Ammonia and nitrous oxide emissions from soils under ruminant urine patches and the effects of biochar amendment on these emissions and plant nitrogen uptake

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Arezoo Taghizadeh-Toosi

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Urine and dung from ruminants contributes to emissions of both nitrous oxide (N_2O) and ammonia (NH_3). These are important for a variety of agronomic and environmental reasons. Urine contributes a much larger fraction than dung to the total NH_3 and N_2O emissions. Nitrous oxide is a potent greenhouse gas and its emission from grazing animal excreta is considered a major loss pathway for N_2O emitted from agricultural soils. Ammonia can be volatilised from the soil surface shortly after ruminant urination. In addition, a fraction of this NH_3 may be converted into N_2O after NH_3 is redeposited onto the soil.

Biochar is produced as a by-product of the low temperature pyrolysis of biomass during bioenergy extraction. Incorporation of biochar into soil is of global interest as a potential carbon sequestration tool. Nitrogen (N) transformations in soil can be affected by the presence of biochar.

This current research has been conducted to determine the effects of incorporating biochar into soil, on: ruminant urine-derived N_2O fluxes, NH_3 volatilisation, N uptake by pasture, and pasture yield.

The first experiment examined the effects of biochar incorporation (0, 15 and 30 t ha^{-1}) on N_2O emissions during an 86-day spring-summer field study following ^{15}N-labelled ruminant urine application. The results showed that N_2O fluxes were reduced by 70%, after incorporating 30 t ha^{-1} of biochar. The uptake of NH_3 by biochar was proposed as a possible mechanism for the reduced N_2O emissions. No differences occurred, due to biochar addition, with respect to dry matter yields, herbage N content, or herbage recovery of ^{15}N.

In the second experiment the capacity of four biochar types to take up NH_3 was determined in order to define a possible mechanism for reduced N_2O emissions observed under 30 t ha^{-1} biochar in first experiment. The subsequent plant availability of biochar adsorbed NH_3-N was then determined. The results showed that NH_3-N adsorbed by biochar was stable, but readily plant available when placed in the soil. Plant dry matter yields were 2 to 3 times greater and N uptake by plants doubled when biochar containing adsorbed NH_3 was incorporated into soil over a 25-day incubation study.

The third experiment was conducted to measure NH_3 volatilisation in-situ using micrometeorological methods during a 6-day summer field study. Soil temperature, pH, ammoniacal-N and moisture were measured in the top soil to provide input parameters for a volatilisation model. Cumulative NH_3 volatilisation was 25.7 (± 0.5) % of the N applied. The results from this experiment were used as a maximum and in-situ measure of the NH_3 volatilisation rate following urine application.

The fourth experiment was conducted to determine if incorporating biochar (0, 15 and 30 t ha^{-1}) actually reduced NH_3 volatilisation from soil under ruminant urine application, and assessed the subsequent plant availability of this biochar adsorbed NH_3-N. The NH_3 volatilisation from ^{15}N-labelled ruminant urine, applied to soil, was reduced by 45% after incorporating either 15 or 30 t ha^{-1} of biochar. When these urine-treated biochar particles were placed in fresh soil, subsequent plant growth was not affected but the uptake of ^{15}N in plant tissues increased, indicating that the adsorbed-N was plant available.

This thesis demonstrates incorporating biochar into the soil can significantly decrease NH_3 and N_2O emissions from ruminant urine with the captured N recycled to crops while simultaneously sequestering carbon in soils.

Keywords: nitrogen, nitrous oxide, ammonia, ruminant urine, urine patch, biochar, ryegrass, ^{15}N stable isotope, mitigation.

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Finally, I dedicate this thesis to the memory of my grandfather, who loved everything about science. I always believe in his advice: “Only science could take you to remote and exciting parts of the world”.

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Table of Contents

List of Tables

List of Figures

List of Equations

Chapter 1
Introduction

1.1 Background

A great deal of attention has been focused on mitigating the rising atmospheric concentrations of greenhouse gases (e.g. (IPCC, 2007)).
One mitigation option, biochar, has created international interest. Biochar is a by-product formed during the pyrolysis of biomass. It has been mooted that this relatively inert material can be used to sequester carbon (C) in soils and ultimately lead to a lowering of global carbon dioxide (CO_2) concentrations. In addition, the incorporation of biochar into soil has potential implications for N cycling since it appears to have impacts on soil N_2O fluxes and possibly nitrification processes.

This current Ph.D. research has been conducted to assess the ability of biochar to mitigate NH_3 and N_2O emissions under one of the largest NH_3 and N_2O emitting sources in anthropogenic agricultural systems: the ruminant urine patch.

1.2 Research objectives

The objectives of this project were to:

1. Determine the physical and chemical properties of biochar made from *Pinus radiata* under different manufacturing processes; Chapters 4 and 5.
2. Establish field experiments to (a) investigate the impacts of topsoil biochar amendment and urine addition on dry matter yield and N uptake, N_2O and NH_3 emissions, and soil physical and chemical properties, and (b) measure the maximum extent of NH_3 emissions following urine application to pasture in the absence of added biochar; Chapters 4 and 6.
3. Conduct a series of laboratory incubation experiments to investigate the possible mechanisms of biochar's effects on dry matter yields, soil N dynamics, and NH_3 volatilisation following urine amendment, including the influence of biochar type, and rate; Chapters 5 and 7.

1.3 Thesis structure

This thesis consists of 8 chapters, including:

Chapter 1 This chapter provides an outline of the thesis research.

Chapter 2 This chapter reviews the literature on the N cycling in grazed pastures, the various methods to measure and mitigate NH_3 and N_2O emitted from soils, and the known effects of biochar, to date, on N cycling in soils. In addition, research opportunities are highlighted.

Chapter 3 This chapter details the materials and methods used during the four experiments.

Chapter 4 This chapter describes the effects of biochar amendment on N_2O emissions from pasture soils following urine application. The ^{15}N-enriched urine was applied to a pasture field trial and the fate of ^{15}N examined.

Chapter 5 This chapter examines the potential for different types of biochar to adsorb NH_3-^{15}N. Then the fate of N adsorbed in/on biochar was investigated in a plant-soil system.

Chapter 6 This chapter was performed in order to measure NH_3 volatilisation rates from a realistic farming situation using a micrometeorological method in the field.

Chapter 7 This chapter examined the mitigation potential of biochar on NH_3 volatilisation following ^{15}N-enriched urine application in a laboratory experiment using aspirated chambers. In addition, fresh and urine-treated biochars were used as soil amendments to investigate the subsequent influence of the biochar on N cycling in a soil-plant system.

Chapter 8 This chapter summarises previous chapters and presents future research recommendations.

Chapter 2
Literature Review

2.1 Introduction

Grazing ruminants alter N cycling dynamics in pasture ecosystems and enhance gaseous N emissions such as NH_3 and N_2O. In this review, the literature on N cycling processes in grazed pasture systems, especially the interaction with urine-N, the different methods used to evaluate NH_3 volatilisation and N_2O emission rates, and the various approaches to decrease the negative-effect of NH_3 and N_2O emissions, along with potential research opportunities are discussed. Biochar is reviewed in detail as a potential mitigation option for sequestrating C and altering N cycling in soil. In addition, the current understanding of the effects of biochar on soil physical and biochemical properties are discussed.

2.2 Cycling of N in grazed pasture system

2.2.1 Sources of N

Nitrogen occupies a unique position among the elements that are essential for plant growth due to the significant amounts required by most agricultural crops (Stevenson, 1982). Total soil N contents range from < 0.1% to > 4% in desert and organic soils, respectively (Haynes, 1986b). Gains in soil N occur not only through fixation of molecular N_2 by microorganisms but also via atmospheric deposition, deposition of animal excreta, and the use of N fertilisers (Stevenson, 1982).

2.2.1.1 Biological N fixation

In New Zealand, legumes traditionally provide the main source of N for pastures via biological N fixation (BNF), which substantially reduces N fertiliser requirements, and improves feed quality for grazing animals (McLaren & Cameron, 1990).

Biological N fixation is performed by free-living micro-organisms or by micro-organisms which live in symbiosis with higher plants (McLaren & Cameron, 1990). These organisms may be phototrophic or chemotrophic, autotrophic or heterotrophic (Gallon & Chaplin, 1987).

Rates of BNF in legume - grass pastures have been reported to range from 35 to 296 kg N ha^{-1} yr^{-1} (Ledgard & Steele, 1992).

In white clover mowing trials throughout New Zealand, BNF equated to 45 - 142 and 76 - 105 kg N ha^{-1} yr^{-1} when measured using ^{15}N isotope dilution and acetylene reduction assays, respectively. Other reported values from grazed trials equate to 82 - 291 kg N ha^{-1} yr^{-1} measured using the ^{15}N isotope dilution (Crush et al., 1983; Edmeades & Goh, 1978; Hoglund et al., 1979; Ledgard et al., 1987; Ledgard et al., 1990).

Ruminant urine has been shown to decrease BNF rates 80% and 50% in late autumn and early spring, respectively (Ledgard et al., 1982).

In pastures, BNF is influenced by legume persistence, legume production, soil N content, and competition with the other grasses. Moreover, legume persistence and production is affected by factors such as soil water content, soil acidity, soil fertility, pests and disease (Ledgard & Steele, 1992). Biochar application into soil can influence BNF (Rondon et al., 2007).

2.2.1.2 Fertiliser

In Europe, agricultural production has historically depended on regular applications of N fertiliser to pastures. In contrast, New Zealand pastures have traditionally depended mostly on BNF and low rates of N fertiliser ranging from 30 to 100 kg N ha^{-1} yr^{-1} (Ledgard & Steele, 1992; McLaren & Cameron, 1990). However, there has been an increase in synthetic N fertiliser application in New Zealand from 59,265 tonnes in 1990 to 328,157 tonnes in 2008 which equates to an approximate five-fold increase in fertiliser use (Kelliher & de Klein, 2006), leading to an increase in annual N application rates.

2.2.1.3 Ruminant excreta

There are four main ruminant population categories grazing the 7.75 million hectares of pasture land in New Zealand: dairy cattle, beef cattle, sheep and deer. Dairy cow numbers in New Zealand equate to 5.3 million animals, grazing 1.7 million hectares of pasture ("Statistics New Zealand," 2008a; "Statistics New Zealand," 2008b). The N intake of a ruminant is redistributed into meat, milk, wool, urine, dung or retained in the animal (Hutton et al., 1965; Hutton et al., 1967). Urine contains compounds such as urea (50 - 93% of excreted N), heterocyclic bases, amino acids and peptides (Bathurst, 1952; Bristow et al., 1992; Doak, 1952) that alter the chemical, physical and biological properties of the soil onto which it is deposited thus altering nutrient cycling, including the production of greenhouse gases (GHGs) over a short period of time (Bathurst, 1952; Bristow et al., 1992; Doak, 1952). Dung contains large quantities of organic matter with significant organic-N, but the negative

environmental effects of dung on soil are less than urine (Saarijärvi & Virkajärvi, 2009; Williams & Haynes, 1995).

Calculations of the quantity of N in animal waste management systems are based on the N excreted per head per day multiplied by the livestock population (IPCC, 2006). Nitrogen excretion rates depend on the type of animal, the volume of dry matter eaten, N concentration of the dry matter and water consumption (Betteridge et al., 1986; Bristow et al., 1992; Doak, 1952; Haynes & Williams, 1993; Luo et al., 2008c). Typically, more than 40 - 53% of the consumed N is excreted as urine, and this value increases to 75% in dairy cow urine which normally contains between 2 to 20 g N L^{-1} (Haynes & Williams, 1993). This urine is deposited unevenly on the pastures with rates up to 1000 kg N ha^{-1} in dairy cow urine patches especially in stock camps, near shelter-belts, and around water troughs (Betteridge et al., 1986; Bristow et al., 1992; Doak, 1952; Gillingham & During, 1973; Haynes & Williams, 1993; Hilder, 1966; Luo et al., 2008c; Snaydon, 1981). The size of a urine patch is approximately 0.16 to 0.49 m^2 (the affected area averages 0.68 m^2 due to both urine diffusion through the soil and because plants outside the patch may have roots in the urine patch able to take up urine-N) and depends on the volume of urine excreted, soil moisture status, wind, slope, and soil compaction (During & McNaught, 1961; Haynes & Williams, 1993; Langtina et al., 1987; Williams et al., 1990). The previous studies have shown that the majority of urine (ca. 85%) remains in the top 100 mm of the soil column and the remnant of it may penetrate to a depth of 400 mm (Williams & Haynes, 1994). The environmental impacts of urine patches are discussed in Section 2.2.4.

2.2.1.4 Atmospheric deposition

Atmospheric N compounds may be added to the pasture through wet or dry deposition (Galloway et al., 1994). Wet deposition carries the dissolved inorganic-N (NH_3, ammonium (NH_4^+), and nitrate (NO_3^-)) and dissolved organic-N by precipitation. Dry deposition includes various interactions between airborne N compounds and water, plant, soil, stone or rock by gravitational settling, turbulent transport, impaction and molecular diffusion. The input from atmospheric deposition is considered to be < 15 kg N ha^{-1} yr^{-1} in rural New Zealand (Floate, 1987; Porter et al., 1999; Whitall & Paerl, 2001).

2.2.2 Transformations of N

The N transformation processes in soils are well known and are not specific to grassland systems. However, grazing animals are stocked at higher densities on pastures than natural

systems and contribute to N cycling through the deposition of dung and urine conspicuously (Snaydon, 1981; White et al., 2000).

2.2.2.1 Mineralisation and Immobilisation

Biological conversion of organic N into inorganic forms occurs via mineralisation, while the opposing process of immobilisation removes inorganic-N into the organic-N pools (Stevenson, 1982).

Mineralised N will be assimilated by plants and/or microorganisms. Microbial N uptake, leads to a build up of N in the soil which may be converted to more stable humus forms (Stevenson, 1994). Plants provide energy for micro-organisms and also compete for any N mineralised (Haynes, 1986a). Mineralisation and immobilisation are affected by the interactions of plants, soil physical and chemical properties, climate and the soil's C to N ratio (Haynes, 1986b; McLaren & Cameron, 1990).

2.2.2.2 Nitrification

Nitrification is the aerobic conversion of NH_3 into nitrite (NO_2^-) and NO_3^- by nitrifying bacteria including autotrophic bacteria, heterotrophic organisms and methylotrophs (Haynes, 1986b; Schmidt, 1982; Suzuki et al., 1974). Chemical nitrification may occur in soil, however, it is thought to be of minor importance (Bartlett, 1981; Haynes, 1986b). Hydroxylamine (NH_2OH), nitroxyl (NOH) or its dimer, hyponitrite ($N_2O_2^{2-}$) and NO_2^- are intermediate compounds and are not stable under normal pasture soil conditions (Bremner & Shaw, 1958; Nicholas, 1978; Sherlock et al., 2008).

$$\begin{array}{c} \quad\quad NO \quad N_2O \\ \quad\quad \nearrow \quad \searrow \quad \uparrow \\ NH_4^+ \leftrightarrow NH_3 \rightarrow NH_2OH \rightarrow (HNO) \rightarrow NO_2^- \rightarrow NO_3^- \end{array} \qquad \textit{[Equation 2.1]}$$

Nitrification rates tend to increase linearly with increasing soil NH_4^+ concentrations and microbial nutrient supply such as phosphorus (P) (Kirk & Kronzucker, 2005; White & Reddy, 2003), and decrease under low (moisture stress) and high (less oxygen, O_2, availability) soil moisture contents due to their effects on biological activity. The typical range of soil moisture suitable for nitrification is -10 to -33 kPa (Malhi & McGill, 1982). Also, nitrification rates increase exponentially with increasing temperature and stabilise when soil temperature exceeds the site specific average high temperature for the warmest month of the year (Smith et al., 2003). Nitrification rates are not limited when the pH is > neutral (the optimal pH range is between 7 and 9) but decrease exponentially as soils become acidic

(Bartlett, 1981; Haynes & Sherlock, 1986). Moreover, herbicides, pesticides, acetylene and heavy metals may inhibit nitrification (Liang & Tabatabai, 1978). Biochar has been considered as a material which might affect nitrification rates (Section 2.3.4).
In grazed pasture soils, the population of nitrifying bacteria reportedly increases following the application of urine due to the resulting soil pH increase, which shortens the generation time of nitrifying bacteria (Robinson, 1953). Other factors (e.g. NH_4^+ concentrations, soil moisture, and pH) also change over time in urine patches and may cause fluctuations in nitrification rates before a constant rate of nitrification is reached (Haynes & Williams, 1992).

2.2.2.3 Denitrification

Biological denitrification [Equation 2.2] is a microbially mediated reduction process that occurs under partially to completely anaerobic soil conditions, whereas chemodenitrification occurs primarily in acidic soils (pH < 5.0) that also contain NO_2^-, present as the result of denitrification or nitrification. Nitrite reacts within soils and nitrogenous gases such as N_2, NO, NO_2 and N_2O may be subsequently emitted (Blackmer & Bremner, 1978a; Blackmer & Bremner, 1978b; Haynes & Sherlock, 1986; Nelson, 1982; Sherlock et al., 2008).

$$NO_3^- \xrightarrow{\text{nitrate reductase}} NO_2^- \xrightarrow{\text{nitrite reductase}} NO \xrightarrow{\text{NO-reductase}} N_2O \xrightarrow{\text{N2O-reductase}} N_2 \qquad \textit{[Equation 2.2]}$$

Various factors affect denitrification rates. These include soil water content and its effect on soil anoxic conditions, availability of organic C, the soil pH, temperature and substrate supply. If any of these conditions are not optimal then the denitrification rate is compromised. Increasing soil water content progressively fills soil pores and limits the diffusion of O_2 into the soil, and the subsequent movement, distribution and relative proportion of denitrification gases (Kumar et al., 2000). The O_2 content of a soil is largely influenced by rainfall, irrigation, groundwater table, soil texture and biological O_2 consumption. Generally, denitrification rates increase after rainfall or irrigation (denitrification is the dominant process when the water-filled pore space (WFPS) is > 70%) and decrease again when the soil dries out (Bothe et al., 2007; Debbie et al., 1999). Anoxic conditions and anoxic micro sites are most probable in wet soils with a high respiration rate. However, high local rates of O_2 consumption (e.g. near a source of easily degradable organic matter) may cause enhanced denitrification in micro sites in soil, even in dry soils (Bothe et al., 2007).

The C supply influences biological denitrification since the microbes are heterotrophic. Hence, the lack of C can limit denitrification (Firestone, 1982). Manures contain easily degradable C and application of manures to pasture soils increases denitrification activity and N_2O emissions (Ellis et al., 1998; Velthof et al., 2003).

Denitrification rates are generally lower at acidic pH values and increase as pH increases to the optimum pH (7.0 - 8.5). The denitrification process tends more toward completion (N_2 increase relative to N_2O) as pasture soil pH increases due to an increase in denitrifying bacteria activity (Cŭhel et al., 2010) .

Soil temperature results in changes to the N_2O:N_2 ratio with N_2O dominating at lower and N_2 at higher temperatures due to the contribution of non-biological reactions (Haynes & Sherlock, 1986; Vinther, 1990). Small inputs of NO_3^- affect the anaerobic reduction of N_2O to N_2 if the denitrifying microorganisms in the soil have not had a chance to adapt to anaerobic reduction of N_2O to N_2 (Blackmer & Bremner, 1978b). The N_2O:N_2 ratio favours the production of N_2O and N_2 when NO_3^- concentrations are high and low, respectively (Blackmer & Bremner, 1978a; Blackmer & Bremner, 1978b; Bothe et al., 2007; Carter & Gregorich, 2008). Biochar has been considered as a material which might influence soil denitrification rates (Section 2.3.4).

Urine and dung deposition on pastures causes hot spots of high N and C concentrations, high moisture and high pH, which promotes denitrification (over 0.6 g N m^{-2} d^{-1} following urine deposition) (de Klein & van Logtestijn, 1994). One previous study showed 70% of the denitrification loss came from the 14% of the area affected by sheep excreta in grazed pastures (Colbourn, 1993).

2.2.2.4 Nitrifer–Denitrification

Ammoniacal-N is transformed to NO_2-N and N_2O-N by autotrophic bacteria in nitrifier-denitrification (Casciotti & Ward, 2005; Garbeva et al., 2006; Schmidt et al., 2004; Shaw et al., 2006). High concentrations of organic N, low organic C availability, and low oxygen pressure can cause up to 44% of the N_2O-N emitted to evolve via the nitrifer-denitrification pathway over 24 h following the addition of ^{15}N-labelled ammonium nitrate to silt loam soil at 50% WFPS (Kool et al., 2011; Wrage et al., 2001; Wrage et al., 2005). The likelihood of nitrifier-denitrification happening is quite high in urine patches mostly due to the relatively large NO_2^- content of a soil (Koops et al., 1997) . However, further studies are necessary to assess the significance of N_2O-N via nitrifier-denitrification in pasture soils (Wrage et al., 2001).

2.2.2.5 The fixation and adsorption of NH_4^+

Clay minerals in soil contain negative charges which occur as a result of isomorphous substitution of $A1^{3+}$ for Si^{4+} (or Mg^{2+} for $A1^{3+}$) and the dissociation of H^+ from -OH groups bound to Si and/or Al (Nommik & Vahtras, 1982). Ammonium can be fixed in lattice structures of clay minerals and thus be non-exchangeable (Stevenson, 1994). Soil organic matter also contains negative charges at typical pasture soil pH, which originates from the dissociation of the functional groups such as carboxyl (COOH) and phenolic (OH) groups (Hayes & Swift, 1978). The total negative charge of a soil represents the ability of a soil to hold positively charged ions, such as NH_4^+. The NH_4^+ adsorption capacity of soil is affected by soil pH and humification of organic matter (Stevenson, 1994) and is a reversible pathway where NH_4^+ on exchange sites and in soil solution are in dynamic equilibrium. Hence, NH_4^+ in such a form is available to plants but effectively protected against leaching (Cameron & Haynes, 1986). Urine deposition increases NH_4^+ concentrations rapidly (Crush & Evans, 1988). Potential effects of biochar on NH_4^+ adsorption are discussed below (Section 2.3.4).

2.2.2.6 Effects of fauna and flora

There are various fauna and flora in soil systems including micro-, meso- and macro-fauna. The microfauna are protozoa (unicellular eukaryotes), nematodes, and some arthropods. Meso-fauna are larger and include earthworms, mites, termites, snails, mice, and large nematodes. Macro-fauna includes burrowing mammals like moles and rabbits (Lepage et al., 2006). Micro-fauna and meso-fauna are accompanied by micro-flora, which includes algae, bacteria, fungi and yeasts. Meso-fauna usually fragment organic matter to finer particles which give rise to surface areas for microbial colonisation, and subsequently speeds the decomposition and mineralisation processes (Curl, 1988; Salt et al., 1948). Pasture management (e.g. fertiliser application and ruminant stocking rate and density) has a complicating effect on soil organisms (Schon et al., 2008). Furthermore, some macro-fauna such as earthworms can contribute to the N cycle through N consumption, digestion, respiration, excretion and worm cast egestion, which constitutes an important micro-site for denitrification and causes spatial heterogeneity of N gas fluxes (Bhadauria & Reamarkrishan, 1996; Buse, 1990; Elliot et al., 1990; Robinson et al., 1992; Svensson et al., 1986). Nitrogen is available for plants in various forms including inorganic-N (NH_4^+-N and predominantly NO_3^--N which are the most important forms under agricultural conditions), organic nitrogen (amino acids, peptides, etc. when mineralisation rates are low due to acid pH, low temperature and reduced microbial activity), and gaseous N (NH_3 and NO_x when N

supply for plants is low) (von Wirén et al., 1997). Isotope studies have found 22 - 78% of the urea-N in urine to be retained in living plant biomass, while 20 - 30% remained in the soil organic matter (SOM) (Clough et al., 1998; Lehmann et al., 2003). Plants may also play a passive role in N uptake by intercepting applied fertiliser solution before it reaches the soil surface and undergoes transformation. Crop height and density are important factors in this process (Yamada et al., 1965).

Factors influencing the plant uptake of NH_4^+ and NO_3^- include pH, temperature, photosynthesis rates, and the presence of other ions. Maximum and minimum adsorption of NH_4^+ occurs at pH = 7 - 8 and pH = 4 - 5, respectively. At low temperature, NO_3^- uptake is reduced relative to NH_4^+. Photosynthesis provides energy from shoot to root for the uptake processes (Haynes, 1986a). Other cations or anions compete with NH_4^+ or NO_3^- during active uptake (Clarkson & Warner, 1979; Haynes, 1986b; von Wirén et al., 1997).

The plant rhizosphere also affects micro-flora and fauna. Its rich C source causes an increase in the microbe-grazing fauna population which occurs as a result of the presence of root exudates and sloughed root cells (Kuzyakov, 2002). An increase in the microbial population and hence microbial activity results in the release of inorganic nitrogen and phosphorus (Curl, 1988).

2.2.3 Losses of N

Nitrogen losses occur as a result of crop removal, leaching, gaseous losses due to microbial processes (e.g. N_2O) and chemical processes such as NH_3 volatilisation (Stevenson, 1982).

2.2.3.1 Leaching

Various factors influence leaching processes such as convection, diffusion, hydrodynamic dispersion, chemical and biological processes, soil physical properties and soil charge (Cameron & Haynes, 1986; Gaines & Gaines, 1994). The leaching of dissolved organic N (DON) or the NH_4^+-N form of inorganic nitrogen is considered low due to soil adsorption, soil fixation, soil microbial immobilisation, nitrification process, plant uptake and NH_3 volatilisation. Nitrogen leaching and the rates vary from 0.3 kg N ha^{-1} yr^{-1} to 127 kg N ha^{-1} yr^{-1} following urine application in pasture (Cho, 1971; Khanna, 1981; Misra et al., 1974; van Kessel et al., 2009).

In grazed pastures such as those in New Zealand, ruminant urine creates areas with an excess of plant N demand over supply and leaching can occur in the range of 50 - 200 kg N ha^{-1} yr^{-1}

which can be effected by soil type and the presence of a water table (Cameron & Haynes, 1986; Clough et al., 1996; Di & Cameron, 2002).

2.2.3.2 NH_3 volatilisation

Ammonia is generated at the soil surface in abundant quantities shortly following the surface application of nitrogenous fertilisers that raise soil pH (especially urea), various ammoniacal wastes to soil, and ruminant urination (Sherlock et al., 1995). However, NH_3 volatilisation rates can vary extensively (e.g. from 1.7% to 56% of the applied urea N) depending on the crop, cultural conditions, dung or urine deposition rates and method of fertiliser application etc. (Sherlock et al., 1989).

Volatilisation of NH_3 from ruminant urine is formed when the major N-containing species in urine, namely urea, $CO(NH_2)_2$, is catalytically hydrolysed by the ubiquitous soil enzyme, urease, and ammonium carbonate ($(NH_4)_2CO_3$) is produced. Then, carbonate ions (CO_3^{2-}) are hydrolysed and hydroxide and bicarbonate ions (HCO_3^-) are produced. These processes are complex and involve a series of biochemical and physicochemical steps (Avnimelech & Laher, 1977; Sherlock & Goh, 1984a, 1984b; Vlek & Craswell, 1981). Urea hydrolysis is a biochemical reaction and NH_3-N formation is a chemical reaction, which requires an alkaline pH (Court et al., 1964; Saggar et al., 2004). The high pH of a urine patch occurs due to generation of hydroxide (OH^-) and HCO_3^- ions and in conjunction with the increase in the soil NH_4^+-N concentration, provides ideal conditions for NH_3-N production (Haynes & Sherlock, 1986; Haynes & Williams, 1993). As the pH rises, the proportion of NH_4^+-N converted to NH_3-N increases (Bates & Pinching, 1950; Court et al., 1964). The NH_3-N in soil solution is in equilibrium with atmospheric NH_3-N [Equation 2.5], and can be volatilised (Whitehead, 1995).

$$CO(NH_2)_2 + 2H_2O \xrightarrow{urease} 2NH_4^+ + CO_3^{2-} \quad [Equation\ 2.3]$$

$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^- \quad [Equation\ 2.4]$$

$$NH_4^+ + OH^- \rightarrow NH_{3(g)} + H_2O \quad [Equation\ 2.5]$$

Peak soil NH_4^+-N concentrations often occur within 24 h of urine deposition, since urea hydrolysis may be complete within 48 h (Holland & During, 1977; Petersen et al., 1998); although NH_4^+-N concentrations remain elevated for at least 2 - 3 weeks after urine deposition, depending on the nitrification rate (Ball et al., 1979; Lovell & Jarvis, 1996; Petersen et al., 1998; Sherlock & Goh, 1984a, 1984b; Whitehead & Raistrick, 1993) .

The various physical, chemical and biological factors that affect NH_3 volatilisation from surface applied urea, include environmental factors (soil moisture and rainfall, temperature (Acquaye & Cunningham, 1965; Ernst & Massey, 1960; Lyster et al., 1980; McGarry et al., 1987; Prasad, 1976; Volk, 1959; Watkins et al., 1972) wind speed, atmospheric NH_3 concentration and plant cover), physical factors (soil texture), chemical factors (pH, cation exchange capacity (CEC), H^+ ion buffer capacity, presence of soluble cations and organic matter), biological factors (microbial activity) and agronomic factors (rate, form and mode of urea application, liming, irrigation and the presence or absence of livestock) (Fenn & Hossner, 1985; Freney & Black, 1988; Freney et al., 1981; Freney et al., 1983). Soil organic-C is the soil property that principally controls urease levels in soil. Since the surface soil layers are highly enriched with organic matter, urease activity is highest in these layers and decreases sharply with increasing soil depth (Dalal, 1975; Dash et al., 1981; Gould et al., 1973; McGarity & Myers, 1967; Simpson, 1968; Zantu et al., 1977). Those soil factors which are positively correlated with organic-C are also correlated with urease activity. These include: CEC, high nutrient availability, total-N, clay contents (Dalal, 1975; Dash et al., 1981; Frankenberger & Dick, 1983; O'Toole et al., 1982; Verstraeten, 1978; Zantu et al., 1977), soil microbial activity (Frankenberger & Tabatabai, 1982), and increasing urea concentrations (Kumar & Wagenet, 1984; Overrein & Moe, 1967; Tabatabai & Bremner, 1972). Some soil properties which are negatively correlated with organic-C are also negatively correlated with urease activity. These include soil pH and sand content (Dash et al., 1981; Frankenberger & Dick, 1983; Kumar & Wagenet, 1984; Rao & Ghai, 1985; Singh & Bajwa, 1986). The soil pH and CEC are the main factors that influence NH_3-N volatilisation (Whitehead & Raistrick, 1993; Zhenghu & Honglang, 2000). However, NH_3-N volatilisation may correlate better with the soil pH after urine deposition than with the initial soil pH (Whitehead & Raistrick, 1993). Hydrolysis of urea is optimal at pH > 6.5, and can cause a localised increase in the soil pH (Haynes & Sherlock, 1986; Jarvis & Pain, 1990). Although the high pH in a urine patch increases the NH_3-N: NH_4^+-N ratio, potentially enhancing NH_3-N volatilisation, it may also increase the negative surface charge of the CEC, so that more NH_4^+ is adsorbed, which could reduce NH_3-N volatilisation (Haynes & Williams, 1993).

Increasing air and soil temperature, moisture content and wind movement are dominant factors controlling NH_3 loss. Application of urea to dry soil prevents urea hydrolysis and conditions suitable for NH_3 volatilisation. Contrastingly, NH_3 losses from urea increase in soils with high moisture content (Bacon et al., 1986; Bouwmeester et al., 1985; Catchpoole et

al., 1983; Ernst & Massey, 1960; Humphreys et al., 1988). Moreover, increasing air and soil temperatures increase the NH_3 volatilisation due to increasing rates of urease activity (Moyo et al., 1989); and decreasing air and soil temperatures decrease NH_3 volatilisation and required a longer duration to sustain the rate of losses achieved at higher temperatures. Wind movement increases the removal of $NH_{3(g)}$ from the soil-air interface which increases the NH_3 diffusion rate from the soil or solution in response to a large NH_3 partial pressure gradient (Haynes & Sherlock, 1986; Nelson, 1982; Vlek & Craswell, 1981). During periods of very low wind movement, the concentration of ammoniacal-N increases in the soil solution, and subsequently the potential for high fluxes increases with resuming high wind speeds (Sherlock & Goh, 1984a, 1984b).

❖ *NH_3 volatilisation measurement methods*

Several field methods for determining NH_3 volatilisation have been developed and assessed. These include N-balance methods, static chambers, non-static (aspirated) chambers and wind-tunnels, and micrometeorological methods (Sherlock et al., 2008).

I. N-balance method

In N-balance methods, the sum of N in all measured pools is subtracted from the known amount of N-added, with amount of N unaccounted for defined as the NH_3 loss (Sherlock et al., 2008).

II. Static chambers

Static chambers (or enclosures) consisting of short cylinders of sufficient basal diameter to cover the actively volatilising surface are used in conjunction with dilute aqueous acid traps to determine volatilised NH_3 (Sherlock et al., 2008).

III. Non-static chambers and wind-tunnels

Non-static chambers are similar to static chambers, but the outside air is drawn through the chamber headspace (aspiration) passing the NH_3-laden air through to an external acid trap. A wind tunnel is another form of the aspirated chamber which attempts to minimise the modifying influence of chambers by using fans to better approximate a typical ambient wind-speed during the time of measurement (Sherlock et al., 2008).

IV. Micrometeorological method

In the micrometeorological method, gas concentration gradients, wind speeds and other micrometeorological phenomena are employed to calculate surface $NH_{3(g)}$ fluxes from a much larger area than can be calculated from the other methods. Energy Balance, Eddy

Correlation and Aerodynamic procedures have all been used to determine the exchange of a range of soil-derived gases (e.g. CO_2 and H_2O). Ammonia losses from plots can be measured using one of a number of micrometeorological methods as below (Sherlock et al., 2008):

1. Full mass balance method;
   (a) NH_3 and wind speed measured independently,
   (b) NH_3 and wind speed measured together using "Leuning" sampler,
2. Wilson method (abbreviated mass balance method), and
3. Backward Lagrangian Stochastic models (BLS).

Potential effects of biochar on NH_3 volatilisation are discussed in Section 2.3.4.

2.2.3.3 N_2O emissions

Direct N_2O emissions occur from synthetic fertiliser, animal waste, and the decomposition of plant residues left on agricultural soils (Lyon et al., 1989; Muzio et al., 1989; Rochette & Janzen, 2005). Indirect N_2O emissions may occur off the site of direct N inputs by conversion of N compounds into N_2O following volatilisation, leaching or runoff processes (Eggleston et al., 2006).

In soil, N_2O is produced via biotic and abiotic processes including nitrification (both autotrophic and heterotrophic), biological denitrification and chemo-denitrification (Hutchinson & Mosier, 1981; Tortoso & Hutchinson, 1990). The ability to distinguish between the sources (denitrification and nitrification) of N_2O produced in soils remains difficult because (a) denitrification and nitrification processes may occur at the same time in the soil aggregates (Arah & Smith, 1990), (b) denitrification rates depend on NO_2^- and NO_3^- concentrations which are produced in nitrification processes and (c) no efficient inhibitor exists for the initial step of denitrification in soils (Garrido et al., 2002).

Spatial and temporal variability of soil impacts upon N_2O emissions. The high spatial variability observed in pasture N_2O fluxes is associated with irregular distribution of particulate organic matter, inorganic-N concentrations, and denitrification micro-sites in the soil (Parkin, 1987). It has previously been noted that animal trampling can cause soil compaction which is known to change the physical properties of a soil including bulk density, porosity, and WFPS under grazed pastures; and consequently increase N_2O emissions. Stocking rate, stock type and the grazing regime of pastures all affect N_2O emissions and an increase in small and compact aggregates in the soil results in maximum N_2O emissions (Luo et al., 2008b; Uchida et al., 2008). The temporal variation in N_2O fluxes is considerable and can vary both diurnally and seasonally. Diurnal variation is largely temperature induced with

maximum N_2O fluxes in the afternoon or early evening hours (Sherlock et al., 1992). Seasonal variation in N_2O emission patterns also appears to be driven largely by soil temperature and rainfall (Sherlock et al., 1992), growth season of plants, and after incorporation of plant residues (Pu et al., 1999; van der Weerden et al., 1999; Wang et al., 2008).

❖ *N_2O emissions measurement methods*

Several methods for determining N_2O fluxes have been developed; including:

I. Static chambers (Denmead, 1979);

II. Aerodynamic gradient methods (Christensen et al., 1996; Hargreaves et al., 1996; Smith et al., 1994); and,

III. Eddy correlation (Christensen et al., 1996; Hargreaves et al., 1996; Smith et al., 1994).

The use of static chambers dominate in N_2O emissions research and offer an efficient way for measuring N_2O emissions in various in-situ field and laboratory studies. However, several replications of each treatment are required due to the spatial and temporal variability of N_2O production (Jones et al., 2011). In this thesis, static chambers are used to quantify N_2O emissions. Potential effects of biochar on N_2O emissions are discussed in Section 2.3.4.

2.2.4 Environmental implications of N losses

Ammonia volatilisation from agricultural soils is implicated as a dominant factor in (a) the formation of atmospheric secondary aerosols due to NH_3 reacting with nitric and sulphuric acids in the atmosphere, (b) NH_3 contributing to the formation of acid rain, (c) acidification of soils, (e) acidification and eutrophication of lakes and rivers (Bobbink et al., 1992), (f) decreasing biodiversity in terrestrial Ecosystems (Martikainen, 1985; Mosier et al., 1998), (g) decreased methane (CH_4) oxidation rates in soils (Mosier et al., 1996), and (h) acting as an indirect source of N_2O (Barthelmie & Pryor, 1998; Bobbink et al., 1992; VanDer Eerden, 1982).

Nitrous oxide is a potent greenhouse gas with a 150 year atmospheric lifetime and a global warming potential 298 times that of CO_2 over a 100 year time horizon (Forster et al., 2007). The atmospheric concentration of N_2O is increasing linearly (0.26% yr^{-1}) and was 319 ± 0.21 μL L^{-1} in 2005 (Forster et al., 2007). This N_2O gas reacts indirectly with ozone in the stratosphere and therefore contributes to the catalytic destruction of the ozone layer that

protects us against UV light (Bothe et al., 2007; Crutzen, 1974; Duxbury et al., 1993; Robertson, 1993) . It has been theorised that a doubling of the atmospheric N_2O concentration could lead to a 12% decrease in total ozone in the stratosphere (de Correa et al., 1996).

From an environmental point of view, the conversion of NH_4^+ to NO_3^- in nitrification processes has advantages and disadvantages. Nitrification may reduce NH_4^+ toxicity, decrease the potential for NH_3 volatilisation, and help the release of fixed NH_4^+. However, it increases the mobility of N in the soil, potentially increasing N availability to plants, but also potentially increasing soil acidification and the availability of N for denitrification and leaching (Burden, 1982; Cameron & Haynes, 1986; Carter & Gregorich, 2008; Hogg, 1981; Ravishankara et al., 2009; Steele et al., 1984). Nitrate and dissolved organic nitrogen (DON) losses could account for 19 - 26% of the total soluble N in pasture and might affect ground-water quality (van Kessel et al., 2009). Denitrification has both positive and negative effects as well. The positive effect of denitrification is that it decreases the potential leaching of NO_3^- to ground and surface waters. The negative effect is that incomplete denitrification is a major source of the greenhouse gas N_2O and is a loss of N, otherwise available for the growth of plants.

Nitrate leaching and emissions of nitrogenous gases are two predominant pathways in grazed pastures, the common land use in New Zealand, and these N losses reduce the N efficiency in intensively grazed pasture systems (Haynes & Williams, 1993).

Globally, about 40% of anthropogenic N_2O emissions come from agricultural sources with livestock production contributing 30% of this total (Davidson & Mosier, 2004; Denman et al., 2007; Oenema et al., 2001). In New Zealand, the GHG inventory, when expressed as CO_2 equivalents, comprises 17% as N_2O with ruminant urine contributing 50% of the total N_2O emissions (de Klein et al., 2001; Petrie et al., 2007). In addition, NH_3 volatilisation dominates as an indirect source of N_2O which is represented as the released proportions of manure-N ($Frac_{GASM}$; the IPCC default of 0.2 or 20%[1]) and fertiliser-N ($Frac_{GASF}$; the IPCC default of 0.1 or 10%) to the atmosphere (Sherlock et al., 2008). Also N_2O emissions from grazing pastures are generally high, reaching up to 0.6 mg N m^{-2} h^{-1} after deposition of animal excreta in winter (de Klein et al., 2003).

In 2008, New Zealand's total GHGs were 13.9 Mt CO_2-e (22.8%) higher than the 1990 total GHGs level of 60.8 Mt CO_2-e (UNFCCC, 2008). This has consigned New Zealand to either

[1] Sherlock et al. (2008) recommended a New Zealand specific value of 0.1 could be considered for adoption (Sherlock et al., 2008).

reducing GHGs emissions for the period 2008 - 2012 to the 1990 level or taking the responsibility for any emissions over these levels (IPCC, 2006).
Biochar has not been assessed as a mitigation tool for any of these environmental consequences of N losses in pasture systems.

2.2.5 Mitigation of N losses

Several techniques have been used to mitigate gaseous N losses to date. One method is inhibiting urease activity by adding compounds such as N-(n-butyl) thiophosphorictriamide (NBPT), which is recognised as a urease inhibitor, to the urea fertiliser which slows the rate of hydrolysis. However, this can lead to the downwards movement of urea in the soil before hydrolysis (Chen et al., 2008). Applying NBPT on to grassland resulted in a reduction of NH_3 volatilisation by around 50%, however, the level of reduction was influenced by the NBPT concentrstion used (Watson et al., 1994a; Watson et al., 1994b).

Another approach is to use nitrification inhibitors such as nitrapyrin, dicyandiamide (DCD) and dimethylpyrazole phosphate (DMPP). Previous studies have shown the performance of nitrapyrin, DCD and DMPP can reduce the emission of N_2O by 51, 41 and 49%, respectively (Skiba et al., 1993; Weiske et al., 2001; Wolt, 2004). The use of inhibitor such as DCD does not increase N_2O emissions measured in New Zealand, when applied to pastures (Cameron et al., 2000; de Klein et al., 2001). On the contrary, more recent New Zealand studies have clearly shown that DCD applied as a fine particle suspension to urine patches results in an average reduction in N_2O emissions of 70% (Di et al., 2007). However, soil moisture, texture and temperature influence the effectiveness of these inhibitors. The effectiveness of DMPP, like DCD, is influenced by temperature, soil texture, and moisture (Barth et al., 2001; Merino et al., 2005; Pasda et al., 2001).

Improved nutrient management, i.e. reducing N inputs to the animal using forage-based options, and collecting animal waste to avoid times when N_2O emissions are potentially high, has also been assessed as a possible mitigation option (Luo et al., 2008a). The potential to use biochar as a mitigation tool is discussed in Section 2.3.4.

2.3 Biochar

Biochar is a by-product of the pyrolysis of organic material (e.g. wood, plants, and plant waste) (Lehmann & Joseph, 2009). The manufactured biochar can be used as a soil amendment while volatile gases and fluids can be captured and used as energy (Davies, 2007;

DeLuca et al., 2006). The most straightforward effect of applying biochar to soil is a net withdrawal of CO_2 from the atmosphere, due to the embodied C in the biochar (Lehmann, 2007a; Wardle et al., 2008).

2.3.1 Pyrolysis

Pyrolysis is the decomposition of organic material in a combustion and gasification process with little or no available O_2. The products of pyrolysis processes can be solids, liquids or gases (Lehmann, 2007a; Winsley, 2007). During pyrolysis, mass is mostly lost in the form of volatile organics. Chemically bound moisture in organic materials can be lost by thermal decomposition at temperatures above 120°C. Hemicelluloses are degraded at 200°C to 260°C, cellulose at 240°C to 350°C, and lignin at 280°C to 500°C (Sjöström, 1993).
Approximately 50% of the feedstock carbon content is converted to biochar; depending on the type of feedstock, and the other 50% is volatilised as gases; mainly, H_2, CO_2 and CO, but also CH_4 and other volatile hydrocarbons. There are some methods available which can condense these gases into bio-oils or bio-gas products that can be used by industry as crude fuels. This crude fuel can be cleaned and processed to produce either higher quality fuel such as diesel or gasoline fuel or refined to produce chemical feed stocks such as wood tar, aromatic oils, and pyroligneous liquor (Baum & Weitner, 2001; Benites et al., 2007; Chan et al., 2007; Claudia et al., 2007; Knicker, 2007; Libra et al., 2011).

2.3.2 Biochar properties

The type of feedstock which is used to manufacture biochar influences the biochar carbon content. Properties such as CEC, pH, electrical conductivity and surface area of fresh biochar are all dependent on the type of feedstock, composition of organic and mineral fractions, and process conditions under which the biochar is produced (Figure 2.1). Increasing the pyrolysis temperature raises biochar pH, CEC and surface area of the biochar. This is associated with a decrease in C yield from the original plant biomass (Chan & Xu, 2009; Lehmann, 2007a; Singh et al., 2010).

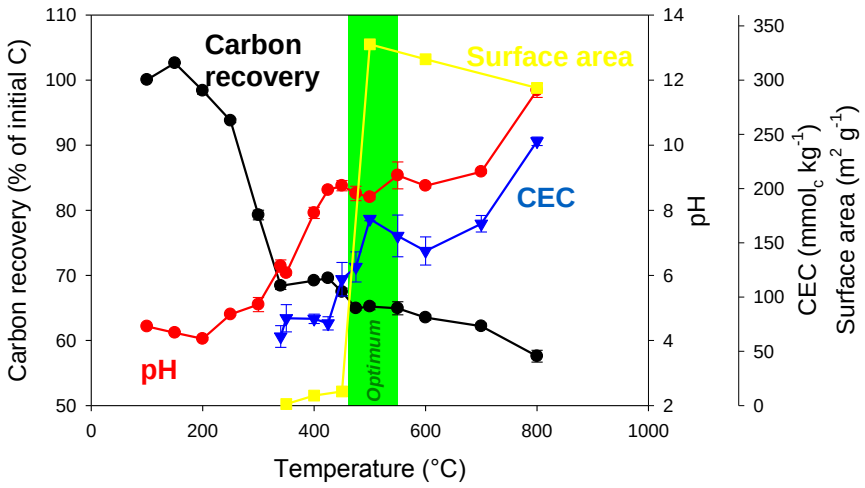


Figure 2.1 The effect of pyrolysis temperature on C recovery, CEC (measured at pH 7), pH, and surface area. Wood pyrolysed in closed containers (0.025 m^3) with a heating rate of 0.4°C per minute area (Lehmann, 2007a).

The chemical composition of the biomass feedstock has a direct impact upon the physical nature of the biochar produced. Biochars prepared at higher temperatures (500°C to 700°C) are dominated by aromatic (aryl) C; well carbonized and plant-characteristic functional group C structures are removed, as indicated by low H/C ratios and low O content, and also have a high surface area. In contrast, biochars formed at lower temperatures (300°C to 400°C) still contain the characteristics of the original plant material and are only partially carbonized, with high H/C ratios and O contents, and have a lower surface area. The conversion of aliphatic C to aromatic C, a gradual decrease in the amounts of OH and CH_3 and an increase in C=C with increasing temperature (150°C to 550°C), during pyrolysis is accompanied by a reduction in biochar C mineralisation rates. This reduction in mineralisation of biochar C also suggests a reduction in the availability of nutrients such as N, P and S in biochar that are bound in the organic structure. Volatile organic compounds (VOCs) remain on the biochar surfaces immediately following pyrolysis; and can be energy sources for microbes (Spokas, 2010). However, these compounds might be turned over in one or two seasons and are unlikely to remain on biochar for a long period (Lehmann & Joseph, 2009; Ogawa, 1994; Zackrissona et al., 1996). As a consequence, low-temperature biochars are found to have higher amounts of amphoteric (acid-basic) surface functional groups (Lehmann & Joseph, 2009). Various functional groups on the surfaces of biochar can either donate or withdraw electron density to or from the arene structures present in the biochar. The pi (π) bonds present in these arene rings and influence the surface charge on the biochar and its sorption

capacity. Electron resonance can be further modified due to the presence of either electron donating or electron withdrawing functional groups bound to the arene rings. An electron-donating resonance effect is observed when functional groups such as OH, NH_2, OR or O(C=O)R with a lone pair of electrons are directly bonded to an arene ring. In contrast, an electron-withdrawing resonance effect is observed when groups such as (C=O)OH, (C=O)H or NO_2 draw electron density from an arene ring (Lehmann & Joseph, 2009; Smith, 2005).

Biochar may reduce either the concentration of phenolic compounds in the soil solution which might cause the immobilisation of inorganic N, P or S; or the activity of the compounds that could inhibit the nutrient transformation (Paavolainen et al., 1998; Schimel et al., 1996; Stevenson & Cole, 1999; Ward et al., 1997; White, 1991).

Biochar can show basic or acidic (i.e. amphoteric) pH values in aqueous dispersions. A high O content of the functional groups on biochar results in low pH (acidity) and high CEC, while low O contents of the functional groups on biochar result in basic surface properties and anion exchange (AEC) behaviour (Cheng et al., 2008).

Biochar has two types of density: the solid density and the bulk or apparent density. Solid density is the density at the molecular level, related to the degree of packing of the C structure. Bulk density is that of the material consisting of multiple particles and includes the macroporosity within each particle and the inter-particle voids (Lehmann & Joseph, 2009). Bulk density is closely related to porosity, penetrability, drainage and aeration of biochar materials (Brady & Weil, 2002).

The feedstock and process conditions can potentially be used to predict these biochar properties. However, each type of biochar should be tested based on a biochar classification scheme before application to the soil for its various impacts on soil properties (BioEnergy, 2008).

2.3.3 Effects of biochar on soil physical and biochemical properties

Incorporation of biochar into soil may provide benefits for the physical, chemical and biological properties of the soil and sustainable agriculture (Lehmann & Joseph, 2009). Indications exist that biochar might have not only the same benefit as other organic amendments in the soil, but might also sequester more C than other organic matter amendments and retain exchangeable cations and/or plant available nutrients better than these organic matters in soil, while decreasing environmental pollution and eutrophication risks (Chan et al., 2007; Glaser et al., 2002) . In addition, biochar might also retain organic matter within its pore and preserve it from degradation (Zimmerman et al., 2011).

Biochar can store C in soil due to its polycyclic aromatic structure which makes it stable in the environment (DeLuca et al., 2006). Biochar applications to soil increase soil aeration (Laird, 2008), water holding capacity, penetrability and drainage (Brady & Weil, 2002; Glaser et al., 2002; Isobe et al., 1996; Steiner et al., 2007; Warnock et al., 2007).
Soil pH may increase following the addition of alkaline biochar, and consequently bio-available P and base cations increase which can lead to increases in plant yield responses (Chan et al., 2007; Van Zwieten et al., 2007; Warnock et al., 2007) . One previous study showed the application of biochar with pH = 9.4 to acid soil increased soil pH from 4.20 to 5.93 and from 4.20 to 5.73 with and without fertiliser, respectively (Van Zwieten et al., 2007).
Biochar applications can potentially improve CEC and soil base saturation (Glaser et al., 2002; Lehman, 2007b; Major et al., 2010a; Major et al., 2010b) reflecting an increase in the retention of nutrients (e.g. N) in the soil and nutrient use efficiency (Koide et al., 2011; Lehmann, 2007a). Subsequently, root development and crop yield may increase (Chan et al., 2007; Glaser et al., 2002; Lehmann et al., 2003; Novak et al., 2009; Steiner et al., 2007; Warnock et al., 2007). In addition, biochar might reduce leaching in the soil (Laird et al., 2010). Biochar applications can also potentially create a suitable environment for the growth of beneficial microbes and symbionts (Lehman, 2007b; Noguera et al., 2010; Warnock et al., 2007). Ishii and Kadoya (1994) proposed that elongation of roots and the intensity of vesicular arbuscular mycorrhiza infection increases in biochar treated soils (Ishii & Kadoya, 1994). Moreover, biochar incorporation into soils can confine soil microbial populations that contribute to plant disease (Nerome et al., 2005). Biochar additions may reduce phyto-toxicity of soil pollutants due to adsorption or immobilisation of these compounds (Beesley et al., 2010; Novak et al., 2009; Smernik, 2009).

2.3.4 Biochar and the N cycle

Besides potentially sequestering C, biochar may also provide agronomic benefits (Sohi et al., 2010) and alter the N transformation rates within the soil (Clough and Condron, 2010; and references therein). Examples of N fluxes and transformations affected by biochar addition include inorganic-N leaching (Singh et al., 2010), NH_3 volatilisation (Steiner et al., 2010), N-fixation (Rondon et al., 2007), nitrification (Ball et al., 2010), and N_2O emissions (Spokas et al., 2009). However, to our knowledge, there has not been any study that has examined the effects of biochar incorporation on soil N dynamics and N_2O emissions following either urine or N fertiliser application under pasture conditions.

Several laboratory-based studies have documented the suppression of N_2O emissions as a result of biochar addition to soils (e.g. (Bhandral et al., 2007; Renner, 2007; Rondon et al., 2005). Rondon et al. (2005) showed N_2O emissions reductions of up to 50% and 80% on soybean and grass pots following biochar addition, respectively (Rondon et al., 2005). This suppression of the N_2O emissions was considered to be due to either (a) the alkaline nature of the biochar and thus the potential to increase N_2O reduction via denitrification, (b) the porous structure of biochar particles affecting the biochar water absorption capacity and consequently affecting O_2 availability in the soil which might have influence on denitrification and N_2O emissions from soil, and (c) lower mineralisation as a result of a higher C:N ratio or lower C quality leading to lower nitrification rates (Yanai et al., 2007). Furthermore, following pyrolysis of biomass, some microbially toxic compounds such as furan may be retained on or in the biochar (Knicker, 2007). Also, a majority of biochars exhibit ethylene production which might reduce GHGs such as N_2O due to inhibition of microbial NH_4^+ nitrification (Porter, 1992). This ethylene could be produced in the soil in association with microbial communities on biochars (Spokas et al., 2010).

Cayuela et al. (2010) demonstrated that biochar, produced from green waste and poultry manure, reduced soil N_2O emissions relative to a control soil when incubated for 60 days at 20^{o}C and 80% WFPS (Cŭhel et al., 2010). An experiment by Singh et al. (2010) used repacked soil columns, into which were mixed poultry and wood waste biochars. After several wetting drying cycles the N_2O emissions were reduced by up to 72% (Singh et al., 2010). Using an unweathered biochar in a laboratory experiment, Clough et al. (2010) found the effect of biochar incorporation (20 t ha^{-1}) initially stimulated N_2O emissions in the presence of ruminant urine, although the cumulative N_2O flux over time was not significantly different from a urine-only treatment. Thus biochar incorporation into soil can affect N_2O fluxes but detailed field data are lacking. There have, to our knowledge, been no reports of in-situ work performed in pastures under either fertiliser or urine treatments.

On the other hand, Deluca et al. (2006) showed that the addition of field collected charcoal and NH_4^+ on pine forest soils increased nitrification rate due to the alleviation of the factors inhibiting the nitrifying microbial community. However, these mechanisms might be specific to forest soils with ectomycorrhizal trees and/or ericaceous shrubs which produce phenolic compounds; and the scenario is potentially different in agricultural soil (DeLuca et al., 2006; Lehmann et al., 2006; Lepage et al., 2006; Warnock et al., 2007).

Biochar additions have also significantly increased Biological Nitrogen Fixation (BNF) and improved biomass production of common beans at different rates, possibly as a result of (a)

lowering inorganic N availability in the soil due to the high C/N ratio of the biochar, (b) the availability of the other macro nutrients other than N or micronutrients and higher pH, and (c) the biochar enhanced mycorrhizal infection (Rondon et al., 2007).

2.4 Research needs

It has been estimated that current net emissions of CO_2, CH_4 and N_2O could be reduced by 12% per annum if biochar was used to sequester C into soil (Woolf et al., 2010). However, any positive or negative environmental effects of sequestering C as biochar need to be understood and quantified (Lehmann et al., 2006).

Several mechanisms may be potentially affected by biochar thus biochar incorporation could lead to decreases in NH_3 and N_2O emissions from soil: (a) biochar can uptake and adsorb NH_3, (b) biochar addition to soils results in altered levels of soil porosity, water holding capacity and/or other in soil physical properties that could affect N_2O emissions, (c) biochar in soil alters levels of nutrient availability leading to altered plant growth and microbial biomass and activity which could affect biotic and abiotic processes of N_2O production in soil (Bailey et al., 2011; Durenkamp et al., 2010; Smith et al., 2010), and (d) biochar may reduce N availability due to either adsorption of inorganic-N or immobilisation during initial decomposition of biochar which is an N-poor product (Warnock et al., 2007; Yanai et al., 2007). It must be noted that biochar might be oxidised with increasing biochar residence time in soil and this might result in altered biochar properties (Abiven et al., 2011 ; Brodowski et al., 2005; Hilscher & Knicker, 2011). These changes in properties over time need to be determined with respect to biochar's effectiveness as a soil amendment.

Much of the research at the time of writing this literature review has been unfocused and spread over many aspects of N cycling, N fluxes and biochar, with none having examined grazed pasture systems. An improved understanding is required with respect to N cycling and potential mitigation of environmentally detrimental N losses of the grazed pastoral ecosystem's response to biochar application.

Chapter 3
Materials and Methods

3.1 Soil collection and analyses

3.1.1 Soil collection and storage

Soil collection processes are detailed in the 'Materials and Methods' sections of the following chapters. Within 24 h of each experiment starting, soils were collected and sieved, and then stored at 4°C, until use. Major physicochemical properties of the soils used in the following experiments are presented in the related chapters.

3.1.2 Bulk density

Soil bulk densities were determined using a core method. Soil cores (0.073 m diameter × 0.075 m deep) were taken and dried in the oven at 105°C. Bulk density was determined using Equation 3.1 (Topp & Ferré, 2002):

$$\rho_b = \frac{M_S}{V_t} \qquad \textit{[Equation 3.1]}$$

where;

ρ_b : bulk density (g cm^{-3})

M_S : mass of oven dry soil (g)

V_t : core volume (cm^3)

3.1.3 Gravimetric and volumetric soil moisture contents

Moist soil cores were weighed and then oven dried at 105°C. After cooling, these cores were reweighed, and the mass of water lost as a percentage of the mass of the dried soil was calculated using Equation 3.2 (Topp & Ferré, 2002):

$$\theta_g = \frac{M_W}{M_S} \qquad \textit{[Equation 3.2]}$$

where;

θ_g : gravimetric soil moisture content (g water g^{-1} oven dry soil)

M_W : mass of water (g) equals mass of field moist soil (g) – mass of oven dry soil (g)

Volumetric soil water content was calculated as follows (Topp & Ferré, 2002):

$\theta_v = \theta_g \times \rho_b$ *[Equation 3.3]*

where:.

θ_v : volumetric water content (cm^3 water cm^{-3} soil)

3.1.4 Soil porosity and water-filled pore space

Total porosity, ∅, was calculated as follows (Topp & Ferré, 2002):

$\emptyset = 1 - (\frac{\rho_b}{\rho_p})$ *[Equation 3.4]*

where;

ρ_p : soil particle density (g soil cm^{-3} soil) [assumed to be 2.65 g cm^{-3}]

Then, soil water-filled pore space (WFPS) was calculated as follows:

$WFPS = \frac{(\theta_g \times \rho_b)}{\emptyset} \times \frac{100}{1}$ *[Equation 3.5]*

where;

WFPS : water-filled pore space (%)

3.1.5 Soil pH

Soil surface pH was measured using a Hanna HI 9025C portable pH meter fitted with a soil surface probe (Broadley-James Corporation, Irvine, CA, USA). The pH meter was calibrated with pH 4, pH 7 and pH 9 buffer solutions prior to performing analyses. To take a reading, the tip of the pH electrode probe was pressed gently against a small subsample that was wetted with one drop of deionised water. A stable reading was typically obtained within 10-15 seconds.

3.1.6 Inorganic-N

Soil inorganic-N was extracted with a 2 M KCl solution (Mulvaney, 1996) at a 1:10 ratio of soil: solution. The extractant was then centrifuged at 2000 rev min^{-1} (480 × *g*) for 10 min, and filtered (Whatman No. 41) into 30 mL screw-top plastic containers before storage at 4°C until analysis.

The KCl extracts were analysed for three forms of soil-extractable inorganic-N: ammonium-N (NH_4^+-N), nitrite-N (NO_2^--N) and nitrate-N (NO_3^--N) (Blakemore et al., 1987). These analyses were performed on a twin-channel flow injection analyser (Model FS 3000, Alpkem, College Station, Texas). Ammonium-N was determined spectrophotometrically at 590 nm

following NaOH addition and diffusion as $NH_{3(aq)}$ through a gas diffusion membrane and reduction with a proprietary colour-forming reagent. The NO_3^--N was reduced to NO_2^--N using a cadmium reduction coil (open-tubular cadmium reactor) and the total NO_2^--N then reacted with sulphanilamide/NED to form an azo-dye compound. The colour intensity of this compound was determined spectrophotometrically at 540 nm. Initial NO_2^--N was determined as above but without the cadmium reduction coil. The NO_3^--N ion concentration was then calculated from the difference between the two NO_2^--N measurements.
Equation 3.6 was used to calculate the inorganic-N concentration of each sample:

$$N_S = \frac{(N_e \times V)}{M_S} \qquad \textit{[Equation 3.6]}$$

where;

N_S : inorganic N content (mg kg^{-1} dry soil)

N_e : inorganic N concentration of extract (mg L^{-1})

V : volume of solution (KCl + soil moisture) (L)

M_S : mass of oven dry soil (kg)

3.1.7 Total-N and atom% ^{15}N

Total-N and atom% ^{15}N at both enriched and natural abundance level were determined using Continuous Flow Isotope Ratio Mass Spectrometer (CFIRMS) (Sercon, U.K.). Samples (soil, biochar or plant) were initially combusted at 1000°C, using a Cr_2O_3 catalyst and a pulse of pure O_2 in a helium (He) carrier in an automated Dumas style elemental analyser which was linked to a PDZ Europa 20-20 stable isotope ratio mass spectrometer. During the combustion process, N_2, NO_x, CO_2 and H_2O were produced. Hot Cu reduced NO_x to N_2 and removed excess O_2. Water was removed using the desiccant trap, $Mg(ClO_4)_2$. A packed-GC column separated N_2 from trace impurities for analysis of ^{15}N and total-N (Boutton & Yamasaki, 1996).

3.2 Urine collection and analyses

The details of the urine collection for each study are included in the 'Materials and Methods' sections of the following chapters. For all experiments, urine was collected from multiple dairy cows one day prior to application. The urine was bulked and analysed for total-N, using Continuous Flow Isotope Ratio Mass Spectrometer (CFIRMS) (PDZ Europa Ltd, Crewe,

UK), by pipetting 10 µL of urine onto a bed of chromosorb-W inside a tin capsule. Samples were analysed in triplicate.

3.3 Headspace gas sampling and analyses

In general, samples for N_2O were taken from ambient air at time zero, immediately before placing the headspace chambers on the chamber rings. The headspace chambers were then sampled at 0.25 and 0.50 hourly intervals. To take the gas samples, a glass syringe equipped with a 3-way stopcock and a 0.5 mm gauge needle were used. The syringe was flushed with ambient air and then injected into the chamber headspace, whereupon the syringe was gently flushed twice. A 10 mL gas sample was then taken and injected into a pre-evacuated 6 mL Exetainer® (Labco Ltd, High Wycombe, UK) causing the vials to be over-pressurised to prevent diffusion of external air into the sample. The vials were reduced to ambient pressure immediately prior to analysis using a double-ended needle to release the extra pressure into a beaker of water.

3.3.1 Gas chromatographic analysis

Nitrous oxide samples were analysed using a gas chromatograph (GC) (8610, SRI Instruments, CA) interfaced to a liquid autosampler (Gilson 222XL, Middleton, WI) as described by Clough et al. (2006) and similar to the configuration used by Mosier and Mack (1980). To enable gas analyses a purpose-built double concentric injection needle replaced the liquid sample sipper on the autosampler, allowing rapid purging of the gas sample for injection. A 1 m long pre-column preceded a 6 m long analytical column, both 3 mm OD stainless steel packed with Haysep Q. An automated 10-port gas-sampling valve on the GC sent the oxygen-free N_2 carrier gas (40 mL min^{-1}) through both the pre-column and analytical column in series (in inject mode) or back-flushed the pre-column. At the posterior end of the analytical column a 4-port gas sampling valve was synchronised to send the gas stream to the detector. Nitrous oxide concentrations were quantified with a hot (313°C) ^{63}Ni electron capture detector (ECD). Each batch of N_2O samples was preceded by a series of standards (0, 1.4, 2.9, and 5.8 µL L^{-1}) and interspersed with 1.2 µL L^{-1} reference standards. Emissions were calculated using Equation 3.7 (Anthony & Hutchinson, 1990; Hutchinson & Mosier, 1981):

$$F_{N_2O} = \left\{ \frac{V_c (C_1 - C_0)^2}{2(C_1 - C_2 - C_0)} \times ln\left[\frac{C_1 - C_0}{C_2 - C_1}\right] \times \frac{P C_{ha} C_D M_{N_2}}{[G_c (T_K + T_{C^\circ})] A_c t_1} \right\} \times 10^2 \qquad \textit{[Equation 3.7]}$$

where;

F_{N_2O} : N_2O flux (µg N_2O-N m^{-2} d^{-1})

C_0 : N_2O concentration at time 0 (µL L^{-1})

C_1 : N_2O concentration at time 1 (µL L^{-1})

C_2 : N_2O concentration at time 2 (µL L^{-1})

P : atmospheric pressure (Pa) [101325]

V_c : chamber volume (m^3)

A_c : chamber area (m^2)

G_c : gas constant (J K^{-1} mol^{-1}) [8.314]

T_K : absolute temperature at 0°C (K) [273.15]

T_{C° : air temperature at 5 cm height (°C)

C_{ha} : conversion factor m^2 to ha [10000]

C_D : minutes per day (min) [1440]

M_{N_2} : molecular weight of N_2O-N (g mol^{-1}) [28.0314]

t_0 : start of cover period (min)

t_1 : gas sample time 1 (min)

t_2 : gas sample time 2 (min)

3.3.2 Continuous flow isotope ratio mass spectrometry (CFIRMS)

3.3.2.1 The collection and isotopic enrichment of urine

Urea, enriched with ^{15}N (98.0 atom% $^{15}N_2$-urea; Isotec, Miamisburg, Ohio), was added to the urine to allow isotope trace analysis of urea-N, the dominant N form applied in urine. The final ^{15}N enrichment of the urine is indicated in the following chapters. All isotopically labelled urine samples were analysed (Section 3.2).

3.3.2.2 N_2O-^{15}N sampling and analyses

I. $^{15}N_2O$ sampling

In general the headspace chambers remained in position for 2 h whereupon 15 mL gas samples were taken and placed into pre-evacuated 12 mL Exetainers® (This ensured that the Exetainers® were over-pressurised, to prevent sample contamination.), for subsequent determination of the N_2O-^{15}N enrichment (Clough et al., 2006).

II. CFIRMS analysis

Immediately prior to analysis, the Exetainers® were reduced to ambient pressure using a double-ended needle to release the extra pressure into a beaker of water. The gas samples were automatically injected into the TGII trace gas system and the N_2O in the gas sample was concentrated by cryo-trapping and focusing. The gas was then carried in the He carrier flow to the CFIRMS. Each run was bracketed by N_2O standards (35 µL L^{-1}) and interspersed with 2 standards after every 9 samples analysed.

3.3.2.3 NH_4^+-^{15}N and NO_3^--^{15}N analyses

I. Reagents

A 2.5 M solution of potassium hydrogen sulphate ($KHSO_4$) was prepared by adding 7 mL of concentrated sulphuric acid (H_2SO_4) and 22 g of potassium sulphate (K_2SO_4) to distilled water (DI) to make 100 mL final volume. Heavy magnesium oxide (MgO) powder was heated in a muffle furnace at 600°C for 2 h, cooled, and then stored in an airtight container. Lengths of stainless steel wire (7 cm) were bent to allow them to be wedged across the top of a 120 mL plastic screw-top container. A 5 mm disk, cut from glass filter paper with a hole punch, was threaded onto each wire and 10 µL of 2.5 M $KHSO_4$ was pipetted onto the disk.

II. Method

The diffusion method of Brooks et al. (1985) was used to determine NH_4-^{15}N (Brooks et al., 1989). It involved creating a basic environment to convert all NH_4^+ to NH_3 gas, which was then trapped on the acidified filter paper. Sufficient 2 M KCl soil extract or 0.1 M H_2SO_4 from the NH_3 traps were used so that a total of 50 µg NH_4^+-N was pipetted into each container and 0.2 g MgO was added. Immediately thereafter a wire and acidified disk were inserted across the top of the container and the lid was replaced. The contents of each container were carefully swirled with a glass bead so that the ingredients would mix without splashing the disk. The containers were incubated at room temperature (20 - 25°C) for 6 days. After this time the wires were removed with tweezers and placed into a drying rack, with the end of the wire in contact with the tweezers being put into the hole in the rack. This ensured that the disks could be pushed off the uncontaminated end of the wire when dry. After 24 h in a 50°C oven the dried disks were removed and placed into individual tin capsules for CFIRMS analysis. For the NO_3^--^{15}N procedure, the NH_4-^{15}N method was carried out and after removing disks for analysis, a new set of disks were prepared and placed onto wires. Then Devarda's alloy was added in order to reduce the NO_3^- to NH_4^+. Immediately a wire and acidified disk were inserted across the top of the container and the lid was replaced. The

contents of each container were carefully swirled with a glass bead so that the ingredients would mix without splashing the disk. The containers were incubated at room temperature (20 - 25°C) for 6 days.

III. CFIRMS analysis

The encapsulated diffusion disks were placed into an autosampler tray atop a GSL automated Dumas system and the samples were dropped in sequence into a combustion tube and combusted at 1000°C in an oxygen atmosphere. The gases generated were carried onto a GC column for separation and then into the CFIRMS for analysis. Each run commenced with a series of calibration standards, wheat flour (Elemental Microanalysis, 2.12% N, 1.66‰ ^{15}N), which had been calibrated against IAEA N-1 (0.4‰ vs. N_2 in air). In addition, two reference standards were included after every 10 samples, and the first sample of every nine was analysed in duplicate.

3.4 NH_3 measurements

In general NH_3 volatilisation was measured using either an array of "Leuning samplers" (Leuning et al. 1985), which are devices that point into the wind and trap the NH_3 in the air passing through the device in the field (Chapter 6), field-based static chambers (Chapter 4) or laboratory-based dynamic chambers with acid traps (Chapter 7). The ammonia collection processes are detailed in the 'Materials and Methods' sections of those chapters.

The measurement of the NH_3 concentration in the samples was carried out using an ion specific electrode (HUN model ISE-10-10-00). The NH_3 electrode is a modified pH electrode consisting of a normal 'glass' pH electrode with a water-repelling Teflon® membrane stretched tightly over its active surface. After addition of 10 M NaOH to convert NH_4^+ ions in samples to un-ionised NH_3, the NH_3 then diffuses through the Teflon® membrane to interact with the glass electrode surface providing a voltage reading proportional to the logarithm of the NH_3 concentration. The response of the electrode was linear with respect to the log of the NH_4^+-N concentration over a wide dynamic range from ~ 0.02 µL L^{-1} NH_4^+-N to ~100 µL L^{-1} NH_4^+-N. Below 0.015 µL L^{-1} NH_4^+-N, the electrode response deviated from linearity and equilibration times (the time for the reading to stabilise) became excessive. Thus, the lowest NH_4^+-N concentration that could be determined with any reliability was ~ 0.02 µL L^{-1}. Fresh standards were prepared (0.02 µL L^{-1} to 100 µL L^{-1}) to provide a standard curve. The NH_3 concentration of each sample was calculated using

standards that were produced at the beginning and at the end of each sample measurement period.

3.5 Plant measurements and analyses

3.5.1 Dry matter yield

In Chapter 4, 5 and 7 the herbage was harvested by hand. Roots (Chapters 4 and 7) were rinsed with distilled water, to remove soil particles, then leaves and roots were dried at 60°C for 48 h, and weighed.

3.5.2 Total plant N and isotopic composition

The oven dried plant samples were ground (< 200 µm) prior to determination of total-N and ^{15}N enrichment by CFIRMS (Section 3.1.7).

3.5.3 Total ^{15}N recovery

Shoot and root recovery of ^{15}N applied was also determined as follows (Cabrera & Kissel, 1989):

$$N\ recovered\ (\%) = \frac{100 \times (p \times (c-b))}{(f \times (a-b)} \qquad [Equation\ 3.8]$$

where;

p : moles of N in the labelled sample

f : moles of N applied

c : atom % ^{15}N abundance in the labelled sample

a : atom % ^{15}N abundance of N applied

b : atom % ^{15}N abundance of unlabelled sample (natural abundance)

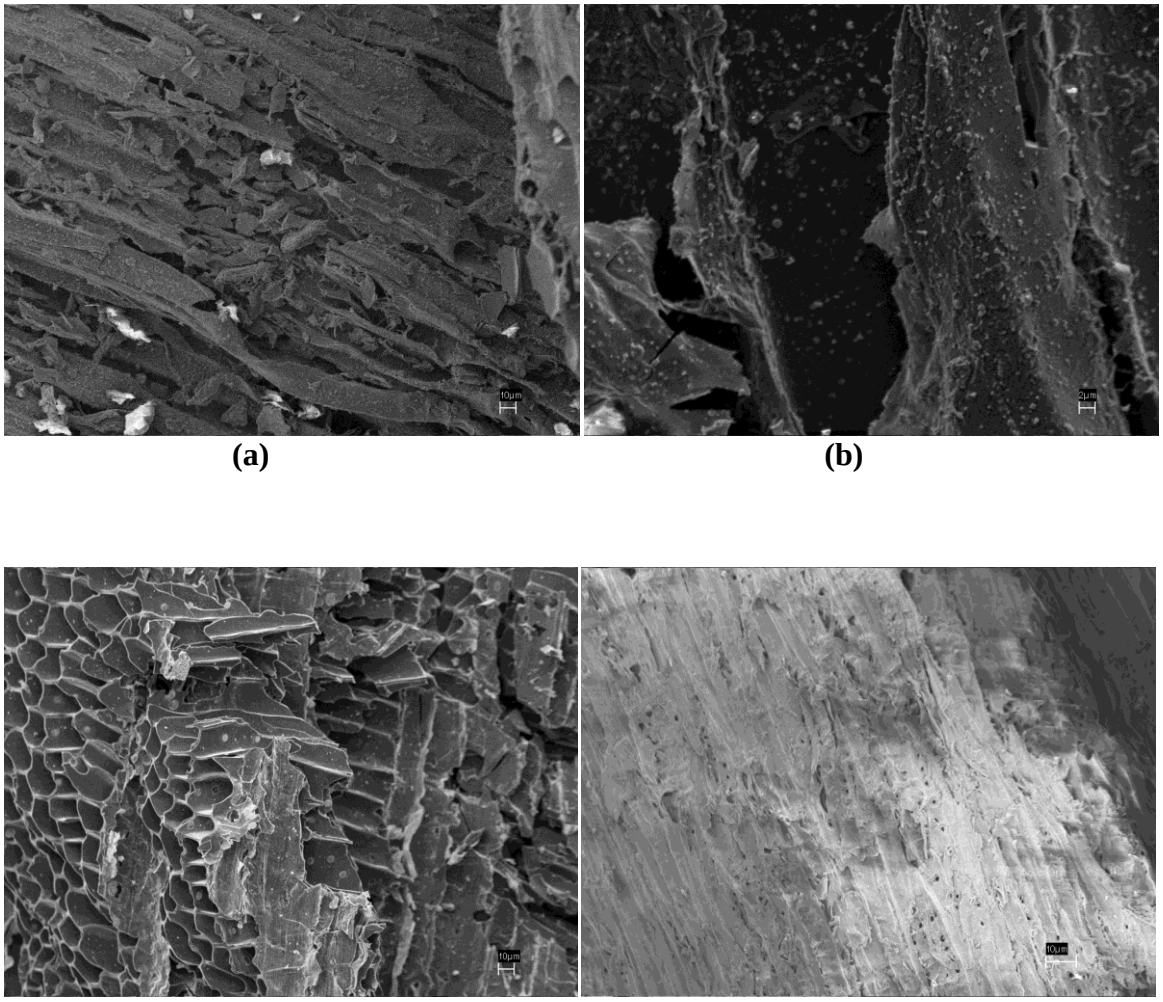
3.6 Biochar analyses

Four biochars manufactured from *Pinus radiata* (Table 3.1), and supplied by Carbonscape™ Limited and Slater Limited, were characterised for pH, electrical conductivity (EC), cation exchange capacity (CEC), anion exchange capacity (AEC), elemental analysis, the presence of VOCs, specific surface area, surface acidity, particle and bulk densities, as described below.

Table 3.1 Biochar treatments

Biochar	Description
	Feedstock : Radiata Pine
BC_1	Pyrolysis Temperature : 300°C,
	Heating Rate : Fast 5°C min^{-1}
	Pyrolysis Vacuum : Shallow 75 kPa
	Agitation factor[o] : -
BC_2	Feedstock : Radiata Pine
	Pyrolysis Temperature : 300°C,
	Heating Rate : Fast 5°C min^{-1}
	Pyrolysis Vacuum : Shallow 75 kPa
	Agitation factor[o] : +
BC_3	Feedstock : Radiata Pine
	Pyrolysis Temperature : 350°C
	Heating Rate : Not known
	Pyrolysis Vacuum : Not known
	Agitation factor[o] : -
BC_4	Feedstock : Radiata Pine
	Pyrolysis Temperature : 500°C
	Heating Rate : Fast 5°C min^{-1}
	Pyrolysis Vacuum : Deep 10 kPa
	Agitation factor[o] : -

[o]+ Agitation factor (putting into motion by stirring) applied. - Agitation factor did not apply.



(a) **(b)**

(c) **(d)**

Figure 3.1 SEM images (2000 ×) of four different biochars; (a) BC_1, (b) BC_2, (c) BC_3, and (d) BC_4. Scale bar equals 10 µm.

3.6.1 pH

Biochar pH (in both water and 0.01M $CaCl_2$) was measured using the method of Blakemore et al. (1987). This involved adding 10 mL of DI water or 0.01 M $CaCl_2$ to 1 g of air-dried biochar (1:10 biochar : solution ratio), stirring and leaving the mixture to stabilise overnight (for 24 h).

Then, the pH was measured with a SevenEasy pH meter (Mettler Toledo, Columbus, OH, USA) which had been calibrated using buffers pH 4 and pH 7.

3.6.2 Electrical conductivity

Electrical conductivity (EC) was determined based on the method of Blakemore et al. (1987). Air-dried biochar was shaken with DI water at a 1:10 biochar: water ratio for 30 min at 20°C. The mixture was then centrifuged at 1500 rev min^{-1} (270 × *g*) for 5 min (Blakemore et al., 1987). Conductivity was then immediately measured, using a Radiometer Copenhagen CDM 83 electrical conductivity meter that was calibrated using a 0.01 M KCl solution. The conductivity readings (μS cm^{-1}) were divided by 1000 for conversion into dS m^{-1} (Miller & Curtin, 2008).

3.6.3 Particle density

Particle density of samples was defined as the ratio of biochar mass (m_{BC}) to the volume of the biochar particle enclosed within the outer surface of the particle (V_{BC}). The volume of oven-dried biochar was determined using a conventional pycnometer (density bottle) by volume displacement with kerosene. The following Equations were used to calculate biochar particle density of each sample.

The mass of the biochar was calculated using the formula:

$$m_{BC} = m_2 - m_1 \qquad \textit{[Equation 3.9]}$$

where;

m_{BC} : mass of biochar sample (g)

m_1 : mass of density bottle (g)

m_2 : mass of density bottle plus biochar sample (g)

The volume of the biochar sample was determined using the formula (Gupta, 2002; Rasul et al., 1999):

$$V_{BC} = \frac{m_k - (m_4 - m_3)}{\rho_k} \qquad \textit{[Equation 3.10]}$$

where;

V_{BC} : volume of biochar sample (cm^3)

m_k : mass of kerosene to fill pycnometer (g)

m_3 : mass of pycnometer plus biochar (g)

m_4 : mass of pycnometer plus biochar plus kerosene in (g)

ρ_k : density of kerosene at 25.0°C ± 0.1°C (g cm^{-3})

Finally, the density of biochar sample was defined using the formula (Gupta, 2002; Rasul et al., 1999):

$$\rho_p = \frac{m_{BC}}{V_{BC}} \qquad \textit{[Equation 3.11]}$$

where;

ρ_p : particle density of biochar (g cm^{-3})

m_{BC} : mass of biochar (g)

V_{BC} : volume of biochar (cm^3)

3.6.4 Bulk density

Bulk density of biochar material consists of multiple particles and includes the macroporosity within each particle and inter-particle voids (Lehmann & Joseph, 2009). The volume of oven-dried biochar was determined using mercury (Hg) volume displacement (Pastor-Villegas et al., 2006). Using a measuring cylinder a biochar particle of known mass was immersed into a known volume of of Hg. Equation 3.12 was used to determine the biochar bulk density:

$$\rho_b = \frac{m_{BC}}{v_t} \qquad \textit{[Equation 3.12]}$$

where:

ρ_b : bulk density of biochar (g cm^{-3})

m_{BC} : mass of biochar (g)

v_t : total biochar volume (cm^3), equal to the displaced volume of Hg

3.6.5 Cation exchange capacity

I. Reagents and standards

Cation exchange capacity was measured using a silver thiourea (AgTU) method (Blakemore et al., 1987). Thiourea reagent was made by dissolving 30 g thiourea in DI water and making up to 2 L. The 0.01 M AgTU was made by dissolving 15 g thiourea in about 300 mL of DI water in a 1 L container and then dissolving 1.699 g

silver nitrate in about 500 mL of DI water and adding it to the thiourea solution and making up to 1 L with DI water.

Standards were prepared in five 100 mL volumetric flask as below:

1. 100 mL thiourea,
2. 25 mL AgTU and 75 mL thiourea,
3. 50 mL AgTU and 50 mL thiourea,
4. 75 mL AgTU and 25 mL thiourea, and
5. 100 mL AgTU.

These corresponded to 0, 0.25×10^{-2} M, 0.50×10^{-2} M, 0.75×10^{-2} M, and 1.0×10^{-2} M Ag TU.

II. Method

The extractions were performed by adding 35 mL 0.01 M AgTU to 0.70 g biochar. The extraction tubes were shaken on an end-over-end shaker for 16 h, followed by centrifugation at 2000 rpm for 10 min and filtered through Whatman No. 40 filter paper into a 30 mL plastic vial.

The standards and samples were analysed on a Varian 720-ES Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) fitted with an SPS-3 auto-sampler and ultrasonic nebuliser (Varian, Melbourne, Australia) for silver (Ag). Equation 3.14 was used to calculate CEC:

$$CEC=(1-N)\times 50 \qquad \textit{[Equation 3.13]}$$

where:

CEC : cation exchange capacity ($cmol_c\ kg^{-1}$)

N : Ag concentration ($\mu g\ mL^{-1}$)

3.6.6 Anion exchange capacity

I. Reagents

The saturating solution, 0.2 M $BaCl_2$/0.2 M NH_4Cl, was prepared by dissolving 48.9 g $BaCl_2.2H_2O$ and 10.7 g NH_4Cl in DI water and making up to 1 L with DI water. The 0.05 M $BaCl_2$ solution was made by dissolving 12.2 g $BaCl_2.2H_2O$ in DI water and making up to 1 L with DI water. The equilibrating solution, 0.002 M $BaCl_2$, was prepared by dissolving 0.4889 g $BaCl_2.2H_2O$ and making up to 1 L with DI water. The reacting solution, 0.005 M $MgSO_4$, was made by dissolving 1.2340 g

$MgSO_4.7H_2O$ and making up to 1 L with DI water. The 0.05 M $MgSO_4$ solution was made by dissolving 12.3240 g $MgSO_4.7H_2O$ and making up to 1 L with DI water. Ionic strength reference solution, 0.0015 M $MgSO_4$, was prepared by dissolving 0.3697 g $MgSO_4.7H_2O$ and making up to 1 L with DI water.

II. Method

Anion exchange capacity (AEC) was assessed using the Compulsive Exchange Method (Sparks, 1996). An air-dried biochar sample (2 g) was weighed out into a centrifuge tube (30 mL) of known mass, reweighed to determine the exact biochar mass, then deionised water (10 mL) was added and the sample shaken for 1 h. The EC of the suspension was measured and showed biochar samples contained low amounts of soluble salts (EC < 4dS m^{-1}). The supernatant solution was kept for determining water extractable ions. Then, 10 mL 0.2 M $BaCl_2$/0.2 M NH_4Cl solution was added, and the sample shaken for a future 2 h, centrifuged and decanted. Next 20 mL 0.05 M $BaCl_2$ was added to the tube, mixed thoroughly with a Vortex stirrer, centrifuged and the supernatant discarded. Care was taken to avoid loss of biochar material. In order to bring the biochar to the standard 0.006 M ionic strength, samples were washed three times with 20 mL portions of 0.002 M $BaCl_2$ solution. To determine AEC, the supernatant was retained for Cl^- determination (variable C_2). The tubes and contents were weighed to estimate the volume of entrained $BaCl_2$ solution (variable V_1). Then, 10 mL 0.005 M $MgSO_4$ solution was added to begin the compulsive exchange of Mg for Ba. The samples were mixed thoroughly and allowed to stand for 1 h. The EC of the suspension was compared with that of the reference 0.0015 M $MgSO_4$ solution. The conductivity ratio ($CR = EC_{susp}/EC_{ref}$) was > 1.0 and $pH_{susp} = pH_{BaCl2}$ showed that the appropriate conditions of pH and EC had been established. Next the tubes were reweighed (variable V_2), centrifuged and Cl^- concentrations (variable C_1) were determined in the supernatant and the solution retained above on a Dionex DX-120 Ion Exchange Chromatograph (Dionex Corp., Sunnyvale, CA, USA) fitted with a Dionex AAS50 Autosampler. The system was suppressed with an Anion Self-Regenerating Suppressor (ASRS®-Ultra). The ions were separated on a Dionex Ion-Pac® AS9-SC column (250 mm length × 4 mm ID), and the eluent was a weak sodium carbonate/ sodium bicarbonate (2 mM Na_2CO_3/ 0.75 mM $NaHCO_3$) solution at a flow rate of 2 mL min^{-1}. Equation 3.15 was used to calculate AEC:

$$AEC = 50(C_1V_2 - C_2V_1) \qquad \textit{[Equation 3.14]}$$

where:

AEC : anion exchange capacity ($cmol_c\ kg^{-1}$)

C_1 : concentration of Cl^- in final solution ($mmol\ mL^{-1}$)

C_2 : concentration of Cl^- in entrained solution ($mmol\ mL^{-1}$)

V_2 : final volume which was the weight of final $BaCl_2$ (g)

V_1 : entrained volume which was the weight of entrained $BaCl_2$ (g)

3.6.7 Specific surface area

I. Reagents

Sodium thiosulphate ($Na_2S_2O_3$), 0.0394 M, was prepared by dissolving 9.7779 g $Na_2S_2O_3.5H_2O$ in 1L DI water. Iodine (I_2) solution (0.0473 M) was made by diluting 5 M iodine in DI water using standard volumetric concentrate iodine (M & B Volucon, Dagenham, England).

Thiodène 1% was used as starch indicator which made the end of titration easy for tracking the disappearance of the iodine color.

Before the titration, the reagents were standardised prior the application. A 25 mL aliquot of diluted I_2 solution ($0.0473\ mol\ L^{-1}$) was pipetted into a 100 mL Erlenmeyer flask and titrated with the 0.0394 M Na_2SO_3 solution.

II. Method

The oven-dried biochar sample (0.5 g) was placed into the glass vial, and 25 mL of a 0.0473 M I_2 solution was added to the sample and capped immediately. The vials were shaken for 1 min at a minimum of 240 strokes min^{-1}, and centrifuged at 2000 rpm for 3 min. The supernatant was decanted immediately into clean vials and capped immediately. Then, the 20 mL of solution was pipetted into a 250 mL Erlenmeyer flask and titrated with standardized 0.0394 M sodium thiosulphate solution using a glass Buret (ASTM, 2009). As the titration proceeds, the iodine color fades from light brown to pale yellow. It was difficult to see the last traces of iodine color. To make the endpoint easier to detect, once it was near the endpoint, a small amount of starch solution (Thiodène) was added to the titration flask. Starch reacts with a mixture of iodine and iodide ions to form a noncovalent dark blue complex which occured at the end point of the titration. The iodine adsorption number was calculated to the nearest 0.1 g kg^{-1} as follows:

$$I=\left[\frac{B-S}{B}\right]\times\left(\frac{V}{W}\right)\times N\times 126.91 \qquad \textit{[Equation 3.15]}$$

where:

I : iodine adsorption number, g I_2 kg^{-1} biochar (g kg^{-1})

B : sodium thiosulphate required for the blank (mL)

S : sodium thiosulphate required for the sample (mL)

V : calibrated volume of the 25-mL iodine pipette or dispenser

W : mass of biochar sample (g)

N : normality of the iodine solution (meq mL^{-1})

126.91 : equivalent mass of iodine (mg meq^{-1})

Using Equation 3.15 results in units of mg I_2 g^{-1} biochar, which is equivalent to g I_2 kg^{-1} biochar.

3.6.8 Biochar surface acidity

I. Reagents

Sodium hydroxide was prepared using 0.1 M NaOH solution (JT Baker, Netherlands). A 0.1 M solution of hydrochloric acid (HCl) was prepared by diluting 8.3 mL of 37% HCl in DI water to make a 1 L final volume. The titration indicator consisted of 0.1 g of phenolphthalein powder dissolved in 50 mL of ethanol and 50 mL of water. It is colorless in acidic solutions and pink in basic solutions.

II. Method

The method was based on the Boehm method (Boehm, 1994). The method involved suspending 0.5 g of biochar in 25 mL of a 0.1 M NaOH solution, stirring in a closed vessel for ~20 - 24 h. After this time, the slurry was filtered and a 10 mL aliquot was added to 15 mL of 0.1 M HCl. The HCl neutralized the unreacted base and kept further reaction between atmospheric CO_2 and the various bases from occurring. The solution was then back-titrated with 0.1 M NaOH.

The volume of NaOH required to neutralise the sample was converted to titrable negative surface charge. The results were expressed as mmol H^+ equivalent g^{-1} biochar.

3.6.9 Elemental composition

Total elemental analyses on biochar were carried out for: carbon (C), nitrogen (N), phosphorus (P), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron

(Fe), manganese (Mn), chromium (Cr) , copper (Co), boron (B), cadmium (Cd), aluminium (Al) and lead (Pb).

3.6.9.1 Water extractable ions

The solutions containing water extractable ions were prepared as described using the AEC method. The elements were measured using ICP-OES (as described in 3.6.5).

3.6.9.2 Total elements

Total elemental analyses of the biochars were performed using microwave digestion (Microwave Solvent Extraction Labstation (Ethos SEL), Italy), where 2 g of biochar was digested in 6 mL of 10.2 M HCl, 2 mL of 11.0 M HNO_3, and 1 mL of 30% H_2O_2 (Kovacs et al., 2000). Digests were filtered through Whatman No. 52 filter paper into 25 mL volumetric flasks diluted to volume with water then analysed using ICP-MS (as described in 3.6.5).

3.6.9.3 Total N and C

Total C and N were analysed in biochar material using an Elementar Vario-Max CN Elemental Analyser (Elementar GmbH, Hanau, Germany). The samples were combusted at 900°C in an oxygen atmosphere. The combustion process converted any elemental C and N into CO_2, N_2 and NO_x. The NO_x was subsequently reduced to N_2. These gases were then passed through a thermal conductivity cell to determine CO_2 and N_2 concentrations and the %C and %N is calculated from the sample weights.

3.6.10 Volatile organic compounds

A qualitative analysis of VOCs in the biochar samples was determined using an automated headspace solid-phase micro-extraction (SPME) procedure in conjunction with gas chromatography-mass spectrometry (GC-MS). Biochar samples were placed into 20-mL SPME vials and quickly capped. A CTC Combi-Pal auto sampler (CTC Analytics AG, Zwingen, Switzerland) incubated the vials at 40°C for 40 min while the enclosed headspace of the vial was exposed to a 2-cm-long DVB/CAR/PDMS combination SPME fibre (Supelco, Bellefonte, PA), which was preconditioned for 10 min at 250°C under a helium (He) atmosphere before use. Desorption of the headspace volatiles occurred when the fibre was inserted into the heated injection port (250°C for 5 min) of a Shimadzu GC-MS-QP2010 GC-MS equipped with two gas chromatograph columns in series, namely an Rtx-Wax 30-m by 0.25- mm i.d. by 0.5-μm film thickness (polyethylene glycol, Restek, Bellefonte, PA) and an Rxi-1ms 15-

m by 0.25-mm i.d. by 0.50-µm (100% dimethylpolysiloxane, Restek). Helium was used as the carrier gas with the GC-MS set to a constant linear velocity of 32.3 cm s^{-1}. The injector was operated in splitless mode for 5 min, then switched to a 20.5:1 split ratio. The column oven was held at 40°C for 5 min (during desorption of the SPME fibre), then heated to 250°C at 4°C min^{-1} and held at this temperature for 15 min. The total run time was 72.5 min. The interface and mass spectrometry source temperatures were set at 250 and 200°C, respectively. The mass spectrometer was operated in electron impact mode at an ionisation energy of 70 eV and a mass range of 33 to 403 $m\ z^{-1}$. The data acquisition software used was GC-MS solutions (version 5.0, Shimadzu) in full scan mode. Volatile organic compounds were identified by matching mass spectra with the spectra of reference compounds in the National Institute of Standards and Technology EPA/NIH Mass Spectral Library database (Clough et al., 2010).

3.7 Statistical analysis

Statistical analysis of data was performed using Minitab® version 15.1 and 16. One-way analysis of variance (ANOVA) was used to determine if treatment means differed, and when differences occurred the comparison between means was made using Tukey's method ($p < 0.05$). The General Linear Model was used to perform univariate (one response) analysis of variance for each response variable, and to determine the interactions of different factors on these variables ($p < 0.05$).
Data were tested for normality before performing analyses of variance (ANOVAs), with 95% confidence limits ($p < 0.05$) used to indicate levels of significance. Nitrous oxide flux data were log transformed (ln+1) following tests for skewness. Error bars are standard errors of mean (s.e.m.). Pearson correlations were also performed.
Graphing was carried out using SigmaPlot Version 8.02 and 11 (© 1986-2001, SPSS Inc.).

Chapter 4
Effect of biochar amendment on N_2O emissions from soil under ruminant urine application[2]

4.1 Introduction

Globally, N_2O emissions from grazing animal excreta are estimated to be responsible for 1.5 Tg of the total 6.7 Tg of anthropogenic N_2O emissions. Several laboratory-based studies have documented the suppression of N_2O emissions as a result of biochar addition to soils (Section 2.3.4). Thus biochar incorporation into soil can affect N_2O fluxes but detailed field data are still lacking. However, to our knowledge, there have been no reports of in-situ work performed in pastures under either fertiliser or urine treatments. Furthermore, as outlined in Section 2.3.4 biochar has the potential to influence soil inorganic-N concentrations. Therefore this experiment focused on the impact of adding biochar to a pasture soil and the subsequent effects on N cycling including changes in ruminant urine-derived N_2O fluxes, N uptake by pasture, and pasture yield following urine deposition, with the hypotheses being:

1. that biochar rate would impact on pasture yield and N uptake due to changes in inorganic-N pools, and
2. that soil inorganic-N concentrations and N_2O fluxes would be affected by biochar rates.

4.2 Materials and Methods

4.2.1 Pasture establishment

A run-out perennial ryegrass (*Lolium perenne L.*) pasture situated at Lincoln University (43^{o}38.902S, 172^{o}27.793E), on a Templeton silt loam soil (Hewitt, 1998), was surrounded by an electric fence and renovated in May (autumn) 2009 (Table 4.1).

[2] A variant of this Chapter was published as:
Taghizadeh-Toosi, A., Clough, T. J., Condron, L. M., Sherlock, R. R., Anderson, C. R., & Craigie, R. A. (2011). Biochar incorporation into pasture soil suppresses in situ nitrous oxide emissions from ruminant urine patches. *Journal of Environmental Quality*, *40*(2), 468-476.

The pasture was cultivated to a depth of 0.30 m, using a rotocultivator. The field plots were arranged in a randomized block split-plot design (9 m^2 in area (3 × 3 m)) and unweathered biochar, manufactured from *Pinus radiata* (BC_3 in Table 3.1), was then incorporated to a depth of 0.10 m at rates of either 0, 15 or 30 t ha^{-1}, according to experimental treatment (Table 4.2, Figure 4.1, and Figure 4.2). This was achieved by spreading the biochar material onto the plots and then making a shallow pass with the rotocultivator. The trial area was then rolled with a Cambridge roller to produce a fine seed-bed tilth, prior to sowing with a forage perennial ryegrass (cultivar 'Samson') at a rate of 12.5 kg ha^{-1} in rows 0.14 m apart. After ryegrass emergence, urea fertiliser was applied twice; 83 kg ha^{-1} on 9th September 2009 and then 50 kg ha^{-1} on 28th October 2009. To suppress broadleaf weed growth a selective herbicide (Jaguar®, Bayer CropScience) was applied (1.5 L ha^{-1}) on the 21st October. A fungicide to prevent stem rust (Proline®, Bayer CropScience) was applied on 19th November (0.2 L ha^{-1}).

Table 4.1 General soil properties at the experimental site pre-cultivation°.

pH	5.5
Total N (g kg^{-1})	2.52
Total C (g kg^{-1})	28
Olsen P (mg kg^{-1})	28.4
K ($cmol_c$ kg^{-1})	0.84
Ca ($cmol_c$ kg^{-1})	3.6
Mg ($cmol_c$ kg^{-1})	0.90
Na ($cmol_c$ kg^{-1})	0.12
CEC ($cmol_c$ kg^{-1})	14
Total Base Saturation (%)	39
Available N (kg ha^{-1})	45
Anaerobically Mineralisable N (µg g^{-1})	31

°Soil tests were performed commercially by Hill Laboratories, Hamilton, New Zealand. Soil sample depth was 0 - 0.075 m. Thirty soil cores were taken from the site, bulked, and submitted for analysis, n = 1.

Table 4.2 Biochar (BC_3) physical and chemical properties.

Analysis	Result
CEC ($cmol_c\ kg^{-1}$)	8.0
AEC ($cmol_c\ kg^{-1}$)	4.0
$pH_{(H2O)}$	7.8
$pH_{(CaCl2)}$	7.4
Electrical conductivity ($dS\ m^{-1}$)	0.5
Particle density ($Mg\ m^{-3}$)	1.1
Bulk density ($Mg\ m^{-3}$)	0.4
Surface acidity (moles $H^+\ kg^{-1}$)	1.4
Specific surface area ($mg\ g^{-1}$)	127.4
N content ($mg\ g^{-1}$)	0.65
C content ($mg\ g^{-1}$)	772
C: N ratio	1187
Volatile organics found	Ethanol
Biochar particle size fractions (% by weight)	45-15 mm (24.1%)
	15 mm - 7 mm (33.8%)
	7 mm - 5.6 mm (1.13%)
	5.6 mm – 4 mm(10.6%)
	4 mm – 2 mm (15.2%)
	2 mm - 1mm (4.7%)
	≤1mm (10.6%)

4.2.2 Treatments and experimental design

On 13th November 2009, headspace chamber bases (diameter 0.39 m, stainless steel), which protruded 0.10 m into the soil, were installed. These contained an annular water trough. During gas sampling events, insulated, stainless steel headspace covers with 0.10 m high walls created an 11.6 L headspace when they were placed on the bases. The headspace cover, which was insulated with polystyrene foam to avoid temperature perturbations during gas flux measurements, sat on the annular water-filled trough creating a gas-tight seal. There were two chambers on the 0 t ha^{-1} plots and one chamber on the 15 t ha^{-1} and 30 t ha^{-1} plots. All treatments were replicated five times giving a total of 20 chambers.

Located immediately adjacent to each gas sampling chamber was a soil sampling plot (0.37 m × 0.43 m) which received the same rate of urine or water (Figure 4.1.).

Four biochar-urine treatments, replicated 5 times, were set-up on the field trial area. Two of these treatments, consisting of 'nil biochar plus nil urine' (control), and 'nil biochar plus urine' (0U), were positioned on the 0 t ha^{-1} biochar plots (Figure 4.1). The 'biochar at 15 t ha^{-1} plus urine' (15U), and 'biochar at 30 t ha^{-1} plus urine' (30U) treatments were sited on the 15 and 30 t ha^{-1} plots respectively (Figure 4.1). Prior to urine application, pasture was cut to a height of 0.05 m to simulate grazing.

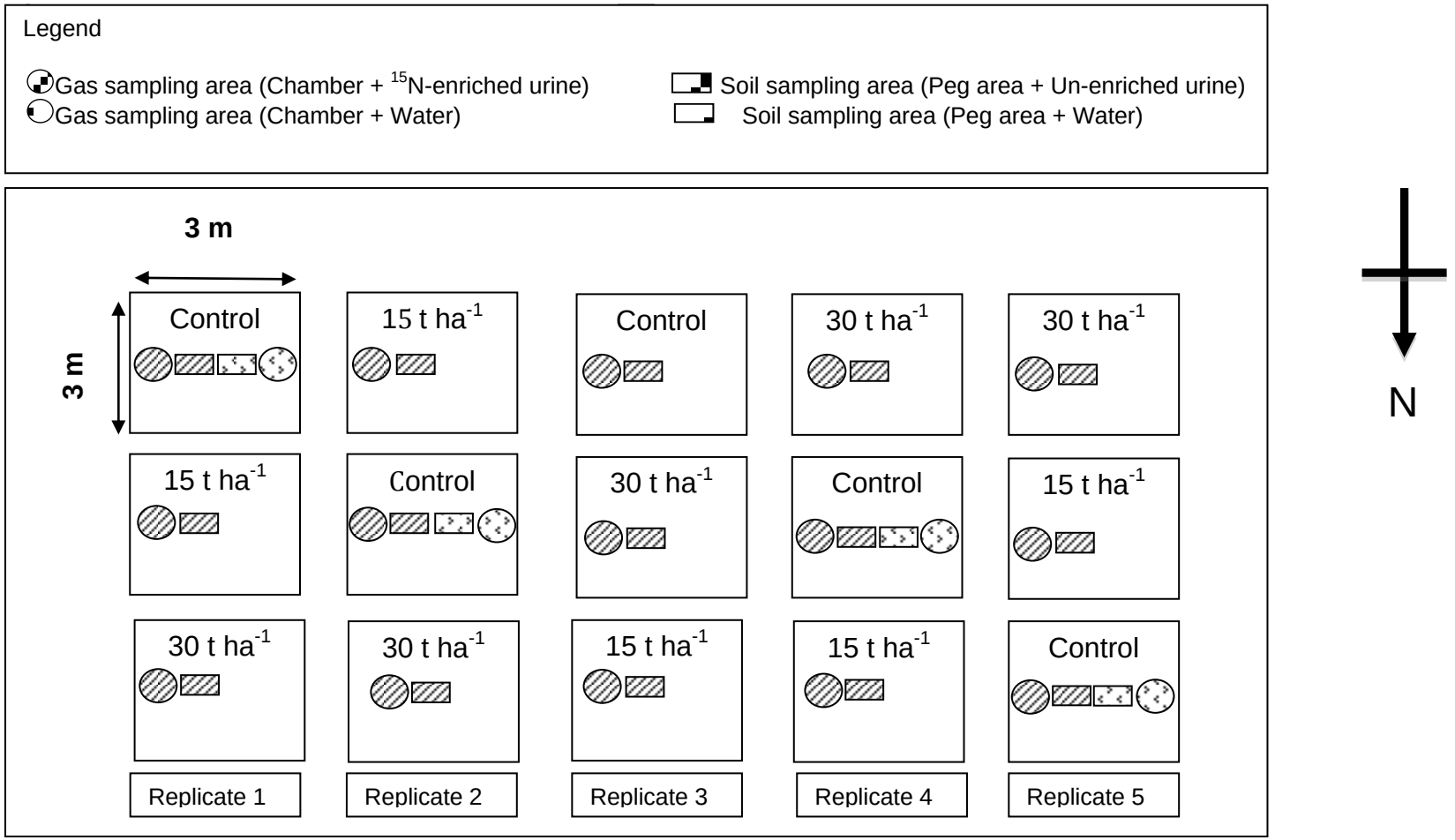


Figure 4.1 Field trial layout showing the position of the gas chambers and soil sampling plots within the biochar treated plots (0, 15 or 30 t ha^{-1}).

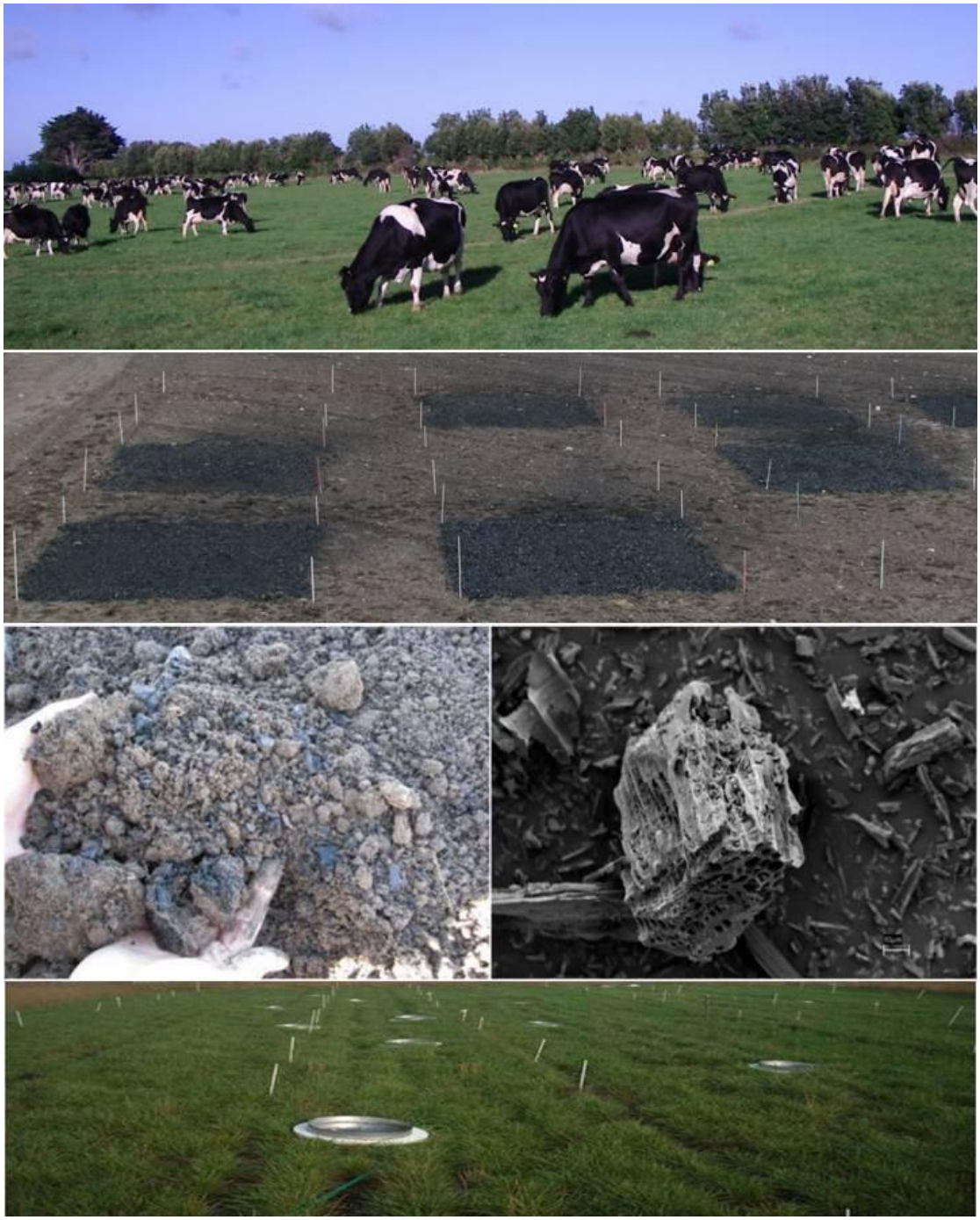


Figure 4.2 Photo showing the field trial layout, incorporated biochar in the soil, biochar SEM, and the pasture 3 months after sowing.

4.2.3 Urine collection, amendment and application

Urine was collected from Friesian cows at the Lincoln University dairy farm (43^{o} 38.522S, 172^{o} 26.450E) that had been grazing perennial ryegrass/white clover (*Trifolium repens*) pasture and it was immediately analysed for total-N by CFIRMS (Section 3.2). The urine contained 5.0 g N L^{-1} when collected. Urine was stored overnight at 4°C. The next day this urine was split into two portions. One portion was enriched with ^{15}N-labelled urea (98.0 atom% $^{15}N_2$-urea; Isotec, Miamisburg, Ohio) to 4.963 atom% ^{15}N, with a final urinary-N concentration of 10 g N L^{-1}, prior to applying it only to pasture within the gas sampling chambers at a rate of 930 kg N ha^{-1}. The second portion had urea, at natural abundance, added so that the concentration of urinary-N also equalled 10 g N L^{-1}. This additional urea was added to raise the N content of the urine to the upper bounds of that found during bovine urinary-N deposition (Haynes & Williams, 1993).

Once formulated the urine treatments were immediately transported to the field and applied at 13:00 h on the 26th of November. Enriched urine (1 L) was decanted into a measuring cylinder and slowly poured onto the chamber area using a watering can to ensure even distribution (chambers prevented the spread of enriched urine outside the area.). Urine was added to the soil sampling plots, adjacent to the gas sampling chambers, at the same N rate (1.440 L).

4.2.4 Field sampling, analyses and micrometeorological measurements

4.2.4.1 Soil bulk density

Soil bulk densities (Mg m^{-3}) of the main plots were determined 5 months after pasture renovation on 15th October 2009. These were determined by taking a soil core (0.073 m diameter × 0.075 m deep) and drying the soil at 105^{o}C for 48 h to determine gravimetric moisture content (θ_g) of the sample and calculating the bulk density (Section 3.1.2).

4.2.4.2 Soil surface pH

Soil surface pH measurements were taken on 33 occasions following urine-treatment application, from 2 days prior to urine application until 86 days after urine application (on days -2, -1, 0, 1, 2, 3, 4, 7, 9, 11, 13, 16, 18, 21, 24, 26, 28, 29, 33, 35, 38, 41,42, 44, 47, 49, 51, 54, 56, 61, 63, 70, and 86), using a flat-surface pH electrode, calibrated with appropriate buffer solutions (Section 3.1.5).

4.2.4.3 Soil sampling for moisture content, inorganic-N and CEC

Soil cores (0.025 m diameter × 0.075 m depth) were taken on 17 occasions (days -2, 1, 4, 7, 9, 11, 16, 18, 21, 26, 33, 41, 44, 47, 51, 56, 65) over the course of the study in order to monitor θ_g and inorganic-N concentrations (Sections 3.1.3 and 3.1.6, respectively). On each occasion, two soil cores were taken from each soil sampling plot, bagged and mixed, and then immediately transported to the laboratory.

In order to determine if gross CEC, defined as soil plus or minus biochar, had increased over time, further soil cores (0.025 m diameter × 0.075 m depth) were taken from each biochar plot, in non-urine affected areas, after the biochar had been in-situ for 14 months, and the CEC was determined (Section 3.6.5).

4.2.4.4 Plant sampling

Samples of ryegrass herbage were collected inside the chambers and rectangular areas at a height of 0.05 m separately on days 21, 43, 56 and 58, by hand harvesting at the soil surface.

The samples were immediately placed in paper bags, and dried (60°C). Dry matter yield, total N content and atom% ^{15}N enrichment were determined (Section 3.5).

4.2.4.5 Headspace N_2O and N_2O-^{15}N sampling

Headspace gas samples were taken for N_2O-N on 33 occasions over the 86 d study (on days -2, -1, 0, 1, 2, 3, 4, 7, 9, 11, 13, 16, 18, 21, 24, 26, 28, 29, 33, 35, 38, 41,42, 44, 47, 49, 51, 54, 56, 61, 63, 70, and 86). Further headspace gas samples were collected for ^{15}N analysis of N_2O-^{15}N on 13 occasions (days 7, 11, 16, 21, 24, 28, 33, 38, 41, 44, 47, 51, 61; Section 3.2.2.2).

4.2.4.6 Headspace NH_3 sampling

Headspace NH_3 samples were collected during N_2O-N sampling periods over the 12 d study (on days 0, 1, 2, 3, 4, 7, 9, 11, 13, 16, 18, 21) by putting an acid trap screw-top glass bottle filled with 1M H_2SO_4 (20 mL) in each chamber area before placing the headspace chamber on the chamber rings. After 2 h the acid trap bottles were removed and the lids screwed on tightly. The solution was transferred to the laboratory and analysed (Sections 3.4).

4.2.4.7 Meteorological data

Meteorological data (air temperature and rainfall) and the soil temperature at 0.10 m soil depth were obtained from the nearby meteorological station (The Lincoln University Meteorological Station).

4.2.5 Statistical analysis

Statistical analysis was performed using Minitab®. Tests for normality showed the N_2O flux data were skewed and so these were log transformed (In(flux+1)) prior to statistical analysis, with N_2O fluxes compared on individual sampling days and for cumulative fluxes over the sampling period. Analyses of variance (ANOVA) was used to test for differences in treatment means and Tukey's Test was used to determine those treatments responses for differences, with 95% confidence limits ($p < 0.05$). Graphing was carried out using SigmaPlot®.

4.3 Results

4.3.1 Soil water content and meteorological measurements

Gravimetric soil moisture (θ_g) did not differ significantly due to biochar or urine treatments for any given sampling day, averaging 18.8, 19.3, 17.9, and 16.7% for the control, 0U, 15U and 30U treatments; respectively, with maximum and minimum mean values of 26.7 and 6.5%, respectively, corresponding to WFPS values of 67 and 16% (Figure 4.3). As the summer season progressed, θ_g declined over time, due to evapotranspiration exceeding sporadic and infrequent rainfall, but θ_g increased following irrigation or substantial rainfall events. The average daily soil temperature (0.10 m depth) ranged from 13.2 to 25.6°C, following trends in the average daily air temperature which ranged from 8.7 to 23.6°C (Figure 4.3).

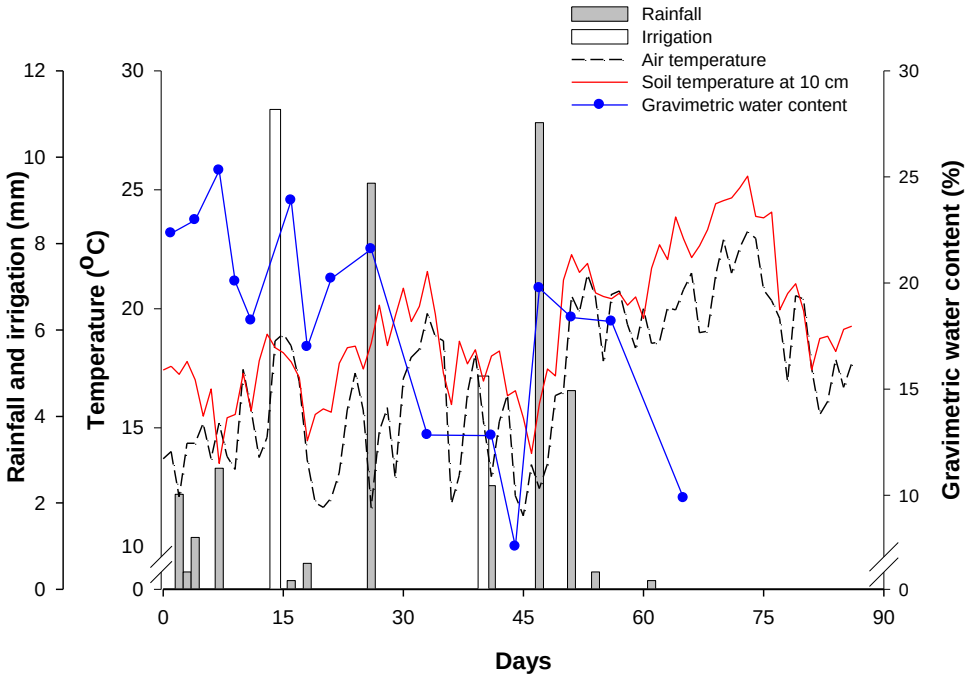


Figure 4.3 Rainfall, soil and air temperatures over the 86 day experimental period at the meteorological station site 3 km from the trial site. Gravimetric water content values were determined from in-situ sampling at the field site.

4.3.2 Soil surface pH

Surface soil pH became elevated following urine application, and it remained higher ($p < 0.01$) in plus-urine treatments, when compared to the control, until day 86 (Figure 4.4). When comparing only the three urine treatments, there was a trend for the soil surface pH to be higher with increasing biochar rate when sampled between days 7 to 47 but statistically significant differences ($p \leq 0.05$) only occurred on day 16 and between days 38 to 47, when the 30U treatment had a surface soil pH higher than in the 0U treatment (Figure 4.4).

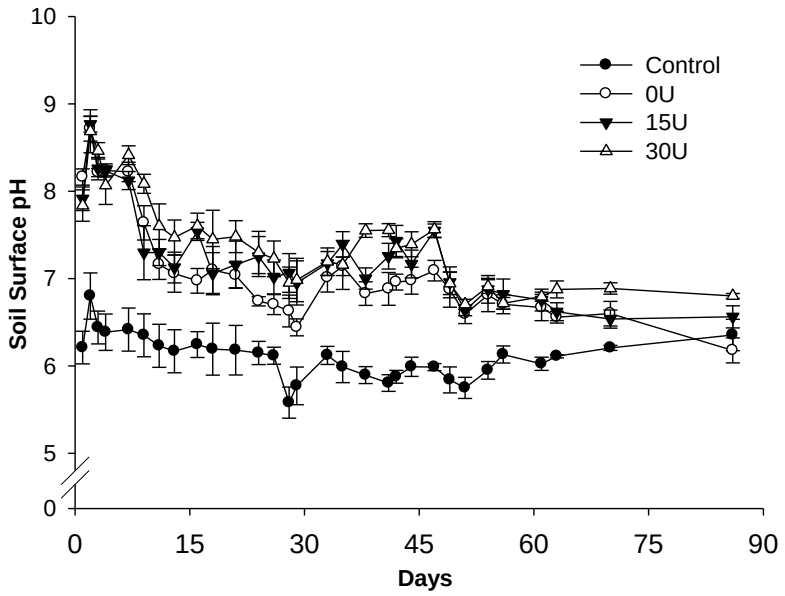


Figure 4.4 Soil surface pH over time, following urine application (error bars = ± s.e.m., n = 5).

4.3.3 Soil bulk density

Soil bulk density did not change significantly, despite the addition of biochar, with an overall average of 1.29 ± 0.08 (SD) Mg m^{-3} (Figure 4.5).

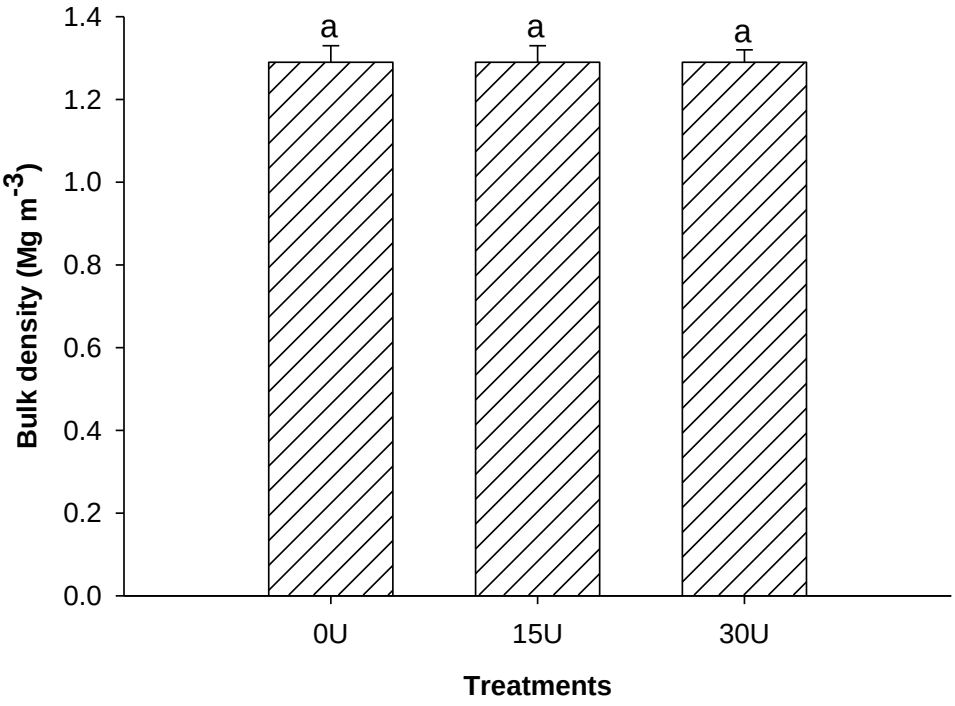


Figure 4.5 Soil bulk density determined 5 months after pasture renovation (error bars = + s.e.m., n = 5).

4.3.4 Biochar CEC

After 14 months the CEC of the soil cores (± biochar) taken from the biochar treated plots averaged 6.0 ± 0.2, 6.9 ± 0.2, 6.1 ± 0.3 (± s.e.m., n = 5) for the control, 15 t ha^{-1}, and 30 t ha^{-1} plots, respectively, with no difference due to biochar treatment.

4.3.5 Inorganic-N

The inorganic-N concentrations increased significantly ($p < 0.01$) with urine addition, and NH_4^+-N reached maximum mean concentrations of 180 to 234 μg g^{-1} dry soil between days 4 to 7 (Figure 4.6a). There was a trend for soil NH_4^+-N concentrations in the urine treatments to increase after day 21, with increasing rates of biochar, but this was not statistically significant ($p \geq 0.09$, Figure 4.6a). By day 65 soil NH_4^+-N concentrations were still elevated in the 30U treatment when compared with the 0U treatment ($p < 0.05$, Figure 4.6a). Mean soil NO_3^--N concentrations were significantly higher ($p < 0.05$) under urine treatments than in the control, from day 9 onwards, peaking at 72 μg g^{-1} dry soil in the 0U treatment on day 26. Between days 11 to 44, there was a trend for soil NO_3^--N concentrations to be lower with increasing biochar rate and this was statistically significant on days 11, 18, and 26 (Figure 4.6b).

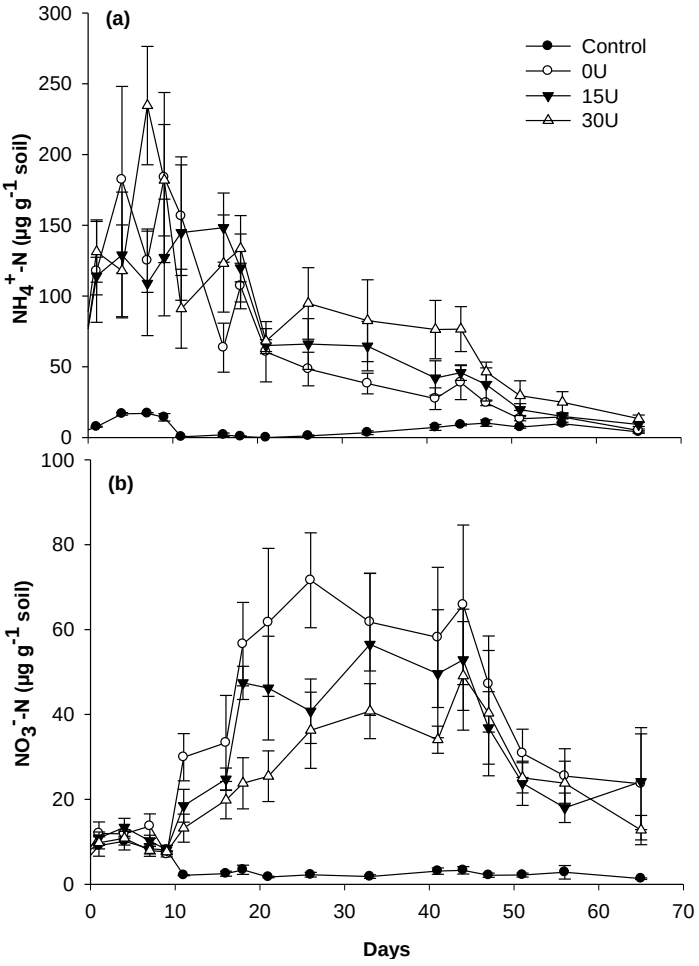


Figure 4.6 Soil (a) NH_4^+-N, and (b) NO_3^--N concentrations over time, following urine application (error bars = ± s.e.m., n = 5).

4.3.6 Herbage yields and N contents

4.3.6.1 Dry matter yield

No statistical differences in dry matter yield (DMY), due to treatment, occurred on day 21, although the trend was for higher DMY when urine was present (Table 4.3). By day 43 dry matter yields were higher ($p < 0.01$) under urine treatments than in the control, but by day 58 only the 15U treatment had a higher ($p < 0.05$) DMY than in the control (Table 4.3). When comparing just the urine treated plots, increasing the biochar rate had no significant effect on DMYs (Table 4.3). There was insufficient growth for harvesting of dry matter at day 86 when the final N_2O flux measurements were made.

4.3.6.2 N content

At day 21 only the herbage in the 0U and 15U treatments had an N percentage higher than in the control ($p < 0.05$), but by days 43 and 58 all urine-treated herbage had higher ($p < 0.01$) N percentages than in the control (Table 4.3). Comparing only the biochar treatments under urine, there were no significant differences in dry matter N percent as biochar rate was increased, at any time, although the trend was for lower N contents with increasing biochar rate on days 21 and 43 (Table 4.3).

4.3.6.3 Total N uptake

Nitrogen uptake by the herbage, a function of N percentage and DMY, was higher ($p \leq 0.05$), on all occasions, when urine was present, ranging from 1.3 to 9.0 g m^{-2} (Table 4.3). The addition of biochar to the soil had no effect on N uptake in the presence of urine (Table 4.3).

4.3.6.4 ^{15}N enrichment and recovery

The atom% ^{15}N enrichment of the herbage in the control was at natural abundance while in the urine treatments the atom% ^{15}N enrichment was significantly higher ($p < 0.01$), ranging from 3.55 to 3.99 with ^{15}N enrichment in the herbage decreasing over time (Table 4.3). Recovery of applied ^{15}N in the herbage did not vary due to the addition of biochar at any time with total ^{15}N recovery in the herbage after 58 days ranging from 14.3 to 17.5% (Table 4.3).

Table 4.3 Biochar and urine effects on dry matter yields, herbage N content, N uptake, ^{15}N enrichment and %^{15}N recovery.

Variable	Day	Treatment[°]				ANOVA result. (* = $p < 0.1$, ** = $p < 0.05$, ns = not significant)	
		Control	0U	15U	30U	All Treatments[▪]	Urine treatments only[⊙]
Dry matter yield (g m^{-2})	21	153 ±67	199 ±40	199 ±31	242 ±41	ns	ns
	43	103 ±15	224 ±53	185 ±57	182 ±46	*	ns
	58	44 ±15	91 ±19	104 ±31	82 ±34	*	ns
Herbage N content (%)	21	2.7 ±0.3	4.5 ±0.3	4.5 ±0.2	3.3 ±1.8	*	ns
	43	2.5 ±0.2	4.1 ±0.3	3.9 ±0.4	3.7 ±0.2	**	ns
	58	2.9 ±0.2	4.3 ±0.3	3.9 ±0.3	4.1 ±0.4	**	ns
N uptake (g m^{-2})	21	4.0 ±1.7	8.87 ±1.60	8.89 ±1.07	8.28 ±4.77	*	ns
	43	2.5 ±0.3	9.03 ±1.73	7.27 ±2.84	6.70 ±1.73	**	ns
	58	1.3 ±0.5	3.88 ±0.81	3.97 ±1.01	3.30 ±1.99	**	ns
Atom%^{15}N	21	0.41 ±0.02	3.62 ±0.16	3.72 ±0.11	3.55 ±0.23	**	ns
	43	0.38 ±0.01	3.94 ±0.06	3.99 ±0.10	3.89 ±0.09	**	ns
	58	0.38 ±0.01	3.72 ±0.14	3.77 ±0.12	3.69 ±0.10	**	ns
^{15}N recovery (%)	21	-	6.85 ±1.24	7.05 ±0.99	6.11 ±3.53	-	ns
	43	-	7.63 ±1.49	6.26 ±2.54	5.60 ±1.53	-	ns
	58	-	3.09 ±0.75	3.20 ±0.82	2.60 ±0.94	-	ns

[°] Treatments: Control (nil urine & nil biochar); 0U (urine & nil biochar); 15U (urine & biochar at 15 t ha^{-1}); 30U (urine & biochar at 30 t ha^{-1});
[▪] ANOVA comparing all treatments; [⊙]ANOVA comparing only the urine treatments (0U, 15U and 30U).

4.3.7 N_2O-N fluxes

Fluxes of N_2O were significantly higher ($p \leq 0.05$) than in the control when urine was present, in all or some of the urine treatments from days 1 to 35, and days 44, 47, and 54 (Figure 4.7a). Between days 4 to approximately day 35, when comparing only the urine treatments, there was a trend for N_2O fluxes to decrease with increasing rates of biochar, with N_2O fluxes from the 30U treatment being statistically lower ($p \leq 0.05$) than from the 0U treatment during this period on days 4, 7, 13, 24, and 26 (Figure 4.7a). The cumulative N_2O fluxes, were higher under urine deposition when compared to the control, and when the urine treatments were compared against each other, the N_2O fluxes from the 30U treatment were lower ($p < 0.05$) than in either the 0U or 15U treatments (Figure 4.7b). When the mean cumulative N_2O-N fluxes were expressed as a percentage of urine-N applied the 0U, 15U and 30U treatments had respective emissions of 0.15, 0.16, and 0.07%, with statistically lower emissions from the 30U treatment ($p < 0.05$). When expressed as an emission factor (N_2O-N from the urine treatment in question, minus the N_2O-N from the control, divided by the urine-N applied) the 0U, 15U and 30U treatments had respective mean emission factors of 0.12, 0.13, and 0.04%; respectively.

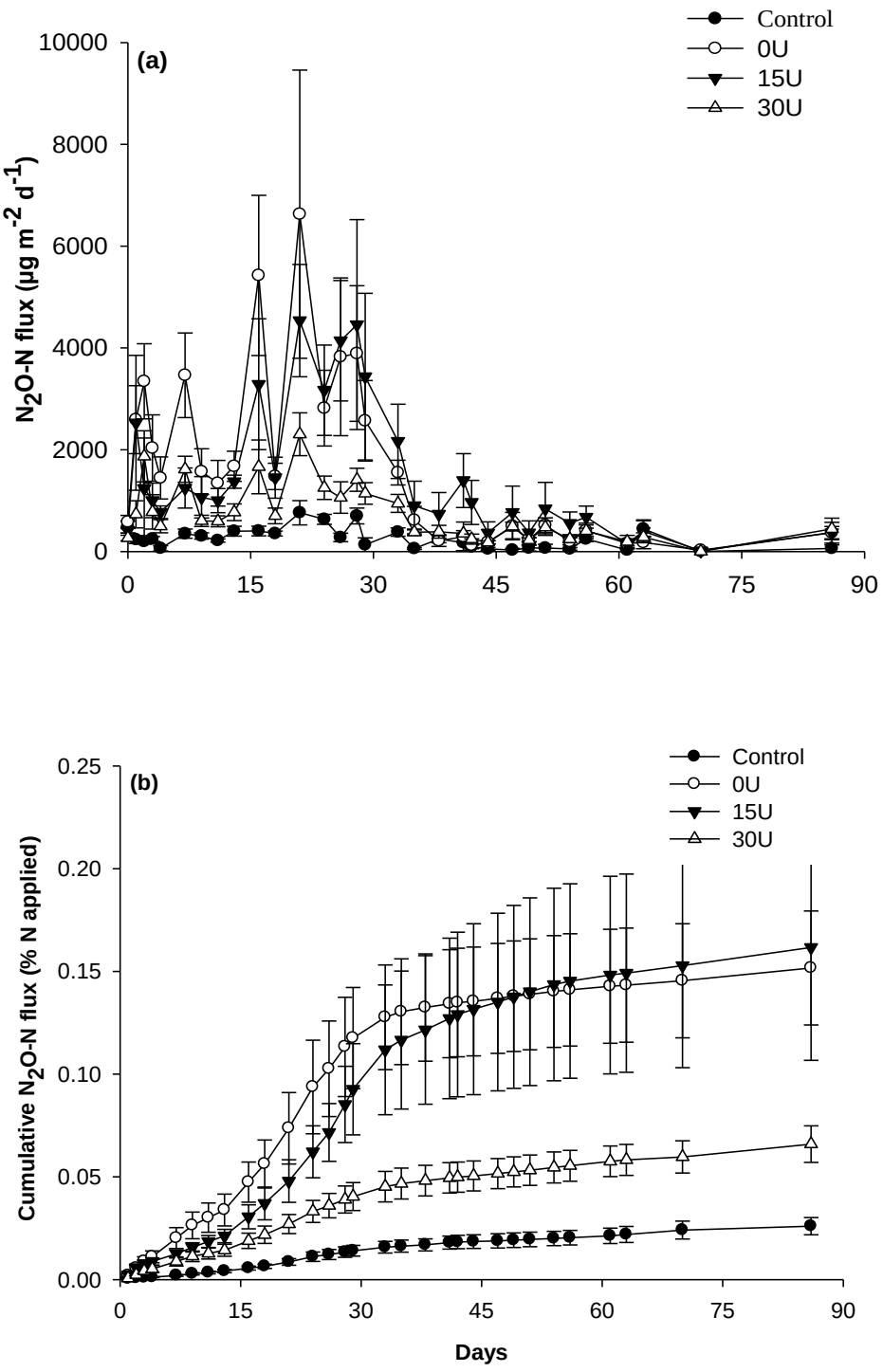


Figure 4.7 (a) Geometric mean N_2O emissions from different treatments following urine application (error bars = ± one geometric standard error of the mean, n = 5), (b) Non-transformed cumulative loss of N_2O from urine-treated soils, showing the amount of N emitted as N_2O-N as a percentage of the total N applied to the plots. 'Nil urine' is also plotted to show "control" emissions to put the magnitude into perspective (error bars = ± s.e.m., n = 5).

4.3.8 N_2O-^{15}N enrichment

The ^{15}N enrichment of the N_2O from the urine-^{15}N treated plots remained higher than in the control treatment throughout the entire period of the study (Figure 4.8). When comparing the urine-biochar treatments there was a trend for the atom% ^{15}N enrichment of the N_2O to be lower with increasing rates of biochar from day 11 to day 33, and this was statistically significant ($p \leq 0.05$) on days 16 and 28 (Figure 4.8). The mean percentage recovery of ^{15}N applied, as N_2O-N, equated to 0.86 ± 0.43, 0.88 ± 0.84, and 0.23 ± 0.10 atom %, with no statistical difference between these values ($p = 0.15$).

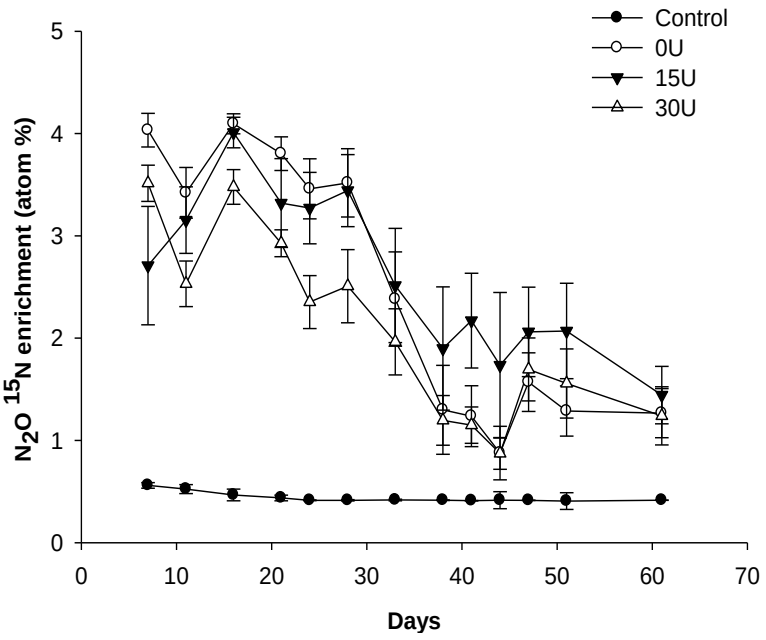


Figure 4.8 The determined atom% enrichment of N_2O-^{15}N for treatments (error bars = ± s.e.m., n = 5).

4.3.9 NH_3 fluxes

Ammonia concentrations in the acid traps were analysed using flow injection analysis and an ion specific electrode (Sections 3.1.6 and 3.4). But, NH_3 concentrations were below the detection limit in both of the methods.

4.4 Discussion

4.4.1 Soil N dynamics and N_2O emissions

Increases in soil surface pH, and the duration of the pH increase, were typical of what is expected following ruminant urine deposition onto pasture (Jarvis & Pain, 1990). This increase occurs due to urea hydrolysis while the subsequent decline in pH is a result of H^+ ions being released during NH_3 volatilisation (Sherlock, 1984a, 1984b) and nitrification (Wrage et al., 2001).

Elevation of soil NH_4^+-N concentrations resulted from the hydrolysis of urine-derived urea. The small increase in soil NH_4^+-N concentrations in the control treatment (water only) was possibly due to mineralisation of organic matter. Soil NH_4^+-N is in chemical equilibrium with aqueous NH_3 in the soil and significant volatilisation of NH_3 may occur when the soil pH is elevated (> 7.0), as occurs under urine patches. Ammonia volatilisation fluxes were not of sufficient magnitude to be measured in this experiment, probably due to an artefact of the experimental technique. This artefact could have arisen from either the lack of appropriate air flow inside the chamber area and/or insufficient time to collect a measurable amount of NH_3[3]. Hence, we can only speculate what the loss of NH_3 was, but for urine patches in grazed pasture it is commonly thought to be 10 - 20% of urine-N deposited (Sherlock et al., 2008). The remaining soil NH_4^+ pool can be taken up by pasture plants, become immobilised by soil microbes or be oxidised further to NO_3^--N. The latter process explains the observed increase in soil NO_3^--N concentrations under the urine treatments.

In this current study, biochar addition clearly influenced soil inorganic-N dynamics, with lower NO_3^--N concentrations at the highest rate of biochar (30U), when compared with the 0U treatment, and trends for higher NH_4^+-N under the 30U treatment. It is well recognized that biochar materials are able to promote adsorption of NH_3 ((Clough & Condron, 2010) and references therein). Thus biochar in the soil under a urine patch potentially creates a sink for the NH_3. We propose that one possible mechanism for the reduced NO_3^- concentrations and lower N_2O emissions observed under the 30U treatment was the uptake and adsorption of NH_3 by the biochar. Adsorption of urinary derived NH_3 by the biochar would have increased with increasing biochar rate. This would serve to reduce the soil NH_4^+-N pool available to nitrifiers and the NO_3^--N pool subsequently formed. If such adsorbed NH_3 is extractable with

[3] In chapter 6, NH_3 volatilisation was determined in a control circular large plot using a micrometeorological method.

2 M KCl it would explain the observed trend for higher NH_4^+-N concentrations at the highest biochar rate (30U).

A further factor demonstrating that biochar altered the soil inorganic-N pool was the reduced ^{15}N enrichment of the N_2O flux in the 30U treatment, indicating that the source of the inorganic-N that the N_2O was derived from came from an inorganic-N pool with a lower proportion of urine-N than in the 0U treatment.

Thus, we propose that under the highest rate of biochar, NH_3 formation and its subsequent adsorption onto and/or into the biochar, reduced the inorganic-N pool available for nitrifiers and thus NO_3^--N concentrations were reduced. Then, since the NO_3^--N pool had a lower concentration the ^{14}N dilution arising from soil mineralisation was relatively greater, thus lowering the ^{15}N enrichment of the N_2O source pool(s). Consistent with this is the lower N_2O flux from the 30U treatment, as a % of urine-N applied, and as a cumulative N_2O flux. Soil N_2 fluxes were not measured in this study and the relatively alkaline nature of biochar, when compared to soil, may possibly have favoured further reduction of N_2O to N_2. This could explain a lower N_2O flux but not the differences in ^{15}N enrichment observed.

An alternative theory to explain lower soil NO_3^--N concentrations in the presence of biochar was drawn by (Singh et al., 2010): following the incorporation of either poultry or woodchip derived biochar to soil columns and a 5 month incubation with 3 wetting-drying cycles differences in NH_4^+-N and NO_3^--N leaching were observed. They concluded that these differences could have been due to increases in the sorptive properties of the biochars. Our results showed no substantial increase in the CEC of the soil plus biochar matrix after 14 months. Other studies have demonstrated increases in biochar CEC over longer periods (Liang et al., 2006), but such a mechanism cannot be responsible for the observed reductions in soil NO_3^- concentrations noted here.

Chemicals that inhibit nitrification lead to a prolonged occurrence of NH_4^+-N in the soil and lower NO_3^--N concentrations. A trend observed in the 30U treatment in the current study. It has been previously noted that biochar contains VOCs that are known nitrification inhibitors. For example, Clough et al. (2010) found α-pinene in an unweathered biochar. Ethanol was the only VOC detected in the biochar, in the current study, prior to its incorporation into the soil, which was several months prior to urine deposition and so it is assumed that its effect if any would have been negligible by the time urine treatments were applied. Other non-volatile microbially inhibiting compounds may have existed in the biochar. Spokas et al. (2010) hypothesised that ethylene, a known microbial inhibitor, was microbially produced from

biochars and that ethylene may be the cause of the observed changes in microbial dynamics and N_2O suppression (Spokas et al., 2010). This interesting theory needs testing with respect to longevity of ethylene production in the soil. The biochar in the current study had been in the soil for approximately 7 months (May to November), prior to urine treatment.

The addition of fire-derived charcoal to forest soils has been shown to enhance native soil organic matter mineralisation (Wardle et al., 2008a) highlighting the current lack in our understanding of how biochar might affect native soil carbon pools (Wardle et al., 2008b). At the high rate of biochar used here the dilution of the ^{15}N pool, supplying the N_2O flux, could potentially have occurred as a result of enhanced mineralisation of soil organic matter. But had this been the case we might have expected to observe an increase in the size of the inorganic-N pool under the 30U treatment and this was not observed.

The soil microbial-^{15}N pool was not measured in the current study and this should be included in future studies to further elucidate the mechanisms of biochar perturbance of the N cycle.

Soil moisture conditions were consistent with summer soil conditions, and given that denitrification is expected to dominate at WFPS values in excess of 60%, the soil moisture conditions predominately favoured nitrification mechanisms as N_2O forming pathways. However, denitrification and nitrifier-denitrification may still operate at anaerobic microsites under aerobic soil conditions (Müller et al., 2004; Russow et al., 2009). During days 15 - 35, when the N_2O-^{15}N enrichment was generally lower in the 30U treatment, there was also considerable rainfall or irrigation, and the highest N_2O fluxes occurred. Denitrification of NO_3^--N as an N_2O production mechanism is definitely plausible under these conditions. As a percentage of the urine-N applied, the cumulative N_2O fluxes were low when compared with the New Zealand specific N_2O emission factor for urine-N that is currently set at 1.0% of urine-N excreted. This was most likely a function of the drier summer soil conditions, despite the irrigation, and this study needs to be repeated under winter conditions to see if similar reductions in cumulative N_2O emissions occur under the 30U treatment.

4.4.2 Pasture growth

The fact that dry matter yields were not detrimentally affected by biochar addition indicates that there are no apparent negative effects of biochar incorporation under the conditions of this trial. The lower percentage N of the dry matter harvested from the 30U treatment on day 21 is consistent with what was seen in the inorganic-N pool at this time, with less inorganic-N equalling less N uptake at this time. Had this been under a grazing regime this would have

meant a positive feed-back in terms of reducing subsequent urinary-N derived N_2O emissions since subsequent urination events would have consisted of urine with lower N contents.

4.4.3 Bulk density of soil-biochar treatments

Surprisingly, the addition of biochar did not translate into statistically different soil bulk densities. At the highest rate of biochar (30 t ha^{-1}) the area of the soil-sampling core, used to determine bulk density, would have received 12.6 g of biochar, which at its measured bulk density of 0.4 Mg m^{-3} equates to 3.13×10^{-5} m^3 of biochar. Assuming all this was equally distributed within the target depth of 0.10 m, the soil bulk density core, with a depth of 0.075 m, could have contained 2.35×10^{-5} m^3 of biochar. A theoretical bulk density under such ideal mixing, and using the nil biochar soil as a reference, would equal 1.23 Mg m^{-3}, which is within one standard deviation of the bulk density determined at 30 t ha^{-1}. Thus, further replication of the bulk density sampling, or changes in the method, are required if changes in bulk density at biochar rates up to 30 t ha^{-1} are to be detected.

4.5 Conclusions

Under the field conditions experienced here the addition of biochar at 30 t ha^{-1} reduced cumulative N_2O emissions by ca. 50%. Due to the season the N_2O emissions, when expressed as a percentage of N applied were relatively low (0.2% of N applied). Nevertheless, once N_2O fluxes from the controls are considered the 30 t ha^{-1} treatment yielded a N_2O emission factor of just 0.04% compared to 0.12% from the urine only treatment. Thus, 30 t ha^{-1} of biochar reduced the N_2O emission factor from urine by around 70%. Inorganic-N levels were modified in an environmentally desirably manner, by the highest rate of biochar applied, with lower NO_3^- concentrations in the soil, indicating that had NO_3^--N leaching occurred there would have been lower NO_3^--N losses at the highest rate of biochar used. The lack of NH_3 results from this field experiment suggested a new study focus to explain the possible mechanism of adsorption of NH_3 by the biochar which would be performed in following chapters. Also, the results from the current study are strongly encouraging and warrant further intensive work under winter conditions when leaching and N_2O emissions are higher. In summary this study has demonstrated that biochar, can reduce N_2O emissions from ruminant urine patches in-situ. Thus if other studies confirm the relatively long residence time expected of biochar in the soil then the ‘win-win’ situation of both sequestering carbon while reducing N_2O emissions may prove achievable. However, further study is still required

to determine seasonal effects and the effects of repeated deposition onto soil-biochar matrices, that vary with biochar size, rate and soil type.

Chapter 5
Adsorption of NH_3 gas on biochar samples and its subsequent plant availability[4]

5.1 Introduction

Mineralisation rates of organic N contained in biochar are expected to be low since the stability of biochar is high; and the inorganic and organic N content of biochar determines its value as a slow-release N fertiliser (Lehmann & Joseph, 2009).

The incorporation of biochar into the soil is feasible on those soils where cultivation events already occur, such as intensively managed systems. These intensive agroecosystems also receive the highest rates of N inputs via fertiliser or excreta, often in forms that generate NH_3, such as urea, anhydrous NH_3 fertilisers or ruminant urine. It is well recognized that adsorption of NH_3 on/in to biochar can occur (Asada et al., 2002; Clough & Condron, 2010), with rates ranging from 0.2 to 4 mg g^{-1}, and varying due to temperature, humidity, pressure, presence of CO_2 and biochar materials (Day et al., 2005; Kastner et al., 2009; Li et al., 2003; Rodrigues et al., 2007). However, the bioavailability of this N has not been assessed (Lehmann et al., 2006). In the previous experiment it was hypothesised that adsorption of NH_3 was responsible for the reductions in N_2O fluxes and their associated ^{15}N enrichments, and the changes in observed soil inorganic-N concentrations. This being the case it raises the question: "How bioavailable is biochar adsorbed NH_3 when placed in the soil matrix?"

In this chapter, to further our understanding of the bioavailability of biochar-adsorbed NH_3 in soils, four biochar materials were exposed to ^{15}N enriched NH_3, subsequently referred to as eBC_1 to eBC_4. These biochars were added to soil and plants were grown to assess the bioavailability of the biochar-adsorbed ^{15}N. The effect of the same biochar materials that had not been exposed to ^{15}N enriched NH_3 (BC_1 to BC_4) was also assessed against a soil + ryegrass treatment (nBC). The stability of the adsorbed NH_3 in ambient air was also assessed over time.

[4] A variant of this chapter was published as:
Taghizadeh-Toosi, A., Clough, T. J., Sherlock, R. R., & Condron, L. M. (2011). Biochar adsorbed ammonia is bioavailable. *Plant and Soil, In Press*. doi:10.1007/s11104-011-0870-3.

The hypotheses tested were:

1. that biochar type would affect NH_3 adsorption and plant availability, and
2. that NH_3-N adsorbed on/in biochar is bioavailable.

5.2 Materials and Methods

5.2.1 Experimental design and treatments

I. The 1st experiment was conducted using five experimental treatments with four replicates in a randomised block design. The treatments consisted of 4 biochar types referred to as BC_1, BC_2, BC_3, BC_4 (Table 3.1) and blank.

II. The 2nd experiment was focused on the stability of NH_3 adsorbed-^{15}N on biochar particles, using four replicates of NH_3-^{15}N labelled biochar (eBC_1).

III. The 3rd experiment was carried out in a randomised block design with four replicates. Ten treatments consisted of: soil only, soil + perennial ryegrass (*Lolium perenne L.*), soil + unenriched biochar + perennial ryegrass , referred to as BC_1 to BC_4, and soil+^{15}N enriched biochar + perennial ryegrass, referred to as eBC_1 to eBC_4, which were replicated four times (Table 5.1).

Table 5.1 Treatment abbreviations.

	Treatments°	Definition	Replicates
1	S	Soil	4
2	nBC	Soil + ryegrass	4
3	BC_1	Soil + ryegrass + BC_1	4
4	BC_2	Soil + ryegrass + BC_2	4
5	BC_3	Soil + ryegrass + BC_3	4
6	BC_4	Soil + ryegrass + BC_4	4
7	eBC_1	Soil + ryegrass + eBC_1	4
8	eBC_2	Soil + ryegrass + eBC_2	4
9	eBC_3	Soil + ryegrass + eBC_3	4
10	eBC_4	Soil + ryegrass + eBC_4	4

°BC= ^{15}N-unenriched treatments, and eBC= ^{15}N-enriched treatments.

5.2.2 Soil and biochar characterisation

A Temuka silt loam soil (Hewitt, 1998) was collected from a grazed pasture at Lincoln University, air-dried, sieved to 2 mm and analysed (Table 4.1). Four biochars manufactured from *Pinus radiata* were characterised for pH, EC, CEC, AEC, total elemental analysis, water extractable ions, the presence of VOCs, specific surface area, surface acidity, particle and bulk densities as described in Section 3.6 (Tables 5.3, 5.4, and 5.5)

Table 5.2 Biochar physical and chemical properties (Mean ± s.e.m., n = 2).

	BC_1	BC_2	BC_3	BC_4
CEC ($cmol_c\ kg^{-1}$)	3.09 ± 0.00	2.67 ± 0.05	7.99 ± 0.11	3.86 ± 0.05
AEC ($cmol_c\ kg^{-1}$)	3.32 ± 0.02	5.19 ± 0.01	4.03 ± 0.01	4.96 ± 0.01
$pH_{(H2O)}$	5.15 ± 0.01	5.97 ± 0.00	7.77 ± 0.05	6.64 ± 0.06
$pH_{(CaCl2)}$	5.74 ± 0.00	5.56 ± 0.01	7.39 ± 0.01	6.71 ± 0.01
EC ($dS\ m^{-1}$)	0.01 ± 0.00	0.01 ± 0.00	0.53 ± 0.00	0.02 ± 0.00
ρ_p ($g\ cm^{-3}$)	1.55 ± 0.04	1.29 ± 0.02	1.09 ± 0.02	1.60 ± 0.03
ρ_b ($g\ cm^{-3}$)	0.09 ± 0.02	0.13 ± 0.02	0.41 ± 0.02	0.08 ± 0.03
Surface acidity (mmoles $H^+\ g^{-1}$)	1.75 ± 0.05	1.30 ± 0.10	1.35 ± 0.05	1.35 ± 0.15
Iodine adsorption° ($mg\ g^{-1}$)	21.35 ± 1.67	22.00 ± 1.44	127.35 ± 12.90	56.32 ± 4.29
N content ($mg\ g^{-1}$)	0.40 ± 0.05	1.40 ± 0.02	0.65 ± 0.02	2.20 ± 0.01
C content ($mg\ g^{-1}$)	621.65 ± 0.04	757.6 ± 0.01	771.8 ± 0.02	826.4 ± 0.00
C: N ratio	1554.1	541.1	1187	375.6
VOCs detected	- Carboxylic acids (8)▪ -Alcohols (6) -Aldehydes (9) -Esters (11) -Ethers (5) -Hydrocarbons (16) -Ketones (21) -Phenols (7)	-Carboxylic acids (9) -Alcohols (13) -Aldehydes (9) -Esters (10) -Ethers (8) -Hydrocarbons (22) -Ketones (29) -Phenols (16)	-Ethanol	-Carboxylic acids (5) -Alcohols (2) -Aldehydes (7) -Esters (9) -Ethers (4) Hydrocarbons (4) -Ketones (12) -Phenols (3)

°An indirect measurement of specific surface area.
▪ The number of different compounds detected for each chemical class in bracket.

Table 5.3 Biochar total elemental composition (mg kg^{-1} ± s.e.m., n = 2).

	BC_1	BC_2	BC_3	BC_4
Al	195 ± 20	141 ± 25	782 ± 31	450 ± 32
As	0.7 ± 0.1	0.3 ± 0.02	2 ± 0.1	0.7 ± < 0.1
B	4 ± 0.4	4 ± 1	29 ± 0.2	6 ± 0.2
Ca	795 ± 89	1524 ± 183	8994 ± 172	685 ± 51
Co	nd°	nd	nd	nd
Cd	0.3 ± 0.2	0.1 ± 0.01	nd	0.1 ± 0.0
Cr	2 ± 0.3	2 ± 0.1	3 ± < 0.1	5 ± 0.01
Cu	4 ± 0.6	2 ± 0.4	9 ± 0.5	5 ± 0.2
Fe	486 ± 134	610 ± 53	1437 ± 2	2238 ± 60
P	82 ± 10	49 ± 10	525 ± 1	73 ± 7
Zn	18 ± 2	128 ± 17	41 ± 1	28 ± 2
S	74 ± 19	38 ± 3	466 ± 18	7 ± 3
Rb	0.5 ± 0.1	0.1 ± 0.1	nd	0.9 ± 0.0
Pb	0.4 ± 0.1	0.6 ± 0.1	nd	0.6 ± 0.0
K	1128 ± 121	671 ± 76	1713 ± 11	1472 ± 97
Mg	267 ± 29	208 ± 48	1206 ± 2	180 ± 22
Mn	60 ± 7	65 ± 15	95 ± 0.3	50 ± 4
Mo	nd	nd	nd	0.1 ± 0.0
Na	55 ± 5	65 ± 7	654 ± 1	85 ± 3
Ni	1 ± 0.2	1 ± 0.1	2 ± 0.0	2 ± 0.1
N	400 ± 35	1400 ± 14	650 ± 18	2200 ± 11
C	621650 ± 27	757600 ± 8	771800 ± 14	826400 ± 2

°nd = These matrices were not detected and were below the level of detection (As < 5.0 µg L^{-1}; Cd < 0.3 µg L^{-1}; Co < 0.5 µg L^{-1}; Cu < 0.6 µg L^{-1}; Li < 0.1 µg L^{-1}; Mo < 0.8 µg L^{-1}; Ni < 1.3 µg L^{-1}; Pb < 3.0 µg L^{-1}).

Table 5.4 Water extractable ions (mg kg^{-1} ± s.e.m., n = 2).

	BC_1	BC_2	BC_3	BC_4
Al	2 ± 0.0	0.6 ± 0.0	2 ± 0.0	1.2 ± 0.0
As	0.05 ± 0.0	nd	0.4 ± 0.0	nd
B	0.4 ± 0.0	0.2 ± 0.0	3 ± 0.05	1 ± 0.0
Ca	34 ± 0.4	102 ± 1	538 ± 1	51 ± 1
Co	nd	nd	nd	nd
Cd	nd	nd	0.1 ± 0.0	nd
Cr	0.01 ± 0.0	0.02 ± 0.0	0.2 ± 0.0	0.02 ± 0.0
Cu	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.05 ± 0.0
Fe	1 ± 0.0	1 ± 0.0	19 ± 0.4	2 ± 0.0
P	10 ± 0.3	2.4 ± 0.0	14 ± 0.0	9 ± 0.0
Zn	0.6 ± 0.0	1 ± 0.0	1 ± 0.0	1 ± 0.0
S	15 ± 0.1	5 ± 0.1	365 ± 1	28 ± 0.2
Rb	nd	nd	nd	0.3 ± 0.0
Pb	nd	nd	0.4 ± 0.0	nd
K	349 ± 1	219 ± 1	400 ± 0.2	882 ± 1.1
Mg	16 ± 0.1	7 ± 0.2	311 ± 0.2	14 ± 0.0
Mn	2 ± 0.0	1.2 ± 0.0	4 ± 0.0	1 ± 0.0
Mo	nd	nd	nd	nd
Na	23 ± 0.1	19 ± 1	295 ± 1	32 ± 0.3
Ni	0.04 ± 0.0	0.03 ± 0.0	nd	0.03 ± 0.0

° nd = These matrices were not detected and were below the level of detection (As < 5.0 µg L^{-1}; Cd < 0.3 µg L^{-1}; Co < 0.5 µg L^{-1}; Cu < 0.6 µg L^{-1}; Li < 0.1 µg L^{-1}; Mo < 0.8 µg L^{-1}; Ni < 1.3 µg L^{-1}; Pb < 3.0 µg L^{-1}).

5.2.3 N-enriched biochar production - 1st experiment.

5.2.3.1 Reagents

Ammonium sulphate ($(NH_4)_2SO_4$), 0.05 M, was made by dissolving 6.27 g $(NH_4)_2SO_4$ and 0.343 g $(^{15}NH_4)_2SO_4$ in deionised water to make a 1 L final volume. The $(^{15}NH_4)_2SO_4$ was ^{15}N-enriched (98.0 atom %^{15}N; Isotec, Miamisburg, Ohio) and produced a final $(NH_4)_2SO_4$-^{15}N enrichment of 5.36 atom %. Sodium hydroxide (NaOH), 0.1 M, was prepared by dissolving 4.0 g NaOH in 1 L DI water.

5.2.3.2 Method

The biochar samples were ^{15}N enriched by exposing them to the ^{15}N enriched NH_3, that was generated by reacting excess 0.1 M NaOH with ^{15}N enriched $(NH_4)_2SO_4$. Glass vials (20 mL) were filled up with glass sand and attached to petri dishes (50 mm diameter × 10 mm height) with epoxy resin. Next, petri dishes containing oven-dried biochar (1.5 g), < 2 mm, were placed in Mason jars containing 55 mL of 0.1 M NaOH. Four other mason jars were used as a control treatment, containing just the petri dishes without any biochar. Gas-tight lids were put on prior to injecting 25 mL of 0.05 M $(NH_4)_2SO_4$ via a septa in the lid using a syringe and 38 mm 16 gauge hypodermic needles (Precision-Glide, Becton-Dickinson, NJ, USA). The NH_3 was generated according to:

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O \qquad \textit{[Equation 5.1]}$$

Jars were left sealed for one week. Then excess 0.1 M H_2SO_4 was injected to neutralise the solution in the jars and allow any remaining NH_3 gas to be absorbed by the acid solution [Equation 5.2]. The jars were then left for a further 2 h.

$$2NaOH + H_2SO_4 \rightarrow Na_2SO_4 + 2H_2O \qquad \textit{[Equation 5.2]}$$

Figure 5.1 Photo of the biochar ^{15}N enrichment process where biochar is exposed to the ^{15}N enriched NH_3 that was generated by reacting excess NaOH with ^{15}N enriched $(NH_4)_2SO_4$. Note empty jar, one of four run as experimental blanks.

5.2.3.3 Sampling and analyses

All the ^{15}N enriched biochar samples were stored in sealed vials prior to analysis. Then, the biochar samples (BC and eBC) were analysed for total N and ^{15}N enrichment as explained for soil samples (Section 3.1.7) three days after labelling. Biochar inorganic-N concentrations (NH_4^+-N and NO_3^--N) were determined with a 1 h, 2 M KCl extraction (biochar: solution ratio of 1:25). The procedures for biochar total N, and ^{15}N enrichment and inorganic-N were the same as described earlier (Sections 3.1.7 and 3.1.6, respectively).

5.2.4 Stability of N-adsorbed on biochar particles - 2nd experiment.

The stability of the adsorbed NH_3 was assessed using the eBC_1 material since it contained a significant amount of adsorbed ^{15}N. Samples were placed under a continuous ambient air-flow of 0.65 m s^{-1} in a fume cabinet at room temperature for 12 days. Biochar subsamples were taken every other day and analysed, using CFIRMS, for total N content and ^{15}N

enrichment (Section 3.1.7). Also, SEM magnification of eBC_1 material was performed in order to see any difference in the biochar material after biochar was exposed to the NH_3.

5.2.5 Effect of biochars on plant N uptake - 3rd experiment.

5.2.5.1 Method

To determine the plant availability of biochar adsorbed NH_3, the four biochar materials, with and without ^{15}N enriched NH_3 were incorporated with the air-dried soil (50 g soil:1 g biochar), within 4 days of ^{15}N labelling, and placed into 60 mL pots made from plastic syringe bodies (ME-738/2, BD Drogheda, Ireland). The pot had Whatman No.42 filter paper at the base to prevent soil loss. The soil was then brought to field capacity (30% gravimetric water content (θ_g), WFPS = 48%) using deionised water prior to planting 5 perennial ryegrass seeds into the surface soil of each pot, except for the soil only treatment. These pots were subsequently kept in a growth cabinet for 25 days that had an alternating day/night temperature regime of 20°C/15°C, a relative humidity of 70%, and a 12 h day length (HPL340, 6 klux at plant level). Each pot was weighed on a daily basis and any water loss due to evapotranspiration was replaced with deionised water.

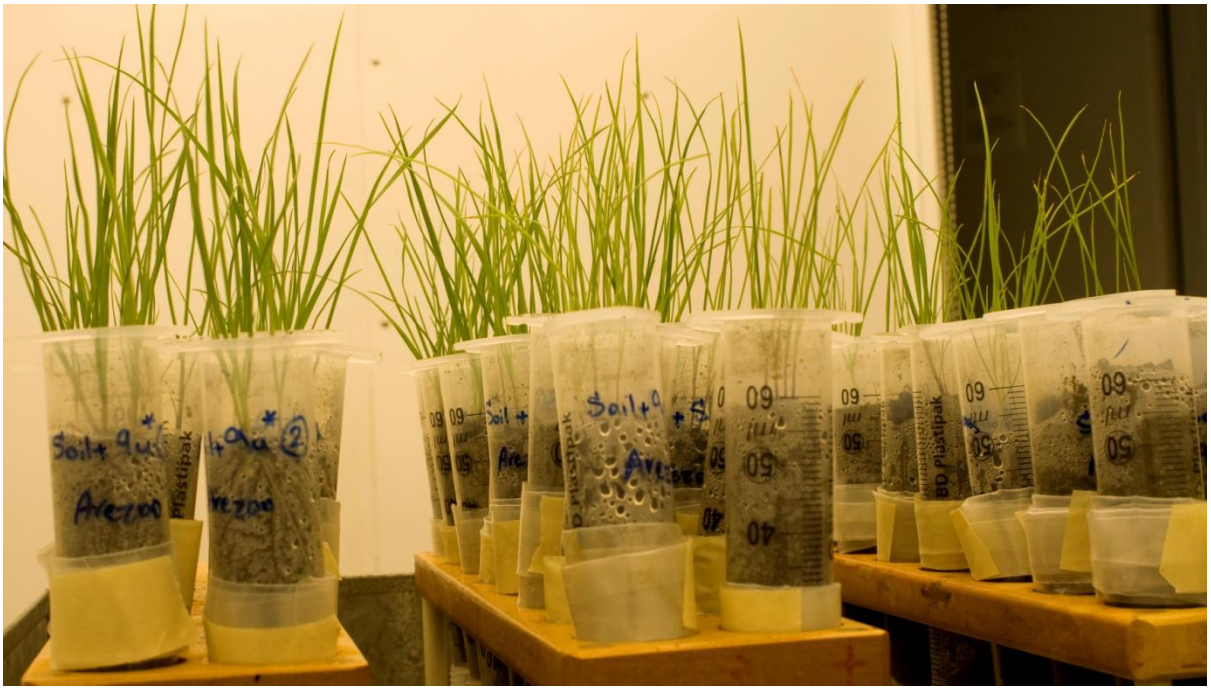


Figure 5.2 Photo of the pots and plants growing in growth cabinet during the 3rd experiment.

5.2.5.2 Sampling and analysis

After 25 days the pots were destructively sampled with ryegrass plants harvested and separated into leaf and root tissues. Roots were rinsed with distilled water, to remove soil particles, then leaves and roots were dried at 60°C for 48 h, weighed, and then ground (< 200 µm) prior to determination of total-N and ^{15}N enrichment by CFIRMS (Section 3.5.2). Biochar was separated from the soil and the gravimetric moisture content (Section 3.5.2) of soil and biochar determined. Biochar subsamples were rinsed with deionised water and dried (60°C), to remove any visible soil fragments. Subsamples of soil and biochar were taken for inorganic-N analyses using 2 M KCl extraction (10 g soil: 50 mL 2 M KCl; 0.2 g biochar:5 mL 2 M KCl) as described in Section 3.1.6. Further biochar subsamples were ground to < 200 µm prior to determination of total N content and ^{15}N enrichment (Section 3.1.7). Recoveries of ^{15}N applied in plant, soil, and biochar fractions were calculated in a routine manner (Section 3.5.3). The variance of the total ^{15}N recovered was calculated as being equal to the sum of the variances of each N pool plus twice the covariance of all two-way combinations of the N pools (Legg & Meisinger, 1982).

5.2.6 Statistical analysis

Statistical analyses were performed using Minitab®. Analyses of variance (ANOVA) was used to determine differences between treatment means. When differences occurred Tukey's method was used, with 95% confidence limits ($p < 0.05$), to compare treatment means. Linear regression was performed to determine relationships between variables. Graphing was carried out using SigmaPlot®.

5.3 Results

5.3.1 First experiment

5.3.1.1 Total N content and ^{15}N enrichment - 1st experiment.

Total N content (mg g^{-1}eBC) and atom% ^{15}N increased significantly (Figure 5.3) following the exposure of biochar to NH_3 gas, ranging from 7.8 - 10.0 and 3.37 - 4.88 respectively (p < 0.001).

Recovery of $(NH_4)_2SO_4$-^{15}N varied significantly with biochar material (p = 0.003), and equated to 25.6 (± 2.0), 20.6 (± 2.2), 13.4 (± 0.7), and 18.1 (± 1.5) percent in the eBC_1, eBC_2, eBC_3 and eBC_4 materials, respectively (s.e.m. in parentheses).

Linear regression showed that the N contents of the eBC materials were strongly related to their initial pH (r^2 = 0.92, p < 0.05) and their surface acidity (r^2 = 0.74, p < 0.14). Other measured variables did not correlate with increases in biochar N content (Table 5.5, and Figure 5.4).

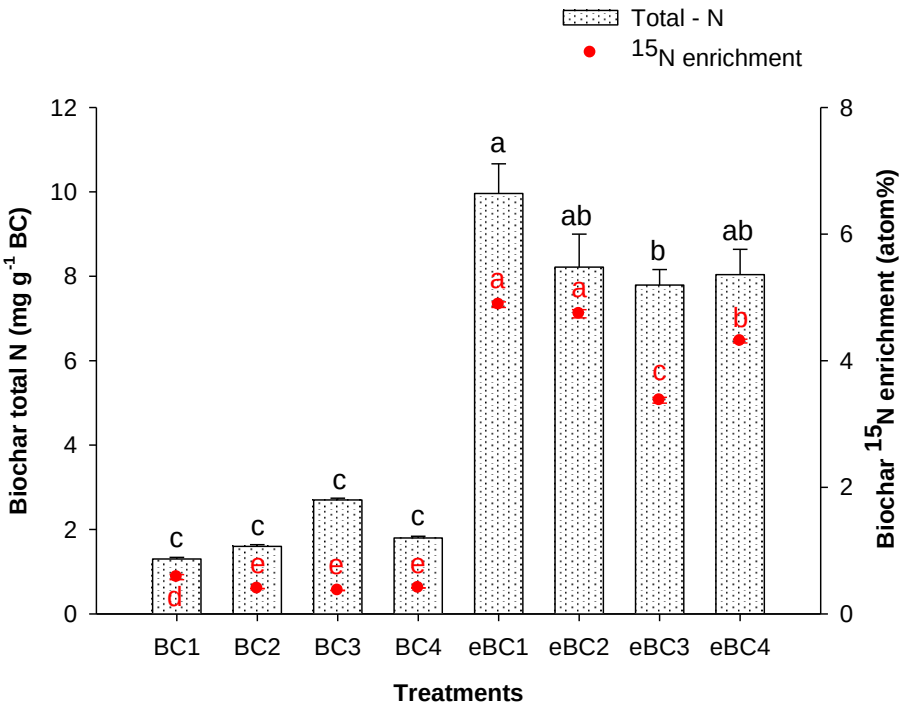


Figure 5.3 Total N content and ^{15}N enrichment of biochar materials pre and post expose to NH_3-^{15}N (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, p < 0.05). NOTE differing scales on y axes.

Table 5.5 Regression coefficients, with *p*-values, for the relationship between total N of eBC and measured biochar variables.

	R^2 (%)	*p* value
CEC	53.2	0.27
AEC	23.1	0.52
pH[°]	**91.6**	**0.04**
EC	50	0.29
S.A.[■]	74.1	0.14
I.A.[⊙]	65.4	0.19
Al	60.9	0.22
Ca	50.8	0.29
Fe	37	0.39
P	45	0.33
Zn	3.7	0.81
S	37	0.39
K	26.4	0.49
Mg	41.6	0.35
Mn	33.7	0.42
Na	52.3	0.28
N	10.4	0.68
C	69.4	0.17
As	34.4	0.41
B	52	0.28
Cr	21.3	0.54
Cu	46.1	0.32
Zn	3.7	0.81
Ni	19.1	0.56

[°]pH = pH (H_2O),

[■]S.A. = Surface Acidity, and

[⊙]I. A. = Iodine Adsorption.

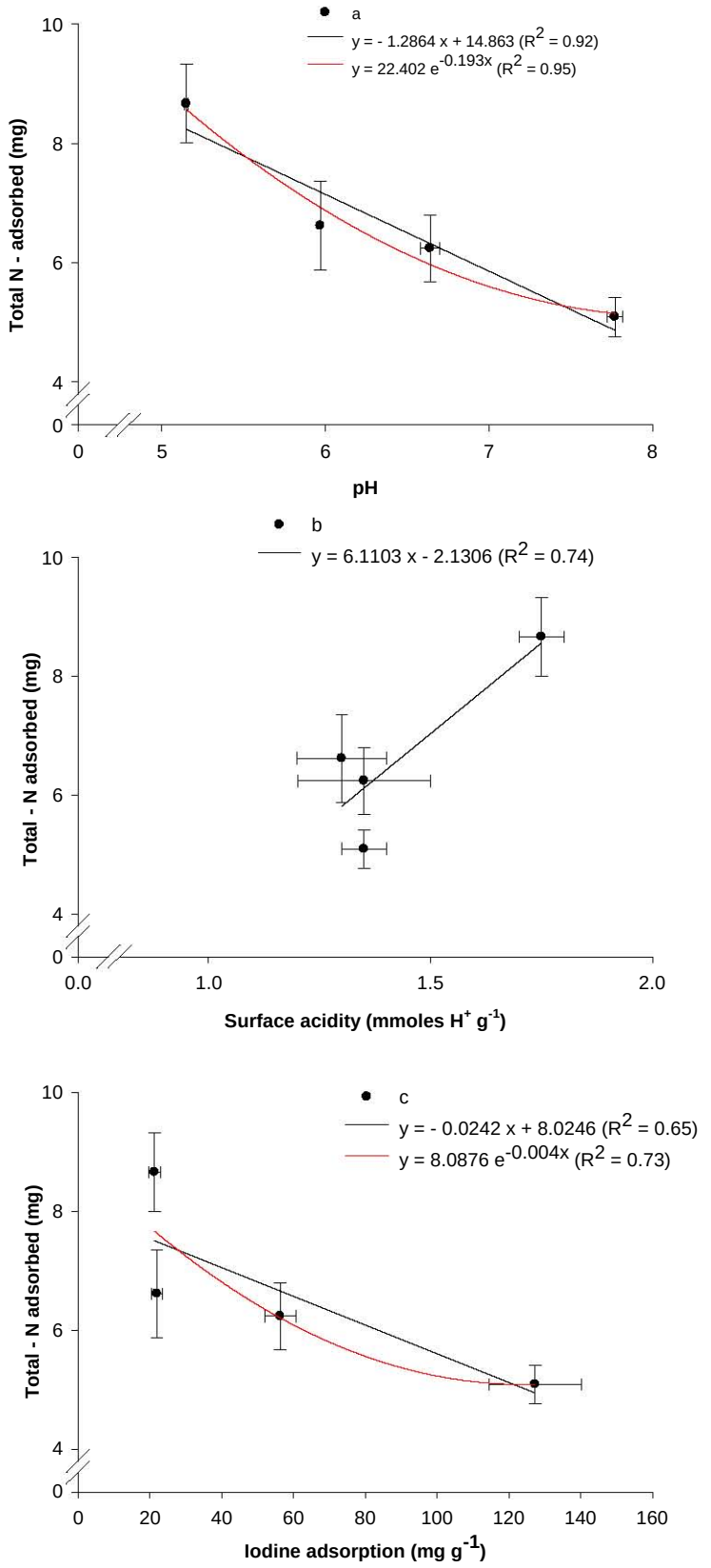


Figure 5.4 Relationships between total-N adsorbed and (a) initial biochar pH, (b) biochar surface acidity, and (c) iodine adsorption (error bars = ± s.e.m., n = 4). NOTE differing scales on x axes.

5.3.1.2 Inorganic-N - 1st experiment.

Extraction of eBC materials with 2 M KCl showed that their NH_4^+-N concentrations had increased following exposure to NH_3 (Figure 5.5), with BC and eBC materials containing an average 40 (± 1) and 760 (± 153) µg g^{-1} of NH_4^+-N, with biochar not affecting NH_4^+-N concentration (Figure 4.5). The initial NO_3^--N concentrations of the BC materials averaged 290 (± 1) µg g^{-1} of biochar, varying with the BC material. Inorganic-N accounted for 4 - 23% of total-N. However, post ^{15}N labelling NO_3^--N was undetectable in the eBC materials (Figure 5.5).

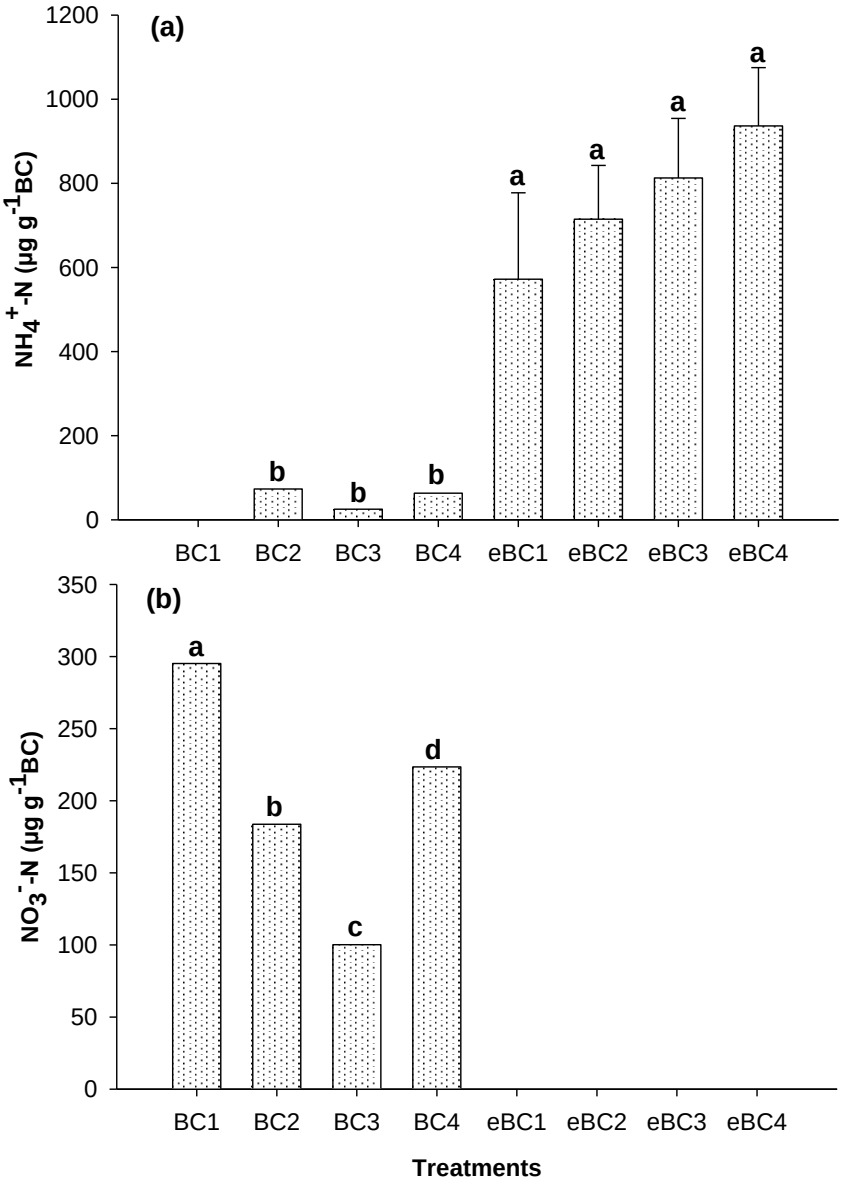


Figure 5.5 Mean biochar (a) NH_4^+-N and (b) NO_3^--N concentrations determined in the biochar materials pre and post expose to NH_3-^{15}N (error bars = + s.e.m.; n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). <u>NOTE</u> differing scales on y axes.

5.3.2 Second experiment

5.3.2.1 Stability of N-adsorbed on biochar particles - 2nd experiment.

The total N content and atom% ^{15}N of the eBC_1 material showed no significant changes occurred when leaving the samples in the fume cabinet over time ($p > 0.05$) with values of 8.5 mg g^{-1} and 4.4 atom % ^{15}N, respectively, when averaged over all days. It was demonstrated that the ^{15}N-biochar matrix was stable under ambient conditions (Figure 5.6). The SEM (2000 ×) showed no observable difference in the biochar materials pre- and post-exposure to NH_3 gas (Figure 5.7).

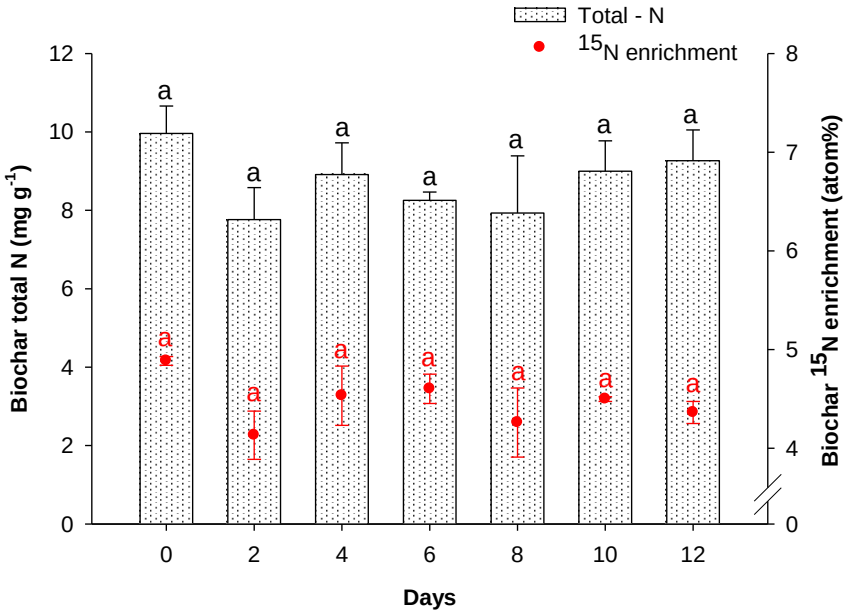
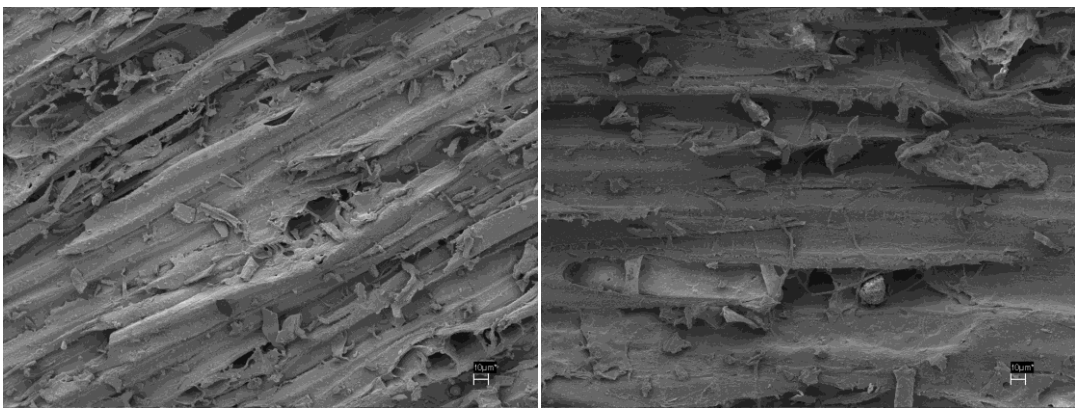


Figure 5.6 Total N content and ^{15}N enrichment of eBC_1 material over 12 days under an ambient air flow of 0.65 m s^{-1} (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scales on y axes.



(a) **(b)**

Figure 5.7 SEM images (2000 ×) of e BC_1, (a) pre- and (b) post-expose to NH_3-^{15}N. Scale bar equals 10 µm.

5.3.3 Third experiment

5.3.3.1 Dry matter yield - 3rd experiment.

In the nBC and BC treatments the leaf dry matter yield (DMY) averaged 27 ± 2.7 mg DM pot^{-1}. Root DMYs in the nBC and BC treatments were 38 ± 4.9 mg DM pot^{-1}. When the BC treatments were compared with the nBC treatment, there was no difference in yield (Figure 5.8). After 25 days the eBC treatments (Figure 5.8) had higher leaf DMYs (range 60 - 90 mg DM pot^{-1}) and root DMYs (100 - 110 mg DM pot^{-1}) than in the nBC and BC treatments (p < 0.01).

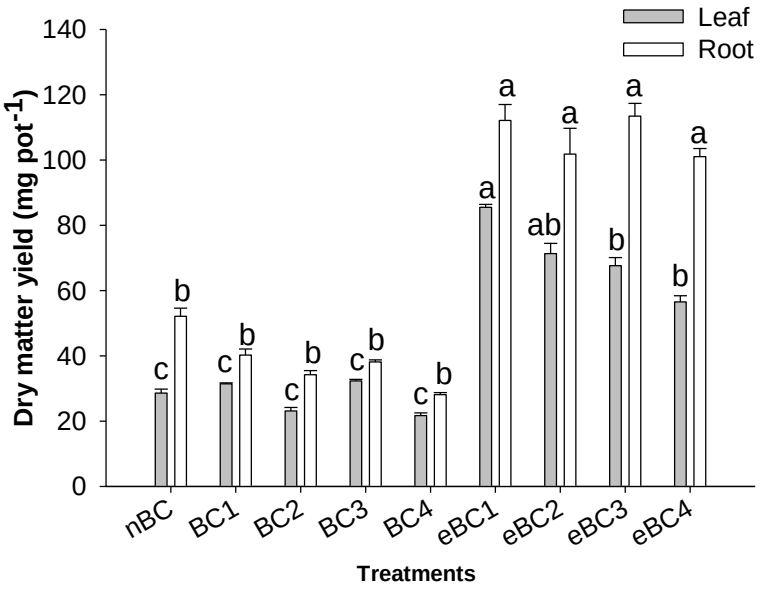


Figure 5.8 Leaf and root dry matter yields following the 25 day growth cabinet study (error bars = + s.e.m., n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$).

5.3.3.2 Total N content and ^{15}N enrichment in plants - 3rd experiment.

The N content of the leaves in the BC treatments did not differ from the leaves in the nBC treatment (Figure 5.9a) with average values ranging from 17 to 22 mg g^{-1}. Root N contents were higher (p < 0.05) in the BC treatments than in the nBC treatment (Figure 5.9b).
In the eBC_1 and eBC_2 treatments leaf N contents were higher (p < 0.001) than in the nBC treatment and were 41, 36 and 17 mg N g^{-1} herbage, respectively. In the eBC_3 and eBC_4 treatments the leaf N contents were elevated when compared to the nBC treatment (both 25 mg N g^{-1} herbage) but not statistically different (Figure 5.9a). The N content of the root tissues in the eBC treatments differed between treatments (p < 0.05) and were 9.6, 10.9, 6.2,

and 7.7 mg N g^{-1} root for the eBC_1, eBC_2, eBC_3, and eBC_4, respectively (Figure 5.9b), and N content was higher in the eBC_1 and eBC_2 treatments (Figure 5.9b).

The ^{15}N enrichment of the leaf material in the BC treatments averaged 0.37 atom% and did not vary with BC treatment or differ from the nBC treatment (0.37 atom% ^{15}N). Leaf atom% ^{15}N values were higher ($p < 0.01$) in the eBC_1 and eBC_2 treatments (3.56 and 3.35, respectively) than in the eBC_3 and eBC_4 treatments (2.88 and 2.65 atom%, respectively) and these were all elevated when compared to the BC or nBC treatment (Figure 5.9a). Root atom% ^{15}N values in the BC and nBC treatments averaged 0.371 atom% with no treatment differences (Figure 5.9b). The eBC treatments resulted in higher root atom% ^{15}N values than in the BC treatments, with higher values in the eBC_1 and eBC_2 treatments than in the eBC_3 and eBC_4 treatments (Figure 5.9b).

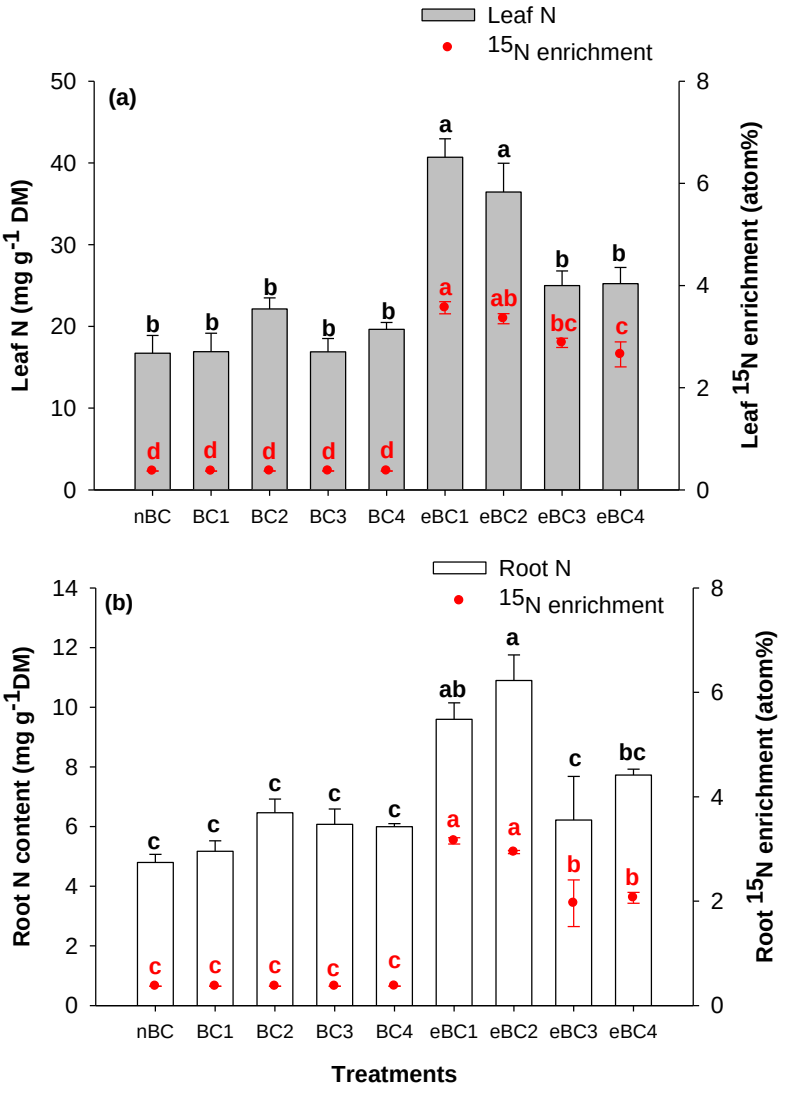


Figure 5.9 The N content and ^{15}N enrichment in (a) leaf and (b) root tissues following the 25 day growth cabinet study (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scales on y axes.

5.3.3.3 Uptake of N by plants - 3rd experiment.

The total leaf N uptake in the nBC and BC treatments did not differ due to treatment averaging 0.5 ± 0.1 mg g^{-1} (Figure 5.10). The total leaf N uptake in the eBC treatments was higher ($p < 0.01$) than in the nBC and BC treatments and also differed ($p < 0.01$) between eBC treatments, with higher uptake in the eBC_1 and eBC_2 treatments than in the eBC_3 and eBC_4 treatments (Figure 5.10).

The total N root uptake in nBC and BC treatments did not differ significantly between treatments (0.2 ± 0.02 mg g^{-1} root, when averaged over all treatments), but root N uptake was elevated in the eBC treatments, with no difference between eBC treatments (Figure 5.10).

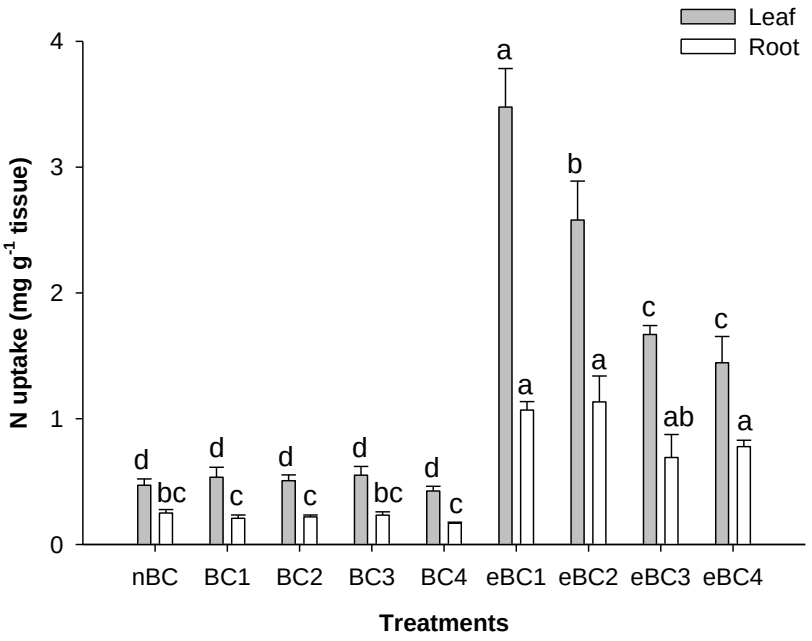


Figure 5.10 The N uptake by leaf and root tissues following the 25 day growth cabinet study (error bars = + s.e.m., n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$).

5.3.3.4 Recovery of ^{15}N in plant tissues - 3rd experiment.

The ^{15}N recovery in the leaf DM of the eBC treatments, did not differ between the eBC_1 (26 ± 3 %), eBC_2 (23 ± 2 %) and eBC_3 (19 ± 1 %) treatments (Figure 5.11). However, the eBC_1 and eBC_2 treatments had higher ($p < 0.01$) ^{15}N recoveries in the leaf DM than the eBC_4 treatment (11 ± 1.1 %). The ^{15}N recovery by root DM averaged 7 ± 2 % and did not vary with eBC treatment (Figure 5.11).

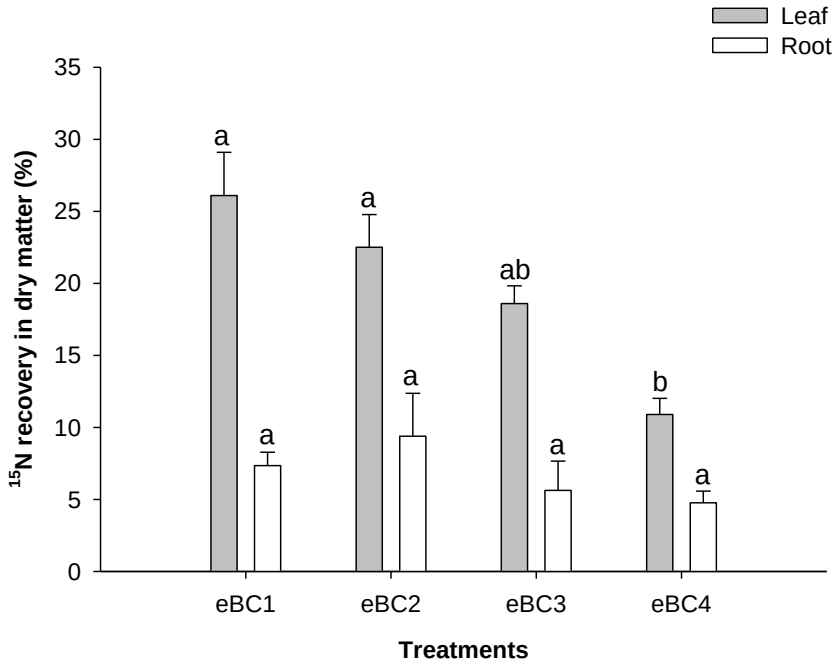


Figure 5.11 Recovery of ^{15}N applied in leaves and roots as a % of N applied in the biochar materials following the 25 day growth cabinet study (error bars = + s.e.m., n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$).

5.3.3.5 Biochar and soil total-N content and ^{15}N enrichment - 3rd experiment.

At the end of the experiment the biochar extracted from the BC treatments had N contents and ^{15}N enrichments ranging from 1.1 - 1.9 mg g^{-1} and 0.37 - 0.38 atom % ^{15}N, respectively (Figure 5.12). Higher values occurred in the eBC treatments ($p < 0.001$) where N contents (mg g^{-1}) and ^{15}N enrichments ranged from 2.2 - 4.8 mg g^{-1} and 2.13 - 4.50 atom% ^{15}N, respectively. The recovery of ^{15}N applied in the eBC_1, eBC_2, eBC_3 and eBC_4 materials, taken from the soil, equated to 11 (± 1), 10 (± 1.4), 3 (± 0.4), and 5 (± 0.4) %; respectively (Figure 5.13), with greater recovery in eBC_1 and eBC_2 treatments than in the eBC_3 and eBC_4 treatments ($p < 0.001$).

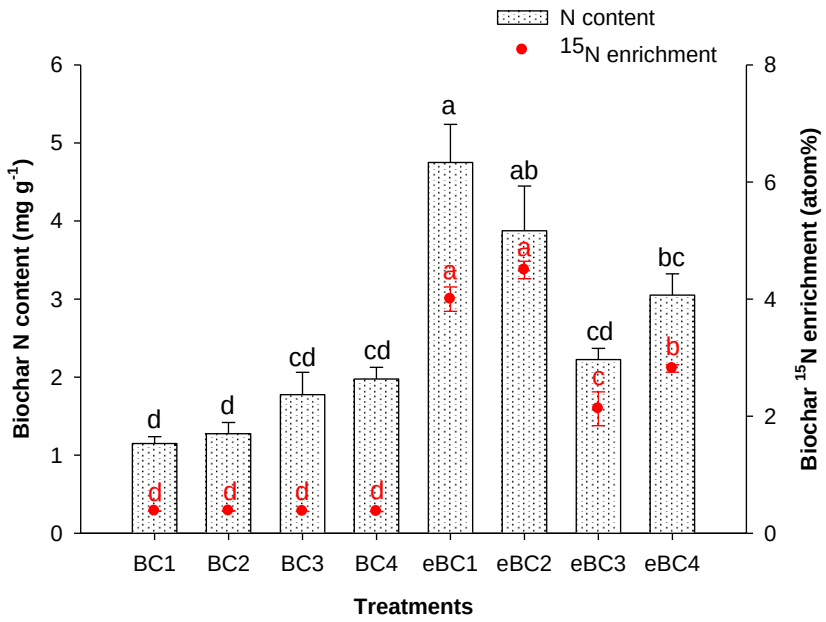


Figure 5.12 The N contents and ^{15}N enrichments of the biochar materials recovered from the soil following the 25 day growth cabinet study (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scales on y axes.

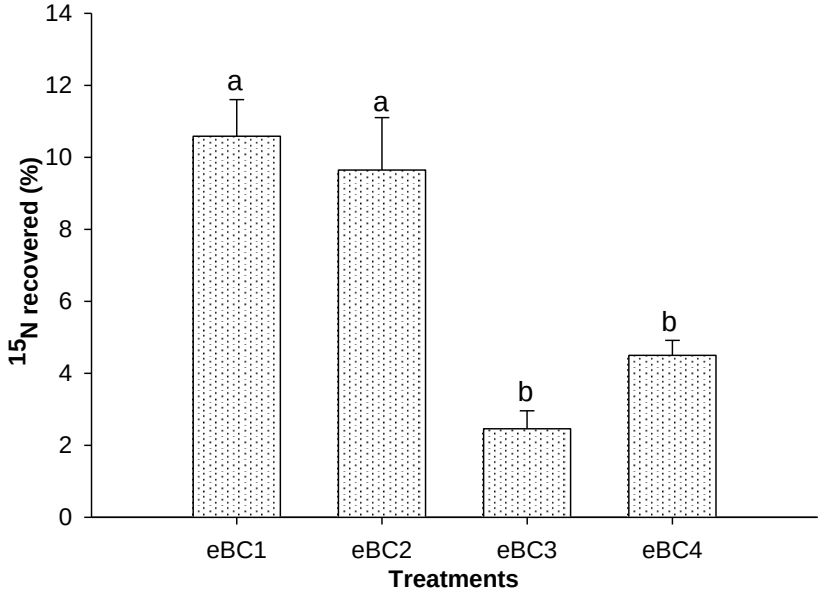


Figure 5.13 Recovery of ^{15}N applied in eBC treatments following the 25 day growth cabinet study (error bars = + s.e.m., n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$).

Total soil-N (organic + inorganic N (mg g^{-1})) at the end of experiment did not differ due to treatment with an average value of 2.4 (± 0.06) over all treatments (Figure 5.14). The atom% ^{15}N values (Figure 5.14) were higher in the eBC treatments (average 0.47 ± 0.01) in comparison to BC, S, and nBC treatments (average 0.37 ± < 0.1, $p < 0.001$). This reflected the presence of the ^{15}N enriched inorganic-N pool resulting from eBC addition. Mean recoveries of ^{15}N from the total soil-N pool in the eBC_1, eBC_2, eBC_3 and eBC_4 treatments were 45 (± 2), 29 (± 4), 47 (± 1), and 35 (± 6) %; respectively.

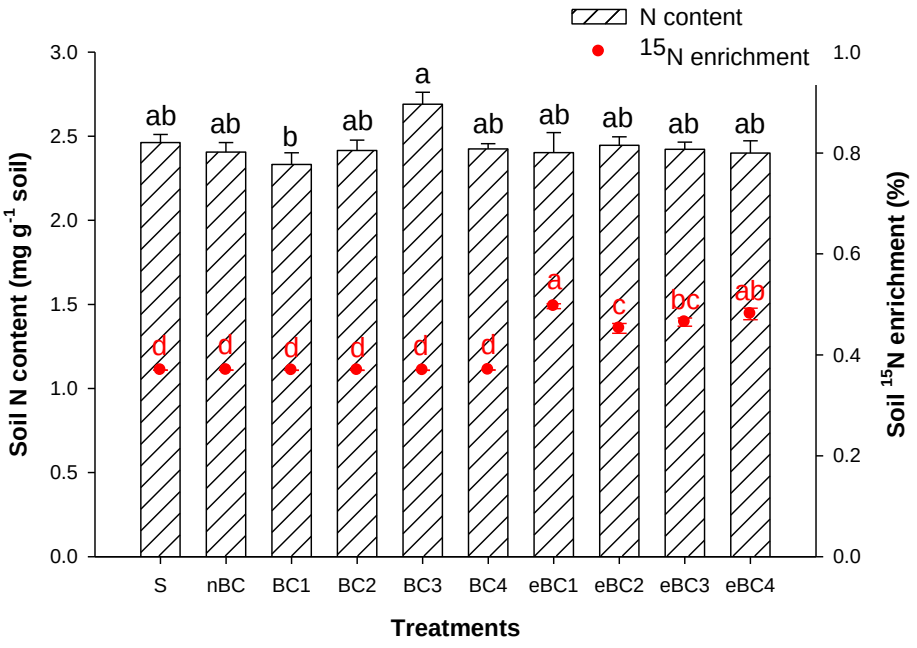


Figure 5.14 The N content and ^{15}N enrichment in soil fractions following the 25 day growth cabinet study (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). <u>NOTE</u> differing scales on y axes and S referred to soil treatment.

5.3.3.6 Inorganic-N in biochar and soil fractions - 3rd experiment.

The KCl extraction of the biochar particles removed from the soil at the end of the experiment showed that NH_4^+-N concentrations (µg NH_4-N g^{-1} biochar) did not differ due to biochar type (Figure 5.15a). However, the mean of NH_4^+-N concentrations did differ ($p < 0.05$) depending on whether biochar had been exposed to NH_3 with mean values of 1350 (± 702) and 430 (± 350) µg NH_4^+-N g^{-1} biochar in the eBC and BC treatments, respectively ($p < 0.05$). The NO_3^--N concentrations did not differ significantly ($p > 0.05$) between treatments (Figure 5.15b).

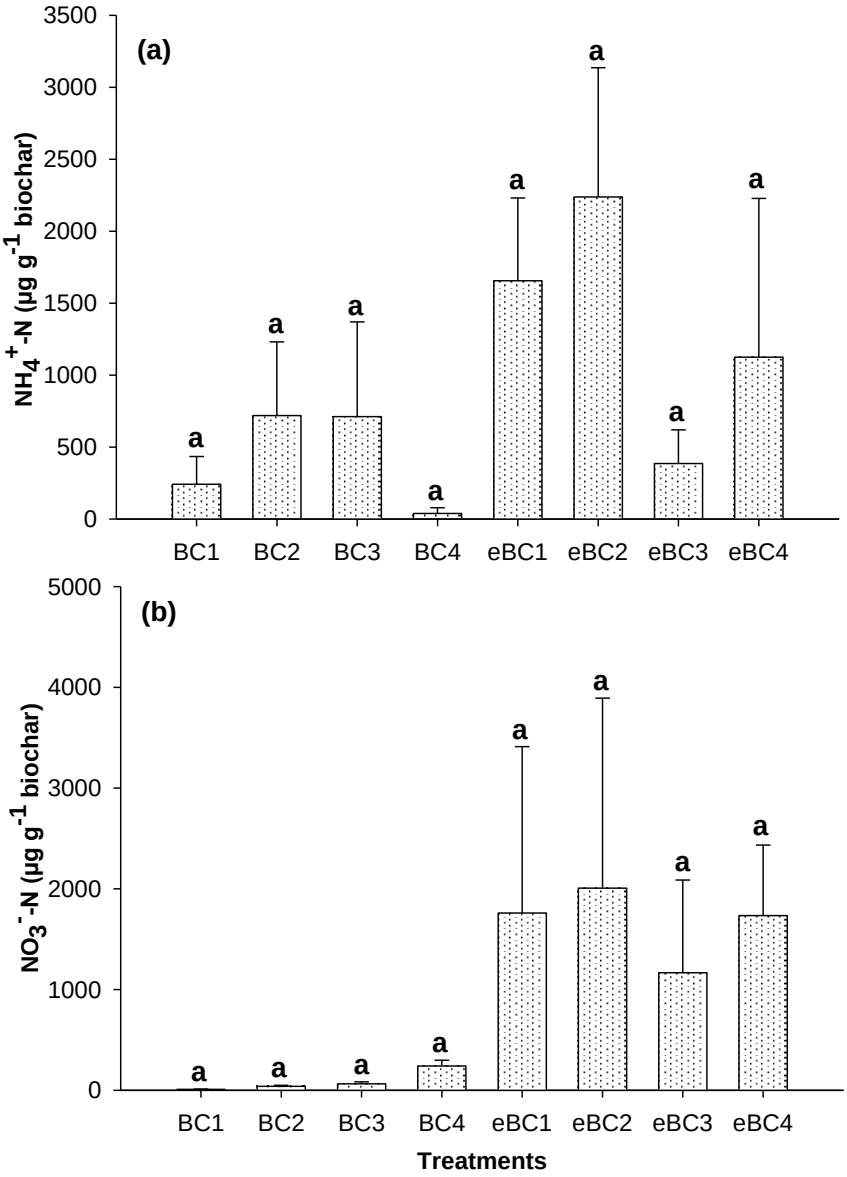


Figure 5.15 Mean biochar (a) NH_4^+-N and (b) NO_3^--N concentrations determined in the biochar particles recovered from the soil after 25 days (error bars = + s.e.m., n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scales on y axes.

The NH_4^+-N concentrations in the soil fraction of the S and nBC treatments were below the detection limit while the respective average NO_3^--N concentrations in these treatments differed ($p < 0.01$) and were 21 and 1.2 mg g^{-1} soil, respectively. In the BC treatments the soil NH_4^+-N concentrations were also under the detection limit while the soil NO_3^--N concentrations did not differ between BC treatments, ranging from 0.8 to 1.6 mg g^{-1} soil (Figure 5.16). In the eBC treatments soil NH_4^+-N concentrations were also under the detection limit, while soil NO_3^--N concentrations averaged 10.9 ± 7.4 mg g^{-1} soil but they did not differ statistically from the mean of the BC treatments (1.2 mg g^{-1} soil), neither did they differ due to eBC treatments (Figure 5.16).

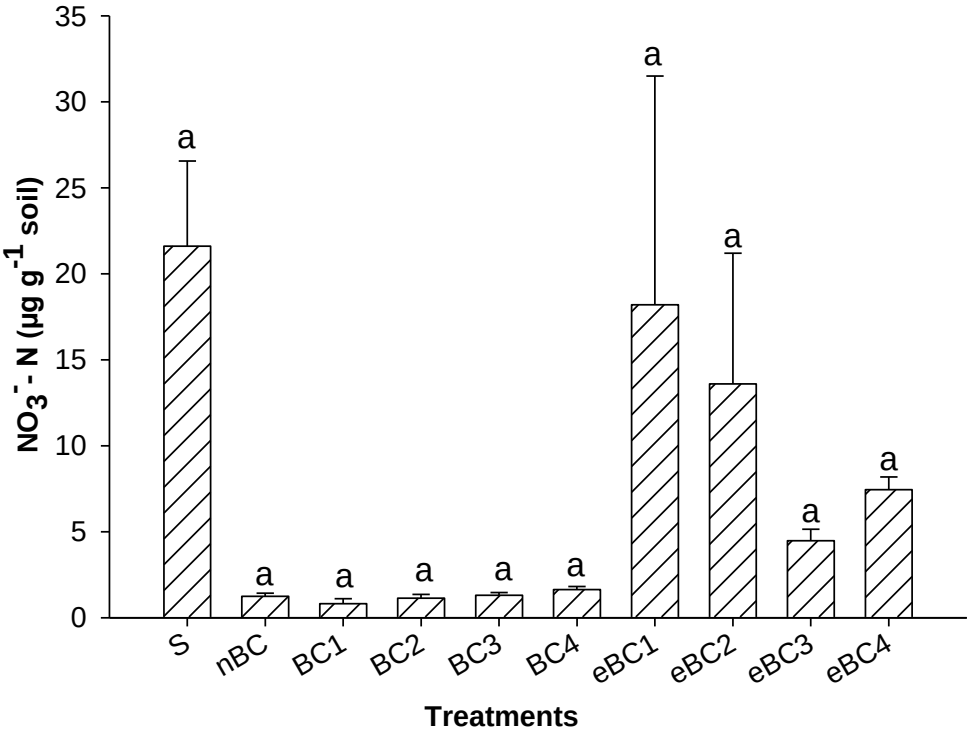


Figure 5.16 Mean soil NO_3^--N concentrations determined in the soil fractions after 25 days (error bars = + s.e.m., n = 4). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$).

5.3.3.7 Total ^{15}N recovery in plant, soil and biochar - 3rd experiment.

Mean total ^{15}N recovery (leaf + root + soil + biochar removed from soil) differed significantly ($p < 0.05$) with treatments; 89 ± 2, 71 ± 8, 73 ± 2 and 56 ± 6 % in eBC1, eBC_2, eBC_3 and eBC4; respectively. It is assumed unrecovered ^{15}N was lost in N gaseous forms.

5.4 Discussion

5.4.1 Biochar adsorption property

This study showed biochar materials capturing up to 8.7 mg of NH_3-N g^{-1} of biochar. This was higher than in previously summarised studies (Clough & Condron, 2010) where rates of the order of 0.2 to 1.8 mg of NH_3-N g^{-1} of biochar were noted. The difference may be a function of biomass type (feedstock) used, biochar pyrolysis conditions and/or the NH_3 concentration the biochars were exposed to. To date the literature suggests that, acid functional groups are a key factor in a biochar's ability to adsorb NH_3 (Clough & Condron, 2010). Other experiments that have exposed biochar to ozone, resulted in increased NH_3 adsorption, reinforcing the theory that acid functional groups were responsible for NH_3 adsorption (Kastner et al., 2009). The close relationship observed here between either the biochars pH and surface acidities and the amount of NH_3-N adsorbed supports this idea.

Li et al. (2003) demonstrated that flue-gas CO_2 could be removed via formation of ammonium carbonate (NH_4HCO_3) onto biochar surfaces when NH_3 was present (Li et al., 2003). Following this Day et al. (2005) used scanning electron microscopy (SEM) to observe the formation of a white powder both inside and on the surface of biochar material produced at 400°C and exposed to NH_3 in the presence of CO_2 (Day et al., 2005). Using the same SEM magnifications as Day et al. (2005) on the BC and eBC materials, no visible difference in the biochar materials was observed. This doesn't rule out the possibility of NH_4HCO_3 formation, since experimental conditions and substrate rates used here will have differed. However, it raises the possibility of other mechanisms sequestering the NH_3. The close relationship between biochar pH and surface acidity, and the stability of the eBC_1 material over time suggest that NH_3 was sequestered into the biochar with the resulting product in an NH_4^+ form. The stability of the eBC material tested in terms of its N content and ^{15}N enrichment showed that the N compound formed on the biochar was not subject to sublimation.

Increasing pyrolysis temperature results in a decrease in acidic functional groups (Asada et al., 2002; Kastner et al., 2009). The results of the current study used a limited range of biochar manufacturing temperatures but ^{15}N adsorption by biochar was lower in the biochar made at the highest manufacturing temperature (BC_4). Ammonia concentrations of 41 μL L^{-1} have been recorded in the headspace above synthetic urine patches after 5 minutes (Clough et al., 2003), and soil atmosphere concentrations will likely be larger.

Assuming that NH_3 adsorption occurs in-situ, and there are no reasons to suggest otherwise, then biochar previously incorporated into the soil may act as a slow release N pool for plants once NH_3 has been produced and adsorption occurs; therefore reducing the leakage of N from

agricultural systems. Ammonia volatilisation losses have also been shown to be reduced during the composting of animal waste with biochar (Steiner et al. 2010) and it may be possible to recycle N from animal housing facilities if biochar adsorbed NH_3 proves to be bioavailable following composting.

5.4.2 Effect of biochar and N-adsorbed biochar on plant

Nitrogen uptake is a function of plant DM production and N concentration in the DM produced. The use of ^{15}N stable isotope unequivocally demonstrates that NH_3 adsorbed onto biochar can provide a source of N for plants when the biochar-NH_3 complex is placed in the soil matrix. Increases in dry matter yield were a consequence of the increased soil N availability under the eBC treatments, as demonstrated by the uptake of ^{15}N isotope in the grass and root leaf tissues. Nitrogen uptake was typical of what might be expected following N fertiliser application with leaf N contents in the order of 40 mg g^{-1} of leaf tissue. Higher average recovery in eBC_1 probably was due to more ^{15}N being combined with acidic functional groups. The eBC material which had the least effect on leaf dry matter yields was in fact the eBC_3 material which had the highest pH.

Thus biochar has the potential to act not only as a passive scrubber of NH_3 but also as a N fertiliser, enhancing the benefit of sequestering biochar-embodied C into the soil.

In order to maximise the potential uptake of NH_3, biomass pyrolysis conditions need to be tuned to enhance the acidity of the biochar material produced. Further testing must now be performed to ascertain in-situ biochar uptake rates of NH_3 under conditions where anthropogenic NH_3 emissions occur and to also examine the fate of this biochar-adsorbed-NH_3 in terms of plant availability.

5.5 Conclusions

This study provides support for the hypothesis developed in Chapter 4 where it was assumed that adsorption of NH_3 was responsible for the observed reduction in N_2O fluxes and changes in their ^{15}N enrichment. The 78% increase in DM yield in eBC treatments supports the hypothesis of this experiment, that biochar adsorbed NH_3 is bioavailable. However, this study now raises more questions which require future studies to investigate: (a) the efficiency of a biochar-N input versus traditional synthetic fertilisers, (b) NH_3 adsorption by biochar in the urine patch or under urea fertiliser applications and subsequent plant N uptake, (c) the delivery mechanism(s) and factors affecting biochar NH_3 adsorption in-situ, and (d) the quantity of N released and its duration; from biochar adsorbed-NH_3 over time.

Chapter 6
NH_3 emissions from a regular pattern of cattle urine patches[5]

6.1 Introduction

Urine and dung from farm animals are both sources of both N_2O and NH_3. Of these two sources, urine contributes a much larger fraction than dung to the total NH_3 emissions (Jarvis et al., 1989; Ryden et al., 1987; Sherlock et al., 2008). Following the hydrolysis of the urea in the urine voided by grazing animals onto pasture, between 20 and 80 g m^{-2} of ammoniacal-N (NH_4^+-N + NH_3-N) is found within a typical urine patch (Oenema et al., 1997). The relative proportions of each form of ammoniacal-N is dependent on the soil pH, temperature, and NH_3-N volatilisation rate (Haynes & Sherlock, 1986). As the NH_3 is emitted, it is transported away from the patch by the wind both vertically and horizontally. These conditions are far removed from those typically experienced under laboratory conditions and consequently emissions under field conditions can differ substantially from NH_3 volatilisation losses measured in the laboratory (Fenn & Hossner, 1985). Within the urine patch the highest concentration of urine-N is in the top 15 mm of soil with 75% of urea typically located within that depth, although both unhydrolysed urea and ammoniacal-N can move deeper into the soil after rainfall (Holland & During, 1977).

In this experiment, plot area, urine patch number and urine volume were designed to provide a realistic simulation of the urine load created by about a dozen dairy cattle grazing an area of pasture for about 24 h. This grazing time and the stocking density are typical for rotational grazing practices on Canterbury dairy farms.

The objective of the agency (Manaki-Whenua Landcare Research) which supported this particular study was to obtain new experimental data to determine $Frac_{GASM}$ in a controlled, but realistic, farming situation and relate the obtained emission rates to the known chemistry and physics of the NH_3 volatilisation process (see section 2.2.3.2). The main objective for carrying out this particular NH_3 volatilisation study for this current thesis was to provide a

[5] This work described in this Chapter is included in the following:
Laubach, J., Taghizadeh-Toosi, A., Sherlock, R. R., & Kelliher, F. M. (2011). Ammonia emissions from a regular pattern of cattle urine patches. *Agricultural and Forest Meteorology. (Submitted).*

direct in-field measurement of the likely maximum extent of NH_3 emissions following urine application to pasture in the absence of added biochar. Such a measurement is an essential link between earlier static chamber attempts at measuring NH_3 emissions (Chapter 4) and later laboratory-based dynamic chamber (Chapter 7) measurements which, in both cases, employed zero biochar as one of the treatments. In this experiment, a grid pattern of cattle urine patches was created within a circular area and NH_3 emissions from this area measured for several days.

6.2 Materials and methods

6.2.1 Paddock establishment

This NH_3 volatilisation field experiment was conducted from 24 February to 5 March 2010 on a paddock 1 km west of Lincoln University, South Island (43° 38.56' S, 172° 27.34' E). The soil is classified as an Eyre stony fine sandy loam (Hewitt, 1998). The general soil properties are shown in Table 6.1. Sheep grazing on the experimental area ceased 14 days prior to the experiment to ensure the base level of NH_3 emissions were as small as possible, and the paddock was mown the following day. The surrounding terrain consisted of similar flat paddocks. A circle with a 15 m radius was marked as the experimental plot such that the nearest shelterbelts were at ca. 150 m distance to the NE and S, and farther away in other directions. It should be noted that the need for a circular large plot was the reason to choose this paddock with these soil properties which were similar to the soil properties of the other chapters (Table 4.2).

Table 6.1 General soil properties at the experimental site°.

pH	5.9
Total N (mg kg^{-1})	2.82
Total C (mg kg^{-1})	26.7
Olsen P (mg kg^{-1})	15.84
K ($cmol_c$ kg^{-1})	0.46
Ca ($cmol_c$ kg^{-1})	10.5
Mg ($cmol_c$ kg^{-1})	0.71
Na ($cmol_c$ kg^{-1})	0.15
CEC ($cmol_c$ kg^{-1})	15
Total Base Saturation (%)	77
Available N (kg ha^{-1})	98
Anaerobically Mineralisable N (μg g^{-1})	65
Bulk density (g cm^{-3})	1.01

°Soil test was performed commercially by Hill Laboratories, Hamilton, New Zealand. Soil sample depth was 0 - 0.075 m. Thirty soil cores were taken from the site, bulked, and submitted for analysis, n=1.

6.2.2 Integrated Horizontal Flux method

A micrometeorological mass balance procedure that can be carried out over relatively small areas, and is particularly suited to measuring $NH_{3(g)}$ volatilisation, is the 'Integrated Horizontal Flux' (IHF) method. This method was first proposed by Beauchamp et al. (1978) and was employed to study the NH_3 volatilisation rates from downwind concentration profiles, emitted from sewage sludge applied to a field, in order to calculate emission rates (Beauchamp et al., 1978; Denmead, 1995). The method assumes that all the NH_3 gas emitted from control sources within a defined ground area leaves via the downwind side, carried by the wind. To make the method independent of wind direction, the source area is given a circular shape and the horizontal flux of NH_3 is measured in the centre of the circular plot (20 to 30 m in radius). Air arriving at the centre will always have travelled along some radius of the circle, regardless of wind direction, thus a constant fetch (equal to the plot radius) is maintained. A mast is located in the centre of the treated plot and devices are placed at various heights on that mast to measure the product of wind speed, u, and $NH_{3(g)}$ concentration, ρ, i.e. the horizontal NH_3 flux. By using passive samplers of appropriate design (Leuning et al, 1985) $\overline{u\rho}$ can be measured directly (where the overbar denotes the time

average over the collection period) (Figure 6.1). To obtain the total flux, the products, $\overline{u\rho}$ are integrated over the full height of the resulting NH_3 plume (Figure 6.2).

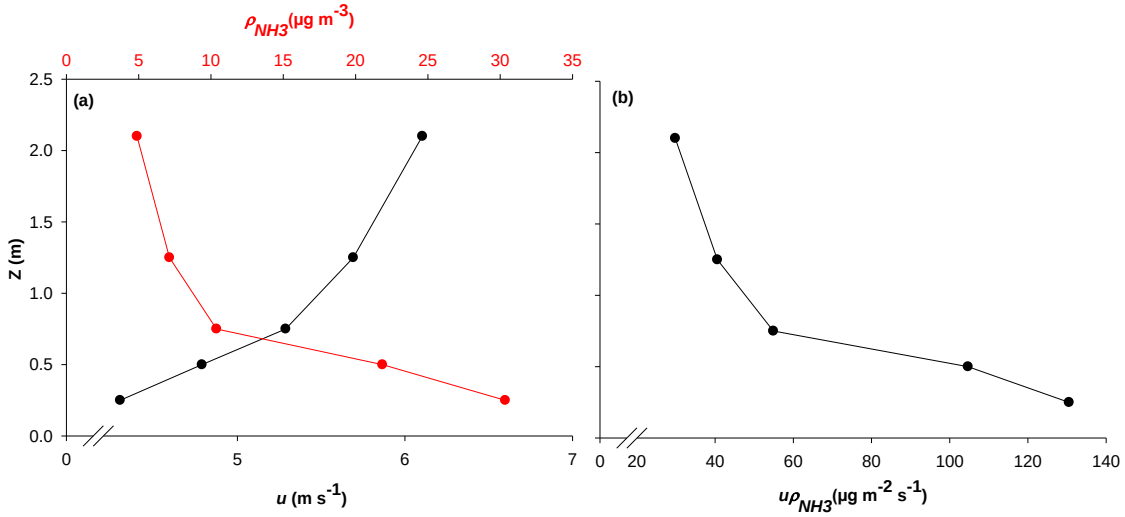


Figure 6.1 (a) Example of vertical profiles of wind speed, *u*, and NH_3 concentration, *ρ* ,and (b) profile of the horizontal flux (product of *u* and *ρ*).

According to Leuning et al., (1985) $\overline{u\rho}$ is obtained by:

$$\overline{u\rho} = \frac{M}{At} \qquad \textit{[Equation 6.1]}$$

where;

M : the measured mass of NH_3 collected (µg NH_3-N)

t : sampling period (s)

A : the effective cross-sectional area which is determined by wind-tunnel calibrations ($2.42\times10^{-5}m^2$)

The vertical flux density, F, can be expressed as:

$$F = \frac{1}{X}\int_0^z (\overline{u\rho} - \overline{u\rho_b})dz \qquad \textit{[Equation 6.2]}$$

where;

F : vertical flux density (µg NH_3-N m^{-2} s^{-1})

X : the distance travelled by the wind over the treated area, i.e. the radius of the circular plot (15 m)

$\overline{u\rho}$: horizontal NH_3-N flux at each sampling height, z, (µg NH_3-N m^{-2} s^{-1})

$\overline{u\rho_b}$: mean horizontal background NH_3-N flux (µg NH_3-N m^{-2} s^{-1})

The integral in Equation 6.2 is an acceptable approximation of the integrated horizontal flux during each sampling period obtained by calculating the sum of the shaded areas as depicted in Figure 6.2 below, i.e.:

$$\int_0^z (\overline{u\rho} - \overline{u\rho_b})dz = \sum A + B + C + D + E \qquad \textit{[Equation 6.3]}$$

However, it must be noted that the extrapolation of the horizontal flux to zero at zero height is likely to be more complex than shown below (Figure 6.2a) since ρ keeps growing towards the ground while u approaches zero rather more slowly. Allowing for this effect would increase the size of the bottom horizontal bar in Figure 6.2a and further contribute to the overall integrated horizontal flux. A conservative approach to estimating this effect is to simply double the width of the bottom horizontal bar (Figure 6.2b), and this is the approach used for each measurement period in this experiment. In addition, the horizontal flux might not reach zero at the highest measurement level (Figure 6.2) leading to a small error. For simplicity, this small error has been neglected in Figure 6.2.

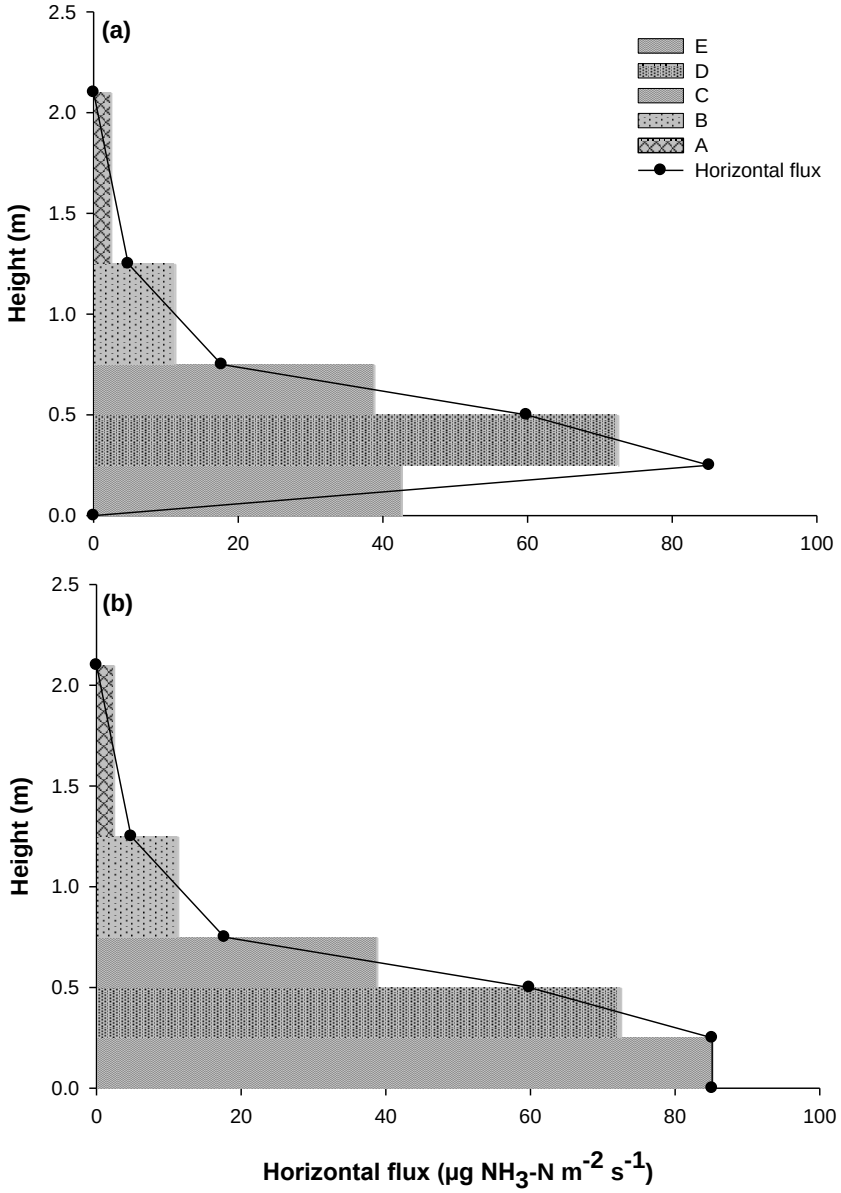


Figure 6.2 The area beneath the horizontal flux curve (the hatched rectangles, A to E) was used to calculate the vertical flux during a typical sampling period; (a) the graph with extrapolation of the horizontal flux to zero at zero height and (b) the graph with modification of the horizontal flux at zero height.

6.2.3 Micrometeorological conditions

Weather conditions before and during the experiment were generally warm and dry. An area of ca. 40 m by 70 m including the marked circle was irrigated repeatedly prior to the experiment, first on 12 Feb and last on 23 Feb, providing an estimated total of 40 mm. Only one light rainfall event occurred during the experiment itself, providing 2.5 mm of

precipitation, over night from 25 to 26 Feb between 23:30 and 02:30 (36 to 39 h after urine application).

6.2.4 Experiment setup geometry

To ensure that the concentration profile is always measured downwind of the emission source, a setup with rotational symmetry was used with the NH_3 profile mast in the centre of a circular emission source so that the length of the emitting source (fetch) was constant and independent of wind direction.

Sampling heights were 0.25, 0.50, 0.75, 1.25 and 2.10 m above ground on the mast (Figure 6.3). Four masts were set up at 50 (± 5) m distance from the circle's centre to the NW, NE, SE and SW. Dependent upon wind direction forecast, for any given collection period one of these masts was anticipated to be upwind of the circular plot, and an additional NH_3 sampler was mounted at 2.1 m height on this mast to determine the upwind background concentration. The horizontal location of the background sampler is the only parameter in the setup that depended on wind direction. A wind profile mast with cup anemometers at the same five heights as the NH_3 samplers was installed 35 m to the NNW from the circle's centre (Figure 6.4).

Figure 6.3 Photos of wind profile (left) and NH_3 profile (right) masts.

Within the circular plot, a rectangular grid of 132 points was marked with locations for urine patches. The distance between neighboring patches was 2.3 m in order to provide a homogenous source of NH_3. The central grid point (location of the NH_3 mast) and the four grid points nearest to it were omitted, to provide an access area where an operator could change the NH_3 samplers without disturbing any urine-treated soil. This effectively defined an inner radius for the urine patch area (Figure 6.4). In addition, at 1 m distance outside the perimeter of the 15 m circle, 24 further urine patch locations were placed, at equal distances of 4.2 m from each other, for the purpose of repeated soil sampling during the experiment. This soil sampling inevitably constituted a potential disturbance to the NH_3 emissions process. While the 24 disturbed urine patches are accounted for as sources in the computation of the emission rates, their placement at the perimeter of the circle ensured that they provided only a minor fraction of the NH_3 collected at the circle's centre. The major fraction originated from the 132 patches within the 15 m radius. One additional urine patch was created at 20 m distance from the circle's centre, still within the irrigated area, for the purpose of taking horizontal transects of soil samples across a urine patch. This single patch is not accounted for in the emission rate computations, and the error from this is considered negligible.

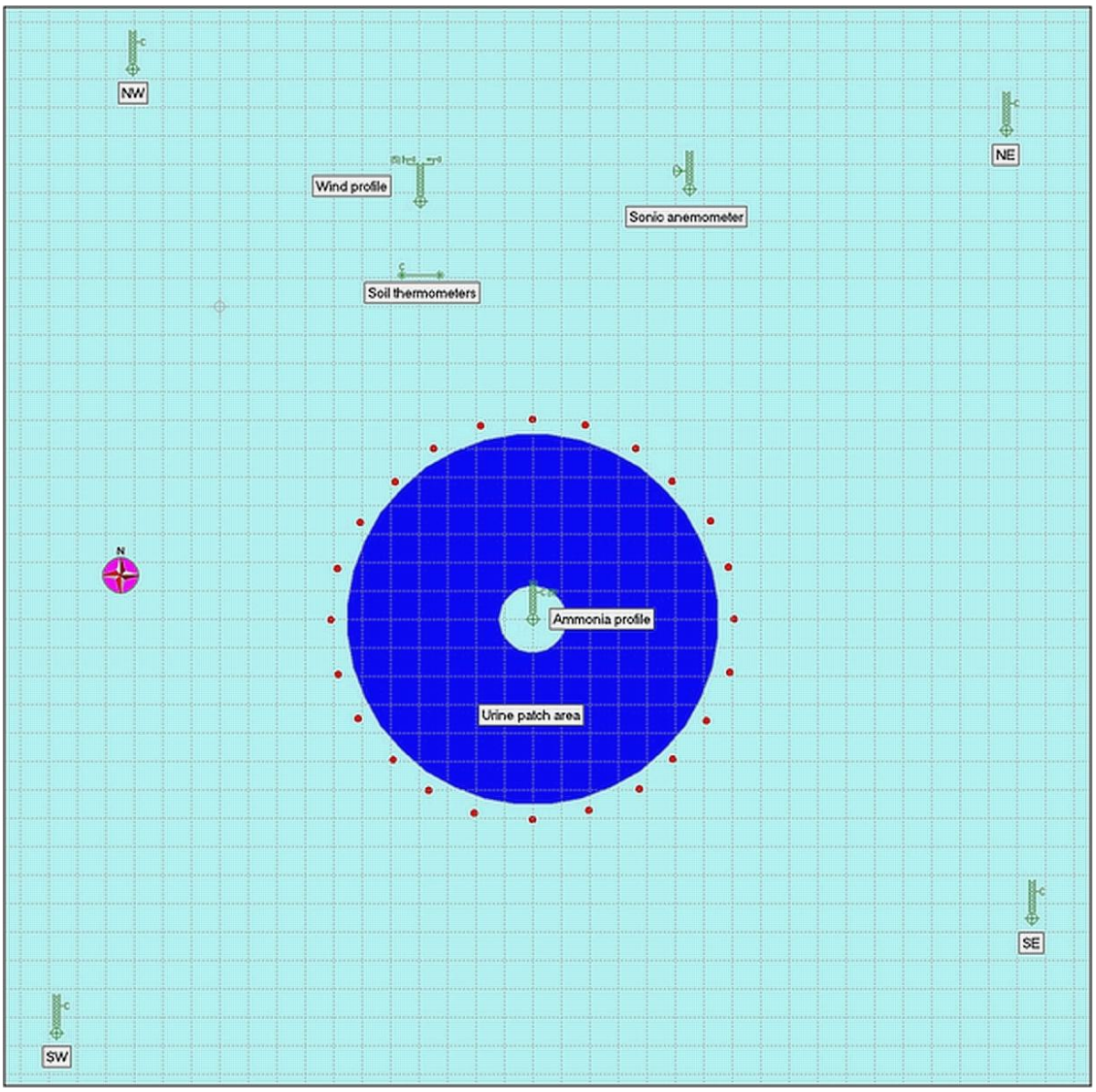


Figure 6.4 Scale diagram of the experimental setup. 132 urine patches were created within the dark blue area, one at each grid point (grid cell size is 2.3 by 2.3 m). From 24 extra urine patches (red dots), soil samples were repeatedly taken. Ammonia was collected at five heights in the centre of the dark blue area, and at one of the four locations labelled NW, NE, SE, and SW (the one closest to the anticipated upwind direction).

6.2.5 Urine collection, amendment and application

Two days prior to the initiation of the experiment, 235 L of cattle urine was collected, by a team of personnel, from dairy cows during the afternoon milking at the Lincoln University Dairy Farm. The urine was collected directly from the cows in 5 L plastic jugs as the animals urinated. It was then transferred into a single large (270 L) plastic drum, and from there dispensed into 12 separate 20 L PVC containers for easy overnight storage in a refrigerated room at 4°C. The N content of the urine determined early the following day on duplicate subsamples using a C-N analyser (Section 3.1.7), was 5.07 g L^{-1}. Following overnight storage, the contents of the 20 L containers were poured back into the 270 L drum, water (5 L) was added to obtain the required volume of 240 L, and then the N content was boosted to 10.0 g L^{-1} by addition of urea (2.683 kg). The granulated urea was added with stirring and after it had dissolved the modified urine was transferred in 1.5 L portions into 160 PVC milk bottles (2 L). These bottles were placed into bins for easy overnight storage in the refrigerated room at 4°C.

From 10:45 to 11:30 the following day, the 1.5 L urine samples were then poured onto each of the 157 previously marked locations within the experimental plot in a controlled fashion, using funnel devices with tubing attached to the outlet. The outlets of these devices were held at 1.2 m above ground. This pouring method was designed to realistically simulate the shape and size of real cattle urine patches, aiming for about 0.3 m radius. In practice, the patches were not necessarily circular because gusty NW winds during the pouring period, caused considerable scatter in aiming accuracy. The total amount of N applied to the test plot was equivalent to a uniform application of 33 kg N ha^{-1} over all the entire area or 600 kg N ha^{-1} on actual urine patches.

6.2.6 Soil analyses

6.2.6.1 Urine patch size and penetration depth

In order to assess the penetration depth and breadth of the urine in typical urine patches, four patches of brilliant-blue dye FCF in aqueous solution were created about 5 m south of the urine patch circle, within the irrigated area. For each of these patches, 7.5 g of brilliant-blue dye was added to 1.5 L of water and delivered to the soil with the same pouring method as used to create the urine patches.

Figure 6.5 Photo of urine patch size simulation using brilliant-blue dye in aqueous solution.

6.2.6.2 Soil temperature

Soil temperature was measured at two depths (2 and 5 cm) in two replicates, using thermocouples. These were buried 1 m apart from each other, 6 m south of the wind profile mast, well within the irrigated area.

6.2.6.3 Soil sampling

On each occasion when the ‘Leuning samplers’ were changed (section 6.2.7.2) except the last change of the day, a sub-set of 6 soil cores was sampled from the urine patches located around the plot area. Also, once each day, soil samples were taken at 8, 16, 24, 32, and 40 cm in a horizontal transect from the centre of the one additional urine patch which was created at 20 m distance from the circle's centre. The soil sampling was carried out by pushing a sharp-edged metal ring of 6.5 cm diameter to about 5 mm depth into the ground and the entire soil

and herbage content inside the ring was then removed and returned immediately to the laboratory for analyses of pH, moisture and inorganic N content as described in Chapter 3.

6.2.7 NH_3 measurements

6.2.7.1 Charging and discharging NH_3 passive samplers

Profiles of NH_3 concentration were measured with an array of 'Leuning samplers' (Leuning et al., 1985), which are collection devices that point into the wind and completely remove the NH_3 contents from the air entering the device (Figure 6.6). In order to continue sampling at the end of a collection period, each Leuning sampler must be replaced by an identical one. There were 12 samplers available for this project, allowing the installation of six for simultaneous collection, while the other six are subjected to extraction of the previously collected NH_4^+ and prepared for another collection period. One sampler is needed to measure upwind concentration, leaving up to five available for a profile.

Figure 6.6 Leuning NH_3 sampler(s) on the mast.

Each sampler contains a long length of stainless steel foil wrapped into a spiral around the central axis of the sampler thereby forming an elaborate array of surfaces that are coated with a thin film of solid oxalic acid. Charging of the samplers was carried out by firstly replacing the detachable tail section with a large polypropylene plug and then adding approximately 30 mL of a solution of oxalic acid in acetone (3% W/V) to the sampler through the front inlet. The inlet was then stoppered using a rubber bung and the sampler was shaken vigorously for 30 seconds to ensure complete wetting of all interior surfaces. After 30 seconds, the stopper

was removed and the excess solution was allowed to drain, with the residual acetone quickly evaporating to leave a thin film of solid oxalic acid crystals coating the sampler's interior surfaces. The inlet was then re-stoppered and the tail assembly was attached, ensuring that the fins were in the same plane as the pivots so that the sampler accurately tracked the wind direction. In this current study this charging process was carried out in a fume cupboard in a laboratory, although in earlier studies the procedure has been carried out in the field (Sherlock et al., 1989; Sherlock et al., 1995; Sherlock et al., 2002; Van der Weerden, 1990). The sealing of the sampler was important at this stage to avoid contamination with any NH_3 present in the ambient air. The charged samplers were taken to the paddock, mounted on the masts and their positions recorded. Insulation tape covering the outlet in the tail section was then removed, followed by the inlet bung. The time was then recorded for the beginning of a new sampling period.

As the airstream impacts on these coated surfaces, the NH_3 molecules react with the solid oxalic acid, forming ammonium oxalate ($(NH_4)_2C_2O_4$). At the end of the collection period, this $(NH_4)_2C_2O_4$ together with unreacted excess oxalic acid must be washed out and analysed for NH_4^+ concentration. In a fume cupboard back in the laboratory, the tail assembly was removed and replaced by the large polypropylene plug previously employed during the charging procedure. Using a measuring cylinder, 30 mL of deionised water was accurately measured and poured into the inlet of the sampler once the rubber bung had been removed. The sampler was then re-bunged and shaken and rotated vigorously for at least 30 seconds to dissolve the oxalic acid and ammonium oxalate crystals. Each sampler was drained into a measuring cylinder to record the volume of the extract obtained. This volume was then made up to 30 mL using deionised water and then poured into plastic 100 mL bottles for storage at $4^{\circ}C$ and subsequent analysis for NH_4^+ content using an ion specific electrode (Section 3.4).

Before the samplers could be charged again for use in the next sampling period, it was necessary to wash the interior surfaces to remove any trace levels of $(NH_4)_2C_2O_4$. To wash a sampler, approximately 30 mL of deionised water was added, it was re-bunged, and shaken for about 30 seconds, then drained into a bucket. Finally, approximately 30 mL of acetone was added and shaken vigorously for about 15 seconds to remove the excess water. That acetone was then drained and discarded.

6.2.7.2 Data collection and averaging periods

Collection of gaseous NH_3 at the circle's centre and at the background mast began immediately after the urine was applied and continued for the following 213 h (nearly 9 days) which spanned a total of 34 collection periods (9 long overnight and 25 daytime periods).

Each collection period was planned to be long enough to collect a measurable amount of NH_3, but short enough to provide meaningful time resolution. This was achieved for the first 6 days following urine application. Daytime collections were 2 h long on the day of the urine application and the following day. From the 3rd to 5th days after urine application this was extended to 3 h, and thereafter to 4 h. Over night, the samplers were left unchanged for 14 to 16 h. The long nighttime collection periods provided increased analytical accuracy of the collected amounts of NH_3, but at the expense of increased contamination risk of the background sampler when wind direction changed during the night, as well as poor resolution of the temporal evolution of the emission rates.

6.2.7.3 Correction for background concentration

In order to correctly convert the vertical profile of measured horizontal NH_3 fluxes to the emission rate of the urine-treated area, it is necessary to first subtract the background horizontal flux upwind of the urine plot. The concentration at the upwind sampler location is a true "background", which implies height-constancy, provided that it is influenced only by sources and sinks so far away that their contributions are evenly mixed throughout the surface layer. The NH_3 sampler placed on the separate mast upwind of the urine plot provides a measurement of the horizontal flux of NH_3 at one height, $\overline{u\rho}$ (2.1 m). Dividing this flux by the wind speed at that height therefore gives the required background concentration.

For each NH_3 sampler in the vertical profile, the background concentration is then multiplied by the correct wind speed for the sampler's height and the product subtracted from the horizontal flux measured by the sampler. The resulting net horizontal fluxes are then height-integrated [Equation 6.2].

It was found that occasionally the subtracted background flux exceeded the horizontal flux for a given sampler, resulting in a negative net horizontal flux at that particular height. In order to deal with the negative net horizontal flux, the difference between two sampler concentrations determined by the ion-specific electrode was checked to determine whether it was below the resolution limit of the NH_3 analysis method (0.02 $ugNH_3\ mL^{-1}$) or not. If it was lower than the resolution limit, the background sampler concentration was replaced by the concentration of the rogue sampler; in consequence, the net flux from that height would be zero and the net fluxes at the other heights were increased insignificantly. If the difference was greater than resolution limit, the location of the background sampler was checked using wind direction records to find out if it was really downwind of the urine plot or not, resulting in possible contamination of the background measurement by NH_3-laden air from the treated plot. Then, the background concentration was replaced by the minimum concentration in the vertical

profile typically the concentration at 2.1 m. Furthermore, if either of those possible explanations could not explain the negative net horizontal flux measurements, the accidental contamination of NH_3 samplers during the laboratory analyses or the presence of unexpected sources near upwind of the background sampler, might have occurred. Such periods (8 out of 34 periods) were excluded from the analysis.

6.2.8 Meteorological measurements and data collection

Wind speed was measured by five cup anemometers (Model A101M, Vector Instruments, Rhyl, Clwyd, UK) with matched calibrations. Wind speed data were stored as 5-min averages by a datalogger (Model 21X, Campbell Scientific, Logan, Utah).

6.2.9 Estimating NH_3 volatilisation by an indirect method

The need for large untreated circular treated plots in the IHF micrometeorological method has severely limited its use in multi-treatment field experiments (Sherlock et al., 1995). However, a physico-chemical model used in combination with a single IHF method plot (Sherlock et al., 1995) could be a possible way to estimate NH_3 volatilisation from small treated plots. This model relies on the difference between the NH_4^+-N concentrations in the soil aqueous phase and NH_3 concentrations in atmosphere. Wind speed, soil surface pH and temperature are dominant factors which affect the equilibrium between NH_4^+-N and NH_3-N and subsequently NH_3 volatilisation rate in this model. These parameters were used to determine the relationship between the measured vertical NH_3 flux and the calculated NH_3 gas concentration (Sherlock et al., 1995).

$$F = ku_z(\rho_0 - \rho_z) \qquad \textit{[Equation 6.4]}$$

where;

F : the calculated vertical NH_3 flux (µg N m^{-2} s^{-1})

k : a dimensionless exchange coefficient

u_z : the mean windspeed at some reference height (m s^{-1})

ρ_z : the NH_3 concentration at a reference height (z) (µg N m^{-3})

ρ_0 : the equilibrium NH_3 concentration at the soil surface (µg N m^{-3}); and calculated as

$$\rho_0 = [NH_3]_{soln} 10^{\left(1.6937 - \frac{1477.7}{T}\right)} \qquad \textit{[Equation 6.5]}$$

where;

$[NH_3]_{soln}$: the concentration of NH_3(aq) present within the soil solution at the soil surface (µg m^{-3}); and calculated as below

T : temperature (°K)

$$[NH_3]_{soln} = \frac{[NH_4^+ + NH_3]_{soln}}{1 + 10^{\left(0.09018 + \frac{2729.92}{T} - pH\right)}} \qquad \textit{[Equation 6.6]}$$

where:

$[NH_4^+ + NH_3]_{soln}$: the total ammoniacal-N concentration within the soil solution (determined by 2 M KCl extraction) (µg NH_4^+-N m^{-3})

pH : soil surface pH

6.3 Results

6.3.1 NH_3 volatilisation

Ammonia volatilisation was defined clearly for the first 18 collection periods (up to 90 hours following urine application which included 4 nights) with the largest rates in the first 4 h after the urine application of about 7 µg N m^{-2} h^{-1}. The fractions of applied N emitted as NH_3 then decreased over the next 6 days, and were 8.3, 5.0, 4.4, 4.7, 1.8 and 1.6%, respectively. After 97 h (sampling period 20) following urine application, the NH_3 volatilisation fluxes had dropped markedly. It should be noted that the background sampler contained more NH_3 than the profile samplers in sampling period 7 and 19 for unknown reasons. For these periods, the NH_3 emissions were interpolated between neighboring periods. In addition, in the sampling periods 26 to 31, large amounts of NH_3 were detected in all samplers which did not make sense in terms of the profile shape and profile-background differences. The data for these periods were discarded due to the possibility of laboratory contamination. Hence, cumulative fractional NH_3 loss of 25.7 (± 0.5[6]) % (Figure 6.7) was obtained over the first 6 days.

[6] Former studies gave an estimated relative error of 10 to 15% for the IHF method (Denmead & Raupach, 1993; Harper & Sharpe, 1998). In this experiment, the error estimate in parentheses was based on the statistical relative error of the mass-budget method, propagated from the measurement errors involved. This error was obtained as 10 (± 5) % for individual collection periods. The standard relative error of the cumulative flux is smaller than that, due to the rules of error propagation.

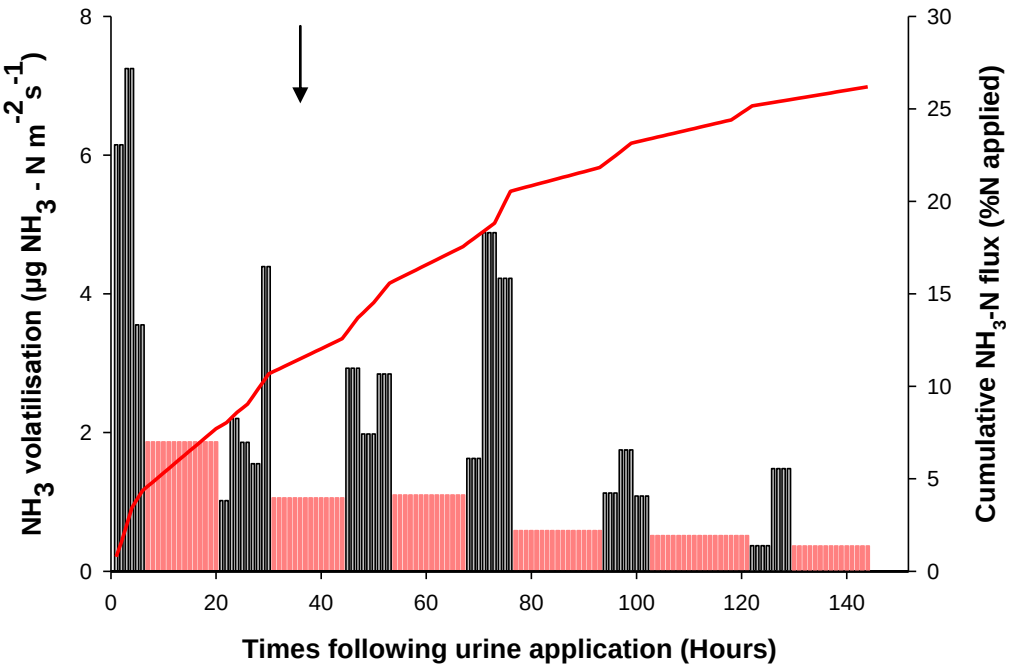


Figure 6.7 The histogram shows the NH_3-N volatilisation rates over the 6 days following urine application determined with the IHF mass budget method (black and pink bars define day- and night-time periods, respectively); and the line shows the cumulative loss of NH_3-N as a percentage of the total N applied to the plot. The time of the only rain event (2.5 mm precipitation) is indicated by the arrow.

6.3.2 Soil results

6.3.2.1 Soil temperature

The soil temperature followed the usual diurnal cycle, with a typical day-night span of 12°C (Figure 6.8). The mean temperature during the first six days after urine application was 17.6°C at 2 cm depth (minimum 11.5°C, maximum 28.2°C) and 18.0°C at 5 cm depth (minimum 12.4°C, maximum 28.7°C). The mean temperature of the first two days which accounted for about half of the NH_3 emissions (Figure 6.9) was higher, 19.3°C at 2 cm depth and 19.7°C at 5 cm depth. The daily air temperature ranged from 13.2 to 29°C over the experiment (Figure 6.8).

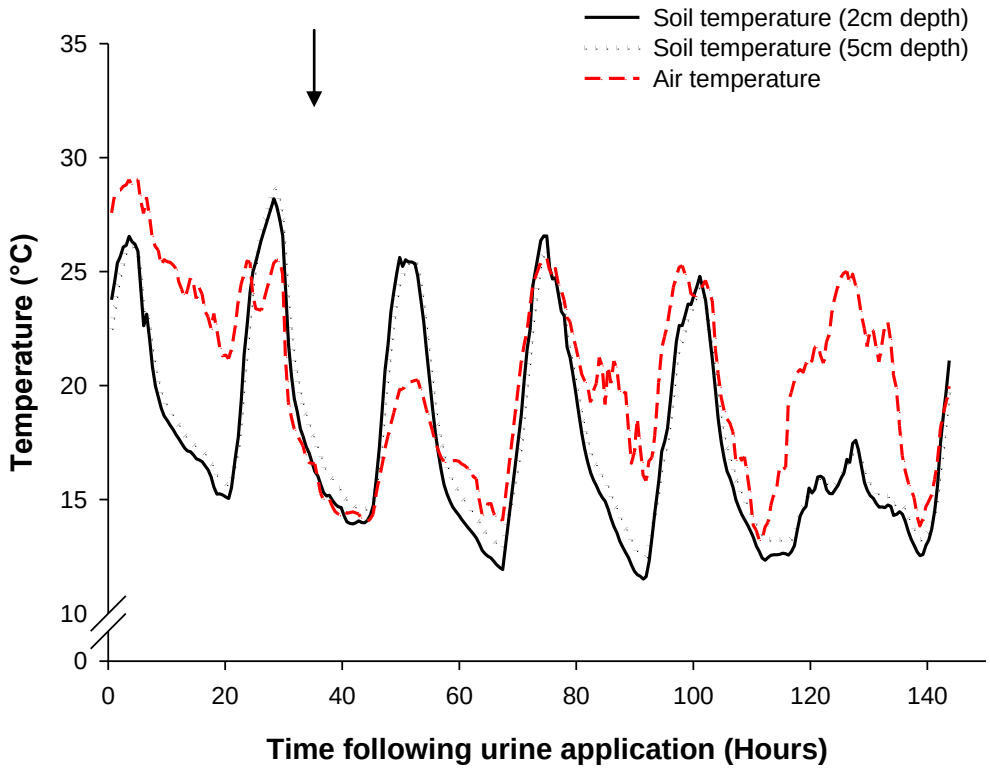


Figure 6.8 Soil and air temperatures over the 6 day experimental period.

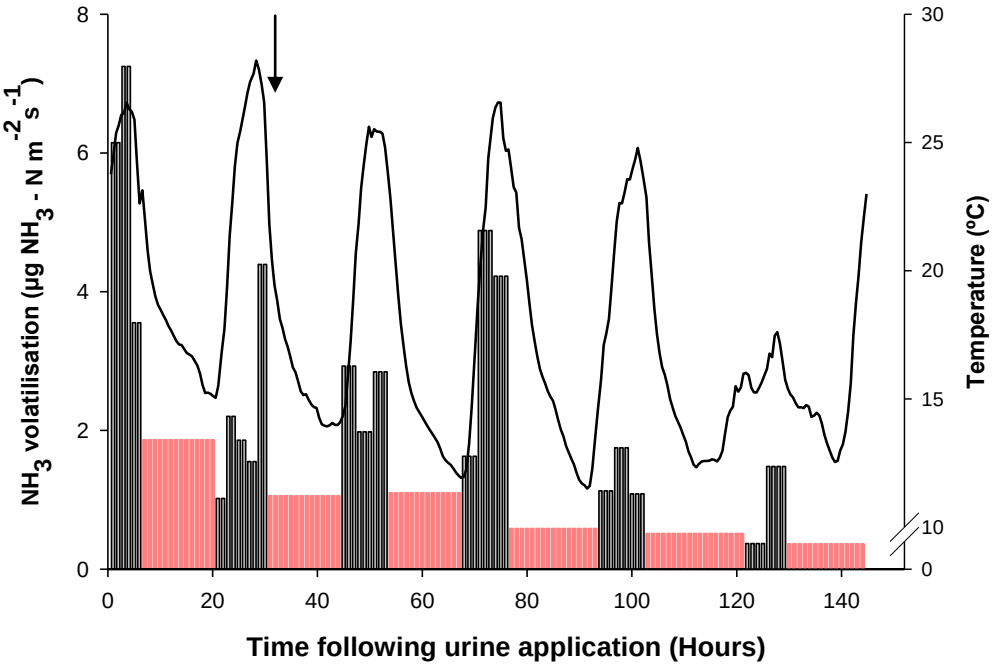


Figure 6.9 The histogram shows the NH_3-N volatilisation rates over the 6 days following urine application determined with the IHF mass budget method (black and pink bars defined day- and night-time periods, respectively ; and the line shows the soil temperature at 2 cm depth. The time of the only rain event (2.5 mm percipitation) is indicated by the arrow. NOTE differing scales on y axes.

6.3.2.2 Soil pH

The soil surface (0 – 5 mm depth) pH was 6.74 (s.e.m. = 0.12) before urine application and increased, 2 h following urine application to 8.53 (± 0.05), declining after 23 h to 7.91 ± (0.13) on the following day (Figure 6.10). There was also a clear temporal increase from 7.9 to 8.4 after the rain event on day 2. Apart from that, the pH changed relatively little during any given day. By 193 h after urine application, the soil pH values consistently equalled the initial value which is not shown here (pH = 6.77).

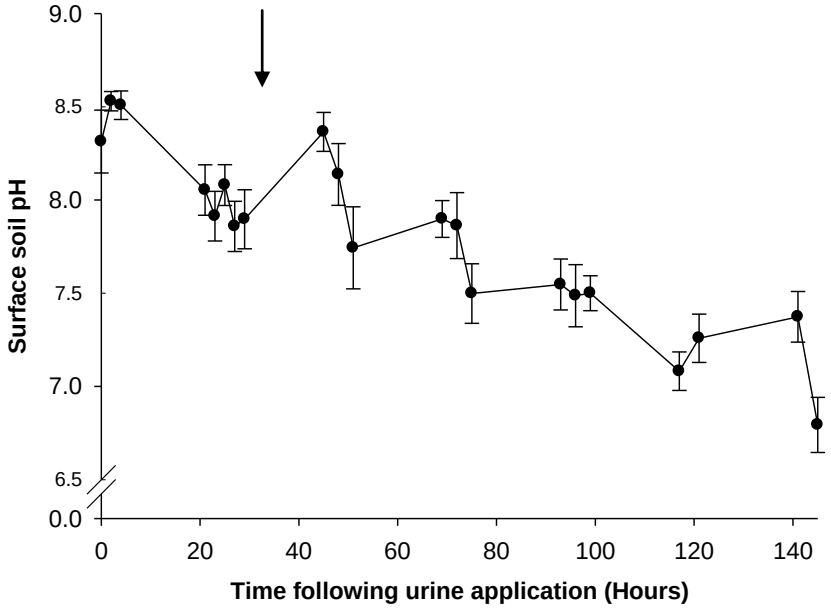


Figure 6.10 Soil surface pH over 6 days following urine application (error bars = ± s.e.m., n = 6). The time of the only rain event (2.5 mm precipitation) is indicated by the arrow.

6.3.2.3 Water content

Soil moisture content was determined on subsamples of the soil used for pH measurements. In the first 29 h after urine application, the soil dried steadily from initially 40 to finally 21% gravimetric water content (Figure 6.11). Following the overnight rainfall event during the second night, soil moisture recovered to 40% and then fell continuously to a minimum of 10% after 6 days (No further drying was observed on Days 7 to 9, with values between 11 to 18% recorded).

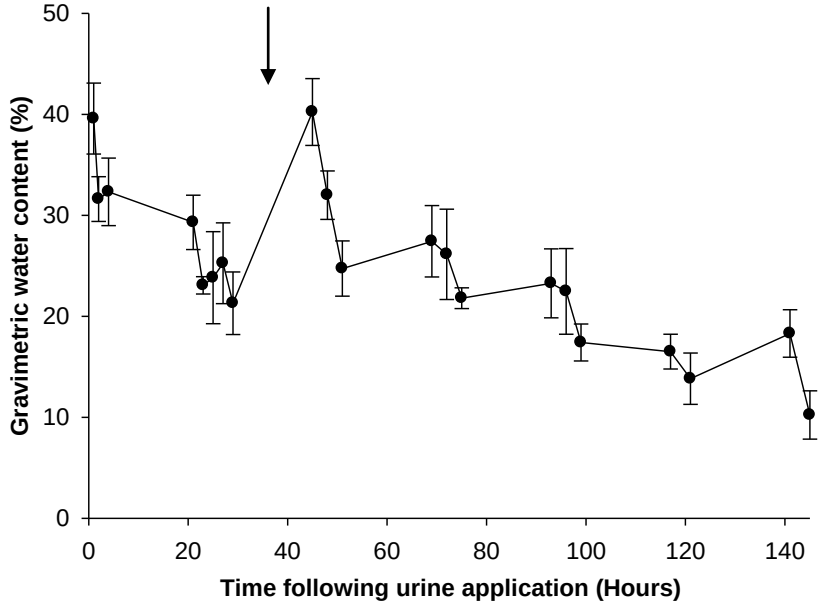


Figure 6.11 Gravimetric water content values were determined from in-situ sampling at the field site over the 6 days following urine application (error bars = ± s.e.m., n = 6). The time of the only rain event (2.5 mm precipitation) is indicated by the arrow.

6.3.2.4 Inorganic nitrogen

Ammonium-N concentrations, which were strongly correlated to pH ($r^2 = 0.77$, $p < 0.05$), increased after 4 hours following urine application and dropped off afterwards. A second peak was observed the following day but that was followed by a much larger peak on day 3 following the overnight rainfall event (Figure 6.12a). Nitrate-N concentrations were negatively correlated to pH ($r^2 = 0.77$, $p < 0.05$). Nitrite-N (NO_2^--N) was 1 - 2 magnitudes less abundant than NO_3^--N (and 2 - 3 magnitudes less than NH_4^+-N) and weakly positively correlated with pH ($r^2 = 0.19$, $p > 0.05$).

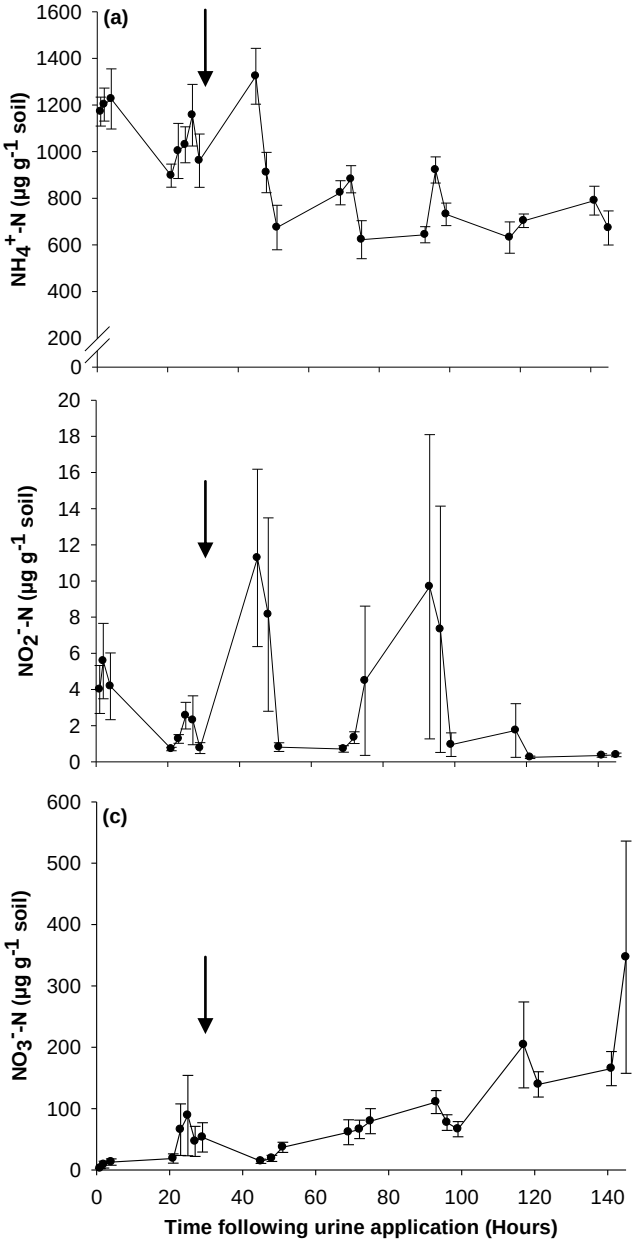


Figure 6.12 Soil (a) NH_4^+-N, (b) NO_2^--N and (c) NO_3^--N concentrations over the 6 days following urine application (error bars = ± s.e.m., n = 6). NOTE differing scales on y axes. The time of the only rain event (2.5 mm precipitation) is indicated by the arrow.

6.3.2.5 *Estimated NH_3 volatilisation using an indirect method*

The results from calculations of the parameters in Equations 6.3 to 6.5 are summarised in Figure 6.13. A strong relationship between the measured vertical NH_3 flux, and $u_{1.2}\rho_0$, which was the product of wind speed at 1.25 m above the soil surface, and the calculated NH_3 gas concentration in equilibrium with the soil solution ($r = 0.76$ or $r^2 = 0.58$), was observed (Figure 6.14).

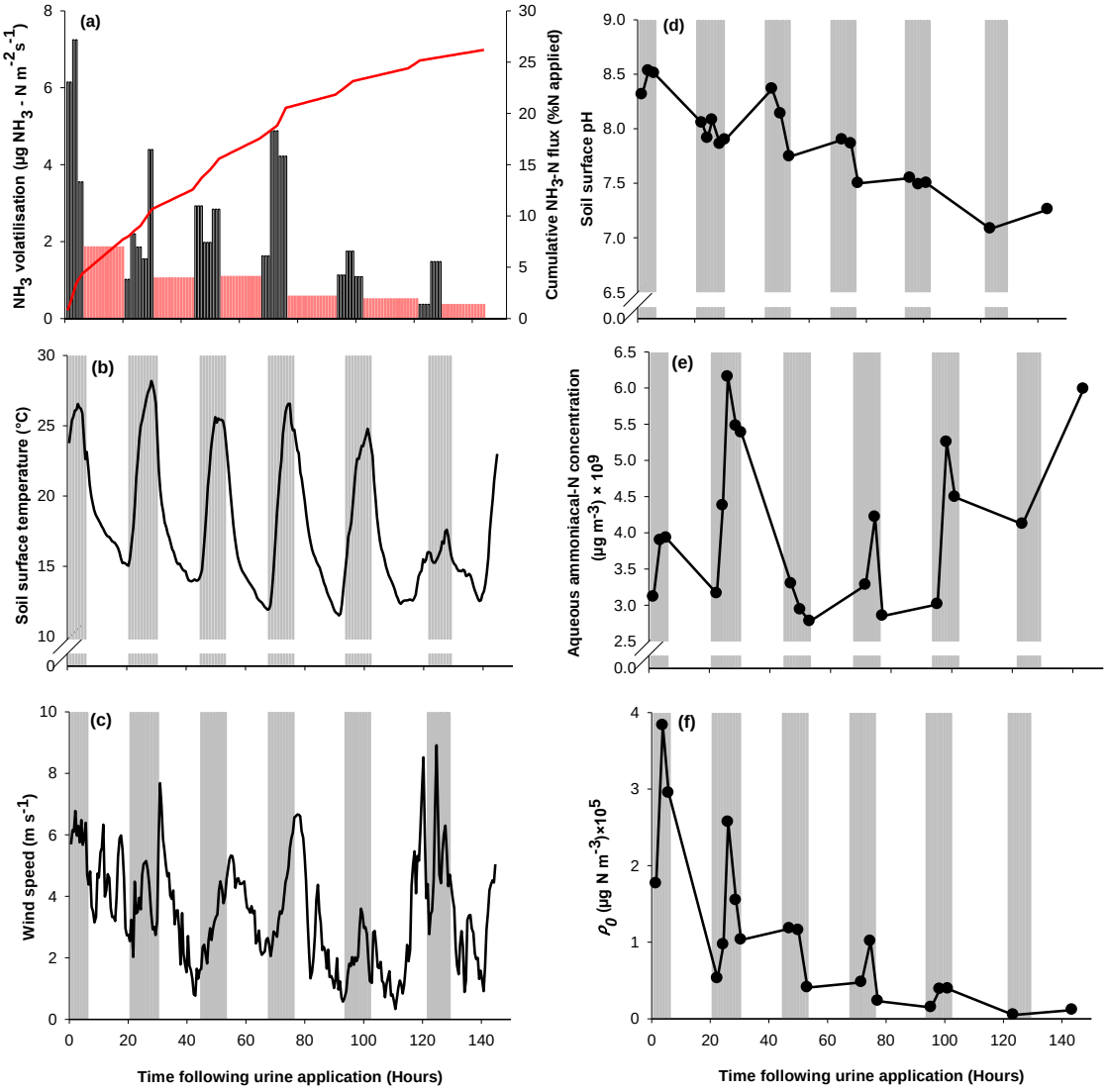


Figure 6.13 (a) NH_3 flux and cumulative NH_3 emissions, (b) soil temperature (2 cm depth), (c) wind speed at 1.2 m, (d) soil surface pH, (e) aqueous ammoniacal-N concentration (0 - 5 mm depth), and (f) soil surface equilibrium $NH_{3(g)}$ concentration; over the 6 days following urine application. NOTE differing scales on y axes and grey and white background define day- and night-time periods, respectively, in graphs (b), (c), (d), (e) and (f).

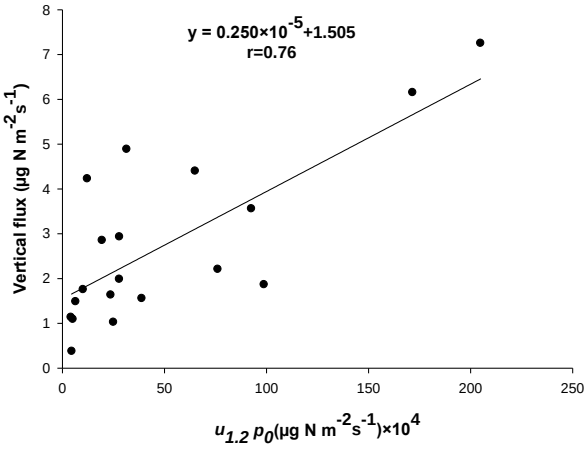


Figure 6.14 The relationship between vertical NH_3 flux, and the product of $u_{1.25}$ and ρ_0 following urine application.

6.3.2.6 Urine patch size and N-balance

The results from the brilliant-blue dye plots showed firstly, that the average dyed patch area was 0.25 m^2, so for a circle, the corresponding radius would be 0.28 m, very close to the anticipated 0.3 m; secondly, that the average dye penetration depth was 1.5 cm, so it is justified to assume that the N delivered with the urine stayed within that top soil layer. These results can be used to quantify the amounts of NH_4^+-N and NO_3^--N accounted for in the urine patches over the 6 day experimental period. When the accumulated volatilised NH_3-N is included a crude urine-N recovery assessment is possible (Figure 6.15).

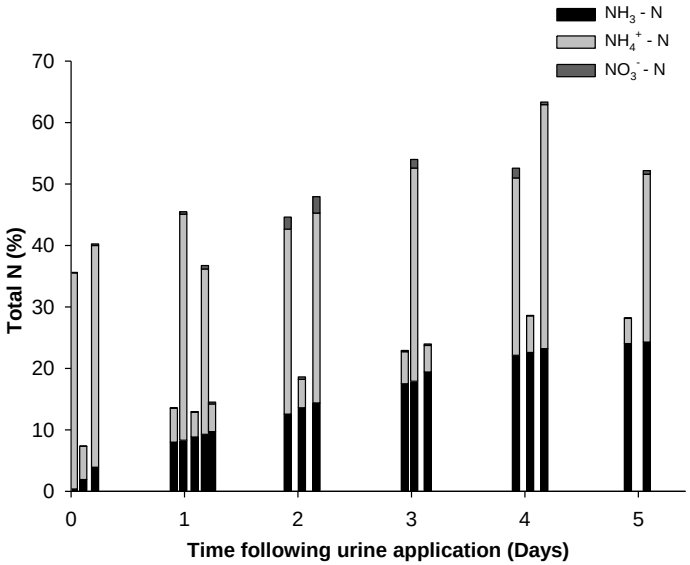


Figure 6.15 Total N (% of N applied) accounted for as volatilised NH_3-N and soil NO_3^--N and NH_4^+-N over the 6 days following urine application.

6.3.2.7 Single Urine patch

The unreplicated results from the daily sampling of the single urine patch showed how soil surface pH values decreased both horizontally across a urine patch and with time following urine application (Figure 6.16). A smoothing algorithm was employed in the generation of this 3-D figure to simply improve its overall appearance and to better visualise trends, and the graph is presented here for that purpose only. Similar results were recorded for NH_4^+-N concentrations which were highest close to the centre of the patch and declined outwards to the patch edge. The opposite trend with distance was observed for NO_3^--N (Figure 6.17).

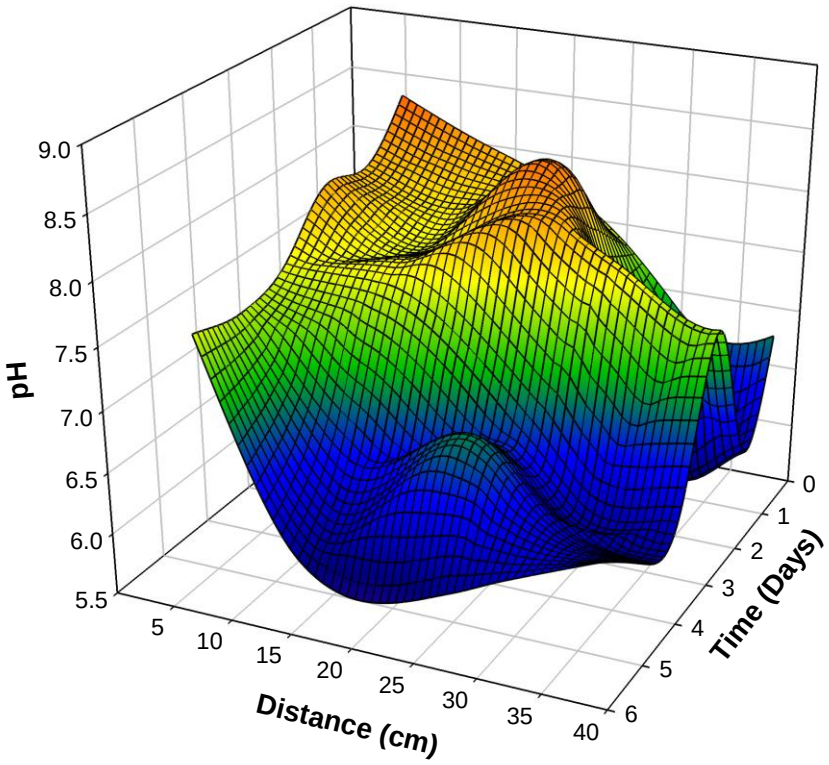


Figure 6.16 Changes in soil surface pH in a single urine patch horizontally from the centre of the urine patch with time following urine application.

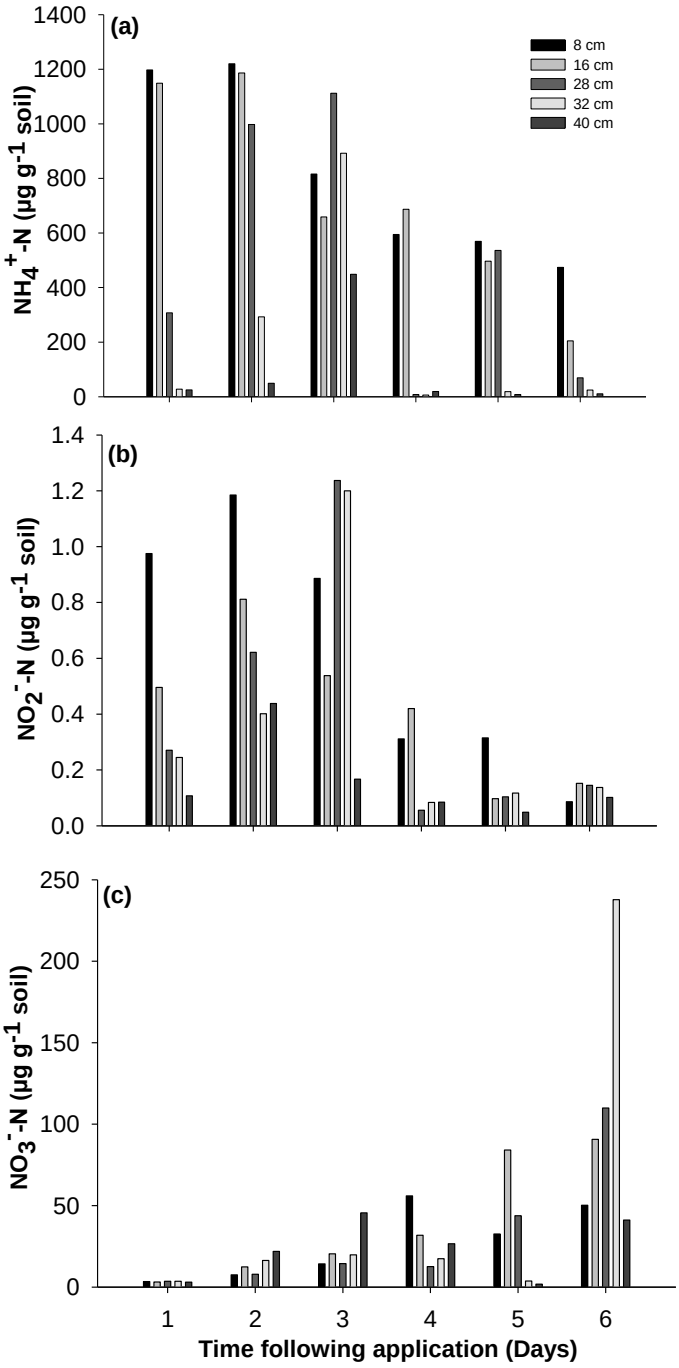


Figure 6.17 Soil (a) NH_4^+-N, (b) NO_2^--N and (c) NO_3^--N concentrations at different distance from the centre of a single urine patch following urine application. <u>NOTE</u> differing scales on y axes.

6.4 Discussion

6.4.1 NH_3 volatilisation

The extent of NH_3 volatilisation from dung tends to be much less than from urine with reported N losses from dung averaging just 1.5% from studies in England (Ryden et al., 1987) and Finland (Saarijarvi et al., 2006) and 4.5% from chamber studies carried out in New Zealand (Sugimoto et al., 1992). The proportion of total urinary-N volatilised as NH_3-N is typically 10 - 40% and the total N losses from real animal deposits (urine and dung combined) under the warm summer conditions can therefore be expected to be of the order of 15%, and less for the cooler seasons (Ball et al., 1979; Bol et al., 2004; Whitehead & Raistrick, 1991; Whitehead et al., 1989). However, measurements from 3 to 52% have been reported (Petersen et al., 1998). Sherlock and Goh (1984) measured NH_3-N emissions for urine treated plots of 22.2%, 24.6%, and 12.2% of total urinary-N in summer, autumn, and winter; respectively. Whitehead and Raistrick (1992) detected between 23% and 39%, with emissions higher on bare soil and lower in the presence of ryegrass. This was despite Doak's (1952) supposition that interception of urine by pasture plants may be a major contributor to NH_3-N emissions (Doak, 1952).

In the current study, the NH_3 volatilisation rate in a controlled experiment simulating a realistic New Zealand farming situation was equivalent to a loss of 25.7 (± 0.5) % of the applied N following urine application. Emissions peaked 4 h after application and then generally declined, with superimposed diurnal variations. Ammonia volatilisation is affected by various factors: especially soil surface pH, soil moisture, soil ammoniacal-N content, air and soil temperature (Section 2.2.3.2). The rate of urea hydrolysis and the effect of these factors on volatilisation rates also depends on the time elapsed after urine application (Sherlock & Goh, 1984a, 1984b). Valuable information on how the effects of these factors influence volatilisation rates may be overlooked, when only the cumulative volatilisation after a certain time following urine application is considered. Therefore, the different sampling periods were planned to be long enough to collect a measurable amount of NH_3, but short enough to provide meaningful time resolution. This approach enabled the 'volatilisation profile' to be determined and examination of this profile and the factors mentioned above, helps explain more about the volatilisation process following the application of urine.

The warm soil temperature during this study would certainly have facilitated rapid urea hydrolysis (not measured) which in turn would have helped to promote NH_3-N loss. Low soil temperatures have been seen to retard NH_3-N volatilisation (Sherlock and Goh, 1984). High temperatures induce rapid production of NH_4^+-N, a rapid increase in soil surface pH leading

in turn to a more rapid increase in NH_3-N emissions and also potentially to the faster accumulation of NO_3^--N by nitrification in the plot area.

The largest volatilisation rates of about 7 µg N m^{-2} h^{-1} were found in the first 4 h after the urine application, and were a consequence of both the high initial increase in pH of the urine treated areas due to the rapid hydrolysis of urea, and the resulting high soil NH_4^+-N levels. Emissions dropped rapidly over the next two collection periods to about 2 µg N m^{-2} h^{-1}. A second increase in NH_3 emission rate for three consecutive daytime collection periods, two days after urine application, may have been in response to a light rainfall event during the preceding night (2.5 L m^{-2} in 3 h). Nocturnal rainfall may have stimulated the hydrolysis of any remaining unhydrolysed urea and/or facilitated the diffusion of volatilisable dissolved ammoniacal-N from deeper than 5 mm into the soil surface, and ammoniacal-N concentration dropped during the following day rapidly due in part to loss via volatilisation. Similar rainfall-induced increases in NH_3 fluxes have been observed in IHF method measurements reported elsewhere (Sherlock et al., 1995).

The other temporary increases in emission rates were short-lived and restricted to afternoon collection periods and were likely driven by elevated soil temperatures. From then onwards the general trend was a steady further decline due to drying of the soil surface which would inhibit the hydrolysis of any remaining unhydrolysed urea, with emission rates roughly halving every 2 days (Black et al., 1985)

In general, diurnal NH_3 variations correspond with fluctuations in topsoil pH, NH_4^+-N concentrations, moisture and especially temperature (Sherlock & Goh, 1984a, 1984b) with maximum emission rates in the midday and afternoon, and minimum rates during the night. Previous studies such as those of Beauchamp et al. 1978, Sherlock et al. 1995 showed similar trends.

6.4.2 Evaluating NH_3 volatilisation model and N-balance

The linear relationship obtained between the measured vertical NH_3 flux and the $u_{1.25}\rho_0$ product agreed well with previous experiments which were carried out using different sources and rates of N (Sherlock et al., 1995). In one of those experiments, also conducted near Lincoln, synthetic urine was applied to a 1256 m^2 area (20 m radius circular plot) uniformly at the rate of 500 kg N ha^{-1} (Sherlock et al., 1995). In the current experiment, urine was applied at the rate of 600 kg N ha^{-1} and the urine affected area was 39 m^2 (each urine patch was 0.25 m^2 and there were 156 urine patches), a factor 32 smaller. This provides a reasonable explanation for why the slope found by Sherlock et al. (1995), representing *k* in Eq. 6.4, was a factor of 31 larger than the current one in Fig. 6.14 (7.5×10^{-5} vs. 0.24×10^{-5}): by accounting

for the effective emitting area, the results from the two experiments, on similar soil and in similar weather conditions, are quantitatively consistent.

As seen in Figure 6.15, only around 50% of the applied urine-N can be accounted for over the 5 days following urine application. Some of the applied urine-N is likely to be rapidly immobilised by soil micro-organisms and this would not be accounted for using the methods employed here. Previous studies using ^{15}N-labelled urine have enabled immobilised-N to be determined and found it to account for 7.2% of N applied (Adams & Adams, 1983; Whitehead & Bristow, 1990). Also during the initial 24 to 48 h following urine application, some unhydrolysed urea-N would also be present and this too would be unaccounted for by the sampling and analytical techniques used (Sherlock & Goh, 1984a, 1984b). The NO_3^--N that is ultimately formed in the patches could be subject to leaching and/or denitrification which may have resulted in emissions of both N_2O and N_2. These nitrogenous gases' emissions are also possible during those 6 days but are unlikely to be large compared to the other three N-forms. The results for the urine patch size from the brilliant-blue dye experiment might have underestimated the depth of urine penetration, since the penetration of brilliant-blue dye might be slower than urine due to formation of ion pairs with Ca^{2+} in dye. Subsequently, dye might be retarded relative to the penetration of the water in which it was dissolved (Flury & Flühler, 1995). A more comprehensive attempt is required to evaluate the full nitrogen budget.

It should be noted here that another similar experiment was carried out subsequently by myself and colleagues from Manaki-Whenua Landcare Research and Lincoln University to measure the NH_3 volatilisation rate from a real-world distribution of animal excreta including dung and urine from a group of 12 cattle. The objective was to obtain a more realistic determination of $Frac_{GASM}$ than was possible using just urine. However, consideration of that particular experiment is beyond the scope of this current thesis.

6.5 Conclusions

An intentional point of difference of this experiment to similar past experiments was that the urine was applied to the soil in a pattern of many realistically-poured, -sized and -spaced urine patches, rather than by uniform spraying (e.g. Sherlock et al. 1995) or a few single urine patches underneath measurement chambers (e.g. Sherlock & Goh 1984) which might result in higher gaseous loss from grazed pastures due to lack of correct patchiness (Mosier et al., 2004). Nevertheless, the observed amounts of volatilised NH_3 were typical for the warm summer conditions of this current experiment and agree with expectations based on those past observations, for the temperature and moisture conditions encountered. It therefore appears

that the lack of correct ‘patchiness’ in previous experiments did not bias the past results. Future study is still required to: (a) quantify the urine-N pathway in order to achieve 100% N recovery in the soil-plant-atmosphere system, and (b) determine possible ways to preserve N in soil, and subsequently increase N availability for plants.

Chapter 7

Effects of biochar amendment on NH_3 volatilisation from soil under ruminant urine application and its subsequent plant availability[7]

7.1 Introduction

The majority of the NH_3-N emitted following a ruminant urination event is derived from urea-N (Section 2.2.3.2). As urea hydrolysis takes place NH_4^+ forms. Subsequent transformation of NH_4^+ to NH_3 in the soil occurs when the soil pH increases due to generation of hydroxide and bicarbonate ions as a result of the subsequent hydrolysis of the carbonate ion as described in Section 2.2.3.2 (Avnimelech & Laher, 1977; Holland & During, 1977; Petersen et al., 1998).

Biochar application to soil has been shown to affect N transformations and retention processes in soil that can influence the 'sink' capacity for greenhouse gas emissions (Lehmann & Joseph, 2009). However, there exist no studies demonstrating the potential effects of biochar on NH_3 volatilisation (Lehmann & Joseph, 2009), with the exception of the work published from this thesis (Chapter 5 (Taghizadeh-Toosi, et al. 2011b)).

In chapter 4, uptake of NH_3 by biochar was unequivocally demonstrated using ^{15}N, however, NH_3 volatilisation measurements were not successful in-situ (Chapter 4), probably due to an artefact of the experimental technique, as discussed in Section 4.4.1. In chapter 5, the amount of NH_3-N captured by biochar was quantified by exposing biochars to ^{15}N enriched NH_3 with the results showing one gram of biochar could capture up to 8.7 mg of NH_3-N. In chapter 6, NH_3 volatilisation was determined in-situ using a micrometeorological method, showing that NH_3 volatilisation could account for 25.7% of the urine-N deposited onto pasture and modelling showed soil $NH_{3(g)}$-N concentrations could reach 0.4 mg N L^{-1} soil solution. The presence of NH_3 in urine patches and the proximity of biochar under such conditions could lead to changes in N cycling and transformation which have not been adequately assessed in the previous chapters. Thus this current laboratory experiment was designed to examine

[7] A variant of this Chapter was published as:

Taghizadeh-Toosi, A., Clough, T. J., Sherlock, R. R., & Condron, L. M. (2011c). A wood based low-temperature biochar captures NH_3-N generated from ruminant urine-N, retainig its bioavailability. *Plant and Soil, In Press*. doi:10.1007/s11104-011-1010-9.

biochar effects on the fate of NH_3-N volatilisation under a urine patch with biochar present, and the subsequent fate of the urine-derived biochar-adsorbed NH_3-N in a soil and plant system.

The hypotheses tested, using two experiments, in this section of the current study were:

1) that the incorporation of biochar into a soil would suppress urine-induced NH_3 volatilisation due to adsorption of NH_3 by biochar, and
2) that biochar adsorbed-N, initially urine-derived, would subsequently be plant available.

7.2 Materials and methods

7.2.1 Experimental design and treatments

I. The 1st experiment was designed to investigate the impact of biochar incorporation on NH_3 volatilisation specifically from within a urine patch. It was conducted using four experimental treatments, with five replicates, arranged in a randomised block design. The four treatments consisted of a control (soil only); soil plus urine with no biochar (0U); soil plus urine with biochar previously incorporated, prior to urine addition, at either 15 or 30 t ha^{-1} (15U and 30U, respectively). In addition, empty incubation vessels were run as blanks (n = 2). There were a total of 20 incubation vessels (Table 7.1). Soil and urine details are provided below.

Table 7.1 Treatments and abbreviations for the 1st experiment.

Treatments	Abbreviations	Replicates
Soil	Control	5
Soil + 0 t ha^{-1} biochar + Urine	0U	5
Soil + 15 t ha^{-1} biochar + Urine	15U	5
Soil + 30 t ha^{-1} biochar + Urine	30U	5

II. The 2nd experiment was performed to assess the bioavailability of the biochar adsorbed NH_3-N, that originated from the urine-N in the 1st experiment above. Again, a randomised block design was used with five replicates. The 6 treatments (Table 7.2), described below, consisted of soil only (S); soil planted with perennial ryegrass, *Lolium perenne* (SP); soil with biochar incorporated at 20 t ha^{-1} (20S); soil with biochar incorporated at 20 t ha^{-1} and planted with perennial ryegrass (20SP). The biochar used in treatments 20S and 20SP had not previously been incorporated into

soil or been exposed to urine. It was the same biochar as initially added to the soil in the 1st experiment. In the remaining treatments the biochar used was extracted from the soil that had received urine in the 1st experiment, and it is subsequently termed 'urine-treated biochar'. Thus, urine-treated biochar was incorporated with soil at 20 t ha^{-1}, without plants (20US) and with perennial ryegrass at 20 t ha^{-1} (20 USP).

Table 7.2 Treatments and abbreviations for the 2nd experiment where BC_3 is the biochar previously described (Table 3.1).

Treatments	Abbreviation	Replicates
Soil	S	5
Soil + perennial ryegrass	SP	5
Soil + 20 t ha^{-1} fresh BC_3	20S	5
Soil + 20 t ha^{-1} fresh BC_3 + perennial ryegrass	20SP	5
Soil + 20 t ha^{-1} urine-treated° BC_3	20US	5
Soil + 20 t ha^{-1} urine-treated BC_3 + perennial ryegrass	20USP	5

°Urine treated-biochar retrieved from 30U treatment of 1st experiment.

7.2.2 Effect of biochar on NH_3 volatilisation rates - 1st experiment.

7.2.2.1 Urine collection, amendment and application

Cow urine (500 mL) was collected from Friesian dairy cows and stored overnight at 4°C. A subsample of the urine was analysed using a C-N analyser (Section 3.2). It contained 7.3 g N L^{-1}. In order to trace the urine-N applied, additional ^{15}N labelled urea (98.0 atom% $^{15}N_2$-urea; Isotec, Miamisburg, Ohio) was added and dissolved in the collected urine immediately prior to its application, to take the urine N concentration to 10 g N L^{-1} and with a final ^{15}N enrichment of 20 atom %.

7.2.2.2 Soil collection and treatment preparation

A Temuka silt loam soil (Hewitt, 1998) was collected from the Iverson field, Lincoln University, New Zealand (43°38.8'S, 172°29.1'E), from under a perennial ryegrass pasture (Table 4.2). Soil properties demonstrate that the soil was of good fertility and not lacking in terms of nutrients required for ryegrass growth. Characteristics of the fresh biochar used in this experiment, which was BC_3 biochar, were previously shown in Tables 5.2, 5.3, and 5.4. The soil and biochar particles were sieved to < 4 mm and 4 - 6 mm, respectively. Biochar was incorporated with 185 g sieved soil at the treatment rates based on jar surface area (0, 15 and 30 t ha^{-1}) and placed into 0.5 L Mason jars. The soil and biochar occupied approximately 0.25

L of the Mason jars. Each jar had 22.5 mL of urine pipetted onto the soil surface at an application rate equivalent to 509 kg N ha^{-1}, while the control (C) treatment cores received 22.5 mL of deionised water. The jars were arranged randomly and incubated at 17.7°C throughout the experiment. Gas-tight lids were put on the jars immediately following urine application. Each lid contained two rubber septa, each fitted with a 3-way stopcock. Two needles, a 16G × 1.8 cm, and a 21G × 5.0 cm, (Precision-Glide, Becton-Dickinson, NJ, USA and BD Drogheda, Ireland; respectively), were attached to the 3-way stopcocks, acting as entry and exit ports for air flow into and out of the jar headspace, respectively (Figure 7.1). The 21G needle was connected to a plastic tube (4 cm) that directed NH_3-scrubbed air into the jar and towards the soil surface.

Four manifolds were employed to distribute airflow. Each manifold contained six small and two larger terminals. The small terminals were connected to the jars while the two large terminals were attached to a manifold and air compressor. Air from the compressed air reservoir was swept through the system at 220 mL min^{-1}, which equated to a flow of 10 mL min^{-1} for each jar or approximately 0.04 headspace changes per minute (Figure 7.1). Airflow was checked and monitored each day using a flow meter (GAP Meter, G A Platon Ltd, England). Two NH_3 scrubbing units containing a 0.3 M boric acid solution, with bromocresol green-methyl red indicator, were placed upstream of the larger terminals to scrub any NH_3 present in the compressed air. Vials containing 20 mL of 0.1 M H_2SO_4, were placed after each incubation jar to collect any volatilised NH_3 (Figure 7.1) as the air flow bubbled through the H_2SO_4 solution.

Ammonia collection periods were either 16 or 8 h long during the first 24 h following urine application and for the following 6 days. During days 7 to 10 the NH_3 collection period was extended to 24 h, and thereafter to 48 h until day 12. These NH_3 collection periods were 83 h from days 13 to 22, and 173 h from day 22 until day 29, by which time NH_3 emissions from the urine treatments were not significantly different from the C treatment. Subsamples of H_2SO_4 solution were taken and analysed for both NH_4^+-N concentration and the ^{15}N enrichment of the NH_4^+-N (Section 3.3.2.3).

Figure 7.1 Photo of the incubation jars, during experiment 1, connected to the incoming NH_3 scrubbed air and the downstream acid traps for capturing NH_3 volatilised from the incubation jars. Note empty jar, one of two run as experimental blanks.

7.2.2.3 Maintenance of soil water content

The soil moisture was maintained at 30% gravimetric water content (θ_g), the water content post treatment application, by daily weighing the jars and replacing any lost mass with deionised water using a hypodermic syringe connected to the 21G × 5 cm needle.

7.2.2.4 Soil and biochar sampling post urine application

After 29 days, biochar and soil were removed from the jars and sieved through a 4 mm sieve to separate the soil and biochar. Sub samples were taken to determine θ_g of both soil and biochar (Section 3.1.3). Further biochar subsamples were rinsed with deionised water, to remove any visible soil fragments. Then soil and biochar samples were dried (60^oC) and ground, separately, to < 200 µm in order to determine their total N contents and ^{15}N enrichments (Section 3.1.7). Subsamples of the sieved soil and biochar materials were also taken to determine their inorganic-N concentrations, using 2 M KCl extraction (10 g soil: 100 mL 2 M KCl; 0.5 g biochar: 5 mL 2 M KCl), and their inorganic-^{15}N enrichments (Section 3.1.6 and 3.3.2.3, respectively).

7.2.3 Effect of urine-treated biochar on plants - 2nd experiment.

7.2.3.1 Soil collection and treatment preparation

Fresh soil was collected as described above (Section 7.2.2.2) and sieved to ≤ 6 mm. Any aggregates > 6 mm, stones and organic matter remaining on the sieve were discarded. Oven dried (60°C) biochar recovered from the 'urine-treated' soil which was stored in sealed vials for a week, and hereafter called 'urine-treated' biochar, and 'fresh' biochar, which was biochar with no prior contact with soil, were incorporated with soil (124 g soil: 3.92 g biochar, for 20 t ha^{-1} treatment) within 10 days of the 1st experiment ending. Poly-vinyl chloride (PVC) pots (85 mm high × 55 mm ID) were filled with the sieved soil-biochar mixture to a depth of 60 mm. The pots had nylon mesh (< 0.5 mm) attached to their bases to prevent soil loss. The soil was then brought to field capacity (30% θ_g), 47% WFPS, using deionised water. Then 5 perennial ryegrass seeds were planted into the surface soil of those treatments where perennial ryegrass was required to be present.

All pots for each treatment (Table 7.2) were placed in a growth cabinet for 30 days, run under an alternating day/night temperature and light regime of 20/15°C, 12/12 h (HPL340, 6 klux at plant level), respectively, and a relative humidity of 70%. Each pot was weighed daily and any water loss due to evapotranspiration was replaced by pipetting deionised water onto the soil surface (Figure 7.2).

Figure 7.2 Photo of the pots and plants growing in the growth cabinet during the 2nd experiment.

7.2.3.2 Plant sampling and analyses

After 30 days the pots were destructively sampled. Ryegrass plants were harvested and separated into leaf and root tissues. Roots were rinsed with distilled water, to remove soil particles, then leaves and roots were dried at 65°C for 48 h, weighed, and then ground, separately (< 200 μm), prior to determination of plant tissue total-N and ^{15}N enrichment (Section 3.5.2).

7.2.3.3 Soil and biochar sampling during destructive pot sampling

Biochar was separated from the soil, by sieving, and θ_g of soil and biochar were determined (Section 3.1.3). Then biochar subsamples were rinsed with deionised water, to remove any visible soil fragments. Then soil and biochar samples were dried (65°C) and ground, separately to < 200 μm, in order to determine their total N contents and ^{15}N enrichments (Section 3.1.7). Subsamples of fresh soil and biochar were also taken for inorganic-N analyses (Section 3.1.6), using 2 M KCl extraction (3 g soil: 30 mL 2 M KCl; 0.9 g biochar: 10 mL 2 M KCl), and determination of the ^{15}N enrichments of the inorganic-N species in the 2 M KCl extracts was also performed (Section 3.1.6).

7.3 Statistical analysis

Statistical analysis was performed using Minitab®. Analyses of variance (ANOVA) were used to test for differences in treatment means and Tukey's Test was used to determine which treatments differed, with 95% confidence limits ($p < 0.05$). The General Linear Model was used to perform univariate (one response) analysis of variance for each response variable, and to determine the interactions of different factors on these variables ($p < 0.05$). Graphing was carried out using SigmaPlot®.

7.4 Results

7.4.1 First Experiment

7.4.1.1 Volatilisation losses of NH_3 - 1st experiment.

The NH_3 fluxes (from the non-urine treatments) were under the electrode resolution limit (0.02 μg mL^{-1}) as were NH_3 fluxes from the blanks. At the end of the experimental period (day 29), when considering only the urine treatments, total NH_3 losses were significantly lower due to biochar addition ($p < 0.05$). The 15U and 30U treatments did not differ, losing only 0.79 ± 0.14 and 0.78 ± 0.09 % of the urine-N applied, whilst the 0U treatment lost 1.42 ± 0.26 %. However, all treatments showed similar NH_3 flux trends following urine application (Figure 7.3).

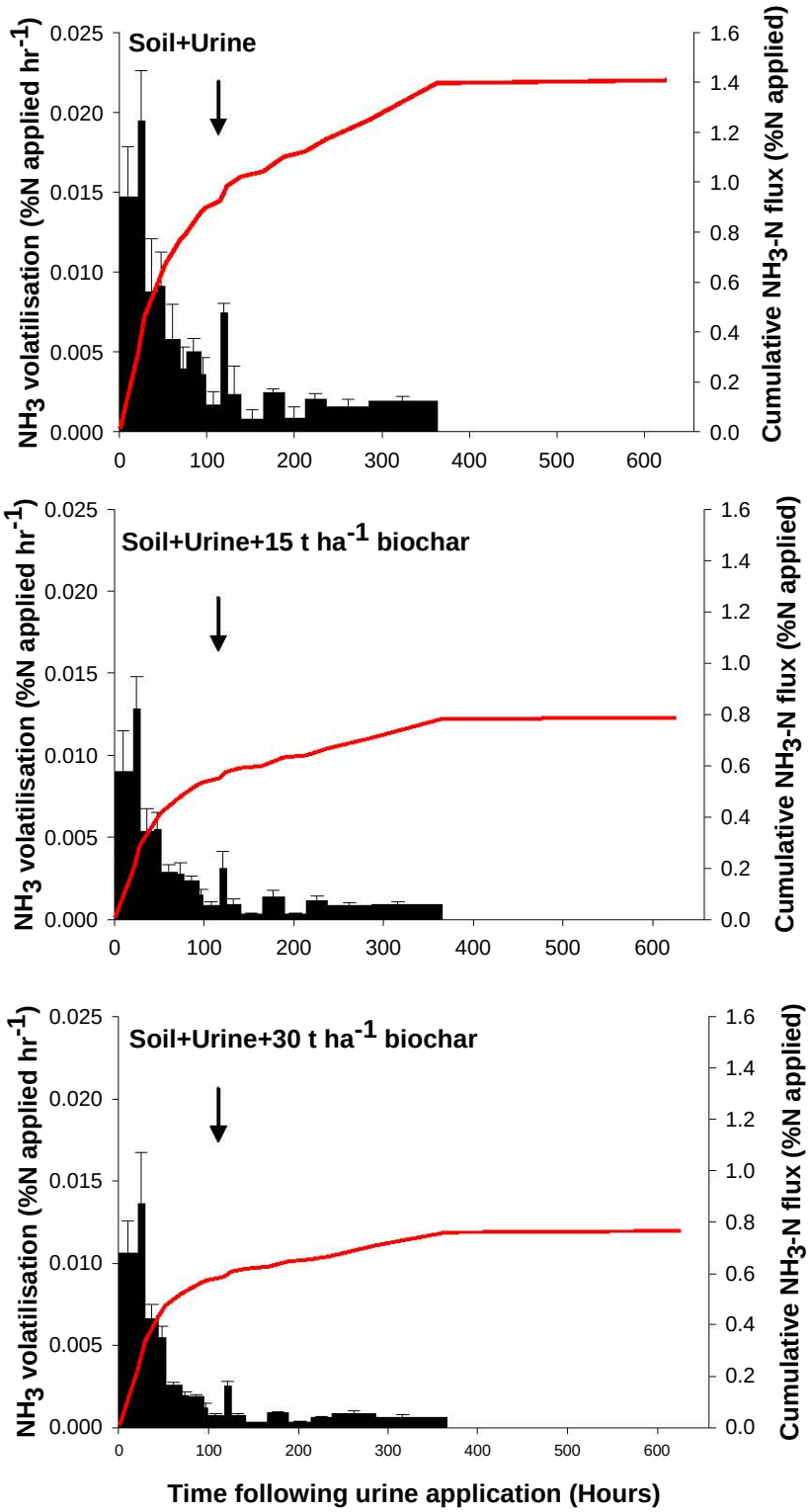


Figure 7.3 Histograms showing the NH_3-N volatilisation rates and line showing cumulative loss of NH_3-N as a percentage of urine applied. The only time water was added, to maintain the initial mass, is indicated by the arrow. NOTE differing scales on y axes.

7.4.1.2 ^{15}N enrichment of NH_3 flux - 1st experiment.

After 24 h the atom% ^{15}N enrichment of the NH_3-N volatilised was higher ($p < 0.01$) in the 0U treatment (7.88 atom %, Figure 7.4). However, after 52 h, the NH_3-^{15}N enrichment was lower ($p < 0.01$) in the 0U than in either the 15U or 30U treatments. As time progressed the ^{15}N enrichment of the NH_3-N decreased gradually over time, in all treatments, with no significant differences at 125 or 365 h (Figure 7.4).

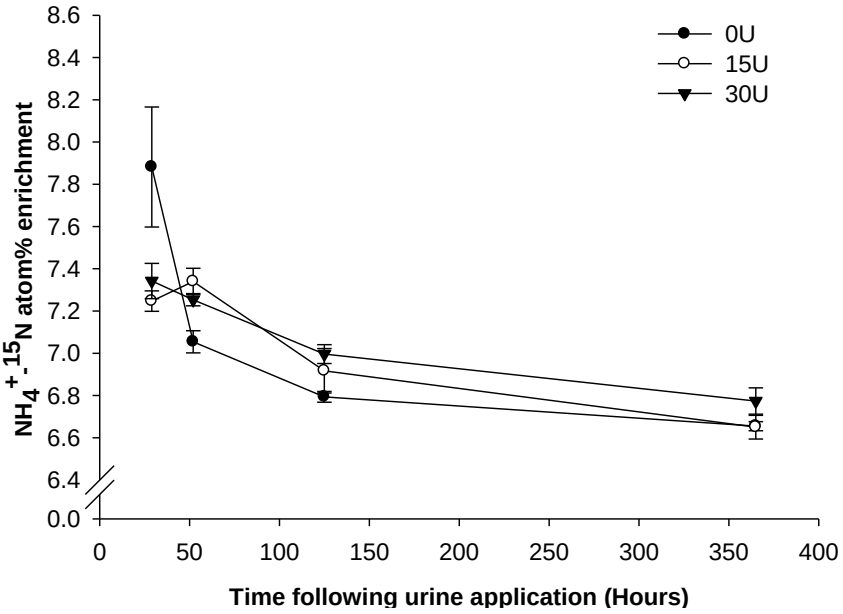


Figure 7.4 Mean ^{15}N enrichment of the NH_3-N flux over time (error bars = ± s.e.m., n = 5).

7.4.1.3 Biochar N content and ^{15}N enrichment - 1st experiment.

After 29 days of incubation under the applied urine, the N contents of the biochars in the 15U and 30U treatments (± s.e.m.) were 5.12 ± 1.09, and 8.56 ± 1.26 mg g^{-1}, respectively, with biochar N content in the 30U treatment significantly higher ($p < 0.01$) than in the biochar originally added to the soil, 2.68 ± 0.04 mg g^{-1} (Figure 7.5). The ^{15}N enrichments of the biochar materials also varied after 29 days, having increased ($p < 0.01$) under the urine treatments and were 0.370 ± 0.00, 4.02 ± 0.62, and 4.16 ± 0.75 atom % in the initial biochar material, 15U and 30U treatments, respectively with no difference in biochar ^{15}N enrichment in the 15U and 30U treatments (Figure 7.5).

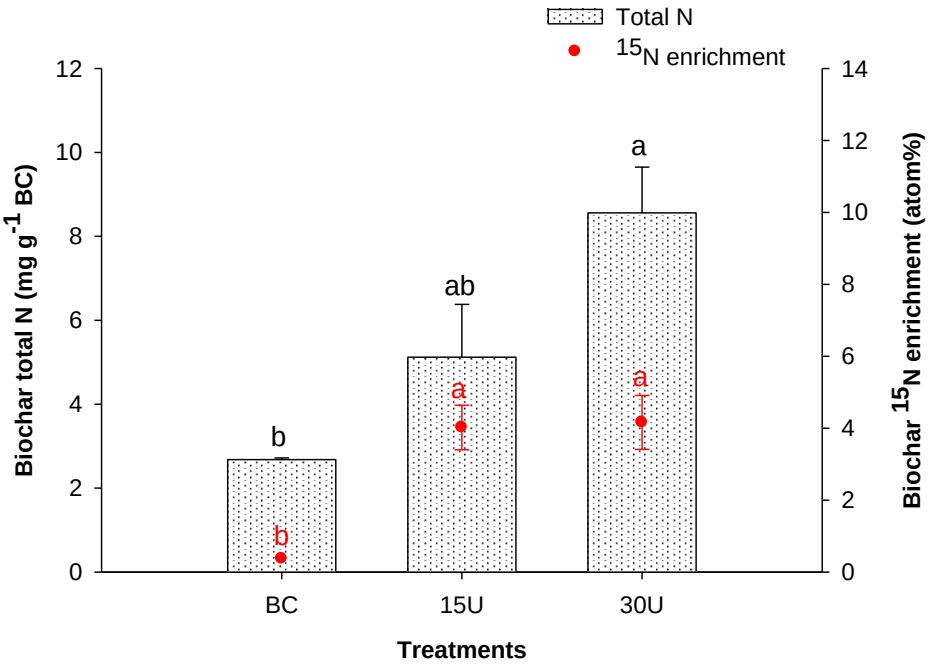


Figure 7.5 Mean total N contents and ^{15}N enrichments of the initial biochar material applied and the biochar recovered from under the 15U and 30U treatments 29 days after urine application (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scales on y axes.

7.4.1.4 Soil N content and ^{15}N enrichment - 1st experiment.

Soil total N contents increased ($p < 0.01$) following urine application when compared with the control treatment, with values (± s.e.m.) of 1.90 ± 0.08, 2.66 ± 0.03, 2.68 ± 0.04, and 2.70 ± 0.03 mg g^{-1}soil for the control, 0U, 15U and 30U treatments, respectively. However, biochar rate did not affect soil N content when urine was present (Figure 7.6). Soil ^{15}N enrichment increased significantly under urine treatments and values were 0.37 ± 0.00, 3.29 ± 0.06, 3.49 ± 0.04, 3.43 ± 0.09 atom % ^{15}N in the control, 0U, 15U and 30U treatments, respectively, with no statistical difference in soil ^{15}N-enrichment due to biochar addition at any rate (Figure 7.6).

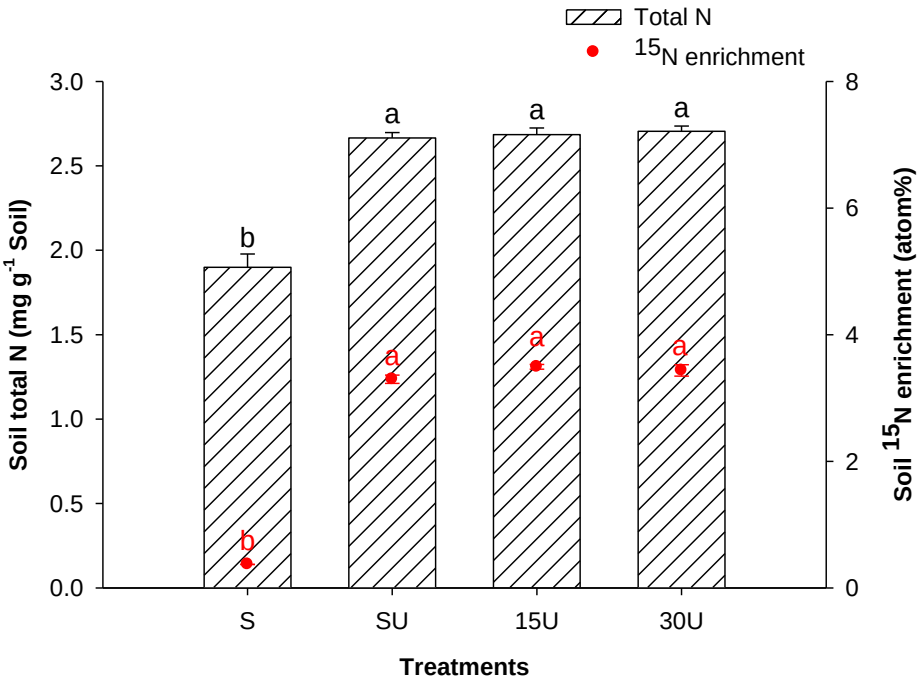


Figure 7.6 Mean total N contents and ^{15}N enrichments of soil recovered from the S, 0U, 15U and 20U treatments 29 days after urine application (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scale on y axes.

7.4.1.5 Biochar inorganic N - 1st experiment.

The concentrations of the NH_4^+-N extracted from the biochar particles were 312 ± 36 and 167 ± 53 µg g^{-1} biochar in the 15U and 30U treatments, respectively, $p = 0.05$ (Figure 7.7a). No significant differences were observed with respect to the extracted biochars' NO_2^--N and NO_3^--N concentrations, and the mean values for the 15U and 30U treatments were 1.1 ± 0.3 and 111 ± 20 µg g^{-1} biochar, respectively (Figure 7.7b & c).

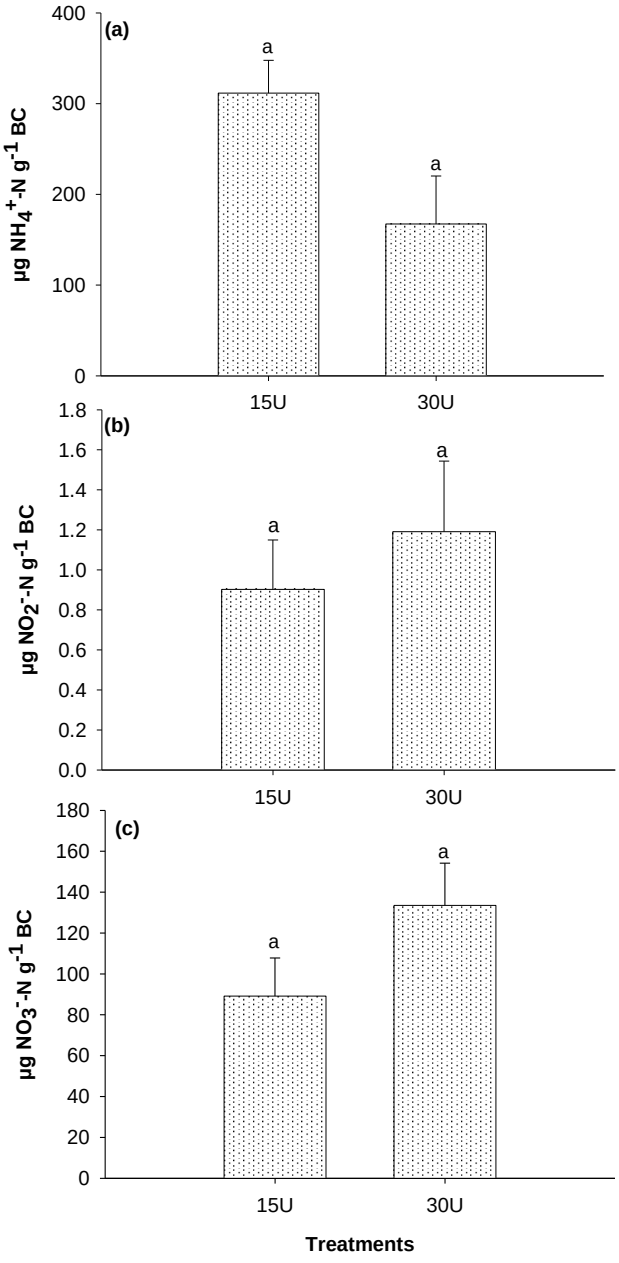


Figure 7.7 Mean biochar inorganic-N concentrations (a) NH_4^+-N, (b) NO_2^--N, and (c) NO_3^--N determined using 2 M KCl extractions on rinsed biochar extracted from the soil 29 days after urine application (error bars = + s.e.m., n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scale on y axes.

7.4.1.6 Soil inorganic N - 1st experiment.

Soil NH_4^+-N, NO_2^--N and NO_3^--N concentrations were elevated ($p < 0.001$) after 29 days in those treatments where urine was applied (Figure 7.8). Concentrations of NH_4^+-N were lower ($p < 0.05$) in the 30U treatment than in the 0U treatment (Figure 7.8a). The NO_2^--N concentrations did not differ under urine with increasing biochar rate, averaging 0.2 ± 0.1 µg g^{-1} soil (Figure 7.8b). Soil NO_3^--N decreased as biochar rate increased ($p < 0.01$) with concentrations of 204 ± 6, 176 ± 4, and 160 ± 4 µg g^{-1} soil in the 0U, 15U and 30U treatments, respectively (Figure 7.8c).

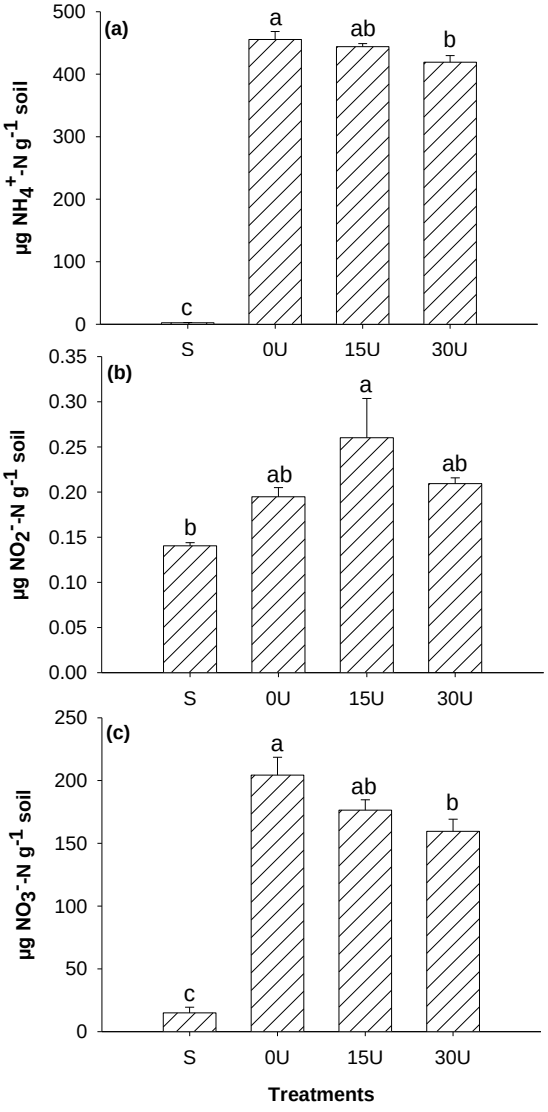


Figure 7.8 Mean soil (a) NH_4^+-N, (b) NO_2^--N, and (c) NO_3^--N concentrations determined in the soil fraction 29 days after urine application (error bars = + s.e.m., n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scale on y axes.

7.4.1.7 Soil inorganic ^{15}N enrichment - 1st experiment.

The atom % ^{15}N enrichment of both the soil NH_4^+-N and NO_3^--N increased under all urine treatments, but increasing the rate of biochar had no significant effect ($p > 0.05$) on these values with overall mean enrichments of 8.13 ± 0.03, and 6.60 ± 0.10 atom % ^{15}N, respectively (Figure 7.9).

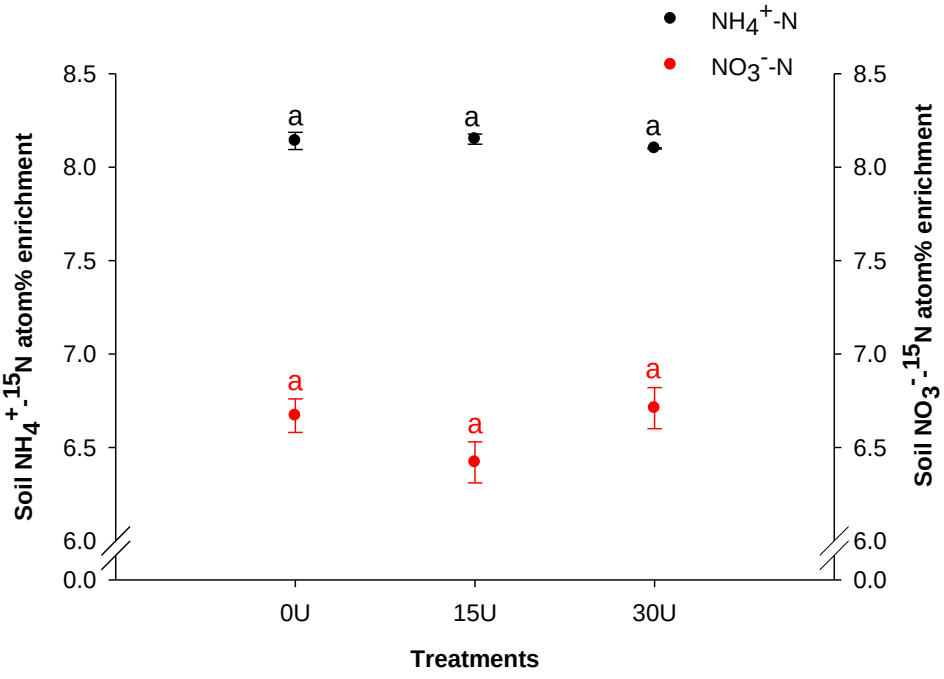


Figure 7.9 Soil inorganic-N atom % ^{15}N enrichment determined for ^{15}N enriched urine various treatments (error bars = ± s.e.m., n = 5).

7.4.2 Second experiment

7.4.2.1 Dry matter yield - 2nd experiment.

The leaf dry matter yield (DMY) averaged 72 ± 13 mg pot^{-1} with no significant ($p > 0.05$) differences between treatments (Figure 7.10). Biochar type (fresh versus urine-treated) had no significant effects on leaf DMYs ($p > 0.05$), when comparing only the biochar amended treatments.

Root DMY did not differ between treatments ($p > 0.05$), and averaged 333 ± 43 mg pot^{-1} (Figure 7.10). Biochar type had no significant effect on root DMYs ($p > 0.05$), 298 ± 51 and 315 ± 53 in urine-treated and fresh biochar treatments, respectively.

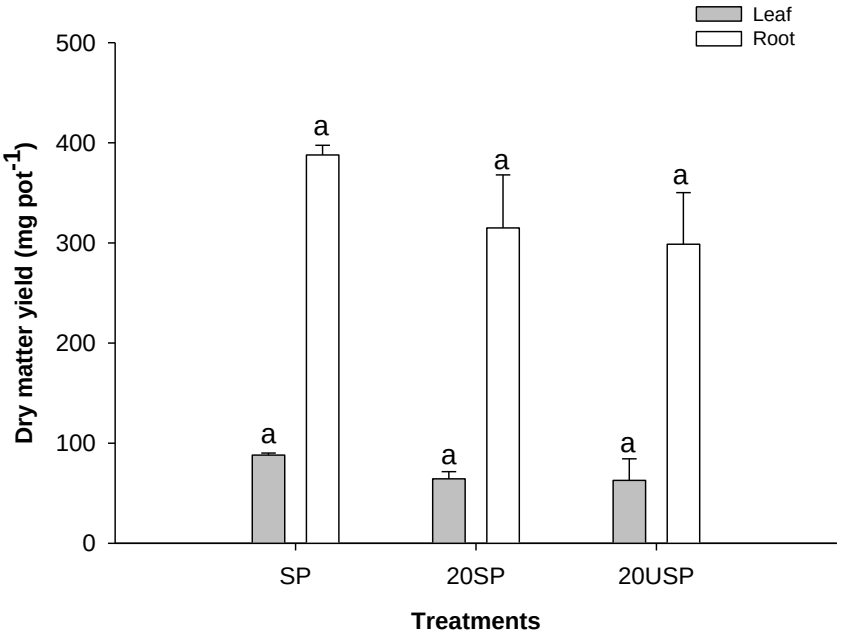


Figure 7.10 Mean leaf and root dry matter yields after 25 days (error bars = + s.e.m.; n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$).

7.4.2.2 *Plant total N content and ^{15}N enrichment - 2nd experiment.*

The N contents of the leaves and roots in the treatments did not differ significantly with treatment and on average N contents were 38.6 ± 2.5 and 12.9 ± 0.9 mg g^{-1}, respectively (Figure 7.11). The urine-treated biochar did not alter (p > 0.05) leaf N contents (40.75 ± 0.97 mg g^{-1}) when compared to the fresh biochar (36.79 ± 0.37 mg g^{-1}) (Figure 7.11a). Root N content did not vary when either urine-treated or fresh biochar was applied (Figure 7.11b), 12.48 ± 1.37 and 12.52 ± 0.16 mg g^{-1}, respectively (p > 0.05). The treatments receiving urine-treated biochar had significantly higher atom% ^{15}N enrichment in leaves and roots when compared to the treatments receiving fresh biochar content (Figure 7.11). The ^{15}N enrichment of the leaf and root materials increased to 2.40 ± 0.14 and 1.97 ± 0.15 atom % following addition of urine-treated biochar in the 20USP treatment (Figure 7.11b).

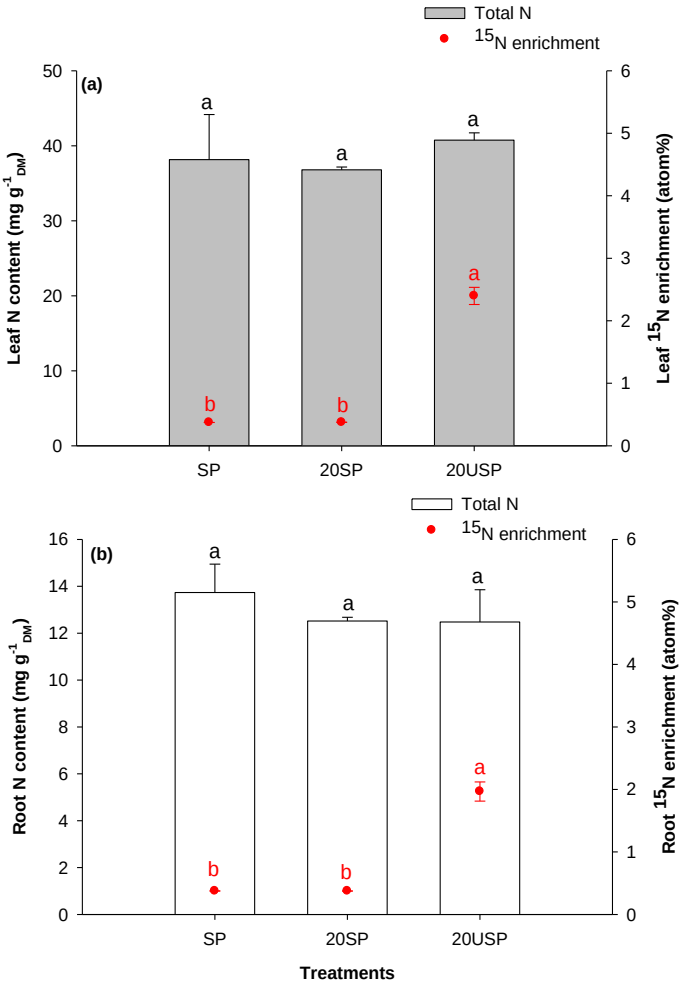


Figure 7.11 Mean N content and ^{15}N enrichment in (a) leaf and (b) root tissues over 30 days (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, p < 0.05). NOTE differing scales on y axes.

7.4.2.3 Plant N uptake and ^{15}N recovery - 2nd experiment.

The total leaf N uptake did not differ with treatments ($p > 0.05$) and was equal to 2.78 ± 0.62 mg g^{-1} leaf when averaged over all treatments (Figure 7.12). Biochar type, urine-treated versus fresh biochar, did not affect leaf N uptake, 2.58 ± 0.89 and 2.38 ± 0.27 mg g^{-1} leaf, respectively ($p > 0.05$). The same scenario was observed for root N uptake ($p > 0.05$) with an average of 4.33 ± 0.62 mg g^{-1} root (Figure 7.12). Root N uptake did not vary with biochar type ($p > 0.05$), 3.65 ± 0.57 and 3.97 ± 0.69 mg g^{-1}, in urine-treated and fresh biochar treatments, respectively. The leaf ^{15}N recovery was 4.35 ± 1.53 atom % in the 20USP treatment. Root ^{15}N recovery was 5.16 ± 0.90 atom % in the 20USP treatment.

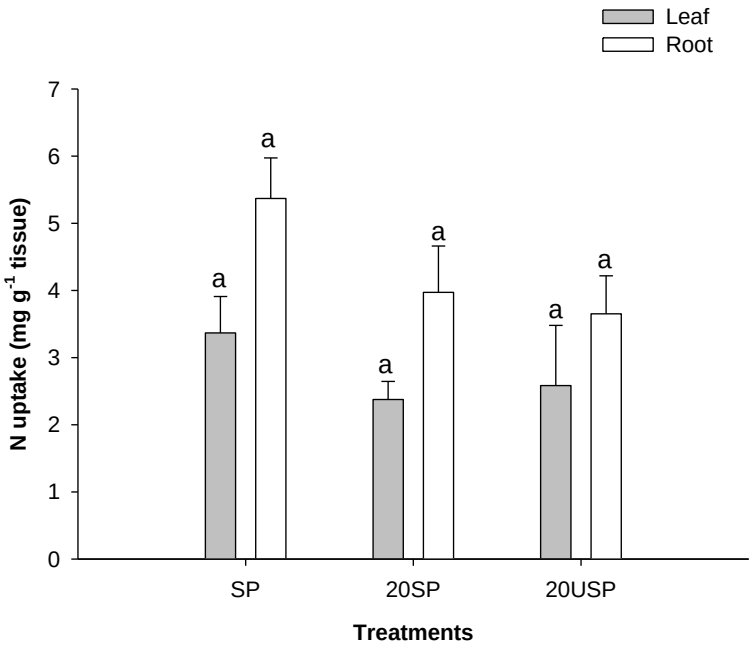


Figure 7.12 Mean N uptake by leaf and root tissues (error bars = + s.e.m., n = 5). For each variable, lower case letters indicate significant difference between means (Tukey's Test, $p < 0.05$).

7.4.2.4 Biochar N content and ^{15}N enrichment - 2nd experiment.

After 30 days the N contents of the ^{15}N enriched urine-treated biochar recovered from the 20US and 20USP treatments were 2.8 ± 0.2 and 3.5 ± 2 mg g^{-1} biochar, respectively (Figure 7.13). On average the N content of the urine treated biochar had decreased by 4.7 mg g^{-1} biochar over the 30 days since addition to the soil. Biochar total N contents did not differ when comparing urine-treated biochar and fresh biochar types ($p > 0.05$). Plants had no effect on N content of the biochars ($p > 0.05$), 3.2 ± 1.1 and 2.1 ± 0.3 mg g^{-1} biochar when comparing plant and non-plant treatments, respectively, and averaging values across biochar types (Figure 7.13). There was no interaction due to biochar type and plants on biochar N content ($p > 0.05$).

Atom % ^{15}N of the ^{15}N enriched urine-treated biochar remained higher ($p < 0.05$) in the 20US and 20USP treatments, averaging 2.54 ± 0.33, when compared with the ^{15}N enrichment of the fresh biochar recovered from the 20S and 20SP treatments, after 30 days, which averaged 0.37 ± 0.00 atom % (Figure 7.13). Plant presence had no effect on biochar ^{15}N enrichment ($p > 0.05$), averaging 1.25 ± 0.44 and 1.67 ± 0.68 atom % when plants were present and absent in biochar amended treatments, respectively (Figure 7.13). No interaction occurred due to biochar type and plant presence with respect to ^{15}N enrichment ($p > 0.05$).

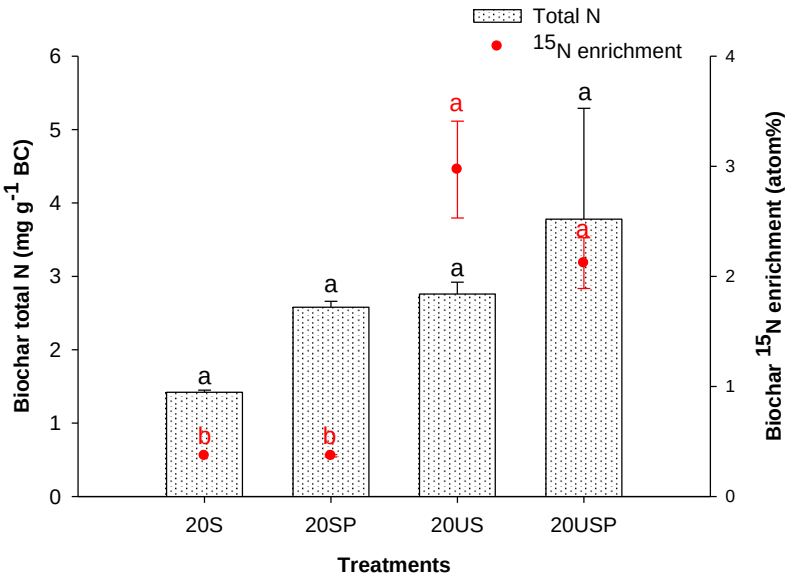


Figure 7.13 Mean total N content and ^{15}N enrichment of biochar after 30 day incubation (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scales on y axes.

7.4.2.5 Soil N content and ^{15}N enrichment - 2nd experiment.

Soil total N content ranged from 1.91 to 2.33 mg g^{-1} soil (Figure 7.14) and differed with treatment ($p = 0.001$). Soil total N contents did not differ due to biochar type ($p > 0.05$) (Figure 7.15), but plants had a significant effect on soil N content ($p < 0.05$) which equalled 2.01 ± 0.07 and 2.25 ± 0.06 mg g^{-1} soil in the absence and presence of plants in biochar amended treatments, respectively (Figure 7.15). There was no interaction between biochar type and plant treatments with respect to soil N content ($p > 0.05$).

Atom % ^{15}N varied significantly between all treatments ($p = 0.00$), equalling natural abundance (0.37 ± 0.00 atom %) in the S, SP, 20S, and 20SP treatments. However this value was higher in the 20US and 20USP treatments, equating to 0.48 ± 0.01 atom % when averaged over these two treatments (Figure 7.14). Plants had no effect on soil atom % ^{15}N ($p > 0.05$), 0.41 ± 0.04 and 0.44 ± 0.04 % when averaged over the plant and non-plant treatments, respectively, when comparing only biochar amended treatments (Figure 7.15). No interaction was observed between biochar type and plant treatments on soil ^{15}N enrichment ($p > 0.05$).

The %^{15}N recovery in the soil was 27.7 ± 11 and 16.1 ± 2.8 % in the 20US and 20USP treatments, respectively ($p > 0.05$).

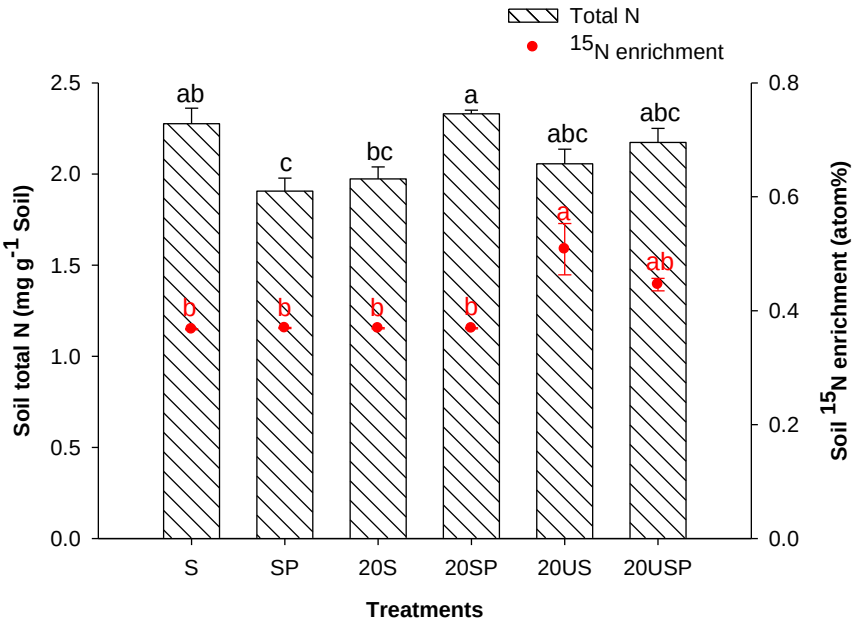


Figure 7.14 Mean soil total N content and ^{15}N enrichment over the 30 day incubation experiment when comparing all treatments (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scales on y axes.

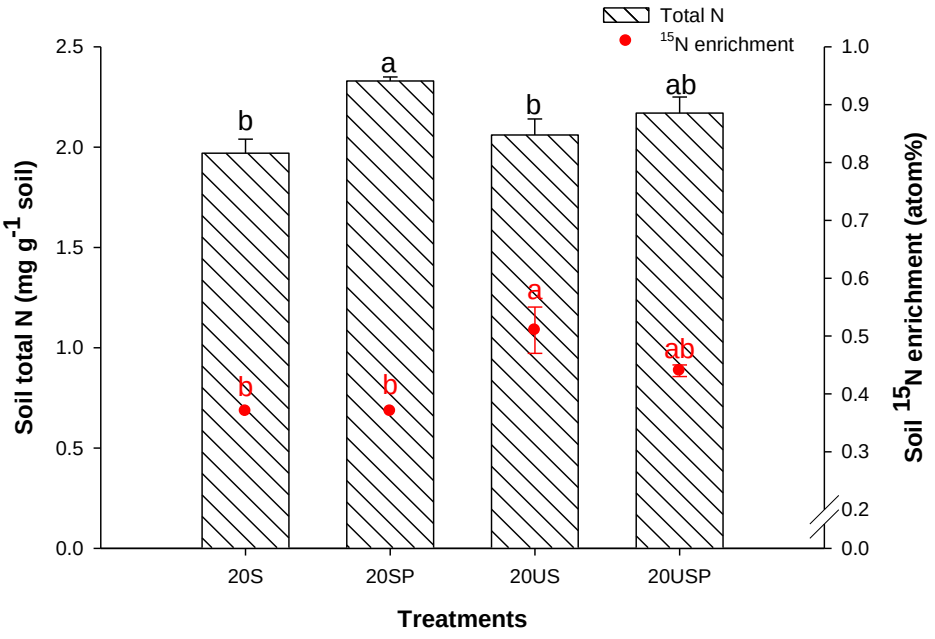


Figure 7.15 Mean soil total N content and ^{15}N enrichment after 30 day incubation when comparing only biochar treatments (error bars = + s.e.m. for total N and ± s.e.m. for ^{15}N enrichment, n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$) . NOTE differing scales on y axes.

7.4.2.6 Biochar inorganic N - 2nd experiment.

Extracted biochar NH_4^+-N concentrations ranged from 0.0 to 30.8 µg g^{-1} biochar with significant difference between treatments (p = 0.00) (Figure 7.16a). Concentrations of NH_4^+-N were higher (p < 0.01) in the urine-treated biochar compared with fresh biochar, and were 30.2 ± 3.0 and 3.7 ± 1.8 µg g^{-1} biochar, respectively (Figure 7.16a). Plants had no effect on biochar NH_4^+-N concentrations (p > 0.05), averaging 19.1 ± 6.0 and 14.8 ± 7.2 µg g^{-1} biochar in plant and no plant treatments, respectively, when averaging across biochar amended treatments (Figure 7.16a). No interaction was observed due to biochar type and plant presence on biochar NH_4^+-N concentrations (p > 0.05).

The results of biochar NO_2^--N concentrations (Figure 7.16b) showed significant differences (p < 0.05) due to higher concentrations of NO_2^--N in the 20US treatment. Plants caused biochar treatments to have lower (p < 0.05) NO_2^--N concentrations than non-plant treatments (0.1 ± 0.0 and 0.4 ± 0.1 µg g^{-1} biochar, respectively) when comparing means across the biochar amended treatments (Figure 7.16b). No significant interaction was observed due to biochar type and plant presence on NO_2^--N concentrations (p > 0.05).

The NO_3^--N concentrations also differed significantly between treatments (p = 0.00) and ranged from 4.8 ± 0.1 to 109.1 ± 23.3 µg g^{-1} biochar (Figure 7.16c). However, concentrations of NO_3^--N differed (p < 0.05) when the urine-treated biochar and fresh biochar treatments were compared and were 88.2 ± 19.7 and 36.4 ± 14.9, respectively, when averaged over plant treatments (Figure 7.16c). Plants had no effect on biochar NO_3^--N concentrations (p > 0.05), averaging 67.6 ± 7.1 and 56.9 ± 29.1 µg g^{-1} biochar in plant and no plant treatments respectively, when comparing biochar amended treatments (Figure 7.16c). There was a significant interaction due to biochar type and plants on biochar NO_3^--N concentrations (p < 0.05) with lower NO_3^--N concentrations with plant presence in urine-treated biochar, and higher concentrations in the presence of plants with fresh biochar.

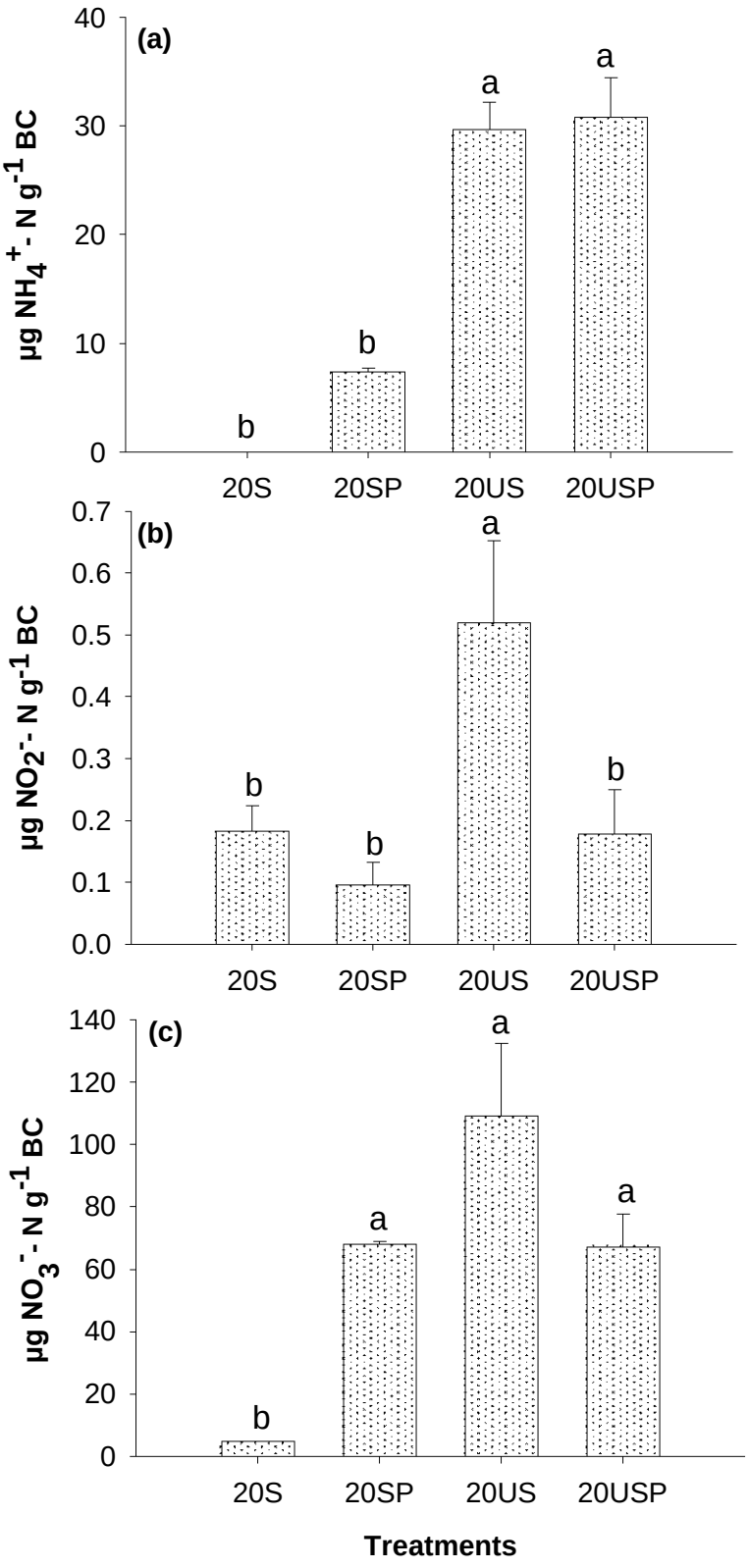


Figure 7.16 Mean biochar (a) NH_4^+-N, (b) NO_2^--N, and (c) NO_3^--N concentrations determined in biochar samples over the 30 day incubation experiment (error bars = + s.e.m., n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scales on y axes.

7.4.2.7 Soil inorganic N - 2nd experiment.

The resulting soil NH_4^+-N concentrations (Figure 7.17a) did not differ between treatments and ranged from zero to 0.7 ± 0.5 µg g^{-1}soil ($p > 0.05$). Concentrations of NH_4^+-N did not differ ($p > 0.05$) due to biochar type (Figure 7.18a). Plants had no effect on soil NH_4^+-N concentrations ($p > 0.05$), averaging 0.3 ± 0.1 and 0.6 ± 0.4 µg g^{-1} biochar in plant and no plant treatments, respectively (Figure 7.18a). There was no interaction between biochar type and plant treatments on soil NH_4^+-N concentrations ($p > 0.05$).

The NO_2^--N concentrations varied significantly ($p < 0.05$) between treatments ranging from 0.1 ± < 0.1 to 0.37 ± < 0.1 µg g^{-1}soil (Figure 7.17b). However, NO_2^--N concentrations did not differ ($p > 0.05$) with biochar type or plant presence (Figure 7.18b). There was a significant interaction between biochar type and plants on soil NO_2^--N concentrations ($p < 0.05$). The NO_2^--N concentrations increased with plant presence in the fresh biochar treatment, but no difference was observed on NO_2^--N concentrations of urine-treated biochar in the presence of plants (Figure 7.18b).

The NO_3^- -N concentrations (Figure 7.17c) ranged from 16.0 ± 0.2 to 67.5 ± 2.2 differing with treatments ($p = 0.00$). The NO_3^- -N concentrations did not differ ($p > 0.05$) due to biochar type (Figure 7.18c). Plants caused biochar treated soils to have a lower ($p < 0.05$) NO_3^- -N concentrations (25.0 ± 4.3) than the non-plant treatments (61.23 ± 6.98) (Figure 7.18c). There was no interaction due to biochar and plant treatments on soil NO_3^- -N concentrations ($p > 0.05$) (Figure 7.18c).

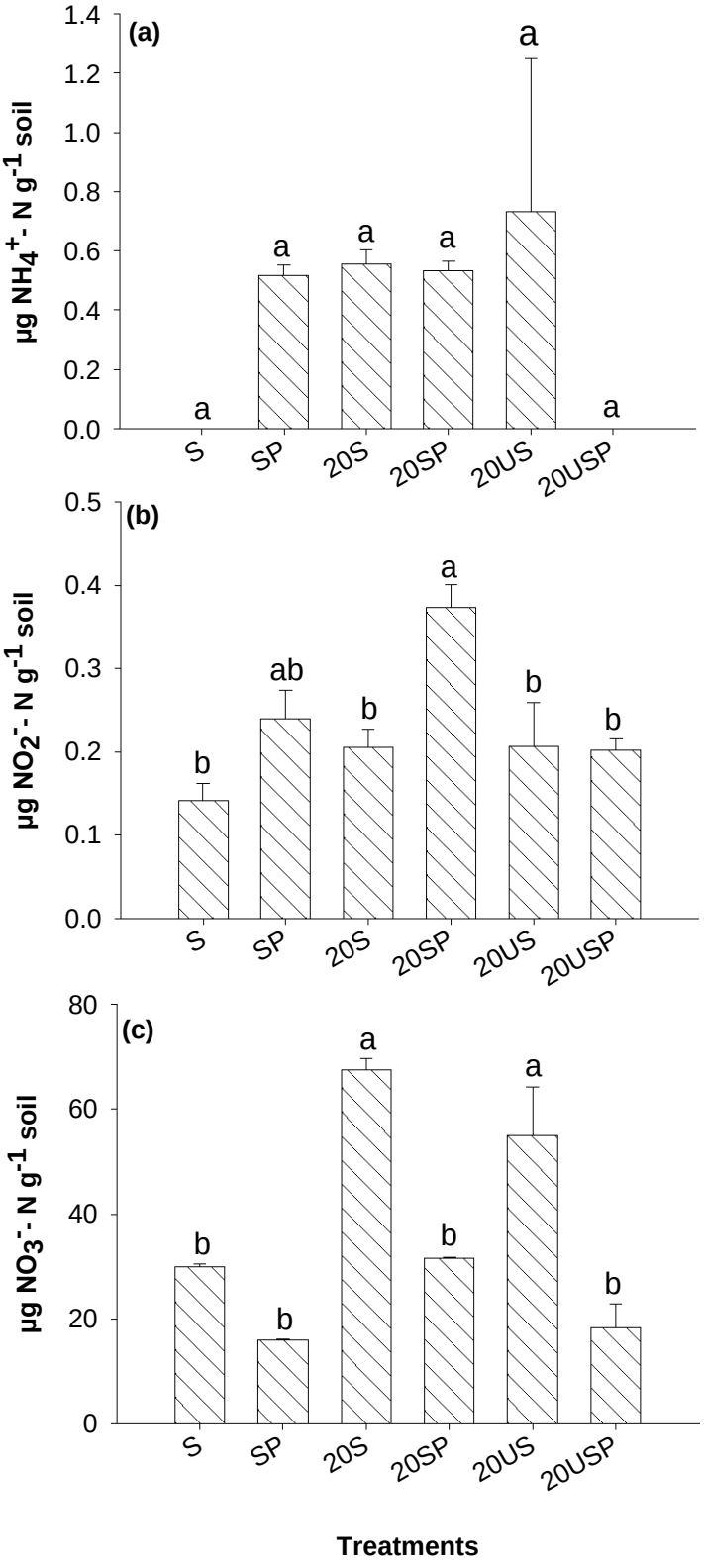


Figure 7.17 Mean soil (a) NH_4^+-N, (b) NO_2^--N, and (c) NO_3^--N concentrations determined in biochar samples over the 30 day incubation when comparing all treatments (error bars = + s.e.m., n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scales on y axes.

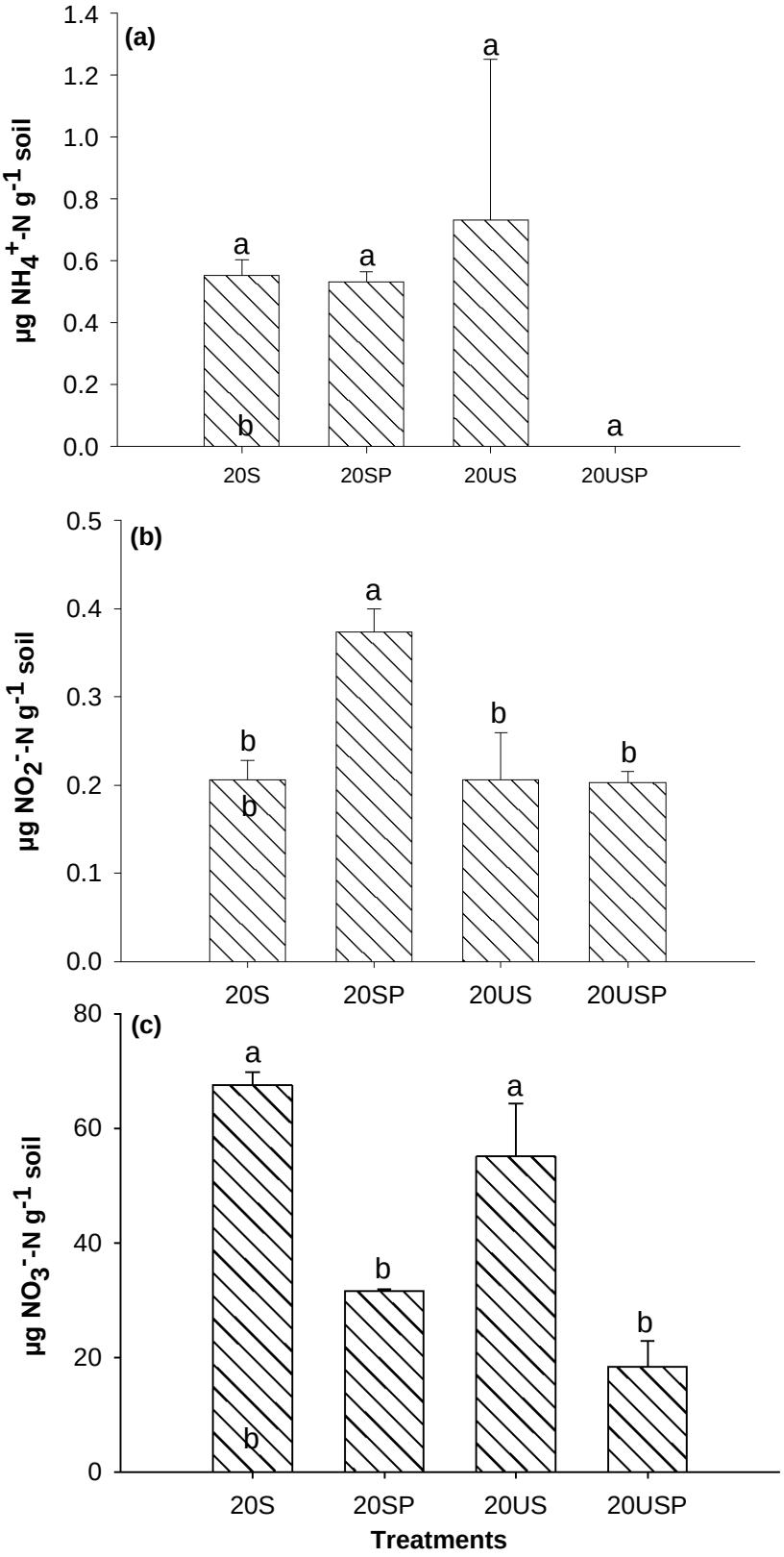


Figure 7.18 Mean soil (a) NH_4^+-N, (b) NO_2^--N, and (c) NO_3^--N concentrations determined after 30 day incubation when comparing only biochar treatments (error bars = + s.e.m., n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$). NOTE differing scales on y axes.

7.4.2.8 Biochar and soil inorganic-^{15}N enrichment - 2nd experiment.

There was insufficient sample remaining for ^{15}N analyses of either biochar NH_4^+-^{15}N, biochar NO_3^--^{15}N or soil NH_4^+-^{15}N.

The atom % ^{15}N enrichment of the soil NO_3^--^{15}N in the urine treatments were 2.02 ± 0.12 and 1.80 ± 0.14 in 20US and 20USP treatments, respectively ($p > 0.05$) with plants not affecting the ^{15}N enrichment (Figure 7.19).

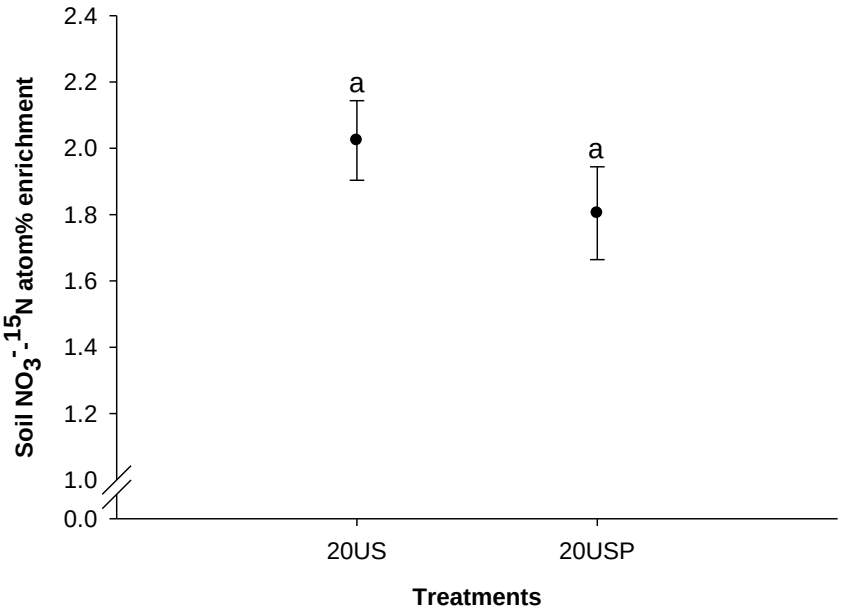


Figure 7.19 Mean atom% ^{15}N enrichment determined for soil NO_3^--N (error bars = ± s.e.m., n = 5). For each variable, lower case letters indicate significant differences between means (Tukey's Test, $p < 0.05$).

7.5 Discussion

7.5.1 Effect of biochar on soil NH_3 volatilisation and soil inorganic-N

Results of the first experiment show that, as hypothesised, the inclusion of biochar within the soil reduced NH_3 emissions. However, it should be noted that NH_3 gas only accounted for 1.4% of the N applied in the 0U treatment which is relatively low when considering other studies, for example, the field study (Chapter 6). The soil properties were similar to previous laboratory and field experiments and the low NH_3 flux recorded here may have been due to the application of different methods for NH_3 measurements. In aspirated chambers, as used in the current experiment, low air flow rates (≈ 0.04 air exchange min^{-1}) are likely to cause an underestimate of NH_3 volatilisation rate (Gao & Yates, 1998). A more realistic air flow rate (e.g. 17 air exchange min^{-1} (Sherlock & Goh, 1984a)) was not feasible in the current experimental set up.

Nevertheless, NH_3 volatilisation from ^{15}N-labelled ruminant urine was reduced by 45% after incorporating either 15 or 30 t ha^{-1} of biochar. The lower atom% ^{15}N enrichment of the NH_3 volatilised, after 24 h, in the presence of biochar indicates less urine-^{15}N contributed to the NH_3 flux which was consistant with biochar adsorbing some of the urinary-N derived NH_3. The elevated total N content of the biochar and its ^{15}N enrichment indicated that urine-^{15}N had been adsorbed on the biochar. This clearly demonstrates that the presence of biochar in the soil, under a urine patch, could potentially act as a sink for NH_3 and/or NH_4^+. This experiment has not distinguished between biochar recovering urine-^{15}N as either NH_4^+-N or NH_3-N. However, given the CEC of the biochar material, 8 $cmol_c$ kg^{-1}, (Table 4.2) the theoretical maximum increase in biochar N content (as NH_4^+-N) would be 1.36 g N kg^{-1} of biochar. However, this only represents 56 and 23% of the increase in biochar total N seen under the 15U and 30U treatments, respectively. Such an effect of biochar CEC on uptake of urine-N as NH_4^+ would also be reduced due to the influence of other urine-derived cations such as K^+ swamping and competing for CEC sites. Thus it is reasonable to assume that adsorption of NH_3 was the dominant mechanism by which urine-N was removed from the soil matrix under the applied urine.

In this current study the increase in biochar total N content, 2.44 and 5.88 mg g^{-1} in the 15U and 30U treatments, respectively, is comparable to rates seen in Chapter 5. This is despite the different sources of NH_3 and method of N application (urine versus NH_3 gas). As mentioned in section 5.4.1, the presence of acidic functional groups are considered a main factor involved in the adsorption of NH_3 by biochar. The amount of KCl extractable NH_4^+-N on the biochar taken out of the urine affected soil averaged 0.24 mg g^{-1} biochar which was only a

fraction of the N adsorbed on/in biochar particles. Again these results are in good agreement with the extractable NH_4^+-N values in Chapter 5.

After 29 days the soil inorganic-N was, as expected, elevated where urine-N had been applied but interestingly the concentration of both NH_4^+ and NO_3^--N were trending down as biochar rate increased, which for NO_3^--N parallels what was seen in the field experiment under urine (Chapter 4). Resources precluded a more in depth examination of the N dynamics here over time but future studies need to examine the temporal dynamics in the concentrations of inorganic-N extracted from biochar and soil following urine addition, in order to fully understand the mechanism(s) of N release from the biochar materials.

7.5.2 Effect of fresh and urine-treated biochar on soil-plant N dynamics

Interestingly the application of either fresh or urine-treated biochar had no effect on herbage dry matter yields (root or leaf) or N concentration in the herbage tissues (root or leaf) when compared to soil without biochar. Thus plant N uptake was not influenced by either biochar presence or biochar type, supporting the idea from the earlier experiment (Chapter 4) that biochar application did not cause any negative effect on plant growth. However, the ^{15}N enrichment of plant tissues became elevated in the urine-treated biochar treatment. This clearly demonstrates that biochar derived ^{15}N was not only bioavailable but actually contributed to the N pool taken up by the plants. This begs the question as to why the plants preferentially took up urine-treated biochar derived N as opposed to native soil-N. Presumably, because of the lack of treatment differences in plant yields N was not limiting plant growth in the fresh biochar treatment, and neither were other nutrients or growing conditions limiting yield, although these later factors are considered unlikely to have been limiting. Therefore assuming equal N demand in all treatments, plants presumably took up the urine-treated biochar N preferentially because it was easier to access, and readily moving into soil solution, as opposed to native soil N needing to first be mineralized. This current experiment has not considered competition for urine-treated biochar N between the microbial community and plants. Again this would be an interesting study to examine the temporal dynamics and competition between these N demanding populations. After 30 days there was still more NH_4^+-N extracted from the urine-treated biochar than the fresh and had the experiment been run longer differences in plant-N uptake may have been seen as a result of further N release in the urine-treated biochar treatment. This assumes of course that all biochar adsorbed-N is ultimately plant available. Again this needs to be further evaluated.

7.6 Conclusion

This study has shown that biochar addition to soil can reduce the NH_3 volatilisation loss from ruminant urine treated soil. Furthermore, this study has demonstrated that urine-N adsorbed by biochar under a urine patch can be available to plants. In this current study there were few differences between using 15 or 30 t ha^{-1} but optimal rate effects need to be further evaluated. It is concluded that there is the potential for biochar to be used in intensively grazed pastures to decrease NH_3 volatilisation and act as a transient store of plant available N. However, future studies are still required, as mentioned above, and to verify this result at a more realistic scale using micrometeorological methods. In addition the impacts of seasonality and soil moisture may also need to be considered.

Chapter 8
Conclusions and recommendations

8.1 Introduction

The results obtained in the preceding experiments are summarised in this chapter. Conclusions are drawn with respect to the findings and future research opportunities are proposed.

8.2 Overall summary

8.2.1 Effects of ruminant urine and biochar on N_2O emissions from pasture soil

A single ruminant urine deposition creates "hot spots" where soil N concentrations exceed the pasture plants' immediate demands (Haynes & Williams, 1993). Transformation of this urinary-N leads to the creation of an inorganic-N pool which results in N_2O being produced via nitrification, nitrifier-denitrification, or denitrification (Wrage et al., 2001). Prior to the current thesis being undertaken, several laboratory-based studies had documented the suppression of N_2O emissions as a result of biochar addition to soils (Section 2.3.4). In this thesis (Chapter 4) the effect of incorporating biochar into soil on urinary-N transformations, plant-N uptake and N_2O fluxes was investigated in-situ for the first time.

The biochar treatment of 30 t ha^{-1} reduced N_2O fluxes by 70% and also reduced soil NO_3^--N concentrations. A hypothesis put forward to explain these results proposed that this was due to some of the urine-N being removed from the N pool immediately available for microbial transformation, with removal occurring due to NH_3 adsorption onto the biochar.

The reductions in the in-situ emissions of N_2O were unable to be examined further in the suite of experiments that followed, due to other scientific tangents being developed. However, there are clear and urgent research needs.

- Diffusion of a gas through the soil depends on soil water content and temperature so there is a need to determine if biochar induced reductions in N_2O fluxes are dependant on soil moisture and temperature. These are of course driven by season and management e.g. irrigation.
- The proposed mechanism for NH_3 adsorption involves the reaction of carboxylic acid groups on the biochar surface (Section 2.3.2). These are a function of the biochar manufacturing properties. Questions that arise include how permanent or replaceable

these biochar functional groups are over time in the soil and how this affects NH_3 adsorption. Can the biochar, once exposed to NH_3, adsorb NH_3 a second time? Would N_2O fluxes be reduced for a second time if a urine patch event occurred where one had previously been? These ideas need to be tested to further evaluate the efficacy of biochar incorporation in soil as a N_2O mitigation method.

- Furthermore, if NH_3 adsorption is the major factor causing the reduction in N_2O then the subsequent potential release of the adsorbed N and its potential to subsequently contribute at a later 'moment' to N_2O fluxes should also be examined.

8.2.2 Effects of biochar on plant N uptake and plant growth

Since NH_3 adsorption onto the biochar was hypothesised as being a removal process for microbial-available N an experiment was performed to examine the capacity for biochar materials to adsorb NH_3 (including the biochar used in the in-situ field experiment (Chapter 4) and the subsequent bioavailability of the NH_3 adsorbed. The use of ^{15}N labelled NH_3 unequivocally demonstrated the biochar materials could take up NH_3 and that it became bioavailable once placed in a soil with plants present (Chapter 5). Recoveries of ^{15}N in the plant tissues were comparable to recoveries previously achieved from under synthetic fertiliser or urine patches. This study demonstrated a possible method for mitigating NH_3 from agricultural operations and an exciting potential use for biochar that contains adsorbed NH_3. However, this study also raised several research questions that need to be addressed before the potential of this study can be fully exploited.

- There is a need to examine the mechanism of NH_3 adsorption in greater detail. While the current study identified pH and surface acidity as being important, and this concurred with previous literature, the actual product formed on the biochar was not identified. Further studies need to positively elucidate the mechanism of NH_3 adsorption under the conditions that would be found in the soil or in other agricultural operations where NH_3 forms.
- Other issues raised by this study include understanding the length of time it takes for biochar adsorbed NH_3 to be released once it is placed in the soil, and what soil factors affect this. Due to the nature of the experiments conducted here, long term studies were not undertaken. Thus there is an unresolved question. Is all the biochar adsorbed NH_3 eventually released to the soil?
- The role that biochar adsorbed NH_3 can play as a fertiliser has been demonstrated in the current thesis. However, we still need to know - What is the efficacy of the biochar

adsorbed NH_3 when compared to traditional synthetic fertilisers? What is the rate of N release?

- And finally, how does biochar adsorbed NH_3 affect microbial communities involved in soil N transformations?

8.2.3 Effects of ruminant urine and biochar on NH_3 volatilisation from pasture soil

The field experiment in Chapter 6 demonstrated, using an in-situ study, that emissions of NH_3 from urine could be as high as 25.7% of the urine-N applied. Furthermore the study showed that soil concentrations of NH_3 could be sufficiently high to be adsorbed and impacted by biochar. While the study in Chapter 5 showed that NH_3 could be adsorbed by biochar it was not proven that it occurred under ruminant urine and the hypothesis established in Chapter 4 'That reductions in N_2O emissions under a urine patch were due to NH_3 adsorption' had not been fully proven. Thus Chapter 7 attempted to resolve this issue by being designed to measure NH_3 volatilisation with and without biochar amended soils exposed to urine. It was seen that biochar did reduce NH_3 emissions and that the N contained on/in the biochar, when transferred to fresh soil, was again bioavailable. Again further questions and studies are now required to pursue this research thread.

- Can reduction of NH_3 volatilisation be demonstrated in-situ? If so what is the scope of achievable reductions and what is the optimal rate of biochar? And where should biochar be placed to optimise the effect?

8.2.4 Effects of biochar adsorbed N on plant N dynamics.

In both chapters 5 and 7 fragments of ^{15}N labelled biochar were transplanted into fresh previously untreated soil and plants were sown and allowed to grow, and then harvested with uptake of biochar derived ^{15}N clearly demonstrated. In Chapter 5 uptake of biochar derived N clearly increased yields. However, this was not the case in Chapter 7, possibly for reasons outlined in Section 7.5.2. Clearly biochar derived N is plant available but the contrasting plant yield dynamic arising from these two studies leads to other research questions concerning the longevity of the biochar N supply and competition for this supply.

- How rapidly is biochar N made available to plants? Are microbes competing for this N in a similar manner as would occur in the absence of biochar or does biochar presence favour either plant-N or microbial-N uptake? Studies examining the dynamics and interactions of the microbial N pool and plants are required.

8.3 Conclusions

The science of biochar is a rapidly advancing field of soil research and even during the conduct of this thesis (2008 - 2011) there have been over 200 publications dealing with biochar. This thesis has made important key contributions to this literature by demonstrating for the first time that:

- Biochar can mitigate N_2O emissions in-situ under ruminant urine grazing and influence soil inorganic-N concentrations.
- The resulting hypothesis for these effects was proven to be true using a laboratory study that showed biochar taking up N in a urine affected soil.
- Ammonia adsorbed onto biochar has been shown to be bioavailable and to have a potential role as a mitigation tool, where NH_3 emissions need to be captured, and subsequently as a potential N fertiliser.
- There are several key areas of biochar-nitrogen research that must be studied further to advance the scientific discoveries made here.

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