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Ammonia oxidizing microbial communities and relationships with nitrous oxide emissions as affected by different land uses

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Master of Agricultural Science

at
Lincoln University
by
Chaoyu Li

Lincoln University
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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Agricultural Science.

Ammonia oxidizing microbial communities and relationships with nitrous oxide emissions as affected by different land uses

by

Chaoyu Li

Nitrous oxide (N\textsubscript{2}O) emissions in New Zealand largely come from agriculture through the processes of nitrification and denitrification as a part of the nitrogen cycle. Ammonia oxidising bacteria (AOB) and ammonia oxidising archaea (AOA) are the main drivers of ammonia oxidation (the first step of nitrification) in soil thus they affect N\textsubscript{2}O emissions. It is thought that different land uses and land use change can impact on ammonia oxidising (AO)-populations and N\textsubscript{2}O emissions, however, the influence of different land uses on AO-populations and N\textsubscript{2}O emissions are not fully understood. Therefore the objectives of this thesis were to: 1) Determine the impact of three different land uses (pine tree plantation, dairy farming and sheep farming) in the same geographic location with the same soil type on the abundance of AOB and AOA; 2) Determine the effect of urine application on AO community abundance in the three soils; and 3) Determine the effect of urine application on N\textsubscript{2}O emissions from soils from the three different land uses.

Two projects were conducted to assess the effects of different land uses and the effect of urine addition on AO communities and N\textsubscript{2}O emissions.

In Project 1, AO-popuations in the Templeton silt loam soil under three different land uses were analysed. The soils were collected from three adjacent sites: pine tree plantation, dairy farm and sheep farm. The results showed that AOB abundance was higher in the dairy ($P < 0.05$) and sheep ($P < 0.05$) farming soils than in the pine tree soil. When the AOB and AOA abundance was compared at each site, AOB abundance was higher than AOA abundance in the dairy farming soil ($P < 0.05$), however, AOA abundance was higher than AOB abundance in the sheep farming soil ($P < 0.05$). In addition, the AOA abundance in the pine tree soil was lower.
than that in the dairy \((P < 0.001)\) and sheep \((P < 0.05)\) pasture soils. These results support the hypothesis that AOB prefer higher N environments whilst AOA prefer lower N environments. However, the reason for the lower AOA abundance in the pine tree soil compared to the dairy and sheep farm soils requires further research.

Project 2 was an incubation study, where cow urine was applied to the three different soils and incubated at 20°C for 126 days in jars for gas sampling and in pottles for soil sampling. The \(\text{N}_2\text{O}\) emission trends in the urine-treated dairy and sheep farm soils were similar. They reached a peak shortly after urine application and then decreased rapidly to almost background levels afterwards. However, \(\text{N}_2\text{O}\) emissions in the urine-treated pine tree soil increased gradually and reached a peak at a much delayed time at day 91. The total amount of \(\text{N}_2\text{O}\) emitted from the urine-treated pine tree soil \((P < 0.05)\) was the highest of the three soils investigated. A large amount of available carbon in the urine-treated pine tree soil was probably the main reason for the high \(\text{N}_2\text{O}\) emission. The results from the analysis of microbial populations also supported the hypothesis that urine application will increase AOB abundance, but not that of AOA. AOB abundance in the urine-treated dairy and sheep farm soil increased and reached peak abundance at day 60. The rapid increase in AOB abundance in these two soils following urine application probably reflected the influence of past land use history on the AO communities where the AOB populations had adapted to the higher nitrogen environment in these two land uses where nitrogen is applied as fertilisers and animal excreta returns. AOB abundance in the urine-treated pine tree soil increased more slowly but continued for an extended period until the end of the incubation study.

These results suggest that land use history can have a major effect on AO microbial population abundance and these effects have implications on \(\text{N}_2\text{O}\) emissions.

**Keywords:** Land use, nitrous oxide emissions, ammonia oxidising bacteria, ammonia oxidising archaea, ammonium, nitrate, urine.
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Chapter 1
Introduction

Greenhouse gases (GHG) capture the radiant heat from the sun in the lower atmosphere of the Earth, forcing the Earth’s atmosphere to a higher temperature than natural levels (Claire et al., 2006). There are three main GHGs that contribute to the global warming, namely carbon dioxide (CO$_2$), methane (CH$_4$) and nitrous oxide (N$_2$O). The levels of these greenhouse gases have increased since pre-industrial times.

In New Zealand, agriculture is the largest GHG contributor accounting for more than 46% of the total emissions. GHG emissions from agriculture in New Zealand have increased since 2008 (MfE 2014b), as a result of rising dairy production. Average dairy farm areas have increased and some of this increase has been the conversion of forestry land to dairy farming (MfE, 2014a).

In 2012, N$_2$O emissions accounted for 14.3% of total GHG emissions in New Zealand with 97.1% of the total N$_2$O emissions coming from agriculture (MfE 2014a). Most of these N$_2$O emissions from agriculture come from pastoral based farming. Nitrogen (N) fertiliser application and animal excretion during grazing are the main causes for increased N$_2$O emissions. Generally, N$_2$O is released from the processes of nitrification and denitrification. Ammonia oxidisation, as the first step of nitrification, provides the substrate for the production of N$_2$O. The process of ammonia oxidisation is carried out by ammonia oxidising bacteria (AOB) and archaea (AOA). Normally AOB play a dominant role in the soils with high fertility (Di et al., 2009), whereas AOA are more abundant in soils with low pH and low fertility (Di et al., 2010b; Leininger et al., 2006). Soils under different land uses have different properties. These differences in soil properties can alter the populations of AOA and AOB, and further affect N$_2$O emissions. The causes of differences in the abundance of AOA and AOB in different soils are not clear in the literature and require further investigation.

N$_2$O emissions have been reported to change when soil properties such as soil aeration status, soil pH, temperature, organic carbon levels and mineral N concentrations change (de Klein et al., 2001) and land use has a significant influence on these soil properties. It is therefore thought that a change in land use may lead to changes in N$_2$O emissions. However, few previous studies have reported the impact of land use change on N$_2$O emissions, especially when the land use is changed from forestry to dairy farming. Similarly few studies have reported the change in AOA and AOB abundance when land use is changed from forestry to dairy farming.
A review of the literature has identified significant gaps in knowledge and understanding about the impacts of three different land uses (forestry, dairy and sheep farming) and urine-N inputs on AOB and AOA abundance from the same soil type and geographic location. There is also a lack of knowledge and understanding of the effects of different land uses on N\textsubscript{2}O emissions from soils.

1.1 Goals and Objectives

The goal of this research was to study the impact of three different land uses (pine tree plantation, dairy farming and sheep farming) on the abundance of ammonia oxidising (AO) microbial communities (AOB and AOA), and the effect of urine-N application on their population abundance in these soils. In addition, the effect of different land uses and urine-N inputs on nitrous oxide emissions was measured.

These goals were achieved by accomplishing the following objectives:

1. Determine the impact of three different land uses (pine tree plantation, dairy farm and sheep farm) in the same geographic location with the same soil type on the abundance of ammonia oxidisers (ammonia oxidising bacteria and ammonia oxidising archaea).

2. Determine the effect of urine application on AO community abundance in the three soils.

3. Determine the effect of urine application on N\textsubscript{2}O emissions from soils from the three different land uses.

1.2 Hypotheses

It is hypothesized that:

1. Land use significantly affects the abundance of AOB and AOA.

2. AOB will be dominant in both the dairy farm soil and the sheep farm soil. However, AOA will dominate in the pine tree soil, due to low pH and lower N content.

3. Application of urine will increase AOB, but not AOA population abundance.

4. Nitrous oxide emissions will be significantly affected by land use and nitrogen input.
5. Nitrous oxide emissions will be higher in the dairy farm soil than in the sheep and pine tree soils.
Chapter 2
Review of the Literature

2.1 Introduction

New Zealand agriculture produces about 35.0 million tonnes of carbon dioxide equivalent (Mt CO$_2$-e) greenhouse gases and is New Zealand’s largest GHG contributor accounting for over 46% of the total emissions (Figure 2.1) (MfE, 2014b). There are three main GHGs emitted from New Zealand agriculture: (i) carbon dioxide (CO$_2$), (ii) methane (CH$_4$) and (iii) nitrous oxide (NO$_2$). The relative contribution from each of the GHG gases to the total GHG emissions is shown in Figure 2.2 (de Klein & Ledgard, 2005).

Nitrous oxide emissions represent approximately 14% of total GHG emissions in New Zealand. These emissions largely come from agricultural areas, accounting for 97% of total N$_2$O emissions (MfE, 2014a). Nitrous oxide has an atmospheric lifetime of 114 years and has a global warming potential almost 298 times greater than carbon dioxide (IPCC, 2007). There are three main contributors of nitrous oxide GHG emissions from New Zealand agricultural systems. Animal excreta deposition is the biggest contributor and accounts for over 80% of nitrous oxide GHG emissions in the whole agricultural system (de Klein, et al., 2006), followed by fertiliser applications and effluent which represent at 14% and 3% respectively. Since 2008, GHG emissions from the agriculture sector have increased, mainly as a result of the expansion of dairy farming in New Zealand.
Figure 2.1 Sources of greenhouse gas emissions in New Zealand (MfE, 2014b).

Figure 2.2 The main greenhouse gases in New Zealand’s GHG inventory (de Klein & Ledgard, 2005).
Under the Kyoto Protocol, New Zealand has committed to reducing the total GHG emissions to 1990 levels. New Zealand as a country is making major research efforts in trying to increase the agricultural production efficiency but decrease GHG emissions.

Nitrous oxide is generated in soil mainly through the process of nitrification and denitrification. Ammonia oxidising microbial communities are central to the process of nitrification (and ultimately to the process of denitrification through the production of nitrate).

2.2 The nitrogen cycle and N$_2$O production

A large proportion of the N in soil is in organic forms and cannot be used directly by plants. The availability of this organic N is determined by its conversion to available mineral forms, such as ammonium and nitrate by mineralization. N inputs to soil include nitrogen fixation, fertiliser application and grazing animal excreta returns in agricultural soils. Ammonium from these N inputs is converted to nitrate by nitrifying bacteria by the process of nitrification. Apart from plant and animal uptake, mineral nitrogen can return to the soil organic matter by the process of immobilisation. In addition, there are gaseous losses to the atmosphere by volatilization and by denitrification. Another loss pathway is nitrate leaching. The nitrogen cycle is shown in Figure 2.3.

![Diagram of the nitrogen cycle](image)

Figure 2.3 The nitrogen cycle (McLaren & Cameron, 1996).
2.2.1 Nitrification

Nitrification is a significant biological process in the nitrogen cycle. Autotrophic ammonia oxidising bacteria (AOB), such as *Nitrosospira* and *Nitrosomonas*, and ammonia oxidising archaea (AOA) are involved during the first step of nitrification, converting ammonium (NH$_4^+$) to nitrite (NO$_2^-$) (Bremner, 1997). Although the function of the ammonia monooxygenase (*amoA*) enzymes produced by AOB and AOA is the same, the genes encoding for the enzymes are significantly different in their nucleotide sequences. The physiological characteristics of archaeal species are not yet fully understood, because their cultivation in the laboratory is difficult (Killham & Prosser, 2007). However, very often the abundance of AOA *amoA* genes detected in soils is significantly higher than that of AOB (Leininger et al., 2006). Moreover, it has been suggested that their roles in nitrification are more important than AOB, especially where there are low nutrients and low pH (Erguder et al., 2009). However, Di et al. (2009) found that AOB are more important in high fertility agricultural soils.

AOB are classified into five genera: *Nitrosomonas*, *Nitrosopococcus*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus* (Koops et al., 1991). AOB are chemolithoautotrophs that use ammonia as a source of reductant and energy (Hooper et al., 2005). Ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) from autotrophic AOB, are two key enzymes generating the substrate and electrons during the process of ammonia oxidation (Bothe et al., 2000). The subunits of AMO are encoded by *amoA*. Thus, an a-subunit of AMO gene, *amoA*, is often selected as a target for primers and probes to discriminate between ammonia and methane oxidisers (Alzerreca et al., 1999).

AOA, play a significant, but less understood role in the global nitrogen cycle (Francis et al., 2005). Many AOA sequences are unique to their sampling locations (Francis et al., 2005). A study on the genome of AOA from the low-salinity sediments in San Francisco Bay shows that the ammonia oxidation pathway genes appear in the AOA genome and the pathway is different from that of AOB (Blainey et al., 2011). Moreover, in the North Sea and North Atlantic, the numbers of archaeal *amoA* gene copies were 10-1000 times as many as those of AOB (Wuchter et al., 2006). Another study demonstrated that in some pristine and agricultural soils of specific climate zones, archaeal *amoA* gene copy were 3000 times higher than betaproteobacteria (Leininger et al., 2006). The rate of ammonia oxidation is often correlated with the *amoA* gene expression (Treusch et al., 2005).
Nitrite oxidising bacteria (NOB), such as *Nitrobacter*, complete the second step of nitrification, converting nitrite (NO$_2^-$) to nitrate (NO$_3^-$). This step is very rapid, so nitrite does not accumulate in the soil.

### 2.2.2 Denitrification

The denitrification process uses the substrate from nitrification for denitrifiers. It mainly releases some gases, such as N$_2$O and N$_2$ through the reduction of NO$_3^-$ or NO$_2^-$ (Cameron et al., 2002; van Spanning, et al., 2007). The process of denitrification is normally carried out under anaerobic conditions. The bacteria involved in the oxidation of organic matter N use NO$_3^-$ or NO$_2^-$ as the electron acceptor instead of O$_2$ (Delwiche, 1981; Sherlock, 1992; van Spanning et al., 2007). They finally produce N$_2$O and N$_2$ under low O$_2$ conditions (Robertson & Groffman, 2007). Generally, anaerobic conditions in soil usually occur after rainfall events because the saturation of water pores increases and the penetration of O$_2$ in the soil is low.

Various denitrifying bacteria are involved in denitrification by synthesising specific metalloenzymes in each step of the denitrifying pathway (van Spanning et al., 2007). Some denitrifiers (e.g. *Pseudomonas* species) participate in the entire process and release N$_2$ as the end product (Coyne, 1999), whereas other species produce only some of the enzymes required for the complete denitrification and release N-oxides as the final products (Robertson & Groffman, 2007). The deficiency of an intermediate in the process, adverse soil conditions and insufficient number of microbes producing N$_2$O reductases lead to the occurrence of incomplete or partial denitrification (Ingraham, 1981). Thomson et al. (2012) found that AOB also have the ability to denitrify during the process of nitrifier denitrification.

N$_2$O emissions generally come from the processes of nitrification and denitrification (Figure 2.4). Nitrate is denitrified to nitrite, nitric oxide, nitrous oxide and di-nitrogen by denitrifying bacteria. Much of the N$_2$O escapes as gas during these steps.
The autotrophic ammonia oxidising bacteria are sensitive to the changes in soil conditions. Under low O₂ concentrations, the nitrate reductase enzyme of AOB may produce N₂O by displacing O₂ with NO₃⁻ as the terminal electron acceptor (Haynes & Sherlock, 1986). A large volume of nitrous oxide gas can therefore be emitted before converting to di-nitrogen under field conditions. Apart from low O₂ conditions, high concentrations of NO₃⁻ in soil solution, a warm temperature for the activities of microbial communities and adequate carbon content in the soil lead to increased losses of nitrogen via denitrification (Firestone, 1982; Saggar et al., 2009). Moisture content is also an important factor affecting the production of N₂O (Smith et al., 1998). Further studies are needed to reveal the relative contribution of different microbial communities to N₂O emissions under different soil conditions.

2.3 Microbial communities under different soil conditions

Agricultural management practices, such as animal grazing, fertiliser use, irrigation, tillage and crop rotation, have a big influence on microbial communities in soils, affecting their abundance, structure and activities. The degree of the influence depends on the changes in physicochemical characteristics of the soil (e.g., mineral N, pH, moisture, bulk density and nutrient contents) and
in the quantity, quality and distribution of crop residues (Hayden et al., 2010; Kong et al., 2010). In turn, the changes in the soil microbial communities will affect inorganic N availability, and as a result, plant productivity (Feng et al., 2003; Parfitt et al., 2010).

Ammonia-oxidising bacteria (AOB) and archaea (AOA) are the main drivers of the ammonia oxidation, which is the first step of nitrification in soil (Cabello et al., 2009). The abundance and activities of AOB and AOA are affected by soil conditions, such as moisture, pH and nutrient content (Table 2.1).

Table 2.1 The soil conditions affecting AO-populations.

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<tr>
<th>Factors</th>
<th>AO - populations</th>
<th>Reference</th>
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<td>Soil moisture</td>
<td>AO populations increase under wet conditions.</td>
<td>(Avrahami &amp; Bohannan, 2007; Di et al., 2014)</td>
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<td>Aeration status</td>
<td>AOB are more abundant under oxic conditions and AOA are more abundant under anoxic conditions.</td>
<td>(Santoro et al., 2008)</td>
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<td>Soil pH</td>
<td>The optimal pH for AOB is close to neutral. AOA are more abundant in low pH conditions.</td>
<td>(Erguder et al., 2009; Shammas, 1986; Šimek et al., 2002)</td>
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<td>Temperature</td>
<td>The optimal temperature for microbial activity is between 25°C and 30°C.</td>
<td>(McLaren &amp; Cameron, 1996)</td>
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<td>Mineral N concentrations</td>
<td>AOB are more abundant under high N concentrations, whereas AOA are more abundant under low N concentrations.</td>
<td>(Di et al., 2009)</td>
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</table>
2.3.1 Soil moisture and aeration

The abundance of AOB in soil normally increases when soil moisture is close to field capacity (-10 kPa) and this is the moisture content when the highest rate of nitrification occurs in soil (Avrahami & Bohannan, 2007; McLaren & Cameron, 1996). Di et al. (2014) found soil moisture content significantly affected the growth of ammonia oxidising communities. The AOB functional genes increased with increasing soil moisture content. Moreover, ammonia oxidizers can grow under extremely wet soil conditions (Di et al. 2014). Seasonal changes in rainfall, which affect the moisture and aeration status in soils, therefore affect AO-populations. High temperature and rainy weather in summer create moist and warm conditions for microbial communities and stimulate their activities (Sherlock, 1992). Moreover, rain on dry soils also leads to a sharp increase in nitrification (McLaren & Cameron, 1996). He et al. (2007) found higher copy numbers of both AOB and AOA amoA genes in summer than in winter in Chinese upland red soils, due to wetter weather in summer.

In general, the process of ammonia oxidation requires molecular O₂. Cultivation normally increases soil aeration and stimulates the activities of nitrifying bacteria, resulting in an accumulation of nitrate (McLaren & Cameron, 1996). However, several genera of AOB can oxidise ammonia in the presence of pyruvate and under anoxic conditions (Schmidt et al., 2002) and at low O₂ concentrations can compete for ammonia with anaerobic ammonia-oxidising (ANAMMOX) bacteria (Strous et al., 2006). In the habitats like O₂-depleted zones, AOB and ANAMMOX bacteria can deplete ammonia simultaneously. Some studies also reported high concentrations of AOA under low oxygen conditions (Lam et al., 2007; Park et al., 2006; Santoro et al., 2008). Santoro et al. (2008) found that AOA were 30 times less abundant under oxic conditions and 10 times more abundant under anoxic conditions, compared with AOB.

2.3.2 Soil pH

Soil pH has a significant influence on the population abundance and activities of AOB and AOA (He et al., 2007), and the distribution and composition of microbial communities (Liu et al., 2010; Parkin et al., 1985). The optimum soil pH for the nitrifiers is close to neutral (Shammas, 1986; Šimek et al., 2002) and AOB are more abundant in neutral soils. Stephen et al. (1996) found that Nitrosospira cluster 3, one of AOB genera, was dominant in neutral soils. When the pH is lower than about 5.5, nitrification rates, especially ammonia oxidation, decreases dramatically (de Boer & Kowalchuk, 2001). In contrast, NH₃ may reach a toxic level
in alkaline soils and the nitrifiers’ activities also slow down (McLaren & Cameron, 1996). Therefore, neutrality is suitable for AOA/AOB growth.

Some studies demonstrated that AOA contribution can be higher than AOB in low fertility and low pH environments (Erguder et al., 2009). Nicol et al. (2008) found that the expression of archaeal and bacterial amoA gene had similar rates to a maximum pH at 6.9. Then bacterial amoA abundance increased with increasing soil pH, whereas archaeal amoA abundance decreased with increasing pH. He et al. (2007) also reported that AOA played a more important role in ammonia oxidation in acidic soils compared with AOB (Figure 2.5).

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**Figure 2.5** Quantification of archaeal and bacterial amoA gene copies in soil with pH values ranging from 4.5 to 7.5 (He et al., 2007).

### 2.3.3 Temperature

Temperature directly influences nitrification rates and microbial activities. The optimal temperature for nitrification and nitrifying activity in soil is between 25 °C and 30 °C (McLaren & Cameron, 1996). Previous studies have shown that ammonia oxidisers cannot tolerate too low or too high temperatures (Avrahami & Conrad, 2005). Avrahami et al. (2003) found that only one cluster belonging to *Nitrosospira* species dominated at low temperature between 4°C and 10°C, but it totally disappeared when the temperature reached 30 °C.
Temperature has a larger impact on AOB abundance compared with AOA abundance. For example, Szukics et al. (2010) found that AOB were relatively abundant when soil temperature increased, whereas AOA abundance was not affected as much by elevated temperatures between 5 °C and 25 °C. Tourna et al. (2008) found archaeal ammonia oxidisers gradually increased with increasing incubation temperatures between 5 °C and 30 °C. Szukics et al. (2010) also reported that AOA were more active than AOB at the start of their incubation when gradually elevating temperatures occurred from 5 °C to 25 °C. In contrast, the AOB communities required several weeks to adapt a higher soil temperature.

Based on the information above, the optimal temperature for incubation studies involving microbial communities should be set up at a moderate level (Adair & Schwartz, 2008), for example 20 °C.

2.3.4 Ammonia and nitrate concentrations

It has been reported that AOB are more abundant under high ammonia concentrations, whereas AOA prefer growing under low ammonia substrate conditions (Di et al., 2010b). With increasing NH₄⁺ concentrations, only several AOB species are involved in nitrification. They changed on a physiological level to adapt to high ammonia concentrations (Avrahami et al., 2002). AOA are relatively abundant in soil, but their contribution to nitrification in soils under high nitrogen concentrations appears to be relatively small compared to AOB (Di et al., 2009).

2.4 N₂O emissions affected by different soil properties

Soil properties have a major influence on nitrous oxide emissions (Choudhary et al., 2002). The main properties that have an effect, include: soil moisture, temperature, texture, pH and the concentration and availability of organic matter, nitrate and ammonium (de Klein et al., 2001). Under anaerobic conditions, denitrification is the dominant process producing N₂O and this process is also affected by various soil properties. Table 2.2 shows some of the key factors affecting N₂O emission rates from New Zealand soils. Both high spatial and temporal variability in N₂O emissions are strongly related to these factors (Choudhary et al., 2002; de Klein et al., 2001).
Table 2.2 Factors affecting N$_2$O emissions in soils.

<table>
<thead>
<tr>
<th>Factors</th>
<th>The change of N$_2$O emissions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aeration status</strong></td>
<td>N$_2$O emissions increase under anaerobic conditions.</td>
<td>(Luo et al., 2007; Sherlock, 1992; Smith et al., 1998)</td>
</tr>
<tr>
<td><strong>Soil pH</strong></td>
<td>Emission rates decrease with pH increase and the highest rates are occur between pH 5-7.</td>
<td>(Chalamet, 1985; Rolston, 1981; Sherlock, 1992)</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>Emission rates show a bell shape response to temperature with the maximum emissions at 15°C.</td>
<td>(Rafique et al., 2011; Sherlock, 1992)</td>
</tr>
<tr>
<td><strong>Organic carbon levels</strong></td>
<td>Denitrification and N$_2$O emissions increase with carbon content.</td>
<td>(Burford &amp; Bremner, 1975; Luo et al., 2010b; Sherlock, 1992)</td>
</tr>
<tr>
<td><strong>Mineral N concentrations</strong></td>
<td>Emission rates increase with the increase in nitrate and ammonium concentration.</td>
<td>(Avrahami et al., 2002; Fillery, 1983; Luo et al., 2007; Sherlock, 1992)</td>
</tr>
</tbody>
</table>

2.4.1 Moisture and aeration

N$_2$O emissions are affected by soil moisture content. Wet soil conditions produce anaerobic conditions which result in the process of denitrification, causing N$_2$O and N$_2$ emissions (Sherlock, 1992). Incomplete denitrification occurs in moist soil with a low water-filled pore space (WFPS) and a large amount of N$_2$O can be released before conversion to N$_2$. However, over time complete denitrification occurs in completely anaerobic conditions with high WFPS because N$_2$O is further reduced to N$_2$. This occurs because the N$_2$O-reductase, which converts N$_2$O to N$_2$, requires low O$_2$ concentrations (Thomson et al., 2012). Research of Smith et al. (1998) showed that N$_2$O emissions increased in a clay loam soil when the WFPS was increased.
to 80%, but the emissions decreased again when WFPS was increased over 90%. Szukics et al. (2010) also found soil moisture content was the main factor related to N₂O emissions due to denitrification. NO emissions were the highest at 30% WFPS, while N₂O emissions were the highest at 55% WFPS and N₂O was further reduced to N₂ at 70% WFPS (Szukics et al. 2010). The optimal soil moisture content of the N₂O emissions from different studies was slightly different probably due to other soil properties (such as soil texture, pH and mineral N concentrations).

In addition to the “true” denitrification, N₂O can also be released during the process of nitrification through an individual reductive side reaction under low O₂ conditions. Ammonia oxidising communities can use NO₂⁻ as the electron acceptor taking place of O₂ during this process (Sherlock, 1992). The process is called nitrifier denitrification. Nitrifier denitrification plays a major role on N₂O emissions in soils (Baggs, 2011). In addition, nitrification indirectly produces N₂O and N₂ by providing the substrate for denitrifiers. Denitrification is the same, enzymatically, as nitrifier denitrification (Baggs, 2011). Therefore, N₂O emissions may rise, when the abundance of ammonia oxidisers in soils increase.

Net rainfall (i.e. rain minus evapotranspiration) changes soil moisture content and this increases N₂O emissions from soil. Rainfall in autumn and winter causes an increase in N₂O emissions because of the increasing WFPS inducing greater denitrification rates during this period. Over 85% of N₂O emissions from grazed pasture soils in New Zealand when the WFPS is greater than 50% (de Klein et al., 2006). The N₂O emissions continue to increase and reach a disproportional amount when high WFPS is widespread (de Klein et al., 2006). Saggiar et al. (2004) considered that grazing after a rainfall event stimulated N₂O emissions in summer, which was 5-10 times higher than in winter. Rafique et al. (2011) also found a positive correlation between WFPS and N₂O emissions and suggested that restricted grazing on seasonally wet soils would reduce N₂O emissions. Smith et al. (1998), however, thought that N₂O emissions were lower in summer. However, the soil conditions they studied were drier. Therefore, soil moisture is a significant factor in seasonal changes and impacts on N₂O emissions.

2.4.2 Soil pH

The optimum pH for denitrification and microbial communities involved in denitrification is from pH 7 to 8 (Rolston, 1981; Sherlock, 1992). Complete denitrification rates are lower in acidic soils (Šimek et al., 2002), but N₂O production increases (Wijler & Delwiche, 1954).
Weslien et al. (2009) studied the relationship between N$_2$O emissions and pH in forest organic soils, which showed a strong negative correlation (Figure 2.6). The maximum N$_2$O emissions at pH 3.7 was 5 times higher than the minimum N$_2$O emissions at pH 5.9 (Weslien et al., 2009).

Thomson et al. (1994) found that the intermediates of denitrification, NO$_2^-$ and N$_2$O, were produced by the denitrifiers at pH 5.5. The intermediate concentrations decreased when pH was increased to 8.5 because of the complete denitrification converting NO$_3^-$ to N$_2$.

### 2.4.3 Temperature

High soil temperatures lead to an increase in denitrification and autotrophic nitrification rates and the optimum temperature for these processes is 30°C (Sherlock, 1992). However, New Zealand’s soils hardly reach this temperature. Smith et al. (1998) found that N$_2$O emissions increased with the increasing temperatures in the soils at both 5 and 10 cm depths (Figure 2.7).
Figure 2.7 The relationship between N$_2$O emissions and temperatures at 5 and 10 cm depth (Smith et al., 1998).

The N$_2$O emissions were still at a high level even below 15°C, due to incomplete denitrification and only a smaller proportion of N$_2$O being converted to N$_2$. Keeney et al. (1979) reported that the N$_2$O emissions at temperatures lower than 15°C were equal to the emissions at 25 °C. The denitrification rate was low, but incomplete denitrification was predominant at 15°C. Rafique et al. (2011) also found that N$_2$O emissions increased with the increasing temperatures. In contrast, this positive relationship disappeared above 17°C. The authors suggested that the influence of moisture content and N availability on N$_2$O emissions was greater than temperatures after 17 °C.

However, Maag and Vinther (1996) showed that the nitrous oxide produced by nitrification decreased with increasing temperature. The percentage N$_2$O-N at 5°C (0.49%) was higher than at 20°C (0.17%).

2.4.4 Soil texture

Soil texture is classified by different particle size distribution of the solid inorganic material in soils (McLaren & Cameron, 1996). Therefore, the drainage system and aeration under different soil textures vary. Luo et al. (2010a) reported that poorly or imperfectly drained soil, such as clay soils, can emit up to five times more N$_2$O than free drainage soils, such as sandy soils. A higher percentage of denitrification occurs in clay soils, due to anaerobic conditions.
Wiodarczyk et al. (2011) studied three different soil types, including silt, loam and sand. Silty soils had the highest \( \text{N}_2\text{O} \) emissions and sandy soils the lowest. They explained that different soil textures lead to the differences in pore size distribution and further effects on WFPS.

Maag and Vinther (1996) also found that the nitrous oxide produced by nitrification in the loamy soils was also significantly higher than in the sandy soils. However, Rafique et al. (2011) stated that gley soils with a poor draining system could emit lower amount of \( \text{N}_2\text{O} \) than the podzols with free drainage. Their explanation was that waterlogging in gley soils caused a high WFPS and the fully anaerobic conditions caused more complete denitrification, reducing \( \text{N}_2\text{O} \) to \( \text{N}_2 \); compared to the less anaerobic conditions in the free draining podzols.

### 2.4.5 Organic carbon content

Organic matter is involved in the respiratory processes of both denitrification and nitrification, and is oxidised during these processes. The amount of C in organic matter impacts on the amount and rate of denitrification. Weier et al. (1993) found that denitrification rates increased at high N concentrations when available C increased. Burford and Bremner (1975) found a positive correlation between \( \text{N}_2\text{O} \) emissions and water soluble organic C (Figure 2.8). Water soluble C is a necessary substrate for denitrifying bacteria growth. Myrold and Tiedje (1985) found that limiting the supply of C caused denitrification rates to decrease and lowered nitrate concentrations in a loam soil. Luo et al. (2010b) found that higher \( \text{N}_2\text{O} \) emissions occurred from a draining soil with high carbon concentration compared to a similar soil with a lower carbon content. In addition, peat soils were reported to release 97-165 kg \( \text{N}_2\text{O}-\text{N}/\text{ha/year} \), in comparison to mineral soils, which only released 1-4 kg \( \text{N}_2\text{O}-\text{N}/\text{ha/year} \) (Sherlock, 1992).
The production of nitrous oxide is affected by plants through their effects on total and soluble organic matter in the soils. Plants can release low molecular weight organic compounds into the rhizosphere. These compounds increased N₂O emission from the topsoils (Thomson et al., 2012).

2.4.6 Ammonium and nitrate concentration

Ammonium and nitrate provide the substrate to nitrifiers and denitrifiers. These microbial communities contribute to N₂O emissions through both nitrification and denitrification. N₂O emissions are higher, when NH₄⁺ concentrations increase (Avrahami et al., 2002). Brevik (2013) reported that both the rates of denitrification and generation of N₂O in the soil increased with an increase of ammonia concentrations following fertiliser application (Figure 2.9). However, Wetselaar et al. (1972) found nitrification was negatively correlated with high N environments and was inhibited under ammonium concentrations over 3000 ppm.
High nitrate concentrations can promote denitrification rates in soils when organic C is not limited (Sherlock, 1992). The effect of nitrate on N₂O emissions show up in the conversions from N₂O to N₂ (Burford & Bremner, 1975). Low NO₃⁻ concentrations delay the conversion from N₂O to N₂. High NO₃⁻ concentrations inhibit the N₂O reductase enzyme and cause incomplete denitrification (Fillery, 1983). Then, the reduction of N₂O to N₂ is restrained and large amounts of N₂O are produced.

2.5 Different land uses affecting microbial communities and N₂O emissions

2.5.1 Forestry

New Zealand has 1.7 million hectares of plantation forests, accounting for around 6% of the total land area (Ministry for primary industries. 2012). Radiata pine (*Pinus radiata*), as the main plant, occupies nearly 90% of New Zealand’s plantation forests (McLaren & Cameron, 1996). The soil affects plant growth and the plants also change soil properties. McDonald and Laacke (1990) considered that deep sandy loams with a thick surface layer are the best soil type for the growth of *Pinus radiata*. The soil pH generally is low in forest soils (Osman, 2013). It has been
reported that high concentrations of nitrogen exist in forest soils (Osman, 2013), however, available S and Olsen P status in forest soils are usually lower than under other land uses (Osman, 2013; Sparling & Schipper, 2002).

Szukics et al. (2010) found that microbial biomass was high in a forest soil (Austria), due to strong internal nutrient cycling. The microbial communities for nitrification and denitrification varied widely and their activities were rapid. Moreover, the AOA abundance was higher than AOB in these forest soils, which was consistent with some previous reports (Chen et al., 2008; He et al., 2007; Leininger et al., 2006).

Ying et al. (2010) studied ammonia–oxidising bacteria and archaea under different land uses including restoration plots growing various plants without human disturbance including pine tree in Hunan province, China. The results showed that N mineralization in the forest soil was higher than in some cultivated soils, such as pasture. AOB in the forest soil was also higher than in the cultivated soil. Less human disturbance in the forest soils was probably the main reason for the higher mineral N and AOB, compared with pasture (Carney et al., 2004; Compton & Boone, 2000).

Bruns et al. (1999) found that there was greater AOB richness in native soil than in cultivated soils. In addition, the changes in the AOB community compositions were positively correlated to the changes in N bioavailability under forest sites (Hynes & Germida, 2012). However, the study of Carney et al. (2004) found the opposite. They reported that the soils with human disturbance have a higher AOB diversity than in the plantation and forest plots. It is noteworthy that the human-managed soil plots examined by Carney et al. (2004) were low intensity and had less fertiliser application, which were different from another two studies with high intensive management and high nutrient inputs. Human management can have a significant influence on soil pH, temperature and soil characteristics. The change of these factors leads to the difference of AOB abundance under different land uses (Avrahami et al., 2003; Kowalchuk, et al., 2000; Webster et al., 2002).

Melillo et al. (2001) found that N₂O emissions in a Brazilian region increased at first when tropical forest was converted to pasture, and then the emissions decreased.

### 2.5.2 Dairy and sheep farms

Cattle and sheep grazing, especially under moist conditions, can damage soils. High stocking rates on dairy or sheep farms can reduce soil aeration and infiltration (Climo & Richardson,
1984; Singleton & Addison, 1999). High stocking rates reduce pore space and continuity which further reduces soil permeability (Drewry et al., 2000). Drewry and Paton (2000) noted that macroporosity decreased 70% in grazed soils compared with ungrazed soils in Southland, New Zealand. Air permeability and hydraulic conductivity also decreased in the grazed treatments. Mulholland and Fullen (1991) found that the bulk density on areas heavily trodden by cows was 21% higher than areas less trodden. The reduction of macroporosity was also observed on sheep-grazed plots (Climo & Richardson, 1984), but soil compaction on dairy farms generally is higher than on sheep farms (Drewry et al., 2000). Sparling and Schipper (2002) compared the soil properties in different land use in New Zealand and found that the bulk density and macroporosity of dairy and sheep farms were relatively low and macroporosity in dairy farming land was even lower than 10% (Figure 2.10).

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Figure 2.10 Box plots revealing medians and quartiles for soil physical properties (bulk density and macroporosity), arranged by different land use (Sparling & Schipper, 2002).

Chemical properties in dairy farming soils are also significantly different to other land uses. Most of New Zealand’s undeveloped soils are acidic with pH values lower than 5.5 (Parfitt et al., 1997; Schipper & Sparling, 2000). However, most agricultural soils have a pH higher than 5.5, due to the application of lime (Cornforth & Sinclair, 1982). Higher C and N contents also occur in the soils of dairy/ sheep farms compared with other land uses.

Di et al. (2010b) found that the abundance and activity of AOB rapidly increased in the topsoils collected from intensively grazed dairy pasture in New Zealand, especially the ones that received cow urine. However, AOA only grew in the soils without urine treatments. Therefore, they suggested that the difference of N conditions has a significant influence on the populations
and activities of AOB and AOA even under the same soil property. AOB prefer growing in high-fertility soils with high nitrogen load, but AOA grow well under low-fertility soil conditions (Erguder et al., 2009; Valentine, 2007). Offre et al. (2009) also reported high abundance of AOA and ammonia oxidation rate in a Scottish site without N fertiliser.

The majority of \( \text{N}_2\text{O} \) emissions in New Zealand’s agricultural system comes from animal excreta (de Klein & Ledgard, 2005). Di and Cameron (2000) found that the deposition in some urine patches is equal to 1000 kg N/ha. The reason of these high N concentrations is that dairy cows cannot completely metabolise the N they ingest and about 75% of the N is excreted (Reijnen, 2002). The pasture and soil microbes in dairy farming system cannot take up all the nitrogen from urine patches, so large amounts of nitrogen are lost through leaching, denitrification and \( \text{NH}_3 \) volatilization (Eckard et al., 2010). Most of the excreted N is in the form of urea and this is converted into \( \text{NH}_4^+ \) through hydrolysis (Di et al., 2010a). Then, the \( \text{NH}_4^+ \) is converted into \( \text{NO}_3^- \) during the process of nitrification. The large amount of \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) that accumulate in urine patches provide optimum conditions for \( \text{N}_2\text{O} \) emissions through denitrification and autotrophic nitrification (Sherlock, 1992). \( \text{N}_2\text{O} \) emissions increase on New Zealand’s dairy farms during autumn and winter, due to the rainy weather (de Klein et al., 2006). Soil WFPS in these farm lands become higher and this creates anaerobic conditions and denitrification.

The influence of sheep farming on the nitrogen cycle generally is less than dairy farming, because sheep urine patches contain less nitrogen than dairy patches (Di & Cameron, 2002). Nevertheless, sheep urine also contributes to the increase of \( \text{N}_2\text{O} \) emissions. Mahmood and Prosser (2006) studied grassland soil collected from the Sourhope Research Station located in the Borders region of Scotland. They added different concentrations of synthetic sheep urine as the treatment. During the period of incubation, they found that \( \text{NH}_4^+ \) concentrations immediately increased and reached a peak at the 7th day, then reduced gradually until the end of the experiment. The \( \text{NH}_4^+ \) concentrations were higher in the soil samples containing high concentrations of the urine than the ones containing lower concentrations. Soil \( \text{NO}_3^- \) concentrations only had a slight increase after a short lag period in the control treatment, however soil \( \text{NO}_3^- \) concentrations in the soils following high urine concentrations significantly increased. There was also an increase in pH after sheep urine treatment. The analysis of ammonia oxidising microbial communities in the soils with sheep urine, showed a general increase in microbial activities and the changes of microbial structures were related not only to ammonia concentrations, but also pH and \( \text{NO}_3^- \) concentrations. Variability was greatest during the lag phase, which produced ammonia, but this was not converted into nitrate.
2.6 Conclusions

Nitrous oxide emissions in soils mainly come from the process of nitrification and denitrification. Ammonia oxidation, as the first step of nitrification, provides the necessary substrate for denitrification and is a key driver of N₂O production. Ammonia oxidation is carried out by AOA and AOB. Generally, bacteria dominate the process of ammonia oxidation in high fertility soils (Di et al., 2009), but archaea are probably more important under low fertility and low pH conditions (Di et al., 2010b; Leininger et al., 2006).

The abundance and community structures of AOA and AOB are different under different soil conditions. Field capacity (-10kPa) is the ideal soil moisture content for the process of nitrification to occur. In drier soils, when field capacity is lower than -1500kPa (permanent wilting point), plants cannot survive and the nitrogen cycle cannot proceed. The wetter soils lead to the limitation of oxygen and also restrict nitrification (McLaren & Cameron, 1996). Nevertheless, AOA populations generally are much higher than AOB when O₂ concentrations are low (Santoro et al., 2008). The optimal pH for microbial activity is from 4.5 to 7.5 (McLaren & Cameron, 1996). In low pH environment, the populations of AOA are reported to be higher than AOB (Erguder et al., 2009; He et al., 2007). The optimal temperature is from 25°C to 30°C. The mineral N concentrations and nitrifying microbes also have a positive correlation. AOA are more abundant than AOB in low nutrients (Di et al., 2009, 2010b).

N₂O emissions are affected by complex soil properties, which are positively or negatively correlated with rates of both nitrification and denitrification. The main factors affecting N₂O emissions in soils are soil moisture, pH, temperature, organic matter content and mineral N concentrations. The rates of denitrification increase under moist conditions with limited O₂ concentrations. Thus, N₂O emissions increase with the occurrence of incomplete denitrification (Smith et al., 1998). However, under extremely wet soil conditions, where WFPS is over 70%, leads to the production of N₂O-reductase and complete denitrification converting N₂O into N₂, so N₂O emissions are reduced (Szukics et al., 2010; Thomson et al., 2012).

The optimal pH for N₂O emissions is between 5 and 7 (Rolston, 1981; Sherlock, 1992). N₂O emissions generally are higher under lower pH conditions (Weslien et al., 2009), due to the inhibition of N₂O-reductase in acidic soils (Fillery, 1983). N₂O emissions increase with an increase in the temperatures in soils and reach a peak at 15°C (Sherlock, 1992). Even though the rate of denitrification is low below 15 °C, incomplete denitrification predominates and more N₂O is produced at lower temperatures (Keeney et al., 1979). The trend is reversed above 17
°C. Soil moisture and available N are affected by temperature and play more important roles in nitrous oxide emissions at higher temperatures (Rafique et al., 2011). Both organic matter and mineral N provide the necessary substrate for microbial communities involved in nitrification and denitrification. Hence, there is a positive correlation between N₂O emissions and organic matter, as well as mineral N (Burford & Bremner, 1975; Fillery, 1983; Sherlock, 1992).

The specific properties of forest soils affect the populations of AOA and AOB and N₂O emissions. Forest soils are acidic in New Zealand. The macronutrient concentrations (e.g., N, P and S) in forest soils generally are lower than dairy or sheep farming soils. Low pH and low nutrients lead to higher AOA populations compared with AOB populations (Erguder et al., 2009). The N₂O emissions from forest soils are relatively low, due to low N contents.

However, farming changes soil properties in dairy and sheep farms. Soil pH increases with the application of lime (Cornforth & Sinclair, 1982) and animal grazing creates urine patches containing high N concentrations in these soils (de Klein et al., 2006). Therefore, N₂O emissions are thought to be higher in agricultural systems compared with forestry.

This review of the literature had identified significant gaps in our knowledge of ammonia oxidising microbial communities and relationships with N₂O emissions as affected by different land uses. Therefore the objectives of this thesis are to:

1. Determine the impact of three different land uses (pine tree plantation, dairy farm and sheep farm) in the same location under same soil type on the abundance of ammonia oxidisers (ammonia oxidising bacteria and ammonia oxidising archaea) (Chapter 4, project 1).
2. Determine the effect of urine application on AO community abundance in the three soils to understand the changes of ammonia oxidising abundance as the land use changes from pine tree or sheep farm to dairy farm (Chapter 5, project 2).
3. Determine the effect of urine application on N₂O emissions from soils from the three different land uses to understand the changes of N₂O emissions as the land use changes from pine tree or sheep farm to dairy farm (Chapter 5, project 2).
Chapter 3

Materials and methods

3.1 Project 1

3.1.1 Experimental plan

The goal of this experiment was to determine the effect of different land uses on soil properties and the composition and abundance of ammonia oxidising organisms (AO). Approximately 300 g samples of the Templeton silt loam were taken from random locations at three different land use areas. There were five replicates for each land use. Soil properties including pH, moisture, mineral N, and nutrient content were determined. The DNA concentrations and the abundance of AO populations were quantified by qPCR analysis.

3.1.2 Site selection

Soil samples were collected from three adjacent sites under three different land uses in the study area.

i. Pine trees
ii. Dairy farming
iii. Sheep farming

The soil type at all three sites was a Templeton silt loam (Typic Immature Pallic Soil). The three sites were located in close proximity to the northwest of Lincoln University about 20 km south of Christchurch, in Canterbury, in the South Island of New Zealand. The coordinates of the selected sites were as follows: dairy farm site (43°38'26.95"S, 172°26'37.85"E), sheep farm site (43°38'38.01"S, 172°27'25.36"E) and pine tree plantation (43°38'38.02"S, 172°27'29.01"E)
(Figure 3.1). The sheep farm site and the pine tree site has been under the respective land use for more than 30 years. The dairy farm was converted from sheep farming about 12 years ago.

![Figure 3.1 Locations of the three study sites.](image)

### 3.1.3 Soil sampling

The soil samples for project 1 were collected on 28th July 2014. Five soil samples were randomly collected from 0-10 cm depth from each location (Figure 3.2). Each sample consisted of approximately 10 soil cores randomly sampled and bulked together. Urine patches were avoided when collecting soil samples from the dairy and sheep sites. Soil samples from the tree area were collected in close proximity (3-5 meters) to the pine trees. The samples were placed into plastic bags and brought to the laboratory. The samples were then sieved through a 5mm sieve removing roots, stones and worms, and mixed well. Immediately after this subsamples were taken for molecular-biology analysis, while the rest of the soil samples were kept refrigerated (4-6°C) overnight, and the rest of the analysis was performed next day.
3.1.4 Extractable ammonium and nitrate

A 5 ± 0.05 g of soil was weighed into a 50 mL centrifuge tube and combined with 25 mL of 2M potassium chloride (KCl) solution. The samples were then shaken using a Ratek Platform Mixer for one hour and centrifuged at 4000 rpm for 10 min. Finally, the supernatant was filtered through 110 mm–diameter filter paper into 30 mL vials (Figure 3.3) and the collected filtrate frozen at -20°C until analysed by using a flow injector analyser (FIA) (FOSS FIAstar 5000 triple channel analyser) with SoFIA software version 1.30 (Foss Tecator AB, Sweden).
3.1.5 Soil pH

A 10 ± 0.05 g soil subsample was weighed into a 70 mL vial. Twenty five millilitres of deionised water were then added to the vial, stirred well and then left overnight. The pH of the solution was analysed by using a Mettler Toledo Seven Easy pH Meter (Mettler Toledo, Switzerland).

3.1.6 Soil moisture

Subsamples were randomly taken from the sieved soil samples to determine soil moisture. Approximately 10 g of soil was weighed in a paper cup, and dried at 105°C for 24 hours before reweighing (Figure. 3.4) (Blakemore, 1987). Soil moisture was calculated using the following equation:

\[
\text{Soil moisture (\%)} = \frac{\text{(wet soil (g)} - \text{dry soil (g)})}{\text{dry soil (g)}} \times 100
\]
3.1.7 AOA/AOB assays

3.1.7.1 DNA extraction

Soil genomic DNA was extracted using NucleSpin® Soil Kit (Macherey-Nagel, Düren, Germany) following the manufacturer’s instructions. Each 0.25 g soil subsample was put into a NucleSpin® bead tube. Then, 700 µL solution Buffer SL2 and 150 µL Enhancer SX were added, followed by homogenisation using MP Fast prep-24 (MP Biomedicals, USA) at 4.0 m/s speed for 1 min. The next step was to centrifuge the soil samples at 11,000 g for 2 min, add 150 µL Buffer SL3 and vortex for 5 sec to mix well. After 5 min incubation at 4°C, the samples were centrifuged for 1 min at 11,000 g. The supernatants were kept and transferred to a NucleSpin® Inhibitor Removal Column in a collection tube. The collection tubes were centrifuged for 1 min at 11,000 g and the flow-through was kept for further analysis. The flow-through was treated to adjust the binding conditions. 250 µL Buffer SB was added to the flow-through and vortexed for 5 sec again to mix well. A NucleoSpin Soil Column was placed in a new collection tube and 550 µL of sample was added to the column, then centrifuged 1 min at 11,000 g and the flow-through discarded. The remaining samples were treated by repeating the last step until no sample was left. In order to wash the silica membrane, 500 µL Buffer SB was loaded to the column, centrifuged for 30 sec at 11,000 g and the flow-through discarded. 550 µL Buffer SW1 was used instead of Buffer SB to repeat the last step. The next step was to vortex for 2 sec, centrifuge 30 sec at 11,000 g and discard the flow-through, after adding 700 µL Buffer SW2 to the column. This step was carried out twice. The column was dried by
centrifuging for 2 min at 11,000 g. To enable DNA elution, the column was put into a new collection tube and 100 µL of Buffer SE was added. The lids of the new tubes remained open during 1 min incubation at room temperature, then closed to centrifuge for 30 sec at 11,000 g. The eluted DNA sample was stored at -20°C before further analysis.

3.1.7.2 DNA Quantification

Quality and quantity of the extracted DNA were estimated by three methods according to the manufacturers’ instructions: (1) spectrophotometrically using Nanodrop®; (2) fluorometrically using Qubit® fluorometer (Invitrogen, NZ); and (3) by agarose gel electrophoresis.

3.1.7.3 Quantitative PCR (qPCR) analysis

The extracted DNA was diluted 10 times with deionised water by using a CAS-1200 Robotic liquid handling system (Corbett Life Science, Australia). Ammonia monooxygenase (amoA) gene abundance was quantified using primers Arch-amoAF/arch-amoAR and amoA-1F-Mod/amoAr-i for ammonia oxidising archaea (AOA) and ammonia oxidising bacteria (AOB) respectively (Hornek et al., 2006; Stephen et al., 1996). Twenty microliter reaction mixture was prepared for each sample using CAS-1200, containing 0.4 µL primer (final concentration 0.2 uM), 1.5 µL of 1:10 diluted template soil genomic DNA and 10 µL SYBR Premix Ex Taq (TaKaRa, Nori Biotech, Auckland, New Zealand).

The qPCR amplification conditions are described in details in Di et al. (2010b). A melting curve analysis was performed following amplification by continuously measuring the fluorescence during the temperature increase between 50°C and 99°C. The temperature profiles used for running the PCR were shown in Table 3.1. Data was then analysed using the Rotor Gene 6000 series software 1.7.

<p>| Table 3.1 PCR temperature profiles used for AOA and AOB (Di et al. 2009). |
|----------------|----------------|----------------|
|                 | AOA &amp; AOB      |
| First denaturing | 94 °C          | 2 minutes      |
| Denature         | 94 °C          | 20 seconds     |</p>
<table>
<thead>
<tr>
<th>Anneal</th>
<th>55 °C</th>
<th>30 seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extend</td>
<td>72 °C</td>
<td>30 seconds</td>
</tr>
</tbody>
</table>

### 3.1.8 Statistical analysis

Mean values and standard errors of the means (SEM) for mineral N concentrations, pH, moisture content and microbial community (AOA and AOA) abundance were calculated based on the five replicates for each land use, using Microsoft Excel 2010 (Microsoft Corporation, USA). P-values for checking the significant difference between different land uses’ mean values were calculated following one-way analysis of variance (ANOVA) using SigmaPlot 12.3 (Systat, San Jose, CA, USA). All the values were log-transformed except pH for the statistical analysis.
3.2 Project 2 - Incubation study

3.2.1 Experimental design

An incubation study was carried out to test the proposed hypotheses, that N₂O emissions and ammonia oxidising community growth in response to urine application will vary in the soils of different land uses.

Soils for the incubation experiment were collected from the same locations as described in section 3.1.2. Approximately 30 kg soil from each site was sieved, mixed and stored at 4°C. Before starting the incubation, the field capacity of each soil was determined as described in Section 3.2.2. Other soil properties were analysed at Analytical Research Laboratories Limited (ARL, Napier, NZ). There were three soils (pine tree plantation, dairy farm and sheep farm), two treatments (Control and Urine (700 kg urine-N/ha)) and each treatment had four replicates. For each replicate, there were two sets of samples, one set for subsampling and analysis of mineral N, and the other set for measuring N₂O emissions. Bulk density for each soil was determined and the soil in the jars and pottles was packed to the bulk density of 1.0 g/cm³. A 700 g soil sample was packed to half way in gas sampling jars for N₂O sampling and a 600 g soil was packed into soil sampling pottles for subsequent subsampling. The volume of air above the soil surface in each gas jar was about 350 mL. Each incubation jar or pottle was sealed with a lid with two ventilation holes of 1 cm diameter. Both sets of vessels were randomly placed inside an incubator set at 20°C. Over the duration of the experiment the soil moisture was maintained at field capacity by adjusting the weight of the vessels twice a week. Urine was collected from the Lincoln University Research Dairy Farm (LURDF), analysed for N concentration and the right volume was applied to the surface of gas sampling jars and pottles (Figure 3.5). The same volume of water was applied to the Control. The soils were incubated from 6th August 2014 to 12th February 2015.
3.2.2 Estimation of soil field capacity

Field capacity was defined by Veihmeyer and Hendrickson (1931) as “the amount of water held in the soil after the excess gravitational water has drained away and after the rate of downward movement of water has materially decreased.”

Each soil field capacity was determined as follows. A soil core was packed into a round container with a volume of 416 cm³ to a bulk density of 1.0 g/cm³. The soil cores were then placed in a water tray for water absorption for about 24 h. The saturated soil subsamples were transferred onto a tension table at 1m suction (-1 bar) to allow drainage of excess water for 4 or 5 days (i.e. until no water comes out from the suction) (Figure 3.6). The soils were weighed after drainage and placed into a 105°C oven for 48 h and weighed again to calculate the field capacity (FC) by the following formula:

\[ FC (%) = \frac{\text{wet soil (g)} - \text{dry soil (g)}}{\text{dry soil (g)}} \times 100 \]
Deionised water was added to incubation jars and pottles twice a week to maintain the samples at field capacity. Before soil sampling, the weight of the pottles were checked. Approximately 30g of soil was taken from the pottles for soil analysis, including mineral N, pH, moisture and the abundance of microbial communities at certain time intervals (day 1, day 7, day 15, day 30, day 60, day 90 and day 120). After each soil sampling, the pottles were reweighed. The addition of water next time for maintaining the soil samples at field capacity was based on the reweighed pottles.

### 3.2.3 Nitrous oxide measurement

For N₂O sampling the gas sampling jar lids were replaced with a gas sampling lid which had a three-way valve to allow gas sampling using a syringe (Figure 3.7). During a period of 30 minutes, two 20 mL headspace samples were collected into 6 mL vials at time 0 and 30 minutes. A preliminary study has shown a linear response of N₂O emissions over the sampling period, therefore a two sampling protocol was adopted. The gas samples were analysed using gas chromatography (SRO8610 linked to a Gilson 222XL autosampler) using an Electron Capture Detector (ECD) (SRI Instruments, USA) and quantified using stored ambient air samples. Elevated concentrations were achieved using ethylene and acetylene in stored air samples. Sampling was carried out twice a week for the duration of the trial.
For each sampling time, hourly N$_2$O emissions were calculated using the following equation:

$$
\text{N}_2\text{O flux} = \frac{(c_2 - c_1) \times HH \times P \times M_{\text{N}_2\text{O}}}{(t_2 - t_1) \times R \times T_K}
$$

N$_2$O flux = Hourly N$_2$O emissions (mg N$_2$O-N m$^{-2}$ hr$^{-1}$)

c$_1$ = N$_2$O concentration at $t_1$ (mg L$^{-1}$)

c$_2$ = N$_2$O concentration at $t_2$ (mg L$^{-1}$)

HH = Headspace height (m)

P = Atmosphere pressure (1 atm)

M$_{\text{N}_2\text{O}}$ = Molecular weight of N in N$_2$O (28 g mol$^{-1}$)

t$_1$ = Time 1st sample taken (0 hours)

t$_2$ = Time 2nd sample taken (0.5 hours)
\[ R = \text{Universal gas constant (0.082057 atm mol}^{-1}\text{ K}^{-1}) \]

\[ T_K = \text{Temperature (K)*} \]

* Temperature (K) = Temperature (the incubator set at 20°C) + 273.15

### 3.2.4 Changes in soil ammonium and nitrate concentrations

Soil samples were taken on day 1, day 7, day 15, day 30, day 60, day 90 and day 120. For each sampling date the bottles were removed from the incubator. About 25 g subsample was taken from each pottle from the surface right down to the bottom of the soil layer from one side of the container, using a soil corer. Each sample was uniformly mixed for physicochemical analyses, including extractable ammonium and nitrate (5 g), soil pH (10 g) and moisture (10 g).

A 5 ± 0.05 g sample was placed into a tube and extracted with KCl to determine changes in NH₄⁺ and NO₃⁻, as described in section 3.1.4.

### 3.2.5 Soil pH and moisture

At the time of soil sampling, soil pH and moisture content were also determined using the methods described in Section 3.1.5 (pH) and Section 3.1.6 (moisture).

### 3.2.6 AOA/AOB assays

Real-time qPCR was used to determine the abundance of AOA and AOB amoA gene as described in section 3.1.7.3. The time intervals of the soil sampling from the pottle were day 1, day 7, day 14, day 30, day 60, day 90 and day 120. DNA was extracted and either analysed immediately after sampling, or otherwise stored at -80°C as described in Section 3.1.7.1.

### 3.2.7 Statistical analysis

Mean values and standard errors of the means (SEM) for microbial community (AOA and AOA) abundance, mineral N concentrations, N₂O emissions and pH were calculated based on the four replicates for each land use, using Microsoft Excel 2010 (Microsoft Corporation, USA). P-values for checking the significant difference between control and urine treatment in each land use’s mean values were calculated following t-test analysis of variance (ANOVA) using SigmaPlot 12.3 (Systat, San Jose, CA, USA). All the values were log-transformed except pH for statistical analysis.
Chapter 4
The impact of three different land uses on ammonia oxidising community abundance

4.1 Introduction

Human activities have changed land-use patterns in New Zealand which in turn has affected a range of microbiological, chemical and physical soil properties. Between 1990 and 2008, the area of forest land increased by 586,600 hectares in New Zealand, whereas the area of grassland decreased by 484,500 hectares. Most of the new forest area is plantation forestry which has been established from grassland. Nevertheless, grassland was still the largest land use (dairy, sheep and cattle farms, etc.), accounting for approximately 54% in New Zealand’s total land use in 2008. Forestry is the second largest land use, accounting for over 37% of total land use (MfE, 2011).

Radiata pine (Pinus radiata), as the main forestry species planted in New Zealand, can tolerate the conditions of low soil pH condition (McLaren & Cameron, 1996; Sparling & Schipper, 2002) and most forest soils in New Zealand have a low pH. In contrast to forest soils, soils under dairy and sheep farming have a higher pH and are affected by fertiliser applications and animal excretion which result in higher C and N contents in soils of dairy and sheep farms compared with forestry. Sparling and Schipper (2002) found that organic N in dairy farming soils was the highest ranging from 0.18 to 380 μg N cm⁻³. The main reason was that cow urine is rich in nitrogen and, once deposited to soil, can lead to immobilisation the soil organic matter (de Klein et al., 2006). Soil pH increases after the establishment of these farms, due to the applications of lime (Cornforth & Sinclair, 1982).

The abundance and community structures of AOA and AOB are significantly influenced by soil properties with high populations of AOB generally found in soil with a high nitrogen content (Di et al., 2009). However, AOA are more abundant than AOB in low fertility soils (Di et al., 2009, 2010b). Therefore, as soil conditions change under different land use, ammonia oxidising communities might also change, affecting nitrogen cycling in different land use systems. However, there is a lack of information in the literature on how ammonia oxidising communities are affected by different land uses.
The objectives of this experiment were therefore to determine the influence of different land uses on 1) chemical soil properties, especially ammonia and nitrate concentrations and 2) ammonia oxidising bacteria (AOB) and archaea (AOA).

It was hypothesised that: 1) land use would significantly affect the abundance of AOB and AOA; and 2) AOB would be abundant in both dairy farm soil and sheep farm soil, whereas, AOA would be dominant in pine tree soil, due to lower N content.
4.2 Methods

4.2.1 Site location

Detailed descriptions of the materials and methods have been given in Chapter 3 (see section 3.1). A brief description is provided here for completeness.

This project was set up to determine the effect of different land uses on soil properties and ammonia oxidising communities.

The soil type at all three sites was a Templeton silt loam (Typic Immature Pallic Soil). The three sites were located in close proximity at the northwest of Lincoln University about 20 km south of Christchurch, in Canterbury, in the South Island of New Zealand: pine tree plantation (43°38'38.02"S, 172°27'29.01"E), dairy farm site (43°38'26.95"S, 172°26'37.85"E) and sheep farm site (43°38'38.01"S, 172°27'25.36"E) (Figure 4.1 A to C).

4.2.2 Soil sampling and analysis

The project was set up on 28th July 2014. Bulk soil samples were randomly taken from 0-10 cm depth from three adjacent sites under different land uses: 1) Pine trees 2) Dairy farming and 3) Sheep farming. Approximately 10 soil cores were taken from each site and bulked together to create a 300 g sample. Five such samples were collected as replicates in each site (Figure 4.1 D to E). Urine patches were avoided when collecting soil samples from the dairy site. Soil samples from the tree area were collected in close proximity (3-5 meters) to the pine trees. The samples were then placed into plastic bags and brought to the laboratory. The samples were then sieved through a 5 mm sieve removing roots, stones and worms. The sieved soil was mixed well and refrigerated (4-6°C) until analysis.
Soil properties from the different land uses were determined, including pH, moisture content, mineral N and nutrient content. The DNA concentrations and the abundance of AO- populations were tested by qPCR analysis.

The extractions of ammonia and nitrate were carried out with the addition of 25 mL of 2M potassium chloride (KCl) solution into a subsample of 5 ± 0.05 g of soil. The samples were then shaken using a Ratek Platform Mixer for one hour and centrifuged at 4000 rpm for 10 mins. Finally, the samples were filtered through 110 mm filter paper into 30 mL vials (Figure 3.3) and the collected filtrate frozen at -20°C until analysed by using a flow injector analyser (FIA) (FOSS FIAstar 5000 triple channel analyser) with SoFIA software version 1.30 (Foss Tecator AB, Sweden).

Soil pH was determined using the following method. A 10 ± 0.05 g soil subsample was weighed into a 70 mL vial. Twenty five millilitres of deionised water were then added to the vial, stirred
well and then left overnight. The pH was analysed using a Mettler Toledo Seven Easy pH Meter (Mettler Toledo, Switzerland).

Soil moisture was tested for each subsample. Approximately 10 g of soil was weighed in a paper cup, and dried at 105°C for 24 hours and reweighed (Blakemore, 1987). Soil moisture was calculated using the following equation: (Soil moisture (%) = (wet soil (g) - dry soil (g))/dry soil (g) x 100).

4.2.3 AOB and AOA assays

A subsample of 0.25 g was taken from each soil sample and DNA extracted using NucleSpin® Soil Kit (Macherey-Nagel, Düren, Germany). Quality and quantity of the extracted DNA were estimated by three methods according to the manufacturers’ instructions: (1) spectrophotometrically using Nanodrop®; (2) fluorometrically using Qubit® fluorometer (Invitrogen, NZ); and (3) using Enduro® agarose gel electrophoresis.

4.2.3.1 PCR analysis

Polymerase chain reaction’s (PCR) were set up by using a CAS-1200 Robotic liquid handling system (Corbett Life Science, Australia). The extracted DNA was diluted 10 times with deionised water. Ammonia monoxygenase (amoA) gene abundance was quantified using primers Arch-amoAF/arch-amoAR and amoA-1F-Mod amoAr-i for ammonia oxidising archaea (AOA) and ammonia oxidising bacteria (AOB) respectively (Hornek et al., 2006; Stephen et al., 1996). 20μL reaction mixture was prepared for each sample by using the CAS-1200, containing 0.4 μL primer (final concentration 0.2 uM), 1.5 μL of 1:10 diluted template soil genomic DNA and 10 μL SYBR Premix Ex Taq (TaKaRa, Nori Biotech, Auckland, New Zealand).

A Rotor-GeneTM 6000 (Corbett Life Science) was used for real-time PCR to amplify and produce a melting curve to measure the fluorescence continuously when the temperature was between 50°C and 99°C. The AOA and AOB amoA genes, and copy numbers were detected by standard curves of real-time PCR based on the study of Di et al. (2010b). All reagents used during the process were uncontaminated.

4.2.4 Statistical analysis

Mean values and standard error of the mean (SEM) for mineral N concentrations, pH, moisture content and microbial community (AOA and AOA) abundance were calculated based on the
five replicates for each land use, using Microsoft Excel 2010 (Microsoft Corporation, USA). One-way analysis of variance (ANOVA) was used to determine if the differences between the mean values were statistically significant. The statistical analysis was performed using SigmaPlot 12.3 (Systat, San Jose, CA, USA). All the values were log-transformed except pH prior to the statistical analysis.
4.3 Results

4.3.1 Soil ammonium concentrations

NH$_4^+$ concentration was low in all soils with concentrations of 2.29, 1.23 and 3.54 mg NH$_4$-N/kg in the pine tree, dairy and sheep farm soils, respectively. However, there were no significant differences between these values ($P > 0.05$) (Figure 4.2).

![Soil ammonium concentration](image)

Figure 4.2 Soil ammonium concentration for pine tree, dairy farming and sheep farming soils. Error bars indicate SEM.
4.3.2 Soil nitrate concentrations

The nitrate concentration was also low in all land uses (Figure 4.3). No NO₃⁻ was detected in pine tree soil, and there were no significant differences between dairy farm soil (4.89 mg NO₃-N/kg) and sheep farm soil (0.79 mg NO₃-N/kg) ($P > 0.05$).

![Figure 4.3 Soil nitrate concentration for pine tree, dairy farming and sheep farming soils. Error bars indicate SEM.](image-url)
4.3.3 Soil pH and moisture

All soils were acidic. The pH in the pine tree soil (pH = 5.3) was lower than the dairy (pH = 6.0) and sheep farm soils (pH = 6.4) (Figure 4.4).

Figure 4.4 Soil pH for pine tree, dairy farming and sheep farming soils. Error bars show standard error of the mean (SEM).
The moisture content of pine tree soil (17%) was less than the moisture content of the dairy (24%) and sheep (29%) farm soils. (Figure 4.5).

**Figure 4.5** Soil moisture for pine tree, dairy farming and sheep farming soils. Error bars show standard error of the mean (SEM).
4.3.4 Ammonia oxidising community abundance

AOB *amoA* gene abundance was significantly higher in the dairy pasture soil and the sheep pasture soil with $2.40 \times 10^7$ copy numbers/g dry soil and $1.44 \times 10^7$ copy numbers/g dry soil, respectively, compared with the pine tree soil which had $9.64 \times 10^5$ copy numbers/g dry soil ($P < 0.05$). There was no significant difference between AOB *amoA* gene abundance in the dairy and the sheep farm soils ($P > 0.05$) (Figure 4.6A).

The AOA *amoA* gene abundance in the sheep farm soil was $3.38 \times 10^7$ copy numbers/g dry soil and was significantly higher than that in the dairy farm soil and pine tree soil ($P < 0.05$). In addition, the AOA *amoA* gene abundance in dairy farm soil with $5.34 \times 10^6$ copy numbers/g dry soil was significantly higher than that in the pine tree soil with $2.44 \times 10^5$ copy numbers/g dry soil ($P < 0.001$) (Figure 4.6B).

Overall, the AOB *amoA* gene abundance was significantly higher than the AOA abundance in dairy farm soil ($P < 0.05$), but in the sheep farm soil, the AOA *amoA* gene abundance was significantly higher than the AOB abundance ($P < 0.05$). There was no statistically significant difference between AOA *amoA* gene abundance and AOB *amoA* gene abundance in pine tree soil ($P > 0.05$).
Figure 4.6 Average AOB (A) and AOA (B) amoA gene abundance (copy numbers/g of soil). Error bars indicate SEM. The different letters above the bars denoted significant difference and the same letters denoted no significant difference.
4.4 Discussion

The results showed that NH$_4^+$ and NO$_3^-$ concentrations were low in the soils of all land uses. The previous study from Di et al. (2010a) found that NH$_4^+$ accumulated in urine patches and NH$_4^+$ concentrations in dairy farming soils were high. However, urine patch areas were specifically excluded in the sampling protocol for the soil in this study. Soil NH$_4^+$ in the pine tree soil was similar to the dairy and sheep farm soils, but no NO$_3^-$ was detected in the pine tree soil. This was probably caused by the lower AO-populations in the pine tree soil resulting in lower nitrification rates. Low pH was probably also one of the impacting factors since de Boer and Kowalchuk (2001) have reported that nitrification rates were significantly reduced when the pH is lower than 5.5.

Both pH and moisture content in the pine tree soil were lower than in the dairy and sheep farming soils. This agrees with the results of Sparling and Schipper (2002), where pH and moisture content was low in pine tree soils. McLaren and Cameron (1996) explained that radiata pine (Pinus radiata), as the main tree species in New Zealand’s forest, can tolerate the low pH conditions. Lime would not have been applied in the pine tree area. However, lime would have been applied in dairy and sheep farming soils, which would have increased the soil pH in those areas (Cornforth & Sinclair, 1982).

Land use types (such as forestry, dairy farming and sheep farming) may have a significant impact on soil microbial communities. The results of AOA and AOB abundance analysis from this study support this hypothesis. The results show that dairy farming soil and sheep farming soil share higher microbial population abundance compared with the pine tree soils.

Higher AOB abundance in the dairy pasture soil and sheep pasture soil was most likely because of the continued nitrogen inputs in the form of nitrogen fertilisers and animal excreta returns. These N inputs stimulated the growth of AOB in the dairy pasture soil and sheep pasture soil. AOB prefer a high N environment and this is reflected in the higher total N concentrations in the dairy and sheep pasture soils (Appendix 1).

The higher AOA abundance than AOB abundance in the sheep pasture soil was probably because of lower N inputs. Sheep farming soils in New Zealand usually receive much less N fertiliser input compared with dairy farming soils. This is in agreement with previous findings of Di et al. (2009) and Di et al (2010a) who found AOB growth was favoured by high N environments, whereas AOA growth was favoured by low N environments.
However, the hypothesis of higher AOA abundance in pine tree compared with dairy and sheep pasture soil has to be rejected. The reasons for the higher AOA abundance in the dairy pasture soil and sheep pasture soil than in the pine tree soil ($P < 0.001$) requires further investigation. Previous studies by other researchers have shown that AOA growth was favoured in low pH environments (Di et al., 2010a; Erguder et al., 2009). The pH in the pine tree soil was lower than that in dairy pasture soil and sheep pasture soil, but AOA abundance was also lower in the pine tree soil than dairy and sheep pasture soil. Therefore, it is likely that other factors have also influenced the AOA abundance in the pine tree soil.

4.5 Conclusions

Results from this study clearly show that land use can have a major impact on soil properties such as pH and ammonia oxidising communities. The lower soil pH in the tree plantation compared with the dairy farm and sheep farm soils could be caused by the deposition of pine tree needles which would have acidified the pine tree soil and no application of lime in this land use. The finding of greater AOB amoA gene abundance in the dairy and sheep farming soils than in the pine tree soil also confirms the hypothesis that AOB growth is favoured by higher N inputs in the dairy and sheep farm soils. Similarly, the higher N inputs also resulted in higher AOB abundance than AOA abundance in the dairy pasture soil because AOB prefer higher N environment to grow whereas AOA prefer a low N environment. Therefore, the lower N inputs in the sheep farm soil led to its higher AOA than AOB abundance in this land use. However, the reasons for higher AOA abundance in the dairy farming soil and sheep farming soil than in the pine tree soil were not very clear and require further research. It is possible that other factors (other than N status and pH) also affected AOA growth in the pine tree soil.
Chapter 5
The effect of urine addition on ammonia oxidising populations and N$_2$O emissions – an incubation study

5.1 Introduction

Nitrous oxide emissions in soils are influenced by different anthropogenic land uses. Agricultural systems are a major source of greenhouse gases, especially N$_2$O. Nitrous oxide emissions represent approximately 14% of total GHG emissions in New Zealand. They largely come from agricultural areas which account for 97% of the total N$_2$O emissions (MfE, 2014a).

N$_2$O emissions generally come from the processes of nitrification and denitrification (Wrage et al., 2001). Much of the N$_2$O escapes as gas when nitrate is denitrified to nitrite, nitric oxide, nitrous oxide and di-nitrogen by denitrifying bacteria. The rates of these processes are affected by a complex interaction of soil properties, including soil moisture, pH, temperature, organic matter content and mineral N concentrations. The production of N$_2$O-reductase is a critical factor to N$_2$O emissions. N$_2$O-reductase is the enzyme that catalyses the reduction of nitrous oxide to dinitrogen. If N$_2$O-reductase is inhibited by soil properties, denitrification will not be completed, with N$_2$O being produced rather than N$_2$. However, in extremely wet soil environments, where the water-filled pore space (WFPS) is over 70%, N$_2$O-reductase is produced and complete denitrification occurs, i.e., converting N$_2$O into N$_2$, so N$_2$O emissions are reduced (Szukics et al., 2010; Thomson et al., 2012).

The optimal pH for N$_2$O emissions is between 5 and 7 (Rolston, 1981; Sherlock, 1992). N$_2$O emissions generally are high under low pH conditions (Weslien et al., 2009), due to the inhibition of N$_2$O-reductase in acidic soils. This leads to the production of N$_2$O instead of further converting to N$_2$ (Fillery, 1983). The optimal temperature for N$_2$O emissions is 15°C (Sherlock, 1992). Keeney et al. (1979) found that N$_2$O emissions increased with the increasing temperature up to 15°C, due to the predominant role of incomplete denitrification and only a small proportion of N$_2$O being converted to N$_2$. However, the N$_2$O emissions did not increase and remained constant when the temperature was above 17 °C (Rafique et al., 2011).
In addition, carbon and mineral N, encourage the production of N$_2$O, because the microbial communities involved in nitrification and denitrification require one or both of these for their growth and development (Burford & Bremner, 1975; Fillery, 1983; Sherlock, 1992).

High animal stocking rates, soil compaction, animal excretion (particularly urine) and the use of nitrogen fertilisers result in high N concentrations in the soils of intensively grazed pastures (de Klein et al., 2006; Di et al., 2009) and can further increase N$_2$O emissions. In general, when different farm systems are compared, the soils in dairy farms release more N$_2$O emissions than in sheep farms, due to greater urine loads excreted by cattle (Drewry et al., 2000). However, our understanding of the impact of different soil, environmental and land use practices is still limited, and this poor understanding is an obstacle for the development of management practices to mitigate N$_2$O emissions.

Therefore, the objectives of this project were to determine the influence of different land uses on: 1) N$_2$O emissions; 2) ammonia oxidising bacteria (AOB) and archaea (AOA); 3) ammonia and nitrate concentrations, and 4) the impact of the addition of animal urine on N$_2$O emissions and ammonia oxidisers in soils taken from three different ecosystems: pine tree soil, dairy pasture soil and sheep farm soil. It was hypothesised that: 1) nitrous oxide emissions would be significantly affected by land use and nitrogen input; 2) N$_2$O emissions would be higher in the dairy farm soil than in the sheep and forestry soils; and 3) application of urine would increase AOB, but not increase AOA population abundance.
5.2 Materials and Methods

5.2.1 Incubation trial set up

A detailed description of the materials and methods have been given in Chapter 3 (see section 3.2). A brief description is provided here for completeness.

The incubation study was set up to determine the impact of different land uses, and the addition of urine on ammonia oxidising populations and N\textsubscript{2}O emissions.

The soils were collected on 6\textsuperscript{th} August 2014 from three different sites, which are located in close proximity at the northwest of Lincoln University about 20 km south of Christchurch, in Canterbury, in the South Island of New Zealand: dairy farm site (43°38'26.95"S, 172°26'37.85"E ), sheep farm site (43°38'38.01"S, 172°27'25.36"E) and pine tree plantation (43°38'38.02"S, 172°27'29.01"E). The soils were sieved through a 5.0 mm sieve, mixed well and stored at 4˚C until trial set up. Soil chemical properties were analysed at Analytical Research Laboratories Limited (ARL, Napier, NZ). Soil moisture content was adjusted to field capacity. Then, a 700 g soil sample was packed in gas sampling jars for N\textsubscript{2}O collection and measurement. In addition, 600 g of soil was packed into soil sampling pottles for subsequent subsampling and analysis of mineral N. The soil in both types of vessels was packed to a bulk density of 1.0 g/cm\textsuperscript{3}. The incubation jars or pottles were sealed with lids with a breathing hole of 1 cm diameter (Figure 5.1). Both sets of samples were placed inside an incubator set at 20˚C for one week to equilibrate the microbial communities.

![Figure 5.1 Gas sampling jars with incubation lids (left), and soil sampling pottles (right).](image)

All treatments were applied to the soil surface in the incubation jars and pottles on 22\textsuperscript{th} September 2014. The urine was collected from dairy cows grazing at the Lincoln University
Research Dairy Farm (LURDF). There were two treatments for the study: Control and Urine (700 kg urine-N/ha) and each treatment had four replicates for each soil type.

Soil moisture was maintained at field capacity by adjusting the weight of the vessels twice a week. The trial period for the whole incubation study was about 4 months, from 24th September 2014 to 27th January 2015.

5.2.2 Determination of the field capacity

A soil core was packed into a round container with a volume of 416 cm$^3$ to a bulk density of 1.0 g/cm$^3$. The soil cores were then placed in a water bath for water absorption for about 24 h. The saturated soil cores were transferred onto a tension table at 1m suction (-1 bar) to allow drainage of excess water for 4 or 5 days (i.e. until no water came out from the suction). The soils were weighed after drainage and placed into a 105°C oven for 48 h and weighed again to calculate the field capacity (FC): FC (%) = (wet soil (g) - dry soil (g))/dry soil (g) *100). Based on field capacity estimates, soil moisture was maintained twice a week using deionised water.

5.2.3 Nitrous oxide sampling

Gas sampling was taken twice weekly from 24th September 2014 (Day 1) to 27th January 2015 (Day 126). For N$_2$O sampling the gas sampling jar lids were replaced with a gas sampling lid which contained a septum, three way tap and needle. During a period of 30 minutes, two samples were collected in 6 mL vials at time 0 and 30 minutes. Then, the gas samples were analysed using gas chromatography (SRO8610 linked to a Gilson 222XL autosampler) using an Electron Capture Detector (ECD) (SRI Instruments, USA) and quantified using stored ambient air samples. Elevated concentrations were achieved using ethylene and acetylene in stored air samples.

5.2.4 The analysis of soil properties

For each soil sampling date, the pottles were removed from the incubator Approximately 30 g of the soil was taken out from each pottle and mixed well. Subsamples were placed into four different containers to determine ammonia and nitrate concentrations, pH, moisture and ammonia oxidising communities (Figure 5.2).
Figure 5.2 Centrifuge tubes for extraction of mineral N (top left); the vials with pink lids for soil pH analysis (bottom left); paper cups for soil moisture analysis (top right) and the vials with white lids for ammonia oxidising community analysis (bottom right).

Mineral N was extracted for each of the 24 samples by weighing a subsample of 5 ± 0.05 g of soil into a 50 mL disposable centrifuge tube and combined with 25 mL of 2M potassium chloride (KCl) solution. The samples were then shaken using a Ratek Platform Mixer for one hour and centrifuged at 4000 rpm for 10 mins. Finally, the samples were filtered through a 110 mm filter paper into 30 mL vials and the collected filtrate frozen at -20°C until analysed by using a flow injector analyser (FIA) (FOSS FIAstar 5000 triple channel analyser) with SoFIA software version 1.30 (Foss Tecator AB, Sweden).

Soil pH was tested during the period of the incubation. A 10 ± 0.05 g soil subsample was weighed into a 70 mL vial. Twenty five millilitres of deionised water were then added to the vial, stirred well and then left overnight. The pH was analysed by using a Mettler Toledo Seven Easy pH Meter (Mettler Toledo, Switzerland).

The soil moisture content was measured in order to maintain the levels of soil moisture at field capacity. Approximately 10 g of soil was weighed in a paper cup, and dried at 105°C for 24
hours and reweighed (Blakemore, 1987). Soil moisture was calculated using the following equation: Soil moisture (%) = (wet soil (g) - dry soil (g))/dry soil (g) x 100.

5.2.5 AOB and AOA assays

Subsamples of soil were collected at Day 1, 7, 15, 30, 60, 90, 120 to determine ammonia mono-oxygenase (amoA) gene copy numbers of AOB and AOA. The soil samples were stored at -80°C before extraction.

The DNA extraction was carried out using NucleSpin® Soil Kit (Macherey-Nagel, Düren, Germany). The extracted DNA was detected and quantified by three methods: (1) spectrophotometrically using Nanodrop®; (2) fluorometrically using Qubit® fluorometer (Invitrogen, NZ); and (3) using agarose gel electrophoresis.

5.2.5.1 PCR analysis

Polymerase chain reaction (PCR) were set up by using a CAS-1200 Robotic liquid handling system (Corbett Life Science, Australia). The extracted DNA was diluted 10 times with deionised water. Ammonia monooxygenase (amoA) gene abundance was quantified using primers Arch-amoAF/arch-amoAR and amoA-1F-Mod/amoAr-i for ammonia oxidising archaea (AOA) and ammonia oxidising bacteria (AOB) respectively (Hornek et al., 2006; Stephen et al., 1996). A 20 µL reaction mixture was prepared for each sample by using the CAS-1200, containing 0.4 µL primer (final concentration 0.2 uM), 1.5 µL of 1:10 diluted template soil genomic DNA and 10 µL SYBR Premix Ex Taq (TaKaRa, Nori Biotech, Auckland, New Zealand).

A Rotor-GeneTM 6000 (Corbett Life Science) was used for real-time PCR to measure the fluorescence continuously when the temperature was between 50°C and 99°C. The AOA and AOB amoA genes, and copy numbers were detected by standard curves of real-time PCR based on the study of Di et al. (2010b).

5.2.6 Statistical analysis

Mean values and standard errors of the means (SEM) for microbial community (AOA and AOA) abundance, mineral N concentrations, N₂O emissions and pH were calculated from four replicates for each treatment using Microsoft Excel 2010 (Microsoft Corporation, USA). P-values for checking the significant difference between control and urine treatment in each land use’s mean values were calculated following t-test analysis of variance (ANOVA) using
SigmaPlot 12.3 (Systat, San Jose, CA, USA). All the values except pH were log-transformed for statistical analysis.
5.3 Results

5.3.1 Ammonia oxidising community abundance

5.3.1.1 Ammonia oxidising bacteria

The growth of AOB in pine tree soil following urine application was initially slow, but started to increase rapidly at day 15 and reach a peak at day 120 with a value of $7.13 \times 10^7$ copies/g dry soil (Figure 5.3). The AOB populations in dairy farming soil grew rapidly after urine application reaching a peak of $6.18 \times 10^7$ copies/g dry soil at day 60. The AOB population abundance in sheep farming soil also grew rapidly after urine treatment, but the growth rate was lower than that in dairy farming soil and reached a peak of $4.41 \times 10^7$ copies/g dry soil at day 60.

![Figure 5.3 AOB amoA gene abundance for the incubation study. Error bars indicate SEM.](image-url)
In the “control” treatment, AOB abundance in the dairy farming soil was higher than in the pine tree soil and sheep farming soil throughout the incubation period. The AOB abundance in the “sheep control” was higher than in the “pine tree control” (Figure 5.3).

The application of urine had a significant effect on AOB amoA gene abundance in all land uses compared to the controls. AOB abundance in urine-treated pine tree soil \((2.37 \times 10^7 \text{ copies/g dry soil})\) was almost 12 times higher \((P < 0.05)\) than the “tree control” treatment \((2.01 \times 10^6 \text{ copies/g dry soil})\) at day 30 and the difference between “tree urine” and “tree control” treatment became more significant after that date \((P < 0.05)\). AOB abundance in the urine-treated dairy farm soil increased significantly at day 7 with \(3.85 \times 10^7 \text{ copies/g dry soil}\), which was approximately 1.8 times higher \((P < 0.05)\) than the AOB abundance in the “dairy control” at day 7 with \(2.09 \times 10^7 \text{ copies/g dry soil}\). However, the AOB abundance returned to almost background levels and there was no significant difference between “dairy urine” and “dairy control” at day 120 \((P > 0.05)\). AOB abundance in the urine-treated sheep farm soil increased significantly \((P < 0.05)\) at day 15 with \(4.03 \times 10^7 \text{ copies/g dry soil}\) compared with the AOB abundance in the “sheep control” treatment \((1.30 \times 10^7 \text{ copies/g dry soil})\). The significant difference between the “sheep urine” and “sheep control” treatments remained until the end of the incubation trial.
5.3.1.2 Ammonia oxidising archaea

The AOA population abundance did not increase with urine treatment in pine tree soil and dairy farm soil (Figure 5.4). The AOA abundance in the sheep farm soil following urine treatment slightly decreased to $4.04 \times 10^6$ copies/g dry soil at day 30, but then subsequently increased to reach a peak of $1.24 \times 10^7$ copies/g dry soil at day 120.

Generally, there was no significant difference between control and urine treatments in all land uses. Only at day 120, the AOA abundance in urine-treated sheep farm soil with $1.24 \times 10^7$ copies/g dry soil was about 1.9 times higher than that in the “sheep control”.

The AOA abundance remained higher in the sheep pasture soil with or without urine addition than pine tree or dairy farm soils, and the AOA abundance in the dairy farm soil remained higher than in the pine tree soil.

![Figure 5.4 AOA amoA gene abundance for the incubation study. Error bars indicate SEM.](image-url)
5.3.2 Soil ammonium concentrations

The addition of urine had a significant effect on NH$_4^+$ concentrations (Figure 5.5). The dairy and sheep farming soils followed similar trend and had higher NH$_4^+$ concentrations than pine tree soil at the beginning. However, the NH$_4^+$ concentrations decreased rapidly in the dairy farming soil, with the NH$_4^+$ concentration decreasing from 671 mg NH$_4^+$-N/kg of dry soil at day 1 to 93 mg NH$_4^+$-N/kg of dry soil at day 120. The NH$_4^+$ concentrations in sheep farming soil had a small fluctuation following the urine treatment application and started a significant decline after 30 days, reducing from 609 mg NH$_4^+$-N/kg of dry soil at day 30 to 208 mg NH$_4^+$-N/kg of dry soil at day 120. The highest NH$_4^+$ concentrations in the pine tree soil was observed at day 7 with a peak of 698 mg NH$_4^+$-N/kg of dry soil, with the concentrations in pine tree soil decreasing after 15 days from 673 mg NH$_4^+$-N/kg of dry soil to 115 mg NH$_4^+$-N/kg of dry soil at day 120. When comparing the average reduction in NH$_4^+$ concentrations throughout the trial period, the dairy farming soil had the highest average reduction of 85% with the sheep farming and pine tree soil having average reduction of 68% and 58%, respectively.

Figure 5.5 Soil ammonium concentration. Error bars indicate SEM.
In the control treatments, there were no significant differences in ammonium concentrations between all land uses. $\text{NH}_4^+$ concentrations in all land uses were low and they remained stable during the whole period of incubation (Figure 5.5).

There were significant differences between urine and control treatments in all land uses throughout the whole incubation study ($p<0.05$), despite the decreasing trends in all the urine treatments.
5.3.3 Soil nitrate concentrations

In control treatments, all land uses showed a steadily increasing trend. The NO$_3^-$ concentrations in dairy farming soil was the highest over the incubation period. However, the highest NO$_3^-$ concentration in dairy farm soil was only 118 mg NO$_3$-N/kg of dry soil at day 120 (Figure 5.6). The addition of urine had a significant influence on NO$_3^-$ concentrations in all land uses. NO$_3^-$ concentrations in the dairy farm soil with urine treatment had the fastest increase from 26 mg NO$_3$-N/kg of dry soil at day 1 to 511 mg NO$_3$-N/kg of dry soil at the end of the trial. It was significantly higher (P< 0.001) than “dairy control” after day 7.

![Figure 5.6 Soil nitrate concentrations. Error bars indicate SEM.](image)

Nitrate concentrations in the pine tree soil with urine treatment had a significant increase (P<0.001) after 30 days, compared with “tree control”. The NO$_3^-$ concentrations in the urine treatment increased from 84 mg NO$_3$-N/kg of dry soil at day 30 to 414 mg NO$_3$-N/kg of dry soil at day 90.

The increasing trend for NO$_3^-$ concentrations in the sheep farming soil following urine treatment was slower compared with the dairy pasture soil and pine tree soil. However, NO$_3^-$
concentrations in the sheep farming soil still increased from 61 mg NO$_3$-N/kg of dry soil at day 15 to 295 mg NO$_3$-N/kg of dry soil at day 120 and was significantly higher (p<0.001) than that in the “sheep control” after day 15.
5.3.4 N₂O emissions

5.3.4.1 Daily N₂O emissions

Urine treatment, overall, had a significant impact on N₂O emissions (Figure 5.7). In the urine treatment, dairy and sheep farms followed similar N₂O emission patterns. In contrast, the N₂O emissions from the pine tree soil was different. When urine was applied on the 24th September 2014 (day 1), the N₂O emissions in dairy and sheep farming soils increased immediately, reaching a peak of 1.63 mg N₂O-N/m²/hr and 2.02 mg N₂O-N/m²/hr, respectively before rapidly decreasing to lower levels. However, the N₂O emission peak of the pine tree soil appeared 90-days after urine application. The N₂O emissions in the pine tree soil were low initially. After day 35, the pine tree soil emissions started a gradual increase and there was a significant difference (P < 0.05) between the “tree urine” (0.048 mg N₂O-N/m²/hr) and the “tree control” (0.002 mg N₂O-N/m²/hr) at day 35. The pine tree soil with urine treatment reached a peak at day 91 of 1.35 mg N₂O-N/m²/hr, and then decreased to 0.064 mg N₂O-N/m²/hr at day 126. In the control treatments, the N₂O flux in all land uses remained almost below 0.003 mg N₂O-N/m²/hr.

Figure 5.7 N₂O emissions for the incubation study. Error bars indicate SEM.
5.3.4.2 Total N₂O emissions

The total N₂O emissions in the urine-treated pine tree soil (5.338 kg N₂O-N/ha) were significantly higher (P<0.001) than those in the dairy farming (2.201 kg N₂O-N/ha) and sheep farming soils (0.992 kg N₂O-N/ha) (Figure 5.8). The control treatments, on average, only emitted small amounts of N₂O over the incubation period. The total N₂O emissions from the control dairy farming soil were the highest among the control treatments at 0.374 kg N₂O-N/ha.

With the addition of urine, the total N₂O emissions in all land uses significantly increased (P<0.05). The total N₂O emissions in the pine tree soils following urine treatment had the most significant increase by approximately 204 times, from 0.026 kg N₂O-N/ha in the control treatment to 5.338 kg N₂O-N/ha in the urine treatment.

![Figure 5.8 Total N₂O gas during the incubation period. Standard errors show SEM.](image-url)
5.3.5 Soil pH

With the addition of urine, soil pH increased significantly first, and then decreased in all land use types (Figure 5.9). The pH in dairy farming soil showed a decreasing trend from 7.45 (day 1) to 4.56 (day 120). However, 7 days following urine application, in both the pine tree and sheep farm soils, pH had a slight increase of 0.42 units and 0.14 units, respectively, and then decreased over time to 4.81 and 5.34, respectively at day 120. In the control treatments, soil pH did not fluctuate significantly throughout the trial.

![Figure 5.9 Soil pH after urine application (day 1). Error bars show standard error of the mean (SEM).](image-url)
5.4 Discussion

5.4.1 Ammonia oxidising bacteria

The results from this study showed that urine-N input had a major influence on ammonia oxidisers’ growth in all the different soils. Different land uses also had a major effect on the rate of ammonia oxidisers’ growth. This is in agreement with the hypothesis that application of urine will increase AOB abundance. The rapid growth of AOB in the dairy pasture soil following urine application was probably related to the initially high AOB abundance in this soil, resulting from high N inputs from nitrogen fertiliser and animal excreta returns in dairy production systems (Di & Cameron, 2002; Di et al., 2010b).

The initially slow growth of AOB in the pine tree soil following urine application was probably because of the extremely low initial AOB abundance in the soil. This finding is in agreement with previous studies which found AOB abundance in forest soils was relatively low, compared with AOA abundance (Chen et al., 2008; He et al., 2007; Leininger et al., 2006). However, the AOB growth in the urine-treated pine tree soil continued throughout the incubation period. This suggests that the AOB communities in the pine tree soil have the capacity to utilize the nitrogen from the urine and to grow after an initial period of exposure to the urine nitrogen. This is similar to the findings of Hynes and Germida (2012) that there was a positive correlation between AOB abundance and N bioavailability under forest sites. However, further research is required to improve our understanding of the factors involved in the growth pattern of AOB in the pine tree soil.

It is interesting that the AOB abundance in the pine tree soil was initially lower than the AOB abundance in the dairy pasture soil and sheep pasture soil. However, the AOB abundance in the pine tree soil exceeded the AOB abundance in the sheep pasture soil after half of the incubation period (after 60 days) (Figure 5.3). The intermediate AOB abundance in the urine-treated sheep pasture soil may reflect a history of lower N inputs in the sheep pasture soil compared with the dairy farming soil. These results suggest that AOB growth is not only affected by the amount of nitrogen applied during the experiment but also related to the past land use history.

5.4.2 Ammonia oxidising archaea

The minimum impact of urine application on AOA abundance in all three soils supports the original hypothesis that AOA abundance will not increase with urine treatment. This strongly
suggests that AOA growth is not related to the supply of available N in the form of animal urine (Figure 5.4). This is in agreement with the finding of Di et al. (2010b) who found AOA abundance did not increase in the soils with urine treatment. However, the AOA abundance was higher in the sheep pasture soil, with or without urine addition. The higher AOA abundance in the sheep pasture soil, with or without urine addition, is probably because of the lower soil N fertility status compared to the dairy pasture soil. This agrees with findings from previous studies that AOA growth was favoured by low soil N content (Erguder et al., 2009; Valentine, 2007). Offre et al. (2009) also reported that AOA abundance was high in the soils without N fertiliser.

The extremely low AOA abundance with or without urine in the pine tree soil is probably due to other factors which limited the growth of AOA in this soil. Further studies are required to understand the reasons for the extremely low AOA abundance in the pine tree soil.

**5.4.3 Ammonia and nitrate concentrations**

The rapid increase in the nitrate concentration in dairy pasture soil following urine application (Figure 5.6) corresponded well with the initial rapid AOB growth in this soil (Figure 5.3). This indicates that the rapid AOB growth in the dairy pasture soil following urine application resulted in high nitrification rates (Cabello et al., 2009). Di et al. (2009) also found that the increasing NO$_3^-$ concentrations in grassland soil treated with animal urine was strongly related with the AOB abundance.

The nitrate concentrations in the urine-treated dairy farm soil was the highest during the whole incubation period. This is in agreement with the finding of Drewry et al. (2000) who found that NO$_3^-$ concentrations in dairy farming soils were generally higher than in sheep farming soils; due to greater amounts of urine excreted by cows, compared with sheep.

Similarly the nitrate concentration increases in the sheep farming and pine tree soils also followed the AOB growth patterns in these two soils. These results suggest that AOB abundance is a determining factor in the nitrification rate of the urine-treated soils.

In general, NH$_4^+$ concentrations in all land uses with urine treatments decreased with time, whereas NO$_3^-$ concentrations initially increased. This reflects the conversion of NH$_4^+$ into NO$_3^-$ of the urine-N applied during the incubation period. The AOB promoted nitrification rates and the production of NO$_3^-$. 
5.4.4 Nitrous oxide emissions

The findings of this study support the hypothesis that N$_2$O emissions would be significantly affected by different land uses and high nitrogen inputs. However, the hypothesis regarding the higher N$_2$O emissions in dairy farm soil than sheep farm and pine tree soil has to be rejected. It is very interesting that despite the slow start, the N$_2$O emission was the highest from urine-treated pine tree soil compared with those in the corresponding treatments of the dairy and sheep pasture soils. The high N$_2$O emissions from the urine-treated pine tree soil was probably due to the relatively high amounts of available carbon in the pine tree soil compared with the sheep and dairy pasture soil (Appendix 1). Burford and Bremner (1975) stated that N$_2$O emissions would increase when water soluble organic C was higher. This is similar to Luo et al. (2010b) who also found high N$_2$O emissions in the soil with high carbon concentrations. These results have major implications for N$_2$O emissions when pine tree forests are cleared for dairy farming. It means that if pine tree forestry is converted to dairy farm, there is potential for huge increases in N$_2$O emissions due to animal urine nitrogen inputs.
5.5 Conclusions

Land use and urine-N input significantly affected the N$_2$O emissions and microbial community abundance from the Templeton silt loam soil used in this study. The application of urine had a significant effect on AOB amoA gene abundance in all land uses compared to the controls. With urine addition, AOB abundance in the dairy farm soil and sheep farm soil showed a similar trend with high peaks at day 60. The AOB abundance in dairy farm soil was higher than in sheep farm soil throughout the whole incubation study. The application of nitrogen fertiliser and animal excreta returns were probably the main reasons for rapid growth of AOB in the dairy and sheep farm soil (Di & Cameron, 2002; Di et al., 2010b). The AOB abundance in the pine tree soil was low initially, but increased continuously until the end of the study. The AOB population in pine tree soil probably needed a period of exposure to adapt to the urine nitrogen. However, the reason for the AOB abundance increase needs further study.

Unlike AOB abundance, AOA abundance was not affected by urine-N input. This supports the hypothesis that application of urine will increase AOB population abundance, but not AOA. The AOA abundance in sheep farm soil was significantly higher than in dairy farm soil and pine tree soil. Lower N fertility in sheep farm soil may have led to the higher AOA abundance compared with dairy farm soil (Erguder et al., 2009; Valentine, 2007).

N$_2$O emissions were significantly affected by the different land uses and by urine-N input. With the application of urine, N$_2$O emissions in dairy farm soil and sheep farm soil immediately reached a peak, then sharply decreased to close to the background level. However, N$_2$O emissions in the urine-treated pine tree soil had a delay before it reached a peak at day 91. It is worth mentioning that the total N$_2$O emissions in the pine tree soil following urine treatment were significantly higher (P<0.001) than the dairy farm and sheep farm soils (Figure 5.8). Therefore, the hypothesis that N$_2$O emissions will be higher in the dairy farm soil than in the sheep farm soil and pine tree soil must be rejected. The high N$_2$O emission in the pine tree soil following urine treatment is probably caused by large amounts of available carbon.
Chapter 6
General conclusions and directions for future research

6.1 General conclusions

6.1.1 N$_2$O emissions

In the incubation trial (Chapter 5), different land uses and urine-N inputs had a significant effect on nitrous oxide (N$_2$O) emissions from the Templeton silt loam soil. N$_2$O emissions in the urine-treated pine tree soil increased rapidly after day 52 and reached a peak at day 91. N$_2$O emissions in the urine-treated dairy farm and sheep farm soils reached a peak at the early stages of the trial and then quickly decreased to a low level, but they were still higher than in their respective controls. In the control treatments, N$_2$O emissions in these three land uses remained at a low level throughout the incubation period.

The total N$_2$O emissions from the pine tree soil following urine treatment was the highest of the three different land uses following urine-N inputs. Thus, the hypothesis that N$_2$O emissions will be higher in the dairy farm soil, compared with the sheep farm soil and pine tree soil, has to be rejected. The N$_2$O emissions in the urine-treated dairy farm soil was the second highest, followed by the emissions in the urine-treated sheep farm soil. The total N$_2$O emissions in urine treatments were significantly higher ($P < 0.05$), compared with control treatments in all land uses. The total N$_2$O emissions in the “pine tree urine” treatment were 204 times higher than the “pine tree control”. The higher N$_2$O emissions from the pine tree soil following urine treatment may be due to the higher concentrations of available carbon in pine tree soil compared with sheep and dairy farm soil (Appendix 1). N$_2$O emissions were higher when water soluble organic C was higher which is in agreement with Burford and Bremner (1975) and Luo et al. (2010b).

These results suggest that if forest land is converted to dairy farm, there would potentially be high N$_2$O emissions following urine deposition by the grazing animal. Therefore, the conversion from forestry to dairy farm would result in double negative impacts on climate change, the removal of carbon sink by the forest and increased N$_2$O emissions from animal urine.
6.1.2 Ammonia oxidising communities

In project 1 (Chapter 4), different land uses had a significant effect on ammonia oxidising bacteria (AOB) and ammonia oxidising archaea (AOA) amoA gene abundance, and so the hypothesis that land use significantly affects the AOB and AOA abundance can be accepted. However, AOA amoA gene abundance in the sheep farm and dairy farm soils was significantly higher than in the pine tree soil \((P < 0.05)\). The hypothesis that AOA abundance was higher in pine tree soil than in dairy and sheep farm soils must be rejected. Previous studies showed that AOA growth was favoured by low pH and low N environments (Di et al., 2009; Di et al., 2010a; Erguder et al., 2009). Despite lower pH and total N in the pine tree soil compared with the dairy and sheep farm soils, AOA abundance was still lower in the pine tree soil. Therefore, other factors may impact on AOA amoA gene abundance in the pine tree soil.

AOB amoA gene abundance was significantly higher in the dairy and sheep farm soils than in the pine tree soil \((P < 0.05)\). This supports the hypothesis that AOB will be more abundant in dairy and sheep farm soils than in pine tree soil. However, AOB amoA gene abundance in the dairy and sheep farm soil were not significantly different \((P > 0.05)\). AOB growth is favoured by high N environments, and the dairy and sheep farm soils received continued nitrogen inputs from nitrogen fertilisers and animal excreta returns (Di et al., 2009; Di et al., 2010a).

The comparison of AOB and AOA amoA gene abundance in each land use showed that AOB abundance was higher than AOA abundance in the dairy farm soil, but AOA abundance was higher than AOB abundance in the sheep farm soil. The reason probably was lower N inputs in sheep farm soil than in dairy farm soil. This is in agreement with Di et al. (2009) and Di et al. (2010a) who showed that AOB preferred a high N environment, but AOA preferred a low N environment.

In project 2 – the incubation trial (Chapter 5), the addition of urine increased AOB abundance, but not AOA abundance in all land uses, supporting the hypothesis that application of urine will increase AOB population, but not AOA population to be accepted. AOB abundance in the urine-treated pine tree soil rapidly increased after 15 days and the AOB growth continued until the end of the incubation study. The peaks of AOB abundance in the urine-treated dairy farm soil and sheep farm soil appeared at day 60 and the peak of AOB in the dairy farm soil was slightly higher than the sheep farm soil. In the “control” treatment, AOB abundance did not change in all land uses. The results of AOB abundance in all land uses with urine-N inputs were consistent with the N\(_2\)O emissions from different soils. Therefore, AOB communities probably plays a
significant role on N$_2$O emissions. The increase of AOB abundance in the urine-treated pine tree soil was delayed probably because the AOB population required a period of exposure to adapt to the high urine nitrogen environment or the AOB growth was initially inhibited by the high NH$_4^+$ concentrations. The rapid increase of AOB abundance in the urine-treated dairy and sheep farm soils was probably due to the adaptation of AOB communities to the high N environments in the dairy farm system and to a lesser extent, sheep farm system (compared with the pine tree system).

The AOA populations remained constant in all urine-treated land uses and there was no significant difference between control and urine treatments in all land uses. The only exception was AOA abundance in the urine-treated sheep farm soil which increased at day 120. The AOA abundance was higher in the sheep farm soil with or without urine application than the pine tree and dairy farm soils throughout the whole incubation study, and the AOA abundance in the dairy farm soil was higher than in the pine tree soil. Similarly, Di et al. (2010b) found that AOA abundance did not increase in the urine-treated soils.

The AOB abundance increased in all urine-treated land uses, but the AOA abundance remained unchanged. Therefore, these results again support the hypothesis that AOB growth is favoured by high N environments such as that in a urine patch soil, but AOA growth is not favoured by the high N environment in a urine patch soil.

6.1.3 Soil NO$_3^-$ and NH$_4^+$ concentrations

The urine application had a significant effect on NO$_3^-$ concentrations in all land uses. NO$_3^-$ concentrations in the urine-treated dairy farm soil had the fastest increase, followed by the NO$_3^-$ concentration in the urine-treated pine tree soil which also had a significant increase and reached a peak at day 90. NO$_3^-$ concentrations in the urine-treated sheep farm soil had a slower increase, compared with the pine tree and dairy farm soil. The increasing trend of nitrate concentrations in all land uses followed the AOB growth patterns. This would suggest that AOB growth is very important to the nitrification process in the urine-treated soils.

The NH$_4^+$ concentrations in all urine-treated land uses decreased rapidly. Despite a rapid increase from day 1 to day 7, NH$_4^+$ concentrations in the urine-treated pine tree soil still declined quickly after day 7. The increasing trend of NO$_3^-$ concentrations and decreasing trend of NH$_4^+$ concentrations in all land uses following urine treatments demonstrated the conversion of NH$_4^+$ into NO$_3^-$ during the process of nitrification.
6.2 Future research

In view of the results from the project 1 (Chapter 4), it is suggested that future research would analyse the factors that impact on AOA abundance in pine tree soils. It is hypothesized that AOA abundance is high in low pH and low N environments. The pH and nitrogen concentrations were lower in the pine tree soil, but the AOA abundance was still very low in this soil. Therefore, some other factors may affect the AOA abundance in the pine tree soil.

In the incubation study (Chapter 5), the reason for the increasing trend of N$_2$O emissions in the urine-treated pine tree soil requires further research. It is hypothesized that N$_2$O emissions will be higher in the urine-treated dairy farm soil than in the sheep and pine tree soils. However, the study showed that total N$_2$O emissions in the urine-treated pine tree soil was the highest, followed by the urine-treated dairy farm and sheep farm soils, and the reasons for this require further investigation. The available carbon in the pine tree soil may be an important deciding factor. Thus, soluble carbon concentrations in the soils should be measured during the period of incubation. The reason for the continuous increase of AOB abundance in the urine-treated pine tree soil above those in the dairy and sheep pasture soils also needs further investigation.

In future research, field trials should be set up to better understand the change of N$_2$O emissions and ammonia oxidising communities when land use is changed.
Appendices

Appendix 1: Soil characteristics

Table A 1.1 Soil characteristics of the different land uses used in the project 1 and project 2. Results from Analytical Research Laboratory.

<table>
<thead>
<tr>
<th>Analysis (Unit)</th>
<th>Pine tree</th>
<th>Dairy farm</th>
<th>Sheep farm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>5.3</td>
<td>6.1</td>
<td>6.2</td>
</tr>
<tr>
<td>Olsen P (µg/mL)</td>
<td>17</td>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td>Sulphate Sulphur (µg/g)</td>
<td>32</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>CEC (me/100g)</td>
<td>19</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Potassium (me/100g)</td>
<td>0.37</td>
<td>0.36</td>
<td>0.56</td>
</tr>
<tr>
<td>Calcium (me/100g)</td>
<td>5.9</td>
<td>9.7</td>
<td>8.1</td>
</tr>
<tr>
<td>Magnesium (me/100g)</td>
<td>3.29</td>
<td>1.69</td>
<td>0.77</td>
</tr>
<tr>
<td>Sodium (me/100g)</td>
<td>1.43</td>
<td>0.19</td>
<td>0.11</td>
</tr>
<tr>
<td>Organic matter (% W/W)</td>
<td>7.0</td>
<td>6.4</td>
<td>4.9</td>
</tr>
<tr>
<td>Total N (% W/W)</td>
<td>0.22</td>
<td>0.33</td>
<td>0.25</td>
</tr>
<tr>
<td>Total C (% W/W)</td>
<td>4.04</td>
<td>3.69</td>
<td>2.84</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>19</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>
References


Di, H. J., Cameron, K. C., Shen, J. P., Winefield, C. S., O'Callaghan, M., Bowatte, S., & He, J.


of eight new species of ammonia-oxidizing bacteria: *Nitrosomonas communis* sp. nov., *Nitrosomonas ureae* sp. nov., *Nitrosomonas aestuarii* sp. nov., *Nitrosomonas marina* sp. nov., *Nitrosomonas nitrosa* sp. nov., *Nitrosomonas eutropha* sp. nov., *Nitrosomonas oligotropha* sp. nov. and *Nitrosomonas halophila* sp. nov. *Journal of General Microbiology, 137*(7), 1689-1699.


Thomson, A. J., Giannopoulos, G., Pretty, J., Baggs, E. M., & Richardson, D. J. (2012). Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. Philosophical Transactions of the Royal Society B: Biological Sciences, 367(1593),


