Effect of Biochar on Soil Microbial Communities, Nutrient Availability, and Greenhouse Gases in Short Rotation Coppice Systems of Central Alberta

by

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Abstract

Short rotation coppice (SRC) systems using willow (Salix spp.) grown on marginal soil, amended with biochar may represent a promising source of renewable green energy for rural communities of Alberta. The Ohaton Wood Energy project, an agroforestry site located in Camrose County, is one of several ongoing SRC projects in Alberta. This project evaluated the effect of biochar on microbial communities, nutrient availability, and greenhouse gases (GHG) in Solonetzic soils dedicated to agroforestry purposes. The study used both lab incubation and field plots to examine the effect of biochar. In the lab incubation, straw and willow biochars were applied to low and high EC soils. The application rates of biochar were 0, 1, 2.5, 5, and 10% (w/w). Chloroform fumigation extraction and alkali trap methods were used to assess soil microbial biomass and activity. Microbial biomass carbon (MBC) and nitrogen (MBN) increased in the presence of biochar in low EC soil. In high EC soil, the metabolic quotient increased, while MBC was reduced. Nitrate (NO_3^-) availability was reduced with biochar addition. In the field study, willow and conifer biochars were applied at 1 and 2.5% (w/w) application rates, to high and low EC and waste water irrigated and non-irrigated zones. The metabolic quotient increased by 177% with addition of conifer biochar at 2.5% rate in irrigated soil. MBC and MBN didn't change drastically in response to biochar additions. Phospholipid fatty-acid (PLFA) analysis and community level physiological profiling (CLPP) were used to examine soil microbial structure and function. Non-metric multidimensional scaling (NMS) was used to distinguish differences between these microbial profiles. Biochar didn't alter PLFA structure in any of treated soils compared to control, but conifer 2.5% changed CLPP in both high and low EC soils. These results indicate that microbial function can change in a short period of time with addition of biochar, but microbial structure and biomass may need more time to shift. Plant root

simulator probes were applied in-situ to measure soil nutrient bioavailability. NMS was also applied to compare nutrient profiles. The nutrient profiles of conifer 2.5% and willow 1% were significantly different from the control in non-irrigated high EC zone. Photoacoustic multi-gas analyzer was connected to static chambers to measure CO_2 and N_2O emissions from soil. Biochar decreased gas emissions from non-irrigated high EC plots in the first 3 weeks. Establishing a strong link between GHG emissions, soil microbial processes, and nutrient profiles as indicators of ecosystem functions needs further research.

Dedicated to

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Jafar Taghavimehr and Fatemeh Abdollahi Molaei

And my beloved spouse

Sahar Safaei

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Chapter 1: Introduction

In Alberta, there are over 250 small towns and villages, and 64 rural municipal districts with a combined population of 452,000, producing about 150 million litres of wastewater every day (Statistics Canada, 2013). Treatment of this huge amount of wastewater needs strong infrastructure supported with high budget from municipalities and counties. Establishing expensive wastewater treatment facilities needs a lot of money for both construction and maintenance. Irrigation of short rotation coppice (SRC) systems may provide a treatment option with low costs making it more feasible for Alberta rural communities to treat their municipal wastewater and produce biomass for biofuel production (Aasamaa *et al.*, 2010). With this type of system in place, rural areas of Alberta may become self-sufficient in their energy production and waste water treatment. This energy is produced with high efficiency and low costs; moreover, it inputs less carbon dioxide into atmosphere compared to fossil fuels (Cao and Pawlowski, 2013). At the same time, energy transportation costs will be removed and no environmental contamination in the form of oil spill from pipelines will happen. Furthermore, less deforestation takes place as there is no need to cut trees in order to establish pipelines.

1.1 Wastewater

Wastewater irrigation of SRC systems provides opportunities for resource sustainability, environmental quality, and economic stability (Hogg *et al.*, 1997). Currently there are stringent regulations in Alberta for wastewater disposal in surface waters and failure to meet these regulations carries heavy fines. Municipal wastewater which is released into water bodies may create water pollution through eutrophication, which is caused by excessive amounts of nutrients, such as nitrates and phosphates, and leads to hypoxia in aquatic inhabitants through algal bloom (Dillon and Rigler, 1975; Vollenweider, 1976; Schindler *et al.*, 1978). Anthropogenic

eutrophication hazards can become elevated at the end of growing season as both temperature and agricultural activities rise significantly in summer (Schindler and Donahue, 2006). Soil and plants may act well to filter nutrients, therefore wastewater diversion onto lands on which trees are growing may improves groundwater and surface water quality by limiting nutrients movement into these reservoirs (Keesstra *et al.*, 2009). Pathogen hazards in water bodies could also be avoided if wastewater is first filtered via soil (Schindler and Donahue, 2006). Municipal wastewater from rural areas can be stored in lagoons to achieve better quality via microbial decomposition and then irrigated on land to provide enough moisture and nutrients for woody plant growth. It is also reported that water availability may be a crucial growth factor at many willow sites (Lindroth and Cienciala, 1996) and it can be inferred that water consumption of willows is relatively high. The efficiency of these wastewater treatment facilities is high due to the elevated rate of wastewater uptake and its accumulation in plant biomass.

Irrigation of intensive willow plantations with municipal wastewater is viewed as a means of addressing three environmental problems in Alberta - water pollution, climate change, and clean water shortage. Southern Alberta has a semi-arid climate in which the rate of evapotranspiration exceeds precipitation. Irrigation for agriculture is the largest consumer of fresh water in Alberta, accounting for 75% of all water allocated (Alberta Environment, 2002). As urban population grows in Alberta competition for fresh water forces agriculture sector to improve its water efficiency and productivity on irrigated lands (Bjornlund *et al.*, 2009). One way to do this is irrigating woody plants such as willow with municipal wastewater. In the water for Life strategy planned to increase water use efficiency by 30% by 2012 (Alberta Environment, 2003). Water use efficiency in agriculture is described as the amount of irrigated water applied and retained in root zone as a percentage of total water supplied to farm (Bjornlund *et al.*, 2009).

The challenges of fresh water shortage has been elevated to the point where in 2005 the Minister of Environment declared that the department won't issue any further water license in 3 of 4 sub-basins of South Saskatchewan River Basin (Alberta Environment, 2005).

1.2 Short rotation coppice systems

SRC systems using willow (Salix spp.) grown on marginal soil, amended with biochar may represent a promising source of renewable green energy for rural areas in Alberta. The Ohaton Waste to Energy project, located in Camrose County, is one of several ongoing SRC projects in Alberta. In this project the effect of biochar on SRC systems to increase wastewater treatment efficiency and biomass production for bioenergy, marginal soil amendment opportunities, and greenhouse gas emissions mitigation is studied.

Planting in the European style is usually done with a double-row system with intervals of 1.5 m. There is 0.6 m distance between rows which makes a plant density of 13700 – 17800 plants/ha. The willows are coppiced (harvested) in winter after 3 – 5 years (Aronsson *et al.*, 2002).

1.3 Salinity

There is a huge area of marginal lands in southern Alberta mostly consisting of Solonetzic soils which are high in salinity (Alberta Agriculture Food and Rural Development, 2005b). Saline soils cover a major area of the Canadian Prairies with an estimated 4 million ha of salt-affected land (Hangs *et al.*, 2011), mostly occurring in Alberta. These lands are mainly under agricultural activities and show low productivity because of high electrical conductivity (EC) and Sodium Adsorption ratio (SAR) (Alberta Agriculture Food and Rural Development, 2005a). One alternative is land use change to woody plants such as willow which is highly resistant to salinity (Hangs *et al.*, 2011). By this means, the productivity of these lands can increase through

elevating the production. The production of woody biomass can serve as a source of cheap and clean energy for rural areas.

1.4 Climate

The climate of Camrose County is classified as cold semi-arid (Bsk) and mild humid continental (Dfb) to the district lies within the Aspen Parkland ecoregion which is a gradient from grasslands to the boreal forest (Hydrogeological Consultants Ltd. and Canada Prairie Farm Rehabilitation Administration, 2005). The mean annual temperature is 2.8 ˚C and the highest monthly temperature is 16.3 ˚C in July. Mean annual precipitation is 493 mm based on 30 years of data gathered from 1971 to 2000 (Hydrogeological Consultants Ltd. and Canada Prairie Farm Rehabilitation Administration, 2005). The calculated annual potential evapotranspiration is 494 mm which is higher than annual precipitation (Hydrogeological Consultants Ltd. and Canada Prairie Farm Rehabilitation Administration, 2005). Higher evapotranspiration compared to precipitation implies irrigation may be needed for farming activities in Camrose municipal district to increase the crop production.

1.5 Bioenergy

Currently, there is global interest in the use of willow as a feedstock for bioenergy production (Berndes *et al.*, 2003; Sims *et al.*, 2006). The increasing need of bioenergy production and reduction of fossil fuel reliance has been under a lot of focus due to the increasing rate of atmospheric CO_2 concentrations and subsequent consequences such as climate change. This idea has been further improved by biochar addition to bioenergy cropping systems and increasing their GHG emissions mitigation capabilities (IPCC, 2007; McCormack *et al.*, 2013). Biochar will be discussed in more detail later. The coupled processes of biochar making and bioenergy production create a fundamental link. Bioenergy can be achieved through biochar

processing and biochar can be applied to the same bioenergy cropping system (Gaunt and Lehmann, 2008; Sohi *et al.*, 2009). Bioenergy cropping systems might provide one solution. Short rotation coppice (SRC) systems, consisting of intensive willow plantations for example, are highly photosynthetic which can capture atmospheric CO_2 in their biomass and add it to the existing soil carbon stock. Turning this biomass into biochar and addition of it to soil in order to make a slow turn-over carbon pool can contribute to C sequestration and improve soil quality (Tallis, 2010; McCormack *et al.*, 2013). The C sequestration mechanism via biochar application is based on turning of photosynthetically captured CO_2 into stable C structure and its storage in soil stock. Hence, highly resistant substance to decomposition can't be lost through carbon mineralization for very long periods (Field *et al.*, 2013).

1.6 Biochar

Biochar is a high-valued side-product of SRC systems which can be produced in-situ and applied to adjacent agricultural and non-agricultural fields. Biochar is the by-product of organic feedstock heating in an oxygen limited environment (Lehmann, 2007). It is made through the pyrolysis process in which the structure turns into more carbon dominated substance and as temperature elevates more aromatic structures form and make it more suitable for carbon sequestration while lower temperature make it suitable for amendment purposes (Novak *et al.*, 2009b). As mentioned before, it could be used as a soil amendment. Biochar will be discussed in more detail in other chapters. Currently with collaboration of Alberta Innovates Technology Futures (AITF) and Lakeland College two mobile pyrolysis units - ABRI-Tech Unit from Quebec and BigChar Unit from Australia – have been purchased to decrease the costs of biochar production in Alberta. These units can travel to each treatment facility and make biochar at the same location therefor biochar transportation costs will be cut.

As biochar stays in SRC systems for a very long time, it can provide long-term benefits to the system like CEC moderation, pH elevation, and management of organic C supply for soil microorganisms (Lehmann, 2007). Frequently, biochar effects on different soil ecosystems have been reported to be contradictory. But, in many research it is reported that biochar elevates soil water holding capacity (WHC) (Jha *et al.*, 2010; Beck *et al.*, 2011; Liu *et al.*, 2012; Raave *et al.*, 2014; Ulyett *et al.*, 2014). Interestingly, this change can lead to better irrigation management strategies and higher water use efficiency as more water is retained in root zone and wastewater leaching happens less. Consequently, less groundwater contamination is expected to take place. Moreover, nutrient dynamics and microbial activity can change with changes in soil water content as there would be more time for plants to take up nutrients. Mulcahy *et al.* (2013) concluded a less water stress imposed on plants as a result of higher water retention with addition of biochar. In general, biochar effect can be categorized in four categories as literature proposes (1) C sequestration elevation (Glaser *et al.*, 2002; Laird, 2008), (2) plant nutrient levels enhancement (Novak *et al.*, 2009a), (3) soil water retention improvement, and (4) microbial activity enhancement (Steiner *et al.*, 2008; Lehmann *et al.*, 2011).

Biochar addition to soil in these systems can stimulate microbial activity and mineralize nutrients for plant uptake. Hence biochar can act as nutrient management factor in soil and improve soil nutrient dynamics (Lehmann *et al.*, 2011). It can also increase plant root exudates via increase in nutrient bioavailability. Root exudates as dissolved organic carbon (DOC) and nitrogen (DON) are energy sources for microbial metabolism and main constituents for increasing microbial biomass, as a result higher abundance of microbial community can provide higher nutrient availability (Norton and Firestone, 1996). In general, biochar can improve symbiotic relationship between plants and microbes and make soil healthier. Biochar application

in SRC systems retains organic carbon for a longer time (Hua *et al.*, 2014), hence the capability of SRC systems in carbon sequestration could be higher along with biochar application (Galaz, 2012). Recently it has been reported from microcosm research that CO_2 and N_2O emissions from biochar-amended soils were significantly lower than control soils (Aguilar-Chavez *et al.*, 2012; Kammann *et al.*, 2012).

The amount of added biochar to soil generates crucial implications in terms of SOM mineralization and release of greenhouse gases as well. There is an upper boundary of application at which biochar has no more effects or inversely influences soil function. Several studies have found that by increasing biochar application, the release rate of CO_2 and N_2O gases from soil reduces progressively (Spokas *et al.*, 2009; Zhang *et al.*, 2012), and Keith *et al.* (2011) showed a reduction of labile carbon mineralization in form of CO_2 released from soil.

1.7 Novel measurement methods for soil ecosystem

Biochar addition to soil can alter mineralization of native soil organic matter (SOM) through the changes in soil microbial behavior (Prayogo *et al.*, 2014). It is reported that several mechanisms are at play when biochar is added: 1) carbon substrate addition (Smith *et al.*, 2010), 2) production or adsorption of substances which interactively stimulate and prohibit microbial growth (Kasozi *et al.*, 2010; Spokas *et al.*, 2010), 3) physical protection of microbes from predators which create an appropriate habitat for various communities (Pietikainen *et al.*, 2000). Microbial community composition can be changed through the introduction of a wide variety of substances such as ethylene (Spokas *et al.*, 2010), and polycyclic aromatic hydrocarbons (PAHs) (Quilliam *et al.*, 2012). It is discussed that dominant microbial community composition controllers which are pH and substrate availability can change significantly with biochar addition (Fierer *et al.*, 2009). The knowledge on biochar effect on microbial communities is still limited

and microbial structural responses to biochar are dependent on its type and other relevant factors (Steinbeiss *et al.*, 2009). Some studies have discussed the changes in physical and chemical soil properties and their consequent impacts on microbial abundance and community composition (Pietikainen *et al.*, 2000; Kolb *et al.*, 2009; O'Neill *et al.*, 2009; Liang *et al.*, 2010; Anderson *et al.*, 2011). The most important physical and chemical properties are porosity, water holding capacity, pH, and chemical sorption capacity (Downie *et al.*, 2009; Brewer *et al.*, 2011; Novak *et al.*, 2012; Watzinger *et al.*, 2014). Thus far contradictory results have been reported on the effect of biochar on microbial communities. Some studies have reported no effects of biochar on microbial biomass (Castaldi *et al.*, 2011; Dempster *et al.*, 2012), while others indicated significant changes in phosphate solubilizing microbial community (Anderson *et al.*, 2011), and a reduction in microbial diversity (Khodadad *et al.*, 2011). These changes in microbial biomass and structure are attributed to positive (Luo *et al.*, 2011), and negative priming effect on SOM (Jones *et al.*, 2011). The reports on microbial respiration indicate both stimulation and inhibition (Zheng *et al.*, 2012). Respiration could be representative of microbial activity but stimulation or reduction does not necessary mean microbes are under better conditions.

Nitrification can be inhibited with biochar addition while the population of nitrifying microbial community is reduced (Wardle *et al.*, 1998; Elmer and Pignatello, 2011). It is discussed that N immobilization can take place on biochar surface and microbial communities with lower C:N ratios take up substrates with higher C:N ratios as a response to this physical immobilization (Gundale and DeLuca, 2006; Jesus *et al.*, 2010). Therefore, biochar can decrease N availability in marginal N-deficient soils due to rapid elevation in soil C:N ratio (Muhammad *et al.*, 2014). In contrast, DeBoer and Kowalchuk (2001) postulated that elevated pH with presence of biochar can increase the population of ammonia oxidizing bacteria (AOB) which is

specialized in nitrification. Hence, biochar can favor nitrification process and increase nitrate availability assuming ammonium is sufficient. Microbial behavior can change due to the changes in enzyme activity as a result of enzyme adsorption to biochar surface. Different microbial enzymatic activity can lead to drastic changes in C and N cycling and mineralization (Prayogo *et al.*, 2014).

It is postulated that in marginal soils where microbial activity is limited by substrate availability; addition of labile C can make a positive priming effect and induce more microbial activity where C and energy are not limiting factors for soil organic carbon (SOC) decomposition (Hamer *et al.*, 2004). A part of SOC is consisted of black carbon (BC) which is highly resistant to microbial degradation. It is reported that up to 60% of SOC in Canadian great prairies is BC (Ponomarenko and Anderson, 2001). On the other hand, BC can have prohibitive effect of added labile C degradation as some of this C can be adsorbed to the surface of BC (Jonker and Koelmans, 2002). Since biochar has a lot of BC it can prohibit C degradation. The overall productivity and quality of soil media could be defined by the activity of microbial communities and their participation in nutrient cycling, carbon turn-over, and greenhouse gases (Montecchia *et al.*, 2011). Soil microbial structure and activity should be accounted as important parameters affecting soil functionality and health alongside with plant productivity, as plants and soil microbes are in close relationship (Montecchia *et al.*, 2011). A rapid measurement method, called community level physiological profiling (CLPP), for evaluation of metabolic potential of soil microbial community has been applied by several studies (Mader *et al.*, 2002; Esperschuetz *et al.*, 2007). Several approaches exist within CLPP method and this study based on CO_2 detection using sealed microtiter plates along with pH sensitive dye (Campbell *et al.*, 2003). Microbial community composition is estimated by phospholipid fatty acid (PLFA) analysis

based on the definition of a profile of PLFA biomarkers from microbial cell membrane phospholipids (Zelles, 1999).

Prendergast-Miller *et al.* (2014) discussed the indirect biochar-root interactions and introduced rhizosheath as a layer of soil tightly bound to plant roots in P-deficient soils. They reported less accumulation of rhizosheath in biochar amended soils meaning that biochar provided a better supply of P, thus plant roots had no need to create thick rhizosheath for higher uptake of P (Brown *et al.*, 2012). In highly acidic and calcareous conditions the availability of phosphate (PO_4^{3-}) decreases drastically. In acidic conditions high levels of Al and Fe oxides and hydroxides form high energy bonds with PO_4^{3-} as insoluble formations of Fe and Al phosphates (Lindsay, 1979). In calcareous condition which occurs in alkaline environments mostly, PO_4^{3-} precipitates in the form of metal complexes like Ca-P and Mg-P (Amer *et al.*, 1985; Marschner, 1995). Although P contamination in water ecosystems is of high concern but P is less available than N and K in terrestrial ecosystems. It is reported that biochar with its high anion exchange capacity (AEC) (DeLuca *et al.*, 2009), can reduce the concentration of Al and Fe and decrease the sorption of PO_4^{3-} on ferrihydrite ($(Fe^{3+})_2O_3 \bullet 0.5H_2O$) (Cui *et al.*, 2011). Some studies have found an increase in soil P and K with addition of biochar (Lehmann *et al.*, 2003; Schnell *et al.*, 2012). Cao and Harris (2010) reported that some nutrients such as K, Mg, and Mn can release from biochar.

Soil C loss with presence of biochar is dependent on its kind and interaction with soil, climate conditions, plant cover, and length of application period. Since biochar might have significant amounts of labile carbon (Wardle *et al.*, 2008), it can increase the rate of native SOM decomposition in the form of positive priming (Kuzyakov *et al.*, 2009). This should be true for shorter periods and in longer term it is proven that recalcitrant part of biochar has the dominant

role (Jones *et al.*, 2011). With respect to soil C sequestration and GHG emissions mitigation, biomass conversion to biochar via an inexpensive pyrolysis process can provide socio-environmental benefits when applied to bioenergy cropping systems (Wang *et al.*, 2013). Evaluation of N_2O in some studies with respect to biochar amendment showed no effects (Scheer *et al.*, 2011; Xie *et al.*, 2013), while other studies have attributed its reduction to denitrification inhibition and ammonia (NH_3) adsorption by acidic functional groups on biochar surface (Yanai *et al.*, 2007; Lehmann and Joseph, 2009; Taghizadeh-Toosi *et al.*, 2012a, b). The aim of this study was to evaluate the short-term effect of biochar on soil microbial communities, nutrient availability (N, P, K), and GHG emissions in bioenergy cropping systems. Hence, according to aforementioned literature we assumed a shift in microbial structure and activity with a reduction in N availability and elevation in P and K. The changes in nutrient profiles as a result of microbial behavior alteration could result in lower CO_2 and N_2O emissions.

1.8 Research questions

With respect to all the challenges and needs which were addressed before our research revolved around these questions:

Lab incubation:

1. Does biochar improve water holding capacity?
2. Can biochar increase microbial biomass and activity?
3. Does inorganic N availability increase with biochar addition?

We believed that biochar would increase WHC therefore increasing the amount of moisture availabe to plants and microorganisms. Consequently, the biomass and activity of microorganisms could increase allowing for more N mineralization to take place.

Field study:

1. Can biochar increase microbial biomass and activity and change microbial function and community structure?
2. Does soil nutrient availability change with biochar addition?
3. Does biochar addition to soil reduce GHG emission?

We believed that biochar would increase microbial biomass and activity in field leading towards a change in microbial function and structure. The nutrient availability would change as a result of change in soil moisture. Although microbial activity could increase, but due to biochar recalcitrant nature GHG emissions would decrease in field.

Chapter 2: Does Biochar Increase Water Holding Capacity, Microbial Biomass, and Nitrogen Availability in Solonetzic Soils: A Lab Incubation Study

2.1 Introduction

There is an increasing public attention to biochar application in agricultural lands as a means to improve soil quality and fertility (Lehmann, 2007). There are also other potential benefits of adding biochar to soil such as carbon sequestration, greenhouse gas emissions mitigation, biogeochemical activity improvement, increasing crop production, and decreasing the leaching of nutrients (Kolb *et al.*, 2009; Atkinson *et al.*, 2010; Singh *et al.*, 2010; Sohi *et al.*, 2010; Ennis *et al.*, 2012).

Biochar is the recalcitrant by-product of pyrolysis of organic matter in an oxygen limited environment (Goldberg, 1985; Lehmann, 2006). This substance is comprised of labile and recalcitrant pools (Oren, 2001). The labile portion can be mineralized in a short period of time, from days to months, but the recalcitrant portion is more resistant to both biotic and abiotic degradation taking centuries to millennia to completely mineralize (Smith *et al.*, 2010). Biochar benefits can put into two categories: long term carbon (C) sequestration (Spokas, 2010; Zimmerman *et al.*, 2011) and improving soil quality and health, with an emphasis on microbial dynamics (Ogawa *et al.*, 1983; Pietikainen *et al.*, 2000). It is also important to note that via increasing soil C sequestration the quality of soil can improve because soil C plays an important role in feeding microorganisms and provides them energy resources for their activities (Thompson LM, 1978; Stevenson, 1994; Sohi *et al.*, 2010). Biochar can play an important role in sustaining labile organic carbon from excessive microbial decomposition through its porous structure as a shelter for soil organic matter.

The effect of biochar on soil properties can change drastically according to feedstock and pyrolysis conditions (Enders *et al.*, 2012; Cayuela *et al.*, 2014). For example, biochars made from compost-based organic matter can provide more available organic C and N than those produced from woody materials (Van Zwieten *et al.*, 2014). Prayogo *et al.* (2014) linked the lower rate of SOM mineralization with the presence of biochar to physical protection of adsorbed C, release of some toxins, and sorption of microbial enzymes leading microbial community towards lower activity and degradation of SOM. Biochars can also have various values of porosity, pH, surface area, number of functional groups, ash content, and redox properties which consequently alter their effects on soil. Redox properties make the transformation of electrons to some denitrifiers easier (Van Zwieten *et al.*, 2014). Furthermore, biochars produced at lower temperatures have more variable organic matter status encompassing more aliphatic and ligno-cellulose type composition. Microorganisms can break down these structures more easily, hence these readily degradable structures are mineralized by microbes as substrates (Alexander, 1977).

Some other studies have also linked the GHG emissions reduction of biochar to the decrease in SOM degradation (Hammond *et al.*, 2011; Shackley *et al.*, 2012). This is related to the physical protection of SOM by means of biochar, as SOM stays in porous media of biochar and is out of microbial access (Zimmerman *et al.*, 2011). Biochar has been shown to change microbial community structure, increase total biomass of microbial community, and alter microbial enzymatic activity leading towards changes in decomposition processes (Lehmann *et al.*, 2011). Specific adjustments in pH, nutrient availability, and organic matter degradability manipulate some branches of microbial community such as specialized organisms in nitrification and denitrification processes (Anderson *et al.*, 2011; Ducey *et al.*, 2013) and fungal biomass (Warnock *et al.*, 2010).

In agroforestry systems, willow is the leading feedstock for bioenergy production and can turn into a high value product grown on poor quality lands. This woody plant can provide feedstock needed either for energy purposes or biochar. Therefore, the value of biochar should be compared to other by-products of these systems in terms of its benefits and profits from both environmental and economical perspectives (Fletcher *et al.*, 2014). Establishing SRC systems on marginal lands previously under cultivation of agricultural crops includes such benefits as increasing biomass production and elevating the value of these lands, while potentially reducing GHG emissions (Shibu *et al.*, 2012), overcoming issues of water limitation (Dimitriou *et al.*, 2009) and energy challenges (Gruenewald *et al.*, 2007). Biochar addition to these systems can create opportunities for more productivity with respect to the aforementioned objectives.

The lands in central and southern Alberta are Chernozems associated with Solonetzic soils. That means in an area which is mostly focused on agricultural activities, Solonetzic soils appear with patches of saline zones which might greatly influence agricultural productivity. The variation in soil salinity is because of differences in mineral geology found in the parent material of these soils (Yuan *et al.*, 2007). Soil EC is a measurement parameter closely linked to the concentration of dissolved cations and anions in soil solution. Salinity is a major issue in areas with lower rainfall and higher rate of evapotranspiration and affects soil function negatively (Sumner, 1995). Salinity occurs with the upward movement and accumulation of salts in the root zone which increases the osmotic potential. The adverse influence of salinization can be addressed as physical, chemical, and biological (Iwai *et al.*, 2012). Soils with EC more than 4 dS m^{-1} are called saline soils (Elmajdoub *et al.*, 2014). There is a huge area of salt affected lands in the world by 831 million hectares as reported by Mavi *et al.* (2012), hence in order to create appropriate land management practices it is of great importance to understand the biological

processes in these soils with an emphasis on nutrient cycling (Aislabie *et al.*, 2012). The osmotic potential in salt-affected soils decreases and not only makes a huge negative impact on both water and nutrient uptake of plants (Tahira *et al.*, 2011), but also a large adverse influence on the activity and growth of microorganisms. Salt resistant microorganisms produce osmolytes in order to reduce the loss of water from their cells while other sensitive microbes perish. Osmolyte plays an important role in maintaining cell volume. Osmolyte production needs a lot of energy in which more substrate in soil should be assimilated by microbes, thus reduces the growth rate (Anders *et al.*, 2013). As a result of this, the composition of microbial community can change according to the salt concentrations.

As mentioned before, microbial growth and activity is under the effect of the availability of organic C. This happening changes the status of SOM through the change in microbial community (Rietz and Haynes, 2003). There are contradictory reports on the effects of salinity on soil microbial character and both increases and decreases of C and N mineralization have been recorded in several studies (Jones *et al.*, 2010; Artiola *et al.*, 2012; Joseph *et al.*, 2013). Elmajdoub *et al.* (2014) reported a reduction in both microbial biomass C and respiration with addition of soil EC. Soil salinity is in correlation with moisture content as soil water content reduces, salinity increases because the same amount of salts remain in less water content and their concentration increases. By this means, in saline soils with decrease in moisture content the osmotic potential reduces and microbial community goes under stress (Yan and Marschner, 2013). Biochar potentially can reduce these negative impacts via increasing soil water content.

2.2 Research questions

The pace of organic matter mineralization plays an important role on the remediation of poor quality soil through affecting aggregate formation and nutrient turnover (Thompson LM,

1978). Therefore, we set out to test the effect of biochar made from regionally specific feedstocks on the high salinity found in Solonetzic soils. On the other hand, the complicated interaction of biochar with different soil types can lead one study towards different conclusions in terms of soil functionality and properties (e.g. pH, CEC, mineralizable C and N, nutrient cycling, and microbial activity and composition). These different conclusions create variable interpretations of soil-plant-microbe interactions and generate important implications on soil amendment strategies (Graber *et al.*, 2010; Kolton *et al.*, 2011; Lehmann *et al.*, 2011). This research work was designed to investigate the microbial dynamics and N availability changes in two saline and non-saline soils following the addition of two biochars at different rates. We had three research questions:

1. Does biochar improve water holding capacity?
2. Can biochar increase microbial biomass and activity?
3. Can biochar increase inorganic N (NO_3^- and NH_4^+) availability?

In spite of complex interaction between biochar and soil, we hypothesized an increase in microbial biomass and activity and increase in N availability with addition of biochar. We also hypothesized that biochar can reduce the impact of salinity by increasing soil moisture content. On the other hand, it can provide some labile C to a subset of microorganisms to increase their activity and growth even in saline conditions (Yan and Marschner, 2013).

2.3 Methods and materials

2.3.1 Experimental Setup

In late summer of 2012, bulk soil was collected from both low and high EC zones for an incubation study. Treatments were established by addition of straw and willow biochar at 0, 1, 2.5, 5, and 10 percent (w/w) to both low and high EC soils. Each treatment had 4 replicates to

increase the accuracy of statistical analysis. The site from which the bulk samples were collected was a 1 hectare agroforestry research field stablished by Camrose County to serve as bioenergy cropping system. The legal location is SE-22-46-19-W4M and the soil on-site is of the Camrose Association, loam textured, Solodized Solonetz (Vega-Jarquin *et al.*, 2003).

2.3.1.1 Incubation

100 gr of sieved samples were put in 1L glass Mason jars and pre-incubated for 20 days followed by a 20-day incubation. The Moisture content of each treatment was maintained at 60% of water holding capacity (WHC).

2.3.2 Laboratory analysis

2.3.2.1 Water retention

Water retention was measured with pressure-plate apparatus to evaluate water retention curve (Yeates *et al.*, 2002). Soil was put in cores and placed on ceramic plates of different porosities to create different pressures in a pressure vessel. The water content was manipulated until it reached to equilibrium at prescribed pressure. The amount of pressure imposed on soil was equal to the matric potential (negative) of the water-filled pores (Yeates *et al.*, 2002). Four levels of pressure were applied including at 33, 300, 800, and 1500 kPa. Samples were then weighed before and after drying for 24 hours at 105 ˚C (Kalra *et al.*, 1991). The amount of time in the pressure chamber varied from 1 day (33) to 3 days (300 and 800) and 10 days (1500). Treatments were replicated 3 times.

2.3.2.2 Total carbon and nitrogen characterization

Total carbon and nitrogen (TC/TN) were measured on soil samples and biochar types, in order to determine was needed to prescribe how much biochar should be added to soil to stimulate microbial growth (Durenkamp *et al.*, 2010). 1-5 gr of sieved (biochar with 4 mm and

soil with 2 mm sieve) sample was ground with Retsch MM200 ball mill grinder. 20 mg of high EC soil, 5 mg of low EC soil, and 2 mg of biochar was encapsulated in aluminium tin for dry combustion with a thermocouple sensor (Costech Analytical Technologies Inc., Valencia, CA, USA) (Norris *et al.*, 2011; Hahn and Quideau, 2013).

2.3.2.3 pH and EC

Sieved samples were air dried for pH and EC measurements. 20 ml of deionized water was added to 10 gr of each sample (1:2 soil-to-solution ratio) (Kalra *et al.*, 1995; Novak *et al.*, 2007). The mixtures were shaken for 30 minutes and centrifuged for 10 minutes at 2000 rpm. 1413 µS/cm electrolytes and 12.9 mS/cm electrolytes (Orion™ Conductivity Standards, Thermo Scientific™) were used to calibrate the EC meter (Mettler Toledo, Mississauga, Canada) for low and high EC samples, respectively. A 2 point calibration with 4.01, 7.00, and 9.21 pH buffers (Orion™ pH Buffer, Thermo Scientific™) was done to calibrate the pH meter (Mettler Toledo, Mississauga, Canada) for samples. The transparent portion of the solution was poured in a test tube and used for EC measurement by putting the EC sensor in the tube and reading the device. After EC measurement supernatant was returned to the container and used for pH measurement.

2.3.2.4 Microbial biomass

In this study, chloroform fumigation extraction (CFE) was used to examine the changes in microbial biomass (Brookes *et al.*, 1985). Biochar can influence the results from this method by sorbing soil organic C and N (Durenkamp *et al.*, 2010). In order to retain the consistency of the results with other studies we didn't apply any correction coefficient (Swallow *et al.*, 2009). Soil microbial biomass carbon and nitrogen (MBC/MBN) were evaluated using the extraction of carbon and nitrogen in samples by 0.5 M K_2SO_4 (Brookes *et al.*, 1985). After incubation, 25 gr of each soil was put in a 50 ml beaker and extracted with 50 ml of 0.5 M K_2SO_4 (1:2 soil-to-

solution ratio), shaken for 1 hour, and vacuum filtered with Whatman P2 filter papers. Another 25 gr of each soil was fumigated for 3 days. Fumigation was done by putting each set of samples in a dessicator along with chloroform. The dessicator was sealed and vacuum pumped to bring the inner pressure to zero and evaporate the chloroform. Extraction of fumigated soils was done right after the fumigation period.

2.3.2.5 Dissolved organic carbon and nitrogen

Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were measured using a Shimadzu TOC-VTN instrument (Mandel Scientific Company Inc., ON, Canada). Microbial biomass carbon and nitrogen were determined using measured DOC/DON in extracted samples before and after fumigation (Swallow *et al.*, 2009).

2.3.2.6 Microbial respiration

Basal respiration of soil microbial biomass was measured using the alkali trap method (Deenik *et al.*, 2010). Carbon dioxide (CO_2) emitted by microbial activity was trapped in 0.5 sodium hydroxide (NaOH) forming sodium carbonate (Na_2CO_3). Microbial respiration was measured during the incubation period for 20 days. 100 gr of treatment soil was placed in 1L Mason jars. Uncapped scintillation vials holding 20 ml of 0.5 M NaOH were placed in jars. The jars were sealed, and kept under room temperature at 25 ˚C. At the end of incubation period, scintillation vials were taken out and sealed immediately. Solutions were titrated with 0.5 M hydrochloric acid (HCL) to a clear end point (Zibilske, 1994; Sundaravalli and Paliwal, 2000). Metabolic quotient (qCO_2) was calculated as respired carbon to microbial biomass carbon and indicates heterotrophic activity of soil microorganisms (Pirt, 1975; Anderson and Domsch, 1986).

2.3.2.7 Ion-exchange resin membrane

Ion exchange resin (IER) membranes were used to measure soil inorganic nitrogen (NH_4^+ and NO_3^-) availability as described by (Johnson *et al.*, 2005). One anion and one cation IER was buried in each jar during the incubation period. Each treatment was replicated 2 times and due to insufficient resources biochar at 2.5% didn't have any IER. The counter ions on resins were Na^+ and HCO_3^-. Each resin was 10 cm^2.The resins were scrubbed with a brush to remove soil particles and washed with deionized water, then extracted for NH_4^+ and NO_3^- analysis. The resins were extracted by placing in centrifuge tubes with 20 ml of 0.5 M HCL, then each sample was shaken for 0.5 hour. An aliquot of extracts was analyzed calorimetrically for NH_4^+ and NO_3^- by the sodium salicylate/nitroprusside method for ammonium (Mulvaney, 1996) and the cadmium reduction method for nitrate (Mulvaney, 1996) using Smart Chem (Westco Scientific Instruments, Inc.).

2.3.3 Statistical analyses

Multi-way ANOVA test was used to compare treatments based on soil EC, biochar, and percentage of biochar addition to soil. Microbial biomass carbon and nitrogen, dissolved organic carbon and nitrogen, microbial respiration, metabolic quotient, inorganic nitrogen availability data were tested for normality and homogeneity of variance using Shapiro-Wilk and Levene's test, respectively. All data were normally distributed. Mixed model function of SAS version 9.2 (SAS Institute 2010) was used to run ANOVA. R software (Version 2.15.2) was used to graph all data points.

2.4 Results

2.4.1 Water retention

Water retention curve from pressure-plate apparatus showed that biochar increases soil water retention at all water potentials. Both willow and straw biochar increased the water retention of both high and low EC soils. The gravimetric water content at field capacity (33 kPa) for high EC soil increased from 23% to 28% and 30% with 10% addition of straw and willow biochar, respectively. Furthermore, the gravimetric water content at field capacity for low EC soil increased from 24% to 32% and 35% with 10% addition of straw and willow biochar, respectively. There was a drastic reduction of soil water content from 33 to 300 kPa compared to other pressures. In general, low EC soil showed higher water retention in comparison to high EC soil at the same pressure and biochar addition. For instance, field capacity in low EC soil with 10% straw biochar addition was 4% more than high EC soil with the same conditions. On the other hand, willow biochar treatments showed more water retention compared to straw biochar treatments. For instance, 10% addition of willow biochar in low EC soil showed 3% more water retention at field capacity compared to 10% addition of straw in the same soil. Generally, low EC-willow biochar treatments showed the highest water retention and high EC-straw biochar showed the lowest retention. Data variability increased from low EC to high EC soils with increase in standard deviation (Figure 2-1 & 2-2).

2.4.2 Total carbon and nitrogen

Total carbon of high EC soil, low EC soil, straw biochar, and willow biochar was 0.57%, 3.23%, 73.66%, and 81.75% (Table 2-1), respectively, and total nitrogen was 0.05%, 0.33%, 0.58%, and 0.58% (Table 2-1), respectively. Total carbon of the two soils was low indicating marginal conditions. We prescribed 1%, 2.5%, 5%, and 10% addition of biochar to soils, where

10% biochar was an exaggerated amount to potentially drive significant differences. Prior to incubation, total carbon and nitrogen of individual treatments were calculated. Total carbon of high EC soil was increased from 0.57% for control to 8.7% with 10% addition of willow biochar. Total carbon of low EC soil was increased from 3.23% for control to 11.4% with 10% addition of willow biochar (Table 2-2 & 2-3). The results for total nitrogen are shown in the same tables.

2.4.3 pH and EC

Biochar increased the pH of both high and low EC soils. The pH for high EC soil was increased from 7.82 to 7.94 and 8.00 with 10% addition of straw and willow biochars), respectively. The pH for low EC soil was increased from 5.26 to 6.04 and 6.64 with 10% addition of straw and willow biochars (Table 2-2 & 2-3), respectively.

In general, there was a mild decreasing trend for EC with increase in biochar application for both soils, although it is not statistically proven here. The EC for high EC soil was decreased from 4.58 to 4.2 and 4.45 mS/cm with 10% addition of straw and willow biochars, respectively. The EC for low EC soil was decreased from 1.84 to 1.59 and 1.4 mS/cm with 10% addition of straw and willow biochars (Table 2-2 & 2-3), respectively.

In order to bring the moisture content of all treatments to equilibrium which was 60% of field capacity; we needed to measure water content of each treatment and add enough water to each one. The water content of treatments were different due to the level of biochar water contents and different rates of biochar addition (results are not shown here.).

2.4.4 Microbial biomass

Microbial biomass carbon (MB-C) in low EC soil was more than high EC soil (Figure 2-5). Biochar application had contrasting effects on two soils; it increased MB-C in low EC soil and reduced it in high EC soil. Straw biochar didn't show any significant changes in high EC

soil, but in low EC, 10% addition of straw biochar increased MB-C significantly (P=0.028). Significant increase of MB-C in low EC soil started at 2.5% addition (P=0.0001) and all four rates of willow biochar application decreased MB-C in high EC soil. At 10% addition of willow biochar MB-C increased by 19 µg/ g dry soil in low EC soil and decreased by 36 µg/ g dry soil in high EC soil. MB-C in low EC soil was significantly higher at 2.5% and 5% additions of willow biochar compared to straw biochar at the same rates (P=0.0021 and P=0.0061, respectively). All application rates of willow biochar in high EC soil represented lower values regarding MB-C compared to straw biochar at the same rates (P=0.0119, P=0.0015, P<0.0001, and P<0.0001, 1%-10% respectively). In low EC soil, MB-C of willow biochar at 2.5% and 5% rates was 21.3 and 18.8 µg/ g dry soil more than straw biochar at the same application rates, respectively. In high EC soil, MB-C of willow biochar at 1%, 2.5%, 5%, and 10% rates was 17.1, 22.1, 28.1, 27.9 µg/ g dry soil more than straw biochar at the same application rates, respectively. MB-C of control in low EC soil was 58 µg/ g dry soil more than high EC soil (Figure 2-5).

Microbial biomass nitrogen (MB-N) in low EC soil was higher than high EC soil regardless to biochar application. None of straw and willow biochar application rates were successful in making significant changes to MB-N in high EC soil. The significant increase of MB-N in low EC soil for straw biochar started at 5% rate (P=0.0309). On the other hand, the significant increase of MB-N in low EC soil for willow biochar started at 2.5% rate (P=0.0005), and it was significantly higher than straw at the same application rate. MB-N in low EC soil was increased by 6.8 µg/ g dry soil at 10% addition of straw biochar. In both soils, MB-N of willow biochar at 2.5% rate was significantly higher than straw biochar at the same rate (P=0.0112 for high EC soil and P=0.0351 for low EC soil). Willow biochar at 2.5% rate increased MB-N by 3.1

and 3.7 µg/ g dry soil more than straw biochar at the same rates in low EC and high EC soils, respectively. MB-N of control in low EC soil was 9.5 µg/ g dry soil more than high EC soil (Figure 2-6).

Microbial biomass carbon to nitrogen ratio (MB-C/MB-N) in high EC soil was higher than low EC soil (P=0.0006). Straw biochar at 2.5% rate in high EC soil increased MB-C/MB-N ratio significantly (P=0.0101). Other biochar treatments except straw at 1% and 5% rates reduced MB-C/MB-N ratio significantly (P=0.7354 and P=0.0739, respectively). In low EC soil, only straw and willow biochars at 10% rate reduced MB-C/MB-N ratio significantly (P=0.0124 and P=0.0368, respectively). All willow biochar treatments showed less MB-C/MB-N ratios compared to straw biochar treatments at the same rates in high EC soil. No biochar treatment at the same rate (straw vs. willow) was significantly different in low EC soil (Figure 2-7).

2.4.5 Dissolved organic matter and microbial biomass

Dissolved organic carbon (DOC) in high EC soil was more than low EC soil. In low EC soil only straw biochar at 10% application rate (P=0.04) and willow biochar at the same rate (P=0.0002) significantly reduced DOC. In high EC soil, only willow biochar at 10% application rate significantly reduced DOC (P<0.0001). Willow biochar at 10% application rate decreased DOC by 13 µg/ g dry soil in high EC soil and 9.5 µg/ g dry soil in low EC soil. In high EC soil, DOC of willow biochar at 2.5%, 5%, and 10% rates was significantly lower than straw biochar at the same rates (P=0.0192, P=0.0389, P<0.0001, respectively). DOC in willow biochar at 2.5%, 5%, and 10% rates was 5.7, 5, and 10.1 µg/ g dry soil less than straw biochar at the same rates, respectively. DOC of control in high EC soil was 26 µg/ g dry soil more than low EC soil (Figure 2-3).

Dissolved organic nitrogen (DON) in high EC soil was more than low EC soil. In general, all biochar treatments showed lower DON except straw biochar at 2.5% application rate in high EC soil, which was significantly higher than control ($P<0.0001$). In low EC soil, straw biochar significantly reduced DON at 2.5% rate ($P=0.0307$), and willow biochar significantly reduced DON at 5% rate ($P<0.0001$). In high EC soil, straw biochar was only showing significant reduction at 10% rate ($P=0.0002$), and willow biochar started to show significant decrease at 5% rate ($P=0.0423$). Willow biochar at 10% rate in high EC soil decreased DON by 7 µg/ g dry soil and in low EC soil by 8.3 µg/ g dry soil. DON of high EC soil was 8.7 µg/ g dry soil more than low EC soil. In high EC soil, except for straw biochar at 2.5% rate which was increased, DON of willow biochar at 5% and 10% rates was less than straw biochar at the same rates ($P=0.0003$ and $P=0.0117$, respectively). Willow biochar at 5% rate reduced DON by 4 µg/ g dry soil and at 10% rate by 2.7 µg/ g dry soil more than straw biochar at the same rates. In low EC soil, DON of both biochars at the same application rates was not significantly different (Figure 2-4).

2.4.6 Microbial respiration

After 19 days of incubation, soil basal respiration in high EC soil was more than low EC soil. The most significant increase in high EC soil was at 2.5% rate for both straw and willow biochars ($p<0.0001$). Microbial respiration of all biochar treatments in high EC soil was significantly higher than control except for straw biochar at 1% application rate ($P=0.3866$). Microbial respiration of all biochar treatments in low EC soil was significantly higher than control except for straw at 5% and willow at 1% application rates ($P=0.0552$ and $P=0.1279$, respectively). The most significant increase in low EC soil was at 10% rate for both straw and willow biochars ($P<0.0001$). Willow biochar at 2.5% rate in high EC soil increased respiration

by 4 µg C-CO_2 / g dry soil / day and willow biochar at 10% rate in low EC soil increased respiration by 2.3 µg C-CO_2 / g dry soil / day. All willow biochar treatments in high EC soil showed more respiration than straw biochar at the same rates except for 2.5% application rate. None of willow biochar treatments showed significant higher respiration values compared to straw biochar treatments at the same rates. Soil basal respiration of control in high EC soil was 6.1 µg C-CO_2 / g dry soil / day more than low EC soil (Figure 2-8).

Metabolic quotient in high EC soil was more than low EC soil. In high EC soil, all biochar treatments except straw at 1% and 5% rates showed significant larger values (P=0.9559 and P=0.0662, respectively). In low EC soil, metabolic quotient of both straw and willow biochars at 10% application rate was significantly more than control (P=0.0312 and P=0.0345, respectively). Metabolic quotient in high EC soil was increased by 0.028, 0.062, and 0.07 with addition of straw and willow biochar at 2.5% and willow biochar at 10% application rate, respectively. Metabolic quotient in low EC soil was increased by 0.026 with addition of willow biochar at 10% application rate. Metabolic quotient of all willow biochar treatments was significantly more than straw biochar treatments at the same rates in high EC soil, but none of willow biochar treatments showed significant higher metabolic quotient than straw treatments in low EC soil. Willow biochar additions at 1%, 2.5%, 5%, and 10% rates increased metabolic quotient by 0.033, 0.033, 0.045, and 0.045 compared to the same additions of straw biochar, respectively. Metabolic quotient of control in high EC soil was 0.064 more than low EC soil (Figure 2-9).

2.4.7 Ion-exchange resin membrane

Nitrate (NO_3^-) was significantly higher in high EC soil in comparison to low EC soil. In both high and low EC soils, straw biochar at 5% and 10% application rates and willow biochar at

10% rate significantly decreased NO_3^- availability. In high EC soil, straw biochar at 5% and 10% and willow biochar at 10% rates decreased NO_3^- by 26.8, 24.8, and 45.1% compared to control, respectively. In low EC soil, straw biochar at 5% and 10% and willow biochar at 10% rates decreased NO_3^- by 34.7, 50.4, and 54.1% compared to control, respectively. Soil NO_3^- of straw biochar at 5% rate was significantly less than willow biochar at the same application rate in high EC soil (P=0.0011). On the contrary, Soil NO_3^- of straw biochar at 10% rate was significantly more than willow biochar at the same application rate in high EC soil (P=0.0454). None of biochar treatments were significantly different in low EC soil. Soil NO_3^- of control in high EC soil was 55.4% more than low EC soil (Figure 2-10).

Ammonium (NH_4^+) didn't change drastically regarding biochar addition. It was only high EC-control treatment which was significantly lower than biochar treatments except willow biochar at 10% rate, although it was also increased (P=0.0539). Biochar increased soil NH_4^+ availability (for instance, P=0.0368 for straw at 1% rate in high EC soil). Straw biochar at 1% addition in high EC soil increased soil NH_4^+ by 171.8% compared to control (Figure 2-11). Ammonium was much less in both soil compared to nitrate. The ratio of nitrate to ammonium in high EC soil was 31 and biochar decreased this ratio by 78% with addition of willow at 10% rate.

2.4.8 Correlation between soil parameters

Basal respiration (CO_2) was negatively correlated with MBC/MBN in both soils (R=-0.82 for high EC and R=-0.74 for low EC) (Table 2-4 & 2-5).

Soil nitrate NO_3^- showed an interesting behavior versus MBC/MBN in terms of biochar addition and soil EC. Low EC soil didn't represent a lot of change in NO_3^- along with changes in MBC/MBN and the trend was increasing from 100 to 200 μg / 10 cm^2 soil. On the other hand,

high EC soil represented changes in NO_3^- from 150 to 350 µg / 10 cm^2 soil, which was generally higher than low EC soil. NO_3^- increased with increase in MBC/MBN to an optimum value of 9 for MBC/MBN; then it was reduced with increase in MBC/MBN. Apparently in both soils, addition of biochar decreased NO_3^- (Figure 2-12). MBN was negatively correlated with NO_3^- and addition of biochar decreased nitrogen availability and increased MBN in both soils. Low EC soil showed higher MBN and lower nitrogen availability compared to high EC soil (Figure 2-13). NO_3^- was positively correlated with MBC/MBN n both soils (R=0.40 for high EC and R=0.71 for low EC), and it was negatively correlated with basal respiration (R=-0.47 for high EC and R=-0.73 for low EC) (Table 2-4 & 2-5).

2.5 Discussion

2.5.1 Microbial dynamics under EC regimes

This study showed that EC, as expected, was a main factor affecting biochar influence on microbial dynamics. Microbial community abundance represented via MBC showed two contrasting increasing and decreasing trends in low and high EC soils (Figure 2-5). The same happened for MBN as it increased in low EC soil and didn't show any changes in high EC soil. Other soil characteristics such as pH, total organic carbon and nitrogen, organic matter addition were also important as well as some that we didn't measure such as root exudates (Fu and Cheng, 2002). The pH in high EC was measured as basic (pH=7.8) and both TC and TN were lower compared to low EC soil. Higher growth of microorganisms can be attributed to priming effect of biochar. It is proposed that biochar can provide some labile carbon to microbes and increase their growth and activity by more soil organic matter (SOM) decomposition. Hence, low organic matter content can be limiting factor for microbial growth. High EC soils lead to lower plant growth because of lower osmotic potential. Thereafter lower plant growth lead to lower

aboveground biomass (Dijkstra *et al.*, 2006). Consequently, lower amount of carbon in the form of organic matter could be added to soil.

Microorganisms mineralized more C as CO_2 in high EC soil which could be indicative of higher stress on them. These results are confounding the results from (Pankhurst *et al.*, 2001; Yuan *et al.*, 2007; Elmajdoub *et al.*, 2014). They found a decreasing trend of respiration with addition of EC and attributed this to lower availability of easily available C substrates, but in contrast, our results showed that elevated EC increased both C substrate availability and activity shown via respiration. In this study, high availability of dissolved organic carbon (DOC) is accompanied by higher activity of organisms. Higher activity of organisms in high EC conditions is mostly due to the synthesis of the organic osmolytes. The generation of osmolytes needs a lot of energy (Oren, 2001; Hagemann, 2011). Supposedly, organisms set a priority for osmolyte synthesis to overcome saline conditions. This stressful condition can be supported by lower microbial abundance and growth in high EC conditions of this study. Mavi and Marschner (2013) found that MBC was more sensitive to increasing salinity than was cumulative respiration. Hence, C is more preferentially utilized for energy rather than growth and this leads to lower efficiency of C utilization.

This study found a higher availability of both DOC and DON with increasing EC meaning that microbes were less able to utilize available substrate, which is in agreement with Mavi and Marschner (2012). On the other hand, higher amounts of DOC and DON could be due to lysed cells of some sensitive organisms to salinity (Mavi and Marschner, 2013). These results are also in agreement with higher nitrate availability in high EC soil compared to low EC soil indicative of lower capability of microbes to utilize inorganic N for growth. Vega-Jarquin *et al.* (2003) also found that in saline soils microbes can immobilize inorganic N.

2.5.2 Microbial dynamics and N turnover under biochar regimes

Biochar addition increased water retention in all cases and matric potentials. This is indicative of higher potential of microbial activity and growth, although the water content of all treatments were mediated after addition of biochar and before incubation started. Biochar also increased pH from 5.26 to 6.64 for low EC soil and from 7.8 to 8 for high EC soil. The change in pH can make a huge difference in living conditions for microbes. Moreover, biochar increased soil organic carbon stock, since it is highly rich in C. On the other hand, it didn't change soil EC drastically and there was a very slight reduction (Table 2-2 & 2-3).

It is reported that biochar can increase microbial biomass and activity through elevation of pH in acidic soils (Pietri and Brookes, 2008). The low EC soil was fairly acidic (pH=5.26) and addition of biochar increased MBC, MBN, respiration and metabolic quotient concurrently making the soil more neutral (pH=6.6). Lehmann *et al.* (2011) noted that biochar can potentially produce alkaline micro-habitats leading to favourable niche environments for microbial populations. In this acidic soil, no change in ammonium was observed and nitrate availability was reduced with addition of biochar meaning that biochar didn't change net mineralisation but increased microbial immobilization of nitrogen. This result was partly in contrast to what Dempster *et al.* (2012) found. They attributed their results to the changes in ammonia oxidiser community structure because of a significant reduction in rates of nitrification, and particularly ammonia oxidation as basicity increased with addition of biochar (Deboer *et al.*, 1988). In high EC soil, irrespective of rate, biochar addition increased NH_4^+ significantly, meaning that mineralization of nitrogen was elevated but no change was observed in low EC soil (Figure 2-11). Introduction of some labile C might have stimulated some ammonifiers to mineralize some N and since there was little SOM, higher rates of biochar addition didn't increase NH_4^+

availability (Gundale and DeLuca, 2007). The amount of NH_4^+ was lower compared to NO_3^- indicative of higher nitrification rates and faster turnover of ammonium. Nitrification is more dominant in lower pH, so with increase in pH nitrification becomes less dominant and nitrate production slows down (Ulyett *et al.*, 2014) . On the other hand, biochar addition can absorb some NO_3^- in its micro-pores and make it less available to microbial utilisation. Bacteria typically inhabit in pores larger than 0.6 μm (Strong *et al.*, 1998). As long as biochar continues to aging, positive charge is likely to increase as negative charge elevates (Cheng *et al.*, 2008). Some studies reported a high C sorption capacity of biochar which is 1 to 3 times larger than sorption capacity of SOM (Koelmans *et al.*, 2006; Pignatello *et al.*, 2006). That means N cycling microbes have less access to easily decomposable C and need to use more energy to synthesis more enzymes to metabolise more complex C structures such as liginin and cellulose (Mavi and Marschner, 2013). Furthermore, sorption efficiency is dependent on the type of biochar, age of biochar, feedstock, and pyrolysis conditions (Zackrisson *et al.*, 1996; James *et al.*, 2005; Brown *et al.*, 2006). This likely made differences between two biochars. Cation exchange capacity of biochar can potentially increase due to external oxidation in soil (Liang *et al.*, 2010), and it can lose some of its sorptive properties as some non-polar binding sites get blocked (Zackrisson *et al.*, 1996). This incubation study was done in 20 days and results are contingent to longer periods to some point.

In high EC soil no change in MBN was observed and MBC was even decreased with addition of willow biochar. This could be attributed to the concurrent influence of the priming effect of biochar and status of SOM. Biochar possibly have provided some labile C to microbes and also toxins. More SOM can absorb higher amounts of toxins without letting microbes to get damage and grow more. On the other hand, microbes can assimilate provided labile C and

decompose more SOM. Lower SOM in high EC soil can't protect microbes from released toxins. Consequently, more biochar addition leads to lower MBC in high EC soil. Lower MBC can lead to lower plant shoot biomass (Dempster *et al.*, 2012) or higher shoot biomass (Chan *et al.*, 2008; Steiner *et al.*, 2008). This study was done in lab incubation and there was no plant effect and possibly no positive rhizosphere priming effect with addition of root exudates. But it is possible to mention that with lower aboveground biomass there is less C substrate for microorganisms and the negative effect of biochar would be exacerbated. It is also important to note that straw biochar didn't change MBC in high EC soil at all. It could be a result of less volatile organic compounds (VOC's) such as benzene and ethylene (Spokas *et al.*, 2010) which made it less toxic to organisms (Girvan *et al.*, 2005; Deenik *et al.*, 2010). This study didn't do any measurements on volatile compounds and more investigation is needed to distinguish between two biochars in terms of toxicity.

MBC/MBN was reduced in both soils with addition of biochar except for straw biochar below 5% rate in high EC zone. This is in agreement with the results from other studies (Joergensen and Brookes, 1990; Durenkamp *et al.*, 2010). One reason for this was that biochar had higher affinity towards absorbing C containing organic compounds (Cornelissen *et al.*, 2005). Thus, more dissolved organic nitrogen was accessible for microbes to become immobilized, as DON was highly negatively correlated with MBN (R=-0.67 for high EC & R=-0.73 for low EC) (Table 2-4 & 2-5). The change in MBC/MBN suggests a change in microbial community structure (Yoo and Kang, 2012). A reduction in MBC/MBN indicates that bacteria are becoming more dominant in microbial biomass (Freppaz *et al.*, 2012). In high EC soil, straw biochar at lower application rates even increased MBC/MBN ratio. It could be indicative of how willow biochar made better habitat for bacteria against grazing predators and better colonization.

Visser *et al.* (1983) reported that in low nutrient regimes and highly stressed disturbed soils in Alberta, Canada, bacteria were enhanced more than fungi and actinomycetes. Higher activity of microorganisms with addition of biochar and a concurrent unavailability of nitrogen can push microbes under stress and increase the abundance of bacteria over fungi and actinomycetes. This is what happened in this study. Furthermore, bacterial abundance can inhibit the decomposition of more complex organic material in saline soils, since fungi are specialized in breaking down lignin and cellulose (Harper and Lynch, 1985). Consequently, reduction in MBC/MBN wouldn't sound good for biochar mediated marginal soils as decomposition of more complex compounds become slower, but in terms of C sequestration it opens a very different scenario.

There was a negative correlation between MBN and NO_3^- suggesting that most of the nitrate was immobilized by microorganisms. Less nitrate availability and more MBN were in the realm of higher rates of biochar addition (Figure 2-13). This is another proof which tells lower C availability drives microbes towards N utilisation with addition of biochar. N_2O is a greenhouse gas emission which is not measured in this lab incubation and will be discussed in chapter 3. But higher rates of microbial and physical immobilization of N can slow down N turnover in soil and decrease nitrification and denitrification processes in soil. Hence, N_2O which is mainly produced from these processes (Pathak, 1999) can decrease to a significant degree with biochar addition.

Different confounding results from different studies could be a consequence of different methodologies (Dempster *et al.*, 2012). In this study microbial abundance and growth was measured by CFE in which microbe cells are lysed during fumigation and dissolved organic matter is extracted from samples before and after fumigation. Biochar has high sorption capacity and during fumigation can absorb a significant amount of dissolved organic carbon and nitrogen released from died organisms. This happening can underestimate the abundance of microbial

population (Durenkamp *et al.*, 2010). The level of underestimation can be more in low EC soil as there is potentially more abundance of microbes. Dempster *et al.* (2012) found that there was no effect of biochar on the recovery of ^{14}C labelled microbial biomass or sorption of amino acids during the CFE process.

Microbial respiration was measured using alkali traps put in incubation jars and NaOH trapping emitted CO_2. Mineralised C could be from both biotic and abiotic sources. Yoo and Kang (2012) proposed that some of evolved CO_2 was absorbed by biochar. It is reported that biochar can reduce native soil organic carbon through the process of mineralisation (Steinbeiss *et al.*, 2009). Dempster *et al.* (2012) reported a 2.5% carbonate existing in biochar which increased CO_2 after dissolution in soil solution. In this study, CO_2 was rate dependent except 2.5% in high EC soil (Figure 2-8), meaning that more addition of biochar provided more HCO_3^- and more evolution of CO_2, although carbonates weren't measured in this study. Some studies showed repressions in biotic CO_2 emission after biochar application (Jones *et al.*, 2011; Keith *et al.*, 2011). The results of this study were in agreement with Kolb *et al.* (2009) as basal respiration was increased. Smith *et al.* (2010) did a lab incubation study of young biochar addition to pasture soil and found an initial increase in CO_2 evolution only for a few days. They concluded that biochar carried some labile C and the early phase of C mineralisation was a result of this introduced labile C. In high EC soil, respiration was negatively correlated with DOC, DON, NO_3^-, MBC and MBC/MBN ratio. The negative correlation between respiration and MBC/MBN could indicate higher availability of N to microbes and more preferential metabolic activity of N over C (Table 2-4). On the other hand, in low EC soil respiration was still negatively correlated with MBC/MBN, but positively correlated with MBC. This might mean microbes were under less stress but still nitrogen was more available to them.

Metabolic quotient (qCO_2) which was indicative of microbial activity followed the same trends as respiration. Biochar addition increased qCO_2 and willow biochar showed more pronounced results. Metabolic quotient is a good measurement of stress on microbial community (Iwai *et al.*, 2012). A change in metabolic quotient is representing a shift in microbial community structure as a consequence of environmental stress (Rasul *et al.*, 2006). Low qCO_2 suggests that microbial community are more energetically efficient and are able to assign more carbon for growth rather than for maintenance (Zak *et al.*, 1994). Sakamoto and Oba (1994) reported that less efficient bacterial communities can convert less substrate C into biomass C than fungi in salt affected soils. All of the results of this study about the shifts in microbial communities are supportive for each other. Increase in bacterial communities lead to a reduction in MBC/MBN ratio and increased metabolic quotient which was indicative of more stress. Addition of biochar changed the microbial communities to less efficient in C utilisation (Figure 2-5 & 2-7 & 2-9). Figure 2-12 shows a correlation between NO_3^- availability and MBC/MBN. According to this correlation a balance can be seen between bacteria and fungi population where MBC/MBN is between 8 and 10. Since availability of nitrate is highest it is assumed that there is less competition between two groups of organisms over nitrogen resources.

2.6 Conclusion

In highly stressed soil, biochar mostly decreased microbial abundance and drove it towards a bacterial dominance. It also increased the immobilization of N and decreased its availability. Other parameters such as DOC and DON which are important sources for microbial utilisation were reduced. Metabolic quotient was also increased which showed more stress on microbial communities. Biochar increased pH towards a more basic environment which may have made the conditions even worse. In a more healthy soil, it generally increased total

population and at exaggerated rate of 10% only showed a significant increase in metabolic quotient. But it also decreased nitrogen availability and dissolved organic matter but made the soil neutral in terms of pH. This study was a short term lab incubation and the results can change significantly in longer term. More research is needed to characterize the applied biochar in order to investigate the complex interactions between biochar and soil. There was no plant or any other confounding factors besides biochar which could change the results. According to these short term results biochar is not proposed for marginal soils as it can worsen the conditions for microbial communities.

Table 2-1: Total carbon and nitrogen of soils and biochars

	Total C ((w/w)%)	Total N ((w/w)%)
High EC soil	0.56	0.05
Low EC soil	3.22	0.33
Straw biochar	73.66	0.58
Willow biochar	81.75	0.58

Table 2-2: Basic characteristics of treatments made with different rates of biochar addition

	EC (mS/cm)	pH	Calculated Pre-Incubation Total C ((w/w)%)	Calculated Pre-Incubation Total N ((w/w)%)
High EC - Control	4.58	7.82	0.56	0.050
High EC - Straw1%	4.62	7.83	1.30	0.056
High EC - Straw2.5%	4.48	7.91	2.41	0.064
High EC - Straw5%	4.50	7.90	4.24	0.079
High EC - Straw10%	4.20	7.94	7.93	0.108
High EC - Willow1%	4.59	7.86	1.38	0.056
High EC - Willow2.5%	4.44	7.82	2.61	0.064
High EC - Willow5%	4.35	7.93	4.65	0.079
High EC - Willow10%	4.45	8.00	8.74	0.108
Low EC - Control	1.84	5.26	3.22	0.332
Low EC - Straw1%	1.46	5.47	3.96	0.338
Low EC - Straw2.5%	1.58	5.28	5.07	0.347
Low EC - Straw5%	1.77	5.82	6.91	0.361
Low EC - Straw10%	1.59	6.04	10.59	0.390
Low EC - Willow1%	1.74	5.43	4.04	0.338
Low EC - Willow2.5%	1.82	5.55	5.27	0.347
Low EC - Willow5%	1.42	5.58	7.31	0.361
Low EC - Willow10%	1.40	6.64	11.40	0.390

Table 2-3: Correlation between measured soil parameters of both high and low EC soil. The bottom left belongs to high EC and the top right belongs to low EC.

High EC	MBC/ MBN	NO_3^-	DOC	DON	MBC	MBN	CO_2	Low EC
MBC/ MBN	1	0.71	0.74	0.79	-0.37	-0.85	-0.74	MBC/ MBN
NO_3^-	0.40	1	0.63	0.91	-0.50	-0.72	-0.73	NO_3^-
DOC	0.66	0.66	1	0.71	-0.19	-0.64	-0.71	DOC
DON	0.77	0.63	0.83	1	-0.44	-0.73	-0.84	DON
MBC	0.76	0.38	0.47	0.64	1	0.80	0.24	MBC
MBN	-0.82	-0.40	-0.67	-0.67	-0.29	1	0.59	MBN
CO_2	-0.82	-0.47	-0.54	-0.57	-0.69	0.64	1	CO_2

Table 2-4: ANOVA table for main effects and interactions. Numbers are P values.

	DOC	DON	MBC	MBN	MBC/ MBN	CO_2	qCO_2	NO_3^-	NH_4^+
EC	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9933
Biochar	<.0001	<.0001	0.0540	<.0001	<.0001	<.0001	<.0001	<.0001	0.4219
EC*Biochar	0.0072	0.0002	<.0001	0.2066	<.0001	<.0001	<.0001	0.0978	0.0473

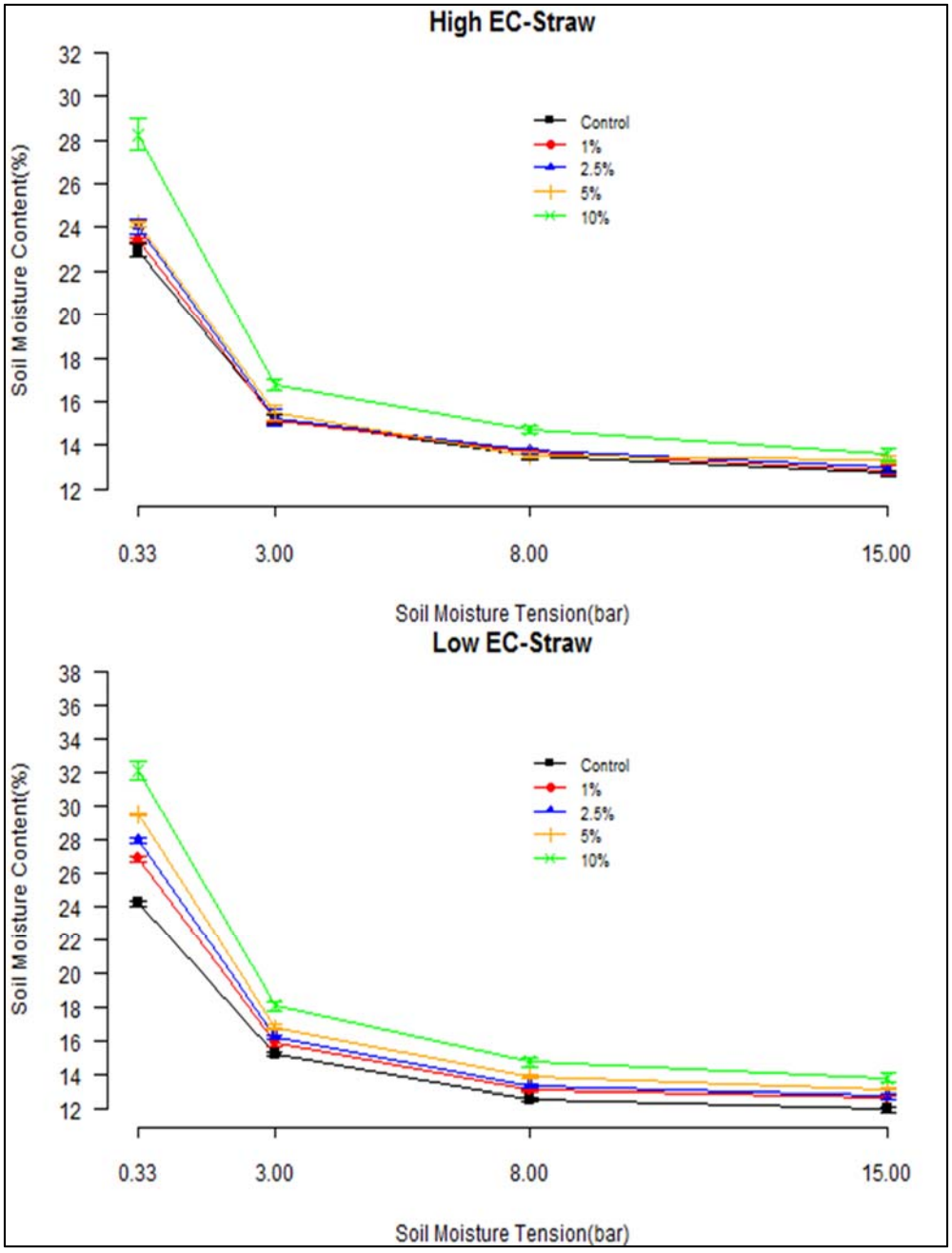

Figure 2-1: Pressure plate apparatus results for straw biochar treatments at different matric (negative) potentials. Moisture contents at 0.33 bar is called field capacity. Soil moisture content is volumetric and each bar is 100 kPa.

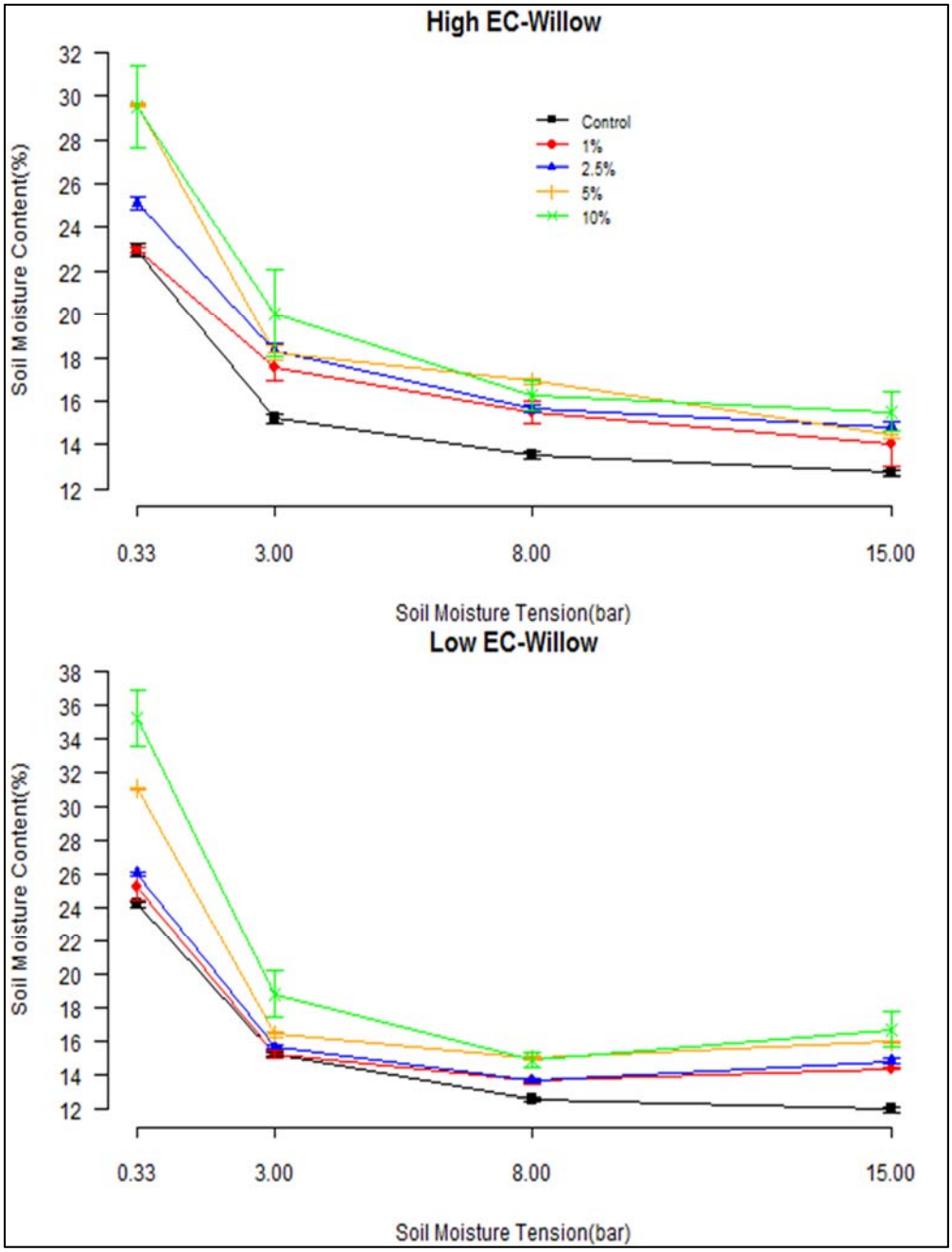

Figure 2-2: Pressure plate apparatus results for willow biochar treatments at different matric (negative) potentials. Soil moisture content is volumetric and each bar is 100 kPa.

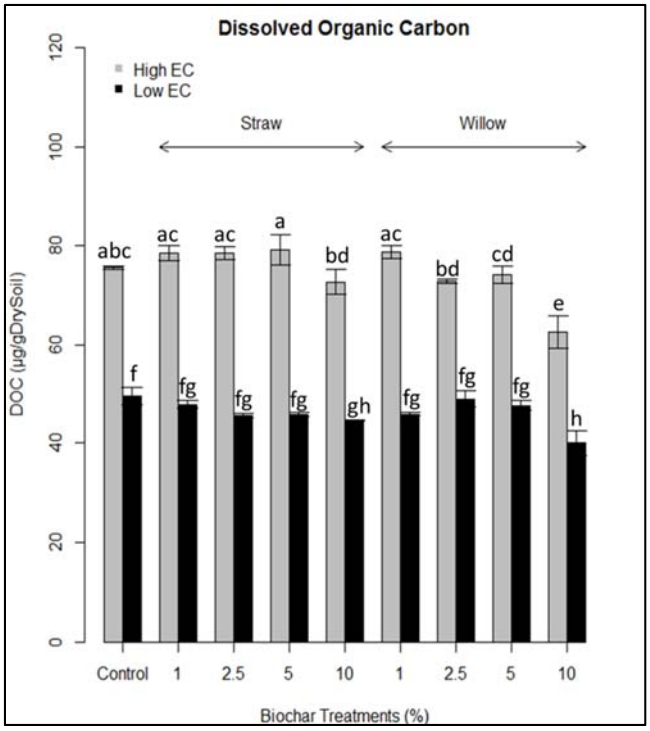

Figure 2-3: Dissolved organic carbon (µg/ g dry soil) of treatments after incubation. The same letters are indicative of no significant change in dependent variable after pairwise comparison.

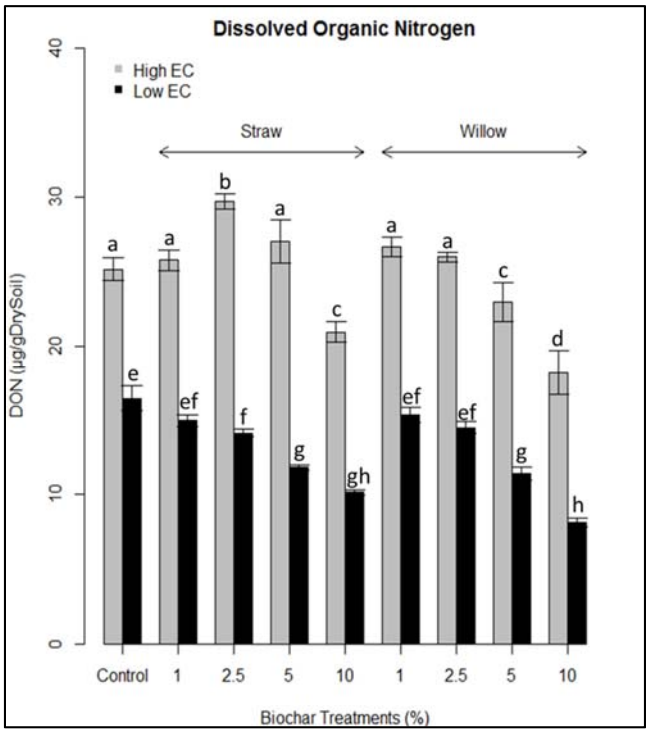

Figure 2-4: Dissolved organic nitrogen (µg/ g dry soil) of treatments after incubation. The same letters are indicative of no significant change in dependent variable after pairwise comparison.

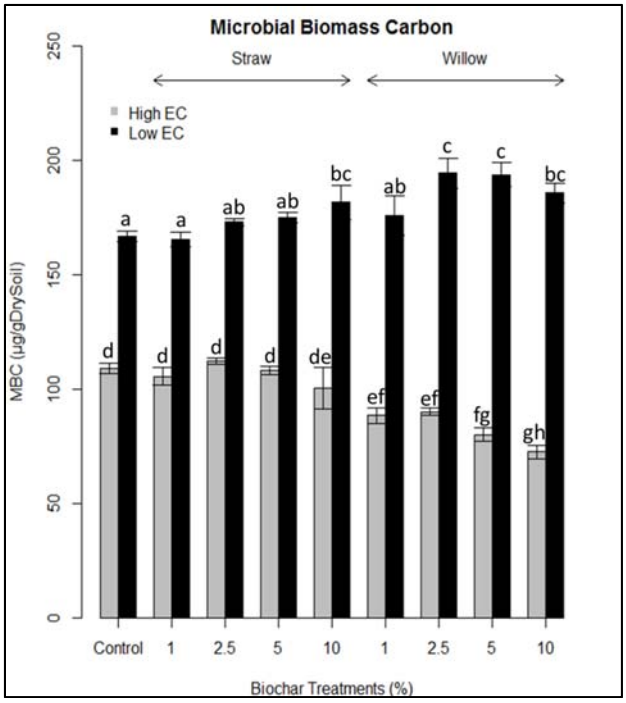

Figure 2-5: Soil microbial biomass carbon (µg/ g dry soil) of treatments after incubation. The same letters are indicative of no significant change in dependent variable after pairwise comparison.

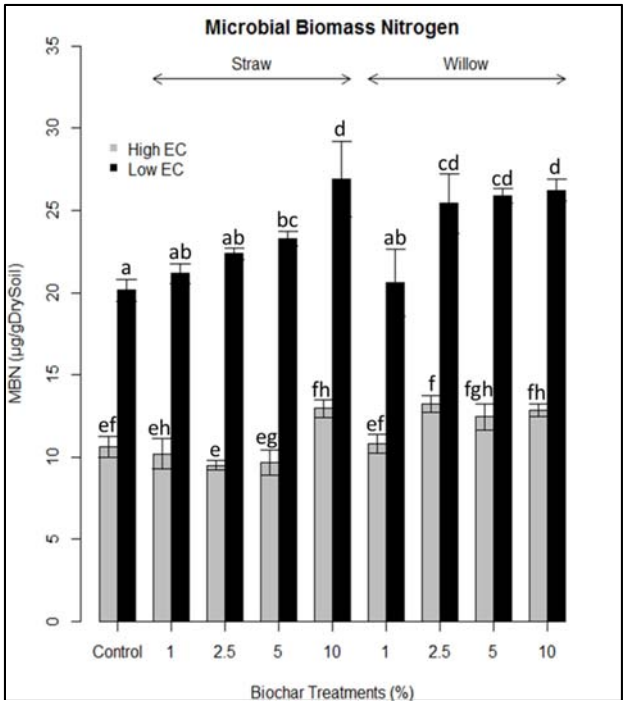

Figure 2-6: Soil microbial biomass nitrogen (µg/ g dry soil) of treatments after incubation. The same letters are indicative of no significant change in dependent variable after pairwise comparison.

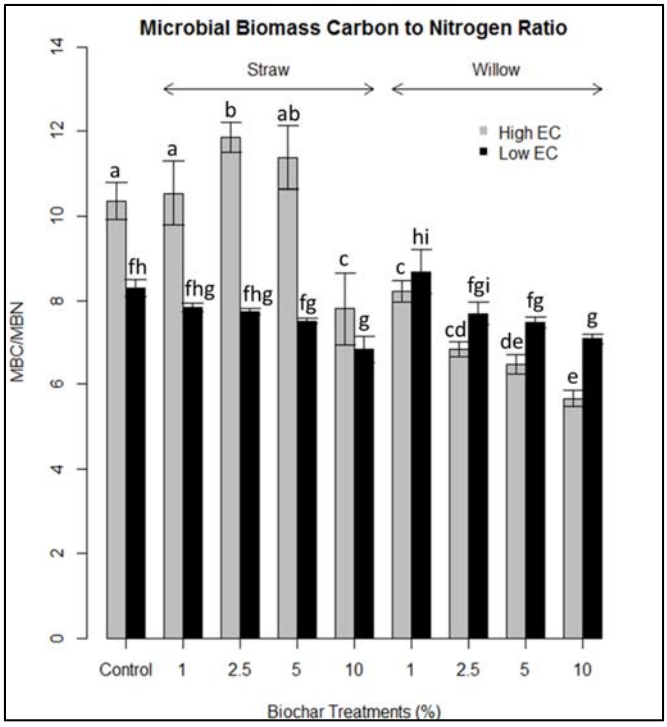

Figure 2-7: Microbial biomass carbon to nitrogen ratio (MBC/MBN) after incubation period. The same letters are indicative of no significant change in dependent variable after pairwise comparison.

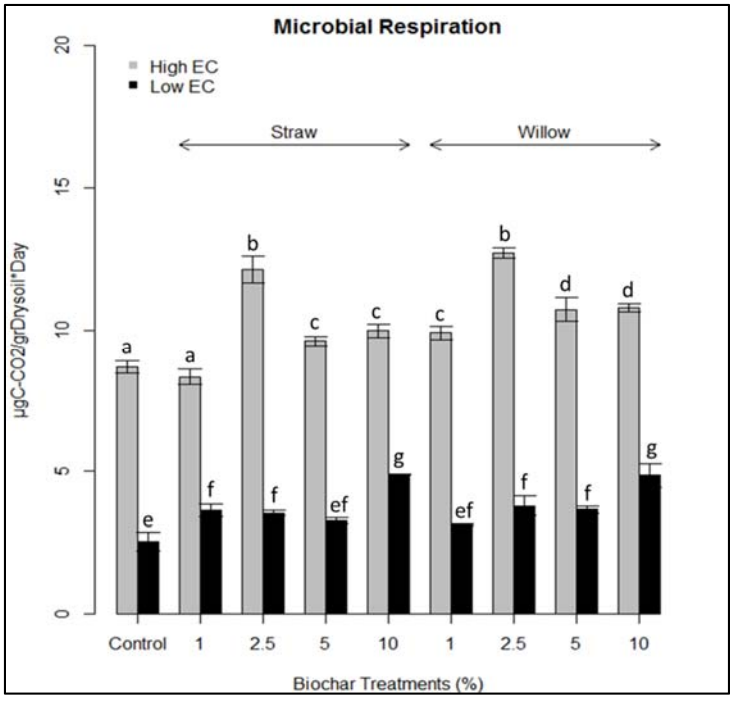

Figure 2-8: Microbial respiration measured in µg C-CO_2 / g dry / day soil. The same letters are indicative of no significant change in dependent variable after pairwise comparison.

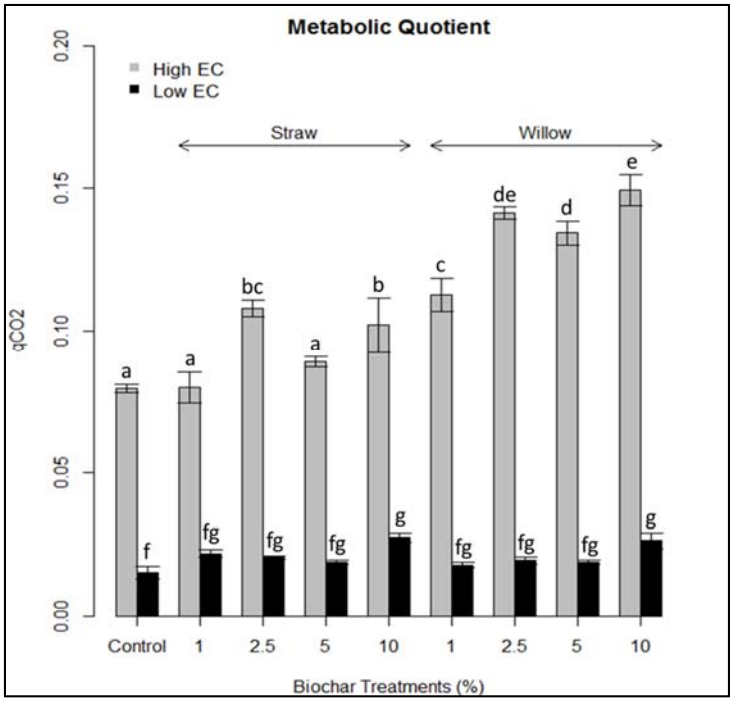

Figure 2-9: Metabolic quotient (qCO_2) as ratio of respiration to biomass representative of metabolic activity of microbes. The same letters are indicative of no significant change in dependent variable after pairwise comparison.

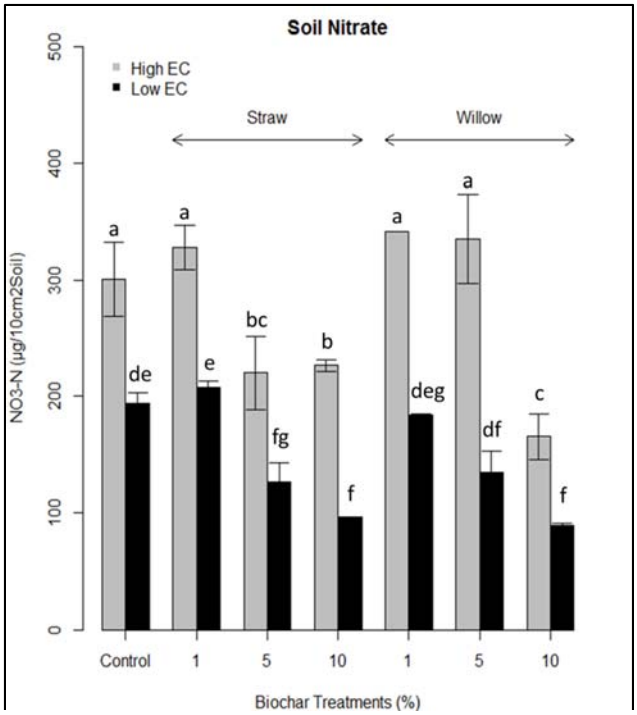

Figure 2-10: Soil nitrate (NO_3^-) measured in µg / 10 cm^2 soil after incubation period. The same letters are indicative of no significant change in dependent variable after pairwise comparison.

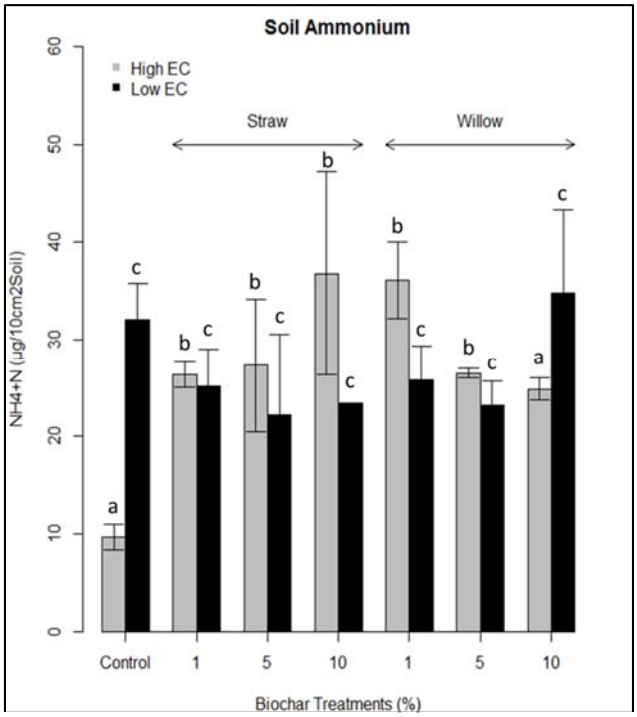

Figure 2-11: Soil ammonium (NH_4^+) measured in µg / 10 cm^2 soil after incubation period. The same letters are indicative of no significant change in dependent variable after pairwise comparison.

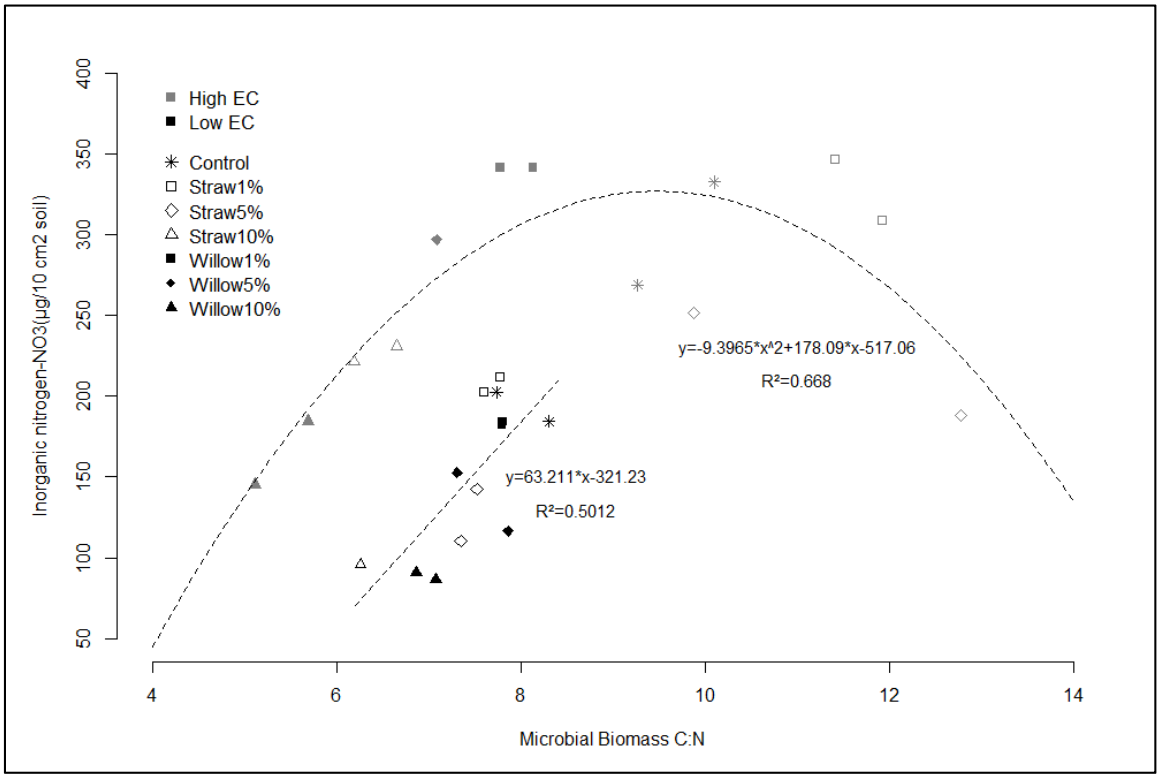

Figure 2-12: Soil nitrate (NO_3^-) versus microbial biomass C:N ratio after incubation period. Interestingly, in high EC soil the highest concentration of NO_3^- appeared with microbial biomass C:N ratio at 8-10. The data of low EC soil was not distributed as high EC soil data in terms of biochar application.

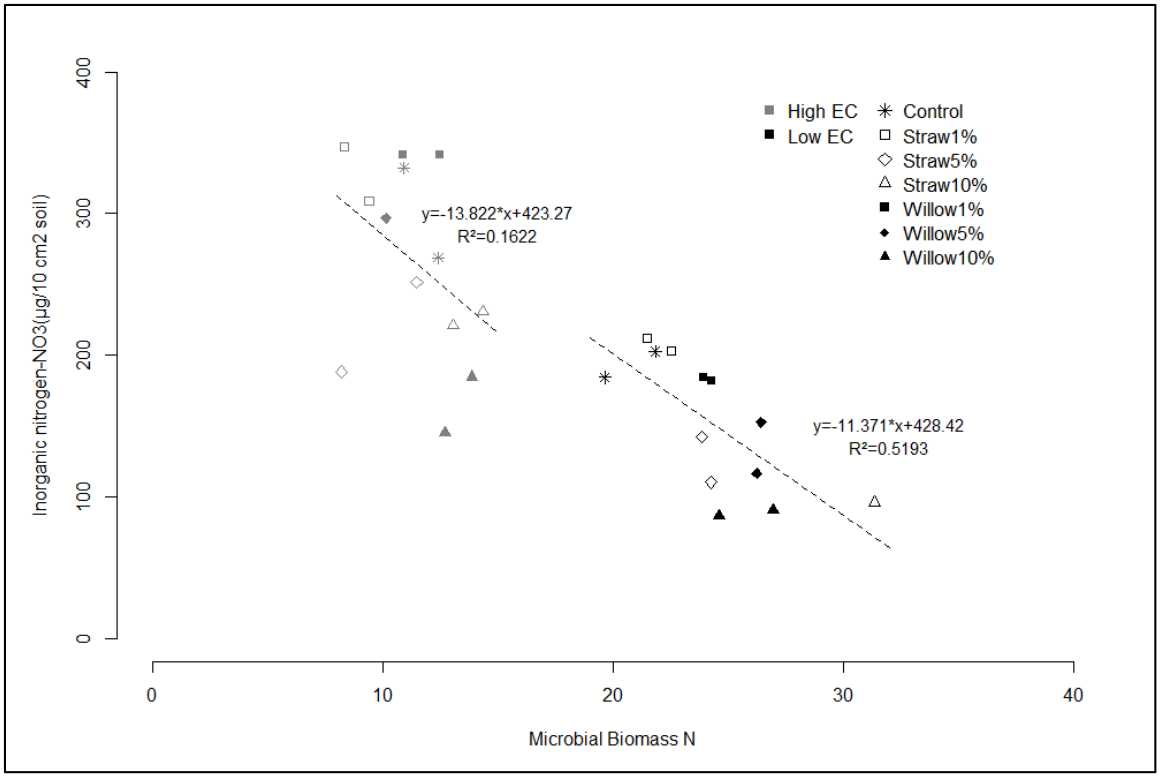

Figure 2-13: Soil nitrate (NO_3^-) versus microbial biomass N. MBC was negatively correlated with NO_3^-. With addition of biochar more nitrate was immobilized in microbial biomass. Low EC soil showed higher MBN and lower nitrogen availability and in both soils biochar decreased nitrogen availability.

Chapter 3: Biochar Application in Short Rotation Coppice Systems: Effects of Microbial Dynamics, Nutrient Availability, and Greenhouse Gases

3.1 Introduction

The Canada-Wide Strategy for the Management of Municipal Wastewater Effluent, which was endorsed by the Canadian Council of Ministers of the Environment in 2009, sets standards and objectives for the 3,500 wastewater facilities in Canada. The strategy will be implemented over a 30 year time frame, at an estimated cost of $10 - $13 Billion. Small communities in rural Canada have limited financial capacity, and the Strategy explicitly recognizes that alternatives to costly infrastructure investments will be determined on a case by case basis in order to give those communities the flexibility to meet the new standards (Canadian Council of Ministers of the Environment, 2009). In addition, the Alberta Municipal Wastewater Management Program, which regulates large wastewater systems (approx. 80% of the Province's population) does not include farms (Alberta Municipal Water/Wastewater Partnership, 2009). These two exclusions, small rural communities and farms, will require creative approaches such as proposed in this research to develop sustainable, efficient agricultural technologies and processes to turn wastewater and marginal lands into assets that can be used to improve productivity and to mitigate GHG emissions. The latter is an important potential source of income, as Alberta's Carbon Offset System currently has a protocol in place related to agroforestry, and this Project's research outcomes can contribute to protocols currently under consideration related to lagoons, biochar, soil amendments, renewable energy systems, water use efficiency and perennial cropping systems, all of which have the potential to qualify for carbon offset credits and become complementary sources of income in rural communities (Alberta Environment and Sustainable Resource Development, 2015).

In Alberta, there are over 250 small towns and villages and 64 rural municipal districts, with a combined population of 452,000, producing about 150 million litres of wastewater every day (Statistics Canada, 2013). Most communities use lagoons to treat sewage wastewater and face costly challenges as their populations fluctuate or water treatment quality standards for wastewater discharges to surface water bodies get more stringent. Irrigation is becoming a desired alternative to wastewater disposal. Alberta Environment has issued Guidelines for Municipal Wastewater Irrigation that acknowledges the advantage of using wastewater for irrigation, but stresses the need to test for potentially harmful physical, biological and chemical constituents (Alberta Environment, 2000). To date sub-surface irrigation installations have been the preferred method of ameliorating these risks, but these systems are double the cost of point source surface flood irrigation. Surface irrigation systems are currently in use in Europe, but are new to Alberta and Canada and need to be tested under local conditions. Five wastewater-irrigated willow and poplar sustainable woody crop plantation demonstration sites were established throughout Alberta. The plantations total 30 hectares and are located in the Town of Whitecourt, the Hamlet of Ohaton in Camrose County, the Town of Beaverlodge and the Hamlet of Clairmont in the County of Grande Prairie, and near the City of Edmonton. An agreement was also reached with Sturgeon County to establish a sixth site at Villeneuve. Project collaborators include 2 towns, the City of Edmonton, 3 counties, 6 private sector companies, 2 post-secondary institutions, 2 research organizations, 2 Alberta government departments, 2 Federal government organizations and the Edmonton Waste Management Centre of Excellence. Project partners have contributed an additional $227K in cash and over $450K in contributions to the research.

South and east-central portions of Alberta have a semi-arid climate, where evaporation exceeds precipitation (Alberta Water Portal, 2013b). Irrigation for agriculture is the largest user

of water in Alberta, accounting for 60 - 65% of all water consumed. 1.6 M acres (two thirds of all irrigation development in Canada) generate 20% of the province's gross agriculture production, worth $5 Billion to the local economy (Alberta Water Portal, 2013a). As urban populations grow, competition for fresh water - the traditional source for irrigation purposes - will force the agriculture sector to improve its water use, efficiency and productivity. One way to do this is by using wastewater for irrigation of non-food crops such as willow, a fast-growing woody perennial agroforestry crop not widely grown in Alberta due to its high water demand (Doody and Benyon, 2011).

The work also has application to the 7.8 Million hectares of solonetzic (sodic) agricultural soils in Canada, of which 4 - 5 Million hectares are located in Alberta and 1.8 Million hectares in Saskatchewan (Alberta Agriculture Food and Rural Development, 1993). If certain clones of willow can flourish in solonetzic soil conditions, or help to remediate salty soils, this outcome would have a beneficial impact on productivity and agricultural diversification of non-food crops.

The Agricultural Greenhouse Gases Program was created in 2009. Canada was one of the founding members of this group, along with New Zealand, US, Australia, Denmark, France, Germany, Ireland, Japan, Netherlands, Norway, UK, Spain, Sweden and Switzerland. Developing country member states include: Argentina, Chile, Columbia, Ghana, India, Indonesia, Malaysia, Mexico, Pakistan, Peru, the Philippines, Uruguay and Vietnam. Brazil, China and South Korea attend as observers. As a part of this program University of Alberta has received $598,400 of total $20.3 million to study carbon sequestration and greenhouse gas emissions mitigation in different soil-climate conditions with respect to agroforestry systems (Agriculture and Agri-Food Canada, 2014). All of member countries would have a potential

interest in wastewater-irrigated agroforestry systems that have environmental, social and economic benefits.

Results should be able to be scaled down to individual farms. Most rural residents have septic fields that could potentially be used as a source of wastewater for irrigation of fast-growing woody species. In addition, livestock producers generate large quantities of nutrient-rich wastewater that must be disposed of in environmentally acceptable ways. Dairy farms are among the largest wastewater generators, with water produced from washing milking equipment, holding pens and exit alleys. In 2007, there were approximately 15,000 dairy farms in Canada (75,000 in the US) (Natural Sciences and Engineering Research Council of Canada, 2011). Farm-based wastewater could be recycled for biomass production for local (farm-level) energy production or be sold to the municipality. Wastewater and marginal lands could therefore be turned into productive assets, GHG emissions minimized and a new source of income generated, as these agricultural technologies have the potential to qualify for carbon offset credits and become complementary sources of income in rural communities.

The proposed project will involve installing a point source flood irrigation system on the Ohaton site and irrigating the willow plantation with treated wastewater from the hamlet's sewage lagoon, to determine optimum surface irrigation properties and water use efficiency of fast growing woody species. Applying biochar has the potential to increase biomass yields, effect the nutrient stabilization and carbon sequestration in soils and to mitigate GHG emissions, but to date there is little information on any of these processes in Alberta.

Biochar is a black carbon substrate that results from the pyrolysis of organic matter. Evidence suggests that biochar has the potential to reduce GHG emissions by 500% (Van Zwieten *et al.*, 2010), however, this effect is dependent on the type of feedstock used, the

temperatures attained during carbonization, and the geographic location of the test plots. Biochar also increases the water holding capacity of a soil and may reduce N2O emissions. This project will test the efficacy of biochar's ability to improve soils, especially the solonetzic soils at the Ohaton site. Our comprehension of biochar interactions with soil in terms of microbial communities, nutrient dynamics, and greenhouse gases remains limited, in spite of a large expansion in research on the effects of this substance on soil media (Spokas, 2010; Jeffery *et al.*, 2011; Biederman and Harpole, 2013). The potential benefit of biochar application in soil as a stable carbon stock has been further fortified by its possible positive impacts on soil biota and fertility (Sohi *et al.*, 2010). Biochar application to bioenergy cropping systems can create better opportunities for carbon sequestration, global warming mitigation, and remediation of marginal lands.

Short rotation coppice (SRC) systems, consisting of intensive willow plantations for example, are highly photosynthetic which can capture atmospheric CO_2 in their biomass and add it to the existing soil carbon stock. Turning this biomass into biochar and addition of it to soil in order to make a slow turn-over carbon pool can contribute to C sequestration and improve soil quality (Tallis, 2010; McCormack *et al.*, 2013). Establishing SRC systems in ex-arable soils remediated with biochar and irrigated with municipal wastewater is beneficial from several aspects. These systems may provide soils with higher organic carbon due to less tillage practices, more litter additions, extensive rooting systems, more organic matter addition from wastewater, and less carbon loss (Lemus and Lal, 2005). Co-application of biochar and municipal wastewater in these systems may also increase yield and cut fertilizer needs (Laird, 2008). The synergistic interactions of biochar and fertilizers are evaluated in other studies (Chan *et al.*, 2008; Lau *et al.*, 2008).

This research will enhance the understanding and accessibility of surface wastewater-irrigated woody biomass agroforestry systems and make that technology available to Canadian farmers and rural municipalities. It will turn wastewater and marginal lands into assets, reduce fossil fuel expenditures and impacts, assess crop productivity gains and the carbon sequestration/GHG emission mitigation potential of the addition of biochar to marginal lands. It will also generate opportunities for new rural revenue sources through newly-developed Carbon Offset protocols. The protocols themselves could be transferrable to other domestic and international jurisdictions, as would be the surface-irrigated agroforestry system.

3.2 Research questions

1. Can biochar increase microbial biomass and activity and change microbial function and community structure?
2. Does soil nutrient availability increase with biochar addition?
3. Does biochar addition to soil reduce GHG emissions and increase soil carbon sequestration?

3.3 Methods and materials

3.3.1 Site Description

The study site was located next to the small hamlet of Ohaton, Camrose County, Alberta, part of the 35-community Battle River Alliance for Economic Development. The site was located 15 kilometers east of the city of Camrose. This site was established in 2009 by Camrose County as a municipal wastewater treatment facility which has the capability to provide biofuels as sustainable energy resources for rural communities in Alberta. The coordinates of the site were 52.97619° N and 112.66851° W and the soil on-site was of the Camrose Association, loam textured, Solodized Solonetz (Vega-Jarquin *et al.*, 2003). There was a hard B_{nt} layer at lower

horizon due to high salt concentrations, specifically sodium (Vega-Jarquin *et al.*, 2003). Water infiltration and root penetration was prohibited because of this hard pan. Collapsed soil structure, high salt concentrations, and prohibited root penetration have made the soil marginal and the land low value in terms of agricultural activities (Jindo *et al.*, 2012; Lu *et al.*, 2014). Solonetzic soils are closely associated with other soil orders such as Chernozems in Canadian Prairies (Prayogo et al., 2014). The study site was located in prairies and showed patches of saline soils. These patches were the locations of high electrical conductivity (EC) soil with higher concentrations of sodium in B horizon. The soil on site was divided into two groups of low and high EC (Figure 3-1). In 2009, gypsum ($CaSO_4 \cdot 2H_2O$) was applied to soil as calcareous soil amendment. Nordby et al. (1981) reported that these amendments can improve plant growth by increasing water infiltration into the soil. The soil was also deep tilled to B horizon in order to eliminate the hard pan hindering plant root growth, but in 2013 this hard pan existed due to re-accumulation of sodium. The hard pan started at 15-20 cm depth showing grayish bright color and highly condensed soil structure.

Before Fall of 2009 the land was under canola cultivation, thereafter the site was dedicated to this multi-purpose wood-energy project. Willows were planted in the early Summer of 2010, in the standard European two-row bed design, at a density of 15,000 stems per hectare. Four clones - 3 salt tolerant clones from previous study were planted on site (Muhammad *et al.*, 2014). The clones were Tully, India, Owasco, and SX61. Multiple clones were used to test variability in productivity. Some clones such as SX61 showed low aboveground biomass in high EC area, but salt tolerant clones like Tully Champion and India showed high productivity in this zone.

Camrose County climate is varied between cold semi-arid (Bsk) and mild humid continental (Dfb) and the district lies within Aspen Parkland ecoregion which is a gradient from boreal forest to grasslands. The annual temperature is averaged 2.8 ˚C and the highest monthly temperature reaches to 16.3 ˚C in July. Mean annual precipitation is 493 mm based on 30 years data gathered from 1971 to 2000. Calculated annual potential evapotranspiration using Thornthwaite method (Thornthwaite et al., 1960) is 494 mm which is higher than annual precipitation (Hydrogeological Consultants Ltd. and Canada Prairie Farm Rehabilitation Administration, 2005).

The site consisted of two fields. The larger field area was 8470 m^2 with dimensions of 110x77 m and the smaller field area was 2783 m^2 with dimensions of 55x50.6 m. Two willow rows, called beds, were planted 60 cm from each other and beds were 2.2 m from each other to improve access. There were 3 zones of irrigation, 36 beds of clones, and 72 rows of willows in the larger field. Also, there was 1 zone of irrigation, 24 beds of clones, and 48 rows of willows in the smaller field (Figure 3-1).

3.3.2 Experimental setup

3.3.2.1 Salinity measurement

In late Summer of 2012, EM-38 was utilized to measure apparent soil EC (Federle *et al.*, 1986b). The Geonics EM-38 (G-EM38; Geonics Inc., Mississauga, ON, Canada), a commercially produced electromagnetic induction instrument (EMI) was applied to evaluate the spatial variability of soil salinity (Tunlid *et al.*, 1989a). The readings were done with intervals of 6 m alongside the rows and 6.5 m perpendicular to the rows. By collecting 329 data points and using SigmaPlot software (Version 11), soil EC contour map was created (Figure 3-1). By

having EC contour map; we were able to include EC factor in the experimental design. EM-38 provided the EC numbers in terms of mS m^{-1} which is 10 times larger than dS m^{-1}.

3.3.2.2 Biochar application

On July 18th and 19th biochar was added to soil, simply being spread on the surface. A rototiller was used to mix biochar with soil to 10 cm depth. In order to unify the disturbance effect, all control plots were tilled as well. Willow biochar was applied at 1% (w/w) rate and conifer biochar at 1% (10500 kg ha^{-1}) and 2.5% (26250 kg ha^{-1}) rates to soil. Willow biochar was made with feedstock from another SRC system located in Whitecourt, Alberta. Alberta Biochar Initiative (ABI) and Canadian Wood Fiber Centre (CWFC) produced biochar with the mobile pyrolysis unit located in Vegreville, Alberta. Willows were transformed into chips to make the process easier and faster. Quenching was done at the end of production to cool down the biochar. There were 3 bags of willow biochar and each one showed high variation as well, from 85% to 336%. We added biochar based on its dry weight to the soil. Conifer biochar was purchased from The Prasino Group located in Calgary. This biochar was originally from South Carolina, USA, 2013.

3.3.2.3 Irrigation system

Four lagoons were established next to the site in order to be filled with municipal wastewater coming from Ohaton. Anaerobic microbial digestion happened in two small lagoons and after quality improvement the wastewater was transferred to the third and fourth lagoons where aerobic activity of microorganisms dominated. The irrigation system was established in Summer 2013. There were 4 zones of irrigation on the site. Wastewater was pumped from the last lagoon and transported through underground pipes to 4 zones. The system was supposed to irrigate zones 1 and 3, thus 2 irrigated and 2 non-irrigated zones were present on site. Surface

irrigation started on August 10, 2013, 52 days after biochar application. In each zone wastewater was conveyed alongside the rows through 3 PVC pipes. Holes were drilled on each pipe by 10 m intervals, so wastewater could come out of each hole via hydraulic pressure produced by electric pump. There was a slight gradient from south west to north east of the site, therefore the water moved in this direction both above and below ground after gushing out of pipe holes. In Summer of 2013 the system was programmed to irrigate both zones 1 and 3 for 26 minutes three times per day, 8 hours apart. Approximately, 35 cubic meters of wastewater was removed from the final pond daily. Over the summer with 75 days of irrigation, 2650 cubic meters of wastewater were consumed.

3.3.2.4 Experimental Design

The experimental design was completely randomized and biochar treatments were assigned to each experimental plot randomly (Figure 3-1). Each experimental plot consisted of two 4x1 m sub-plots to which biochar was added and a bed of willow clone with dimensions of 4x0.6 m in between of two sub-plots. Biochar was not added to the space inside the clone beds because of operational limitations. The space was so small that no tiller could reach inside. The treatments were established based on soil EC, irrigation, and biochar application. EC was divided in low and high; irrigation in irrigated and non-irrigated; and biochar in control (no biochar), willow 1%, conifer 1%, and conifer 2.5%. The combination of different levels of EC, irrigation, and biochar variables made our research treatments (Table 3-1).

3.3.3 Soil sampling

In September 2013, composite soil samples were collected from the field for lab analysis. Due to flaw in irrigation system some of the plots were flooded, therefore sampling from the 1st zone of irrigation was not done. Approximately, 50 gr of top 10 cm of soil was collected from

each of 6 different spots of each plot and mixed in the bag for microbial analysis. Each bag was sealed, labeled, and transferred to a fridge at -4 ˚C. A subset of composite samples was separated to be freeze dried and stored in a super freezer at -80 ˚C for PLFA analysis. Another set of bulk samples were collected with a soil core to assess bulk density changes with respect to biochar application.

3.3.4 Water content and soil temperature

Water content and soil temperature was measured in field with ML3 ThetaProbe (Delta-T Devices Ltd., Burwell, UK) and VWR® dual channel thermometer (Radnor, PA).

3.3.5 Lab analyses

3.3.5.1 pH and EC

Sieved samples were air dried for pH and EC measurements. 20 ml of deionized water was added to 10 gr of each sample (1:2 soil-to-solution ratio) (Kalra *et al.*, 1995; Novak *et al.*, 2007). The mixtures were shaken for 30 minutes and centrifuged for 10 minutes at 2000 rpm. 1413 µS/cm electrolytes and 12.9 mS/cm electrolytes (Orion™ Conductivity Standards, Thermo Scientific™) were used to calibrate the EC meter (Mettler Toledo, Mississauga, Canada) for low and high EC samples, respectively. A 2 point calibration with 4.01, 7.00, and 9.21 pH buffers (Orion™ pH Buffer, Thermo Scientific™) was done to calibrate the pH meter (Mettler Toledo, Mississauga, Canada) for samples. The transparent portion of the solution was poured in a test tube and used for EC measurement by putting the EC sensor in the tube and reading the device. After EC measurement supernatant was returned to the container and used for pH measurement.

3.3.5.2 Bulk density

Undisturbed soil was collected from top 10 cm depth using a steel cylinder. Each cylinder was 10 cm deep, 7.5 cm in diameter, and 442 cm^3 in volume. Bulk samples were carefully

collected and packed in ziplock bags in order not to lose moisture. Each sample was weighed and then oven-dried for 24 h at 105 ˚C and weighed again. Bulk density was calculated with the core volume and weight of dry soil (Mckenzie *et al.*, 2004).

3.3.5.3 Microbial biomass

In this study, chloroform fumigation extraction (CFE) was used to examine the changes in microbial biomass (Brookes *et al.*, 1985). Biochar can influence the results from this method by sorbing soil organic C and N (Durenkamp *et al.*, 2010). In order to retain the consistency of the results with other studies we didn't apply any correction coefficient (Swallow *et al.*, 2009). Soil microbial biomass carbon and nitrogen (MBC/MBN) were evaluated using the extraction of carbon and nitrogen in samples by 0.5 M K_2SO_4 (Brookes *et al.*, 1985). After incubation, 25 gr of each soil was put in a 50 ml beaker and extracted with 50 ml of 0.5 M K_2SO_4 (1:2 soil-to-solution ratio), shaken for 1 hour, and vacuum filtered with Whatman P2 filter papers. Another 25 gr of each soil was fumigated for 3 days. Fumigation was done by putting each set of samples in a dessicator along with chloroform. The dessicator was sealed and vacuum pumped to bring the inner pressure to zero and evaporate the chloroform. Extraction of fumigated soils was done right after the fumigation period.

3.3.5.4 Microbial respiration

Basal respiration of soil microbial biomass was measured using the alkali trap method (Deenik *et al.*, 2010). Carbon dioxide (CO_2) emitted by microbial activity was trapped in 0.5 sodium hydroxide (NaOH) forming sodium carbonate (Na_2CO_3). Microbial respiration was measured during the incubation period for 20 days. 100 gr of treatment soil was placed in 1L Mason jars. Uncapped scintillation vials holding 20 ml of 0.5 M NaOH were placed in jars. The jars were sealed, and kept under room temperature at 25 ˚C. At the end of incubation period,

scintillation vials were taken out and sealed immediately. Solutions were titrated with 0.5 M hydrochloric acid (HCL) to a clear end point (Zibilske, 1994; Sundaravalli and Paliwal, 2000). Metabolic quotient (qCO_2) was calculated as respired carbon to microbial biomass carbon and indicates heterotrophic activity of soil microorganisms (Pirt, 1975; Anderson and Domsch, 1986).

3.3.5.5 Phospholipid fatty acid analysis

Microbial community composition was characterized using phospholipid fatty acid (PLFA) analysis based on a modified version of (Bligh and Dyer, 1959) extraction. This procedure was described in detail by (Hannam *et al.*, 2006) and (Frostegard and Baath, 1996). Briefly, field samples were freeze dried and 2 gr of freeze-dried sample was prepared for PLFA analysis. Lipids were extracted from soil samples with a one-phase mixture (1:2:0.8 v/v/v) of chloroform, methanol, and citrate buffer (0.15 M, pH 4.0), and polar lipids (containing phospholipids) were separated from neutral lipids and glycolipids using pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE). The MIDI peak identification software (MIDI, Inc., Newark, DE) was used to distinguish fatty acids in soil samples (Degens and Harris, 1997). A total 37 different PLFAs were differentiated and identified. 30 PLFAs were considered to be microbial community biomarkers as mentioned in literature (Nordby *et al.*, 1981; Federle *et al.*, 1986a; Sezgin *et al.*, 1988; Tunlid *et al.*, 1989b; Frostegard *et al.*, 1993; Baath *et al.*, 1995; White *et al.*, 1996; Kieft *et al.*, 1997; Zelles, 1997; Zogg *et al.*, 1997; Bossio and Scow, 1998; Fierer *et al.*, 2003; Denef *et al.*, 2007).

3.3.5.6 Community level physiological profiling analysis

MicroResp^{TM} was utilized as a system including two detachable parts, one as deep-well plate encompassing 96 wells storing soil samples along with substrates and the other as detection

plate encompassing the detection gel made of cresol red (12.5 ppm, w/w), potassium chloride (150 mM), and sodium bicarbonate (2.5 mM) set in 150 µl of purified agar (3%) (Campbell *et al.*, 2003). These plates were placed face to face via a perforated rubber gasket and sealed by clamps. Community level physiological profiles (CLPPs) were assessed using the procedures fully described by (Campbell *et al.*, 2003) and (Lalor *et al.*, 2007). Carbon substrates were chosen based on (Degens and Harris, 1997) and (Stevenson *et al.*, 2004) recommendations to simulate root exudates in rhizosphere. In general, 15 substrates consisted of 4 amino acids (L-alanine, L-arginine, L-cysteine, and L-lysine), 6 carbohydrates (D-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, and D-trehalose), 4 carboxylic acids (ascorbic acid, α-ketobutyric acid, malic acid, and citric acid) , and 1 polymer (tween 80) were used to make stock solutions with concentration of 80 g L^{-1}. In order to add the substrate to soil based on 30 mg of C g of soil $water^{-1}$ concentration, each stock solution was diluted with deionized water.

Before starting the experiment, a calibration curve was produced by incubation of 10 different soil samples at different timings (1, 3, 6, and 16 h). Soil samples were placed in 1L glass jars with septums on lids and attached to an infra-red gas analyzer (IRGA) (Model LI-8100A LI-COR Inc., Lincoln, Nebraska). The curve was based on the absorbance rates of detection wells (A_{570}) measured by a microplate reader (Model Synergy™ HT BioTek® Instruments, Inc., Winooski, Vermount) and the CO_2 evolved from each sample measured by IRGA. The best fit for calibration curve was as follows: $CO_2 = 687.72 \times A^{-2.264}$, where A is the absorbance rate at 570 nm. The model was well fitted to data points with a high R^2 of 0.93 (Table 3-2).

The moisture content of sieved soil samples was measured in order to calculate the concentration of substrate aliquots. Each sample was transferred on a third equipment made from

a 300-µl well microtiter plate accompanied by a removable Perspex sheet at the bottom. Then, the third device was placed on the deep-well and the sliding sheet was removed and the soil was transferred to the corresponding well. The deep-well plate was tapped gently in order to bring a good contact between soil and substrate. The detection plate was then sealed on the deep-well plate with clamps and gasket. Then, the system was incubated for 6 h at 25 ˚C. The absorbance rate of each plate was read before and after incubation and the readings were standardized based on the average value of the readings before incubation (Stevenson *et al.*, 2004). A template was used to remember the location of each substrate and sample during the incubation (Campbell *et al.*, 2003). In total, one substrate was replicated in 3 wells for each sample and 2 samples were applied to each plate (48 wells dedicated to each sample). Control wells didn't get any substrate to measure the basal respiration of each soil. The moisture content of samples was not altered in order to estimate the numbers affected by field conditions, although commonly it was around 10% (approximately 40% of WHC).

3.3.6 On-site measurements

3.3.6.1 Ionic resin analysis

Plant root simulator (PRS^{TM}) probes (Western Ag Innovations Inc., Saskatoon, SK) were used and are designed to capture nutrient anions and cations inside soil solution. These probes provide the measurable concentration of bioavailable nutrients which can be taken up by plant roots during *in situ* incubation period. Each probe has a resin membrane framed inside a plastic applicator handle and covers 10 cm^2 of soil with 215 meq of approximate surface area. Two pairs of cation and anion probes were installed at two depths (5 and 15 cm). Each experimental plot comprised 3 replicates of probe-pairs at each depth (3×2×2=12). In general, 672 probes (56 plots × 12 probes) were buried on August 14th, 2013, and left in situ for 19 days. Probes burial was

done 56 days after biochar application and 4 days after irrigation system became operational. After burial, the probes were removed and soil particles were removed with a clean brush and rinsed with deionized water, then returned to Western Ag Innovations Inc. for elution with 0.5 M HCl and nutrient analysis. Ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) were quantified colorimetrically on a segmented flow Autoanalyzer III (Bran and Lubbe, Inc., Buffalo, NY). Potassium (K^+), sulfate (SO_4^{2-}), calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), and boron (B^+) were quantified by ICP-OES (PerkinElmer Optima 3000-DV, PerkinElmer Inc., Shelton, CT) (MacKenzie and Quideau, 2010).

3.3.6.2 Greenhouse gas measurements

A photoacoustic infrared multi-gas analyzer (Innova model 1312; www.innova.dk) was used in the field to measure soil CO_2 and N_2O fluxes. Basically, the analyzer along with static chambers which were previously installed at each plot made a non-steady state closed system (Figure 3-4), which has been fully described by (Rochette, 2008). The dimensions of each chamber were 65 cm × 16 cm × 15 cm which made a rectangular shape. Top 10 cm of the chamber collar was taped in order to inhibit sunlight penetration inside the chamber and the bottom 5 cm was buried inside the soil. In total, 44 chambers were installed to make measurements of all replicates of all treatments except for SX61 willow clones. The measurements were done in time intervals of 6 minutes and repeating 4 times for each chamber (0, 6, 12, and 18 minutes). The CO_2 and N_2O measurements were done once a week from July 19th to September 2nd, which included 3 weeks of measurements before the start of irrigation and 3 weeks of measurement after irrigation. During each measurement, the analyzer was connected to the chamber for 1 minute via plastic tubings. The following formula suggested by (Rochette, 2005) was used to calculate gas concentration regarding time: $F = (dG/dt) * V/A$, where F = Gas

flux (mg m^{-2} min^{-1}), dG/dt = change in gas concentration with time (mg m^{-3} min^{-1}), V = volume of chambers (m^3), A = area covered by chambers (m^2).

3.3.7 Statistical analysis

Data were visually tested in SAS version 9.2 (SAS Institute 2010) to determine if it was normally distributed or not. Residuals were examined and depending on the scatter plot, their normality was judged. If they were not normally distributed, then a log transformation was applied to the real dataset. Levene's test was performed to test the homogeneity of variance.

Due to the multivariate nature of the dataset for microbial community and nutrient availability ordination analysis was applied. Non-metric multi-dimensional scaling (NMS) is a method that reduces multivariate dataset to highlight general patterns and distinguish potential ecosystem function indicators (Kruskal, 1964). Since the main goal of this research was to identify the effects of biochar in different soil environments, the treatments were classified in different categories regarding soil EC, irrigation, and depth for nutrient analysis.

Patterns in PLFA, CLPP, and ion resin data were examined using PCORD software (Version 6, MjM Software Design, Gleneden Beach, OR, USA). The main matrix of PLFA consisted of all 30 PLFAs identified in literature as community biomarkers, while the secondary matrix consisted of nutrients and microbial communities such as bacteria, actinomycetes, and fungi. The main matrix of CLPP contained all added 15 carbon substrates and the control, while the secondary matrix contained only pH and EC values measured on the same samples. All 15 quantified elements by resins were put in the main matrix for nutrient availability ordination, while moisture, temperature, carbon dioxide, nitrous oxide, bulk density, pH, and EC were put in secondary matrix. The secondary matrix of nutrient ordination comprised carbon dioxide and nitrous oxide as well due to concurrent in situ measurements of these gases. Before log-square

root transformation and analysis, all PLFA biomarkers and CLPP with less than seven non-zero numbers were eliminated to remove the influence of rare molecules (MacKenzie and Quideau, 2010). All elements were kept for ordination analysis of the nutrient data. The Sorensen (Bray-Curtis) distance measure along with an autopilot function set to 'slow and thorough' was used. The multiple response permutation procedure (MRPP) was also used to compare distances in ordination space between points corresponding to different biochar applications within each combination of soil ECs, irrigation types, and depths for nutrients. MRPP was used in order to identify whether treatments were statistically different in ordination space (Legendre, 1998).

3.4 Results

3.4.1 Soil properties

Addition of biochar caused an increasing trend in pH (Table 3-3). In both non-irrigated and irrigated high EC zones, conifer biochar at 2.5% rate increased pH significantly (Table 3-3). An increase in biochar addition from 1% to 2.5% elevated pH and it was significant for non-irrigated high EC soil (P=0.01). Willow biochar had higher pH compared to conifer biochar at the same application rate, though the differences were insignificant (Table 3-3). There was no effect of biochar addition on EC and no trend was found with biochar application rate. Irrigation increased EC significantly (P=0.001) (Table 3-3). Biochar increased water content, which was significant for 2.5% application rate in all zones. Irrigation did not increase water content for biochar treatments (Table 3-3). There was no effect of biochar on soil temperature. Moreover, irrigation didn't have any effect on temperature (Table 3-3). Biochar reduced bulk density in all zones, but the reduction was only significant in non-irrigated high EC zone with addition of 2.5% (Table 3-3). High EC soil showed more compaction with higher bulk density (Table 3-3).

3.4.2 Microbial respiration and biomass

Microbial respiration increased significantly in the irrigated high EC zone. Conifer biochar at 1 and 2.5% (P=0.004 and P=0.013, respectively) increased respiration by 61 and 48%, respectively compared to control in irrigated high EC soil. High EC soil respiration was significantly higher in irrigated zone compared to non-irrigated zone (P=0.0004) by 290% irrespective of biochar effect (Figure 3-5). Metabolic quotient increased significantly in irrigated high EC zone with the addition of conifer biochar at 2.5% (P=0.027) and was 177% higher compared to control. Addition of biochar had no significant effect in the other zones (Figure 3-6).

There was no significant effect of biochar on microbial biomass or MB-C/MB-N ratio. In non-irrigated high EC zone, the decreasing trend with addition of biochar was more apparent. Irrigation decreased MB-C/MB-N significantly irrespective of biochar effect (P=0.004) (Figure 3-4).

3.4.3 Phospholipid fatty acid analysis

The NMS ordination of soil microbial communities provided a two-dimensional solution in which microbial community structure wasn't different with respect to biochar addition (data not shown here). It was however different under different irrigation and EC values. Microbial structure of non-irrigated high EC soil was highly correlated with cyclopropyl / monoenoic fatty acids and microbial communities of irrigated high EC soil were highly correlated with calcium. Microbial structure of non-irriagted low EC soil was highly correlated with bacteria, actinomycetes, and potassium.

3.4.4 Community level physiological profiling analysis

The NMS ordination of CLPP data showed significant difference between control and conifer 2.5% in both high and low EC soils (Figure 3-8 a&b), meaning that biochar changed the metabolic activity of the microbial community. The data in low EC soil was more variable, hence it was not possible to point out the dominating factors in each treatment.

3.4.5 Ionic resin analysis

The NMS ordination of the nutrient profiles in non-irrigated high EC, irrigated high EC, and irrigated low EC showed significant difference between control and conifer 2.5%. No significant results were detected in non-irrigated low EC zone (Figure 3-9 a&b, 3-10 a&b). Nutrient profile of conifer biochar at 2.5% in non-irrigated high EC soil was highly correlated with bacteria and actinomycetes as indicated by the 2nd matrix.

Biochar addition to soil reduced nitrate (NO_3^-) availability and the reduction was significant for conifer 2.5% in non-irrigated high EC soil (Figure 3-11). PO_4^- was significantly increased in non-irrigated high EC zone with addition of conifer biochar at 2.5% rate and irrigated high EC zone with addition of willow biochar at 1% rate (Figure 3-13). K^+ was significantly increased with addition of willow biochar in all zones, but conifer biochar at 2.5% increased it in non-irrigated zones as well (Figure 3-14).

3.4.6 Greenhouse gas measurements

All biochar applications reduced both CO_2 and N_2O in high EC soil in the 3rd week of measurements (Figure 3-15). Results are shown from the 3rd week, since disturbance effects are lowest and irrigation was not started. An uneven distribution of wastewater increased the variability of data after the 4th week, making it more difficult to interpret and no trend was detected thereafter. No biochar application showed significant reduction in low EC soil for either

gas, but it was significant in the first 2 weeks (data not shown here). CO_2 and N_2O were highly correlated with R^2=0.83 (Figure 3-16).

Correlation between NO_3^- and N_2O showed that changes in nitrate didn't lead to any significant change in nitrous oxide gas. R^2 in low EC soil was higher than high EC soil and the parameters were positively correlated in non-irrigated zone and negatively correlated in irrigated zone. The variability of nitrate was much higher in non-irrigated high EC soil (between 0 and 80). Biochar apparently reduced nitrate availability in non-irrigated high EC soil, but data points of low EC soil were less variable (Figure 3-17 & 3-18).

3.5 Discussion

3.5.1 Biochar

3.5.1.1 Microbial biomass

Biochar application didn't change microbial biomass significantly, however MBC/MBN ratio showed a decreasing trend in non-irrigated high EC zone. This trend was in agreement with lab results from chapter 2. The similar trends in high EC soil can be attributed to elevated pH after biochar addition (Table 3-3). We believe that biochar increased bacterial population over fungal population, since the ratio was reduced (Figure 3-4). Watzinger *et al.* (2014) suggested that an imposed short-term stress via change in soil characteristics supports rapidly growing microorganisms, such as Gram-negative bacteria. High MBC/MBN ratio in non-irrigated high EC soil compared to irrigated high and low EC soil (Figure 3-4) with value of 15 can prove high value of water stress on microorganisms as discussed in chapter 2. Truu *et al.* (2009) found an increasing amount of alkaline phosphatase in irrigated samples and attributed that to the increasing number of bacterial communities because of better water and nutrient supply. Non-irrigated low EC samples were also collected from areas where more aboveground biomass

existed and possibly more carbon and nutrients were provided to microbes. It is also suggested that biochar creates a more favourable habitat for some bacterial populations (Khodadad *et al.*, 2011). In contrast, biochar had very low pronounced effect in irrigated soil, although it increased pH significantly (Table 3-3). Elevated pH could favor the production of aforementioned alkaline phosphatase in irrigated samples. There were no significant differences between biochar treatments in terms of EC and temperature (Table 3-3 & 3-6), so the possible changes to microbial communities should be attributed to pH and water content (Table 3-3 & 3-5). It should be noted that the EC of high EC samples were not actually higher than low EC samples, as soil EC was measured with EM-38 at 30 cm depth and samples were collected from top 10 cm.

3.5.1.2 Microbial respiration

Long term application of wastewater in SRC systems can result in elevated microbial growth and activity (Peng *et al.*, 2011). Our respiration data from irrigated samples are in agreement to aforementioned statement (Figure 3-5). Biochar addition to non-irrigated high EC soil didn't increase microbial heterotrophic respiration. It is assumed that after two months, biochar had given most of the degradable C to microorganisms in field and during the incubation no labile C is left for decomposition (Smith *et al.*, 2010). Hence microbes didn't show any changes in activity with respect to biochar addition. This is in agreement with the results from chapter 2, as incubation started right after treatments were made with biochar addition to bulk soil. It is postulated that high surface area of biochar may lead to faster decomposition of added organic compounds (Grayston and Campbell, 1996; Rousk *et al.*, 2010; Santos *et al.*, 2012), through the enhanced growth of fast growing species (Grayston *et al.*, 1998). Since MBC/MBN didn't change and respiration increased significantly, it is believed that biochar addition in irrigated high EC zone increased metabolism rather than changes in microbial structure. It is

possible to attribute higher metabolic quotient in irrigated zone to more stress as EC was fairly higher (Table 3-3). Increase in water content might have put microorganisms under more stress and elevated metabolic quotient. According to the results from chapter 2, water content at FC was about 23% and microbes are more efficient at 60% of WHC. Water content increase to 40% can apply more stress on microbes and make their metabolism less optimized (Figure 3-6).

3.5.1.3 Phospholipid fatty acid analysis

Multivariate analysis of PLFA data showed no significant differences between biochar treatments (data not shown). The results were in agreement with Prayogo *et al.* (2014) indicating that addition of biochar didn't change microbial biomass. They added biochar at 2% rate and after 30 days found no significant difference in microbial PLFA's including bacteria, fungi, and actinomycetes. Lehmann *et al.* (2011) and Steinbeiss *et al.* (2009) postulated that fungal biomass has the better ability to decompose more complex compounds in biochar and biochar application can increase fungal population rather than bacterial. On the other hand, using culture-dependent approaches, it is reported that actinomycetes can thrive better in biochar mediated environments (Rousk *et al.*, 2010; Anderson *et al.*, 2011; Khodadad *et al.*, 2011). Prayogo *et al.* (2014) reported a significant difference between PLFA biomarkers from 30 and 90 days incubation, meaning that time can change microbial community structure. It wouldn't be hard to imagine that biochar could change microbial community structure in longer period. Farrell *et al.* (2013) found that after 75 days total microbial PLFA's showed a significant increase with addition of biochar. It is suggested that pH is one of the main and most important properties which can have a huge impact on microbial community composition (Prayogo *et al.*, 2014). It is dependent on charring material and pyrolysis conditions, and also can increase significantly if the temperature is elevated (Peng *et al.*, 2011). Although the biological implications of pH in soil is not totally

clear but Jones *et al.* (2011) attributed its influence to the changes in decomposition rates. In this study biochar increased pH but it is believed that either it may needed more time to change the biology of the soil or the changes weren't so drastic. A multivariate analysis of PLFA data from non-irrigated high EC, non-irrigated low EC, and irrigated high EC showed a significant difference irrespective of biochar application (Figure 3-7). Bacterial communities were positively correlated with potassium and negatively correlated with phosphrous and micro-nutrients.

3.5.1.4 Community level physiological profiling analysis

Multivariate analysis of microbial functional diversity (CLPP) showed a significant change with biochar addition at 2.5% rate in both high and low EC soils (Figure 3-8 a&b). These results are in agreement with Dempster *et al.* (2012), as they found a significant change at 25 t ha^{-1} (2.27% (w/w)) rate. Their lower rate was 5 t ha^{-1} , which was equal to 0.45% (w/w). It is postulated that all utilized substrates are metabolizable and these results are indicative of a physiological change in community towards respiration rather assimilation. Some communities have higher growth yield efficiency (GYE) which can accumulate more biomass as they reach substrates (Montecchia *et al.*, 2011). Respiration response to substrate addition was greater in soils amended with biochar and possibly biochar has favored r-strategists over k-strategists (Esperschuetz *et al.*, 2007).

CLPP was done using MicroResp procedure and activity was measured before any significant growth happened (Chapman *et al.*, 2007). Alkali trap results were measurements of cumulative soil basal respiration over a 19 days period and microbial growth influenced the measurement of CO_2 contrary to CLPP having a short incubation period (6 h) in which minimal growth occurs (Garland *et al.*, 2010). It is reported that CO_2 from arginine substrate is positively

correlated with pH (Grayston *et al.*, 2004). In this study, biochar increased both pH and CO_2 from arginine. The same study also reported a positive correlation between pH and both gram negative and positive bacteria. Meharg and Killham (1990) reported an increase in bacterial growth with elevation of pH. More CO_2 evolution from biochar treated soils can also be attributed to both priming effect of biochar and more plant rhizodeposition (Grayston and Campbell, 1996; Grayston *et al.*, 1998). More plant rhizodeposits favor bacterial communities dominate fungi, since fertile soil media are bacterially dominated (Grayston *et al.*, 2004). These results are partially in agreement with MBC/MBN ratio results as it was reduced with biochar addition in high EC soil (Figure 3-4).

3.5.1.5 Ionic resin analysis

In this study, biochar application rate was an important driving factor in changing soil nutrient profiles. Conifer biochar at 2.5% was effective in alteration of 12 nutrients altogether in 3 out of 4 zones (Figure 3-9 a&b, 3-10 a). These changes can be most attributed to liming effect of biochar (Table 3-3) (Rondon *et al.*, 2007; Biederman and Harpole, 2013). Lin *et al.* (2013) attributed the changes in NH_4^+ to the contribution of carboxylic functional groups and forming N-C bonds on the surface of biochar. Carboxylic functional groups are highly acidic and can be removed with increase in pyrolysis temperature (Arriagada *et al.*, 1994). Although no measurement on functional groups was done in this study, but supposedly high temperature (400 ˚C) removed these groups and increased pH of biochar. Hence, the ammonium status was mostly remained unchanged with addition of biochar except for a significant increase in irrigated low EC zone. It is also postulated that high concentration of DOC might decrease the affinity of clay and biochar and lead to more plant available N in the form of NH_4^+ (Lin *et al.*, 2013). The relationship between biochar provided DOC and availability of NH_4^+ remains questionable and

needs future research. It is reported that labile fraction of biochar C is less than 1% (Zimmerman, 2010; Cross and Sohi, 2011), thus addition of biochar at higher rates can release more substantial amounts of labile C to soil. Biederman and Harpole (2013) did a meta-analysis by analyzing the results of over 371 independent experiments and showed that biochar increases total soil N, supposedly by providing N within its structure and not most of it is available to plants. In our study, we found a decreasing trend in NO_3^- and a significant decrease for non-irrigated high EC soil. This decrease can be attributed to the increase in C:N ratio and passing the limit of 25 in which microbial biomass start to immobilize nitrogen, but we didn't find any significant changes in MBN (Chapin *et al.*, 2011). Other possible explanation could be attributed to plant uptake, but NO_3^- was only significantly reduced in non-irrigated high EC zone which showed less aboveground plant biomass. This hypothesis is also in contrast to the findings from Borchard *et al.* (2012). They found a reduced plant uptake by 24% with addition of beech and oak biochar. The only mechanism which would be more likely is the physical immobilization of NO_3^- onto the surface of biochar (Mizuta *et al.*, 2004). Taghizadeh-Toosi *et al.* (2011) found the same decreasing trend in NO_3^- with increase in biochar addition. The leaching of NO_3^- is less probable as biochar can improve moisture retention and keep more NO_3^- in rhizosphere. There was also a hard pan at 20 cm which prohibited the fast leaching of NO_3^-. No relation between microbial structure and nitrogen dynamics in field can be constructed, as there wasn't any significant change in microbial community composition with addition of biochar. On the contrary, a potential affinity between microbial functionality and nitrogen dynamics can be made in future research. The increase in PO_4^{3-} and K^+ is in line with the meta-analysis of Biederman and Harpole (2013). Biochar can act as the source of P and K to soil by keeping these nutrients in its labile organic compounds and provide them during the weathering process (Topoliantz and

Ponge, 2005; Yamato *et al.*, 2006; Rajkovich *et al.*, 2012). The increase in PO_4^{3-} can be achieved by four mechanisms. The first is through the liming effect as biochar increases pH and eliminates the mobility of substances like Al and Fe (Cui *et al.*, 2011). These elements form bonds with phosphate mostly in acidic conditions and make it less available to plants. The pH of soils was between 5 and 8 and its elevation to neutral conditions can make PO_4^{3-} more available. The second is the reduction in leaching processes in which P can be absorbed to biochar surface and released gradually (Laird *et al.*, 2010; Beck *et al.*, 2011). The third is provided DOC by biochar and its sorption onto clay particles and prohibition of PO_4^{3-} adsorption by them (Haynes and Mokolobate, 2001); hence the availability of phosphate in soil solution increases. The fourth is the release of humic substances and their influence on PO_4^{3-} availability by prohibiting the formation of calcium phosphate crystal phases (Alvarez *et al.*, 2004). It is reported that biochar can retain distinctive amounts of phosphorous after pyrolysis (Bridle and Pritchard, 2004; Hossain *et al.*, 2011; Wu *et al.*, 2011). The complex interaction of biochar mediated substances and phosphate leaves questions for future research. The higher availability of K^+ with biochar addition is in line with other studies (Gaskin *et al.*, 2010; Silber *et al.*, 2010; Yao *et al.*, 2010; Schulz and Glaser, 2012). Lehmann *et al.* (2002) reported that biochar can act as the direct source of P and K. On the other hand, P and K content depend on feedstock (Gaskin *et al.*, 2008). Our results indicate that willow biochar was more effective towards K^+ addition to soil in both irrigated and non-irrigated zones. The amount of leachable K^+ in biochar can reduce over time, hence the rate of biochar application and feedstock play an important role in K^+ availability. Higher rate and some feedstocks can provide enough K^+ which still can be released after longer periods. Sun *et al.* (2013) did a physical separation of biochar in their treatments and observed that K^+ was more leachable than P and N. The high amounts of released K^+ can also attributed to

high pyrolysis temperature as more ash could be produced on biochar. It is also important to mention that the soil condition is also of high importance as conifer biochar at 2.5% left K^+ unchanged in irrigated zones. There might be a precipitation mechanism via substances in wastewater that didn't let K^+ to stay in soil solution freely. On the other hand, willow biochar may supplied high rates of K^+ that wastewater didn't have precipitation capacity for all of it.

3.5.1.6 Greenhouse gas measurements

CO_2 and N_2O emissions were measured weekly for 6 weeks in situ. The first week of measurements was 1 week after biochar addition in which soil was disturbed totally to 10 cm depth. It is believed that disturbance impact was remained for at least 2 weeks as GHG emissions for control plots dropped by 50% in low EC and 39% in high EC zone. These droppings suggest that how disturbance can impose drastic negative impact specifically in less marginal soils because of possibly higher organic carbon stock. On the other hand, a flush of irrigation on whole site increased GHG emissions in week 4 by 114% in low EC and 85% in high EC zone for control plots. Between week 4 and 6, emissions dropped by 43% in low EC and 50% in high EC zone for control plots. Wastewater was not distributed evenly on site and made the whole data highly variable and distinguishing differences was almost impossible (data not shown). Other studies have documented the impact of spatial variety on GHG emissions (Chadwick *et al.*, 2000; Fangueiro *et al.*, 2008; Angst *et al.*, 2014). Hence, only data from the third week with the least disturbance and irrigation effects is portrayed (Figure 3-15 to 3-27). In the third week, no biochar application showed significant decrease in GHG emissions in low EC soil, but all biochar applications showed significant reduction in high EC soil. Spokas and Reicosky (2009) reported the diverse effects of biochar on GHG emissions with respect to different soil conditions. Possibly less soil organic C in high EC soil favoured negative priming of biochar and gas fluxes

remained low. As mentioned in chapter 2, biochar is capable of adsorbing labile C which is an energy source for microbes. Constructing a strong linkage between microbial properties and GHG emissions is not possible as there was a large time gap between soil sampling and gas flux measurements. The subject of future research shall be finding the concurrent impact of biochar on microbial communities and gas fluxes and how they follow each other. Both CO_2 and N_2O followed a very similar pattern as they were highly correlated in this agroforestry system. It is possible to continue data collection for consecutive growing seasons and find a more real correlation factor between gases and limit data collection to one gas. Thus, other gas can be calculated from the former. There was no strong correlation between NO_3^- and N_2O in both irrigated and non-irrigated zones, however in non-irrigated zone the correlation was positive and in irrigated zone it was negative. These results suggest that wastewater application can have a slight change in nitrification and denitrification processes. The changes of N_2O along with NO_3^- were more pronounced suggesting a well thrived microbial community in low EC soil and more plant productivity.

3.5.2 Irrigation

Soil EC was increased with addition of wastewater meaning that wastewater elevated soil salinity (Table 3-3). No other soil characteristics were changed with addition of wastewater. Wastewater decreased MBC/MBN ratio possibly due to anaerobic conditions imposing stress on microbial community (Figure 3-4). Higher metabolic activity as a response to wastewater addition is in agreement with MBC/MBN ratio reduction showing more metabolic activity to survive in harsh conditions (Figure 3-6). Bacterial communities are less efficient in carbon substrate utilization, though they probably had more access to nutrients and carbon with addition of wastewater. The lower MBC/MBN ratio in low EC soil was in agreement with PLFA data

showing that microbial structure was highly correlated with bacterial communities (Figure 3-4 & 3-8). The availability of nitrogen and phosphorous was lower in both soil EC zones with addition of biochar and potassium showed a slight increase (Figure 3-11 & 3-13 & 3-14). NO_3^- and N_2O were negatively correlated with presence of wastewater possibly due to anaerobic conditions and elevation of denitrification processes (Figure 3-18).

3.6 Conclusion

Significant reduction of GHG emissions in the third week of measurements in high EC soil suggests that biochar would be more beneficial in C sequestration and GHG emissions mitigation in marginal soils. But according to the goals of establishing agroforestry systems, improving soil nutrient dynamics needs more investigation as biochar decreased total inorganic N and increased PO_4^{3-} and K^+ in non-irrigated high EC soil. Biochar ability in increasing PO_4^{3-} and K^+ extended to both irrigated and low EC zones as its feedstock and processing conditions were other critical factors affecting nutrient availability. No significant changes in microbial biomass and structure were observed in short term but the functionality was altered with addition of conifer biochar at 2.5% application rate. Microbial function is a quick responsive metric as any change in soil environment such as pH, moisture, and labile C can significantly change it in short term. The complex interactions of biochar and soil regarding organo-mineral processes can show vastly diverse results in agroforestry systems and longer period research is essential to make the results highly conclusive.

Table 3-1: Classified independent variables applied to the field experiment

Electrical Conductivity (EC)	Irrigation	Biochar (w/w)
High	Irrigated	Control (No biochar)
		Willow biochar at 1%
Low	Non-irrigated	Conifer (Prasino) biochar at 1%
		Conifer (Prasino) biochar at 2.5%

Table 3-2: Deep-well plate layout as shown above, it is important to notice that detection plate has an inverse order of naming as it is placed face to face with deep-well plate.

	Sample 1					Sample 2					
Alanine	Alanine	Alanine	Arginin	Arginin	Arginin	Alanine	Alanine	Alanine	Arginin	Arginin	Arginin
Cysteine	Cysteine	Cysteine	Glucose	Glucose	Glucose	Cysteine	Cysteine	Cysteine	Glucose	Glucose	Glucose
Fructose	Fructose	Fructose	Lysine	Lysine	Lysine	Fructose	Fructose	Fructose	Lysine	Lysine	Lysine
Trehalos	Trehalos	Trehalos	Malic	Malic	Malic	Trehalos	Trehalos	Trehalos	Malic	Malic	Malic
Arabinos	Arabinos	Arabinos	Ascorbi	Ascorbi	Ascorbi	Arabinos	Arabinos	Arabinos	Ascorbi	Ascorbi	Ascorbi
Mannose	Mannose	Mannose	Citric A	Citric A	Citric A	Mannos	Mannos	Mannose	Citric A	Citric A	Citric A
GABA	GABA	GABA	Galacto	Galacto	Galacto	GABA	GABA	GABA	Galacto	Galacto	Galacto
Tween	Tween	Tween	Control	Control	Control	Tween	Tween	Tween	Control	Control	Control

Table 3-3: Basic characteristics of treatments consisting of pH, electrical conductivity (dS m^{-1}), volumetric water content (%), temperature (°C), and bulk density (gr cm^{-3}). The level of significance is shown for pairwise comparison of biochar treatments with control at each zone. The numbers are mean values for each characteristic.

Irrigation	Soil	Biochar	pH	EC	VWC	Temp	Bulk Density
Non Irrigated	**High EC**	**Control**	6.16	0.57	19.51	17.5	1.116
		Willow 1%	6.61	0.44	17.88	18.1	1.075
		Conifer 1%	6.41	0.71	26.52	17.3	1.038
		Conifer 2.5%	7.31**	0.95	42.75***	17.1	0.876*
	Low EC	**Control**	5.61	0.63	14.13	16.3	0.997
		Willow 1%	6.3	0.44	17.11	14.9	0.846
		Conifer 1%	5.91	1.01	24.78*	16.7	0.965
		Conifer 2.5%	6.26	0.91	38.72***	16.2	0.944
Irrigated	**High EC**	**Control**	6.08	2.43	20.07	17.4	1.220
		Willow 1%	6.71	1.35	17.96	16.0	1.141
		Conifer 1%	6.33	1.94	28.49	16.9	1.174
		Conifer 2.5%	7.14**	2.13	37.79***	17.5	1.056

**Significant at $p < 0.05$*

***Significant at $p < 0.01$*

****Significant at $p < 0.001$*

Table 3-4: ANOVA table of nutrient data. Numbers are P values.

	NO_3^-	NH_4^+	PO_4^{3-}	K^+
Irrigation	0.0231	0.0519	0.0412	0.2926
EC	0.0108	0.0350	0.0001	0.0035
Irrigation*EC	0.1087	0.8117	0.8207	0.1617
Biochar	0.0842	0.2215	0.0359	<.0001
Irrigation*Biochar	0.3996	0.8200	0.1597	0.0122
EC*Biochar	0.1921	0.1353	0.1703	0.2777
Irrigation*EC*Biochar	0.1443	0.3535	0.0122	0.1748

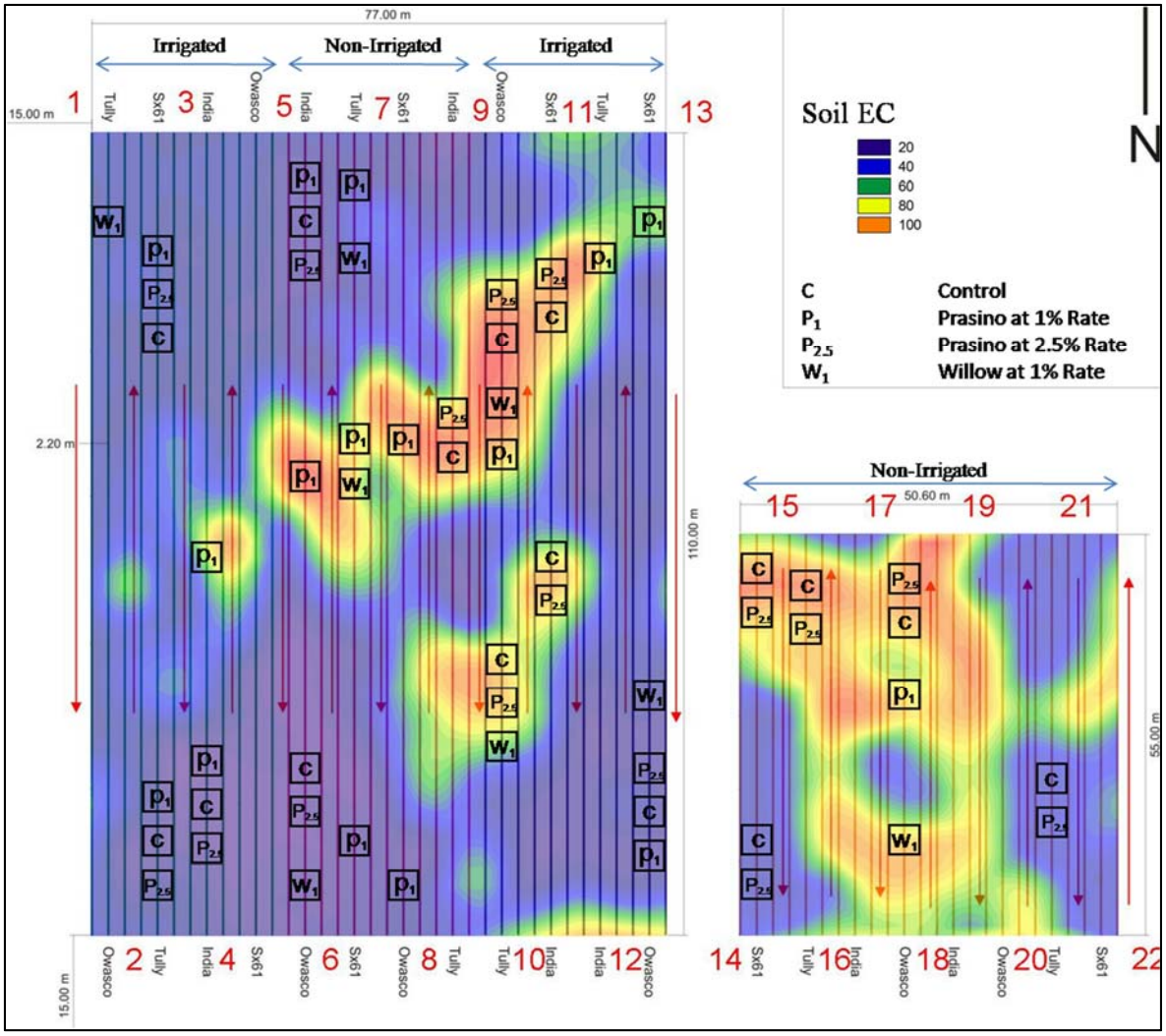

Figure 3-1: Site map layout showing irrigation zones, willow clone names, high and low EC zones, and experimental plots completely randomized on the whole site.

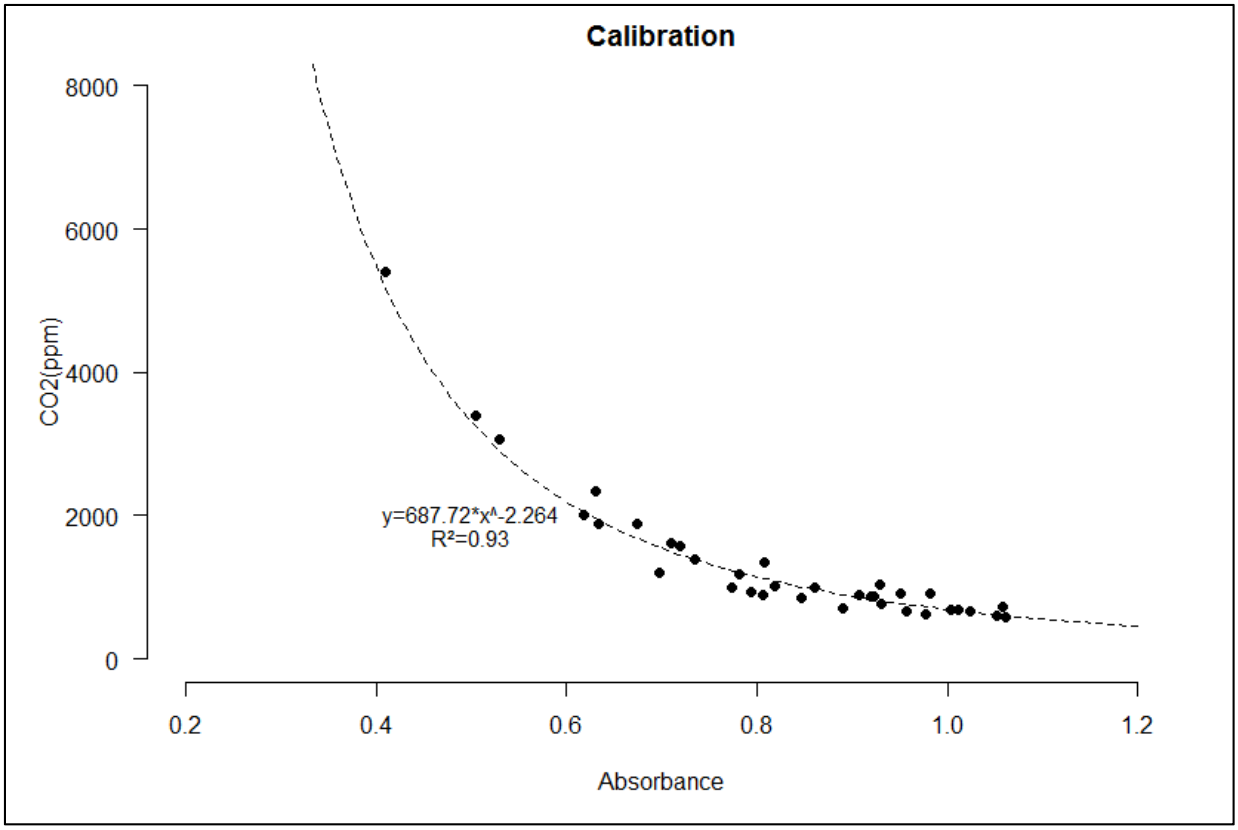

Figure 3-2: The calibration curve used to convert the absorbance numbers to CO_2 concentrations.

Figure 3-3: The non-steady closed chamber system comprised of both photoacoustic monitor and static chamber in connection.

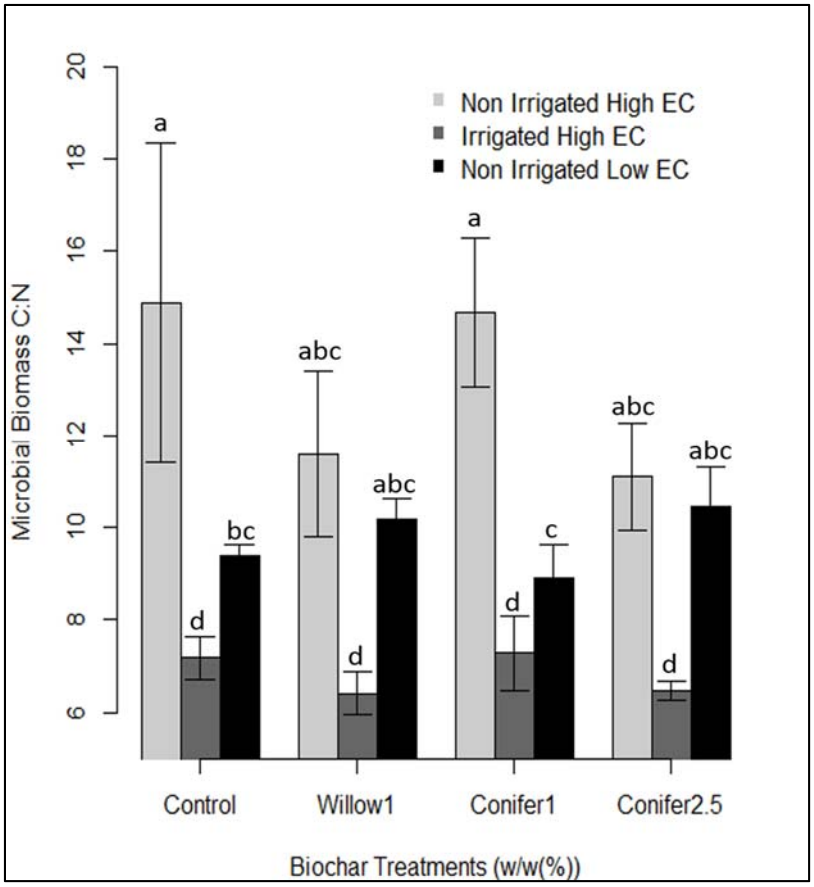

Figure 3-4: Microbial biomass carbon to nitrogen (MB-C/MB-N) ratio.

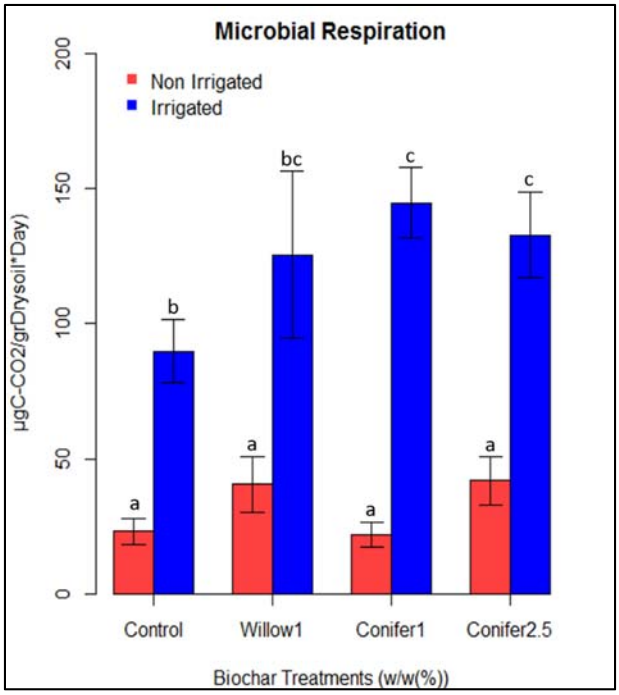

Figure 3-5: Microbial respiration of high EC samples for non-irrigated and irrigated treatments

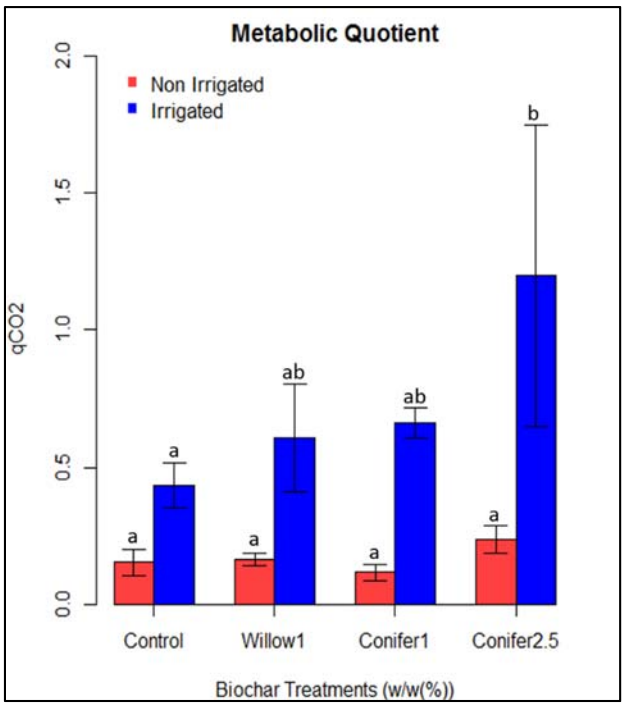

Figure 3-6: Metabolic quotient of high EC samples for non-irrigated and irrigated treatments

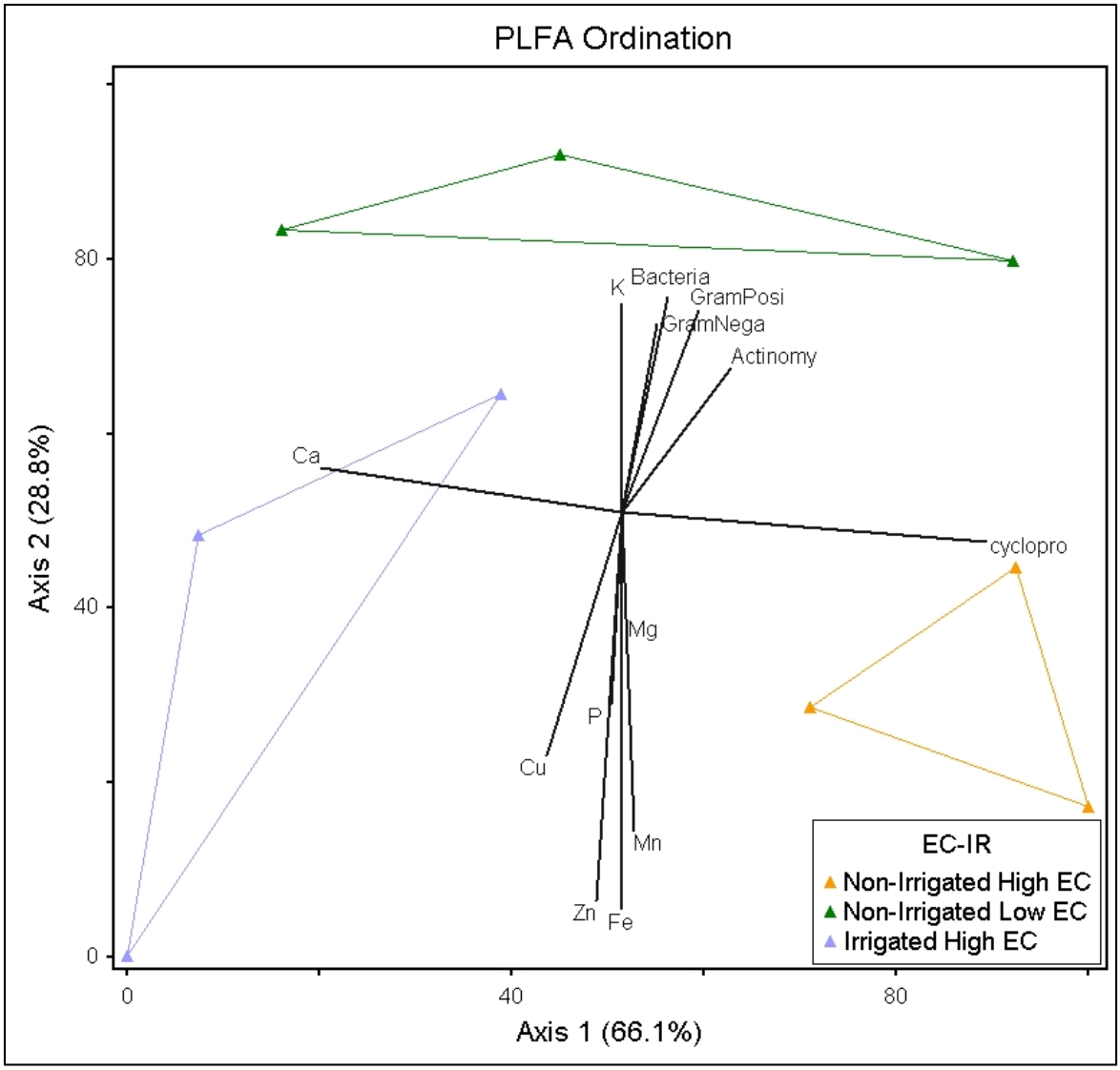

Figure 3-7: Non-metric multidimensional scaling ordination of microbial phospholipid fatty acids (PLFA) data in non-irrigated high EC, non-irrigated low EC, and irrigated high EC samples irrespective of biochar addition. The proportion of variance explained by each axis is based on the correlation between distance in the ordination space and distance in the original space. It was 66.1% for axis 1 and 28.8% for axis 2. Multiple response permutation procedure (MRPP) was done to do pairwise comparisons between treatments. PLFA profile of non-irrigated high EC soil was significantly different from the PLFA profiles of non-irrigated low EC and irrigated high EC soils with P=0.034 and P=0.024, respectively. Non-irrigated soil was dominated by cyclopropyl fatty acids / monoenoic precursors and irrigated soil was dominated by calcium. Low EC soil was dominated by bacteria, actinomycetes, and potassium. High EC soil was dominated by phosphorous, magnesium, and micro nutrients.

a) b)

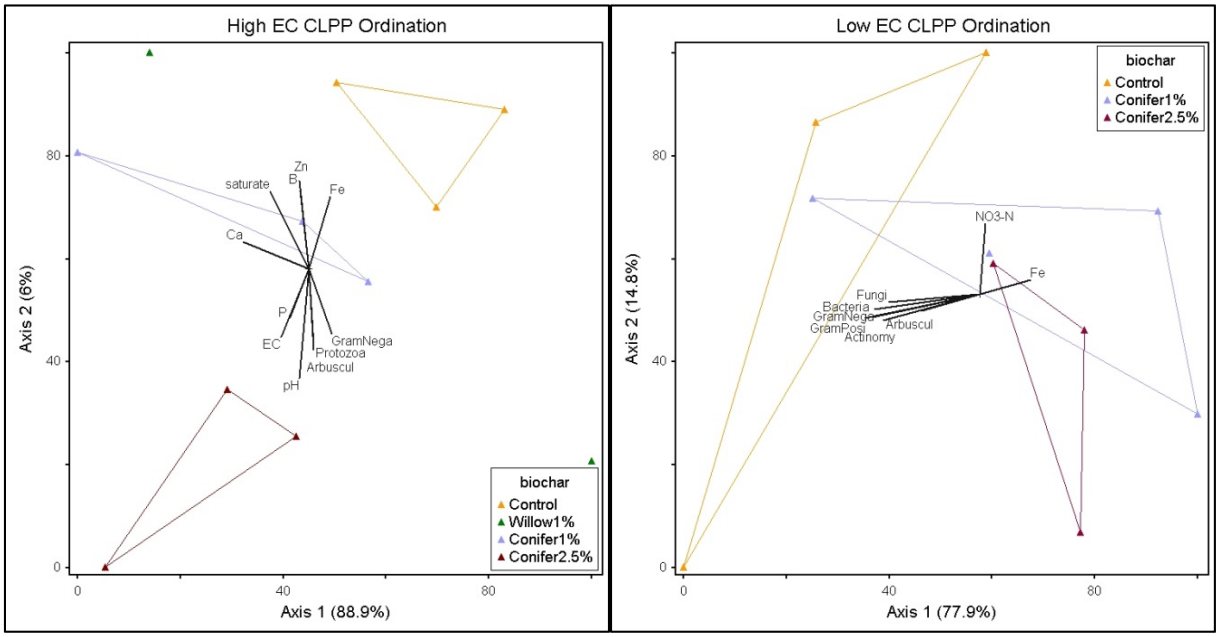

Figure 3-8:

a) Non-metric multidimensional scaling ordination of microbial community level physiological profiling (CLPP) data in high EC soil. All CLPP data were from non-irrigated zone, since there was flooding in irrigated zones which made CLPP experimentation impossible. The variance explained by axis 1 was 88.9% and by axis 2 was 6%. CLPP of conifer 2.5% was significantly different from CLPP of control with P=0.023.

b) Non-metric multidimensional scaling ordination of microbial community level physiological profiling (CLPP) data in low EC soil. . The variance explained by axis 1 was 77.9% and by axis 2 was 14.8%. Data was highly variable as data polygons were highly distributed in ordination space. CLPP of conifer 2.5% was significantly different from CLPP of control with P=0.033.

a) b)

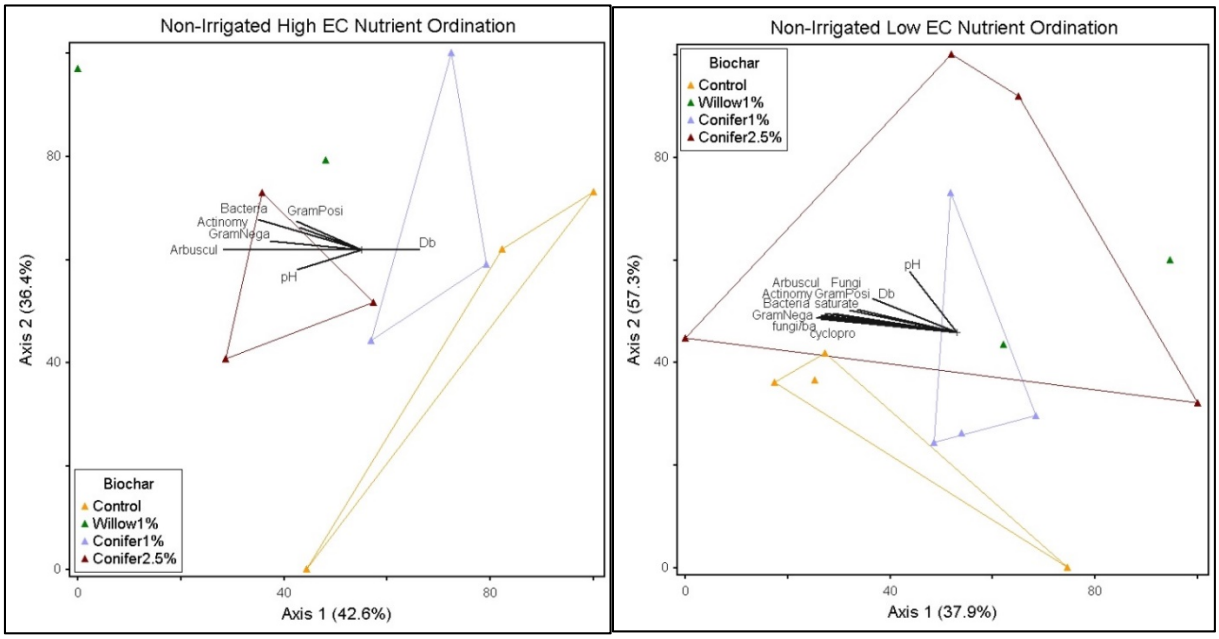

Figure 3-9:

a) Non-metric multidimensional scaling ordination of nutrient data in non-irrigated high EC zone. 42.6% of variance was explained by axis 1 and 36.4% was explained by axis 2. Nutrient profile of conifer 2.5% was significantly different from nutrient profile of control with P value of 0.036. Conifer 2.5% was dominated by Bacteria and actinomycetes.

b) Non-metric multidimensional scaling ordination of nutrient data in non-irrigated low EC zone. 37.9% of variance was explained by axis 1 and 57.3% was explained by axis 2. In low EC zone data was highly variable and biochar didn't affect nutrient profiles significantly.

a) b)

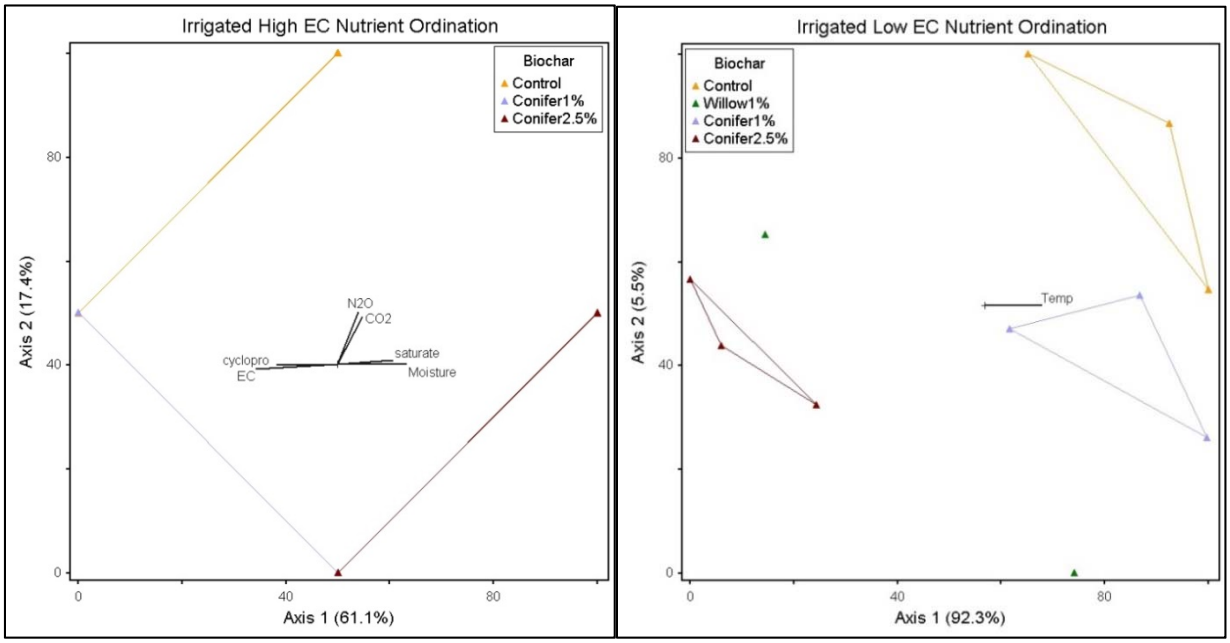

Figure 3-10:

a) Non-metric multidimensional scaling ordination of nutrient data in irrigated high EC zone. 61.1% of variance was explained by axis 1 and 17.4% was explained by axis 2. Repeatedly, data was highly variable, but nutrient profile of conifer 2.5% was significantly different from the nutrient profile of control with P value of 0.016.

b) Non-metric multidimensional scaling ordination of nutrient data in irrigated low EC zone. 92.3% of variance was explained by axis 1 and 5.5% was explained by axis 2. Nutrient profile of conifer 2.5% was significantly different from the nutrient profile of control with P value of 0.023.

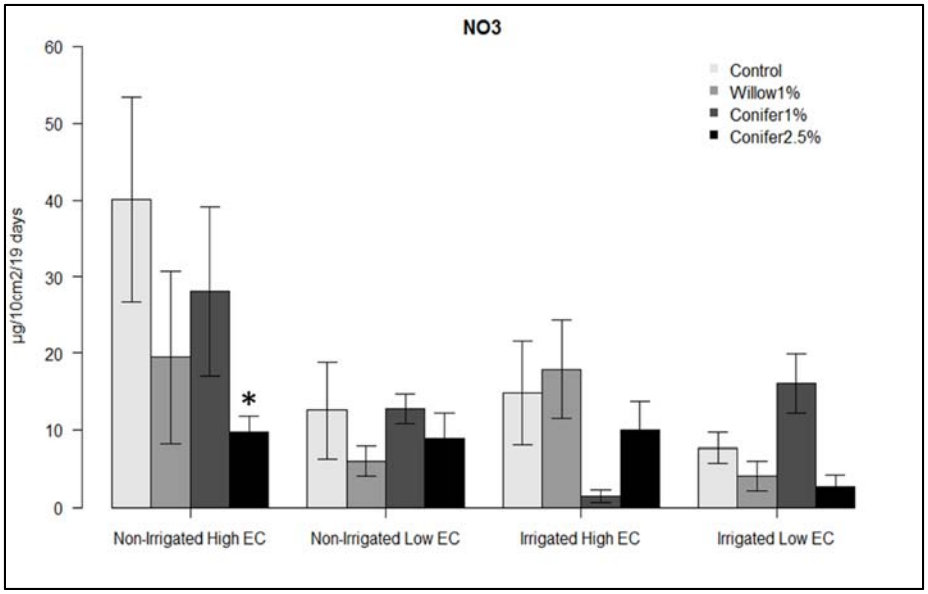

Figure 3-11: Nitrate (NO_3^-) availability at 5 cm depth. It was significantly reduced in non-irrigated high EC zone at 2.5% conifer biochar (P=0.0008). A marginal effect of willow biochar addition was also observed (P=0.052).

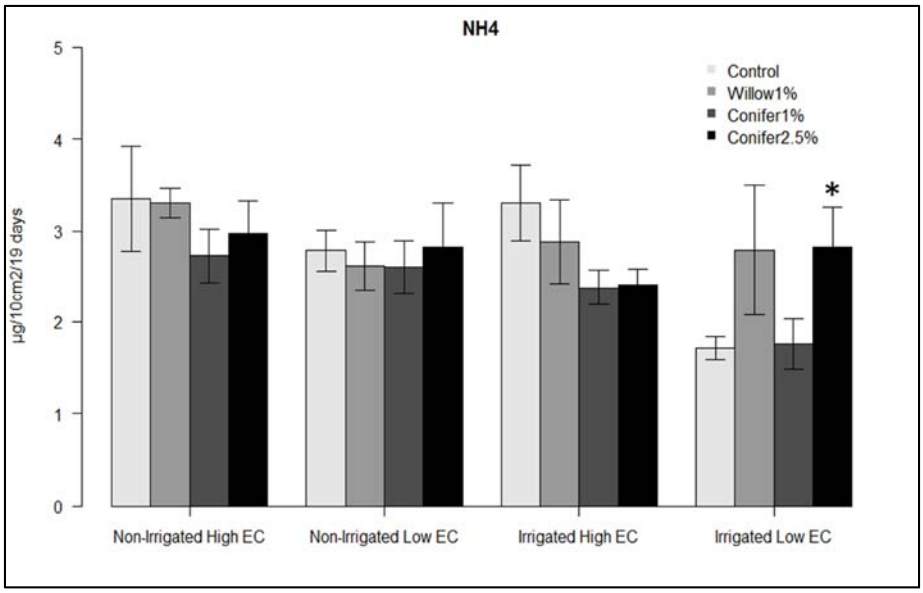

Figure 3-12: Ammonium (NH_4^+) availability at 5 cm depth. NH_4^+ was significantly increased with conifer biochar at 2.5% rate in irrigated low EC zone (P=0.043). A marginal effect of willow biochar was observed in the same zone (P=0.096). Conifer biochar at 1 and 2.5% marginally decreased NH_4^+ in irrigated high EC zone (P=0.066 and P=0.075, respectively).

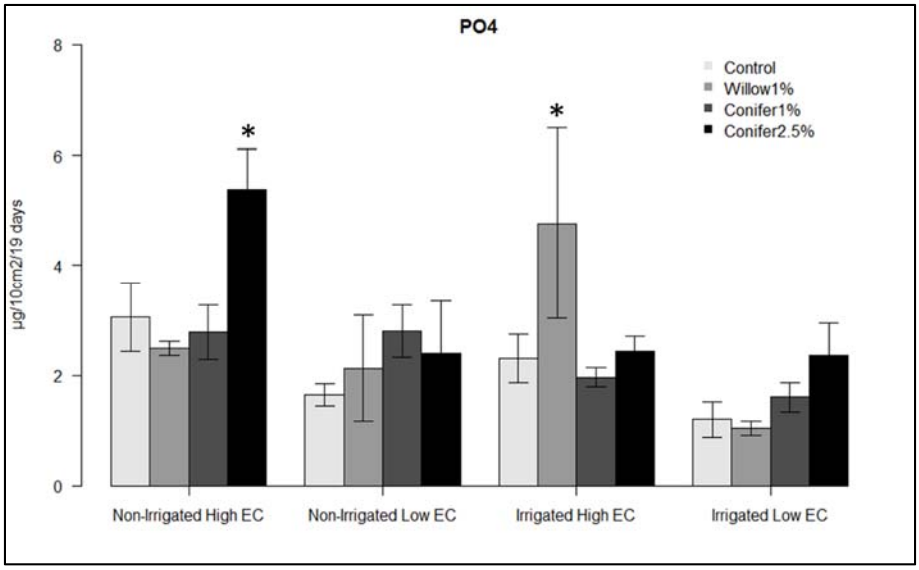

Figure 3-13: Phosphate (PO_4^{3-}) availability at 5 cm depth. PO_4^{3-} was significantly increased in non-irrigated high EC zone with addition of conifer biochar at 2.5% rate (P=0.004). It was also significantly increased in irrigated high EC zone with addition of willow biochar at 1% rate (P=0.011).

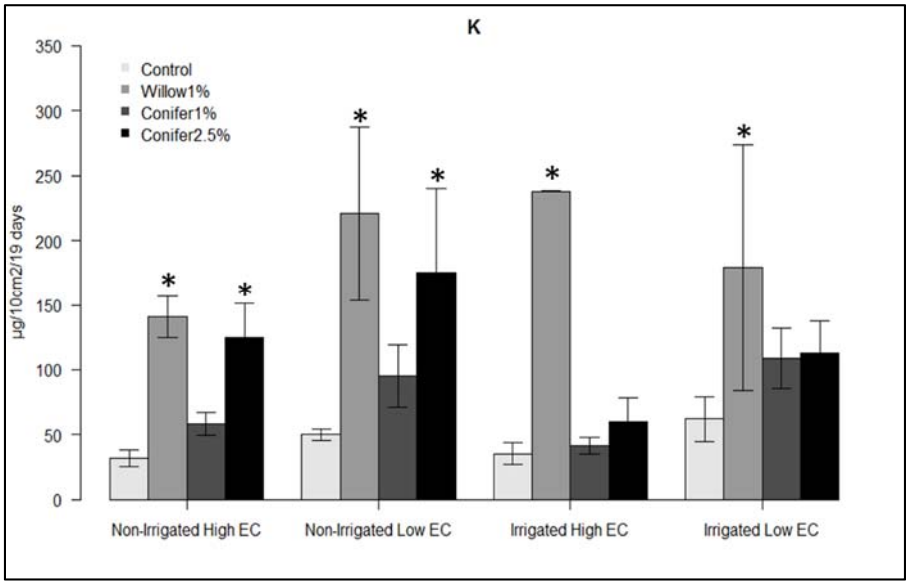

Figure 3-14: Potassium (K^+) availability at 5 cam depth. Willow biochar significantly increased K^+ in all zones and conifer biochar at 2.5% rate increased it in non-irrigated zones. Conifer biochar at 1% rate didn't change it significantly.

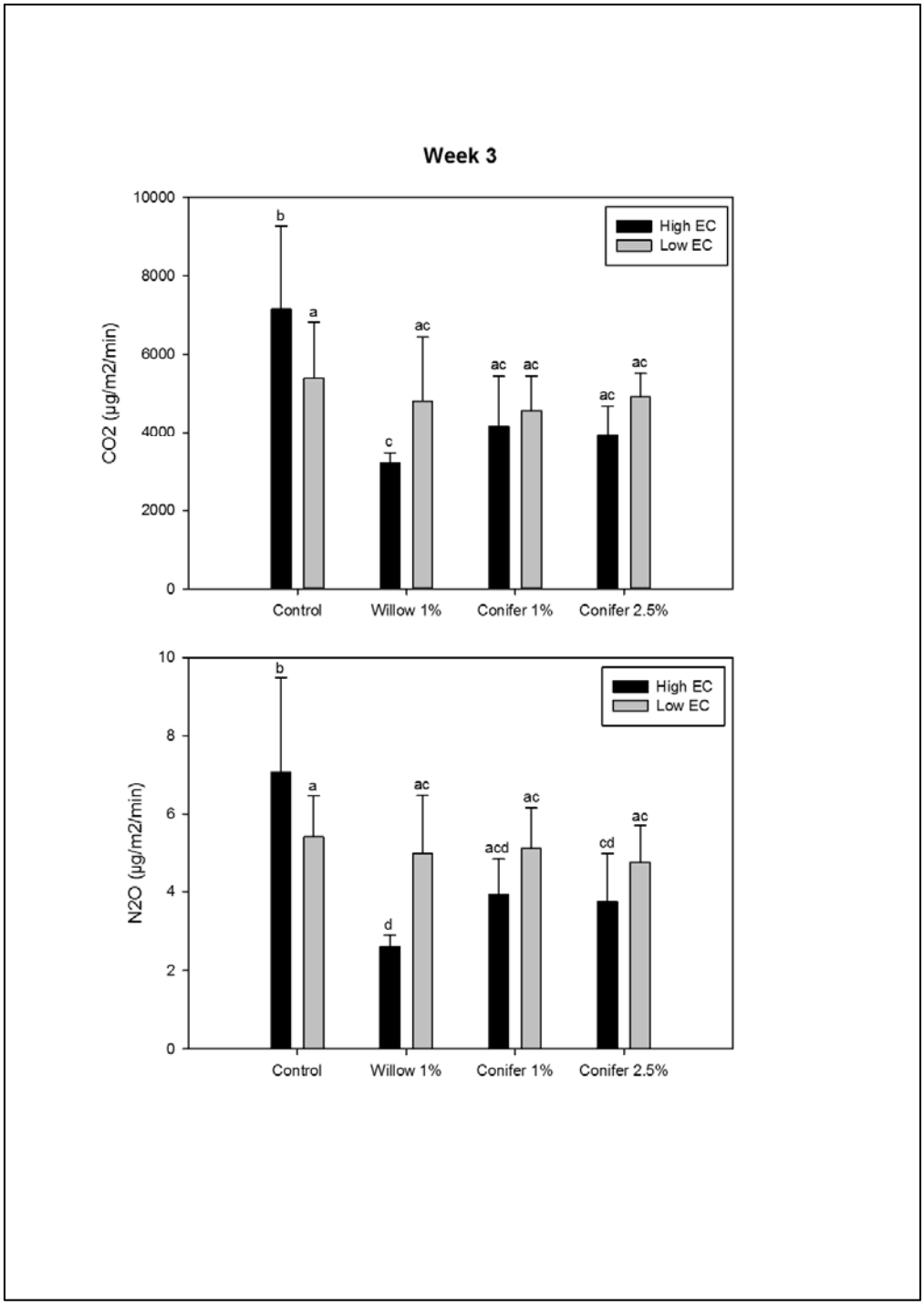

Figure 3-15: Gas fluxes (CO_2 and N_2O) measured in the 3rd week when the effect of soil disturbance was lower and right before irrigation started. In high EC soil all biochar applications decreased both gas fluxes, but in low EC soil biochar had no significant effect. A slight reduction in GHG emissions can be seen in low EC soil with addition of biochar.

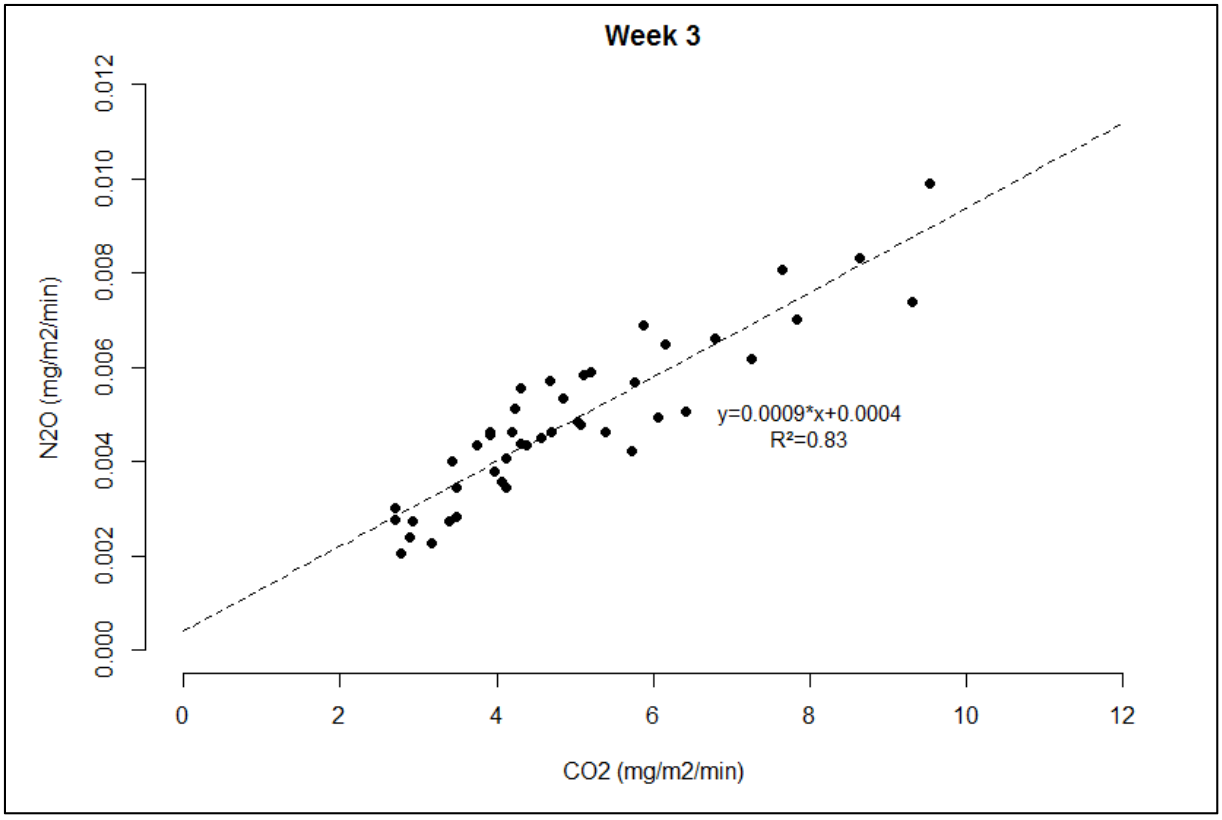

Figure 3-16: Correlation between CO_2 and N_2O with a high R^2=0.83. Two gases were highly correlated.

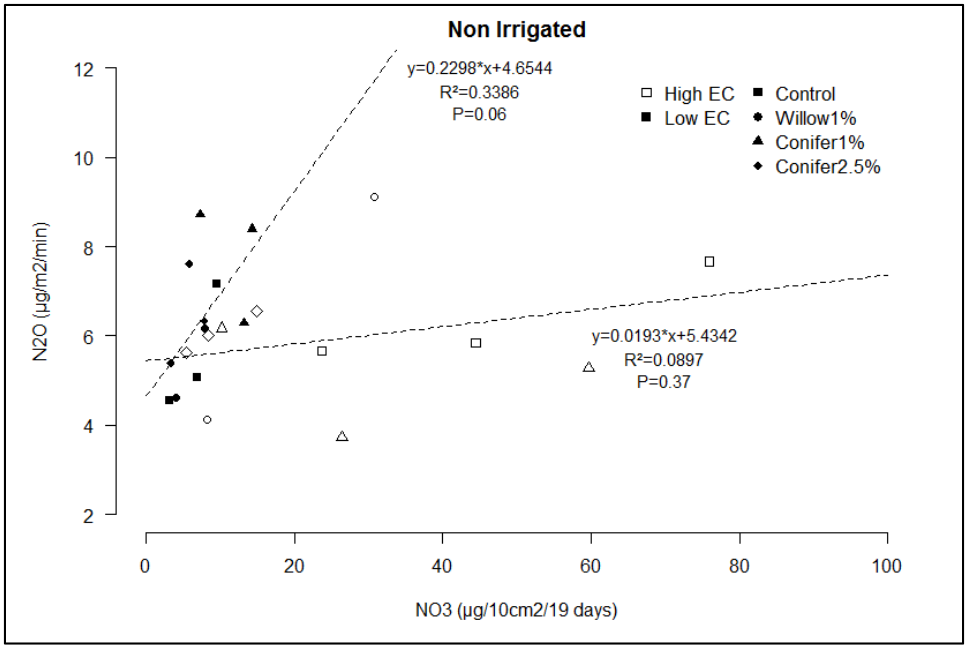

Figure 3-17: Correlation between N_2O and NO_3^- from non-irrigated zone. N_2O was positively correlated with nitrate.

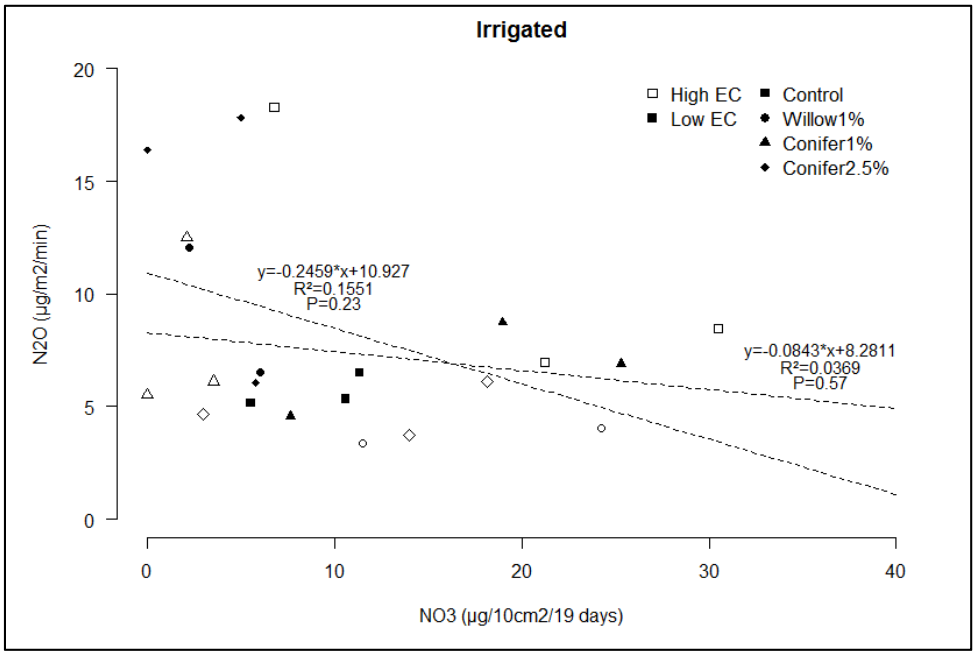

Figure 3-18: Correlation between N_2O and NO_3^- from irrigated zone. N_2O was negatively correlated with nitrate.

Chapter 4: Tech Transfer

4.1 Summary

This study was conducted under both lab and field conditions at two different time periods. The lab samples were better indicative of salinity effects as high EC soil showed electrical conductivity more than 4 dS/m, meaning it lay within saline category. High EC soils from field showed no EC more than 1 dS/m and it was irrigation which increased it to 2.5 dS/m; undoubtedly, wastewater added some salinity to soil. The reason for this discrepancy was in situ EC measurements and soil sampling for different depths. EC measurements were done with EM-38 which gave results for a soil profile of 50 cm and field samples were collected from top 10 cm of soil profile. The compacted soil layer showed itself at shallower depth in high EC zone indicative of more negative effects of concentrated salts in that hard pan.

In this project no plant measurements were done in order to assess the effect of biochar on photosynthesis and growth. Biochar addition was done on small plots of 4x2 m and its impact was at small scale. I recommend that biochar will be added to the half of the site at 2.5% application rate. Consequently, the growth and plant biomass production can be measured as well.

4.2 Experimental limitations

During the field experiment other challenges were diagnosed which are believed to have confounding impacts on the research results. These confounding factors might have reduced the significance of treatment differences specifically in microbial analysis such as PLFA and CFE. Microbial community structure and abundance are more sensitive to changes in soil biogeochemical properties and if these changes are confounding with experimental treatments, then as mentioned before the level of significance would be reduced. As it was the first time that

irrigation system was being initiated on site, in order to test the functionality of the system, both non-irrigated and irrigated zones were irrigated with wastewater for several minutes. Regardless of pre-planned experimental design, the whole experimental plots including non-irrigated ones went under irrigation. The other confounding factor was uneven distribution of wastewater on site. Presumably, all irrigated plots needed to achieve wastewater as equal as it was possible, but weekly observations done at site proved this unevenness. Some plots were totally dry and some were totally water saturated. Moreover, hydraulic head at the end of rows was higher and at the end of field season when sampling was being done, some plots at the end of one non-irrigated zone were unintentionally affected by wastewater. Due to the high pressure, wastewater was emitted more at the end of that irrigated zone and made its way to non-irrigated zone because of the gradient. I believe that perforated pipelines can't provide a well-established irrigation system with high efficiency. The idea of uneven distribution of wastewater was further strengthened by high variability in GHG emissions results after irrigation system became fully operational (Chapter 3). This issue can cause groundwater contamination as deep percolation might happen in highly saturated areas. On the other hand, wastewater irrigated bioenergy cropping systems wouldn't be highly efficient in terms of wastewater treatment and biomass production if all plants and soil don't acquire equal moisture and nutrients. This heterogeneity can cause further problems such as excessive CO_2 and N_2O emissions from soil as plants and microbes need limited amounts of nutrients. I recommend that subsurface or drip irrigation should be substituted with current system or at least before establishing the surface irrigation it was needed to do all calculations for surface gradient in all directions. Consequently, instead of perforated pipelines, well-engineered furrows with respect to the surface gradients were needed to establish between willow rows. This method of irrigation could cause removal of biochar from experimental plots

due to the plausible erosion. Since there could be some functional limitations for field measurements and also disease hazards, drip irrigation and well-engineered surface irrigation are recommended for research and industrial sites, respectively. The planted willows included 4 different hybrid clones to monitor clonal variability under saline conditions and pick the best ones for better productivity. Studying clonal variation was not a part of this project. Due to some spatial limitations, each willow clone was accounted as a replicate for each treatment. It is also believed that clonal variability was a confounding factor impacting treatment effects. Some clones were more resistant to harsh conditions and showed more aboveground biomass and more biomass could result in higher organic matter accumulation, higher root biomass and exudates, more nutrient uptake, and more microbial abundance and activity as a response to plant's stronger presence.

Alkali trap method might not be an ideal method for measuring microbial activity specifically of biochar treated saline soils. Biochar can abiotically emit some CO_2 due to the presence of carbonates on its surface. Furthermore, saline soils can abiotically emit some ammonia and emitted ammonia can neutralize some of alkali liquid (Werth and Zamanian, 2011). CLPP method had also its own limitations as it was not possible to put the saturated samples in wells due to huge disturbance on samples and relevant bias in results.

Generally, in situ measurement of soil nutrients with PRS probes had the least operational limitations as it was fast and no matter if soil was saturated or not. But still, soil nutrients were possibly affected by aforementioned environmental variability.

4.3 Recommendations

Biochar increased soil moisture content in both lab and field studies. Plants in SRC fields have higher chance to survive in arid conditions specifically in southern Alberta. More water

retention with presence of biochar gives plants more time to uptake nutrients via their roots as nutrients such as nitrate can stay in root zone for longer periods. Nitrate reduction with addition of biochar might be indicative of N immobilization which can be used by microbes later. This is beneficial in terms of GHG emissions mitigation and cut of fertilizer applications for the next growing seasons.

In general with all limitations and challenges, biochar showed some good trends and significant results in both lab and field experiments. It reduced nitrogen availability, and increased phosphorus and potassium availability. Higher potassium could be relevant to introduced ash on biochar surface. Furthermore, biochar treated soils showed significant dissimilarities to untreated soils in ordinations spaces in regards to nutrient and CLPP profiles. Microbial abundance was mostly affected in lab rather than field and I believe more time is needed to observe changes in microbial community composition and abundance in the field. More research is needed in this area with samples collected from the field.

Biochar effect on GHG emissions was shortly studied and no solemn conclusion could be taken out of the study, but in short term (Chapter 3) it was successful in reducing both gases from soil in marginal soil after 3 weeks. More research is highly needed on this topic and carbon sequestration in Alberta's SRC systems. At larger scale, agricultural sector contributes to 15% of global anthropogenic GHG emissions (FAO, 2008). SRC systems as mentioned before are established in agricultural sites in order to possibly reduce GHG emissions from these farmlands.

4.4 Potential C credits

Using Greenhouse Gas Equivalencies Calculator of U.S. Environmental Protection Agency (EPA) gives a cumulative carbon offset number which encompasses both CO_2 and N_2O measured on this site (EPA, 2014). Presumably, if biochar reduces CO_2 with a value of 3

mg/min/m^2 and N_2O with a value of 3 μg/min/m^2 from this agroforestry field (Numbers are achieved from chapter 3 for the third week of measurements in high EC zone.), then according to the calculator it would be equal to 20.5 tons of CO_2 per one hectare annually (Figure 4-1). According to current Alberta's carbon pricing under Specified Gas Emitters Regulation (SGER), GHG emissions reduction is priced $15 per tonne. Annually, this rate brings a revenue of $307.5 for one hectare of biochar treated SRC field to each farmer.

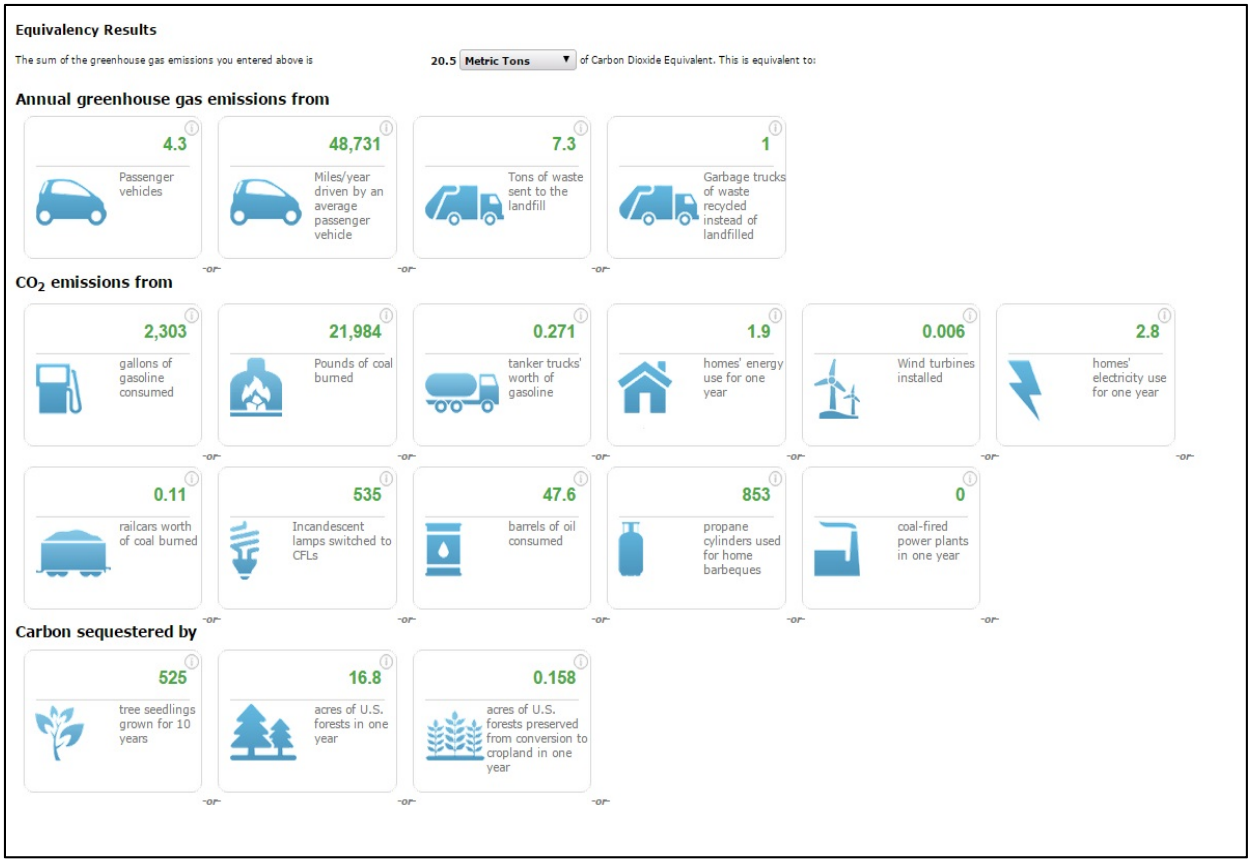

Figure 4-1: Equivalencies calculated based on EPA calculator which shows the results for annual emissions from different sources of emissions.

Chapter 5: Literature Cited

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Appendix

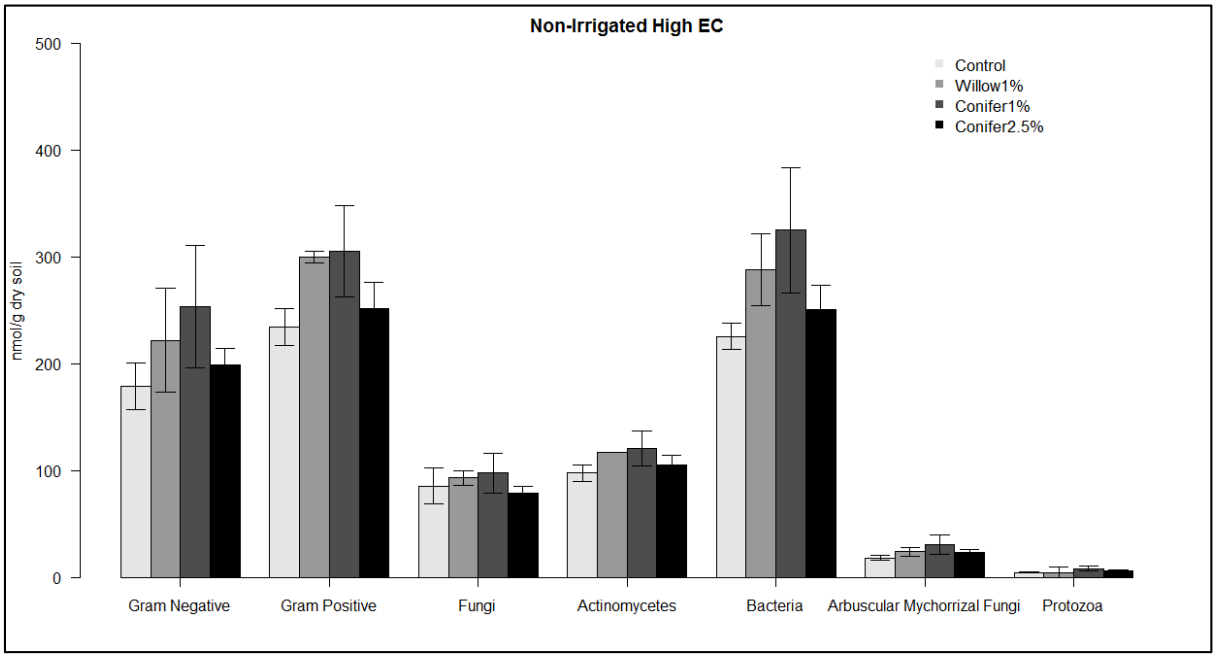

Figure A-1: Microbial communities from non-irrigated high EC samples. Biochar addition had no significant effect on communities, though a slight increasing trend could be seen.

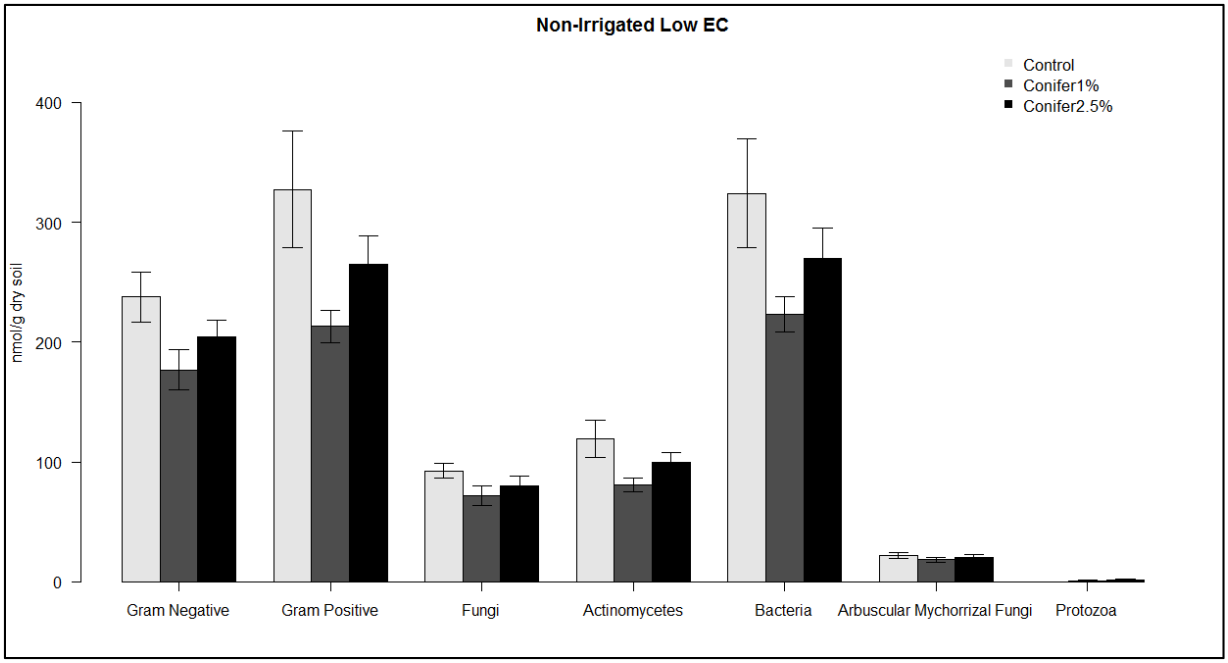

Figure A-2: Microbial communities from non-irrigated low EC samples. Biochar insignificantly reduced microbial communities.

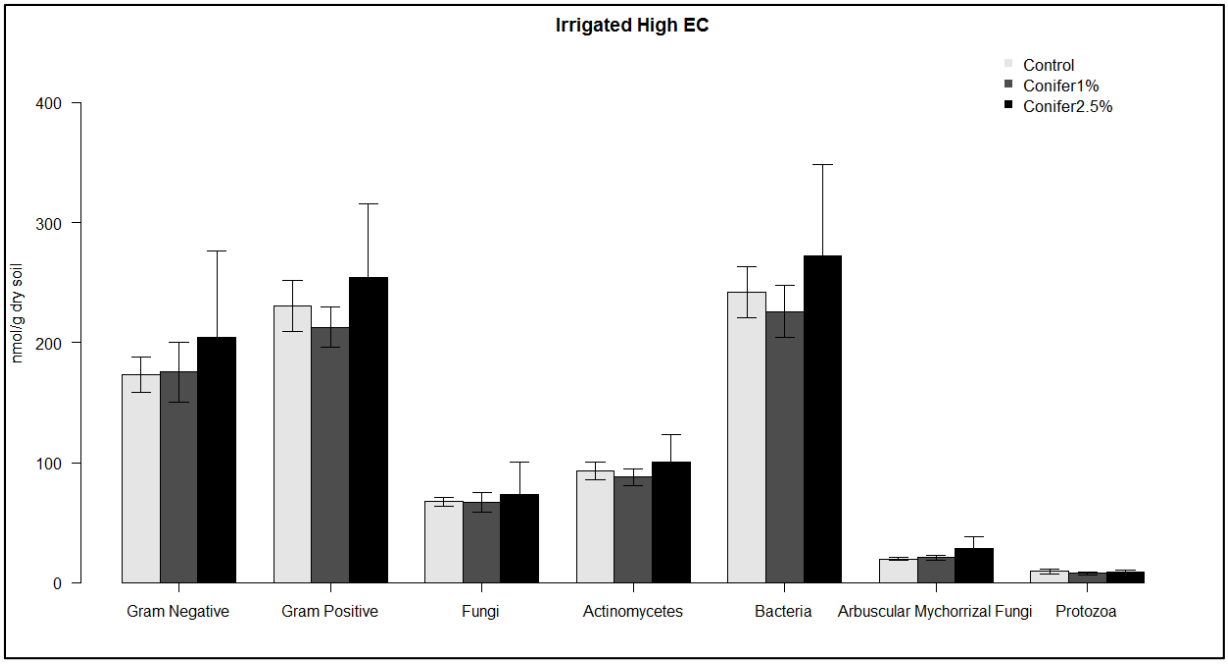

Figure A-3: Microbial communities from irrigated high EC samples. Biochar didn't show any trend on microbial communities and data was highly variable.

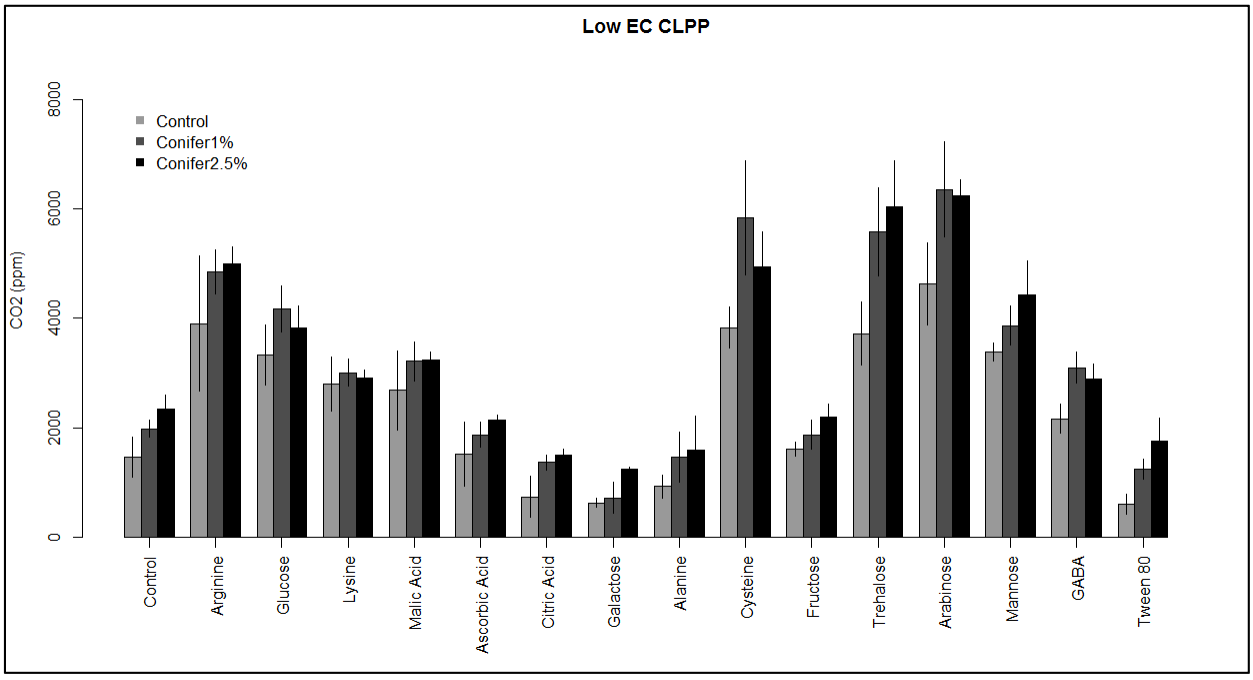

Figure A-4: Cumulative CO_2 (ppm) emission from 6 hours incubation after carbon substrate addition to soil samples in 96 deep wells Microresp plates. Water content of treatments was not adjusted. Biochar addition increased CO_2 emission but the variability of dataset was almost high.