometer (Ultraspec 200, Pharmacia Biotech, USA) at wavelengths of 480 (A_{480}), 645 (A_{645}) and 663 nm (A_{663}). Pigments were calculated as follows:

Chlorophyll a (mg g^{-1}) = $(12.72 * A_{663} - 2.58 * A_{645})(E_t / F_w)$

Chlorophyll b (mg g^{-1}) = $(22.87 * A_{645} - 4.67 * A_{663})(E_t / F_w)$

Carotenoids (mg g^{-1}) = $(A_{480} + 0.114 * A_{663} - 0.638 * A_{645})(E_t / F_w)$

3.2.6 Long Term Growth Chamber Experiment

3.2.6.1 Experimental Setup

The long term experiment was conducted using 7 inch (180 mm) pots filled with tailings of each treatment (See section 3.2.3). Four pots of the same treatment rate were placed in a seeding flat one for each species. Each treatment was replicated 4 times. Pots containing the amended tailings were incubated in the growth chamber for 4 weeks allowing chemical and physical changes to occur. Following incubation, 20 seeds of *B. juncea* and *M. sativa* and 25 seeds of *A. trachycaulum* and *F. pratensis* were seeded in separate pots within the seeding flats. Following seeding, pots were covered with 1 cm of moist peat and placed in a growth chamber at 22 °C, 12 hours of ~350 µmol m^{-2} s^{-1} light. Clear plastic wrap was placed over the seeded pots and removed on the 6^{th} day post seeding. Following seedling emergence the total number of seedlings per pot was reduced to 9. One hundred mL (4.2 g L^{-1}) of Plant Prod ™ fertilizer was added to each pot at 1, 3, 5, 7 and 9 weeks post seeding. Watering was conducted every other day by adding 100 mL of water to the surface of the pot. After 30 days post seeding, water was added as needed when the surface appeared dry. Water was added to the seedling flat once every 2 weeks in order for water to be drawn upwards into the pots.

3.2.6.2 Tailings Analysis

Tailings samples were collected post amendment application and at harvest. Two samples from each pot were collected at both time periods. One sample was taken from the upper 3 cm of the pot and the second taken from the lower 10 cm of the pot. Soil analysis followed the same process and methodology as described in section 3.2.5.2.

Conductivity of the filtrate was measured using a conductivity meter (Traceable, Control Company, Texas, USA).

3.2.6.3 Growth

Pots of *M. sativa* and *B. juncea* were thinned to avoid competition within pots at 4 and 5 weeks with 3 plants retained for analysis. Three plants were left to mature until harvest at 12 weeks post seeding. Pots of *F. pratensis* and *A. trachycaulum* were thinned at 5 weeks to avoid competition with 4 plants retained for analysis. Four plants were left to mature until harvest at 12 weeks post seeding. Plant processing following harvest and methodology for measuring biomass and height followed the same procedure as section 3.2.5.3. Leaf area was measured for *B. juncea* during the 4 and 12 week harvests while leaves from *P. pratensis, F. pratensis*, *M. sativa* and *A. trachycaulum* were not collected due to their small size and difficulty in obtaining accurate total plant leaf area. All *B. juncea* leaves were removed from the stem of the 3 harvested plants for leaf area measurement using an area meter (Li-300, LiCor, Nebraska, USA).

3.2.6.4 Photosynthetic Pigment Content

Three harvested plants of each species were randomly selected for pigment extraction from each treatment pot at 5 and 12 weeks post seeding. Leaf tissue was sampled using 0.25 cm^2 leaf disks from *M. sativa* and *B. juncea*. Three mature healthy leaves were chosen at random and 1 disk taken from each leaf. Leaf tissue was sampled from 3 leaves of *F. pratensis* and *A. trachycaulum* by removing the top 3 cm of a blade and then discarding the tip (1 cm) of the blade. The 3 samples taken from an individual plant were then pooled and the combined fresh weight recorded. Pigment extraction and quantification followed the same process and methodology as described in section 3.2.5.4.

3.2.6.5 Anothocyanin Content

Stems of *M. sativa* and *B. juncea* were selected for anthocyanin pigment extraction at 12 weeks post seeding. Lyophilized tissue was utilized following an

anthocyanin extraction and quantification method based on a procedure outlined by Jiang and Joyce (2003). The lower 2 cm of the stem was collected with the woody tissues removed. The sample was weighed then ground (mortar and pestle) in 10 ml extraction buffer (50% methanol, 1% HCl, in H_20) at 4 °C. Samples were kept on ice following grinding and then centrifuged for 20 min at 4900 *g*. Absorbance of the supernatant was measured using a spectrophotometer (Ultraspec 200, Pharmacia Biotech, USA) at 530 nm and quantity expressed on an absorbance/dry tissue ratio.

3.2.6.6 Electrolyte Leakage

Electrolyte leakage in leaf tissue of *M. sativa* and *B. juncea* was measured at 4 and 12 weeks while *F. pratensis* and *A. trachycaulum* were measured at 12 weeks post seeding. Electrolyte leakage was not sampled at 4 weeks for *F. pratensis* and *A. trachycaulum* due to the small size of the leaves. Three plants per pot were chosen at random and 1 mature leaf from each was randomly selected. Leaf tissue was sampled using a 0.25 cm^2 leaf disk for *M. sativa* and *B. juncea* and a 0.10 cm^2 leaf disk for *F. pratensis* and *A. trachycaulum*. The technique followed for electrolyte leakage was based on a modified protocol by Renault et al. (1998). Each leaf disk was placed in a plastic centrifuge tube filled with 20 mL of de-ionized water. Leaf disks were washed for 1 hour then placed in a second centrifuge tube containing 20 mL of de-ionized water (conductivity a_0). The centrifuge tube containing the leaf disks were then shaken gently for 5 hours with the leaf disks carefully flipped after 2.5 hours of shaking. Leaf disks were then removed from the centrifuge tube and the conductivity of the water (a) measured using a conductivity meter (Traceable, Control Company, Texas, USA). Leaf disks were subjected to 4 cycles of freezing and thawing using liquid nitrogen. Disks were placed back in their respective centrifuge tubes. The centrifuge tubes were gently shaken for an additional 5 hours and the total conductivity (b) measured with a conductivity meter (Traceable, Control Company, Texas, USA). Electrolyte leakage was expressed as followed:

Electrolyte leakage (%) = $[(a- a_0)/(b- a_0)] \times 100$

3.2.6.7 Transpiration

Transpiration for all species was measured prior to harvest at 12 weeks post seeding. Three plants per pot were randomly selected. One mature leaf was randomly chosen for transpiration measurements using a steady state porometer (Model Li-1600, LiCor, Nebraska, USA) between 9:30 and 11:00 AM. Leaf area was measured using an area meter (Li-300, LiCor, Nebraska, USA).

3.3 Field Experiments

3.3.1 Site Description

Field experiments were conducted to verify and validate results of the growth chamber experiments in a natural and realistic setting. Two separate field experiments were conducted on the east side of highway #304 at the Central Manitoba tailings impoundment. Field season 2003 was conducted over the summer of 2003 and field season 2004 conducted over the summer of 2004. Experimental sites were located at 50° 54' 16" N 95° 20' 6" W (Figure 1). Field sites were selected near the center of the north south axis of the impoundment and appeared to be relatively free of surface salt crusting. No established vegetation existed in either site prior to treatment and seeding of selected species.

3.3.2 Field Season 2003

3.3.2.1 Experimental Setup

Mine tailings were tilled to a depth of 15 cm using a rotor tiller. Water soluble modified humic substances (soluble 80) from BlackEarth Humates Ltd. were added in field season 2003 and 2004 to the surface of the tilled tailings in treatment rates of 0, 7.5, 11.3 and 15.0 grams amendment kg^{-1} tailings which corresponded to 0, 15,000, 22,000 and 30,000 kg $hectare^{-1}$. Amendment was applied in these rates in order reach concentrations of 0, 2, 3 and 4 g C kg^{-1} tailings respectively. Bulk density of the tailings (1,347 kg m^3) was estimated by placing 200 g of air dried tailings into a graduated cylinder allowing the tailings to settle with the volume recorded. Random block design was used for the placement of different treatments. Four treatment blocks in field season 2003 represent one replicate and were repeated 4 times for a total of 16 treatment blocks

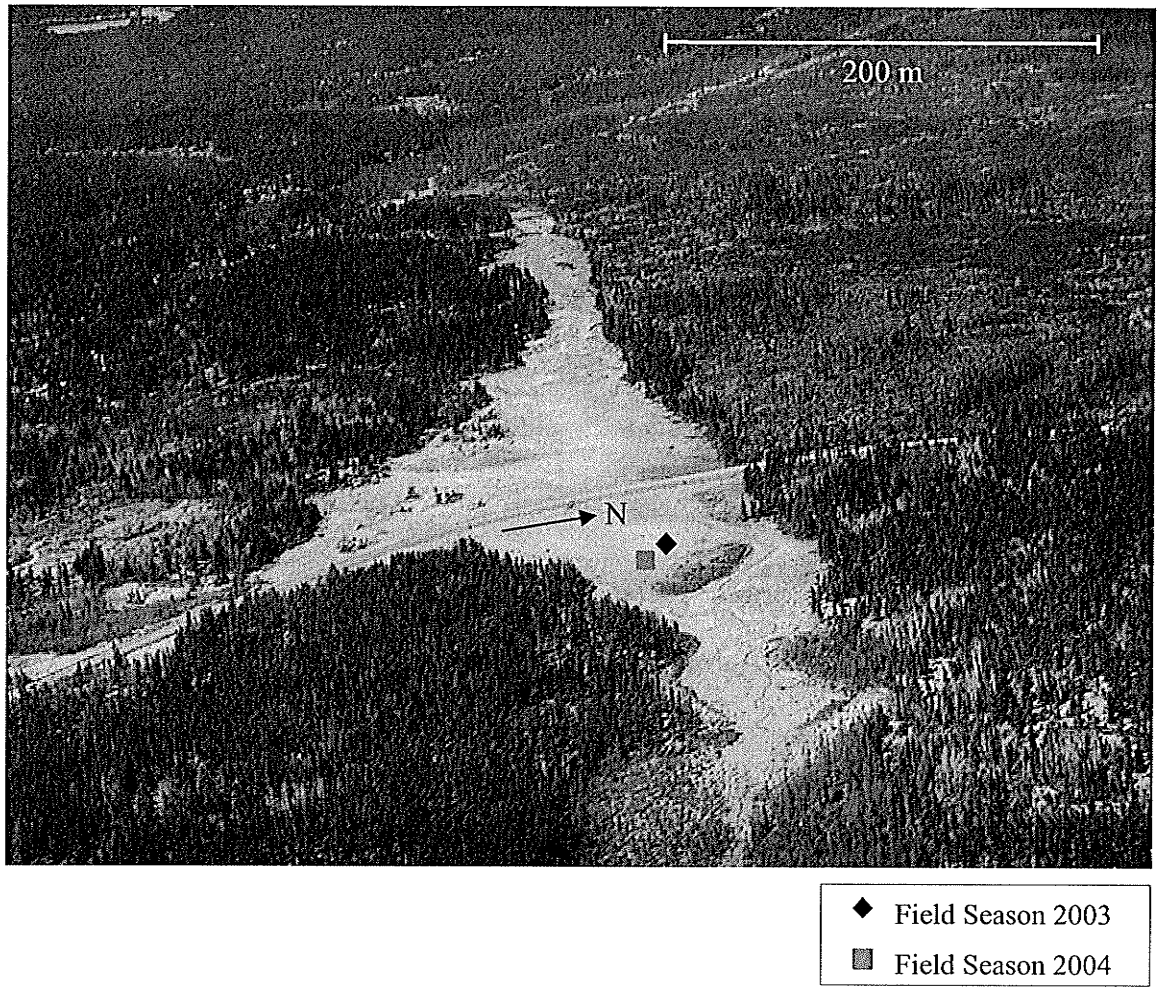


Figure 1. Field season 2003 and 2004 locations at the Central Manitoba mine tailings site. (Image courtesy of Dr. Barbara Sherriff, 2001)

(Figure 2). Amended tailings were left to incubate for 4 weeks in order to allow chemical and physical property changes of the tailings to occur before seeding took place.

Following incubation, seeding plots (0.5 m x 0.5 m) for *M. sativa*, *B. juncea, P. pratensis, F. pratensis* and *A. trachycaulum* where established randomly within each treatment block (0.7 m x 3 m) (Figure 3). One hundred seeds of each selected species were placed on the tailings surface of each plot. Following seeding approximately 3 cm moist peat was applied to the surface of each treatment block. Each treatment block was fertilized with 7.5 L (3.8 g L^{-1}) of Plant Prod ™ soluble fertilizer 20/20/20 (N/P/K) with chelated micronutrients (iron 0.1%, manganese 0.05%, zinc 0.05%, and copper 0.05%) and unchelated micronutrients (boron 0.02% and molybdenum 0.0005%). Garden anti-bird netting was placed over top of the peat to help protect the surface from wind erosion. Watering over the field season was conducted approximately once a month adding 12.5 L of water per treatment block. A second fertilizer addition of 12.5 L (2.3 g L^{-1}) was conducted 1 month after seeding.

3.3.2.2 Tailings Collection and Analysis

Four tailings samples from each treatment block were collected using a Dutch auger at pre treatment, post treatment, the time of seeding, the time of harvest and 1 year post treatment. These samples were taken along a transect through each treatment block at approximately 0.75 m apart. Samples were collected to a depth of 15 cm and placed in a cooler until transport. Samples were air dried and then stored at 5 °C until further analysis.

An extract of the tailings was collected by saturating 30 grams air dry tailings with distilled water and filtering the liquid through Whatman #1 filter paper. The pH of the filtrate was measured using a Dual Channel pH/Ion meter (Accumet AR25, Fisher Scientific, Georgia, USA) and the conductivity measured using a conductivity meter (Traceable, Control Company, Texas, USA). Soil organic carbon of each sample was measured at the times of pre treatment, post treatment, harvest and 1 year post treatment. Organic carbon was determined using the reduction of chromic acid by organic carbon determination technique as outlined by Walkley (1946). Tailings samples of less than 1

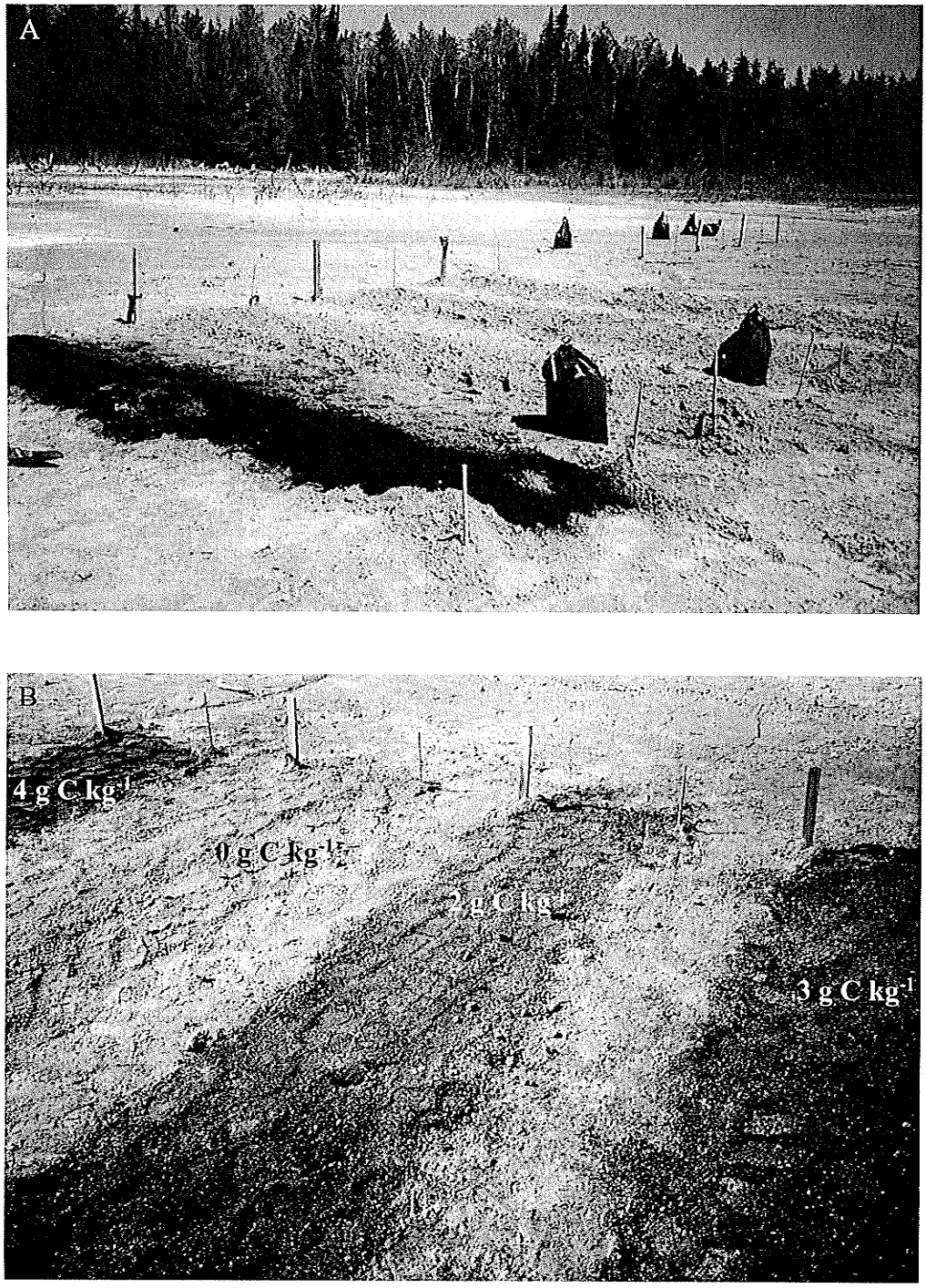


Figure 2. A) Humic amendment application to the surface of the treatment block and B) Treatment block following mixing of amendment evenly with the tailings.

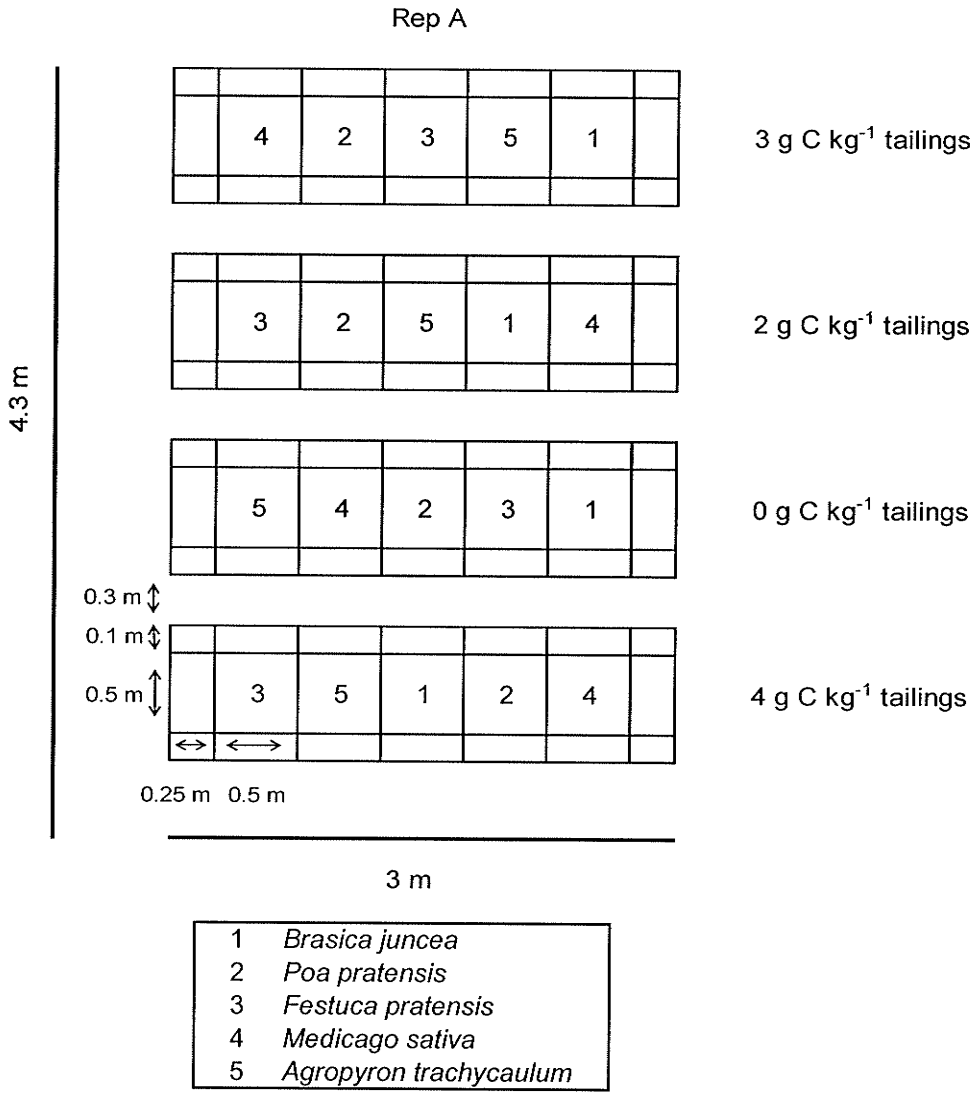


Figure 3. Field plots of a single replicate in field season 2003.

gram were combined with 10 ml of potassium dichromate ($K_2Cr_2O_7$) (1N) and mixed evenly. Concentrated sulphuric acid (20 ml) was added to the dichromate tailings mixture to rapidly digest the organic material. Following digestion, flasks were allowed to stand for 30 minutes. Concentrated phosphoric acid (10 ml) and 200 ml of water were added to each sample. Excess un-reacted chromate ions ($Cr_2O_7^{2-}$) were titrated with ferrous sulphate ($FeSO_4$) using o-phenanthroline-ferrous complex as an endpoint indicator. Organic carbon was calculated as follows where $f = 1.30$:

Organic Carbon % = f * (meq $K_2Cr_2O_7$ – meq $FeSO_4$)(0.003)(100)/g water free soil

Water stable aggregation was measured based on the method outlined by Angers and Mehuys (1993). Water stable aggregates were measured from 4 size fractions >2.0, 2.0-1.0, 1.0-0.5, and 0.5-0.25 mm. Two random samples (40 g air dry tailings) were selected from each treatment block. Thirty grams were placed on a wet sieving apparatus and 10 g were dried at 105 °C to determine water content of each sample. Samples were placed on the top of the sieve stack (2-1-0.5-0.25 mm sieves) and lowered into the water quickly. Sieving occurred in the vertical direction for 20 minutes with 640 oscillations. Aggregates in the uppermost sieve were kept below the surface of the water during upstroke of the oscillation. Size fractions were collected, dried at 105 °C and weighed.

3.3.2.3 Seedling Emergence, Survival and Growth

Seedling emergence was measured 2 and 4 weeks post seeding. Survival was recorded at the time of harvest 12 weeks post seeding. Ten plants (when available) from each plot were randomly harvested 12 weeks post seeding in September. Harvested plants were washed with distilled water prior to root and shoot separation and fresh biomass measurement. Tissues were lyophilized and dry biomass of roots and shoots recorded. Plant height was measured in *M. sativa* and *B. juncea* from the base of the stem to the shoot apical meristem. *Poa pratensis, F. pratensis* and *A. trachycaulum* shoot height was measured from the base of the stem to the tip of the tallest leaf.

3.3.2.4 Photosynthetic Pigments

Three harvested plants of each species were randomly selected for pigment extraction. Three mature healthy leaves were chosen at random and 1 disk (0.25 cm^2)

taken from each leaf. The 3 samples taken from an individual plant were then pooled and the combined fresh weight recorded then lyophilized. Pigment extraction and quantification followed the same process and methodology as described in section 3.2.5.4.

3.3.2.5 Elemental Analysis

Three random plants, for each species, from each plot (with roots and shoots separated) were ground and pooled for elemental analysis. Samples (0.1 to 1 g) were digested with 10 ml concentrated hydrochloric acid over 30 minutes. Four millilitres of perchloric acid was then added and the solution heated to 150-200 °C for 1 hour. The solution was then diluted up to 25 mL with deionized water. Analysis of *M. sativa*, *A. trachycaulum* and soil was conducted via inductively coupled plasma optical emission spectrometry (ICP-OES) on an ICP-Emission Spectrometer (Liberty 200, Varian, USA), as outlined by Thompson and Walsh (1983).

3.3.3 Field season 2004

3.3.3.1 Experimental Setup

Plots for field season 2004 were established approximately 10 meters south east from the plots of field season 2003. Amendment of mine tailings with soluble 80 followed the same procedure and treatment rates as mentioned in field season 2003 (see section 3.3.3.1). Field season 2004 included a side experiment (Appendix A) using unmodified humic materials (mini granule) from BlackEarth Ltd applied in treatment rates of 4.0 and 7.9 grams amendment kg^{-1} tailings which corresponded to 9,500 and 19,000 kg amendment $hectare^{-1}$. Amendment was applied in these rates to reach concentrations of 2 and 4 g C kg^{-1} tailings. Random block design was used for the placement of different treatments. Six treatment blocks in field season 2004 constituted one replicate and were repeated 4 times. Tailings treated with modified humic substances were left to incubate for 4 weeks in order to allow chemical and physical property changes of the tailings to occur before seeding took place.

Following incubation, seeding plots (0.5 m x 0.5 m) for *M. sativa*, *F. rubra, F. pratensis, A. elongatum* and *A. trachycaulum* where established randomly within each

treatment block (0.7 m x 3 m) (Figure 4.). One hundred seeds of each selected species were placed on the tailing surface of each plot. Following seeding approximately 3 cm moist peat was applied to the surface of each treatment block. Each treatment block was fertilized with 7.5 L of 20/20/20 (N/P/K) (7.4 g L^{-1}) Plant Prod ™ soluble fertilizer 20/20/20 (N/P/K) with chelated micronutrients (iron 0.1%, manganese 0.05%, zinc 0.05%, and copper 0.05%) and unchelated micronutrients (boron 0.02% and molybdenum 0.0005%). Garden anti-bird netting was placed over top of the peat to help protect the surface from wind erosion. Watering over the field season was conducted approximately once a month by adding 12.5 L of water per treatment block. A second fertilizer addition of 12.5 L (4.6 g L^{-1}) 20/20/20 (N/P/K) was conducted 1 month after seeding.

3.3.3.2 Tailings Collection and Analysis

Tailings samples were collected at the same times and analyzed for the same parameters as described in section 3.3.2.2.

3.3.3.3 Seedling Emergence, Survival and Growth

Emergence times, survival counts and growth measurements followed the same process and methodology as described in section 3.3.2.3 with the exception of 12 plants per plot being harvested for analysis.

3.3.3.4 Photosynthetic Pigments

Three harvested plants of each species were randomly selected for pigment extraction following the same procedure as field season 2003 (see section 3.3.2.4). Leaf tissue was sampled from 3 leaves of *F. pratensis* by removing the top 3 cm of a blade and then discarding the tip (1 cm) of the blade. The 3 samples taken from an individual plant were then pooled and the combined fresh weight recorded then lyophilized. Samples were prepared as outlined in field season 2003 (see section 3.3.2.4) with pigments extracted and quantified followed the same procedure and methodology as described in section 3.2.5.4.

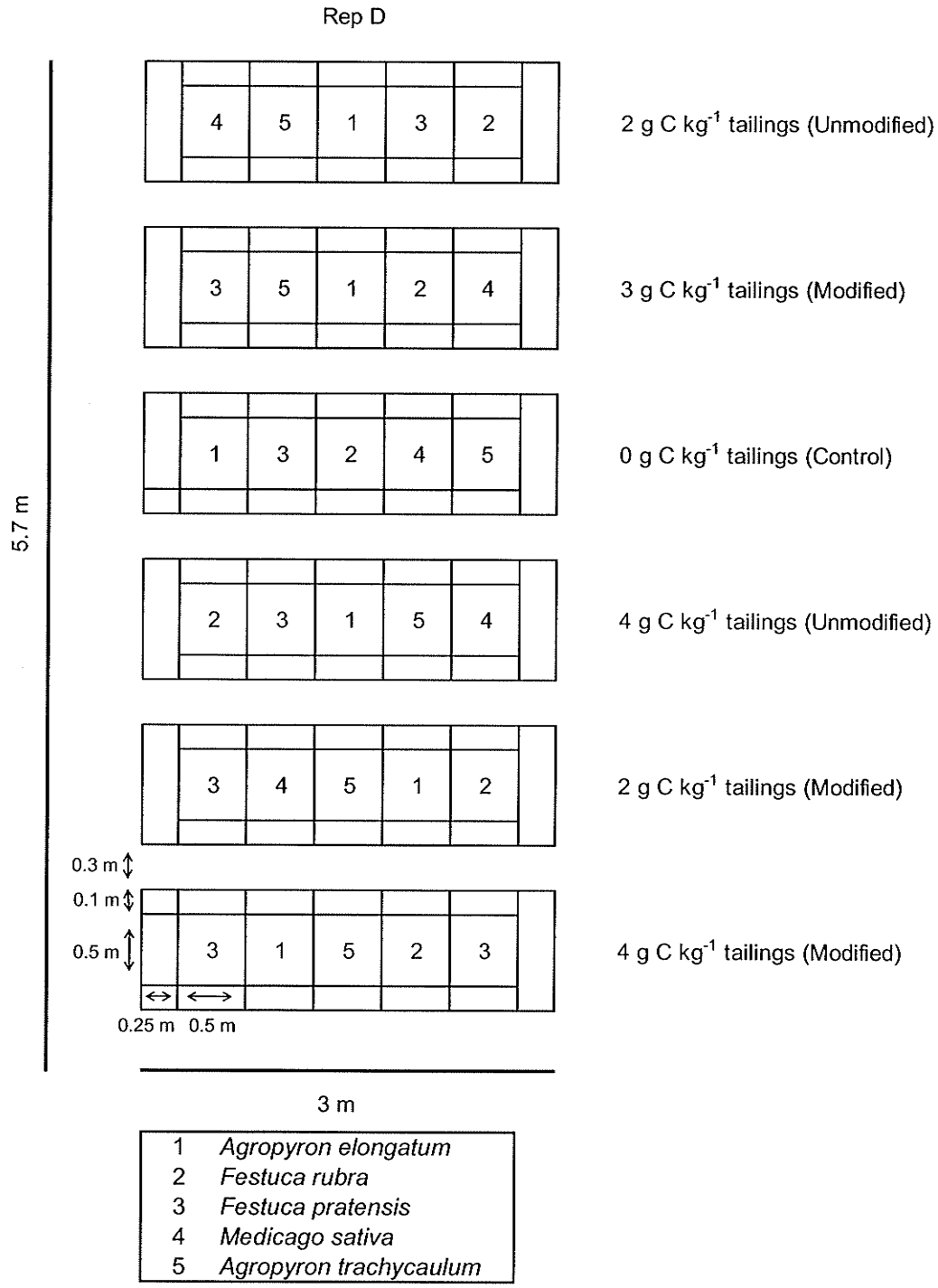


Figure 4. Field plots of a single replicate in field season 2004.

3.3.3.5 Elemental Analysis

Elemental analysis of plant (*M. sativa*, *F. pratensis* and *A. trachycaulum*) and tailings samples followed the same process and methodology as described in section 3.3.2.5.

3.4 Data Analysis

Data were analyzed with a general linear model (GLM) using a nested (inclusion of sub-samples) one-way analysis of variance (ANOVA) comparing treatments for biomass, pigment contents, electrolyte leakage, transpiration, pH, conductivity and soil structure. Sub-samples were compared and considered for outlier (greater or less than 3 standard deviations from the mean) removal prior to analysis of variance of the sample means (Moore D. 1995). Emergence, survival and elemental contents were analyzed using a one-way analysis of variance to compare treatments. Means were compared with the Duncan multiple range test using SPSS (SPSS version 13, SPSS Inc., Chicago, USA).

4. Results

4.1 Tailings Analysis

4.1.1 Short Term Growth Chamber Experiment

4.1.1.1 pH

The pH of the mine tailings averaged 7.8 with a standard error of ± 0.1 prior to the addition of amendment. No significant differences in tailings pH were observed among the treatments prior to the addition of modified humic substances and at the time of harvest. The tailings pH did not significantly change over the course of the experiment.

4.1.2 Long Term Growth Chamber Experiment

4.1.2.1 pH

The pH was not significantly different pre treatment or following the application of humic substances (Table 1).

4.1.2.2 Conductivity

Conductivity of the tailings prior to the application of humic amendment were not significantly different between the treatments (Table 1). Following application of modified humic substances the conductivity was increased relative to the control. At the time of harvest a similar trend to the post treatment was observed and no significant difference between treatments occurred over the two sample times.

4.1.2.3 Elemental Analysis

Elemental analysis of modified humic substances showed that the elements phosphorus and potassium were abundant with concentrations of 30,519 $\mu g\ g^{-1}$ and 157,040 ppm respectively (Table 2). Analysis of tailings prior to the addition of modified humic substances showed copper and iron at concentrations of 3,535 $\mu g\ g^{-1}$ and 31,140 $\mu g\ g^{-1}$ respectively.

Table 1. Conductivity and pH of mine tailings amended with modified humic substances at pretreatment, post treatment and harvest (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Conductivity (dS m^{-1})	pH
0 weeks (Pre Treatment)		
0 g C kg^{-1} tailings (Control)	2.15 ± 0.02 [a]	7.39 ± 0.03 [a]
2 g C kg^{-1} tailings	2.17 ± 0.03 [a]	7.40 ± 0.02 [a]
4 g C kg^{-1} tailings	2.13 ± 0.03 [a]	7.38 ± 0.04 [a]
0 weeks (Post Treatment)		
0 g C kg^{-1} tailings (Control)	2.17 ± 0.03 [a]	7.40 ± 0.03 [a]
2 g C kg^{-1} tailings	4.93 ± 0.16 [b]	7.49 ± 0.04 [a]
4 g C kg^{-1} tailings	7.81 ± 0.19 [c]	7.53 ± 0.05 [a]
12 weeks (Harvest)		
0 g C kg^{-1} tailings (Control)	2.13 ± 0.10 [a]	7.37 ± 0.02 [a]
2 g C kg^{-1} tailings	4.93 ± 0.21 [b]	7.45 ± 0.08 [a]
4 g C kg^{-1} tailings	7.62 ± 0.20 [c]	7.63 ± 0.09 [b]

Table 2. Copper iron, phosphorus, potassium, and sodium content in modified humic substances (Mean ± SE n = 3) and mine tailings prior to treatment with modified humic substances (Mean ± SE n = 2). * Sodium levels were not measured in the tailings.

Sample	Cu ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)	P ($\mu g\ g^{-1}$)	K ($\mu g\ g^{-1}$)	Na ($\mu g\ g^{-1}$)
Humic amendment	3.0 ± 0.4	3878 ± 114	30519 ± 1002	157040 ± 1056	3038 ± 78
Tailings	3535 ± 21	31140 ± 431	134 ± 18	56 ± 3	*

4.1.3 Field Experiments

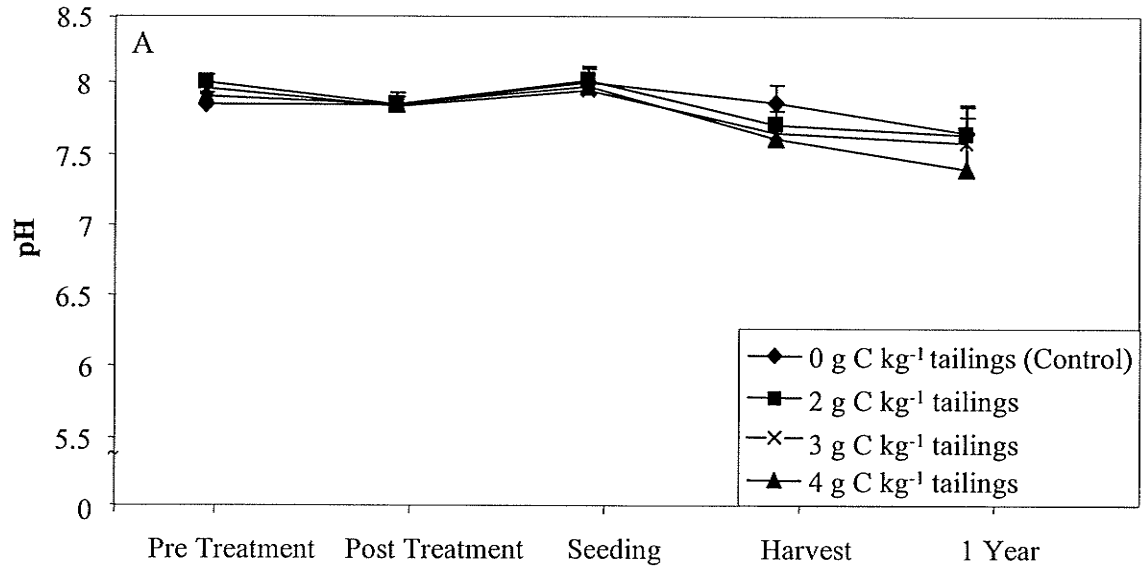
4.1.3.1 Weather Conditions

Field conditions at the time of amendment application in the two field seasons were different. Precipitation recording from the closest weather station in Beausejour gave an approximation for the type of moisture conditions present in the tailings prior to amendment application until harvest in September (Appendix B). Field season 2003 moisture conditions over the growing season were lower than field season 2004. During preparation of the field site in season 2003, tailings were tilled and amendment applied into the tailings was relative ease as tailings were dry with the water table deep below the surface. Field season 2004 treatment conditions were different from field season 2003 due to presence of a high water table which was at the surface of the tailings. As a result of the spring conditions and greater precipitation over the summer months, field season 2004 had noticeably more moisture over the entire growth season compared to field season 2003.

4.1.3.2 pH

In field season 2003, average pH of the tailings before the application of modified humic substances was 7.9 (Figure 5). Following amendment addition there was a small but significant (~0.1 pH) decrease in pH in all the amended treatments compared to pre treatment measurements (Appendix C). The pH fluctuated over the growing season either significantly increasing or decreasing at various times independent of treatment.

The average pH in the treatment blocks in field season 2004, before the application of modified humic substances, was lower compared to field season 2003. Tailings average pH ranged between 6.40 in 4 g C kg^{-1} treatment and 6.85 in the 3 g C kg^{-1} treatment (Figure 5). However, individual samples exhibited heterogeneity with samples as low as 3.78 and as high as 8.05 (Appendix D). Following amendment addition, pH was significantly increased in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments. The pH in individual treatments fluctuated over the growing season either significantly increasing or decreasing at various times independent of treatment (Appendix C).



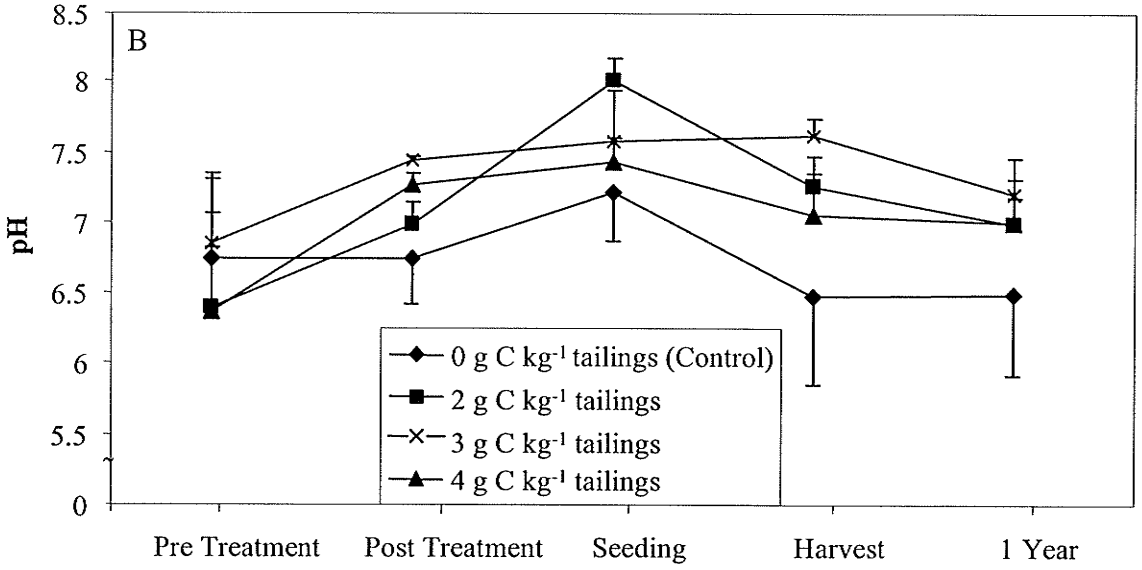


Figure 5. The pH of mine tailings amended with modified humic substances at specific times over a 1 year period for (A) field season 2003 and (B) field season 2004 (Mean ± SE, n = 4).

4.1.3.3 Elemental Analysis

Total background copper and iron content in the tailings was 3,661 $\mu g\ g^{-1}$ and 39,308 $\mu g\ g^{-1}$ respectively in field season 2003 (Table 3). Copper and iron content were not affected by the addition of modified humic substances. Background phosphorus and potassium content was 139 $\mu g\ g^{-1}$ and 9,292 $\mu g\ g^{-1}$ respectively. Addition of modified humic substances significantly increased phosphorus content but no significant differences in potassium content were observed between the treatments.

In field season 2004, the selected site was higher in background copper and iron levels compared to field season 2003 with an average copper and iron content of 4,713 $\mu g\ g^{-1}$ and 47,685 $\mu g\ g^{-1}$ respectively. Like field season 2003, copper and iron content were not affected by the addition of amendment. Background phosphorus and potassium contents were lower than field season 2003 with values of 94 $\mu g\ g^{-1}$ and 7,001$\mu g\ g^{-1}$. Addition of humic substances increased both phosphorus and potassium content in the tailings with an increasing trend with increasing treatment rate.

4.1.3.4 Conductivity

The average conductivity of the tailings in field season 2003 before application of modified humic substances was 2.11 dS m^{-1}. Following application of modified humic substances the conductivity of each treatment increased (Figure 6). At seeding there were no significant changes in conductivity from post treatment (Appendix C). However, at harvest there was a significant decrease in conductivity in the amendment treatments compared to measurements observed at seeding while the control did not change significantly. One year following treatment the conductivity of the amended tailings was significantly lower than the measurements at harvest and closer to the original pre treatment conductivity. At that time, only the 3 g C kg^{-1} and 4 g C kg^{-1} treatments had significantly larger conductivity values compared to the control.

The average conductivity of the tailings before application of modified humic substances was 3.28 dS m^{-1} in field season 2004. A similar trend of increasing conductivity following the application of modified humic substances was observed in field season 2004.

Table 3. Copper (Cu), Iron (Fe), Phosphorous (P), and Potassium (K) content in mine tailings amended with modified humic substances immediately following application for field season 2003 and field season 2004 (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

Treatment	Cu ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)	P ($\mu g\ g^{-1}$)	K ($\mu g\ g^{-1}$)
Field Season 1				
0 g C kg^{-1} tailings (Control)	3,661 ± 197 [a]	39,308 ± 3984 [a]	139 ± 20 [a]	9,292 ± 1056 [a]
2 g C kg^{-1} tailings	3,702 ± 220 [a]	38,108 ± 3364 [a]	443 ± 38 [b]	11,320 ± 1508 [a]
3 g C kg^{-1} tailings	3,667 ± 158 [a]	36,965 ± 1968 [a]	683 ± 106 [b]	11,310 ± 1521 [a]
4 g C kg^{-1} tailings	3,812 ± 249 [a]	39,651 ± 3799 [a]	706 ± 119 [b]	11,693 ± 948 [a]
Field Season 2				
0 g C kg^{-1} tailings (Control)	4,713 ± 446 [a]	47,685 ± 2614 [a]	94 ± 23 [a]	7,001 ± 849 [a]
2 g C kg^{-1} tailings	4,626 ± 329 [a]	47,535 ± 2945 [a]	323 ± 31 [b]	9,860 ± 885 [b]
3 g C kg^{-1} tailings	5,023 ± 394 [a]	46,676 ± 1692 [a]	494 ± 73 [c]	11,130 ± 158 [bc]
4 g C kg^{-1} tailings	4,931 ± 615 [a]	47,972 ± 4088 [a]	738 ± 15 [d]	12,110 ± 694 [c]

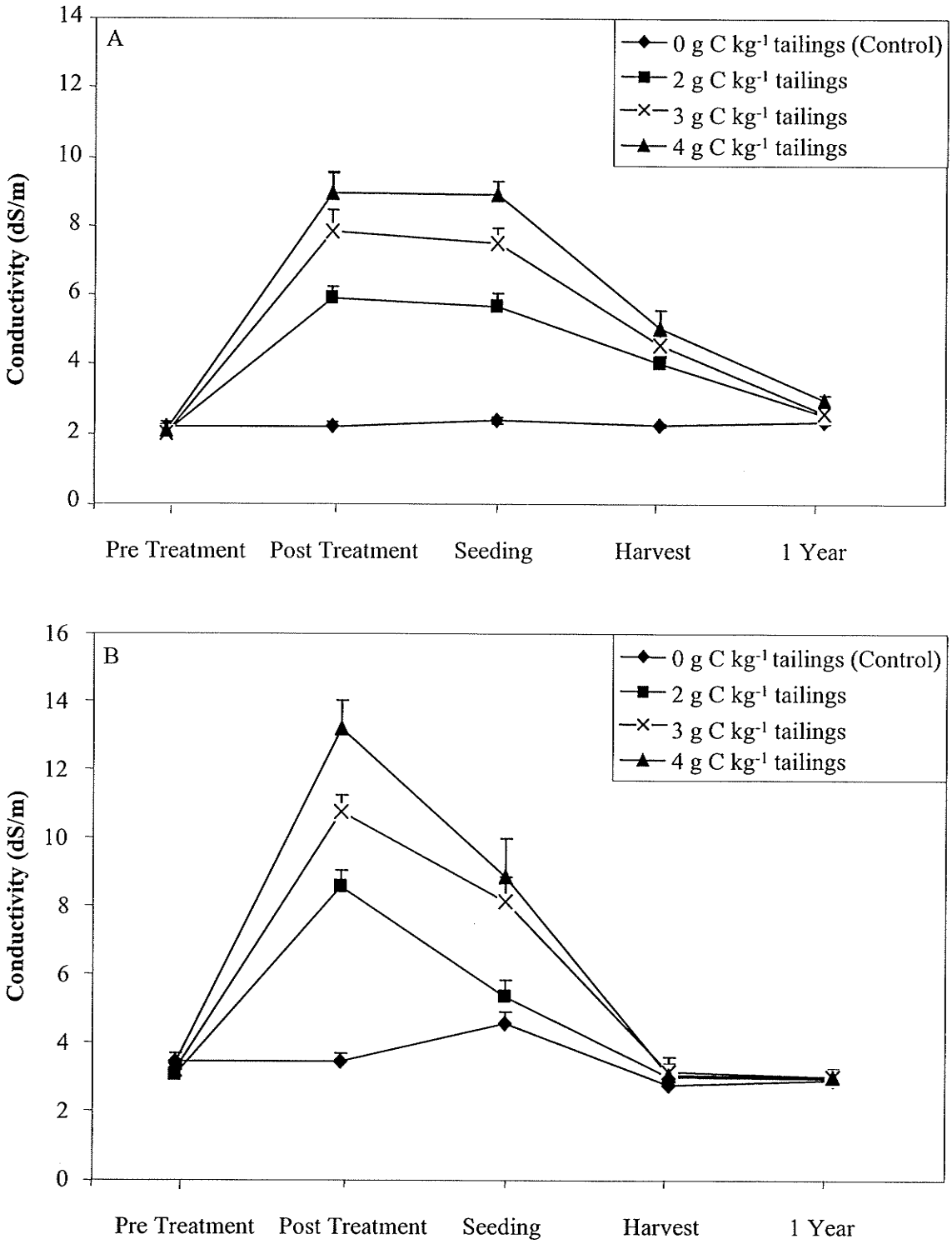


Figure 6. The conductivity of mine tailings amended with modified humic substances at specific times over a 1 year period for (A) field season 2003 (B) field season 2004 (Mean ± SE, n = 4).

However, increases in conductivity were significantly larger than field season 2003 (Figure 6). At seeding a significant decrease in conductivity was observed in treatments amended with modified humic substances such that only the 3 g C kg^{-1} and 4 g C kg^{-1} treatments were significantly greater than the control. The control was the only treatment to increase in conductivity at the time of seeding. At harvest no significant difference in conductivity between the treatments was observed with the exception of the 3 g C kg^{-1} modified humic amendment treatment which remained greater than the control. At harvest all treatments had a significant reduction in conductivity compared with seeding and were similar to their pre-treatment conductivity values. One year following amendment application, no differences were observed between the treatments and furthermore the conductivity of the treatments was not significantly different from the pre treatment or harvest values.

4.1.3.5 Organic Carbon Content

Field season 2003 average carbon content of the tailings before application of humic substances was 0.47% (Figure 7). Following the addition of humic substances the carbon content exhibited an increasing trend with treatment rate with values of 0.80%, 0.99% and 1.26% in the 2 g C kg^{-1}, 3 g C kg^{-1}, and 4 g C kg^{-1} treatments respectively. No significant changes were observed within treatments between the post treatment and harvest sample periods (Appendix C). One year following amendment application the organic carbon content of the amendment treatments was significantly higher in all treatments compared to any other sample period. No significant differences were observed between the amended treatments at 1 year but all treatments remained significantly higher than the control. One year following treatment visual observations showed a reduction in the depth of the amended zone. No profiles of the amended zone were obtained 1 year following treatment but profiles of the treatment zone 2 years following treatment show a similar reduction in the size of the amended zone with less than 5 cm depth in some cases (Figure 8).

Average carbon content before the application of amendment in field season 2004 was 0.41% (Figure 7). Following the addition of humic substances the carbon content exhibited an increasing trend with treatment rate with values of 0.70%, 1.09%, and 1.43%

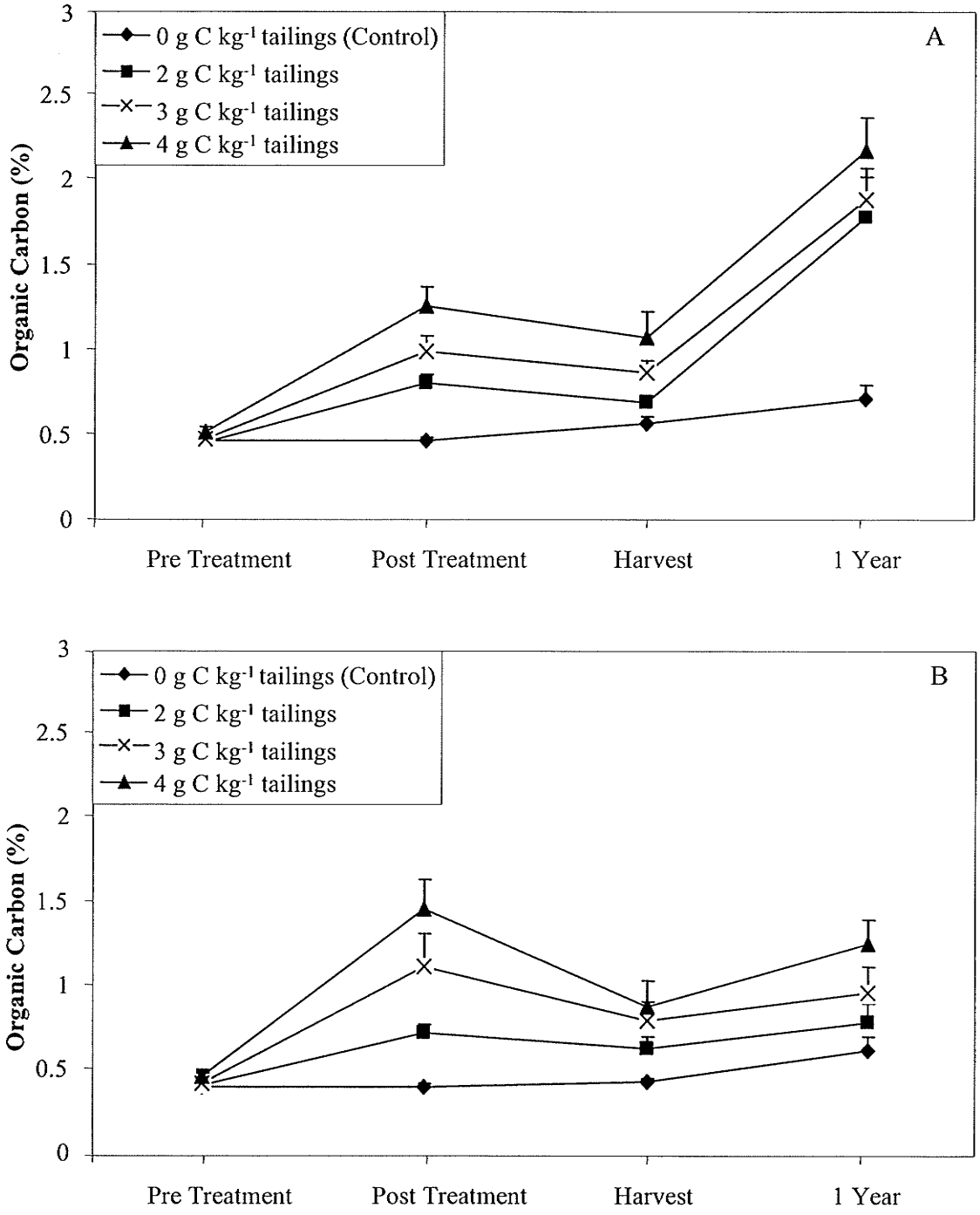


Figure 7. The organic carbon (%) of mine tailings amended with modified humic substances at specific times over a 1 year period for (A) field season 2003 and (B) field season 2004 (Mean ± SE, n = 4).

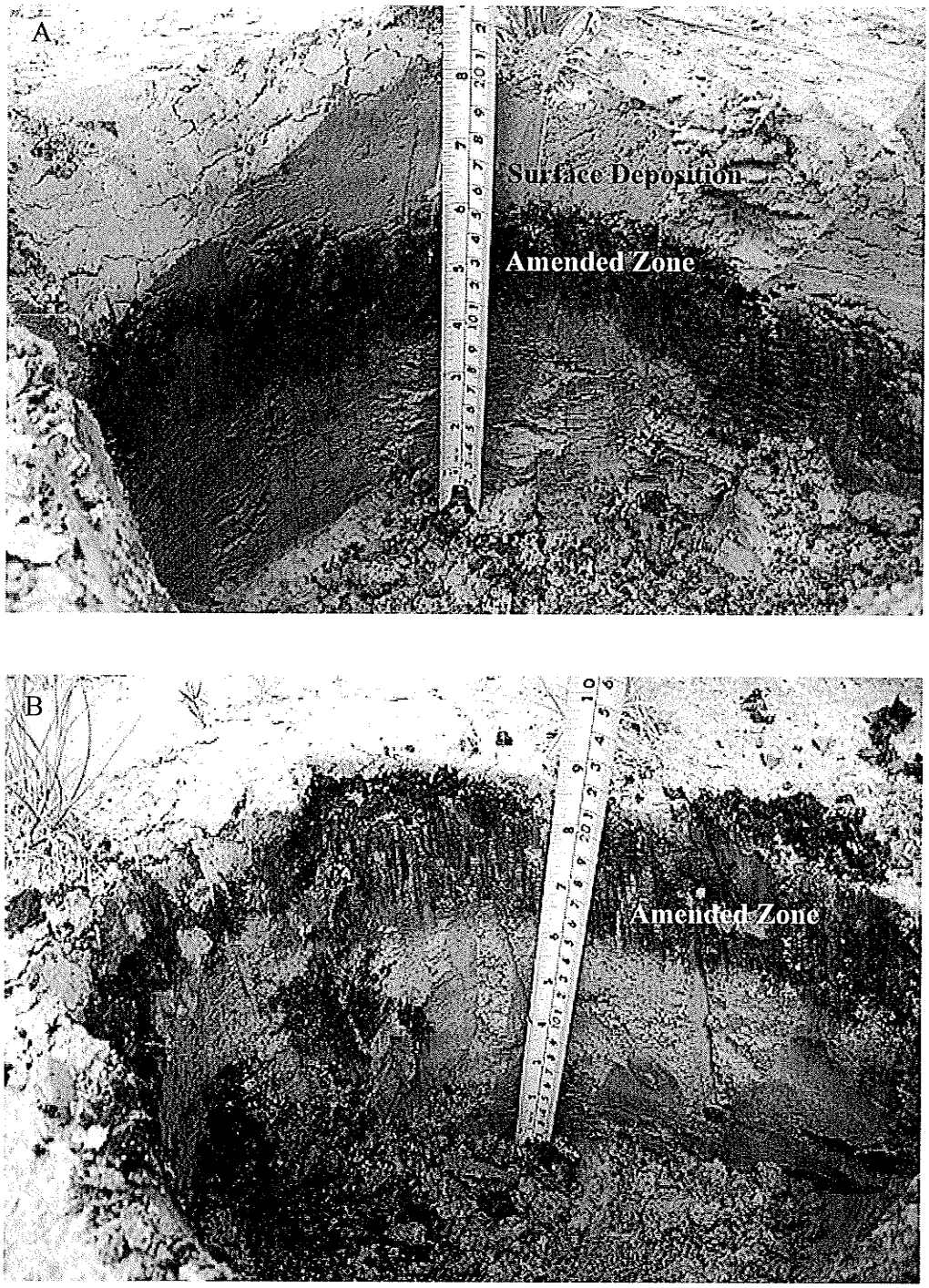


Figure 8. Depth of amended zone within tailings 2 years following amendment application in field season 2003 A) exhibiting accumulation of tailings above the amended zone and B) exhibiting no surface accumulation of tailings.

in the 2 g C kg^{-1}, 3 g C kg^{-1} and 4 g C kg^{-1} treatments respectively with carbon content not significantly different from field season 2003 (Appendix C). Unlike the previous field season at harvest carbon content was reduced in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments compared to pre treatment measurements. However, despite the reduction in carbon content the amendment treatments remained significantly greater than the control. Unlike field season 2003, carbon contents 1 year following treatment did not increase in the amended treatments from the previous harvest sample (Appendix C).

4.1.3.6 Tailings Structure

Pre treatment total macro aggregation was 8.44% across all treatments (Table 4). Following amendment application and a one month incubation total aggregation was significantly increased by ~100% in the amended treatments while no change occurred in the control (Table 4). Increases in aggregation following incubation were observed in the 0.25 – 0.5 mm, 0.5 – 1.0 mm and 1.0 – 2.0 mm size fractions (Appendix E). However, no significant differences in total aggregation or specific size fractions were observed between the amended treatments themselves (Table 4). Total aggregation remained constant such that by harvest no significant changes occurred from the previous sample time with the exception of the 2 g C kg^{-1} treatment (Appendix E). Changes in aggregate distribution between seeding and harvest did occur in the 2 g C kg^{-1} and 3 g C kg^{-1} treatments though no clear trend was apparent. However, both treatments exhibited a significant increase in the >2.0 mm fraction compared to the control over the same time interval (Appendix E). No significant change in aggregate distribution between harvest and seeding occurred in the 4 g C kg^{-1} treatment.

In field season 2004, tailings had a background structure slightly higher than field season 2003 with a total macro aggregation of 10.63% (Table 5). Following amendment application and 1 month incubation total aggregation was increased by ~250% in the 2 g C kg^{-1} and 3 g C kg^{-1} and as much as 350% in the 4 g C kg^{-1} treatment. Like field season 2003 the increases in aggregation were observed in the 0.25 – 0.5 mm, 0.5 – 1.0 mm and 1.0 – 2.0 mm size fractions. An increase in the >2.0 mm fraction also occurred in the

Table 4. Distribution of water stable aggregates in field season 2003 in mine tailings amended with modified humic substances at pretreatment, seeding and harvest (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$).

	Aggregate Distribution				
	> 2.0 mm (%)	1.0 - 2.0 mm (%)	0.5 - 1.0 mm (%)	0.25 - 0.5 mm (%)	Total > 0.25 mm (%)
Pretreatment					
0 g C kg^{-1} tailings	0.46 ± 0.20 ^{ab}	1.62 ± 0.23 ^{a}	2.41 ± 0.37 ^{a}	4.01 ± 0.48 ^{a}	8.50 ± 0.78 ^{a}
2 g C kg^{-1} tailings	0.36 ± 0.08 ^{ab}	1.80 ± 0.31 ^{a}	2.65 ± 0.23 ^{a}	3.57 ± 0.28 ^{a}	8.37 ± 0.76 ^{a}
3 g C kg^{-1} tailings	0.75 ± 0.44 ^{b}	1.92 ± 0.41 ^{a}	2.25 ± 0.47 ^{a}	3.60 ± 0.74 ^{a}	8.52 ± 1.87 ^{a}
4 g C kg^{-1} tailings	0.39 ± 0.14 ^{ab}	1.87 ± 0.35 ^{a}	2.34 ± 0.43 ^{a}	3.77 ± 0.56 ^{a}	8.37 ± 1.40 ^{a}
Seeding					
0 g C kg^{-1} tailings	0.60 ± 0.09 ^{a}	1.70 ± 0.23 ^{a}	2.07 ± 0.19 ^{a}	3.71 ± 0.32 ^{a}	8.08 ± 0.72 ^{a}
2 g C kg^{-1} tailings	1.01 ± 0.25 ^{a}	3.21 ± 0.29 ^{b}	4.59 ± 0.40 ^{b}	7.15 ± 0.50 ^{b}	15.96 ± 0.81 ^{b}
3 g C kg^{-1} tailings	0.77 ± 0.11 ^{a}	3.78 ± 0.37 ^{b}	4.96 ± 0.38 ^{b}	8.03 ± 0.67 ^{b}	17.54 ± 1.06 ^{b}
4 g C kg^{-1} tailings	1.04 ± 0.21 ^{a}	3.44 ± 0.53 ^{b}	4.80 ± 0.66 ^{b}	7.27 ± 0.99 ^{b}	16.55 ± 2.30 ^{b}
Harvest					
0 g C kg^{-1} tailings	0.36 ± 0.12 ^{a}	1.49 ± 0.18 ^{a}	2.56 ± 0.22 ^{a}	4.56 ± 0.15 ^{a}	8.97 ± 0.56 ^{a}
2 g C kg^{-1} tailings	1.36 ± 0.36 ^{b}	4.43 ± 0.59 ^{b}	5.54 ± 0.24 ^{b}	6.89 ± 0.62 ^{b}	18.22 ± 1.05 ^{b}
3 g C kg^{-1} tailings	1.38 ± 0.22 ^{b}	3.84 ± 0.63 ^{b}	4.99 ± 0.44 ^{b}	6.35 ± 0.76 ^{b}	16.55 ± 1.96 ^{b}
4 g C kg^{-1} tailings	0.93 ± 0.31 ^{ab}	4.37 ± 0.87 ^{b}	5.27 ± 0.63 ^{b}	6.81 ± 0.76 ^{b}	17.37 ± 2.21 ^{b}

Table 5. Distribution of water stable aggregates in field season 2004 in mine tailings amended with modified humic substances at pretreatment, seeding and harvest (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$).

	Aggregate Distribution				
	> 2.0 mm (%)	1.0 - 2.0 mm (%)	0.5 - 1.0 mm (%)	0.25 - 0.5 mm (%)	Total > 0.25 mm (%)
Pretreatment					
0 g C kg^{-1} tailings	0.52 ± 0.09 ^{a}	2.27 ± 0.51 ^{a}	3.09 ± 0.31 ^{a}	4.82 ± 0.62 ^{a}	10.70 ± 1.26 ^{a}
2 g C kg^{-1} tailings	0.50 ± 0.14 ^{a}	2.15 ± 0.58 ^{a}	3.38 ± 0.60 ^{a}	4.12 ± 1.14 ^{a}	10.16 ± 2.40 ^{a}
3 g C kg^{-1} tailings	0.46 ± 0.11 ^{a}	2.25 ± 0.68 ^{a}	3.12 ± 0.75 ^{a}	4.79 ± 1.04 ^{a}	10.62 ± 0.52 ^{a}
4 g C kg^{-1} tailings	0.49 ± 0.09 ^{a}	2.49 ± 0.28 ^{a}	3.50 ± 0.31 ^{a}	4.58 ± 0.33 ^{a}	11.06 ± 0.83 ^{a}
Seeding					
0 g C kg^{-1} tailings	0.36 ± 0.10 ^{a}	1.63 ± 0.09 ^{a}	4.42 ± 0.78 ^{a}	6.16 ± 0.56 ^{a}	12.57 ± 1.15 ^{a}
2 g C kg^{-1} tailings	3.36 ± 2.01 ^{ab}	6.81 ± 1.63 ^{b}	12.13 ± 1.57 ^{b}	9.74 ± 0.84 ^{ab}	32.04 ± 3.80 ^{b}
3 g C kg^{-1} tailings	2.83 ± 1.37 ^{ab}	7.05 ± 1.36 ^{b}	10.77 ± 1.21 ^{b}	12.07 ± 1.18 ^{b}	32.71 ± 1.83 ^{b}
4 g C kg^{-1} tailings	6.57 ± 2.32 ^{b}	12.00 ± 0.60 ^{c}	13.78 ± 1.24 ^{b}	11.75 ± 1.04 ^{b}	44.10 ± 1.82 ^{c}
Harvest					
0 g C kg^{-1} tailings	0.46 ± 0.19 ^{a}	2.44 ± 0.55 ^{a}	4.21 ± 1.26 ^{a}	5.97 ± 0.98 ^{a}	13.08 ± 2.86 ^{a}
2 g C kg^{-1} tailings	6.11 ± 1.40 ^{b}	11.66 ± 2.03 ^{bc}	6.94 ± 0.74 ^{b}	8.56 ± 0.47 ^{a}	33.27 ± 1.79 ^{b}
3 g C kg^{-1} tailings	6.76 ± 1.44 ^{b}	9.78 ± 1.24 ^{b}	7.22 ± 1.04 ^{b}	7.11 ± 0.43 ^{a}	30.88 ± 1.84 ^{b}
4 g C kg^{-1} tailings	12.14 ± 1.61 ^{c}	12.71 ± 0.91 ^{c}	7.63 ± 0.99 ^{b}	6.62 ± 0.34 ^{a}	39.09 ± 1.95 ^{b}

4 g C kg^{-1} treatment (Appendix E). No differences in total aggregation or specific size fractions were observed between the 2 g C kg^{-1}, 3 g C kg^{-1}, 4 g C kg^{-1} treatments with the exception of the 1.0 – 2.0 mm size fraction in the 4 g C kg-1 treatment (Table 5). Like field season 2003, at harvest no significant change in total aggregation occurred from the previous sample time (Appendix E). The only exception was the 4 g C kg^{-1} treatment where a decrease in total aggregation from the previous time was observed. Changes in aggregate distributions between seeding and harvest did occur while no change in the control aggregate distribution was observed (Appendix E). Generally, an increase in >2.0 mm and 1.0 – 2.0 mm aggregate fractions was observed while a decrease in the percentage of aggregates in the 0.5 – 1.0 mm and 0.25 – 0.5 mm size fractions. However, the 4 g C kg^{-1} treatment showed little change in the 1.0 – 2.0 mm fraction while the >2.0 mm fraction was significantly increased from the previous sample period (Appendix E).

4.2 Plant Analysis

4.2.1 Short Term Growth Chamber Experiment

4.2.1.1 Seedling Emergence and Survival

The total seedling emergence after the 21 days was greater than 80 % for *F. pratensis, M. sativa*, and *B. juncea* and greater than 60 % for *A. trachycaulum* across all treatments. Final seedling emergence was not significantly affected by the addition of amendment to the tailings with the exception of *P. pratensis* (Figures 9a and 9b). A significant delay in emergence was observed between 3 and 8 days post seeding in all species with the exception of *A. trachycaulum* (Figures 9a and 9b). Emergence values of *P. pratensis* were less then 20 %. Seed viability was tested via standard germination test on moist filter paper and poor seed viability was the source of the low emergence. *Poa pratensis* was thus abandoned from the experiment.

4.2.1.2 Growth

Growth responses to the different treatments was not the same for all species. *Medicago sativa* dry shoot and root biomass were higher than the control in both the 2 g C kg^{-1} and 4 g C kg^{-1} treatments with an increase in biomass as much as 137 % in the

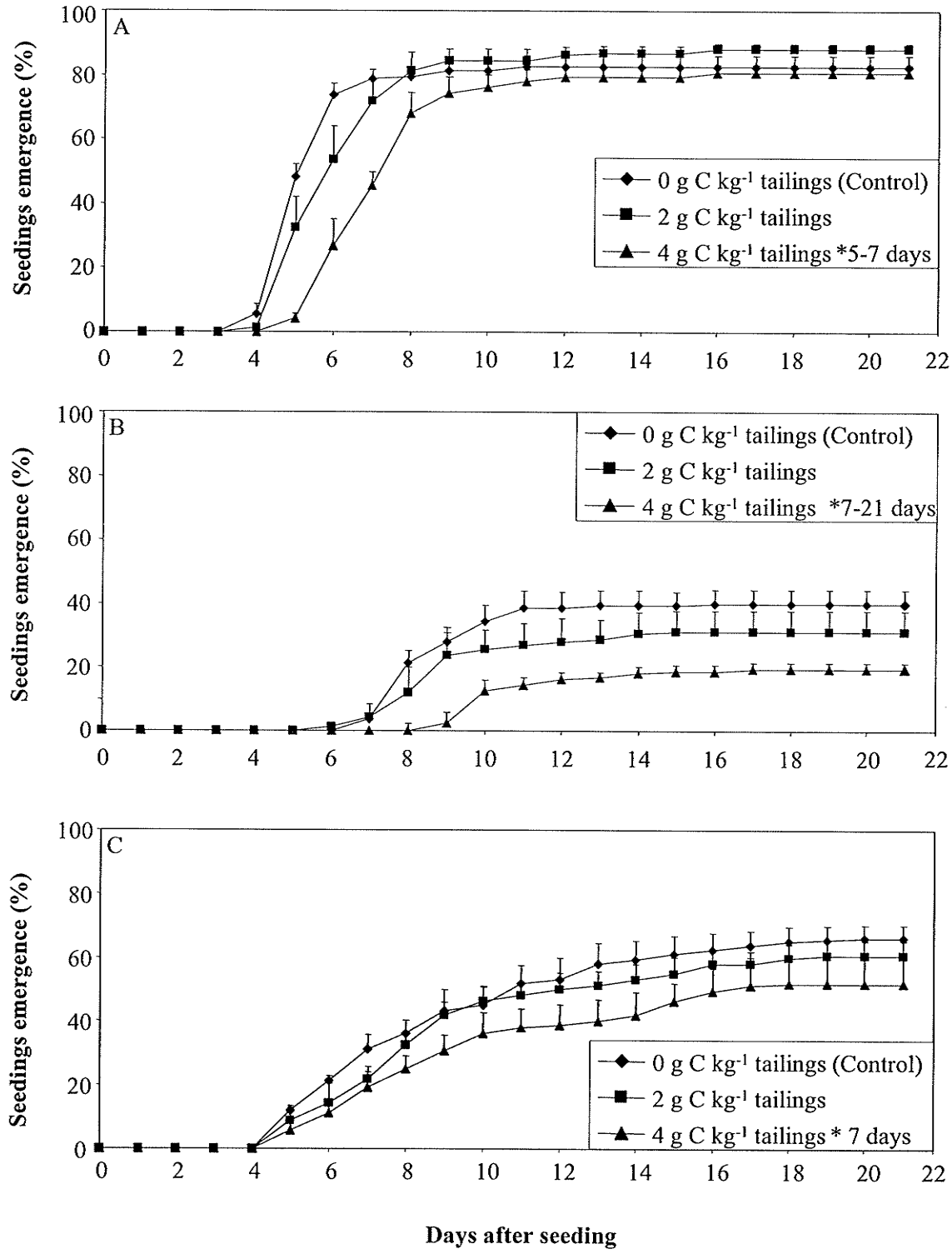


Figure 9a. Emergence of A) *Festuca pratensis,* B) *Poa pratensis* and C) *Agropyron trachycaulum* seedlings over a 21 day growth period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). * Indicates a significant difference observed in a treatment during a certain time interval ($p < 0.05$).

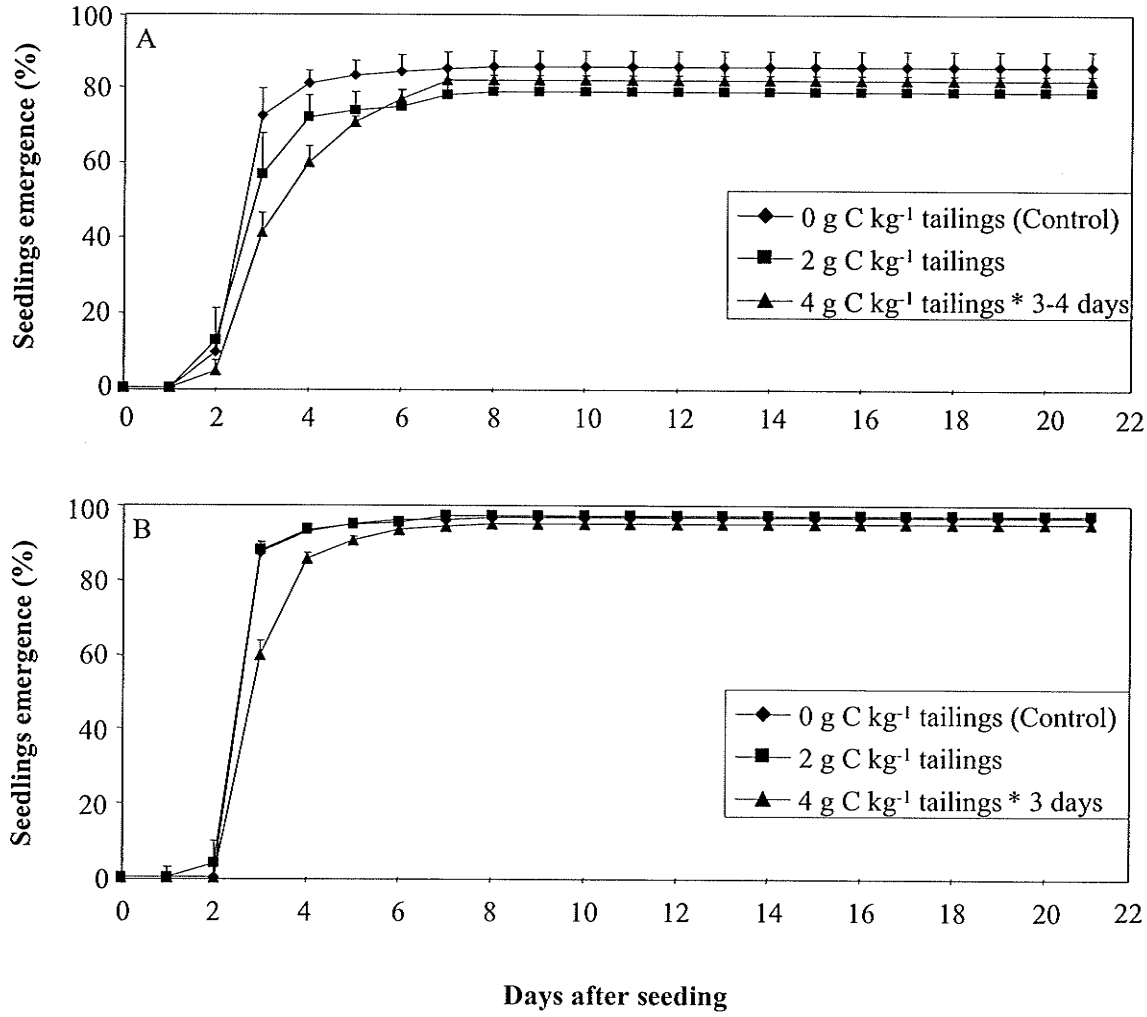


Figure 9b. Emergence of A) *Medicago sativa* and B) *Brassica juncea* seedlings over a 21 day growth period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). * Indicates a significant difference observed in a treatment during a certain time interval ($p < 0.05$).

shoot and 109 % in the root (Table 6). *Brassica juncea* and *F. pratensis* had significant decreases in dry shoot biomass with increasing humic amendment treatment rate with a decrease of 158 % for *F. pratensis* and 36 % for *B. juncea* in the 4 g C kg^{-1} treatment. Root biomass for *F. pratensis* was significantly lower in the 4 g C treatment compared to the control while *B. juncea* exhibited no significant differences in biomass among the treatments. *Poa pratensis* and *A. trachycaulum* exhibited no significant differences in root or shoot biomass between the treatment rates. *Festuca pratensis* is the only species that showed a significant increase in its root to shoot ratio in the 4 g C kg^{-1} treatment (Table 6). Plant shoot height tended to follow similar trends as biomass with *M. sativa* being the only species to show a significant increase (68 %) in height due to the application of humic material in the 2 g C kg^{-1} treatment (Table 6). *Festuca pratensis, P. pratensis,* and *B. juncea* shoot heights were not significantly different in the 2 g C kg^{-1} treatment relative to the control but a significant decrease in shoot height was observed in the 4 g C kg^{-1} treatment. *Agropyron trachycaulum* was the only species to have no significant differences between the amended treatments relative to the control but the 2 g C kg^{-1} treatment was significantly taller than the 4 g C kg^{-1} treatment.

4.2.1.3 Photosynthetic Pigments

Medicago sativa, *P. pratensis* and *A. trachycaulum* showed no significant changes in chlorophyll a and b contents between the treatments (Table 7). However, significantly lower chlorophyll a and b contents relative to the control were observed in the 2 g C kg^{-1} and 4 g C kg^{-1} treatments for *B. Juncea* while only in the 4 g C kg^{-1} treatment for *F. pratensis*. Carotenoid content was unaffected by the amendment addition in *A. trachycaulum*, *M. sativa* and *B. juncea* (Table 7). However, *F. pratensis* carotenoid content was 29 % lower in the 4 g C kg^{-1} tailings treatment relative to the control. *Poa pratensis* also exhibited a significant decrease in carotenoid content with a 31 % reduction in the 2 g C kg^{-1} treatment.

Table 6. Dry root and shoot biomass, root to shoot ratio, and shoot height of *Medicago sativa, Agropyron trachycaulum, Festuca pratensis, Brassica juncea a* nd *Poa pratensis* after a 21 day growing period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	Root to shoot ratio	Height (cm)
Medicago Sativa				
0 g C kg^{-1} tailings (Control)	19.5 ± 1.83 ^{a}	8.0 ± 0.75 ^{a}	0.43 ± 0.06 ^{a}	2.2 ± 0.1 ^{a}
2 g C kg^{-1} tailings	46.1 ± 2.57 ^{c}	16.8 ± 1.22 ^{c}	0.37 ± 0.04 ^{a}	3.8 ± 0.5 ^{c}
4 g C kg^{-1} tailings	32.2 ± 1.77 ^{b}	12.8 ± 0.22 ^{b}	0.40 ± 0.03 ^{a}	2.8 ± 0.3 ^{b}
Agropyron trachycaulum				
0 g C kg^{-1} tailings (Control)	8.0 ± 0.66 ^{a}	5.3 ± 0.62 ^{a}	0.66 ± 0.05 ^{a}	12.6 ± 0.5 ^{ab}
2 g C kg^{-1} tailings	10.3 ± 1.66 ^{a}	6.5 ± 0.84 ^{a}	0.64 ± 0.08 ^{a}	13.6 ± 0.8 ^{b}
4 g C kg^{-1} tailings	6.4 ± 0.81 ^{a}	4.5 ± 0.56 ^{a}	0.70 ± 0.03 ^{a}	12.1 ± 0.7 ^{a}
Festuca pratensis				
0 g C kg^{-1} tailings (Control)	32.3 ± 2.50 ^{c}	9.4 ± 1.02 ^{b}	0.30 ± 0.03 ^{a}	20.2 ± 0.8 ^{b}
2 g C kg^{-1} tailings	26.7 ± 1.90 ^{b}	9.2 ± 0.53 ^{b}	0.35 ± 0.03 ^{a}	20.3 ± 0.4 ^{b}
4 g C kg^{-1} tailings	12.5 ± 0.90 ^{a}	5.5 ± 0.50 ^{a}	0.45 ± 0.01 ^{b}	14.6 ± 0.2 ^{a}
Brassica juncea				
0 g C kg^{-1} tailings (Control)	144.1 ± 7.35 ^{c}	7.8 ± 1.60 ^{a}	0.05 ± 0.01 ^{a}	4.9 ± 0.3 ^{b}
2 g C kg^{-1} tailings	115.6 ± 8.07 ^{b}	7.5 ± 1.29 ^{a}	0.06 ± 0.01 ^{a}	4.6 ± 0.2 ^{b}
4 g C kg^{-1} tailings	92.8 ± 9.37 ^{a}	6.1 ± 0.46 ^{a}	0.07 ± 0.01 ^{a}	3.8 ± 0.2 ^{a}
Poa pratensis				
0 g C kg^{-1} tailings (Control)	2.2 ± 0.16 ^{a}	0.8 ± 0.12 ^{a}	0.33 ± 0.06 ^{a}	7.4 ± 0.1 ^{b}
2 g C kg^{-1} tailings	2.6 ± 0.47 ^{a}	0.7 ± 0.26 ^{a}	0.25 ± 0.08 ^{a}	7.3 ± 0.4 ^{b}
4 g C kg^{-1} tailings	1.7 ± 0.44 ^{a}	0.6 ± 0.25 ^{a}	0.46 ± 0.13 ^{a}	6.2 ± 0.4 ^{a}

Table 7. Chlorophyll and Carotenoid content of leaf tissue of *Medicago sativa, Agropyron trachycaulum, Festuca pratensis, Brassica juncea* and *Poa pratensis* after a 21 day growing period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Chlorophyll a (mg g^{-1} fresh weight)	Chlorophyll b (mg g^{-1} fresh weight)	Carotenoids (mg g^{-1} fresh weight)
Medicago Sativa			
0 g C kg^{-1} tailings (Control)	1.30 ± 0.15 ^{a}	0.47 ± 0.03 ^{a}	0.038 ± 0.008 ^{a}
2 g C kg^{-1} tailings	1.31 ± 0.07 ^{a}	0.43 ± 0.02 ^{a}	0.042 ± 0.004 ^{a}
4 g C kg^{-1} tailings	1.28 ± 0.05 ^{a}	0.44 ± 0.03 ^{a}	0.036 ± 0.001 ^{a}
Agropyron trachycaulum			
0 g C kg^{-1} tailings (Control)	1.76 ± 0.14 ^{a}	0.52 ± 0.05 ^{a}	0.075 ± 0.004 ^{a}
2 g C kg^{-1} tailings	1.94 ± 0.36 ^{a}	0.54 ± 0.10 ^{a}	0.080 ± 0.013 ^{a}
4 g C kg^{-1} tailings	2.11 ± 0.43 ^{a}	0.63 ± 0.11 ^{a}	0.087 ± 0.019 ^{a}
Festuca pratensis			
0 g C kg^{-1} tailings (Control)	1.96 ± 0.16 ^{a}	0.56 ± 0.05 ^{a}	0.088 ± 0.070 ^{b}
2 g C kg^{-1} tailings	1.90 ± 0.14 ^{a}	0.55 ± 0.04 ^{a}	0.085 ± 0.005 ^{b}
4 g C kg^{-1} tailings	1.28 ± 0.34 ^{b}	0.35 ± 0.09 ^{b}	0.063 ± 0.012 ^{a}
Brassica juncea			
0 g C kg^{-1} tailings (Control)	1.25 ± 0.03 ^{a}	0.41 ± 0.01 ^{a}	0.054 ± 0.002 ^{a}
2 g C kg^{-1} tailings	1.01 ± 0.08 ^{b}	0.34 ± 0.02 ^{b}	0.046 ± 0.005 ^{a}
4 g C kg^{-1} tailings	0.92 ± 0.06 ^{b}	0.31 ± 0.02 ^{b}	0.044 ± 0.004 ^{a}
Poa pratensis			
0 g C kg^{-1} tailings (Control)	2.47 ± 0.31 ^{a}	0.78 ± 0.11 ^{a}	0.106 ± 0.009 ^{a}
2 g C kg^{-1} tailings	1.65 ± 0.15 ^{a}	0.49 ± 0.05 ^{a}	0.074 ± 0.008 ^{b}
4 g C kg^{-1} tailings	1.72 ± 0.18 ^{a}	0.51 ± 0.04 ^{a}	0.083 ± 0.005 ^{ab}

4.2.2 Long Term Growth Chamber Experiment

4.2.2.1 Growth

Growth response of the selected plant species grown in tailings amended with modified humic substances varied according to species and time of sampling. *Medicago sativa* shoot and root biomass at 4 weeks was significantly higher in the 2 g C kg^{-1} (306%) and 4 g C kg^{-1} (223%) treatments relative to the control while in the case of shoot biomass the 2 g C kg^{-1} treatment exhibited the greatest increase in biomass (Table 8a). However, by 12 weeks post seeding no significant differences between the treatments were observed. *Brassica juncea* shoot and root biomass followed a decreasing trend in biomass with amendment rate but by 12 weeks no significant differences were observed between the treatments from the control. The slower growing grass species were thinned at 5 weeks. Shoot and root biomass for *F. pratensis* exhibited decreasing trends in biomass with increasing treatment rate while *A. trachycaulum* root and shoot biomass was lower in 4 g C kg^{-1} treatment relative to the control (Table 8b). However, at 12 weeks shoot and root biomass for *A. trachycaulum* and *F. pratensis* was lower in the 2 g C kg^{-1} and 4 g C kg^{-1} treatments relative to the control with no difference between the treatments with the exception of root biomass in the 4 g C kg^{-1} treatment for *F. pratensis*. Root to shoot ratio for *M. sativa* was significantly increased in the 2 g C kg^{-1} and 4 g C kg^{-1} treatments respectively relative to the control at 4 weeks (Table 8a). At 12 weeks an increase in the root to shoot ratio in the 2 g C kg^{-1} was observed. No differences in root to shoot ratio were observed among the treatment for *B. juncea* at 4 weeks. At 12 weeks, *B. juncea* root to shoot ratio in the 2 g C kg^{-1} treatment was significantly greater than the control. *Agropyron trachycaulum* root to shoot ratio did not differ among the treatments at 5 weeks while at 12 weeks both the 2 g C kg^{-1} and 4 g C kg^{-1} treatments were significantly increased by 51 % and 41 % respectively (Table 8b). The root to shoot ratio for *F. pratensis* at 5 weeks was significantly higher in the 4 g C kg^{-1} treatment relative to the control while at 12 weeks the root to shoot ratio was significantly lower in the 4 g C kg^{-1} treatment relative to the control. *Medicago sativa* shoot height at 4 weeks was stimulated in the amended treatments while at 12 weeks no significant differences were observed between the treatments (Table 8a). *Brassica juncea* shoot height exhibited

Table 8a. Shoot and root biomass, root to shoot ratio and height of *Medicago sativa and Brassica juncea* at 4 and 12 week post seeding in mine tailings amended with humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Shoot biomass (g dry weight)	Root biomass (g dry weight)	Root to shoot ratio (dry weight ratio)	Height (cm)
Medicago Sativa				
4 weeks				
0 g C kg^{-1} tailings (Control)	0.029 ± 0.008 ^{a}	0.005 ± 0.001 ^{a}	0.280 ± 0.042 ^{a}	2.6 ± 0.2 ^{a}
2 g C kg^{-1} tailings	0.088 ± 0.009 ^{c}	0.009 ± 0.001 ^{b}	0.111 ± 0.020 ^{b}	8.9 ± 1.3 ^{b}
4 g C kg^{-1} tailings	0.064 ± 0.002 ^{b}	0.010 ± 0.001 ^{b}	0.154 ± 0.013 ^{b}	7.0 ± 0.7 ^{b}
12 weeks				
0 g C kg^{-1} tailings (Control)	1.543 ± 0.134 ^{a}	1.668 ± 0.117 ^{a}	1.058 ± 0.064 ^{ab}	29.1 ± 3.1 ^{a}
2 g C kg^{-1} tailings	1.981 ± 0.115 ^{a}	2.131 ± 0.258 ^{a}	1.134 ± 0.064 ^{b}	32.3 ± 2.3 ^{a}
4 g C kg^{-1} tailings	1.794 ± 0.126 ^{a}	1.493 ± 0.111 ^{a}	0.885 ± 0.077 ^{a}	28.7 ± 2.6 ^{a}
Brassica juncea				
4 weeks				
0 g C kg^{-1} tailings (Control)	0.335 ± 0.037 ^{c}	0.021 ± 0.004 ^{c}	0.064 ± 0.006 ^{a}	3.5 ± 0.2 ^{ab}
2 g C kg^{-1} tailings	0.230 ± 0.019 ^{b}	0.012 ± 0.001 ^{b}	0.054 ± 0.008 ^{a}	3.9 ± 0.2 ^{b}
4 g C kg^{-1} tailings	0.098 ± 0.014 ^{a}	0.005 ± 0.001 ^{a}	0.052 ± 0.003 ^{a}	2.9 ± 0.3 ^{a}
12 weeks				
0 g C kg^{-1} tailings (Control)	4.003 ± 0.284 ^{a}	0.988 ± 0.057 ^{a}	0.245 ± 0.021 ^{ab}	12.1 ± 1.9 ^{a}
2 g C kg^{-1} tailings	3.853 ± 0.439 ^{a}	1.015 ± 0.093 ^{a}	0.275 ± 0.012 ^{b}	13.8 ± 2.6 ^{a}
4 g C kg^{-1} tailings	3.529 ± 0.738 ^{a}	0.752 ± 0.178 ^{a}	0.202 ± 0.017 ^{a}	10.6 ± 1.4 ^{a}

Table 8b. Shoot and root biomass, root to shoot ratio and height of *Agropyron trachycaulum* and *Festuca pratensis* at 5 and 12 weeks post seeding in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Shoot biomass (g dry weight)	Root biomass (g dry weight)	Root to shoot ratio (dry weight ratio)	Height (cm)
Agropyron trachycaulum				
5 weeks				
0 g C kg^{-1} tailings (Control)	0.067 ± 0.014 ^{b}	0.017 ± 0.008 ^{b}	0.291 ± 0.058 ^{a}	17.9 ± 1.8 ^{a}
2 g C kg^{-1} tailings	0.052 ± 0.005 ^{ab}	0.014 ± 0.004 ^{ab}	0.273 ± 0.042 ^{a}	17.5 ± 1.5 ^{a}
4 g C kg^{-1} tailings	0.039 ± 0.002 ^{a}	0.009 ± 0.002 ^{a}	0.244 ± 0.032 ^{a}	14.2 ± 1.2 ^{a}
12 weeks				
0 g C kg^{-1} tailings (Control)	1.274 ± 0.171 ^{b}	0.656 ± 0.133 ^{b}	0.527 ± 0.052 ^{a}	32.9 ± 1.4 ^{b}
2 g C kg^{-1} tailings	0.506 ± 0.058 ^{a}	0.398 ± 0.048 ^{a}	0.798 ± 0.045 ^{b}	28.9 ± 1.6 ^{a}
4 g C kg^{-1} tailings	0.418 ± 0.099 ^{a}	0.223 ± 0.013 ^{a}	0.742 ± 0.051 ^{b}	26.1 ± 2.3 ^{a}
Festuca pratensis				
5 weeks				
0 g C kg^{-1} tailings (Control)	0.302 ± 0.029 ^{c}	0.053 ± 0.012 ^{c}	0.172 ± 0.023 ^{a}	32.9 ± 1.4 ^{b}
2 g C kg^{-1} tailings	0.140 ± 0.036 ^{b}	0.024 ± 0.011 ^{b}	0.181 ± 0.049 ^{a}	31.1 ± 1.7 ^{b}
4 g C kg^{-1} tailings	0.069 ± 0.033 ^{a}	0.014 ± 0.011 ^{a}	0.234 ± 0.055 ^{b}	18.3 ± 2.3 ^{a}
12 weeks				
0 g C kg^{-1} tailings (Control)	2.213 ± 0.593 ^{b}	0.779 ± 0.086 ^{c}	0.438 ± 0.098 ^{b}	44.7 ± 1.9 ^{b}
2 g C kg^{-1} tailings	0.860 ± 0.198 ^{a}	0.395 ± 0.123 ^{b}	0.386 ± 0.084 ^{ab}	34.8 ± 2.2 ^{a}
4 g C kg^{-1} tailings	0.479 ± 0.089 ^{a}	0.147 ± 0.050 ^{a}	0.251 ± 0.028 ^{a}	31.9 ± 3.9 ^{a}

no significant differences between the amended treatments and the control at 4 weeks or 12 weeks (Table 8a). No differences in *A. trachycaulum* shoot height were observed at 5 weeks but at 12 weeks the 2 g C kg^{-1} and 4 g C kg^{-1} treatments were 12 % and 21 % shorter, respectively in comparison to the control (Table 8b). *Festuca pratensis* shoot height at 5 weeks was reduced by 44 % in 4 g C kg^{-1} treatment relative to the control while at 12 weeks both the 2 g C kg^{-1} (22 %) and the 4 g C kg^{-1} (29 %) were significantly shorter than the control. Leaf area for *B. juncea* at 4 weeks was 64 % lower in the 4 g C kg^{-1} treatment relative to the control (Table 9). At 12 weeks no significant differences in leaf area were observed.

4.2.2.2 Photosynthetic Pigments

Medicago sativa showed no significant changes in chlorophyll a and b content in any of the treatments at both 5 and 12 weeks (Table 10a). Chlorophyll a and b content in *B. juncea* was significantly reduced in the 4 g C kg^{-1} treatment by 21 % and 30 % at both 5 weeks and 12 weeks. A 28 % decrease in chlorophyll b was also observed in the 2 g C kg^{-1} treatment at 12 weeks post seeding. *Agropyron trachycaulum* and *F. pratensis* had lower chlorophyll a and b content in the 2 g C kg^{-1} and 4 g C kg^{-1} treatments relative to the control at 5 weeks post seeding (Table 10b). At 12 weeks though, *A. trachycaulum* exhibited no significant differences in chlorophyll a or b content among the treatments. *Festuca pratensis* had a reduction of 33 % and 32 % in chlorophyll a and b content respectively in the 4 g C treatment relative to the control.

Medicago sativa exhibited no significant differences in carotenoid content between the treatments relative to the control at 5 weeks but at 12 weeks carotenoid content was 20 % and 26 % lower than the control in both the 2 g C kg^{-1} and 4 g C kg^{-1} treatments respectively (Table 10a). *Brassica juncea* and *A. trachycaulum* had significant decreases in carotenoid content after 5 weeks in the 4 g C kg^{-1} treatment while after 12 weeks no significant differences among the treatments were observed (Table 10a and 10b). *Festuca pratensis* exhibited reductions in carotenoid content at 5 weeks in both the 2 g C kg^{-1} and 4 g C kg^{-1} treatments with values of 28.7 $\mu g\ g^{-1}$ fresh weight and 31.5 $\mu g\ g^{-1}$ fresh weight respectively relative to the control of 55.8 $\mu g\ g^{-1}$ fresh weight while after 12 weeks there were no differences among the treatments.

Table 9. Total leaf area of *Brassica juncea* at 4 and 12 week growth periods in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$). Leaf area was difficult to measure accurately and therefore no values are reported for the other species.

	Leaf Area ($cm^{2)}$
Brassica juncea	
4 weeks	
0 g C kg tailings (Control)	77.9 ± 7.6 [b]
2 g C kg^{-1} tailings	62.0 ± 5.7 [b]
4 g C kg^{-1} tailings	28.2 ± 5.1 [a]
12 weeks	
0 g C kg tailings (Control)	364 ± 45 [a]
2 g C kg^{-1} tailings	343 ± 51 [a]
4 g C kg^{-1} tailings	349 ± 51 [a]

Table 10a. Chlorophyll and Carotenoid content in leaf tissue of *Medicago sativa* and *Brassica juncea* at 5 and 12 weeks post seeding in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Chlorophyll a (mg g^{-1} fresh weight)	Chlorophyll b (mg g^{-1} fresh weight)	Carotenoids (µg g^{-1} fresh weight)
Medicago Sativa			
5 weeks			
0 g C kg^{-1} tailings (Control)	1.50 ± 0.35 ^{a}	0.71 ± 0.17 ^{a}	63.9 ± 10.2 ^{a}
2 g C kg^{-1} tailings	1.83 ± 0.18 ^{a}	0.78 ± 0.14 ^{a}	75.1 ± 4.3 ^{a}
4 g C kg^{-1} tailings	1.91 ± 0.09 ^{a}	0.62 ± 0.05 ^{a}	68.9 ± 5.7 ^{a}
12 weeks			
0 g C kg^{-1} tailings (Control)	2.08 ± 0.17 ^{a}	0.81 ± 0.08 ^{a}	92.4 ± 7.2 ^{b}
2 g C kg^{-1} tailings	1.91 ± 0.14 ^{a}	0.63 ± 0.05 ^{a}	74.1 ± 5.6 ^{a}
4 g C kg^{-1} tailings	1.76 ± 0.15 ^{a}	0.63 ± 0.05 ^{a}	68.6 ± 5.8 ^{a}
Brassica juncea			
5 weeks			
0 g C kg^{-1} tailings (Control)	1.26 ± 0.09 ^{b}	0.42 ± 0.04 ^{b}	50.9 ± 3.7 ^{b}
2 g C kg^{-1} tailings	1.24 ± 0.07 ^{b}	0.42 ± 0.02 ^{b}	54.8 ± 2.3 ^{b}
4 g C kg^{-1} tailings	0.63 ± 0.19 ^{a}	0.21 ± 0.05 ^{a}	31.3 ± 6.7 ^{a}
12 weeks			
0 g C kg^{-1} tailings (Control)	1.04 ± 0.10 ^{b}	0.38 ± 0.03 ^{b}	48.9 ± 4.5 ^{a}
2 g C kg^{-1} tailings	0.80 ± 0.09 ^{ab}	0.28 ± 0.03 ^{a}	39.5 ± 4.1 ^{a}
4 g C kg^{-1} tailings	0.74 ± 0.07 ^{a}	0.24 ± 0.03 ^{a}	37.1 ± 3.7 ^{a}

Table. 10b Chlorophyll and Carotenoid content in leaf tissue of *Agropyron trachycaulum* and *Festuca pratensis* at 5 and 12 weeks post seeding in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Chlorophyll a (mg g^{-1} fresh weight)	Chlorophyll b (mg g^{-1} fresh weight)	Carotenoids (µg g^{-1} fresh weight)
Agropyron trachycaulum			
5 weeks			
0 g C kg^{-1} tailings (Control)	1.93 ± 0.28 ^{b}	0.55 ± 0.08 ^{b}	76.8 ± 13.3 ^{b}
2 g C kg^{-1} tailings	1.15 ± 0.09 ^{a}	0.32 ± 0.03 ^{a}	49.2 ± 3.9 ^{ab}
4 g C kg^{-1} tailings	1.27 ± 0.21 ^{a}	0.33 ± 0.05 ^{a}	57.5 ± 9.8 ^{a}
12 weeks			
0 g C kg^{-1} tailings (Control)	1.44 ± 0.32 ^{a}	0.47 ± 0.10 ^{a}	57.6 ± 11.5 ^{a}
2 g C kg^{-1} tailings	1.48 ± 0.14 ^{a}	0.43 ± 0.05 ^{a}	62.4 ± 5.6 ^{a}
4 g C kg^{-1} tailings	1.63 ± 0.18 ^{a}	0.45 ± 0.05 ^{a}	67.9 ± 7.7 ^{a}
Festuca pratensis			
5 weeks			
0 g C kg^{-1} tailings (Control)	1.37 ± 0.08 ^{b}	0.39 ± 0.02 ^{b}	55.8 ± 3.2 ^{b}
2 g C kg^{-1} tailings	0.80 ± 0.12 ^{a}	0.20 ± 0.03 ^{a}	38.7 ± 4.5 ^{a}
4 g C kg^{-1} tailings	0.53 ± 0.07 ^{a}	0.15 ± 0.02 ^{a}	31.5 ± 5.1 ^{a}
12 weeks			
0 g C kg^{-1} tailings (Control)	0.65 ± 0.07 ^{b}	0.27 ± 0.03 ^{b}	36.0 ± 4.0 ^{a}
2 g C kg^{-1} tailings	0.56 ± 0.07 ^{b}	0.22 ± 0.03 ^{ab}	34.2 ± 2.7 ^{a}
4 g C kg^{-1} tailings	0.44 ± 0.07 ^{a}	0.18 ± 0.03 ^{a}	30.5 ± 5.0 ^{a}

4.2.2.3 Anthocyanin

Anthocyanin content for *M. sativa* and *B. juncea* was not significantly different among the treatments at 12 weeks (Figure 10).

4.2.2.4 Electrolyte Leakage

At 4 weeks post seeding *M. sativa* and *B. juncea* exhibited 62 % and 212 % greater electrolyte leakage in the 4 g C kg^{-1} treatment relative to the control (Table 11a). No significant differences in electrolyte leakage between the treatments and the control were observed at 12 weeks for *M. sativa.* At 12 weeks, *B. juncea* also showed no significant differences between the amended treatments and the control but electrolyte leakage was significantly greater in the 4 g C kg^{-1} treatment relative to the 2 g C kg^{-1} treatment. At 12 weeks, both *M. sativa* and *B. juncea* leaf tissue electrolyte leakage in the 4 g C kg^{-1} treatment was significantly lower than the measurements taken at 4 weeks. *Agropyron trachycaulum*, and *F. pratensis* leaf tissue, only measured at 12 weeks, exhibited no significant differences in electrolyte leakage between the treatments (Table 11b).

4.2.2.5 Transpiration

Medicago sativa, A. trachycaulum, and *F. pratensis* tissue exhibited no differences in transpiration between the treatments (Table 12). *Brassica juncea* had significantly lower leaf transpiration in the 2 g C kg^{-1} and 4 g C kg^{-1} treatments with values of 3.36 mmol H_2O s^{-1} and 3.98 mmol H_2O s^{-1} respectively relative to the control value of 5.43 mmol H_2O s^{-1}.

4.2.3 Field Experiments

4.2.3.1 Seedling Emergence

Brassica juncea had over 70 % emergence in all treatments at 2 weeks post seeding with no significant differences between the treatments. At 4 weeks all treatments had a reduced number of seedlings compared to 2 weeks (Figure 11). In addition at 4 weeks the 3 g C kg^{-1} treatment was significantly lower in emergence compared to the control. *Poa pratensis* exhibited limited emergence (< 3%) at both 2 and 4 weeks post

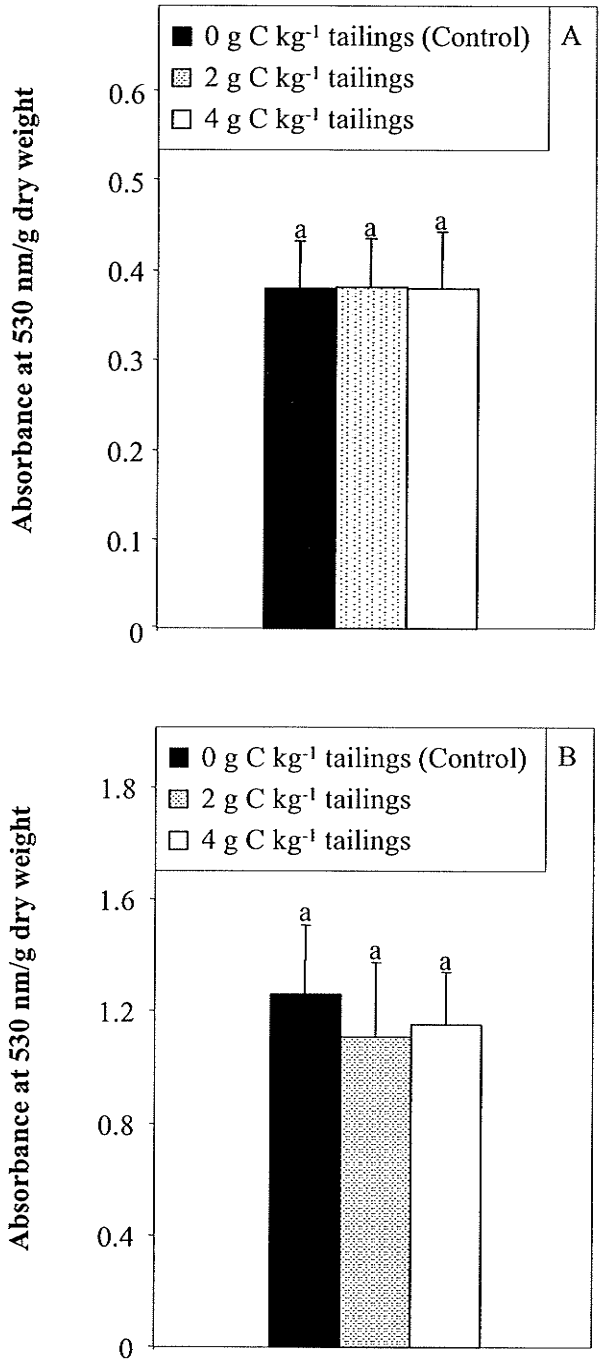


Figure 10. Anthocyanin absorbance/gram dry tissue weight in A) *Medicago sativa* and B) *Brassica juncea* stem tissue after a 12 week growth period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

Table 11a. Electrolyte leakage of leaf tissue of *Medicago sativa* and *Brassica juncea* after a 4 and 12 week growth period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Ion leakage %
Medicago Sativa	
4 weeks	
0 g C kg^{-1} tailings (Control)	6.10 ± 0.91 [a]
2 g C kg^{-1} tailings	6.64 ± 0.71 [a]
4 g C kg^{-1} tailings	9.90 ± 1.59 [b]
12 weeks	
0 g C kg^{-1} tailings (Control)	3.69 ± 0.44 [a]
2 g C kg^{-1} tailings	2.71 ± 0.33 [a]
4 g C kg^{-1} tailings	3.86 ± 0.48 [a]
Brassica juncea	
4 weeks	
0 g C kg^{-1} tailings (Control)	6.26 ± 0.40 [a]
2 g C kg^{-1} tailings	6.54 ± 0.46 [a]
4 g C kg^{-1} tailings	19.51 ± 4.40 [b]
12 weeks	
0 g C kg^{-1} tailings (Control)	4.48 ± 1.27 [ab]
2 g C kg^{-1} tailings	2.97 ± 0.64 [a]
4 g C kg^{-1} tailings	5.50 ± 1.21 [b]

Table 11b. Electrolyte leakage of leaf tissue of *Agropyron trachycaulum* and *Festuca pratensis* after a 12 week growth period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Ion leakage %
Agropyron trachycaulum	
12 weeks	
0 g C kg^{-1} tailings (Control)	6.03 ± 0.69 [a]
2 g C kg^{-1} tailings	4.99 ± 0.59 [a]
4 g C kg^{-1} tailings	6.07 ± 0.55 [a]
Festuca pratensis	
12 weeks	
0 g C kg^{-1} tailings (Control)	5.47 ± 1.02 [a]
2 g C kg^{-1} tailings	4.66 ± 0.86 [a]
4 g C kg^{-1} tailings	5.36 ± 0.42 [a]

Table 12. Leaf transpiration in *Medicago sativa, Brassica juncea, Agropyron trachycaulum* and *Festuca pratensis* after a 12 week grow period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Transpiration (mmol H_20 s^{-1})
Medicago Sativa	
0 g C kg^{-1} tailings (Control)	2.53 ± 0.51 [a]
2 g C kg^{-1} tailings	2.32 ± 0.37 [a]
4 g C kg^{-1} tailings	2.22 ± 0.42 [a]
Brassica juncea	
0 g C kg^{-1} tailings (Control)	5.43 ± 0.80 [a]
2 g C kg^{-1} tailings	3.36 ± 0.75 [b]
4 g C kg^{-1} tailings	3.98 ± 0.70 [b]
Agropyron trachycaulum	
0 g C kg^{-1} tailings (Control)	4.11 ± 0.54 [a]
2 g C kg^{-1} tailings	3.31 ± 0.44 [a]
4 g C kg^{-1} tailings	3.23 ± 0.29 [a]
Festuca pratensis	
0 g C kg^{-1} tailings (Control)	1.71 ± 0.29 [a]
2 g C kg^{-1} tailings	2.07 ± 0.34 [a]
4 g C kg^{-1} tailings	2.16 ± 0.46 [a]

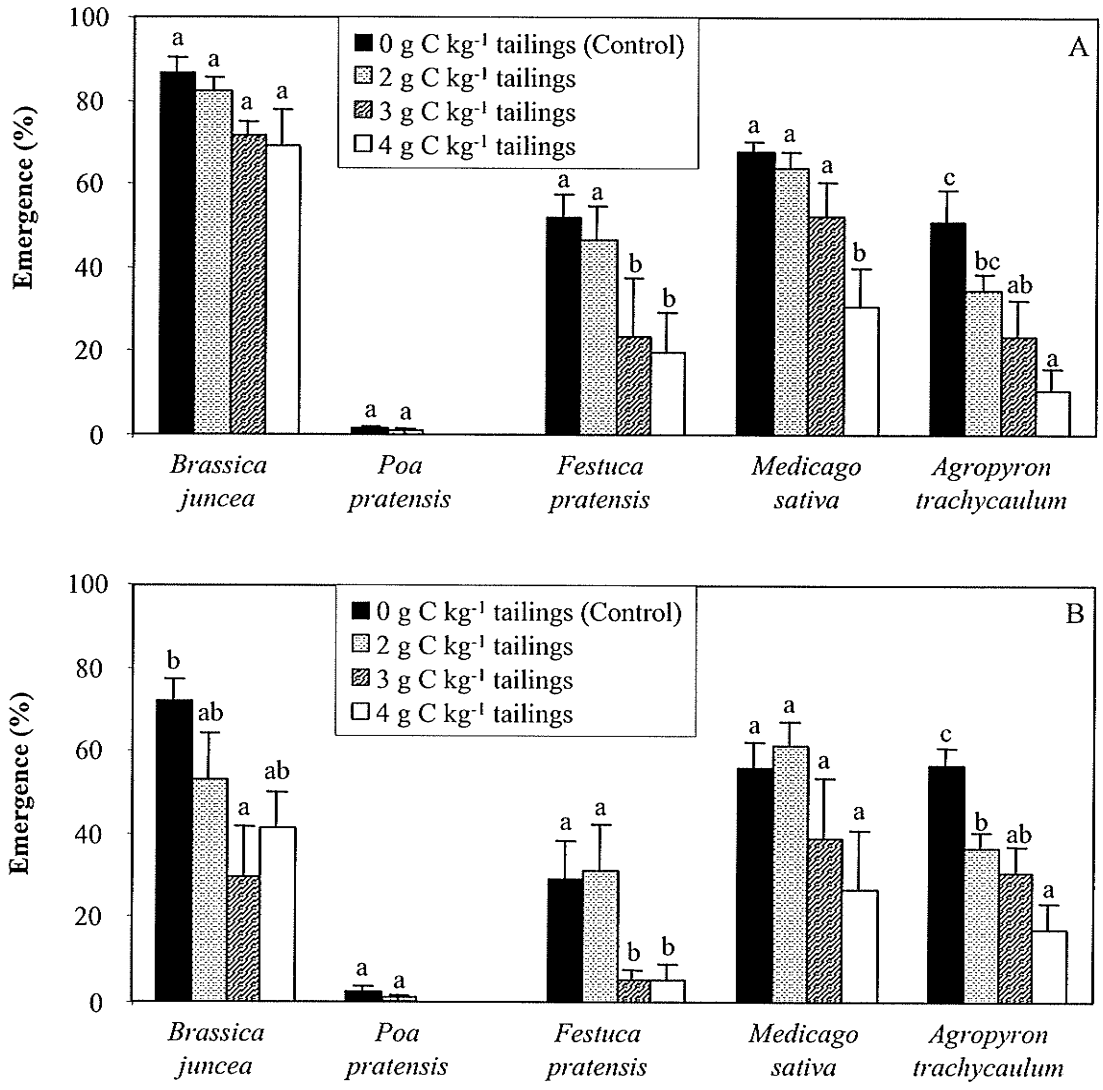


Figure 11. Field season 2003 seeding emergence at (A) 2 weeks and (B) 4 weeks post seeding in mine tailings amended with modified humic substances (Mean ± SE, n=4). Error bars with different letters indicate a significant difference ($p < 0.05$).

seeding. Emergence of seedlings occurred in the control and 2 g C kg^{-1} treatments at 2 weeks while at 4 weeks seedlings were only observed in the control treatment. *Festuca pratensis* emergence was highest in all treatments at 2 weeks post seeding compared to 4 weeks, with over 50% emergence in both the control and 2 g C kg^{-1} treatment. No significant difference was observed between the 2 g C kg^{-1} and control treatments at either 2 or 4 weeks post seeding. The 3 g C kg^{-1} and 4 g C kg^{-1} treatments were significantly lower than the control at both 2 and 4 weeks with less than 30% emergence. *Medicago sativa* emergence values at 4 weeks were not significantly lower than emergence at 2 weeks post seeding. At 2 weeks post seeding, emergence was not significantly different than the control in the 2 g C kg^{-1} and 3 g C kg^{-1} treatments while emergence in the 4 g C kg^{-1} treatment was significantly lower. However, at 4 weeks no significant difference in emergence among the treatments was observed. *Agropyron trachycaulum* emergence at 4 weeks was not significantly different from 2 weeks. *Agropyron trachycaulum* emergence at 2 and 4 weeks was greatest in the control (>50%). Emergence was significantly reduced in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments at 2 weeks with the lowest emergence values (<20%) observed in the 4 g C kg^{-1} treatment. At 4 weeks emergence in the 2 g C kg^{-1}, 3 g C kg^{-1} and 4 g C kg^{-1} treatments were significantly less than the control with the lowest emergence in the 4 g C kg^{-1} treatment (<30%).

In field season 2004, unlike field season 2003, emergence of seeds at 2 and 4 weeks was not affected by the rate of humic amendment addition for all species (Figure 12). On an individual species basis *A. elongatum* emergence was greater than 60% in all treatments at 2 weeks post seeding. At 4 weeks post seeding reductions in emergence were observed in both the 2 g C kg^{-1} treatment compared to 2 weeks post seeding. *Festuca rubra* had emergence of less than 40% in all treatments at 2 weeks while increases in emergence were observed in 2 g C kg^{-1} treatment at 4 weeks. *Festuca pratensis* and *M. sativa* showed no significant changes in emergence from 2 to 4 weeks post seeding. *Agropyron trachycaulum* emergence was significantly higher in the 2 g C kg^{-1} treatment at 4 weeks compared to emergence measured at 2 weeks post seeding.

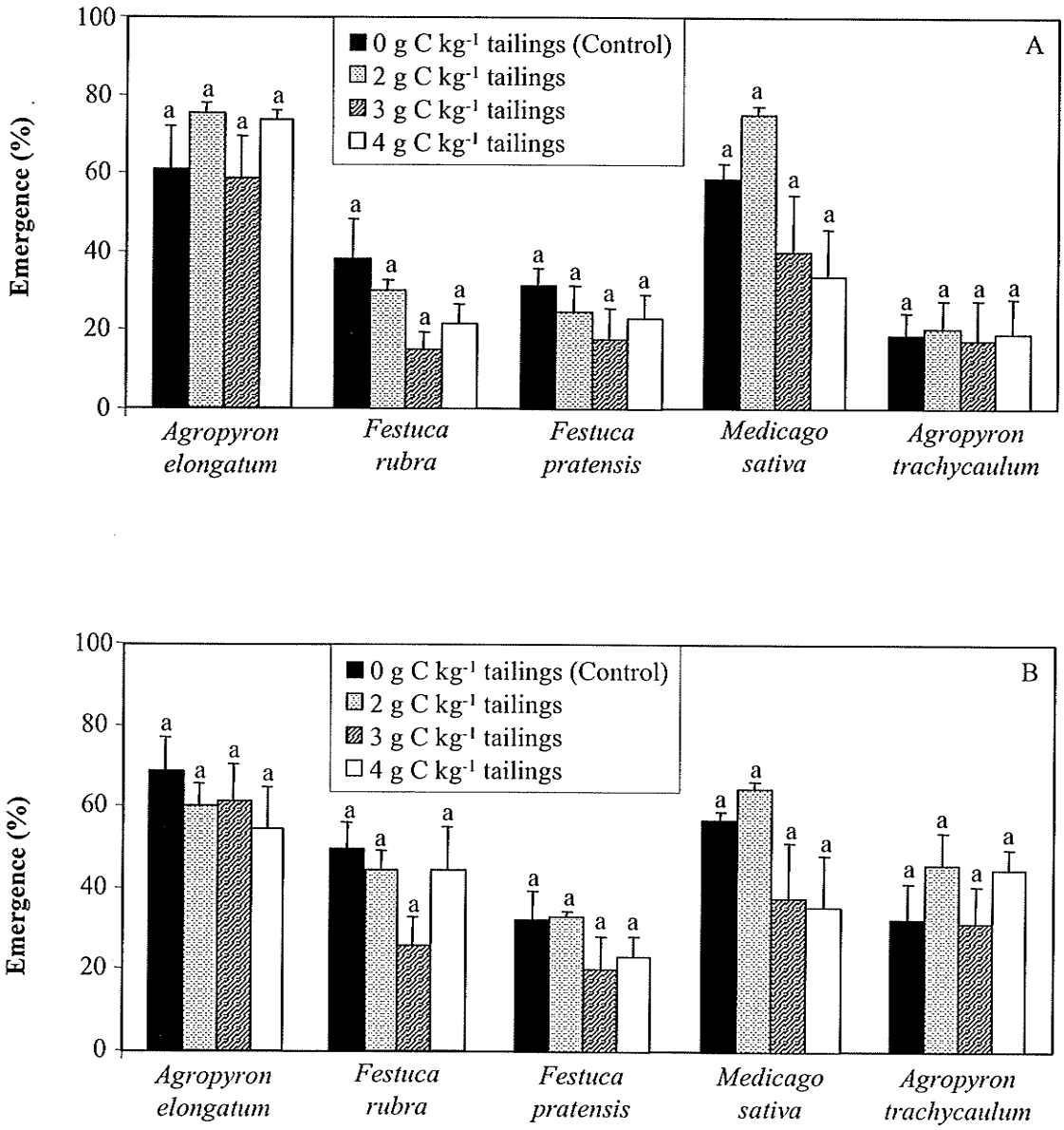


Figure 12. Field season 2004 seeding emergence at (A) 2 weeks and (B) 4 weeks post seeding in mine tailings amended with modified humic substances (Mean ± SE, n=4). Error bars with different letters indicate a significant difference ($p < 0.05$).

4.2.3.2 Survival

In field season 2003 no plants of either *B. juncea or P. pratensis* survived until harvest (Figure 13). *Brassica juncea* was completely defoliated by members of the *Phyllotreta* genus "Flee Beetles" while *P. pratensis* suffered from poor seed viability and reduced seedling emergence. Survival in *F. pratensis, M sativa* and *A. trachycaulum* followed similar patterns as seedling emergence with an overall reduction in number of plants surviving until harvest from the initial emergence measurements. *Festuca pratensis* and *A. trachycaulum* had significantly lower survival rate in the 4 g C kg^{-1} treatment relative the control. *Medicago sativa* survival exhibited no significant differences among the treatments.

In field season 2004, the survival at harvest for all species was not affected by the rate of humic amendment addition (Figure 13). Survival of *A. elongatum* was less than 37 % with *F. rubra* and *M. sativa* survival less than 27 % and 31 % respectively. *Festuca pratensis* survival was less than 13 % and survival for *A. trachycaulum* was less than 32 % in all treatments. A general trend in decreased survivorship at harvest compared to emergence values was also observed.

4.2.3.3 Growth

Medicago sativa shoot biomass was significantly greater in the 4 g C kg^{-1} treatment with 51% greater biomass than the control in field season 2003. However, no differences in root biomass were observed between the amended treatments (Table 13). *Agropyron trachycaulum* and *F. pratensis* dry shoot and root biomass exhibited no significant differences between the 2 g C kg^{-1} treatment and the control. *Agropyron trachycaulum* had significantly lower shoot biomass in both the 3 g C kg^{-1} (33%) and 4 g C kg^{-1} (75%) treatments relative to the control. Root biomass followed a similar trend where both the 3 g C kg^{-1} and 4 g C kg^{-1} treatments were significantly lower than the control. *Festuca pratensis* survival was low in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments preventing accurate statistical analysis of biomass. Root to shoot ratio for *M. sativa* was highest in the control compared to the amended treatments (Table 13). No significant differences in root to shoot ratio were observed in *A. trachycaulum* or *F. pratensis.*

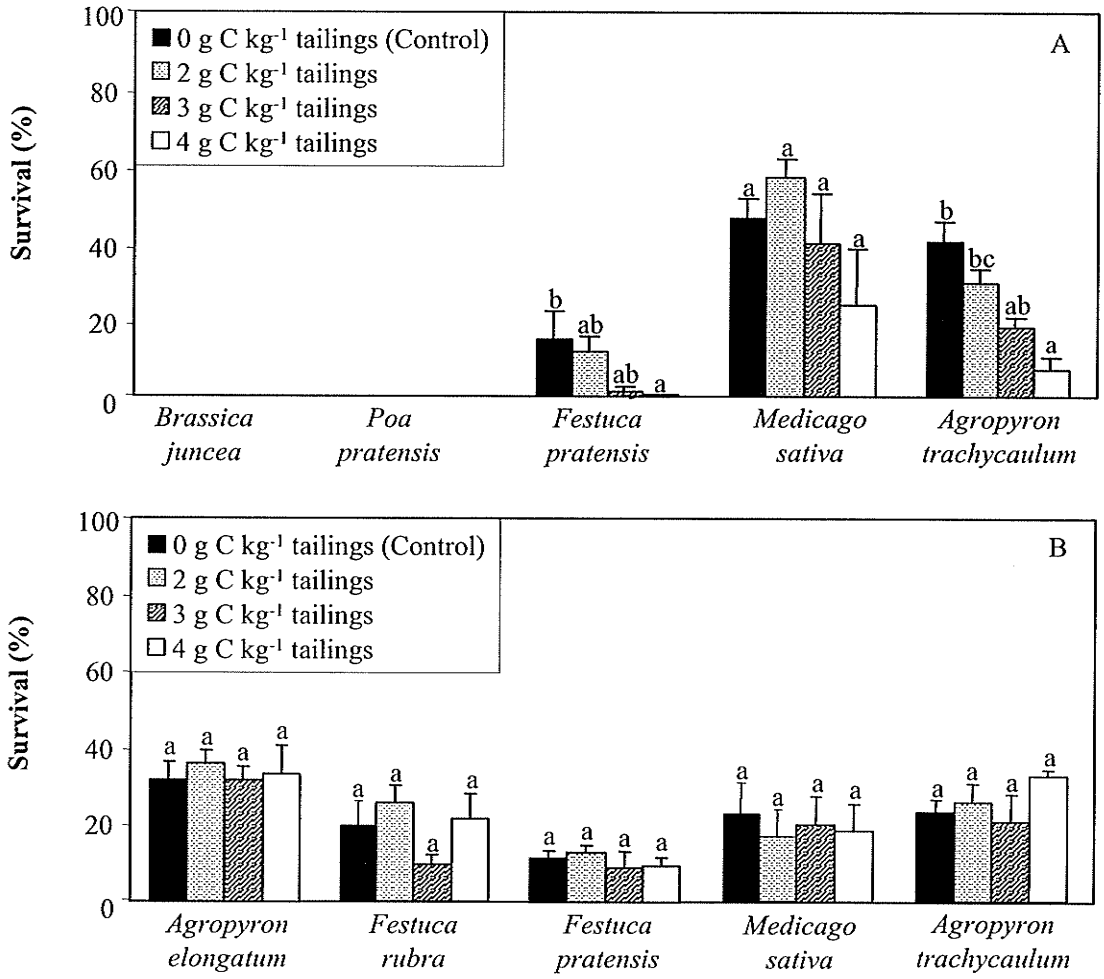


Figure 13. Survival at 3 months post seeding in mine tailings amended with modified humic substances in (A) field season 2003 (B) field season 2004 (Mean ± SE, n = 4). Error bars with different letters indicate a significant difference ($p < 0.05$).

Table 13. Field season 1 dry root and shoot biomass, root to shoot ratio, and shoot height of *Medicago sativa* , *Agropyron trachycaulum* and *Festuca pratensis* after a 3 month growing period in mine tailings amended with humic materials (mean ± SE, n = 4). Different letters represent significant differences (p = 0.05). * indicates insuficient data to conduct statistical analysis (n = 2).

	Shoot biomass g dry weight	Root biomass g dry weight	Root to shoot ratio	Height cm
Medicago Sativa				
0 g C/kg tailings	0.716 ± 0.086 ^{a}	0.500 ± 0.057 ^{a}	0.841 ± 0.091 ^{b}	14.5 ± 0.4 ^{a}
2 g C/kg tailings	0.995 ± 0.080 ^{ab}	0.493 ± 0.041 ^{a}	0.518 ± 0.023 ^{a}	15.8 ± 0.9 ^{a}
3 g C/kg tailings	0.838 ± 0.229 ^{ab}	0.465 ± 0.090 ^{a}	0.605 ± 0.052 ^{a}	16.4 ± 0.9 ^{a}
4 g C/kg tailings	1.085 ± 0.171 ^{b}	0.477 ± 0.088 ^{a}	0.437 ± 0.028 ^{a}	15.7 ± 2.1 ^{a}
Agropyron trachycaulum				
0 g C/kg tailings	0.597 ± 0.079 ^{c}	0.291 ± 0.039 ^{c}	0.540 ± 0.068 ^{a}	23.4 ± 2.9 ^{b}
2 g C/kg tailings	0.634 ± 0.077 ^{c}	0.299 ± 0.037 ^{c}	0.464 ± 0.038 ^{a}	24.8 ± 1.7 ^{b}
3 g C/kg tailings	0.401 ± 0.060 ^{b}	0.179 ± 0.027 ^{b}	0.508 ± 0.159 ^{a}	24.3 ± 4.6 ^{b}
4 g C/kg tailings	0.148 ± 0.034 ^{a}	0.074 ± 0.017 ^{a}	0.497 ± 0.133 ^{a}	16.2 ± 2.0 ^{a}
Festuca pratensis				
0 g C/kg tailings	1.379 ± 0.221 ^{a}	0.634 ± 0.103 ^{a}	0.540 ± 0.036 ^{a}	17.1 ± 2.2 ^{a}
2 g C/kg tailings	1.355 ± 0.234 ^{a}	0.591 ± 0.115 ^{a}	0.498 ± 0.031 ^{a}	18.3 ± 2.2 ^{a}
3 g C/kg tailings	0.116 ± 0.097 *	0.043 ± 0.031 *	0.508 ± 0.044 *	8.5 ± 3.9 *
4 g C/kg tailings	0.735 ± 0.697 *	0.458 ± 0.444 *	0.473 ± 0.027 *	13.7 ± 6.9 *

Medicago sativa and *F. pratensis* had no significant differences in height between the treatments with average heights of 15.6 cm and 17.7 cm respectively. *Agropyron trachycaulum* had shorter shoots in the 4 g C kg^{-1} treatment (29%) in comparison to the control.

In field season 2004, *M. sativa* shoot biomass was significantly greater (90%) in the 3 g C kg^{-1} treatment relative to the control. In addition *M. sativa* showed no changes in root biomass due to amendment application (Table 14a). In field season 2004, like season 2003, *A. trachycaulum* shoot biomass was significantly lower than the control with 38% and 37% lower biomass in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments respectively. Significant reductions in root biomass (~27%) were also observed in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments relative to the control. *Festuca pratensis* shoot biomass was lower (37 %) in the 4 g C kg^{-1} treatment relative to the control while with no other treatments showed any significant differences from the control. In addition, no significant differences in root biomass was observed between the amended treatments relative to the control. *Agropyron elongatum* and *F. rubra* shoot biomass was not significantly different among the treatments (Table 14b). *Agropyron elongatum* root biomass was significantly higher in the 2 g C kg^{-1} (19%) and 4 g C kg^{-1} (35%) treatment while no significant differences in root biomass among the treatments were observed for *F. rubra*. Root to shoot ratios in amended treatments for *M. sativa*, *A. trachycaulum* and *F. rubra* were not significantly different from the control. *Festuca pratensis* root to shoot ratio was increased relative to the control in the 2 g C kg^{-1} and 4 g C kg^{-1}. The root to shoot ratio for *A. elongatum* was significantly greater in all amended treatments relative to the control with an increase in 2 g C kg^{-1}, 3 g C kg^{-1}, and 4 g C kg^{-1} treatments though no significant differences were observed between the amended treatments (Table 14a). *Agropyron trachycaulum* shoot height averaged 25.5 cm and was not significantly affected by the addition of modified amendment. *Medicago sativa* and *F. pratensis* exhibited changes in height with *M. sativa* showing an increase in the 3 g C kg^{-1} treatment and *F. pratensis* a decrease in the 3 g C kg^{-1} treatment. *Agropyron elongatum* shoot height was also lower in the 3 g C kg^{-1} treatment relative to the control. *Festuca rubra* shoot height was significantly lower in

Table 14a. Field season 2004 dry root and shoot biomass, root to shoot ratio, and shoot height of *Medicago sativa, Agropyron trachycaulum* and *Festuca pratensis* after a 3 month growing period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Shoot biomass (g dry weight)	Root biomass (g dry weight)	Root to shoot ratio	Height (cm)
Medicago Sativa				
0 g C kg^{-1} tailings (Control)	0.634 ± 0.084 ^{a}	0.432 ± 0.056 ^{ab}	0.732 ± 0.051 ^{ab}	21.1 ± 1.0 ^{a}
2 g C kg^{-1} tailings	0.814 ± 0.141 ^{a}	0.480 ± 0.074 ^{ab}	0.861 ± 0.097 ^{b}	21.2 ± 1.6 ^{a}
3 g C kg^{-1} tailings	1.226 ± 0.154 ^{b}	0.662 ± 0.067 ^{b}	0.648 ± 0.048 ^{a}	26.9 ± 1.2 ^{b}
4 g C kg^{-1} tailings	0.707 ± 0.089 ^{a}	0.349 ± 0.048 ^{a}	0.551 ± 0.043 ^{a}	21.3 ± 1.0 ^{a}
Agropyron trachycaulum				
0 g C kg^{-1} tailings (Control)	0.628 ± 0.083 ^{b}	0.221 ± 0.021 ^{b}	0.439 ± 0.024 ^{a}	25.0 ± 1.3 ^{a}
2 g C kg^{-1} tailings	0.562 ± 0.062 ^{b}	0.212 ± 0.021 ^{b}	0.434 ± 0.038 ^{a}	25.1 ± 0.9 ^{a}
3 g C kg^{-1} tailings	0.390 ± 0.044 ^{a}	0.160 ± 0.023 ^{a}	0.410 ± 0.025 ^{a}	24.4 ± 1.3 ^{a}
4 g C kg^{-1} tailings	0.398 ± 0.051 ^{a}	0.161 ± 0.017 ^{a}	0.445 ± 0.021 ^{a}	24.9 ± 1.0 ^{a}
Festuca pratensis				
0 g C kg^{-1} tailings (Control)	0.931 ± 0.096 ^{b}	0.379 ± 0.038 ^{a}	0.438 ± 0.021 ^{a}	22.0 ± 0.7 ^{b}
2 g C kg^{-1} tailings	0.882 ± 0.106 ^{ab}	0.434 ± 0.048 ^{a}	0.519 ± 0.026 ^{b}	20.7 ± 0.9 ^{b}
3 g C kg^{-1} tailings	0.693 ± 0.149 ^{ab}	0.312 ± 0.051 ^{a}	0.470 ± 0.036 ^{ab}	16.8 ± 0.8 ^{a}
4 g C kg^{-1} tailings	0.588 ± 0.063 ^{a}	0.325 ± 0.036 ^{a}	0.578 ± 0.027 ^{b}	20.1 ± 0.6 ^{b}

Table 14b. Field Season 2004 dry root an shoot biomass, root to shoot ratio, and shoot height of *Agropyron elongatum* and *Festuca rubra* after a 3 month growing period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Shoot biomass (g dry weight)	Root biomass (g dry weight)	Root to shoot ratio	Height (cm)
Agropyron elongatum				
0 g C kg^{-1} tailings (Control)	0.978 ± 0.101 ^{a}	0.290 ± 0.026 ^{a}	0.341 ± 0.017 ^{a}	28.6 ± 1.4 ^{b}
2 g C kg^{-1} tailings	0.810 ± 0.063 ^{a}	0.345 ± 0.026 ^{bc}	0.439 ± 0.014 ^{b}	24.1 ± 1.1 ^{ab}
3 g C kg^{-1} tailings	0.749 ± 0.070 ^{a}	0.319 ± 0.025 ^{ab}	0.462 ± 0.016 ^{b}	22.7 ± 0.9 ^{a}
4 g C kg^{-1} tailings	0.891 ± 0.085 ^{a}	0.394 ± 0.037 ^{c}	0.487 ± 0.025 ^{b}	27.8 ± 1.5 ^{b}
Festuca rubra				
0 g C kg^{-1} tailings (Control)	0.327 ± 0.049 ^{a}	0.172 ± 0.035 ^{a}	0.517 ± 0.039 ^{a}	14.3 ± 0.5 ^{c}
2 g C kg^{-1} tailings	0.302 ± 0.043 ^{a}	0.144 ± 0.019 ^{a}	0.545 ± 0.026 ^{a}	12.5 ± 0.4 ^{b}
3 g C kg^{-1} tailings	0.238 ± 0.033 ^{a}	0.133 ± 0.018 ^{a}	0.584 ± 0.053 ^{a}	11.2 ± 0.4 ^{a}
4 g C kg^{-1} tailings	0.291 ± 0.044 ^{a}	0.159 ± 0.026 ^{a}	0.541 ± 0.029 ^{a}	12.8 ± 0.4 ^{b}

all the amended treatments relative to the control with the lowest height observed in the 3 g C kg^{-1} treatment.

4.2.3.3 Photosynthetic Pigments

Medicago sativa chlorophyll a and b content was not significantly different between the treatments (Table 15). *Agropyron trachycaulum* had a significant increase in both chlorophyll a and b content in the 3 g C kg^{-1} treatment. *Medicago sativa* had no significant differences in carotenoid content between the treatments. *Agropyron trachycaulum* exhibited increased carotenoid content in the 3 g C kg^{-1} treatment.

Like field season 2003, *M. sativa* chlorophyll a content was not significantly different between the treatments in field season 2004 (Table 16a). However, in field season 2004, *M. sativa* chlorophyll b content was significantly lower in the amended treatments relative to the control. In field season 2004, *A. trachycaulum* chlorophyll a and b content was significantly lower in the amended treatments with an average decrease of 28% for chlorophyll a and 38% for chlorophyll b. *Festuca pratensis* chlorophyll a and b content in field season 2004 was also significantly lower in the amended treatments relative to the control. *Agropyron elongatum* chlorophyll a and b content exhibited no significant difference among the modified humic amendment treatments (Table 16b). Chlorophyll a and b content for *F. rubra* was significantly lower in the amended treatments relative to the control. Carotenoid content for *M. sativa, A. trachycaulum*, and *A. elongatum* exhibited no significant difference between the control and amendment treatments (Table 16a and 16b). However, *F. pratensis* had significant reductions of carotenoid content in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments relative to the control. *Festuca rubra* also exhibited a significant decrease in carotenoid content relative to the control in the amended treatments.

4.2.3.4 Elemental Analysis

Medicago sativa copper and iron content in both the root and shoot tissue was not affected by the addition of modified humic substances (Table 17). Copper content in *M. sativa* averaged 82 μg g^{-1} in the shoot and 129 μg g^{-1} in the root. Iron content in *M. sativa* averaged 470 μg g^{-1} in shoot tissue and 400 μg g^{-1} in the root tissue. *Agropyron*

Table 15. Field Season 2003 chlorophyll and carotenoid content in leaf tissue of *Medicago sativa, Agropyron trachycaulum* and *Festuca pratensis* after a 2 month growing period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$). * indicates insuficient data to conduct statistical analysis (n = 2).

	Chlorophyll a (mg g^{-1} fresh weight)	Chlorophyll b (mg g^{-1} fresh weight)	Carotenoids (mg g^{-1} fresh weight)
Medicago Sativa			
0 g C kg^{-1} tailings (Control)	1.20 ± 0.16 [a]	0.35 ± 0.04 [a]	0.046 ± 0.007 [a]
2 g C kg^{-1} tailings	1.14 ± 0.08 [a]	0.36 ± 0.02 [a]	0.035 ± 0.004 [a]
3 g C kg^{-1} tailings	1.22 ± 0.15 [a]	0.36 ± 0.04 [a]	0.045 ± 0.005 [a]
4 g C kg^{-1} tailings	1.03 ± 0.12 [a]	0.30 ± 0.03 [a]	0.036 ± 0.004 [a]
Agropyron trachycaulum			
0 g C kg^{-1} tailings (Control)	1.45 ± 0.10 [a]	0.41 ± 0.03 [a]	0.076 ± 0.005 [a]
2 g C kg^{-1} tailings	1.38 ± 0.09 [a]	0.36 ± 0.02 [a]	0.068 ± 0.005 [a]
3 g C kg^{-1} tailings	2.11 ± 0.24 [b]	0.57 ± 0.07 [b]	0.105 ± 0.010 [b]
4 g C kg^{-1} tailings	1.27 ± 0.04 [a]	0.34 ± 0.03 [a]	0.060 ± 0.002 [a]
Festuca pratensis			
0 g C kg^{-1} tailings (Control)	1.03 ± 0.09 [a]	0.32 ± 0.02 [b]	0.062 ± 0.005 [a]
2 g C kg^{-1} tailings	0.96 ± 0.11 [a]	0.26 ± 0.03 [a]	0.058 ± 0.005 [a]
3 g C kg^{-1} tailings	0.64 ± 0.14 [*]	0.17 ± 0.04 [*]	0.043 ± 0.005 [*]
4 g C kg^{-1} tailings	0.77 ± 0.11 [*]	0.16 ± 0.01 [*]	0.041 ± 0.005 [*]

Table 16a. Field Season 2004 chlorophyll and carotenoid content of leaf tissue of *Medicago sativa, Agropyron trachycaulum* and *Festuca pratensis* after a 3 month growing period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Chlorophyll a ($mg\ g^{-1}$ fresh weight)	Chlorophyll b ($mg\ g^{-1}$ fresh weight)	Carotenoids ($mg\ g^{-1}$ fresh weight)
Medicago Sativa			
0 g C kg^{-1} tailings (Control)	0.88 ± 0.09 [a]	0.33 ± 0.04 [b]	0.046 ± 0.005 [a]
2 g C kg^{-1} tailings	0.71 ± 0.06 [a]	0.20 ± 0.02 [a]	0.037 ± 0.003 [a]
3 g C kg^{-1} tailings	0.72 ± 0.08 [a]	0.22 ± 0.02 [a]	0.039 ± 0.003 [a]
4 g C kg^{-1} tailings	0.80 ± 0.08 [a]	0.25 ± 0.03 [a]	0.038 ± 0.004 [a]
Agropyron trachycaulum			
0 g C kg^{-1} tailings (Control)	1.06 ± 0.10 [b]	0.38 ± 0.03 [b]	0.041 ± 0.005 [a]
2 g C kg^{-1} tailings	0.74 ± 0.12 [a]	0.23 ± 0.05 [a]	0.030 ± 0.003 [a]
3 g C kg^{-1} tailings	0.76 ± 0.06 [a]	0.23 ± 0.02 [a]	0.038 ± 0.005 [a]
4 g C kg^{-1} tailings	0.80 ± 0.06 [a]	0.24 ± 0.02 [a]	0.037 ± 0.003 [a]
Festuca pratensis			
0 g C kg^{-1} tailings (Control)	1.25 ± 0.10 [b]	0.37 ± 0.03 [b]	0.063 ± 0.004 [b]
2 g C kg^{-1} tailings	0.92 ± 0.13 [a]	0.28 ± 0.04 [a]	0.052 ± 0.007 [a]
3 g C kg^{-1} tailings	0.82 ± 0.08 [a]	0.24 ± 0.02 [a]	0.044 ± 0.004 [a]
4 g C kg^{-1} tailings	0.92 ± 0.10 [a]	0.28 ± 0.03 [a]	0.047 ± 0.004 [a]

Table 16b. Field season 2004 chlorophyll and carotenoid content of leaf tissue of *Agropyron elongatum* and *Festuca rubra* after a 3 month growing period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$)

	Chlorophyll a (mg g^{-1} fresh weight)	Chlorophyll b (mg g^{-1} fresh weight)	Carotenoids (mg g^{-1} fresh weight)
Agropyron elongatum			
0 g C kg^{-1} tailings (Control)	0.90 ± 0.07 ^{a}	0.30 ± 0.02 ^{a}	0.044 ± 0.003 ^{a}
2 g C kg^{-1} tailings	0.74 ± 0.11 ^{a}	0.24 ± 0.04 ^{a}	0.036 ± 0.003 ^{a}
3 g C kg^{-1} tailings	0.77 ± 0.05 ^{a}	0.23 ± 0.01 ^{a}	0.040 ± 0.003 ^{a}
4 g C kg^{-1} tailings	0.93 ± 0.05 ^{a}	0.28 ± 0.02 ^{a}	0.046 ± 0.003 ^{a}
Festuca rubra			
0 g C kg^{-1} tailings (Control)	1.28 ± 0.06 ^{c}	0.45 ± 0.06 ^{b}	0.056 ± 0.003 ^{c}
2 g C kg^{-1} tailings	0.90 ± 0.09 ^{b}	0.25 ± 0.03 ^{a}	0.046 ± 0.004 ^{b}
3 g C kg^{-1} tailings	0.72 ± 0.09 ^{ab}	0.21 ± 0.03 ^{a}	0.038 ± 0.003 ^{a}
4 g C kg^{-1} tailings	0.78 ± 0.06 ^{a}	0.23 ± 0.02 ^{a}	0.042 ± 0.002 ^{ab}

Table 17. Field Season 2003 copper, iron, phosphorous, and potassium content in *Medicago sativa* and *Agropyron trachycaulum* shoot and root tissue after a 3 month growth period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$).

	Cu ($\mu g\ g^{-1}$ dry weight)	Fe ($\mu g\ g^{-1}$ dry weight)	P ($\mu g\ g^{-1}$ dry weight)	K ($\mu g\ g^{-1}$ dry weight)
Medicago sativa				
Shoot Tissue				
0 g C kg^{-1} tailings (Control)	74 ± 6 ^{a}	409 ± 64 ^{a}	1,170 ± 70 ^{a}	14,330 ± 270 ^{a}
2 g C kg^{-1} tailings	95 ± 7 ^{a}	501 ± 47 ^{a}	2,163 ± 136 ^{b}	14,871 ± 515 ^{a}
3 g C kg^{-1} tailings	81 ± 20 ^{a}	499 ± 158 ^{a}	2,151 ± 56 ^{b}	14,307 ± 836 ^{a}
4 g C kg^{-1} tailings	77 ± 5 ^{a}	470 ± 90 ^{a}	2,435 ± 273 ^{b}	13,178 ± 109 ^{a}
Root Tissue				
0 g C kg^{-1} tailings (Control)	134 ± 45 ^{a}	313 ± 87 ^{a}	703 ± 89 ^{a}	8,957 ± 900 ^{a}
2 g C kg^{-1} tailings	134 ± 19 ^{a}	391 ± 85 ^{a}	2,769 ± 150 ^{b}	12,535 ± 822 ^{b}
3 g C kg^{-1} tailings	142 ± 24 ^{a}	488 ± 152 ^{a}	2,724 ± 114 ^{b}	13,143 ± 223 ^{b}
4 g C kg^{-1} tailings	107 ± 16 ^{a}	407 ± 154 ^{a}	2,902 ± 222 ^{b}	14,414 ± 2,055 ^{b}
Agropyron trachycaulum				
Shoot Tissue				
0 g C kg^{-1} tailings (Control)	209 ± 75 ^{a}	938 ± 330 ^{a}	932 ± 55 ^{a}	11,737 ± 701 ^{a}
2 g C kg^{-1} tailings	195 ± 18 ^{a}	1,016 ± 139 ^{a}	2,565 ± 210 ^{b}	13,373 ± 114 ^{ab}
3 g C kg^{-1} tailings	219 ± 63 ^{a}	1,090 ± 302 ^{a}	3,013 ± 239 ^{b}	14,120 ± 510 ^{ab}
4 g C kg^{-1} tailings	173 ± 29 ^{a}	753 ± 152 ^{a}	3,950 ± 231 ^{c}	16,226 ± 2,745 ^{b}
Root Tissue				
0 g C kg^{-1} tailings (Control)	575 ± 43 ^{a}	1,557 ± 281 ^{a}	608 ± 80 ^{a}	12,015 ± 473 ^{a}
2 g C kg^{-1} tailings	951 ± 162 ^{ab}	2,328 ± 474 ^{a}	2,468 ± 402 ^{b}	13,516 ± 233 ^{ab}
3 g C kg^{-1} tailings	1,179 ± 166 ^{b}	1,627 ± 249 ^{a}	2,672 ± 226 ^{b}	18,233 ± 2,828 ^{b}
4 g C kg^{-1} tailings	1,180 ± 191 ^{b}	1,909 ± 368 ^{a}	3,352 ± 340 ^{c}	29,444 ± 1,875 ^{c}

trachycaulum iron content was not affected by the addition of modified humic substances with an average value of 949 μg g^{-1} in the shoot and 1,855 μg g^{-1} in the root. Copper shoot content was not significantly different between the treatments with an average of 199 μg g^{-1}. However, a significant increase in root copper content (105%) was observed in both the 3 g C kg^{-1} and 4 g C kg^{-1} treatments relative to the control. *Medicago sativa* phosphorus content was increased in both the shoot (92%) and root (300%) tissue relative to the control in the 2 g C kg^{-1}, 3 g C kg^{-1}, and 4 g C kg^{-1} treatments. *Medicago sativa* potassium content in the shoot tissues was not significantly different between the treatments. However, potassium content in the roots tissue was higher in the amended treatments relative to the control. *Agropyron trachycaulum* shoot phosphorus content was increased relative to the control in the 2 g C kg^{-1}, 3 g C kg^{-1} and 4 g C kg^{-1} treatments with the 4 g C kg^{-1} treatment having the largest increase (324%) in phosphorus content. *Agropyron trachycaulum* root phosphorus content was significantly higher in the 2 g C kg^{-1}, 3 g C kg^{-1} and 4 g C kg^{-1} treatments relative to the control with the largest increase in the 4 g C kg^{-1} treatment (616%). *Agropyron trachycaulum* shoot tissue potassium content was significantly higher in the 4 g C kg^{-1} treatment with no differences observed between the amended treatments. Root tissue potassium content exhibited a significant increase in both the 3 g C kg^{-1} (52%) and 4 g C kg^{-1} (145%) treatments relative to the control with the largest increase observed in the 4 g C kg^{-1} treatment.

Like field season 2003, *M. sativa* copper and iron content in shoot and root tissue was not affected by the addition of modified humic substances in field season 2004 (Table 18a). Copper content in *M. sativa* averaged 81μg g^{-1} in the shoot and root content averaged 125 μg g^{-1}. Iron content in *M. sativa* averaged 439 μg g^{-1} in shoot tissue and root tissue content was lower than field season 2003 with an average of 231 μg g^{-1}. *Agropyron trachycaulum* copper content was not affected by the addition of modified humic with no significant differences between treatments in field season 2004 with an average value of 143 μg g^{-1} in the shoot and 562 μg g^{-1} in the root. Iron shoot content was not significantly different between the treatments with an average of 333 μg g^{-1}. However, a significant increase in root iron content of 65 % in the 2 g C kg^{-1} was observed. *Agropyron elongatum* copper content was not affected by the addition of

Table 18a. Field season 2004 copper, iron, phosphorous, and potassium content in *Medicago sativa* and *Agropyron* trachycaulum shoot and root tissue after a 3 month growth period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$).

	Cu ($\mu g\ g^{-1}$ dry weight)	Fe ($\mu g\ g^{-1}$ dry weight)	P ($\mu g\ g^{-1}$ dry weight)	K ($\mu g\ g^{-1}$ dry weight)
Medicago sativa				
Shoot Tissue				
0 g C kg^{-1} tailings (Control)	76 ± 15 ^{a}	360 ± 38 ^{a}	1,840 ± 190 ^{a}	16,508 ± 2,045 ^{a}
2 g C kg^{-1} tailings	62 ± 117 ^{a}	441 ± 112 ^{a}	2,410 ± 236 ^{a}	21,687 ± 1,168 ^{ab}
3 g C kg^{-1} tailings	109 ± 29 ^{a}	579 ± 283 ^{a}	2,167 ± 150 ^{a}	25,221 ± 1,295 ^{b}
4 g C kg^{-1} tailings	79 ± 26 ^{a}	375 ± 114 ^{a}	2,168 ± 205 ^{a}	20,521 ± 1,487 ^{ab}
Root Tissue				
0 g C kg^{-1} tailings (Control)	120 ± 19 ^{a}	190 ± 31 ^{a}	1,440 ± 107 ^{a}	11,621 ± 207 ^{a}
2 g C kg^{-1} tailings	156 ± 30 ^{a}	247 ± 33 ^{a}	2,892 ± 344 ^{b}	13,859 ± 1,220 ^{ab}
3 g C kg^{-1} tailings	119 ± 17 ^{a}	281 ± 83 ^{a}	2,290 ± 319 ^{b}	14,347 ± 401 ^{b}
4 g C kg^{-1} tailings	106 ± 15 ^{a}	208 ± 72 ^{a}	2,404 ± 113 ^{b}	13,595 ± 846 ^{ab}
Agropyron trachycaulum				
Shoot Tissue				
0 g C kg^{-1} tailings (Control)	145 ± 61 ^{a}	293 ± 25 ^{a}	1,557 ± 174 ^{a}	16,061 ± 1,923 ^{a}
2 g C kg^{-1} tailings	142 ± 54 ^{a}	402 ± 90 ^{a}	1,977 ± 184 ^{a}	15,280 ± 1,761 ^{a}
3 g C kg^{-1} tailings	154 ± 30 ^{a}	380 ± 76 ^{a}	2,257 ± 342 ^{a}	18,283 ± 3,085 ^{a}
4 g C kg^{-1} tailings	130 ± 53 ^{a}	256 ± 90 ^{a}	2,049 ± 352 ^{a}	16,917 ± 2,121 ^{a}
Root Tissue				
0 g C kg^{-1} tailings (Control)	545 ± 141 ^{a}	290 ± 20 ^{a}	1,186 ± 76 ^{a}	16,840 ± 1,909 ^{a}
2 g C kg^{-1} tailings	672 ± 256 ^{a}	478 ± 33 ^{b}	1,714 ± 128 ^{ab}	16,773 ± 2,411 ^{a}
3 g C kg^{-1} tailings	459 ± 94 ^{a}	287 ± 38 ^{a}	2,234 ± 328 ^{b}	18,862 ± 2,079 ^{a}
4 g C kg^{-1} tailings	573 ± 220 ^{a}	314 ± 95 ^{a}	1,800 ± 249 ^{ab}	17,514 ± 2,425 ^{a}

Table 18b. Field season 2004 copper, iron, phosphorous, and potassium content in *Agropyron elongatum* shoot and root tissue after a 3 month growth period in mine tailings amended with modified humic substances (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$).

	Cu ($\mu g\ g^{-1}$ dry weight)	Fe ($\mu g\ g^{-1}$ dry weight)	P ($\mu g\ g^{-1}$ dry weight)	K ($\mu g\ g^{-1}$ dry weight)
Agryopyron elongatum				
Shoot Tissue				
0 g C kg^{-1} tailings (Control)	80 ± 9.1 [a]	272 ± 22 [a]	1568 ± 255 [a]	19,415 ± 2,047 [a]
2 g C kg^{-1} tailings	120 ± 26 [a]	332 ± 44 [a]	2304 ± 186 [b]	21,638 ± 1,674 [a]
3 g C kg^{-1} tailings	108 ± 22 [a]	403 ± 147 [a]	2373 ± 226 [b]	17,332 ± 2,120 [a]
4 g C kg^{-1} tailings	86 ± 6.5 [a]	352 ± 69 [a]	2331 ± 224 [b]	19,699 ± 1,344 [a]
Root Tissue				
0 g C kg^{-1} tailings (Control)	542 ± 113 [a]	754 ± 158 [a]	1052 ± 193 [a]	13,139 ± 512 [a]
2 g C kg^{-1} tailings	485 ± 130 [a]	610 ± 179 [a]	1652 ± 188 [b]	19,397 ± 676 [b]
3 g C kg^{-1} tailings	668 ± 124 [a]	833 ± 259 [a]	1699 ± 161 [b]	18,413 ± 1,842 [b]
4 g C kg^{-1} tailings	693 ± 183 [a]	1039 ± 188 [a]	1793 ± 86 [b]	18,532 ± 987 [b]

modified humic substances with an average value of 98 μg g^{-1} in the shoot and 597 μg g^{-1} in the root (Table 18b).

Iron content was not significantly different between the treatments with an average shoot content of 340 μg g^{-1} and average root content of 809 μg g^{-1}. Unlike field season 2003, *M. sativa* phosphorus content in the shoot tissue was not significantly different between the treatments. Root phosphorus content was significantly higher in the amended treatments. Potassium shoot and root contents in *M. sativa* were significantly higher in the 3 g C kg^{-1} treatment, while all other modified treatments showed no significant difference from the control. Unlike field season 2003, *A. trachycaulum* shoot phosphorus content was not different between the amended treatments. However, amended treatments were significantly greater than the control while root phosphorus content was 88 % greater than the control in the 3 g C kg^{-1} treatment. *Agropyron trachycaulum* shoot and root potassium contents exhibited no significant differences between the treatments. *Agropyron elongatum* shoot and root phosphorus content was significantly higher than the control in amended treatments. However, only root potassium contents were significantly higher in the amended treatments relative to the control while no significant differences in shoot potassium were observed among the treatments.

5. Discussion

5.1 Tailings

5.1.1 Short Term Growth Chamber Experiment

5.1.1.1 pH

An initial pH of 7.8 was observed in the tailings prior to the application of amendments. No significant change in pH was observed after addition of amendment in either the 2 g C kg^{-1} tailings or the 4 g C kg^{-1} tailings treatments over the course of the experiment. However, Ibrahim and Goh (2004) observed an increase in pH of mine tailings from Central Manitoba mine site treated with 4 g C kg^{-1} modified humic substances. The primary reason for an increase in pH was due to neutral (pH ~7) BlackEarth modified humic amendment being applied to highly acidic tailings (4.4 – 5). The absence of a pH change in the tailings used in this experiment can likely be attributed to the fact that the tailings were already neutral/slightly basic pH prior to the application of the amendment. A number of locations within the tailings and areas centered around the original deposition site contain lower pH values (Sherriff, personal communication, 2006). Tailings collected for the experiment were over 300 m from the original deposition site and thus likely had a higher calcite to sulfide mineral content buffering acid generation preventing a decrease in pH.

5.1.2 Long Term Growth Chamber Experiment

5.1.2.1 pH

Tailings used in the long term experiment had a pH of 7.4 prior to the addition of modified humic substances and as in the short term experiment no significant change in pH was observed following the addition of modified humic substances.

5.1.2.2 Elemental Analysis

Total copper and iron content of the tailings prior to amendment application was 3,535 ug g^{-1} and 31,140 ug g^{-1} respectively and was comparable to other studies by Renault et al. (2000) and Londry and Sherrif (2005). Analysis of the modified humic substances resulted in low copper, iron and sodium content. Therefore, the application of

amendment should not have significantly altered the amounts of these elements in the tailings.

Typical copper levels across a broad range of soil types tend to vary from 13 to 30 $\mu g\ g^{-1}$ (Baker, 1990; Kabata-Pendias and Pendias, 1992). Copper levels in the tailings far exceeded normal soil amounts by a factor of ~190. Proposed maximum/acceptable soil concentrations of copper are less than 100 $\mu g\ g^{-1}$ to avoid potential toxic effects on plants (Kabata-Pendias and Pedias, 1992). Despite a high total copper content in the tailings the amount of plant available copper was likely lower. Insoluble copper in crystalline form was present in the primary sulfide mineral chalcopyrite (Salzsauler, 2001). While this mineral remains susceptible to oxidation over time, copper in this form would have not been plant available in the short term. Plant available copper is also directly linked to soil pH, organic matter content, clay content, hydroxide, oxide and phosphate contents (McLaren and Crawford, 1973). Under neutral pH soil conditions, like those observed in the tailings of this study, soluble copper precipitates out of soil solution complexing with sulfates, carbonates and hydroxides (Kabata-Pendias and Pendias, 1992). These minerals and other metallic carbonates and hydroxides have the ability to adsorb copper ions and remove them from soil solution due to their negative surface charge. Thus at higher pH these minerals remain stable and possess more charge sites that are free to bind/sorb copper ions decreasing the amount of copper in soil solution (Kabata-Pendias and Pendias, 1992). The low organic carbon and clay minerals content of the tailings (Ibrahim and Goh, 2004) along with the neutral pH observed would suggest that the majority of copper (not in primary mineral form) would be contained in or sorbed to the surface of carbonates and oxide/hydroxide minerals (McLaren and Crawford, 1973). However, copper contained within and sorbed to these minerals remains highly sensitive to dissolution by acidity under lower pH conditions (McBride, 1981). Thus due to the elevated levels of copper found within the tailings even under higher pH conditions the potential for toxicity remains through rhizosphere interactions and root released organic acids. These soil root interactions generating acidity are likely to contribute to the solubilization of copper carbonates and can remove sorbed copper within the rhizosphere (Lombi et al., 2001). The addition of modified humic substances contributed a significant amount of organic matter to the tailings. Humic substances have the ability to

form both soluble and insoluble complexes with copper and application of organic matter is thought to be an effective mechanism of copper retention in soils due to the strong stable complex that forms (McBride, 1981; Schnitzer and Kahn, 1978). Organic matter such as humic substances specifically humic and fulvic acids have been shown to have a maximum sorption of 160 mg of Cu per gram of humic acid (Stevenson and Fitch, 1981). Thus the addition of the modified humic substances leads to a decrease in the soluble plant available copper in solution compared to the untreated tailings (Senkiw and Goh, 2006). Furthermore soluble copper content might be lowered by the presence of phosphate (delivered along with the amendment) which has been suggested by some authors to remove heavy metals such as copper, cadmium, cobalt and especially lead from solution through precipitation (Sugiyama et al., 2003).

Typical iron levels across a broad range of soil types varies from 5,000 to 50,000 $\mu g\ g^{-1}$ (Kabata-Pendias and Pendias, 1992). As a plant growth medium, the tailings were not outside the normal soil range of total iron. Like copper, insoluble iron exists in primary mineral form with pyrite, chalcopyrite and pyrrhotite being the more common minerals found in sulfide tailings (Richardson and Ostry, 1996). Oxidation of these minerals over time releases iron into soil solution along with acidity but the majority of iron in this form remains plant unavailable in the short term. Plant available iron is largely dependent on pH/redox potential, organic matter content to a lesser degree than copper, adsorption to carbonates, oxides/hydroxides and clay minerals (McLaren and Crawford, 1973). Soluble iron content in soils is typically low in relation to the total iron content as soluble iron forms insoluble oxide and hydroxide minerals (Shuman, 1985). The neutral pH, low organic matter content and absence of clay minerals in the Central Manitoba tailings (Goh and Ibrahim, 2004) would suggest that the vast majority of iron present, not in primary minerals, is contained in or sorbed to the surface oxides and hydroxides (McLaren and Crawford, 1973). Due to the pH of the tailings, it is unlikely that soluble iron and more specifically Fe^{3+} would be in high enough concentration to have had any toxic effects on growing plants. Like copper, organic matter also binds iron forming soluble and insoluble complexes though iron tends to form mobile organic complexes and chelates (Kabata-Pendias and Pendias, 1992). The humic substance iron complexes are much less stable than the copper humic complexes allowing for greater

movement of iron in soil solution (Schnitzer and Khan, 1978). Thus the addition of modified humic substances in the treated tailings may allow for greater iron movement and delivery to the root surface resulting in increased iron uptake.

Elemental analysis of the BlackEarth modified humic amendment revealed a high potassium content of 157,040 $\mu g\ g^{-1}$ and a phosphorus content of 30,519 $\mu g\ g^{-1}$. The soluble salt like nature of the modified humic amendment results in these nutrients being released into the soil solution in the form of K^+ and PO_4^{3-} as the humic material disassociates in the presence of water. While both of these nutrients are not known to be toxic in high concentrations (Jones, 1998) any ion in significant quantity can induce osmotic stress on plants.

5.1.2.3 Conductivity

Conductivity of tailings prior to amendment with modified humic substances averaged 2.15 dS m^{-1} which according to the SPAC (1999) indicates a slightly saline soil. The likely source of the conductivity is from the oxidation of sulfide minerals releasing metals into the soil solution. Another source of ions if due to the basin like nature of the tailings deposit, collecting runoff/dissolved minerals from the surrounding area. Precipitation of minerals on the soil surface during dry conditions is common and salt crusts can be observed in some locations (Appendix F). The addition of modified humic substances significantly increases the conductivity of the tailings due to the dissolution of the modified humic substances releasing the charged humic substances (humate), and potassium and phosphate ions into soil solution (see section 5.1.2.2). Conductivity measured in the 2 g C kg^{-1} was 4.93 dS m^{-1} and in the 4 g C kg^{-1} a conductivity of 7.81 dS m^{-1} was recorded. Conductivity within this range (4.1 – 8.0 dS m^{-1}) indicates moderately saline conditions (SPAC, 1999). Moderate saline conditions may reduce plant available water and may lead to osmotic stress on emerging seedlings depending on species sensitivity. At 12 weeks post seeding, similar trends in conductivity were observed between the treatments with no significant changes in conductivity from the previous sample period. This result suggests that despite the mobile nature of the ions no change in conductivity will occur unless there is significant leaching.

5.1.3 Field Experiments

5.1.3.1 Weather

Many soil chemical and physical property differences observed were likely related to the moisture differences between the 2 field seaons (Appendix B). Plant performance, such as survival, growth and overall stress, is also affected by environmental conditions and will be discussed in the plant section (see section 5.2.3)

5.1.3.2 pH

The pH of the tailings in field season 2003 was homogenous across the treatments with an average pH of 7.9 before amendment application. Reasons for the neutral pH observed were discussed in section 5.1.1.1. The pH of the tailings in field season 2004 was more heterogeneous across treatments with an average range in pH between 6.4 - 6.9. Individual samples and in some cases entire treatments were representative of the heterogeneity with a wide range of pH values as low as pH 3.8 and as high as 8.1. Low pH values are due to the exhaustion of calcite by a higher local proportion of sulfide minerals (Sherriff, personal communication, 2006). Exhaustion of the pH buffering calcite and various other carbonates leads to pH values as low as 4 (Blows and Ptacek, 1994).

Small (0.1 pH) but significant fluctuations in pH were observed following amendment application in field season 2003 but no clear trends related to amendment rate were present. The absence of major pH changes is likely a function of high initial pH prior to treatment. Field season 2004 showed significant increases in pH in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments following amendment addition while no change was observed in the 2 g C kg^{-1} treatment. However, this result is somewhat misleading due to the heterogeneity of pH within the individual treatments. When considered on an individual replicate basis the results show that treatments with low initial pH values prior to treatment showed an increase in pH following amendment application whereas no change occurred following amendment application in treatments that had initially a neutral pH. Major fluctuations in pH were observed within individual sample zones between sampling periods in field season 2004. The heterogeneity of the tailings and the minor differences in sample location are more likely the source of large changes (up to 4 units)

in pH observed within sample times and not major changes in pH itself (Appendix D). This result suggests that distances as little as 0.5 meter is all that is required to experience large pH shifts within the tailings.

Field seasons 2003 and 2004 exhibited minor changes in pH with significant differences observed within treatments over the growing season and between treatments. These fluctuations during the growing seasons appeared to be independent of amendment application and may be due to mineral composition (heterogeneity) and topographical features affecting local environmental conditions. Environmental conditions including oxidation state and water status (saturation) in the tailings over the growing season. These conditions are likely to influence these pH fluctuations with varying amounts of mineral precipitates being formed or the rate of sulfide minerals being oxidized. Biological influences from both plants and microorganisms are likely to cause a decrease in pH over the growing seasons due to release of organic acids in an attempt to increase metal availability for micronutrient uptake (Lombi et al., 2001). Mine tailings also contain a diverse assemblage of bacteria that are capable of increasing the background rate of sulfide mineral oxidation leading to the release of acidity (Johnson et al., 2002). However, tailings also contain a number of metal and sulfate reducing bacteria that may lead to pH increases (Johnson et al., 2002). Application of fertilizer during experimentation may also have caused changes in pH. Soluble Plant Prod™ fertilizer contained ammonium, nitrate and urea. Biological interactions with nitrogen can lead to increases or decreases in soil pH depending on the nature of the nitrogen source. Plant uptake of ammonium tends to decrease soil pH while nitrate uptake can cause an overall increase in soil pH (Smiley, 1974; Nye, 1981). Breakdown of urea by soil organisms causes a rapid increase in soil pH (Qing-ru et al., 2005). Depending on the various rates of uptake, microorganism composition, breakdown and usage of nitrogen over the growing season it is difficult to draw any conclusions on the overall effect of nitrogen fertilization though one may be present.

5.1.3.3 Elemental Analysis

Total copper content of the tailings prior to amendment application was 3,661 μg g^{-1} in field season 2003 while the content in field season 2004 was higher at 4,713 μg g^{-1}.

Iron content was also higher in season 2004 with 47,685 μg g^{-1} compared to field season 2003 with 39,308 μg g^{-1}. Tailings heterogeneity is likely responsible for the differences between the 2 field sites. Copper and iron content did not significantly change following amendment application as expected due to the low copper and iron content of the amendment. Forms of iron and copper, their interaction with organic materials and their availability in tailings have been discussed in section 5.1.2.2. However, some treatments and specific samples within plots of field season 2004 had low pH values (less than pH 4) suggesting the potential for increased soluble iron and copper concentrations and increased potential for toxicity for plants.

The solubility and related availability increase of both iron and copper with decreasing soil pH is due to two primary causes. The first being a reduction in the surface charge of particles (that sorb the metallic ions) and the second being the dissolution of the metal containing minerals such as copper carbonate and iron hydroxides/oxides (Sposito, 1989). Reductions in soil pH leads to a decrease in the number of negatively charged sites on minerals surfaces as the sites are filled by protons. The effect is a net reduction in overall surface negative charge with increasing acidity as the protons prevent the sorption of soluble metals from solution (Sposito, 1989). Protons may also be substituted for existing sorbed metals releasing them into soil solution further increasing the amount of available iron and copper. Prevention of metal precipitation and consumption of existing copper and iron minerals under lower pH conditions also causes an increase in the amount of available iron and copper in soil solution. Tailings with a pH lower than 5 prevent carbonate mineral (metallic carbonate) formation that would otherwise occur under higher pH conditions and likely leads to an increase in soluble contents of both iron and copper (Blowes and Ptacek, 1994). At pH values less than pH 4, precipitation of soluble iron in the form of hydroxides/oxides is prevented and thus dissolution of existing iron hydroxides/oxide minerals occurs further increasing soluble iron in soil solution (Blowes and Ptacek, 1994).

Typical levels of phosphorus in soil range from 50 μg g^{-1} to 1,000 μg g^{-1} with up to 30-50% in organic forms in mineral soils (Foth and Ellis, 1997). Tailings total phosphorus was on the lower end of the mineral soil range with a total of 139 μg g^{-1} in season 2003 and 94 μg g^{-1} in season 2004. With low organic matter content in the tailings

(Ibrahim and Goh, 2004) the majority of phosphorus was likely contained within mineral form (Foth and Ellis, 1997). Soluble phosphorus is typically very low in all soil types with young soils (weakly weathered, similar to tailings) containing mostly primary mineral forms and while amounts of phosphorus in the surface sorbed fraction are typically reduced and thus in the absence of fertilization a deficiency for plants might occur (Foth and Ellis, 1997). Elemental analysis of BlackEarth modified humic substances showed that they contained 30,519 $\mu g\ g^{-1}$ phosphorus (soluble). Following application there was a significant increase in phosphorus content in all treatments relative to the control. No trend with amendment rates were observed in field season 2003, while field season 2004 exhibited a strong trend of increasing phosphorus content with increasing treatment rate. The large standard error observed, especially in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments, in field season 2003 may suggest that the degree of mixing during amendment application may not have been sufficient. Forms of phosphorus are strongly influenced by pH where phosphorus availability tends to be highest in the acidic range of soil pH between 4.5 and 6.5 while at low pH less than <4.5 and above neutral pH the availability of phosphorus is reduced (Lucas and Davis, 1961). This would suggest that in field season 2003, where the pH was homogenous, the amount of available phosphorus would have been similar among the individual treatments with solubilization of phosphorus occurring within the rhizosphere. The heterogeneity of pH observed in field season 2004 likely had an influence on phosphorus availability. Samples ranged in neutral pH to highly acidic and this likely lead to greater or lesser amounts of available phosphorus within individual areas of the tailings as the form of phosphorus and quantities of available phosphorus would change depending on location.

Typical levels of total soil potassium range from 3,000 $\mu g\ g^{-1}$ and 26,000 $\mu g\ g^{-1}$ in most soils types. Mineral soils tend to fall towards the upper end while organic soils fall on the lower end of total soil potassium (Foth and Ellis, 1988). The mine tailings, being mineral in nature, were expected to have a high total potassium content as K-feldspar and mica were identified by Salzsauler (2001) as the second and fourth most abundant primary minerals within the tailings. However, total potassium was lower than anticipated with values of 9,292 $\mu g\ g^{-1}$ in field season 2003 and 7,001 $\mu g\ g^{-1}$ in field season 2004. Based on average mineral soils and due to the nature of the tailings the

majority of potassium in the tailings was likely insoluble in primary K-feldspar and mica mineral form. Only a minor amount of potassium is typically plant available either free in solution or exchangeable sorbed to the surface of charged particles (Foth and Ellis, 1988). Elemental analysis of BlackEarth humic materials previously discussed in section 5.1.2.2 showed a potassium content (soluble) of 157,040 $\mu g\ g^{-1}$. It was expected that following application, a significant increasing trend with amendment rate would be observed due to the relatively high amount of soluble potassium within the amendment. However, this trend was only observed in field season 2004 while in field season 2003 no significant differences were observed between the amended tailings and the control following treatment. Similar to phosphorus the absence of any increasing trends with increasing treatment rate and a high standard error may suggest that the degree of mixing was not sufficient.

5.1.3.4 Conductivity

Conductivity of tailings prior to amendment with modified humic substances averaged 2.11 dS m^{-1} in field season 2003 and 3.28 dS m^{-1} in field season 2004, both of which according to the SPAC (1999) indicated a slightly saline soil. Sources of conductivity within the tailings has been discussed in section 5.1.2.3. Differences in pre treatment conductivity can be attributed to differences in tailings mineralogy, topography and drainage or the difference in moisture and environmental conditions prior to amendment application. The high water table observed in field season 2004 and in the presence of a high rate of evaporation at the surface was the likely source of the increased conductivity as ions were drawn upwards and deposited near the surface.

The addition of modified humic substances significantly increased the conductivity of the tailings in both field seasons. Dissolution of the modified humic substances released the charged humic substances, potassium and phosphate ions into soil solution and as expected the greater the treatment rate the larger the increase in conductivity. The results from field season 2003 indicate moderately saline levels in the 2 g C kg^{-1} and 3 g C kg^{-1} treatments with a strongly saline condition the 4 g C kg^{-1} treatment. Field season 2004 results indicated moderately saline conditions for the 2 g C kg^{-1}, while both the 3 g C kg^{-1} and 4 g C kg^{-1} treatments were strongly saline.

Differences between the field seasons are likely related to depth of amendment application and related concentration due to the weather condition in field season 2004 preventing proper depth of tilling. Depth of tilling was more difficult to achieve in season 2004 due to the saturated nature of the tailings and "dough" like consistency of the tailings. Desired depth was likely not reached and as a result a lower volume of tailings were treated resulting in a greater rate of application. This could be a likely explanation for the higher concentration of ions present. As discussed in 5.1.2.3 conductivity values observed may lead to a reduction in plant available water within the tailings and lead to osmotic stress on germinating seeds, establishing and growing plants depending on the sensitivity of the individual species.

In both field seasons conductivity of the treatments decreased over time indicating mobility of the ions added during amendment application. The most probable explanation for the decrease in conductivity over time is due to the leaching of ions through inputs of surface water though rainfall and watering. Field season 2004, despite larger initial conductivity values, experienced a much more rapid decrease in conductivity compared to field season 2003. Noticeable differences between the field seasons were observed at the time of seeding, where in field season 2004 conductivity decreased from the previous sample period while season 2003 remained unchanged from the previous sample period. Further differences between field season 2003 and 2004 in regards to changes in conductivity over the growing season were also observed at harvest and 1 year following treatment. In field season 2004, only the 3 g C kg^{-1} treatment was significantly higher than the control at harvest with no significant differences between the treatments observed after 1 year. In contrast, harvest conductivity in the amended treatments for field season 2003 was significantly higher than the control and after 1 year both the 3 g C kg^{-1} and 4 g C kg^{-1} treatment remained higher than the control. The greater amount of precipitation is likely to have been the source for the more rapid decrease in conductivity observed in field season 2004 as the rate of leaching and ion movement would have been higher (Appendix B). As the high conductivity values following treatment were likely to cause osmotic stress of vegetation the decrease in conductivity over time is also likely to cause a decrease in stress levels of any vegetation established post seeding. Furthermore, the near "normal" or background levels observed

in all treatments 1 year following amendment application also suggest that any vegetation that had established or attempted to establish the following year would not experience any osmotic stress due to treatment of modified humic substances. An increase in conductivity was observed in the control treatment for field season 2004 between post treatment and seeding, while no parallel change was observed in field season 2003. A potential source of this difference may be related to the saturated nature of the tailings in field season 2004. During amendment application where dissolved ions in amendment treatments allowing more horizontal movement instead of downward into the tailings and thus contaminate the control treatment. Visual observations made post treatment noted horizontal surface movement of amendment dissolved in water following application.

5.1.3.5 Organic Carbon

Organic carbon content in the tailings was low prior to amendment application and not significantly different between field seasons with a value of 0.47% in field season 2003 and 0.41% in field season 2004. Typical organic carbon levels vary greatly with soil type and geographic location. Carbon content in boreal forests varies but a recently cleared boreal forested luvisol had an organic carbon level of ~4.0% (Soon and Arshad, 1994). Other examples of soils include a permanently established grassland which possessed a carbon content of 3.1% while adjacent agricultural land averaged ~1.7% organic carbon (Johnston, 1991). Soil organic carbon plays an important role in soil development and fertility through nutrient cycling, retention, and supply of nutrients. Soils or tailings in this case that are low in organic carbon are prone to surface erosion, possess poor soil or degraded soil structure and have reduced nutrient quality (Swift, 2001). Background carbon levels in both field seasons were slightly higher than the 0.23 % carbon in Central Manitoba mine tailings determined by Ibrahim and Goh (2004) with the likely explanation for the difference being due to heterogeneity in carbon levels as tailings were sampled from different locations within the site.

The addition of modified humic amendment significantly increased the carbon content along an increasing trend with amendment rate in both field seasons. Carbon content in both field seasons increased to levels above the theoretical values where as the expected increase from background levels by 0.2%, 0.3%, and 0.4% in their respective

treatments such that in field season 2003 the predicted increase in the 4 g C kg^{-1} treatment following treatment should have been 0.87%. This result may suggest that plots in both field seasons were not tilled to a sufficient depth or that field conditions during the time of application were sufficiently different from laboratory settings in which estimates of bulk density were taken. One or both of these factors may have resulted in a lower volume of tailings amended and caused higher than intended rates of amendment application. A further, albeit less likely, possibility was that the carbon % of the amendment was higher than the specified 29 % carbon in the data sheet provided by BlackEarth Humates Ltd.

Changes in carbon content over the growing season did occur in field season 2004, with both the 3 g C kg^{-1} and 4 g C kg^{-1} treatments experiencing a reduction in carbon at harvest from the previous sample period. However, no change was observed in the 2 g C kg^{-1} treatment. Treatments in field season 2003 had no significant changes during the same time interval. A reduction in carbon was also found by Ibrahim and Goh (2004) where a 4 g C kg^{-1} treatment, in a growth chamber study, exhibited a reduction in organic carbon over 24 weeks due to microbial activity and breakdown of the amendment. Differences between the 2 field seasons can be attributed to moisture conditions where in field season 2003 tailings conditions were dryer, perhaps reducing microbial activity compared to field season 2004 which had more moisture available. Another source of difference may be due to the increased nutrient application in field season 2004 causing increased microbial growth and thus increased breakdown of the organic materials.

Increases in carbon content between harvest and 1 year following treatment in all treatments was observed for field season 2003. Field season 2004 carbon values one year following treatment were only higher than the previous sample period in the 4 g C kg^{-1} treatment and control. The small increase in carbon of the control treatments in both field seasons might be attributed to carbon sequestering through plant debris breakdown, plant and microbial exudates, microbial growth, break down of peat and infiltration of soluble organic compounds from the peat cover layer into the tailings. In field season 2003 one year following treatment the carbon levels in the amended treatments were nearly double their original post treatment and harvest values. It is unlikely that increases in carbon of

this magnitude up a 104 % (over 10 g C kg^{-1}) was due to carbon sequestering in the amendment treatments. Conant et al. (2005) utilized the DAYCENT model for measuring carbon sequestering and was able to predict carbon sequestering from various soils using different theoretical management practices from 4 sites in Canada and the US. Typical modeled carbon increases were less than 5% annually with the highest increase no more than 25% annually (Conant et al., 2005). A potential reason for the increase in the tailings may be related to the reduced size of the treatment zone where the amendment or aggregates and soil particles may have been redistributed into a more compact zone (Figure 8). The resulting compacted layer appears to have occurred in all treatments and even in regions where burial with new material did not occur. In field season 2004, the 4 g C kg^{-1} treatment was the only amended treatment to increase in organic carbon content but did not experience the same magnitude of increase as field season 2003. However, it should be noted that depth of the amended zone after 1 year was not recorded in field season 2004.

5.1.3.6 Tailings Structure

Low levels of soil organic carbon (low organic matter) were likely responsible for the reduced soil structure. Total macro aggregation was low in the tailings with a background total macro aggregation of 8.5% and 10.7% in field season 2003 and 2004 respectively. Ibrahim and Goh (2004) described the tailings from the Central Manitoba minesite as a highly degraded soil due to reduced levels of macro aggregation in tailings samples of ~13%. Soil structure was significantly improved by double or triple the background levels in field season 2003 and 2004 respectively due to the addition of modified humic substances. A similar increase in total aggregation was observed by Ibrahim and Goh (2004) using a 4 week incubation period in mine tailings amended with modified humic substances. Whiteley (1993) found that materials high in sand and low in clay were unaffected by ammonium humate amendment (a similar organic salt material) and Piccolo et al. (1997) suggested that it was the formation of clay humic complexes that increased aggregate stability within 3 different soils. Considering the low clay content (~4%) and high sand content (~48%) of the tailings reported by Ibrahim and Goh (2004), results from this study suggest that the tailings have a sufficient amount of

charged mineral surfaces (non clay) that are bound together by modified humic substances and lead to the formation of water stable aggregates. Individual differences between the improvement in total macro aggregation of two field seasons are likely due to differences in environmental and chemical factors that promote soil aggregation (Kooistra, 1996). The more favorable conditions in field season 2004 resulted in a larger increase in total macro aggregation, in comparison to field season 2003. As mentioned previously moisture conditions of the tailings during amendment application and over the incubation period were different between the field seasons and are likely the primary source of structure forming differences.

The rate of amendment application appeared to have little effect on total macro aggregation in field season 2003 and in field season 2004 where only the 4 g C kg^{-1} treatment was significantly greater than the other amended treatments with no clear trend present. Total aggregation as well did not change significantly between harvest and seeding with maximum aggregation present following incubation for most treatments with the exception of the 2 g C kg^{-1} (season 2003) which increased and the 4 g C kg^{-1} (season 2004) which decreased. These results differs from Ibrahim and Goh (2004), which found that higher rates of modified humic substances promote increased macro aggregate formation and an increase in aggregation over time. The difference may be related to a number of factors including a different mode of application as Ibrahim and Goh (2004) applied liquid modified humic substances to tailings crusting soil particles into the liquid amendment, they had a more accurate rate of amendment application, and a controlled growth chamber setting with different environmental conditions. Application of amendment in a dry powder, as it was done in this study and then mixing prevented crusting of soil particles into "globs" of amendment as observed by Ibrahim and Goh (2004). Secondly, the higher than theorized rates of amendment added in this study may have contributed excess potassium ions. It has been suggested by Auerswald et al. (1996), that potassium can act in a similar manner as sodium such that potassium can interact with charged surfaces and promote dispersion of soil particles. However, decreases in conductivity over time would suggest that aggregate formation could have continued as any dispersive effects of potassium would have been reduced as the season progressed to harvest. However, there were no increases in the structure of the higher

treatment rates as time progressed. Thirdly environmental conditions including moisture, wet/dry cycles, and other pre-mentioned aggregate forming factors were different between the two studies and may have led to the differences observed.

Following incubation the largest increases in aggregation were observed in the 0.25 – 0.5 mm, 0.5 – 1.0 mm and 1.0 – 2.0 mm size fractions with no change observed within the >2.0 mm fraction. However, by harvest time changes in the distribution of aggregates, especially in field season 2004, did occur with samples showing an increase in the >2.0 mm fraction while a general reduction in the percent of aggregates in the 0.25 – 0.5 mm and 0.5 – 1.0 mm fractions was observed. This result suggests that smaller fractions of macro aggregates are being bound together in larger aggregates over time by modified humic substances and/or other organic materials such as polysaccharides exuded by plant and microbial activity. In field season 2003, both the 2 g C kg^{-1} and 3 g C kg^{-1} treatments showed increases in the >2.0 mm fraction over the same timer interval with only the 3 g C kg^{-1} treatment showing any similar reduction in the 0.25 – 0.5 mm and 0.5 – 1.0 mm fractions. These differences between field seasons again may be related to the environmental conditions, in which the field season 2003 was much dryer with less water available in soil solution and this likely had an effect on the rate and nature of aggregate formation during soil development.

5.2 Plant

5.2.1 Short Term Growth Chamber Experiment

5.2.1.1 Seedling Emergence and Survival

The 2 g C kg^{-1} tailings treatment had no significant effect on the emergence rate or total number of seeds emerged for any of the selected species. However, the 4 g C kg^{-1} tailings treatment rate had an inhibitory effect by delaying emergence in *F. pratensis*, *M. sativa* and *B. juncea* and delaying emergence as well as reducing the total number of seed that emerged for *P. pratensis*. The only species to have no delay in emergence was *A. trachycaulum,* suggesting that the emergence response to the amended concentration is likely species specific. Other studies using humic extracts have produced varying results with both stimulation and no effect on emergence rate and overall emergence %. Humic fractions have been shown to influence processes such as protein synthesis, enzymatic

rate and respiration rate within seeds during germination increasing germination rate (Smidova, 1962). Ayuso et al. (1996a) using humic substance extracts from leonardite also found a positive effect on germination rate of *Nicotiana tobacum* (tobacco), *Lepidium sativum* (watercress), and *Hordeum vulgare* (barley) seeds. Other studies have found no effects such as Piccolo et al. (1993) where coal derived humic substance extracts, applied in aquas solution, had no measurable effect on germination rate and germination percentage using seeds of *Lactuca sativa* (lettuce) *and Lycopersicum esculentum* (tomato). Ayuso et al. (1996a) suggested that an optimum concentration of humic substances largely depends on the origin of the humic material and seed in question. The lack of any increase in emergence for *F. pratensis*, *M. sativa* and *B. juncea* and *P. pratensis* may in part be due to concentrations of humic substances above the range of stimulation for those species. Stimulation of germination for most species has been found to be in the range of 0.03 to 0.5 g C L^{-1} (Smidova, 1962; Dixit and Kishore, 1967, Ayuso et al, 1996a). Treatments in this study were likely to have exceeded the limit for stimulation as the literature suggests very low rates of application. Inhibition of the emergence rate for *F. pratensis, M. sativa and B.juncea* and total number of seeds emerged for *P. pratensis* may also be due to a reduction in available water. Application of modified humic substances released a significant amount of potassium, phosphate and humic ions into the soil which increased conductivity and thus decreased the osmotic potential of the soil (see section 5.1.2.3). Decreased osmotic potential is likely to decrease the amount of water available for germination and emergence such that the higher the rate of amendment addition, the greater the reduction in available water and thus the greater the reduction in emergence rate. Results then suggest that *P. pratensis* is the least tolerant species while *A. trachycaulum* would be the most tolerant to osmotic stress during initial emergence.

Under the high moisture and constant temperature of the growth chamber overall emergence across the treatments was greater than 80 % for *F. pratensis, M. sativa*, and *B. juncea* and greater than 60 % for *A. trachycaulum*. This might indicate that under the neutral pH and high moisture conditions the germination of the selected species, with the exception of *P. pratensis* due to its poor seed viability, can be relatively successful in mine tailings. Elemental analysis of the tailings showed a high total copper content

within the tailings (see section 5.1.2.2). Copper chloride has been shown to reduce the number of germinated *Triticum* sp. (wheat) seeds by nearly 20% with concentrations as low as 5 mM while 8 mM reduced the germination to less than 60% (Munzuroglu and Geckil, 2002). It is likely that soluble copper remains higher in the tailings compared to average soil concentrations despite the neutral pH of the tailings in the experiment (see section 5.1.2.2). Results from Munzuroglu and Geckil (2002) suggest that plant sensitivity to copper may play a role in inhibiting emergence of the selected species despite the neutral pH of the tailings especially in the control treatments. In the amended treatments the addition of organic matter and phosphate is likely to have reduced soluble copper, potentially reducing any toxic effects (see section 5.1.2.2).

5.2.1.2 Growth

Growth responses of the selected species to the amendment application was species and treatment specific with positive, negative, and no growth responses observed after 1 month of growth. The 2 g C kg^{-1} and 4 g C kg^{-1} treatment stimulated growth in *M. sativa* with the highest growth response in the 2 g C kg^{-1} treatment while little effect of either treatment was observed on the growth of *A. trachycaulum* and *P. pratensis*. Furthermore the 2 g C kg^{-1} and 4 g C kg^{-1} treatments inhibited the growth response of *F. pratensis* and *B. juncea* with the greatest degree of inhibition observed in the 4 g C kg^{-1} treatment. These growth responses could be due to a number of factors including: interactions of plants with humic substances, osmotic stress due to increased conductivity following amendment application, exposure to high ion concentrations over the duration of the experiment, responses to improved soil structure and potential effects of high copper levels present in the tailings. The physiological and biochemical differences between the selected species and their response to these factors, is likely to affect growth in different ways. Furthermore the high density of seedlings in the seedling flats and the shallow depth of soil in the trays relative to the pots may also be factors effecting growth response of the selected species.

Humic substances that remained in soil solution and did not participate in binding with soil particles were free to interact with establishing plant roots. Humic substances have been shown to have a wide range of direct (within cells) and indirect effects (in soil)

on plants that may either stimulate, have no effect, or inhibit plant growth depending on concentration. Stimulation of growth includes the indirect effects such as the promotion of macronutrient (N, P, K) and micronutrient (Fe, Cu, Zn, etc) uptake (Dormaar, 1975; Lee and Bartlett, 1976; Rauthan and Schnitzer, 1981) while direct growth stimulation relates to activity within cells such as enhanced protein synthesis (Bukvova and Tichy, 1967), direct auxin like activity (O'Donnell, 1973), inhibition of indole-3-acetic acid oxidase activity (Mato et al., 1971), enhancement of photosynthesis (Vaughan, 1969) and increased respiration rate (Sladky and Tichy, 1959). Humic substances also play a role in reducing soil solution elements such as copper, solubilization of micronutrients from mineral form, solubilization of some macronutrients such as K, Ca and P within the soil, and they have also been noted for their stimulation of the soil microbial population which can also promote growth (Chen and Aviad, 1990). Inhibition of growth has been suggested to occur by way of indirect effects that have been attributed to excessive concentrations of humic substances. Excessive levels of humic substances may decrease micronutrient uptake possibly due to excess ligands that bind the micronutrients and make them unavailable for plant uptake (Ayuso et al., 1996b; Pertuit et al., 2001). Direct inhibitory effects involve the reduction of enzyme activity, protein synthesis and decreases in photosynthetic and respiratory rates (Vaughan and Malcolm, 1985; Chen and Aviad, 1990). Most studies on the effects of soluble humic substances have focused on different humic derived sources such as peat, lignite, compost, soil, or sewage sludge with different concentrations from different sources having different effects on plants. However, humic substances from all sources have been shown by various authors to stimulate both root growth and elongation and shoot growth (Vaughan and Malcolm, 1985; Chen and Aviad 1990). Humic substances typically stimulate root growth, which is enhanced to a greater degree relative to shoot growth (Chen and Aviad, 1990). Humic substances have commonly been obtained from lignite sources such as leonardite and a number of experiments have focused on the concentration required to stimulate growth for a variety of species. Humic substances extracted from leonardite, in a hydroponic experiment, were found to stimulate the root and shoot growth of *Hordeum vulgare* (barley) at a maximum concentration of 5 mg C L^{-1} while concentrations of 10, 50 and 100 mg C L^{-1} caused inhibition of root and shoot growth (Ayuso et al., 1996b). A further

study by Ayuso et al. (1996a) carried out using a calcarious soil, suggested an addition of extracted humic substances at an optimal rate (highest biomass) of 50 mg C kg^{-1} but concentrations up to 200 mg C kg^{-1} were successful in increasing biomass of *Hordeum vulgare* (barley) while concentrations over 200 mg C kg^{-1} had an inhibitory effect. Piccolo et al. (1993) used seeds of *Lactuca sativa* (lettuce) and exposed them to humic substances extracted from sub-bituminous coal and found stimulation of total dry biomass up to 5 g L^{-1}. Whiteley and Williams (1993) suggested that soluble extracts from lignite (peroxide solubilization) applied in mine tailings (~36 g kg^{-1}) had no positive effect and inhibited both root and shoot growth of *Agrostis capillaris* (Bent grass). The majority of authors have suggested that concentrations of humic substances from a variety of sources should be applied in concentrations of 50 – 300 mg L^{-1} in order to stimulate root and/or shoot growth (Chen and Aviad, 1990). The concentration of modified humic substances present in soil solution following the incubation period was not determined but the amendment was applied in rates of 2 g C kg^{-1} (7.5 g kg^{-1}) and 4 g C kg^{-1} (15.0 g kg^{-1}). These concentrations exceeded most of the recommended rates of application for an experiment conducted in soil or soil like medium.

High concentrations of ions within the soil (see section 5.1.2.3) decreases soil water potential resulting in increased difficulty of water uptake by plants. The net effect on plants is a reduction in available water causing osmotically induced water stress leading to decreased growth and will be discussed in the longer term experiment in section 5.2.2.1. Furthermore increased levels of potassium, phosphate and copper within the soil can impair the uptake of other nutrients inducing deficiency and in the case of copper can cause toxicity inhibiting the growth of plants. These factors will also be discussed in the long term experiment (see section 5.2.2.1). Changes in soil structure due to the application of amendment were not measured in the short term growth chamber experiment but the effect on plant growth will be discussed in the field experiments (see section 5.2.3.3).

5.2.1.3 Photosynthetic Pigments

Medicago sativa, *P. pratensis*, and *A. trachycaulum*, species which showed positive and neutral growth responses, exhibited no significant changes in their chlorophyll a and b contents. However, species with negative growth responses to amendment application such as *B. juncea* and *F. pratensis* showed reductions in both their chlorophyll a and b contents. Carotenoid content was not effected by the amendment in most species and treatments. However, both the 2 g C kg^{-1} and 4 g C kg^{-1} treatments for *F. pratensis* and the 2 g C kg^{-1} treatment for *P. pratensis* experienced reductions in carotenoid content. Similar to the growth responses, it is difficult to attribute changes in pigment content to any one particular factor. The factors influencing the pigment content of the selected species are likely to include: interactions of plants with humic substances, osmotic stress due to increased conductivity following amendment application, exposure to increased ion concentration over the duration of the experiment, and potential effects of the high copper levels present in the tailings.

Few studies have investigated the effect of humic substances on plant pigments but it has been suggested that humic substances uptaken into plants increase the chlorophyll contents in leaf tissue through a direct effect on the biochemical cycles such as chlorophyll synthesis (Chen and Aviad, 1990). However, little is known on the exact influence of humic substances on the photosynthetic apparatus and chlorophyll production (Vaughan and Malcolm, 1985). Studies have also shown increases in chlorophyll content does not necessarily result in higher plant yields (Chen and Aviad, 1990). Sladky and Tichy, (1959) applied 600 mg of humic substances onto *Begonia semperflorens* using a foliar spray, and determined an increase in total chlorophyll content by 11 to 24 % depending on individual responses. Another study by Sladky (1959) applied soil derived humic and fulvic acids at a rate of 50 mg L^{-1} to a hydroponic solution. *Solanum lycopersicum* (tomato) plants showed an increase a 63% in total chlorophyll content using humic fractions and 69 % in the fulvic fraction relative to control treatments. Increases in carotenoid and xanthophylls contents have also been observed (Sladky, 1967).

Deficiencies of nutrients such as magnesium and iron have the potential to develop due to excess chelation by humic substances (Ayuso et al., 1996b; Pertuit et al.,

2001). Magnesium acts as the central core of the chlorophyll molecule residing in the porphryin ring portion of the molecule. Thus any impairment in the uptake of magnesium creating a deficiency is likely to result in a decrease in the chlorophyll content of the leaf (Bennett, 1993). Furthermore as magnesium is also an essential co factor in a number of enzymes such as transphosphorylase, dehydrogenase, and carboxylase deficiencies can further disrupt biochemical pathways (Bennett, 1993). Iron is essential for the synthesis of chlorophyll and the role of iron has been suggested to be related to porphyrin ring formation. Activity of heme-containing enzymes and the contents of both heme and chlorophyll are influenced in a similar manner by the iron content suggesting that a step in chlorophyll synthesis is linked to iron supply (Bogorad, 1960). Studies with plants subjected to iron deficiency result in a decreased rate of conversion of eoproporphyrinogen III to protoporphyrin. Iron then plays an important role in the formation of porphyrin, a key component of the chlorophyll molecule (Lascelles, 1956). Thus deficiencies in iron will lead to decreased formation of porphyrin molecules anda may be responsible for the reduced chlorophyll content.

Osmotic stress induced by the high concentration of phosphate, potassium and humate ions added following amendment application (see section 5.1.2.3) may have caused water stress for some species. Water stress and subsequent oxidative stress may have lead to pigment breakdown and the disruption of pigment synthesis and will be discussed in the long term experiment (see section 5.2.2.2). Elevated phosphate, potassium and copper levels in soil solution may also impair uptake of micronutrients leading to a deficiency disrupting chlorophyll synthesis. The role of these elements will also be discussed in the long term growth chamber experiment (see section 5.2.2.2)

5.2.2 Long Term Growth Chamber Experiment

5.2.2.1 Growth

Growth response of the selected species in the long term growth chamber experiment was similar to those found at 4 weeks in the short term growth chamber experiment with a small number of exceptions discussed below. The growth response of the selected species to modified humic substances was previously discussed in section 5.2.1.2 but further discussion on osmotic stress due to increased conductivity following

amendment application, exposure to high ion concentrations over the duration of the experiment and the potential effects of the high copper levels present in the tailings will be covered in this section.

Tailings used in the long term experiment were slightly saline prior to amendment application and increased to moderate conductivity levels in the 2 g C kg^{-1} (4.93 dS m^{-1}) and 4 g C kg^{-1} (7.91 dS m^{-1}) treatments (see section 5.1.2.3) due to the addition of potassium, phosphate and humate ions (see section 5.1.2.2). Salt tolerance of the selected species varies according to published source and likely indicates some variation based on species variety, environmental conditions, and soil type used. According to the US Department of Agriculture (1969), *M. sativa* and *F. pratensis* can tolerate salinity between 4 - 12 dS m^{-1}. Swift (1997) suggested that *A. trachycaulum* and *M. sativa* have moderate saline tolerance between 4 - 8 dS m^{-1} while *P. pratensis* has low salt tolerance. In addition Hardy BBT limited (1989) lists the saline tolerance of *A. trachycaulum* as 8 - 15 dS m-1, *M. sativa* as 4 - 8 dS m^{-1}, and *P. pratensis* as low and Sharma and Manchanda, (1997) described *B. juncea* as having saline tolerance of 4 - 6 dS m^{-1}. Growth of the selected species was somewhat unexpected based on saline tolerances. Conductivity values above the range of saline tolerance for the selected species were expected to result in decreased growth. Species such as *B. juncea* which did have a decreased growth response is likely due to conductivity values above its range of tolerance. However, species such as *A. trachycaulum* were grown in tailings within the suggested saline tolerance range but had a decreased growth response relative to the control at 12 weeks. These results suggest that for most species a response to humic substances and salinity was likely present.

Osmotic stress in the amended treatments was likely present with increasing stress with treatment rate while species responses were dependant on sensitivity to osmotic stress. Water stress, induced by an osmotic imbalance between the plant and the soil and an inability to uptake water from its environment, affects many complex physiological and biochemical processes including: transpiration, metabolic changes, ion movement, nutrient uptake, membrane integrity, signaling pathways, protein metabolism, transpiration, photosynthetic and respiration rates and cell elongation (Lambers et al., 1998). Depending on the degree of stress and the species involved growth responses to

osmotic stress typically show a reduction in both root and shoot growth, with shoot growth usually inhibited to a greater degree. Thus root to shoot ratios of plants typically change due to water stress (Munns and Termatt, 1986). Growth limitations are thought to be in part due to interference and disruption of the carbon balance between photosynthesis and respiration and a reduction in energy and carbohydrate available for growth. Carbon balance is primarily disrupted by reductions in photosynthetic rate which may decrease by up to 100% while respiration rate may either increase or decrease but never becomes completely impaired resulting in imbalance between the two (Flexas et al., 2005). Reduction in photosynthetic rate is primarily due to decreased carbon assimilation as a result of stomatal closure and under extreme water stress secondary oxidative stress can also occur providing less carbohydrate available for growth. Respiration rate typically decreases due to reduced phytosynthate assimilation though respiration rate can increase under severe water stress (Flexas et al., 2005). Further effects of water stress include a reduced rate of leaf emergence and an overall reduction in leaf size (Munns, 2002).

One major problem associated with high ion concentration in soils is the disruption of nutrient uptake. High concentrations of soluble potassium in the soil can induce nutrient deficiencies in other elements due to competition for uptake on transport proteins within the root cell membranes. Deficiencies of elements such as magnesium and calcium have been reported due to excess amounts of potassium in the soil (Locascio, 1993). It has also been suggested that increased levels of phosphate can also reduce soluble content of iron and zinc levels in soil solution and lead to a deficiency of both metals in plants (Sinclair, 1993). Interference on normal nutrient balance and the creation of deficiencies can result in disruption of biochemical/metabolic pathways and proper enzyme/protein function. Deficiencies in magnesium and iron are particularly damaging as magnesium is part of the chlorophyll molecule and iron is involved in its synthesis serving as an electron carrier in the photosynthetic apparatus (Bogorad, 1960; Bennett, 1993). The disruption of biochemical reactions due to nutrient deficiencies and reduced production of chlorophyll will lead to less photosynethetic output and decreased growth.

A possible contributing growth factor is the presence of copper. Soluble copper content in the tailings soil solution was not measured but high total levels of copper (see section 5.1.2.2) may have had an effect on the growth of the selected species. Copper toxicity is dependant on the sensitivity of individual species with sensitive species showing growth reduction at soluble concentrations as low as 15 – 20 $\mu g\ g^{-1}$ (Kabata-Pendias and Pendias, 1992), with general estimates on toxicity around 20 to 100 $\mu g\ g^{-1}$ (Jones, 1998). Root malformation and decreased root development and biomass is one of the common symptoms of copper toxicity (Kabata-Pendias and Pendias, 1992). Copper is also known to reduce photosynthetic output due to a disruption of the photosynthetic pathways and pigment synthesis. Impairment of a plants photosynthetic machinery is likely to a decrease in the growth response of the selected species (see section 5.2.2.2). In the amended treatments the addition of organic matter and phosphate is likely to have reduced soluble copper with increased reduction with increasing treatment rate potentially reducing any toxic effects (see section 5.1.2.2).

A small number of differences between the short term and long term experiments were observed. In the short term experiment at 4 weeks post seeding *A. trachycalum* exhibited an absence of any growth response to amendment application while in the long term experiment at 5 weeks a significant reduction of root and shoot biomass was observed in the 4 g C kg^{-1} treatment. This result may be due to changes in saline tolerance of *A. trachycaulum* at different growth stages as many plant species vary in terms of their saline tolerance with growth stage. Some plant species such as *Zea mays* (corn) *Hordeum volgare* (barley) and *Triticum aestivum* (wheat) are more resistant to saline stress during germination and during establishement saline tolerance decreases (CDA, 1977). Other species such as *M. sativa* (alfalfa) and *Beta vulgaris* (sugar beat) are sensitive to saline stress during germination and become more saline tolerant as they establish and mature (CDA, 1977). However, no published material exists on the degree of saline tolerance for *A. trachycaulum* at varying growth stages. In the long term experiment, root biomass and shoot height for *M. sativa* were stimulated but no significant differences were observed between the 2 g C kg^{-1} and 4 g C kg^{-1} treatments where as in the short term experiment stimulation was larger in the 2 g C kg^{-1} treatment than in the 4 g C kg^{-1} treatment. Root to shoot ratio of *M. sativa* in the amended

treatments of the long term experiment was significantly lower than the control, while in the short term experiment no change was observed in the amended treatments. This results suggests greater shoot growth proportionally to root growth in the long term experiment. Changes in growth of *M. sativa* may be related to the fact that there was a limited number of plants in pots (long term growth chamber experiment) compared to the short term growth chamber experiment with more crowded flats with little soil depth. *Medicago sativa* and *B. juncea* showed a 20-50% increase in shoot biomass in the long term experiment compared to the short term experiment at 4 weeks. The different aspect of the growth conditions may have led to this overall increase in biomass. such that changes to the competitive factors for light and soil depth may be responsible for some of the growth response of the selected species.

The growth response of *M. sativa* and *B. juncea* at 12 weeks was dramatically different from the response at 4 weeks despite that there were no significant changes in conductivity of the treatments. No significant differences in root or shoot biomass, root to shoot ratio, or height for *B. juncea* and *M. sativa* were observed between the treatments at 12 weeks. At 12 weeks *A. trachycaulum* and *F. pratensis* both continued to show a negative growth response with amendment application exhibiting a significant reduction in shoot height and root and shoot biomass in the 2 g C kg^{-1} and 4 g C kg^{-1} treatments. The results of *M. sativa* suggest that the greatest degree of growth stimulation by humic substances occurred during the early growth stages following emergence while at 12 weeks as the plants grew and established, the degree of growth stimulation was reduced yielding no significant differences between the amended treatments and control. Few studies have considered how the stimulatory effects of humic substances vary with the growth stage of the species; most studies conducted investigating specific germination or longer term yield responses (Chen and Aviad, 1990). Results from leaf area, biomass and height for *B. juncea* at 12 weeks strongly suggest an early recovery in growth due to the development of tolerance mechanisms to saline stress within the plants. Recovery in growth is likely due to the development of osmotic balancing between the plant and tailings and induction protective mechanisms within the plant (Xiong and Zhu, 2002). However, the growth response of *A. trachycaulum* and *F. pratensis* at 12 weeks suggests

a greatly delayed recovery or an absence of recovery from osmotic stress for these species.

5.2.2.2 Photosynthetic Pigments

The pigment content of the selected species was influenced by a similar series of factors as outlined in section 5.2.1.3. Pigment contents of the selected species at 4 and 5 weeks were similar to the results of the short term experiment with minor differences which will be discussed below. The effect of modified humic substances on pigment content of the selected species was previously discussed in section 5.2.1.3. Further discussion on pigment content and the effect of osmotic stress due to increased conductivity following amendment application, exposure to high ion concentrations over the duration of the experiment and high copper levels present in the tailings is discussed in this section.

Water stress can affect pigment content and the overall photosynthetic apparatus. Water stress induced by high concentrations of solutes disrupts biochemical processes within plants and is likely to disrupt the synthesis of chlorophyll and carotenoid pigments (Lambers et al., 1998). Water stress typically reduces the photosynthetic rate by way of stomatal closure due to decreased CO_2 intake but also leads to an increased potential for oxidative stress from a buildup of excess excitation energy (Smirnoff, 1993). The excess excitation energy leads to an increased production of activated oxygen species and can ultimately lead to photo bleaching of pigments molecules themselves (Demmig-Adams and Adams, 1992). Production of activated oxygen species such as superoxide, hydrogen peroxide and hydroxyl radical are capable of damaging membranes, photosynthetic pigments, proteins, lipids, and nucleic acids (Asada, 1994). Damaging effects of reactive oxygen species and photobleaching induced by water stress as well as a reduction in the rate of synthesis are likely to reduce both carotenoid and chlorophyll pigment contents. The presence of reactive oxygen species stimulates the detoxification process by increasing the activity of antioxidant compounds such as ascorbic acid, glutathione, thioredoxin, carotenoids and reactive oxygen scavenging enzymes (Xiong and Zhu, 2002).

Deficiencies of magnesium and iron due to the addition of humic substances and the effect of these deficiencies on pigments were discussed in section 5.2.1.3. Deficiencies of these two nutrients may also develop due to a high concentration of potassium interfering with magnesium uptake and high levels of phosphate which have been suggested to induce iron deficiency (see section 5.2.2.1). Furthermore copper has also been shown to have a number of negative effects on the production of pigments and the entire photosynthetic mechanisms. Copper specifically has been shown to have a phytotoxic effect on the photosynthetic electron transport system of *Spinacia oleracea* (spinach) (Sandmann and Boger, 1980). Copper also has the potential to induce an iron deficiency which may lead to chlorosis of the leaves (Jones, 1998). Toxic concentrations of copper have also been suggested to inhibit iron and manganease metabolism in the shoot tissues resulting in disruption of the photosynthetic pathway by inhibiting the synthesis of chlorophyll (Maksimiec and Baszynski, 1996).

A couple of minor differences between the pigment response of the long term growth chamber experiment compared to the short term growth chamber experiment were noted. In the long term growth chamber experiment, *B. juncea* exhibited decreases in chlorophyll a and b contents in 4 g C kg^{-1} treatment at 4 weeks but in the short term growth chamber experiment reductions were also observed in the 2 g C kg^{-1} treatment. Furthermore, a significant reduction in carotenoid content was observed in the 4 g C kg^{-1} treatment while no similar response was observed in the short term growth chamber experiment. Similarly for the growth response, pigment differences between the long term and short term growth chamber experiments may be related to reduced competitive factors which may result in increased stress. Pigment content response to amendment application for *A. trachycaulum* at 5 weeks post seeding was similar to the growth response, such that a stress response leading to decreased biomass and pigment production was observed. Reduction in chlorophyll contents were observed in the 2 g C kg^{-1} and 4 g C kg^{-1} treatments relative to the control, while a reduction in carotenoid content was observed in the 4 g C kg^{-1} treatment. However, in the short term experiment no similar responses were observed. Like the growth response, this might suggest differences in response to humic substances, micronutrient deficiencies or osmotic stress according to growth stage or competitive effects in the more crowded flats. In the long

term growth chamber experiment at 5 weeks, chlorophyll contents of *F. pratensis* exhibited reductions in chlorophyll content in the 2 g C kg^{-1} treatment and 4 g C kg^{-1} treatment while in the short term growth chamber experiment at 4 weeks reductions were only noted in the 4 g C kg^{-1} treatment. Like *A. trachycaulum* competitive factors or growth staging differences may be responsible for the differences such that susceptibility to osmotic stress increased, ability to uptake micronutrients or response to humic substances changed.

At 12 weeks post seeding, chlorophyll content remained reduced in a number of species. However, *M. sativa* was the exception where at 12 weeks no significant differences in chlorophyll content were observed between the treatments. *Brassica juncea* continued to show reduced chlorophyll a content the 4 g C kg^{-1} treatment and reduced chlorophyll b content in the 2 g C kg^{-1} and 4 g C kg^{-1} treatments despite a recovery in growth response. This result suggests that impairment in chlorophyll production remained despite the apparent development of tolerance mechanisms as observed by the lack of significant differences in the growth response from the control. Another unexpected result was that *A. trachycaulum* chlorophyll a and b contents were not significantly different between the treatments by 12 weeks. While the growth response continued to show reduced growth, response of the pigments would suggest that normal synthesis of pigments had resumed or that antioxidant production reduced and prevented oxidative stress. However, carotenoid production remained impaired in the 4 g C kg^{-1} treatment suggesting the pathway of synthesis remained inhibited or that degradation still occurred. Chlorophyll a and b content of *F. pratensis* at 12 weeks continued to exhibit a reduction in the 4 g C kg^{-1} treatment relative to the control. However, by 12 weeks no significant differences between the 2 g C kg^{-1} treatment and the control were observed.

5.2.2.3 Anthocyanin

Anthocyanins play an important role in light attenuation in response to high light stress which can be induced due to closure of stomata during osmotic stress (Krol et al, 1995). Anthocyanins have been shown to function in the reduction of photoinhibition and photobleaching of chlorophyll in high light conditions (Steyn et al., 2002). Visual

observation of *B. juncea* and *M. sativa* stem tissue appeared to show a relationship between presence of anthocyanin (red colouration) and treatment. Stem tissue was selected due to visual appearance of differences in red colouration between different treatments. However, the stem tissue obtained from *M. sativa* and *B. juncea* did not show any significant differences in anthocyanin content among the treatments at 12 weeks. The absence of any anthocynanin content differences in *M. sativa* was not unexpected as growth response and chlorophyll contents do not suggest the presence of any inhibitory effects, suggesting the absence of any major stresses. *Brassica juncea* on the other hand, despite exhibiting signs of increased tolerance by way of growth recovery still had lower chlorophyll content and decreased transpiration in the 4 g C kg^{-1} treatment at 12 weeks compared to the control. The source of tissue is likely to have played a role in the absence of any significant differences between the treatments for *B. juncea* as stem tissue typically plays a much lesser role in photosynthesis, with leaves being a more suitable choice for anthocyanin extraction.

5.2.2.4 Electrolyte Leakage

Under stress conditions membrane damage or disruption of the normal composition of the membrane can occur and result in membrane dysfunction (Sullivan and Ross, 1979). Damage or disruptions typically lead to increased membrane permeability and thus electrolyte leakage from cells may occur. It has been suggested that electrolyte leakage is a good indicator for environmental stresses such as osmotic (Sullivan and Ross, 1979), low temperatures (Saelim and Zwiazek, 2000) and salt stress (Chen et al., 1999). Osmotic stress and saline stress have been shown to cause increased electrolyte leakage while no research has been conducted on humic substances and their effects on membrane stability (Sullivan and Ross, 1979). *Medicago sativa* and *B. juncea* exhibited higher electrolyte leakage in the 4 g C kg^{-1} treatment relative to the control at 4 weeks. However, by 12 weeks no significant differences were observed between the treatments for either species. These results suggest that at 4 weeks both species showed the signs of stress in the 4 g C kg^{-1} treatment. Growth response results of *M. sativa* indicated a stimulation in growth in the 2 g C kg^{-1} and 4 g C kg^{-1} treatments. The 2 g C kg^{-1} treatment exhibited greater increases in biomass relative to the 4 g C kg^{-1} treatment

and electrolyte leakage may provide some insight as to why. Osmotic stress was more likely to occur in 4 g C kg^{-1} treatment due to a higher concentration of ions with the modified humic amendment. Thus, osmotic stress and the subsequent production of reactive oxygen species (free radicals) produced under high light stress may be responsible for inducing the higher electrolyte leakage in the amended treatments relative to the control treatment. Furthermore, a greater degree of osmotic stress may be responsible for the lower performance of the higher 4 g C kg^{-1} treatment relative to the 2 g C kg^{-1} treatment. The electrolyte leakage response of *B. juncea* was unexpected, as growth reductions followed a trend of decreasing biomass with increasing treatment rate suggesting that both the 2 g C kg^{-1} treatment and 4 g C kg^{-1} treatment might show signs of membrane dysfunction. Thus, this result might indicate that some tolerance mechanisms had begun to develop and that membrane repair may have occurred by the time 4 week measurements were taken. Furthermore, results of both species at 12 weeks may suggest that membrane repair had occurred yielding no differences between the 2 g C kg^{-1} and 4 g C kg^{-1} treatments and the control. Electrolyte leakage from *A. trachycaulum* and *F. pratensis* were only measured at 12 weeks with no comparisons from 4 or 5 weeks available. Results for these species also indicate a relative absence of membrane damage in both treatments at 12 weeks. This result, coupled with growth results of *A. trachycaulum* and *F. pratensis,* suggest that membrane damage may have been present during the earlier stages of growth.

5.2.2.5 Transpiration

Osmotic stress typically induces a reduction in transpiration rate due to stomatal closure or partial closure (Heath et al., 1985). Results from the transpiration measurements show no significant differences between treatments at 12 weeks for *M. sativa*, *A. trachycaulum* and *F. pratensis*. *Brassica juncea* was the only species to show a significant reduction in transpiration at 12 weeks, with a decrease in transpiration in both the amended treatments relative to the control. The response of *B. juncea* is somewhat unexpected as growth and electrolyte leakage responses suggest an early recovery from stress. Furthermore, the response of *A. trachycaulum* and *F. pratensis*, coupled with their electrolyte leakage values may indicate a degree of recovery by these species despite an

overall loss of growth. The fact that photosynthetic production remained impaired for both *A. trachycaulum* and *F. pratensis* further suggests that any recovery due to stress was not completely overcome. Thus in combination, these results may also suggest that recovery took a significant amount of time to occur and that the growth of these species was delayed enough to have resulted in differences between the control and treatments even by 12 weeks.

5.2.3 Field Experiments

5.2.3.1 Seedling Emergence

Emergence values at 2 weeks in season 2003 tended to be higher than emergence at 4 weeks for the majority of the treatments while there was very little difference between 2 and 4 weeks in field season 2004. Decreased emergence between 2 and 4 weeks in field season 2003 was an indication of mortality, likely due to the dryer conditions and not enough moisture for successful germination. However, field season 2004 conditions were excessively wet during seeding and may have led to decreased oxygen content within the tailings inhibiting germination. Hypoxia can reduce germination rate and may have occurred within the tailings (Benvenuti and Macchia, 1995). Therefore, it is possible that hypoxia played a role in the emergence in field season 2004. In addition the low pH of areas within the treatments of field season 2004 likely led to decreased emergence. Typically germination of most seeds is inhibited at pH values less than 3 (Salter and McIlvaine, 1920). At these low pH values the availability of essential mineral nutrients is decreased and the availablility of toxic metals is increased leading to an inhibition of cellular processes (Adams 1984). Emergence of the selected species was measured and with visual observations of the tailings and comparrions with the pH map (Appendix D) suggested that pH values less than 5 reduced emergence. The reduction in emergence despite pH values that were not extremely acidic could be related to the high concentrations and availibity of copper (see section 5.1.2.2).

Emergence of the selected species in both field seasons at 2 weeks was lower than the emergence of the same selected species used in the growth chamber experiment. Lower emergence values are likely related to less than ideal moisture conditions and resulting osmotic stress, variable temperature, and likely higher soluble concentrations of

humic substances inhibiting emergence (see sections 5.1.3.4 and 5.2.1.1). In field season 2003, emergence of *P. pratensis* was poor in all treatments while *M. sativa, F. pratensis* and *A. trachycaulum* were moderately successful with emergence between 20 – 60% in all treatments. *Brassica juncea* emergence was the highest of the plant species with 70% or higher emergence in all treatments. Due to low survival of *P. pratensis* and *B. juncea* in field season 2003, the species were replaced with *A. elongatum* and *F. rubra* for field season 2004. Results were similar to field season 2003 with *M. sativa*, *F. pratensis* and *A. trachycaulum* showing moderately successful emergence with values between 20 and 60% in all treatments. *Agropyron elongatum* was the most successful species in season 2004 with greater than 60% emergence, while *F. rubra* had less than 40% in all treatments. Overall seedling emergence is likely related to saline tolerance of the selected species during the emergence stage of growth. In addition to the saline tolerance of the selected species outlined in section 5.2.2.1, *A. elongatum* has been suggested to tolerate between 11 to 16 dS m^{-1} while saline tolerance of *F. rubra* has been suggested to varying widely based on cultivar type with a range between 5 to 9 dS m^{-1} (Hardy BBT limited 1989).

In field season 2003, amendment application tended to result in decreased emergence as treatment rate increased. At 2 and 4 weeks, a decrease in seedling emergence relative to the control was observed for *F. pratensis* and *A. trachycaulum* in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments, while *M. sativa* showed a reduction in emergence in the 4 g C kg^{-1} treatment. At 4 weeks, *B. juncea* had decreased emergence relative to the control in the 3 g C kg^{-1} treatment and *M. sativa* showed no significant differences between the treatments. However, in field season 2004 no effect of amendment on emergence for any species was observed at either 2 or 4 weeks. Increasing conductivity with increasing levels of amendment application, with less than ideal field conditions likely led to reduced emergence in the amended treatments. The absence of any significant differences in field season 2004 despite a similar conductivity as season 2003 is likely due to more favorable moisture conditions relative to field season 2003 which was much drier.

5.2.3.2 Survival

In both field seasons, survival results had similar trends as the emergence results from 2 and 4 weeks but with increased mortality of individuals over time in all treatments with the control treatment generally having the greatest survival. The exception was the survival of *B. juncea* and *P. pratensis* in field season 2003, all *B. juncea* individuals were lost to herbivory and all of *P. pratensis* to post emergence mortality. Survival in field season 2003 was highest in *M. sativa* with up to 60% while survival was lower for *F. pratensis* (20%) and *A. trachycaulum* (40%). In field season 2003 decreased survival with increasing treatment rate was observed for *F. pratensis* and *A. trachycaulum* while no significant differences between treatments were observed for *M. sativa*. Survival for *F. pratensis* was reduced in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments with only 2-3 plants surviving until harvest, thus removing the potential for statistical comparison with the other treatments. In field season 2004, amendment application had no significant affect on survival in any of the treatments with a survival rate between 15 - 40 % for all species. During the initial growth stages, tolerance to saline conditions, dependant on cultivar and species (see sections 5.2.2.1 and 5.2.3.1) is likely to play a major role in the performance of individual species, affecting their final survival. Decreases in conductivity over time in the field seasons did occur (see section 5.1.3.4) and as a result osmotic stress over the field seasons decreased with greatly reduced levels of osmotic stress present by harvest. In field season 2003, plants experienced dryer environmental conditions relative to field season 2004, a factor that likely contributed to the decreased survival of *F. patensis* and *A. trachycaulum*. The higher and more favorable moisture levels in field season 2004 and rapid decrease in conductivity over the growing season likely contributed to lower osmotic stress levels and may be responsible for the absence of differences in survival between the treatments. However, despite greater moisture levels and a more rapid decrease in conductivity over the field season 2004 growth period, lower overall survivorship relative to field season 2003 was observed. Field season 2004 did contained areas of significantly low pH, a condition that was not present in field season 2003 (see section 5.1.3.2 and Appendix D). Low pH values tended to decrease the survival and growth of species but due to the sampling protocol individual pH values associated with plots were not known. General comparisons and estimates using information contained

within Appendix D provides some clues on which areas were lower in pH. With this in consideration, it was thought that where survival was unexpectedly low pH was the likely cause. It should be noted that despite the presence of species such as *F. rubra,* that have shown some acidic tolerance (Hardy BTT limited, 1989) little to no survival of any species regardless of treatment was noted in regions of low pH (<4.5). The greater mortality in these regions is likely due to increased soluble levels of iron and more specifically copper causing toxicity (see section 5.1.2.2) and/or reduced nutrient availability at low pH.

Increases in soil structure in theory should have led to more favorable soil conditions such as ease of root penetration, water infiltration and drainage and increased water holding capacity. However despite improved structure due to modified humic substance application (see section 5.1.3.6) no significant increase in survival was observed for any species.

Visual observations of remaining species after field season 2003 and 2004 were made (Appendix G). *Medicago sativa* was successful in establishing, flowering and setting seed in all treatments. Germination of *M. sativa* seedlings released from pods was also noted 2 years following initial seeding. Nodulation within the rooting zone was also observed and plants did not appear to be suffering from stress in subsequent years, likely due to their ability to fix sufficient quantities of nitrogen. *Agropyon trachycaulum* in field season 2003 and 2004 and *A. elongatum* were successful in maintaining their presence in the plots, individuals did flower and set seed in the second year following seeding. However, seed viability was not checked and no obvious sign of seedling emergence was noted. Signs of plant regression were present with little growth in subsequent years and leaf chlorosis noted likely due to depletion of supplemented nitrogen 1 year following seeding. Similar effects in tailings without continuous nitrogen supplementation have been noted by Johnson and Bradshaw (1977). *Festuca pratensis* planted in field season 2003 and 2004 and *F. rubra* planted in season 2004 had limited growth in subsequent years. Neither species flowered and both showed similar signs of regression as in the *Agropyron* species. However, grass species appeared to be somewhat successful in vegetative reproduction in some locations. A halo of weedy species in and around the treatment plots were observed, some possibly introduced and others naturally occurring

vegetation including: *Taraxacum officinale* (dandelion), *Atriplex sp.* (saltbush), *Solidago sp.* (goldenrod), *Cirsium arvense* (Canadian thistle) *Plantago sp.* (plantain), *Aster sp.* (aster), *Salix sp.* (willow), *Populus balsamifera* (balsam poplar), and *Melilotus sp.* (sweet clover). Observations also suggest that rotor tilling and sufficient fertilizer application in subsequent years following seeding may lead to the long term establishment of plants and promote the colonization of naturally growing species within the higher pH portions of the tailings.

5.2.3.3 Growth

In field season 2003 application of modified humic substances resulted in a range of growth responses with stimulation, inhibition and no effect. *Medicago sativa* was the only species to show stimulation of growth with the 4 g C kg^{-1} treatment showing a significant increase in shoot biomass while no other treatment showed any changes from the control. The growth response of *A. trachycaulum* was similar to the long term growth chamber experiment, though the 2 g C kg^{-1} treatment was not significantly different from the control in the field season 2003. However, a decrease in biomass of both shoots and roots within the 3 g C kg^{-1} and 4 g C kg^{-1} treatments was observed. Survival of *F. pratensis* was so poor in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments that analysis could not be conducted but this result did suggest that the soil conditions generated in the higher treatment rates was not favorable to the growth of the species. Survival for *F. pratensis* was high in the 2 g C kg^{-1} treatment and the growth response was not significantly different from the control. Growth responses in field season 2004 were similar to field season 2003, where *M. sativa* was the only species to show an increase in growth, with the 3 g C kg^{-1} treatment having greater shoot and root biomass as well as height compared to the control. The growth response of *A. trachycaulum* was also similar to field season 2003 with decreased growth in the 3 g C kg^{-1} and 4 g C kg^{-1} treatments. In field season 2004, the survival rate of *F. pratensis* was higher in the amended treatments compared to field season 2003 and statistical analysis was possible. Results from the growth responses showed no significant differences in the amended treatments relative to the control with the exception of lower shoot biomass in the 4 g C kg^{-1} treatment. The growth responses of *A. elongatum* and *F. rubra* showed little effect of the amendment on

growth. *A. elongatum* had a slight increase in root biomass in the amended treatments while *F. rubra* showed a significant reduction in height.

The effect of humic substances, osmotic stress and potential effects of high soil copper levels previously discussed in the growth chamber experiments (see section 5.2.1.2 and 5.2.2.1), are all likely to contribute to the growth responses in the field experiments. In addition the decreasing conductivity levels over the growth season likely lead to lower osmotic stress as the growing seasons progressed (see section 5.1.3.4) affecting the growth response differently at different times depending on species sensitivity. Visual observations and meteorological data both suggest differing environmental conditions between the 2 field seasons and was likely to have profound effect on the growth responses of the selected species. Furthermore potassium and phosphate deficiencies are likely to have contributed to decreased growth and increased stress in the control treatments (see section 5.2.3.5). Increases in soil structure (see section 5.1.3.6) as a result of humic application in both field seasons, especially in field season 2004, may have led to more favorable soil properties that may have facilitated the growth response of the selected species. Field season 2004 also exhibited heterogenous pH within treatments. The pH ranged from neutral to less than pH 4. The effect of pH on increased heavy metal availability and decreased nutrient availability cannot be over looked as a growth effecting factor well as the direct effect of significant acidity on the plant itself. Unfortunately, sampling protocol from the field experiments did not allow for direct correlations between the growth response and pH sampled. As a result, the exact role of pH on growth could not be determined. Comparrisons made between the pH map (Appendix D) in relation to where emergence, survival and biomass data show values exceedingly low and unexpected are suggestive of a low pH effect. All of these above mentioned factors contributed to the growth response of the selected species. However, these factors are confounded such that the effect of any one factor and the extent of its effect on the growth response remains difficult to interpret.

5.2.3.4 Photosynthetic Pigments

The response of plant pigment contents to amendment application in field season 2003 varied according to species. *Medicago sativa* chlorophyll a, chlorophyll b and carotenoid contents showed no significant changes due to amendment application in any treatments. *Agropyron trachycaulum* response showed a significant increase in all pigments but only in the 3 g C kg^{-1} treatment. *Festuca pratensis* exhibited significant reductions in chlorophyll a, chlorophyll b and carotenoid content in the amended treatments relative to the control. In field season 2004, *M. sativa* and *A. elongatum* exhibited no significant differences in pigment content between the treatments. Unlike field season 2003, *A. trachycaulum* showed decreased chlorophyll a and b contents in the amended treatments in field season 2004. However, *F. pratensis* exhibited a similar trend as field season 2003 with significant reductions in all pigments in the amended treatments relative to the control. Decreased pigment content in the amended treatments in field season 2004 was also noted for *F. rubra*.

Similar to the growth response, pigment contents were influenced by a series of factors: the effect of humic substances on the production of pigments, nutrient deficiencies induced by high ion concentrations or excessive humic substance binding to metallic nutrients, osmotic stress and the potential effects of high soil copper levels have been discussed in the growth chamber experiments (see sections 5.2.1.3 and 5.2.2.2). In addition, potassium and phosphorus deficiencies were observed in both field seasons within the control treatments (see section 5.2.3.5) and likely lead to decreased synthesis of pigments. Furthermore, the higher moisture conditions of field season 2004 compared to field season 2003 may have lead to lower levels of osmotic stress resulting in greater pigment production and reduced levels of pigment destruction in the amended treatments (see section 5.2.2.2). It remains unclear why pigment contents in the amended treatments of *A. trachycaulum* in field season 2004 would show reductions relative to control treatments while in field season 2003, which appears to be a more stressful growing season, no reductions were observed. In addition, areas of lower pH in field season 2004 is likely to have influenced the pigment levels as plants growing in areas of low pH would have suffered from increased metal toxicity and likely micronutrient deficiencies,

as copper and iron would compete with micronutrients for uptake (see section 5.2.1.3 and 5.2.2.2).

5.2.3.5 Elemental Analysis

Humic substances have a wide range of effects on the uptake of nutrients and have been suggested to enhance growth by increasing macro and micro nutrient uptake (see section 5.2.1.2). Other authors have suggested that high concentrations of humic substances can lead to micronutrient deficiency due to excessive ligand binding of various metallic nutrients (see section 5.2.1.2). Furthermore, in relation to tailings, it has also been suggested that high instances of mortality of non metal tolerant cultivars of *Agrostis capillaris* grown in humic amended mine tailings was due to increased metal uptake causing toxicity (Whiteley and Williams, 1993).

Medicago sativa and *A. elongatum* under field conductions showed no significant changes in copper or iron content with amendment application in either shoot or root tissues. *Agropyron trachycaulum* showed no significant changes in copper or iron content in root or shoot tissue in both field seasons, with the exception of field season 2003 where shoot copper content in 3 g C kg^{-1} and 4 g C kg^{-1} treatments was higher than the control treatment. Copper and iron levels typically varied by species but contents from the selected species were generally much higher than expected for tissues. Leaf copper content is typically expected to range from 5 to 30 $\mu g\ g^{-1}$, with toxicity between 20 and 100 $\mu g\ g^{-1}$ (Jones, 1998). Iron in leaf tissue typically ranges from 100 to 500 $\mu g\ g^{-1}$, with a poorly defined range for toxicity but is general considered to occur between 500 and 1000 $\mu g\ g^{-1}$ (Jones, 1998). Results from the 2 field seasons typically showed higher root copper contents relative to shoots with copper content higher in the *Agropyron* species relative to *M. sativa.* All species possessed shoot copper levels above 70 $\mu g\ g^{-1}$ up to 154 $\mu g\ g^{-1}$ while root tissue results indicated levels as high as 693 $\mu g\ g^{-1}$. Iron content was high based on typical levels but generally not considered excessive with the exception of *A. trachycalum* in field season 2003, where iron levels were 1,090 $\mu g\ g^{-1}$ and 2,328 $\mu g\ g^{-1}$ in the shoot and root, respectively. Tissue copper content at this level, without the presence of visual tissue injury, suggests that the majority of copper was not likely within the cells. However, the potential for internal cellular concentrations to have

been high enough for copper to impair or disrupt biochemical pathways, effect nutrient uptake, pigments and growth response remains a possibility (see sections 5.2.2.1 and 5.2.2.2). The elevated copper levels could be in part due to contamination of above ground tissues by way of tailings dust entering leaf tissue through open stomata, becoming bound within the cuticle, or in crevices within the shoot structure. Other factors related to elevated root levels may include appoplastic movement of copper and iron into the root tissue with these ions becoming bound to cell walls, precipitated within the appoplast and possibly bound to the external root surfaces (Greger, 1999). Differences between the species iron and copper contents in the shoot and root tissues are likely related to differences at the level of uptake in the root membrane and the inherent ability to translocate metals from their roots to their shoot portions and the physical structural differences between the species in trapping tailings dust (Greger, 1999).

Phosphorus and potassium contents within shoot and root tissue were affected by humic amendment application with an increase in both elements observed. The increase was most pronounced in field season 2003 with only *M. sativa* showing no change in shoot potassium. Results from field season 2004 exhibited no clear trends with instances of increases in specific treatments. However, *M. sativa* phosphate content in root tissue, *A. elongatum* phosphate content in shoot and root tissue and *A. elongatum* potassium root content were all increased relative to the control in the amended treatments in field season 2004. Due to the presence of significant quantities of phosphate and potassium added along with the humic amendment application (see section 5.1.2.2), it is difficult to determine if the humic substances facilitated an increased uptake of potassium and phosphate or if the higher tissue content was a result of higher initial levels of phosphate and potassium in the amended treatments. Phosphate content in shoots typically ranges from 1,500 $\mu g\ g^{-1}$ to 10,000 $\mu g\ g^{-1}$ with a sufficiency level considered at 4,000 $\mu g\ g^{-1}$. Deficiency is considered to occur at levels less than 2,000 $\mu g\ g^{-1}$ and contents greater than 10,000 $\mu g\ g^{-1}$ are considered in excess (Jones, 1998). Potassium contents in shoot tissue range from 10,000 $\mu g\ g^{-1}$ to 50,000 $\mu g\ g^{-1}$ of dry leaf weight. Sufficienct levels are typically considered at 30,000 $\mu g\ g^{-1}$, while deficiency occurs at levels less than 15,000 $\mu g\ g^{-1}$ with toxicity occuring at levels above 50,000 $\mu g\ g^{-1}$ (Jones, 1998). Potassium deficiency is likely to lead to reduced growth, and problems with water status, as

potassium is involved in opening and closing of stomata and the maintenance of turgor pressure (Jones, 1998). In addition phosphate deficiency typically stunts growth as phosphorus is a critical component of some enzymes, ATP, RNA, and DNA (Jones, 1998). As a result the growth of the control treatments was likely affected by a deficiency in these nutrients

Results suggest that control treatments in field season 2003 for all species were greatly deficient in phosphorus, while in field season 2004 a smaller magnitude of deficiency is exhibited and may be related to the greater rate of fertilizer application in field season 2004 or from lateral movements of potassium and phosphate from amended plots into the controls due to the saturated nature of the tailings (see section 5.1.3.4). Application of an amendment containing soluble phosphate appears to have eliminated any potential for deficiency in the amended treatments. Similar to phosphorus, potassium was typically deficient in the control treatments for the selected species in field season 2003 and slightly above deficiency levels in field season 2004. The addition of amendment tended to increase plant potassium contents though shoot contents were only raised to levels just above deficiency or to levels still considered deficient for potassium. It should be noted that these results provided a "snap shot" at the time of harvest on the nutrient status of the plants, after significant leaching of ions had occurred over the growing season. Furthermore, in field season 2004 potassium and phosphate contents in the controls tended to be higher than field season 2003, while conversely amended treatment contents tended to be lower than field season 2003. These differences may be related to the higher rate of fertilizer application in field season 2004, which attempted to overcome any potential deficiencies observed in the control treatments of season 2003. Another likely source is lateral movement of ions from amended plots into the control treatment increasing the phosphate and potassium contents in the control treatments. In field season 2004 selected species in the amended treatments did not contain phosphorus and potassium levels as high as field season 2003, this may be attributed to the greater rate of conductivity reduction over field season 2004. Such a reduction is the result of a greater rate of leaching, leading to less readily available potassium and phosphate compared to field season 2003.

6. Conclusion

Mine tailings from the Central Manitoba mine site have a higher pH than anticipated, with neutral values present in tailings obtained for the growth chamber experiments as well as the field experiments. However, field season 2004 demonstrated the pH heterogeneity present in some locations within the tailings. Large changes in pH present over short distances, with changes of up to 4 pH units in less than 0.5 meters was observed. The addition of modified humic substances had no effect on the pH of tailings that were neutral, while pH values within the acidic range were raised following amendment application. Elemental analysis of the tailings showed that copper is likely a more significant problem compared to iron in the establishment of plants, as values of total copper exceeded toxic levels for most plants. Under lower pH conditions both metals are likely to have posed a problem to plant establishment and growth as they become more biologicaly available. The addition of modified humic substances significantly increased the conductivity of the tailings following a trend of increasing conductivity with increasing treatment rate. Conductivity was elevated to 2.17 dS m^{-1} in the 2 g C kg^{-1}, 4.93 dS m^{-1} in the 3 g C kg^{-1}, and 7.81 dS m^{-1} in the 4 g C kg^{-1} treatments in the long term growth chamber experiment. Difficulty in rotor tilling and mixing the amendment to the desired tailings depth in the field experiments led to conductivity values in all treatments higher than those in growth chamber experiments. Conductivity values were as high as 8.96 dS m^{-1} and 13.1 dS m^{-1} in 4 g C kg^{-1} treatment for field season 2003 and 2004, respectively. In the field experiments, precipitation was the most probable reason for a reduction in conductivity over the growing season as ions were leached away from the site both horizontally and vertically. Organic carbon prior to amendment application was very low and as expected, carbon levels were raised following the addition of modified humic substances. Carbon decreased over the growing season, likely due to microbial degradation and with some loss possibly due to leaching. Carbon contents increased dramatically between harvest and 1 year following treatment after an over wintering period in both field seasons. Carbon sequestering alone seems unlikely to account for the overall magnitude of the increase. Further investigation is needed to determine why such large carbon increases occurred in the amended treatments. The addition of modified humic substances to mine tailings did significantly

improve the structure of the tailings, with an increase in total aggregation that was maintained over the growing season. However, the extent of the improvement was likely dependent on moisture conditions following application and over the field season. Changes in aggregate fractions occurred over the field seasons where the total aggregation remained constant from the time of seeding to harvest, but an increase in the > 2 mm fraction with reductions in the proportion of smaller fractions was observed.

In the growth chamber experiments modified humic substances at 2 g C kg^{-1} and 4 g C kg^{-1} tailings had little effect on emergence rate and number of emerged seedlings, with a slight delay noted for some species in the emergence experiment. Growth and pigment responses also varied according to species with only *M. sativa* showing stimulation in growth with an increase in shoot and root biomass and height. The remaining species exhibited no significant benefit from the addition of the modified humic substances with *B. juncea* and *F. pratensis* exhibiting a negative response with an inhibition of growth. However, by 12 weeks *B. juncea* and *M. sativa* showed no significant differences in growth between the treatments while *A. trachycaulum* and *F. pratensis* showed an inhibition of growth. Modified humic substances caused an increase in electrolyte leakage of leaf tissue for both *M. sativa* and *B. juncea* in the short term, which is likely attributed to osmotic stress due to the high conductivity in the amended treatments. However, after 12 weeks of growth no differences in electrolyte leakage were observed between the treatments suggesting that *M. sativa* and *B. juncea* were able to recover from the initial osmotic stress. Field experiments had reduced seedling emergence compared to the growth chamber experiments. In field season 2003 modified humic substances had no effect on emergence or had an inhibitory effect while in field season 2004 no effect of amendment on emergence was observed. Survival followed the same trends between the two field seasons with environmental conditions likely responsible for the differences. In the field studies the growth response of the selected species to the humic amendment was similar to the growth chamber studies though *M. sativa* continued to show a benefit on its growth at harvest.

Overall the absence of any significant stimulatory effects of the amendment on growth of some species may be attributed to a high concentration of humic substances that are directly or indirectly impairing the growth of some of the selected species.

Another factor may be osmotic stress induced by high concentrations of ions in the amended tailings. High conductivity may have led to an impairment of biochemical and physiological processes within the plants as well as cause water stress. However, it would appear that *M. sativa* is capable of tolerating the high conductivities and able to take advantage of favourable humic substance concentrations in soil solution resulting in an increased growth response.

Rotor tilling, 2 fertilizer applications and regular watering over the growing season in the control treatments was successful in establishing species in the tailings in the first year of growth and may suggest a possible method of reclamation for the Central Manitoba site. The poor nutrient quality of the tailings resulted in regression of the grass species, *A. elongatum*, *A. trachycaulum*, *F. rubra*, and *F. pratensis* in subsequent years while the ability of *M. sativa* to fix nitrogen likely allowed the plants to remain successful for at least 3 years following initial seeding. With continued fertilizer application growth promotion of grass species could be accomplished. *Brassica juncea* and *P. pratensis* were less successful in the tailings and thus appear to be unsuitable for further revegetation efforts. Furthermore alternative species mixes may also be investigated using native or naturalized species along with tilling and fertilizer applications. In addition, *M. sativa* after 2 years of growth was able to act as a wind break likely able to reduce surface wind erosion. *Medicago sativa* plants were able to trap leaf litter and other organic debris in and around the base of the plants creating an organic cover in which small *M. sativa* seedlings were growing. Grass species in both field seasons were able to trap small amounts of organic material but are likely to have a much greater impact in preventing water erosion due to their fibrious rooting systems.

Additional research with modified humic substances in tailings should investigate their use as a nutrient supplement to promote growth instead of facilitating the development of soil structure. Application of humic substances in lower concentrations alone with an alternative organic amendment may prove more beneficial to the establishment and survival of plants in the tailings. Treatment rates applied in this study were likely above the range of stimulation and created osmotic stress on the selected species. Alterative organic amendments might include paper mill sludge, peat or leaf litter to increased water retention capacity, improve the soil aeration and promote the

development of structure. Further research related to this study could also involve the determination of soluble copper levels within the tailings as opposed to the absolute amount. This information would help determine the degree or potential of copper to pose a problem for plant growth. In addition a concentration determination of soluble humic substances after the incubation period would be valuable. Such information would be useful in finding a more suitable rate of humic amendment addition in order to stimulate a broader range of species and avoid the inhibitory effects due to excessively high concentrations of humic substances and high conductivity.

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Appendix A Unmodified Humic Substances Field Experiment

1 Introduction

The effect of unmodified humic substances (mini granule) on tailings chemical and physical properties and plant responses were investigated at the request of BlackEarth Ltd during field season 2004. Unmodified humic substances share the same parent material (lignite) as modified humic substances but have not undergone any modification procedure to alter the solubility or chemistry of the material. Unmodified humic substances contain humic acid, fulvic acid, and humin possessing: low water solubility, low pH (3.9), and a high cation exchange capacity (BlackEarth Humates Ltd, 2004).

The effects of humic substances on plants are typically investigated in which the humic substances are in a soluble form (Chen and Aviad, 1990). Coal derived humic substances are typically treated with an extraction agent such as hydroxides or peroxides to solublize the humic substances (Vaughan and Malcolm, 1985). Unmodified humic substances do not contain significant quantities of phosphorus or potassium but are high in carbon. The first objective of the experiments was to assess the effect of unmodified humic substances applied in rates of 2 g C kg^{-1} and 4 g C kg^{-1} on tailings physical and chemical properties. The second objective was to study the growth and physiological responses of the selected species growing in tailings amended with unmodified humic substances. It was hypothesized that unmodified humic substances would promote aggregation, increase the organic carbon, have little effect on the pH or conductivity and stimulate the growth of the selected plant species.

2 Methods and Materials

2.1 Experimental Setup

Field season 2004 included the addition of unmodified humic substances (mini granule) from BlackEarth Ltd in treatment rates of 4.0 and 7.9 grams amendment kg^{-1} tailings which, corresponds to 9,500 and 19,000 kg $hectare^{-1}$. The amendment was mixed to a depth of 15 cm of the tilled tailings. Amendment was applied in these rates to reach concentrations of 2 and 4 g carbon kg^{-1} tailings. A random block design was used for the placement of different treatments along with the modified humic treatments. Unmodified amendment treatments had no incubation period due to problems obtaining the materials with seeding following application. Fertilizer was applied to unmodified treatments as described in section 3.3.3. Seeding plots were established as outlined in section 3.3.3.1.

2.4 Tailings Collection and Analysis

Soil samples were collected as described in section 3.3.3.2. Samples were taken along with the modified humic amendment collection periods, with the exeption that pre treatment samples were collected a month before umodified humic amendment treatment application. Samples were analyzed for conductivity, pH, organic carbon and soil structure as described in section 3.3.3.2. Samples for elemental analysis were also collected and analyzed as described in section 3.3.3.5.

2.5 Emergence, Survival and Growth

Seedling emergence, plant survival until harvest and growth response was measured as described in section 3.3.3.3.

2.6 Data Analysis

Data was analyzed as outlined in section 3.4.

3 Results

3.1 Tailings Analysis.

Elemental analysis of the unmodified humic substances showed potassium and phosphate contents with values of 25.0 $\mu g\ g^{-1}$ and 88.2 $\mu g\ g^{-1}$, respectively. Iron and copper concentrations were also low with an average of 16 $\mu g\ g^{-1}$ for copper and 4637 $\mu g\ g^{-1}$ for iron.

The average conductivity of the treatments before application of modified and unmodified humic substances was of 3.28 $dS\ m^{-1}$. The 2 g C kg^{-1} unmodified and 4 g C kg^{-1} unmodified treatments were sampled following application (also considered time of seeding) of unmodified humic substances and conductivity was found to have increased from the background levels to 4.33 $dS\ m^{-1}$ and 5.46 $dS\ m^{-1}$, respectively. By the time of harvest, both unmodified treatments had lower conductivity values with no significant difference from their pre treatment values. One year following amendment application no differences were observed between the unmodified treatments and the control or between the unmodified treatment and the modified treatments. The tailings pH was not significantly affected by the addition of unmodified humic substances with treatments exhibiting chaotic changes in pH over the growing season.

Carbon content in the 2 g C kg^{-1} unmodified and 4 g C kg^{-1} unmodified treatments was increased to 0.70% and 1.15% respectively from the background level of 0.47%. No significant changes in carbon content were observed over the growing season. However, carbon content did decrease in the 4 g C kg^{-1} unmodified treatment 1 year following treatment.

Soil structure was not measured at the time of seeding due to the absence of an incubation period. Aggregation was measured from prior to amendment application and at harvest and compared against modified humic treatments samples from those times (Table A1). No significant differences were observed between treatments prior to amendment application. By harvest an increase in total soil aggregation was found in the 2 g C kg^{-1} unmodified treatment relative to the control while the 4 g C kg^{-1} unmodified treatment showed no significant difference from the control. In addition the modified treatments resulted in the largest increase in total soil aggregation. In consideration to

individual aggregate distributions the 2 g C kg^{-1} and 4 g C kg^{-1} unmodified treatments both had exhibited a significant increase in the 0.5 – 1.0 mm fraction and the 2 g C kg^{-1} treatment showed an increase in the 1.0 – 2.0 mm fraction relative to the control.

3.2 Plant Analysis

Emergence was measured at 2 and 4 weeks with emergence percentage higher at 4 weeks. Like the amended treatment, no significant differences from the control treatment were noted in emergence or survival until harvest in either the 2 g C kg^{-1} or 4 g C kg^{-1} unmodified treatments.

Unmodified humic amendment had no significant effect on the on shoot and root biomass for *A. trachycaulum, A. elongatum*, *F. pratensis* and *F. rubra* with the exception of *M. sativa* which did show a significant increase in shoot biomass in the 4 g C kg^{-1} unmodified treatment relative to the control (Table A2 and A3). Unmodified humic amendment had no significant effect on shoot height for *A. trachycaulum* and *A. elongatum*. A variable effect in shoot height was observed for the remaining species with stem heights slightly higher than the control or lower than the control in both the 2 g C kg^{-1} and 4 g C kg^{-1} unmodified treatments with no clear trends.

4 Discussion

4.1 Tailings

Unlike the modified humic amendment, unmodified humic substances did not contain significant quantities of phosphate and potassium. Iron and copper levels were also low and not likely to alter background levels. An increase in conductivity was observed in the unmodified humic substance treatments following amendment application although the actual increase in conductivity due to amendment itself is likely small. A decrease in conductivity was observed in the modified humic treatments between post treatment and seeding was likely the result of downward and lateral movement of ions. An increase in conductivity in the control treatments suggests that movement of ions from modified humic amendment treatment blocks into neighboring treatments was occurring. The control treatment increased by 1.12 dS m^{-1} by the time of seeding which was the time in which the unmodified amendment was applied. The 2 g C kg^{-1} and 4 g C kg^{-1} treatments increased in conductivity from base levels by 1.27 dS m^{-1} and 1.74 dS m^{-1}, respectively. Unfortunately, pre treatment samples were measured one month before amendment application allowing for lateral movements of ions to enter the plots. Thus it is possible that some of the conductivity increase associated with the unmodified treatment is due to ions moving into the unmodified treatments from neighboring modified humic amended treatments. As expected, conductivity levels decreased over the growing season and reached background levels by the end of the growing season with no significant differences from the control. The pH of the unmodified humic amendment (mini granule) is reported by BlackEarth Humates Ltd (2004) to be 3.9. Application of the unmodified amendment did not significantly alter the pH of the tailings. Carbon content of the unmodified treatments did not significantly change over the growing season. Decreases in the carbon content of mine tailings amended with modified humic substances were attributed to microbial degradation (Ibrahim and Goh, 2004). However, the soluble nature and reduction in conductivity would suggest leaching of soluble modified humic substances may have also occurred. The absence of any change in carbon content in the unmodified humic amended treatments over the growing season may suggest little microbial breakdown or that the insoluble nature of the unmodified amendment prevented leaching. The decrease in

carbon content from harvest to 1 year following treatment in the 4 g C kg^{-1} unmodified treatment but absence in the 2 g C kg^{-1} treatment was unexpected as no reduction is observed in the 2 g C kg^{-1} treatment.

Total aggregation was significantly greater in the 2 g C kg^{-1} treatment but no significant differences from the control were observed for the 4 g C kg^{-1} treatment at harvest. Individual fraction increases relative to the control was observed for both treatments in the 0.5 – 1.0 mm fraction, while only the 2 g C kg^{-1} treatment showed an increase in the 1.0 – 2.0 mm fraction. Modified humic substances resulted in larger increase in total aggregation which can likely be attributed to the differences in solubility and thus activity and interaction with soil particles.

4.2 Plant

Emergence and survival was not significantly affected by the addition of unmodified humic substances. No effect on the shoot or root biomass of *A. trachycaulum*, *A. elongatum*, *F. pratensis* and *F. rubra* was observed though *M. sativa* did exhibit a significant increase in shoot biomass in the 4 g C kg^{-1} treatment. The insoluble nature of the amendment provides less interaction between humic substances and the growing plants than the modified humic amendment. The majority of studies as noted by Vaughan and Malcolm (1985) and Chen and Aviad (1990) that investigate the effects of humic substances on plants used soluble humic substance extracts. The amount of humic substances in the unmodified humic treatments present in the soil solution is likely low due to the nature of the material. However, soluble content was not measured and only can be assumed to have been low. The effects of any soluble humic substances that were present are described in sections 5.2.1.2 and 5.2.2.1

5 Conclusions

The addition of unmodified humic substances resulted in a change in the tailings structure and thus would imply that the unmodified humic substances are able to bind particles and promote structure formation. However, amendment with modified humic substances produced a greater increase in soil aggregation than unmodified humic substances. Over a longer period of time, due to weathering and interaction with the environment, it is likely that the unmodified humic substances will play a larger chemical and physical role in the tailings and may lead to greater structural development.

Tailings treated with unmodified humic substances did not have the disadvantages associated with modified humic substances in that: unmodified humic substances did not substancially increase the conductivity to extreme levels following amendment application and had low potential for inhibitory concentrations of soluble humic substances. However, the nature of unmodified humic substances, being relatively insoluble, is also likely to reduce the potential for positive stimulatory effects in the short term. Despite the improvement in soil structure and lower conductivity associated with the unmodified humic substance amendment, no significant benefit to plant growth (biomass or height) was observed over the 3 month growth period.

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Table A1. Distribution of water stable aggregates in field season 2004 (% of mine tailings) in mine tailings amended with modified humic substances and unmodified humic substances at Pre Treatment and Harvest (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$).

	Aggregate Distribution				
	> 2.0 mm	1.0 - 2.0 mm	0.5 - 1.0 mm	0.25 - 0.5 mm	Total (> 0.25 mm)
Pre Treatment					
0 g C kg^{-1} tailings (Control)	0.52 ± 0.10 ^{a}	2.27 ± 0.37 ^{a}	3.09 ± 0.37 ^{a}	4.82 ± 0.56 ^{a}	10.70 ± 1.26 ^{a}
2 g C kg^{-1} tailings (modified)	0.52 ± 0.15 ^{a}	2.30 ± 0.42 ^{a}	3.46 ± 0.52 ^{a}	4.43 ± 0.89 ^{a}	10.71 ± 2.40 ^{a}
3 g C kg^{-1} tailings (modified)	0.46 ± 0.13 ^{a}	2.25 ± 0.63 ^{a}	3.12 ± 0.97 ^{a}	4.79 ± 0.84 ^{a}	10.62 ± 0.52 ^{a}
4 g C kg^{-1} tailings (modified)	0.49 ± 0.11 ^{a}	2.49 ± 0.29 ^{a}	3.50 ± 0.29 ^{a}	4.58 ± 0.46 ^{a}	11.06 ± 0.83 ^{a}
2 g C kg^{-1} tailings (unmodified)	0.78 ± 0.31 ^{a}	2.62 ± 0.95 ^{a}	3.39 ± 0.68 ^{a}	4.84 ± 0.69 ^{a}	11.63 ± 2.50 ^{a}
4 g C kg^{-1} tailings (unmodified)	0.44 ± 0.25 ^{a}	2.89 ± 1.07 ^{a}	3.80 ± 0.78 ^{a}	4.57 ± 1.00 ^{a}	10.24 ± 2.28 ^{a}
Harvest					
0 g C kg^{-1} tailings (Control)	0.46 ± 0.14 ^{a}	2.44 ± 0.38 ^{a}	4.21 ± 0.85 ^{a}	5.97 ± 0.79 ^{a}	13.08 ± 2.86 ^{a}
2 g C kg^{-1} tailings (modified)	6.11 ± 1.06 ^{b}	11.66 ± 1.45 ^{d}	6.94 ± 0.74 ^{b}	8.56 ± 0.98 ^{a}	33.27 ± 1.79 ^{cd}
3 g C kg^{-1} tailings (modified)	6.76 ± 1.84 ^{b}	9.78 ± 1.16 ^{cd}	7.22 ± 0.90 ^{b}	7.11 ± 0.74 ^{a}	30.88 ± 1.84 ^{cd}
4 g C kg^{-1} tailings (modified)	12.14 ± 1.22 ^{c}	12.71 ± 0.75 ^{d}	7.63 ± 0.71 ^{b}	6.62 ± 0.53 ^{a}	39.09 ± 1.95 ^{d}
2 g C kg^{-1} tailings (unmodified)	1.75 ± 0.57 ^{a}	7.53 ± 1.72 ^{bc}	8.97 ± 0.96 ^{b}	9.15 ± 0.52 ^{a}	26.46 ± 4.17 ^{bc}
4 g C kg^{-1} tailings (unmodified)	1.11 ± 0.50 ^{a}	5.12 ± 1.94 ^{ab}	6.90 ± 1.57 ^{b}	7.78 ± 0.82 ^{a}	20.91 ± 4.38 ^{ab}

Table A2. Dry root and shoot biomass, root to shoot ratio, and shoot height of *Medicago sativa* , *Agropyron trachycaulum* and *Festuca pratensis* after a 3 month growing period in mine tailings amended with modified humic substances and unmodified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Shoot biomass (g dry weight)	Root biomass (g dry weight)	Root to shoot ratio	Height (cm)
Medicago Sativa				
0 g C kg^{-1} tailings (Control)	0.634 ± 0.084 ^{a}	0.432 ± 0.056 ^{a}	0.73 ± 0.05 ^{cd}	21.1 ± 1.0 ^{a}
2 g C kg^{-1} tailings (modified)	0.814 ± 0.141 ^{ab}	0.480 ± 0.074 ^{a}	0.86 ± 0.10 ^{d}	21.2 ± 1.6 ^{a}
3 g C kg^{-1} tailings (modified)	1.226 ± 0.154 ^{c}	0.662 ± 0.067 ^{b}	0.65 ± 0.05 ^{bc}	26.9 ± 1.2 ^{b}
4 g C kg^{-1} tailings (modified)	0.707 ± 0.089 ^{ab}	0.349 ± 0.048 ^{a}	0.55 ± 0.04 ^{ab}	21.3 ± 1.0 ^{a}
2 g C kg^{-1} tailings (unmodified)	0.591 ± 0.094 ^{a}	0.331 ± 0.046 ^{a}	0.61 ± 0.04 ^{abc}	19.4 ± 1.0 ^{a}
4 g C kg^{-1} tailings (unmodified)	1.025 ± 0.163 ^{bc}	0.426 ± 0.056 ^{a}	0.49 ± 0.03 ^{a}	27.5 ± 1.4 ^{b}
Agropyron trachycaulum				
0 g C kg^{-1} tailings (Control)	0.628 ± 0.083 ^{b}	0.221 ± 0.021 ^{a}	0.44 ± 0.02 ^{a}	25.0 ± 1.3 ^{a}
2 g C kg^{-1} tailings (modified)	0.562 ± 0.062 ^{ab}	0.212 ± 0.021 ^{a}	0.43 ± 0.04 ^{a}	25.1 ± 0.9 ^{a}
3 g C kg^{-1} tailings (modified)	0.390 ± 0.044 ^{a}	0.160 ± 0.023 ^{a}	0.41 ± 0.03 ^{a}	24.4 ± 1.3 ^{a}
4 g C kg^{-1} tailings (modified)	0.398 ± 0.051 ^{a}	0.161 ± 0.017 ^{a}	0.44 ± 0.02 ^{a}	24.9 ± 1.0 ^{a}
2 g C kg^{-1} tailings (unmodified)	0.565 ± 0.053 ^{ab}	0.209 ± 0.017 ^{a}	0.41 ± 0.02 ^{a}	26.2 ± 1.0 ^{a}
4 g C kg^{-1} tailings (unmodified)	0.520 ± 0.093 ^{ab}	0.216 ± 0.040 ^{a}	0.44 ± 0.04 ^{a}	27.3 ± 1.4 ^{a}
Festuca pratensis				
0 g C kg^{-1} tailings (Control)	0.931 ± 0.096 ^{b}	0.379 ± 0.038 ^{ab}	0.44 ± 0.02 ^{a}	22.0 ± 0.7 ^{c}
2 g C kg^{-1} tailings (modified)	0.882 ± 0.106 ^{ab}	0.434 ± 0.048 ^{ab}	0.52 ± 0.03 ^{bc}	20.7 ± 0.9 ^{c}
3 g C kg^{-1} tailings (modified)	0.693 ± 0.149 ^{ab}	0.312 ± 0.051 ^{a}	0.47 ± 0.04 ^{ab}	16.8 ± 0.8 ^{a}
4 g C kg^{-1} tailings (modified)	0.588 ± 0.063 ^{a}	0.325 ± 0.036 ^{a}	0.58 ± 0.03 ^{c}	20.1 ± 0.6 ^{bc}
2 g C kg^{-1} tailings (unmodified)	0.913 ± 0.100 ^{b}	0.509 ± 0.060 ^{b}	0.56 ± 0.02 ^{c}	18.4 ± 0.5 ^{ab}
4 g C kg^{-1} tailings (unmodified)	0.942 ± 0.119 ^{b}	0.439 ± 0.071 ^{ab}	0.44 ± 0.02 ^{a}	27.7 ± 1.3 ^{d}

Table A3. Dry root an shoot biomass, root to shoot ratio, and shoot height of *Agropyron elongatum* and *Festuca rubra* afte a 3 month growing period in mine tailings amended with modified humic substances and unmodified humic substances (Mean ± SE, n = 4). Different letters represent significant differences ($p < 0.05$).

	Shoot biomass (g dry weight)	Root biomass (g dry weight)	Root to shoot ratio	Height (cm)
Agropyron elongatum				
0 g C kg^{-1} tailings (Control)	0.978 ± 0.101 ^{a}	0.290 ± 0.026 ^{a}	0.34 ± 0.02 ^{a}	28.6 ± 1.4 ^{cd}
2 g C kg^{-1} tailings (modified)	0.810 ± 0.063 ^{a}	0.345 ± 0.026 ^{ab}	0.44 ± 0.01 ^{b}	24.1 ± 1.1 ^{ab}
3 g C kg^{-1} tailings (modified)	0.749 ± 0.070 ^{a}	0.319 ± 0.025 ^{ab}	0.46 ± 0.02 ^{b}	22.7 ± 0.9 ^{ab}
4 g C kg^{-1} tailings (modified)	0.891 ± 0.085 ^{a}	0.394 ± 0.037 ^{b}	0.49 ± 0.02 ^{b}	27.8 ± 1.5 ^{bcd}
2 g C kg^{-1} tailings (unmodified)	1.003 ± 0.094 ^{a}	0.325 ± 0.029 ^{ab}	0.37 ± 0.03 ^{a}	26.1 ± 1.9 ^{abc}
4 g C kg^{-1} tailings (unmodified)	0.991 ± 0.102 ^{a}	0.339 ± 0.034 ^{ab}	0.38 ± 0.03 ^{a}	31.7 ± 1.8 ^{d}
Festuca rubra				
0 g C kg^{-1} tailings (Control)	0.327 ± 0.049 ^{a}	0.172 ± 0.035 ^{a}	0.52 ± 0.04 ^{a}	14.3 ± 0.5 ^{d}
2 g C kg^{-1} tailings (modified)	0.302 ± 0.043 ^{a}	0.144 ± 0.019 ^{a}	0.54 ± 0.03 ^{a}	12.5 ± 0.4 ^{b}
3 g C kg^{-1} tailings (modified)	0.238 ± 0.033 ^{a}	0.133 ± 0.018 ^{a}	0.58 ± 0.05 ^{a}	11.2 ± 0.4 ^{a}
4 g C kg^{-1} tailings (modified)	0.291 ± 0.044 ^{a}	0.159 ± 0.026 ^{a}	0.54 ± 0.03 ^{a}	12.8 ± 0.4 ^{b}
2 g C kg^{-1} tailings (unmodified)	0.271 ± 0.040 ^{a}	0.143 ± 0.026 ^{a}	0.54 ± 0.03 ^{a}	12.6 ± 0.4 ^{b}
4 g C kg^{-1} tailings (unmodified)	0.239 ± 0.030 ^{a}	0.132 ± 0.019 ^{a}	0.55 ± 0.03 ^{a}	13.2 ± 0.4 ^{bc}

Appendix B - Meteorological Data

Table B1. Meteorological data from Beausejour monitoring station for 2003. The Green Lane, Environment Canada. 2007. Environment Canada Weather Office: Online National Climate Data and Information Archive. Internet Source.

Monthly Data Report for 2003

Month	Mean Max °C	Mean Temp °C	Mean Min °C	Extr Max °C	Extr Min Temp °C	Total Rain mm	Total Snow cm	Total Precip mm	Snow Grnd cm
Jan	-10.6	-15.4	-20.2	7	-32.5	T	14	14	13
Feb	-12.7	-18.8	-24.9	-1	-37.5	T	10	10	20
Mar	-1.8	-7.8	-13.7	14.5	-40.5	8.2	8	16.2	0
Apr	13.3	5.8	-1.7	25.5	-10	21	14	35	0
May	20.8	12.9	4.8	27.5	-6	64.8	0	64.8	0
Jun	23.9	17.1	10.2	31	3.5	84.7	0	84.7	0
Jul	26.9	20	13	32	6	31	0	31	0
Aug	28.4	21.6	14.7	35.5	5	81.6	0	81.6	0
Sep	18	12.4	6.8	30	-2	96.8	T	96.8	0
Oct	12.5	6.8	1	28.5	-7	7	18	25	0
Nov	-2.2	-6.5	-10.8	8.5	-22	3.6	9	12.6	2
Dec	-4	-9	-13.9	4	-31.5	15.2	25	40.2	15
Entire Year						413.9	98	511.9	
Spring and Growing Season						388.1	22	410.1	

Table B2. Meteorological data from Beausejour monitoring station for 2004. The Green Lane, Environment Canada. 2007. Environment Canada Weather Office: Online National Climate Data and Information Archive. Internet Source.

Monthly Data Report for 2004

Month	Mean Max °C	Mean Temp °C	Mean Min °C	Extr Max °C	Extr Min Temp °C	Total Rain mm	Total Snow cm	Total Precip mm	Snow Grnd cm
Jan	-16.1	-21.9	-27.7	-5	-44.5	0	57	57	58
Feb	-5.7	-11	-16.2	7	-35	0	2	2	0
Mar	0.6	-5.3	-11.1	11.5	-24.5	49	25	74	12
Apr	9.7	3.9	-2	20	-9	16.6	T	16.6	0
May	13.3	7.7	2.1	25	-7.5	78.7	53	131.7	0
Jun	20.1	14.2	8.2	29.5	2.0S	84.4	0	84.4	0
Jul	24.5	18.4	12.2	29.5	4.5	58.4	0	58.4	0
Aug	20.3	14.6	8.8	26	2.0S	106.8	0	106.8	0
Sep	20.3	15.1	9.8	31	2	187.2	0	187.2	0
Oct	10.6	6.2	1.7	27	-10	52.2	0	52.2	0
Nov	5.1	-0.1	-5.2	16	-17.0S	1.8	15	16.8	2
Dec	-8.1	-14.7	-21.3	4.5	-36.0S	T	56	56	40
Entire Year						635.1	208	843.1	
Spring and Growing Season						581.1	78	659.1	

Appendix C – Field Seasons 2003 and 2004 Conductivity, pH, and Organic Carbon Statistical Analysis

Table C1. The conductivity, pH and carbon content of mine tailings amended with modified humic substances for field season 2003 at specific times over a 1 year period (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$). Note comparisons between treatments. * Carbon content was not measured at time of seeding.

	Conductivity ($dS\ m^{-1}$)	pH	Carbon (%)
Pre Treatment			
0 g C kg^{-1} tailings (Control)	2.22 ± 0.13 ^{a}	7.84 ± 0.08 ^{a}	0.45 ± 0.03 ^{a}
2 g C kg^{-1} tailings	2.07 ± 0.06 ^{a}	7.99 ± 0.06 ^{b}	0.45 ± 0.03 ^{a}
3 g C kg^{-1} tailings	2.02 ± 0.05 ^{a}	7.95 ± 0.04 ^{ab}	0.47 ± 0.02 ^{a}
4 g C kg^{-1} tailings	2.12 ± 0.06 ^{a}	7.90 ± 0.06 ^{ab}	0.52 ± 0.02 ^{b}
Post Treatment			
0 g C kg^{-1} tailings (Control)	2.28 ± 0.10 ^{a}	7.86 ± 0.08 ^{a}	0.45 ± 0.03 ^{a}
2 g C kg^{-1} tailings	5.93 ± 0.35 ^{b}	7.83 ± 0.08 ^{a}	0.80 ± 0.05 ^{b}
3 g C kg^{-1} tailings	7.85 ± 0.59 ^{c}	7.83 ± 0.07 ^{a}	0.99 ± 0.09 ^{c}
4 g C kg^{-1} tailings	8.96 ± 0.58 ^{d}	7.84 ± 0.06 ^{a}	1.26 ± 0.11 ^{d}
Seeding			
0 g C kg^{-1} tailings (Control)	2.41 ± 0.09 ^{a}	7.99 ± 0.12 ^{a}	*
2 g C kg^{-1} tailings	5.72 ± 0.35 ^{b}	8.01 ± 0.09 ^{a}	*
3 g C kg^{-1} tailings	7.55 ± 0.43 ^{c}	7.94 ± 0.05 ^{a}	*
4 g C kg^{-1} tailings	8.94 ± 0.39 ^{d}	7.96 ± 0.05 ^{a}	*
Harvest			
0 g C kg^{-1} tailings (Control)	2.27 ± 0.06 ^{a}	7.85 ± 0.14 ^{b}	0.56 ± 0.04 ^{a}
2 g C kg^{-1} tailings	4.04 ± 0.27 ^{b}	7.70 ± 0.09 ^{a}	0.69 ± 0.04 ^{ab}
3 g C kg^{-1} tailings	4.58 ± 0.31 ^{b}	7.63 ± 0.06 ^{a}	0.86 ± 0.08 ^{b}
4 g C kg^{-1} tailings	5.05 ± 0.56 ^{b}	7.60 ± 0.10 ^{a}	1.07 ± 0.16 ^{c}
1 Year			
0 g C kg^{-1} tailings (Control)	2.36 ± 0.06 ^{a}	7.64 ± 0.20 ^{b}	0.71 ± 0.09 ^{a}
2 g C kg^{-1} tailings	2.57 ± 0.08 ^{ab}	7.62 ± 0.21 ^{b}	1.79 ± 0.29 ^{b}
3 g C kg^{-1} tailings	2.61 ± 0.11 ^{b}	7.56 ± 0.19 ^{b}	1.90 ± 0.13 ^{b}
4 g C kg^{-1} tailings	3.00 ± 0.13 ^{c}	7.39 ± 0.19 ^{a}	2.19 ± 0.20 ^{b}

Table C2. The conductivity, pH and carbon content of mine tailings amended with modified humic substances for field season 2003 at specific times over a 1 year period (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$). Note comparisons made across sample times. * Carbon content was not measured at time of seeding.

	Conductivity ($dS\ m^{-1}$)	pH	Carbon (%)
0 g C kg^{-1} tailings (Control)			
Pre Treatment	2.22 ± 0.13 ^{a}	7.84 ± 0.08 ^{b}	0.45 ± 0.03 ^{a}
Post Treatment	2.28 ± 0.10 ^{a}	7.86 ± 0.08 ^{b}	0.43 ± 0.02 ^{a}
Seeding	2.41 ± 0.09 ^{a}	7.99 ± 0.12 ^{c}	*
Harvest	2.27 ± 0.06 ^{a}	7.85 ± 0.14 ^{b}	0.56 ± 0.04 ^{a}
1 Year	2.36 ± 0.06 ^{a}	7.64 ± 0.20 ^{a}	0.71 ± 0.09 ^{b}
2 g C kg^{-1} tailings			
Pre Treatment	2.07 ± 0.06 ^{a}	7.99 ± 0.06 ^{c}	0.45 ± 0.03 ^{a}
Post Treatment	5.93 ± 0.35 ^{c}	7.83 ± 0.08 ^{b}	0.80 ± 0.05 ^{b}
Seeding	5.71 ± 0.34 ^{b}	7.94 ± 0.05 ^{c}	*
Harvest	4.04 ± 0.27 ^{a}	7.70 ± 0.09 ^{a}	0.69 ± 0.04 ^{ab}
1 Year	2.57 ± 0.08 ^{a}	7.62 ± 0.21 ^{a}	1.79 ± 0.29 ^{c}
3 g C kg^{-1} tailings			
Pre Treatment	2.02 ± 0.05 ^{a}	7.95 ± 0.04 ^{c}	0.47 ± 0.02 ^{a}
Post Treatment	7.85 ± 0.59 ^{c}	7.83 ± 0.07 ^{b}	0.99 ± 0.09 ^{b}
Seeding	7.55 ± 0.42 ^{c}	7.94 ± 0.22 ^{c}	*
Harvest	4.58 ± 0.31 ^{b}	7.63 ± 0.06 ^{a}	0.86 ± 0.08 ^{b}
1 Year	2.61 ± 0.11 ^{a}	7.56 ± 0.19 ^{a}	1.90 ± 0.13 ^{c}
4 g C kg^{-1} tailings			
Pre Treatment	2.12 ± 0.06 ^{a}	7.90 ± 0.06 ^{cd}	0.52 ± 0.02 ^{a}
Post Treatment	8.96 ± 0.58 ^{c}	7.84 ± 0.06 ^{c}	1.26 ± 0.11 ^{b}
Seeding	8.94 ± 0.39 ^{c}	7.96 ± 0.05 ^{d}	*
Harvest	5.05 ± 0.56 ^{b}	7.60 ± 0.10 ^{b}	1.07 ± 0.16 ^{b}
1 Year	3.00 ± 0.13 ^{a}	7.39 ± 0.19 ^{a}	2.19 ± 0.20 ^{c}

Table C3. The conductivity, pH and carbon content of mine tailings amended with modified humic substances for field season 2004 at specific times over a 1 year period (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$). Note comparisons between treatments. * Carbon content was not measured at time of seeding.

	Conductivity ($dS\ m^{-1}$)	pH	Carbon (%)
Pre Treatment			
0 g C kg^{-1} tailings (Control)	3.41 ± 0.26 ^{a}	6.75 ± 0.26 ^{a}	0.38 ± 0.02 ^{a}
2 g C kg^{-1} tailings	3.03 ± 0.19 ^{a}	6.43 ± 0.43 ^{a}	0.39 ± 0.04 ^{a}
3 g C kg^{-1} tailings	3.15 ± 0.16 ^{a}	6.85 ± 0.26 ^{a}	0.40 ± 0.02 ^{a}
4 g C kg^{-1} tailings	3.34 ± 0.12 ^{a}	6.40 ± 0.31 ^{a}	0.44 ± 0.04 ^{a}
Post Treatment			
0 g C kg^{-1} tailings (Control)	3.49 ± 0.26 ^{a}	6.72 ± 0.19 ^{a}	0.38 ± 0.02 ^{a}
2 g C kg^{-1} tailings	8.54 ± 0.50 ^{b}	6.92 ± 0.11 ^{a}	0.70 ± 0.04 ^{b}
3 g C kg^{-1} tailings	10.73 ± 0.47 ^{c}	7.41 ± 0.05 ^{b}	1.09 ± 0.17 ^{c}
4 g C kg^{-1} tailings	13.16 ± 0.83 ^{d}	7.24 ± 0.08 ^{ab}	1.43 ± 0.18 ^{d}
Seeding			
0 g C kg^{-1} tailings (Control)	4.53 ± 0.36 ^{a}	7.19 ± 0.18 ^{a}	*
2 g C kg^{-1} tailings	5.30 ± 0.53 ^{a}	7.78 ± 0.11 ^{b}	*
3 g C kg^{-1} tailings	8.11 ± 0.72 ^{b}	7.54 ± 0.22 ^{ab}	*
4 g C kg^{-1} tailings	8.83 ± 1.12 ^{b}	7.40 ± 0.12 ^{a}	*
Harvest			
0 g C kg^{-1} tailings (Control)	2.74 ± 0.22 ^{a}	6.50 ± 0.30 ^{a}	0.41 ± 0.02 ^{a}
2 g C kg^{-1} tailings	2.98 ± 0.20 ^{a}	6.79 ± 0.28 ^{ab}	0.60 ± 0.07 ^{b}
3 g C kg^{-1} tailings	3.13 ± 0.22 ^{a}	7.57 ± 0.09 ^{c}	0.77 ± 0.11 ^{c}
4 g C kg^{-1} tailings	3.04 ± 0.14 ^{a}	7.04 ± 0.18 ^{b}	0.85 ± 0.16 ^{c}
1 Year			
0 g C kg^{-1} tailings (Control)	2.89 ± 0.12 ^{a}	6.52 ± 0.35 ^{a}	0.59 ± 0.08 ^{a}
2 g C kg^{-1} tailings	2.93 ± 0.23 ^{a}	6.52 ± 0.50 ^{a}	0.76 ± 0.12 ^{a}
3 g C kg^{-1} tailings	3.04 ± 0.17 ^{a}	7.18 ± 0.07 ^{a}	0.93 ± 0.15 ^{ab}
4 g C kg^{-1} tailings	3.01 ± 0.23 ^{a}	6.99 ± 0.16 ^{a}	1.22 ± 0.15 ^{b}

Table C4. The conductivity, pH and carbon content of mine tailings amended with modified humic substances for field season 2004 at specific times over a 1 year period (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$). Note comparisons made across sample times. * Carbon content was not measured at time of seeding.

	Conductivity ($dS\ m^{-1}$)	pH	Carbon (%)
0 g C kg^{-1} tailings (Control)			
Pre Treatment	3.41 ± 0.26 ^{a}	6.75 ± 0.26 ^{ab}	0.38 ± 0.02 ^{a}
Post Treatment	3.49 ± 0.26 ^{a}	6.72 ± 0.19 ^{ab}	0.38 ± 0.02 ^{a}
Seeding	4.53 ± 0.36 ^{b}	7.19 ± 0.18 ^{b}	*
Harvest	2.74 ± 0.22 ^{a}	6.50 ± 0.30 ^{a}	0.41 ± 0.02 ^{a}
1 Year	2.89 ± 0.12 ^{a}	6.52 ± 0.35 ^{a}	0.59 ± 0.08 ^{b}
2 g C kg^{-1} tailings			
Pre Treatment	3.03 ± 0.19 ^{a}	6.43 ± 0.43 ^{a}	0.39 ± 0.04 ^{a}
Post Treatment	8.54 ± 0.50 ^{c}	6.92 ± 0.11 ^{a}	0.70 ± 0.04 ^{b}
Seeding	5.30 ± 0.53 ^{b}	7.78 ± 0.11 ^{b}	*
Harvest	2.98 ± 0.20 ^{a}	6.79 ± 0.28 ^{a}	0.60 ± 0.07 ^{b}
1 Year	2.93 ± 0.23 ^{a}	6.52 ± 0.50 ^{a}	0.76 ± 0.12 ^{b}
3 g C kg^{-1} tailings			
Pre Treatment	3.15 ± 0.16 ^{a}	6.85 ± 0.26 ^{a}	0.40 ± 0.02 ^{a}
Post Treatment	10.73 ± 0.47 ^{c}	7.41 ± 0.05 ^{b}	1.09 ± 0.17 ^{b}
Seeding	8.11 ± 0.72 ^{b}	7.54 ± 0.22 ^{b}	*
Harvest	3.13 ± 0.22 ^{a}	7.57 ± 0.09 ^{b}	0.77 ± 0.11 ^{b}
1 Year	3.00 ± 0.17 ^{a}	7.18 ± 0.07 ^{ab}	0.93 ± 0.15 ^{b}
4 g C kg^{-1} tailings			
Pre Treatment	3.34 ± 0.12 ^{a}	6.40 ± 0.31 ^{a}	0.44 ± 0.04 ^{a}
Post Treatment	13.16 ± 0.83 ^{c}	7.24 ± 0.08 ^{b}	1.43 ± 0.18 ^{b}
Seeding	8.83 ± 1.12 ^{b}	7.40 ± 0.12 ^{b}	*
Harvest	3.04 ± 0.14 ^{a}	7.04 ± 0.18 ^{b}	0.85 ± 0.16 ^{c}
1 Year	3.00 ± 0.23 ^{a}	6.99 ± 0.16 ^{b}	1.22 ± 0.15 ^{c}

Appendix D - Tailings pH Map

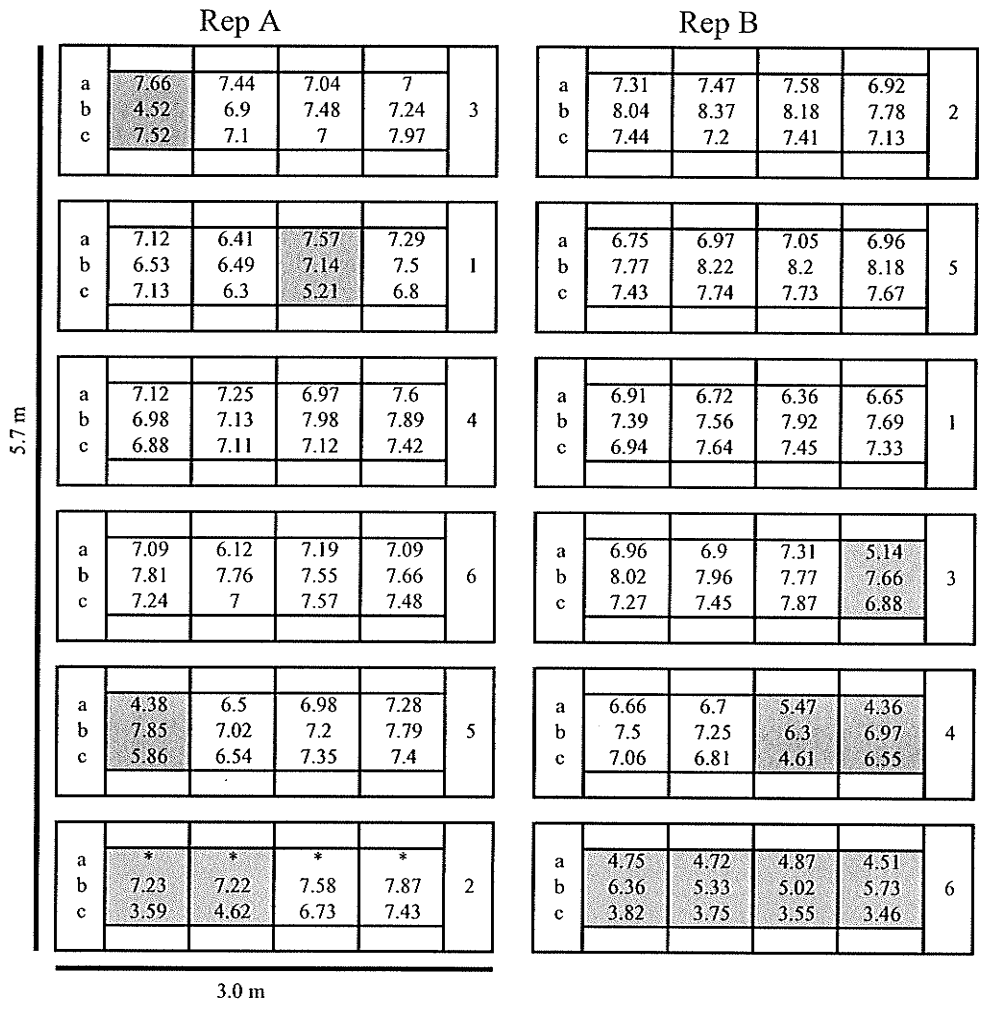

Figure D1. Field season 2004 Rep A and B pH map, showing pH from different sample periods with areas of lower and higher pH highlighted.

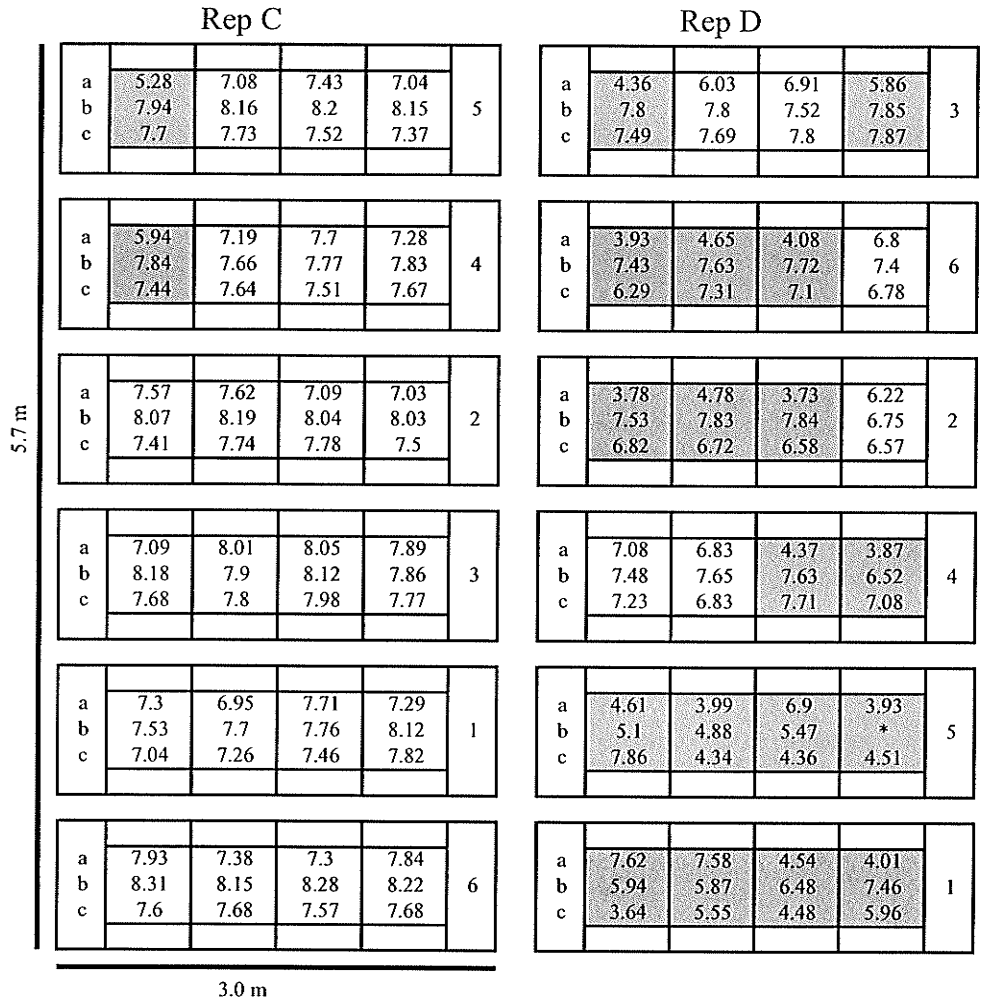

Figure D2. Field season 2004 Rep C and D pH map, showing pH from different sample periods with areas of lower and higher pH highlighted.

Appendix E – Distribution of Aggregates Statistical Analysis

Table E1. Distribution of water stable aggregates in field season 2003 in mine tailings amended with modified humic substances at pre treatment, seeding and harvest (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$). Note comparrisons made across sample times.

	Aggregate Distribution				
	> 2.0 mm (%)	1.0 - 2.0 mm (%)	0.5 - 1.0 mm (%)	0.25 - 0.5 mm (%)	Total > 0.25 mm (%)
0 g C kg^{-1} tailings					
Pre Treatment	0.46 ± 0.20 ^{a}	1.62 ± 0.23 ^{a}	2.41 ± 0.37 ^{a}	4.01 ± 0.48 ^{a}	8.50 ± 0.78 ^{a}
Seeding	0.60 ± 0.09 ^{a}	1.70 ± 0.23 ^{a}	2.07 ± 0.19 ^{a}	3.71 ± 0.32 ^{a}	8.08 ± 0.72 ^{a}
Harvest	0.36 ± 0.12 ^{a}	1.49 ± 0.18 ^{a}	2.56 ± 0.22 ^{a}	4.56 ± 0.15 ^{a}	8.97 ± 0.56 ^{a}
2 g C kg^{-1} tailings					
Pre Treatment	0.36 ± 0.08 ^{a}	1.80 ± 0.31 ^{a}	2.65 ± 0.23 ^{a}	3.57 ± 0.28 ^{a}	8.37 ± 0.76 ^{a}
Seeding	1.01 ± 0.25 ^{ab}	3.21 ± 0.29 ^{b}	4.59 ± 0.40 ^{b}	7.15 ± 0.50 ^{b}	15.96 ± 0.81 ^{b}
Harvest	1.36 ± 0.36 ^{b}	4.43 ± 0.59 ^{c}	5.54 ± 0.24 ^{c}	6.89 ± 0.62 ^{b}	18.22 ± 1.05 ^{c}
3 g C kg^{-1} tailings					
Pre Treatment	0.75 ± 0.44 ^{a}	1.92 ± 0.41 ^{a}	2.25 ± 0.47 ^{a}	3.60 ± 0.74 ^{a}	8.52 ± 1.87 ^{a}
Seeding	0.77 ± 0.11 ^{a}	3.78 ± 0.37 ^{b}	4.96 ± 0.38 ^{b}	8.03 ± 0.67 ^{c}	17.54 ± 1.06 ^{b}
Harvest	1.38 ± 0.22 ^{b}	3.84 ± 0.63 ^{b}	4.99 ± 0.44 ^{b}	6.35 ± 0.76 ^{b}	16.55 ± 1.96 ^{b}
4 g C kg^{-1} tailings					
Pre Treatment	0.39 ± 0.14 ^{a}	1.87 ± 0.35 ^{a}	2.34 ± 0.43 ^{a}	3.77 ± 0.56 ^{a}	8.37 ± 1.40 ^{a}
Seeding	1.04 ± 0.21 ^{a}	3.44 ± 0.53 ^{b}	4.80 ± 0.66 ^{a}	7.27 ± 0.99 ^{b}	16.55 ± 2.30 ^{b}
Harvest	0.93 ± 0.31 ^{a}	4.37 ± 0.87 ^{b}	5.27 ± 0.63 ^{a}	6.81 ± 0.76 ^{b}	17.37 ± 2.21 ^{b}

Table E2. Distribution of water stable aggregates in field season 2004 in mine tailings amended with modified humic substances at pre treatment, seeding and harvest (Mean ± SE, n = 4). Different letters indicate significant differences ($p < 0.05$). Note comparrisons made across sample times.

	Aggregate Distribution				
	> 2.0 mm (%)	1.0 - 2.0 mm (%)	0.5 - 1.0 mm (%)	0.25 - 0.5 mm (%)	Total > 0.25 mm (%)
0 g C kg^{-1} tailings					
Pre Treatment	0.52 ± 0.09 ^{a}	2.27 ± 0.51 ^{b}	3.09 ± 0.31 ^{a}	4.82 ± 0.62 ^{a}	10.70 ± 1.26 ^{a}
Seeding	0.36 ± 0.10 ^{a}	1.63 ± 0.09 ^{a}	4.42 ± 0.78 ^{a}	6.16 ± 0.56 ^{a}	12.57 ± 1.15 ^{b}
Harvest	0.46 ± 0.19 ^{a}	2.44 ± 0.55 ^{b}	4.21 ± 1.26 ^{a}	5.97 ± 0.98 ^{a}	13.08 ± 2.86 ^{b}
2 g C kg^{-1} tailings					
Pre Treatment	0.50 ± 0.14 ^{a}	2.15 ± 0.58 ^{a}	3.38 ± 0.60 ^{a}	4.12 ± 1.14 ^{a}	10.16 ± 2.40 ^{a}
Seeding	3.36 ± 2.01 ^{ab}	6.81 ± 1.63 ^{b}	12.13 ± 1.57 ^{c}	9.74 ± 0.84 ^{b}	32.04 ± 3.80 ^{b}
Harvest	6.11 ± 1.40 ^{b}	11.66 ± 2.03 ^{c}	6.94 ± 0.74 ^{b}	8.56 ± 0.47 ^{b}	33.27 ± 1.79 ^{b}
3 g C kg^{-1} tailings					
Pre Treatment	0.46 ± 0.11 ^{a}	2.25 ± 0.68 ^{a}	3.12 ± 0.75 ^{a}	4.79 ± 1.04 ^{a}	10.62 ± 0.52 ^{a}
Seeding	2.83 ± 1.37 ^{ab}	7.05 ± 1.36 ^{b}	10.77 ± 1.21 ^{c}	12.07 ± 1.18 ^{b}	32.71 ± 1.83 ^{b}
Harvest	6.76 ± 1.44 ^{b}	9.78 ± 1.24 ^{c}	7.22 ± 1.04 ^{b}	7.11 ± 0.43 ^{a}	30.88 ± 1.84 ^{b}
4 g C kg^{-1} tailings					
Pre Treatment	0.49 ± 0.09 ^{a}	2.49 ± 0.28 ^{a}	3.50 ± 0.31 ^{a}	4.58 ± 0.33 ^{a}	11.06 ± 0.83 ^{a}
Seeding	6.57 ± 2.32 ^{b}	12.00 ± 0.60 ^{b}	13.78 ± 1.24 ^{c}	11.75 ± 1.04 ^{b}	44.10 ± 1.82 ^{c}
Harvest	12.14 ± 1.61 ^{c}	12.71 ± 0.91 ^{b}	7.63 ± 0.99 ^{b}	6.62 ± 0.34 ^{a}	39.09 ± 1.95 ^{b}

Appendix F – Salt Crusts

Figure E1. Salt crusts on tailings surface with presence dependant on environmental conditions.

Appendix G - Vegetation in Field Sites

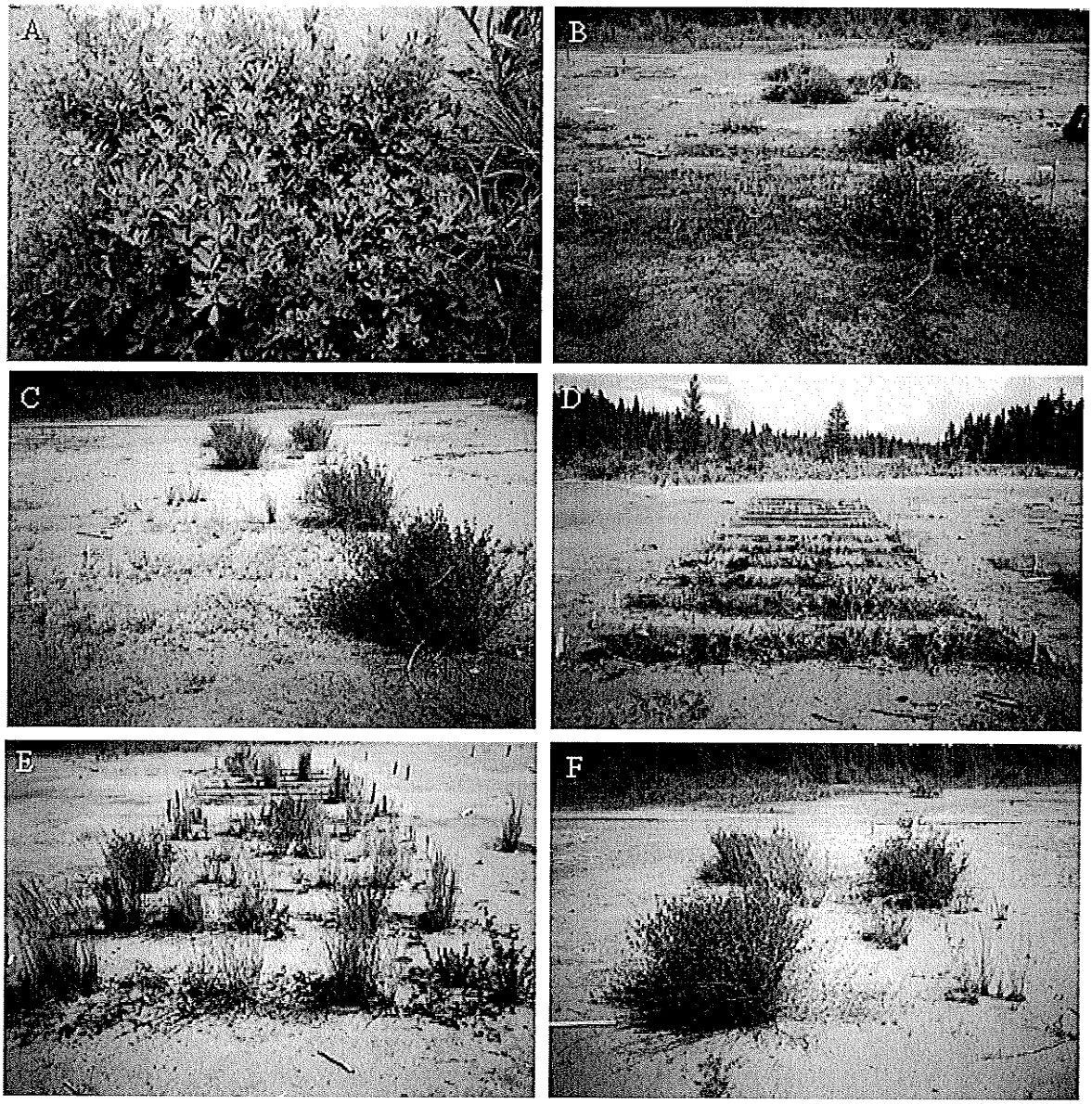

Figure G1. A) *Medicago sativa* 3 months following seeding in field season 2003, B) Summer of 2nd year following seeding from field season 2003. Note presence of vegetation within and around replicate and establishment and flowering of *M. sativa*. C) Same location as picture B, in spring of 3rd year of growth, grass species very sparse, *M. sativa* well established. D) Summer of field season 2004, 1st year of growth, relatively low biomass per species. E) Summer of 2nd year following seeding from field season 2004. Note chlorosis of leaf tissue in grass species. F) Fall of 2nd year following seeding from field season 2004, regression of grass species but *M. sativa* not showing signs of regression.

Appendix H – Long Term Growth Chamber Experiment

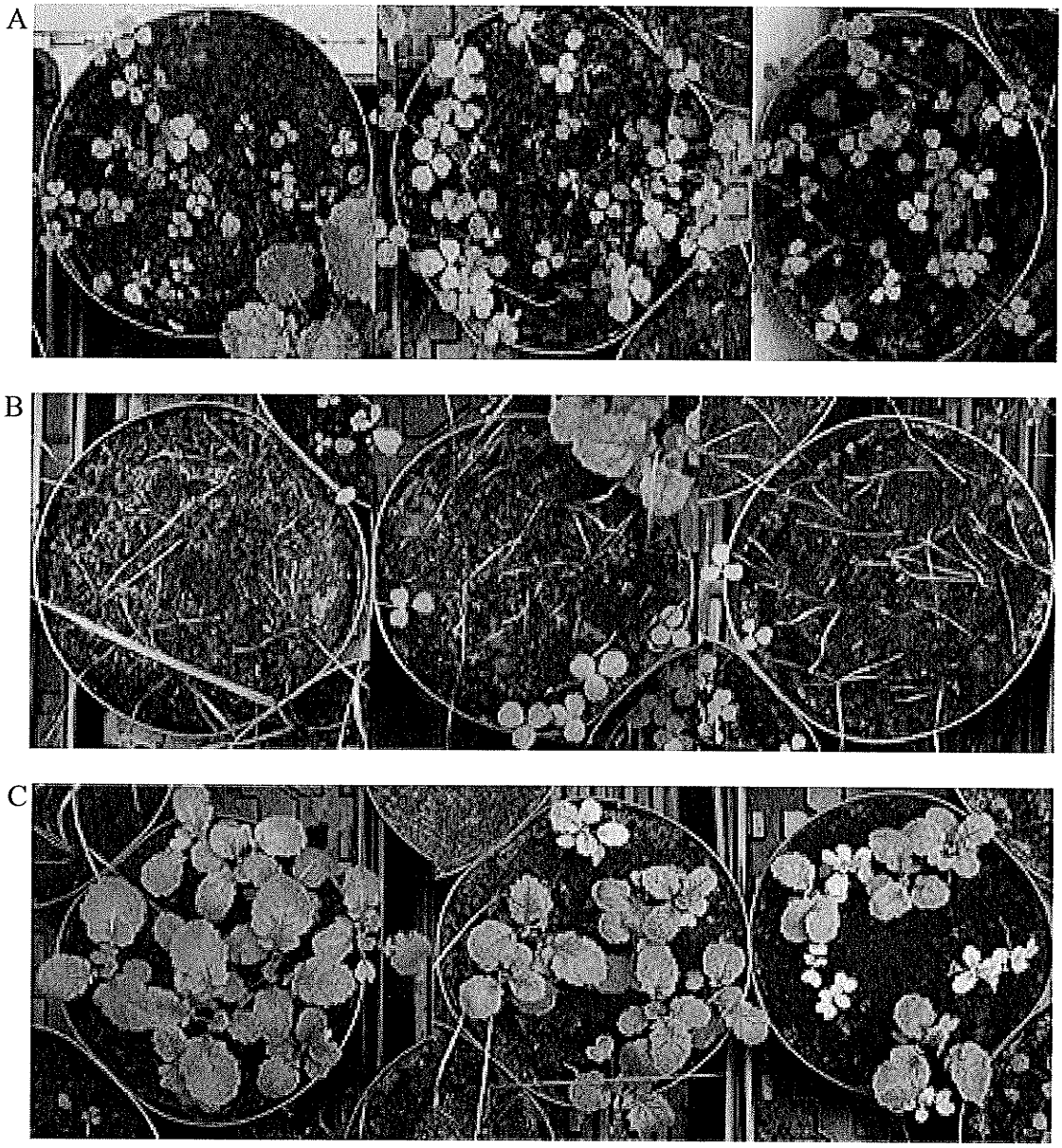

Figure H1. *Medicago sativa* (A), *Agropyron trachycalum* (B), and *Brassica juncea* (C) at 4 weeks post seeding in tailings amended with modified humic substances. Note enchanced biomass of *M. sativa* in the 2 g C kg^{-1} treatment, while decreased growth for *B. juncea* in both amended treatments.