THE USE OF MICROBIAL AND ORGANIC AMENDMENTS IN THE REVEGETATION OF SMELTER-AFFECTED SOILS NEAR FLIN FLON, MB

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ABSTRACT

The boreal forest area around Flin Flon, MB, and Creighton, SK, has been the site of a metal mining and smelting complex since the 1930s. Smelter emissions, coupled with forest logging, forest fires, and subsequent soil erosion, have led to severe vegetation dieback and the development of soils containing a mixture of metals in varying concentrations. In affected areas, existing vegetation typically is stunted. Limestone applications to affected soils have served to increase pH and, in some instances, the vegetation has responded positively; however, in some areas limestone application has failed to restore vegetation, leading to an interest in examining the suitability of other soil amendments to affect revegetation in these areas. Typically revegetation programs focus on aboveground vegetation responses; however, healthy plant growth often is dependent on the presence of an equally healthy soil microbial community. Thus, this study attempted to link revegetation success with responses of the soil microbial community structure to various soil amendments.

Two studies were conducted to determine the influence of soil amendments (biochar, municipal and manure compost, glauconite, and an arbuscular mycorrhizal/ectomycorrhizal inoculant) on plant growth and microbial community structure in two soils from the Flin Flon area, classified as containing high and low metal concentrations. The two studies evaluated the growth of boreal forest understory species American vetch (*Vicia americana*) and tufted hairgrass (*Deschampsia caespitosa*) and overstory species jack pine (*Pinus banksiana)* and trembling aspen (*Populus tremuloides*) after addition of soil amendments, and the subsequent effects on microbial community structure. Greenhouse experiments evaluated plant growth for a period of 8 weeks (understory species) or 19 weeks (overstory species), after which plants were analyzed for changes in biomass and metal accumulation in plant tissue. Soils were analyzed for available metal concentrations, as well as microbial biomass carbon and nitrogen, and phospholipid fatty acid concentration, which is a measure of microbial community structure. Significant effects were seen on plant growth and microbial community structure due to the metal concentrations in the soil, but no one amendment consistently impacted plant growth or metal uptake, or any measured microbial parameter. The results of this study indicate the variability of plant growth and microbial functioning in soils from the study site, as well as the

inherent challenges associated with revegetating heavy metal affected soils, and underline the need for further research on plant growth and microbial community structure at this site.

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AM	Arbuscular mycorrhizal / ectomycorrhizal inoculant
AMF	Arbuscular mycorrhizal fungi
BMB	Bone meal biochar
CM	Composted manure
F:B	Fungal:Bacterial biomass ratio
Gr+	Gram positive
Gr-	Gram negative
GC	Glauconite
LSD	Least significant difference
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MRPP	Multi-response permutation procedures
MuC	Municipal Compost
NMDS	Non-metric multidimensional scaling
PLFA	Phospholipid fatty acid
SOM	Soil organic matter

1. INTRODUCTION

In Canada there are twelve base metal smelters located in six provinces throughout the country – British Columbia, Alberta, Saskatchewan, Ontario, Quebec, and New Brunswick (Environment Canada, 2010). These production sites refine and smelt various metals. During the smelting process a range of substances are emitted, some of which are toxic, including sulfur dioxide (SO_2), particulate matter, heavy metals, acidic compounds (such as hydrogen chloride [HCl] and sulfuric acid [H_2SO_4]), ammonia (NH_3), and various greenhouse gases (Environment Canada, 2010). Modern regulatory practices have reduced emissions using technological means, but often the surrounding environment remains affected by the aerial discharge.

Areas around metal smelters often are devoid of vegetation, and the term 'industrial barrens' has been applied to denote these areas (Kozlov and Zvereva, 2007). This term is used to describe sites that have developed due to airborne deposition of anthropogenically-sourced metal smelting particulates. Vegetation, if present, often is stunted and deformed. The revegetation of industrial barrens is a challenging task, and factors such as soil pH, soil organic matter (SOM) content, hydrology, and native flora and soil fauna must be taken into account in any type of revegetation program.

Flin Flon, MB, and Creighton, SK, are located on the Saskatchewan-Manitoba border in the Churchill River Upland Boreal Ecoregion. This ecoregion is dominated by a mixed forest community composed of pine, spruce, fir, poplar, and aspen (Henderson and McMartin, 1995; Henderson et al., 1998). A metal mining and smelting complex owned and operated by HudBay Minerals Inc. (formerly Hudson Bay Mining and Smelting Co., Ltd.) has dominated the landscape around these two communities since the 1930s. The first smokestack was 30 m high, and since then it has changed height numerous times. A more recent smokestack was built in 1974 and is 251 m high, and was in active use until July 2010, when the copper smelting operations were terminated. Zinc processing is still actively done at the facility. Until the closure, the smelter produced cadmium (Cd), copper (Cu), and zinc (Zn) from ores mined in the vicinity. Since the smelter's closure, mined copper ores are shipped elsewhere for processing. Particulate emissions from the stack have decreased due to stricter environmental standards over the years,

but material deposition includes arsenic (As), Cd, chromium (Cr), cobalt (Co), Cu, iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), sulfur (S), and Zn (Henderson and McMartin, 1995).

Deposition of smelter-related particulates is highly variable around the area of the stack, but the results of the emissions can be seen up to several kilometers from the smelter (Henderson et al., 1998). According to Henderson and McMartin (1995), soils in the area surrounding Flin Flon/Creighton are shallow and commonly sandy. Due to decades of a combination of smelter emissions, soil erosion, forest fires, and logging, soils are poorly formed and contain a mixture of metal oxides and have high concentrations of Cu and Zn. Organic soils in the area are large stockpiles of heavy metals from decades of smelter emissions; however, mineral soils exhibit the same type of depositional structure. Nearest the smelter, concentrations of metals such as As, Cd, Cu, Hg, Ni, Pb and Zn are highest, but smelter-related metals can be found in humus and till samples up to 35 km from the stack (Henderson et al., 1998; Winterhalder, 2003).

Soil pH typically is low, and vegetation is either non-existent or stunted and malformed. The expected climax ecosystem for this area is a spruce *(Picea sp.)* forest, but few spruce grow in the affected areas (Henderson and McMartin, 1995). Vegetation common to areas of high soil metal content includes a metal tolerant, non-native bentgrass *(Agrostis capillaris)* community, and stunted species of willow *(Salix sp.)*, birch *(Betula sp.)*, and poplar *(Populus sp.)*. Effects on soil and vegetation in the area related to aerial particulate deposition and historical logging and forest fires include high tree mortality, reduced growth and species diversity, and soil erosion (Henderson and McMartin, 1995).

A community-based greening program was initiated in Flin Flon in 1999 (Winterhalder, 2003). Using a volunteer-based work force, the group has applied local limestone to over 34 ha of affected land, in the hopes that the limestone would raise the pH of the soil and enable the vegetation to re-establish, regardless of the metal concentration of the soil. Since its inception, the project has had varying degrees of success. While many areas have responded to the limestone applications, other areas have not – even where soil testing shows the pH is at a level typically conducive to plant growth (Winterhalder, 2003).

The lack of plant response to liming alone, even with a change in soil pH, has led to interest in examining the suitability and potential of other soil amendments, including various types of compost, fertilizer, clay minerals, and fungal amendments. The use of microorganisms

and their relationships with plant roots largely has not been studied, especially in the context of smelter-affected soil restoration. Indeed, the criterion for evaluating success of plant growth in smelter-affected soils is often largely visual, and does not take into account the highly complex and dynamic below-ground microbial community and the influences that it might have on soil restoration and forest revegetation (Mummey et al., 2002). Examining the influence of various amendments on the relationships between plants and soil microorganisms might enable more successful reintroduction of native plant varieties into smelter-affected sites. In a larger context, understanding the microbial communities involved in shifting the structure of a disturbed landscape to one of a more natural forest is essential to ensure that a functioning forest community will develop as revegetation proceeds.

The overall objective of this study was to determine how the addition of various organic and microbial amendments to smelter-affected soils in the Flin Flin-Creighton area alters the microbial community, thus promoting consequent revegetation growth. Specific objectives and hypotheses are as follows:

Objective 1: Assess the microbial community structure in two soils with high and low metal concentrations, and relate community structure to soil quality (soil pH), plant-available metal concentrations, and ease of revegetation (total plant biomass).

Hypothesis 1: Soils with higher concentrations of available heavy metals will have lower microbial abundance.

Hypothesis 2: Amendments that shift the microbial community to a more abundant one correlate to increased plant growth (total biomass or plant height).

Hypothesis 3: Amendments that promote fungal-dominated microbial communities correlate with an increase in the total biomass of forest tree seedlings.

Objective 2: Identify the amendment or combination of amendments that effectively promote the growth of understory and climax species in smelter-affected soil.

Hypothesis 4: Plants inoculated with endo- or ectomycorrhizal fungi will have a higher total biomass due to symbiotic relationships.

Hypothesis 5: Amendments that decrease plant metal uptake into shoot and root biomass will correlate with increased growth of understory and climax species.

1.1 Organization of the Thesis

The research described in this thesis is presented in two (2) chapters, each of which was written as a stand alone manuscript for publication. Each chapter begins with a preface that describes how the chapter relates to the thesis as a whole and includes a brief summary of the research (i.e., abstract). Each chapter then follows a standard journal paper (Soil Sci. Am. J.) format with (i) a brief introduction including a review of the relevant literature; (ii) a detailed materials and methods section containing enough detail that other researchers could repeat the work; (iii) a summary of the results and the statistical treatment of the data; and (iv) a discussion of the results, relating them to the original research questions and placing them into context with the current published literature, also including a discussion of the implications of the research.

Chapter 3 presents the work related to the investigation of the influence of various soil amendments on the growth and metal uptake of two understory forest species, as well as these amendments' influences on microbial community structure and soil metal concentration. Chapter 4 similarly investigates the influence of the same amendments on the growth and metal uptake of two climax forest tree species, as well as the amendments' influences on microbial community structure and soil metal concentration.

The research chapters (Chapters 3 and 4) are followed by a synthesis chapter (Chapter 5) that connects the individual chapters, summarizes major findings and implications of the research, as well as suggests directions for future research on the subject. Literature cited throughout the thesis is compiled in the Literature Cited section that follows after Chapter 5. Limestone requirements for the soils used in the experiment, Pearson product moment correlation coefficient 'r' tables, and information on germination of tree seed in the soils used in this study can be found in Appendices A to C.

2. LITERATURE REVIEW

2.1 Revegetation Strategies for Heavy Metal Affected Soils

Inorganic pollutants appear as natural elements on earth, but can be concentrated and released into the atmosphere by anthropogenic means. Mining, industry, traffic, agriculture, and military activities can lead to the buildup of these pollutants into the soil, air and water (Pilon-Smits, 2005). Metals such as As, Cu, Ni, Pb, and Zn often are found in concentrations higher than are tolerable for plant growth (Kozlov and Zvereva, 2007). Some metals, such as Pb and As, are problematic at lower concentrations, and their speciation and mobility are influenced by soil factors such as pH and reduction reactions (O'Day and Vlassopoulos, 2010). Unlike organic pollutants, metals cannot be degraded, only stabilized or transformed. This affects how metals behave in the soil system, and how they affect plant growth.

Historical forms of soil cleanup often are invasive, cost-prohibitive and destructive (Fletcher, 2006). These environmental cleanups are often labeled *'ex-situ'*, because they take place outside of the area that needs to be cleaned. *Ex-situ* cleanups often are not acceptable to the public and have been labeled, on occasion, as more destructive than the contaminants themselves (Fletcher, 2006). *In-situ* forms of remediation that involve revegetation are much less destructive, more environmentally and cost-friendly than *ex-situ* forms, and they have the added bonus of not disturbing native plant and microbial communities (Fletcher, 2006). Plant-based forms of remediation also are solar-powered via photosynthesis, using less fossil-fuel energy than traditional forms of cleanup. Plants can be used to remediate a contaminated area by restricting the movement of contaminants and preventing them from getting into the water table as a consequence of uptake and transpiration of contaminants in the plant, or by modifying passive movement of the contaminant in the evapotranspiration stream, collectively termed *phytovolatilization* (Fletcher, 2006; Ruttens et al., 2006). Additionally, plant roots can extract mobile ions from the soil and store them in roots and shoots (*phytoextraction*). Some plant enzymes also have the ability to degrade organic pollutants (*phytostimulation/phytodegradation*) (Fletcher, 2006; Ruttens et al., 2006). Soil microbiota in the form of bacteria and fungi also can influence organic pollutant degradation in the root zone (*rhizodegradation*), while plant roots

also stabilize the soil and prevent it from further erosion by wind or water (Pilon-Smits, 2005; Ruttens et al., 2006). *Phytostabilization* is highly dependent on the effectiveness of a plant root system and can occur by sorption of the metals to the roots, the precipitation of the metals into an immobile form, complexation of the metals, or the transformation of metals into forms that are less plant available (Ghosh and Singh, 2005).

Plants differ greatly in their capacity for phytoremediation. Root physiology, stress and drought tolerance, associated microorganisms, climate, toxicity levels, pollutant availability, and soil properties such as organic matter content and particle size, all are influential in the level of success of a revegetation program (Pilon-Smits, 2005; Ruttens et al., 2006). In general, plants that work well for phytoremediation are fast-growing, metal tolerant, hardy and competitive (Pilon-Smits, 2005). Plants with large, dense root systems will facilitate quicker rhizodegradation, in part by promoting higher microbial growth.

Understanding the complex role of plants, roots, bacteria and fungi in soil is important in any revegetation program (Khan, 2006). Plant roots promote symbiosis with bacteria and fungi, which can transform and degrade contaminants using various enzymes (Gerth, 2000). Plant roots also add various carbon (C) sources to the rhizosphere, which increases the bacterial activity in the area directly adjacent to the plant roots. This 'rhizosphere effect' results in a microbial density that is one to four orders of magnitude higher than densities in the bulk soil (Salt et al., 1998). Roots also have the potential to translocate and collect metals from the soil, concentrating them in the root tissue. These metals must be available for uptake, but once they are taken up they are removed from the soil cycling and can create a less toxic soil environment, creating a better environment for growth of less tolerant plants and microorganisms (Gerth, 2000). In addition, plant root exudates can cause precipitation reactions of the metal contaminants with soil constituents, further removing contaminants from the mobile and available nutrient pool (Ruttens et al., 2006).

2.2 Response of Plant Vegetation to Soils Containing Heavy Metals

The manifestation of metal toxicity in plants occurs through a number of means. Heavy metals can replace the cations in some enzymes, causing reduced activity, mortality and mutation (Lambers et al., 2008). Effects of metal toxicity are seen in the roots, where stunting occurs. Depending on soil conditions such as organic matter content and texture, pH, metal species,

fertilization, plant species, fungi, and soil redox potential, the availability of metals to plants may differ (Cheng, 2003; Nieminen, 2004).

Metals have differing effects on plant species. For example, Mn, Zn and Cd affect photosynthesis. Zinc also affects water uptake and Cu has a tendency to collect in root tissue and subsequently stunt growth (Nieminen, 2004; Lambers et al., 2008). Often metals in combination will affect bioavailability of one or more of the metals, and can affect the nutrient status of seedlings (Cheng, 2003; Nieminen, 2004). Paschke et al. (2000) suggests that damaging effects from metal uptake can influence water uptake, photosynthesis, and cell wall permeability.

Using grasses to limit the movement of metals out of an affected area and stabilize the soils is one technique for revegetation of industry-affected areas. Seeding a grass-legume mix allows for rapid growth resulting in an aesthetically pleasing green mat that also provides soil and microclimate stabilization, traps snow for increased moisture and has the potential to introduce biologically fixed nitrogen (N) into the system (Winterhalder, 1995). Tufted hairgrass (*Deschampsia caespitosa)* is a metal tolerant grass that has the ability to colonize areas of low pH (less than 4.0) (Winterhalder, 1995). It is used for revegetation purposes in restoring cover to disturbed stream banks and canals, and reclaiming mine sites (Darris and Gonzalves, 2009). Paschke et al. (2000) found that tufted hairgrass readily accumulated Zn, and stored it in both roots and shoots. The researchers also found that root growth of this grass was more susceptible to Zn toxicity (indicated by stunted growth) than was shoot growth. Ryegrass (*Lolium sp.*) was found to take up Pb and Zn and store it in both roots and shoots, but applications of green compost, wood bark, and cork were observed to reduce this effect (Rate et al., 2004; Nwachukwu and Pulford, 2009). Often, N deficiencies challenge revegetation efforts, and the addition of plants capable of biological N fixation into the revegetation strategy often is the key to success (Winterhalder, 1995). Frerot et al. (2006) found that the use of a legume in various mixtures of grasses increased soil N, thereby increasing soil cover and biomass in a soil contaminated with high total soil concentrations of Cd, Pb, and Zn (1 382, 92 700, and 161 000 mg kg^{-1}, respectively).

The influence of heavy metals on tree species is similar to that of grasses; root and shoot growth is reduced, and often survival is compromised with increasing soil metal concentrations (Jones et al., 1984; Nieminen, 2004). Helmisaari et al. (2007) found that white birch (*Betula pubescens*) and Scots pine (*Pinus sylvestris*) naturally colonized a soil affected by Ni

(exchangeable concentration of 250 mg kg^{-1}) from a smelter when mulch was used as a ground cover. In greenhouse experiments, seedlings of Scots pine were found to accumulate both Cu and Ni in roots, but researchers noted that the mobility of these two metals within the plant differed (Cu is significantly less mobile than Ni, and will tend to stay within the plant roots) (Nieminen, 2004). The movement of metals within the plant tissues can determine if they are returned into the soil system (i.e., through dropped leaves) (Nwachukwu and Pulford, 2008). Aspen (*Populus tremuloides)* is potentially useful for revegetation work, as this species can produce clones readily, and thereby colonize rapidly. Aspen typically is associated with many different types of ectomycorrhizae, some of which have been found to be metal tolerant, including *Laccaria* and *Tricholoma* species (Cripps and Miller, 1993; Cripps, 2003). According to Wotten et al. (1986) seedling and root growth of jack pine (*Pinus banksiana)* and black spruce *(Picea mariana)* trees were significantly affected by atmospheric deposition of metals and soil metal concentrations, although seed quality and germination was not significantly affected by pollutant deposition. Jack pine needles were shown to contain elevated concentrations of Cu, Pb, and Zn from smelter deposition in Sudbury, ON, even after airborne metal deposition was reduced, suggesting that this species actively accumulated these metals (Gratton et al., 2000). According to Jones et al. (1984), jack pine roots have been shown to accumulate Ni and Cu; however, these metals can significantly impede healthy root development.

2.3 Microbial Community Response to Soils Containing Heavy Metals

Microbial communities can be sensitive indicators of the success of a revegetation strategy, as changes in community composition and size can occur when communities are stressed or recovering. The microbial community often can be an indicator of effects from both chemical processes as well as bioavailability of contaminants within the soil (Hinojosa et al., 2005). The fatty acids found in a soil vary depending on the microbial communities present within it. This variation among soil microfauna is important because it allows a specific 'fingerprint' to be assigned to various microorganisms, and thus changes in microbial abundance in response to a stressor can be easily traced (Kelly et al., 2003; Hinojosa et al., 2005). In projects that involve revegetation, often the microbial population is overlooked in favor of a more visual representation (i.e., plant growth); thus, there is a limited understanding of microbial ecology on disturbed and reclaimed sites (Dmitriu et al., 2010).

Phospholipid fatty acids (PLFA) are an important component of all microbial membranes. Analysis of these fatty acids gives information on living biomass, community composition and microbial activity. Although PLFA analysis is not an indicator of microbial diversity, it is a rapid indicator of both microbial community structure and composition (Bardgett and McAlister, 1999). When the fatty acids of bulk soil microbial communities are assessed using PLFA analysis, there are important biomarkers that allow differentiation between microbial and non-microbial fatty acids, which allows researchers to assess changes in the microbial community size and composition.

The biomarkers that researchers have used to determine microbial community structure using the PLFA method differ greatly; however, some biomarkers have been shown to be particularly useful in indicating rapid changes in the microbial community, especially in the context of the disturbance of a system. The fatty acid 16:1ω5c is an important component in the cell walls of AMF, and is known to be particularly responsive to metal concentrations in soil (Hinojosa et al., 2005). Another fungal marker, 18:2ω6,9, has been useful in monitoring the impact of metals on fungal populations, and was found to increase with decreasing Cu concentrations, indicating it is a responsive indicator in terms of metal concentration (Kiikkila et al., 2001). Other components of the soil microbial biomass also have been shown to negatively respond to soil metal concentrations, including actinomycetes and Gram-positive (Gr+) bacteria (Kelly et al., 2003).

The fungal:bacterial (F:B) ratio also is purportedly an important soil quality indicator, as undisturbed ecosystems tend to have a higher proportion of fungal to bacterial biomass (Hinojosa et al., 2005). Shifts in this ratio to one that is more fungal dominated can indicate a conversion of the soil microbial community to one that is more reliant on natural soil biological processes, such as organic matter decomposition, that are increasingly present in a natural, undisturbed system (Bardgett and McAlister, 1999). Pennanen et al. (1996) found that this ratio changed along a pollution gradient, where the fungal community was more sensitive to pollution. Fungal communities have been observed to respond negatively to disturbance, perhaps due to the lack of fine root systems available for colonization (Bardgett and McAlister, 1999). Dmitriu et al. (2010) concluded that reclamation imposes shifts in microbial community structure by changing the relative amounts of fungal and bacterial components contributing to the microbial biomass. Thus, the F:B ratio may be indicative of microbial community change.

Another method to determine microbial community size is the chloroform fumigation-extraction method (Vance et al., 1987). This method compares the amount of C and N released between a fumigated sample and a non-fumigated control. The difference in C and N released on fumigation is taken to be proportional to the size of the microbial community, as extracted C comes from the cell walls of soil microorganisms, and thus can be used to monitor microbial responses to environmental stress (Vance et al., 1987).

Changes in a microbial community – whether in quality, diversity, or abundance – can be the result of increased tolerance of the microbial community or the die-off of non-tolerant species. In either case, it is an important indicator of biologically significant changes in the environment and can help researchers identify and implement successful revegetation strategies. The addition of amendments to soils containing heavy metals not only can improve plant growth, but can improve microbial community function and shift community structures to those more representative of non-contaminated areas by changing soil pH and immobilizing metals, thereby creating a more favorable environment for microbial growth (Kelly et al., 2003).

2.4 The Impact of Soil Amendments on Soil Quality in the Presence of Heavy Metals

The addition of organic matter to the soil in various forms has been used to increase soil aggregation, soil fertility and plant growth, as well as improve soil aeration, water and nutrient holding capacities, resistance to compaction and erosion, maintenance of soil pH, and the supply of nutrients for soil and microbial processes (Henry and Bergeron, 2005; Gadepalle et al., 2007). Soil structure is perhaps the most important physical property influenced by SOM. The interaction of soil mineral particles with SOM creates a soil structure that is preferred for plant growth, and allows proper root development. This aggregation also decreases erosion and resists compaction, decreasing soil strength (Dick and McCoy, 1993). Total porosity can increase with increasing SOM levels, leading to larger numbers of large pores, and fewer small pores (Dick and McCoy, 1993). Chemically, SOM has numerous adsorption sites for plant available nutrients, improving nutrient retention and supply. The most resistant part of SOM is the most chemically active, and greatly influences cation exchange capacity (Bot and Benites, 2005). Organic matter also reduces the effects of acidic soil by increasing the concentrations of calcium (Ca) ions in the soil and displacing aluminum (Al) and hydrogen (H) ions (Farrell et al., 2010a).

Biologically, the increased nutrients attributable to the various affects of the SOM promote the proliferation of many different types of soil microbial communities.

Recently, it has been shown that the addition of organic matter can influence the bioavailability of contaminants in the soil, including metals (Brown et al., 2003; Farrell et al., 2010a). This can influence the leaching potential of metal contaminants, metals' persistence in the soil, as well as the potential for plant uptake of metals. These results can be positive or negative, depending on the desired outcomes of the revegetation program.

2.4.1 Compost

Compost is the product of decomposition of organic matter by soil microorganisms. Compost can be made out of many organic materials, including yard waste, and manure (Henry and Bergeron, 2005), and can include various plant-essential nutrients, including N, potassium (K), magnesium (Mg), and Ca (Borken et al., 2002). It is abundant, relatively inexpensive, is generally regarded by the public as 'environmentally friendly' (Ros et al., 2006), and affects soil physical properties such as organic matter content, aeration, water and nutrient holding capacity, and electroconductivity (Farrell et al., 2010b; Gadepalle et al., 2007). As such, it is being used in numerous revegetation projects to increase vegetation growth on sites as well as to buffer vegetation (whether pre-existing or newly planted) from the effects of heavy metals (Borken et al., 2002; Brown et al., 2003; Gadepalle et al., 2007; Nwachukwu and Pulford, 2008; Farrell et al., 2010). It is important to note that the compost itself must have low levels of metals, or further additions of compost may increase total and plant available metals, and may increase uptake of metals into roots and shoots (Pinamonti et al., 2007). Additionally, high rates of amendments might be required to achieve significant progress in revegetation (Nwachukwu and Pulford, 2009; Baker et al., 2011).

Composts have been used as amendments with the goal of remediating metal affected soils. Typically, the addition of this material is intended to immobilize the metals and provide nutrients to enhance plant growth. The addition of compost often allows plants to establish a root system to prevent soil erosion. The compost also provides binding sites for heavy metal immobilization (Brown et al., 2003). The heavy metals then become unavailable for plant use, and form complexes with organic matter, oxides and carbonates within the soil (Gadepalle et al., 2007). Cordova et al. (2011) found that adding compost to Cu-affected soils significantly

increased both the quantity of Cu bound to the organic fraction, and the availability of plant nutrients to encourage plant growth. The Cu-organic matter complexes that were formed also resulted in a reduction of leaching of metals through the soil profile. Other researchers found that the application of compost decreased the leaching of Cu, Pb, and Zn throughout the profile due to complexation with organic matter (Farrell et al., 2010b).

Adding compost to heavy metal affected soils has the potential to decrease plant accumulation of heavy metals by buffering seedlings from high soil concentrations of S, As and other metals (Helmisaari et al., 2007; Farrell et al., 2010a). Seedlings planted in mulch pockets are protected from drought, and blown-in seeds have the protection they need to develop strong root systems and explore the environment (Helmisaari et al., 2007). Farrell et al. (2010a) observed the growth of roots in heavy metal affected soils below an added compost layer and suggested that this observation indicates the potential for future site stability.

Although organic amendments can reduce bioavailability of some metals, the positive effects of organic matter addition may decline after organic matter decomposes. For example, in one case the addition of organic matter was negated after only two years, at which time the organic matter was decomposed, causing immobilized metals to re-enter solution (Borken et al., 2002). In addition, high background salt concentrations in the soil as well as combinations of metals in soil can contribute to a reduction in the amount of metals sorbed (Nwachukwu and Pulford, 2008).

Compost provides an influx of healthy microbial communities into the soil, and also provides a ready energy source for all native soil microorganisms and fungi (Dick and McCoy, 1993; Henry and Bergeron, 2005). Perucci (1990) found that organic C was highly correlated with biological activity, indicating a link between compost addition to soil and microorganism response. Borken et al. (2002) reported that compost additions to soils affected soil respiration and microbial biomass C, but only in the upper mineral layers. Microbial N, S, and phosphorus (P) also are positively affected by compost additions (Perucci, 1990). Farrell et al. (2010b) found that compost additions to soils containing heavy metals resulted in a significant increase in bacterial and fungal diversity and activity as compared to a control. Baker et al. (2011) applied high rates of compost to contaminated mine wastes and observed a significant increase in total microbial biomass. Microbial community size can increase with application of compost (Kiikkila et al., 2001), but functional activity may not increase substantially (Farrell et al., 2010b).

Microbial communities exposed to metal-affected soils but buffered from toxic effects with a layer of compost may increase tolerance to that metal, as Kiikkila et al. (2001) found when examining Cu-affected soil. These effects on soil microbial communities may not last. For example, Borken et al. (2002) found that microbial respiration increased with compost additions, but lessened in the second year. Similarly, Perucci (1990) reported that the application of compost only increased microbial biomass for three months.

2.4.2 Biochar

Biochar has the ability to increase long-term soil C concentrations and improve crop growth due to its chemical recalcitrance and high sorption properties. It is the product of thermal decomposition of biomass produced by pyrolysis, and can be made out of many different types of organic matter, including biomass energy crops and residues, agricultural waste, compost (both municipal and kitchen), animal waste, and sewage sludge (Sohi et al., 2009; Namgay et al., 2010). These partially combusted plant or C-sourced materials have highly variable chemical and physical properties, depending on the source of organic materials used to create them. Biochar has a long residence time in the soil, and is highly resistant to decomposition (Warnock et al., 2007) and there is evidence for low levels of oxidation, transformation, and biological degradation losses (Preston and Schmidt, 2006). Due to these properties, biochar is being screened as a source for increasing C stocks in soils (C sequestration) to buffer against climate change (Preston and Schmidt, 2006; Sohi et al., 2009). These same properties could also make biochar a favorable amendment for remediating metal affected soils.

Within the soil, biochar additions create numerous effects, including increasing the organic matter content, increasing pH, and sorbing toxic metals making them less available (Beesley et al., 2010; Namgay et al., 2010). Biochar is highly aromatic, making it very stable. It also has the ability to increase cation exchange capacity in soils to retain key exchangeable plant nutrients (Sohi et al., 2009). The large number of pores created when organic matter is combusted helps to promote soil moisture retention, influencing moisture and nutrient release over longer time periods.

According to Warnock et al. (2007), biochar additions to the soil can affect microbial communities citing in particular, the impact on mycorrhizal fungi – a beneficial soil microorganism. There are numerous ways by which biochar contributes the potential to influence

mycorrhizal abundance and functioning (Warnock et al., 2007). By altering soil physical and chemical properties, nutrient concentrations in the rhizosphere are changed, greatly affecting the potential for mycorrhizal colonization. Biochar can also influence the activity of other microorganisms, which similarly can have beneficial or detrimental effects on mycorrhizal growth. Biochar also can absorb plant allelochemicals that are toxic to mycorrhizae, and may also provide a refuge for fungi and bacteria, allowing them to multiply more easily (Sohi et al., 2009).

Beesley et al. (2010) found that using biochar on a metal-affected soil significantly decreased the water-soluble concentrations of Cd and Zn, making them less plant available and therefore less phytotoxic. Namgay et al. (2010) investigated the impact of biochar on plant-available metals (As, Cd, Cu, Pb, and Zn) and found that applications of biochar significantly decreased the contents of As, Cd, and Cu in maize shoots. The amount of metals available for extraction also was influenced by biochar application, with As and Zn increasing, Pb decreasing, and Cu unchanged. Chen et al. (2006) found that with the application of increasing amounts of biochar, concentrations of Pb in both shoots and roots of Chinese cabbage *(Brassica rapa)* were significantly reduced (from 28 mg kg^{-1} to as low as 7 mg kg^{-1}). The high sorption capacity of biochar, coupled with its ability to influence pH, may be the key to its ability to manipulate uptake of metals into plant tissue, although it is important to take into account the source and makeup of the biochar and soil factors (Warnock et al., 2007; Namgay et al., 2010).

2.4.3 Mineral amendments

The practice of amending contaminated soils with minerals for immobilizing soil contaminants has been quickly gaining acceptance as a way to remediate metal affected soils. Commonly used materials include clay minerals, carbon, silica, phosphates, limestone, and combustion by-products such as fly ash (O'Day and Vlassopoulos, 2010). These minerals either adsorb contaminants to their surface, or incorporate them into structures, immobilizing them and subsequently taking them out of the soil system.

Depending on the crystalline structure, clay minerals that succeed at adsorbing metals typically have high cation exchange capabilities. However, Van Herwijnen et al. (2007) found that mineral amendments had limited effects on metal mobility when mixed with composts. They attributed this to the low levels of mineral-compost mixtures added, and indicated that when

organic matter declines, metals may become more available. This may increase the importance of minerals in a revegetation system since the impact of organic amendments may be temporary.

Glauconite is an iron-rich form of the clay mineral illite, with the chemical formula $(K,Na)(Fe^{3+},Al,Mg)_2((Si,Al)_4O_{10})(OH)_2$. Glauconite commonly has a greenish color, and is normally formed in shallow marine environments (Minkina et al., 2011). This mineral, like many other clay minerals, has a high content of exchangeable potassium and iron, and has excellent ion exchange, buffering, and sorptive qualities (Minkina et al., 2011). Glauconite's high ion exchange capacity resulted in a decrease in Pb content of radishes by almost 200% when applied as an amendment (Petkova et al., 2000).

Liming is used in agricultural landscapes to reduce soil acidity. It also is used in an industrial capacity to reduce acidity caused by mining, smelting, or refining. Adding limestone neutralizes the acidity in the soil by precipitating metals such as Al and Mn, adding Ca and Mg to the soil, and ameliorating the effects of Zn, Cu, or Ni on plants (Atkinson, 1964). The increase in soil pH associated with limestone application also creates a better environment for microbial activity, which in turn increases the cycling of organic matter and nutrients within the soil. At the Harjavalta Cu-Ni smelter in Finland, liming was found to decrease mobile Cu and Ni concentrations, increase plant-available Ca and Mg concentrations, decrease metal leaching, and increase tree growth and survival (Kiikkila, 2003).

Sudbury, ON has been the site of a Cu-Ni smelter since the 1800s. Impacts from logging and fires, as well as atmospheric deposition from the smelter led to a significant decline in tree cover and soil erosion, until approximately 100 km^2 of the area was completely barren (Lautenbach, 1987). Limestone added since a remediation program was initiated in 1978 has allowed reclamation of many key areas that once were devoid of vegetation and topsoil. Agricultural grade limestone applied at a rate of 11,000 kg ha^{-1} followed by seeding with a grass/legume mixture resulted in a green vegetation mat by the next spring. Over 12 km^2 have been reclaimed in this manner since the program's induction (Winterhalder, 2003). Winterhalder (1995) suggests that the addition of limestone to these soils worked to ameliorate metal effects in a number of ways including precipitation of Cu and Ni; reduced Al toxicity due to complexation with hydroxyl ions; reduction in metal uptake; increased available soil P, which acts as both a nutrient and buffer; and enhanced root membrane strength.

2.4.4 Fungal amendments

Many plants form symbiotic relationships with root mycorrhizae in natural and agricultural ecosystems. In fact, up to 90% of land plants in both natural and agricultural ecosystems form relationships with AMF (Khan, 2006). These fungi are important for the establishment of seedlings through root colonization, as well as for accessing otherwise unavailable nutrient sources, which greatly benefit plant growth and nutrition (Simard, 2009). Mycorrhizal fungi significantly increase the absorptive area of the plant root, allowing for optimum water and nutrient uptake, especially in soils affected by industry (Turnau et al., 2010). Under stressful conditions, symbiotic fungi are able to help support plant growth.

These relationships help both directly and indirectly in increasing plant tolerance to heavy metals, including acting as a buffer between the root and the heavy metal affected soil and reducing metal translocation into the shoots by either containing the metals in the roots or within the mycorrhizal structures themselves, increasing the plants tolerance against drought and nutrient stress thereby improving plant establishment, as well as affecting the aggregation of soil particles and increasing the uptake of soil pollutants into small pores (Leyval et al., 2002; Vosatka et al., 2006). In acidic soils, AMF may have a role in capturing base cations and slowly releasing them, as well as releasing nutrients through mineral weathering (Finlay et al., 2004).

Relatively few studies have been conducted to assess the influence of the soil-plant-microbe relationships in revegetation in metal-affected soils. However, there is potential to exploit these favorable relationships. It has been suggested that the ability to withstand pollution stress might be due to colonization of roots by mycorrhizae that help reduce metal toxicity (Wilkinson and Dickinson, 1995). Audet and Charest (2007) suggest that the mechanism for increased plant tolerance to heavy metals is due to fungal-metal binding processes that reduce availability of metals to plant roots. This modification of the root system allows the plant to tolerate stressful conditions such as low fertility, low pH, and metal toxicity (Cordell et al., 2000). Plants inoculated with AMF typically had increased plant biomass and root length, and influenced the development of rhizosphere community structures when coupled with a compost amendment (Solis-Dominguez et al., 2011).

Sequestration of metals within the plant roots also has been observed to increase with fungal associations. For example, Frey et al. (2000) found that Cd and Zn were sequestered within the fungal and root cells of Norway spruce *(Picea abies)* associated with the

ectomycorrhizal species *Hebeloma*. They reported a decrease in the transfer of Zn into the roots, and phytotoxic Cd was transformed into a non-toxic form. This indicates that the fungal hyphal net had a significant impact on the movement of these two metals within the plant-root system. Similarly, Krupa and Kozdroj (2007) found that inoculation of pine seedlings with fungi significantly reduced the translocation of Zn, Cd, and Pb from roots to shoots.

Given the role that fungi play in heavy metal affected soils, it is likely that successful restoration of plant communities is dependent upon fungal symbiosis with the plants, and the establishment of new mycorrhizal networks is often helped by contact from established networks nearby. Where native AMF are lacking, the introduction of strains adapted to metal toxicity and the existing climate may stimulate the natural process of symbiosis (Jeffries et al., 2003; Harris, 2009). Introducing non-native plant and mycorrhizae species into a landscape for the purpose of revegetation may put native species at risk of extinction by exotic or invasive introduced fungi (Cripps, 2003). Also, inoculation with non-native, expensive or generic fungi may not be precise enough to garner the desired results (Cripps, 2003). Native fungi adapted to a specific ecosystem have the potential to be much more efficient and cost-effective.

Harris (2009) suggests that in the context of disturbed landscapes, microbial communities are there to be manipulated. As an increase in the ratio of fungal to bacterial biomass is often observed as the forest progresses in establishment (Lauber et al., 2008), it stands to reason that the establishment of an appropriate (fungal-dominated) mycorrhizal community could be a precursor to the development of a target plant community.

3. THE EFFECT OF MICROBIAL AND ORGANIC AMENDMENTS ON THE GROWTH OF UNDERSTORY SPECIES *DESCHAMPSIA CAESPITOSA* AND *VICIA AMERICANA* AND RELATED MICROBIAL COMMUNITIES IN TWO METAL-AFFECTED SOILS

3.1 Preface

This initial experiment was conducted to examine the impact of soil amendments on plant growth and microbial community structure in metal-affected soils. The aim was to investigate whether a change in the microbial community could act as a precursor to positively influence plant growth. Plant biomass growth of two understory species was evaluated. Additionally, plant metal uptake, soil metal concentrations, and microbial community structure were investigated. This experiment also served to identify those amendment treatments that increased plant biomass the greatest amount over the control. These amendments were subsequently used in a further experiment in which treated soils were planted with forest tree species (Chapter 4).

3.2 Introduction

Growth of understory species in areas affected by metal smelting is fundamental in ensuring the success of a revegetation program (Helmisaari et al., 2007). Understory species are often used to stabilize the soil and are prized for their fast growth, resulting in a visually pleasing green landscape (Winterhalder, 1995). A lack of understory growth can enhance soil erosion, decrease soil water-holding capacity, and may increase leaching of metals (Helmisaari et al., 2007). However, metal uptake into plant tissues can cause reduced growth, mortality and plant mutation, depending on the availability of metals in the soil (Cheng, 2003; Lambers et al., 2008). Although most revegetation programs use a visual indicator of success (i.e., plant growth), soil microbial communities also can be affected by soil metal concentrations, and thus can be used as an indicator of community change as revegetation proceeds (Hinojosa et al., 2005). Key microbial biomarkers – of AMF, fungi, and Gr+ bacteria – have been shown to change with increasing or decreasing levels of soil disturbance (Kiikkila et al., 2001; Kelly et al., 2003; Hinojosa et al., 2005).

The addition of soil amendments to a metal-affected soil has the potential to reduce recovery time and enhance revegetation efforts (Helmisaari et al., 2007). Soil amendments such as compost and biochar can increase both soil water holding and cation exchange capacity, improve soil aggregation and contribute to the ability of the soil to adsorb or complex with metal ions, altering their availability to plants (Bot and Benites, 2005; Farrell et al., 2010a). Biological amendments, such as AMF, similarly can contribute to soil conditions that promote revegetation. The important symbiotic relationship between mycorrhizae and plant root systems can act as a 'buffer' between the plant and metal-affected soil, improving establishment; it also can increase root sequestration of metals (Frey et al., 2000; Leyval et al., 2002).

The objectives of this experiment were to determine which, if any, of the trial soil amendments increase the growth of two understory plants in metal-affected soil and to determine the effects of these amendments on various microbial indicators. The consequent impact of amendments on metal availability in the soil and metal uptake to plant tissues was assessed.

3.3 Materials and Methods

3.3.1 Site description and soil sampling

Soils used in this study were collected from areas surrounding Flin Flon, MB, and Creighton, SK, located in the Churchill River Upland Boreal Ecoregion. A mixed forest community composed of pine, spruce, fir, poplar, and aspen (Henderson and McMartin, 1995) is the expected climax ecosystem for the region. Hudbay Minerals Inc. has been operating a Cu and Ni smelter in the area since the 1930s. For decades, particulate emissions from the smelter, coupled with soil erosion and logging has resulted in weakly formed soils high in various metals, including Al, As, Cd, Cu, Pb, S, and Zn (Henderson and McMartin, 1995). Vegetation in the area is stunted and in many cases, native vegetation is non-existent.

Soils were collected from the area in August 2010 and subsequently used in a growth chamber experiment. Samples were collected from five sites previously identified as having either high or low metal concentrations during a 2008 survey of an area within 1 km of the area surrounding the former smelter (Fig. 3.1). Soil samples were from areas that had not previously been treated with other amendments, including limestone (unpublished data) (Table 3.1). Classification of the sampling points as either high or low metal soils was based on levels of Cu,

determined in October 2009 (unpublished data). Specifically, soils having greater than 300 μg g^{-1} available Cu were classified as high metal soils. Soils were collected from sites dominated by soils in the Brunisolic and Luvisolic Orders. Mineral soil samples were collected from below the organic matter layer, and included both the A and B horizons. Depth varied for each sample, depending on the depth of the horizons. The samples collected from the various sites were bulked according to the metal concentration (i.e., high and low), and were homogenized by thorough mixing. This type of sampling is known as 'composite sampling' and it is often used where knowledge of the mean is more important than knowledge of specific site variability (Byrnes, 2009). In this case, specific site characteristics were deemed less important than finding a treatment that performed well on a generally high or low metal soil representative for the Flin Flon area. The low metal soil had an average pH of 4.18, whereas the high metal soil had an average pH of 4.34.

Table 3.1. A description of the Flin Flon sites sampled in August 2010 for use in greenhouse trials, including soil type, metal concentrations and vegetation present at the site.

Site†	Soil Type	Metal Concentration‡	Vegetation Present
3.3	Thin Eluviated Dystric Brunisol	Low	Aspen, bentgrass
3.7	Eluviated Dystric Brunisol	Low	Aspen, bentgrass
4.9	Thin Eluviated Dystric Brunisol	High	Bentgrass
5.22	Orthic Grey Luvisol	Low	Alder, pine, birch, berries
6.5	Thin Eluviated Dystric Brunisol	High	Bentgrass

†Sample site numbers correspond to sites characterized during a 2008 soil survey of the area surrounding the smelter (unpublished data).
‡Based on 2009 copper concentration data (unpublished).

3.3.2 Soil preparation

Soils were transported in coolers and kept cool using icepacks. The soils were then air-dried and passed through an 8 mm sieve size to remove rocks, roots and stones. Sieved soils with the same metal concentration level were mixed in a large cement mixer to ensure homogeneity. Three soils were mixed to form the 'low metal' soil (collected from sites 3.3, 3.7, and 5.22), and two soils were mixed to prepare the 'high metal' soil (collected from sites 4.9 and 6.5). A reference forest sample (unaffected by metals) was used in one set of analyses (i.e., microbial

community profiling) to compare with the two soils used in the experiment. This soil was gathered from a non-metal affected forest location near Sherridon, MB (Table 3.2).

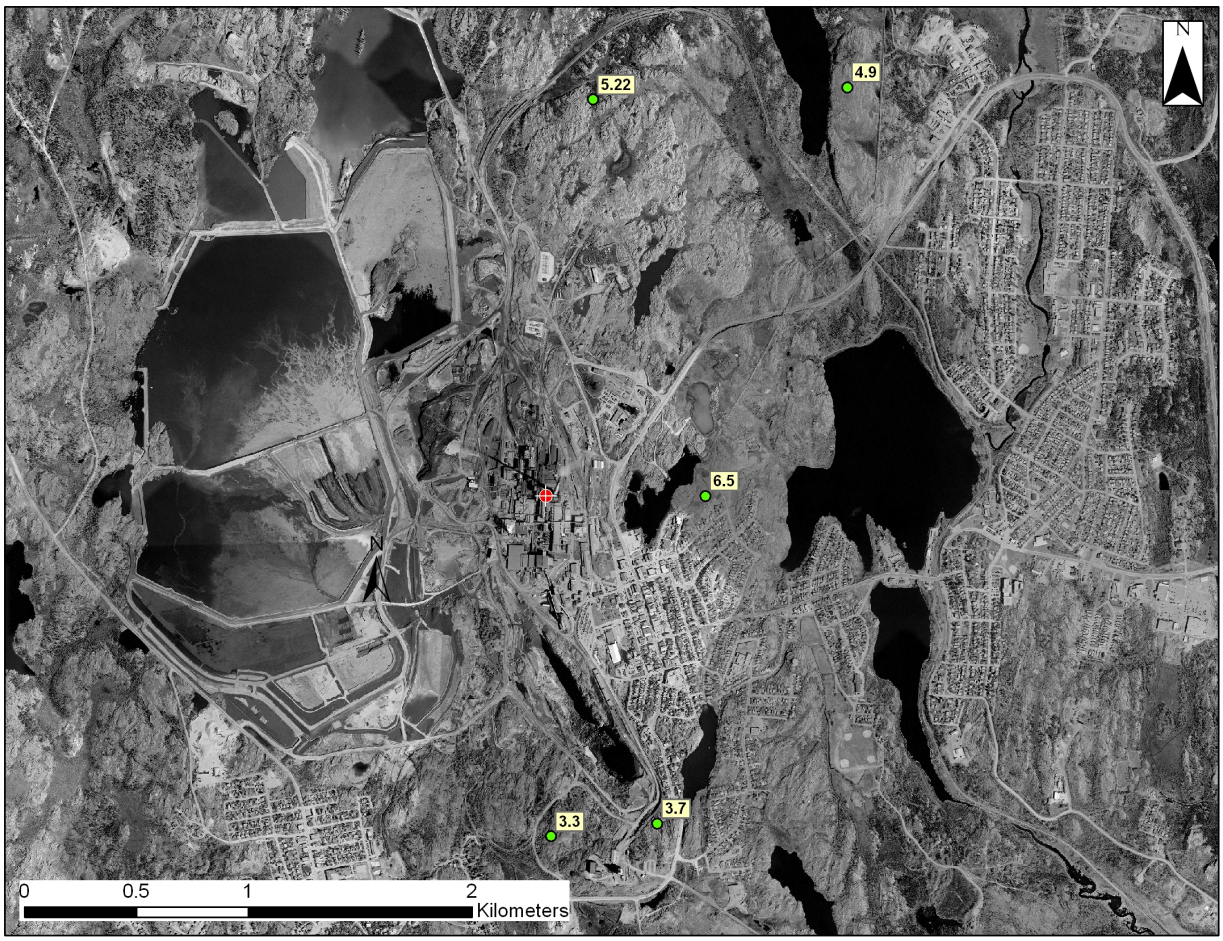

Fig. 3.1. A map showing Flin Flon, MB, and the location of sites used in this study in relation to the smelter stack (red). Green points indicate sites of soil collection. Source material used in the creation of the map provided by HudBay Minerals Inc.

A mite avoidance test was conducted by John Owojori, Department of Soil Science, Saskatoon, SK, according to Owojori et al. (2011) to verify that the level of toxicity in the metal affected soil retained its properties following bulking and processing of the soils.

Prior to use in the growth chamber experiment, dolomitic limestone (lime) was added to raise the pH of each bulked soil to 5.5. The rate of application of limestone was determined from limestone response curves calculated by ALS Labs (Saskatoon, SK) (Appendix A). The

dolomitic limestone was collected at the same time as soil collection, and was collected from a location in the same area from which the soils were collected. It is the same limestone source as is currently used as a field amendment for soils in the area. Limestone was ground with a heavy wooden roller to a particle size of 2 mm.

Table 3.2. Chemical characteristics of the low and high metal soils and control soil bulked properties, including pH, Al, Cd, Cu, and Zn contents.

		Soil Properties‡					
Soil Contents		**pH**	**OC**	**Al**	**Cd**	**Cu**	**Zn**
			%	mg kg^{-1}			
Low Metal	3.3†	4.18	7.45	22	1	19	322
	3.7						
	5.22						
High Metal	4.9	4.34	13.25	88	6	170	688
	6.5						
Unaffected Forest Control Soil	--	4.16	--	33	0	1	2

† Soils mixed together to create the composite soil sample (See Table 3.1).
‡ A subsample of soil was used to measure various soil properties; pH was determined before limestone additions; OC=Organic Carbon content.

3.3.3 Amendments and plant species used

The first trial was conducted under controlled conditions in a phytotron facility and was intended to assess amendments previously screened on similar soils and identified as having potential in revegetation strategies (data not published). The amendments chosen were composted manure (Moo Poo™, Westland, Ltd., Calgary, AB; CM), city compost (City of Saskatoon compost depot, Saskatoon, SK; MuC), meat and bone meal biochar (Titan Clean Energy, Saskatoon, SK; BMB), glauconite (Department of Soil Science, Saskatoon, SK; GC) and a mycorrhizal inoculant (Myke®Pro Landscape, Premier Tech Biotechnologies Ltd., Quebec, Canada; AM) (Table 3.3). Metal concentrations in each amendment were low, with the exception of Zn concentration in the AM treatment, and Al and Zn concentrations in the composted manure.

Two understory plant species were used in the growth chamber experiment - tufted hairgrass, and a legume, American vetch. These plant species have been shown to be metal tolerant; additionally, both species are desired in the area and American vetch is native to the study area (Winterhalder, 1995; Paschke et al., 2000; Frerot et al., 2006).

Table 3.3. Amendments used in Experiment #1, classified by source, type of amendment, pH and Al, Cd, Cu, and Zn concentrations.

Amendment	Trade Name	Source	Amendment Type	pH	Metal Concentrations			
					Al	Cd	Cu	Zn
					——— mg kg^{-1} ———			
Composted Manure; CM	Moo Poo™	Westland, Ltd., Calgary, AB	Organic	6.50	5660†	0.42	12	49
Bone Meal Biochar; BMB	--	Titan Clean Energy, Saskatoon, SK	Organic	9.45	0	0	3	0
Glauconite; GC	--	Department of Soil Science, University of Saskatchewan, Saskatoon, SK	Mineral	6.48	0	0	0	0
Municipal Compost; MuC	--	City of Saskatoon Compost Depot, Saskatoon, SK	Organic	6.97	0	0	3	0
Mycorrhizal Inoculant; AM	Myke®Pro Landscape	Premier Tech Biotechnologies Ltd., Quebec, Canada	Fungal‡	6.80	0	0	1	21

† Compost Manure (Al, Cd, Cu, and Zn) and mycorrhizal inoculant (Zn) were analyzed by ALS Laboratories, Inc., Saskatoon, SK.
‡ Fungal content (approximate spores g^{-1}): Endomycorrhizal fungi: *Glomus intraradices* (15); Ectomycorrhizal fungi: *Pisolithus tinctorius* (100,000), *Scleroderma cepa* (7500), *Scleroderma citrinni* (7500), *Rhizopogon roseolus* (3750), *Rhizopogon subscaerelescens* (3750), *Rhizopogon villosulus* (3750), *Rhizopogon vulgaris* (3750), *Laccaria laccata* (2250).

3.3.4 Experiment setup and harvest

The experiment was conducted using a fully randomized, factorial design with four replicates (Table 3.4). Four controls were included in the experiment, one for each plant and soil combination for a total of 256 cone-tainers™ (Stuewe and Sons, Inc., Tangent, OR). Cone-tainers™ are plastic, tapered, cylindrical planters, 3.8 cm in diameter by 21 cm in length that allow for maximum plant root expansion. Amendment treatments and combinations are shown in Table 3.5.

Table 3.4. Plant, soil, and amendment combinations used in Experiment #1. Four replicates were included.

Plant	Soil	Amendment Treatment†
Tufted hairgrass	Low metal	1 to 16
Tufted hairgrass	High metal	1 to 16
American vetch	Low metal	1 to 16
American vetch	High metal	1 to 16

†See Table 3.5 for treatment (amendment) descriptions.

Table 3.5. Treatments and amendment combinations used in Experiment #1.

Number	Amendment Treatment
1	Control (no amendment added)
2	Composted Manure (CM)
3	Bone Meal Biochar (BMB)
4	Glauconite (GC)
5	Municipal Compost (MuC)
6	Granular Arbuscular Mycorrhizal – Ectomychorrizal Inoculant (AM)
7	CM + BMB
8	CM + GC
9	CM + MuC
10	CM + AM
11	BMB + GC
12	BMB + MuC
13	BMB + AM
14	GC + MuC
15	GC + AM
16	MuC + AM

A 10% w/w (amendment/soil) amendment rate was used for all treatments except the mycorrhizal inoculant, which was applied at a manufacturer suggested rate of 2 g per cone-tainer™. Soils were mixed with treatments and then placed in cone-tainers™, for a total mass of ninety grams of treated soil. Mycorrhizal inoculant was applied to the manufacturer's specifications and placed in a layer just below the planting depth of each seed to ensure

maximum root contact. The soil in all cone-tainers™ was kept at 80% field capacity by weight, using repeated applications of reverse osmosis water, as required. Five American vetch seeds were placed at a depth of 3 cm, and were thinned to two plants per cone-tainer™ following emergence. Approximately 10 tufted hairgrass seeds were placed on the surface of the soil and covered with a very thin layer of loose soil. White polypropylene beads (2mm spherical diameter) were placed on the surface after germination and emergence to reduce moisture evaporation from the soil surface. Plants were maintained in a growth chamber with a 16/8 hour day/night cycle and day/night temperatures of 24°C/21°C.

All plants were harvested 8 wk after planting, at which time shoots were cut at the soil surface and roots were gently separated from the soil by hand, and washed in tap water. Plant material was weighed, dried at 40°C, weighed again, ground, and then stored for future metal concentration analysis. Soil, previously removed from each cone-tainer™ and separated from the roots, was thoroughly mixed and subsequently subsampled for metal concentration analysis and various microbial analyses. Subsamples were either air dried for storage, freeze dried for microbial community analysis, or refrigerated at 5^{o} C for microbial biomass analysis. Only samples with treatments of the control, BMB, BMB+GC, BMB+MuC and BMB+AM were used for further metal and microbial analysis, as these were determined to have the greatest biomass increases of the fifteen combinations.

3.3.5 Soil, plant, and microbial analyses

Soluble metals (Al, Cd, Cu, and Zn) were extracted from the soil samples based on the extraction method of Wightwick et al. (2010). Briefly, air dry soil (2 g) was weighed into a 200 mL polypropylene container to which 20 mL of 0.01 *M* $CaCl_2$ extraction reagent was added to achieve 1:10 soil:extraction solution ratio. The containers of soil and extraction reagent were shaken overnight at room temperature (~20ºC). Supernatant was vacuum filtered through a 0.2 µm millipore filter (Millipore Corporation, Billerica, MA). Microwave plasma – atomic emission spectroscopy (MAP-AES 4100; Agilent Technologies, Mississisauga, ON, Canada) was used to analyze elements contained in the extracts. Wavelengths monitored were: Al (396.15 nm), Cd (228.80 nm), Cu (324.75 nm), and Zn (213.86 nm).

Elements (Al, Cd, Cu, Ni, and Zn) in plant tissues were determined using a procedure adapted from Ippolito and Barbarick (2000) and Lesniewicz and Zyrnicki (2000). Briefly, ~0.1 g

ground plant sample was measured into 100 mL glass digestion tubes and 6 mL HNO_3 was added and heated to 90ºC. After 75 min, 5 mL H_2O_2 was added and the mixture was left to digest for an additional 30 min. After cooling, the solution was brought up to 25 mL using distilled water and filtered through a Whatman #5 filter. Blanks free of plant material were included in the digestions to provide information on background metal concentrations. Prior to analyses using MAP-AES, solutions were syringe filtered to 0.1µm (Whatman, Piscataway, NJ). Wavelengths monitored were: Al (396.15 nm); Cd (228.80 nm); Cu (324.75 nm); Ni (352.45 nm); Zn (213.86 nm).

Quantitative biomass measurements of fungal and bacterial communities were measured by phospholipid fatty acid analysis (PLFA). This analysis was performed using a modified method from Helgason et al. (2010), which was based on methods by White et al. (1979) and Bligh and Dyer (1959). Briefly, fatty acids were extracted from 4.0 g of freeze-dried, ground mineral soil, and separated on a 0.50 g silicon solid phase extraction column (Varian Inc., Mississauga, ON). After extraction, phospholipids were methylated and analyzed using a Hewlett Packard 5890 Series II gas chromatograph with a 23.85m x 0.2mm x 0.3µm film thickness Ultra 2 column, and a flame ionization detector (GC-FID) with a temperature of 300°C (J&W Scientific). Injector temperature was set at 250°C, and the analysis temperature program was as follows: oven temperature 170°C, ramping to 260°C at 5°C min^{-1} and then to 310°C at 40°C min^{-1} for a run time of 20.75 minutes. MIDI identification software (MIDI Inc., Newark, DE) was used to identify peaks, which were quantified using the concentration of an internal standard (methyl nonadecanoate [19:0]). Total microbial biomass was calculated as the sum of all identified PLFA peaks, relative to a known concentration of standard (Helgason et al., 2010). Biomarkers used to represent Gr+ bacteria were i140:0, i15:0, a15:0, i16:0, i17:0, and a17:0. Biomarkers used to represent Gram negative bacteria (Gr-) were 16:1ω7t, 16:1ω9c, 16:1ω7c, 18:1ω7c, 18:1ω9c, cy17:0, and cy19:0. Fungal biomass represented by 18:2ω6c and 16:1ω5c was used to indicate the presence of AMF (Frostegaard and Baath, 1996; Hamel et al., 2006; Helgason et al., 2010). Physiological stress biomarkers (labeled as Stress 1 and Stress 2) represent the ratios of cy17:0 to 16:1ω7c and cy19:0 to 18:1ω7c, respectively (Grogan and Cronan, 1997). The relative change in F:B biomass can indicate shifts in the microbial community influenced by stress, including metal concentration (Pennanen et al., 1996). Samples of unlimed, untreated, and unplanted soil were included as a baseline to determine changes in

microbial biomass and community composition after treatment additions. Calculations on peak areas to determine total microbial biomass values were done using equations found in Hedrick et al. (2005).

A modified version of the chloroform fumigation-extraction method (Voroney et al., 2008) was used to determine microbial biomass C (MBC) and N (MBN). Briefly, four portions of moist soil (~15 g) were weighed, giving two replicates of soil to be fumigated with $CHCl_3$, and two unfumigated replicates (controls). The controls were extracted with 0.5 *M* K_2SO_4 immediately, while the other samples were placed in a desiccators under vacuum and fumigated for 24 h in darkness. Following fumigation, extractant (0.5 *M* K_2SO_4) was added to the samples at a ratio of 1:3 oven dry soil weight (g) to extractant volume (mL), and samples were shaken for 1 h and subsequently filtered (Whatman GF 934-AH, Piscataway, NJ). The filtrate was refrigerated at 5^{o} C until analysis for total C and N (TOC-V, Shimadzu Scientific Instruments, Columbia, MD). Microbial biomass carbon and MBN were calculated according to Voroney et al. (2008). Values used for k_{EC} and k_{EN} (representing the efficiency of the extraction for C and N) were 0.35 and 0.5, respectively (Joergensen, 1996 and Joergensen and Mueller, 1996).

3.3.6 Statistical analysis

Data residuals were used as an indicator of normalcy and to determine if data transformation was necessary (Goodall, 1993). If necessary, data sets were either square root transformed (total biomass), or log(x+1) transformed (all plant and soil metal concentration data and all PLFA data) (Little and Hills, 1978; Steel and Torrie, 1980). Data points attributed to faulty machine analysis were removed (one data point in soil MBN analysis for experiment 2).

Differences between treatments were determined using an analysis of variance (ANOVA) in CoStat (CoHort Software, Monterey, CA). Fisher's least significant difference (LSD) was used as a means separation test. Correlations between variables (i.e., plant growth versus metal uptake) were determined using the Pearson Product Moment Correlation Coefficient, 'r'. Significant differences are reported for those means that differ at the $P<0.05$ level, unless otherwise indicated. Orthogonal contrasts were used to find differences at significance levels of $P<0.10$, 0.05, and 0.01 to compare several groupings of treatments, as described in data tables.

Interpretation of the PLFA data (mol %) using non-metric multidimensional scaling (NMDS) was done using PC-ORD version 6.0 (MJM Software Design, Gleneden Beach, OR).

Nonmetric multidimensional scaling is an ordination method that is well suited to data that is non-normal, and thus handles ecological data sets well (McCune and Grace, 2002). Final ordinations presented are those with minimized final stress. Percentages displayed on the x- and y-axis represent the amount of variance in the solution that is represented by each axis (McCune and Grace, 2002). Multi-response permutation procedures (MRPP) were performed using the Sørensen distance measure to test for differences between groups within the ordination plots. The chance-corrected within-group agreement (*A*) describes within-group homogeneity as compared to random expectation through a gradient of 0 (i.e., heterogeneity within groups is equivalent to chance expectation) to 1 (i.e., all items are identical within groups) (McCune and Grace, 2002). The statistic (*p)* represents the statistical significance of *A*. Relationships between measured variables and ordination scores are represented by r, the correlation of the variable with the ordination, and appear as vectors on each ordination plot (McCune and Grace, 2002). Fatty acid methyl esters with less than 1% representation of total concentration of PLFA were not included in ordinations.

3.4 Results

Mite avoidance measurements resulted in a 58% preference for the low metal soil and 32% preference for the high metal soil (data not shown). This indicates that the soil retained its properties following bulking and processing of the soils.

3.4.1 Plant biomass

American vetch and tufted hairgrass total biomass growth differed significantly between treatments (Fig. 3.2 and Fig. 3.3). Overall, American vetch produced more biomass than tufted hairgrass, and both plants had significantly higher biomass growth in the low metal soils as compared to the high metal soils. While the treatments that increased plant biomass by the largest amount over the control varied between plant species and soil type, in general treatments that included biochar (BMB, BMB+CM, BMB+GC, BMB+MuC, and BMB+AM) increased plant biomass the most over the control.

Contrast analysis on total biomass growth was done to compare the effects of using biochar and AMF inoculation as amendments alone and in combination with other amendments (Table 3.6). For American vetch plants grown in either low or high metal soil, biochar treatments

were significantly higher from the control. Additionally, the solo biochar treatments were significantly different from the treatments that contained biochar combined with another amendment. Biochar contrasts for tufted hairgrass were less conclusive, as were contrasts on AM inoculation for both plants.

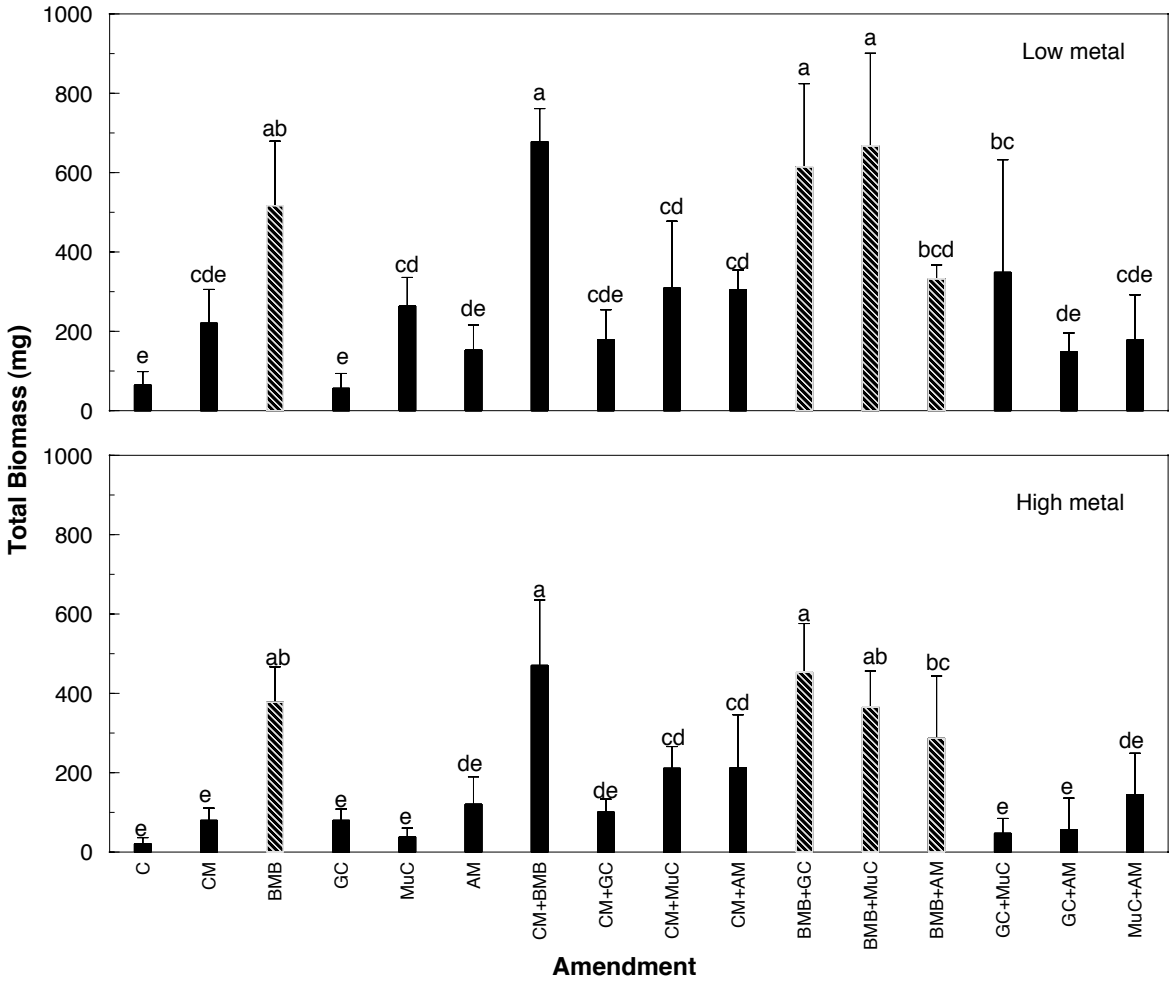

Fig. 3.2. Total biomass growth (mg) of American vetch after 8 wk of growth in low and high metal soils from Flin Flon, MB. Hatched bars indicate treatments containing biochar. Letters that differ within each separate graph are significantly different at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4). Treatment information can be found in Table 3.5.

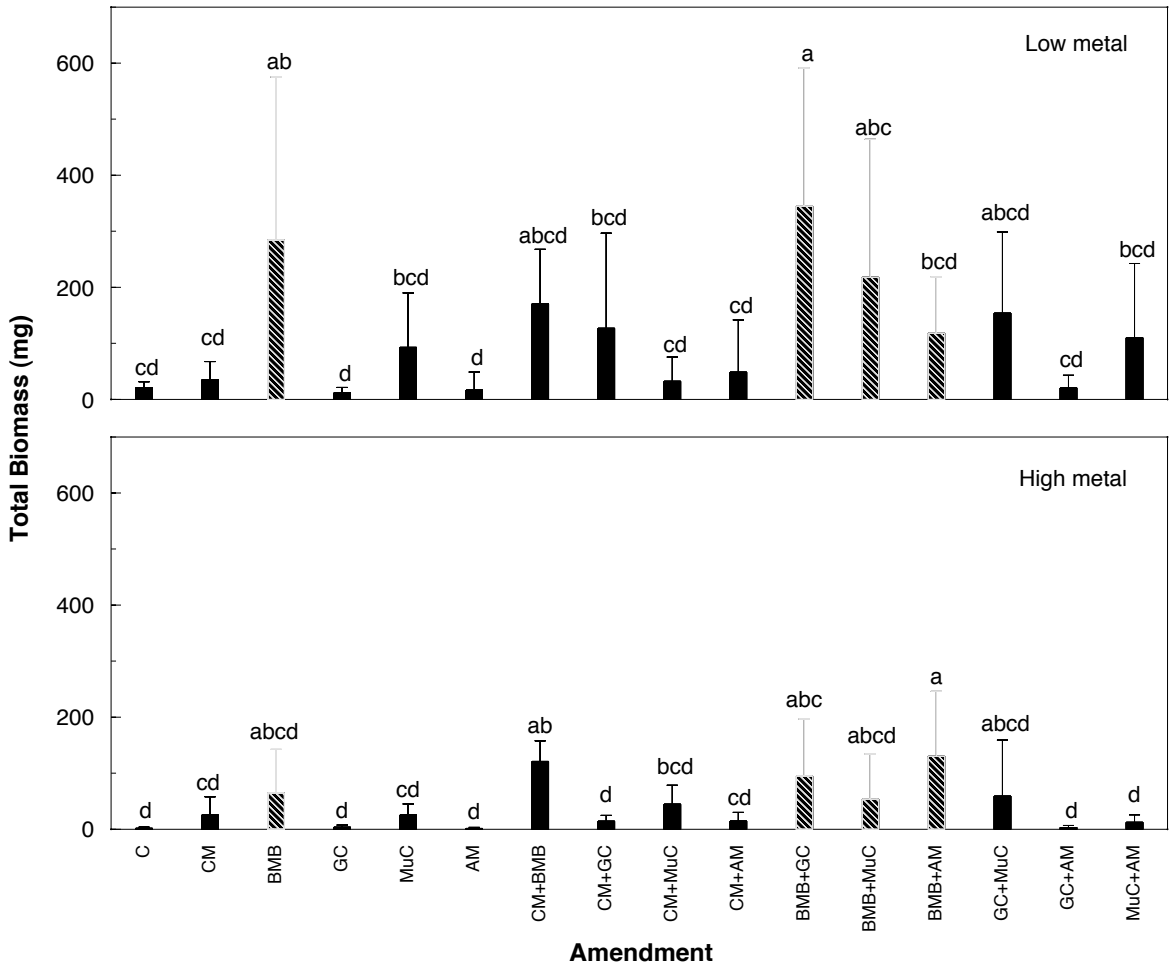

Fig. 3.3. Total biomass growth (mg) of tufted hairgrass after 8 wk of growth in low and high metal soils from Flin Flon, MB. Hatched bars indicate treatments containing biochar. Letters that differ within each separate graph are significantly different at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4). Treatment information can be found in Table 3.5.

Table 3.6. Means comparisons (LSD) and contrast analyses comparing total biomass from two plant species, American vetch and tufted hairgrass, grown in high and low metal content soils with various treatments.

		American vetch		Tufted hairgrass	
Treatment†	**Treatment Number**	**Low metal soil**	**High metal soil**	**Low metal soil**	**High metal soil**
		Total biomass (mg)			
C	1	64.5	20.6	20.9	1.7
CM	2	220.3	79.6	34.8	25.3
BMB	3	516.7	377.2	284.4	64.3
GC	4	56.2	79.9	11.6	3.5
MuC	5	263.8	38.2	92.8	25.3
AM	6	152.7	120.3	16.6	1.4
CM+BMB	7	677.7	470.2	170.4	120.9
CM+GC	8	178.6	100.4	126.7	14.2
CM+MuC	9	308.8	210.8	32.3	44.5
CM+AM	10	304.7	212.0	48.6	14.5
BMB+GC	11	614.3	453.6	344.9	94.7
BMB+MuC	12	667.3	365.5	217.6	53.7
BMB+AM	13	332.1	286.7	118.0	129.8
GC+MuC	14	348.4	47.1	153.8	58.9
GC+AM	15	148.2	56.5	20.4	3.1
MuC+AM	16	177.9	144.7	109.9	12.4
LSD‡ (0.05)		*189.6*	*148.9*	*199.8*	*80.3*
Contrasts	**Treatments Compared**	**Differences Between Means**			
AM, CM+AM, BMB+AM, GC+AM, MuC+AM - C	6, 10, 13, 15, 16 - 1	158.7 *	143.4 *	41.8	30.5
BMB, CM+BMB, BMB+GC, BMB+MuC, BMB+AM - C	3, 7, 11, 12, 13 - 1	497.1 ***	370.0 ***	206.1	90.9
CM+BMB, BMB+GC, BMB+MuC, BMB+AM – BMB	7, 11, 12, 13 - 3	56.2 ***	16.8 ***	-71.6	35.5 **
CM+AM, BMB+AM, GC+AM, MuC+AM - AM	10, 13, 15, 16 - 6	88.1	54.7	57.7	38.5

*, **, and *** represent differences at significance levels of $P \leq 0.10$, 0.05, and 0.01, respectively

† Refer to Table 3.5 for a complete description of treatments.

‡ LSD results are conservative estimates where some treatments had n<4 due to missing values.

3.4.2 Soil microbial biomass carbon and nitrogen

Soil microbial biomass carbon, determined at the termination of the experiment, ranged from ca. 100 to 260 μg g^{-1} (Fig. 3.4). None of the amendments significantly enhanced MBC over the control. Application of BMB+MuC resulted in a significant reduction in MBC in low metal soil in which American vetch was grown, whereas BMB+AM application resulted in reduced MBC in both low and high metal soils where American vetch was grown. Amendment with BMB reduced MBC by almost 50% in low metal soils planted with tufted hairgrass. Contrast analysis of MBC for biochar treatments indicated that for all plant and soil combinations except the American vetch/high metal soil, the treatment containing biochar by itself was significantly different (P<0.1 or 0.05) than those treatments that combined biochar with another amendment (Tables 3.7 to 3.10).

Analysis of MBN yielded negative values (data not displayed), invalidating the data. It is possible that the negative values are an artefact of the procedure, due to the content of natural organic carbon or biochar amendments in the samples. Durenkamp et al. (2010) similarly observed negative MBN values and suggested that organic carbon has the potential to adsorb microbial C and N released after fumigation.

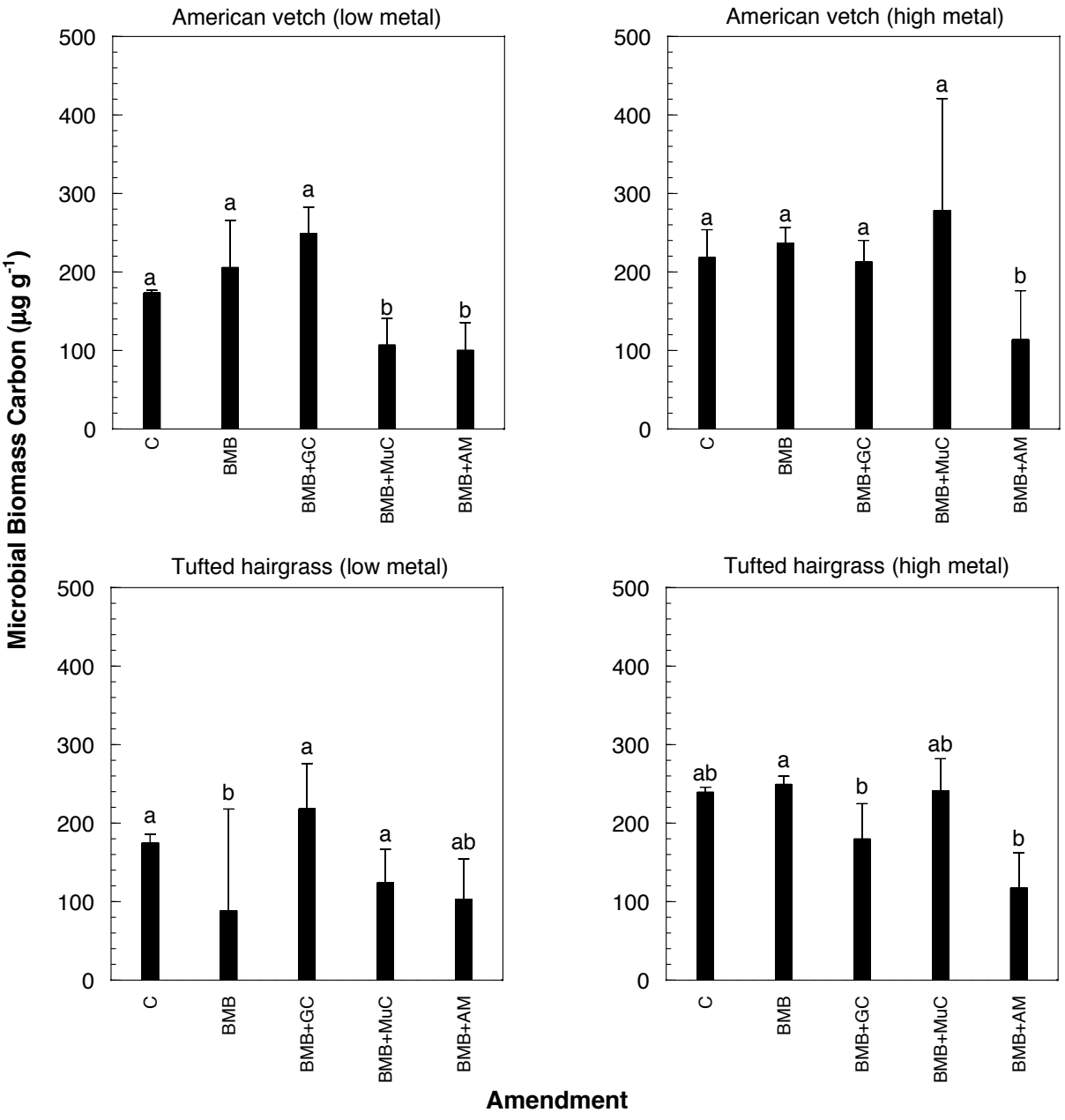

Fig. 3.4. Microbial biomass carbon (µg g^{-1}) as determined by chloroform fumigation-extraction for American vetch (upper) and tufted hairgrass (lower) after 8 wk of growth in low and high metal content soils. Letters that differ within each separate graph are significantly different at $P<0.05$. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4). Treatment information can be found in Table 3.5.

Table 3.7. Means comparisons (LSD) and contrast analysis comparing treatments of low metal concentration soil planted with American vetch.

Treatment†	Treatment Number	MBC	Soil Al	Soil Cd	Soil Cu	Soil Zn	Total PLFA	Gr+	General Fungi	Gr -
		µg g^{-1}	mg kg^{-1}				nmol g^{-1}			
C	1	173.41	5.00	1.25	8.25	270.25	7.36	1.10	1.52	0.65
BMB	2	205.55	0.00	0.00	0.00	48.75	7.24	1.05	1.40	0.63
BMB+GC	3	249.04	0.00	0.00	0.00	47.25	7.25	0.89	1.37	0.59
BMB+MuC	4	106.75	0.00	0.00	0.25	45.75	7.46	1.14	1.61	0.59
BMB+AM	5	99.99	0.50	0.25	1.50	78.00	13.15	1.74	3.62	1.14
LSD‡ (0.05)		*57.14*	*1.51*	*0.48*	*2.87*	*104.11*	*9.38*	*1.24*	*3.07*	*0.85*
Contrasts	**Treatments Compared**	**Difference Between Means**								
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	-8.08	-4.88 ***	-1.19 ***	-7.81 ***	-215.31 ***	1.42	0.11	0.48	0.09
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	-53.62*	0.17	0.08	0.58	8.25	2.05	0.21	0.80	0.14

Treatment†	Treatment Number	F:B	Stress 1	Stress 2	Plant Al	Plant Cd	Plant Cu	Plant Ni	Plant Zn
					mg kg^{-1}				
C	1	0.45	0.92	2.35	2605.52	39.69	424.69	20.71	4409.32
BMB	2	0.45	0.91	1.80	6562.95	15.68	447.98	2.22	1390.23
BMB+GC	3	0.49	0.88	1.68	7640.73	15.13	571.46	6.09	787.22
BMB+MuC	4	0.51	0.98	1.39	4417.62	10.89	343.40	23.85	847.19
BMB+AM	5	0.54	0.76	2.33	4584.50	10.26	394.00	31.93	981.24
LSD‡ (0.05)		*0.15*	*0.36*	*0.41*	*4132.27*	*16.91*	*448.91*	*26.57*	*3022.76*
Contrasts	**Treatments Compared**	**Difference Between Means**							
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	0.05	-0.04	-0.55 **	3195.93	-26.70 ***	14.52	-4.69	-3407.85 **
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	0.06	-0.04	0.08	-1015.33	-3.59	-11.69	18.40	-518.35

*, **, and *** represent differences at significance levels of $P \leq 0.10$, 0.05, and 0.01, respectively.

† Refer to Table 3.5 for a complete description of treatments.

‡ LSD results are conservative estimates where some treatments had n<4 due to missing values.

Table 3.8. Means comparisons (LSD) and contrast analysis comparing treatments of high metal concentration soil planted with American vetch.

Treatment†	Treatment Number	MBC	Soil Al	Soil Cd	Soil Cu	Soil Zn	Total PLFA	Gr+	General Fungi	Gr -
		µg g^{-1}	mg kg^{-1}				nmol g^{-1}			
C	1	218.21	34.75	3.00	89.25	251.75	30.76	5.13	3.67	3.02
BMB	2	236.53	3.50	1.00	21.25	70.25	31.58	5.61	3.95	3.42
BMB+GC	3	212.60	3.75	1.00	23.00	74.25	25.84	4.05	3.96	2.55
BMB+MuC	4	278.37	3.25	1.00	15.25	62.00	32.32	5.94	3.75	3.36
BMB+AM	5	113.56	3.75	0.75	23.50	103.00	26.59	4.06	3.40	2.55
LSD (0.05)‡		*110.04*	*12.98*	*1.49*	*26.47*	*93.41*	*12.74*	*2.18*	*1.64*	*1.34*
Contrasts	**Treatments Compared**	**Difference Between Means**								
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	-7.95	-31.19 ***	-2.06 **	-68.50 ***	-174.38 ***	-1.68	-0.22	0.10	-0.05
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	-35.02	0.08	-0.08	-0.67	9.50	-3.33	-0.93	-0.25	-0.60

Treatment†	Treatment Number	F:B	Stress 1	Stress 2	Plant Al	Plant Cd	Plant Cu	Plant Ni	Plant Zn
					mg kg^{-1}				
C	1	0.25	0.57	1.64	0.00	116.46	6098.01	1281.54	11026.44
BMB	2	0.25	0.63	1.61	5029.59	16.93	1147.14	71.98	1446.39
BMB+GC	3	0.32	0.61	1.61	4105.99	10.79	720.57	33.04	1012.70
BMB+MuC	4	0.23	0.67	1.45	6182.66	15.30	1111.46	62.16	1279.79
BMB+AM	5	0.29	0.69	1.64	2721.68	11.99	838.11	85.39	1065.50
LSD (0.05)‡		*0.08*	*0.09*	*0.24*	*5426.39*	*115.38*	*5293.09*	*843.66*	*7565.37*
Contrasts	**Treatments Compared**	**Difference Between Means**							
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	0.02	0.08	-0.06	4509.98 *	-102.71 *	-5143.69 *	-1218.40 **	-9825.35 **
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	0.03	0.03	-0.04	-692.81	-4.24	-257.09	-11.78	-327.06

*, **, and *** represent differences at significance levels of $P \leq 0.10$, 0.05, and 0.01, respectively.

† Refer to Table 3.5 for a complete description of treatments.

‡ LSD results are conservative estimates where some treatments had n<4 due to missing values.

Table 3.9. Means comparisons (LSD) and contrast analysis comparing treatments of low metal concentration soil planted with tufted hairgrass.

Treatment†	Treatment Number	MBC	Soil Al	Soil Cd	Soil Cu	Soil Zn	Total PLFA	Gr+	General Fungi	Gr -
		μg g^{-1}	mg kg^{-1}				nmol g^{-1}			
C	1	174.82	1.25	0.25	2.25	170.50	16.08	1.59	3.44	0.95
BMB	2	88.45	0.00	0.00	0.03	64.75	15.86	1.82	3.06	1.07
BMB+GC	3	218.24	0.00	0.00	0.03	23.50	11.91	1.08	2.69	0.68
BMB+MuC	4	123.79	0.00	0.00	0.00	24.25	11.42	1.27	3.06	0.72
BMB+AM	5	102.93	8.50	0.75	27.25	107.50	10.77	1.07	2.88	0.65
LSD (0.05)‡		*105.73*	*11.47*	*1.07*	*35.86*	*129.42*	*5.85*	*0.74*	*1.79*	*0.43*
Contrasts	**Treatments Compared**	**Difference Between Means**								
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	-41.47	0.88	-0.06	4.58	-115.50 *	-3.59	-0.28	-0.52	-0.17
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	59.87 *	2.83	0.25	9.07	-13.00	-4.49	-0.68 *	-0.18	-0.39

Treatment†	Treatment Number	F:B	Stress 1	Stress 2	Plant Al	Plant Cd	Plant Cu	Plant Ni	Plant Zn
					mg kg^{-1}				
C	1	0.51	0.62	1.69	2797.36	208.92	10337.16	513.86	16134.44
BMB	2	0.62	0.64	1.47	38823.28	161.26	6939.43	538.93	11849.85
BMB+GC	3	0.83	1.09	1.44	7185.58	13.83	675.83	71.69	1227.33
BMB+MuC	4	0.69	0.69	1.26	3696.93	16.63	746.86	92.18	1574.85
BMB+AM	5	0.66	0.66	1.97	2036.95	7.31	684.43	279.31	1434.91
LSD (0.05)‡		*0.19*	*0.51*	*0.66*	*44588.64*	*304.51*	*14413.57*	*656.77*	*22659.19*
Contrasts	**Treatments Compared**	**Difference Between Means**							
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	0.19 *	0.15	-0.16 **	10138.33	-159.16	-8075.52	-268.33	-12112.71
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	0.11	0.17	0.09	-34516.79	-148.67	-6237.06	-391.20	-10437.49

*, **, and *** represent differences at significance levels of $P \leq 0.10$, 0.05, and 0.01, respectively.

† Refer to Table 3.5 for a complete description of treatments.

‡ LSD results are conservative estimates where some treatments had n<4 due to missing values.

Table 3.10. Means comparisons (LSD) and contrast analysis comparing treatments of low metal concentration soil planted with tufted hairgrass.

Treatment†	Treatment Number	MBC	Soil Al	Soil Cd	Soil Cu	Soil Zn	Total PLFA	Gr+	General Fungi	Gr -
		µg g^{-1}	mg kg^{-1}				nmol g^{-1}			
C	1	239.16	1.67	0.00	0.23	1.80	27.48	4.87	3.31	2.52
BMB	2	249.23	0.00	0.00	0.08	0.81	43.39	7.76	5.21	3.15
BMB+GC	3	179.64	0.25	0.00	0.11	1.85	30.89	4.20	3.26	1.48
BMB+MuC	4	241.33	0.00	0.00	0.07	1.87	26.48	2.89	2.29	2.21
BMB+AM	5	117.25	0.00	0.00	0.07	0.72	23.24	4.13	3.74	2.48
LSD (0.05)‡		*51.48*	*1.08*	*0.00*	*0.15*	*1.11*	*27.15*	*4.52*	*3.50*	*1.23*
Contrasts	**Treatments Compared**	**Difference Between Means**								
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	-42.30	-1.61 **	0.00	-0.15 *	-0.49	3.52	-0.13	0.32	-0.19
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	-69.82 **	0.08	0.00	0.00	0.67	-16.52	-4.02	-2.11	-1.09

Treatment†	Treatment Number	F:B	Stress 1	Stress 2	Plant Al	Plant Cd	Plant Cu	Plant Ni	Plant Zn
					mg kg^{-1}				
C	1	0.26	0.54	1.73	0.00	0.00	0.00	0.00	0.00
BMB	2	0.24	0.59	1.64	2400.90	42.23	4086.15	276.25	3757.97
BMB+GC	3	0.68	0.49	1.69	4678.93	80.04	3650.77	184.84	3607.05
BMB+MuC	4	0.28	1.46	1.64	910.83	7.39	1148.89	420.14	1312.07
BMB+AM	5	0.34	0.57	1.85	41.80	30.68	2821.17	107.18	2781.52
LSD (0.05)‡		*0.44*	*1.60*	*0.32*	*4540.03*	*56.24*	*4666.82*	*443.37*	*4481.89*
Contrasts	**Treatments Compared**	**Difference Between Means**							
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	0.13	0.24	-0.02	2008.12	40.09	2926.75	247.10	2864.65
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	0.19	0.25	0.09	-523.71	-2.86	-1545.87	-38.86	-1191.09

*, **, and *** represent differences at significance levels of $P \leq 0.10$, 0.05, and 0.01, respectively.

† Refer to Table 3.5 for a complete description of treatments.

‡ LSD results are conservative estimates where some treatments had n<4 due to missing values.

3.4.3 PLFA analyses

Relative and absolute abundances of Gr+, Gr-, and AMF were affected by soil type (i.e., metal concentration) for both plant species (Table 3.11). Amendment treatments affected the relative abundance of Gr+ bacteria and AMF when American vetch was grown, and the absolute abundance of Gr- when tufted hairgrass was grown. Interaction effects between amendment treatments and soil metal concentration were non-significant for all three biomarkers.

Table 3.11. Two-way analysis of variance (ANOVA) for absolute and relative abundance of Gr+, Gr- and AMF PLFA biomarkers in soils planted with American vetch and tufted hairgrass.

	Gram +		**Gram -**		**AMF**	
	nmol g^{-1}	mol%	nmol g^{-1}	mol%	nmol g^{-1}	mol%
American vetch						
Amendment	NS†	***	NS	NS	NS	*
Soil	***	***	***	***	***	***
A*S	NS	NS	NS	NS	NS	NS
Tufted hairgrass						
Amendment	NS	NS	*	NS	NS	NS
Soil	***	*	***	*	*	***
A*S	NS	NS	NS	NS	NS	NS

*, **, *** Significant at $P<0.05$, 0.01, and 0.001, respectively.
† NS= Not significant

Total PLFA biomass was not significantly affected by amendment treatments irrespective of plant species grown (Fig. 3.5 and Fig. 3.6) High metal soils had higher total PLFA biomass (ca. 30 nmol g^{-1} soil) than low metal soils for both plant species (ca. 8 to 10 nmol g^{-1} soil). This may be due to the presence of a different plant community or higher levels of soil organic matter on high metal soil collection sites, which could have created conditions that promoted microbial biomass community growth (Table 3.1). In high metal soils grown with American vetch, Gr+ and Gr– biomarkers were significantly higher than the same biomarkers in low metal soils although total PLFA biomass levels in soils treated with amendments were not significantly different than the control (Fig. 3.5).

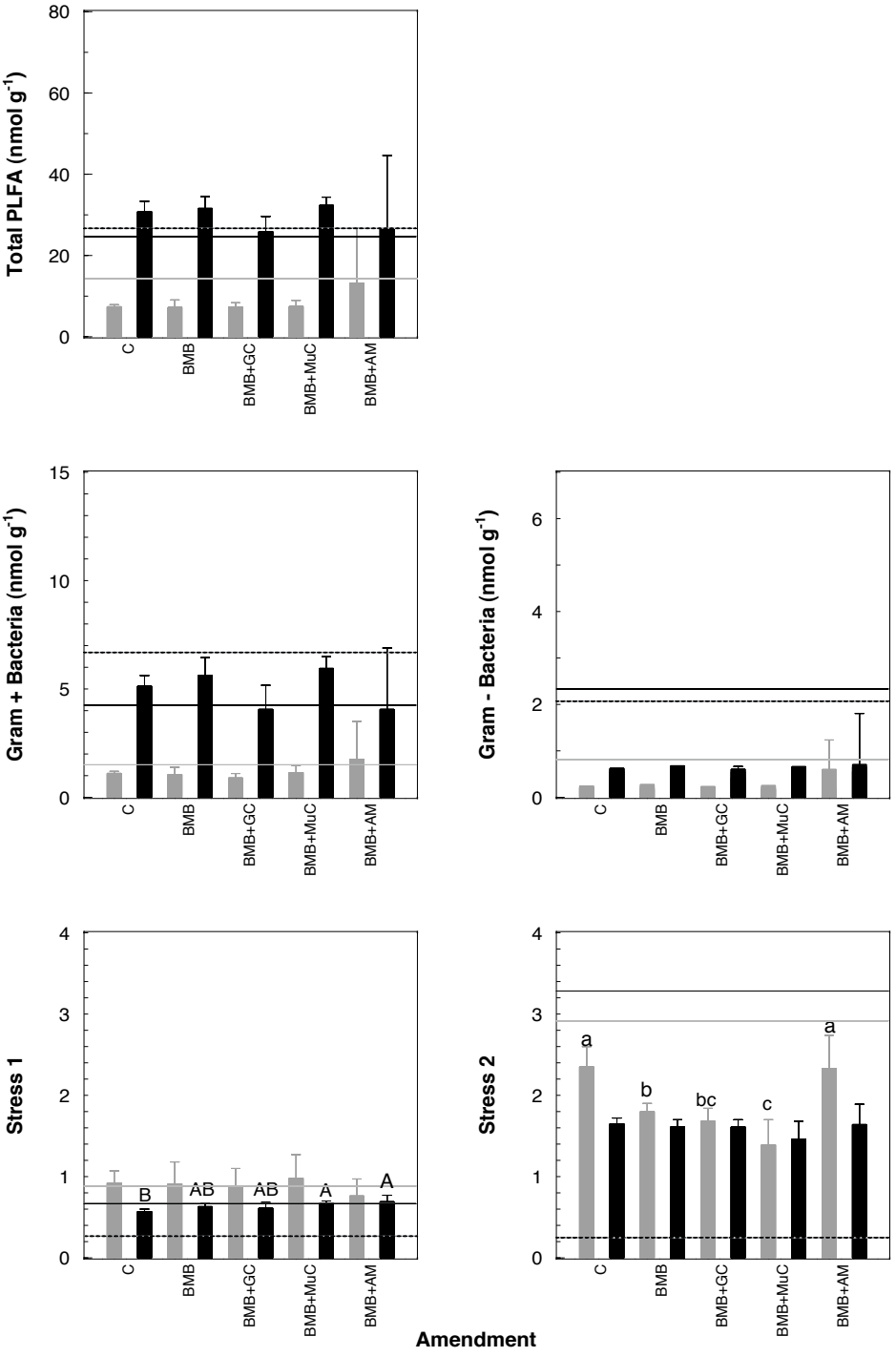

Fig. 3.5. Post-harvest PLFA biomarkers for low (grey bars) and high (black bars) metal soils planted with American vetch after 8 wk of growth. Solid grey line = untreated low metal soils; solid black line = untreated high metal soil; dashed line = control forest soil. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

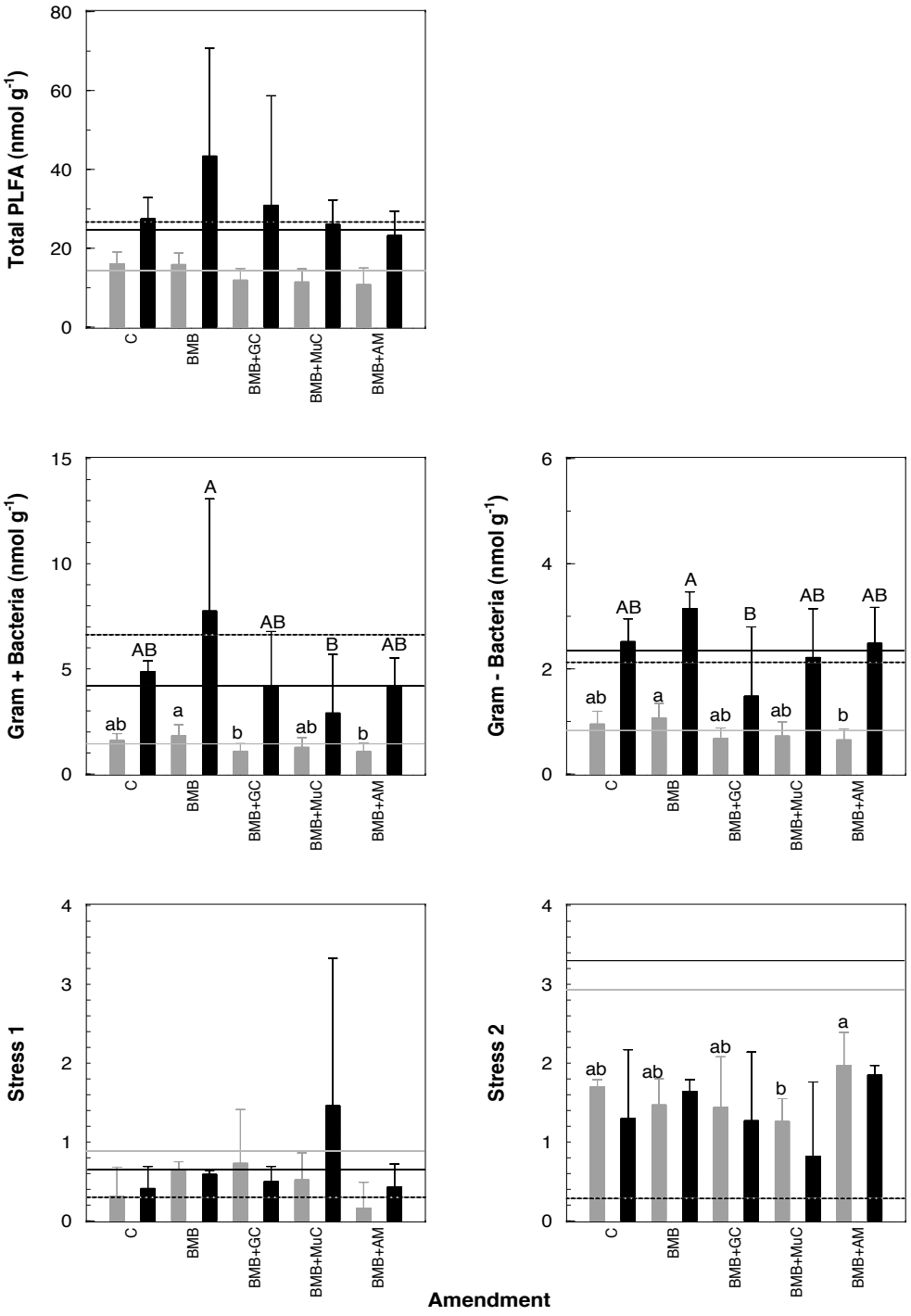

Fig. 3.6. Post-harvest PLFA biomarkers for low (grey bars) and high (black bars) metal soils planted with tufted hairgrass after 8 wk of growth. Solid grey line = untreated low metal soils; solid black line = untreated high metal soil; dashed line = control forest soil. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at $P<0.05$. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

Arbuscular mycorrhizal fungi biomass did not differ between soils and was not significantly different for any amendment (Table 3.12). Contrast analysis for soils planted with American vetch showed no clear differences in the various biomarkers in either soil (Tables 3.7 and 3.8).

Table 3.12. AMF biomarker concentrations for two plant species grown in soils containing low and high concentrations of metals with various amendment treatments added.

	American vetch		Tufted hairgrass	
Amendment	**Low metal**	**High metal**	**Low metal**	**High metal**
	AMF nmol g^{-1} soil			
C	0.10	0.24	0.02 b†	0.18
BMB	0.11	0.30	0.10 a	0.37
BMB+GC	0.09	0.19	0.00 b	4.62
BMB+MuC	0.12	0.35	0.03 b	0.28
BMB+AM	0.14	0.22	0.00 b	0.24
LSD‡ (0.05)	*0.11*	*0.15*	*0.03*	*5.97*

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
‡ LSD results are conservative estimates where some treatments had n<4 due to missing values.

Stress biomarkers 1 and 2 differed depending on soil metal concentration and amendment treatments. Stress 1 values were <1 in both low and high metal soils whereas Stress 2 values ranged from 1.3 to 2.3 in low and high metal soils. Amendment treatment BMB+MuC in the high metal soil significantly increased Stress 1 relative to the control (from 0.56 to ca. 0.70), while in low metal soils, amendments BMB, BMB+MuC, and BMB+GC significantly reduced Stress 2 relative to the control (from 2.34 to ca. <1.8). Contrast analysis indicated that Stress 2 was significantly different (P<0.05) in low metal soil where biochar was added as the sole amendment as compared to treatments that combined biochar with another amendment (Table 3.7).

Total PLFA levels in both low and high metal soils were not significantly affected by amendment treatments where tufted hairgrass was grown (Fig. 3.6). Amendments did not significantly alter the concentrations of Gr+ and Gr- bacteria relative to the control. Arbuscular mycorrhizal fungi biomass was not significantly affected in high metal soils, but in low metal

soils, treatment BMB increased AMF concentrations above the control (Table 3.12). Concentrations of AMF ranged from ca. 0.1 to 1.1 nmol g^{-1} soil.

Contrast analysis on the impact of biochar on PLFA biomarkers indicated that for low metal soils planted with tufted hairgrass (Table 3.9), Gr+ and Gr- bacteria were significantly different ($P<0.1$) for solo biochar treatments as compared to treatments where biochar was combined with other amendments.

Stress biomarkers were not significantly different between soil types when planted with tufted hairgrass, and amendment treatment differences were not detected for Stress 1 in either soil. Similarly, amendment treatments did not elicit significant differences in Stress 2 relative to the control in low metal soils. Contrast analysis indicated that Stress 2 was significantly different ($P<0.05$) in low metal soil when the soil was amended with biochar when compared to treatments that combined biochar with other amendments (Table 3.9).

For soils planted with either American vetch or tufted hairgrass, general fungal and bacterial concentrations were not significantly different (Fig. 3.7). Low metal soils had a significantly higher F:B ratio than high metal soils for soils planted with American vetch (0.52 vs. 0.28). For soils planted with tufted hairgrass, F:B ratios were significantly higher in low metal soils than in high metal soils (0.65 vs. 0.34). Within low metal soils, the F:B ratio was increased by treatments BMB+MuC and BMB+GC (Fig. 3.7). Contrast analysis of F:B ratios indicated that in low metal soils planted with tufted hairgrass, treatments containing biochar were significantly different than the control (Table 3.9).

Analysis of microbial community structure using NMDS resulted in a two-dimensional solution that separated microbial communities by soil metal concentration along Axis 1 and by plant species along Axis 2, representing 58% and 38% of the variability in the solution, respectively (Fig. 3.8). The final stress of this ordination was 9.16. American vetch and tufted hairgrass in low metal soils had different microbial communities; those same plants in high metal soils had similar microbial communities. Low metal soils were strongly correlated (r=0.7) with the F:B ratio and percentage of general bacteria. High metal soils were strongly correlated (r=0.7) with AMF, Gr+, and Gr– biomarkers. Untreated low and high metal soils were different from all treatment combinations, and all plant and soil combinations were different from the unaffected forest control soil.

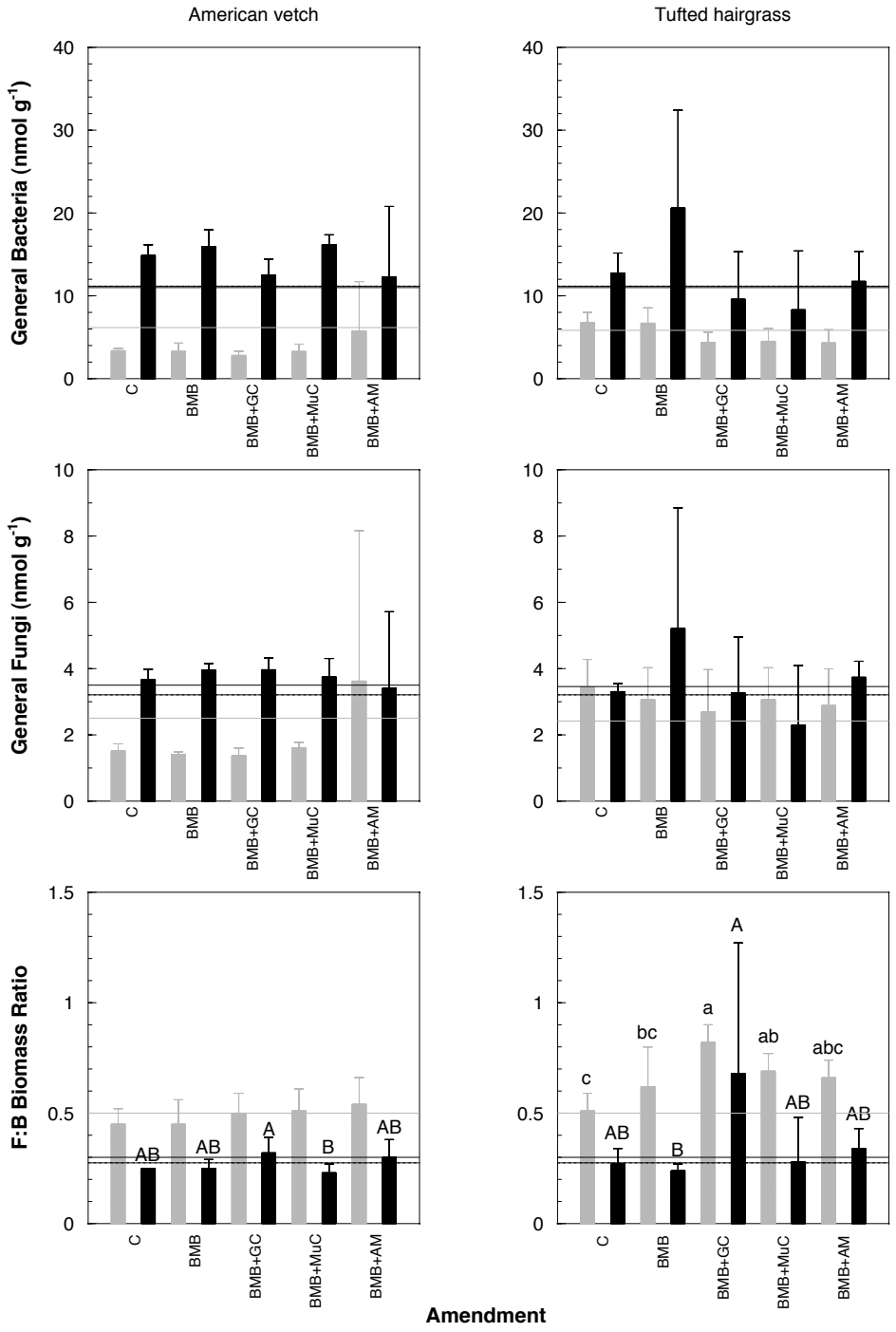

Fig. 3.7. Fungal and bacterial biomarker concentrations and fungal:bacterial (F:B) biomass ratios for American vetch and tufted hairgrass grown in soils with low and high metal concentrations (grey and black bars, respectively). Solid grey line = untreated low metal soil; solid black line = untreated high metal soil; dashed line = control forest soil. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at $P<0.05$. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

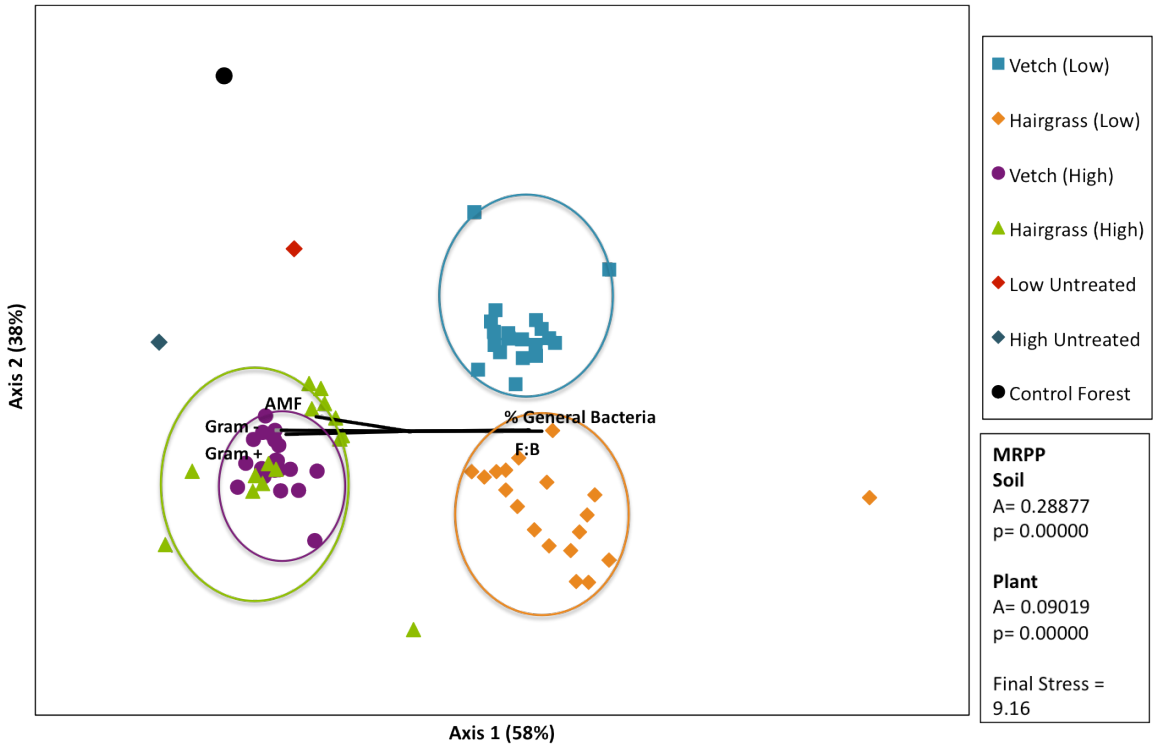

Fig. 3.8. Non-metric multidimensional scaling (NMDS) analysis (final stress = 9.16) and multiple response permutation procedure (MRPP) analysis of soil and plant effects on PLFA profiles (mol%) of American vetch and tufted hairgrass in low and high metal soils as compared to untreated soils and a control forest soil. *A*= chance-corrected within-group agreement; *p*= statistical significance of *A*. Vectors represent correlation (r=0.7) to various biomarkers.

3.4.4 Plant and soil metal concentrations

Concentrations of Al, Cd, Cu, Ni, and Zn in plant tissue did not differ significantly, irrespective of the soil metal concentrations, and plant tissue concentrations of all five metals were highly variable for both plant species. For American vetch plants, concentrations of Al ranged from ca. 1300 to 5800 mg kg^{-1}, Cd ranged from 11 to 78 mg kg^{-1}, Cu from ca. 600 to 3200 mg kg^{-1}, Ni from ca. 20 to 650 mg kg^{-1}, and Zn from ca. 900 to 7700 mg kg^{-1} (Fig. 3.9). Concentrations of Cd, Cu, Ni, and Zn were significantly higher in the control plants than in any plant from a treated soil. Concentrations of Al in American vetch were higher than the control in treatment BMB+GC for low metal soils, and treatment BMB+MuC for high metal soils. Contrast analysis indicated that for both soils, plant metal concentrations were significantly different in biochar treated soils as compared to the control (Tables 3.7 and 3.8).

For tufted hairgrass plants, concentrations of Al ranged from ca. 1000 to 20,000 mg kg^{-1}, Cd from ca. 10 to 100 mg kg^{-1}, Cu from ca. 900 to 5500 mg kg^{-1}, Ni from ca. 130 to 407 mg kg^{-1}, and Zn from ca. 1400 to 8000 mg kg^{-1} (Fig. 3.10). Concentrations of all five metals were not significantly different irrespective of soil metal concentrations, and none of the amendment treatments significantly affected plant tissue concentrations of Al when plants were grown in low metal soils, or plant tissue concentrations of Cd, Cu, Ni, and Zn when plants were grown in either of the soil types (i.e., high or low metal concentrations). Amendment treatment BMB+GC increased plant tissue Al content as compared to the control in high metal soils.

Soil available metal concentrations (Al, Cd, Cu) at the termination of the experiment were significantly higher in soils planted with American vetch as compared to concentrations found in tufted hairgrass, although Zn concentrations were not significantly different between plant species (Fig. 3.11 and Fig. 3.12). High metal soils planted with American vetch had significantly more available Al, Cd, and Cu at the termination of the experiment, whereas high metal soils planted with tufted hairgrass had significantly more available Zn than high metal soils planted with American vetch.

All amendment treatments in both low and high metal soils planted with American vetch resulted in a significant reduction in available metal concentrations of Al, Cd, Cu, and Zn at (P<0.05) at the termination of the experiment relative to the control treatment (Fig. 3.11). Available Al was very low in all low metal soils, irrespective of amendment treatment (<5 mg kg^{-1}). Available Al in high metal soils ranged from ca. 4 mg kg^{-1} in the amended treatments to 34 mg kg^{-1} in the control. Available Cd levels were low in both high and low metal soils at the termination of the experiment, and ranged from 0 to 3 mg kg^{-1}. In the low metal soils, Cu levels ranged from 8 mg kg^{-1} in the control to 0 mg kg^{-1} in soils amended with BMB and BMB+MuC. In high metal soils, Cu levels were much higher, ranging from 15 to 90 mg kg^{-1} at the termination of the experiment, and Zn concentrations were the highest of all metals measured. Metal concentrations in low metal soils ranged from 45 mg kg^{-1} in BMB+GC to 270 mg kg^{-1} in the control. High metal soils had similar ranges of soil metal concentrations, with soils treated with BMB+GC containing 60 mg kg^{-1} available Zn and the control soils containing 250 mg kg^{-1} available Zn.

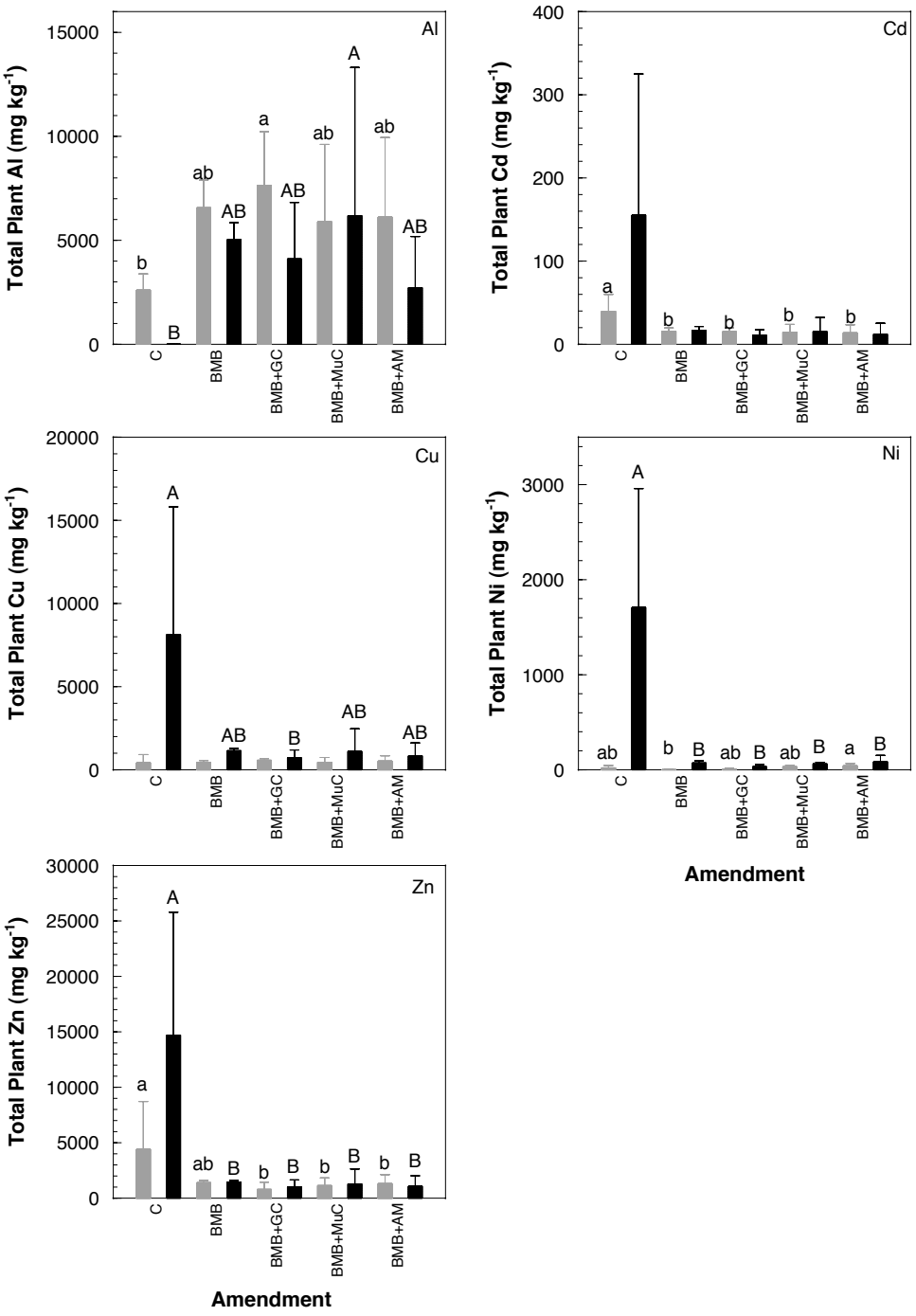

Fig. 3.9. Plant metal concentrations for combined root and shoot biomass of American vetch plants grown in low (grey bars) and high (black bars) metal soils after 8 wk of growth. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

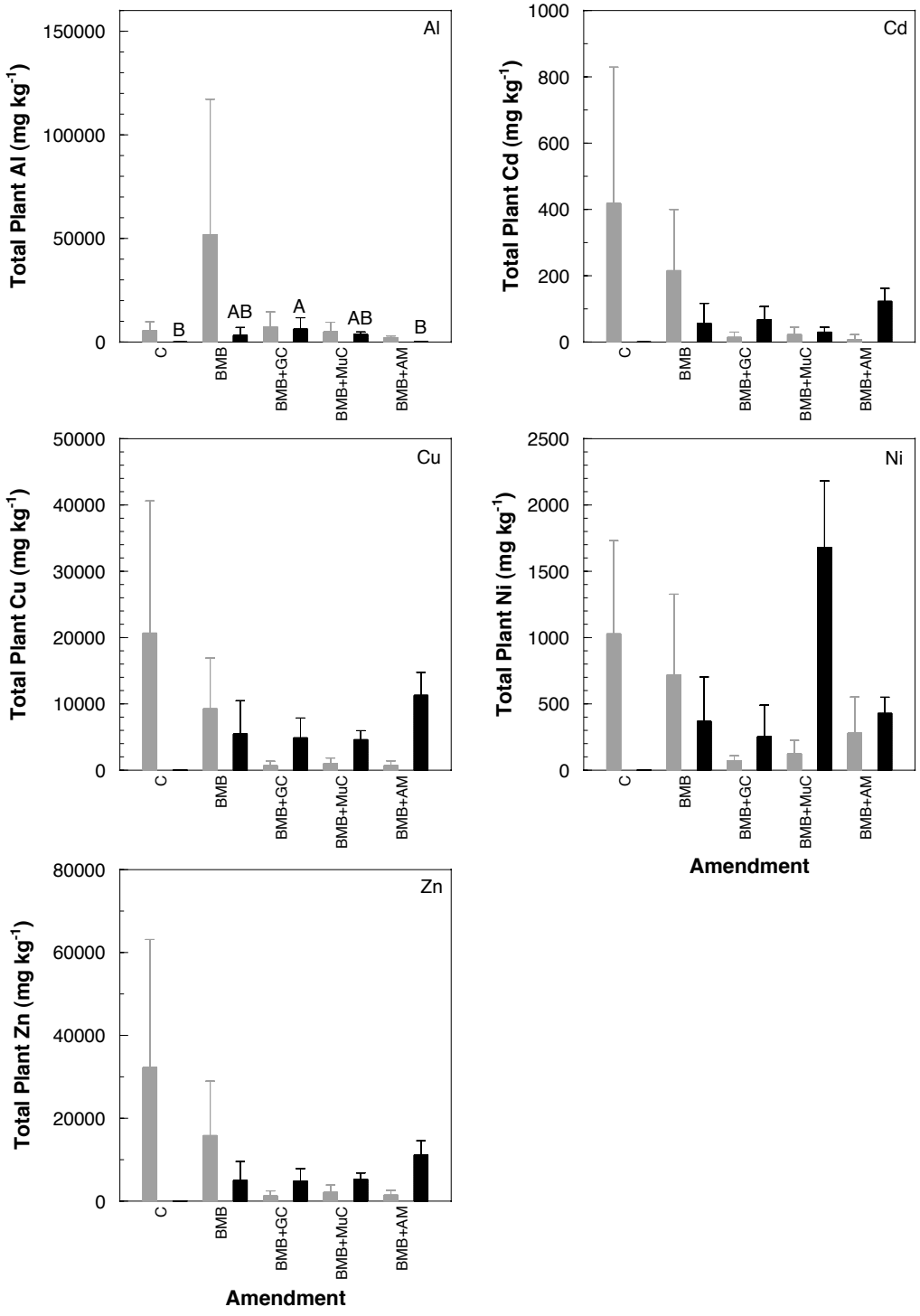

Fig. 3.10. Plant metal concentrations for combined root and shoot biomass of tufted hairgrass plants grown in low (grey bars) and high (black bars) metal soils after 8 wk of growth. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

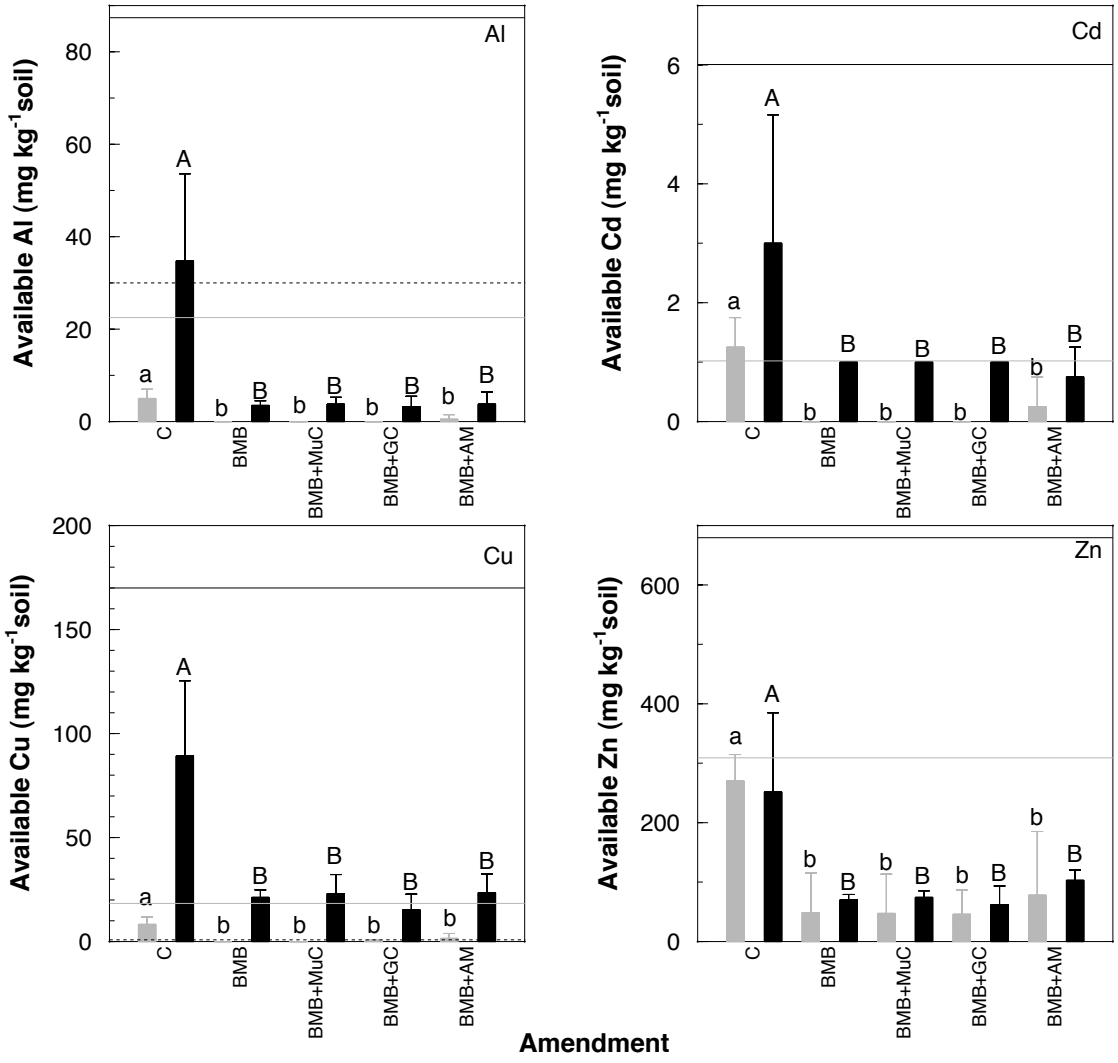

Fig. 3.11. Post-harvest soil metal concentrations for low (grey bars) and high (black bars) metal soils planted with American vetch after 8 wk of growth. Initial soil metal concentrations are show as: solid grey line = untreated low metal soil; solid black line = untreated high metal soil; dashed line = control forest soil. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

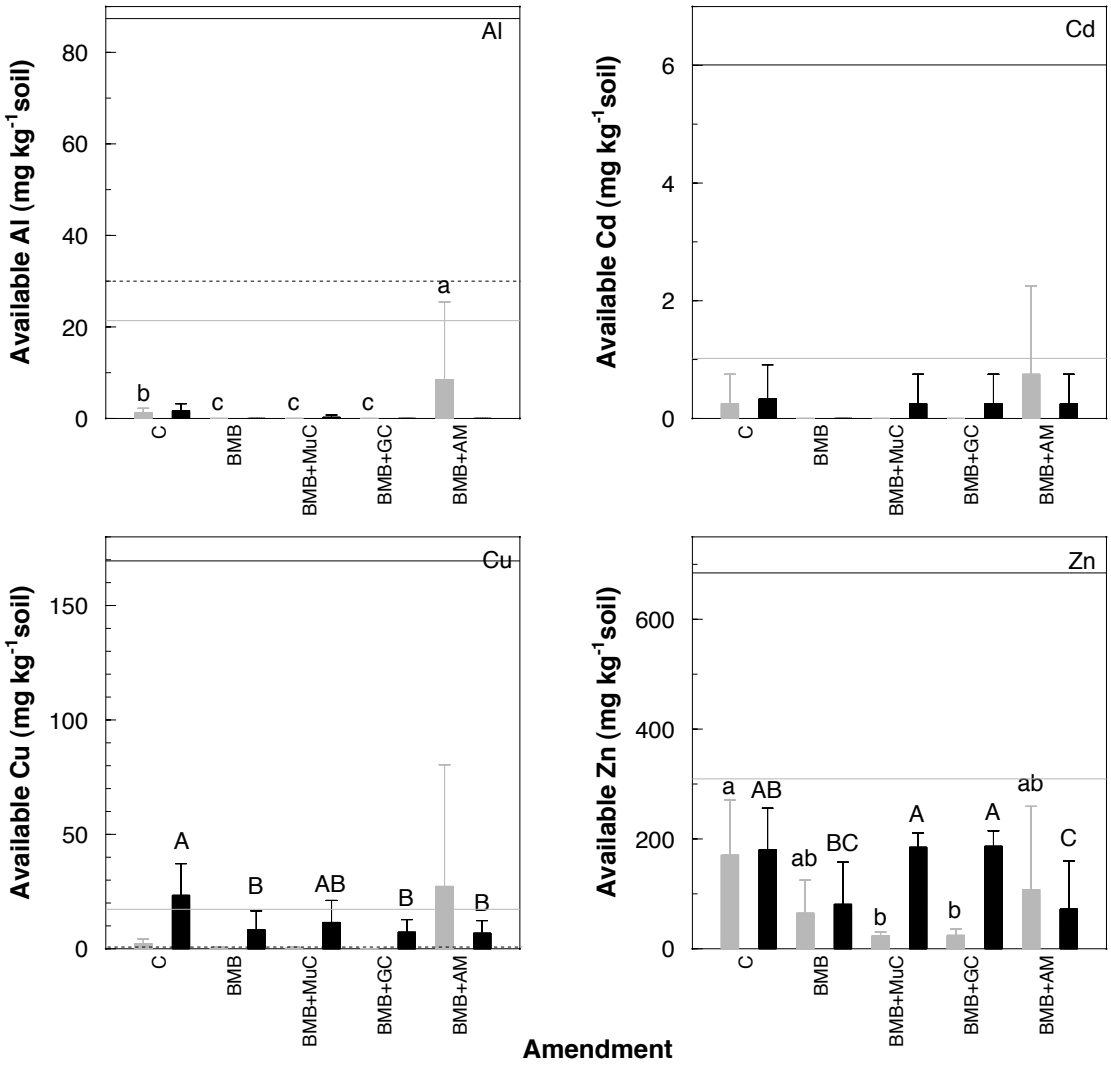

Fig. 3.12. Post-harvest soil metal concentrations for low (grey bars) and high (black bars) metal soils planted with tufted hairgrass after 8 wk of growth. Initial soil metal concentrations are show as: solid grey line = untreated low metal soil; solid black line = untreated high metal soil; dashed line = control forest soil. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at $P<0.05$. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

Available metal concentrations for soils planted with tufted hairgrass were highly variable (Fig. 3.12). At the termination of the experiment, soil available Al concentrations did not show significant amendment treatment differences as compared to the control; the control treatment in high metal soils had significantly more available Al than any other soils receiving an amendment treatment but concentrations were low and did not exceed 2 mg kg^{-1}. Differences in available Cd concentrations were not significant for either soil type. Differences in the concentrations of available Cu were not significant in low metal soils, but amendment treatments BMB, BMB+GC, and BMB+AM reduced available Cu concentration in high metal soils from 23 mg kg^{-1} to as low as 7 mg kg^{-1} as compared to the control at the termination of the experiment. Available Zn concentration was highest in the control treatment for low metal soils (170 mg kg^{-1}) and was significantly reduced by treatments BMB+MuC and BMB+GC to 24 mg kg^{-1}. Available Zn concentrations in high metal soils were significantly reduced from 186 mg kg^{-1} in the control to ca. 80 mg kg^{-1} by amendment treatments BMB and BMB+AM.

3.4.5 Pearson's correlations between plant biomass, plant tissue metal concentration, microbial parameters, and soil metal concentrations

Total plant biomass for American vetch grown in both soils was negatively correlated with Al, Cd, Cu, and Zn soil metal contents at the termination of the experiment ($P<0.05$). Total plant biomass for low metal soils also was negatively correlated with the Stress 2 biomarker. Total biomass of tufted hairgrass was not correlated with any variables for either soil (Tables B.1 to B.4). The MBC associated with American vetch plants grown in low metal soils was negatively correlated with Gr+ bacteria; no other correlations were significant with MBC for either plant species. Total PLFA in soils planted with either plant species was positively correlated with other microbial community variables (Tables B.1 to B.4). Other soil microbial variables had varying degrees of correlation in soils grown with either plant. Stress 2 values for low metal soils planted with American vetch were significantly positively correlated with all soil metal contents ($P<0.05$). The F:B ratio was negatively correlated with other soil lipid biomarkers in high metal soils planted with American vetch (Table B.2), was negatively correlated with Gr+ and Gr- bacteria in low metal soils planted with tufted hairgrass (Table B.3), and positively correlated with AMF in high metal soil (Table B.4). Correlations of other soil and plant characteristics with plant metal concentrations were generally not significant, although American

vetch metal contents in both soils were correlated with soil metals (Tables B.1 and B.2). This was not the case for tufted hairgrass.

3.5 Discussion

Ideally, amendments are intended to stimulate biomass production; however, amendments used in this study varied in their ability to promote plant growth. In general, treatments containing BMB (including BMB, BMB+CM, BMB+GC, BMB+MuC and BMB+AM) increased biomass the most for both American vetch and tufted hairgrass, in both low and high metal soils. Nwachukwu and Pulford (2009) found that grasses grown in soils treated with compost grew better than in soils treated with other amendments. Both Brown et al. (2003) and Farrell et al. (2010b) indicated that amendment of soils contaminated with heavy metals with compost significantly increased plant biomass.

Biochar was the most effective treatment for enhancing plant growth. Biochar has a high affinity for adsorbing unwanted contaminants from soil (Beesley et al., 2011), but it is non-selective; soil nutrients may also be sorbed, decreasing the soils' total nutrient content. Biochar has the ability to increase water holding capacity and soil structure - important characteristics when revegetating an area. Increased porosity associated with biochar amendments may also provide areas for complex root-microorganism-biochar interactions to occur. These can include redox reactions or contaminant sorption (Joseph et al., 2010). However, many of these characteristics are only seen when biochar is applied with an organic amendment, suggesting it is less suitable as a solo amendment (Beesley et al., 2011). In this study, contrast analysis suggested that reductions in plant growth response existed when biochar amendment was applied solely as compared to treatments where biochar was applied with another amendment.

It was postulated that in addition to enhancing plant growth, the various amendments were likely to also elicit a response in the microbial biomass. Concentrations of MBC in the metal contaminated soils ranged from ca. 100 to 260 $\mu g\ g^{-1}$, comparable to levels in metal-contaminated soils reported by Baker et al. (2011) (ca. 70 to 300 $\mu g\ g^{-1}$) and Perez-de-Mora et al. (2006) (ca. 20 to 400 $\mu g\ g^{-1}$). These levels are lower than MBC values found for other Canadian boreal forest sites in Alberta (ca. 800 $\mu g\ g^{-1}$; McMillan et al., 2007) and British Columbia (ca. 200 to 550 $\mu g\ g^{-1}$; Tan et al., 2008), which may be indicative of the low soil quality associated with metal contamination and other compounding factors in the Flin Flon area. Heavy metals have a significant effect on MBC. Wang et al. (2007) found that MBC decreased significantly

with increasing levels of Zn and Cu concentration. Although this study indicated low levels of MBC, it was not correlated with soil metal contents.

Soil amendments had no significant effect on MBC as compared to the control, in contrast to Borken et al. (2002) and Babalola et al. (2012), who found that the application of compost increased MBC up to 47% in mineral soils. Two treatments, BMB+MuC and BMB+AM actually reduced MBC relative to the control by almost 50%. Although much research has been done on soil amendments and their ability to bind metals and prevent them from entering the soil system (Farrell et al., 2010a), other researchers have shown that some amendments increase the solubility of heavy metals (Rate et al., 2004). This could result in increased toxicity to microorganisms, and negatively affect MBC (Wang et al., 2007). Although biochar has the ability to sorb soluble C, Durenkamp et al. (2010) found that the addition of biochar to soil did not decrease the amount of extractable C, but notes that it is dependent on both the type of biochar and the type of soil. Jin (2010) found that extractable MBC increased up to 40% with a high biochar application rate. It may be that the eight weeks of this study was not long enough to see any potential increases in MBC caused by biochar in the amended soils.

This study found that higher metal soils had more total PLFA, which is in contrast to findings from a metal smelter-affected soil in Pennsylvania (Kelly et al., 2003) and a mine spill site in Spain (Hinojosa et al., 2005). It is possible that the higher metal content of the soil supports a microbial community more tolerant of heavy metal presence, and thus more likely to increase in size over less tolerant communities or bacterial groups (Pennanen et al., 1996). Additionally, the presence of a different plant community on high and low metal soils, as well as higher organic carbon content in high metal soils, may have contributed to a higher microbial biomass in high metal soils, by creating conditions that favor microbial biomass growth.

The presence of soil amendments has the potential to increase soil PLFA levels, although no treatment differences were seen in this study. Baker et al. (2011) found that high rates of compost applied to metal contaminated soils increased total PLFA, as well as Gr+ and fungal communities. Farrell et al. (2010b) suggest that the presence of microorganisms from the composts themselves may influence the structure of the microbial community. Indeed, the BMB+MuC treatment had one of the higher levels of total PLFA and various lipid biomarkers in the soils planted with American vetch (but not significantly so); however, this was not the case in soils planted with tufted hairgrass.

This experiment found high metal soils had higher numbers of both Gr+ and Gr- bacteria relative to low metal soils. Concentrations of Gr- bacteria have been shown to be more prevalent in stressful conditions such as high temperature, low pH, or heavy metal concentrations (Kaur et al., 2005; Fernandez et al., 2010), and the unaffected forest control sample used in this study has less Gr- bacteria than found in high metal soils, but higher than amounts found in low metal soils. Similarly, Gr+ concentrations from the unaffected forest control are higher than Gr+ values in both high and low metal soils. This may be attributed to the total PLFA being generally lower in low metal soils, and indicates that microbial communities in these soils are not yet at an equilibrium approximating their past unaffected state. Concentrations of Gr- and Gr+ bacteria and their changes relative to soil metal concentrations, however, are variable. Baker et al (2011) found that liming of a soil shifted the microbial community to one more in favor of Gr- bacteria, while the addition of compost shifted it towards Gr+. Frostegaard et al. (1993) similarly found that the addition of limestone to a metal contaminated soil resulted in a microbial community in favor of Gr- bacteria. The porous nature of biochar and ability to sorb soluble C may provide growth substrate for bacteria (Beesley et al., 2011), but Lehmann et al. (2011) suggest that as many negative effects on microbial abundance may occur from biochar additions as positive ones such as pH changes or sorption of plant signaling compounds. Total microbial community shifts may appear after amendment application. For example, Jin (2010) found that increasing rates of biochar application to a bulk soil resulted in it approaching the microbial community structure of a non-amended rhizosphere counterpart soil. Additionally, Steinbess et al. (2009) found that the type of biochar strongly influenced the growth of different types of bacterial and fungal species. It was not clear in the ordination of PLFA community profiles (Fig 3.8) from the current experiment if any amendment treatment influenced a microbial community change – ordinations separated by treatment did not show any clear distinctions; rather, microbial communities appeared to differ based on plant and soil type.

Fungal communities were significantly higher in high metal soils. These observations are in contrast to findings that show lower fungal biomass in soils with high metal concentrations, specifically Zn levels (Kelly et al., 2003; Hinojosa et al., 2005). Baker et al. (2011) also suggested that high soil Zn levels could suppress fungal growth, although there is evidence that fungi may be more resistant to heavy metals than bacteria (Pennanen et al., 1996; Kelly et al., 1999). Arbuscular mycorrhizal fungi levels were low in both soils in which both American vetch and tufted hairgrass were grown, and were not a significant part of the overall microbial

community. It is possible that this biomarker was present in low levels due to a lack of plant roots with which to form a symbiotic relationship (Pennanen et al., 1996). Low AMF levels may also be due to the application of biochar to the soil. Warnock et al. (2010) found that biochar application significantly reduced AMF abundance and suggested it may be due to inhibitions on growth, alterations in P availability, or pH alteration.

Physiological stress biomarkers "Stress 1 and 2" represent the ratios of cy17:0 to 16:1ω7c and cy19:0 to 18:1ω7c, respectively (Grogan and Cronan, 1997). Higher ratios are indicative of a soil microbial community under high stress. Shifts in these ratios are caused by stresses in the soil system, including high temperatures, water stress, low pH and heavy metal presence in the soil (Kaur et al., 2005). Stress 1 values for this experiment were less than 1 for both plant species, and Stress 2 values ranged from 1.3 to 2.3. These are higher than those documented by Tischer et al (2008) who found stress ratios between 0.47 and 0.66 for two heavy metal contaminated sites in Germany. Frostegaard et al. (1993) found a higher Stress 1 value (0.46) and a lower Stress 2 value (0.75) in limed forested plots as compared to control plots (0.37 and 1.45, respectively). When the stress values from treatments in this experiment were compared to the unaffected forest soil, all stress values are higher than the unaffected forest stress values, which are 0.4 and 0.26 for Stress 1 and 2, respectively. Stress 2 amended soil values specifically are almost 10x higher than the unaffected forest control, indicating an environment under extreme stress. When the stress values are compared to the unamended soil values, Stress 1 values are not much different. Stress 2 values show a large decrease with treatment (even with only limestone, as per the experiment's control treatment), indicating the Stress 2 variable is responsive and is a good indicator of change in these communities. As all treatments were assessed in the presence of growing plants, the decrease in stress level could be attributed to the presence of plant species and could have helped buffer the microbial community from the heavy metal presence in the soil. As Zhang et al. (2006) reported, stress values may decrease with time as the environment returns to a more natural state.

Using the F:B ratio of soils has been shown to be a good indicator of stress in environmental systems as it is correlated with soil organic matter content and pH (Frostegaard and Baath, 1996; Pennanen et al., 1996; Baker et al., 2011). Higher ratios indicate a larger fungal biomass. When American vetch was grown, F:B ratios were similar and non-significant for low metal soils, irrespective of amendment treatment. Similarly, amendment treatments were not

different from the control in high metal soils. For tufted hairgrass, treatments BMB+GC and BMB+MuC resulted in an increase in the F:B relative to the control. It is possible that increased C from these amendments may be integral in allowing fungal biomass to increase, as was suggested by Hinojosa et al. (2005). Increased SOM has been linked to an increased F:B (Frostegaard and Baath, 1996). High metal soils (both treated and the control) had a similar F:B to the unaffected forest soil (ca. 0.25), while low metal soils had higher F:B ratios (ca. 0.5 to 1.3). Pennanen et al. (1996) indicated that F:B ratios increased with decreasing distance from a metal smelter. Hinojosa et al. (2005) found F:B ratios in metal contaminated soil ranging from 0.27 in heavy metal polluted soils to 0.48 in non-polluted soils, a similar range to what is reported for this experiment.

There have been no reported tissue concentrations of Al, Cd, or Ni in either American vetch or tufted hairgrass. Tissue concentrations of Cu and Zn in both American vetch and tufted hairgrass were quite high but concentrations in both plants were similar to concentrations reported previously (Paschke et al., 2000). These researchers found high tolerance levels in both plant species for both Cu and Zn.

All amendment additions to soils appeared to decrease the amount of Cd, Cu, Ni, and Zn uptake in American vetch plants, which is similar to what Farrell et al. (2010) found with the addition of compost to Cu and As contaminated soils. Biochar application has been show to decrease plant tissue metal uptake (Namgay et al., 2010); contrast analyses of this experiment showed that amendments containing biochar significantly decreased plant metal uptake from the control. Additionally, Paschke et al. (2000) found that tufted hairgrass was able to take Zn into both roots and shoots, but this experiment showed no significant differences in Zn uptake between the amended soils and the control soil. The data did, however, trend towards lower Zn uptake in amended soils. This trend is similar to one found for a similar grass species where applications of compost and other soil amendments decreased Zn uptake (Nwachukwu and Pulford, 2009).

Soil available concentrations of Al, Cd, Cu, and Zn were variable but were higher in high metal soils. For both soil types planted with American vetch, all treatments significantly reduced available metals in the soil. As all treatments were limed at the beginning of the experiment, it appears that the significant reduction in soil metal concentrations for both soils planted with American vetch could be due to the impact of biochar, which was present in all of the added amendment treatments. Tufted hairgrass did not show this same trend, however. Although the

addition of limestone to the soil will increase soil pH and thus decrease metal availability in soil (Winterhalder, 1995), it appears to be the addition of other soil amendments that significantly reduced metal availability relative to the control treatment, which received only limestone. It is important to note that in order to keep soil metals immobilized, either large amounts of amendment must be added, or it must be reapplied at a future time (Kelly et al., 2003; Baker et al., 2011).

Contrast analysis indicated that the treatments containing biochar and all other amendment treatments did not differ significantly from the solo biochar treatment; likewise, the ANOVA did not suggest one treatment was better at reducing metal availability than another. It could be that biochar was having the largest effect on soil metal availability; it could also be that the addition of organic matter of any type will improve these soils. Both biochar and compost have been shown to decrease soil metal availability in contaminated soils (Helmisaari et al., 2007; Beesley et al., 2010; Farrell et al., 2010). Reichman et al. (2002) found that the availability of Zn increases as pH decreases, and that Cu availability was strongly influenced by the addition of organic matter. In this study, it is possible that both biochar and compost could reduce the availability of all metals, not only Zn.

3.6 Conclusion

This work indicated that the addition of soil amendments to soil that has been affected by various heavy metals resulted in significant affects on the soil characteristics, plant growth, and on the microbial community. Changes in both plant growth and soil microbial characteristics can be attributed to the impact of treatments to the heavy metal contents in soil. However, the high variability of these characteristics may have contributed to a lack of measureable significant differences between treatments for this experiment. Results were not specific to one soil amendment, and were not consistent between plant species or soil metal concentration. A longer trial may have shown more definitive results, especially in terms of microbial community shifts. The variability of these results indicate the challenges and realities of revegetating heavy metal affected soils and highlight the need for further research on the subject.

4. THE EFFECT OF MICROBIAL AND ORGANIC AMENDMENTS ON THE GROWTH OF OVERSTORY SPECIES *PINUS BANKSIANA* AND *POPULUS TREMULOIDES* AND RELATED MICROBIAL COMMUNITIES IN TWO METAL-AFFECTED SOILS

4.1 Preface

Addition of various amendment treatments to soils in Chapter 3 indicated that treatments containing biochar (alone or in combination with another amendment) had the greatest positive impact on growth of understory species. In this subsequent experiment, the focus was on the impact of amendments on tree species grown on metal-affected soils. Specifically, biochar amendments were again added to metal-affected soils, and two forest tree species, jack pine and trembling aspen, were grown under controlled conditions for nineteen weeks. The affect of these biochar amendments on plant growth, microbial parameters, and soil and plant metal uptake characteristics where assessed.

4.2 Introduction

The growth of various tree species in metal affected soils has been studied by numerous researchers (Wotton et al., 1986; Cripps and Miller, 1993; Niemenen, 2004; Helmisaari et al., 2007). Typically, seedling survival as well as root and shoot growth is reduced with increasing soil metal concentrations (Jones et al., 1984). Depending on the availability, type, and mobility of metals in the soil, trees may take up metals and store them in either root or shoot tissues (Jones et al., 1984; Gratton et al., 2000; Niemenen, 2004). Trees that colonize rapidly and have mycorrhizal associations, such as trembling aspen, can cover ground quickly and fungal associations may increase plant metal tolerance (Cripps, 2003). Ectomycorrhizal root associations can act as a 'buffer' between the plant and metal-affected soil and influence seedling establishment and subsequent uptake of metals into plant tissues (Frey et al., 2000). Microbial communities also are affected by soil metal concentrations and can be ideal indicators of community stress or change as revegetation proceeds, although understanding of microbial communities in distressed soils is still incomplete (Kelly et al., 2003; Hinojosa et al., 2005; Dmitriu et al., 2010). The addition of amendments to soils can alter various soil properties, and

when added to metal-stressed soils can alter bioavailability and thus persistence and uptake of soil metals (Farrell et al., 2010).

The objectives of this experiment were to determine which, if any, soil amendments would increase the growth of two tree species in metal-affected soil and to determine the effects of these amendments on various soil microbial indicators. The consequent impact of amendments on metal availability in the soil as well as metal uptake in plant tissues was assessed in a growth chamber experiment.

4.3 Materials and Methods

4.3.1 Soil collection and preparation

Soils used in this experiment were collected from the Flin Flon area surrounding the smelter location, as previously described in Chapter 3 (Table 3.1), and prepared as previously described (Sections 3.3.1 and 3.3.2). Metal contents and bulked soil properties are reported in Table 3.2 (Chapter 3). Briefly, soils were transported in coolers and kept cool using icepacks, then air-dried and passed through an 8 mm sieve size to remove rocks, roots, and stones. Soils collected from areas with similar metal concentrations were homogenized with a large cement mixer to form the 'low metal' and 'high metal' soils (sites 3.3, 3.7, and 5.22, and sites 4.9 and 6.5, respectively). A reference forest sample that has not been affected by metal deposition was used in various analyses to compare with the two soils used in the experiment. This soil was gathered from a location near Sherridon, MB and was subject to the same sampling and processing procedures as for the metal-affected soils.

Prior to the initiation of the growth chamber experiment, dolomitic limestone (lime) was added to the soils to raise the pH of each bulked soil to 5.5. This was done to simulate limestone application that currently is used as a remediation strategy for soils in the Flin Flon area. The dolomitic limestone is locally available in the Flin Flon area and was collected at the same time as soil collection. The limestone was ground with a heavy wooden roller to a particle size of 2 mm. The rate of application of limestone was done according to lime response curves calculated by ALS Labs (Saskatoon, SK) (Appendix A).

4.3.2 Amendments and plant species used

Amendments used in this experiment were similar to those used in Chapter 3 with the exception that composted manure was not used in this experiment (Table 4.1). Two tree species were used – one year old jack pine (~18 cm tall) and one year old trembling aspen seedlings (>50 cm tall). Tree seedlings were acquired from Tree Time Services Inc. (Edmonton, Alberta, Canada), and were rooted plugs. Jack pine and trembling aspen seeds used for germination tests were acquired from Pacific Regeneration Technologies (Prince Albert, SK, Canada), and Western Native Seed (Coaldale, Colorado), respectively (Appendix C). These plant species have been shown to be metal tolerant, and both jack pine and aspen are native to the Flin Flon area (Wotton et al., 1986; Cripps et al., 2003).

Table 4.1. Treatments used in Experiment #2, examining the impact of amendments on jack pine and aspen survival and growth.

Treatment Number	Contents
1	Control (no treatment added)
2	BMB†
3	BMB + GC
4	BMB + MuC
5	BMB + AM

† Refer to Table 3.3 for explanation of amendment abbreviations

4.3.3 Experiment setup and harvest

This experiment was conducted using a fully randomized, factorial design that included four replicates. Treatments were chosen from those previously tested in Experiment #1 (Chapter 3) based on the positive biomass increase relative to the control (Table 4.2). Four controls were used in this experiment, one for each plant and soil combination. A 10% w/w (amendment/soil) treatment application rate was used. Soils were mixed with amendments and 900 g of treated soil was placed into 15cm diameter plastic pots. Each tree seedling had a root ball of peat material attached. This was loosened before planting but was not fully removed. Mycorrhizal inoculant was placed around the root ball to ensure maximum root contact at a manufacturer-suggested rate of 15 g per pot. One jack pine or trembling aspen seedling pet pot was placed in the soil, and pots were kept at 80% field capacity (Topp et al., 2008) for the duration of the experiment. White

polypropylene beads were placed on the soil surface to reduce moisture loss. Plants were maintained in a growth chamber with a 16/8 h day/night cycle with a day/night temperature range of 24°C/21°C.

Plants were harvested after 19 wk. Above ground biomass was collected by clipping the trees at the soil surface. The roots were carefully extracted from the bulk soil, washed in tap water, rinsed in reverse osmosis water, blotted dry, and weighed. Both above- and below-ground biomass was dried at 40°C for a minimum of 48 h, weighed again, and stored for further analysis. Soil was mixed to create a bulk sample, and was then subsampled by hand into plastic bags for subsequent analysis of metal content and various microbial parameters, as previously described in Section 3.2.4.

Table 4.2. Plant, soil and amendment treatment combinations used in Experiment #2. Replicates of four were included for a total of 80 pots.

Plant	Soil	Amendment treatment†
Jack pine	Low metal	1 to 5
Jack pine	High metal	1 to 5
Trembling aspen	Low metal	1 to 5
Trembling aspen	High metal	1 to 5

† Refer to Table 4.1 for treatment explanations

4.3.4 Soil, plant, and microbial analyses

Soil, plant, microbial, and statistical analyses were carried out as in Sections 3.3.5 with a few exceptions. Specifically, because the trees seedlings included a root ball with associated planting medium, the contribution of the planting material to the initial seedling weight had to be estimated in order to calculate bare root and shoot biomass. Thus, change in biomass was calculated for tree seedlings using data collected from unplanted seedlings (‘reference trees’) at the start of the experiment. Specifically, a representative sample of 45 tree seedlings of each species were weighed and then destructively separated in to shoot and root ball. The shoots and root balls were oven dried and the mean shoot and root weights were determined and assumed to represent the average shoot and root weights, and proportion of each, at the initiation of the experiment. These values were used to calculate relative increases in shoot and root weight. The reference trees were used to estimate the mean tree weights at planting without the attached root

ball. Water contents of reference trees were then used to estimate the dry planting weight of seedlings. These weights were then compared to tree dry weights taken at harvest to determine changes in total biomass, as well as root and shoot biomass.

Tree roots were analyzed for evidence of ectomycorrhizal colonization using the gridline intersect method as per Bundrett et al. (1996). Briefly, a sample of unstained root tissue was dispersed in a 9 cm diameter Petri plate with 0.5 cm grid lines. The number of mycorrhizal root tips was determined by visual observation and was recorded and compared to the number of non-mycorrhizal roots to arrive at a percentage of root tips colonized. Plant roots and shoots were separately analyzed for metal concentrations of Al, Cd, Cu, and Zn. Plant tissue digestion was carried out as in Section 3.3.5, using 1.0 ± 0.2 g of sample.

4.3.5 Statistical analysis

Transformations of the data were determined necessary by calculation of the residuals, which were then used to indicate data normalcy (Goodall, 1993). If necessary, data sets were either square root transformed (MBC data) or log(x+1) transformed (all PLFA data and root colonization data). Statistical comparisons were not made between plant species. Differences between the various treatments were determined using an analysis of variance (ANOVA) in CoStat (CoHort Software, Monterey, CA). Fisher's least significant difference (LSD) was used to test means separation. Correlations between variables (i.e., total PLFA versus soil metal concentrations) were determined using the Pearson Product Moment Correlation Coefficient, 'r'. Significant differences are reported for those means that differ at the $P<0.05$ level, unless it is otherwise indicated. To compare several groupings of treatments, orthogonal contrasts were used to find significance levels of $P<0.10$, 0.05, and 0.01, as described in data tables.

4.4 Results

4.4.1 Plant metal stress, biomass, and height measurements

After nineteen weeks of growth, both species showed signs of metal toxicity in leaves and needles. Visual signs of heavy metal accumulation in leaves in trembling aspen begins with uniform stippling on the leaf surface followed by necrotic leaf margins (Hermle et al., 2007). These characteristics were clearly observed in this experiment (Fig. 4.1), and were discernable as

early as 6 wk after planting in all treatments. In jack pine, needles showing heavy metal stress (specifically Cu or Zn) often show chlorosis of lower needles and subsequent necrosis (Reichman, 2002); these signs were less clearly seen in this experiment, but were detected as early as 10 weeks after planting, in all treatments.

Fig. 4.1. Leaf chlorosis and subsequent necrosis caused by metal stress on trembling aspen leaves in this experiment at 6 (left) and 12 wk (right) after planting.

Change in root biomass from planting to harvest was not significant for jack pine or trembling aspen trees grown in soils containing low or high metal concentrations (Fig. 4.2). Shoot biomass of jack pine planted in low metal soil and of trembling aspen planted in high metal soils indicated that all treatments increased biomass over the control, which showed a negative growth trend. Treatment BMB+AM increased jack pine shoot growth over the control in high metal soils. The low amount of significant differences seen are attributed to a high level of variance within the plant species in terms of growth, likely due in part to uncertainty regarding the estimation of initial plant weights at time of planting due to the attached peaty root ball. Percent change in plant height from planting to harvest also was compared between amendment treatments for all plant and soil combinations (Fig. 4.3). Although jack pine had significantly more relative change in height at the termination of the experiment than trembling aspen, no amendment treatment significantly increased plant height over the control for either plant species. Although the lack of significant responses may signify that the amendments were not effective in promoting plant growth, it is also possible that real treatment differences were

obscured by a high degree of variability in the data. Plant height change over 19 weeks of growth ranged from ca. 5 to 55%.

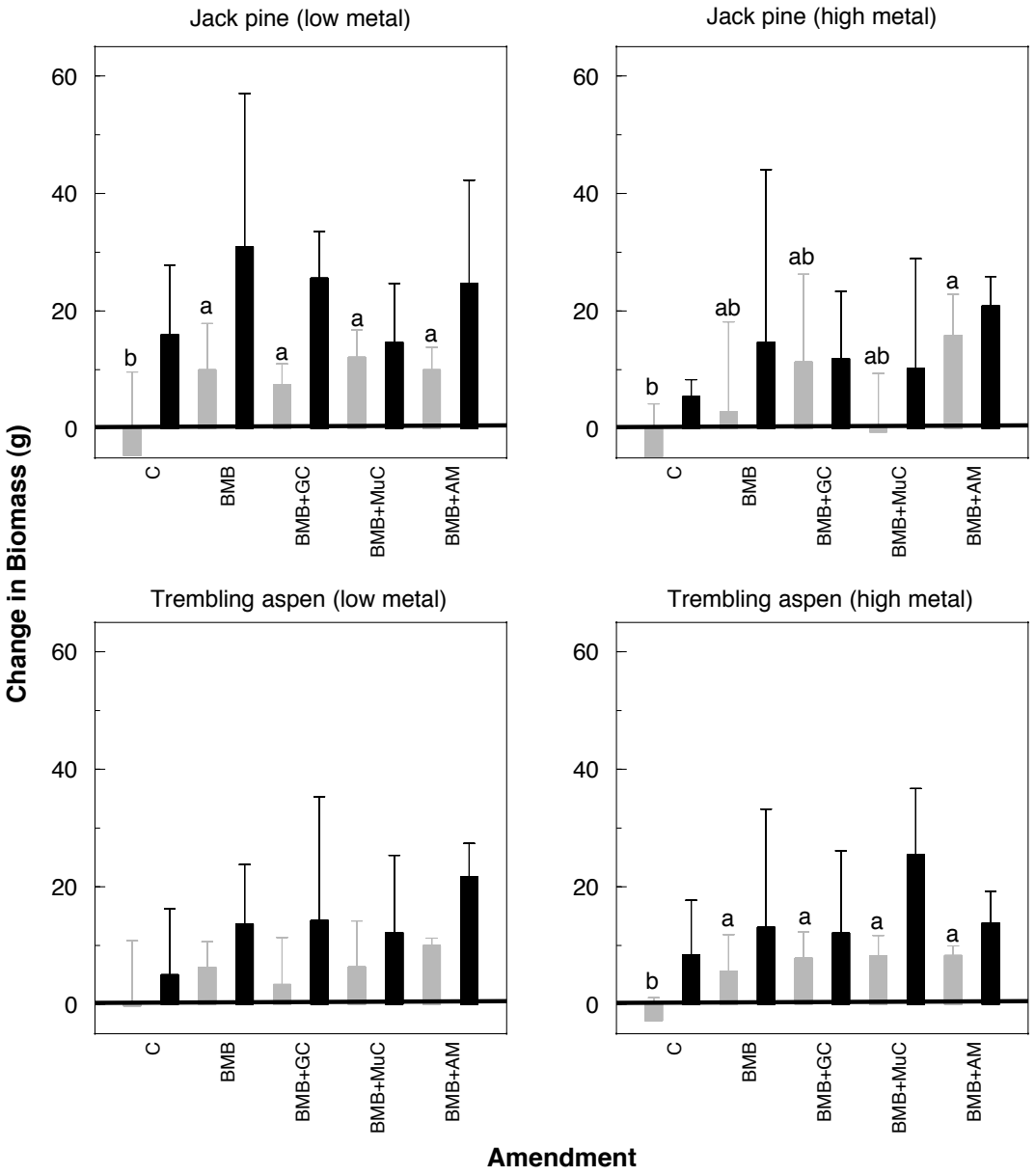

Fig. 4.2. Change in biomass (g) after 19 wk of growth in low and high metal soils of jack pine and trembling aspen in shoots (grey bars) and roots (black bars). Letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

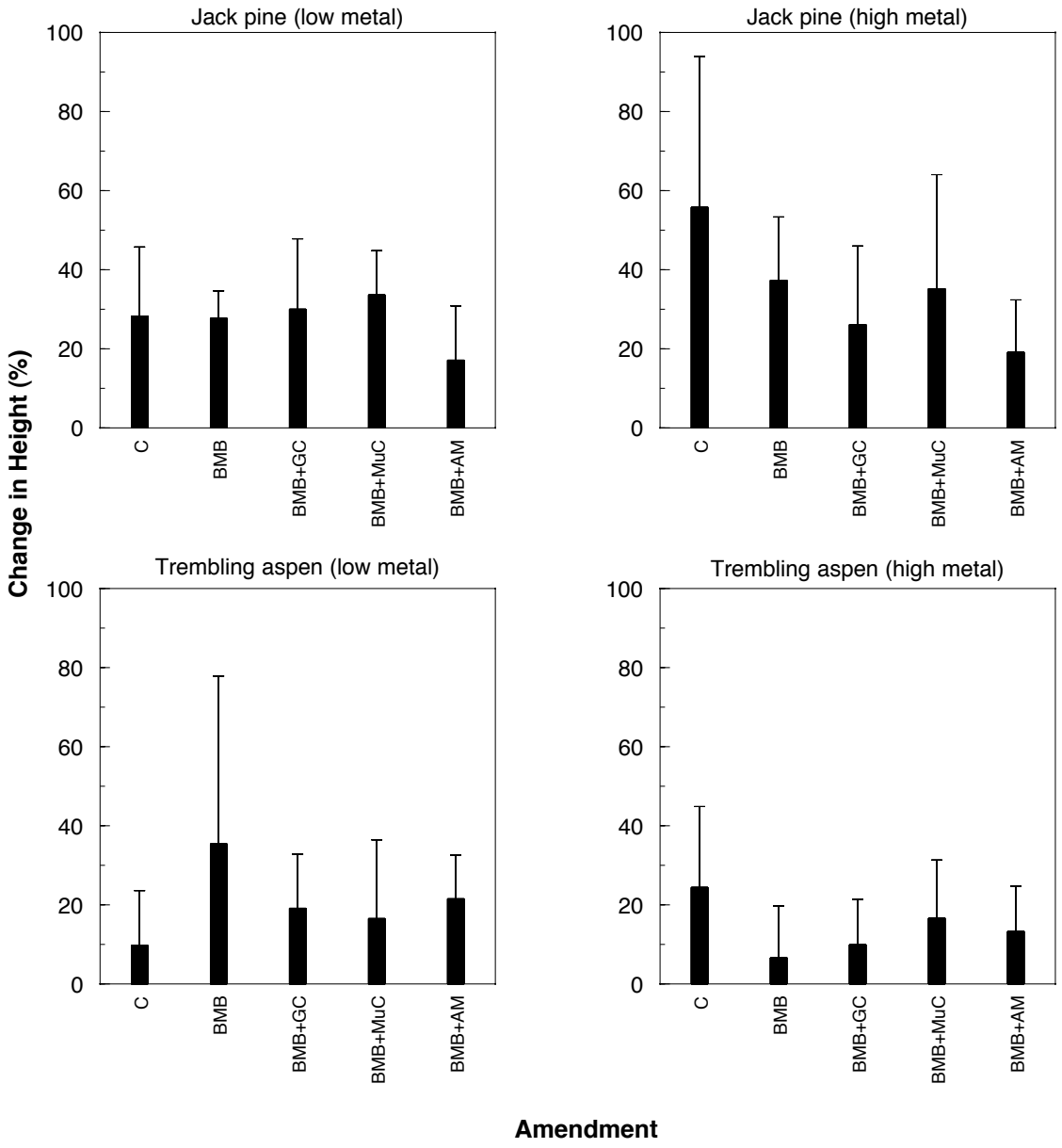

Fig. 4.3. Percent change in height over 19 wk of growth in low and high metal soils of jack pine and trembling aspen. Error bars represent standard error of the mean (n=4). Treatments were not statistically different (P<0.05). Letters represent separate statistical comparisons within each separate graph and are significant at P<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

4.4.2 Soil microbial biomass carbon and nitrogen

Concentrations of soil MBC ranged from 108.4 to 214.9 μg g^{-1}. There was no detectable difference in the soil MBC values at the termination of the experiment, irrespective of the plant species grown ($P<0.05$). Similarly, no detectable differences in soil MBC were observed between high or low metal soils for trembling aspen; however, when jack pine was grown, treatment differences were seen in high metal soils (Fig. 4.4). Differences associated with the application of the various amendments were not significant in either soil in which jack pine was grown or in low metal soil in which trembling aspen was grown. Where amendments were applied in high metal soils and trembling aspen was grown, differences in MBC were detected between amendments but none of the amendments resulted in differences in MBC relative to the control.

Soil MBN concentrations ranged from 16.0 to 24.0 μg g^{-1}. Concentrations of MBN did not differ significantly between plant species, not were significant differences detected between amendment treatments in the low metal soils when either jack pine or trembling aspen were grown (Fig. 4.5). High metal soils planted with jack pine had significantly higher MBN than low metal soils. Treatment differences in low metal soils planted with jack pine were not significant, but treatments BMB+MuC, and BMB+AM significantly increased MBN in high metal soils in which jack pine soils was grown to 27.8 and 26.0 μg g^{-1}, respectively, as compared to the control which supported 11.4 μg g^{-1} MBN. Treatment differences in low metal soil planted with trembling aspen were not significant. In high metal soil, treatment BMB+MuC increased soil MBN from 16.91 μg g^{-1} in the control to 24.77 μg g^{-1} in high metal soils. Contrast analysis suggests that for trembling aspen trees grown in high metal soils, the treatment containing biochar with other amendments significantly increased MBC as compared to biochar by itself; contrast analysis for other plant and soil combinations were not significant (Tables 4.3 to 4.6).

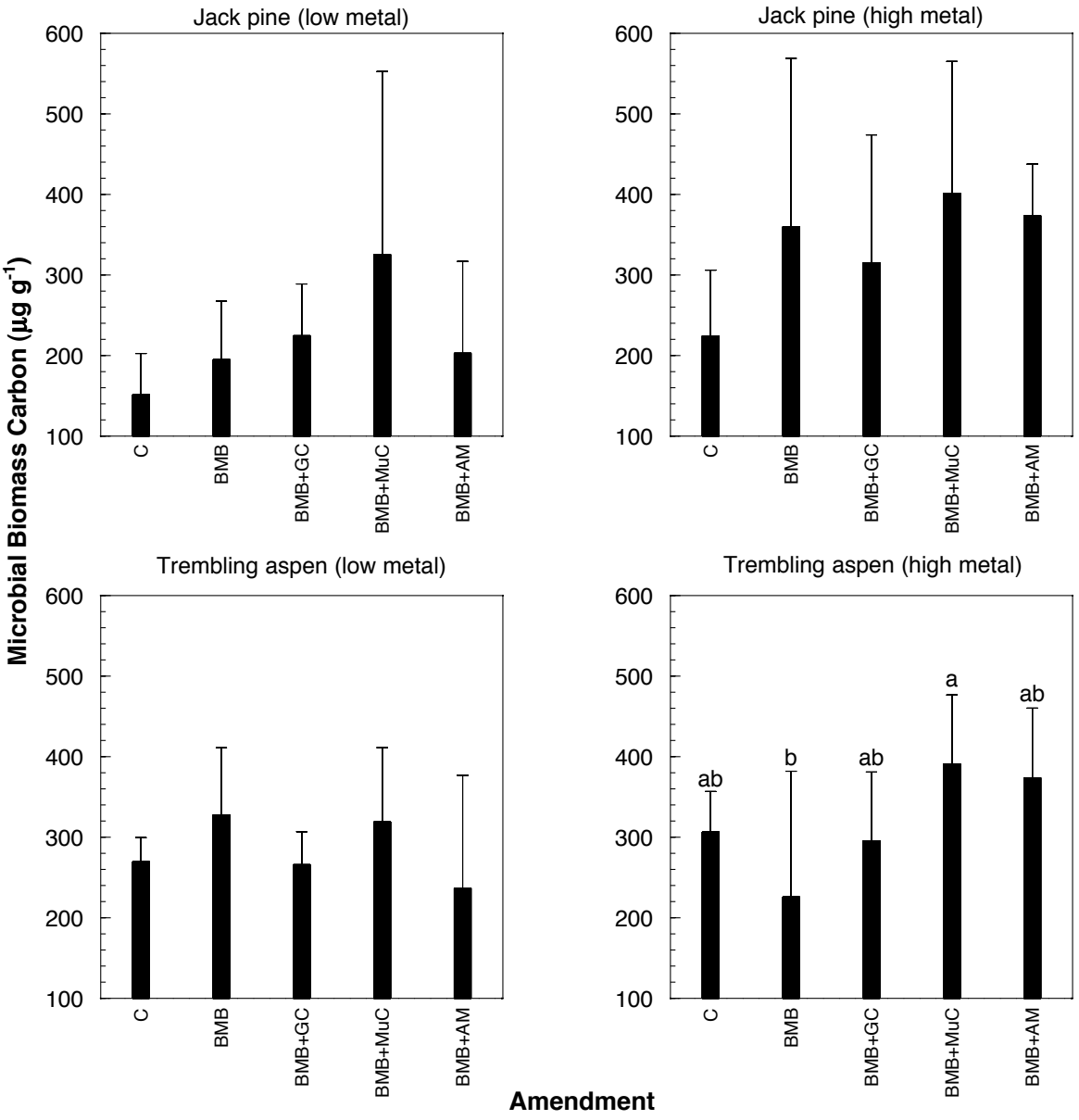

Fig. 4.4. Microbial biomass carbon concentrations (µg g^{-1}) in low and high metal soils after 19 wk of growth of jack pine and trembling aspen. Letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

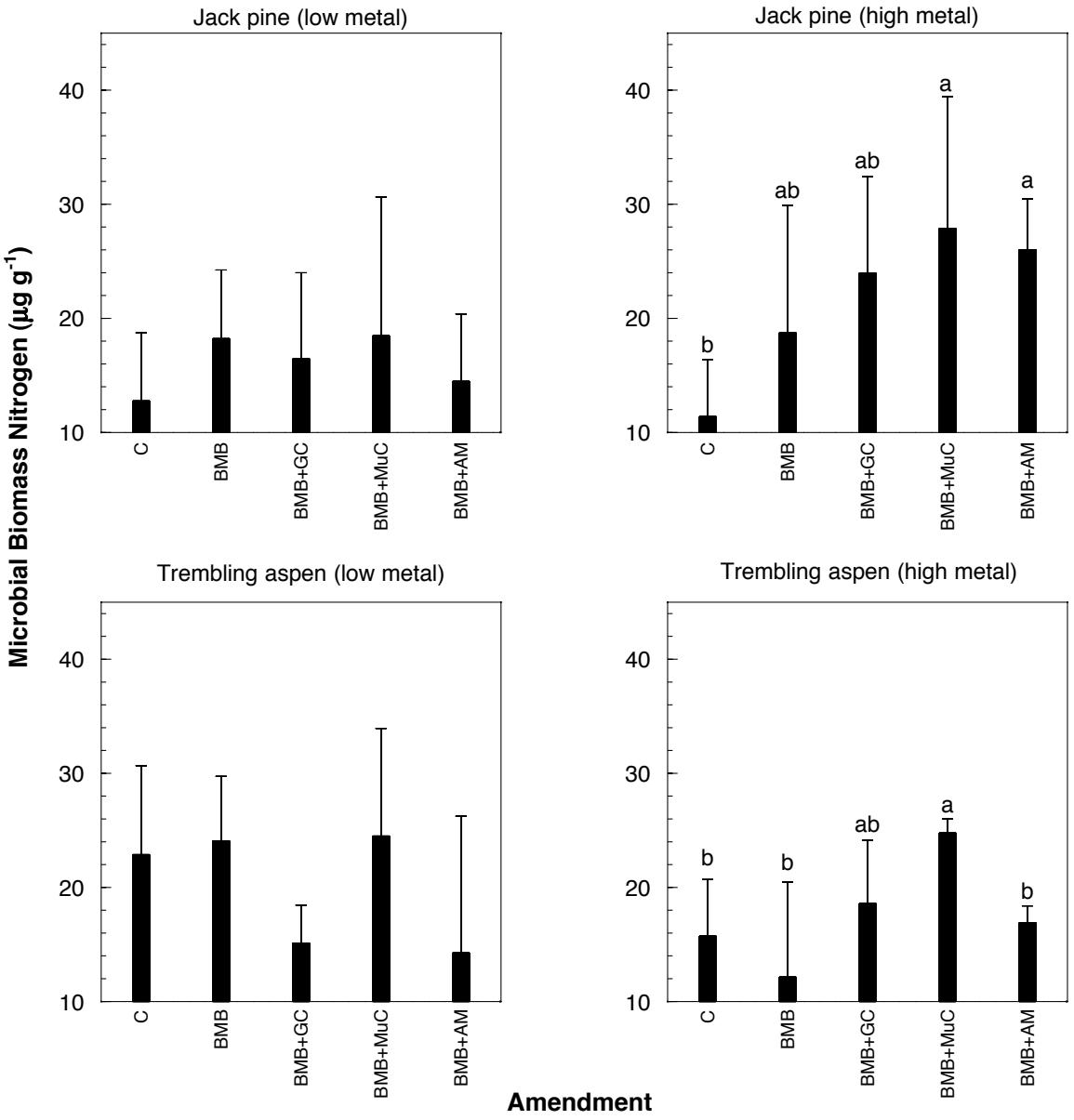

Fig. 4.5. Microbial biomass nitrogen concentrations (µg g^{-1}) in low and high metal soils after 19 wk of growth of jack pine and trembling aspen. Letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

Table 4.3. Means comparison (LSD) and contrast analysis comparing treatments of low metal concentration soil planted with jack pine.

Treatment†	Treatment Number	Height Change	Root Colo.	MBC	MBN	Total PLFA	Gr+	General Fungi	Gr -	Stress 1	Stress 2	F:B
		——— % ———		—— µg g^{-1} ——		———— nmol g^{-1} ————						
C	1	28.30	28.86	151.44	12.77	48.03	11.05	5.14	6.06	0.78	2.10	0.18
BMB	2	27.70	12.97	195.09	18.22	40.96	8.81	3.91	4.81	0.73	1.97	0.17
BMB+GC	3	30.00	9.26	224.55	16.46	35.36	7.12	4.93	4.17	0.76	2.17	0.24
BMB+MuC	4	33.60	11.85	325.29	19.04	69.64	13.29	19.26	5.86	0.79	1.36	1.57
BMB+AM	5	17.08	14.42	202.92	14.48	25.99	5.44	3.26	3.19	0.73	2.09	0.24
LSD (0.05)‡		*21.14*	*12.56*	*186.54*	*10.93*	*23.76*	*4.56*	*14.14*	*3.00*	*0.09*	*0.77*	*1.74*
Contrasts	**Treatments Compared**	**Differences Between Means**										
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	-1.21	-16.74	85.52	4.28	-5.04	-2.39	2.70	-1.55	-0.03	-0.20	0.38
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	-0.81	-1.13	55.83	-1.56	2.70	-0.19	5.24	-0.40	0.03	-0.10	0.51

Treatment Number	Soil Al	Soil Cd	Soil Cu	Soil Zn	Shoot Al	Shoot Cd	Shoot Cu	Shoot Ni	Shoot Zn	Root Al	Root Cd	Root Cu	Root Ni	Root Zn
	——— mg kg^{-1} ———													
1	51.00	-	-	3.00	833.98	1.65	30.34	52.37	742.48	1400.76	1.59	169.31	81.49	524.77
2	30.00	-	-	1.75	137.70	5.21	151.93	54.76	409.44	2202.18	1.21	225.19	97.08	517.80
3	43.50	-	-	6.50	121.18	0.00	16.03	19.40	527.49	1541.98	2.10	154.95	68.87	519.32
4	50.25	-	-	4.00	85.43	0.00	1284.00	19.07	411.98	1555.55	2.45	212.53	94.57	620.84
5	32.75	-	-	3.50	114.18	0.00	15.46	15.85	468.26	819.65	1.59	102.11	68.18	393.44
LSD (0.05)‡	*30.63*	-	-	*3.87*	*896.98*	*5.87*	*175.35*	*9.28*	*287.27*	*776.27*	*2.41*	*70.38*	*51.35*	*284.35*
Treatments Compared	**Differences Between Means**													
2,3,4,5-1	-11.88	-	-	0.94	-719.36 *	-0.35	336.52	-25.10 ***	-288.19 *	129.08	0.25	4.38	0.69	-11.92
3,4,5-2	12.17	-	-	2.92	-30.77	-5.21 *	286.57	-36.65 ***	59.80	-896.45 **	0.84	-68.66 *	-19.87	-6.60

*, **, and *** represent differences at significance levels of $P \leq 0.10$, 0.05, and 0.01, respectively.

† Refer to Table 3.5 for a complete description of treatments.

‡ LSD results are conservative estimates where some treatments had n<4 due to missing values.

Table 4.4. Means comparison (LSD) and contrast analysis comparing treatments of high metal concentration soil planted with jack pine.

Treatment†	Treatment Number	Height Change	Root Colo.	MBC	MBN	Total PLFA	General Fungi Gr+	General Fungi Gr -	Stress 1	Stress 2	F:B
		%		µg g^{-1}		nmol g^{-1}					
C	1	55.83	17.55	224.47	11.40	28.82	6.41	2.64	2.84	0.64	1.90	0.20
BMB	2	37.30	17.63	359.90	18.75	55.46	12.35	5.31	7.04	0.57	1.74	0.15
BMB+GC	3	26.08	24.39	315.18	23.97	34.29	7.26	3.52	4.55	0.65	1.84	0.17
BMB+MuC	4	35.20	17.48	401.44	27.80	48.78	11.45	5.37	6.46	0.57	1.70	0.15
BMB+AM	5	19.13	31.03	373.56	26.02	25.85	5.77	2.18	3.44	0.63	1.81	0.15
LSD (0.05)‡		*38.52*	*12.71*	*219.82*	*13.04*	*35.04*	*7.61*	*5.09*	*4.29*	*0.09*	*0.35*	*0.10*
Contrasts	**Treatments Compared**	**Differences Between Means**										
BMB, BMB+GC, BMB+MuC, BMB+AM – C	2,3,4,5-1	-26.40	5.08	138.05	12.74 *	12.28	2.80	1.46	2.53 *	-0.04	-0.13	-0.05
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	-10.50	6.67	3.49	7.18	-19.15	-4.19	-1.62	-2.22	0.05	0.04	0.01

Treatment Number	Soil Al	Soil Cd	Soil Cu	Soil Zn	Shoot Al	Shoot Cd	Shoot Cu	Shoot Ni	Shoot Zn	Root Al	Root Cd	Root Cu	Root Ni	Root Zn
	mg kg^{-1}													
1	115.00	0.00	0.25	19.00	58.99	-	12.03	12.86	407.47	928.13	4.36	263.61	92.41	841.11
2	139.00	0.00	0.50	23.75	73.90	-	15.86	9.51	261.18	1113.85	5.25	357.43	94.17	648.65
3	78.25	0.00	0.00	12.00	40.14	-	9.04	11.14	221.15	840.63	3.79	290.18	91.77	537.76
4	29.25	0.25	2.75	58.25	208.36	-	42.64	4.39	422.54	5800.72	18.51	529.03	48.22	1410.70
5	83.25	0.00	0.00	13.25	55.07	-	12.10	8.30	433.85	1774.91	5.00	239.26	0.45	613.61
LSD (0.05)‡	*77.63*	*0.34*	*3.74*	*71.82*	*165.66*	-	*36.99*	*6.65*	*265.09*	*4274.25*	*12.71*	*262.89*	*38.37*	*764.07*
Treatments Compared	**Differences Between Means**													
2,3,4,5-1	-32.56	0.06	0.56	7.81	35.38	-	7.88	-4.53	-72.79	1454.40	3.78	90.37	-33.76*	-38.43
3,4,5-2	-75.42*	0.08	0.42	4.08	27.29	-	5.40	-1.57	98.00	1691.57	3.85	-4.61	-47.36***	205.37

*, **, and *** represent differences at significance levels of $P \leq 0.10$, 0.05, and 0.01, respectively.

† Refer to Table 3.5 for a complete description of treatments.

‡ LSD results are conservative estimates where some treatments had n<4 due to missing values.

Table 4.5. Means comparison (LSD) and contrast analysis comparing treatments of low metal concentration soil planted with trembling aspen.

Treatment†	Treatment Number	Height Change	Root Colo.	MBC	MBN	Total PLFA	Gr+	General Fungi	Gr -	Stress 1	Stress 2	F:B
		%		μg g^{-1}		nmol g^{-1}						
C	1	9.70	36.08	269.79	22.87	24.23	5.36	2.89	3.17	0.93	2.16	0.18
BMB	2	35.43	29.96	327.74	24.08	40.21	8.53	6.89	4.48	0.64	1.53	0.31
BMB+GC	3	19.12	15.20	265.93	15.11	36.81	8.07	4.74	4.32	0.67	1.89	0.25
BMB+MuC	4	16.55	21.64	319.04	24.49	43.79	9.29	7.25	4.61	0.71	1.39	0.30
BMB+AM	5	21.47	22.32	236.75	14.27	49.24	9.95	4.23	5.75	0.79	1.79	0.15
LSD (0.05)‡		*35.03*	*24.05*	*130.67*	*12.35*	*16.75*	*3.91*	*3.67*	*2.08*	*0.24*	*0.73*	*0.13*
Contrasts	**Treatments Compared**	**Differences Between Means**										
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	13.44	-13.80	17.58	-3.38	18.28*	3.60**	2.89*	1.62*	-0.23*	-0.51	0.07
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	-16.38	-10.24	-53.83	-6.12	3.07	0.57	-1.48	0.41	0.08	0.16	-0.08

Treatment Number	Soil Al	Soil Cd	Soil Cu	Soil Zn	Shoot Al	Shoot Cd	Shoot Cu	Shoot Ni	Shoot Zn	Root Al	Root Cd	Root Cu	Root Ni	Root Zn
	mg kg^{-1}													
1	101.75	-	0.00	10.75	32.99	0.00	15.66	10.55	1225.79	581.79	3.45	57.87	32.99	355.53
2	79.75	-	0.00	8.00	75.08	4.75	25.75	34.88	581.51	768.19	4.55	95.96	75.08	589.37
3	59.25	-	0.00	7.00	31.38	5.71	13.85	93.23	435.77	794.06	2.26	67.75	31.38	262.43
4	74.67	-	0.00	7.67	27.88	7.08	12.13	94.68	887.38	359.34	3.58	53.65	27.89	201.66
5	132.00	-	0.50	12.75	29.43	2.96	9.36	97.68	954.24	723.03	5.05	75.69	29.43	347.02
LSD (0.05)‡	*108.96*	-	*0.81*	*10.17*	*87.03*	*7.49*	*18.26*	*60.13*	*745.59*	*1075.48*	*4.52*	*104.09*	*87.03*	*419.19*
Treatments Compared	**Differences Between Means**													
2,3,4,5-1	-15.33	-	0.13	-1.90	7.95	5.13	-0.39	69.57**	-511.07	79.37	0.41	15.39	7.96	-5.41
3,4,5-2	8.89	-	0.17	1.14	-45.52	0.50	-13.97	60.32*	177.62	-142.71	-0.92	-30.26	-45.51	-319.00

*, **, and *** represent differences at significance levels of $P \leq 0.10$, 0.05, and 0.01, respectively.

† Refer to Table 3.5 for a complete description of treatments.

‡ LSD results are conservative estimates where some treatments had n<4 due to missing values.

Table 4.6. Means comparison (LSD) and contrast analysis comparing treatments of high metal concentration soil planted with trembling aspen.

Treatment†	Treatment Number	Height Change	Root Coloniz	MBC	MBN	Total PLFA	Gr+	General Fungi	Gr -	Stress 1	Stress 2	F:B
		%		µg g^{-1}		nmol g^{-1}						
C	1	24.38	23.07	306.44	61.76	24.10	4.34	1.29	3.06	0.59	2.37	0.11
BMB	2	6.57	5.25	225.95	12.15	27.12	4.88	1.53	3.19	0.57	2.06	0.12
BMB+GC	3	9.92	22.16	295.95	18.59	30.94	5.77	2.28	3.37	0.57	1.62	0.14
BMB+MuC	4	16.62	21.16	391.02	24.78	62.57	11.35	6.77	6.67	0.61	0.44	0.23
BMB+AM	5	13.35	32.74	373.69	16.91	54.31	9.35	5.24	6.09	1.24	2.07	0.17
LSD (0.05)‡		*22.02*	*21.67*	*149.02*	*62.48*	*33.33*	*6.21*	*4.78*	*3.68*	*0.83*	*0.54*	*0.11*
Contrasts	**Treatments Compared**	**Differences Between Means**										
BMB, BMB+GC, BMB+MuC, BMB+AM - C	2,3,4,5-1	-12.77	-2.74	15.21	-43.65	19.64	3.50	2.67	1.77	0.16	-0.82*	0.06
BMB+GC, BMB+MuC, BMB+AM - BMB	3,4,5-2	6.73	20.10**	127.60*	7.94	22.15	3.94	3.23	2.19	0.24	-0.68	0.06

Treatment Number	Soil Al	Soil Cd	Soil Cu	Soil Zn	Shoot Al	Shoot Cd	Shoot Cu	Shoot Ni	Shoot Zn	Root Al	Root Cd	Root Cu	Root Ni	Root Zn
	mg kg^{-1}													
1	82.25	0.00	0.50	10.50	47.11	2.36	26.78	191.56	1000.00	377.39	4.15	101.46	1.64	442.90
2	31.50	0.00	0.00	4.50	15.99	4.32	5.16	49.76	482.67	163.72	1.69	42.73	0.19	154.99
3	37.25	0.00	0.00	6.00	105.18	3.42	11.22	73.52	876.78	699.41	6.52	184.71	1.49	557.17
4	57.25	0.25	1.25	24.00	22.94	4.96	11.12	95.87	1215.52	871.96	6.62	206.78	1.11	585.85
5	0.00	0.00	7.25	73.50	99.52	3.02	27.19	73.19	782.50	927.00	7.14	235.81	0.93	733.64
LSD (0.05)‡	*43.05*	*0.34*	*3.82*	*24.06*	*148.02*	*6.17*	*29.51*	*136.52*	*709.56*	*908.83*	*6.56*	*256.96*	*1.35*	*664.00*
Treatments Compared	Differences Between Means													
2,3,4,5-1	-50.75	0.06	1.63	16.50	13.80	1.57	-13.11	-118.48*	-160.63	288.13	1.34	66.05	-0.71	65.02
3,4,5-2	0.00	0.08	2.83	30.00**	59.89	-0.52	11.35	31.10	475.59	669.07	5.07	166.37	0.99	470.56

*, **, and *** represent differences at significance levels of $P \leq 0.10$, 0.05, and 0.01, respectively.

† Refer to Table 3.5 for a complete description of treatments.

‡ LSD results are conservative estimates where some treatments had n<4 due to missing values.

4.4.3 PLFA analysis

Where jack pine was grown, only the absolute biomass of Gr+ bacteria was significantly affected by any of the amendment treatments, irrespective of the soil metal concentrations (Table 4.7). In contrast, several PLFA biomarkers were affected by both the soil metal concentration and the amendment treatments (i.e., AMF relative biomass, and Gr- relative and absolute biomass) when trembling aspen was grown. Interaction effects between amendment treatments and soil were non-significant for any of the three biomarkers.

Table 4.7. Two-way analysis of variance (ANOVA) for absolute and relative abundance of Gr+, Gr- and AMF PLFA biomarkers in soils planted with jack pine and trembling aspen.

	Gram +		Gram -		AMF	
	nmol g^{-1}	mol%	nmol g^{-1}	mol%	nmol g^{-1}	mol%
Jack pine						
Amendment	NS†	NS	NS	NS	NS	NS
Soil	*	NS	NS	NS	NS	NS
A*S	NS	NS	NS	NS	NS	NS
Trembling aspen						
Amendment	NS	***	NS	NS	NS	*
Soil	**	NS	*	*	*	NS
A*S	NS	NS	NS	NS	NS	NS

*, **, *** Significant at $P<0.05$, 0.01, and 0.001, respectively.
† NS= Not significant

Total PLFA (nmol g^{-1}) concentrations were not significantly different between jack pine and trembling aspen, and total PLFA concentrations did not differ significantly between high and low metal soils for either tree species (Fig. 4.6 and Fig. 4.7). Initial total PLFA measurements of untreated soils indicates more PLFA in high metal soils, which may be attributed to plant communities each sample site is associated with, as well as higher soil organic carbon (Table 3.1). Amendment treatments in low metal soils planted with jack pine did not increase total PLFA over the control (Fig. 4.6). In high metal soils planted with jack pine, treatment differences were not significant. All amendments increased total PLFA over the control in low metal trembling aspen soils, and BMB+GC and BMB+AM increased total PLFA over the control in high metal soils planted with trembling aspen.

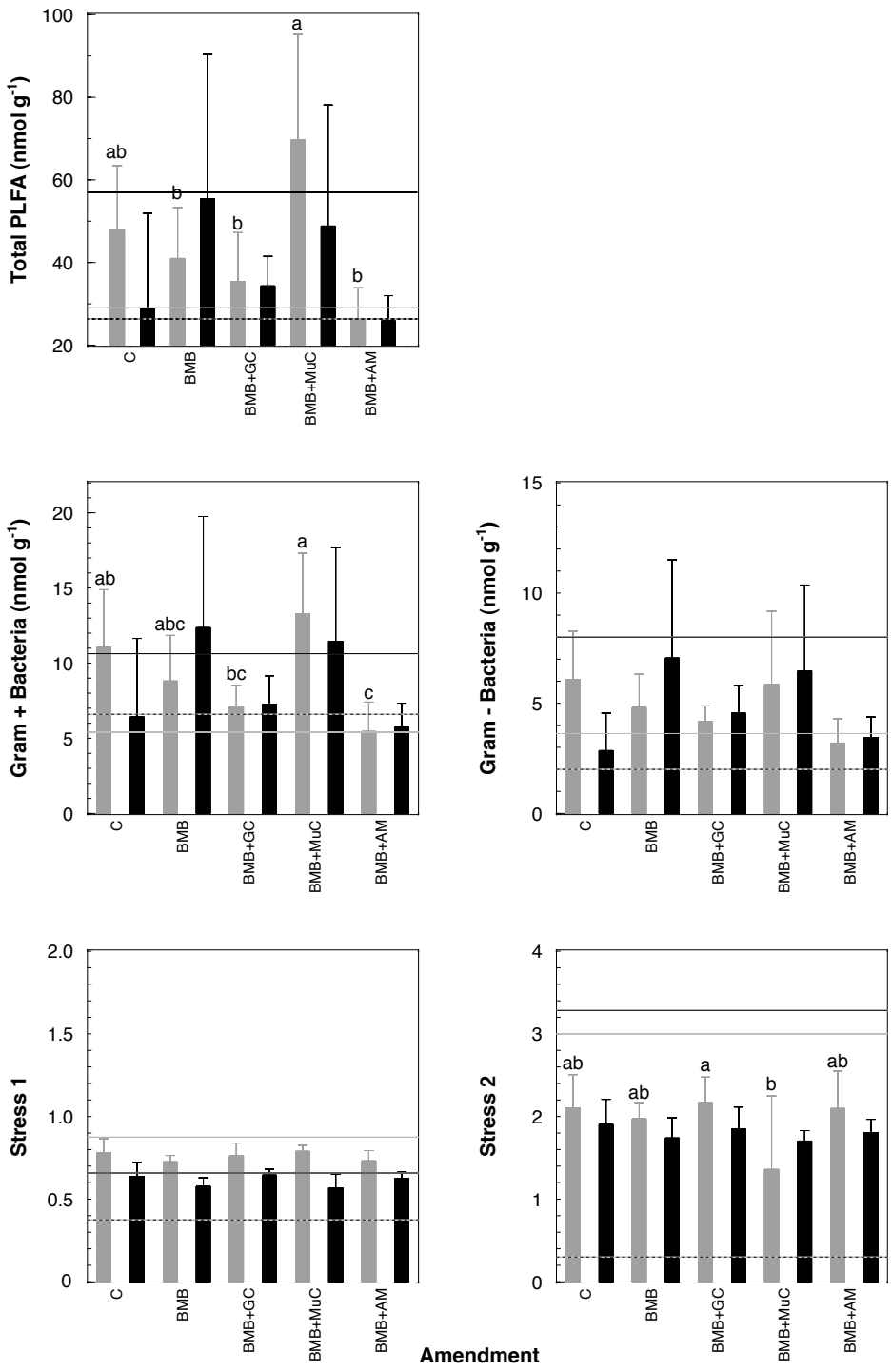

Fig. 4.6. Post-harvest PLFA biomarkers for low (grey bars) and high (black bars) metal soils after 19 wk growth of jack pine. Solid grey line = untreated low metal soils; solid black line = untreated high metal soil; dashed line = control forest soil. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at $P<0.05$. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

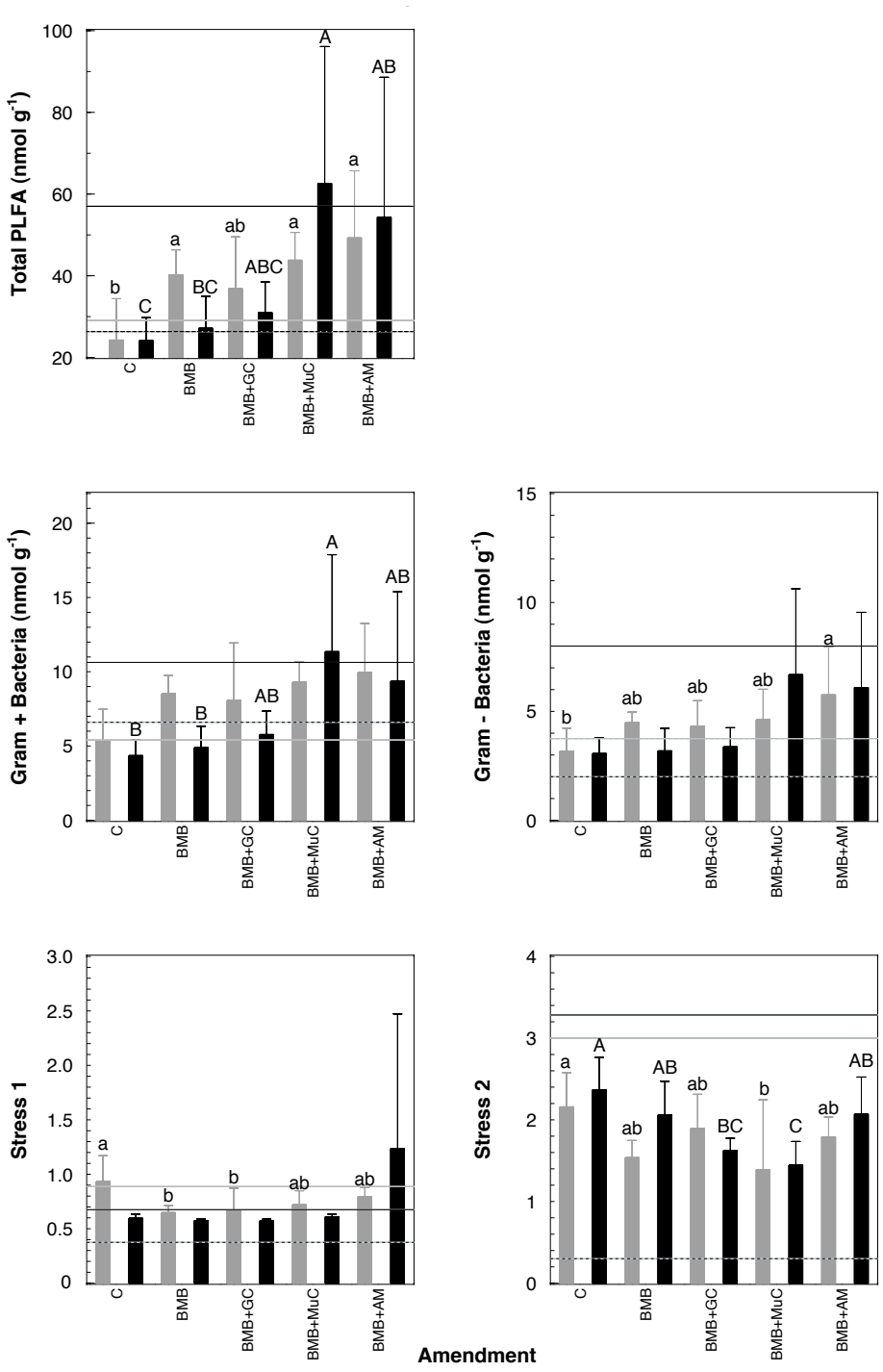

Fig. 4.7. Post-harvest PLFA biomarkers for low (grey bars) and high (black bars) metal soils after 19 wk growth of trembling aspen. Solid grey line = untreated low metal soils; solid black line = untreated high metal soil; dashed line = control forest soil. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at $P<0.05$. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

Biomarkers of Gr+ and Gr- were not significantly different between soils when planted with jack pine. Amendment treatment BMB+AM decreased Gr+ bacterial concentrations in low metal soils. Concentrations of Gr- bacteria were not significantly different for any treatment in either soil. Biomarker concentrations differed with respect to the control forest soil (unaffected by metals) in both soils. Stress 1 values were higher in low metal soils than in high metal soils (0.75 and 0.61, respectively) and both were higher than the unaffected control (0.39). Amendment treatment differences were not seen for Stress 1 values. Stress 2 values were also higher in low metal soils as compared to high metal soils and Stress 2 values for both high and low metals soils (1.94 and 1.79, respectively) were significantly higher than the unaffected control (0.26).

In soils planted with trembling aspen, amendment treatments BMB+MuC and BMB+AM increased Gr+ bacteria in high metal soils, and Gr- bacteria in low metal soils (Fig. 4.7). Biomarker concentrations differed with respect to the control forest soil in both metal affected soils. Treatments BMB and BMB+GC decreased Stress 1 values relative to the control (from 0.93 to less than 0.71) in low metal soils. Stress 2 values were reduced from 2.15 to 1.38 by treatment BMB+MuC in low metal soils, and were reduced in high metal soils from 2.37 to 1.62 and 1.44 by treatments BMB+GC and BMB+MuC, respectively (Fig. 4.7). Amendment BMB+MuC increased AMF concentration over the control in low metal soils planted with trembling aspen; other treatments did not show a significant difference in AMF concentration (Table 4.8).

Fungal:Bacterial ratios were significantly higher in low metal soils for both species (Fig. 4.8). In high metal soils planted with trembling aspen, treatment BMB+MuC increased the F:B ratio significantly over the control from 0.10 to 0.22. General bacterial biomass and F:B ratios in soils planted with jack pine did not show significant treatment differences. Treatment BMB+MuC increased general fungal biomass over the control in low metal soils planted with jack pine. In low metal soils planted with trembling aspen, treatment BMB+AM increased bacterial biomass above the control; treatment BMB+MuC had a similar effect in high metal soils. Treatment BMB+MuC also increased general fungal biomass over the control in both low and high metal soils, and increased the F:B ratio in high metal soils. Treatment BMB also increased fungal biomass over the control in high metal soils. Fungal and bacterial concentrations varied with respect to concentrations found in the unaffected forest soil.

Microbial community structure was analyzed using NMDS, and resulted in a two-dimensional solution that separated microbial communities by plant species along Axis 1. This represents 76% and 11% of the variability in the solution along Axis 1 and 2, respectively (Fig. 4.9). The final stress value of this ordination was 16.84. Microbial communities in low metal soil in which jack pine and trembling aspen were grown were similar to each other, but different from microbial communities in the untreated low metal control soil. Microbial communities from high metal soils in which jack pine and trembling aspen were grown were not similar; only the communities in high metal soil in which jack pine was grown were different from the untreated high metal control. Microbial communities in low metal soils were positively correlated (r=0.6) with F:B and the percentage of general bacterial biomass. An unaffected forest control sample was analyzed but were very dissimilar to the data presented and thus does not appear on the ordination.

Table 4.8. AMF biomarker concentrations in soils containing low and high concentrations of metals amended with treatments following growth of jack pine and trembling aspen.

	Jack pine		Trembling aspen	
Amendment	**Low metal**	**High metal**	**Low metal**	**High metal**
	AMF nmol g^{-1} soil			
C	0.79	0.52	0.40 b†	0.38
BMB	0.70	0.94	0.91 ab	0.48
BMB+GC	0.62	0.56	0.94 ab	0.46
BMB+MuC	0.89	1.90	0.99 a	0.92
BMB+AM	0.46	0.41	0.90 ab	0.59
LSD§ (0.05)	0.51	0.73	1.32	0.53

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
§ LSD results are conservative estimates where some treatments had n<4 due to missing values.

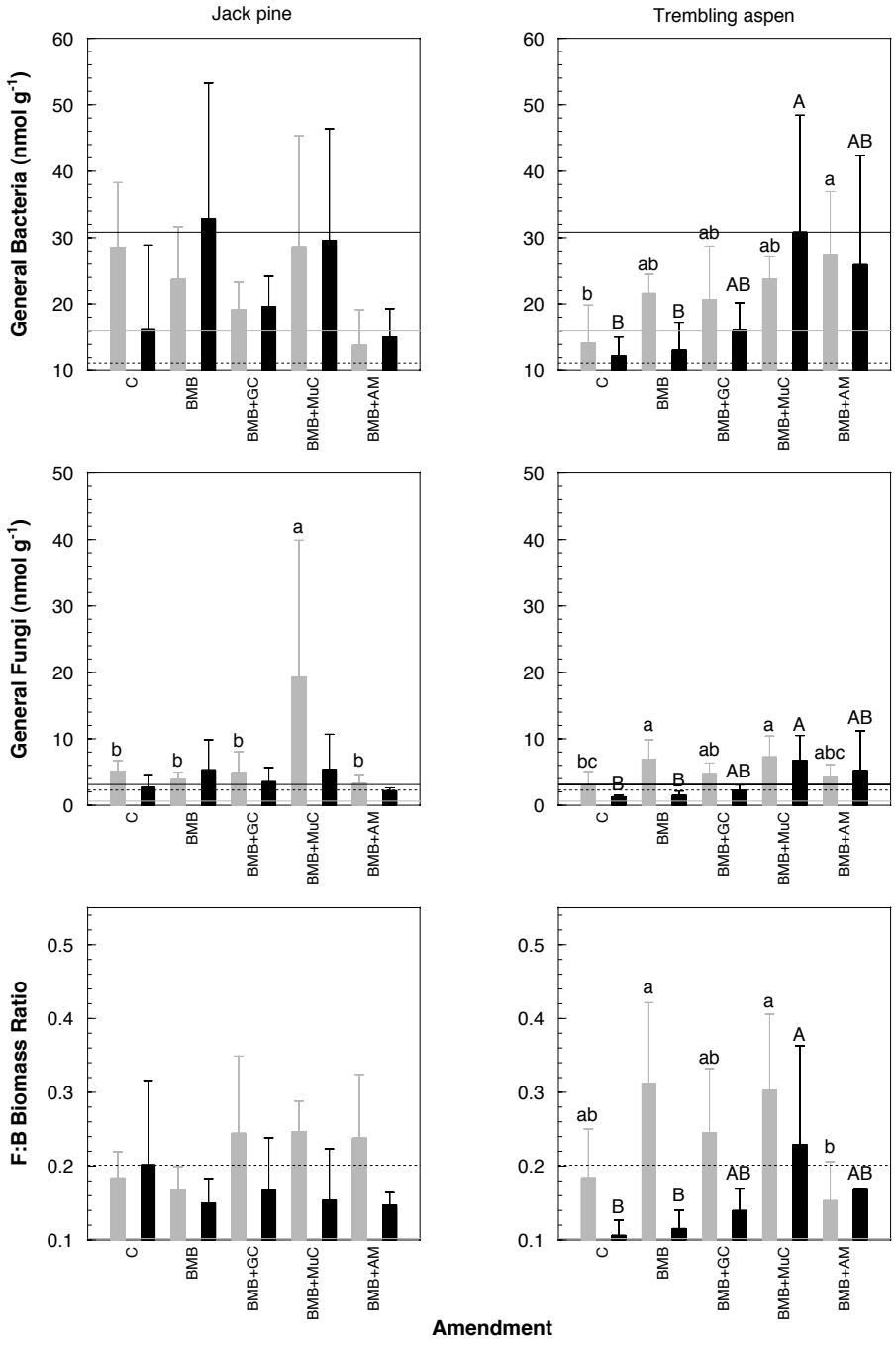

Fig. 4.8. Fungal and bacterial biomarker concentrations and fungal:bacterial (F:B) biomass ratios for jack pine and trembling aspen grown in soils with low and high metal concentrations (grey and black bars, respectively). Solid grey line = untreated low metal soil; solid black line = untreated high metal soil; dashed line = control forest soil. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

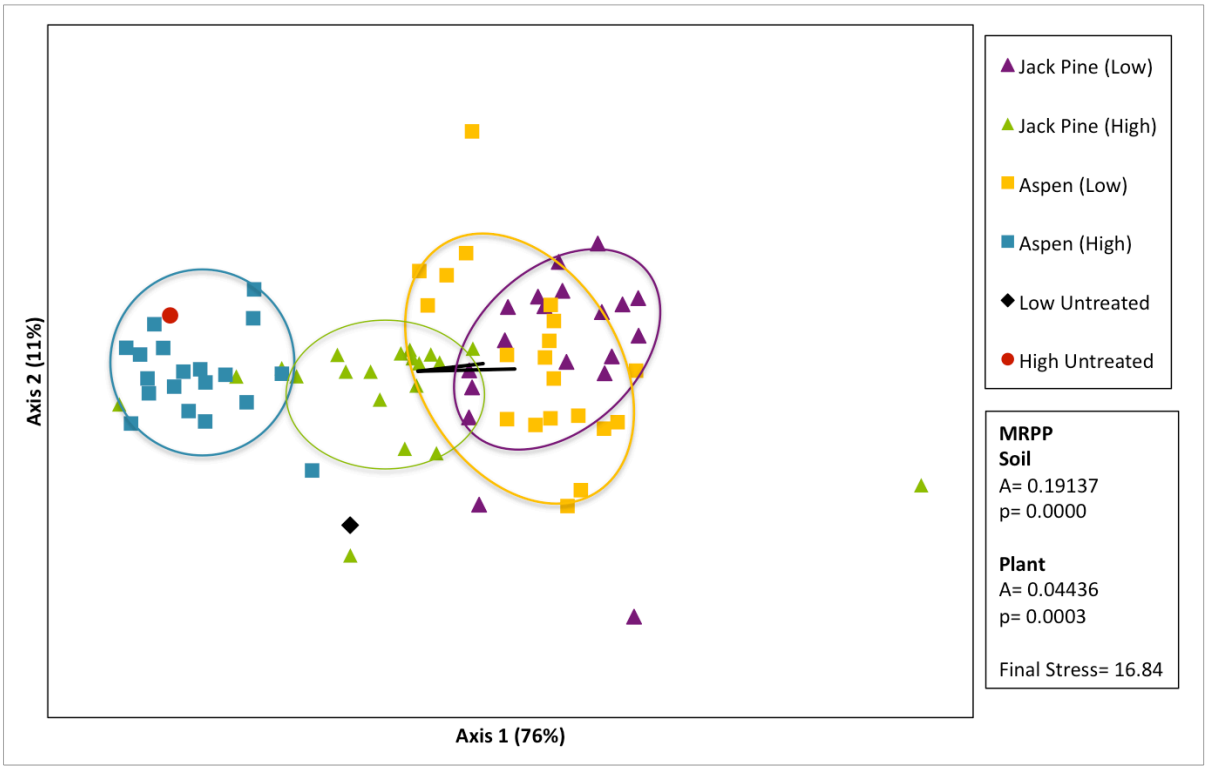

Fig. 4.9. Non-metric multidimensional scaling (NMDS) analysis (final stress = 16.84) and multiple response permutation procedure (MRPP) analysis of soil and plant effects on PLFA profiles (mol %) of jack pine and trembling aspen in low and high metal soils from Flin Flon, MB. *A*= chance-corrected within-group agreement; *p*= statistical significance of *A*. Vectors represent correlation (r=0.6) to various biomarkers.

4.4.4 Plant and soil metal concentrations

Differences in the concentrations of Al, Cd, and Cu in jack pine shoot tissue were not significant between soil types (i.e., low or high metal concentrations) whereas tissue concentrations of Ni and Zn in jack pine grown in low metal soils were significantly higher than those of jack pine grown in high metal soils (Fig. 4.10). Shoot concentrations of Al, Cd, or Cu of jack pine grown in low metal soils were unaffected by amendment treatments. All amendment treatments except BMB reduced shoot Ni content relative to the control, from ca. 54 to 15 mg kg^{-1}, whereas amendment with BMB and BMB+MuC reduced shoot Zn content from ca. 740 to 400 mg kg^{-1}. Cadmium concentrations were negligible in shoots grown in high metal soils. Cu concentrations in jack pine grown in high metal soils varied (9 to 42 mg kg^{-1}) although significant amendment treatment differences were not detected. Shoot concentrations of Ni

ranged from ca. 4 to 12 mg kg^{-1} when grown in high metal soils, and amendment treatment BMB+MuC reduced tissue concentrations significantly relative to the control.

The concentrations of Cd, Cu, and Zn in root tissue of jack pine grown in high metal soils were significantly higher than jack pine grown in low metal soils, although concentrations of Al and Ni were not significantly different between soils. In jack pine seedlings grown in low metal soils, concentrations of Cd, Ni, and Zn showed no significant treatment differences, and concentrations of Cu in amended soils were not significantly different from the control (Fig. 4.11). Treatment BMB increased root concentrations of Al. In roots of jack pine grown in high metal soils, treatment BMB+MuC significantly increased the concentrations of Al, Cd, and Cu, while treatment BMB+AM significantly reduced the concentration of root Ni from 94 to 0.5 mg kg^{-1}.

Soil type (i.e., soil metal concentration) did not significantly affect metal concentrations in trembling aspen shoots. Furthermore, none of the amendments significantly affected shoot tissue concentrations of Al, Cd, or Cu. In contrast, the concentration of Ni in aspen shoots grown in low metal soils increased from ca. 10 mg kg^{-1} to 97 mg kg^{-1} when soils were amended with BMB+AM, whereas BMB significantly reduced shoot concentrations of Zn (Fig. 4.12). Shoot Ni concentrations in aspen trees grown in high metal soils decreased from 49 to 191 mg kg^{-1} in response to the addition of BMB. Shoot concentrations of Zn did not differ between amendments.

Tissue concentrations of Al, Cd, Ni, and Zn in the roots of trembling aspen did not differ significantly between soil types. In contrast, plants grown in high metal soils had higher concentrations of Cu in root samples (Fig. 4.13). For plants grown in low metal soils, none of the amendments significantly affected root metal concentrations for any of the metals. Similarly, root concentrations of Al, Cd, Cu, and Zn in plants grown in high metal soils were not significantly affected by application of any of the amendments. Treatment BMB reduced root Ni concentration but concentrations were quite low in all soils (ca. 1 mg kg^{-1}). Contrast analysis of plant tissue metal concentrations were not consistent but did show significant differences between the control soil and all treatments, as well as differences between biochar treated soil versus soil treated with biochar and another amendment in terms of root tissue concentrations of the various metals (Tables 4.3 to 4.6).

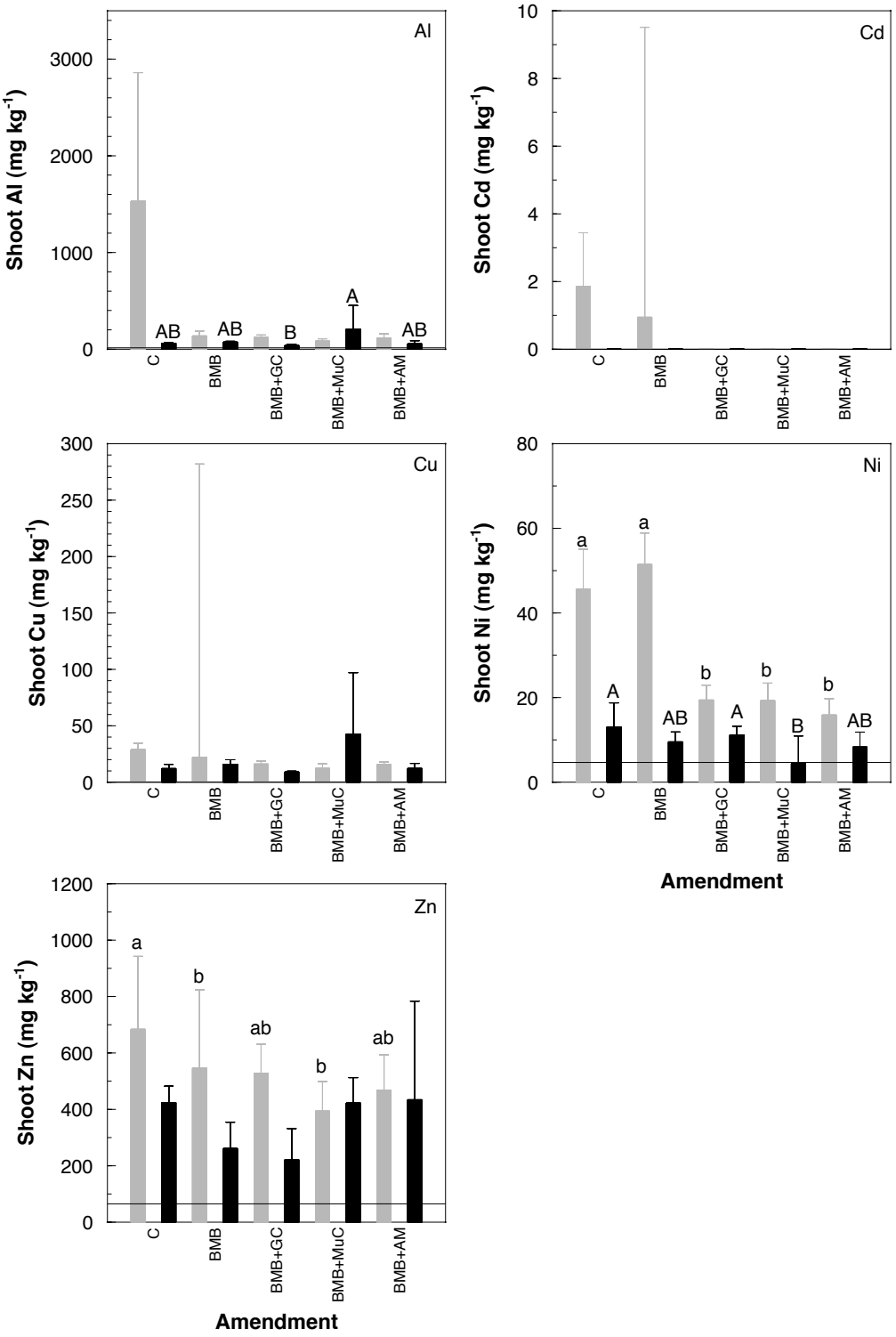

Fig. 4.10. Shoot tissue metal concentrations for jack pine plants grown in low (grey bars) and high (black bars) metal soils after 19 wk of growth. Solid black line = metal concentration of reference tree sample. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at $P<0.05$. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

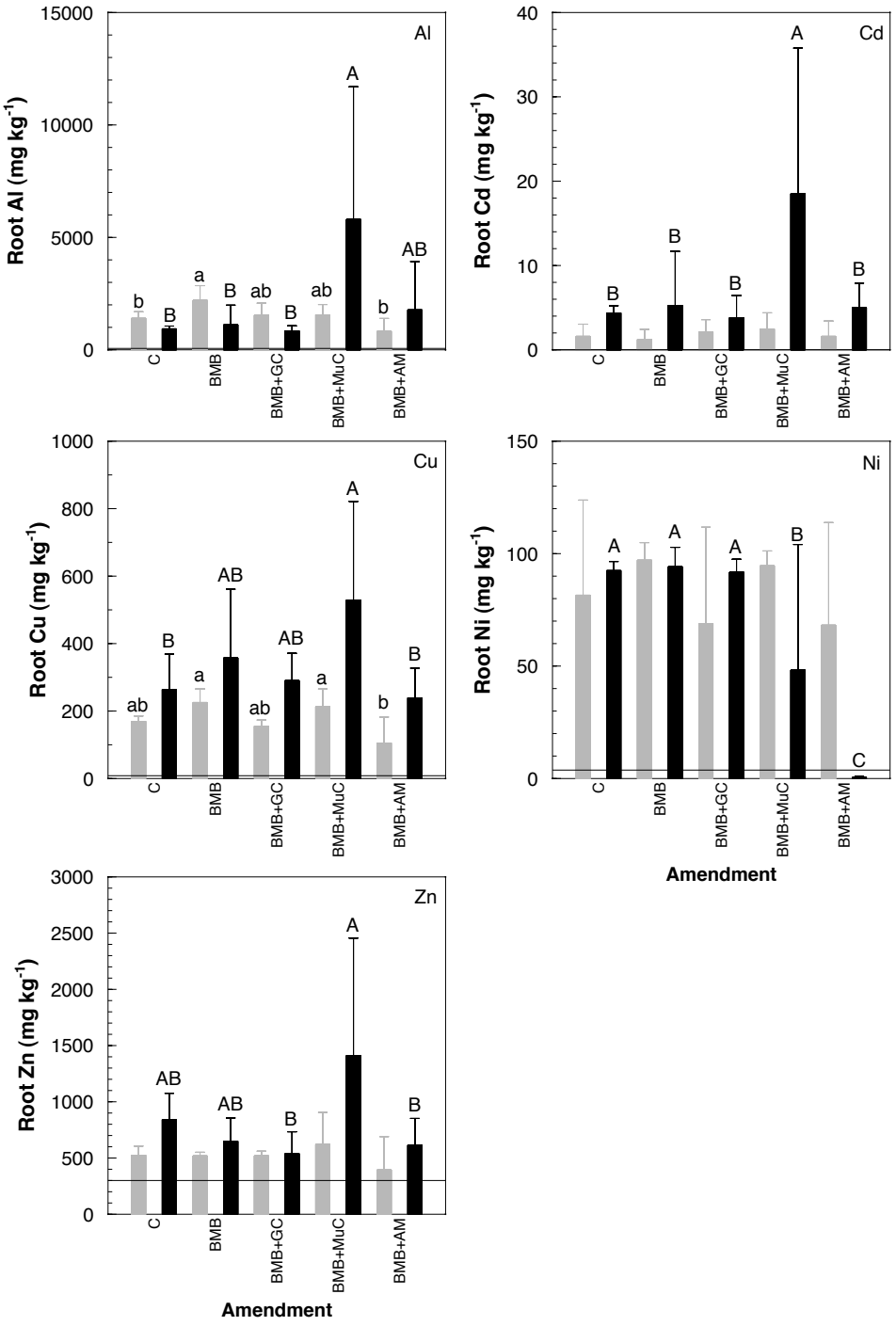

Fig. 4.11. Root tissue metal concentrations for jack pine plants grown in low (grey bars) and high (black bars) metal soils after 19 wk of growth. Solid black line = metal concentration of reference tree sample. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at $P<0.05$. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

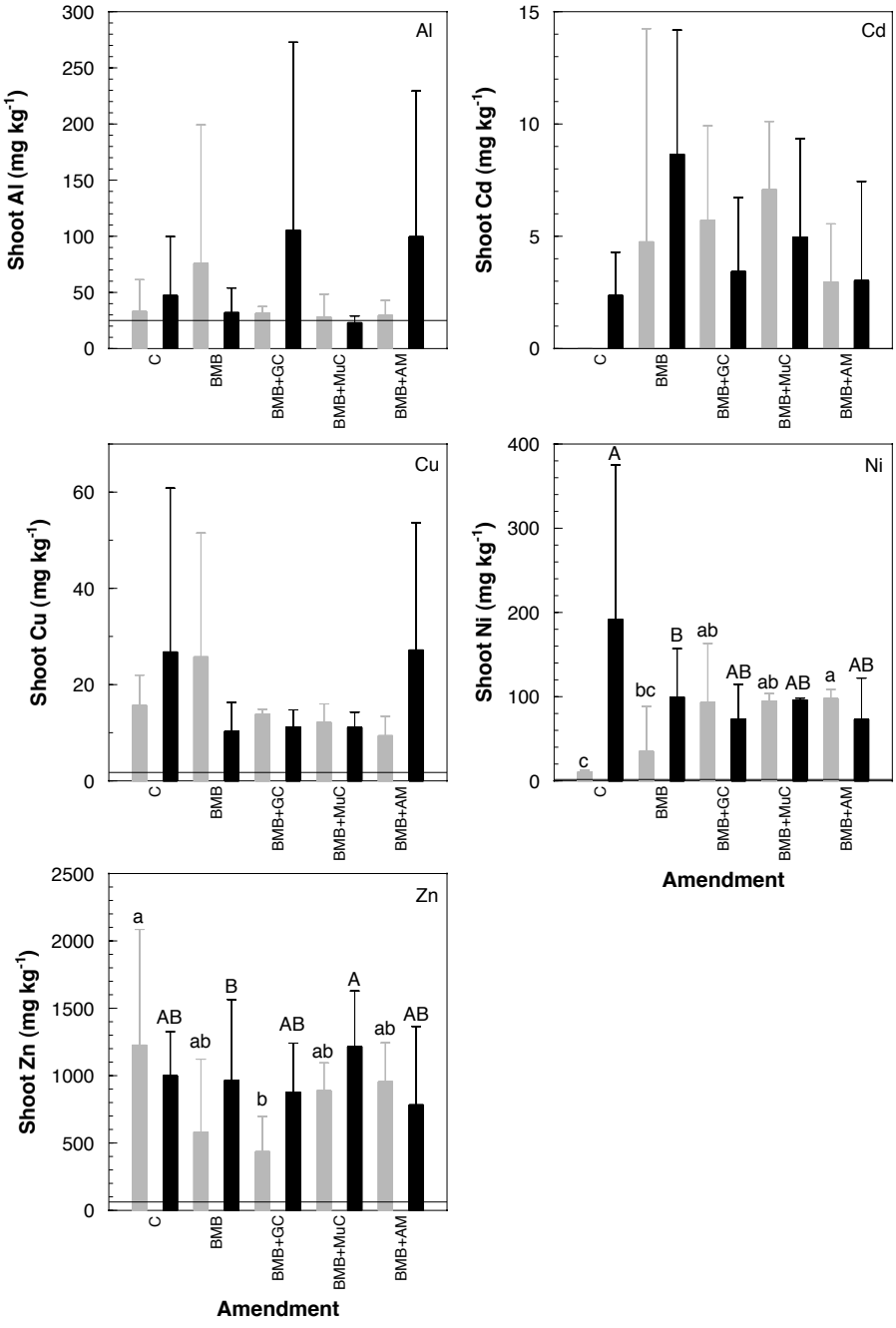

Fig. 4.12. Shoot tissue metal concentrations for trembling aspen plants grown in low (grey bars) and high (black bars) metal soils after 19 wk of growth. Solid black line = metal concentration of reference tree sample. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

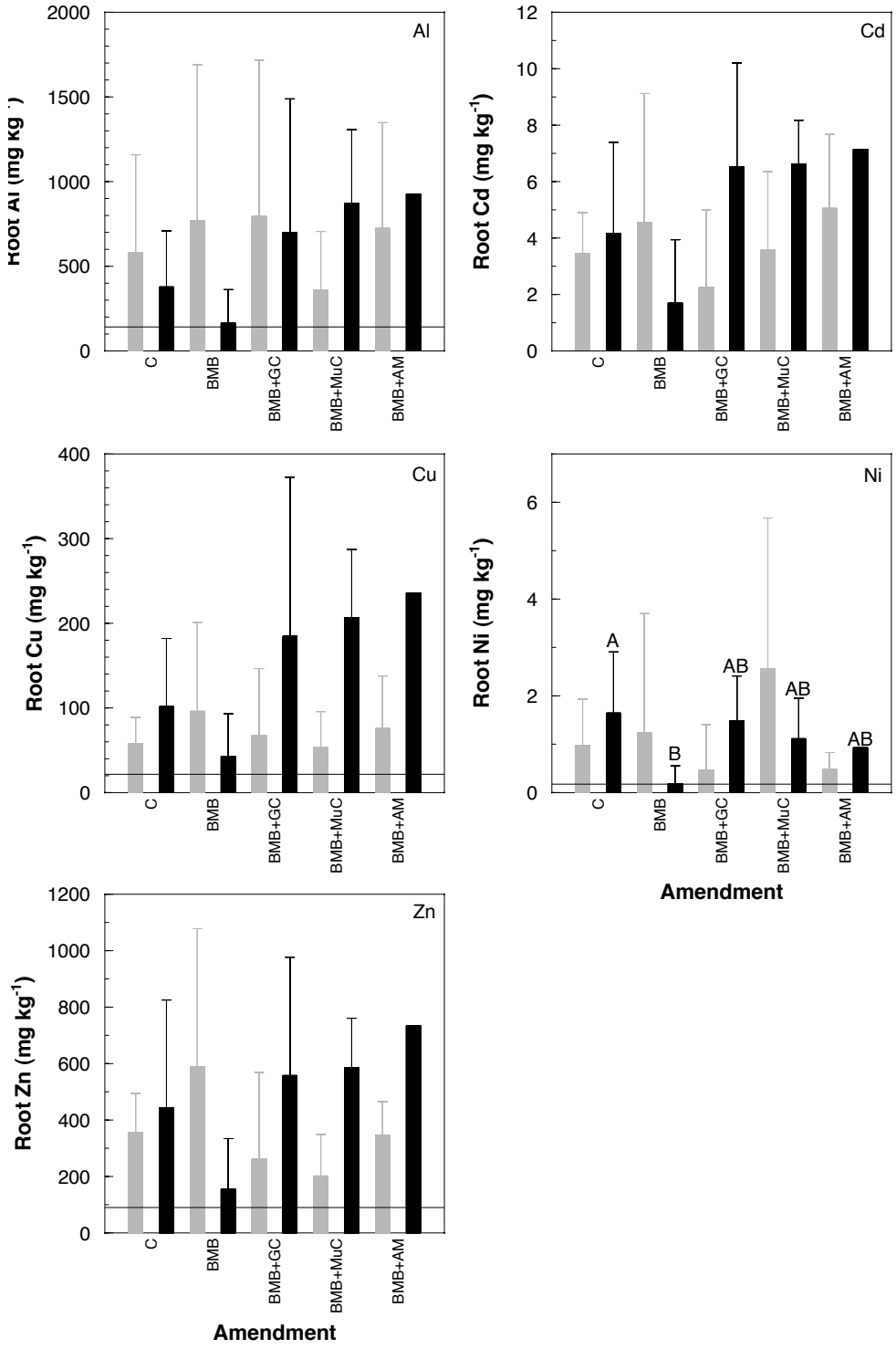

Fig. 4.13. Root tissue metal concentrations for trembling aspen plants grown in low (grey bars) and high (black bars) metal soils after 19 wk of growth. Solid black line = metal concentration of reference tree sample. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

At harvest, available soil concentrations of Al ranged from 30 to 140 mg kg^{-1}; concentrations of available Zn ranged from ca. 60 to <1 mg kg^{-1} (Fig. 4.14). Available Al and Zn concentrations were significantly higher in high metal soils. In soils planted with jack pine, soil Cd and Cu concentrations were low (<3 mg kg^{-1}) and significant differences were not detected between low and high metal soils. Treatment BMB+MuC reduced available Al significantly in high metal soils. Levels of available soil Zn did not differ significantly between the control and any of the remaining treatments.

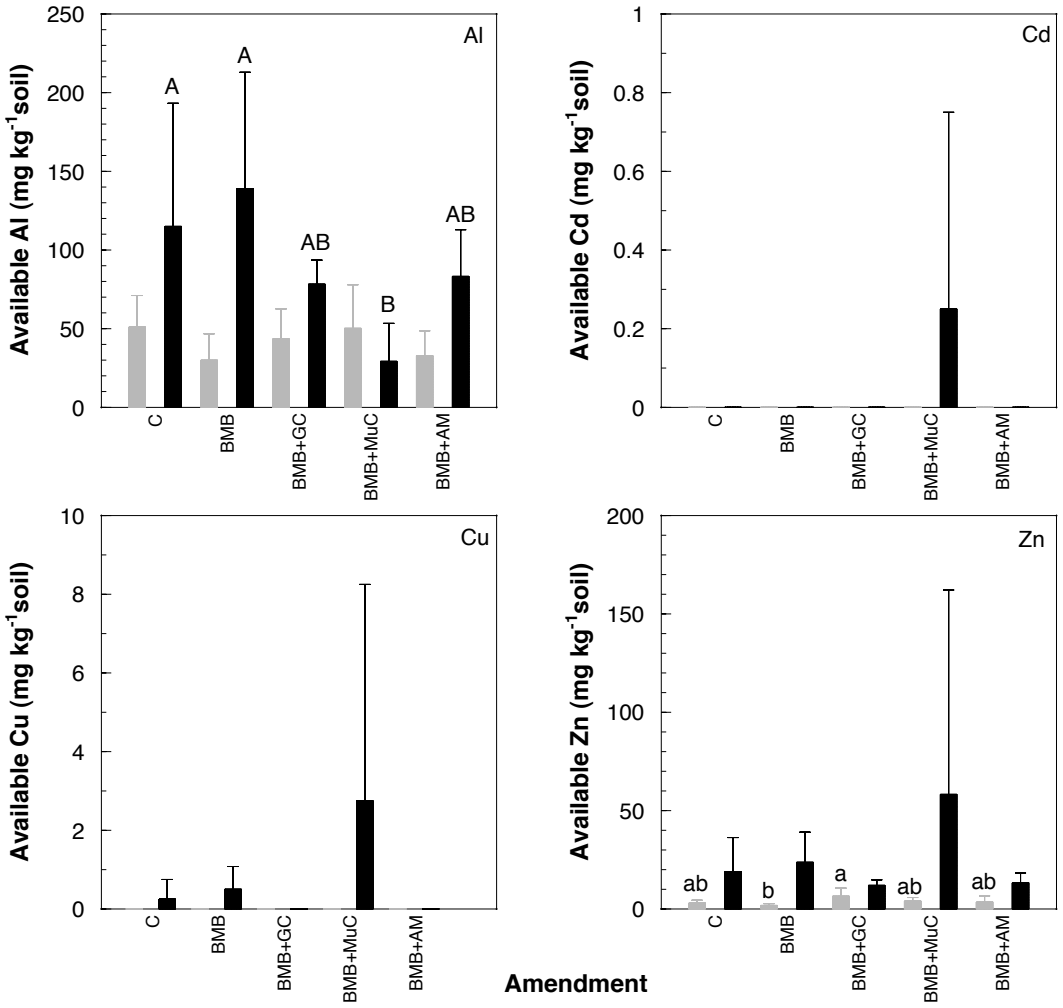

Fig. 4.14. Post-harvest soil metal concentrations for low (grey bars) and high (black bars) metal soils planted with jack pine after 19 wk of growth. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at *P*<0.05. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

In soils planted with trembling aspen, concentrations of Cd and Cu were low (0 to 8 mg kg^{-1}) (Fig. 4.15). Concentrations of available Cu and Zn were significantly higher in high metal soils, with available Zn concentrations ranging from ca. 7 to 80 mg kg^{-1}. In high metal soils, amendment treatment BMB+AM increased available Zn and Cu over the control. Available Al was higher in low metal soils. Amendment treatments BMB, BMB+GC, and BMB+AM reduced available Al in high metal soils. For both tree species, contrast analysis was not conclusive and did not indicate differences between treatment groups (Tables 4.3 to 4.6).

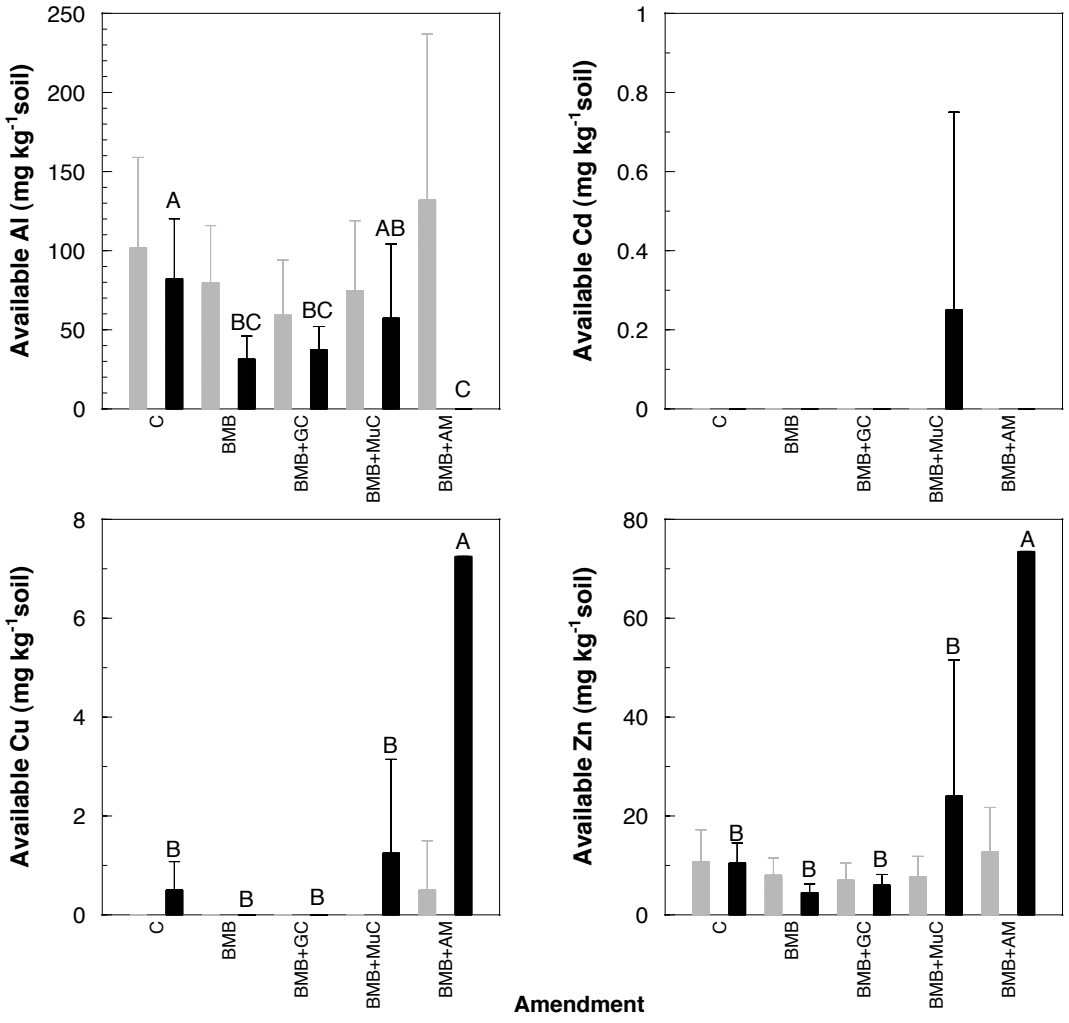

Fig. 4.15. Post-harvest soil metal concentrations for low (grey bars) and high (black bars) metal soils planted with trembling aspen after 19 wk of growth. Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at $P<0.05$. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

4.4.5 Mycorrhizal root colonization

Ectomycorrhizal root colonization (%) was not significantly different between plant species (Fig. 4.16). Within plant species, trembling aspen colonization was not significantly affected by soil metal concentration, whereas jack pine grown in high metal soils had significantly more mycorrhizal colonization than when grown in low metal soils (ca. 21 and 14%, respectively). Amendment treatments did not significantly affect root colonization of jack pine as compared to untreated (control) jack pine grown in low metal soils (Fig. 4.16). Root colonization of trembling aspen grown in high metal soils was not significantly increased by amendments, whereas treatment BMB significantly reduced root colonization over the control by ca. 20% in low metal soils. Contrast analysis of root colonization was not consistent and only for aspen grown in high metal soils did the solo biochar treatment cause a significant difference in colonization from the other treatments (Tables 4.3 to 4.6).

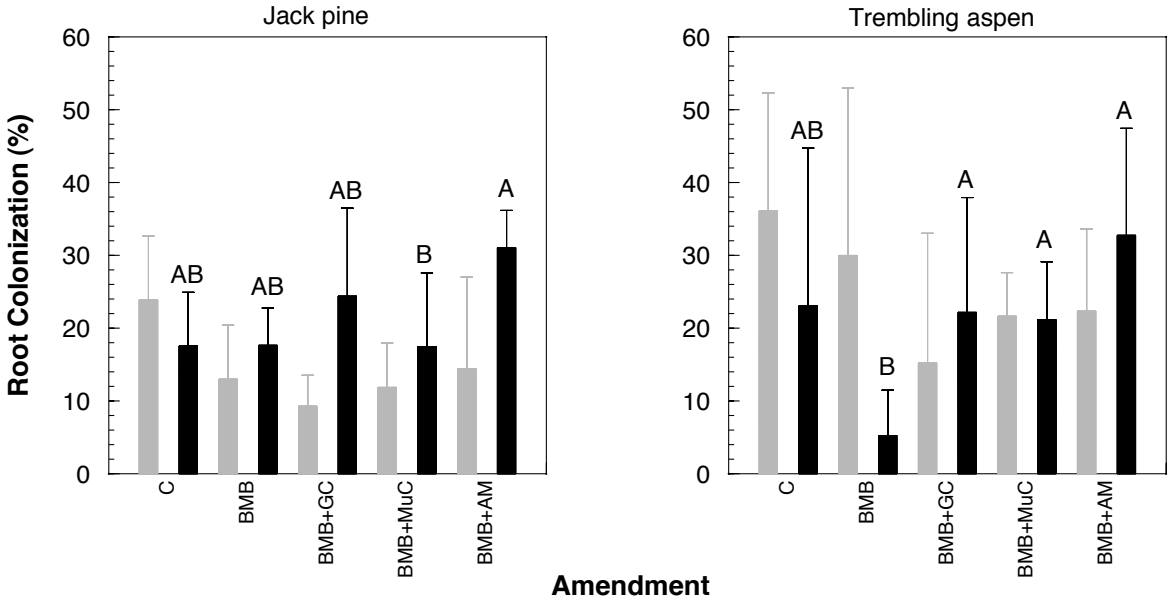

Fig. 4.16. Root colonization (%) in jack pine and trembling aspen grown in soils with low and high metal concentrations (grey and black bars, respectively). Upper and lowercase letters represent separate statistical comparisons within each separate graph and are significant at $P<0.05$. Statistical significance is reported for the transformed data; however, means and accompanying error bars represent non-transformed data. Error bars represent non-transformed standard error of the mean (n=4).

4.4.6 Pearson's correlations of plant growth, soil characteristics, soil and plant tissue metal concentrations, microbial characteristics, and ectomycorrhizal root colonization

No two variables were consistently correlated across all four plant and soil combinations (Tables B.5 to B.8), and low Cd and Cu soil concentrations and shoot Cd concentrations did not enable correlations for these variables. Correlations of particular interest included Stress variables 1 and 2 significantly correlating with MBC, MBN, F:B, and root Cd concentrations in Jack pine grown in low metal soils. Furthermore, these biomarkers correlated significantly (P<0.01) with root metal concentrations in trembling aspen planted with high metal soils. Soil metal contents were for the most part not correlated with any other variables, including plant tissue metal concentrations.

4.5 Discussion

Plant root biomass after 19 weeks of growth did not show a significant change due to treatment, irrespective of the initial soil metal concentration level. Shoot growth of both jack pine and trembling aspen did show significant differences, specifically that plants grown as a 'control' were more negatively affected than those plants grown in treated soils. Although these observations suggest that the effect of the amendments is unclear, it also is possible that significant differences were not detected due to the inherent variability in the plant growth. This, in part, reflects the problems associated with estimating the initial tree seedling weights. Tree seedlings were planted with a large root ball that had an inconsistent amount of peat contained within. Thus, it was difficult to determine if the consequently high variability was masking a trend in plant growth due to soil amendment use. What was suggested by the results is that seedling growth was stunted by the metal concentrations in the metal-affected soil, especially without any treatment at all. The poor root and shoot growth observed visually is similar to the observations reported by Jones et al. (1984) who examined jack pine and black spruce grown in soils near a metal smelter near Thompson, MB. These researchers found significant positive correlations between growth inhibition and soil metal content, and made visual observations of root and shoot stunting. They attributed poor plant growth to ion exchange competition with heavy metals at the root surface, and elevated metal levels in tissue causing direct or indirect toxic effects, such as dwarfism, chlorosis, and necrosis (Jones et al., 1984). In the current

experiment, no correlations were detected between growth and soil metal content, but it is possible that the high variability of the plant growth obscured any correlations that may have existed.

Concentrations of MBC in this experiment, ranging from ca. 150 to 400 μg g^{-1}, were slightly higher compared to the first experiment where concentrations ranged from ca. 100 to 260 μg g^{-1}. These numbers are similar to MBC concentrations for stressed soils reported by Perez-de-Mora et al. (2006) (ca. 20 to 450 μg g^{-1}) and Baker et al. (2011) (ca. 70 to 300 μg g^{-1}), but are much lower than values found for other Canadian Boreal forest sites, which can range from ca. 200 to 800 μg g^{-1} (McMillan et al., 2007; Tan et al., 2008). This likely reflects the compromised quality of the soil at the Flin Flon study site. Amendment treatments did not have a measurable effect on MBC as compared to the control. This is in contrast to Borken et al. (2002) and Jin (2010), who found that additions of compost and biochar increased soil MBC. High levels of available soil metals have been shown to reduce MBC (Wang et al., 2007), and it is possible that the addition of some soil amendments can facilitate an increase in metal availability, thus decreasing MBC in the soil (Rate et al., 2004).

Soil MBN concentrations ranged from ca. 16 to 24 μg g^{-1} soil, with high metal soils having significantly higher MBN than low metal soils. These ranges are quite low, but are similar to those reported by McMillan et al. (2007) in soils contaminated with oil sands tailings (ca. 50 μg g^{-1}), as well as ranges reported by Baker et al. (2011) for soils contaminated with Pb and Zn mine waste (ca. 5 to 100 μg g^{-1}). Although the soils in this experiment were not compared to a control forest sample in the vicinity, it is worth noting that McMillan et al. (2007) found that even after reclamation, MBN concentrations in the soil were still not as high as an unaffected, natural soil, indicating the sensitive nature of this indicator.

This experiment did not reveal consistent differences in total PLFA concentrations associated with soil metal concentrations, which is in contrast to observations by Kelly et al. (2003), who found that soils containing higher amounts of metals had lower total PLFA. This is also in contrast to findings from the previous experiment using understory plant species (Experiment #1, Chapter 3) in which higher amounts of total PLFA in the high metal soils were observed. Initial numbers of total PLFA on untreated soils were, however, higher in high metal concentration soils, which may be indicative of the plant community in the soil creating an environment favorable to microbial biomass growth, as well as higher levels of organic carbon in

high metal soils. No single treatment affected PLFA biomarkers consistently for all soil and plant combinations. Gram+ bacteria has been found in increased levels in soils with compost additions (Baker et al., 2011), although it has been suggested that Gr- bacteria dominate soils that are stressed by temperature, pH, or heavy metal concentrations (Kaur et al., 2005; Tischer et al., 2008). It also has been suggested that Gr- bacteria are stimulated when soil is amended with lime (Frostegaard et al., 1993). These observations likely indicate that the metal-affected soil is not yet near to its natural equilibrium, although what the ratios of Gr- to Gr+ bacteria should be are unclear. The addition of biochar to these soils may have had a positive effect on the microbial community by increasing the amount of C available (Beesley et al., 2011), but it may also cause changes in pH and interfere with plant signaling compounds, affecting microbial community structure and growth (Lehmann et al., 2011).

Similar to the previous experiment on understory species (Chapter 3), fungal species were more abundant in soils with a higher metal content as compared to the low metal soil. This is in contrast to Kelly et al. (2003) and Hinojosa et al. (2005), who both found that higher concentrations of metals (especially Zn) in soil typically reduce fungal growth. Other research suggests that the development of resistance to heavy metals may increase the fungal microbial group population relative to other microbial groups (Pennanen et al., 1996; Kelly et al., 1999). Fungal resistance to heavy metals over the decades of smelter emissions may be driving the higher fungal populations in this soil. Soil AMF populations were relatively low, and did not make up a significant portion of the microbial biomass – this is likely because AMF do not colonize trees. Populations of AMF in the control forest soil were higher, but not significantly so, than the metal-affected soils. It is possible that low or stunted root growth from all plants in the area (due to soil metal content) led to low AMF populations because AMF are dependent on host plant resources for growth. Low AMF populations may also be due to the application of biochar to the soil. Warnock et al. (2010) suggests that biochar may decrease AMF populations by growth inhibition, alteration of P availability, or pH changes.

Stress biomarkers 1 and 2 are good indicators of microbial community stress, which can be a function of temperature, water, pH or heavy metal presence (Kaur et al., 2005). Stress values in this experiment were higher than those found by Tischer et al. (2008) for heavy metal contaminated sites in Germany. Both Stress 1 and 2 values in the amended soils were significantly different from the control forest soil. This indicates a soil microbial community that

is not at equilibrium. Stress 2 values show an intriguing decrease with all treatments, even if only with lime (as per the control treatment). The soil microbial community appears to be responsive to the presence of heavy metals and thus is a good indicator of change in these soils. Additionally, all treatments were planted and the reduction in stress level could be attributed to the presence of plant species helping to buffer the microbial community from stress created by the heavy metal presence in the soil. Stress values may continue to change as the environment returns to a more natural state (Zhang et al., 2006).

The ranges of F:B ratios observed in this experiment were higher in low metal soils and ranged from ca. 0.1 to 0.3, which are similar to values reported by Hinojosa et al. (2005) for soils affected with heavy metals. This is an encouraging discovery, as Pennanen et al. (1996) indicated that F:B ratios increased with decreasing distance from the smelter, suggesting that higher F:B ratios may be associated with 'healthier' soils. As noted previously, however, higher fungal biomass in the metal affected soils may simply be a function of fungal adaptation to soil heavy metal concentrations over decades of smelter deposition. Only one treatment significantly affected this ratio over the control. High metal soils planted with trembling aspen and amended with BMB+MuC increased the F:B. This shift in the F:B ratio towards a higher fungal contribution could be due to a fungal response to increased SOM (Frostegaard and Baath, 1996), in this case supplied by both the biochar and the municipal compost.

Concentrations of metals in plant tissues were not affected consistently by soil type, plant species, or amendment, although it has been shown that both jack pine and trembling aspen will readily accumulate metals into both roots and shoots (Jones et al., 1984; Kahle, 1993; Hutchinson and Hayward, 2010; Cloutier-Hurteau et al., 2011). Concentrations of Al, Cd, Cu, Ni in plant tissues observed in the current experiment were similar to concentrations reported by the above researchers; Zn concentrations were higher than typically is reported but this is likely due to the high amount of Zn found in the test soils. Jones et al. (1984) found significant correlations between soil metal concentrations and jack pine tissue concentrations. In the current experiment, similar correlations were not detected; however, Zn concentrations were associated with a high degree of variability and thus correlations were inconsistent and any real relationships may have been obscured. Although Giasson et al. (2006) found that Zn content was higher in plants treated with mycorrhizae, the inoculated treatment (BMB+AM) did not have significantly different levels of Zn in either roots or shoot tissue for either plant species. It is important to note that field

concentrations of tissue metals may be lower, as plant roots seek out less contaminated areas (Kahle, 1993).

The residual levels of Al, Cd, Cu, and Zn remaining in the soil at the termination of the experiment were inconsistent as compared to those in the previous experiment (Chapter 3). Concentrations of available metals remaining in the soil were higher in soils initially identified as "high metal", with the exception of Al concentrations in soils planted with trembling aspen. Treatment differences were inconsistent and few significant treatment differences were detected. Concentrations of available Cd were negligible in all treatments except for high metal soils treated with BMB+MuC. This treatment, as was the case for all the treatments, was associated with a high amount of variability. The effect of biochar on metal-affected soils is variable, often decreasing the availability of some metals while increasing the availability of others (Chen et al., 2006; Beesley et al., 2010; Namgay et al., 2010). Without clear and consistent results from these treatments, it is difficult to say which, if any, would be best for application in the study site area in terms of reducing or altering available metal concentration levels.

The inoculation of tree roots with ectomycorrhizal species has been shown to increase plant biomass and influence metal uptake into shoots and roots (Kahle, 1993; Krupa and Kozdroj, 2007; Polanco et al., 2008). Mycorrhizal colonization of roots is known to increase root biomass production and the association acts as a buffer between soil metals and plant roots. Unfortunately, colonization and its related influences on root growth decrease as soil metals increase and ectomycorrhizal fungi application has been found to be less effective in soils with high metal contents (Kahle, 1993). Additionally, increased metal contents can result in the loss of fine tree roots, where colonization takes place. This may result in lower colonization rates (Pennanen et al., 1996). This study found that jack pine planted in high metal soils had significantly higher root colonization than low metal soils although overall colonization was quite low. Moreover, although there were no significant treatment differences detected, the amendment containing AMF/ectomycorrhizae (BMB+AM) trended toward a higher percentage of colonized roots. The success of a fungal association may be time dependent and thus further studies should investigate the impact of fungal application over a longer time frame.

4.6 Conclusion

The work done examining the influence of soil amendments on plant growth and microbial community structure suggests that heavy metals have a significant influence on these characteristics. Visual changes in plant health, as well as alterations in microbial community biomass and structure can be attributed to the presence of excess levels of heavy metals within the soil. However, the high variability of the measured indicators in this study, specifically of plant growth and soil and tissue metal contents, resulted in inconsistent results and did not suggest that any one amendment was superior to another. A trial conducted over a longer duration, or a field-scale trial may produce more definitive results. It appears that revegetation of these soils is possible, but may be slow and more research is certainly needed.

5. SUMMARY AND CONCLUSIONS

This study was initiated to examine the impact of various organic and microbial amendments to smelter-affected soils in the Flin Flon-Creighton area on both plant growth and the soil microbial community. It was hypothesized that changes to the soil microbial community might be linked to revegetation success, and that the addition of amendments to soils gathered from the Flin Flon area with varying levels of metal concentrations would affect plant growth and microbial community structure. Experiments were carried out with the intention of investigating this hypothesis with both under- and over-story forest species, and by measuring various microbial, soil, and plant parameters. The soils used in the experiment were comprised of soil samples from more than one site and bulked to provide two experimental soils, one with high and one with low metal concentrations. Soils were limed to raise the pH to make it more hospitable for plant seedlings, and to simulate liming treatments occurring in the area at present. Amendments were identified that may aid in increasing plant growth and microbial community structure, and two greenhouse trials were carried out, using forest understory species (American vetch and tufted hairgrass), and overstory species (jack pine and trembling aspen) as the test plants. Specific objectives for the project included: (i) assessing microbial community structure in soils, and relating community structure to soil quality, plant-available metal concentrations, and plant biomass response; and (ii) identifying the amendment(s) that were able to promote the growth of understory and climax species in the smelter-affected soils.

In general, higher microbial abundance was not found in soils that had lower metal concentrations (*Hypothesis 1)*. Numbers of total PLFA were either higher in high metal concentration soils, or did not differ between soils. This is in contrast to a number of studies that have found higher microbial biomass in soils with lower metals (Kelly et al., 2003; Hinojosa et al., 2005). This may be due to the high metal soils supporting a microbial community more tolerant of heavy metal presence – functional groups may be more likely to increase over time over less tolerant groups (Pennanen et al., 1996). Additionally, higher organic carbon in high metal soils, as well as the presence of a different plant community on high and low metal

sampling sites may have contributed to an environment enabling proliferation of the microbial community in high metal soils.

Amendments applied to soils from the Flin Flon area that had been planted with various types of vegetation did not show a consistent effect on microbial community structure or plant growth (*Hypothesis 2)*. Additions of amendment treatments had varying and inconsistent impacts on soil microbial community structure, microbial biomass, and plant growth. In general, soils with lower metal concentrations supported higher amounts of plant and microbial biomass. In terms of shifting the microbial community structure to one that more closely approximates a forest soil unaffected by metals, amendment treatments did not have a consistent effect and differed depending on which soil type and plant species to which they were applied. In both experiments, all soil metal concentration and plant species combinations supported a microbial community that was different than the unaffected forest soil. This indicates that these metal affected soils are not supporting communities traditionally associated with a healthy forest. In terms of amendment treatment effects on total microbial abundance (total PLFA concentration), only soils planted with trembling aspen showed any significant treatment differences with amendment treatment, and correlations between these two variables were low, indicating that soil amendments, in this context, did not influence microbial community abundance.

In general, increases in the ratio of F:B biomass caused by amendment treatments were inconsistent and did not strongly correlate with total plant biomass (*Hypothesis 3)*. Fungal biomarker concentrations in both experiments were more abundant in soils with a higher metal concentration. This is in contrast to Kelly et al. (2003) and Hinojosa et al. (2005), who both found that higher soil concentrations of metals typically reduce the presence of fungal indicators. However, fungal microbial groups have been shown to develop resistance to heavy metals, and this may be related to the increased fungal population in soils with a high metal concentration (Pennanen et al., 1996; Kelly et al., 1999). Inoculation of plants with the AMF/ectomycorrhizal inoculant did not significantly increase total biomass in any of the studied plant species (*Hypothesis 4*). Ectomycorrhizal fungi colonization of roots was low and may be related to the metal concentration in the soil (Kahle, 1993). Additionally, a lack of mycorrhizal vegetation in the area could have resulted in the die-out of native mycorrhizal soil species. These species would have been adapted to site-specific soils and related metal concentrations (Jeffries et al.,

2003). Fungal colonization of roots, over time, may increase root biomass production as well as buffer plants from metal uptake into tissues (Kahle, 1993; Polanco et al., 2008).

All plant species studied have been shown to take up metals into tissues; however, this experiment showed that no amendment had a consistent effect on tissue metal uptake, irrespective of plant species grown or soil metal concentration (*Hypothesis 5)*. Additionally, significant correlations between plant tissue metal uptake into roots or shoots and total biomass growth were inconsistent, and in the case of the overstory species (jack pine and trembling aspen), potential significant correlations may be been masked by total biomass measurements with a high degree of variability.

Although there have been previous studies done on plant growth and soil parameters of metal-affected soils in the Flin Flon area (Jones et al., 1984; Wotton et al., 1986; Henderson et al., 1998; Winterhalder, 2003), this is the first to study how the addition of soil amendments may affect these parameters. Additionally, no work on microbial community structure has been done previous to this study in this area. The results from this study indicate that the soils and associated microbial communities located in the Flin Flon area are very diverse, the latter appear to have adapted to their local conditions. Amendment of the soil may help increase plant growth and microbial diversity or community structure, but the diversity of the study site will make it difficult to predict outcomes for any specific area. Notably, the presence of any vegetation growing in the soils appeared to have an important effect on microbial communities, by reducing the stress biomarkers. This indicates the importance of having a revegetation program that focuses on the success of growing plants in these soils. This will further reduce stress on the microbial community, which will allow more plant species to proliferate, furthering the greening of Flin Flon.

This study provided valuable information on the makeup of the microbial community in this area, although further research is necessary to fully understand the scope and diversity of these communities in the metal-affected areas. A more in-depth study characterizing the exact microbial species found in Flin Flon soils would enable a greater understanding of which microbial groups play an important functional role in these soils. This information could be used to further revegetation efforts. As well, further field studies on amendment use and its effects on plant growth and the microbial community are suggested. This would enable field-level effects to be observed – perhaps stronger differences between amendments could be observed on a larger

scale. A longer-term study (either in a greenhouse or on a field scale) would allow root colonization of trees to be studied in terms of how they might be affected by metal levels and amendments; no effects were observed in this study but a longer establishment term might provide valuable information on colonization. It certainly can be argued that the development of the microbial community in the Flin Flon area to one that more closely approximates a site unaffected by metals could be the key to achieving revegetation in a shorter term. Lastly, ensuring that measurements of revegetation are not only visual, and also take into account shifts in the below ground microbial community as well, can ensure a more successful and thorough revegetation program.

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APPENDIX A

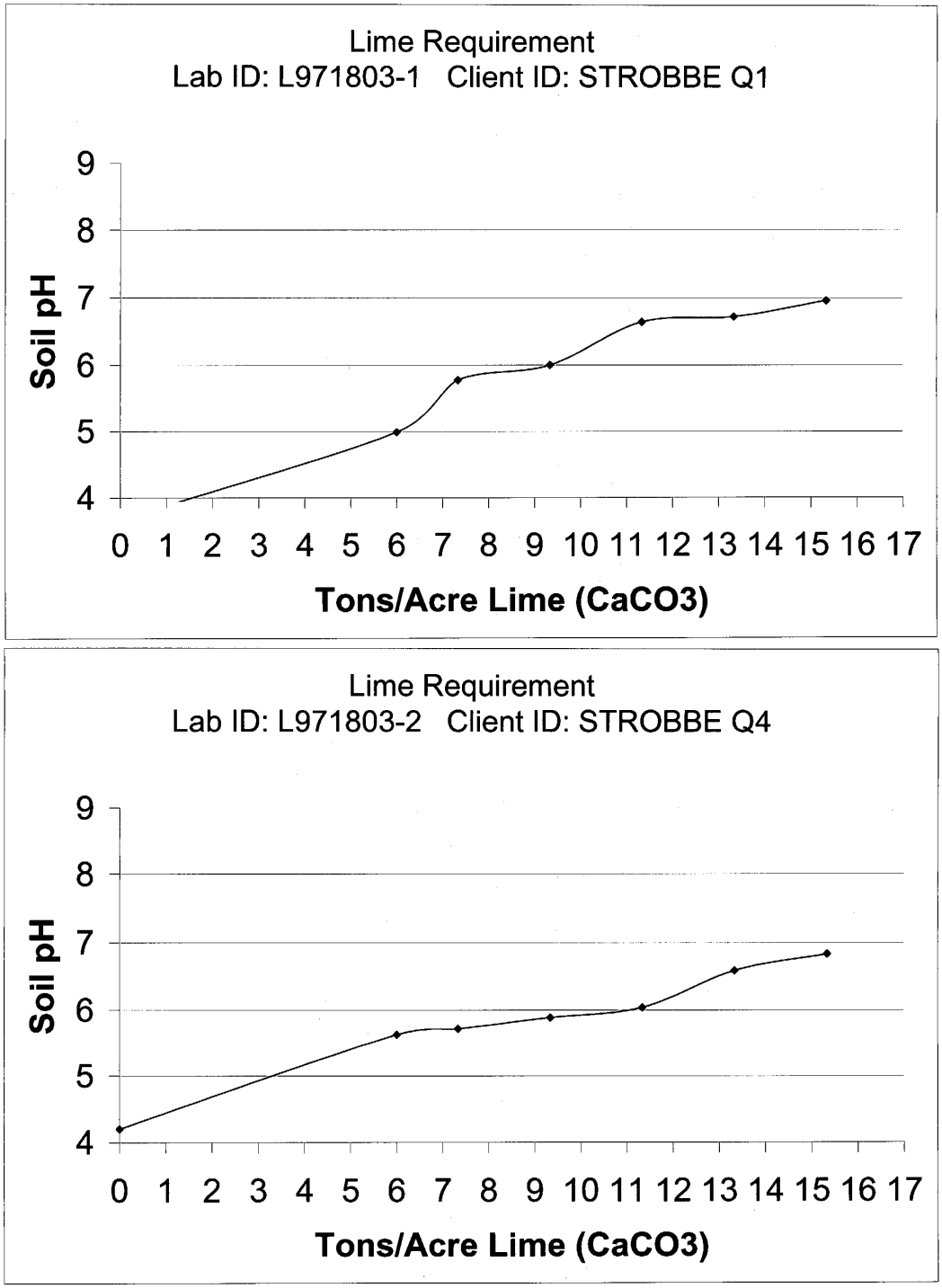

Fig. A.1 Lime response curves for soil containing low (upper) and high (lower) metal concentrations from Flin Flon, MB as calculated by ALS Laboratories, Saskatoon, SK.

APPENDIX B

Table B.1. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for American vetch grown in low metal soils.

	MBC	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Total Biomass	0.03	0.09	0.17	-0.72 ***	-0.06	-0.01	-0.11	-0.12	-0.68 ***	-0.70 ***	-0.68 ***	-0.74 ***
MBC	1.00	-0.39	-0.10	-0.01	-0.40	-0.41	-0.45 *	-0.39	0.11	0.10	0.07	0.13
F:B		1.00	-0.31	-0.08	0.26	0.24	0.18	0.26	-0.18	-0.15	-0.12	-0.06
Stress 1			1.00	-0.15	0.10	0.04	0.08	0.05	0.08	0.09	0.03	0.05
Stress 2				1.00	0.01	-0.15	-0.01	0.09	0.52 *	0.71 ***	0.61 **	0.62 **
Total PLFA					1.00	0.96 ***	0.98 ***	-0.99 ***	-0.07	-0.11	-0.03	-0.16
AMF						1.00	0.98 ***	0.95 ***	-0.15	-0.21	-0.11	-0.23
Gr+							1.00	0.97 ***	-0.02	-0.08	0.02	-0.11
Gr-								1.00	-0.05	-0.09	-0.02	-0.13
Al									1.00	0.81 ***	0.95 ***	0.84 ***
Cd										1.00	0.90 ***	0.89 ***
Cu											1.00	0.88 ***
Zn												1.00

Table B.1. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for American vetch grown in low metal soils (continued).

	Total Biomass	MBC	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Plant Al	0.32	0.20	-0.03	-0.21	-0.38	-0.09	-0.06	-0.13	-0.14	-0.50 *	-0.53 *	-0.52 *	-0.48 *
Plant Cd	-0.51 *	0.26	-0.48 *	-0.12	0.23	-0.10	-0.11	-0.03	-0.08	0.57 **	0.45 *	-0.52 *	0.45 *
Plant Cu	0.01	0.17	-0.18	-0.31	-0.18	-0.45	-0.03	-0.05	-0.05	-0.20	-0.25	-0.22	-0.18
Plant Ni	-0.07	0.17 *	-0.09	-0.27	0.01	0.14	0.14	0.20	0.12	0.04	-0.05	0.06	-0.06
Plant Zn	-0.46 *	0.10	-0.36	-0.10	0.26	-0.06	-0.07	-0.01	-0.025	0.42	0.40	0.41	0.42

Table B.2. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for American vetch grown in high metal soils.

	MBC	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Total Biomass	-0.08	0.08	0.16	-0.37	-0.09	0.04	-0.05	-0.03	-0.72 ***	-0.57 **	-0.76 ***	-0.63 **
MBC	1.00	-0.01	-0.21	-0.42	0.04	0.01	0.04	-0.04	0.18	0.32	0.16	-0.05
F:B		1.00	-0.25	-0.13	-0.38	-0.68 **	-0.56 *	-0.54 *	-0.17	-0.12	-0.17	-0.18
Stress 1			1.00	0.15	0.09	0.35	0.15	0.16	-0.34	-0.38	-0.30	-0.41
Stress 2				1.00	0.47 *	0.16	0.37	0.41	0.11	-0.06	0.18	0.25
Total PLFA					1.00	0.69 ***	0.97 ***	0.96 ***	0.10	0.08	0.10	0.10
AMF						1.00	0.84 ***	0.83 ***	-0.08	-0.02	-0.09	-0.03
Gr+							1.00	0.99 ***	0.09	0.09	0.08	0.09
Gr-								1.00	0.07	0.07	0.07	0.06
Al									1.00	0.87 ***	0.99 ***	0.51 *
Cd										1.00	0.87 ***	0.45 *
Cu											1.00	0.56 *
Zn												1.00

Table B.2. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for American vetch grown in high metal soils (continued).

	Total Biomass	MBC	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Plant Al	0.31	0.26	0.18	0.24	-0.29	0.14	0.35	0.17	0.16	-0.42	-0.30	-0.44	-0.47 *
Plant Cd	-0.44	0.17	-0.10	-0.40	-0.03	0.12	0.07	0.13	0.10	0.52 *	0.81 ***	0.54 *	0.53 *
Plant Cu	-0.48 *	0.18	-0.12	-0.39	-0.01	0.15	0.11	0.16	0.13	0.51 *	0.78 ***	0.54 *	0.53 *
Plant Ni	-0.59 **	0.03	-0.16	-0.48 *	0.17	0.09	-0.02	0.08	0.04	0.31	0.33	0.36	0.39
Plant Zn	-0.56 *	0.14	-0.14	-0.47 *	0.06	0.13	0.06	0.13	0.10	0.47 *	0.67 **	0.51 *	0.52 *

Table B.3. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for tufted hairgrass grown in low metal soil.

	MBC	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Total Biomass	-0.12	0.45	0.40	-0.29	-0.02	-0.05	-0.03	-0.02	0.05	0.01	0.05	-0.08
MBC	1.00	-0.28	-0.09	0.22	-0.06	-0.20	-0.08	-0.04	-0.08	-0.06	-0.08	-0.12
F:B		1.00	0.14	-0.40	-0.33	-0.42	-0.47 *	-0.54 *	0.13	0.07	0.16	-0.22
Stress 1			1.00	-0.65 **	0.48 *	0.26	0.58 *	0.57 *	-0.29	-0.36	-0.29	-0.23
Stress 2				1.00	-0.40	-0.09	-0.37	-0.28	-0.02	-0.01	-0.02	0.10
Total PLFA					1.00	0.39	0.94 ***	0.89 ***	-0.04	-0.06	-0.06	0.12
AMF						1.00	0.58 **	0.58 **	-0.14	-0.17	-0.14	-0.17
Gr+							1.00	0.97 ***	-0.11	-0.14	-0.13	0.01
Gr-								1.00	-0.22	-0.25	-0.23	-0.02
Al									1.00	0.96 ***	0.99 ***	0.69 ***
Cd										1.00	0.96 ***	0.74 ***
Cu											1.00	0.65 **
Zn												1.00

Table B.3. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for tufted hairgrass grown in low metal soil (continued).

	Total Biomass	MBC	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Plant Al	-0.13	0.07	-0.15	-0.31	-0.06	0.09	0.31	0.09	0.14	-0.08	-0.07	-0.07	-0.09
Plant Cd	-0.29	-0.04	-0.39	-0.32	0.04	0.23	0.43	0.24	0.25	-0.05	-0.12	-0.07	0.27
Plant Cu	-0.29	-0.04	-0.39	-0.30	0.06	0.22	0.40	0.22	0.24	-0.05	-0.11	-0.06	0.25
Plant Ni	0.45 *	-0.20	-0.41	-0.41	0.04	0.22	0.44	0.23	0.23	-0.08	-0.07	-0.09	0.18
Plant Zn	-0.30	-0.06	-0.39	-0.31	0.06	0.22	0.42	0.22	0.23	-0.05	-0.12	-0.07	0.25

Table B.4. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for tufted hairgrass grown in high metal soil.

	MBC	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Total Biomass	-0.43	-0.19	-0.18	0.18	-0.12	-0.15	-0.04	0.13	-0.31	-0.38	-0.32	-0.36
MBC	1.00	-0.16	0.19	-0.27	0.35	0.06	0.06	0.05	0.22	0.14	0.33	0.39
F:B		1.00	0.04	-0.24	-0.22	0.61 **	-0.09	-0.61 **	0.02	0.37	0.20	0.30
Stress 1			1.00	-0.39	0.10	-0.18	-0.30	0.03	-0.09	0.34	-0.11	0.18
Stress 2				1.00	0.25	-0.33	0.55 *	0.69 ***	0.02	-0.48 *	-0.11	-0.35
Total PLFA					1.00	0.50 *	0.55 *	0.23	0.17	0.19	0.13	-0.18
AMF						1.00	0.38	-0.31	0.18	0.40	0.31	0.17
Gr+							1.00	0.68 **	0.14	-0.08	0.12	-0.32
Gr-								1.00	-0.05	-0.32	-0.15	-0.37
Al									1.00	-0.04	0.28	0.16
Cd										1.00	0.61 **	0.53 *
Cu											1.00	0.61 **
Zn												1.00

Table B.4. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for tufted hairgrass grown in high metal soil (continued).

	Total Biomass	MBC	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Plant Al	0.34	0.11	-0.46	-0.02	-0.06	0.34	0.06	0.34	0.18	-0.16	-0.13	-0.02	0.04
Plant Cd	-0.26	-0.09	-0.12	-0.17	-0.33	0.53	0.39	0.54 *	0.05	-0.14	-0.08	-0.06	0.27
Plant Cu	0.25	-0.08	0.11	0.02	-0.29	0.50 *	0.24	0.45 *	-0.02	-0.20	-0.08	-0.09	-0.27
Plant Ni	-0.13	0.28	0.00	0.62 **	-0.12	0.35	0.26	-0.03	0.16	-0.16	0.30	0.05	0.20
Plant Zn	0.27	-0.09	-0.10	0.05	-0.30	0.49 *	0.21	0.42	0.17	0.22	-0.08	-0.13	-0.28

Table B.5. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for jack pine grown in low metal soils.

	MBC	MBN	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
% Change in Height	0.20	0.18	-0.13	0.067 *	0.16	0.62 **	0.40	0.61 **	0.40	0.43	-	-	0.16
MBC	1.00	0.78 ***	0.63 **	0.20	-0.60 **	0.04	-0.46 *	-0.09	-0.51 *	0.09	-	-	0.19
MBN		1.00	0.49 *	0.34	-0.54 *	0.16	-0.25	0.09	-0.27	-0.34	-	-	-0.02
F:B			1.00	0.26	-0.801 ***	0.45 *	-0.23	0.27	-0.40	-0.02	-	-	-0.03
Stress 1				1.00		-0.15	-0.10	0.10	-0.01	-0.10	-	-	-0.15
Stress 2					1.00	-0.51 *	-0.02	-0.40	0.21	0.02	-	-	-0.02
Total PLFA						1.00	0.72 ***	0.96 ***	0.62 **	-0.05	-	-	0.00
AMF							1.00	0.80 ***	0.91 ***	-0.04	-	-	-0.01
Gr+								1.00	0.76 ***	0.01	-	-	-0.04
Gr-									1.00	0.02	-	-	-0.02
Soil Al										1.00	-	-	0.40
Soil Cd											1.00	-	-
Soil Cu												1.00	-

Table B.5. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for jack pine grown in low metal soils (continued).

	% Change in Biomass	MBC	MBN	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Shoot Al	0.14	0.26	0.08	-0.09	0.39	0.21	0.21	0.12	0.32	0.36	-0.22	-	-	-0.24
Shoot Cd	-0.11	-0.22	-0.07	-0.10	-0.31	0.10	0.20	0.41	0.24	0.23	-0.21	-	-	-0.26
Shoot Cu	-0.13	-0.22	-0.04	-0.09	-0.23	-0.09	0.19	0.39	0.23	0.23	-0.28	-	-	-0.25
Shoot Ni	0.07	-0.33	-0.17	-0.23	-0.14	0.15	0.16	0.27	0.24	0.34	-0.02	-	-	-0.39
Shoot Zn	0.09	-0.15	-0.26	-0.07	0.05	0.11	-0.13	-0.13	-0.13	-0.08	0.32	-	-	0.02
Root Al	-0.05	0.04	0.08	0.17	-0.30	-0.36	0.42	0.27	0.41	0.20	0.01	-	-	0.04
Root Cd	-0.26	-0.05	-0.09	0.47 *	0.06	-0.52 *	0.38	0.20	0.27	-0.05	0.00	-	-	0.12
Root Cu	0.36	0.25	0.23	0.11	-0.08	-0.34	0.43	0.27	0.47 *	0.31	0.41	-	-	0.12
Root Ni	-0.15	0.23	0.17	0.15	-0.21	0.44	0.18	0.12	0.17	0.01	0.18	-	-	-0.06
Root Zn	0.24	-0.07	0.10	0.21	-0.21	-0.49 *	0.66 **	0.53 *	0.69 ***	0.47 *	0.00	-	-	0.27
% AMF Colon.	0.26	0.07	0.08	0.10	-0.21	-0.17	0.12	0.04	0.22	0.11	0.40	-	-	-0.07

Table B.6. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for jack pine grown in high metal soils.

	MBC	MBN	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
% Change in Height	-0.32	-0.52 *	0.61 **	-0.47	0.25	-0.10	0.08	-0.06	-0.18	0.32	-0.31	-0.30	-0.26
MBC	1.00	0.86 ***	0.15	-0.10	-0.32	0.39	0.36	0.39	0.41	-0.38	0.00	-0.05	-0.12
MBN		1.00	0.04	-0.08	-0.26	0.31	0.25	0.32	0.38	-0.32	-0.06	-0.10	-0.16
F:B			1.00	-0.35	0.15	-0.19	0.09	-0.17	-0.17	-0.05	-0.30	-0.34	-0.35
Stress 1				1.00	0.29	0.15	-0.38	-0.20	-0.15	0.18	0.05	0.06	0.11
Stress 2					1.00	-0.39	0.90 ***	-0.41	-0.39	0.44	-0.09	-0.05	0.07
Total PLFA						1.00		0.99 ***	0.96 ***	-0.13	-0.13	-0.13	-0.16
AMF							1.00	0.93 ***	0.89 ***	-0.22	-0.07	-0.09	0.12
Gr+								1.00	0.96 ***	-0.14	-0.08	-0.09	-0.11
Gr-									1.00	-0.17	-0.12	-0.12	-0.15
Soil Al										1.00	-0.35	-0.23	-0.12
Soil Cd											1.00	0.99 ***	0.97 ***
Soil Cu												1.00	0.99 ***

Table B.6. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for jack pine grown in high metal soils (continued).

	% Change in Biomass	MBC	MBN	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Shoot Al	-0.23	0.00	-0.05	-0.27	-0.09	-0.17	-0.09	0.02	-0.03	-0.07	-0.34	0.98 ***	0.97 ***	0.95 ***
Shoot Cd	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shoot Cu	-0.28	0.02	-0.04	-0.32	-0.04	-0.15	-0.07	0.00	-0.02	-0.06	-0.32	0.99 ***	0.98 ***	0.96 ***
Shoot Ni	0.35	-0.12	-0.21	0.47 *	0.06	0.35	-0.38	-0.24	-0.37	-0.39	0.38	0.09	0.12	0.17
Shoot Zn	0.05	0.14	-0.11	-0.01	0.07	0.22	-0.13	-0.10	-0.16	-0.16	-0.23	0.11	0.09	0.07
Root Al	0.04	0.29	0.48*	-0.07	-0.06	-0.10	0.12	0.08	0.15	0.16	-0.26	-0.09	-0.12	-0.15
Root Cd	0.06	0.33	0.47 *	-0.04	-0.04	-0.11	0.09	0.06	0.11	0.11	-0.32	-0.01	-0.05	-0.09
Root Cu	-0.03	0.36	0.41	-0.24	0.00	-0.21	0.27	0.18	0.27	0.26	-0.32	0.04	0.01	-0.04
Root Ni	0.29	-0.21	-0.38	0.21	-0.11	0.07	0.13	0.24	0.12	0.10	0.20	0.14	0.18	0.19
Root Zn	0.16	0.25	0.35	0.01	0.00	0.05	0.10	0.08	0.12	0.08	-0.22	-0.07	-0.10	-0.13
% AMF Colon.	0.15	-0.18	0.00	0.05	0.11	0.46 *	-0.18	-0.25	-0.22	-0.16	0.25	-0.61 **	-0.62 **	-0.57 **

Table B.7. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for trembling aspen grown in low metal soils.

	MBC	MBN	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
% Change in Height	0.14	0.04	0.45 *	-0.16	-0.01	0.39	0.34	0.36	0.31	-0.06	-	0.02	-0.02
MBC	1.00	0.73 **	0.41	-0.19	-0.29	-0.21	-0.20	-0.23	0.39	0.19	-	-0.07	0.17
MBN		1.00	0.50 *	-0.16	-0.42	-0.15	-0.19	-0.14	-0.37	0.10	-	-0.26	0.17
F:B			1.00	-0.38	-0.56 **	0.30	0.10	0.26	0.03	-0.19	-	-0.18	-0.17
Stress 1				1.00	0.69 ***	-0.48 *	-0.73 ***	-0.52 *	-0.19	0.15	-	0.08	0.17
Stress 2					1.00	-0.50 *	-0.61 **	0.58 **	-0.07	0.23	-	0.09	0.27
Total PLFA						1.00	0.76 ***	0.97 ***	0.87 ***	0.02	-	0.34	0.02
AMF							1.00	0.84 ***	0.62 **	-0.03	-	0.18	-0.05
Gr+								1.00	0.84 ***	-0.01	-	0.30	0.02
Gr-									1.00	0.20	-	0.44	0.22
Soil Al										1.00	-	0.73 ***	0.96 ***
Soil Cd											1.00	-	-
Soil Cu												1.000	0.64 **

Table B.7. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for trembling aspen grown in low metal soils (continued).

	% Change in Biomass	MBC	MBN	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Shoot Al	0.74 ***	0.14	-0.01	0.25	-0.17	-0.12	0.02	0.01	-0.05	-0.04	-0.14	-	0.03	-0.22
Shoot Cd	0.67 **	0.30	0.18	0.45 *	-0.21	-0.21	0.16	0.01	0.05	0.01	-0.13	-	0.03	-0.15
Shoot Cu	0.71 ***	0.24	0.16	0.31	-0.17	-0.06	-0.12	-0.10	-0.17	-0.10	-0.10	-	-0.03	-0.14
Shoot Ni	0.21	-0.13	-0.32	0.00	0.458 *	-0.33	0.58 **	0.58 **	0.56 *	0.44	-0.19	-	0.20	-0.20
Shoot Zn	-0.15	0.04	0.37	-0.04	0.28	0.19	-0.24	-0.41	-0.24	-0.23	0.27	-	-0.03	0.34
Root Al	-0.09	-0.06	0.21	-0.11	-0.12	-0.09	-0.12	-0.08	-0.11	-0.17	-0.06	-	-0.12	-0.03
Root Cd	0.37	0.06	0.15	-0.09	-0.13	-0.14	-0.10	-0.11	-0.16	-0.24	0.00	-	0.13	-0.07
Root Cu	-0.19	0.01	0.19	-0.23	-0.12	-0.04	-0.20	-0.05	-0.17	-0.22	-0.03	-	-0.10	-0.04
Root Ni	0.34	0.07	0.10	0.38	-0.27	-0.50 *	-0.01	0.09	-0.05	-0.34	-0.30	-	-0.40	-0.34
Root Zn	0.55	0.04	0.12	-0.01	-0.06	0.02	-0.24	-0.18	-0.28	-0.25	-0.13	-	-0.03	-0.16
% AMF Colon.	0.26	-0.06	0.25	0.23	0.32	0.08	-0.18	-0.48 *	-0.26	-0.21	0.02	-	-0.08	0.02

Table B.8. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for trembling aspen grown in high metal soils.

	MBC	MBN	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
% Change in Height	0.29	0.41	0.32	-0.02	-0.14	-0.03	-0.03	-0.03	-0.03	0.13	-0.23	0.03	-0.04
MBC	1.00	0.17	0.31	0.04	-0.22	0.30	0.29	0.30	0.29	-0.13	-0.05	0.13	0.19
MBN		1.00	-0.08	-0.04	-0.03	-0.22	-0.19	-0.19	-0.18	-0.04	-0.01	-0.11	-0.13
F:B			1.00	-0.04	-0.52 *	0.53 *	0.42	0.49 *	0.43	-0.11	-0.04	-0.08	0.02
Stress 1				1.00	-0.21	0.10	-0.36	0.06	0.05	-0.26	-0.04	0.22	0.35
Stress 2					1.00	-0.34	-0.20	-0.35	-0.20	0.12	-0.18	0.29	0.17
Total PLFA						1.00	0.86 ***	0.99 ***	0.99 ***	0.11	-0.24	0.00	0.12
AMF							1.00	0.88 ***	0.88 ***	0.30	-0.21	-0.17	-0.13
Gr+								1.00	0.986 ***	0.14	0.24	-0.04	0.07
Gr-									1.00	0.15	-0.29	0.03	-0.13
Soil Al										1.00	0.26	-0.48 *	-0.54 *
Soil Cd											1.000	0.14	0.32
Soil Cu												1.00	0.93 ***

Table B.8. Pearson product moment correlation coefficient 'r' correlations between total plant biomass, soil microbial characteristics, and soil metal concentrations for trembling aspen grown in high metal soils (continued).

	% Change in Biomass	MBC	MBN	F:B	Stress 1	Stress 2	Total PLFA	AMF	Gr+	Gr-	Soil Al	Soil Cd	Soil Cu	Soil Zn
Shoot Al	-0.24	0.19	-0.11	0.09	-0.08	0.04	0.21	0.19	0.21	0.22	-0.12	-0.11	-0.07	0.06
Shoot Cd	-0.28	-0.45 *	-0.10	-0.15	0.37	-0.15	0.17	0.08	0.17	0.16	0.11	0.03	-0.09	-0.08
Shoot Cu	-0.28	0.24	-0.09	0.09	0.00	0.16	-0.37	0.36	0.37	0.41	0.10	-0.08	0.01	0.15
Shoot Ni	-0.14	0.02	-0.01	-0.19	0.00	0.20	-0.04	0.04	-0.04	0.01	0.40	0.00	0.05	-0.02
Shoot Zn	0.19	-0.09	0.27	0.04	-0.31	-0.20	0.19	0.08	0.19	0.19	0.22	0.08	0.04	0.02
Root Al	0.29	0.11	0.02	0.20	0.65 ***	-0.49 *	0.33	-0.10	0.31	0.25	-0.07	0.00	0.05	0.19
Root Cd	0.24	-0.07	0.05	0.08	0.74 ***	-0.41	0.09	-0.33	0.07	0.02	-0.15	0.15	0.13	0.25
Root Cu	0.27	0.01	0.04	0.12	0.75 ***	-0.45 *	0.17	-0.27	0.14	0.09	-0.12	0.06	0.09	0.22
Root Ni	0.19	0.05	0.15	-0.02	0.25	0.09	-0.03	-0.02	-0.01	-0.01	0.12	0.27	-0.10	0.02
Root Zn	0.28	-0.02	0.01	0.06	0.75 ***	-0.32	0.09	-0.35	0.06	0.03	-0.12	0.09	0.14	0.27
% AMF Colon.	0.38	-0.02	-0.01	0.33	0.10	-0.14	0.23	0.02	0.21	0.20	-0.12	0.05	0.31	0.34

APPENDIX C

Objective

The area of land that the metal smelter in Flin Flon, MB has affected is likely to be recolonized by tree species by natural reseeding – through wind and water deposition. This experiment was intended to determine the germination rates and potential for seed growth in limed, amended soil. These plant species have been shown to be metal tolerant, and both jack pine and aspen are native to the Flin Flon area (Wotton et al., 1986; Cripps et al., 2003).

Materials and Methods

Jack pine and trembling aspen seeds used for germination tests were acquired from Pacific Regeneration Technologies, Prince Albert, SK, Canada, and Western Native Seed, Coaldale, Colorado, respectively. Eight treatments were studied – three controls (filter paper, non-affected forest soil, non-limed affected control (low and high metal), and a limed affected control (low and high metal). The four treatments from experiment two were also tested (BC, BC+MuC, BC+GC, and BC+AMF). Treatments were mixed together in a bag and placed on 8.5cm petri dishes in replicates of four. Ten seeds of each species were placed on the soil surface and kept moist for eight weeks (Kemball et al., 2010).

Results

After 8 weeks, all dishes planted with jack pine had germinated seed (Table C.1), while none of the trembling aspen seeds germinated. There were no significant differences among amendment treatments for the jack pine results.

Table C.1. Germination of jack pine after 8 weeks

Treatment	% Germinated
Control – filter paper	90 ± 12
Control – Unaffected Forest Soil	80 ± 16
Control – Affected Low Metal	85 ± 17
Control – Affected High Metal	85 ± 10
Control – Affected Low Metal (Limed)	93 ± 10
Control – Affected High Metal (Limed)	95 ± 10
BMB – Low Metal	80 ± 16
BMB – High Metal	80 ± 22
BMB+MuC – Low Metal	88 ± 15
BMB+MuC – High Metal	80 ± 8
BMB+GC – Low Metal	70 ± 10
BMB+GC – High Metal	83 ± 21
BMB + AM – Low Metal	80 ± 16
BMB + AM – High Metal	78 ± 22

Discussion

It is likely that the trembling aspen seeds were not viable, and this is the reason they did not germinate. Jack pine seeds appeared to be unaffected by soil type (i.e., metal concentration) and amendment treatments did not show significant differences. Although germination rates for these jack pine seeds were high, germinated seeds often die before full establishment in the soil. A field scale trial is suggested with a broad dispersal of both jack pine and trembling aspen seeds to determine possible treatment effects and establishment rates of these tree species in metal affected soils.