# The effect of biochar on soil health and greenhouse gas emissions in a conventional temperate agricultural system

by

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**Author's Declaration**

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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**Statement of Contributions**

A. Chapter 2 was a collaborative effort between candidate and coauthors: Maren Oelbermann, Will Jiang, Laura McFarlan, Teressa Silverthorn and Lily de Loë. The collection and analysis of in-situ data was completed by the candidate and co-authors 2-4. The written manuscript chapter 2 was completed by the candidate and edited by coauthor 1 and 2.

Chapter 3 was a collaborative effort between candidate and coauthors: Maren Oelbermann and Will Jiang. The collection and analysis of in-situ data was completed by the candidate and co-author 2. The written manuscript chapter 3 was completed by the candidate and edited by coauthors.

B. Chapter 2 contributions: Primary in situ data collection and analysis (75% candidate, 25% co-authors 2-4). Written manuscript completed by candidate (90%) with editing by co-authors (10%).

Chapter 3 contributions: Primary in situ data collection and analysis (80% candidate, 20% co-author 2). Written manuscript completed by the candidate (90%) with editing by co-authors (10%).

**Abstract**

Biochar has been present in tropical agriculture as a soil amendment for millennia. Its ability to alter soil physical and chemical characteristics has been utilized to improve soil health and crop productivity. The use of biochar in temperate agricultural soils is a new concept, and has been practiced for about a decade. To date, few long-term field studies have quantified the temporal effects biochar has on soil health or greenhouse gas emissions (GHGs) of temperate agricultural soil. The objectives of this study were to quantify differences in soil characteristics of biochar and non-biochar amended soil, to determine the relationship between emissions and soil characteristics, and to determine temporal variations in GHG emissions in temperate agricultural soils following biochar additions. The treatments were (1) 6 t/ha poultry manure plus 135 kg/ha of nitrogen (urea) fertilizer (**MN**), (2) 3 t/ha poultry manure plus 3 t/ha biochar (**MB**) and (3) 3 t/ha poultry manure, 3 t/ha biochar plus 135 kg/ha fertilizer (**MNB**). It was found that the vast majority of analyzed attributes were unchanged by biochar additions. Soil moisture, temperature, and $PO_4^{3-}$ were however significantly greater ($P<0.05$) in the conventional treatment, while C/N ratios and light fraction distribution within the soil were temporarily altered by additions. Though not significant, biochar soils, MB and MNB, appeared favorable for corn yield and aboveground biomass accumulation (2016). The opposite effect was found for soybean yield (2017), although this was still not significant. Soil $CO_2$ and $N_2O$ emissions were not significantly different ($P<0.05$) among the conventional treated soils and biochar-amended soils in 2016 and 2017. $CO_2$ and $N_2O$ emission rates were similar in both field seasons. Results from this study revealed that a low biochar addition rate had few, or temporary impacts on soil health and greenhouse gas emissions.

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## 1. Literature Review and Thesis Objectives

### 1.1 Biochar in Agriculture

Climate change (CC) has become closely associated with industries such as agriculture, which account for 10-12% of anthropogenic greenhouse gas (GHG) emissions (IPCC, 2014). While contributing to CC, agriculture is as well acutely vulnerable to increasing CC impacts, such as frequent drought events which damage crop yields (Motha & Baier, 2003). With the ever growing concern over CC, it is of great interest to academics, policymakers, and farmers to find feasible methods of addressing the impacts agriculture and CC have on each other. In temperate agriculture, biochar is being investigated as a potential solution to both slow the rate of climate change and ameliorate the severe effects CC may pose to soil health and crop production (Ameloot et al., 2013; Ajayi & Horn, 2017; Jones et al., 2013).

Biochar is merely charcoal added to soil with the intent of improving soil health and its characteristics, such as nutrient retention, for increased crop productivity (Kloss et al., 2012; Lehmann & Joseph, 2009; Lone et al., 2015). The sustainable nature of biochar is another essential defining feature. To qualify as biochar, the char feedstock must be sourced sustainably, and contribute to improved environmental outcomes for agriculture (Lehmann & Joseph, 2009). Like charcoal, it is comprised of thermally decomposed (pyrolyzed) biomass, which causes carbon to form a large body of polycyclic aromatic hydrocarbon structures (Kloss et al., 2012). Unlike graphite, which has a purely aromatic crystalline structure, biochar has additional functional groups and is therefore amorphous (Atkinson et al., 2010). These functional groups give the char heterogeneous chemical properties (Lehmann & Joseph, 2009; Atkinson et al., 2010). The functional groups can form regions of acidity and alkalinity, hydrophobicity and hydrophilicity, and locations of cation and anion exchange on the same body of char (Atkinson et

al., 2010; Lehmann & Joseph, 2009). The individual pieces of char, which have variable chemical makeup, can be chemically interactive with soil (Atkinson et al., 2010; Lehmann & Joseph, 2009). The characteristics of biochar are determined by three primary factors; the biochar feedstock, the temperature of pyrolysis, and the residence time in a kiln (Lehmann & Joseph, 2009; Glaser et al., 2002). The desired properties of the biochar can be controlled using these three factors (Glaser et al., 2002).

Despite variability among individual chars, biochar is broadly characterized by its high surface area and stable carbon (Lehmann & Joseph, 2009; Smith et al., 2010). As the carbon restructures during pyrolysis, other molecules are lost as smoke/tar, and pore spaces are formed giving biochar its characteristically large surface area (Keiluweit et al., 2010). The number and size of pores, however, depends heavily on pyrolysis temperature (Glaser et al., 2002). Typically, higher burn temperatures result in larger pore spaces and greater surface area (Lehmann & Joseph, 2009). Pore spaces are important as they function in many of the soil-biochar interactions (Rogovska et al., 2014).

Biochar is comprised of extremely stable carbon structures and undergoes little change in a variety of environments, which is a useful property (Schneider et al., 2011; Glaser et al., 2002). A study by Schneider et al. (2011) found that after a century of weathering in a tropical climate there was no significant change in the physical or chemical composition of biochar. Even after centuries of rain, the Terra Preta soils in the Amazon Basin are considered to have maintained their original stable biochar (Glaser et al., 2001, Petter & Madari, 2012). The anthropogenic nature of the Terra Preta soil shows that biochar has been a tropical soil amendment used for thousands of years (Petter & Madrari, 2012). Treating agricultural land with fire is not unique to South America: nearly 40% of global soils having been fire altered (Atkinson et al., 2010; Karer

et al., 2013). Globally, biochar has primarily been introduced by the "slash and burn" technique of land-clearing. Slash and burn was very popular in tropical climates to improve soil organic carbon (SOC), which allowed ash and other by-products to be quickly mineralized (Atkinson et al., 2010). Along with ash, deposits of charcoal are formed and build up in the soil after each burn event (Schneider et al., 2011). However, large amounts of charcoal have also been intentionally added to soil, such as in Zambia and the Amazon where charcoal was produced in hearths for agricultural amendments (Spokas et al., 2012).

Tropical soils can possess properties that limit agricultural productivity due to weathered mineral components and their long history of intensive land use (Glaser et al., 2002; Lehmann & Rondon, 2006). Low cation exchange capacities (CEC), low pH, and rapid carbon mineralization in tropical soil results in low carbon content and reduced yields (Lehmann et al., 2003; Güereña et al., 2013). These factors make it difficult for land to remain productive over long periods of time, especially now amidst CC (Lehmann & Rondon, 2006). For example, issues such as water scarcity will increase as the climate changes (Karer et al., 2013). Increasing water retention is one way that biochar can reduce CC detriments (Dil et al., 2014). Biochar has been an effective amendment within the tropics to improve a variety of soil physical and chemical characteristics (Lehmann et al., 2003; Glaser et al., 2002; Karer et al., 2013).

While biochar has been used for millennia in the tropics, it has only recently begun to be explored in temperate agriculture (Atkinson et al., 2010). Research on the use of biochar in temperate agriculture is a developing field, with studies focusing on biochar beginning in the 2000s (Lehmann et al., 2011). Up to 2010, only a moderate body of research had been conducted within laboratory settings (Atkinson et al., 2010). However, within the past five years, field experiments have begun in temperate climates across the globe (Nelissen et al., 2015). The

outcomes of these experiments have varied greatly depending on how the local soil and biochar properties interact. Light textured soils especially have benefitted from higher temperature biochars, which due to their high porosity improve retentive properties of the soil (Dil et al., 2014).

## 1.2 Impacts of biochar on soil health in temperate agriculture

### *1.2.1 Soil Physical Characteristics*

Soil health is comprised of the physical, chemical, and biological soil components that contribute to or maintain, soil environmental and agronomic productivity (Lone et al., 2015; Lal, 2011). Healthier soils can sustain greater agricultural productivity and ecological integrity (Lal, 2011). The critical physical characteristics which contribute to soil health are; texture, structure, drainage, and water retention (Lal, 2011). By influencing these characteristics, biochar can affect soil health. In both tropical and temperate agricultural projects, biochar has been found to alter soil moisture and water interactions (Borchard et al., 2014; Hammond et al., 2013). Specifically, biochar can improve soil water infiltration and water-holding capacity (Karer et al., 2013 Borchard et al., 2014). These improvements are a result of biochar's pore space (Lehmann et al., 2011). Pore space increases water-holding capacity (WHC), which reduces the effects of water stress and can also facilitate oxygen diffusion (Dil et al., 2014; Lehmann et al., 2011; Jones et al., 2011b). Biochars produced at low temperatures (200-400°C) have more, but smaller, micropores and lack nanopores (Zimmerman et al., 2011; Lehmann & Joseph, 2009). High-temperature biochars (500-700°C) have nanopores and fewer but larger micropores, as pores become conjoined (Zimmerman et al., 2011; Lehmann & Joseph, 2009). These pores physically absorb and retain water. As a result, treating the soil with biochar not only increase water infiltration

rates but also available moisture (Hammond et al., 2013; Karer, 2013). Though rarely observed, among some soil and biochar combinations, water may be removed from the soil and held within the biochar structure, making the soils drier (Steiner et al., 2010). The absorption of soil water has been seen to improve aeration and therefore aerobic microbial communities. Though Steiner et al. (2010) did not investigate crop yields in their study, it follows that negative implications for crops could exist, especially in dry years, following moisture loss to biochar.

The high porosity of biochar also makes it lighter than many other soil constituents, and when biochar is incorporated in soil it can decrease bulk density (Bamminger et al., 2016). Biochar can also improve soil structure, and thereby soil health, by promoting stable soil aggregate formation (Liu et al., 2012). It can do so by acting as a binding agent for soil organic matter, the critical constituent which binds all other soil particles together (Browdowski et al., 2006; Liu et al., 2012; Bronick & Lal, 2005). The bound soil particles then alter other physical characteristics by improving air and water transportation, and resisting compaction. Soil structure not only exerts influence on other physical characteristics but chemical processes as well (Bronick & Lal, 2005; Liu et al., 2012).

### *1.2.2 Soil Chemical Characteristics*

The capacity of soil to sustain chemical reactions is a crucial attribute of healthy soils (Lal, 2011). The mechanisms behind how biochar influences soil chemical properties remain poorly understood (Atkinson et al., 2010; Glaser et al., 2002). One commonly observed effect has been biochar's increase of tropical soil pH, which has been one of the significant benefits of its use in these systems (Lehmann & Joseph, 2009; Atkinson et al., 2010). Though less frequent and less permanent, increases to temperate soil pH have also been observed, primarily by the use

of high temperature biochars (Ander et al., 2013; Anderson et al., 2011; Bamminger et al., 2014; Glaser et al., 2015; Imparato et al., 2016). These increases have been attributed to by-products of pyrolysis (alkaline minerals) and the additional functional groups on biochar (Lehmann et al., 2011). Though biochar has an array of side-chain functional groups, it is mostly comprised of hydrocarbons (Lehmann et al., 2011).

Soil organic carbon (SOC) levels are strong determinants of soil health, as they influence other chemical, physical, and biological characteristics (Lal, 2011). SOC is often lost from agricultural soils for reasons including; lower biomass returns, altered temperature and moisture regimes which increase decomposition, and susceptibility to leaching and erosion (Lal, 2011). Biochar research has investigated how SOC can be built and the carbon cycle altered following additions. Labile biochar carbon, those that form side-chain functional groups, can interact with more active C pools, like the free-light fraction or hot water extractable carbon (Vasilyeva et al., 2011; Liang et al., 2008). SOC, however, is a large soil reservoir, and typically biochar effects are indistinguishable in the first several years of studies (Dil et al., 2014). Depending on the type of soil and biochar, SOC can be impacted in two ways; recalcitrant portions can accumulate, or labile components can trigger the decomposition of pre-existing SOC (Lentz & Ippolito, 2012; Luo et al., 2011; Jones et al., 2011b). As already mentioned with the formation of stable aggregates, biochar's reactive components can bind and retain SOC, leading to carbon accumulation within the soil (Liu et al., 2012; Lentz & Ippolito, 2012). The remaining body of biochar is aromatic hydrocarbons, which are recalcitrant and will not breakdown in soil, therefore contributing to even greater carbon storage within the soil (Ippolito et al., 2012). The portion of labile carbon is readily consumed by microbial communities (Domene et al., 2014). Some researchers have hypothesized that biochar's ability to retain soil nutrients can optimize

soil nutrient ratios, allowing microbes to decompose previously inaccessible soil carbon (Lone et al., 2015).

Surface functional groups on biochar create localized regions of cation and anion exchange (Lehmann et al., 2011). Due to the larger surface area of biochar, there are many opportunities for interactions with soil nutrients (Atkinson et al., 2010). In this way, biochar can improve soil health since nutrient retention is a critical factor of soil health. In general, cation exchange capacity is increased in biochar soil, enhancing the availability of nutrients such as $NH_4^+$, $Mg^{2+}$, and $K^+$ (Atkinson et al., 2010; Glaser et al., 2002). When biomass is burned at high temperatures to produce high porosity, nitrogen and other macro- and micronutrients are burned off as tars (Lehmann & Joseph, 2009). Following the addition of this porous and nutrient depleted matter to the soil, it can quickly absorb these nutrients from the rhizosphere (Ding et al., 2010; Borchard et al., 2014; de la Rosa & Knicker, 2011). This absorption can prevent nutrient leaching within the soil, but also reduce plant access to these nutrients (Güereña et al., 2013). Nutrient availability is the dominant limiting factor for temperate agriculture (de la Rosa & Knicker, 2011). Therefore, newly pyrolyzed biochar can temporarily decrease crop yields by absorbing micro- and macronutrients (Borchard et al., 2014). As previously mentioned, biochar feedstock and pyrolysis-temperature will determine the extent of these effects (Lehmann & Joseph, 2009). Desirable attributes, such as high pore space (which allow for more microbes, water holding capacities, and nutrient exchange), require high pyrolysis temperatures (Lehmann & Joseph, 2009).

### *1.2.3 Soil Biology*

Changes to soil physical and chemical characteristics are expected to have an impact on soil biology (Lehmann et al., 2011). Soil biology is the final key component of soil health (Lal, 2011). Healthy soils must be free from both pests and pathogens, maintain high biodiversity and biomass, as well as contribute to beneficial physical and chemical properties (Lal, 2011). Macrofauna is an essential component of soil biology, as they influence soil biogeochemical processes, the soil food web, and plant-available nutrients (Marks et al., 2014; Lehmann et al., 2011; Domene et al., 2015). Soil invertebrates can also indicate soil quality and ecotoxicity, along with altering the ecosystem conditions (Lehmann et al., 2011). For example, soil macrofauna often modifies soil structures and therefore material exchange in the soil (Domene et al., 2015). In this way, soil macrofauna can ultimately impact crop production (Decaëns et al., 2006). Soil health and crop management also regulate macrofauna populations, which respond to soil pH, nutrient availability, and crop residues (Domene et al., 2015). These, in turn, alter soil moisture and C/N ratios (Domene et al., 2015; Reibe et al., 2015; Lavelle et al., 2006). Unfortunately, very little temperate research has been carried out to date on biochar interactions with soil macrofauna (Reibe et al., 2015; Castracani et al., 2015; Ameloot et al., 2013). Biochar impacts a variety of soil characteristics, and how invertebrates react will be highly dependent upon soils and amendment schemes (Domene et al., 2015). One suggested way that biochar influences macrofauna is by affecting their food source (Domene et al., 2015). Increased bacterial numbers, which can be improved by biochar, generally enhance macrofauna grazing (Domene et al., 2015). However, species that do not specifically graze on rhizosphere bacteria, such as some earthworms, have still been observed to prefer biochar soils for yet unknown reasons (Lehmann et al., 2011). Interactions with earthworms are of interest since they can

consume and excrete mineral enriched biochar and distribute it within the soil profile (Marks et al., 2014; Domene et al., 2015; Lehmann et al., 2011). On the whole, the specific interactions between soil invertebrates and microbial biota in biochar treated soil are not well understood (Lehmann et al., 2011; Ameloot et al., 2013; Marks et al., 2014; Domene et al., 2015).

Within temperate biochar projects, the majority of biological research has focused on microbial communities (Domene et al., 2014). Both microbial biomass and activity generally increase with greater biochar additions, but to a degree community nutrient efficiency can also be improved (Bamminger et al., 2016; Domene et al., 2015). The labile components of biochar are held partially responsible for these changes, along with the alterations to aeration and pH which will favour particular microbial species (Bamminger et al., 2016; Lehmann et al., 2011). The general increase in soil microbial activity as a result of biochar can mean increases in metabolic-by-products, which impact soil aggregation and nutrient cycling (Lehmann et al., 2011). The fundamental shift in community composition following biochar additions also effects soil health as nutrient cycles change, for example, biochar can decrease nitrifier populations (Luo et al., 2011; Mitchell et al., 2015; Bamminger et al., 2014; Anderson et al., 2011). In other studies, the addition of biochar favoured fungi and gram-positive bacteria, which can also impact soil nutrient levels (Bamminger et al., 2014; Gomez et al., 2014; Mitchell et al., 2015).

### *1.2.4 Crop Yield and Biomass*

The impact of biochar on crop yields are of paramount concern for agricultural producers. Improvements to crop yields have been observed in temperate biochar studies and can range from increases of 5% to upwards of 100% (Hammond et al., 2013). Biochar benefits crops by improving nutrient and moisture retention (Atkinson et al., 2010). However, improvements are

not universally experienced (Borchard et al., 2014; Hammond et al., 2013). Many temperate studies found no impact or even initial decreases in crop yield (Hammond et al., 2013; Karer et al., 2013; Borchard et al., 2014). Nutrient availability is the primary limiting factor of agriculture, fortunately in temperate agriculture nutrient unavailability is not further exasperated by low CEC and pH, which decrease nutrient retention (Güereña et al., 2013; Anderson et al., 2011). As noted above, nitrogen loss is reduced in biochar systems, but this does not mean that it is more available to crops (Lehmann et al., 2003). At times, the nitrogen is tightly bound to biochar and unavailable (Borchard et al., 2014). In this way, it can contribute to an initial decrease in crop yields (Borchard et al., 2014). However, research by Kloss et al. (2014), Schulz et al. (2013), and Glaser et al. (2015) showed that adding nutrient sources (i.e., fertilizers, composts, manure) ameliorated these initial adverse effects. The majority of studies that did not find a reduction in yields had several other factors in common: the biochar was formed in traditional kilns, the feedstock was high in plant nutrients, and they were implemented in coarse-textured soils (Haefele et al., 2011; Spokas et al., 2012, Glaser et al., 2002; Borchard et al., 2014).

Nearly all biochar studies have focused on its soil chemical impacts and the effects to crop yields (Lehmann et al., 2011; Ameloot et al., 2013). As a result of this, and the immaturity of this field of study, there remain many gaps in research on the full effects of biochar, as noted above. Just as other amendments have optimal application rates for specific crop and soil type combinations, biochar ought to be appropriately applied (Schulz et al., 2011; Ameloot et al., 2013). However, due to limited studies to inform ideal rates, these prescriptions have not been determined for individual crops or soil types, let alone the combinations thereof (Ameloot et al., 2013). This knowledge would allow farmers to access biochar's variety of benefits, including

carbon sequestration, without sacrificing soil productivity in their agricultural system (Atkinson et al., 2010).

## 1.3 Biochar and GHG emissions in temperate agricultural systems

The addition of biochar to lower soil GHG emissions is another relatively new concept (Hüppi et al., 2015). As a result of this, the mechanisms by which biochar alters soils components, and therefore GHG emissions is unclear (Spokas & Reicosky, 2009; Kuzyahov et al., 2014; Anderson et al., 2011). Sohi et al. (2010) and Clough & Condron (2010) identified no existing peer-reviewed field studies investigating GHG emissions before 2010. However, since then, several field studies have been undertaken in temperate environments (Appendix A); still few resembled typical Canadian agroecosystem management practices (Appendix A). The majority of GHG biochar studies have been conducted using micro- and mesocosms, which do not capture temporal variations in emissions (Clough & Condron, 2010). It is important to understand temperate soil responses to varying quantities and types of biochar additions and common agronomic fertilizer or manures. As well, it is important to uncover how these additions impact GHG emissions (Glaser, 2015).

### *1.3.1 Carbon Dioxide Emissions*

Not only does biochar impact the carbon cycle by storing once atmospheric carbon in the soil, but it also alters soil conditions causing a positive feedback loop (Jones et al., 2011b). Soil moisture, pH, and nutrient availability impact microbial activity and therefore soil carbon cycling (Lehmann et al., 2013). However, the amount and type of carbon in the soil (i. e. labile or recalcitrant) strongly determined microbial interactions and the rate of $CO_2$ emissions (Smith et

al., 2010). Newly incorporated biochar has portions of labile carbon which are consumed by soil microbes (Luo et al., 2011; Ameloot et al., 2013: Liang et al., 2008). In most temperate studies there were initial peaks in $CO_2$ release following biochar incorporation into the soil, these peaks were most distinct when larger portions of biochar were used (Mitchell et al., 2015; Gomez et al., 2014). These initial peaks were followed by emission decreases as biochar aged and labile compounds were entirely consumed (Jones et al., 2011b; Zimmerman et al., 2011; Cross & Sohi, 2011; Steinbeiss et al., 2009; Liang et al., 2008; Kammann et al., 2012). In many temperate biochar projects, no changes to $CO_2$ were also commonly observed (Kuzyakov et al., 2009; Knoblaunch et al., 2011). In some cases, as seen with Bamminger et al. (2014) $CO_2$ emissions decrease following biochar additions. Their decrease was the result of a biochar-induced fungal dominant microbial community (Bamminger et al., 2014).

### *1.3.2 Nitrous Oxide Emissions*

Presently ~60% of $N_2O$ emissions are a result of agricultural practices (Smith et al., 2007; Nelissen et al., 2014). Nitrous oxide ($N_2O$) is a very potent GHG, 265 times more potent than $CO_2$, and greater amounts have been released recently due to agricultural fertilizers and soil disturbances (Bamminger et al., 2014; USEPA, 2007). Some studies have identified biochar's potential to decrease $N_2O$ emissions, though the exact mechanisms promoting this are still undetermined (Case et al., 2015). Some studies propose physical changes (i.e., moisture and aeration) are behind the change in emissions, while others indicate it is chemical effects (i.e., lower $NH_4$ availability), and others still say ecological (i.e., shifts in microbial communities) (Deng et al., 2015; Anders et al., 2013; Case et al., 2015). Ultimately, biochar has been documented to increase soil aeration, soil pH, and N immobilization; thereby suppressing $N_2O$

emissions (Cayuela et al., 2013; Signor & Cerri, 2013; Condron et al., 2013). While the majority of studies have observed decreased emissions, many have noted a short-term emission peak from temperate soil amended with biochar (Yuan et al., 2017; Smith et al., 2010). Increases have been attributed to ecological changes and enhanced enzyme activity among microbial communities (Yuan et al., 2017). Whether biochar's direct impact is to physical or chemical characteristics, the soil biota that drives the nitrogen cycle will be impacted. Chemically emissions can increase as a result of increased N retention within soils (Singh et al., 2010).

### 1.4 Knowledge Gaps

It is yet undetermined whether biochar is an effective amendment to soils used for temperate agriculture (Atkinson et al., 2010). Biochar results are variable and depend on biochar type (feedstock composition, pyrolysis temperature, and available oxygen during pyrolysis) and the soil type it is added to (Imparato et al., 2016; Atkinson et al., 2010). For this reason, to identify patterns in the impact of biochar, further studies are needed to evaluate interactions between different biochar and soils types. Long-term studies as well are required to characterize the long-term relationships between soil health, greenhouse gas emissions, and biochar. The greatest gaps among all soil and biochar types, are the effects on crop growth, macrofauna, microbial communities, and the driving factors behind greenhouse gas emission (Ameloot et al., 2013; Castracani et al., 2015; Anderson et al., 2011; Spokas & Reicosky, 2009; Hüppi et al., 2015). While physical and chemical changes have been documented, these interactions with biological systems are complex. For this reason, while observed soil changes have been theorized to benefit plants and soil fauna, such effects have not always been observed (Ameloot et al., 2013; Anderson et al., 2011; Hüppi et al., 2015; Spokas & Reicosky, 2009). The poor

understanding of soil microbial communities in response to biochar may be contributing to the lack of knowledge surrounding macrofauna, crop yields, and greenhouse gas emissions (Domene et al., 2014; Domene et al., 2015; Bamminger et al., 2014).

### 1.5 Thesis Objectives and Hypothesis

To address the aforementioned knowledge gaps, this study aimed to assess the suitability of biochar as a soil amendment in temperate agricultural practices, both as a tool for achieving GHG neutral agriculture and for promoting soil health. The specific objectives of this study therefore were:

1) Determine effects of biochar amendment on soil physical, chemical, and biological characteristics,

2) Quantify effects of biochar amendment on crop grain yields and biomass production, and

3) Quantify effects of biochar amendment on temporal GHG emissions.

These objectives are motivated by the goal to contribute new science on how to successfully utilize biochar in temperate agricultural systems. The following manuscripts presented in this thesis will address these objectives and relate to the following hypothesis:

The biochar design used in this study, of a low addition rate, will store carbon in the soil without negatively impacting soil health, crop growth, or stimulating GHG release from Ontario agricultural soils.

The first manuscript in this thesis investigates two years of effects on soil health, crop yields/ biomass productivity, and biological impacts following biochar additions. These characteristics were analyzed from 2016-2017. Macrofauna analysis was carried out once a season (spring, summer, and fall) to determine the interacting effects of biochar and season. At

harvest, soil characteristics and crop production were measured. These analyses were carried out to observe the short-term impacts of biochar, as well as, anticipate future changes to soil chemistry. The second manuscript is comprised of the results of a two-year bi-weekly GHG sampling experiment. $CO_2$ and $N_2O$ sampling were carried out in conjunction with bi-weekly soil analysis. This study was implemented to distinguish the influence of biochar on GHG emissions across seasons and to determine which soil characteristics were the most impactful for these emissions. One biochar-altered soil element alone was not identified to dominate these processes. The conclusions drawn are most appropriately interpreted in the context of temperate sandy-textured soils.

### 1.6 Literature Review Summary

In summary, biochar has a potential to impact soil health and greenhouse gas emissions within temperate agricultural systems. However, due to the variability of impacts dependent on biochar quantity and quality, its suitability for temperate systems this is not certain. This project is needed to determine whether negative effects can be avoided and if any benefits can be accessed in an Ontario agricultural system. This project is valuable as well in contributing much-needed information as to how biochar, directly and indirectly, impacts crop productivity, soil macrofauna, and microbial communities. As well as, this project weighs in on the value of biochar as a carbon-negative farming practice.

## 2. Effect of biochar on soil health and crop growth in temperate agriculture

### 2.1 Overview

While biochar is commonly used in tropical agriculture to improve soil health, its addition to temperate agricultural soils represents relatively new territory. The objectives of this study were to evaluate soil health and crop productivity in a conventional agricultural production system amended with biochar. The treatments, established on a sandy loam soil in Southern Ontario, consisted of (1) poultry manure (6 t/ha) and nitrogen fertilizer (urea) (135 kg/ha) (**MN**); (2) poultry manure (3 t/ha) and biochar (wood source material) (3 t/ha) (**MB**); and (3) poultry manure (3 t/ha), fertilizer (135 kg/ha), and biochar (3 t/ha) (**MNB**). Following the treatments, several tests were conducted at harvest to determine the effects of biochar on soil health and crop growth. Results showed that biochar additions significantly ($P>0.05$) lowered soil moisture in the first and second year of the project. Moisture was 12.7 % (s.e.= 0.39), 10.1 % (s.e.= 0.34), and 10.7 % (s.e.= 0.36) in MN, MB, and MNB treatments, respectively. Soil C/N ratios and microbial diversity were the only characteristics affected in the first year of this project. Soil C/N was 9.2 in MN, 7.8 in MB, and 7.5 in MNB. In 2016, soil richness was found to increase in MB and MNB, though diversity decreased. These differences were not sustained through the second year of the project. $PO_4^{3-}$ concentrations were significantly lower in biochar treated soil within the first year of this study, but by the second year were higher than the conventional treatment. In year 1 (2016), mean bicarbonate extractable phosphate-P ($PO_4^{3-}$P) levels were 55.4 mg P/kg, 44.8 mg P/kg, 48.8 mg P/kg, in MN, MB, and MNB treatments, respectively. In year 2 (2017), the concentrations were 76.9, 80.6, and 90.2 mg P/kg in MN, MB, and MNB respectively. None of these changes resulted in significant differences ($P<0.05$) to corn (2016) or soybean (2017) grain yield, shoot or root biomass.

## 2.2 Introduction

Biochar is a charcoal-based soil amendment that aims to improve soil health and productivity (Ameloot et al., 2013). This carbon-rich material is known to modify both soil's physical and chemical characteristics, and subsequently plant growth (Atkinson et al., 2010). Biochar has been an amendment in tropical agricultural systems for millennia, however, only within the last decade has research explored how biochar modifies soil characteristics and processes in temperate agriculture (Cayuela et al., 2013; Atkinson et al., 2010). Despite the growing field of research, it is difficult to identify the interacting mechanisms and predict biochar's impacts within temperate soils (Atkinson et al., 2010; Borchard et al., 2014; Luo et al., 2013).

Changes to soil physical properties directly impact soil health, by contributing to material exchange and structural integrity, but also indirectly by influencing soil chemistry and biology (Lal, 2011). Physical characteristics are commonly modified in temperate soils after biochar additions (Atkinson et al., 2010). Increased infiltration rates and moisture retention are widely observed due to biochar's porosity (Karer et al., 2013; Nelissen et al., 2015; Bamminger et al., 2016). The outcome of treatments is dependent mainly on the type and quantity of biochar, as well as the nature of recipient soils. Though most studies found increased moisture and retention, a few studies including Steiner et al. (2010) observed a decrease in soil moisture. In addition to this, biochar porosity has also decreased soil bulk density, and therefore lowers temperate soil's susceptibility to compaction (Karer et al., 2013; Bamminger et al., 2016). A lower bulk density is beneficial for soil ecology and crop root growth (USDA). Biochar has also contributed to increasing soil stability, preventing erosion and preserving ecological niches, thereby enhancing soil biodiversity (Martinsen et al., 2014; Jones et al., 2012; Nelissen et al., 2015). The physical changes explored here are especially beneficial for coarse-textured soils, which retain little

moisture and do not naturally form strong soil aggregates (Rogovska et al., 2014; Ajayi & Horn, 2017). Many of these changes are further helpful amidst climate change. With climate change, Ontario is likely to see an increase in extreme weather events (Reid et al., 2007). These extreme events will mean extended periods without precipitation and infrequent but heavy rainfall that can destroy aggregates and increase soil erosion (Reid et al., 2007). Biochar can potentially benefit agriculture by storing soil moisture during drought periods and stabilizing soil particles (Karer et al., 2013; Lone et al., 2015).

Impacts on chemical conditions can be more variable than those on physical characteristics. pH is one chemical property that consistently increases among biochar studies (Prendergast-Miler et al., 2011; Schomberg et al., 2014; Rutigiano et al., 2014; Luo et al., 2011). An elevated pH is desired in agriculture because it increases the availability of essential nutrients, such as P and K, as well as increases soil fertility and microbial diversity (Atkinson et al., 2010; Anders et al., 2013). pH is not a great concern in Southern Ontario, which has calcareous soils that buffer against high acidity (Lentz & Ippolito, 2011). Temperate studies, many of which also have calcareous soils, saw no pH change or just observed an initial increase that settled back to normal over time (Güereña et al., 2013; Jones et al., 2012). pH drives nutrient availability and will impact biota, favouring certain species (Domene et al., 2015). Unlike pH, soil organic carbon (SOC) and total nitrogen (TN) experienced a greater variety of effects in temperate studies (Jones et al., 2012; Karer et al., 2013; Lentz & Ippolito, 2012; Luo et al., 2011). Some studies have observed biochar stabilizing these nutrients and contributing to the accumulation of C and N in soil (Lentz & Ippolito, 2012; Yuan et al., 2017; Liang et al., 2008). However, others have concluded that biochar can trigger mineralization of SOC (Luo et al., 2011; Qayyum et al., 2012; Mitchell et al., 2015). The ratio between SOC and TN (C/N ratio) is

a vital soil health quality indicator that generally increases following biochar additions (Atkinson et al., 2010; Nelissen et al., 2015; Anders et al., 2013). C/N ratio increases are attributed to biochar contributing carbon or stabilizing native SOC (Atkinson et al., 2010; Nelissen et al., 2015). SOC is a substantial component of soil and large portion it is stable, therefore it is difficult to observe long-term changes to SOC (Awale et al., 2017). Studies investigating effects 1-2 years after biochar additions may not capture or convey the result of biochar additions (Dil et al., 2014). Soil free light fraction (FLF) was analyzed to observe short-term contributions to future SOC and TN. FLF is a portion of soil made up of newly incorporated biomass which can be consumed by soil biota or overtime convert into a stable, inactive portion of SOC and TN (Gosling et al., 2013; Liang et al., 2008). Investigating soil FLF accumulation is an approach to project an increase or decrease of SOC and TN (Gosling et al., 2013). In the literature, it has been found that biochar contributes the most to the heavy-fraction of soil, with only ~30% becoming incorporated in the FLF (Vasilyeva et al., 2011). While few studies that have explored this, it has been suggested that biochar will change the distribution of FLF carbon and nitrogen in the soil horizon (Vasilyeva et al., 2011).

Biochar's modifications to nutrient cycles have been a focus within literature (Clough et al., 2013). The biochar pyrolysis temperature and feedstock type; exert a considerable influence on the degree of effects to soil nutrients (Akinson et al., 2010). In temperate agriculture, biochar has appeared to modify the nitrogen cycle, impacting biotic and abiotic interactions within the soil and on the surface of biochar (Clough & Condron, 2010). Some studies have seen no impact on $NH_4^+$ and $NO_3^-$, with nitrogen absorption offset by reduced N losses (Jones et al., 2012; Nelissen et al., 2015). Many studies found short-term absorption of N by biochar, which reduces the concentration of bioavailable N species but also reduces leaching (Anders et al., 2013;

Bamminger et al., 2014). Among studies with low temperature (<400°C) biochar (Anders et al., 2013), N was found to be stabilized and retained in the rhizosphere of soil, thereby increasing soil fertility (Qayyum et al., 2012; Prendergast-Miller et al., 2011). The broad concepts of biochar-N interactions are understood: biochar retains N through ion exchange, absorbing $NH_3$, immobilizing $NO_3^-$ (Clough et al., 2013). As well, biochar can create conditions that do not favour denitrifying bacteria, these interactions can reduce rates of soil denitrification (Anderson et al., 2011; Clough et al., 2013). However, the degrees to which these processes are observed depends on the source material, the pyrolysis temperature of biochar, and to which soil they are added (Clough et al., 2013; Joseph et al., 2010). Phosphorous is another primary limiting nutrient for temperate plant growth (De Luca et al., 2009). Though biochars are noted to function as an effective P fertilizer, decreases in bioavailable P have been observed in temperate biochar projects, especially if there was an initial increase in soil pH due to biochar (Wang et al., 2012; Atkinson et al., 2010). $PO_4^{-3}$ can become less accessible to plants if biochar increasing P solubility, adsorbs P, or alters enzyme activity (De Luca et al., 2009; Zhang et al., 2016; Madiba et al., 2016). In soils with low P sorption capacities, biochar can retain and initiate increases to available P (Zhai et al., 2015; Novak et al., 2009; Lehmann et al., 2003). Low sorptive soils, such as sandy soils, have poor nutrient retention and can benefit from biochar additions (Uzoma et al. 2011; Dil et al., 2014). The reduction in nutrient leaching is especially crucial for agricultural systems, which lose significant amounts of nutrients into the surrounding environment (Kulyk, 2012). These fluxes have been detrimental to natural and human systems causing eutrophication and well-water contamination (Galloway et al., 2008).

Biochar impacts on soil biota are not well understood, and little research has been carried out concerning macrofauna (Atkinson et al., 2010; Domene et al., 2014). Available research has

focused on a few insect species. Impacts range from increased reproduction (Marks et al., 2014) to biomass loss (Li et al., 2011). The small number of studies, with their variable outcomes, make it difficult to understand to what degree biochar impacts macrofauna physically (i.e., desiccation, improved aeration), chemically (i.e., toxicity, nutrient availability), or ecologically (i.e., food sources). One study has observed the impact of biochar on earthworms, and saw an increase in soil water content (Hardie et al., 2014). Therefore, insects can also act as an intermediary for indirect biochar impacts (Burrell et al., 2016). In regards to soil biota, more information is available in on soil microbial life, which generally benefits from biochar (Bamminger et al., 2014). Biochar has driven changes regarding diversity, community structures, efficiency, and total microbial biomass (Bamminger et al., 2016; Anderson et al., 2011; Domene et al., 2014). Much like chemical and physical characteristics, not all impacts have been consistent across studies (Domene et al., 2014; Marks et al., 2014). While some saw increases in species diversity and colonization, especially among lower temperature biochars (Lou et al., 2013; Mitchell et al., 2015; Bamminger et al., 2014), others found that biochar favoured a narrow array of species (Khodadad et al., 2011; Quilliam et al., 2013; Mitchell et al., 2015). Some applications favoured fungi (Bamminger et al., 2014; Yuan et al, 2017), while others saw more gram-negative bacteria (Gomez et al., 2014), and yet others gram-positive bacteria (Mitchell et al., 2015). Likewise, some studies saw an initial decrease in activity, though most found short-term increases in activity (Luo et al., 2011; Rutigiano et al., 2014; Mitchell et al., 2015; Bamminger et al., 2014). Changes to microbial communities impact nutrient cycling and therefore continued investigations are essential (Knicker, 2011).

The aforementioned changes to soil structure and nutrient availability, and the resulting changes to soil biota, strongly impact crop productivity. Due to a naturally higher pH, cation

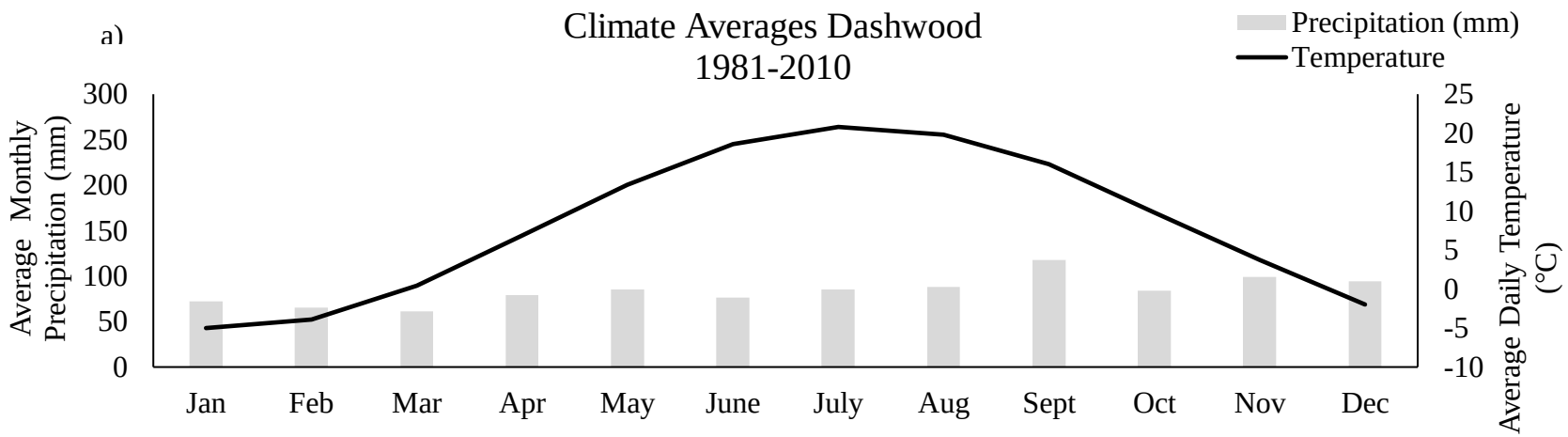
exchange capacity (CEC), and SOC content, temperate soils need fewer alterations to soil conditions for productivity (Atkinson et al. 2010). With many needs already met, biochar does not always improve temperate soil characteristics (Appendix B). Very rarely has biochar resulted in a straightforward increase in crop productivity within temperate systems. However, certain temperate soil types and land uses can benefit (Kloss et al., 2014). Biochars can aid soils that are naturally less effective at retaining moisture and nutrients (such as coarse-textured soils) during droughts (Glaser et al., 2014; Hammond et al., 2013). Many sandy soil projects have observed impacts to crop yield (Borchard et al., 2014; Nelissen et al., 2015; Güereña et al., 2013; Hammond et al., 2013). However, many temperate studies found an initial or sustained decrease in crop growth (Karer et al., 2013; Anders et al., 2013; Borchard et al., 2014; Kloss et al., 2014; Lentz & Ippolito, 2012). In several cases, these impacts were corrected with additional fertilizers (Glaser et al., 2015; Hammond et al., 2013). The degree of influence on crop yield was found to vary between biochar types; especially among wood biochars, manure biochars, and grass biochars (Kloss et al., 2014; Anders et al., 2013). The pyrolysis temperature of biochar influenced crop productivity. Typically, low-temperature biochars were found to be more beneficial for improving soil fertility (Qayyum et al., 2012; Luo et al., 2013; Yuan et al., 2017). It is apparent once again that the outcomes of biochar additions will vary significantly depending on biochar makeup (original material and pyrolysis temperature), the amount that is added, and the soil type it is being added to (Atkinson et al., 2010). This study aimed at contributing to an understanding of how a high-temperature biochar addition might impact soil health and crop productivity in a course textured Southern Ontario agricultural soil. Crop yield differences between biochar and non-biochar soil in southern Ontario were quantified.

## 2.3 Methods

### *2.3.1 Site Description*

This study was carried out in Bayfield, Ontario, Canada (43°34’45.8”N, 81°39’52.2”W). The site was located 183 m above sea level with a 1.5% slope, and the soil has been classified as a uniform Grey-Brown Luvisol (Burford loam). The nearest historical weather station in Dashwood, ON (43°22’00.0”N, 81°37’00.0”W) reported an average annual temperature of 8.2°C and average annual precipitation of 1006.8 mm (Government of Canada). Historically, July has been the warmest month while January the coldest, with an average temperature of 20.8°C and -5.0°C, respectively. September has been the wettest and March the driest month at this site, with an average of 117.9 and 60.9 mm precipitation, respectively. Prior to initiating this study, the site received poultry manure with switchgrass bedding on a three-year rotation and fertilizer additions when supporting cash crops [(maize (*Zea mays* L.)-soybean (*Glycine max* Merr. L.) rotation). The first field season was initiated in 2016, beginning with maize crop followed by soybean crop the next growing season, 2017. Both field seasons ended after crop harvest. The 2016 field season terminated in early November and 2017 in early October.

a)


Climate Averages Dashwood
1981-2010
Precipitation (mm)
Temperature
Average Monthly Precipitation (mm)
Average Daily Temperature (°C)
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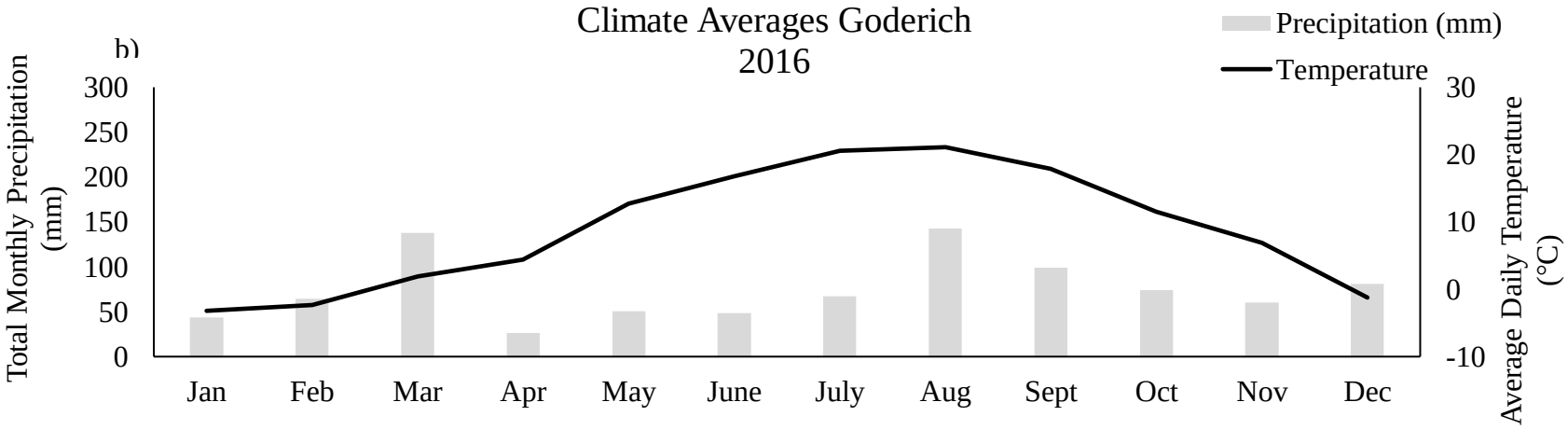
b)


Climate Averages Goderich
2016
Precipitation (mm)
Temperature
Total Monthly Precipitation (mm)
Average Daily Temperature (°C)
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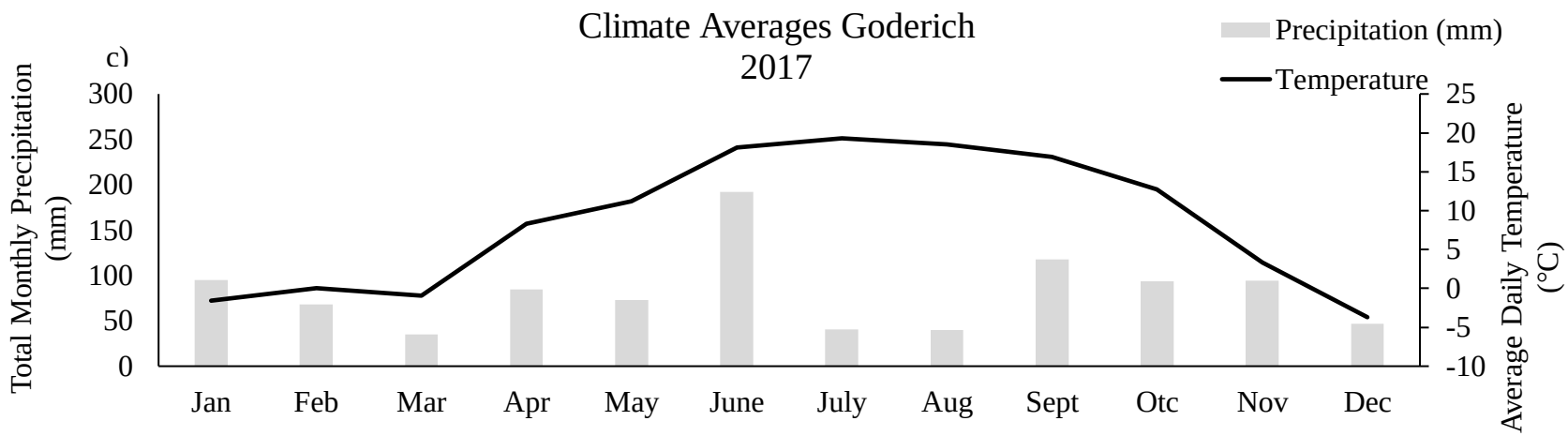


**Figure 2.1** Historic a monthly climatic conditions from a) the nearest historic weather station in Dashwood, Ontario from 1981-2010, b) the nearest present station in Goderich, Ontario in 2016, and c) 2017.

The experimental design was a complete randomized design (CRD) with three treatments each replicated three times, yielding nine plots in total (10 m x 10 m plot size). A three-meter buffer zone was established between each treatment plot to account for edge effects. No measurements were taken within the outer meter of each treatment plot to avoid border effects. The treatments were: 6 t/ha poultry manure (M) with switchgrass bedding plus 135 kg/ha nitrogen (N) fertilizer (urea) (MN); 3 t/ha poultry manure plus 3 t/ha biochar (B) (MB); 3 t/ha poultry manure, 135 kg/ha nitrogen (N) fertilizer, and 3 t/ha biochar (MNB). Biochar was only added once at the onset of the study using a drop spreader. The biochar was provided by Titan Carbon Smart Technologies (Saskatoon, Saskatchewan, Canada). The biochar feedstock was 50/50 pine (*Pinus* spp.) and spruce (*Picea* spp.), and underwent slow pyrolysis and reached peak temperature at 550°C for 15min (Table 2.2). The site was under minimum tillage using a disc harrow, which incorporated the manure and biochar into the soil to a depth of 8 cm in May 2016. Conventional commercial farm management operations, such as herbicide additions (N-phosphonomethyl glycine (Glyphospate)), and N fertilizer application rates were considered standard agronomic practices for this region within Southern Ontario. Soil sampling was primarily carried out on three dates, baseline (May 5$^{th}$, 2016), harvest 2016 (November 7$^{th}$, 2016) and harvest 2017 (October 7$^{th}$, 2017). Soil baseline conditions are given below in Table 2.1.

**Table 2.1** Pre-experiment characteristics of the project soil

| | **Burford Loam Soil (0-10 cm)** |
|---|---|
| Classification | Grey-Brown Luvisol |
| Landuse | Corn-Soybean 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) |
| Bicarbonate ext. P (mg P $kg^{-1}$) | 52.6 (1.32) |

Standard errors are given in parentheses. All values are expressed on a dry weight basis.

**Table 2.2** Pre-experiment characteristics of the biochar and manure used in this study

| | **Titan Carbon Smart Technologies Biochar** | **Poultry Manure with switchgrass bedding** |
|---|---|---|
| Pyrolysis Type | 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 μg/g | 13725 mg/kg |
| Ca | 0.68 μg/g | 14200 mg/kg |
| Mg | 0.23 μg/g | 4500 mg/kg |
| S | 0.03 μg/g | 3600 mg/kg |

All values are expressed on a dry weight basis. Biochar properties were supplied by Titan Industries.

*2.3.2 Soil Sampling and Analysis*

*2.3.2.1 Soil Physics*

Infield analysis, such as infiltration rate measurement, were carried out the day of baseline, harvest 2016, and harvest 2017 soil collection. Infiltration was measured using a 2800 Guelph Permeameter, Model 09.07 (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). Raw data were converted into an infiltration rate using the Guelph Permeameter Calculations spreadsheet recommended in the 2800 Guelph Permeameter, Model 09.07 Operating Instructions Manual (soilmoisture.com). Several soil characteristics, including soil moisture, and temperature, were sampled biweekly for the entire growing season of each year at two randomly selected points in each treatment replicate, coinciding with greenhouse gas sampling (cf. Chapter 3). Soil temperature and moisture were measured to a 10 cm depth using an HH2-WET sensor within 1 m radius of each sample point from 10 a.m. and 3 p.m. from May to November 2016 and 2017 (Delta T Devises, Cambridge, UK). Before crop harvest, soil samples were collected at five random points using a spade to collect soils between the depths of 0-10cm, 10-20cm and 20-30cm within each plot. Unless analyzed immediately, soil samples were frozen for later analyses. At these same points a bulk density ring was driven horizontally into the middle of the three depths. The soil held in the ring was then oven-dried at 105°C for 48 hrs, weighed to determine dry weight and quantity bulk density. At the time of sample collection, five additional soil samples were combined at the shared depths of 0-10 cm, 10-20 cm, and 20-30 cm. Therefore, each treatment had three combined replicates from each of treatment replicate of the three depths. These combined samples were used for the analysis of aggregate stability and all chemical characteristics.

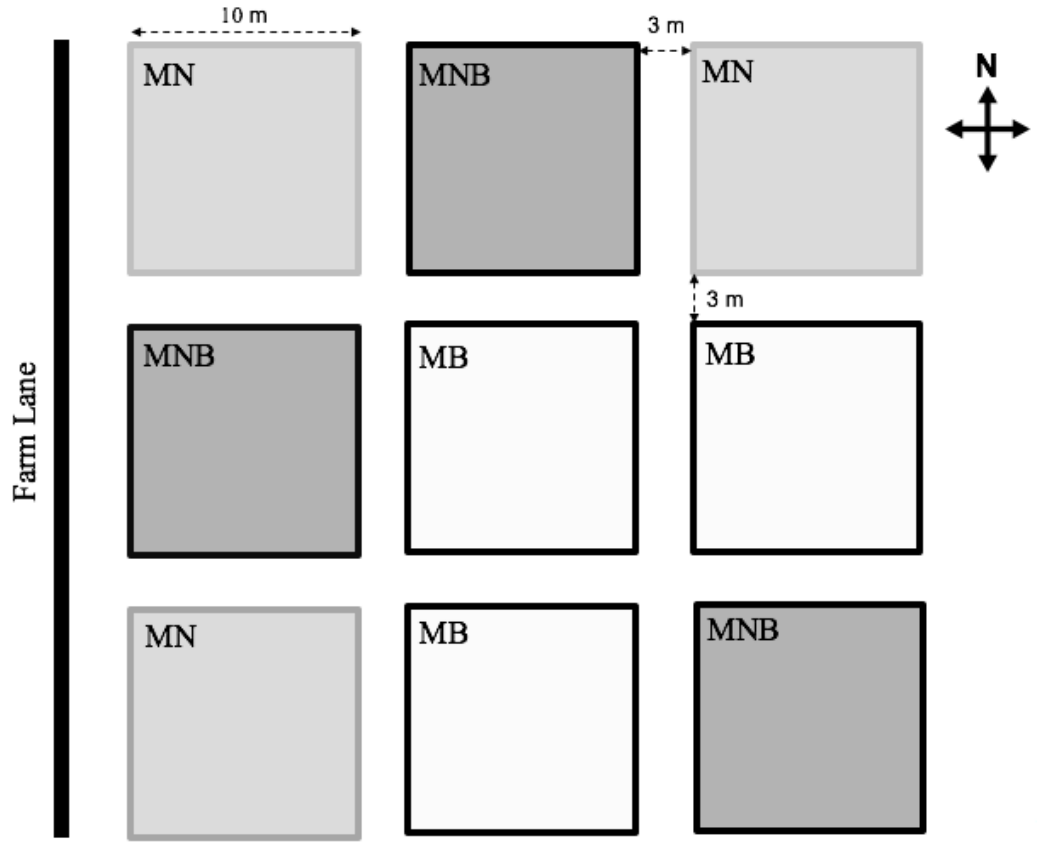


**Figure 2.2** Schematic diagram of project's complete randomized design (CRD) plots at H & N Baker Farm, Bayfield Ontario, Canada.

Aggregate stability was measured using a protocol adapted from Carter et al. (2002) and Mehuys et al. (2007). To minimize slaking, the soil was moistened to approximately 50% field capacity with a mist bottle 5 minutes before sieving. Air-dried soil samples were passed through a 2-mm sieve and ~10 g of each sample was placed onto a 250 μm sieve. The initial weight was recorded (W1). The sieves were then gently lowered into a tub of de-ionized water and moved up and down approximately 4 cm 30 times/minute. After 10 minutes of wet sieving, the soil remaining on the sieve was rinsed off with de-ionized water into tins and oven-dried at 105°C overnight. The dried soil represents weight 2 (W2). W2 subtracted from W1 gives the weight of particles and aggregates >250 μm. After weighing, the remaining soils were shaken in a 50 ml solution of 0.5% sodium hexametaphosphate solution on a reciprocating shaker at 180 rpm for

45 minutes. The mixtures were then poured through a 250 μm sieve, a flat plate was used to gently break up the remaining aggregates, which were further rinsed with de-ionized water. The remaining coarse particles were transferred with de-ionized water into tins and were oven-dried at 105ºC overnight. The coarse particles were subsequently weighed (W3). Carter et al.'s (2002) calculation for the percentage of water-stable macro-aggregates was used:

Eq. [2.1] $$\%\text{WSA} = 100 * \frac{W_2 - W_3}{W_1 - W_3}$$

In addition, the percent of unstable aggregates (%UA) was calculated with

Eq. [2.2] $$\%\text{UA} = 100 * \frac{W_1 - W_2}{W_1}$$

and percent of stable aggregates (%SA) with

Eq. [2.3] $$\%\text{SA} = 100 * \frac{W_2 - W_3}{W_1}$$

*2.3.2.2 Soil Chemical Analysis*

Following soil collection, after crop harvest, the combined soil samples (from 5 points) at 0-10 cm, 10-20 cm, and 20-30 cm were air-dried. Soil pH was determined using a 1:1 soil to ultra-pure water paste using a pH meter (Fisher Scientific, Pittsburg, USA). Before soil elemental SOC and TN analysis, carbonates were removed from the air-dried soil through acid washing (Dyer et al., 2012). 2.0 g of soil was treated with 50.0 ml of 0.5 M HCl and was shaken three times over 24 hrs on a reciprocating shaker at 200 rpm (Heidolpj Unimax 1010 DT, Schwabach, Germany). Following a settling period, the acid solution was removed using a pipette. The soils were washed by adding 50 ml ultrapure water and shaking the soils at 200 rpm daily for four days. The soils were dried at 40°C for 2 days and then ground in a ball mill (Retsch® ZM1, Haan, Germany) to 250 μm. Approximately 15 mg of the ground soil was weighed and packaged into tin capsules (Costech, 5 x 9 mm), to be further analyzed in an Elemental Analyzer (Costech

4010, Cernusco, Italy) for soil organic carbon and total nitrogen (%). From these results, the C/N ratio was calculated.

The free light fraction was isolated from soils using the method of Carter and Gregorich (2007). 50 ml of NaI solution (with a specific gravity of 1.7) was added to 25 g of air dried soil. Each sample was first hand-shaken for 10 seconds to ensure mixing and subsequently shaken at 250 rpm for an hour on a reciprocating shaker (Heidolpj Unimax 1010 DT, Schwabach, Germany). Following mixing, the soil was then settled for 48 hrs at room temperature. The light fraction of the soil was stratified on the surface of the solution and aspirated onto microfiber glass filters (Whatman 934-AH, Buckinghamshire, UK) using the vacuum filter unit illustrated in Carter and Gregorich's methodology (2007). The filters were pre-weighed for each sample and weighed once more after drying the FLF samples on the filters. The FLF was then gently removed from the filter surface, ground, weighed, packaged into tin capsules (Costech, 5 x 9 mm), and finally analyzed in an Elemental Analyzer (Costech 4010, Cernusco, Italy) for FLF carbon and FLF nitrogen (%). From these results the FLF C/N ratio was calculated.

To quantify changes in $NH_4^+$ and $NO_3^-$, biweekly soil samples were collected over 24 weeks during the growing season between May - November 2016 and 2017, which coincided with greenhouse gas sampling (cf. Chapter 3). Approximately 250 g of soil were removed from a depth of 0-10 cm from the 18 locations (Estefan & Sommer, 2013) and stored frozen (~ -15°C). As well, at harvest soil was collected at 0-10 cm, 10-20 cm, and 20-30 cm for analysis. Before each analysis, portions of soil samples were thawed, air-dried, sieved (2 mm), and extracted in 5 g batches with 25 ml of 2 M KCl. This mixture was shaken at 180 rpm for 15 minutes on a reciprocating shaker (Heidolpj Unimax 1010 DT, Schwabach, Germany), and filtered through Whatman 42 filter paper. The resulting supernatants were used to analyze $NH_4^+$ and $NO_3^-$

content. $NH_4^+$ was analyzed on a Shimadzu 1800 UV-Vis Spectrophotometer (Shimadzu Corp., Kyoto, Japan) at a wavelength of 650 nm following an hour of colour development at room temperature (Verdow et al., 1978; Foster, 1995). $NO_3^-$ was analyzed at a wavelength of 540 nm over 12 hrs after colour development using the same UV-Vis Spectrophotometer (Miranda et al., 2001; Doane & Horwath, 2003). Soil samples collected at harvest at the three depths were analyzed each year for $NH_4^+$, $NO_3^-$, and ortho-phosphate ($PO_4^{-3}$). Extractable P was determined using the Olsen P method (Amacher et al., 2003) and analyzed on a Shimadzu 1800 UV-Vis Spectrophotometer (Shimadzu Corp., Kyoto, Japan) at a wavelength of 880 nm following the Ascorbic Acid method (Amacher et al., 2003).

*2.3.2.1 Soil Biology*

*2.3.2.1.1 Macrofauna Invertebrates*

In May 2016, a random point was selected in each plot and macrofauna were collected within 1m of this point on the sample date. In spring, summer, and autumn of both years soil monoliths were dug at this point to a dimension of 25 cm x 25 cm x 30 cm with a spade to collect soil invertebrates. The monoliths were then hand-sorted for insects the day of collection or bagged, transported to the lab, and hand-sorted within one week of collection. Macrofauna were counted and identified to an order level, to investigate soil invertebrate populations and diversity.

*2.3.2.1.2 Microfauna*

After field collection, portions of the combined soil samples from each site were frozen. Before analysis, the soil was moved into a 4°C refrigerator to thaw for a week. Once thawed, a portion was used for soil microbial biomass (SMB) and another for microbial diversity analysis. For the SMB process, the thawed soils were moistened to a ~50% water holding capacity, incubated at room temperature for seven days, and sieved (2 mm) to remove large rocks (Allison,

2008). After seven days the soil was divided into three 30 g portions. The first portion was oven-dried for 24 hrs to determine the moisture content of the soil, the second portion was immediately extracted, and the third portion was fumigated for 24 hrs with $CHCl_3$ before extraction (Allison, 2008). Before being extracted the third 30 g portion of soil was fumigated in a vacuum desiccator with 50 ml of chloroform, as outlined by Voroney et al. (2008) and Allison (2008).

The oven-dried soil was used to determine how much 0.05 M $K_2SO_4$ solution was needed to extract each 30 g wet samples. For every gram of the wet soil's dry weight, 2 ml of solution was added. The mixture was then shaken at 200 rpm for 1 hour and filtered through Whatman GF 934-AH filter paper. All filtrates were then freeze-dried (Mandel ModulyoD-115, Ashville, NC) before being oven dried at <40°C overnight. Following this, ~10 mg was packaged in tin capsules (Costech, 5 x 9 mm) and analyzed in the elemental analyzer (Costech 4010, Cernusco, Italy).

The following calculations were used to calculate SMB-C (Voroney et al., 2008).

Eq. [2.4] $$SMB - C = \frac{C_F - C_U}{K_{EC}}$$

$C_F$ was the total weight of extractable C from the fumigated soils (μg/ g soil), as given by the elemental analyzer. $C_U$ was the total weight of extractable C from the unfumigated soils (μg/ g soil). $K_{EC}$ was the conversion factor of 0.35 (Voroney et al., 2008). Soil microbial biomass nitrogen (SMB-N) was calculated similarly but used a conversion factor ($K_{EN}$) of 0.5 (Voroney et al., 2008).

Soil microbial diversity was determined following the methodology performed by Garland (1996). Ecoplates™ were incubated for five days at room temperature and read on a EL800 Microplate Reader (BioTek Instruments, Inc. 2011, Bad Friedrichshall, Germany)

approximately every 12 hrs. Well-colour development peaked at 95 hrs. The measurements from 95 hrs were then used to calculate the average well colour development (AWCD), species richness, and Shannon Diversity Index. AWCD was calculated as the average microplate well optical density (OD) at 590 nm, excluding the control wells [Equation 4] (Garland, 1996).

Eq. [2.5] $$\text{AWCD} = \frac{\Sigma OD_i}{31}$$

Richness was counted from the wells which had an OD greater than 0.25 at 590 nm. The Shannon Diversity Index ($H_s$) gives a value to compare the diversity and evenness of the microbial communities within each substrate. It was quantified using Equation 2.6. Within Equation 2.6, $p_i$ is the ratios of the substrates activity ($OD_i$) to the sum of activity within all the substrates ($\Sigma OD_i$).

Eq. [2.6] $$\text{Hs} = -\Sigma p_i \, (\ln p_i)$$

*2.3.3 Crop Sampling and Analysis*

On November 7th, 2016, 40 cm x 2 m strips were randomly chosen from each treatment replicate. From these strips, all maize and soybean yields, shoot biomass (stalks, leaves, tassels, husks), and root biomass was collected. Out of interest to the farmer, all maize cobs were collected from the entire 10 m x 10 m plots. The maize was collected in a bin, and a wet weight was determined in the field using a Manual Poultry scale BAT 1 (VEIT Electronics, Moravany, Czech Republic). The 40 cm x 2 m samples of maize grain were weighed as well and brought to the lab. All soybean plant matter within the 40 cm x 2 m strip was collected on October 7th, 2017 and transported to the lab for further analysis. Plant aboveground biomass was considered all plant matter above the first aerial roots, excluding the grain. Loppers were used to cut the biomass into sections to weigh in the field. Root biomass was collected after carefully digging 20 cm around the crop rows and loosening the soil. The soil was light enough that the roots could be

lifted out and gently shaken with minimum root loss. The roots were then weighed in the field and brought back to the lab for cleaning. Each root mass was carefully cleaned in water to remove all remaining soil particles. A 2 mm sieve was used to collect additional root loss during cleaning. The cobs, aboveground biomass, and cleaned roots were then heated in an oven at 72°C for 48 hrs (Oelbermann & Voroney, 2007). The final weights were then collected on a Sartorius 6200 D electronic top load digital scale (Göttingen, Germany). Total plot biomasses were subsequently inferred for each treatment using these numbers. During the biweekly sampling, soil temperature and moisture were measured as well using an HH2-WET Sensor (Delta T Devices, Cambridge, UK).

*2.3.4 Statistical Analysis*

All statistical analyses were conducted on SPSS Statistics for Windows Version 23 and had a P>0.05 threshold probability level. One-way analyses of variances (ANOVA) were carried out in conjunction with Tukey's *post hoc* HSD to test for emissions homogeneity of variance and normality. One-way ANOVA analyses were conducted to quantify the impact of the three treatments (MN, MB, MNB) on soil characteristics, soil biology, and crop yields. All characteristics were analyzed between years (2016, 2017) and/or among seasons [spring (May 19$^{th}$ to June 21$^{st}$), summer (June 22$^{nd}$ to September 2$^{nd}$), and autumn (September 23$^{rd}$ to November 5$^{th}$)]. Two-way ANOVAs were used to determine whether time (year or season) and treatment type were interacting to significantly influence soil characteristics mentioned earlier (Kloss et al., 2014; Lutes et al., 2016). Distribution normality was determined using the Shapiro-Wilk Test and Kolmogorov-Smirnov Test. Any value distributions that were not normal (p>0.05) were given as medians. Significant differences among means were determined with Tukey's *post hoc* HSD tests. Pearson's two-tailed correlation analysis was used for each treatment to establish

the relationship between crop yields and soil characteristics. A principal components analysis was carried out using the AWCD values collected from the microbial Ecoplates to examine how similar microbial communities were (Dil et al., 2014).

## 2.4 Results

### *2.4.1 Soil Characteristics*

#### *Soil Physics*

Soil infiltration treatment effects were not significant (P= 0.876) at any time during this study. As well, there were no significant changes from baseline measurements to 2016, or from 2016 to 2017 (P=0.102). In 2017, biochar soils appeared to have increased infiltration rates, but these were not statistically significant (Table 2.4). Infiltration rates did no change following additions of biochar, as well there were no treatment-by-year interactions (P>0.05).

Both year (P<0.001) and treatment (P=0.034) were found to significantly impact bulk density levels, though there was no interaction for treatment-by-year (P=0.145). When considering an LSD interaction, a significant difference (P=0.035) was identified between MN-MB and MN-MNB (P=0.017), with MN having a lower bulk density of ~0.1 g/cm$^3$. A significant difference, however, only existed between MN-MNB (P=0.045) using Tukey's comparison. As well, bulk density was different across each sampling date. 2017 was significantly lower than in 2016 by 0.132 g/cm$^3$ (P=0.022).

Water-stable soil aggregates only saw significant change between sampling depths (P=0.017). As sampling depth increased so did the percent of water-stable aggregates within each treatment and across dates. The 10-20 cm depth had an average of 44.0% water-stable aggregates and 0-10 cm had 37.3% (P=0.017). 2017 appeared to have the highest percent of water-stable aggregates, followed by baseline and then 2016, although there were no significant

differences identified. No significant difference was found among treatments, though MN had the highest total of aggregate stability, followed by MB and MNB. As well, differences among treatments were not observed for stable aggregate particles greater than 250μm. The difference between treatments was negligible when considering this portion of water-stable soil aggregates. The aggregate particles >250μm levels were far more similar between depths (10-20 cm was slightly higher, P=0.075) and had a greater temporal change (P<0.001) than when considering the total water-stable soil aggregates. However, the temporal pattern remained the same for this subset of stable-aggregates with 2017 having the greatest percent followed by baseline and finally 2016.

**Table 2.3** Mean ($\bar{\chi}$) baseline physical soil characteristics in temperate soil amended from three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada. Standard errors are given in parentheses.

| **Soil Physical Characteristics** | | **Baseline** | | |
|---|---|---|---|---|
| | | MN | MB | MNB |
| Wet Stable Aggregates % | 0-10cm | 35.1 (2.15)$^{A,a}$ | 40.3 (5.16)$^{A,a}$ | 45.9 (8.49)$^{A,a}$ |
| | 10-20cm | 45.8 (4.50)$^{A,a}$ | 44.4 (1.04)$^{A,a}$ | 53.1 (6.99)$^{A,a}$ |
| Wet Stable Aggregates % >250 μm | 0-10cm | 84.7 (1.59)$^{A,a}$ | 86.3 (3.22)$^{A,a}$ | 87.0 (2.46)$^{A,a}$ |
| | 10-20cm | 85.4 (1.56)$^{A,a}$ | 86.8 (1.50)$^{A,a}$ | 89.6 (2.99)$^{A,a}$ |
| Bulk Density g/cm$^2$ | 0-10cm | 1.3 (0.03)$^{A,a}$ | 1.2 (0.04)$^{A,a}$ | 1.3 (0.05)$^{A,a}$ |
| | 10-20cm | 1.3 (0.07)$^{A,a}$ | 1.3 (0.06)$^{A,a}$ | 1.3 (0.05)$^{A,a}$ |
| | 20-30cm | 1.3 (0.04)$^{A,a}$ | 1.3 (0.03)$^{A,a}$ | 1.3 (0.03)$^{A,a}$ |
| Infiltration cm/s | 0-10cm | 0.006 (0.002)$^{A}$ | 0.005 (0.000)$^{A}$ | 0.004 (0.001)$^{A}$ |

$^{A}$ Values followed by the same upper case letters are not significantly different among treatments (at p< 0.05). $^{a}$ Values followed by the same lower case letters are not significantly different among depths (at p<0.05).

**Table 2.4** Mean ($\bar{\chi}$) yearly (2016 & 2017) physical soil characteristics in temperate soil amended from three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada. Standard errors are given in parentheses.

| **Soil Physical Characteristic** | | **2016** | | | **2017** | | |
|---|---|---|---|---|---|---|---|
| | | MN | MB | MNB | MN | MB | MNB |
| Wet Stable Aggregates % | 0-10cm | 33.7 (3.75)$^{A,a}$ | 21.6 (4.50)$^{A,a}$ | 23.3 (7.60)$^{A,a}$ | 49.0 (2.23)$^{A,a}$ | 51.0 (3.09)$^{A,a}$ | 45.1 (4.82)$^{A,a}$ |
| | 10-20cm | 38.3 (2.28)$^{A,a}$ | 37.3 (3.04)$^{A,b}$ | 29.3 (4.29)$^{B,a}$ | 51.2 (2.73)$^{A,a}$ | 52.2 (6.92)$^{A,a}$ | 55.4 (4.98)$^{A,a}$ |
| Wet Stable Aggregates % >250µm | 0-10cm | 81.9 (0.86)$^{A,a}$ | 81.2 (0.83)$^{A,a}$ | 81.1 (3.28)$^{A,a}$ | 88.9 (1.54)$^{A,a}$ | 89.5 (1.32)$^{A,a}$ | 86.9 (0.68)$^{A,a}$ |
| | 10-20cm | 85.4 (1.07)$^{A,b}$ | 85.7 (0.55)$^{A,b}$ | 83.9 (4.54)$^{A,a}$ | 90.1 (0.63)$^{A,a}$ | 88.8 (1.79)$^{A,a}$ | 88.1 (1.48)$^{A,a}$ |
| Bulk Density g/cm$^2$ | 0-10cm | 1.3 (0.04)$^{A,a}$ | 1.3 (0.04)$^{A,a}$ | 1.3 (0.04)$^{A,a}$ | 1.1 (0.02)$^{A,a}$ | 1.1 (0.03)$^{B,a}$ | 1.1 (0.02)$^{AB,a}$ |
| | 10-20cm | 1.3 (0.10)$^{A,a}$ | 1.3 (0.03)$^{A,a}$ | 1.3 (0.02)$^{A,a}$ | 1.1 (0.05)$^{A,a}$ | 1.1 (0.05)$^{A,a}$ | 1.1 (0.06)$^{A,a}$ |
| | 20-30cm | 1.3 (0.07)$^{A,a}$ | 1.3 (0.03)$^{A,a}$ | 1.3 (0.02)$^{A,a}$ | 1.1 (0.04)$^{A,a}$ | 1.1 (0.07)$^{A,a}$ | 1.1 (0.01)$^{A,a}$ |
| Infiltration cm/s | 0-10cm | 0.008 (0.001)$^{A}$ | 0.004 (0.001)$^{A}$ | 0.006 (0.001)$^{A}$ | 0.006 (0.002)$^{A}$ | 0.008 (0.003)$^{A}$ | 0.008 (0.001)$^{A}$ |
| Moisture % | 0-10cm | 13.0 (0.58)$^{A}$ | 10.3 (0.50)$^{B}$ | 10.8 (0.50)$^{B}$ | 12.3 (0.50)$^{A}$ | 9.8 (0.43)$^{B}$ | 10.5 (0.52)$^{B}$ |
| Temperature °C | 0-10cm | 19.4 (8.72)$^{A}$ | 19.2 (8.83)$^{A}$ | 19.6 (8.83)$^{A}$ | 22.3 (7.55)$^{A}$ | 21.2 (7.55)$^{A}$ | 21.7 (7.55)$^{A}$ |

$^{A}$ Values followed by the same upper case letters are not significantly different among treatments (at $p < 0.05$). $^{a}$ Values followed by the same lower case letters are not significantly different among depths (at $p<0.05$).

**Table 2.5** Variance analysis of soil physical characteristics under three soil treatments (MN, MB, MNB) in temperate soil at H & N Baker Farm, Bayfield, ON, 2016-2017. F is the F-statistic, in the brackets P is the significance given as a P-value.

| **Fixed Effect** | **Agg. Stab. % Wet Stable** | **Agg. Stab. % >250μm** | **Bulk Den. g/cm$^2$** | **Infiltration cm/s** | **Moisture %** | **Temperature °C** |
|---|---|---|---|---|---|---|
| | F (P>F) | F (P>F) | F (P>F) | F (P>F) | F (P>F) | F (P>F) |
| Date | **59.8 (0.00)** | **24.9 (0.00)** | **37.1 (0.00)** | 2.0 (0.18) | 0.23 (0.634) | 1.47 (0.226) |
| Depth | **6.6 (0.02)** | 3.5 (0.08) | 0.5 (0.63) | - | **67.18 (0.000)** | **179.17 (0.000)** |
| Treatment (Trt) | 1.1 (0.34) | 0.8 (0.48) | **3.7 (0.03)** | 0.1 (0.88) | **19.67 (0.000)** | 0.63 (0.533) |
| Date*Depth | 0.7 (0.43) | 1.8 (0.19) | 0.0 (1.00) | - | - | - |
| Date*Trt | 1.4 (0.28) | 0.0 (0.95) | 2.0 (0.15) | 1.6 (0.23) | 0.46 (0.635) | 0.51 (0.604) |
| Depth*Trt | 0.4 (0.68) | 0.0 (0.99) | 0.1 (0.97) | - | - | - |
| Date*Depth*Trt | 1.1 (0.34) | 0.3 (0.78) | 0.2 (0.93) | - | 1.31 (0.266) | 1.03 (0.394) |

Bolded values indicate significant factors.

*Soil Chemistry*

A slightly lower pH level was found in biochar treatments, though this difference was marginal and not significant across either 2016 and 2017. MN was at its lowest at baseline and saw the greatest increase following its manure and urea additions. The pH was significantly impacted by depth-year interaction (P=0.034). The 0-10 cm depth was significantly lower than 10-20 cm (P=0.004) and 20-30 cm (P<0.001) in the second year. The pH was more evenly spread among the three depths in 2016. Due to the second year change, 0-10 cm was significantly lower than 10-20 cm and 20-30 cm (P<0.005).

SOC was not significantly different among any treatments (P=0.701). MB and MNB had very similar percentages and were only found to be slightly higher than MN, most noticeably in 2016, after biochar additions. The treatment-time interaction was also not significant (P=0.241). Only 2017 was found to differ significantly from the baseline measurement (P=0.002) and was higher by 0.46%. While there were no interactions between date and depth (P=0.854), depth resulted in significant differences (P=0.021). As depth increased, SOC decreased; 0-10 cm had 0.55% more carbon than 20-30 cm (P=0.012). Much like SOC, total nitrogen (TN) had only slight and insignificant differences between biochar soil and MN, MB and MNB had a greater amount than MN. As well, TN mirrored SOC by decreasing with increased depth. 0-10 cm TN 0.06% greater than 20-30 cm (P= 0.001) by 0.06%. 2016 was found to have the greatest TN level, followed by 2017, and then the baseline. When compared by LSD, the baseline was significantly lower (-0.025%) than harvest 2016 (P=0.027). This difference did not exist within the Tukey's comparison. Treatment-by-year interacted to produce a significant impact on the carbon: nitrogen ratio (C/N) among treatments (P=0.002). C/N was greatest in MN, followed by MB, and finally MNB in 2016; 9.2, 7.8, and 7.5 respectively. In 2017 MNB and MB ha the greatest total C/N ratio; 12.5, 12.0, and 14.9, respectively. However, treatment alone did not

produce differences (P>0.05). Each treatment altered the distributions among depths. In 2016, MNB had the lowest average value at its 0-10 cm horizon and the highest at 20-30 cm. For every other treatment and date, the 20-30 cm sampling depth had the greatest ratio, followed by 10-20 cm, then 0-10 cm. The greatest C/N was observed in 2017, followed by the baseline reading and 2016, which were more similar. 2017 had a ratio significantly higher than 2016 (P<0.001) (Table 2.7).

Although biochar soils had higher soil light fraction carbon (FLF-C) content, these differences were not significant (P=0.474). Treatment-by-depth-by-depth interactions were, however, significant (P=0.01). The depth where most FLF-C was held changed for each treatment from baseline to harvest 2016 and to harvest 2017 (Table 2.7). Before treatments were applied, MN and MNB stored more FLF-C in the top 0-10 cm of soil, while MB held it in the 10-20 cm. However, six months following soil additions, MN held far less FLF-C in the top 10 cm, and biochar treatments retained more carbon in the top 10 cm. Following another twelve months (2017), all treatments had similar FLF-C readings. Over the two years, it seemed that biochar treatments held carbon in 0-10 cm of soil, while MN had more in the 10-30 cm. Unlike FLF-C, light fraction nitrogen (FLF-N) had no significant impacts from factors or their interactions. All values between treatments were very close with no significant difference; the same was true for depth (Table 2.7). 2016 and 2017 exhibited nearly identical total amounts of FLF-N, though 2017 was slightly lower and was significantly lower than baseline. Like FLF-C, a trend appeared in which biochar-treated soil held more FLF-N in the top 0-10 cm while MN held more in 10-30 cm. Due to the differences among soil FLF-C, the FLF carbon: nitrogen ratio (FLF-C/N) also had a significant year-by-treatment interaction (P=0.002). In 2016 MN had a significantly lower FLF-C/N than the biochar treatments, both of which were quite similar. However, in 2017, this

ratio rose and exceeded MNB and MB. Even further, biochar treated soils' FLF-C/N increased to such an extent that 2016 was significantly greater than baseline (P=0.003) and even more in 2017 (P=0.001). In the end, MNB had the greatest total ratio, followed by MB and finally MN. MNB's ratio was only significantly greater than MN's (P=0.015). Depth did not have a significant impact and did not interact with other factors. However, like FLF-C and FLF-N, MN had a higher C/N ratio at the lower while biochar treated soils had a higher C/N at 0-10 cm.

Soil ammonium ($NH_4^+$) and nitrate ($NO_3^-$) did not differ significantly among treatments. MB only had slightly higher concentrations of $NH_4^+$ but lower $NO_3^-$ values that MNB and MN (P>0.05). Depth as well had no significant impact on nitrogen concentrations; values were similar among depths for $NH_4^+$ though $NO_3^-$ appeared to have greater levels in 0-10 cm. Date was found to be a significant interaction. Both 2016 and 2017 $NH_4^+$ concentrations differed from baseline concentrations (2016, P=0.054; 2017, P<0.001), as well as from each other. It was found that in the first year $NH_4^+$ fell significantly (P<0.001). For $NO_3^-$ it was the interaction of depth-by-year that caused the greatest variation (P=0.048). In 2016, the 20-30 cm depth had the greatest $NO_3^-$ concentration. However, over the course of the next year, this depth lost the greatest amount of $NO_3^-$. The 0-10 cm appeared to lose very little, and 10-20 cm lost only slightly more (Figure 2.3).

Soil phosphate ($PO_4^{3-}$) was one of the few soil characteristics significantly affected by biochar additions. The treatment-by-year interaction was significant (P=0.012) therefore all treatment differences must be discussed in a temporal context. While MN had the greatest amount of $PO_4^{3-}$ in 2016, in the following year it accumulated less phosphorus than MB or MNB. MNB, which had the second highest concentration in 2016 and the greatest in 2017, significantly greater than MN. MB, which had the lowest concentration in 2016, had a nearly identical $PO_4^{3-}$

concentration to MN in 2017. By the second year, MN had a 38%, MB 84%, and MNB 80% increase in P stocks. All concentrations for depth were very similar and did not vary significantly (P>0.05).

**Table 2.6** Mean ($\bar{\chi}$) baseline (May 2016) carbon: nitrogen characteristics, including free light fraction (FLF) values, before treatment application in Bayfield, Southern Ontario, Canada. Standard errors are given in parentheses.

| **Carbon: Nitrogen Characteristics** | | **Baseline** | | |
|---|---|---|---|---|
| | | MN | MB | MNB |
| SOC | 0-10 cm | 1.2 (0.09)$^{A,b}$ | 1.1 (0.08)$^{AB,a}$ | 0.8 (0.02)$^{B,a}$ |
| % | 10-20 cm | 0.9 (0.11)$^{A,a}$ | 0.9 (0.02)$^{A,a}$ | 1.3 (0.21)$^{A,a}$ |
| | 20-30 cm | 0.7 (0.16)$^{A,a}$ | 0.9 (0.10)$^{A,a}$ | 1.0 (0.26)$^{A,a}$ |
| TN | 0-10 cm | 0.2 (0.01)$^{A,a}$ | 0.1 (0.01)$^{A,a}$ | 0.1 (0.01)$^{B,a}$ |
| % | 10-20 cm | 0.1 (0.00)$^{A,ab}$ | 0.1 (0.00)$^{A,a}$ | 0.1 (0.02)$^{A,a}$ |
| | 20-30 cm | 0.1 (0.01)$^{A,b}$ | 0.1 (0.01)$^{A,a}$ | 0.1 (0.03)$^{A,a}$ |
| C:N | 0-10 cm | 7.5 (0.36)$^{B,a}$ | 9.2 (0.22)$^{A,a}$ | 9.1 (0.03)$^{A,a}$ |
| | 10-20 cm | 6.5 (0.38)$^{B,ab}$ | 8.8 (0.53)$^{A,a}$ | 9.4 (0.16)$^{A,a}$ |
| | 20-30 cm | 5.9 (0.54)$^{B,b}$ | 10.8 (1.58)$^{A,a}$ | 8.5 (0.58)$^{AB,a}$ |
| FLF-C | 0-10 cm | 27.7 (13.11)$^{A,a}$ | 23.5 (5.45)$^{A,a}$ | 23.8 (1.34)$^{A,a}$ |
| % | 10-30 cm | 16.3 (1.34)$^{A,a}$ | 28.3 (3.10)$^{A,a}$ | 19.1 (5.00)$^{A,a}$ |
| FLF-N | 0-10 cm | 2.8 (1.47)$^{A,a}$ | 3.0 (0.67)$^{A,a}$ | 3.3 (0.12)$^{A,a}$ |
| % | 10-20 cm | 2.0 (0.10)$^{A,a}$ | 3.5 (0.16)$^{B,a}$ | 2.6 (0.64)$^{AB,a}$ |
| FLF- | 0-10 cm | 10.3 (0.47)$^{A,a}$ | 7.7 (0.12)$^{B,a}$ | 7.1 (0.16)$^{B,a}$ |
| C/N | 10-30 cm | 8.0 (0.30)$^{A,b}$ | 8.0 (0.60)$^{A,a}$ | 7.4 (0.12)$^{A,a}$ |

[A] Values followed by the same upper case letters are not significantly different among treatments (at p< 0.05). [a] Values followed by the same lower case letters are not significantly different among depths (at p<0.05).

**Table 2.7** Mean ($\bar{\chi}$) yearly (2016 & 2017) carbon: nitrogen characteristics, including free light fraction (FLF) values, in temperate soil amended from three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada. Standard errors are given in parentheses.

| **Carbon: Nitrogen Characteristics** | | **2016** | | | **2017** | | |
|---|---|---|---|---|---|---|---|
| | | MN | MB | MNB | MN | MB | MNB |
| SOC | 0-10cm | 1.2 (0.02)$^{A,a}$ | 2.2 (0.96)$^{A,a}$ | 1.2 (0.15)$^{A,a}$ | 1.8 (0.40)$^{A,a}$ | 1.4 (0.11)$^{A,a}$ | 1.8 (0.27)$^{A,a}$ |
| % | 10-20cm | 1.1 (0.10)$^{A,a}$ | 1.2 (0.33)$^{A,a}$ | 1.2 (0.28)$^{A,a}$ | 1.7 (0.14)$^{A,a}$ | 1.5 (0.22)$^{A,a}$ | 1.3 (0.23)$^{A,a}$ |
| | 20-30cm | 0.7 (0.05)$^{A,b}$ | 1.0 (0.16)$^{AB,a}$ | 1.1 (0.02)$^{B,a}$ | 1.2 (0.32)$^{A,a}$ | 1.3 (0.21)$^{A,a}$ | 1.1 (0.06)$^{A,a}$ |
| TN | 0-10cm | 0.1 (0.00)$^{A,a}$ | 0.2 (0.07)$^{A,a}$ | 0.2 (0.01)$^{A,a}$ | 0.15 (0.04)$^{A,a}$ | 0.12 (0.01)$^{A,a}$ | 0.15 (0.03)$^{A,a}$ |
| % | 10-20cm | 0.1 (0.01)$^{A,a}$ | 0.2 (0.02)$^{A,a}$ | 0.1 (0.02)$^{A,ab}$ | 0.15 (0.02)$^{A,a}$ | 0.13 (0.02)$^{A,a}$ | 0.11 (0.02)$^{A,a}$ |
| | 20-30cm | 0.1 (0.01)$^{A,b}$ | 0.1 (0.01)$^{B,a}$ | 0.1 (0.01)$^{AB,b}$ | 0.10 (0.03)$^{A,a}$ | 0.10 (0.02)$^{A,a}$ | 0.09 (0.01)$^{A,a}$ |
| C:N | 0-10cm | 9.3 (0.22)$^{A,a}$ | 8.6 (1.05)$^{B,a}$ | 5.0 (2.50)$^{C,a}$ | 11.9 (0.14)$^{A,a}$ | 12.1 (0.03)$^{A,a}$ | 11.7 (0.26)$^{A,a}$ |
| | 10-20cm | 9.4 (0.13)$^{A,a}$ | 7.4 (0.94)$^{B,b}$ | 8.1 (1.18)$^{C,b}$ | 11.6 (0.43)$^{A,a}$ | 11.4 (0.51)$^{A,a}$ | 12.2 (0.54)$^{A,a}$ |
| | 20-30cm | 8.8 (0.11)$^{AB,b}$ | 7.4 (0.81)$^{B,b}$ | 9.5 (0.61)$^{C,c}$ | 12.2 (0.30)$^{A,a}$ | 12.3 (0.32)$^{A,a}$ | 13.7 (1.81)$^{A,a}$ |
| FLF-C | 0-10cm | 9.4 (4.31)$^{A,a}$ | 39.1 (12.22)$^{B,a}$ | 29.1 (5.02)$^{B,a}$ | 17.4 (0.99)$^{A,a}$ | 15.8 (1.04)$^{A,a}$ | 16.4 (0.74)$^{A,a}$ |
| % | 10-30cm | 26.2 (13.04)$^{A,b}$ | 12.1 (4.79)$^{B,b}$ | 23.3 (3.72)$^{A}$ | 16.5 (1.26)$^{A,a}$ | 15.6 (1.13)$^{A,a}$ | 15.4 (0.37)$^{A,b}$ |
| FLF-N | 0-10cm | 1.6 (0.17)$^{A,a}$ | 2.7 (0.77)$^{A,a}$ | 2.0 (0.33)$^{A,a}$ | 2.0 (0.08)$^{A,a}$ | 2.4 (0.27)$^{A,a}$ | 2.4 (0.21) $^{A,a}$ |
| % | 10-20cm | 3.3 (1.64)$^{A,a}$ | 1.5 (0.68)$^{A,a}$ | 2.3 (0.43)$^{A,a}$ | 2.0 (0.05)$^{A,a}$ | 2.4 (0.36)$^{A,a}$ | 1.9 (0.08) $^{A,a}$ |
| FLF- | 0-10cm | 5.3 (2.30)$^{A,a}$ | 14.2 (0.48)$^{B,a}$ | 14.5 (0.72)$^{B,a}$ | 8.6 (0.36)$^{A,a}$ | 6.8 (0.84)$^{A,a}$ | 6.8 (0.36)$^{A,a}$ |
| C/N | 10-30cm | 7.9 (0.05)$^{A,a}$ | 9.9 (2.04)$^{A,a}$ | 10.7 (3.58)$^{A,a}$ | 8.1 (0.42)$^{A,a}$ | 6.7 (0.52)$^{A,a}$ | 7.9 (0.53)$^{A,a}$ |

$^{A}$ Values followed by the same upper case letters are not significantly different among treatments (at $p< 0.05$). $^{a}$ Values followed by the same lower case letters are not significantly different among depths (at $p<0.05$).

**Table 2.8** Mean ($\bar{\chi}$) baseline (May 2016) mineral N and P concentrations before treatment application in Bayfield, Southern Ontario, Canada. Standard errors are given in parentheses.

| **Nutrient Characteristics** | | **Baseline** | | |
|---|---|---|---|---|
| | | MN | MB | MNB |
| $NH_4^+$ mg N $kg^{-1}$ | 0-10cm | 8.9 (1.11)$^{A,a}$ | 11.0 (1.96)$^{A,a}$ | 10.6 (0.93)$^{A,a}$ |
| | 10-20cm | 7.2 (0.70)$^{A,a}$ | 7.5 (1.33)$^{A,a}$ | 8.9 (0.67)$^{A,a}$ |
| | 20-30cm | 10.5 (2.93)$^{A,a}$ | 6.2 (1.13)$^{A,a}$ | 9.7 (1.49)$^{A,a}$ |
| $NO_3^-$ mg N $kg^{-1}$ | 0-10cm | 5.4 (0.68)$^{A,a}$ | 5.34 (0.50)$^{A,a}$ | 4.70 (1.11)$^{A,a}$ |
| | 10-20cm | 2.9 (0.50)$^{A,b}$ | 2.84 (0.05)$^{A,b}$ | 2.00 (0.57)$^{A,ab}$ |
| | 20-30cm | 2.3 (0.54)$^{A,b}$ | 2.08 (0.27)$^{A,a\b}$ | 1.64 (0.71)$^{A,b}$ |
| $PO_4^{3-}$ mg P $kg^{-1}$ | 0-10cm | 56.0 (2.33)$^{A,a}$ | 49.5 (0.51)$^{A,a}$ | 51.1 (4.20)$^{A,a}$ |
| | 10-20cm | 54.9 (2.50)$^{A,a}$ | 50.2 (4.15)$^{A,a}$ | 60.0 (5.16)$^{A,a}$ |
| | 20-30cm | 57.0 (1.43)$^{A,a}$ | 50.0 (7.04)$^{A,a}$ | 48.8 (4.31)$^{A,a}$ |

$^{A}$ Values followed by the same upper case letters are significantly different among treatments (at $p< 0.05$). $^{a}$ Values followed by the same lower case letters are not significantly different among depths (at $p<0.05$).

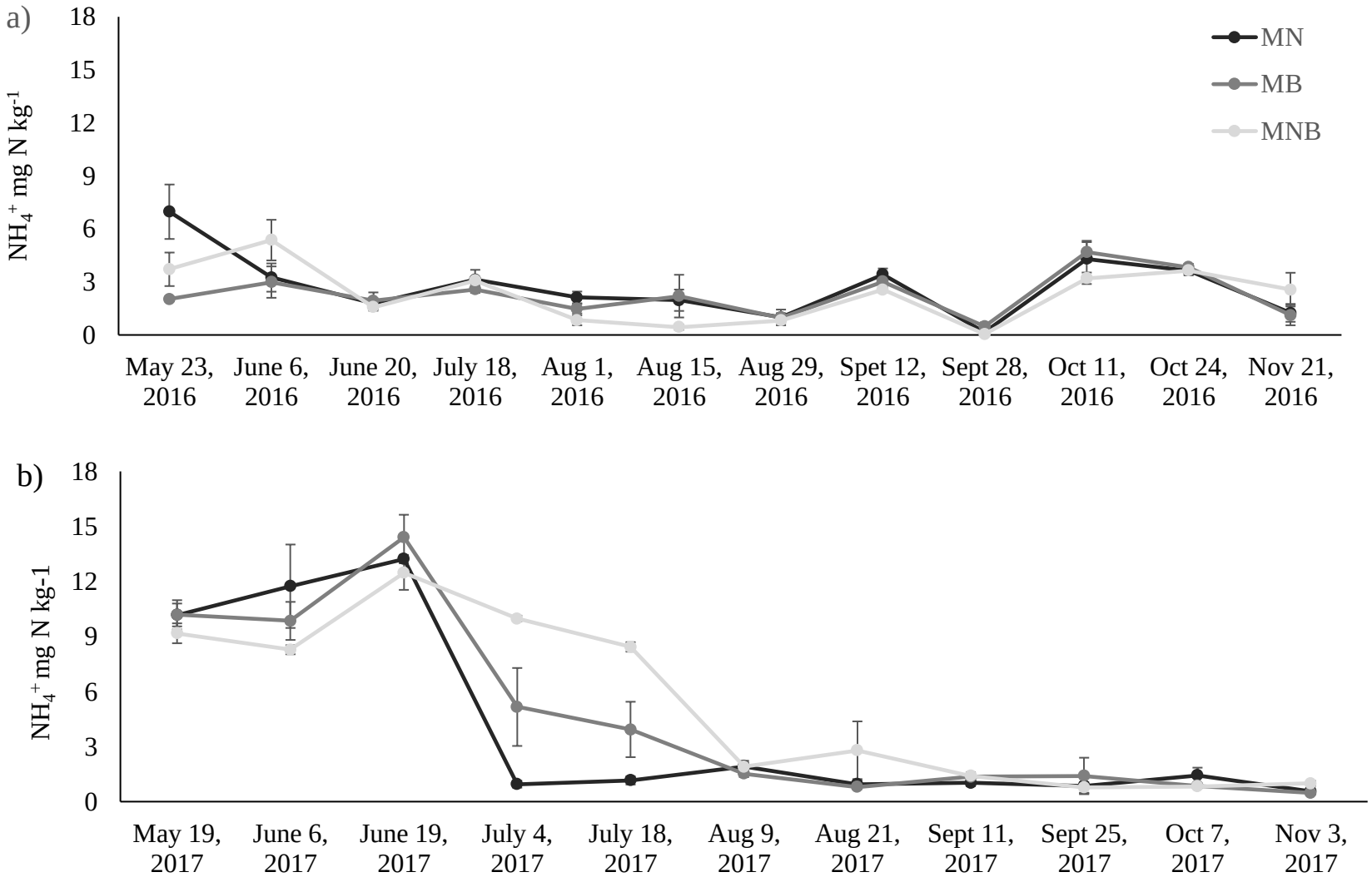


**Figure 2.3** Mean monthly 0-10 cm depth ammonium concentrations ($NH_4^+$ mg N $kg^{-1}$) in a) 2016 and b) 2017 from temperate soil amended from three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada.

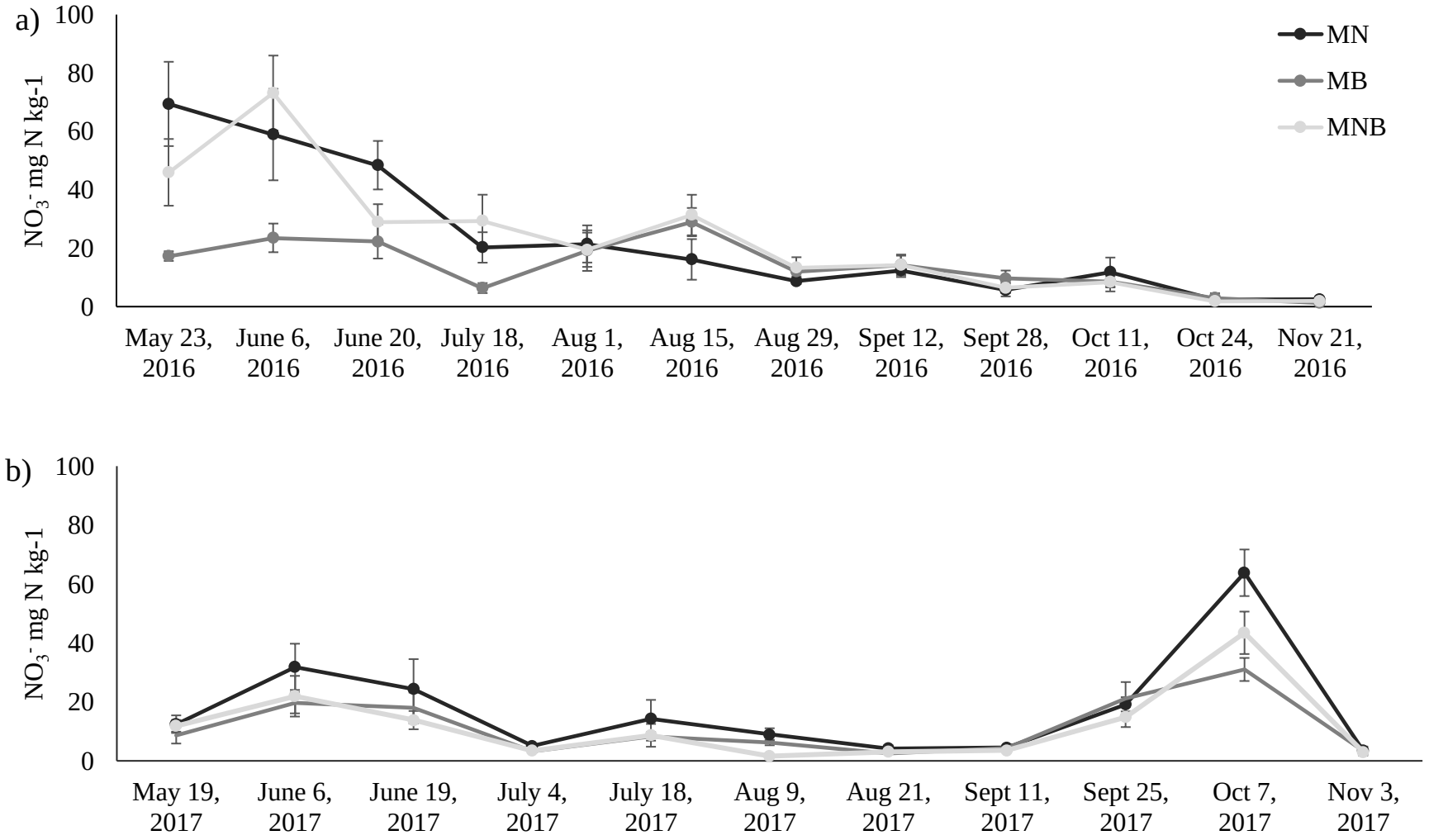


**Figure 2.4** Mean monthly 0-10 cm depth nitrate concentrations ($NO_3^-$ mg N $kg^{-1}$) in a) 2016 and b) 2017 from temperate soil amended from three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada.

**Table 2.9** Mean ($\bar{\chi}$) yearly (2016 & 2017) nutrient concentrations in temperate soil amended from three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada. Standard errors are given in parentheses.

| | | 2016 | | | 2017 | | |
|---|---|---|---|---|---|---|---|
| | | MN | MB | MNB | MN | MB | MNB |
| $NH_4^+$ mg N $kg^{-1}$ | 0-10 cm | 7.4 (1.71)$^{A,a}$ | 7.8 (1.00)$^{A,a}$ | 10.3 (2.83)$^{A,a}$ | 0.5 (0.08)$^{A,a}$ | 0.8 (0.12)$^{AB,a}$ | 1.2 (0.21)$^{B,a}$ |
| | 10-20 cm | 7.8 (2.56)$^{A,a}$ | 8.0 (1.25)$^{A,a}$ | 7.5 (0.81)$^{A,a}$ | 0.3 (0.09)$^{A,ab}$ | 0.7 (0.18)$^{A,a}$ | 0.8 (0.26)$^{A,a}$ |
| | 20-30 cm | 7.3 (0.91)$^{A,a}$ | 8.7 (1.63)$^{A,a}$ | 4.9 (0.50)$^{A,a}$ | 0.2 (-)$^{A,b}$ | 0.5 (0.10)$^{A,}$ | 0.9 (0.08)$^{C,a}$ |
| $NO_3^-$ mg N $kg^{-1}$ | 0-10 cm | 2.0 (0.13)$^{A,a}$ | 1.9 (0.31)$^{A,a}$ | 2.6 (0.44)$^{A,a}$ | 2.7 (0.39)$^{A,a}$ | 3.8 (0.61)$^{B,a}$ | 5.9 (1.07)$^{B,a}$ |
| | 10-20 cm | 1.9 (0.64)$^{A,a}$ | 0.8 (0.07)$^{A,b}$ | 2.8 (1.34)$^{A,a}$ | 1.7 (0.45)$^{A,ab}$ | 3.5 (0.89)$^{B,a}$ | 3.8 (1.29)$^{A,a}$ |
| | 20-30 cm | 3.7 (2.09)$^{A,a}$ | 2.4 (0.74)$^{A,c}$ | 3.1 (1.76)$^{A,a}$ | 1.2 (-)$^{A,b}$ | 2.7 (0.52)$^{A,a}$ | 4.6 (0.38)$^{C,a}$ |
| $PO_4^{3-}$ mg P $kg^{-1}$ | 0-10 cm | 57.6 (1.88)$^{A,a}$ | 44.7 (2.81)$^{B,a}$ | 48.3 (6.30)$^{AB,a}$ | 80.0 (8.99)$^{A,a}$ | 81.5 (4.00)$^{A,a}$ | 98.6 (8.33)$^{A,a}$ |
| | 10-20 cm | 57.0 (5.74)$^{B,ab}$ | 43.7 (1.26)$^{A,b}$ | 52.8 (2.31)$^{B,a}$ | 73.6 (3.14)$^{A,a}$ | 76.4 (5.98)$^{A,b}$ | 83.7 (8.06)$^{A,a}$ |
| | 20-30 cm | 51.8 (0.66)$^{A,b}$ | 45.9 (7.59)$^{A,b}$ | 45.5 (7.23)$^{A,a}$ | 77.0 (6.29)$^{A,a}$ | 83.7 (5.44)$^{A,a}$ | 88.4 (5.03)$^{A,a}$ |

$^{A}$ Values followed by the same upper case letters are significantly different among treatments (at $p< 0.05$). $^{a}$ Values followed by the same lower case letters are not significantly different among depths (at $p<0.05$).

**Table 2.10** Variance analysis of carbon and nutrient characteristics under three soil treatments (MN, MB, MNB) in temperate soil at H & N Baker Farm, Bayfield, ON, 2016-2017. F is the F-statistic, in the brackets P is the significance given as a P-value.

| **Fixed Effect** | **SOC %** | **TN %** | **C:N** | **FLF-C %** | **FLF-N %** | **FLF-C:N** | **$NH_4^+$ mg N $kg^{-1}$** | **$NO_3^-$ mg N $kg^{-1}$** | **$PO_4^{3-}$ mg N $kg^{-1}$** |
|---|---|---|---|---|---|---|---|---|---|
| | F (P>F) | F (P>F) | F (P>F) | F (P>F) | F (P>F) | F (P>F) | F (P>F) | F (P>F) | F (P>F) |
| Trt | 0.4 (0.70) | 1.4 (0.26) | 0.8 (0.48) | 0.8 (0.47) | 0.0 (0.85) | **3.9 (0.04)** | 0.3 (0.77) | 0.5 (0.01) | 2.2 (0.12) |
| Year (Yr) | 3.0 (0.09) | 2.5 (0.12) | **80.0 (0.00)** | **6.9 (0.02)** | 0.0 (0.84) | **13.8 (0.00)** | **165 (0.00)** | 4.8 (0.04) | **153 (0.00)** |
| Dep | **4.4 (0.02)** | **7.6 (0.00)** | 1.4 (0.27) | 1.3 (0.27) | 0.0 (0.97) | 1.2 (0.28) | 0.9 (0.44) | 1.1 (0.36) | 0.8 (0.46) |
| Trt*Yr | 1.5 (0.24) | **3.9 (0.03)** | 2.2 (0.12) | 1.4 (0.27) | 0.6 (0.58) | **9.1 (0.00)** | 0.2 (0.80) | **3.6 (0.04)** | **5.0 (0.01)** |
| Dep*Yr | 0.2 (0.85) | 0.5 (0.62) | 0.3 (0.71) | 0.8 (0.40) | 0.6 (0.45) | 1.6 (0.22) | 0.5 (0.64) | 2.0 (0.15) | 1.3 (0.28) |
| Depth*Trt | 0.2 (0.92) | 0.1 (0.98) | **2.7 (0.05)** | **5.4 (0.01)** | 2.4 (0.11) | 1.5 (0.24) | 1.1 (0.38) | 0.1 (1.00) | 0.3 (0.86) |
| Trt*Dep*Yr | 1.1 (0.37) | 1.0 (0.41) | 0.7 (0.59) | **5.7 (0.01)** | 2.3 (0.12) | 2.8 (0.08) | 1.1 (0.38) | 0.6 (0.67) | 0.2 (0.88) |

Bolded values indicate significant factors.

*2.4.2 Soil Biology*

Total macrofauna counts were unaffected by season, treatments, or any interaction of those factors ($P>0.05$). Year had an impact on total counts, with 2017 having significantly more macrofauna observed ($P<0.001$). When each year was considered in isolation the only difference that emerged was that spring 2016 had a significantly higher species count than summer 2016 ($P=0.036$, Table 2.11). As a general trend, in both years MB had a higher total count, though again this was not significantly greater. When specific species were analyzed in isolation, specific effect emerged. Treatment significantly impacted the *Haplotaxida* family (earthworms) in 2017 ($P=0.023$). In this year, MN had the highest count, followed by MNB, and finally MB. MB counts were significantly lower than MN ($P=0.007$) (Table 2.11). Within the *Enchytraeida* family (whiteworms), only year, season, and year-by-season had impacts on mean counts. Most whiteworms were collected in 2017 when seasonal impacts were also the most impactful ($P=0.005$). As a trend in both years, MB had the greatest mean count of whiteworms (Table 2.11). Similarly, there were greater total counts of the *Julida* family (an order of soil millipedes) in 2017 ($P<0.001$), along with seasonal variation ($P=0.030$). The difference among treatments were not large and were insignificant ($P>0.05$). Finally, like the whiteworms and millipedes, the highest *Coleoptera* (beetles) counts came from 2017, where there was a seasonal variation ($P=0.022$). In 2017, summer had the greater counts of whiteworms, millipedes, and beetles ($P<0.05$). This was followed by autumn for all species, though autumn and spring were not significantly different.

**Table 2.11** Total (2016, 2017) mean macrofauna counts under three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada. Standard errors are given in parentheses.

| | | Total Count | Whiteworms | Earthworms | Millipedes | Beetles |
|---|---|---|---|---|---|---|
| **Spring** | **MN** | 34.3 (15.86) | 0.7 (0.67) | 7.7 (2.40) | 0.7 (0.67) | 1.3 (1.33) |
| | **MB** | 54.0 (47.52) | 5.7 (3.84) | 2.3 (1.20) | 3.3 (3.33) | 0.3 (0.33) |
| | **MNB** | 37.0 (26.58) | 2.7 (2.67) | 6.0 (2.08) | 0.3 (0.33) | - |
| **Summer** | **MN** | 37.0 (7.09) | - | 5.7 (2.33) | - | - |
| | **MB** | 60.7 (28.42) | - | 3.7 (1.76) | 0.3 (0.33) | - |
| | **MNB** | 37.0 (11.50) | - | 4.2 (1.06) | - | - |
| **Autumn** | **MN** | 44.3 (11.84) | - | 7.0 (1.15) | 1 (1) | - |
| | **MB** | 26.7 (10.17) | - | 1.3 (1.33) | 0.7 (0.33) | - |
| | **MNB** | 26.3 (20.03) | 1.3 (1.33) | 3.7 (0.67) | 0.7 (0.33) | 0.7 (0.67) |

Total insect counts had no significant factors or interactions among treatments.

Over the two years of this project soil conditions significantly impacted the microbial community; both species richness (P=0.018) and species diversity (P=0.266). Date, nor date-by-treatment interactions, were significant (P>0.05). However, when each year was considered in isolation, 2016 had the greatest differences in values among treatments, and only these differences were significant. Species richness was significantly higher in biochar treated soils. In 2016, MN had an average species count of 12, in 2017 this hardly changed and was 12.3. In 2016 biochar treated soils averaged 15 species, which lowered to 12.8 in 2017. While biochar treated soils had a greater richness, they scored lower on the Shannon Diversity Index (Hs). In 2016 MN had a Hs value of 1.04, again in 2017 this hardly changed to 1.03. In 2016 biochar treated soil averaged a value of 0.90, which increased to 1.00 in 2017.

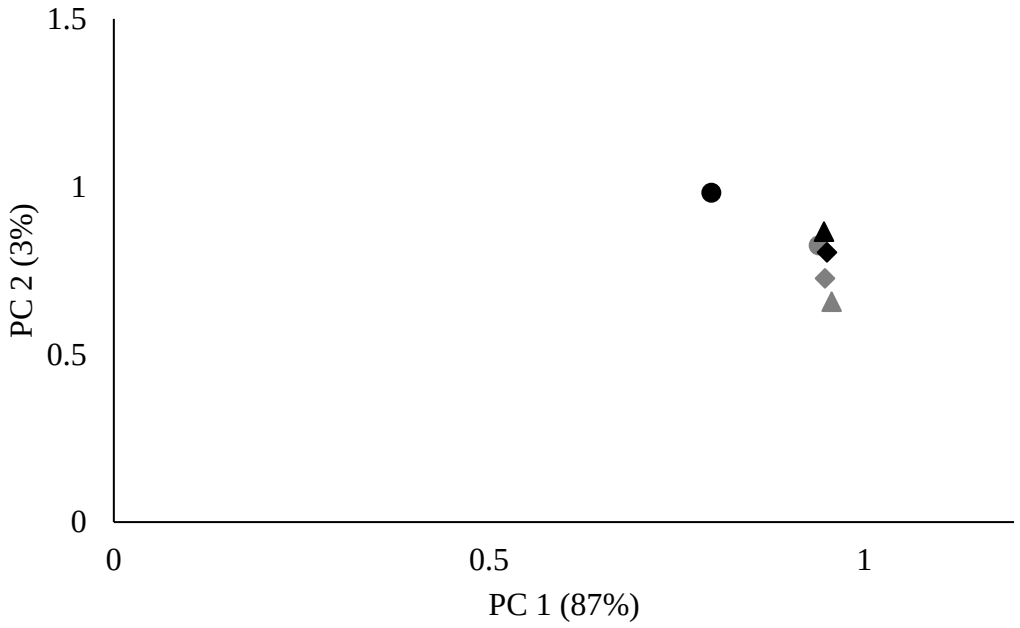


**Figure 2.5** This principal component analysis is associated with microbial activity values, carbon utilization, on Biolog Ecoplates. Visually the first Principal Component 1 (PC 1) measures treatment effects, while Principal Component 2 (PC 2) measures year. Percent of variance is given in brackets. The soils used were accessed from Bayfield, Ontario in 2016 (black markers) and 2017 (grey markers) from soils treated with poultry manure and N fertilizer (MN- •), poultry manure and biochar (MB- ♦), and poultry manure, N fertilizer and biochar (MNB- Δ ) in Bayfield, Southern Ontario, Canada.

**Table 2.12** Mean values for 2017's Soil Microbial Biomass (SMB) values from temperate soil amended with poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada.

| 2017 SMB | | **MN** | **MB** | **MNB** |
|---|---|---|---|---|
| Total Carbon (μg C/g) | 0-10cm | 527.6 (81.97)$^{A,a}$ | 774.3 (129.37)$^{A,a}$ | 481.0 (68.22)$^{A,a}$ |
| | 10-20cm | 235.7 (118.57)$^{A,b}$ | 397.1 (128.80)$^{A,a}$ | 308.6 (106.41)$^{A,a}$ |
| | 20-30cm | 256.2 (19.95)$^{A,b}$ | 544.8 (337.85)$^{A,a}$ | 233.3 (103.18)$^{A,a}$ |
| Total Nitrogen (μg N/g) | 0-10cm | 97.3 (10.73)$^{A,a}$ | 92.0 (30.00)$^{A,a}$ | 64.0 (10.00)$^{A,a}$ |
| | 10-20cm | 40.0 (14.19)$^{A,b}$ | 36.7(17.68)$^{A,a}$ | 31.3 (13.38)$^{A,a}$ |
| | 20-30cm | 36.0 (9.87)$^{A,b}$ | 84.7 (58.15)$^{A,a}$ | 37.3 (11.10)$^{A,a}$ |
| C/N Ratio | 0-10cm | 5.4 (0.61)$^{A,a}$ | 9.8 (1.34)$^{B,a}$ | 7.6 (0.91)$^{AB,a}$ |
| | 10-20cm | 7.9 (1.83)$^{A,a}$ | 12.5 (2.02)$^{A,a}$ | 10.5 (1.25)$^{A,a}$ |
| | 20-30cm | 8.1 (1.70)$^{A,a}$ | 8.4 (2.46)$^{A,a}$ | 5.6 (1.99)$^{A,a}$ |

$^{A}$ Values followed by the same upper case letters are significantly different among treatments (at $p< 0.05$). $^{a}$ Values followed by the same lower case letters are not significantly different among depths (at $p<0.05$).

**Table 2.13** Variance analysis of SMB values under three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada. Standard errors are given in parentheses. F is the F-statistic, in the brackets P is the significance given as a P-value.

| **Fixed Effect** | **SMB Carbon** (μg C/g) | **SMB Carbon** (μg C/g) | **SMB C/N Ratio** |
|---|---|---|---|
| | F (P>F) | F (P>F) | F (P>F) |
| Treatment (Trt) | 2.39 (0.12) | 0.89 (0.43) | 2.45 (0.12) |
| Depth | 3.13 (0.07) | 2.93 (0.08) | 2.68 (0.10) |
| Trt*Depth | 0.18 (0.95) | 0.49 (0.74) | 0.80 (0.54) |

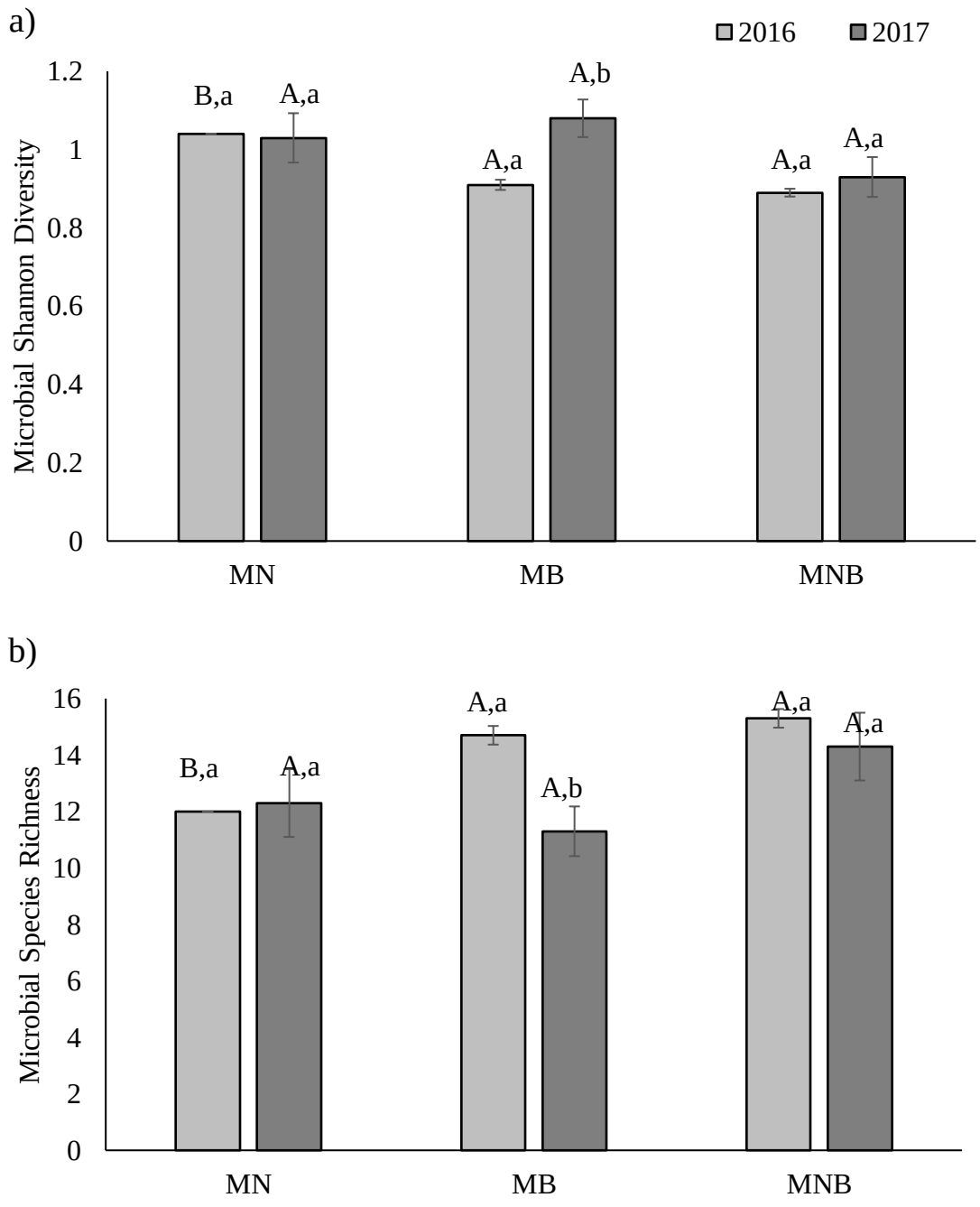


**Figure 2.6** Mean a) Shannon Diversity Indexes (Hs) and b) soil microbial richness (R) counts and from temperate soil amended with three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in 2016 and 2017 from Bayfield, Southern Ontario, Canada.
[A] Values followed by the same upper case letters are significantly different among treatments (at $p < 0.05$). [a] Values followed by the same lower case letters are not significantly different among depths (at $p<0.05$).

*2.4.3 Crop Yield and biomass productivity*

In the 2016 growing season, maize grain yields, shoot and root biomasses, shoot/root ratios, and grain/biomass ratios were not significantly different among treatments (P=0.128). As a general trend for grain yield, MB produced the most corn followed by MN and then MNB (Figure 2.3). MNB produced the most shoot biomass followed by MB and then MN, though the differences were not significant (P>0.005). Treatment did not significantly impact biomass accumulation (P=0.503). A reverse of the grain yield-biochar trend was observed for root biomass accumulation. Though it was still not significant (P=0.823) MNB produced the most roots, followed by MN, then MNB. The shoot/root ratio was found to be highest in MNB, followed closely by MN. While MB had the lowest ratio, there was no significant treatment effect or difference between treatments (P>0.05) (Figure 2.3). In reverse, MB had the highest grain: biomass ratio followed by MN and MNB, though again there were no significant differences between the mean ratio and treatment (P=0.238).

Much like the 2016 growing season, soil treatments did not have a significant impact on crop yield and biomass productivity in 2017 (P>0.05). As a trend, biochar did not increase soybean yields or biomass production. MN had the greatest grain yield, shoot & root biomass, shoot/root ratio, and grain/biomass ratio. MNB had the second greatest measures in each of these, except for the shoot: root ratio and grain: biomass ratio, where it had the lowest numbers. For these ratios, MB had far more similar results to MN, though again none of these differences were found to be significant (P>0.05). Both maize and soybean shoot biomass were significantly correlated (P>0.005) with $NH_4^+$, while at a P=0.05 confidence interval soy grain was negatively correlated with it (P=0.043). No other crop characteristics were significantly correlated with SOC, TN, C/N ratio, $NH_4^+$, $NO_3^-$, $PO_4^{3-}$.

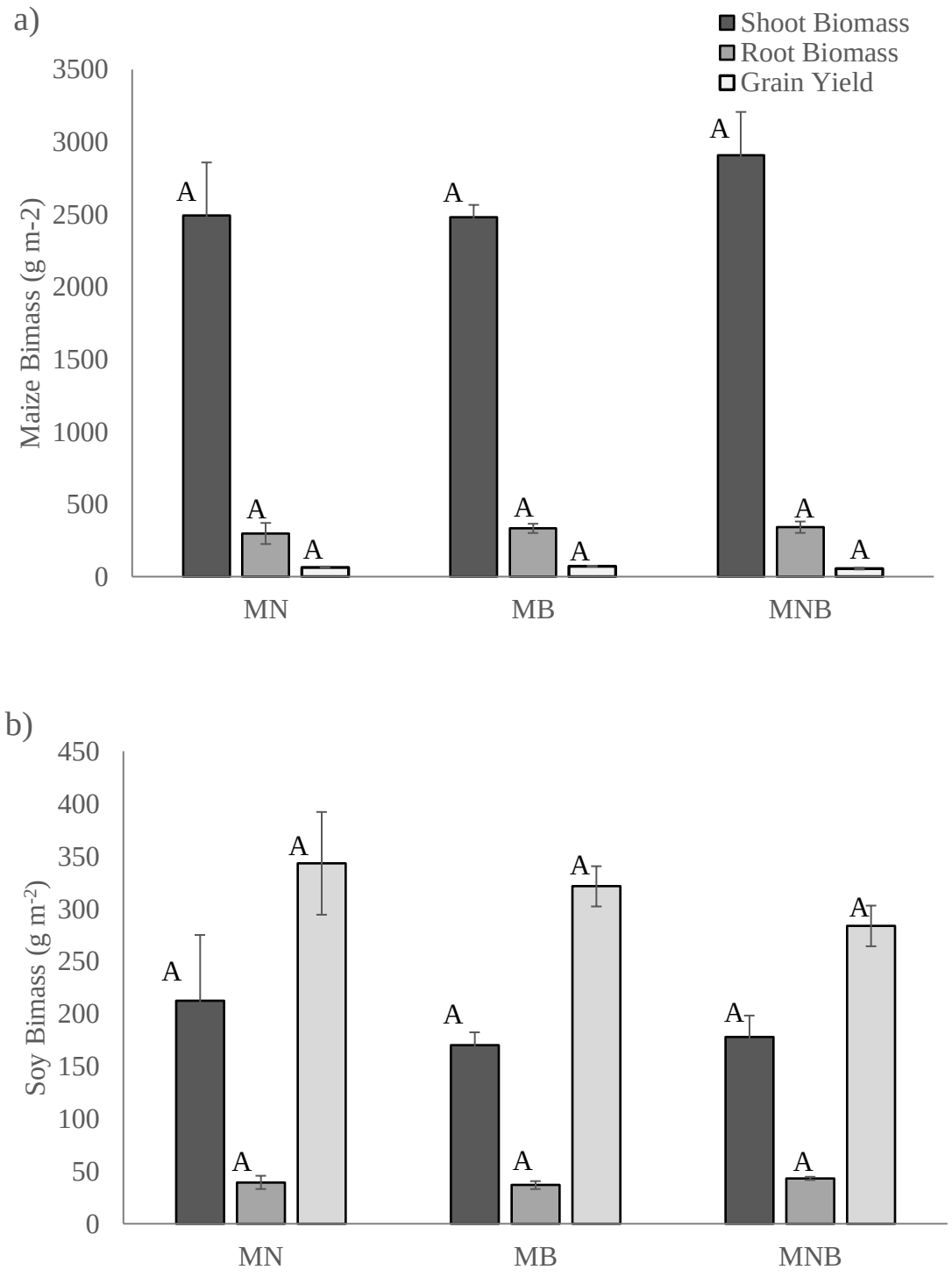


**Figure 2.7** Mean grain yield, shoot biomass, and root biomass of a) maize in 2016 and b) soybean in 2017 from temperate soil amended with three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada. [A] Values followed by the same upper case letters are significantly different among treatments (at $p < 0.05$).

## 2.5 Discussion

### *2.5.1 Biochar effects on soil health*

Soil health is a measure of the ability of soil to support biotic life, crop productivity, improve air and water quality, and resist degradation (Doran & Zeiss, 2000). It includes both physical and chemical characteristics, and it has a reciprocal relationship with soil biology (Doran & Zeiss, 2000). Biochar is known to impact all of these characteristics (Atkinson et al., 2010). Greater changes to soil conditions occur when greater amounts of biochar are incorporated into soils (Appendix A). In this study, only a small portion of biochar was added infield, as this represented the amount of biochar farmers were comfortable applying and could afford (Farrell et al., 2014). Among temperate studies biochar addition rates range from 10 -100 t/ha, while this study added 3 t/ha (Jones et al., 2011b). As expected, few physical changes were observed following this amount of added biochar. The general trend in our results showed that time and sampling depth had a greater effect on these characteristics, and therefore soil health

The one exception to this was soil moisture, which decreased as found to be lower in biochar treated soils as a result of porosity. This result, however, will be discussed in greater detail in Chapter 3 (Clough et al., 2013). Infiltration was one physical characteristic completely unaltered by treatments, or over time. If a change were to have occurred, it would have been expected that infiltration rates increase due to the porosity of biochar and improvements to the course soil structure (Karer et al., 2013; Nelissen et al., 2015; Bamminger et al., 2016, Steiner et al., 2010). No significant changes occurred to aggregate stability following biochar additions; therefore, it is not surprising that infiltration was unaffected as well. The low addition rate of this study resulted in low impacts to soil conditions and did not change soil structure in either year.

Even though bulk density (BD) values appeared nearly identical between the three treatments, a statistical difference was uncovered. The differences were small, but the conventional treatment (MN) had a significantly lower BD than the biochar treated soils. This effect was likely a spurious observation due to sampling or analytical error. In most studies biochar has been found to decrease bulk density, compaction, and erosion by improving soil structure (Karer et al., 2013; Borchard et al., 2016; Nelissen et al., 2015). In this way, biochar has been able to improve soil health. In more than a dozen biochar projects not a single one reported increased bulk density (Appendix A). In 2016 soil was collected following maize remover, causing soils to be compacted by machinery, and bulk density values were slightly higher ($P<0.001$) (Hamza & Anderson, 2005). The scale of compaction was minimized, likely due to the coarse-texture of the soil, and had no apparent impact on soil health (Hamza & Anderson, 2005).

Like bulk density, aggregate stability is an important physical characteristic of healthy soil as it promotes seedling growth, root penetration, gas diffusion, and water transport (Hartley et al., 2016). Among sandy temperate soils, biochar has been found to enhance aggregate stability (Abujabhah et al., 2016; Hartley et al., 2016). As already mentioned no significant differences emerged among treatments. Only small differences existed in 2016; Borchard et al. (2016) similarly saw this in their 15 t/ha application of biochar. In the first year, biochar soils had slightly lower aggregate stability but by 2017 this improved, though no changes emerged among treatments. Nelissen et al. (2015) also saw a more distinct improvement in aggregate stability due to biochar in their second year. However, since MN had increased stability along with MB and MNB, it was likely land use management, such as different crop grown, that resulted in the improved stability (Lal, 2011). The general trend among all physical

characteristics was that small additions of biochar could only bring about small changes. The potential benefits of biochar described in the literature were not observed. At the same time, negative impacts to soil physical properties were not seen either.

Physical and chemical characteristics continually interact. Just as many physical characteristics were unaffected by biochar additions, so were many chemical characteristics. While biochar frequently raises pH within temperate studies, this project did not observe any impact to soil pH (Prendergast-Miler et al., 2011; Luo et al., 2011; Rutigiano et al., 2014; Schomberg et al., 2014). Güereña et al. (2013) saw no change to pH either from their 3, 12, or 30 t/ha additions to a silt-loam in New York. However, Jones et al. (2012) saw an initial increase after adding 50 t/ha to a sandy-clay loam in Wales, which was already acidic. The increase was only short-lived and dropped as biochar aged (Jones et al., 2012). Once again, the amount of biochar added in our study appeared too little to impact pH. As well, lower temperature biochars, such as the biochar used by Luo et al. (2011) and Schomberg et al. (2012), have been found to be more reactive and more likely to result in observable impacts. This study's biochar is considered a high pyrolysis temperature biochar, and therefore less likely to affect pH. Furthermore, this region in southern Canada has very neutral calcareous soils, which resist pH fluctuation (Ippolito, Laird, Busscher, 2012). Song et al. (2018) and Lentz & Ippolito (2012) saw no effect after adding even upwards of 30 t/ha of high pyrolysis temperature biochar to similar soils calcareous soils.

Other chemical characteristics that exert strong influences on soil productivity are soil organic carbon (SOC), total nitrogen (TN) and the ratio between them (C/N). Soil carbon and nitrogen have a reciprocal relationship, where soil nitrogen expands the ability of carbon pools to grow and be maintained, while soil carbon is necessary to retain and store soil nitrogen (Knicker,

2011). Therefore, both must be monitored together. In several temperate studies, biochar has been observed to stabilize or enhance SOC and increase the availability of TN (Lentz & Ippolito, 2012). One proposed way biochar stabilizes SOC is through the inhibition of carbon mineralization (Lentz & Ippolito, 2012; Qayyum et al., 2012). However, this is a point of contention, and biochars produced under lower temperatures have been suggested to cause an initial loss in SOC by stimulating mineralization with labile carbon inherent to new biochar (Mitchell et al., 2015; Qayyum et al., 2012; Luo et al., 2011). Temperate studies have also observed this spectrum of effects (Nelissen et al., 2015; Yuan et al., 2017). Moreover, large amounts of biochar can also immobilize TN, causing plant N deficiency (Atkinson et al., 2010; Steiner et al., 2010). In this study, a loss of SOC and TN was not observed in the first year; both measures were found to be higher in biochar treatments following the first growing season. This increase was not statistically significant, and became far less distinct in the second year of this study. Only depth was an impactful factor for both of these measures. The SOC and TN content at lower depth increased as expected since natural processes easily move biochar-C and nutrients down through sandy soil (Major et al., 2010; Major et al., 2009).

Not only are individual C and N quantities important to monitor, but their ratio is essential as well. C/N ratios indicate the quality of organic matter in the soil, decomposition levels and determines microbial activity potential (Batjes, 1996). A ratio between 10 to 12 can be found in most soils, with higher ratios making soil increasingly prone to N immobilization and resistant to degradation (Atkinson et al., 2010). As a trend, this project's biochar-treated soils had slightly higher ratios than MN. To effect C/N, biochar projects added between 10-90 t/ha (Gaijć & Koch, 2012; Kloss et al., 2014). The only significant impact on C/N in this study was the redistribution of C/N between depths among treatments. C/N ratios were more even distributed

in the biochar treated soils than MN. Each depth, 0-10 cm, 10-20 cm, and 20-30 cm, had fairly even ratios in biochar treated soil. MN had much lower 10-20 cm and 20-30 cm C/N ratios. The higher ratios of C/N among all depths in biochar treated soils indicated greater degrees of microbial activity and breakdown throughout the soil profile (Batjes, 1996). Soil texture as well impacts the vertical distribution of the C/N ratio, where sandy soils are unable to retain more SOC in the upper layers of soil (Hobley et al., 2013). Biochar has been observed to sorb SOC and reduce loss (Zimmerman et al., 2011). Such redistribution was also observed by Naisse et al. (2015) in sandy soil in France, which was attributed to different microbial activities and soil retention capacity. Naisse et al. (2015) measured an increase in stable SOM in deeper subsoils.

The free light fraction (FLF) of soil represents an important portion of soil nutrients (Demisie et al., 2014). FLF represents the relatively young, labile pool, of SOM (Demisie et al., 2014). FLF is an important fraction of SOM since it contributes to SOC and TN turnover within soil and quickly reacts to environmental changes (Vasilyeva et al., 2011; Demisie et al., 2014). FLF was analyzed in this study to forecast changes to SOC and TN. While biochar did not impact the amount of SOC or TN, there was the potential that changes could have been observed within the FLF (Vasilyeva et al., 2011). However, biochar did not significantly alter total FLF-C or FLF-N. This was unsurprising since such a small portion of biochar was added to the soil, and only ~30% of biochar contributes to the FLF (Vasilyeva et al., 2011). Demisie et al. (2014), which used wood biochar pyrolyzed at a high temperature, only observed a ~5% contribution to FLF-C. Our study used a similar biochar type, and in the first year, it appeared to increase FLF-C in upper layers of soil slightly. Demisie et al. (2014), concluded that even a small increase in FLF-C was favorable for improving soil quality; improving aggregation and carbon sequestration. As a result of the FLF-C increase, the total FLF C/N ratio was significantly higher for biochar soils,

especially in the first year of this study. Biochar's FLF-C contributions were labile and were consumed by microbes before harvest 2017; in this way there appeared to be biologically facilitated biochar aging (Ameloot et al., 2013). Keith et al. (2011) also observed this quick loss of labile FLF-C to the microbial community. By 2017 labile FLF had decreased, as well as was more evenly distributed within the biochar-treated soil. Similar to Vasilyeva et al. (2011), this study found that biochar treated soils appeared contribute to mobile FLF, which became distributed throughout several depths, not just the top 10 cm. Vasilyeva et al., (2010) found that biochar originating FLF moved down the soil horizon.

One conventional indicator of soil health is macronutrient concentrations; it is a common limiting factor for productivity. Following the single amendment addition, Spring 2016 had the greatest $NH_4^+$, $NO_3^-$, and $PO_4^{3-}$ concentrations. From then on concentrations fell across that year. Much like Jones et al. (2012), biochar's impact on $NH_4^+$ concentrations in sandy soil was not significant, though trends were observed. In spring 2016, $NH_4^+$ concentrations were initially lower in biochar treated soils, as a result of absorption (Muchow, 1998; Clough & Condron, 2010). This absorption of $NH_4^+$ by biochar was commonly observed in other temperate studies (Bamminger et al., 2014; Anders et al., 2013; Clough & Condron et al., 2013). After the initial absorption of $NH_4^+$, concentration decreases were less extreme among biochar treated soil in 2016. By the end of the growing season, MB and MNB had very similar concentrations to MN (Figure 2.3). The ability of biochar to hold $NH_4^+$ and $NO_3^-$ depends on the feedstock and pyrolysis temperature of the biochar (Atkinson et al., 2010). Being composed of pine/spruce and pyrolyzed at a high temperature the char pore size is expected to be reasonably large, indeed greater than 0.30 nm. For this reason, $NH_4^+$ ions, which have a diameter of 0.286 mm, can be readily absorbed into the pores of biochar (Späth, 2010). Clough et al., (2013) however did not

find that pyrolysis temperature trends impacted $NH_4^+$ availability, but rather decreased ammonia lose was the result of biochar feedstock materials, which determined surface CEC rates (Kloss et al., 2014). Clough & Condron (2010) found short-term CEC increases with biochar amended soils, which only improved after weathering, increasing its ability to hold onto $NH_4^+$ and enhance soil fertility. It is unclear whether aged biochar was able to retain more $NH_4^+$ in 2017, or if the increased concentrations were solely the result of the soybean crop. In 2017, there were increased $NH_4^+$ and $NO_3^-$ content. In 2017, $NH_4^+$ was greatest at mid-June, when soybean nodules were most active in producing ammonia ($NH_3$) (Garg & Geetanjali, 2007). $NH_4^+$ peaked then and decreased steadily until August as soybean crop matured and required less nitrogen fixation (Figure 2.2). $NO_3^-$ concentrations began to rise in early September, due to the shedding and decomposition of the soybean leaves. In early October, these concentrations rapidly fell.

Nitrate also underwent an extreme drop in concentration following spring 2016, but unlike $NH_4^+$, the depth-by-treatment trends were significant (Figure 2.3). Biochar treated soil had the lowest concentrations in the first year, again, likely the result of adsorption (Sarkhot et al. 2011; Bamminger et al., 2014). Biochar is known to temporarily immobilize $NO_3^-$, decreasing its availability to plants (Atkinson et al., 2010). Though the biochar treated soil had the lowest concentrations they held the greatest proportion of their $NO_3^-$ in the top portion of soil. $NO_3^-$ was nearly evenly distributed in MN among all measured depths. Prendergast-Miller et al. (2011) similarly saw $NO_3^-$ localized in the rhizosphere of biochar treated soils. Prendergast-Miller et al. (2011) attributed the accumulation to reduced leaching and increased biological use-efficiency of $NO_3^-$. The extent of leaching-prevention can equal or overcome losses from adsorption, allowing biochar treated soils' $NO_3^-$ concentrations to exceed those in conventional treatments (Clough et al., 2013; Haider et al., 2017). High-temperature biochars reduce organic N built into the char

structures, but are made more porous, and therefore able to retain onto both $NH_4^+$ and $NO_3^-$ (Clough, 2013). Biochar also increases areas of cation and anion exchange, thus further altering $NH_4^+$ and $NO_3^-$ movements in soil (Kloss et al., 2014; Atkinson et al., 2010).

Phosphate ($PO_4^{3-}$) was one of the few soil characteristics affected by biochar. In 2016, the outcome resembled that of Rogovska et al. (2014); biochar resulted in initial lower $PO_4^{3-}$ concentrations (Zhang et al., 2016). Like $NH_4^+$ and $NO_3^-$ concentrations in 2016, MN and MNB began with the highest concentration of $PO_4^{3-}$ due to fertilizer additions. However, biochar appeared to absorb phosphorous since MNB had a lower concentration than MN (Rogovska et al., 2014; Laird et al., 2010). MNB was not much lower than MN since wood-sourced high pyrolysis temperature biochars have moderate to low sorption capacities (Riddle et al., 2018). However, in 2017 $PO_4^{3-}$ accumulated across all treatments and a new trend emerged. This apparent increase in year two was likely in part due to soybean being grown instead of corn. While corn has greater amounts of residue and therefore P stored within its residue (83.1 lb $P_2O_5$/ acre); corn loses minimal P (31%) between maturity and harvest (Oltmans & Mallarion, 2011). In contrast, soybean can lose upwards of 60% of residue-P between maturity and harvest, the vast majority of this takes place between September and October, which is when soil sampling took place in 2017 (Oltmans & Mallarion, 2011). It is thought that this caused the observed increase in $PO_4^{3-}$ concentrations in 2017. While all treatments had increased P in 2017, the increase was not even among treatments. Biochar treated soils accumulated far more available P (Table 2.9). This may be due to lower pore absorption rates, which no longer off-setting biochar's ability to reduce P leaching (Lehmann et al., 2011; Joseph et al., 2010; Liang et al., 2006). Biochar pores readily absorbs nutrients and soil materials when initially added to the soil; over time these pores become filled and the rate of absorption is decreased, as seen in 2016

(Lehmann et al., 2011; Joseph et al., 2010; Liang et al., 2006; Domene et al., 2014, Laird et al., 2010). Side functional groups on the surface of biochar increase its anion exchange capacity (AEC) and reduce nutrient loss (Domene et al., 2014; Sarkhot et al., 2011; Laird et al., 2010; Chintala et al., 2013). Large adsorption rates may have initially hidden surface AEC effects, once absorption decreased the biochar treated soils retained more $PO_4^{3-}$ than the conventional treatment. Furthermore, wood-sourced, high pyrolysis temperature biochar, which was also used in this study, has been found to act as effective P fertilizers, even after 6 seasons (Wang et al., 2012; Riddle et al., 2018; Zhang et al., 2016; Khodadad et al., 2011; Zhai et al., 2015). Sandy soils are especially susceptible to phosphorous loss, and calcareous soils can limit biotic access of P, such soils can benefit from biochar which decreases leaching and increases biotic sorption (Zhai et al., 2015; Domene et al., 2014; Chintala et al., 201). The ability of biochar to prevent leaching and retain nutrients is among its most impactful for supporting soil biology (Atkinson et al., 2010).

*2.5.2 Biochar effects on soil biology*

The only significant result within macrofauna came in the second year, where *Haplotaxida* (earthworms) counts decreased in biochar treated soils. It is thought that earthworms perhaps graze on microbes growing on the surface of biochar (Lehmann et al., 2011). Though it is still unclear how microbial communities impact earthworm populations, it has been found that earthworms in biochar treated soils can also alter microbial communities (Bamminger et al., 2014). Given that no differences existed within the microbial community structure or soil microbial biomass (Table 2.13; Figure 2.6) in 2017, it is unlikely that food source alone caused the decrease in the second year. While Lehmann et al. (2011) saw weight loss of earthworms, they also observed them deliberately ingest biochar for an unknown reason.

2017 was, as well, a wetter year than 2016; therefore desiccation was unlikely impacting the worms as it had for Li et al. (2011). While toxic by-products have been proposed as an earthworm deterrent, those were observed in treatments that had upwards of 90 t/ha additions or used low pyrolysis temperature biochars (at 10 cm depth) (Li et al., 2011; Iqbal et al., 2015). As well, these compounds would have exerted a more significant effect in 2016 than 2017. It is possible that due to 2016's dry year too few earthworms were present to observe treatment impacts. On average only 14 earthworms were counted among all plots and depths at each sampling events. Most of the biochar impacts had diminished by the second year of this study, except moisture, so it is yet unknown which effects induce earthworm avoidance. Earthworms are an important component of biochar transport and contribute to its movement between soil horizons (Joseph et al., 2010; Lehmann et al., 2011). The relationship between biochar and macrofauna - let alone biochar, microbes, and macrofauna - is still poorly understood.

Biochar additions did not impact total soil macrofauna. Most soil characteristics were unchanged by biochar, thus it is no surprise that insect diversity or abundance was not greatly affected (Domene et al., 2014). Although not significant, there was a general trend that MB had the greatest abundance of macrofauna, while MN had the greatest diversity (Hs). Interestingly, this trend was mirrored by soil microbial diversity. It is possible that microbial community changes influenced the Hs and richness of macrofauna communities since the microbial changes were significant (Marks et al., 2014). Another trend observed in the first-year was that biochar-treated soils saw more *enchytraeids* (whiteworms), which are soil indicators sensitive to disturbances (Pelosi & Römbke, 2016). Whiteworms are very sensitive to nutrient additions in agriculture, explaining why they were not seen in MN (Pelosi & Römbke, 2016). MB had the greatest number of whiteworms, though MNB had a similar total; therefore, the biochar perhaps

buffered the impacts of urea fertilizer. Unlike other macrofauna species, whiteworms do not feed on soil microbes (Marks et al., 2014; Domene et al., 2015).

Soil health impacts soil biology, and while biochar altered few soils characteristics, some impacts to soil microbial diversity were still observed (Nelissen et al., 2015). In year one of the study, biochar-treated soils had the highest diversity of species (Figure 2.6). However, the elevated levels dropped by the second year, which may have been the result of FLF-C and N difference from biochar (Anders et al., 2013; Demisie et al., 2014). Labile C and N from biochar may have supported the initial increase in microbial species richness (Luo et al., 2013, Jones et al., 2012). Microbes consumed labile compounds within the first year of the study. By 2017 biochar FLF-C was halved, and the extra species were lost; species diversity was equal among all treatments. Potentially, some of the microbial changes could have been the result of the crop change between 2016 and 2017 Imparato et al. (2016) observed increases in soil microbial diversity following low biochar additions. When considering the evenness of microbial species distributions, it was found that MN had the greatest Shannon Diversity index (Figure 2.6). While biochar-treated soils had the most number of unique species, only a couple of species dominated; which is another impact commonly observed (Rutigiano et al., 2014; Khodadad et a., 2010). Mitchell et al. (2015), Bamminger et al. (2016), and Rutigiano et al. (2014) also observed short-term impacts to microbial community structures lasting between 4 to 14 months, depending on biochar type and addition rate. In the second year of this study, the Shannon index and total soil microbial biomasses were very similar among treatments; it was in the first year that biochar favoured specific microbial groupings as it did for Rutigiano et al. (2014) and Bamminger et al. (2016). The PCA also illustrated this change (Figure 2.5). In 2016, the MN microbial communities were very different from the biochar treated microbial communities. By 2017 all

communities resembled one another and were only slightly different from the previous year. At this time, it is unknown which groups were favoured in the first year, whether it was fungi, as was the case for Luo et al. (2011), or gram-positive bacteria as was the case for Mitchell et al. (2015). Along with Hs and richness evening out by 2017, SMB measurements were similar among all the treatments. MB did have the greatest SMB carbon though. As seen, in the PCA (Figure 2.5), the three 2017 communities were slightly different from 206, but all more similar among themselves. The PCA seems to indicate available nutrients impacted the microbial communities since MN 2016, which had the greatest concentrations stood apart from all communities. By 2017 the nitrogen concentrations had dropped, most drastically in MN, and evened out, making all the communities similar.

*2.5.3 Biochar effects on crop growth*

Soil health also drives crop growth; it is little surprise that following very few changes to soil characteristics, productivity was not affected by biochar in 2016 and 2017. Crop biomass, (2016 maize and 2017 soybean), was not significantly different among treatments (Figure 2.7). Deng et al. (2015) also observed no significant changes to maize yield after adding 25 t/ha of biochar in Tennessee. Hüppi et al. (2015) also saw no impact after adding 20 t/ha in Switzerland. An amount of biochar far greater than 3 t/ha would have been needed to impact our yields (Borchard et al., 2014; Bamminger et al., 2016). In order to observe decreases to maize yield, biochar additions of 72-300 t/ha were needed (Karer et al., 2013; Borchard et al., 2014). Though, as a trend, despite lower available moisture and phosphate, the biochar-treated soils produced slightly greater root biomass, shoot biomass, and MB produced the greatest grain yield. In our study, phosphate was not the most important soil characteristic determining crop growth. Only $NH_4^+$ had a significant correlation with maize shoot biomass; which is uptaken by shoot biomass early

on in maize development (Bender et al., 2013). Grain yield, which is primary concern agriculture, did not appear to be affected by the soil chemical characteristics observed in this study.

The slight increase in grain yield in the biochar plots did not carry over into soybean production. Though not significant, it appeared that biochar soils produced a lower average grain yield in comparison to the conventional treatment. In the literature, additions of as little as 5 t/ha significantly affected soybean yield additions of (Glaser et al., 2002; Kishimoto & Sugiura, 1985). Soybean, which seemed to be negatively impacted by biochar in this study, is more sensitive than corn to soil characteristics such as pH and micronutrient deficiencies (Glaser et al., 2002; Kloss et al., 2014). Macronutrients did not appear to be the leading cause of all the decreases, since $NH_4^+$, $NO_3^-$, and $PO_4^{3-}$ were not significantly correlated with grain yield or root biomass. $NO_3^-$ was however correlated with soybean shoot biomass, in fact, $NO_3^-$ was lowest in MB treatments and yielded the lowest shoot biomass. The biochar may have limited $NO_3^-$ availability during shoot growth by absorbing what was converted from soybean $NH_3$ production. The more biochar added, the greater the effect (Glaser et al., 2002). Kloss et al. (2014) saw a decrease in soybean production only in the first year following 30 t/ha and 90 t/ha additions. Lentz & Imppolito (2012) saw an increase in crop yields in their first year after a 22.4 t/ha biochar addition but a decrease in yield in their second year, a pattern resembling our own study.

**2.6 Conclusions**

In conclusion, biochar affected few soil conditions in this project as a result of the low amounts integrated with the field. When using a tenth of the average biochar temperate study addition, only moisture, $PO_4^{3-}$, light fraction C/N ratios, microbial richness, and microbial

Shannon diversity were significantly impacted. Just the moisture differences remained in 2017. The degree of variation was still small between treatments. In 2016, both moisture and $PO_4^{3-}$ were on average lower in biochar treated soil due to biochar's absorptive quality. For moisture, there was simply a consistently lower level, but for $PO_4^{3-}$ there was an initial decrease but also a reduction in P loss. Once minerals and particles filled biochar pore spaces, they no longer absorbed and lowered available soil phosphate. At this point, phosphate began to accumulate in biochar treated soils. Labile C and N on new biochar resulted in light fraction differences. These labile compounds were consumed by soil microbes, temporarily stimulating richness, but also reducing diversity. After the first year, labile nutrients were consumed, and FLF-C/N levels and microbial communities normalized. Regardless of these changes, there were no significant effects to corn or soybean yields in the first two years of this study. Therefore, the implemented biochar design of this project was an economically feasible way to store previously atmospheric carbon in the soil.

# 3. Impact of biochar on greenhouse gas emissions from temperate agriculture: A field study

## 3.1 Overview

Biochar has demonstrated influence over greenhouse gas (GHG) emission rates in short-term laboratory and short-term field studies. Few long-term field studies exist in temperate systems that investigate biochar effects on GHGs. The objective of this study was to determine the long-term effects of biochar on GHG emissions from biochar-amended agricultural soil in Ontario. The treatments, established on sandy loam soil in Southwestern Ontario, consisted of; (1) poultry manure (6 t/ha) and nitrogen (urea) fertilizer (135 kg/ha) (**MN**); (2) poultry manure (3 t/ha) and biochar (3 t/ha) (**MB**); and (3) poultry manure (3 t/ha), fertilizer (135 kg/ha), and biochar (3 t/ha) (**MNB**). Following the implementation of the treatments, various tests were conducted bi-weekly to determine the effects of biochar on GHG emissions and the soil characteristics that influence emissions. Sampling took place within two growing seasons, from 2016-2017. Soil moisture, temperature, and $NO_3^-$ were significantly lower in biochar soils ($P>0.05$). Soil $CO_2$ and $N_2O$ emission rates were not significantly different among treatments in either 2016 or 2017. Only $N_2O$ emission appeared to be impacted by treatments in 2016 ($P=0.04$), however, no treatments were significantly different from one another in total or within individual seasons ($P>0.05$). Both GHG emissions did appear to be different among seasons ($P>0.05$). $CO_2$ and $N_2O$ emissions were frequently affected by soil moisture, temperature, and available soil nitrogen. In some seasons moisture was a strong influence on emission rates. This study determined that complex interactions among soil and soil microbial communities affect soil characteristics and drive GHG emissions.

## 3.2 Introduction

Biochar is charcoal, pyrolytically altered biomass that is used as a soil amendment to improve soil health and crop yields (Ameloot et al., 2013; Lehmann et al., 2006). Biochar has been used as an amendment in tropical agricultural soils for millennia; its highly conjugated and aromatic carbon structure has enabled biochar to resist degradation and remain there for thousands of years (Atkinson et al., 2010). Biochar aided in improving the physical, chemical, and biological characteristics of tropical soils. Biochar has been used to increase soil water holding capacity, nutrient retention and exchange, and pH (Atkinson et al., 2010; Van Zwieten et al., 2010; Sohi et al., 2010; Lehmann et al., 2006; Kloss et al., 2014). Temperate soils often have a naturally higher soil pH than tropical soils, as well more soil organic matter (SOM), different microbial communities, a unique mineral composition, and higher nutrient retention; therefore, they do not receive the same benefits from biochar (Kloss et al., 2014). However, biochar has an additional capability to reduce greenhouse gas emissions from agricultural soil (Bamminger et al., 2014). At this time is it unsure how temperate systems will respond to biochar additions; research on temperate biochar responses is still in its infancy and comprehensive long-term studies are limited (Schomberg et al., 2012).

Based on available research, it has been proposed that biochar can be used as a carbon-negative soil amendment to mitigate climate change in temperate agroecosystems (Hammond et al., 2013; Borchard et al., 2014). Agriculture represents a notable 12% of anthropogenic GHG release (Smith et al., 2014). Reliance on manure and fertilizers contribute to substantial greenhouse gas emissions. In the US, nitrogen fertilizers and cropping practices result in 78% of total national nitrous oxide ($N_2O$) emissions (Smith, 2017; Ameloot et al., 2013). Globally it 60% of $N_2O$ emissions (Smith et al., 2007; Nelissen et al., 2014). Carbon dioxide ($CO_2$) and $N_2O$

are two agriculturally produced GHGs of concern; $CO_2$ due to its prevalence and $N_2O$ due to its potency (Smith et al., 2014; Smith, 2017). Biochar can affect atmospheric concentrations of these gases in two ways. Firstly, up to 50% of plant carbon (C) (sequestered from the atmosphere) can be converted, not lost during pyrolysis, into carbon-rich biochar (Zhao et al., 2014). Biochar is then added to soils where it is stored and remains for hundreds of years (Jones et al., 2011a). Secondly, biochar alters microbial ecosystems and the availability of nutrients to the microbiome (Lehmann et al., 2011; Anders et al., 2013). The resulting microbial communities have lower $CO_2$ and $N_2O$ emissions, which can occur for example if the new communities are more efficient or are no longer dominated by denitrifiers (Lehmann et al., 2011; Bamminger et al., 2014; Smith et al., 2014; Zimmerman et al., 2011). It is unknown to what extent biochar alters soils physical characteristics (i. e. soil structure, soil moisture), chemical characteristics (i. e. pH, nutrient concentrations), and edaphic processes (i. e. decomposition, nitrification/denitrification), and how these influence GHG emissions (Lehmann et al., 2006; Lehman et al., 2011). The exact mechanisms of interactions differ among soils, climates, and biochar types (feedstock and pyrolysis temperature) (Lehmann et al., 2006; Lehman et al., 2011). Spokas and Reicosky (2010) observed that among 16 different biochar studies: a third increased $CO_2$ emissions, a third decreased them, and the remaining studies caused no change.

Biochar has only recently begun to be used in temperate agriculture and therefore it lacks a substantial body of literature (Lehmann et al., 2011). As well the majority of temperate biochar research has been conducted in the laboratory, rather than field; this is true of $CO_2$, and especially $N_2O$, research. In 2010, Sohi et al. and Clough & Condron (2010) identified no existing peer-reviewed in-field biochar studies investigating $N_2O$ emissions. Though a handful of

field condition temperate experiments have been carried out for $CO_2$, few resemble a typical Canadian agricultural system (Atkinson et al., 2010).

Few studies assessing biochar's impact on GHGs explore the exact interacting mechanisms initiating the quantified changes (Lehmann et al., 2011). Soil moisture is one proposed characteristic that, under the influence of biochar, impacts microbial communities and therefore emissions (Rondon et al., 2005; Yanai et al., 2007; Liesch et al., 2010). Biochar has been found to either increase or decrease soil moisture, depending on the parent material and biochar type (Glaser et al., 2002; Clough et al., 2013; Kulyk, 2012). Typically, sandy soils experience the greatest increase in available moisture (Rogovska et al., 2014; Hammond et al., 2013). Soil temperature is another characteristic proposed to impact emissions, though little evidence supports that biochar significantly influences soil temperature (Kulyk, 2012; Krull et al., 2004; Anderson et al., 2011). Chemically, $NH_4^+$ and $NO_3^-$ are known to directly influence $N_2O$ emissions (Kulyk, 2012; Anderson et al., 2011; Signor & Cerri, 2013). Soil pH is another chemical factor that strongly influences microbial emission rates and can be increased by biochar (Lehmann et al. 2006). As well, soil C/N ratios affect GHG soil emissions since they influence biotic activity and nutrient cycles (Cayuela et al., 2014). Newly produced biochar is partially comprised of labile carbon residues (Jones et al., 2011a). These residues are thought to be quickly mineralized, and therefore a short-term increase in GHG emissions (Jones et al., 2011a; Zimmerman et al., 2011). After the labile carbon was decomposed emission rates fell (Atkinson et al., 2010; Zimmerman et al., 2011; Cross & Sohi, 2011). Zimmerman et al. (2011) reported biochar sorbing SOM onto its surface and thereby reduce its degradation and GHG release. As well, biochar pyrolysis forms aromatic and polycyclic hydrocarbons that do not support, or will even suppress, microbial biological processes and reduce GHG emissions (Gomez et al., 2014).

Likely, a combination of the above factors impact soil microbial communities, and in conjunction, alter GHG levels (Lehmann et al., 2006; Lehmann et al., 2011; Ball et al., 2010; Mitchell at al., 2015).

No consensus has been reached on the exact process behind biochar's influence of GHG emission rates. Biochar alters the interacting physical and biochemical characteristics in soils (Lehmann et al., 2006; Lehmann et al., 2011). It is likely that for each individual soil and biochar mixture, a different soil characteristic or interaction will dominate emission release (Spokas & Reicosky, 2009). Agricultural systems should not be presumed equal within biochar studies. It is essential to build an extensive body of knowledge for temperate biochar research. Canadian biochar research contributes to improving the understanding of temperate soil responses to biochar (Glaser et al., 2015). Therefore, the objective of this study was to evaluate temporal variation in GHG emissions in a biochar-amended soil over several seasons, across two years. This study provides novel information on biochar impacts to GHG emissions of calcareous soil in a southern Canada.

## 3.3 Methods

### *3.3.1 Site Description*

Refer to *Chapter 2.3.1*. Note that GHG collection continued for another month after 2016 and 2017 harvests.

### *3.3.2 GHG Sampling and Analysis*

Two PVC chamber bases (10 cm diameter, 25 cm length) were inserted at two randomly selected points to a depth of 10 cm for biweekly gas sampling (total of 18 ports) in each treatment replicate (Parkin & Venterea, 2010). The chamber bases were installed in May and

remove briefly for crop harvest and removed again at the end of the season in November in 2016 and 2017. Following installation, the open gas ports were left for several days to allow the soils to regain equilibrium following the installation disturbance. Chamber caps were covered with reflective insulation and had a 1 cm rubber septum for air extraction and 10 cm long vent tube (3 mm inner diameter) to release internal pressure buildup (Dyer et al., 2012; Smith et al., 2003).

Since GHG emissions have large temporal variations, sampling occurred biweekly (Parkin et al., 2012). Samples were extracted between 10 a.m. and 3 p.m. to account for diurnal fluctuations and minimize sampling biases (May through November 2016 and 2017). At sampling, the open ports were capped and gas samples were collected with a 10 ml syringe. Originally, the gas was collected at 0, 10, 20, 30 min from May-September 2016. However, in September 2016 this was reduced 0, 15, 30 min when it was found that four sampling points did not yield more precise rates of gas emissions. 10 ml of gas was collected from each chamber headspace, manually injected into a 3 ml evacuated vial, and stored at room temperature.

$CO_2$ and $N_2O$ concentrations (ppm) were quantified with an Agilent 6890 Gas Chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA), using a capillary column attached to a micro-electron capture detector (ECD). The GHG emission rates were calculated using an equation developed by Hutchinson and Mosier (1981). An initial equation was used to determine if the GHG release resembles a linear or curvilinear response

Eq. [3.1] $$\frac{C_1-C_0}{C_2-C_1}$$

where $C_0$, $C_1$, and $C_2$ are the flux values at time 0, 15 and 30 min [ppm (v)], respectively. If Eq. 1 is <1, a linear regression slope is used to solve for GHG flux. If the ratio of Eq. 1 yields a result >1, another Hutchinson & Mosier equation is used [Equation 3.2].

Eq. [3.2] $$f_{(0)} = \frac{V(C_1-C_0)^2}{[A*t_1(2*C_1-C_2-C_0)*\ln[\frac{C_1-C_0}{C_2-C_1}]}$$

In this equation $C_0$, $C_1$ and $C_2$ are the flux values at time 0, 15 and 30 min (ppm (v)), V is the volume of the capped chamber ($cm^3$), A is the soil surface area covered by the chamber ($m^2$), and $t_1$ is the time interval between sampling events (min) (Hutchinson & Mosier, 1981). The equation yields flux ($f_0$) are in μL of GHG gas ($CO_2$ or $N_2O$) $m^{-2}$ $min^{-1}$ (Hutchinson & Mosier, 1981). Values were then converted into μmol $m^{-2}$ $h^{-1}$ of trace gas using the ideal gas law, and molecular masses were used to adjust the flux to μg $m^{-2}$ $h^{-1}$ of trace gas (Lutes et al., 2016). The atmospheric pressure and temperature for the ideal gas law were collected from historical weather data from the Goderich Weather Station (43°45'58.8"N, 81°43'01.1"W).

*3.3.3 Soil Sampling and Analysis*

The same two sample points for GHG collection were used for $NH_4^+$ and $NO_3^-$ analysis. Biweekly, soil samples were taken within 1 m radius of each sample point. Between 10 a.m. and 12 p.m. soil temperature and moisture were measured using a HH2-WET sensor biweekly during soil collection at these same sample points (Delta T Devises, Cambridge, UK). Changes in $NH_4^+$ and $NO_3^-$ were quantified using the same methodology described in *Chapter 2.3.2.* Samples were collected biweekly for 24 weeks during the growing season between May- November 2016 and 2017 and coincided with the gas collection.

*3.3.4 Statistical Analysis*

All statistical analyses were conducted as described in *Chapter 2.3.4.* GHGs and soil characteristics were analyzed between years (2016, 2017) or among seasons [spring (May 19$^{th}$ to June 21$^{st}$), summer (June 22$^{nd}$ to September 2$^{nd}$), and autumn (September 23$^{rd}$ to November 5$^{th}$)]. Two-way ANOVAs were used to determine whether time (year or season) and treatment type were interacting to significantly influence emissions or soil characteristics (Kloss et al., 2014;

Lutes et al., 2016). Distribution normality was determined using the Shapiro-Wilk Test and Kolmogorov-Smirnov Test. Any value distributions that were not normal ($p>0.05$) were given as medians. Pearson's two-tailed correlation analysis was used for each treatment to determine the relationship between $CO_2$, $N_2O$ and soil characteristics (moisture, temperature, $NH_4^+$, and $NO_3^-$).

## 3.4 Results

### *3.4.1 GHG Emissions*

The interactive effect of treatment-by-season was not significant for $CO_2$ emissions. Neither was treatment alone a significant effect on $CO_2$ emissions in 2016 or 2017 ($P>0.05$). In 2016, while there was no significant difference among soil treatments, MN had the greatest mean rate in the spring and autumn (Table 3.2). In 2016, MB had the greatest emission rate in the summer. Though 2017 saw no significant difference among treatments, in this year MNB had the greater mean $CO_2$ emission rate; specifically, in spring and summer. The total emissions were not significantly different from 2016 to 2017 ($P=0.098$). In both 2016 and 2017, $CO_2$ emissions among seasons were significantly different ($P<0.001$). Emissions were significantly greater in the springs followed by summers then autumns ($P<0.05$). In 2016, $CO_2$ emissions were significantly correlated with several soil characteristics. $CO_2$ emissions in 2016 had the strongest correlation with temperature across all seasons. Soil moisture significantly and positively correlated with $CO_2$ in the dry summer of 2016 (for MN and MB), but negatively in the wet autumn of 2017 (for MN and MNB). $CO_2$ emissions were significantly and positively correlated with soil temperature in autumn 2016 (with MB and MNB) and in autumn 2017 for all three treatments ($r^2=0.419$; $P<0.001$). $CO_2$ emissions were significantly and positively correlated with soil $NH_4^+$ concentrations in autumn 2016 (for MNB) and autumn 2017 (for MB and MNB). $CO_2$

emissions were not correlated with soil $NO_3^-$ during any season. However, $NO_3^-$ had a positive correlation within the total (2016 and 2017) means ($r^2$=0.34, P< 0.001).

Unlike $CO_2$, the treatment-by-season-by-year interaction was significant for $N_2O$ soil emissions (μm $N_2O$-N $m^{-2}$ $h^{-1}$) (P=0.006). Biochar treated soils produced less $N_2O$ emissions in spring 2016, but more in spring 2017. In both these years, the summers these rates were equal to or lower than the conventional treatment (Table 3.3). As seen with $CO_2$ emissions, total $N_2O$ emissions were significantly higher in the spring, followed by summer and then autumn. In both years, seasonality was a strong driver of $N_2O$-N emissions, with emissions sharply decreasing as the seasons progressed from spring to autumn. 2016 and 2017 emissions were not very different (P>0.05). 2016 appeared to produce initially more $N_2O$ but had the sharper decrease across seasons. In 2016, the biochar-amended soils produced lower emissions; most notably in spring 2016, though in autumn they produced slightly more. In 2017, the biochar-treated soils had a slightly greater emissions rate, due to increased emissions in spring 2017 (Table 3.4). Like $CO_2$, $N_2O$ emissions correlated with several soil characteristics. $N_2O$ emissions were significantly and positively correlated to soil moisture in summer 2016, spring 2017 and summer 2017 (for MN), and for all three treatments in autumn 2017. $N_2O$ emissions were significantly and positively correlated to soil $NO_3^-$ concentration in spring 2017 for MNB and as a total among year 1 treatments (P<0.01). A significant positive correlation was observed between $N_2O$ emissions with $NH_4^+$, $NO_3^-$, and soil temperature when all data from each year and season were considered together (Table 3.5). However, these were largely driven by strong correlations in autumn 2017.

**Table 3.1** Variance analysis of total soil physical characteristics and GHG emissions under 3 soil treatments (MN, MB, MNB) in temperate soil at H & N Baker Farm, Bayfield, ON, 2016-2017.

| **Fixed Effect** | **Moisture** | **Temperature** | **$NH_4^+$** | **$NO_3^-$** | **$CO_2$** | **$N_2O$** |
|---|---|---|---|---|---|---|
| | F (P>F) | F (P>F) | F (P>F) | F (P>F) | F (P>F) | F (P>F) |
| Year | 0.23 (0.63) | 1.47 (0.23) | 109.7 (0.00) | 19.09 (0.00) | **2.75 (0.10)** | 0.827 (0.36) |
| Season | **67.2 (0.00)** | **179.2 (0.00)** | 204.2 (0.00) | 55.0 (0.00) | **41.5 (0.00)** | 68.12 (0.00) |
| Treatment (Trt) | **19.7 (0.00)** | 0.63 (0.53) | 0.28 (0.76) | 12.13 (0.00) | 0.40 (0.67) | 3.441 (0.03) |
| Year*Trt | 0.46 (0.64) | 0.51 (0.61) | 1.88 (0.15) | **3.30 (0.04)** | 0.47 (0.63) | 1.95 (0.14) |
| Season*Trt | 0.99 (0.42) | 0.38 (0.83) | **3.54 (0.01)** | **5.70 (0.00)** | 0.41 (0.80) | 0.98 (0.42) |
| Year*Season*Trt | 1.31 (0.27) | 1.03 (0.39) | **7.04 (0.00)** | **4.74 (0.00)** | 0.37 (0.83) | **3.67 (0.01)** |

Bolded values represent significant factors.

**Table 3.2** Median ($\bar{\chi}$) seasonal (spring, summer, and autumn) soil $CO_2$ emissions (mg $CO_2$-C $m^{-2}$ $h^{-1}$) from soil amended with poultry manure and N fertilizer (MN); poultry manure and biochar (MB); and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada. 95% confidence intervals are given in parentheses.

| | **MN** | **MB** | **MNB** | **Season ($\bar{\chi}$)** |
|---|---|---|---|---|
| **Spring 2016** | 225.4 (79.46)$^{A,a}$ | 208.0 (75.89)$^{A,a}$ | 147.5 (51.96)$^{A,a}$ | **192.8 (40.24)*** |
| **Summer 2016** | 79.6 (12.41)$^{A,b}$ | 79.6 (12.99)$^{A,b}$ | 77.9 (11.47)$^{A,b}$ | **78.7 (7.02)*** |
| **Autumn 2016** | 40.6 (13.25)$^{A,c}$ | 31.0 (7.62)$^{A,c}$ | 34.8 (7.64)$^{A,c}$ | **34.8 (5.94)*** |
| **2016($\bar{\chi}$)** | 72.4 (21.95)* | 59.0 (22.54) | 66.9 (16.78)* | |
| **Spring 2017** | 103.2 (32.44)$^{A,a}$ | 145.3 (36.48)$^{A,a}$ | 154.9 (47.96)$^{B,a}$ | **125.2 (23.31)*** |
| **Summer 2017** | 106.5 (14.41)$^{A,b}$ | 115.9 (17.58)$^{A,b}$ | 127.2 (20.21)$^{A,b}$ | **117.3 (10.24)*** |
| **Autumn 2017** | 69.0 (24.75)$^{A,c}$ | 69.4 (23.36)$^{A,c}$ | 64.2 (19.23)$^{A,c}$ | **67.0 (12.64)*** |
| **2017($\bar{\chi}$)** | 98.5 (13.57)* | 101.8 (15.13) | 107.2 (19.17)* | |
| **Spring ($\bar{\chi}$)** | 136.3 (52.92) | 163.0 (53.86) | 147.5 (58.37) | **151.1 (31.60)** |
| **Summer ($\bar{\chi}$)** | 93.0 (21.54) | 87.5 (31.38) | 89.6 (22.99) | **90.2 (14.86)** |
| **Autumn ($\bar{\chi}$)** | 41.9 (15.31) | 35.4 (16.54) | 44.3 (13.41) | **41.5 (8.64)** |
| **Treatment($\bar{\chi}$)** | 84.4 (18.15) | 82.3 (21.76) | 84.4 (20.08) | |

$^{A}$ Values followed by the same upper case letters are significantly different among treatments (at $p< 0.05$). $^{a}$ Values followed by the same lower case letters are not significantly different among seasons (at $p<0.05$). Values followed by *are significantly different between the two years within treatments.

**Table 3.3** Median ($\bar{\chi}$) seasonal (spring, summer, and autumn) soil $N_2O$ emissions (μg $N_2O$ -N $m^{-2}$ $h^{-1}$) from soil amended with poultry manure and N fertilizer (MN); poultry manure and biochar (MB); and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada. 95% confidence intervals are given in parentheses.

| | **MN** | **MB** | **MNB** | **Season ($\bar{\chi}$)** |
|---|---|---|---|---|
| **Spring 2016** | 106.5 (74.74)$^{A,a}$ | 54.3 (56.87)$^{A,a}$ | 159.2 (70.69)$^{A,a}$ | 102.2 (36.72) |
| **Summer 2016** | 14.2 (7.24)$^{A,b}$ | 7.9 (6.59)$^{A,b}$ | 20.0 (7.25)$^{A,b}$ | 14.2 (4.15)* |
| **Autumn 2016** | 6.7 (7.52)$^{A,b}$ | 12.8 (7.77)$^{A,b}$ | 11.0 (10.60)$^{A,b}$ | 10.5 (4.94)* |
| **2016($\bar{\chi}$)** | 13.3 (12.07) | 18.3 (14.28) | 21.0 (12.61) | |
| **Spring 2017** | 32.4 (23.52)$^{A,a}$ | 52.6 (27.68)$^{A,a}$ | 47.8 (22.68)$^{A,a}$ | 45.1 (14.70) |
| **Summer 2017** | 22.8 (14.17)$^{A,b}$ | 14.9 (7.31)$^{A,b}$ | 27.2 (8.60)$^{A,b}$ | 22.7 (6.17)* |
| **Autumn 2017** | 38.1 (17.76)$^{A,c}$ | 42.3 (18.03)$^{A,c}$ | 34.7 (15.46)$^{A,c}$ | 38.7 (9.76)* |
| **2017($\bar{\chi}$)** | 25.8 (10.29) | 28.1 (11.43)$^{A}$ | 31.1 (8.47)$^{A}$ | |
| **Spring ($\bar{\chi}$)** | 44.6 (34.53) | 54.2 (28.48) | 72.2 (34.53) | 56.5 (18.16) |
| **Summer ($\bar{\chi}$)** | 21.6 (6.84) | 13.1 (9.17) | 22.6 (7.30) | 21.0 (4.42) |
| **Autumn ($\bar{\chi}$)** | 12.9 (9.10) | 19.5 (11.24) | 20.0 (9.88) | 16.7 (6.74) |
| **Treatment($\bar{\chi}$)** | 22.7 (10.85) | 24.8 (8.91) | 27.2 (10.21) | |

$^{A}$ Values followed by the same upper case letters are significantly different among treatments (at $p< 0.05$). $^{a}$ Values followed by the same lower case letters are not significantly different among depths (at $p<0.05$). Values followed by *are significantly different between the two years within treatments.

**Table 3.4** 2016 and 2017 linear regression coefficient of determination ($r^2$) values for $CO_2$-C and $N_2O$-N emissions and soil characteristics from soil amended with poultry manure and N fertilizer (MN); poultry manure and biochar (MB); and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada.

| **Season** | **GHG** | **2016** | | | | **2017** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **$NO_3^-$** | **$NH_4^+$** | **Soil Temp** | **Soil Moist** | **$NO_3^-$** | **$NH_4^+$** | **Soil Temp** | **Soil Moist** |
| **Spring** | $CO_2$-C | 0.119 | 0.061 | 0.014 | 0.059 | 0.052 | 0.104 | 0.223 | -0.143 |
| | $N_2O$-N | 0.161 | **-0.284**$^*$ | **0.345**$^{**}$ | -0.171 | 0.103 | 0.154 | 0.191 | 0.181 |
| **Summer** | $CO_2$-C | 0.116 | 0.022 | -0.037 | **0.304**$^{**}$ | 0.147 | **0.365**$^{**}$ | **0.465**$^{**}$ | **-0.262**$^*$ |
| | $N_2O$-N | -0.067 | 0.049 | -0.091 | **0.177**$^*$ | 0.022 | 0.046 | -0.077 | **0.221**$^*$ |
| **Autumn** | $CO_2$-C | 0.234 | -0.138 | **0.419**$^{**}$ | 0.053 | 0.207 | -0.135 | **0.728**$^{**}$ | **-0.765**$^{**}$ |
| | $N_2O$-N | 0.246$^*$ | -0.098 | **0.671**$^{**}$ | **-0.437**$^{**}$ | 0.027 | 0.**320**$^*$ | **0.741**$^{**}$ | **-0.640**$^{**}$ |

*Denotes a statistically significant relationship (P<0.05); **denotes a statistically significant relationship (P<0.01).

**Table 3.5** Two-year total linear regression coefficient of determination ($r^2$) values for $CO_2$-C and $N_2O$-N emissions and soil characteristics from soil amended with poultry manure and N fertilizer (MN); poultry manure and biochar (MB); and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada.

| | **GHG** | **$NO_3^-$** | **$NH_4^+$** | **Soil Temp** | **Soil Moist** |
|---|---|---|---|---|---|
| **Total** | $CO_2$-C | **0.285**$^{**}$ | **0.182**$^{**}$ | **0.320**$^{**}$ | **-0.140**$^*$ |
| | $N_2O$-N | **0.351**$^{**}$ | -0.023 | **0.340**$^{**}$ | 0.040 |

*Denotes a statistically significant relationship (P<0.05); **denotes a statistically significant relationship (P<0.01).

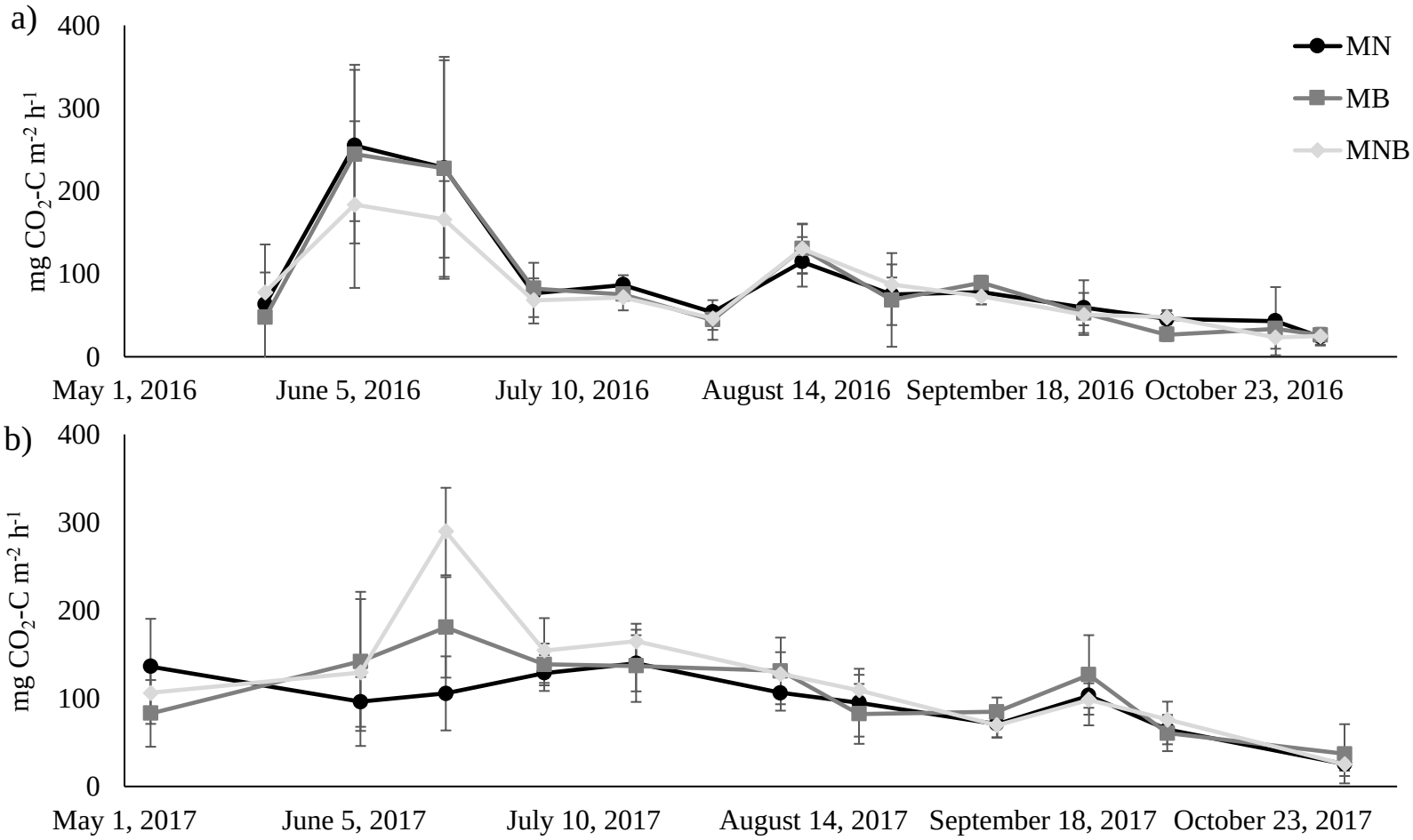


**Figure 3.1** Median $CO_2$ emissions (mg $CO_2$-C $m^{-2}$ $h^{-1}$) from temperate soils amended with three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in a) 2016 and b) 2017 from Bayfield, Southern Ontario, Canada. 95% confidence interval bars are given for each median value

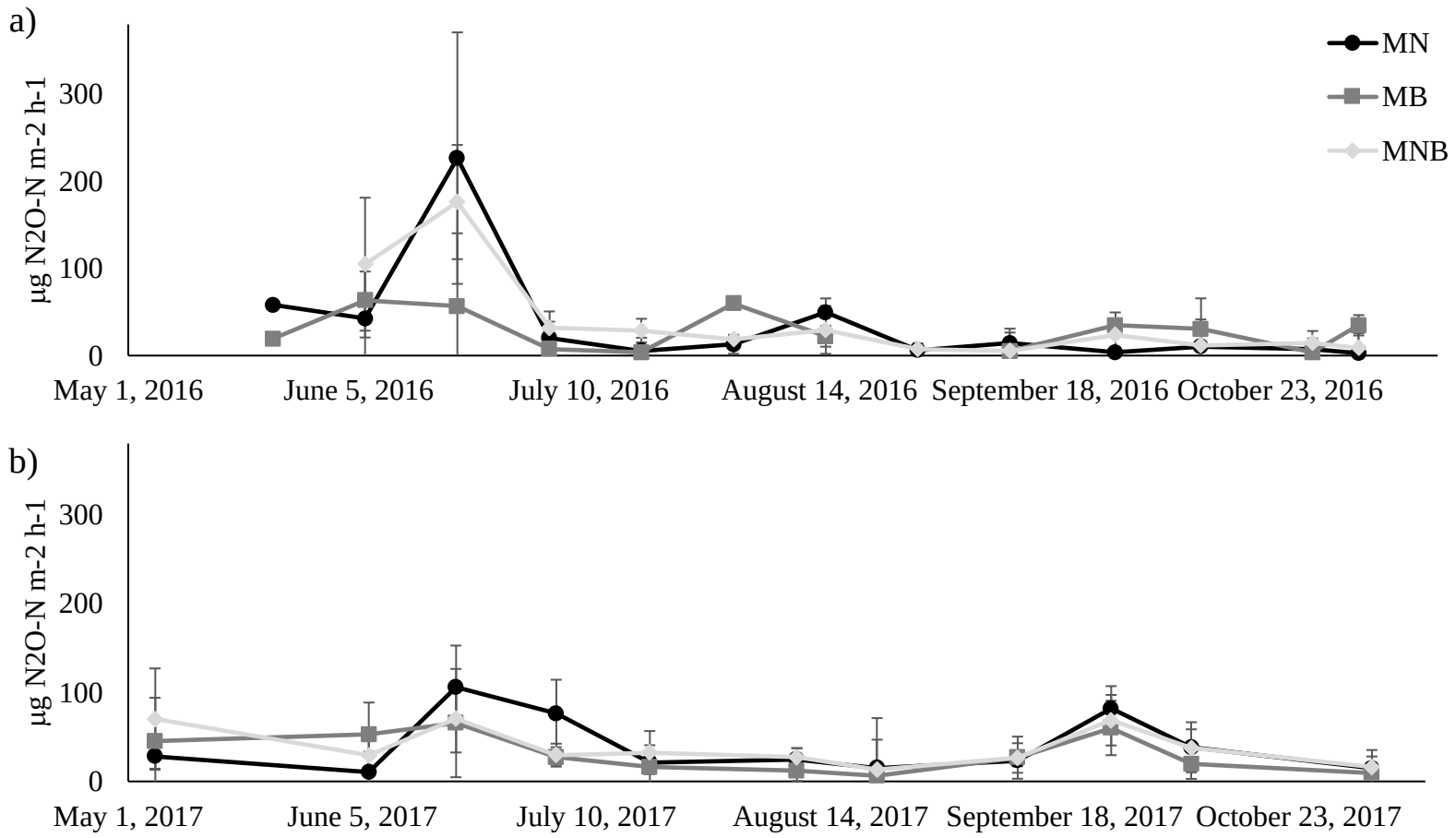


**Figure 3.2** Median $N_2O$ emissions (µg $N_2O$ -N $m^{-2}$ $h^{-1}$) from temperate soils amended with three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in a) 2016 and b) 2017 from Bayfield, Southern Ontario, Canada. 95% confidence interval bars are given for each median value.

*3.4.2 Soil Characteristics*

Soil moisture was significantly different among treatments ($P<0.001$) in both 2016 and 2017. The lowest average soil moisture was found in the MB (10.4 %) followed by the MNB (11.1 %) and MN (13.3 %) treatments. Though very similar temperatures were seen in all three treatments, MB and MNB were significantly lower in temperature than MN (-2.9ºC and -2.2ºC; $P<0.001$). (Figure 3.4). Both moisture and temperature were affected by climatic conditions and were inversely related throughout the two growing seasons.

In 2016 and 2017, one of the few significant interactions for ammonium ($NH_4^+$) was treatment-by-season-by-year ($P<0.001$). Among seasons, $NH_4^+$ was significantly higher in spring 2016 and summer 2016. In the spring, MN had significantly higher $NH_4^+$ values, followed by MNB, but by summer the differences between all treatments were not statistically significant (Table 3.1). In autumn 2016 the differences remained insignificant, and the soils treated with biochar had similar concentrations to MN (Figure 2.3). 2017 continued to see no statistical difference among treatments($P=0.756$); however, differences arose among seasons. During 2017, soybean growth (spring and early summer) elevated $NH_4^+$ concentrations. $NH_4^+$ was quickly consumed, and the concentration dropped during soybean maturation. $NO_3^-$ as well had a significant treatment-by-season-by-year interaction ($P=0.001$). The greatest differences among treatments existed in Spring 2016 ($P<0.001$). In this season MN was significantly greater than MB ($P<0.001$), though this difference diminished by summer concentrations were very similar by autumn 2016. In 2017, the treatments were still statistically similar until autumn 2017, when MN accumulated the greatest soil $NO_3^-$ concentration following the shedding of soybean foliage. In October $NO_3^-$ concentrations peaked and MN was significantly greater than MB ($P=0.007$) and MNB ($P=0.025$). In both years there were consistently lower concentrations of $NO_3^-$ in soils treated with biochar (Figure 2.4).

**Table 3.6** $NH_4^+$ (mg N $kg^{-1}_{soil}$) and $NO_3^-$ (mg N $kg^{-1}_{soil}$) treatment and seasonal median concentrations [treatment ($\bar{x}$); season ($\bar{x}$)] from soil amended with poultry manure and N fertilizer (MN); poultry manure and biochar (MB); and poultry manure, N fertilizer and biochar (MNB) in Bayfield, Southern Ontario, Canada over 28 weeks in 2016 and 24 weeks in 2017 growing season. Standard errors are given in parentheses.

| | | MN | MB | MNB | Season ($\bar{x}$) |
|---|---|---|---|---|---|
| $NH_4^+$ mg N $kg^{-1}_{soil}$ | Spring | 7.94 (0.74)$^{A,a}$ | 5.45 (0.73)$^{B,a}$ | 7.50 (0.74)$^{A,a}$ | **7.60 (0.43)$^{a*}$** |
| | Summer | 1.50 (0.57)$^{C,b}$ | 1.63 (0.57)$^{B,b}$ | 1.85 (0.55)$^{B,b}$ | **1.62 (0.31)$^{b*}$** |
| | Autumn | 1.10 (0.67)$^{A,b}$ | 1.05 (0.67)$^{A,b}$ | 1.10 (0.67)$^{A,c}$ | **1.10 (0.39)$^{c}$** |
| | Treatment($\bar{x}$) | **1.95 (0.39)$^{A}$** | **1.86 (0.37)$^{A}$** | **2.35 (0.39)$^{A}$** | |
| $NO_3^-$ mg N $kg^{-1}_{soil}$ | Spring | 32.78 (5.00)$^{A,a}$ | 16.93 (5.15)$^{C,a}$ | 17.79 (5.00)$^{B,a}$ | **20.57 (2.92)$^{a*}$** |
| | Summer | 8.15 (4.02)$^{A,b}$ | 7.34 (3.98)$^{A,b}$ | 7.00 (4.06)$^{A,b}$ | **7.58 (2.32)$^{b*}$** |
| | Autumn | 5.30 (4.68)$^{A,b}$ | 6.35 (4.76)$^{B,b}$ | 4.11 (4.68)$^{B,b}$ | **4.93 (2.72)$^{b*}$** |
| | Treatment($\bar{x}$) | **9.88 (2.65)$^{A*}$** | **9.47 (2.69)$^{A*}$** | **9.38 (2.65)$^{A}$** | |

Values followed by the same uppercase letter, comparing differences among treatments (MN, MB, MNB) within season, are not significantly different ($p<0.05$). Values followed by the same lowercase letter, comparing differences among seasons and within treatments (MN, MB, MNB) within season, are not significantly different ($p<0.05$). Values followed by * represent differences when comparing grand means ($\bar{x}$) between seasons within treatments ($p<0.05$).

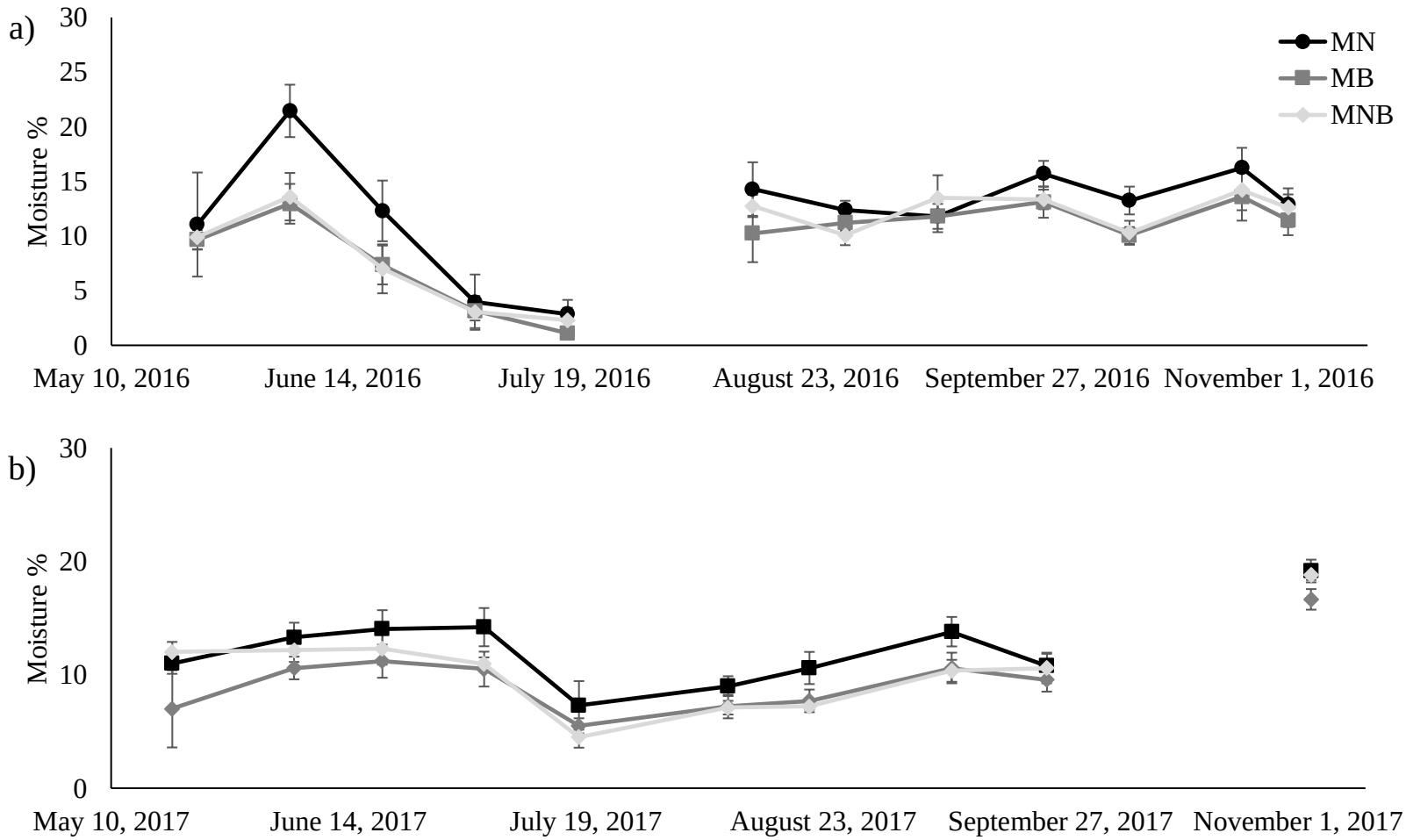


**Figure 3.3** Median moisture (%) at 0-10cm (with confidence intervals error bars) from temperate soils amended with three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in a) 2016 and b) 2017 from Bayfield, Southern Ontario, Canada.

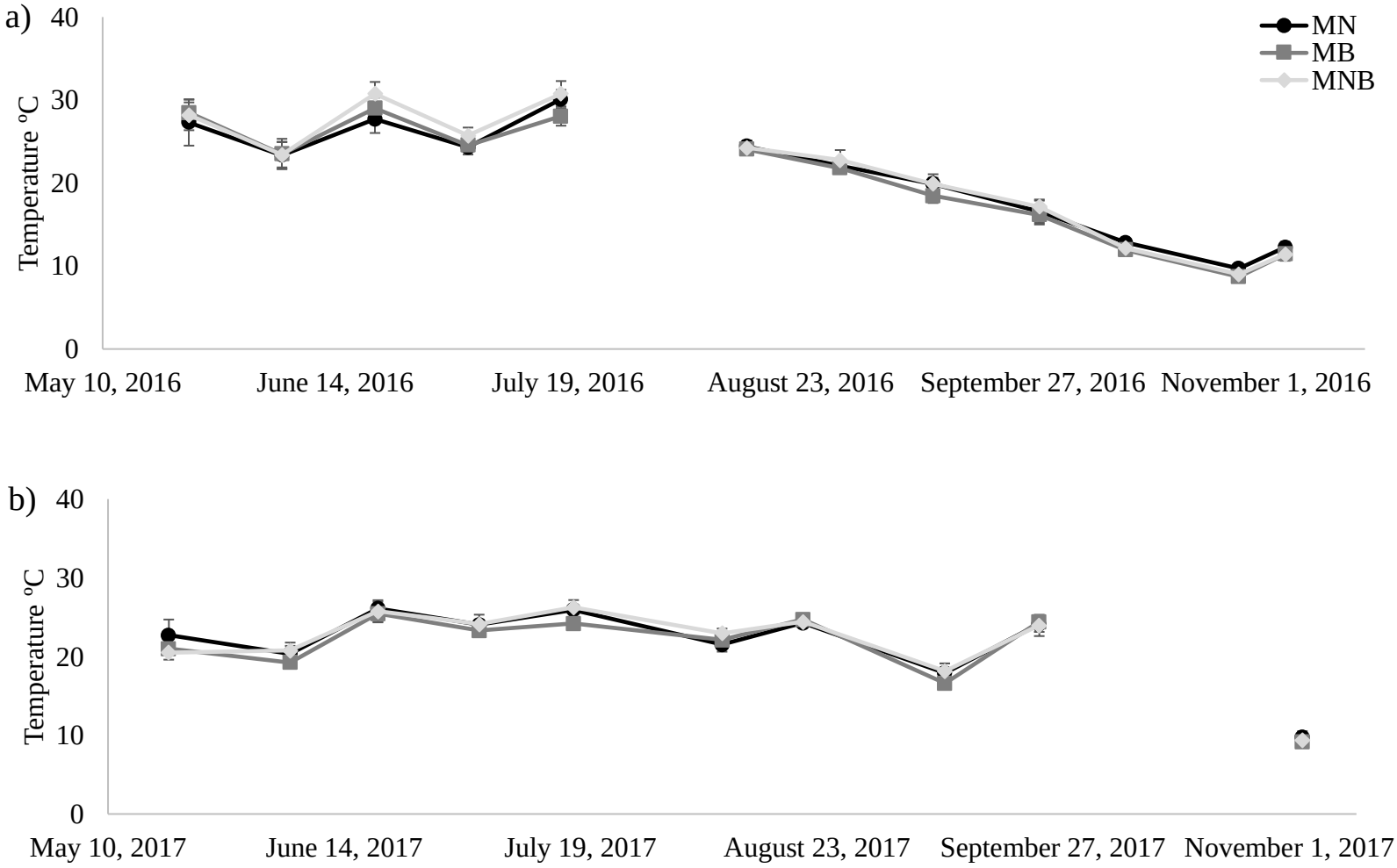


**Figure 3.4** Median temperatures (°C) at 0-10cm (with confidence intervals error bars) from temperate soils amended with three treatments: poultry manure and N fertilizer (MN), poultry manure and biochar (MB), and poultry manure, N fertilizer and biochar (MNB) in a) 2016 and b) 2017 from Bayfield, Southern Ontario, Canada.

### 3.5 Discussion

Soil conditions strongly influence soil GHG emission rates. Therefore, it is important to discuss biochar impacts on soil physical, chemical, and biological conditions before exploring GHG changes. Despite low biochar addition rates, soil moisture was still affected by biochar in this study. While changes to soil moisture vary among biochar studies, this project saw a decrease in moisture. This decrease was similar to Uzoma et at. (2011) and Devereux et al. (2012), who attributed the lower moisture to biochar's high surface area, which readily absorbs water (Barnes et al., 2014; Yao et al., 2012). Most microbial organisms have a narrow optimal soil moisture range (USDA, 2009); thus biochar initiating a 15-20% decrease in soil moisture was expected to impact microbial GHG emissions. Related to soil moisture, soil temperature impacts vary among biochar studies; some observing increases (Genesio et al. 2012), decreases (Zhang et al., 2013), or no effect (Nelissen et al., 2014). Biochar darkens soil, increasing albedo, and therefore increase soil temperature (Genesio et al. 2012). Temperature decreases have been associated with altered soil textures, which absorb less infrared radiation and insulate soil from ambient temperature changes (Zhang et al., 2013).

The most substantial change within soil $NH_4^+$ and $NO_3^-$ concentrations occurred seasonally. Nitrogen concentrations were unsurprisingly greater within MN and MNB treatments due to N fertilizer input in spring 2016. Nitrogen fertilizer was added in spring 2016 to increase maize yield. Nitrogen concentrations quickly dropped across summer and autumn of 2016; this decrease was a result of the growing maize and soil biota, which promptly consumed available soil N (Cayuela et al., 2013; Regehr et al., 2015). In 2017, soybean production affected $NH_4^+$ concentration, elevating it during the early growing season, as rhizobacteria produced $NH_3$ (Garg & Geetanjali, 2007). Once the plants had matured the concentration quickly fell, at which time $NO_3^-$ rose due to soybean foliage, considered a high-quality residue, shedding and decomposition

(Yang & Cai, 2005; Ball et al., 1999; McDaniel et al., 2014). At that point, a significant difference briefly emerged due to biochar's adsorption of available N (Clough et al., 2013). Differences among treatments were not as apparent as differences among seasons. Only when excess N became available in the soil did $NH_4^+$ and $NO_3^-$ concentrations treatment differences emerge. Initially, in spring 2016, MB had the lowest concentrations, not having received fertilizer additions. Both $NO_3^-$ and $NH_4^+$ were lower in biochar treated soils; even MNB, which received fertilizer, as a result of adsorption (Clough et al., 2013). Biochar is known to have regions of cation and anion exchange and therefore can absorb both $NO_3^-$ and $NH_4^+$ (Clough et al., 2013). $NO_3^-$ and $NH_4^+$ adsorption is a common effect of woody-biochars and was found to increase as biochar pyrolysis temperatures increase (Spokas & Reicosky, 2009; Clough & Condron, 2010; Barnes et al., 2014; Kerré et al., 2017; Yao et al., 2012; Anders et al., 2013). Similar to the studies which observed N absorption, this study's biochar was both wood-sourced and pyrolyzed at a high temperature. However, the absorption was limited in this study by the low biochar addition rate. Small, negligible changes to soil $NH_4^+$ and $NO_3^-$ following small biochar additions, were also observed in several temperate studies (Clough & Condron, 2010; Backer et al., 2017; Hangs et al. 2016). Since N absorption has been seen to decrease $N_2O$ emissions, it was unlikely that the minimal changes in soil N would result in altered $N_2O$ release (Anders et al., 2013). Nutrient concentrations, however, are not often the sole driving force behind microbial respiration in temperate agriculture; properties such pH, moisture, and temperature change are greatly impactful for $CO_2$-C emissions (Kloss et al., 2014).

Biochar can directly influence $CO_2$ emissions by affecting soil physical or chemical characteristics (Ameloot et al., 2013). Indirectly, biochar can create favorable environments for microbial communities, these communities contribute a large portion of GHG emissions (Jones

et al., 2011a; Bamminger et al., 2014; Khodadad et al., 2011; Dil et al., 2014; Luo et al., 2015; Domene et al., 2015). Enhanced microbial activity can accelerate the decomposition of soil SOM, which leads to increased $CO_2$ emissions (Kuzyakov et al., 2000). Biochar has also created more efficient microbial communities, which produce less $CO_2$ (Bamminger et al., 2014). No significant effect of biochar on soil $CO_2$ emissions was observed among treatments in either field season; only significant seasonal effects were observed. The greatest $CO_2$ emissions occurred in spring 2016, due to the incorporation of soil amendments and warm/moist conditions. A greater amount of labile C and N were also available at that time and accessible to the microbial community (Cross & Sohi, 2011). Following spring 2016, and the consumption of labile C, only recalcitrant C compounds remained and therefore $CO_2$ emissions decreased (Zimmerman et al., 2011; Cross & Sohi, 2011).

As seen in this study, it was typical for short-term releases of $CO_2$ to follow soil amendment additions (Jones at al., 2011a; Yoo & Kang, 2012). In spring 2016, MNB and MN yielded the highest GHG emissions. However, it had nearly a double emission rate in Summer 2016. There was potentially a partial breakdown of biochar's carbon during this season since as observed in Chapter 2, free light fraction carbon (FLF-C) decreased between 2016 and 2017 (Jones at al., 2011a; Yoo & Kang, 2012; Demisie et al., 2014; Table 2.7). For Jones et al. (2011a) this rate increase was most notable in the first month and steadily decreased over 15 months. Cross & Sohi (2011) observed similar effects to those of Jones et al. (2011a) and proposed that labile compounds were consumed and emissions lowered over time once only recalcitrant biochar carbon remained. The initial spike of $CO_2$ released in summer 2016 was perhaps a result of bioavailable carbon residues being decomposed since $CO_2$ emissions were not correlated with any physical or nutrient soil conditions at this time. C/N ratios are a known

indicator of soil productivity which in turn directly contributes to $CO_2$ production. Biochar additions increase C/N ratios which can decrease microbial activity within the soil (Atkinson et al., 2010). C/N ratios in this study were more evenly distributed in biochar treated soils and had a higher ratio in lower depths. Despite the changes to soil moisture, temperature, and nitrogen species, $CO_2$ emissions from this study in Bayfield, ON were not significantly different among the three treatments, Manure and Nitrogen (MN), Manure and Biochar (MB), Manure, Nitrogen, and Biochar (MNB) (Table 3.1). As mentioned earlier, this study incorporated comparatively small amounts of biochar. Therefore, drastic changes from the biochar additions were unlikely. While the differences between treatments were not extreme, there were significant changes across seasons, especially in spring 2016 (Figure 3.1). The decrease after spring 2016 could also have been a result of soil acclimatization; since as nutrient differences and water content normalize, after a year more correlations between soil characteristics and emissions were seen (Zimmerman et al., 2011).

In summer and autumn, soil moisture and temperature also affected $CO_2$ emissions. This is because of moisture and temperature influences on microbial activity (Banerjee et al., 2016). It has been proposed that by absorbing soil moisture, biochar can facilitate $O_2$ diffusion which increases microbial respiration, and therefore greater $CO_2$ emissions (Jones et al., 2011a). This effect was most evident in summer 2017 when $CO_2$ had the greatest a negative correlation to moisture. In 2016's dry soils, aeration was not a limiting factor, therefore moisture did not exert a strong influence on $CO_2$ emissions (Agriculture and Agri-Food Canada, 2017). However, a slight negative correlation also existed across the two years despite different levels of precipitation, as 2017 was unusually wet (Agriculture and Agri-Food Canada, 2018). In this study, biochar treated soils were the driest and produced slightly more $CO_2$. However, the pattern observed could also

have been the result of the inverse relationship between soil moisture and temperature. As soil moisture increases soil temperatures decrease, and this causes $CO_2$ emissions to decrease (Davidson et al., 1998; Smith et al., 2003). In autumn 2017, when temperatures were lowest, temperature was positively correlated to $CO_2$ emissions. Temperature can be an important limiting factor for microbial growth (Zanchi et al., 2014). Not only do physical conditions affect $CO_2$ emissions, but nutrient concentrations as well (Liang et al., 2015). Elevated soil nitrogen levels have not been known to cause $CO_2$ emission from soils directly, however, $NH_4^+$ can stimulate soil enzyme activity, and thereby enhance $CO_2$ emissions (Liang et al., 2015).

The specific qualities of biochar are often observed to affect GHG emissions from soils; pyrolytic temperature and source material determine nutrient exchange and adsorption rates, as well as amounts of labile compounds (Atkinson et al., 2010). Only seven in-field investigations of biochar and poultry manure impacts on $N_2O$ emissions are available (Agegnehu et al., 2015; Angst et al., 2014; Felber et al., 2014; Karhu et al., 2011; Verhoeven & Six, 2014; Wanatabe et al., 2014; Rose et al., 2016). Of these, only the tropical study resulted in decreased $N_2O$ emissions (Agegnehu et al., 2015). Other amendment schemes have resulted in decreased $N_2O$ emissions as well, but those were seen in laboratory settings and with high-temperature biochars (>700°C) (Cayuela et al., 2014; Anders et al., 2013; Bamminger et al., 2014). Biochars pyrolyzed at temperatures above 600°C adsorb large amounts of soil $NO_3^-$, as a result of greater porosity, which decreases access for both the nitrifiers and/or denitrifiers, and therefore decrease $N_2O$ emission (Smith et al., 2014). Woody feedstock chars also stabilize N and slow N mineralization (Van Zwieten et al., 2013). Newly pyrolyzed biochars contain ethylene and polycyclic aromatic hydrocarbons residues which also inhibit denitrifiers (Gomez et al., 2014; Zimmerman et al., 2011; Clough & Condron, 2010). Given the low addition rate of biochar in

this study, few residues would have been present in our biochar treatments to suppress $N_2O$ production. Considering the quantity of application, subtle responses were expected. Indeed, $N_2O$ emissions were not found to differ significantly in any season among any of the three treatments of this study (Table 3.2). However, when all seasons and years were combined together it appeared that biochar soils produced less $N_2O$ than the conventional treatment. This has been an observed outcome of other biochar studies, since emissions often result from nitrogen availability for microbes to convert and biochar can reduce available nitrogen (Clough & Condron, 2010; Troy et al., 2013). This interaction was illustrated further by $N_2O$'s correlation to soil $NO_3^-$, which was at times responsible for ~35% of the total observed emission (Table 3.5). At the same time, soil temperature was responsible for ~30% of the total $N_2O$ emissions (Table 3.5). These two characteristics explained more than half of total $N_2O$ emissions. The degree to which these two factors affected emissions also depended on weather conditions and nutrient additions. 2016 showed a greater difference among treatment emissions than 2017 since the greatest differences in soil conditions developed in the first year. The total emissions rate was slightly lower and more even in 2017 than in 2016. This decrease was attributed to cooler weather patterns and lower $NO_3^-$ concentrations. The initial spring flush of $N_2O$-N was much lower in the second year since no fertilizers were added. Autumn emissions elevated slightly in 2017 from the summer readings. This was likely the result of $NO_3^-$ production from decomposing soybean leaves (McDaniel et al., 2014). It was found that in summer and autumn 2017, $N_2O$ emissions were positively correlated to moisture availability. Maximum $N_2O$ emissions rates exist between the range of 80-95% water-filled pore space (Schindlbacher et al., 2004). Throughout the frequent rainfall events in 2017, ideal water-filled pore space conditions for denitrification were often present, resulting in a greater total time of peak emissions. While

pore size has been attributed to decreased $N_2O$ emissions in several studies, in this case, it may have facilitated increased emissions (Atkinson et al., 2010). Aeration is a significant factor in $N_2O$ generation and appeared to impact $N_2O$ emission in this study (Cayuela et al., 2014; Atkinson et al., 2010). The greatest emissions occurred in the driest year and moisture was strongly correlated with gas production in the wettest year. As well, moisture determines soil temperature (McLain & Martens, 2006). $N_2O$ has been found to be affected by soil temperature in many land-uses, as well as in this study (Schindlbacher et al., 2004). $N_2O$ emissions were significantly correlated with temperature during autumn 2017, the coldest season. The lack of consistent correlations with $NH_4^+$ and $NO_3^-$ was observed in other studies (Verdouw et al., 1978; Troy et al., 2013), and indicates a complexity of soil emission drivers. The most influential factors in this study were nitrogen availability in 2016 and physical conditions in 2017 (Butterbach-Bahl et al., 2013; Felber et al., 2014). Spring 2016 to summer 2016 had a 60% decrease in mean $N_2O$-N. In 2017 that seasonal decrease was only 50%. The least drastic reductions were found in MN soils, suggesting that aging of biochar induced some reduction in emissions (Clough & Condron, 2010). As seasons progressed in both years, nutrient levels, labile carbon biochar residues, and emissions all decreased together.

### 3.6 Conclusion

The amount of biochar added to this temperate agricultural soil was low and did not significantly impact GHG soil emissions. Results showed that adding biochar with poultry manure or with mineral N fertilizer and/or poultry manure did not induce greater greenhouse gas emissions in any given season. These results also demonstrated that complex soil interactions changed seasonally and influenced GHG emissions, with or without biochar. Physical soil

characteristics had the most consistent and greatest impact on emissions. As a result, weather conditions heavily influenced emissions; with more extreme conditions resulting in stronger effects. $NO_3^-$ was identified as a factor that can strongly influence $N_2O$ emissions as well. Since biochar ages following its addition, future research should focus on long-term field-scale GHG emissions. More growing seasons, with their unique weather patterns, would aid in determining the full influence of physical characteristics over GHG emissions in biochar soil. It is important to now decide what optimal amounts of biochar can be economically utilized by agricultural producers while still mitigating GHG emissions. Though GHG emissions were statistically identical among treatments in each season, as a trend, $CO_2$ and $N_2O$ were initially lower in biochar treated soil. To move agriculture towards a carbon-neutral status it is important to know whether one large addition of biochar or several small additions would result in the lowest initial $CO_2$ release. This low application-rate strategy was able to bypass the initial significant increase in $CO_2$ seen in early-stages of larger biochar additions. It is expected that in the following 5 years, the emissions of our site will remain lower in our biochar treated plots. Continual observation would be beneficial to determine what the final long-term impacts are going to be of biochar treatments.

## 4. Conclusion

In previous studies biochar has demonstrated its potential to change soil characteristics in a variety of ways, and in doing so impact; soil health, crop growth and GHG emissions. However, few of the biochar projects have taken place infield to validate its effect on soil systems. As well, little research exists to date on its effects on soil ecology and the mechanics by which it alters GHG emissions. This study provided a unique design, of low additions, in a Canadian agriculture system as an attempt to fill the aforementioned knowledge gaps. This study did not observe many of the results of previous works.

Chapter 2 demonstrated that low biochar additions result in few changes to soil physical, chemical, biological characteristics, or crop growth. Biochar's small-scale changes to C/N distribution, as well as microbial diversity and richness were together short-lived. Microbial communities were able to access initial labile components of biochar in this temperate system. As biochar aged labile carbon was consumed, the differences between microbial communities diminished. While soil microbial richness and diversity appeared temporarily affected by biochar, these results ultimately did not affect crop productivity or macrofauna populations. Similarly, phosphate was only significantly decreased by biochar within the first growing season following its addition and then accumulated within biochar-amended soil within the second growing season. As Spokas et al. (2011) observed, the aging processes can drastically change the chemical surface or pore space composition of biochar. However, physical and chemical composition are not equally altered during the aging process of biochar (Yuan et al., 2017). Only soil moisture was consistently lower in 2016 and 2017, as a result of biochar, in this temperate agricultural study. At every biweekly sampling event, biochar soils had significantly lower moistures. This revealed that as biochar aged in its first season it underwent more chemical changes than physical. Nelissen et al. (2015) also observed an unequal degree of change between

soil physical and chemical characteristics as biochar aged. These effects were, however, not severe enough to drive changes to soil productivity. Based on soil productivity indicators, soil health was not altered by biochar. In conclusion, this chapter demonstrated that an economically feasible portion of biochar can be used in temperate soils without compromising soil health or crop productivity.

Chapter 3 showed that low-addition biochar schemes do not impact $CO_2$ but potentially impacted general $N_2O$ emission rate trends. However there were no significant difference season to season each year. In studying $N_2O$ emissions this chapter was novel; it is among the first biochar field projects in Canada to do so. Similar infield experiments have been carried out in agricultural fields in Germany (Bamminger et al., 2014) and Switzerland (Hüppi et al., 2015), though addition rates exceeded economically feasible levels. Much like Chapter 2, altered soil characteristics did not result in secondary impacts, in this case, GHG emission. While weather changes had the largest influence on emissions, only weak correlations existed between soil characteristics and GHG. Biochar ultimately did not definitively trigger or lower GHG emissions, as it had in other studies (Yaun et al., 2017; Case et al., 2015; Deng et al., 2015). Some laboratory settings observed no changes to $CO_2$ as well (Knoblaunch et al., 2011; Steiner et al., 2010). It is likely that this lack of effect was again due to the low biochar addition rates. Most projects required ~20t/ha before effects were observed (Bamminger et al., 2014; Hüppi et al., 2015; Mitchell et al., 2015). The increased complexity of field setting may have contributed to this as well and made it harder to identify which effects were driving the emission system.

In the future, more biochar project designs or biochar types should be implemented in temperate settings. It would be of value to identify which addition amounts or length of aging time might result in beneficial changes to crop yields or GHG emissions. Moderate amounts of

biochar and higher pyrolysis temperatures would likely result in more profound decreases in emissions (Zimmerman et al., 2011). As well, different feedstocks would likely result in different impacts to soil health and emissions. Continuing this project would provide important information on the continued aging of biochar; to determine if the physical changes persist and whether differences in C/N or among microbial communities reemerge. This study could benefit as well from an analysis of collected soils to determine how much biochar remained in our soil following year one, and now after year two. At the very least, it would be beneficial to continue this project for several more crop rotations; in order to provide a greater amount of information on crop responses to biochar, but also biochar responses to an array of weather patterns. With this extra information, farmers will be able to customize biochar use to meet the specific needs of their farm and our shared climate.

**References**


Abujabhah, I. S., Bound, S. A., Doyle, R., Bowman, J. P. (2016). Effects of biochar and compost amendments on soil physico-chemical properties and the total community within a temperate agricultural soil. *Applied Soil Ecology, 98,* 243-253.

Ajayi, A.E., Horn, R. (2017). Biochar-induced changes in soil resilience: Effects of soil texture and biochar dosage. *Pedosphere: An International Journal*, *27*(2), 236-247.

Agegnehu, G., Bass, A.M., Nelson, P.N., Muirhead, B., Wright, G., Bird, M.I. (2015). Biochar and biochar-compost as soil amendments: effects on peanut yield, soil properties and greenhouse gas emissions in tropical North Queensland, Australia. *Agriculture, Ecosystems & Environment*, *213*, 72-85.

Agriculture and Agri-Food Canada. (2017). 2016 annual report of agroclimate conditions across Canada. Government of Canada. Retrieved January 2017 from http://www.agr.gc.ca/resources/prod/doc/pdf/agroclimate_ar_2016-en.pdf.

Agriculture and Agri-Food Canada. (2018). 2017 Annual report of agroclimate conditions across Canada. Government of Canada. Retrieved June 2017 from http://www.agr.gc.ca/eng/programs-and-services/drought-watch/2017-annual-report-of-agroclimate-conditions-across-canada/?id=1519400741748#a3

Allison, Steve. (2008). *Chloroform fumigation direct extraction (CFDE) protocol for microbial biomass carbon and nitrogen*. Adapted from: http://www.stanford.edu/group/Vitousek 5 May 1998, S. E. Hobbie.

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.

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

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.

Anderson, C. R., Leo, M., Condron, T. J., Clough, M. F., Stewart, A., Hill, R. A., Sherlock, R. R. (2011). Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia*, *54*(5-6), 309-320.

Angst, T. E., Six, J., Reay, D. S., Sohi, S. P. (2014). Impact of pine chip biochar on trace greenhouse gas emissions and soil nutrient dynamics in an annual ryegrass system in California. *Agriculture, Ecosystems & Environment*, *191*, 17-26.

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.

Awale, R., Emeson, M. A., Machado., S. (2017). Soil organic carbon pools as early indicators for soil organic matter stock changes under different tillage practices in inland pacific northwest. *Frontiers in Ecology and Evolution*. Retrieved May 2017 from https://www.frontiersin.org/articles/10.3389/fevo.2017.00096/full

Ball, B. C., Parker, J. P., Scott, A. (1999). Soil and residue management effects on cropping conditions and nitrous oxide fluxes under controlled traffic in Scotland. *Soil and Tillage Research, 52*(3-4), 191-201.

Ball, P.N., MacKenzie, M.D., De Luca, T.H., Holben, W.E. (2010). Wildfire and charcoal enhance nitrification and ammonium-oxidizing bacteria abundance in dry montane forest soils. *Journal of Environmental Quality, 39*, 1243-1253.

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.

Banerjee, S, Helgason, B., Wang, L., Winsley, T., Ferrari, B. C., Siciliano, S. D. (2016). Legacy effects of soil moisture on microbial community structure and $N_2O$ emissions. *Soil Biology and Biochemistry, 95*, 40-50.

Barnes, R. T., Gallagher, M. E., Masiello, C., A., Liu, Z., Dugan., B. (2014). Biochar-induced changes in soil hydraulic conductivity and dissolved nutrient fluxes constrained by laboratory experiments. *PLoS One, 9*(9), e108340.

Batjes, N. H. (1996). Total carbon and nitrogen in soils of the world. *European Journal of Soil Science, 47*, 151-163.

Bender, R. R., Haegele, J. W., Ruffo, M. L., Below, F. E. (2013). Modern corn hybrids' nutrient uptake patterns. *Better Crops, 97*(1), 7-10.

Borchard, N., Siemens, J., Ladd, B., Möller, A., Amelung, W. (2014). Application of biochars to sandy and silty soil failed to increase maize yield under common agricultural practice. *Soil and Tillage Research, 144*, 184-194.

Bronick, C. J., Lal, R. (2005). Soil structure and management: a review. *Geoderma, 124*(1-2), 3-22.

Brodowski, S., John, B., Flessa, H., Amelung, W. (2006). Aggregate-occluded black carbon in soil. *European Journal of Soil Science, 57*, 539-546.

Burrell, L., D., Zehetner, F., Rampazzo, N., Wimmer, B., Soja, G. (2016). Long-term effects of biochar on soil physical properties. *Geoderma*, *282*, 96-102.

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., Zechmeister-Boltenstern. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*.

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

Case, S. D. C., McNamara, N. P., Reay, D. S., Stott, A. W., Grant, K., Whitaker, J. (2015). Biochar suppresses $N_2O$ emissions while maintaining N availability in a sandy loam soil. *Soil Biology and Biochemistry*, *81*, 178-185.

Castracani, C., Maienza, A., Grasso, D. A., Genesio, L., Malcevschi, A., Miglietta, F., Vaccari, F. P., Mori, A. (2015). Biochar-macrofauna interplay: Searching for new bioindicators. *Science of the Total Environment*, *536*, 449-456.

Cayuela, M. L., van Zwieten, L., Singh, B. P., Jeffery, S., Roig, A., Sànchez-Monedero, M. A. (2014). Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. *Agriculture, Ecosystems and Environment*, *191*, 5-16.

Chintala, R., Schumacher, T. E., McDonald, L. M., Clay, D. E., Malo, D. D., Paplernik, S. K., Clay, S. A., Julson, J. L. (2013). Phosphorus sorption and availability from biochars and soil/biochar mixtures. *Clean- Soil, Air, Water*, *42*(5), 626-634.

Clough, T. J., Condron, L. M. (2010). Biochar and the nitrogen cycle: Introduction. *Journal of Environmental Quality*, *39*(4), 1218-23.

Clough, T. J., Condron, L. M., Kammann, C., Müller, C. (2013). A review of biochar and soil nitrogen dynamics. *Agronomy*, *3*, 275-293.

Cross, A., Sohi, S. P. (2011). The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. *Soil Biology and Biochemistry*, *43*(10) 2127-2134.

Davidson, E. A., Belk, E., Boone, R. D. (1998). Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Global Change Biology*, *4*, 217-227.

Decaëns, T., Jiménez, J. J., Gioia, C., Measey, G. J., Lavelle, P. (2006). The values of soil animals for conservation biology. *European Journal of Soil Biology*, *42*, S23-S38.

de la Rosa, J. M., Knicker, H. (2011). Bioavailability of N released from N-rich pyrogenic organic matter: An incubation study. *Soil Biology and Biochemistry 43*(12), 2368-2373.

De Luca, T. H., MacKenzie, M. D., Gundale, M. J., Jones D. L. (2009). *Biochar effects on soil nutrient transformation.* Chapter 14. In: Lehmann, J., Joseph, S. (eds) Biochar for environmental management science and technology. London, UK: Earthscan, 251-280.

Demisie, W., Liu, Z., Zhang, M. (2014). Effect of biochar on carbon fractions and enzyme activity of red soil. *Catena*, *121*, 214-221.

Deng, Q., Hui, D., Wang, J., Iwuozo, S., Yu, C., Jima, T., Smart, D., Reddy, C., Dennis, S. (2015). Corn yield and soil nitrous oxide emission under different fertilizer and soil management: A three-year field experiment in middle Tennessee. *PLoS ONE*, *10*(4), e0125406.

Devereux R. C., Sturrock, C. J., Mooney, S. J. (2012). The effects of biochar on soil physical properties and winter wheat growth. *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, *103*(1), 13-18.

Dil, M., Oelbermann, M., Xue, W. (2014). A An evaluation of biochar pre-conditioned with urea ammonium nitrate on maize (*Zea mays* L.) production and soil biochemical characteristics. *Canadian Journal of Soil Science*, *94*(4), 551-562.

Ding, Y., Liu, Y. X., Wu, W. X., Shi, D. Z, Yand, M., Zhong, Z. Z. (2010). Evaluation of biochar effects on nitrogen retention and leaching in multi-layered soil columns. *Water, Air, & Soil Pollution, 213*(1), 47-55.

Doane, T., Horwath, W.R. (2003). Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters*, *36*, 2713-2722.

Doran, J. W., Zeiss, M. R. (2000). Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology*, *15*(1), 3-11.

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 Biology and Biochemistry, 72*, 152-162.

Domene, X., Hanley, K., Enders, A., Lehmann, J. (2015). Short-term mesofauna responses to soil additions of corn stover biochar and the role of microbial biomass. *Applied Soil Ecology, 89*, 10-17.

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. doi:10.1002/jpln.201100167.

Estefan, G., Sommer, R., Ryan, J. (2013). *Methods of Soil, Plant, and Water Analysis: A Manual for the West Asia and North Africa Region* (3rd ed.). Beirut, Lebanon: ICARDA.

Farrell, M., Macdonald, L. M., Butler, G., Chirino-Valle, I., Condron, L. M. (2014). Biochar and fertiliser applications influence phosphorus fractions and wheat yield. *Biology and Fertility of Soils*, *50*, 169-178.

Felber, R., Leifeld, J., Horak, J., Neftel, A. (2014). Nitrous oxide emission reduction with greenwaste biochar: comparison of laboratory and field experiments. *European Journal of Soil Science*, *65*, 128-138.

Foster, J.C. (1995). *Soil Nitrogen.* In: Alef, K., Nannipieri, P. (eds) Methods in applied soil microbiology and biochemistry. Academic Press, San Diego, CA.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z. C., Freney, J. R., Martinelli, L. A., Seitzinger, S. P., Sutton, M. A. (2008). Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science*, *320*, 889-892.

Garg, N., Geetanjali. (2007). Symbiotic nitrogen fixation in legume nodules: process and signaling. A review. *Agronomy for Sustainable Development*, *27*(1), 59-68.

Genesio, L., Migiletta, F., Lugato, E., Baronti, S., Pieri, M., Vaccari, F. P. (2012). Surface albedo following biochar application in durum wheat. *Environmental Research Letters*, *7*, 1-8.

Glaser, B., Haumairer, L., Geggenberger, G., Zech, W. (2001). The 'Terra Preta' phenomenon: a model for sustainable agriculture in humid tropics. *Naturwissenschaften*, *88*(1), 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.

Glaser, B., Wiedner, K., Seelig, S., Schmidt, H. P., Gerber, H. (2015). Biochar organic fertilizers from natural resources as substitute for mineral fertilizers. *Agronomy for Sustainable Development*, *35*(2), 667-678.

Gomez, J. D., Denef, K., Stewart, C. E., Zheng, J., Cotrufo, M. F. (2014). Biochar addition rate influences soil microbial abundance and activity in temperate soils. *European Journal of Soil Science*, *65*, 28-39.

Gosling, P., Parsons, N., Bending, G. D. (2013). What are the primary factors controlling the light fraction and particulate soil organic matter content of agricultural soils? *Biology and Fertility of Soils*, *49*, 1001-1014.

Government of Canada. Canadian Climate Normals 1981-2010 Station Data: Dashwood. Retrieved January 2018 from http://climate.weather.gc.ca/climate_normals/index_e.html

Gregorich, E.G., Beare, M.H. (2007). Physically uncomplexed organic matter. In Carter, M.R. and Gregorich, E.G., Eds., Soil Sampling and Methods of Analysis, 2nd Edition, CRC Press, Boca Raton, FL.

Güereña, D., Lehmann, J., Hanley, K., Enders, A., Hyland, C., Riha, S. (2013). Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. *Plant and Soil*, *365*(1-2), 239-254.

Haefele, S.M., Konboon, Y., Wongboon, W., Amarante, S., Maarifat, A. A., Pfeiffer, E. M., Knoblauch, C. (2011). Effects and fate of biochar from rice residues in rice-based systems. *Field Crops Research*, *121*(3), 430-440.

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, cause and possible solutions. *Soil & Tillage Research, 82*, 121-145.

Hangs, R. D., Ahmed, H. P., Schoenau, J. J. (2016). Influence of willow biochar amendment on soil nitrogen availability and greenhouse gas production in two fertilized temperate prairie soils. *BioEnergy Research*, *9*(1), 157-171.

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

Hartley, W., Riby, P., Waterson, J. (2016). Effects of three different biochars on aggregate stability, organic carbon mobility and micronutrient bioavailability. *Journal of Environmental Management*, *181*, 770-778.

Hobley, E., Willgoose, G. R., Frisia, S., Jacobsen, G. (2013). Environmental and site factors controlling the vertical distribution and radiocarbon ages of organic carbon in sandy soil. *Biology and Fertility of Soils*, *49*(8), 1015-1026.

Hüppi, R., Felber, R., Neftel, A., Six, J., Leifeld, J., (2015). Effect of biochar and liming on soil nitrous oxide emissions from a temperate maize cropping system. *Soil*, *1*(2), 707-717.

Hutchinson, G. L., Mosier, A, R. (1981). Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal*, *45*, 311-315.

IBM Corp. *IBM SPSS statistics for windows, Version 22.0*. IBM Corp, Armonk, 2013.

Imparato, V., Hansen, V., Santos, S. S., Nielsen, T. K., Giagnoni, L., Hauggaard-Nielsen, H., Johansen, A., Renella, G., Winding, A. (2016) Gasification biochar has limited effects on functional and structural diversity of soil microbial communities in a temperate agroecosystem. *Soil Biology and Biochemistry*, *99*,128-136.

IPCC. (2014). *Climate change 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, Pachauri, R. K., Meyer, L. A. (eds.)] IPCC, Geneva, Swizerland, 151.

https://www.ipcc.ch/report/ar5/syr/

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

Iqbal, H., Garcia-Perez, M., Flury, M. (2015). Effect of biochar on leaching of organic carbon, nitrogen, and phosphorus from compost in bioretention systems. *Science of the Total Environment*, *521-522*, 37-45.

Jones, D. L., Edwards-Jones, G., Murphy, D. V. (2011a). Biochar mediated alterations in herbicide breakdown and leaching in soil. *Soil Biology and Biochemistry*, *43*(4), 804-813.

Jones, D. L., Murphy, D. V., Khalid, M., Ahmad, W., Edwards-Jones, G., De Luca, T. H. (2011b). Short-term biochar-induced increase in soil CO2 release is both biotically and abiotically mediated. *Soil Biology and Biochemistry*, *43*(8), 1723-1731.

Jones, D. L., Rousk, J., Edwards-Jones, G., De Luca, T. H., Murphy, D. V. (2012). Biochar-mediated changes in soil quality and plant growth in a three year field trial. *Soil Biology and Biochemistry*, *45*, 113-124.

Joseph, S.D., Camps-Arbestain, M., Lin, Y., Munroe, P., Chia, C.H., Hook, J., van Zwieten, L., Kimber, S., Cowie, A., Singh, B.P., Lehmann, J., Foidl, N., Smernik, R.J., Amonette, J.E., (2010). An investigation into the reactions of biochar in soil. *Australian Journal of Soil Research*, *48*, 501-515.

Kammann, C., S. Ratering, C. Eckhard, and C. Müller. (2012). Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, methane) fluxes from soils. *Journal of Environmental Quality*, *41*(4), 1052-1066.

Karer, J., Wimmer, B., Zehetner, F., Kloss, S., Soja, G. (2013). Biochar application to temperate soils effects on soil fertility and crop yield. *Geophysical Research Abstracts*, *14* (May), 1. Retrieved from http://meetingorganizer.copernicus.org/EGU2012/EGU2012-6432-1.pdf

Karhu, K., Mattila, T., Bergstrom, I., Regina, K. (2011) Biochar addition to agricultural soil increased $CH_4$ uptake and water holding capacity-results from a short-term pilot field study. *Agriculture, Ecosystems, and Environment*, *140*, 309-313.

Keiluweit, M., Nico, P. S., Johnson, M. G., Kleber, M. (2010). Dynamic molecular structure of plant biomass-derived black carbon (biochar). *Environmental Science & Technology*, *44*(4), 1247-1253.

Kerré, B., Willaert, B., Smolders, E. (2017). Lower residue decomposition in historically charcoal-enriched soils is related to increased adsorption of organic matter. *Soil Biology and Biochemistry*, *104*, 1-7.

Khodadad, C. L. M., Zimmerman, A. R., Green, S. J., Uthandi, S., Foster, J. S. (2011). Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. *Soil Biology and Biochemistry, 43*(2), 385-392.

Kishimoto, S., Sugiura, G. (1985). Charcoal as a soil conditioner, In: *Symposium on Forest Products Research, International Achievements for the Future*, *5*, 12-23.

Kloss, S., Zehetner, 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.

Knicker, H. (2011). Soil organic N - An under-rated player for C sequestration in soils? *Soil Biology & Biochemistry*, *43*, 1118-1129.

Knoblauch, C., Maarifat, A. A., Pfeiffer, E. M., Haefele. S. M. (2011). Degradability of black carbon and its impact on trace gas fluxes and carbon turnover in paddy soils. *Soil Biology and Biochemistry, 43*(9), 1768-1778.

Krull, E. S., Skjemstad, J. O., Baldock, J. A. (2004). Functions of soil organic matter and the effect on soil properties. *In: Grains Research and Development Corporation, 129*. http://www.grdc.com.au/uploads/documents/cso000291.pdf

Kulyk, N. (2012). Cost-Benefit analysis of the biochar application in the U. S. cereal crop cultivation. *Center for Public Policy Administration Capstones*, *12*.

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X. (2009). Black carbon decomposition and incorporation into soil microbial biomass estimated by 14-C labeling. *Soil Biology Biochemistry, 41*, 210-219.

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.

Laird, D., Fleming P., Wang, B, Q., Horton, R., Karlen, D. (2010). Biochar impact on nutrient leaching from a Midwestern agricultural soil. *Geoderma*, *158*, 436-442.

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.

Lavelle, P., Decaën, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi, J. P. (2006). Soil invertebrates and ecosystem services. *European Journal of Soil Biology, 42*, S3-S15.

Lehmann, J., Pereira da Silva Jr., J., Steiner, C., Nehls, T., Zech, W., Glaser, B. (2003). Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil*, *249*, 343-357.

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

Lehmann, J., Rondon, M. (2006). Biochar soil management on highly weathered soils in the humid tropics. In: Uphoff, N., Ball, A. S., Herren, H., Husson, O., Laing, M., Palm, C., Pretty, J., Sanchez, P., Sanginga, N., Thies, J. (Eds.), *Biological Approaches to Sustainable Soil Systems*. Taylor & Francis, Boca Raton, Florida, 517-530.

Lehmann, J., Joseph, S. (2009) *Biochar for Environmental Managemen*t. London, UK; Earthscan.

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.

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.

Li, D., Hockaday, W. C., Masiello, C. A., Alvarez, P. J. J. Earthworm avoidance of biochar can be mitigated by wetting. *Soil Biology & Biochemistry*, *43*, 1732-1737.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luizäo, F. J., Petersen, J., Neves, E. G. (2006). Black carbon increases cation exchange capacity in soils. *Soil Science Society of America Journal*, *70*, 1719-1730.

Liang, B., Lehmann, J., Solomon, D., Sohi, S., Thies, J. E., Skjemstad, J. O., Luizao, F. J., Engelhard, M. H., Neves, E. G., Wirick, S. (2008). Stability of biomass-derived black carbon in soils. *Geochimica et Cosmochimica Acta*, *72*(24), 6069-6078.

Liang, G., Houssou, A. A., Wu, H., Cai, D., Wu, X., Gao, L., Li, J., Wang, B., Li, S. (2015). Seasonal Patterns of Soil Respiration and Related Soil Biochemical Properties under Nitrogen Addition in Winter Wheat Field. *PLOS ONE*, *10*(12), e0144115.

Liesch, A. M., Weyers, S. L., Gaskin, J. W., Das, K. C. (2010). Impact of two different biochars on earthworm growth and survival. *Annals of Environmental Science*, *4*, 1-9.

Liu, X., Han, F., Zhang, X. (2012). Effect of biochar on soil aggregates in the Loess Plateau: Results from incubation experiments. *International Journal of Agriculture and Biology*, *14*, 975-979.

Lone, A. H., Najar, G. R., Ganie, M. A., Sofi, J. A., Ali, T. (2015). Biochar for sustainable soil health: A review of prospects and concerns. *Pedosphere*, *25*(5), 639-653.

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P. C. (2011). Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biology and Biochemistry*, *43*(11), 2304-2314.

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 Biology and Biochemistry*, *57*, 513-523.

Luo, C., Lü, F., Shao, L., He, P. (2015). Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes. *Water Research*, *68*, 710-718

Lutes, K., Oelbermann, M., Thevathasan, N. V., Gordon, A. M. (2016). Effect of nitrogen fertilizer on greenhouse gas emissions in two willow clones (*Salix miyabeana and S. dasyclados*) in Southern Ontario, Canada. *Agroforestry Systems*, *90*(5), 785-796.

Major, J., Steiner, C., Downie, A., Lehmann, J. (2009). *Biochar Effects on Nutrient Leaching.* Chapter 15. In: Lehmann, J., Joseph, S. (eds) Biochar for environmental management science and technology. Earthscan, London, 251-280.

Major, J., Lehmann, J., Rondon, M., Goodale, C. (2010). Fate of soil-applied black carbon: downward migration, leaching and soil respiration. *Global Change Biology*, *16*(4), 1366-1379.

Marks, E. A. N., Mattana, S., Alcañiz, J. M., Domene, X. (2014). Biochars provoke diverse soil mesofauna reproductive responses in laboratory bioassays. *European Journal of Soil Biology*, *60*, 104-111.

Martinsen, V., Mulder, J., Shitumbanuma, V., Sparrevik, M., Børresen, T., Cornelissen, G. (2014). Farmer-led maize biochar trials: Effect on crop yield and soil nutrients under conservation farming. *Journal of Plant Nutrition and Soil* Science, *117*, 681-695.

McDaniel, M. D., Grandy, A. S., Tiemann, L. K., Weintraub, M. N. (2014). Crop rotation complexity regulates the decomposition of high and low quality residues. *Soil Biology and Biochemistry*, *78*, 243-254.

McLain, J. E., Martens, D. A. (2006). Moisture controls on trace gas fluxes in semiarid riparian soils. *Soil Science Society of America Journal*, *70*, 367-377.

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

Miranda, K.M., Espey, M.G., Wink, D.A. (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, *5*, 62-71.

Mitchell, P. J., Simpson, A. J., Soong, R., Simpson, M. J. (2015). Shifts in microbial community and water-extractable organic matter composition with biochar amendment in a temperate forest soil. *Soil Biology & Biochemistry*, *81*, 244-254.

Morales, M. M., Comerford, N., Guerrini, I. A., Flcao, N. P. S., Reeves, J. B. (2013). Sorption and desportion of phosphate on biochar and biochar-soil mixtures. *Soil Use Management*, *29*, 306-314.

Motha, R. P., Baier, M. (2005). Impacts of present and future climate change and climate variability on agriculture in the temperate regions: North America. *Climate Change*, *70*, 137-164.

Naisse, C., Girardin, C., Davasse, B., Chabbi, A., Rumpel, C. (2015). Effect of biochar addition on C mineralization and soil organic matter priming in two subsoil horizons. *Journal of Soils Sediments*, *15*, 825-832.

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

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.

Oelbermann, M., Voroney, R. P. (2007). Carbon and nitrogen in a temperate agroforestry system: Using stable isotopes as a tool to understand soil dynamics. *Ecological Engineerin*g, *29*, 342-349.

Oltmans, R. R., Mallarino, A. P., (2011). Phosphorus and potassium removal and leaching from residue in corn and soybean. *North Central Extention-Industry Soil Fertility Conference*, *27*, 197-202. Des Moines, IA.

Parkin, T, B., Venterea ,R, T. (2010). *USDA-ARS GRACEnet Project Protocols Chapter 3: chamber-based trace gas flux measurements*. http://www.ars.usda.gov/SP2UserFiles/Program/ 212/Chapter%203.%20GRACEnet%20Trace%20Gas%20 Sampling%20Protocols.pdf.

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.Pelosi, C., Römbke., J. (2016). Are Enchytraeidae (Oligochaeta, Annelida) good indicators of agricultural management practices? *Soil Biology and Biochemistry*, *100*, 255-263.

Petter, F. A., Madari, B. E. (2012). Biochar: Agronomic and environmental potential in Brazilian savannah soils. *Revista Brasileira de Engenharia Agrícola E Ambiental*, *16*(7), 761–768.

Prendergast-Miller, M. T., Duvall, M., Sohi, S. P. (2011). Localisation of nitrate in the rhizosphere of biochar-amended soils. *Soil Biology & Biochemistry, 43*, 2243-2246.

Qayyum M. F., Steffens, D., Reisenauer, H. P., Schubert, S. (2012). Kinetics of carbon mineralization of biochars compared with wheat straw in three soils. *Journal of Environmental Quality, 41*, 1210-1220.

Quilliam, R. S., Glanville, H. C., Wade, S. C., Jones, D. L. (2013). Life in the 'charosphere' – Does biochar in agricultural soil provide a significant habitat for microorganisms? *Soil Biology & Biochemistry, 65*, 287-293.

Regehr, A., Oelbermann, M., Videla, C., Echarte, L. (2015). Gross nitrogen mineralization and immobilization in temperate maize-soybean intercrops. *Plant and Soil, 391*(1-2), 353-365.

Reibe, K., Gotz, K., Roß, C, Döring,T. F., Ellmer, F., Ruess, L. (2015). Impact of quality and quantity of biochar and hydrochar on soil Collembola and growth of spring wheat. *Soil Biology and Biochemistry, 83*, 84-87.

Reid, S., Bellivaeu, S., Smit, B, Cadwell, W. (2007). Vulnerability and adaptation to climate risks in southwestern Ontario farming systems. In Wall, E., Smit, B., Wandel, J. (Ed.), *Farming in a Changing Climate: Agricultural Adaptation in Canada*. Vancouver, BC: UBC Press.

Riddle, M., Cederlund, H., Schmieder, F., Bergström, L. (2018). Magnetite-coated biochar as a soil phosphate filter: From laboratory to field lysimeter. *Geoderma*, *327*, 45-54.

Rogovska, N., Laird, D. A., Rathke, S. J., Karlen, D. L. (2014). Biochar impact on Midwestern Mollisols and maize nutrient availability. *Geoderma*, *230–231*, 34-347.

Rutigliano, F. A., Romano, M., Marzaioli, R., Baglivo, I., Baronti, S., Miglietta, F., Castaldi, S. (2014). Effect of biochar addition on soil microbial community in a wheat crop. *European Journal of Soil Biology, 60*, 9-15.

Rondon, M., Ramirez, A., Lehmann, J. (2005). Charcoal additions reduce net emissions of greenhouse gases to the atmosphere. *In: Proc. of the 3rd Symp. on Greenhouse Gases and Carbon Sequestration*, 208, Baltimore, MA: USDA.

Rose, T. J., Keen, B., Morris, S. G., Quin, P., Rust, J., Kearney, L., Kimber, S., Van Zwieten, L. (2016). Application of woody biochar and woody mulch to mitigate nitrous oxide emissions from a poultry litter-amended soil in the subtropics. *Agriculture, Ecosystems, & Environment, 228*, 1-8.

Sarkhot, D. V., Berhe, A. A., Ghezzehei, T. A. (2012). Impact of biochar enriched with dairy manure effluent on carbon and nitrogen dynamics. *Journal of Environmental Quality 41*(4), 1107-1114.

Schneider, M. P. W., Lehmann, J., Schmidt, M. W. I. (2011). Charcoal quality does not change over a century in a tropical agro-ecosystem. *Soil Biology and Biochemistry, 43*(9), 1992-1994.

Schindlbacher, A., Zechmeister-Boltenstern, S., Butterbach-Bahl, K. (2004). Effects of soil moisture and temperature on NO, $NO_2$, and $N_2O$ emissions from European forest soils. *Journal of Geophysical Research, 109*, D17.

Schomberg, H. H., Gaskin, J. W., Harris, K., Das, K. C., Novak, J. M., Busscher, W. J., Watts, D. W., Woodroof, R. H., Lima, I. M., Ahmedna, M., Rehrah, D., Xing. B. (2012). Influence of biochar on nitrogen fractions in a Coastal Plain soil. *Journal of Environmental Quality, 41*, 1087-1095.

Schulz, H., Dunst, G., Glaser, B. (2013). Positive effects of composted biochar on plant growth and soil fertility. *Agronomy for Sustainable Development, 33*(4), 817-827.

Signor, D., Cerri, C. E. P. (2013). Nitrous oxide emissions in agricultural soils: a review. *Agricultural Research in the Tropics, 43*(3), 322-338.

Singh, B. P., Hatton, B. J., Singh, B., Cowie, A. L., Kathuria, A. (2010). Influence of biochars on Nitrous Oxide emission and nitrogen leaching from two contrasting soils. *Journal of Environmental Quality, 39*(4), 1224-35.

Smith, K. A., Ball, T., Conen, F., Dobbie, K. E., Massheder, J., Rey, A. (2003). Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. *European Journal of Soil Science, 54*, 779-791.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O. (2007). *Agriculture. In Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [B. Metz, O.R. Davidson, P.R. Bosch, R. Dave, L.A. Meyer (eds)], Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

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

Smith, P., Bustamante, M., Ahammad, H. (2014) In: Edenhofer, O., Pichs-Madruga, R., Sokona Y. (Eds.), *Climate Change 2014 – Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press, 811-922.

Smith, K. K. (2017). Changing views of nitrous oxide emissions from agricultural soil: key controlling processes and assessment at different spatial scales. *European Journal of Soil Science, 68*, 137- 155.

Sohi, S. P., Krull, E., Lopez-Capel, E., Bol, R. (2010). A review of biochar and its use and function in soil. *Advances in Agronomy*, *105*, 47-82.

Song, D., Tang, J., Xi, X., Zhang, S., Liang, G., Zhou, W., Wang, X. (2018). Responses of soil nutrients and microbial activities to additions of maize straw biochar and chemical fertilization in a calcareous soil. *European Journal of Soil Biology*, *84*, 1-10.

Spokas, K.A., Reicosky, D. C. (2009). Soil, impacts of sixteen different biochars on production, greenhouse gas. *Annual Environmental Science*, *3*(612), 179-193.

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. *Journal of Environmental Quality*, *41*(4), 973-989.

Steiner, C., Das, K. C., Melear, N., Lakly, D. (2010). Reducing nitrogen loss during poultry litter composting using biochar. *Journal of Environmental Quality*, *39*(4), 1236-1242. http://doi.org/10.2134/Jeq2009.0337

Troy, S. M., Lawlor, P. G., O'Flynn, C. J., Healy, M. G. (2013). Impact of biochar addition to soil on greenhouse gas emissions following pig manure application. *Soil Biology and Biochemistry*, *60*, 173-181.

USDA. (n.d.) Soil Bulk Density/ Moisture/ Aeration. Natural Resources Conservation Service. Retrieved May 2018 from https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_053260.pdf

USDA. (2009). Soil quality indicators: Soil respiration. *USDA Natural Resources Conservation Service*.

Uzoma, K. C., Inoue, M., Andry, H., Fujimaki, H., Zahoor, A., Nishihara, E. (2011). Effect of cow manure biochar on maize productivity under sandy soil conditions. *Soil Use and Management*, *27*(2), 205-212.

Van Zwieten, L., Kimber, S., Morris, S., Chan, K., Downie, A., Rust, J., Cowie, J. A. (2010). Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant & Soil*, *327*, 235-246.

Vasilyeva, N., Abiven, S., Milanovskiy, E. Y., Hilf, M., Rizhkov, O. V., Schmidt, M. W. I. (2011). Pyrogenic carbon quantity and quality unchanged after 55 Years of organic matter depletion in a chernozem. *Soil Biology and Biochemistry*, *43*(9), 1985-88.

Verdouw, H., Van Echteld, C. J. A., Dekkers, E. M. J. (1978). Ammonia determination based on indophenol formation with sodium salicylate. *Water Research*.

Verhoeven, E., Six, J. (2014). Biochar does not mitigate field-scale $N_2O$ emissions in a Northern

California vineyard: an assessment across two years. *Agriculture, Ecosystems, & Environment*, *191*, 27-38.

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. Boca Raton: Taylor & Francis Group.

Wanatabe, A., Ikeye, K., Kanazaki, N., Makabe, S., Sugiura, Y., Shibata, A. (2014). Five crop seasons' records of greenhouse gas fluxes from upland fields with repetitive applications of biochar and cattle manure. *Journal of Environmental Management*, *144*, 168-175.

Wang, T., Camps-Arbestain, M., Hedley, M., Bishop, P. (2012). Predicting phosphorus bioavailability from high-ash biochars. *Plant soil*, *357*, 173-187.

Yanai, Y., Toyota, K., Okazaki, M. (2007). Effects of charcoal addition on $N_2O$ emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. *Soil Science and Plant Nutrition*, *53*, 181-188.

Yang, L., Cai, Z. (2005). The effect of growing soybean (*Glycine max. L.*) on $N_2O$ emission from soil. *Soil Biology and Biochemistry*, *37*(6), 1205-1209.

Yao, Y., Gao, B., Zhang, M., Inyang, M., Zimmerman, A. R. (2012). Effect of biochar amendment on sorption and leaching of nitrate, ammonium, and phosphate in a sandy soil. *Chemosphere*, *89*(11), 1467-1471.

Yoo, G., Kang, H. (2012). Effects of biochar addition on greenhouse gas emissions and microbial responses in a short-term laboratory experiment. *Journal of Environmental Quality*, *41*(4), 1193-1202.

Yuan, Y., Chen, H., Yuan, W., Williams, D., Walker, J. T., Shi, W. (2017). Is biochar-manure co-compost a better solution for soil health improvement and $N_2O$ emissions mitigation? *Soil Biology & Biochemistry*, *113*, 14-25.

Zanchi, F. B., Meesters, A. G. C. A., Waterloo, M. J., Kruijt, B., Kesselmeier, J., Luizao, F. J., Dolman, A. J. (2014) Soil $CO_2$ exchange in seven pristine Amazonian rain forest sites in relation to soil temperature. *Agricultural and Forest Meteorology*, *192*, 96-107.

Zhai, L., Zhuoma, C. J., Liu, J., Wang, H., Ren, T., Gai, X., Xi, B., Liu, H. (2015). Short-term effects of maize residue biochar on phosphorous availability in two soils with different phosphorous sorption capacities. *Biology and Fertility of Soils*, *51*(1), 113-122.

Zhang, Q., Wang, Y., Wu, Y., Wang, X., Du, Z., Liu, X., Song, J. (2013). Effects of biochar amendment on soil thermal conductivity, reflectance, and temperature. *Soil Science Society of America Journal*, *77*, 1478-1487.

Zhang, H., Chen, C., Gray, E. M., Boyd, S. E., Yang, H., Zhang, D. (2016). Roles of biochar in improving phosphorus availability in soils: A phosphate adsorbent and a source of available phosphorus. *Geoderma*, *276*, 1-6.

Zhao, L., Cao, X., Zheng, W., Kan, Y. (2014). Phosphorus-assisted biomass thermal conversion: Reducing carbon loss improving biochar stability. *PLoS One*, *9*(12), e115373.

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

**Appendix A.** Summary of notes collected on reviewed biochar studies, including; study site location, biochar type (feedstock and temperature), the amount of biochar utilized, and the observed outcomes.

*Bolded author names indicate that the study was carried out in temperate soils.*

| **Biochar Effects on Soil Characteristics, Soil Biology, and Crop Yields** | | | | |
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| **Author** | **Amount** | **Type** | **Temps °C slow,** | **Impacts** |
| **Anders et al, 2013**<br><br>Greenhouse and field (Austria) | 24 t/ha<br>72 t/ha | Vineyard pruning<br>Wheat straw<br>Mixed wood<br>Beech hardwood | 400<br>525<br>500 | • No impact on SMB<br>• Shift in family, genera, and species<br>• Increased pH, CEC, C/N, EC, $C_{org}$<br>• Decreased EC in first 7 months<br>• Increased C/N favours fungi+ limits bacteria<br>• Field: C/N increased, everything else not impacted<br>• Field: Lower wheat yield without additional fertilizer |
| **Anderson et al, 2011**<br><br>Missouri (Silt-loam) | 15 t/ha<br>30 t/ha | Pine | | • Biochar decreased abundance of microbes associated with nitrification of $NH_4$ +$NO_2$<br>• Increased mycobacterial nitrate reduction to $NH_4$, and $N_2$ fixation<br>• Promotes phosphate solubilizing bacteria<br>• Increased abundance of bacteria families that can degrade recalcitrant C compounds<br>• Potentially decreased bacterial plant pathogens<br>• Few changes to total microbe abundance and activity<br>• Impact pH and water holding capacity (WHC), therefore decreased $N_2O$ emissions |

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| **Bamminger et al, 2014**<br><br>Germany<br>(Silty ag soil) | 30 t/ha<br>30cm | *Miscanthus giganteus* | 600 slow | • Reduced NH4 and NO3 concentrations<br>• Increased microbial biomass, favoured fungi<br>• More efficient microbial community |
| **Bamminger et al, 2016**<br><br>Germany | 30 t/ha<br>30cm | *Miscanthus giganteus* | 600 slow | • No impact to shoot biomass<br>• Decreased bulk density<br>• Increased pH<br>• Decreased $NO_3$<br>• No impact on microbial biomass |
| **Borchard et al, 2014**<br><br>Germany<br>(sandy-silt) | 45 t/ha<br>300 t/ha | Hardwood<br>Softwood | 500slow & gas<br>1100Flash | • The 45 t/ha had no impact to maize yield<br>• 300 t/ha decreased maize yield<br>• Flash=decline in germination rate (organic phytotoxins)<br>• Slow= increased WHC 20%<br>• 45 t/ha did not impact WHC or aggregate stability<br>• 300 t/ha increased WHC, C/N, K<br>• 300 t/ha changed K/MG, K/N, and P/K |
| **Bornø et al., 2018** | | Softwood<br>Rice husk<br>Oil seed rape | 550 | • Increased available P<br>• Did not interact with fertilizers<br>• Affected the microbial community and phosphatase activity<br>• Crop residue biochars increase fertilizing effect |
| **Brunn et al., 2014** | 51 t/ha<br>102 t/ha<br>204 t/ha<br>408 t/ha | Straw<br><br>Hardwood | Lowgas<br><br>slow | |
| **Chintala et al., 2013**<br><br>Acidic clay (S. Dakota)<br>Calcareous (Minnesota) | | Corn stover<br>Ponderosa pine<br>Wood residue + switchgrass | 650 | • Pine increased available P (43% of sorbed P), especially bicarbonate extractable P<br>• Biochar increased P sorption in calcareous soils<br>• Biochar increased AEC |

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| | | | | • Laboratory experiment, says in-field needed |
| de la Rosa & Knicker et al, 2011 | 150 mg in 150 g soil at 15cm | Rye grass (imitating wildfire) | 350 | • Increased biomass with biochar<br>• Biochar partially decomposed (N) nutrient source for microbes (SMB)<br>• Biochar increased total C+N |
| de la Rosa et al, 2018<br><br>Mediterranean (Sandy-clay calcareous) | 15 t/ha | Pine<br>Paper sludge<br>Sewage sludge<br>Vineyard cuttings<br>Woodchips | | • C content decreased over 24 months, partially attributed to mineralization (initial loss). Most in first year, statistically insignificant after that.<br>• Decreased N in first 6 months for wood, increase onward<br>• Initial pH rose then fell back<br>• Feedstock important for how biochar ages/breaks apart<br>• Biochar increased slow C pool without increasing $CO_2$ |
| Dempster et al, 2012<br><br>Australia, Mediterranean, Wales (Eutic cambisol) | 25 t/ha<br>10cm | Mixed hardwood | 450<br>48hrs | • Short+ long term no major shift in C turn over or partitioning in SMB<br>• No big impact on organic N mineralization<br>• Biochar aged/change considerably over 3 years in Wales |
| **Domene et al, 2014**<br><br>New York (Sandy-loam) | 3 t/ha<br>12 t/ha<br>30 t/ha<br>1 t/ha/yr | Corn stover | $600^{slow}$ | • Medium term effect paper<br>• Biochar increased microbial abundance (especially as biochar increased)<br>• Mesofauna+ leaf litter unaffected (trend increased)<br>• Biochar increased $NO_2$ + $NO_3$ mineralization, decreased $SO_4$ +Cl |
| **Domene et al, 2015** | 0.2<br>0.5<br>2 | Corn stover | $600^{slow}$ | • Short term effect paper |

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| New York (Sandy-loam) | 7<br>14%<br>w/w | | | • Microbial biomass increased as biochar increased, decreased after 61 days<br>• Collembolan avoided biochar, enchtraids did not<br>• This avoidance disappeared when increased microbial biomass of $NH_4$. Therefore not toxic |
| **Durenkamp et al, 2010**<br><br>UK and China (Silty-clay-loam, Loamy sand, Red loam) | 3.5 mg/g<br>28 mg/g | Beech for UK<br><br>Corn+ wood for China | 500<br><br>350-400 | • Biochar did not decrease extraction efficiency of biomass C or N<br>• CEC increase over time |
| **Gaijć & Koch, 2012**<br><br>Germany (Silty) | 10 t/ha 15cm<br>30 t/ha pot | Sugar beet pulp | 190 | • Biochar decreased initial beet growth<br>• Likely microbial N immobilization<br>• Immobilization increased with increased C/N ratios from biochar<br>• Increased C/N ratio biochar decreased leaching |
| **Glaser et al., 2002** | 5 t/ha | | | • Decrease soybean and maize yield |
| **Glaser et al, 2014**<br><br>Germany (Sandy) | 1 t/ha<br>10 t/ha<br>40 t/ha | Green cuttings | 650 | • Biochar +fertilizer more yield increase than just fertilizer<br>• Biochar increased K, Mg, Zn uptake; decreased Na, Cu, Ni, Cd uptake<br>• Can increase WHC. More biochar= increased pH<br>• Even 1 t/ha increased efficiency of nutrient use |
| **Gomez et al, 2014** | 1 %<br>5<br>10<br>20 | Oak pellet | 550 Fast | • Biochar increased microbial abundance and favour Gram - |

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| Colorado, Iowa, Michigan, Minnesota | | | | over fungi and + (increased as biochar amount increased)<br>• Biochar decreased extractable phospholipid fatty acids<br>• Biochar-C incorporated into SMB |
| **Güereña et al, 2013**<br><br>New York (Silt-loam) | 3 t/ha<br>12 t/ha<br>30 t/ha<br>1 t/ha/yr | Maize stover | 600[slow] | • No impact to maize yield or plant N uptake<br>• N decreased after biochar, no change to pH<br>• Biochar increased microbial biomass N 3-fold + increased retention of N in topsoil |
| **Haider et al., 2017**<br><br>Germany Temperate sandy soil<br>4 year | 15 t/ha<br>30 t/ha | Norwary spruce and European beech | 550-600 | • 30 initial decrease in Mn in first maize<br>• Reduced NO3 leaching<br>• Higher moisture<br>• Decreased crop yield N deficiency<br>• No increase to crop yields in 4 years |
| **Hammond et al (2013)**<br><br>UK (Silty-clay-loam, loamy-sand) | 10 t/ha<br>20 t/ha<br>40 t/ha | Mixed wood | 500[slow] | • Most cases no impact to crops<br>• Increased yield w 1-20 t/ha biochar, most with 10 t/ha decrease with 40 t/ha<br>• Increased biochar= increased yield if fertilizer added<br>• Biochar increased soil moisture retention<br>• Beyond 30 t/ha can produce negative effects<br>• 10 t/ha sufficient for benefits<br>• Shallow incorporation for this effect |
| **Hardie et al (2014)**<br><br>Austria Planosol and Chernozem | 3%, top 30cm | Mixed wood<br>Wheat straw<br><br>Vineyard | 525<br>525<br><br>525<br>400 | • Biochar decreased bulk density<br>• Improved aggregate stability<br>• Improved available plant water in planosol soil by 38%<br>• Course texture have most to gain |

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| **Hartley et al., 2016**<br><br>UK | 5% | Green waste<br>Shrub<br>Softwood | 440[steam] | • Aggregate stability only improved with oversize biochar<br>• Micronutrients reduced in wheat grain<br>• Biochar increased dissolved organic carbon (doc) in pore waters compared to control<br>• Biochar enhanced soil respiration |
| Hilscher & Knicker, 2011 | | See GHG sheet | | |
| **Imparato et al, 2016**<br><br>Danish<br>(agri sandy) | 0.8-1.4 t/ha<br>6-8 t/h | Wheat straw | 700-750[gas] | • Biochar did not impact ATP content, soil toxicity or catabolic community profiles<br>• Increasing biochar increased phenol oxidase activity and pH and decreased cellulose activity<br>• Low bichar additions increased diversity of soil microbes<br>• Biochar had limited impact to functional and structural diversity of microbial community |
| **Jones et al, 2011b**<br><br>Wales<br>Australia | 10 t/ha<br>100 t/ha | Hardwood | 450 [48hrs]<br>600 [24hrs] | • Sorbs herbicide reducing leaching, available to microbes and biodegradation<br>• Herbicide mineralization inverse with biochar particle size<br>• Biochar reduces herbicide dissipation therefore environmental contamination and human exposure<br>• May impact efficiency of soil applied herbicides |
| **Jones et al, 2012**<br><br>Wales (Sandy-clay loam) | 25 t/ha<br>50 t/ha<br>20cm | Hardwood | 450 [48hrs] | • Biochar increases foliar N (yr 2) and biomass, maize shoot biomass (yr 3), fungal + bacterial growth/turn over (yr 2), soil respiration |

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| | | | | • Biochar favours bacterial decomposer community<br>• No impact to DOC, $NO_3^-$, $NH_4^+$, N mineralization, $NH_4^+$volitization, density, or sorption (short or long term)<br>• Impacts not same between field and lab<br>• No impact on crop (maize) in first year<br>• Initial pH increases then fall as ages |
| **Karer et al, 2013**<br><br>Austria (Silt loam, clay loam) | 24 t/ha<br>72 t/ha | Beech | 550 slow | • During drought biochar increases WHC and yield with highest Biochar addition<br>• Maize + grain decreases with highest biochar without fertilizer. No impacts when fertilizer added too<br>• Biochar caused N deficiency, N released too slow<br>• Biochar decrease bulk den, increases infiltration, decrease compaction + erosion |
| Khodadad et al, 2010<br><br>Florida (Sandy) | 300 mg/ 3g soil | Oak<br>Grass | 250 3hrs<br>650 | • Biochar decreases microbial diversity and increases specific taxa |
| Kloss et al, 2014<br><br>Planosol, Cambisol, Chernozem | 30 t/ha<br>90 t/ha | Wheat straw<br>Mixed wood<br>Vineyard pruning | 525 slow 60m<br><br>400 | • C/N increases at 90 t/ha<br>• Initial decreases crop yields by biochar (decreases Cu, Fe, Mn, Zn, increases Mo in plant tissue)<br>• Strong variation of impact with soil type |
| **Koide et al, 2011**<br><br>Penn State (Silt-loam) | 56.8 t/ha | Hardwood | slow | • Just method for determining how much biochar really is in field |

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| **Kuzyakov et al, 2009**<br><br>Germany | 108g biochar in 45g soil | Ryegrass | 400 | • Very slow biochar mineralization, decreases during incubation. No significant $CO_2$ emit<br>• Direct incorporation of biochar-C into SMB |
| **Lentz & Ippolito, 2012**<br><br>Idaho | 22.4 t/ha | Hardwood | 500 fast | • Biochar increases available Mn, TC, and TOC<br>• 1st year biochar increases corn silage, not impact grain<br>• 2nd year biochar decreases silage, yield, and S concentrations<br>• No pH change<br>• Biochar may inhibit mineralization of manure C |
| Li et al, 2011<br><br>Artificial soil | 9 t/ha<br>90 t/ha<br>180 t/ha | Applewood sawdust | 400-525 slow | • Biochar=weight loss in earthworms but no impact on reproduction<br>• Due to water-loss, not nutrient deficiency or toxins |
| Liang et al, 2008<br><br>Brazil (Anthrosols) | | | | • Biochar increases physical, stability, stable ortho-mineral C, oxidation on biochar surface<br>• Biochar no difference between amount 1st year and 600-8700 years<br>• Older biochar no impact on $CO_2$, younger biochar increased $CO_2$ |
| **Luo et al, 2011**<br><br>UK (Silty-loam) | 50 mg C/ g soil | *Miscanthus giganteus* | 350<br>700 | • Both biochar temperatures increases biomass C concentration + soil microbe ATP (decreases bio available C as temp increases), most in 90 days but continued into 180<br>• Biomass C linear relationship with $CO_2$ evolution<br>• Biochar mineralization a biological process<br>• Increases pH (more with higher temp) |

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| | | | | • Low temp biochar most colonized with bacteria + fungi |
| **Luo et al, 2013**<br><br>UK (Clay-loam) | 50 mg/g | *Miscanthus giganteus* | 300<br><br>700 | • 300 increases biomass C and ATP in 1st 90 days then decreased. Not likely correlated with biochar though<br>• 700 less C in biomass<br>• microbe colonization attributed to biochar-C avail + surface area (more in low temp)<br>• Biochar mineralization a biological process<br>• Biochar makes C, N, and micronutrients available and reduces toxics of Al+Mn<br>• Biochar increases nutrient solubility due to pH increases<br>• More benefits when soil pH lower |
| Ma et al., 2016 | 7.8 t/ha | Maize straw and peanut hull | | • Improved macro-aggregates<br>• Improved SOC. No effect on C/N ratio<br>• Major (2012) no impact on water holding capacity<br>• Jeffery (2015) no impact to water retention, aggregate stability in sandy soil |
| Madiba et al., 2016<br><br>Australia Loamy-sand | 5 t/ha<br>10 t/ha | Chicken manure<br>Wheat chaff | 450 | • Increased plant P uptake, P availability to microbes, and P sorption<br>• Favoured mycorrhizae<br>• 5 t/ha increased wheat yield the most, 10 t/ha slightly<br>• Biochar decreased relative leaching, but increased water holding capacity of soil so P lost in solution too |
| Marks et al, 2014 | 0.5%<br>1.3<br>3.2 | Poplar<br><br>Slude | ~500 slow<br>~500 fast<br>~500 slow | • Pine gas char inhibited invertebrates, likely increase carbonate, Ca content, and pH |

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| Spain | 8<br>20<br>50 | Pine | ~500 slow<br>~500 fast<br>~500 gas | • High amount of biochar decrease number of inverts<br>• Wood char stimulated reproduction for 1 species, not other |
| **Mitchell et al, 2015**<br><br>Ontario (Brunisol) | 5 t/ha<br>10 t/ha<br>20 t/ha | Maple wood | 500 | • Pyrolysis at temp convers cellulose, hemi-cellulose, and lignin aromatic<br>• Biochar increase $CO_2$<br>• Initial depression of gram +, -, and actinomycetes In first 16 weeks. Then recover (adapt)<br>• Favour gram + over gram – and fungi<br>• Biochar increases activity of specific microbes= increases $CO_2$ and decreases labile SOM |
| **Naisse et al., 2015** | 0.3g Biochar/ 30g soil | Poplar wood<br>Maize silage | 250<br>1200gas | • Chemical stability of both biochars unaffected by physical weathering<br>• High temper gas led to protection of native SOM (negative priming)<br>• Low temperature led to positive priming (stimulated native soil OM mineralization) |
| **Nelissen et al, 2015**<br><br>Belgium (Sandy loam) | 20 t/ha | Hard+ soft wood | 480 slow | • Slight impact to soil microbe community in 1st yr<br>• No impact to N+ P uptake or crop yield in first 2 yr<br>• Stable C applied<br>• No N immobilization observed<br>• Improved water storage capacity and soil stability after some aging<br>• Increase SOC<br>• No Impact to spring barley crop yield |

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| **Prendergast-Miller et al, 2011**<br><br>UK | 20 t/ha<br>60 t/ha | Mixed wood | | • Biochar increases wheat root length<br>• Biochar decreases root N uptake<br>• Plant biomass and N content similar between treatment<br>• $NO_3$ localized in rhizosphere with biochar, decreases loss increases efficiency<br>• Increases pH<br>• No impact to crop biomass or root architecture |
| **Qayyum et al, 2012**<br><br>Germany | 50 t/ha | Bark<br><br>Sewage sludge | 200<br><br>400 | • 200 higher nutrient concentration but loss of C in alfisol<br>• 400 lowest CO2 emissions after 270 days. Stabilizes SOM<br>• 400 Charcoal shows accumulation of $NO_3$-N<br>• High temperature biochar better for C-sequestration<br>• Low temperature biochar better for increasing soil fertility |
| **Quilliam et al, 2013**<br><br>Wales (Sand-clay loam) | 50 t/ha<br>20cm | Hardwood mix | 450[48] | • In short term no significant habitat for microbes<br>• Labile compounds impact microbe activity and structure<br>• Biochar 5x greater surface area than soil<br>• Biochar only sparsely colonized by microbes<br>• Sorption can prevent microbes from penetrating pores<br>• Glucose mineralizes faster on + in biochar than soil |
| Riddle et al., 2018 | | Hardwood/spruce mix coated in magnetite | 380-430 | • Wood BC low source of P and poor P sorption capacities<br>• Coating reduced P loss |
| Rutigiano et al, 2014 | 30 t/ha<br>60 t/ha | Wood | 500 $^{slow}$ | • No impact on $C_{organic}$, $C_{extractable}$, $C_{SMB}$, or genetic diversity |

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| Italy, Tuscany (Silty-loam) | | | | • Increase pH, specific microbe activities and change in functional diversity after 3 months but none after 14 (expect pH, a little higher)<br>• Positive stimulation to soil microbe activity short lived |
| Sarkhot et al, 2011<br><br>California Orchard | 10 g/kg 15 cm | Hardwood | 300 slow | • Biochar 68-75% decrease in net nitrification<br>• Biochar 221-229% decrease in net ammonification<br>• Reduced $NH_4$+ $NO_3$ concentrations- Adsorption rather than immobilization<br>• Source of K for 20 days<br>• Decreases N + P leaching ~70% |
| Schneider et al, 2011<br><br>Kenya | | Burns | | • Charcoal resists chemical degradation after 100 years |
| Schomberg et al, 2012<br><br>South Carolina | 40 t/ha | Peanut hull<br><br>Pecan shell<br><br>Poultry litter<br><br>Switchgrass<br><br>CQest | 400<br>500<br>350<br>500<br>350<br>700<br>250<br>500<br>500 | • No increase in mineralizable N fraction (no stimulation of microbial biomass)<br>• Decrease in resistant N fraction<br>• Volatization of N as $NH_3$<br>• 350 Poultry increased soil N<br>• Low temp increase pH (higher ash)<br>• All biochar increases soil C<br>• High temperature decreases N leaching<br>• High temperature shell, hull, and litter had net N loss to volatilization<br>• Little impact on microbial biomass |
| **Steinbeiss et al, 2009** | 30% C w/w | Glucose-derived<br><br>Yeast-derived | 850 | • See GHG sheet |

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| Germany (aerable and forest)<br>28 | | | | |
| Sun & Lu, 2014<br><br>Clayey soil<br>Greenhouse | 20 g/ka<br>40<br>60 | Straw<br>Waste-sludge | 500 | • Increased macroaggregate formation<br>• Increased aggregate stability, resistance to slaking and inter particular cohesion<br>• Straw biochar increased available water contents of soil and pore space |
| **Vasilyeva et al, 2011**<br><br>Russia (Chernozem) | | Wild fire | | • Charcoal stock did not change or decrease over time<br>• Move down horizons- stock lost form FLF<br>• Water repellant |
| Wang et al, 2012<br><br>Acid washed sand<br>Tokomaru silt loam | 1:1 | Eucalyptus | 250<br>350<br>450<br>550 | • Hydro stable (labile pool in biochar) N decreases as pyrolysis temperature increase<br>• C + N increase stable as pyro temperature increases<br>• N restructures as pyro temperature increases<br>• N availability decreases as pyro temperature increases |
| **Yuan et al, 2017**<br><br>North Carolina (Loamy-sand) | 1%<br>5%<br>120ml container | Harwood/Rice hull (added to chicken manure) | Low [gas] | • Enhanced total C+N, (in)organic N (especially $NO_3^-$), SMB C+N cellulose enzyme activity, N2O bacteria +fungi, and N2O+CO2 emissions<br>• Significantly lower than just chicken manure alone<br>• SOC more stable with biochar<br>• Short term N, SMB+ activity |
| **Zhai et al., 2015**<br><br>Yunnan province (red earth) | 2%<br>4%<br>8% | Maize straw | 400 | • 8% biochar increased soil Olsen-phosphorous in red earth and fluvo-aquic soil<br>• 8% increase SMB-phosphorus in both soils |

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| Beijing (fluvo-aquic/ haplic luvisol) | | | | • Increases due to ash fraction, 77% of total biochar P is ash |
| Zhang et al., 2016<br><br>Queensland, Australia<br><br>Clay-loam | | Mallee<br>Wild-fire burn<br>Jarrah<br>Greenwaste<br>Sugarcane bagasse<br>Blady grass<br>Pine<br>Peanut shell<br>Timber | 750<br>350-500<br>750<br>450<br>350<br><br>450<br>750<br>450<br>750 | • Amount and form of P made available in soil depended on biochar type<br>• 500 wood biochars had highest Olsen-P values<br>• Can be a P source and absorb P<br>• Can act as fertilizer<br>• Grass had highest extractable P, provided 90% of crop needs |

| **Biochar Impacts on GHG Emissions** | | | | |
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| **Authors and Date** | **Amount** | **Type** | **Temp** | **Impacts** |
| **Anders et al, 2013**<br><br>Greenhouse and field (Austria) | 24 t/ha<br>72 t/ha | Vineyard pruning<br>Wheat straw<br>Mixed wood<br>Beech hardwood | 400<br>525<br>500 | • Biochar enhances organisms involved in N cycling in soil. Especially those than can decrease $N_2O$ through promoting denitrification to $N_2$ or by absorbing $NH_4$ |
| **Bamminger et al, 2014**<br><br>Germany (Silty ag soil) | 30 t/ha<br>30cm | Miscanthus giganteus | 600 $^{slow}$ | • Reduced $CO_2$ (up to 43%) and $N_2O$ (up to 42%)<br>• Reduced NH4 and NO3 concentrations<br>• Increases microbe biomass, favoured fungi<br>• More efficient microbe community |
| **Bamminger et al, 2016**<br><br>Germany | 30 t/ha<br>30cm | Miscanthus giganteus | 600 $^{slow}$ | • Microbe abundance unaffected<br>• Initial decomposition by fungi with warmer temperatures (none after 7-12 months)<br>• Increases microbe biomass, fungi favoured<br>• More efficient microbial community, no change to SMB |

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| | | | | • No change to total aboveground biomass |
| **Case et al., 2015**<br><br>UK (Sandy loam) | 28 t/ha | Hardwoods | 180-400 slow | • Reduced denitrification by 37% (85-95% of soil N2O emissions)<br>• Increased soil N mineralization by269% and nitrification by 34%<br>• Therefore alters N transformation, not just limits NH4 and NO3 |
| Cross et al, 2011<br><br>Australia (sand) | 2g BC/ 19 g Sand | Sugar cane | 10 types of slow | • C mineralization often higher in biochar due to rapid utilization of biochar labile compounds<br>• Biochar does not prime for losses of natural SOM<br>• Negative priming in biochar soil due to stabilization of labile compounds<br>• Largest CO2 from lowest biochar temp |
| de la Rosa et al, 2018 | 15 t/ha | Pine<br>Paper sludge<br>Sewage sludge<br>Vineyard cuttings<br>Woodchips | | • C content decrease over 24 months, partially attributed to mineralization (initial loss). Most in first year, stat insignificant after that<br>• Decreases N in first 6 months for wood, increase onward<br>• Biochar increases slow C pool without increase $CO_2$ |
| **Deng et al, 2015**<br><br>Tennessee | 2.5 $kg/m^2$<br><br>25 t/ha | Wood | | • Decrease $N_2O$ emissions, correlated with decrease water filled pore space<br>• Highest $NH_4$ concentration<br>• No sig diff to corn yield<br>• Biochar decreases $N_2O$ sensitivity to WFPS |
| Feng et al, 2013 | 24 t/ha | Corn stalk | 300<br>400<br>500 | • Decrease $CH_4$ emissions<br>• Biochar change microbial community |
| **Gomez et al, 2014** | 1 %<br>5<br>10<br>20 | Oak pellet | 550 Fast | • Biochar increases microbe abundance and favour Gram - over |

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| Colorado, Iowa, Michigan, Minnesota | | | | fungi and + ( increase as biochar amount increase)<br>• Biochar decreases extractable phospholipid fatty acids<br>• More $CO_2$ emission as biochar increase<br>• Biochar-C incorporated into SMB, especially where emission is largest |
| **Harter et al, 2016**<br><br>Switzerland (Loamy-sand) | 10% w/w 10cm | Green waste | 700 Slow | • Significantly decreased $N_2O$ emissions. Alters abundance and activity of denitrifier marker genes<br>• Biochar increase anaerobic bacteria + including chitin-aromatic C degrading microbes<br>• 3 generations more abundant in biochar all strict or facultative anaerobes compatible with denititrifiers + fixing molecular N<br>• Counter Anders et al (2011), Chen (2015), Xu (2014)<br>• Abiotic sorption of $N_2O$ onto biochar + chemo-denitrification decrease $N_2O$ |
| **Hilscher & Knicker, 2011**<br><br>Switzerland | 120g soil/ 400 mg biochar | Grass | | • Different degradation + humification dynamics at work (Biochar ages and chemicals alter)<br>• Microbes access and degrade biochar-N |
| **Hüppi et al, 2015**<br><br>Switzerland | 20 t/ha | Green waste | 650 slow | • Biochar cumulative decrease in $N_2O$, not statistically significant<br>• Decrease not caused by pH<br>• Biochar no impact on yield or biomass<br>• Biochar no impact on N or P uptake |
| **Jones et al, 2011a**<br><br>North Wales (Sandy clay loam) | 100 t/ha | Hardwood | 450 48hrs | • Increase $CO_2$ efflux. Breakdown of organic C and the release of inorganic C in biochar<br>• Release small compared to C stored in biochar |

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| 36 | | | | • Short term release (48hrs – 1 month) |
| Kastner, 2014 | 5 t/ha<br>10 t/ha | | | |
| **Knoblaunch et al, 2011**<br><br>Germany | 2.5% w/w | Rice husk | | • Initial $CH_4$ increase<br>• No impact on $CO_2$ (slightly lower) |
| **Luo et al, 2011**<br><br>UK (Silty-loam) | 50 mg C/ g soil | *Miscanthus giganteus* | 350<br>700 | • 350 caused increase priming (mineralization) un low and high pH<br>• Priming effect decreases as temperature increases<br>• $CO_2$-C released, SOC decrease with biochar. But more carbon in char then released<br>• Biochar significant decomposition of SOC |
| **Mitchell et al, 2015**<br><br>Ontario (Brunisol) | 5 t/ha<br>10 t/ha<br>20 t/ha | Maple wood | 500 | • Biochar increase $CO_2$<br>• Biochar increases activity of specific microbes= increases $CO_2$ and decreases labile SOM |
| Sarknot et al, 2011 | | | | • 67-68% decrease $CO_2$ flux<br>• 26% decrease $N_2O$ flux |
| Singh et al, 2010<br><br>Australia (Alfisol, vertisol) | | Wood<br>Poultry manure | 400<br>500 | • Initial (4 months) greater $N_2O$ emissions+ leaching from poultry biochar. Wood biochar had no impact<br>• Overtime all decreased $N_2O$emissions in both soils (oxidation on biochar increase sorption)<br>• $NH_4$ leaching reduced overtime<br>• Reductions over time attributed to increase soprtion<br>• Largest emissions following additions |

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| **Smith et al, 2010**<br><br>Washington State (Silt-loam, sand) | 11.2 t/ha<br>22.4 t/ha<br>44.8 t/ha | Switch-grass | 500 considers this low | • Increase respiration as biochar addition increase short term<br>• Short-term mineralization increases soil C<br>• No extra emissions after 6 days following 50 days<br>• Labile C pool for young biochar (bio oil) |
| **Steinbeiss et al, 2009**<br><br>Germany (aerable +forest) | 30% C w/w | Glucose derived<br>Yeast derived | 850 | • Yeast biochar increases $CO_2$ at beginning, decomposition slowed after 4 months. No impact from glucose<br>• No difference after 12 weeks<br>• Increase SOC loss (high yeast, moderate glucose)<br>• Impact same for both soils |
| Steiner et al, 2010<br><br>North Georgia | 5%<br>20% | Pine | 400 | • No poultry litter mass loss with biochar<br>• Decreases moisture, increases pH, increases $CO_2$+temp, decreases N with high biochar application<br>• Biochar decreases $H_2S$, increase aeration<br>• Sorption of $NH_4$<br>• Initial $CO_2$ peak in 20% biochar then no difference<br>• Increase compost speed with biochar |
| Too & Kang, 2012 | 20 t/ha 10cm | Barley<br><br>Manure | 350<br><br>600-800 | • 350 No $CO_2$, $CH_4$ change, decreases $N_2O$ in pasture<br>• 350 increased fungal biomass in paddy<br>• 600 including $N_2O$ emission in rice paddy ( increase N mineralization)<br>• Initial burst of $CO_2$ then appear decreases in $CO_2$ and $CH_4$<br>• Biochar provides sufficient available N |
| **Yuan et al, 2017** | 1%<br>5% | Harwood/Rice hull (added to | Low [gas] | • Enhanced total C+N, (in)organic N (especially NO3), SMB C+N |

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| North Carolina (Loamy-sand) | 120ml container | chicken manure) | | cellulose enzyme activity, $N_2O$ bacteria + fungi, and $N_2O$+ $CO_2$ emissions<br>• Significantly lower than just chicken manure alone<br>• Biochar suppressed N2O from bacteria denitrification and/or nitrifier denitrification<br>• SOC more stable with biochar<br>• Short term increase of N, SMB and activity<br>• Reduced CO2 efflux |
| **Zhou et al, 2017**<br><br>Subtropical China (Sandy-silty clay) Temperate forest | 10 t/ha<br>30 t/ha | Bamboo | 800 Slow | • Biochar significantly increases (by 20%) soil respiration in temperate<br>• Respiration + correlated with SMB-C and with DOC<br>• Increase temperature sensitivity of respiration in biochar in both ecosystems |
| Zimmerman et al, 2011<br><br>Quartz Sand Northern Florida (Alfisols, Entisols, Mollisol) | 90 t/ha | Oak<br>Pine<br>Bubinga<br>Eastern Gamma Grass<br>Bagasse (sugar cane) | 250<br>400 considers this low<br>650 | • More C released from biochar<br>• C mineralization increases with decreasing pyro temperature<br>• C mineral higher in grass than hardwood<br>• Positive priming with low temperature + grass biochar in 1st 90 days. Negative priming later (250-500 days)<br>• Soil OM stimulates mineralization of labile biochar components over short term<br>• Long term biochar enhances soil C storage through OM sorption to biochar<br>• Agricultural soil had more negative priming (lower SOC to begin with(greater portion of total mineralizable SOM), more sorption to biochar) |

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| | | | | • Over time SOM progressively sorbed onto biochar, win pores protected from degradation<br>• Estimate annually sequester C~12% current anthropogenic $CO_2$ emissions |