Effect of Biochar on Ammonification and Nitrification in a Coarse Sandy Soil

by

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Abstract

The addition of biochar to soil is believed to have positive effects on soil nutrient retention. Enhanced cation exchange capacity, water holding capacity and soil aeration are thought to be some of the benefits provided by biochar. In Alberta, reclamation of disturbed sites may be hastened by the addition of soil amendments and biochar is being studied as one possible option. More conventional amendments such as chemical fertilizer, compost, peat and forest floor material have been previously studied and compared in a reclamation setting.

The objectives of the work presented in this thesis are to determine the effects of biochar on: 1) the fate of nitrogen applied to a nutrient-deficient, coarse-textured forest soil in the form of both inorganic and organic fertilizers; 2) the biological processes of ammonification and nitrification 3) the physical attributes responsible for nitrogen retention such as sorption of organic nitrogen and ammonium by negatively charged sites.

The results of the experiments summarized in this thesis found that biochar reduced nitrogen leaching at an application rate of 25 tonne ha^{-1} and that biochar increased soil retention of nitrogen fertilizer, however the biological effects of biochar on ammonification and nitrification of soil organic nitrogen, can lead to nitrogen losses from soil, offsetting the increased storage capacity. The alteration of soil biogeochemistry by biochar in this experiment resulted in increased nitrification.

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Special thanks to my loving and supportive parents, on whom I’ve relied countless times to help out, fill in and step up. I intend to spend more of my time enjoying your company and trying to pay you back for a lifetime of care. And finally, a big thanks to my wife, Melany who has listened to me talk excitedly about soil since our first date and who has nodded and smiled ever since.

To anyone continuing the study of intricate details of soil science: the energy you invest pursuing your work in whatever subject is important to you is what propels science forward. No contribution is too small.

“That which can be asserted without evidence, can be dismissed without evidence.”
—Christopher Hitchens

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Chapter 1. Introduction

1.1. Disturbance and Reclamation in Upstream Oil and gas

The boreal forest covers 713,787 km^2 in Canada. Of this, 381,444 km^2 is located within Alberta`s borders (NCC, 2014) and about 31% of the Canadian boreal zone has been accessed for industrial development (Anielski and Wilson, 2009).

Both natural and anthropogenic disturbances in Canada's boreal forests are common. Oil and gas activity, forestry, agriculture and mining all contribute to disturbances of varying scale (Government of Alberta, 2012). The nature of disturbances vary widely based on the type of development being undertaken. Disturbances caused during oil and gas exploration and production range from spatially limited soil compaction during selective logging undertaken during seismic exploration (Revel et al. 1984), to removal of vegetation and top soil over a one-hectare lease (landform scale), to removal of vegetation, topsoil and meters of mineral soil during surface mining activities in oil sands production on a landscape scale. The Alberta Government requires companies responsible for development and disturbance areas to reclaim disturbed lands. The scale of the disturbance is a key component in determining the objectives and metrics for it reclamation.

Environmental study and research has allowed for greater understanding of ecosystems and has provided greater insight into the complexity and interconnectedness of their components. Soils, hydrology, vegetation, and wildlife are no longer seen as separate fields of study and must be considered holistically when planning, developing and reclaiming a given area following disturbance.

Upstream oil and gas development in Alberta takes place across all ecoregions (Powter et al. 2011), therefore reclamation practices must be adjusted to account for both the specific site conditions and ecological functions as well as the desired future use of the area to be reclaimed.

Owing to the location of resource rich areas in Alberta, much of the province's resource extraction development takes place on forested lands in the green zone. Provincial regulations require reclamation of forested areas to land capability equivalent to pre-disturbance conditions. Equivalent capability is defined as "t*he ability of the land to support various land uses after conservation and reclamation similar to the ability that existed prior to an activity being conducted on the land. The individual land uses will not necessarily be identical."* (Alberta Environment, 2000).

Alberta was the first province to enact land reclamation regulations in 1963 (Powter et al. 2011). Since that time, changes in regulations and research have spawned innovation and both reclamation practices and regulations have become more prescriptive as industry, regulators and the public perceive a need for reclamation and have endeavored to improve environmental performance over time.

Measurement of previous reclamation efforts (Macyk and Drozdowski, 2008) and study of recovery from other disturbances (such as fires) have helped to improve reclamation practices (Errington and Pinno, 2015). However, disturbance from development of oil and gas resources has proceeded beyond the range of variability historically associated with natural disturbances (Pickell et al. 2015).

While reclamation practices have improved, predicting the long term effects of various practices remains hampered by uncertainty owing to the complex ecological functions of various systems, uncertainty of the effects of external perturbations such as climate change and difficulty in determining what constitutes reclamation success. The specifics of reclamation regulations include conservation of topsoil, removal and/or decompaction of drilling pads and compacted soils, drainage, erosion control, contouring, and ensuring that vegetation quality and quantity are similar to control areas (Alberta Environment 2007). Compliance may be measured and evaluated against criteria consisting of a mixture of absolute values as well as relative values determined from nearby undisturbed areas (Alberta Environment 2007). Topsoil thickness, organic matter content, and vegetative cover are examples of measurements which may be used to evaluate reclamation status. As with reclamation objectives, reclamation metrics vary with the scale of the disturbance.

During oil and gas exploration and production, oilsands mining and installation of hydrocarbon pipelines, forested areas may be cleared and surface organic and mineral soils removed. Removal and storage or reuse of organic layers during disturbance is a common practice and upon decommissioning of oil and gas facilities, a portion of the topsoil may be recovered and replaced, however the replacement of soil profiles following disturbance unavoidably alters the soil characteristics which were developed over geologic time scales. As a result, many sites exhibit poor soil properties caused by physical circumstances (e.g. compaction) or deficiencies of needed components (e.g. soil organic matter or specific nutrients) and contain soils which do not function at a level equivalent to undisturbed areas which may inhibit full recovery of these sites to a productive state. These soils may be less able to retain nutrients, or may be structurally poor thereby limiting plant growth. The amount of organic matter present is generally associated with soil fertility as organic matter is the source of plant nutrients and also affects moisture regime, soil chemical properties such as buffering capacity and cation exchange capacity. These qualities are important in controlling nutrient supply, retention and availability for plant uptake (Kornakova, 1966).

Revegetation outcomes are largely dependent on soil management practices, hence the emphasis on soil properties within Alberta's reclamation criteria. The water and nutrient status of a site are foundational to reclamation, however, the loss of local seed and bud stores can also impact primary succession on disturbed sites resulting in the establishment of invasive species or the persistence of disturbance features on the landscape for several decades. (MacFarlane 1999, Osko and MacFarlane, 2001).

1.2. Reclamation Approaches for Forest Ecosystems

Reclamation of Forest ecosystems is a complex task. Before reclamation begins it is important to determine endpoints and indicators which will define when reclamation is achieved. This can be difficult because the future uses and expectations for forested areas are not always well defined. The future proposed land use, potential for logging, and adaptation to future scenarios such as climate change must all be considered when planning reclamation and defining success in the short term (Audet et al. 2014). Restoration of the ecosystem and thus reclamation success is achieved when disturbed areas are indistinguishable from undisturbed. However, because this may not occur within a timeframe that allows for management strategies to be validated, these considerations must be accounted for in reclamation planning (Pinno and Hawkes, 2015). Therefore, indicators must demonstrate not only success but also provide the trajectory that will allow prediction of the reclamation timeline and outcome. This difficulty in setting indicators and endpoints grows as the size of the disturbance grows and undisturbed adjacent areas become spatially distant and dissimilar to that of the area to be reclaimed. It should be recognized that reclamation approaches also depend upon the metrics imposed by the standard or regulations which are being referenced in order to determine reclamation trajectory and success. The type of industry, property ownership and scale of the disturbance may trigger different types of standards and regulations being applied to the site. For example mining operations in Alberta are subject to different closure and reclamation standards than conventional oil and gas operations. These differences are often due to variability in scale. For example reclamation of a 1 hectare oil lease can be assessed against the immediately surrounding area to determine the differences in “on site” (disturbed) versus “off site” (undisturbed) areas.

The time frame for revegetation of forests is highly variable depending upon factors such as climate, hydrological regime and elevation. Short growing seasons tend to slow recovery in boreal forests. Achieving canopy closure with diverse species is important but difficult in short timeframes on relatively unproductive sites (Strong, 2000; Huang et al. 2013). As well, a major challenge in reclamation is the need for early practices to set a course for long-term success. Therefore, in order to predict the effects of reclamation strategies significant research is required.

A number of innovative techniques have been developed to improve reclamation of forested sites. These include planning techniques such as preservation of the forest floor layer by using soil cover as opposed to stripping and replacing topsoil (Bachmann et al. 2014) or conservation of small stands to serve as propagule banks across large disturbances (Lamas et al. 2015). Physical techniques such as decompaction and mounding improve soil characteristics and provide microsites for vegetation establishment. Practices which improve soil structure will speed reclamation by ensuring that plant roots can penetrate soils, sufficient water is contained within the soils to support desired vegetation, and ensure that the soil is stable and resistant to erosion. Adding vegetation through seeding or planting is valuable as it establishes soil cover to prevent erosion and begins nutrient cycling (Sheoran et al.2010). As well, the addition of materials to assist ecosystem recovery has been practiced for a number of years (Sheoran et al. 2010). Adding nutrient rich materials such as organic amendments can improve physical characteristics and add plant available nutrients. Chemical fertilizers may also be used to add nutrients to soil. Given sufficient time, most disturbed sites will revert back to a more “natural” productive state, however functioning at a lower level during this recovery time means that ecosystem services may not be provided at the same level as an undisturbed site.

Choosing effective reclamation approaches for forest sites is made difficult by the dynamic and interdependent nature of forest ecosystem components which lead to constantly changing conditions. This makes determination of reclamation trajectories difficult. For example, the removal of trees results in higher transmission of light to the forest floor thus increasing mineralization and nutrient loss (Hart and Chen, 2006). As canopy closure proceeds mineralization rates will alter with a changing light and temperature regime.

Soil moisture status resulting from the effects of evapotranspiration, water holding capacity and precipitation patterns is a key variable in predicting vegetation species, rate of litter decomposition, forest turnover and thus the buildup of soil organic matter (Osman, 2013). However, other factors such as soil pH and nutrient availability which have been found to be predictive of microbial activity and changes in plant species composition (Brocket et al. 2012). These factors may be less influential than moisture on overall productivity but still controlling of vegetative growth, nutrient cycling and soil development for reclamation (Macdonald et al. 2012). Chapin (1983) estimated that the annual turnover of biomass and nutrients in boreal understory vegetation is 34–43% compared to only 2–5% in trees thus, as succession proceeds, nutrient cycling and storage changes with species present. Generally, amendment addition is undertaken to alter soil moisture, soil chemistry and nutrient status and the net effect of adding amendments may be hard to predict and, given complex dynamics, it can be difficult to determine whether or not a soil amendment should be used, how quickly it will release nutrients into soil, and how long these effects might persist and whether the result is beneficial.

1.3. Nitrogen in Forest Ecosystems

Limiting nutrients are those which are exhausted first and will limit cellular growth (either in plants or soil organisms). Nitrogen is often the focus of soil nutrient studies as it is considered the most important element in plant nutrition and growth and is often considered the limiting nutrient in forest ecosystems (Tamm, 1991). Previous work has indicated that carbon may be limiting in clay soils and nitrogen may more often be limited in coarse soils and that lack of available nitrogen may limit mineralization and slow nitrogen cycling in sandy soils (Bimuller et al. 2014). The concept of limiting nutrients is often valuable in study of agrology as it relates to agricultural systems. In forest ecosystems there may be wide variability in the availability, speciation and cycling of nutrients. In response the character and nature of the forest may be substantially different owing to nutrient status among other attributes major examples of this would include moisture regime, topographical features (elevation, aspect and slope) surrounding ecosites and plant animal interactions (Xiaomei, et al 2016; Pulla et al. 2016). In other words, the nature of the ecosite is an adaptation to the site conditions. Nitrogen dominates forest nutrition (Fisher and Binkley, 2013), therefore, having a fuller understanding how management techniques promote or restrict availability of nitrogen for capture and uptake by plants is beneficial in adaptive management of reclamation activities.

Nutrient pools and fluxes are an important concept when considering ecosystems. With respect to nitrogen, pools are the forms in which it is stored such as proteins within plants or as N_2 within the atmosphere while fluxes consist of the biotic and abiotic process which alter the forms of nitrogen, transferring nitrogen between pools such as decomposition of plant matter or fixation of atmospheric nitrogen. Residence time may be highly variable between nitrogen pools, for example nitrogen in wood and lignin attached to phenolic and cyclic compounds is much more difficult to mineralize than that coming from soil organisms (Paul and Clark, 1998) therefore availability of nitrogen depends on the recalcitrance of the pool.

The process of nutrient movement through functioning ecosystems is cyclical, with plants and microbes taking up the inorganic forms of nitrogen, incorporating these into biomass and eventually releasing organic nitrogen as litter fall, excretions or dead cells/tissues at the end of life. The soil environment (including factors such as pH, temperature, oxygen level, water status etc.) directly impacts these fluxes by influencing available energy, oxygen and water available to the microbes which are primarily responsible for these processes in soil.

Industrial disturbance often represents an interruption to these cycles with entire reservoirs of nitrogen being removed and/or reintroduced at various scales. Revegetation of disturbed sites dramatically alters nitrogen cycling depending upon the type of vegetation present. During forest reclamation, nitrogen cycling and availability can be used as a metric by which undisturbed and disturbed forests may be compared to determine reclamation trajectory and success. Transformation between oxidation states, organic and inorganic forms and thus location in soils is mediated by soil organisms. Nitrogen is readily found and commonly transformed between the inorganic forms NO_2^- NO_3^- and NH_4^+ (Paul and Clark 1998). Organic nitrogen molecules may be water soluble or found within the structures of soil organic matter or microbial biomass. The water soluble, inorganic forms of nitrogen are more mobile and may be taken up by plants. While a number of processes are responsible for nitrogen transformation, two of the most significant processes are ammonification (the depolymerisation of nitrogen containing polymers into nitirogen's reduced form (ammonia) and nitrification. Rather than focusing on the entire nitrogen cycle, the focus of this thesis will be on these two major nitrogen fluxes (ammonification and nitrification). Mineralization is the process by which organic molecules contained in decomposing plant material and soil biomass is converted from organic to inorganic forms. Ammonification may be differentiated from mineralization by it specificity toward nitrogen rather than conversion of organic molecules to inorganic forms. Nitrification is the oxidation of available ammonium in the soil to nitrite and, subsequently to nitrate. These two processes are depicted within the context of the nitrogen cycle in figure 1-1.

The fluxes associated with the nitrogen cycle may be occurring simultaneously resulting in net fluxes which are the result opposing gross fluxes. The soil conditions which dictate the microbial activity associated with these processes account for the magnitude of these fluxes however, some of these transformations may also occur via abiotic mechanisms.

As it is a microbial process, ammonification is altered by soil conditions which directly impact microbial activity. As well, depending upon the origin of the material, the substrate, soil organic matter, is comprised of different chemical constituents which decompose at different rates. Soil organic matter also contains mineral constituents of varying particle sizes which affect stability of soil organic matter. In estimating rates of ammonification, rough calculations may be made which determine C:N ratio in litter and soil organic matter. Generally, high C:N ratios are associated with low rates of mineralization as there is not sufficient nitrogen to support microbial processes (Xiong et al. 2014). This effect however, depends upon the degradability of the organic matter. Mineralization in heavy organic matter fractions (considered less degradable) was found to increase with increased C:N, while light fraction mineralization decreases with increasing C:N due to immobilization (Swanston et al. 2004). As well, seasonality impacts mineralization rates and availability of soil nitrogen with mineralization increasing during snow melt and microbial biomass and available inorganic nitrogen decreasing from January to October (Duran et al. 2014, Freppaz et al. 2014).

Nitrification is the oxidation of NH_4^+ to NO_2^- which is then be further oxidized to NO_3^-. These reactions are primarily microbially driven as archaea and bacteria use these reactions to derive energy. Similar to mineralization/ammonification, the conditions which effect nitrification and denitrification include pH, oxygen level and water, however oxygen concentration plays a dominant role in these reactions with nitrification primarily occurring in aerobic conditions and denitrification occurring in anaerobic conditions. Speciation is a major factor in sorption of nitrogen in soil, positively charged NH_4^+ and dissolved organic nitrogen are adsorbed onto non-polar the negatively charged and polar organic soil sites while negatively charged NO_2^- and NO_3^- reside in soil pore water and, remain unadsorbed and are transported with water flow. Nitrification of ammonium is an acidifying process resulting in lower soil pH (De Vries and Breeuwsma,1987).

1.4. Soil amendments in Forest Reclamation

Using soil amendments for reclamation is an area of continued study. The physical and chemical properties of amendments are simple to determine, and the positive or negative short term outcomes may be measured easily on the amended sites and comparisons may be made against desired values and/or un-amended control sites. However, the long-term physical, chemical and biological soil properties changes resulting from application of amendments are less well understood and require more study as these properties become interrelated over time. Ideally, enhanced plant growth on disturbed sites will capture nutrients provided by reclamation amendments thus preventing nutrient losses through leaching and re-establishing the nutrient cycle on the site (Pinno et al. 2014). However selecting amendments, and the frequency and rates of application is difficult due to poor understanding of long term effects. Additional uncertainty occurs with external perturbations such as deposition of nitrogen from anthropogenic and natural sources.

Chemical fertilizers and organic amendments have both been used to hasten reclamation. Chemical fertilizers add nutrients while organic amendments are added to improve soil structure as well as adding nutrients (Watts et al. 2012). Often, the use of organic soil amendments on reclamation sites will provide a substitute for naturally formed soil organic matter rather than waiting for natural processes to form SOM through the addition of organic material by plant litter.

The rates of nutrient release are important for forest growth. Forest fertilization experiments have shown that low rates of nutrient application over longer periods of time result in better tree growth responses (Fisher and Binkley, 2012) and that slower release of nutrients and the improvement of soil structure may provide long term benefit to forests (Larcheveque et al. 2011), however other work has shown that addition of nitrogen to N limited systems may result in microbial immobilization (Bengtsson & Bergwall 2000) indicating that not all amendments have positive effects and their use requires optimization. Further, nitrogen limited ecosytems

1.4.1.Chemical Fertilizers

The use chemical fertilizer to add nutrients (primarily nitrogen and phosphorus) has been researched extensively particularly in commercial forestry applications. Fertilizer addition often achieves the short term goal of vegetative growth however, the longer term effects on species and succession is less certain as chemical fertilization may also support competing understorey vegetation rather than desired tree species (Jacobs et al. 2005). Fertilizer effectiveness in reclamation is also highly dependent upon soil type and which nutrients are limiting (Pinno et al. 2014). As well, chemical fertilizers do not add organic matter or structure to deficient soils. This means that physical aspects such as water holding capacity or root penetration are not immediately improved however, soil physical properties may improve over longer terms owing to improved vegetative growth and the resulting organic matter inputs. Losses from leaching may be substantial if the physical soil properties do not support plant growth and nutrient storage.

1.4.2. Compost

Compost has been used for reclamation of boreal forest sites for a number of years. It is considered desirable as it improves both physical structure of soils, increases microbial activity and provides nutrients (Bresson et al. 2001; Vangronsveld et al. 1996). The release of nitrogen from compost is gradual as some of the organic matter is quite recalcitrant. The rate of ammonification from composts is slower and generally follows first order kinetics with the release of nitrogen occurring at a rate proportional to the concentration of organic nitrogen forms in the soil (Chalk et al. 2013). However, as with most amendments, the C:N ratios of the soil/compost mixture will also affect the ammonification rate. Wolkowski (2003) found that N fertilizer provided more available nitrate than compost as only 6-17% of the nitrogen in compost became available in the first year.

As compost is often created from waste materials, chemical contaminants such as metals or physical contaminants such as unseparated glass or plastic may be present and could have negative effects on vegetation (Francis et al. 2013).

Soil physical characteristics may be improved by compost addition, however. Whezlan et al. (2013) found that even at high rates of application and deep incorporation, compost may not drastically improve hydraulic functioning (i.e. improvement of drainage in clay soils or increased water retention of sandy soils). While compost is used to add nitrogen to soil it has also been demonstrated to reduce nitrogen leaching (Elbl et al. 2014) but contains inorganic constituents which result in elevated electrical conductivity. Other research has shown that MSW compost can successfully replace peat additions in a growing medium showing similar water holding capacity and porosity (Garcia et al 1998).

1.4.3. Forest Floor Material

Forest floor material has been evaluated as a soil amendment largely in oil sands mining operations as organic material from areas to be mined can be removed and used for reclamation at former mined areas (Errington and Pinno, 2015). Forest floor is often compared to peat moss as a reclamation amendment as both are available for use nearby developments. Previous research indicates that forest floor material compares favorably against peat as microbial communities are consistent with those of upland forests. As well, forest floor materials will act as a source of propagules aiding the establishment of understory and canopy plant species (Errington and Pinno, 2015). Forest floor material is a limited resource and only somewhat plentiful in areas where further disturbance necessitates its removal but it may be impractical for use on remote sites.

1.4.4. Peat

Peat has been used for reclamation of disturbed sites for many years as it is readily available in large quantities. In conventional oil and gas, peat may be harvested and hauled to reclamation sites. In oilsands mining, a mixture of peat and mineral soils is created by overstripping peat deposits (Macyk and Drozdowski 2008). In recent years, the use of peat has come under scrutiny as harvesting of peat from non-minable areas represents additional disturbance in ecologically sensitive areas which do not recover quickly (Wilhelm, et al. 2015). As well, peat amended sites have also been found to exhibit substantially different qualities than natural sites including increased organic material mineralization, and altered microbial community structure (MacKenzie and Quideau, 2012). Peat mineral soil mixtures will not contain the plant propagules which allow for faster establishment of native species on reclaimed sites.

1.4.5. Biochar

Biochar is a soil amendment comprised of pyrolized organic matter produced by pyrolyzing organic biomass such as wood, crop waste or manure in low oxygen environments (Mohan et al. 2006). Previous studies have demonstrated that biochar can improve a variety of soil qualities including water and nutrient holding capacity, soil aeration, root penetration, microbial habitat and functioning, and contaminant sequestration (Lehmann and Joseph, 2015). Biochar has been proposed as a soil amendment for use in both agricultural and reclamation settings however, feedstock material and pyrolysis conditions affect the properties of biochar and thus its effects. Biochar is able to influence the nutrient status of soils by altering both biotic and abiotic factors. There are several ways in which biochar may impact the nitrogen cycle. Stimulating active soil microbial biomass responsible for mineralization of soil organic matter by altering the physical or chemical environment. These alterations include changes in pH and soil chemistry, hydrological properties of soil such as water holding capacity, which may intern affect oxygen content (Lehmann and Joseph, 2015). Biochar may also sequester chemicals including nutrients, biologically toxic contaminants, signalling enzymes and allelopathic agents and provides habitat for microbes (Bonanomi et al. 2015). As well, soil organic matter may be protected from mineralization within pores of biochar which are too small to host microbes responsible for nutrient cycling (Pignatello et al. 2006).

The nutrient holding capacity of biochar amended soil relates to not only how much nutrient the soil can store but also how quickly nutrients are released, and what chemical form they take (Uleyette, 2014). Previous work has shown that slower release fertilizer performs better than immediately available fertilizers in reclamation settings (Ciccarese et al. 2012). If biochar is able to improve physical soil characteristics and promote slow release of nutrients from soil it may be presumed to be a beneficial soil amendment. Further study is needed to account for the effect of these processes in biochar amended soil.

Previous studies have found that biochar may impact both mineralization and nitrification in soil however the direction of these effects is not settled (Leduc and Rothstein, 2007; Ameloot et al. 2014).

Disturbances in the boreal forest may be natural as well as anthropogenic. Fires are the dominant natural disturbance in the boreal forest (Johnson, 1992). Study of fire affected forests can provide insight into patterns of recovery following disturbance and may be used to study reclamation practices (Errington & Pinno, 2015). Studying the effects of biochar in soil in this context may be helpful understanding value of biochar as char residue from fires may have analogous effects to biochar.

1.5. Thesis Objectives

This study focuses on coarse soils with low SOM. Reclamation on sites with coarse soils may be especially difficult as available nutrients and water holding capacity are limited by low soil organic matter and thus fertility is inhibited (Norisada et al 2004). Chemical fertilization and/or use of organic soil amendments to add SOM is often employed to promote vegetative grown however, these may have limited effectiveness as the mineral soil has generally lower capacity to hold the nutrients provided by amendments.

Previous studies of biochar as a soil amendment or co-amendment have shown promising results in improving soil characteristics by catalyzing important ecological soil processes. Improved cation exchange capacity, water holding capacity and microbial biomass and activity may be well suited to the improvement of deficient coarse textured soils.

Biochar is believed to alter nitrogen cycling and reduce leaching and gaseous nitrogen emissions from soil. The mechanisms and net fluxes involved require further investigation. This study will look at biochar’s impact on nitrogen processes in soil and compare its effect when used as a soil amendment in conjunction with other soil amendments. The underlying premise of this work is that soil changes imparted by biochar on nitrogen fluxes (i.e. ammonification and nitrification) are more significant than changes in nitrogen pools as measured by microbial biomass and extractable nitrogen.

These experiments evaluate the effects of different soil amendments on nitrogen dynamics. The study soils chosen for this work were obtained from a conventional oil and gas well site however, the site shares characteristics with many other disturbed sites in northern boreal forests including both mineral and oil sands mining.

Chapter two looks at the interactions of biochar with added chemical fertilizer in a sterilized and unsterilized coarse soil, and inert Ottawa sand. Nitrogen leaching and retention and calculated net nitrification the main measurements used to determine biochar's effect in soil.

Chapter three examines carbon and nitrogen mineralization from soil amended with two different types of organic amendments (compost and forest floor material) and two different biochar treatments (raw biochar and a biochar reclamation pellet developed by Alberta Innovates – Technology Futures).

Chapter four summarizes the major findings of the previous two chapters and looks at the overall results in the context of similar research highlighting some potential areas of future study.

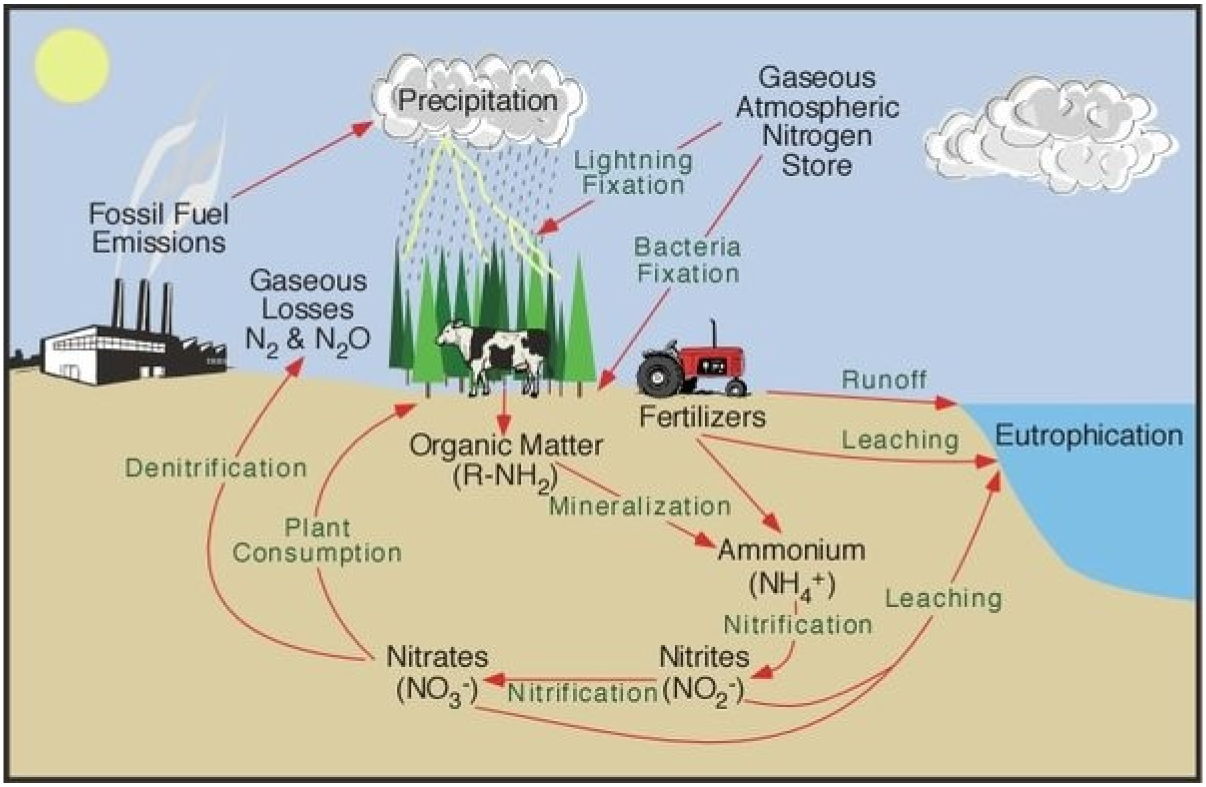

Figure 1-1 Nitrogen Cycle (Source: Pidwirny, 2006)

Chapter 2. Biochar's Effect on Nitrogen Cycling in Forest Soils

2.1. Introduction

2.1.1. Reclamation of Coarse-Textured Forest Soils

Successful reclamation of forested areas is subject to a number of factors including resource availability, propagule presence and viability, species performance and species interactions (Bhatti and Vitt, 2012). The specific challenges of low soil fertility, non-optimal soil pH, salinity, soil compaction, competition from weeds, and animal browsing can slow the speed at which sites recover (Bussler et al. 1984; Andersen et al. 1989; Casselman et al. 2006). Monitoring key soil functions such as nutrient cycling and moisture regulation can provide information which may be used to assess and predict site recovery. Nutrient losses, commonly following disturbance (Turner, 2010), inhibit soil function and may confound revegetation efforts. Soil organic matter (formed from plant and animal residues) is the primary reservoir for soil nutrients and, therefore, mediates soil nutrient fluxes and transformations.

While reclamation practitioners rely on the outcomes of vegetative growth to assess reclamation success, other more fundamental measures, such as nutrient cycling, can predict effects of disturbance and identify limitations which may impair site reclamation (Maynard et al. 2014). Site characteristics such as topsoil depth and soil texture are basic indicators of soil health and have been included by Alberta's regulators as mandatory information when assessing reclamation on a landform scale (Alberta Environment, 2008). Disturbance of forest soils can often result in loss of organic material through poor conservation practices or stored topsoil degradation. Prior to 1983, topsoil conservation was not a regulatory requirement for well site construction in Alberta (Osko and Glasgow, 2010) and topsoil loss, unavoidably encountered during disturbances, can result in loss of ecosystem function including nutrient cycling, water retention, and microbial habitat (Pyper et al. 2013) . The addition of soil amendments to compensate for nutrient losses at degraded sites is one solution for restoring ecological function and thus the ability to support vegetation for site reclamation

Sites with coarse, sandy soils are inherently low in soil organic matter prior to disturbance and are therefore sensitive to any losses of organic matter incurred during disturbance. In addition, poor cation exchange capacity and water holding capacity of sandy soils limits nutrient and water availability to plants making these sites less productive and thus, more difficult to reclaim (Maynard et al. 2014). Using amendments to improve soil properties and add additional resources to soil can, in turn, support plants and soil fauna. However, the use of amendments requires an understanding of the dynamics associated with the nutrients that are to be added to achieve the desired effect.

2.1.2. The Nitrogen Cycle in Disturbed Forests

Soil nitrogen dynamics are defined by the pools and fluxes within the system. Generally, nitrogen, is considered a limiting nutrient in boreal forest ecosystems (Bosatta and Staaf, 1982; Tamm et al. 1982). Undisturbed forests are efficient at retaining and recycling nitrogen and nitrogen losses are small relative to internal nitrogen fluxes (Bashkin, 2006). However, the concept of limiting nutrients may not be as singularly valuable in forest reclamation as it is in agricultural applications. It should be recognized that spatial heterogeneity in nutrient concentrations results from specific features such as topography (e.g. slope, aspect altitude) (Xiaomei, et al 2016) and local scale lithology (Pulla et al. 2016). Therefore, quantifying specific fluxes pools and fluxes may be valuable at the ecosite level while only relative measures in fluxes and pools may be helpful at larger scales.

Soil organic matter is a controlling factor in soil nitrogen dynamics (Priha et al. 2001) and removal of topsoil, the largest repository of soil organic matter, can damage or destroy microbial communities responsible for nitrogen flux making them less diverse and reducing function. Further, coarse soils have a low surface area to volume ratio, large pore size and few charged surfaces (Czaban et al. 2013). These physical and chemical characteristics make these sites difficult to revegetate following disturbances and coarse soil sites are less resilient to relatively small alterations in the physical and chemical environment. Nitrogen fluxes and pools are dependent upon physical, chemical and biological processes in soil and nitrogen dynamics may be grossly affected by disturbance. Mineralization of organic matter and nitrification of inorganic nitrogen are dominant processes in soil which control plant available nitrogen. Generally, disturbance appears to increase soil nitrogen loss through increased mineralization and nitrification leading subsequently to leaching and volatilization (Tamm et al. 1982; Maynard et al. 2014). This may be caused by changes in soil moisture, aeration and soil temperature increases.

The mechanisms controlling nitrogen dynamics are complex and interrelated.. Diminished nitrogen uptake by plants and reduced nitrogen lost in gaseous affect plant available nitrogen in soil (Leduc and Rothstein, 2007; Kreutzweiser et al.2008).

Other factors such as soil chemistry may be affected by disturbance. For example, soil pH, a factor normally limiting to nitrifying bacteria, may change following disturbances acting as negative feedback however in forests following a disturbance, nitrification tends to increase even in acidic soils (Binkley and Fisher 2013). Increased soil temperature following clearing also increases decomposition rates of soil organic material and alters soil moisture regimes which can affect the speciation and thus location of nitrogen in soil as oxidation state and chemical form influences physical characteristics such as sorption and mobility (Kreutzweiser et al. 2008).

Following disturbance, high gross nitrification rates and increases in microbial assimilation dramatically alter nitrogen pools in forest soils (Westbrook and Devito, 2004; Hart and Stark, 1997).

Improving retention of soil nitrogen in forms which become plant available may prove beneficial to reclamation activities. However, depending upon the soil type and the desired effect, adding amendments which alter nitrification in a coarse soil with low organic matter have the potential to increase leaching and gaseous N losses. Means by which nutrient cycling following anthropogenic disturbance may mimic disturbance following wildfires may be helpful in developing reclamation practices. Induced canges in nitrogen availability should promote the growth of desirable native species rather than competing weeds or invasive species. Understanding the dynamics and fate of nitrogen in forest soils will inform how best to add nitrogen and look at the effects of amendments such as biochar on nitrogen pools and fluxes. Broad indicators such as C:N ratio in soils are founded in agricultural study however and are too simplistic to be toward forest reclamation but can be used to predict nitrogen leaching from a simple soil system (Cools et al. 2014). As noted above, additional factors such as aeration, soil temperature, soil texture and water holding capacity also influence soil nitrogen transformation and these factors need to be considered when developing best management practices with respect the nutrient regime of sites undergoing reclamation.

2.1.3.Biochar's Influence on Nitrogen Cycling

Studies have suggested that gross nitrification and mineralization increase in natural systems exposed to fire more frequently which may be important in early succession. Biochar has been suggested as an amendment which may improve soil properties regarding nutrient retention, water holding capacity and soil aeration. The influence of biochar on soil nitrogen dynamics is complex and does not easily allow for predictions to be made which rely on simple models. In using biochar for forest soil reclamation, understanding the characteristics of both the biochar and the site to which it will be applied is important. The putative positive effects of biochar are numerous and have implications for soil nutrients. Adsorption of cations and effects on nutrient cycling microbes are particularly important. A summary of the effects of biochar can be found in Table 2-1.

Biochar is understood to influence soil nitrogen dynamics in a number of indirect ways both physically and chemically (Clough et al. 2013). Ammonia oxidizing bacteria are believed to be present with greater abundance in soils with greater charcoal content (Ball et al. 2010). Rather than being significantly decomposed in soil, biochar is generally thought to be a recalcitrant form of carbon which is not utilized as either a food source or an electron donor/acceptor by microbes as it is resistant to enzymatic degradation (Ladygina and Rineau, 2013). Since little of the carbon in biochar used as a substrate, there is no requirement for additional nitrogen for building microbial biomass. Rather, biochar provides surface area for reactions and sorption as well as physical structure altering aeration and water holding capacity in soil. Biochar has a large surface area to volume ratio and it has a relatively high density of sites (i.e. functional groups) which are active in the soil environment (Lehman and Joseph, 2015).

These physicochemical characteristics of biochar may help predict the impact on nitrogen dynamics. In term of properties which retain soil nitrogen sorption of organic molecules and CEC of biochar may be considered major factors. Sorption of non-polar nitrogen containing compounds (dissolved organic nitrogen) and biochar’s large cation exchange capacity would indicate that nitrogen might be retained in organic and NH_4^+forms in biochar-amended soil (Mukherjee, 2014).

There are however, properties of biochar which may result in loss of nitrogen in soil. The pH of biochar is generally basic which may have a liming effect in soils, raising the overall pH of the soil, as well functional groups on biochar surfaces may buffer soil acidity (Yuan et al. 2011). This could enhance nitrification. Nitrification in soil is process by which NH_4^+ is converted (by ammonia oxidizing bacteria) into NO_3^- . The reaction produces H^+ which acidifies soil and provides negative feedback to ammonium oxidizing bacteria the buffering and liming capacity of biochar interrupts the negative feedback of free H^+

in the soil and promotes nitrification (Ball et al. 2010) and, potentially, promote leaching of a form of N that is poorly retained in soil NO_3^-.

Additional surface area, micropores and aeration may also result in additional microbial biomass and thus, nitrogen immobilization. However, other studies have shown that by preventing nutrient leaching in soils, black carbon (a biochar analogue) has been shown to increase microbial activity in nutrient rich soil. However, in nutrient poor soils, it may sequester available nutrients thereby reducing microbial activity (Kuzyakov et al. 2009). Mineralization and nitrification have been shown to be affected by biochar, but the direction of this effect seems to vary; both increased and decreased mineralization and nitrification has been observed under different conditions (Prommer et al. 2014), O

Aeration, soil moisture and soil temperature all tend to affect nitrification suggesting nitrification might be easily altered by changes in soil conditions, however, antecedent nitrogen dynamics in the soil also impact biochar's influence. For example, DeLuca et al. (2006) observed that nitrification rates are unaffected by char in soils with already high rates of nitrification.

Increased nitrification should result in increased leaching of nitrate/nitrite, however, other studies have observed reduced nitrate leaching and reduced N_2O emissions from biochar-treated soils supporting the idea that the overall N pool increases when biochar is added to soil though there may be smaller offsetting nitrogen "sinks", removing NO_3^- from the system.

Previous experiments have indicated that controlled release fertilizers may be applied at lower rates to achieve similar vegetation results to immediately available fertilizers applied at higher rates (Sloan and Jacobs. 2013) supporting the view that retention of nutrients and release to plant available forms in timeframes consistent with plant growth is key. As biochar is purported to improve soil nutrient retention (particularly nitrogen), the experiment proposed here was intended to examine biochar's effect on soil nitrogen dynamics when applied to coarse-textured soils low in organic matter.

The conditions of the study are controlled and intended to maximize nitrification in soils – that is, C:N ratios lower than 20 and water filled pore space is near 60% (Paul and Clark, 1998). The goal of this study is to determine the net influence biochar exerts on plant available forms of nitrogen and differentiate purely chemical effects (i.e. increased CEC and organic adsorption) from biological effects resulting from altered soil conditions (i.e. pH, aeration, moisture content) which promote nitrification.

2.2. Research Objectives and hypotheses

The literature indicates that the effect of biochar on nitrogen cycling is dependent upon a number of variables associated with the soil and the biochar. The goal of this experiment was to look at these effects in a common reclamation scenario and address the question: over a short time period, what effect does biochar have on leaching and retention of added nitrogen in a coarse-textured forest soil? A sandy forest soil low in organic matter was amended with biochar. Overall leaching and storage of nitrogen were broken down into their component vectors such as nitrification, ammonification and immobilization. Measurement of microbial response in the form of respiration and biomass is used to examine biochar's influence on soil microbial activity.

It is hypothesized that biochar reduces nitrogen leaching by physically adsorbing nitrogen on active sites , however the effects of biochar on soil conditions which control microbial activity may also have a strong influence on nitrogen location and speciation and may be much more significant than the relationship between soil NH_4^+ and altered CEC. In order to examine this, soil retention and leaching of nitrogen species was measured along with other biological indicators (carbon and nitrogen in microbial biomass and respiration). These measurements were used to infer the important nitrogen controlling vectors of ammonification and nitrification and examine biochar's net effects, attributing them between biological and abiotic factors with the underlying hypothesis that biochar's effects on biological processes is more significant than direct physicochemical interactions between nitrogen and biochar. Understanding the nitrogen dynamics in this way will allow us to better predict the fate of nitrogen which has been mineralized from organic matter as well as nitrogen added as fertilizer.

2.3. Materials and Methods

2.3.1. Study Soil

The soil used in this experiment was obtained from an abandoned well site located approximately 230 km east of Peace River, AB (12-08-82-13 west of the 6th meridian, elevation 700 m) within the central mixedwood subregion of the Boreal Forest natural area. The soil texture was loamy sand with little organic matter present and is mapped as a Regosol. After abandonment, the site was recontoured and topsoil replaced. In June 2012, the entire area was seeded with mixtures of fall rye and native grasses (awned wheat grass, slender wheat grass, fringed brome and ticklegrass) to help stabilize the soil and provide protection for both planted and naturally established trees. The site is surrounded by Jack pine with patches of aspen in the overstory and the understory is dominated by lichens and ericaceous shrubs. The organic forest floor horizon in the natural stand was very thin and was mixed with the underlying sand during site work. The result of the topsoil replacement was largely homogenized loamy sand with some fine organics present. The site was visited by AITF from May 27-31, 2013 and soil samples were obtained by hand from shallow pits. The soil was air dried at 40°C and ground to 2 mm prior to homogenization by the cone and quarter method using 5 passes (Schumacher et al. 1990). Soil chemical properties are summarized in Table 2-1. Samples of the soil were oven dried at 105°C for 24 hours and then weighed to measure water content. The density and porosity were measured by placing the dried soil in a cup and gently tamping to settle, then measuring the mass of the soil with density (g cm^{-3}) = Dry soil weight (g)/Soil volume (cm^3). The moisture content (by mass) was calculated by the following formula: Water (%) by mass = [wet mass - dry mass)/dry mass]×100. Porosity was calculated by wetting a known volume of the dried soil to saturation and inferring the saturation volume as volumetric pore content then dividing this by the overall volume of soil used. Additional analysis including total nitrogen, extractable ammonium nitrogen (NH_4-N), extractable nitrate nitrogen ($N0_3$-N), total carbon, total organic carbon and microbial biomass carbon and nitrogen were performed at the Natural Resources Analytical Laboratory at the University of Alberta (NRAL) prior to the leaching experiment.

The raw biochar was produced by AITF in Vegreville, Alberta using pinchips from debarked spruce wood. This feedstock was selected as it is unlikely to contain contaminants such as heavy metals. AITF's Auger Retort Carbonizer was used to produce the biochar required at a maximum feedstock temperature of 600°C and an average retention time of 9 min (AITF, 2015) Biochar chemical properties (pH, conductivity, CEC) were provided by AITF and can be found in Table 2-2. The Ottawa sand, used as an inert material in this experiment, was obtained from Fisher Scientific.

2.3.2. Experimental Design and Preparation

This experiment used an incomplete factorial design with four replicates of each treatment to examine the effects of biochar on nitrogen transformation and leaching and partition these effects between biotic and abotic mechanisms. In order to study these physicochemical and biological effects the treatments used in the experiment included: 1) Autoclaved and non-autoclaved treatments were intended to compare soils where biological activity associated with nutrient cycling is allowed to carry on uninhibited (non-autoclaved) versus inhibiting the processes such as building microbial biomass, ammonification, nitrification and denitrification (autoclaved). 2) Soil from the Peace River site and

Ottawa sand were compared to help distinguish sources of carbon and nitrogen species (i.e., fertilizer versus mineralized OM) in the leachate as well as to allow the physicochemical characteristics of the biochar to regulate nitrogen retention and transport in absence of other sources of SOM. 3) Nutrient amendment (ammonium nitrate solution) at two levels – 0 and 11 kg ha^{-1} allowed for comparison of contributions from nitrogen already in the soil to nitrogen added in the amendment. 4) Biochar was added to all soil used in the experiment in 0, 10 and 25 tonne ha^{-1} applications to examine the effects of two biochar treatment levels compared to a control.

2.3.3.Soil Mixture Preparation and pre-Incubation

A workflow diagram in Fig. 2-1 summarizes the soil preparation and incubation procedures. The soil mixtures used for the experiment were prepared by treating the study soil with 0, 10 and 25 tonne ha^{-1} of raw biochar. In order to simulate weathering through the physical processes associated with freeze/thaw, a mortar and pestle was used for one minute to increase the surface area of the raw biochar (Cheng et al 2008). Aging biochar by this process is thought to alter biochar pH, CEC, and oxygen-carbon ratio (Hale et al. 2013). The ground biochar was mixed with soil in the proportions to be used in the experiment and sufficient water was added to achieve 60% water filled porosity in large containers. The mixtures were placed in a freezer set at -8°C for 4 days.

The sealed containers were then placed in an incubator set to 34.5°C. for 5 days before being transferred to drying trays. The mixtures were then dried for 10 days at 34°C. The soil was then transferred into sealed containers and stored at -8°C until used in the experiment. CEC and pH was then measured on the weathered soil mixtures and these results can be found in Table 2-3.

2.3.3.1. Autoclave Treatment

An autoclave was used to sterilize half of the soil. The method selected was dry sterilization described by Lotrario et al (1994) as it minimally disrupts soil properties. The soil was dried at the conclusion of the weathering process described above then stored at -8°C prior to being autoclaved three consecutive times at 121°C and 15 psi pressure in shallow Pyrex^{TM} pans. Likewise, all lab material including soil vessels, filters, and distilled water (for leaching and ammonium nitrate addition) were autoclaved once prior to beginning each replicate.

2.3.3.2. Nutrient Treatment and Leaching Procedure

The leaching experiment was carried out using 500 mL Nalgene^{TM} Reusable Filter Holders (part number 300-4050). Prior to sterilization, a 20 micron filter was added to each vessel. The vessels were loaded with 500 g of soil. In order to wet the soil, 144 ml of water was added to each vessel and removed with vacuum after 30 minutes. Half of the samples were then treated with 80 mL of nutrient amendment solution containing 0.34625 g L^{-1} NH_4NO_3 dissolved in distilled water to provide nitrogen equivalent to 11 kg N ha^{-1} resulting in a C:N ratio of 18 (conditions favorable to nitrification). The non-amended samples were treated with water. Leachate was collected under vacuum the following day. Leaching of the vessels was carried over four consecutive days by adding 80 mL of water added to each vessel and removing it by vacuum after 30 minutes. In order to minimize denitrification while maximizing microbial activity, the experiment was run with water filled pore space near 60%. The work process is summarized in Figure 2-1.

2.3.4. Sampling and Measurements

2.3.4.1. N_2O and CO_2 Flux

Soil flux of N_2O and CO_2 was measured using an Innova 1312 photoaccoustic infrared portable gas analyzer. The headspace above the vessel was purged with outside air then sealed. Readings were taken at 0, 1, 2 and 3 minutes to establish the rate of increasing or decreasing CO_2 and N_2O concentrations in the headspace of the vessels. Flux measurements were performed immediately prior to application of liquids (nutrient solution or water) to the vessels.

2.3.4.2. Leachate Analysis

A total of six leachate samples were collected from each vessel with the pH and electrical conductivity being measured on each leachate sample as it was collected. To minimize nitrogen transformation in the stored leachate, all samples were placed in a freezer for storage prior to further handling. The initial saturating sample (leachate sample 1), and the nutrient addition sample (leachate sample 2) were submitted for analysis individually. The four water leaching samples were used to make a single composite sample (leachate sample 3). These three samples were submitted for total carbon, and total nitrogen using a Shimadzu Total Carbon and Nitrogen Analyzer (Shimadzu, 2001). NH_4-N and NO_3-N analysis was performed using a SmartChem 200 spectrophotometer.

2.3.4.3. Soil Analysis

At the conclusion of the leaching experiment, the soil from the vessels was collected in sealed Ziploc™ bags and analyzed for total carbon, total organic carbon, total nitrogen, extractable NH_4-N and NO_3-N and microbial biomass carbon and nitrogen. Total carbon, total organic carbon and total nitrogen samples were analyzed using a Costech 4010 Elemental Analyzer System (Costech Analytical Technologies Inc., Valencia, CA, USA).

Samples submitted for total exchangeable NH_4-N, and exchangeable NO_3-N were prepared using KCl extraction (Maynard and Karla, 1993) and analyzed using a SmartChem 200. Soil microbial biomass carbon and nitrogen measurements were obtained using the chloroform fumigation extraction method (Voroney et al. 2008).

2.3.4.4. Calculation of Nitrogen Pools and Fluxes

For the purposes of this experiment, retained nitrogen in soil was calculated as total extractable NH_4-N, plus NO_3-N plus microbial biomass N as these represent the most readily available forms of nitrogen:

$$N_{Retained} = NH_4\,(N)_{extractable} + NO_3(N)_{extractable} + microbial\ N$$

[1]

Dissolved organic nitrogen (DON) was calculated as total nitrogen in the leachate minus NH_4-N and NO_3-N in the leachate:

$$DON = TN_{Leached} - [NO_3(N) + NH_4(N)]_{Leached} \qquad [2]$$

Net ammonification was estimated as the sum of extractable and leached inorganic nitrogen at the conclusion of the experiment minus inorganic nitrogen added in the form of nutrient amendment minus extractable NH_4^+-N and NO_3-N in soil before leaching:

$$Net\ Ammonification = \qquad [3]$$

$$[NO_3(N)_{extractable} + NH_4(N)_{extractable} + NO_3(N)_{leached} + NH_4(N)_{leached}]_{final} - [NO_3(N)_{extractable} + NH_4(N)_{extractable} + NO_3(N)_{added} + NH_4(N)_{added}]_{inital}$$

Changes in NO_3-N must be included in the ammonification estimate because NH_4^+ produced by ammonification may be quickly nitrified. Nitrification of NH_4-N in the soil at time 0 would cause a slight over-estimation of ammonification. Ammonification of soil organic matter (i.e., gross ammonification) is calculated as mineralized N minus the change in microbial biomass N:

$$SOM\ Ammonification\ = \qquad [4]$$

$$[NO_3(N)_{extractable} + NH_4(N)_{extractable} + NO_3(N)_{leached} + NH_4(N)_{leached}]_{final} - [NO_3(N)_{extractable} + NH_4(N)_{extractable} + NO_3(N)_{added} + NH_4(N)_{added}]_{inital} - \Delta MBN$$

Net nitrification was estimated as the difference of the sum of extractable and leached NO_3-N at beginning and conclusion of the experiment:

$$Net\ Nitrification =$$

$$[NO_3(N)_{extractable} + NO_3(N)_{leached}]_{final} - [NO_3(N)_{extractable} + NO_3(N)_{added}]_{inital} \qquad [5]$$

A nitrification ratio was estimated by comparing the ratio of extractable plus leached NH_4-N to extractable plus leached NO_3-N:

$$Nitrification\ Ratio\ =$$

$$[NO_3(N)_{leached} + NO_3(N)_{extractable}]\ /\ [NH_4(N)_{leached} + NH_4(N)_{extractable}] \qquad [6]$$

Using the measurements obtained in leachate and soil a nitrogen budget was created which describes the pools and species of nitrogen prior to and following incubation in order to calculate the fluxes between pools.

2.3.5.Statistical Analysis

There were four fixed factors used in this experiment: autoclave treatment (autoclaved or non-autoclaved), soil type (study soil or Ottawa sand), biochar treatment (0, 10 or 25 tonne ha^{-1}), and nitrogen addition (amended or non-amended). While the overall study was an incomplete factorial design, two models were used in this experiment both of which had balanced designs based on combining factors. The first model (Model 1) examines nutrient-amended samples only (non-amended samples were ignored). Comparisons are made between the Ottawa sand (O), non-autoclaved Peace River soil (NA) and autoclaved Peace River soil (A) to examine the physical chemical effects of biochar in each soil type. The second model (model 2) considers the amendment factor (nutrient (N) or water (W)) as well as the autoclaving factor (autoclaved (A) or non-autoclaved (NA)) in the Peace River soil (ignoring the Ottawa sand samples), each with varying biochar treatments in order to differentiate biotic and abiotic mechanisms in soil nitrogen cycling. ANOVA testing on main factors and interactions was performed using R and statistical significance was declared where $P \leq \alpha = 0.05$. Pairwise comparisons were performed using Tukey's post hoc comparisons were performed where ANOVA results were significant. Relevant interaction effects were also tested using Tukey's post hoc testing. The post hoc comparisons were considered significant where $P \leq \alpha = 0.05$. Where assumptions of normality and homoscedasticity were violated permutational ANOVA was used for data analysis as log, square root and inverse transformations were mostly ineffective.

2.4. Results

2.4.1.Nitrogen Budget

The primary objective of this experiment was to quantify the influence of biochar and sterilization (i.e., autoclaving) on the fate of added fertilizer N in the Peace River soil. With respect to the added N, the following equation represents a mass balance:

$$Added\ NH_4NO_3(N) = \Delta(N\ retained) + \Delta(N\ lost) \quad [6]$$

Where $Added\ NH_4NO_3 - N$ (kg N ha^{-1}) is the amount of added ammonium nitrate, $\Delta(N\ retained)$ is the difference in the sum of extractable NH_4^+ and NO_3^- and microbial biomass N between the nutrient- and water-amended soil samples at each biochar rate, and $\Delta(N\ lost)$ is the difference in the sum of leached NH_4^+, NO_3^-, DON and gaseous N_2O losses between the nutrient- and water-amended soil samples. Essentially, this equality assumes that all added N is partitioned to the soil N pool (N retained), is lost through leaching (leached N) or gaseous emission as N_2O (denitrified N).

Tables 2-4, 2-5 and 2-6 show the overall nitrogen budget for each treatment. The amount of nitrogen measured in the system at the conclusion of the experiment was higher across all treatments than the extractable and added nitrogen in the soil at the beginning of the experiment indicating some ammonification of organic nitrogen in soil. For the autoclaved and non-autoclave Peace River soils, the amount of extractable ammonium following the incubation is equal to or greater than the initial extractable and added amounts demonstrating net ammonification, but there was still a significant amount of nitrate leaching, indicating that most mineralized organic matter was quickly nitrified.

The last three columns of Tables 2-4, 2-5 and 2-6 summarize the differences in N retention ($\Delta(N\ retained)$ and N losses ($\Delta(N\ lost)$) between the nutrient- and water- amended soils for the autoclaved soil, non-autoclaved soil, and Ottawa sand respectively.

Regardless of sterilization treatment and biochar rate, average values of $\Delta(N\ retained) + \Delta(N\ lost)$ are all close to 13 kg N ha^{-1}. The amount of NH_4NO_3-N added to the nutrient-amended treatments was 11 kg N ha^{-1}. This suggests that the observations nearly conform to the equation presented in Eq. [6]. The difference between of 2 kg N ha^{-1} $Added\ NH_4NO_3 - N$ and $\Delta(N\ retained) + \Delta(N\ lost)$ is likely a result of not all N pools being measured before and after the incubation such as labile organic N fractions – light fraction organic N and DON in the soil pore water for example. As well, the ammonification of organic nitrogen in the soil and potentially the labile components of the biochar added nitrogen to the soil during incubation which was later measured in the lost or retained pools. Because of the near reconciliation of the observations and Eq. [6], inferences regarding the influence of biochar amendments on retention of added N fertilizers in the Peace River soil can be made.

When comparing the differences between nutrient- and water-amended soils, the Peace River soil (both autoclaved and non-autoclaved) appeared to retain the NH_4-N which was added in the nutrient solution. However, the 25 tonne ha^{-1} treated non-autoclaved Peace River soil displayed less extractable NH_4 –N and a corresponding increase in extractable NO_3-N which may be a result of nitrification. However, the sum of the retained pool was lowest in the 25 tonne ha^{-1} treatments indicating that biochar did not increase the overall amount of nitrogen retained in inorganic form when comparing differences in nutrient- and water- amended samples.

In the non-autoclaved samples, there was some evidence to show ammonification was increasing with increasing biochar. The difference in ammonification between the nutrient-amended and non-amended soils was negative (indicating potential immobilization) in the 0 tonne ha^{-1} treatment, less negative in the 10 tonne ha^{-1} treatment and positive at 25 tonne ha^{-1}. While these changes are relatively small, a corresponding decrease in microbial biomass nitrogen and increased CO_2 respiration is indicative of biochar's positive effect on mineralization (and thus ammonification) in the non-autoclaved soils observed over the relatively short incubation period of five days.

The biochar impact in altering ammonification was relatively modest with the 10 and 25 tonne ha^{-1} applications increasing ammonification 0.11 kg N ha^{-1} and 0.54 kg N ha^{-1} respectively above the control value of -0.34 kg N ha^{-1} (Table 2-4).

The addition of 10 and 25 tonne ha^{-1} resulted in an change in nitrification of -0.12 and 2.79 kg N ha^{-1} respectively from the control value of 0.47 for these two treatments. When trying to predict the effect that biochar has on soil nitrogen and its availability, the nitrification effect may be much more important. The biochar effects on ammonification were less apparent in the less biologically active vessels (Ottawa sand and autoclaved soil). Similarly, for net nitrification, the non-autoclaved samples were significantly affected by biochar treatment, while Ottawa Sand and autoclaved Peace River soil behaved quite similarly with little change in nitrification – it is likely that autoclaving decreased nitrification. It is apparent that biochar's alteration of the soil environment is more impactful to biological processes and that physicochemical changes in soil which control nitrogen are less enhanced. Observed differences in nitrogen leaching, retention and transformation between treatments in the Nitrogen Budget are examined for statistical significance in the following sections.

2.4.2.Biochar and Nitrogen leaching

Tables 2-7 summarizes the ANOVA results for Model 1. Pairwise comparisons are summarized in Table 2-8. The 25 tonne ha^{-1} treatment leached less total nitrogen than the 10 tonne ha^{-1} treatment though neither the 10 nor the 25 tonne ha^{-1} treatments were significantly different than the control. The reduction in leaching between the nutrient amended and non-amended soil was greatest at 25 tonne ha^{-1} in the non-autoclaved soil (Table 2-4 and 2-5). Biochar rates of 10 and 25 tonnes ha^{-1} in nutrient-treated Ottawa sand leached successively less ammonium nitrogen as expected likely owing to increased CEC. Nutrient-amended soil leached more nitrogen in the order of O<A<NA, indicating that soil processes are either producing or transforming nitrogen into mobile forms and that these are biologically mediated.

Increasing biochar significantly reduced NH_4^+ leaching in the Ottawa sand and this effect was also observed in the non-autoclaved soil (Figure 2-3). NO_3^- leaching was lowest in the Ottawa sand followed by the autoclaved and non-autoclaved soil (Figure 2-4).

Tables 2-9 summarizes model 2 ANOVA (autoclaved and non-autoclave soils with and without fertilizer additions) results and treatment means including pairwise comparisons are summarized in Table 2-10. The autoclaved soil and non-autoclaved soil leached significantly more total nitrogen than the Ottawa sand (Figure 2-2).

The 10 tonne ha^{-1} treatment leached significantly more total nitrogen than the 25 tonne ha^{-1} while the 0 tonne tonne ha^{-1} treatment leached similar amounts of nitrogen to the 10 and 25 tonne ha^{-1} treatments (Figure 2-6).

Dissolved organic nitrogen (DON) leaching was reduced with increasing biochar rates and DON leaching was increased in autoclaved samples (Table 2-8) though this was likely DON input from dead microorganisms. At 25 tonnes Biochar ha^{-1}, more DON may have been adsorbed by the biochar and reduced concentrations in the leachate. This finding may be relevant as DON may also account for substantial soil nitrogen losses (Binkley and Fisher 2013) and sorbed DON was not measured in this experiment.

Inorganic nitrogen leaching (calculated by adding leached NH_4^+ and NO_3^-) was not significantly affected by biochar addition, however inorganic N leaching significantly increased in non-autoclaved soil samples when compared with autoclaved soil. NH_4^+ leaching was significantly decreased with increasing biochar and NO_3^- leaching increased with biochar addition in nutrient-amended non-autoclaved samples. The autoclaved samples leached less NO_3^- overall (Table 2-10).

In both models 1 and 2, soil treated with biochar at a rate of 10 tonne ha^{-1} leached significantly more nitrogen than the 25 tonne ha^{-1} treatments and leached nitrogen similarly to the untreated control regardless of nutrient amendment or autoclave treatment (Fig. 2-2 and 2-6). In all treatments, autoclaved soils leached less nitrate and more ammonium. The non-autoclaved nutrient-treated soil leached more nitrogen when compared to the autoclaved samples likely because nitrification was impeded by autoclaving. This effect appeared to be enhanced by biochar. With increasing biochar rate, non-autoclaved, nutrient-amended samples showed greater NO_3^- N leaching from the samples owing to biochar supporting microbial activity including nitrification (though not increasing microbial biomass).This increases in nitrogen leaching at 10 tonne ha^{-1} and decrease at 25 tonne ha^{-1} was not expected. The changes in nitrification were consistent with previous

2.4.3.Biochar and Retained Nitrogen

Retained soil nitrogen was not significantly increased with increasing biochar application in any of the three soils in model 1 (Table 2-7). On average, the extractable NH_4^+ was significantly higher in the 10 t ha^{-1} biochar treatment across all soil types, but when averaged by soil type, much larger differences were apparent with the highest levels observed in the autoclaved soil followed by non-autoclaved soil and then Ottawa sand (Table 2-8). Extractable nitrate showed higher levels with increasing biochar additions in all soils. Biochar increased extractable NO_3^- in all three soil types and was highest in non-autoclaved soils. (Figure 2-5). Significant increases in extractable NO_3^- were evident with increasing biochar rate across all model 1treatments without a corresponding significant increase in leached NO_3^- and could be the result of nitrification or abiotic oxidation of retained ammonium being enhanced by biochar treatment. A related reduction in extractable NH_4^+ is also seen in non-autoclaved samples (both nutrient-amended and non-amended) however this relationship with biochar is not observed in the autoclaved soil (Tables 2-4 and 2-5).

Biochar did not appear to exert a significant effect on overall retained soil nitrogen (Tables 2-9 and 2-10). However, there was a significant biochar effect on which nitrogen species were present in the soils of model 2. Significant increases in extractable NO_3^- with increasing biochar treatment were evident across all soil treatments and extractable NH_4^+ decreased with biochar treatment only in the non-autoclaved samples (Table 2-10).

In both autoclaved and non-autoclaved soils, microbial biomass nitrogen increased with increasing biochar rate without added N, but this apparent biochar-induced difference disappeared when N was added to the soil (Table 2-10). Though not statistically significant, this may indicate that when nitrogen was a limiting nutrient in microbial growth, biochar increased soil N used to build biomass by retaining microbially available nitrogen.

The non-autoclaved nutrient-amended samples did not contain significantly more nitrogen as microbial biomass (table 2-10) which suggests that in nutrient-treated samples, nitrogen was no longer the limiting nutrient in building microbial biomass. This is consistent with increased nitrogen availability potentially resulting from reduced nitrogen immobilization because of less labile carbon availability or perhaps immobilization of NO_3^+ not being able to capture all of the NO_3^+ being produced by greater gross nitrification (Westbrook and Devito, 2014). However in the non-autoclaved, non-amended samples, more nitrogen was stored as microbial biomass with increasing biochar treatment.

The theoretical calculated increase in soil mineral nitrogen based upon biochar's cation exchange capacity of 53 meq 100 g^{-1} in the 10 and 25 tonne ha^{-1} biochar treatments is 20 and 45 kg N ha^{-1} as NH_4^+-N respectively. The addition of 11 kg N ha^{-1} nitrogen as NH_4NO_3 corresponding to 5.6 kg NH_4-N ha^{-1}

could, theoretically, have been retained by the biochar amended soil by adsorption of NH_4^+ on negatively charged sites and the measured increase in exchangeable NH_4-N following fertilizer addition was within this range. However, CEC increase is only one of the effects which biochar exerts in soil and while the impact of additional CEC was observed in the Ottawa sand treatments

2.4.4.Biochar and Microbial Processes

Microbial respiration and microbial biomass carbon were significantly increased (Figure 2-8) with biochar treatment, however microbial biomass carbon was only observed to increase at the 10 tonne ha^{-1} rate (Tables 2-4, 2-5, 2-9). Differences in ammonification were not statistically significant upon ANOVA analysis of individual treatments though it was calculated to be larger in autoclaved samples (Table 2-9). Net nitrification (as measured by nitrification: ammonification ratio) was significantly increased by biochar addition in non-autoclaved samples and there was a significant interaction between autoclave and biochar treatments with transformation ratio increasing with biochar in the non-autoclaved samples (Figure 2-10). Perhaps this effect was the result of biochar's enhancement of conditions which support nitrifying bacteria. In the autoclaved samples microbial respiration was not reduced, however, nitrification was diminished, presumably resulting from the inhibition of nitrifying bacteria by autoclaving the soil. Increased biomass carbon and respiration could mean assimilation of NO_3^+ produced by nitrification is occurring, however microbial biomass increases were significant only in non-amended soils.

2.5. Conclusions

Based on comparisons between autoclaved and non-autoclaved soils, changes in physical and chemical conditions which influence biotic processes are more significant in explaining the net effect of biochar treatment on nitrogen speciation and thus disposition. Nitrogen added to the soil as well as mineralized from SOM was nitrified and thus both present in soil and leachate in the plant available form of NO_3^-. Biochar's other observed biological effects include increasing microbial respiration and nitrification at 10 and 25 tonne ha^{-1} and increased ammonification and microbial biomass C at 10 tonne ha^{-1}. The physicochemical effects of biochar manifesting as increased CEC (and possibly, DON sorption) at 25 tonne ha^{-1} was able to retain soil N whereas at 10 tonne ha^{-1} the alterations in the soil environment which enhance biological processes may not have been offset by physicochemical effects.

Other research has found that soil nitrogen dynamics may be affected by biochar in different ways. Prommer et al. (2014) observed that extractable NO_3^- decreased with biochar addition whereas this study found the opposite. Steinbeiss (2009) found that biochar increased mineralization of organic matter and Prayogo (2014) found the opposite though at higher application rates than were used in this study. Overall, the amount of leached nitrogen and soil N does not change as one might predict from the addition of CEC to the soil, however nitrogen speciation and microbial activity significantly changes with biochar. In this study biochar's enhancement of nitrification was likely more significant in determining nitrogen location and speciation, with ammonification and physicochemical absorption of nitrogen species being less impactful. These effects could be due to changes in the soil environment such as water holding capacity and aeration, or due to chemical changes in soil attributed to biochar such as acidity buffering or a liming affect. Assimilation of the NO_3^+ produced by nitrification may have been limited by available carbon in the soil.

The effect that biochar appeared to exert in this experiment (altering ammonification and nitrification) were not linear with biochar application rate. While the physicochemical sorption of nitrogen by biochar should be linear with biochar application rate as CEC is measure on a charge per mass basis, the biological effects observed here were not linear. The apparent net effect of these relationships was an increase in nitrogen leaching at an application rate of 10 tonne ha^{-1} and a decrease in leaching at 25 tonne ha^{-1}. The overall reduction in nitrogen loss was about 3.5% in nutrient treated soils and 13% in non-nutrient treated soils at the 25 tonne ha^{-1} application rate. The overall conclusion of this experiment is that the biochar seemed to impact biogeochemistry and, in this coarse textured soil, an increase in nitrification was the result. This may have the potential to result in a loss of nutrients in soils or simply provide more plant available nitrogen for uptake. In this experiment the absolute differences in nitrogen leaching were small, about 1 kg N ha^{-1} in 25 tonne ha^{-1} treatments versus the control or about 10% of added N. Over long periods of time this may be environmentally significant, but without information on how plants respond to co-application of fertilizers and biochar, it isn't clear how long-lived the biochar effect will be. Other studies have shown decadal increases in nitrification (greater than 10 years) following fire and these changes have been (at least partially) attributed to the addition of charcoal (Ganzlin et al. 2016).

The impact of plants in a forest ecosystem may alter a number of soil characteristics which were not accounted for in this 5 day incubation. This includes plant uptake of nutrients, effects on soil aeration and pH (Binkley and Fisher, 2013). Other field conditions such as moisture regime and temperature could have substantial effects on biological conditions and physical processes, As well, the testing of biochar amended soils in nitrification promoting conditions (C:N near 18:1) represent extraordinary conditions which might not be expected in a field study.

The results of this study would suggest that adding biochar to soil may hold some benefit as the observations here were similar to observations following a fires (increased nitrification). However, fire affected forests also exhibit other properties which are not found in anthropogenic disturbances and that following fire nitrogen no longer appears to be a limiting nutrient (Romme et al. 2009). Alterations in the thermal properties of the forest floor, increased hydrophobicity of soils and alterations of the propagule bank (Smithwick et. al, 2005) are examples of conditions which may be dramatically different between fire affected forests and sites disturbed for development. It would be unreasonable to imagine that the recovery of anthropogenic disturbances could perfectly mimic recovery from fires by applying biochar, however the changes in nitrification observed here were similar to observations from studies of fire affected sites.

Table 2-1 Summary of Biochar Effects on Nitrogen Dynamics

		Effect on Nitrogen dynamics
Physical	Increased water holding capacity	Various depending on water content
	Aeration	Enhanced or reduced mineralization Enhanced nitrification
	Creation of structure and microsites for microbes	More diverse and abundant microbial community
	Increased fine soil penetrability	Increased nutrient uptake
Chemical	Enhanced CEC	Increased exchangeable ammonium nitrogen levels
	Adsorption of organic molecules	Proteins not broken down Removal of allelopathic or inhibitory chemicals
	Increased acidity buffering	Enhanced nitrification

Source: Lehmann and Joseph, 2015

Table 2-2 Analysis of soil and biochar chemical properties prior to incubation

Parameter	Units	Untreated Soil	Ground Biochar
Microbial biomass N	(ug g-1 soil)	5.638	-
Microbial biomass C	(ug g-1 soil)	123.1	-
TOC	(wt%)	0.566	-
Organic Matter	(wt%)	1.082	-
Extractable NH4-N	(mg kg-1)	0.0342-	0.447
Extractable NO3-N	(mg/kg -1)	000986	0.769
Total Nitrogen	(wt%)	0.023	4.65
Total Carbon	(wt%)	0.572	89.91
Cation Exchange Capacity	meq 100g-1	-	53
Electrical Conductivity	dS m-1	-	1.68
pH		-	9.1

Table 2-3 Analysis of biochar amended soil and Ottawa sand after weathering of mixtures

Parameter	Soil 0 t ha^{-1}	Soil 10 t ha^{-1}	Soil 25 t ha^{-1}	Ottawa 0 t ha^{-1}	Ottawa 10 t ha^{-1}	Ottawa 25 t ha^{-1}
CEC (meq 100 g-1)	4.9	5.5	5.2	<4.0	<4.0	<4.0
pH	6.8	7	7.1	7.3	8.3	9.1

Table 2-4 Nitrogen budget comparing N pools and leaching in nutrient- and water-amended non-autoclaved Peace River Soil samples before and after incubation and leaching

	Nutrient Amendment	Nutrient Amended			Non-Amended			Difference (Amended – Non-amended)		
	Biochar Treatment	0	10	25	0	10	25	0	10	25
Soil Prior to Leaching	Extractable NH_4-N kg ha^{-1}	1.29	1.28	1.35	1.29	1.28	1.27			
	Extractable NO3-N kg ha^{-1}	0.90	0.90	0.89	0.90	0.90	0.89			
	Microbial biomass N kg ha^{-1}	3.13	3.12	3.09	3.13	3.12	3.09			
Added Nutrient	NH_4-N Added kg ha^{-1}	5.49	5.48	5.49	0.00	0.00	0.00			
	NO3 Added (kg ha^{-1}	5.49	5.48	5.49	0.00	0.00	0.00			
Total N Before Leaching		**16.51**	**16.47**	**16.51**	**5.54**	**5.51**	**5.46**			
Soil after Leaching	Extractable NH_4-N remaining kg ha^{-1}	7.54	7.07	3.94	2.92	1.72	1.05	4.62	5.35	2.89
	Extractable NO_3-N remaining kg ha^{-1}	0.89	1.91	4.62	0.80	2.15	2.96	0.08	-0.23	1.67
	Microbial Biomass N in Vessel kg ha^{-1}	1.92	1.90	1.95	1.39	1.62	2.03	0.52	0.28	-0.08
	Total Retained	10.34	10.88	10.51	5.12	5.48	6.03	5.23	5.41	4.48
Leached Nitrogen	TOTAL_NH_4-N kg ha^{-1}	0.42	0.31	0.05	0.36	0.31	0.06	0.06	0.01	0.00
	TOTALNO_3-N kg ha^{-1}	10.94	11.46	11.81	5.07	5.86	5.20	5.88	5.59	6.61
	Theoretical DON Leached kg ha^{-1}	5.20	5.12	3.99	2.79	2.66	1.85	2.41	2.46	2.14
Emitted Nitrogen	N_2O-N emitted kg ha^{-1}	0.12	0.17	0.20	0.12	0.10	0.14	0.00	0.06	0.06
Total Lost kg ha^{-1}		16.68	17.06	16.06	8.33	8.93	7.24	8.35	8.13	8.81
Total N after Leaching (lost + retained)		**27.03**	**27.94**	**26.56**	**13.45**	**14.41**	**13.27**	**13.58**	**13.53**	**13.29**
Net Ammonification		**6.62**	**7.62**	**7.28**	**6.96**	**7.85**	**7.09**	**-0.34**	**-0.23**	**0.20**
Net Nitrification		**5.44**	**7.00**	**10.05**	**4.97**	**7.11**	**7.26**	**0.47**	**-0.12**	**2.79**
Nitrification ratio (net nit/net min)		**0.82**	**0.92**	**1.38**	**0.71**	**0.91**	**1.02**	**0.11**	**0.01**	**0.36**
SOM Mineralized (min $-\Delta MBN$)		**7.84**	**8.63**	**8.42**	**8.70**	**9.35**	**8.14**	**-0.86**	**-0.52**	**0.28**

Table 2-5 Nitrogen budget comparing N pools and leaching in nutrient- and water-amended autoclaved Peace River Soil samples before and after incubation and leaching

	Nutrient Amendment	Nutrient Amended			Non-Amended			Difference (Amended – Non-amended)		
	Biochar Treatment	0	10	25	0	10	25	0	10	25
Soil Prior to Leaching	Extractable NH_4-N kg ha^{-1}	1.29	1.28	1.27	1.29	1.28	1.27			
	Extractable NO_3-N kg ha^{-1}	1.11	1.11	1.10	1.11	1.11	1.10			
	Microbial biomass N kg ha^{-1}	3.13	3.12	3.09	3.13	3.12	3.09			
Added Nuttrient	NH_4-N Added kg ha^{-1}	5.49	5.49	5.49	0.00	0.00	0.00			
	NO3 Added kg ha^{-1}	5.49	5.49	5.49	0.00	0.00	0.00			
Total N Before Leaching		**16.52**	**16.49**	**16.44**	**5.54**	**5.51**	**5.46**			
Soil after Leaching	Extractable NH_4-N remaining kg ha^{-1}	10.24	11.43	10.10	5.54	6.56	4.78	4.695	4.865	5.323
	Extractable NO_3-N remaining kg ha^{-1}	0.31	0.84	2.17	0.38	0.63	1.07	-0.070	0.211	1.092
	Microbial Biomass N in Vessel kg ha^{-1}	2.16	1.01	1.07	0.71	1.13	1.15	1.455	-0.113	-0.081
	Total Retained	12.70	13.28	13.34	6.63	8.32	7.00	6.08	4.96	6.33
Leached Nitrogen	Leached NH_4-N kg ha^{-1}	0.69	1.05	0.71	0.59	0.72	0.59	0.098	0.333	0.112
	Leached NO_3-N kg ha^{-1}	8.17	7.74	8.10	2.81	2.83	3.13	5.363	4.911	4.967
	Theoretical DON Leached kg ha^{-1}	6.90	7.31	6.13	4.81	5.12	3.75	2.097	2.190	2.376
Emitted Nitrogen	N_2O-N emitted kg ha^{-1}	0.16	0.14	0.17	0.10	0.12	0.13	0.062	0.016	0.034
Total Lost kg ha^{-1}		15.92	16.24	15.10	8.30	8.79	7.61	7.62	7.45	7.49
Total N after Leaching (lost+retained)		**28.63**	**29.51**	**28.44**	**14.93**	**17.10**	**14.61**	**13.70**	**12.41**	**13.82**
Net Ammonification		**6.23**	**7.89**	**7.93**	**7.13**	**8.56**	**7.41**	**-0.90**	**-0.66**	**0.51**
Net Nitrification		**2.09**	**2.19**	**3.88**	**2.29**	**2.56**	**3.31**	**-0.20**	**-0.37**	**0.57**
Nitrification ratio (net nit/net min)		**0.34**	**0.28**	**0.49**	**0.32**	**0.30**	**0.45**	**0.01**	**-0.02**	**0.04**
SOM Mineralized (min $-\Delta MBN$)		**7.20**	**10.00**	**9.94**	**9.55**	**10.55**	**9.35**	**-2.35**	**-0.55**	**0.59**

Table 2-6 Nitrogen budget comparing N pools and leaching in nutrient- and water-amended Ottawa sand samples before and after incubation and leaching

	Nutrient Amendment	Nutrient Amended			Non-Amended			Difference (Amended – Non-amended)		
	Biochar Treatment	0	10	25	0	10	25	0	10	25
Soil Prior to Leaching	Extractable NH_4-N kg ha^{-1}	0.000	0.001	0.004	0.000	0.001	0.004			
	Extractable NO_3-N kg ha^{-1}	0.000	0.002	0.006	0.000	0.002	0.006			
	Microbial biomass N kg ha^{-1}	0.000	0.000	0.000	0	0	0			
Added Nuttrient	NH_4-N Added kg ha^{-1}	5.48	5.49	5.49	0	0	0			
	NO_3-N Added kg ha^{-1}	5.48	5.49	5.49	0	0	0			
Total N Before Leaching		**10.96**	**10.99**	**10.98**	**0.00**	**0.00**	**0.01**			
Soil after Leaching	Extractable NH4 remaining kg ha^{-1}	0.862	1.040	0.662	0.426	0.567	0.658	0.436	0.473	0.004
	Extractable NO_3-N remaining kg ha^{-1}	0.328	0.428	0.654	0.271	0.415	0.368	0.057	0.012	0.286
	Microbial Biomass N in Vessel kg ha^{-1}	0.044	-0.146	0.179	-0.109	-0.022	-0.119	0.153	-0.124	0.298
Total Retained		1.23	1.32	1.50	0.59	0.96	0.91	0.65	0.36	0.59
Leached Nitrogen	Leached NH_4-N kg ha^{-1}	6.217	4.961	3.471	0.033	0.012	0.007	6.184	4.948	3.464
	Leached NO_3-N kg ha^{-1}	5.759	5.747	4.753	0.111	0.063	0.061	5.648	5.683	4.692
	Theoretical DON Leached kg ha^{-1}	2.062	3.995	3.669	0.132	0.098	0.221	1.930	3.897	3.448
Emitted Nitrogen	N_2O-N emitted kg ha^{-1}	-0.035	-0.037	-0.028	-0.032	-0.048	-0.029	-0.003	0.011	0.000
Total Lost		14.00	14.67	11.86	0.24	0.13	0.26	13.76	14.54	11.60
Total N after Leaching (lost+retained)		**2.20**	**1.18**	**-1.44**	**0.84**	**1.05**	**1.09**	**1.36**	**0.13**	**-2.53**
Net Ammonification		**0.61**	**0.68**	**-0.08**	**0.38**	**0.48**	**0.42**	**0.22**	**0.20**	**-0.51**
Net Nitrification		**0.27**	**0.57**	**0.06**	**0.45**	**0.45**	**0.39**	**-0.18**	**0.12**	**-0.33**
Nitrification ratio (net nit/net min)		**2.16**	**1.33**	**-1.62**	**0.95**	**1.08**	**1.21**	**1.21**	**0.25**	**-2.83**
SOM Mineralized (min $-\Delta MBN$)		**2.20**	**1.18**	**-1.44**	**0.84**	**1.05**	**1.09**	**1.36**	**0.13**	**-2.53**

Table 2-7 P-values for factors in ANOVA Model 1 in nutrient-amended soils with biochar treatments. ψ Denotes Permutational ANOVA Bold lettering indicates statistical significance where p≤α=0.05.

	Total N Leaching	**Leached DON**	**Leached NH4**$^{\psi}$	**Leached NO3**	**Retained Soil N**	**Extractable NH4**$^{\psi}$	**Extractable NO3**$^{\psi}$
Soil	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.001**
Biochar	**0.048**	0.38	**≤0.001**	0.97	0.71	**0.03**	**≤0.001**
Soil×Biochar	0.80	0.48	**≤0.001**	0.37	0.99	0.07	**≤0.001**

Table 2-8 Pairwise comparisons of Nitrogen pools and fluxes from Model 1 in nutrient-amended soils with biochar treatments of 0, 10 and 25 tonne ha-1 in three soil types: autoclaved soil (A), non-autoclaved soil (NA), and Ottawa Sand (O). Uppercase letters indicate significant differences between soil types. Symbols indicate significant differences between biochar treatments. Lower case letter indicate significant differences between soil × biochar treatments. Significant main effects indicated by bold letters. Significant interactions (soil × biochar) indicated by italics. Significance declared where $p \leq \alpha =$ 0.05 using Tukey`s Post Hoc comparison. ψ Denotes permutational ANOVA

Soil	**Biochar**	**N Leach**	**Std. Dev**	**DON**	**Std. Dev**	**Leach NH4 ψ**	**Std. Dev**	**Leach NO3**	**Std. Dev**	**Retained Soil N**	**Std. Dev**	**Ext NH4 ψ**	**Std. Dev**	**Ext NO3ψ**	**Std. Dev**
		kg ha^{-1}`		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}	
A	0	15.76	0.90	6.90	1.43	*0.69*^{d}	0.07	8.17	1.11	12.70	1.60	10.24	1.39	*0.31*^{ad}	0.08
	10	16.10	1.14	7.31	1.09	*1.05*^{d}	0.54	7.74	0.62	13.28	2.04	11.43	2.59	*0.84*^{cd}	0.15
	25	14.93	1.40	6.13	1.12	*0.71*^{ef}	0.42	8.10	1.23	13.34	2.00	10.10	1.35	*2.17*^{b}	0.41
	Average	**15.60**^{A}	1.17	**6.78**^{a}	1.22	**0.81**^{A}	0.40	**8.00**^{A}	0.95	**13.11**^{A}	1.73	**10.59**^{A}	1.80	**1.10**^{A}	0.85
NA	0	16.56	1.10	5.20	1.58	*0.42*^{ef}	0.15	10.94	0.95	10.34	1.09	7.54	0.91	*0.89*^{b}	0.21
	10	16.89	1.24	5.12	2.13	*0.31*^{f}	0.13	11.46	1.69	10.88	0.87	7.07	0.95	*1.91*^{b}	0.21
	25	15.85	1.30	3.99	2.26	*0.05*^{g}	0.02	11.81	1.02	10.51	0.86	3.94	2.06	*4.62*^{b}	1.78
	Average	**16.44**^{B}	1.19	**4.77**^{b}	1.91	**0.26**^{B}	0.19	**11.40**^{B}	1.21	**10.58**^{B}	0.89	**6.18**^{B}	2.10	**2.47**^{B}	1.90
O	0	14.04	1.22	2.06	1.04	*6.22*^{a}	0.56	5.76	0.33	1.23	0.36	0.86	0.25	*0.33*^{ad}	0.07
	10	14.70	1.49	4.00	1.14	*4.96*^{bc}	0.33	5.75	0.46	1.32	1.10	1.04	1.05	*0.43*^{ad}	0.08
	25	11.89	3.40	3.67	2.28	*3.47*^{ac}	0.44	4.75	0.95	1.50	0.38	0.66	0.06	*0.65*^{b}	0.19
	Average	**13.54**^{A}	2.40	**3.24**^{b}	**1.69**	**4.88**^{C}	**1.24**	**5.42**^{C}	0.76	**1.35**^{C}	0.65	**0.85**^{C}	0.59	**.47**^{A}	0.18
Average	0	**15.45***†	1.47	4.72	2.43	**2.44***	2.81	8.29	2.35	8.09	5.27	**6.21***†	4.21	**.51***	0.31
	10	**15.90***	1.51	5.47	1.99	**2.11***	2.16	8.32	2.66	8.49	5.55	**6.51***	4.71	**1.06***	0.67
	25	**14.23**†	2.70	4.60	2.11	**1.41**†	1.58	8.22	3.16	8.45	5.40	**4.90**†	4.29	**2.48**†	1.96

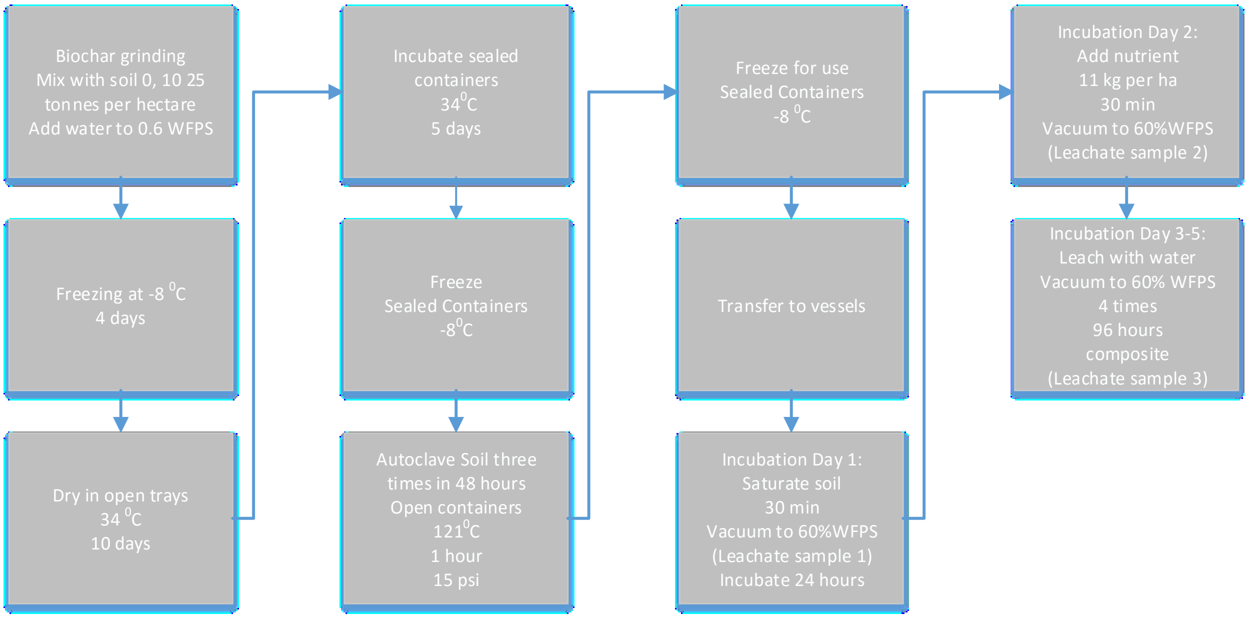

Figure 2-1 Workflow showing amendment and soil handling for the experiment

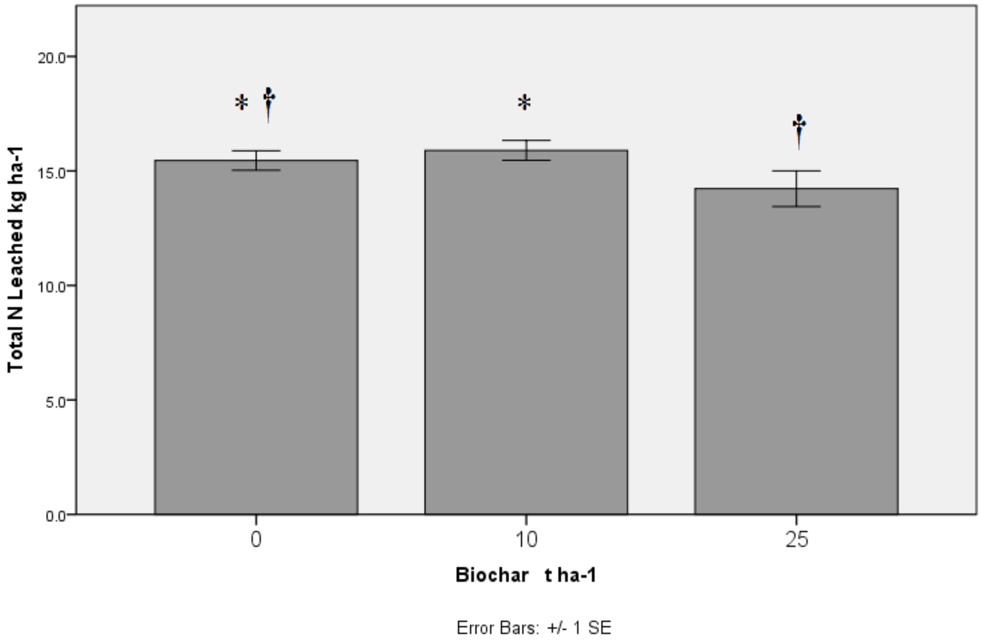

Figure 2-2 Total nitrogen leached from nutrient-amended Ottawa sand and Peace River soil with biochar treatments 0, 10 and 25 tonnes ha^{-1} Symbols indicate significant differences between biochar treatments. Significance declared where p≤α= 0.05 using Tukey`s Post Hoc comparison

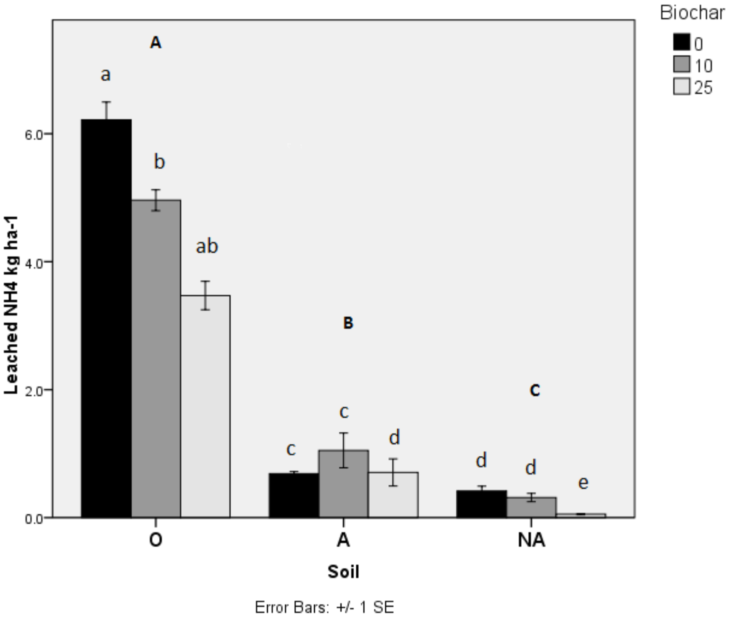

Figure 2-3 Total ammonium leached from nutrient-amended soils with biochar treatments 0, 10 and 25 tonnes ha^{-1} in three soil types: Ottawa Sand (O), autoclaved Peace River soil (A) and non-autoclaved Peace River soil (NA). Uppercase letters above groups indicate significant differences between soil types. Lower case letter indicate significant differences between soil-biochar combinations. Significance declared where p≤α= 0.05 using Tukey`s Post Hoc comparison.

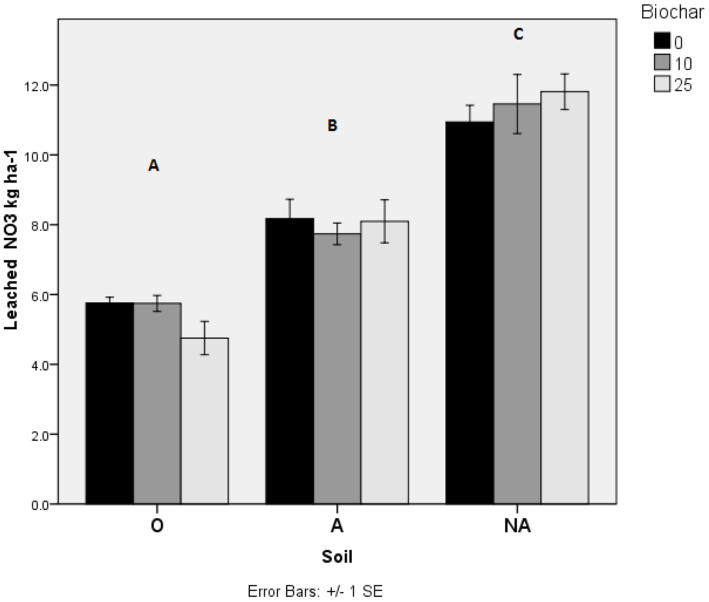

Figure 2-4 Cumulative nitrate leached from nutrient-amended soils with biochar treatments 0, 10 and 25 tonnes ha^{-1} in three soil types: Ottawa Sand (O), autoclaved Peace River soil (A) and non-autoclaved Peace Rver soil (NA). Uppercase letters above groups indicate significant differences between soil types. Significance declared where p≤α= 0.05 using Tukey`s Post Hoc comparison.

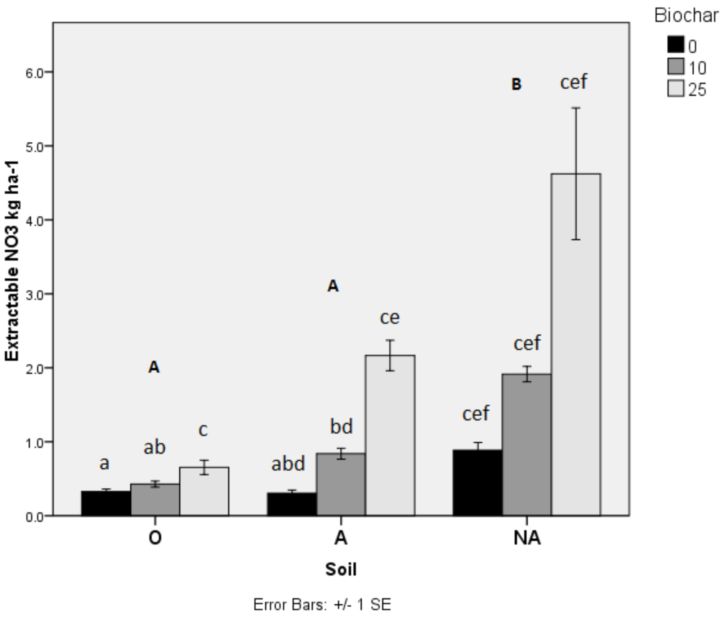

Figure 2-5 Extractable nitrate in nutrient-amended soils with biochar treatments 0, 10 and 25 tonnes ha^{-1} in three soil types: Ottawa Sand (O), autoclaved Peace River soil (A) and non-autoclaved Peace River soil (NA) . Uppercase letters above groups indicate significant differences between soil types. Lower case letter indicate significant differences between soil-biochar combinations. Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison.

Table 2-9 P-values for factors in ANOVA Model 2 in nutrient-amended soils with biochar treatments. Bold lettering indicates statistical significance where $p \leq \alpha = 0.05$. $^{\psi}$ Denotes Permutational ANOVA

	N Leaching	**DON** $^{\psi}$	**DIN**	**Leach NH4** $^{\psi}$	**Leach NO3**	**Retained Soil N**	**MBN** $^{\psi}$	**Ext NO3** $^{\psi}$	**Ext NH4** $^{\psi}$	**CO2 Respiration**	**MBC** $^{\psi}$	**Min** $^{\psi}$	**Nitrif**	**NIT:MIN Ratio** $^{\psi}$
		kg ha^{-1}\`	kg ha^{-1}\`	kg ha^{-1}	kg ha^{-1}\`	kg ha^{-1}	kg ha^{-1}\`	kg ha^{-1}	kg ha^{-1}\`	kg ha^{-1}	kg ha^{-1}\`	kg ha^{-1}	kg ha^{-1}\`	kg ha^{-1}
Autoclave	0.322	0.134	**0.001**	**≤0.0001**	**≤0.0001**	**≤0.0001**	**0.013**	**≤0.0001**	**≤0.0001**	0.084	0.072	0.824	**<0.0001**	**<0.0001**
Biochar	**0.030**	**≤0.0001**	0.544	**0.028**	0.691	0.277	0.859	**<0.0001**	**0.001**	**0.019**	**0.030**	0.213	**<0.0001**	**<0.0001**
Nutrient	**≤0.0001**	0.189	0.306	0.110	**≤0.0001**	**<0.0001**	0.158	**0.023**	<0.0001	0.198	0.663	0.710	0.1776	**0.0148**
Autocl:Bioch	0.975	0.891	0.762	0.383	0.510	0.718	0.490	**0.005**	**0.039**	0.206	0.261	0.660	0.0798	**0.0415**
Autocl:Nutr	0.243	0.858	0.895	0.293	0.125	0.355	0.699	0.808	0.379	0.838	0.960	0.922	0.1765	0.0981
Bioch:Nutr	0.930	0.199	0.614	0.902	0.760	0.894	0.137	**0.007**	0.560	0.842	0.843	0.800	0.1059	0.2231
Autocl:Bioch:Nutr	0.934	0.943	0.820	0.660	0.712	0.512	0.486	0.566	0.259	0.826	0.562	0.887	0.5432	0.565

Table 2-10 Pairwise comparisons of Nitrogen totals from Model 2 in soil treated with biochar at 0, 10 and 25 tonnes ha^{-1} in non-autoclaved (NA) and autoclaved (A) Peace River soil (averaging nutrient amended and non-amended treatements). Uppercase letters indicate significant differences between autoclave treatments. Lower case letter indicate significant differences between biochar treatments. Significant main effects indicated by bold letters. Significant interactions (autoclave × biochar) indicated by italics. Significance declared where p≤α= 0.05 using Tukey`s Post Hoc comparison. $^{\psi}$ Denotes permutational ANOVA

Bioch	**Autocl.**	**N Leaching**	**Std. Dev**	**DON**$^{\psi}$	**Std. Dev**	**DIN**	**Std. Dev**	**Leach** $NH_4$$^{\psi}$	**Std. Dev**	**Leach** NO_3	**Std. Dev**	**Retained Soil N**	**Std. Dev**	**MBN**$^{\psi}$	**Std. Dev**
		kg ha^{-1}`		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}	
0	NA	12.39	4.62	4.00	1.81	8.39	3.31	0.39	0.13	8.01	3.25	7.73	2.89	1.66	0.65
	A	11.98	4.09	5.85	1.49	6.13	3.04	0.64	0.17	5.49	3.02	9.67	3.47	1.44	0.88
	Average	**12.19**^{ab}	4.22	**4.92**^{a}	1.87	7.26	3.28	**0.51**^{a}	0.20	6.75	3.30	8.70	3.24	1.55	0.76
10	NA	12.86	4.71	3.89	2.02	8.97	3.43	0.31	0.13	8.66	3.36	8.18	2.99	1.76	0.42
	A	12.38	4.10	6.21	1.40	6.17	2.88	0.88	0.47	5.28	2.69	10.80	3.15	1.07	0.52
	Average	**12.62**^{a}	4.27	**5.05**^{a}	2.06	7.57	3.38	**0.60**^{b}	0.44	6.97	3.42	9.49	3.26	1.41	0.58
25	NA	11.47	4.78	2.92	1.91	8.56	3.61	0.05	0.02	8.50	3.60	8.27	2.51	1.99	0.49
	A	11.20	4.17	4.94	1.58	6.26	2.94	0.65	0.33	5.61	2.81	10.17	3.92	1.11	1.44
	Average	**11.34**^{b}	4.34	**3.93**^{b}	1.99	7.41	3.39	**0.35**^{ab}	0.38	7.06	3.46	9.22	3.33	1.55	1.13
Avg.	NA	12.24	4.53	3.60	1.90	**8.64**^{A}	3.30	**0.25**^{A}	0.18	**8.39**^{A}	3.27	**8.06**^{A}	2.69	**1.80**^{A}	0.53
	A	11.86	3.97	5.67	1.52	**6.19**^{B}	2.82	**0.72**^{B}	0.35	**5.46**^{B}	2.72	**10.21**^{B}	3.40	**1.20**^{B}	0.99

Table 2-10 continued Pairwise comparisons of Nitrogen totals from Model 2 in soil treated with biochar at 0, 10 and 25 tonnes ha^{-1} in non-autoclaved (NA) and autoclaved (A) Peace River soil (averaging nutrient amended and non-amended treatements). Uppercase letters indicate significant differences between autoclave treatments. Lower case letter indicate significant differences between biochar treatments. Significant main effects indicated by bold letters. Significant interactions (autoclave × biochar) indicated by *italics*. Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison. ᵠ Denotes permutational ANOVA

Bioch	**Autocl.**	Ext NO$_3$ ᵠ	Std. Dev	Ext NH$_4$ ᵠ	Std. Dev	CO$_2$ Respiration	Std. Dev	MBC ᵠ	Std. Dev	Min ᵠ	Std. Dev	Nitrif	Std. Dev	NIT:AMM ᵠ	Std. Dev
		kg ha^{-1}`		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}			
0	NA	*0.84*^{z}	0.20	*5.23*^{z}	2.56	94.54	37.03	13.18	4.61	6.79	0.73	5.21	0.96	*.767*^{y}	.115
	A	*0.34*^{z}	0.08	*7.89*^{y}	2.74	85.63	32.72	11.27	4.71	6.68	1.27	2.19	1.03	*.323*^{w}	.155
	Average	**0.59**^{a}	0.30	**6.56**^{a}	2.91	**90.08**^{a}	34.06	**12.22**^{a}	4.61	6.73	1.00	3.70a	1.83	**.545**^{a}	.264
10	NA	*2.03*^{y}	0.40	*4.39*^{xz}	2.95	101.38	29.90	19.19	5.64	7.73	1.28	7.06	1.70	*.904*^{z}	.085
	A	*0.73*^{z}	0.17	*9.00*^{y}	3.25	132.35	43.80	18.25	9.56	8.22	2.75	2.38	0.77	*.302*^{x}	.071
	Average	**1.38**^{b}	0.73	**6.69**^{a}	3.82	**116.87**^{ab}	39.60	**18.72**^{b}	7.60	7.98	2.09	**4.72**^{a}	2.73	**.603**^{b}	.320
25	NA	*3.79*^{x}	1.56	*2.49*^{x}	2.09	113.23	33.41	22.43	7.00	7.18	0.63	8.66	2.01	*1.208*^{z}	.283
	A	*1.62*^{yz}	0.91	*7.44*^{y}	2.99	157.67	62.27	13.61	8.28	7.67	2.12	3.60	1.37	*.460a*^{wx}	.103
	Average	**2.70**^{c}	1.66	**4.97**^{b}	3.57	**135.45**^{b}	53.46	**18.02**^{b}	8.70	7.43	1.53	**6.13**^{b}	3.10	**.834**^{ab}	.438
Avg.	NA	**2.22**^{A}	1.53	**4.04**^{A}	2.71	103.05	33.03	18.27	6.81	7.23	0.97	**6.97**^{A}	2.11	**.960**^{A}	.257
	A	**0.90**^{B}	0.75	**8.11**^{B}	2.94	125.22	54.95	14.38	8.01	7.52	2.14	**2.72**^{B}	1.22	**.362**^{B}	.131

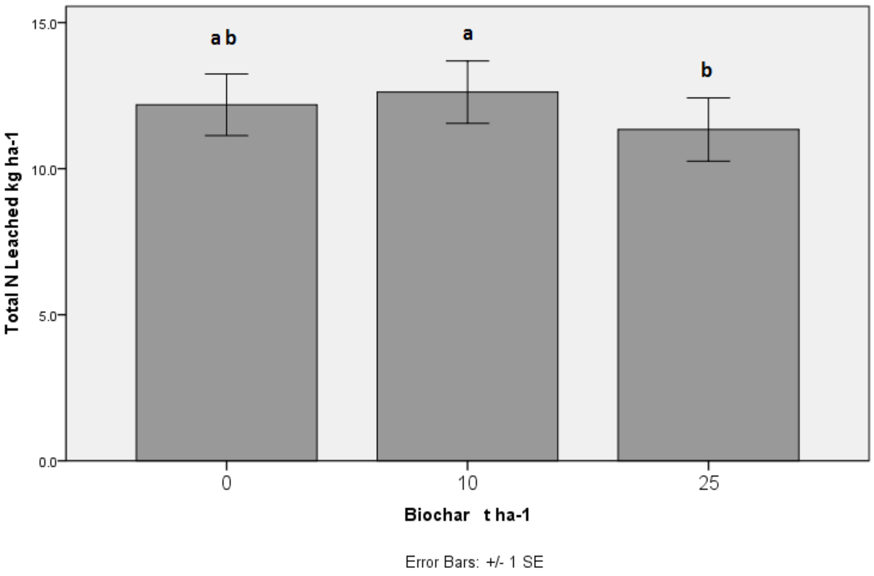

Figure 2-6 Total nitrogen leaching in Peace River soil with respect biochar treatments of 0, 10 and 25 tonnes ha^{-1}, averaged across autoclave and nutrient-amendment treatments. Letters indicate significant differences between biochar treatments. Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison.

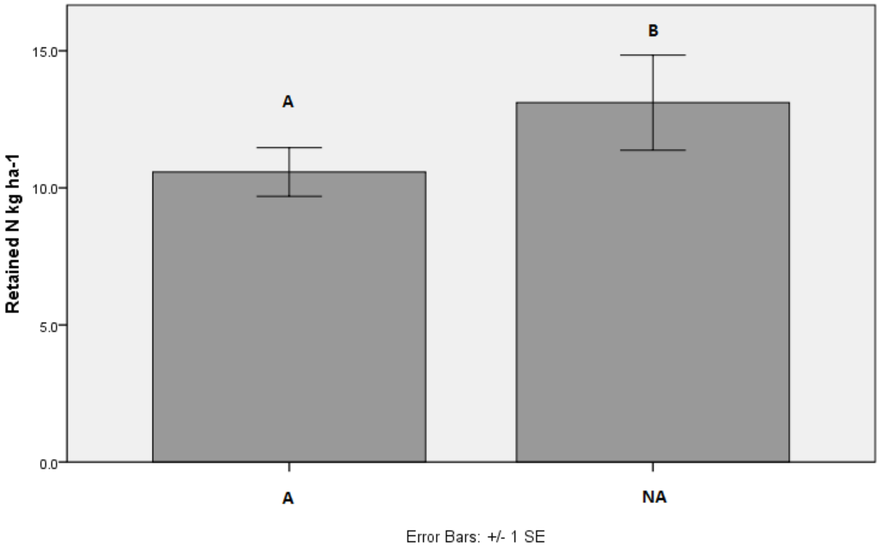

Figure 2-7 Retained Nitrogen in Peace River soil with respect to autoclave (A) and non-autoclaved (NA) treatments averaged over nutrient-amendment and biochar treatments Letters indicate significant differences between autoclave treatments. Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison.

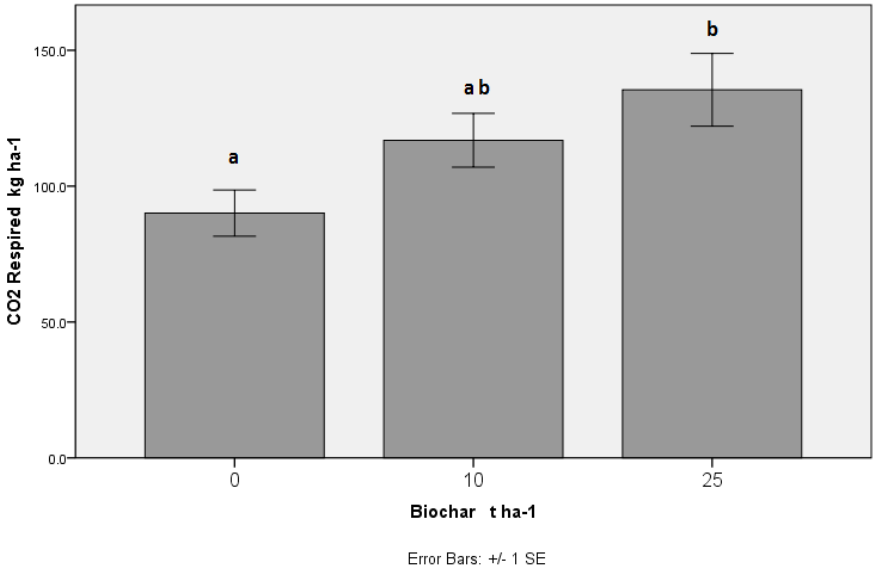

Figure 2-8 Total CO_2 respiration in Peace River soil with respect biochar treatments of 0, 10 and 25 tonnes ha^{-1}, averaged across autoclave and nutrient-amendment treatments. Letters indicate significant differences between biochar treatments. Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison.

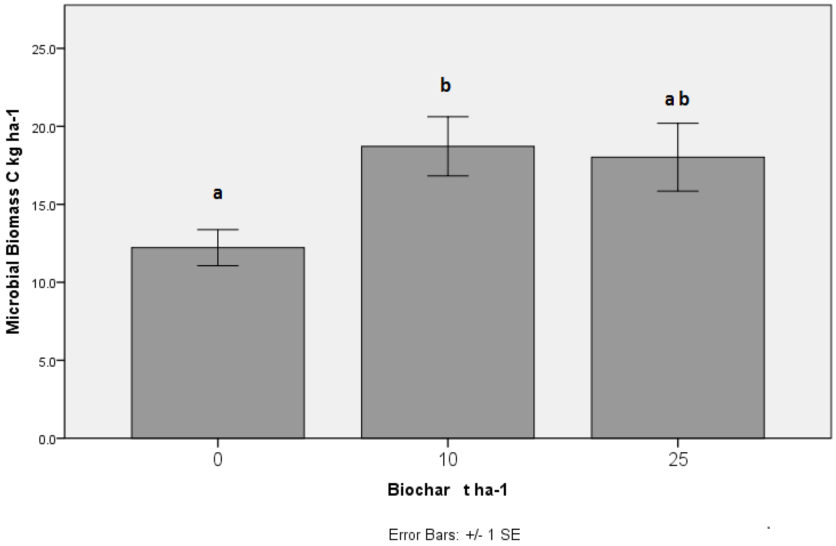

Figure 2-9 Microbial biomass in Peace River soil with respect biochar treatments of 0, 10 and 25 tonnes ha^{-1}, averaged across autoclave and nutrient-amendment treatments. Letters indicate significant differences between biochar treatments. Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison.

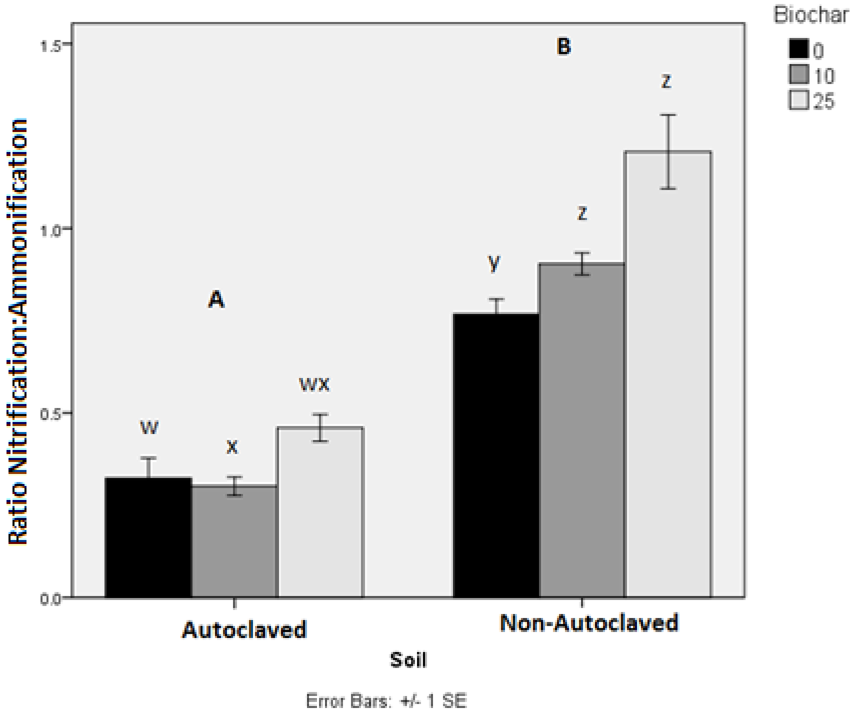

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Figure 2-10 Nitrification:Ammonification ratio in soil treated with biochar at 0, 10 and 25 tonnes ha^{-1} in autoclaved versus non-autoclaved soilUppercase Letters indicate significant differences between autoclave treatments. Lowercase letters indicate significant differences between biochar × autoclave treatments. Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison.

Chapter 3. Use of Soil Amendments in Forest Ecosystem Reclamation

3.1. Introduction

In the upstream oil and gas sector, sites which are constructed for exploration and production must be abandoned and reclaimed. Construction practices often require the removal of topsoil from well sites and, invariably, some portion of this material is lost due to accelerated topsoil decomposition during storage or poor conservation practices. This may result in less organic matter being present on reclaimed well sites.

Soil deficiencies such as low organic matter affect more than just the location and speciation of nutrients. Soil attributes such as water holding capacity and penetrability may impact revegetation of sites following disturbance. The addition of organic soil amendments such as compost can improve these soil qualities as well as providing required nutrients for plant growth. It would be advantageous to understand the behaviour of organic amendments when added to a forest ecosystem particularly with respect to nitrogen dynamics as nitrogen is often thought of as limiting in forests ecosystems (Bosatta and Staaf, 1982; Tamm et al. 1982). However, it should be recognized that nutrient mass is not evenly distributed in any forest ecosystem (REF) and that differentials in nutrient {content??] result in adaption by plants and unique ecosites. If organic amendments are very recalcitrant in forest soils they may decompose too slowly to add required nutrients. Conversely, if they decompose too quickly, they may release large concentrations of nutrients over a short period of time resulting in nutrient losses from the soil in by leaching, soil gas flux or may support undesirable nitrophillic species instead of desired native species. Ideally, nutrients are conserved and continually cycled within the forest system. Understanding these dynamics would allow reclamation practitioners to choose between amendments, selecting the type, quantity and combination of materials which would allow reclamation to mimic the nutrient dynamics and succession trajectories of natural systems. The addition of amendments would be sufficient to supply nutrients to match the nutrient uptake of vegetation at the site. In this situation, organic amendment decomposition would facilitate the formation of the O horizon soil resulting in the eventual replacement of amendment organic matter in the soil with organic matter produced by plant decomposition.

3.1.1.Mineralization and Biochar

Mineralization is the process by which nutrients derived from plant residues are transformed into forms which are plant available. In the case of nitrogen, proteins, amino sugars and nucleic acids contained in soil organic matter are degraded into NH_4^+ -one of the mineral forms of nitrogen (Paul and Clark 1998). This process is undertaken by a variety of microbes and environmental conditions such as pH, moisture, temperature and available oxygen are influential in their activity. While microbially mediated nutrient transformation is important to determining plant available nutrients, mineralization of organic matter may be considered a limiting factor in nutrient availability. The rate at which added organic material mineralizes is an important determinant of reclamation trajectory. Mineralization of organic matter is mediated by microbes which respond to environmental conditions, however synergistic and antagonist effects have both been observed when adding nutrient-rich organic amendments to soil. High levels of nutrient have been found stimulate decomposition of recalcitrant native organic materials (Dijkstra, et al. 2009) and other research has shown that elevating nitrogen levels suppresses the formation of lignin decomposing enzymes (Carreiro et al., 2000; Saiya-Cork et al., 2002).

Biochar as a co-amendment has been associated with increased organic matter mineralization and nitrogen immobilization (Hamer et al. 2004 Novak et al., 2009). These effects appear to be variable and determined by a number of factors such soil type, water holding capacity, type of biochar, and type of organic substrate. For example, previous work has shown that mineralization is increased in soil amended with biochar produced at low temperatures (250 and 400°C) and decreased with biochar produced at higher temperatures (525 and 650ºC) (Zimmerman et. al, 2011). Biochar may affect nitrogen leaching and retention and immobilization by microbes. The mineralization of organic matter is only part of the overall picture when determining biochar's impact on plant available nutrients (Sika and Hardie, 2014). When considering using amendments for reclamation, the balance between formation of SOM and release of nutrients for plant growth is required in order to correctly apply exogenous organic materials. The microbes responsible for this are sensitive to environmental conditions (Turrion et al. 2012) and biochar has been shown to influence a number of these conditions thereby influencing mineralization rates and nutrient cycling processes.

3.2. Research Objectives and Hypotheses

The goal of this experiment is to determine effect of biochar on organic matter mineralization and determine the net effect of different soil amendments applied alone or in combination with biochar on available nitrogen. Raw biochar produced at AITF and a biochar reclamation amendment (BCRA) are both evaluated as biochar amendments. Compost and a forest floor amendment are used as organic matter additions. This information may be useful in selecting amendments and determining application rates in order to meet the nutrient requirements of vegetation which desired for reclamation of disturbed areas.

As soil priming is often encountered with biochar addition, it was predicted that the addition of biochar would stimulate release of carbon and nitrogen from the soil. Further, it was predicted that non-amended soil, followed by forest floor amended soil, followed by compost amended soil will each release successively more carbon and nitrogen given the increasing amount of organic carbon and nitrogen in each of these substrates.

3.3. Materials and Methods

3.3.1. Study Soil

The soil used in this experiment was obtained from an abandoned well site located approximately 230 km east of Peace River, AB (12-08-82-13 W6M elevation 700m) within the central mixedwood subregion of the Boreal Natural Area. The soil texture consists principally of sand with little organic matter present and is mapped as a Regosol. After abandonment, the site was recontoured and topsoil replaced. In June 2012, the entire area was seeded with mixtures of fall rye and native grasses (awned wheat grass, slender wheat grass, fringed brome and ticklegrass) to help stabilize the soil and provide protection for planted and naturally established trees. The site is surrounded by Jack pine with patches of aspen in the overstory and understory dominated by lichens and ericaceous shrubs. The organic forest floor horizon in the natural stand was very thin and was mixed with the underlying sand during site work. The result of the topsoil replacement was largely sand with some fine organics present. The site was visited by AITF from May 27-31, 2013 and soil samples were obtained by hand from shallow pits. The study soil was air dried at 40ºC and ground to 2 mm prior to homogenization by the cone and quarter method using 5 passes (Schumacher et al. 1990). The site was visited a second time from July 15-19, 2013 in order to collect additional soil samples for this study. The soil appeared slightly coarser and lighter in color than the soils previously collected in May, 2013. The soil collected during the second visit was used for the lower 20 cm of the soil columns.

Soil chemical properties were provided by AITF and can be found in Table 3-1. Samples of the soil were oven dried at 105°C for 24 hours and then weighed to measure water content. The bulk density and porosity were measured by placing the dried soil in a cup and gently tamping to settle then measuring the mass of the soil. Density (g/cm^3) = Dry soil weight (g)/Soil volume (cm^3). The moisture content (by mass) was calculated by the following formula: Water (%) by mass = [(wet mass - dry mass)/dry mass]×100. Porosity was calculated by wetting a known volume of the dried soil to saturation and inferring the saturation volume as volumetric pore content then dividing this by the overall volume of soil used. Additional analysis including total nitrogen, extractable ammonium nitrogen (NH_4-N), extractable nitrate nitrogen (NO_3-N), total carbon, total organic carbon and microbial biomass carbon and nitrogen were performed at the Natural Resources Analytical Laboratory at the University of Alberta (NRAL) prior to the leaching experiment.

The raw biochar was produced by AITF in Vegreville, Alberta using pinchips from debarked spruce wood. This feedstock was selected as it is unlikely to contain contaminants such as heavy metals. AITF's Auger Retort Carbonizer was used to produce the biochar required at a maximum feedstock temperature of 600ºC and an average retention time of 9 min (AITF, 2015)

The Biochar Reclamation Amendment (BCRA) was produced at AITF in Vegreville by pelletizing a mixture of compost and biochar (70% and 30% respectively). The raw biochar and BCRA physical and chemical properties (pH, conductivity, CEC) were provided by AITF and can be found in Table 3-1.

The compost was provided by the City of Edmonton's Waste Management Centre and the forest floor mix was retrieved from the undisturbed area surrounding the Peace River site by hand excavating the top 2-3 cm of organic material on the soil. Results of the chemical analysis for all amendments analysis can be found in Table 3-1.

3.3.2. Experimental Design and Preparation

This experiment used a factorial design with four replicates of each treatment. The first factor consisted of 2 organic amendments applied at a rate of 25 t/ha or no amendment. The treatments included compost, forest floor mix and non-amended soils. The second factor consisted of two types of biochar: raw biochar, BCRA (pelletized biochar and compost). Biochar and BCRA were added to non-amended and organic-amended soils used in the experiment in 0, 10 and 25 tonne per hectare (tonne ha^{-1}) applications to examine the effects of two biochar treatment levels compared to a control.

A second experiment was run concurrently in order to determine whether the BCRA produced by AITF performed similarly to the raw component products. One treatment of raw compost and raw biochar was blended in the same proportions as BCRA (70% compost, 30% biochar) and applied at 25 tonne ha^{-1}.

3.3.3. Incubation Procedure

The soil incubation was carried out over 12 weeks at Alberta Innovates Technology Futures lab in Edmonton, Alberta. The soil was contained cylindrical soil columns measuring 60 cm in height and 10 cm in diameter. The bottom 20 cm of each column was filled with the study soil from the Peace River Site. A 10 cm layer of soil amended according to the experimental design was added to each to simulate addition of organic amendments which are tilled into the top 10 cm of soil (see Figure 3-1).

500 mL of water was added to the columns initially to wet the soil. Over the course of the study water was added to the columns weekly. The total volume of water added to each column (not including initial wetting) was 930 mL over 12 weeks, equivalent to the average May to October weekly precipitation in the Peace River area, about 10.7 mm per week (Environment Canada). Water was collected weekly from the bottom of the columns weighed and frozen for later analysis.

3.3.4. Analysis

3.3.4.1. N_2O and CO_2 Flux

Soil N_2O and CO_2 flux was measured using an Innova 1312 photoaccoustic infrared portable gas analyzer. The headspace above the vessel was purged with outside air then sealed. Readings were taken seven times in 10 minutes to establish the rate of increasing or decreasing CO_2 and N_2O concentrations in the headspace of the vessels. CO2 flux was also measured using a Licor 8100 Soil Gas flux system taking three readings from each column. Flux measurements were performed twice weekly using each of the two instruments.

3.3.5.Leachate Analysis

The leachate samples from each column were used to create three composite samples consisting of leachate collected from weeks 1-5, weeks 6-9 and weeks 10-12. These composite samples were submitted for total carbon, total organic carbon, and total nitrogen analysis using a Shimadzu Total Carbon and Nitrogen Analyzer (Shimadzu, 2001). NH_4-N and NO_3-N analysis was performed using a SmartChem 200 sphectrophotometer.

3.3.6.Soil Analysis

At the conclusion of the leaching experiment, the top 10 cm of soil from the vessels was collected in sealed Ziploc^{TM} bags and analysed for extractable NH_4-N and NO_3-N using the KCl extraction method (Maynard et al. 2008). Only two of the four soil replicates were run for extractable NH_4-N and NO_3-N.

3.3.7.Statistical Analysis

ANOVA testing on main factors and interactions was performed using R and statistical significance was declared where $p \leq \alpha = 0.05$. Pairwise comparisons were performed using Tukey's post hoc comparisons were performed where ANOVA results were significant. Relevant interaction effects were also tested using Tukey's post hoc testing. The post hoc comparisons were considered significant where $p \leq \alpha = 0.05$. When assumptions of normality and homoscedasticity were violated, permutational ANOVA was used for data analysis as log, square root and inverse transformations were largely unsuccessful at creating a normal distribution.

3.4. Results

3.4.1.Raw Biochar

Tables 3-2 summarizes ANOVA results and treatment means including pairwise comparisons are summarized in Table 3-3. Generally, compost-amended soils leached significantly more nitrogen as nitrate than soils amended with forest floor material or non-amended soils. The addition of compost to soil increased nitrogen leaching by 26.7 and 27.2 kg ha^{-1} over forest floor and non-amend soil respectively and had a significant effect on extractable nitrogen contained within the soil. This data supports the use of organic amendments such as compost to add nitrogen to soils low in organic matter.

Raw biochar application had a significant effect on CO_2 respiration with pairwise comparison showing a significant effect between the 0 and 25 tonne ha^{-1} treatments (Figure 3-2) perhaps demonstrating the positive effects of biochar on soil microbes. While an associated increase in mineralization of organic matter would also be expected given the increase in respiration, it is also possible that the observed increase in CO_2 respiration was a result of the initial decomposition of labile fractions within biochar, rather than from soil organic matter as found by in other experiments (Smith and Collins, 2010; Cross and Saran 2011).

Raw biochar appeared to exert different effects in amended versus non-amended soils with respect to carbon and nitrogen leaching, however these results were not significant. In non-amended soil, increasing the biochar rate to 25 tonne ha^{-1} was observed to increase nitrogen soil loss over the 0 and 10 tonne ha^{-1} treatments, this is not-consistent with findings in part I where 10 tonne ha^{-1} treatments leached the most nitrogen. This might indicate that the impact of biochar changes with time (i.e. a 12 week versus a 5 day experiment). As well, the increase in extractable NO_3-N in the non-amended soils might indicate enhanced nitrification as observed in Chapter 2.

Raw biochar did not have a significant effect on N_2O production, nitrogen leaching, carbon leaching or extractable nitrogen storage in soil. The application rate of the biochar may have been too low to observe a discernible effect on these variables. This is consistent with the findings of Prommer et al. (2014) that biochar did not significantly impact mineralization.

Total carbon and total organic carbon leaching were not significantly affected by amendment type or raw biochar addition. In non-amended soils both carbon and nitrogen leaching was increased with biochar treatment, while in soils amended with compost or forest floor material, carbon and nitrogen leaching was reduced with increasing biochar, however these findings were not statistically significant. ANOVA testing showed that extractable nitrogen was significantly affected by amendment type however, pairwise comparison showed no significant effect between the individual amendment types There was an increase in the extractable NO_3-N in the non-amended samples, (Table 3-3) however it was not statistically significant as the small number of extractable nitrogen samples submitted (n=2) did not provide sufficient statistical power to detect an effect during pairwise comparison.

3.4.2.BCRA

Tables 3-4 summarizes ANOVA results and treatment means for BCRA treated soil. Pairwise comparisons are summarized in Table 3-5. As with raw biochar, compost-amended soils leached significantly more nitrogen as nitrate than soils amended with forest floor material or non-amended soils. Nitrogen leaching was greatest in soil amended with 25 tonne ha^{-1} BCRA, though this is unsurprising as BCRA is comprised of 70% compost. CO_2 respiration, total carbon and organic carbon leaching was not significantly affected by amendment type or BCRA addition. Extractable nitrogen (as both NH_4^+ and NO_3^-) was significantly increased with BCRA amendment. A significant BCRA application rate × Amendment interaction occurred for extractable nitrogen in the 25 tonne ha^{-1} BCRA treated soils without compost or forest floor. This treatment having significantly more extractable nitrogen than all of the other treatments (Figure 3-3-and 3-4). When comparing BCRA and compost/biochar blend, they behaved similarly in terms of respiration and carbon loss (Tables 3-6 and 3-7). The nitrogen lost and extractable nitrogen remaining in soil of the BCRA pellet treatments, were significantly different from control, but this was not the case for the compost/biochar blend (Tables 3-6, 3-7 and Figure 3-5).

BCRA application to the soil did not show a significant effect on respiration and leaching, however, exactable nitrogen in soil was significantly increased in the 10 and 25 tonne ha^{-1} treatments (5.8 and 11.3 kg ha^{-1} respectively) when compared to non-BCRA treated soils (Figure 3-4).

The BCRA pellets appeared to perform better than the biochar/compost blend in terms of both reduced nitrogen losses and more plant available nitrogen remaining in soil (Figure 3-5).

3.5. Conclusions

As the addition of biochar did not appear to significantly alter N mineralization we may conclude that biochar may not induce the release of plant available nitrogen however the increase in CO_2 respiration may hint at accelerated decomposition of organic matter.

Addition of organic amendments can add nitrogen to soils; however, understanding the rate of plant available nutrient release is important in determining application rates and types of amendments selected. While some previous work indicates that organic matter mineralization is affected by biochar (Wardle, 2008), this experiment has not supported this hypothesis in a clear way. Understanding whether biochar accelerates or retards mineralization would be of assistance in assessing its use as a reclamation amendment and further study may be required to determine the effects of soil type, biochar characteristics and application rate. While biochar's impact on mineralization may not be confirmed, other considerations such as nitrogen transformation, nutrient retention and water holding capacity may warrant the use of biochar in reclamation. These factors are relevant to the use of organic amendments as nitrogen immobilization and transformation play a significant role in nitrogen dynamics (Prommer et. al, 2014).

Depending upon the nutrient requirements of the site in question, using compost appears to be a means of providing plant available nitrogen to sites deficient in organic matter. While extractable inorganic nitrogen was not significantly increased in the forest floor or compost amended soils, nitrogen leaching was increased. Increased leaching associated with biochar treatment appears similar to the findings of Chapter 2 though here it was observed in the 25 tonne ha^{-1} treatments of non-amended soil rather than the 10 tonne ha^{-1} treatment. This might indicate that biochar and organic amendments may be useful co-amendments to reduce nitrogen loss in soil and that BCRA pellets may be useful in this regard as it has the potential to provide extractable nitrate to soil and mitigate nitrogen losses.

Table 3-1 Analysis of soil, organic amendment and biochar chemical properties

Parameter	Units	Untreated Soil	Ground Biochar	BCRA	Compost	Forest Floor
Microbial biomass N	(ug g-1 soil)	5.638	-	-	715.7	509.62
Microbial biomass C	(ug g-1 soil)	123.1	-	-	4005.8	4150.12
TOC	(wt%)	0.566	-	39.86	19.48	3.62
Organic Matter	(wt%)	1.082	-	-	-	-
Extractable NH4-N	(mg kg^{-1})	0.0342	0.447	169.68	656.4	5.72
Extractable NO3-N	(mg kg^{-1})	0.0986	0.769	1033.1	1542.5	0.244
Nitrogen	(wt%)	0.023	4.65	-	2.59	0.36
Total Carbon	(wt%)	0.572	89.91	39.45	21.72	4.73
C:N Ratio		24.9	-	-	8.4	13.1
Cation Exchange Capacity	meq 100g-1	-	53	-	-	-
Electrical Conductivity	dS m-1	-	1.68	13.2	17.1	0.114
pH		7.36	9.10	7.83	7.39	5.15

Table 3-2 ANOVA testing of fluxes and pools following incubation in soil treated with raw biochar at 0, 10 and 25 tonnes ha^{-1} in compost amended, forest floor amended and non-amended soils. Bold lettering indicates statistical significance where $p \leq \alpha = 0.05$. *Denotes Permutational ANOVA $^{\Psi}$denotes n=2

	N flux d^{-1}	CO_2 d^{-1}	Leach NH4*	Leach NO3	Leach TN*	DIN	Total N	TC*	TOC*	TIC*	Total C*	Ext NH_4*$^{\Psi}$	Ext NO_3*$^{\Psi}$	Ext N*$^{\Psi}$
Biochar	0.629	**0.042**	0.092	0.821	0.686	0.817	0.706	0.3607	0.6356	1	0.1516	0.146	0.3725	0.4326
Amend	0.627	0.187	0.4362	**≤0.005**	**≤0.005**	**≤0.005**	**≤0.005**	0.6649	0.9608	0.902	0.6863	0.248	0.0556	**0.050**
Biochar × Amend	0.99	0.885	0.6062	0.402	0.538	0.415	0.533	0.7086	0.9889	0.9686	0.9559	0.343	0.434	0.4377

Table 3-3 Pairwise comparisons of nitrogen and carbon average concentrations. Soil treated with raw biochar at rates of 0, 10 and 25 tonnes ha^{-1} in compost amended, forest floor amended and non-amended soils. Uppercase letters (A,B,C) indicate significant differences between amendments Lower case letters (a,b,c) indicate significant differences between biochar treatments. Significant main effects indicated by bold letters. Significant interactions (amendment × biochar) indicated by italics (w,x,y,z). Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison. *denotes Permutational ANOVA $^{\psi}$denotes n=2

Amend	**Biochar**	**N flux d^{-1}**	**Std. Dev**	**CO_2 Flux d^{-1}**	**Std. Dev**	**Leach NH_4***	**Std. Dev**	**Leach NO_3**	**Std. Dev**	**Leach TN**	**Std. Dev**	**DIN**	**Std. Dev**	**Total N Lost**	**Std. Dev**
Compost	0	.0365	.0236	35.56	4.20	0.116	0.102	73.20	22.76	72.39	22.69	73.32	22.82	75.09	23.41
	10	.0429	.0115	34.45	2.34	0.022	0.042	66.68	10.07	63.86	4.48	66.71	10.05	66.96	5.26
	25	.0435	.0071	38.29	3.37	0.045	0.047	62.97	7.95	61.02	11.79	63.02	7.98	64.14	11.18
	Average	.0410	.0146	36.10	3.50	0.061	0.075	**67.62**^{A}	14.34	**65.76**^{A}	14.46	**67.68**^{A}	14.38	**68.73**^{A}	14.65
Forest	0	.0367	.0146	34.58	2.89	0.037	0.041	41.06	1.98	40.46	4.27	41.10	1.97	43.13	3.12
	10	.0370	.0093	34.85	2.09	0.038	0.036	39.43	4.55	39.19	7.27	39.46	4.58	41.87	6.47
	25	.0402	.0075	37.58	5.22	0.030	0.043	38.56	3.51	38.38	6.74	38.59	3.53	41.25	6.26
	Average	.0380	.0100	35.67	3.59	0.035	0.036	**39.68**^{B}	3.35	**39.34**^{B}	5.71	**39.72**^{B}	3.37	**42.08**^{B}	5.04
None	0	.0343	.0053	30.94	4.55	0.066	0.065	36.81	4.83	37.21	6.60	36.87	4.89	39.67	6.10
	10	.0369	.0094	33.43	3.81	0.029	0.044	37.75	5.06	35.57	11.09	37.78	5.09	38.23	10.25
	25	.0377	.0082	36.15	2.66	0.012	0.011	45.33	4.65	44.11	8.78	45.34	4.66	46.83	8.09
	Average	.0363	.0073	33.51	4.06	0.036	0.048	**39.96**^{B}	5.92	**38.96**^{B}	9.02	**40.00**^{B}	5.94	**41.58**^{B}	8.49
All Amendment Treatments	0	.0358	.0148	*33.70*^{a}	4.13	0.073	0.075	50.36	20.89	50.02	20.79	50.43	20.94	52.63	20.96
	10	.0389	.0096	*34.25*^{ab}	2.65	0.030	0.038	47.95	15.24	46.21	15.03	47.98	15.23	49.02	15.02
	25	.0405	.0073	*37.34*^{b}	3.65	0.029	0.037	48.95	11.92	47.84	13.12	48.98	11.93	50.74	12.89

Table 3-3 cont. Pairwise comparisons of nitrogen and carbon average concentrations. Soil treated with raw biochar at rates of 0, 10 and 25 tonnes ha^{-1} in compost amended, forest floor amended and non-amended soils. Uppercase letters (A,B,C) indicate significant differences between amendments Lower case letters (a,b,c) indicate significant differences between biochar treatments. Significant main effects indicated by bold letters. Significant interactions (amendment × biochar) indicated by italics (w,x,y,z). Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison. *denotes Permutational ANOVA $^{\psi}$denotes n=2

Amend	**Biochar**	**TC***	**Std. Dev**	**TOC***	**Std. Dev**	**TIC***	**Std. Dev**	**Total C Lost***	**Std. Dev**	**Ext NH_4^{ψ}**	**Std. Dev**	**Ext NO_3^{ψ}**	**Std. Dev**	**Ext N^{ψ}**	**Std. Dev**
Compost	0	67.86	21.47	58.94	18.84	8.92	7.18	2607.80	215.78	1.29	.01	24.08	4.22	25.38	4.21
	10	59.62	6.00	52.37	2.96	7.25	7.45	2537.83	297.87	1.86	.26	85.74	79.45	87.60	79.71
	25	57.00	7.83	48.11	10.36	8.90	5.48	2813.40	393.44	2.07	.22	34.42	2.55	36.50	2.33
	Average	61.49	13.25	53.14	12.25	8.36	6.17	2653.01	306.65	1.74	.39	48.08	46.26	49.82	46.44
Forest	0	60.30	2.20	52.10	9.42	8.20	7.51	2532.22	59.18	1.85	.57	21.48	3.55	23.33	4.12
	10	60.18	5.05	51.76	11.93	8.42	7.82	2567.80	304.60	2.03	.31	18.68	1.74	20.71	2.06
	25	53.94	6.06	42.52	9.39	11.42	4.83	2763.67	514.08	2.19	.12	25.81	3.65	28.00	3.78
	Average	58.14	5.28	48.80	10.42	9.35	6.39	2621.23	331.11	2.02	.34	21.99	4.01	24.01	4.25
None	0	58.39	9.60	49.12	12.93	9.27	9.39	2293.07	459.88	1.87	.35	21.51	2.40	23.38	2.76
	10	54.82	8.03	45.84	12.56	8.98	5.33	2464.42	412.71	1.81	.08	27.48	12.49	29.29	12.58
	25	60.16	6.98	52.01	13.94	8.15	7.73	2681.81	374.95	1.79	.08	22.71	3.10	24.50	3.02
	Average	57.79	7.83	48.99	12.19	8.80	6.95	2479.76	412.41	1.82	.17	23.90	6.50	25.72	6.55
All Amendment Treatments	0	62.18	13.05	53.39	13.60	8.80	7.33	2477.69	301.60	1.67	.42	22.36	3.00	24.03	3.09
	10	58.21	6.38	49.99	9.68	8.22	6.34	2523.35	313.07	1.90	.21	43.97	48.55	45.87	48.61
	25	57.04	6.86	47.55	11.08	9.49	5.74	2752.96	394.77	2.02	.22	27.65	5.95	29.67	6.02

Table 3-4 ANOVA testing of fluxes and pools following incubation in soil treated with BCRA at 0, 10 and 25 tonnes ha-1 in compost amended, forest floor amended and non-amended soils. Bold lettering indicates statistical significance where $p \leq \alpha = 0.05$. *Denotes Permutational ANOVA $^{\psi}$denotes n=2

	N flux d-1	**CO2 d-1**	**Leach NH4***	**Leach NO3***	**Leach TN**	**DIN***	**Total N**	**TC**	**TOC***	**TIC***	**Total C**	**Ext NH4*ψ**	**Ext NO3*ψ**	**Ext N*ψ**
Biochar	0.39	0.33	1.000	0.38	0.3344	0.21	0.3009	0.808	0.6889	0.7917	0.5677	**0.0425**	**0.0297**	**0.02486**
Amend	0.97	0.34	0.422	**≤0.005**	**≤0.005**	**≤0.005**	**≤0.005**	0.234	0.5222	0.9398	0.6897	0.2431	0.06641	0.06513
Biochar × Amend	0.73	0.32	0.894	0.506	0.67621	0.498	0.629	0.996	1.000	1.000	0.7255	0.1103	**0.00194**	**0.00226**

Table 3-5 Pairwise comparisons of nitrogen and carbon average concentrations. Soil treated with BCRA at rates of 0, 10 and 25 tonnes ha^{-1} in compost amended, forest floor amended and non-amended soils. Uppercase letters (A,B,C) indicate significant differences between amendments Lower case letters (a,b,c) indicate significant differences between biochar treatments. Significant main effects indicated by bold letters. Significant interactions (amendment × biochar) indicated by italics (w,x,y,z). Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison. *denotes Permutational ANOVA $^{\psi}$denotes n=2

Amend	Biochar	N flux d^{-1}	Std. Dev	CO_2 Flux d^{-1}	Std. Dev	Leach NH_4	Std. Dev	Leach NO_3	Std. Dev	Leach TN	Std. Dev	DIN	Std. Dev	Total N Lost	Std. Dev
	0	.037	.024	35.56	4.20	.116	.102	73.20	22.76	72.39	22.69	73.32	22.82	75.09	23.41
	10	.048	.006	37.41	4.39	.095	.083	75.24	13.28	73.54	17.57	75.33	13.35	76.95	17.18
Compost	25	.033	.009	34.30	2.95	.071	.070	68.64	14.91	68.50	18.86	68.71	14.97	70.87	18.67
	Average	.039	.015	35.76	3.77	.094	.080	**72.36^{A}**	16.07	**71.48^{A}**	18.07	**72.45^{A}**	16.13	**74.30^{A}**	18.22
	0	.037	.015	34.58	2.89	.037	.041	41.06	1.98	40.46	4.27	41.10	1.97	43.13	3.12
	10	.041	.007	36.04	3.87	.045	.051	48.28	6.51	47.61	10.42	48.32	6.56	50.52	9.95
Forest	25	.039	.012	33.47	2.41	.068	.078	54.33	9.56	54.68	13.24	54.40	9.64	57.52	12.20
	Average	.039	.011	34.70	3.02	.050	.055	**47.89^{B}**	8.35	**47.58^{B}**	10.92	**47.94^{B}**	8.39	**50.39^{B}**	10.39
	0	.034	.005	30.94	4.55	.066	.065	36.81	4.83	37.21	6.60	36.87	4.89	39.67	6.10
	10	.039	.008	34.03	1.23	.062	.073	47.89	8.14	48.56	11.43	47.95	8.21	51.38	10.76
None	25	.041	.010	36.00	2.89	.058	.059	51.01	8.35	51.58	11.65	51.06	8.40	54.51	10.94
	Average	.038	.008	33.66	3.61	.062	.060	**45.23^{B}**	9.16	**45.78^{B}**	11.24	**45.30^{B}**	9.20	**48.52^{B}**	10.90
	0	.036	.015	33.70	4.13	.073	.075	50.36	20.89	50.02	20.79	50.43	20.94	52.63	20.96
All Amendment Treatments	10	.042	.007	35.82	3.44	.067	.067	57.13	16.02	56.57	17.52	57.20	16.07	59.61	17.41
	25	.038	.010	34.59	2.73	.065	.063	57.99	12.98	58.25	15.52	58.06	13.02	60.97	14.95

Table 3-5 Cont. Pairwise comparisons of nitrogen and carbon average concentrations. Soil treated with BCRA at rates of 0, 10 and 25 tonnes ha^{-1} in compost amended, forest floor amended and non-amended soils. Uppercase letters (A,B,C) indicate significant differences between amendments Lower case letters (a,b,c) indicate significant differences between biochar treatments. Significant main effects indicated by bold letters. Significant interactions (amendment × biochar) indicated by italics (w,x,y,z). Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison. *denotes Permutational ANOVA $^{\Psi}$denotes n=2

Amendment		**TC**	**Std. Dev**	**TOC**	**Std. Dev**	**TIC**	**Std. Dev**	**Total C Lost**	**Std. Dev**	**Ext NH$_4$ $^{\Psi}$**	**Std. Dev**	**Ext NO$_3$ $^{\Psi}$**	**Std. Dev**	**Ext N $^{\Psi}$**	**Std. Dev**
Compost	0	67.86	21.47	58.94	18.84	8.92	7.18	2607.80	215.78	1.29	.01	*24.08*^{z}	4.22	25.38^{z}	4.21
	10	67.74	11.41	58.87	15.91	8.86	6.52	2785.34	497.28	2.30	.63	*27.87*^{z}	1.88	30.17^{z}	1.25
	25	69.02	11.17	58.54	15.54	10.48	5.14	2557.33	385.18	1.79	.07	*20.26*^{z}	7.03	22.05^{z}	7.10
	Average	68.21	13.99	58.79	15.22	9.42	5.79	2650.16	361.99	1.80	.53	24.07	5.07	25.86	5.22
Forest	0	60.30	2.20	52.10	9.42	8.20	7.51	2532.22	59.18	1.85	.57	*21.48*^{z}	3.55	23.33^{z}	4.12
	10	59.71	6.49	52.62	12.92	7.08	7.19	2676.98	457.82	1.83	.21	*33.63*^{yz}	11.75	35.47^{yz}	11.95
	25	63.91	12.10	54.57	15.04	9.34	5.45	2490.76	345.38	2.71	.14	*24.08*^{z}	4.94	26.79^{z}	5.08
	Average	61.31	7.52	53.10	11.52	8.21	6.20	2566.65	312.41	2.13	.53	26.40	8.23	28.53	8.27
None	0	58.39	9.60	49.12	12.93	9.27	9.39	2293.07	459.88	1.87	.35	*21.51*^{z}	2.40	23.38^{z}	2.76
	10	61.36	10.35	53.72	16.85	7.64	6.56	2516.38	122.88	2.06	.08	*21.79*^{z}	2.54	23.85^{z}	2.62
	25	62.58	10.00	53.46	16.24	9.12	6.60	2672.44	379.59	2.20	.24	*54.79*^{y}	5.57	56.99^{y}	5.33
	Average	60.78	9.22	52.10	14.14	8.68	6.95	2493.96	357.12	2.05	.25	32.70	17.37	34.74	17.48
All Amendment Treatments	0	62.18	13.05	53.39	13.60	8.80	7.33	2477.69	301.60	1.67^{a}	.42	*22.36*^{a}	3.00	*24.03*^{a}	3.09
	10	62.94	9.45	55.07	14.14	7.86	6.17	2659.57	376.88	2.06^{ab}	.36	*27.76*^{ab}	7.59	*29.83*^{ab}	7.57
	25	65.17	10.47	55.52	14.31	9.65	5.25	2573.51	344.14	2.23^{b}	.43	*33.04*^{a}	17.54	*35.28*^{b}	17.56

Table 3-6 ANOVA testing of nitrogen and carbon fluxes and pools following incubation in untreated soil and soil treated with BCRA at 25 tonne ha^{-1}, and raw biochar compost blend at 25 tonne ha^{-1} Bold lettering indicates statistical significance where p≤α= 0.05. *Denotes Permutational ANOVA $^{\psi}$denotes n=2

	N flux d-1	**CO2 d-1**	**Leach NH4***	**Leach NO3***	**Leach TN**	**Leach DON**	**Total N Lost**	**TC**	**TOC**	**TIC**	**Total C Lost**	**Ext N$^{\psi}$**
Pelletizing	0.646	0.104	0.7321	0.2251	**0.0507**	0.381	**0.032**	0.643	0.887	0.874	0.325	**0.0129**

Table 3-7 Pairwise comparisons of nitrogen and carbon average concentrations. Untreated soil and soil treated with BCRA at 25 tonne ha^{-1}, and raw biochar compost blend at 25 tonne ha^{-1}. Lower case letters (a,b,c) indicate significant differences between treatments. Significance declared where p≤α= 0.05 using Tukey`s Post Hoc comparison. *denotes Permutational ANOVA $^{\psi}$denotes n=2

Biochar Treatment	**Biochar Type**	**CO2 d-1**	**N flux d-1**	**Leach NH4***	**Leach NO3***	**Leach TN**	**Leach DON**	**Total N Lost**	**TC**	**TOC**	**TIC**	**Total C Lost**	**Ext N$^{\psi}$**
Control – No treatment	Mean	30.94	0.0343	0.0657	36.81	**37.21^{a}**	0.33	**39.67^{a}**	58.39	49.12	9.27	2293.07	**23.38^{a}**
	Std. Dev.	4.55	0.0053	0.0651	4.83	6.60	2.72	6.10	9.60	12.93	9.39	459.88	2.755
BCRA	Mean	36.00	0.0406	0.0577	51.01	**51.58^{ab}**	0.52	**54.51^{ab}**	62.58	53.46	9.12	2672.44	**56.99^{b}**
	Std. Dev.	2.89	0.0101	0.0591	8.35	11.65	4.57	10.94	10.00	16.24	6.60	379.59	5.33
Raw Biochar Compost	Mean	36.40	0.0397	0.0397	52.73	**58.39^{b}**	5.62	**61.28^{b}**	56.08	49.17	6.91	2690.06	**28.56^{ab}**
	Std. Dev.	2.94	0.0132	0.0718	17.45	12.36	8.50	11.27	9.42	13.50	4.58	341.78	0.815

Figure 3-1 Soil columns for amendment and biochar study. The lower 20 cm was comprised of non-amended soil. Amendments were added to the top 10 cm of the soil.

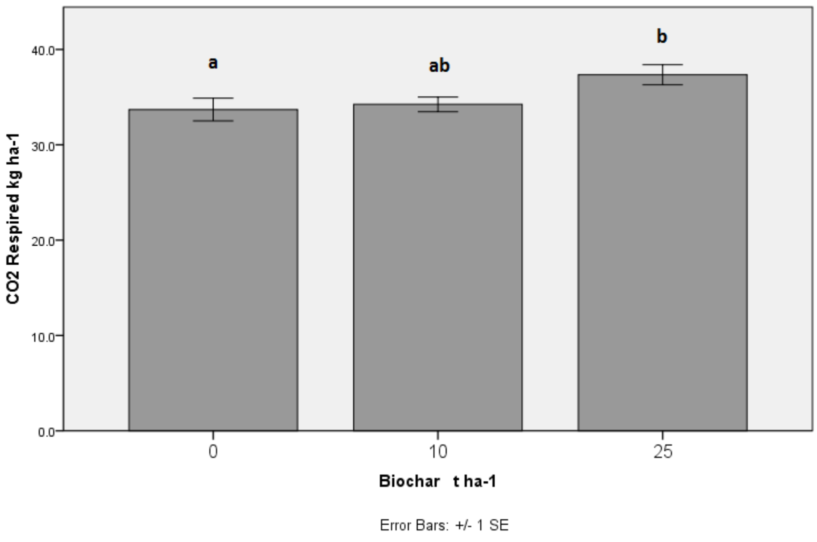

Figure 3-2 CO_2 respiration leached from soil treated with 0, 10 and 25 tonnes ha^{-1} of raw biochar. Letters above groups indicate significant differences between treatments. Significance declared where p≤α= 0.05 using Tukey`s Post Hoc comparison

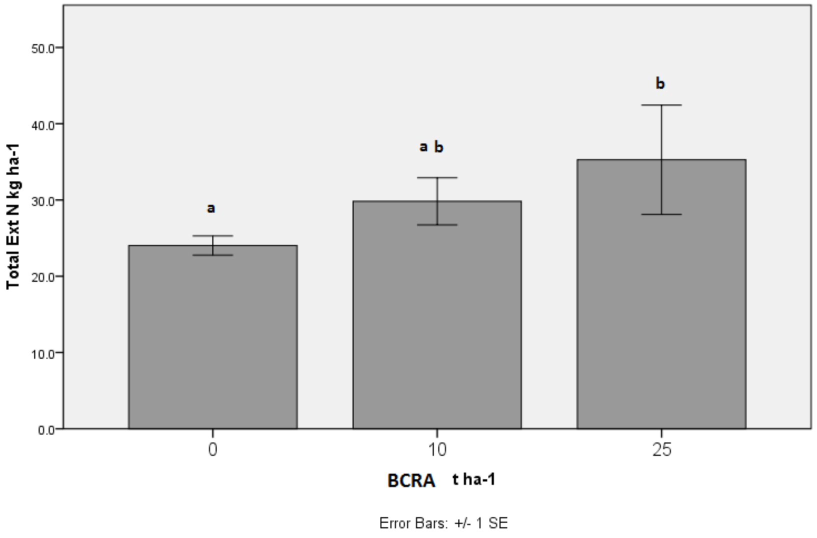

Figure 3-3 Total extractable nitrogen remaining in top 10 cm of soil treated with 0, 10 and 25 tonnes ha^{-1} of BCRA . Letters above groups indicate significant differences between treatments. Significance declared where p≤α= 0.05 using Tukey`s Post Hoc comparison (n=2)

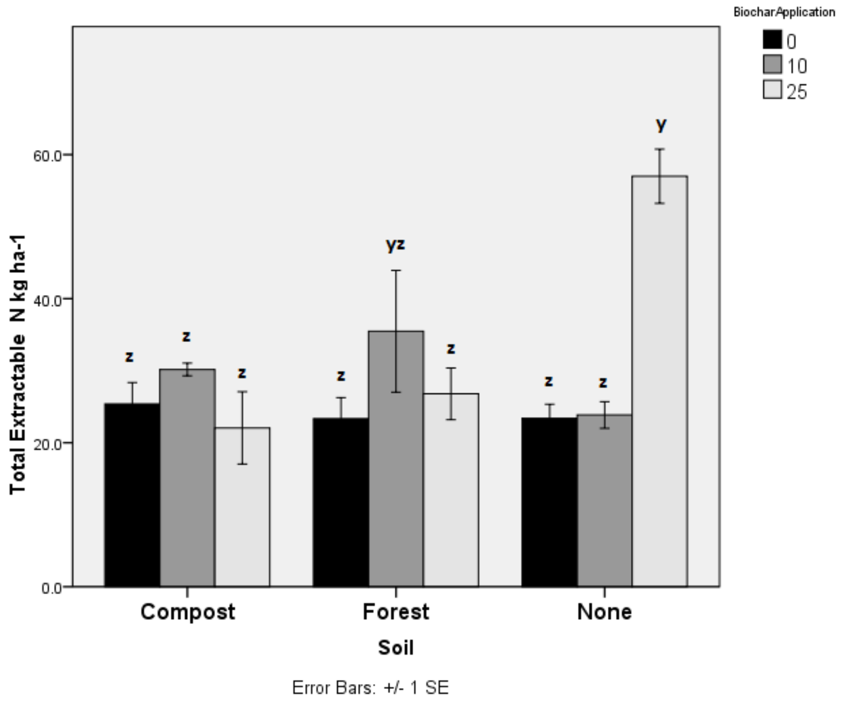

Figure 3-4 Total extractable nitrogen remaining in soil treated with 0, 10 and 25 tonnes ha^{-1} of BCRA in compost amended, forest floor amended and non-amended soils. . Letters above groups indicate significant differences between treatments. Significance declared where p≤α= 0.05 using Tukey`s Post Hoc comparison (n=2)

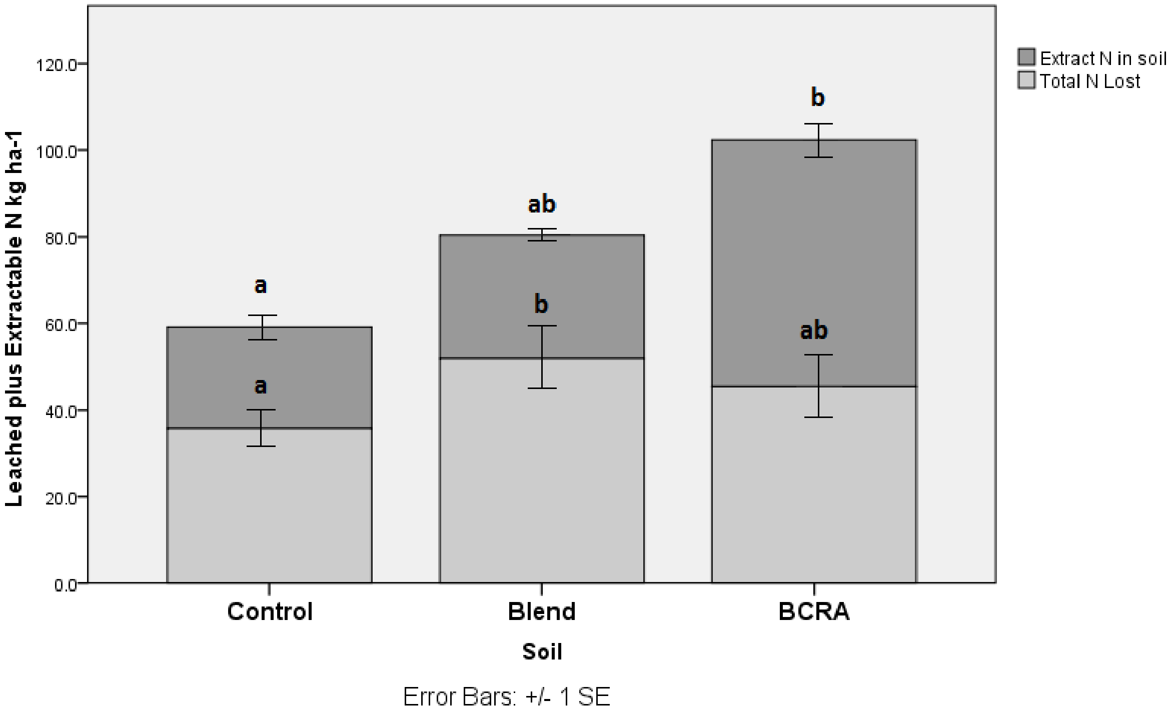

Figure 3-5 Total extractable nitrogen remaining in soil and total nitrogen lost from soil treated with 0 tonnes ha^{-1} of BCRA (control) a blend of compost and raw biochar at 25 tonne ha^{-1} and the BCRA at 25 tonne ha^{-1}. Letters above groups indicate significant differences between treatments. Significance declared where $p \leq \alpha = 0.05$ using Tukey`s Post Hoc comparison (extractable N sample size n=2)

Chapter 4. Summary of Findings and Future Research

4.1. Summary of findings

The use of soil amendments to provide nutrients and structure to degraded soils is a possibility for improving reclamation outcomes. However, soil amendments require study over the long term order to assess their ultimate effects. Peat, compost, forest floor material and biochar are have all been proposed to hasten reclamation, however the mechanisms and effects of these materials, both individually and when used as co-amendments are not well understood. Organic amendment decomposition and the disposition of the nutrients derived from this process are important when predicting reclamation outcomes.

Nitrogen cycling provides a single lens through the effects of soil amendments might be understood. Mineralization and nitrification are two processes which greatly influence nitrogen dynamics and the effects on these processes were observed in these experiments. It was found that while the changes in nitrogen loss and retention from soils are easily measured and are affected by amendments the mechanisms and transfers amongst the various nitrogen pools are complex and difficult to predict. However it appeared that biotic processes and resulting fluxes are more significant than physical pool increase of stored nitrogen and that these changes may be linear with biochar application rate in the case of CEC and sorption but nonlinear in the case of biological effects.

The physical increase in CEC added by biochar to soil contributed to nitrogen storage appeared to be confirmed by the Ottawa sand portion of the experiment however, this is not the entire story. Literature review suggested that changes in mineralization of organic matter are not consistently observed and this experiment was not able to clarify this. Biochar related increases in nitrification are supported by literature and were supported by this experiment however, the magnitude of these changes had varying results on the net effects of nitrogen leaching and storage.

Biochar's effect on nitrogen mineralization did not appear consistent. In part I this experiment nitrogen mineralization was highest at 10 tonne ha^{-1} and lowest at 25 tonne ha^{-1} irrespective of nutrient addition. In Chapter 3, Nitrogen loss was lower with increasing biochar treatments in soil which was amended but increased in non-amended soils though these findings were not significant. CO_2 respiration was generally increased with biochar addition in both experiments.

Compost as a soil amendment appeared to provide significantly more nitrogen to soil over the long term and the use of chemical fertilizer and organic amendments may achieve similar results. However, the addition of biochar to soil may induce changes in microbial growth, activity and function which may further alter nitrogen dynamics in unintended ways. These changes are likely the result of biochar effects on the soil microbial environment.

4.2. Direction of Future Research

Use of nitrification and mineralization as a measure of nutrient dynamics remains a valid means of understanding the effects of soil amendments. Study of these two important processes is ongoing and the body of research around biochar is continuing to expand. Further study in which biochar properties or pyrolysis conditions are linked with effects in soil would inform reclamation practitioners and other users as to which biochar products might be suitable based on the desired outcome.

This experiment was conducted under controlled conditions in the absence of environmental factors such as unpredictable moisture regimes, freeze/thaw cycles and uptake and influx of nutrients from plants. The next logical step application of this work is to field study. Other field mineralization and nitrification studies have been undertaken to look at biochar effects on mineralization over the medium term (Ameloot et al. 2014) and can provide guidance as to methodology. However, the use of biochar as a reclamation amendment necessitates an approach which accounts for the changing dynamics of a maturing forest rather than a more steady state sought by agricultural applications.

The finding that nitrification increased with biochar application was limited to a rather short duration and was not confirmed by the longer term organic amendment study. However, similar results between this experiment, other biochar experiments and studies of fire affected forests provides some confidence as to biochar's effect on nitrification. As well, the combined effects of biochar and organic amendments were did not easy to resolve in this experiment. Therefore, future efforts might focus on this particular combination especially since the use of the BCRA pellet appeared to show some promise as a nitrogen rich soil amendment.

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Appendix 1 Complete Peace River Soil Results

Parameter	Autoclave	Nutrient Treatment	Biochar Treatment	Mean	Std. Deviation	Parameter	Autoclave	Nutrient Treatment	Biochar Treatment	Mean	Std. Deviation
N Leaching	NA	N	0	16.562	1.099	Ext NH4	Live	N	0	7.541	.910
			10	16.892	1.237				10	7.069	.954
			25	15.851	1.295				25	3.938	2.059
		W	0	8.213	1.469			W	0	2.918	.495
			10	8.827	2.609				10	1.716	.507
			25	7.098	0.742				25	1.046	.595
	A	N	0	15.763	0.903		Sterile	N	0	10.237	1.394
			10	16.099	1.142				10	11.428	2.592
			25	14.933	1.400				25	10.103	1.354
		W	0	8.205	0.485			W	0	5.543	.915
			10	8.665	1.064				10	6.563	1.452
			25	7.477	1.285				25	4.781	.455
DON	NA	N	0	5.203	1.579	Ext NO3	Live	N	0	.886	.210
			10	5.119	2.127				10	1.915	.208
			25	3.986	2.263				25	4.622	1.780
		W	0	2.789	1.118			W	0	.804	.219
			10	2.656	0.957				10	2.145	.535
			25	1.847	0.619				25	2.955	.807
	A	N	0	6.902	1.427		Sterile	N	0	.305	.083
			10	7.308	1.090				10	.839	.147
			25	6.130	1.123				25	2.165	.412
		W	0	4.806	0.440			W	0	.375	.081
			10	5.118	0.401				10	.627	.121
			25	3.754	0.890				25	1.073	.973
Leach NH4	NA	N	0	0.416	0.152	CO2 Respiration	Live	N	0	95.480	47.105
			10	0.314	0.128				10	109.694	42.578
			25	0.053	0.019				25	124.646	38.855
		W	0	0.357	0.128			W	0	93.593	31.265
			10	0.307	0.154				10	93.060	9.389
			25	0.055	0.030				25	101.817	27.349
	A	N	0	0.686	0.069		Sterile	N	0	93.054	35.290
			10	1.051	0.545				10	150.118	52.164
			25	0.706	0.425				25	160.850	66.899
		W	0	0.588	0.237			W	0	78.205	33.246
			10	0.718	0.370				10	114.590	30.229
			25	0.593	0.259				25	154.493	67.428

Parameter	Autoclave	Nutrient Treatment	Biochar Treatment	Mean	Std. Deviation	Parameter	Autoclave	Nutrient Treatment	Biochar Treatment	Mean	Std. Deviation
Leach NO3	NA	N	0	10.944	0.955	MBC	Live	N	0	11.999	5.049
			10	11.459	1.693				10	19.248	7.355
			25	11.811	1.020				25	22.328	10.238
		W	0	5.068	0.857			W	0	14.355	4.521
			10	5.865	1.624				10	19.137	4.479
			25	5.196	0.313				25	22.532	3.080
	A	N	0	8.174	1.109		Sterile	N	0	13.167	2.242
			10	7.740	0.622				10	16.924	10.191
			25	8.096	1.231				25	11.491	6.382
		W	0	2.811	0.917			W	0	9.364	6.098
			10	2.829	0.667				10	19.568	10.232
			25	3.130	0.642				25	15.737	10.361
Retained Soil N	NA	N	0	10.344	1.093	Mineral n	Live	N	0	7.998	1.474
			10	10.884	0.871				10	9.257	1.480
			25	10.509	0.861				25	8.193	1.956
		W	0	5.116	0.252			W	0	5.923	1.233
			10	5.478	0.819				10	7.031	2.443
			25	6.032	0.768				25	5.861	0.897
	A	N	0	12.705	1.602		Sterile	N	0	9.309	2.354
			10	13.279	2.037				10	11.721	3.190
			25	13.339	1.998				25	10.980	2.809
		W	0	6.626	0.978			W	0	8.112	0.900
			10	8.315	1.587				10	10.198	2.570
			25	7.005	2.268				25	8.093	2.544
DIN	NA	N	0	11.360	1.099	Nitrification	Live	N	0	1.502	0.226
			10	11.773	1.820				10	1.851	0.484
			25	11.865	1.033				25	7.100	7.867
		W	0	5.425	0.933			W	0	1.845	0.532
			10	6.172	1.773				10	4.185	1.585
			25	5.252	0.328				25	9.490	5.337
	A	N	0	8.860	1.056		Sterile	N	0	0.793	0.205
			10	8.791	0.717				10	0.715	0.152
			25	8.802	1.631				25	0.956	0.102
		W	0	3.400	0.728			W	0	0.542	0.238
			10	3.547	0.706				10	0.481	0.088
			25	3.723	0.578				25	0.772	0.272

Parameter	Autoclave	Nutrient Treatment	Biochar Treatment	Mean	Std. Deviation
MBN	NA	N	0	1.917	0.878
			10	1.900	0.173
			25	1.949	0.197
		W	0	1.395	0.169
			10	1.616	0.571
			25	2.031	0.723
	A	N	0	2.162	0.596
			10	1.012	0.675
			25	1.070	1.713
		W	0	0.708	0.243
			10	1.125	0.399
			25	1.151	1.378