The effect of biochar amendment on the health, greenhouse gas emission, and climate change resilience of soil in a temperate agroecosystem

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Biochar has been a successful soil amendment in tropical agriculture for thousands of years. Biochar's intrinsic chemical and physical properties benefit agriculture in terms of soil health, environmental pollution, and crop productivity. The effect of biochar as a soil amendment in temperate agriculture faces unique challenges and is still in its infancy. The objectives of this study were to determine the effect of a wood-biochar in a temperate agricultural soil in terms of soil health, greenhouse gas (GHG) emissions, and resilience against warming and CO_2 fertilization. This study consisted of three triplicated soil treatments: 6t/ha poultry manure and 135 kg/ha urea-N fertilizer (MN), 3t/ha poultry manure and 3t/ha biochar (MB), and 3 t/ha poultry manure, 135 kg/ha urea-N fertilizer, and 3t/ha biochar (MNB). The field study found a significantly greater fraction of stable macroaggregates in MB than MN and MNB (p=0.040), lower NH_4^+-N in MB than MN and MNB (p < 0.001), and higher soil microbial biomass carbon in MNB than MN and MB (p = 0.002). The temporal soil GHG emission study found significantly lower CO_2 and trends in lower N_2O (not significant) emissions with biochar amendment (p = 0.031). However, the seasonal factor (e.g. soil moisture) had a greater influence on soil GHG emission. The climate change resilience study introduced climate condition as a second fixed factor including: ambient (AMB), elevated temperature (TEMP), CO_2 fertilization (fCO$_2$), and elevated temperature plus CO_2 fertilization (fCO$_2$×TEMP). Results showed biochar behaved independently of the climate condition factor for vast majority of soil and soybean characteristics. MNB responded poorly compared to MN and MB in many soil and plant characteristics suggesting conflicting urea-biochar interactions. Soybeans matured quicker under warming effect but developed abnormal physical traits. Findings from these studies suggest biochar can be a valuable implementation to temperate soil to improve soil health and mitigate environmental stress that leads to and results from climate change.

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Chapter 1: Literature Review

1.1 Soil Health and Agricultural Sustainability

As a primary foundation that provides for humans and society on earth, creating and maintaining healthy and arable soil is imperative to sustain the rapidly growing human population (Worster 1990). Sustainability in agriculture is not only limited to keeping food production up to scale to meet our current needs, but also ought to be guided with educated foresight in preparation for the future generations (Abrol & Sangar 2006). However, rapid soil degradation and the resultant decreased arability of soil are a direct result of natural causes such as heavy rainfall and strong winds as well as the intensification of agricultural practices and lack of sustainable farming operations (Osman 2014). While changes in land use and intense agricultural management practices directly contribute to climate change via the emission of agricultural greenhouse gases (GHG), soil degradation is also directly facilitated by the changing climate (IPCC 2014). As a result, proper land management is crucial to mitigating effects of, and factors contributing to climate change (IPCC 2014).

1.2 Biochar in Agriculture

Terra Preta do Indio, also commonly known as Amazonian Black or Dark Earth, was created over 2000 years ago by pre-Columbian Indians in the Brazilian Amazon (Sombroek 1966). Various studies over the past 20 years have confirmed that this particularly fertile black earth was likely unintentionally created by introducing charred organic matter of wood, plant matter, animal bones, and ceramics into otherwise infertile soil. (Sombroek 1966; Smith 1980; Kern & Kampf 1989; Glaser et al. 2001). These soil additives are referred to as black carbon and a form of biochar which greatly improves soil organic matter (SOM) (Glaser et al. 2001; Lucheta

et al. 2017). This form of biochar facilitated the immobilization of labile carbon and promoted the accumulation of SOM which would otherwise have been lost in some oxidized form (Atkinson et al. 2010). Since then, researchers have become interested in biochar's ability as a soil amendment to increase soil health and fertility outside of the tropical regions of the world (Ameloot et al. 2013; Atkinson et al. 2010).

Biochar, by traditional definition, is charcoal used as a soil amendment for agricultural or environmental purposes. A recent definition of biochar by the International Biochar Initiative (IBI) defines it as "The solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment" (IBI 2015). More specifically, biochar is a carbon-rich, highly porous, and inert charcoal that acts as a long-term additive that can improve soil health and fertility by enhancing nutrient retention time, soil aeration, and provides habitat for soil microbes (Kloss et al. 2014; Smith et al. 2010).

Biochar is comprised mainly of pyrogenic carbon produced from sustainable sources of feedstock, such as agricultural residues and agroforestry biomass, via an oxygen-limiting process, termed pyrolysis, that is carried out under high temperature conditions (Woolf et al. 2010). Such processes produce charcoals with intrinsic chemical and physical properties rich in highly substituted polycyclic aromatic hydrocarbons which renders the char to be exceptionally amorphous, porous, and inert (Kloss et al. 2012; Lehmann & Joseph 2009). The specific biochar generated is also highly variable depending on numerous factors such as feedstock material and quality, pyrolysis temperature, residence time, and oxygen availability (Spokas et al. 2012; Atkinson et al. 2010). Engineers exploit various combinations of these factors to produce specific types of biochar that vary in density, surface area, porosity, carbon content, pH,

hydrophobicity, ion exchange capacity (typically cationic for essential macro- and micro-nutrients) among other traits (Tripathi et al. 2016).

The starting material for biochar production typically comes from environmentally friendly and sustainable sources such as common compost, agricultural waste, and agricultural and agroforestry biomass (Fischer & Glaser 2012; Woolf et al. 2010; Dil 2011). Compost and crop residue application to soil is a conventional agricultural practice which replenish the SOM pool (Fischer & Glaser 2012). However, due to the abundance of microbial activities, a huge portion of the labile organic carbon added is often lost via aerobic decomposition (Fischer & Glaser 2012). The conversion of this nutrient-rich organic matter to a more recalcitrant form of black carbon such as biochar can therefore improve SOM recycling while reducing agricultural GHG emissions (Fischer & Glaser 2012; Woolf et al. 2010). Wood can be part of the biofuel production process that offers great renewable alternatives to fossil fuel where high quality wood biochar is often produced as a side-product (Ronsse et al. 2012; Dil 2011). Biochar characteristics such as ash content, chemical structure, pore size distribution, surface area, and functional groups are often of a direct result based on the choice of the feedstock material (Gai et al. 2014). For instance, poultry manure-based biochar tends to have a larger porosity and therefore larger surface area than biochar produced starting from wheat-straw under identical pyrolysis settings (Sun et al. 2011). Additionally, manure biochar is typically nutrient-rich and therefore better at improving soil nutrients than wood sourced biochar, but wood biochar has shown greater capacity for carbon sequestration and nutrient retention against leaching (Domingues et al. 2017). The difference originates from wood biochar consisting of more highly substituted aromatic hydrocarbons and higher C:H ratio than nutrient-rich biochar types (Domingues et al. 2017). It is believed that wood-based biochar is a high-quality product for soil

amendment due to its low toxin and ash content, while being rich in carbon and promoting

nutrient retention (Kloss et al. 2014).

Pyrolysis temperature also determines the porosity and ash content of the char produced

where higher temperatures (above 500°C) tend to result in larger pore sizes and therefore lower

densities and higher surface areas which are ideal for biochar as a soil amendment (Lehmann &

Joseph, 2009). However, high temperature pyrolysis also yields higher toxic ash content, which

is often undesirable (Lehmann & Joseph, 2009). Porous biochar interacts with soil to increase the

aeration, soil nutrient and water retention while decreasing soil bulk density (BD), this increases

overall crop productivity and facilitates crop root growth (Pandey et al. 2016). To balance the

positive and negative influences of pyrolytic temperature on the biochar produced, relatively low

temperature chars (~400 °C) are typically used as a conservative approach when generating

biochar for application in agricultural systems (Anders et al. 2013).

Residence time is the length of time the feedstock material is left to be pyrolyzed in the

kiln or reactor to produce biochar. The pyrolytic process is often divided into slow pyrolysis and

fast pyrolysis (Bruun et al. 2012). Slow pyrolysis traditionally involves a residence time of hours

up to days and is often less technologically advanced compare to fast pyrolysis (Bruun et al.

2012). Slow pyrolysis is typically carried out in dirt pits, simple kilns, and pyrolizer tanks under

controlled conditions to produce relatively equal amounts of liquid biofuel, syngas, and the solid

biochar where biochar is typically the desired product (Bruun et al. 2012; Dickinson et al. 2013).

On the other hand, fast pyrolysis typically employs a residence time within seconds which is

done in specialized reactors which converts the vast majority of the feed stock into bio-oil as it is

often the desired product in the biofuel industry (Bruun et al. 2012; Dickinson et al. 2013).

Residence time also has a significant interactive effect with pyrolytic temperature on the physical

and chemical properties of the char produced (Novak et al. 2009). For instance, Sun et al. (2017) finds that biochar yield, pH, and sorption decreases as residence time increases at low temperatures (~300°C) and only sorption decreases as residence time increases at high temperatures (~600°C). Depending on the feedstock material, the 2-hour to 4-hour range is determined to be the most appropriate residence time to produce biochar for agricultural purposes where biochar yield and nutrient retention are maximized using a common laboratory muffle furnace (Sun et al. 2017).

1.2.1 Biochar and Soil Physics

Physical soil characteristics typically have the largest effect on agroecosystems since soil BD, texture, macro-and micro-structure can be manipulated to directly influence the microcosmic environment of the pedosphere (Haynes & Naidu 1998; Lal 2011). These physical attributes determine soil moisture, aeration, and rate of water infiltration and in turn affect soil chemistry in terms of ion exchange rate, reaction surface for nutrient retention and toxin chelation, and nutrient availability (Basso et al. 2012). Consequently, soil biology such a biodiversity, cropping diversity, and crop growth are also greatly affected by soil physical and chemical characteristics (Chan et al. 2008).

For example, low soil water holding capacity (WHC) and high infiltration rates are common problems associated with sandy soils due to the lack of micropores and microaggregates (Gentile et al. 2013). The abundant presence of macropores and macroaggregates means sandy soils often offer low soil surface area which is an undesirable trait in terms of soil water retention, nutrient retention and exchange, and biological activities (Zhang & You 2013). To combat these issues, wood-based and low temperature biochars with a high surface area and abundance of micropores have been added to sandy soils (Pastor-Villegas et al.

2010). Results from this research showed that biochar treated soils had an increased capacity to hold water against gravitational percolation and also increased soil moisture retention as a long-term effect (Hammond et al. 2013).

Biochar's high porosity also contributes to soil aeration and improves the exchange rate of essential gases such as oxygen, which directly benefits microbial, macrofaunal, and crop root metabolism (Case et al. 2012). The low density and firm physical structure of biochar, due to its porous macrostructure, not only improves soil gaseous exchanges, but also provides physical support to decease soil BD and offers resistance to soil compaction for all soil types (Mukherjee & Lal 2013). Soils with greater tilth promote crop root growth, which often significantly improves agronomical yields (Dam et al. 2005; Abiven et al. 2015). Abiven et al. (2015) reports that corn root surface area and branching are improved in soils amended with biochar that have significantly lower BD and cation exchange capacity (CEC) than unamended soils; the enhanced root biomass likely contribute to the increased grain yield in biochar treated soils in a tropical region. A study on soil BD and crop yield in a sandy loam soil in central Canada also concludes a strong link between an increase in crop production and lower BD (Dam et al. 2005). As a result, various soil physical characteristics that have been positively affected by biochar in tropical soils may be extrapolated to temperature agricultural soil (Atkinson et al. 2010).

1.2.2 Biochar and Soil Chemistry

SOM plays a crucial role in the formation and maintenance of soil structure as well as soil fertility as it is a key component in the aggregation of soil particles (Beare et al. 1994; Brady & Weil 1999). SOM originates from the microbial decomposition of plant and animal residues which are important in the storage and cycling of essential nutrients such as nitrogen, phosphorus and many micronutrients (Tipping et al. 2016). Soil organic carbon (SOC) is the major

component of SOM and the specific fraction can vary depending on the soil type (Jain et al. 1997). Therefore, SOC is often an indicator and proxy for SOM to evaluate soil health and fertility (Périé and Ouimet 2008). SOM is one of the largest carbon reserves on earth; however, modern and intensive agricultural practices often result in the loss of SOM and therefore, the loss of SOC (Lefebvre et al. 2011). For instance, soil erosion due to water and wind, climate change, and intensive soil tillage breaks up soil particles (aggregates), increases rates of SOM decomposition, and therefore loss of SOC (Lefebvre et al. 2011; Lal 2011).

The idea behind implementing biochar into soil to improve soil health and fertility lies heavily on the fact that biochar often tends to facilitate the accumulation of the recalcitrant portion of SOM, and therefore, the long-term sequestration of carbon in soil (Kimetu & Lehmann 2010). Scientists now wish to determine whether temperate agricultural soil can also benefit from similar effects of biochar addition (Atkinson et al. 2010). It is likely that temperate soils will not improve with biochar amendment to the extent that tropical soils do due to the existing nutrient cycle that helps with SOM recovery in temperate agriculture and due to the fundamental differences in soil chemistry between tropical and temperate soil, such as pH and cationic exchange capacity (Tiessen et al. 1994).

Biochar in soil has demonstrated profound ability to retain inorganic nitrogen species typically in the form of ammonium (Mia et al. 2017). For example, Yang et al. (2016) finds that higher pyrolytic temperature biochars improves nitrate adsorption. However, the extent to which biochar is able to fix nitrate in soil via sorption is still limited compared to soil particles (Yang et al. 2017). Phosphate retention is also reported but only under certain conditions depending on biochar production settings (such as source feedstock and pyrolysis temperature), as well as the chemical conditions of the soil (Trazzi et al. 2016). Sachdeva et al. (2019) finds that temperate

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soil samples containing wood biochar aged for 3 years are able to retain significantly more total phosphorus than the same soil without biochar. Additionally, the abundant highly functional reaction surface of biochar often increases soil CEC, especially sandy soils (Liang et al. 2006). Higher CEC allows for better retention of various common essential cationic nutrients, such as ammonium and potassium in a sandy soil (Liang et al. 2006). However, since temperate soils are often higher in pH than tropical soils, the improvement in CEC is expected to be not as pronounced with biochar addition in temperate agriculture (Robertson et al. 1999). The improved SOM accumulation and inorganic nutrients retention from biochar amendment then promotes the development of crops and local microbial communities in the treated soil (Lehmann & Joseph 2009; Luo et al. 2013).

Lastly, large additions of biochar have been shown to increase soil pH, which helps with alkalization or liming of acidic soils common in the tropics. However, the change in pH as a result of biochar addition is often very miniscule in soils with close to neutral or basic pH values such as temperate soils (Smider & Singh 2014). The mechanism behind biochar increasing soil pH is often a result of the immobilization of heavy metal cations by intra-particle diffusion as well as a liming effect (Rees et al. 2013; Smider & Singh 2014; Jeffrey et al. 2011). High temperature biochars (> 500 °C) were shown to increase temperate soil pH (Lehmann et al. 2011). Biochar produced at high pyrolytic temperatures are typically significantly higher in alkaline metals such as potassium, calcium and magnesium as well as ash content. These alkaline materials can offer a temporary increase in soil pH upon biochar addition, but the effect decreases with time (Lehmann et al. 2011).

1.2.3 Biochar and Soil Biology and Agronomic Productivity

Vast numbers of biological communities exist through anthropogenic manipulation in agricultural soils (Benton et al. 2003; Dorrough et al. 2007). The effect of biochar on soil microbial dynamics, including microbial activity, microbial communities, and microbial biomass (SMB) remains controversial. While earlier research suggested biochar addition improved SMB and microbial activity, recent studies conclude that biochar addition often leads to a decrease in SMB and overall microbial activity due to decreased SOM decomposition and nitrogen mineralization (Dempster et al. 2012; Li et al. 2018). Another study found that with increasing biochar application rates, decreases in SMB occurred but bacterial diversity increased (Li et al. 2018). Chemical engineers have been aspiring to create novel types of biochar that selectively inhibit soil-borne pathogens. In a grand literature review, 85% of the biochars studied showed significant suppression of soil-borne pathogens such as *Fusarium* spp., *Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani*, *Sclerotinia* spp., *Sclerotium* spp., and *Verticillium dahlia* while only 3% reported a significant increase in soil pathogens (Bonanomi et al. 2015). Biochar addition to soil can also improve crop yield by promoting mycorrhizal growth in soil (Johnson et al. 1997). Mycorrhizal relationships are crucial in temperate agriculture where soils tend to have a basic pH, and chemical and physical immobilization of phosphates and iron often prevents uptake by unsupported plant roots (Li et al. 2006). The application of biochar promotes mycorrhizal fungi colonization in combination with conventional soil additives such as inorganic fertilizers and manure (Chan et al. 2007; van Zwieten et al. 2009). Madiba et al. (2016) also found that biochar amendment in a sandy loam soil in Australia significantly favoured the formation and maintenance of mycorrhizae in wheat (*Triticum aestivum* L. var. Wyalkatchem).

Additionally, meso- and macrofauna play a significant role in soil since they increase soil porosity, reduce soil crusting, improve soil aggregation, and release bioavailable nutrients for plants (Kwaad et al. 1998; Blanchart et al. 2007). Although mites, ants, and earthworms are abundant in temperate regions, they are also sensitive to changes in their habitat (Cole et al. 2006). However, Lehmann et al. (2011) reported that biochar addition promoted soil macrofaunal diversity and activity, but the mechanisms for this remain unknown. On the contrary, a short-term field study in northern Italy found no interaction between biochar and soil meso- and macrofauna, with the exception to one species of ant, when wood-derived biochar was added to soil (Castracani et al. 2015).

For agronomical productivity, the vast majority of research showed a positive effect on soil due to biochar addition (Novak et al.2012; Mukherjee & Lal 2013; Jeffery et al. 2011). These studies generally attributed the increased crop productivity to multiple interactive physical, chemical, and biological factors (Novak et al.2012; Mukherjee & Lal 2013; Jeffery et al. 2011; Lehmann et al. 2011). For example, better water retention, as a result of improved soil physical characteristics including resistance to soil compaction and enhanced soil aeration from biochar addition, facilitates crop growth (Novak et al. 2012; Mukherjee & Lal 2013). Chemically, the liming effect of biochar addition especially in acidic temperate and tropical soil (Jeffery et al. 2011) in addition to improved soil nutrient retention (Kimetu & Lehmann 2010; Mia et al. 2017) will benefit microbial activity and mycorrhizal species, which ultimately enhances agricultural productivity (Lehmann et al. 2011).

1.3 Biochar and the Environment

Recently, environmental applications of biochar have been investigated by scientists typically for its ability to sequester labile carbon, act as a long-term carbon sink in soil, and

reduce agricultural GHG production (Lehmann 2007). Biochar, while it can have diverse physicochemical properties, is typically comprised of highly interlinked hydrocarbon chemical structures that are exceptionally stable under various climatic conditions (Glaser et al. 2002). The increasing need to reduce anthropogenic GHG emission and increase the overall sustainability of agricultural land use has sparked an interest of scientists to investigate the role of biochar as a soil amendment in environmental management and remediation (Lal 2004; Lal 2011). Such studies reported biochar's ability to sequester carbon as part of the pyrogenic carbon cycle and reduce nutrient runoff and therefore minimizing issues involving freshwater eutrophication (Ngatia et al. 2017). Additionally, the production of biofuels and biochar is considered an overall carbon-negative process where less carbon dioxide (CO_2) is released to the atmosphere than removed from the overall process (Lal 2011; Lee et al. 2018; Lee 2010). This makes the biofuel industry a promising sector with agricultural and environmental benefits while offering a sustainable alternative to alleviate the current reliance on the limited fossil fuel reserve (Laird 2008; Bhattarai et al. 2011).

Agricultural emission is a common source contributing to the overall anthropogenic GHG emission pool, where approximately 11% of all GHG emissions globally is from land-use CO_2 alone (IPCC 2014). The majority of total global carbon is also stored within the pedosphere, and soils possess even greater storage capacity upon biochar amendment (Zomer et al. 2017; Lehmann et al. 2006). As a result, agricultural lands have become a tangible target for the source reduction and global sinks of atmospheric GHG to combat climate change (Ippolito et al. 2012). However, the mechanisms behind biochar-induced source reduction of various agricultural GHGs are still unclear (Kuzyahov et al. 2014). There is a consensus on biochar-mediated reduction of CO_2 and N_2O by promoting microbial inorganic nutrient immobilization and

suppression of microbial species involved in the denitrification processes (Barrett & Burke 2000; Qiu et al. 2016; Zwieten et al. 2014). As for methane (CH_4), Feng et al (2012) discovered that rice paddy fields in China amended with biochar emitted significantly lower CH_4 than those without biochar. However, in fields with biochar, methanogenic proteobacterial and archaeal growths were not inhibited and were instead promoted over methanotrophic species indicating biochar reduced agricultural CH_4 emission by means other than microbial contrary to prior knowledge (Feng et al. 2012).

Leachate retention, as mentioned earlier, is another advantage of biochar application contributing both agricultural and environmental benefits. Biochar's capacity to retain leached nutrients can play a significant role in minimizing eutrophication and additionally increase bioavailable nutrients for plant uptake (Kimetu & Lehmann 2010; Mia et al. 2017). For example, a recent study conducted by Mia et al. (2017) concluded that soil amended with aged wood biochar significantly retained more recoverable soil ammonium (NH_4^+) than those without biochar. Mia et al. (2017) suggested that this was likely due to cationic exchange on biochar surface in a sandy loam soil. They also postulated that this effect could play a significant role in minimizing nitrous oxide (N_2O) emission via nitrification (Mia et al. 2017).

1.3.1 Biochar and Climate Change

Potential adverse effects of climate change on agriculture and the long-term security of food are imminent (ECO 2016; IPCC 2007; FAO 2009). Some of these effects have already been observed in various regions of the world where extreme weather conditions are suppressing crop yields, and climate change induced extreme climatic events have becoming more frequent in the past century and are projected to continue (Najafi et al. 2018; Asadieh et al. 2016; IPCC 2007). One commonly observed effect of climate change is increasing aridity, typically in the form of

heat waves, as a result of the changing climate (Brown 2006; Schimel 2010; Trenberth et al. 2013). Local governments and agricultural producers typically respond with irrigation practices (Trenberth et al. 2013). However, long-term reliance on irrigation depletes surface and ground water which often leads to water shortages globally, where over 70% of fresh water is used for agriculture (FAO 2007). Biochar can promote soil water retention and therefore reduce the reliance on irrigation and climate-induced damage to crop yield (Sun & Lu 2014, NRC 2019). For instance, sandy soils that received wood biochar was able to significantly improve the resistance of tomato (*Lycopersicon esculentum*) seedlings against wilting under drought events in northern USA and northern Italy (Mulcahy et al. 2013). However, Mulcahy et al. (2013) highlighted that the quantity of biochar required to produce such biological significance was very high where sandy loam soil and sandy soil required 15% v/v and 30% v/v of biochar added, respectively.

1.4 Knowledge Gaps

Due to the massive variability in the types of biochar (e.g., feedstock source and pyrolysis process) and application rates, a large knowledge gap exists on the effect of biochar on soil physical, chemical, and biological characteristics in temperate soil (Atkinson et al. 2010; Mechler et al. 2018). Using biochar amended soil as a carbon sink to combat environmental issues by mitigating GHG emissions and climate change was plausible and our interest now focuses on whether it can be realized in temperate soils (Lehmann 2007; Mechler et al. 2018).

Additionally, long-term in-field studies on biochar in agriculture are scarce in temperate agriculture. This furthers our current knowledge gap on the long-term effect of biochar on physical, chemical, and biological characteristics and its response to conventional as well as sustainable agroecosystem management practices. Environmentally, since anthropogenic GHG

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emissions are of great global concern, long-term studies on the interaction between biochar amended soil and its ability to mitigate or exacerbate GHG emissions from temperate agricultural soil remains elusive (Bamminger et al., 2014).

The rising threats presented due to climate change, which is closely linked to food insecurity, are currently recognized globally (Lobell et al. 2008; Tai et al. 2014). Therefore, it is vital that we improve our understanding on how the agroecosystem may respond to rising atmospheric temperature and GHG concentrations. Determining the role that biochar plays in conventional temperate agriculture under projected climate conditions could prove to be a valuable mitigating strategy against any adverse effect of climate change.

1.5 Objectives and Hypotheses

The goal of this study is to determine if biochar amendment can improve soil health, mitigate GHG emissions, and influence the effect of climate change on soil health. The specific objectives of this study are:

To determine and compare changes in soil health by evaluating soil physical, chemical, and biological characteristics in soil amended with biochar and without biochar

To determine and compare changes in crop productivity in soil amended with biochar and without biochar.

To quantify and compare greenhouse gas emissions and their relationship to soil chemical and physical characteristics in soil amended with biochar and without biochar.

To determine the impact of warming and CO_2 fertilization associated with climate change on soil health by evaluating soil physical, chemical, biological, and crop characteristics in soil amended with and without biochar.

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It is hypothesized that soil amended with biochar will have improved soil health, crop productivity, and reduced greenhouse gas emissions. It was also hypothesized that soil amended with biochar is more resilient against effects of climate change.

Chapter 2: The effect of biochar on soil health and fertility in temperate agriculture

2.1 Abstract

Biochar has been created to successfully increase soil fertility in tropical lands for thousands of years. Now, scientists and producers in temperate agriculture also seek to take advantage of biochar as to improve soil health and agronomic yield. The goal of this study is to investigate the effect biochar as a soil amendment under conventional farm management in southern Ontario in the year 2018. The study site is comprised of 3 triplicated treatment plots: 6t/ha poultry manure and 135 kg/ha urea-N fertilizer (MN), 3t/ha poultry manure and 3t/ha biochar (MB), and 3 t/ha poultry manure, 135 kg/ha urea-N fertilizer, and 3t/ha biochar (MNB). Key findings include: MB contained the greatest fraction of stable macroaggregates at 78.9 w/w than MN at 70.4 % w/w and MNB at 73.9 % w/w (p = 0.040). MB had the lowest sample mean soil ammonium at 1.94 mg N/kg_{soil} whereas MN and MNB were significantly higher at 2.71 mg N/kg_{soil} and 3.13 mg N/kg_{soil} respectively (p < 0.001). MNB contained substantially higher soil microbial biomass carbon at 202 µg C/g_{soil} than MN and MB at 68 µg C/g_{soil} and 90 µg C/g_{soil} respectively (p = 0.002). The significantly greater soil microbial biomass in MNB was likely due to the urea retention favouring certain microbial species. The dry year of 2018 suppressed biological growth and crop yield. Microbial biomass was greatly reduced compared to the previous year with a grand average of 120 µg $C_{microbial}$/g_{soil} in 2018 and 418 µg $C_{microbial}$/g_{soil} in 2017 (p < 0.001). Grain yield was approximately 520 g/m^2 which was better than when corn was first grown (grand average grain yield of 64 g/m^2) in the extremely dry year of 2016. Findings from this study suggest biochar was able to partially alleviate soil additive reliance. However, the current state of biochar industry does not offer an economically feasible option for agricultural producers to incorporate biochar at an effective rate.

2.2 Introduction

Agricultural producers have always been looking for means to improve soil health and fertility. Soil additives such as fertilizers and organic wastes have been some of the most common supplements mixed into soil to achieve better crop yields (Noble 2011; Chan et al. 2008). Until recently, biochar has only been utilized in tropical regions for its pronounced beneficial effects on acidic and metal-rich types of soil (Lehmann & Rondon 2006). Now, biochar has gained attention of scientists and agricultural producers outside of the tropical regions for its reported abilities to promote soil health under various conditions, but to different extents (Zimmerman et al. 2011; Jeffrey et al. 2011).

Biochar conditions soil by altering its physical, chemical and biological properties (Atkinson et al. 2010). Specifically, biochar amended soil makes use of biochar's intricate physical attributes such as its high porosity, low density, and firm structure to obtain better water holding capacity (WHC), water retention, slower rate of leaching, and lower soil bulk density (BD) (Atkinson et al. 2010, Bamminger et al. 2016). However, these improvements are usually most strongly observed in sandy soils since WHC and nutrient leaching are a common issue with low soil aggregate surface area due to large soil particles (Basso et al. 2013). The extent to which biochar affects the physical characteristics of a given soil is also heavily dependent on the char type. For instance, Sun and Lu (2013) discovered that straw biochar was able to significantly promote the formation of soil macroaggregates, resulting in an increase in macro- and meso-pores that led to a greater available water content of clayey soil (Vertisol). Woodchip biochar, on the other hand, was not able to increase the formation of stable aggregates in the same clayey soil. (Sun & Lu 2013). However, biochar addition to all soil types tends to contribute to soil stability typically as a result of the reinforced soil structural integrity thereby reducing soil

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degradation by weathering and thus creating a physiologically buffered agroecosystem beneficial for biological growths (Nelissen et al. 2015, Sun & Lu 2013).

Chemical characteristics of soil are often complex when it comes to soil-biochar interactions (Rajkovich et al. 2012). In general, acidic soils benefit the most from the liming effect from biochar amendment (Rees et al. 2014). In sandy soils, inorganic nutrient leaching and poor soil organic matter (SOM) buildup are common problems (Basso et al. 2013). Biochar has demonstrated the ability to promote SOM accumulation and retention such as decomposed plant and animal matters to enhance soil fertility and soil health (Plaza et al. 2016). Biochar was also shown to greatly improve inorganic nutrient retention such as ammonium (NH_4^+-N) (Gai et al. 2014). The highly porous nature of biochar allows for a massive reaction surface area per volume ratio available for ionic exchange in soil (Mukome et al. 2013). This can be beneficial in temperate agriculture as nutrient retention is a common issue due to extreme variabilities in climate conditions resulting in annually inconsistent agronomical yields (Atkinson et al. 2010). Biochar can also be used for heavy metal sorption in soil which contributes to the pH effect mentioned above but can also be effective in reducing the concentration of toxic substance notably in heavy mental and macro-organic pollutants contaminated soils (Zhang et al. 2013, Rees et al. 2013). The toxin adsorptive nature of biochar in turn promotes biological activity and often agronomic yields (Zhang et al. 2013, Rees et al. 2013).

In addition to physical and chemical benefits of implementing biochar into agricultural lands, biochar also provides biological benefits. Biochar is capable of directly influencing the ecology of many soil systems. Previous studies have shown that the overall effect of biochar on soil biota is complex and less information exists on the interaction of soil biota with biochar than biochar's effect on soil physical and chemical properties (Atkinson et al. 2010, Lehmann et al.

2011). Microbial communities have generally benefited from biochar in most studies with respect to total soil microbial activity and biodiversity (Lehmann et al. 2011, Domene et al. 2014, Luo et al. 2013). Research also suggests that biochar promotes fungal populations such as mycorrhizal fungi more than bacteria (Bamminger et al. 2014). Proposed mechanisms of biological benefits from biochar mostly involved increased bioavailable nutrients, nutrient retention, water retention, and suppression of soil toxins (Bamminger et al. 2016, Domene et al. 2014, Luo et al. 2013). However, studies have also observed non-significant or negative effects of biochar on soil biology (Dempster et al. 2012). Lehmann et al. suggested that these phenomena could be due to the sorptive nature of biochar which influences the extraction type of biological assays, as well as low quality biochar produced from low quality feedstock or pyrolytic procedures (Lehmann et al. 2011; Maroušek et al. 2017). For instance, heavy metal contaminants are common in sewage sludge biochar which require additional steps in the biochar production process to avoid heavy metal induced toxicity (Maroušek et al. 2017). Overall, more research is required to further understand the biological benefits of biochar as a soil amendment in order to properly evaluate its advantages and disadvantages.

Lastly, it is also important to evaluate the effect of biochar on crop productivity, especially grain yield, since economic gains or at least partially offsetting the amendment cost, is crucial for agricultural producers prior to incorporating biochar into their conventional farming operations (Kulyk 2012). Biochar tends to be less effective in temperate soils since temperate soils are often higher in pH, allowing for better cation exchange capacity (CEC), and the existing nutrient cycle in temperate soils makes them more arable than tropical soils (Robertson & Grandy 2006; Tiessen et al. 1994). Tiessen et al (1994) proposed that this was due to tropical soils being under constant weathering and agricultural use while temperate soils received annual

breaks to re-accumulate inorganic nutrients and SOM (Tiessen et al. 1994). Other studies found that the difference in SOM between temperate and tropical soils could be miniscule based on factors such as the increased weathering of soil particles due to the freeze-thaw cycle in temperate regions (Tiessen et al. 1994; Greenland et al. 1992; Robertson & Grandy 2006).

With respect to the farm owners' concern and request regarding new soil additives, a very small amount of biochar was incorporated in this study. Also, considering the current cost of high-quality biochar, treatment plots only received 3 tons of biochar per hectare to reflect the economic feasibility of biochar (Soja et al. 2014). This rate of application was near the very low end of most biochar studies typically ranging from 1 to about 40 t/ha and averaging 20 t/ha (Mechler et al. 2018). For instance, Gomez et al. (2014) found that soil microbial abundance and activity were improved with increasing wood biochar addition rates in temperate soil especially at the highest rate tested (20% w/w). Another study found a statistical increase in soil water content at a 6% w/w addition rate, but no statistical difference at a 3% w/w application rate in a sandy soil (Basso et al. 2013). Conservatively assuming a general BD of 1.2 g/cm^3 and an application depth of 15 cm, a 1% w/w biochar addition rate is roughly equal to 18 t/ha. As a result, very limited but realistic results are expected from this biochar study. The goal of this study is focused on contributing knowledge to better understand the impact of a high temperature, slow pyrolysis, and wood-based biochar on soil health and crop productivity of a coarse sandy loam conventional agricultural soil in southern Ontario, Canada.

2.3 Methodology

2.3.1 Study Site

The study site was located in Bayfield, Huron County, Ontario, Canada (43°34'45.8"N, 81°39'52.2"W). The site was located 183 meters above sea level with a 1.5% slope. The site consisted of an area (42m × 42m) of a conventional commercial agricultural farmland generously provided by farm owners of H&N Baker Farm for the biochar soil amendment research. The soil was classified as a uniform calcareous Grey-Brown Luvisol, and its association was a Burford sandy loam (Table 2.1). Historical temporal weather data were obtained from nearby weather stations situated in Dashwood (43°22'00.0"N, 81°37'00.0"W), ON indicating an average annual temperature of 8.2 °C (maximum average of 20.8 °C in July and minimum average of 5.0 °C in January), and an average annual precipitation of 1006.8mm (maximum monthly average of 117.9 mm in September and minimum average of 60.9 mm in March) (Environment Canada 2019). The farmland was primarily used for cash crop farming of maize (*Zea mays* L.) and soybean (*Glycine max* Merr. L.) on an annual rotation. The study site receives on-farm sourced poultry manure with switchgrass bedding as well as commercial urea-N based fertilizer every other year when corn is produced.

Table 2.1 Baseline soil characteristics prior to the addition of biochar in Bayfield, Ontario, 2016. Standard errors are given in parentheses. Data obtained from Mechler (2018).

	Burford Loam Soil (0-10 cm)
Classification	Grey-Brown Luvisol
Land-use	Corn-Soybean Annual Rotation
Texture	Sandy Loam
Bulk Density (g/cm^3)	1.26 (0.01)
pH	7.07 (0.03)
Total Organic C (%)	1.07 (0.05)
Total N (%)	0.12 (0.01)
C/N	8.35 (0.37)
Olsen P (mg P kg^{-1})	52.6 (1.32)

All values are expressed on a dry weight basis.
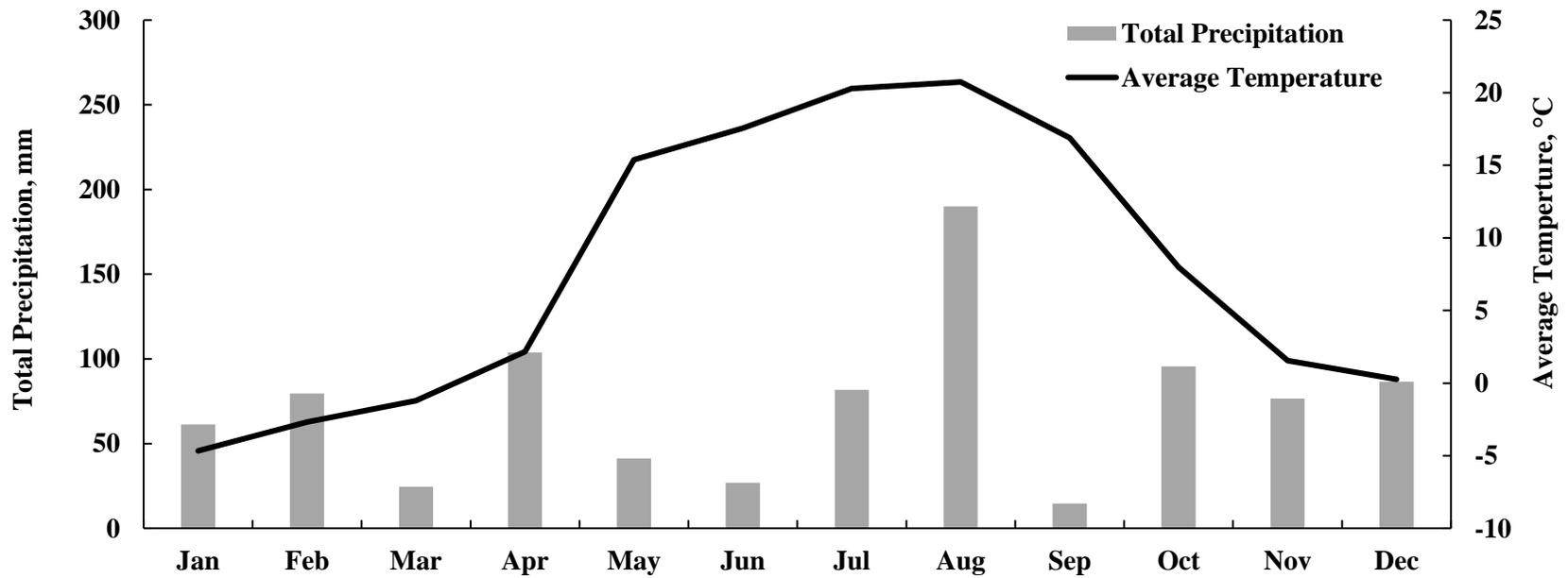
Figure 2.1 Monthly climate data from nearest weather station to Bayfield in Goderich for the study year 2018. Average total precipitation is 1000 mm (830 mm rainfall) from recent decade historical norms, and 880 mm (580 mm rainfall) in 2018.

2.3.2 Experimental Design

This study employed a complete randomized design (CRD) with 3 soil treatment groups each replicated 3 times. The plot size for each treatment replicate was 10m × 10m, and 3-meter buffer was placed between plots and on the outside of outer plots (Figure 2.2) in order to minimize edge effects. A 1 m buffer within each plot was also used to minimize edge effects during sample extraction. The three treatments include 6t/ha poultry manure and 135 kg/ha urea-N fertilizer (MN), 3 t/ha poultry manure and 3 t/ha biochar (MB), and 3 t/ha poultry manure, 135 kg/ha urea-N fertilizer, and 3 t/ha biochar (MNB). Treatments containing biochar have received a one-time addition of Mayan Gold™ biochar (Titan Carbon Smart Technologies, Saskatchewan, Canada) at the beginning of the study in May 2016 using a drop spreader. The biochar was a 50-50 mix of pine (*Pinus* spp.) and spruce (*Picea* spp.) feedstock generated with slow pyrolysis at 550 °C (Table 2.2). All plots were subjected to commercial farming operations such as minimal tillage with a disc harrow and application of glyphosate herbicide. For this study, soil and crop harvest took place on October 15, 2018. The base-line conditions (prior to amendment addition in April 2016) were provided by Mechler (2018) and are presented in Table 2.1.
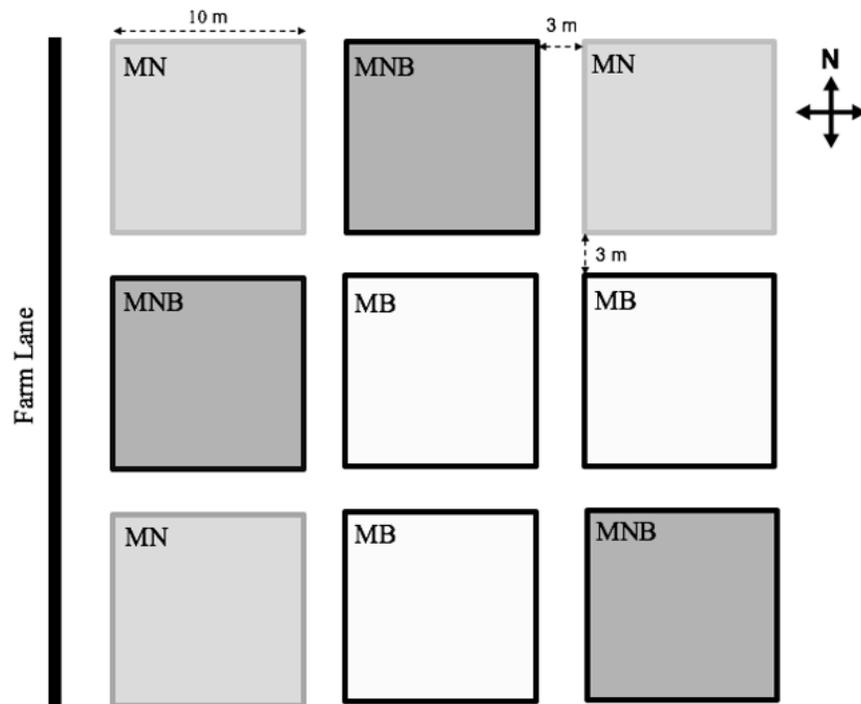
Figure 2.2 Schematic diagram of project's complete randomized design (CRD) plots at H & N Baker Farm, Bayfield Ontario, Canada.

Table 2.2 Characteristics of the biochar and manure used as soil treatment in this study

	Titan Carbon Smart Technologies Biochar	Poultry Manure with Switchgrass Bedding
Pyrolytic Method	Slow Pyrolysis, 550°C	-
Feedstock	Pine/Spruce	-
Water Content (%)	1.7	34.1
pH	7.2	7.9
Total Organic C (%)	80	30.3
Total N (%)	0.5	3.2
C/N	170	9.5
Ash content (%)	12	-
P	0.03	0.83
K	0.30 mg/kg	13725 mg/kg
Ca	0.68 mg/kg	14200 mg/kg
Mg	0.23 mg/kg	4500 mg/kg
S	0.03 mg/kg	3600 mg/kg

All values are expressed on a dry weight basis. Data provided by Titan Carbon Smart Industries, Saskatchewan.

2.3.3 Physical Soil Health Characteristics

A total of five samples were collected to 10 cm, 10-20 cm, and 20-30 cm depths from each treatment replicate (n=135). Bulk Density was collected by inserting a BD ring (inner diameter: 4.5 cm, height: 5.1 cm) horizontally into the undisturbed side of a pit. The soil inside the rings were then oven-dried at 105 °C for 48 hours. The dry weight of the soil divided by the inner volume of the BD ring yielded the BD values (McKenzie et al. 2002).

To determine soil physical, chemical, and biological characteristics, soil samples were collected at 5 random points within each plot and bulked together at 3 depths, top 10 cm, 10-20 cm, and 20-30 cm, totaling to 27 soil samples. These samples were used for various laboratory analyses at the University of Waterloo and stored at -18°C until needed. Aggregate stability was determined using a modified protocol from Carter et al. (2002) and Mehuys et al. (2007). An initial weight of 10 g (W1) of sieved (2 mm) air-dried soil samples were each placed in aluminum weigh-boats and slowly brought to ~50% WHC to avoid slaking effect 10 minutes prior to sieving. The soil was then sieved through a 250 µm sieve inside a bucket of distilled water by uniform raising and lowering of the sieve by 4 cm 30 times per minute for 10 minutes. The portion remaining in the sieve was washed into aluminum weight-boats and oven-dried at 105 °C for 24 hours or until no more weight loss was observed; the oven-dried weight is denoted as W2. These soil samples were then individually shaken in 50-mL centrifuge tubes containing 50 mL of 0.5% w/w sodium hexametaphosphate (a dispersion agent) on a reciprocating shaker (Heidolpj Unimax 1010 DT) at 180 rpm for 45 minutes, and the mixtures were then sieved through the 250 µm sieve again identical to before with the exception of a final gentle physical breakup of the particles using a flat surface. The remaining content inside the sieve was against rinsed into aluminum weight-boats and over-dried for weighing (W3). The stable macro- (> 250

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μm) and micro- (<250 µm) aggregate contents (% w/w) were determined according to Carter et al. (2002) where the formulae are as follows:

Stable macro aggregates (> 250 μm) = (W3 / W1) × 100%   [1]

Stable micro aggregates (< 250 μm) = [(W2 – W1) / W1] × 100%   [2]

Soil water infiltration rate was measured using a 2800 Guelph Permeameter, model 09.07 (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) in the top 8 cm of soil surface on the day of harvest. Natural ground water was obtained on-farm and used. Timed data were recorded and converted to a rate of infiltration (cm/s) using the Guelph Permeameter Calculations Excel spreadsheet provided as part of 2800 Guelph Permeameter Model 09.07 Operating Instructions Manual.

2.3.4 Chemical Soil Health Characteristics

Soil organic carbon (SOC) and total nitrogen (TN) were quantified using 2 g of sieved (2 mm) and air-dried soil. The inorganic carbonate content was removed by adding ~50 mL of 0.5M HCl to the soil inside 50 mL centrifuge tubes and shaken reciprocally at 200 rpm for 30 minutes 3 times over 24 hours. After an 8-hour of settling period, HCl was removed by pipetting. The soils were then washed by ~50 mL of deionized Ultrapure water (18.2 MΩ·cm at 25 °C) by mixing and draining in the same manner as before daily for 4 days. The soils were then oven-dried at 40 °C until no more weight loss was observed (Dyer et al. 2012). The oven-dried soils were ground to a fine powder using a ball mill (Retsch ZM1), the powdered samples were then packaged in tin capsules (Costech, 5 × 9 mm) and subjected to a combustion-gas chromatography elemental analyzer with thermal conductivity (TCD) endpoint detection

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(Costech ECS 4010) to determine net C% and N%; soil C/N ratio was derived based on SOC and TN values.

Hot-water extractable carbon (HWC) was quantified by first adding 30 mL of ultrapure water to 3g (dry-weight equivalent) fresh soil shaken at 200 rpm on a reciprocal shaker for 30 minutes at room temperature, then centrifuged at 1450 G for 20 minutes inside 50 mL centrifuge tubes, and the supernatant was discarded. The remaining sediment was re-suspended in 30 mL of ultrapure water and then placed inside a hot water bath at 80 °C for 16 hours. The mixtures were shaken and centrifuged as before, and the supernatant was filtered through a cellulose nitrate membrane filter (0.45 µm) (Ghani et al. 2003) and freeze-dried. The solid particles remaining were packed and run through the elemental analyzer to determine total carbon content.

Light-fraction organic matter (LFOM) was determined according to Gregorich and Ellert (1993). 50 mL of NaI solution (specific density of 1.7 g/mL) was added to each 25 g sample of sieved (2 mm) and air-dried soil. These mixtures were then briefly hand-shaken, then shaken at 250 rpm on a reciprocal shaker for 1 hour. The mixtures were then allowed to settle for 48 hours at room temperature. The light fraction of the soil was suctioned and isolated onto a glass microfibre filter (Whatman GF 934-AH, 1.5 µm) using the vacuum suction unit described by Gregorich and Ellert (1993). The contents were then washed by ~75 mL of 0.01M CaCl solutions and >75 mL of ultrapure water to remove NaI. The cleaned-up LFOM was then oven-dried at 60 °C until no more weight was lost, ground to a fine powder and analyzed in the elemental analyzer as before for %C, %N, from which LF-C/N ratio was also calculated.

The concentrations of soil inorganic nutrients, NH_4^+-N, nitrate (NO_3^--N) and ortho-phosphate (PO_4^{3-}-P) were determined by colorimetry according to protocols adapted from Maynard and Kalra (1993) and Kuo (1996). For nitrogen species analysis, 5 g of a sieved (2 mm)

and air-dried soil sample were extracted using 25 mL of 2.0 M KCl solution by mixing on a reciprocal shaker at 180 rpm for 15 minutes. The extractants were filtered through a paper filter (Whatman 42, 2.5 µm). Vanadium catalyzed quantitative reduction of NO_3^--N and Berthelot reaction of ammonia were performed, and the colorimetric solutions were measured on a UV-Vis spectrophotometer (Shimadzu UV-1800) at wavelengths 650 nm and 540 nm for NH_4^+-N and NO_3^--N respectively. 2.5 g of each sieved (2 mm) and air-dried soil sample was extracted using 50 mL of 0.5M $NaHCO_3$ (pH 8.5) and shaken at 180 rpm for 15 minutes on a reciprocal shaker for PO_4^{3-}-P quantification. The Olsen-phosphorus colorimetry (ascorbic acid method) of PO_4^{3-}-P was performed and measured at 680 nm (Amacher et al., 2003). Soil pH was determined by creating a 50% (w/v) fresh soil mixture with ultrapure water and measured with a pH meter (Fisherbrand, Accumet).

2.3.5 Biological Soil Health Characteristics and Crop Productivity

SMB was determined according to Voroney et al. (2008). Fresh soil samples were sieved (2mm) and kept at 50% WHC at room temperature for 5 days. 30 g of the soil samples were extracted using $2\times$ oven-dried weight equivalent in volume (approximately 51mL) of 0.05 M K_2SO_4, shaken at 200 rpm for 60 minutes on a reciprocal shaker. The mixtures were then filtered through a glass microfibre filter (VWR 961, 1.5µm), the filtrates were then freeze-dried, packed and run on an elemental analyzer for non-fumigated SMB (nfSMB) carbon and SMB nitrogen. Another set of 30 g of the soil samples was exposed to chloroform fumigation inside desiccators under high vacuum for 24 hours, the chloroform was discarded, and chloroform vapor was removed by 5-minute periods of vacuum pump suction for 6 periods totaling to over 30 minutes of vacuum suctioning. The chloroform fumigated soil samples were then extracted, freeze-dried, analyzed for fumigated SMB (fSMB) carbon and nitrogen like before. SMB carbon and nitrogen

contents, and SMB C/N ratio are calculated by the following equations respectively (Voroney et al. 2008):

$$SMB\text{-}C = (fSMB\text{-}C - nfSMB\text{-}C) / 0.35 \qquad [3]$$

$$SMB\text{-}N = (fSMB\text{-}N - nfSMB\text{-}N) / 0.50 \qquad [4]$$

$$SMB\ C/N = SMB\text{-}C / SMB\text{-}N \qquad [5]$$

Soil microbial community structure was determined using Biolog EcoPlates™ according to Garland and Mills (1991). 1 g of each sieved (2 mm) fresh soil sample was suspended into 10 mL of 0.85% w/w NaCl solution. This mixture was then further diluted by a factor of 10000 via serial dilution, and then incubated into a 96-well Ecoplate™. The EcoPlates™ were incubated at 25 °C for 10 days and changes in well colour were quantified twice per day using a microplate reader (BioTek EL 800). The time at which the maximum peak colour development occurred (t = 7.5 days) was chosen as the dataset used to calculate average well colour development (AWCD), richness of species (R), and Shannon Diversity index (Hs).

AWCD was calculated as a function of an average microplate well optical density (OD) measured spectrophotometrically at 590 nm correcting for the control well containing just water in an equation as follows:

$$AWCD = \Sigma(OD_i - OD_{control})/31 \qquad [6]$$

where OD_i is the optical density at i^{th} well, $CO_{control}$ is the OD of the control well. The sum is divided by 31 because the 96-well microplate includes 3 replications. R of species is simply the number of wells that had a positive response (purple colour development). Hs is an estimation of

microbial biodiversity of the soil sample taking the number of species and population evenness into consideration calculated from the following equation:

$$Hs = -\Sigma[p_i \times \ln(p_i)] \qquad [7]$$

where p_i is the ratio between substrate response (OD_i) to the sum of total substrate response (ΣOD_i).

Crop (maize) sampling coincided with soil sampling on October 15, 2018. Crop biomass (grain yield, shoot, and root biomass) was sampled from a 2 m x 0.4 m area that was randomly selected within each treatment replicate. Roots were collected in a 20 cm $\times$ 20 cm square and cleaned with water to remove soil particles with a 2-mm sieve to retain fragmented roots. All components of the maize biomass were oven-dried at 72°C until no further weight loss is observed. After oven drying, a 50-50 mixture of stems and leaves subsampled from shoots were ground up and analyzed with the elemental analyzer to determine carbon and nitrogen content from which the C/N ratio was also quantified.

2.3.6 Statistical Analyses

All statistical analyses were performed computationally on IBM SPSS™ for Windows, Version 25. All tests were conducted with an overall type I error rate (alpha level) of 0.05 including two-factor within-group pair-wise mean contrast procedures when an interaction term was significant ($p < 0.05$). Two-way analysis of variance (ANOVA) was performed for most test results with treatment and depth as fixed factors, except for those with only one valid independent variable, such as infiltration rate and microbial community structural analyses due to the depth factor having only one level. Tukey's *post hoc* pair-wise t-tests were performed for factors or interaction terms that had significant effects on tested variables. Shapiro Wilk's test

was performed (n<2000) to check for normality of data. Mean values were still strictly used for statistical analyses for consistency.

2.4 Results

2.4.1 Physical Soil Health Characteristics

Soil treatment and depth did not have an interactive effect on any of the soil physical characteristics measured (Figure 2.3). BD differed significantly among treatments ($p = 0.029$) where MN soils had the lowest BD, and MNB had the highest with MB being in the middle and not significantly different from either MN or MNB sample groups. BD also significantly differed among depths ($p = 0.049$) where soil in the top 10 cm had the lowest density, soil from 10 cm to 20 cm were the most densely compacted, and soil from 20 cm to 30 cm was in the middle and not significantly different from either of the other two depths (Figure 2.4).

Soil stable macro-aggregates (> 250 μm) differed only significantly among treatments ($p = 0.040$) (Figure 2.3). Soil samples from the MN treatment contained the lowest fraction of stable macro-aggregate by dry weight while MB had the highest fraction. MNB was in the middle and not significantly different from either of the other two treatment sample groups (Table 2.4). Soil stable micro-aggregates (< 250 μm) were not significantly different among treatments (Figure 2.3); MNB displayed consistent trends in having the highest fraction of stable micro-aggregates compared to the other two sample groups but not significantly higher due to the large standard errors (Table 2.4). Soil infiltration rates did not significantly differ across either fixed factor (Figure 2.3), these values also varied greatly from one treatment replicate to another and therefore massive standard errors (up to 44% RSD) were associated with each treatment group mean (Table 2.4).

Table 2.3 Two-way analyses of variance on soil physical characteristics across treatments (MN, MB, MNB) and depths (top 10, 20, 30 cm) of a temperate agricultural farm. H&N Baker Farm, Bayfield, ON, 2018.

Fixed Factors	Bulk Density	Stable Aggregate > 250µm	Stable Aggregate < 250µm	Infiltration Rate*
	F (p > F)	F (p > F)	F (p > F)	F (p > F)
Treatment	**4.317 (0.029)**	**3.884 (0.040)**	1.561 (0.237)	3.077 (0.120)
Depth	**3.573 (0.049)**	0.025 (0.976)	0.319 (0.731)	-
Treatment × Depth	0.636 (0.644)	0.131 (0.969)	0.297 (0.876)	-

*One-way ANOVA was performed for soil surface infiltration rate since depth was not a factor. Significant terms are in bold (α = 0.05).

Table 2.4 Mean values, their associated standard error, and pair-wise Tukey comparison on physical soil health characteristics across treatments (MN, MB, MNB) and depths (0-10, 10-20, 20-30 cm) of a temperate agricultural soil collected in Bayfield, ON, 2018.

		MN	MB	MNB	Depth Overall
		$\bar{x}\ (\sigma_{\bar{x}})$	$\bar{x}\ (\sigma_{\bar{x}})$	$\bar{x}\ (\sigma_{\bar{x}})$	$\bar{x}\ (\sigma_{\bar{x}})$
Bulk	**0-10 cm**	1.12 (0.02)[Aa]	1.21 (0.01)[Aba]	1.22 (0.02)[Ba]	**1.18 (0.02)[a]**
Density	**10-20 cm**	1.18 (0.05)[Ab]	1.30 (0.02)[ABb]	1.34 (0.01)[Bb]	**1.28 (0.03)[b]**
g/cm³	**20-30 cm**	1.21 (0.02)[Aab]	1.23 (0.11)[ABab]	1.24 (0.01)[Bab]	**1.23 (0.03)[ab]**
	Treatment Overall	**1.17 (0.02)[A]**	**1.25 (0.04)[AB]**	**1.27 (0.02)[B]**	
Stable	**0-10 cm**	70.7 (1.7)[Aa]	79.0 (1.00)[Ba]	73.3 (3.7)[ABa]	74.3 (1.7)[a]
Aggregates	**10-20 cm**	70.7 (1.3)[Aa]	77.0 (1.00)[Ba]	74.7 (5.3)[Aba]	74.1 (1.9)[a]
(> 250µm)	**20-30 cm**	70.0 (5.9)[Aa]	80.7 (1.20)[Ba]	73.7 (6.4)[Aba]	74.8 (3.0)[a]
%w/w dry	**Treatment Overall**	**70.4 (1.8)[A]**	**78.9 (0.8)[B]**	**73.9 (2.6)[AB]**	
Stable	**0-10 cm**	8.0 (1.0)[Aa]	6.7 (0.9)[Aa]	9.0 (0.6)[Aa]	7.9 (0.5)[a]
Aggregates	**10-20 cm**	8.3 (2.0)[Aa]	8.7 (0.3)[Aa]	9.3 (1.8)[Aa]	8.8 (0.8)[a]
(< 250µm)	**20-30 cm**	6.8 (1.9)[Aa]	7.3 (1.9)[Aa]	10.0 (1.5)[Aa]	8.1 (1.0)[a]
%w/w dry	**Treatment Overall**	**7.7 (0.9)[A]**	**7.6 (0.7)[A]**	**9.4 (0.7)[A]**	
Infiltration Rate cm/s	**0-10 cm**	0.10 (0.03)[A]	1.41 (0.62)[A]	0.59 (0.19)[A]	

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments (α = 0.05).
[a] Values followed by the same lower-case letter denote sample means that are statistically similar among depths (α = 0.05).

2.4.2 Chemical Soil Health Characteristics

Soil treatment and depth did not have any interactive effect on any of the soil chemical characteristics measured. SOC did not differ significantly among treatments, though MNB did have the lowest amount of SOC at every depth compared to MN and MB. SOC decreased significantly at each increment in depth (p < 0.001) (Tables 2.5 and 2.6). HWC was the highest for MN, then MB, and lowest for MNB consistent at all depths; however, the pattern was not significant at the specified alpha level (p = 0.059). HWC differed significantly by depth, where the top 10 cm contained the highest HWC, second highest was from 10 cm - 20 cm, and lowest at 20 cm - 30 cm deep (Tables 2.5 and 2.6). Light-fraction organic carbon (LF-C) did not vary significantly by soil treatments or depths (Tables 2.5 and 2.6). Soil carbon to nitrogen ratios (C/N) differed significantly at p < 0.001 among treatment groups and not by depth (Table 2.5). MN contained the highest soil C/N ratio compared to the other two treatments containing biochar, and the biochar groups were statistically similar themselves (Table 2.6). L-F C/N ratios increased with depth and did not differ among soil treatments (Table 2.6)

TN, though did not significantly differ among treatments, was consistently higher in MB than MN and MNB (Table 2.7 and 2.8a). Soil TN differed significantly by depths, where the top 10 cm contained the highest amount of TN followed by 10 - 20 cm, and soil from depth 20 cm - 30 cm contained the least amount of TN (Table 2.8a). Soil NH_4^+-N was the lowest in the MB treatment group compared to the other two (p = 0.001). Soil NH_4^+-N content did not differ significantly between MN and MNB. Soil NO_3^--N on the other hand, while it did not differ across treatments or depths, followed a similar trend as soil TN where MB showed a fairly consistent higher NO_3^--N content. Soil NH_4^+-N and NO_3^--N were also the most abundant in the top 10 cm, decreasing as depth increased just like TN, however, the trend was not statistically

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significant (Tables 2.7 and 2.8a). LF-N content significantly decreased with depth alone (Table 2.8a). A lot of random variation was observed in LFOM overall (Tables 2.6 and 2.8a). Contrary to soil nitrogen species, soil PO_4^{3-}-P content followed a reverse pattern where PO_4^{3-}-P was the lowest in MB compared to MN and MNB, soil PO_4^{3-}-P content also increased with respect to depth instead opposite of soil nitrogen. However, these sample mean differences were not significant (Tables 2.7 and 2.8a). Soil pH were consistently slightly basic and did not differ across treatments nor depths (Tables 2.7 and 2.8b).

Table 2.5 Two-way analyses of variance on soil carbon characteristics across treatments (MN, MB, MNB) and depths (top 10, 20, 30 cm) of a temperate agricultural farm. H&N Baker Farm, Bayfield, ON, 2018.

Fixed Factors	Soil Organic Carbon	Hot-water-extractable Carbon	Light-fraction Organic Carbon	Soil C/N Ratio	Light-fraction C/N Ratio
	$F (p > F)$	$F (p > F)$	$F (p > F)$	$F (p > F)$	$F (p > F)$
Treatment (Trt)	2.694 (0.095)	3.327 (0.059)	3.655 (0.058)	**15.159 (<0.001)**	2.802 (0.100)
Depth	**23.155 (<0.001)**	**19.200 (<0.001)**	1.806 (0.204)	0.998 (0.388)	**5.686 (0.034)**
Trt × Depth	0.900 (0.484)	60.168 (0.129)	3.447 (0.066)	2.487 (0.080)	3.617 (0.059)

Significant terms are in bold ($\alpha = 0.05$).

Table 2.6 Mean values, their associated standard error, and pair-wise Tukey comparison on soil carbon characteristics across treatments (MN, MB, MNB) and depths (0-10, 10-20, 20-30 cm) of a temperate agricultural soil collected in Bayfield, ON, 2018.

		MN	MB	MNB	Depth Overall
		$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$
Soil Organic	**0-10 cm**	1.18 (0.08)^{Aa}	1.16 (0.02)^{Aa}	1.09 (0.12)^{Aa}	**1.15 (0.04)^a**
Carbon	**10-20 cm**	0.90 (0.04)^{Ab}	0.96 (0.09)^{Ab}	0.85 (0.03)^{Ab}	**0.90 (0.03)^b**
(% w/w dry)	**20-30 cm**	0.89 (0.06)^{Ac}	0.71 (0.09)^{Ac}	0.61 (0.09)^{Ac}	**0.74 (0.06)^c**
	Treatment Overall	0.94 (0.06)^A	0.94 (0.08)^A	0.85 (0.08)^A	
Hot-water-	**0-10 cm**	153 (19)^{Aa}	131 (8)^{Aa}	124 (12)^{Aa}	**136 (8)^a**
extractable	**10-20 cm**	114 (8)^{Ab}	106 (13)^{Ab}	86 (11)^{Ab}	**102 (7)^b**
Organic Carbon	**20-30 cm**	83 (8)^{Ac}	73 (19)^{Ac}	61 (6)^{Ac}	**73 (7)^c**
(mg C/kg soil)	**Treatment Overall**	117 (12)^A	103 (11)^A	90 (10)^A	
Light-fraction	**0-10 cm**	13.7 (0.4)^{Aa}	15.0 (0.2)^{Aa}	11.9 (1.1)^{Aa}	13.6 (0.6)^a
Organic Carbon	**10-20 cm**	28.7 (8.2)^{Aa}	12.1 (1.1)^{Aa}	12.0 (3.2)^{Aa}	17.6 (3.8)^a
(% w/w dry)	**Treatment Overall**	21.2 (5.0)^A	13.6 (0.8)^A	12.0 (1.9)^A	
Soil C/N Ratio	**0-10 cm**	11.7 (1.23)^{Aa}	10.8 (0.27)^{Ba}	11.1 (0.05)^{Ba}	11.2 (0.39)^a
(w/w)	**10-20 cm**	12.9 (0.07)^{Aa}	10.6 (0.32)^{Ba}	10.4 (0.40)^{Ba}	11.3 (0.43)^a
	20-30 cm	14.5 (1.18)^{Aa}	10.2 (0.26)^{Ba}	10.8 (0.18)^{Ba}	11.9 (0.76)^a
	Treatment Overall	**13.0 (0.64)^A**	**10.5 (0.16)^B**	**10.8 (0.16)^B**	
Light-fraction	**0-10 cm**	5.8 (1.4)^{Aa}	7.3 (0.5)^{Aa}	7.8 (1.5)^{Aa}	**7.0 (0.7)^a**
C/N Ratio	**10-20 cm**	33.1 (13.0)^{Ab}	5.8 (0.6)^{Ab}	14.4 (3.1)^{Ab}	**17.8 (5.6)^b**
(w/w)	**Treatment Overall**	19.5 (8.5)^A	6.6 (0.5)^A	11.1 (2.1)^A	

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).

[a] Values followed by the same lower-case letter denote sample means that are statistically similar among depths ($\alpha = 0.05$).

Table 2.7 Two-way analyses of variance on soil nitrogen, phosphorus, and pH characteristics across treatments (MN, MB, MNB) and depths (top 10, 20, 30 cm) of a temperate agricultural farm. H&N Baker Farm, Bayfield, ON, 2018.

Fixed Factors	Soil Total Nitrogen	Light-fraction Organic Nitrogen	Soil Ammonium	Soil Nitrate	Soil Ortho-phosphate	Soil pH
	F ($p > F$)	F ($p > F$)	F ($p > F$)	F ($p > F$)	F ($p > F$)	F ($p > F$)
Treatment, Trt	2.127 (0.148)	2.882 (0.095)	**10.567 (0.001)**	2.162 (0.144)	1.816 (0.191)	0.233 (0.795)
Depth	**21.904 (<0.001)**	**6.855 (0.022)**	1.844 (0.187)	0.835 (0.450)	2.342 (0.125)	1.746 (0.203)
Trt × Depth	0.523 (0.720)	2.481 (0.125)	0.406 (0.802)	0.423 (0.790)	0.679 (0.616)	0.132 (0.969)

Significant terms are in bold ($\alpha = 0.05$).

Table 2.8a Mean values, their associated standard error, and pair-wise Tukey comparison on soil nitrogen and phosphorus species across treatments (MN, MB, MNB) and depths (0-10, 10-20, 20-30 cm) of a temperate agricultural soil collected in Bayfield, ON, 2018.

		MN	MB	MNB	Depth Overall
		$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$
Soil Total Nitrogen	**0-10 cm**	$0.103\ (0.010)^{Aa}$	$0.108\ (0.003)^{Aa}$	$0.099\ (0.011)^{Aa}$	**0.103 (0.005)^a**
(% w/w, dry)	**10-20 cm**	$0.070\ (0.003)^{Ab}$	$0.091\ (0.009)^{Ab}$	$0.082\ (0.005)^{Ab}$	**0.081 (0.004)^b**
	20-30 cm	$0.062\ (0.004)$	$0.070\ (0.010)$	$0.056\ (0.008)$	**0.063 (0.004)^c**
	Treatment Overall	$0.078\ (0.007)^{A}$	$0.090\ (0.007)^{A}$	$0.079\ (0.007)^{A}$	
Light-fraction	**0-10 cm**	$2.59\ (0.48)^{Aa}$	$2.07\ (0.14)^{Aa}$	$1.66\ (0.34)^{Aa}$	**2.10 (0.221)^a**
Organic Nitrogen	**10-20 cm**	$1.148\ (0.36)^{Ab}$	$2.09\ (0.110)^{Ab}$	$0.98\ (0.36)^{Ab}$	**1.40 (0.230)^b**
(% w/w, dry)	**Treatment Overall**	$1.87\ (0.42)^{A}$	$2.08\ (0.080)^{A}$	$1.32\ (0.27)^{A}$	
Soil Ammonium,	**0-10 cm**	$3.02\ (0.21)^{Aa}$	$2.22\ (0.09)^{Ba}$	$3.33\ (0.26)^{Aa}$	$2.86\ (0.19)^a$
NH$_4^+$	**10-20 cm**	$2.64\ (0.13)^{Aa}$	$1.72\ (0.76)^{Ba}$	$3.34\ (0.20)^{Aa}$	$2.57\ (0.33)^a$
(mg N/kg $_{soil}$)	**20-30 cm**	$2.48\ (0.22)^{Aa}$	$1.89\ (0.12)^{Ba}$	$2.71\ (0.32)^{Aa}$	$2.36\ (0.17)^a$
	Treatment Overall	**2.71 (0.13)^A**	**1.94 (0.24)^B**	**3.13 (0.17)^A**	
Soil Nitrate, NO$_3^-$	**0-10 cm**	$7.52\ (5.27)^{Aa}$	$11.75\ (2.49)^{Aa}$	$4.55\ (1.28)^{Aa}$	$7.94\ (2.01)^a$
(mg N/kg $_{soil}$)	**10-20 cm**	$6.72\ (3.63)^{Aa}$	$8.97\ (2.95)^{Aa}$	$3.72\ (0.73)^{Aa}$	$6.47\ (1.56)^a$
	20-30 cm	$5.87\ (1.23)^{Aa}$	$5.26\ (1.30)^{Aa}$	$4.42\ (0.49)^{Aa}$	$5.18\ (1.72)^a$
	Treatment Overall	$6.70\ (1.90)^{A}$	$8.66\ (1.51)^{A}$	$4.23\ (0.47)^{A}$	
Soil Ortho-	**0-10 cm**	$43.3\ (2.5)^{Aa}$	$39.2\ (2.5)^{Aa}$	$40.1\ (2.4)^{Aa}$	$40.8\ (1.5)^a$
phosphate, PO$_4^{3-}$	**10-20 cm**	$42.3\ (0.9)^{Aa}$	$41.5\ (2.7)^{Aa}$	$46.1\ (7.7)^{Aa}$	$43.3\ (2.5)^a$
(mg P/kg $_{soil}$)	**20-30 cm**	$51.9\ (2.9)^{Aa}$	$40.5\ (2.9)^{Aa}$	$51.3\ (6.6)^{Aa}$	$47.9\ (2.9)^a$
	Treatment Overall	$45.8\ (2.0)^{A}$	$40.4\ (1.4)^{A}$	$45.8\ (3.4)^{A}$	

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).

[a] Values followed by the same lower-case letter denote sample means that are statistically similar among depths ($\alpha = 0.05$).

Table 2.8b Mean values, their associated standard error, and pair-wise Tukey comparison on physical soil pH across treatments (MN, MB, MNB) and depths (0-10, 10-20, 20-30 cm) of a temperate agricultural soil collected in Bayfield, ON, 2018.

		MN	MB	MNB	Depth Overall
		$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$
Soil pH	**0-10 cm**	7.2 (0.2)[Aa]	7.1 (0.1)[Aa]	7.1 (0.2)[Aa]	7.1 (0.1)[a]
	10-20 cm	7.4 (0.1)[Aa]	7.3 (0.1)[Aa]	7.3 (0.1)[Aa]	7.3 (0.1)[a]
	20-30 cm	7.3 (0.1)[Aa]	7.3 (0.1)[Aa]	7.2 (0.1)[Aa]	7.3 (0.1)[a]
	Treatment Overall	7.3 (0.1)[A]	7.2 (0.1)[A]	7.2 (0.1)[A]	

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).

[a] Values followed by the same lower-case letter denote sample means that are statistically similar among depths ($\alpha = 0.05$).

2.4.3 Biological Soil Health Characteristics and Crop Productivity

Soil treatment and depth did not have any significant interactive effect on soil biology. Soil microbial biomass carbon and nitrogen varied considerably between sample groups, and therefore had large standard errors. MNB mean SMB-C was significantly higher than MN and MB while MN and MB did not differ significantly from each other ($p = 0.002$). SMB-N did not differ significantly across soil treatments. Both SMB-C and SMB-N differed across depths ($p = 0.004$) where only the top 10 cm had significantly higher SMB carbon and nitrogen by mass. SMB C/N ratios did not differ across treatments or depths, however MNB had the highest C/N ratio consistently at all depths (Tables 2.9 and 2.10). Soil microbial community structural analyses did not differ significantly between AWCD, R, or Hs. However, MN having the largest values followed by MB then by MNB was a common trend for AWCD, R, and Hs measurements (Tables 2.9 and 2.10). Large variations were observed for crop productivity characteristics except for shoot carbon and nitrogen. Contrasts between treatment sample means were not significant for maize crop yield, above-ground biomass, or below-ground biomass (Tables 2.11 and 2.12).

Table 2.9 Two-way analyses of variance on soil microbial characteristics across treatments (MN, MB, MNB) and depths (top 10, 20, 30 cm) of a temperate agricultural farm. H&N Baker Farm, Bayfield, ON, 2018.

Fixed Factors	Soil Microbial Carbon Biomass	Soil Microbial Nitrogen Biomass	Soil Microbial C/N Ratio	Average Well Colour Development*	Richness*	Shannon Diversity Index, Hs*
	F (p > F)	F (p > F)	F (p > F)	F (p > F)	F (p > F)	F (p > F)
Treatment, Trt	**8.791 (0.002)**	1.927 (0.174)	2.537 (0.110)	2.953 (0.128)	4.000 (0.079)	0.019 (0.981)
Depth	**7.618 (0.004)**	**6.958 (0.006)**	0.067 (0.935)	-	-	-
Trt × Depth	0.208 (0.930)	1.274 (0.317)	0.605 (0.665)	-	-	-

*One-way ANOVA was performed instead since depth was not a factor.
Significant terms are in bold ($\alpha = 0.05$).

Table 2.10 Mean values, their associated standard error, and pair-wise Tukey comparison on biological soil health characteristics across treatments (MN, MB, MNB) and depths (0-10, 10-20, 20-30 cm) of a temperate agricultural soil collected in Bayfield, ON, 2018.

		MN	MB	MNB	Depth Overall
		$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$
Soil Microbial	**0-10 cm**	124 (63)[Aa]	185 (40)[Aa]	281 (27)[Ba]	**197 (32)[a]**
Carbon Biomass	**10-20 cm**	47 (36)[Ab]	40 (13)[Ab]	176 (18)[Bb]	**88 (25)[b]**
μg C/g soil	**20-30 cm**	34 (34)[Ab]	45 (24)[Ab]	148 (77)[Bb]	**76 (31)[b]**
	Treatment Overall	**68 (27)[A]**	**90 (28)[A]**	**202 (32)[B]**	
Soil Microbial	**0-10 cm**	43 (11)[Aa]	131 (56)[Aa]	83 (5)[Aa]	**86 (21)[a]**
Nitrogen Biomass	**10-20 cm**	19 (16)[Ab]	35 (16)[Ab]	43 (7)[Ab]	**32 (8)[b]**
μg N/g soil	**20-30 cm**	22 (9)[Ab]	20 (12)[Ab]	33 (16)[Ab]	**25 (7)[b]**
	Treatment Overall	28 (7)[A]	62 (24)[A]	53 (9)[A]	
Soil Microbial	**0-10 cm**	3.3 (2.2)[Aa]	1.8 (0.7)[Aa]	3.4 (0.2)[Aa]	2.8 (0.7)[a]
C/N Ratio	**10-20 cm**	1.2 (1.2)[Aa]	1.9 (0.8)[Aa]	4.2 (0.4)[Aa]	2.6 (0.6)[a]
w/w	**20-30 cm**	0.9 (0.9)[Aa]	2.4 (0.4)[Aa]	5.4 (3.2)[Aa]	2.9 (1.3)[a]
	Treatment Overall	1.9 (0.9)[A]	2.0 (0.4)[A]	4.3 (1.0)[A]	
Average Well Colour Development	**0-10 cm**	0.339 (0.118)[A]	0.174 (0.087)[A]	0.048 (0.005)[A]	0.187 (0.060)[A]
Richness counts	**0-10 cm**	15.7 (3.8)[A]	11.0 (2.5)[A]	5.0 (0.6)[A]	10.6 (2.0)[A]
Shannon Diversity Index, Hs	**0-10 cm**	2.94 (1.19)[A]	2.93 (0.28)[A]	2.77 (0.22)[A]	2.88 (0.36)[A]

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).

[a] Values followed by the same lower-case letter denote sample means that are statistically similar among depths ($\alpha = 0.05$).

Table 2.11 One-way analyses of variance on crop characteristics across treatments (MN, MB, MNB) from the top 10 cm of a temperate agricultural soil. H&N Baker Farm, Bayfield, ON, 2018.

Fixed Factors	Corn Cob Yield	Above-ground Biomass	Below-Ground biomass	Shoot Carbon	Shoot Nitrogen	Shoot C/N Ratio
	F (p > F)	F (p > F)	F (p > F)	F (p > F)	F (p > F)	F (p > F)
Treatment	0.257 (0.781)	0.569 (0.594)	2.685 (0.147)	0.333 (0.729)	0.032 (0.968)	0.167 (0.850)

Significant terms are in bold ($\alpha = 0.05$).

Table 2.12 Mean values, their associated standard error, and pair-wise Tukey comparison on crop productivity across treatments (MN, MB, MNB) and depths (0-10, 10-20, 20-30 cm) of a temperate agricultural soil collected in Bayfield, ON, 2018.

	MN	MB	MNB	Depth Overall
	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$
Corn Cob Yield, g	710 (452)[A]	490 (98)[A]	740 (72)[A]	650 (140)
Above-ground Biomass, g	1180 (216)[A]	1380 (116)[A]	1170 (109)[A]	1240 (84)
Below-ground Biomass*, g	280 (96)[A]	660 (182)[A]	360 (57)[A]	430 (85)
Shoot Carbon %w/w dry	46.2 (0.23)[A]	46.7 (0.61)[A]	46.2 (0.62)[A]	46.3 (0.27)
Shoot Nitrogen %w/w dry	1.6 (0.72)[A]	1.9 (0.69)[A]	1.8 (0.78)[A]	1.8 (0.37)
Shoot C/N Ratio w/w	59 (38.4)[A]	35 (15.8)[A]	50 (30.6)[A]	48 (15.3)

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).

2.5 Discussion

The low rate of biochar application (3 t/ha) likely caused minimal but reflected economically realistic changes in soil characteristics contrary to most biochar studies that uses large amounts of biochar inflating the effects of biochar amendment (Mechler et al. 2018). The study site was under an inherent spatial bias with improving soil characteristics from east to west which further masked the effect of biochar as the west-most column contained two non-biochar (MN) plots (Figure 2.2). This topographical bias favouring plots closest to the farm lane could be explained by the heterogeneity nature of soil as well as the line of trees planted as a windbreak providing shading for the study site in the afternoon and slight reduction in soil erosion by wind (Wilkinson 1999). This was supported by the consistently higher soil moisture and various soil characteristics measured around and within the three treatment plots by the lane, one of which was MNB and had better soil characteristics than the other two MNB treatment replicates (cf. Chapter 3, Figure 3.4). To address this issue, statistical analyses using median values instead of sample means were considered and attempted. However, this further exaggerated the inherent spatial differences associated with the plots and was therefore not applied.

The study site consisted mainly of a sandy loam type of soil without irrigation. As a result, the study plots along with the rest of the farm suffered greatly from the weather conditions, drier than the decadal average, in 2018. This was presented in Figure 2.1 where the beginning of the growing season was extremely dry with very little precipitation, followed by heavy rainfall in August, and then the driest month of the year in September. It should be noted that the spatial bias associated with the land and the contrasting weather patterns likely impacted the findings from this study.

Soil physical attributes are fundamental to soil functionality and can usually be improved by biochar applications (Lehmann et al. 2011; Hardie et al. 2014). Soil BD and pore-size distribution are often improved upon biochar addition which lead to enhancements of soil aeration, root penetration, and soil water content (Hardie et al. 2014; Downie et al. 2009). In this study, soil BD increased with depth as seen in Table 2.4 which was expected because greater forces that cause the soil to compact with depth naturally occur in association with compaction generated by farm machinery (Hamza & Anderson 2005). Interestingly, MN samples were significantly lower in BD and higher (consistently but not significantly) in soil water content (Chapter 3) than MB and MNB sample groups which was contrary to many research findings on biochar amendment (Nelissen et al. 2015; Hardie et al. 2014). This was likely due to the small biochar addition making little difference compared to the effect of 3 t/ha more manure addition to MN plots as well as the spatial biases favoring the soil conditions in 2 MN and 1 MNB treatment plots. Poultry manure with switch grass bedding could lower soil bulk density, resist compaction, and was shown to significantly increase soil water retention suggesting that biochar was not able to replace poultry manure in terms of physical soil enhancements (Samson et al. 2016; Ould Ahmed et al. 2010). Few studies mentioned any long-term effects on soil moisture or field capacity when low rates of biochar additions were employed (Agusalim et al. 2010; Laird et al. 2010; Karhu et al. 2011). Furthermore, few studies exist that have studied the impact of biochar on soil physical characteristics at low application rates especially when soil physical characteristics tend to improve linearly up to very high application rates (100% v/v) (Githinji 2013).

Soil stable macroaggregates provide crucial soil macrostructure such as macropores that allow for excessive water drainage and air exchange (Downie et al. 2009). From this study, MB

treatment plots contained the highest fraction of soil stable macro- (>250μm) aggregates by

weight which is in compliance with most literature on biochar addition and soil aggregate

formation (Sun & Lu 2013; Downie et al. 2009; Ouyang et al. 2013; Jien & Wang 2013).

However, MNB was significantly lower in stable macroaggregates than MB which suggests

biochar and the urea fertilizer may have some an interactive effect. Urea fertilizer is a solid soil

additive usually in the form of pellets containing a very high fraction of urea which could have

saturated the available reaction surface of biochar counteracting its intended functions outside of

urea retention (Simha et al. 2016; Hu & Zhang 2019). Simha et al. (2016) found that various

types of biochar possessed a large capacity to interact with and retain urea, and they suspected

this interactive effect was driven by biochar's high chemical affinity for urea. As a result, the

saturation of biochar's reaction surface with urea could hinder biochar-soil particle interactions

to form macroaggregates. Soil microaggregates serve to form the micropores in soil responsible

for water and nutrient exchange (Angers et al. 2007, Sun & Lu 2013). This study found no

statistical significance for stable micro- (<250μm) aggregates between treatments or depths

likely due to sandy soil containing an amount of microaggregates too miniscule to make a

significant difference between sample means (Basso et al. 2013) -- at about an order of

magnitude lower than macroaggregates shown in Table 2.4. This was in agreement with the

study by Hardie et al. (2014), where they also found no statistical difference in soil aggregates

even at a much higher biochar application rate (47 t/ha of acacia whole tree green waste) in a

sandy loam soil in Tasmania, Australia. Mukherjee and Lal (2013) suggested that improvements

in soil physics are highly soil and biochar specific. For example, even though the majority of

biochars consisted of large fractions of micropores, research found that only 25 out of 60 soil-

biochar combinations yielded positive results in related physical characteristics such as WHC

(Downie et al. 2009; Streubel et al. 2011). The contradicting results in the literature indicate that the compatibility between soil and biochar types is complex and therefore require further research.

Large random variations existed within and between treatment groups for infiltration rate. Extremely fast water drainage was common across all treatment replicates. Therefore, no significant conclusions could be made based on the data from this study. Higher biochar and manure addition rates could potentially reduce the undesired rapid percolation common in sandy soils (Downie et al. 2009). However, there is a lack of reliable solution to obtain an economically feasible rate of biochar addition to significantly improve soil physical traits (Herath et al. 2013).

SOC is the most commonly used indicator for estimating SOM content and soil health as the SOC content of soil often directly correlates to crop productivity and the sustainability of a given agricultural land (West & Post 2002; Jobbágy & Jackson 2000). Thus, the idea of implementing biochar into soil as a long-term strategy to promote the buildup and maintenance of SOM can be of great interest for agricultural producers (Plaza et al. 2016, Hua et al. 2013). From this study, SOC content decreased with increasing depth but not by treatment as shown in Table 2.6. This was unexpected as the consensus in literature points to improvements in SOC retention with biochar addition (Atkinson et al. 2010; Kloss et al. 2014). This was likely due to the additional 3 t/ha manure in MN which offset the difference between treatment replicates containing biochar and those without. Manure contains bioavailable organic matter for microbial uptake while biochar contains highly stable black carbon which promotes accumulation of SOM but is not available for decomposition itself (Hadas et al. 1996; Schmidt et al. 2011). Since biochar provides the sites where SOC accumulation takes place (Hua et al. 2013), there was likely an underestimation of SOC for MB and MNB treatments as large chunks of biochar

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(>2mm) were sieved out during the SOC/TN procedure. Though not significant, MNB contained the lowest level of SOC than MN and MB, consistent at each depth, suggesting biochar and urea fertilizer may have an interactive effect. Simha et al. (2016) and Hu & Zhang (2019) have found and proposed mechanisms for biochar's great capacity to adsorb urea up to a ratio of 1:1 w/w for highly porous chars such as wood-derived biochar. Microbial mineralization of urea is common in soils containing low SOC and inorganic N such as sandy soils as well as soils that are under long-term inorganic N applications (Han et al. 2004; Bandick & Dick 1999; Cusack et al. 2011;). Since microbes are primarily responsible for SOM decomposition, this could explain why MNB contained the least amount of SOC (Fontaine et al. 2003). Though, there exists research with contradicting results. For instance, Moran et al. (2005) found that soil mineralized nitrogen facilitated residue decomposition and stable SOM formation at high soil N content. Soil C/N ratio often dictates the rate of organic residue decomposition and nitrogen cycling (Qiu et al. 2016). Typically, a C/N ratio of 20:1 is desired, that is, 20 unit of carbon to 1 unit of nitrogen by mass in soil; this comes from the fact that microbes require a minimum C/N ratio of about 8 to sustain life and an additional C/N ratio of 16 is optimal for maximized microbial activity totaling to 24:1 (USDA 2011; Bengtsson et al. 2003). Similar to SOC, soil C/N ratio was significantly higher for MN than MB and MNB which again could be explained by the higher rate of manure addition in MN and the removal of biochar chunks during the process of determining SOC (Hadas et al. 1996; Schmidt et al. 2011). Unfortunately, sandy soils tend to have the lowest SOM content compared to other types of soil (Gai et al. 2014), an average C/N ratio of just above 10:1 was observed in this study (Table 2.6). Longer study periods should be employed to further investigate whether aging of biochar leads to better SOC accumulation since the chemistry and

morphology of biochar change from prolonged exposures to agricultural and environmental elements (Uchimiya et al. 2010).

LFOM is the light solid fraction of organic compost in soil that recently started decomposing or was about to begin the decomposition process (Janzen et al. 1992; Gregorich & Janzen 1996; Gregorich & Ellert 1993). The LFOM content is comprised of litterfall, crop and animal residues that can function as a sensitive indicator of the effect of farming practices on SOM cycling (Janzen et al. 1992; Gregorich & Janzen 1996). LFOM varied by depth where deeper LFOM contained more carbon and surface LFOM was more nitrogen-rich by mass, therefore a drastically higher LF C/N ratio is observed in the lower level of soil. This could be a direct result of higher density of soil microbial population near the soil surface (Table 2.10) metabolizing the organic carbon content of LFOM (Carter 1992). No significant differences were observed for LF-C and -N contents across treatment effects (Table 2.6), this indicates that the land management practices did not negatively impact the soil.

Ghani et al. (2003) showed that HWC content in soil was strongly correlated to soil CO_2 emission which suggested that HWC presents the portion of SOC that is readily available for microbial uptake. Similar to SOC, HWC is the labile portion of SOC which is a sensitive indicator of SOM quality and therefore is also considered a sensitive indicator of soil health (Ghani et al. 2003; Hamkalo & Bedernichek 2014). In this study, as expected, WHC followed a similar declining pattern as SOC with respect to increasing depth (Table 2.6). Though not significant, MN again contained the highest HWC content consistently at every soil depth which could be again due to the manure addition, absence of biochar adsorption, or the removal of biochar during sieving. Similar to before, MNB plots, though not significant, contained the lowest HWC content consistent at each depth (Table 2.6). This could again be owing to the

biochar-urea interactive effect promoting microbial decomposition of SOM as suggested by the highest microbial biomass C observed for MNB (Table 2.10) (Simha et al. 2016; Ghani et al. 2003; Fontaine et al. 2003).

Nitrogen and phosphorus are common limiting nutrients in the soil that determine agricultural yields provided the soil contains a healthy level of SOM (Wang et al. 2009). Soil nitrogen and phosphorus typically exist in a concentration gradient as they interact with environmental and biological aspects of the pedosphere (Zhang & McGrath 2004). The common trends for soil TN, NH_4^+-N, and NO_3^--N where they decreased in concentration with respect to increasing depth (Table 2.8). This suggests that the topsoil was better at retaining nitrogen species than the lower horizons, consistent with the fact that the site only contained about ~ 20 cm of organic and to subsoil horizons followed immediately by a rougher substratum horizon of ferrous rocky sand underneath. Microbial denitrification also occurs near the fine roots where oxygen can be limited under soil resulting in lower nitrogen species at lower depths (Cook et al. 2013). MB was significantly lower in soil NH_4^+-N concentration than both MN and MNB, and though not significant, MNB had slightly higher soil NH_4^+-N than MN. Considering the additional manure addition to MN and low rate of biochar application, this suggests that biochar did play a role in nitrogen nutrient retention in this type of temperate soil. While the results indicate that this level of biochar addition was not able to replace the use of urea fertilizer, the observed effect of biochar on NH_4^+-N retention should alleviate N fertilizer reliance as suggested in many studies (Lehmann et al. 2011; Biederman & Harpole 2013). Interestingly, a study found an increased reliance on external nutrient source, typically in soils that are poor in inorganic nutrient content, followed by biochar addition due to N and P immobilization by biochar (Gul &

Whalen 2016). As a result, biochar and fertilizer applications should be done with soil types and conditions in consideration.

No significant differences were observed across treatment groups for soil NO_3^--N or PO_4^{3-}-P even though MN plots received more poultry manure and MB received the least amount of soil nutrient additives (Table 2.8). This suggests an overuse in nitrogen fertilizers and nutrient-rich manures where an excessive amount of inorganic nutrients was applied to and then immediately lost from the soil, which has often led to eutrophication as observed in southern Ontario (Smith et al. 1999; Yang et al. 2007; Good & Beatty 2011). Soil pH did not change across soil treatments or depths which was expected as the soil was already slightly basic (Table 2.6) and the commonly observed liming effect of biochar addition to acidic soil was not observed in this study (Rees et al. 2013). It is also possible that the calcareous nature of soil in Ontario and biochar have an interaction since biochar has high CEC and affinity for calcium in soil as found in a study by Jien & Wang (2013) on wood-based biochar similar to the case of biochar-urea interaction mentioned before. This can be a potential drawback to biochar implementation in temperate soils which are often basic and calcareous (Lentz & Ippolito 2012).

From nutrient cycling, toxin filtration, and microclimate management to biomaterial, biofuel, and food production, soil biology is an essential aspect of agriculture (Altieri 1999; Gonthier et al. 2014). Microbial activity and diversity directly promote soil health and vice versa and are often sensitive to changes in the microenvironment of the soil (Elsgaard et a. 2001; Renella et al. 2005). The most notable pattern in the soil biological analyses is the decrease in SMB, both SMB-C and SMB-N, with increasing depth (Table 2.10). This is consistent with the patterns of SOC and inorganic nutrients in Tables 2.6 and 2.8 where microbes preferentially thrived in the nutrient-rich topsoil (SARE 2012). MNB treatment contained significantly and

substantially higher SMB-C by mass than MN and MB which could be explained by the underlining interactive effect between urea-nitrogen fertilizer and biochar favouring microbial species responsible for the urease activity in soil (Cusack et al. 2011). Nitrogen is often a limiting nutrient in most ecosystems which can be provided in great excess by nitrogen fertilizers (Dawson & Hilton 2011). However, huge portions of the added nitrogen are often lost via leaching after the saturation of soil sorption sites (Zhang et al. 2015). The observed microbial bloom in MNB soil samples could therefore be owing to the direct benefit of the biochar retaining a portion of the large urea nitrogen dump at the beginning of the growing season (Simha et al. 2016; Taghizadeh-Toosi et al. 2012). Taghizadeh-Toosi et al. (2012) showed that low temperature wood biochar was able to significantly retain urea-derived ammonia in soil against volatile nitrogen losses. Collectively speaking, SMB was a lot lower this year compared to the year 2016 of the in-field study (Mechler 2018) likely due to the fact that the 2018 growing season experienced more severe weather conditions (Figure 2.1).

Further microbial community assays were performed including AWCD and R which are measures of diversity of microbial species based on nutrient source metabolization, and Hs which is a measure of biodiversity but with population density adjustment (Garland 1991). Again, though not significant, MN had the highest average AWCD, R, and Hs, followed by MB and lastly MNB. This was likely due to MN receiving more poultry manure which is rich in labile organic matter and inorganic nutrients benefitting microbial activities (Welbaum et al. 2010). MNB had the lowest biodiversity but also highest SMC-C indicating the N-fertilizer and biochar treatment promoted the thriving growth of one or a small group of species of microbes, likely those with urease activity (Table 2.10) (Cusack et al. 2011). Large urea addition to soil

often results in a decrease in soil microbial diversity likely due to the sudden pH shift from the volatilization of urea (Fan & Mackenzie 1993; Zhang et al. 2008).

Various crop measurements were studied, and no significant soil treatment effect was observed in terms of crop yield, biomass, or elemental makeup due to the low rate of biochar application. This was in compliance with various studies on corn yield with wood biochar. For example, Gaskin et al. (2010) found that corn yield decreased with biochar application at 22 t/ha in the first year, but the decrease did not persist in subsequent years. Karer et al. (2013) found that corn yield increased only when 72 t/ha biochar was added (and not lower) where additional fertilizer was also a necessary cofactor likely due to the immobilization of N in soil as a result of large biochar addition. As a result, substantially more biochar would be required to expect an increase in crop productivity which is currently economically unfeasible for agricultural producers (Herath et al. 2013). Additionally, Borchard et al. (2014) discovered that maize yield decreased when biochar addition exceeded 300 t/ha in a sandy loam soil in Germany and this effect persists for more than 2 years suggesting an upper limit to biochar amendment. It is worth noting that the study site produced a very low grain yield this year at 520 g/m^2 compared to the annual average of approximately 1200 g/m^2 in Ontario (Agricorp 2019). The study site also produced even a much lower maize grain yield at 64 g/m^2 in 2016 (Mechler 2018). This was likely due to the unusually dry weathers experienced in southern Ontario in 2018 (Figure 2.1) and even worse in 2016 that sandy soils responded poorly against (Basso et al. 2013).

2.6 Conclusion

In general, the small biochar addition resulted in very limited differences in soil parameters. A slight but significant increase in stable soil macroaggregates in MB suggests biochar contributed to the formation of soil macroaggregates. WHC, SOC and HWC were the highest in MN likely due to the higher SOM input from the additional poultry manure application; they were the lowest in MNB which suggests that the urea-biochar interaction facilitated the microbial decomposition of SOM. The improved retention of urea by biochar likely favoured urease-producing bacteria since SMB-C was the highest in MNB while microbial community structure showed MNB contained the lowest microbial biodiversity likely due to the deleterious effect of large urea addition in conjunction with biochar-urea interactions. Additionally, treatment replicates containing biochar (MB and MNB) contained the highest soil NH_4^+-N content confirming biochar's ability to retain mineralize nitrogen species (especially cationic) in a sandy soil. No statistical differences were observed in soil nitrate and phosphorus between any soil treatments even though additional manure was supplied to MN and no N fertilizer was added to MB suggesting the conventional farming practices employ an excessive amount of nutrient-rich soil additives where majority are likely lost as agricultural pollutants. The low level of biochar addition did not influence crop productivity among treatments as expected. Lastly, crop productivity and microbial activities were greatly suppressed likely due to the extreme climatic patterns in this study year. Longer periods of study and larger biochar additions should be implemented to observe any potential long-term effect associated with the physical, chemical, and biological changes to the soil under a temperate climate.

Chapter 3: The effect of biochar on temperate agricultural greenhouse gas emissions

3.1 Abstract

Amidst rapidly rising atmospheric greenhouse gas (GHG) concentrations, scientists are interested in utilizing biochar to reduce agricultural GHG production as a long-term soil amendment. The goal of the study was to investigate the effects of biochar and selected soil characteristics (NH_4^+-N, NO_3^--N, temperature, and moisture) on soil GHG production on a conventional farm in southern Ontario. The study site consisted of three triplicated treatment plots: 6 t/ha poultry manure and 135 kg/ha urea-N fertilizer (MN), 3 t/ha poultry manure and 3 t/ha biochar (MB), and 3 t/ha poultry manure, 135 kg/ha urea-N fertilizer, and 3 t/ha biochar (MNB). Temporal data, Pearson correlations, and multiple linear regressions on soil CO_2, and N_2O emissions, temperature, moisture, ammonium (NH_4^+-N), and nitrate (NO_3^--N) were summarized to investigate potential links to GHG emission. Overall, MNB and MB emitted less CO_2 than MN, and the difference was significant between MNB and MN (163.9 mg CO_2-C m^{-2} h^{-1}, 137.5 and 127.4 mg CO_2-C m^{-2} h^{-1} for MN, MB and MNB respectively). Though not significant, biochar treatments also emitted less N_2O than MN. Season had the much greater effect on soil GHG emissions ($p < 0.001$ for CO_2 and N_2O) compared to treatment effects ($p = 0.031$ for CO_2 and $p = 0.067$ for N_2O) due to the drastic soil moisture levels as a result of the severe weathers experienced in 2018. Following soil moisture, soil temperature was the second-best predictor for soil CO_2 emission and soil NH_4^+-N was the second-best predictor for soil N_2O emission based on the number of significant Pearson correlations and multiple linear regression coefficients. Findings from this study showed that biochar was able to suppress soil GHG emissions even at low biochar addition rates representative of what is currently economically feasible.

3.2 Introduction

One of the key obstacles of climate change is dealing with the rising concentration of atmospheric greenhouse gases (GHGs) (IPCC 2007). A GHG is a relatively stable gas in the atmosphere that contributes to a greenhouse effect on a globally scale. Increases in the concentration of GHG trap heat emitted from the sun on earth, then directly lead to noticeable increases in atmospheric temperature (IPCC 2014, ECO 2016). Some notable members of GHGs include water vapor - the most abundant GHG on earth, carbon dioxide (CO_2) - the most abundant non-water GHG on earth, methane (CH_4), and nitrous oxide (N_2O) - two of the most potent GHGs contributing to the warming effect (ECO 2016). CO_2 is the most oxidized form of carbon emitted from a wide range of natural and human processes such as open air burning of agricultural waste, fossil fuel burning, and cement production (ECO 2016). While CO_2 has the least warming potential on a per molecule basis, its extensive residence time of over 200 years and great abundance in the atmosphere make CO_2 the top contributor to the greenhouse effect and therefore a common indicator for GHG levels (Lashof & Ahuja 1990). N_2O, while not nearly as abundant as CO_2, is estimated to be over 300 times more potent than CO_2 at causing the greenhouse warming effect (Lashof & Ahuja 1990; Portmann et al. 2012). When enough thermal energy is supplied, N_2O is a strong oxidizer and can act as a catalyst that facilitate the destruction of the ozone layer which in turn increases UV light exposure on earth's surface, further contributing to the warming effect among other health effects (Portmann et al. 2012).

With approximately 11% of all anthropogenic GHG emission being produced from the agricultural sector (IPCC 2014), various mitigation strategies have been implemented to reduce CO_2 emission in the agricultural sector such as minimal tillage (ECCC 2016). However, N_2O emission has been on the rise as a result of increased nitrogen fertilizer usage (Mosier et al. 1998,

ECCC 2016). Agriculture is the largest source of N_2O emission, responsible for over 60% of all N_2O globally, and is a constant source of anthropogenic N_2O due to food production requirements (Nelissen et al. 2014; Reay et al. 2012).

Biochar has been gaining interest due to its observed abilities to function as a permanent carbon sink while sequestering atmospheric CO_2 and reducing agricultural GHG emissions (Batjes 1998; Spokas & Reicosky 2009; Agegnehu et al. 2016). There are numerous proposed mechanisms behind how biochar sequestered carbon and reduced GHG emissions in the soil. First, carbon sequestration is achieved by converting biomass to recalcitrant biochar as a long-term soil amendment instead of allowing for its complete decomposition such as agricultural crop and animal waste (Woolf et al. 2010; Smith 2016). Biochar often facilitates the accumulation of humic soil organic carbon (SOC) (Hua et al. 2013; Li et al. 2018). Ball (1997) suggests that this in turn increases soil C/N ratio and can alter plant nutrient uptake and increase plant lignin content. Ball (1997) suspects the increased lignin content reduces the rate of decomposition and therefore decreases agricultural emission of CO_2. More recently, research emphasizes that an increase in C/N ratio promotes microbial inorganic nutrient immobilization which results in less bioavailable soil nitrogen species for soil microbial activities and ultimately leading to reduced soil N_2O emission (Barrett & Burke 2000; Qiu et al. 2016). Research also suggests a priming effect associated with biochar in soil catalyzing the turn-over rates of fine roots and root exudation of micromolecular organic matter to the rhizosphere thereby stabilizing labile carbon via surface exchange reactions with soil particles (Paterson et al. 1997). Biochar is shown to directly stabilize labile carbon and nitrogen species that often limits substrate availability for microbial substrate breakdown and denitrification processes. This again leads to a reduced output of CO_2 and N_2O from biochar amended soil (Zwieten et al. 2014). Liu et al.

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(2014) discovers that the frequently observed reduction in soil N_2O emission in biochar amended soil coincides with a reduction in ammonia- and nitrite-oxidizing bacteria as well as a decrease in the number of ammonia monooxygenase gene amoA and nitrite reductase gene nirS. However, the long-term effect of biochar on temperate soil GHG emissions is still in its infancy where few studies exist on soil CO_2 emission and even fewer for soil N_2O emission especially for a conventional temperate agricultural system (Clough & Cordron 2010; Atkinson et al. 2010). The purpose of this study is to investigate the potential effect of biochar on soil CO_2 and N_2O emissions of a temperate soil under conventional farming operations in southern Ontario. Soil CH_4 is not monitored in this study due to the abundance of methanotrophic species relative to methanogenic species in temperate agricultural soils as a result of the relatively low soil temperature, and high soil pH, and a lack of flooded rice fields in temperate regions (Dunfield et al. 1993; Ueyama et al. 2015).

3.3 Methodology

3.3.1 Study Site

See Chapter 2, section *2.3.1 site information*.

3.3.2 Experimental Design

The same study plots were used for the temporal study on GHG emissions as described in Chapter 2, section *2.3.2 Experimental Design*. GHG emissions (for CO_2 and N_2O), soil (for NH_4^+-N and NO_3^--N), and soil physical characteristics (temperature and moisture) were sampled and determined concurrently on a biweekly basis from May 21st, 2018 to November 12th, 2018 for a total of three data sets in spring (May 21th to June 25th), five data sets in summer (July 10th to September 4th), and five data sets in autumn (September 17th to November 12th). Two random

sampling locations, within each plot, were chosen totaling to 3 treatment groups with 6 replicates each – 18 sets of samples biweekly.

3.3.3 Greenhouse Gas Emission

A Polyvinyl chloride (PVC) chamber (inner diameter: 10 cm, height: 25 cm) was inserted 10 cm into the surface of the soil at each of the sampling points for gas sampling a week prior to the first sampling date allowing for soil to stabilize and regain equilibrium. Chamber caps covered in a reflective material with a sampling septum and a 10-cm long ventilation tube (inner diameter: 3 mm) to offset any built-up pressure were used as insulation for and only during gas extraction (ports were open outside of the sampling events) (Parkin & Venterea, 2010). Approximately 10 mL of gas was collected from the headspace inside each gas chamber at 0, 15, and 30 minutes following chamber capping. The gaseous sample was stored in a 3-mL evacuated glass vials at room temperature.

Due to equipment limitations, only CO_2 and N_2O were measured for CO_2's great abundance and N_2O's great potency and great relevance to agricultural soils (Lashof & Ahuja 1990; Portmann et al. 2012). Atmospheric concentrations of CO_2 and N_2O from each gas sample were measured by gas chromatography (Agilent 6890N) using 250 µL injection volume, 30-meter capillary column, and thermal conductivity (TCD) and electron capture detectors (ECD) for CO_2 and N_2O respectively. The atmospheric concentrations of CO_2 and N_2O (in ppm) were then used to calculate the net flux of CO_2 and N_2O emissions using the following equations proposed by Hutchinson and Mosier (1981):

$$\text{Order of flux} = (C_1 - C_0) / (C_2 - C_1) \qquad [6]$$

where the order of the rate of emission was determined by the atmospheric concentrations of a given GHG at time = 0 (C_0), 15 (C_1), and 30 (C_2) minutes (ppm). From eq. 6, a value < 1 meant a linear model (linear slope) was used to determine the soil GHG flux, and a valve > 1 would imply that Hutchinson & Mosier equation was used to model the soil GHG flux (f):

$$f = V (C_1 - C_0)^2 / \{A \times t (2 \times C_1 - C_2 - C_0) \ln[C_1 - C_0 / C_2 - C_1]\} \qquad [7]$$

where V is the volume of the head space inside the gas chamber, A is the surface area of the soil inside the chamber, t is the time interval between each sampling event (15 min). The resultant f value is then a measure of a volume of a given GHG per area per unit time. These flux values are then converted to a measure of a mass of a given GHG per area per unit time using the Ideal Gas Law and molecular masses of CO_2 and N_2O as follows (Lutes et al. 2016):

$$PV = nRT \qquad [8]$$

where P is the pressure, V is the volume, n is the number of moles of a given GHG, R is the Ideal Gas Law constant, and T is temperature.

3.3.4 Soil Ammonium and Nitrate

Soil samples were collected biweekly alongside GHG sampling within a 1 m radius of each chamber location and were used for the determination of soil ammonium and nitrate content. See *2.3.4 Soil Chemistry* for quantitative analyses of soil NH_4^+-N and NO_3^--N.

3.3.5 Soil Physical Characteristics

Soil temperature (°C) and moisture (% w/w) in the top 10 cm were measured biweekly alongside GHG sampling, within a 1 m radius of each chamber location, using a portable sensor (Delta T HH2-WET).

2.3.6 Statistical Analyses

All statistical analyses were performed computationally on IBM SPSS™ for Windows, Version 25. All tests were conducted with an overall type I error rate (alpha level) of 0.05 including two-factor within-group pair-wise mean contrast procedures when an interaction term was significant ($p < 0.05$). Two-way analysis of variance (ANOVA) was performed for most test results with treatment and season as fixed factors. Tukey's *post hoc* pair-wise t-tests were performed for factors or interaction terms that had significant effects on tested variables (except for temperature and moisture since they are addressed in chapter 2). Two-tailed Pearson correlations were performed for each of the two GHG emissions to every soil chemical and physical measurement (NH_4^+-N, NO_3^--N, temperature, and moisture) in each season and all year. Multiple linear regressions were also performed to model each GHG emission as the dependent variable with soil chemical and physical measurement (NH_4^+-N, NO_3^--N, temperature, and moisture) as independent variables. Sample groups that were not normally distributed as determined by Shapiro Wilk's test (n<2000) were incorporated as medians in graphs, though mean values were strictly used for statistical analyses for consistency.

3.4 Results

3.4.1 Soil Treatment and Seasonal Effects

Soil treatment and season did not have an interactive effect on soil GHG emissions, chemical, or physical characteristics. All measured soil characteristics differed significantly by the seasonal fixed effect ($p < 0.001$). However, only CO_2 emission differed significantly by the treatment effect ($p = 0.031$) (Table 3.1). CO_2 emission was the highest in spring and summer, and significantly lower in autumn. CO_2 emission was also the highest in the MN treatment, whereas MNB had significantly lower emission while MB was in the middle and not significantly different from either MN or MNB treatments (Table 3.2 and Figure 3.1). N_2O emissions were significantly higher in spring than summer and autumn. While not significant ($p = 0.067$) N_2O emission was also the highest in MN treatment, and was relatively consistent in all seasons (Table 3.3 and Figure 3.2). Soil ammonium was the highest in the spring season and decreased significantly at each subsequent season. Similarly, soil nitrate (NO_3^--N) was the highest in the first two seasons, and significantly lower in autumn (Table 3.4 and Figure 3.5).

Table 3.1 Two-way analyses of variance on GHG emissions, physical and chemical characteristics across treatments (MN, MB, MNB) and seasons (Spring, Summer, Autumn) of temperate agricultural soil. H&N Baker Farm, Bayfield, ON, 2018.

	CO_2	N_2O	Temperature	Moisture	NH_4^+-N	NO_3^--N
	F (p > F)	F (p > F)	F (p > F)	F (p > F)	F (p > F)	F (p > F)
Season	**49.154 (<0.001)**	**11.652 (<0.001)**	**242.53 (>0.001)**	**18.953 (<0.001)**	**38.492 (<0.001)**	**88.976 (<0.001)**
Treatment, Trt	**3.528 (0.031)**	2.754 (0.067)	0.301 (0.740)	1.533 (0.218)	0.559 (0.573)	2.173 (0.116)
Season × Trt	0.550 (0.700)	1.191 (0.317)	0.083 (0.987)	0.671 (0.613)	0.610 (0.656)	0.881 (0.476)

Significant terms are in bold (α = 0.05).

Table 3.2 Mean values, their associated standard error, and pair-wise Tukey comparison on CO_2 emissions (mg CO_2-C m^{-2} h^{-1}) across treatments (MN, MB, MNB) and seasons (Spring, Summer, Autumn) of a temperate agricultural soil collected in Bayfield, ON, 2018.

	MN	MB	MNB	Seasonal Overall
	x̄ (se)	x̄ (se)	x̄ (se)	x̄ (se)
Spring 2018	220.3 (20.2)[Aa]	179.9 (19.6)[ABa]	153.7 (21.1)	**184.6 (11.5)[a]**
Summer 2018	194.9 (15.4)[Aa]	174.5 (15.7)[ABa]	165.7 (15.4)[Ba]	**178.4 (9.0)[a]**
Autumn 2018	76.5 (16.6)[Ab]	58.0 (15.7)[ABb]	62.7 (15.4)[Bb]	**65.7 (9.2)[b]**
Treatment Overall	**163.9 (10.1)[A]**	**137.5 (9.9)[AB]**	**127.4 (9.9)[B]**	

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments (α = 0.05).
[a] Values followed by the same lower-case letter denote sample means that are statistically similar among seasons (α = 0.05).

Table 3.3 Mean values, their associated standard error, and pair-wise Tukey comparison on N₂O emissions (μg N$_2$O-N m^{-2} h^{-1}) across treatments (MN, MB, MNB) and seasons (Spring, Summer, Autumn) of a temperate agricultural soil collected in Bayfield, ON, 2018.

	MN	MB	MNB	Seasonal Overall
	$\overline{x}$ (se)	$\overline{x}$ (se)	$\overline{x}$ (se)	$\overline{x}$ (se)
Spring 2018	273.1 (49.4)^{Aa}	135.6 (47.9)^{Aa}	235.9 (51.0)^{Aa}	**214.9 (28.5)^a**
Summer 2018	40.3 (38.7)^{Ab}	40.6 (44.2)^{Ab}	58.5 (41.2)^{Ab}	**46.5 (23.9)^b**
Autumn 2018	145.2 (47.9)^{Ab}	17.6 (47.9)^{Ab}	22.4 (41.2)^{Ab}	**61.7 (26.4)^b**
Treatment Overall	152.9 (26.3)^A	64.6 (26.9)^A	105.6 (25.8)^A	

^{A} Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).
^{a} Values followed by the same lower-case letter denote sample means that are statistically similar among seasons ($\alpha = 0.05$).

Table 3.4 Mean values, their associated standard error, and pair-wise Tukey comparison on ammonium and nitrate concentrations across treatments (MN, MB, MNB) and seasons (Spring, Summer, Autumn) of a temperate agricultural soil collected in Bayfield, ON, 2018.

		MN	MB	MNB	Seasonal Overall
		$\overline{x}$ (se)	$\overline{x}$ (se)	$\overline{x}$ (se)	$\overline{x}$ (se)
NH₄⁺	**Spring 2018**	6.26 (0.65)^{Aa}	5.91 (0.65)^{Aa}	7.22 (0.67)^{Aa}	**6.46 (0.38)^a**
mg N kg^{-1}$_{soil}$	**Summer 2018**	4.08 (0.52)^{Ab}	4.18 (0.50)^{Ab}	3.91 (0.50)^{Ab}	**4.06 (0.29)^b**
	Autumn 2018	2.61 (0.50)^{Ac}	1.93 (0.50)^{Ac}	2.31 (0.50)^{Ac}	**2.28 (0.29)^c**
	Treatment Overall	4.32 (0.32)^A	4.01 (0.32)^A	4.48 (0.33)^A	
NO₃⁻	**Spring 2018**	56.15 (5.22)^{Aa}	39.18 (5.22)^{Aa}	47.54 (5.22)^{Aa}	**47.62 (3.01)^a**
mg N kg^{-1}$_{soil}$	**Summer 2018**	44.45 (4.04)^{Aa}	38.18 (4.04)^{Aa}	43.20 (4.04)^{Aa}	**41.94 (2.33)^a**
	Autumn 2018	4.13 (4.04)^{Ab}	4.46 (4.18)^{Ab}	3.60 (4.18)^{Ab}	**4.07 (2.39)^b**
	Treatment Overall	34.91 (2.58)^A	27.27 (2.61)^A	31.45 (2.61)^A	

^{A} Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).
^{a} Values followed by the same lower-case letter denote sample means that are statistically similar among seasons ($\alpha = 0.05$).

3.4.2 Greenhouse Gas Emissions and Correlation to soil Characteristics

Considering only significant two-tailed Pearson correlations, CO_2-C emission moderately correlated to soil moisture (r = 0.598) and weakly positively correlated to soil NH_4^+-N (r = 0.275) in spring; CO_2-C weakly negatively correlated to soil temperature (r = -0.246), weakly positively correlated to soil moisture (r = 0.382), and weakly positively correlated to soil NO_3^--N content (r = 0.020) in summer; in autumn, CO_2-C moderately positively correlated to soil temperature (r = 0.612), weakly negatively correlated to soil moisture (r = -0.383), and weakly positively correlated to soil NO_3^--N (r = 0.289). Overall, soil CO_2-C emission nearly moderately positively correlated to soil temperature (r = 0.543), weakly positively correlated to soil NH_4^+-N (r = 0.336) and NO_3^--N (r =0.433) (Table 3.5).

Again, considering only significant two-tailed Pearson correlations, N_2O-N emission moderately positively correlated to soil moisture (r = 0.631) and weakly positively to soil NH_4^+-N (r = 0.404) in spring; N_2O-N emission weakly positively correlated to soil moisture (r = 0.295) and to soil NH_4^+-N (r = 0.262) in summer; N_2O-N emission only weakly correlated to soil moisture (r = 0.268) in autumn. Overall, soil N_2O-N emission weakly correlated to soil moisture (r = 0.409) and to soil NH_4^+-N (r = 0.356) (Table 3.5).

At a type one error rate of 5% and allowing for the effect of all fixed effects (Soil temperature, moisture, NH_4^+-N, and NO_3^--N), soil CO_2-C emission increased by an average of 11.9 ± 5.32 mg CO_2-C m^{-2} h^{-1} for every 1 °C increase in soil temperature, and 17.4 ± 3.40 mg CO_2-C m^{-2} h^{-1} for every 1% increase in soil moisture in spring. Soil CO_2-C emission increased by an average of 6.3 ± 2.08 mg CO_2-C m^{-2} h^{-1} for every 1 % increase in soil moisture, and 0.8 ± 0.24 mg CO_2-C m^{-2} h^{-1} for every 1 mg N kg^{-1}_{soil} increase in soil NO_3^--N in summer. Soil CO_2-C emission increased by an average of 5.5 ± 1.08 mg CO_2-C m^{-2} h^{-1} for every 1 °C increase in soil

temperature in autumn. Overall, soil CO_2-C emission increased by an average of 7.2 ± 0.67 mg CO_2-C m^{-2} h^{-1} for every 1 °C increase in soil temperature, 11.5 ± 1.28 mg CO_2-C m^{-2} h^{-1} for every 1 % increase in soil moisture, and 5.4 ± 1.77 mg CO_2-C m^{-2} h^{-1} for every 1 mg N kg^{-1}_{soil} increase in soil NH_4^+-N.

At a type one error rate of 5% and allowing for the effect of all fixed effects (Soil temperature, moisture, NH_4^+-N, and NO_3^--N), soil N_2O-N emission increased by an average of 37.3 ± 7.81 µg N_2O-N m^{-2} h^{-1} for every 1 % increase in soil moisture, and 26.0 ± 10.79 µg N_2O-N m^{-2} h^{-1} for every 1 mg N kg^{-1}_{soil} increase in soil NH_4^+-N in spring; soil N_2O-N emission increased by an average of 5.7 ± 1.49 µg N_2O-N m^{-2} h^{-1} for every 1 % increase in soil moisture, and 5.2 ± 1.51 mg µg N_2O-N m^{-2} h^{-1} for every 1 mg N kg^{-1}_{soil} increase in soil NH_4^+-N in summer; soil N_2O-N emission increased by an average of 30.1 ± 11.59 µg N_2O-N m^{-2} h^{-1} for every 1 % increase in soil moisture in autumn; overall, soil N_2O-N emission increased by an average of 4.5 ± 1.81 µg N_2O-N m^{-2} h^{-1} for every 1 °C increase in soil temperature, 27.2 ± 3.44 µg N_2O-N m^{-2} h^{-1} for every 1 % increase in soil moisture, and 28.5 ± 4.78 µg N_2O-N m^{-2} h^{-1} for every 1 mg N kg^{-1}_{soil} increase in soil NH_4^+-N.

Table 3.5 Two-tailed Pearson linear correlation coefficient of determination for CO_2-C and N_2O-N emissions by soil physical and chemical characteristics. H&N Baker Farm, Bayfield, ON, 2018.

Season	GHG*	Temperature (°C)	Moisture (% w/w)	NH_4^+ (mg N kg^{-1}$_{soil}$)	NO_3^- (mg N kg^{-1}$_{soil}$)
		r (p)	r (p)	r (p)	r (p)
Spring 2018	CO_2	0.192 (0.173)	**0.598 (<0.001)**	**0.275 (0.048)**	-0.052 (0.716)
	N_2O	-0.061 (0.681)	**0.631 (<0.001)**	**0.404 (0.004)**	-0.115 (0.437)
Summer 2018	CO_2	**-0.246 (0.023)**	**0.382 (<0.001)**	0.020 (0.856)	**0.250 (0.020)**
	N_2O	-0.129 (0.290)	**0.295 (0.014)**	**0.262 (0.031)**	0.153 (0.210)
Autumn 2018	CO_2	**0.612 (<0.001)**	**-0.383 (<0.001)**	0.197 (0.075)	**0.289 (0.010)**
	N_2O	-0.008 (0.950)	**0.268 (0.044)**	-0.036 (0.791)	0.076 (0.584)
Overall 2018	CO_2	**0.543 (<0.001)**	0.073 (0.278)	**0.336 (<0.001)**	**0.433 (<0.001)**
	N_2O	-0.007 (0.925)	**0.409 (<0.001)**	**0.356 (<0.001)**	0.083 (0.279)

* Units for soil CO_2 and N_2O emissions are mg CO_2-C m^{-2} h^{-1} and µg N_2O-N m^{-2} h^{-1} respectively.
Significant terms are in bold ($\alpha = 0.05$).

Table 3.6 Multiple Linear regression coefficient (b), coefficient standard error, and significance (p) for CO_2-C and N_2O-N emissions by soil physical and chemical characteristics allowing for all predictor variables. H&N Baker Farm, Bayfield, ON, 2018.

Season	GHG*	Temperature (°C)	Moisture (% w/w)	NH_4^+ (mg N kg^{-1}soil)	NO_3^- (mg N kg^{-1}soil)
		b ± SE (p)	b ± SE (p)	b ± SE (p)	b ± SE (p)
Spring 2018	CO_2 [a]	**11.9 ± 5.32 (0.030)**	**17.4 ± 3.40 (<0.001)**	7.1 ± 4.72 (0.141)	0.7 ± 0.76 (0.354)
	N_2O [b]	1.3 ± 12.21 (0.917)	**37.3 ± 7.81 (<0.001)**	**26.0 ± 10.79 (0.021)**	0.3 ± 1.74 (0.868)
Summer 2018	CO_2 [c]	-2.3 ± 3.09 (0.458)	**6.3 ± 2.08 (0.003)**	0.7 ± 2.10 (0.734)	**0.8 ± 0.24 (0.001)**
	N_2O [d]	2.8 ± 2.23 (0.214)	**5.7 ± 1.49 (<0.001)**	**5.2 ± 1.51 (0.001)**	0.1 ± 0.17 (0.653)
Autumn 2018	CO_2 [e]	**5.5 ± 1.08 (<0.001)**	0.621 ± 2.19 (0.778)	7.4 ± 6.69 (0.275)	-1.6 ± 1.20 (0.197)
	N_2O [f]	6.0 ± 5.72 (0.303)	**30.1 ± 11.59 (0.012)**	-7.7 ± 35.35 (0.829)	4.9 ± 6.36 (0.443)
Overall 2018	CO_2 [g]	**7.2 ± 0.67 (<0.001)**	**11.5 ± 1.28 (<0.001)**	**5.4 ± 1.77 (0.002)**	0.4 ± 0.22 (0.075)
	N_2O [h]	**4.5 ± 1.81 (0.014)**	**27.2 ± 3.44 (<0.001)**	**28.5 ± 4.78 (<0.001)**	-0.4 ± 0.61 (0.508)

* Units for soil CO_2 and N_2O emissions are mg CO_2-C m^{-2} h^{-1} and μg N_2O-N m^{-2} h^{-1} respectively.
Significant terms are in bold (α = 0.05).
[a] r^2 = 0.453, adj. r^2 = 0.406;
[b] r^2 = 0.477, adj. r^2 = 0.429;
[c] r^2 = 0.272, adj. r^2 = 0.235;
[d] r^2 = 0.278, adj. r^2 = 0.232;
[e] r^2 = 0.398, adj. r^2 = 0.365;
[f] r^2 = 0.132, adj. r^2 = 0.061;
[g] r^2 = 0.520, adj. r^2 = 0.511;
[h] r^2 = 0.378, adj. r^2 = 0.363.
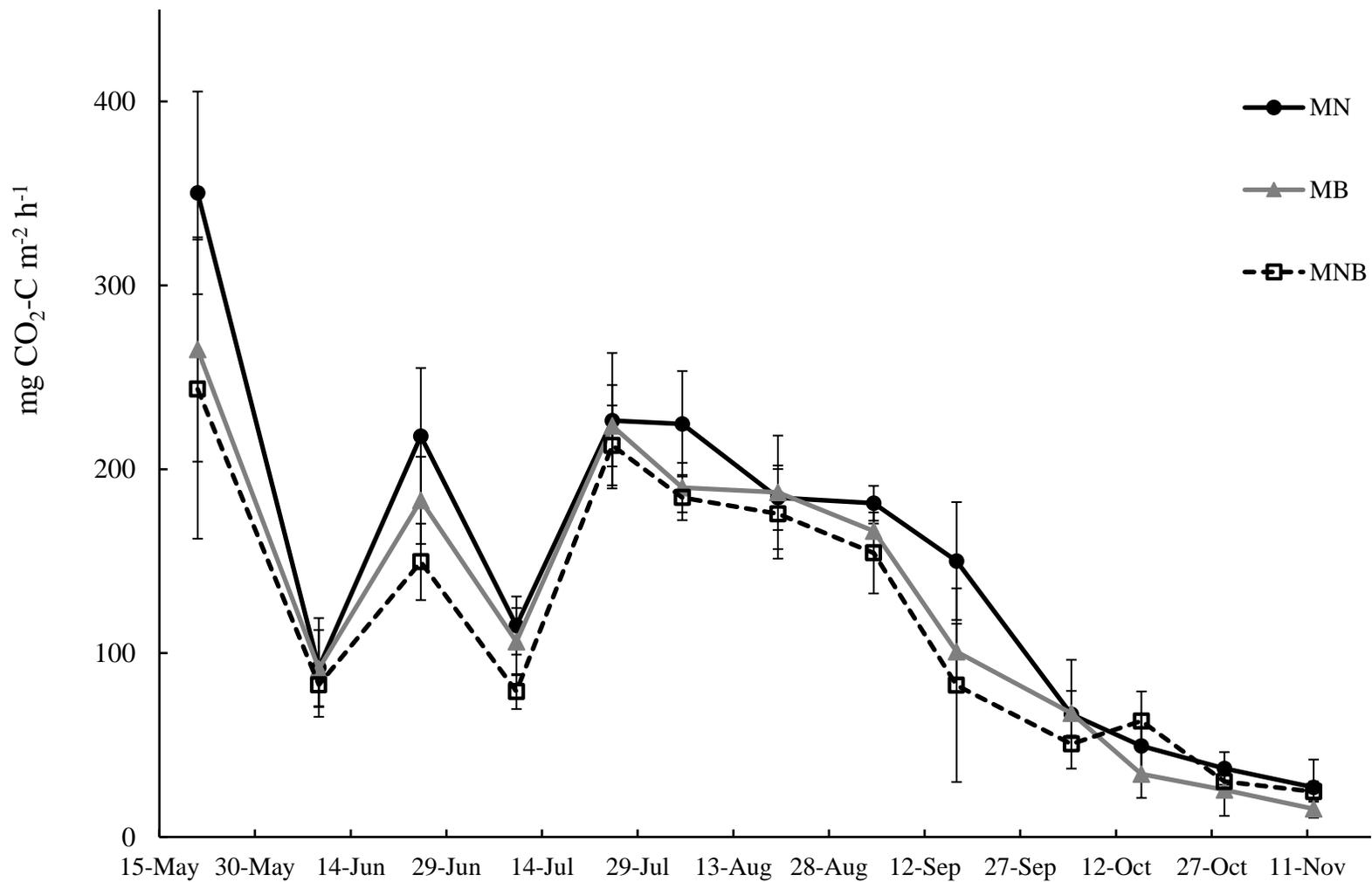
Figure 3.1 Mean (with median corrections) and standard errors of CO_2 emissions (mg CO_2-C m^{-2} h^{-1}) from temperate soil amended with three treatment groups: poultry manure and N-fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N-fertilizer and biochar (MNB). H&N Baker Farm, Bayfield, ON, 2018
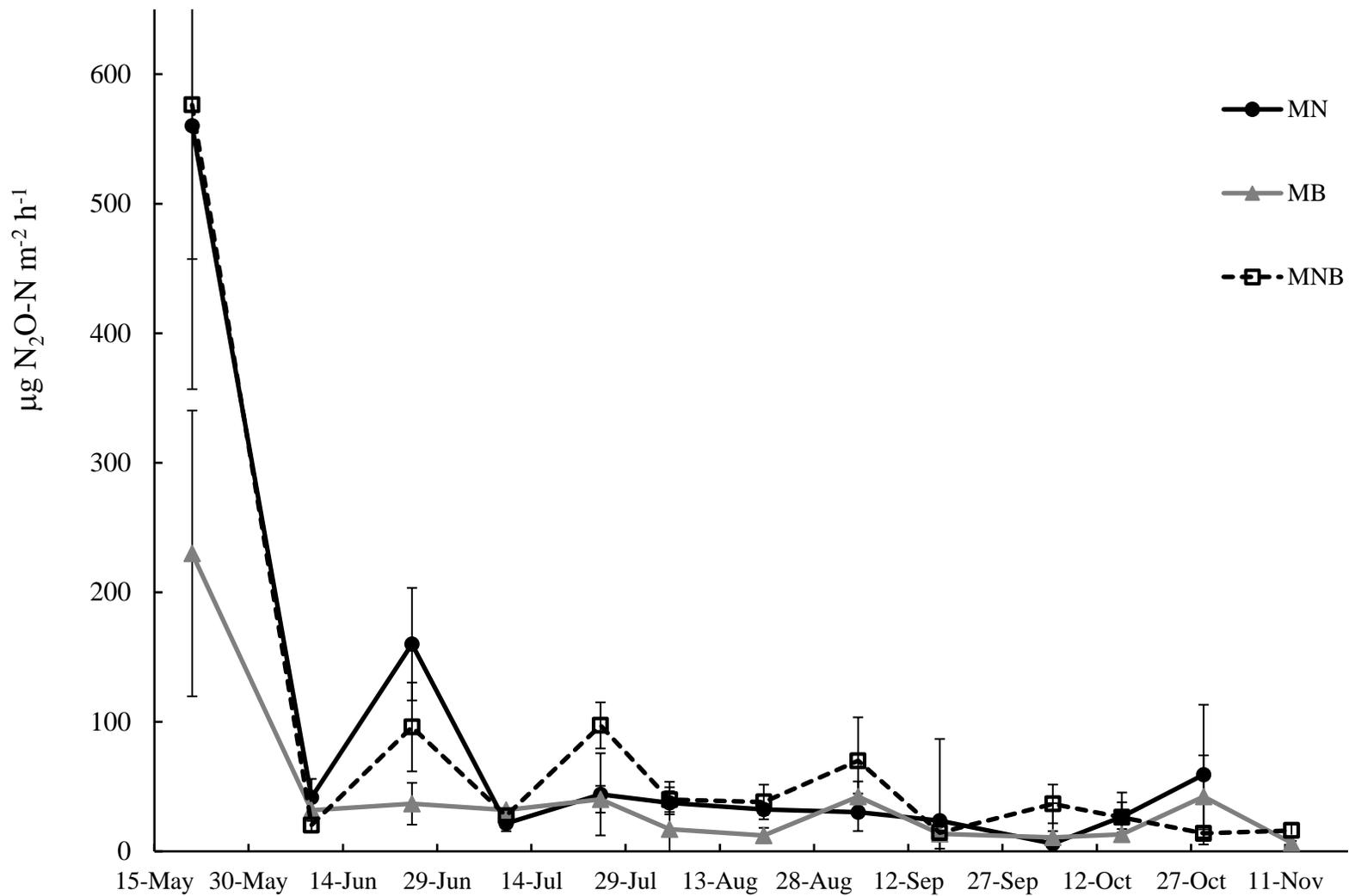
Figure 3.2 Mean (with median corrections) and standard errors of N_2O emissions (μg N_2O -N m^{-2} h^{-1}) from temperate soil amended with three treatment groups: poultry manure and N-fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N-fertilizer and biochar (MNB). H&N Baker Farm, Bayfield, ON, 2018
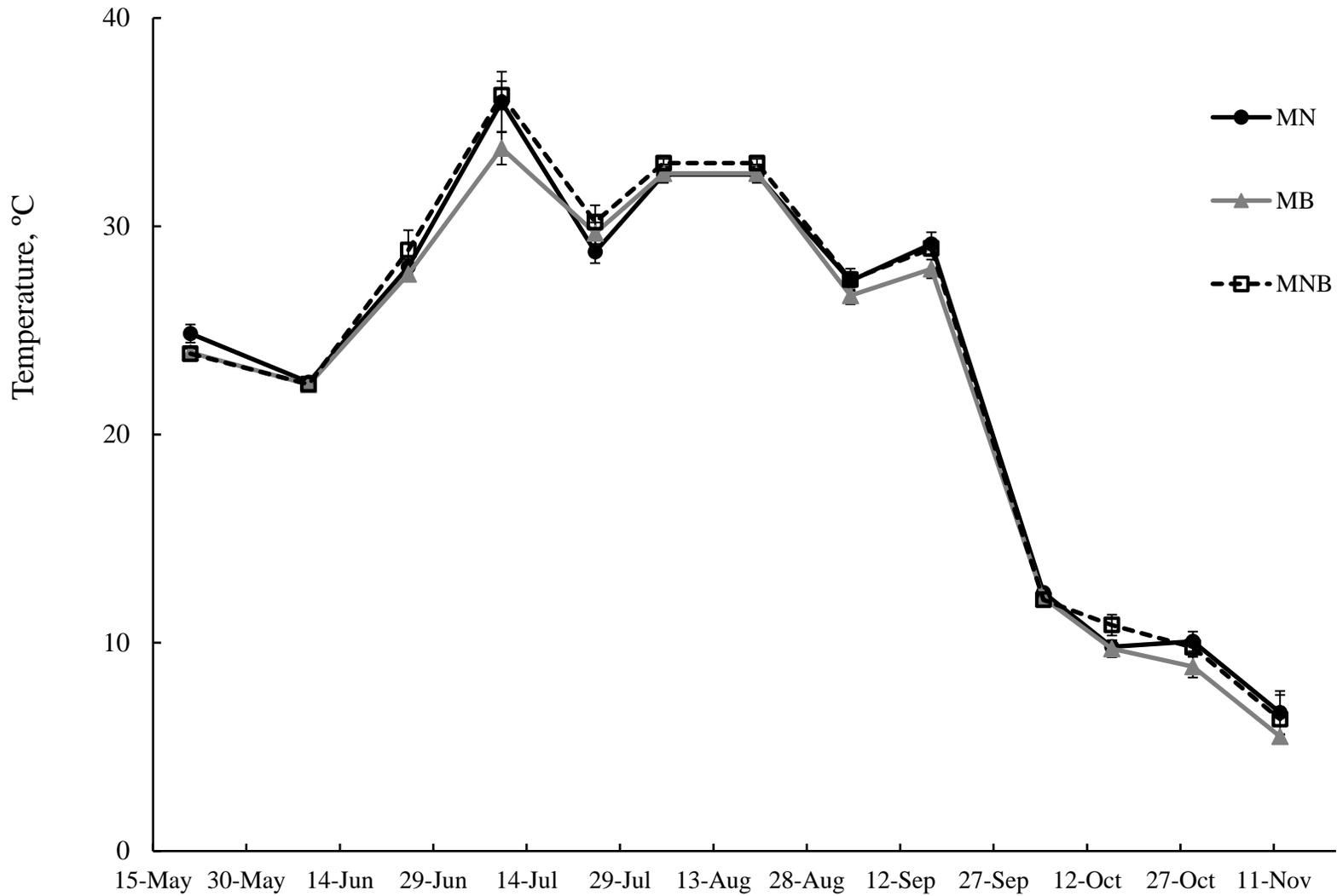
Figure 3.3 Mean (with median corrections) soil temperature (ºC) and standard errors of temperate soil amended with three treatment groups: poultry manure and N-fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N-fertilizer and biochar (MNB). H&N Baker Farm, Bayfield, ON, 2018
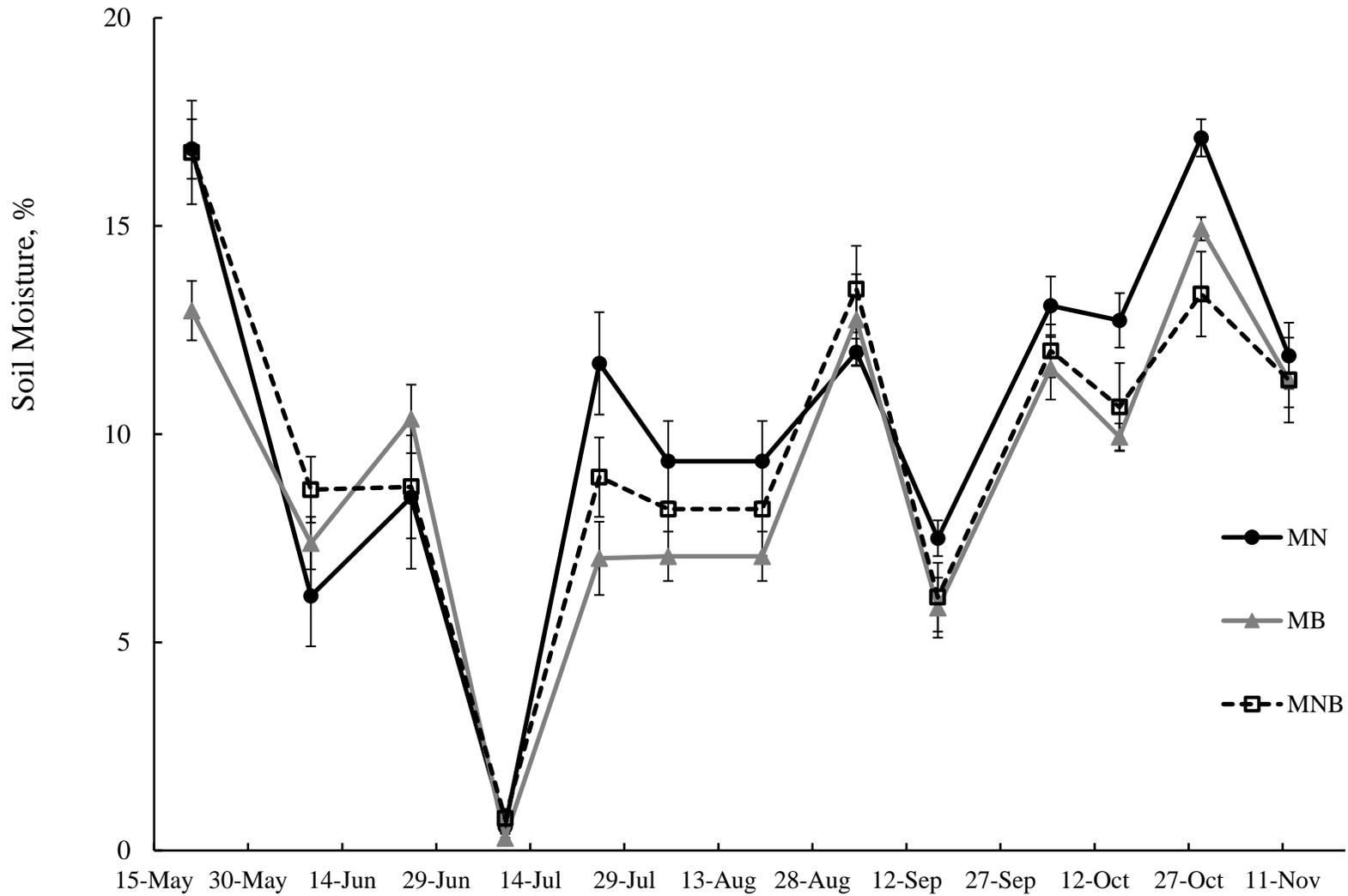
Figure 3.4 Mean (with median corrections) soil moisture (% w/w) and standard errors of temperate amended with three treatment groups: poultry manure and N-fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N-fertilizer and biochar (MNB). H&N Baker Farm, Bay field, ON, 2018
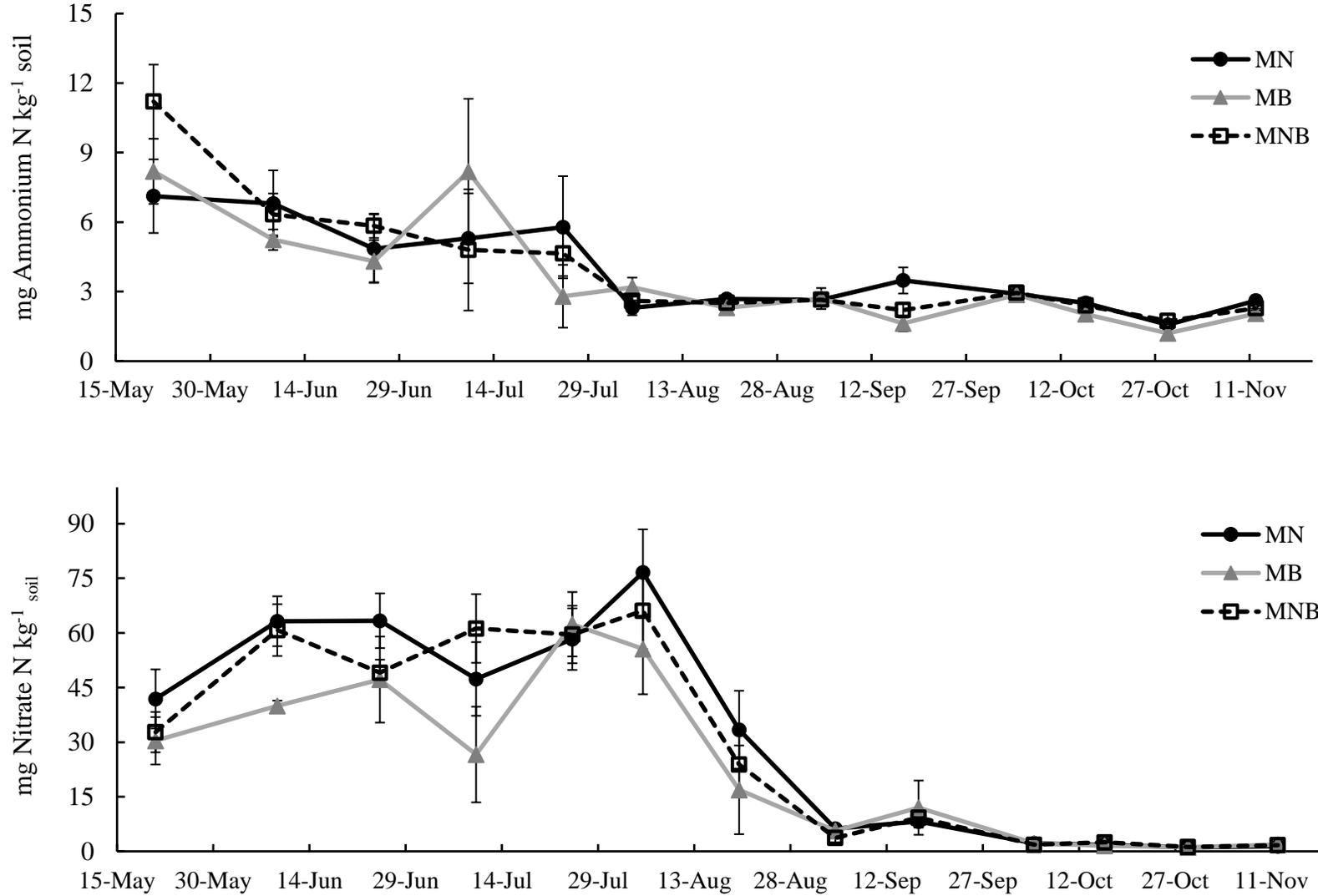
Figure 3.5 Mean (with median corrections) and standard errors of soil ammonium (mg N kg$^{-1}_{soil}$, top) and nitrate (mg N kg$^{-1}_{soil}$, bottom) contents from temperature soil amended with three treatment groups: poultry manure and N-fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N-fertilizer and biochar (MNB). H&N Baker Farm, Bayfield, ON, 2018

3.5 Discussion

Soil CO_2 and N_2O were the highest in the MN treatment indicating that biochar played a part in reducing soil GHG emissions. MNB emitted the least amount of CO_2 even though MNB had the highest SMB-C (but lowest microbial diversity) (Table 2.10), this suggests that the addition of biochar in conjunction with urea-N fertilizer favored specific microbial species, likely those involved in the urea-derived ammonium nitrification processes (Singh et al. 2013). This was also supported by the higher N_2O emission in MNB compared to MB (though not significant) and the statistically similar inorganic N among all treatments even though MN and MNB received doubled amount of nutrient-rich poultry manure and additional urea fertilizer respectively (Tables 3.3 and 3.4). It is likely that urea addition alone was responsible for the increase in soil N_2O emission in MN and MNB since MN produced more N_2O than MNB with double the N fertilizer input. As well, research suggests that biochar reduces soil N_2O emission from urea N fertilizer. For instance, a study on a maize field with calcareous loamy soil in China found significant reductions in N_2O emission with 20 t/ha wheat straw biochar and 300 kg urea-N fertilizer (Zhang et al. 2012). They suspected that the increase in soil C/N ratio, improved soil aeration, and decreased bulk density likely suppressed nitrification activity which was well documented in literature (Zhang et al. 2012; Cavigelli & Robertson 2001; Zwieten et al. 2009).

Findings from this study supporting biochar-induced reduction in soil GHG emissions are in agreement with the literature. For example, a study by Song et al. (2016) found urea and biochar addition, even at the lowest addition rate (0.5% w/w), to a calcareous soil in northern China resulted in significantly decreased soil CO_2 emission even though SOC and TN did not change. Other research also found reduced CO_2 and increased N_2O emissions with biochar (at 1.5% w/w) and urea addition in a loam soil from north Italy (Fiorentino et al. 2019).

Additionally, Fiorentino et al. (2019) found that biochar-urea addition favored urea-derived NH_4^+ in soil and counteracted soil N immobilization from labile organic matter addition. Zwieten et al. (2014) found that when added without urea, biochar induced immobilization of available nutrients for microbes that were involved in the denitrification processes in various soil types from Australia. Aside from biochar, the significantly higher soil CO_2 emission in MN could be explained by the additional 3 t/ha poultry manure added since manure was rich in labile organic C and shown to greatly increase soil water holding capacity (WHC) and C/N ratio which would promote microbial decomposition of SOM (Ould Ahmed et al. 2010; Hadas et al. 1996; Welbaum et al. 2010). The agreement between this study and the literature suggests biochar amendment can offset manure- and urea-N fertilizer derived agricultural greenhouse gases in temperate Canadian agriculture especially when no significant differences in crop yield was observed (cf. Ch 2).

The rates of microbial CO_2 and N_2O production are determined by microbial decomposition and nitrification processes, respectively in the soil, which are directly affected by climatic conditions, namely rainfall and temperature, and agricultural practices such as tilling (Schaufler et al. 2010; Flechard et al. 2007; Gritsch et al. 2015). As expected in the temperate region of Ontario with minimal tillage at the beginning of May, most of the biological activity took place in the spring and early summer as reflected by the decrease in CO_2 and N_2O emissions shortly after May (Tables 3.2 and 3.3) (Mechler et al. 2018; Philippe et al. 2018). Additionally, the study site experienced an atypical lengthy dry period starting in May 2018 as well as an upsurge in air temperature which further explained the GHG emission patterns (Figures 2.1, 3.3, and 3.4). There was a huge spike followed by a rapid drop in N_2O emission within the first two weeks of planting as shown in Figure 3.2. Soil CO_2 followed a similar pattern which suggests

that soil disturbances from amendment application, tilling, and crop seeding had a much greater effect on soil GHG emissions, especially N_2O, than climatic events. Many studies linked significantly and substantially increased soil GHG production, especially CO_2, to physical soil disturbances such as tilling and seeding owing to the increased SOM oxidation in the soil (Reicosky 1997; Scala et al. 2006). Soil N_2O production has often been connected to the use of organic and inorganic nitrogen fertilizers in agricultural soil which could explain the substantially higher initial surge of N_2O emission in MN and MNB than MB in Figure 3.2 (Bouwman 1996; Ding et al. 2013).

Soil NH_4^+-N and soil NO_3^--N did not change in the first three months of the growing period, then declined rapidly in August as shown in Figure 3.5. This again was likely due to the extremely dry and warm months from May to early August 2018, where biological activities were greatly suppressed in the soil (Schaufler et al. 2010; Burri et al. 2018). This period included the annual lowest recorded soil moisture level and highest soil temperature on July 10th, where the top 10 cm of the soil were nearly completely dry (<1% w/w water content) (Figure 3.3 and 3.4). Leaching due to the heavy precipitation events in August, which had the highest monthly total precipitation in the year 2018 by a large margin (Figure 2.1), likely contributed to the sudden drop in soil inorganic N contents along with the enhanced microbial activity (Schaufler et al. 2010; Flechard et al. 2007; Gritsch et al. 2015). Soil moisture (Figure 3.4) did not fully reflect this, since sampling during and few days after rain events were avoided to prevent damage to the gas chromatography apparatus.

Soil biological activities are the primary contributors to soil GHG emissions. Thus, living conditions and available nutrients are often primary keys to dictating how much soil GHGs are emitted (Serrano-Silva et al. 2011, Bond-Lamberty et al. 2016). Table 3.5 lists the Pearson linear

correlation coefficients of determination (r) between the emission of each GHG and every soil physical and chemical variable tested. First, soil moisture was a dominant factor related to variation in emission of both soil GHGs, with statistically significant low to moderate strengths of Pearson r's, which was expected since soil moisture is often the primary factor controlling soil microbial activities (Ould Ahmed et al. 2010; Fu. et al. 2018). Additionally, given the extremely dry months experienced in 2018, it was no surprise that soil moisture was the primary factor in predicting soil GHG emissions in this study. For example, in the previous crop season (2017), Mechler et al. (2018) found that soil moisture was less of a dominating factor and soil temperature was more of a competitive factor at predicting soil GHG emissions. In this study, soil temperature was the second-best predictor for CO_2 emission, but soil NH_4^+-N was the second-best predictor for N_2O emission judging by Pearson's r (Tables 3.5). This was expected as SOM decomposition usually occurs in the topsoil where soil temperature is sensitive to air temperature and soil moisture, and nitrification of ammonium is the rate limiting step in the nitrogen cycle yielding nitrite which is a precursor to N_2O by denitrification (Rivera et al. 2012; Qin et al. 2011). When factoring all predictor variables into a multiple linear regression model, very similar results were observed (Table 3.6) in comparison to Pearson correlations. As the best indicator of available inorganic N nutrients, the decent correlation between soil NH_4^+-N and soil GHG emissions again suggests that soil organic and inorganic nutrient input is key in controlling agricultural GHG production.

3.6 Conclusion

The trends in soil GHG emission data suggested that biochar was able to reduce soil GHG emission even at a low application rate in the temperate region of southern Ontario. This may have been due to nutrient immobilization to reduce microbial decomposition and ammonium nitrification. Poultry manure addition in MN resulted in the significantly higher CO_2 emission due to improved WHC and labile organic and inorganic nutrient contents. Though not statistically significant, the presence of nitrogen fertilizer in MNB led to urea-induced elevation in soil N_2O emission potentially due to the promotion of urea-derived ammonium nitrification. The seasonal factor was dominant in soil GHG emission likely due to the extreme dry months in 2018. Aside from soil moisture, soil temperature was the second-best predictor for soil CO_2 emission while soil NH_4^+-N was the second-best predictor for soil N_2O emission likely due to intrinsic differences in soil characteristics by depth. Findings from this study solidifies that biochar can be implemented as a long-term soil amendment to reduce soil GHG emissions where increasing addition rates of biochar could enhance the reduction but at an impractical cost.

Chapter 4: The effect of biochar as soil amendment on climate change resilience

4.1 Abstract

Climate change due to elevated atmospheric greenhouse gas concentrations is projected to continue to warm the globe for the next century. Sustainable agroecosystems management practices, including the use of biochar as a soil amendment, may help mitigate effects of climate change. The objective of this study was to investigate how biochar amended soil responds to elevated CO_2 and temperature that southern Ontario is predicted to experience in 2050 based on IPCC projections (average 4°C increase in atmospheric temperature and 250 ppm increase in atmospheric CO_2 concentration). The study consisted of three triplicated treatments: 6 t/ha poultry manure and 135 kg/ha urea-N fertilizer (MN), 3 t/ha poultry manure and 3 t/ha biochar (MB), and 3 t/ha poultry manure, 135 kg/ha urea-N fertilizer, and 3 t/ha biochar (MNB). Each treatment replicate was growing soybean (*Glycine max* Merr. L.) under four climate conditions: ambient (AMB), elevated temperature (TEMP), enriched CO_2 concentration (fCO_2), and elevated temperature plus CO_2 fertilization (fCO_2×TEMP) over a 90-day period with light intensity, humidity, and soil moisture held constant. Results showed no statistical interaction between treatment and climate condition fixed factors (except for microbial species richness). MNB was 0.2% and 0.16% lower in soil organic carbon content than MN and MB respectively, MNB contained 0.024% lower total nitrogen than MN, MNB contained the least amount of SMB-C (480 ug C/g $_{soil}$), and lowest crop yield (9.1 g/plant) suggesting conflicting urea-biochar interactions. Soybean yield was the highest under fCO_2×TEMP (13.4 g/plant), and second highest under TEMP (11.2g/plant). However, undesired drooping of beanstalk was observed under warming conditions. Results showed that biochar behaved independently of induced climate effects and was not able to offset the effects of fCO_2 and warming.

4.2 Introduction

Climate change is often characterized by distinct global temperature increases as a result of rapidly increasing atmospheric greenhouse gas (GHG) emissions (IPCC 2007). This climatic shift is unequivocally more than random terrestrial temperature fluctuations in the solar system considering the available historical weather data for over at least 800,000 years (Solomon et al. IPCC 2007, FCO 2016). The scientific definition of climate change has evolved since first proposed in 1966 by the World Meteorological Organization (WMO). Currently, the term "climate change" evokes an observable long-term (over decades to millennia) change to the current climatic pattern often due to human activities (Hulme 2016).

The impact of climate change on agriculture is not well understood as climate change can impact meteorological, hydrological, and physiological aspects of the agroecosystems on a global scale and studying this enormously complex system is one of the greatest challenges to scientists today (Gornall et al. 2010). Climate has always strongly influenced agricultural success; farmers have adapted to the weather conditions of their region in order to maximize yield (Gornall et al. 2010). Now that climate change is in effect, farmers are faced with more complications to maintain necessary environmental conditions for conventional crop growing (Niles et al. 2016).

One of the most detrimental components of climate change is the warming of average global temperature, which has already surpassed the initial projection by Intergovernmental Panel on Climate Change (IPCC 2007). This increase in average global temperature is as a sum of extreme climatic fluctuations in temperate regions and prolonged periods of heat waves in both temperate and tropical regions of the world (Rosenzweig et al. 2001; Schär et al. 2004). The overall warming of the globe severely impacts the hydrological cycle in many of these regions

(Rosenzweig et al. 2001). Specifically, the frequency and intensity of droughts and floods are expected to increase as the warming effect worsens (Rosenzweig et al. 2001). Few crops can tolerate these prominent warming effects, and yields diminish (Liu et al. 2013). This phenomenon is often associated with wilting of crops and pollen infertility (Young et al. 2014; Zinn et al. 2010). Additionally, research suggests that the effect of heat stress on the physiological development of plants is independent of any other abiotic stresses. Instead, the susceptibility to heat stress significantly varies between developmental stages of a given plant species (Barnabás et al. 2008; Sakata & Higashitani 2008). Therefore, mitigation strategies against the warming aspect of climate change in agriculture is key to food security.

It has been suggested that global food production will have to increase by approximately 70% to meet the demand of an expected global population of around 9 billion by 2050 (FAO 2009). The threat of climate change, and many of its predicted and observed threats to agriculture, are major obstacles to achieve the goal of sufficient global food production. To tackle the source of the problem, increasing global atmospheric GHG concentrations, scientists and policy makers have determined that agricultural practices and forestry have the potential to be the most cost-effective sector to combat climate change (Smith & Olesen 2010; Conant 2011). Managed soil can be a long-term carbon sink that not only contributes to GHG abatement, but could also be utilized for biomass feedstock production in the generation of biofuel which produces biochar as a by-product that in turn can be returned to the soil to further enhance carbon sequestration (Smith & Olesen 2010). Existing agricultural adaptations for climate change aim to achieve a few key points, such as reducing soil erosion and nutrient leaching. These practices influence the carbon and nitrogen cycles in the direction of overall reduced CO_2, CH_4, and N_2O emissions and boosted agronomical yields (Christiensen et al. 2007; Olesen et al.

2004). Improvement in soil moisture is another crucial goal since agricultural yield directly depends on maintaining a healthy level of soil moisture under a changing climate, while in turn soil moisture also positively influences carbon storage and promote N_2O emission when N is added in excess to crop needs (Smith & Olesen 2010). Therefore, it is important to invest in appropriate soil management, including soil moisture and temperature, as it directly protects the agroecosystem against extreme weather conditions. This is because sudden shifts in regional climatic patterns directly challenge global food security as local agricultural producers often struggle to adapt to and mitigate the various effects of climate change to farmlands (Thornton et al. 2014).

A common black carbon by-product in the biofuel production process, can often be engineered to amend soil for agricultural purposes, this is often referred to as biochar (Spokas et al. 2012). The anoxic production of biochar via pyrolysis is a bio-energy conversion technique where solid, liquid, and gaseous fuel products are generated from various sources of biomass feedstock. Biofuel production is considered overall carbon-negative where the net amount of carbon dioxide (CO_2) generated and released into the environment is less than what has been sequestered during the process (Lee et al. 2018; Lee 2010). In addition to biochar being a valuable soil amendment (cf. Chapter 2), recent studies have reported a potential for biochar in agriculture to function as a climate change mitigation strategy against environmental factors that contribute to and result from climate change (Woolf et al. 2010; Brassard et al. 2016). Such factors include increased soil water content and abatement of agricultural GHG emissions to reduce the effect of climate change which often elevates soil temperature and decreases soil moisture (Mukherjee & Lal 2013; Parkin et al. 2012; Brassard et al. 2016). Biochar directly impacts the soil chemistry and biology with typical decreases in soil biological activity and

therefore decreases in soil organic matter accumulation and crop yield (Liang et al. 2014). Biochar's large porosity and reaction surface, as a result of its highly substituted aromatic chemical structure, allow it to improve water holding capacity (WHC) (Atkinson et al. 2010). Biochar can also alter soil water content, which can ameliorate the warming and drying of soil due to climate change (Paetsch et al. 2018). Paetsch et al. (2018) also found that aging of biochar further improved soil hydraulic conditions for protection against drought.

The goal of this study is to investigate climate change resilience of biochar amended soil under laboratory settings where the same treatment groups from the previous chapters are subjected to warming and CO_2 fertilization climate conditions. Since the soil samples in this study were obtained directly from the field study plots (c.f. Chapter 2), the low but economically feasible rate of biochar addition is also a factor in this study. This will again likely produce minor but realistic results compared to the climate condition factor due to economic feasibility of quality biochar (Herath et al. 2013). However, since the atmospheric conditions are under constant monitoring using laboratory-grade growth chambers, the spatial bias observed in-field (c.f. Chapter 2) should be much less prominent.

4.3 Methodology

4.3.1 Experimental Design

All soil samples used were collected in October 2017 from the same study site as mentioned in previous chapters. Soil was collected by bulking soil samples from three randomly selected points per treatment replicate (MN, MB, and MNB, n = 3) to a 20 cm depth. Each treatment replicate was subjected to four climate conditions totaling to 36 unique sample groups. The four climate conditions include ambient temperature and CO_2 concentration (AMB),

elevated temperature and ambient CO_2 concentration (TEMP), ambient temperature and enriched CO_2 concentration (fCO_2), and elevated temperature plus CO_2 fertilization (f$CO_2\times$TEMP). Ambient conditions were based on historical weather data in the recent decade from Environment Canada. Climate conditions that had warming in effect were 4 °C warmer than the ambient temperature at all times and climate conditions that had CO_2 fertilization in effect were 250 ppm higher in atmospheric CO_2 concentration from the ambient CO_2 level at all times (Table 4.1). Other environmental parameters such as light intensity, humidity, and soil water content were kept constant among all climate conditions (Tables 4.2). Only two environmental chambers (Conviron PGR-15, Controlled Environments Inc., Winnipeg, MB) were available at the time of the study. Each growth chamber was used twice for the total of 4 climate conditions tested resulting in a pseudo-replicated split plot experimental design. External instruments were used to calibrate and offset inherent variations between the two growth chambers such as light intensity, CO_2 concentration, humidity, temperature, and soil moisture. Approximately 5 kg of soil for each climate condition and treatment replicate were potted and seeded with four soybean seed and placed with even spacing inside each growth chamber. All nine pots (three treatments and three replicates) inside each growth chamber were systematically rotated weekly to account for any potential spatial variations inside each chamber (see Figure 4.1). All soybean seeds were removed, except for the first to germinate. Every climate condition was allowed a 90-day growth period, soil and crop samples were harvested on the 90th day of each growth period for analyses.

Figure 4.1 Experimental setup inside each Conviron PGR-15 environmental chamber.

Table 4.1 Conviron PGR-15 environmental chamber climate conditions according to IPCC projections in 2050 (IPCC 2014), over 90 days with 3 replicates of 3 treatments (MN, MB, MNB). Soybean was cultivated in each pot containing soil samples collected in Bayfield, ON. Ambient conditions adapted from Environment Canada.

Climate \ Treatment	Manure + N fertilizer MN	Manure + biochar MB	Manure + N fertilizer + Biochar MNB
Ambient, AMB	CO_2 = 400 ppm Day temperature = 25 °C Night temperature = 15 °C	CO_2 = 400 ppm Day temperature = 25 °C Night temperature = 15 °C	CO_2 = 400 ppm Day temperature = 25 °C Night temperature = 15 °C
Warming, TEMP	CO_2 = 400 ppm Day temperature = **29 °C** Night temperature = **19 °C**	CO_2 = 400 ppm Day temperature = **29 °C** Night temperature = **19 °C**	CO_2 = 400 ppm Day temperature = **29 °C** Night temperature = **19 °C**
CO_2 fertilization, fCO_2	CO_2 = **650 ppm** Day temperature = 25 °C Night temperature = 15 °C	CO_2 = **650 ppm** Day temperature = 25 °C Night temperature = 15 °C	CO_2 = **650 ppm** Day temperature = 25 °C Night temperature = 15 °C
Combined, fCO_2×TEMP	CO_2 = **400 ppm** Day temperature = **29 °C** Night temperature = **15 °C**	CO_2 = **400 ppm** Day temperature = **29 °C** Night temperature = **15 °C**	CO_2 = **400 ppm** Day temperature = **29 °C** Night temperature = **15 °C**

Day time was assumed to be from 7:00 to 19:00, night-time was assumed to be from 21:00 to 5:00. A middle temperature value in between day and night times for 2 hours was employed to avoid rapid heating/cooling inside the environment chambers.

Table 4.2 Conviron PGR-15 environmental chamber fixed climate conditions using average climate data adapted from Goderich weather station in Goderich, ON (Environment Canada). Soil water content arbitrarily kept at 50% field capacity.

Variable \ Time	5:00 – 7:00	7:00 – 19:00	19:00 – 21:00	21:00 – 5:00
Light (µmol m^{-2} s^{-1}*)	300	450	300	0
Humidity, (%*)	70	60	70	80
Soil Water %, (%WHC)**	< 50	< 50	< 50	50

* Lighting and humidity schemes are identical among all levels of the climate conditions.

** Soil water content was held at 50% maximum water holding capacity, all samples were watered in the evening every other day.

4.3.2 Soil Analyses

Soil organic carbon (SOC),C/N ratio, hot-water-extractable carbon (HWC), total nitrogen (TN), ammonium (NH_4^+-N), nitrate (NO_3^--N), ortho-phosphate (PO_4^{3-}-P), were analyzed employing the same methodology as outlined in Chapter 2 (c.f. *2.3.4 Soil Chemistry).*

Soil microbial biomass carbon (SMB-C), microbial biomass nitrogen (SMB-N), microbial C/N ratio, microbial community structural analyses, including average well colour development (AWCD), richness (R), and Shannon Diversity index (Hs) were analyzed employing the same methodology as outlined in Chapter 2 (c.f. *2.3.5 Soil Biology and Crop Productivity).*

4.3.3 Plant Analyses

All plant sample harvests and measurements took place on day 90. Final plant height (cm) was measured and recorded (stems were straightened if droopy during measurement). Soybean pods from each climate condition and treatment replicate were harvested, dried, and weighed (g/plant). Soybean shoots were severed from the roots at 1 cm above the soil's surface. The shoots were then dried and weighed as above-ground biomass (g/plant). The remaining biomass was cleaned of soil (by washing), dried, and weighed as below-ground biomass (g/plant). Soybean shoot C/N ratio was determined, and the drying processes of all plant samples were performed employing the same methodology as outlined in Chapter 2 (c.f. section *2.3.5 Soil Biology and Crop Productivity).*

4.3.4 Statistical Analyses

All statistical analyses were performed computationally on IBM SPSS™ for Windows, Version 25. All tests were conducted with an overall alpha level of 0.05 including two-factor

within-group pair-wise mean contrast procedures when an interaction term was significant ($p < 0.05$). Two-way analysis of variance (ANOVA) was performed for most test results with treatment and climate condition as fixed factors (depth was not a factor in this project). Tukey's *post hoc* pair-wise t-tests were then performed for factors or interaction terms that had significant effects on tested variables. Shapiro Wilk's test was performed ($n<2000$) to check for normality of data. Mean values were still strictly used for statistical analyses for consistency.

4.4 Results

4.4.1 Chemical Soil Health Characteristics

Treatment and climate condition did not have a significant interactive effect on any soil chemical characteristics (Tables 4.3 and 4.5). Soil organic carbon (SOC) significantly differed with treatment ($p = 0.003$) where MNB contained the least amount of SOC %, w/w compared to MN and MB treatments. Soil C/N ratio significantly differed among treatment (< 0.001) and climate condition ($p = 0.034$) factors. Soil C/N was significantly greater in the MB than in the MN and MNB treatments. Soil C/N ratio was also greater under the fCO_2 climate condition than the TEMP climate condition, whereas AMB and $fCO_2 \times$TEMP climate conditions were intermediate to but not statistically different from either fCO_2 or TEMP climate conditions. Hot-water extractable organic carbon (HWC) only significantly differed by climate conditions ($p = 0.036$). For example, TEMP climate condition had the highest mg HWC / kg_{soil} while fCO_2 climate condition had the lowest, AMB and $fCO_2 \times$TEMP were intermediate to TEMP and fCO_2 climate conditions but not significantly different from TEMP or fCO_2 climate conditions (Tables 4.3 and 4.4).

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Total N (%, w/w) differed significantly by treatment ($p < 0.001$) but not climate conditions

where MN had the highest TN content and MNB had the lowest, whereas MB was not

statistically different from either MN or MNB (Tables 4.5 and 4.6). Soil NH_4^+-N (mg N/kg) only

differed significantly among climate conditions ($p < 0.001$) where AMB and TEMP contained

significantly higher NH_4^+-N content than fCO_2 and $fCO_2 \times TEMP$. Soil NO_3^--N significantly

differed by both treatment ($p = 0.023$) and climate condition ($p < 0.001$) factors. MB had the

highest soil NO_3^--N content, MNB had the lowest while MN was intermediate and not

significantly different from either MB or MNB. Among climate conditions, TEMP had

significantly higher soil NO_3^--N content than the other three climate conditions which were

statistically similar themselves. Soil PO_4^{3-}-P content varied significantly among climate

conditions ($p < 0.001$) where fCO_2 and $fCO_2 \times TEMP$ climate conditions contained significantly

higher PO_4^{3-} than AMB and TEMP climate conditions (Tables 4.5 and 4.6).

Table 4.3 Two-way analyses of variance on soil carbon characteristics among treatments (MN, MB, MNB) and climate conditions (AMB, TEMP, $fCO_2 \times TEMP$) of a soil collected in Bayfield, ON.

Fixed Factors	Soil Organic Carbon	Soil C/N Ratio	Hot-water-extractable Organic Carbon
	F (p > F)	F (p > F)	F (p > F)
Treatment (Trt)	**7.555 (0.003)**	**16.638 (<0.001)**	0.719 (0.497)
Climate	0.438 (0.728)	**3.415 (0.034)**	**3.333 (0.036)**
Trt × Climate	0.108 (0.995)	0.909 (0.505)	1.233 (0.325)

Significant terms are in bold ($\alpha = 0.05$).

Table 4.4 Mean values, their associated standard error, and pair-wise Tukey comparison on soil carbon characteristics among treatments (MN, MB, MNB) and climate conditions (AMB, TEMP, $fCO_2\times$TEMP) of a soil collected in Bayfield, ON.

	Climate Condition	MN	MB	MNB	Climate Overall
		$\overline{x}$ ($\sigma_{\overline{x}}$)	$\overline{x}$ ($\sigma_{\overline{x}}$)	$\overline{x}$ ($\sigma_{\overline{x}}$)	$\overline{x}$ ($\sigma_{\overline{x}}$)
Soil Organic Carbon (% w/w dry)	**AMB**	$1.18\ (0.12)^{Aa}$	$1.18\ (0.11)^{Aa}$	$1.01\ (0.06)^{Ba}$	$1.12\ (0.06)^{a}$
	TEMP	$1.21\ (0.02)^{Aa}$	$1.13\ (0.05)^{Aa}$	$0.98\ (0.07)^{Ba}$	$1.10\ (0.04)^{a}$
	fCO₂	$1.19\ (0.09)^{Aa}$	$1.10\ (0.11)^{Aa}$	$0.95\ (0.04)^{Ba}$	$1.08\ (0.06)^{a}$
	fCO₂×TEMP	$1.12\ (0.08)^{Aa}$	$1.11\ (0.06)^{Aa}$	$0.94\ (0.07)^{Ba}$	$1.06\ (0.05)^{a}$
	Treatment Overall	$\mathbf{1.17\ (0.04)^{A}}$	$\mathbf{1.13\ (0.04)^{A}}$	$\mathbf{0.97\ (0.03)^{B}}$	
Soil C/N Ratio (w/w)	**AMB**	$8.97\ (0.06)^{Aab}$	$9.83\ (0.241)^{Bab}$	$9.43\ (0.27)^{Aab}$	$\mathbf{9.41\ (0.16)^{ab}}$
	TEMP	$8.92\ (0.10)^{Aa}$	$9.68\ (0.288)^{Ba}$	$9.16\ (0.24)^{Aa}$	$\mathbf{9.25\ (0.16)^{a}}$
	fCO₂	$9.57\ (0.07)^{Ab}$	$10.09\ (0.093)^{Bb}$	$9.39\ (0.13)^{Ab}$	$\mathbf{9.69\ (0.12)^{b}}$
	fCO₂×TEMP	$9.40\ (0.12)^{Aab}$	$9.81\ (0.078)^{Bab}$	$9.27\ (0.08)^{Aab}$	$\mathbf{9.49\ (0.09)^{ab}}$
	Treatment Overall	$\mathbf{9.21\ (0.09)^{A}}$	$\mathbf{9.85\ (0.08)^{B}}$	$\mathbf{9.31\ (0.09)^{A}}$	
How-water-extractable Organic Carbon (mg C/kg soil)	**AMB**	$280\ (79)^{Aab}$	$370\ (58)^{Aab}$	$300\ (54)^{Aab}$	$\mathbf{320\ (35)^{ab}}$
	TEMP	$310\ (55)^{Ab}$	$350\ (23)^{Ab}$	$380\ (47)^{Ab}$	$\mathbf{340\ (24)^{b}}$
	fCO₂	$240\ (32)^{Aa}$	$260\ (21)^{Aa}$	$170\ (49)^{Aa}$	$\mathbf{220\ (23)^{a}}$
	fCO₂×TEMP	$380\ (36)^{Aab}$	$280\ (17)^{Aab}$	$250\ (77)^{Aab}$	$\mathbf{300\ (32)^{ab}}$
	Treatment Overall	$300\ (28)^{A}$	$310\ (20)^{A}$	$270\ (34)^{A}$	

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).

[a] Values followed by the same lower-case letter denote sample means that are statistically similar among climate conditions ($\alpha = 0.05$).

Table 4.5 Two-way analyses of variance on soil nitrogen and phosphorus characteristics among treatments (MN, MB, MNB) and climate conditions (AMB, TEMP, fCO$_2$×TEMP) of a soil collected in Bayfield, ON.

Fixed Factors	Total Nitrogen	Ammonium	Nitrate	Ortho-phosphate
	F (p > F)	F (p > F)	F (p > F)	F (p > F)
Treatment (Trt)	**9.603 (0.001)**	1.187 (0.322)	**4.410 (0.023)**	2.457 (0.107)
Climate	1.198 (0.332)	**152.207 (<0.001)**	**11.331 (<0.001)**	**75.765 (<0.001)**
Trt × Climate	0.173 (0.982)	1.387 (0.260)	1.613 (0.187)	0.735 (0.626)

Significant terms are in bold (α = 0.05).

Table 4.6 Mean, standard error of the mean, and pair-wise Tukey comparison on soil inorganic chemical characteristics among treatments (MN, MB, MNB) and climate conditions (AMB, TEMP, fCO$_2$×TEMP) of a soil collected in Bayfield, ON.

	Climate Condition	MN	MB	MNB	Climate Overall
		$\overline{x}$ ($\sigma_{\overline{x}}$)	$\overline{x}$ ($\sigma_{\overline{x}}$)	$\overline{x}$ ($\sigma_{\overline{x}}$)	$\overline{x}$ ($\sigma_{\overline{x}}$)
Total Nitrogen	**AMB**	0.131 (0.013)^{Aa}	0.120 (0.009)^{ABa}	0.107 (0.004)^{Ba}	0.120 (0.006)^{a}
(%w/w dry)	**TEMP**	0.136 (0.004)^{Aa}	0.117 (0.008)^{ABa}	0.106 (0.005)^{Ba}	0.120 (0.005)^{a}
	fCO$_2$	0.124 (0.009)^{Aa}	0.109 (0.011)^{ABa}	0.101 (0.003)^{Ba}	0.111 (0.005)^{a}
	fCO$_2$×TEMP	0.119 (0.007)^{Aa}	0.113 (0.005)^{ABa}	0.102 (0.007)^{Ba}	0.111 (0.004)^{a}
	Treatment Overall	**0.128 (0.004)^{A}**	**0.115 (0.004)^{AB}**	**0.104 (0.002)^{B}**	
Ammonium, NH$_4$$^+$	**AMB**	11.3 (0.4)^{Aa}	13.1 (0.9)^{Aa}	11.8 (0.5)^{Aa}	**12.1 (0.4)^{a}**
(mg N/kg $_{soil}$)	**TEMP**	12.4 (0.7)^{Aa}	12.6 (1.1)^{Aa}	12.2 (0.4)^{Aa}	**12.4 (0.4)^{a}**
	fCO$_2$	5.7 (0.6)^{Ab}	4.4 (0.6)^{Ab}	4.9 (0.2)^{Ab}	**5.0 (0.3)^{b}**
	fCO$_2$×TEMP	5.3 (0.5)^{Ab}	5.1 (0.4)^{Ab}	3.9 (0.3)^{Ab}	**4.8 (0.3)^{b}**
	Treatment Overall	8.7 (1.0)^{A}	8.8 (1.3)^{A}	8.2 (1.2)^{A}	
Nitrate, NO$_3$$^-$	**AMB**	3.0 (0.4)^{ABa}	3.1 (0.5)^{Aa}	2.5 (0.5)^{Ba}	**2.9 (0.2)^{a}**
(mg N/kg $_{soil}$)	**TEMP**	4.4 (0.5)^{ABb}	5.5 (1.0)^{Ab}	3.1 (0.6)^{Bb}	**4.3 (0.5)^{b}**
	fCO$_2$	2.4 (0.2)^{ABa}	2.8 (0.2)^{Aa}	1.6 (0.1)^{Ba}	**2.3 (0.2)^{a}**
	fCO$_2$×TEMP	2.4 (0.4)^{ABa}	2.6 (0.2)^{Aa}	2.9 (0.2)^{Ba}	**2.7 (0.2)^{a}**
	Treatment Overall	**3.0 (0.3)^{AB}**	**3.5 (0.4)^{A}**	**2.5 (0.2)^{B}**	
Ortho-phosphate,	**AMB**	69 (2)^{Aa}	67 (2)^{Aa}	69 (1)^{Aa}	**68 (1)^{a}**
PO$_4$$^{3-}$	**TEMP**	70 (1)^{Aa}	68 (1)^{Aa}	72 (1)^{Aa}	**70 (1)^{a}**
(mg P/kg $_{soil}$)	**fCO$_2$**	106 (3)^{Ab}	96 (7)^{Ab}	94 (5)^{Ab}	**99 (3)^{b}**
	fCO$_2$×TEMP	103 (5)^{Ab}	96 (4)^{Ab}	99 (4)^{Ab}	**99 (3)^{b}**
	Treatment Overall	87 (5)^{A}	82 (5)^{A}	83 (4)^{A}	

^{A} Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).

^{a} Values followed by the same lower-case letter denote sample means that are statistically similar among climate conditions ($\alpha = 0.05$).

4.4.2 Biological Soil Health Characteristics

Soil microbial biomass carbon (SMB-C) only differed significantly among treatment (p = 0.001) where MNB had significantly lower SMB-C than MN and MB treatments. Though not significant (p = 0.280), SMB nitrogen (SMB-N) and the C/N ratio of the soil microbial biomass (SMB-C/N) were also the lowest in MNB followed by MN and MB treatments (Tables 4.7 and 4.8a). Average well colour development (AWCD) only differed among climate conditions (p = 0.014) where AMB was significantly higher than the other climate conditions except for the $fCO_2 \times TEMP$ climate condition (Tables 4.7 and 4.8a). Treatment and climate condition factors had an interactive effect for microbial richness (F = 4.289, p = 0.004). Microbial richness was not significantly different in the MN treatment for all climate conditions. Microbial richness was greater in the AMB and fCO_2 climate conditions compared to TEMP; and $fCO_2 \times TEMP$ was intermediate and not statistically different within the MB treatment. Microbial richness was the greatest in the AMB climate condition followed by $fCO_2 \times TEMP$, TEMP, and fCO_2 in the MNB treatment. However, $fCO_2 \times TEMP$ was not statistically lower than AMB nor statistically higher than TEMP, fCO_2 was only statistically lower than AMB and $fCO_2 \times TEMP$. Microbial richness did not vary significantly among the treatment factor under $fCO_2 \times TEMP$ climate condition. MNB had a greater microbial richness than MN, and MB was intermediate and not significantly different from either MN or MNB in the AMB climate condition. MN had significantly greater microbial richness than MB, and MNB was intermediate and statistically similar to both MN and MB in the TEMP climate condition. MB had significantly greater microbial richness count than MNB where MN was intermediate and not statistically different from MB or MNB treatments in the fCO_2 climate condition (Tables 4.7 and 4.8b). Contrasts among treatment groups for Shannon

diversity index (Hs) was significant (p = 0.001), but not among climate conditions, where MN

had higher Hs than MB and MNB treatments (Table 4.7 and 4.8b).

Table 4.7 Two-way analyses of variance on soil microbial characteristics among treatments (MN, MB, MNB) and climates (AMB, TEMP, fCO$_2$×TEMP) of a soil collected in Bayfield, ON.

Fixed Factors	Soil Microbial Carbon Biomass	Soil Microbial Nitrogen Biomass	Soil Microbial C/N Ratio	Average Well Colour Development	Richness	Shannon Diversity, Hs
	F (p > F)	F (p > F)	F (p > F)	F (p > F)	F (p > F)	F (p > F)
Treatment (Trt)	**9.094 (0.001)**	1.341 (0.280)	0.625 (0.544)	1.059 (0.362)	1.663 (0.211)	**9.828 (0.001)**
Climate	1.999 (0.141)	0.711 (0.555)	0.383 (0.766)	**4.364 (0.014)**	**5.565 (0.005)**	0.580 (0.634)
Trt × Climate	1.727 (0.158)	0.822 (0.564)	0.485 (0.813)	1.783 (0.145)	**4.289 (0.004)**	0.540 (0.772)

Significant terms are in bold (α = 0.05).

Table 4.8a Mean, standard error of the mean, and pair-wise Tukey comparison on soil microbial characteristics among treatments (MN, MB, MNB) and climate conditions (AMB, TEMP, fCO$_2$×TEMP) of a soil collected in Bayfield, ON.

	Climate Condition	MN	MB	MNB	Climate Overall
		$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$
Soil Microbial	**AMB**	580 (89)^{Aa}	940 (155)^{Aa}	460 (30)^{Ba}	660 (89)^a
Carbon Biomass	**TEMP**	570 (94)^{Aa}	670 (120)^{Aa}	340 (78)^{Ba}	530 (69)^a
(µg C/g soil**)**	**fCO$_2$**	700 (51)^{Aa}	660 (95)^{Aa}	470 (3)^{Ba}	610 (47)^a
	fCO$_2$×TEMP	740 (120)^{Aa}	680 (14)^{Aa}	640 (48)^{Ba}	690 (40)^a
	Treatment Overall	**650 (45)^A**	**740 (58)^A**	**480 (38)^B**	
Soil Microbial	**AMB**	93 (14)^{Aa}	137 (9)^{Aa}	75 (18)^{Aa}	102 (12)^a
Nitrogen Biomass	**TEMP**	107 (19)^{Aa}	113 (36)^{Aa}	63 (17)^{Aa}	94 (15)^a
(µg N/g soil**)**	**fCO$_2$**	125 (9)^{Aa}	99 (32)^{Aa}	111 (43)^{Aa}	112 (16)^a
	fCO$_2$×TEMP	113 (23)^{Aa}	124 (9)^{Aa}	121 (4)^{Aa}	120 (8)^a
	Treatment Overall	110 (8)^A	118 (12)^A	92 (13)^A	
Soil Microbial	**AMB**	6.3 (0.6)^{Aa}	6.8 (1.1)^{Aa}	6.7 (1.4)^{Aa}	6.6 (0.5)^a
C/N Ratio	**TEMP**	5.5 (0.8)^{Aa}	6.5 (0.9)^{Aa}	5.8 (1.5)^{Aa}	5.9 (0.6)^a
(w/w)	**fCO$_2$**	5.6 (0.1)^{Aa}	7.4 (1.2)^{Aa}	5.5 (1.5)^{Aa}	6.2 (0.6)^a
	fCO$_2$×TEMP	6.6 (0.3)^{Aa}	5.6 (0.4)^{Aa}	5.3 (0.5)^{Aa}	5.8 (0.3)^a
	Treatment Overall	6.0 (0.3)^A	6.6 (0.4)^A	5.8 (0.6)^A	
Average Well	**AMB**	0.14 (0.03)^{Aa}	0.14 (0.04)^{Aa}	0.28 (0.09)^{Aa}	**0.19 (0.037)^a**
Colour	**TEMP**	0.16 (0.04)^{Ab}	0.07 (0.02)^{Ab}	0.07 (0.01)^{Ab}	**0.10 (0.020)^b**
Development,	**fCO$_2$**	0.08 (0.03)^{Ab}	0.08 (0.05)^{Ab}	0.10 (0.01)^{Ab}	**0.09 (0.016)^b**
AWCD	**fCO$_2$×TEMP**	0.12 (0.02)^{Aab}	0.10 (0.03)^{Aab}	0.10 (0.01)^{Aab}	**0.11 (0.010)^{ab}**
	Treatment Overall	0.13 (0.02)^A	0.10 (0.02)^A	0.14 (0.03)^A	

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments (α = 0.05).

[a] Values followed by the same lower-case letter denote sample means that are statistically similar among climate conditions (α = 0.05).

Table 4.8b Mean, standard error of the mean, and pair-wise Tukey comparison on soil microbial characteristics among treatments (MN, MB, MNB) and climate conditions (AMB, TEMP, fCO$_2$×TEMP) of a soil collected in Bayfield, ON.

	Climate Condition	MN	MB	MNB	Climate Overall
		$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$
Microbial	**AMB**	**18.0 (1.2)^{Aa}**	**19.0 (0.01)^{ABa}**	**22.0 (0.6)^{Ba}**	19.7 (0.7)
Richness	**TEMP**	**18.7 (0.9)^{Aa}**	**15.0 (0.6)^{Bb}**	**17.0 (0.6)^{ABbc}**	16.9 (0.6)
(counts)	**fCO$_2$**	**17.0 (0.6)^{ABa}**	**18.7 (1.2)^{Aa}**	**15.3 (0.9)^{Bc}**	17.0 (0.7)
	fCO$_2$×TEMP	**19.0 (1.5)^{Aa}**	**16.3 (0.9)^{Aab}**	**19.3 (1.5)^{Aab}**	18.2 (0.8)
	Treatment Overall	18.2 (0.5)	17.2 (0.6)	18.4 (0.9)	
Shannon Diversity	**AMB**	4.1 (0.6)^{Aa}	2.6 (0.3)^{Ba}	2.5 (0.3)^{Ba}	3.1 (0.3)^{a}
Index, Hs	**TEMP**	4.0 (0.4)^{Aa}	2.7 (0.1)^{Ba}	2.8 (0.6)^{Ba}	3.2 (0.3)^{a}
	fCO$_2$	3.6 (0.6)^{Aa}	3.2 (0.5)^{Ba}	3.1 (0.6)^{Ba}	3.3 (0.3)^{a}
	fCO$_2$×TEMP	4.7 (0.2)^{Aa}	3.2 (0.5)^{Ba}	2.8 (0.5)^{Ba}	3.6 (0.4)^{a}
	Treatment Overall	**4.1 (0.2)^{A}**	**2.9 (0.2)^{B}**	**2.8 (0.2)^{B}**	

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).

[a] Values followed by the same lower-case letter denote sample means that are statistically similar among climate conditions ($\alpha = 0.05$).

4.4.3 Soybean Plant Characteristics

There was no statistically significant interactive effect for treatment and climate condition factors on any of the soybean measurements (Table 4.9). However, soybean pod biomass and above-ground (AG) shoot biomass differed significantly among treatments ($p = 0.021$ and < 0.001 respectively) and climate conditions ($p = 0.038$ and < 0.001 respectively). MN had significantly greater AG biomass than MNB, MB was intermediate and not significantly different from either MN or MNB. Pod biomass was the greatest in the $fCO_2 \times TEMP$ climate condition, followed by the TEMP, fCO_2, and AMB climate conditions. Aboveground biomass was significantly lower in MNB than MN and MB treatments. AG biomass was also significantly lower in the TEMP climate condition compared to the other climate conditions (Tables 4.9 and 4.10a). Belowground (BG) root biomass differed significantly only among climate conditions ($p = 0.005$), where fCO_2 and $fCO_2 \times TEMP$ had the greatest amount of BG biomass, whereas TEMP had the lowest BG biomass, and AMB was not significantly different from any climate conditions (Table 4.9 and 4.10a). Shoot/root ratio only significantly differed by climate conditions ($p < 0.001$), AMB and $fCO_2 \times TEMP$ had significantly greater shoot/root ratios than TEMP and fCO_2 climate conditions. Shoot heights varied significantly by climate conditions ($p < 0.001$), and not treatments. For example, $fCO_2 \times TEMP$ had the tallest soybean plants, followed by TEMP, AMB and fCO_2 climate conditions. Lastly, shoot C/N ratio differed significantly by climate conditions ($p = 0.005$) and not treatments where AMB contained a higher C/N ratio than the other climate conditions (Tables 4.9 and 4.10b).

Table 4.9 Two-way analyses of variance on soybean crop characteristics among treatments (MN, MB, MNB) and climate conditions (AMB, TEMP, fCO$_2$×TEMP) grown from a soil collected in Bayfield, ON.

Fixed Factors	Soybean Pod Biomass	Above-ground Biomass	Below-ground Biomass	Shoot/Root Ratio	Shoot Height	Shoot C/N Ratio
	F (p > F)	F (p > F)	F (p > F)	F (p > F)	F (p > F)	F (p > F)
Treatment (Trt)	**4.573 (0.021)**	**3.761 (0.038)**	2.976 (0.070)	0.316 (0.732)	1.978 (0.160)	0.827 (0.449)
Climate	**25.641 (<0.001)**	**13.384 (<0.001)**	**5.465 (0.005)**	**14.450 (<0.001)**	**33.979 (<0.001)**	**5.640 (0.005)**
Trt × Climate	0.347 (0.904)	0.661 (0.681)	1.486 (0.225)	1.161 (0.359)	0.911 (0.504)	0.826 (0.562)

Significant terms are in bold (α = 0.05).

Table 4.10a Mean, standard error of the mean, and pair-wise Tukey comparison on soybean crop characteristics (measured on 90th day) among treatments (MN, MB, MNB) and climate conditions (AMB, TEMP, fCO$_2$×TEMP) grown from a soil collected from Bayfield, ON.

	Climate Condition	MN	MB	MNB	Climate Overall
		$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$
Soybean Pod	**AMB**	7.9 (0.7)^{ABa}	7.4 (0.8)^{Aa}	6.8 (0.9)^{Ba}	**7.4 (0.4)^a**
Biomass	**TEMP**	11.8 (0.9)^{ABb}	11.9 (0.6)^{Ab}	9.8 (1.1)^{Bb}	**11.2 (0.6)^b**
(g/plant, dry)	**fCO$_2$**	9.3 (1.0)^{ABa}	9.7 (0.6)^{Aa}	8.0 (0.5)^{Ba}	**9.0 (0.5)^a**
	fCO$_2$×TEMP	13.3 (1.4)^{ABc}	14.8 (0.9)^{Ac}	12.0 (0.9)^{Bc}	**13.4 (0.7)^c**
	Treatment Overall	**10.6 (0.8)^{AB}**	**10.9 (0.9)^A**	**9.1 (0.7)^B**	
Above-ground	**AMB**	5.2 (0.2)^{Ac}	6.2 (0.6)^{Ac}	5.2 (0.3)^{Ac}	**5.5 (0.3)^c**
Biomass	**TEMP**	10.3 (1.8)^{Aab}	9.9 (0.8)^{ABab}	7.6 (1.2)^{Bab}	**9.3 (0.8)^{ab}**
(g/plant, dry)	**fCO$_2$**	8.7 (1.0)^{Abc}	7.0 (0.1)^{ABbc}	6.4 (0.5)^{Bbc}	**7.4 (0.5)^{bc}**
	fCO$_2$×TEMP	10.3 (1.3)^{Aa}	9.7 (0.7)^{ABa}	8.6 (0.3)^{Ba}	**9.5 (0.5)^a**
	Treatment Overall	**8.6 (0.8)^A**	**8.2 (0.6)^{AB}**	**7.0 (0.5)^B**	
Below-ground	**AMB**	7.2 (0.6)^{Aab}	7.8 (0.7)^{Aab}	6.2 (0.9)^{Aab}	**7.1 (0.44)^{ab}**
Biomass	**TEMP**	5.9 (0.8)^{Ab}	6.0 (0.1)^{Ab}	5.1 (0.5)^{Ab}	**5.7 (0.31)^b**
(g/plant, dry)	**fCO$_2$**	10.5 (1.7)^{Aa}	7.4 (0.2)^{Aa}	7.0 (0.5)^{Aa}	**8.3 (0.76)^a**
	fCO$_2$×TEMP	7.3 (1.0)^{Aa}	8.4 (1.0)^{Aa}	7.1 (0.7)^{Aa}	**7.6 (0.48)^a**
	Treatment Overall	7.7 (0.7)^A	7.4 (0.4)^A	6.4 (0.4)^A	
Shoot/root ratio	**AMB**	0.93 (0.16)^{Ab}	1.02 (0.11)^{Ab}	1.03 (0.06)^{Ab}	**0.99 (0.06)^b**
(w/w per plant)	**TEMP**	1.40 (0.15)^{Aa}	1.28 (0.07)^{Aa}	1.22 (0.04)^{Aa}	**1.30 (0.06)^a**
	fCO$_2$	0.85 (0.06)^{Ab}	0.95 (0.03)^{Ab}	0.93 (0.10)^{Ab}	**0.91 (0.04)^b**
	fCO$_2$×TEMP	1.41 (0.01)^{Aa}	1.17 (0.06)^{Aa}	1.23 (0.09)^{Aa}	**1.27 (0.05)^a**
	Treatment Overall	1.15 (0.09)^A	1.11 (0.05)^A	1.10 (0.05)^A	

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments (α = 0.05).

[a] Values followed by the same lower-case letter denote sample means that are statistically similar among climate conditions (α = 0.05).

Table 4.10b Mean, standard error of the mean, and pair-wise Tukey comparison on soybean crop characteristics among treatments (MN, MB, MNB) and climate conditions (AMB, TEMP, $fCO_2 \times$TEMP) grown from a soil collected in Bayfield, ON.

	Climate Condition	MN	MB	MNB	Climate Overall
		$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$	$\overline{x}\ (\sigma_{\overline{x}})$
Shoot Height	**AMB**	45 (4)^{Aa}	48 (4)^{Aa}	45 (4)^{Aa}	**46 (2)^{a}**
(cm/plant)	**TEMP**	75 (10)^{Ab}	71 (6)^{Ab}	68 (7)^{Ab}	**72 (4)^{b}**
	fCO$_2$	55 (2)^{Aa}	56 (4)^{Aa}	50 (1)^{Aa}	**54 (2)^{a}**
	fCO$_2$×TEMP	114 (13)^{Ac}	94 (12)^{Ac}	87 (1)^{Ac}	**98 (7)^{c}**
	Treatment Overall	72 (9)^{A}	68 (6)^{A}	63 (5)^{A}	
Shoot C/N	**AMB**	42 (10)^{Aa}	43 (10)^{Aa}	29 (1)^{Aa}	**38 (5)^{a}**
Ratio	**TEMP**	22 (3)^{Ab}	23 (2)^{Ab}	26 (6)^{Ab}	**24 (2)^{b}**
	fCO$_2$	27 (4)^{Ab}	28 (3)^{Ab}	22 (2.)^{Ab}	**26 (2)^{b}**
	fCO$_2$×TEMP	25 (3)^{Ab}	24 (3)^{Ab}	25 (3)^{Ab}	**25 (1)^{b}**
	Treatment Overall	29 (3)^{A}	30 (3)^{A}	26 (2)^{A}	

[A] Values followed by the same upper-case letter denote sample means that are statistically similar among treatments ($\alpha = 0.05$).

[a] Values followed by the same lower-case letter denote sample means that are statistically similar among climate conditions ($\alpha = 0.05$).

4.4.4 Qualitative Observations

      Notable variations in the expression of phenotypic traits were qualitatively observed in soybean plants when subjected to different climate conditions. While no prominent differences were observed between treatment groups, climate conditions containing a warming effect (e.g. TEMP and $fCO_2 \times$TEMP) produced taller shoots, higher pod biomass, lower root biomass, and more fallen leaves. The taller shoots were all drooping under TEMP and $fCO_2 \times$TEMP climate conditions. Plants produced visibly lusher leaves in terms of greenness and size of leaflets under AMB and TEMP conditions than climate conditions containing fCO_2 effect (fCO_2 and $fCO_2 \times$TEMP) (Figure 4.2).

Figure 4.2 Physical comparison between soybean qualitative phenotypical traits under: AMB (top-left), TEMP (top-right), fCO_2 (bottom-left), and $fCO_2 \times TEMP$ (bottom-right) climate conditions.

4.5 Discussion

Similar to the results discussed in previous chapters of this thesis, the treatment effect was substantially smaller in comparison to the climate condition effect on soil and plant (soybean) properties as well in this chapter. Again, this pattern was likely due to the low rate of biochar application in these studies. There was also a lack of interactive effect between the treatment and climate condition factors with the only exception to soil microbial richness (Table 4.7), which indicates that the effect of biochar functions independently of common climate change conditions such as warming and CO_2 fertilization (or the combined effect of warming and CO_2 fertilization). This was expected since soil moisture is usually the primary factor that drives soil biological activities, and therefore soil nutrient availability and crop nutrient uptake and productivity (Drenovsky et al. 2004; Wildung et al. 1975; Klotzsche et al. 2018). Furthermore, it should be noted that limited research on the interactive effect of biochar as a soil amendment and induced environmental conditions such as CO_2 fertilization and warming is currently available.

Soil organic carbon (SOC) is key to determining soil health and soil fertility (Cuevas & Chacon 1994), and various studies have concluded biochar's ability to promote SOC accumulation and retention (Hua et al. 2013; Yi et al. 2018). Surprisingly, MNB contained an overall statistically lower SOC content than MN, and the same was observed for SMB-C (Table 4.4 and 4.8a). This may be due to the removal of solid biochar macroparticles (sieving) during the analytical process of SOC determination; similar to the case observed in Chapter 2. Considering the biochar-urea interactions, as mentioned in previous chapters, the intended functionality of biochar was likely hindered by the saturation of the reaction surface of biochar with urea (Simha et al. 2016; Hu & Zhang 2019). This was backed up by the significantly lower SOC content in MNB than MB where MB contained a similar amount of SOC as MN. The SOC

values were also in agreement with soil microbial biomass carbon (SMB-C) and crop characteristics summarized in Table 4.8a and Table 4.10a where MNB contained significantly lower SMB-C and crop productivity in terms of soybean pod and above-ground (shoot) biomasses in comparison to MN and MB. Interestingly, SOC did not vary among climate conditions. Normally, the rate of SOC decomposition follows a first order reaction kinetics with respect to soil temperature (Frøseth et al. 2015; Reichstein & Janssens 2009).

The soil C/N ratio was higher in MB than MN and MNB as expected since MB samples did not receive any urea-N fertilizer. The lack of statistical difference between MN and MNB sample means for soil C/N ratio was surprising since biochar is mostly carbon by weight. This could be due to the sieving process that removed majority of the biochar chunks from soil samples amended with biochar. Hot-water-extractable carbon (HWC) is believed to represent the portion of organic carbon that is readily available for microbial uptake (Ghani et al. 2003) which is therefore potentially a sensitive estimator for soil health. Though HWC was statistically the lowest in fCO_2 by a small margin (considering the size of the standard errors), SMB was not the lowest for fCO_2 which indicates that HWC did not reflect soil microbial activity as well as SOC, and the significant difference observed for HWC could be a fluke considering the relatively large standard errors (Tables 4.4 and 4.8a). Recent research suggests that HWC is a promising measure to detect changes in soil organic matter (SOM) dynamics typically due to drastic changes in land use such as a conversion of grassland to cropland (Spohn & Giani 2011). This could explain the lack of meaningful pattern in HWC in this study as the soils did not experience excessive manipulations.

When comparing to the in-field study in Chapter 2, soil inorganic chemical properties were influenced differently by the biochar treatment effect under growth chamber conditions.

This may be because many environmental parameters were kept constant under laboratory settings such as light intensity, moisture, and soil water content (Table 4.1). MN contained significantly higher total soil nitrogen than MB which was expected due to the high rate of urea-N fertilizer addition. However, MNB contained the least amount of soil total nitrogen (TN) out of the three treatment groups. This was likely due to the interaction between biochar and urea fertilizer as reported by various researchers (Nelissan et al. 2014; Simha et al. 2016). Biochar has a straightforward adsorptive effect on urea via surface electrostatic interactions, and the uptake equilibrium follows a Dubinin-Radushkevich isotherm model which is a relatively new applied adsorption model in adsorption equilibrium studies that apply to many adsorption equilibria in nature (Simha et al. 2016; Hu & Zhang 2019). While the specific maximum adsorption uptake varies with the char type (i.e. source feedstock, pyrolysis temperature), it is estimated to be about 1000 mg urea per gram of biochar as determined by Simha et al. (2016). Simha et al. (2016) are currently determining biochar's specific adsorption affinity to urea. Biochar's high adsorption capacity and possibly high affinity for urea are therefore likely key factors in the observed lower TN in MNB sample group since large biochar residues (diameter > 2 mm) were removed (sieving) during the soil TN analysis. Soil NO_3^--N was also statistically the lowest for MNB which contributed to the lower TN content for MNB, but the mean differences in soil NO_3^--N were very small compared to the mean differences in TN (Table 4.6). The difference in the patterns of TN values by treatment between this study and the field study in Chapter 2 could be explained by the fact that no additional urea-N or manure were reapplied prior to the start of this study. Thus, the bioavailable portion of soil N were likely used up during the sampling period in 2018 (c.f. Chapter 2) by biological denitrification activity. Soil TN was slightly lower in climate

conditions with CO_2 fertilization which could be explained by significantly lower soil NH_4^+-N in fCO$_2$ and fCO$_2\times$TEMP climate conditions.

NH_4^+ oxidation (nitrification) is a major and often the rate limiting step of the nitrogen cycle (Rivera et al. 2012; Qin et al. 2011). A recent study by Pratscher et al. (2011) found that NH_4^+ oxidation is often coupled to CO_2 fixation by archaea and bacteria in agricultural soil via DNA-stable isotope probing (SIP). Utilizing DNA-SIP, Pratscher et al. (2011) showed that the genes responsible NH_4^+ oxidation and autotrophic CO_2 assimilation were expressed simultaneously for a dynamic and wide range of microbial and archaeal communities in agricultural soil. This indicates that increasing atmospheric concentrations of CO_2 would likely lead to an increase in agricultural N_2O emission. Research had shown that the nitrogen cycle has significant temperature dependency as biological production of mineralized NH_4^+ and the subsequent enzymatic nitrification of NH_4^+ to NO_3^- are highly temperature sensitive in the soil (Barnard et al. 2005; Veraart et al. 2011). However, there was practically no difference in soil NH_4^+-N between climate conditions with and without the warming effect (AMB, fCO$_2$ vs. TEMP and fCO$_2\times$TEMP) suggesting that the temperature dependency of the nitrification processes was likely influenced by the nitrogen fixing soybean rhizobia species. A study by Indrasumunar et al. (2012) found that soil high in calcium and pH heavily promoted the N_2 fixation by rhizobia in the soybean root nodules to ammonia which could be the dominating factor in controlling soil NH_4^+-N content in this study.

The higher available PO_4^{3-}-P content observed in soil samples under CO_2 fertilization effect could be explained by the increase in soybean's demand for bioavailable P as proposed by Jin et al. (2015). They highlighted a key mechanism in CO_2 induced P demand in plants due to the stimulation in photosynthesis as a result of increased atmospheric CO_2 concentration. This in

turn likely induced a change in root exudates that eventually led to P mobilization from otherwise less soluble phosphorus-containing complexes in soil (Jin et al. 2015). This P mobilization could explain the higher PO_4^{3-}-P content in fCO_2 and $fCO_2{\times}TEMP$ sample groups since the Olsen-P method only detects free PO_4^{3-}-P in soil (Coventry et al. 2001).

Aside from the aforementioned pattern noted for SMB-C among treatments, SMB-N as well as soil microbial C/N ratio largely remained statistically similar between treatments and climate conditions. However, the microbial community analyses revealed niche differences that SMB analyses failed to discover (Tables 4.8a and 4.8b). Specifically, average well colour development (AWCD) showed that microbial activity was the highest under ambient conditions than all other climate conditions. This was expected as microbial communities tend to be very sensitive to changes in the environment which often result in irregular living conditions that require intensive adaption for existing species and communities (Waldrop & Firestone 2006). Judging from AWCD alone, it may seem as if CO_2 fertilization and warming effects had similar impacts on the overall microbial activities, the richness of biodiversity indicates otherwise. Under constant ambient conditions, MNB had the highest count towards species richness, this could be explained by the fact that MNB treatment groups received more variety of soil additives than MB and MN, therefore hosting the most diverse population of microbes (Liu et al. 2016). Richness was significantly lower for MB samples under the warming effect which could mean that urea plays a role in microbial heat tolerance, though there's a lack of literature supporting this hypothesis. The general trend in species richness is that MN remained largely unaffected by induced climatic conditions, MB responded poorly against warming effects (TEMP and $fCO_2{\times}TEMP$), and MNB responded poorly under fCO_2. This was unexpected as biochar's ability to maintain and promote soil water content and soil organic matter should benefit biological

growth (Atkinson et al. 2010; Plaza et al. 2016), but instead the opposite was observed and confirmed by the Shannon Diversity Index (Hs) which accounts for both population density as well as species diversity summarized in Table 4.8b.

Largest statistical differences in crop characteristics arose from the induced climate conditions seen in Tables 4.10a and 4.10b. Generally, warming produced the largest effects, and CO_2 fertilization to a lesser extent, on crop growth and maturity as soybean pod biomass and plant height were the highest in TEMP and even higher in $fCO_2 \times$TEMP. This was in complete agreement with the literature and reasonably so since agriculture in the North had historically suffered from relatively cold weathers unsuitable for many crop species to cultivate easily if possible at all (Maracchi et al. 2005; IPCC 1998; AAFC 2015; Sionit et al. 1987). The crops had reached a later stage of maturity under warming conditions as shown by the higher final shoot height, soybean pod biomass as well as the browning and litterfall of the soybean plants for TEMP and $fCO_2 \times$TEMP sample groups presented in Tables 4.10a, 4.10b, and Figure 4.2. While the promoted growth is evident under warming conditions, beanstalks with hastened growth suffered from a lack of physical structural integrity as seen in Figure 4.2 where the beanstalks cultured under TEMP and $fCO_2 \times$TEMP conditions were drooping. Rapid growth spurts could be the reason behind this undesired phenotypical trait. The malformed beanstalks would be difficult to harvest using existing equipment and can cause entangling when grown in high density on a field. The significantly higher shoot/root ratio observed in TEMP and $fCO_2 \times$TEMP indicates that the soybean plants were coping with physical stress regardless of the higher soybean pod yield (Agathokleous et al. 2018). This may pose a problem outside of controlled laboratory settings where naturally occurring weather conditions such as droughts could severely suppress yield. For instance, Mechler et al. (2018) found greatly diminished corn yield in the extremely dry year of

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2016, whereas the same study site produced close to an order of magnitude higher grain yield in the wetter year of 2018 (c.f. Chapter 2). Shoot C/N ratios were significantly higher for AMB sample group but likely due to random errors since it is very unlikely for a plant to have such drastically altered elemental makeup. Crop shoot/root ratio is a sensitive indicator of environmental stress on plants by chemical and/or physical means (Agathokleous et al. 2018). In ontogeny, plant roots typically develop first in preparation for adequate nutrient uptake for the subsequent development of shoots (Lohier et al. 2014).

4.6 Conclusion

There was a lack of interactive effect between treatment effect and climate condition effect suggesting biochar behaves independently of climate conditions as a soil amendment. The urea-biochar interaction could be hindering biochar's intended functionality in a nutrient-limiting condition as seen by the poorly performing MNB treatment group. The only exception to this was microbial species richness which was relatively high for MNB under many climate conditions suggesting the greater variety of soil additives led to greater soil biodiversity. Soil NH_4^+-N was significantly lower under CO_2 fertilization conditions suggesting an interaction between soil NH_4-N and atmospheric CO_2 likely due to microbial and archaeal NH_4^+ oxidation coupled to their autotrophic CO_2 fixation. Soybean crop yield was greatly enhanced by the warming effect and CO_2 fertilization to a lesser extent. However, undesired wilting of beanstalks manifested under warming conditions likely due to rapid growth spurts resulting in unbalanced plant development. This was evident from the significantly higher shoot/root ratio observed for soybean plants under warming effects indicative of the plant's coping mechanism against environmental stress such as heat. Future studies could look to study different types of biochar considering economic feasibility and other common crops such as corn for comparison.

5. Grand Conclusion

In summary, the 3 t/ha biochar addition in these projects were drastically lower than a typical biochar study averaging to approximately 20 t/ha. Given the current state of the biochar production industry, the cost of agricultural quality biochar would make biochar an economically unfeasible method of soil amendment and as carbon sink. The 3 t/ha rate of biochar addition employed in this project gives a much more realistic estimate on how biochar would assimilate into temperate agriculture as a soil amendment than other studies. As a result, the secondary factors such as seasonal and environmental effects on selected soil parameters and GHG emissions were much more prominent than soil treatment effects.

In the third-year of the three-year in-field study, biochar amended soil plots contained a significant increase in stable macroaggregates as expected from biochar-catalyzed macroparticle formation in soil. However, WHC, SOC and HWC were the highest in MN likely due to the higher SOM input from the additional poultry manure application and biochar was not able to fully offset the effects of reduced poultry manure addition. The lack of substantial difference in soil inorganic N and P nutrients between study plots containing higher manure addition and plots containing biochar suggested that biochar did improve inorganic nutrient retention in the soil. Treatment plots that received biochar and urea (MNB) selectively promoted specific groups of microbes likely due to the biochar-induced adsorptive retention of urea and/or volatile urea-derived ammonium in soil. Lastly, crop yield was not affected at this rate of biochar application. Longer periods of study and larger biochar additions should be implemented to observe any potential long-term effect associated with the physical, chemical, and biological changes to the soil in a temperate agricultural ecosystem.

The GHG emission study showed that biochar was able to reduce soil GHG emission at the low application rate employed in this study site consisting of a sandy loam Luvisol in southern Ontario. This was likely due to nutrient immobilization, which reduced microbial decomposition and nitrification processes. Poultry manure addition in MN resulted in the significantly higher CO_2 emission due to improved WHC and labile organic and inorganic nutrient contents immediately available for microbial uptake. MNB produced more N_2O emission than MB due to the urea nitrogen fertilizer addition, however there was no sign showing that urea retained by biochar caused higher N_2O emission after the immediate fertilizer application. Aside from soil treatments, soil moisture was the best predictor for both CO_2 and N_2O emissions. Soil temperature and NH_3-N were the second-best predictors for CO_2 and N_2O emissions respectively which was in agreement with existing literature. Findings from this study solidifies that biochar can be implemented as a long-term soil amendment to achieve soil GHG abatement where increasing addition rates of biochar could enhance the reduction but at an impractical cost.

From the climate change resilience growth chamber study, there was a lack of interactive effect between the soil treatment factor and climate condition factor meaning biochar behaved independently of induced CO_2 fertilization and warming conditions as a soil amendment. MNB fared poorly in terms of soil organic carbon, nitrogen, microbial, and crop parameters. This was likely due to the urea-biochar interaction as seen in Chapter 1 as well where the excess urea may have saturated biochar's reaction surfaces. Under induced climate conditions, microbial and archaeal NH_4^+ oxidation coupled autotrophic CO_2 fixation could explain the significantly lower soil NH_4^+-N observed under CO_2 fertilization conditions. The rate of soybean plant maturation was greatly enhanced by the warming effect, though, the growth spurts caused undesirable

phenotypes, namely drooping beanstalks. The significantly higher shoot:root ratios under warming conditions indicated a coping mechanism against environmental stress. Future studies could investigate different biochars, N_2O fertilization, as well as different crop systems to better understand how biochar treated soils respond.

References

Abiven S., Hund A., Martinsen V., Cornelissen G. (2015). Biochar amendment increases maize root surface areas and branching: a shoveloimics study in Zambia. *Plant and Soil,* 395 (1-2), 45-55.

Abrol I.P. and Sangar S. (2006). Sustaining Indian agriculture – conservation agriculture the way forward. *Current Science,* 91 (8), 1020-1025.

Agathokleous E., Belz R.G., Kitao M., Koike T., Calabrese E.J. (2018). Does the root to shoot ratio show a hermetic response to stress? An ecological and environmental perspective. *J. For. Res.,* URL: https://doi.org/10.1007/s11676-018-0863-7

Agegnehu G., Bass A.M., Nelson P.N., Bird M.I. (2016). Benefits of biochar, compost and biochar–compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. *Science of The Total Environment,* 543 (A), 295-306.

Apricorp. (2019). Maps show 2018 corn yields. Access: June 2019. URL: http://www.agricorp.com/en-ca/News/2019/Pages/PI-MapShows2018CornYields.aspx

Agriculture and Agri-Food Canada, AAFC. (2015). Impact of climate change on Canadian agriculture. Government of Canada, Retrieved May 2019 from http://www.agr.gc.ca/eng/science-and-innovation/agricultural-practices/climate-change-and-agriculture/future-outlook/impact-of-climate-change-on-canadian-agriculture/?id=1329321987305

Agriculture and Agri-Food Canada, AAFC. (2019). 2018 Annual report of agroclimate conditions across Canada. Government of Canada. Retrieved April 2019 from http://climate.weather.gc.ca/index_e.html

Agusalim M., Wani H.U., Syechfani M.S. (2010). Rice husk biochar for rice based cropping system in acid soil 1. The characteristics of rice husk biochar and its influence on the properties of acid sulfate soils and rice growth in West Kalimantan, Indonesia. *Journal of Agricultural* Science, 2 (1), 39-47.

Alteiri M.A. (1999). The ecological role of biodiversity in agroecosystems. *Practical Use of Invertebrates to Assess Sustainable Land Use,* 19-31.

Amacher M.C., O'Neill K.P., Dresbach R., Palmer C. (2003). *Forest Inventory & Analysis: Manual of soil analysis methods.*

Ameloot N., Graber E. R., Verheijen F. G. A., De Neve S. (2013). Interactions between biochar stability and soil organisms: Review and research needs. *European Journal of Soil Science*, 64 (4), 379-390.

Anders E., Watzinger A., Rempt F., Kitzler B., Wimmer B., Zehetner F., Stahr S., Zechmeister-Boltenstern S., Soja G. (2013). Biochar affects the structure rather than the total biomass of microbial communities in temperate soils. *Agricultural and Food Science*, 22(4) 404-423.

Asadieh B., Krakauer N. Y., Fekete B. M. (2016). Historical trends in mean and extreme runoff and streamflow based on observations and climate models. *Water*, 8 (5), 189.

Atkinson C.J., Fitzgerald J.D., Hipps,N.A. (2010). Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. *Plant and Soil*, 337(1), 1-18.

Ball A.S. (1997). Microbial decomposition at elevated CO2 levels: effects of litter quality. *Global Change Biol.* 3, 379–386.

Bamminger C., Zaiser N., Zinsser, P., Lamers, M., Kammann, C., Marhan, S. (2014). Effects of biochar, earthworms, and litter addition on soil microbial activity and abundance in a temperate agricultural soil. *Biology and Fertility of Soils*, 50 (8), 1189-1200.

Bandick A.K., Dick R.P. (1999). Field management effects on soil enzyme activities. *Soil Biol. Biochem.,* 31, 1471-1479.

Baranick M., McElwee D., Zazycki M., Biochar feasibility study: Exploring the environmental, social and economic value of a biochar business in the methow valley.

Barnabás B., Jäger K., Fehér A. (2008). Review: The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell. Environ.*, 31(1), 11-38.

Barnard R., Leadley P.W., Hungate B.A. (2005). Global change, nitrification, and denitrification: A review. *Global Biogeochem.,* Cy 19: GB1007.

Barrett J.E., Burke I.C. (2000). Potential nitrogen immobilization in grassland soils across a soil organic matter gradient. *Soil Biol. Biochem.,* 32, 1707-1716.

Basso A.S., Miguez F.E., Laird D.A., Horton R., Westgate M. (2013). Assessing potential of biochar for increasing water-holding capacity of sandy soils. *GCB Bioenergy,* 5(2), 132-143.

Batjes N.H. (1998). Mitigation of atmospheric CO_2 concentrations by increase carbon sequestration in the soil. *Biol. Fertil. Soils,* 27, 230-235.

Beare M.H., Hendrix P.F., Cabrera M.L., Coleman D.C. (1994). <u>Aggregate-Protected and Unprotected Organic Matter Pools in Conventional- and No-Tillage Soils</u>. *Soil Science Society of America Journal*, 58 (3), 787.

Beesley L., Moreno-Jiménez E., Gomez-Eyles J.L., Harris E., Robinson B., Sizmur T. (2011). A review of biochar's potential role in the remediation, revegetation and restoration of contaminated soils. *Pollution,* 159, 3269-3282.

Bengtsson G., Bengtson P., Månsson K.F. (2003). Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biol. Biochem.,* 35 (1), 143-154.

Beniston M., Tol R. S. J., Delcolle R., Hörmann G., Iglesias A., Innes J., McMichael A. J., Martens A. J. M., Nemesova I., Nicholls R. J. and Toth F. L.: 1998, 'Europe', in Watson, R. T., Zinyowera, M. C. and Moss, R. H. (eds.), *The Regional Impacts of Climate Change – An Assessment of Vulnerability*, Special Report of Working Group II of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, pp. 289–324.

Benjamin J.G., Nielsen D.C. (2006). Water deficit effects on root distribution of soybean, field pea and chickpea. *F Crop Res,* 97, 248–253.

Benton T.G., Vickery J.A., Wilson J.D. (2003). Farmland biodiversity: is habitat heterogeneity the key? *Trends in Ecology & Evolution,* 18 (4), 182-188.

Bhattarai K., Stalick W.M., McKay S., Geme G., Bhattarai N. (2011). Biofuel: an alternative to fossil fuel for alleviating world energy and economic crises. *J Environ. Sci. Health A. Tox. Hazard. Subst. Eng.,* 46 (12), 1424-1442.

Biederman L.A., Harpole W.S. (2013). Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *Glob. Change Biol. Bioenergy*, 5, 202-214.

Blanchart E., Bernoux M., Sarda X., Siqueira Neto M., Cerri C.C., Piccolo M., Douzet J.M., Scopel E., Feller C. (2007). Effect of direct seeding mulch-based systems on soil carbon storage and macrofauna in central Brazil. *Agric. Conspec. Sci.*, 72, 81-87

Bonanomi G., Antignani V., Pane C., Scala F. (2007). Suppression of soilborne fungal diseases with organic amendments. *Journal of Plant Pathology*, 89, 311-324.

Bonanomi G., Ippolito F., Scala F. (2015). A "black" future for plant pathology? Biochar as a new soil amendment for controlling plant diseases. *Journal of Plant Pathology,* 97 (2), 223-234.

Bon-Lamberty B., Smith A.P., Bailey V.L. (2016). Temperature and moisture effects on greenhouse gas emissions from deep active-layer boreal soils. *Biogeosciences,* 13(24), 6669-6681.

Borchard N., Siemens Jan, Ladd B.,

Brady N.C., Weil R.R. (1999). The nature and properties of soils. *Pearson Edication, ISBN: 978-0133254488.*

Brassard P., Godbout S., Raghavan V. (2016). Soil biochar amendment as a climate change mitigation tool: Key parameters and mechanisms involved. *J. Environ. Management,* 181, 484-497.

Brown L.R. (2006) Plan B 2.0. Rescuing a Planet Under Stress and a Civilization in Trouble. New York, US. *W.W. Norton Press*.

Bruun E.W., Ambus P., Egsgaard H., Haunggaard-Nielsen H. (2012). Effects of slow and fast pyroslysis on soil C and N turnover dynamic. *Soil Biol. Biochem.,* 46, 73-79.

Burri S., Niklaus P.A., Grassow K., Buchmann N., Kahmen A. (2018). Effects of plant productivity and species richness on the drought response of soil respiration in temperate grasslands. *PLoS ONE,* 13 (12), e0209031, DOI 10.1371/journal.pone.0209031
Carter M.R. (1992). Influence of reduced tillage systems on organic matter, microbial biomass, macro-aggregate distribution and structural stability of the surface soil in a humid climate. *Soil and Tillage Research,* 23 (4), 361-372.

Carter M.R. (2002). Soil quality for sustainable land management: Organic matter and aggregation interactions that maintain soil functions. *Agronomy Journal*, 94, 38-47.

Cavigelli M.A., Robertson G.P. (2001). Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. *Soil Biol. Biochem.,* 33, 297-310.

Case S.D.C., McNamara N.P., Reay D.S., Whitaker J. (2012). The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil – The role of soil aeration. *Soil biol. Biochem.,* 51, 125-134.

Chan K.Y., van Zwieten L., Meszaros I., Downie A., Joseph S. (2007). Agronomic values of greenwaste biochar as a soil amendment. *Aust J Soil Res* 45, 629-634.

Chan K.Y., Zwieten L.V., Meszaros I., Downie A., Joseph S. (2008). Agronomic values of greenwaste biochar as a soil amendment. *Australian Journal of Soil Research,* 45(8), 629-634.

Christensen J. H., Hewitson B., Busuioc A., Chen A., Gao, X., Held I., Jones R., Kolli R. K., Kwon W.T. et al. (2007). Regional climate projections. In Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Avery, M. Tigor & H. L. Miller), pp. 847–940. Cambridge, UK: Cambridge University Press.

Clough T.J., Cordron L.M. (2010). Biochar and the nitrogen cycle: Introduction, *J. Environ. Qual.,* 39 (4), 1218-23.

Cole L., Bradford M.A., Shaw P.J.A., Bardgett R.D. (2006). The abundance, richness and functional role of soil meso- and macrofauna in temperate grassland – A case study. *Applied Soil Ecology,* 33 (2), 186-198.

Conant R.T. (2011). Sequestration through forestry and agriculture. *WIREs Clim. Change,* 2, 238-254.

Cook F.J., Knight J.H., Kelliher F.M. (2013). Modelling oxygen transport in soil with plant root and microbial oxygen consumption: depth of oxygen penetration. *Soil Research,* 51 (6), 539-553.

Coventry J.L., Halliwell D.J., Nash D.M. (2001). The orthophosphate content of bicarbonate soil extracts. *Aus. J. Soil Res.,* 39 (2), 415-421.

Cusack D.F., Silver W.L., Torn M.S., Burton S.D., Firestone M.K. (2011). Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. *Ecology*, 92, 621-632.

Dam R.F., Mehdi B.B., Burgess M.S.E., Madramootoo C.A., Mehuys G.R., Callum I.R. (2005). Soil bulk density and crop yield under eleven consecutive years of corn with different tillage and residues practices in a sandy loam soil in central Canada.

Dawson C.J., Hilton J. (2011). Fertiliser availability in a resource-limited world: Production and recycling of nitrogen and phosphorus. *Food Policy, 36 (1),* S14-S22.

Dempster D.N., Gleeson D.B., Solaiman Z.M., Jones D.L., Murphy D.V. (2012). Decreased soil microbial biomass and nitrogen mineralization with Eucalyptus biochar addition to a coarse textured soil. *Plant and Soil,* 354 (1-2), 311-324.

Dil M. (2018). The effects of nitrogen-enriched biochar on maize (zea mays) productivity and soil organic carbon (Published M.Sc. Thesis). University of Waterloo, Waterloo, Canada.

Ding W., Luo J., Li J., Yu H., Fan J., Liu D. (2013). Effect of long-term compost and inorganic fertilizer application on background N_2O and fertilizer-induced N_2O emissions from an intensively cultivated soil. *Science of the Total Environment,* 465, 115-124.

Domene X., Mattana S., Hanley K., Enders A., Lehmann J. (2014) Medium-term effects of corn biochar addition on soil biota activities and functions in a temperate soil cropped to corn. *Soil Biol. Biochem.,* 72, 152-162.

Domingues R.R., Trugilho P.F., Silva C.A., de Melo I.C.N.A, Melo L.C.A., Magriotis Z.M., Sanchez-Monedero M.A. (2017). Properties of biochar derived from wood and high-nutrient biomasses with the aim of agronomic and environmental benefits. *PLOS one,* 12 (5), e0176884.

Dorrough J., Moll J., Crosthwaite J. (2007). Can intensification of temperate Australian livestock production systems save land for native biodiversity? *Agri. Ecosys. Environ.,* 121 (3), 222-232.

Downie A., Crosky A., Munroe P. (2009). Physical properties of biochar. In: Lehmann J., Joseph S. (eds). *Biochar for Environmental Management: Science and Technology*. Earthscan, London, UK.

Drenovsky R.E., Vo D., Graham K.J., Scow K.M. (2004). Soil water content and organic carbon availability are major determinants of soil microbial community composition. *Microbial Ecology,* 48 (3), 424-430.

Dunfield P., Kowles R., Dumont R., Moore T.R. (1993). Methane production and consumption in temperate and subarctic peat soils: Responses to temperature and pH. *Soil Biol. Biochem.,* 25 (3), 321-326.

Dyer L., Oelbermann M., Echarte L. (2012). Soil carbon dioxide and nitrous oxide emissions during the growing season from temperate maize-soybean intercrops. *Journal of Plant Nutrition and Soil Science*, 175(3), 394-400.

Elsgaard L., Petersen S.O., Debosz K. (2001). Effects and risk assessment of linear alkylbenzene sulfonates in agricultural soil. 1. Short-term effects on soil microbiology. *Environ. Toxicol. Chem.,* 20 (8), 1656-1663.

Environmental and Climate Change Canada, ECCC. (2016). National Inventory Report 1990-2014: Greenhouse Gas Sources and Sinks in Canada, Part 1.

Environment Canada. Canadian Climate Normals 1981-2010 Station Data: Dashwood. Retrieved: January 2019, from http://climate.weather.gc.ca/climate_normals/index_e.html

Environmental Commissioner of Ontario, ECO. (2016). Facing climate change: Greenhouse Gas Progress Report 2016. *Environmental Commissioner of Ontario, Ontario, Canada.*

Fan M.X., Mackenzie A.F. (1993). Urea and phosphate interactions in fertilizer microsites: ammonia volatilization and pH changes. *Sci. Soc. Am. J.,* 57 (3) 839-845.

Feng Y., Xu Y., Yu Y., Xie Z., Lin X. (2012). Mechanisms of biochar decreasing methane emission from Chinese paddy soils. *Soil Biol. Biochem.,* 46, 80-88.

Flechard C.R., Ambus P., Skiba U., Rees R.M., Hensen A., van Amstel A., van den Polvan Dasselaar A., Soussana J.F., Jones M., Clifton-Brown J., Raschi A., Horvath L., Neftel A., Jocher M., Ammann C., Leifeld J., Fuhrer J., Calanca P., Thalman E., Pilegaard K., Di Marco C., Campbell C., Nemitz E., Hargreaves K.J., Levy P.E., Ball B.C., Jones S.K., van de Bulk W.C.M., Groot T., Blom M., Domingues R., Kasper G., Allard V., Ceschia E., Cellier P., Laville P., Henault C., Bizouard F., Abdalla M., Williams M., Baronti S., Berretti F., Grosz B. (2007). Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. *Agriculture. Ecosyst. Environ.,* 121, 135-152.

Fiorentino N., Sánchez-Monedero M.A., Lehmann J., Enders A., Fagnano M., Cayuela M.L. (2019). Interactive priming of soil N transformations from combining biochar and urea inputs: A 15N isotope tracer study. *Soil Biol. Biochem.,* 131, 166-175.

Fontaine S., Mariotti A., Abbadie L. (2003). The priming effect of organic matter: a question of microbial competition? *Soil Biol. Biochem.,* 35 (6), 837-843.

Food and Agriculture Organization of the United Nations (FAO). (2008). Climate change and food security: a framework document, Rome, Italy. *Interdepartmental working group on climate change.* Retrieved: June 2019.

Food and Agriculture Organization of the United Nations (FAO). (2009). Global agriculture towards 2050. Rome, Italy. *High-level Expert Forum: How to Feed the World 2050.* Retrieved: May 2019.

Frøseth R.B., Bleken M.A. (2015). Effect of low temperature and soil type on the decomposition rate of soil organic carbon and clover leaves, and related priming effect. *Soil Biol. Biochem.,* 80, 156-166.

Fu C., Wang G., Bible K., Goulden M.L., Saleska S.R., Scott R.L., Cardon Z.G. (2018). Hydraulic redistribution affects modeled carbon cycling via soil microbial activity and suppressed fire. *Global Change Biology,* 24 (8), 3472-3485.

Gai X., Wang H., Liu J., Zhai L., Liu S., Ren T., Liu H. (2014). Effects of Feedstock and Pyrolysis Temperature on Biochar Adsorption of Ammonium and Nitrate. *PLoS ONE,* 9(12): e113888. URL: https://doi.org/10.1371/journal.pone.0113888

Garland J.L., Mills A.L. (1991). Classification and characterization of heterotrophic microbial communities on the basis of patterns of community level sole-carbon-source utilization. *Applied and Environmental Microbiology,* 57, 2351-2359.

Gentile R.M., Vavlauwe B., Six J. (2013). Integrated Soil Fertility Management: Aggregate carbon and nitrogen stabilization in differently textured tropical soils. *Soil Biol. Biochem.,* 67 (2013) 124-132.

Gaskin J.W., Speir R.A., Harris K., Das K.C., Lee R.D., Morris L.A., Fisher D.S. (2010). Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status, and yield. *Agron. J.,* 102 (2), 623-633.

Ghani A., Dexter M., Perrott K.W. (2003). Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilization, grazing and cultivation. *Soil Biol. Biochem.*, 35, 1231-1243.

Glaser B., Haumaier L., Guggenberger G., Zech W. (2001). The "Terra Preta" phenomenon: A model for sustainable agriculture in the humid tropics. *The Science of Nature*, 88, 37–41

Glaser B., Lehmann J., Zech W. (2002). Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - A review. *Biology and Fertility of Soils*, 35(4), 219-230.

Githinji L.J.M. (2013). Effect of biochar application rate on physical and hydraulic properties of a sandy loam. *Archives of Agronomy and Soil Science,* 60 (4), 1-14.

Gonthier D.J., Ennis K.K., Farinas S., Hsieh H., Iverson A.L., Batary P., Rudolphi J., Tscharntke T., Cardindale B.J., Perfecto Ivette. (2014). Biodiversity conservation in agriculture requires a multi-scale approach. *Proc. Biol. Sci.,* 281 (1791), 20141358.

Gornall J., Betts R., Burke E., Clark R., Camp J., Willett K., Wiltshire A. (2010). Implications of climate change for agricultural productivity in the early twenty-first century. *Philos. Trans. R. Soc. Lond. Biol. Sci.,* 365(1554), 2973-2989.

Good A.G. and Beatty P.H. (2011) Fertilizing Nature: A Tragedy of Excess in the Commons. *PLoS Biology,* 9 (8), e1001124. https://doi.org/10.1371/journal.pbio.1001124

Greenland D.J., Wild A., Adams D. (1992). Organic matter dynamics in soils of the tropics: From myth to complex reality, in: Myths and Science of Soils of the Tropics, Lal, R. and Sanchez, P.A., Eds., *Soil Science Society of America*, Madison, WI, 17-34, Special Publication 29.

Gregorich E.G. and Ellert B.H. (1993). Light fraction and microorganic matter in mineral soils. In Carter M.R., Soil Sampling and Methods of Analysis. *Canadian Society of Soil Science,* 397-407.

Gregorich E.G. and Janzen H.H. (1996). Storage of Soil Carbon in the Light Fraction and Macro-Organic Matter. In: Carter, M.R. and Stewart, B.A., Eds., *Structure and Soil Organic Matter Storage in Agricultural Soils*, CRC Press, Boca Raton, 167-190.

Gritsch C., Zimmermann M., Zechmeister-Boltenstern S. (2015). Interdependencies between temperature and moisture sensitivities of CO 2 emissions in European land ecosystems. *Biogeosciences,* 12 (20) 5981-5993.

Gul S., Whalen J.K. (2016). Biochemical cycling of nitrogen and phosphorus in biochar-amended soils. *Soil Biol. Biochem.,* 103, 1-15.

Hadas A., Kautsky L., Portnoy R. (1996). Mineralization of composted manure and microbial dynamics in soil as affected by long-term nitrogen management. *Soil Biol. Biochem.,* 28 (6), 733-738.

Hamkalo Z., Bedernichek T. (2014). Total, cold and hot water extractable organic carbon in soil profile: impact of land-use change. *Zemdirbyste-Agriculture,* 101 (2), 125-132.

Hammond J., Shackley S., Prendergast-Miller M., Cook J., Buckingham S., Pappa V. (2013). Biochar field testing in the UK: Outcomes and implications for use. *Carbon Management,* 4, 159-70.

Hamza M.A., Anderson W.K. (2005). Soil compaction in cropping systems: A review of the nature, causes and possible solutions. *Soil and Tillage Research,* 82 (2), 121-145.

Han K., Choi W., Han G., Yun S., Yoo S., Ro H. (2004). Urea-nitrogen transformation and compost-nitrogen mineralization in three different soils as affected by the interaction between both nitrogen inputs. *Biology and Fertility of Soils,* 39 (3), 193-199.

Hardie M., Clothier B., Bound S., Oliver G., Close D. (2014). Does biochar influence soil physical properties and soil water availability? *Plant and Soil,* 376 (1-2), 347-361.

Haynes R.J., Naidu R. (1998). Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. *Nutrient Cycling in Agroecosystems.,* 51 (2), 123-137.

Herath H.M.S.K., Camps-Arbestain M., Hedley M. (2013). Effect of biochar on soil physical properties in two contrasting soils: An Alfisol and an Andisol. *Geoderma,* 209-210, 188-197.

Hua L., Lu Z., Ma H., Jin S. (2013). Effect of biochar on carbon dioxide release, organic carbon accumulation, and aggregation of soil. *Environmental Progress & Sustainable Energy,* 33 (3), 941-946.

Hulme M. (2016). Climate change (concept of). *The International Encyclopedia of Geography,* 2016.

Hu Q., Zhang Z. (2019) Application of Dubinin–Radushkevich isotherm model at the solid/solution interface: A theoretical analysis. *J. Mol. Liq.,* 277, 646-648.

Indrasumunar A., Menzies N.W., Dart P.J. (2012). Calcium affects the competitiveness of acid-sensitive and acid-tolerant strains of Bradyrhizobium japonicum in nodulating and fixing nitrogen with two soybean cultivars in acid soil. *Soil Biol. Biochem.,* 46, 115-122.

International Biochar Initiative (IBI). (2015). Standardized Product Definition and Product Testing Guidelines for Biochar That Is Used in Soil. URL: http://www.biochar-international.org/characterizationstandard

Ippolito J. A., Laird D. A., Busscher W. J. (2012). Environmental benefits of biochar. *Journal of Environmental Quality*, 41 (4), 967-972.

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jain T. B., Graham R. T., Adams D. L. (1997). Carbon to organic matter ratios for soils in Rocky Mountain coniferous forests. *Soil Sci. Am. J.,* 61, 1190-1195.

Janzen H.H., Campbell C.A., Brandt S.A., Lafond G.P., Townley-Smith L. (1992). Light-Fraction Organic Matter in Soils from Long-Term Crop Rotations. *Sci. Soc. Am.,* 56 (6), 1799-1806.

Jeffery S., Verheijen F.G.A., Van Der Velde M., Bastos A.C. (2011). A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture Ecosystems & Environment*, 144, 175-187.

Jin J., Tang C., Sale P. (2015). The impact of elevated carbon dioxide on the phosphorus nutrition of plants: a review. *Ann. Bot.,* 116 (6), 987-999.

Jobbágy E.G., Jackson R.B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications,* 10 (2), 423-436.

Johnson N.C., Graham J.H., Smith F.A. (1997). Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist*. 135 (4), 575–585.

Karhu K., Mattila T., Bergstrom I., Regina K. (2011). Biochar addition to agricultural soil increased CH4 uptake and water holding capacity — results from a short-term pilot field study. *Agriculture, Ecosystems and Environment*, 140 (1-2), 309-313.

Kern D.C., Kampf N. (1989). Antigos assentamentos indígenas na formação de solos com Terra Preta Arqueológica na Região de Oriximiná, Pará. *Revista Brasileira de Ciência do Solo,* 13, 219–25.

Kimetu J.M., Lehmann J. (2010). Stability and stabilisation of biochar and green manure in soil with different organic carbon contents. *Australian Journal of Soil Research,* 48 (7), 577-585.

Kloss S., Zehetner F., Dellantonio A., Hamid R., Ottner F., Liedtke V., Schwanninger M., Gerzabel M.H., Soja G. (2012). Characterization of slow pyrolysis biochars: Effects of feedstocks and pyrolysis temperature on biochar properties. *J. Environ. Qual.,* 41, 990-1000.

Kloss S., Zehtner F., Wimmer B., Buecker J., Rempt F., Soja G. (2014). Biochar application to temperate soils: Effects on soil fertility and crop growth under greenhouse conditions. *Journal of Plant Nutrition and Soil Science,* 177(1), 3-15.

Klotzsche A., Jonard F, Looms M.C., van der Kruk J., Huisman J.A. (2018). Measuring soil water content with ground penetrating radar: a decade of progress. *Vadose Zone Journal,*

Kulyk N. (2012). Cost-Benefit Analysis of the Biochar Application in the U.S. Cereal Crop Cultivation. *University of Massachusetts, School of Public Policy Capstones, Amherst, US.*

Kuo S. (1996). Phosphorus. *Methods of Soil Analysis,* 869-919.

Kuzyakov Y., Bogomolova I., Glaser B. (2014). Biochar stability in soil: Decomposition during eight years and transformation as assessed by compound-specific ^{14}C analysis. *Soil Biology and Biochemistry*, 70, 229-236.

Kwaad F.J.P.M., Zijp M.V.D., Dijk P.M.V. (1998). Soil conservation and maize cropping systems on sloping loess soils in the Netherlands. *Soil Till. Res.*, 46, 13-21.

Laird D.A. (2008). The charcoal vision: a win-win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. *Agron. J.,* 100, 178.

Laird D.A., Fleming P., Davis D.D., Horton R., Wang B., Karlen D.L. (2010). Impact of biochar amendments on the quality of a typical midwestern agricultural soil. *Geoderma*, 158 (3-4), 443-449.

Lal R. (2004). Soil carbon sequestration impacts on global climate change and food security. *Science*, 304(5677), 1623-1627.

Lal R. (2011). Soil health and climate change: An overview. *Soil Health and Climate Change*, 29, 3-24.

Lal R., Kimble J.M., Follett R.F., Stewart B.A. (1998). *Soil Process and the Carbon Cycle.* US, CRC Press.

La Scala N., Bolonhezi D., Pereira G.T. (2006). Short-term soil CO_2 emission after conventional and reduced tillage of a no-till sugar cane area in southern Brazil. *Soil and Tillage Research,* 91 (1-2), 244-248.

Lashof D.A., Ahuja D.R. (1990). Relative contributions of greenhouse gas emissions to global warming. *Nature,* 344, 529-531.

Lee C.H., Wang C.C., Lin H.H., Lee S.S., Tsang D.C.W., Jien S.H., Ok Y.S. (2018). In-situ biochar application conserves nutrients while simultaneously mitigating runoff and erosion of an Fe-oxide-enriched tropical soil. *Sci. Total. Environ.* 619-620, 665-671.

Lee J.W., Hawkins B., Day D.M., Reicosky D.C. (2010). Sustainability: the capacity of smokeless biomass pyrolysis for energy production, global carbon capture and sequestration. *Energy & Environ. Sci.,* 3(11), 1695-1705.

Lefebvre A., Ellers W., Chunn B. (2011). Environmental sustainability of Canadian agriculture. Ottawa, Ontario. *Agriculture and Agri-Food Canada.* Retrieved: May 2019.

Lehmann J. (2007). A handful of carbon. *Nature,* 447, 143-144.

Lehmann J., Joseph S. (2009) Biochar for environmental management: science and technology. *Routledge,* London UK. *Earthscan.* Retrieved: April 2019.

Lehmann J., Rillig M. C., Thies J., Masiello C. A., Hockaday W. C., Crowley D. (2011). Biochar effects on soil biota - A review. Soil Biology and Biochemistry, 43(9), 1812-1836.

Lehmann J. and Rondon M. (2006). Bio-Char Soil Management on Highly Weathered Soils in the Humid Tropics. Biological Approaches to Sustainable Soil Systems, 36, 518-527.

Lehmann J., Gaunt J. Rondon M. (2006). Bio-char sequestration in terrestrial ecosystems – a review. *Mitigation and Adaptation Strategies for Global Change,* 11 (2), 403-427.

Lentz R. D., Ippolito J. A. (2012). Biochar and manure affect calcareous soil and corn silage nutrient concentrations and uptake. *Journal of Environment Quality*, 41 (4), 1033-1043.

Liang B., Lehmann J., Solomon D., Kinyangi J., Grossman J., O'Neill B., Skjemstad J.O., Thies J., Luizao F.J., Petersen J., Neves E.G. (2006). Black carbon increases cation exchange capacity in soils. *Sci. Soc. Am. J.,* 70 (5), 1719-1730.

Liang C., Zhu X., Fu S., Méndez A., Gascó G., Paz-Ferreiro J. (2014). Biochar alters the resistance and resilience to drought in a tropical soil. *Enviro. Res. Lett.,* 9, 064013.

Li H., Smith S.E., Holloway R.E., Zhu Y., Smith F.A. (2006). Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. *New Phytol.,* 172 (3), 536-543.

Li Q., Lei Z., Song X., Zhang Z., Ying Y., Peng C. (2018). Biochar amendment decreases soil microbial biomass and increases bacterial diversity in Moso bamboo (Phyllostachys edulis) plantations under simulated nitrogen deposition. Environmental Research Letters, 13, URL: https://iopscience.iop.org/article/10.1088/1748-9326/aab53a/pdf

Liu B., Liu L., Tian L., Cao W., Zhu Y., Asseng S. (2013). Post-heading heat stress and yield impact in winter wheat of China. *Global Change Biology,* 20 (2), 372-381.

Liu L., Shen G., Sun M., Cao X., Shang G., Ping C. (2014). Effect of biochar on nitrous oxide emission and its potential mechanisms. *Journal of the Air & Waste Management Association,* 64 (8), 894-902.

Liu T., Chen X., Hu F., Ran W., Shen Q., Li H., Whalen J.K. (2016). Carbon-rich organic fertilizers to increase soil biodiversity: Evidence from a meta-analysis of nematode communities. *Agri., Ecosyst. Environ.,* 232, 199-207.

Li Y., Li Y., Chang S.X., Yang Y., Fu S., Jiang P., et al. (2018). Biochar reduces soil heterotrophic respiration in a subtropical plantation through increasing soil organic carbon recalcitrance and decreasing carbon-degrading microbial activity. *Soil Biol. Biochem.,* 122, 173-185.

Lobell D.B., Burke M.B., Tebaldi C., Mastrandrea M.D., Falcon W.P., Naylor R.L. (2008). Prioritizing climate change adaptation needs for food security in 2030. *Science,* 319 (5863), 607-610.

Lucheta A.R., de Souza Cannavan F., Tsai S.M., Kuramae E.E. (2017). Amazonian Dark Earth and its black carbon particles harbor different fungal abundance and diversity. *Pesosphere,* 27 (5), 832-845.

Luo Y. Durenkamp M., De Nobili M., Lin Q., Devonshire B.J. Brookes P.C. (2013). Microbial biomass growth, following incorporation of biochars produced at 350 °C or 700 °C, in a silty-clay loam soil of high and low pH. *Soil Biol. Biochem.* 57, 513-523.

Maracchi G., Sirotenko O., Bindi M. (2005). Impacts of Present and Future Climate Variability on Agriculture and Forestry in the Temperate Regions: Europe. *Climate Change,* 70 (1-2), 117-135.

Maroušek J., Vochozka M., Plachý J., Žák J. (2017). Glory and misery of biochar. *Clean Technologies and Environmental Policy.* 19 (2), 311-317.

Maynard D.G., Kalra Y.P. (1993) Nitrate and exchangeable ammonium nitrogen. *Soil Samplingand Methods of Analysis,* 25-38.

McKenzie N., Coughlan K. and Cresswell H. (2002). Soil Physical Measurement and Interpretation for Land Evaluation. *CSIRO Publishing,* Collingwood, Victoria.

McNeill J.R. and McNeill W. (2003). The Human Web: A Bird's-Eye View of World History. New York, *W. W. Norton & Company*, Retrieved: April 2019.

McNutt M. (2013). Climate change impacts. *Science,* 341 (6145), 435.

Mechler M.A.A. (2018). The effect of biochar on soil health and greenhouse gas emissions in a conventional temperate agricultural system (Published M.Sc. Thesis). University of Waterloo, Waterloo, Canada.

Mechler M.A.A., Jiang R.W., Silverthorn T., Oelbermann M. (2018). Impact of biochar on soil characteristics and temporal greenhouse gas emissions: A field study from southern Canada. *Biomass and Bioenergy,* 118, 154-162.

Mehuys G. R., Angers D. A., Bullock M. S. (2007). Aggregate stability to water. *Soil Sampling and Methods of Analysis, Second Edition*. CRC Press.

Mia S., Singh B., Dijkstra F. A. (2017). Aged biochar affects gross nitrogen mineralization and recovery: a ^{15}N study in two contrasting soils. *Bioenergy,* 9, 1196-1206.

Mosier A., Kroeze C., Nevison C., Oenema O., Seitzinger S., Cleemput O. (1998). Closing the global N2O budget: nitrous oxide emissions through the agricultural nitrogen cycle. *Nutrient Cycling in Agroecosystems,* 52 (2-3), 225-248.

Mukherjee A., Lal R. (2013). Biochar impacts on soil physical properties and greenhouse gas emissions. *Agronomy,* 3 (2), 313-339.

Mukome F.N.D., Zhang X., Silva L.C.R., Six J., Parikh S.J. (2013). Use of Chemical and Physical Characteristics to Investigate Trends in Biochar Feedstocks. *J. Agric. Food Chem.,* 61 (9), 2196-2204.

Najafi E., Devineni N., Khanbilvardi R.M., Kogan F. (2018). Understanding the Changes in Global Crop Yields Through Changes in Climate and Technology. *Earth's Future,* 6 (3), 410-427.

Natural Resources Canada (NRC). (2019). Impacts and Adaptation: Equipping Canadians to Adapt to Climate Change. *Government of Canada.* URL: https://www.nrcan.gc.ca/environment/impacts-adaptation10761. Retrieved: June 2019.

Nelissen V., Ruysschaert G., Manka'Abusi D., D'Hose T., De Beuf K., Al-Barri B., Cornelis W., Boeckx P. (2015). Impact of a woody biochar on properties of a sandy loam soil and spring barley during a two-year field experiment. *European Journal of Agronomy,* 62, 65-78.

Nelissen V., Saha B.K., Ruysschaert G., Boeckx P. (2014). Effect of different biochar and fertilizer types on N2O and NO emissions. *Soil Biol. Biochem.,* 70, 244-255.

Ngatia L.W., Hsieh Y.P., Nemours D., Fu R., Taylor R.W. (2017). Potential phosphorus eutrophication mitigation strategy: Biochar carbon composition, thermal stability and pH influence phosphorus sorption. *Chemosphere,* 180, 201-211.

Niles M.T., Brown M., Dynes R. (2016). Farmer's intended and actual adoption of climate change mitigation and adaption strategies. *Climate Change,* 135 (2), 277-295.

Noble R. (2011). Risks and benefits of soil amendment with composts in relation to plant pathogens. *Australasian Plant Pathology,* 40 (2), 157-167.

Novak J.M., Lima I., Xing B., Gaskin J.W., Steiner C., Das K.C., Ahmedna M., Rehrah D., Watts D.W., Busscher W.J. (2009). Characterization of designer biochar produced at different temperatures and their effects on a loamy sand. *Ann. Environ. Sci.,* 3,195–206.

Novak J.M., Busscher W.J., Watts D.W., Amonette J.E., Ippolito, J.A., Lima, I. M., Gaskin J., Das K.C., Steiner C., Ahmedna M., Rehrah D., Schomberg H. (2012). Biochar's impact on soil-moisture storage in an ultisol and two aridisols. *Soil Science*, 177, 310-320.

Olesen J. E., Rubæk G., Heidmann T., Hansen S., Børgesen C. D. (2004). Effect of climate change on greenhouse gas emission from arable crop rotations. *Nutrient Cycling in Agroecosystems*, 70, 147-160.

Ouyang L., Wang F., Tang J., Yu L., Zhang R. (2013). Effects of biochar amendment on soil aggregates and hydraulic properties. *J. Soil Sci. Plant Nutr.,* 13 (4), 991-1002.

Ould Ahmed B.A., Inoue M., Moritani S. (2010). Effect of saline water irrigation and manure application on the available water content, soil salinity, and growth of wheat. *Agricultural Water Management,* 97 (1), 165-170.

Paetsch L., Mueller C.W., Kögel-Knabner I., von Lützow M., Girardin C., Rumpel C. (2018). Effect of in-situ aged and fresh biochar on soil hydraulic conditions and microbial C use under drought conditions. *Scientific Reports,* 8, 6852.

Pandey V., Patel A., Patra D.D. (2016). Biochar ameliorates crop productivity, soil fertility, essential oil yield and aroma profiling in basil (Ocimum basilicum L.). *Ecological Engineering,* 90, 361-366.

Parkin T. B., Venterea R. T., Hargreaves S. K. (2012). Calculating the detection limits of chamber- based soil greenhouse gas flux measurements. *Journal of Environmental Quality*, 41, 705-715.

Pastor-Villegas J., Rodríguez J.M.M., Pastor-Valle J.F., Rouquerol J., Denoyel R., García G.M. (2010). Adsorption–desorption of water vapor on chars prepared from commercial wood charcoals, in relation to their chemical composition, surface chemistry and pore structure. *J. Anal. Appl. Pyrolysis,* 88, 124–133.

Paterson E., Hall J.M., Rattray E.A.S., Griffiths B.S., Ritz K. (1997). Effect of elevated CO2 on rhizosphere carbon flow and soil microbial processes. *Global Change Biol.,* 3, 363– 377.

Périé C., Ouimet R. (2008). Organic carbon, organic matter, and bulk density relationships in boreal forest soils. *Canadian Journal of Soil Science*, 88 (3), 315-325.

Plaza C., Giannetta B., Fernandez J., Lopez-de-Sa E., Polo A., Gasco G., Mendez A., Zaccone C. (2016). Response of different soil organic matter pools to biochar and organic fertilizers. *Agriculture, Ecosystems & Environment,* 225, 150-159.

Portmann R.W., Daniel J.S., Ravishankara A.R. (2012). Stratospheric ozone depletion due to nitrous oxide: influences of other gases. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.,* 367 (1593), 1256-1264.

Pratscher J., Dumont M. G., Conrad R. (2011). Ammonia oxidation coupled to CO2 fixation by archaea and bacteria in an agricultural soil. *PNAS,* 108 (10), 4170-4175.

Qin H., Xu Q., Cao Z., Wong M. (2011). Population size and nitrification activity of soil ammonia-oxidizing bacteria in long-term intensive managed phyllostachys praecox stands. *Botanical Review,* 77 (3), 313-319.

Qiu S., McComb A.J., Bell R.W. (2016). Ratios of C, N and P in soil water direct microbial immobilization–mineralization and N availability in nutrient amended sandy soils in southwestern Australia. *Agri. Eco. Environ.,* 127, 93-99.

Osman K.T. (2014). Soil Degradation, Conservation and Remediation. Chittagong, Bangladesh. *Springer.* Retrieved: April 2019.

Rajkovich S., Enders A., Hanley K., Hyland Charles., Zimmerman A.R., Lehmann J. (2012). Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. *Biology and Fertility of Soils,* 48 (3), 271-284.

Reay D.S., Davidson E.A., Smith K.A., Smith P., Melillo J.M., Dentener F., Crutzen P.J. (2012). Global agriculture and nitrous oxide emissions. *Nature Climate Change,* 2, 410-416.

Rees F., Simonnot M.O., Morel J. L. (2013). Short-term effects of biochar on soil heavy metal mobility are controlled by intra-particle diffusion and soil pH increase. *European Journal of Soil Science,* 65 (1), 149-161.

Reichstein M., Janssens I.A. (2009). Semi-empirical modelling of the response of soil respiration to environmental factors in laboratory and field conditions. In: Kutsch W.L., Bahn M., Heinemeyer A. (Eds.), *Soil Carbon Dynamics: An Integrated Methodology*. Cambridge University Press, Cambrigde, 207-220.

Reicosky D.C. (1997). Tillage-induced CO_2 emission from soil. *Nutrient Cycling in Agroecosystems,* 49 (1-3), 273-285.

Renella G., Mench M., Landi L., Nannipieri P. (2005). Microbial activity and hydrolase synthesis in long-term Cd-contaminated soils. *Soil Biol. Biochem.*, 37, 133–139.

Robertson G.P., Coleman D.C., Sollins P., Bledsoe C.S. (1999). Standard Soil Methods for Long-term Ecological Research. *LTER*, 106-114.

Robertson G.P., Grandy A.S. (2006). Soil System Management in Temperate Regions. *Biological Approaches to Sustainable Soil Systems,* 27-39.

Ronsse F., van Hecke S., Dickinson D., Prins W. (2012). Production and characterization of slow pyrolysis biochar: influence of feedstock type and pyrolysis conditions. *GCB Bioenergy,* 5 (2), 104-115.

Rosenzweig C., Iglesias A., Yang X.B., Epstein P.R., Chivian E. (2001). Climate change and extreme weather events; implications for food production, plant diseases, and pests. *Global Change and Human Health,* 2 (2), 90-104.

Sakata T., Higashitani A. (2008). Male sterility accompanied with abnormal anther development in plants–genes and environmental stresses with special reference to high temperature injury. *Int. J. Plant Dev. Biol.*, 2, 42-51.

Samson R., Delaquis E., Deen B., DeBruyn J., Eggimann U. (2016). Switchgrass agronomy. *Ontario Biomass Producers Co-operative INC.* URL: https://www.agrireseau.net/documents/Document_93992.pdf

Schär C., Vidale P.L., Lüthi D., Frei C., Häberli C., Liniger M.A., Appenzeller C. (2004). The role of increasing temperature variability in European summer heatwaves. *Nature,* 427, 332-336.

Schaurfler G., Kitzler B., Schindlbacher A., Skiba U., Sutton M.A., Zechmeister-Boltenstern S. (2010). Greenhouse gas emissions from European soils under different land use: effect of soil moisture and temperature. *Euro. J. Soil Sci.,* 61(5), 683-696.

Schimel D. (2010). Drylands in the earth system. *Science,* 327, 418-419.

Schmidt M.W.I, Torn M.S., Abiven S., Dittmar T., Guggenberger G., Janssens I.A., Kleber M. et al. (2011). Persistence of soil organic matter as an ecosystem property. *Nature,* 478, 49-56.

Serrano-Silva N., Luna-Guido M., Fernandez-Luqueno F., Marsch R., Dendooven L. (2011). Emission of greenhouse gases from an agricultural soil amended with urea: A laboratory study. *Applied Soil Ecology,* 47 (2), 92-97.

Simha P., Yadav A., Pinjari D., Pandit A.B. (2016). On the behavior, mechanistic modelling and interaction of biochar and crop fertilizers in aqueous solutions. *Resource-Efficient Technologies,* 2(3), 133-142.

Singh J., Kunhikrishnan A., Bolan N.S., Saggar S. (2013). Impact of urease inhibitor on ammonia and nitrous oxide emissions from temperate pasture soil cores receiving urea fertilizer and cattle urine. *Science of the Total Environment,* 465, 56-63.

Sionit N., Strain B.R., Flint E.P. (1987). Interaction of temperature and co2 enrichment on soybean: photosynthesis and seed yield. *Can. J, Plant Sci.,* 67 (3), 629-636.

Smider B. and Singh B. (2014). Agronomic performance of a high ash biochar in two contrasting soils. *Agriculture, Ecosystems and Environment,* 191, 99-107.

Smith J.L., Collins H.P., Bailey V.L. (2010). The effect of young biochar on soil respiration. *Soil Bio. Biochem.,* 42 (12), 2345-2347.

Smith N. J. H. (1980) Anthrosols and human carrying capacity in Amazonia. *Annals of the Association of American Geographers,* 70 (4), 553-566.

Smith P. (2016). Soil carbon sequestration and biochar as negative emission technologies. *Global Change Biology,* 22 (3), 1315-1324.

Smith P. et al. (AFOLU). In: Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Edenhofer O., Pichs-Madruga R., Sokona Y., Farahani E., Kadner S., Seyboth K., Adler A., Baum I., Brunner S., Eickemeier P., Kriemann B., Savolainen J., Schlömer S., von Stechow C., Zwickel T. and Minx J.C. (eds.)]. *Cambridge University Press*, Cambridge, United Kingdom and New York, NY, USA.

Smith P. (2016). Soil carbon sequestration and biochar as negative emission technologies. *Global Change Biology,* 22 (3), 1315-1324.

Smith P. and Olesen J.E. (2010). Synergies between the mitigation of, and adaptation to, climate change in agriculture. *J. Agri. Sci.,* 148, 543-552.

Smith V.H., Tilman G.D., Nekola J.C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution,* 100 (1-3), 179-196.

Soja G., Bücker J., Gunczy S., Kitzler B., et al. (2014). Economic feasibility of biochar application to soils in temperate climate regions. *Geophysical Research Abstracts,* 16. EGU General Assembly.

Solomon S., Qin D., Manning M., Chen Z., Marquis M., Averyt K.B., Tignor M., Miller H.L. (2007). Understanding and Attributing Climate Change. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change (IPCC).

Sombroek W. G. (1966). Amazon soils: a reconnaissance of the soils of the Brazilian Amazon region. A dissertation, Wageningen University, Wageningen, Netherlands.

Song D., Xi X., Huang S., Liang G., Sun J., Zhou W., Wang X. (2016). Short-term responses of soil respiration and c-cycle enzyme activities to additions of biochar and urea in a calcareous soil. *PLoS ONE,* 11 (9), e0161694 DOI 10.1371/journal.pone.0161694

Spohn M., Giani L. (2011). Total, hot water extractable, and oxidation-resistant carbon in sandy hydromorphic soils-analysis of a 220-year chronosequence. *Plant Soil,* 338, 183-192.

Spokas K.A., Cantrell K.B., Novak J.M., Archer D.W., Ippolito J.A., Collins H.P., Boateng A.A., Lima I.M., Lamb M.C., McAloon A.J., Lentz R.D., Nichols K.A. (2012) Biochar: A synthesis of its agronomic impact beyond carbon sequestration. J. Environ. Qual., 41, 973-989.

Spokas K.A., Reicosky D.C. (2009). Impacts of sixteen different biochars on soil greenhouse gas production. *Annals of Environmental Science,* 3, 179-193.

Sun F., Lu S. (2014). Biochars improve aggregate stability, water retention, and pore-space properties of clayey soil. *Journal of Plant Nutrition and Soil Science,* 177 (1), 26-33.

Sun J., He F., Pan Y., Zhang Z. (2017). Effects of pyrolysis temperature and residence time on physicochemical properties of different biochar types. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science,* 67(1), 1-11.

Sun K., Ro K., Guo M.X., Novak J., Mashayekhi H., et al. (2011) Sorption of bisphenol A, 17a–ethinyl estradiol and phenanthrene on thermally and hydrothermally produced biochars. *Bioresour Technol,* 102, 5757–5763.

Sustainable Agriculture Research and Education (SARE) (2012). Soil Microorganisms. *University of Maryland, Maryland, USA.* Retrieved: April 2019.

Swift M.J., Heal O.W., Anderson J.M. (1979). Decomposition in Terrestrial Ecosystems. Oxford, UK. *Blackwell Scientific.* Retrieved: June 2019.

Taghizadeh-Toosi A., Clough T.J., Sherlock R.R., Condron L.M. (2012). A wood based low-temperature biochar captures NH3-N generated from ruminant urine-N, retaining its bioavailability. *Plant and Soil,* 353 (1-2), 73-84.

Tai A.P.K., Val Martin M., Heald C.L. (2014). Threat to future global food security from climate change and ozone air pollution. *Nature Climate Change,* 4, 817-821.

Tiessen H., Cuevas E., Chacon P. (1994). The role of soil organic matter in sustaining soil fertility. *Nature,* 371, 783-785.

Tipping E., Somerville C.J., Luster J. (2016). The C:N:P:S stoichiometry of soil organic matter. *Biogeochemistry,* 130 (1-2), 117-131.

Thornton P.K., Ericksen P.J., Herrero M., Challinor A.J. (2014). Climate variability and vulnerability to climate change: a review. *Glob. Chang. Biol.,* 20 (11), 3313-3328.

Trazzi P.A., Leahy J.J., Hayes M.H.B., Kwapinski W. (2016). Adsorption and desorption of phosphate on biochars. *J. Environ. Chem. Eng.,* 4 (1), 37-46.

Trenberth K.E., Dai A., van der Schrier G., Jones P.D., Barichivich J., Briffa K.R., Sheffield J. (2013). Global warming and changes in drought. *Nature Climate Change,* 4, 17-22.

Tripathi M., Sahu J.N., Ganesan P. (2016). Effect of process parameters on production of biochar from biomass waste through pyrolysis: A review. *Renewable and Sustainable Energy Reviews,* 55, 467-481.

Uchimiya M., Lima I.M., Klasson T., Wartelle L.H. (2010). Contaminant immobilization and nutrient release by biochar soil amendment: Roles of natural organic matter. *Chemosphere,* 80 (8), 935-940.

Ueyama M., Takeuchi R., Takahashi Y., Ide R., Ataka M., Kosugi Y., Takahashi K., Saigusa N. (2015). Methane uptake in a temperate forest soil using continuous closed-chamber measurements. *Agricultural and Forest Meteorology,* 213, 1-9.

United States Department of Agriculture, USDA. (2011). Carbon to Nitrogen Ratios in Cropping Systems, *USDA NRCS,* URL: https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcseprd331820.pdf

Van Zwieten L., Singh B.P., Kimber S.W.L., Murphy D.V., Macdonald L.M., Rust J., Morris S. (2014). An incubation study investigating the mechanisms that impact N_2O flux from soil following biochar application. *Agriculture, Ecosystems & Environment*, 191, 53-62.

Veraart A.J., de Klein J.J.M., Scheffer M. (2011). Warming can boost denitrification disproportionally due to altered oxygen dynamics. *PLoS One,* 6 (3), e18508.

Voroney R. P., Brookes P. C., Beyaert R. P. (2008). Soil microbial biomass C, N, P, and S. In M. R. Carter & E. G. Gregorich (Eds.), *Soil Sampling and Methods of Analysis* (2nd ed.) 637-651.

Waldrop M.P., Firestone M.K. (2006). Response of microbial community composition and function to soil climate change. *Microb. Eco.,* 52 (4), 716-724.

Wang B., Qiu Y.L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, 16 (5), 299-363.

Wang G., Huang W., Mayes M.A., Liu X., Zhang D., Zhang Q., Han T., Zhou G. (2019). Soil moisture drives microbial controls on carbon decomposition in two subtropical forests. *Soil Biol. Biochem.,* 130, 185-194.

Wang Y., Zhang X., Huang C. (2009). Spatial variability of soil total nitrogen and soil total phosphorus under different land uses in a small watershed on the Loess Plateau, China. *Geoderma,* 150, 141-149.

West T.O., Post W.M. (2002). Soil organic carbon sequestration rates by tillage and crop rotation: a global data analysis. *Soil Sci Soc. Am. J.,* 66, 1930-1946.

Welbaum G.E., Sturz A.V., Dong Z., Nowak J. (2010). Managing soil microorganisms to improve productivity of agro-ecosystems. *Critical Reviews in Plant Sciences.* 23 (2), 175-193.

Whiffin V.S., van Paassen L.A., Harkes M.P. (2007). Microbial Carbonate Precipitation as a Soil Improvement Technique. *Geomicrobiology Journal,* 24 (5), 417-423.

Wildung R.E., Garland T.R., Buschborn R.L. (1975). The interdependent effects of soil

temperature and water content on soil respiration rate and plant root decomposition in arid grassland soils. *Soil Biol. Biochem.,* 7 (6), 373-378.

Wilkinson A.G. (1999). Poplars and willows for soil erosion control in New Zealand. *Biomass and Bioenergy,* 16 (4), 263-274.

Woolf D., Amonette J.E., Stree-Perrott F. A., Lehmann J., Joseph S. (2010). Sustainable biochar to mitigate global climate change. *Nature Communications,* 1, 56.

Worster D. (1990). Transformations of the Earth: Toward an agroecological perspective in history. *Journal of American History,* 76, 1092.

Yang C., Prasher S.O., Wang S., Kim S.H., Tan C.S., Drury C., Patel R.M. (2007). Simulation of nitrate-N movement in southern Ontario, Canada with DRAINMOD-N. *Agricultural Water Management,* 87 (3) 299-306.

Yang J., Li H., Zhang D., Wu M., Pan B. (2017). Limited role of biochars in nitrogen fixation through nitrate adsorption. *Science of the Total Environment,* 592, 758-765.

Yong L., Wu J., Shen J., Liu S., Wang C., Chen D., Huang T., Zhang J. (2016). Soil microbial C:N ratio is a robust indicator of soil productivity for paddy fields. *Sci. Rep.,* 6, 35266.

Young L.W., Wilen R.W., Bonham-Smith P.C. (2004). High temperature stress of Brassica napus during flowering reduces micro- and megagametophyte fertility, induces fruit abortion, and disrupts seed production. *J. Exp. Bot.*, 55(396), 485-495.

Zhang A., Liu Y., Pan G., Hussain Q., Li L., Zheng J., Zhang X. (2012). Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. *Plant Soil,* 351, 263-275.

Zhang C., McGrath D. (2004). Geostatistical and GIS analyses on soil organic carbon concentrations in grassland of southeastern Ireland from two different periods. *Geoderma.* 119, 261-275.

Zhang J., Li H., Shen H., Chen Y., Fang J., Tang Z. (2015). Effects of Nitrogen Addition on Nitrogen Resorption in Temperate Shrublands in Northern China. *PLoS ONE, 10(6): e0130434 DOI 10.1371/journal.pone.0130434*

Zhang J., You C. (2013). Water holding capacity and absorption properties of wood chars. *Energy Fuel*, 27, 2643–2648

Zhang N., Wan S., Li L., Bi J., Zhao M., Ma K. (2008). Impacts of urea N addition on soil microbial community in a semi-arid temperate steppe in northern China. *Plant and Soil,* 311 (1-2) 19-28.

Zimmerman A.R., Gao B., Ahn M. (2011). Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biol. Biochem.* 43 (6), 1169-1179.

Zinn K.E., Tunc-Ozdemir M., Harper J.F. (2010). Temperature stress and plant sexual reproduction: uncovering the weakest links. *J Exp Bot.*, 61(7), 1959-1968.

Zwieten V.L., Singh B., Joseph S., Kimber S., Cowie A., Chan Y.K. (2009). Biochar and emissions of non-CO2 greenhouse gases from soil. In: Lehmann J, Joseph S (eds) Biochar for Environmental Management Science and Technology. *Earth-scan Press*, UK, 227-249.