EVALUATION OF BIOCHAR ON REDUCING NITROGEN LEACHING FROM A TYPICAL SOIL IN NOVA SCOTIA

by

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ABSTRACT

Biochar has been recently used as a soil amendment on reducing the nitrogen (N) leaching from agricultural soils to the surface and ground waters. A laboratory study using column leaching tests to determine the effectiveness of different types of biochars under different dosages on reducing leaching of nitrate and ammonium from an agricultural soil in Nova Scotia. A significant ($p<0.05$) decrease in the amount of nitrate leached from 'fertilized' soil was observed on biochar treatments comparing to the control treatment. Poultry manure derived biochars were more effective on reducing nitrate leaching than wood derived biochars. Poultry manure biochars produced at high temperature showed the lowest cumulative nitrate leaching than other types of biochars. Biochar applied at rate of 5 g kg^{-1} of soil showed non-significant difference on reducing nitrate leaching compared with application rate of 10 g kg^{-1} of soil except for MH biochar, which reduced more nitrate from leaching at higher application rate. Soil received wood-derived biochars showed non-significant lower ammonium leaching than soil received non-biochar. Poultry manure derived biochars prone to increase the ammonium leaching over times.

LIST OF ABBREVIATIONS USED

N	Nitrogen
N_2	Nitrogen Gas
NO_2^--N	Nitrite-Nitrogen
NO_3^--N	Nitrate-Nitrogen
NH_3-N	Ammonia-Nitrogen
NH_4^+-N	Ammonium-Nitrogen
HTT	Highest Treatment Temperature
CEC	Cation Exchange Capacity
AEC	Anion Exchange Capacity
SOM	Soil Organic Matter
WHC	Water Holding Capacity
MH	Mixed Hardwood
WW	Wood Waste
MP	Poultry manure heated at medium temperature
HP	Poultry manure heated at high temperature
Ca	Calcium
$CaCl_3$	Calcium Chloride
Na	Sodium
K	Potassium
NH_4Cl	Ammonium Chloride
$NaNO_3$	Sodium Nitrate
M	Mole
MAC	Maximum Acceptable Concentration
RMA	Repeated Measures Analysis
CBA	Cost-Benefit Analysis
LSM	Least Square Means

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CHAPTER 1: INTRODUCTION

1.1 Background

Nova Scotia is a world leader in agricultural research and production for products including carrots, wild blueberries, and strawberries. (Nova Scotia Department of Agriculture, 2012). Nova Scotia has 403,044 ha of total farm area including crop and pasture land, summer fallow, natural land for pasture, woodlands, wetlands, and Christmas tree area. In 2012, the agriculture and food industry in Nova Scotia accounted for about 2% of the provincial total Gross Domestic Product (GDP) (Agriculture and Agri-Food Canada, 2014). Agriculture in Nova Scotia is concentrated mainly in the Cumberland, Colchester, Kings, and Hants counties (called "agricultural triangle"), which contribute to over 60% of agricultural revenues in the province (Nova Scotia Department of Agriculture, 2012).

Vegetable crops such as carrot and potato are the main crops in Nova Scotia, which required the relatively high amount of nitrogen (N) for crop growth (111 kg N/ha for carrot and 185 kg N/ha for potato) (Laboski et al., 2006; Nova Scotia Department of Agriculture, 2012). However, soils in Nova Scotia are naturally acidic and infertile, which require the use of fertilizer to increase the soil fertility and crop yield in Nova Scotia (Canada Department of Agriculture, 1972). About 60.8% and 46.1% of farming areas in Nova Scotia apply commercial fertilizer and manure, respectively (Scott et al., 2002). The use of fertilizer may increase the potential of nutrient, especially N, leaching from agriculture fields to the surface and ground waters (Haverstock, 2010).

Nutrients, such as nitrogen (N), leaching from agricultural areas to ground and surface waters could cause the negative environmental impacts and economic loss to farmers, and pose health risks to human (Jiao et al., 2004; Tabatabai et al., 2005). Nitrate (NO_3^--N) can be a health concern in water wells used for drinking water if nitrate concentrations exceeds the Health Canada Maximum Acceptable Concentration (MAC) of NO_3^--N for drinking water (10 mg/L) (Health Canada, 2014). NO_3^--N levels at or above MAC have been known to cause a potentially fatal blood disorder named methemoglobinemia or "blue-baby" syndrome, a decline in the oxygen carrying capacity of blood in infants under six months of age (Scott et al., 2002). Nova Scotia Environment (2012) reported about 15% to 25% of the water wells in Nova Scotia were found to have excessive nitrate, over the limit of MAC in any given year since 1989 to 2011; 10% of the water wells had an upward trend in nitrate levels. Recent studies in Thomas Brook Watershed indicate that high NO_3^--N concentrations in groundwater can also cause pollution in surface water systems, especially during summer months (Gauthier et al., 2009). Nitrate-N can also be discharged to a surface water body as base flow, high nitrate levels in surface water will contribute to eutrophication (Nova Scotia Environment, 2012), which results in a reduction of available oxygen for aquatic life and a decline of productivity of fishery (Zheng et al., 2013). In Nova Scotia, blooms of algae have been reported in several locations, which thrive in areas where the water is shallow, warm, slow-moving and high in nutrients such as nitrate, like ponds in the late summer or early fall (Nova Scotia Environment, 2011). These examples demonstrate the consequences of nutrients leaching especially N into the environment in Nova Scotia.

Therefore, it is necessary to introduce soil nutrient management practices to retain nutrients especially N in the root zone and prevent them from loss through leaching. There are several approaches that may be employed to reduce nutrient leaching, such as using cover crops, which can reduce nitrate leaching and soil erosion as well as soil compaction, and increase soil organic matter (SOM) (Lehmann & Schroth, 2003; Hoorman, 2009; Schroeder, 2012). However, these conventional nutrient management approaches may require extra costs on hiring labor, planting crops and buying farming tools (Nova Scotia Department of Agriculture, 2012).

Recently, there has been much interest in biochar as a soil conditioner to reduce the nutrient leaching, and to improve and maintain the soil fertility and the crop yield (Lehmann et al., 2003; Chan et al., 2008; Major, 2009[a]; Singh et al., 2010[a]; Filiberto & Gaunt, 2013). Biochar has large surface area due to its porous structure, which enables the adsorption of molecules such as N and P (Laird et al., 2010). Furthermore, its high negative surface charge enables biochar to hold cations such as NH_4^+ by increasing soil's cation exchange capacity (CEC), and the numerous pores improve microbial activity in soil that are important for nutrient cycling (Bruun et al., 2012). Biochar amendment in soil will increase soil's water holding capacity (WHC) and soil's pH, reduce soil bulk density, and enhance soil aggregation, thus increasing soil fertility (Yao et al., 2012).

Experimental work which explores the beneficial effects of biochar as a soil amendment in terms of reduced soil nutrients leaching and increased crop yield is limited. Some work has been conducted under laboratory conditions as well as at field scale but the results have been highly variable and sometimes inconsistent (Yamato et al., 2006; Hyland, 2010; Laird et al., 2010; Bruun, 2011; Major et al., 2012; Jien & Wang, 2013; Zheng et al., 2013; Guo et al., 2014). Hyland (2010) found biochar produced from both oak and paper mill waste could both reduce NO_3^--N leaching from soil. However, biochar produced from poultry manure under the same laboratory condition increased the NO_3^--N leaching when applied in soil along with sawdust. Similarly, Bruun (2011) reported biochars produced under fast pyrolysis condition slightly decreased NO_3^--N leaching, whereas slow pyrolysis biochars did not reduce NO_3^--N leaching. Hyland (2010) also found increasing application rates of biochars produced from oak and corn respectively resulted in the lower NO_3^--N leaching. These contrary findings suggest biochar's effectiveness on nutrient leaching could be affected by its parent material, different pyrolysis conditions such as temperature and pyrolysis rate, and the application rate of biochar in soil.

1.2 Objectives

Most of the biochar research on nutrient leaching have been conducted on highly weathered tropical and subtropical soils. No information is available about the effect of biochar application on N retention of Nova Scotian soil, which is characterized as cool, moist and acidic due to Nova Scotia's modified-continental climate (Davis & Browne, 1996). Contradictory results from previous studies indicated that biochar's effectiveness on nutrient leaching attributes to factors such as the original feedstocks of biochar, the biochar making conditions such as temperature, and the dosage of biochar applied in soil. **The overall goal** of this project is to investigate whether or not the tested biochars, which were produced from different parent materials and at different pyrolysis temperatures, and at different dosage will be effective on mitigating the leaching of NO_3^--N and NH_4^+-N from a Nova Scotian soil. **Specific objectives** for this research goal are to:

i. Study the ability of biochars on reducing the leaching of NO_3^--N and NH_4^+-N from a testing soil, fertilized with relatively high concentration of $NaNO_3$ and NH_4Cl solution by conducting weekly leaching experiment for 28 weeks;

ii. Compare the effectiveness of different types of biochar under the same application rate on reducing NO_3^--N and NH_4^+-N leaching from soils fertilized with nutrient solutions;

iii. Compare the effectiveness of different application rate of the same type of biochar on decreasing the loss of NO_3^--N or NH_4^+-N through leaching;

iv. Recommend the optimum biochar type, which helps soil to obtain the highest retention rate of NO_3^--N and NH_4^+-N respectively.

1.3 Thesis Structure

This thesis is divided into five chapters: introduction, a review of the literature, the design of leaching experiment and experimental procedure, the experimental results and detailed discussions, and general conclusion and recommendations for future work.

To explore the project goal and specific research objectives, the following series of topics with disclosure of the experimental work and the resultant outcomes will be covered in this thesis. **Chapter 1** presents the background information of Nova Scotian agriculture issues including nutrients leaching and adoption of biochar as a soil amendment and the objectives of this research. **Chapter 2** covers a general introduction of literature review relevant to this research area following the detailed information on nutrients leaching, current management of nutrient retention, mitigation of nutrients leaching using biochar, and the uncertainties of using biochar. In **Chapter 3**, the experimental design, procedures, the methodology of testing the concentrations of NO_3^--N and NH_4^+-N in the leachate samples, and the statistical method for data analysis are described. **Chapter 4** reports the detailed experimental results and the discussion of results by answering the research questions relevant to research objectives. **Chapter 5** gives a summary and conclusion of the research and the recommendations for future research.

CHAPTER 2: LITERATURE REVIEW

This chapter is to provide the reader with the background information for this thesis. In this section, some basic context relevant to nutrients leaching (especially nitrogen leaching), and the management options of nutrients leaching in agriculture will be covered. This will be followed by a review of the effects of using biochar as an emerging soil amendment on nitrogen leaching reduction, which includes the characteristics of biochar relevant to its nutrients retention capability, the mechanisms of biochar on nitrate retention and ammonium retention, and the uncertainties of using biochar as a soil amendment.

2.1 Nitrogen Leaching

In agriculture, soil provides plant essential nutrients such as nitrogen (N), phosphorus (P), potassium (K) and sulphur (S), which are dissolved in water and absorbed by plant's root (Alberta Agriculture and Food, 2008; Nova Scotia Department of Agriculture, 2012). However, these nutrients are not usually high enough in soil for optimum plant growth, especially N, which is typically the most deficient and first limiting nutrient, followed by P, K, and S (Province of Manitoba, 2013). Therefore, application of fertilizers or animal manures in agricultural soil supplies the needed nutrients for crop growth.

Plants take up essential nutrients from the soil in soluble and inorganic forms except for some metal elements, which can be absorbed as organic complexes (Alberta Agriculture and Food, 2008). However, when percolating water carries those dissolved and mobile nutrients away from the rooting zone where plant can absorb nutrients, leaching of nutrients occur (Major et al., 2009). Nutrient losses through leaching are an important aspect of nutrient cycling in agriculture (Brady & Weil, 2008). For nutrients dissolved in the soil solution a migration of negatively charged nutrients called 'anions' will always follow by an equivalent migration of positively charged nutrients called 'cations' due to the electro-neutrality (Major et al., 2009). In such case, the leaching of NO_3^--N after organic matter mineralization or N fertilization will accompany by the leaching of cations such as calcium (Ca), potassium (K), and magnesium (Mg) (Lehmann & Joseph, 2009). In such case, it is not only NO_3^--N is being leached out but also the cations are being lost from soil. In agriculture, N is one of the essential nutrients to support plant growth, whereas Nitrate-N is the most vulnerable N to leaching through soil. It is the objective of the next section to explain the nitrogen cycling and nitrogen leaching in soil.

2.1.1 Nitrogen Cycling

Nitrogen (N) is an essential nutrient for plant growth, which influences the production of plant proteins such as enzymes, mitochondria and carrier, storage and structural proteins (Alberta Agriculture and Food, 2008). NO_3^--N is the primary inorganic form of leached N and NH_4^+-N is the second major form of inorganic N, which is mild leachable in soil solution (International Plant Nutrition Institute, 2015 [b]). Nitrate and ammonium are the only two inorganic forms of N that plants can directly utilize for growth. Organic N compounds are the important source of N for crops, which are components of soil organic matter (SOM), must undergo decomposition before N is plant available (Alberta Agriculture and Food, 2008).

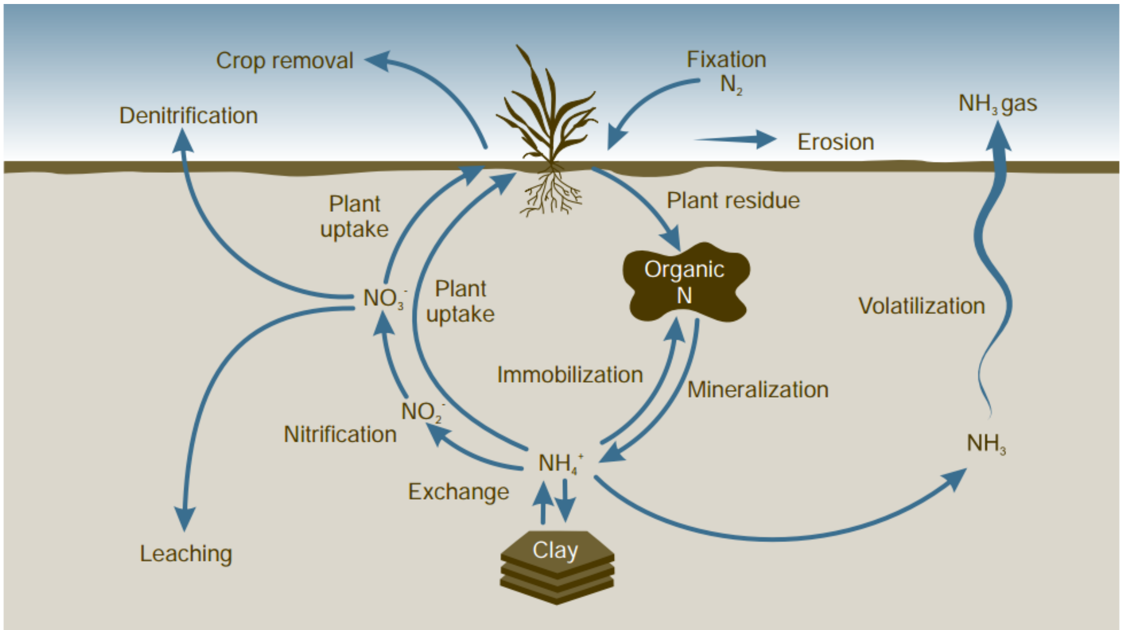


Figure 2.1 The agricultural nitrogen cycle. Source: Retrieved from Alberta Agriculture and Food (2008).

N exists in different forms in N cycling, which involves gains, losses and transformation of N among pools in soil (Fig. 2.1). There are seven forms of N that involved in the N cycle, including nitrogen gas (N_2), ammonia (NH_3^+), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrogen oxide gases (NO, N_2O) and organic N (Jones & Jacobsen, 2005[a]). In soil, organic N from animal manures, synthetic fertilizers, decaying plants or composts is broken down into simpler forms through mineralization by soil organisms such as insects, small animals, and microorganisms (SESL Australia, 2012). During the N mineralization, organic N compounds are converted to NH_4^+, which can be partly absorbed by plants for growth partly because NH_4^+ will be quickly converted to NO_2^- and NO_3^- through nitrification for plant uptake (Jones & Jacobsen, 2005[b]).

N can be lost from soil through the leaching of mobile N (mainly NO_3^- and mildly NH_4^+), run off or erosion with surface water during rain events or irrigation, volatilization from NH_4^+ to NH_3 gas and the denitrification from NO_3^--N to N_2 gases (International Plant Nutrition Institute, 2015[a]). NH_4^+-N is the positively charged N, which can be also absorbed temporarily into negative charged sites of soil particles through cation exchange. NH_4^+ can be also converted to organic N by microbial biomass through immobilization since the decomposition of crop residues requires N from either residue or soil solution (Alberta Agriculture and Food, 2008). Therefore, the leaching of N in soil solution refers to mainly the nitrate leaching because NO_3^--N is one of the most mobile N in soil, which is the major contributor of eutrophication in open water resources, although in some cases leaching of NH_4^+ may occur (Correl, 1998; Havlin et al., 1999). Otherwise, NH_4^+ in soil solution is nitrified to NO_3^--N or volatilized to gas, or to be held in clay and soil organic matter.

In soils, N transformation is affected by soil additives such as biochar, organic fertilizers, animal waste and compost (DeLuca et al., 2015). Biochar has been recently treated as a soil amendment to improve soil fertility and crop yields (Major, 2009[a]) but the literatures regarding the influence of biochar on soil N transformation are limited. It has been reported that applying biochar into forest soils could directly affect N transformations in acidic phenol-rich soils of both temperate (DeLuca et al., 2006; Gundale & DeLuca, 2006; MacKenzie & DeLuca, 2006) and boreal (DeLuca et al., 2002; Berglund et al., 2004). Prommer et al. (2014) reported that biochar addition in soil could increase the gross nitrification rates in soil, which could due to biochar's liming effect (Ball et al., 2010; Nelissen et al., 2012). Biochar has the potential to raise the pH of acidic soils due to its neutral to alkaline pH with a concurrent positive response of soil nitrifiers whose pH optimum is slightly acid to neutral pH (Stienstra et al., 1994; Prosser & Nicol, 2012). However, biochar could decrease rather than increase the pH of soil that is slightly alkaline (Prommer et al., 2014). Biochars influence N mineralization depending on the types of parent materials of biochars along with impacts on the C/N ratio of the soil microbial community (Prommer et al., 2014). Biochars contain high C and low N such as beech wood (C/N ratio = 200/1) could reduce the N mineralization through N limitation enhancement, which results in the retention of NH_4^+-N in the N-limited micro-flora (Schimel & Bennett, 2004). In contrary, biochars contain high N and low C such as manure-biochars could increase N mineralization by facilitating the microbial C limitation, resulting in the excess of N to be mineralized (Wang et al., 2012).

Little direct evidence exists showing the effect of biochar on N immobilization, volatilization or denitrification (DeLuca et al., 2015). Biochar could induce the net immobilization of inorganic N already present in the soil solution or applied as fertilizer because some decomposition occurs when biochar is freshly applied in soil (Schimel et al., 1996; Liang et al., 2006). This could explain the reduction of inorganic N leaching, which will discuss in the further section. Biochar could cause a reduction of volatilization from NH_4^+-N in soil due to the decrease of N mineralization or adsorption of NH_4^+-N by biochar, which lowers the potential for NH_3 volatilization (Le Leuch & Bandosz, 2007). Biochar influence the denitrification in soil by catalyzing the reduction of N_2O to N_2, indicating the potential of greenhouse gas emission reduction of biochar (DeLuca et al., 2015).

Biochar application into soil could affect the N transformation in soil, which demonstrates the mechanisms of N retention by biochar and the reduction of N leaching in the soil.

2.1.2 Factors Affect Nitrogen Leaching

In general, nitrogen leaching can be affected by soil properties (such as texture), climate (such as rainfall intensity), and application rate and timing of fertilizer and manure (Lehmann & Joseph, 2009).

Soil Properties

Soil properties determine the movement of water in soil, making it the key impact factor of N leaching (International Plant Nutrition Institute, 2015[a]). Soil texture, which is classified as the proportion of sand, silt, and clay, plays an essential role in plant nutrition because it influences the nutrients and water retention ability (Alberta Agriculture and Food, 2008). In soil, the rate of rain infiltrating into soil and moving nutrients away from rooting zone depends on the soil porosity, which is determined by soil texture and structure (Lehmann & Joseph, 2009). Soils dominated by clay have small pores that help to retain soil solution and have very high surface areas, ranging up to 90 acres per pound of soil, which provide numerous binding places for nutrients, thus reducing N leaching (Jones & Jacobsen, 2005[a]). Soils dominated by sand have large pore spaces between particles and low surface area, which can lead to bypass flow, thus accelerating N leaching (Renck & Lehmann, 2004). In addition, soil texture and structure determines soil permeability, which is the measurement of the water movement through soil pores of a saturated soil (International Plant Nutrition Institute, 2015[a]). Soil has coarser texture tend to have the higher permeability and experience the faster flow down the soil profile than that of finer-textured soil (Lehmann & Joseph, 2009). Sandy soil holds less soil water and nutrients holding capacity is low due to the larger soil pores (macro-pores) as well as high soil permeability (Fig. 2.2). In short, coarser-texture soil has a higher susceptibility to N leaching because the presence of large pores with resulting fast water movement and N carried away from rooting zone.

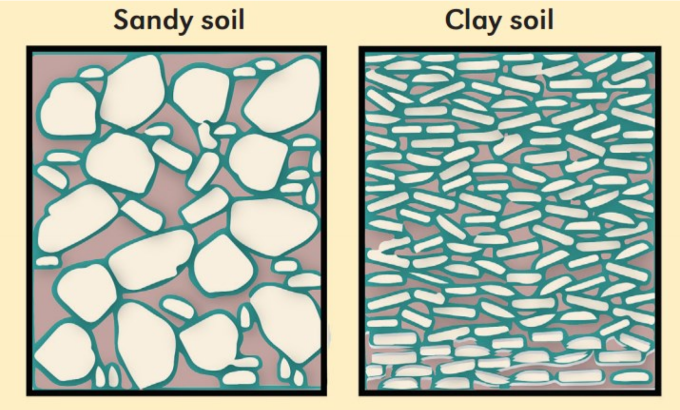


Figure 2.2 Pore size and pore distribution of sandy soil and clay soils. Source: Retrieved from International Plant Nutrition Institute (2015[a]).

The chemistry of clays, soil minerals such as metal oxides, and organic matter are also the impact factors of N leaching (Lehmann & Joseph, 2009). Cation exchange capacity (CEC) is the total negative charge on soil, which measures the ability of a soil to attract and supply nutrients to a crop (Jones & Jacobsen, 2005[b]). Similar to the CEC, the anion exchange capacity (AEC) is the ability of soil particles to absorb and store cations (measured in meq/100g soil) (Jones & Jacobsen, 2005[a]). Some typical values of CEC for various soil textures are shown in Table 2.1. Soil with high clay content has greater negatively charged sites and higher CEC, which can help soil to hold positively charged nutrients such as NH_4^+-N, hence reducing NH_4^+-N leaching (Lehmann & Schroth, 2003; Brady & Weil, 2008). The unit of CEC is milliequivalents (or meq) of negative charge/100 g of soil (Jones & Jacobsen, 2005[a]).

Table 2.1 Cation Exchange Capacity (CEC) for a range of soil textures. Source: Retrieved from Brady (1984).

Soil Texture	CEC Range (meq/100g soil)
Sand	2-4
Sandy loam	2-17
Loam	8-16
Silt loam	9-26
Clay	5-58

Similar to clay, soil organic matter (SOM) also provides negatively charge sites and has as high as 215 meq/100g of CEC, which can attract positively charged ions (cations) into soil (Jones & Jacobsen, 2005[a]; Mengel, 2011). Soil pH affects the availability of nutrients, thus influencing the CEC and AEC of soil. The lower soil pH usually leads to the lower CEC of clay particles and SOM due to the increase of H^+ ions in soil solution, which will neutralize the negatively charges on clays and organic matter (Fig.2.3). In short, acidic soils have the lower CEC and less available 'base' cations (such as Na^+, K^+, Ca^{2+}, Mg^{2+}) than that in alkaline soils.

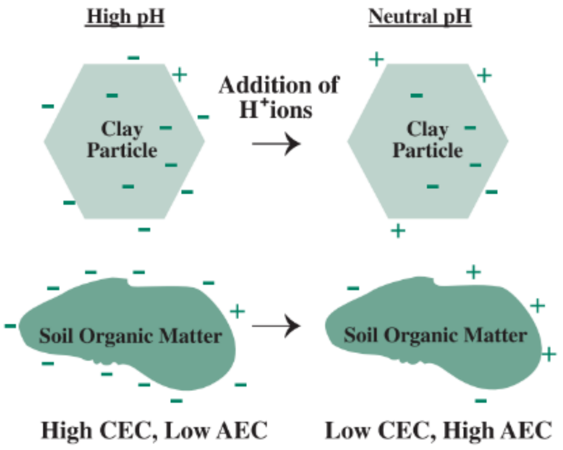


Figure 2.3 Effect of pH on CEC and AEC of clay particles and soil organic matter. Source: Retrieved from Jones & Jacobsen (2005[a]).

In Nova Scotia, *Gibraltar soil*, *Cobequid* soil and *Queens* soil are the dominant soil types, which are mainly forested, although *Queens* soil can be used for hay, pasture, and planting some grains (Canada Department of Agriculture, 1972). There are two major agricultural soils in Nova Scotia, the *Tormentine* soil (422,000 acre) and the *Stewiacke* soil (36,000 acre), which are located mainly in Stewiacke, Truro, Berwick, Hansford, Merigomish, Pugwash, Tormentime and Woodville. These two soils have been widely used for agriculture on growing various crops (Canada Department of Agriculture, 1972). The soil texture of these two soils is characterized as moderately and medium coarse, which are dominated by sandy loam, loam, silt loam and silt (Environment Canada, 2013). Besides, acidity is found in both *Tormentine* soil and Stewiacke soil in soil depth of 0-20 inches (Canada Department of Agriculture, 1972). Therefore, we can expect that the soil water and nutrients retention abilities of these two agricultural soils in Nova Scotia are relatively low due to the acidity of soil, indicating the low CEC which can be one of the contributors of nutrients leaching in these areas. This is mainly due to the acidity of soil and the sandy texture of soil in Nova Scotia, which indicated the low SOM and CEC of natural Nova Scotian soil.

Rainfall Amount

Rainfall amount (or irrigation) is one of the impact factors of nutrients leaching in agricultural areas. In general, if water inputs such as rainfall or irrigation is over the evapotranspiration, which means soil water content exceeds field capacity, nutrient leaching will occur (Lehmann & Schroth, 2003; International Plant Nutrition Institute, 2015[a]). Nutrient leaching will happen more often in areas with humid climate than that with arid climate (Havlin et al., 1999). Nova Scotia has a humid climate, which receives abundant rainfall and snowfall during rainy seasons and winter. It seems that nutrient leaching is prone to happen in autumn, winter and early spring because soil has low plant cover and receives abundant percolating waters from rainfall and snowfall (Jiao et al., 2004).

Cropping System and Fertilization

Over-fertilization or over use of manure could also contribute to the leaching of nutrients from soils (Al-Kaisi & Yin, 2003). If application of fertilizers or manure are higher than the crop needs, nutrients are vulnerable to leaching especially nitrate, which is highly mobile (Lehmann & Schroth, 2003; International Plant Nutrition Institute, 2015[a]). Nutrient-use efficiency varies among crop species and varieties, which could lead to greater nutrient losses through leaching when the nutrient-use efficiency is low (Major et al., 2009). Nutrient leaching is generally greatest under fertilized row crops such as maize or horticultural crops. Deep-rooted plants such as trees can recycle leached nutrients that have migrated to deeper soil horizons (Rowe et al., 1998; Allen et al., 2004). The amounts, chemical form, timing and placement of fertilizers greatly affect nutrient leaching (Melgar et al., 1992; Cahn et al., 1993; van Es et al., 2002).

2.2 Management Practices for Nutrient Retention

Nutrients leaching will cause losses of plant essential nutrients such as nitrate, it is important for farmers to reduce nutrients leaching from agricultural soils. Several management practices can effectively reduce nutrient leaching and potential for water contamination.

Cover crops can take up nitrate residual in soil for their own growth and utilize available soil moisture, hence decreasing water available for nutrients leaching (Schroeder, 2012). Cover crops are beneficial to soil by reducing N leaching and soil erosion, increasing water infiltration and soil organic carbon, and promoting soil aggregation stability and soil structure (Lehmann & Schroth, 2003). However, cover crops have disadvantages such as an increase in costs for planting crops, potential risks of disease and insect pests and an increase in labor cost (Hoorman, 2009).

Crop rotation is a practice that grows different crops in the same area in sequential season, which has been suggested as another effective management practices to not only reduce nutrient leaching especially for N but also increase soil productivity in agriculture (Havlin et al., 1990). Crop rotation can improve soil structure, hence improving soil organic matter and nutrient pool due to the enhancement of root structure and the increase of various macro-pores (Tilman et al., 2002). Al-Kaisi (2001) found disadvantages of crop rotation including the requirement of new skills and different equipment to introduce new crops, an increase of labor costs.

Other management practices such as increasing fertilizer use efficiency can be effective to reduce nutrient leaching and increase soil fertility (Tilman et al., 2002). Fertilizer application rates can be adjusted precisely to meet crops needs by dividing application in two or more time intervals during the cropping season or by placing fertilizers at the zone of maximum root activity of crops (Lehmann & Schroth, 2003).

Although the conventional strategies are able to reduce nutrient leaching in some way, there are some issues associated with them. The requirement of labor and advanced technologies increase the expense to farmers. Some strategies also bring side effects such as increased risks of pest and disease. Comparing the conventional management practices, there are some new development of nutrient management strategies, which bring both economic and environmental benefits such as using biochar as a soil amendment. For example, using biochar as a soil amendment is one of these novel strategies, which can help to reduce nutrients leaching from soil, displace conventional fossil fuel based fertilizers, and sequester carbon to reduce greenhouse gas emission. The benefits and physicochemical properties of biochar will be discussed in the next section.

2.3 Mitigation of Nitrogen Leaching Using Biochar

Biochar is the carbon-rich product obtained when biomass is pyrolyzed with little or no available oxygen (Ventura et al., 2013). Biochar has been used as one of the new nutrient management practice to mitigate nutrients leaching along with environmental and economic benefits (Lehmann & Joseph, 2009).

Biochar has a porous structure, which is beneficial to microbial activity and aids nutrient recycling in the soil by providing habitats and creating a stable soil carbon pool to sequestrate carbon. Biochar has a high concentration of carbonates, which will have a liming effect on acidic soils by increasing pH of soil (Sohi et al., 2010; Lehmann et al., 2011). Soils amended with biochar improve soil's nutrient retention capacity and CEC. Biochar helps to increase soil fertility by adsorbing soil organic matter and providing nutrients from high mineral-ash biochars like manure biochar. Biochar enables the enhancement of soil WHC and other physical properties such as soil aggregation and bulk density (Lehmann & Joseph, 2009).

2.3.1 Characteristics of Biochar

2.3.1.1 Physical Properties

Operating parameters such as Highest Treatment Temperature (HTT), heating rate and pressure during the pyrolysis process influence the resultant physical properties of biochar of any given parent materials including surface area and porosity (Downie et al., 2009). Of these parameters, HTT referring to the final charring temperature which the biomass is subjected to in the pyrolysis reactor has been found to have the greatest overall influence on the final product characteristics (Antal & Gronli, 2003; Lua et al., 2004; Özçimen & Ersoy-Meriçboyu, 2008).

Surface area is an important factor to affect improvements in fertility such as water, air, nutrient cycling and microbial activity (Sohi et al., 2010). The surface area of biochars typically increase with the HTT until it reaches the temperature at which deformation of biochar's structure occurs, leading to subsequent decreases in surface area (Downie et al., 2009). Low HTT (e.g. 450^{o}C) results in surface area of less than 10 m^2 g^{-1}, whereas intermediate temperatures (600-700^{o}C) result in surface area of about 400 m^2 g^{-1} (Fig.2.7). However, the surface areas decrease progressively from 500^{o}C to 800^{o}C due to the decomposition and softening of some volatile fractions to form an intermediate melt in the biochar structure (Fig.2.7) (Lehmann & Joseph, 2009).

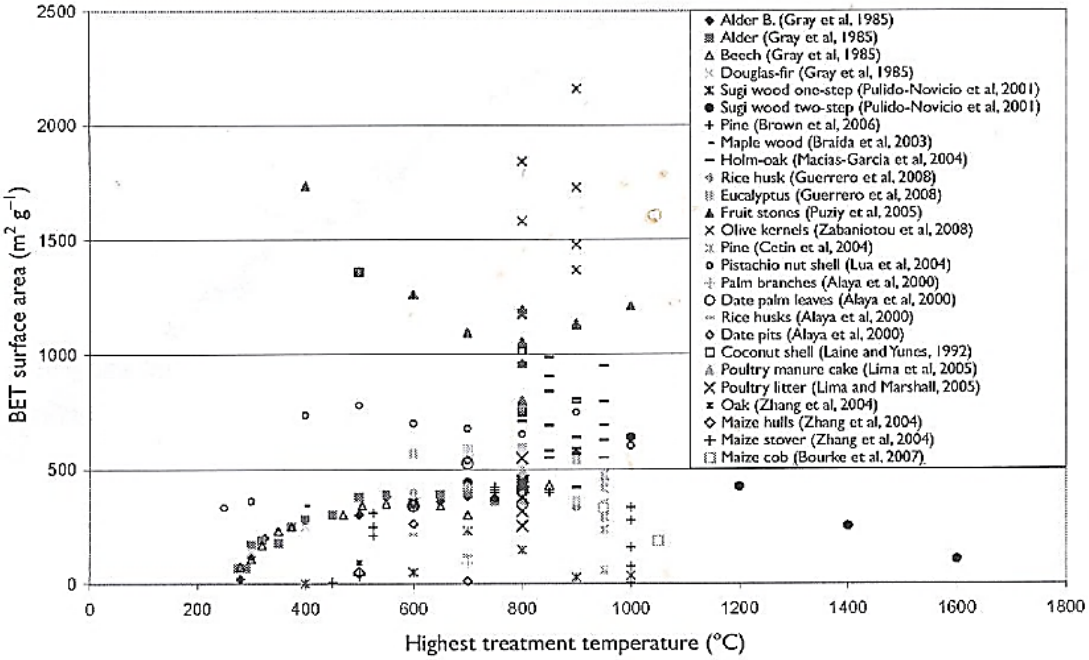


Figure 2.4 Biochar surface area plotted against highest treatment temperature (°C). Source: Retrieved from Downie et al. (2009).

Surfaces area of biochar are generally greater than that of sand (0.01-0.1 m^2g^{-1}) and comparable to or even greater than that of clay (5-750 $m^2 g^{-1}$), which consequently increases the overall soil-specific surface with biochar amendment (Bagreev et al., 2001).

Micro-pores (<2nm) contribute to the surface area of biochars and are responsible for the high retention capacities of molecules of small dimensions such as common solvent and gases, which indicates that biochars with high porosity has greater WHC and will cause an increase of overall soil WHC (Pietikäinen et al., 2000). Tryon (1948) found that biochar addition in sandy soil increased the soil water content due to greater WHC, whereas clay soil would experience a decrease in water content with biochar amended due to the increase of plant biomass and evaporative surfaces. Meso-pores (2-50 nm) in biochar play an important role in liquid-solid adsorption process, and Macro-pores (>50 nm) in biochar are relevant to movement of roots through soil, vital soil function like aeration and hydrology, and also provide habitats for soil microbes (Sohi et al., 2010). High HTT will also enlarge pores of biochar since the walls between adjacent pores are destroyed, which leads to an increase of total pore volume and contributes to improved soil function and soil microbial activities (Zhang et al., 2013). Biochars obtained under different temperatures would result in different surface areas, pore volume, and porous structure, which directly affect the effectiveness of biochar on nutrient leaching reduction.

Pyrolysis heating rate also affect the biochar's effectiveness on nutrient retention in soil. Biochars produced from slow pyrolysis condition (typical heating rates between 1 and 30 °C min^{-1}) (Lua et al., 2004) have different physical properties from those made under fast pyrolysis (typical heating rate greater than 200°C sec^{-1}) (Ronsse et al., 2013). Boateng (2007) reported switchgrass biochars produced under fast pyrolysis condition have low surface areas typically between 7.7 to 7.9 m^2 g^{-1}. Verheijen et al. (2010) stated that slow pyrolysis would be better than fast pyrolysis because it can maximize the yield of biochar and produce most stable of the pyrolysis end products with larger surface area and pore volumes when biochar is used as soil amendment. It should be noted that these results are only directly relevant for their given feedstock and process conditions (Downie et al., 2009).

In short, the higher HTT (<600 ^{o}C) resulting in greater surface area and porosity of biochar although when HTT is higher than 600^{o}C the surface area will decrease. So final charring temperature of 600 ^{o}C results in highest surface area, whereas temperature that is lower 400^{o}C results in biochar with more available N for plant uptake than higher temperature. Moreover, the slow pyrolysis produces biochar with higher surface area and pore volume than fast pyrolysis, which is more stable when used as soil amendment.

Biochar produced from different biomass materials can affect its physical properties. Biochar from wood is likely to have larger surface area and more meso- and macor-pores than other biochars. Lehmann & Joseph (2009) reported Oak, maize hull and maize stover biochars different surface areas of 92m^{2} g^{-1}, 48m^{2} g^{-1}, and 38m^{2} g^{-1}, and total pore volumes of 0.1458 cm^{3} g^{-1}, 0.0581cm^{3} g^{-1}, and 0.0538cm^{3} g^{-1}respectively. However, biochar with higher ash content like manure will experience an increase of porosity as the ash is leached out over time, hence the nutrient retention ability and WHC will increase over time (Downie et al., 2009). Tryon (1948) also found biochar with high ash content caused a greater increase in pH of soil. Furthermore, application of biochar can increase the aggregate stability of soil due to the presence of humic substances in biochar, hence decreasing soil bulk density. Above all, wood biochars produce the best pore structure and manure biochars can be used not only as soil amendment but also as soil fertilizers. Therefore, the investigation of different effects of wood biochar and manure biochar on nutrient leaching reduction is important to obtain the optimum parent materials of biochar.

2.3.1.2 Chemical Properties

Chemical properties of biochar such as cation exchange capacity (CEC) are important on nutrient availability in soil, which depend on the feedstock properties, pyrolysis conditions and residence time of biochar in soil.

Chan & Xu (2009) found that the composition and charge properties of biochar will change during the charring process, implying a progressive decrease of hydroxyl and methyl groups with increase of HTT as indicated with decreased H/C and O/C ratios, and less amounts of acid-basic surface functional groups.

In general, biochar derived from 500 ^{o}C to 700 ^{o}C is well carbonized, which has lower H/C ratios and low O content (<10%) than that obtained at temperatures of 300^{o}C to 400^{o}C because the carbonization is partial (Hammes & Schmidt, 2009). Higher charring temperature (<600 ^{o}C) results in higher CEC, which indicates more cations like NH_4^+-N are retained in soil for plant uptake, whereas once temperatures are higher than 600 ^{o}C the CEC will decline (Lehmann, 2007). Liang et al. (2006) also reported that once biochar is amended in soil over time (‘aged biochar’) the CEC will be higher because more negative charges and charged organic matter are on the surface of biochar and oxidation reactions occur, which are promoted by microbial activity. Compared with aged biochar, newly applied biochar may also absorb anions. CEC and AEC of biochars are most likely to be determined by overall soil pH, age and weathering environment of biochar, which highlights the importance of incubation of biochar and soil during both laboratory or outdoor experiment (Lehmann & Joseph, 2009).

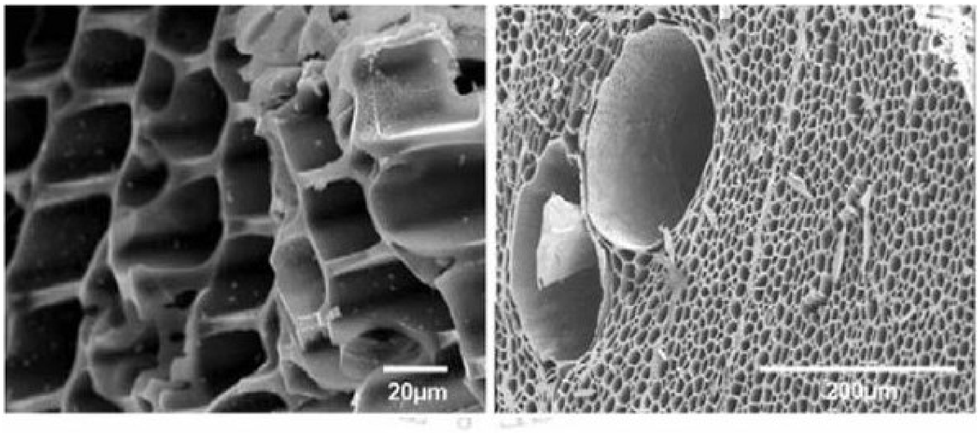
Slow pyrolysis involves longer heating time than fast pyrolysis, which results in biochar with higher carbon retention capacity as proved by Bruun (2011) who found that carbon components were fully retained in the topsoil amended with slow pyrolyed biochar but a potential risk of C leaching on sandy soil amended with fast pyrolysis biochar. This again indicates that biochar produced under slow pyrolysis can be more effective on nutrient retention than that from fast pyrolysis. The nutrient content of biochar is mainly determined by the nature of parent materials. Biochar produced from woody biomass has low ash contents of less than 1%, while manure-based biochar contains high mineral ash contents of up to 15%, which can supply considerable amounts of nutrients to crop or plants (Bruun, 2011).

Because of different physical and chemical properties of biochars may affect their nutrient holding capability, there is a need to study the effectiveness of biochars obtained from varying materials and under different pyrolysis conditions on nutrient leaching reduction.

2.3.1.3 Biological Properties

In soil, food web is the complex mixture of bacteria, fungi, protozoa, nematodes and micro-arthropods, which control the nutrients cycling within an ecosystem (Scott et al., 2002).

The application of biochar in soil can greatly improve the soil microbial activity by providing a suitable habitat for the colonization, growth and reproduction of soil microbes especially for bacteria, actinomycetes and arbuscular mycorrhizal fungi (Warnock et al., 2007). This attributes to the porous structure (Fig.2.8) and high internal surface area of biochar, and its ability to adsorb soluble organic matter, gases and inorganic nutrients (Thies & Rillig, 2009).



20µm 200µm

Figure 2.5 The porous structure of biochar invites microbial colonization. Source: Retrieved from Lehmann & Joseph (2009).

In Nova Scotia, the infertile agricultural soils are characterized by an excess of fungi relative to bacteria (Fungi: Bacterial >1), indicating the acidity of soils because fungi are dominant in acidic soil conditions, which can result in the decline of productivity of agricultural soils and availability of certain essential plant nutrients (Scott et al., 2002). It has been found that acidic soils incorporated with biochar show the increase of pH, which significantly increased the population of bacterial since bacterial prefers neural soil environment, and altered soil function by influencing enzyme activities, thus, overall microbial activity (Fierer & Jackson, 2006; Thies & Rillig, 2009).

In short, biochar application in agricultural soil can not only improve soil microbial activity, but also increase soil function and, thus, soil fertility as well as crop growth.

2.3.2 Mechanisms of Biochar on Nitrogen Retention

Several studies found the effectiveness of biochar on reducing NO_3^--N leaching from soils. At present, the physicochemical mechanisms that make biochar able to reduce NO_3^--N leaching are still unclear and literature about biochar effect on N leaching is still scarce (Laird et al., 2010). However, several hypotheses can be proposed (Kameyama et al., 2012; Ventura et al., 2013): (1) decreased water percolation through increased soil water holding capacity (WHC); (2) NO_3^--N adsorption by base functional groups of biochar; (3) microbial immobilization and denitrification of NO_3^--N; (4) increased N uptake from plants; and/or (5) absorption of NH_4^+-N and inhibition of nitrification activity, as a result of reduction in NO_3^--N percolation.

<u>Increased Water Holding Capacity</u>

The reduction of NO_3^--N may attribute to the increase of WHC, which was reported by Kameyama et al. (2012) who found the higher saturated hydraulic conductivity (K_s) from soils amended with 5 and 10% (w/w) bagasse charcoal than that of non-biochar treatment. But no significant difference was found between non-biochar treatment and treatment with 1 and 3% bagasse charcoal respectively (Kameyama et al., 2012). The porous structure of biochar can help soil to hold more waters, which may extend the residence time of NO_3^--N in the root zone of crop, thus increasing the opportunity of NO_3^--N to be adsorbed by crop without being leached out from soil.

NO_3^--N Adsorption by Base Functional Groups

Kondo et al. (2001) suggested that biochar's surface functional properties are affected by pyrolysis temperature of making biochar; lower temperature forms acid functional groups and higher temperatures forms base functional groups. Kameyama et al. (2012) also found that bagasse charcoals formed at high pyrolysis temperature has high pH, which indicated the generation of base functional groups at high temperature. Therefore, the reduction of NO_3^--N leaching may due to the adsorption of NO_3^--N by base function groups of biochar that produced at high temperature. Mukherjee et al. (2011) also stated that the anions such as NO_3^--N may be attracted by means of bridge-bonding with divalent cations such as Ca^{2+}, Mg^{2+} or other metals like Al^{3+} or Fe^{3+}.

Microbial Immobilization and Denitrification of NO_3^--N

In addition, biochar can also reduce NO_3^--N leaching through net immobilization of inorganic N already present in the soil solution or applied as fertilizer, which might result from the decomposition occurred in soil when fresh biochar is incorporated with soil (Liang et al., 2006). During the immobilization process, a temporary reservoir of organic N was built, which could reduce the potential of nitrate leaching in highly leached soil (Steiner et al., 2007). Furthermore, lower NO_3^--N leaching can also attribute to the denitrification loss of NO_3^--N being stimulated by the additional carbon (Clough et al., 2013), which indicates that NO_3^--N loss can be in other form such as nitrogen gas through denitrification.

Adsorption of NH_4^+-N or Inhibition of Nitrification

Because biochar addition can help soil to increase soil organic matter (SOM), thus increasing negatively charged surface sites of soil (Alberta Agriculture and Food, 2008). This indicates the higher NH_4^+-N adsorption by biochar's negatively charged sites and lower nitrification process or lower available NH_4^+-N to be nitrified into NO_3^--N (Berglund et al., 2004; Taghizadeh-Toosi et al., 2011). So, biochar can reduce NO_3^--N leaching through enhancing NH_4^+-N adsorption and inhibiting the nitrification of NH_4^+-N to NO_3^--N.

Unlike NO_3^--N, NH_4^+-N is the positively charged ion (cation), which is usually adsorbed in the negatively charged site of soil such as SOM or clay content. NH_4^+-N leaching reduction can attribute to increase of the negatively charged clays and soil organic matter (Lehmann & Joseph, 2009). NH_4^+-N is the positively charged nutrient (cation), which is primarily adsorbed by negatively charged biochar incorporated soil through an electrostatic attraction process (Zheng et al., 2010).

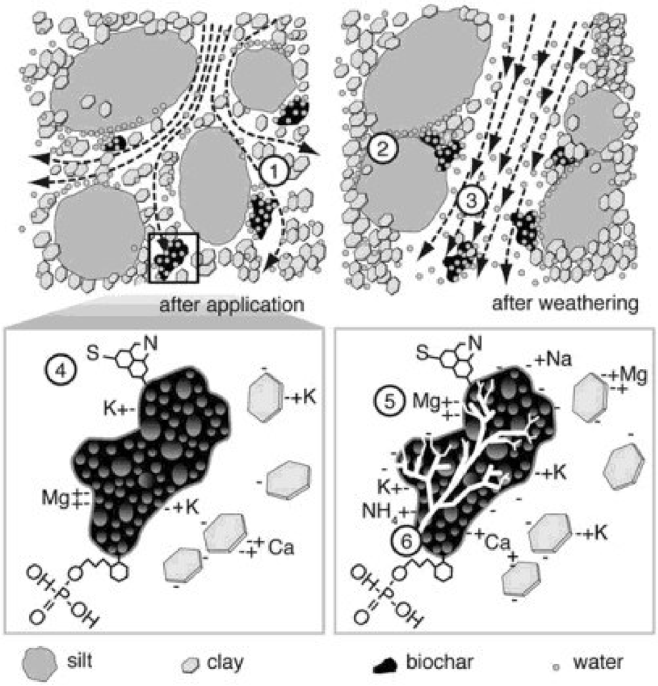


Figure 2.6 Schematic representation of biochar effects on nutrient leaching. Source: Retrieved from Lehmann & Joseph (2009).

After application of biochar in soil, the surface charge of soil will be increased due to the increase of soil organic matter and clay content, thus improving CEC (Liang et al., 2006). The schematic representation of biochar on reducing nutrients leaching is shown in Fig.2.9, which addresses the increase of soil WHC and CEC after application of biochar, attributing to the improvement of soil biota and nutrients retention. Moreover, biochar amendment improves the soil aggregation over time because of the binding between biochar and the other soil constituents, and the presence of preferential flow of water as well as the facilitate transport of biochar particles (Major et al., 2009). In addition, biochar applied in soil will increase the pH of soil, which can provide more negatively charged sites of soil such as SOM and clay contents, thus improving CEC (Jones & Jacobsen, 2005[b]).

2.4 Biochar Critique and Uncertainties

Biochar is not only beneficial to soil but also to the atmosphere. It can capture the carbon in the soil and reduce the carbon dioxide (CO_2) and other greenhouse gas emissions such as methane (CH_4) and nitrous oxide (N_2O) (Van Zwieten et al., 2009). As an emerging soil amendment technology (Dickinson et al., 2015), however, application of biochar into farming practices has some challenges, uncertainties, and risks (Kulyk, 2012). The physical properties of biochar affect the application method of biochar, which includes density, fineness ('dustiness') and fire hazard and health risks.

Of a wide range of soil additives, biochar probably has the lowest density (Blackwell et al., 2009). Spontaneous combustion can occur if a significant amount of biochar dust accumulates in an enclosed space, or if biochar contains a big amount of volatiles (Werther et al., 2000). This risk can be reduced through densification and application of water or fire retardants (Kulyk, 2012). It also need to be concerned while storing, transporting and applying biochar since biochar particles can be easily flown around by wind due to its dustiness (Major., 2009[a]). Besides, some of biochar such as rice husks biochar can contain crystalline material (cristobalite and tridymite) that is toxic (Ibrahim & Helmy, 1981; Stowell & Tubb, 2003). Hence, the use and production of such biochar, the quality control need to be ensured, and the health and safety precautions during handling and application to soil must be employed (Blackwell et al., 2009). Safety recommendations can only be very general because biochar properties varied according to parent materials and pyrolysis procedure (Blackwell et al., 2009).

In Nova Scotia, the leaching of N especially nitrate from soil into surface and ground water due to the use of fertilizers has become an issue. Biochar application to soils has gained interest as a soil amendment on reducing N leaching. However, using biochar have some uncertainties due to the contradictory findings. The significant decrease of nutrient leaching was reported, which suggested that using biochar could effectively help the soil to retain nutrients (Lehmann et al., 2003; Steiner et al., 2007; Steiner et al., 2008; Laird et al., 2010). While some of researches found using biochar could even accelerate the leaching of nutrients or has no effect on nutrient leaching reduction (Lehmann et al., 2003; Hyland et al., 2010; Bruun, 2011). Besides, the mechanisms responsible for the effects of biochar on N cycling and N availability is still poorly understood. The effect of biochar on N leaching is varied due to the physio-chemical properties of different types of biochar and the different pyrolysis conditions of producing biochar. To gain better insight into the effect of biochar derived from various biomass and at different pyrolysis conditions on N leaching, a laboratory experiment using column leaching tests will be conducted.

CHAPTER 3: DESIGN OF EXPERIMENT

This experiment was to determine whether using biochar as a soil amendment on Nova Scotian soils would reduce nitrate and ammonium leaching. This experiment was also to investigate the effect of biochar, which was produced from different parent materials and at different pyrolysis temperature and applied at different rates, on soil nitrate and ammonium leaching. A Nova Scotian soil and four types of biochars were tested in this experiment.

3.1 Description of Soil & Biochar

Surface soil (0-15cm) was collected from a field at the Dalhousie Bio-environmental Engineering Centre (BEEC) (45°40'N, 62°50'W), Truro, Nova Scotia where the Tormentine soil (Orthic Humo-Ferric Podzol) was present (Environment Canada, 2013). Soil samples were transferred immediately after collection and processed according to the following procedures. All plant residues such as roots or litter and macro fauna such as earthworm were removed. Then soil samples were grounded to pass through a 2 mm sieve to remove large size of soil particles. The soil nutrient content (e.g. nitrate and ammonium) and the CEC as well as SOM of soil were analyzed by a commercial laboratory.

Four types of biochar were used, which were produced from: 1) mixed hardwood (such as oak) (>600 ^{o}C); 2) wood waste (mainly from shipping pallets and construction)

(>600 °C); 3) poultry manure produced at medium temperature (400 °C to 600 °C); and 4) poultry manure produced at high temperature (>600°C) (Fig. 3.1). All tested biochars were obtained from commercial suppliers. Before adding the tested biochar into the soil samples, biochars were firstly grounded by a mixer into smaller particles (<0.5mm) because particle size of biochar could affect soil differently. Samples of biochars were sent to commercial laboratory to analyze basic properties such as nutrient content (nitrate and ammonium).

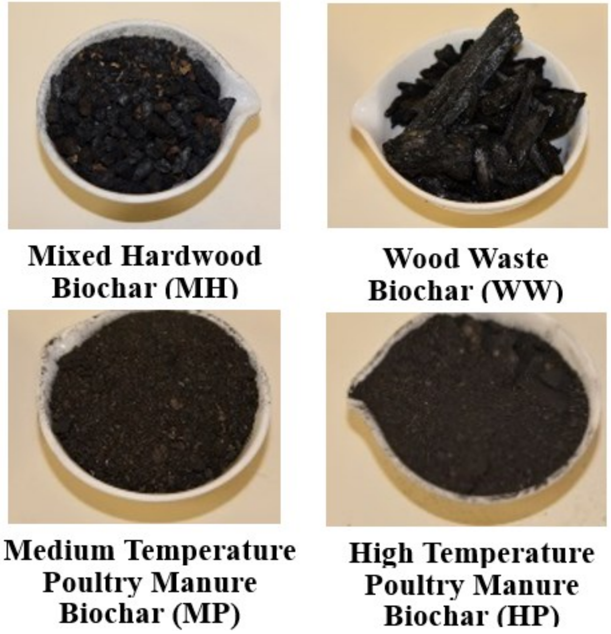


Figure 3.1 Picture of four biochars used in the experiment.

3.2 Experimental Procedure

3.2.1 Preparation of Soil Columns

Soil columns were made of polyvinyl chloride (PVC) tube (length: 25 cm; internal diameter: 7.7cm, volume=1164cm^3). Before adding the soil mixture, a cheese cloth and 100 g of coarse sand (2-5 mm) were placed at the bottom of each column (Fig. 3.2) to create a free drainage and to prevent soil loss. The bottom of each column was covered with a cap with a drilled hole at the center of cap. A 3 mm diameter of drainage tube was inserted into the hole at the end of each column for leachate collection (Fig. 3.2).

One kg of oven-dried weight soil (or 1.3 kg of field-moist soil) was added into the column along with biochar at application rate of 0, 5 or 10 g kg^{-1} of soil (oven-dried weight). 1 kg of oven-dried weight soil was equivalent to about 1.3 kg of field-moist soil according to its moisture content (23.13 %). Before adding the soil mixture into the column, the biochar was placed evenly on the surface of the soil, and a shovel was used to stir the soil mixture evenly for 20 times. Then a plastic funnel was used to add the soil mixture into the column in small portions to prevent the soil losses while adding into the column and to obtain the uniform packing. This can also help to ensure all columns received the relatively uniform height (22-23 cm) of soil mixture to reduce experimental uncertainty.

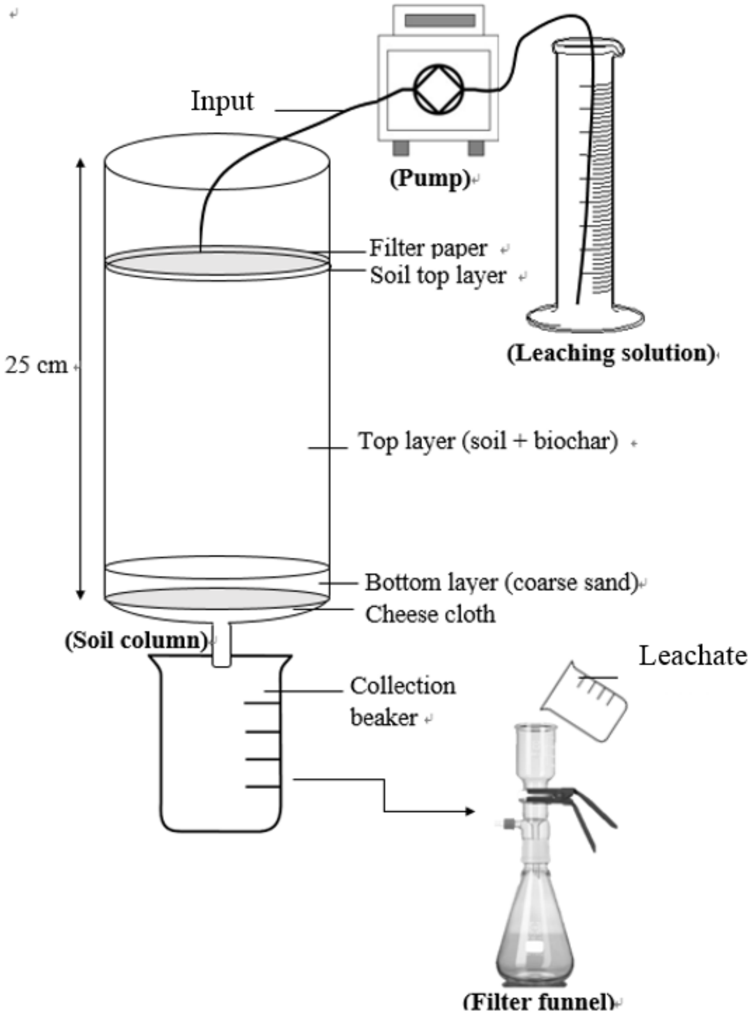


Figure 3.2 Schematic diagram of leaching experiment.

The top of soil layer covered by a filter paper to distribute water evenly over the surface and avoid disturbance of soil surface by the “rain” drops (Fig. 3.3). A plastic film cover was placed at each column to avoid evaporation during the experiment and to secure the tube at the top of column. There were 27 soil columns in this experiment, including 8 biochar treatments and 1 treatment without adding biochar (T_0), and each of treatment was triplicated (Table 3.1). T_{5MH} and T_{10MH} refer to treatment received 5 and 10 g mixed hardwood biochar per kg of soil respectively. T_{5WW} and T_{10WW} refer to treatment received 5 and 10 g wood waste biochar per kg of soil respectively. T_{5MP} and T_{10MP} stand for treatment received 5 and 10 g poultry manure biochar produced at medium temperature per kg of soil respectively. T_{5HP} and T_{10HP} refer to treatment received 5 and 10 g poultry manure biochar produced at high temperature per kg of soil respectively.

Table 3.1 List of experimental treatments and combinations of soil columns.

Treatment	Biochar Application Rate (g kg^{-1} of soil)	Parent Material of Biochar	Replicates	Column #
T_0	0	/	3	1, 2, 3
T_{5MH}	5	Mixed Hardwood (MH)	3	4, 5, 6
T_{10MH}	10		3	7, 8, 9
T_{5WW}	5	Wood Waste (WW)	3	10, 11, 12
T_{10WW}	10		3	13, 14, 15
T_{5MP}	5	Medium Temperature Poultry Manure (MP)	3	16, 17, 18
T_{10MP}	10		3	19, 20, 21
T_{5HP}	5	High Temperature Poultry Manure (HP)	3	22, 23, 24
T_{10HP}	10		3	25, 26, 27

Figure 3.3 Picture showing columns filled with soil and biochar.

3.2.2 Weekly Leaching Experiment

After packing, all soil columns were saturated by distilled water once a day for 2 days. Leachates were collected and recorded every day until the amount of leachates received from each column was constant. Saturation removes air bubbles entrapped during the packing process and to obtain uniformity of moisture in the columns before experiment.

All columns were held at room temperature during the study period. Leaching events were conducted weekly by adding 300 mL of 0.001 M $CaCl_2$ to the surface of soil drop-wise with the aid of pump (Fig. 3.4) for approximately 1 h for all columns. Using diluted $CaCl_2$ helps to preserve the soil structure and the weekly addition of calcium accelerates leaching of other elements from the columns. The first 5 weeks called ‘incubation period’ observed stabilization of NO_3^--N concentrations in the leachate of the control treatment, which suggested that the microbial population had adjusted to the new environment (Laird et al., 2010). On week 6 and 22, soil columns were 'fertilized' with 300 mL of $NaNO_3$ and NH_4Cl solutions respectively, containing 20.25 mg of NO_3^--N and 12.69 mg of NH_4^+-N. This was to observe the effects of 'nitrate-fertilizer' and 'ammonium fertilizer' on the leaching of NO_3^--N and NH_4^+-N from soil incorporated with and without biochar over times.

Figure 3.4 Leaching of 300 mL of 0.001 M $CaCl_2$ solution using pumps.

The weekly leaching events simulated a 1h, 67 mm rainfall event. Leachate from each column was collected until flow stops. Leachate samples were filtrated through the 0.45 μm filter (Fig. 3.2), and the volume of each sample was recorded. The concentration of NO_3^--N and NH_4^+-N in the leachate samples were measured by using a UV-Vis spectrophotometer (*HACH DR-6000*).

3.2.3 Tests of Nitrate and Ammonium

Nitrate-N (NO_3^--N) in the leachates were determined using a Standard Operation Method #8039 by DR6000 Spectrophotometer of HACH called '*Cadmium Reduction Method'* (Table 3.2). To test NO_3^--N, a reagent containing cadmium metal was used to firstly reduce NO_3^--N in the leachate sample to nitrite (NO_2^--N). The nitrite ion reacts with sulphanilic acid in an acidic medium to form an intermediate diazonium salt. Meanwhile, the salt couples with gentisic acid to form an amber colored solution. Ideally, the more nitrate present in the sample, the darker amber color will be shown. Finally, the nitrate is measured under wavelength of 500 nm for spectrophotometer.

The Standard Operation Method 10031 and 10023 by HACH were used respectively to different range of NH_4^+-N in the samples. The detailed information of these testing methods including detection range are shown in Table 3.2. NH_4^+-N present in the sample couples with chlorine in the reagent to form monochloramine. Then salicylate in the reagent reacts with monochloramine to form 5-aminosalicylate. Meanwhile, the 5-aminosalicylate is oxidized after addition of cyanurate to form a blue colored compound. After 20 minutes of reaction time, the blue color turns to yellow color from the excess reagent present and to form a green-colored solution. NH_4^+-N is measured under wavelength of 655 nm using the DR6000 Spectrophotometer.

Table 3.2 Specific information of nutrients test method.

Nutrient	**Method**	**Measurement Range**	**Standard**	**Precision (95% Confidence Interval)**	**Sensitivity Concentration change per 0.010 Abs change**	**Measurement Wavelength**
NO_3^--N	Cadmium Reduction Method	0.3-30.0 mg/L NO_3^--N	10 mg/L NO_3^--N	9.3-10.7 mg/L NO_3^--N	0.3 mg/L at 0 ppm, 0.5 mg/L at 10 ppm, 0.8 mg/L at 30 ppm NO_3^--N	500 nm
NH_4^+-N High Range (HR)	Salicylate Method for HR	0.4-50 mg/L NH_3^+-N	40.00 mg/L NH_3^+-N	38.1-41.9 mg/L NH_3^+-N	0.312 mg/L NH_3^+-N	655 nm
NH_4^+-N Low Range (LR)	Salicylate Method for LR	0.02-2.50 mg/L NH_3^+-N	1.00 mg/L NH_3^+-N	0.90-1.10 mg/L NH_3^+-N	0.014 mg/L NH_3^+-N	655 nm

The method used in both tests were technique-sensitive, which means that shaking time and technique will affect the color development. Therefore, before running tests for samples, a test of a standard solution (e.g. 1 mg/L of NO_3^--N) that was within the test range was always conducted several times to ensure accurate results. Each sample was tested twice to ensure the accuracy of measurement.

3.3 Statistical Analysis

This experiment was designed as a repeated measures experiment, which is a type of factorial experiment, with treatment and time as the two factors. In this experiment, we aimed to analyze the response (the mass of nitrate or ammonium in the leachate) trends over times, and compare responses from different treatments over times. The comparison of times within a treatment was also examined. For example, the nitrate leaching from the same treatment might be significantly different at different time points, which can help to explain and demonstrate the change of nitrate leaching during a long time period of leaching experiment.

The statistical analysis method called Repeated Measures Analysis (RMA) was used. RMA is defined as the measurement of responses that from the same experimental units, which are correlated especially in close time point (Littell et al., 1998). *Proc Mixed Model* Method was tested by SAS, which is one of the methods of RMA, to examine and compare response trends over times. Least Square Means (LSM) test was used to distinguish significant differences among treatment means. All samples in this experiment were run in random order in order to minimize the bias and to balance out effects of “lurking” variables. Normality of data distribution was also run by SAS. RMA only can measure maximum of 15 time points, therefore, this test reduced the number of time points to 14. For example, measuring the values at odd week, and measuring the values at even week.

CHAPTER 4: RESULTS & DISCUSSIONS

4.1 Laboratory Analysis of Soil and Biochar

4.1.1 Nutrients in Biochar

Soil and biochar nutrients tests were conducted by the Analytical Lab of Nova Scotia Department of Agriculture. The nutrients content such as NO_3^--N and NH_4^+-N of four biochar samples (MH, WW, MP and HP) before being applied into soil were tested. The SOM, CEC and the pH of soil samples from all treatments, which were collected before and after 28 weeks of leaching experiment, were also tested.

MP biochar has the highest amount of NO_3^--N and NH_4^+-N compared with the rest of tested biochars, followed by the HP biochar (Table 4.1). The high N content of MP and HP biochar is probably due to the high N content of their parent material (poultry manure) (Joseph & Lehmann, 2009). Adding these amounts of NO_3^--N and NH_4^+-N into soil from biochar could affect the N cycle and N availability of soil, thus influencing the leaching of NO_3^--N and NH_4^+-N in soil. In this situation, biochar can be considered as a nutrient source, which can provide the relatively high amounts of nutrients, especially N into the testing columns. However, excessive amounts of nitrate or ammonium might also increase the leaching nitrate or ammonium during weeks of leaching experiment.

Table 4.1 The mass of nutrient elements of biochar (mg/g) and soil (mg/kg).

Parameter	**pH**	**Unit**	**Elemental composition**						
			NO_3^--N	NH_4^+-N	TN	P	Ca	K	Mg
Mixed Hardwood (MH)	6.1	mg/g	1.4×10^{-3}	0.4	2.5	0.4	5.9	2.4	0.8
Wood Waste (WW)	8.8	mg/g	1.2×10^{-3}	<0.1	1.6	0.1	3.7	1.1	0.7
Medium Temperature Poultry Manure (MP)	8.2	mg/g	2.7	2.1	37.6	28.2	48.3	47.9	13.3
High Temperature Poultry Manure (HP)	9.1	mg/g	0.6	1.1	26.9	42.7	77.0	77.2	20.2
Soil	6.4	mg/kg	168	/	2200	186.7	2289	269.2	309.5

Note: the amount of NH_4^+-N of soil is unavailable because the method for NH_4^+-N testing was based on the sample type being a soil amendment, which couldn't assure the value and accuracy of other sample material using this method for analysis.

4.1.2 Soil Organic Matter & Cation Exchange Capacity

Before the weekly leaching experiment, the addition of fresh MP and HP biochars (un-oxidized biochar) slightly increased the pH of soil (Table 4.2), which might due to the alkalinity and high pH of biochar (Table 4.1). After 28 weeks of leaching experiment, no significant different pH between biochar treatments and control treatment although the pH of MP and HP treatments was slightly higher than control treatment (Table 4.2).

Table 4.2 SOM & CEC of treatments before and after the leaching experiment.

Treatment	**Before Leaching**			**After 28 weeks of leaching**		
	SOM (%)	**CEC (meq/100g)**	**pH**	**SOM (%)**	**CEC (meq/100g)**	**pH**
T_0	3.5	15.8	6.4	3.4	14.6	6.51
T_{5MH}	4.6	17	6.24	3.6	15.0	6.55
T_{10MH}	4.7	17.2	6.25	3.8	14.5	6.57
T_{5WW}	4.2	17.3	6.27	3.4	15.0	6.52
T_{10WW}	4.6	15.4	6.28	3.5	14.7	6.50
T_{5MP}	4.4	18.3	6.48	3.4	14.6	6.66
T_{10MP}	4.4	20.2	6.63	3.5	15.2	6.67
T_{5HP}	4.7	21.3	6.62	3.4	14.8	6.77
T_{10HP}	4.2	22.5	6.74	3.8	15.9	6.92

It has been stated that higher CEC of soil usually due to the higher SOM (Liang et al., 2006), however, no relation was found between SOM and CEC in this experiment by observing the SOM and CEC of biochar treatments before and after leaching.

<u>Before leaching experiment</u>

Before the leaching experiment started, the addition of biochar slightly increased the SOM and CEC of soil except for T_{10WW}, which had the lower CEC (15.4 meq/100g) than T_0 (15.8 meq/100g) for unknown reason. Abedin (2015) found SOM and CEC were higher at soil received 40 tones of biochar per ha (SOM= 4.7%; CEC= 10.5 meq/100g) than soil received 10 tones of biochar per ha (SOM= 3.3%; CEC= 9.9 meq/100g). In this experiment, no positive effect of biochar application rate on SOM and CEC of soil was found before weekly leaching experiment. The SOM of WW biochar treatment increased at higher biochar application rate, whereas the CEC of soil decreased with the increase of biochar application rate. In contrast, T_{10HP} had the lower SOM than T_{5HP}, while the CEC of T_{10HP}

was higher than T_{5HP}. No increase of SOM was observed between T_{5MP} and T_{10MP}, but the CEC of soil in MP biochar treatment increased when MP biochar application rate increased from 5 to 10 g per kg of soil. Only MH biochar treatment was found to have higher SOM and CEC of soil with the increase of MH biochar application rate. Soil pH of MH and WW biochar treatments was lower than T_0 before leaching, whereas MP and HP biochar treatments had higher soil pH than T_0.

After leaching experiment

In general, the addition of biochar slightly increased the CEC of soil after 28 weeks of leaching except for T_{10MH}, which had the lower CEC (14.5 meq/100g) than T_0 (14.6 meq/100g) (Table 4.2). Most of biochar treatments had the higher SOM than T_0 except for T_{5WW}, T_{5MP} and T_{5HP}, which had the same SOM as T_0 (Table 4.2). After 28 weeks of leaching experiment, SOM of all treatments was lower than SOM before leaching and SOM of biochar treatments decreased much more than T_0, which could due to the increased mineralization of SOM by increased of soil microbial activity with addition of biochar (Hammes & Schmidt, 2009). In general, the SOM of biochar treatments improved with the increase of biochar application rate, at the same time, CEC increased with the increase of SOM except for MH and WW biochar treatment, which had the lower CEC at higher biochar application rate. All treatments had higher soil pH after 28 weeks of leaching than that before leaching, especially MP and HP biochar treatment, which always had much higher soil pH than T_0. The pH of MH and WW biochar treatments was higher than T_0 after

28 weeks of leaching experiment, while T_{10WW} was an exception, which had lower pH than T_0. Liang et al. (2006) stated the CEC of soil increases with the increase of time biochar was applied in the soil due to the oxidation of the surface of biochar and the increase of acid functional groups (Liang et al., 2006). However, this experiment found CEC of all treatments after 28 weeks of leaching experiment was lower than CEC before leaching experiment.

4.2 Nitrogen Leaching Over Weeks

4.2.1 Nitrate-Nitrogen

The statistic test result, obtained by using RMA to test the effect of all biochar treatments on NO_3^--N leaching compared with control treatment after 28 weeks of leaching, is shown in Table 4.3. '*Treatment*' refers to biochar treatment effect (MH, WW, MP and HP) on NO_3^--N leaching compared with control treatment. '*Time*' stands for the effect of timing (experimental weeks) on NO_3^--N leaching (Table 4.3). '*Treatment* × *Time*' stands for the interaction effect between biochar treatments and experimental weeks on NO_3^--N leaching (Table 4.3). In this experiment, the null hypothesis of the test is the biochar treatments had significant different NO_3^--N leaching from control treatment as time progressed. '*P-value*' measures the whether or not the test effect is higher or lower than significance level of the test (α) (usually α= 0.05 or 0.1). If $p < \alpha$ (0.05), the test effect is significant, meaning biochar treatments have significantly different NO_3^--N leaching than control treatment over time. If $0.05 < p < 0.1$, the test effect is marginally significant.

Table 4.3 Treatment × Time effect on nitrate leaching among treatments from week 1 to 28.

Effect	P-value
Treatment	0.0731
Time	<.0001
Treatment × Time	<.0001

In this experiment, NO_3^--N leaching on biochar treatments was marginally significant ($0.05<p<0.1$) lower than T_0 without considering the effect of time (Table 4.3, from Fig. 4.1 to Fig. 4.4). Biochar treatments showed statistically significant (p-value < 0.05) lower NO_3^--N leaching (Table 4.3; from Fig. 4.1 to Fig. 4.4)) than T_0 over time. The interactions between biochar and soil are significant, complex and can drastically modify the chemical and physical characteristics of biochar surfaces and, thus, its interaction with nutrients (DeLuca et al., 2015). These interactions require further study, which should also consider the other impact factors such as the crop management in the field.

Incubation period

In this experiment the incubation period was from week 1 to 5, which observed the leaching of NO_3^--N (mg/L) and NH_4^+-N (mg/L) in the leachates from all treatments. The addition of biochar affected the NO_3^--N leaching during the first 5 weeks prior to addition of $NaNO_3$ solution (Fig. 4.1). NO_3^--N concentration in the leachate from all biochar treatments was fluctuated during the first 5 weeks of leaching experiment (incubation period) (from Fig. 4.1 to Fig. 4.4). Similar finding was observed by Laird et al. (2010), who assumed the microbial populations in the soil were adapting to the new environments during this early period. These results are supported by Joseph et al. (2010) who found newly applied biochar in the soil resulted in the numerous oxidation and adsorption reactions.

Treatments receiving non-biochar (T_0) tended to have the overall higher NO_3^--N (mg/column) (3.4 to 2.3 mg) leaching compared with treatments received 5 g biochar/kg of soil (T_{5MH}) and 10 g biochar/kg of soil (T_{10MH}) of Mixed-Hardwood biochar from week 1 to 5 (Fig 4.1). When MH biochar was freshly added in the soil, significant (p-value < 0.05) lower NO_3^--N leaching than T_0 occurred in MH biochar treatments on week 1 or 2 (Table A1 & Table A2). This is probably because the presence of base functional groups on the biochar's surface could help soil to adsorb negatively charged ions such as NO_3^- when biochar was freshly applied in soil (Amonette & Joseph, 2009). Besides, MH biochar in this experiment was reported as an N-depleted material, which could induce net immobilization of inorganic N already present in the soil solution (DeLuca et al., 2015) then reduce NO_3^--N leaching when MH biochar was freshly added into soil (Liang et al., 2006). Nelissen et al. (2012) also found soil experienced an increased short-term immobilization of NH_4^+-N after biochar application, indicating a decreased nitrification of NH_4^+-N to NO_3^--N and a reduced NO_3^--N leaching potential. During the following weeks of incubation (week 3-5), no significant difference was found between T_0 and MH biochar treatments.

Similar finding was observed in the treatment received Wood Waste biochar at application rate of 5g (T_{5WW}) and 10 g (T_{10WW}) per kg of soil respectively. At week of 1, T_{5WW} experienced a significant lower (with a p-value < 0.05) NO_3^--N leaching (Table A3) than T_0 but no significant difference was found during the following weeks of incubation (week 2-5) (Fig. 4.2). T_{10WW} also had a lower NO_3^--N leaching (Fig. 4.2) at first 2 weeks of incubation compared with T_0 even though the difference was not statistically significant (p-value <0.05) (Table A4).

Poultry manures produced at medium temperature (400 ^oC – 600 ^oC) (MP) contained about 2.7 mg NO_3^--N per g of biochar of which was about 4.5 time higher than the biochar produced at high temperature (>600 ^oC) (HP) (Table 4.1). Shinogi (2004) also found a decreased total N (%) of sewage sludge biochar from 3.8% at 400 ^oC to 0.94% at 950 ^oC. The loss of total N at higher temperature also accompanied by a change in the chemical structure of the remaining N in the biochar, which causes a reduced N availability present in the biochar (Bagreev et al., 2001; Chan & Xu, 2009).

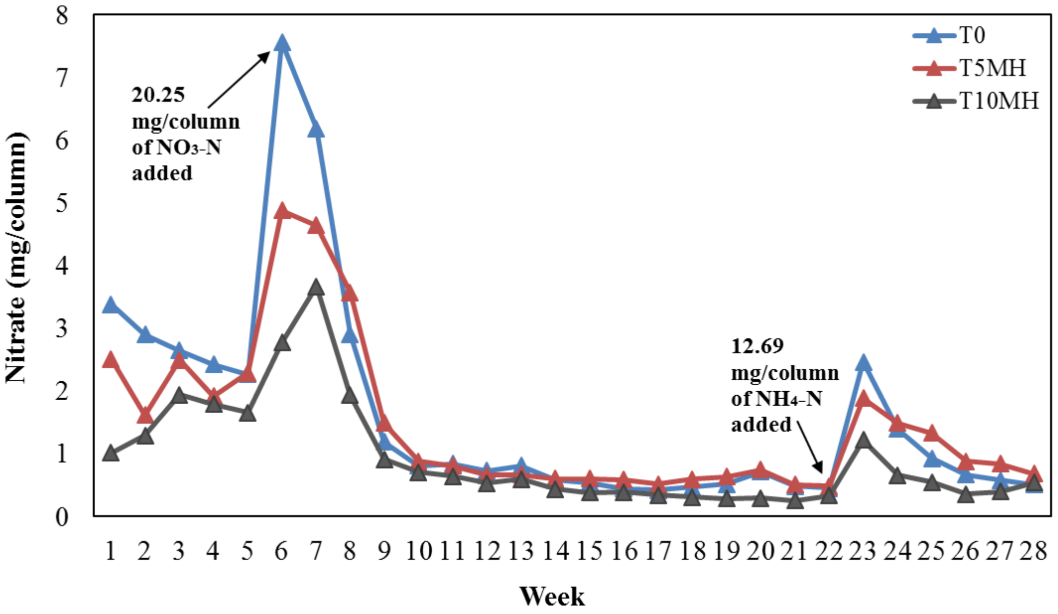


Figure 4.1 Weekly leaching of nitrate (mg/column) from T_0, T_{5MH} and T_{10MH} respectively.

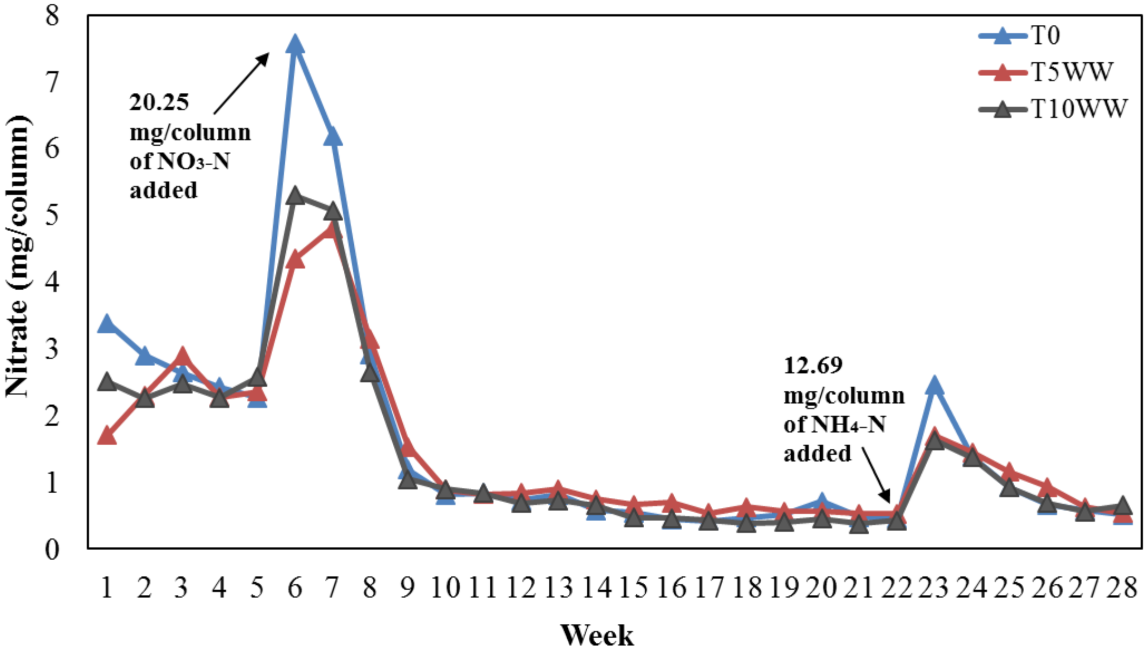


Figure 4.2 Weekly leaching of nitrate (mg/column) from T_0, T_{5WW} and T_{10WW} respectively.

MP biochar treatments prone to have a significant higher (p-value <0.05) NO_3^--N leaching than T_0 at week 1 and 2 (Table A5 & Table A6). This is because the addition of

MP biochar in soil brought additional N into the soil column, which in turns increased the potential NO_3^--N leaching during the first 2 weeks of incubation. As the application rate of MP biochar increased from 5g to 10g biochar per kg of soil, soil experienced even higher NO_3^--N leaching from week 1 to 3 (Fig. 4.3). As time progressed, MP biochar treatment had the lower NO_3^--N leaching than T_0 from week 4 to 5 (Table A6). T_{10MP} even had the lower NO_3^--N leaching than T_{5MP} by week 4 (Fig. 4.3). This probably attributed to the adsorption of NO_3^--N by positively charged sites on the surface of MP biochar (Amonette & Joseph, 2009). NO_3^--N leaching from soil received HP biochar at application rate of either 5g biochar (T_{5HP}) or 10g (T_{10HP}) biochar per kg of soil was fluctuated but decreasing during the first few weeks of incubation (Fig.4.4). During the first 3 weeks, no dramatic difference of NO_3^--N leaching was found between T_0 and HP treatment. As time progressed, there was a clear difference of NO_3^--N leaching between T_0 and HP biochar treatment, where HP biochar treatment had the lower NO_3^--N leaching than T_0 by week 5. MP biochar contained about 4 times higher amount of NO_3^--N than HP biochar, which could explain the much higher leaching of NO_3^--N during the incubation period on MP biochar treatment.

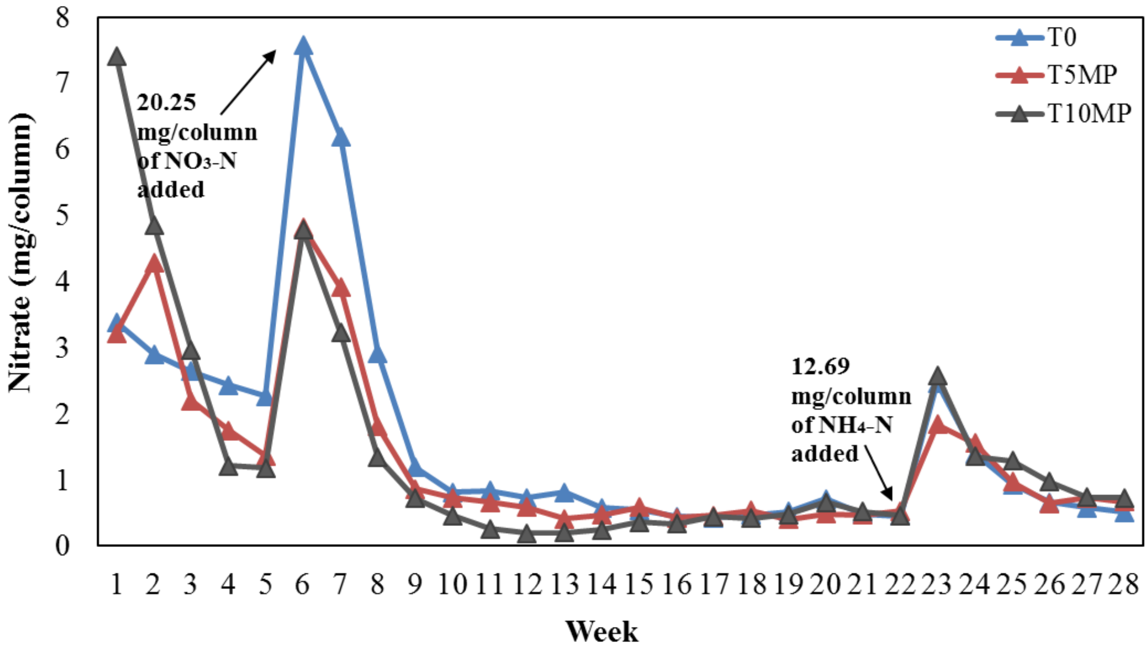


Figure 4.3 Weekly leaching of nitrate (mg/column) from T_0, T_{5MP} and T_{10MP} respectively.

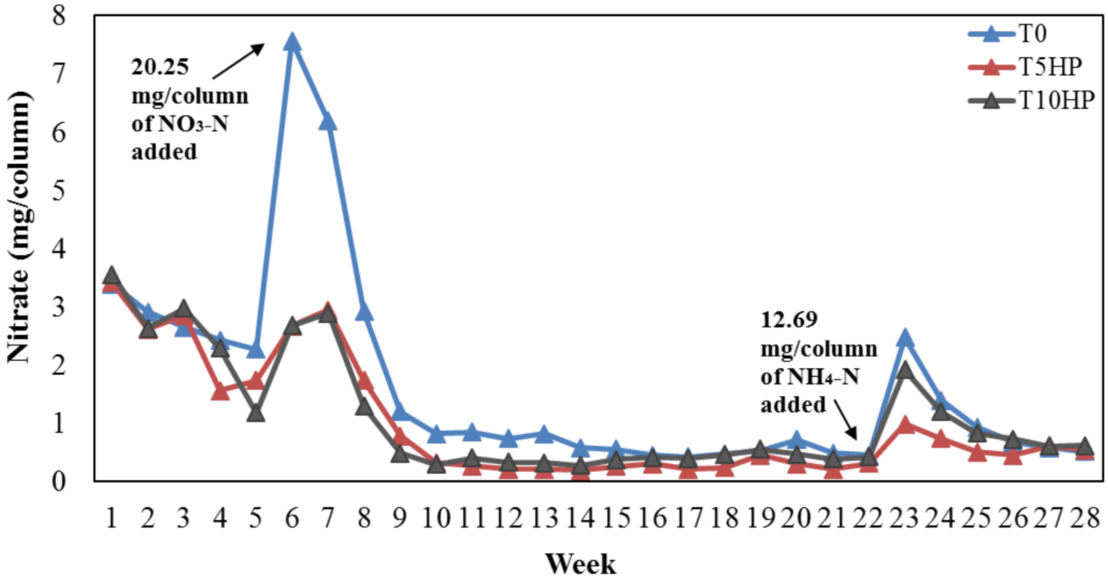


Figure 4.4 Weekly leaching of nitrate (mg/column) from T_0, T_{5HP} and T_{10HP} respectively.

MH and WW biochar treatments had the lower NO_3^--N leaching than T_0 from week 1 to 2, while the difference of NO_3^--N leaching between T_0 and two biochar treatments (MH and WW) reduced as time progressed by week 5 (from Fig. 4.1 to Fig. 4.2). On the first 3 weeks of incubation, no dramatic difference of NO_3^--N leaching was found between HP biochar treatment and T_0 (Fig. 4.4) due to the high NO_3^--N content of HP biochar (Table 4.1). While the difference was getting clear and HP biochar treatment had the lower NO_3^--N leaching than T_0 from week 4 to 5 because the NO_3^--N from HP biochar had been leached out as time progressed (Fig. 4.4). MP biochar treatment had the much higher NO_3^--N leaching than T_0 on the first 2 weeks of incubation (Fig. 4.3) because MP biochar brought the highest amounts of NO_3^--N into soil compared with the rest of 3 biochars (Table 4.1). But MP biochar started to work over time when NO_3^--N from MP biochar leached out as time progressed (week 3-5).

The different effects could due to the different physiochemical properties of their original materials such as the different functional groups of different fresh biochar, which could influence the biochar's ability on nutrients retention (Hammes & Schmidt, 2009). The relative concentration of each type of functional group depends upon the initial composition of the biomass and final reaction temperature for example (Elizalde-Gonzalez et al., 2007). Biochar with more acid functional groups such as carboxyl groups, preferring alkaline pH, would increase the CEC of soil and help soil to retain more cations (Hammes & Schmidt, 2009). While biochar with more basic functional groups such as quinine and phenol would help soil to retain more anions (Brennan et al., 2001). So far, no specific research has investigated the functional groups of different types of biochar. In this research, it is the fresh MH or WW biochar contained more basic functional groups than biochar made from poultry manures, which help the soil to adsorb NO_3^--N from leaching during the incubation period. Further research on exploring the functional groups of biochar is warranted.

Weekly leaching after fertilization ($NaNO_3$) (week 6-21)

On week of 6, $NaNO_3$ solution containing 20.25 mg of NO_3^--N per column was added into all treatments, which caused a peak of NO_3^--N leaching for all treatments by week 6 or 7 (from Fig. 4.1 to Fig. 4.4). Laird et al (2010) also found the manure addition on week 12 resulted in a broad NO_3^--N leaching peak and reached a maximum on week 18. In this experiment, all biochars showed the NO_3^--N retention ability after fertilization on week 6. When $NaNO_3$ solution was added into the soil on week 6, a significant lower NO_3^--N leaching than T_0 was observed in all biochar treatments (from Table A1 to Table A8), but different biochar treatments affected NO_3^--N leaching differently.

On week 6, the NO_3^--N leached out from T_0 dramatically increased from 2.27 mg to 7.57 mg. MH biochar treatment experienced a milder increase of NO_3^--N leaching than T_0 from 2.30 mg to 4.88 mg at 5g biochar per kg of soil and from 1.66 mg to 2.78 mg at 10g biochar per kg of soil (Fig. 4.1). Nitrate leaching from WW biochar treatment increased by 1.99 mg at 5g biochar per kg of soil and 2.73 mg at 10g biochar per kg of soil from week 5 to week 6 (Fig. 4.2). NO_3^--N leaching from MP biochar treatment increased right after

$NaNO_3$ addition from 1.36 to 4.82 mg at 5g biochar per kg of soil and 1.18 mg to 4.78 mg at 10g biochar per kg of soil (Fig. 4.3). Compared with the other 3 types of biochars, HP biochar had the lowest NO_3^--N leaching after addition of $NaNO_3$ solution, which was from 1.73 mg to 2.66 mg at 5 g biochar per kg and 1.17 mg to 2.67 mg at 10 g biochar per kg of soil between week of 5 and 6 (Fig. 4.4).

MH biochar treatment had the significant lower NO_3^--N leaching than T_0 at week 6 to 7 (Table A1 & Table A2) when soil received fertilization on week 6. While the difference of NO_3^--N leaching between T_0 and MH biochar treatment reduced from week 7 to 22 as NO_3^--N from $NaNO_3$ solution leached out over times (Fig. 4.1). T_{10MH} always had the lower NO_3^--N leaching than T_0, while T_{5MH} had the slightly higher NO_3^--N leaching than T_0 from week 8 to 9 and week 15 to 20 (Fig. 4.1). This suggested that the higher application rate of MH would help soil to reduce more NO_3^--N from leaching in this experiment. Similar finding was reported by Sika & Hardie (2014), who found the higher application rate of pine wood biochar had a lower cumulative leaching of NO_3^--N from soil. Laird et al. (2010) also found soil amended with higher application rate of mixed hardwood (mainly oak) biochar had the lower total NO_3^--N leaching than soil with biochar at lower rate. Similar to MH biochar, MP biochar treatment had the significant lower NO_3^--N leaching than T_0 from week 6 to 7 (Table A5 & Table A6), while the difference of NO_3^--N leaching between T_0 and WW biochar treatment decreased from week 8 to 22 (Fig. 4.3). T_{10MP} had the lower NO_3^--N leaching than T_{5MP} from week 7 to 18, which indicated soil incorporated with MP biochar at higher application rate would experience the lower NO_3^--N leaching over time in this experiment.

WW biochar treatment had the significant lower ($p < 0.05$) NO_3^--N leaching than T_0 right after fertilization from week 6 to 7 (Table A3 & Table A4), while the difference of NO_3^--N leaching between T_0 and WW biochar treatment reduced and T_{5WW} had higher NO_3^--N leaching than T_0 from week 8 to 9 and week 12 to 18 (Fig. 4.2). The NO_3^--N (mg/column) of T_{10WW} had overlap with T_0 from week 8 to 18, but the NO_3^--N leaching of T_{10WW} was lower than T_0 from week 19 to 21. T_{10WW} had the overall lower NO_3^--N leaching than T_{5WW} although no dramatic difference of NO_3^--N leaching was found between T_{10WW} and T_0. Hyland et al. (2010) reported the NO_3^--N leaching from soil decreased when the application rate of biochar (paper mill waste biochar & corn biochar) increased from 5g to 20 g per kg of soil or from 20 to 70 g per kg of soil. Biochar made from poultry manure and sawdust biochar had the similar result, while more NO_3^--N was leached out when application rate increased from 20 to 70 g per kg of soil (Hyland et al, 2010). This would suggest the future study to increase the biochar application rate (such as 20 g/kg of soil) and to identify whether or not the higher biochar application rate would result in the lower NO_3^--N leaching than the lower application rate.

HP biochar treatment had the significant lower ($p < 0.05$) NO_3^--N leaching than T_0 after fertilization from week 6 to 8 (Table A7 & Table A8), while the difference of NO_3^--

N leaching between HP biochar treatment and T_0 reduced from week 9 to 21 (Fig. 4.4). T_{5HP} always had the lower NO_3^--N leaching than T_0 from week 6 to 21, while T_{10HP} had overlap of NO_3^--N leaching with T_0 from week 16 to 19 (Fig. 4.4). After week 19 the NO_3^--N leaching of T_{10HP} was again lower than T_0 until week 21 (Fig. 4.4). This showed the lower application rate of HP biochar would help soil to reduce more NO_3^--N from leaching over times in this experiment.

The experimental data (from Fig. 4.1 to Fig. 4.4) showed that parent material of biochar did have effects on biochar's ability on NO_3^--N leaching reduction. This will be discussed further in section 4.3. Biochar application along with fertilization would affect NO_3^--N leaching in soil differently from biochar application without fertilization. In this experiment, MH biochar treatment had the overall lowest NO_3^--N leaching than the rest of biochar treatments before fertilization. As time progressed, NO_3^--N from biochar was leached out and the difference of NO_3^--N leaching between T_0 and 4 biochar treatments was reducing. After soil received fertilizer, HP biochar treatment had the overall lowest NO_3^--N leaching than the other 3 biochars and T_0. This would recommend MH biochar as the most effective soil amendment on reducing NO_3^--N leaching if fertilizer isn't need in the future study. While HP biochar will be recommended to be used to reduce NO_3^--N leaching from soil in the future if fertilizer is required. Guo et al. (2014) found soil along with biochar made from cow dung before fertilization had the lower cumulative NO_3^--N leaching than soil without biochar and fertilizer. But once soil was applied with biochar and ‘fertilizer’ (biogas slurry), the cumulative NO_3^--N leaching was higher at biochar treatments with increasing rates of biogas slurry compared with treatment without biochar (Guo et al., 2014). While Lehmann et al. (2002) found soil along with biochar or biochar plus fertilizer both had significantly higher cumulative NO_3^--N leaching than control treatment (no biochar and no fertilizer).

By looking at the overall NO_3^--N leaching after 21 weeks of leaching, the cumulative NO_3^--N leaching of T_0, T_{5MH} and T_{10MH} was about 38.87 mg, 33.26 mg and 22.20 mg respectively (Fig. 4.5). This indicated MH biochar was effective on reducing NO_3^--N leaching on ‘fertilized’ soil after 21 weeks of leaching. Before fertilization (week 1-5), MH biochar treatments had the lower cumulative NO_3^--N leaching than T_0 with the increase of MH biochar application rate. When soil hasn’t received fertilization (from week 1 to 5), T_0 had a slight higher cumulative NO_3^--N leaching than T_{5WW} and T_{10WW} (Fig. 4.6). After fertilization, WW biochar had the much lower cumulative NO_3^--N leaching than T_0 compared with that before fertilization. The cumulative NO_3^--N leaching from T_{5WW} and T_{10WW} by week of 21 was 33.77 mg and 32.98 mg respectively (Fig. 4.6), which was significant (p-value < 0.05) (Table A3 and Table A4) lower than T_0. There was no significant different cumulative NO_3^--N leaching observed between T_0 and MP or T_0 and HP biochar treatments by week 5 except for T_{10MP} (Fig. 4.7 & Fig. 4.8), which had higher cumulative NO_3^--N leaching by week 5 than T_0. While after fertilization (week 6-21), both MP (32.27 mg and 30.43 mg at 5 g and 10 g biochar per kg of soil respectively) and HP

(24.57 mg and 23.39 mg at 5 g and 10 g biochar per kg of soil respectively) biochar treatments had statistically significant (p-value <0.01) lower accumulated NO_3^--N leaching than T_0 (from Table A5 to Table A8).

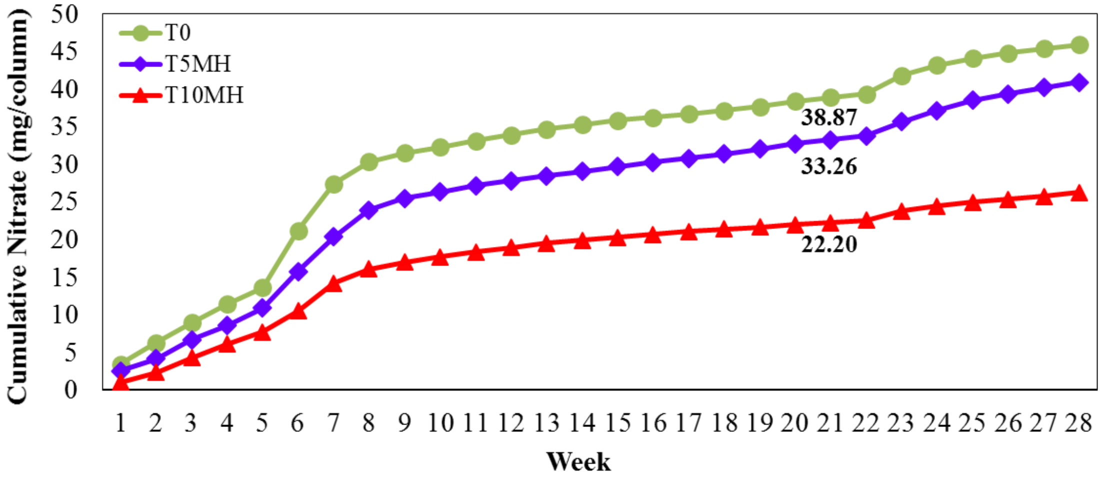


Figure 4.5 Cumulative nitrate (mg/column) leaching from T_0, T_{5MH} and T_{10MH} from 1 to 28 weeks.

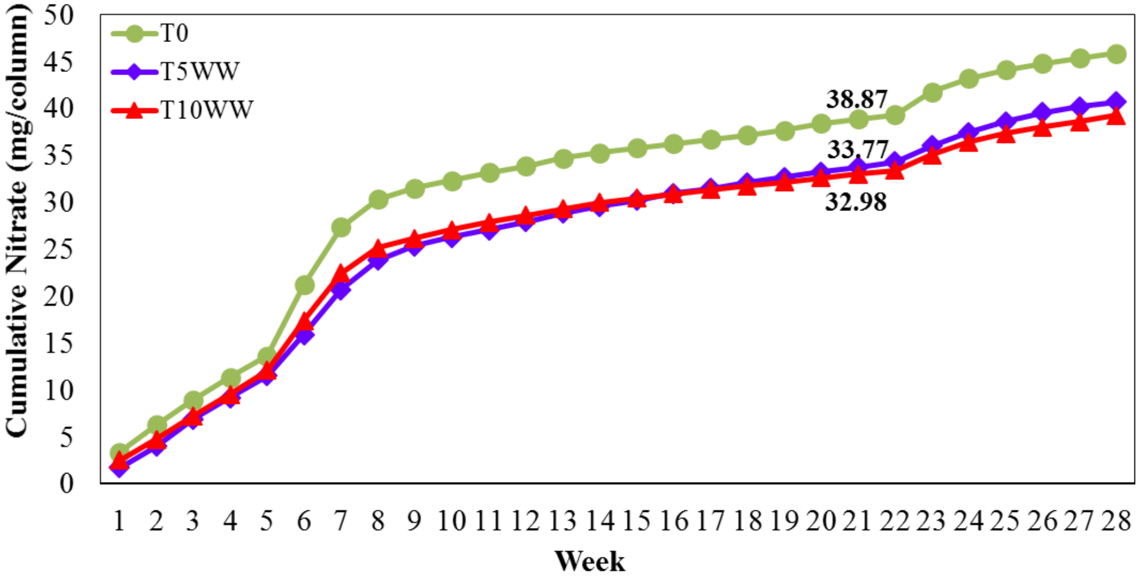


Figure 4.6 Cumulative nitrate (mg/column) leaching from T_0, T_{5WW} and T_{10WW} from 1 to 28 weeks.

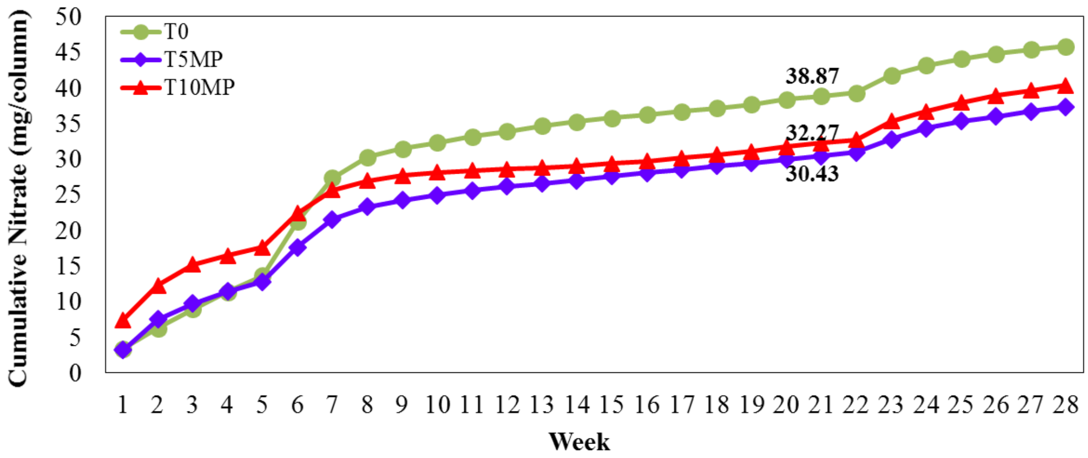


Figure 4.7 Cumulative nitrate (mg/column) leaching from T_0, T_{5MP} and T_{10MP} from 1 to 28 weeks.

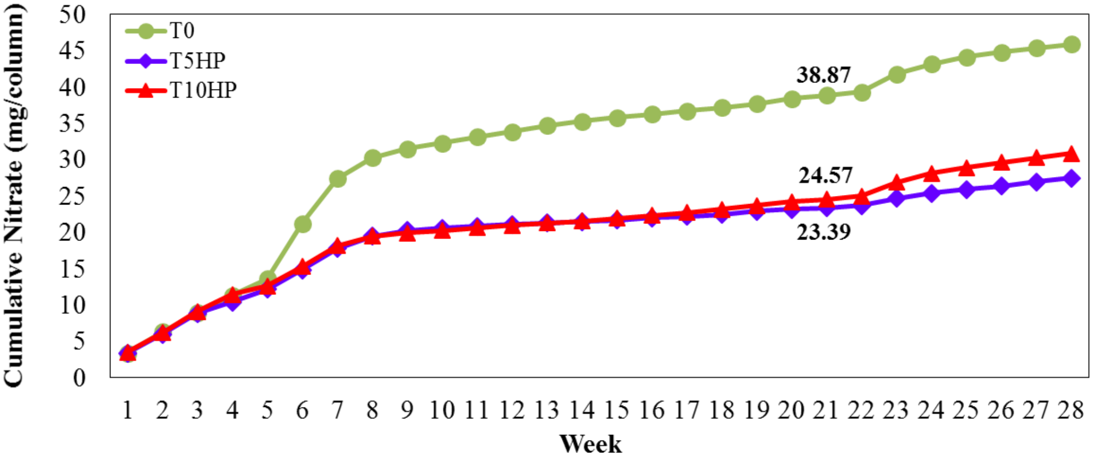


Figure 4.8 Cumulative nitrate (mg/column) leaching from T_0, T_{5HP} and T_{10HP} from 1 to 28 weeks.

Weekly leaching after fertilization (NH_4Cl) (week 22-28)

By week 22, 300 mL of NH_4Cl solution containing 12.69 mg of NH_4^+-N was applied into all treatments. The addition of NH_4^+-N from NH_4Cl solution would affect NO_3^--N in the soil, which resulted in a short-term peak of NO_3^--N leaching at week of 23 (from Fig. 4.1 to Fig. 4.4). Unlike the addition of $NaNO_3$ solution, the peak of NO_3^--N (mg/column) leaching occurred one week later for all treatments, which was probably because NH_4^+-N was partly converted into NO_3^--N during this week.

MH biochar treatment had the lower NO_3^--N leaching than T_0 on week 23, while as time progressed T_{5MH} had higher NO_3^--N leaching than T_0 from week 24 to 28. Unlike T_{5MH}, T_{10MH} always had the lower NO_3^--N leaching than T_0 from week 23 to 28, but the difference of NO_3^--N leaching between T_0 and MH biochar treatment reduced over times. This indicated MH biochar at application rate of 10 g per kg of soil was more effective on reducing NO_3^--N leaching than application rate of 5 g per kg of soil after soil received NH_4Cl fertilizer.

WW biochar treatment showed the lower NO_3^--N leaching on week 23, while no dramatic difference of NO_3^--N leaching was found between T_0 and WW biochar treatment from week 24 to 28. T_{5WW} even had the higher NO_3^--N leaching than T_0 from week 24 to 26, while the difference of NO_3^--N leaching between T_0 and T_{5WW} decreased after week 27.

T_{5MP} had the lower NO_3^--N leaching than T_0 on week 23, whereas T_{10MP} didn’t show the lower NO_3^--N leaching than T_0 after fertilization on week 22. As time progressed, T_{5MP} tended to have the relatively close NO_3^--N leaching than T_0, while T_{10MP} had the higher NO_3^--N leaching than T_0 from week 25 to 27. This indicated that MP biochar at application rate of 10 g per kg of soil was more effective on reducing NO_3^--N leaching than MP biochar at rate of 5 g per kg of soil when soil received NH_4Cl fertilizer.

HP biochar treatments had the lower NO_3^--N leaching than T_0 after fertilization of NH_4Cl (week 23-28). T_{5HP} had the lower NO_3^--N leaching than T_{10HP} from week 23 to 28. So HP biochar at application rate of 5 g per kg of soil would be recommended to be used to reduce NO_3^--N leaching when soil fertilized with ammonium fertilizer.

Comparing the four types of biochars in this research, HP biochar showed the lowest cumulative NO_3^--N leaching than other biochar treatments with increasing leaching times. Lehmann et al. (2002) stated the creation of sites for electrostatic adsorption could be one of the reasons for NO_3^--N adsorption by biochar. However, this usually happens when biochar was freshly applied. With time, the increase of acid functional groups on the surface of biochar (mainly including carboxyl groups, but also phenolic, hydroxyl, carbonyl or quinone C forms) results in an evolution of surface negative charge by replacing surface positive charge of the particles (Cheng et al., 2006). In other words, anion exchange capacity (AEC) of biochar decreases rapidly with oxidation within the soil (Cheng et al., 2008). This research observed the adsorption of NO_3^--N in the first few weeks on some biochar treatments and increased CEC after biochar addition, which could imply the decrease of AEC over times of leaching. The further study on the evolution of functional groups on the surface of biochar is needed to identify the mechanism of NO_3^--N adsorption by biochar through AEC.

Biochar's retention of NO_3^--N could also due to the retention of soil water and therefore nutrients contained in it (Lehmann et al., 2003; Kammann et al., 2011; Dempster et al., 2012). Biochar can increase the residence time for NO_3^--N in the soil solution due to the higher WHC, which may give a higher chance for plant uptake of NO_3^--N (Clough et al., 2013). While the adsorption of NO_3^--N by biochar could be weak since NO_3^--N could be desorbed by water infiltration (Kameyama et al., 2012). In this experiment, no plants were grown in the soil column, which couldn't prove that more NO_3^--N was retained by soil and taken up by plants rather than being leached out due to the increase of soil WHC. Kameyama et al. (2012) did found the increase of soil water content on bagasse biochar amended soil but only when biochar was applied at rate of 50 g and 100 g per kg of soil. There was no significant difference between saturated hydraulic conductivities (K_s) of non-biochar amended soil and that of soils receiving 10 g and 30 g bagasse biochar per kg of soil (Kameyama et al., 2012). The biochar application rate in this experiment was also small (5g and 10 g biochar per kg of soil), therefore, the increase of NO_3^--N retention of soil was not due to the increase of WHC after biochar addition in this experiment. Therefore, it is recommended in the future study to test the effect of biochar on NO_3^--N retention at higher application rate.

Warnock et al. (2007) also indicated that biochar could improve the microbial activity such as the enhancement of mycorrhizal communities in the rhizosphere, which could promote the nutrient uptake such as NO_3^--N by associated plants, hence potentially decreasing leaching. In this research, no evidence would directly show the improved soil microbial activity on biochar treatments. The N cycle is affected by microbial activity, which means the enhancement of microbial activity would affect N transformation. This research found the decreased SOM after 28 weeks of leaching experiment on biochar treatments. This could because biochar improved soil microbial activity, thus increasing the mineralization of SOM. The change of N transformation in soil after biochar addition with time would affect the leaching of NO_3^--N. Future study on exploring the effect of biochar on microbial activity is needed to better understand how the biochar affect NO_3^--N leaching biologically.

Differences in the magnitude of the reduced NO_3^--N leaching among different types of biochar may due to the various physiochemical properties of parent materials used for making biochar and the pyrolysis conditions such as temperature of making biochar (Glaser et al., 1998; Schmidt & Noak, 2000). In this experiment all biochars were produced from different parent materials and pyrolysis conditions such as temperature, which showed the different effects on NO_3^--N leaching over times. MH, WW and HP biochar were produced at high temperature (> 600 ^{o}C), while WW biochar treatment had the higher NO_3^--N leaching than the rest of biochar treatments (from Fig. 4.1 to Fig. 4.4). MP and HP biochar were produced from the same parent material (poultry manure) but at different temperature, which affected NO_3^--N leaching differently. HP biochar treatment had lower NO_3^--N leaching than MP biochar treatment over times (Fig. 4.3 to Fig. 4.4), which indicated the biochar produced from same parent material but at higher temperature could help the soil retain more the NO_3^--N from leaching than that at lower temperature in this experiment.

This could be explained by Kondo et al. (2010) who found biochar produced at higher pyrolysis temperature had more base functional groups, which helped soil to retain more anions such as NO_3^--N, than biochar produced at lower temperature. But this couldn't conclude that biochar produced at lower temperature would always have the lower NO_3^--N retention ability than that at higher temperature because parent material of biochar is the other impact factor. In this experiment, MP biochar was produced at lower temperature than MH and WW biochars, while MP biochar treatment had the lower cumulative NO_3^--N leaching than MH and WW biochar treatment except for T_{10MH} (from Fig. 4.5 to Fig. 4.7). Similarly, Yao et al. (2012) found only biochars (sugarcane bagasse, Peanut hull, Brazilian pepperwood and bamboo) produced at temperature of 600 ^oC showed ability to reduce NO_3^--N leaching compared with biochars produced at temperature of 300 ^oC and 450 ^oC. Hyland (2010) found biochar (70 g/kg of soil) produced from poultry manure mixed with sawdust (300 ^oC) had increased NO_3^--N than non-biochar amended soil. While NO_3^--N leaching was reduced in the biochar treatment for both 300 ^oC oak and 300 ^oC at application rate of 70 g biochar per kg of soil.

The mechanisms that explain NO_3^--N retention by biochar require investigation because this information will probably allow the production of specific biochar for particular uses (e.g. for nutrient management in acid or degraded soil) (DeLuca et al., 2009). Soils in Nova Scotia are naturally acidic and low in organic matter, while the soil samples tested in this research had a neutral pH and relatively high SOM. It is possible that this agricultural soil samples were limed or fertilized before.

In this research, all biochars significantly reduced the leaching of NO_3^--N compared with T_0. HP biochar treatment experienced the lowest cumulative NO_3^--N leaching than either T_0 or the rest of biochar treatments (from Fig. 4.5 to Fig. 4.8). HP biochar helped the soil to reduce higher amount of NO_3^--N from leaching than MP biochar, which suggested the poultry manure produced at high temperature was more effective as the soil amendment on NO_3^--N retention than poultry manure produced at medium temperature. The application rate of biochar did not affect the biochar's effectiveness on NO_3^--N leaching reduction significantly (p-value<0.05) except for the MH biochar and MP biochar. MH biochar or MP biochar at application rate of 10 g biochar/kg of soil had significantly lower cumulative NO_3^--N leaching than biochar at rate of 5 g kg^{-1} soil over times. Inorganic or commercial fertilizers are applied into the soil in order to increase the fertility of soil and to achieve higher crop yield. However, the use of fossil fuel based fertilizers produce the greenhouse gas emissions while making fertilizers such as nitrogen fertilizers (Filiberto & Gaunt, 2013). Biochar has been considered as a potential soil amendment and carbon sequestration medium. Our research has found using biochars would effectively reduce NO_3^--N leaching under fertilized soil ($NaNO_3$ and NH_4Cl solutions). Therefore, application of biochar along with fertilizer in agricultural soils has the potential to reduce NO_3^--N leaching significantly ($p < 0.05$). In addition to other benefits that have been found, such as carbon sequestration.

4.2.2 Ammonium-Nitrogen

In this experiment, the statistic test result, showing the effect of biochar treatments (MH, WW, MP and HP) on NH_4^+-N leaching over time, is shown in Table 4.4. '*Treatment*' refers to biochar treatment effect (MH, WW, MP and HP) on NH_4^+-N leaching compared with control treatment. '*Time*' stands for the effect of timing (experimental weeks) on NH_4^+-N leaching (Table 4.4). '*Treatment* × *Time*' is the interaction effect between biochar treatments and experimental weeks on NH_4^+-N leaching (Table 4.4). In this experiment, the null hypothesis of this test is the biochar treatments had significant different NH_4^+-N leaching from control treatment after 28 weeks of leaching experiment. If $p < \alpha$ (0.05), the test effect is significant, meaning biochar treatments have significantly different NH_4^+-N leaching than control treatment over time. If $0.05 < p < 0.1$, the test effect is marginally significant, indicating biochar treatments have marginal significant different NH_4^+-N leaching than control treatment over time.

Table 4.4 Treatment × Time effect on ammonium leaching from week 1 to 28.

Effect	**P-value**
Treatment	0.2346
Time	<.0001
Treatment × Time	<.0001

Biochar treatment didn't significantly $p < \alpha$ (0.05) affect NH_4^+-N leaching without considering the effect of time (experimental weeks), as indicated by p= 0.234, which is greater than significant level of α=0.05 (Table 4.4). However, all biochar treatments had significantly different NH_4^+-N leaching from control treatment over times (Table 4.4). While different biochar treatment would affect NH_4^+-N leaching differently, which was demonstrated by observing the weekly leaching of NH_4^+-N leaching and cumulative NH_4^+-N leaching over times on all treatments (from Fig. 4.9 to Fig. 4.16).

Incubation period

During the incubation period, treatment received 5 g MH biochar per kg of soil had a significant ($p< 0.05$) higher NH_4^+-N leaching (Table B1) than T_0 on week 1, while the difference of NH_4^+-N leaching between T_{5MH} and T_0 reduced as time progressed (week 2-3) (Fig. 4.9). After week 4, the NH_4^+-N leaching from T_{5MH} was lower than T_0 until week 5 (Fig. 4.9). T_{10MH} had a slight higher NH_4^+-N leaching than T_0 on week 1, while NH_4^+-N leaching from T_{10MH} started to be lower than T_0 from week 2 to 5 (Fig. 4.9 & Table B2). The higher NH_4^+-N leaching observed on MH biochar treatment on week 1 could because MH biochar NH_4^+-N brought about 0.4 mg NH_4^+-N per g of biochar into soil column (Table 4.1). MH biochar at application rate of 10 g per kg of soil had the lower NH_4^+-N leaching than MH biochar at rate of 5 g per kg of soil from week 1 to 4. This could due to the higher application rate of MH contained more acid functional groups, which play important role in NH_4^+-N retention by biochar. But the NH_4^+-N retention by MH biochar wasn't dramatic compared with T_0 from week 1 to 5, which could be explained by Kondo (2010), who found biochar produced at higher pyrolysis temperature has more base functional groups but less acid functional groups.

T_{5WW} had a higher NH_4^+-N leaching than T_0 on week 1 but lower NH_4^+-N leaching from week 2 to 5 (Fig. 4.10 & Table B3). T_{10WW} had lower NH_4^+-N leaching than T_0 during the incubation period (week 1-5) except for an overlap of NH_4^+-N leaching with T_0 on week 3 (Fig. 4.10 & Table B4). The lower NH_4^+-N leaching observed on WW biochar treatment than MH biochar treatment could because WW biochar only contained less than 0.1 mg NH_4^+-N per g of WW biochar, which means only very mall additional NH_4^+-N was brought from WW biochar into soil column. Similar to MH biochar treatment, T_{10WW} had lower NH_4^+-N leaching than T_{5WW} (Fig. 4.10), which could due to more acid functional groups of WW biochar at higher application rate.

During the first week of incubation, addition of MP biochar into soil resulted in a higher NH_4^+-N leaching than T_0, but a slight lower NH_4^+-N leaching than T_0 on week 2 (Fig. 4.12). From week 3 to 5, MP biochar had dramatic higher leaching of NH_4^+-N than T_0 especially when MP biochar was applied at higher rate (10 g per kg of soil) (Fig. 4.12 & Table B5 & Table B6). This was because MP biochar brought about 2.1 mg NH_4^+-N per g of biochar into soil column (Table 4.1). The higher NH_4^+-N leaching on T_{10MP} than T_{5MP} from week 3 to 5 (Fig. 4.12) was because more NH_4^+-N from MP biochar was added into soil column at higher MP biochar application rate.

Similarly, HP biochar treatment received about 1.1 mg NH_4^+-N per HP biochar when HP biochar was freshly added into soil column, which caused the higher NH_4^+-N leaching on week 1 than T_0. The difference of NH_4^+-N leaching between HP biochar treatment and

T_0 reduced from week 2 to 3, but HP biochar started to have higher NH_4^+-N leaching than T_0 from week 4 to 5 during the incubation period. The higher NH_4^+-N leaching on HP biochar treatment during the incubation period (week 1-5) could due to the high amount of NH_4^+-N from HP biochar brought into soil column.

In this experiment, wood-derived biochars (MH and WW) showed the lower NH_4^+-N leaching than manure-derived biochar (MP and HP), which could due to the high NH_4^+-N content of MP and HP biochar. As time progressed, MH and WW did show the ability to reduce NH_4^+-N leaching compared with T_0 from week 4 to 5.

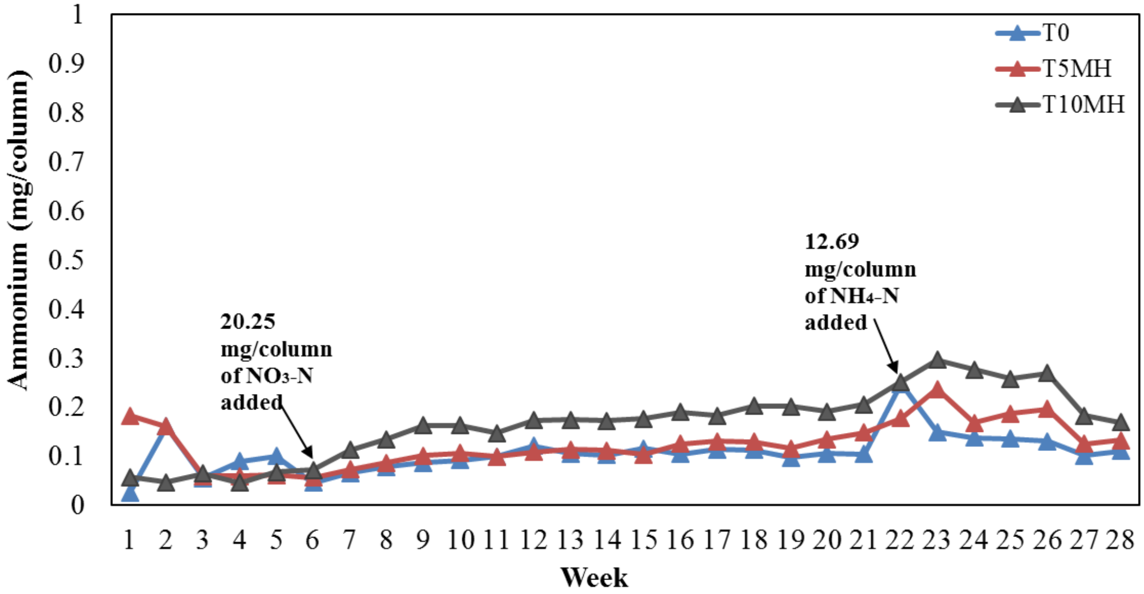


Figure 4.9 Weekly leaching of ammonium (mg/column) from T_0, T_{5MH} and T_{10MH} respectively.

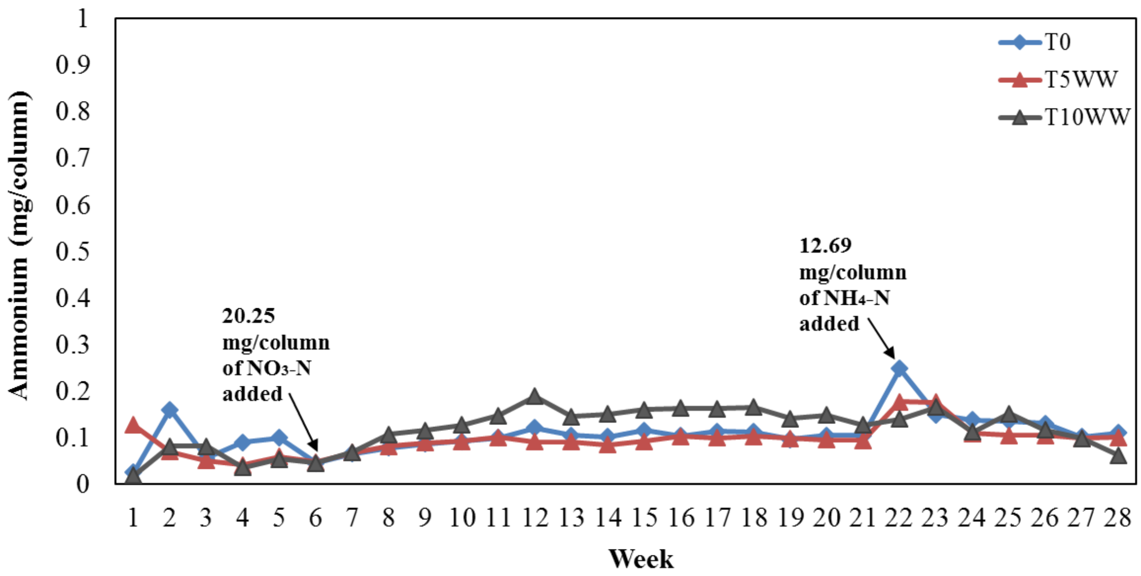


Figure 4.10 Weekly leaching of ammonium (mg/column) from T_0, T_{5WW} and T_{10WW} respectively.

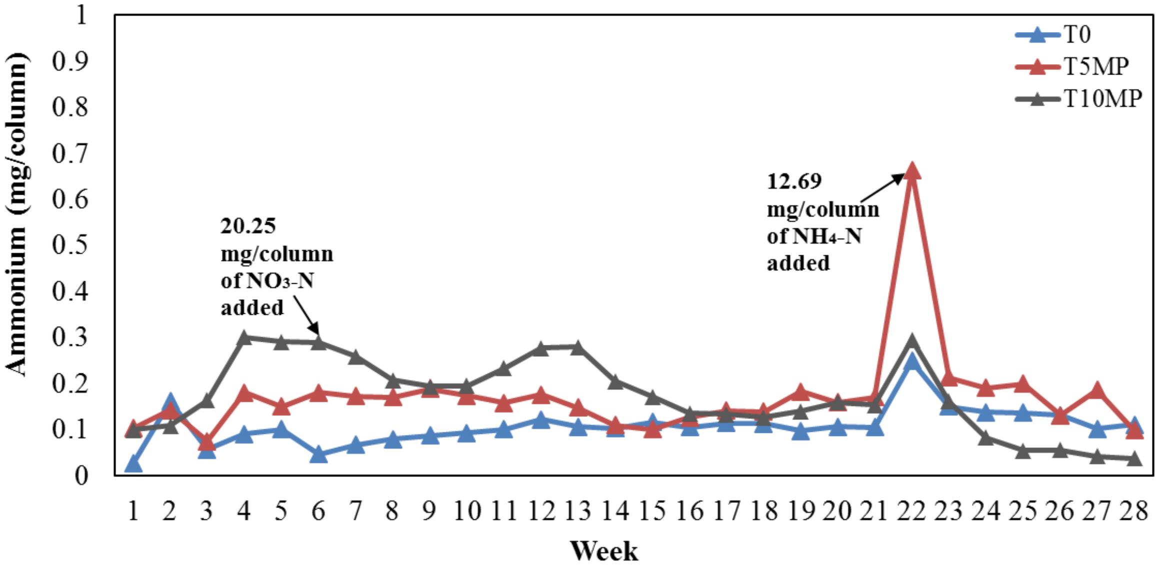


Figure 4.11 Weekly leaching of ammonium (mg/column) from T_0, T_{5MP} and T_{10MP} respectively.

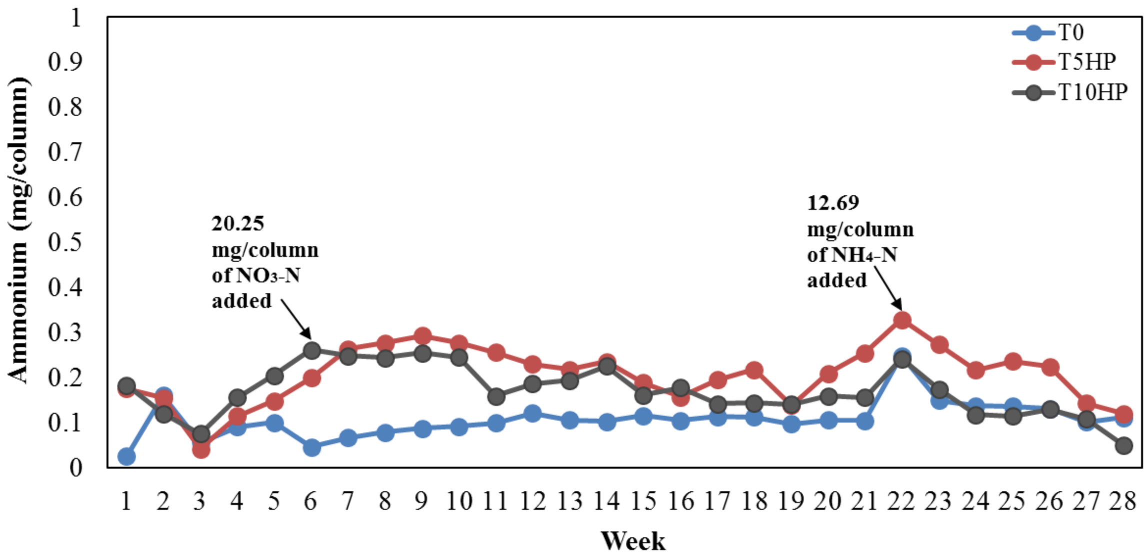


Figure 4.12 Weekly leaching of ammonium (mg/column) from T_0, T_{5HP} and T_{10HP} respectively.

Weekly leaching after fertilization ($NaNO_3$) (week 6-21)

When soil received fertilization on week 6, MH biochar treatment had overall higher NH_4^+-N leaching than T_0 from week 6 to 21, especially for T_{10MH} (Fig. 4.9). From week 11 to 15, T_{5MH} had overlap of NH_4^+-N leaching with T_0 (Fig. 4.9). When MH biochar firstly added into soil, MH biochar treatment had lower NH_4^+-N leaching T_0 (Fig. 4.9) due to the increase of SOM and CEC after MH biochar addition (Table 4.2). With time progressed, the presence of MH biochar would increase the mineralization of SOM and release NH_4^+-N into soil, which could explain the higher NH_4^+-N leaching on MH biochar treatment. This is proved by the decreased SOM and soil CEC observed on MH biochar treatment after 28 weeks of leaching, especially for T_{10MH}, which had the lower CEC than T_{5MH} and higher NH_4^+-N leaching from week 6 to 21 (Table 4.2).

After application of fertilizer ($NaNO_3$) on week 6, WW biochar treatment had an overlap of NH_4^+-N leaching with T_0 between week 6 and 11 for T_{5WW} and from week 6 to 7 for T_{10WW} (Fig. 4.10). The higher NH_4^+-N leaching than T_0 was observed on T_{10WW} from week 8 to 21, while T_{5WW} had lower NH_4^+-N leaching than T_0 from week 12 to 21 (Fig. 4.10). T_{10WW} had the overall higher NH_4^+-N leaching than T_{5WW} due to the lower CEC of soil on T_{10WW} (Table 4.2). Comparing with two wood-derived biochars in this experiment, WW biochar treatment had the overall lower weekly NH_4^+-N leaching than MH biochar treatment from week 6 to 21 when biochar was applied at the same application rate (Fig. 4.9 & Fig. 4.10).

MP biochar had the higher NH_4^+-N leaching after fertilization from week 6 to 21 than T_0 especially for T_{10MP} (Fig. 4.11). This was because MP biochar contained the relatively high NH_4^+-N content (Table 4.1), which brought additional NH_4^+-N into soil column. However, NH_4^+-N leaching on T_{10MP} was increasing from week 10 to 13, but decreased after week 13 and was in the relative same amount as T_0 and T_{5MP} from week 16 to 21 (Fig. 4.11). T_{5MP} had the lower NH_4^+-N leaching than T_{10MP} but higher NH_4^+-N leaching than T_0 from week 6 to 21, while the difference between T_{5MP} and T_0 decreased because NH_4^+-N from MP biochar was leached out as time progressed from week 6 to 18. But from week 18 to 21, the difference of NH_4^+-N leaching between T_{5MP} and T_0 was increasing. Both T_{5MP} and T_{10MP} experienced a short peak of NH_4^+-N leaching on week 19 for T_{5MP} and on week 12 and 13 for T_{10MP}. This could due to the increased mineralization of SOM after MP biochar addition, which released more NH_4^+-N into soil than T_0 and increased the potential of NH_4^+-N leaching on MP biochar treatment. This was proved by the higher reduction of SOM on MP biochar treatment and the lower reduction of SOM on T_0 after 28 weeks of leaching experiment compared with SOM before leaching (Table 4.2). However, the SOM mineralized more slowly with the increasing doses of MP biochar in this experiment, which could explain the lower NH_4^+-N leaching on T_{10MP} than T_{5MP} from week 17 to 21. This is consistent to the finding by Bruun & EL-Zehery (2012), who also reported the slower mineralization of organic matter on soil received biochar at higher application rate. In the early period of the experiment (week 6-16) (Fig. 4.11), the higher NH_4^+-N leaching on T_{10MP} mainly because soil received more NH_4^+-N from MP biochar applied at higher application rate than MP biochar applied at lower dosage.

HP biochar treatment had the higher weekly NH_4^+-N leaching than T_0, while the difference of NH_4^+-N leaching between HP biochar treatment and T_0 decreased as time progressed from week 6 to 21 (Fig. 4.12). This could due to the high NH_4^+-N content of HP biochar, which brought additional NH_4^+-N into soil column. T_{10HP} had the overall lower NH_4^+-N leaching than T_{5HP} from week 6 to 21, which could due to the slower mineralization of SOM over times. T_{5HP} had a higher reduction of the SOM than T_{10HP} after as time progressed (Table 4.2), which again indicated the slower mineralization of SOM with the increase of biochar content in soil.

Comparing four types of biochars in this experiment, manure-derived biochar (MP and HP) had the higher weekly NH_4^+-N leaching than wood-derived biochar (MH and WW).

WW biochar applied at rate of 5 g biochar per kg of soil did show the lower NH_4^+-N leaching from week 6 to 21 (Fig. 4.10) but not significantly ($p < 0.05$ *or* $0.05 < p < 0.1$) lower than T_0 (Table B3).

Weekly leaching after fertilization (NH_4Cl) (week 22-28)

When soil received fertilizer (NH_4Cl solution) on week 22, an increase of NH_4^+-N leaching occurred for T_0 on week 22 and for MH biochar treatment on week 23. After week 23, MH biochar treatment had the higher NH_4^+-N leaching than T_0 until week 28, while the difference of NH_4^+-N leaching between MH biochar treatment and T_0 reduced as time progressed (Fig. 4.9). T_{10MH} had the higher NH_4^+-N leaching than T_{5MH} from week 22 to 28.

After fertilization of NH_4Cl solution, a slight increase of NH_4^+-N leaching was observed on T_{5WW} and T_{10WW} at week of 22 and 23 respectively (Fig. 4.10). While the increase of NH_4^+-N leaching observed on WW biochar treatment was lower than the peak of NH_4^+-N leaching observed on T_0. As time progressed, T_{5WW} still had the lower NH_4^+-N leaching than T_0 until week 28, whereas T_{10WW} had the overlap of NH_4^+-N leaching with T_0 from week 23 to 27 and the lower NH_4^+-N leaching than T_0 on week 28 (Fig. 4.10). This indicated that WW biochar could help soil to reduce NH_4^+-N leaching when soil received high amount of NH_4^+-N from NH_4Cl solution over times especially at application rate of 5 g per kg of soil.

MP biochar treatment had an increase of NH_4^+-N leaching on week of 22 right after fertilization, especially T_{5MP}, which had the significantly (Table B5) higher NH_4^+-N leaching than T_0 and T_{10MP}. T_{10MP} also had higher NH_4^+-N leaching than T_0 from week 22 to 23, but the dramatic lower NH_4^+-N leaching than T_0 was observed on T_{10MP} from week 24 to 28. This indicated MP biochar at application rate of 10 g per kg of soil was effective on reducing NH_4^+-N leaching from soil received ammonium fertilizer than T_0 over times. NH_4^+-N leaching on HP biochar treatment increased after addition of NH_4Cl especially T_{5HP}, which had the higher NH_4^+-N leaching than T_0. While T_{10HP} had the relatively same NH_4^+-N leaching as T_0 from week 22 to 27, and a lower NH_4^+-N leaching was observed on week 28 on T_{10HP}. This would suggest the future study to choose higher HP biochar application rate to test whether not the higher HP content in soil would have lower NH_4^+-N leaching from soil with ammonium fertilizer than T_0.

MP biochar showed the higher ability on NH_4^+-N leaching reduction from week 22 to 28 than HP biochar when biochar application rate is 10 g per kg of soil in this experiment, which could because more acid functional groups of biochar formed at lower pyrolysis temperature (Kondo, 2010). For MP and HP biochar, application rate of 10 g biochar per kg of soil was more effective than application rate of 5 g biochar per kg of soil due to its lower NH_4^+-N leaching from week 22 to 28. On contrast, T_{10MH} had the higher NH_4^+-N leaching than T_{5MH}. No dramatic difference of NH_4^+-N leaching was observed between T_{5WW} and T_{10WW}.

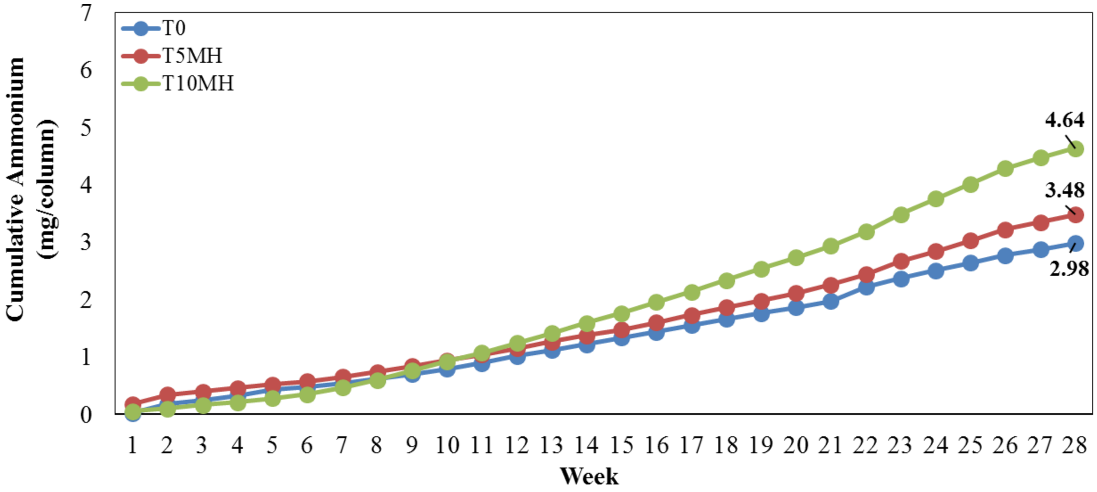


Figure 4.13 Cumulative ammonium (mg/column) from T_0, T_{5MH} and T_{10MH} from 1 to 28 weeks.

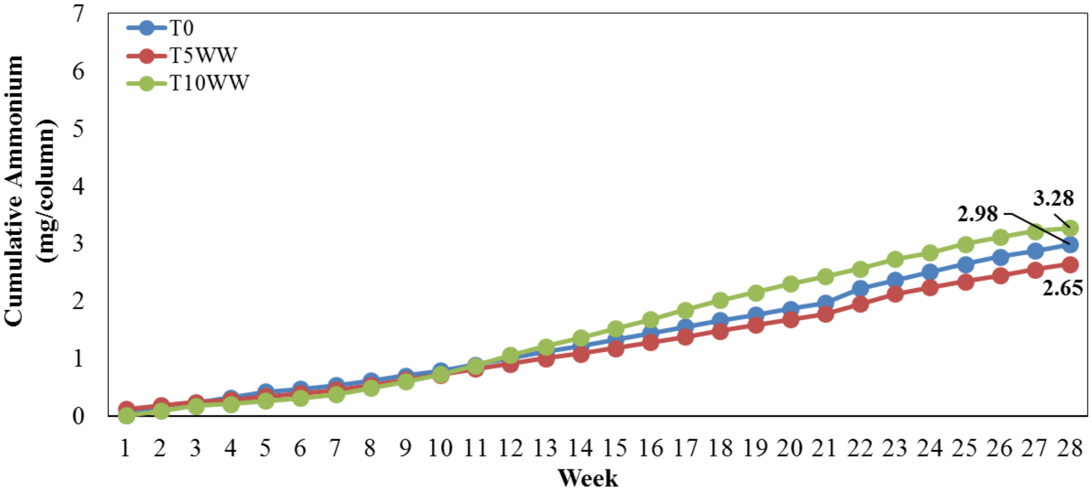


Figure 4.14 Cumulative ammonium (mg/column) from T_0, T_{5WW} and T_{10WW} from 1 to 28 weeks.

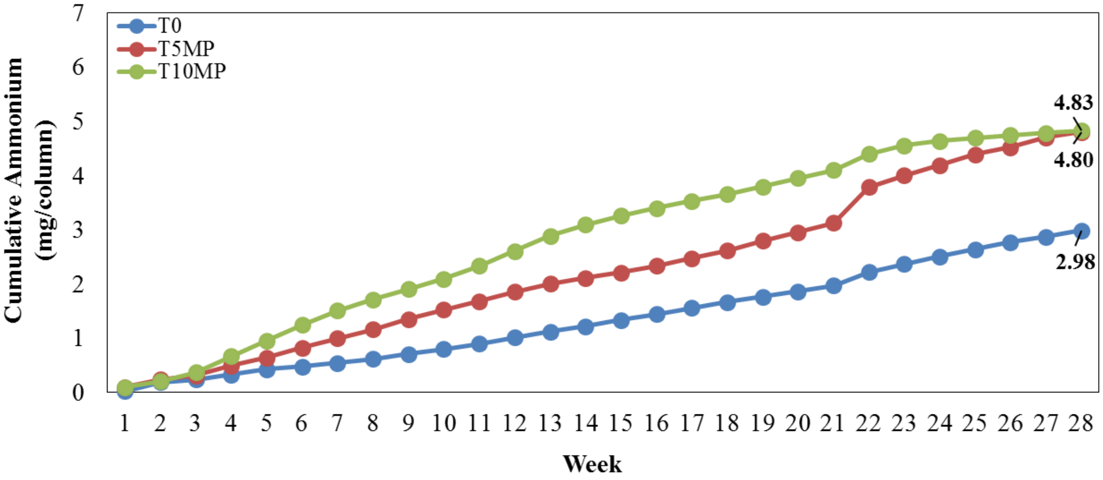


Figure 4.15 Cumulative ammonium (mg/column) from T_0, T_{5MP} and T_{10MP} from 1 to 28 weeks.

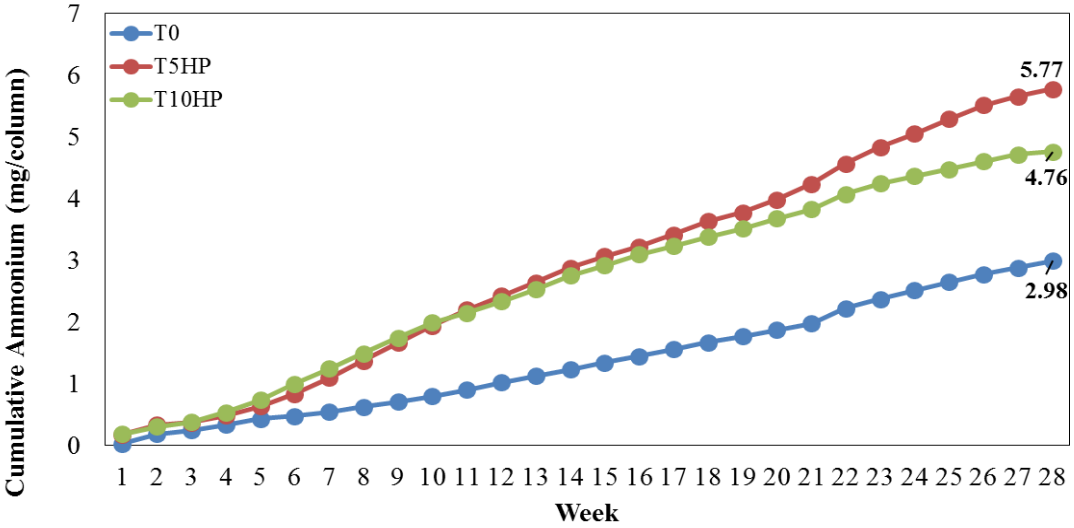


Figure 4.16 Cumulative ammonium (mg/column) from T_0, T_{5HP} and T_{10HP} from 1 to 28 weeks.

In this study, different types of biochar showed different effects on NH_4^+-N leaching after addition of NH_4Cl solution at week of 22. By looking at the cumulative NH_4^+-N leaching after 28 weeks of leaching experiment, MH biochar treatment had the higher cumulative NH_4^+-N leaching than T_0 especially when MH biochar was applied at higher rate (10 g/kg soil) (Fig. 4.13). WW biochar treatment had the lower cumulative NH_4^+-N leaching than T_0 when it was applied at rate of 5 g per kg of soil. The higher application rate of WW biochar didn't help soil to reduce more NH_4^+-N from leaching (Fig. 4.14). From week 4 to 28, a dramatic higher cumulative NH_4^+-N leaching was observed on MP biochar treatment (Fig. 4.15). T_{10MP} had the higher cumulative NH_4^+-N leaching than T_{5MP}, while the difference of cumulative NH_4^+-N leaching between T_{5MP} and T_{10MP} reduced from week 22 to 28, and they tended to have very close amount of cumulative NH_4^+-N leaching by week 28 (Fig. 4.15). This was due to the lower weekly NH_4^+-N leaching fromT_{10MP} and much higher weekly NH_4^+-N leaching from T_{5MP} from week 22 to 28 (Fig. 4.11). Cumulative NH_4^+-N leaching on HP biochar treatment was higher than T_0 from week 1 to 28, and the gap of cumulative NH_4^+-N leaching between T_0 and HP biochar treatment increased as time progressed (Fig. 4.16). From week 1 to week 21, no dramatic difference of cumulative NH_4^+-N leaching was found between T_{5HP} and T_{10HP}, while after soil received NH_4Cl solution, T_{5HP} tended to have higher cumulative NH_4^+-N leaching than T_{10HP} (Fig. 4.16). This was due to the higher weekly NH_4^+-N leaching observed on T_{5HP} after fertilization on week 22 (Fig. 4.12). In summary, WW biochar applied at rate of 5 g per kg of soil showed the lower cumulative NH_4^+-N leaching than T_0 even though it contained little NH_4^+-N content (Table 4.1). T_{10MP} had the higher cumulative NH_4^+-N leaching than T_0 by week 28 (Fig. 4.15) due to the high NH_4^+-N content of MP biochar (Table 4.1). However, MP biochar applied at rate of 10 g per kg of soil did show the good NH_4^+-N leaching reduction than T_0 from week 22 to 28. It is recommended in the future study to test the effect of MP biochar at higher biochar dosage on NH_4^+-N leaching over times. MH and HP biochar treatment didn't have lower cumulative NH_4^+-N leaching than T_0 after 28 weeks of leaching. Unlike NH_4^+-N, all biochars showed the high effectiveness on NO_3^--N leaching reduction (from Fig. 4.1 to Fig. 4.4). This illustrates the choice of biochar type and pyrolysis conditions affect biochar properties, which will affect their nutrient retention ability as a result.

4.3 Retention Rates

4.3.1 Nitrate Retention by Biochar

In this research, all biochars showed the ability to retain NO_3^--N from soils 'fertilized' with $NaNO_3$ solution. But the effectiveness of biochar on nitrate leaching reduction varied depends on types of biochar and the level of biochar applied. Mass balance analysis was used to estimate the percentage of NO_3^--N from biochar recovered in the leachate using:

$$\mathrm{mg}_{\text{retained by biochar addition}} = \mathrm{m}_{\mathrm{L-c}} - (\mathrm{m}_{\mathrm{L-b}} - \mathrm{m}_{\text{from biochar}}) \qquad \text{(Equation 1)}$$

$$\mathrm{R}_{\text{retained by biochar addition}}(\%) = \frac{\mathrm{mg}_{\text{retained by biochar addition}}}{\mathrm{m}_{\mathrm{L-c}}} \times 100 \qquad \text{(Equation 2)}$$

$\mathrm{mg}_{\text{retained by biochar addition}}$ is the amount (mg) of nitrate that biochar helps to retain. $\mathrm{m}_{\text{from biochar}}$ is the amount (mg) of nitrate from biochar. $\mathrm{m}_{\mathrm{L-c}}$ stands for the cumulative amount (mg) of nitrate leached out from control treatment after 21 weeks of leaching (prior to addition of NH_4Cl). $\mathrm{m}_{\mathrm{L-b}}$ equals to the cumulative amount (mg) of nitrate leached out from treatments received biochar after 21 weeks of leaching (prior to addition of NH_4Cl). $\mathrm{R}_{\text{retained by biochar addition}}$

is the nitrate retention rate (%) that biochar helps to retain after 21 weeks of leaching (prior to addition of NH_4Cl).

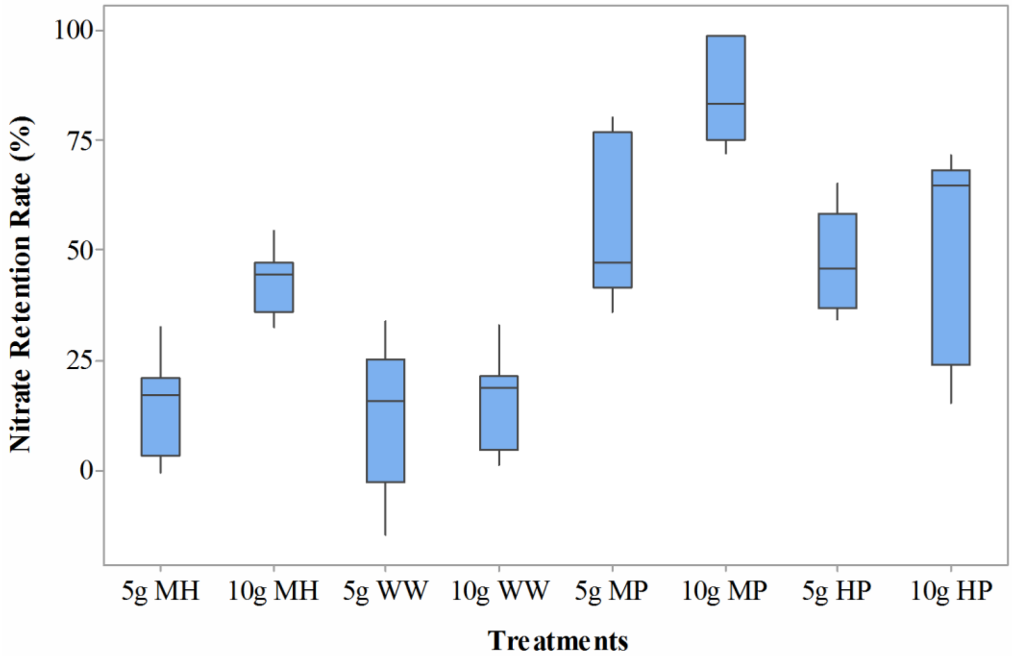


Figure 4.17 Nitrate retention rate (%) that biochar helps to retain from biochar treatments compared with T_0 respectively over 21 weeks of leaching experiment.

After 21 weeks of leaching experiment, an average of 14% of NO_3^--N were retained in soil 'fertilized' with $NaNO_3$ solution along with MH biochar at application rate of 5 g kg^{-1} soil (Fig. 4.17). When MH biochar application rate increased from 5 g to 10 g kg^{-1} of soil, an average of 43% of NO_3^--N were retained in soil. This indicated the higher MH biochar application rate could help the 'fertilized' soil to reduce more NO_3^--N from leaching with time. However, only 2 application rates were tested in this research, which require the further study on testing the higher application rates of biochars' effects on NO_3^--N retention. Similar finding was observed on MP biochar treatments, which reduced about 56 % of NO_3^-

from leaching at rate of 5 g kg^{-1} of soil and 86% at rate of 10 g kg^{-1} of soil in soil 'fertilized' with $NaNO_3$ solution with times (Fig. 4.17).

HP biochar had the relatively high NO_3^--N retention rates (%) with the increase of application rate, which were about 47% (average) at rate of 5 g kg^{-1} of soil and 52% (average) at 10 g kg^{-1} of soil (Fig. 4.17). This result is consistent to the lowest overall weekly and cumulative NO_3^--N leaching on HP biochar treatments prior to the addition of NH_4Cl solution (Fig. 4.4 & Fig. 4.8). It seems that MP biochar had the highest NO_3^--N retention rate (%) than other biochar treatments. Although HP biochar has the lowest cumulative NO_3^--N leaching with times, MP biochar was considered to have the highest NO_3^--N retention rate (%) if the amount of NO_3^--N from biochar was taken consideration. MP contains about 4.5 times higher (Table 4.1) amount of NO_3^--N than HP biochar, which increases the N availability of the soil and could increase the potential of NO_3^--N leaching. But the NO_3^--N leached out from MP and HP biochar treatments were relatively close. In such case, MP biochar was considered as the most effective soil amendment on recovering NO_3^--N in soil without being leached out.

It has been found that WW biochar treatments had the lowest NO_3^--N retention rate (%) after 21 weeks of leaching experiment. About 13% (average) and 15% (average) of nitrate were recovered at application rate of 5 and 10 g kg^{-1} of soil respectively (Fig. 4.17). The negative nitrate retention rate indicated the WW biochar could even accelerate the leaching of NO_3^--N compared with soil without biochar amendment with times.

Singh et al. (2010^b) reported a lower NO_3^--N leaching from soil amended with biochar produced from chipped stemwood at 400 ^oC (W400) compared with soil with no biochar. Singh et al. (2010^b) also reported a significant ($p < 0.1$) higher NO_3^--N leaching from the soil along with poultry manure in the rice hull litter produced at 400 ^oC (PM400) than soil without biochar addition in the first leaching event. Bruun et al. (2012) found that no significant difference ($p <0.05$) of NO_3^--N leaching between biochar treatments and treatment without biochar addition. The contrary results suggested the effect of biochar on reducing NO_3^--N leaching could depend upon the different soil properties that tested, the parent materials of biochar and the pyrolysis temperature of making biochar. Besides, the calculation of NO_3^- recovery rate (%) varies among experiments, which could lead to a slight different result.

In conclusion, MH, MP and HP worked well on reducing NO_3^--N leaching. WW biochar showed the lowest NO_3^--N retention rate than the rest of biochars. Biochar made from poultry manure, characterizing as high mineral content (ash) and low carbon, has shown the higher ability to retain NO_3^--N in soil from leaching than wood-based biochar (low mineral content (ash) and high carbon). HP biochar treatment had the lowest cumulative NO_3^--N leaching than the other treatments. While MP biochar treatment has the highest NO_3^--N retention rate (%) from soil ‘fertilized’ with $NaNO_3$ solution than the other treatments when the amount of NO_3^--N contained in biochar was taken consideration.

4.3.2 Ammonium Retention by Biochar

Mass balance analysis was used to estimate the percentage of ammonium added with the biochar recovered in the leachate using:

$$\mathrm{mg}_{\mathrm{retained\ by\ biochar\ addition}} = \mathrm{m}_{\mathrm{L-c}} - (\mathrm{m}_{\mathrm{L-b}} - \mathrm{m}_{\mathrm{from\ biochar}}) \qquad \text{(Equation 3)}$$

$$\mathrm{R}_{\mathrm{retained\ by\ biochar\ addition}}(\%) = \frac{\mathrm{mg}_{\mathrm{retained\ by\ biochar\ addition}}}{\mathrm{m}_{\mathrm{L-c}}} \qquad \text{(Equation 4)}$$

Where $\mathrm{mg}_{\mathrm{retained\ by\ biochar\ addition}}$ is the amount (mg) of ammonium that biochar helps to retain. $\mathrm{m}_{\mathrm{from\ biochar}}$ is the amount (mg) of ammonium from biochar. $\mathrm{m}_{\mathrm{L-c}}$ stands for the cumulative amount (mg) of ammonium leached out from control treatment after 28 weeks of leaching. And $\mathrm{m}_{\mathrm{L-b}}$ equals to the cumulative amount (mg) of ammonium leached out from treatments received biochar after 28 weeks of leaching. In addition, $\mathrm{R}_{\mathrm{retained\ by\ biochar\ addition}}$ is the ammonium retention rate that biochar helps to retain (%) after 28 weeks of leaching.

According to the SAS test that all tested biochars were not effective on reducing NH_4^+-N leaching from NH_4Cl fertilized soils after 28 weeks of leaching experiment. No significant lower NH_4^+-N leaching was observed between T_0 and biochar treatments. MP and HP biochar even increase the leaching of NH_4^+-N compared with T_0. However, if the additional input of NH_4^+-N from biochar into the soil was considered, the significant high NH_4^+-N retention rate (%) was observed from biochar treatments compared with T_0 (Fig. 4.18).

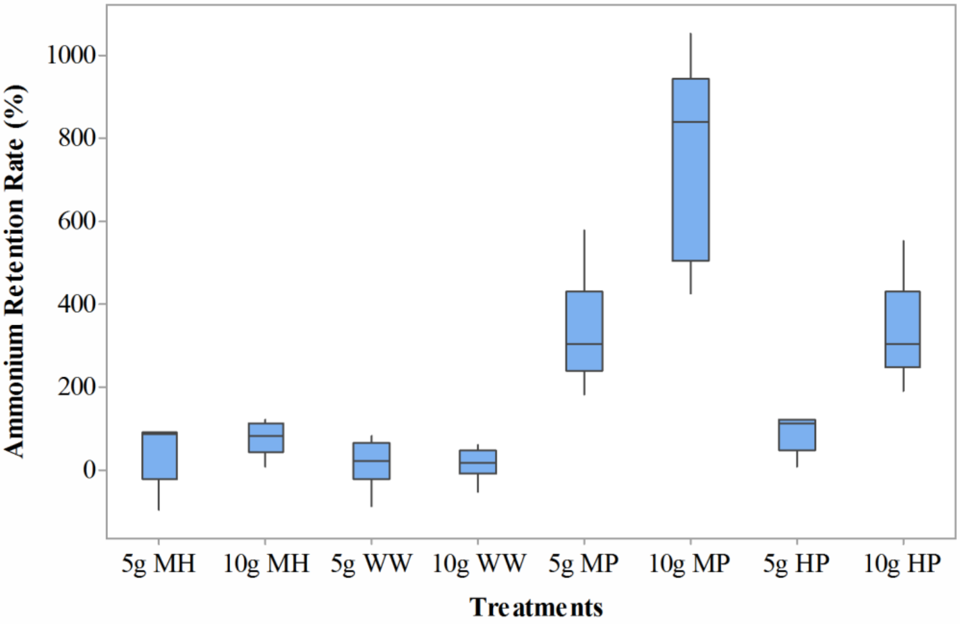


Figure 4.18 Ammonium retention rate (%) that biochar helps to retain from biochar treatments compared with T_0 respectively over 28 weeks of leaching experiment.

T_{5MH} retained an average of 44% of NH_4^+-N (Fig. 4.18). With the increase of MH biochar application rate from 5g to 10g per kg of soil, the NH_4^+-N retention rate (%) increased to 76%. This suggested the higher application rate of MH could result in the higher percentage of NH_4^+-N adsorption with times. Similarly, the higher application of MP and HP biochar, the greater NH_4^+-N adsorption in soil with nitrate and ammonium fertilization. However, WW biochar retained the lower percentage of NH_4^+-N with the increase of application. Average of 335% of NH_4^+-N were recovered from T_{5MP} and about 752% of NH_4^+-N were retained from T_{10MP} compared with T_0 (Fig.4.18). Soil along with HP biochars recovered about 90% of NH_4^+-N at application rate of 5 g kg^{-1} and 337% at rate of 10 g kg^{-1} of soil respectively. Poultry manure derived biochar has overall higher

NH_4^+-N retention rate, indicating the parent materials of biochar affected the effect of biochar on nutrient retention. The NH_4^+-N retention rate (%) from MP or HP biochar treatments was extremely higher than 100%. According to the mass balance equation, the numerator is the difference of the NH_4^+-N (mg) between control treatment and specific biochar treatment by considering the amount of NH_4^+-N (mg) in the soil from biochar. The amount of NH_4^+-N (mg) from MP and HP biochar was up to 4 times higher than cumulative NH_4^+-N (mg) leached out from both control and biochar treatments. The denominator of the equation is the amount of NH_4^+-N (mg) leached out from control treatment, which is much lower than numerator. For example, the average $m_{L\text{-}c}$ and $m_{L\text{-}b}$ from T_{5MP} was about 2.98 mg and 4.8 mg respectively; $m_{\text{from biochar}}$ was about 10.5 mg. So $mg_{\text{retained by biochar addition}}$ was about 8.68 mg, which was about 3 times higher than denominator of 2.98 mg. This mass balance equation didn’t consider the amount of NH_4^+-N in soil before and after leaching, which could obtain the incomplete result. It is recommended in this future experiment to test the amount of NH_4^+-N in soil before and after leaching experiment, which should be considered in the mass balance equation.

Although the leaching of NH_4^+-N of biochar at application rate of 5g or 10g per kg of soil was higher than T_0, which might suggest biochar was not effective on reducing NH_4^+-N leaching. However, considering the total input of NH_4^+-N from biochar, all biochars in this experiment, especially MP and HP biochars, showed the ability of NH_4^+-N retention in soil. Therefore, MP and HP biochar have the higher NH_4^+-N retention ability than MH and

WW biochar by considering the high input of NH_4^+-N from MP and HP biochar into soil. This experiment found no significant increase of SOM and CEC on biochar treatments compared with T_0 (Table 4.2) after 28 weeks of leaching, which might explain non-significant lower NH_4^+-N leaching on biochar treatments than T_0. In sandy soil, the biochar application may significantly increase CEC, but may be also insignificant (Schulz et al., 2012). Further study need to report on the change in freshly incorporated biochar CEC values over time for biochars that have been placed in situ.

4.4 Economic Value of Biochar

This study has found that biochar could greatly reduce NO_3^--N leaching from soil over times, which suggested biochar has benefit to the soil. The ability of biochar on retaining up to 86% of NO_3^--N in the soil could increase the crop yield and reduce the fertilizer cost, which indicated biochar has economic benefit to farmers.

Table 4.5 An example of the estimation of the cost of Ammonium Nitrogen (AN) fertilizer saved for the following year when soil received 10g MP biochar per kg of soil.

Crops	crop area (hectares)	N application rates guidelines for crops (kg N per ha)	Total cost of AN fertilizer for crops (CAD)	Total cost of AN fertilizer for next year (CAD)	**Cost of fertilizer saved (CAD)**	**Cost of fertilized saved (CAD/ha)**
Carrot	1028	111	107162	92160	**$15002**	**$15/ha**
Potato	809	185	140555	120877	**$19678**	**$24/ha**

This study found about 86% of NO_3^--N could be retained in soil amended with 10 g MP biochar per kg of soil (Fig. 4.17). An example of showing the cost of AN fertilizer saved for the next growing season is shown in Table 4.5. Carrots and potatoes are the main vegetable crops in Nova Scotia (Nova Scotia Department of Agriculture, 2012), which have high requirement of N for crop growth (Laboski et al., 2006). If the growing field of carrot in Nova Scotia is about 1028 hectares in the first year, which required about 111 kg N per hectare of carrot growing field according to Laboski et al. (2006). The local price of Ammonium Nitrate (AN) fertilizer is about $324 Canadian dollar (CAD) per 1000 kg of AN fertilizer. So, the total cost of AN fertilizer for this year is $107,162 (CAD) (Table 4.5). If soil received MP biochar at application rate of 10 g/kg of soil, which can help soil retained 86% of NO_3^--N (Fig. 4.17) in soil for next year, so the total cost of AN fertilizer for next year is about $92,160 (CAD) (Table 4.5). It means adding MP biochar (10g/kg of soil) into carrot growing field in the first year can save about $15,002 (CAD) of AN fertilizer for the next growing season. For carrots, the total cost of AN fertilizer saved for the next year is about $19,678 (CAD), which is a significant economic benefit.

Table 4.5 only showed the cost saved for fertilizer without considering the other expenses such as biochar price and the transportation. Profitability of biochar application in soil depends on various parameters such as the different geo-economic agricultural scenarios, the biochar feedstock and crop, biochar production technology and the time-span of evaluation. This experiment found high NO_3^--N retention rate which indicated the lower fertilizer is needed for the following year and the fertilizer cost could be decreased. Besides, if use poultry-manure as the biochar's parent material, the biochar cost and fertilizer can be reduced because it is an animal waste and it contained the relatively high N content. But the assessment of economic value of biochar requires more information, which can be explored in the future study.

CHAPTER 5: CONCLUSION

5.1 Summary and Conclusions

This study tests the effect of four biochars (MH, WW, MP and HP) on NO_3^--N and NH_4^+-N leaching from a Nova Scotian soil over 28 weeks of leaching experiment. It has been found all biochars in this experiment significantly ($p < 0.05$) reduced the leaching of NO_3^--N and greatly retained NO_3^--N in the soil incorporated with 'fertilizers'. Poultry manure-derived biochar had up to 73% higher NO_3^--N retention rate than wood-derived biochar. Besides, poultry manure produced at high temperature showed the lower cumulative NO_3^--N leaching (T_{5HP} =27.50 mg; T_{10HP} = 30.87 mg) than produced at medium temperature (T_{5HP} =37.36 mg; T_{10HP} = 40.39 mg) by week 28. There was no significant difference of cumulative NO_3^--N leaching between T_{5HP} and T_{10HP} before week 19, while a higher cumulative NO_3^--N leaching was observed on T_{10HP} from week 19 to 28. Therefore, poultry manure biochar especially HP biochar was recommended as the most effective soil amendment on reducing NO_3^--N leaching from soil with fertilization over times. Poultry manure as parent material is a lower cost choice compared with other parent materials such as wood. Furthermore, poultry manure-derived biochar contained the relatively high N content, which can be used as the fertilizer and reduce the cost of fertilizer for farmers. MH applied at rate of 10 g kg^{-1} soil may be recommended if no fertilizer is needed and only NO_3^--N leaching reduction is concerned because it showed excellent NO_3^--N leaching reduction without adding additional NO_3^--N to the soil.

No significant lower cumulative NH_4^+-N leaching was observed on MH, MP and HP biochar treatments than soil without biochar addition. While WW biochar applied at rate of 5 g per kg of soil showed the lower cumulative NH_4^+-N leaching than non-biochar amended treatment. T_{10MP} also had the lower weekly NH_4^+-N leaching than non-biochar amended treatment after fertilization from week 22 to 28, which indicated the ability to reduce NH_4^+-N leaching from soil received ammonium fertilizer over times. The higher cumulative NH_4^+-N leaching MP or HP biochar treatment was mainly due to the high amount of NH_4^+-N contained in MP and HP biochar. However, if the additional source of NH_4^+-N was considered all biochars showed high NH_4^+-N retention ability especially for MP or HP biochar.

Overall, this study determined that potential N retention ability of various types of biochar in soil over 28 consecutive weeks of leaching. The experimental results indicate that biochar has great promise to reduce nutrient leaching from agriculture to environment in Nova Scotia. Further, the results were indicative of considerable potential for reducing costs for farmers or agricultural industry in Nova Scotia. These benefits are in addition to other benefits that have been found for biochars, such as carbon sequestration, and soil quality improvement.

5.2 Recommendations for Future Research

Based on the current findings from this research, further studies to better identify the biochar's effect on N leaching from soils in Nova scotia are recommended:

1) The application rate of biochar in soil could be further increased to 20 and 40 g kg^{-1} of soil, thereby exploring the ability of biochar on the NO_3^--N and NH_4^+-N leaching reduction at higher rates. Higher the biochar application rate might result in the lower or higher NO_3^--N and NH_4^+-N leaching according to the previous studies soil (Singh et al., 2010^b; Dempster et al., 2012; Yao et al., 2012; Clough et al., 2013).

2) This research found biochar was able to retain NO_3^--N in soil from leaching with times. However, the mechanisms of N retention by biochar were unclear. Some of studies suggest the functional groups present on surface of the biochar, the increased surface area and porosity by biochar were the contributors of N retention. Therefore, it is recommended in the further study to observe the change of the functional groups on the surface of biochar with times, to test the surface area and porosity of soil after biochar addition and to better understand the mechanism of NO_3^--N retention of biochar.

3) Only one soil type was tested in this research. In the future study, it is suggested to test biochar's effect on N leaching reduction from different soil types in Nova Scotia since the properties of is one of the key impact factors on soil nutrient leaching. It is recommended to compare soils have low and CEC to better understand the adsorption of NH_4^+-N by biochar from soil with high and low CEC.

4) The soils samples were taken from the agricultural field where the soils were fertilized or limed before soil collection. Most of soils in Nova Scotia was naturally acidic and has

low fertility. The further study on identifying the effectiveness of biochar on reducing N from acidic agricultural soils in Nova Scotia is required.

5) The variability among replicates in this project was found in a high range, which might because of inconsistency of soil packing. The mixture of soil and biochar was conducted by stirring soil with biochar in the same way for 20 times. However, this method couldn't assure that biochar were evenly distributed in the soil among replicates. Therefore, in the future experiment, it is recommended to add biochar into the soil at the same depth of soil layer among soil columns.

6) This experiment used $NaNO_3$ and NH_4Cl solutions as the fertilizers. It is recommended in the future study to investigate the biochar's effect on N leaching from soil incorporated with commercial fertilizers such as Ammonium-Nitrate or animal manures.

7) Field experiment is recommended in the future study by planting crops to investigate whether or not biochar can increase the crop production.

8) The assessment of economic value of biochar as a soil amendment can be investigated in the future study.

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Appendix A: Differences of Least Squares Means of Nitrate concentration between control treatment and biochar treatments at all experimental weeks

Table A1 Differences of Least Squares Means of Nitrate between T_0 and T_{5MH}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
Treatment × Time	T_0	1	T_{5MH}	1	0.1237
Treatment × Time	T_0	2	T_{5MH}	2	0.0073
Treatment × Time	T_0	3	T_{5MH}	3	0.8625
Treatment × Time	T_0	4	T_{5MH}	4	0.3627
Treatment × Time	T_0	5	T_{5MH}	5	0.8652
Treatment × Time	T_0	6	T_{5MH}	6	0.0006
Treatment × Time	T_0	7	T_{5MH}	7	0.0309
Treatment × Time	T_0	8	T_{5MH}	8	0.2625
Treatment × Time	T_0	9	T_{5MH}	9	0.4698
Treatment × Time	T_0	10	T_{5MH}	10	0.7882
Treatment × Time	T_0	11	T_{5MH}	11	0.9235
Treatment × Time	T_0	12	T_{5MH}	12	0.7779
Treatment × Time	T_0	13	T_{5MH}	13	0.6392
Treatment × Time	T_0	14	T_{5MH}	14	0.8772
Treatment × Time	T_0	15	T_{5MH}	15	0.6847
Treatment × Time	T_0	16	T_{5MH}	16	0.4057
Treatment × Time	T_0	17	T_{5MH}	17	0.5277
Treatment × Time	T_0	18	T_{5MH}	18	0.6401
Treatment × Time	T_0	19	T_{5MH}	19	0.6942
Treatment × Time	T_0	20	T_{5MH}	20	0.9876
Treatment × Time	T_0	21	T_{5MH}	21	0.9529
Treatment × Time	T_0	22	T_{5MH}	22	0.9012
Treatment × Time	T_0	23	T_{5MH}	23	0.2826
Treatment × Time	T_0	24	T_{5MH}	24	0.9249
Treatment × Time	T_0	25	T_{5MH}	25	0.4093
Treatment × Time	T_0	26	T_{5MH}	26	0.7058
Treatment × Time	T_0	27	T_{5MH}	27	0.4040
Treatment × Time	T_0	28	T_{5MH}	28	0.6542

Table A2 Differences of Least Squares Means of Nitrate between T_0 and T_{10MH}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
Treatment × Time	T_0	1	T_{10MH}	1	<.0001
Treatment × Time	T_0	2	T_{10MH}	2	0.0005
Treatment × Time	T_0	3	T_{10MH}	3	0.1516
Treatment × Time	T_0	4	T_{10MH}	4	0.2122
Treatment × Time	T_0	5	T_{10MH}	5	0.2296
Treatment × Time	T_0	6	T_{10MH}	6	<.0001
Treatment × Time	T_0	7	T_{10MH}	7	0.0002
Treatment × Time	T_0	8	T_{10MH}	8	0.0331
Treatment × Time	T_0	9	T_{10MH}	9	0.3560
Treatment × Time	T_0	10	T_{10MH}	10	0.6692
Treatment × Time	T_0	11	T_{10MH}	11	0.4569
Treatment × Time	T_0	12	T_{10MH}	12	0.4012
Treatment × Time	T_0	13	T_{10MH}	13	0.3973
Treatment × Time	T_0	14	T_{10MH}	14	0.5510
Treatment × Time	T_0	15	T_{10MH}	15	0.5323
Treatment × Time	T_0	16	T_{10MH}	16	0.7888
Treatment × Time	T_0	17	T_{10MH}	17	0.6819
Treatment × Time	T_0	18	T_{10MH}	18	0.3275
Treatment × Time	T_0	19	T_{10MH}	19	0.1672
Treatment × Time	T_0	20	T_{10MH}	20	0.0709
Treatment × Time	T_0	21	T_{10MH}	21	0.2898
Treatment × Time	T_0	22	T_{10MH}	22	0.6012
Treatment × Time	T_0	23	T_{10MH}	23	0.0104
Treatment × Time	T_0	24	T_{10MH}	24	0.0240
Treatment × Time	T_0	25	T_{10MH}	25	0.1890
Treatment × Time	T_0	26	T_{10MH}	26	0.1026
Treatment × Time	T_0	27	T_{10MH}	27	0.3595
Treatment × Time	T_0	28	T_{10MH}	28	0.8957

Table A3 Differences of Least Squares Means of Nitrate between T_0 and T_{5WW}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
Treatment × Time	T_0	1	T_{5WW}	1	0.0016
Treatment × Time	T_0	2	T_{5WW}	2	0.2650
Treatment × Time	T_0	3	T_{5WW}	3	0.7264
Treatment × Time	T_0	4	T_{5WW}	4	0.8594
Treatment × Time	T_0	5	T_{5WW}	5	0.7455
Treatment × Time	T_0	6	T_{5WW}	6	<.0001
Treatment × Time	T_0	7	T_{5WW}	7	0.0320
Treatment × Time	T_0	8	T_{5WW}	8	0.7600
Treatment × Time	T_0	9	T_{5WW}	9	0.3873
Treatment × Time	T_0	10	T_{5WW}	10	0.8645
Treatment × Time	T_0	11	T_{5WW}	11	0.8956
Treatment × Time	T_0	12	T_{5WW}	12	0.7479
Treatment × Time	T_0	13	T_{5WW}	13	0.8805
Treatment × Time	T_0	14	T_{5WW}	14	0.5514
Treatment × Time	T_0	15	T_{5WW}	15	0.6019
Treatment × Time	T_0	16	T_{5WW}	16	0.3157
Treatment × Time	T_0	17	T_{5WW}	17	0.6724
Treatment × Time	T_0	18	T_{5WW}	18	0.5508
Treatment × Time	T_0	19	T_{5WW}	19	0.9298
Treatment × Time	T_0	20	T_{5WW}	20	0.5547
Treatment × Time	T_0	21	T_{5WW}	21	0.8215
Treatment × Time	T_0	22	T_{5WW}	22	0.7112
Treatment × Time	T_0	23	T_{5WW}	23	0.1242
Treatment × Time	T_0	24	T_{5WW}	24	0.9692
Treatment × Time	T_0	25	T_{5WW}	25	0.4258
Treatment × Time	T_0	26	T_{5WW}	26	0.6357
Treatment × Time	T_0	27	T_{5WW}	27	0.9967
Treatment × Time	T_0	28	T_{5WW}	28	0.6753

Table A4 Differences of Least Squares Means of Nitrate between T_0 and T_{10WW}.

Effect	**Treatment**	**Week**	**-Treatment**	**-Week**	**P-value > |t|**
Treatment × Time	T_0	1	T_{10WW}	1	0.2087
Treatment × Time	T_0	2	T_{10WW}	2	0.3551
Treatment × Time	T_0	3	T_{10WW}	3	0.7876
Treatment × Time	T_0	4	T_{10WW}	4	0.8861
Treatment × Time	T_0	5	T_{10WW}	5	0.4459
Treatment × Time	T_0	6	T_{10WW}	6	0.0029
Treatment × Time	T_0	7	T_{10WW}	7	0.1169
Treatment × Time	T_0	8	T_{10WW}	8	0.4609
Treatment × Time	T_0	9	T_{10WW}	9	0.6748
Treatment × Time	T_0	10	T_{10WW}	10	0.7625
Treatment × Time	T_0	11	T_{10WW}	11	0.9314
Treatment × Time	T_0	12	T_{10WW}	12	0.9070
Treatment × Time	T_0	13	T_{10WW}	13	0.7589
Treatment × Time	T_0	14	T_{10WW}	14	0.7087
Treatment × Time	T_0	15	T_{10WW}	15	0.8838
Treatment × Time	T_0	16	T_{10WW}	16	0.8694
Treatment × Time	T_0	17	T_{10WW}	17	0.8811
Treatment × Time	T_0	18	T_{10WW}	18	0.6783
Treatment × Time	T_0	19	T_{10WW}	19	0.6143
Treatment × Time	T_0	20	T_{10WW}	20	0.3696
Treatment × Time	T_0	21	T_{10WW}	21	0.8206
Treatment × Time	T_0	22	T_{10WW}	22	0.9407
Treatment × Time	T_0	23	T_{10WW}	23	0.1266
Treatment × Time	T_0	24	T_{10WW}	24	0.9637
Treatment × Time	T_0	25	T_{10WW}	25	0.9131
Treatment × Time	T_0	26	T_{10WW}	26	0.9499
Treatment × Time	T_0	27	T_{10WW}	27	0.7946
Treatment × Time	T_0	28	T_{10WW}	28	0.6963

Table A5 Differences of Least Squares Means of Nitrate between T_0 and T_{5MP}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
Treatment × Time	T_0	1	T_{5MP}	1	0.8255
Treatment × Time	T_0	2	T_{5MP}	2	0.0034
Treatment × Time	T_0	3	T_{5MP}	3	0.6357
Treatment × Time	T_0	4	T_{5MP}	4	0.1429
Treatment × Time	T_0	5	T_{5MP}	5	0.0302
Treatment × Time	T_0	6	T_{5MP}	6	0.0004
Treatment × Time	T_0	7	T_{5MP}	7	0.0003
Treatment × Time	T_0	8	T_{5MP}	8	0.0146
Treatment × Time	T_0	9	T_{5MP}	9	0.2034
Treatment × Time	T_0	10	T_{5MP}	10	0.5772
Treatment × Time	T_0	11	T_{5MP}	11	0.2861
Treatment × Time	T_0	12	T_{5MP}	12	0.2323
Treatment × Time	T_0	13	T_{5MP}	13	0.0456
Treatment × Time	T_0	14	T_{5MP}	14	0.5198
Treatment × Time	T_0	15	T_{5MP}	15	0.8583
Treatment × Time	T_0	16	T_{5MP}	16	0.9055
Treatment × Time	T_0	17	T_{5MP}	17	0.7384
Treatment × Time	T_0	18	T_{5MP}	18	0.7870
Treatment × Time	T_0	19	T_{5MP}	19	0.3938
Treatment × Time	T_0	20	T_{5MP}	20	0.1821
Treatment × Time	T_0	21	T_{5MP}	21	0.9877
Treatment × Time	T_0	22	T_{5MP}	22	0.7293
Treatment × Time	T_0	23	T_{5MP}	23	0.3055
Treatment × Time	T_0	24	T_{5MP}	24	0.8973
Treatment × Time	T_0	25	T_{5MP}	25	0.9764
Treatment × Time	T_0	26	T_{5MP}	26	0.8711
Treatment × Time	T_0	27	T_{5MP}	27	0.6897
Treatment × Time	T_0	28	T_{5MP}	28	0.6136

Table A6 Differences of Least Squares Means of Nitrate between T_0 and T_{10MP}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
Treatment × Time	T_0	1	T_{10MP}	1	<.0001
Treatment × Time	T_0	2	T_{10MP}	2	0.0002
Treatment × Time	T_0	3	T_{10MP}	3	0.4301
Treatment × Time	T_0	4	T_{10MP}	4	0.0164
Treatment × Time	T_0	5	T_{10MP}	5	0.0193
Treatment × Time	T_0	6	T_{10MP}	6	0.0005
Treatment × Time	T_0	7	T_{10MP}	7	<.0001
Treatment × Time	T_0	8	T_{10MP}	8	0.0004
Treatment × Time	T_0	9	T_{10MP}	9	0.1642
Treatment × Time	T_0	10	T_{10MP}	10	0.1490
Treatment × Time	T_0	11	T_{10MP}	11	0.0071
Treatment × Time	T_0	12	T_{10MP}	12	0.0053
Treatment × Time	T_0	13	T_{10MP}	13	0.0026
Treatment × Time	T_0	14	T_{10MP}	14	0.0787
Treatment × Time	T_0	15	T_{10MP}	15	0.4025
Treatment × Time	T_0	16	T_{10MP}	16	0.3776
Treatment × Time	T_0	17	T_{10MP}	17	0.6953
Treatment × Time	T_0	18	T_{10MP}	18	0.8533
Treatment × Time	T_0	19	T_{10MP}	19	0.7001
Treatment × Time	T_0	20	T_{10MP}	20	0.7854
Treatment × Time	T_0	21	T_{10MP}	21	0.8856
Treatment × Time	T_0	22	T_{10MP}	22	0.9571
Treatment × Time	T_0	23	T_{10MP}	23	0.7722
Treatment × Time	T_0	24	T_{10MP}	24	0.7639
Treatment × Time	T_0	25	T_{10MP}	25	0.3083
Treatment × Time	T_0	26	T_{10MP}	26	0.3089
Treatment × Time	T_0	27	T_{10MP}	27	0.6298
Treatment × Time	T_0	28	T_{10MP}	28	0.4796

Table A7 Differences of Least Squares Means of Nitrate between T_0 and T_{5HP}.

Effect	Treatment	Week	-Treatment	- Week	P-value > |t|
Treatment × Time	T_0	1	T_{5HP}	1	0.9520
Treatment × Time	T_0	2	T_{5HP}	2	0.7950
Treatment × Time	T_0	3	T_{5HP}	3	0.6361
Treatment × Time	T_0	4	T_{5HP}	4	0.0708
Treatment × Time	T_0	5	T_{5HP}	5	0.3480
Treatment × Time	T_0	6	T_{5HP}	6	<.0001
Treatment × Time	T_0	7	T_{5HP}	7	<.0001
Treatment × Time	T_0	8	T_{5HP}	8	0.0095
Treatment × Time	T_0	9	T_{5HP}	9	0.2107
Treatment × Time	T_0	10	T_{5HP}	10	0.0518
Treatment × Time	T_0	11	T_{5HP}	11	0.0084
Treatment × Time	T_0	12	T_{5HP}	12	0.0061
Treatment × Time	T_0	13	T_{5HP}	13	0.0112
Treatment × Time	T_0	14	T_{5HP}	14	0.0226
Treatment × Time	T_0	15	T_{5HP}	15	0.2068
Treatment × Time	T_0	16	T_{5HP}	16	0.5372
Treatment × Time	T_0	17	T_{5HP}	17	0.1950
Treatment × Time	T_0	18	T_{5HP}	18	0.1621
Treatment × Time	T_0	19	T_{5HP}	19	0.7135
Treatment × Time	T_0	20	T_{5HP}	20	0.0852
Treatment × Time	T_0	21	T_{5HP}	21	0.1478
Treatment × Time	T_0	22	T_{5HP}	22	0.5652
Treatment × Time	T_0	23	T_{5HP}	23	0.0024
Treatment × Time	T_0	24	T_{5HP}	24	0.0384
Treatment × Time	T_0	25	T_{5HP}	25	0.0956
Treatment × Time	T_0	26	T_{5HP}	26	0.4093
Treatment × Time	T_0	27	T_{5HP}	27	0.9664
Treatment × Time	T_0	28	T_{5HP}	28	0.9234

Table A8 Differences of Least Squares Means of Nitrate between T_0 and T_{10HP}.

Effect	Treatment	Week	-Treatment	- Week	P-value > |t|
Treatment × Time	T_0	1	T_{10HP}	1	0.9550
Treatment × Time	T_0	2	T_{10HP}	2	0.5533
Treatment × Time	T_0	3	T_{10HP}	3	0.4121
Treatment × Time	T_0	4	T_{10HP}	4	0.9432
Treatment × Time	T_0	5	T_{10HP}	5	0.0163
Treatment × Time	T_0	6	T_{10HP}	6	<.0001
Treatment × Time	T_0	7	T_{10HP}	7	<.0001
Treatment × Time	T_0	8	T_{10HP}	8	0.0003
Treatment × Time	T_0	9	T_{10HP}	9	0.0077
Treatment × Time	T_0	10	T_{10HP}	10	0.0063
Treatment × Time	T_0	11	T_{10HP}	11	0.0697
Treatment × Time	T_0	12	T_{10HP}	12	0.0270
Treatment × Time	T_0	13	T_{10HP}	13	0.0220
Treatment × Time	T_0	14	T_{10HP}	14	0.0644
Treatment × Time	T_0	15	T_{10HP}	15	0.3716
Treatment × Time	T_0	16	T_{10HP}	16	0.7896
Treatment × Time	T_0	17	T_{10HP}	17	0.7460
Treatment × Time	T_0	18	T_{10HP}	18	0.5507
Treatment × Time	T_0	19	T_{10HP}	19	0.8099
Treatment × Time	T_0	20	T_{10HP}	20	0.2035
Treatment × Time	T_0	21	T_{10HP}	21	0.6730
Treatment × Time	T_0	22	T_{10HP}	22	0.8110
Treatment × Time	T_0	23	T_{10HP}	23	0.2125
Treatment × Time	T_0	24	T_{10HP}	24	0.4338
Treatment × Time	T_0	25	T_{10HP}	25	0.6534
Treatment × Time	T_0	26	T_{10HP}	26	0.9392
Treatment × Time	T_0	27	T_{10HP}	27	0.9961
Treatment × Time	T_0	28	T_{10HP}	28	0.8429

Appendix B: Differences of Least Squares Means of Ammonium concentration between control treatment and biochar treatments at all experimental weeks

Table B1 Differences of Least Squares Means of Ammonium between T_0 and T_{5MH}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
Treatment × Time	T_0	1	T_{5MH}	1	0.0046
Treatment × Time	T_0	2	T_{5MH}	2	0.9569
Treatment × Time	T_0	3	T_{5MH}	3	0.9377
Treatment × Time	T_0	4	T_{5MH}	4	0.4174
Treatment × Time	T_0	5	T_{5MH}	5	0.0505
Treatment × Time	T_0	6	T_{5MH}	6	0.7711
Treatment × Time	T_0	7	T_{5MH}	7	0.8686
Treatment × Time	T_0	8	T_{5MH}	8	0.9575
Treatment × Time	T_0	9	T_{5MH}	9	0.8626
Treatment × Time	T_0	10	T_{5MH}	10	0.7423
Treatment × Time	T_0	11	T_{5MH}	11	0.9440
Treatment × Time	T_0	12	T_{5MH}	12	0.8139
Treatment × Time	T_0	13	T_{5MH}	13	0.8839
Treatment × Time	T_0	14	T_{5MH}	14	0.9188
Treatment × Time	T_0	15	T_{5MH}	15	0.9136
Treatment × Time	T_0	16	T_{5MH}	16	0.7933
Treatment × Time	T_0	17	T_{5MH}	17	0.7141
Treatment × Time	T_0	18	T_{5MH}	18	0.8469
Treatment × Time	T_0	19	T_{5MH}	19	0.7614
Treatment × Time	T_0	20	T_{5MH}	20	0.8011
Treatment × Time	T_0	21	T_{5MH}	21	0.7298
Treatment × Time	T_0	22	T_{5MH}	22	0.3697
Treatment × Time	T_0	23	T_{5MH}	23	0.5174
Treatment × Time	T_0	24	T_{5MH}	24	0.7268
Treatment × Time	T_0	25	T_{5MH}	25	0.7806
Treatment × Time	T_0	26	T_{5MH}	26	0.7215
Treatment × Time	T_0	27	T_{5MH}	27	0.9715
Treatment × Time	T_0	28	T_{5MH}	28	0.9795

Table B2 Differences of Least Squares Means of Ammonium between T_0 and T_{10MH}.

Effect	**Treatment**	**Week**	**-Treatment**	**-Week**	**P-value > |t|**
Treatment × Time	T_0	1	T_{10MH}	1	0.3871
Treatment × Time	T_0	2	T_{10MH}	2	0.0169
Treatment × Time	T_0	3	T_{10MH}	3	0.8633
Treatment × Time	T_0	4	T_{10MH}	4	0.1687
Treatment × Time	T_0	5	T_{10MH}	5	0.6763
Treatment × Time	T_0	6	T_{10MH}	6	0.4480
Treatment × Time	T_0	7	T_{10MH}	7	0.3222
Treatment × Time	T_0	8	T_{10MH}	8	0.2803
Treatment × Time	T_0	9	T_{10MH}	9	0.2287
Treatment × Time	T_0	10	T_{10MH}	10	0.2086
Treatment × Time	T_0	11	T_{10MH}	11	0.4063
Treatment × Time	T_0	12	T_{10MH}	12	0.4343
Treatment × Time	T_0	13	T_{10MH}	13	0.2882
Treatment × Time	T_0	14	T_{10MH}	14	0.2584
Treatment × Time	T_0	15	T_{10MH}	15	0.3376
Treatment × Time	T_0	16	T_{10MH}	16	0.2210
Treatment × Time	T_0	17	T_{10MH}	17	0.2719
Treatment × Time	T_0	18	T_{10MH}	18	0.1624
Treatment × Time	T_0	19	T_{10MH}	19	0.0892
Treatment × Time	T_0	20	T_{10MH}	20	0.0928
Treatment × Time	T_0	21	T_{10MH}	21	0.1305
Treatment × Time	T_0	22	T_{10MH}	22	0.8449
Treatment × Time	T_0	23	T_{10MH}	23	0.0869
Treatment × Time	T_0	24	T_{10MH}	24	0.0462
Treatment × Time	T_0	25	T_{10MH}	25	0.0986
Treatment × Time	T_0	26	T_{10MH}	26	0.0425
Treatment × Time	T_0	27	T_{10MH}	27	0.3021
Treatment × Time	T_0	28	T_{10MH}	28	0.7305

Table B3 Differences of Least Squares Means of Ammonium between T_0 and T_{5WW}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
Treatment × Time	T_0	1	T_{5WW}	1	0.0511
Treatment × Time	T_0	2	T_{5WW}	2	0.1128
Treatment × Time	T_0	3	T_{5WW}	3	0.9361
Treatment × Time	T_0	4	T_{5WW}	4	0.1788
Treatment × Time	T_0	5	T_{5WW}	5	0.4667
Treatment × Time	T_0	6	T_{5WW}	6	0.9693
Treatment × Time	T_0	7	T_{5WW}	7	0.9722
Treatment × Time	T_0	8	T_{5WW}	8	0.9406
Treatment × Time	T_0	9	T_{5WW}	9	0.9376
Treatment × Time	T_0	10	T_{5WW}	10	0.9154
Treatment × Time	T_0	11	T_{5WW}	11	0.9157
Treatment × Time	T_0	12	T_{5WW}	12	0.4944
Treatment × Time	T_0	13	T_{5WW}	13	0.7945
Treatment × Time	T_0	14	T_{5WW}	14	0.7076
Treatment × Time	T_0	15	T_{5WW}	15	0.6794
Treatment × Time	T_0	16	T_{5WW}	16	0.8936
Treatment × Time	T_0	17	T_{5WW}	17	0.8248
Treatment × Time	T_0	18	T_{5WW}	18	0.8643
Treatment × Time	T_0	19	T_{5WW}	19	0.9122
Treatment × Time	T_0	20	T_{5WW}	20	0.9112
Treatment × Time	T_0	21	T_{5WW}	21	0.9074
Treatment × Time	T_0	22	T_{5WW}	22	0.3886
Treatment × Time	T_0	23	T_{5WW}	23	0.8697
Treatment × Time	T_0	24	T_{5WW}	24	0.8210
Treatment × Time	T_0	25	T_{5WW}	25	0.5578
Treatment × Time	T_0	26	T_{5WW}	26	0.6640
Treatment × Time	T_0	27	T_{5WW}	27	0.6400
Treatment × Time	T_0	28	T_{5WW}	28	0.4209

Table B4 Differences of Least Squares Means of Ammonium between T_0 and T_{10WW}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
Treatment × Time	T_0	1	T_{10WW}	1	0.7935
Treatment × Time	T_0	2	T_{10WW}	2	0.2584
Treatment × Time	T_0	3	T_{10WW}	3	0.4907
Treatment × Time	T_0	4	T_{10WW}	4	0.1200
Treatment × Time	T_0	5	T_{10WW}	5	0.4578
Treatment × Time	T_0	6	T_{10WW}	6	0.9966
Treatment × Time	T_0	7	T_{10WW}	7	0.9017
Treatment × Time	T_0	8	T_{10WW}	8	0.5942
Treatment × Time	T_0	9	T_{10WW}	9	0.5469
Treatment × Time	T_0	10	T_{10WW}	10	0.4392
Treatment × Time	T_0	11	T_{10WW}	11	0.3954
Treatment × Time	T_0	12	T_{10WW}	12	0.3006
Treatment × Time	T_0	13	T_{10WW}	13	0.4682
Treatment × Time	T_0	14	T_{10WW}	14	0.4090
Treatment × Time	T_0	15	T_{10WW}	15	0.4156
Treatment × Time	T_0	16	T_{10WW}	16	0.3566
Treatment × Time	T_0	17	T_{10WW}	17	0.3938
Treatment × Time	T_0	18	T_{10WW}	18	0.3261
Treatment × Time	T_0	19	T_{10WW}	19	0.3478
Treatment × Time	T_0	20	T_{10WW}	20	0.2339
Treatment × Time	T_0	21	T_{10WW}	21	0.4820
Treatment × Time	T_0	22	T_{10WW}	22	0.3601
Treatment × Time	T_0	23	T_{10WW}	23	0.6627
Treatment × Time	T_0	24	T_{10WW}	24	0.9708
Treatment × Time	T_0	25	T_{10WW}	25	0.7860
Treatment × Time	T_0	26	T_{10WW}	26	0.9763
Treatment × Time	T_0	27	T_{10WW}	27	0.6786
Treatment × Time	T_0	28	T_{10WW}	28	0.1715

Table B5 Differences of Least Squares Means of Ammonium between T_0 and T_{5MP}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
trt*time	T_0	1	T_{5MP}	1	0.1155
trt*time	T_0	2	T_{5MP}	2	0.9817
trt*time	T_0	3	T_{5MP}	3	0.6640
trt*time	T_0	4	T_{5MP}	4	0.2292
trt*time	T_0	5	T_{5MP}	5	0.3523
trt*time	T_0	6	T_{5MP}	6	0.0200
trt*time	T_0	7	T_{5MP}	7	0.1586
trt*time	T_0	8	T_{5MP}	8	0.2452
trt*time	T_0	9	T_{5MP}	9	0.2597
trt*time	T_0	10	T_{5MP}	10	0.3379
trt*time	T_0	11	T_{5MP}	11	0.3511
trt*time	T_0	12	T_{5MP}	12	0.4734
trt*time	T_0	13	T_{5MP}	13	0.4733
trt*time	T_0	14	T_{5MP}	14	0.9607
trt*time	T_0	15	T_{5MP}	15	0.7621
trt*time	T_0	16	T_{5MP}	16	0.9142
trt*time	T_0	17	T_{5MP}	17	0.8226
trt*time	T_0	18	T_{5MP}	18	0.8615
trt*time	T_0	19	T_{5MP}	19	0.2619
trt*time	T_0	20	T_{5MP}	20	0.5578
trt*time	T_0	21	T_{5MP}	21	0.4321
trt*time	T_0	22	T_{5MP}	22	0.9230
trt*time	T_0	23	T_{5MP}	23	0.6045
trt*time	T_0	24	T_{5MP}	24	0.4772
trt*time	T_0	25	T_{5MP}	25	0.5069
trt*time	T_0	26	T_{5MP}	26	0.9004
trt*time	T_0	27	T_{5MP}	27	0.8821
trt*time	T_0	28	T_{5MP}	28	0.3233

Table B6 Differences of Least Squares Means of Ammonium between T_0 and T_{10MP}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
Treatment × Time	T_0	1	T_{10MP}	1	0.0999
Treatment × Time	T_0	2	T_{10MP}	2	0.3352
Treatment × Time	T_0	3	T_{10MP}	3	0.0568
Treatment × Time	T_0	4	T_{10MP}	4	0.0020
Treatment × Time	T_0	5	T_{10MP}	5	0.0075
Treatment × Time	T_0	6	T_{10MP}	6	0.0004
Treatment × Time	T_0	7	T_{10MP}	7	0.0058
Treatment × Time	T_0	8	T_{10MP}	8	0.0439
Treatment × Time	T_0	9	T_{10MP}	9	0.0805
Treatment × Time	T_0	10	T_{10MP}	10	0.0407
Treatment × Time	T_0	11	T_{10MP}	11	0.0180
Treatment × Time	T_0	12	T_{10MP}	12	0.0160
Treatment × Time	T_0	13	T_{10MP}	13	0.0154
Treatment × Time	T_0	14	T_{10MP}	14	0.1442
Treatment × Time	T_0	15	T_{10MP}	15	0.5009
Treatment × Time	T_0	16	T_{10MP}	16	0.9608
Treatment × Time	T_0	17	T_{10MP}	17	0.9579
Treatment × Time	T_0	18	T_{10MP}	18	0.8900
Treatment × Time	T_0	19	T_{10MP}	19	0.6130
Treatment × Time	T_0	20	T_{10MP}	20	0.9254
Treatment × Time	T_0	21	T_{10MP}	21	0.7005
Treatment × Time	T_0	22	T_{10MP}	22	0.8371
Treatment × Time	T_0	23	T_{10MP}	23	0.9318
Treatment × Time	T_0	24	T_{10MP}	24	0.4064
Treatment × Time	T_0	25	T_{10MP}	25	0.1466
Treatment × Time	T_0	26	T_{10MP}	26	0.1843
Treatment × Time	T_0	27	T_{10MP}	27	0.1113
Treatment × Time	T_0	28	T_{10MP}	28	0.0410

Table B7 Differences of Least Squares Means of Ammonium between T_0 and T_{5HP}.

Effect	Treatment	Week	-Treatment	-Week	P-value > |t|
Treatment × Time	T_0	1	T_{5HP}	1	0.0015
Treatment × Time	T_0	2	T_{5HP}	2	0.9794
Treatment × Time	T_0	3	T_{5HP}	3	0.6874
Treatment × Time	T_0	4	T_{5HP}	4	0.6325
Treatment × Time	T_0	5	T_{5HP}	5	0.3093
Treatment × Time	T_0	6	T_{5HP}	6	0.0041
Treatment × Time	T_0	7	T_{5HP}	7	0.0049
Treatment × Time	T_0	8	T_{5HP}	8	0.0064
Treatment × Time	T_0	9	T_{5HP}	9	0.0060
Treatment × Time	T_0	10	T_{5HP}	10	0.0042
Treatment × Time	T_0	11	T_{5HP}	11	0.0195
Treatment × Time	T_0	12	T_{5HP}	12	0.0930
Treatment × Time	T_0	13	T_{5HP}	13	0.0809
Treatment × Time	T_0	14	T_{5HP}	14	0.0751
Treatment × Time	T_0	15	T_{5HP}	15	0.2006
Treatment × Time	T_0	16	T_{5HP}	16	0.3045
Treatment × Time	T_0	17	T_{5HP}	17	0.1084
Treatment × Time	T_0	18	T_{5HP}	18	0.1318
Treatment × Time	T_0	19	T_{5HP}	19	0.3843
Treatment × Time	T_0	20	T_{5HP}	20	0.0734
Treatment × Time	T_0	21	T_{5HP}	21	0.0169
Treatment × Time	T_0	22	T_{5HP}	22	0.3138
Treatment × Time	T_0	23	T_{5HP}	23	0.0673
Treatment × Time	T_0	24	T_{5HP}	24	0.2307
Treatment × Time	T_0	25	T_{5HP}	25	0.1548
Treatment × Time	T_0	26	T_{5HP}	26	0.1511
Treatment × Time	T_0	27	T_{5HP}	27	0.7125
Treatment × Time	T_0	28	T_{5HP}	28	0.8727

Table B8 Differences of Least Squares Means of Ammonium between T_0 and T_{10HP}.

Effect	**Treatment**	**Week**	**-Treatment**	**-Week**	**P-value > |t|**
Treatment × Time	T_0	1	T_{10HP}	1	0.0052
Treatment × Time	T_0	2	T_{10HP}	2	0.2292
Treatment × Time	T_0	3	T_{10HP}	3	0.7896
Treatment × Time	T_0	4	T_{10HP}	4	0.2781
Treatment × Time	T_0	5	T_{10HP}	5	0.2007
Treatment × Time	T_0	6	T_{10HP}	6	0.0019
Treatment × Time	T_0	7	T_{10HP}	7	0.0132
Treatment × Time	T_0	8	T_{10HP}	8	0.0207
Treatment × Time	T_0	9	T_{10HP}	9	0.0144
Treatment × Time	T_0	10	T_{10HP}	10	0.0214
Treatment × Time	T_0	11	T_{10HP}	11	0.2783
Treatment × Time	T_0	12	T_{10HP}	12	0.2117
Treatment × Time	T_0	13	T_{10HP}	13	0.1629
Treatment × Time	T_0	14	T_{10HP}	14	0.1134
Treatment × Time	T_0	15	T_{10HP}	15	0.5033
Treatment × Time	T_0	16	T_{10HP}	16	0.3168
Treatment × Time	T_0	17	T_{10HP}	17	0.7090
Treatment × Time	T_0	18	T_{10HP}	18	0.6378
Treatment × Time	T_0	19	T_{10HP}	19	0.6850
Treatment × Time	T_0	20	T_{10HP}	20	0.7819
Treatment × Time	T_0	21	T_{10HP}	21	0.8081
Treatment × Time	T_0	22	T_{10HP}	22	0.9845
Treatment × Time	T_0	23	T_{10HP}	23	0.8827
Treatment × Time	T_0	24	T_{10HP}	24	0.7134
Treatment × Time	T_0	25	T_{10HP}	25	0.7118
Treatment × Time	T_0	26	T_{10HP}	26	0.8160
Treatment × Time	T_0	27	T_{10HP}	27	0.7492
Treatment × Time	T_0	28	T_{10HP}	28	0.1096