Utility of Wood Ash, Paper Sludge and Biochar Amendments for the Mitigation of Greenhouse Gas Emissions from Acid Boreal Soils

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ABSTRACT

Newfoundland and Labrador (NL) soils are characterized with low fertility, shallow, and acidic soils which impede agricultural productivity. Developing agriculture is a goal for the provincial government of NL and to promote soil fertility and crop productivity, manure and inorganic fertilizers application are required which may lead to higher greenhouse gas emissions (GHGE) from agricultural fields or cropping systems. Efforts to reduce reliance on synthetic soil amendments while taking advantage of abundant locally sourced industrial waste by-products such wood ash and paper sludge available in the province must be examined. Therefore, I hypothesized that wood ash, paper sludge and biochar may be a good source of soil amendments for improving soil health or fertility of boreal soils. I assessed the microbial activities leading to nitrogen losses and availability and functional state of the bacterial and archaeal genes (*nap*A, *nar*G, *nir*S, *nirK*, and *nosZ*) driving nitrogen transport as putative proxy indicators to produce CO_2 and NOx emissions. This study provided insights into the recommendations on the suitability and potential utility of wood ash and paper sludge for improving soil quality, health, and thus agricultural productivity.

GENERAL SUMMARY

Wood ash (WA) may have a long-lasting effect on the ability ameliorating acids in boreal soils compared to paper sludge (PS). Generally, WA and PS increased soil microbial biomass and basal respiration resulting in higher net mineralization. However, no significant effect of biochar was observed as the impact was short-lived. Co-application of urea to WA or PS led to higher net mineralization compared to when used independently. Wood ash or PS contributed significantly to CO_2 emissions. However, addition of biochar was shown to reduce CO_2 emissions in all cases except in WA amended soils. No significant differences were observed in CH_4 emissions across all treatments.

Shift in abundance of N functional genes correlated with soil basal respiration due to change in microbial biomass. WA and PS increased the abundance of 16S bacterial, archaea and denitrifying genes (*nap*A, *nar*G, *nir*S, *nirK*, and *nosZ*). The highest 16S bacterial, *nap*A, *nar*G, *nir*S, and *nirK* genes counts were reported in paper sludge when pH was ≥ 6. The abundance of denitrifying genes was strongly pH-dependent and also affected by soil organic matter, nitrates, ammonium, and EC.

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List of Abbreviations

Ammonia Oxidizing Bacteria (AOB)

Ammonium (NH_4)

Archaeal Ammonia Oxidizing (AOA)

Bulk Density (BD)

Cation Exchange Capacity (CEC)

Carbon (C)

Carbon Dioxide (CO_2)

Deoxyribonucleic Acid (DNA)

Dinitrogen (N_2)

Droplet Digital PCR (ddPCR)

Electrical Conductivity (EC)

General Linear Model (GLM)

Gram (g)

Greenhouse Gases Emission (GHGE)

Megagram per Hectare (Mg/ha)

Metabolic Quotient (qCO_2)

Methane (CH_4)

Microbial Biomass (MB)

Microlitre (uL)

Milligram (mg)

Nanogram (ng)

Newfoundland and Labrador (NL)

Nitrate (NO_3)

Nitrogen (N)

Nitrous Oxide (N_2O)

Paper Sludge (PS)

Polymerase Chain Reaction (PCR)

Principal Component Analysis (PCA)

Revolution per Minute (rpm)

Soil Basal Respiration (SBR)

Soil Organic Carbon (SOC)

Soil Organic Matter (SOM)

Soil Water Content (SWC)

Tonnes per Hectare (t/ha)

Water Holding Capacity (WHC)

Wood Ash (WA)

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CHAPTER 1

1.1 INTRODUCTION

In the North Atlantic Ocean, Newfoundland and Labrador (NL) is located in the boreal forest zone. Because of the province's position, the weather is heavily impacted by cool ocean currents, resulting in temperatures that are insufficient for crop growth (NL Department of Natural Resources, 2004). The land is dominated by coniferous plants and low fertility, shallow, acidic soil further impeding agricultural productivity. Agricultural development is a goal for the provincial government of NL and has been recognized as a component in the province's action plan for economic growth. However, this development may result in higher greenhouse gas emissions (GHGE) from agriculture; agriculture is the source for 18.4% of global anthropogenic GHGE (Ritchie and Roser, 2020) and these emissions are increasing at a pace of about 1% per year (Lamb et al., 2016). Limiting the reliance on costly synthetic soil amendments must be examined. Novel ways to utilize forestry and related industrial waste by-products such wood ash (WA) and paper sludge (PS) may facilitate these goals; if proven safe as agricultural soil amendments, these by-products may be a way to reduce agriculture reliance on synthetic fertilizer. These soil amendments can help with nutrient retention and availability to plants by improving soil quality and health. Soil microorganisms control the majority of soil-based ecosystem services (Hallin et al. 2009), which are influenced by the physical and chemical status of the soil. Any amendment that alters soil physical and chemical characteristics is likely to have an impact on microorganism-mediated activities such as nutrient cycling and speciation, as well as their transport across soils (Lehmann et al. 2011). Previous research on these amendments led me to believe that they would have a favourable impact on soil quality and thus increasing nutrient

availability such as nitrogen. In addition, recycling these waste products in agriculture lowers the forestry industries disposal expenses.

1.2 Literature Review

1.2.1 Wood Ash as Soil Amendment

Wood ash is the residue produced after the combustion of wood and is the most abundant waste product of pulp and paper mill industry, and it is usually landfilled (Hannam et al. 2018). The residual feedstock combusted by pulp and paper mill industries may include roots, branches, tree bark, sawdust and wood chips not used for pulping. The physical and chemical properties of ash, and thus its uses, are related to the properties of the feedstock material, dependent on tree species, and the type of combustion process including the combustion temperature, boiler efficiency, method of collection (Muse and Mitchell, 1995; Campbell, 1990). It commonly retains most of the inorganic nutrients and trace elements of the oil used for wood burning. Ash has been also proposed for use in agriculture as liming agent for acidic soils and as a good source of macronutrients such as potassium, phosphorus, calcium, magnesium, micronutrients such as iron, manganese, boron, copper, and zinc (Naylor and Schmidt, 1986; Kukier and Sumner, 1996; Demeyer, Voundi Nkana, and Verloo, 2001; Saarsalmi et al. 2004; Adekayode and Olojugba, 2010; Sharifi et al. 2013)

1.2.1.1 Physical and Chemical Properties of Wood Ash

Wood ash varies widely: Etiegni et al. (1991) and Etiegni and Campbell, (1991), reported that over 80% of wood ash has particle sizes less than 1.0 mm; Muse and Mitchell (1995) reported distribution of particles to be 8.98% > 2, 25.1% > 0.50, 34.3% > 0.25 and 47.3% >0.106 mm for ashes from pulp and paper mill industry. Wood ash bulk density usually ranged between 0.27 g/cm^3 for ash derived from wood (Huang et al. 1992) such as spruce pine, european beech to 0.51 g/cm^3

for those derived from pulp and paper waste (Muse and Mitchell, 1995). The higher bulk density of ash derived from pulp and paper waste was attributed to the addition of clay and salt to pulp during paper production. Typically, combustion temperatures between 500°C to 900°C release most of the nutrients available in ash without heavy metal volatilization (Pitman, 2006). Etiegni and Campbell (1991) evaluated the effect of combustion temperature on ash yield and chemical properties: increase in temperature from 538°C to about 1100°C, leads to increased metal content but lower ash yield by 45% and decreased ash carbonate driven alkalinity. The concentrations of important nutrients such as potassium (K), sodium (Na), zinc (Zn) and carbonate (CO_3) decreased while the concentrations of heavy metals increased with increasing temperature. The ash pH was reported to range between 9 to 13.5.

The chemical composition of wood ash is determined by type of combustion feedstock (types of plant, either hardwood or softwood), combustion temperature, the distribution of material in the burn chamber, and storage method (Demeyer et al. 2001). Wood ash derived from branch and root of trees contains abundant micro and macronutrients (Hakkila, 1989; Werkelin et al. 2005). Etiegni et al (1990) showed that the composition of ash changes under storage and that CO_2 and moisture often react with ash to form carbonates, bicarbonates, and hydroxides. The liming effect of wood ash depends majorly on the content of carbonates, bicarbonate and hydroxide in the ash. Etiegni et al. (1990) used X-ray diffraction to evaluate the different compounds present in both wet and dry ash. The main oxides identified in the wood ash were lime (CaO), calcite ($CaCO_3$), portlandite ($Ca(OH)_2$) and calcium silicate (Ca_2SiO_4). The $CaCO_3$ concentration of ash varies from 26% to 59%, depending on the feedstock type (Ohno and Erich, 1990). Wood ash combusted with petroleum or coal contain heavy metals such as cadmium (Cd), lead (Pb), copper (Cu) or mercury (Hg) but ash produced by wood-based fuels are typically low in heavy metals

(Grammelis et al. 2006). Vance and Mitchell (2000) found the average nutrient value of WA derived from various sources to be 180 g/kg for calcium, 27.9 g/kg for potassium, 9.7g/kg for magnesium, 4.2g/kg for phosphorus and 0.6 g/kg for nitrogen. Nitrogen is often present in WA in low concentration or completely absent due to volatilization during combustion.

1.2.1.2 Impact of Wood Ash on Soil Parameters and Agronomic Outcomes

Typically, the application of wood ash to agricultural and forest soils have been reported to have profound effects on several soil physical and chemical properties. It has been reported that the application of wood ash can increase soil pH in acidic soils, increase soil solution's electrical conductivity, and nutrients availability (Augusto et al. 2008; Demeyer et al. 2001; Ohno and Erich 1990; Pitman 2006). Decreased Aluminium (Al^{3+}) toxicity, increased pH and concentration of base cations have been also reported (Bramryd and Fransman 1995; Ludwig et al. 2002). Huotari et al. (2015) found that the addition of wood ash increased the forest soil pH between 0.5 and 3.0 units depending on the application rate. A soil is considered acidic if the pH is 5.5 or less (Kochian et al. 2004). Soils with pH from 6.5 to 6.1 are slightly acidic, pH 6.0 to 5.5 are moderately acidic, 5.0 to 5.1 strongly acidic, and 5.0 to 4.4 extremely acidic (McFarland et al. 2011). Acidic soils are less fertile and are often phosphorus (P) deficient; Al is often present in soil solution as phytotoxic Al^{3+} (Feng Ma et al. 2001; Miyasaka et al. 2016). Al^{3+} toxicity prevents root development, decreasing water and nutrients uptake in plants and thereby leading to poor plants growth (Kanyanjua et al. 2002). Increasing the soil pH with WA amendments lowers Al^{3+} toxicity and increases P availability to plants (Demeyer et al. (2001) and Mbah et al. (2010). Etiegni and Campbell (1991) found that ash particles can also expand as a result of hydration of silicates which clog up soil pores leading to decreased aeration and increased water holding capacity in soils. It has been reported that addition of wood ash to soil decreases soil bulk density and improve soil porosity

(Farhain et al. 2022; Mbah et al. 2021). Reduced bulk density aids plant development by distributing air and water to the roots, allowing them to function properly.

Wood ash applied at 30, 40, and 50 dry Mg/ha resulted in 49, 57, and 64% increase in the bean stems and leaves, and dry matter yield, respectively compared to the control without ash (Krejsl and Scanlon, 1996). It was also found that application rate at 30 dry Mg/ha, equivalent to the recommended agronomic lime rate of 7.4 Mg/ha, increased the oat shoots dry matter yields by 45%. Patterson et al. (2004) conducted a 3-yr field study to evaluate the impacts of application rate of 6, 12.5, and 25 Mg/ha wood ash to Grey Luvisols in Alberta: they found an increase of 72 and 50% in dry matter and grain yield of barley, respectively. It was estimated that when soil was amended with 12.5 or 25 Mg/ha WA with N fertilizer, the wood ash led to a 124% increase in canola oil seed yield.

1.2.1.3 Impact of Wood Ash Amendment on Soil Microorganisms

Soil microorganisms are crucial as the base trophic levels in the soil food web and play a key role in ecosystem activities such as decomposition, nutrient cycling, and mineralization (Ronn et al. 2012). Soil microbes have shown to respond to changes caused by wood ash application in soil (Aronsson and Ekelund, 2004; Huotari et al. 2015). However, some studies have also reported no changes in microbial composition following wood ash addition in soil (Fritze et al. 1994).

The increased soil pH that results from ash application can have profound impacts on microbial communities and activity. For instance, Rousk et al. (2010), Rousk and Baath, (2011) and Cruz-Paredes et al. (2017) found that bacterial abundance is favoured at elevated pH, while fungal growth may decrease or remain unaffected (Mahmood et al. 2003; Majdi et al. 2008; Noyce et al. (2016). The soil pH is known to be the main driver of soil microbial communities'

composition (Fierer and Jackson, 2006; Kaiser et al. 2016; Kim et al. 2016; Malik et al. 2016); this can also be related to the soil electrical conductivity (Lozupone and Knight, 2007; Kim et al. 2016). An increase of soil dissolved organic carbon (DOC) following wood ash affects microbial community composition (Jokinen et al. 2006).

It was reported that adding wood ash at rates of under 22 Mg/ha to acidic forest soil result to increased bacterial numbers, microbial biomass, richness, diversity, community composition, growth rate of soil microbes and microbial activity such as mineralization of organic matter (Zimmermann and Frey, 2002; Andreasen et al. 2017); however, most of these parameters declined at high wood ash application rate of 167 Mg/ha (Andreasen et al. 2017). Similar declines were identified by Baath et al. (1995) in podzol soil but at low application rates between 1 and 5 Mg/ha. Positive changes were attributed to elevated pH and electrical conductivity following wood ash application, rather than by wood ash itself (Vestergard et al. 2018). On the other hand, some studies have shown no significant change on microbial community following wood ash application, even at moderate rates (Fritze et al. 1994; Bjork et al. 2010). Increased soil respiration following wood ash addition was also reported (Baath and Arnebrant 1994; Fritze et al. 2000; Perkiomaki and Fritze, 2002; Zimmermann and Frey, 2002). The increase in respiration is often linked to increased mineralization rates and nutrient cycling although in rare cases, the increased respiration maybe an indicator of stress response and inefficient carbon utilization (Anderson, 1994; Wardle and Ghani, 1995). The soil metabolic quotient respiration (qCO_2), an indicator of microbial efficiency or stress, was not significantly affected following wood ash application (Zimmermann and Frey, 2002; Insam et al. 2009).

Boreal forests, and their soils pose unique challenges. In NL, the boreal soils are largely podzolic in nature having low pH and are poor in fertility, thereby making them unsuitable for

cultivation of crops (FAO, 2017). Most studies done on boreal soils demonstrated that wood ash addition led to enhanced microbial activities, as recorded via mineralization and respiration rates (Baath and Arnebrant 1994; Fritze et al. 1995), and growth rate of microbes (Baath and Arnebrant 1994; Hagerberg and Wallander 2002). This is an indication that wood ash greatly impacts microbial activity in soil. However, it is important to note that the rate of change in microbial properties is greatly dependent on the time since last application, rate and form of ash used. Perkiomaki (2004) still observed increase in microbial activity, community and composition 18 years following wood ash application.

1.2.2 Biochar as Soil Amendment

Biochar is an organic material rich in carbon produced by biomass heating in the presence of low oxygen or no oxygen condition. Several advantages of using biochar as soil amendment include an increase in water holding capacity and nutrient retention, improved soil fertility and agricultural productivity, and reduction of GHGE (Lal, 2009; Laird et al. 2010; Lehmann and Joseph 2015; Kuppusamy et al. 2016; Singh et al. 2010). However, contrasting results were also reported: no improvement in crop productions, increased GHGE, and excessive rise in soil pH (Lehmann and Joseph., 2015). Results are dependent on the type of biochar used as result of varying properties (feedstock material), application rate, soil type and climatic conditions (Verhejien et al. 2010).

1.2.2.1 Physical and Chemical Characteristics of Biochar

Biochar is comprised mainly of recalcitrant organic black carbon with micro and macro nutrients contents retained from the feedstock. Feedstock of varying types such as rice husks, nutshells, wood, sewage sludge, pine shavings, straws, orange peels and manure (Chen and Chen,

2009; Spokas and Reicosky, 2009; Mukome et al. 2013) have been employed in the production of biochar. The vast range of feedstocks can be linked to the inevitability of using locally accessible waste biomass. The organic carbon in biochar is stored in aromatic chemical forms and not easily decomposed when used as soil amendment (Sohi et al. 2010; Gurwick et al. 2013), a property which is argued to favour carbon sequestration in soils. Biochar's composition varies with feedstock materials, method and temperature of pyrolysis (Gaskin et al. 2008; Stewart et al. 2013; Shaheen et al. 2019). The carbon content of biochar varies from 172 g/kg to 905 g/kg, nitrogen from 1.8 g/kg to 56.4 g/kg, total phosphorus from 2.7 g/kg to 480 g/kg and total potassium from 1.0 g/kg to 58 g/kg (Lima and Marshall 2005; Chan et al. 2008). Biochar constituents also include variable concentrations of oxygen, hydrogen, sulfur, nitrogen, phosphorus, cations, and heavy metals (Preston and Schmidt 2006). Furthermore, biochar has a wide range of pH, from 4 to 12 (Lehmann et al. 2011), determined by the feedstock material and pyrolysis conditions such as temperature, chemical activation, and atmospheric condition (Downie et al. 2009; Mukherjee et al. 2011). The production of biochar at < 400 °C often result to acidic biochar but increasing temperature leads to alkaline biochar (Lehmann and Joseph 2015). Biochar produced at high pyrolysis temperature have been reported to be effective in mitigating soil acidity and improving retention of nutrients in soil (Dai et al. 2017), while those produced at lower temperature were reported to promote soil cation exchange capacity (Mukherjee et al. 2011). The latter, on the other hand, has minimal effect on acidic soil pH and can slightly lower pH in alkaline soils (Laghari et al. 2015). After biochar application to the soil, oxidation often occurs at its surface because of their interaction with water, oxygen, and other soil agents (Lehmann 2007). Newly produced biochar often has low cation exchange capacity (CEC) but increases with time under storage in the presence of oxygen and water (Liang et al. 2006; Cheng et al. 2008). Furthermore, biochar has a

bulk density of approximately 0.3 g/cm^3 which is far lower than a soil bulk density of 1.3 g/cm^3 (Atkinson et al. 2010; Brady and Weil 2010; Laird et al. 2010).

Feedstock type can be a better estimator of variations in the content of biochar ash content and C/N ratio, rather than pyrolysis temperature (Mukome et al. 2013). Mukome et al. (2013) reported that wood derived biochar has lower ash content compared to those derived from non-wood feedstock. Furthermore, there have been some concerns about noxious waste present in biochar that might percolate into the soil; these include potential heavy metal that pose risk but are commonly found in sewage sludge and treated wood feedstocks (Lievens et al. 2009). Therefore, presence of heavy metals in biochar could be linked to the source of the feedstocks and the process of conversion. Some biochar pollutants may change during pyrolysis and be eliminated or converted into benign compounds, but others retained in the biochar could potentially pose risk when added to the soil. Furthermore, polycyclic aromatic hydrocarbons, which are also noxious, have been found to be produced during pyrolysis. Polycyclic aromatic hydrocarbons are usually derived from carbonaceous feedstocks (Zhurinsh et al. 2005) and are capable of inhibiting nitrogen mineralization and nitrification by decreasing enzymatic activities relevant to nitrogen cycling (Adamczyk et al. 2015, 2017). Hence, it is crucial to understand the compositional contents of feedstock used and biochar to prevent possible environmental risks that could arise after application to the soil.

1.2.2.2 Impact of Biochar Amendment on Soil Parameters and Agronomic Outcomes

Biochar addition could change the physical properties of the soil including soil structure, pore size and soil bulk density with positive increase in soil aeration, water holding capacity, plant production and enhanced microsites that serve as a habitat for soil microorganisms (Downie et al. 2009; El-Naggar et al. 2019), resulting in increased soil fertility (Koide et al. 2011). In addition,

biochar could increase the soil net surface area, which concurrently improves soil water holding capacity, nutrient retention (Chan et al. 2008, Downie et al. 2009), and soil aeration, mainly in fine textured soils (Kolb 2007). The biochar's low bulk density may reduce the soil total bulk density, a preferable outcome for crop production as it improves root development and growth and thus soil aeration (Atkinson et al. 2010; Brady and Weil 2010; Laird et al. 2010). The retention of moisture in soil after application of biochar is often linked to changes in soil structure and aggregation (Brodowski et al. 2006). By interacting with soil organic matter and soil organisms, biochar alters aggregation and availability of minerals (Lehmann and Joseph, 2009) including on boreal forest soils (Gundale et al. 2016). The availability of nutrients could be linked to the increased cation exchange capacity which in turn increase the soil's pH and affects the direct release of nutrients from biochar. Many studies have been carried out on the effect of biochar on tropical and temperate regions agricultural soils, however little information is known about effect of biochar in boreal forest soils (Liu et al. 2015; Bruckman et al. 2016). In boreal soils, most soil nitrogen is stored in organic forms and low nitrogen mineralization rates lead to plant growth to be nitrogen limited (Sponseller et al. 2016). Biochar might increase nitrogen mineralization rates (Ameloot et al. 2015, Case et al. 2015; Gundale et al. 2015).

Biochar could be used as liming source (Rinklebe et al. 2016; Zhang et al. 2017) minimizing the excessive availability of toxic elements such as Al and Fe that restrict plant growth in acid soils. Phosphorus held as Al^{3+} and Fe^{2+} phosphates at acid pH becomes available as acidity decreases (Cui et al. 2011). The alkaline effect of biochar arises from carbonates and chlorides of potassium and calcium present in the biochar ash (Montes-Moran et al. 2004). José Antonio et al. (2014) reported a significant increase in soil pH after addition of different biochars.

These effects of biochar addition to soil can lead to improved soil productivity, especially when added to nutrient deficient, low fertility soils (Laghari et al. 2015; Zhang et al. 2017; Van Zwieten et al. 2010). Its efficiency is not always substantial in fertile soils (Van Zwieten et al. 2010; Hussain et al. 2017). Laghari et al. (2015) observed an 18% to 22% increase in sorghum dry weight on a degraded desert soil after application of biochar derived from pine sawdust. Raboin et al. (2016) have reported that eucalyptus charcoal-derived biochar added to an acidic soil increased yield significantly for both maize and common beans. Several reports have shown that livestock manure biochars have high nutrient content and higher cation exchange capacity than plant feedstock derived biochars. Poultry litter biochar added to an acidic arenosol (Macdonald et al. 2014) or acidic ferralsol (Slavich et al. 2013) was effective in increasing shoot, root and grain biomass, mainly due to the provision of nutrients by biochar. Abbasi and Anwar (2015) have also reported better shoot and root development and significantly higher grain yield after poultry manure biochar compared to biochar derived from white clover residues. The increased plant growth and yield with poultry manure biochar was attributed to increase mineralization rates and its high nutrient content.

1.2.2.3 Impact of Biochar on Soil Microorganisms

Soil microorganisms drive nutrients cycling and are essential for ecosystem functioning (Costanza et al. 1997). Biochars have been shown to change fungal and bacterial diversity and abundance, although without apparent trend (Thies et al. 2015). Currently, information available on the effect of biochar on microbial communities is ambiguous. Effects ranging from negative, positive and no changes have been reported on soil microbial communities and composition following biochar addition depending on the biochar used and soil type (Steiner et al. 2008; Spokas et al. 2009; Lehmann et al. 2011; Galvez et al. 2012; Jones et al. 2012). Some polycyclic aromatic

hydrocarbons such as phenolics and polyphenolics present in biochar may adversely affect soil microorganisms (Warnock et al. 2010).

Rutigliano et al. (2014) reported a significant increase in microbial activity recorded as substrate induced respiration (SIR) and certain catabolic activities linked to succinic, citric, L-ascorbic, gluconic, α-ketoglutaric and fumaric acids, and L-asparagine. However, contradictory results are reported in the literature, from no effect on basal respiration and rates of feeding of soil biota (Domene et al. 2014; Woolf and Lehmann 2012) to an increase in C mineralization (Smith et al. (2010). Biochar was reported to increase the activity and abundance of methanotrophic bacteria responsible for the reduction of CH_4 emission (Feng et al. 2012). In fresh biochar, soil microbes may possibly produce ethylene, which could be connected to the reduction on N_2O and CO_2 emissions. Labile carbon present in biochar constituents may possibly act as substrate for the growth of certain taxa bacterial communities and enhance their activities (Farrell *et al*., 2013; Gomez et al. 2014; Khodadad et al. 2011). Nevertheless, these compounds may be mineralized rapidly (Cheng et al. 2006), thus having only a short-term impact. Microbial biomass carbon (MBC) decreases following biochar addition have been linked to the occurrence of noxious organic compounds (Deenik et al. 2010).

There are no apparent trends of the effects of biochar application on total microbial biomass. For instance, previous studies reported either no change (Anders et al. 2013) or increased (Gomez et al. 2014) or decreased (Dempster et al. 2012) MBC or N following biochar addition. Nevertheless, a meta-analysis deduced that MBC usually increases following biochar application (Biederman and Harpole, 2013). This could signify the independent impacts of several biochar types, indicating that microbial impacts could be firmly dependent on soil. The ways through which biochar increases soil microbial biomass involves absorption of labile organic C contained

in biochar (Bruun et al. 2012), provision of nutrients for the microorganisms from biochar surfaces (Cheng et al. 2008), and biochar's ability to increase the decomposition rate of soil organic matter, thus enhancing microbial activity (Wardle et al. 2008). Furthermore, due to biochar pores which may be less than 5μm (Glaser et al. 2007) could act as habitation for soil microorganisms and protection from grazers (Pietikainen et al. 2000).

Metagenomic assessments suggest that biochar increase bacterial diversity (Hu et al. 2014). However as bacterial diversity increases the fungal diversity might decrease (O'Neill et al. 2009). These differences indicate that biochar favours bacterial C channels over fungal channels (Lehmann et al. 2011). Liang et al. (2008) and Thies and Rillig (2012) suggested that the difference in bacterial and fungal diversity could have resulted from bacteria being more able to make use of the available nutrients and mineral elements by inhabiting the pores of biochar or adsorption to biochar surface. Furthermore, greater microbial diversities are observed in soils amended with biochar for a long term (Kim et al. 2007; Grossman et al. 2010), but lesser bacterial diversities are reported in soil amended with biochar over short terms (Jin 2010; Khodadad et al. 2011). However, some biochars could also decrease total microbial diversity by favouring specific phyla (Khodadad et al. 2011). These reports underline the necessity for further studies that can distinguish between biochars produced with a range of feedstock and pyrolysis temperature, the variable parameters of the receiving soil, and distinct climatic conditions (Ameloot et al. 2013).

1.2.3 Paper Sludge as Soil Amendment

Paper sludge (PS) is an organic by-product produced from the treatment of wastewater during paper production by pulp and paper mill industry. Application of PS as soil amendments to agricultural soils have proved to increase the soil organic matter, soil pH, improving water holding

capacity, soil structure, supply of nutrients and decrease bulk density (Beyer et al. 1997; Cabral et al. 1998; Fierro et al. 1999; Gallardo et al. 2007; Ribeiro et al 2010).

1.2.3.1 Physical and Chemical Characteristics of Paper Sludge

The physical and chemical compositions of PS including the nutrients, heavy metals and organic composition varies considerably, depending mainly on the paper manufacturing processes employed, including the fiber source and treatment processes (Camberato et al. 1997; Pervaiz and Sain 2015). Generally, PS constituents include slowly decomposable organic matter mainly in form of cellulose fibers, calcium, nitrogen, phosphorus, potassium, magnesium, sodium, trace elements, and heavy metals (Cabral et al. 1998). During paper making process, three types of paper mill sludges are produced: primary, secondary, and deinking or tertiary sludge (Mahmood and Elliott, 2006; Monte et al. 2009). Primary sludges are produced by clarification, using sedimentation and flotation, and are mainly composed of high C:N ratio (Faubert et al. 2016). Secondary sludges, also known as biological sludges, are often produced via microbial decomposition following addition of nitrogen and phosphorus in other to maintain the microbial activity. The biological activity helps to reduce the organic pollutants and biochemical oxygen demand in the wastewater. The secondary sludge produced is usually less in quantity than the primary sludge as most of the leftover fibers and inorganic substances are removed during the clarification processes. Primary and deinking sludges are generally low in available nutrients, mostly nitrogen, and have high C:N ratio. Secondary sludges have a high content of nitrogen (N) and phosphorus (P), and a smaller C:N ratio compared to primary sludges (Ziadi et al. 2013), due to the addition of N and P during wastewater treatment to augment the microbial decomposition (Camberato et al. 1997).

Generally, the physical appearance of PS is like that of a chewed paper and includes aggregates of variably pulverized fibers with the particle sizes ranging from 1 to 20 mm (Wallace and Terry, 1998). The electrical conductivity of sludge ranges from 0.09 to 3.9 mS/cm (Zhang et al. 1993; Trepanier et al. 1996; Abdullahi et al. 2016). Electrical conductivity values of < 2 mS/cm are considered desirable for plant growths; however, most plants can tolerate electrical conductivities between 3 and 4 mS/cm (Abdullahi et al. 2016). Studies have also reported the cation exchange capacity (CEC) of paper sludge to vary considerably, ranging from 5.3 to 297 cmol (+) kg^{-1} (Field et al. 1996; Campell et al. 1995; Cavaleri et al. 2004). Such variability in the CEC was linked to varying composition and organic matter contents in the paper sludges (Bellamy et al. 1995). The pH value of PS is often quite alkaline, and varies considerably from 6 to 9 (Thacker, 1986). The alkalinity of PS is due to the caustic substances such as sodium hydroxide, sodium silicate and calcium carbonate used in paper making process (Ferguson, 1992; Abdullahi et al. 2016). Thus, sludge that includes $CaCO_3$ may act as liming agent thereby neutralizing acidic soils and also improve the soil fertility due to their high fiber content which hold up moisture in soil. Organic matter contents, C:N ratio and bioavailability of nutrients to plant also varies with paper sludge. Abdullahi et al. (2016) reported that PS organic matter proportion ranges between 32.54% and 57.91% with an average of 44.0%. The high proportion of organic matter in sludges will enhance the soil fertility and improve the physical properties of the soil. The C:N ratio of PS varies between 6 to 115:1 (Feagley et al. 1994).

It is crucial to understand the chemical composition of PS to prevent possible environmental risk that could arise after application of PS as soil amendment because of their heavy metal concentration. However, numerous studies have shown that PS have low heavy metal concentrations. Abdullahi *et al*. (2016), Cabral and Vasconcelos (1993) and Watson *et al* (1985)

have reported that heavy metal concentration is significantly low in PS and will not have adverse effect on application rates. Barclay (1991) also suggested that application of PS is more suitable as soil amendments compared to municipal waste in terms of their heavy metal contents. Many other researchers have also reported PS as having low heavy metal contents (Feagley et al. 1994; Trépanier and Gallichaud 1994; Bellamy et al. 1995; Camberato et al. 2006). Thus, PS with low heavy metal contents could be used as a soil conditioner.

1.2.3.2 Impacts of Paper Sludge on Soil Parameters and Agronomic Outcomes

The high organic matter content of sludges makes them ideal for enhancing the soil physical properties. PS application to soil as form of carbon could have constant impacts in improving soil properties (Camberato et al. 2006). The decomposition of PS takes place in two-stages with a fast immediate degradation followed by a slow degradation rate over extended period; Chantigny et al. (1999) and Fierro et al. (2000) have shown that over 40% of the paper sludge persists in soil after 2 yrs of application. Cellulose decomposition occurs mainly in the fast decomposition stage, while lignin is accountable for the slow decomposition stage (Chantigny et al. 2000).

Reports have shown that addition of paper mill sludges to soil could modify the physical, chemical, and biological properties of the soil. Application of PS to the soil have improved the soil physical properties such as reducing the bulk density, and increased aggregation of the soil reflecting the beneficial increase in soil organic matter and microbial activity (Camberato et al. 2006). Fierro et al. (1999) reported that bulk density reduced from 1.7 to 1.3 g/cm^3 in sandy mine soil for 2 years following the addition of PS at 105 Mg/ha. Primary sludge used at rate of 160 Mg/ha to loam potato soil also reduced the bulk density from 1.21 to 1.01 Mg/m^3 with increase in soil porosity and saturated hydraulic conductivity (Chow et al. 2003); this enhancement in soil

properties resulted in 23 to 71% decrease in runoff. Trepanier et al. (1998) reported that there was 20% increase in the structural stability of the soil following two yearly additions of sludge, although this impact persisted for less than a year following the final addition. Nemati et al. (2000) also observed 15 to 17% increase in wet aggregate stability in silty clay and loamy soil after addition of combined sludge (deinking and secondary sludge).

Previous studies have shown that application of PS could also increase the water holding capacity of the soil (Fierro et al. 1999; Larney and Angers, 2012), mostly for coarse textured soil. Trepanier et al. (1996) reported water holding capacity of deinking paper sludge to be 0.36 cm^3 cm^{-3} at –33 kPa and 0.26 cm^3 cm^{-3} at –1500 kPa, which is far larger than water holding capacity of most mineral soils. Chantigny et al. (2000) observed increase in soil water contents from 271 to 726 days following sludge addition in silty clay and clay loam soil. The increase in soil water holding capacity could provide a suitable state for soil microorganism, especially in dry season (Chantigny et al. 2000). Zibilske et al. (2000) also reported 49% increase in water content in sandy loam following five yearly additions of sludge. Several researchers concluded that the increased water holding capacity upon sludge addition could be linked to the slow decomposition process and undecomposed sludges in the soil (Aitken et al. 1998; Chantigny et al. 2000; Baziramakenga et al. 2001). Hence, the water holding capacity decline as the sludge decompose in the soil. The efficiency of sludge to improve water holding capacity would be more obvious in soils with relatively low water holding capacity compared with those with high water holding capacity.

The alkalinity of PS as described above resulted from the caustic substances such as NaOH, $NaSiO_3$, and $CaCO_3$ used in the paper making process. This could be particularly useful as liming agent in ameliorating the acidic pH in soil (Torkashvand 2010). Legendre et al. (2004) reported addition of primary sludge to silt loam soil at application rate of 13 and 26 Mg/ha increased the

soil pH by 0.4 and 0.8 units, respectively. Kost et al. (1997) also reported increase in soil pH from 2.9 to 7.3 upon addition of sludge to an unused coal mine soil. Fierrol et al. (1997) observed an increase in soil pH from 4.7 to greater than 6.0 in an abandoned sandpit after addition of deinking sludge after 2 years of addition.

Organic substance with high C:N ratios (greater than 20-30:1) usually cause nitrogen immobilization in soil (Alexander 1977), and primary paper mill sludges usually exceed this proportion. Therefore, it is crucial to consider the consequence of the paper mill sludges C:N ratio on nitrogen immobilization in soil, which directly or indirectly influences the yield of crops (Camberato et al. 2006). The quantity and period of immobilized nitrogen during the degradation of sludge are reliant on factors such as the quantity and C:N ratio of the sludge, soil inorganic, nitrogen source and soil type (Camberato et al. 2006). The excessive nitrogen immobilization in soil upon sludge application can be overcome by the addition of mineral nitrogen fertilizer in combination with paper sludges or use of legumes since they are less dependent on soil nitrate and ammonium for growth (Camberato et al. 2006). The application of the mineral fertilizer will act as supplement nitrogen to counteract for the sludge low nitrogen content. The supplementary nitrogen could come from farm manures with high nitrogen content and low C:N ratios such as swine and poultry manure (Gagnon et al. 2004) and inorganic fertilizer such as urea, ammonium nitrate. However, ability to co-compost paper sludge with nitrogen rich waste product such as fish waste may generate additional agriculturally feasible amendments and alleviate possible contamination of soil and ground water while producing a valuable resource from two waste streams (Hazarika and Khwairakpam, 2018).

1.4 Nitrogen Cycling and Activity of Nitrogen Cycling Genes

N-cycling is a complex biogeochemical cycle with numerous nitrogen transformations steps such as nitrogen fixation, nitrification, and denitrification (Kuypers et al. 2018; Stein and Klotz, 2016) which are mainly driven by diverse microorganisms. Nitrification and denitrification are the main processes leading to nitrogen losses in soil via nitrate leaching and nitrogen off gassing (Philippot et al. 2007; Norton and Stark, 2011). Nitrogen cycling involve a variety of microbes carrying functional genes liable for nitrogen transformation. The most commonly N-cycling genes used to explain the abundance and diversity of soil bacterial communities responsible for nitrogen transformation processes are *nifH* (which encode nitrogenase reductase converting N_2 to NH_3) (Gaby and Buckley, 2011; Ducey et al. 2013), *amoA* (which encodes ammonia monooxygenase oxidizing NH_3) (Leininger et al. 2006; Ducey et al. 2013), *nirK* and *nirS* (which encodes nitrite reductase converting nitrite to nitric oxide) (Braker et al. 1998; Ducey et al. 2013), and *nosZ* (which encode nitrous oxide reductase reducing N_2O to N_2) (Henry et al. 2006; Ducey et al. 2013).

1.4.1 Nitrogen fixation genes

Nitrogen fixation helps to balance nitrogen losses from nitrification or losses that also include loss of nitrate through leaching and denitrification by consistently replenishing the bioavailability of nitrogen pool via converting atmospheric dinitrogen (N_2) into organic nitrogen ($R\text{-}NH_2$) (Jetten, 2008); the *nifH* genes that encode the nitrogenase enzyme (Harter et al. 2014) are mostly restricted to prokaryotes of the Archaea and Bacteria domains.

1.4.2 Nitrifying Genes

Nitrification is the oxidation of ammonium to nitrate via nitrite (Harter et al. 2014; Paul, 2007). The major group of microorganisms that facilitate nitrification processes are chemolithoautotrophic nitrifiers including ammonium-oxidizing bacteria [AOB], ammonium-oxidizing archaea [AOA], and nitrite oxidizers (Harter et al. 2014). Nitrification starts with ammonia oxidation resulting to hydroxylamine [NH_2OH] which is usually carried out by the AOB and AOA that produce the ammonia monooxygenase enzymes through the gene *amoA* (Braker and Conrad, 2011; Hu et al. 2015). In the next stage, the hydroxylamine oxidoreductase enzymes produced by AOB catalyzes the hydroxylamine [NH_2OH] to nitrite [NO_2]. Lastly, nitrite [NO_2] is converted to nitrate [NO_3] in the final stage by nitrite oxidizers as a result of their nitrite oxidoreductase enzymes (Braker and Conrad, 2011; Hu et al. 2015).

1.4.3 Denitrifying Genes

Denitrification is the stepwise reduction of nitrate and nitrite to gaseous products including nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) (Groffman, 2012; Harter et al. 2014). Denitrification begins with nitrate-reducers which produce nitrate reductase encoded by *narG* and *napA* reducing nitrate [NO_3] to nitrite [NO_2]. In the next stage, two nitrite reductases encoded by *nirK* or *nirS* genes reduce nitrite to NO (Harter et al. 2014). At the third stage, NO reducers produce the nitric oxide reductase encoded by *cnorB* and *qnorB* which facilitate the transformation of NO to N_2O. Lastly, N_2O is converted to N_2 which is facilitated by nitrous oxide reductase encoded by the *nosZ* genes (Canfield et al. 2010; Braker and Conrad, 2011).

1.4.4 Impact of Biochar on N- cycling and Activity of N-cycling Genes

The effect of biochar on microbially-mediated N-cycling processes such as nitrogen fixation, nitrification and denitrification have been previously studied in several systems (Ducey et al. 2013; Anderson et al. 2014; Liu et al. 2017). The effect of biochar on abundance of N-cycling genes varies from negative to positive results (Ducey et al. 2013; Wang et al. 2015; Zhang et al. 2017) and depends mainly on biochar feedstock, method and temperature of pyrolysis, soil physical and chemical properties. (Liu et al. 2014; He et al. 2015; Hagemann et al. 2017).

Biochar application in agricultural and forest soil have shown to increase rate of nitrification (Prommer et al. 2014; Case et al. 2015). Xu et al. (2014) evaluated biochar impact on N-cycling in acidic soil and found an increased nitrification rates and decrease in N_2O emission. Biochar that is alkaline may produce a better condition for nitrifiers which in turn increase nitrification rates because of their liming effect (Prommer et al. 2014; Ulyett et al. 2014). Denitrification rate is optimal at a pH range of 7.0 to 8.0 but increasing the pH of acidic soil will result to increased rates of denitrification (Peterjohn, 1991). Simek and Cooper (2002) suggested that biochar may increase the activity of nitrous oxide reductases encoded by the *nosZ* gene due to increased soil pH, thus enhancing N_2 production from N_2O. Xu et al. (2014) concluded that decrease in N_2O emission because of biochar addition could be linked to increased reduction rate of N_2O to N_2.

Several studies have reported that biochar can alter the abundance of functional genes connected to nitrogen fixation (Ducey et al. 2013; Harter et al. 2014). Ducey et al. (2013) evaluated the effect of switchgrass biochar on N-cycling genes have shown that the abundance of genes involved in nitrogen fixation (*nifH*) was 793% more at application rate of 10% (w:w) than soil not amended with biochar. Similar results were also reported by Harter et al. (2014). These increased

in abundance of nitrogen fixation genes suggested that biochar could be another means of increasing the amount of nitrogen fixed periodically in soils for plant availability. However, contrasting results were reported by Xiao et al. (2019) that no correlation was observed between biochar and nitrogen fixation gene (*nifH*).

Biochar has been shown to increase the abundance of ammonia oxidizers (Taketani and Tsai, 2010; Song et al. 2014) that carry the *amoA* gene. Xiao et al. (2019) reported biochar to significantly increase the abundance of archaea ammonia oxidisers (AOA) (by 25.3%) but no significant effect was observed in the abundance of AOB. Similarly, Xu et al. (2014) reported that the increase in nitrification rate after biochar addition to an acidic soil may have been contributed more by archaea than by bacteria, as assessed by the abundances of archaeal and bacterial *amoA*. In contrast, Xiao et al. (2019) reported that biochar significantly increased abundance of AOB in acidic soil but decreased abundance of AOA in neutral soils. Similarly, Nicol et al. (2008) reported that abundance of AOB increased as pH increased from 4.9 to 6.9 while AOA decreased. Van Zwieten et al. (2014) reported that that biochar addition increased both AOA and AOB, while Harter et al. (2014) observed no significant change in the ratios of AOA and AOB upon biochar addition. Biochar derived from wood significantly increased abundance of AOB compared to biochar derived from manure which led to decreased AOB abundance (Xiao et al. 2019).

Denitrification is catalyzed by a series of enzymes which may be affected by biochar. Both *nirK* and *nirS* are vital genes for reducing nitrite to nitrous oxide and are considered as key genes for quantifying denitrification in soil (Braker 2000; Kuypers et al. 2018). Xiao et al. (2019) observed increase in narG abundance upon biochar addition but contrasting results were reported by Bai et al. (2015). Ducey et al. (2013) and Xiao et al. (2019) reported that the abundance of *nirK* and *nirS* genes significantly increased with biochar addition. However, Harter et al. (2014), Xu et

al. (2014) and Bai et al. (2015) observed no significant correlation between biochar and gene *nirK* and *nirS*.

Previous studies have demonstrated that biochar increases the abundance of *nosZ* gene (Harter et al. 2014; Xu et al. 2014; Xiao et al. 2019). Addition of biochar at rates of 10 and >40 Mg/ha significantly increased the abundance *nosZ* by 29.8% and 16.6% (Xiao et al. 2019). The increase in *nosZ* gene following biochar application might explain for the decreased in N_2O emission in soil after biochar addition (Harter et al. 2014).

1.5 SIGNIFICANCE OF THE RESEARCH

This research was designed to provide the understanding of the impact of WA and PS on microbial activities in NL podzolic soils. Because soil microbes are essential component of ecosystem processes and the characterisation of their functions in this project is fundamental to guiding recommendations on the suitability and potential utility of WA and PS for improving soil quality, health and thus agricultural productivity.

1.5.1 Questions

The following research questions are addressed:

1. Do wood ash and paper sludge change soil microbial biomass and alter the decomposition of soil organic matter and mineralization of nitrogen?
2. Do wood ash, biochar and paper sludge mitigate greenhouse gases emission?
3. What abiotic factors correlate to the abundance of denitrification-related N cycling genes?

1.5.2 Objectives

1. To assess the soil microbial activity correlated to potential impacts on soil N transformations (i.e., N losses and N availability).
2. To determine the effect of WA and PS on the functional state of the bacterial and archaeal genes (*nap*A, *nar*G, *nir*S, *nirK*, and *nosZ*) driving N speciation as putative proxy indicators for the production of CO_2 and NOx emissions.
3. To evaluate possible relationships between changes in functional N-cycling genes abundance and change in soil organic matter, NH_4^+-N, NO_3^--N, EC, and pH.

1.5.3 Tested Hypotheses

1. Mineralization of PS organic matter will supply N during growth at rates that might overcome the N uptake by plants.
2. Compared to WA, PS will contribute to GHG emissions due to increased mineralization of sludge organic matter or due to its high C:N ratio. However, biochar addition will be a way to mitigate GHG emissions following wood ash and paper sludge application.
3. The shift in N functional gene abundance correlate with soil basal respiration; this is related to changes in microbial biomass and composition.
4. Wood ash will be a stronger liming agent than paper sludge due to their high calcium carbonate equivalent (CCE) values.
5. Addition of urea to wood ash and paper sludge increases accumulation of net mineral N, but variably across amendment treatments.

CHAPTER 2

METHODOLOGY

2.1 Collection and Characterization of Soil Sample

The soil used in this experiment was taken from a depth of 0-15 cm from a recently converted forest located at the Centre of Agriculture and Forestry Development in Wooddale, (49.0249^{o} N, 55.5488^{O} W) Central NL, Canada during summer 2020. The collected soil was air dried, passed through a 2 mm sieve and homogenized. A composite soil subsample was analysed for standard soil parameters (**Table 2.1**) such as pH, soil texture, organic matter, soil organic carbon, total N, mineral N (NH_4^+ and NO_3^-), EC and cation exchange capacity at Soil, Plant and Feed Laboratory, St John's NL.

2.2 Collection and Characterization of WA, PS and Biochar

Paper sludge and wood ash were collected from Corner Brook Pulp and Paper Mill, NL, Canada consecutively for 15 days and were dried on plastic sheets. Thirty (30) PS and WA samples were mixed to form one composite of PS and WA used in this study. Pine biochar used in this study was purchased from Air Terra Inc. (Alberta, Canada). The biochar was made by slowly pyrolyzing pine wood at 500°C for 30 min. The physical and chemical properties of PS, WA and biochar are shown in **Table 2.1**.

Table 2.1. *Physicochemical characteristics of soil, wood ash, biochar and paper sludge used for this study*

Parameter	Unit (dry weight basis)	Soil	Biochar	Wood ash	Paper sludge
pH		4.8	9.39	12.60	8.20
CCE	%	ND	1.62	12.9	10.40
EC	dS/m	0.02	0.644	9.52	0.32
Total carbon	%	2.88	86.8	5.09	40.10
Total nitrogen	%	0.162	ND	0.03	1.04
NO_3-N	mg/kg	ND	12.9	5.09	2.00
NH_4^+-N	mg/kg	ND	ND	0.74	876
Organic carbon	%	ND	86.6	3.45	39.9
Bulk density	g/cm^3	1.44	0.213	1.16	0.12
Dry matter	%	96.18	87.44	97.79	68.12
CEC	cmol/kg	9.9	ND	ND	ND
C: N	Ratio	17:1	ND	ND	38:1
Texture	Silt%	57.7	ND	ND	ND
	Clay%	22.8			
	Sand%	19.5			

EC: electrical conductivity, CCE: calcium carbonate equivalent, NO_3^--N: nitrate nitrogen, NH_4^+-N: ammonium nitrogen, CEC: cation exchange capacity, ND: not determined

2.3 Experimental Design and Treatments

The laboratory experiment was conducted in a randomized, complete block design (RCBD) with twenty (20) treatments (**Table 2.2**) and three replicates. A 21-day preliminary experiment was conducted to the determine the appropriate application rate of PS, WA and limestone to increase soil pH to the target of 6.3; 16.25 Mg/ha, 40 Mg/ha and 75 Mg/ha (oven dry weight basis) of application rates were used for limestone, WA and PS respectively. Biochar application rate (20 Mg/ha) was used according to Liu et al. (2012). Each of the soil-amendment mixtures listed below were prepared with or without urea fertilizer (N) applied at a rate equivalent to 115 kg N/ha.

Table 2.2. *Experimental treatments and ratios of amendment mixture used in this study*

Treatment ID	Treatment description
Soil	Soil only (Negative control)
L	100% Lime (Positive control)
WA	100% Wood Ash (WA)
PS	100% Paper Sludge (PS)
B	100% Biochar (B)
L+B	100%L+ 100%B
WA+PS	80%WA + 20%PS
WA+B	100%WA + 100%B
PS+B	100%PS + 100%B
PS+WA+B	80%WA + 20%PS +100%B
Soil+N	Soil + urea N
L+N	100%Lime + urea N
WA+N	100%WA + urea N
PS+N	100%PS + urea N
B+N	100%B + urea N
L+B+N	100%L + 100%B + urea N
WA+PS+N	80%WA + 20%PS + urea N
WA+B	100%WA + 100%B + urea N
PS+B	100%PS + 100%B
PS+WA+B+N	80%WA + 20%PS +100%B + urea N

Abbreviations: PS- paper sludge, WA-wood ash, B-biochar, N-nitrogen, L-lime

2.4 Incubation Trial

A controlled incubation experiment was carried out for 123 days. All soil cores were prepared by packing 137.5 g dry weight soil or soil-amendment mixture into a 5-cm diameter, 5-cm height PVC ring and compacting it to a dry bulk density of 1.40 g/cm^3. The rings were enclosed into jars with a total volume of 250 mL. This density corresponds to the field's average near-surface bulk density. A previous study which assessed core length and its influence on soil respiration and denitrification led to the decision of a 5-cm height for the core (Guo et al. 2013). Soil cores were brought to 60% water holding capacity (WHC) with a liquid N fertilizer solution (urea equivalent

to a field application of 115 kg N ha^{-1}) (**Figure 1**) or with the equivalent amount of water for the treatments without N fertilizer. To maintain an aerobic atmosphere and minimise soil water loss, the jars were covered with parafilm pierced with pinholes. All cores were incubated in the dark at a temperature of 20 ± 1 ˚C and relative humidity of 75 ± 5%. All cores were preincubated for three days to stabilize the microorganisms and reduce the pulse effect (Fierer and Schimel, 2002). The constant soil water content in the cores was kept in check by weighing the jars every 3 days and distilled water was added as needed. Soil pH was monitored on each sampling day. The experimental set-up allowed for repeated gas sampling, and N dynamic analysis during the entire incubation. With exception to the soil cores used for gas sampling during the incubation, the rest of soil cores were sampled destructively to analyze soil mineral N, microbial biomass and N functional genes by molecular fingerprinting procedures.

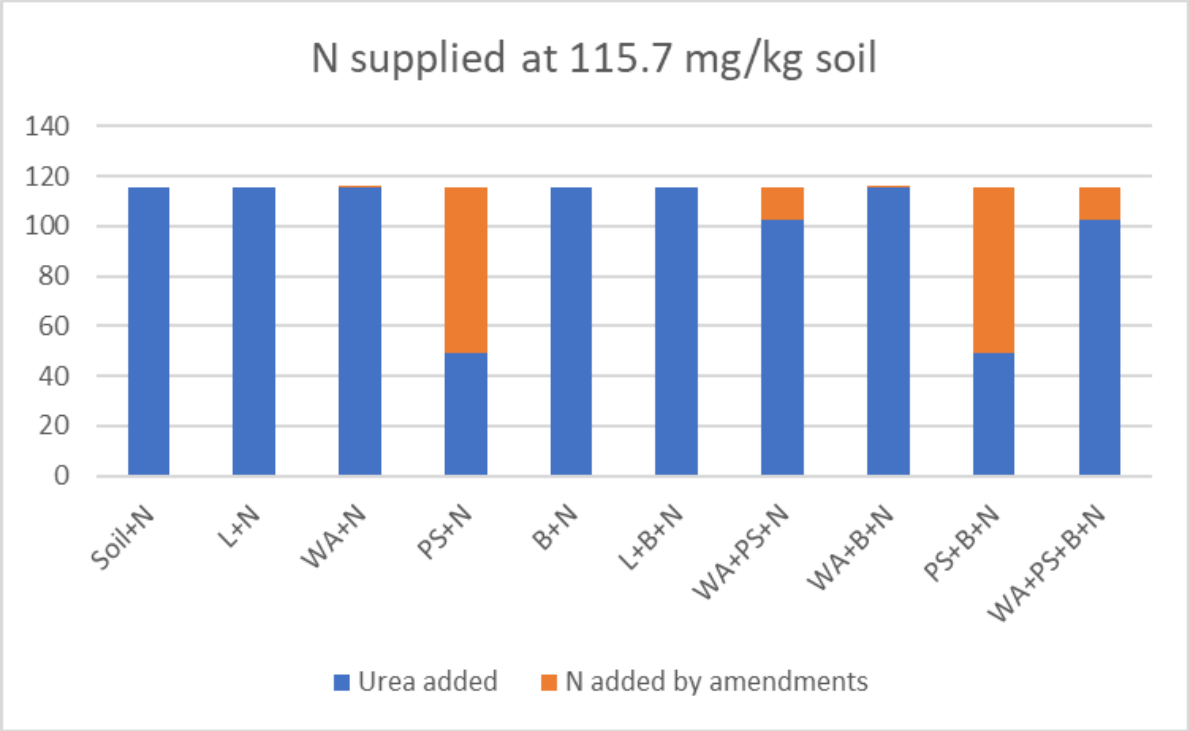

Figure 1. Showing the proportion of N supplied by amendments and urea at start of the incubation.

2.4.1 Soil Physicochemical Properties

2.4.1.1 Mineral Nitrogen

A subset of cores was destructively sampled for mineral N (NH_4^+-N and NO_3-N) on day 1, 4, 7, 15, 22, 36, 56, 93 and 123. Soil samples (10 g dry weight basis) were extracted using 50 mL of 2 M KCl solution and shaken for 60 min on a rotary shaker at 225 rpm (Carter and Gregorich, 2006). Both NH_4^+-N and NO_3^--N in the extracts were determined with Lachat 8500 series Continuous Flow AutoAnalyzer. Net mineralization (representing the sum of ammonification and nitrification minus denitrification) was calculated as the difference between the soil mineral N after last day sampling period and at the first sampling time.

2.4.1.2 Soil pH and Electrical Conductivity

Soil pH and EC was tested using 1:2 of soil to deionized water according to Brady and Weil (2008) with a EC-pH metre (HANNA-HI9813-6 with CAL check) immersed into the supernatant consisting of 10 g soil and 20 mL deionized water after intermittent shaking for 1 h at 160 rpm (Carter and Gregorich 2007).

2.4.1.3 Soil Bulk Density

Bulk density (BD) was determined through core method according to Carter and Gregorich (2007). A soil core was removed with a double cylinder drop hammer sampler. The soil in the core was oven dried at 105 °C for 48 h to remove all moisture. The BD was calculated using the equation below:

$$BD = \frac{total\ mass\ of\ air\ dried\ soil\ (g) - mass\ of\ gravel\ in\ core\ (g)}{volume\ of\ core\ cylinder\ (cm3)}$$

2.4.1.4 Soil Water Content (SWC)

Gravimetric soil water content was determined according to Gardner, (1965) on each sampling day. Ten grams (10 g) each of moist soil samples were oven dried for 24 h at 105 °C to remove all moisture. SWC was calculated using the equation below

$$SWC(\%) = \frac{mass\ of\ wet\ soil\ (g) - mass\ of\ oven\ dried\ soil(g)}{mass\ of\ oven\ dried\ soil\ (g)}\ x\ 100$$

2.4.1.5 Water Holding Capacity (WHC)

Soil samples were put in 5-cm-high, 5-cmsdiameter cylinder cores and immersed in water for 24 h. The cores were weighted after being drained for 48 h and then oven dried for 24 h at 105°C (Lowery et al. 1991). The top of the cores was covered to minimize evaporative loss of water during the 2-day draining. The retained water content per unit dry weight of these cores was determined to be 100 percent WHC.

$$WHC(\%) = \frac{moisture\ held\ in\ drained\ soil}{moisture\ of\ oven\ dried\ soil\ (g)}\ x\ 100$$

2.4.2 Greenhouse Gases Sampling (GHG) and Flux Measurement

GHG emissions from soil cores was measured on Day 1, 4, 7, 15, 22, 36, 56, 93, and 123 of the incubation by measuring the gas (CO_2, and CH_4) concentration accumulated in the jar headspace during the time elapsed between 0 h to 2 h. Briefly, the jars were sealed with airtight lids designed to allow for gas sampling using Gasmet DX 4015 FTIR analyzer. The concentration of CO_2, and CH_4 emission was measured at time 0, 10, 20, and 30 min using Gasmet DX 4015 FTIR analyzer. The lids were then removed and replaced with parafilm punctured with pin holes until the next gas sampling period. Gas flux rates of CO_2, and CH_4 were determined from the slope of linear change in gas concentrations in the headspace over time (Rochette and Bertrand 2007)

and any slopes with $r^2 < 0.5$ were assumed to be zero (Danevcic et al. 2010). The GHG emissions fluxes were calculated according to Pelster et al. (2017) using the equation (1).

$$F = \frac{b \times M_w \times V_{ch} \times 60 \times 10^6}{A_{ch} \times V_m \times 10^9} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq\ (1)$$

F is the flux rate (mg C/m^2/day) for (CO_2, and CH_4), b (ppm/min) is slope of change in linear regression, M_w is the gas molecular weight (g/mol), V_{ch} is the total jar volume minus the volume of the PVC ring with soil (m^3), A_{ch} is the chamber area (m^2), V_m is the corrected standard gas molar volume (m^3/mol) with equation (2)

$$V_m = 22.4 \times 10^{-3} m^3 mol^{-1} \times \frac{273.15 + Temp}{273.15} \times \frac{Air\ pressure}{1013} \ldots\ldots\ldots\ldots\ldots\ldots eq\ (2)$$

Temp (°C) is the chamber air temperature at the time of sampling, while air pressure (hPa) is the atmospheric pressure recorded by the Gasmet DX 4015 FTIR Analyzer.

Cumulative CO_2 and CH_4 was quantified over the course of a 123-day incubation trial by interpolating between data points and integrating over time while assuming a steady flux (Lemke et al. 1999).

2.4.3 Biological Analyses

2.4.3.1 Microbial Biomass (MB)

A subset of cores was destructively sampled for soil MB on Day 1, 36 and 123 of incubation. MB was determined on the fresh moist soil samples by substrate induced respiration (Anderson and Domsch, 1978). The MB was determined by weighing 25 g (dry weight basis) soil samples into 250 mL Erlenmeyer flask and all soil samples were amended with glucose concentration (6 mg per g dry weight) in solution. Soil samples were mixed briefly, and each flask capped with a septum and incubated at 22 °C ± 0.58 for 1 h. After 1 h, the CO_2 accumulated in the headspace of the flask was sampled by extracting 30 mL gas sample each with a syringe. The gas

samples in the syringe were analyzed through injection method on Gas Chromatography (SCION 456-GC) equipped with Thermal Conductivity Detector (TCD) for CO_2 measurements. Microbial biomass was calculated using Anderson and Domsch, (1978) equation as described below:

$$mg\ biomass\ C\ 100g^{-1}\ soil\ =\ 40.04\ \times\ (mL\ CO_2h^{-1}\ 100g^{-1}\ soil) + 0.37$$

2.4.3.2 Basal Respiration (BR)

Soil BR was measured from the soil samples to allow for calculation of metabolic quotients. The BR was determined by weighing 25 g (dry weight basis) soil samples into 250 mL Erlenmeyer flask and each flask capped with a septum and incubated at 22 °C ± 0.58 for 1 h. After 1 h, the CO_2 accumulated in the headspace of the flask was sampled by extracting 30 mL gas sample each with a syringe. The gas samples in the syringe were analyzed through injection method on Gas Chromatography (SCION 456-GC) equipped with Thermal Conductivity Detector (TCD) for CO_2 measurements.

2.4.3.3 Metabolic Quotient (qCO_2)

The qCO_2 is calculated according to Anderson and Domsch (1978) equation shown below.

$$qCO_2\ =\ \frac{Basal\ respiration}{Respiring\ biomass}$$

2.4.3.4 Soil DNA Extraction

DNA was extracted from 0.25 g of soil by using DNeasy PowerSoil Pro DNA Isolation Kit™ according to the manufacturer's instructions. The concentrations (quality and quantity) of the extracted DNA were determined through ThermoScientific™ NanoDrop 2000 (serial no. M125). The DNA was stored at -20 °C for later use.

2.4.3.4.1 Absolute Quantification of Bacterial and Archaeal Genes Relevant to Nitrogen Cycling Genes

Quantitative Polymerase Chain Reaction (qPCR) was carried out on some selected treatments **(Table 2.4)** on Day 1, 36 and 123. Quantitative PCR were carried out for a number of genes relevant to N cycle allowing for determination of gene abundance. Absolute quantification of the targeted genes was performed through PCR amplification using specific primers outlined in **Table 2.4**, the Bio-Rad QX200™ Droplet Digital PCR (ddPCR™) system (serial no. 771BR1304) after Bio-Rad's QX200™ ddPCR™ Evagreen® Supermix instructions. The ddPCR used 20 µL reaction mixtures which contains 10 µL of Evagreen Supermix, 2.5 µL of forward primer (150nM), 2.5 µL of reverse primer (150nM), 3 µL of PCR water, and 2 µL of DNA (20 ng/mL). A PCR master mix was prepared for each set of primer. The master mix accounted for the sum of all of DNA samples including an additional 15% for pipetting error. After the preparation of the PCR reaction mixtures, 20 µL of each reaction was transferred into individual sample wells of a DG8™ Cartridge for Bio-Rad QX200™ Droplet Digital PCR (ddPCR™) system (serial no. 771BR1304) followed by addition of 70 µL Bio-Rad QX200™ Droplet Generation Oil for Evagreen™ into the oil wells, following the QX200™ Generation Manual. Once the droplet generation is completed, 40 µL of the droplets were loaded carefully to a new 96 well PCR plate, sealed with PX1 PCR Plate Sealer (serial no. 770BR1714) and amplified in a Bio-Rad C1000 Touch Thermal Cycler. The thermocycling conditions recommended for Bio-Rad's QX200™ ddPCR™ Evagreen™ Supermix was adopted: (i) activation of enzyme at 95°C for 5 min, (ii) 40 cycles of denaturing at 95°C for 30 s, (iii) 40 cycles of annealing/extension for 60 s at varying temperature following the targeted set of primer (**Table 2.5**), (iv) signal stabilization at 4°C for 5 min, and at 90°C for 5 mins to increase specificity of amplification for 30 sec, with a ramp rate of 2 °C s^{-1}. All quantitative PCR were

performed on three biological replicates and two technical replicates were designed for each sample. Two technical replicates were carried out for no template (negative control) and all resulted in no product amplification. After the thermocycling, samples were transferred into a droplet reader (QX200™ serial no. 771BR1304) for absolute quantification data acquisition through QuantaSoft™ software (Version, 1.4, Bio-Rad).

Table 2.4. *Selected treatments used for quantitative PCR of N cycling genes*

Treatment number	Treatment ID
T1	*Soil*
T2	*L*
T3	*WA*
T4	*PS*
T11	*Soil + N*
T12	*L + N*
T13	*WA + N*
T14	*PS + N*

Abbreviations: PS- paper sludge, WA-wood ash, L- Limestone, N-nitrogen

Table 2.5. *Set of targeted genes used for ddPCR relevant to nitrogen cycle*

Gene name	Abbreviation	Primer ID	Primer sequence (5' to 3')	Annealing temperature (°C)	Reference
Proteobacterial membrane-bound nitrate reductase	narG	narG-F	TCGCCSATYCCGGCSATGTC	58	Bru et al., 2007
		narG-R	GAGTTGTACCAGTCRGCSGAYTCSG		
Nitrous oxide reductase	nosZ	nosZ1F	WCSYTGTTCMTCGACAGCCAGG	61	Henry et al., 2006
		nosZ1R	ATGTCGATCARCTGVKCRTTYTC		
Proteobacterial periplasmic nitrate	napA	V17m	TGGACVATGGGYTTYAAYC	61	Henry et al., 2006
		napA4R	ACYTCRCGHGCVGTRCCRCA		
Denitrifying nitrite Reductase	nirS	nirSCd3aF	AACGYSAAGGARACSGG	57	Throwback et al., 2004
		nirSR3cd	GASTTCGGRTGSGTCTTSAYGAA		
Denitrifying nitrite ss reductase;	nirK	nirK876	ATYGGCGGVCAYGGCGA	59	Henry et al., 2006
		nirK1040	GCCTCGATCAGRTTRTGGTT		
Archaea 16S	Archaea	ARCH1-1369F	CGG TGA ATA CGT CCC TGC	59	Suzuki et al., 2000
		PROK1541R	AAG GAG GTG ATC CRG CCG CA		
Bacterial 16S	Bacteria	BACT1369F	CGG TGA ATA CGT TCY CGG	56	
		PROK1492R	CGW TAC CTT GTT ACG ACT T		

2.5 Statistical Analysis

All data were checked for normality using Anderson-Darling test at a significant level of 0.05 (Minitab 20.1.2). Any data that do not follow the normal distribution was log transformed prior to analysis. All graphical representation were performed in Origin statistical package 2022 SR1 (9.9.0.225). As the research design was not orthogonal, the effect of soil amendments (paper sludge, wood ash, limestone, biochar, and urea) on soil biotic and abiotic parameters and denitrifying gene abundances was determined using a combination of one-way ANOVA with post-hoc Tukey comparison and two-way ANOVAs and general linear models (GLM) in Minitab 20.1.2 (Minitab 20 statistical package, 2020) with $\alpha = 0.05$. However, one-way ANOVA with LSD was used for cumulative GHG emissions dataset. Pearson correlation matrices were performed with $\alpha = 0.05$ for all abiotic and biotic parameters.

Principal Component Analysis was performed in PAST3 (version 3.2.2) to summarize all data into eight vectors to help recognize the similarities of the variability in the response variables.

CHAPTER 3

RESULTS

3.1 Incubation Climate Conditions

For each sampling day, Hobo® data recorders recorded the daily temperatures and relative humidity. During the incubation, the average daily temperature was between 20.04 to 21.02 °C (**Table 3.1**). The lowest and highest average daily temperature were recorded on day 15 and day 93, respectively (**Table 3.1**). There was a slight rise in average daily relative humidity from day 1 to 123 (88.64 ± 1.57 to 93.31 ± 1.01 %) (**Table 3.1**).

Table 3.1. *Average daily incubation temperatures and relative humidity for each sampling day.*

Day	Temperature (°C)	Relative Humidity (%)
1	20.87 ± 0.42	88.64 ± 1.57
4	20.78 ± 0.22	90.36 ± 0.55
7	20.51 ± 0.07	89.34 ± 1.14
15	20.04 ± 0.21	91.09 ± 0.46
22	20.38 ± 0.21	89.43 ± 0.75
36	20.89 ± 0.04	89.6 ± 1.23
56	21.02 ± 0.02	90.75 ± 0.72
93	21.05 ± 0.04	90.68 ± 0.76
123	21.02 ± 0.05	93.31 ± 1.01

3.2 Soil Properties during the Incubation

3.2.1 Incubation dynamics of Basal Respiration (BR), Microbial Biomass (MB), Metabolic Quotients (qCO_2), EC, NO_3, NH_4, pH and GHG emissions

For all treatments, BR, MB, qCO_2, EC, NO_3, NH_4, pH and GHG emissions changed with time. The changes in the biotic and abiotic parameters were, in most case, significantly affected by time (**Table 3.2**). The most noticeable changes between Day 1 and Day 36 were an overall increase in NH_4, NO_3, MB, BR, EC and decrease in qCO_2, GHG emissions in all treatments. Soil pH for all treatments decreased from Day 7 to 123 (**Figure 3.1 a-d**). There was an overall decrease in MB, BR for all treatments and increase in qCO2 (except wood ash and paper sludge treated soil) from Day 36 to 123.

Table 3.2 *Two-way ANOVA to examine the significances of the treatments, sampling days and their interactions for the differences in average biotic and abiotic parameters.*

Factor	BR (mL CO_2)	mg biomass	qCO_2	EC	NO_3^- (mg/kg soil)	NH_4^+ (mg/kg soil)	pH	$mgCO_2$-C m^{-2} h^{-1}	$mgCH_4$-C m^{-2} h^{-1}
Treatments	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	-
Day	0.159	0.161	0.186	0.163	0.023	0.024	0.026	0.024	0.063
Treatment*Day	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
N	180	180	180	180	540	540	540	540	540

3.2.1.2 Soil pH

There were significant treatment-day interactions effects on the soil pH ($p \leq 0.01$). There were significant differences between all treatments from Day 1 to 123 (**Figure 3.1; post-hoc analysis in tables S1, S2 and S3**). Addition of urea to the soil elevated soil pH temporarily. For example, soil control without amendments and urea had a mean pH of 4.73, whereas soil with urea had a mean pH of 5.2. During incubation, the highest pH values were reported in wood ash amended soils, with or without urea and biochar (between 6.0 to 6.4). Soil pH increased from Day 1 to 7, then gradually fell up to Day 123 for all treatments. However, there was no significant variation in pH from day 36 to 123 for wood ash amended soil solely with or without urea (average pH 6.0), whereas the pH of the soil control remained stable from day 1 to 36 but gradually decreased from Day 36 to 123, with a mean pH of 4.7 to 4.0. When the pH of soil control is averaged throughout the sampling days, it is significantly different from the pH of other treatments (**Figure 3.1; posthoc analysis in tables S1, S2 and S3).**

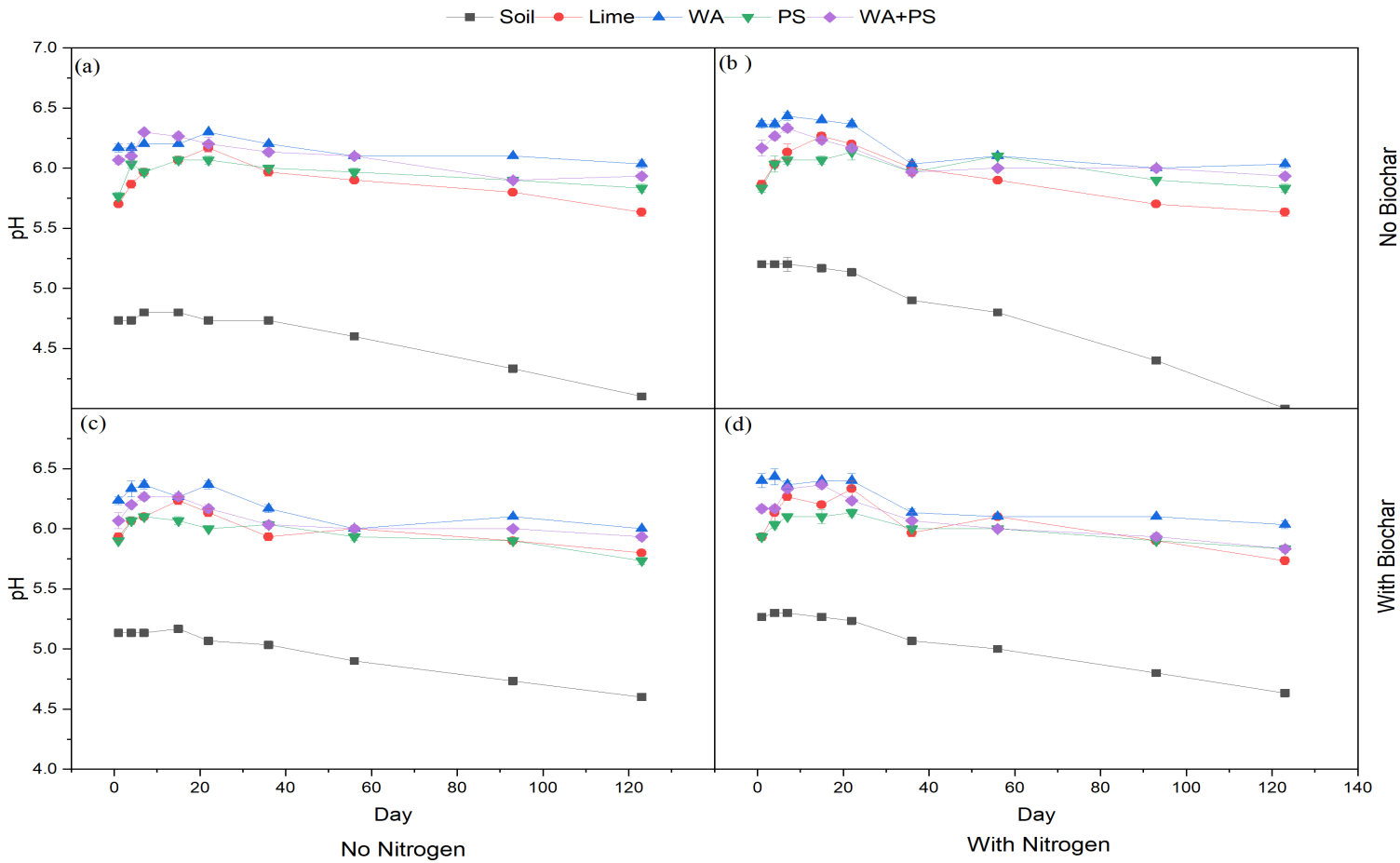

Figure 3.1. *Mean soil pH for treatments with no biochar nor nitrogen (a), treatments with nitrogen only (b), treatments with biochar only (c), treatments with biochar and nitrogen (d) during incubation. Error bars denotes standard error, n = 3.*

3.2.1.3 Soil Inorganic Nitrogen (NO_3^- and NH_4^+)

Significant treatment-day interactions effects were found for soil NH_4^+ concentrations ($p \leq 0.01$). There were statistical differences between treatments on each sampling day from Day 1 to 123 (**Figure 3.2; posthoc analysis in tables S1, S2 and S3**). For all treatments, NH_4 increased significantly from day 1 to 36 ($delta_{36\text{-}1}$ NH_4^+) followed by a statistical plateau (i.e., slower increase, statistically not significant) from Day 56 to 123 (**Figure 3.2**). However, NH_4^+ concentrations were significantly higher in urea amended treatments compared to treatments without urea. General linear model (GLM) was performed for $delta_{36\text{-}1}$ NH_4^+. The GLM showed that application of paper sludge and urea increased NH_4^+ significantly up to day 36 ($p = 0.003, < 0.001$ respectively; model $R^2 = 76.80\%$). Another GLM was further performed on the differences between Day 36 and Day 123 ($delta_{123\text{-}36}$ NH_4^+). It was found that $delta_{123\text{-}36}$ NH_4^+ increased significantly for the treatments receiving paper sludge, wood ash, lime and urea ($p = 0.001, < 0.001, 0.025, < 0.001$, respectively; model $R^2 = 85.6\%$). Biochar had no significant effect ($p = 0.89$).

A significant treatment-day interactions effect was found on soil NO_3^- ($p \leq 0.01$). Soil NO_3^- were significantly different between treatments. Just as reported in NH_4^+, NO_3^- concentrations increased significantly from day 1 to day 36 and plateaued afterwards (i.e., slower increase, statistically not significant) (**Figure 3.3**). NO_3^- concentrations were more significant where urea was added. A GLM analysis found that wood ash, paper sludge and urea increased $delta_{36\text{-}1}$ NO_3^- significantly ($p = 0.012, < 0.001, < 0.001$, respectively; model $R^2 = 75.9\%$). The same analysis for $delta_{123\text{-}36}$ NO_3^- has shown that only paper sludge and urea increased it significantly ($p \leq 0.001, < 0.001$; model $R^2 = 86.2\%$). No significant effect of biochar was observed on delta NO_3^- at $p = 0.6$.

At the end of the incubation, considering the calculated net mineralized soil inorganic N between treatments, paper sludge with and without urea (*PS+N* and *PS*) was significantly different from other treatments, with a mean of 35.55 ± 0.0225 and 24.68 ± 0.772 mg/kg compared to soil control with or without urea with mean of 10.26 ± 0.349 and 7.74 ± 0.269 mg/kg and biochar with or without urea with mean of 18.2 ± 0.0525 and 12.61 ± 0.152 mg/kg, respectively (**Figure 3.4**). Where urea was added the final mineral N likely included urea N; however, while for soil the urea led to a 2.53 ± 0.44 mg N kg^{-1} soil (mean and std) (**Figure 3.5**), for the paper sludge, wood ash and lime treatments the increase due to the addition of urea was over 8 mg N kg^{-1} soil (**Figure 3.5**). This compares to the total urea added N of 115.7 mg N kg^{-1} soil. Such change in the urea treated soils can be thus attributed both to added urea but also to the capacity of treatments other than soil-only to retain, or rather minimise losses of urea-N.

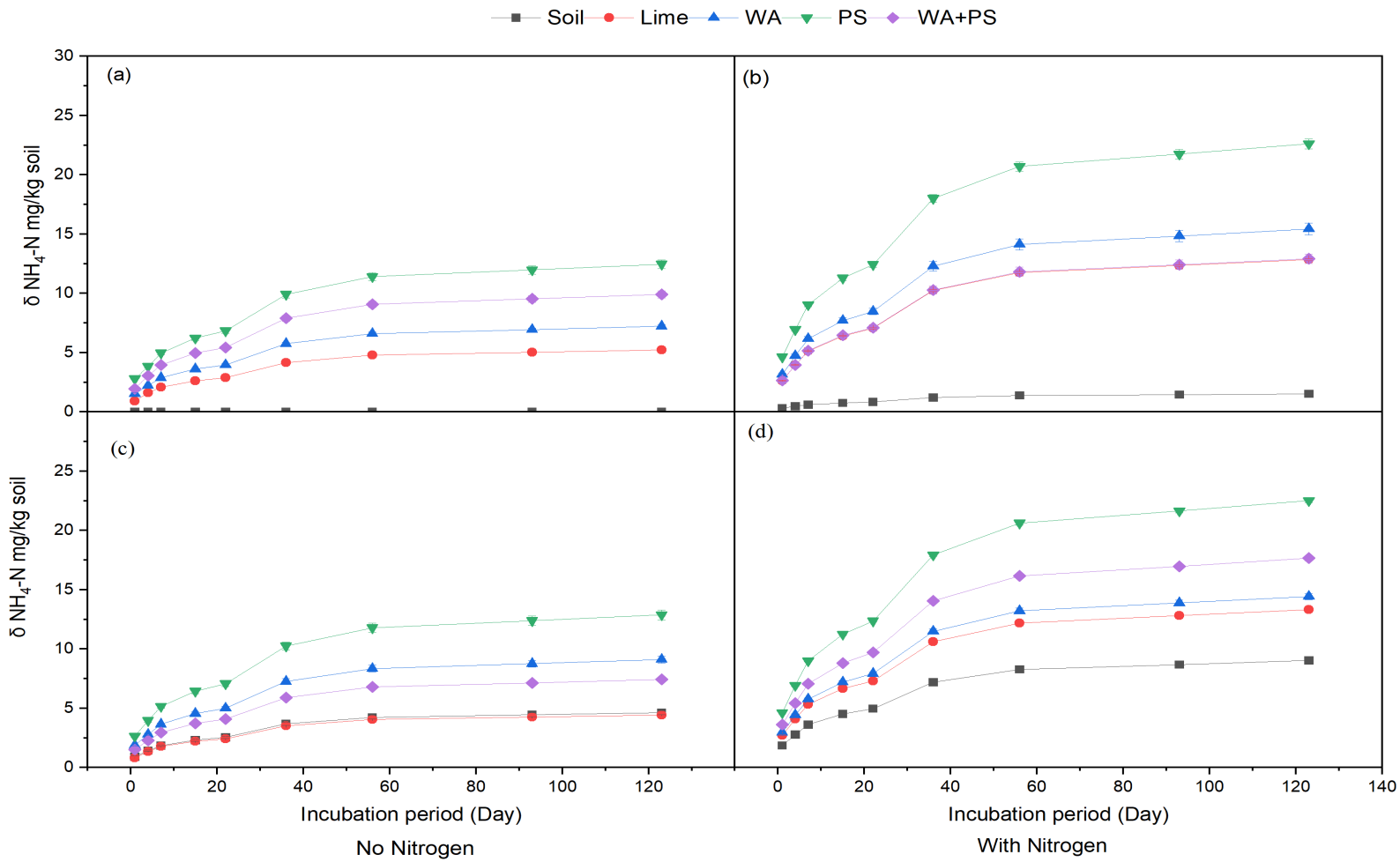

Figure 3.2 *Mean soil δ NH_4^+ (calculated as difference between each treatment and soil control) for treatments with no biochar nor nitrogen (a), treatments with nitrogen only (b), treatments with biochar only (c), treatments with biochar and nitrogen (d) during incubation. Error bars denotes standard error, n = 3.*

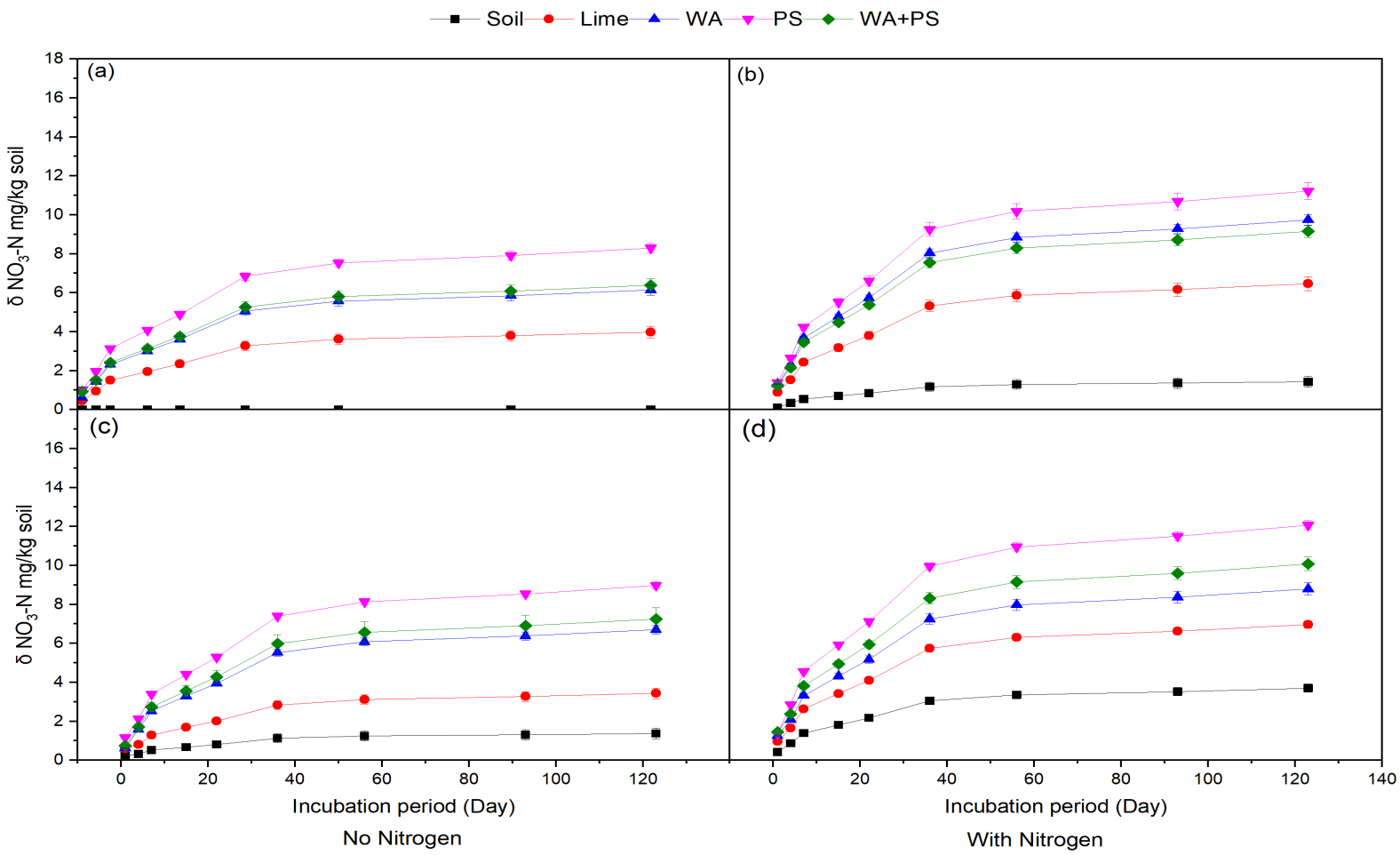

Figure 3.3. *Mean soil δ NO_3^- (calculated as difference between each treatment and soil control) for treatments with no biochar nor nitrogen (a), treatments with nitrogen only (b), treatments with biochar only (c), treatments with biochar and nitrogen (d) during incubation. Error bars denotes standard error, n = 3.*

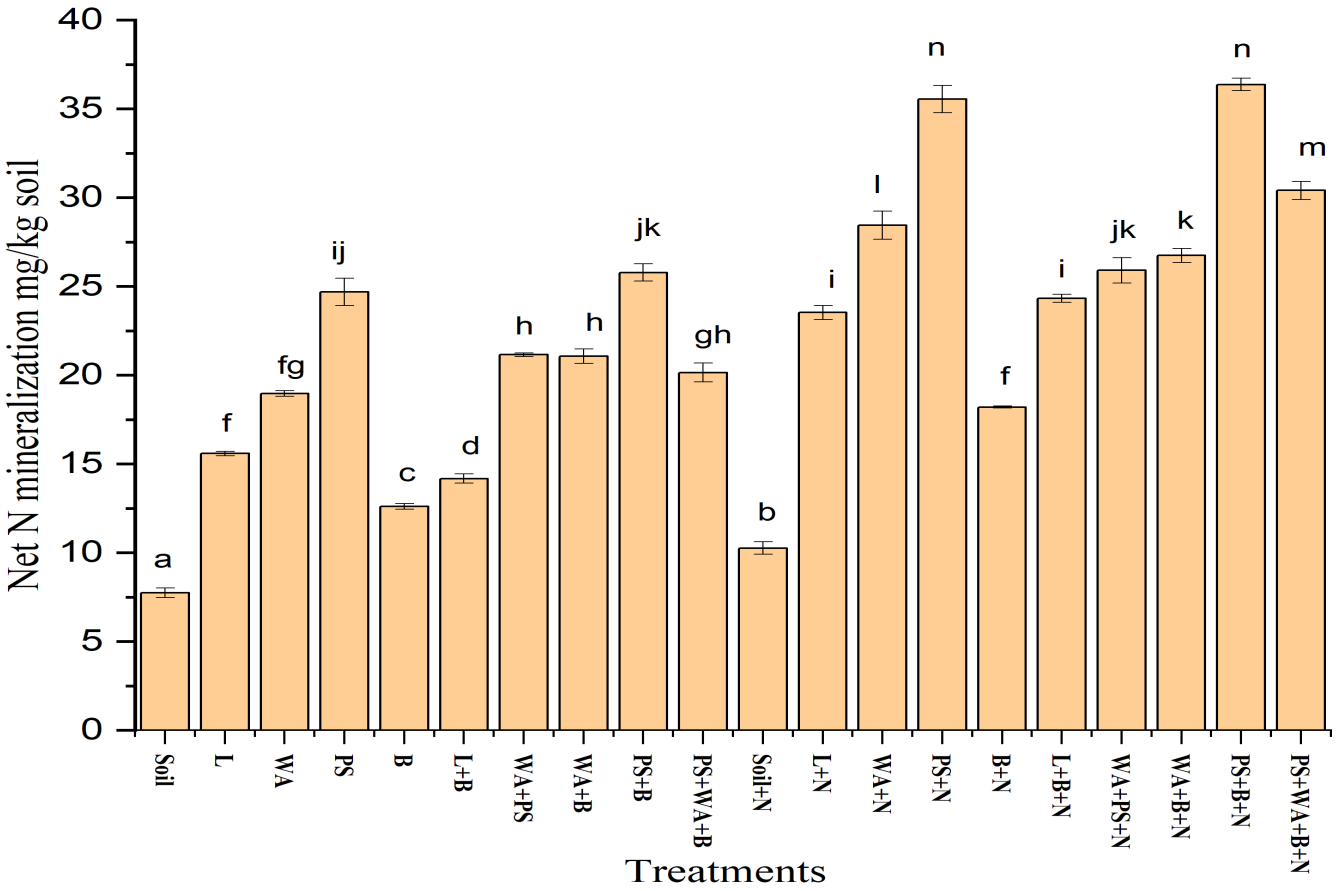

Figure 3.4. *Average net N mineralization for all treatments. mean and CI 95 (error bars). Means that do not share same letter are statistically different.*

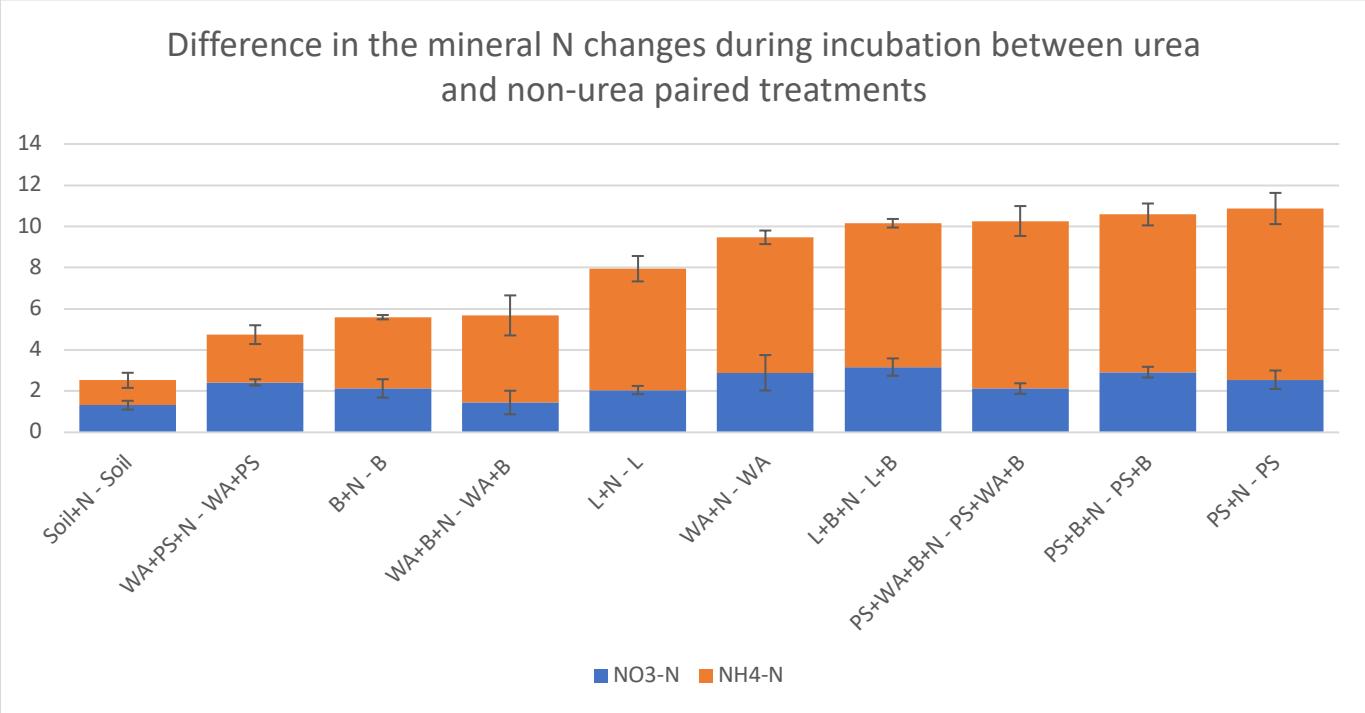

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Figure 3.5. Calculated differences in the mineral N between paired urea and no urea treatments during incubation.

3.2.1.4 Microbial Biomass (MB)

Significant treatment-day interactions effects were found on soil MB ($p \leq 0.01$). There were statistical differences between treatments on Day 1, 36 and 123 (**Figure 3.6; posthoc analysis in tables S1, S2 and S3**). For all treatments, MB increased significantly from day 1 to 36 followed by decreased by Day 123 (**Figure 3.6**). The highest MB value was found in paper sludge amended soil. ANOVA further showed that no significant differences were found when paper sludge was used alone (PS) or in combination with biochar or urea (PS+B or PS+N or PS+B+N) (**posthoc analysis in Tables S1, S2 and S3**). GLM was performed on $delta_{36\text{-}1}$ MB. The GLM showed that paper sludge increased MB significantly up to day 36 ($p \leq 0.001$, model $R^2 = 74.10\%$) compared to other treatments. No significant effect ($p = 0.56$) of urea was found. Another GLM was further performed on $delta_{123\text{-}36}$ MB to estimate the rate of decrease in the biomass. It was found that $delta_{123\text{-}36}$ MB in wood ash and lime treatments changed significantly ($p \leq 0.001, 0.039$ respectively, model $R^2 = 52.20\%$). Biochar showed no significant effect ($p = 0.24$).

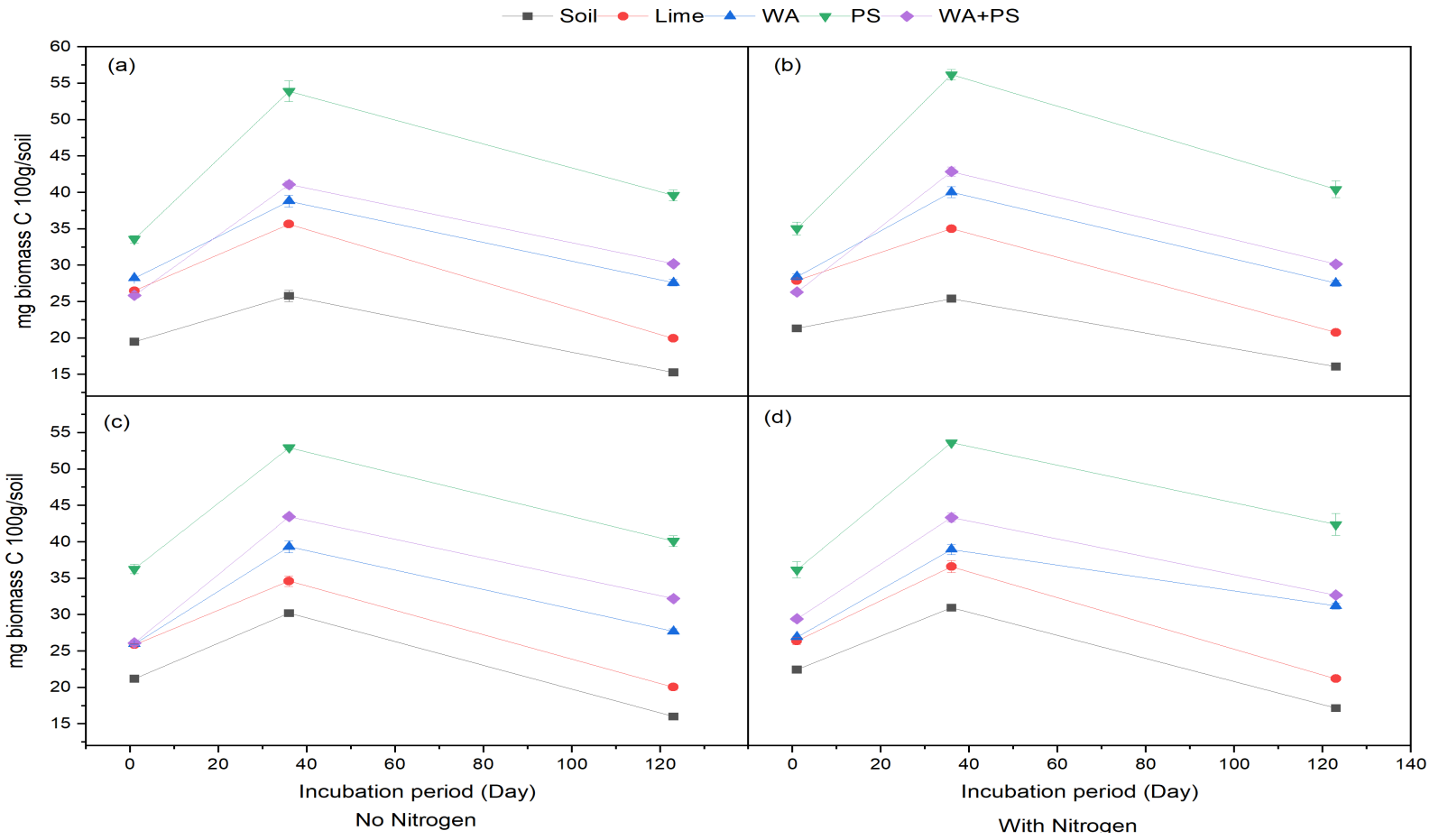

Figure 3.6. *Changes in the microbial biomass for treatments with no biochar nor nitrogen (a), treatments with nitrogen only (b), treatments with biochar only (c), treatments with biochar and nitrogen (d) during incubation. Error bars denotes standard error, n = 3.*

3.2.1.5 Soil Basal Respiration (BR)

Significant treatment-day interactions effects were found on soil BR ($p \leq 0.01$). There were statistical differences between treatments on Day 1, 36 and 123 (**Figure 3.7; posthoc analysis in tables S1, S2 and S3**). For all treatments, BR increased from day 1 to 36 followed by decreased by Day 123 (**Figure 3.7**). The highest BR value was found in paper sludge amended soil. However, GLM performed on $delta_{36\text{-}1}$ BR found that only lime significantly increased respiration up to day 36 ($p = 0.039$, model $R^2 = 26.30\%$) compared to biochar, paper sludge, wood ash and urea ($p = 0.052, 0.351, 0.065, 0.66$ respectively, model $R^2 = 26.30\%$). GLM carried out on $delta_{123\text{-}36}$ BR found that basal respirations in WA and biochar were significantly reduced ($p \leq 0.001, 0.03$. respectively, model $R^2 = 42\%$).

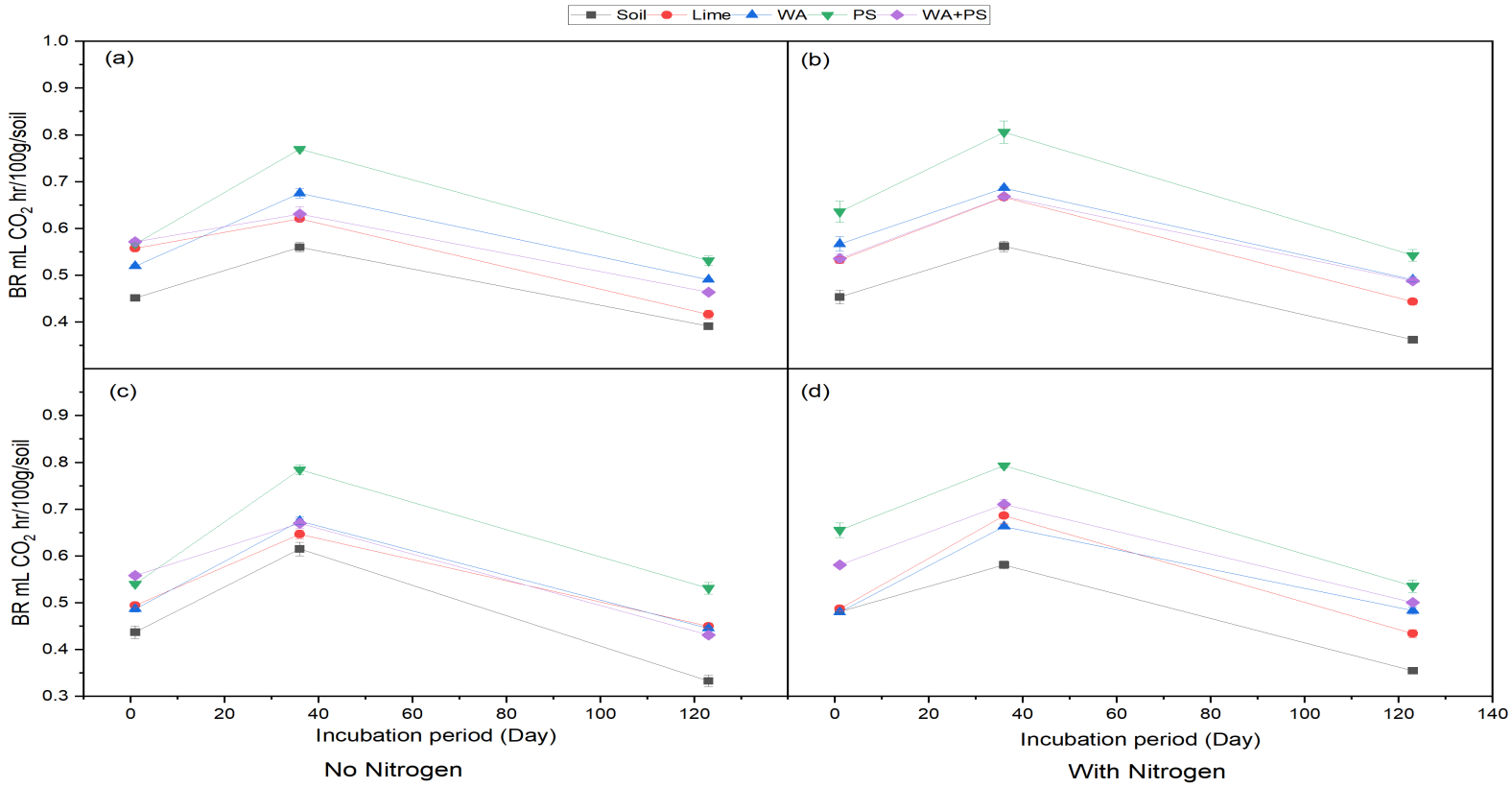

Figure 3.7 *Mean basal respiration for treatments with no biochar nor nitrogen (a), treatments with nitrogen only (b), treatments with biochar only (c), treatments with biochar and nitrogen (d) during incubation. Error bars denotes standard error, n = 3.*

3.2.1.6 Metabolic Quotients (qCO_2)

Significant treatment-day interactions effects were found on qCO_2 ($p \leq 0.01$). For all treatments, qCO_2 decreased significantly from Day 1 to 36 followed by a slight rise by Day 123 except for paper sludge and wood ash (used alone or in combination with biochar or urea). There were significant differences between all treatments on Day 1, 36 and 123 (**Figure 3.8 and posthoc analysis in tables S1, S2 and S3**). The lowest values of qCO_2 were found in treatments with paper sludge and wood ash acting as intermediates. GLM carried out on $delta_{36\text{-}1}$ qCO_2 found that lime, wood ash, paper sludge significantly decreased qCO_2 up to Day 36 ($p = 0.039, < 0.001, < 0.001$ respectively, model $R^2 = 57.5$). Urea and biochar had no significant effect on qCO_2 ($p = 0.852$, 0.731 respectively, model $R^2 = 57.5$). Another GLM performed on $delta_{123\text{-}36}$ qCO_2 showed that qCO_2 increased significantly in biochar and lime ($p = 0.014, < 0.001$ respectively, model $R^2 = 60.70$) while in paper sludge decreased significantly ($p = 0.015$, model $R^2 = 60.70$).

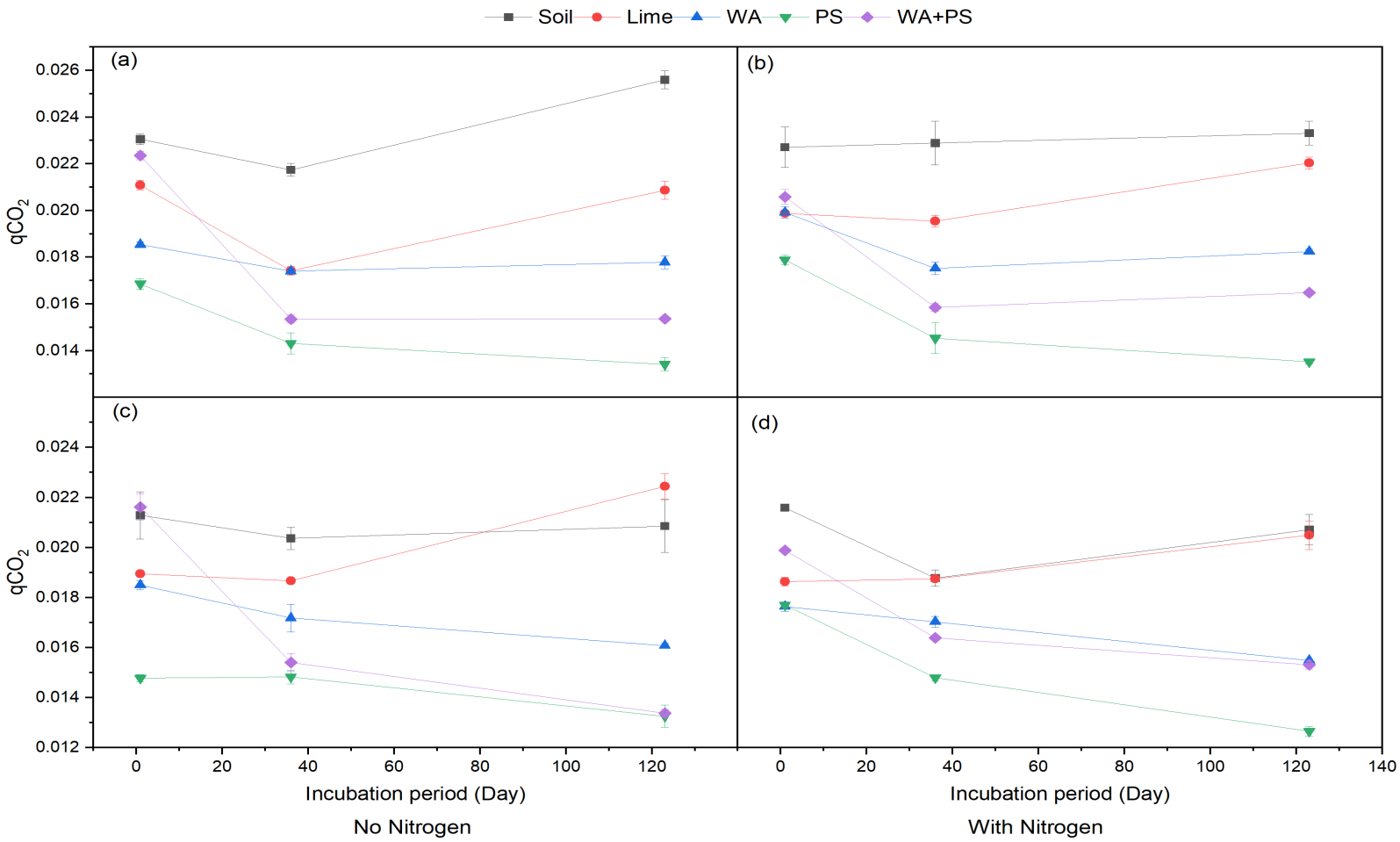

Figure 3.8 *Metabolic quotients for treatments with no biochar nor nitrogen (a), treatments with nitrogen only (b), treatments with biochar only (c), treatments with biochar and nitrogen (d) during incubation. Error bars denotes standard error, n = 3.*

3.2.1.7 Greenhouse Gases Emissions

There were significant treatment-day interactions for the soil CO_2 emissions ($p = 0.01$). Higher temporal CO_2 emissions were found on Day 1 and 4 for all treatments, but gradually decreased up to day 36 (**Figure 3.9**). CO_2 emissions remained steady for most treatments from Day 56 through the end of the incubation (**Figure 3.9**). The impacts of paper sludge, wood ash, and limestone alone, as well as in combination with biochar or urea, on cumulative and temporal CO_2 emissions were significant ($p< 0.05$). There were higher cumulative CO_2 emissions from paper sludge amendments used alone or in combination with urea (64.9 ± 10.2 and 63.6 ± 11.4 kg C ha^{-1} respectively); lowest emissions were found when biochar was used alone or in combination with urea (6.49 ± 1.34 and 14.33 ± 1.9 kg C ha^{-1} respectively) comparable to the soil control without or with urea fertilizer (10.73 ± 1.29 and 17.19 ± 2.22 kg C ha^{-1} respectively) (**Table 3.3**). However, no significant differences were found for the cumulative CO_2 emissions when paper sludge was used alone or in combination with urea (**Table 3.3**). Addition of biochar to paper sludge versus paper sludge used alone or in combination with urea decreased paper sludge treatments' cumulative CO_2 emissions by 28.91 and 25.77% respectively.

Addition of urea to wood ash increased wood ash cumulative CO_2 emissions from 19.61 ± 0.68 to 25.44 ± 3.32 kg C ha^{-1} (**Table 3.3**) accounting for a 22.93% increase in CO_2 emissions comparable to the soil control without or with urea fertilizer (10.73 ± 1.29 and 17.19 ± 2.22 kg C ha^{-1} respectively) (**Table 3.3**). It was further observed that addition of biochar to wood ash or to wood ash and urea increased wood ash cumulative CO_2 emissions by 34.18 and 17.34 % respectively. In contrast, biochar decreased CO_2 emissions when added to the lime, or lime and

urea treatments, by 23.97 and 26.97%, respectively. Biochar addition to soil or soil with urea decreased cumulative CO_2 emissions by 39.52 and 16.64% respectively.

A GLM was performed on cumulative CO_2 emissions data using biochar, paper sludge, wood ash, limestone, and urea as fixed factors. It was found that paper sludge significantly increased cumulative CO_2 emissions ($p \leq 0.001$, $R^2 = 36.19\%$).

There were no statistical differences in temporal and cumulative CH_4 emissions across all treatments ($p > 0.05$) (**Figure 3.10, Table 3.3**).

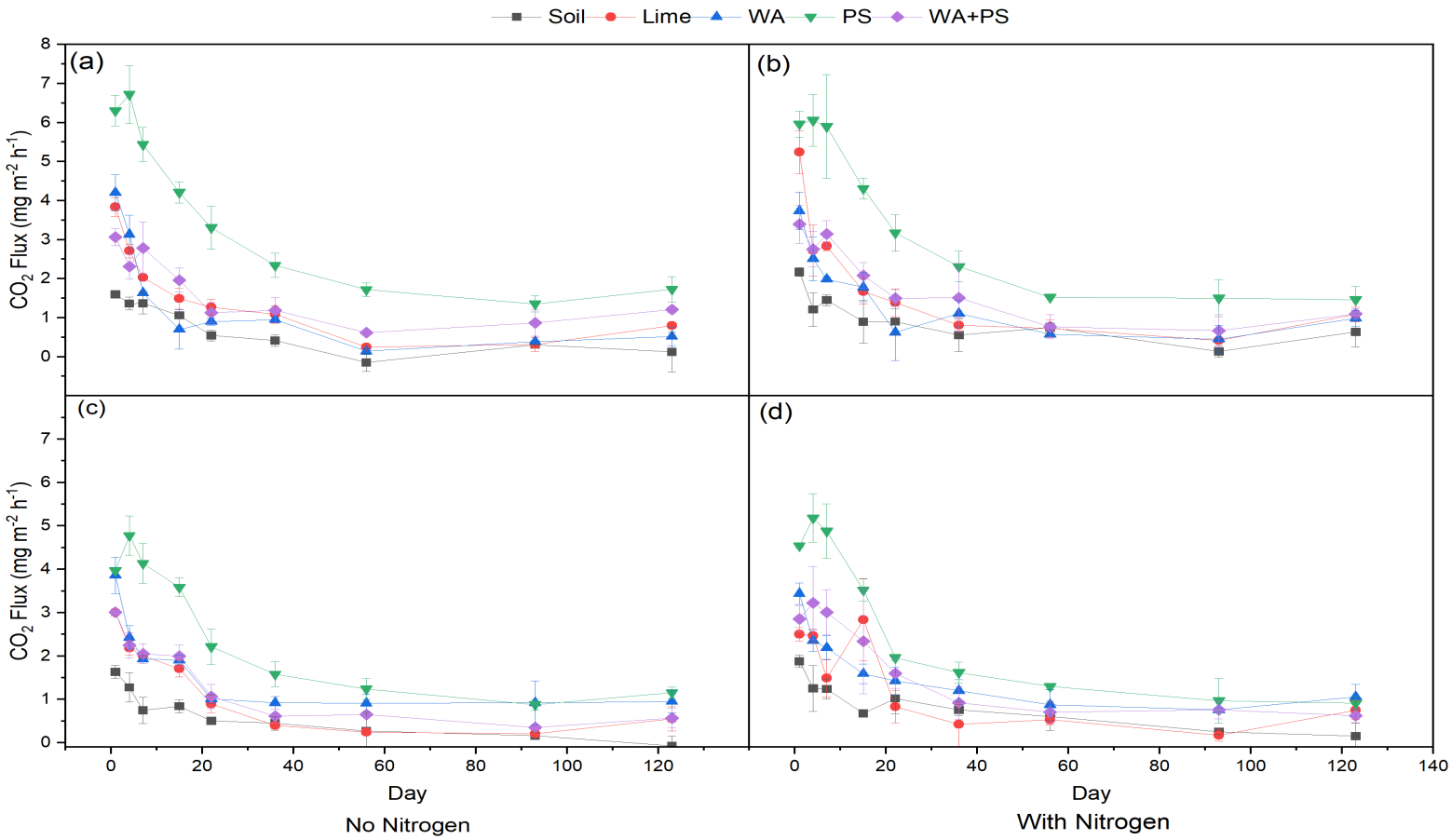

Figure 3.9 *Soil CO_2 emissions for treatments with no biochar nor nitrogen (a), treatments with nitrogen only (b), treatments with biochar only (c), treatments with biochar and nitrogen (d) during incubation. Error bars denotes standard error, n = 3.*

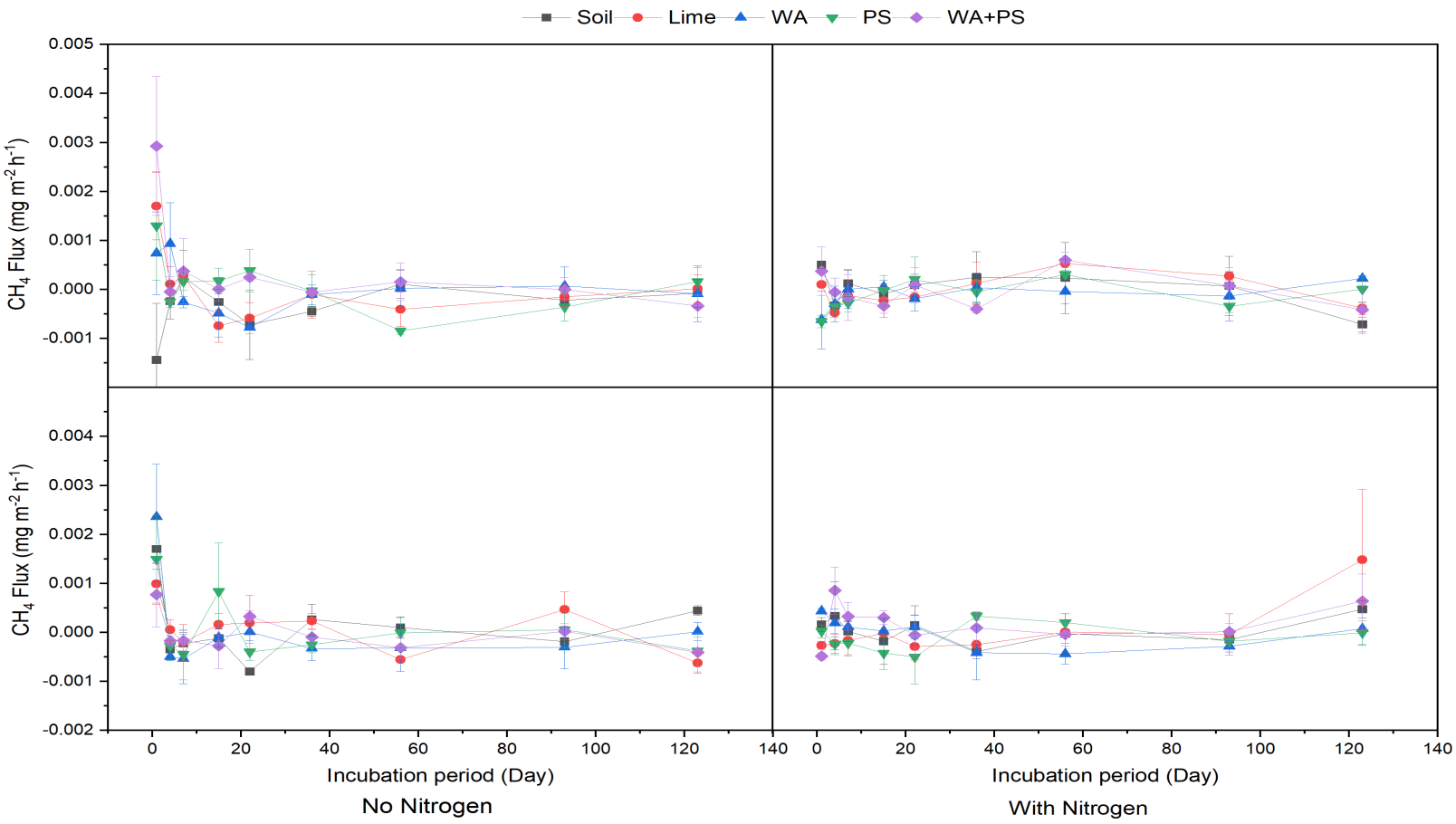

Figure 3.10 *Soil CH_4 emissions for treatments with no biochar nor nitrogen (a), treatments with nitrogen only (b), treatments with biochar only (c), treatments with biochar and nitrogen (d) during incubation. Error bars denotes standard error, n = 3.*

Table 3.3 *Cumulative GHG Emission (CO_2 and CH_4) during the 123 days of incubation*

S/N	Treatment	CO_2 kg C ha^{-1}	CH_4 kg C ha^{-1}
1	Soil	10.73 ± 1.29fg	-0.006 ± 0.002a
2	L	23.78 ± 0.66cdef	-0.0043 ± 0.0048a
3	WA	19.61 ± 0.68defg	-0.0023 ± 0.003a
4	PS	64.90 ± 10.20a	-0.003 ± 0.0015a
5	WA+PS	31.39 ± 6.15cd	0.002 ± 0.0023a
6	B	6.49 ± 1.34g	0.0007 ± 0.0029a
7	L+B	18.08 ± 0.98defg	-0.0017 ± 0.0035a
8	WA+B	29.79 ± 4.42cd	-0.0043 ± 0.0012a
9	PS+B	46.14 ± 5.15b	-0.0017 ± 0.0029a
10	PS+WA+B	22.82 ± 1.43cdef	-0.0027 ± 0.0029a
11	Soil+N	17.19 ± 2.22 defg	0.0003 ± 0.0044a
12	L+N	29.19 ± 0.96cd	0.001 ± 0.004a
13	WA+N	25.44 ± 3.32cde	-0.001 ± 0.004a
14	PS+N	63.6 ± 11.40a	-0.0013 ± 0.0013a
15	WA+PS+N	34.18 ± 8.96bc	-0.0007 ± 0.0012a
16	B+N	14.33 ± 1.90efg	-0.0003 ± 0.003a
17	L+B+N	21.32 ± 2.01cdef	0.0027 ± 0.0052a
18	WA+B+N	30.78 ± 4.13cd	-0.0043 ± 0.0034a
19	PS+B+N	47.21 ± 6.87b	-0.001 ± 0.0017a
20	PS+WA+B+N	30.81 ± 3.26cd	-0.001 ± 0.004a

Mean value of three replicates of the SE, means that do not share same letter are statistically different at 5% significant level

3.3 Relationships Between Biotic and Abiotic Parameters

A correlation analysis was used to determine the relationships between biotic and abiotic characteristics as measured at day 1, 36, and 123. This provided significant evidence for the putative relationships between biotic and abiotic parameters. The results revealed a strong positive correlation between microbial biomass and CO_2 emissions, basal respirations, pH, soil water content, NH_4^+ and NO_3^- (**Table 3.4**). However, microbial biomass and soil pH were strongly negatively correlated with the qCO_2 (**Table 3.4**).

Principal Component Analysis (PCA) was performed on $delta_{36\text{-}1}$ datasets. Component 1 explains ≥41.45% of the variations in the biotic and abiotic parameters, while component 2 explains ≥19.25% of the variation (**Figure 3.11**). All parameters (MB, BR, CO_2 emission, CH_4 emission, pH, EC, NH_4^+ and NO_3^-) increased with paper sludge, according to PCA analysis. In paper sludge-treated soil, however, the metabolic quotient (qCO_2) declined over time (**Figure 3.11**). The effect of wood ash had an intermediates effect.

Table 3.4 *Pearson correlation matrices between biotic and abiotic parameters for days 1, 36 and 123 (α = 0.05. *p ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0.001).*

Correlation between biotic and abiotic parameters for Day 1									
	NO3-N mg kg^{-1} soil	**EC (mS cm^{-1})**	**pH**	**mgCH$_4$- m^2 h^{-1}**	**mgCO$_2$-C m^2 h^{-1}**	**qCO2**	**mg biomass**	**BR mL CO$_2$**	**SWC(%)**
NH4-N mg kg^{-1} soil	0.915***	0.452***	0.557***	-0.204	0.581***	-0.601***	0.792***	0.746***	0.482***
NO3-N mg kg^{-1} soil		0.591***	0.749***	-0.134	0.53***	-0.612***	0.771***	0.716***	0.416***
EC (mS cm^{-1})			0.824***	-0.053	0.215	-0.313**	0.279	0.278	0.079
pH				0.111	0.431***	-0.489***	0.501***	0.457***	0.062
mgCH$_4$- m^2 h^{-1}					-0.015	-0.041	0.003	-0.082	0.08
mgCO$_2$-C m^2 h^{-1}						-0.622***	0.753***	0.611***	0.471***
qCO$_2$							-0.81***	-0.334**	-0.551***
mg biomass C 100g^{-1} soil								0.787***	0.68***
BR mL CO$_2$ h^{-1} 100^{-1} soil									0.439***
Correlation between biotic and abiotic parameters for Day 36									
	NO3-N mg kg^{-1} soil	**EC (mS cm^{-1})**	**pH**	**mgCH$_4$- m^2 h^{-1}**	**mgCO$_2$-C m^2 h^{-1}**	**qCO2**	**mg biomass**	**BR mL CO$_2$**	**SWC(%)**
NH4-N mg kg^{-1} soil	0.93***	0.59***	0.575***	0.044	0.512***	-0.696***	0.781***	0.803***	0.524***
NO3-N mg kg^{-1} soil		0.709***	0.751***	0.017	0.544***	-0.825***	0.859***	0.84***	0.532***
EC (mS cm^{-1})			0.763***	-0.159	0.213	-0.497**	0.434**	0.462**	0.103
pH				-0.022	0.323**	-0.743***	0.657***	0.629***	0.214
mgCH$_4$- m^2 h^{-1}					0.068	0.053	0.023	0.084	0.053
mgCO$_2$-C m^2 h^{-1}						-0.565***	0.649***	0.59***	0.414**
qCO$_2$							-0.923***	-0.748***	-0.526***
mg biomass C 100g/soil								0.928***	0.722***
BR mL CO$_2$ h/100/soil									0.73***
Correlation between biotic and abiotic parameters for Day 123									
	NO3-N mg kg^{-1} soil	**EC (mS cm^{-1})**	**pH**	**mgCH$_4$- m^2 h^{-1}**	**mgCO$_2$-C m^2 h^{-1}**	**qCO$_2$**	**mg biomass**	**BR mL CO$_2$**	**SWC(%)**
NH4-N mg kg^{-1} soil	0.93***	0.357**	0.603***	0.181	0.453***	-0.707***	0.763***	0.749***	0.385**
NO3-N mg kg^{-1} soil		0.458***	0.776***	0.081	0.533***	-0.839***	0.872***	0.859***	0.457***
EC (mS cm^{-1})			0.517***	0.182	0.132	-0.237	0.167	0.26	0.109
pH				0.006	0.485***	-0.677***	0.657***	0.746***	0.311**
mgCH$_4$- m^2 h^{-1}					-0.096	-0.005	-0.04	-0.058	0.04
mgCO$_2$-C m^2 h^{-1}						-0.473***	0.538***	0.574***	0.278
qCO$_2$					.		-0.937***	-0.73***	-0.513***
mg biomass C 100g^{-1} soil								0.891***	0.587***
BR mL CO$_2$ h^{-1} 100^{-1} soil									0.554***

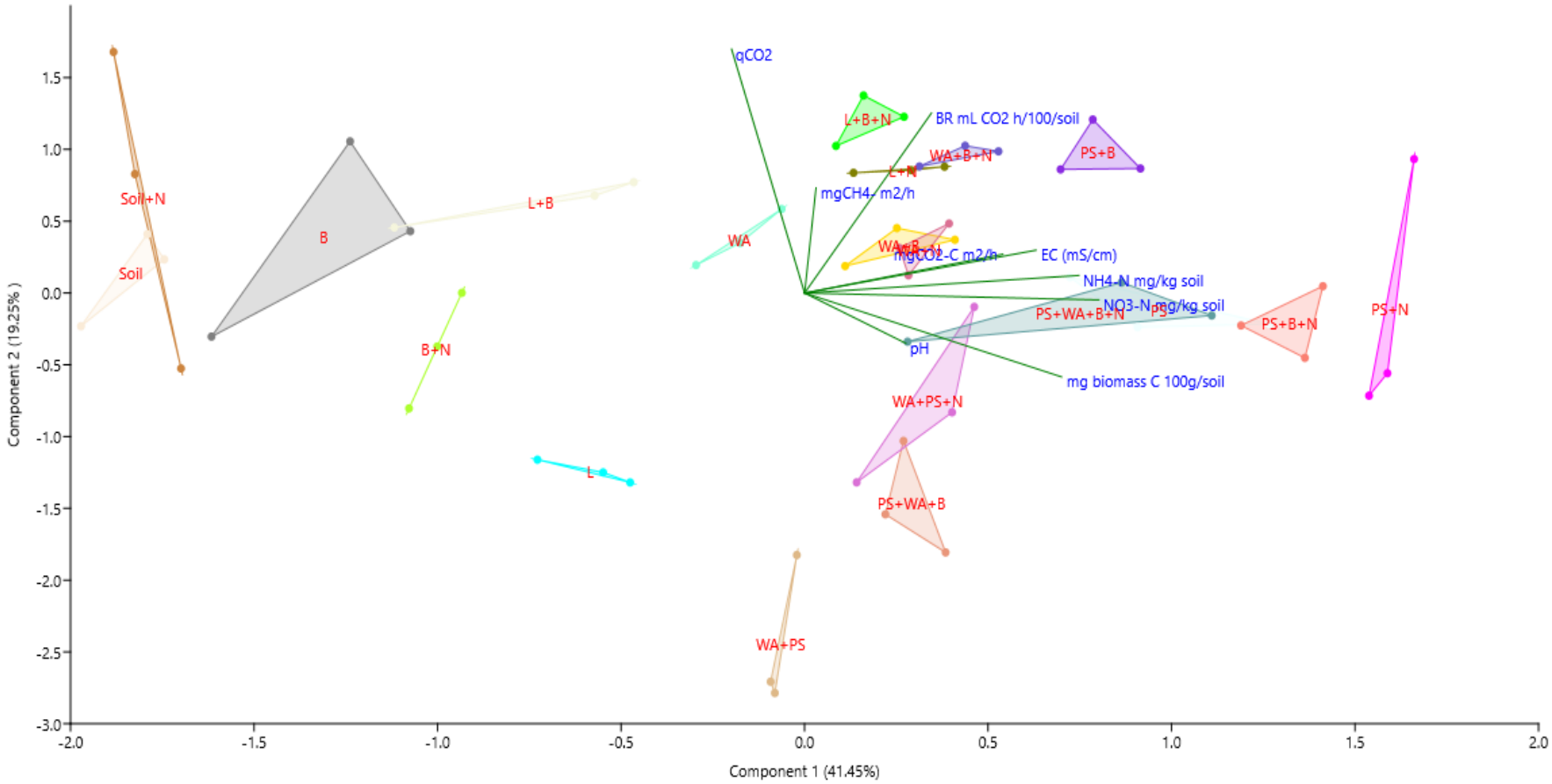

Figure 3.11 *Principal Component Analysis biplots based on rate of change from day 1 to 36 (delta$_{36-1}$) showing the effect of treatments on soil biotic and abiotic parameters.*

3.4 Soil Nitrogen Cycling Genes Abundance

The count of the soil N-cycling genes per mass dry soil varied with treatments and sampling day; the counts were affected significantly by the treatment*day interactions ($p \leq 0.01$) (**Table 3.5**). The N-cycling gene counts increased significantly to day 36, followed by a slight decrease by day 123, for all treatments. On day 36, the highest increase in the counts of 16S bacteria, *NapA*, 16S-archaea, *NarG, NirK* and *NirS* were found in paper sludge treatments with or without urea (**Figure 3.12-3.13**). On day 123, however, the wood ash treatments, with or without urea, had higher levels of *NapA*, archaea, *NarG*, *NirS,* and *NirK* (**Figure 3.12-3.13**). For all sampling days, *NosZ* was significantly higher when wood ash was added but lower for paper sludge applications (**Figure 3.13**). A GLM was performed for the $delta_{36\text{-}1}$ datasets. The GLM showed that paper sludge, wood ash, lime and urea increased significantly $delta_{36\text{-}1}$ 16S bacteria up to day 36 ($p \leq 0.001$, < 0.001, 0.01 and 0.003, respectively; model $R^2 = 97\%$). $delta_{36\text{-}1}$ *NarG* was significantly affected by paper sludge, wood ash, and urea ($p = \leq 0.001$, 0.003, and < 0.001 respectively, model $R^2 = 94\%$). It further revealed that paper sludge, wood ash, and lime increased $delta_{36\text{-}1}$ *NapA* significantly ($p \leq 0.001$, 0.002, and 0.032 $R^2 = 79.90\%$ respectively). Paper sludge, wood ash, lime and urea significantly increased $delta_{36\text{-}1}$ *NirS* and $delta_{36\text{-}1}$ *NirK* ($p \leq 0.001$, < 0.001, 0.028, < 0.001 respectively, model $R^2 = 98.7\%$ and $p \leq 0.001$, < 0.001, 0.014, 0.001 respectively, model $R^2 = 94.7\%$). $delta_{36\text{-}1}$ Archaea were significantly affected by wood ash, paper sludge and urea ($p \leq 0.001$, < 0.001, and < 0.001 $R^2 = 97.4\%$). $delta_{36\text{-}1}$ *NosZ* was also significantly affected by paper sludge, wood ash, lime and urea ($p = 0.001$, < 0.001, 0.001 and < 0.001, $R^2 = 97.7\%$). Another GLM was further performed for the $delta_{123\text{-}36}$ datasets; $delta_{123\text{-}36}$ 16S bacteria was still significantly higher in paper sludge and wood ash treatments ($p \leq 0.001$ and 0.011 $R^2 = 95.9\%$ respectively). It

was also found that delta$_{123\text{-}36}$ *NosZ* was still significantly higher where wood ash was added ($p \leq 0.001$, $R^2 = 96\%$) compared to other treatments.

Table 3.5 *Two-way ANOVA to examine the significances of the treatments, sampling days and their interactions for the differences in average N-cycling genes.*

Factor	16S Bacteria	*NapA*	*NarG*	*NirS*	*NirK*	*NosZ*	Archaea
Treatments	0.036	0.035	0.035	0.039	0.043	0.037	0.038
Day	0.161	0.161	0.161	0.161	0.162	0.16	0.16
Treatment*Day	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
N	180	180	180	180	180	180	180

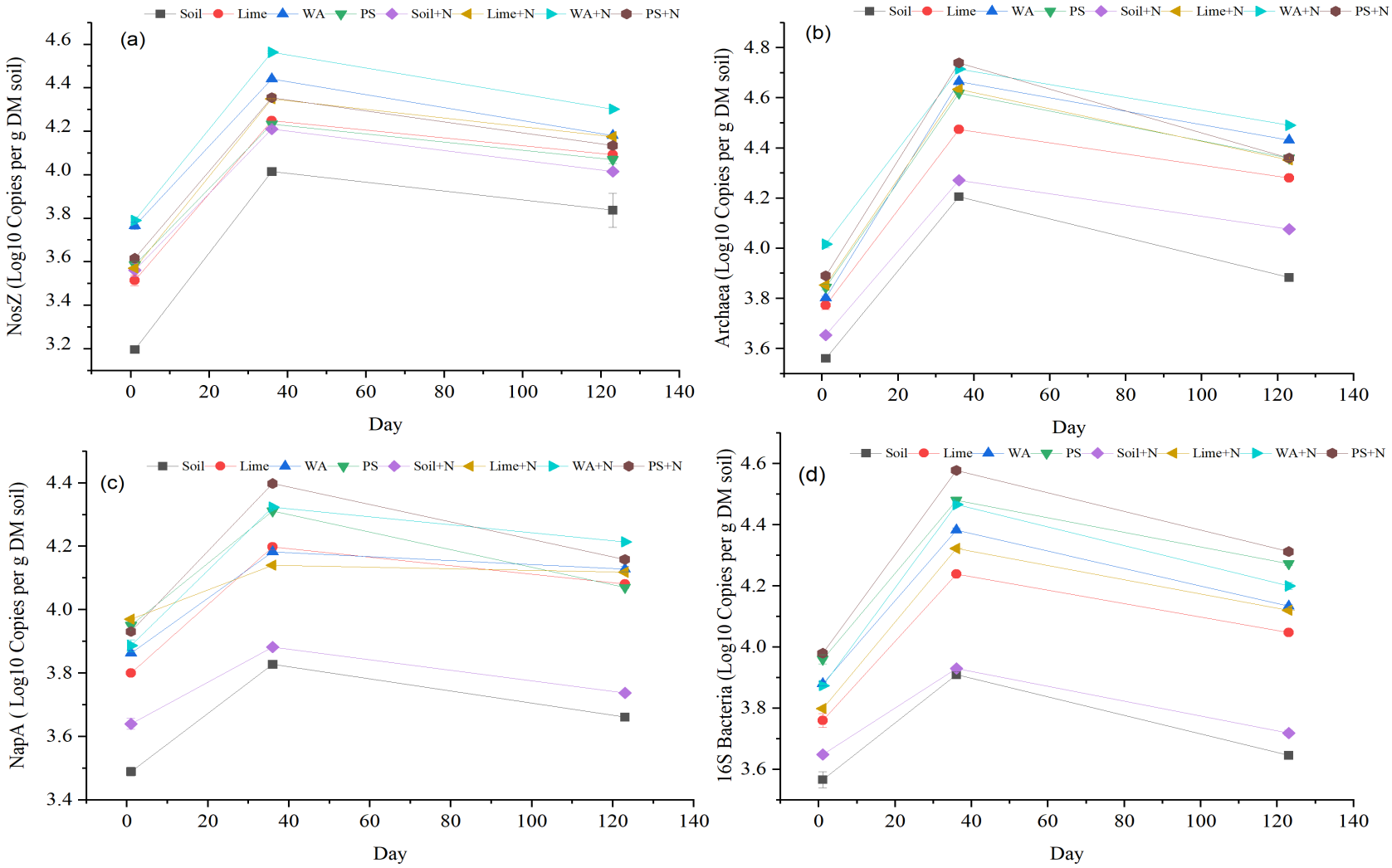

Figure 3.12 *NosZ (a), 16S-archaea (b), NapA (c), and 16S-bacteria (d) gene counts (log_{10} transformed).*

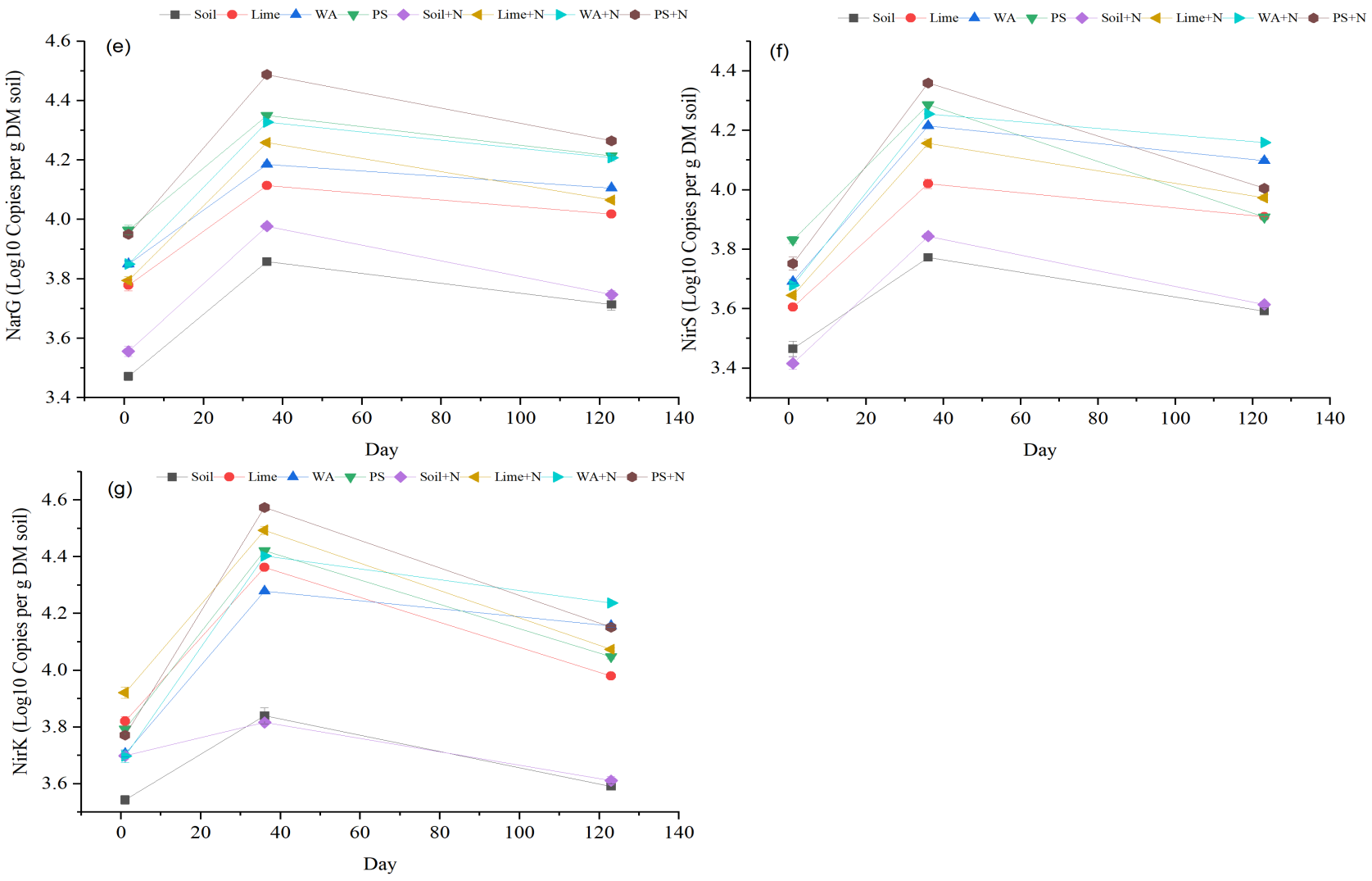

Figure 3.13 *NarG (e), NirS (f), and NirK (g)gene counts (log_{10} transformed).*

3.4.1 Relationships Between Biotic, Abiotic, and Nitrogen Cycling Genes Abundance.

Pearson's correlation matrices were performed on $delta_{36-1}$ and the dataset for day 36 to determine the relationships between biotic and abiotic characteristics on the abundance of N-cycling genes. The Pearson correlation matrices for $delta_{36-1}$ data indicated that the change in abundance of most N-cycling genes was closely correlated with change in microbial biomass, NH_4^+, NO_3^-, EC and soil organic matter (SOM) ($p \leq 0.001$) (**Table 3.7, Figure 3.14**). It also revealed that not all change in genes abundance correlated with change basal respiration. However, change in 16S bacteria, *NarG, NirS* and archaea correlated with change in soil basal respirations ($p \leq 0.05$ or < 0.01). The change in *NosZ* abundance did not show any correlation with change in microbial biomass, basal respiration, and SOM. Furthermore, using the absolute dataset, the Pearson correlation matrices indicated that the abundance of most N-cycling genes positively correlated with microbial biomass, basal respiration, pH, EC, NH_4^+, NO_3^-, and CO_2 emissions ($p \leq 0.01$ or < 0.001) (**Table 3.7**). In addition, all genes were positively correlated with each other.

Principal Component Analysis (PCA) was further performed on rate of change dataset for day 36 subtracted from day 1. Component 1 explains ≥83.92% of the variations in the N-cycling genes abundance, while component 2 explains ≥9.65% of the variation (**Figure 3.15**). Most N-cycling genes increased with sludge according to PCA analysis except for *NosZ* which increased with wood ash.

Table 3.6 *Pearson correlation matrices between biotic, abiotic parameters and N-cycling genes on delta* $_{day\ 36\text{-}1}$ *(Pearson correlation matrix α = 0.05. *P≤ 0.05, ** P≤ 0.01, ***P≤ 0.001)*

	BR mL CO_2	mg biomass	pH	EC (mS/cm)	16S Bacteria	NarG	NapA	NirS	NirK	Archaea	NosZ
16S Bacteria	0.516**	0.855***	0.234	0.652***							
NarG	0.481*	0.791***	0.145	0.618***	0.925***						
NapA	0.34	0.848***	0.174	0.462*	0.908***	0.829***					
NirS	0.571**	0.807***	0.172	0.649***	0.982***	0.918***	0.854***				
NirK	0.381	0.738***	0.425*	0.753***	0.918***	0.898***	0.789***	0.89***			
Archaea	0.477*	0.683***	0.167	0.754***	0.942***	0.84***	0.768***	0.962***	0.886***		
NosZ	0.084	0.17	-0.38	0.689***	0.592*	0.471*	0.507*	0.629***	0.493*	0.736***	
NO_3	0.502**	0.787***	0.149	0.712***	0.982***	0.917***	0.869***	0.98***	0.91***	0.954***	0.676***
NH_4	0.424*	0.749***	0.167	0.72***	0.946***	0.979***	0.823***	0.94***	0.946***	0.901***	0.582**
SOM	0.821***	0.892***	0.357	0.143	0.73***	0.75***	0.697***	0.72***	0.571**	0.473*	-0.06

Table 3.7 *Pearson correlation matrices between biotic, abiotic parameters and N-cycling genes determined on absolute data. (Pearson correlation matrix α = 0.05. *P≤ 0.05, ** P≤ 0.01, ***P≤ 0.001)*

	BR mL CO_2	mg biomass	qCO_2	$mgCO_2$-C m^2/h	$mgCH_4$- m^2/h	pH	EC (mS/cm)	16S Bacteria	NarG	NapA	NirS	NirK	Archaea	NosZ
16S Bacteria	0.955***	0.939***	-0.909***	0.738***	0.055	0.784***	0.701***							
NarG	0.944***	0.918***	-0.846***	0.744***	0.071	0.675***	0.635**	0.966***						
NapA	0.912***	0.92***	-0.921***	0.731***	0.045	0.785***	0.69***	0.973***	0.94***					
NirS	0.954***	0.927***	-0.889***	0.714***	0.077	0.791***	0.705***	0.992***	0.955***	0.945***				
NirK	0.847***	0.807***	-0.803***	0.627***	0.1	0.806***	0.739***	0.875***	0.899***	0.871***	0.861***			
Archaea	0.844***	0.781***	-0.787***	0.55**	0.074	0.861***	0.857***	0.937***	0.885***	0.892***	0.947***	0.862***		
NosZ	0.389	0.306	-0.382	0.12	0.138	0.667***	0.827***	0.583***	0.489**	0.576**	0.594**	0.46**	0.779***	
NO_3	0.91***	0.871***	-0.848***	0.685***	0.12	0.795***	0.777***	0.978***	0.959***	0.959***	0.974***	0.888***	0.962***	0.687***
NH_4	0.886***	0.841***	-0.781***	0.654***	0.095	0.679***	0.705***	0.939***	0.98***	0.912***	0.928***	0.921***	0.909***	0.571**

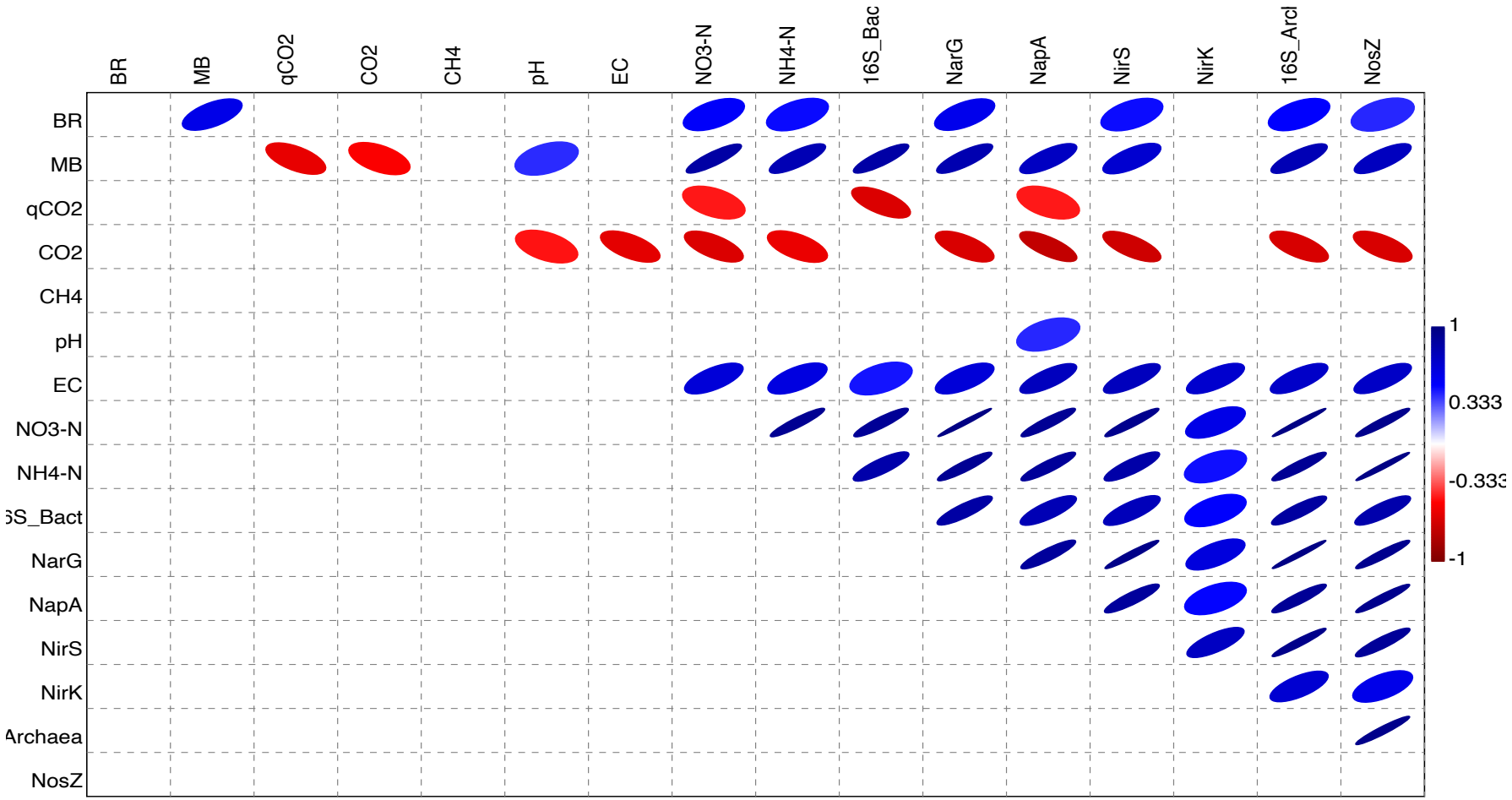

Figure 3.14 *Correlation matrices between biotic, abiotic parameters and N-cycling genes determined on delta* $_{day\ 36\text{-}1}$

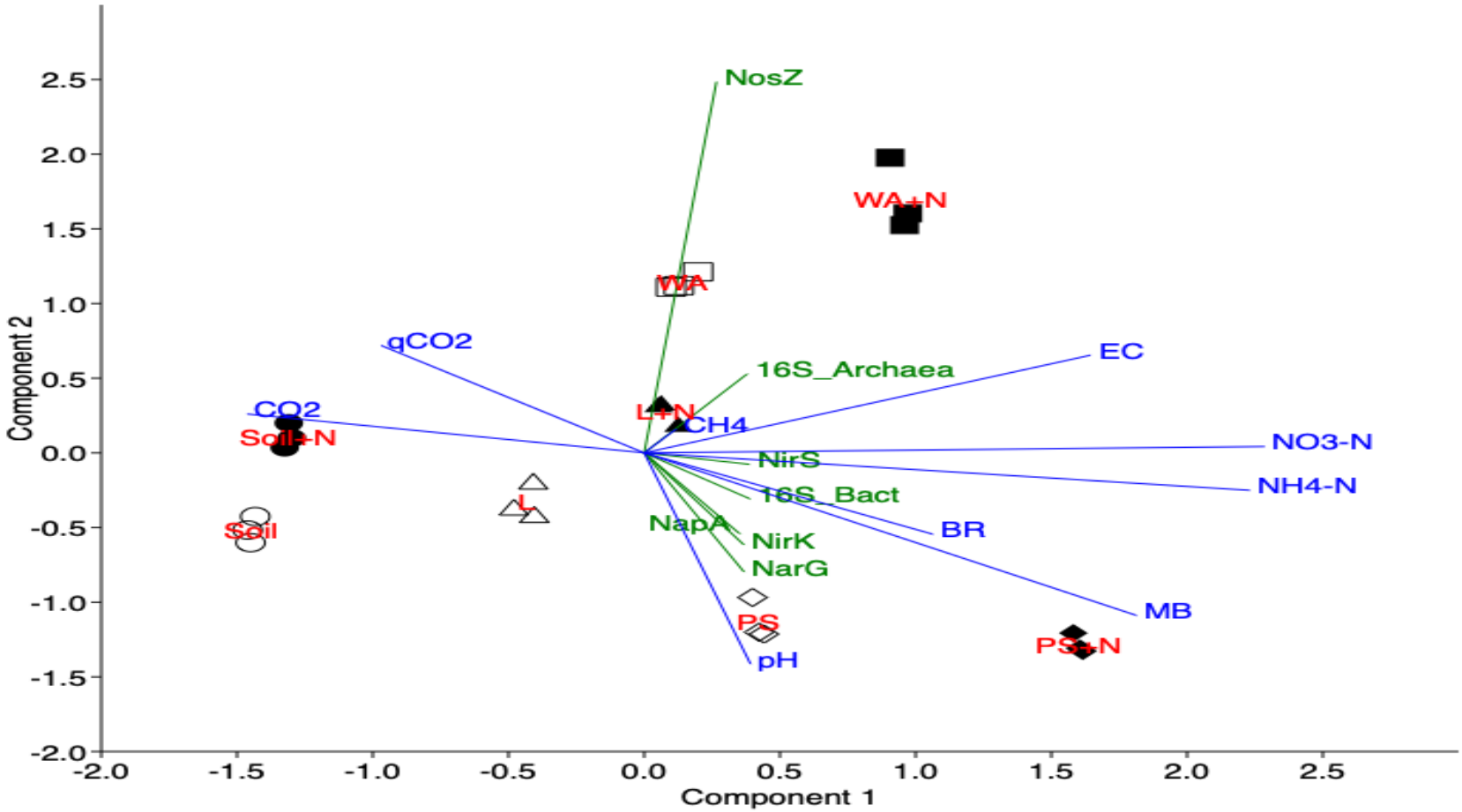

Figure 3.15 *Principal Component Analysis biplots based on rate of change from day 1 to 36 (delta$_{36-1}$) showing the effect of treatments on soil biotic and abiotic parameters.*

CHAPTER 4

DISCUSSION

4.1 Effect of Sludge, Wood Ash and Biochar on Soil Abiotic and Biotic Properties

4.1.1 Soil pH and Inorganic Nitrogen (NO_3^- and NH_4^+)

This study support previous reports (Demeyer et al. 2001; Torkashvand, 2010; Jose Antonio et al. 2014) indicating that addition of WA, PS, limestone, and biochar significantly increases the soil pH ($p = 0.05$) by 1.2–2.0, 1.0–1.7, 1.0–1.5, and 0.5–0.6 units, respectively. The oxides, carbonates and hydroxides that resulted after combustion of WA and those used in the papermaking process may have contributed to the elevated pH in soils amended with WA and PS respectively (Etiegni et al. 1991: Abdullahi et al. 2016). Adding urea led to a brief increase in soil pH, attributable to urea hydrolysis (Rochette et al. 2013); this was consistent across treatments. Hydrolysis of urea often results to the production of NH_3 and CO_2 as by-products which is facilitated by urease enzymes (Cabrera et al. 1991). The NH_3 produced during urea hydrolysis alkalinizes the soil medium and thus increase the soil pH. It was hypothesized that wood ash will be a stronger liming agent compared to paper sludge. As such, by the end of the incubation all treatments, except for WA, showed a reduction in pH. The exact cause has not been determined, but fast mineralization and NH_4^+ removal from the soil, either through losses of nitrification, could be a contributing component (Xu et al. 2006). This was supported by significant correlations between pH and soil inorganic nitrogen (NO_3^- and NH_4^+) ($p \leq 0.001$; Table 4.4). In this study, the predominant pool of mineral N was NH_4^+ persisting throughout the entire incubation period; this may further support speculations on the mechanisms for pH shifts. On the other hand, Dowdy et al. (1991) discovered that the decline in soil pH following the addition of sewage sludge might be attributed to the impacts of organic acids created during sludge decomposition. In addition, aerobic

biological activity also generates CO_2, which reacts with water to form carbonic acid may also have contributed to the decline in pH seen in this study. In general, this study showed that WA may be used as a good source of liming material as it reacts faster and may have a long-lasting effect in the soil. However, future research is needed to be conducted over a long period of time to assess the long-lasting effect of WA in boreal soil as my experiment was conducted over a short period of time.

N mineralization and immobilisation are important processes in N cycle. The process of converting organic N to inorganic N is known as nitrogen mineralization, and the process of converting inorganic N to organic N is known as nitrogen immobilisation (Alexander, 1977). Both activities occur at the same time in soil, with the magnitudes determining if the overall result is net N mineralization or net N immobilisation. Plants make use of mainly inorganic forms of N (NO_3^- and NH_4^+) for their growths i.e incorporation of N into plant macromolecules such as protein and nucleic acids, which are needed to produce more plant biomass. The result supports the hypothesis that addition of urea to WA and PS increases accumulation of net mineral N but variable across amendment treatments as I observed that urea addition increased putative net mineralization, calculated as the difference between the soil mineral N after last day sampling period and first sampling time across all treatments compared to when amendments are used alone. This can be a combination of the treatments' capacity to both initially fix N and then eventually mineralize newly formed organic matter, or due to a net priming effect. Such an increase might occur when substrates are N limited. Zhang et al. (2008) deduced that application of carbon without enough N or application of N without carbon may cause soil microorganisms to be deficient in nitrogen or carbon. Alternatively, increasing availability of nitrogen and carbon may promote microbiological activity for prolonged periods. Organic nitrogen can only be mineralized in a biologically

favourable environments with optimal pH, C:N ratio, moisture and temperature. Furthermore, in this study there were significant correlations between MB and soil inorganic nitrogen (NO_3^- and NH_4^+) ($p \leq 0.001$). Addition of PS and WA, even in the absence of urea, significantly increased soil NO_3^- and NH_4^+; this was more significant in PS amended soil likely due to increased mineralization of PS organic matter (Metzger and Yaron, 1987). Weber *et al*. (1985) also reported that wood ash can lead to a significant increase in net N mineralization. Organic wastes of various sources do commonly lead to increased net nitrogen mineralization and nitrogen availability to crops absorption (Paul and Beauchamp, 1996; Cordovil et al. 2007). Thus, nitrogen immobilization associated with carbon rich sludge application was not notable in this study. Application of sludge with C:N ratio >20 limits the buildup of soil NO_3^- (N'Dayegamiya et al. 2004).

Furthermore, co-application of biochar with paper sludge showed no significant increase in net N mineralization when urea was added i.e *PS+B+N versus PS+N*; but, in the absence of urea the increase was statistically different from PS only *i.e PS+B versus PB*. This contrasts with the findings of Manirakiza et al. (2019) who reported significant decrease in the NO_3^-, NH_4^+, and net N mineralization following co-application of biochar (produced at a pyrolysis temperature of 700 °C) and paper sludge. Biochar produced at higher temperatures is typically characterised by a larger surface area, high CEC, and high porosity (Xu et al. 2012; Yue et al. 2016), which can improve biochar adsorption capacity (Xu et al. 2012). A stronger adsorption of NH_4^+ on biochar particles can decrease net mineralization (Manirakiza et al. 2019). Biochar used in this study was produced at a pyrolysis temperature of 500 °C. Despite this, there was a considerable reduction in NO_3^-, NH_4^+, and net N mineralization when biochar and lime were combined *(L+N versus L+B+N OR L versus L+B)*, either alone or with urea, as well as WA and biochar with urea *(WA+N versus*

WA+B+N). This suggests that combining lime or WA with stable carbon, like biochar, could help increase carbon sequestration while preventing nutrient leaching in soil.

Net mineralization in PS and WA treated soils as seen in this study was mainly pH dependent. Net mineralization was significantly reduced in soils that are acidic (i.e., 4 vs > 5.6). This may be due to reduced microbial activity at low pH, which slows the net mineralization process. A rapid accumulation of mineral nitrogen was seen when the pH was elevated. The substantial relationships between soil inorganic nitrogen (NO_3 and NH_4), microbial biomass and pH revealed in this study ($p = 0.001$) support this. Mineralization in PS and WA-treated soil were strongly influenced by the C:N ratio, soil pH, and nutrient availability.

4.1.3 Microbial Biomass, Basal Respiration and Metabolic Quotient (qCO_2)

In a number of terrestrial settings, measures of MB have been employed in investigations of soil organic matter (SOM) dynamics and nutrient cycling. Soil MB quantifies the amount of live MB in the soil which often accounts for approximately 1 to 5% of total SOM in arable soils (Jenkinson 1988; Smith and Paul 1990). The soil MB as used in this study provides an "early warning" of the effects of stresses on the soil ecosystem, before they are apparent by other metrics, due to its sensitivity to changing soil conditions (Aceves et al. 1999). Another significant indication for determining changes in soil health or quality is soil basal respiration (BR). Basal respiration accounts for the constant rate of respiration in soil caused by the mineralization of organic matter (Pell et al. 2006) and is often measured using CO_2 production or O_2 consumption (Dilly and Zyakun, 2008). There was a strong positive correlation between soil microbial biomass, basal respirations, and pH ($p \leq 0.001$). When a microbial community runs inefficiently it diverts a larger proportion of carbon to maintenance requirements than to biosynthesis and thus the metabolic quotient rises. Such factors affecting qCO_2 includes soil acidic (Blagodatskaya and

Anderson, 1999), heavy metal concentrations (Blagodatskaya et al. 2008), and soil temperature (Wardle and Ghani, 1995). The metabolic quotient (qCO_2) was strongly and negatively correlated with microbial biomass and pH; the increase in pH and MB has been previously reported to be associated with a decrease in qCO_2 (Wardle and Ghani, 1995). Wood ash significantly increased soil MB and respiration (Baath and Arnebrant 1994; Fritze et al. 2000; Perkiomaki and Fritze, 2002; Zimmermann and Frey, 2002). The increase in respiration is commonly due to increased mineralization rates and nutrient cycling and may be an indicator of stress response and inefficient carbon assimilation (Anderson, 1994; Wardle and Ghani, 1995). Manirakiza et al. (2019) also reported an increase in MB following PS application. However, they also reported that MB was further increased when biochar and PS were co-applied. In my trials biochar supplementation led to a short-lived peak in MB, around day 36, but MB eventually decreased towards the end of the incubation to levels similar to the no-biochar treatments (**Figure 3.6, Tables S1, S2, S3**). It is unclear if this was due to any labile carbon in biochar that might be used as substrate by the soil microbes (Farrell et al. 2013; Gomez et al. 2014; Khodadad et al. 2011). Nevertheless, such labile biochar compounds may be rapidly mineralized (Cheng et al. 2006), and thus having only a short-term impact that waned by the end of the incubation. Furthermore, at the end of the incubation the MB in *Biochar* and *Soil* were not significantly different. The highest MB and BR were measured in sludge treated soil **(Figure 3.6, 3.7, Tables S1, S2, S3**), the only amendment that provided significant degradable organic matter known to facilitate accelerated MB accumulation, including the MB added with the PS (N'Dayegamiya et al. 2004; Tripathy et al. 2008). However, the metabolic quotient in PS amended treatments decreased significantly with time, an indication of more efficient carbon sequestrations by the MB. Wood ash had an intermediate effect: at the end of the incubation, there was a slight increase in the metabolic quotient in the *Soil*, *L*, *L+N*, *B*, *B+N*,

L+B, L+B+N treatments. This may be correlated to the associated decrease in pH. In addition, the MB and qCO_2 data generated in this study are moderately representative of values reported by Zimmermann and Frey (2002).

4.1.4 Greenhouse Gases Emissions (CO_2 and CH_4)

Except for the negative control *(Soil only)*, all soils amended had high CO_2 emissions during the early stage of the incubation period (Fidel et al. 2019 and Sarfaraz et al. 2020) suggesting that microbial biomass is rapidly increasing and utilizing the easily decomposable soil organic carbon (Rochette et al. 2006). The CO_2 emissions positively correlated with pH, MB, NO_3^- and NH_4^+ ($p < 0.01$). Wood ash, PS and limestone increased CO_2 emissions compared to soil control. However, there was a significant decrease in CO_2 emissions when biochar was used alone or in combination with *PS* or limestone. The result of this study supports the hypothesis that *PS* will contribute significantly to GHG emissions compared to WA due to mineralization of sludge organic matter. The highest CO_2 fluxes were reported in *PS* amended soils. *PS and PS+N* increased soil cumulative CO_2 emissions by 84% compared to the unamended soil. Biosolids do enhance CO_2 emissions by supplying decomposable carbon to boost microbial respiration and in turn may increase denitrification and thus N_2O emissions by reducing the quantity of oxygen available in the soil (Zhu-Barker et al. 2015).

In general biochar did reduce the CO_2 losses except in *WA+B* and *WA+B+N*. Adding biochar with paper sludge (*PS+B*) reduced cumulative CO_2 emissions by 35% versus *PS*, similar to previous reports of the utility of biochar in reducing sludge and biosolids associated respiratory losses (Lu et al. 2020; Robbie et al. 2014). Biochar addition to soil (*B*) reduced cumulative CO_2 emissions by 39.51%.

Addition of biochar with limestone alone or with urea (*L* or *L+N*) reduced limestone cumulative CO_2 emissions by 23.97% and 26.97% respectively, similar to previous reports of the impacts of rice husk biochar in reducing limestone associated respiratory losses (Mosharrof et al. 2021). However, Mosharrof et al. (2021) also reported increased CO_2 emissions after limestone and biochar derived from oil palm empty bunches were co-applied.

Wood ash addition increased soil cumulative CO_2 emissions by 45% compared to soil control, which contradicts previous studies which reported that wood ash did reduce CO_2 emissions in both acidic and alkaline soils (Zhao et al. 2021). Furthermore, adding biochar to wood ash increased cumulative CO_2 emissions by 63.98% compared to when used alone, suggesting that the increased associated respiratory losses may be related to biochar feedstock not suitable when co-applied with wood ash. Thus, there is a need for further research to investigate the impact of various biochar feedstocks on soil respiratory losses after wood ash addition to boreal soils.

The labile carbon fraction sorption found on the surface or between the pore space of biochar may be the reason for biochar's ability to reduce the soil respiratory losses (Lehmann et al. 2011; Cross et al. 2011) in paper sludge and limestone amended soils. Overall, a positive priming effect, which happens after organic waste or chemical fertilizer application to soil promote decomposition of SOM and mineralization, resulting in an increased respiratory loss (Chen et al. 2014; Fiorentino et al. 2019), might be related to the CO_2 emissions found in my study. The positive correlations between CO_2 emissions and pH, microbial biomass, NO_3^- and NH_4^+ support this.

I found no significant differences in cumulative CH_4 emissions across all treatments (**Figure 3.10, Table 3.3**), similar to previous reports which found no significant difference in CH_4 emissions using urea, urea in conjunction with charcoal, wastewater sludge alone and sludge in

conjunction with charcoal at application rate of 150 kg N ha^{-1} (Diaz-Rojas et al. 2014). Anaerobic conditions are required for production of CH_4 emissions (Willen et al. 2016; Brachmann et al. 2020) which were not present in my study, as the soils during the incubation was at 60% WHC. High moisture content which may create anoxic conditions in the soil, and thus stimulate the production of CH_4 (Yoshida et al. 2015) may be attributed to the increase CH_4 fluxes reported in *L*, *B*, *WA*, and *WA+B* amended soil in the early stage of incubation. In contrast, this situation was unlikely to be true as their moisture content were much lower than soil amended with paper sludge.

4.2 Effect of Treatments on the Abundance Soil Nitrogen Cycling Genes

N-cycling is a complex biogeochemical cycle including a variety of nitrogen transformation activities such as nitrogen fixation, nitrification, and denitrification (Kuypers et al. 2018; Stein and Klotz, 2016), all of which are primarily driven by microorganisms. The primary processes contributing to nitrogen losses in soil via nitrate leaching and nitrogen off gassing are nitrification and denitrification (Philippot et al. 2007; Norton and Stark, 2011). The effect of WA and PS on microbially-mediated N-cycling processes such as nitrogen fixation, nitrification and denitrification have not been extensively studied as there is limited information available.

Urea increased the abundance of all denitrifying genes. Urea addition to soil alters biological mediated processes that are crucial to the N cycle and transformations (Paul and Beaucham, 1996; Raiesi, 2004; Zhang et al. 2008). Paper sludge significantly enhanced the counts of bacterial 16S and all denitrifying genes (*narG, napA, nirS, nirK*) when compared to other treatments and soil control. However, *nosZ* was found in greater abundance in WA amended soil. In comparison to limestone, WA significantly increased 16S bacteria, archaea, and all denitrifying genes, whereas soil without amendment had the lowest denitrifying genes. The overall increase in abundance of denitrifying genes may be correlated to increased soil pH. Previous studies have reported soil pH to significantly impact the abundance of N cycling genes (Hallin et al. 2009; Hu et al. 2013; Liu et al. 2010; Prosser and Nicol, 2012). However, some studies have found no influence of pH on denitrifying populations (Kandeler et al. 2006). The highest abundance of 16S bacteria and most denitrifying genes were measured in PS amended soils (**Figure 3.12, 3.13**), the only amendments that provided significant degradable organic carbon known to facilitate abundance of N-cycling genes (Bru et al. 2011; Hallin et al. 2009; Prosser and Nicol, 2012; Wang et al. 2017). Moreover, many microbial groups involved in the N cycle are heterotrophic and the

addition of organic matter might supply abundant organic carbon that is favorable to their growth (Chen et al. 2012).

The strong positive correlation between pH, organic matter, respiration, microbial biomass, and genes involved in denitrification process support the hypothesis that when microbial biomass increases, the number of microorganisms participating in N-cycling would likely rise and thus correlate with soil respiration, particularly for heterotrophs like denitrifying bacteria. However, no correlation was found between *nosZ* and SOM. This is distinct from published reports that suggest that *nosZ* abundance does correlate to soil organic matter (Kandeler et al. 2006; Tao et al. 2022). This could be because SOM is only one parameter that affected *nosZ* abundance in this study, and the diverse chemistries across the various amendments might have imposed other selective stresses independent on the SOM. For example, the highest *nosZ* counts were measured in WA amended soils (**Figure 3.12**); it is unclear if this was due to presence of heavy metals which enhance their growth (Ke Tan et al. 2021). Furthermore, abundance of denitrifying genes positively correlated with NO_3^- contents, in contrast to previous studies (Attard et al. 2011; Morales et al. 2010; Throback et al. 2004). However, the correlation found suggests that NO_3^- concentration might contribute to denitrifying genes abundance rather than activities that reduce nitrates itself.

The abundance of *nirK* denitrifiers might be more crucial to denitrification process compared to *nirS* denitrifiers because *nirK* counts were greater than *nirS* abundance across all treatments. Both *nirK* and *nirS* are vital genes for reducing nitrite to nitric oxide and are considered as key genes for quantifying denitrification in soil (Braker 2000; Kuypers et al. 2018). Several studies have found higher number *nirK* than *nirS* (Chen et al. 2010; Dandie et al. 2011); nevertheless, greater number of *nirS* than *nirK* have also been observed (Attard et al. 2011), and in a study done on boreal forest soils (Petersen et al. 2012). An increase in SOM and thus organic

carbon has been the most important factor connected to *nirK* abundance (Kandeler et al. (2006) suggesting that PS application in agricultural soils may provide a perfect environment for *nirK* abundance.

At the end of the incubation, there was decrease in denitrifying genes abundance in *PS*, *PS+N, L, L+N* compared to *WA* and *WA+N*. This may be attributed to decrease in pH. Moreover, a meta-analysis conducted on agricultural lands revealed that inorganic or organic fertilisers have less of an impact on N cycling microbes in soils with pH less than 6 compared to soils with pH greater 6 (Ouyang *et al*., 2018). Overall, the abundance of *narG, napA, nirS, nirK* and *nosZ* increases with increase in pH, organic matter (except *nosZ*), EC, NO_3^- and NH_4^+. Soil conditions and features including SOM, NO_3^-, feedstock supply and type (organic carbon and if available in labile form), pH, and nitrate impact both the mitigation and emission of N_2O (Borno et al. (2020).

The high abundance of *nosZ* in WA amended soil may further allow us to hypothesize that wood ash could be a suitable amendment for the mitigation of N_2O emissions. However, it is crucial to highlight that the abundance of *narG, napA, nirK, nirS*, or *nosZ* genes does not imply that the gene with the highest abundance increased denitrification more than the other during our incubation. As a result, further research integrating functional gene expression metrics with microbial community and composition studies will offer more information on the abundance of nitrogen cycling genes with denitrifying activity.

CHAPTER 5

CONCLUSION

Overall, wood ash and paper sludge are good liming materials for boreal soils. Wood ash increased soil pH significantly more than paper sludge. This indicates wood ash may have a long last effect ameliorating acidity in boreal soils compared to paper sludge, but further research is needed to investigate their long-lasting effect on boreal soil pH as this incubation study was conducted over short period of time. Wood ash and paper sludge generally increased the soil microbial biomass and respiration which in turn led to increase in net mineralization. However, co-application of inorganic nitrogen (N) such as urea to wood ash or paper sludge improved soil microbial activity resulting in higher accumulation of net mineral N. The highest net mineralization was measured in sludge amended soils. This indicated that mineralization of sludge organic matter might supply N at rates that might overcome the N uptake by plants, and eventual lead to greenhouse gas (GHG) losses. This was confirmed as the highest respiratory losses were found in PS amended soils and likely linked to a positive priming effect on organic matter degradation due to addition of PS. Wood ash also contributed significantly to GHG emissions (CO_2 emissions) and also attributed to increase in microbial activity on organic matter degradation.

In general, biochar reduced CO_2 emissions from paper sludge and limestone treatments but significantly increased CO_2 fluxes when co-applied with wood ash. This suggests a variable impact of biochar likely both retaining charged compounds but also facilitating GHG producing microbial activities. Further research is needed to investigate suitability of biochar's of variable feedstocks, and the combination of biochar and organic or mineral amendments for reducing CO_2 fluxes in boreal soils.

The change in abundance of N functional genes positively correlated with soil basal respiration, a result indicating changes in microbial biomass. Paper sludge significantly increased the abundance of bacterial 16S, *napA, narG, nirK and nirS* at pH 6 compared to other treatments. However, *nosZ* was more abundant in wood ash amended soil suggesting that wood ash addition in boreal soil may be a way to alleviate N_2O emissions, by facilitating full denitrification to dinitrogen (N_2). The overall increase in gene counts suggests that pH plays a significant role in improving abundance of N-cycling genes. Other factors impacting N cycling gene abundance includes soil organic matter and inorganic N availability. Overall, the N-cycling genes were positively corelated with soil nitrate and ammonium. Future studies integrating functional gene expression metrics with microbial community diversity investigations will provide greater insight into the relationship between the abundance of nitrogen cycling genes and related, measurable denitrifying activity. In conclusion, future study is necessary to elucidate the effects of these amendments in boreal field condition over a long period of time as it will provide more robust understanding as to how these amendments will respond under ideal field conditions.

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SUPPLEMENTARY

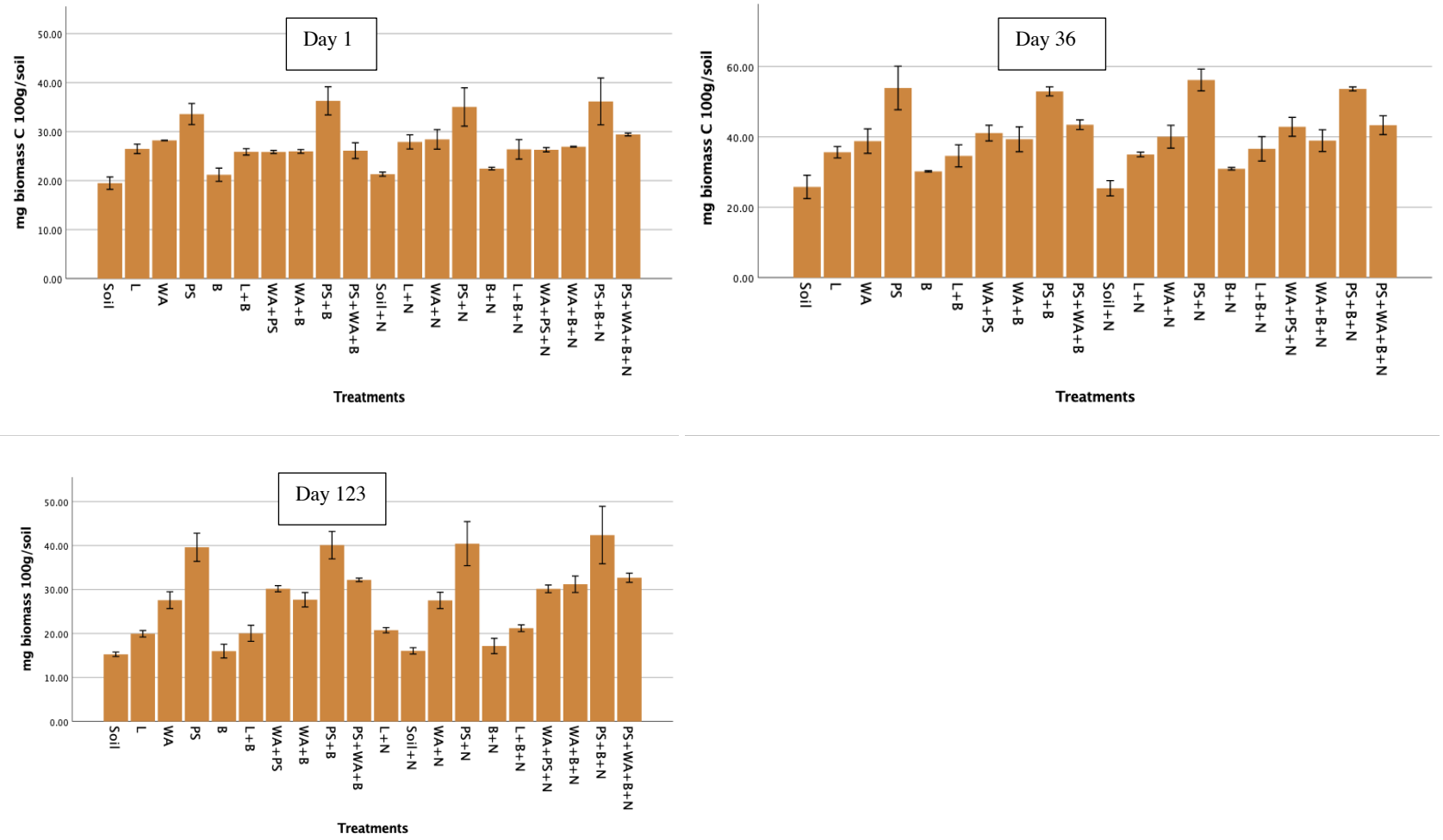

Figure S1: Mean bar chart for microbial biomass (per 100g dry soil; mean and CI95 (error bars); details, including post-hoc analysis in the Supplementary data, Table S1, S2, and S3. CI: Confidence interval

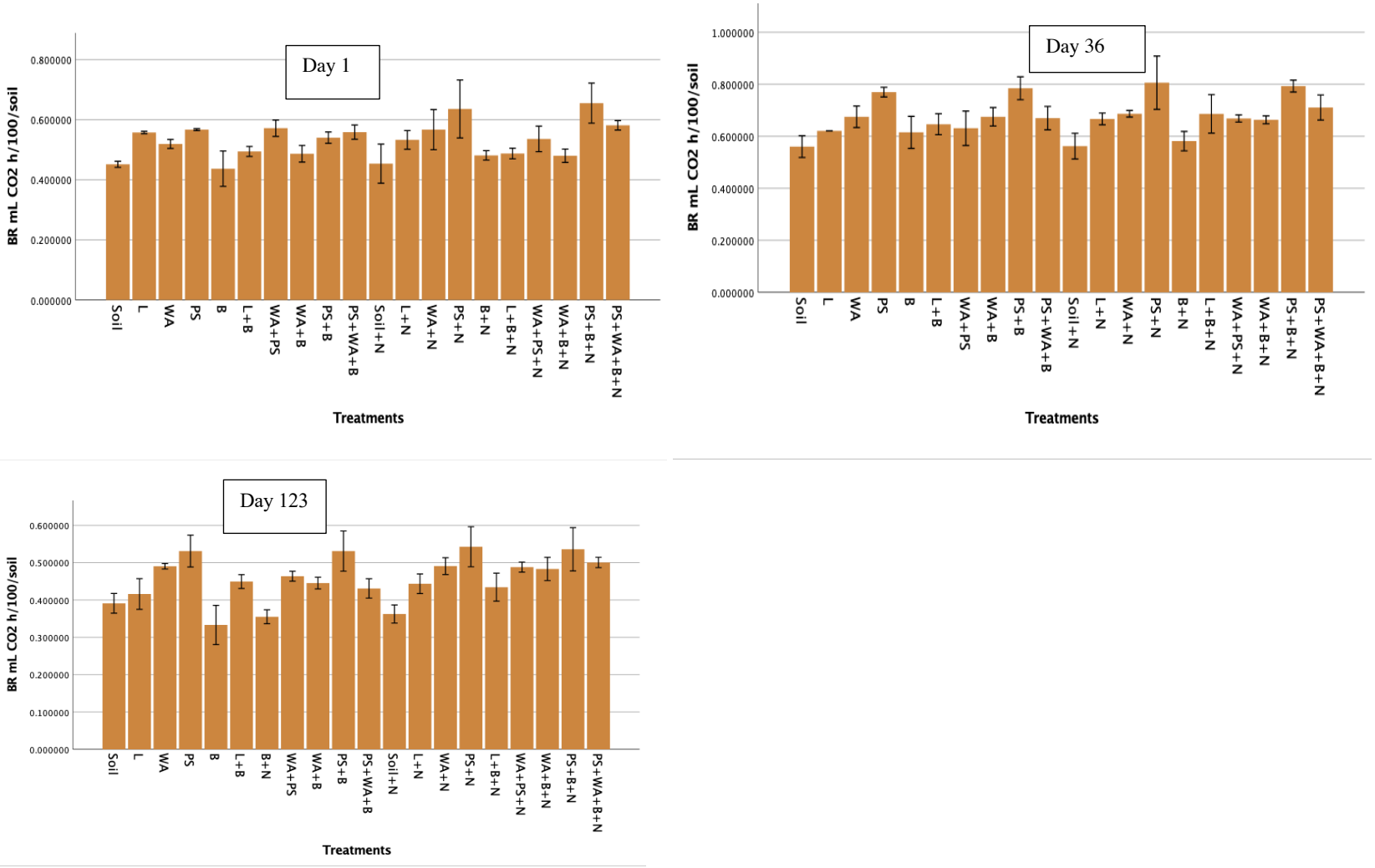

Figure S2: Mean bar chart for basal respiration (per 100g dry soil; mean and CI95 (error bars); details, including post-hoc analysis in the Supplementary data, Table S1, S2, and S3. CI: Confidence interval

Table S1: Effects of treatments on measured biotic and abiotic parameters for Day 1; Letters are post-hoc Tukey test results $\alpha = 0.05$. Means that do not share a letter are significantly different.

Treatments	BR mL CO2 h/100/soil*	mg biomass C 100g/soil*	qCO2*	NO3-N mg/kg soil*	NH4-N mg/kg soil*	pH**	EC (mS/cm)**
Soil	0.4516 gh	19.49 h	0.023054 a	0.45 k	1.01 j	4.73 a	0.2 a
L	0.5576 bc	26.49 def	0.021088 bcd	0.91 i	1.92 h	5.7 c	0.3 abc
WA	0.5195 cdef	28.22 cde	0.018535 efgh	1.04 h	2.54 g	6.17 hij	0.4 efg
PS	0.5670 bc	33.60 b	0.016862 h	1.47 e	3.8d e	5.77 cd	0.3 abcd
B	0.4372 h	21.21gh	0.021284 abc	0.68 j	1.92 h	5.13 b	0.23 ab
L+B	0.4945 defg	25.88 f	0.018957 efg	1.05 h	1.83 h	5.93 efgh	0.3 bcde
WA+PS	0.5718 b	25.86 f	0.022359 ab	1.38 ef	2.97 f	6.07 fghi	0.33 bcdefg
WA+B	0.4868 efgh	25.97 ef	0.018497 efgh	1.08 h	2.88 f	6.23 ijk	0.4 cdefg
PS+B	0.5404 bcd	36.29 a	0.014776 i	1.61 d	3.65 e	5.9 defg	0.3 bcd
PS+WA+B	0.5589 bc	26.13 def	0.021616 abc	1.19 g	2.54 g	6.07 ghij	0.33 bcdefg
Soil+N	0.4539 gh	21.33 gh	0.021997 ab	0.56 k	1.33 i	5.2 b	0.23 ab
L+N	0.5229 bcde	27.91 cdef	0.019356 def	1.33 f	3.65 e	5.87 def	0.3 abc
WA+N	0.5670 bc	28.42 cd	0.019393 def	1.76 bc	4.18 c	6.37jk	0.4 defg
PS+N	0.6359 a	35.04 ab	0.017496 gh	1.83 ab	5.65 a	5.83 cde	0.3 bcdef
B+N	0.4814 fgh	22.47 g	0.02159 abc	0.87 i	2.87 f	5.27 b	0.27 abc
L+B+N	0.4876 efgh	26.39 def	0.018641 efgh	1.41 ef	3.74 de	5.93 efg	0.33 bcdefg
WA+PS+N	0.5362 bcde	26.31 def	0.020004 cde	1.71 cd	3.66 e	6.17 hij	0.37 bcdefg
WA+ B+N	0.4802 fgh	26.93 def	0.017639 fgh	1.71 cd	3.97 cd	6.4 k	0.47 g
PS+B+N	0.6553 a	36.17 a	0.017698 fgh	1.79 bc	5.63 a	5.93 defg	0.33 bcdefg
PS+WA+B+N	0.5815 b	29.43 c	0.019885 cde	1.9 a	4.63 b	6.17	0.43 fg

**Kruskal-Wallis performed on unstandardized data

*One way ANOVA and Turkey test

Table S2: Effects of treatments on measured biotic and abiotic parameters for Day 36; Letters are post-hoc Tukey test results α = 0.05. Means that do not share a letter are significantly different.

Treatments	BR mL CO2 h/100/soil*	mg biomass C 100g/soil*	qCO2*	NO3-N mg/kg soil*	NH4-N mg/kg soil*	pH**	EC (mS/cm)**
Soil	0.5604 g	25.79 h	0.021744 a	3.50 l	3.94 j	4.73 a	0.2 a
L	0.6209 def	35.65 ef	0.017418 cde	6.78 j	8.11 h	5.97 efg	0.5 bcde
WA	0.6752 bcd	38.81 cde	0.017401 cde	8.56 i	9.69 g	6.2 i	0.6 bcdef
PS	0.7699 a	53.91 a	0.014306 h	10.34 f	13.86 e	6.0 efgh	0.5 bcd
B	0.6152 efg	30.21 g	0.020362 ab	4.64 k	7.63 h	5.03 c	0.27 a
L+B	0.6465 cdef	34.63 f	0.018676 bcd	6.33 j	7.47 h	5.93 ef	0.53 bcdef
WA+PS	0.6309 cdef	41.08 bc	0.015353 fgh	8.77 hi	11.83 f	6.13 ghi	0.57 bcdef
WA+B	0.6750 bcd	39.34 cd	0.017181 cdef	9.03 ghi	11.20 f	6.17 hi	0.77 efg
PS+B	0.7848 a	52.94 a	0.014827 gh	10.9 def	14.20 e	6.03 efghi	0.67 cdefg
PS+WA+B	0.6699 bcde	43.48 b	0.015413 fgh	9.48 g	9.86 g	6.03 efghi	0.67 cdefg
Soil+N	0.5604 g	25.41 h	0.02216 a	4.68 k	5.15 i	4.9 b	0.3 ab
L+N	0.6669 bcde	35.02 f	0.019046 bc	8.83 ghi	14.17 e	6.0 efghi	0.67 cdefg
WA+N	0.6867 bc	40.05 bcd	0.017157 cdef	11.53 cd	16.23 c	6.03 efghi	0.73 defg
PS+N	0.8062 a	56.18 a	0.014366 h	12.75 b	21.95 a	5.97 efg	0.57 bcde
B+N	0.5814fg	30.96 g	0.018779 bcd	6.55 j	11.14 f	5.06 cd	0.4 bc
L+B+N	0.6863 bc	36.61 def	0.018744 bcd	9.24 gh	14.55 de	5.97 efg	0.7 defg
WA+PS+N	0.6685 bcde	42.87 b	0.015597 efgh	11.04 de	14.23 e	5.97 efg	0.63 cdef
WA+ B+N	0.6633 bcde	38.96 cde	0.017036 def	10.75 ef	15.46 cd	6.13 fghi	0.93 g
PS+B+N	0.7933 a	53.64 a	0.01479 gh	13.45 a	21.87 a	6.0 efgh	0.63 cdefg
PS+WA+B+N	0.7106 b	43.34 b	0.016394 efg	11.82 c	18.00 b	6.07 efghi	0.9 fg

**Kruskal-Wallis performed on unstandardized data

*One way ANOVA and Turkey test

Table S3: Effects of treatments on measured biotic and abiotic parameters for Day 123; Letters are post-hoc Tukey test results $\alpha = 0.05$. Means that do not share a letter are significantly different.

Treatments	BR mL CO2 h/100/soil*	mg biomass C 100g/soil*	qCO2*	NO3-N mg/kg soil*	NH4-N mg/kg soil*	pH**	EC (mS/cm)**
Soil	0.3912 ij	15.28 f	0.025598 a	4.25 l	4.95 j	4.0 a	0.37 ab
L	0.4163 hi	19.95 de	0.020863 b	8.22 j	10.18 h	5.63 d	0.5 c
WA	0.4906 cde	27.60 c	0.177785 c	10.38 i	12.17 g	6.03 j	0.6 d
PS	0.5313 abc	39.62 a	0.013417 efg	12.54 f	17.41 e	5.83 fghi	0.5 c
B	0.3333 k	16.01 f	0.02086 b	5.62 k	9.58 h	4.6 c	0.3 a
L+B	0.4497 efgh	20.05 de	0.022443 b	7.67 j	9.37 h	5.80 efgh	6 c
WA+PS	0.4638 defg	30.19 bc	0.015358 def	10.64 hi	14.86 f	5.93 hij	0.4 ab
WA+B	0.4455 fgh	27.70 c	0.016085 cd	10.95 ghi	14.07 f	6.0 ij	0.6 d
PS+B	0.5312 abc	40.11 a	0.013257 fg	13.22 def	17.84 e	5.73 defg	0.4 b
PS+WA+B	0.4311 ghi	32.21 b	0.013382 efg	11.50 g	12.38 g	5.93 ghij	0.7 e
Soil+N	0.3626 jk	16.08 f	0.022562 b	5.67 k	6.47 i	4.1 b	0.5 c
L+N	0.4437 fgh	20.77 d	0.021364 b	10.71 ghi	17.80 e	5.63 de	0.6 d
WA+N	0.4910 cde	27.54 c	0.017827 c	13.99 cd	20.38 c	6.03 j	0.7 e
PS+N	0.5428 a	40.45 a	0.013424 efg	15.46 b	27.56 a	5.8 fgh	0.5 c
B+N	0.3552 jk	17.17 ef	0.020713 b	7.95 j	13.99 f	4.63 c	0.5 c
L+B+N	0.4345 gh	21.22 d	0.020487 b	11.21 gh	18.28 de	5.73 def	0.7 e
WA+PS+N	0.4883 de	30.17 bc	0.016185 cd	13.92 de	17.87 e	5.93 hij	0.5 c
WA+ B+N	0.4834 def	31.21 b	0.15486 de	13.04 ef	19.38 cd	6.03 j	0.8 f
PS+B+N	0.536 ab	42.4 a	0.012653 g	16.32 a	27.46 a	5.83 fgh	0.5 c
PS+WA+B+N	0.5007 bcd	32.69 b	0.015318 def	14.33 c	22.61 b	5.83 fgh	0.7 e

**Kruskal-Wallis performed on unstandardized data

*One way ANOVA and Turkey test

Table S4: GLM ANOVA for rate of change from day 1 to 36 parameters (calculated as delta change in day 36 from day 1) with $\alpha = 0.05$ results for biotic parameters.

	Source	DF	F-value	P	R-Sq (%)
BR mL CO2 h/100/soil	pH	1	0.758	0.388	26.30
	EC mS/cm	1	4.255	0.044	
	Biochar	1	3.96	0.052	
	Lime	1	4.491	0.039	
	Wood Ash	1	3.565	0.065	
	Paper Sludge	1	0.884	0.351	
	Urea N	1	0.196	0.66	
	Error	52			
	Total	60			
mg biomass C 100g/soil	pH	1	5.114	0.028	74.10
	EC mS/cm	1	0.405	0.527	
	Biochar	1	0.882	0.352	
	Lime	1	2.677	0.108	
	Wood Ash	1	1.721	0.195	
	Paper Sludge	1	43.018	< 0.001	
	Urea N	1	0.337	0.564	
	Error	52			
	Total	60			
qCO2	pH	1	0.058	0.811	57.5
	EC mS/cm	1	10.103	0.002	
	Biochar	1	0.12	0.731	
	Lime	1	4.461	0.039	
	Wood Ash	1	19.581	< 0.001	
	Paper Sludge	1	28.664	< 0.001	
	Urea N	1	0.035	0.852	
	Error	52			
	Total	60			
mgCO2-C m2/h	pH	1	2.783	0.101	24.20
	EC mS/cm	1	0.256	0.615	
	Biochar	1	2.606	0.113	
	Lime	1	1.011	0.319	
	Wood Ash	1	0.419	0.52	
	Paper Sludge	1	0.159	0.691	
	Urea N	1	0.537	0.467	
	Error	52			
	Total	60			
mgCH4- m2/h	pH	1	0.176	0.676	25.70
	EC mS/cm	1	1.625	0.208	
	Biochar	1	0.024	0.877	
	Lime	1	0.139	0.711	
	Wood Ash	1	0.229	0.634	
	Paper Sludge	1	0.169	0.683	
	Urea N	1	7.884	0.007	
	Error	52			
	Total	60			
NO3-N mg/kg soil	pH	1	8.329	0.006	75.90
	EC mS/cm	1	7.804	0.007	
	Biochar	1	0.711	0.403	
	Lime	1	1.235	0.272	
	Wood Ash	1	6.74	0.012	
	Paper Sludge	1	16.532	< 0.001	
	Urea N	1	24.496	< 0.001	
	Error	52			
	Total	60			
NH4-N mg/kg soil	pH	1	14.213	< 0.001	76.80
	EC mS/cm	1	4.602	0.037	
	Biochar	1	3.007	0.089	
	Lime	1	2.719	0.105	
	Wood Ash	1	1.166	0.285	
	Paper Sludge	1	9.508	0.003	
	Urea N	1	58.217	< 0.001	

	Error	52			
	Total	60			

Table S5: GLM ANOVA for rate of change from day 36 to 123 parameters (calculated as delta change in day 123 and day 36) with $\alpha = 0.05$ results for biotic parameters

	Source	DF	F-value	P	R-Sq (%)
BR mL CO2 h/100/soil	pH	1	3.852	0.055	42 %
	EC mS/cm	1	0.098	0.756	
	Biochar	1	9.83	0.003	
	Lime	1	1.852	0.179	
	Wood Ash	1	18.14	< 0.001	
	Paper Sludge	1	0.697	0.407	
	Urea N	1	0.068	0.796	
	Error	52			
	Total	60			
mg biomass C 100g/soil	pH	1	14.789	< 0.001	52.20
	EC mS/cm	1	1.187	0.281	
	Biochar	1	1.407	0.241	
	Lime	1	4.467	0.039	
	Wood Ash	1	19.319	< 0.001	
	Paper Sludge	1	0.2	0.656	
	Urea N	1	0.642	0.427	
	Error	52			
	Total	60			
qCO2	pH	1	0.296	0.589	60.70
	EC mS/cm	1	1.791	0.187	
	Biochar	1	6.433	0.014	
	Lime	1	13.909	< 0.001	
	Wood Ash	1	0.251	0.619	
	Paper Sludge	1	6.333	0.015	
	Urea N	1	1.44	0.236	
	Error	52			
	Total	60			
mgCO2-C m2/h	pH	1	0.694	0.409	14.9
	EC mS/cm	1	0.069	0.794	
	Biochar	1	0.213	0.646	
	Lime	1	5.29	0.025	
	Wood Ash	1	3.58	0.064	
	Paper Sludge	1	0.052	0.821	
	Urea N	1	0	0.998	
	Error	52			
	Total	60			
mgCH4- m2/h	pH	1	0.03	0.863	7.2
	EC mS/cm	1	0.88	0.353	
	Biochar	1	0.934	0.338	
	Lime	1	0.05	0.824	
	Wood Ash	1	0.002	0.969	
	Paper Sludge	1	0.957	0.332	
	Urea N	1	0.785	0.38	
	Error	52			
	Total	60			
NO3-N mg/kg soil	pH	1	49.698	< 0.001	86.2
	EC mS/cm	1	10.819	0.002	
	Biochar	1	0.278	0.6	
	Lime	1	1.544	0.22	
	Wood Ash	1	1.088	0.302	
	Paper Sludge	1	23.491	< 0.001	
	Urea N	1	60.663	< 0.001	
	Error	52			
	Total	60			
NH4-N mg/kg soil	pH	1	48.006	< 0.001	85.6
	EC mS/cm	1	13.359	0.001	
	Biochar	1	0.021	0.885	
	Lime	1	5.312	0.025	
	Wood Ash	1	18.483	< 0.001	
	Paper Sludge	1	11.528	0.001	
	Urea N	1	114.508	< 0.001	
	Error	52	48.006		
	Total	60			

Table S6: GLM ANOVA for absolute data for day 36 with α = 0.05 results for biotic parameters

	Source	DF	F-value	P	R-Sq (%)
mg biomass C 100g/soil	EC mS/cm	1	0.2	0.657	95.40
	pH	1	171.316	< 0.001	
	SWC	1	0	0.983	
	Biochar	1	0.09	0.766	
	Lime	1	94.135	< 0.001	
	Wood Ash	1	91.201	< 0.001	
	PaperSludge	1	43.948	< 0.001	
	Urea N	1	1.551	0.219	
	Error	51			
	Total	60			
BR mL CO2 h/100/soil	EC mS/cm	1	0.921	0.342	88.0
	pH	1	64.341	< 0.001	
	SWC	1	3.414	0.07	
	Biochar	1	0.305	0.583	
	Lime	1	30.743	< 0.001	
	Wood Ash	1	40.065	< 0.001	
	PaperSludge	1	0.682	0.413	
	Urea N	1	2.383	0.129	
	Error	51			
	Total	60			
qCO2	EC mS/cm	1	0.569	0.454	89.20
	pH	1	72.415	< 0.001	
	SWC	1	3.968	0.052	
	Biochar	1	0.272	0.605	
	Lime	1	22.289	< 0.001	
	Wood Ash	1	13.433	0.001	
	PaperSludge	1	38.421	< 0.001	
	Urea N	1	0.152	0.698	
	Error	51			
	Total	60			
mgCO2-C m2/h	EC mS/cm	1	3.815	0.056	56.50
	pH	1	8.358	0.006	
	SWC	1	4.653	0.036	
	Biochar	1	11.853	0.001	
	Lime	1	19.311	< 0.001	
	Wood Ash	1	22.387	< 0.001	
	PaperSludge	1	3.648	0.062	
	Urea N	1	0.004	0.948	
	Error	51			
	Total	60			
mgCH4- m2/h	EC mS/cm	1	3.685	0.06	9.3
	pH	1	1.281	0.263	
	SWC	1	0.001	0.98	
	Biochar	1	0.913	0.344	
	Lime	1	0.108	0.744	
	Wood Ash	1	0.011	0.919	
	PaperSludge	1	0.096	0.757	
	Urea N	1	1.594	0.213	
	Error	51			
	Total	60			
NO3-N mg/kg soil	EC mS/cm	1	1.798	0.186	95.20
	pH	1	128.994	< 0.001	
	SWC	1	0.11	0.742	
	Biochar	1	0.405	0.527	
	Lime	1	46.093	< 0.001	
	Wood Ash	1	17.219	< 0.001	
	PaperSludge	1	14.301	< 0.001	
	Urea N	1	112.035	< 0.001	
	Error	51			
	Total	60			
NH4-N mg/kg soil	EC mS/cm	1	0.277	0.601	90.30
	pH	1	69.301	< 0.001	
	SWC	1	1.488	0.228	
	Biochar	1	1.709	0.197	
	Lime	1	34.6	< 0.001	
	Wood Ash	1	32.835	< 0.001	
	PaperSludge	1	6.412	0.014	
	Urea N	1	118.995	< 0.001	
	Error	51			
	Total	60			

Table S7: GLM ANOVA for absolute data for day 123 with α = 0.05 results for biotic parameters

	Source	DF	F-value	P	R-Sq (%)
mg biomass C 100g/soil	pH	1	199.9	< 0.001	95.90
	EC mS/cm	1	14.833	< 0.001	
	SWC	1	1.649	0.205	
	Biochar	1	2.086	0.155	
	Wood Ash	1	110.66	< 0.001	
	Paper Sludge	1	76.924	< 0.001	
	Lime	1	131.916	< 0.001	
	Urea N	1	0.374	0.544	
	Error	51			
	Total	60			
BR mL CO2 h/100/soil	pH	1	74.604	< 0.001	85.50
	EC mS/cm	1	0.807	0.373	
	SWC	1	5.383	0.024	
	Biochar	1	14.561	< 0.001	
	Wood Ash	1	18.968	< 0.001	
	Paper Sludge	1	4.366	0.042	
	Lime	1	12.781	0.001	
	Urea N	1	1.747	0.192	
	Error	51			
	Total	60			
qCO2	pH	1	96.76	< 0.001	93.0
	EC mS/cm	1	7.693	0.008	
	SWC	1	0.693	0.409	
	Biochar	1	4.123	0.048	
	Wood Ash	1	26.702	< 0.001	
	Paper Sludge	1	45.231	< 0.001	
	Lime	1	77.197	< 0.001	
	Urea N	1	0.716	0.401	
	Error	51			
	Total	60			
mgCO2-C m2/h	pH	1	10.736	0.002	45.3
	EC mS/cm	1	0.194	0.661	
	SWC	1	0.028	0.868	
	Biochar	1	9.722	0.003	
	Wood Ash	1	4.04	0.05	
	Paper Sludge	1	0.75	0.391	
	Lime	1	1.222	0.274	
	Urea N	1	0.31	0.58	
	Error	51			
	Total	60			
mgCH4- m2/h	pH	1	0.002	0.962	9.5%
	EC mS/cm	1	0.349	0.557	
	SWC	1	0.085	0.772	
	Biochar	1	1.511	0.225	
	Wood Ash	1	0.168	0.684	
	Paper Sludge	1	0.169	0.683	
	Lime	1	0	0.984	
	Urea N	1	0.339	0.563	
	Error	51			
	Total	60			
NO3-N mg/kg soil	pH	1	343.407	< 0.001	96.9
	EC mS/cm	1	3.863	0.055	
	SWC	1	0.002	0.962	
	Biochar	1	0.001	0.97	
	Wood Ash	1	52.805	< 0.001	
	Paper Sludge	1	31.26	< 0.001	
	Lime	1	74.604	< 0.001	
	Urea N	1	134.859	< 0.001	
	Error	51			
	Total	60			
NH4-N mg/kg soil	pH	1	0	< 0.001	93.0
	EC mS/cm	1	0.254	0.254	
	SWC	1	0.514	0.514	
	Biochar	1	2.472	0.122	
	Wood Ash	1	46.809	< 0.001	
	Paper Sludge	1	3.02	0.088	
	Lime	1	39.801	< 0.001	
	Urea N	1	159.876	< 0.001	
	Error	51			
	Total	60			

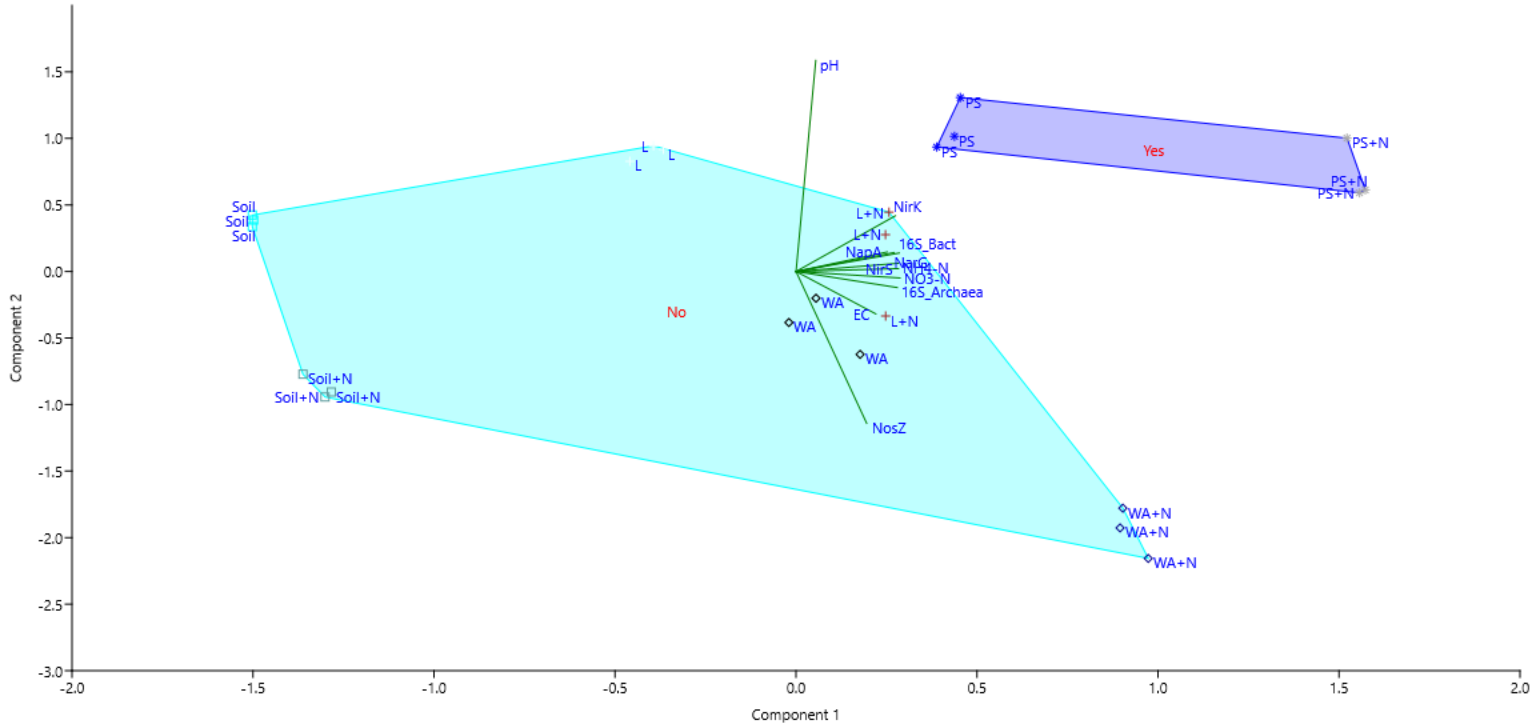

Figure S4: PCA for rate of change from day 1 to 36 (calculated as day 36 subtracted from day 1) for all abiotic parameters including all N-cycling genes. Note: Yes/No refers to sludge

Table S8: GLM ANOVA for rate of change from day 1 to 36 parameters (calculated as day 36 subtracted from day 1) with $\alpha = 0.05$ results for N-cycling genes

	Source	DF	F-value	P	R.sq (%)
16S Bacteria	EC mS/cm	1	0.026	0.874	97.0
	pH	1	0.833	0.374	
	Lime	1	8.464	0.01	
	Wood Ash	1	75.727	< 0.001	
	Paper Sludge	1	72.318	< 0.001	
	Urea N	1	11.993	0.003	
	Error	17			
	Total	24			
NarG	EC mS/cm	1	0.183	0.674	94.0
	pH	1	1.485	0.24	
	Lime	1	0.713	0.41	
	Wood Ash	1	12.343	0.003	
	Paper Sludge	1	23.619	< 0.001	
	Urea N	1	24.339	< 0.001	
	Error	17			
	Total	24			
NapA	EC mS/cm	1	1.026	0.325	79.90
	pH	1	1.352	0.261	
	Lime	1	5.451	0.032	
	Wood Ash	1	14.079	0.002	
	Paper Sludge	1	20.812	< 0.001	
	Urea N	1	0.052	0.822	
	Error	17			
	Total	24			
NirS	EC mS/cm	1	0.24	0.63	98.7
	pH	1	19.013	< 0.001	
	Lime	1	5.783	0.028	
	Wood Ash	1	202.693	< 0.001	
	Paper Sludge	1	116.951	< 0.001	
	Urea N	1	85.437	< 0.001	
	Error	17			
	Total	24			
NirK	EC mS/cm	1	0.224	0.642	94.7
	pH	1	5.104	0.037	
	Lime	1	7.487	0.014	
	Wood Ash	1	27.123	< 0.001	
	Paper Sludge	1	24.881	< 0.001	
	Urea N	1	17.689	0.001	
	Error	17			
	Total	24			
Archaea	EC mS/cm	1	0.835	0.373	97.4
	pH	1	15.311	0.001	
	Lime	1	3.02	0.1	
	Wood Ash	1	117.565	< 0.001	
	Paper Sludge	1	32.346	< 0.001	
	Urea N	1	39.455	< 0.001	
	Error	17			
	Total	24			
NosZ	EC mS/cm	1	0.251	0.623	97.7
	pH	1	2.86	0.109	
	Lime	1	17.909	0.001	
	Wood Ash	1	165.187	< 0.001	
	Paper Sludge	1	18.16	0.001	
	Urea N	1	19.28	< 0.001	
	Error	17			
	Total	24			

Table S9: GLM ANOVA for rate of change from day 36 to 123 parameters (calculated as day 123 subtracted from day 36) with $\alpha = 0.05$ results for N-cycling genes

	Source	DF	F-value	P	R.sq (%)
16S Bacteria	EC mS/cm	1	6.681	0.019	95.9
	pH	1	0.454	0.51	
	Lime	1	1.539	0.232	
	Wood Ash	1	8.132	0.011	
	Paper Sludge	1	22.927	< 0.001	
	Urea N	1	6.681	0.019	
	Error	17			
	Total	24			
NarG	EC mS/cm	1	4.291	0.054	92.2
	pH	1	1.087	0.312	
	Lime	1	0.661	0.428	
	Wood Ash	1	1.598	0.223	
	Paper Sludge	1	2.94	0.105	
	Urea N	1	58.308	< 0.001	
	Error	17			
	Total	24			
NapA	EC mS/cm	1	0.002	0.965	89.1
	pH	1	2.365	0.142	
	Lime	1	0.961	0.341	
	Wood Ash	1	0.665	0.426	
	Paper Sludge	1	4.939	0.04	
	Urea N	1	0.915	0.352	
	Error	17			
	Total	24			
NirS	EC mS/cm	1	0.251	0.623	97.6
	pH	1	1.047	0.32	
	Lime	1	2.788	0.113	
	Wood Ash	1	2.836	0.11	
	Paper Sludge	1	73.847	< 0.001	
	Urea N	1	12.107	0.003	
	Error	17			
	Total	24			
NirK	EC mS/cm	1	1.528	0.233	96.1
	pH	1	5.169	0.036	
	Lime	1	13.347	0.002	
	Wood Ash	1	1.584	0.225	
	Paper Sludge	1	10.994	0.004	
	Urea N	1	31.369	< 0.001	
	Error	17			
	Total	24			
Archaea	EC mS/cm	1	3.03	0.1	89.5
	pH	1	2.501	0.132	
	Lime	1	0.01	0.92	
	Wood Ash	1	0.277	0.606	
	Paper Sludge	1	2.206	0.156	
	Urea N	1	16.382	0.001	
	Error	17			
	Total	24			
NosZ	EC mS/cm	1	1.353	0.261	96.0
	pH	1	0.913	0.353	
	Lime	1	1.983	0.177	
	Wood Ash	1	28.66	< 0.001	
	Paper Sludge	1	1.846	0.192	
	Urea N	1	58.171	< 0.001	
	Error	17			
	Total	24			

Table S10: Soil Textural Analysis

Soil Health Test Report
22-Dec-2021

PEI Analytical Laboratories
PEI Department of Agriculture and Land
23 Innovation Way
PO Box 2000, Charlottetown, PE C1A 7N8
Fax: (902)-368-6299
Telephone: (902)-620-3300

Prince Edward Island CANADA

Memorial University of Newfoundland
Ayodeji Medaiyese
20 University Drive
Corner Brook, NL
A2H 5G4

Client No: 2112100002
Accession No: SH211210001
Samples Reported: 22-Dec-2021
Samples Received: 10-Dec-2021

Soil Health #: SH211210001-1	**Soil #:**	**Sample ID:** 1
Tillage Depth:	**Cropping System:**	**Amendments Applied (manure, etc):** ☐ Yes ☐ No
Yield:		

Soil Texture

Sand (%) 19.5
Silt (%) 57.7
Clay (%) 22.8
Soil Texture Class: Silt Loam

Test	Results	Score (out of 100)	Rating
Organic Matter			
Active Carbon			
Soil Respiration	mg/g		
Aggregate Stability	%		
Biological Nitrogen Availability	mg/kg		
pH			
Phosphorous Index (P/Al)	%		
C:N Ratio			
Total Carbon			
Total Nitrogen			

Soil & Plant Program 2013 NAPT Participating Laboratory

Standards Council of Canada Accredited Laboratory Scope of Accreditation 424 Conseil canadien des normes Laboratoire accrédité Portée d'accréditation 424 TM

http://www.princeedwardisland.ca/labservices

Dates of analysis available upon request.
Organic Matter is calculated from Total Carbon.

ND** - CN ratio could not be accurately calculated due to Total Nitrogen or Total Carbon being below detection limit

Copies To: Memorial University of Newfoundland

Approved By: AS
SHDC

Methods: SHL_1M Active Carbon; SHL_2M Soil Respiration; SHL_3M Biological Nitrogen Availability; SHL_5M Texture; SHL_4M Wet Aggregate Stability; SFL_30M Nitrogen; SFL_22M - pH*; SFL_23M - Organic Matter*; SFL_24M - Nutrients*
* Accredited and NAPT Certified Method