Effects of biochar on rhizosphere processes and biochar co-application with nitrification inhibitor on GHG emissions and microbial and enzymatic activities

by

Prem Pokharel

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Department of Renewable Resources
University of Alberta

Abstract

Production of biochar and its use has a wide implication in waste management, climate change mitigation, soil health enhancement and energy production. The strategy to produce biochar and its application to soil aims to replace waste biomass (by-product of photosynthesis) in soil in a stabilized C form which would otherwise be degraded easily and returned to the atmosphere as CO_2. Pyrolyzing of waste into biochar and putting that into soil plays significant role in mitigating climate change through reduction in greenhouse gas (GHG) emissions from soil and building up of soil organic carbon (SOC). Biochar is a heterogenous material resulting from varieties of feedstocks, pyrolysis conditions and pre-and post-pyrolysis modifications. Understanding of its effects on soil processes and functions across soil types is important as different biochars behave differently with land use types, soil properties, geographical locations and management practices. Despite having a high opportunity of using biochar in forest, grassland and agricultural land in the Canadian prairie region, the inclusion of biochar in the management practices is meagre because of lack of data to demonstrate the benefits of biochar application in different land use types in this region. So, the main objective of this research was to explore the benefits of biochar application in some of the soil types of this region taking an account of rhizosphere processes, and its interaction with nitrification inhibitor on GHG emissions, microbial and enzymatic activities, nutrient mineralization and crop production.

The results of the first study demonstrated that biochar produced from pine sawdust decreased carbon dioxide (CO_2) emission and global warming potential of the emissions from forest but not from grassland soil. Pine sawdust biochar decreased nitrous oxide (N_2O) emission from both forest and grassland soils with no significant effect on methane emissions. Biochar pyrolyzed at 550 °C was more effective than at 300 °C in reducing GHG emissions while post pyrolysis modification by steam activation did not produce significant change in GHG emissions despite having different biochar properties in steam-activated biochar as compared to non-activated one. Second study showed that biochars made from manure pellet and woodchips decreased soil respiration in the rhizosphere (a region of close vicinity to roots) but not from bulk soil. Manure pellet biochars decreased microbial biomass carbon despite increase in dissolved organic carbon and nitrogen. Manure pellet and its biochar had a positive, but woodchips and its biochar had a negative effect on crop production. Third study assessed N transformation using

^{15}N isotope labelling in the rhizosphere and bulk soils after biochar amendment and demonstrated that net N mineralization rates were greater in the biochar amended rhizosphere than bulk soil. Biochar had contrasting effects on gross nitrification rates between rhizosphere and bulk soil and the research demonstrated the importance of gross N transformation processes in understanding the rhizosphere-biochar interactions.

The fourth and fifth studies assessed the interactions between biochar and nitrapyrin (a commonly used nitrification inhibitor) in affecting nitrification rates, N_2O emissions, microbial and ecoenzymatic activities. Manure pellet biochar significantly interacted with nitrapyrin and reduced the efficacy of nitrapyrin in lowering nitrification rates and N_2O emissions. Manure compost biochar increased microbial biomass and C-, N- and P- cycling enzymes while nitrapyrin decreased N- and P-cycling enzymes with no significant interaction between manure compost biochar and nitrapyrin in any of the soil processes studied. The sixth study assessed overall effects of biochar in soil microbial biomass and enzymatic activities across biochar and soil factors from the secondary data using meta-analysis technique and demonstrated that biochar was more effective in acidic soil and with low organic matter and finer textured soil for enhancing microbial activities. Biochars produced at a temperature lower than 550 °C, with pH >10 and C/N ratio less than 10 produced the highest impact on increasing soil microbial and enzymatic activities.

Overall, biochar is beneficial in decreasing GHG emissions, increasing crop production and nutrient limitations for microbial growth and plant uptake in the Canadian prairie region. Its potential in improving soil health was also demonstrated by the increased microbial and enzymatic activities in some of the soils studied. However, the extent of the impact of biochar varied with the feedstock used in pyrolysis, rates of biochar used, land use types and its interaction with other management practices such as nitrification inhibitors. Future research should account for life cycle assessment of biochar production and its application to forest, grassland and agricultural land to determine economic feasibility that will be supportive to farmers interested in using biochar in their management practices in this region.

Preface

This thesis is an original work conducted by Prem Pokharel. A version of chapter two of this thesis has been published as "Pokharel, P., Kwak, J.H., Ok, Y.S. and Chang, S.X. 2018. Pine sawdust biochar reduces GHG emission by decreasing microbial and enzyme activities in forest and grassland soils in a laboratory experiment. **Science of the Total Environment**, 625:1247-1256". A version of chapter three has been published as "Pokharel, P. and Chang, S.X. 2019. Manure pellet and woodchip biochar reduce carbon dioxide emission from rhizosphere but not bulk soils. **Science of The Total Environment**, 659: 463-472". A version of chapter four has been published as "Pokharel, P., Qi, L. and Chang, S.X. 2021. Manure-based biochar decreases heterotrophic respiration and affects gross N transformation rates in rhizosphere soil. **Soil Biology and Biochemistry** 154: 108147". A version of chapter five has been published as "Pokharel, P., and Chang, S.X. 2021. Biochar decreases the efficacy of the nitrification inhibitor nitrapyrin in mitigating nitrous oxide emissions at different soil moisture levels. **Journal of Environmental Management** 295: 113080". A version of chapter six has been published as "Pokharel, P. and Chang, S.X. 2023. Biochar decreases and nitrification inhibitor increases phosphorus limitation for microbial growth in a wheat-canola rotation. **Science of the Total Environment** 858: 159773". A version of chapter seven has been published as "Pokharel P., Ma Z., and Chang, S.X. 2020. Biochar increases soil microbial biomass and changes some extra- and intracellular enzymes activities in a global meta-analysis. **Biochar**, 2: 65-79". I was responsible for conducting the experiment, data collection and analysis as well as manuscript writing. Kwak, J.H., OK, Y.S., Ma, Z., and Qi, L., assisted with editing of manuscript. Chang, S.X. was the supervisory author, contributed to the design of the research and edited the manuscripts.

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Chapter 1. Introduction

1. Research background

Climate change mitigation has been a big challenge worldwide due to the continued increase in greenhouse gas (GHG) emissions and the difficulties both in reducing GHG emissions and in increasing carbon dioxide (CO_2) removal. Anthropogenic activities such as excessive use of fossil fuels, over-exploitation of natural resources, and reduction of forested areas are responsible for the increased GHG emissions and the resultant global climate change. It has also become necessary to increase food production from the limited land resources to feed the growing global population, which has put pressure on converting forest and grassland into agricultural land, increasing the production of chemical fertilizers using fossil fuels resulting in agricultural intensification that has caused a further increase in GHG emissions. Agriculture, forestry, and other land use activities such as the conversion of forest lands and grasslands to cropland and pasture make a substantial contribution to the total GHG emissions on a global scale (about 24%) which is second to the emission from fossil fuel combustion (Smith et al., 2014; Wollenberg et al., 2016). Therefore, we urgently need to develop farming practices to manage the competing interests of increasing crop production and reducing GHG emissions from agriculture.

In general, agricultural land is considered to have a net neutral effect on CO_2 (which is the major GHG for global warming) contribution to the atmosphere because of a balance between CO_2 released from the soil and CO_2 uptake by the crop. However, some intensive farming practices such as tillage have been reported to significantly reduce soil C storage and increase the net release of CO_2 to the atmosphere. In addition, agricultural activities make a substantial contribution to releasing non-CO_2 GHGs such as methane (CH_4) and nitrous oxide (N_2O); these are the other two major GHGs causing global warming with their global warming potential (GWP) of 27-30 and 273 times, respectively, that of CO_2 on a 100-year time scale (EPA, 2022). The agricultural sector accounts for about 56% of total non-CO_2 GHG emissions, representing the highest contribution from anthropogenic activities (EPA, 2013; Smith et al., 2014). In the forestry and other land use sectors, GHG emissions from anthropogenic activities are dominated by CO_2 fluxes, accounting for a third of total global anthropogenic CO_2 emissions from 1750 to 2011 (Smith et al., 2014). Therefore, agricultural practices and land-use conversion from forestry to annual crop production

are major sources of GHG emissions; reducing GHG emissions from these sectors has been identified as one of the most relevant strategies to the goal of limiting global warming to 1.5 °C by the end of the century (Wollenberg et al., 2016).

One of the potential management practices to limit global warming to 1.5 °C is biochar production and its application to agricultural, forest and grassland soils. Biochar is a biomass-derived char produced by thermal decomposition in a partial or total absence of oxygen (Lehmann and Joseph, 2009). Unlike conventional charcoal, biochar is produced by pyrolysis and intended specifically for application to the soil for climate change mitigation and food production globally (Sohi et al., 2010). The strategy to produce biochar and the application of biochar to soil aim to replace waste biomass (a product of photosynthesis) in the soil in a stabilized C form which would otherwise be degraded easily and returned to the atmosphere as CO_2. In addition to its contribution to waste management, biochar has several other benefits, including climate change mitigation, carbon sequestration, increment of crop production, remediation of contaminated land, and enhancement of soil health. Although several past studies have shown positive results in achieving the above-mentioned benefits, biochar's effects on soil processes and functions are not universal across land use types, soil types, geographical locations. Biochar also interacts with other management practices to affect soil processes. One of the underlying causes for that is the heterogeneous nature of biochar (Verheijen et al., 2010) produced from different feedstocks, under different production conditions and modifications applied for certain objectives. The heterogeneous nature of biochars makes it harder to generalize the benefits of biochar application in agricultural, forest and grassland soils. Biochar with different physical and chemical properties affect soil processes and functions differently. Because of these factors, studies dealing with biochar are increasing exponentially in recent years to identify the best biochar for the desired benefit, as biochars with a specific range of properties can yield only certain desired benefits in a particular soil type and geographical location.

The Canadian prairie region has a very high opportunity of using biochar in forest, grassland and agricultural soils. Firstly, the local areas have readily available feedstocks, including manure from livestock, crop residues and forest byproducts. Secondly, most agricultural land has depleted SOC and adding biochar to these lands can increase the amount of C in the soil. Thirdly, agriculture in Canada produces 70% of total anthropogenically produced N_2O, the prairie region

accounting for about 80% of Canada's arable land, could substantially reduce N_2O emissions by the soil application of biochars.

1.1. Biochar properties based on pyrolysis conditions and feedstocks

One of the major aspects of biochar production is that it can help in waste management. Various wastes can be used to produce biochar ranging from crop residues from agriculture, sawmill wastes from forestry, municipal solid waste, and food and animal manure (Yaashikaa et al., 2020). The type of feedstock chosen in biochar production plays an important role in determining physical (particle size, total pore volume and specific surface area and chemical characteristics, including pH, cation exchange capacity, macro-and micro-nutrient concentrations, and calcium carbonate equivalent (Ippolito et al., 2020; Pariyar et al., 2020). In a meta-analysis, Ippolito et al. (2020) have shown that biochars produced from wood-based feedstocks have greater specific surface area and pore volume than in the manure and biosolid-based feedstocks, while crop residues, manure and biosolids produced biochar with a greater cation exchange capacity and pH. When these biochars with different properties are applied to the soil, their effects on soil microbial growth, community composition, and enzymatic activities differ. So, it is essential to produce biochars from different feedstocks readily available in this region and assess their effects on achieving the benefits of GHG emission reduction and increasing crop production.

The pyrolysis condition of biochar is another important factor in determining the physical and chemical properties of biochar. Mostly, pyrolysis is carried out at a temperature below 700 °C, but ranging between 300 and 700 °C, producing biochars with contrasting characteristics. In general, SSA, ash content and pH increase with pyrolysis temperature (Zhang et al., 2017). If the goal of biochar use is to enhance C sequestration, higher pyrolysis temperature yields greater aromaticity with lower O/C and H/C ratios (Spokas, 2010; Wang et al., 2016). However, biochars produced at lower temperatures contain a greater amount of volatile organic matter and nutrients for microbial growth and plant uptake. Pre-and post-pyrolysis activation also causes substantial differences in biochar's properties (Panwar and Pawar, 2020). Biochar activation enhances the surface area, pore structure and surface functional groups, thereby increasing adsorption capacity for many caions and anoins and reactivity in the soil (Ahmad et al., 2014). Different activation methods, including physical, chemical and impregnation techniques, have been studied to optimize biochar's adsorption capacity, particularly those used for environmental remediation (Tan et al.,

2015). Pallarés et al. (2018) examined the effects of steam activation in biochar and showed that the biochar, after activation, reduced H, C and O content on the surface with lower H/C, O/C and N/C molar ratios. There is a need to assess biochar produced under different pyrolysis conditions (i.e., pyrolysis temperature and steam activation) to know whether these biochars effectively reduce GHGs from the soil in different land use types such as forest, grassland and agricultural land in this region.

1.2. Biochar and climate change mitigation

Loss of soil organic carbon (SOC) pool of cropland caused by inappropriate land use and management practices such as tillage operations, residue removal and excessive use of fertilizers and pesticides is another critical environmental issue that needs to be addressed (Lal, 2011). The depletion of SOC pool is causing a significant reduction in soil fertility and crop productivity (Luo et al., 2010). The loss of SOC and excessive use of chemical fertilizers are causing soil acidification and leaching loss of nutrients, which can significantly impact groundwater contamination. Applying biochar can help restore SOC in croplands, thereby supporting soil health and crop production. The potential of biochar to sequester C in the soil and its stability in the soil has been reviewed by Wang et al. (2016). The study revealed that crop residue-derived biochars get decomposed faster than wood-derived biochars in the soil. Weng et al. (2017) have demonstrated that biochar has the potential to increase SOC by stabilizing root-derived C and facilitating negative priming effects of C mineralization in the soil.

The potential of biochar amendment to reduce GHG emissions from agricultural soil has been revealed in many studies (e.g., Bamminger et al., 2014; Borchard et al., 2014; Jeffery et al., 2016; Kammann et al., 2017). The effects of biochar amendment on CO_2 (Liu et al., 2016), methane (CH_4) (Jeffery et al., 2016) and nitrous oxide (N_2O) (Cayuela et al., 2014) have been reviewed in their meta-analyses. In these meta-analyses, CO_2 emissions were found to be neutral, while CH_4 and N_2O emissions were found to be significantly reduced by biochar amendment to soil. The decrease in CH_4 emissions was mainly attributed to the increase in soil aeration and pH that enhances CH_4 oxidation in flooded and acidic soils (Van Zwieten et al., 2009) and the decrease in N_2O emission was attributed to the abiotic mechanisms of sorption capacity of biochar or biotic alterations of nitrifying and denitrifying bacterial activities in the soil (Clough and Condron, 2010; Cayuela et al., 2013). The use of biochar on a global scale can reduce 12%

of current anthropogenic CO_2-C equivalent emissions (Woolf et al., 2010), with a global potential emission reduction of 1-1.8 Pg CO_2-C equivalent per year (Paustian et al., 2016). Biochar has also been shown to increase SOC content by 40% and microbial biomass carbon (MBC) by 18% globally (Liu et al., 2016).

1.3. Biochar and crop production

Positive effects of biochar on crop production have been demonstrated in several individual greenhouse, field experiments, and meta-analytical studies. Since the effects are well correlated to the rate of biochar applied, type of biochar and soil, and the crop itself, selecting the best biochar with optimal rate has always been a challenge across soil types. The effects of biochar amendment on crop productivity and nutrient cycling have been reviewed and critically analyzed by Atkinson et al. (2010), Biederman and Harpole (2013), Crane-Droesch et al. (2013) and Jeffery et al. (2011) in their meta-analyses. Biederman and Harpole (2012) have shown that the addition of biochar significantly increased aboveground productivity, crop yield, soil phosphorus, potassium and nitrogen in the soil but did not affect belowground productivity and plant tissue N concentration. Crane-Droesch et al. (2013) showed that biochar pH, C content and pyrolysis temperature are strong predictors of yield response. In a meta-analysis, Nguyen et al. (2017) have demonstrated that inorganic N in the soil decreases following biochar addition to the soil, ammonium by 11% and nitrate by 10% compared to the soil without biochar amendment. The effects of biochar on improving soil fertility were reviewed by Ding et al. (2016) showed that the biochars having a high surface area with many functional groups and high nutrient content are more effective in improving soil fertility. The study also demonstrated that feedstock, pyrolysis temperature, pH and application rates of biochar are major factors for determining soil fertility in biochar-amended soils. However, there are few studies that have assessed the impact of these factors on soil fertility and crop production in the Canadian prairie region, so we have limited knowledge on how beneficial biochar could be in meeting farmers' expectations in terms of crop production in this region.

1.4. Biochar and rhizosphere processes

One of the most important questions around biochar application that have been surprisingly ignored in past research is biochar-rhizosphere interaction. Most past studies on biochar

application are focused on bulk soil. The rhizosphere, the root-soil interface, is the most active and dynamic hotspot of microbial decomposition of organic matter in the soil (Philippot et al., 2013). The properties of the soil in the rhizosphere are modified by a range of processes caused by the release of root exudates that make it different from the bulk soil. About 15 to 25% of belowground allocated carbon in the plant is exuded from the roots, and these exuded organic substances induce fast C and nutrient mineralization in the rhizosphere (Jones et al., 2009). Therefore, rhizosphere processes are important contributors to emissions of CO_2 and other GHGs and the fast turnover of SOC. In terms of nutrient uptake by plants, this is the most influential region in the soil as the limitation of nutrients in this region severely affect the yield of crops. It is thus important to understand how biochar application impacts the soil processes in this region to help increase nutrient availability in the vicinity of roots and its positive and negative priming effects on the mineralization of labile C (e.g., rhizodeposits). So, understanding biochar-rhizosphere processes is essential in nutrient management, GHG emissions and C sequestration. However, our knowledge of biochar effects on rhizosphere processes is limited. Only a few studies, including Weng et al. (2015 and 2017) in a Ferralsol, have critically analyzed biochar's effect on rhizosphere processes.

1.5. Biochar co-applied with nitrification inhibitor

Biochar has been widely recommended to use in agricultural land for different purposes. One of the attributes of biochar that is often discussed is to increase nitrogen use efficiency by lowering nitrification rate and N loss via leaching of NO_3^-, NH_3 volatilization (Sha et al., 2019) and gaseous loss of NO_2 and N_2 (Liu et al., 2018). Nitrification inhibitors (NIs) are also applied for similar purposes, and there is a long history of application in North America. Co-application of these two management practices in cropland is more likely to be seen in the future because of the widespread use of biochar in the cropland for several other purposes in addition to increasing nutrient use efficiency. It is still not very clear how these two management practices interact with each other; whether they have synergistic, antagonistic or neutral effects on each other. Since biochar has a high adsorption capacity, there is a possibility that biochar can adsorb NI on its surface (similar to other organic compounds) and limits the potential impact of NI in reducing nitrification rates. Some studies have shown such behavior of wood biochar with some NIs (Keiblinger et al., 2018; Fuertes-Mendizábal et al., 2019).

2. Objectives of the research

The overall aim of this research was to gain more insight into the benefits of biochar application to the soil in mitigating climate change and increasing crop production. As mentioned earlier, biochar application has a very high potential for its use in forest, grassland and agricultural lands in the Canadian prairie region. Because of the short growing period in this region, agricultural management practice is different from those in other regions in North America and elsewhere in the world, which makes the soil and crop production systems in this region unique. Although biochar amendment has been tested widely around the world, the use of biochar in this region is falling behind as there is little data to demonstrate its benefits across different land use types in the region. Therefore, this thesis aimed to demonstrate the benefits of biochar amendment in some of the soil types in this region, taking into account the rhizosphere process and its interaction with nitrification inhibitors on GHG emissions, microbial and enzyme activities, and nutrient mineralization and crop production. The findings in this thesis will support sustainable agriculture for farmers who want to use these practices in their agriculture management in this region.

The main objectives of this thesis research were to: (i) examine the effects of biochars produced under different pyrolysis temperatures and steam activation on GHG emissions, enzyme and microbial activities in forest and grassland soils, (ii) assess the effects of unpyrolyzed biomass (manure pellet and woodchips) and their biochars on crop production and soil respiration in bulk and rhizosphere soils, (iii) examine the effects of unpyrolyzed manure pellet and its biochar on gross and net N mineralization in bulk and rhizosphere soils, (iv) examine the effects of interactions between biochar and NI on nitrification rate and N_2O emissions under different soil moisture conditions, (v) assess the effects of interactions between biochar and NI on microbial biomass, ecoenzymatic activities and their stoichiometry to determine microbial nutrient limitations in a wheat-canola rotation, and (vi) review and critically analyze soil microbial and enzyme activities in response of biochar amendment on a global scale.

3. Structure of the thesis

The thesis consists of eight chapters. Chapter one (this chapter) provides background information about the study, and Chapter eight is about the summary of results, conclusions, and

recommendations for further study. A version of Chapter two entitled "Pine sawdust biochar reduces GHG emission by decreasing microbial and enzyme activities in forest and grassland soils in a laboratory experiment" has been published in **Science of the Total Environment**. A version of Chapter three entitled "Manure pellet, woodchip and their biochars differently affect wheat yield and carbon dioxide emission from bulk and rhizosphere soils" has been published in **Science of the Total Environment**. Chapter four is entitled "Manure-based biochar decreases heterotrophic respiration and increases gross nitrification rates in rhizosphere soil". A version of Chapter four has been published in **Soil Biology and Biochemistry**. Chapter five is entitled "Biochar decreases the efficacy of the nitrification inhibitor nitrapyrin in mitigating nitrous oxide emissions from soil at different moisture levels". A version of Chapter five has been published in the **Journal of Environmental Management**. A version of Chapter six entitled "Biochar decreases and nitrification inhibitor increases phosphorus limitation for microbial growth in a wheat-canola rotation" has been submitted to **Science of the Total Environment.** Chapter seven is entitled "Biochar increases soil microbial biomass with changes in extra- and intracellular enzyme activities: a global meta-analysis". A version of Chapter seven has been published in the journal **Biochar.**

Chapter 2. Pine sawdust biochar reduces GHG emission by decreasing microbial and enzyme activities in forest and grassland soils in a laboratory experiment

1. Introduction

Agriculture, forestry and other land use (AFOLU) activities such as conversion of forest lands and grasslands to croplands and pasture are releasing a significant amount of greenhouse gases (GHGs) including carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). The AFOLU activities contribute 24% of total GHG emission on a global scale (Smith et al., 2014; Wollenberg, et al., 2016). In the agricultural sector, non-CO_2 GHG emission is often highlighted as the CO_2 emission is considered neutral because of carbon fixation and oxidation through photosynthesis. But this sector, being the largest contributor to the global anthropogenic non-CO_2 GHGs, accounts for 56% of total anthropogenic emission (EPA, 2013; Smith et al., 2014). The rate of agricultural non-CO_2 emission is increasing (0.9% $year^{-1}$ between 1990 and 2010; Tubiello et al., 2013). In the forestry and other land use sectors, the GHG emission from anthropogenic activities is dominated by CO_2 fluxes, accounting for a third of total global anthropogenic CO_2 emission from 1750 to 2011 (Smith et al., 2014). Agriculture and forestry are therefore the major sources of GHGs and have been a major cause of the rise in global average temperature.

The Paris Agreement of the United Nations Framework Convention on Climate Change (UNFCC) set a goal of limiting global warming to less than 2 °C relative to the preindustrial era and a consensus was reached that the goal can be achieved by reducing anthropogenic sources of GHG emissions (Chabbi et al., 2017). Reducing GHG emission from agriculture and forestry sectors was identified as one of the most relevant strategies to meet the goal of limiting global warming to less than 2 °C (Wollenberg et al., 2016). Soil amendment with biochar, a biomass derived char produced by thermal decomposition in partial or total absence of oxygen, has been proposed as an effective means to reduce GHG emissions from agriculture and forest soils (Smith et al., 2014; Kammann et al., 2017) and help mitigate global warming (Mandal et al., 2016; Smith et al., 2016). The potential of biochar to reduce GHG emissions from agricultural soils has been revealed in many recent studies (e.g., Bamminger et al., 2014; Borchard et al., 2014; Jefferey et al., 2016; Kammann et al., 2017). Woolf et al. (2010) estimated that the use of biochar on a global scale can reduce 12% of current anthropogenic CO_2-C equivalent emissions.

Although the economic and management constraints of biochar application have not been fully assessed yet, biochar application has been estimated to have a global technical potential emission reduction of 1-1.8 Pg CO_2-C equivalent per year (Paustian et al., 2016).

Biochar amendment of soil can sequester carbon (C) and reduce CO_2 emission from soils (Lehmann, 2007; Zhang and Ok, 2014) because of its slow decomposition and long mean residence time. When the biomass is pyrolyzed, biologically and chemically recalcitrant biochar is formed which is highly stable in soils (Paustian et al., 2016). The mean residence time of biochar in soils (calculated by two-component first-order decay equations) has been shown to be several decades to 200 years under the optimal conditions of laboratory experiment (Hamer et al., 2004; Kuzyakov et al., 2009) and up to 2000 years under natural conditions if the decomposition of biochar is considered 10 times slower in natural conditions as compared to optimal laboratory conditions (Kuzyakov et al., 2009). The net CH_4 flux from biochar amended soils depends on its effect on increasing soil aeration and thus increasing CH_4 oxidation (Van Zwieten et al., 2009; Jeffery et al., 2016). Biochar amendment has a potential to reduce N_2O emission from the soil by adsorbing ammonium (NH_4^+) and nitrate (NO_3^-) on the biochar surface because of increased cation and anion exchange capacities during biochar production (Mandal et al., 2016). Biochar can also reduce N_2O emission by decreasing total nitrogen (N) denitrified in the soil (Cayuela et al., 2013).

Previous studies have shown large variability in GHG production from suppression to stimulation when biochar is amended to soils and the variability has been linked to several factors (Mandal et al., 2016). Among them, the basic soil properties are the most important because they can significantly affect soil-biochar-microorganism interactions that result in the variability in GHG emission in soils (Lehmann et al., 2011). The net N mineralization, microbial biomass C and dehydrogenase activity were greater in biochar-amended soils with higher soil organic matter (SOM) than lower SOM content soils (Ameloot et al., 2015; Thomazini et al., 2015). A meta-analysis shows that the response of CO_2 flux and microbial activity to biochar amendment also varies with other soil properties such as soil texture and pH, and land-use types such as forests, grasslands and rice paddies (Liu et al., 2016).

Another important factor is the pyrolysis conditions for biochar production that significantly changes biochar properties that cause variations in GHG emission from biochar-amended soils (Spokas and Reicosky, 2009; Singh et al., 2010; Streubel et al., 2011). The

pyrolysis condition such as the temperature and activation methods used during biochar production changes surface area, cation exchange capacity (CEC), pH, ash content and aromaticity of biochar (Ahmad et al., 2012; Ahmad et al., 2014; Rajapaksha et al., 2016). Pyrolysis temperature has contradictory effects on CEC of biochar with both increasing (Lehmann, 2007; Zhang et al., 2015) or decreasing CEC (Gaskin et al., 2008; Singh et al., 2010; Kloss et al., 2012) with increasing temperature being reported. The pH, surface area and ash content of the biochar are also increased at higher pyrolysis temperatures (Kloss et al., 2012; Lou et al., 2016a). Steam activation during biochar production can also increase the surface area, porosity and adsorption capacity of biochar by removing volatile organic compounds (Azargohar and Dalai, 2006; Rajapaksha et al., 2014). These characteristics of biochar produced at higher pyrolysis temperature and steam activation can have significant decrease on C and N mineralization rate (Awad et al., 2012; Ameloot et al., 2015), enzyme and microbial activities (Awad et al., 2012; Ouyang et al., 2014) and increase in adsorption of NH_4^+ and NO_3^- (Ippolito et al., 2012) in the soil resulting in net reduction of GHG emissions from the biochar-amended soils. However, the studies on the effects of these properties of biochar on GHG emission are more focused on cropland soils. The effect of biochar application on GHG emission from forest and grassland soils has been little studied despite them being important sources of GHG emission.

Economical disposal of sawdust, a by-product of the sawmill industry, has been a problem of growing concern to the wood industries (Daian and Ozarska, 2009) as these industries produce a huge amount of sawdust annually; for instance, 5,355,054 metric tons of sawdust was produced in 2004 in Canada (NRC, 2006). In recent years, sawdust has been used as a raw material in bioenergy based industries. Producing biochar from sawdust would be an alternative method of disposing waste material and use of that biochar in soil amendment could have significant effect on mitigating GHG emission from the soil (Lee et al., 2017). The objectives of this study were to: (i) examine the effects of pine sawdust biochar produced under different pyrolysis conditions (two temperatures (300 and 550 °C) and with or without steam activation) on GHG emission and microbial and enzyme activities in the forest and grassland soils in a laboratory incubation experiment lasting for 100 days and (ii) evaluate the effect of these biochar amendments on global warming potential (GWP) of GHG emission from the forest and grassland soils. It is hypothesized that application of biochars produced at a higher

temperature (550 °C) with steam activation would reduce GHG emission from forest and grassland soils due to the higher recalcitrance, pH, porosity and ash content as compared to the biochars produced at a lower temperature (300 °C) without steam activation. Pine sawdust biochars produced at the higher temperature with steam activation would decrease microbial and enzyme activities in the soils resulting in a decreased GHG emission from forest and grassland soils. In this study, a laboratory incubation experiment was performed to avoid confounding effects that could occur under field conditions. Despite the limitation of laboratory incubation experiments being conducted under artificial conditions, laboratory experiments are very useful for high throughput screening and selecting biochar treatments to be further tested in field trials.

2. Material and methods

2.1. Soil and biochar

Two soil samples were collected from north-central Alberta, Canada: a forest soil (Orthic Gray Luvisol) near Breton (53°07′N, 114°28′W; elevation 832 m) and a grassland soil (Orthic Black Chernozem) near Ellerslie (53°25′N, 113°33′W; elevation 690 m). The texture was silty loam (30% sand, 56% silt and 14% clay) in forest soil and clay loam (29% sand, 36% silt and 35% clay) in grassland soil. To account for some of the field variation, soils (from each site) were collected from four sampling plots, at 50 m apart from each other. The samples were collected from the top mineral soil layer (0-10 cm) using a corer (6 cm diameter). Each sample was a composite of ten soil cores collected from each sampling plot and was used as a replicate for the laboratory incubation experiment as described below.

Roots and plant litter were removed from the fresh soils and the soils were passed through a 2-mm sieve. A sub sample of each fresh soil was air-dried and was used in analyzing of total C and total N concentrations after the soil was ground using a ball mill (Mixer Mill MM 200, Thomas Scientific, Swedesboro, NJ). The total C and total N concentrations were analyzed by an automated elemental analyzer (NA-1500 series, Carlo Erba, Milan, Italy). Soil NH_4^+-N and NO_3^--N were analysed colorimetrically in the soil extract (extracted with 2 mol L^{-1} KCl at 1:5 (m:v) ratio). The indophenol blue method was used for NH_4^+-N (Keeney and Nelson, 1982) and the vanadium oxidation method for NO_3^--N analysis (Doane and Horwath, 2003). Soil pH was measured in a $CaCl_2$ solution (0.01 mol L^{-1}) at a 1:2 (m:v) ratio by using a pH meter (Orion,

Thermo Fisher Scientific Inc., Beverly, MA, USA). Soil texture was measured by the hydrometer method and soil bulk density was determined by measuring the oven-dry weight of soil collected using a steel corer (100 cm^3). The properties of the soils are given in Table 1.

Biochars used in this study were produced from Korean pine (*Pinus koraiensis* Siebold & Zucc.) sawdust at different pyrolysis temperatures (300 and 550 °C) with or without steam activation. The sawdust was air-dried after washing with deionized water and ground to < 2.0 mm size before pyrolysis. The biochars were produced by heating the biomass at a heating rate of 7 °C min^{-1} for 2 hours in limited oxygen supply. For steam activation, the biochars were treated with steam at 5 mL min^{-1} for an additional 45 minutes at the peak temperature. The four biochars used in this experiment were: biochar produced at 300 °C without steam activation (BC300), biochar produced at 300 °C with steam activation (BC300-S), biochar produced at 550 °C without steam activation (BC550) and biochar produced at 550 °C with steam activation (BC550-S). The detail production method is described in Lou et al. (2016a and 2016b) and the physico-chemical properties of these biochars are given in Table 2.

2.2. Experimental design and incubation procedure

The experimental design was a completely randomized block design with five treatments, soil only (CK), soil + BC300, soil + BC300-S, soil + BC550 and soil + BC550-S, for each forest and grassland soil with four replications. Each field soil sample from each soil type was used as a replicate (block) for the incubation experiment; in other words, each field soil sample was used to set up one replicate of the laboratory incubation experiment. Two separate incubation experiments (for each soil type) were set up in parallel: one for measuring GHG emissions and the other for analyzing enzyme and microbial activities.

In the first experiment for GHG emission measurement, 50 g (oven dry weight equivalent) of air-dried (at 22 °C) soil was placed in 250 mL Erlenmeyer flasks. In the second experiment for the analysis of enzyme and microbial activities, 100 g (oven dry weight equivalent) of air-dried soil was placed in 1 L Mason jars. The biochars were mixed with each of the forest and grassland soil at the rate of 1.5% (w/w) on an oven dry weight basis; this application rate is equivalent to ~21 Mg ha^{-1} for the forest and ~17 Mg ha^{-1} for the grassland soil (bulk densities of the forest and grassland soils were 1.42 and 1.15 g cm^{-3}, respectively; Table 1) incorporated in the 0-10 cm surface soil. The rate of 1.5% was chosen because the 1-2% of

biochar application rate was found to significantly change the physical quality of the soil and GHG emissions (Mukherjee and Lal, 2013). Moisture content of the soil was brought to 40% water holding capacity (WHC) by adding deionized water which was determined separately for control and biochar-amended soils before starting lab incubation. Then the control and biochar-amended soils were pre-incubated at 25 °C in the dark for five days to stabilize microbial populations. After pre-incubation, moisture content was brought to 60% WHC which was maintained throughout the incubation period by adding deionized water weekly. The soils were incubated at 25 °C in the dark for 100 days.

2.3. Gas sampling and analysis

For GHG measurement, the flasks were sealed tightly with rubber septum stopper between sampling times that allowed accumulation of gases in the headspace. Gas samples were collected from the flasks daily for the first 6 days, then once every 2 days until day 20 and once every 7 days until day 100.The frequency of the gas sampling was decided based on the CO_2 emission pattern observed in a previous incubation study on a biochar-amended Chernozemic soil (Wu et al., 2013). Daily measurement for the first few days was intended to capture the relatively high variation in GHG flux that can be anticipated at the beginning of the incubation. The variation decreases over time of incubation (Wu et al., 2013) and the frequency of measurement of GHG flux was reduced accordingly. At each sampling, gas samples were taken at 0 and 6 h after the closure of the stopper by a 20 mL gas syringe and the gas samples were transferred to pre-evacuated 10 mL soda glass Isomass Exetainers to provide a positive pressure in the Exetainer. The assumption of linear increase of gas concentration in the headspace was tested (for 2, 4 and 6 hours) in a preliminary experiment with biochar-soil mixtures before the real incubation experiment and the assumption was found to be true until 6 hours after the flask was sealed airtight. The CO_2, CH_4 and N_2O concentrations were analyzed using a Varian CP-3800 gas chromatograph (GC, Varian Canada, Mississauga, Canada). The chromatograph was equipped with a thermal conductivity detector, a flame ionization detector and an electron capture detector. The GHG emission rates were calculated by the change in gas concentration between two samplings. Total GWP in CO_2-C equivalent per g of soil was calculated using following equation (Watson et al., 1996):

$$GWP = R(CO_2) + 25 \times R(CH_4) + 298 \times R(N_2O) \quad (1)$$

where R (CO_2), R (CH_4) and R (N_2O) are the cumulative emission of CO_2, CH_4 and N_2O (μg g^{-1} soil) in the 100-day incubation. The default molecular GWP of CH_4 and N_2O in a 100-year time frame of 25 and 298, respectively, was used in the calculation, while the GWP value for CO_2 was 1 (IPCC, 2007).

2.4. Analyses of soil enzyme activities

Soil samples were collected from the mason jars 1, 10, 50 and 100 days after the commencement of the incubation. The activities of extracellular hydrolytic enzymes including β-1,4-glucosidase and β-1,4-N-acetyl glucosaminidase were analyzed using moist soil samples by fluorimetric method following Sinsabaugh et al. (2003) and German et al. (2011). Briefly, a soil suspension was prepared using 1 g of moist soil sample in a 250 mL Nalgene bottle by adding 125 mL of sodium acetate buffer (50 mmol L^{-1}, pH = 5). The soil suspension was homogenized in a shaker (250 rpm) for 30 minutes. Then a 200 μL aliquot (from the continuously homogenized soil suspension in a stirrer for 2 minutes) was pipetted into 8 wells of black 96 well plates with 50 μL of each substrate (200 μM). For soil background and standard quench, 50 μL of buffer and 50 μL of 4-methylumbelliferyl, respectively, were added with 200 μL soil aliquot into 4 wells each. The plates were incubated at 20 °C in the dark for 3 hours to analyze for the β-1,4-glucosidase and β-1,4-N-acetyl glucosaminidase activities. The concentration of substrate, pH of acetate buffer, and the time for incubation were based on previous studies, which optimized parameters for enzymatic activities in forest and grassland soils (German et al., 2011; Brockett et al., 2012; Hewins et al., 2015). The reaction was stopped by adding 20 μL of 0.5 mol L^{-1} NaOH solution after incubation. Then the fluorescence in the aliquot was measured using a Synergy microplate reader (Synergy HT, Bio-Tek Instruments, Winoosky, VT, USA) with 365 nm excitation and 450 nm emission filters.

2.5. Determination of soil microbial biomass carbon and nitrogen, and available N

Soil microbial biomass carbon (MBC) and nitrogen (MBN) in soil samples collected after 1, 10, 50 and 100 days of incubation were analyzed by the chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). For the analysis of MBC and MBN, 20 g of moist soil

samples were collected and fumigated with chloroform in an evacuated desiccator for 24 hours. Fumigated and non-fumigated soil samples were extracted with 0.5 M K_2SO_4 solution. For the extraction, moist soil samples (20 g) were mixed with 50 mL K_2SO_4 solution, shaken for 1 h in a reciprocating shaker (250 rpm) and filtered. Extractable C and N in the soil extracts were analyzed using a TOC-V analyzer connected to a TN module (Shimatzu Corporation, Kyoto, Japan). The MBC and MBN were calculated by the differences in extractable C and N, respectively, between fumigated and non-fumigated soil samples. Extracts of the non-fumigated soils were also used to analyze available N (by the procedure described above).

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used to test the significance of the effect of five biochar treatments on cumulative CO_2, CH_4 and N_2O emission, and GWP of forest and grassland soils separately using the PROC MIXED model in the SAS (version 9.2, SAS Institute Inc., NC, USA). The biochar effects on soil MBC, MBN, β-1,4-glucosidase and β-1,4-N-acetyl glucosaminidase activities in the forest and grassland soils were also tested separately for each sampling time (after 1, 10, 50 and 100 days of incubation) using one-way ANOVA. A repeated measures ANOVA was used to examine the effects of biochar treatments over time of incubation on soil MBC, MBN, β-1,4-glucosidase and β-1,4-N-acetyl glucosaminidase activities in each soil type. Before statistical analysis, all data mentioned above were tested for normality of distribution with the Shapiro-Wilk test in residuals using UNIVARIATE and homogeneity of variance with Levene's test. Soil MBC and MBN data after 10 and 50 days of incubation in the forest soil was log-transformed (base 10) to perform ANOVA as the residuals of these data were not normally distributed but back-transformed data of these parameters were presented in the results section. Pearson correlation analysis was used to determine the relationship among soil parameters analyzed (regardless of the treatment effect) from their mean values of soil MBC, MBN, β-1,4-glucosidase and β-1,4-N-acetyl glucosaminidase, available N analyzed at 100-day of incubation sampling time and cumulative GHG emission in the entire 100-day incubation. The statistical significance of difference of all treatment effects was determined on the mean values by an $\alpha < 0.05$ in all analyses and the means of the variables were separated using Tukey's test.

3. Results

3.1 Greenhouse gas emissions

The CO_2 emission rates from the soil were increased (irrespective of the biochar treatment) with time initially and then decreased sharply until day 16 in the forest soil and until day 20 of incubation in the grassland soil (Fig. 2-1). Then, the CO_2 emission rates became almost stable until the end of incubation in both soils. The maximum effect of biochar treatments on CO_2 emission appeared to take place in the first 50 days (approximately) of incubation after which CO_2 emissions from all treatments were similar. In the forest soil, cumulative CO_2 emission in the 100-day incubation was reduced ($P = 0.011$) by 16.4% by BC550 as compared to the control, with no significant effects of other treatments (Fig. 2-1; Appendix 2-2). However, the cumulative CO_2 emission was not affected by biochar treatments in the grassland soil. Biochar treatments did not have significant effects on CH_4 flux in both forest and grassland soils in the 100-day incubation (Appendix 2-2).

The effect of biochar treatment on N_2O flux persisted until the end of the 100-day incubation in both soils (Fig. 2-2). The cumulative N_2O emission from the soil in the 100-day incubation was reduced by biochar amendment in both forest ($P = 0.033$) and grassland soil ($P = 0.004$) (Fig. 2-2; appendix 2-2). In the forest soil, biochar amendment with BC550 reduced cumulative N_2O emission by 27.5% and BC550-S by 31.5% as compared to the control. Similarly, in the grassland soil, BC550 biochar treatment reduced the cumulative N_2O emission by 14.8% and BC550-S by 11.7% relative to the control. Cumulative N_2O emission in the grassland soil amended with BC550 was 11.8% less than that amended with BC300 and 4.5% less with BC550-S than with BC300-S (Appendix 2-2).

The total GWP of CO_2, CH_4 and N_2O fluxes were similar to that of CO_2 emission rates as the CH_4 uptake and N_2O emission rates were very small as compared to the CO_2 emission rate (Fig. 2-3). The BC550 reduced GWP by 17.2 % ($P = 0.010$) relative to the control in the forest soil, while other biochar treatments did not affect GWP. In the grassland soil, GWP was not affected ($P > 0.050$) by any biochar treatments.

3.2. Enzyme activities and microbial biomass C and N

Biochar treatment affected β-1,4-glucosidase ($P = 0.014$) and β-1,4-N-acetyl glucosaminidase activities ($P = 0.027$) after 10 days of incubation in the forest soil (Fig. 2-4; Appendix 2-2). In the grassland soil, β-1,4-glucosidase activity was affected after 50 days ($P = 0.043$) and β-1,4-N-acetyl glucosaminidase activity after 10 ($P = 0.041$) and 50 days of incubation ($P = 0.022$) (Fig. 2-4; Appendix 2-2). The BC500 and BC550-S reduced β-1,4-glucosidase by 26 and 35%, respectively, in the forest soil after 10 days and by 13 and 18% respectively in the grassland soil after 50 days of incubation relative to the control. The BC300 and BC300-S treatments did not significantly affect enzyme activities in both forest and grassland soils relative to the control except a significant reduction in β-1,4-N-acetyl glucosaminidase by BC300-S after 50 days of incubation in the forest soil (Fig. 2-4; Appendix 2-2).

The BC550-S significantly reduced soil MBC and MBN after 10 and 50 days of incubation in the forest and MBC after 50 days of incubation in the grassland soil as compared to the control, BC300 and BC300-S (Fig. 2-5; Appendix 3-3). The BC550 reduced soil MBN by 28% ($P = 0.001$) after 10 days of incubation and 39% ($P = 0.002$) after 50 days of incubation in the forest and MBC by 24% ($P < 0.001$) after 10 days of incubation in the grassland soil as compared to the control. The BC300 and BC300-S did not affect soil MBC and MBN except BC300-S reduced MBN after 50 days of incubation in the forest soil as compared to the control.

Repeated measures ANOVA showed that the enzyme activities were greatly influenced by incubation time (Fig. 2-4; Appendix 2-4). In the forest soil, β-1,4-glucosidase activities were the highest at 10-day of incubation, then decreased until the end of the incubation ($P < 0.001$) while the β-1,4-N-acetyl glucosaminidase activities were increased consistently over time ($P < 0.001$). In the grassland soil, however, activities of β-1,4-glucosidase increased until 50-day of incubation but the activities of β-1,4-N-acetyl glucosaminidase reached maximum at the end of the incubation. Microbial biomass C and N were also varied significantly with incubation time (Fig. 2-5, Appendix 2-4).

3.3 Relationships between GHG emission, MBC, MBN, enzyme activities and soil available N

A strong positive relationship exists between CO_2 emission and soil MBC and MBN in the forest soil while the relationship was not significant in the grassland soil (Table 2-3). Emission of N_2O was positively correlated with soil MBN, β-1,4-glucosidase, β-1,4-N-acetyl glucosaminidase and NO_3^--N in the forest soil and MBC, β-1,4-N-acetyl glucosaminidase and NO_3^--N in the grassland

soil. The β-1,4-glucosidase activities were positively correlated with soil MBC and MBN in the forest soil while the β-1,4-N-acetyl glucosaminidase activities were positively correlated with soil MBC and available N in the grassland soil.

4. Discussion

The decrease in cumulative CO_2 emission from the forest soil by BC550 and N_2O emission from the forest and grassland soils by BC550 and BC550-S while there was no significant effect of BC300 and BC300-S on cumulative CO_2 and N_2O emissions from both soils supports our hypothesis that the pine saw dust biochar produced at higher temperature can reduce GHG emission from forest and grassland soils. The reduction of CO_2 and N_2O emissions by biochar amendment in this study is consistent with previous studies (Yanai et al., 2007; Spokas and Reicosky, 2009; Cayuela et al., 2010; Mandal et al., 2016). Only BC550 significantly reduced cumulative CO_2 emission relative to the control, indicating that this biochar would be effective in reducing CO_2 flux from the soil. The lower CO_2 emission rate in BC550 relative to the control was accompanied by lower microbial biomass and enzyme activities (at 10 and 50-day of incubation). The reduction in CO_2 emission by biochar (produced at 550 °C) treatment in the soil having low SOM has been reported in many studies (Awad et al., 2013; Awad et al., 2016). The change in CO_2 emission due to changes in the SOM mineralization process in the soil by biochar application is often caused by priming effects of biochar added to the soil (Kuzyakov et al., 2009; Zimmermann et al., 2011; Awad et al., 2012). Mineralization of SOM can be enhanced by positive priming effects or decreased by negative priming effects of biochar on soil organic matter (Novak et al., 2010; Zimmerman et al., 2011; Bamminger et al., 2014). Mineralization of SOM is likely to be inhibited when the biochars have a surface area greater than 200 $m^2 g^{-1}$ (Ameloot et al., 2013). These biochars can adsorb soluble constituents from the organic matter and physically protect them against further mineralization because of their pores being inaccessible for microorganisms (Ameloot et al., 2013). Pine sawdust biochar produced at 550 °C had a surface area of 293 $m^2 g^{-1}$ (values averaged for steam and non-steam-activated biochars) (Lou et al., 2016a). So, the decrease in total CO_2 emission from soils amended with biochar produced at 550 °C could be attributed to the decrease in soluble constituents of SOM available for mineralization. But, in the grassland soil, biochar amendment was not effective to reduce CO_2 emission likely due to the very high SOM content in the soil; in this case the biochar

application was not effective in reducing the soluble constituents of SOM available for mineralization. The reduction in CH_4 flux from biochar-amended soils is often linked to the increase in soil aeration and porosity particularly in anoxic conditions resulting in the oxidation of CH_4 (Van Zwieten et al., 2009; Jeffery et al., 2016). However, in this experiment, both forest and grassland soils were kept at aerobic conditions (60% WHC) during the entire incubation period and biochar amendment did not significantly affect soil aeration or CH_4 flux in these soils.

The BC550 and BC550-S showed their highest potential to reduce N_2O emission from the forest as well as grassland soils. Relative to the effects of the studied biochars on CO_2 emission, the BC550 and BC550-S biochars reduced N_2O emission from both soils and the effect persisted until the end of the incubation, indicating that these biochars were more effective in reducing N_2O than CO_2 emission. Since N_2O is a more potent GHG than CO_2, this indicates that to properly evaluate the effectiveness of a biochar in mitigating climate change we need to study all three trace GHGs as otherwise we could be making an incorrect conclusion. The reduction of N_2O emission by BC550 and BC550-S could be attributed either to abiotic mechanisms such as the sorption capacity of the biochar or biotic alteration of nitrifying and denitrifying bacterial activities in the soil (Clough and Cordron, 2010; Cayuela et al., 2013).

Sorption of NH_4^+-N and NO_3^--N on biochar surface could be a possible mechanism for the reduction of soil N_2O production in biochar-amended soils (Laird, 2008). Spokas et al. (2012) and Jassal et al. (2015) suggested that physical entrapment of NH_4^+-N in biochar pores is mainly responsible for the adsorption of NH_4^+-N when CEC of biochar is relatively low as observed in BC550 and BC550-S biochars (6.05 and 6.74 cmol kg^{-1}, respectively) vs relatively high CEC in BC300 and BC300-S (29.04 and 32.02 cmol kg^{-1}, respectively; Table 2). In pine saw dust biochar, the surface area was increased from < 1 m^2 g^{-1} to 293 m^2 g^{-1} (values obtained from average of steam and non-steam-activated biochar) when the pyrolysis temperature was increased from 300 °C to 550 °C (Lou et al., 2016a). The increase in surface area could potentially enhance the physical adsorption of NH_4^+-N in biochar pores. In incubation experiments if available N in the soil exceeds microbial demand with no possibility of N uptake by plants, that excess N (either in the NH_4^+ or NH_3 form) could be bound with biochar and thus effectively reduce the inorganic-N pool available for subsequent nitrification and denitrification induced losses of N_2O (Clough and Cordon, 2010).

Under biological mechanism, emissions of N_2O from soils are primarily driven by nitrification and denitrification process that could be affected by the properties of biochar associated with pyrolysis condition. Yanai et al. (2007) reported that the reduction of N_2O emission was linked to the reduced denitrification rate due to increase in soil aeration by biochar amendment. The positive correlation between N_2O emission and NO_3^--N concentration in the soil by biochar addition suggested that the biochar might reduce NO_3^--N availability which would indeed decrease the total N denitrified (Cayuela et al., 2013). The greater ash content and higher pH of BC550 and BC550-S (Table 2) relative to BC300 and BC300-S could have played a significant role in reducing N_2O emission from the soil (Cayuela et al., 2013). The liming effects of ash content and higher pH of BC550 and BC550-S had the potential to increase N_2O reducing activity of denitrifying bacteria that shifts the main microbial source of N_2O from NH_4^+ oxidation to denitrification (Yanai et al., 2007; Baggs et al., 2010; Wu et al., 2013).

The decrease in GWP of the emissions by amending pine sawdust biochar such as BC550 in the forest soil supports earlier findings that biochar application can mitigate global warming by reducing GHG emission from the soil. The application of pine sawdust biochar was not effective in mitigating global warming from the grassland soil because of the lack of significant effect of biochar amendment on CO_2 emission despite significant reduction in N_2O emission. This is because the contribution of N_2O to the total GWP of the emissions from the soil was negligible relative to CO_2 due to much lower N_2O emission and CH_4 uptake as compared to CO_2 emission from the soil. The higher rate of biochar application in the grassland soil could result in a significant reduction in CO_2 emission that would lead to significant effect on GWP of the emissions from the biochar-amended grassland soil (Spokas et al., 2009).

The effects of BC550 and BC550-S on enzyme activities in soils in this experiment were similar to the findings in Ouyang et al. (2014). The greater enzyme activities in the soil amended with BC300 relative to BC550 could be attributed to the greater amount of labile biochar-C present in BC300 (Awad et al., 2012; Masto et al., 2013). Pine saw dust biochar pyrolyzed at low temperature (300 °C) had more aliphatic C-H bonds (Lou et al., 2016a) which could be degraded more easily than the biochar produced at high temperature (550 °C) with less aliphatic C-H bonds. In contrast, biochars pyrolyzed at 550 °C had more aromatic C-C bonds as demonstrated by FT-IR spectra (Lou et al., 2016a). These biochars had a lower amount of degradable matter with higher chemical recalcitrance and stability (Bruun et al., 2011). The addition of labile C

retained in the biochar pyrolyzed at low temperature can be a good source for microorganisms resulting in an increase in microbial activities. The decreased enzyme activities with biochar produced at high temperature may be due to sorption or blocking of either enzyme or substrate (Bailey et al., 2011). The biochar produced at high temperature had greater surface area and porosity (Lou et al., 2016a) to adsorb more substrate that could potentially inhibit enzymatic activities by reducing the availability of highly soluble substrates for soil enzymes (Lammirato et al., 2011).

The treatment effect of sawdust biochar amendment on enzyme activities and microbial biomass did not last until the end of the incubation. The difference of β-1,4-glucosidase activity in the forest soil and β-1,4-N-acetyl glucosaminidase in grassland soil between low and high temperature biochar treatment was diminished after 10 days of incubation. The activity of these enzymes, indicative of microbial activities was highly affected by pyrolysis temperature immediately after biochar amendment. After the addition of biochar, either SOM or the added labile organic matter present in low temperature biochar provides substrates for increased enzyme activity. Bailey et al. (2011) observed an increase in β-1,4-glucosidase and β-1,4-N-acetyl glucosaminidase enzyme activities for a period of 7 days after the addition of 2% (mass basis) low temperature biochar having a 40% labile organic fraction but decreased considerably after 7 days. Once the labile portions of biochar are metabolized and removed, biochars provide no energy for the soil microbes to have long term stimulation of enzyme activity in the soil (Cheng et al., 2008).

The microbial population size in the soil was influenced by BC550 and BC550-S as demonstrated by the decrease in microbial biomass C and N observed at 10- and 50-day of incubation relative to the control in forest soil. The large effect of BC550-S (at 10- and 50-day of incubation) in decreasing soil MBC and MBN may also be linked to the higher recalcitrant C content in the activated biochar produced under high pyrolysis temperature (Azargohar and Dalai, 2006). Steam activation removes the volatile matters from the biochar surface (Azargohar and Dalai, 2006) that reduces the availability of substrates for microbial activities (Zhang et al., 2015). The strong relationship between CO_2 and N_2O emission from the soil and enzyme 1activities as well as microbial biomass suggests that the reduced enzyme activities greatly contributed to the suppression of C and N mineralization in the BC550 and BC550-S amended soils.

5. Conclusions

We conclude that BC550 application was effective in reducing cumulative CO_2 emission and GWP of the emissions from the forest soil and BC550 and BC550-S applications were effective in reducing cumulative N_2O emission from the forest and grassland soils by decreasing extracellular enzyme activities and microbial biomass in these soils under laboratory condition. The greater surface area, porosity, increased aromaticity and ash content of BC500 and BC550-S than in the other biochars made them effective in reducing GHG emissions. The persistent effect of BC500 and BC550-S in reducing N_2O emission from the soils in a 100-day incubation experiment can be an indicative of long-term effect on mitigating N_2O emission from the real ecosystems. The BC550 and BC550-S showed the highest potential of mitigating GHG emission under laboratory condition; they should be tested in long-term field trials before being used in ecosystem management strategies aiming at GHG emission mitigation.

Table 2-1. Selected physical and chemical properties of soils.

Land use	Soil classification		Texture	pH	TC	TN	C/N	NH_4^+-N	NO_3^--N	BD
	Canadian system	FAO WRB system			(g kg^{-1})	(g kg^{-1})		(mg kg^{-1})	(mg kg^{-1})	(g cm^{-3})
Forest	Orthic Gray Luvisol	Albic Luvisol	Silty loam	5.7 (0.2)	21.3 (3.1)	2.2 (0.2)	9.4 (0.7)	2.85 (0.31)	0.34 (0.04)	1.42 (0.27)
Grassland	Orthic Black Chernozem	Calcic Chernozem	Clay loam	6.3 (0.2)	97.2 (11.9)	9.0 (1.9)	10.5 (0.4)	0.81 (0.29)	0.78 (0.06)	1.15 (0.20)

Values are means with standard errors in the parentheses (n = 4).

Abbreviations: TC = total carbon, TN = Total nitrogen, C/N = carbon to nitrogen ratio, NH_4^+-N = ammonium, NO_3^--N = nitrate, and BD = bulk density.

Table 2-2. Properties of biochars produced under different pyrolysis conditions (at 300 and 550 °C with and without steam activation, 'S' represents steam activation of biochar).

Biochar	pH	Resident matter (%)	Ash	Surface area (m^2 g^{-1})	CEC	Molar H/C	Molar O/C	Molar (O+N)/C
BC300	4.92	39.31	2.70	<1	29.04	0.78	0.32	0.33
BC300-S	4.82	38.36	3.92	<1	32.02	0.71	0.29	0.29
BC550	8.16	62.61	6.39	189.2	6.05	0.35	0.10	0.10
BC550-S	7.46	71.64	5.37	397.1	6.74	0.36	0.10	0.10

Data including pH, resident matter, ash, surface area, molar H/C, molar O/C and molar (O+N)/C obtained from Lou et al. (2016a) and CEC (cation exchange capacity) from Yang et al. (2017).

Table 2-3. Pearson correlation coefficient and significance among soil variables in forest and grassland soils (n = 20).

Variable	MBC	MBN	GLU	NAGase	NH_4^+-N	NO_3^--N	CO_2
Forest soil							
MBN	0.69**						
GLU	0.65**	0.65**					
NAGase	0.29	0.16	0.49*				
NH_4^+-N	-0.11	-0.1	0.03	0.04			
NO_3^--N	-0.26	-0.01	-0.05	-0.36	-0.26		
CO_2	0.49*	0.49*	0.43	0.19	0.01	0.27	
N_2O	0.44	0.53*	0.61*	0.64*	-0.04	0.51*	0.11
Grassland soil							
MBN	0.33						
GLU	0.56*	0.23					
NAGase	0.63*	0.01	0.49*				
NH_4^+-N	0.2	0.04	0.28	0.71**			
NO_3^--N	-0.31	0.15	-0.38	0.58*	-0.21		
CO_2	-0.2	-0.13	0.37	0.49*	-0.25	0.25	
N_2O	0.62*	-0.26	0.31	0.71*	0.31	0.72*	-0.37

Variables: MBC = microbial biomass C, MBN = microbial biomass N, GLU = β-1, 4-glucosidase, NAGase = β-1, 4-N-acetylglucosaminidase, NH_4^+-N = ammonium and NO_3^--N = nitrate. * = $P < 0.05$, ** = $P < 0.01$.

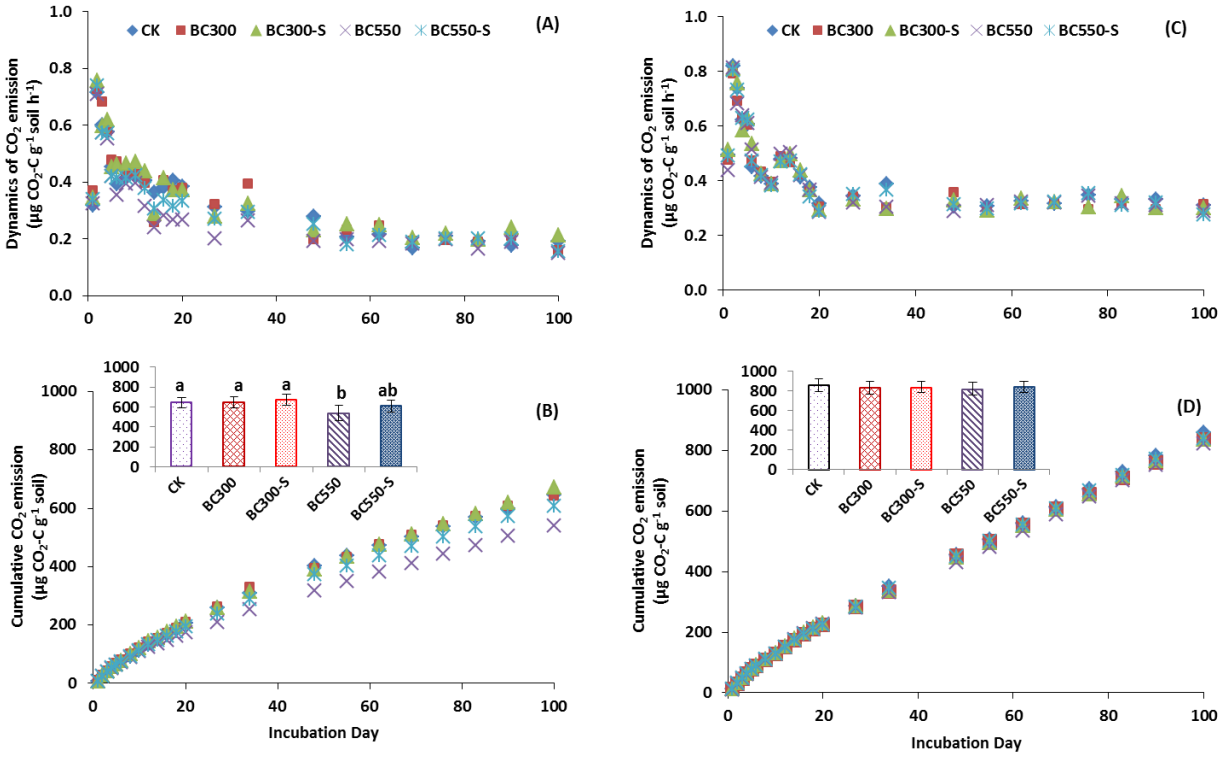

Fig. 2-1. The effects of biochar amendments on CO_2 emission from the forest and grassland soils in a 100-day incubation. (A) the dynamics of CO_2 emission from the forest soil, (B) the cumulative CO_2 emission from the forest soil, (C) the dynamics of CO_2 emission from the grassland soil and (D) the cumulative CO_2 emission from the grassland soil. The inserts are the cumulative CO_2 emission at the end of 100-day incubation (mean ± SE, n = 4). Treatment codes are CK: soil only (control), BC300: soil+ biochar produced at 300 °C without steam activation, BC300-S: soil + biochar produced at 300 °C with steam activation, BC550: soil + biochar produced at 550 °C without steam activation and BC550-S: soil+ biochar produced at 550 °C with steam activation.

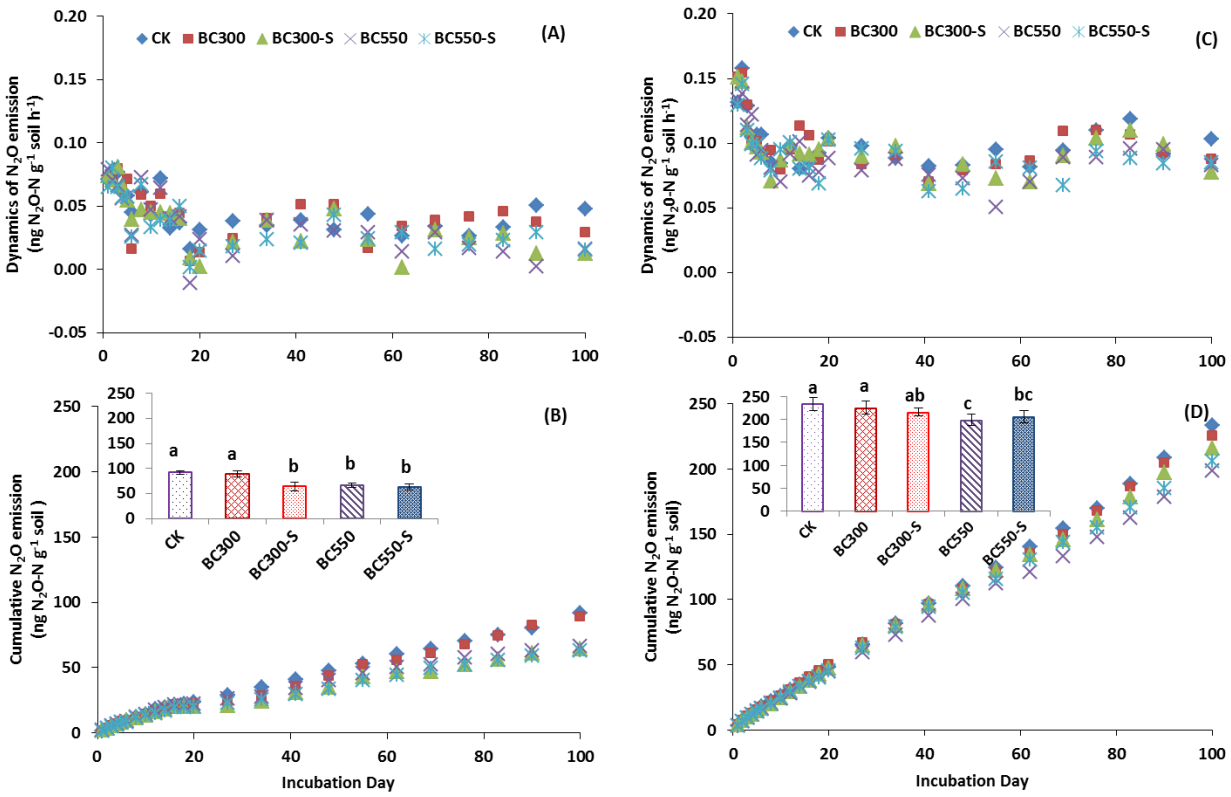

Fig. 2-2. The effects of biochar amendments on N_2O emission from the forest and grassland soils in a 100-day incubation. (A) the dynamics of N_2O emission from the forest soil, (B) the cumulative N_2O emission from the forest soil, (C) the dynamics of N_2O emission from the grassland soil and (D) the cumulative N_2O emission from the grassland soil. The inserts are the cumulative N_2O emission at the end of 100-day incubation (mean ± SE, n = 4). Treatment codes are the same as in Fig.2.1.

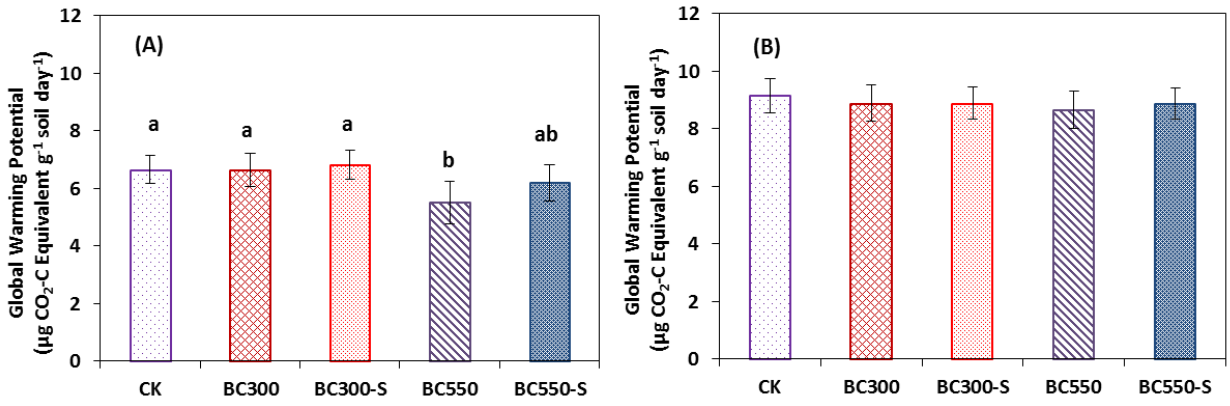

Fig. 2-3. The effects of biochar amendments on the global warming potential of greenhouse gas emissions from (A) the forest soil and (B) the grassland soil. Treatment codes are the same as in Fig. 2.1.

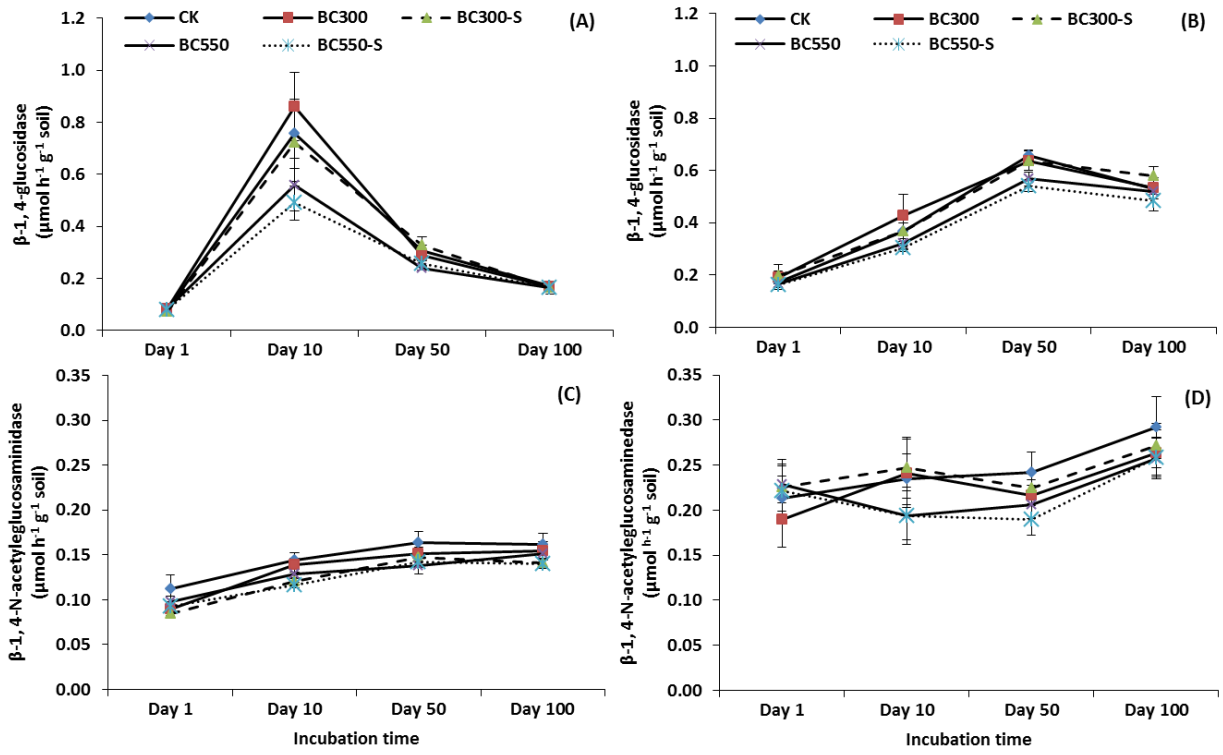

Fig. 2-4. The effects of biochar amendment on extracellular enzyme activities (mean ± SE, n = 4) in the forest and grassland soil; β-1,4-glucosidase in (A) the forest and (B) the grassland soil, and β-1,4-N-acetyl glucosaminidase in (C) the forest and (D) the grassland soil. Treatment codes are same as in Fig. 2.1

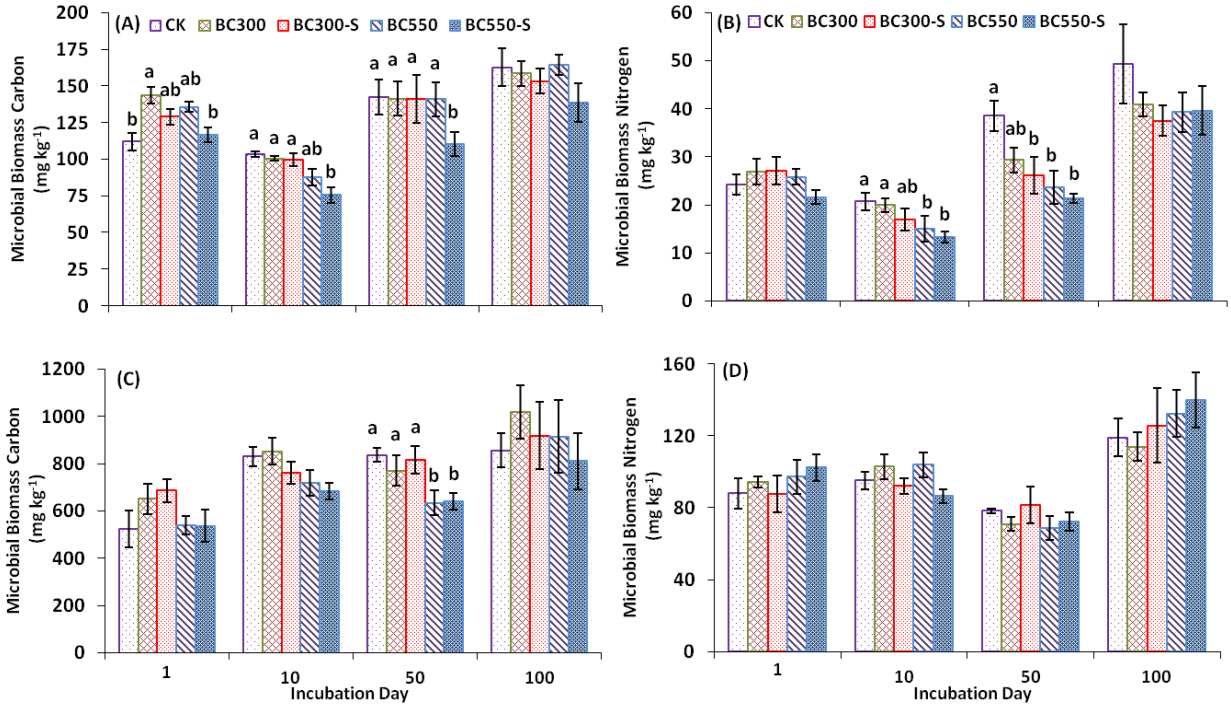

Fig. 2-5. The effects of biochar amendment on microbial biomass carbon and nitrogen (mean ± SE, n = 4) in (A) and (B) the forest soil, and (C) and (D) the grassland soil. Treatment codes are the same as in Fig. 2.1.

Chapter 3. Manure pellet, woodchip and their biochars differently affect wheat yield and carbon dioxide emission from bulk and rhizosphere soils

1. Introduction

Agricultural lands store more than 10% of global soil organic carbon (SOC) and have a large carbon (C) sequestration potential (Smith, 2004; Amundson et al., 2015) but the SOC pool of cropland is being depleted rapidly in recent years because of inappropriate land use and management practices such as tillage operations, residue removal and excessive use of fertilizers and pesticides (Lal, 2011). Increasing the SOC pool in cropland soils is a good strategy to improve soil fertility, to support the resilience of agroecosystems, and to mitigate climate change (Smith et al., 2008; Lal, 2010; Sohi, 2012). The size of the SOC pool can be increased by increasing organic matter input (e.g., application of organic residues or retention of crop residues on site) or by reducing soil SOC decomposition (e.g., decreasing heterotrophic respiration) (Paustian et al., 2016). Application of organic residues could help maintain the levels of SOC in croplands (Badia et al., 2013); however, it may also increase greenhouse gas (GHG) emission (Thangarajan et al., 2013) that can cause global warming, and temporary immobilization of soil nutrients during residue decomposition, which can decrease crop productivity (Prochazkova et al., 2003).

Biochar, a product of pyrolysis of organic residues under low oxygen availability, has been used in croplands to increase crop production and SOC storage, as well as to suppress carbon dioxide (CO_2) efflux from the soil. However, biochar application has been reported to have positive, negative and neutral effects on soil CO_2 emission (Cross and Sohi, 2011; Singh et al., 2012; Pokharel et al., 2017). The discrepancies in the CO_2 flux from biochar-amended soils have been shown to be linked to the physical and chemical properties of biochar (mainly determined by the feedstock type and pyrolysis condition used) and that of the soil (Wang et al., 2016). Biochar, being recalcitrant in nature, has been found to affect heterotrophic respiration by reducing SOC decomposition in the long term (Lehmann, 2007). The negative priming effect of biochar on SOC decomposition also decreases the rate of turnover of both existing SOC and rhizodeposits (Keith et al., 2015). The application of organic residues and their pyrolyzed products (biochars) on soil CO_2 efflux has been widely studied (Wu et al., 2013); however, we

lack comparative experimental data on the effects of the application of unpyrolyzed organic residues and their biochars on cropland soil CO_2 efflux.

Carbon dioxide efflux from cropland soils comes from two major sources: i) autotrophic respiration by roots, and ii) heterotrophic respiration by microbial decomposition of existing and root-derived SOC (Subke et al., 2006). The SOC decomposition in the soil in the vicinity of living roots (the rhizosphere) is significantly greater than that in the bulk soil (Kuzyakov, 2002). Soil respiration from the bulk soil represents the CO_2 emission from heterotrophic decomposition of SOC while soil respiration from the rhizosphere accounts for the sum of root respiration, and the heterotrophic decomposition of existing and root-derived SOC.

The rhizosphere is one of the most active and dynamic hotspots of microbial decomposition of SOC in the world (Kuzyakov and Domanski, 2000; Philippot et al., 2013). The properties of the soil in the rhizosphere are modified by a range of processes caused by the release of root exudates including various organic and inorganic C (Farrar et al., 2003; Hinsinger et al., 2006). About 15 to 27% of belowground allocated C in plants is exuded from the roots which would amount to 400-600 kg C ha^{-1} for grasses and cereals in a growing season (Jones et al., 2009). These exuded organic compounds can induce fast C turnover in the rhizosphere, which is called the priming effect (Jones et al., 2009). Microorganisms in the rhizosphere utilize these substances as easily available C and energy sources for fast growth and reproduction leading to greater CO_2 effluxes than that in the bulk soil. The SOC decomposition rate can be stimulated by up to three folds in soils incubation with roots as compared to those without roots (Zhu and Cheng, 2011). Therefore, the heterotrophic decomposition of root-derived C in the rhizosphere is an important contributor of total CO_2 efflux in the cropland.

Management practices aiming to increase the SOC pool in cropland should also consider reducing heterotrophic decomposition of root-derived SOC. Since biochar has the potential to reduce heterotrophic mineralization of native SOC to CO_2 (Lehmann, 2007), it may also reduce root-derived SOC mineralization leading to an increase in SOC pool as well as a decrease in total soil respiration in the rhizosphere (Cheng et al., 2017). Most studies dealing with biochar amendment to reduce GHG emission and to enhance C sequestration in soil are focused in bulk soil; the study of biochar-rhizosphere interaction has largely been ignored in the past (Weng et al., 2015), limiting our ability to have a mechanistic understanding of the mechanisms involved in biochar effects on soil respiration. A few studies that dealt with biochar-rhizosphere

interaction have shown significant effects of biochar amendment on C and nitrogen (N) mineralization in the rhizosphere. For instance, a recent study by Weng et al. (2017) showed that biochar amendment in a planted Ferralsol decreased SOC degradation by sorption of root exudates on biochar surfaces and enhanced organo-mineral protection. However, the effect of biochar amendment on total soil respiration and microbial activities in the rhizosphere has not been studied.

In this study, we conducted a rhizobox experiment in a greenhouse with two unpyrolyzed organic residues (manure pellet and willow woodchip) and their pyrolyzed products (biochars). These two organic residues are easily available waste materials in Alberta, Canada and biochar production can be a viable option for waste management. In addition, the two feedstocks and the biochars made from these feedstocks (wood-based vs manure-based) had substantially different properties such as pH, C/N ratio, surface area and recalcitrance (Zhao et al., 2013; Jeffery et al., 2017) that can significantly impact on crop yield and soil respiration. Our objectives were: (1) to examine the effect of soil amendment (unpyrolyzed organic residues and their biochars) on wheat biomass and grain yield; (2) to evaluate the effect of these soil amendments on soil respiration in rhizosphere and bulk soils; and (3) to investigate the changes in microbial biomass and dissolved organic C and N caused by the application of unpyrolyzed organic residues and their biochars to the soil. Results from this research will provide data for decision making between the use of raw or unpyrolyzed biomass and the use of biochar derived from such biomass and offer a more mechanistic understanding of the mechanisms involved in the effect of unpyrolyzed and pyrolyzed biomass applications on soil processes, particularly soil GHG emissions.

2. Material and methods

2.1. Soil and amendments

The soil was collected from the 0-10 cm mineral layer in an agricultural field near Leduc (53°11' 33" N, 113°59' 18"), in Alberta, Canada. The soil is classified as an Orthic Black Chernozem (Calcic Chernozem in FAO-WRB system of classification) and has a clay loam texture. Roots were removed from the soil after it was air dried. The soil has the following properties: pH 5.12 (1:5 $CaCl_2$), total C (TC) 32.16 g kg^{-1}, total N (TN) 3.4 g kg^{-1}, C/N 9.42, available NH_4^+-N 2.55

mg kg^{-1}, NO_3^--N 26.14 mg kg^{-1}, PO_4^{3-}-P 25.28 mg kg^{-1}, K 217.53 mg kg^{-1}, extractable S 0.04%, Mg 0.26%, Ca 0.32% and Al 12.3 µg g^{-1} (see below for the methods for soil analysis). The air-dried soil was sieved using a 2-mm sieve and used for the greenhouse experiment.

Unpyrolyzed manure pellet and willow woodchip and their biochars were used as a soil amendment in this experiment. The manure pellets were obtained from EarthRenew Corporation, Calgary, AB, Canada and the willow woodchips from InnoTech Alberta, Vegreville, AB, Canada. Manure pellets and woodchips were pyrolyzed at InnoTech Alberta. The woodchip biochar was produced in an auger retort carbonizer (retort system, InnoTech Alberta) and the manure pellet biochar was produced in a batch drum carbonizer (InnoTech Alberta-designed carbonizer). The pyrolysis temperature ranged between 500 and 550 °C for both biochars with a heating rate of 85-100 °C min^{-1} for woodchip biochar and 9-10 °C min^{-1} for manure pellet biochar with a dwell time at the maximum temperature of 90 and 45 minutes for woodchip and manure pellet biochar, respectively. The distribution particle size in the < 2, 2-4 and < 4 mm fractions was 14.8, 65.9 and 19.3% (by weight), respectively, for unpyrolyzed manure pellet, 15.8, 71.7 and 12.5%, respectively, for manure pellet biochar, 7.3, 68.4 and 24.3%, respectively, for unpyrolyzed woodchip, and 18.6, 70.5 and 11.9%, respectively, for woodchip biochar. Selected properties of the unpyrolyzed manure pellet, woodchip and their biochar samples are given in Table 3-1 (see below for the methods of analyses). The scanning electron micrographs (SEMs) of biochars obtained by using scanning electron microscopy (SEM, Zeiss EVO MA10, Jena, Germany) are given in Appendix 3-1.

2.2. Experimental design

The experiment used a split-plot design with soil amendment treatment as the whole-plot factor and root zone (rhizosphere vs bulk soil) as the sub-plot factor. Soil amendment treatments were: control (no addition of any amendment (CK)), addition of unpyrolyzed manure pellet (MP), addition of manure pellet biochar (MB), addition of unpyrolyzed willow woodchip (WW) and addition of woodchip biochar (WB). The treatments were replicated four times.

The experiment was performed in a greenhouse using rhizoboxes. The rhizobox technique has been widely used to compare the soil processes between bulk and rhizosphere soils (Youssef and Chino, 1988; Fang et al., 2013). The rhizobox was constructed by polyvinyl chloride (PVC) boards with the following dimensions: height 12 cm, length 20 cm and breadth

15 cm. The rhizobox was divided into bulk and rhizosphere soil compartments by a nylon net (300 meshes) which prevented roots from entering into the bulk soil but allowed the movement of water and nutrients through. A thin layer of soil buffer zone (1.5 cm wide, separated by a nylon net) was kept in between the rhizosphere and bulk soil compartments to avoid the edge effect of rhizosphere zone into the bulk soil region. Unpyrolyzed manure pellet and woodchip, and their biochars were mixed with air-dried soil at the rate of 5% (w/w) which is equivalent to 57 t oven-dried biomass ha^{-1} incorporated in the 0-10 cm surface soil. This rate was chosen because Spokas et al. (2009) observed significant increase in sorption of added organic C when the soil was amended with biochar at 5% (w/w). This rate lies in the upper end of biochar application rates (20 to 60 t ha^{-1}) recommended for agricultural application (Baronti et al., 2010), even higher rates have been used in many studies such as 90 t ha^{-1} (Zimmerman et al., 2011) and 100 t ha^{-1} (Jones et al., 2011) in lab incubation experiments, and 180 t ha^{-1} (Zavalloni et al., 2011) in pot experiment to allow the detection of appreciable changes in the parameters examined (Zavalloni et al., 2011).

The rhizoboxes were filled with the soil-amendment mix to 10 cm height with a bulk density (1.15 g cm^{-3}) similar to that in the agricultural land from where the soil used for the experiment was collected. The water holding capacity (WHC) of the mixture was determined separately for unpyrolyzed manure pellet and woodchip and their biochars prior to the experiment. The moisture content of the mixture in the rhizobox was maintained at 40% WHC for 5 days to stabilize the microbial populations in the soil. In each rhizosphere compartment, 14 seeds (7 seeds in each of two rows) of spring wheat (*Triticum aestivum* L. Var GP168) were sown in each rhizobox on the 6^{th} day of the experiment. The WHC of the soil was brought to 50% after the seeds were sown, the WHC was maintained throughout the experiment by adding deionized water at three-day intervals until the wheat seedlings were 7 days old, then daily until the wheat was harvested. The amount of water to be added to each rhizobox in the experiment was determined by using reference rhizoboxes (prepared separately for each treatment) with wheat grown in identical conditions to that of the experiment in which WHC was measured by time domain reflectometry (TDR) probes (Model Theta Probe ML2X, Delta-T Devices, Cambridge, UK).

Five days after seed germination, 2 seedlings from each of the two rows were removed; the remaining 5 seedlings in each of two rows in each rhizobox were grown until the plants were

mature for harvesting. Rhizoboxes were kept in a standard greenhouse (Biological Science Building, University of Alberta, AB, Canada) condition where the temperature was maintained between 23 and 25 °C, relative humidity was maintained between 65 and 85% and the photoperiod was 16 h with light intensity at 400 µmol m^{-2} s^{-1} provided by sodium vapor lamps. A commercial water-soluble fertilizer (Plant-Prod 20-8-20 plus micronutrients, Master Plant-Prod Inc., Ontario, Canada) was applied to the soil at the rate of 90 kg N (nitrate and ammoniacal N) ha^{-1}, 36 kg P ha^{-1} and 90 kg K ha^{-1}. The readily available N (nitrate and ammoniacal N) added to the soil by the soil amendment was 9.2 kg ha^{-1}, 0.6 kg ha^{-1}, 1.1 kg ha^{-1} and 0.2 kg ha^{-1} for manure pellet, manure pellet biochar, woodchip and woodchip biochar treatments, respectively.

2.3. Analysis of soil and amendment properties

Soil pH was measured in a $CaCl_2$ solution (0.01 mol L^{-1}) at a 1:5 (m:v) ratio using a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA). Available N (NH_4^+-N and NO_3^--N) was analyzed colorimetrically in a 2 mol L^{-1} KCl extract following the Indophenol blue method for NH_4^+-N and the Vanadium oxidation method for NO_3^--N. For the analyses of available P and K, soils were extracted by the modified Kelowna extraction method and analyzed by colorimetric method. The total C (TC) and N (TN) concentrations of soil were determined in ball-milled samples using an elemental analyzer (Elementar Vario Micro Cube, Elementar Enalysensysteme GmbH, 63452 Hanau, Germany). The pH, available N, TC and TN concentrations of unpyrolyzed organic residues and their biochars were determined in the same way as that for the soil. Particle size distribution (by weight) in unpyrolyzed organic residues and their biochars was determined on oven-dried biomass using 2 and 4-mm sieves. The cation exchange capacity was determined by a Salicylate-Hypoclorite method (Bower and Holm-Hansen, 1980). Surface area was determined by N isotherms at 77K using a gas adsorption analyzer (Quantachrome Autosorb 1 MP, Quantachrome, Boynton Beach, FL, USA).

2.4. Measurement and calculation of carbon dioxide fluxes

The rhizosphere-derived CO_2 (sum of root respiration and rhizo-microbial respiration) and bulk soil CO_2 fluxes (basal respiration) were measured in rhizosphere and bulk soil compartments, respectively, using static (plexiglass) chambers (3 × 18 × 8 cm), inserted two cm into the soil. The chambers were lined with reflective aluminum foil to maintain the ambient air temperature

in the chamber headspace during measurement. In the rhizosphere compartment, the chambers were inserted into the soil in between two rows of wheat seedlings. During gas sampling, the chamber was covered tightly with a lid (lined with reflective aluminum foil) fitted with a butyl rubber septum for gas sampling. In each sampling, gas samples were collected at 0, 10, 20, 30 minutes after closing the chamber using a 5-mL syringe and injected into pre-evacuated 3 mL glass vials (exetainers) fitted with a butyl rubber septum (Labco Ltd., Lampeter, Wales, UK). The concentration of CO_2 in the gas samples was determined with a gas chromatograph (Varian CP-3800 GC, Varian Inc., USA). The CO_2 efflux in rhizosphere and bulk soils was measured from the day of seed sowing (from the 6th day of experiment) to the day before wheat harvesting (wheat was harvested after 90 days of sowing the seed). Gas samples were collected from the chamber daily for the first eight days, then once every four days until day 52 of seed sowing and after that once every seven days until the wheat was harvested. In each sampling, gas samples were collected between 11:00 and 15:00 h of the day to minimize diurnal variations in CO_2 emission. The rate of CO_2 emission was calculated using the modified ideal gas law equation (Collier et al., 2014):

$$\text{Efflux} = \frac{S \times P \times V}{R \times T \times A} = \frac{S \times P \times h}{R \times T} \quad (1)$$

Where efflux is CO_2 flux rate (μmol m^{-2} s^{-1}), S is the slope obtained from the regression analysis of CO_2 concentration measured at 0, 10, 20 and 30 minutes after closing the chamber (μL L^{-1} s^{-1}); P is the pressure of the gas (Pa), R is the gas constant (m^3 Pa K^{-1} mol^{-1}); V, A and h are the volume (m^3), surface area covered (m^2) and height (m) of the gas chamber, respectively; and T is the air temperature (K) measured at the time of gas sampling. The cumulative CO_2 emission was calculated for 87 days (the period from seed sowing to plant harvest). The relativized cumulative CO_2 emission (g CO_2-C kg^{-1} C m^{-2}) for each treatment was calculated as:

Relativized cumulative CO_2 emission = Cumulative CO_2 emission/total C in amended soil (2)

Where total carbon in amended soil was the sum of total C content of the soil and C content added by the soil amendment.

The autotrophic respiration (R_a) in rhizosphere soil was calculated as:

R_a = total respiration in rhizosphere soil – total respiration in bulk soil (3)

2.5. Plant and soil sampling and analyses

Wheat plants were harvested after maturity at 90 days after seed sowing. Harvesting involved the cutting of the plant at the base of the stem, dismantling the rhizobox, separating bulk and rhizosphere soils and removing the plant roots from the rhizosphere compartment. The aboveground plant was separated into spikes, stem and leaves. Wheat biomass was measured after the components of the plants were oven-dried at 60 °C for 48 hours. For the analysis of microbial biomass, water soluble organic C and N, pH and available N in the rhizosphere compartment, soil samples were collected by separating the soil from roots by gentle shaking (the most common method for collecting rhizosphere soils) and used those soils to represent the rhizosphere soil (Fang et al., 2013; Philippot et al., 2013). Even though we consider the rhizosphere soil we collected using this method represents the bulk soil in the rhizosphere compartment, the reader is cautioned that there is a potential bias as the soil respiration measurement from the rhizosphere compartment and the rhizosphere soil collected above were not entirely the same. The rhizosphere soil collected above may contain small roots broken off from the main root system when it was collected; we thus also caution the reader that the root tips contained in the rhizosphere soil may cause an artifact in the measurement of the properties of the rhizosphere soil. Therefore, comparisons with the literature should be only based on studies that used a similar method for rhizosphere soil collection. After careful separation and collection of the rhizosphere soil, the roots were washed with deionized water and oven-dried for biomass measurement. Sub-samples of rhizosphere and bulk soils were stored in plastic bags at -20 °C for microbial analysis. The remaining soil samples were air-dried for other analyses.

Soil microbial biomass C (MBC) and N (MBN) in rhizosphere and bulk soils were analyzed by the chloroform fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987). For fumigation, 20 g of moist soil sample was fumigated in chloroform in a desiccator for 24 hours. Soil extracts were obtained by mixing 20 g of moist soil with 50 mL of 0.5 mol L^{-1} K_2SO_4 solution, shaking for 1 hour in a reciprocating shaker (250 rpm) and filtering through Whatman no. 42 filter papers. The soil extractions were analyzed for extractable C and N by a TOC-V analyzer connected to a TN module (Shimatzu Corporation, Kyoto, Japan). Dissolved organic C (DOC) was the extracted C in non-fumigated soil samples and dissolved organic N (DON) was calculated by the difference between extractable N and available N (sum of NH_4^+-N

and NO_3^--N) of non-fumigated samples. Microbial biomass C and N were calculated by the differences between the C extracted from fumigated and non-fumigated soil samples and N extracted from fumigated and non-fumigated soils samples, respectively (after dividing by a K_{EC} factor of 0.45). The pH, available N, TC and TN were measured in the air-dried soil samples (by the methods described above).

2.6. Statistical analysis

The experiment used a split-plot design to assess the effect of soil amendment treatment (whole-plot factor) and root zone (sub-plot factor) on soil processes. All statistical analyses were performed using the PROC MIXED Procedure in SAS (version 9.4, SAS Institute Inc., NC, USA). Data were checked for normality of distribution prior to analysis of variance (ANOVA), the cumulative CO_2 emission data were log (10) transformed prior to performing analysis of variance (ANOVA) since the residuals of the data were not normal but the back-transformed data were presented in the results section. One-way ANOVAs were used to test the significance of the effect of amendment treatment on wheat biomass production and two-way ANOVAs were used to test the effect of soil amendment and root zone treatments on other parameters. The means of the variables were compared using LSD test with an $\alpha < 0.05$ in all analyses. The linear model used for the split-plot design was:

$$\boldsymbol{Y}_{ijk} = \mu + \alpha_i + \gamma_k + \eta_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where $\boldsymbol{Y}_{ijk}$ is a dependent variable, μ is the overall mean, α_i is the fixed effect of i^{th} soil amendment, γ_k is the random effect of k^{th} block, η_{ik} is the random effect of whole-plot error, β_j is the fixed effect of j^{th} root zone, $(\alpha\beta)_{ij}$ is the fixed effect of interaction between soil amendment and root zone and ε_{ijk} is the random effect of split-plot error.

3. Results

3.1 Effects of amendments on wheat growth

Wheat biomass production was affected by soil amendment treatment ($P < 0.05$). The manure pellet and woodchip biochars had contrasting effects on biomass production (Fig. 3-1), with the former increased grain yield by 16.1% and total biomass by 8.5% while the latter decreased grain yield and total biomass by 18.2 and 26.7%, respectively. In contrast, the former did not affect

root biomass production but the latter reduced root biomass by 32.2%, as compared to the control. The highest grain yield ($P = 0.01$) and total biomass production ($P = 0.01$) was in the unpyrolyzed manure pellet treatment with 36.1 % greater than the control, and the lowest root and total biomass, and grain yield was with the unpyrolyzed woodchip treatment (all $P < 0.01$).

3.2. Effect of amendment on soil pH, dissolved organic C and N

Unpyrolyzed manure pellet, its biochar and woodchip biochar increased pH in both rhizosphere and bulk soils (both $P < 0.01$; Tables 3-2 and Appendix 3-2). Rhizosphere soil had a greater pH and DOC and DON than that in the bulk soil in all soil amendment treatments. Soil amendment with both unpyrolyzed manure pellet and woodchip significantly increased DOC and DON than that of their respective biochar and control treatments. However, the ratio of DOC to DON was not different between the rhizosphere and bulk soils ($P > 0.05$) but was greater in both manure pellet and woodchip biochar-amended soils than in the control and their unpyrolyzed biomass-amended soils in both rhizosphere and bulk soils (Tables 3-2 and Appendix 3-2).

3.3 Effect of amendment on CO_2 emission from rhizosphere and bulk soils

Unpyrolyzed manure pellet increased cumulative CO_2 emission in the entire growing period of wheat from 395.3 to 735.4 g CO_2-C m^{-2} from the rhizosphere soil and from 228.6 to 555.0 g CO_2-C m^{-2} from the bulk soil as compared to the control (Fig. 3-2; Table S1). Unpyrolyzed woodchip also increased cumulative CO_2 emission by 126.5 and 248.1% in rhizosphere and bulk soils, respectively (Fig. 3-3; Table S1). Both manure pellet and woodchip biochars significantly reduced cumulative CO_2 emission from the rhizosphere soil as compared to the control. Manure pellet biochar reduced the cumulative CO_2 emission by 24.6% while woodchip biochar reduced it by 29.7% as compared to the control in the rhizosphere soil while both biochars didn't affect CO_2 emission from the bulk soil. Cumulative CO_2 emission was greater ($P < 0.01$) in rhizosphere than in the bulk soil in all soil amendment treatments (Tables 2-2 and Appendix 3-2).

Relativized CO_2 emission was affected by soil amendment and differed between root zones (both $P < 0.01$). Relativized CO_2 emission was greater in rhizosphere than that in bulk soil (Table 2-2 and Appendix 3-2). Relativized CO_2 emission was increased by 97.1% by manure pellet application and was decreased by 25.4% by manure pellet biochar application as compared

to the control in bulk soil while it was increased by 51.1% and decreased by 34.5%, respectively, in the rhizosphere soil. Woodchip biochar also significantly decreased relativized CO_2 emission by 56.9 and 63.8% in bulk and rhizosphere soils, respectively, as compared to the control. Manure pellet biochar significantly decreased R_a from 166.7 g CO_2-C m^{-2} (in control) to 101.7 g CO_2-C m^{-2} while unpyrolyzed manure pellet increased R_a to 233.3 g CO_2-C m^{-2} in the entire wheat growing period.

3.4 Microbial biomass C and N in rhizosphere and bulk soils

Both manure pellet and woodchip biochars reduced MBC and MBN as compared to the control in the rhizosphere soil but in the bulk soil the effect was not significant (Fig. 3-4; Table Appendix 3-2). The rhizosphere soil had greater MBC and MBN ($P < 0.05$) than the bulk soil in the control while in the biochar-amended soils (with both biochars), MBC and MBN were not significantly different between rhizosphere and bulk soils. In the soils amended with unpyrolyzed manure pellet and woodchip, MBC and MBN were greater ($P < 0.05$) in the rhizosphere than in the bulk soil except for MBN in the unpyrolyzed manure pellet amendment treatment (Fig. 3-4).

4. Discussion

This study shows contrasting effects of unpyrolyzed organic residues and their biochars on plant biomass and grain yield and soil processes. The result of positive and neutral effects of biochar on reducing CO_2 emission from rhizosphere and bulk soils respectively provides an insight on the mechanistic understanding of biochar induced C dynamics in plant-soil-biochar system.

The variable effects of biochar amendments on plant biomass and grain yield observed in this study are similar to that of previous studies (Spokas et al., 2012; Crane-Droesch et al., 2013; Sun et al., 2017). Vaccari et al. (2011) reported an increase in wheat yield by up to 30% by 30 and 60 Mg ha^{-1} of biochar plus 122 kg N ha^{-1} of fertilizer application. The increase in total wheat biomass and grain yield in manure pellet biochar and the decrease in woodchip biochar application compared to the control can be attributed to the difference in nutrient availability caused by those two amendments (Spokas et al., 2012). Jeffery et al. (2017) categorized biochars into two types based on the potential of biochar to provide nutrients to plants: *nutrient biochar* (produced from manure) and *structural biochar* (produced mainly from plant-derived materials

such as wood) and showed that the crop yield was increased by the application of *nutrient biochars* by up to three-fold as compared to the application of *structural biochars*. Higher TN (and inorganic N) concentrations led to a lower C/N ratio in manure pellet biochar might have positively affected crop yield (Sadaf et al., 2017). Prendergast-Miller et al. (2011) observed increased N mineralization in the rhizosphere by biochar treatment followed by increased wheat production. However, in our experiment we did not observe significant difference in available N between control and biochar-amended soils in the rhizosphere. Some previous studies have also recognized the nutrient retention capacity of biochar leading to a reduction in nutrient leaching (Prendergast-Miller et al., 2011; Clough et al., 2013), making more N available to increase crop production.

Woodchip biochar, on the other hand, had a much higher C/N ratio (105) than that of manure pellet biochar (17) that might lead to immobilization of soil mineral N (Bengtsson et al., 2003) and negatively affect plant biomass and grain yield. In an incubation experiment, Bruun et al. (2012) reported a significant increase in soil N immobilization when soil was amended with wheat straw biochar (C/N = 50) at the rate of 5% (w/w) and Ameloot et al (2013 b) observed net N immobilization with willow wood biochar with a C/N ratio of 75. Our study shows that wheat yield is negatively affected by the application of woodchip biochar at a high rate with a low rate of fertilizer application and suggests that adequate fertilizer N should be added with such biochars when applied at a high rate to improve crop production.

Unpyrolyzed manure pellet application increased wheat biomass and grain yield as compared to the control, because of increased nutrient availability through manure addition to the soil (Barzegar et al., 2002; Lv et al., 2011). Unpyrolyzed woodchip, on the other hand, decreased crop production (root biomass, total biomass and grain yield) because of its high C/N ratio (68). The decomposition of organic residues with high C/N ratios often lead to immobilization of N followed by the decrease in nutrient availability to the crop plants and the reduction in crop yield (Ambus et al., 2001; Kaewpradit et al., 2009).

Biochar application can substantially affect pH of acidic soils with low SOC content as SOC content is linked to the pH buffering capacity of the soil (Stewart et al., 2013). In addition, biochar application has extensively been reported to increase soil pH. In this study, the effect of biochar application on soil pH was similar between bulk and rhizosphere soils although rhizosphere soils had consistently greater pH relative to that of bulk soils in all soil amendment

treatments, likely because of the accumulation of calcium and magnesium in the rhizosphere (Youssef and Chino, 1988). In general, biochar amendment increases microbial biomass in the bulk soil (Gul et al., 2015; Abujabhah et al., 2016), but the effect in rhizosphere soil is not clear. Despite that biochar amendment increased DOC, it decreased MBC and MBN in the rhizosphere soil; this result is consistent with Dempster et al. (2012) who reported a significant decrease of MBC (from 145 to 116 mg C kg^{-1}) by 25 t ha^{-1} of biochar application to a planted soil. In our study, MBC was negatively correlated with DOC:DON ($r^2 = 0.68$, $P < 0.001$, data not shown), suggesting that DOC:DON is a major determinant of microbial biomass in the biochar-amended rhizosphere soil because the growth of microbial population can be inhibited when the N content of readily mineralizable (water-soluble) organic matter is decreased (Filep and Szili-Kovács, 2010).

Both manure pellet and woodchip applications increased cumulative CO_2 emission as well as relativized cumulative CO_2 emission as compared to the control in both bulk and rhizosphere soils. Addition of organic residues to the soil increases nutrient availability, organic matter content, and water holding capacity of soil and also soil pH when organic residues with a pH greater than an acidic soil are applied (Bolan et al., 2004). All the above factors favor microbial activity in these soils for mineralization of the added organic C, leading to a greater CO_2 emission from the soil (Abbas and Fares, 2009; Thangarajan et al., 2013). Manure pellet and woodchip biochar did not significantly affect cumulative CO_2 emission but decreased relativized cumulative CO_2 emission from the bulk soil. The decrease in relativized cumulative CO_2 emission by biochar application was due to the addition of recalcitrant C to the soil (Wang et al., 2016). Manure pellet and woodchip biochar amendments thus play significant roles in soil C sequestration by reducing relativized CO_2 emission from the agricultural soil.

The initially greater CO_2 emission from woodchip biochar-amended soils (both in bulk and rhizosphere soils) can be partly attributed to the labile organic C contained in the biochar (Luo et al., 2011; Zimmerman et al., 2011) and the abiotic release of C from biochar (Zimmerman, 2010). Water soluble labile C released from biochar can be mineralized rapidly by the soil microbial community, leading to a higher CO_2 emission rate from biochar-amended soils in the initial period (Smith et al., 2010; Jones et al., 2011). Although measurement of CO_2 emission began 5 days after the incubation commenced in this experiment to avoid the initial flush of CO_2 emission immediately after wetting the soil, the CO_2 emission was greater in the

biochar applied soil than in the control, likely because of the mineralization of the labile C contained in the biochar that could last for several days (Jones et al., 2011). However, the C sequestration potential of biochar application would not be compromised as only 1-5% of the C contained in the biochar could be readily mineralized (Kuzyakov et al., 2009; Wang et al., 2016) while the rest is sequestered into the soil with half-lives ranging from 114 to 1120 years (Singh et al., 2012; Zimmerman and Gao, 2013).

The result shows that rhizosphere CO_2 emission was higher from the rhizosphere soil than that from the bulk soil, with cumulative CO_2 and relativized cumulative CO_2 emission about two-fold greater in the rhizosphere than in the bulk soil. Rhizosphere priming effect has been recognized for stimulating CO_2 emission from the rhizosphere soil (Zhu et al., 2014). The greater availability of soluble organic carbon in the rhizosphere promotes microbial growth and activities that enhance soil C mineralization leading to an increase in CO_2 emission from rhizosphere soil as compared to that from the bulk soil (Kuzyakov and Domanski, 2000). Biochar amendment has been found to affect C turnover of both existing SOC (Ameloot et al., 2013b) and newly formed root-derived C compounds (Weng et al., 2017). The most important factors affecting soil respiration by biochar amendment are its priming effect on SOC mineralization, both positive (Luo et al., 2011) and negative (Whitman et al., 2014; Keith et al., 2015; Weng et al., 2015), and the physical protection of SOC in the biochar pores.

In our study, the cumulative soil respiration was not affected by either of the biochar amendments in the bulk soil, indicating that the priming effect of the biochars was minimal and thus it did not cause a significant difference in the respiration from the bulk soil. But manure pellet biochar application significantly decreased total soil respiration in the rhizosphere without affecting root biomass, suggesting that the decrease in total respiration in the rhizosphere was most likely due to reduction in heterotrophic decomposition of organic C. A significant decrease in R_a in manure pellet biochar treatment compared to the control also suggests that the biochar reduced the rhizosphere priming effect. While in this experimental setting, it was not possible to distinguish the effects of biochar on native SOC and root-derived organic C mineralization, we suggest that the decrease in total soil respiration was due to enhanced stabilization of root exudates released to the soil by biochar amendment via formation of organo-mineral complexes (Cross and Sohi, 2011; Weng et al., 2015), and the negative priming effect in the rhizosphere (Dempster et al., 2012; Keith et al., 2015; Weng et al., 2017). The addition of labile plant

material can enhance microbial biomass and soil respiration in the control soil, but potential adsorption of labile C in the pores of biochar that are inaccessible to decomposers may inhibit preferential substrate utilization by microbial organisms and lead to negative priming in the biochar-amended soil (Joseph et al., 2010; Ameloot et al., 2013a).

5. Conclusions

This study concludes that applying biochars produced from different organic residues to agricultural land can reduce CO_2 emission from the soil. Pyrolysis of manure pellet and its application to soil was beneficial in increasing wheat biomass and grain yield as well as decreasing soil respiration in the rhizosphere while woodchip biochar reduced soil respiration in the rhizosphere and wheat yield. Both manure pellet and woodchip biochars showed neutral (in bulk soil) and positive (in rhizosphere) effect on reducing cumulative CO_2 emission from the agricultural soil. Reduction of relativized cumulative CO_2 emission by both biochar amendments from bulk and rhizosphere soils demonstrates the potential to apply biochar to increase soil C sequestration in the agricultural field. Soil amendment with manure pellet and woodchip biochars increased dissolved organic C and N but decreased microbial biomass in the rhizosphere. The potential use of the biochars produced from these organic residues should be examined in field experiments for the assessment of their long-term impact on increasing SOC in croplands and supporting resilience of agroecosystems to mitigate climate change. Further experiments using ^{13}C labeling to quantify the contribution of rhizodeposits in total CO_2 emission from rhizosphere would improve our understanding of the process that occur in biochar-amended rhizosphere soils.

Table 3-1. Selected chemical and physical properties of soil amendments

Amendment	pH	TC (g kg^{-1})	TN	C/N	NH_4^+-N (mg kg^{-1})	NO_3^--N	Surface area* (m^2 g^{-1})	CEC* (cmol kg^{-1})
Manure pellet	8.56 (0.05)	188.0 (1.8)	13.03 (0.14)	14.5 (0.26)	161.22 (4.76)	0.23 (0.01)	<1	0.39
Manure pellet biochar	10.01 (0.21)	133.9 (5.9)	7.77 (0.31)	17.2 (0.05)	10.22 (0.46)	nd	13.3	18.38
Woodchip	5.68 (0.11)	461.4 (1.9)	6.76 (0.10)	68.9 (0.08)	18.36 (0.14)	0.15 (0.01)	<1	0.13
Woodchip biochar	8.01 (0.25)	666.1 (3.1)	6.30 (0.10)	105.4 (0.99)	4.01 (0.43)	nd	44.4	0.65

Values are the means with standard errors in the parentheses (n = 3), * indicates the characteristics determined in a single sample.

Abbreviations: TC, total carbon concentration; TN, total nitrogen concentration; C/N, carbon nitrogen ratio; CEC, cation exchange capacity; nd, not detected.

Table 3-2. Effect of soil amendment on pH, dissolved organic carbon (DOC) and nitrogen (DON), and available nitrogen (Avail. N), cumulative carbon dioxide (Cum. CO_2) and relativized cumulative carbon dioxide (Rel. Cum. CO_2) emission in bulk and rhizosphere soils

Soil amendment	pH	DOC (mg kg^{-1})	DON	DOC/DON	Avail. N* (mg kg^{-1})	Cum. CO_2 (g CO_2-C m^{-2})	Rel. Cum. CO_2 (g CO_2-C kg^{-1} C m^{-2})
Bulk soil							
CK	5.26(0.04)	117.73(2.55)	15.88(0.75)	7.47(0.37)	11.39(0.37)	228.60(9.64)	62.14(2.62)
MP	6.44(0.02)	257.33(13.90)	35.02(2.27)	7.36(0.16)	13.54(0.63)	555.03(30.21)	122.48(6.66)
MB	6.46(0.02)	141.38(2.04)	11.50(0.45)	12.35(0.57)	10.01(0.53)	196.31(5.15)	46.33(1.22)
WW	5.54(0.03)	207.37(11.74)	32.30(1.03)	6.45(0.47)	4.05(0.50)	795.72(67.87)	132.09(11.26)
WB	6.10(0.04)	123.60(8.48)	8.54(0.20)	14.43(0.76)	12.28(0.32)	191.28(5.81)	26.77(0.81)
Rhizosphere soil							
CK	5.89(0.03)	153.85(7.75)	18.22(1.35)	8.48(0.27)	8.21(0.56)	395.35(7.44)	107.46(2.01)
MP	6.80(0.06)	392.60(16.26)	54.01(2.80)	7.28(0.20)	4.18(0.54)	735.41(48.58)	162.28(10.72)
MB	6.78(0.01)	200.09(1.73)	16.71(0.47)	11.99(0.32)	6.39(0.44)	298.03(15.55)	70.35(3.67)
WW	5.63(0.03)	267.25(18.25)	43.67(1.86)	6.10(0.20)	2.66(0.27)	895.47(12.41)	148.65(2.06)
WB	6.55(0.01)	210.37(11.41)	19.05(1.65)	11.29(1.22)	7.79(1.05)	277.94(10.96)	38.91(1.53)

Values are the means with standard errors in the parentheses (n = 4).

Abbreviations: CK, control (no amendment); MP, addition of unpyrolyzed manure pellet; MB, addition of manure pellet biochar; WW, addition of unpyrolyzed woodchip; WB, addition of woodchip biochar.

Avail. N* represents the sum of NH_4^+-N and NO_3^--N.

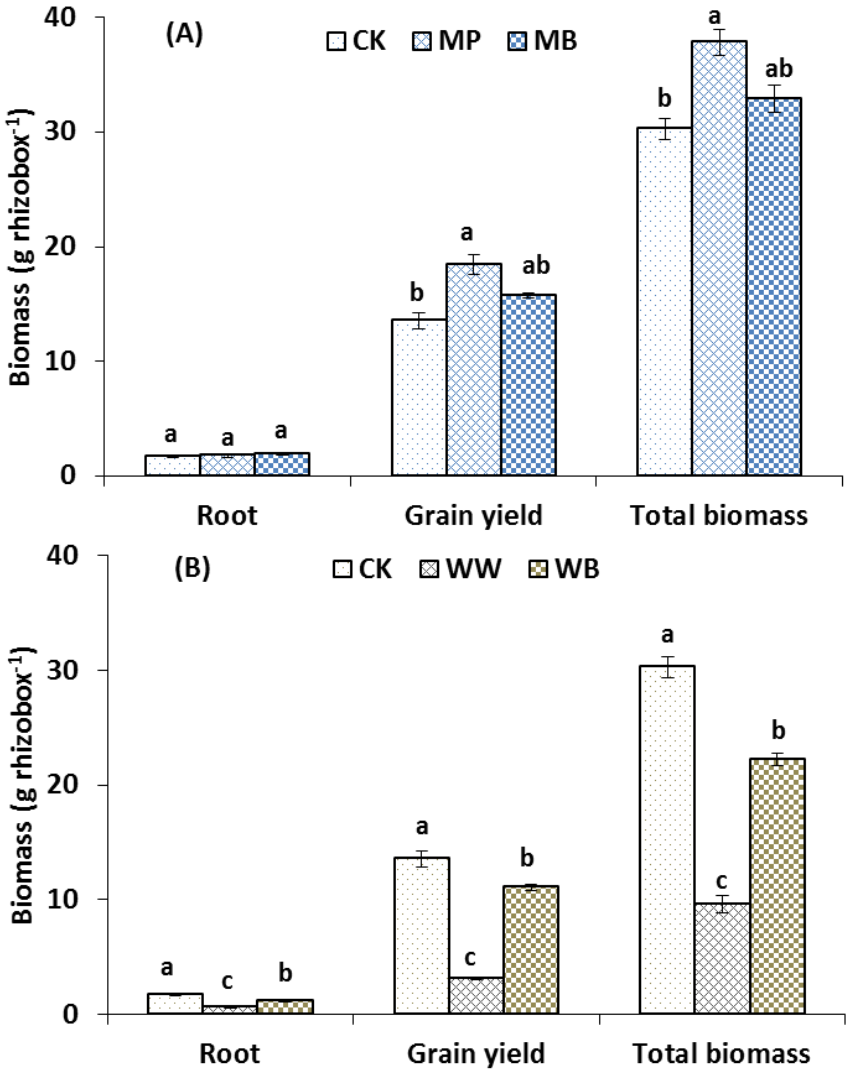

Fig. 3-1. The effects of soil amendments A) manure pellet and B) woodchip on wheat biomass production. Different letters indicate significant differences among the treatments. Treatment codes are CK: control (no amendment), MP: addition of unpyrolyzed manure pellet, MB: addition of manure pellet biochar, WW: addition of unpyrolyzed woodchip, and WB: addition of woodchip biochar.

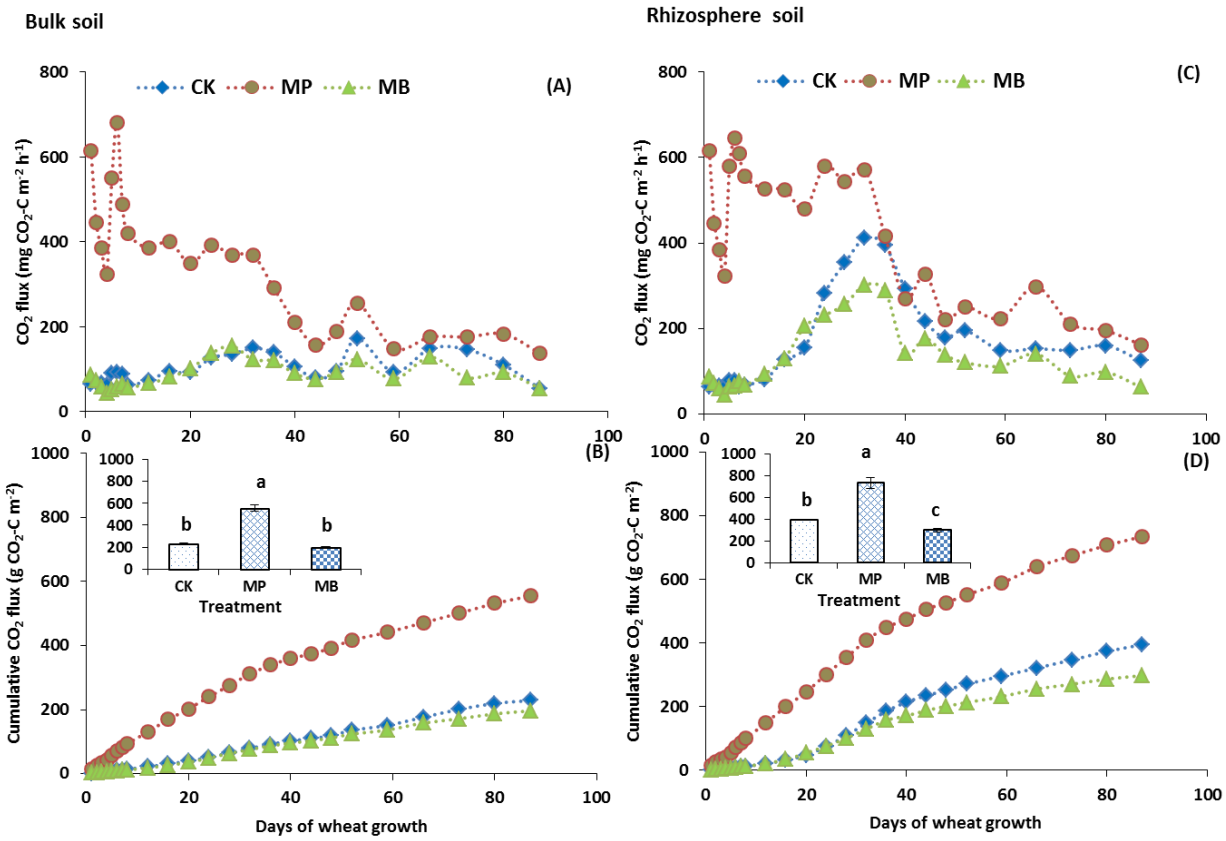

Fig. 3-2. The effects of soil amendments (unpyrolyzed manure pellet and manure pellet biochar) on A) the dynamics of CO_2 emission from the bulk soil, B) the cumulative CO_2 emission from the bulk soil, C) the dynamics of CO_2 emission from the rhizosphere soil and D) the cumulative CO_2 emission from the rhizosphere soil. The insert plots are total CO_2 emission in 87 days. Different letters in the insert plots represent significant differences among the treatments. Treatment codes are CK: control (no amendment), MP: addition of unpyrolyzed manure pellet, MB: addition of manure pellet biochar.

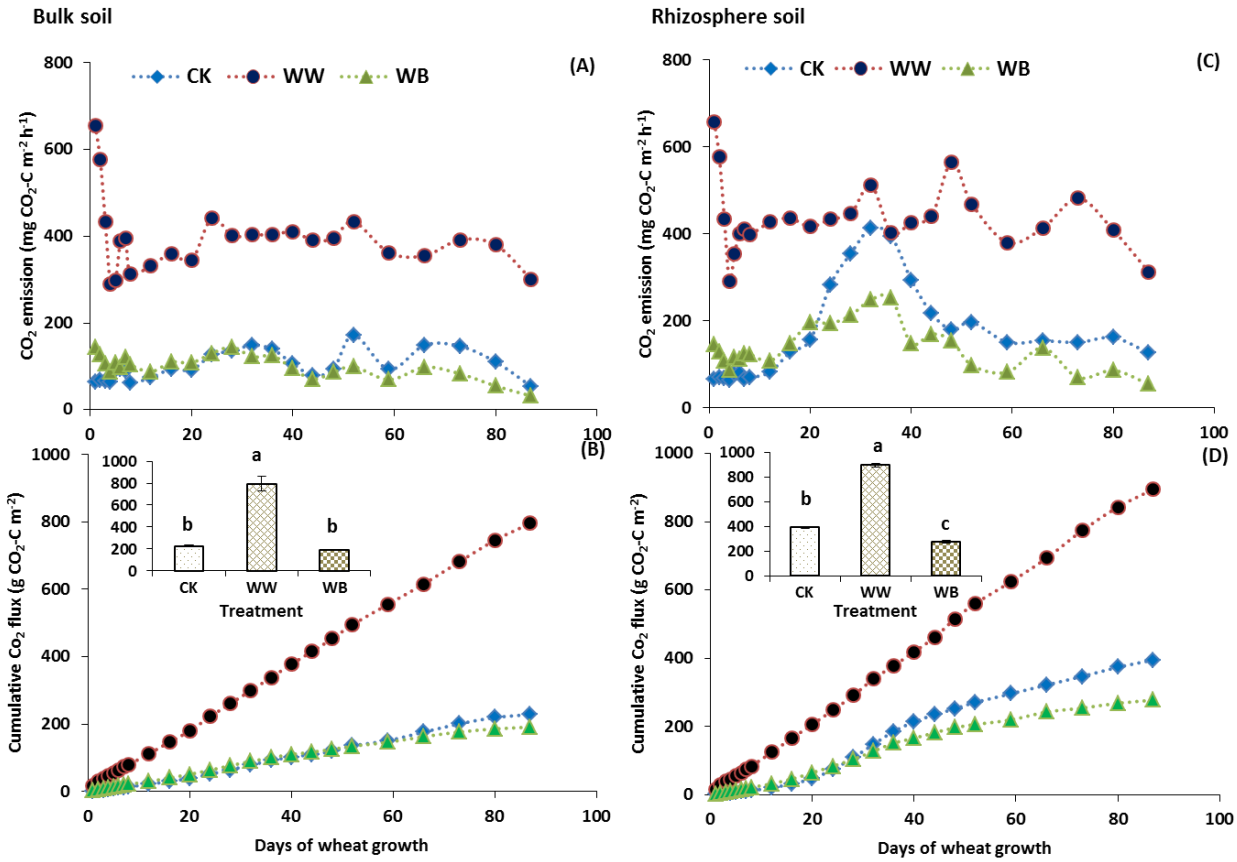

Fig. 3-3. The effects of soil amendments (unpyrolyzed woodchip and woodchip biochar) on A) the dynamics of CO_2 emission from the bulk soil, B) the cumulative CO_2 emission from the bulk soil, C) the dynamics of CO_2 emission from the rhizosphere soil and D) the cumulative CO_2 emission from the rhizosphere soil. The insert plots are total CO_2 emission in 87 days. Different letters in the insert plots represent significant differences among the treatments. Treatment codes are CK: control (no amendment), WW: addition of unpyrolyzed woodchip, and WB: addition of woodchip biochar.

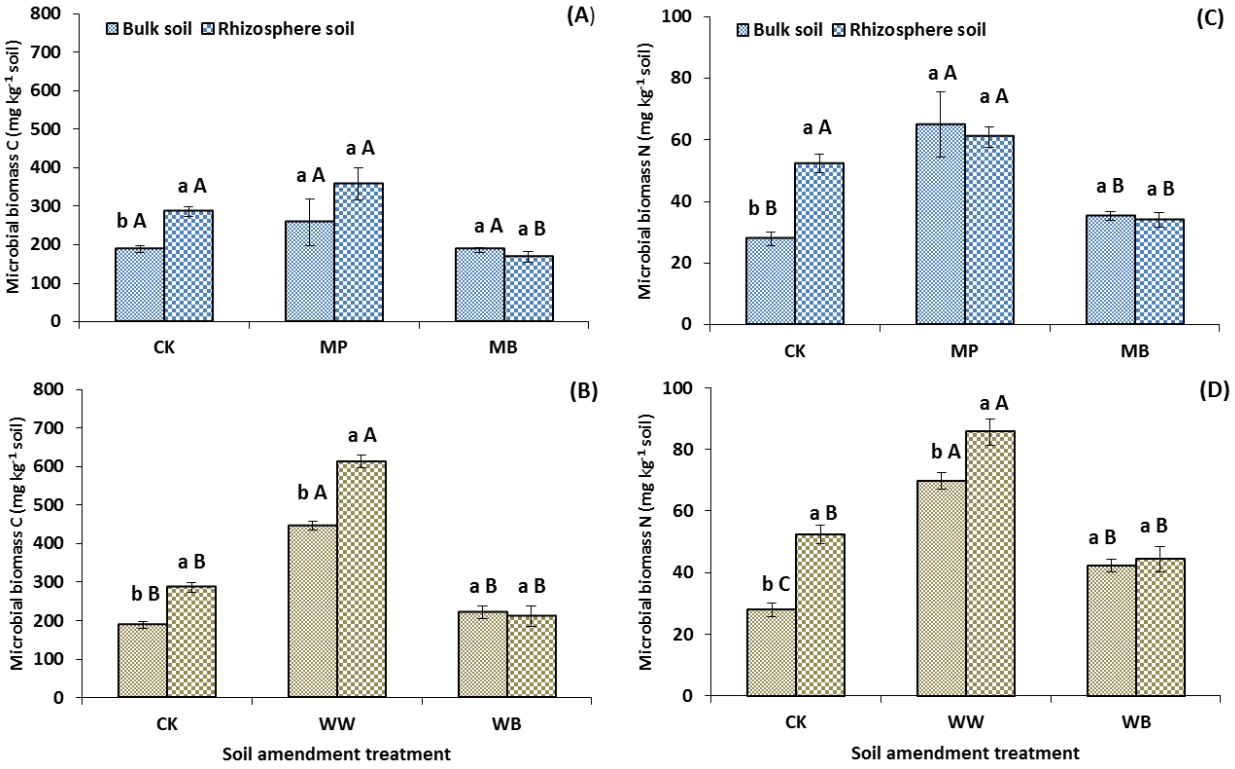

Fig. 3-4. The effects of soil amendments (A and C manure pellet, and B and D woodchip) on microbial biomass carbon (C) and nitrogen (N). Different lowercase letters indicate significant differences between bulk and rhizosphere soil within each soil amendment treatment and uppercase letters indicate significant differences across soil amendment treatment within each root zone. Treatment codes are CK: control (no amendment), MP: addition of unpyrolyzed manure pellet, MB: addition of manure pellet biochar, WW: addition of unpyrolyzed woodchip, and WB: addition of woodchip biochar.

Chapter 4. Manure-based biochar decreases heterotrophic respiration and increases gross nitrification rates in rhizosphere soil

1. Introduction

Pyrolysis of organic material into biochar and adding biochar to cropland soils have been shown to be promising in increasing carbon (C) sequestration and reducing greenhouse gas emissions because of biochar's long-term stability (Woolf et al., 2010). Biochar's chemical and physical characteristics such as high pH, C to nitrogen (N) ratio (C/N), and surface area are crucial factors for biochar to impact soil-microbe interactions that can decrease C and N mineralization (Gul et al., 2015; Zimmerman et al., 2011). In the rhizosphere, C and N mineralization is increased by increasing microbial activity (a process called positive priming) because of rhizodeposits (readily available labile C) that provide the energy for microbes (Zhu et al., 2014), while biochar is known for its negative priming on SOC decomposition in the bulk soil (Zimmerman et al., 2011). It is not known if biochar has similar influence on C and N mineralization in the rhizosphere as compared to that of bulk soil (Whitman et al., 2014), as the rhizosphere substantially differs from the bulk soil in microbial community structure, soil pH and availability of nutrients (Gregory, 2006; Zhu et al., 2014). The effects of biochar on net N mineralization, inorganic N dynamics, and N_2O emissions in bulk soil have been well studied (Clark et al., 2019; Nguyen et al., 2017). A few studies have also determined the effect of biochar on gross N transformation processes that simultaneously determine the productive and consumptive processes of the N cycle in the bulk soil (Hu et al., 2014; Nelissen et al., 2012). This is the first study on comparing gross N transformation processes between rhizosphere and bulk soils when amended with biochar. This study hypothesized that biochar reduces heterotrophic respiration and N_2O emissions from the rhizosphere soil by affecting net and gross N transformation rates and the effects are different between rhizosphere and bulk soils.

2. Material and methods

2.1. Soil and amendment

Soil samples used in this study were collected from a greenhouse experiment; refer to Pokharel and Chang (2019) for details of the greenhouse experiment. Briefly, for the greenhouse

experiment, the soil (Orthic Black Chernozem) was collected from 0-10 cm layer in a cropland near Leduc (53° 11' 33'' N, 113° 59' 18'' W) in Alberta, Canada. The soil was air-dried and mixed with unpyrolyzed manure pellet or manure pellet biochar at the rate of 5% (w/w) and filled in rhizoboxes (20 × 15 × 12 cm). Pellets were made to facilitate the handling, transportation and application of manure. Biochar was produced by heating manure pellets at a temperature range of 500 to 550 °C with a heating rate of 9-10 °C min^{-1} in InnoTech Alberta, Canada; see Pokharel and Chang (2019) for the chemical and physical properties of unpyrolyzed manure pellet and its biochar. Each rhizobox was divided into three compartments separated by nylon layers (300 meshes per inch) before they were filled with soil; wheat (*Triticum aestivum* L.) was grown in the middle compartment of each rhizobox in a greenhouse to produce rhizosphere and bulk soils. Ninety days after seeding, the aboveground wheat plant was harvested, roots were lifted out of the rhizobox and gently shaken to remove the soil adhering to the roots. After all broken roots were removed from the soil, the soil was designated as the rhizosphere soil. The soil collected from the other two compartments of the rhizobox was thoroughly mixed and designated as the bulk soil.

2.2. Experimental design

The experiment used a split-plot design with soil amendment treatment as the whole-plot factor and root zone (rhizosphere vs bulk soil) as the sub-plot factor. Soil amendment treatments were: control (no addition of any amendment (CK)), addition of unpyrolyzed manure pellet (MP), addition of manure pellet biochar (MB), addition of unpyrolyzed willow woodchip (WW) and addition of woodchip biochar (WB). The treatments were replicated four times.

2.3. Measurement of soil heterotrophic respiration and N_2O emissions and soil analysis

For the measurement of heterotrophic soil respiration and N_2O emission (experiment I), 30 g fresh soil (oven-dry equivalent) of control (soil with no addition of manure pellet and biochar in the greenhouse experiment), MP (soil amended with manure pellet in the greenhouse experiment) and MB (soil amended with manure pellet biochar in the greenhouse experiment) treatments from the rhizosphere and bulk soils (with 4 replications) were placed in 250 mL conical flasks. The samples (with moisture content ranging between 31 and 43% water holding capacity (WHC)) were then pre-incubated in the dark at 25 °C for 48 hours to stabilize the

microbial population in the soil. After pre-incubation, de-ionized water was added to the soil to bring the moisture content to 50% WHC of the soil. Gas samples (20 mL) were collected daily for one week at 0 and 24 hours after closing the headspace of the flask with a rubber stopper and injected into the pre-evacuated vials. At each gas sampling, rubber stoppers were removed, and the flasks were left open for 1 hour at room temperature (25 °C) and then flushed with fresh air before closing the flasks with the stoppers for gas sampling. The concentration of CO_2 and N_2O in the gas samples was determined by a GC (Varian CP-3800 gas chromatograph, Mississauga, ON, Canada), and the flux was calculated using the modified ideal gas equation (Pokharel and Chang, 2019). Mean CO_2 and N_2O emission rates for the incubation period was calculated from daily measurement over 7 days. Hot water-extractable organic C (HWEC) and N (HWEN) in the soil were determined following the method of Ghani et al. (2003) and available N (NH_4^+-N and NO_3^--N) in the 2 M KCl extract was determined colorimetrically by indophenol blue and vanadium oxidation methods, respectively.

2.4. Measurement of net and gross N transformation rates

Gross N transformation rates were determined (experiment II) by the ^{15}N isotopic pool dilution method (Hart et al., 1994). Following the procedure described in Hu et al. (2014), 2 mL of an ^{15}N labeled ammonium nitrate solution (either $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$) at 5 atom% was added to 30 g soil (oven-dry equivalent) after pre-incubation of the soil for 48 hours, and the final moisture content was brought to 50% WHC by adding de-ionized water. Soil samples were extracted with a 2 M KCl solution, half of the 96 soil samples (2 soils × 3 amendment treatments × 4 replications × 2 sources of ^{15}N × 2 sampling times) 0.5 hours after and the remaining samples 36 hours after the addition of the ^{15}N labeled ammonium nitrate solution. The concentrations of NH_4^+-N and NO_3^--N in the extracts were determined using MgO and Devarda's alloy, respectively, in a steam distillation system (Vapodest 20, C. Gerhardt, Koningswinter, Germany), followed by titration of the distillates with a 0.01 M NaOH solution in an auto-titrator (719S Titrino, Metrohm AG, Herisau, Switzerland). The distillates were then acidified with 0.05 M H_2SO_4 and dried at 60 °C. The ^{15}N abundances of NH_4^+-N and NO_3^--N in the dried samples were determined by a continuous flow isotope ratio mass spectrometer (Thermo Finnigan Corp, Bremen, Germany) linked to an elemental analyzer (Thermo Fisher Scientific Inc., Bremen, Germany).

Gross N transformation rates, including N mineralization, nitrification and NH_4^+ and NO_3^- consumption, were calculated by the following equations (Hart et al., 1994).

$$m = \frac{M_0 - M_t}{t} \times \frac{Log(H_0 M_t / H_t M_0)}{Log\ (M_0/M_t)}$$

$$C = \frac{M_0 - M_t}{t} \times \frac{Log(H_0/H_t)}{Log\ (M_0/M_t)}$$

Where m is the gross mineralization rate, M_0 is the sum of tracer and non-tracer NH_4^+-N pool at t = 0, M_t is the sum of tracer and non-tracer NH_4^+-N pool at time t (36 hours), H_0 is the ^{15}N pool at t = 0 and H_t is the ^{15}N pool at time t, and C is the consumption rate of NH_4^+ and NO_3^- in time t. Gross nitrification rates were also calculated similar to that of gross mineralization rates where M represents the sum of tracer and non-tracer NO_3^--N pool. Percent recovery of added ^{15}N in NH_4^+-N and NO_3^--N pool was calculated as (Wan et al., 2009):

$$^{15}N\ recovery\ (\%) = \frac{(H_A)_t}{(H_A + H_N)_0} \times 100 \qquad \text{for the } NH_4^+\text{-N pool}$$

$$^{15}N\ recovery\ (\%) = \frac{(H_N)_t}{(H_A + H_N)_0} \times 100 \qquad \text{for the } NO_3^-\text{-N pool}$$

2.5. Statistical analyses

The effects of soil amendment (control, manure pellet biochar and its feedstock application) as a main plot factor and soil zone (rhizosphere and bulk soils) as a subplot factor and their interactions were assessed by two-way analysis of variance (ANOVA) following a split-plot design using the PROC MIXED procedure in SAS (version 9.4, SAS Institute Inc., NC, USA), with least square means separated using the Tukey test at $\alpha = 0.05$. When there is no significant interaction between amendment and soil zone treatments, the effects of soil amendment were further assessed by one-way ANOVA within each soil zone. The data were checked for normality of distribution on residuals; CO_2 and N_2O emissions, and net and gross mineralization and nitrification rates were log-transformed to meet the normality of distribution; however, back-transformed data are presented in the results section.

3. Results and discussion

3.1 Effects of amendments on heterotrophic respiration and N_2O emissions

Hot water extractable C (HWEC) and N (HWEN) were 8.2 and 11.2% greater ($P = 0.006$ and 0.021, respectively) in the rhizosphere than in the bulk soil (Table 4-1). Those conditions make the rhizosphere a more favorable environment for microbial functioning than the bulk soil, leading to enhanced C and N mineralization in the rhizosphere soil (Nannipieri et al., 2007; Prashar et al., 2014). Heterotrophic respiration was significantly affected by the interaction between amendment and soil zone treatments ($P < 0.001$; Fig. 4-1). Compared to the control, manure pellet biochar decreased heterotrophic respiration by 30.2 and 30.5% in rhizosphere and bulk soils, respectively, but the feedstock increased it by 79.8 and 125.6%, respectively (Fig. 4-1). The increase in C/N ratio in the biochar-amended soil (Table 4-1) likely had a dominant effect on lowering CO_2 emissions in that soil, regardless of the soil zone. Manure pellet biochar did not change, but its feedstock increased hot water extractable C and N, which precludes a simple substrate-driven explanation for the reduction of heterotrophic respiration in the biochar-amended rhizosphere and bulk soils. The negative priming effect of biochar in the bulk soil (Jones et al., 2011) and stabilization of rhizodeposits on biochar surfaces (Weng et al., 2017) were mechanisms for the lower heterotrophic respiration in the rhizosphere than in the bulk soil.

The emissions of N_2O were significantly changed by amendment ($P = 0.003$) and soil zone treatments ($P = 0.012$) with no significant interaction between them (Fig. 4-1). Manure pellet biochar did not change N_2O emissions in both soil zones but feedstock increased N_2O emissions as compared to the control, with substantial differences in the increase between rhizosphere and bulk soils (two-fold increase in the rhizosphere and three-fold in the bulk soil; Fig. 4-1). The greater N_2O emissions in the rhizosphere than in the bulk soil is linked to the increased rates of net and gross mineralization and nitrification in the rhizosphere soil (Figs. 4-2 and 4-3), as N_2O emissions were significantly positively correlated with the net and gross mineralization and nitrification rates in the rhizosphere soil (Appendix 4-1). Although soil NO_3^--N concentration plays a major role in producing N_2O via denitrification, the labile organic matter added in the form of root exudates also favors denitrification by stimulating microbial growth and activity, leading to increased oxygen consumption that creates anoxic microsites in the rhizosphere soil (Myrold and Tiedje, 1985). Thus, increased denitrification rates caused by root exudates was likely an additional factor for increasing N_2O emissions in the rhizosphere soil.

3.2. Effect of amendment on net and gross N transformation rates

Net N mineralization and nitrification rates were significantly affected by the interaction between the two treatments (Fig. 4-2). Manure pellet biochar and its feedstock increased net mineralization and nitrification rates in the rhizosphere as compared to that of their bulk soils (Fig. 4-2) because of greater amounts of labile organic C (HWEC) and N (HWEN) and the optimum pH in the rhizosphere soils (Whalen et al., 2001). Amendment and soil zone treatments had significant effects on gross N mineralization, with no significant interaction between them but there was a significant interaction effect on gross nitrification (Fig. 4-3). The availability of NH_4^+ and NO_3^- ions that act as substrates in nitrification and denitrification processes, respectively, in the soil during N_2O production is better reflected by the gross than by the net rates of N transformations (Wan et al., 2009). Rhizosphere soil had higher gross mineralization, and NH_4^+ consumption (Fig.4-3), due to higher microbial activities that resulted from increased dissolved organic C and N in the rhizosphere soil (Landi et al., 2006; Pokharel and Chang, 2019). Manure pellet increased gross N transformation rates (Fig. 4-3) compared to the control and biochar amendment due to increased HWEN (Table 4-1), with significant positive correlations between HWEN and gross N transformation rates in both rhizosphere and bulk soils (Appendix 4-1). Biochar had contrasting effects on gross nitrification rates between rhizosphere and bulk soils, by increasing the rates in the rhizosphere and decreasing them in the bulk soil, as compared to the respective soils of the control treatment (Fig. 4-3). Differential effects of biochar amendment on gross nitrification rates resulted in a lower and higher % recovery of $^{15}NH_4^+$ in the NH_4^+-N pool in the biochar-amended rhizosphere and bulk soils, respectively, as compared to the control treatment (Appendix 4-3). This contrasting biochar effect suggests that different factors that control gross nitrification rates were being affected in rhizosphere and bulk soils. Biochar can adsorb a substantial amount of NH_4^+ ions on its surface near neutral soil pH (Fidel et al., 2018); this would limit the activities of nitrifiers and subsequently reduce gross nitrification rates in the bulk soil by decreasing the availability of NH_4^+ ions as a substrate for nitrifiers (Wan et al., 2009; Wang et al., 2015). However, in the rhizosphere soil, the increased gross nitrification rate under biochar amendment may be caused by the adsorption onto the biochar surface of potential nitrification inhibitors released by plant roots (Subbarao et al., 2015) or rhizosphere-biochar interaction that increased the abundance of nitrifying bacteria (Lehmann et al., 2015).

4. Conclusions

It can be concluded that the application of biochar and its feedstock differentially affected soil heterotrophic respiration, N_2O emissions, and net and gross N transformation rates in rhizosphere and bulk soils. Biochar's contrasting effects on soil processes between rhizosphere and bulk soils demonstrate the need of considering rhizosphere-biochar interactions in understanding N cycle in biochar-amended agricultural soils.

Table 4-1. ANOVA table for the effects of soil amendment and soil zone on soil properties (means ± SE (n = 4)).

Treatment	pH	TC	TN	C/N	HWEC	HWEN
		g kg^{-1}			g kg^{-1}	
Soil amendment						
CK	5.58 (0.12) b	29.71 (0.38) b	2.80 (0.03) c	10.61 (0.08) b	627.26 (15.52) b	49.48 (1.87) b
MP	6.63 (0.07) a	37.54 (1.47) a	3.53 (0.12) a	10.64 (0.08) b	828.59 (16.90) a	85.22 (2.46) a
MB	6.63 (0.06) a	37.63 (1.65) a	3.15 (0.09) b	11.91 (0.20) a	599.11 (8.67) b	52.14 (0.99) b
Soil zone						
RS	6.49 (0.13) a	35.37 (1.42)	3.18 (0.12)	11.12 (0.17)	715.65 (36.06) a	66.31 (5.42) a
BS	6.06 (0.17) b	34.55 (1.61)	3.13 (0.10)	10.99 (0.24)	661.69 (28.36) b	59.65 (4.73) b
ANOVA						
Soil amendment (A)	<0.001	0.006	0.002	0.001	<0.001	<0.001
Soil zone (R)	<0.001	0.576	0.611	0.429	0.006	0.021
A × R	0.004	0.124	0.108	0.443	0.075	0.094

Abbreviations: CK, no amendment; MP, addition of manure pellet; MB, addition of manure pellet biochar; RS, rhizosphere soil; BS, bulk soil; TC, total carbon; TN, total nitrogen; C/N, carbon to nitrogen ratio; HWEC, hot water extractable carbon; HWEN, hot water extractable N.

Different letters in the same column within soil amendment or soil zone indicate significant differences at $\alpha = 0.05$.

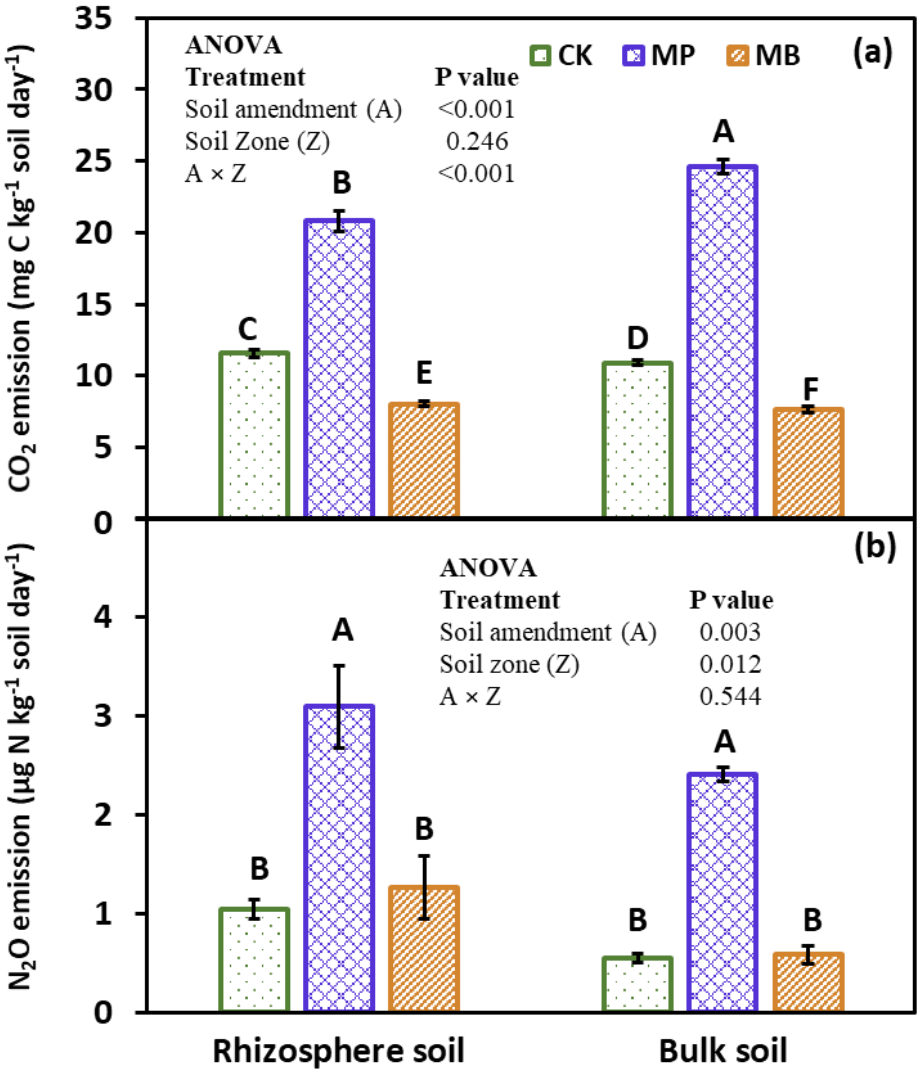

Fig. 4-1. Effects of soil amendment on carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions from rhizosphere and bulk soils. Treatment codes are: CK = no amendment, MP = addition of manure pellet, MB = addition of manure pellet biochar. Means ± SE (n = 4) are separated by different letters among soil amendment and soil zone interactions (a) and among soil amendment treatments within each soil zone type (b) at $\alpha = 0.05$.

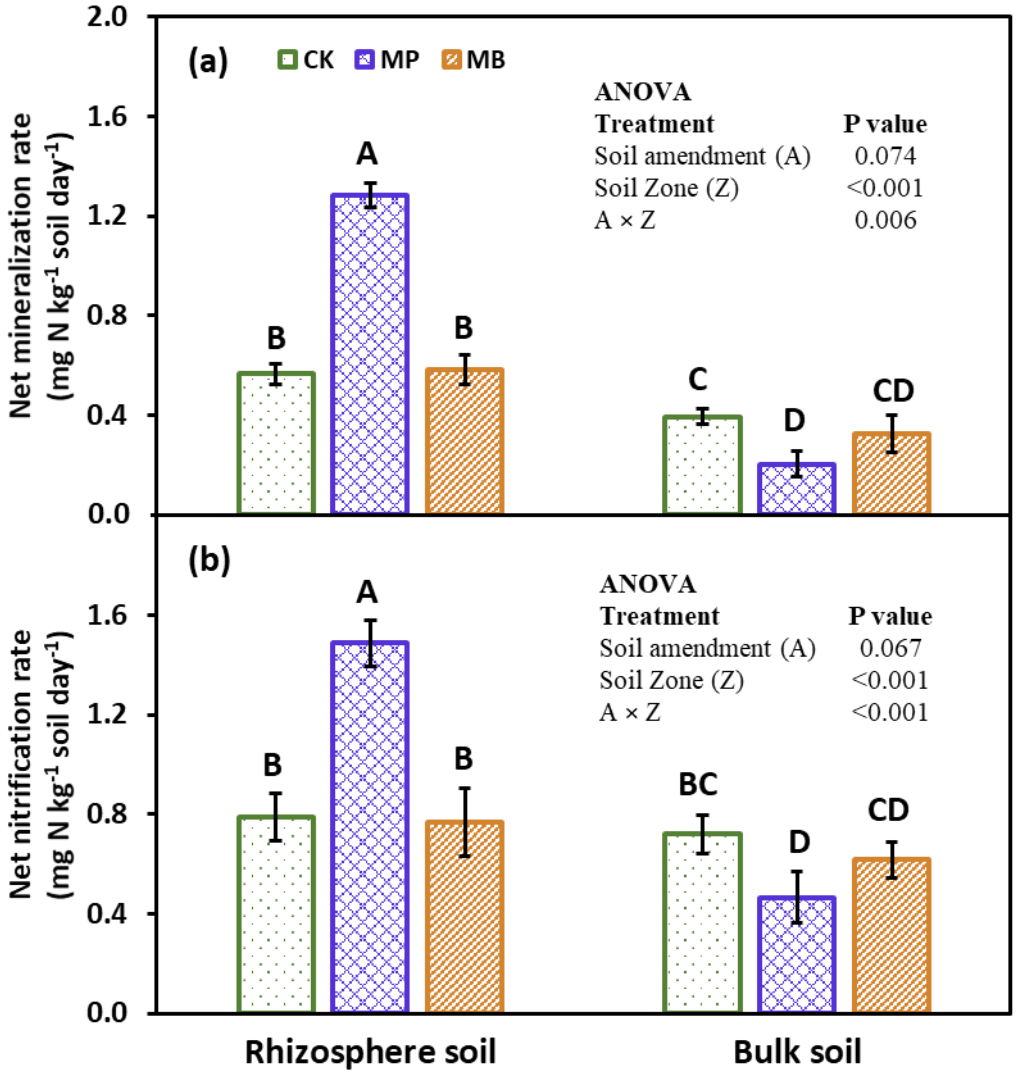

Fig. 4-2. Effects of soil amendment on net nitrogen mineralization and nitrification rates in rhizosphere and bulk soils. Treatment codes are: CK = no amendment, MP = addition of manure pellet, MB = addition of manure pellet biochar. Means ± SE (n = 4) are separated by different letters among soil amendment and soil zone interactions at $\alpha = 0.05$.

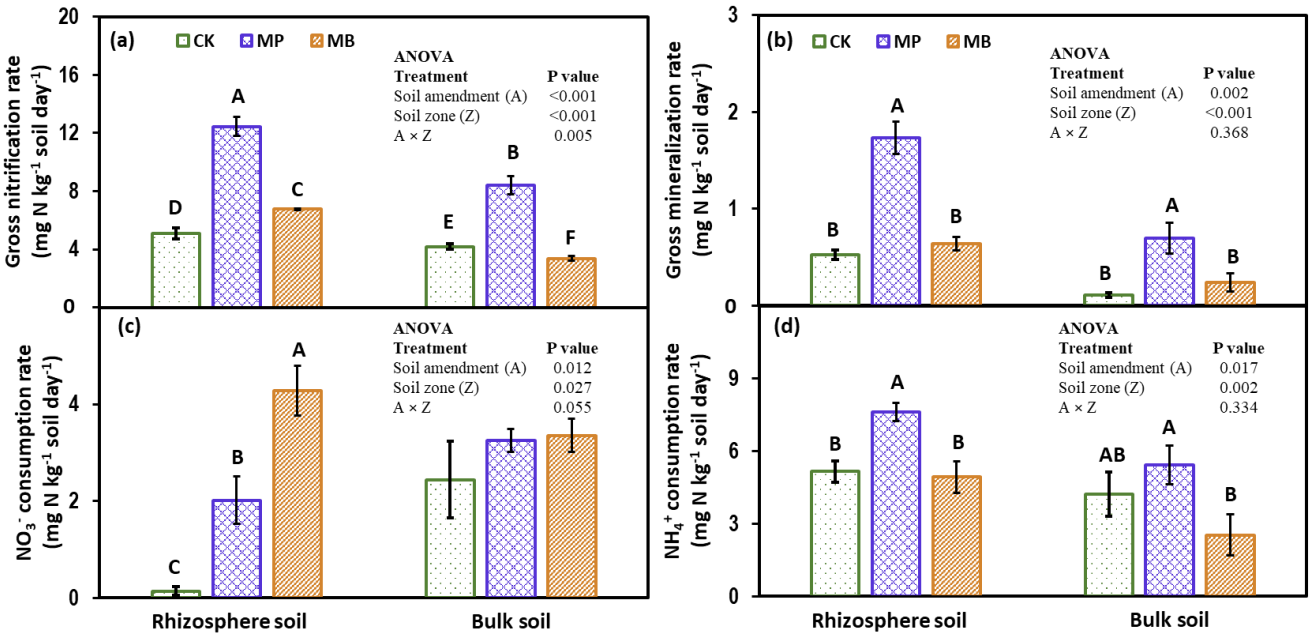

Fig. 4-3. Effects of soil amendment on gross nitrogen transformation rates in rhizosphere and bulk soils. Treatment codes are: CK = no amendment, MP = addition of manure pellet, MB = addition of manure pellet biochar. Means ± SE (n = 4) are separated by different letters among soil amendment and soil zone interactions (a) and among soil amendment treatments within each soil zone type (b, c and d) at $\alpha = 0.05$.

Chapter 5. Biochar decreases the efficacy of the nitrification inhibitor nitrapyrin in mitigating nitrous oxide emissions from soil at different moisture levels

1. Introduction

The increase of nitrous oxide (N_2O) concentration in the atmosphere due to anthropogenic emissions has been attracting significant interest in the last several decades (Butterbach-Bahl et al., 2013; Tian et al., 2020). The concentration of N_2O in the atmosphere is increasing at an alarming rate of 0.95 ppb yr^{-1} for the last 10 years and reached 331.1±0.1 ppb in 2018 (WMO, 2019). Agricultural sources of N_2O emissions, including those from cropland soils due to extensive use of fertilizers and animal waste management systems, contribute significantly to global N_2O emissions (Sutton et al., 2013). Over the past century, cropland has contributed 80% of the global increase of terrestrial N_2O emissions because of increasing rates of synthetic fertilizer use (Tian et al., 2019).

Reducing N_2O emissions from croplands is a critical component of climate change mitigation (Lipper et al., 2014). In cropland soils, major pathways of nitrogen (N) cycling contributing most of the N_2O emissions from the soil are aerobic nitrification, anaerobic denitrification, nitrifier denitrification of ammonium and chemical reduction of nitric oxide and nitrate (Baggs, 2011; Cai et al., 2016). Anthropogenic N_2O emissions from cropland soil can be reduced by adopting various management practices such as biochar application (Cayuela et al., 2014) and the use of nitrification inhibitors (Akiyama et al., 2010; Menendez et al., 2012) which affect the above pathways of N cycling, although different mechanisms have been shown to affect these pathways under different management practices (Duan et al., 2019; Fuertes-Mendizábal et al., 2019). Nitrification inhibitors (NIs) reduce N_2O emissions by lowering nitrification rates through inhibition of ammonia monooxygenase activity (Ruser and Sculz, 2015), while biochar increases soil pH, soil organic carbon (SOC) and soil aeration that subsequently leads to changes in soil N mineralization, immobilization and nitrification rates, and ultimately changes N_2O emissions (Clough et al., 2013; Pokharel et al., 2021). Because of its additional benefits, such as carbon sequestration, nutrient retention and increasing nutrient use efficiency, biochar has been widely recommended for use in the cropland across the world (Hossain et al., 2020; Smith, 2016; Zhou et al., 2021). In this context, it is very important to

understand how biochar interacts with NI in changing soil processes that could potentially affect the efficacy of NI in reducing N_2O emissions from the soil.

The effect of NI in reducing N_2O emissions from biochar-amended soils can be substantially different than from un-amended soils because of increased soil pH and aeration caused by biochar amendment. The increase in soil pH can counteract the potential inhibition of ammonia monooxygenase activities by NI (Che et al., 2015), and an increase in soil aeration can promote aerobic nitrification (Mathieu et al., 2006), both can lead to an increase in N_2O emissions. In addition to this, NI applied to the soil can also be adsorbed on biochar surfaces, making it unavailable for reducing nitrification rates and N_2O emissions (Keiblinger et al., 2018). In the last couple of years, research on interactive effects of biochar with NI in soil N cycling is gaining momentum and the substantial variations in the results depend on the type of biochar, NI and soil characteristics (Fuertes-Mendizábal et al., 2019; Li and Chen, 2020; Li et al., 2021). In a grassland soil, Fuertes-Mendizábal et al. (2019) observed a significant reduction in the efficiency of 3,4-dimethylpyrazole phosphate (DMPP, a NI) by pine dust biochar, while Li et al. (2021) did not observe a significant effect of canola straw biochar on the efficiency of nitrapyrin (a commonly used NI in agricultural soil) in reducing N_2O emissions from an unfertilized agricultural soil. The effect of biochar may be different when nitrapyrin is used with fertilizer application to the soil as N availability for nitrification and denitrification processes differs substantially between these soils. In addition, we still have limited knowledge on biochar's interaction with NI in mitigating N_2O emissions by affecting soil processes, including nitrification rates, availability of ammonium (NH_4^+) and nitrate (NO_3^-) as substrates for nitrification and denitrification processes, respectively, and soil enzyme activities.

One of the key driving factors affecting N_2O emissions from the soil is the moisture level. Its effect on N_2O emissions has been studied extensively under various soil characteristics (Ciarlo et al., 2007; Dobbie and Smith, 2003) and shown that N_2O produced vary substantially under different soil moisture levels with a significant change in aerobic nitrification (that produces N_2O as a byproduct) and anaerobic denitrification (that produces N_2O as an intermediate product) as the dominant pathways of N_2O production (Wu et al., 2013). Soil moisture level controls oxygen availability and determines which pathway of N_2O production becomes dominant (Ciarlo et al., 2007; Wu et al., 2013). Ullah et al. (2016) observed an increase in N_2O emissions when water-filled pore space (WFPS) was increased from 60 to 90% and

attributed to denitrification as a dominant process for the increase in N_2O emissions at 90% WFPS. Since the use of NI may delay the oxidation of NH_4^+ to NO_3^-, thereby reducing N_2O emissions under aerobic conditions with low moisture levels, we have limited knowledge on the effectiveness of NI under high moisture levels. Lin et al. (2021) showed that the response of NI co-applied with manure in reducing N_2O emissions under different WFPS varies between soil types with significant lower reduction at 80 than at 60% WFPS in a Gray Luvisolic soil (characterized by low organic matter content), but the reduction was not apparent in a Black Chernozemic soil (characterized by high organic matter content). The study also showed that the effect of moisture level on the efficacy of NI in reducing N_2O emissions from cropland soils depends on agricultural management practices, including organic soil amendments. The efficacy of NI in reducing N_2O emissions can be substantially different between manure and biochar-applied soils as the immediate availability of mineral N (NH_4^+ and NO_3^-) for nitrification and denitrification processes vary between these soils, the efficacy of NI may further be affected by the soil moisture level. Research is thus required to understand how soil moisture levels significantly affect the efficacy of NI in reducing N_2O emissions from biochar-amended soil for making an appropriate recommendation of the best management practices in order to mitigate global climate change.

In this study, we explored how manure-derived biochar interacts with nitrapyrin (NI) in influencing soil processes and the efficacy of NI in reducing N_2O emissions from a urea-fertilized Gray Luvisolic soil under two moisture levels (60 and 80% WFPS). The objectives of the study were to: (i) examine the effects of biochar and NI on soil properties, N availability and nitrification rates under two moisture levels in the soil over time, (ii) assess the changes in soil enzyme activities in response to biochar and NI applications under two moisture levels, and (iii) analyze the efficacy of NI in reducing N_2O emissions from a biochar-added soil under two moisture levels. In this study, we chose to produce biochar from manure as it is an easily available waste product of livestock farming in Alberta, Canada.

2. Material and methods

2.1. Soil and biochar

The soil was collected using a shovel from the upper 10 cm layer from 4 different places (approx. 50 m apart) in an agricultural land in Breton, Alberta (53°05'19"N, 114°26'29"W). The soil is classified as a Gay Luvisol (Canadian soil classification), a Boralf (USDA soil taxonomy), or an Albic/Gleyed Luvisol (FAO-WRB classification) (Lavkulich and Arocena, 2011) with a loam texture in the surface soil. The bulk density of the soil was determined using a steel corer (100 cm^3). The soil was brought to the laboratory in a cooler (at 4 °C) and passed through a 2-mm sieve after removing stones and visible roots. The soils collected from different places were then mixed well to form a composite sample. The sample was then kept in a refrigerator (at 4 °C), and the moisture content of subsamples of the fresh soil was determined by oven-drying the soil at 105 °C for 24 hours. A subsample of the fresh soil was air-dried and ground by a ball mill (Mixer Mill MM 200, Thomas Scientific, Swedesboro, NJ, USA) to determine total carbon (TC) and N (TN) concentrations using an elemental analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Germany). Other soil properties, including pH, electrical conductivity (EC), dissolved organic C (DOC) and N (DON), hot water extractable C (HWEC) and N (HWEN), available N (NH_4^+-N and NO_3^--N) were determined on the fresh soil (see below for the methods of soil analysis).

The biochar used in this experiment was produced from cattle manure pellet obtained from EarthRenew Corporation, Calgary, Alberta, Canada. The air-dried manure pellets were pyrolyzed in a batch drum carbonizer at InnoTech Alberta, Vegreville, Alberta, Canada. The pyrolysis temperature was 500-550 °C with a heating rate of 9-10 °C and residence time of 45 minutes after reaching the peak temperature. The pellet form of biochar was crushed to < 2 mm size before its use in the soil incubation experiment. The physical and chemical properties of the biochar were determined by methods similar to that used for the soil analysis (physical and chemical properties of the soil and biochar are provided in Table Appendix 5-1). The nitrification inhibitor used in this experiment was an eNtrench Nitrogen Stabilizer (Corteva Agriscience Canada, Calgary, AB, Canada) that had an active ingredient of 200 g nitrapyrin L^{-1}.

2.2. Experimental design and treatments

A laboratory incubation experiment was conducted to examine the effects of manure biochar and nitrapyrin on N_2O emission mitigation, nitrification rates and soil enzyme activities under two

soil moisture levels. The experiment used a factorial design with three factors: (i) manure biochar (with and without biochar added to the soil), (ii) NI (with and without nitrapyrin added to the soil) and (iii) soil moisture level (60 and 80% WFPS) on soil processes, each treatment was replicated four times. Biochar was applied at 30 g biochar kg^{-1} soil (equivalent to 34.2 t ha^{-1} of field application with biochar applied to the upper 10 cm at a soil bulk density of 1.14 g cm^{-3}), and NI was applied at the rate of 950 µg nitrapyrin kg^{-1} soil (equivalent to the rate of 2.7 liter ha^{-1} for field application recommended by the manufacturer). A urea solution was added to the soil at a rate of 90 mg N kg^{-1} soil in all treatments (equivalent to 102 kg N ha^{-1} of field application). The 60% WFPS was selected to ensure optimum moisture level for the microbial activities in the soil while maintaining aerobic condition and 80% WFPS was selected to represent the moisture level of the soil after rainfall event in which aerobic nitrification is limited by the availability of oxygen in the soil.

Two parallel sets of experiments were set up in this experiment, with one set for gas sampling and the other for destructive soil sampling. In the set of experiment used for destructive soil sampling, 40 g soil (oven-dry equivalent) was placed in each Nalgene HDPE bottle (250 mL). A total of 160 bottles were prepared for 5-time soil sampling (8 treatment combinations × 4 replications × 5 sampling times). For the set of experiment for gas sampling, a total of 32 Erlenmeyer flasks (250 mL) were prepared with 40 g soil in each flask. Then 1.2 g biochar was added and homogeneously mixed to the soil in the biochar-addition treatments (1.2 g soil was added in other treatments) before all the soils were incubated at 22 °C for 24 hours in the dark in an incubator. After pre-incubation, 1.9 and 1.96 mL of NI (diluted at 1:10000 with deionized water) was added to the soil uniformly over the surface of the soil in the biochar + NI addition and NI addition treatments, respectively. Soil moisture content was maintained at 60 and 80% WFPS by adding deionized water. The bottles were covered with aluminum foil perforated by a needle to allow oxygen exchange while minimizing water loss. The soil was then incubated in the dark at 22 °C in an incubator for 60 days. The moisture contents of the soil were maintained at 60 and 80% WFPS for the respective treatments throughout the incubation period by adding deionized water in every third day.

2.3. Gas sampling and calculation of nitrous oxide fluxes

Gas samples were collected from the flasks daily for the first 10 days, at every second day for another 10 days and at every third day for the remaining 40 days of the lab incubation. To minimize the effect of water addition on the GHG flux, water was added to the flasks after gas sampling to adjust for soil content. At each sampling time, the flasks were flushed with fresh air for 1 minute before taking a sample. The flasks were then sealed tightly with rubber septa, gas samples were collected at 0 (initial sample) and 22 hours (final sample) using a 20 mL syringe and were injected into 12 mL pre-evacuated soda glass Isomass Exetainers (Labco Limited, Lampeter, Wales, UK). The concentrations of N_2O in the gas samples were determined using a gas chromatograph (Varian CP-300, Varian Canada, Mississauga, Canada). The N_2O emission rates were calculated by the difference in N_2O concentrations between the initial and final concentrations of N_2O in each sampling interval. Cumulative N_2O emissions for the entire 60-day incubation period was calculated by linear extrapolation between consecutive measurements of N_2O emissions. To assess the change in N_2O emissions over time, the emission rates for different time intervals of incubation (0-5, 6-10, 11-20, 21-35 and 36-60 days of incubation) were calculated as the sum of N_2O emissions during that interval divided by the number of days in that interval.

2.4. Soil sampling and analysis

To determine soil physical and chemical characteristics and changes of soil processes over time, 32 bottles (8 treatment combinations × 4 replications) were randomly selected at 5, 10, 20, 35 and 60 days after incubation. Soil pH and EC were determined in soil-water solution in a 1:5 (w:v) ratio using a pH/EC meter (DMP-2 mV, Thermo Orion, USA). Hot water-soluble C was analyzed following the method described in Ghani et al. (2003), with the C concentration determined by a TOC analyzer (TOC-V_{CSN}, Shimadzu, Kyoto, Japan). For the measurement of available N (exchangeable NH_4^+-N and NO_3^--N) concentrations, soil samples were extracted with 2M KCl in 1:5 (w:v/soil: KCl solution) after shaking for 1 hour (200 rpm) in a shaker. Exchangeable NH_4^+-N and NO_3^--N concentrations in the soil extracts were determined by a colorimetric method (Doane and Horwath, 2003; Keeney and Nelson, 1982). Net nitrification rates for different time intervals were calculated by the difference in NO_3^--N concentrations in the soil between initial and final samplings divided by the number of days of lab incubation in

that interval. The net total nitrification rate for the entire incubation period was determined by the difference in NO_3^--N concentrations in soil at the beginning and end of the incubation divided by the total length of the incubation.

The activities of β-1,4-N-acetyl glucosaminidase and urease (soil extracellular enzymes involved in the N cycling processes) were analyzed by a fluorimetric method using 4-methylumbelliferyl (MUF) as a substate (German et al., 2011; Sinsabaugh et al., 2003) and a colorimetric method using urea as a substrate (Sinsabaugh et al., 2000), respectively. Briefly, 1 g of soil was taken in a 125 mL Nalgene HDPE bottle, and 100 mL of 50 mM sodium acetate buffer (pH = 5.5) was added to the soil. The mixture was then shaken for 1 hour in a shaker (at 200 rpm) and poured onto a bowl with a magnetic stirrer. For determining β-1,4-N-acetyl glucosaminidase (NAG) activities, an aliquot of 200 µL soil suspension was pipetted into 16 wells of black 96 plates; 50 µL of 200 µM MUF substrate (4-MUF-N-acetyl-β-glucosaminide) was added into the first 8 wells (soil assay wells), 50 µL of the buffer solution into 4 wells (soil background wells) and 50 µL of 4-MUF in the last 4 wells (soil quench wells) of those 16 wells with soil suspension. This was repeated for all the soil samples for analysis in soil assay plates. In addition, 200 µL of the buffer solution was pipetted into 12 wells, in which 50 µL of the substrate in the first 4 wells (substrate background wells), 50 µL of 4-MUF in the next 4 wells (standard wells) and 50 µL of buffer was added into the last 4 wells (buffer background wells) in the control plate. The plates were incubated at 22 °C in the dark for 3 hours. The fluorescence in the soil suspension and control was measured by a microplate reader (Synergy HT, Bio-Tek Instruments, Winoosky, VT, USA) with 365 nm excitation and 450 nm emission filters after the reaction in the wells was stopped by adding 20 µL of 0.5 M NaOH solution in each well during fluorescence measurement.

For determining the urease activity, an aliquot of 200 µL soil suspension was pipetted into 12 wells of clear 96 plates, 30 µL of urea substrate (150 mM) was pipetted into the first 8 wells (soil assay wells), yielding a final urea concentration of 20 mM, and 30 µL of MQ water was pipetted into the other 4 wells (negative control wells) of the 12 wells with soil suspension. A 200 µL of the buffer and 30 µL of the urea substrate were also pipetted into another 16 wells (substrate control wells). A 200 µL of six standard ammonium chloride (NH_4Cl) solutions ranging from 0-20 µM (0-1080 µg L^{-1}) were pipetted into 8 wells (for each standard solution), and 30 µL of MQ water was added into the NH_4Cl solution (standard wells) in a separate clear

96 plate. The plates were incubated at 22 °C in the dark for 18 hours. For the measurement of ammonium concentration in the soil assay, for the negative control and substrate control, 40 µL of salicylate and cyanurate reagents (Hach Canada, London, Ontario, Canada) were added into the wells, and the absorbance was read at 610 nm by the Synergy microplate reader described earlier.

2.5. Statistical analyses

A repeated-measures analysis of variance (ANOVA) was used to test the treatment effects over time using the PROC MIXED Procedure in SAS (version 9.4, SAS Institute Inc., NC, USA). The treatments were used as fixed effects and sampling times as the repeated measures variables in the data analysis. Data were checked for normality of distribution using Shapiro-wilk test in the residuals prior to ANOVA; reduced cumulative N_2O emissions at 80% WFPS were log-transformed to meet the assumption of normality of distribution; however back-transformed data are presented in the results section. A three-way ANOVA was performed to test the effects of biochar, NI, moisture and their interactions on soil properties, net nitrification rates, enzyme activities and the cumulative N_2O emissions. Statistical significance was set at $\alpha = 0.05$ with mean separation using the LSD test.

3. Results

*3.1 Soil pH, electrical conductivity, and hot water extractable carbo*n

Biochar addition and moisture level, but not NI addition or the interaction among those factors, significantly affected soil pH and EC (Table 5-1). Under both moisture levels, biochar increased soil pH and EC (Table 5-2). Soil pH was greater at 80 than at 60% WFPS, but EC was lower at 80 than at 60% WFPS. Soil pH decreased and EC increased over time in the incubation regardless of the treatment (Appendices 5-2 and 5-3). Soil moisture level had significant effects on HWEC, with HWEC greater at 80% than at 60% WFPS (Tables 5-1 and 5-2).

3.2. Available N and net nitrification rates

Following urea application, exchangeable NH_4^+-N concentration in the soil was significantly changed over time regardless of the treatment, and the changes were significantly different

among treatments within each sampling time (Fig.5-1). Biochar and NI additions, and soil moisture level, with their two-way but not three-way interaction, significantly affected NH_4^+-N concentrations. Compared to the control, biochar and biochar + NI decreased NH_4^+-N concentrations across the samplings by 40.9 and 37.3%, respectively, at 60% WFPS, and 56.7 and 43.1%, respectively, at 80% WFPS, while NI increased the concentrations by 22.1 and 23.3% at 60 and 80% WFPS, respectively (Table 5-2). The concentrations of NO_3^--N were also changed significantly by all three treatments and their two-way interactions (Table 5-1) with greater concentrations at 60 than at 80% WFPS across the samplings (Fig. 5-1). Biochar increased NO_3^--N concentrations by 28.6% while NI decreased it by 20.2% at 80% WFPS; their effects (as compared to the control) were not significant at 60% WFPS (Table 5-2).

Nitrification rates varied significantly over incubation time with the highest rates in the first five days; the rates then consistently decreased until the end of incubation across all treatments (Fig. 5-2). The rates were decreased by biochar ($P < 0.001$) and NI additions ($P = 0.007$), and moisture level ($P < 0.001$). The rates were also affected by biochar × moisture level ($P = 0.02$) and biochar × NI × moisture level interaction (0.031) across the samplings (Table 5-1). Biochar significantly increased the rate on day 5 sampling and decreased it on day 35 and 60 samplings in both moisture levels; biochar was more effective in reducing the rates when the moisture availability was low (Fig. 5-2). Combined application of biochar and NI yielded an additional reduction to that of biochar application alone at 80% WFPS but not at 60% WFPS in overall nitrification rates for entire incubation.

3.3 β-1,4-N-acetyl glucosaminidase and urease activities

The activities of NAG were significantly affected by biochar, NI, moisture level, and biochar × moisture level interaction (Table 5-1); biochar decreased NAG activities by 12.3% at 80% WFPS but did not affect NAG activities at 60% WFPS across the samplings (Table 5-2). The activities of NAG increased over time across biochar and NI application treatments at both moisture levels (Appendix 5-4, Fig. 5-3). Biochar was more effective in decreasing NAG activities at high than at low moisture levels across sampling times with a significant decrease in the activities on all sampling times at 80% WFPS and only on the first two sampling times at 60%WFPS (Fig. 5-3). The mean NAG activities of all sampling times were greater at 80% than at 60% WFPS. Urease activities were also affected by biochar ($P < 0.001$) and soil moisture level ($P = 0.003$) but not by

NI (P = 0.626) and their interactions (Table 5-1). Biochar significantly increased the activities at low but not at high moisture level. Urease activities were greater at 60% than at 80% WFPS and the activities were also changed over time of incubation (Appendix 5-4, Fig. 5-3).

3.3 Nitrous oxide emissions

Soil N_2O emissions fluctuated over the 60-day incubation regardless of the treatment (Fig.5- 4). The emissions reached the peak on day 3 and rapidly decreased until day 10 of incubation then remained relatively stable for the remaining 50 days of the incubation (Fig. 5-4). Biochar did not change (P = 0.839) but NI decreased (P < 0.001) and soil moisture level changed (P = 0.017) cumulative N_2O emissions with significant interactions between biochar and NI, and between NI and moisture (Table 5-2). Biochar did not affect N_2O emissions at 80% WFPS but reduced it by 1.57% at 60% WFPS. Application of NI decreased cumulative N_2O emissions by 31.8% across the moisture levels with a greater reduction of 37.4% at high than 25.1% at low moisture levels as compared to the control (Fig. 5-4). Reduced cumulative N_2O emissions (the difference between the treatments and the control) was significantly lower in biochar + NI than in NI alone at 60% WFPS (Appendix 5-4). Overall, N_2O emissions in the first ten days of incubation account for 72.4 and 76.5% of the total emissions across all treatments at 60 and 80% WFPS, respectively (Figs. 5-4 and 5-5).

4. Discussion

This study evaluated the effects of manure biochar, NI and moisture levels on soil properties, enzyme activities, nitrification rates and N_2O emissions from a cropland soil and showed that these soil processes were affected by the treatments in the incubation. The results also revealed that N_2O emission increases with increase in moisture level from 60 to 80% WFPS; application of biochar and NI are effective practices in reducing N_2O emissions from the soil while applied separately with their effects depending on soil moisture level, however the efficacy of NI in reducing N_2O emissions from the soil are substantially decreased while applied with biochar under both moisture levels.

4.1. Effects of biochar, nitrapyrin and moisture level on soil properties, available N and nitrification rates

Urea application increased soil pH regardless of the treatment due to the hydrolysis of urea and the decrease of the pH over time was due to H^+ produced from nitrification (Barak et al., 1997; Bolan and Hedley, 2003). The alkaline nature (pH = 9.8) of the biochar used in this experiment (Table S1) caused soil pH to increase by biochar addition. Increases in soil pH and SOC by biochar addition improve the quality of the Gray Luvisolic soil which has low pH and SOC, where microbial C and N mineralization are often limited by these factors. Soluble cations such as Ca^{2+}, Mg^{2+}, K^+ and anions such as HCO_3^-, CO_3^{2-} and OH^- are released from the biochar to increase EC in the biochar-added soil (Wang et al., 2015a). Volatile organic matter contained in the biochar can increase HWEC initially, the effect did not persist for long as the volatile organic matter is metabolized by soil microorganisms.

Hydrolysis of urea added to the soil resulted in the peak of NH_4^+-N concentration on day 5, then the concentration decreased over time in all treatments as NH_4^+ is oxidized to hydroxylamine by ammonia monooxygenase (Di and Cameron, 2016). The lower NH_4^+-N concentration in biochar-added soil might be associated with potential adsorption of NH_4^+ on the biochar surface (Zheng et al., 2012) and NH_3 volatilization due to the increase in soil pH (He et al., 2018). Although NH_4^+-N concentrations in the control treatment were greater in the soil at 60 than at 80% WFPS, the effect of soil moisture was opposite under biochar-added treatment indicating a greater impact of biochar in oxidizing NH_4^+-N at high than at low WFPS due to the greater aeration in the latter. Consistent higher NH_4^+-N concentrations in NI applied soil in both moisture levels suggest that NI can reduce nitrification rates effectively in a wide range of moisture conditions. The concentration of NO_3^--N was increased by biochar addition at the early stage of the incubation but was decreased on day 60. Higher concentration of NO_3-N in the biochar-added soil in early days of incubation makes the soil more susceptible to denitrification or NO_3^--N leaching under high moisture condition (Borchard et al., 2019).

Biochar affected net nitrification rates independent of NI across the moisture levels. Biochar's effect on nitrification rate has been shown to be dependent on biochar type and the soil environment, including pH and moisture availability, as they change the availability of substrates for nitrification (Cayuela et al., 2014; Clough et al., 2013). The decrease in overall net nitrification rate by biochar addition for the entire incubation period in this study was caused by

the decrease in the availability of NH_4^+-N as a substrate for nitrification (Yang et al., 2021) and perhaps by the decrease in the abundance and diversity of ammonia-oxidizing bacteria (AOB) caused by the potential phenolic compounds present in the biochar (Wang et al., 2015b). However, a short-term increase in nitrification rate by biochar with urea input at the beginning of incubation was probably associated with its priming effects on nitrifiers' activities (Fiorentino et al., 2019). The longevity of the effect of nitrapyrin in reducing nitrification rates varies with the type of NI, soil temperature and management practices (Cui et al., 2013; Lin et al., 2021). In this study, nitrapyrin reduces the rates only on day 5 and 10, suggesting that its effects in inhibiting nitrification rates in Gray Luvisolic soil were short-lived. The apparent loss of NI's inhibitory effects in biochar-added soil on day 5 and 10 was due to adsorption of NI on biochar surface (Keiblinger et al., 2018; Li et al., 2015).

4.*2. Effects of biochar, nitrapyrin and moisture level on soil enzyme activities*

Recent meta-analyses have shown that biochar increases NAG and urease activities in the soil due to improvement in nutrient supply and liming effects of biochar (Pokharel et al., 2020; Zhang et al., 2019). However, in this study, biochar decreased NAG activity despite the increase in soil pH, possibly due to the increase in EC which can have negative effects on microbial biomass and abundance, and enzyme activities involved in N cycling (Chen et al., 2017; Reitz and Haynes, 2003). In addition, the reduction of NAG activity has also been attributed to the adsorption of the enzyme or the substrate on biochar surfaces (Bailey et al., 2011). Although several feedback mechanisms have been suggested to regulate NAG activities in the soil, the contrasting effects of biochar and NI manifest an inverse relationship between NAG activities and N availability under these treatments (Allison and Vitousek, 2005; Sinsabaugh et al., 2009). The increase in urease activity by biochar amendment in the Gray Luvisolic soil supports results in global meta-analyses (Pokharel et al., 2020; Zhang et al., 2019). The greater activities of urease in the biochar-added soil, as well as the increase of its activity over time, corresponding to the decrease in NH_4^+-N concentration, support the product inhibition mechanism that controls urease activity in this soil (Sinsabaugh and Shah, 2012). However, the decrease in urease activity over time in high WFPS irrespective of biochar and NI application suggests other potential factors such as soil pH and water potential playing dominant roles in determining urease activity in the soil (Sinsabaugh and Shah, 2012).

4.3. Effects of biochar, nitrapyrin and moisture level on N_2O emissions from the soil

The decrease in N_2O emissions by biochar addition at low WFPS was caused by the increase in soil pH that likely increased the *nos*Z and *nir*K gene abundances and their functional N_2O reductase enzyme activities with subsequent increase in the reduction of N_2O to N_2 during denitrification (Aamer et al., 2020; Liu et al., 2014). However, under high WFPS, a lower increase in soil pH by biochar addition (as compared to that of low WFPS) was probably not enough to bring sufficient change in processes described above to achieve a significant reduction in N_2O emissions by biochar addition in comparison to the control. Short-term increase in N_2O emissions (in the first five days of incubation) in biochar-amended soil corresponds to the addition of volatile organic matter added by biochar as an extra source of electron donor in the denitrification process, which was quickly mineralized by microorganisms (Butterbach-Bahl et al., 2013).

Nitrification inhibitor decreased cumulative N_2O emissions under both soil moisture levels indicating that NI is effective in reducing N_2O emissions at different levels of oxygen availability in the soil. Application of NI delays ammonia oxidation (the first step of N transformation of urea) by inhibiting ammonia monooxygenase activity with the reduction of population of AOB (Fuertes-Mendizábal et al., 2019) when oxygen availability is not a limiting factor at 60% WFPS in which aerobic nitrification is the dominant process of N_2O production (Cui et al., 2013; Menendez et al., 2012). In the 60% WFPS treatment, delaying this step of N transformation contributes to slowing down of decomposition of intermediate products such as hydroxylamine or nitrite which produces N_2O by autotrophic ammonia oxidizers (Wrage et al., 2001). The greater reduction in N_2O emissions at 80% WFPS than at 60% WFPS by NI application suggests its additional effects on denitrification process which contributes substantially in total N_2O production under less aerobic condition of 80% WFPS. The decreased rate of NO_3^- production through inhibiting ammonia monooxygenase activity ultimately affects the denitrification process where NO_3^- undergoes stepwise reduction to NO_2^-, NO, N_2O and N_2, with N_2O as an intermediate product (Di and Cameron, 2016). Thus, the inhibition of the rate-limiting step of nitrification, i.e., ammonia oxidation by NI application, was effective in reducing N_2O emissions at low as well as high WFPS.

The results also showed that there was a greater reduction in cumulative N_2O emissions by NI than by biochar application in a 60-day incubation suggesting that NI could be more effective than biochar for short-term mitigation of N_2O emissions. However, it must be noted that the results can be different in the long-term study as biochar continuously decreased N_2O emissions until the end of this experiment, while NI appeared to be effective only in the first few days of application. The short-term effect of NI has been attributed to the microbial degradation of it in the soil (Balaine et al., 2015), while the recalcitrant nature of biochar makes it stable in the soil to effectively reduce N_2O emissions for a long time (Woolf et al., 2010). The effect of NI in reducing N_2O emissions was found to be dependent on biochar addition with a greater reduction in non-added than biochar-added soil under both moisture levels; the result was similar to that of Fuertes-Mendizábal et al. (2019) in which DMPP was found to significantly interact with biochar in reducing N_2O emissions from a grassland soil. Combined application of NI with biochar showed an antagonistic effect as N_2O emissions reduction in their combined application was much lower than the sum of those reductions from their separate applications. The counteracting effect of biochar on the efficacy of NI in reducing N_2O emissions was likely due to the potential effects of biochar on degradation, adsorption, and volatilization of NI that prevented it from inhibiting ammonia monooxygenase activity (Fuertes-Mendizábal et al., 2019; Keiblinger et al., 2018). Biochar likely accelerated the degradation of NI by increasing soil pH and soil aeration that can have direct impact on NI degradation (Balaine et al., 2015; Li and Chen, 2020; Touchton et al., 1979). The increase in soil pH by adding biochar could be beneficial for microbial activities in acidic soil, however its apparent negative impact on NI degradation will likely reduce the efficacy of NI. Selection of appropriate feedstock and pyrolysis conditions during biochar production can be helpful to minimize the impact of biochar in soil pH and aeration which could potentially reduce its impact on NI degradation. Zhang et al. (2020) has shown spontaneous endothermic reaction accompanied by physical adsorption as the mechanism of adsorption of NI on soil and demonstrated that adsorption capacity increases with soil organic matter (SOM) content. Biochar's role in increasing SOM content might have caused substantial increase in adsorption of NI thereby reducing its availability for inhibiting ammonia monooxygenase activity in the soil leading to negative impact on its efficacy in mitigating N_2O emissions. Biochar is known for its high organic pollutant adsorption capacity (Dai et al., 2019), further studies are thus required to explore its effect on NI adsorption and to validate potential

role of biochar on decreasing NI availability through adsorption on its surface. The benefits from prolonged effects of biochar (vs short-term effect of NI) in reducing N_2O emission outweigh the benefits of applying NI suggesting land management with biochar application could be more appropriate than applying NI in terms of N_2O emission mitigation from the cropland.

5. Conclusions

This study concludes that the application of manure biochar and NI can be effective management practices in mitigating N_2O emissions in the studied Gray Luvisolic soil despite substantial differences in the magnitude of their effects between 60 and 80% WFPS. Significant variations in affecting N availability, net nitrification rate and soil enzyme activities over time demonstrate that various mechanisms were involved in reducing N_2O emissions from NI and biochar-added soils under two soil moisture levels. Although NI appeared to be more effective than biochar in reducing N_2O emissions in this short-term study, we should not overlook the persistent effect of biochar in reducing N_2O emissions for the longer period before reaching to any conclusion in comparing their efficacies. Application of NI in biochar-added soil showed that biochar reduced the efficacy of NI under both moisture levels. Biochar is applied to the cropland once in several years, while NI is applied every time with N fertilizer application, NI may interact differently between fresh and aged biochars. So, the efficacy of NI in biochar-amended soil should be further examined in long-term research that allows us to assess NI interaction with aged biochar and the long-term effect on reducing N_2O emissions from agricultural soils.

Table 5-1. Three-way ANOVA (F and P values) for the effects of manure biochar, nitrification inhibitor and moisture regime in soil properties, enzyme activities and nitrous oxide emissions.

	Biochar (MB)		**Nitrapyrin (NI)**		**Moisture (M)**		**MB X NI**		**MB X M**		**NI X M**		**MB X NI X M**	
Variable	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
pH	4018.06	**<0.001**	3.05	0.095	952.04	**<0.001**	1.18	0.289	105.42	**<0.001**	0.68	0.418	1.14	0.297
EC	5093.21	**<0.001**	3.37	0.081	989.04	**<0.001**	0.20	0.659	3.07	0.094	0.02	0.884	0.01	0.988
HWEC	1.93	0.179	0.36	0.555	91.69	**<0.001**	1.36	0.256	1.84	0.189	0.73	0.402	10.82	**0.004**
NH_4^+-N	641.08	**<0.001**	52.02	**<0.001**	152.22	**<0.001**	8.63	**0.007**	55.05	**<0.001**	6.83	**0.016**	0.13	0.725
NO_3^--N	27.04	**<0.001**	8.53	**0.008**	694.17	**<0.001**	0.03	0.865	19.83	**<0.001**	27.52	**<0.001**	0.40	0.531
Nitrification	144.96	**<0.001**	8.95	**0.007**	44.68	**<0.001**	0.07	0.787	5.61	**0.027**	3.16	0.091	5.36	**0.031**
NAG	21.52	**<0.001**	8.91	**0.007**	76.46	**<0.001**	0.20	0.661	8.74	**0.007**	0.82	0.374	0.18	0.673
UR	41.72	**<0.001**	0.24	0.626	10.56	**0.003**	0.36	0.556	2.81	0.108	0.05	0.827	0.48	0.495
Cum. N_2O	0.04	0.839	23.00	**<0.001**	6.80	**0.017**	15.94	**0.007**	0.03	0.868	5.22	**0.033**	0.03	0.854

Abbreviations: EC, electrical conductivity; HWEC, hot water extractable carbon; NH_4^+-N, exchangeable ammoniacal-nitrogen concentration; NO_3^--N, nitrate-nitrogen concentration; NAG, β-1,4-N-acetyl glucosaminidase activities; UR, urease activities; Cum. N_2O, cumulative nitrous oxide emissions.

P values in the bold indicate a significant effect at $\alpha = 0.05$.

Table 5-2. Mean (SE) of soil pH, electrical conductivity (EC), hot water extractable carbon (HWEC), exchangeable ammoniacal-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), β-1,4 N-acetyl glucosaminidase (NAG) and urease (UR) activities in different treatments calculated from five sampling times over a 60-day incubation

Treatment	pH	EC	HWEC	NH_4^+-N	NO_3^--N	NAG	UR
		μS cm^{-1}	mg kg^{-1}			nmol g^{-1} soil h^{-1}	
60% WFPS							
CK	5.51(0.01)c	168.89(2.84)b	382.55(8.92)a	12.48(0.27)b	67.88(1.85)	29.59(0.65)	70.98(8.04)b
MB	6.60(0.01)a	328.65(1.70)a	330.77(9.93)b	7.37(0.14)c	68.19(1.73)	28.13(0.37)	115.74(12.09)a
NI	5.58(0.01)b	171.72(1.23)b	360.96(14.81)ab	15.24(0.21)a	69.89(0.74)	30.28(0.77)	72.99(8.38)b
MB+NI	6.60(0.01)a	333.62(2.64)a	373.27(3.77)a	7.82(0.14)c	71.21(0.75)	29.81(0.89)	116.7(8.38)a
80%WFPS							
CK	6.13(0.05)b	91.81(4.17)b	424.13(8.52)	18.6(0.50)b	39.52(1.15)b	35.52(1.37)ab	65.00(4.82)b
MB	6.89(0.01)a	259.74(5.44)a	439.16(5.63)	8.05(0.67)d	50.85(2.29)a	31.16(0.88)c	83.99(11.53)ab
NI	6.14(0.01)b	95.39(3.52)b	437.54(9.76)	22.94(0.84)a	31.56(0.88)c	37.73(0.94)a	61.87(11.34)b
MB+NI	6.90(0.01)a	265.32(2.79)a	422.07(13.25)	10.58(0.53)c	41.13(1.82)b	33.40(0.73)bc	94.89(12.11)a

Treatment codes are: CK, unamended control; MB, manure biochar addition; NI, nitrification inhibitor addition; MB+NI, manure biochar and nitrification inhibitor addition. Different letters indicate significant differences between the biochar and nitrification inhibitor treatments within each WFPS treatment ($P < 0.05$). Values without letters are not significantly different between the biochar and nitrification inhibitor treatments within each WFPS treatment.

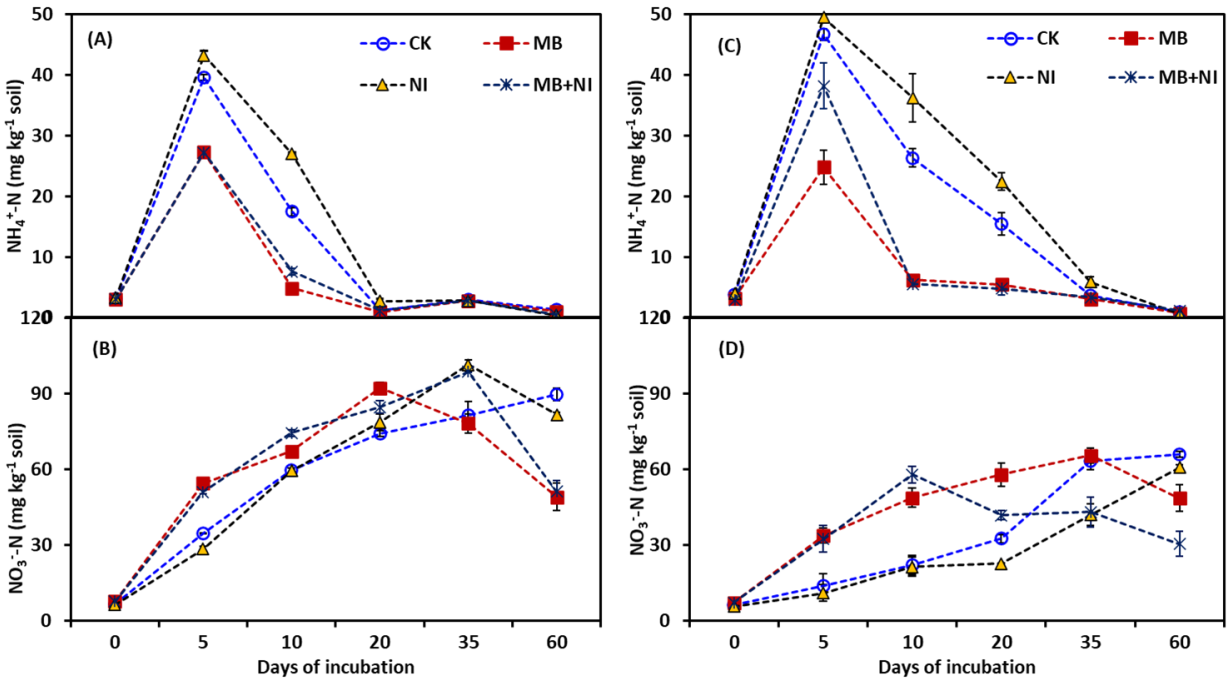

Fig. 5-1. Effects of manure biochar and nitrification inhibitor on exchangeable ammonium (NH_4^+-N) and nitrate nitrogen (NO_3^--N) concentrations in the soil under two moisture contents over a 60-day incubation period. (A) and (B) at 60% WFPS, and (C) and (D) at 80% WFPS. Treatment codes are CK: control (no manure biochar and nitrification inhibitor added), MB: manure biochar added, NI: nitrification inhibitor added, MB+NI: manure biochar and nitrification inhibitor added. Error bars show the standard error of the mean (n = 4).

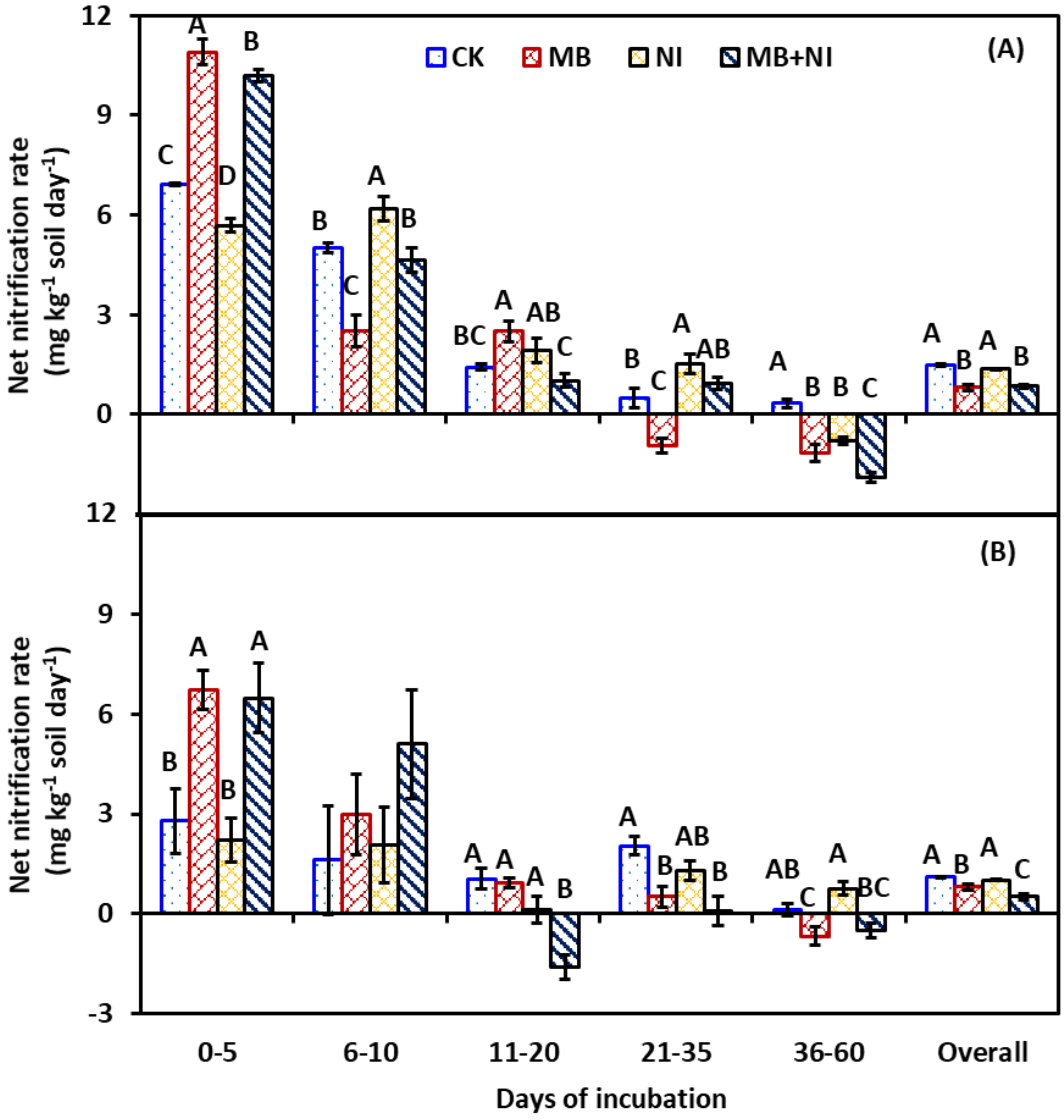

Fig. 5-2. Net nitrification rates at different sampling time over a 60-day incubation period in the soil at 60% WFPS (A) and (B) 80% WFPS. Different letters within each sampling time represent significant differences among the treatments. Treatment codes are CK: control (no manure biochar and nitrification inhibitor added), MB: manure biochar added, NI: nitrification inhibitor added, MB+NI: manure biochar and nitrification inhibitor added. Error bars show the standard error of the mean (n = 4).

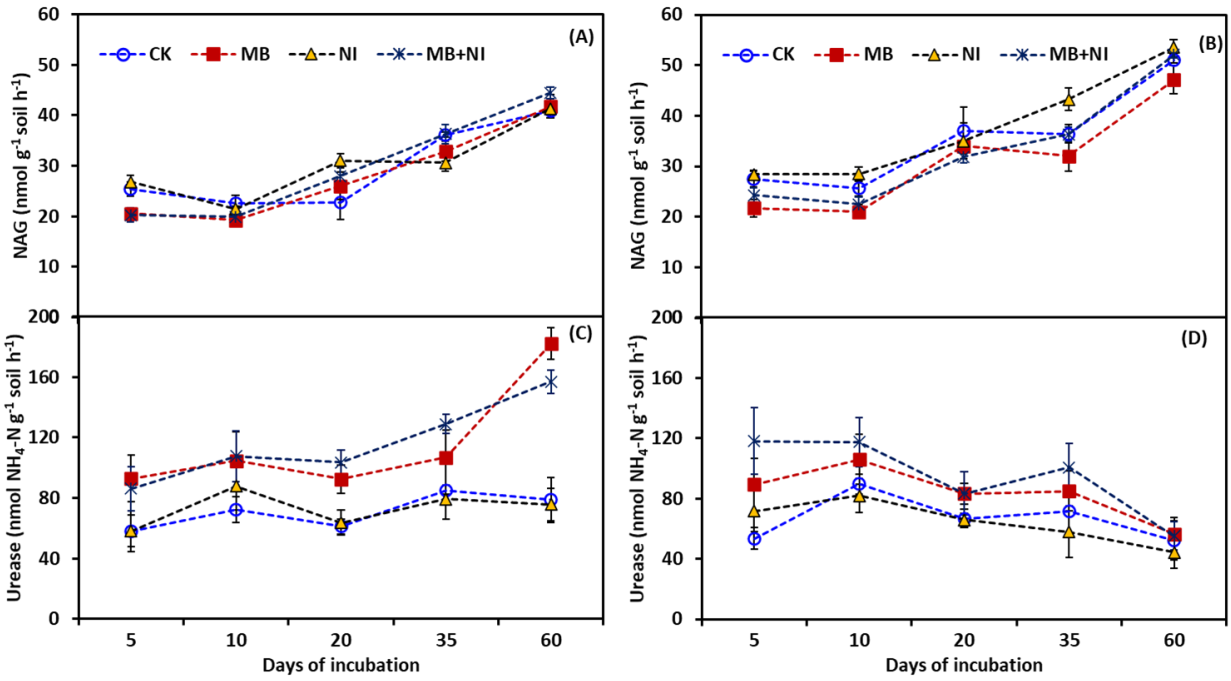

Fig. 5-3. Effects of manure biochar and nitrification inhibitor on soil enzymes (β-1,4 N-acetyl glucosaminidase (NAG) and urease (UR)) activities under two soil moisture contents over a 60-day incubation period. (A) and (C) at 60% WFPS, and (B) and (D) at 80% WFPS. Treatment codes are CK: control (no manure biochar and nitrification inhibitor added), MB: manure biochar added, NI: nitrification inhibitor added, MB+NI: manure biochar and nitrification inhibitor added. Error bars show the standard error of the mean (n = 4).

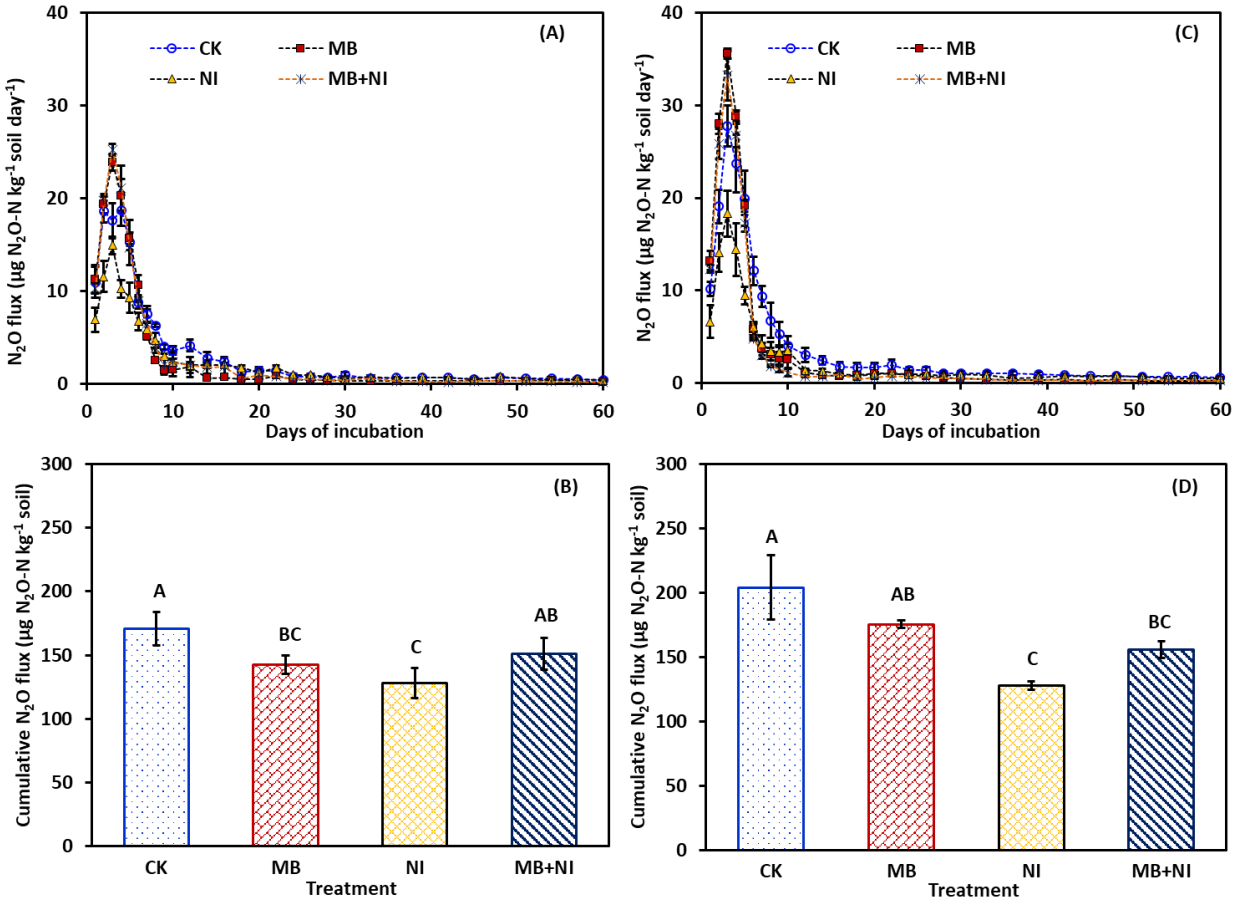

Fig. 5-4. Effects of manure biochar and nitrification inhibitor on N_2O emissions from the soil under two moisture contents over a 60-day incubation period. (A) and (B) are dynamics and cumulative N_2O emissions at 60% WFPS, (C) and (D) are dynamics and cumulative N_2O emissions at 80% WFPS. Different letters in the cumulative N_2O emissions represent significant differences among the treatments. Treatment codes are CK: control (no manure biochar and nitrification inhibitor added), MB: manure biochar added, NI: nitrification inhibitor added, MB+NI: manure biochar and nitrification inhibitor added. Error bars show the standard error of the mean (n = 4).

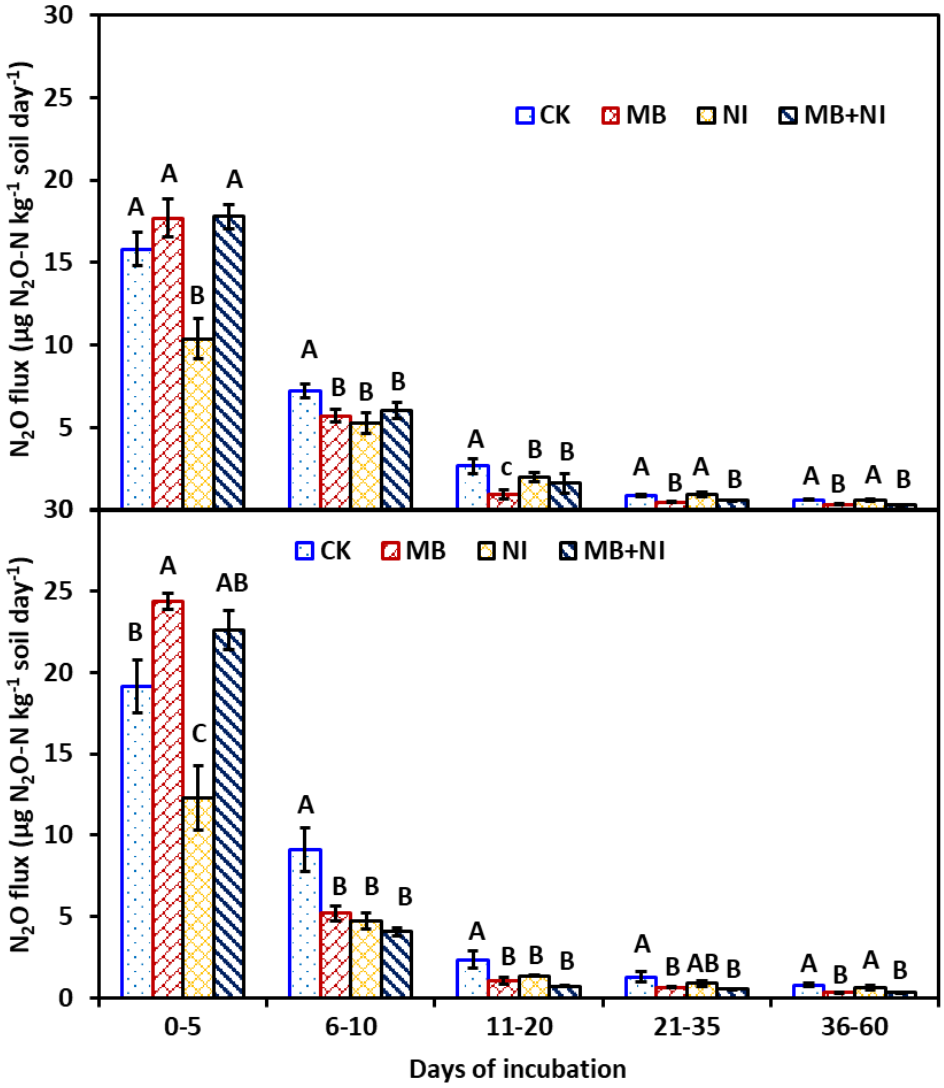

Fig. 5-5. N_2O emissions from the soil calculated for different intervals of time corresponding to the soil sampling time over a 60-day incubation period at 60% WFPS (A) and at 80% WFPS (B). Different letters within each sampling time represent significant differences among the treatments. Treatment codes are CK: control (no manure biochar and nitrification inhibitor added), MB: manure biochar added, NI: nitrification inhibitor added, MB+NI: manure biochar and nitrification inhibitor added. Error bars show the standard error of the mean (n = 4).

Chapter 6. Biochar decreases and nitrification inhibitor increases phosphorus limitation for microbial growth in a wheat-canola rotation

1. Introduction

Different management practices affect soil microbial and ecoenzymatic activities that alter soil organic carbon (SOC) mineralization and soil fertility. Application of biochar and nitrification inhibitors (NI) to cropland are common agricultural management practices recommended for reducing greenhouse gas (GHG) emissions and increasing crop production via changing microbial community compositions (Akiyama et al., 2010; Jeffery et al., 2011; Zhang et al., 2020); however, their impacts on ecological stoichiometry that relates elemental ratios of resources to microbial biomass and ecoenzymatic activities (Schimel and Weintraub, 2003; Griffiths et al., 2012) are not well studied. Ecological stoichiometry can indicate the direction of shift between limitations of carbon (C) and nutrients such as nitrogen (N) and phosphorus (P) for microbial growth and activities. How these management practices affect microbial nutrient limitations in agricultural soils and their subsequent impact on soil fertility and crop production have rarely been studied.

Stoichiometric imbalances between the availability of resources (energy and materials) and microbial metabolism lead to C or nutrient limitations for microbial activities responsible for organic matter decomposition (Tapia-Torres et al., 2015; Cui et al., 2019). According to the ecological stoichiometric theory, microbial metabolism is directly affected by the relative availability of resources (e.g., C, N and P) in the soil. For instance, microbial growth is limited when the availability of N or P falls below the threshold level for optimum microbial growth. When the availability of these nutrients is limited, microorganisms secrete ecoenzymes to decompose complex organic materials such as plant and microbial cell walls into soluble substrates, which are then available for microbial assimilation and plant uptake (Nannipieri et al., 2002; Allison and Vitousek, 2005). Microbial growth and enzymatic activities have a negative feedback mechanism that is regulated by the allocation of their resources to synthesize C, N and P-acquiring enzymes, depending on their relative availability in the soil (Sinsabaugh and Moorhead, 1994; Schimel and Weintraub, 2003). When the microbial demand for a nutrient (e.g., N or P) increases or the availability of that nutrient becomes limiting for microbial growth, microbes allocate more resources to synthesize the enzyme which accelerates the release of

nutrients from organic materials and brings changes in the ecoenzymatic stoichiometry (Sinsabaugh and Moorhead, 1994; Sinsabaugh et al., 2014). Thus, understanding the relationships among resources, microbial biomass and ecoenzymatic stoichiometry can provide insight into the limitation and availability of nutrients for microbial growth and soil fertility in agricultural soils.

Application of biochar (a recalcitrant C produced by pyrolysis of biomass in the absence of oxygen) can increase soil nutrient availability for crop production via enhanced C and nutrient mineralization through the priming effect (Wang et al., 2016). The increased nutrient availability can also enhance microbial metabolism in the soil (Ding et al., 2016; Zhou et al., 2017). Biochar's positive effects on microbial growth, microbial biomass (C and N) and ecoenzymatic activities have been demonstrated in several experimental (Pukalchik et al., 2018; Song et al., 2018) and meta-analysis studies (Pokharel et al., 2020; Zhang et al., 2019). Similar effects of biochar have been reported on microbial biomass P (MBP) (Gao et al., 2018), although a few studies have reported on biochar effects on MBP. There is a lack of understanding of how biochar shifts limitations between C and nutreints for microbial growth and activities. In a recent study, Chen et al. (2022) analyzed ecoenzymatic stoichiometry and demonstrated that biochar increased soil microbial C and N, and decreases P limitations, and there were large variations in these effects among biochar types and soil characteristics with the strongest impact from a wood biochar on a soil with low organic C (< 20 g kg^{-1}) in their study.

Nitrification inhibitors are commonly applied to reduce nitrification rates and soil N_2O emissions and increase soil N (in the ammonium form) availability and nutrient use efficiency (Bremner and Yeomans, 1986; Akiyama et al., 2010; Pokharel and Chang, 2021). There is a potential for NI to change microbial biomass N and activities of N-acquiring enzymes due to increases in soil N availability. We need to explore the impact of NI application on ecoenzymatic stoichiometry and microbial C and nutrient limitations. It is also possible for both biochar and NI to be applied to the same field in the same growing season given the somewhat different purposes of applying biochar and NI in crop fields. We therefore need to understand how biochar and NI affect ecoenzymatic stoichiometry and microbial C and nutrient limitations when they are applied together. Some previous studies have reported lack of (Li et al., 2021) or negative (Keiblinger et al., 2018; Fuertes-Mendizábal et al., 2019; Li and Chen, 2020) impacts of biochar on the efficacy of NI on soil N mineralization and GHG emissions; the negative impacts were

attributed to biochar's potential of adsorption and degradation of NI. However, it is not clear whether biochar interacts with NI in affecting microbial and enzymatic activities and their stoichiometry in alleviating nutrient limitations for microbial metabolism in agricultural soils.

The main objectives of this study were to understand the stoichiometric characteristics of C, N and P in soil and explore nutrient limitations for microbial metabolism in response to agricultural management practices. In this two-year field study, we assessed the interactive effects of biochar and nitrapyrin (a commonly used NI) on microbial biomass C, N and P, activities of extracellular enzymes (C-, N- and P-acquiring enzymes), and their stoichiometry to understand C and nutrient limitations of microbial metabolism in an agricultural soil. Biochar used in this study was produced from locally available manure in Western Canada that had been composted for one year before pyrolysis. We hypothesized that biochar amendment and NI applied to an agricultural soil: (i) increase the availability of soil nutrients (N and P) for microbial metabolism and plant uptake, (ii) increase soil microbial biomass with a significant change in microbial stoichiometry caused by a disproportionate increase among microbial biomass C, N and P, and (iii) affect ecoenzymatic (C-, N- and P-acquiring) activities and their stoichiometry with subsequent change in C and nutrient limitations for microbial metabolism.

2. Material and methods

2.1. Field experiment

The field experiment was set up at the Breton research Plots, a long-term agricultural research site near the town of Breton (53.0895° N, 114.4408° W), Alberta, Canada, in 2019. The area is characterized by long cold winters and short warm summers with a mean annual temperature of 3.59 °C and a mean annual precipitation of 535.2 mm based meterological data between 1991 and 2020. Historically the area was planted with wheat (*Triticum aestivum* L.), canola (*Brassica napus* L)., and barley (*Hordeum vulgare* L.) in rotation. The soil in the research plots is a Gray Luvisol (Canadian Soil Classification System) with a slightly acidic pH and a loam texture (Appendix 6-1). During the 2-year study, the area was cultivated with wheat in the first year and canola in the second year. Two treatments, i.e., soil amendment with a biochar and a nitrification inhibitor, were applied to the field in a randomized complete block design. Blocking was used to account for the slope of the field. The biochar treatment had three levels: BC0 (no biochar

addition), BC10 (biochar added at 10 t ha^{-1}) and BC20 (biochar added at 20 t ha^{-1}) and the NI treatment had two levels: NI0 (no NI added) and NI1 (NI added to the soil at the rate described below). The treatments were replicated four times using plots that were 3.5 × 6.0 m in size, separated by a 0.5 m buffer between the plots, and the blocks were separated by an 8 m buffer. Biochar used in this experiment was produced by pyrolysis of manure compost at 650 °C. Please refer to Gross et al. (2022) for the details of the biochar production. Biochar was uniformly spread on the surface soil at the rate described above and tilled to a depth of 10 cm immediately after biochar application to reduce the loss of biochar by wind. Spring wheat and canola were grown in 2019 and 2020, respectively, with fertilizers applied at the rate of 100 kg N ha^{-1}, 22 kg P ha^{-1} and 46 kg K ha^{-1} in banding between the rows of seeds. The NI used in this study was eNtrench Nitrogen Stabilizer (obtained from Corteva Agriscience Canada, Calgary, AB, Canada), which had an active ingredient of 200 g nitrapyrin L^{-1}. The NI was applied to the soil at the rate of 2.7 L ha^{-1} (as recommended by the manufacturer). The NI was mixed with urea and air-dried before its application to the field. Biochar was applied in the first year of study (in 2019), while NI was applied in both years (in 2019 and 2020), along with fertilizer application at the time of seeding.

2.2. Soil sampling and analysis

For background soil properties of the study area, one soil sample was collected from each of four random locations (that are approx. 20-25 m apart) in May 2019 before experimental plots were set up. In each of the four locations, four random points were selected, and soil samples were collected from the surface soil (0-10 cm) and composited for each location. The soils were then processed and analyzed by the methods described below. To determine the treatment effect, soil samples were collected from individual plots in July and September in both 2019 and 2020, representing Alberta's active growing season. During soil sampling, soils were collected from 0-10 cm surface soil using an auger from five random points and were composited to form a composite sample for each plot. The soil was brought to the laboratory in a cooler (on ice) and stored in a refrigerator (4 °C) until the samples were processed and extracted, within a week of sampling. Before the analyses were performed, stones and visible roots were removed from the soil, and the soil was passed through a 2 mm sieve.

Soil pH and electrical conductivity were determined in a 1:5 soil solution (w:v for soil: water) using a pH/EC meter (DMP-2 mV, Thermo Orion, USA). Total C and N concentrations in the air-dried soil were analyzed by an elemental analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Germany). Available N (sum of exchangeable NH_4^+-N and NO_3^--N), dissolved organic C (DOC), microbial biomass, and enzymatic activities were analyzed using the fresh soil. To determine available N, soil was extracted with 2 M KCl in 1:5 (w:v soil: KCl solution) after shaking for 1 hour in a reciprocal shaker (180 rpm). The concentrations of NH_4^+-N and NO_3^--N in the extract were measured by indophenol blue and vanadium oxidation methods, respectively (Doane and Horwath, 2003; Keeney and Nelson, 1982). Microbial biomass C and N were analyzed following a chloroform fumigation method (Brookes et al., 1985; Vance et al., 1987). Briefly, 10 g of soil (oven-dry equivalent) was fumigated with ethyl alcohol-free chloroform for 24 hours in a desiccator kept in the dark at room temperature and extracted with 0.1 M K_2SO_4 after the soil solution was shaken in a reciprocal shaker at 200 rpm for 1 hour. Unfumigated soil samples were also extracted by a similar method used in the extraction of fumigated samples. Total organic C and N in the soil extracts were measured by a TOC-V analyzer connected to a TN module (Shimatzu Corporation, Kyoto, Japan). Microbial biomass C and N were calculated from the difference in total extracted organic C and N between fumigated and non-fumigated samples and divided by an extraction coefficient of 0.45 (Joergensen, 1996). The total organic C and N in the unfumigated samples also represent dissolved organic C (DOC) while dissolved organic N (DON), was calculated from the difference between dissoved N and mineral N. Microbial biomass P was determined by the method described in Vance et al. (1987), in which the fumigation method is similar to that used for MBC and MBN determination, the soil was extracted with a 0.03M NH_4F-0.025 M HCl solution for MBP determination. Inorganic P in the extract of fumigated and unfumigated samples was determined colorimetrically by the ammonium molybdate-ascorbic acid method. The MBP was calculated by the difference in inorganic P between the fumigated and unfumigated samples divided by an extraction coefficient of 0.45 (Joergensen, 1996). The inorganic P from the unfumigated samples was Olsen-P (Cui et al., 2018).

The activities of extracellular enzymes for C, N and P cycling, β-1,4-glucosidase (BG), β-1,4-N-acetyl glucosaminidase (NAG) and acid phosphatase (AP), respectively, were determined by the fluorometric method described in Sinsabaugh et al. (2003) and German et al. (2011).

Briefly, 1 g (oven dry equivalent) fresh soil was mixed with 100 mL of 50 mM sodium acetate buffer (pH = 5.5) and shaken for 1 hour in a reciprocating shaker (at 200 rpm). The suspension was transferred to a bowl, and an aliquot of 200 µL soil suspension was taken while the suspension was still homogenized by a stirrer in the bowl and pipetted into 16 wells of a black 96 plate in which 50 µL 200 µM MUF (4-Methylumbelliferone) substrates (4-MUF-β-D-glucopyranoside for BG, 4-MUF-N-acetyl-β-glucosaminide for NAG and 4-MUF-phosphate for AP) were added into the first eight wells (soil assay wells). In the remaining eight wells, 50 µL of sodium acetate buffer (in soil background wells) and 50 µL of MUF (10 µM) standard (into soil quench wells) were added to 4 wells each of the black plate. Control plates were also prepared by pipetting 200 µL buffer and 50 µL substrate in the first four wells, 200 µL buffer and 50 µL MUF in the other four wells and 250 µL buffer in another four wells that represent substrate background wells, standard wells and buffer background wells, respectively. The plates were incubated in the dark at room temperature (22 °C) for 3 hours before the fluorescence was measured using a fluorometer (Synergy HT, Bio-Tek Instruments, Winoosky, VT, USA). At the end of the incubation, 20 µL of NaOH (0.5 M) was added to each well by an auto-dispenser in the fluorometer and was shaken well to stop the reaction. The fluorescence was then read at 360 nm excitation and 460 nm emission.

2.3. Calculation of stoichiometric homeostasis, threshold elemental ratio and microbial nutrient limitations

Community level microbial C:N and C:P homeostasis of soil microorganisms was determined by plotting regressions $lnC:N_R$ vs $lnC:N_B$ and $lnC:P_R$ vs $lnC:P_B$, respectively (Sterner and Elser, 2002a; Cui et al., 2018), where $_R$ and $_B$ represent resources and microbial biomass, respectively. Slopes < 1 and >1 represent strong and weak homeostasis, respectively.

Microbial C and nutrient limitations were determined by calculating vector length and the angle of EEA following Moorhead et al. (2013) and Cui et al. (2019).

Vector length = Sqrt $((BG/AP)^2 + (BG/NAG)^2)$

Vector angle = degrees (ataan2((BG/AP), BG/NAP))

Vector length represents C limitations with a longer vector length representing greater limitations, and vector angle represents N/P limitations. The C and nutrient limitations from C-,

N- and P-acquiring enzyme activities were based on stoichiometric and metabolic theories in ecology (Sterner and Elser, 2002; Allison et al., 2010).

Threshold elemental ratio (TER), which is the ratio of elements at which growth shifts between nutrient and energy were calculated following Sinsabaugh et al. (2009) and Cui et al. (2018).

$$TER_{C:N} = (BG/NAG)B_{C:N})/n_0$$

$$TER_{C:P} = (BG/AP)B_{C:P})/p_0$$

Where $TER_{C:N}$ and $TER_{C:P}$ are the threshold elemental ratios for C:N and C:P, respectively, that limits microbial growth. The BG, NAG and AP are the ecoenzymatic activities for C, N and P cycing, respectively, and $B_{C:N}$ and $B_{C:P}$ are the microbial biomass C to N and C to P ratios, respectively. The n_0 and p_0 are the normalization constants determined by the intercept in linear regressions of ln(BG) vs ln(NAG) and ln(BG) vs ln(AP), respectively. A resource C:N below $TER_{C:N}$ indicates excess N for meeting the decomposers' N requirement; thus, decomposers become C or energy limited. On the other hand, a resource C:N above $TER_{C:N}$ indicates N limitation for the decomposers.

2.4. Statistical analyses

Linear mixed effect models were used to determine the effects of treatments in the measured variables. Treatments were treated as fixed effects, and blocks were random effects in the analysis. The effects of biochar and NI application for the first and second year were assessed separately (as different crops were grown in the first and second year of study) by a two-way analysis of variance (ANOVA; factorial design) in SAS (version 9.4, SAS Institute Inc, NC, USA). Prior to ANOVA, data were checked for their normality of distribution and homogeneity of variances in the residuals using Shapiro-Wilk and Levene's tests, respectively. The data of MBN for 2019 and TER for 2019 and 2020 were log-transformed to meet the assumption of normality of distribution and homogeneity of variance before ANOVA; however, back-transformed data are presented in the results section. When the treatments and their interaction effects were significant, means were compared using the LSD test at $\alpha = 0.05$.

3. Results

3.1 Effects of biochar and NI on soil C, N, P and resource elemental ratio

There were no significant interactions between the treatments in affecting soil pH, DOC, available N, Olsen-P, and resource elemental ratio. Biochar amendment increased soil pH in both, first and secondyears ($P < 0.001$ and $= 0.024$, respectively), Olsen-P in the first year ($P = 0.009$) and NO_3^--N in the second year (0.035), while NI application increased NO_3^--N ($P = 0.032$) and total available N ($P = 0.042$) only in the first year of the study (Table 6-1). Soil pH was significantly higher in BC20 than in BC10 in the first year, but not in the second year. The rate of biochar amendment did not affect Olsen-P, but NO_3^--N was lower in BC20 than in BC10. The treatments did not affect other soil properties analyzed in this study in both years (Table 6-1). Biochar amendment significantly decreased DOC:Olsen-P and AN:Olsen-P ratios in the first year, while NI did not affect resource C:N, C:P and N:P ratios in both years (Appendix 6-2).

3.2. Effects of biochar and NI on soil microbial biomass C, N and P and their stoichiometry

Microbial biomass C was increased by biochar amendment in the first and second year ($P = 0.038$ and 0.015, respectively) but was not affected by NI application and their interactions. The BC10 and BC20 increased MBC by 17.1 and 28.8%, respectively, in the first year, and by 59.2 and 74.6%, respectively, in the second year as compared to the control (Fig. 6-1). Similarly, MBP was also increased by biochar amendment in the first and second years ($P = 0.015$ and 0.048, respectively); however, the increases in MBP by biochar amendments were greater in the first than in the second year, unlike the effects on MBC. An increase in MBN by biochar amendment was observed only in the second year. Microbial biomass stoichiometry was also affected by biochar amendment in the first year but not in the second year. The BC20 treatment increased $B_{C:N}$ by 48.7% compared to BC10, and decreased $B_{N:P}$ by 49.5 and 46.4% compared to BC10 and control, respectively (Table 6-2). The NI application and its interaction with biochar amendment did not significantly change microbial biomass stoichiometry (Table 6-2).

3.3 Effects of biochar and NI on soil extracellular enzyme activities and ecoenzymatic stoichiometry

The activities of BG, NAG and AP were significantly affected by biochar and NI applications but not their interactions. Biochar application affected BG and NAG activities only in the second year, and AP activity only in the first year. The increase in BG and NAG activities were

proportionate with the rate of biochar application, while the activities of AP were not significantly different between BC10 and BC20 (Fig. 6-2). The NI application decreased BG activities by 14.6% (P = 0.001), NAG activities by 28.8% (P = 0.002) and AP activities by 11.15 % (P = 0.040) only in the second year (Fig. 6-2). The $TER_{C:N}$ varied from 24 to 31 in the first and 22 to 26 in the second year with no significant effects of NI, while $TER_{C:P}$ was decreased by 43% by BC20 compared to the control in the first year, both parameters were not affected by NI application and its interactions with biochar amendment in both years of study (Fig. 6-3). Regardless of the treatment, $TER_{C:N}$ and $TER_{C:P}$ were both lower in the second than in the first year of study.

Both treatments changed the ratios of activities of C-, N- and P-acquiring enzymes in the second year. Biochar application increased BG:AP and NAG:AP ratios, while NI application increased BG:NAG and decreased NAG:AP ratios (Table 6-3). Vector lengths were not significantly affected, but vector angles were decreased by biochar (P = 0.037) but were increased by NI application (P = 0.043). In general, the vector length ranged between 3.06 and 3.34 under biochar amendment and between 3.11 and 3.42 under NI application, while the vector angle ranged between 79 to 83 across all treatments (Table 6-3). The regressions of C- vs N- and C- vs P-acquiring enzyme activities showed that the relationships of these enzyme activities are weak with the slopes less than one, and the slopes are not consistent among the treatments between the first and second year of the study (Appendix 6-3).

4. Discussion

We show that the microbial metabolism in a wheat-canola rotation was limited by soil P availability regardless of the treatment, while biochar and NI applications affected soil microbial and enzymatic activities. The lack of interaction between NI and manure compost biochar suggests that biochar's potential role in adsorbing and degrading NI, as demonstrated in previous studies (e.g., Fuertes-Mendizábal et al., 2019; Keiblinger et al., 2018), does not substantially affect the efficiency of NI in changing microbial and enzymatic activities. Although both applications substantially affected soil properties and nutrient availability, biochar amendment had a greater effect on ecoenzymatic stoichiometry, alleviating nutrient limitations on microbial metabolism than NI application.

4.1. Effects of biochar and NI applications on soil properties, elemental ratios of C, N and P

The Gray Luvisolic soil in the studied site is slightly acidic that could potentially limit C and nutrient mineralization caused by slow microbial metabolism. The application of alkaline manure compost biochar was useful in increasing soil pH that likelyimproved microbial metabolism. In contrast to the positive effects of biochar on SOC content reported in several previous studies (Smith, 2016; Majumder et al., 2019; Gross et al., 2021), biochar application did not change SOC content in this study The lack of biochar effect on SOC may be linked to the neutralizing effects between increase in SOC content through stabilization of root-derived SOM (Weng et al., 2017) and decrease in SOC through enhanced mineralization rate of existing or biochar-added SOM due to the increase in soil pH (Naisse et al., 2015)

The increase in NO_3^--N in the second year after biochar application demonstrates that aged biochar performs differently than the fresh biochar (applied in the first year) in affecting nitrification rates and NO_3^--N adsorption. Aging of biochar decreases surface area for adsorption of NO_3^--N with a potential increase in nitrification rates resulting in greater NO_3^--N concentrations in the biochar-amended soil (Nguyen et al., 2017). The increase in Olsen-P after biochar amendment was apparent only in the first year, suggesting that the available P added by biochar amendment does not persist for long in the field but is taken up by plants or microorganisms (Glaser and Lehr, 2019). The increase in available nutrients, including NO_3^--N and Olsen-P, suggests that the application of manure compost biochar in the studied Gray Luvisolic soil can be beneficial in increasing soil fertility and crop production. The effect of biochar was more pronounced on Olsen-P as compared to the DOC and AN in soil, which is demonstrated by the substantial decrease in the elemental ratios of soil C:P and N:P. The change in the elemental ratio of resources in the soil is important in determining C and nutrient limitations for microbial metabolism. Although manure compost biochar application had substantial effects on soil nutrient (N and P) availability in the Gray Luvisolic soil, the study demonstrated that the biochar applied at 20 t ha^{-1} did not produce additional benefits in increasing nutrient availability in comparison to the 10 t ha^{-1} rate.

Nitrification inhibitor application did not change NH_4^+-N, indicating that eNtrench Stabilizer (with nitrapyrin as the NI) applied at 2.7 L ha^{-1} was probably not effective in reducing the nitrification rate in the Gray Luvisolic soil. The effectiveness of NI in reducing nitrification rates depends on several factors, including the type and rate of NI applied to the soil, type and

rate of N fertilizer applied, and soil type (Akiyama et al., 2010; Ruser and Schultz, 2015). The result suggests the need for further study with different rates and types of NI application for the intended efficiency of NI in reducing the nitrification rate in the studied soil. The greater amount of NO_3^--N in NI applied plots in the first year could be associated with the decrease in denitrification activities (Bremer et al., 1986). The effect of an increase in NO_3^--N was not apparent in the second year, caused by potential leaching of NO_3^--N due to greater precipitation in the study area in the second year. In addition, an anaerobic condition in the soil caused by greater precipitation in the second year likely reduced nitrification rate and amount of NO_3^--N in all treatments.

4.2. Effects of biochar and NI applications on soil microbial biomass and ecoenzymatic activities

The lack of significant interaction between biochar and NI applications in soil microbial biomass and eco-enzymatic activities suggests that the biochar did not affect the efficacy of NI in changing microbial activities in the studied Gray Luvisolic soil. Our finding is in contrast with the substantial degradation and adsorption of an NI (3, 4-dimethylpyrazole phosphate) on woody biochar surfaces reported in Keiblinger et al. (2018) and Fuertes-Mendizábal (2019). They attributed the reduced efficiency of NI in reducing nitrifying bacterial activities to the degradation and adsorption of the NI on biochar. Our biochar may have different affinities to the nitrapyrin used in this study as the adsorption capacity of biochars for organic materials varies with feedstock type and pyrolysis conditions (Hassan et al., 2020). Biochar increased microbial biomass in both years (except MBN in the first year), which can be attributed to the improvement in soil acidity. The addition of labile C, N and P from biochar may also have contributed to the increase in microbial biomass through positive priming effects; however, the greater increase in MBC in the second year than in the first year indicates that the consistent increase in soil pH in both years played a greater role than the biochar added labile organic matter (OM) because the labile OM released from biochar may become metabolized quickly and does not persist for long (Bruun et al., 2011). Different rates of biochar have been recommended for improving microbial activities; this study showed that the 10 t ha^{-1} rate increased microbial biomass C in this soil with low SOC content. Similar to findings in a meta-analysis (Gao et al., 2018), where biochar amendment substantially increased MBP, corresponding to the increase in

soil available P. However, it should be noted that only the 20 t ha^{-1} application rate significantly increased MBP in this study.

Similar to findings in Pukalchik et al. (2018), Song et al. (2018) and Pokharel et al. (2020), biochar amendment increased C-, N- and P-acquiring enzyme activities in this study; however, the effects were not consistent between the first and second year of the study. Several factors have been attributed to increasing ecoenzymatic activities in biochar-amended soils, including biochar neutralizing acidic soil, the addition of substrates for enzyme activities (Novak et al., 2013) and increases in microbial biomass (Song et al., 2020). Nitrification inhibitor decreased BG, NAG and AP activities in the second year of the study, suggesting that NI had adverse impacts on soil enzyme activities regardless of C-, N- and P-mineralization processes. Maienza et al. (2014) showed that the growth of heterotrophic bacteria and fungi was reduced by a NI (3,4 dimethylpyrazole phosphate), resulting in lower C- and N- acquiring enzyme activities and SOM decomposition. Zhang et al. (2017) also observed a significant decrease in BG and urease activities by the NI despite the negligible impact on bacterial community composition.

4.3. Effects of biochar and NI on soil stoichiometric homeostasis, threshold elemental ratios and microbial nutrient limitations

Based on the slope of $lnC:N_B$ (microbial biomass C:N) vs $lnC:N_R$ (resources C:N) and $lnC:P_B$ vs $lnC:P_R$, the soil in the studied area appears to have a strong stoichiometric homeostasis as the slopes are not significantly different from 0 (all $P > 0.05$) regardless of the treatment and year of study (Table S4). Stoichiometric homeostasis occurs when the microbial community is dominated by heterotrophic organisms, which play greater roles in SOM degradation than communities dominated by autotrophic organisms (Sterner and Elser, 2002). Strong homeostasis in the studied soil suggests faster SOM degradation with the release of nutrients, thereby increasing soil fertility (Griffiths et al., 2012; Cui et al., 2018). The threshold elemental ratio indicates the relative shift between nutrient (N and P) and C limitations in microbial growth and metabolism. Relatively high C:N or C:P ratios indicate nutrient limitations, while low C:N or C:P ratios indicate energy limitation (Sterner and Elser, 2002, Cui et al., 2018). Despite the added labile C through biochar amendment in BC20, $TER_{C:P}$ was reduced compared to the control. The result suggests that microbial growth and metabolism shifted from nutrient (P) limitation in the control towards C limitation in the application of manure compost biochar at 20

t ha^{-1}. The lower $TER_{C:N}$ and $TER_{C:P}$ in the second than in the first year of the study, regardless of the treatment, was likely associated with the lower uptake of nutrients by the crop in the second year, due to the lower canola yield as a result of poor germination in the second year of the study, which would lower the competition for nutrient uptake between microorganisms and plants, alleviating nutrient limitations for microbial growth and metabolism.

The ratio of the activities of C- vs N- and C- vs P-acquiring enzymes in the soil have frequently been used as an indicator of microbial C vs nutrient limitations, with a decreasing ratio indicating nutreint limitations relative to C (Cui et al., 2019; Mori, 2020; Chen et al., 2022). The decrease in BG/NAG and increase BG/AP by BC20 compared to the control in the second year of the study indicates that biochar has contrasting effects on N and P limitations. On the other hand, NI application increased BG/NAG, suggesting the alleviation of N relative to C limitation due to increased availability of N as a result of potential reductions in nitrification rate and leaching loss of N. The vector length calculated from the ratio of BG/AP and BG/NAG suggests that no treatment significantly changed C limitation for microbial metabolism. Since the vector angle is > 45° in all treatments, the soil in the studied area had microbial P limitations relative to N. Biochar and NI applications had significant effects on vector angle, demonstrating their effects on microbial P limitation relative to N, although biochar amendment decreased while NI application increased vector angle suggesting alleviation and aggravation of P limitation relative to N, respectively.

5. Conclusions

We conclude that the applications of manure compost biochar and NI do not significantly interact in affecting soil microbial and ecoenzymatic activities in a Gray Luvisolic soil, indicating minimal impacts of biochar on adsorbing and degrading NI. Manure compost biochar affected microbial and ecoenzymatic stoichiometry more than nitrapyrin. Manure compost biochar increased microbial biomass C, N and P with consecutive increases in C-, N-, and P-acquiring enzyme activities while NI decreased these enzyme activities. The treatment effects on vector angle of ecoenzymatic stoichiometry demonstrate that biochar and NI applications have contrasting effects on nutrient limitations on microbial growth and metabolism; biochar alleviates but NI aggravates microbial P limitation relative to N. The study concludes that manure compost biochar could be beneficial in improving soil health by increasing microbial

growth and activities which are otherwise limited by low P availability for microbial uptake in the studied Gray Luvisolic soil.

Table 6-1. Effects of biochar amendment and NI application on soil properties (means with standard errors in parentheses) (n=4).

Treatment	Soil pH	DOC	DON	NH_4^+-N	NO_3^--N	AN	Olsen-P
		mg kg^{-1}					
2019							
Biochar							
BC0	5.62(0.13) c	59.22(2.93)	18.69(1.74)	10.31(2.37)	13.40(2.95)	23.71(4.5)	3.52(0.24) b
BC10	6.15(0.07) b	57.58(2.52)	17.62(4.69)	9.89(2.08)	10.91(2.55)	20.80(4.32)	5.24(0.57) a
BC20	6.53(0.06) a	61.29(2.45)	13.04(2.85)	8.52(1.26)	13.76(3.08)	22.27(4.11)	5.57(0.47) a
Nitrification inhibitor							
NI0	6.01(0.13)	58.81(2.38)	16.21(3.30)	7.82(0.76)	10.47(1.89) b	18.30(2.48) b	4.64(0.41)
NI1	6.19(0.14)	59.91(1.90)	16.69(2.06)	11.32(1.97)	14.90(2.51) a	26.22(3.86) a	4.92(0.48)
ANOVA							
BC	**<0.001**	0.518	0.119	0.787	0.422	0.801	**0.009**
NI	0.105	0.678	0.597	0.132	**0.032**	**0.041**	0.565
BC × NI	0.691	0.263	0.827	0.381	0.105	0.281	0.297
2020							
Biochar							
BC0	6.22(0.13) b	45.79(1.21)	8.53(0.47)	12.47(1.77)	8.27(1.02) b	20.75(2.54)	2.94(0.27)
BC10	6.43(0.14) ab	55.16(5.27)	7.78(0.55)	13.11(1.67)	10.62(1.31) a	23.73(2.43)	4.24(0.59)
BC20	6.63(0.10) a	47.87(1.17)	8.45(0.43)	10.69(0.85)	7.35(0.61) b	18.05(1.16)	3.96(0.64)
Nitrification inhibitor							
NI0	6.44(0.09)	51.77(3.70)	8.64(0.48)	11.02(0.89)	9.19(1.07)	20.21(1.69)	3.54(0.41)
NI1	6.41(0.12)	47.44(1.18)	7.87(0.26)	13.16(1.42)	8.31(0.71)	21.48(1.97)	3.89(0.49)
ANOVA							

BC	**0.024**	0.103	0.352	0.514	**0.035**	0.180	0.292
NI	0.787	0.262	0.172	0.234	0.370	0.599	0.612
BC × NI	0.429	0.314	0.860	0.512	0.909	0.684	0.863

Abbreviations: BC0, no biochar added; BC10, manure biochar added at 10 t ha^{-1}; BC20, manure biochar added at 20 t ha^{-1}; NI0, no nitrification inhibitor added; NI1, nitrification inhibitor added; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; NH_4^+-N, exchangeable ammoniacal nitrogen; NO_3^--N, nitrate nitrogen; AN, available nitrogen; Olsen-P; inorganic phosphorus.

Different letters indicate significant differences within each factor of biochar and nitrification inhibitor applications ($P < 0.05$). P values in the bold indicate a significant effect at $\alpha = 0.05$.

Table 6-2. Effects of biochar and NI applications on microbial biomass stoichiometry (means with standard errors in parentheses) (n=4).

Treatment	2019			2020		
	$B_{C:N}$	$B_{N:P}$	$B_{C:P}$	$B_{C:N}$	$B_{N:P}$	$B_{N:P}$
Biochar (BC)						
BC0	10.99(1.29) ab	3.62(0.95) a	32.37(3.42)	8.16(0.60)	1.66(0.33)	13.15(2.51)
BC10	8.21(0.99) b	3.84(0.88) a	27.66(5.23)	8.05(0.52)	2.40(0.47)	18.32(2.52)
BC20	12.21(1.34) a	1.94(0.30) b	21.60(2.66)	8.28(0.82)	2.16(0.69)	14.68(2.78)
Nitrification inhibitor (NI)						
NI0	11.05(1.23)	2.70(0.59)	23.75(2.16)	8.45(0.51)	1.96(0.39)	15.57(2.48)
NI1	9.90(0.96)	3.56(0.71)	30.67(4.04)	7.88(0.53)	2.18(0.45)	15.20(1.82)
ANOVA						
BC	**0.041**	**0.033**	0.183	0.967	0.351	0.357
NI	0.402	0.130	0.146	0.458	0.570	0.901
BC × NI	0.151	0.273	0.682	0.612	0.189	0.184

Abbreviations: BC0, no biochar added; BC10, manure biochar added at 10 t ha^{-1}; BC20, manure biochar added at 20 t ha^{-1}; NI0, no nitrification inhibitor added; NI1, nitrification inhibitor added; $B_{C:N}$, microbial biomass carbon: nitrogen; $B_{N:P}$, microbial biomass nitrogen: phosphorus; $B_{C:P}$, microbial biomass carbon: phosphorus. Different letters indicate significant differences within each factor of biochar and nitrification inhibitor applications ($P < 0.05$). P values in the bold indicate a significant effect at $\alpha = 0.05$.

Table 6-3. Effects of biochar and NI applications on ecoenzymatic stoichiometry (means with standard errors in parentheses) (n=4).

Treatment	2019					2020				
	BG:NAG	BG:AP	NAG:AP	Vector length	Vector angle	BG:NAG	BG:AP	NAG:AP	Vector length	Vector angle
					Biochar (BC)					
BC0	3.11	0.48	0.19	3.17	79.04	3.66 a	0.48 b	0.14 b	3.34	82.84 a
	(0.20)	(0.02)	(0.01)	(0.20)	(0.44)	(0.27)	(0.03)	(0.01)	(0.21)	(0.63)
BC10	3.35	0.52	0.17	3.40	80.40	3.19 ab	0.52 ab	0.17 ab	3.30	80.15 ab
	(0.17)	(0.04)	(0.01)	(0.17)	(0.80)	(0.27)	(0.03)	(0.01)	(0.15)	(1.29)
BC20	3.01	0.57	0.18	3.06	79.71	2.99 b	0.57 a	0.19 a	3.06	79.12 b
	(0.18)	(0.04)	(0.01)	(0.17)	(1.15)	(0.15)	(0.02)	(0.01)	(0.10)	(0.68)
					Nitrification inhibitor (NI)					
NI0	3.09	0.53	0.20	3.15	78.90	3.02 b	0.53	0.19 a	3.11	79.51 b
	(0.16)	(0.03)	(0.010)	(0.16)	(0.98)	(0.25)	(0.03)	(0.01)	(0.16)	(1.08)
NI1	3.15	0.52	0.19	3.21	79.12	3.54 a	0.51	0.15 b	3.42	81.50 a
	(0.16)	(0.04)	(0.01)	(0.16)	(0.95)	(0.22)	(0.04)	(0.01)	(0.16)	(0.80)
					ANOVA					
BC	0.4919	0.397	0.525	0.491	0.515	**0.020**	**0.040**	**0.038**	0.2919	**0.037**
NI	0.5892	0.146	0.102	0.628	0.103	**0.026**	0.609	**0.043**	0.0802	**0.043**
BC × NI	0.9721	0.433	0.570	0.970	0.571	0.129	0.315	0.118	0.4972	0.119

Abbreviations: BC0, no biochar added; BC10, manure biochar added at 10 t ha^{-1}; BC20, manure biochar added at 20 t ha^{-1}; NI0, no nitrification inhibitor added; NI1, nitrification inhibitor added; BG, β-1,4-glucosidase; NAG, β-1,4 N-acetyl glucosaminidase; AP,

acid phosphatase activities. Different letters indicate significant differences within each factor of biochar and nitrification inhibitor applications ($P < 0.05$). P values in the bold indicate a significant effect at $\alpha = 0.05$.

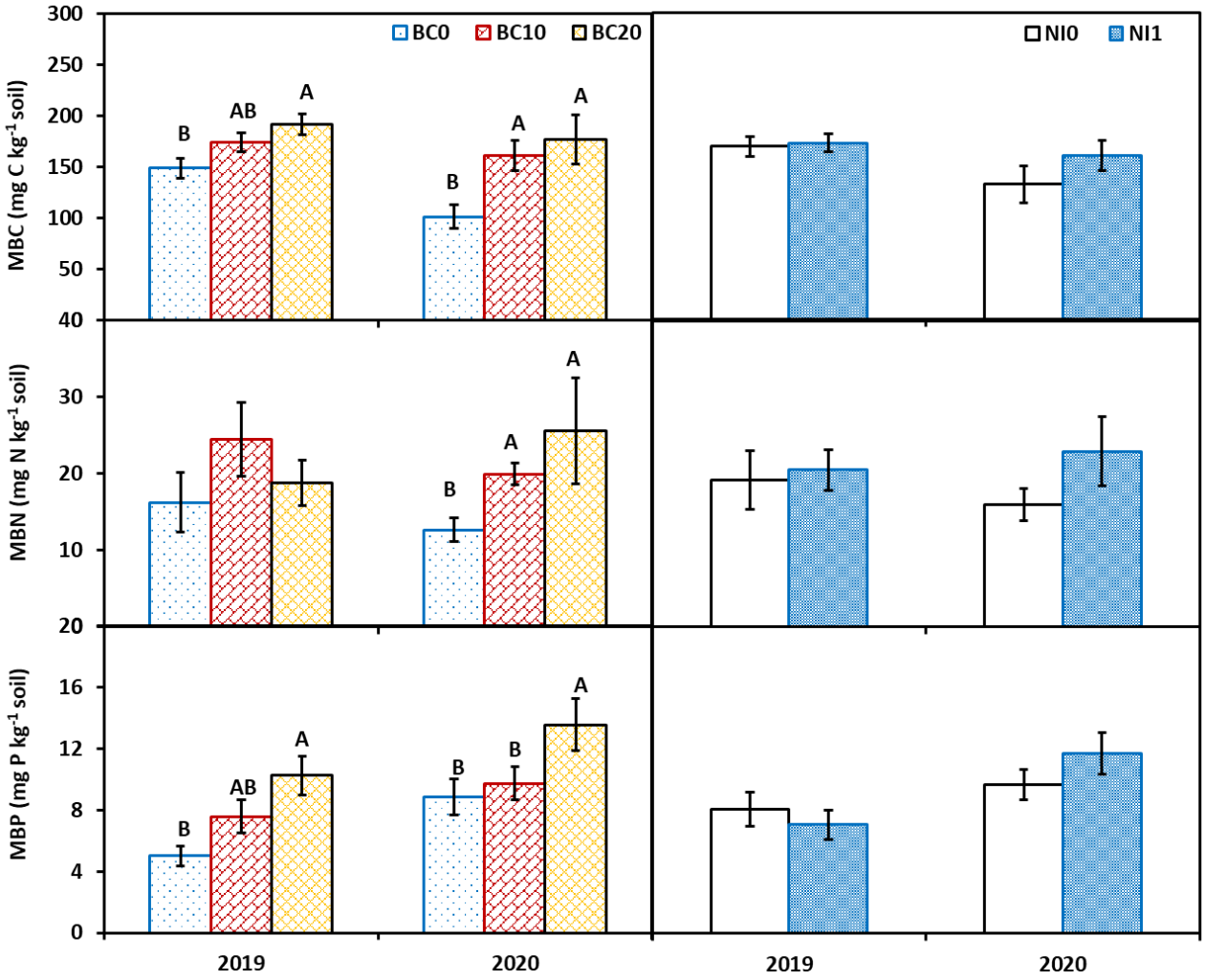

Fig. 6-1. Effects of manure-compost biochar and nitrification inhibitor applications on microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP). Treatment codes are BC0: control (no biochar added), BC10: manure biochar added at 10 t ha^{-1}, BC20: manure biochar added at 20 t ha^{-1}, NI0: no nitrification inhibitor added, NI1: nitrification inhibitor added. Error bars show the standard error of the mean (n = 4).

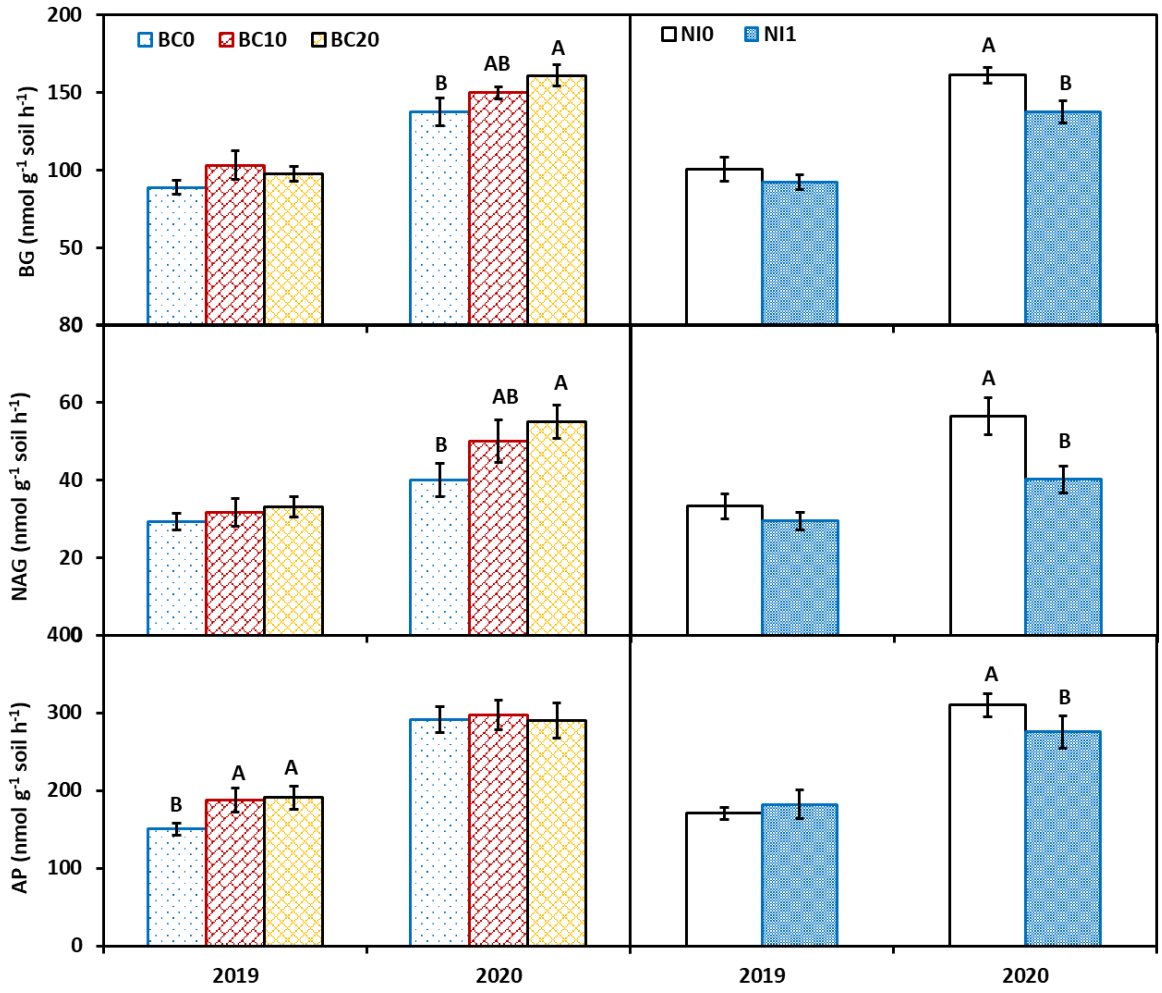

Fig. 6-2. Effects of manure-compost biochar and nitrification inhibitor applications on microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP). Treatment codes are BC0: control (no biochar added), BC10: manure biochar added at 10 t ha^{-1}, BC20: manure biochar added at 20 t ha^{-1}, NI0: no nitrification inhibitor added, NI1: nitrification inhibitor added. Error bars show the standard error of the mean (n = 4).

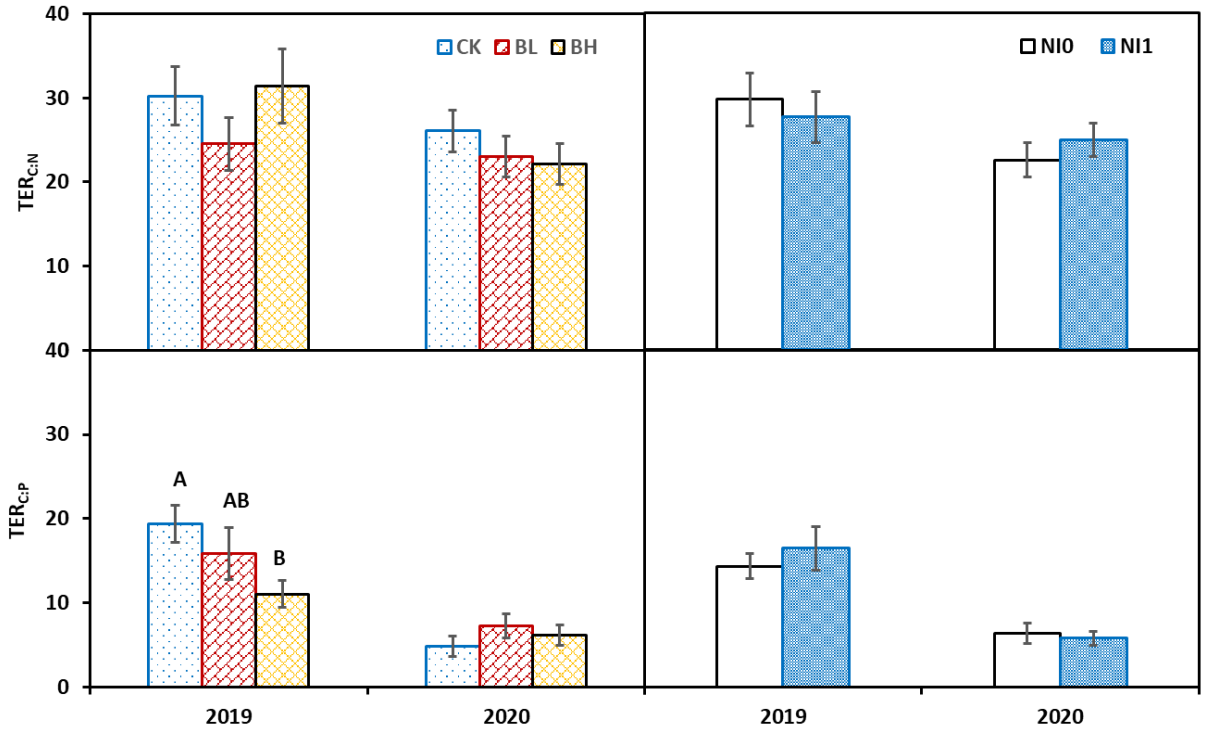

Fig. 6-3. Effects of manure-compost biochar and nitrification inhibitor applications on threshold elemental ratios of carbon to nitrogen ($TER_{C:N}$) and carbon to phosphorus ($TER_{C:P}$). Treatment codes are BC0: control (no biochar added), BC10: manure biochar added at 10 t ha^{-1}, BC20: manure biochar added at 20 t ha^{-1}, NI0: no nitrification inhibitor added, NI1: nitrification inhibitor added. Error bars show the standard error of the mean (n = 4).

Chapter 7. Biochar increases soil microbial biomass with changes in extra- and intracellular enzyme activities: a global meta-analysis

1. Introduction

The soil application of biochar, a product of pyrolysis of biomass in partial or complete absence of oxygen, has been proposed as a potential management strategy to improve soil quality, support the resilience of agroecosystems, and mitigate global climate change by increasing soil organic carbon (C) and fertility (Woolf et al., 2010; Biederman and Harpole, 2013; Lu et al., 2014), and reducing greenhouse gas emissions (Spokas and DC, 2009; Crombie et al., 2015; Liu et al., 2016). These goals can be achieved by changing soil processes such as soil organic matter (SOM) decomposition and nutrient mineralization, in association with changed microbial and enzymatic activities through biochar application (Sohi et al., 2009; Lehman et al., 2011). Biochar application affects microbial and enzymatic activities by changing the availability of resources by adding labile C (Kuzyakov et al., 2009, Zimmerman at al., 2011) to the soil as well as by changing the soil environment (Verheijen et al., 2010). Soil enzymes catalyze the rate limiting steps of SOM decomposition and nutrient cycling, and their activities are very sensitive to changes in the soil environment (Sinsabaugh, 1994; Burns et al., 2013) that can be brought on by biochar application. Therefore, many studies have assessed the effect of biochar application on microbial and enzymatic activities.

A wide range of soil enzymes (extra- and intracellular) has been studied in biochar application experiments, including hydrolases and oxidases that decompose macromolecules of varying composition and complexity into soluble substrates for microbial assimilation. These enzymes target different groups of substrates present in soils for SOM decomposition (Sinsabaugh, 2010) and their activities are substantially influenced by biochar application (Paz-Ferreiro et al., 2013; Zhang et al., 2014; Song et al., 2018). In biochar application studies, the most widely assayed soil hydrolytic enzymes for C cycling (C-acquisition) are β-1,4-glucosidase, β-D-cellobiohydrolase and β-1,4-xylosidase; β-1,4-N-acetyl-glucosaminidase, leucine amino peptidase and urease for N cycling (N-acquisition); and acid phosphatase and alkaline phosphatase for P cycling (P-acquisition) (Chen et al., 2013; Song et al., 2016; Pukalchik et al., 2018). The phenol oxidase and peroxidase are the most studied oxidizing soil enzymes while dehydrogenase is the most studied intracellular enzyme in biochar application studies (Ouyang et

al., 2014; Chen et al., 2017). Most biochar studies that involved the above-mentioned enzyme activities are focused on soils that are severely deteriorated by extensive agricultural practices or contaminated by heavy metals. The low microbial and enzymatic activities often impede nutrient cycling and productivity of these soils (de Mora et al., 2005; Thavamani et al., 2012; Araujo et al., 2013). The use of biochar as a 'soil conditioner' can improve the quality of those soils by increasing microbial growth and enzyme activities that are associated with C and nutrient cycling (Verheijen et al., 2010).

Biochar application can have contrasting effects on soil enzyme activities. For instance, biochar application was found to significantly increase (Pukalchik et al., 2018), decrease (Chen et al., 2013; Zhang et al., 2014; Zheng et al., 2016; Benavente et al., 2018) or not change (Yoo and Kang, 2012; Song et al., 2016) β-1,4-glucosidase activities in upland agricultural soils. Similarly, biochar application has been shown to increase (Song et al., 2018), decrease (Bamminger et al., 2014; Chen et al., 2017) or have no effect (Chen et al., 2019) on the activities of β-1,4-N-acetyl-glucosaminidase, which is involved in N-acquiring activities of microorganisms (Parham and Deng, 2000). The responses of acid and alkaline phosphatases that are associated with the cleavage of P-containing organic compounds to biochar application also varied widely in both the direction and magnitude (Ouyang et al., 2014; Purakayastha et al., 2015). The wide variation in the response of enzyme activities to biochar application is associated with soil type and biochar property (Sohi et al., 2010; Gul et al., 2015).

Biochar application to the soil can change the physical (e.g., soil aeration, aggregation and water holding capacity) and chemical properties (e.g., soil pH, CEC and C/N ratio) of soil (Verheijien et al., 2010; Gul et al., 2015; Wang et al., 2017). Changes in these soil properties eventually alter microbial community composition and enzyme activities in the soil (Zhang et al., 2018a). However, the change in soil properties and their subsequent effects on microbial and enzymatic activities following biochar application is a function of soil texture, land use type and initial soil property (Sohi et al., 2010; Xie et al., 2015). Biochar application increased water holding capacity and enzymatic activities (catalase, dehydrogenase and invertase) in coarse-textured but not in fine-textured soils (Khadem and Raiesi, 2017). Wu et al. (2018) observed an increase in activities of C cycling related enzymes in alkaline soil with no significant change in N cycling related enzyme activities in alkaline and acidic soils following biochar addition.

The biochar-induced changes in soil properties and their subsequent effects on microbial and enzymatic activities also depend on the feedstock type used, the pyrolysis condition and biochar application rate (Singh et al., 2010; Gul et al., 2015). Biochar properties such as pH, C/N ratio, surface area and labile C content that have direct influence on enzyme activities (BřEndová et al., 2012) are functions of feedstock type and pyrolysis condition. High pyrolysis temperature produces biochars with higher pH, surface area and aromatic C and application of such biochars to the soil increases enzymatic activities associated with C cycling in a fluvo-aquic soil (Wang et al., 2015). Biochars produced from manure- and wood-based feedstocks are different in their nutrient content and pH (Lee et al., 2013; Novak et al., 2013). Generally, application of biochars produced from manures and crop residues have higher pH, labile C and nutrient contents than that produced from wood feedstocks (Novak et al., 2013) that can increase activities of enzymes regulating C and N cycling in the soil (Bailey et al., 2011).

The number of studies that assess soil enzymatic and microbial activities in response to biochar application is rapidly increasing but the large number of such studies with contrasting results have made it difficult to reach a conclusion on the potential roles of biochar application in achieving the desired ecological benefits. With the surge in biochar amendment studies in recent years that involve assessment of soil microbial and enzymatic activities, quantitative reviews using meta-analysis procedure are helpful to critically analyze biochar's effects on microbial and enzymatic activities on a global scale. Zhang et al. (2019) showed an increase in N- and P-cycling enzymes by biochar application in their meta-analysis based on data from 43 papers that covered publications prior to 2016. However, Zhang et al. (2019) did not include the assessment of the relationship between change in microbial biomass and enzymatic activities and did not analyze dehydrogenase (intracellular enzyme) activity in biochar-amended soils. Analyzing dehydrogenase activities in the soil is critical to understand the effect of biochar on metabolic activities of microorganisms in the soil (Serra-Wittling et al., 1995). This global meta-analysis is based on more data (from 72 papers) on microbial and enzymatic activities than the Zhang et al. (2019) meta-analysis by including relevant papers published after 2016. This study has the following objectives: i) to quantitatively assess the effect size of biochar application on microbial biomass, activities of intra-and extracellular enzymes that are involved in C, N and P acquisitions, ii) to assess the relationship between changes in microbial biomass and changes in C and N acquisition enzyme activities in biochar-amended soils, and iii) to identify key factors of

soil and biochar that influence the response of intra- and extracellular soil enzymatic activities to biochar application.

2. Material and methods

2.1. Literature search

A literature search was conducted to collect data for this meta-analysis using Web of Science and Google Scholar using the following key words: biochar or char or pyrolyzed char or black carbon and soil and enzyme or enzymatic activities. Papers were selected based on the following criteria: (1) studies having at least three replicates in the experiment, (2) studies with treatment (biochar applied) effects paired with a control (no biochar applied) in the same experimental condition, and (3) studies reporting at least one of the following enzyme activities (given below). Studies that used (i) biochars modified by steam or citric and tartaric acid activation, denaturing stress and photochemical weathering, and (ii) biochars used in combination with other additives such as compost and lime with their control treatment not reported, were excluded. In addition, papers that reported incomplete unit of enzyme activities (such as enzyme activities with no time in the unit) were also excluded. The authors of a few of the papers were contacted to get additional information such as the unit and absolute values of enzyme activities (when only relative values were reported), standard deviation (SD) or standard error (SE) in the data (if not reported in the paper).

2.2. Data collection and compilation

A total of 72 papers (published until February 18, 2019) each with an independent study were selected to collect the data used in this meta-analysis (Appendix 1). Data sets for enzyme activities including mean values with the number of replicates (n) and SD or SE for the control and biochar application treatments were extracted from the tables and figures of the papers. The mean and SD (or SE) were extracted from figures using GetData Graph Digitizer 2.26 (http://getdata-graph-digitizer.com/download.php). The SD was calculated as $SD = SE \times \sqrt{n^2}$. If the experiment included different organic amendments, only the data for the biochar application alone and its control were extracted from that experiment. If there were data from multiple sampling times in a study, we used the data of the last sampling. In addition, in field experiments

that involved multiple depths of soil to examine biochar's effect on enzyme activities, we used only data for the uppermost soil layer to avoid potential bias caused by different soil layers being sampled (Jian et al., 2016) since biochar is generally applied to the upper 10-20 cm soil. The soil pH, total carbon (TC), total nitrogen (TN) and soil texture, and feedstock type, pyrolysis temperature, biochar pH, C/N ratio and biochar application rate (in percentage) data were also extracted from the papers. The latitude and longitude of the study location were also collected to help plot global distribution of study sites in this meta-analysis.

A total of 12 enzymes (11 extracellular and 1 intracellular) that represent the most common hydrolytic and oxidative enzymes in the soil were considered to examine the effect of biochar application on soil enzyme activities (Table 1). The extracellular enzymes included in this study are α-1,4-glucosidase, β-1,4-glucosidase, β-D-cellobiohydrolase, β-1,4-xylosidase, β-1,4-N-acetyl-glucosaminidase, leucine amino peptidase, urease, acid phosphatase, alkaline phosphatase, phenol oxidase, peroxidase and the intracellular enzyme studied is dehydrogenase. The hydrolytic extracellular enzymes were further integrated into C-acquisition (*C-acq*), N-acquisition (*N-acq*) and P-acquisition (*P-acq*) enzymes based on the targeted substrate or nutrients they act on. The activities of *C-acq* represent the average of α- and β-glucosidase, cellobiohydrolase and xylosidase, the activities of *N-acq* represent the average of acetyl-glucosaminidase, leucine amino peptidase and urease, and that of *P-acq* represent the average of acid and alkaline phosphatase activities. Soil microbial biomass C and N data were also extracted from the papers as dependent variables.

The selected soil and biochar data (as independent variables) were categorized into groups to facilitate meta-analysis and to help identify major factors affecting soil microbial and enzymatic activities. Following the classification of soil used in previous meta-analyses of biochar's effects on soil microbial and enzymatic activities (Zhang et al., 2018a and 2019), soil pH was categorized into acidic (< 6.5), neutral (6.5-7.5 inclusive) and alkaline (> 7.5), TC and TN were categorized into three groups (< 10, 10-20 inclusive and > 20 g kg^{-1} for TC and < 1, 1-2 inclusive and > 2 g kg^{-1} for TN). Soil textural classes were divided into three groups: fine (clay, clay loam, silty clay loam and silty clay), medium (silt, loam, silt loam and sandy silt loam) and coarse (sandy loam, sandy clay loam, loamy sand, sand) following the USDA soil classification system. If soil textural classes were not reported but only percentages of the soil particles were given in the paper, the textural classes were determined by the percentage of clay, silt and sand.

The experiments were divided into three types: lab incubation, greenhouse and field experiments. To assess the effect of time since biochar application, studies were categorized based on experiment duration into short- (experiments that span up to 100 days of biochar application), medium- (101-365 days inclusive) and long-term studies (> 365 days). Biochar feedstock types were categorized into wood, crop residue (including rice, wheat and soybean straw, maize silage, rice husk, oil seed rape and weeds), urban wastes (municipal solid waste and sewage sludge) and manure (poultry, cattle and swine). Pyrolysis temperature was categorized into low (< 350 ºC), medium (350-550 ºC inclusive) and high (> 550 ºC); biochar pH was categorized into < 8, 8-10 inclusive and > 10; biochar C/N ratio into < 50, 50-100 inclusive and > 100. Biochar application rate was converted to percentage (w/w) if needed by using bulk density of the soil and depth of soil to which biochar was applied. If soil bulk density was not reported, it was estimated by the standard bulk density calculator based on soil texture (https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils). The biochar application rate was then categorized into four: < 1, 1-3 inclusive, 3-5 inclusive and > 5%.

2.3. Data analysis

To assess the effect size of biochar application on soil enzymatic activities and microbial biomass, we used the natural log-transformed response ratio (ln *RR*: the ratio of treatment over control) as commonly used in other meta-analyses (Jian et al., 2016; Liu et al., 2016) because it improves statistical behaviors (Hedges et al., 1999). The ln *RR* was calculated as:

$$\ln RR = \ln (\bar{X_t}/\bar{X_c})$$

Where $\bar{X_t}$ and $\bar{X_c}$ are the observed values of a selected variable (enzyme activities or microbial biomass C and N) under treatment (biochar application) and control, respectively. Estimation of effect size in meta-analysis largely depends on the weighting of the individual observation that can subsequently affect the inferences that can be made from a meta-analysis (Ma and Chen, 2016). Various weighting functions have been used in previous meta-analyses (Jian et al., 2016; Ma and Chen, 2016; Zhang et al., 2018a). The use of variance estimates in weighting functions are often unreliable because of large variances due to diverse site conditions and small sample sizes (common in many published studies we have considered in this meta-analysis). Following

these previous studies (Ma and Chen, 2016; Zhang et al., 2018b), we used the number of replications for the weighting function of observations as they found that this weighting function assigned less extreme weight and gave less weight to studies with multiple non-independent observations than any other weighting function. The weighting factor was calculated as:

$$W_r = (N_t \times N_c)/(N_t + N_c)$$

Where W_r is the weight associated with ln *RR* of observations of each variable, N_t and N_c are the number of replications in the treatment and control, respectively. The meta-analysis was conducted using maximum likelihood estimation with the *lme4* package in R. We bootstrapped the estimates of weighted response ratio (ln *RR'*) to generate 95% confidence intervals (Liu et al., 2016) by using the 'confint ()' function in the 'boot' package in R (Adams et al., 1997; Canty and Ripley, 2012). The following equation was used to transform the log-transformed weighted response ratio back to the percentage change for ease of interpretation which is commonly used in other meta-analyses (Luo et al., 2006; Jian et al., 2016).

$$\text{Effect size (\%)} = (e^{\ln RR'}-1) \times 100\%$$

We consider the effect of biochar application on enzyme activities and microbial biomass to be significantly different from control if the 95% confidence interval of ln *RR'* does not overlap with zero (Luo et al., 2006).

3. Results

3.1 Overall effects of biochar application on soil enzyme activities and microbial biomass

Biochar application significantly increased the activities of urease, alkaline phosphatase and dehydrogenase by 23.1, 25.4 and 19.8%, respectively, as compared to the control, but did not affect the activities of other enzymes (Fig. 7-1; Table 7-2). In other words, biochar application increased the activities of *N-acq* enzymes by 23.3% but did not affect the activities of *C-acq* and *P-acq* enzymes. Biochar application also significantly increased MBC by 21.7% but had no effect on MBN (Fig. 7-1; Table 7-2). Regression analyses showed that *RR* of MBC had

significant linear relationships with *RR* of *C-acq* and *N-acq* enzymes in acidic soils but not in neutral and alkaline soils ($P < 0.05$). In acidic soils, *RR* of MBC had a negative relationship ($P = 0.002$) with *RR* of *C-acq* and a positive relationship ($P < 0.01$) with *N-acq* enzymes (Fig. 7-2).

3.2. Effect of biochar on activities of urease, alkaline phosphatase, dehydrogenase and N-acq enzymes and MBC in different soils

The effects of biochar on soil enzyme activities were dependent on soil characteristics (Table 7-3). Urease activities were increased by biochar application by 33.3 and 31.2% in soils having TC less than 10 and between 10 and 20 g kg^{-1}, respectively. Biochar also increased urease activities in soils having TN < 2 g kg^{-1} but not in soils having TN > 2 g kg^{-1} or TC > 20 g kg^{-1}. The increase in urease activities by biochar application was significant in fine but not in coarse-textured soils. Similarly, biochar also increased dehydrogenase activities by 40% in neutral soils while there were no effects in acidic and alkaline soils (Table 7-3). In greenhouse experiments, biochar application significantly increased dehydrogenase activities by 31.8% (Table 7-3). The activities of alkaline phosphatase were dependent on soil pH, TC, TN and texture: an increase of 52% in acidic soils, but not in neutral and alkaline soils; increases of 25.7 and 36.6% in soils with TC < 10 and 10-20 g kg^{-1}, respectively; and an increase of 67.4% in field experiments but not in lab incubation and greenhouse experiments (Table 7-4).

The magnitude of biochar's effect on *N-acq* enzyme and MBC was also dependent on soil characteristics (Fig. 7-3). Biochar significantly increased the activities of *N-acq* enzyme by 26.6% in acidic and 27.3% in alkaline soils; by 34.8% in soils with TC < 10 g kg^{-1}; 32.7% in soils with TN < 1 g kg^{-1}; and 23.5 and 21% in field and greenhouse experiments, respectively (Fig. 7-3). The MBC was significantly increased by biochar in most cases in soils with different pH, TC, TN and texture: 16.6 and 38.7% in acidic and neutral soils, respectively; 23.6 and 23.2% in soils with TC < 10 and 10-20 g kg^{-1}, respectively; and 22.1 and 18.6% in coarse and fine-textured soils, respectively (Fig. 7-3).

3.3 Effect of biochar properties on activities of urease, alkaline phosphatase, dehydrogenase and N-acq enzymes and MBC

The effects of biochar on increasing activities of urease, alkaline phosphatase, dehydrogenase and N-acq enzymes and MBC were also dependent on pyrolysis temperature and feedstock type

and the properties of biochar (such as pH and C/N ratio) associated with pyrolysis conditions and feedstock type used while the activities of other enzymes were not significantly affected by those factors (Tables 7-5 and 7-6). Urease activities were increased by 25.9% by biochar produced at high pyrolysis temperature (> 550 ºC), 23.9% by manure-based biochar and 33.5% by biochar with high pH (> 10) with no significant change in urease activities by any biochar application rates. But alkaline phosphatase activities were significantly increased by the biochars produced from crop residues and wood and lower rates of biochar application (< 3%) (Table 7-6). Dehydrogenase activities were increased by biochars produced at low pyrolysis temperature (< 350 °C) and biochars with low C/N ratio (<50) but not affected by any biochar pH ranges, feedstock types and biochar application rates (Table 7-5). Biochar significantly increased urease activities in short term studies while increased alkaline phosphatase activities only in long-term studies.

Significant positive changes in N-*acq* enzymes were observed with the application of manure-based biochars, biochars produced at medium pyrolysis temperatures (350-500 °C), biochars with high pH (> 10), and biochars applied at low rates (< 1%) (Fig. 4). Activities of N-*acq* enzymes were found to be significantly increased by biochars with lower C/N ratios (< 100), and in field and greenhouse but not in lab incubation experiments. Similarly, MBC was significantly increased by biochars produced from crop residues and urban wastes, by biochars with high pH (> 8) and low C/N ratios (< 100), and by all biochar application rates except the rate of 3-5%. The MBC was found to be significantly increased in the field but not in lab incubation and greenhouse experiments (Fig. 7-4).

4. Discussion

We showed that microbial biomass C and activities of urease, alkaline phosphatase, dehydrogenase and the enzymes involved in N-acquiring activities were significantly increased by biochar application to the soil although the magnitude of those increases varied widely with soil properties, the characteristics of biochar associated with feedstock, pyrolysis temperature, biochar application rate and experiment type. This meta-analysis also showed that activities of none of the individual enzymes we studied and *C-, N-* and *P-acq* enzymes were significantly reduced by biochar application although there are studies showing decreases in some of these enzyme activities in published experiments. The neutral and significantly positive effects of

biochar application on soil microbial biomass and enzyme activities shown by this study along with the negative effects of biochar on CH_4 and N_2O emission (e.g., Jeffery et al., 2016 and Borchard et al., 2018) suggest the crucial roles biochars can play in enhancing soil quality while mitigating global climate change.

4.1. Biochar application increases soil microbial biomass C and some extra- and intracellular enzyme activities

In this meta-analysis, biochar application was found to significantly increase microbial biomass C and activities of some extracellular enzymes including N cycling (urease), P cycling (alkaline phosphatase) and intracellular enzyme (dehydrogenase). Similar to the results of Zhang et al. (2019), N-*acq* enzymes activities were found to be significantly increased in this meta-analysis, however, P-*acq* enzymes activities were not significantly changed, the result is different from Zhang et al (2019) where P- *acq* activities were shown to be significantly increased by 11% by biochar application. Probably the inclusion of more data points in our study (166) caused the disappearance of the effects of biochar on P-*acq* activities observed in Zhang et al. (2019) based on 76 data points.

The observed increase in these enzyme activities could be due to the increase in the availability of resources such as labile organic C (Kuzyakov et al., 2009) or the increase in reaction kinetics by improving soil matrix pH through addition of biochar (Van Zwieten et al., 2010; Gul et al., 2015). The increase in microbial and enzyme activities in the soil has also been referred to as the priming effect caused by biochar application to soil (Wardle et al., 2008; Zimmerman et al., 2011). Although the amount of labile C present in the biochar is generally much lower than the recalcitrant C present, the stimulation of short-term microbial growth and enzyme activities by addition of biochar to the soil have been reported in previous studies (Zimmerman et al., 2011; Farrell et al., 2013). The surfaces and pores of biochar provide habitat for microorganisms as well as increase the movement of air, water and nutrients within the soil matrix that can help promote microbial abundance and activities (Gul et al., 2015). The protection of soil microorganisms (bacteria and fungi) from grazers or competitors on biochar pores has also been pointed out for the increase in microbial biomass and the activities of enzymes secreted by these microorganisms (Theis and Rillig, 2009). In addition, the increase in soil temperature by trapping heat due to biochar's black color may speed up microbial growth

and enzyme activities. However, further studies are warranted to assess the effect of biochar on increasing soil temperature that subsequently affected microbial and enzyme activities in the soil.

The increase in alkaline phosphatase activity in biochar-amended soils suggests that (i) microbial demand for P increased, (ii) P availability in soil for microbial growth became limiting, or (iii) a combination of both occurred (Nannipieri et al., 2002; Schimel and Weintraub, 2003) in biochar-amended soils. Dehydrogenase activity that is considered to be a good indicator of metabolic activity was enhanced by the addition of labile organic C through biochar application (Serra-Wittling et al., 1995). The oxidative enzymes that mediate oxidation of phenolic compounds using oxygen were almost unchanged (although phenol oxidase tended to decrease slightly) by biochar application, suggesting that biochar does not play crucial roles in key ecosystem functions of lignin degradation, humification of aromatic ring-containing xenobiotic chemicals and dissolved organic C export (Sinsabaugh, 2010). Phenol oxidase is primarily produced by fungi (Burke and Cairney, 2002), the decreasing tendency of this enzyme activity can potentially be linked to the decrease in fungal biomass due to the increase in soil pH by biochar addition (Rousk et al., 2009).

Biochar addition shows contrasting effects on C- and N- acquiring enzyme activities in response to its effect on microbial biomass increase particularly in acidic soils although the effects were not significant in neutral and alkaline soils (Fig. 2). The decrease in *RR*_C-*acq* enzyme with an increase in *RR*_MBC indicates that the increase in MBC by biochar addition tends to decrease C-*acq* enzyme activities in the soil. Biochar addition increases labile C content in the soil that leads to an increase in microbial biomass. With an increase in easily available C source, microorganism allocate less energy to produce C-*acq* enzymes in order to reduce costs and maximize resource returns (Allison and Vitousek, 2005). Since N contained in the biochar added to the soil is generally not easily available for microbial consumption, microorganisms have to produce more N-*acq* enzymes to meet the increasing microbial demand of N when external N added to the soil (such as through biochar addition) is not readily available (Moorhead and Sinsabaugh, 2006). The increase in N-*acq* enzyme activities by biochar addition indicates that microorganisms in these soils are N-limited (Talbot and Treseder, 2012) possibly caused by high C/N ratios of biochars that can lead to N immobilization in soil (Bengtsson et al., 2003).

4.2. Biochar-induced changes in soil MBC and enzyme activities vary with soil conditions

Similar to results in a previous meta-analysis (Zhang et al., 2019) and other published studies, this meta-analysis also shows that biochar-induced changes in soil MBC and enzyme activities vary widely with soil conditions. Biochar's effect was more pronounced in soils with acidic pH than the soils with neutral and alkaline pH as demonstrated by the significant increase in MBC and *N-acq* in the acidic soils. Most of the biochars have alkaline pH, the addition of biochar thus may increase the pH of the soil by its liming effects (Clough et al., 2013; Gul et al., 2015; Nguyen et al., 2017), making the soil condition more favorable for microbial and enzymatic activities. The increase in *N-acq* enzyme might be linked to the decreased N availability to microorganisms by biochar addition because of high metabolism of microorganisms due to the increased pH as limited N availability can stimulate enzyme production (Allison and Vitousek, 2005). Since enzyme production is N and energy intensive process, microorganisms produce enzymes at the expense of growth and metabolism of microorganisms at lower nutrient availability (Allison and Vitousek, 2005). Contrary to this, the theory of stimulation of enzyme production by addition of complex sources to mobilize nutrients from these sources (Sinsabaugh and Moorhead, 1994) can also explain the reason for increased *N-acq* enzyme in biochar-amended soil. The increase in *N-acq* enzyme activities in acidic soil by biochar application has an important implication in maintaining soil health particularly in agricultural soils that are often severely degraded and acidified because of excessive use of inorganic fertilizer.

The significant increase in alkaline phosphatase (by 53%) but not in acid phosphatase activities by biochar application in acidic soil shows highly sensitive nature of alkaline phosphatase with pH change in biochar-amended soil. Acosta-Martinez and Tabatabai (2000) showed that alkaline phosphatase activities were increased by 97 times with increase of a unit pH change resulting from liming in agricultural soil. On the other hand, dehydrogenase activity was significantly increased only in the soil having pH range between 6.5 to 7.5 with no significant effects on acidic and alkaline soils. Since dehydrogenase activity can be used as an indicator of metabolic activity in the soil (Moeskops et al., 2010), biochar was found to be ineffective to change the metabolic activity in acidic and alkaline soils. Although, soil pH has been found to be the best predictor of dehydrogenase activity in different soils (Quilchano and Maranon, 2002), the result of this meta-analysis suggests that dehydrogenase activities in biochar-amended soils are likely be affected more by other than the liming factor of biochar.

Another important soil factor that significantly affects MBC and enzymatic activities after biochar application is the native SOM. Biochar increased MBC and N-*acq,* urease and alkaline phosphatase activities in soils having relatively lower SOM. Although, we were not able to assess the change in soil organic C and N by biochar application in this meta-analysis as only a few studies (we considered in this study) have reported it, we assume that the addition of biochar might have increased the soil organic C significantly (as shown in a meta-analysis study by Liu et al., 2016) that could increase microbial and enzyme activities in the soils where these activities were limited by low availability of substrate as in the case of soil with low SOM (Ameloot et al., 2015). Soil MBC and N-*acq*, urease, alkaline phosphatase and dehydrogenase activities were found to increase in greenhouse and field experiments but not in lab incubation. In lab incubation, effects of biochar are assessed in controlled environment, but field experiments involve many environmental factors that are not under control such as soil moisture and temperature that can have significant effects on enzyme activities (Steinweg et al., 2012), the effect of increasing soil temperature and moisture by biochar addition might be the cause for the increase in these activities in field experiments.

4.3. Biochar-induced changes in soil MBC and enzyme activities vary with biochar properties

The overall response of biochar application on soil MBC and activities of most of the enzymes (we considered in this study) were positive, but the response differed in magnitude among C, N and P cycling enzymes as well as the biochar types. Biochar itself is a heterogeneous material (Czimczik et al., 2002, Downie et al., 2009, Keiluweit et al., 2010); the variations in biochar's properties are induced by feedstock type and pyrolysis conditions (Kloss et al., 2012). The major variations occur in biochar pH, C/N ratio, surface area and porosity that can substantially change the microbial and enzymatic activities in biochar-amended soil. The multiple regression analysis (data not shown) showed that biochar's pH and C/N ratio and pyrolysis temperature and application rate could explain only a part (3 to 42%) of the total variation in weighted response ratios of microbial and enzyme activity change in biochar-amended soils. This result suggests that other attributes such as surface area, porosity and labile C present in the biochar should also be considered to assess the effect of biochar application on microbial and enzyme activities in the soil. Among the biochar properties we studied in this meta-analysis, biochar's pH has pronounced effect in changing MBC, N-*acq* and urease activities. Biochar with high pH (> 10)

made significant increase but other biochars did not. Increasing pyrolysis temperature often produces biochar with higher pH that might be useful in increasing N-releasing enzyme activity in the soil (Gul et al., 2015). The biochars produced at temperature range of 350-550 °C showed significant increase in these enzymes but not the biochar produced at lower pyrolysis temperature. The activities of dehydrogenase, however, was increased by biochar produced at low temperature (< 350 °C), biochars produced at low temperature can have significant amount of volatile organic matter in the biochar that can stimulate dehydrogenase production to increase metabolic activity of the soil microorganisms for volatile organic matter decomposition (Moeskops et al., 2010).

Another key factor to significantly affect *N-acq*, urease and dehydrogenase is C/N ratio of biochar. Biochar with low C/N ratio had significant positive effects but not the biochars with high C/N ratio. Although C/N ratio of soil is negatively correlated with enzyme activities (Geisseler and Horwath, 2009), addition of biochars (which have generally much higher C/N ratio than that of soil) did not cause significant negative impacts on enzyme activities. In biochar-amended soil, the effect of biochar addition may not be enough to have substantial increase in soil's C/N ratio for the significant negative impact on enzyme activities. Under feedstock type categories, manure-based biochars were found to increase urease, crop residue-based and wood-based biochars to increase alkaline phosphatase activities. One possible mechanism for the increase in these enzymes by adding biochars is the stimulation of corresponding enzyme production due to addition of organic N and P rich biochars to the soil as micro-organisms can produce more enzymes to mobilize mineral N and P from these added organic matters (Allison and Vitousek, 2005).

5. Conclusions

Biochar application increased soil microbial biomass and activities of some of the enzymes we studied although the magnitude of increase in microbial biomass and those enzymatic activities differed widely with soil type and biochar property. Biochar application is not equally useful in increasing microbial biomass and enzymatic activities in the soil over a wide range of soil pH, SOC and soil texture, as this study shows that biochar can increase microbial biomass and enzymatic activities in soils with lower pH, TC and TN, and in fine textured soils but not in neutral, alkaline or coarse-textured soils. Before biochar application, determining some of the

key soil characteristics such as pH, SOC and texture is thus important to achieve the anticipated result of improving soil quality through increasing microbial biomass and stimulating enzymatic activities in biochar-amended soils. Similarly, due to availability of a wide range of feedstock types and pyrolysis conditions, biochars with diverse characteristics have been produced; optimizing biochar characteristics by selecting a particular feedstock and pyrolysis temperature can yield substantial benefit in improving soil quality, as this study shows that biochars with a higher pH, lower C/N ratio or produced at pyrolysis temperatures of 350-550 ºC had greater effects on microbial biomass and enzymatic activities. The increase in *N-acq* and alkaline phosphatase activities by biochar application have important implications for agricultural soils that are extensively cultivated and have low crop productivity as such soils may have limited N and P availabilities.

Table 7-1. Overall effects (ln *RR'*) of biochar application on soil microbial biomass and enzyme activities

No	Enzyme	EC	Abbreviation	Functions
1	α-1,4-glucosidase	3.2.1.20	AG	Hydrolysis of complex carbohydrates, starch and glycogen
2	β-1,4-glucosidase	3.2.1.21	BG	Cellulose degradation
3	β-D-cellobiohydrolase	3.2.1.91	CBH	Cellulose degradation
4	β-1,4-xylosidase	3.2.1.37	BX	Reduction of cellulose from xylan
5	β-1,4-N-acetyl-glucosaminidase	3.2.1.14	NAG	Chitin and peptidoglycan degradation
6	Leucine amino peptidase	3.4.11.1	LAP	Hydrolysis of polypeptides to leucine and other hydrophobic amino acids
7	Urease	3.5.1.5	UR	Hydrolysis of urea to ammonia and CO_2
8	Acid phosphatase	3.1.3.2	ACP	Hydrolysis of phosphosaccharides and phopsholipids to release phosphates
9	Alkaline phosphatase	3.1.3.1	ALP	Hydrolysis of phosphosaccharides and phopsholipids to release phosphates
10	Phenol oxidase	1.10.3.2	PHOx	Extracellular oxidation of lignin
11	Peroxidase	1.11.1.7	PEO	Extracellular oxidation of lignin
12	Dehydrogenase		DEH	Intracellular oxidation of organic molecules during microbial respiration

Table 7-2. Overall effects (ln *RR'*) of biochar application on soil microbial biomass and enzyme activities

Variable	Mean (ln *RR'*)	CI		Sample size (n)	Effect (%)
		Lower	Upper		
MBC	**0.190**	**0.096**	**0.287**	**108**	21.7
MBN	0.154	-0.119	0.396	58	15.2
AG	0.155	-0.404	0.766	17	18.9
BG	-0.058	-0.166	0.057	165	-6.7
CBH	0.103	-0.205	0.360	45	10.1
BX	0.045	-0.250	0.360	25	9.2
NAG	0.142	-0.222	0.536	48	15.4
LAP	0.144	-0.026	0.310	**35**	16.0
UR	**0.202**	**0.061**	**0.345**	**91**	23.1
ACP	-0.068	-0.171	0.052	130	-6.0
ALP	**0.225**	**0.065**	**0.385**	**77**	25.4
PHOx	-0.156	-0.385	0.117	38	-12.6
PEO	-0.051	-0.532	0.375	19	-5.8
DEH	**0.174**	**0.013**	**0.338**	**108**	19.8
C-acq	-0.047	-0.172	0.072	162	-4.8
N-acq	**0.218**	**0.066**	**0.353**	**121**	23.3
P-acq	0.025	-0.064	0.133	161	3.1

Abbreviations: ln *RR'*: weighted response ratio: MBC: microbial biomass carbon; MBN: microbial biomass nitrogen, AG: α-1,4-glucosidase; BG: β-1,4-glucosidase; CBH: β-D-cellobiohydrolase; BX: β-1,4-xylosidase; NAG: β-1,4-N-acetyl-glucosaminidase; LAP: leucine amino peptidase; UR: urease; ACP: acid phosphatase; ALP: alkaline phosphatase; PHOx: phenol oxidase; PEO: peroxidase; DEH: dehydrogenase; *C-acq*: carbon acquisition enzyme; *N-acq*: nitrogen acquisition enzyme; *P-acq*: phosphorus acquisition enzyme; CI: confidence interval at 95%.

Effect size in bold indicates significant effect of biochar application at 95% CI, positive values in effect size indicate positive effect and negative values indicate negative effect.

Table 7-3. Effects of biochar application on the activities of dehydrogenase and urease under different edaphic factors.

Edaphic factor	Dehydrogenase					Urease				
	Mean (ln *RR'*)	CI		Sample size (n)	Effect (%)	Mean (ln *RR'*)	CI		Sample size (n)	Effect (%)
		Lower	Upper				Lower	Upper		
Soil pH										
Acidic	0.160	-0.080	0.454	53	17.2	0.209	-0.001	0.399	68	23.4
Neutral	**0.359**	**0.149**	**0.576**	**24**	**40.2**	0.060	-0.168	0.251	8	5.8
Alkaline	0.016	-0.361	0.432	23	0.8	0.289	-0.003	0.599	11	34.1
Soil TC										
<10	-0.047	-0.448	0.321	22	-8.0	**0.295**	**0.140**	**0.463**	**50**	**33.3**
10-20	0.198	-0.062	0.447	57	21.2	**0.282**	**0.068**	**0.491**	**21**	**31.2**
>20	0.246	-0.272	0.755	15	28.1	-0.023	-0.557	0.402	18	-7.4
Soil TN										
<1	0.046	-0.649	0.851	16	4.1	**0.270**	**0.113**	**0.428**	**35**	**31.3**
1-2	0.250	-0.073	0.561	48	21.6	**0.387**	**0.150**	**0.672**	**26**	**48.1**
>2	0.047	-0.313	0.478	10	3.9	-0.111	-0.567	0.381	16	-8.1
Soil texture										
Coarse	0.198	-0.055	0.438	43	18.5	0.187	-0.016	0.365	46	19.6
Medium	0.078	-0.300	0.436	19	11.6	NA	NA	NA	NA	NA
Fine	-0.039	-0.903	0.805	46	0.5	**0.270**	**0.064**	**0.500**	**32**	**32.2**
Land use										
Dry cropland	0.164	-0.003	0.326	72	17.5	**0.223**	**0.034**	**0.408**	**62**	**24.1**
Forest	-0.042	-0.593	0.515	14	-1.9	NA	NA	NA	NA	NA
Rice paddy	**0.888**	**0.561**	**1.171**	**4**	**139.1**	NA	NA	NA	NA	NA
Grassland	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Abbreviation: TC: total carbon (g kg^{-1}), TN: total N (g kg^{-1}); ln *RR'*: weighted response ratio; CI: confidence interval at 95%. Effect size in bold indicates significant effect of biochar application at 95% CI, positive values in effect size indicate positive effect and negative values indicate negative effect.

Table 7-4. Effects of biochar application on the activities of alkaline phosphatase under different edaphic factors.

Edaphic factor	Alkaline phosphatase				
	Mean (ln *RR'*)	CI		Sample Size (n)	Effect (%)
		Lower	Upper		
Soil pH					
Acidic	**0.432**	**0.243**	**0.626**	**14**	**52.6**
Neutral	0.136	-0.126	0.432	29	16.4
Alkaline	0.010	-0.110	0.127	30	16.4
Soil TC					
<10	**0.215**	**0.009**	**0.457**	**36**	**25.7**
10-20	**0.323**	**0.092**	**0.514**	**29**	**36.6**
>20	-0.035	-0.641	0.675	4	6.3
Soil TN					
<1	0.378	-0.203	0.948	12	44.1
1-2	**0.257**	**0.106**	**0.412**	**33**	**29.8**
>2	0.019	-0.648	0.711	9	8.1
Soil texture					
Coarse	0.258	-0.045	0.526	28	26.5
Medium	**0.247**	**0.028**	**0.438**	**18**	**29.2**
Fine	0.178	-0.043	0.421	19	18.8
Land use					
Dry cropland	**0.181**	**0.027**	**0.308**	**63**	**20.4**
Forest	NA	NA	NA	NA	NA
Rice paddy	**0.512**	**0.361**	**0.630**	**6**	**66.2**
Grassland	NA	NA	NA	NA	NA

Abbreviation: TC: total carbon (g kg^{-1}), TN: total N (g kg^{-1}); ln *RR'*: weighted response ratio; CI: confidence interval at 95%.

Effect size in bold indicates significant effect of biochar application at 95% CI, positive values in effect size indicate positive effect and negative values indicate negative effect.

Table 7-5. Effect of biochar application on the activities of dehydrogenase and urease under different biochar properties and experimental conditions.

Biochar properties	Dehydrogenase					Urease				
	Mean (ln *RR'*)	CI		Sample size (n)	Effect (%)	Mean (ln *RR'*)	CI		Sample size (n)	Effect (%)
		Lower	Upper				Lower	Upper		
Feedstock										
Crop residue	0.110	-0.144	0.361	42	11.5	0.252	-0.006	0.438	40	27.5
Wood	0.162	-0.075	0.435	52	16.6	0.176	-0.081	0.349	40	15.5
Manure	0.229	-0.095	0.522	9	26.8	**0.214**	**0.004**	**0.432**	**4**	**23.9**
Urban waste	0.526	-0.277	1.298	5	69.8	0.363	-0.139	0.724	7	39.3
Pyrolysis temp										
High	-0.006	-0.347	0.290	40	-1.5	**0.226**	**0.104**	**0.371**	**35**	**25.9**
medium	0.163	-0.107	0.404	41	19.1	0.219	-0.014	0.440	51	24.3
Low	**0.487**	**0.213**	**0.765**	**18**	**65.9**	0.121	-0.079	0.299	3	10.7
Biochar pH										
<8	0.363	-0.001	0.746	17	44.9	-0.229	-1.035	0.571	3	-24.2
8-10	0.094	-0.164	0.322	40	7.6	0.209	-0.010	0.399	43	23.2
>10	0.036	-0.349	0.447	27	3.6	**0.279**	**0.118**	**0.414**	**43**	**33.5**
Biochar C/N										
<50	**0.262**	**0.073**	**0.465**	**30**	**29.3**	**0.426**	**0.224**	**0.616**	**25**	**53.3**
50-100	0.271	-0.039	0.661	20	37.1	**0.263**	**0.094**	**0.447**	**32**	**29.4**
>100	-0.027	-0.360	0.336	38	-2.3	0.027	-0.147	0.229	26	3.6
Biochar rate										
<1	0.122	-0.051	0.311	31	13.4	**0.225**	**0.005**	**0.442**	**25**	**22.5**

1-3	0.141	-0.157	0.477	39	13.4	0.194	-0.076	0.434	39	22.6
3-5	-0.024	-0.691	0.814	4	-2.6	0.110	-0.065	0.295	14	11.2
>5	0.214	-0.094	0.539	31	27.1	**0.424**	**0.161**	**0.675**	**9**	**50.6**
Experiment duration										
Short	0.150	-0.044	0.380	58	16.2	**0.258**	**0.038**	**0.470**	**42**	**29.2**
Medium	0.101	-0.221	0.417	21	12.0	0.095	-0.135	0.311	27	11.2
Long	0.108	-0.460	0.621	12	14.9	0.190	-0.250	0.572	20	23.9
Experiment										
Lab incubation	0.070	-0.206	0.354	43	6.5	0.236	-0.077	0.512	39	27.9
Field	0.216	-0.071	0.535	19	24.7	0.206	-0.119	0.487	25	21.2
Greenhouse	**0.288**	**0.059**	**0.501**	**10**	**31.8**	**0.185**	**0.070**	**0.298**	**27**	**19.9**

Abbreviation: C/N: carbon to nitrogen ratio; ln *RR'*: weighted response ratio; CI: confidence interval at 95%.

Effect size in bold indicates significant effect of biochar application at 95% CI, positive values in effect size indicate positive effect and negative values indicate negative effect.

Table 7-6. Effect of biochar application on the activities of alkaline phosphatase under different biochar properties and experimental conditions.

Biochar properties	Alkaline phosphatase				
	Mean (ln *RR'*)	CI		Sample size (n)	Effect (%)
		Lower	Upper		
Feedstock					
Crop residue	**0.211**	**0.025**	**0.4050**	**53**	**21.3**
Wood	**0.228**	**0.098**	**0.3750**	**20**	**25.7**
Manure	NA	NA	NA	NA	NA
Urban waste	NA	NA	NA	NA	NA
Pyrolysis temp					
High	0.350	-0.027	0.694	10	41.5
medium	**0.214**	**0.045**	**0.403**	**53**	**24.9**
Low	NA	NA	NA	NA	NA
Biochar pH					
<8	0.153	-0.068	0.374	16	17.0
8-10	0.268	-0.090	0.579	24	30.2
>10	0.183	-0.006	0.359	23	19.8
Biochar C/N					
<50	0.144	-0.061	0.359	23	14.5
50-100	0.295	-0.066	0.614	27	32.1
>100	**0.227**	**0.057**	**0.415**	**21**	**25.9**
Biochar rate					
<1	**0.341**	**0.058**	**0.598**	**26**	**41.2**

1-3	**0.254**	**0.119**	**0.415**	**32**	**30.2**
3-5	0.029	-0.653	0.570	3	2.2
>5	-0.244	-0.516	0.131	7	-18.9
Experiment duration					
Short	0.004	-0.174	0.162	43	0.3
Medium	**0.218**	**0.006**	**0.399**	**15**	**25.6**
Long	**0.549**	**0.300**	**0.763**	**12**	**74.4**
Experiment					
Lab incubation	-0.024	-0.322	0.283	20	-2.5
Field	**0.503**	**0.309**	**0.719**	**14**	**67.4**
Greenhouse	0.097	-0.027	0.244	43	11.1

Abbreviation: C/N: carbon to nitrogen ratio; ln *RR'*: weighted response ratio; CI: confidence interval at 95%.

Effect size in bold indicates significant effect of biochar application at 95% CI, positive values in effect size indicate positive effect and negative values indicate negative effect.

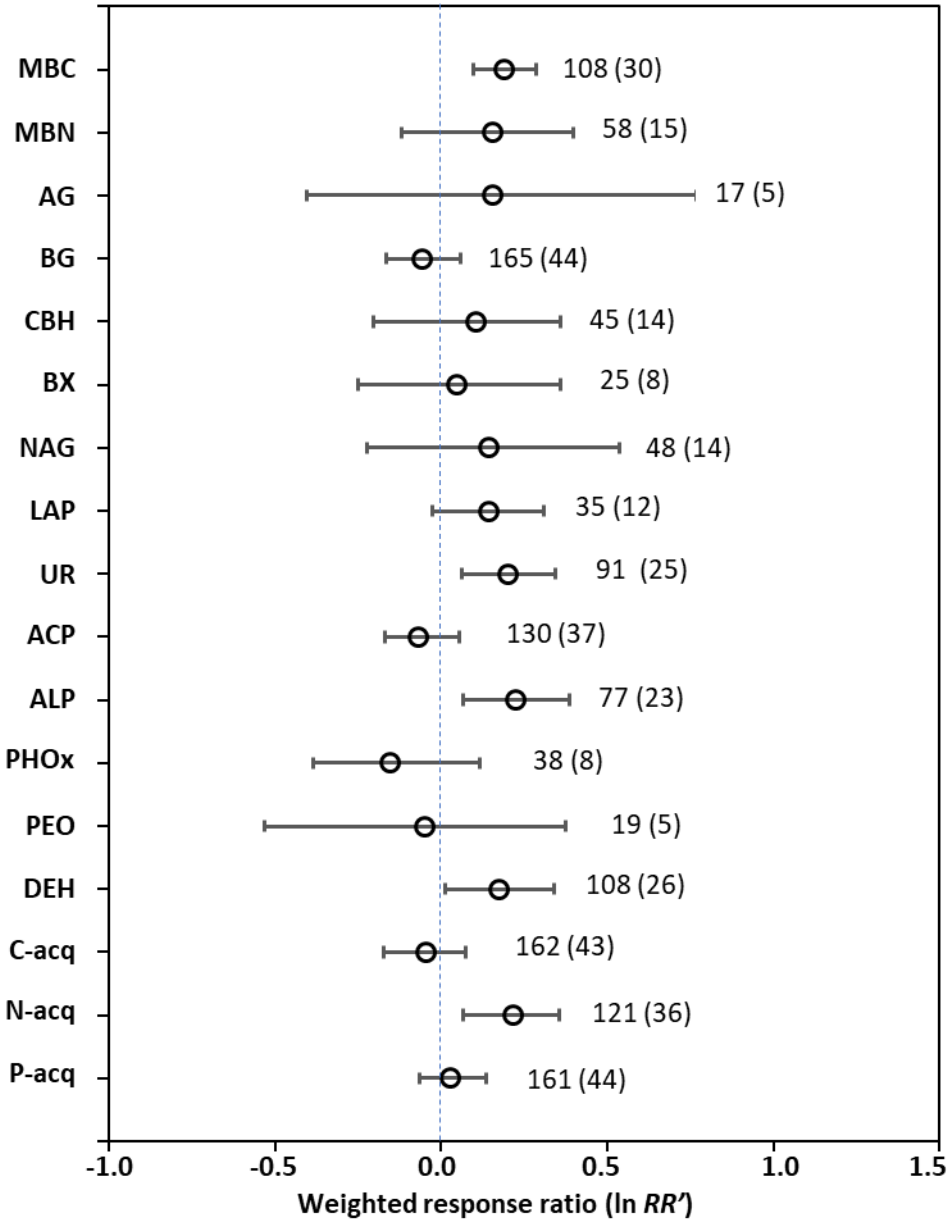

Fig. 7-1. Overall effects of biochar application on soil intra- and extracellular enzyme activities and microbial biomass carbon and nitrogen. The bars represent 95% confidence intervals and the number besides each bar represents sample size with the number of studies noted in parentheses.

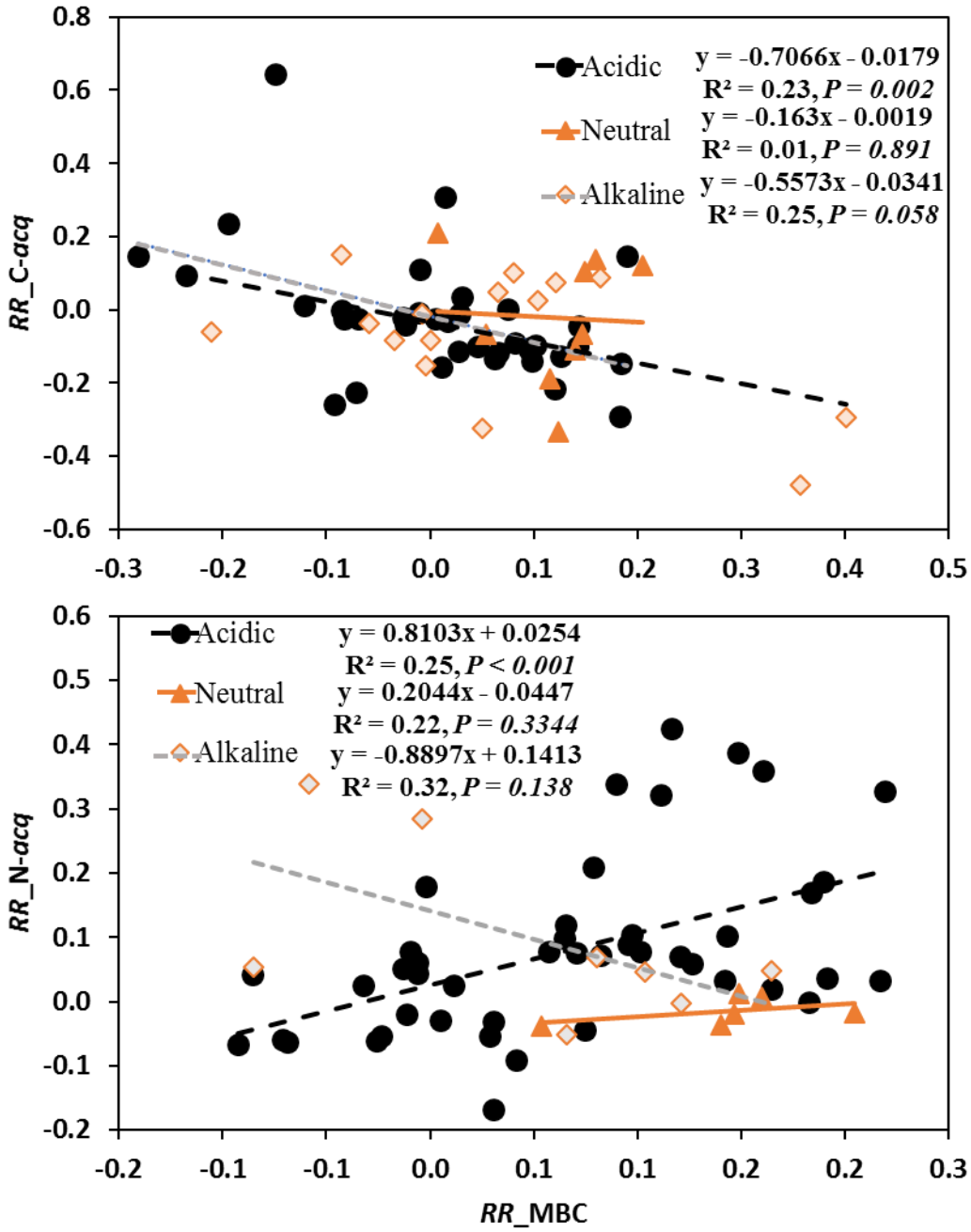

Fig. 7-2. Relationship of response ratio of MBC (*RR*_MBC) with response ratio of C-*acq* (*RR*_C-*acq*) and response ratio of N-*acq* (*RR*_N-*acq*) enzymes in biochar-amended acidic, neutral and alkaline soils.

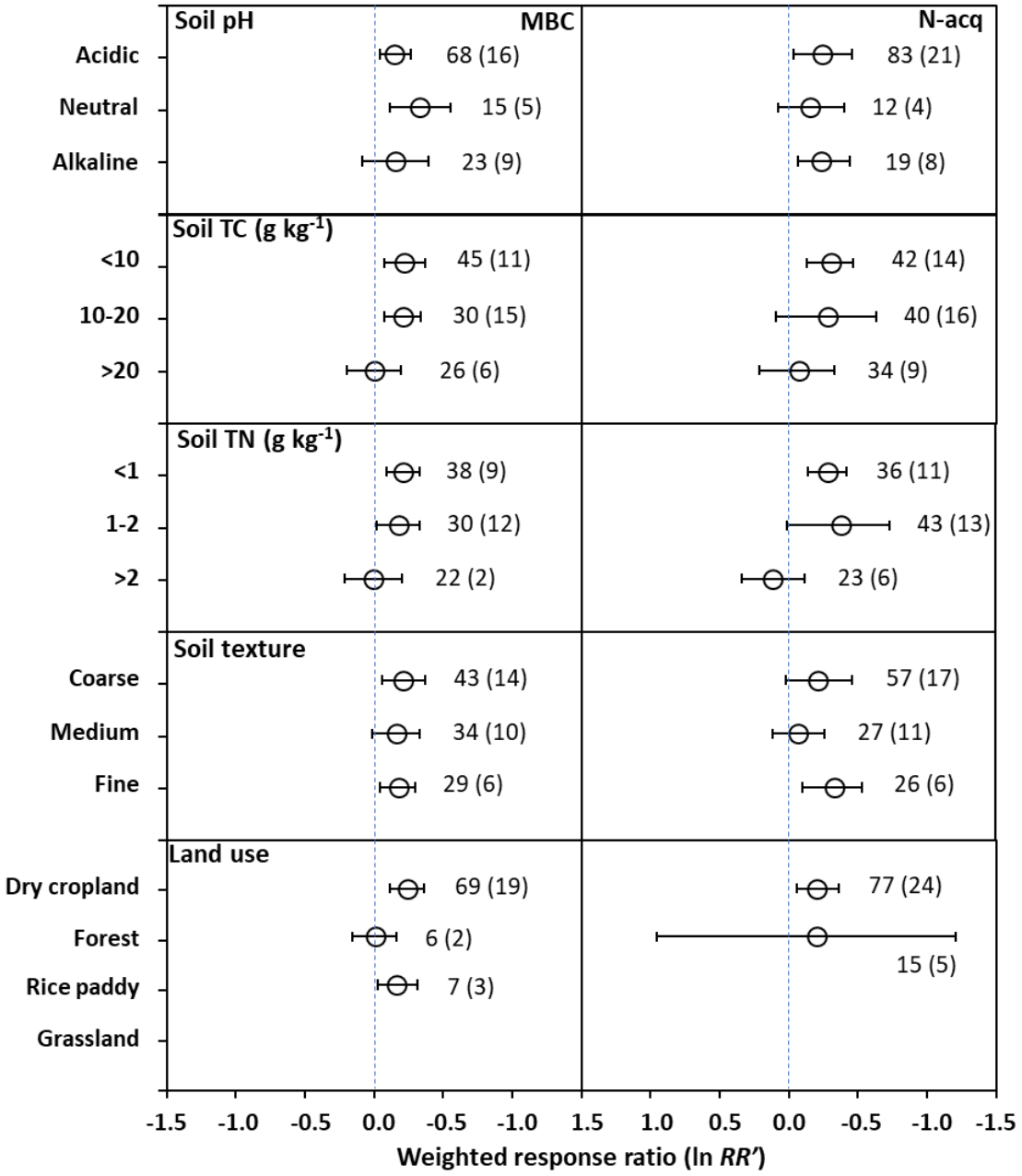

Fig. 7-3. Change in soil nitrogen acquisition (N-*acq*) enzyme activities and microbial biomass carbon (MBC) in biochar amended soils under different edaphic and experimental conditions. The bars represent 95% confidence intervals and the number besides each bar represents sample size with the number of studies noted in parentheses.

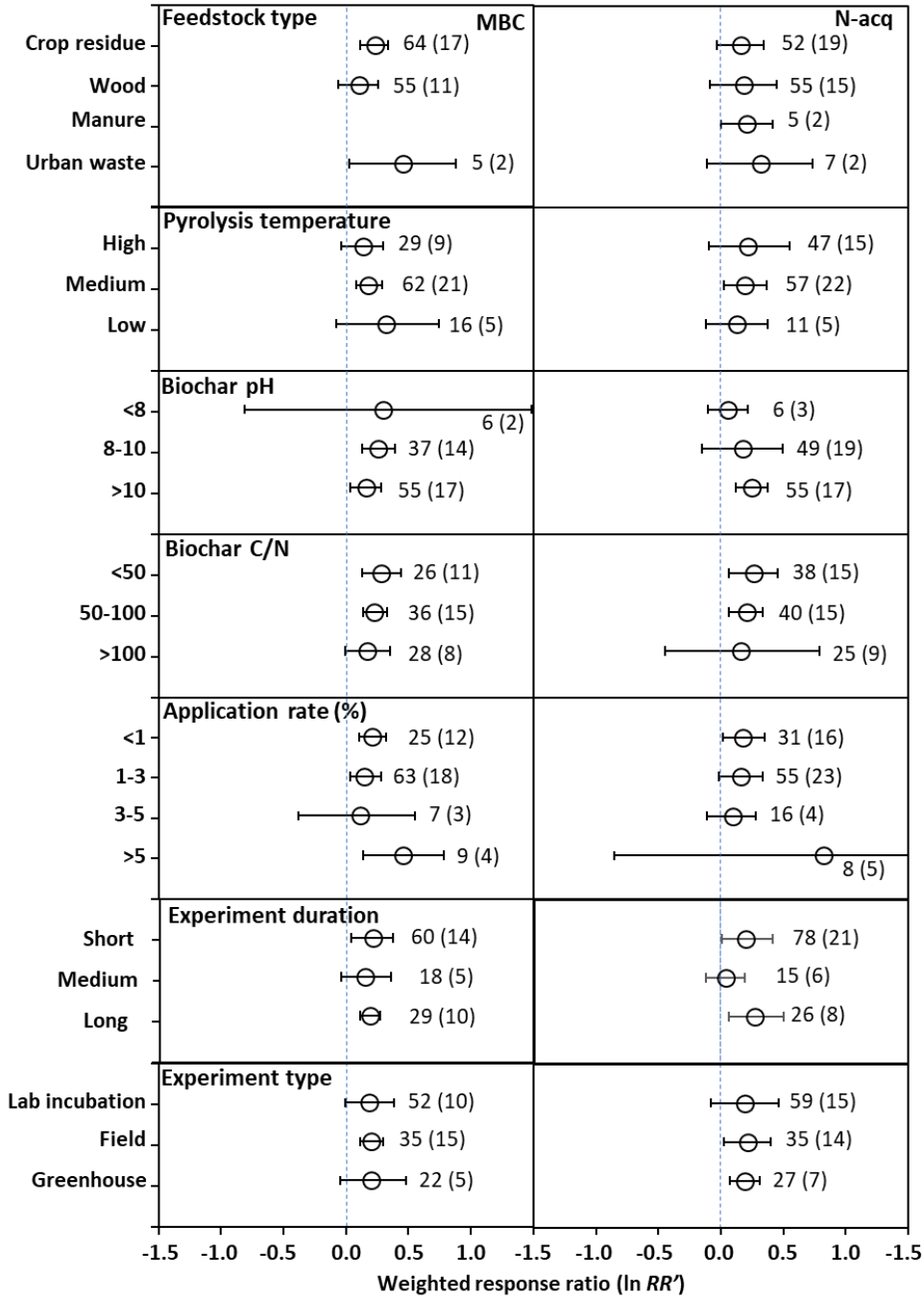

Fig. 7-4. Change in soil nitrogen acquisition (N-*acq*) enzyme activities and microbial biomass carbon (MBC) in soils amended with biochars with different properties. The bars represent 95% confidence intervals and the number besides each bar representing sample size with the number of studies noted in parentheses.

Chapter 8. Summary and future research recommendations

1. Research overview

The overall aim of this research was to gain insight into the benefits of biochar application to the soil in mitigating climate change and increasing crop production. Biochar amendment has been proposed as an effective agricultural management practice for reducing soil GHG emissions, increasing soil fertility and crop production, and improving soil health (Woolf et al., 2010). Biochar itself is a heterogeneous material with a wide variation in its properties based on feedstock, pyrolysis conditions and pre-and post-pyrolysis activations (Verheijen et al., 2010). In addition to that, biochar's effectiveness in achieving targeted benefits around climate change mitigation and crop production varies widely across geographical locations, soil types and their interaction with other management practices (Downie et al., 2009). This is why it becomes very hard to generalize the effectiveness of biochar in achieving these benefits unless we have the data across all these above-mentioned factors. That necessitates a large number of studies that account for various methods of biochar production, applied across different types of soil in different geographical locations and in interaction with different management practices. Because of this, the number of studies on biochar amendment has skyrocketed in the last decade. Despite that, there is still a gap in our understanding of how biochar can be used to enhance our benefits in reducing GHG emissions and crop production in the Canadian prairie region despite having a huge potential for environmental and economic benefits from biochar application in this region.

This thesis research had the main objective of assessing the effects of different types of biochars (based on feedstock type and pyrolysis condition) along with nitrification inhibitors on soil microbial activities and nutrient cycling for enhancing benefits in crop production and reducing GHG emissions from forest, grassland and cropland soils in the Canadian prairie region. To achieve the objective, this thesis research conducted experiments under different settings: (i) laboratory incubation experiments that focused on understanding the mechanism of the change in microbial growth and activity under a controlled environment (ii) greenhouse experiments focusing on the effects on yield of a single crop grown under a controlled environment, (iii) a field experiment with a crop rotation that allowed us to deal with the biochar's effects in interaction with another agricultural management practice (application of nitrification inhibitor) under uncontrolled environmental conditions, and (iv) a meta-analysis

study for assessing the overall effects of biochar in soil microbial and enzymatic activities across various environmental factors in different geographical locations.

2. Summary of research results

2.1. Pyrolysis temperature and steam activation in pine sawdust biochar for mitigating GHG emissions from forest and grassland soils

In this study, biochars were produced from pine saw dust at 300 and 550 °C with post pyrolysis steam activation, mixed with soil from a forest and grassland and incubated in dark at 25 °C in a laboratory for 100 days. The results showed that biochar produced at 550 °C and with steam activation had greater surface area, porosity, ash content and increased aromaticity than the biochar produced at 300 °C and without steam activation. Pyrolysis temperature and steam activation did not have significant interaction in affecting biochar's properties. The effects of pyrolysis temperature and steam activation were not consistent in reducing CO_2, N_2O and CH_4 emissions from forest and grassland soils. Biochars produced at 300 °C was not effective in reducing GHG emissions and changing microbial activities. Biochar produced at 550 °C without steam activation was effective in reducing cumulative CO_2 emission and global warming potential of the emissions from the forest but not in grassland soil while N_2O emissions were reduced by biochars produced at 550°C regardless of steam activation from forest and grassland soils. The persistent effect of biochar produced at 550 °C throughout the incubation for 100 days indicated long-term effect of these biochars in reducing N_2O emissions from forest and grassland soils. The study concluded that biochar produced from pine sawdust at 550 °C could be beneficial in reducing N_2O emissions from forest as well as grassland soils.

2.2. Biochars from manure pellets and woodchips in crop production and soil respiration in bulk and rhizosphere soils

In this study, biochars were produced from manure pellets and woodchips and applied to a cropland soil to assess their effects on crop production and soil respiration in bulk and rhizosphere soils under greenhouse conditions. The study aimed to assess the effects of biochar in the rhizosphere which is the hotspot of microbial activities. The results showed that pyrolyzed manure pellet and woodchips and their raw feedstocks differently affected wheat yield and soil

respiration from bulk and rhizosphere soils. Manure pellets and its their biochar increased while woodchip and its biochardecreased wheat yield. Both biochars decreased soil respiration from the rhizosphere soil but not from bulk soils; but the relativized cumulative CO_2 emissions was decreased by both biochars from both bulk and rhizpshere soils were decreased by both biochars. Although dissolved organic C and N were increased but microbial biomass C and N were decreased by manure pellet biochar application. The study concluded that biochars produced from organic residues have differential impacts on soi processes in the bulk and rhizosphere regions, and thus measurements based on bulk soil alone may result in erroneous conclusions about the effect of biochars on soil CO_2 emission.

2.3. Effects of manure-based biochar on heterotrophic respiration and gross nitrification rates

This study aimed to assess the effects of manure pellet biochar on C and N mineralization in the rhizosphere in a laboratory incubation experiment. Biochar-amended wheat rhizosphere soil was collected from the previous greenhouse experiment and gross N mineralization was examined using ^{15}N isotopic pool dilution method. The results showed that heterotrophic respiration was decreased by biochar but increased by its feedstock. Manure pellet increased N_2O emission from both bulk and rhizosphere soil but the percent increase was greater in the bulk than in the rhizosphere soil. Net N mineralization and nitrification rates were greater in biochar-amended rhizosphere soil than in the bulk soil. Rhizosphere soils had greater gross mineralization rate than in the bulk soil. The study concluded that biochar had similar effects on heterotrophic respiration and N_2O emissions but had contrasting effects on gross nitrification rates between rhizosphere and bulk soils, highlighting the importance of gross N transformation processes in understanding the rhizosphere-biochar interactions.

2.4. Interaction between manure pellet biochar and nitrification inhibitor in N_2O emission

The objective of the study was to assess the interaction between biochar and nitrification inhibitor in reducing N_2O emissions. The experiment was performed in a laboratory with a manure pellet biochar and nitrapyrin at different moisture contents of 60 and 80% water filled pore space (WFPS). The results showed that nitrification rates were significantly affected by biochar, nitrification inhibitor and moisture content interactions. Biochar initially increased and then decreased the rates, resulting in 45.2 and 26.6% overall reductions in low and high WFPS,

respectively while NI reduced the rates only in the first 10 days at 60% WFPS. Biochar decreased and NI increased β-1,4-N-acetyl glucosaminidase activities while urease activities were increased by biochar in both soil moisture contents. Biochar had significant interaction with NI in reducing cumulative N_2O emission with the efficacy of NI being reduced when co-applied with biochar. Cumulative N_2O emissions were greater at high than at low WFPS; the emissions were decreased by biochar at 60% WFPS and NI at both 60 and 80% WFPS. The study concluded that biochar reduces efficacy of nitrapyrin in mitigating N_2O emission and their effects on net nitrification rates, enzyme activities and N_2O emissions are dependent on soil moisture level.

2.5. Manure compost biochar and nitrification inhibitor in ecoenzymatic stoichiometry and microbial nutrient limitation

This study assessed the effect of variable rate of manure-compost with nitrification inhibitor in microbial and ecoennzymatic stoichiometry in a wheat canola-rotation in a field experiment. In this study, biochar produced from manure compost at the rate of 0, 10 and 20 t ha^{-1} with the recommended rate (2.7 L ha^{-1}) of eNtrench Nitrogen Stabilizer (NI) were applied to a Gray Luvisolic soil in a wheat-canola rotation. The results of this 2-year field study demonstrated that biochar did not have significant interaction with NI in affecting soil microbial biomass, enzymatic activities and their stoichiometries. Biochar increased microbial biomass C, N and P regardless of the rate but were not affected by NI. Biochar increased but NI decreased N- and P-cycling enzymatic activities. The studied area showed microbial P limitations regardless of the treatments with biochar had decreasing and NI had increasing P limitations. Biochar decreased the threshold elemental ratio of C:P at which microbial growth limitation switches between nutrient and C limitations, suggesting a shift towards C relative to nutrient (P) limitation. Biochar decreased and NI increased microbial P limitations relative to N. This study concluded that biochar produced from manure-compost can be useful in increasing microbial growth by alleviating P limitations in a wheat-canola crop rotation.

2.6. Biochar application in microbial biomass and eco-enzymatic activities

This study examined the overall effects of biochar in soil microbial biomass and enzymatic activities by a global meta-analysis. This study identified key factors of biochar that are effective

in increasing microbial biomass and enzyme activities under different soil environments using 964 data points from 72 papers. The results showed that the effects of biochar in soil enzyme activities vary in direction and magnitude based on soil type, biochar properties and the type of soil enzyme. Overall, biochar increased microbial biomass C and urease, alkaline phosphatase and dehydrogenase activities. Biochar was more effective in acidic soils, with low soil organic matter and finer texture for increasing microbial activities. In terms of biochar properties, high pH (>10) and C/N ratio less than 50 had the greatest impact on MBC and enzyme activities. Biochar produced at 300-550 °C increased but the pyrolysis temperature higher than 550 °C did not substantially affect soil MBC and enzyme activities. The effects of biochar also varied between laboratory and field studies, short-term (within 100 days biochar application) and long-term (that span longer than 100 days). application) while alkaline phosphatase was increased in long-term studies that span more than one year. The increase in MBC and activities of some soil enzymes in response to biochar application with no negative effects on any hydrolytic and oxidative enzymes illustrate its potential to enhance soil quality, particularly in degraded soils with low nutrient availability and fertility due to limited soil microbial and enzymatic activities. This study also showed that biochars can be designed to achieve specific properties for enhancing microbial and enzymatic activities for specific soils.

3. Conclusions

This thesis concludes that biochar is effective agricultural management practices for reducing GHG emissions, increasing crop production and alleviating microbial P limitations with or without having substantial interaction with a nitrification inhibitor in some of the soil processes. The increase or decrease in the soil microbial and enzymatic activities in biochar applied soil varied well with pyrolysis conditions (temperature and post-pyrolysis activation), feed-stock type, land use type (forest, grassland and agricultural land) and in interaction with management practices, including nitrification inhibitor. One of the easily available feedstocks in in this region is manure, pelletizing manure or composting with woodchips (a waste of sawmill industries) before pyrolysis yielded a biochar that seemed to be effective in soil amendment for mitigating climate change and enhancing soil health. The thesis added on the knowledge on providing a mechanistic understanding of how biochar performs differently between bulk and rhizosphere region. Manipulation of rhizosphere is more important than the bulk soil region in terms of crop

production because rhizosphere is the region where plant roots interact with soil that influences the nutrient uptake of plants most (Kuzyakov and Domanski, 2000; Philippot et al., 2013). This thesis demonstrated significant difference in the impact of biochar on nutrient mineralization and highlighted the importance of taking rhizosphere into account for understanding soil processes and functions after biochar amendment in a cropland soil.

In the context of recommendation for a wide application of biochar for climate change mitigation and increasing crop production across the world in different land use types, we may come across using biochar with other agricultural management practices including NI application that necessitates the understanding of their interactions in the soil. This thesis research showed the results of biochar and NI effects in various soil processes such as soil nitrification and N_2O emissions, microbial and enzymatic activities through laboratory and field experiments and concluded that manure pellet biochar decreases the efficacy of nitrapyrin in reducing nitrification rate and N_2O emissions but manure compost biochar does not impact on the efficacy of nitrapyrin in affecting some other soil processes such as microbial and ecoenzymatic stoichiometry. One of the important benefits of biochar is the improvement of soil health by increasing soil microbial and enzymatic activities. The results of this thesis showed a wide variation on the impacts of soil microbial and enzymatic activities through global meta-analysis and concluded that optimizing biochar properties for enhancing soil health are soil-specific and targeted enzymes for C-, N and P-acquisition. This thesis examined some of the soil processes related to GHG emissions, nutrient mineralization and crop production in response to biochar and a nitrification inhibitor, further studies should explore microbial community composition in different soils of Canadian prairie region to improve our understanding of biochar's potential benefits in this region.

4. Recommendations for future studies

- Long-term studies: Most of the studies in this thesis are short-term studies, including lab incubation, greenhouse and field experiments that collected data for two years. The effects of biochar and nitrification inhibitor in changing soil processes and function are highly dependent on environmental factors (Lehmann, 2007; Akiyama et al., 2010) which has been indicated to some extent in the field experiment in this thesis, the impact of these treatments was different between the first and second year given the difference in precipitation and

temperature in the study area in addition to the difference in the crops grown in the field. So, it is necessary to get the data for multiple years from long-term field studies that account for the variation in weather conditions and crop rotations to make any valid recommendations of using biochar and nitrification inhibitor in agricultural management practices in the Canadian prairie regions.

- Effect of fresh and aged biochar on soil processes: Several researchers have demonstrated substantial differences between fresh and aged biochars in terms of their porosity, CEC and recalcitrance (Tan et al., 2020). Since biochar is persistent in a soil on a centennial scale (Kuzyakov et al., 2009), it is unclear whether the biochar behaves in the same or different manner when it is fresh or field aged after application of several years. Since there has been a history of around 20 years of biochar applied in the prairie region, there is an opportunity to revisit those sites to assess the effects of field-aged biochar in soil processes and functions.
- Biochar's effects on sulfur fertility: Most of the earlier biochar research focusses on N and P fertility (Chien et al., 2011). Sulfur is one of the macro nutrients for crop production and S deficiencies have been frequently found on well-drained and grey wooded soils in the Canadian prairies (Giweta et al., 2014). Application of sulfate-containing fertilizers is susceptible to leaching in coarse-textured soils. Biochar application can be a potential management practice to reduce the leaching loss of sulfate that could be beneficial in increasing nutrient use efficiency and production of high S demanding crops such as canola. Further research is thus necessary to improve our understanding on S and other nutrient use efficiency in response to biochar applications.
- Biochar's effects on micronutrients: Biochar itself is not a fertilizer but plays important role in increasing nutrient availability through physical (by adsorption), chemical (by changing soil pH) and biological (by changing microbial community composition) properties of the soil. There are many parts around the world and in Canadian prairie region with deficiency of micro-nutrients such as Mo, Zn, Cu and B which limit the crop production. Biochar application can enhance availability of those micro-nutrients (either by adding those nutrients from the biochar itself or by changing soil pH) thereby increasing nutrient use efficiency of macro-nutrients and crop yield. This kind of research has not received much attention by the researchers in the past

- Biochar's interaction with other management practices: One of the emerging management practices to help mitigate climate change and increase crop production in the Canadian prairie is using a perennial cropping system (Amiro et al., 2017). This practice has shown a substantial impact on reducing GHG emissions from the soil and increase in SOC and crop production (Kim et al., 2021). Biochar has been proposed as an effective method for making climate-smart soil, but previous studies have shown that biochar may have positive as well as negative priming effects in native soil C mineralization. It is therefore important to study the effect of using a perennial cropping system in biochar-amended soils to know if these two management practices will have synergistic or antagonistic effects in increasing SOC and improving soil health.
- Variable rates of biochar and fertilizer: Many farmers in the Canadian prairie region follow fertilizer recommendations from soil tests and set their target yield to maximize economic return. That encourages farmers to use the fertilizer at the upper end which may sometimes cause greater loss of fertilizer and reduction in nutrient use efficiency. In this context, it would be helpful to test different rates of fertilizer and biochar in different soil types in this region to know the optimal rates of fertilizer and biochar for maximizing nutrient use efficiency and the potential of building up of SOC in the cropland, the data from such studies would be useful to farmer to make a decision on fertilizer and biochar application to their cropland.
- Life cycle assessment of biochar application: After all, the most important driver for farmers is whether they would like to apply biochar into their field or not is the economic return. Thousands of studies have demonstrated the environmental benefits of biochar application to the soil around the world, but it is very hard to convince local farmers to use biochar on their land unless they see an economic return from that. To know whether the application of biochar is economically viable in this region or not should be assessed through life cycle assessment. There have been only a few LCA analysis for biochar production and its application to the soil in Canada, but they are limited to the eastern region only (Dutta and Raghavan, 2014; Homagain et al., 2016). Since, the prairie region has a high potential of easily available feedstocks (manure and crop residues, for instance) in a local area that could substantially reduce the cost of biochar production and application in this region, indicating the economic viability of biochar amendment in this region. It is therefore essential that future studies should focus on LCA of biochar production and application in this region.

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Appendices

Appendix 2-1. Effects of biochar treatment on cumulative emission of CO_2, CH_4 and N_2O from forest and grassland soil in 100-day incubation

Treatment	Forest soil			Grassland soil		
	CO_2 (μg CO_2-C g^{-1} soil)	**CH_4 (ng CH_4-C g^{-1} soil)**	**N_2O (ng N_2O-N g^{-1} soil)**	**CO_2 (μg CO_2-C g^{-1} soil)**	**CH_4 (ng CH_4-C g^{-1} soil)**	**N_2O (ng N_2O-N g^{-1} soil)**
CK	644.72 (49.79)a	-317.09 (52.75)	91.8 (3.24)a	858.37 (65.54)	-563.75 (62.33)	233.17 (13.50)a
BC300	644.07 (56.66)a	-319.24 (24.45)	89.08 (6.35)a	835.30 (66.13)	-569.65 (61.44)	225.37 (13.68)a
BC300-S	670.85 (53.21)a	-357.15 (46.48)	64.15 (8.79)b	837.67 (56.57)	-561.32 (54.33)	215.81 (8.85)ab
BC550	539.30 (74.86)b	-374.34 (47.78)	66.54 (3.72)b	821.63 (67.07)	-588.07 (54.24)	198.60 (11.91)c
BC550-S	608.25 (62.08)ab	-364.83 (50.83)	62.86 (6.53)b	839.33 (56.57)	-583.76 (67.97)	205.93 (12.77)bc
One-way ANOVA[a]	**0.011**	0.0581	**0.033**	0.302	0.299	**0.004**

Values are means with standard errors in the parentheses (n = 4).

[a]Values in bold indicate significance at $P < 0.05$.

Negative values under CH_4 column indicate CH_4 uptake from the soil

Appendix 2-2. Effects of biochar treatment on soil enzyme activities in forest and grassland soils

Soil		GLU (μmol h^{-1} g^{-1} soil)				NAGase (μmol h^{-1} g^{-1} soil)			
	Treatment	Day 1	Day 10	Day 50	Day 100	Day 1	Day 10	Day 50	Day 100
Forest	CK	0.077 (0.01)	0.755 (0.07)ab	0.306 (0.03)	0.173 (0.01)	0.112 (0.02)	0.143 (0.01)a	0.163 (0.01)	0.162 (0.01)
	BC300	0.083 (0.01)	0.860 (0.13)a	0.289 (0.02)	0.170 (0.01)	0.089 (0.01)	0.139 (0.01)a	0.152 (0.01)	0.155 (0.01)
	BC300-S	0.072 (0.01)	0.721 (0.15)ab	0.329 (0.03)	0.165 (0.02)	0.084 (0.01)	0.121 (0.01)b	0.147 (0.01)	0.141 (0.01)
	BC550	0.084 (0.01)	0.560 (0.10)bc	0.240 (0.01)	0.163 (0.02)	0.098 (0.01)	0.128 (0.01)ab	0.137 (0.01)	0.151 (0.01)
	BC550-S	0.079 (0.01)	0.491 (0.06)c	0.257 (0.01)	0.163 (0.01)	0.092 (0.01)	0.116 (0.01)b	0.141 (0.01)	0.140 (0.01)
One-way ANOVA[a]		0.567	**0.014**	0.101	0.973	0.192	**0.0274**	0.416	0.221
Grassland	CK	0.171 (0.01)	0.366 (0.06)	0.658 (0.02)a	0.531 (0.04)	0.213 (0.02)	0.234 (0.02)ab	0.242 (0.02)a	0.291 (0.03)
	BC300	0.191 (0.02)	0.428 (0.08)	0.637 (0.03)ab	0.535 (0.02)	0.190 (0.03)	0.241 (0.04)a	0.216 (0.01)ab	0.264 (0.02)
	BC300-S	0.198 (0.04)	0.366 (0.03)	0.635 (0.04)ab	0.578 (0.03)	0.225 (0.02)	0.247 (0.03)a	0.224 (0.01)ab	0.271 (0.02)
	BC550	0.167 (0.01)	0.322 (0.01)	0.570 (0.02)bc	0.518 (0.03)	0.229 (0.02)	0.194 (0.02)b	0.206 (0.01)bc	0.257 (0.02)
	BC550-S	0.161 (0.01)	0.302 (0.01)	0.541 (0.02)c	0.483 (0.03)	0.221 (0.03)	0.193 (0.03)b	0.189 (0.02)c	0.258 (0.02)
One-way ANOVA[a]		0.538	0.358	**0.043**	0.491	0.671	**0.041**	**0.022**	0.223

Values are means with standard errors in the parentheses (n = 4).

[a]Values in bold indicate significance at $P < 0.05$.

Abbreviations: GLU = β-1, 4-glucosidase, NAGase = β-1, 4-N-acetylglucosaminidase.

Appendix 2-3. Effects of biochar treatment on soil microbial biomass C (MBC) and N (MBN) in forest and grassland soils

Soil		**MBC (mg kg^{-1})**				**MBN (mg kg^{-1})**			
	Treatment	Day 1	Day 10	Day 50	Day 100	Day 1	Day 10	Day 50	Day 100
Forest	CK	112.1(6.0)	103.6 (1.9)	142.4 (12.0)	162.9 (13.0)	24.2 (2.2)	20.7 (1.8)	38.5 (3.1)	49.3 (8.2)
	BC300	143.9 (5.7)	100.6 (1.6)	141.5 (11.6)	158.7 (8.4)	26.9 (2.7)	19.9 (1.5)	29.3 (2.5)	40.9 (2.5)
	BC300-S	129.0 (5.4)	99.8 (4.3)	141.2 (9.3)	153.5 (8.5)	27.1 (2.6)	16.9 (2.3)	26.1 (3.4)	37.6 (3.2)
	BC550	135.7 (3.4)	87.8 (5.4)	141.2 (11.7)	164.8 (6.9)	25.8 (1.6)	14.9 (2.7)	23.6 (3.4)	39.3 (4.2)
	BC550-S	116.8 (5.2)	75.6 (5.4)	110.6 (8.3)	139.0 (13.2)	21.6 (1.5)	13.3 (1.1)	21.3 (1.0)	39.6 (5.1)
One-way ANOVA[a]		**0.006**	**0.001**	**0.042**	0.157	0.244	**0.001**	**0.002**	0.211
Grassland	CK	524.5 (78.5)	830.3 (41.1)	836.6 (29.7)	856.8 (73.0)	88.1 (8.4)	95.2 (5.0)	78.5 (1.3)	119.1 (10.4)
	BC300	651.0 (65.6)	852.2 (56.0)	770.9 (63.9)	1018.0 (113.0)	94.4 (3.2)	103.0 (6.8)	71.1 (3.7)	113.9 (8.0)
	BC300-S	685.8 (50.3)	762.3 (48.0)	817.9 (58.4)	918.9 (141.3)	87.6 (10.1)	92.3 (4.4)	81.7 (10.2)	125.8 (20.8)
	BC550	538.9 (39.6)	718.6 (55.4)	634.6 (51.9)	915.4 (154.3)	97.4 (9.5)	104.0 (7.0)	68.7 (6.5)	132.5 (13.1)
	BC550-S	537.2 (67.7)	685.5 (35.3)	641.4 (36.5)	811.6 (119.3)	102.4 (7.5)	86.5 (3.9)	72.3 (5.0)	140.0 (15.2)
One-way ANOVA[a]		0.149	0.101	**<0.001**	0.315	0.578	0.129	0.194	0.549

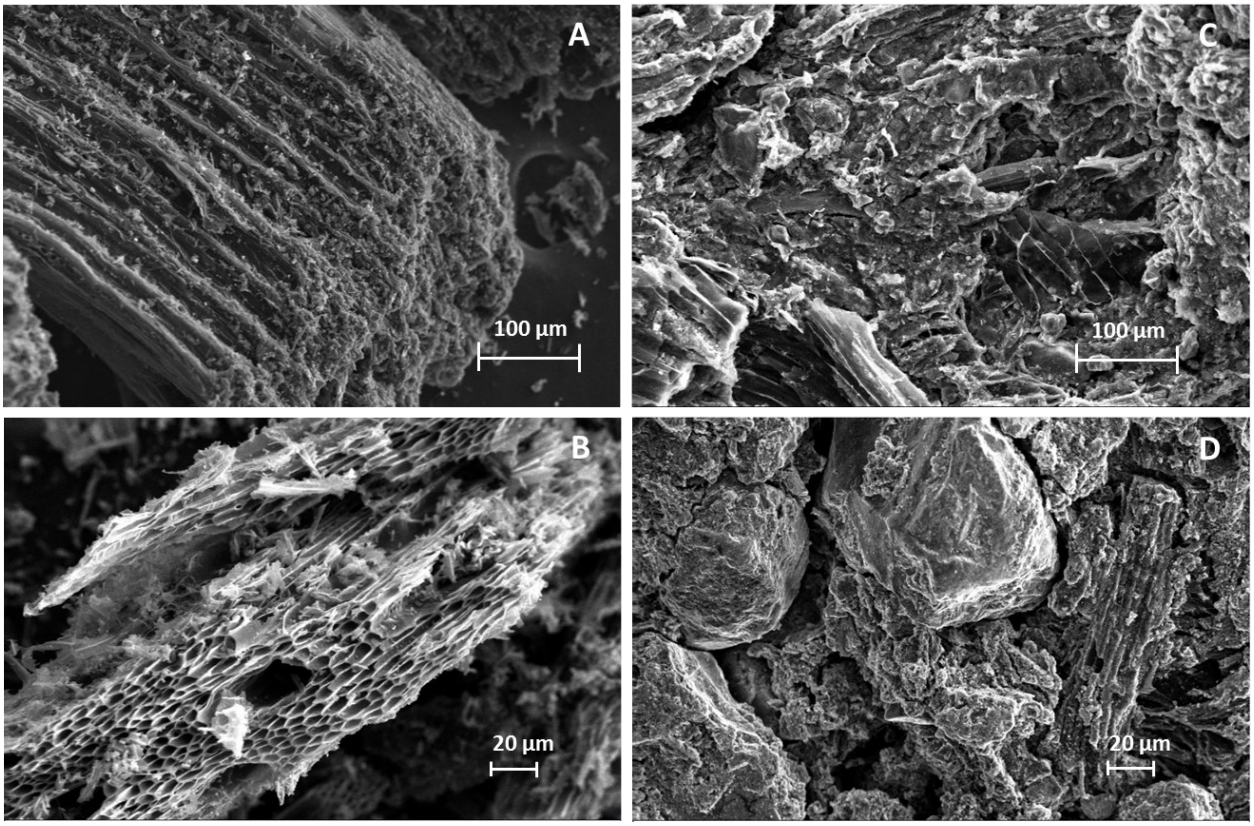
Values are means with standard errors in the parentheses (n = 4).

[a]Values in bold indicate significance at $P < 0.05$.

Appendix 2-4. The effects of biochar treatment, incubation time and their interactions on microbial biomass and enzyme activities in forest and grassland soils.

Soil properties	**Forest soil**						**Grassland soil**					
	Biochar treatment (B)		Incubation time (T)		B × T		Biochar treatment (B)		Incubation time (T)		B × T	
	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value
MBC	4.71	**0.011**	46.71	**<0.001**	0.94	0.518	2.44	0.091	14.83	**<0.001**	0.56	0.863
MBN	2.04	0.241	71.53	**<0.001**	1.11	0.374	0.18	0.943	36.41	**<0.001**	1.08	0.398
GLU	2.08	0.133	119.27	**<0.001**	1.81	0.076	3.15	**0.045**	145.42	**<0.001**	0.49	0.911
NAGase	4.63	**0.012**	56.25	**<0.001**	0.43	0.942	0.35	0.841	11.46	**<0.001**	0.99	0.471

Abbreviations: MBC = microbial biomass C, MBN = microbial biomass N, GLU = GLU = β-1, 4-glucosidase, NAGase = β-1, 4-N-acetylglucosaminidase



Appendix 3-1. Scanning electron micrograph (SEM) images of the biochars at different magnifications: (A) and (B) woodchip biochar, (C) and (D) manure pellet biochar.

Appendix 3-2. ANOVA table for pH, dissolved organic carbon (DOC) and nitrogen (DON), microbial biomass carbon (MBC) and nitrogen (MBN), and cumulative carbon dioxide (Cum. CO_2) and relativized cumulative carbon dioxide (Rel. cum. CO_2) emission as affected by soil amendment and root zone treatments

Soil amendment	pH		DOC		DON		DOC/DON ratio		MBC		MBN		Cum. CO_2		Rel. Cum. CO_2	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Manure pellets																
Amendment treatment (A)	604.12	<0.001	199.66	<0.001	165.77	<0.001	86.24	<0.001	25.78	0.001	14.28	0.005	161.62	<0.001	171.87	<0.001
Root zone treatment (R)	233.02	<0.001	197.54	<0.001	98.61	<0.001	0.74	0.411	4.43	0.073	4.59	0.061	64.76	<0.001	64.7	<0.001
A × R	10.74	0.004	27.54	<0.001	33.24	<0.001	3.36	0.081	8.73	0.012	9.27	0.006	1.69	0.237	1.99	0.192
Woodchips																
Amendment treatment (A)	402.78	<0.001	29.98	0.001	213.46	<0.001	35.79	0.001	195.95	<0.001	85.69	<0.001	121.25	<0.001	229.68	<0.001
Root zone treatment (R)	243.6	<0.001	197.54	<0.001	60.56	<0.001	5.14	0.049	49.8	<0.001	29.83	0.001	13.97	0.004	53.72	<0.001
A × R	41.04	<0.001	27.54	0.037	7.68	0.011	11.44	0.003	18.5	0.001	6.24	0.021	1.08	0.381	9.56	0.005

Appendix 4-1. Pearson correlation co-efficient (r) for the relations among soil characteristics, net and gross nitrogen transformation rates, and carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions

	PH	TC	TN	C/N	HWEC	HWEN	NN	NM	GM	GN	CO_2
Root zone											
TC	0.716*										
TN	0.583	0.917***									
C/N	0.458	0.374	-0.025								
HWEC	0.447	0.414	0.498	-0.1137							
HWEN	0.536	0.529	0.529	-0.173	0.968***						
NN	0.422	0.551	0.751**	-0.363	0.431	0.636*					
NM	0.486	0.559	0.753**	-0.348	0.504	0.697*	0.972***				
GM	0.601	0.711*	0.865***	-0.223	0.676*	0.833**	0.901***	0.937***			
GN	0.743**	0.644*	0.758**	-0.134	0.701*	0.845**	0.831**	0.895***	0.957***		
CO_2	0.113	0.314	0.635*	-0.686*	0.52	0.662*	0.816**	0.805**	0.823**	0.713*	
N_2O	0.466	0.232	0.473	-0.501	0.684*	0.785**	0.711*	0.774**	0.744**	0.818**	0.729*
Bulk soil											
TC	0.756**										
TN	0.806**	0.906***									
C/N	0.503	0.838**	0.530								
HWEC	0.443	0.131	0.496	-0.362							
HWEN	0.609*	0.256	0.598	-0.236	0.966***						
NN	-0.656*	-0.464	-0.778*	0.066	-0.894***	-0.925***					
NM	-0.457	-0.532	-0.769*	-0.065	-0.685*	-0.715*	0.821**				
GM	0.623*	0.236	0.398	-0.013	0.624*	0.678*	-0.584	-0.281			

GN	0.201	-0.090	0.309	-0.572	0.931***	0.841**	-0.764**	-0.527	0.512		
CO_2	0.144	-0.191	0.209	-0.646*	0.925***	0.825**	0.066	-0.501	-0.013	-0.572	
N_2O	0.461	-0.031	0.330	-0.459	0.932*	0.923***	-0.762**	-0.475	0.692*	0.890***	0.891***

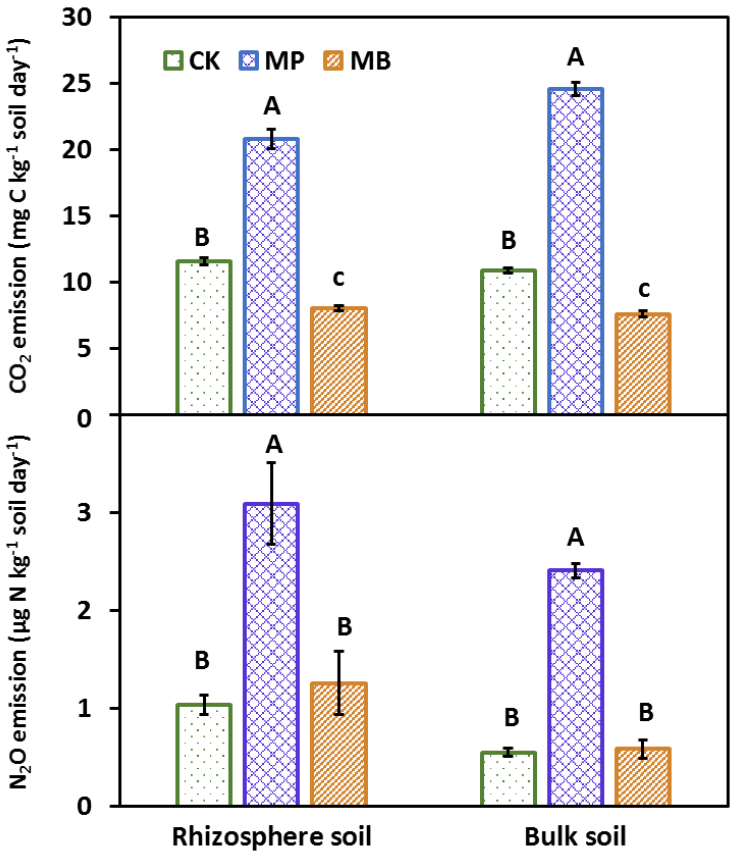
Abbreviations: TC, total carbon; TN, total nitrogen; C/N, carbon to nitrogen ratio; HWEC, hot water extractable carbon; HWEN, hot water extractable nitrogen; NN, net nitrification rate; NM, net mineralization rate; GM, gross mineralization rate; GN, gross nitrification rate. *, ** and *** indicate significant correlations at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Appendix 4-2. Mean separation of treatment effects with significant interaction (at $\alpha = 0.05$) between soil amendment and soil zone treatments (means with standard errors in the parentheses)

Treatment	Soil pH	CO_2 emission	Net mineralization	Net nitrification	Gross nitrification
		mg C kg^{-1} soil day^{-1}	mg N kg^{-1} soil day^{-1}		
CK-RS	5.89 (0.03) c	11.57 (0.28) c	0.57 (0.04) b	0.79 (0.05) b	5.08 (0.37) d
CK-BS	5.26 (0.04) d	10.90 (0.19) d	0.39 (0.03) bc	0.72 (0.04) bc	4.17 (0.19) e
MP-RS	6.80 (0.06) a	20.81 (0.75) b	1.28 (0.05) a	1.49 (0.05) a	12.44 (0.65) a
MP-BS	6.45 (0.02) b	24.58 (0.51) a	0.20 (0.05) d	0.47 (0.05) d	8.41 (0.65) b
MB-RS	6.79 (0.01) a	7.94 (0.22) e	0.63 (0.06) b	0.81 (0.07) b	6.75 (0.17) c
MB-BS	6.46 (0.02) b	7.60 (0.26) f	0.33 (0.07) cd	0.62 (0.04) c	3.36 (0.16) f

Abbreviations: CK, no amendment; MP, addition of manure pellet; MB, addition of manure pellet biochar; RS, rhizosphere soil; BS, bulk soil.

Different letters in the same column indicate significant differences at $\alpha = 0.05$.



Appendix 4-3. Effects of manure pellet biochar and its feedstock on carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions from rhizosphere and bulk soils. Treatment codes are: CK = no amendment, MP = addition of manure pellet, MB = addition of manure pellet biochar. Mean ± SE (n = 4). Different letters within each soil type represent significant differences at $\alpha = 0.05$.

Appendix 5-1. Selected chemical and physical properties of soil and biochar. Values are the means with standard errors in the parentheses (n = 3)

	pH	EC	TC	TN	C/N	DOC	DON	HWEC	HWEN	NH_4^+-N	NO_3^--N
		$\mu S\ cm^{-1}$	$g\ kg^{-1}$			$mg\ kg^{-1}$					
Soil	5.38 (0.05)	52.01 (0.51)	16.97 (0.43)	1.80 (0.20)	9.43 (0.16)	77.10 (0.54)	12.05 (0.59)	565.07 (4.30)	47.27 (1.00)	3.22 (0.10)	6.20 (0.37)
Biochar	9.98 (0.08)	4157 (45.57)	131.62 (2.31)	7.21 (0.26)	18.23 (0.19)	74.38 (4.56)	4.89 (0.34)	221.02 (4.77)	14.02 (0.54)	1.49 (0.24)	0.81 (0.08)

Abbreviations: EC, Electrical conductivity; TC, total carbon; TN, total nitrogen; C/N, carbon-nitrogen ratio; DOC, dissolved organic carbon; DON, Dissolved organic nitrogen; HWEC, hot water extractable carbon; HWEN, hot water extractable nitrogen; NH_4^+-N, exchangeable ammonium nitrogen concentration; NO_3^--N, nitrate-nitrogen concentration

Appendix 5-2. Effect of manure biochar and nitrification inhibitor in soil pH in different sampling time of 60-day incubation

Treatment	pH				
	Day 5	**Day 10**	**Day 20**	**Day 35**	**Day 60**
60% WFPS					
CK	5.96(0.01)C	5.67(0.01)C	5.57(0.01)B	5.54(0.02)B	4.81(0.05)D
MB	6.73(0.01)A	6.69(0.02)A	6.69(0.02)A	6.62(0.01)A	6.28(0.02)B
NI	6.03(0.03)B	5.72(0.01)C	5.49(0.01)C	5.49(0.01)B	5.16(0.04)C
MB+NI	6.72(0.02)A	6.62(0.02)B	6.69(0.02)A	6.57(0.05)A	6.43(0.02)A
80%WFPS					
CK	6.14(0.10)C	6.31(0.01)C	6.21(0.05)B	5.93(0.05)B	5.55(0.12)B
MB	6.84(0.07)A	6.96(0.02)A	6.80(0.03)A	6.78(0.04)A	6.69(0.02)A
NI	6.41(0.05)B	6.36(0.03)C	6.23(0.01)B	5.86(0.04)B	5.35(0.03)B
MB+NI	6.92(0.03)A	6.8 (0.03)B	6.75(0.01)A	6.89(0.04)A	6.69(0.02)A

Treatment codes are: CK, unamended control; MB, manure biochar addition; NI, nitrification inhibitor addition; MB+NI, manure biochar and nitrification inhibitor addition. Different letters indicate significant differences between the biochar and nitrification inhibitor treatments within each WFPS treatment ($P < 0.05$). Values without letters are not significantly different between the biochar and nitrification inhibitor treatments within each WFPS treatment.

Appendix 5-3. Effect of manure biochar and nitrification inhibitor in electrical conductivity (EC) in different sampling time of 60-day incubation

Treatment	EC (μS cm^{-1})				
	Day 5	**Day 10**	**Day 20**	**Day 35**	**Day 60**
60% WFPS					
CK	120.25(1.36)C	148.70(0.80)C	177.68(5.07)B	200.70(6.74)B	197.13(2.79)B
MB	297.98(4.58)A	303.68(4.43)B	342.60(5.70)A	371.38(3.33)A	327.63(5.14)A
NI	95.34(4.06)D	158.18(2.91)C	191.10(4.57)B	212.50(5.85)B	201.50(2.47)B
MB+NI	277.95(2.61)B	354.03(4.62)A	345.98(5.08)A	370.65(2.37)A	319.48(6.91)A
80% WFPS					
CK	69.28(4.94)B	76.40(6.12)B	100.47(2.71)C	128.78(9.02)B	84.12(9.02)C
MB	263.43(6.18)A	300.05(7.92)A	227.83(21.75)B	300.50(13.20)A	230.93(12.12)A
NI	61.01(3.43)B	83.06(6.76)B	95.49(2.84)C	133.94(19.79)B	103.46(7.96)C
MB+NI	257.60(7.86)A	314.33(2.97)A	275.45(11.99)A	290.93(4.89)A	188.28(4.00)B

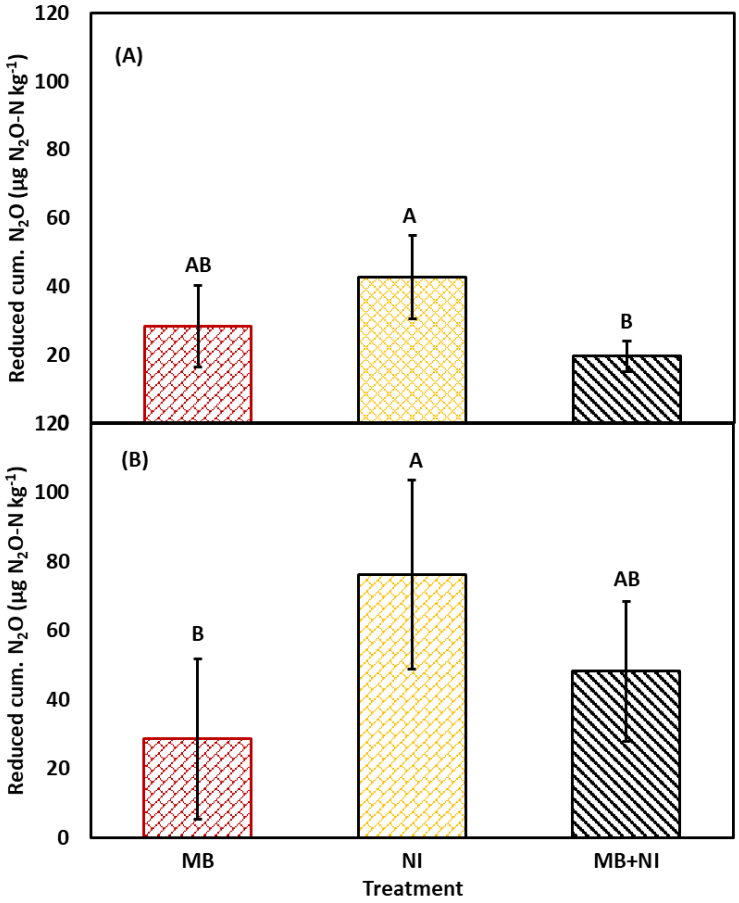
Treatment codes are: CK, unamended control; MB, manure biochar addition; NI, nitrification inhibitor addition; MB+NI, manure biochar and nitrification inhibitor addition. Different letters indicate significant differences between the biochar and nitrification inhibitor treatments within each WFPS treatment ($P < 0.05$). Values without letters are not significantly different between the biochar and nitrification inhibitor treatments within each WFPS treatment.

Appendix 5-4. Repeated measures ANOVA (F and P values) for the effects of manure biochar and nitrification inhibitor in soil properties, available nitrogen, and enzyme activities under two soil moisture levels

Treatment	pH		EC		HWEC		NH_4^+-N		NO_3^--N		NAG		UR	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
60% WFPS														
Biochar (MB)	6887.29	**<0.001**	4000.38	**<0.001**	1.55	0.302	3354.71	**<0.001**	0.22	0.67	1.29	0.338	12.43	**0.039**
Nitrapyrin (NI)	32.47	**0.011**	3.86	0.144	1.31	0.335	33.68	**0.01**	2.04	0.249	2.93	0.186	0.17	0.708
Time (T)	406.13	**<0.001**	201.59	**<0.001**	34.10	**<0.001**	6893.80	**<0.001**	318.89	**<0.001**	116.79	**<0.001**	11.21	**<0.001**
MB × NI	83.57	**0.003**	0.15	0.728	49.18	**0.006**	47.86	**0.006**	0.33	0.606	0.39	0.576	0.01	0.933
MB × T	114.95	**<0.001**	57.44	**<0.001**	2.57	0.092	548.56	**<0.001**	66.63	**<0.001**	2.55	0.094	15.06	**<0.001**
NI × T	19.46	**<0.001**	35.97	**<0.001**	0.45	0.773	28.63	**<0.001**	14.62	**<0.001**	1.78	0.197	1.26	0.337
MB × NI × T	5.32	**0.011**	4.25	**0.023**	9.65	**<0.001**	11.43	**<0.001**	2.30	0.119	6.54	**0.005**	0.94	0.473
80% WFPS														
Biochar (MB)	555.15	**<0.001**	1289.40	**0.018**	0.01	0.985	307.60	**<0.001**	27.58	**0.013**	35.56	**0.009**	8.91	0.096
Nitrapyrin (NI)	0.36	0.592	0.05	0.864	0.03	0.87	26.51	**0.014**	229.98	**<0.001**	5.07	0.11	1.66	0.327
Time (T)	239.55	**<0.001**	43.45	**0.001**	73.18	**<0.001**	314.18	**<0.001**	83.55	**<0.001**	137.91	**<0.001**	5.96	**0.016**
MB × NI	0.01	0.993	0.01	0.94	1.77	0.276	2.16	0.238	3.97	0.14	0.01	0.991	0.92	0.44
MB × T	41.07	**<0.001**	6.51	**0.049**	8.41	**0.002**	50.79	**<0.001**	29.08	**<0.001**	0.33	0.853	2.33	0.143
NI × T	8.42	**0.002**	1.41	0.374	1.25	0.342	4.58	**0.018**	12.74	**<0.001**	1.88	0.179	8.57	**0.005**
MB × NI × T	3.23	0.051	2.00	0.26	0.45	0.772	5.94	**0.007**	0.75	0.577	0.49	0.745	0.41	0.798

Abbreviations: EC, electrical conductivity; HWEC, hot water extractable carbon; NH_4^+-N, exchangeable ammoniacal nitrogen concentration; NO_3^--N, nitrate-nitrogen concentration; NAG, β-1,4-N-acetyl glucosaminidase activities; UR, urease activities; Cum. N_2O, cumulative nitrous oxide emissions.

P values in the bold indicate a significant effect at $\alpha = 0.05$.



Appendix 5-5. Efficacies of biochar and NI in reducing N_2O emissions from the soil at 60% WFPS (A) and 80% WFPS (B). Different letters in the reduced cumulative N_2O emissions represent significant differences among the treatments. Treatment codes are MB: addition of manure biochar, NI: addition of nitrification inhibitor added, MB+NI: addition of manure biochar and nitrification

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Appendix 6-1. Basic properties (means with std errors in parentheses) of soil and biochar used in the study. (n=4).

	pH	EC	TC	TN	NH_4^+-N	NO_3^--N	Olsen-P
		$\mu S\ cm^{-1}$	$g\ kg^{-1}$		$mg\ kg^{-1}$		
Soil	5.46 (0.06)	87.34 (5.76)	117.46 (1.35)	1.87 (0.31)	8.09 (1.71)	5.66 (0.72)	10.92 (0.13)
Biochar	9.43 (0.31)	3498.53 (106.38)	34.7 (1.02)	6.90 (0.20)	11.83 (2.34)	5.02 (0.97)	18.09 (2.46)

Abbreviations: EC, electrical conductivity; TC, total carbon; TN, total nitrogen; NH_4^+-N, exchangeable ammoniacal nitrogen; NO_3^--N, nitrate nitrogen; Olsen-P; inorganic phosphorus

Appendix 6-2. Effects of biochar and NI applications on resource carbon and nutrients (nitrogen and phosphorus) ratio (means with standard errors in parentheses) (n=4).

Treatments	2019			2020		
	$R_{C:N}$	$R_{C:P}$	$R_{N:P}$	$R_{C:N}$	$R_{C:P}$	$R_{N:P}$
Biochar (BC)						
BC0	3.13(0.57)	17.27(1.20) a	7.02(1.28) a	2.45(0.30)	16.39(1.32)	7.31(0.94)
BC10	4.39(1.38)	11.87(1.27) b	3.89(0.64) b	2.42(0.21)	15.12(2.51)	7.54(2.64)
BC20	3.34(0.53)	11.64(1.20) b	4.00(0.58) b	2.74(0.21)	14.07(1.92)	5.45(0.96)
Nitrification inhibitor (NI)						
NI0	4.34(0.91)	13.74(1.16)	4.22(0.67)	2.69(0.20)	15.76(1.12)	6.29(0.71)
NI1	2.90(0.43)	13.45(1.34)	5.73(0.92)	2.38(0.19)	14.62(1.96)	7.25(1.83)

Abbreviations: BC0, no biochar added; BC10, manure biochar added at 10 t ha^{-1}; BC20, manure biochar added at 20 t ha^{-1}; NI0, no nitrification inhibitor added; NI1, nitrification inhibitor added; $R_{C:N}$, resource carbon: nitrogen ratio; $R_{C:P}$, resource carbon: phosphorus ratio; $R_{N:P}$, resource nitrogen: phosphorus ratio microbial biomass carbon. Different letters indicate significant differences within each treatment of biochar amendment and nitrification inhibitor application ($P < 0.05$).

Appendix 6-3. Regression analysis of ecoenzymatic relationship under biochar amendment and NI application treatments

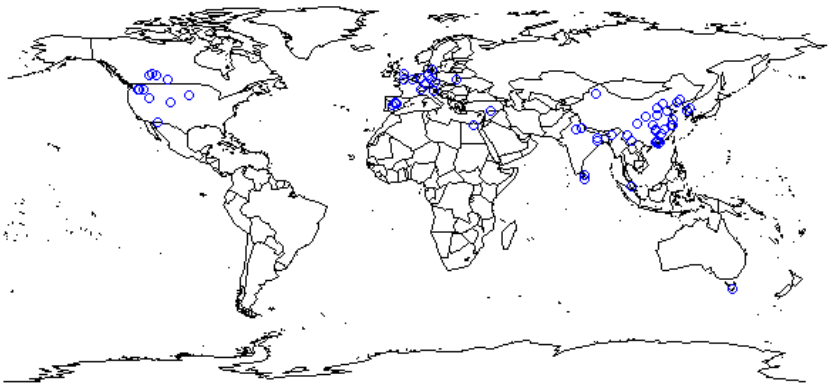
Regression		Treatment	2019				2020			
			Slope	R^2	P	n	Slope	R^2	P	n
		Biochar (BC)								
ln(BG) vs ln(NAG)	C vs N	BC0	0.41	0.65	**0.080**	8	0.49	0.83	**0.012**	8
		BC10	0.64	0.89	**0.003**	8	0.19	0.80	0.179	8
		BC20	0.38	0.55	0.158	8	0.47	0.83	**0.011**	8
ln(BG) vs ln(AP)	C vs P	BC0	0.65	0.72	**0.045**	8	0.52	0.46	0.254	8
		BC10	0.49	0.44	0.278	8	-0.06	-0.24	0.738	8
		BC20	0.23	0.36	0.374	8	0.47	0.77	**0.026**	8
ln(NAG) vs ln AP)	N vs P	BC0	1.26	0.88	**0.004**	8	1.48	0.77	**0.024**	8
		BC10	1.02	0.66	**0.007**	8	-0.43	-0.24	0.573	8
		BC20	-0.02	-0.02	0.953	8	0.67	0.62	0.098	8
		Nitrification inhibitor (NI)								
ln(BG) vs ln(NAG)	C vs N	NI0	0.61	0.79	**0.002**	12	0.25	0.72	**0.008**	12
		NI1	0.40	0.63	**0.027**	12	0.45	0.78	**0.003**	12
ln(BG) vs ln(AP)	C vs P	NI0	0.95	0.59	**0.042**	12	0.07	0.10	0.761	12
		NI1	0.36	0.70	**0.011**	12	0.21	0.27	0.401	12
ln(NAG) vs ln AP)	N vs P	NI0	0.62	0.30	0.341	12	-0.09	-0.04	0.891	12
		NI1	0.62	0.75	**0.005**	12	0.40	0.29	0.357	12

Abbreviations: BC0, no biochar added; BC10, manure biochar added at 10 t ha^{-1}; BC20, manure biochar added at 20 t ha^{-1}; NI0, no nitrification inhibitor added; NI1, nitrification inhibitor added; C, carbon; N, nitrogen; P, phosphorus; BG, β-1,4-glucosidase; NAG, β-1,4 N-acetyl glucosaminidase; AP acid phosphatase activities. P values in the bold indicate a significant effect at $\alpha = 0.05$

Appendix 6-4. Regression analysis of microbial biomass relationship under biochar amendment and NI application treatments

Regression		Treatment	2019				2020			
			Slope	R^2	P	n	Slope	R^2	P	n
		Biochar (BC)								
$lnB_{C:N}$ vs $lnR_{C:N}$	C vs N	BC0	0.25	0.29	0.481	8	0.32	0.50	0.203	8
		BC10	0.09	0.16	0.696	8	-0.19	-0.32	0.439	8
		BC20	0.24	0.32	0.442	8	-0.05	-0.03	0.936	8
$lnB_{C:P}$ vs $lnR_{C:P}$	C vs P	BC0	-1.35	-0.59	0.122	8	-0.10	-0.10	0.822	8
		BC10	0.68	0.50	0.204	8	-0.01	-0.01	0.976	8
		BC20	0.59	0.43	0.292	8	0.19	0.23	0.581	8
		Nitrification inhibitor (NI)								
$lnB_{C:N}$ vs $lnR_{C:N}$	C vs N	NI0	0.12	0.16	0.625	12	0.25	0.31	0.330	12
		NI1	0.10	0.15	0.645	12	-0.14	-0.16	0.613	12
$lnB_{C:P}$ vs $lnR_{C:P}$	C vs P	NI0	0.70	0.47	0.124	12	-0.20	-0.26	0.408	12
		NI1	-0.18	-0.15	0.631	12	0.16	0.27	0.397	12

Abbreviations: BC0, no biochar added; BC10, manure biochar added at 10 t ha^{-1}; BC20, manure biochar added at 20 t ha^{-1}; NI0, no nitrification inhibitor added; NI1, nitrification inhibitor added; $B_{C:N}$, microbial biomass carbon: nitrogen ratio; $B_{C:P}$, microbial biomass carbon: phosphorus ratio; $B_{N:P}$, microbial biomass nitrogen: phosphorus ratio; $R_{C:N}$, resource carbon: nitrogen ratio; $R_{C:P}$, resource carbon: phosphorus ratio; $R_{N:P}$, resource nitrogen: phosphorus ratio.



Appendix 7-1. The global distribution of the study sites used in this meta-analysis.