The application of biochar as a soil amendment in land reclamation

by

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Abstract

Surface mining activities cause severe adverse effects on soils. Scientists across the world have used different physical, chemical and biological reclamation techniques to recover mining disturbed areas. The effectiveness and efficiency of reclamation techniques is crucial to reclamation success. Biochars are biological residues combusted under low oxygen conditions, resulting in a porous, low-density carbon rich material. Research has suggested that biochar can be used as an amendment to improve soil physical, chemical, and biological quality. The present study investigated the application of biochar as a soil amendment for land reclamation. Specifically, the impact of biochar application on aspen growth, microbial biomass, soil respiration, heavy metal adsorption, and metabolic quotient were measured in a greenhouse experiment using land reclamation soils and in a field experiment on a reclaimed coal mine west of Edmonton, AB, Canada. Results of the greenhouse experiment showed that the biochar had the ability to retain the soil nutrients, increase the soil microbial biomass and soil heterotrophic respiration; while the petroleum- coke had a negative impact on tree growth. In the field experiment, the results showed that biochar increased DOC, DON (dissolved organic carbon and nitrogen), MBC and MBN (microbial biomass carbon and nitrogen) and soil heterotrophic respiration. The results are consistent with previous findings, which suggested that biochar can improve soil available nutrient and increase microbial activity.

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Chapter 1: General introduction

1.1 Disturbances

A disturbance is an incident that occurs at various temporal and spatial scales, which causes pronounced change in ecosystem properties (Pickett et al., 1985). According to the initiation, disturbances can be divided into two categories: anthropogenic disturbance- such as mining activities, forest harvesting or grazing; and natural disturbances- such as fire, wind, flood and volcano eruptions, insects and disease (Hastings 1980; Dale et al., 2001).

In Canada, fire, insects and diseases are major natural disturbances in forest ecosystems, while timber harvesting and mining activities are the two main anthropogenic disturbances (Natural Resources Canada, 2014). Disturbances alter the natural components in an ecosystem. For example, fire and deforestation remove aboveground vegetation and reduce moisture uptake by plants, which will lead to increased soil moisture content available to sprouting plants and seedlings; these will further influence soil properties and microbial communities (Certini, 2005; DeBano et al.,1998; Verma and Jayakumar, 2012).

Many anthropogenic disturbances, such as surface mining, severely influence natural ecosystems, altering the development, structure and function of forest plants and their soils (Attiwill, 1994). Surface mining removes all vegetation and surface soil, including any organic carbon in it. Moreover, there is usually substantial waste rock/overburden and tailings deposited at the soil surface after mining, which may cause serious environmental problems such as heavy metal contamination (Bradshaw, 2000).

1.1.1 Surface mining as an anthropogenic disturbance

Surface mining operations exert tremendous adverse impacts on the environment, which disrupt natural ecosystems and causes severe ecological damage to mining areas across the world (Dudka et al., 1997; Rathore et al., 1993; Visser et al., 1979). These mining activities remove all vegetation, forest floor material and surface soils, thus change the nutrient and biological conditions of soils. Reclaimed landscapes are typically constructed with lower soil horizon (often C horizon) substrates placed at the soil surface (Bradshaw, 2000). However, these lower soil horizons are poor in nutrients and have no seeds or vegetation propagules to facilitate vegetation establishment.

Soil surface mining can change the natural landscape and topographic profile, and also changes the hydrological conditions in the mining site. It alters the runoff pattern of the surface and ground water, potentially causing drought or flood, which could further aggravate soil erosion and degradation (Hortenstine et al., 1972). Plants cannot survive on the soil, so the soil loses its original ecological value and the reclamation can be extraordinarily difficult (Bradshaw, 2000).

Moreover, heavy metal contamination could be another problem and it has been documented in coal mine soils around the world (Massey et al., 1972; McLaren et al., 1973). Heavy metals, a loosely defined term, may include the transition metals, some metalloids, lanthanides, and actinides (Gaunt and Lehmann, 2002). A great deal of research has been conducted on mining derived toxicity of heavy metals on plant growth. Elements such as Pb, Zn, Cd, Cu, Mn, Fe, Rb, As, Ti, Cr, Hg and Sr are major heavy metals (or metals which at some level of concentration are toxic to plants) that were

found in reclamation substrate of coal mines (Massey et al., 1972; McLaren et al., 1973; Adamo et al., 1996). The above problems are common ones generated after mining.

1.1.2 Land reclamation after mining disturbance

Land reclamation legislature

In Alberta, the mining areas are required to be reclaimed by the mining companies under governmental policy and regulations- the Environmental Protection and Enhancement Act (Government of Alberta, 2014). Regional guidelines including the Land Capability Classification System for Forest Ecosystems (LCCS) and Guidelines for Reclamation to Forest Vegetation in the Athabasca Oil Sands Region (GRFVOSR) are used in conjunction with the governmental rules (CEMA, 2007). According to these regulations, land capability of the mining site should be reclaimed to its pre- disturbance level. There is no consensus on criteria or indicators of reclamation success across the world, but several criteria are commonly accepted: (1) self- sustainable; (2) ecosystem function rebuilt; (3) recovery of biodiversity; (4) recovery of soil properties; (5) resistance to invasion of non- native/undesirable species (Ewel, 1987; Aronson et al., 1993; Hobbs and Norton, 1996). The ultimate goal of land reclamation is to reestablish stable soils along with plant and microbial abundance and activity, in order to recover the whole ecosystem which has been disturbed by mining activities. In order to achieve these, various techniques can be used in the reclamation processes.

Land reclamation techniques

Reclamation techniques can be categorized into physical, chemical and biological approaches. Physical reclamation is designed to improve the soil physical condition (Tejada et al., 2006). The organic layer on the surface generally holds more moisture than the mineral soil. Thus, being deprived of a surface organic layer, the mineral substrate on the mining site is directly exposed to solar radiation, causing loss of soil moisture and soil crusting (Toy et al., 2002). Adding surface coverage to the soil can provide physical protection for the soil. Mulches, crop straws, wood wastes, and forest floor material are commonly used physical amendments after mining activities (Tejada et al., 2006). They are generally incorporated into the disturbed soil to improve the soil physical properties. These physical amendments could isolate the soil from the severe environment, prevent soil from erosion, increase the water holding capacity (the amount of water that a given soil can hold), decrease the soil bulk density and improve its physical structure (Bradshaw, 2000).

Chemical reclamation is also an effective way to improve soil nutrient conditions after mining (Pichtel et al., 1992). After surface mining, most soils lack nutrients since the entire surface horizons are gone and only the lower horizons are left. The first step before reestablishing the plant communities is to recover the nutrient condition of the soil. Fertilizer, compost and sewage sludge could all be used as soil chemical amendments to provide nutrients to soil (Hortenstine et al., 1972; Pichtel et al., 1992; Seaker et al., 1998). Some of them can also be good chelating agents to mitigate the toxicity of heavy metals and other contaminants (Nowack, 2008).

As to biological remediation, revegetation and introduction of local propagules are two major ways to reclaim the soil (Bradshaw, 2000). Nowadays, inoculation of soil

microorganisms is a new, but not common, way to improve soil microbial properties, which is attracting increasing attention (Sylvia et al., 1990; Herrera et al., 1993).

The selection of reclamation method is crucial to reclamation success, while technical and economic feasibility should also be taken into consideration. Currently, an increasingly common path to reclaim mining sites in Alberta is to salvage highly fertile forest floor materials from undisturbed forests nearby to reclaim the disturbed soil (Fung and Macyk, 2000). Compared to parent geological material in the mining site, these organic substrates are high in nutrient content and low in bulk density, and may also contain propagules of local species (MacKenzie and Naeth, 2007& 2009). When applied to the mining areas, these materials may improve the soil physical, chemical and biological properties (McMillan, et al., 2007; MacKenzie and Naeth, 2007& 2009). For example, in the Athabasca oil sands region, the peat mineral soil and the upland forest floor mineral soil are two types of material being used as soil amendments to reclaim the mining disturbed soil (McMillan, et al., 2007). However, this method is expensive and also limited by the availability of the surface soil material (Bradshaw, 2000).

1.1.3 Fire as a natural disturbance in forest ecosystems

Fire is an important natural disturbance in many forest and prairie ecosystems which alters their physical, chemical and biological properties and processes (Davies et al., 2010; Certini, 2005; Verma and Jayakumar, 2012). Impacts of fire on forests are often difficult to predict because of the heterogeneous community structure, different soil types, variability in weather conditions and the complexity of fire regimes (DeBano et al., 1998; DeLuca et al., 2002; García-Corona et al., 2004). Fire effects on soil, plant and animal species can be variable (Gill and Groves, 1981; Komarek et al., 1969; Lyon et al.,

1978). For example, fire can raise the pH of some acidic soils (Certini, 2005), but can also lead to soil erosion and formation of water repellent layers (Imeson et al., 1992). It can accelerate nutrient cycling in soils (DeBano et al., 1998), which may also lead to leaching losses (Grier et al., 1975).

Impacts of fire on soil physical properties

Forest fire generally causes the loss of organic matter at the surface of soils and to some degree in upper soil mineral layers (Nave et al., 2011). Pyrolysis of soil organic matter (SOM) begins when fire temperature reaches the range of 200 - 250°C and complete consumption begins at approximately 460°C (Giovannini et al., 1988, Garcia-Corona et al., 2004). Combustion of SOM is generally restricted to surface soil layers due to low oxygen concentration and high moisture content in deep soil horizons (Neary et al., 1999). However, ground fires can smolder in deep, moist organic layers, and can also penetrate deep in the soil profile by burning roots (Kirsten, 2010). A direct change to soil physical properties is the formation of a water repellent layer under the soil surface (Imeson et al., 1992) which can reduce the soil hydraulic conductivity substantially (Robichaud et al., 2000). Meanwhile, the combustion of vegetation and litter layer will deplete the soil organic horizons, and makes the soil prone to erosion, which is another detrimental consequence of forest fire (Sevink et al. 1989). But the loss in soil organic horizons can remove the top cover above seeds and facilitate the regeneration of some plants (Gill and Groves, 1981).

Impacts of fire on soil chemical properties

Fire is one of the major disturbances affecting chemical changes in forest soils. Fire volatilizes and pyrolyzes C and N in soil organic matter (SOM) of the forest floor, where 55-92% of SOM losses occur at temperatures of 380- 460°C (Giovannini et al., 1988; García- Corona et al., 2004); this changes nutrient cycling in soils. The major product after natural fires is black carbon (Certini, 2005). Forest fire leads to a substantial decline in total soil nutrient content due to volatilization, but increases the soil available forms of nutrients (Kutiel and Naveh, 1987b). The nutrients in biomass may be lost during volatilization, deposited as ash, or remain in incompletely burned biomass (Boerner, 1982). Wildfire typically consumes the forest floor, which causes losses of total Nitrogen (N) and Phosphorus (P) from the forest floor through volatilization, but the heat-induced degeneration of soil organic N and P increases mineralization of N and P (Johnson et al., 2004; Neary et al., 1999; Raison et al., 1985; White et al., 1973). Available forms of potassium (K), calcium (Ca), and magnesium (Mg) may also increase after fire (Christensen, 1976; Raison, 1979).

Forest fires can increase soil pH, due to the denaturation of organic acids in soils, because oxidation results in some base forming elements such as Ca, Mg, and K in organic soils becoming hydroxides and carbonates after fire (Kutiel and Naveh, 1987a). Simard et al. (2001) showed that the increase of soil pH could last for 14 years after fire. The amount of hydroxides and carbonates generated after fire depends on the fire intensity and the amount of SOM consumed (Certini, 2005).

Impacts of fire on soil biological properties

Soil microbes are vulnerable to forest fire since they are living organisms which have relatively low upper lethal temperature limits (DeBano et al., 1998). A number of

studies showed that fire has a direct impact on soil microorganisms through a reduction in microbial biomass (Acea et al., 1996; Prieto-Fernández et al., 1993). The meso- and microfauna with limited mobility in the surface layer of forest soils tend to be killed during a severe fire when the temperature reaches nearly 250°C at 10 cm depth (Roberts et al., 1965).

The abundance of microorganisms will decrease immediately after fire (Prieto-Fernández et al., 1998), but some studies suggest that microbial communities can recover after several years or decades (Xiang et al. 2014; Fritze et al., 1993). Fritze (1993) conducted a long term study in a coniferous forest which found that the microbial community recovered to pre-burn levels 12 years after fire. A Mediterranean forest study also showed that bacteria and fungi were able to recolonize burned areas immediately after fire, and therefore the impacts of fire on microorganisms tend to be less persistent than for other components of the forest ecosystem (Gema et al., 2011).

The major product of natural fire is charcoal. Biochar is a surrogate for charcoal from wildfire, which now can be produced artificially and be used as a soil amendment (Sohi et al, 2010). It can benefit the soil from different perspectives through improving soil physical, chemical and biological properties.

1.2 Biochar

Biochar is a solid material produced by thermal degradation of organic materials in an oxygen-limited environment, which is contained within the black carbon (BC) continuum (Fig.1.1). Depending on the temperatures reached during combustion and the species identity of the source material, the product after pyrolysis may vary. BC includes the entire spectrum of pyrolyzed carbon materials, ranging from char, charcoal and

biochar, to soot and graphite (Lehmann et al. 2007). Previous studies showed that biochar is resistant to decomposition and can be used as a soil amendment (Kishimoto and Sugiura, 1985; Lehmann et al., 2009). Many different devices have been invented to produce biochar, from pyrolysis kilns, ovens, and stoves used in the household and rural industries, to rotary kilns, and rotary hearth furnaces operated in large- scale industries throughout the world (Joseph and Taylor, 2014).

1.2.1 Properties of biochar

Pyrolysis conditon of biochar

There are a variety of biochars because the material, pyrolysis time and temperature are different (Sohi et al, 2010). The source substrate of biochar can be wood, nut shell, crop straw, sludge or even animal waste. It has shown promise for various applications, including use in soil amelioration, environmental pollution remediation, and sewage treatment. Because of differences in substrate materials, processing techniques, and pyrolysis conditions, biochars have different pH, ash content, water holding capacity, pore structure and specific surface area; this results in further variation in their environmental effects and suitability for different applications (Huang et al., 2006). From the perspective of heating rate and reaction time, pyrolysis methods of biochar could be divided into two categories: slow pyrolysis and fast pyrolysis.

Fast pyrolysis (Sohi, 2010): The bio-material is ground into fine particles and put into fast pyrolysis equipment, and pyrolyzed instantaneously under strict control of pyrolysis rate (10- 200°C/ sec). Through the process, the bio- material will be pyrolyzed into biochar, bio-gas and bio- oil. The aim of fast pyrolysis is to acquire more bio- oil;

thus the production of biochar only accounts for 15- 25% of the bio- material (O'Laughlin et al., 2009; Mohan et al., 2006).

Slow pyrolysis (Sohi et al., 2010): Slow pyrolysis can produce more biochar (accounts for 30%- 35% of the original bio- material) than fast pyrolysis. Slow pyrolysis can be classified into three types according to pyrolysis temperature: (1) Low temperature pyrolysis: 500- 580°C; (2) Medium temperature pyrolysis: 660- 750°C; (3) High temperature pyrolysis: 900- 1100°C. Generally, the lower the pyrolysis temperature is, the higher the yield of biochar from a given amount of the original bio- material will make (Demirbas, 2004).

Elemental content of biochar

The major content of biochar is C, but it also contains small quantities of N, P, K, Ca, Na, Mg, A1 and Cu, etc. (Huang et al., 2006; Yuan et al., 2011). During the process of production, most H, O, N, S will be lost under high temperature pyrolysis, and the major part left in biochar is aromatic C (Huang et al., 2006). The elemental content of biochar, however, also depends on the source material, pyrolysis substrates, time and method (Chan and Xu 2009).

Normally, biochar derived from biosolids is higher in N, P, K, Ca, Na, Mg, Al and Cu than plant material derived biochar. Yuan et al. (2011) found that four leguminous straw derived biochars had more nutrients (Ca, Mg and K) than five kinds of non-leguminous plant derived biochar. On the other hand, the pyrolysis temperature also affects the elemental content in biochar with higher pyrolysis temperature causing lower total N in biochar (Yuan et al., 2011).

Functional groups and pH of biochar

Normally, the surface of biochar is rich in functional groups, including carboxyl, lactone base, phenolic hydroxy, hydroxyl and carbonyl group (Sohi et al., 2010; Yuan et al., 2011). The chemical properties of a biochar greatly depend on type and number of these functional groups. The polarity of these functional groups also affects the affinity for water of biochar (Yuan et al., 2011). Most types of biochar are basic, because they contain basic salts and alkaline metal elements such as K, Ca, Na and Mg, calcite and organic anions; e.g., -COO-, -O- (Yuan et al., 2011; Glaser et al., 2003). The radicals of -COO- and -O- can adsorb H^+, which will cause a higher pH of the biochar than its derived material. This is also a major reason why biochar tends to have a negatively charged surface (Yuan et al., 2011). The pH of a biochar is influenced by pyrolysis temperature. Generally, as the temperature increases, the number of acidic radicals decreases, and the number of alkaline radicals increases correspondingly (Sohi et al., 2010). But the pH will not change until the pyrolysis temperature reaches a threshold value, and this value differs in different studies. The alkaline radicals in biochar increased as the temperature increased, but there was no increase of alkali radicals above temperatures of 800°C (Sohi et al., 2010). Singh et al. (2010) found that the acidic radicals decreased drastically when the pyrolysis temperature increased from 400°C to 550°C. However, some studies suggested that below the temperature of 500°C, the number of acidic radicals in biochar increases as the temperature increases, but decreases when the temperature increases above 600°C (Hao et al., 2010). Thus, the increase or decrease in the quantity of acidic and alkali radicals probably depends on a threshold temperature, but this temperature varies according to the organic substrate used to make

biochar (Sohi et al., 2010). Furthermore, the pH of biochar is greatly influenced by characteristics of the raw materials used to produce biochar. Generally, the pH of biochar made from livestock faeces, sludge, tree leaves is higher than for biochar produced from woody materials (Sohi et al., 2010).

Cation exchange capacity (CEC) of biochar

Cation exchange capacity (CEC) is the maximum amount of cations a soil can hold at a given pH value. It reflects the nutrient retention ability of a soil (Donahue, 1971). The quantity of acid functional groups in biochar directly influences its CEC, because these functional groups provide the positions for anions (Cheng et al., 2006; Cheng et al., 2008). Biochar normally has higher CEC when made under a low pyrolysis temperature (<400°C) than high pyrolysis temperature (>500°C). The reason, as mentioned above, could be that the quantity of acidic functional groups often decreases as pyrolysis temperature increases (Gaskin et al., 2008; Singh et al., 2010). Cheng et al. (2006) found that when biochar was oxidized under the natural condition gradually, the oxygen-containing functional groups increased, which caused a decline in the positive charges and an increase in negative charges in biochar. Consequently, the CEC increased gradually. CEC of biochar can also be influenced by its ash content. As the pyrolysis temperature increases, the ash content in biochar will also increase. Since the ash contains large amounts of alkaline metals (such as K, Na) and dissoluble salts, it will substantially influence the CEC (Fuertes et al., 2010). Singh et al. (2010) compared the biochar free of dissolvable salt with the original biochar, and the results indicated that CEC of the biochar without dissolvable salt decreased as the pyrolysis temperature increased, while CEC of the biochar with dissolvable salt increased as the pyrolysis

temperature increased. Some studies also showed that non- polar bonding was important when the biochar was produced under high temperatures of 1000°C. At this temperature, carbons become hydrophobic and do not adsorb large amounts of polar substances (Yam et al.,1990).

1.2.2 Impacts of biochar on soil

Impacts of biochar on soil physical and chemical properties

Biochar has many potential benefits when applied to soil, depending on its properties such as: pH, ash content, bulk density, volume of pores (Okimori et al., 2003). Since the physical and chemical properties of biochar could be very different when derived from different materials, they could have distinct influences when applied to soils. Different studies indicated that different biochars or pyrolysis temperatures may lead to different pH and CEC of biochar (Cheng et al., 2008; Sohi et al., 2010; Mukherjee et al., 2011). Yuan et al. (2011) compared the liming effect of four crop straw- derived biochars (canola, rice, soybean, and pea straws). The pH of the four types of crop straws ranged between 6.27 and 6.81. But after a low temperature (350°C) and oxygen-limited pyrolysis, their pH increased to between 7.69- 10.26. The biochars increased soil pH by 0.27- 0.5 units at the application rate of 1%, and by 0.47- 1.20 units at 2% application rate (w/w). It was also found that legume straw-derived biochar had a greater liming effect (increasing the pH) on acidic soil than did non-legume biochars. Mukherjee (2011) studied the influence of addition of three different biochar type- Laurel oak (*Quercus lobata*), Loblolly pine (*Pinus taeda*) and Gamma grass (*Tripsacum floridanum*) - on soil. He found that pyrolysis temperature influenced pH of all three types of biochars – resulting in pH values of 3.7±0.7, 6.6±1.4, and 8.6±1.7 at pyrolysis temperatures of 250,

400 and 650°C, respectively. Addition of these biochars to the soil either increased or decreased the soil pH, depending on the pH of soil that need to be amended. Generally, biochars processed under higher temperatures will be better used as a soil amendment for acid soils, while biochar made at lower temperatures would be better for alkaline soils and could also increase the soil cation exchange capacity (CEC) (Mukherjee et al., 2011).

The application of biochar to soil may change soil physical properties such as soil water retention, total pore space, decrease the soil density, and in turn influence plant growth (Downie et al., 2009). Studies have found that more water and nutrients were retained in soil as biochar content increased (Fellet et al., 2011). Biochar has a lower density compared to some mineral soils, and pores in biochar can retain air and water (Downie, et al., 2009), so it can improve the soil physical properties. A study found that biochar prevented nutrient leaching from certain kinds of soil (Steiner et al., 2007). Biochar can also affect the soil chemical property by altering the soil pH when added to soil. Some biochar has a high pH, which may have a liming effect on highly weathered and acidic soils (Luke et al., 2011).

Impacts of biochar on soil biological properties

Biochar, as a soil amendment, can also influence soil microbial communities. Parkhurst et al. (1967) found a positive influence of biochar on microbial growth which stimulated people to look deeper into the mechanism underlying this effect. Their results showed that biochar could provide habitat to microbes and protect them against severe environmental conditions (Figure 1.2). Bacteria could be adsorbed to the surfaces of biochar, making them less vulnerable to be leached away in soils (Pietikäinen et al., 2000) and perhaps also reducing predation since bacteria and fungi are better protected

against grazers by hiding in pores of the biochar (Figure 1.2) (Ezawa et al., 2002; Thies and Rillig, 2009). Biochar may positively affect abundance and/or activity of mycorrhizal fungi (both arbuscular mycorrhizal and ectomycorrhizal fungi) (Makoto et al., 2010; Solaiman et al., 2010). But this finding needs more verification since some other studies had opposite results which showed that biochar had no effect or a negative effect on arbuscular mycorrhizal fungal (AMF) abundance (Warnock et al., 2007; 2010). In a 3 year study, Jones (2012) reported that biochar increased soil respiration, bacterial and fungal growth rate in a cropland. The properties of biochar, such as specific surface area, pore volume, pore size, and functional groups of biochar may all influence the adsorption and immobilization of microbes on it. These properties can be categorized in the following paragraphs.

The surface area, pore volume and pore size of biochar can all influence its absorption capability. Generally, the ability of biochar to adsorb soil organics and heavy metals will increase as the surface area and pore volume increases. Messing et al. (1979) found that the micropore distribution on the biochar surface was a key factor to the adsorption ability, and pore size as large as 1-5 times of the microbal size had the best adsorption and accumulation ability. The adsorption of microbes onto biochar mainly depends on two forces: hydrophobic attraction or electrostatic forces. The adsorption of *E. coli* increased with increasing hydrophobicity of biochar (Rivera-Utrilla et al., 2001). The adsorption of microbes onto biochar happened on the first 1- 2d after the application of biochar and was followed by reproduction and increasing metabolism of microbes and the formation of a biological film. For microbes which are larger than the pore size of biochar, the large surface area can still provide vast habitat for them, for this reason the

adsorption ability of biochar increases as the surface area and pore volume increase (Rivera-Utrilla et al., 2001).

Oxygen-containing functional groups are also an important characteristic of biochar. The type and number of various functional groups on the biochar surface can influence its adsorption ability. The microbial adsorption and biochar type can be affected by many factors. Rivera-Utrilla et al. (2001) used the activated carbon as a carrier and conducted an experiment on *E. coli* adsorption. He found that the more acidic oxygen functional groups a biochar had, the higher hydrophilicity and adsorption ability a biochar had. Liu et al. (2002) used activated carbon fiber as a carrier to conduct an experiment. The result showed that adsorption ability of the activated carbon fiber was crucial to the early stage of immobilization of soil microbes, and the oxygen-containing functional groups on the surface of carbon fiber were important to its adsorption ability. Thus, a proper amount of oxygen-containing functional groups was important to keep the activated carbon's moisture and electronegativity (Liu et al., 2002).

Impacts of biochar on plant growth

Many studies have investigated how biochar influences crop growth and yield. In a 3- year study, Jones et al. (2012) found that biochar increased grass yield and significantly increased foliar N, but had no effect on the growth of maize. However, they found that biochar had little effect on the amount of dissolved organic C (DOC) and N (DON) in agricultural soil. Vaccari et al. (2011) found that application of biochar in an agricultural field increased wheat biomass up to 30%, and high levels of biochar application had no negative impact on crop yield in this 2- year study. This enhancement in crop yield can be attributed to the fact that biochar ameliorated soil acidity and

promoted soil fertility by increasing the concentration of Ca^{2+}, Mg^{2+}, and K^{+} oxides. Chan and Xu (2009) found that biochar addition can increase crop yield by improving utilization of fertilizer efficiency. But until now, there has been limited research on how biochar influences tree growth. A field experiment conducted in Japan showed biochar stimulated Sugi tree growth by 144% - 224% (Kishimoto and Sugiura, 1985).

Impacts of biochar on heavy metal absorption/adsorption

Evidence has shown that biochar can decrease the bioavailability or phytotoxicity of heavy metals or other contaminants in mining polluted soil (Beesley et al., 2010; Park et al., 2011). During the past decades, research has been conducted on the capability of biochar to adsorb heavy metals or organic pollutants in waste water. Fellet et al. (2011) found that biochar influenced bioavailability and leachability of some heavy metal pollutants. Many studies conducted in China found biochar's ability to absorb heavy metals. In a biochar study conducted in 2007, Chen found that with an increased application rate of the bamboo biochar and a decreased biochar particle size (using smaller biochar particles), Cu^{2+} adsorption rate increases. Bamboo biochar is also an ideal adsorption material of zinc, which can effectively remove the Zn^{2+} in waste water. Chen et al. (2006) studied the Pb^{2+} adsorption capacity of biochar, and found that the adsorption effect was influenced by pH, adsorption time and temperature. At the condition of pH 4.0, adsorption time of 30 min and water temperature of 25℃, the adsorption rate of Pb^{2+} by super- fine bamboo biochar was 99.8%. In addition, bamboo biochar also had strong adsorption or removal effect of dichlorophenol and fluorine in water solution (Xu et al., 2002; Zhang et al., 2005).

Impacts of biochar on chemical fertilizer retention

Previous studies (Steiner et al., 2007; Fellet et al., 2011) suggested that more nutrients could be retained in soil with biochar addition. This characteristic of biochar is especially important when applied to land reclamation soils which lack top soils and have low nutrient content. Plants require large amounts of nutrients during growth. But only after the decomposition of litter and coarse woody debris begins will soils be able to supply nutrients to plants, if there is no exterior nutrient amendment being applied (Wilckea et al., 2005). Thus, the nutrients in land reclamation soils are limited in the early stage of revegetation processes and application of fertilizer is an important path to provide extra nutrients for revegetation. The application of biochar together with fertilizer could be an optimal choice to maximize the use of fertilizer because of the special characteristic of biochar - high porosity and surface to volume ratio (Chan et al. 2007). Other studies also suggested that more fertilizer-provided nutrients was retained in soil when the fertilizer was applied with biochar together (Chan et al., 2007; Steiner et al., 2008).

1.3 Summary

Surface mining, as an anthropogenic disturbance, has caused enormous ecological damage to ecosystems across the world during the past centuries (Johnson and Miyanishi, 2008; Mohammad et al., 2010). Because the land surface has to be removed before the mining activities, the surface soils and vegetation are severely disturbed. The post-mining land surface normally often consists of geological parent materials or subsoil poor in nutrients (Bradshaw, 2000). Thus, land reclamation is needed to recover the whole

ecosystem after these disturbances. Charcoal, a product of natural fire disturbance, has drawn increasing attention from scientists around the world due to its special function in soil property improvement and plant growth. Many studies have shown that biochar can improve soil nutrient availability, increase microbial activity, improve soil nutrient retention and adsorb/immobilize heavy metals (Lehmann and Joseph, 2009; Sohi et al., 2010). So this chapter evaluated the basic properties of biochar and its influences on the above functions. But most previous studies focused on the application of biochar in agricultural lands or waste water treatment, so its usage in land reclamation and forest ecosystem reconstruction needs more study. Therefore, we wanted to examine the effects of biochar on improving soil nutrient availability, increasing microbial activity, improving soil nutrient retention and adsorbing/ immobilizing heavy metals.

1.4 Objectives

The overall objective of the research presented in this thesis was to investigate the effectiveness of black carbon (BC) addition on land reclamation soil disturbed by coal mining. It also includes the effect of fertilizer application and the combined effects of biochar and fertilization on land reclamation.

In Chapter 2, I examine the impact of BC additions on five land reclamation soils in a greenhouse study, including the effect of BC addition on aspen growth, soil nutrient availability, microbial biomass, and soil respiration in land reclamation soils.

In Chapter 3, I present the results of a field study, which included the influences of biochar and fertilizer (including two application rates) addition and their combined

effects on soil nutrient availability, adsorption of heavy metals, microbial biomass and soil respiration.

In Chapter 4, the conclusion chapter, I summarize major findings of the thesis research, and provide more information on biochar regarding its properties and its potential for greenhouse gas emission control. This gives direction for future research into the use of biochar in land reclamation after surface mining.

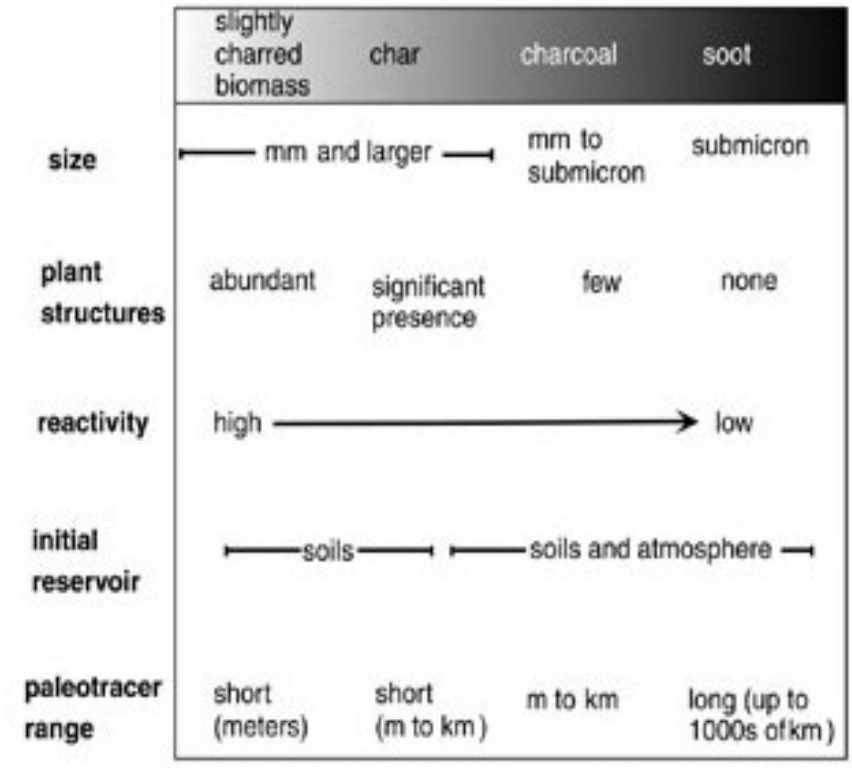


Figure 1.1 Black carbon combustion continuum (Masiello, 2004).

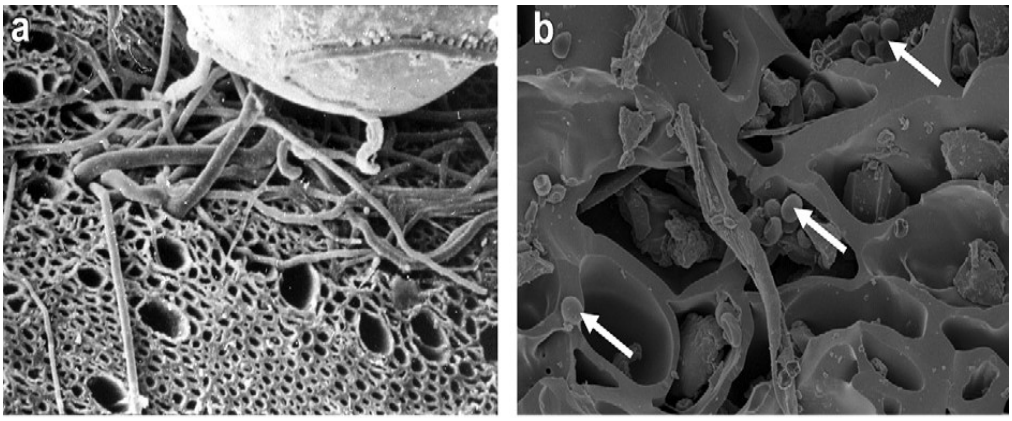


Figure 1.2 (a) Fungal hyphae on biochar (Lehmann and Joseph, 2009);

(b) Microorganisms in pores of corn biochar (arrows) (Jin, 2010).

Chapter 2: Effect of black carbon additions on aspen growth and soil microbial activity in land reclamation soils

2.1 Introduction

Mining sites across the world are being increasingly exploited in order to keep pace with the increasing human demand for energy. Surface mining, as an anthropogenic disturbance, has caused enormous ecological damage to ecosystems across the world during the past century (Adamo et al. 1996; Dudka et al., 1997; Massey et al., 1972; Rathore et al., 1993; Visser et al., 1979). Oil sands mining and bitumen extraction in Canada produces large volume of waste material such as sand, clay, coke and residual bitumen. Currently, oil sands coke and fine tailing are stockpiled on the mining areas. It has been reported that Syncrude and Suncor both produce 3 million tons of coke per year (Scott and Fedorak, 2004; Chung et al. 1996). In Canada these stockpiles should be reclaimed or incorporated into other reclamation options as part of the closure plans of oil sands operators (Nakata et al. 2011), but environmental concerns like leaching, mobility and toxicity in the long term need to be addressed. Mining companies are required to dispose the stockpile of byproducts (such as coke and fine tailings) once the mining activity is terminated (EPEA, Government of Alberta, 2014). Currently, one approach is to incorporate these byproducts into reclamation activities, so the impacts of application of coke or fine tailings on the target areas need further studies (Furimsky, 1998).

The traditional approach to reclaim mining disturbed areas in Canada is to stockpile the surface soil and upper horizon soils together before mining, then re-spread them back to the area once mining activities have terminated. Companies also use approaches that directly salvage peat from wetlands or forest floor material stripped from upland areas,

and use them as a surface amendment to reclaim the post- mining areas (Lucas et al., 1966; Fung and Macyk, 2000). The forest floor material has very low bulk density and high nutrient content, so its use can improve the physical, chemical and biological properties of soil when applied to mining sites (Fung & Mackyk, 2000; Lanoue, 2003).

In recent years, black carbon (BC) has drawn increasing attention from scientists around the world due to its special function in soil property improvement and plant growth (Sohi et al., 2010). BC is formed through the incomplete combustion of fossil fuels, bio-fuels or vegetation biomass under oxygen- limited conditions (Ramanathan, 2008). Many studies have shown that BC can improve soil nutrient availability, increase microbial activity and improve soil nutrient retention in different plant communities (Lehmann and Joseph, 2009; Sohi et al., 2010). Until now, forest floor material, peat or other organic matter have been increasingly used as amendments in mining area reclamation in Alberta and studied by researchers (Fung and Macyk, 2000; MacKenzie and Naeth, 2007& 2009; McMillan et al., 2007). Previous studies of biochar mostly focused on its application in agricultural lands or waste water treatment, so its usage in land reclamation and forest ecosystem reconstruction needs more studies.

BC includes a range of products such as char, charcoal and biochar, coke, etc (Fig.1.1)(Goldberg, 1985; Masiello, 2004). Biochar and coke were two different kinds of BC which will be used in our greenhouse experiment. Biochar is the solid product of thermal degradation of organic materials in an oxygen-limited environment; it is resistant to decomposition and is used as a soil amendment (Lehmann and Joseph, 2009). Petroleum coke (coke) is the solid carbonaceous material derived from oil refinery coker units or other cracking processes (IUPAC, 1997). BC (including biochar and coke) has

many potential benefits to soil pH, ash content, bulk density and volume of pores depending on its properties (Okimori, et al., 2003). However, there is limited research about the effects of coke on the soil nutrient availability, plant growth or soil microbes, so its impacts on the environment are still unclear. Furimsky (1998) found that coke is inert, and its stockpiles did not have significant environmental impacts under low pH.

The application of BC to soil can change soil physical properties such as soil water retention, total pore space, and soil density, in turn influencing plant growth (Downie et al., 2009). Studies have found that more water and nutrients were retained in soil as the biochar content increased, while the bioavailability of toxic metals decreased (Fellet et al., 2011). Biochar has lower density compared to a mineral soil, and pores in biochar can retain air and water (Downie, et al., 2009), and therefore improve soil physical properties. Biochar can also prevent nutrient leaching from soil because of its porous structure (Steiner, 2007). Boichar also benefits soil microorganisms when applied as an amendment to soil. Bacteria could be adsorbed to particle surfaces of biochar, which makes them less vulnerable to be leached away in soils (Pietikäinen et al., 2000) and may reduce predation since bacteria and fungi are protected from grazers by hiding in the pores of biochar (Ezawa et al., 2002; Thies and Rillig, 2009).

For the impact of black carbon on plants, Wasylyshen et al. (2002) and Nakata et al. (2007) found that coke decreased plant biomass and photosynthesis and transpiration rates, which they attributed to nutrient deficiency and metal toxicity in soil. Many studies have investigated how biochar influences the crop growth, but few have examined the relationship between biochar or coke application and tree growth on reclamation soil types. Biochar was found to stimulate Sugi tree growth by 144%- 224% in Japan

(Kishimoto and Sugiura, 1985). However, there is still limited research on how biochar influences the tree growth.

The ultimate goal of reclamation is to reestablish soil, plant and microbial communities and improve soil properties which are disturbed or degraded by mining activity. The objective of this greenhouse experiment was to examine the impact of addition of two types of Black carbons (coke and biochar) on five different land reclamation soils. I hypothesized that the biochar amendment will improve the plant growth, retain the soil available nutrient, and increase microbial biomass and soil respiration in the land reclamation soils, while coke may have some negative impacts on them.

2.2 Materials and methods

Soil types and biochar

The wheat straw biochar used in our experiment was produced by a slow pyrolysis process (400°C- 500°C for 2 hours) by Alberta Innovates- Technology Futures (AITF; Vegreville, AB, Canada). The biochar had a pH of 9.8, bulk density of 78 kg m^{-3}, water holding capacity of 59%, total porosity of 85%, electronic conductivity of 1.2 mS cm^{-2}, total C of 65.6%, total N of 1.1%, total sulphur of 0.1%, total hydrogen of 2.6%, total oxygen of 12.2%, volatile matter of 5.0 %, and ash content of 18.5% on an oven- dry weight basis (Waste Materials Engineering Lab., Alberta Innovates- Technology Futures, Vegreville, AB, Canada).

Five different soil or reclamation substrates were used in our experiment (Table 2.1): Peat mineral- mix (PMM), Forest floor mineral- mix (FFM), B horizon soil I with

high P content (BHP), B horizon soil II with low P content (BLP) and Whitewood parent geological material (WWP). The Peat- mineral mix (PMM) was a soil mixture of approximately 50% salvaged peat obtained from sphagnum bogs and 50% mineral soil. The Forest floor- mineral mix (FFM) is the salvaged material from upland forest including the LFH layer with underlying mineral soil mixed in a volume ratio of 1:5. The BHP and BLP were collected from the B horizon of soils near Utikuma Lake in north-central Alberta in Athabasca oil sands, Alberta in May 2009. The Whitewood parent geological material was collected at the Transalta coal mine area, north of Lake Wabamun, about 70 kilometers west of Edmonton, Alberta in July, 2011. All of the soil materials are no longer natural soils but stockpiled substrates. Each soil was mixed evenly and sieved through a 4mm sieve to remove coarse woody debris, twigs and leaves, and then stored in buckets until use.

The properties of the PMM, FFM, BHP and BLP soils were measured for previous studies conducted by Pinno et al. (2011). For the properties of the WWP, fresh soil samples were taken by both a metal soil probe 2.5cm in diameter (for measurement of Total C (TC), Total N (TN), pH) and soil sample rings 7.5cm in diameter and 10cm in depth (for measurement of bulk density) at the end of August, 2012. For soil probe samples, about 100g soil for each treatment was collected from 0-25cm depth below the soil surface. The samples were transferred to freezer bags (Ziploc) immediately after collection, and then they were put into a cooler with ice packs inside to keep them fresh.

Soil samples from probes were air dried for two weeks at 25°C for pH and total C and N measurement. Soil pH was measured by a pH meter in the laboratory. 5g of dry sample was placed into a beaker; 10g of deionized water was added and then stirred for

10min. After the soil was suspended for 1 hour, a pH meter (Mettler-Toledo, Ohio, USA) was calibrated and used to measure soil pH (Kalra and Maynard, 1991).

For TC measurement, soil samples were ground at the frequency of 15 Hz (900 min^{-1}) for 1 min into 5μm particles (Retsch MM400 mixer mill), and then 10mg of each sample was transferred onto a small tin capsule and wrapped up tightly. Total C content were measured by the dry combustion method (Costech Analytical Technologies Inc., Valencia, CA, USA) (Nelson and Sommers, 1982).

The bulk density was measured on soil samples taken using soil rings. Soil samples in soil rings were transferred to aluminum cases, oven dried at 105°C for 24h and then weighed. The ring height and radius were measured by a ruler in cm to the nearest mm. The bulk density was calculated by the following formula (Cresswell and Hamilton, 2002): a) Soil volume = ring volume (cm^3) = $\pi \times$ radius$^2 \times$ ring height (cm); b) Bulk density (g/cm^3) = Dry soil weight (g)/Soil volume (cm^3). The moisture content (by mass) was calculated by the following formula: Water (%) by mass = (wet mass - dry mass/dry mass)×100.

Experimental design

Overall, the experiment is a complete factorial design. There were three Black Carbon treatments (biochar, coke and control), five soil or reclamation substrates (PMM, FFM, BHP, BLP and WWP soils), two plant treatments (with and without aspen), and four replicates for each treatment. Biochar and coke (10% by soil volume) were added to the five soil types separately. One gallon square pots were then filled with each of the soil types, wet to saturation and incubated one month under ambient conditions (25℃ during

the day and 15°C at night; 18 hour light exposure per day). Aspen seeds were initially planted in peat plugs, and all peat plugs were placed in a plastic tray under ambient conditions (25°Cduring the day and 15°C at night; 18 hour light exposure per day) and watered daily. After germination, the aspen seedlings were left in the peat plugs for five weeks of growth before they were transplanted to the soil pots. In total, 120 pots (60 pots with aspen and 60 control pots without aspen) of soil were incubated for a month, then 60 aspen seedlings in peat plugs were transplanted into four pots of each soil type×BC addition combination.

Ionic resin capsules were installed in each soil pot 10 cm under the soil surface and left to adsorb ions. Each resin capsule was 2 cm in diameter, and contained approximately 10 ml of mixed bed ionic resins (PST-2, Unibest, Bozeman, Montana, USA). All aspen pots and no-aspen pots were placed on two plastic trays separately and watered to field capacity with a two- day interval. All aspen pots were randomly rearranged on the tray to avoid any heterogeneity of the light condition. The experiment was run for six months.

Plant biomass

After six months of growth, all 60 aspen trees were cut at the soil surface, and the stem and leaves of each tree (the aboveground biomass) were collected into clean paper bags. The roots (the belowground biomass; including the fine roots) were dug out from pots, separated from the soil, and carefully washed on a 1mm metal sieve with distilled water. All aboveground and belowground biomass were oven dried at 65°C for 24 hours and then weighed using an electronic scale.

Soil available nutrients analysis

Soil samples (approximately 200g) from all 120 pots were collected at the end of the experiment. 10g of fresh soil sample from each pot was weighed and oven dried at 105°C for 24 hours, and the dry samples were weighed again to calculate moisture content. Resin capsules were collected 16 weeks after the aspen was planted, put into 50ml of 2M KCl solution and shaken for 30 min. The suspension was centrifuged to remove soil particles, and the extracts were analyzed for available N (NO_3-N, NH_4- N) and available P (PO_4^-) (DeLuca et al. 2002; MacKenzie and DeLuca, 2006). A SmartChem™200 Discrete Analyzer (ManDel Scientific Instrument Inc., Canada) was used to analyze NO_3-N and NH_4- N and PO_4-P in the extracts.

Microbial biomass carbon and nitrogen

The chloroform- fumigation extraction (CFE) method was used to determine dissolved organic carbon and nitrogen (DOC and DON) and the soil microbial biomass carbon and nitrogen (MBC and MBN) in the different soil treatments (Vance et al., 1987). Two portions of fresh soils were taken from each pot after the 6 month experiment period, and 25g of each was placed into 100mL glass beakers. One sample from each pot was fumigated with $CHCl_3$ in a dessicator lined with wet filter paper to maintain humidity. Then soil samples were put inside a desicator and about 30mL ethanol-free $CHCl_3$ was added into a small beaker with a few boiling chips in it. The dessicator was evacuated after the $CHCl_3$ boiled for 2 minutes, and then placed in the dark at 25°C with $CHCl_3$ vapor for 48 hours. After 48 hours, the dessicator was evacuated again in order to refill it with $CHCl_3$ vapor and the samples were left for fumigation for another 48 hours.

Both fumigated and unfumigated samples were put in to 150-200mL plastic bottles to which 50mL of 0.5M K_2SO_4 was added. The bottles were placed on a reciprocal shaker and shaken for 30 minutes. The suspension was filtered through a Buchner funnel with Q2 filter paper. The fumigated and unfumigated samples were analyzed for dissolved organic carbon (DOC) by TOC- V_{CSN}, Total Organic Analyzer (ManDel Scientific Instrument Inc., Canada), and dissolved organic nitrogen (DON) by TOC- V_{CSN}, Total Organic Analyzer with a TNM1 accessory. Then MBC and MBN were calculated according the formulas: $MBC = DOC_{fumigated} - DOC_{unfumigated}$; $MBN = DON_{fumigated} - DON_{unfumigated}$.

Soil basal respiration

Soil basal respiration was measured by the alkali trap method after the 6 month experiment. Since the soil respiration in our experiment was tested by alkali trap method, only soil microbial respiration (also called soil basal respiration or heterotrophic respiration) was taken into account, there was no respiration due to plant roots. To quantify soil basal respiration, 50g of soil from each pot was placed into a mason jar along with a vial containing 20mL of 0.5M NaOH. There were two empty jars (blanks) for control with 20mL of 0.5M NaOH inside them. Jars were capped and placed in the dark at 25°C. After one week of incubation, the NaOH solution was titrated to determine how much CO_2 had been trapped. For this, 1ml $BaCl_2$ and 3 drops of phenolphthalein were added into each vial and 0.5M HCl was used to titrate the NaOH solution until it turned clear (when the solution pH was 8.8). Then the amount of CO_2 per vial was calculated according to the amount of 0.5M HCl added, using the formula: CO_2-C (mg/kg

soil) = $(A_1\text{-}A_2)\times N\times E\times D$. A_1 represents titrant added to control vials (ml), A_2 represents titrant added to test sample (ml), N represents concentration of HCl=0.5mol/L, E represents the coefficient, and D represents dilution factor 4 (only 5 out of 20 ml of NaOH for each sample was used).

Metabolic quotient qCO_2

Metabolic quotient (qCO_2) (Anderson and Domsch, 1985) refers to the ratio of soil basal respiration to microbial biomass. The qCO_2 was calculated with the following formula (Anderson and Domsch, 1985): *qCO_2= soil microbial respiration/MBC*

Statistical analysis

We had five soil types, three Black Carbon (BC) treatments (Biochar, Coke and No- BC treatments) and two plant treatments (Tree and No-tree treatments).
To examine differences among the soil types, two-way ANOVAs were done with soil types (five types), plant (Tree and No-tree) and their interaction. In this analysis, the effect of biochar was ignored. These analyses were done on NO_3-N, NH_4- N, PO_4- P, DOC, DON, MBC, MBN, CO_2 and qCO_2. In the ANOVAs, statistical significances were determined at α= 0.1. Post-hoc comparisons of means were done if there were significant effects of soil or a soil× plant treatment interaction. If only soil effect was significant, each soil type was compared for Tree and No-tree treatments separately, and α was adjusted by 0.1/ 10= 0.01; if only tree effect was significant, no further post- hoc test was needed; if there was a significant interaction, soil types were compared for Tree and No-tree treatments and Tree and No- tree treatments were compared for each soil type

respectively, and α was adjusted by 0.1/ 20= 0.005. For tree total biomass, aboveground and belowground biomass, one-way ANOVAs were done separately to determine if there were significant differences among the five soil types ignoring BC treatments; if there was a significant effect of soil, then in the post-hoc test, α is adjusted by 0.1/10= 0.01.

Another two-way ANOVA was done for each soil type separately to determine if there were significant differences in NO_3-N, NH_4- N, PO_4- N, DOC, DON, MBC, MBN, CO_2 or qCO_2 between tree treatments, BC treatments and their interactions; and then one-way ANOVAs for each soil type separately were done to test for effects of BC treatments for tree total biomass, aboveground and belowground biomass.

All residuals of data were examined for the assumptions of normality and homogeneity of variances. A log transformation was conducted before the ANOVA was run when the residual of data was not normal or homogeneous. In the ANOVAs, statistical significances were determined at α= 0.1. Post-hoc comparisons of means were done if there were significant effects of BC or a BC×Plant- treatment interaction. If only BC effect was significant, the three BC treatments were compared for tree and no-tree treatments separately; if only tree effect was significant, no further post- hoc test was needed; if there was a significant interaction, BC treatments were compared for Tree and No- tree treatments and Tree and No- tree treatments were compared for each BC treatment respectively. Alpha values for the post- hoc comparisons were adjusted by the number of comparisons. If there was a tree×BC interaction, I compared Tree to No-tree for a given soil type and BC treatment, and α was adjusted by 0.1/3= 0.033; when the comparisons were made among three black carbon treatments for a given soil type and Tree or No-tree treatment separately, I adjusted α by 0.1/3= 0.033.

2.3 Results

Tree biomass

Overall, there was a significant effect of soil type on the total aspen biomass, aboveground and belowground biomass (Table 2.2, Fig. 2.1&2.2). The total and aboveground biomass was significantly higher in the PMM soil than the rest of four soils; the FFM and BHP soils had significantly higher total and aboveground biomass than BLP and WWP soils. The PMM soil had significantly higher belowground biomass than the BLP and WWP soils; the FFM, BHP and BLP soils had significantly higher belowground biomass than the WWP soils.

There was an effect of Black Carbon (BC) on the total aspen biomass for the PMM, BLP and WWP soils, on the aboveground biomass for PMM, BLP and WWP soils, and on the belowground biomass for BLP and WWP soils (Table 2.3, Fig.2.1). Biochar did not significantly affect aspen growth, but coke sometimes reduced it. The total aspen biomass (aboveground biomass plus belowground biomass) was significantly lower in the coke treatment than in the NoBC PMM and WWP soils while the biochar treatment was intermediate; and lower in the coke treatment than the NoBC and biochar amended BLP soil. Tree aboveground biomass was significantly lower in coke treatment than in the control for PMM and WWP soils, and than in the control and biochar treatments for the BLP soil. The tree belowground biomass in the coke treatment was significantly lower than in the control for the BLP and WWP soil while the biochar treatment was intermediate.

Nitrate (NO_3-N)

There was a significant effect of soil types on the NO_3-N (Table 2.2; Fig. 2.3). In the pots without aspens, the NO_3-N was significantly higher in the organic soils (PMM and FFM) than the mineral soils; for the mineral soils, the BLP soil had higher NO_3-N than the WWP soil while the BHP soil was intermediate. In the pots with aspen, the FFM soil had higher NO_3-N than the BHP, BLP and WWP soils; the PMM soil had higher NO_3-N than the BHP soil.

There was a BC effect on nitrate in all soil types, a plant effect in all soils except WWP, and a BC× Plant interaction on nitrate in all soil types except the FFM soil (Table 2.3; Fig. 2.3). In the NoTree treatments, the NoBC treatments had higher nitrate than the coke treatment in all the soil types; the NoBC treatments had higher nitrate than the biochar treatment in the PMM, BHP, BLP and WWP soils; the biochar treatment had higher nitrate content than the coke treatment in the FFM and WWP soil. For soils with trees, the nitrate was higher in the NoBC treatments than in the coke amended PMM, FFM, BLP and WWP soils, and than in the biochar amended PMM soil; the nitrate was higher in the biochar than in the coke amended WWP soil. In the BLP soil with aspen planted, the biochar treatment was intermediate compared to the control or coke treatments.

There was significantly higher nitrate in the NoTree treatments than the Tree treatments for FFM soil, and in NoBC PMM, BHP, BLP and WWP soils; in the biochar amended PMM soil; as well as in the coke amended BLP and WWP soils.

Ammonium (NH_4-N)

There was a significant effect of soil types on the NH_4-N (Table 2.2; Fig. 2.4). In the pots with and without aspens, the order of the amount of NH_4-N was higher in FFM > PMM > BHP & BLP > WWP.

There was a significant BC effect on NH_4-N in the PMM and FFM soils, and a plant effect on NH_4-N in the FFM soil (Table 2.3; Fig. 2.4). Unlike the trend in the nitrate graph, there were a few differences in the NH_4-N among the black carbon treatments. In the NoTree treatment, the coke amendment significantly decreased the amount of NH_4-N in the FFM soil while the biochar treatment was intermediate. In the soils with aspen planted, the unamended PMM soil had higher NH_4-N than the coke amended soil; the unamended FFM soil had higher NH_4-N than the coke and biochar amended soil. The FFM soil had higher NH_4-N in the NoTree pots than in the tree pots.

Phosphate (PO_4-P)

There was a significant effect of soil types on the PO_4-P (Table 2.2; Fig. 2.5). In the pots without aspens, the order of the amount of NH_4-N was higher in BHP>FFM> WWP>BLP>PMM; in the pots with aspen, the order of the amount of NH_4-N was higher in BHP=FFM= WWP>BLP=PMM.

There was a BC effect on PO_4-P in the PMM and FFM soils, a plant effect on PO_4-P in the WWP and a BC× Plant interaction in the BLP and WWP soils (Table 2.3; Fig. 2.5). In the NoTree treatments, the NoBC and coke amended PMM soils had higher phosphate than the biochar amended PMM soil; the coke amended FFM soil had lower phosphate than the NoBC and biochar amended PMM soils; the NoBC BLP soil had higher phosphate than the biochar amended BLP soil while the coke treatment was intermediate. In the WWP soil with aspen planted, the coke and biochar treatments had

significantly higher phosphate than NoBC treatment. The NoBC BLP soil had higher phosphate in the NoTree than in the Tree treatment, while the biochar amended BLP and coke amended WWP had lower phosphate in the NoTree treatment than Tree treatment.

Dissolved organic carbon (DOC)

There was a significant effect of soil types on the DOC (Table 2.2; Fig. 2.6). In the pots without aspens, the PMM soil had higher DOC than the rest of the four soil types; the FFM, BLP and WWP soils had higher DOC than the BHP soil. In the pots with aspen, the PMM soil had higher DOC than the rest of the four soil types; the FFM soil had higher DOC than the BHP, BLP and WWP soils.

There was a BC effect on DOC in PMM soil, a plant effect on DOC in FFM and BLP soil, and effects of BC, plant and BC× Plant interaction on WWP soil (Table 2.3; Fig. 2.6). There was no significant difference among BC treatments in the NoTree treatment. However, the biochar amended PMM soil had significantly higher DOC than coke treatment in the pots with tree planted, while the NoBC was intermediate. The NoBC soil had significantly higher DOC than the coke and biochar treatments for the WWP soil with trees planted. The No-tree treatment had significantly higher DOC than the Tree treatment in the FFM soil, and in all three BC treatments (including NoBC) for BLP and WWP soils (Fig. 2.6).

Dissolved organic nitrogen (DON)

There was a significant effect of soil types on the DON (Table 2.2; Fig. 2.7). In the pots without aspens, the order of the DON content was higher in the PMM > FFM > BHP

> BLP > WWP. In the pots with aspen, the order of DON content was higher in the PMM > FFM & BHP > BLP > WWP.

There was a BC effect on DON in PMM, FFM, BHP and WWP, a plant effect on DON in PMM, FFM and BHP soils, and a BC× Plant interaction in the PMM and BHP soils (Table 2.3; Fig. 2.7). In the NoTree pots, dissolved organic nitrogen (DON) was significantly lower in the coke treatment than the NoBC and biochar amended PMM and FFM soils; and lower in both BC (coke and biochar) amended BHP and WWP soils than the NoBC soils. In the aspen pots, both coke and biochar amendments decreased the DON in the WWP soil. DON was significantly higher in NoTree than Tree treatments in the FFM soil and in the NoBC and biochar treatments for PMM soil, but significantly lower in NoTree than Tree treatments in the coke amended BHP soil (Fig. 2.7).

Microbial biomass carbon (MBC)

There was a significant effect of soil types on the MBC (Table 2.2; Fig. 2.8). In the pots without aspens, the order of the MBC content was higher in the PMM > FFM > BHP, BLP and WWP. In the pots with aspen, the organic soils (PMM and FFM) had higher MBC than the mineral soils; the WWP soil had higher MBC than the BLP soil while the BHP soil was intermediate.

Overall, there was a plant effect on MBC in the PMM, BLP and WWP soils, and a BC× Plant interaction for the FFM and WWP soils (Table 2.3; Fig, 2.8). In the tree pots, the MBC was significantly higher in the biochar treatment than in the control in the FFM soil while the coke treatment was intermediate; the MBC was higher in the coke treatment than the biochar treatment in the WWP soil while the NoBC was intermediate. MBC was significantly higher in the No- tree treatments than the tree treatments in the

PMM and BLP soils, and higher in the No- tree treatments for the NoBC FFM and coke amended WWP than the tree treatments (Fig 2.8).

Microbial biomass nitrogen (MBN)

There was a significant effect of soil types on the MBN (Table 2.2; Fig. 2.9). In the pots without aspens, the order of the MBN content was higher in the PMM> FFM> BHP> BLP> WWP. In the pots with aspen, the order of the MBN content was higher in the PMM> FFM> BHP> BLP= WWP soils.

There was a BC effect on MBN in the PMM, FFM and BHP soil, a plant effect on MBN in the PMM, BHP, BLP and WWP soil, and a BC× Plant interaction in the PMM and WWP (Table 2.3; Fig. 2.9). In the no-aspen pots, MBN was significantly higher in biochar treatment than NoBC or coke treatments in the PMM soil; coke amended BHP had significantly lower MBN than NoBC or biochar treatment. In the aspen pots, MBN was significantly higher in coke and biochar treatments than in the control for the FFM soil. MBN was higher in NoTree than in Tree treatments for the BHP and BLP soils, for all BC treatments (including NoBC) of the PMM soil, and for NoBC and biochar amended WWP soil (Fig. 2.9).

Soil basal respiration

There was a significant effect of soil types on the soil basal respiration (CO_2 emission; Table 2.2; Fig. 2.10). In the pots without aspens, the order of the soil basal respiration was higher in the PMM > FFM and WWP > BHP and BLP soils. In the pots with aspen, the PMM soil had higher basal respiration than the rest of four soils; the FFM

and WWP soil had higher basal respiration than the BLP soil while BHP soil was intermediate.

There was a BC effect on soil respiration in FFM and WWP, a plant effect on soil respiration in PMM and BHP soil, and a BC× Plant interaction in the PMM and BHP soils (Table 2.3; Fig. 2.10). In the PMM soils without tree planted, NoBC treatment had significantly lower respiration rate than the biochar treatment while the coke treatment was intermediate. In the FFM soil with aspen planted, the biochar and coke amendments had lower respiration rate than NoBC soil, but for the BHP and WWP soil with aspen planted, the opposite was true, where coke and biochar amended soils had higher respiration rate than NoBC soils. The respiration rate was significantly higher in the NoTree than the Tree treatments for WWP soil and for the biochar amended PMM soil, but lower in the NoTree than the Tree treatments for the coke and biochar amended BHP soil.

Metabolic quotient (qCO_2)

There was a significant effect of soil types on the metabolic quotient (qCO_2; Table 2.2; Fig. 2.11). In the pots without aspens, the WWP soil had higher metabolic quotient than the PMM, FFM and BLP soils; the BHP and BLP soils had higher metabolic quotient than the PMM soils. In the pots with aspen, the mineral soils (BHP, BLP and WWP) had higher metabolic quotient than the organic soils (PMM and FFM).

There was a BC effect on qCO_2 in the FFM soil, a plant effect on qCO_2 in the PMM and BLP soils, and a BC× Plant interaction in the PMM and FFM soils (Table 2.3; Fig. 2.11). In pots with aspen planted, the qCO_2 was significantly higher in coke

treatment than in biochar treatments for the PMM soil while the NoBC was intermediate; the qCO_2 was higher in NoBC treatment than in the coke or biochar treatments in the FFM soil. However, in the aspen pots of the WWP, the biochar amended soil had higher qCO_2 than the coke treatment while the NoBC was intermediate.

The qCO_2 was higher in Tree than NoTree treatments for the BLP soil, in the NoBC PMM and FFM soils, and in the coke amended FFM soil; but lower in Tree than NoTree treatments for the NoBC and coke amended WWP soil (Fig. 2.11).

2.4 Discussion

Generally, organic soils (PMM and FFM) had higher aspen biomass, inorganic N, DON, microbial biomass and soil heterotrophic respiration than the mineral soils (BHP, BLP and WWP). This is not surprising since PMM and FFM soils are mixture of organic and mineral soils. Organic matter in peat and forest floor material provided carbon sources for soil microorganisms and led to higher microbial biomass and soil heterotrophic respiration in PMM and FFM soil, compared to mineral soils. During the process of SOM (soil organic matter) decomposition by soil microbes, more nitrate, ammonia, DOC and DON were generated.

When comparing the two organic soil types, PMM had higher DOC and DON, but lower amounts of ammonia, nitrate and PO_4-P than FFM, and there was a higher microbial biomass and soil heterotrophic respiration in PMM than FFM. PMM used in our experiment had a marked higher amount of organic matter (95.2 g kg^{-1} SOC) than FFM (12.2 g kg^{-1} SOC) (Table 2.1), and a significantly higher amount of DOC and DON than FFM soil. Because the higher content of soil organic matter in PMM provided more

carbon source for soil microbes than FFM, there was a higher microbial biomass and microbial respiration in the PMM than FFM (Fig. 2.8& 2.9).

Generally during the process of growth, plants need a large amount of inorganic N, P and K, especially N, so it was surprising that PMM had better plant growth than FFM soil since FFM has a higher amount of available N and P. The reason might be attributed to the benefit from soil microbes. Mycorrhizae are the most widespread mutualistic symbionts between plant and soil microorganisms (Gianinazzi-Pearson, 1996). There were higher microbial biomass in the PMM than FFM, so one possibility was that there were more mycorrhizae in the PMM and they assisted the plant in taking up nutrients which accounted for better growth in PMM despite fewer nutrients. Previous evidence suggested that mycorrhizae facilitae plant roots to absorb more nutrients in soil since mycorrhizal mycelia are much smaller in diameter than the fine roots, and thus can enlarge surface area of nutrient absorption for roots (Smith and Read, 2010). Another reason could be that there was a faster decomposition of organic matter (as indicted by the soil respiration rate, Fig. 2.10) and turnover of nutrients in the PMM than the FFM since there were more microorganisms in PMM, and a higher turnover rate of available nutrients resulted in a better plant growth in the PMM.

Influences of BC on soil available nutrients and aspen growth

As to black carbon effects, coke and biochar showed a marked ability to absorb NO_3^-, but we did not find that black carbon absorbs a significant amount of NH_4^+ in four of the five soil types, which suggested that biochar and coke primarily absorbs NO_3^- rather than NH_4^+. These results were consistent with a previous leaching experiment where Yao et al. (2012) found that the peanut hull derived biochar (pyrolyzed under

600°C) decreased the amount of nitrate and ammonium in the leachates by 34% and 14%, respectively. It proved that biochar had a higher absorption capability of nitrate than ammonium. Comparing my greenhouse study with Yao's study (2012), it is suggested that crop derived biochars have similar absorption ability of inorganic N, but the adsorption characteristic of crop derived biochar may be different from the biochars made from tree species. In the same experiment, Yao (2012) also found that Brazilian pepperwood derived biochar (pyrolyzed under 600°C) had similar absorption ability for nitrate and ammonium, where the biochar decreased the the leaching of nitrate, ammonium, and phosphate by 34.0%, 34.7%, and 20.6%, respectively, compared to the control. The reason of the difference between biochars is not clear and need further study, and it may be attributed to the differences in cellular structure of the feedstock (Yao, 2012).

The aspen pots had a significantly lower amount of nitrate than the no- aspen pots, but this trend was not obvious for the ammonia. Some evidence suggested that plant species show different preferences or abilities to absorb different forms of inorganic N (Glass, 1989; Min et al., 1998). For example, white spruce demonstrated higher rate of NH_4^+ uptake than NO_3^-, while trembling aspen showed a preference for NO_3^- over NH_4^+ (Kronzucker et al., 1995, 1996, 1997; Min et al., 1998). In my experiment, aspen was chosen as the experimental species, and NO_3^- was significantly lower in the pots with aspen planted than the no- aspen soil in the no- black carbon treatment, while the NH_4^+ was only lower in aspen pots of the FFM soil than the no- aspen pots. This demonstrated that the major form of inorganic N uptaken by aspen is NO_3^- rather than NH_4^+, which was consistent with previous studies.

Previous studies (Steiner, 2007; Fellet et al., 2011) suggested that more nutrients could be retained in soil with biochar addition and biochar can also prevent nutrient leaching loss and increase nutrient availability in the soil. Some evidence indicated that nutrients absorbed by black carbon is bio-available and could be taken up by plant and soil microorganisms in the long term (Taghizadeh-Toosi, 2012). However, our 6 month experiment did not show the benefit of biochar retained nutrients (nitrate) on plant growth. This characteristic of biochar is especially important if fertilizer will be applied with biochar together in sandy soils since sandy soils are more susceptible to leaching than silt loam or clay loam soils (Hergert 1986; Nyamangara et al., 2003), and application of biochar together with fertilizer could be one of the options to prevent nutrient losses. However, current studies have mostly been conducted on farmland to test the impact biochar on crop growth. There have been few studies conducted on the impact of biochar or coke application into land reclamation soils, so our results may provide a preliminary evidence for the influence of black carbon addition on nutrient retention and tree growth.

Previous studies suggested that pet-coke has negative effect on plant growth. Wasylyshen (2002) found coke amendment had a negative effect on barley growth; Nakata et al. (2011) found coke decreased the plant biomass, photosynthesis and transpiration rates. In my study, the pet-coke inhibited aspen growth in the PMM, BLP and WWP soils, which is consistent with previous studies.

BC and soil microorganisms

Previous findings showed that biochar had positive effects on soil microorganisms. The huge surface area and numerous inside pores of biochar can provide habitat for microorganisms and protect them against grazers (Ezawa et al., 2002; Pietikäinen et al.,

2000; Thies and Rillig, 2009). Jones (2012) found that biochar addition increased the growth rate of fungi and bacteria. Our results were consistent with previous studies in that biochar increased the soil microbial biomass in PMM and microbial activity-demonstrated by increased soil respiration in WWP. Except for providing shelter for soil microorganisms, application of biochar could also benefit the microbes from some other perspectives. Since the biochar adsorbed dissolvable nutrients are bioavailable (Taghizadeh-Toosi et al., 2012), one possibility for higher microbial activity in PMM soil is that biochar could absorb nitrate and nitrate can be used by soil microbes. There were more microbes surviving and reproducing in the PMM because the nitrogen source was higher in biochar amended PMM soil than control. In the Aspen treatment, microbial biomass (MBC&MBN, Fig. 2.8&2.9) was lower in pots with trees versus without tree planted. This could be due to competition for available nitrogen between the trees and the soil microbes (Bardgett et al., 2003; Harte and kinzig, 1993; Owen and Jones, 2001). In addition, more microbes need more organic matter to support metabolism and reproduction, so there was maybe less DOC in pots without aspen planted than aspen pots because the higher microbial biomass was resulting in faster decomposition of the organic carbon.

Microbial activity, as indicated by soil heterotrophic respiration (Fig. 2.10), was higher in biochar amended PMM soil and higher in coke amended WWP compared to unammended PMM and WWP. This was consistent with the observation of Jones (2012), who found that biochar addition increased soil respiration. The soil heterotrophic respiration data were also consistent with our soil microbial biomass data, which showed that biochar has a positive effect on soil microorganisms. More soil organisms could

generate more CO_2 during the process of metabolism. The qCO_2 data indicated that the microorganisms in the mineral soils had higher use efficiency of the organic substrate than in the organic soils (Fig. 2.11). Evidence suggested that microorganisms living under soil environmental stresses, such as an inappropriate soil pH, have higher qCO2 (microbial use efficiency of the organic substrate) (Anderson and Domsch, 1993; Insam and Haselwandter, 1989). In our case, the nutrient availability may be one of the stresses that resulted in an increase in the microbial use efficiency on the organic substrate. According to our microbial biomass data, there were more soil microbes in notree pots than tree pots, so the CO_2 emission and qCO_2 was also higher in soil without aspen planted.

2.5 Implications and future research

During the process of land reclamation after surface mining activities, the selection of reclamation substrate is very important. Our study suggested that PMM and FFM are two substrates which had higher plant growth, soil available N, microbial biomass and soil basal respiration than the mineral soils. To reclaim the sub-soil in the post-mining areas to a fertile soil which effectively supports re-establishment of plant communities, the PMM and FFM are two possible reclamation substrates directly transfer to the post-mining areas. Biochar and Coke showed the ability to adsorb dissolvable nutrients in soil, but these nutrients did not benefit the aspen growth in this short term experiment. Coke decreased the plant growth in PMM, FFM and WWP soils, but it did not show a negative effect the soil microbes.

One limitation of our experiment is that it only lasted for six month, long term effects of black carbon addition on aspen growth, soil microbial biomass and activity were still not clear. There are limited studies related to the effect of coke on plant and soil microbes and the growth of trees, so its impact requires further study. Previous studies showed that the biochar absorbed nutrients are bioavailable, so future studies could also investigate the long term effect of biochar and other forms of black carbon on the plants, soil and soil microorganisms. Since different biochar has distinct features when used as a soil amendment, future studies could also investigate the effect of different plant species derived biochar and other forms of black carbon on the reclaimed soils.

Table 2.1 Basic properties of five reclamation soils.

Soil type	Total carbon (%)	Total nitrogen (%)	Available phosphorus (mg kg^{-1})	Bulk density $g.cm^{-3}$	pH	Field moisture content %
PMM	9.52	0.38	1.26	0.65	7.1	49.2
FFM	1.22	0.04	20.64	1.41	6.6	6.4
BHP	0.21	0.02	192.03	1.33	6.9	5.1
BLP	0.22	0.01	6.77	1.43	7.4	4.5
WWP	0.25	0.05	17.15	1.29	7.8	13.8

Note: PMM (peat- mineral mix); FFM (forest floor- mineral mix); BHP (B horizion soil with high phosphorous content); BLP (B horizon soil with low phosphorous content); WWP (Whitewood coal mine parent geological material). The properties of the PMM, FFM, BHP and BLP soils were adopted from Pinno et al., 2011.

Table 2.2 Results (*p*- valuess) of two- way ANOVAs examining the effects of five soil types, plant (with or without aspens) and their interactions on the total aspen biomass, above and below ground biomass of aspen, nitrate (NO_3-N), ammonium (NH_4-N), available phosphorus (PO_4-P), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), soil basal respiration (CO_2) and metabolic quotient (qCO_2) after six months of aspen growth.

	Soil[2]	Plant[3]	soil×Plant[4]
Total aspen biomass[1]	**<0.0001**		
Above ground biomass[1]	**<0.0001**		
Below ground biomass[1]	**<0.0001**		
NO_3-N[1]	**<0.0001**	**<0.0001**	**0.0536**
NH_4-N[1]	**<0.0001**	**0.0045**	0.6508
PO_4-P[1]	**<0.0001**	0.5147	0.1993
DOC[1]	**<0.0001**	**<0.0001**	**<0.0001**
DON[1]	**<.0001**	**0.0484**	**0.0004**
MBC[1]	**<.0001**	0.1302	**0.0659**
MBN[1]	**<0.0001**	**<0.0001**	**<0.0001**
Soil basal respiration (CO_2)	**<0.0001**	0.2979	**0.0157**
Metabolic quotient (qCO_2)[1]	**<0.0001**	0.2556	**0.0171**

Note: Bolded and underlined *P*- values denote significant differences at α= 0.1.

[1] The data were Log transformed to meet the assumptions of ANOVA.

[2] 'Soil' represents five soil types used in our experiment- PMM, FFM, BHP, BLP and WWP soils.

[3] 'Plant' denotes soil pots with and without aspen planted.

[4] 'Soil×Plant' means the interaction between soil and plant treatments.

Table 2.3 Results (*p*- valuess) of two- way ANOVAs examining the effects of three BC treatment (NoBC, coke and biochar), plant (with or without aspens) and their interactions on the total aspen biomass, above and below ground biomass of aspen, dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), nitrate (NO_3-N), ammonium (NH_4-N), available phosphorus (PO_4-P), soil basal respiration (CO_2) and metabolic quotient (qCO_2) in the five soils after six months of aspen growth.

	treatment	PMM	FFM	BHP	BLP	WWP
Total aspen biomass	**BC[1]**	**0.0631**	0.4566	0.2773	**0.0106**	**0.0238**
Above-ground biomass	**BC[1]**	**0.0191**	0.3099	0.2719	**0.0013**[4]	**0.0938**
Below-ground biomass	**BC[1]**	0.2513	0.5973[4]	0.1891	**0.0663**	**0.0360**
NO_3-N	**BC[1]**	**<.0001**	**0.0001**[4]	**0.0004**	**<.0001**[4]	**<.0001**[4]
	Plant[2]	**0.0002**	**0.0014**[4]	**0.0015**	**0.0005**[4]	0.4670[4]
	BC×Plant[3]	**0.0588**	0.5339[4]	**0.0124**	**0.0602**[4]	**0.0063**[4]
NH_4-N	**BC[1]**	**0.0182**[4]	**<.0001**	0.2812	0.1559	0.1855
	Plant[2]	0.2086[4]	**0.0026**	0.2192	0.3189	0.3269
	BC×Plant[3]	0.2219[4]	0.8135	0.9234	0.8416	0.2670
PO_4-P	**BC[1]**	**0.0024**[4]	**0.0335**	0.1773	0.1559	0.2789
	Plant[2]	0.5830[4]	0.2010	0.1966	0.6667	**0.0750**
	BC×Plant[3]	0.1510[4]	0.2631	0.8165	**0.0290**	**0.0608**
DOC	**BC[1]**	**0.0053**	0.6476	0.8775	0.6876[4]	**<.0001**
	Plant[2]	0.8754	**0.0012**	0.3013	**<.0001**[4]	**<.0001**
	BC×Plant[3]	0.3942	0.3308	0.2079	0.9746[4]	**<.0001**
DON	**BC[1]**	**0.0041**[4]	**0.0005**[4]	**0.0050**[4]	0.2105[4]	**0.0020**[4]
	Plant[2]	**<.0001**[4]	**0.0025**[4]	**0.0611**[4]	0.4076[4]	0.5490[4]
	BC×Plant[3]	**0.0375**[4]	0.1072[4]	**0.0220**[4]	0.7028[4]	0.9306[4]
MBC	**BC[1]**	0.3564	0.4901	0.3174	0.5414	0.7176[4]
	Plant[2]	**<.0001**	0.9629	0.5119	**0.0651**	**0.0538**[4]
	BC×Plant[3]	0.1919	**0.0558**	0.6089	0.5568	**0.0228**[4]
MBN	**BC[1]**	**0.0003**	**0.0283**[4]	**0.0469**[4]	0.9445	0.6761
	Plant[2]	**<.0001**	0.1063[4]	**0.0008**[4]	**0.0007**	**<.0001**
	BC×Plant	**0.0012**	0.2965[4]	0.1894[4]	0.4738	**0.0435**
Soil basal respiration (CO_2)	**BC[1]**	0.2656	**0.0013**	0.3119	0.9797	**0.0437**
	Plant[2]	**0.0496**	0.7109	**0.0042**	0.9836	**0.0142**
	BC×Plant[3]	**0.0493**	0.1240	**0.0316**	0.2743	0.1270
Metabolic quotient (qCO_2)	**BC[1]**	0.1213	**0.0007**	0.1881	0.4391	0.1080[4]
	Plant[2]	**<.0001**	0.4418	0.1609	**0.0073**	**0.0003**[4]
	BC×Plant[3]	**0.0103**	**0.0008**	0.9101	0.1561	**0.0022**[4]

Note: Bolded and underlined *P*- values denote significant differences at α= 0.1.

[1] 'BC' represents black carbon addition in soils- coke, biochar or NoBC.

[2] 'Plant' denotes soil pots with and without aspen planted.

[3] 'BC×plant' means the interaction between them.

[4] The data were Log transformed to meet the assumptions of ANOVA.

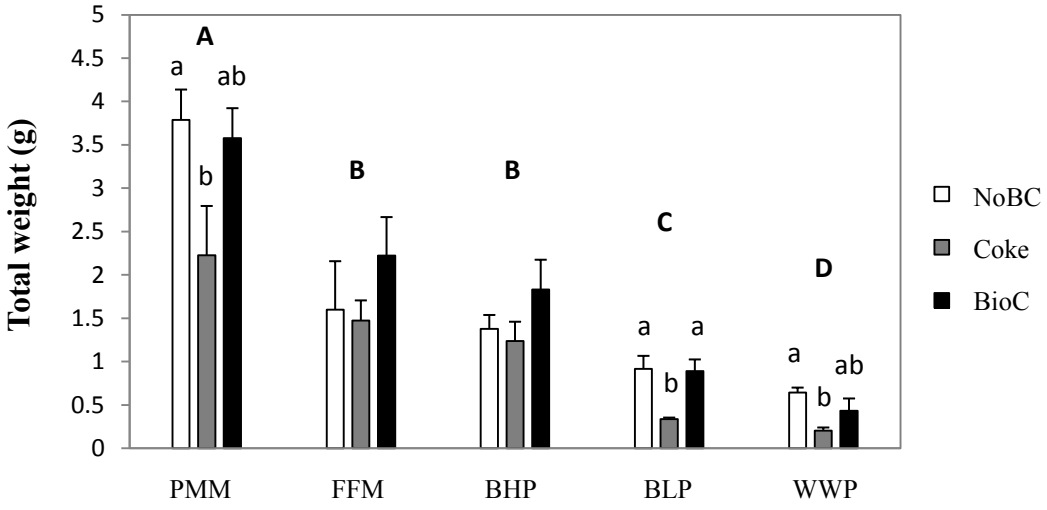


Figure 2.1 Total aspen biomass (mean ± standard error) after six months of growth in the five soil types- PMM, FFM, BHP, BLP and WWP. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at $\alpha = 0.1/10 = 0.01$. Lower case letters above bars denote significant differences among three BC treatments at $\alpha = 0.1/3 = 0.0333$ among BC treatments in a given soil type.

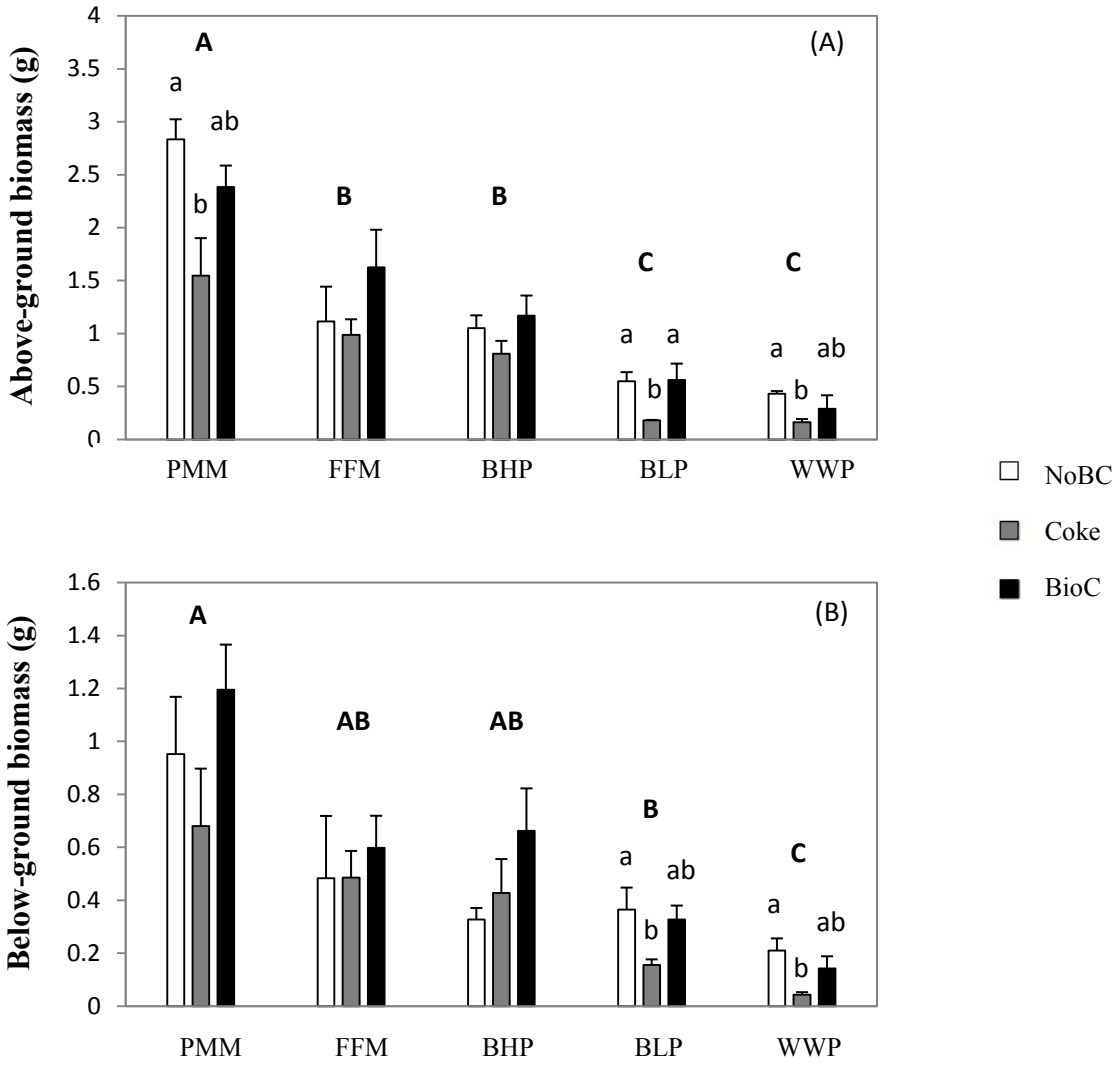


Figure 2.2 (A) above-ground biomass and (B) below-ground biomass of aspen (mean ± standard error) after six months of growth in the five soil types- PMM, FFM, BHP, BLP and WWP. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at $\alpha = 0.1/10 = 0.01$. Lower case letters above bars denote significant differences among three BC treatments at $\alpha = 0.1/3 = 0.0333$ among BC treatments in a given soil type.

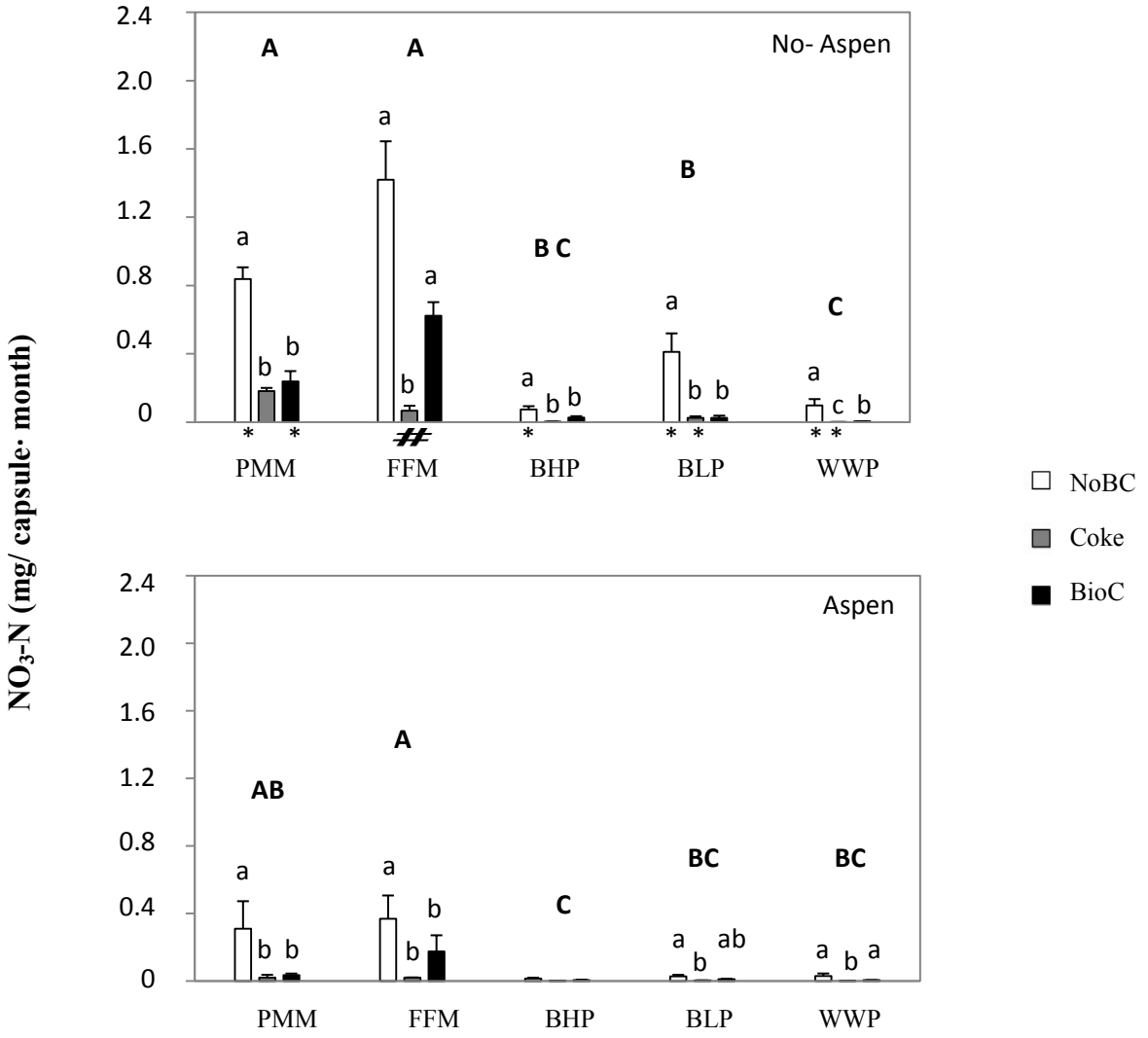


Figure 2.3 NO_3-N (mean ± standard error) in each soil treatment of five soil types- PMM, FFM, BHP, BLP, WWP. 'Aspen' represents soil pots with aspen planted; No-Aspen is pots without aspen planted. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at α= 0.1/10= 0.01; lower case letters above bars denote significant differences among three BC treatments at α=0.1/6= 0.0167 among BC treatments in a given soil type. '*' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type and BC treatment when there is a significant Biochar× Plant interaction; '#' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type, if there is no significant Biochar× Plant interaction.

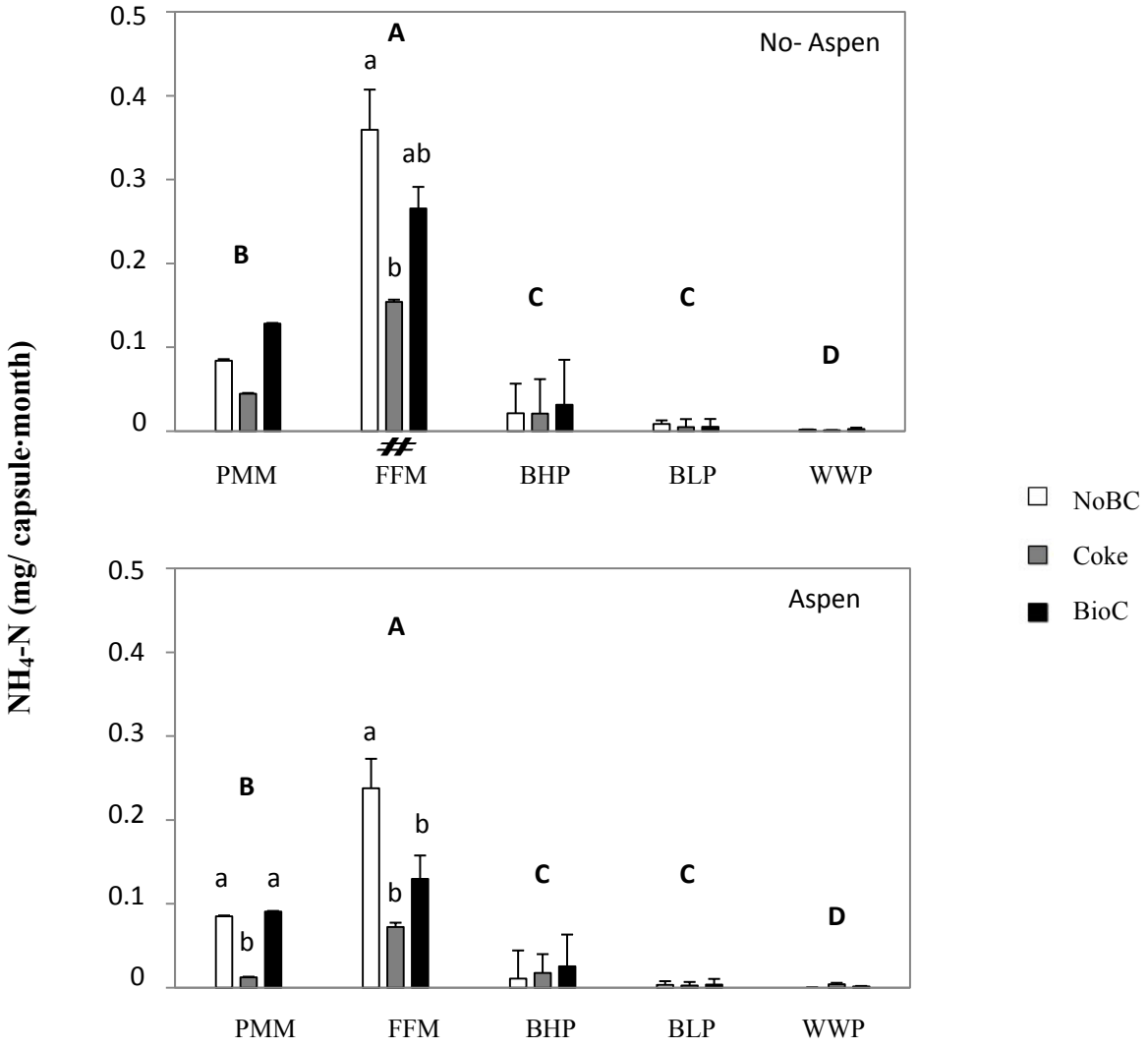


Figure 2.4 NH_4-N (mean ± standard error) in each soil treatment of five soil types- PMM, FFM, BHP, BLP, WWP. 'Aspen' represents soil pots with aspen planted; No-Aspen is pots without aspen planted. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at α= 0.1/10= 0.01; lower case letters above bars denote significant differences among three BC treatments at α=0.1/6= 0.0167 among BC treatments in a given soil type. '*' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type and BC treatment when there is a significant Biochar× Plant interaction; '#' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type, if there is no significant Biochar× Plant interaction.

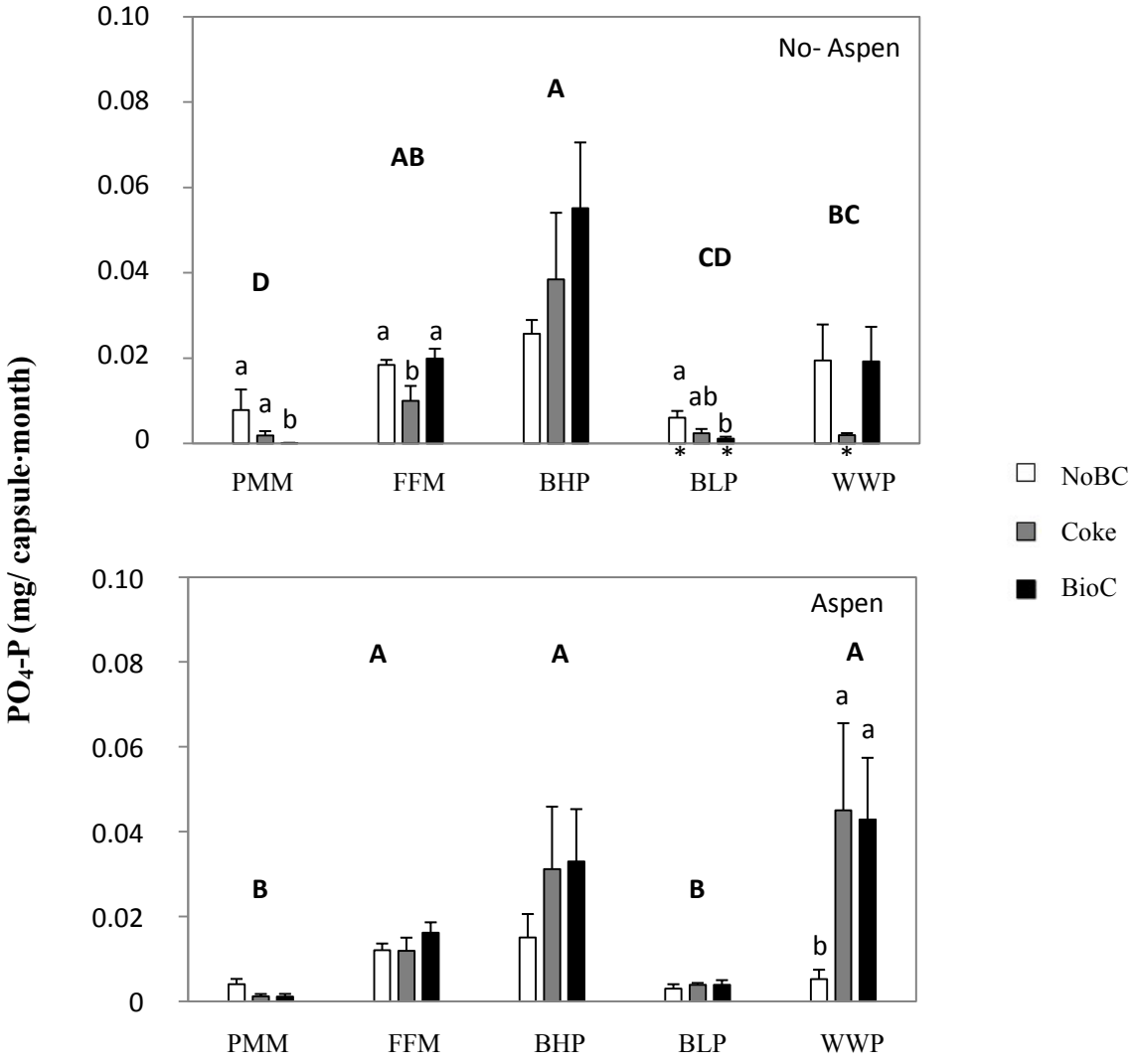


Figure 2.5 PO_4-P (mean ± standard error) in each soil treatment of five soil types- PMM, FFM, BHP, BLP, WWP. 'Aspen' represents soil pots with aspen planted; No- Aspen is pots without aspen planted. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at α= 0.1/10= 0.01; lower case letters above bars denote significant differences among three BC treatments at α=0.1/6= 0.0167 among BC treatments in a given soil type. '*' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type and BC treatment.

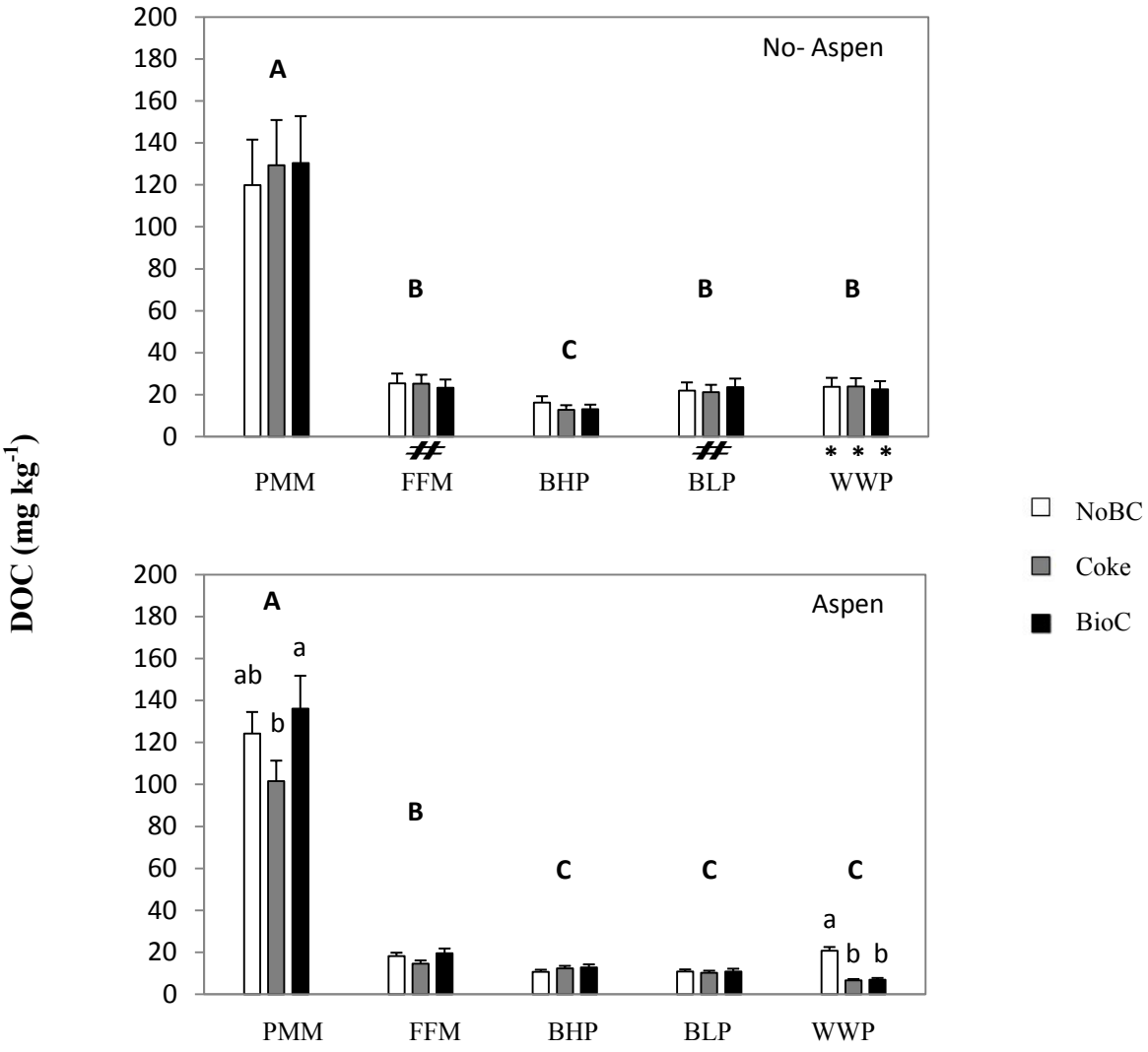


Figure 2.6 Dissolved organic carbon (DOC) (mean ± standard error) in each soil treatment of five soil types- PMM, FFM, BHP, BLP, WWP. 'Aspen' represents soil pots with aspen planted; No- Aspen is pots without aspen planted. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at α= 0.1/10= 0.01; lower case letters above bars denote significant differences among three BC treatments at α=0.1/6= 0.0167 among BC treatments in a given soil type. '*' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type and BC treatment when there is a significant Biochar× Plant interaction; '#' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type, if there is no significant Biochar× Plant interaction.

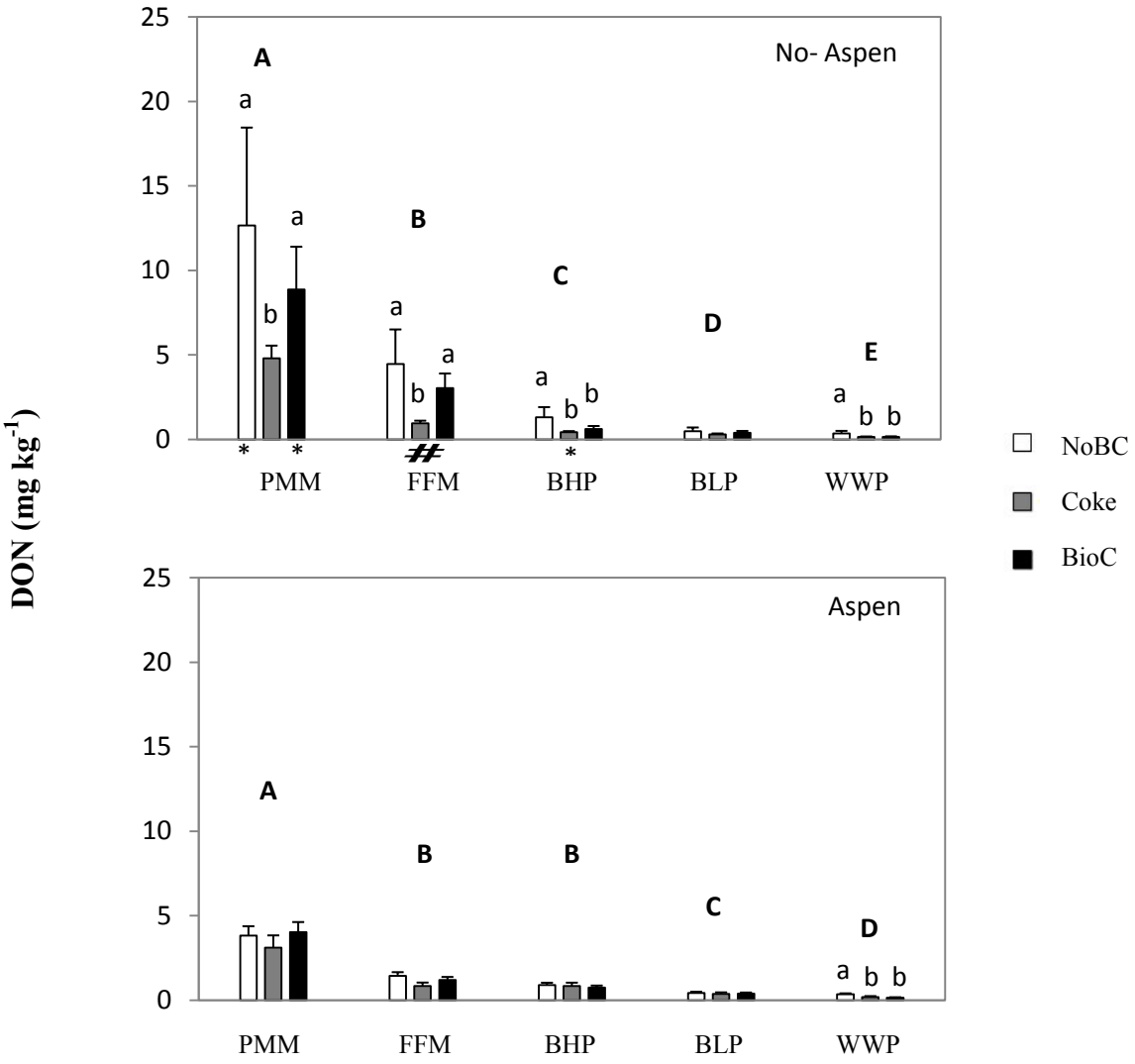


Figure 2.7 Dissolved organic nitrogen (DON) (mean ± standard error) in each soil treatment of five soil types- PMM, FFM, BHP, BLP, WWP. 'Aspen' represents soil pots with aspen planted; No- Aspen is pots without aspen planted. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at α= 0.1/10= 0.01; lower case letters above bars denote significant differences among three BC treatments at α=0.1/6= 0.0167 among BC treatments in a given soil type. '*' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type and BC treatment when there is a significant Biochar× Plant interaction; '#' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type, if there is no significant Biochar× Plant interaction.

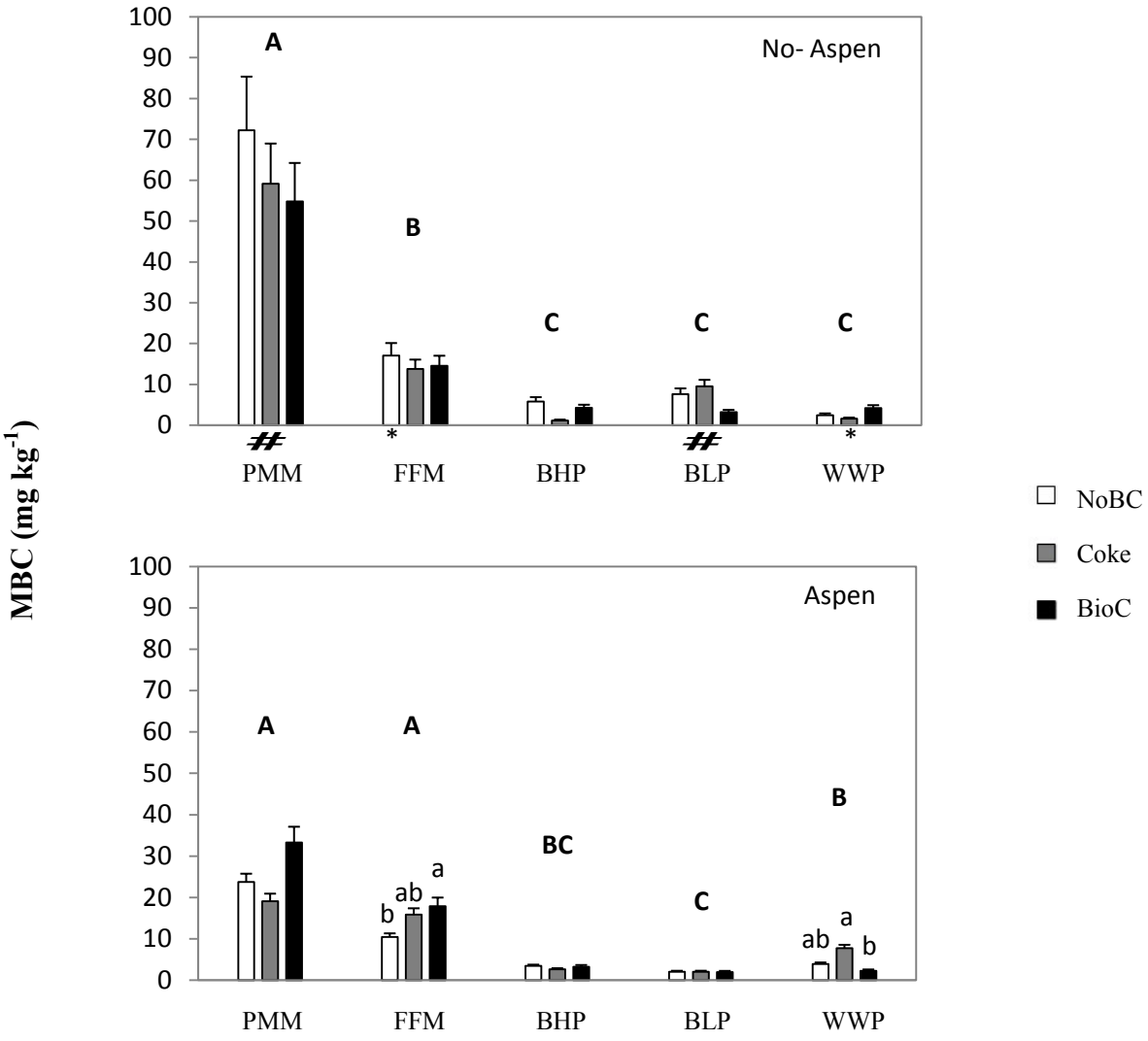


Figure 2.8 Microbial biomass carbon (MBC) (mean ± standard error) in each soil treatment of five soil types- PMM, FFM, BHP, BLP, and WWP. 'Aspen' represents soil pots with aspen planted; No- Aspen is pots without aspen planted. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at α= 0.1/10= 0.01; lower case letters above bars denote significant differences among three BC treatments at α=0.1/6= 0.0167 among BC treatments in a given soil type. '*' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type and BC treatment when there is a significant Biochar× Plant interaction; '#' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type, if there is no significant Biochar× Plant interaction.

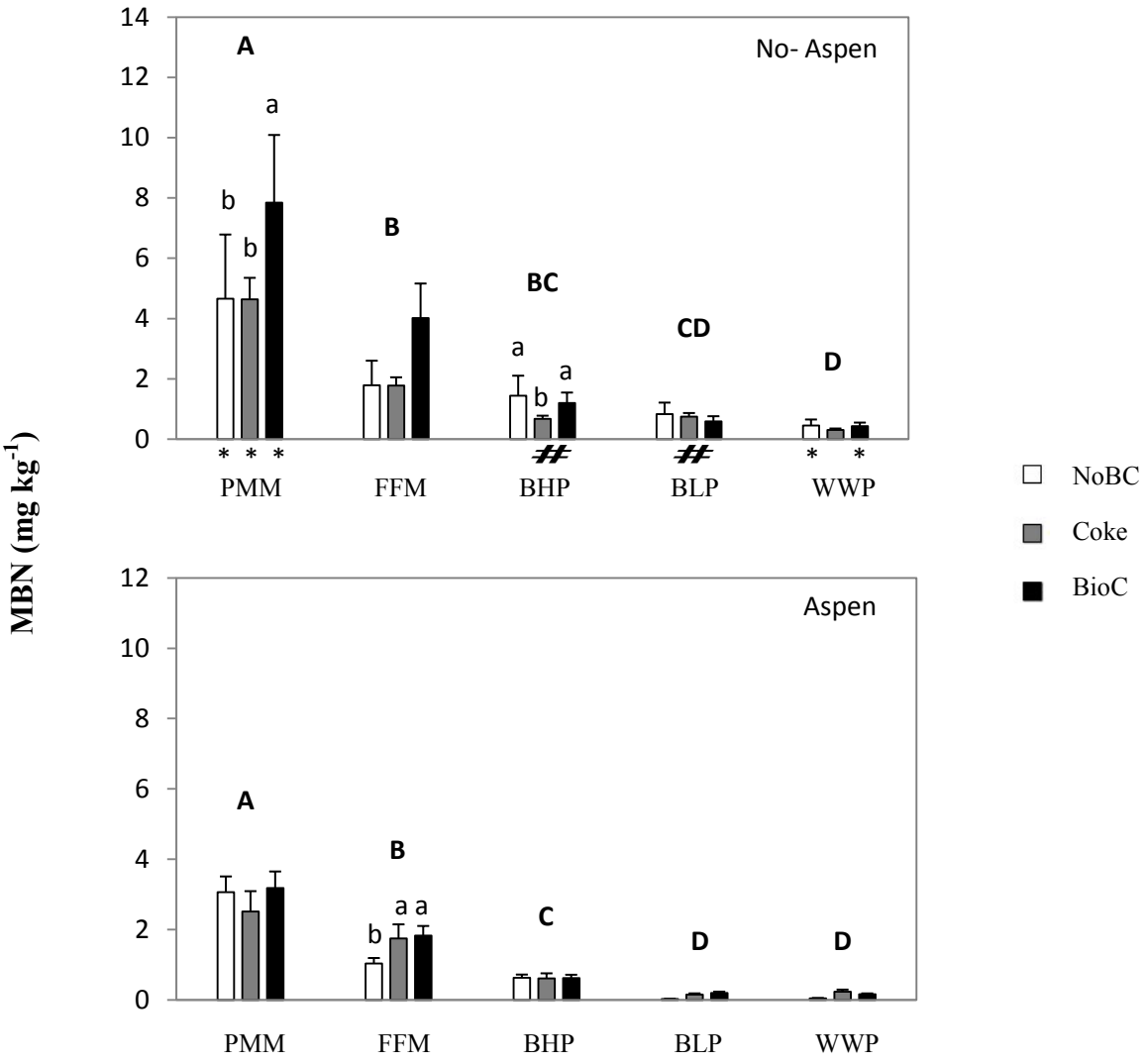


Figure 2.9 Microbial biomass nitrogen (MBN) (mean ± standard error) in each soil treatment of five soil types- PMM, FFM, BHP, BLP, WWP. 'Aspen' represents soil pots with aspen planted; No- Aspen is pots without aspen planted. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at α= 0.1/10= 0.01; lower case letters above bars denote significant differences among three BC treatments at α=0.1/6= 0.0167 among BC treatments in a given soil type. '*' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type and BC treatment when there is a significant Biochar× Plant interaction; '#' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type, if there is no significant Biochar× Plant interaction.

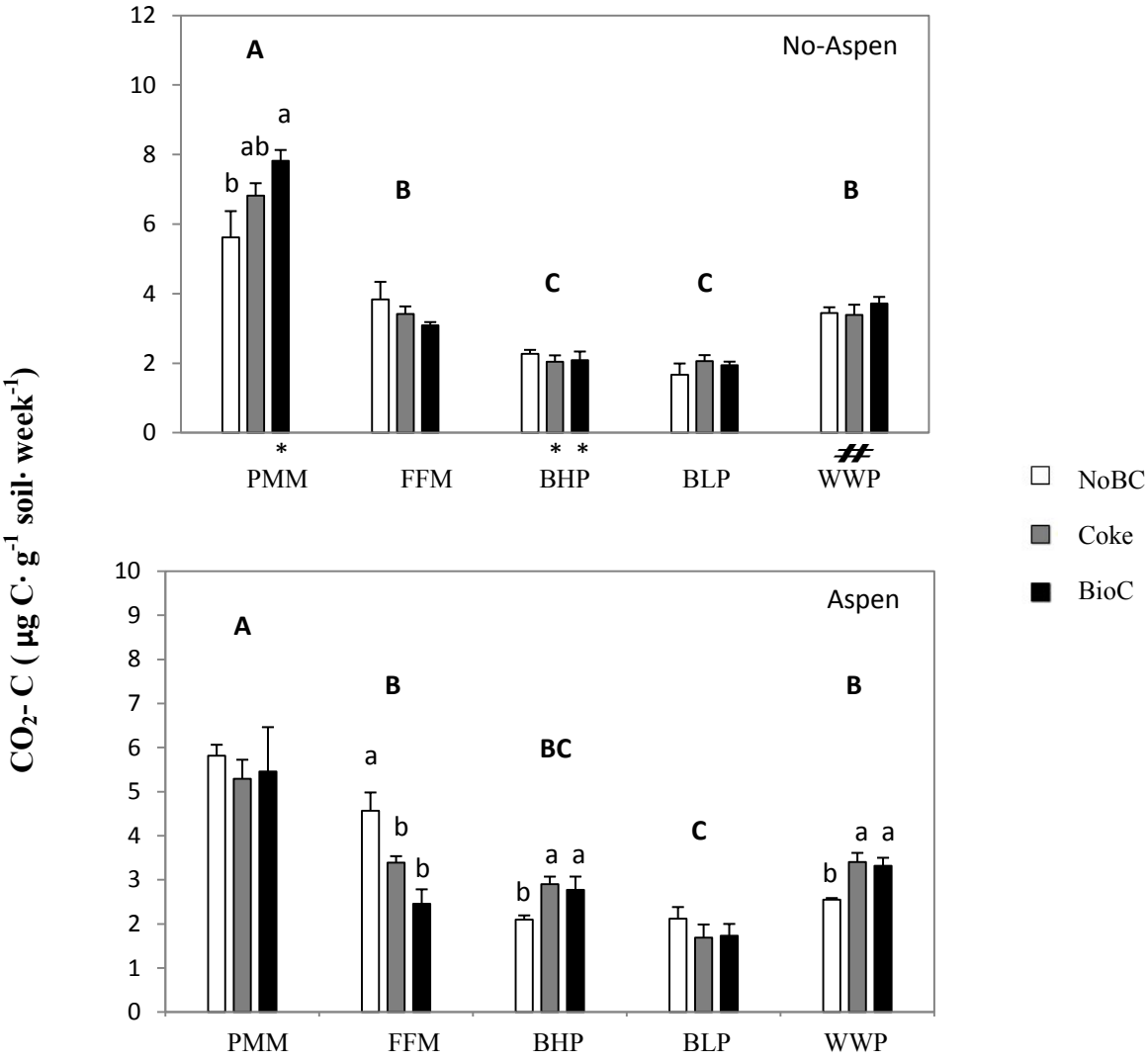


Figure 2.10 Soil heterotrophic respiration (CO_2 emission) (mean ± standard error) in each soil treatment of five soil types- PMM, FFM, BHP, BLP, WWP. 'Aspen' represents soil pots with aspen planted; No- Aspen is pots without aspen planted. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at α= 0.1/10= 0.01; lower case letters above bars denote significant differences among three BC treatments α=0.1/6= 0.0167 among BC treatments in a given soil type. '*' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type and BC treatment when there is a significant Biochar× Plant interaction; '#' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type, if there is no significant Biochar× Plant interaction.

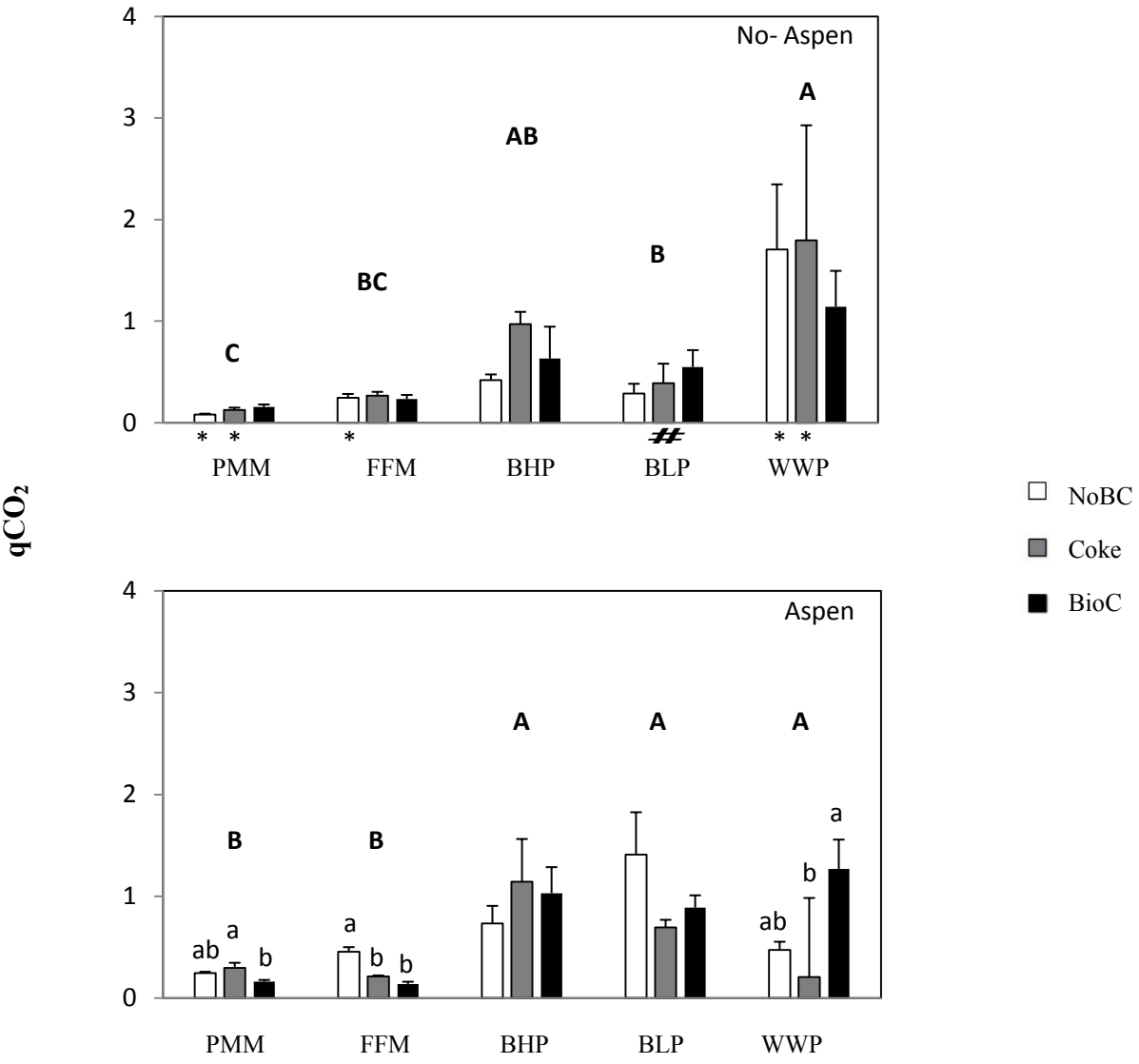


Figure 2.11 Metabolic quotient (qCO_2) (mean ± standard error) of each soil treatment of five soil types- PMM, FFM, BHP, BLP, WWP. 'Aspen' represents soil pots with aspen planted; No- Aspen is pots without aspen planted. 'NoBC' means no black carbon addition in soils; 'Coke' represents soils with petroleum-coke addition; 'BioC' represents soils with biochar addition. Upper case letters above bars denote significant differences among five soil types at α= 0.1/10= 0.01; lower case letters above bars denote significant differences among three BC treatments at α=0.1/6= 0.0167 among BC treatments in a given soil type. '*' denotes significant differences at α= 0.1 between Aspen and No-Aspen treatments for a given soil type and BC treatment when there is a significant Biochar× Plant interaction; '#' denotes significant differences at α= 0.1 between Aspen and No- Aspen treatments for a given soil type, if there is no significant Biochar× Plant interaction.

Chapter 3: Effect of biochar and fertilizer addition on soil nutrients, microbial biomass, microbial activity and heavy metal adsorption in coal mine land reclamation soils

3.1 Introduction

Surface mining has a huge influence on soil and vegetation, by disrupting natural ecosystems and causing severe ecological damage in mining areas around the world (Dudka et al., 1997; Rathore et al., 1993; Visser et al., 1979). By the end of 2012, oil sands mining in the Athabasca region had disturbed 767 km^2 of boreal ecosystems (Alberta Energy, Government of Alberta, 2012), causing tremendous ecological damage to the air, water, soil and plants around it. Over 310 km^2 forest areas in Alberta has been disturbed by coal mining by the end of 2010 (Government of Alberta, 2010). The Environmental Protection and Enhancement Act (EPEA), enacted by the Alberta government, requires mining companies to reclaim disturbed soils so that they are "capable of supporting a diverse, self-sustaining, locally similar boreal forest landscape, regardless of the end land use" (Government of Alberta, 2014). The reclaimed areas are also required to support a land capability equivalent to pre-mining state (Cumulative Environmental Management Association, 2012).

During the past decades, a popular approach to reclaim surface mining sites in Canada is to salvage peat or forest floor material from wetlands and forests nearby (Fung and Macyk, 2000). These organic soil materials have very low bulk density and high nutrients, so it can improve the physical, chemical and biological properties of soil when blended into subsurface geological material (McMillan et al., 2007; MacKenzie and Naeth, 2007& 2009). However, this method is limited by the availability of material.

Biochar is a product derived from oxygen limited combustion of organic materials, and could be used as an amendment for land reclamation (Sohi et al., 2010). Many studies in different plant communities showed that biochar can improve soil nutrient availability, increase microbial activity, improve soil nutrient retention and adsorb/immobilize heavy metals (Lehmann and Joseph, 2009; Sohi et al., 2010). Recently, biochar has been used as an amendment to soil during land reclamation processes (Tejada, Garcia et al. 2006). It benefits the soil by improving the soil physical structure, reducing nutrient leaching loss, and improving plant yield (Fellet, et al, 2011; Lehmann et al., 2006; Krull et al. 2009; Beesley et al, 2011). Biochar could retain nutrients in soil and prevent nutrients from leaching loss (Steiner, 2007; Fellet et al., 2011). The reason could be attributed to biochar's large surface area and porous structure. Because biochar has high porosity and surface to volume ratio (Chan et al. 2007), its application to soil has been shown to significantly decrease the leaching loss of fertilizer- provided nutrients (Lehmann et al., 2003; Chan et al., 2007; Steiner et al., 2008). However, most previous studies focused on the application of biochar to farmland to improve crop growth, so its usage in land reclamation and forest ecosystem reconstruction needs more study.

Coal mining activities have also caused severe heavy metal contaminations in mining areas across the world (Massey et al., 1972; Moffat, 1995; Schmidt, 1997). Land reclamation often focuses on the re-establishment of surface soil and plant communities; however, soil contamination is a potential problem which requires equal attention and needs to be resolved simultaneously. Numerous researchers have found that both surface and underground mining have the potential to contaminate the post- mining areas by bringing toxic heavy metals to the surface, such as lead (Pb), zinc (Zn), cadmium (Cd)

and copper (Cu). These elements can have major adverse effects on vegetation and microorganisms living in soil (Massey et al., 1972; Adamo et al., 1996). These metals in soil are generally difficult to remove and remediation can be very expensive (Moffat, 1995). Further, the residence time of heavy metals in soil can be hundreds or even thousands of years (McGrath, 1987). If mining areas are reclaimed to farmland, heavy metals in soil lead to phytoxicity and can decrease crop yields (Schmidt, 1997). The worse consequence is that the accumulation of heavy metals in crops will enter the food chain and possibly be consumed by animals and humans (Soler-Rovira et al., 1996). Thus, monitoring and removal/immobilization of heavy metals is of great importance during the reclamation process. The essence to resolve the metal pollution problem is to decrease the metal mobilization and plant available metals. Therefore, in order to reclaim mining sites, researchers have recently focused on approaches which can improve the soil properties and solve the heavy metal problem at the same time. It has been well documented that biochar can adsorb and immobilize heavy metals in contaminated soil (Debela et al, 2012; Karam et al, 2011; Uchimiya et al, 2010). Since heavy metals are toxic to plants and microbes, lower availability of heavy metals will also lead to better plant growth, higher microbial biomass and microbial activity (Jonnalagadda et al., 2006).

The objective of this study was to determine whether application of biochar and fertilizer improves soil nutrients, microbial biomass and activity, and decreases the concentration of soil bio-available heavy metals. Since coal mining activities have caused severe heavy metal contamination in mining areas across the world, I expected that surface mining led to severe heavy metals contamination (such as As, Cd, Cr, Pb, etc.) at our Whitewood (WW) experimental site, and biochar amendment will decrease the

amount of available heavy metals in WW coal mine soil. I also expected that biochar could retain fertilize provided nutrients and biochar+fertilizer amendment would confer a greater benefit on microbial activity and soil nutrient availability than applications of biochar or fertilizer alone.

3.2 Materials and methods

Research site and Experimental Design

The research site was located at the Whitewood coal mine, which is north of Lake Wabamun, about 70 kilometers west of Edmonton, Alberta (lat 53°33' N, long 114°29'W). The coal mine was in operation from 1962 until 2010. Since the top soil was removed during the mining processes and used to reclaim an agricultural area nearby, marginal soil was left on the surface of post-mining areas, which is low in soil organic matter and nutrient content. The soil on our experimental site is C horizon soil/parent geological material of a Black- Dark Gray Chenozem or Gray Luvisol based on the Canadian system of soil classification, with the following properties: clay loam texture, pH 8.1, total C 2.4%(confirmed), total N 0.05%(confirmed), available NO_3-N 10.7 mg kg^{-1}, available NH_4-N 1.34 mg kg^{-1}, available P 0.58 mg $kg^{-1}$and available K 16 mg kg^{-1}. The soil was sieved through a 2-mm sieve and homogenized for the incubation experiment.

Six 0.5 ha areas on the mining site were selected as our experimental sites (blocks). The experiment is an incomplete factorial design replicated in blocks. In each block treatments were applied, including: a) application of biochar (two levels: biochar or no biochar); b) application of fertilizer (three levels: high, low, no fertilizer). These two

treatments were applied in a factorial design (2x3=6 treatment combinations). Aspen trees were planted in the entire block except for in one central area which included a 2x2 factorial design with: the two levels of biochar, and two of the fertilizer treatments (low fertilizer and no fertilizer); thus the 'no tree' area had four of the six treatment combinations (Fig.3.1).

The lodgepole pine- derived biochar used in our experiment was produced by a slow pyrolysis process (at 400°C- 500°C for 2 hours). The biochar had a pH of 7.3, total C of 56%, total N of 1.3% and bulk density of 232 kg m^{-3}. Lodgepole pine (*Pinus contorta*)- derived biochar was applied at a rate of 1.67 ton/ha (0.54% by volume) in October, 2011. The biochar was spread manually on the plots and then mixed with the PGM by a tractor with a disk harrow to a depth of 20cm. Aspen seedlings were planted at a density of 6000 trees/ha in early May of 2012. Controlled- release fertilizer was obtained from/ was produced by Agrium Advanced Technologies Direct Solutions (Western Canada- Horticulture, Calgary, AB, Canada). The fertilizer had 19% N (ammonium nitrogen 8.09%, nitrate nitrogen 7.16%, urea nitrogen 3.75%), 6% P (P_2O_5), 13% K (K_2O), 4.7% S, 1% Mg (0.5 % water soluble Mg), 1.2% Fe (1.18 % water soluble Fe and 0.02% chelated Fe), 0.1% Mn (0.079% water soluble Mn), 0.002% Mo (0.002% water soluble Mo), 0.1% Zn (0.079% water soluble Zn) and 0.1 % Cu (0.079% water soluble Cu). At the end of June, the fertilizer (15- 9- 12- 6; N, P, K, S) was applied at two rates (LowFert & HighFert, equivalent to 50 kg Nitrogen/ha and 100 kg N/ha, respectively); the fertilizer was spread by seed spreader manually onto soil but not disked in.

Soil sampling and analyses

Soil samples were taken by both a metal soil probe 2.5cm in diameter (for measurement of Total C (TC), Total N (TN), pH, dissolved organic carbon and nitrogen (DOC and DON), microbial biomass carbon and nitrogen (MBC and MBN), soil respiration and heavy metals) and soil sample rings 7.5cm in diameter and 10cm in depth (for measurement of bulk density only) at the end of August, 2012. For soil probe samples, about 500g soil (for each treatment in each plot, soil was taken from ten random spots, and then was mixed into one sample) was collected from 0-25cm depth below the soil surface. The soil samples (both soil probe samples and soil core samples) were transferred to freezer bags (Ziploc) immediately after collection (each sample was put into one freezer bag), and then they were put into a cooler with ice packs inside to keep them fresh. About 200g of soil probe samples from each treatment was air dried for two weeks at 25°C for pH, total C and N measurement. Soil pH was measured by a pH meter in the laboratory. 5g of dry sample was placed into a beaker, 10g of deionized water was added and then stirred for 10min. After the soil was suspended for 1 hour, a pH meter (Mettler-Toledo, Ohio, USA) was calibrated and used to measure soil pH (Kalra and Maynard, 1991). For TC and TN measurement, soil samples were ground at the frequency of 15 Hz (900 min^{-1}) for 1 min into 5μm particles (Retsch MM400 mixer mill), and then 10mg of each sample was transferred onto a small tin capsule and wrapped up tightly. Total C and N content were measured by the dry combustion method (Costech Analytical Technologies Inc., Valencia, CA, USA)(Nelson and Sommers, 1982). The bulk density was measured by soil samples taken using soil rings (one sample for each treatment was taken in each plot). Soil samples in soil rings were transferred to aluminum cases, oven dried at 105°C for 24h and then weighed. The ring height and radius were

measured by a ruler in cm to the nearest mm. The bulk density was calculated by the following formula (Cresswell and Hamilton, 2002): a) Soil volume = ring volume (cm^3) = $\pi \times$ radius$^2 \times$ ring height (cm); b) Bulk density (g/cm^3) = Dry soil weight (g)/Soil volume (cm^3).

Nutrient availability

Plant Root Simulator (PRS™) probes use an ion exchange membrane (Western Ag Innovations, Inc. Saskatoon, Canada) to assess soil nutrient availability. The probes were used in cation/anion pairs to measure root available ions. Prior to use, ion exchange membranes were saturated with a counter-ion (HCO_3^- for anion probes and Na^+ for cation probes), allowing them to absorb soil ions (Western Ag Innovations Inc., 2014). The probes measure nutrient availability to plant roots by ion exchange with soil across the probe membrane over the burial period. In late June two pairs of plant root simulator (PRS) probes (one pair include one cation and one anion PRS probe) were installed under the soil surface at each of two diagonal corners of each 2×2 m^2 sub-plot; there were two sub-plots in each treatment in the tree areas and one in each treatment in the no-tree areas (described in Fig. 3.1). A soil knife was used to dig a hole in the soil to 15cm depth, and then the probe was pushed vertically into the hole until completely buried, only leaving the a piece of flagging tape above the soil surface. After burial, the soil on top of the probes was back cut with a soil knife to ensure thorough contact with the probe membrane. The probes were collected after 8 weeks of burial, cleaned thoroughly with de-ionized water, placed in ziplock bags and sent back to Western Ag Innovations, Saskatoon, Canada for analysis of available N (NO_3-N, NH_4- N), available P (PO_4-P), K

and S. At Western Ag, the NO_3^--N and NH_4- N were measured colorimetrically by an automated flow injection analysis system using the Lachat QuikChem AE Automated Flow Injection Ion Analyzer (United States Environmental Protection Agency, 1991). Available P (PO_4^-), K and S were measured by inductively-coupled plasma spectrometry (ICP-OES, Thermo Fisher, USA).

Heavy metal analyses

A common method to assess total metal concentration in soils is extraction in nitric acid or *aqua regia* (Sims and Eivazi, 1997*).* Although the measurement of total metal content in soil is important in estimating pollution, it does not provide any information about the metal mobility or toxicity (Levei et al., 2010). In order to measure the bio-available heavy metal in soil, many sequential extraction methods have been developed. The Diethylene Triamine Pentaacetic Acid (DTPA) and Ethylene Diamine Tetraacetic Acid (EDTA) extraction schemes are two most effective methods to measure the available heavy metal in polluted soil. So the available metal in this experiment was measured by EDTA method (Quevauviller, et al., 1996).

The total and bio-available heavy metal levels in soil were determined by the acid extraction and EDTA method, respectively (Quevauviller, et al., 1996). To quantify the heavy metals (As, Cr, Cd and Pb), 20g of soil from each sample was oven-dried at 105◦C for 24 h, then ground in a ball mill (Retsch MM400 mixer mill). To assess total heavy metals, 0.4g of each dry soil sample was put into a test tube, 10ml concentrated nitric acid was added to each test tube and microwave digested for 10 min at 1600W(185°C). Total As, Cd, Cr and Pb concentrations were measured by an inductively coupled plasma optical emissions spectrometer (ICP-OES) (Thermo Fisher, USA). To assess the available

heavy metals (As, Cr, Cd and Pb), 0.4g of each dry soil sample was mixed with the 50 ml 0.5M EDTA solution and shaken for 1 hour on a reciprocal shaker. The suspension was filtered through a Buchner funnel with Q2 filter paper. Available As, Cd, Cr and Pb concentrations were measured by an inductively coupled plasma optical emissions spectrometer (ICP-OES) (Thermo Fisher, USA).

Microbial biomass carbon and nitrogen

The Chloroform- Fumigation Extraction (CFE) method was used to determine the soil microbial biomass (Vance et al., 1987). Two portions of soils (one sample to be fumigated & the other not fumigated) were taken from each treatment, and then 25g of each sample was placed into 100mL glass beakers. One sample was fumigated with $CHCl_3$ in a dessicator lined with wet filter paper to maintain humidity. Then soil samples were put inside a desicator and about 30mL ethanol-free $CHCl_3$ was added into a small beaker with a few boiling chips in it. The dessicator was evacuated after the $CHCl_3$ boiled for 2 minutes, and then placed in the dark at 25°C with $CHCl_3$ vapor for 48 hours. After 48 hours, the dessicator was evacuated again in order to refill $CHCl_3$ vapor in the dessicator and the samples left for fumigation for another 48 hours. Both fumigated and unfumigated samples were put in to 150-200mL plastic bottles along with 50mL of 0.5M K_2SO_4. The bottles were placed on a reciprocal shaker and shaken for 30 minutes. The suspension was filtered through a Buchner funnel with Q2 filter paper. The fumigated and unfumigated samples were analyzed for dissolved organic carbon (DOC) by TOC-V_{CSN}, Total Organic Analyzer (ManDel Scientific Instrument Inc., Canada), and dissolved organic nitrogen (DON) by TOC- V_{CSN}, Total Organic Analyzer with a TNM1 accessory. The microbial biomass carbon and nitrogen (MBC and MBN) were calculated

according the formulas: MBC= $DOC_{fumigated} - DOC_{unfumigated}$; MBN= $DON_{fumigated} - DON_{unfumigated}$.

Soil basal respiration and metabolic quotient (qCO_2)

To quantify soil basal respiration, 50g of soil from each treatment was placed into a mason jar along with a vial containing 20mL of 0.5M NaOH. There were two empty jars for control (blanks) with 20mL of 0.5M NaOH in them. Jars were capped and placed in the dark at 25° C. After one week of incubation, the NaOH solution was titrated to determine how much CO_2 had been trapped. For this, 1ml $BaCl_2$ and 3 drops of phenolphthalein were added into each vial and 0.5M HCl was used to titrate the NaOH solution until it turned clear (when the solution pH was 8.8). Then the amount of CO_2 per vial was calculated according to the amount of 0.5M HCl added, using the formula: CO_2-C (mg/kg soil) = $(A_1\text{-}A_2) \times N \times E \times D$. A_1 represents titrant added to blank (ml), A_2 represents titrant added to test sample (ml), N represents concentration of HCl=0.5mol/L, E represents the coefficient, and D represents dilution factor 4 (only 5 out of 20 ml of NaOH for each sample was used).

Metabolic quotient (qCO_2) refers to the ratio of soil basal respiration to microbial biomass. The qCO_2 was calculated with the following formula (Anderson and Domsch, 1985): *qCO_2= soil microbial respiration/MBC*

Statistical analysis

Since there is a random effect (site or block), PROC MIXED in SAS 9.2 was used to conduct statistical analysis. Three different mixed models were used to examine the

influence of the different factors involved in this trial. There was one random factor- block (site1/2/3/4/5/6), and three fixed factors- aspen (tree/notree), biochar (control/biochar) and fertilizer (control/low/high), which could be included in the models (Table 3.1). As noted above, all three fertilizer levels were included within the area in which trees were planted, whereas only control and low fertilizer were included in the area without tree planted (Fig.3.1). For available nutrients measured by PRS probes, each 2×2 small quadrat had two pairs of PRS probes. We extracted the ions on two pairs (1 for cation and the other for anion in each pair) together and we had two sub- plots within each tree plot, so we averaged the values (available nutrients measured by PRS probes) from the two sub- plots prior to statistical analysis.

We had three models for statistical analysis: Model 1 was set up to examine the influence of planting aspen, biochar, fertilizer (two levels), and their interactions. It was a split -plot design including block (random), aspen (fixed, two levels), biochar (fixed, two levels), and fertilizer (fixed, two levels). In this model, aspen was the main plot, the biochar was the split-plot, and the fertilizer was the split- split- plot. Since there was no high fertilizer in the areas without tree planted, the high fertilizer level was omitted in this model. In Model 1, if there was a significant interaction involving tree (Tree×Biochar, Tree×Fertilizer or a Tree×Biochar×Fertilizer interaction), then a post-hoc analysis was done to compare Tree vs Notree for each Fertilizer×Biochar combination separately (α= 0.1/4= 0.025), otherwise Model 2 and 3 were used to explore further for any significant interactions involving biochar and fertilizer.

Model 2 was set up to examine significant effects of biochar (biochar and CK), fertilizer (0, low and high level of fertilizer) and their interactions (α= 0.1). It included

only the area in which trees were planted. In this model, if only fertilizer was significant, I compared among the three fertilizer levels (α= 0.1/3= 0.033) while ignoring the biochar effect; if only the biochar was significant, then no post-hoc analysis is needed; if there was a significant interaction, then I compared among the six biochar×fertilizer treatment combinations (α= 0.1/15= 0.00667).

Model 3 was set up to examine the significant effects of biochar (biochar and CK), fertilizer (0 and low level of fertilizer application) and their interactions while the high level of fertilizer treatment was not included (α= 0.1). It included only the area in which no tree was planted. In this model, if only the fertilizer or biochar was significant, then no post-hoc analysis is needed; if there was a significant interaction, then I compared among the four biochar×fertilizerertilizer treatment combinations (α= 0.1/4= 0.025).

For these analyses, residuals were examined for the assumptions of normality and homogeneity. If they did not meet the assumptions, these data were subject to a log transformation when necessary to meet these assumptions. Following significant (α= 0.1) main effects in the mixed models, post-hoc comparisons of means were conducted to further examine differences. The α-value for these post-hoc comparisons was adjusted by the number of comparisons. For example, in Model1, if there were significant interaction involving tree treatment, I compared among treatments for Tree and No-tree separately with the α= 0.1; if there was an interaction between Biochar and Tree treatments, I made 6 comparisons- pairwise among the treatment combinations, and I adjusted α by 0.1/6= 0.01667.

3.3 Results

Total C and total N content

The ANOVA result showed that low fertilization (LF) treatments had significantly higher soil total C (TC) and total N (TN) content than unfertilized treatments in Model 1 in which only two fertilizer levels were considered (Table 3.1; Fig 3.2 & 3.3). In Model 2 (model for the tree areas), there was a significant Biochar×Fertilization interaction for TN, but post-hoc analysis did not show any significant difference among the treatments. Model 3 (model for the no-tree areas) suggested that TN was significantly higher in LF treatments than the unfertilized treatments.

Soil pH

The ANOVA result showed significant effects of low fertilizer on soil pH in Model 1 in which only two fertilizer levels were considered (Table 3.1; Fig 3.4). The Low Fertilizer treatment had significantly lower soil pH than the no fertilization treatment (Model 2, Table 3.1). Model 3 showed the same trend that the Low Fertilizer treatment had significantly lower soil pH than the no fertilization treatment in the areas without tree planted (p=0.02, Fig 3.4).

Available nutrients

There was a significant effect of LF treatment and a significant three way interaction on NO_3-N in Model 1 (Model 1, Table 3.1, Fig 3.5a). Model 2 suggested that there was a significant effect of fertilization and a significant biochar×fertilizer interaction on NO_3-N (Model 2, Table 3.1). The post-hoc test suggested that the soil with HF treatment (HF and BC+HF) had significantly higher NO_3-N than the unfertilized soil (CK and BC) in the

tree areas. Model 3 showed that the soil with LF treatment (LF and BC+LF) had significantly higher NO_3-N than the unfertilized soil (CK and BC) in the areas without tree planted (Model 3, Table 3.1).

There was a significant effect of Tree× Fertilization interaction on the NH_4-N in Model 1(Table 3.1, Fig.3.5b), but post-hoc test did not show any significant effect. There were no significant effects on the available P or K in any of the models. For available S, the ANOVA results in Model 1 (Table 3.1) showed the available S in tree areas was significantly higher than in the areas without trees planted (for the CK, BC, BC+LF and LF treatments).

Total and available heavy metals

There were significant effects of fertilization on total and available Cr content, Tree×Biochar interaction on total Cr content, and Tree×Fertilization interaction on the total and available Cd content in Model 1(Table 3.1; Fig 3.6 &3.7). In Model 2, the only significant effect was that the high level of fertilization increased the available Cr content (Table 3.1). Model 3 (areas without trees) suggested that the LF treatment significantly increased the total Cd, Cr and Pb, and available Cd and Pb irrespective of biochar treatment (Table 3.1).

DOC, DON, MBC and MBN

Model 1suggested that DOC was significantly higher in biochar amended treatments than in the un-amended treatments (P=0.02) (Table 3.1, Fig.3.8). Model 2 showed that the DOC and DON were significantly higher in the BC amended soil than in the un-amended soil in areas where trees were planted (Table 3.1, Fig.3.8). In areas

without aspen planted (Model 3), the DOC was also significantly higher in the BC amended soil than in the CK (Table 3.1, Fig.3.8). For the microbial biomass, all models suggested that MBC and MBN were significantly higher in biochar amended treatments than in the un-amended treatments for both tree and No-tree areas (Table 3.1, Fig.3.9).

Soil heterotrophic respiration and qCO2

Model 1 suggested that there were significant effects of Tree×Biochar interaction on soil heterotrophic respiration (CO_2 emission) and metabolic quotient (Table 3.1; Fig 3.10). The biochar application significantly increased the soil heterotrophic respiration in the areas without tree planted (Model 3, Table 3.1). However, Model 2 and 3 showed no significant effect of fertilizer or biochar on the metabolic quotient.

3.4 Discussion

Effect of biochar and fertilizer application on total C, total N content and soil pH

Our experiment suggested that fertilization significantly increased the TC. This could be due to fertilization induced tree growth, which could lead to more fine roots and root exudates in the soil of the fertilized areas increasing the TC content. Fertilization increased TN and decreased the soil pH in the areas without aspen planted, but not in the aspen areas. The influence of fertilization on soil pH could be explained by the fact that the fertilizer applied on our research site contained a large proportion of NH_4NO_3 (15.25% wt), which lowers the soil pH when applied as a soil amendment. The application of biochar (biochar has a pH of 8.4) had the potential to increase the soil pH, but we found no such effect. Since the application rate of biochar on our research site was not high

(1.67 tons/ha), it seemed that the soil had the ability to buffer its pH against any changes due to the biochar application.

In contrast to our hypothesis, biochar application did not increase the soil total C (TC) content. The reason was unclear and there are several possibilities. There were visible coal fragments and residues left on the soil surface after mining and these would have been part of the carbon affecting TC in the soil samples. Biochar was applied to the experimental sites in October, 2011, but soil samples were taken in August, 2012. The wind and water erosion was severe on the research field since there was little plant or organic layer coverage, so another reason could be that soil erosion had removed part of the biochar we applied.

Effect of biochar and fertilizer application on available nutrients

After mining activities, surface horizons are removed leaving the parent geological material on the land surface which leaves the soil in poor nutrient condition. So improving the soil nutrients is the priority before rebuilding the plant and microbial communities. Fertilizer is a commonly used amendment to provide soil nutrients (Pichtel et al., 1992). Our results also suggested that fertilization significantly increased the nitrate and ammonia content in soil. However, the results did not show an increased available P, K or S in post- mining site after fertilization. The reason may be that the fertilizer we used was slow release granules, but the PRS probes were collected 8 weeks after installation, so a large portion of fertilizer might not have been fully released into the soil. Another possibility may be that the P and K are less mobile than N in soil, so we did not find any significant difference in P and K among the treatments.

Previous studies (Steiner, 2007; Fellet et al., 2011) have shown that biochar can help retain nutrients in soil and prevent nutrient leaching losses. Because biochar has a large surface area, it might retain nutrients and prevent leaching loss after the rainfall. Lehmann et al. (2003) found that amendment of biochar significantly decreased the leaching loss of fertilizer provided N. Other studies also suggested that application of biochar together with an N fertilizer will could retain nutrients in biochar particles and prevent losses (Chan et al., 2007; Steiner et al., 2008). This can be attributed to biochar's high porosity and surface to volume ratio (Chan et al. 2007). Our results supported this in the tree areas where soil with the BC+ HF combination had higher nitrate than CK or BC, while HF only was not significantly higher than the CK or BC treatment. In the areas without trees planted, the BC+LF combination had higher nitrate than the BC only or the CK, while LF treatment did not have higher nitrate than the CK or BC treatments. These results suggest that biochar is capable of helping retain fertilizer- provided nutrients in soil.

Effect of biochar and fertilizer application on total and available heavy metals

It is well reported that application of biochar as a soil amendment can reduce heavy metals in soil (Beesley et al., 2011; Gomez-Eyles et al. 2011;). Gomez-Eyles et al. (2011) found biochar can significantly reduce the available Cd and Cu after 1-2 months of biochar application. In a soil column study, Beesley et al. (2011) observed that biochar can significantly reduce the concentration of Cd and Zn in leachates. Biochar with high oxygen functional groups is effective for immobilizing heavy metals, especially Pb^{2+}, Cu^{2+} in acidic and low CEC soils (Wulfsberg, 2000). Other researchers also found functional groups in Biochar can also help to immobilize the heavy metals in

contaminated soil. For example, Uchimiya et al. (2012) found that oxidized biochar that is richer in carboxyl functional groups has significantly greater effects on Pb, Cu, and Zn immobilization compared to untreated biochar. This can be attributed to the fact that cations such as Pb and Cd can combine with the carboxyl functional groups and form complexes on the biochar surface (Utrilla, et al., 2002). These studies also suggested that oxidized biochar will be more recalcitrant and rich in carboxyl functional groups which can persist longer and have a long-term effect on heavy metal absorption. However, according to the CCME (Canadian Council of Ministers of the Environment) Soil and Water Guidelines (Eckford and Gao, 2009), the concentration of the heavy metals I measured- As, Cd, Cr and Pb did not exceed the threshold values for agricultural or parks/residential use; nor did my study show any evidence that biochar can absorb or immobilize total or available heavy metals in soil. For the effect of biochar and fertilizer combination, Karami et al. (2011) found that compost and biochar had a joint effect on reducing Pb concentrations in pore water and plant uptake. But in our study, we did not find any evidence that biochar had combined effect with fertilizer on heavy metal absorption or immobilization.

We found that fertilization increased the total and available Cd, Cr and Pb in the soil without trees planted; in the tree areas, there was only an increase of available Cr in the HF treatment. The reason could be that the fertilizer contained a large proportion of NH_4NO_3, and the NO_3^- could increase the solubility of heavy metals in the soil (Kevresan et al., 1998). There was less NO_3^- in the tree areas because of uptake by aspen, so the increase of the available heavy metals in the tree areas was not evident.

Effect of biochar and fertilizer application on DOC, DON, MBC and MBN

Our result suggested that biochar significantly increased the soil DOC, DON, MBC and MBN. This was consistent with previous studies which also suggested that biochar was able to retain nutrients in soil and increase the available nutrients in soil (Steiner, 2007; Fellet et al., 2011). Laird et al. (2010) also found that biochar at an application rate of 20 g kg^{-1} decreased total N leaching by 11%. Higher DOC and DON provided more nutrients to soil microbes, so in my experiment, the soil microbial biomass data was in accordance with the soil dissolved nutrient data. Steinbeiss et al. (2009) reported that biochar can promote fungi and Gram-negative bacteria in a silty soil. So our result was also consistent with previous findings where application of biochar increased the soil microbial biomass, which was indicated by the increased MBC and MBN.

Effect of biochar and fertilizer application on soil heterotrophic respiration

Previous studies suggested that biochar had many positive effects on soil microorganisms, because the huge surface area and porous structure of biochar provide proper shelter for soil microorganisms, which also makes them less vulnerable to be leached away in soils (Pietikäinen et al., 2000; Fellet et al., 2011). Improved living conditions can increase the soil microbial population and more soil microorganisms can generate more CO_2. Jones et al. (2012) found that biochar addition can increase soil respiration. Our study indicated that the soil microbial respiration was higher after biochar amendment compared with the control soil taken in the areas without trees planted. This was also consistent with our soil microbial biomass results, which suggested that biochar have a positive effect on soil microorganisms, and more soil microbes generated more CO_2. But in the soil taken from the areas with tree planted, we did not observe any significant effect of biochar on CO_2 emission. The soil heterotrophic

respiration also tended to be higher in the areas without trees planted than in the tree areas (Fig. 3.9a), so the increase of the microbial respiration was not significant in the tree areas. The reason was not clear. One possibility could be that the tree and microbes were competing for N and other nutrients in the soil. So there were less microbes in the soil samples due to lack of nutrients, and less microbes generated less CO_2.

3.5 Implications and future research

Land reclamation previously focused on the re-establishment of plant communities and the soil recovery, and paid little attention to the heavy metal immobilization. Even though in our study the concentration of heavy metals in the study area did not exceed the standard in the CCME Soil and Water Guidelines (Eckford and Gao, 2009), the heavy metal is a vital problem in post-mining areas around the world. Reclamation methods which are applied to minimize level of contamination after mining activities are crucial to achieve our goal of land reclamation, and the impact of biochar on decreasing the toxicity of heavy metal and other contaminants left in soils after mining need more studies in the future.

After mining at Whitewood, the subsoil exposed to the surface is a sandy loam, but previous research found that biochar can benefit the sandy soil more than clay soil from preventing nutrient's leaching. Thus, future studies could investigate the effect of biochar on nutrient retention in sandy soils. Further, the wind erosion on the research site is severe due to lack of plant cover, so part of the biochar and fertilizer we applied was blown away. Thus, in the future, the establishment of plant communities should be prior to application of biochar and fertilizer to fix the soil.

Table 3.1 Results of Mixed Model Analyses of Variance (P-values are given) for soil available nutrients at the Whitewood coal mine reclamation site measured by PRS probes. (a) is for Mixed Model 1 including the influence of aspen, biochar, fertilization treatments (none and low level of fertilization) and their interactions; (b) is for Mixed Model 2 including only the areas that were planted with trees and examining the effect of biochar, fertilization treatments (all three levels) and their interactions; (c) is for Mixed Model 3 including the no-tree areas only and examining the effect of biochar, fertilization treatments (none and low level of fertilization) and their interactions.

(a)

	TC	TN	pH	NO_3-N	NH_4-N	P	K	S
T[1]	0.37	0.42	0.40	0.96	0.31	0.62	0.11	**0.08**
B[2]	0.29	0.32	0.90	0.94	0.64	0.65	0.67	0.92
T[1]× B[2]	0.15	0.12	0.59	0.20	0.36	0.66	0.23	0.49
F[3]	**0.07**	**0.07**	**0.03**	**0.02**	0.68	0.63	0.51	0.35
T[1]× F[3]	0.81	0.97	0.28	0.85	**0.06**	0.78	0.55	0.67
B× F[3]	0.32	0.85	0.17	0.95	0.59	0.12	0.67	0.74
T[1]× B[2]× F[3]	0.46	0.18	0.47	**0.05**	0.67	0.63	0.99	0.87

	As(Ttl)	Cd(Ttl)	Cr(Ttl)	Pb(Ttl)	As(Avl)	Cd(Avl)	Cr(Avl)	Pb(Avl)
T[1]	0.59	0.40	0.46	0.44	0.13	0.39	0.71	0.28
B[2]	0.77	0.39	0.82	0.82	0.57	0.31	0.88	0.98
T[1]× B[2]	0.60	0.70	**0.05**	0.67	0.81	0.96	0.68	0.95
F[3]	0.24	0.27	**0.04**	0.31	0.79	0.17	**0.08**	0.35
T[1]×F	0.72	**0.04**	0.30	0.13	0.56	**0.08**	0.48	0.27
B× F[3]	0.76	0.49	0.73	0.87	0.85	0.94	0.97	0.80
T[1]× B[2]× F[3]	0.44	0.26	0.90	0.52	0.38	0.13	0.18	0.52

	DOC	DON[4]	MBC	MBN	CO_2	qCO_2
T[1]	0.63	0.7599	0.63	0.40	0.60	0.79
B[2]	**0.02**	0.1145	**0.03**	**0.03**	0.14	0.67
T[1]× B[2]	0.91	0.8644	0.91	0.78	**0.04**	**0.10**
F[3]	0.65	0.4725	0.36	0.90	0.42	0.26
T[1]× F[3]	0.88	0.2589	0.54	0.61	0.49	0.31
B[2]× F[3]	0.89	0.8588	0.74	0.96	0.96	0.76
T[1]× B[2]× F[3]	0.67	0.3924	0.31	0.11	0.73	0.31

(b)

	TC	TN	pH	NO_3-N	NH_4-N	P	K	S
B^2	0.23	0.41	0.75	1.00	0.86	0.69	0.31	0.75
F^3	0.39	0.41	0.58	**0.07**	0.66	0.68	0.58	0.48
$B^2 \times F^3$	0.19	**0.10**	0.41	**0.08**	0.82	0.19	0.76	0.85

	As(Ttl)	Cd(Ttl)	Cr(Ttl)	Pb(Ttl)	As(Avl)	Cd(Avl)	Cr(Avl)	Pb(Avl)
B^2	0.48	0.90	0.14	0.95	0.69	0.57	0.87	0.97
F^3	0.30	0.79	0.36	0.93	0.82	0.88	**0.03**	0.99
$B^2 \times F^3$	0.58	0.50	0.88	0.87	0.81	0.61	0.55	0.97

	DOC	DON^4	MBC	MBN	CO_2	qCO_2
B^2	**0.02**	**0.02**	**0.07**	0.35	0.92	0.31
F^3	0.91	0.21	0.73	0.59	0.41	0.52
$B^2 \times F^3$	0.72	0.25	0.69	0.57	0.72	0.82

(c)

	TC	TN	pH	NO_3-N	NH_4-N	P	K	S
B^2	0.79	0.52	0.75	0.35	0.29	1.00	0.59	0.57
F^3	0.16	**0.05**	**0.02**	**0.05**	**0.08**	0.89	0.96	0.71
$B^2 \times F^3$	0.23	0.20	0.11	0.13	0.93	0.48	0.78	0.91

	As(Ttl)	Cd(Ttl)	Cr(Ttl)	Pb(Ttl)	As(Avl)	Cd(Avl)	Cr(Avl)	Pb(Avl)
B^2	0.50	0.23	0.24	0.55	0.57	0.31	0.75	0.92
F^3	0.20	**0.01**	**0.05**	**0.03**	0.15	**<0.01**	0.16	**0.04**
$B^2 \times F^3$	0.70	0.66	0.76	0.67	0.25	0.14	0.46	0.33

	DOC	DON	MBC	MBN	CO_2	qCO_2
B^2	**0.07**	0.36	**0.02**	**0.08**	**0.02**	0.16
F^3	0.81	0.79	0.72	0.78	0.94	0.91
$B^2 \times F^3$	0.82	0.50	0.42	0.25	0.78	0.43

Note: Significant P values are bolded. * indicated the data were not normally distributed and were Log transformed.

[1] 'T' represents aspen treatment (plot with or without aspen planted).

[2] 'B' represents biochar treatment.

[3] 'F' represents fertilizer treatment.

[4] Log transformed data.

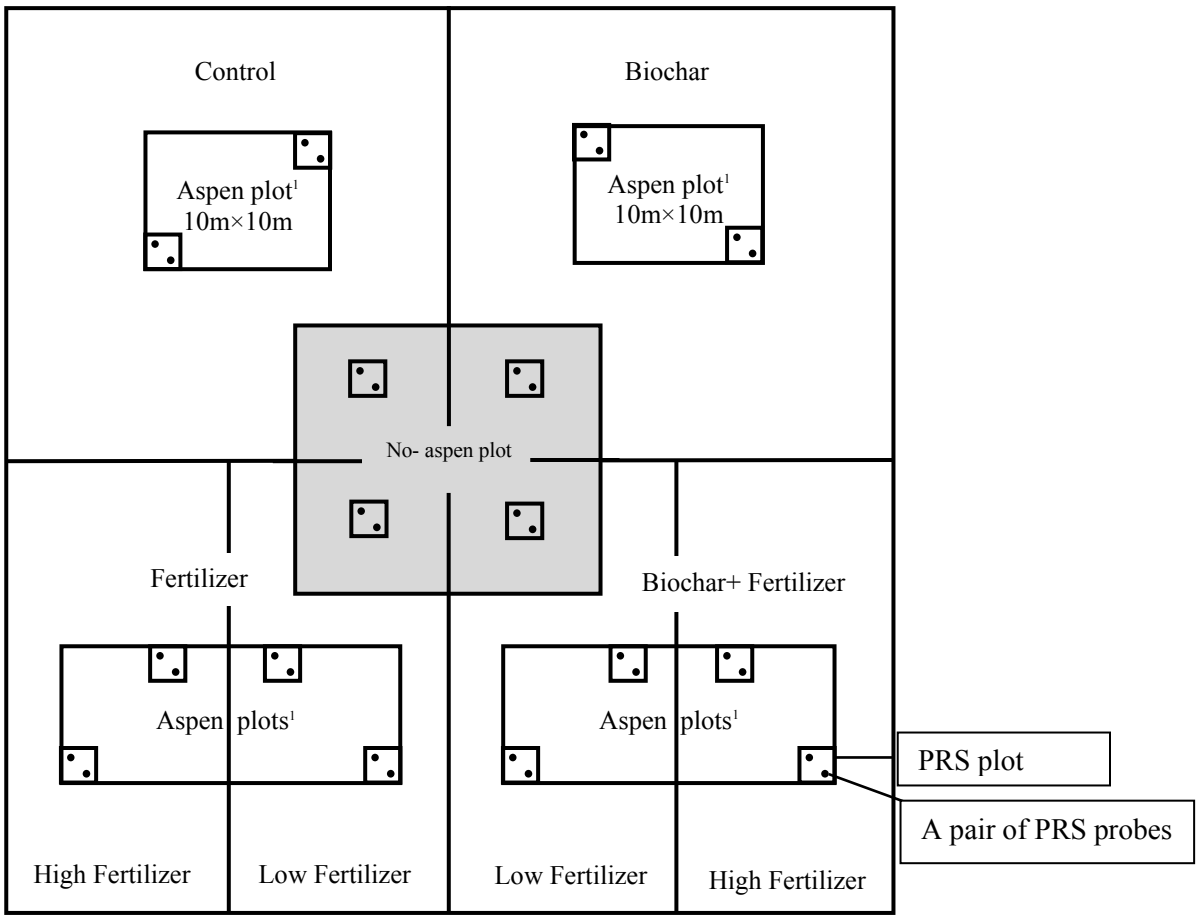


Figure 3.1 Experimental design for plots at the Whitewood mine reclamation site. The entire areas was planted with aspen except for the 'No- aspen plot' area.

[1] The aspen plots were areas within which aspen were being monitored as part of another study.

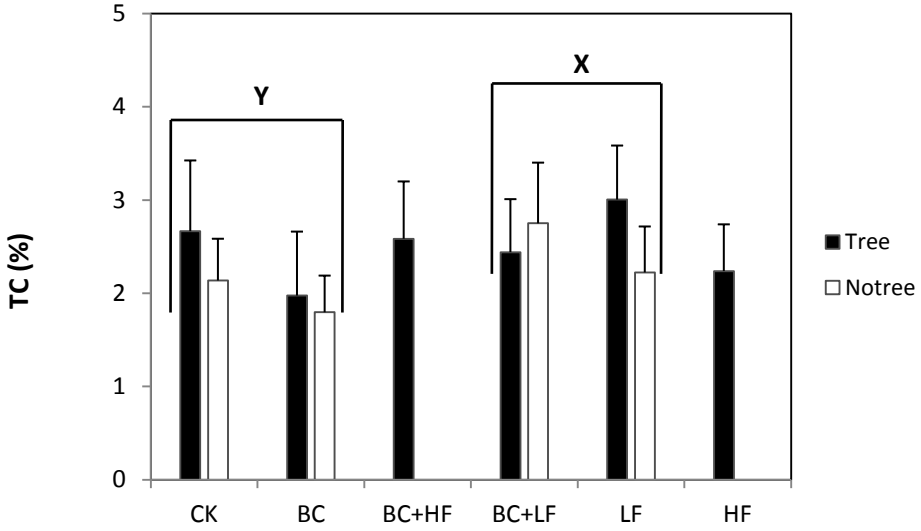


Figure 3.2 Total soil carbon (%) (mean ± standard error) in the soil samples taken from the Whitewood coal mine reclamation site for the different experimental treatments: planting of aspen trees (tree vs no tree), biochar addition, fertilization. CK = control (no biochar, no fertilizer); BC = biochar only, BC+HF = biochar and high fertilizer, BC+LF= biochar and low fertilizer, LF = low fertilizer alone, HF = high fertilizer alone. Upper case letters 'X' and 'Y' above bars denote ANOVA results from Model 1, which show significant effects of fertilizer (0 versus LF) irrespective of tree and biochar.

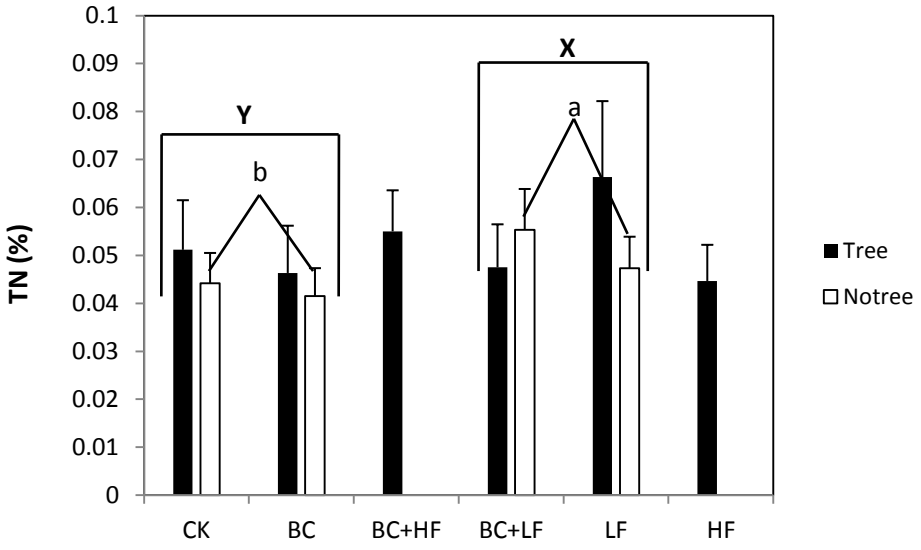


Figure 3.3 Total soil nitrogen (%) (mean ± standard error) in the soil samples taken from the Whitewood coal mine reclamation site for the different experimental treatments: planting of aspen trees (tree vs no tree), biochar addition, fertilization. CK = control (no biochar, no fertilizer); BC = biochar only, BC+HF = biochar and high fertilizer, BC+LF= biochar and low fertilizer, LF = low fertilizer alone, HF = high fertilizer alone. Upper case letters 'X' and 'Y' above bars denote ANOVA results from Model 1, which show significant effects of fertilizer (0 versus LF) irrespective of tree or biochar. Lower case letters 'a' and 'b' above bars denote ANOVA results from Model 3(no-tree areas), which show significant effects of fertilizer (0 versus LF) irrespective of biochar.

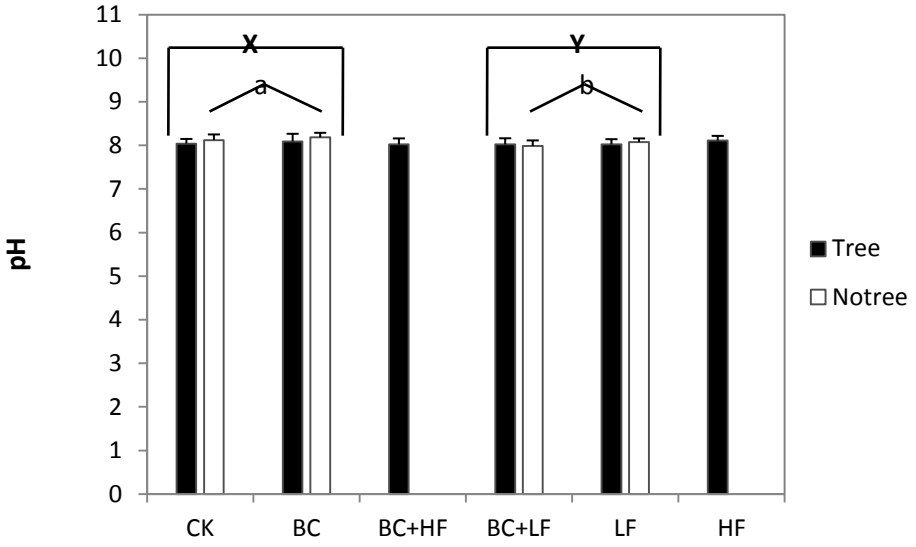
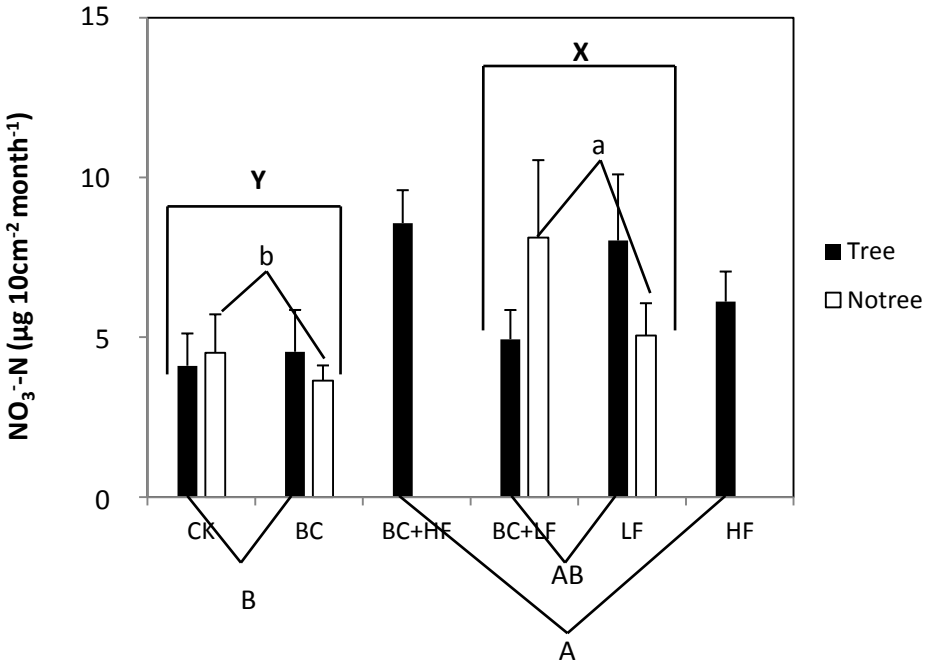
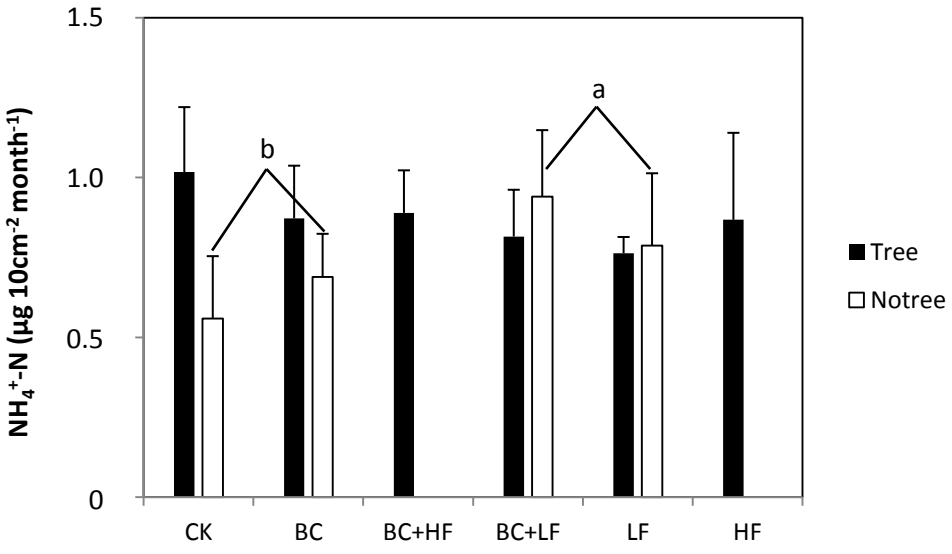


Figure 3.4 pH (mean ± standard error) of the soil samples taken from the Whitewood coal mine reclamation site for the different experimental treatments: planting of aspen trees (tree vs no tree), biochar addition, fertilization. CK = control (no biochar, no fertilizer); BC = biochar only, BC+HF = biochar and high fertilizer, BC+LF= biochar and low fertilizer, LF = low fertilizer alone, HF = high fertilizer alone. Upper case letters 'X' and 'Y' above bars denote ANOVA results from Model 1, which show significant effects of fertilizer (0 versus LF) irrespective of tree or biochar. Lower case letters 'a' and 'b' above bars denote ANOVA results from Model 3(no-tree areas), which show significant effects of fertilizer (0 versus LF) irrespective of biochar.

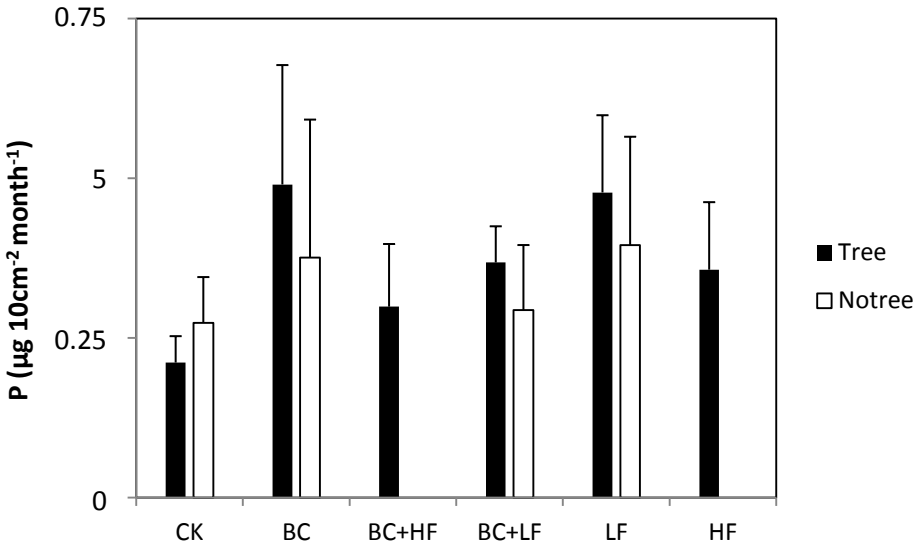
a)


15
10
5
0
NO_3^--N (µg 10cm^{-2} month^{-1})
X
Y
a
b
Tree
Notree
CK
BC
BC+HF
BC+LF
LF
HF
B
AB
A

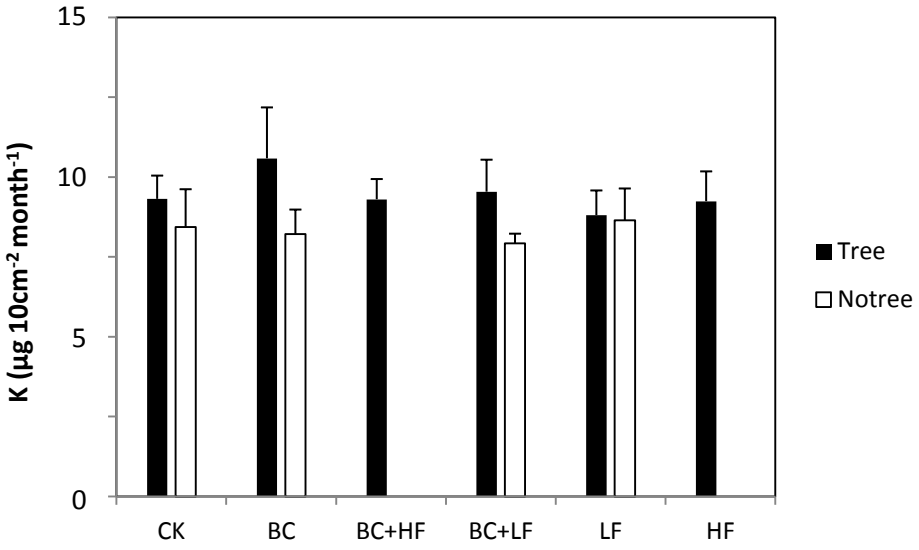
b)


1.5
1.0
0.5
0
NH_4^+-N (µg 10cm^{-2} month^{-1})
a
b
Tree
Notree
CK
BC
BC+HF
BC+LF
LF
HF

c)


0.75
5
0.25
0
P (µg 10cm-2 month-1)
CK
BC
BC+HF
BC+LF
LF
HF
Tree
Notree

d)


15
10
5
0
K (µg 10cm-2 month-1)
CK
BC
BC+HF
BC+LF
LF
HF
Tree
Notree

e)

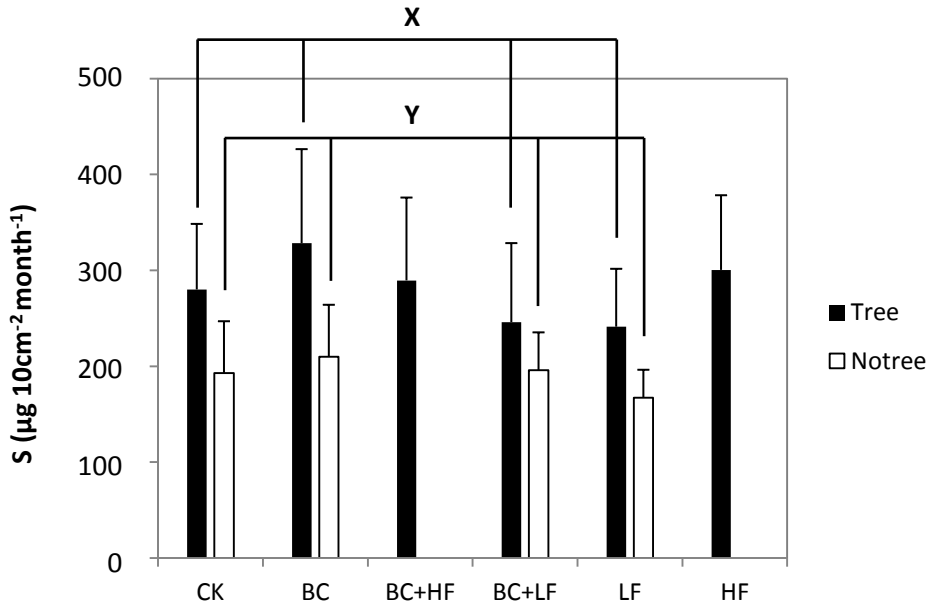
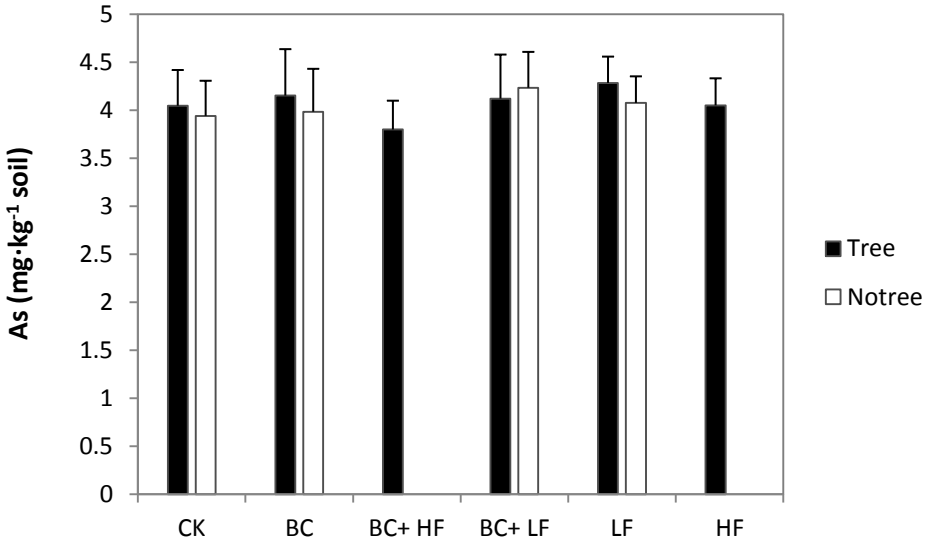
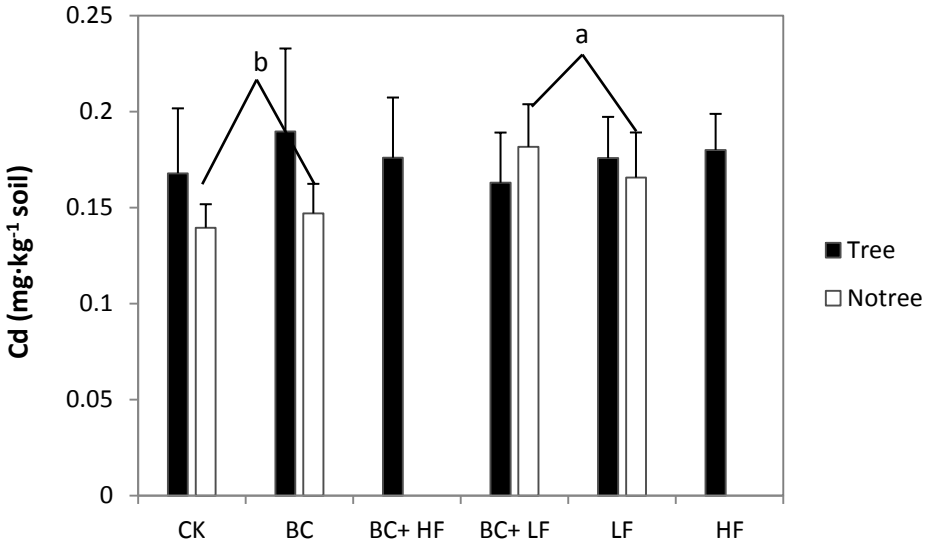


Figure 3.5 Available a) NO_3-N, b) NH_4-N, c) PO_4-P, d)K, e) S (µg/10cm^2) (mean ± standard error) measured by PRS probes in the Tree and Notree model in the Whitewood coal mine reclamation site for the different experimental treatments: planting of aspen trees (tree vs no tree), biochar addition, fertilization. CK = control (no biochar, no fertilizer); BC = biochar only, BC+HF = biochar and high fertilizer, BC+LF= biochar and low fertilizer, LF = low fertilizer alone, HF = high fertilizer alone. Upper case letters 'X' and 'Y' above bars denote ANOVA results from Model 1, which in (a) show significant effects of fertilizer (0 versus LF) irrespective of tree or biochar and in (e) show significant effects of tree, irrespective of fertilizer or biochar. Upper case letters below the bars in (a) denote ANOVA results from Model 2 (tree areas), which show significant effects of fertilizer (0 versus LF or HF) irrespective of biochar. Lower case letters above the bars in (a) and (b) denote ANOVA results from Model 3(no-tree areas), which show significant effects of fertilizer, irrespective of biochar.

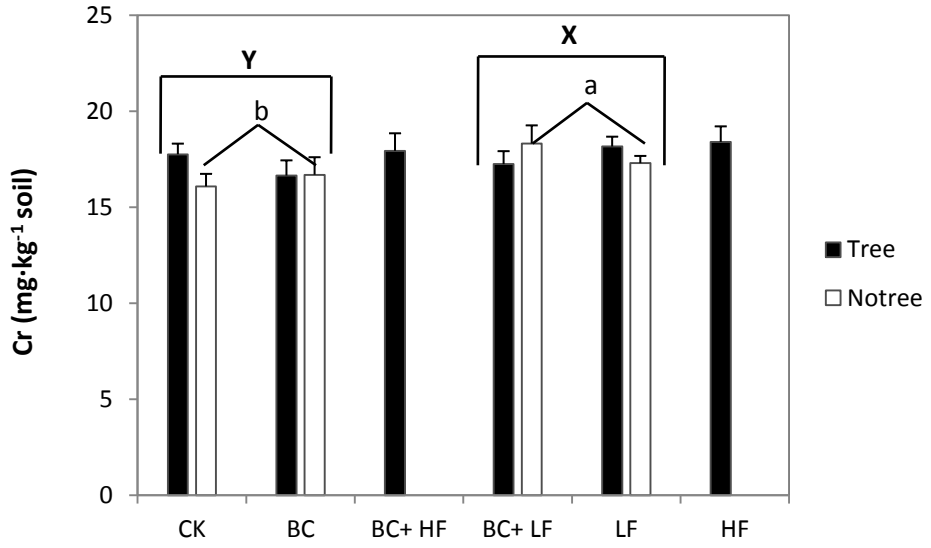
a)


As (mg·kg-1 soil)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
CK
BC
BC+ HF
BC+ LF
LF
HF
Tree
Notree

b)


Cd (mg·kg-1 soil)
0.25
0.2
0.15
0.1
0.05
0
b
a
CK
BC
BC+ HF
BC+ LF
LF
HF
Tree
Notree

c)



d)

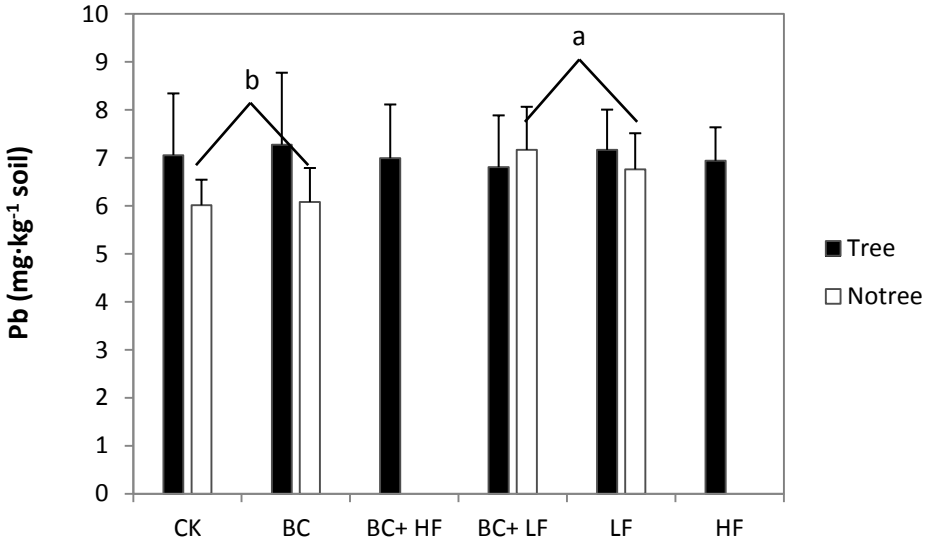
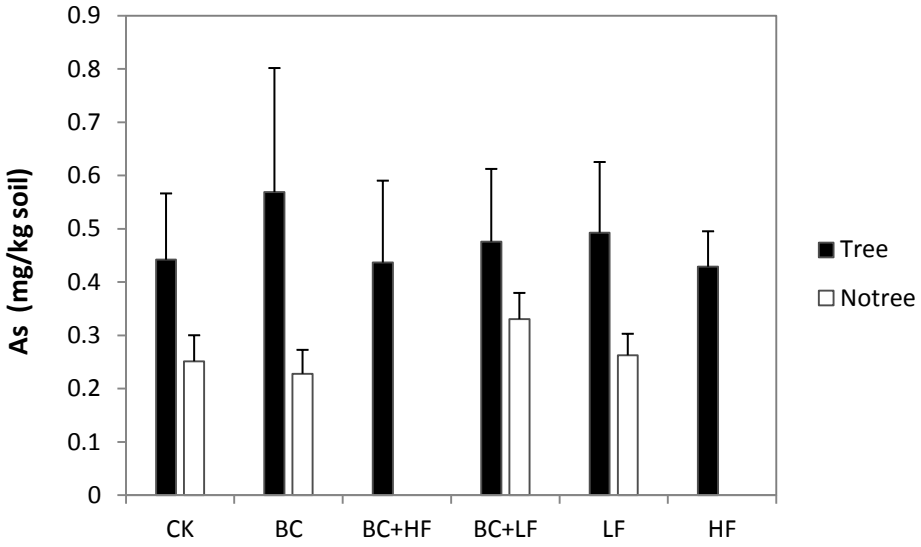
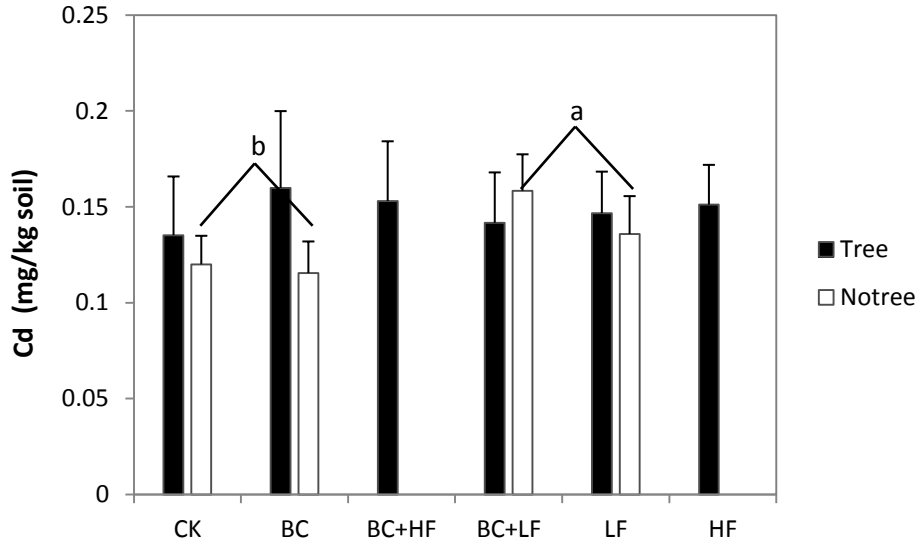


Figure 3.6 Total a)As, b) Cd, c) Cr, d) Pb (mean ± standard error) measured by ICP (mg/kg PGM) in the Whitewood coal mine reclamation site for the different experimental treatments: planting of aspen trees (tree vs no tree), biochar addition, fertilization. CK = control (no biochar, no fertilizer); BC = biochar only, BC+HF = biochar and high fertilizer, BC+LF= biochar and low fertilizer, LF = low fertilizer alone, HF = high fertilizer alone. Upper case letters 'X' and 'Y' above bars in (c) denote ANOVA results from Model 1, which show significant effects of fertilizer (0 versus LF) irrespective of tree or biochar. Lower case letters above the bars in (b), (c) and (d) denote ANOVA results from Model 3(no-tree areas), which show significant effects of fertilizer (0 versus LF), irrespective of biochar.

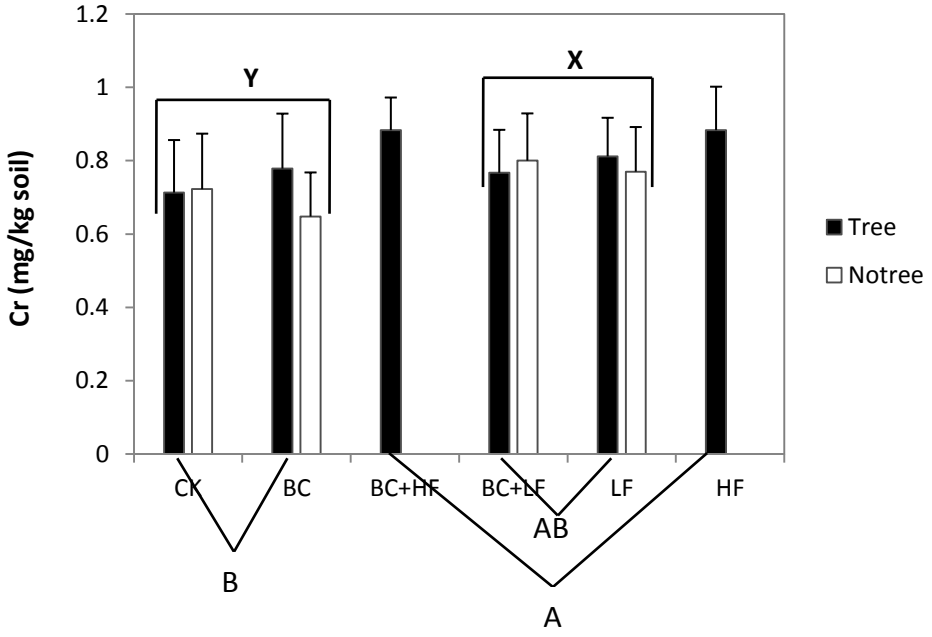
a)


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
As (mg/kg soil)
CK
BC
BC+HF
BC+LF
LF
HF
Tree
Notree

b)


0.25
0.2
0.15
0.1
0.05
0
Cd (mg/kg soil)
b
a
CK
BC
BC+HF
BC+LF
LF
HF
Tree
Notree

c)



d)

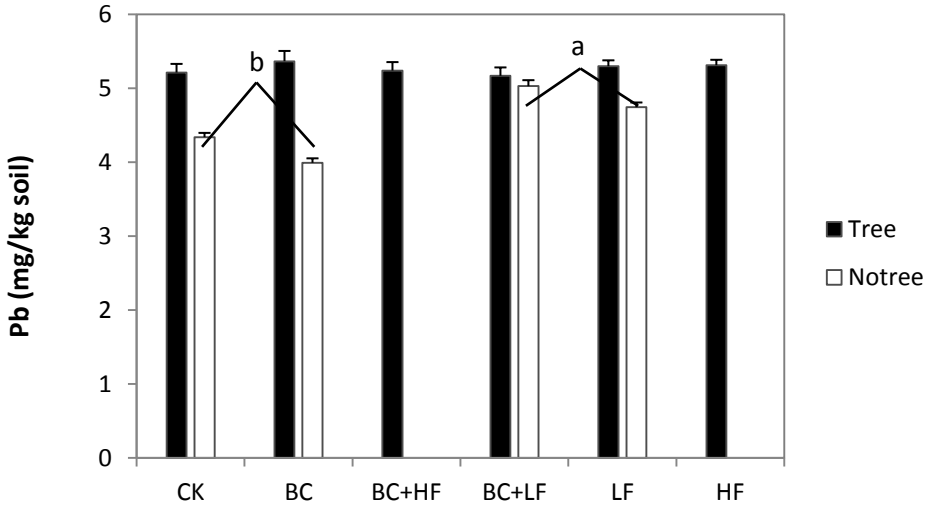
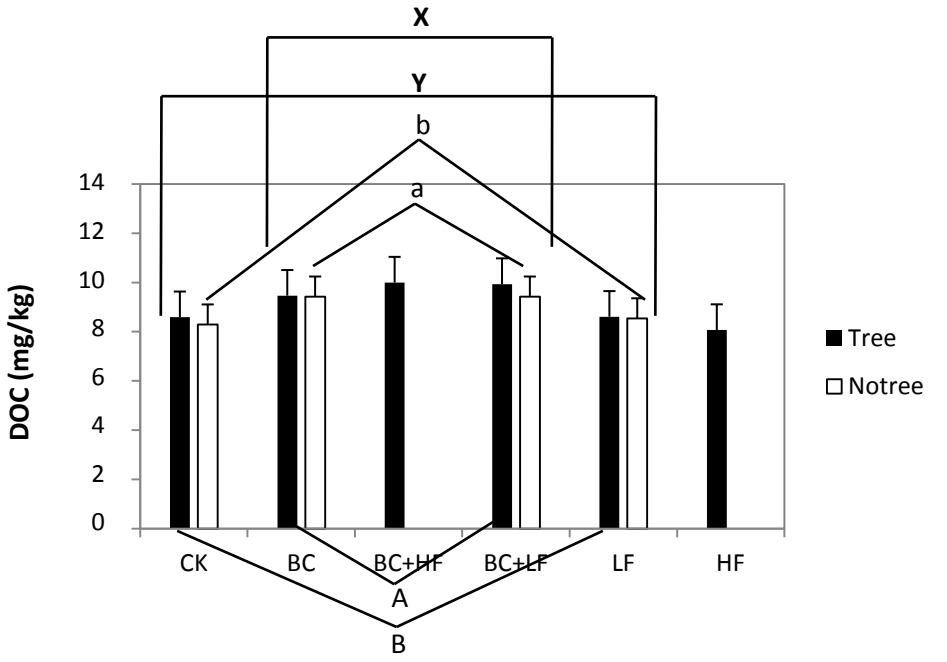


Figure 3.7 Available a)As, b) Cd, c) Cr, d) Pb (mean ± standard error) measured by EDTA method (mg/kg PGM) in the Whitewood coal mine reclamation site for the different experimental treatments: planting of aspen trees (tree vs no tree), biochar addition, fertilization. CK = control (no biochar, no fertilizer); BC = biochar only, BC+HF = biochar and high fertilizer, BC+LF= biochar and low fertilizer, LF = low fertilizer alone, HF = high fertilizer alone. Upper case letters 'X' and 'Y' above bars in (c) denote ANOVA results from Model 1, which show significant effects of fertilizer (0 versus LF) irrespective of tree or biochar. Upper case letters below the bars in (a) denote ANOVA results from Model 2 (tree areas), which show significant effects of fertilizer (0 versus LF or HF) irrespective of biochar. Lower case letters above the bars in (b) and (d) denote ANOVA results from Model 3(no-tree areas), which show significant effects of fertilizer (0 versus LF), irrespective of biochar.

a)



b)

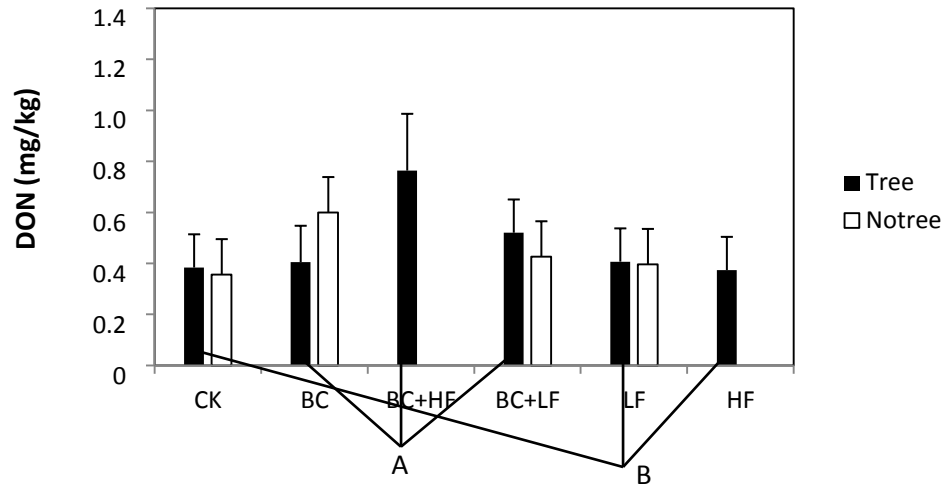
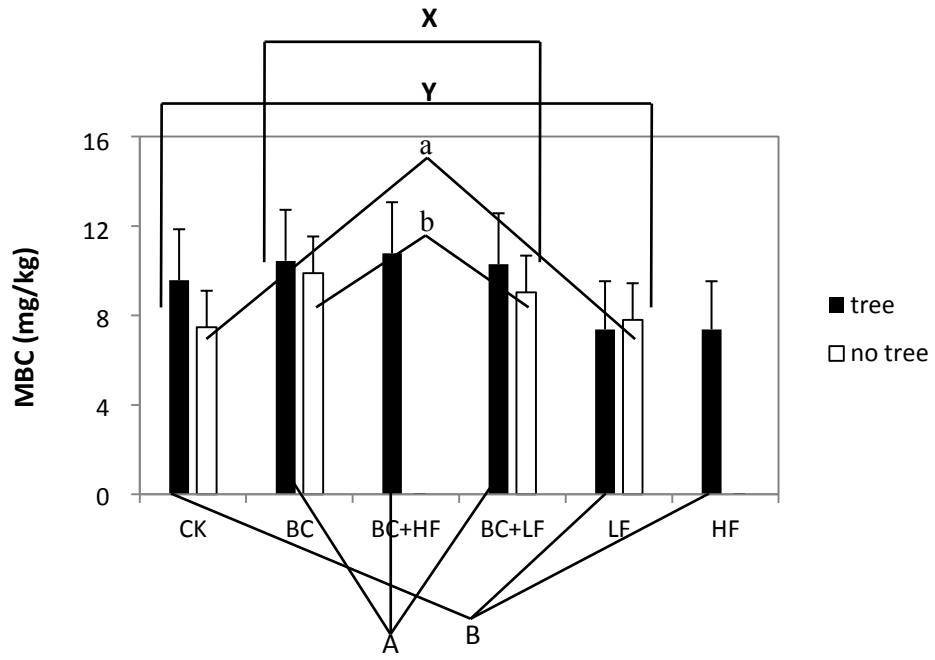


Figure 3.8 Effects of biochar, fertilizer and their combinations on a) dissolved organic carbon (DOC) (mean ± standard error) and b)dissolved organic Nitrogen (DON) (mean ± standard error) measured by CFE method (fresh PGM samples) in the Whitewood coal mine reclamation site for the different experimental treatments: planting of aspen trees (tree vs no tree), biochar addition, fertilization. CK = control (no biochar, no fertilizer); BC = biochar only, BC+HF = biochar and high fertilizer, BC+LF= biochar and low fertilizer, LF = low fertilizer alone, HF = high fertilizer alone. Upper case letters 'X' and 'Y' above bars in (a) denote ANOVA results from Model 1, which show significant effects of biochar (0 and BC) irrespective of tree or fertilizer. Upper case letters below the bars denote ANOVA results from Model 2 (tree areas), which show significant effects of biochar (0 versus BC) irrespective of fertilizer. Lower case letters above the bars in (a) denote ANOVA results from Model 3(no-tree areas), which show significant effects of biochar (0 versus BC) irrespective of fertilizer.

a)



b)

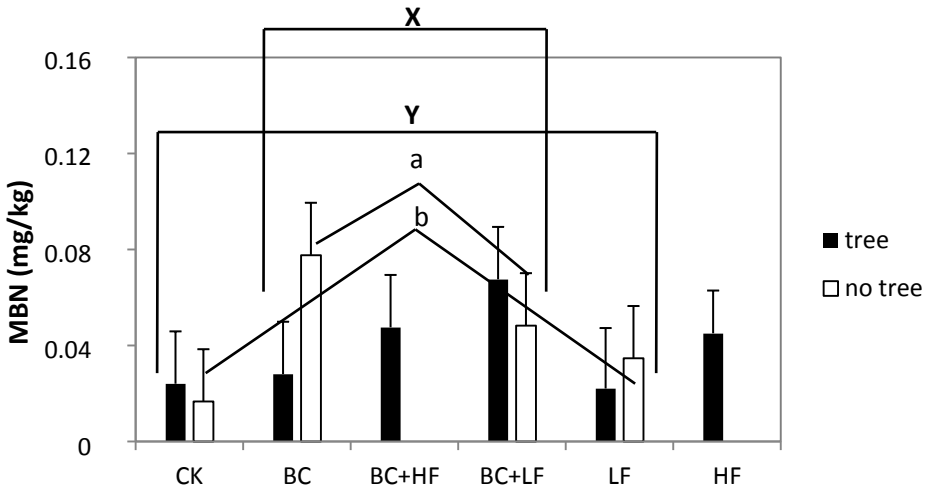
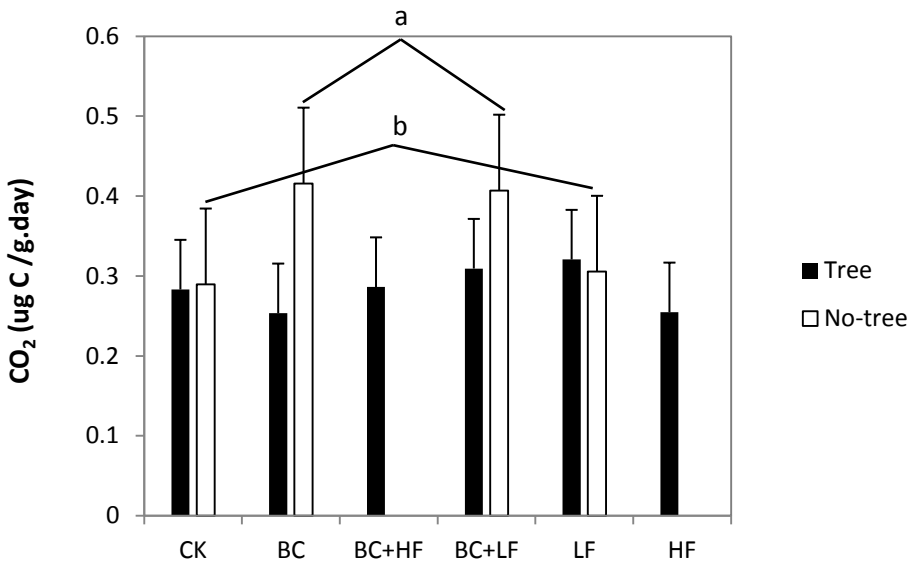


Figure 3.9 Effects of biochar, fertilizer and their combinations on a) microbial biomass carbon (MBC) (mean ± standard error) and b) microbial biomass nitrogen (MBN) (mean ± standard error) measured by CFE method (fresh PGM samples) in the Whitewood coal mine reclamation site for the different experimental treatments: planting of aspen trees (tree vs no tree), biochar addition, fertilization. CK = control (no biochar, no fertilizer); BC = biochar only, BC+HF = biochar and high fertilizer, BC+LF= biochar and low fertilizer, LF = low fertilizer alone, HF = high fertilizer alone. Upper case letters 'X' and 'Y' above bars denote ANOVA results from Model 1, which show significant effects of biochar (0 versus BC) irrespective of tree or fertilizer. Upper case letters below the bars in (a) denote ANOVA results from Model 2 (tree areas), which show significant effects of biochar (0 versus BC) irrespective of fertilizer. Lower case letters above the bars denote ANOVA results from Model 3(no-tree areas), which show significant effects of biochar (0 versus BC) irrespective of fertilizer.

a)



b)

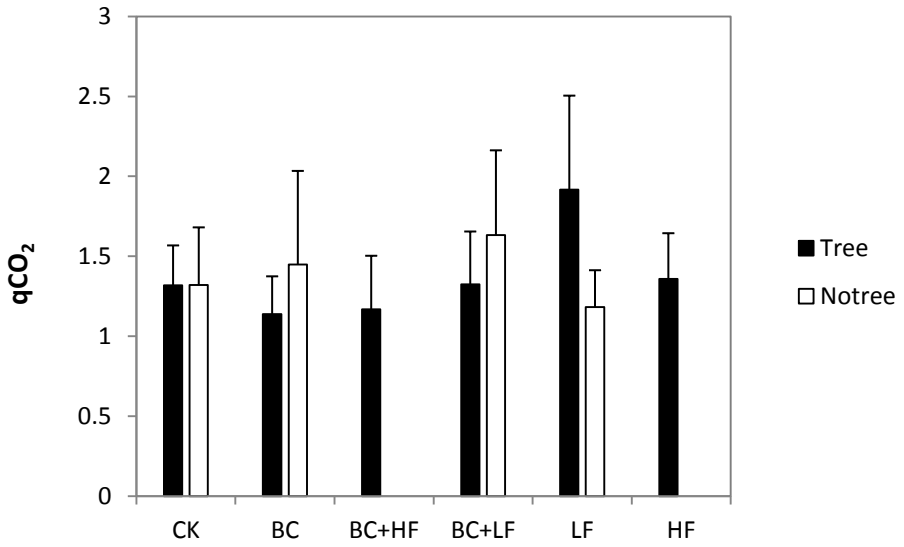


Figure 3.10 Effects of biochar, fertilizer and their combinations on a) soil respiration (mean ± standard error) measured by CFE method and b) metabolic quotient (qCO_2, fresh PGM samples) (mean ± standard error) in the Whitewood coal mine reclamation site for the different experimental treatments: planting of aspen trees (tree vs no tree), biochar addition, fertilization. CK = control (no biochar, no fertilizer); BC = biochar only, BC+HF = biochar and high fertilizer, BC+LF= biochar and low fertilizer, LF = low fertilizer alone, HF = high fertilizer alone. Lower case letters above the bars in (a) denote ANOVA results from Model 3(no-tree areas), which show significant effects of biochar (0 versus BC) irrespective of fertilizer.

Chapter 4: General discussion and conclusions

4.1 Summary

Surface mining, as an anthropogenic disturbance, has caused enormous damage to ecosystems across the world during the past centuries (Dudka et al., 1997; Rathore et al., 1993; Visser et al., 1979). Because the land surface has to be removed before the mining activities, surface soils and vegetation are influenced substantially. The post- mining land surface normally only consists of parent geological materials or subsoil poor in nutrients (Bradshaw, 2000). Another problem is heavy metal contamination in post- mining areas (Massey et al., 1972; Moffat, 1995; Schmidt, 1997).

So land reclamation is needed to recover the whole ecosystem after these disturbances. Biochar, a man- made surrogate for natural fire- generated charcoal, has drawn increasing attention from scientists around the world due to its special function in soil property improvement and plant growth. Many studies showed that biochar can improve soil nutrient availability, increase microbial activity, improve soil nutrient retention and adsorb/ immobilize heavy metals in different plant communities (Lehmann and Joseph, 2009; Sohi et al., 2010). But most previous studies focused on the application of biochar in agricultural lands or waste water treatment, so its use in land reclamation and forest ecosystem reconstruction needs more study. Overall, my thesis provided insights into whether biochar can improve soil nutrient availability, increase microbial activity, improve soil nutrient retention and adsorb/ immobilize heavy metals.

Greenhouse experiment

I expected biochar amended soils to have higher tree biomass, soil nutrient availability, microbial biomass and soil respiration compared to the controls; while coke may have negative effects on these factors. The results showed that biochar decreased the soil available nutrients, especially nitrogen in the land reclamation soil. The reason could be that the nutrients were absorbed by biochar and coke. This implies that the biochar application may benefit the soil in the long term. After biochar amendment, there was no significant improvement of aspen growth, microbial biomass or metabolic quotient in the land reclamation soils, while coke may have a negative impact on the aspen growth in PMM, BLP and WWP soils.

Field experiment

It has been well documented that biochar can adsorb/ absorb and immobilize heavy metals in contaminated soil (Debela et al, 2012; Karam et al, 2011; Uchimiya et al, 2010). Therefore, I expected the amount of heavy metals (As, Cd, Cr, Pb) in WW coal mine soil to be lower in the biochar amended soil than the original soil. But our result did not show a significant effect of biochar on heavy metal adsorption.

Since biochar has the ability to retain nutrients, it is able to protect nutrients applied as fertilizer from leaching. Thus, we expected that application of biochar and fertilizer together would benefit plant growth, microbial activity and soil nutrient availability at the Whitewood site. The field work results showed that biochar increased the DOC, DON, MBC, MBN and soil heterotrophic respiration. This was consistent with previous findings which suggested that biochar can retain soil dissolvable nutrients and increase

soil microbial biomass and basal respiration (Lehmann and Joseph, 2009; Sohi et al., 2010). The biochar did not change the soil pH significantly.

4.2 Future Research

Application of biochar on land reclamation

Prior to surface mining, top horizon soils (including plant propaguale and microbes in soil) have to be removed. The post- mining land surface normally consists of subsoil poor in nutrients and soil microbes. Thus, re-establishment of the soil profile and soil microbial community is of great importance. In our fied study, the biochar application significantly increased the dissoveld organic carbon and nitrogen in soil, which could increase the soil dissolvable nutrients and facilate the reclamation process when it is applied as an amendment. The microbial biomass in the biochar amended soil also increased compared to the unamended soil. So biochar can also help the post-mining soil to recover its microbial property. In our experiment, biochar also had the ability to retain a large amount of dissolvable nutrients, such as nitrate. Previous studies suggested that the biochar retained nutrients is bioavailable, but in our short term experiment the biochar did not assist the aspen growth.

Thus, in future land reclamation processes, biochar could be an optimal amendment to retain soil nutrients and improve soil microbial property. But long term effect of biochar retained nutrients on plant growth may need further studies.

Impacts of different biochar

There are a variety of biochars because the material, pyrolysis time and temperature are different (Sohi et al, 2010). The source substrate of biochar can be wood, nut shell, crop straw, sludge or even animal waste. Since the physical and chemical properties of biochar could be very different when derived from different materials, they could have distinct influences when applied to soils.

Because of differences in substrate materials, processing techniques, and pyrolysis conditions, biochars have different pH, ash content, water holding capacity, pore structure, and specific surface area; these, in turn, result in them having different environmental effects and application fields (Huang et al., 2006).

Biochars processed under higher temperatures will be better used as a soil amendment for acid soils, while biochar made at lower temperatures may increase the soil cation exchange capacity (CEC) (Mukherjee et al., 2011). Biochars pyrolyzed at higher temperatures have higher pH, and thus could be better used in acid soils as an amendment, while biochar made at lower temperatures will have a higher cation exchange capacity (CEC) (Mukherjee, 2011). Different studies indicated that different biochar or pyrolysis temperatures may lead to different pH and CEC of biochar (Cheng et al., 2008; Sohi et al., 2010; Mukherjee et al., 2011). However, the influences of various biochar types, pyrolysis temperature and the application rate on different soils or research sites are hard to predict.

So future studies can be conducted to determine whether different biochar additions (wood, nut shell, crop straw or sludge- derived biochar), created at different pyrolysis temperature (such as 300°C and 800°C), and applied at different rates (for example 5%, 10%, 20%), will benefit the reclaimed soil differently through improving

soil nutrient availability, increasing microbial activity, improving plant growth and adsorbing/ immobilizing heavy metals.

Oxygen-containing functional group (OCFG) on biochar

Previous studies showed that Oxygen-containing functional groups (OCFG) are very important to the absorption ability of biochar. The type and number of various functional groups on the biochar surface can influence its adsorption ability (Rivera-Utrilla et al., 2001; Liu et al., 2002). In previous studies, different biochars or the same biochar produced under different conditions had very different amendment effects on soil (Huang et al., 2006; Sohi et al, 2010). In order to understand how biochar influences the soil (such as nutrient availability, microbial activity and greenhouse gas emission), we need to study the chemical composition and function groups on the biochar surface and the influence of this on its usefulness as a soil amendment.

Impacts of biochar on N_2O emission

N_2O is a trace gas in the atmosphere, but its impact on the global atmospheric environment cannot be neglected because it can deplete the ozone layer (Forster et al., 2007; Ravishankara et al., 2009). N_2O can absorb infrared radiation, and its global warming potential is 298 and 11.9 times as much as CO_2 and CH_4, respectively (Bridle, 2004). Many studies have been conducted on the influence of biochar addition on soil N_2O emission, and most of them showed that biochar reduced soil N_2O emission. (Rondon et al., 2006; Toosi et al., 2011) So, future studies can be conducted to reveal the effect of biochar application on N_2O emission.

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