Potential of manuka and kanuka for the mitigation of nitrous oxide emissions from NZ dairy farms

---

A dissertation
submitted in partial fulfilment
of the requirements for the Degree of
Bachelor of Science with Honours

at
Lincoln University
by
Roshean Fitzgerald

Lincoln University
2012

Supervisors:
Prof. Brett Robinson, Department of Soil and Physical Sciences, Lincoln University, New Zealand
Prof. Timothy Clough, Department of Soil and Physical Sciences, Lincoln University, New Zealand
Prof. Nicholas Dickinson, Department of Ecology, Lincoln University, New Zealand

Abstract of a dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Science with Honours.

Potential of manuka and kanuka for the mitigation of nitrous oxide emissions from NZ dairy farms

by

Roshean Fitzgerald

Nitrous oxide (N_2O) is a greenhouse gas with a global warming potential 298 times that of carbon dioxide. Since pre-industrial times, atmospheric levels of N_2O have increased from 270 to 319 ppb. More than a third of emissions are anthropogenic, most of which are due to agriculture. This loss of nitrogen as N_2O represents an important economic loss to the agricultural industry. N_2O is primarily produced in soils via the processes of nitrification and denitrification, both of which are biologically driven processes. It is possible that these processes may be perturbed by plants, such as the New Zealand natives: *Leptospermum scoparium* (manuka) and *Kunzea ericoides* (kanuka) that are known to contain antimicrobial compounds. Potentially, the strategic planting of manuka or kanuka on NZ dairy farms may reduce N_2O emissions. This study aimed to test whether manuka and kanuka affected soil microbes and specifically altered the production of N_2O.

A greenhouse pot trial was conducted using *Lolium perenne* (perennial ryegrass pasture), manuka and kanuka plants to test whether the latter two reduced the N_2O fluxes from the soil when ~200 kg N ha^{-1} dairy shed effluent was added as a nitrogen source. A closed-chamber method was developed using 20 L plastic buckets to enable collection of gas samples from the whole pot system. N_2O concentrations were determined using gas chromatography fitted with an electron capture detector. A preliminary trial indicated that manuka plants may reduce N_2O fluxes from soil. However, a full-scale trial did not produce measurable N_2O fluxes. This was attributed to the high volume to soil surface area (0.17 m^3:0.02 m^2) of the chambers used, meaning they provided insufficient sensitivity to determine the low N_2O fluxes.

Following the unsuccessful greenhouse study, a field trial was carried out using an already established method of field N_2O collection. Again a closed-chamber method was used, but, these chambers had a much lower volume to soil surface area ratio (0.26 m^3:0.18 m^2). N_2O emissions from soil beneath 5 year-old kanuka trees were compared with bare ground soil (control) treated with either water or dairy shed effluent. Soil samples were taken from adjacent plots which received the same treatments. For effluent-treated plots, N_2O fluxes were higher from the control compared with kanuka plots; cumulative fluxes were 65 and 13 mg N_2O-N m^{-2}, respectively. Soil nitrate levels were higher under kanuka than control plots, mean values were 17.1 and 3.3 µg g^{-1} soil, respectively. These findings may indicate an inhibition of denitrification beneath kanuka.

A further experiment demonstrated that 7 days after inoculation, soil *Escherichia coli* levels in manuka and kanuka pots (average 8 000 cfu g^{-1}) were reduced compared with pasture control pots (65 000 cfu g^{-1}).

These experiments demonstrate that manuka and kanuka affect the functioning and survival of soil microorganisms and reduce N_2O emissions under some conditions. Further research should focus on elucidating the mechanisms responsible for this inhibition, and test the range of environmental conditions where these plants may be used effectively.

Keywords: *Leptospermum scoparium*, *Kunzea ericoides*, *Lolium perenne*, N_2O, antimicrobial, nitrogen cycle, dairy shed effluent, *Escherichia coli*

Acknowledgements

Firstly I would like to acknowledge my supervisor Brett Robinson who has been very supportive throughout the study, for meeting with me on a regular basis to discuss results and progress, and for being so enthusiastic about the study. To my other supervisors: Timothy Clough, and Nicholas Dickinson thank you for all your help, guidance and advice along the way.

A huge thank you to Hannah Franklin for your help with sample collection and set up of both the nitrous oxide trials, I truely valued your advice and your friendly company, particularly out in the field. I appreciate your interest and enthusiasm for the study and thank you for always being there to bounce ideas off and have discussions.

I would like to thank the technicians who provided friendly and useful advice, and for analysis of samples: Manjula Premaratne, Qian Liang, Lynne Clucas, Leanne Hassall. Thank you Neil Smith for your technical expertise in setting up and providing me with a data logger as well as your help with the development of the nitrous oxide collection chambers for pot experiments. For their useful ideas which helped develop the collection chambers for the pot experiment, thank you Carole Barlow, and Stephen Moore . Rob Sherlock thank you for your ideas and guidance, they were much appreciated. I would like to thank the greenhouse staff Brent Richards and Leona Meachen for their help and for accomodating this experiment.

Thank you to the team at the Centre for Soil and Environmental Research (CSER), particularly Roger Atkinson, and Trevor Hendry for lending us equipment for the field trial, your friendly advice and for being so accomodating.

Thank you Jennifer Prosser, and Jacqui Horswell from Environmental Science and Research (ESR) for supplying advice, help and equipment for the antimicrobial experiment.

I would like to thank the New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC) and DairyNZ for providing scholarships for my tuition fees and living costs.

Lastly to thank you to my partner Darren, my friends, flatmates and family for being so supportive, helpful and enthusiastic about this study, I couldn’t have done it without you!

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

1.1 Nitrous oxide (N_2O)

Nitrous oxide (N_2O) is a GHG which has a global warming potential 298 times that of carbon dioxide (van Zwieten *et al.*, 2009). Since pre-industrial times, N_2O levels in the atmosphere have risen from 270 to 319 ppb (Figure 1.1) (IPCC, 2007a). More than a third of all N_2O emissions are anthropogenic and are primarily due to agriculture (IPCC, 2007a). In New Zealand, there has been a 25.5% increase in N_2O emissions from agricultural soils since 1990, caused by nitrogen fertiliser use and an increase in animal excreta (Ministry for the Environment, 2012). The loss of nitrogen in the form of N_2O represents an important economic loss to producers and consumers of agricultural products (van Zwieten *et al.*, 2009).

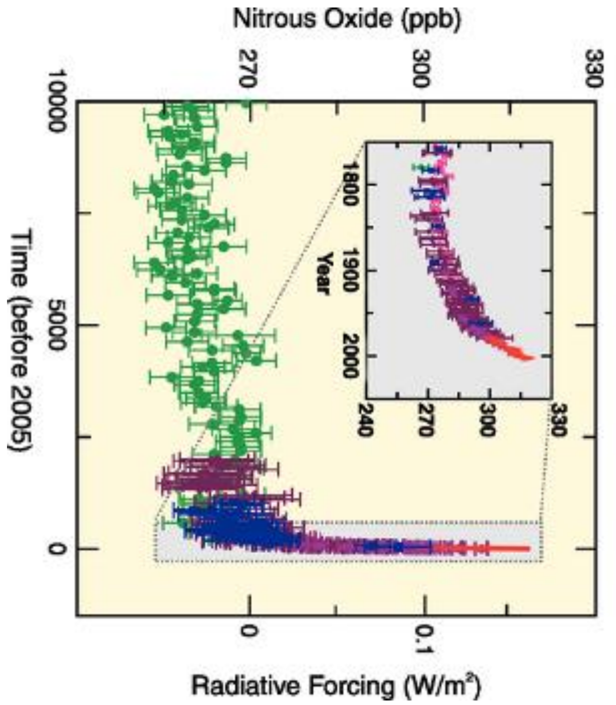

Figure 1.1 Atmospheric concentrations of N_2O over the last 10 000 years and since 1750 (inset) from ice cores and corresponding radiative forcings. From IPCC (2007a).

N_2O is produced in the soil by several processes that may occur simultaneously within different microsites of the same soil (Baggs, 2008). Most N_2O evolved from soils is produced by biological processes such as nitrification and denitrification, and little is produced by chemical processes such as chemodenitrification (Bremner, 1997; de Klein *et al.*, 2001).

Nitrification involves the oxidation of ammonia (NH_3) to nitrate (NO_3^-) which is carried out by autotrophic bacteria and archaea in aerobic conditions (DeLuca *et al.*, 2009). These include: ammonia-oxidising bacteria, autotrophic nitrite oxidising bacteria, ammonia oxidising archaea-bacteria, and heterotrophic nitrifying bacteria and fungi (de Klein *et al.*, 2008). However in low oxygen concentrations nitric oxide (NO) and N_2O can be formed as by-products of NH_4^+ oxidation (Kim & Hollocher, 1983).

Denitrification is the process by which NO_3^- or nitrite (NO_2^-) is reduced to dinitrogen gas (N_2), of which both NO and N_2O are obligate intermediates. This occurs in the absence of oxygen and is carried out by heterotrophic facultative anaerobes (which can use both O_2 and NO_2^- or NO_3^- as electron acceptor). The four enzymes involved are: nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (van Zwieten *et al.*, 2009; Uchida, 2010). Figure 1.4 illustrates soil processes resulting in losses of nitrogen (N) from agricultural systems. The loss pathways with negative environmental consequences are NO_3^- leaching and gaseous losses of N_2O.

1.2 Inhibition of the nitrogen cycle by plants

For many years it has been hypothesized that plants release compounds from their root systems that are capable of inhibiting the nitrification process. A bioassay experiment with exudates from a tropical pasture grass *Brachiaria humidicola* showed a strong inhibitory effect on *Nitrosomonas europaea* (a nitrifying bacteria) function and were shown to inhibit nitrification in air-dried and then rewetted soil. Results showed that the release of biological nitrification inhibition (BNI) compounds had no negative effect on soil microbial populations or plant growth promoting microorganisms, though effectiveness of BNI varied with soil type (Gopalakrishnan *et al.*, 2009). Sorghum (*Sorghum bicolor*) has been shown to have significant BNI capacity, the active constituent was identified as methyl 3-(4-hydroxyphenyl) propionate. BNI compound release from roots is a physiologically active process, stimulated by the presence of ammonium. This may be a useful tool for decreasing the emission of nitrogenous greenhouse gases such as N_2O from soil and reducing off-farm impacts associated with NO_3^- leaching (Zakir *et al.*, 2008).

1.2.1 Manuka and kanuka

Manuka (*Leptospermum scoparium* J. R. Forst. & G. Forst.) and kanuka (*Kunzea ericoides* (A. Rich.) Joy Thomps.) are native New Zealand shrubs that are members of the Myrtaceae family (Stephens *et al.*, 2005) (Figure 1.2). Manuka and kanuka establish rapidly and their success can be attributed to abundant flowering when the trees are young and the copious production of light seeds that are easily dispersed by wind. Manuka is the most abundant New Zealand shrub, and often forms closed stands up to 8 m tall. Kanuka often co-dominates with manuka in seral shrubland but can grow to 20 m tall (Wardle, 2002).

Figure 1.2 a) Manuka flowers, leaves and large fruit capsules. b) Kanuka foliage and flowers. From Wardle (2011).

Inhibition of the nitrogen cycle by manuka and kanuka

Currently there are no studies on nitrification inhibition caused by manuka or kanuka plants. However, inhibition by monoterpenes with a similar molecular structure to the known nitrification inhibitor: nitrapyrin was suggested as a factor in the extremely low nitrification rates observed in coastal redwood forests. One of these: α-pinene, has been found in manuka and kanuka essential oils (Table 2.2). α-pinene significantly inhibited the growth of *Nitrosomonas europaea* in batch culture experiments (Ward *et al.*, 1997). This nitrification inhibition demonstrated by one of the constituents of manuka and kanuka essential oils provides promising evidence to support the hypothesis that these plants may be natural nitrification inhibitors. The molecular structures of both nitrapyrin and α-pinene are shown in Figure 1.3. Clough *et al.* (2010) detected α-pinene in *Pinus radiata* biochar used in their experiments. This indicated that this compound remained after the biochar manufacturing process of pyrolysis. Thus biochar produced from manuka and kanuka could be mixed into agricultural soils on a larger scale to further reduce the effects of NO_3^- leaching and N_2O emissions, in addition to strategic plantings.

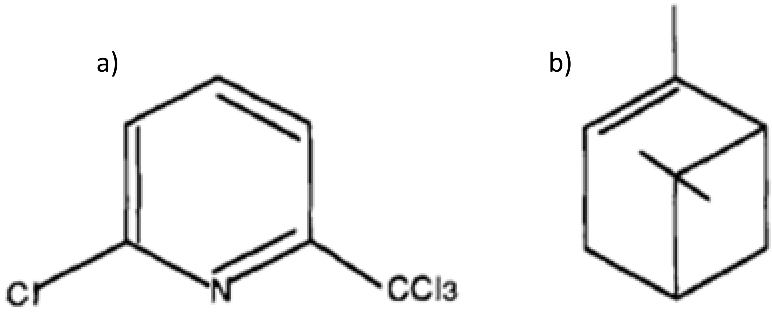

Figure 1.3 a) Structural formula of Nitrapyrin. b) Structural formula of α-pinene.

Ethanol extracts of *Eugenia caryophyllata,* another member of the Myrtaceae family, were found to inhibit nitrification (Haile *et al.*, 2006). Aqueous extracts of litter from another member of the Myrtaceae family: *Melaleuca leucadendron* were found to contain water-soluble compounds with antimicrobial influence on two nitrifying bacteria: *Nitrosomonas* and *Nitrobacter* (Boquel & Suavin, 1972). Table 1.1 summarises the potential benefits provided by the strategic planting of manuka or kanuka on dairy farms in terms of NO_3^- leaching and losses via N_2O emission.

Table 1.1 Summary of potential effects manuka and/or kanuka planted on dairy farms may have on the cycling of nitrogen.

Action	Effect on NO_3^- leaching	Effect on N_2O emissions
Inhibition of mineralisation	Decrease	Decrease
Inhibition of nitrification	Decrease	Decrease
Inhibition of denitrification	Increase	Decrease (or increased if N_2O reduction to N_2 is inhibited)
Increased transpiration	Decrease	Decrease
Decreased infiltration of rainfall due to canopy	Decrease	Decrease

Antimicrobial properties of manuka and kanuka

Since before European settlement in New Zealand, Maori used manuka and kanuka components (bark, leaves, sap, and seeds) for medicinal purposes (Brooker & Cooper, 1961; Stephens *et al.*, 2005). Currently, manuka oil and other manuka products (particularly honey) are sold widely throughout New Zealand as remedies for minor infections and ailments (Visavadia et al., 2008). Manuka extracts and honey products have been shown to have antimicrobial properties (Badet & Quero, 2011; Prosser, 2011).

Water extracts of manuka, were found to significantly reduce the growth of five pathogenic bacterial strains potentially found in biosolids (Prosser, 2011). These were *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli*, *Campylobacter jejuni* and *Clostridium perfringins*. However, when soil was taken from underneath a long-standing manuka forest, effects of the antimicrobial ability on the bacteria from biosolids were not observed. In an attempt to explain this the author suggested that the components that gave rise to the antimicrobial activity in manuka were quickly degraded in the soil; or that manuka plants released antimicrobial agents from roots continuously, which did not remain stable in the soil (Prosser, 2011).

In soils, much of the N cycle is biologically driven by soil bacteria. If the antimicrobial properties of manuka and kanuka are demonstrated in the field, then it is possible that these could have an inhibiting affect on the N cycle in the soil. Figure 1.4 illustrates the many potential sites at which manuka and kanuka could interfere with the N cycle due to inhibition or antimicrobial activity. For example, inhibition of nitrifying bacteria such as *Nitrosomonas, Nitrosospira,* or *Nitrobacter* would reduce the amount of nitrogen present as NO_3^- in the soil and thus reduce its potential to be leached but is also likely to reduce N_2O emissions by reducing the substrate of denitrification. The inhibition of mineralisation of organic N compounds to NH_4^+, which can be taken up by plants, would reduce the negative environmental effects of N, but at the same time less N would be available for plant uptake. Inhibition of denitrifying bacteria would likely reduce the amount of N_2O emitted though may also inhibit the conversion of N_2O to N_2 gas.

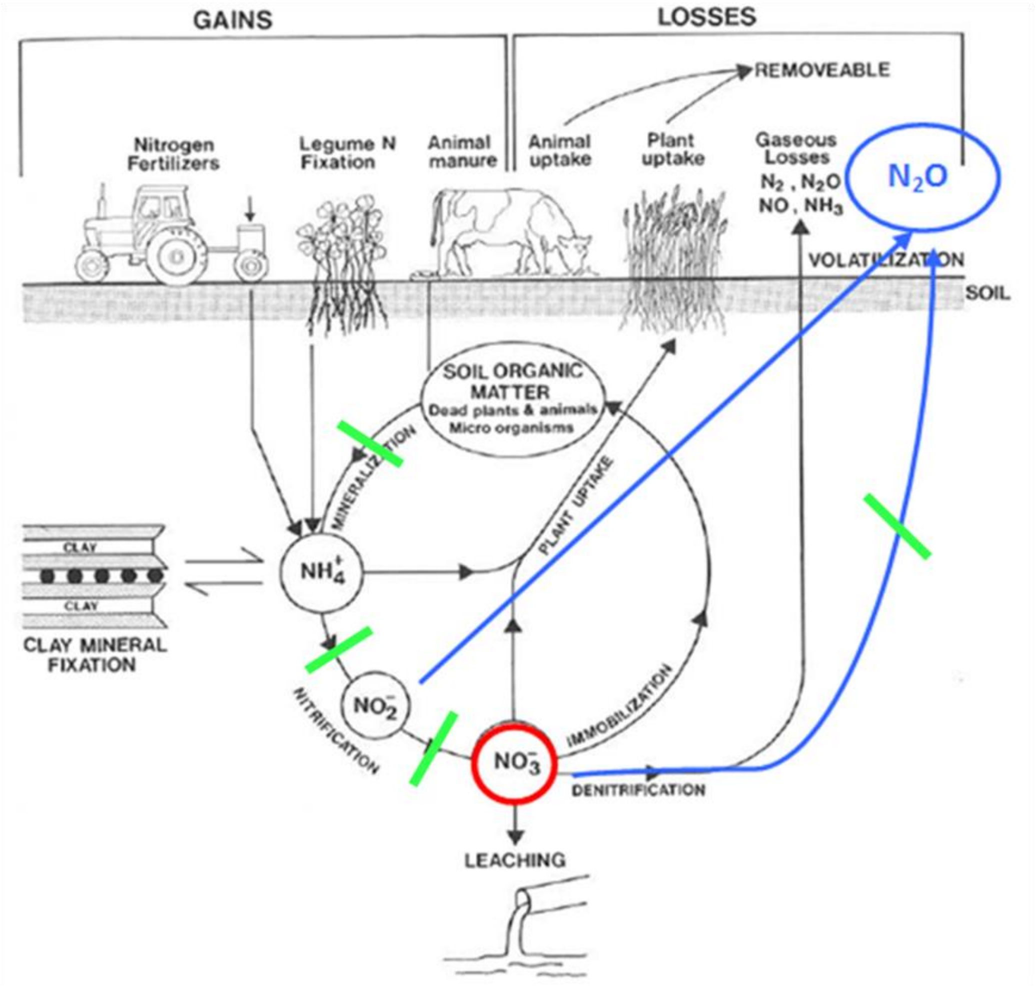

Figure 1.4 The nitrogen cycle in agricultural systems showing sources of nitrogen loss (blue and red) and potential sites of inhibition by manuka and kanuka (green). Adapted from McLaren & Cameron (1996).

Currently there are no studies which have investigated whether manuka or kanuka affect the nitrogen cycle. However, as manuka and kanuka are reported to contain compounds known to affect nitrification as well as antimicrobial compounds which could affect bacteria involved in the N cycle, it is hypothesised that these plants could affect N cycling and reduce N_2O emissions.

1.3 Aim

This study aimed to test whether manuka and kanuka affected soil microbes and specifically altered the production of N_2O.

Chapter 2

Background

Part A

2.1 Greenhouse gases (GHGs)

Greenhouse gases (GHGs) are atmospheric gases that absorb and re-emit long-wave radiation back to the earth's surface (Pinares-Patino *et al.*, 2009). These are critically important for the regulation of the earth's surface temperature, as without them, the average temperature would be -19 ^{o}C instead of the current 14 ^{o}C (IPCC, 2007b). 'Warming of the climate system is unequivocal, as is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice, and rising global average sea levels' (IPCC, 2007a). The IPCC states that 'most of the observed increase in global average temperatures since the mid-20th century is very likely due to the observed increase in anthropogenic GHG concentrations'. They go further to state that in fact, it is likely that increases in GHG concentrations alone would have caused more warming than what is currently observed because some warming has been offset by volcanic and anthropogenic aerosols (IPCC, 2007a). New Zealand is the only developed country where agricultural GHG emissions play a major role in its national emission profile (contributing 47.1% (Ministry for the Environment, 2012)). New Zealand has signed up for the Kyoto Protocol and has to take responsibility for its greenhouse gas emissions above 1990 levels. Thus in order to reduce future total GHG emissions there is a need to find ways to mitigate agricultural emissions.

2.2 Factors affecting N_2O fluxes

The regulation of N_2O emissions is very complex, and involves both proximal and distal factors. Proximal factors include: the rate of nitrification and denitrification, the ratio of the end products of denitrification and the diffusion of N_2O through the soil profile (de Klein *et al.*, 2008). These are affected by distal factors which include those of soil aeration and moisture status, and factors which affect denitrification such as mineral N (particularly NO_3^-), carbon availability, soil pH and temperature (Figure 2.1).

Soil aeration affects both the processes of nitrification and denitrification as well as the diffusion of N_2O through soil. It is a function of rainfall or irrigation, soil compaction and grazing management (de Klein *et al.*, 2008). Increased soil moisture conditions cause air-filled pores to become filled with water and denitrifiers switch from using O_2 to NO_3^- as a terminal electron acceptor (Sherlock, 1992). For denitrification, aerobic conditions severely decrease the activity of N_2O-reductase either because it is unstable or only partially functional, or because its formation is repressed (Bakken & Dorsch, 2007). Reduced soil aeration increases the N_2 to N_2O ratio of denitrification (Bolan *et al.*, 2004). This is likely to be because restricted N_2O diffusion through the soil profile increases the chance of N_2O reduction and its subsequent emission as N_2 from the soil surface (de Klein *et al.*, 2008).

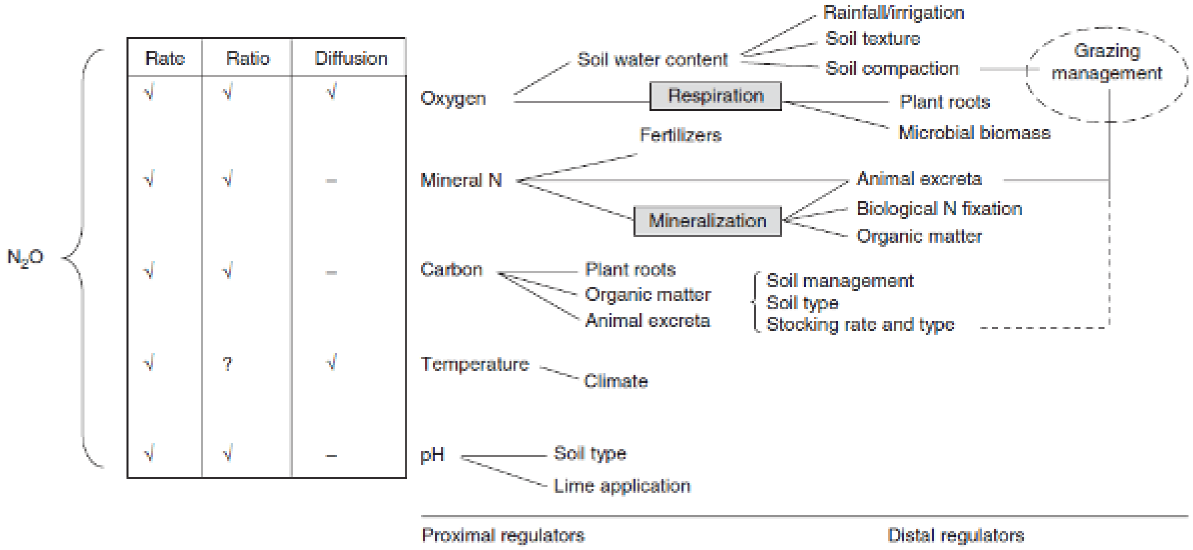

Figure 2.1 Schematic diagram of factors affecting: (i) the rate of nitrification and denitrification; (ii) the N_2/N_2O ratio of denitrification; and (iii) N_2O diffusion from soil (√, affected by this factor; –, not affected by this factor; ?, unclear if affected by this factor). Shaded boxes represent biological processes. From de Klein *et al.* (2008).

Nitrogen inputs from excreta and fertiliser regulate N_2O emissions in grazed pastoral soils (de Klein *et al.*, 2008). Following urine deposition, the N loading under a urine patch can be as high as 1000 kg N ha^{-1} (Di & Cameron, 2012). This exceeds the utilisation capacity of both plants and soil organisms and can cause high levels of NH_4^+ and NO_3^- to accumulate (Sherlock, 1992). In particular, NO_3^- concentration affects the rate of denitrification and can be described by a Michaelis-Menton mathematical function. The availability of NO_3^- for denitrifying bacteria is the first biological step in denitrification, this is dependent on: the rate of nitrification, the rate of N consumption by plants and bacteria, NO_3^- leaching and diffusion rates through the soil. NO_3^- concentration influences the N_2O:N_2 ratio of the gaseous products of nitrification by inhibiting the reduction of N_2O to N_2. Thus at low NO_3^- concentrations N_2 is predominant, whereas N_2O is predominant at high NO_3^- concentrations (Bolan *et al.*, 2004).

Carbon availability, or the supply of readily decomposable organic matter to soil is critical in controlling the rate of denitrification (Bolan *et al.*, 2004). A positive correlation between the water soluble carbon content of soils and denitrification rate has been shown (Sherlock, 1992). The surface layers of permanent pastures are rich in organic matter with potential for denitrification when fertilised or when urine and dung are deposited. Oxygen depletion can also result from high carbon (C) substrate and thus enhance denitrification. It has been suggested that decomposition of plant litter, animal faeces, and root exudates from the perennial plant cover maintain moderate to high levels of available C in grazed pasture soils for denitrification. The accessibility of C supply to bacteria regulates denitrification. Organic C decreases with depth, and often limits denitrification in subsoils. Limited supply of C is likely to cause partial denitrification resulting in the release of NO and N_2O intermediate gases. As C availability increases the N_2O:N_2 ratio decreases (Bolan *et al.*, 2004). Increased N_2O fluxes have been observed under peat soils compared with mineral soils and with the application of sewage sludge materials, containing high levels of water soluble carbon, to land (Sherlock, 1992).

Soil pH affects both the rate of nitrification and denitrification as well as the ratio of N_2O:N_2 produced by denitrification. Although the range for denitrification of is broad, neutral pH conditions (pH 6-8) are optimal for most denitrifying bacteria (Bolan *et al.*, 2004). In contrast to this, a study by Simek *et al.* (2002) found no simple relationship between denitrifying enzyme activity (DEA) and soil pH as high DEA was found both in acid and alkaline soils. They also found that the populations of soil denitfiers were adapted to their natural soil pH. In acidic conditions denitrification tends to slow down, but can still occur at pH values as low as 3.5. The mechanism for pH affect on denitrification is unclear. The proportion of N_2O increases as pH decreases and N_2O is often the dominant product of denitrification in acid soils. This has been suggested to be due to the presence of increasing amounts of NO_2^- at lower pH levels (Bolan *et al.*, 2004). A study by Clough *et al.* (2003) investigated whether liming could mitigate N_2O fluxes from soils amended with urine. It was found that the main effect of lime was to promote nitrification, which markedly affected N_2O fluxes with pH 6.1 (with 500 kg N ha^{-1} synthetic urine) producing more N_2O than any other pH. No optimum soil pH was found for the higher N treatment of 1000 kg N ha^{-1} and denitrification was not found to be enhanced. Sherlock (1992) described the optimal pH for nitrification and denitrification to be between 7.0 and 8.0. It was stated that while most of New Zealand's soils used for pastoral grazing have a pH less than optimal for denitrification, under these more acidic conditions it may be N_2O rather than N_2 which is the end-product for denitrification. Shortly after urine is deposited onto pasture, soil pH may increase to 9.0 or higher. At these high pHs, soil organic matter can become solubilised and thus more readily metabolisable by denitrifiers. This, combined with the high level of N is one of the reasons why urine patches are a major source of N_2O emissions.

Soil temperature affects denitrification through its effect on microbial activity which in general, increases with increasing temperature up to 30 °C (Sherlock, 1992; Bolan *et al.*, 2004). However, denitrification can occur at a wide range of soil temperatures between 0 and 75 °C. Indirect effects of temperature on denitrification include that of O_2 solubility and diffusion in water effects. Temperature is thought to cause temporal fluctuations in denitrification. The optimum temperature for denitrification could differ in different regions. Soil temperature can limit denitrification during winter. In New Zealand, a relatively high denitrification rate is often observed during winter where the soil temperature is below 10 °C, this is associated with high soil moisture contents. The N_2O:N_2 ratio produced by denitrification has been shown to decrease with soil temperature (Bolan *et al.*, 2004) particularly below 15 °C (Sherlock, 1992). Thus the maximum N_2O fluxes may not coincide seasonally with maximum denitrification rates if denitrification goes to completion. Under field conditions, N_2O release rates show a diurnal pattern which closely follows the fluctuations in soil temperature (Sherlock, 1992).

2.2.1 Sources of N_2O

Animal waste

Grazing ruminants utilize relatively little of the N in feed and 75-90% of their dietary N is recycled back into the system via urine and dung. The proportion of N excreted in urine increases as the N content of the diet increases (de Klein *et al.*, 2008). Ruminant urine is considered to be a major source of N_2O emissions in grazed pastoral soils, of which the main N component is urea ($CO(NH_2)_2$), this is rapidly hydrolysed to ammonium (NH_4^+) (Uchida, 2010). The emission factor (N_2O emitted as percentage of N voided) is generally greater for urine than for dung, with median values of 1.5% and 0.2% for cattle urine and dung respectively (de Klein *et al.*, 2008).

Effluent

Dairy farm effluent (DFE) comprises of animal faeces, urine, and wash-down water collected at the milking shed. This is collected daily during the milking season and sprayed onto grazed dairy pastures (Clough & Kelliher, 2005). The intensification of dairying has increased the production of dairy farm effluent. N_2O emissions from effluent application to land tend to be lower than from urine patches, as the N is applied more evenly to the soil, rather than in concentrated urine patches although N_2O emissions from soil manure applied to pastures were similar to those of urine patches (de Klein *et al.*, 2008). Cameron *et al.* (2002) found that when DFE was applied at 300 or 600 kg N ha^{-1} y^{-1} in spring, 8.4-12.2% was lost by denitrification. Dairy effluent has been positively correlated with N_2O emissions (Jezierska-Tys & Frqc, 2007). Similarly irrigation of effluent has been shown to increase N_2O

emissions, which were particularly high when effluent was applied to recently grazed wet soils (Luo *et al.*, 2008; Bhandral *et al.*, 2010).

Nitrogenous fertilisers

N_2O emissions also occur immediately following application of N fertiliser before this N is utilised by the plant/animal system. The emission factor for N fertiliser is affected by the rate, type and timing of N fertiliser applications, with N_2O emissions generally increasing exponentially with the amount of N applied. The type of fertiliser had a relatively small impact with estimated emission factors ranging from 0.8 to 1.1%, and the mean global emission factor for N fertiliser used at typical application rates calculated as 0.9% (de Klein *et al.*, 2008).

Indirect emissions

Indirect emissions from excreta and fertiliser N can occur through N that is lost from the system via NO_3^- leaching or runoff, NH_3 volatilisation and subsequently emitted from surface waters or following re-deposition of NH_3 to land (de Klein *et al.*, 2008). Other indirect emissions include those from human consumption of crops followed by municipal sewage treatment, and those emitted during some food processing operations. Atmospheric deposition of NO_x and NH_4^+ from NH_3 can fertilize soils and surface waters and thus enhance the production of N_2O (Mosier *et al.*, 1998). Oxidation of atmospheric NH_3 and subsequent reaction of the intermediate NH_2 radical with NO_2 can also lead to the indirect production of N_2O (Mosier *et al.*, 1998).

Cultivation

Large N_2O emissions can occur following cultivation of soils with high organic matter content (Histosols). Enhanced emissions due to cultivation are estimated at 2-15 kg N_2O-N ha^{-1} y^{-1} of cultivated Histosol and occur as a result of mineralisation of the N-rich organic matter. The rate at which mineralisation occurs relates to the quality of the N in the soil, the drainage status, management practices and climatic conditions (Mosier *et al.*, 1998).

2.2.2 N_2O methods of measurement from soil

N_2O gas sample collection

A brief review of N_2O literature showed the most common method of N_2O measurement to be the closed/static chamber method. This involves placing a sealed chamber over an area of soil for a defined period of time and taking samples of gas at various intervals. Chambers were made out of a variety of materials including PVC (Clough & Kelliher, 2005; Collins *et al.*, 2011), metal insulated with polystyrene foam (Di & Cameron, 2012), Perspex (Ambus *et al.*, 2007) and stainless steel (Li *et al.*, 2012). These varied in size and shape with some being circular (Smith *et al.*, 2008; Bhandral *et al.*, 2010; Luo *et al.*, 2010; Collins *et al.*, 2011; Di & Cameron, 2012) and others square (Kelly *et al.*, 2008; Li *et al.*, 2012). The headspace height ranged from 10 – 45 cm, but the most common height was around 10 – 15 cm (Ambus *et al.*, 2007; Collins *et al.*, 2011; Di & Cameron, 2012; Li *et al.*, 2012). Sampling intervals varied significantly with samples taken every 15, 20, 30, 40, 60, 120, and 180 minutes in some studies to determine N_2O fluxes, depending on the purpose of the study. Water troughs at the bottom of the chamber were commonly used to provide a gas tight seal (Luo *et al.*, 2010; Di & Cameron, 2012). Laboratory incubation studies were also common, where soil was placed into jars and gas tight lids were used to collect gas samples (Azam *et al.*, 2002; Wrage *et al.*, 2005; Jezierska-Tys & Frqc, 2007; Pereira *et al.*, 2010). Other studies used intact soil columns in the lab (Clough & Kelliher, 2005; Ambus *et al.*, 2007) or lysimeters out in the field (Luo *et al.*, 2010).

An alternative to the closed-chamber method was a continuous flowing method (Kelly *et al.*, 2008). The flow rate was 3 L of gas per minute and the automated chambers were linked directly to the gas measurement system (Fourier transform infrared spectrometer). Measurements were taken continuously from the air stream of each chamber for 30 minutes, with the chambers sampled sequentially for 24 h each day.

N_2O chemical analysis

In the majority of the studies, gas chromatography with an electron capture detector was used to determine the N_2O concentrations in the samples which were then converted into a flux (Azam *et al.*, 2002; Wrage *et al.*, 2005; Ambus *et al.*, 2007; Jezierska-Tys & Frqc, 2007; Smith *et al.*, 2008; Bhandral *et al.*, 2010; Collins *et al.*, 2011; Di & Cameron, 2012; Kammann *et al.*, 2012; Li *et al.*, 2012). Other methods included continuous flow mass spectrometry (Clough & Kelliher, 2005), a Fourier transform infrared spectrometer (Kelly *et al.*, 2008), and a trace gas analyser fitted internal filters (Pereira *et al.*, 2010).

Statistical analysis of N_2O data

Most commonly ANOVA was used with 5% LSDs (Clough & Kelliher, 2005; Wrage *et al.*, 2005; Zaman *et al.*, 2008; Bhandral *et al.*, 2010; Pereira *et al.*, 2010), and data were quite often log transformed prior to statistical analysis (Ambus *et al.*, 2007; Smith *et al.*, 2008; Luo *et al.*, 2010; Kammann *et al.*, 2012). Other methods of statistical analysis included general linear models (Zaman *et al.*, 2008), 2-

sample t-tests (Ambus *et al.*, 2007), Kruskal-Wallis test (Wrage *et al.*, 2005; Kammann *et al.*, 2012), Student Newman Keuls test (Kammann *et al.*, 2012), linear regression (Li *et al.*, 2012), independent sample t-test (Li *et al.*, 2012), correlation (Azam *et al.*, 2002; Jezierska-Tys & Frqc, 2007; Bhandral *et al.*, 2010), and least significant ratios (Luo *et al.*, 2010). Some studies stated that they checked the assumptions for ANOVA (Clough & Kelliher, 2005), or tested for normality and heterogeneity of variances (Wrage *et al.*, 2005; Collins *et al.*, 2011; Kammann *et al.*, 2012), while others provided no information on this. Studies often lacked information about their experimental design.

Part B

2.3 Native plants in NZ farm systems

Wide-scale removal of natural ecosystems has resulted from the introduction of agriculture into pristine environments. In New Zealand, remnants of the original vegetation are still common (Wilson, 1992). It is estimated that over the last 150 years more than 50% of New Zealand's land cover has been converted to grazing land (Nazra, 2011). New Zealand's 1997 State of the Environment report noted that 'Biodiversity decline is New Zealand's most pervasive environmental issue, with 85% of lowland forests and wetlands now gone, and at least 800 species and 200 subspecies of animals, fungi and plants considered threatened'. New Zealand dairy farmers receive little or no support for environmental initiatives and are dependent on milk payments for most of their income. Therefore dairy farmers prioritize production to the neglect of environmental care and conservation of native biodiversity (Jay, 2005). For example, in Canterbury, farms are particularly depauperate in native vegetation. Fire associated with Polynesian settlement resulted in the replacement of native forest by shrub and grassland. Following European settlement in the 1840s, a combination of fire and cultivation saw the majority of the plains converted to an agricultural landscape within 50 years. Today they are characterised by fields of cereal crops; ryegrass, clover pasture; windbreaks and plantations of coniferous and *Eucalyptus* species (Norton & Miller, 2000). The estimated area of the Canterbury Plains covered in native vegetation is less than 0.5% and native birds have almost completely gone due to habitat removal (Walls, 2009). Ecologists and conservationists aim to create a greenway – a network of patches and corridors for native wildlife which spans across the Canterbury Plains from the mountains to the sea (Walls, 2009).

2.3.1 Intensification of agriculture

Significant intensification of dairy farming from 1990 to 2011 is illustrated by an 80% increase in dairy cattle in New Zealand (from 3 440 815 to 6 175 061) (Statistics New Zealand, 2011). This combined with a 15% decrease in land area in grassland (1990 to 2010) (Statistics New Zealand, 2011), a 10% increase in milk solids (MS) production per cow and a 26% increase in MS per hectare, shows the extent of intensification which has been aided by the use of nitrogenous fertilisers and irrigation. Up until the 1990s, pasture production was dependent on the use of phosphorous fertilisers with some potash and N from fixation by legumes. The use of nitrogenous fertilisers has resulted in a diminished role of legumes for the supply of nitrogen. Intensive livestock production on grasslands has been the subject of considerable research to lower N_2O emissions (Pinares-Patino *et al.*, 2009). One of the main concerns with agricultural intensification in terms of N_2O emissions is the increased number of urine patches per hectare associated with a greater stocking rate. The New Zealand economy is reliant upon the dairy industry, as it represents around 25% of export earnings (Burke & Verkerk, 2010). So there is no question that dairy farming is a necessity in New Zealand. However, improvements to the industry could be made in terms of the environment to ensure that the land, lifestyles and clean green image of New Zealand and its people are maintained.

2.3.2 Native plants on NZ dairy farms

As part of the Emissions Trading Scheme (ETS), increased tree planting and more effective management of animal waste, have been suggested as ways in which GHG emissions can be reduced (Ministry for the Environment, 2011). Incorporation of native plants onto dairy farms or as riparian margins may help to meet these requirements.

Another benefit of having native vegetation on dairy farms is improved public perception. A study in the Catlins, Otago, showed farmers had a practical, utilitarian attitude towards remnants of natural ecosystems on farms. However, a growing appreciation of the aesthetic values of native forest on farms was noted amongst some of the landholders, and attitudes were slowly changing (Wilson, 1992). In the Waikato many dairy farmers regarded native forest favourably, although the proportion of farmers who actively conserved their forest was small (Jay, 2005).

There is a growing awareness of the importance of areas for nature conservation outside of protected natural areas. Historically, ecological values have rarely taken precedence over economic values. However protected natural areas alone cannot be relied upon to conserve all aspects of New Zealand biodiversity (Norton & Miller, 2000). Native plants strategically incorporated into New Zealand dairy farms would provide small habitats for native flora and fauna. These small habitat patches (1-6 ha) could perform useful ecological functions provided habitat quality was not diminished. Spatial connectivity could be achieved by spacing these habitat areas at approximately 5 km apart (Meurk & Hall, 2006). Norton and Miller (2000) stated that through integrated land management, it should be possible to sustain both a productive return from the land and the conservation of native biodiversity. This idea was reinforced by Meurk and Hall (2006) who suggested that this type of planting to improve biodiversity in modified landscapes would be relatively modest and compatible with land use and economic constraints in urban and rural landscapes.

2.4 Manuka and kanuka

Manuka is able to grow on a wider range of soils, and on generally less fertile soils than kanuka (Wardle, 2002). The two main ecological roles of manuka in New Zealand vegetation include: permanent dominance of extreme environments, and as a seral or pioneering species (Stephens *et al.*, 2005). The leaves of both species are unpalatable to grazing mammals (Wardle, 2002) so are not usually eaten by livestock. Manuka is non-poisonous to humans (Sykes, 2002) and no toxicity information in relation to livestock was found. Two species of *Kunzea* are recognised in New Zealand and both are endemic. *K. ericoides* is widely distributed and occurs either as a shrub or tree depending on the site. *K. sinclairii* is restricted to Three Kings and Great Barrier Islands where it grows as a prostrate or straggling shrub, often forming extensive thickets (Wardle, 2011). *K. ericoides* is the species discussed here.

2.4.1 Antimicrobial compounds in manuka and kanuka

Many compounds have been identified to be present in manuka and kanuka products and essential oils. Some of these have been found to exhibit antimicrobial activity and include: triketones, and monoterpenes.

Triketones

Triketone-enriched oils are commercially valuable because of their antibiotic activity (Douglas *et al.*, 2001), and thus the economic value of sources of such oils is clear. Manuka essential oils contain β-triketones and variances in composition have been noted depending on the age of the plant (Maddocks-Jennings *et al.*, 2005). Naturally occurring β-triketones from manuka include isoleptospermone and other triketones from plants of the Myrtaceae family include flavesone, leptospermone and grandiflorone (van Klink *et al.*, 1999). Christoph *et al.* (1999) found oil characterising triketones, namely leptospermone, flavesone, and isoleptospermone amounted to about 20% of the composition of manuka oil. The antimicrobial activity of manuka oil has been associated with a fraction containing three major and three trace triketones by Porter and Wilkins (1998).

Leptospermone

Leptospermone is an antibacterial β-triketone (Weston *et al.*, 2000) (Figure 2.2). It is insoluble in water and present in the leaves, twigs and seeds of manuka (Weston *et al.*, 2000; Jeong *et al.*, 2009). Jeong *et al.* (2009) found that leptospermone strongly inhibited the growth of harmful intestinal bacteria: *Clostridium difficile* and *Clostridium perfringens* at 1.0 mg disc^{-1} and moderately inhibited their growth at 0.5 mg $disc^{-1}$. However it did not inhibit the growth of other, beneficial intestinal bacteria tested (Jeong *et al.*, 2009). Levels of leptospermone found in manuka essential oils range from 14% to 16% (v/v) (Porter & Wilkins, 1998; Schnitzler *et al.*, 2008).

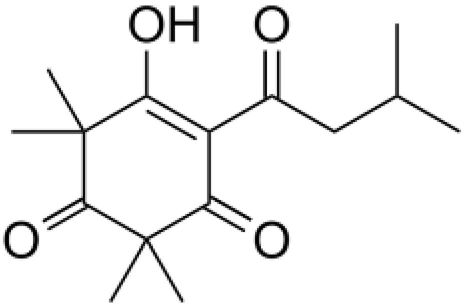

Figure 2.2 Chemical structure of leptospermone (3,5-hydroxy-4-(2-methyl-1-oxopentyl)-2,2,6,6,-tetramethyl-4-cyclohexane-1,3-dione) (Maddocks-Jennings *et al.*, 2005).

Monoterpenes

Monoterpenes have been found in manuka and kanuka essential oils. The amount of monoterpenes such as α-pinene, β-pinene and myrcene increased from less than 1% in young trees to 17-34% in trees that were 3 years older (Maddocks-Jennings *et al.*, 2005) (Table 2.1). In manuka essential oils, monoterpene hydrocarbon levels were found to be in the range of 2.2% to 14.1% (Christoph *et al.*, 1999). Kanuka oil had high levels of α-pinene (55.5%) compared with manuka oil (1.3%) which had low levels of monoterpenes in a study by Porter and Wilkins (1998) (Table 2.2). Similarly, Schnitzler *et al.* (2008) found α-pinene levels of 71% and 1% (v/v) for kanuka and manuka essential oils respectively. At a dilution of 0.001%, α-pinene was found to demonstrate a maximum non-cytotoxic concentration.

Table 2.1 Relative (%) comparison of essential oils from manuka (*Leptospermum scoparium*) and kanuka *(Kunzea ericoides*). From Maddocks-Jennings *et al.* (2005).

	L. scoparium **(North Island)**	***L. scoparium*** **(South Island)**	***K. ericoides*** **(North Island)**	***K. ericoides*** **(South Island)**
α-pinene	1.3	6.3	61.6	72.4
β-pinene	0.5	0.4	0	0.7
Myrcene	0	1.7	-	0.3
p-Cymene	0	0.7	2-5	2.9
Other monoterpenes	33	2.4	<3	<8
Sesquiterpenes	10.5-34	70	<2	7
1,8-Cineole	0	<7	6	5.1
Globulol	0	0	0	0
Terpinen-4-ol	0	0	0	0
Viridiflorol	Trace	0	3.2	?
Geraniol	0	7.2	0	0
Linalool	?Trace	6-20	0	1.4
α-Terpineol	?	0-1.3	1.1	0
Caryophyllene	<3	8.3	0	0
Humulene	?Trace	5.5	0	0
Eudesamol isomers	0	10.11	0	0
Leptospermone	10-20	0	0	0
Iso-leptospermone	2-7	0	0	0
Calamenene	>9	1.5	0	1.1

Table 2.2 GC-MS identification of % peak area contributions of components of commercial essential oils of manuka (*Leptospermum* scoparium) and kanuka *(Kunzea ericoides*). From Porter and Wilkins (1998).

Component	Manuka oil	Kanuka oil
α-pinene (%)	1.3	55.5
β-pinene (%)	0.1	0.6
Myrcene (%)	0.2	0.1
Isoleptospermone	4.6	
Leptospermone	15.54	

2.4.2 Manuka and kanuka potential effect on N_2O emissions

As the majority of N_2O is produced biologically by soil bacteria, and manuka has demonstrable antimicrobial properties a testable hypothesis is that manuka may inhibit the nitrification or denitrification processes in soils by killing off/inhibiting the growth of the bacteria involved in these processes (Figure 2.3). If this is the case, this would add another benefit to encourage the strategic plantings of manuka and kanuka onto New Zealand dairy farms. In the future farmers could be able to claim this in terms of the emissions trading scheme (ETS).

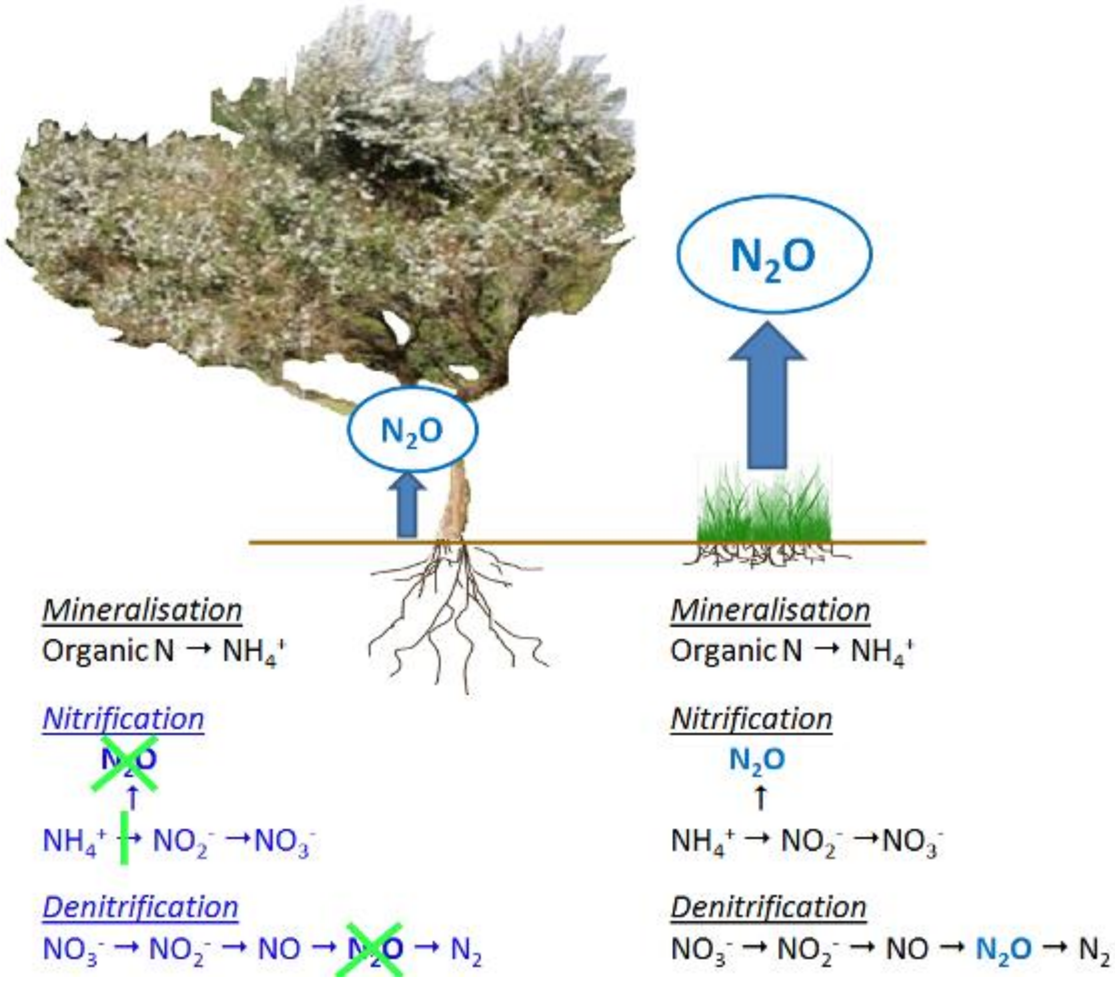

Figure 2.3 Nitrous oxide emissions and potential inhibition by the planting of manuka and kanuka, compared with pasture.

2.5 Potential further benefits of manuka and kanuka on farms

If antimicrobial effects are found in the field, potential benefits of strategically incorporating manuka or kanuka onto dairy farms could include: reduced N_2O emissions, and reduced nitrate leaching through nitrification inhibition. Other benefits could include: increased uptake of N, increased removal of water, reduced intensity of rainfall, carbon sequestration, and the production of manuka honey which sells at a premium. For a more detailed description of these see Appendix A.

Chapter 3

Nitrous Oxide Greenhouse Experiment

3.1 Introduction

This Chapter describes a proof-of-concept experiment set up in a greenhouse at Lincoln University. In greenhouse experiments, variability can be reduced that may occur in the field (e.g. soil, climate, pest, heterogeneity, weeds etc.). However, experimental methodology for N_2O measurement has not been well developed for greenhouse pot trials. Thus a large portion of this Chapter consists of method development and testing.

3.2 Aim

The aim of this experiment was to develop a method to accurately determine the N_2O fluxes from soil in pots planted with manuka or kanuka seedlings as well as pasture.

3.3 Methods

3.3.1 Development of method for collection of N_2O samples from pots

A closed-chamber system of N_2O measurement was used because it allows smaller fluxes to be measured due to the gases emitted by the soil not being continually diluted with external air and less time is required to enrich an air sample beyond the minimum detectable concentration. Hutchinson and Mosier (1981) stated that soil cover methods offer the most useful approach to field measurement of N_2O emissions from soil as they can measure smaller fluxes, avoid the potential error associated with sub-ambient pressure, and minimizes disturbances of the study site that might alter its N_2O emission rate. Similar circumstances are required for pot experiments in the greenhouse which is why this method was chosen for the current experiment.

Collection chambers were set up to measure the N_2O production from the entire pot system. This involved the use of 20 L high density polyethylene (HDPE) buckets inverted and placed in a tray containing ~15 mm deep water to provide an airtight seal. Individual water trays were used for each chamber to avoid cross-contamination and each had a saucer glued to it for the pot to sit without it getting wet. Two holes were made in the top of the chamber, one with a rubber bung, the other a rubber septum through which the sampling needle apparatus was inserted. The sampling needle apparatus consisted of a Baxter three-way large bore stopcock with rotating male luer lock adaptor, a 1.6 by 40 mm BD Microlance$^{\text{TM}}$ (white), and a 0.8 by 0.38 mm BD PrecisionGlide$^{\text{TM}}$ (green) needle joined with a male luer couple (Figure 3.1).

Figure 3.1 Gas collection chamber setup for nitrous oxide measurements (left). Gas collection valve and needles in top of chamber (right).

Samples were collected by placing a 6 mL Exetainer® (Labco Limited, UK) with a septum in the lid onto the green needle. A syringe attached to a Baxter three-way large bore stopcock with rotating male luer lock adaptor and green needle was also inserted into the septum of the Exetainer®. A 20 mL sample of gas was drawn out of the chamber and discarded to flush the ambient air out of the Exetainer®. Next 20 mL was drawn out and pumped 3 times to mix before the 20 mL sample was drawn out, the valve to the chamber was closed and 15 mL of the sample was pumped into the Exetainer®.

3.3.2 Measurement of N_2O

Immediately prior to analysis, gas samples were brought to ambient atmospheric pressure. Nitrous oxide concentration was determined using gas chromatography (GC) (SRI 8610 gas chromatograph; SRI Instruments, CA, USA) fitted with a ^{63}Ni electron capture detector (ECD) (Figure 3.2) and linked to an autosampler (Gilson 222 XL; Gilson Inc., WI, USA). PeakSimple (SRI Instruments, CA, USA) was the software used to control and monitor the ECD.

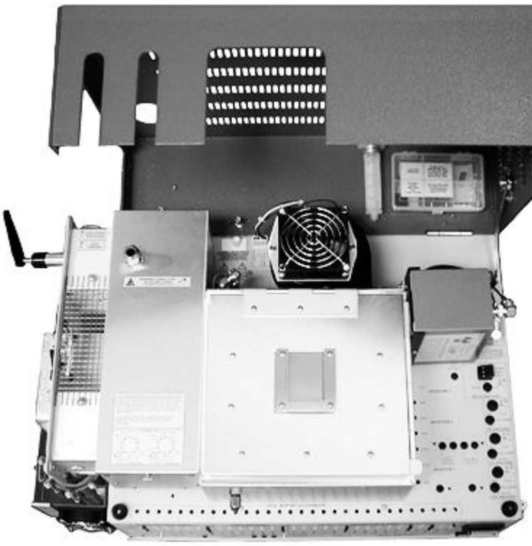

Figure 3.2 The hardware associated with the SRI 8610 Gas Chromatograph.

3.3.3 Method testing and results

To test the reliability of the N_2O collection vessels, a 2 µL L^{-1} standard of N_2O was injected into three chambers. See Appendix B for calculation used to make up standards. Samples were collected 15 minutes and 30 minutes after injection of the standard. No difference ($F_{1,4}$=0.36;*P*=0.582) in N_2O concentration was shown between these two times (2.2 µL L^{-1}) (Figure 3.3).

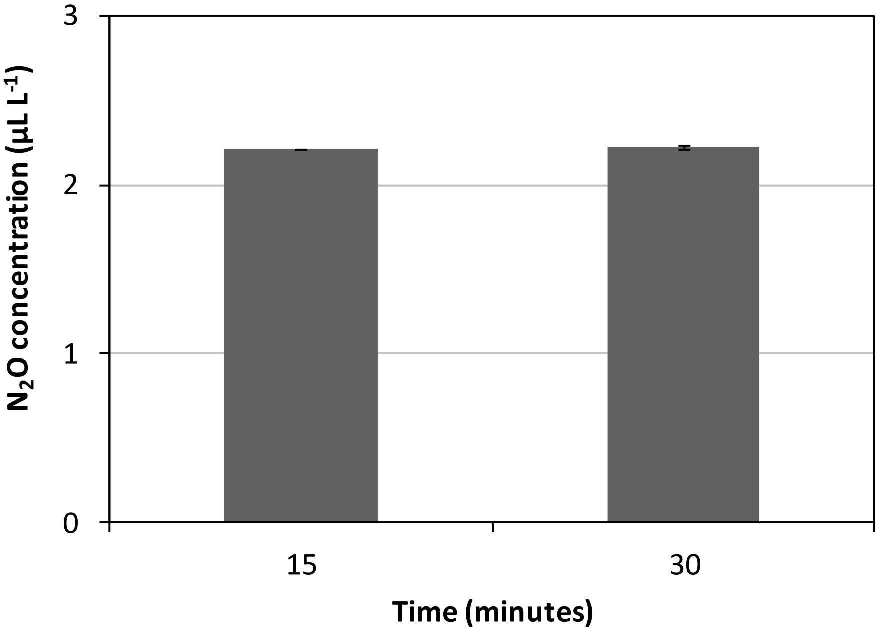

Figure 3.3 Method testing measurements taken 15 and 30 minutes following the injection of 2 µL L^{-1} of N_2O standard into the collection chamber. Error bars are standard error of the mean (n = 3).

Method testing to examine the samples collected 15, 30, 60, 120, 180, 240, and 300 minutes after injection of N_2O standard to 1 µL L^{-1} N_2O showed consistency. No difference between samples taken at each time was found ($F_{6,35}$=0.81;*P*=0.572) and the average N_2O concentration was 2.77 µL L^{-1} (Figure 3.4). Figure 3.5 shows the results of method testing after injection of N_2O standard to make 0.5 µL L^{-1} N_2O compared with blanks of ambient air injected into the chamber, 15, 30 and 60 minutes following injection. Average N_2O concentrations were 0.39 and 0.88 µL L^{-1} for the blank and the 0.5 µL L^{-1} N_2O injected standard, respectively. Again, no difference between N_2O concentrations at each time was observed ($F_{2,11}$=2.22;*P*=0.155).

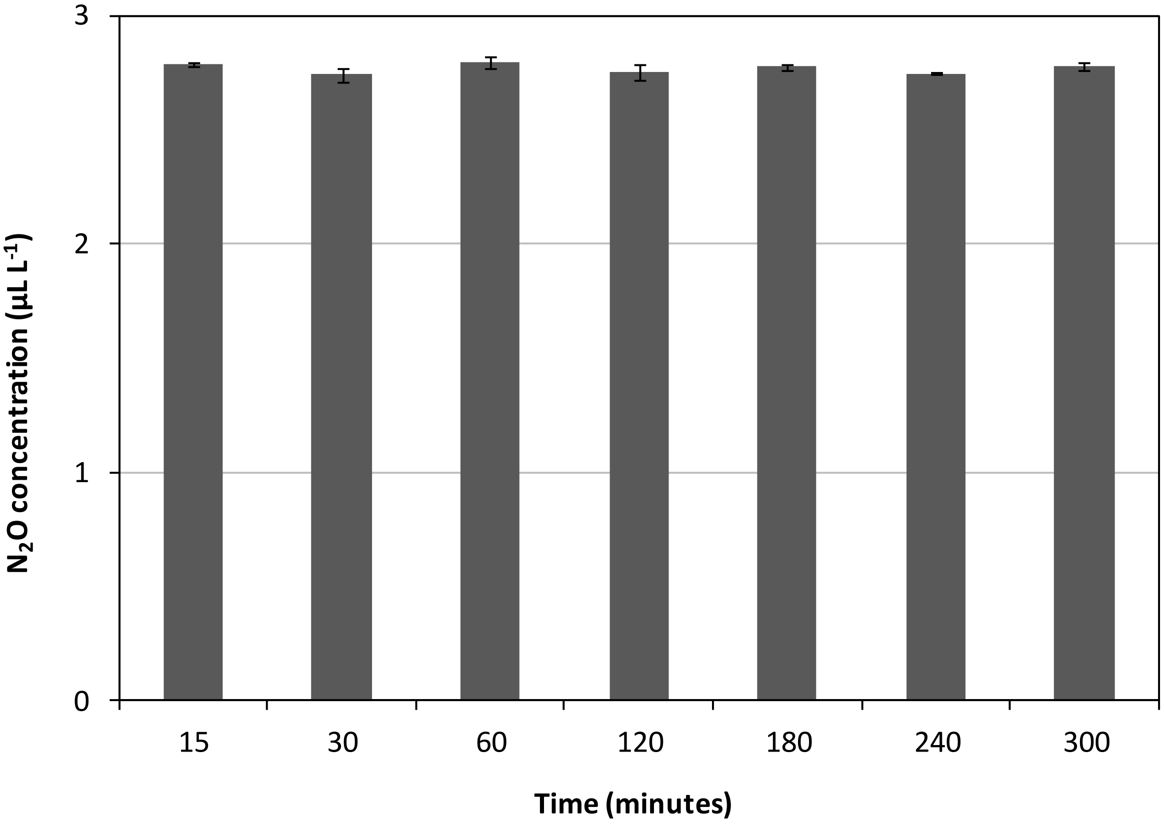

Figure 3.4 Method testing measurements taken at 15, 30, 60, 120, 180, 240 and 300 minutes following the injection of 1 µL L^{-1} of N_2O standard into the collection chamber. Error bars are standard error of the mean (n = 6).

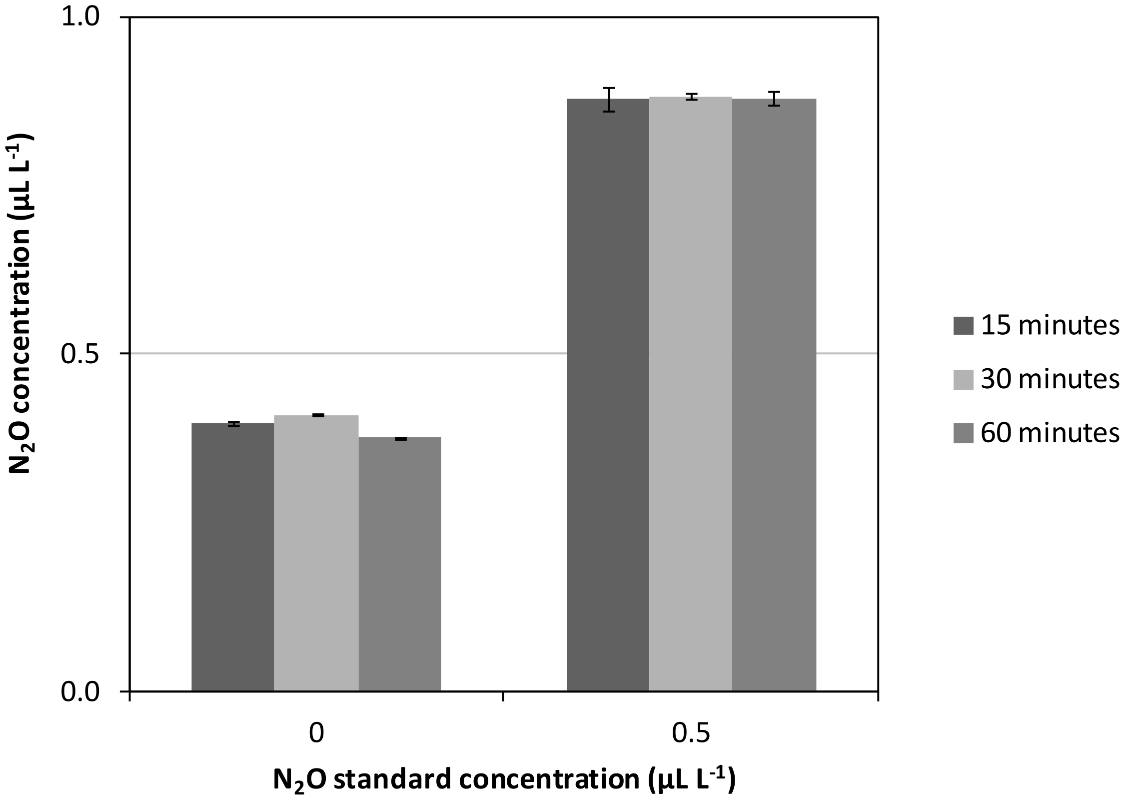

Figure 3.5 Method testing measurements taken at 15, 30, and 60 minutes following the injection of 0.5 µL L^{-1} of N_2O standard into the collection chamber compared to a blank (ambient air). Error bars are standard error of the mean (n = 3).

3.3.4 Soil collection and experimental setup

Templeton silt loam topsoil (top 20 cm) was sourced from the Lincoln University Dairy Farm (43°38’11.70”S, 172°26’17.00”E), previous studies have shown this to have a pH of 5.0, C content of 2.0%, and N content of 0.18% (Table 3.1) (Knowles *et al.*, 2011).

The soil was homogenised using a concrete mixer to break up the large clumps, some roots were removed during this process. Lime was added at a rate of 6.25 g lime L^{-1} soil to raise the pH to around 6 and mixed in the concrete mixer. This was then used to pot up each plant species: Pasture (P) Perennial ryegrass – *Lolium perenne* L. cv. ONE50, endophyte = AR1 (sown 6 December 2011); Manuka (M) – *Leptospermum scoparium* J. R. Forst. & G. Forst.; Kanuka (K) – *Kunzea ericoides* (A. Rich.) Joy Thomps. Manuka seed was sourced from Lake Ohau and was sown on 30 November 2009. Kanuka seed was sourced from a low pH soil and was sown on 3 March 2010.

Table 3.1 Soil chemical properties for the Templeton silt loam. Values in brackets represent the standard error of the mean (n = 3). From Knowles *et al.* (2011).

	Templeton
pH	5.6
CEC (cmol$_c$ kg^{-1})	12.4 (0.5)
C (%)	2.0 (0.1)
N (%)	0.18 (0.01)
P (mg kg^{-1})	518 (25)
S (mg kg^{-1})	193 (15)
Ca (mg kg^{-1})	3005 (101)
Mg (mg kg^{-1})	855 (11)
K (mg kg^{-1})	1401 (119)
Na (mg kg^{-1})	136 (4)
Cd (mg kg^{-1})	0.4 (0.1)
Cr (mg kg^{-1})	11.6 (0.4)
Cu (mg kg^{-1})	4.5 (0.1)
Pb (mg kg^{-1})	12.0 (0.1)
Zn (mg kg^{-1})	43 (1)

Pots were arranged as a completely randomised block design in the greenhouse (Figure 3.6). The experimental blocks were the replicates, of which there were five.

Treatments included: Templeton silt loam (Control, C)
Templeton silt loam + effluent application (Effluent, E)

Figure 3.6 Pots in the greenhouse on 19 December 2011 (left), and 31 January 2012 (right).

Effluent properties and collection

Dairy shed effluent was sourced from the Lincoln University Dairy Farm, Canterbury, New Zealand. This was collected on 9 September 2011 in 20 L plastic containers as it flowed into the storage pond from the milking platform. It was then homogenised in a 200 L plastic drum and stored at 5 °C prior to application. Chemical analysis of the effluent following collection showed that it contained 450 mg N L^{-1}.

Application

Dairy shed effluent was applied at a rate of 200 kg N ha^{-1} to each pot (Figure 3.7). This was carried out over a period of 5 days from 26-30 April 2012. The pots were allowed to dry out prior to effluent application to allow the soil to absorb the added volume of liquid and minimise leaching losses out of the bottom of the pots. The calculation used for an application rate of 200 kg N ha^{-1} with effluent containing 450 mg N L^{-1} was:

200 kg N ha^{-1}/10 000 m^2 ha^{-1} x 1 000 000 mg kg^{-1} x 0.0201 m^2 = 402 mg N pot^{-1}
402 mg N pot^{-1}/450 mg N L^{-1} effluent = 0.9 L effluent pot^{-1}

Figure 3.7 Effluent application to pots, 26-30 April 2012.

N_2O gas sampling

Gas samples were collected 0, 0.5, 1 and 2 hours after the chamber was placed on top of the plant (Figure 3.8). The trial was carried out over a period of two weeks and gas samples were taken on the following dates: 1, 2, 4, 7, 9, 11, and 14 May 2012.

Figure 3.8 Experimental setup, showing plants in greenhouse ready to be sampled (left) and sample chambers in position during gas sampling (right).

Calculation of N_2O Flux (µg N_2O-N m^{-2} h^{-1})

The following equations derived from Muller (1995) were used to calculate the N_2O flux (µg N_2O-N m^{-2} h^{-1}) from the concentration (µL L^{-1}) given after GC analysis:

Equation A, when: $(C_1-C_0)/(C_2-C_1) \leq 1$

$$\frac{[(C_2 - C_0)V \times P]M_{N2}}{[R(T_K + T_{^\circ\mathrm{C}})]A \times t_2}$$

Equation B, when: $(C_1-C_0)/(C_2-C_1) > 1$

$$\frac{V(C_1 - C_0)^2}{(2C_1 - C_2 - C_0)}\, ln\left[\frac{C_1 - C_0}{C_2 - C_1}\right] \frac{P\, M_{N2}}{[R(T_K + T_{^\circ\mathrm{C}})]A \times t_1}$$

where:

C_0, C_1, C_2 = N_2O concentration [µL L^{-1}] at times t_0, t_1, t_2 respectively

P = atmospheric pressure [atm] = 1

V = chamber volume [L]

R = gas constant [L atm K^{-1} mol^{-1}] = 0.08205746

T_K = absolute temperature at 0°C [K] = 273.15

T_{oC} = air temperature [°C]

A = soil surface area [m^2]

t_2 = total cover period [h]

t_1 = $t_2/2$ [h]

M_{N2} = molecular weight of N_2O-N [g mol^{-1}] = 28.01344

3.3.5 Rhizon sampling

Installation

Rhizon samplers (19.21.21 RHIZON MOM, Rhizosphere Research Products, Wageningen, The Netherlands) were installed at a depth of 5 cm into the side of each pot. These had a 10 cm porous part (2.5 mm diameter) with a bulb tip (2.8 mm diameter), glass fiber strengthener wire, 12 cm tubing PVC/PE, and a male luer lock. To install these, a 4 mm hole was drilled through the pot with an electric drill, and a metal rod of similar diameter to the rhizon samplers pushed through to make way for the sampler. Once the porous part of the rhizon sampler was pushed inside the pot, the outside was sealed with silicon sealant. Samplers were left to stabilise for one month before collection commenced.

Sample collection

Soil water samples were collected on 2, 8, and 15 May 2012 using rhizon samplers. To do this, a 30 mL syringe was attached to the rhizon sampler, this was drawn out to 22 mL and held in place by a wooden stopper to create a vacuum. The suction provided caused the soil water to be removed from the pot and collected in the syringe. The syringes were left on for 24 hours. After this they were dismantled, any soil water collected was transferred into 30 mL plastic sample vials which were then frozen until analysis.

Sample analysis for NO_3^- and NH_4^+

Soil water samples were naturally defrosted overnight at room temperature prior to analysis. Soil water nitrate (NO_3^-) and ammonium (NH_4^+) were analysed using a Flow Injection Analyser (FIA) (Alpkem Flow Solution 3000 twin channel with Alpkem Winflow 4.03 software; Alpkem, O.I. Analytical, TX, USA). Further details of this methodology can be found in *Nitrate/nitrite in soils by Cd reduction: Method Abstract* (O.I. Analytical) and *TKN by Gas Diffusion and FIA: Method Abstract* (O.I. Analytical). NO_3^- and NH_4^+ concentrations (mg L^{-1}) were converted to mg per litre of soil by multiplication with the estimated moisture content.

3.3.6 Moisture content estimation

Pots were weighed prior to both N_2O sampling and rhizon sampling to estimate moisture content. After all measurements had been completed, the pots were weighed again and a Hydrosense™ moisture probe (12 cm) (Campbell Scientific Australia Pty. Ltd., QLD, Australia) (Figure 3.9) was used to determine the moisture content across a range of pot weights following wetting and drying periods. A standard curve was made and used to estimate moisture content by pot weight throughout the experiment.

Figure 3.9 Hydrosense$^{\text{TM}}$ moisture probe used to determine soil moisture content (left). Data logger setup for temperature probes (right).

3.3.7 Temperature data

Four temperature probes were set up, one in each plant species at 10 cm soil depth and one air temperature sensor set in the shade (Figure 3.9). A data logger was used to collect temperature information every 10 minutes during the N_2O measurement periods. Hourly and daily average temperatures were also recorded throughout the experiment. Air temperature data were used in the N_2O flux calculations (Equations A and B).

3.3.8 Soil pH

After all other measurements had been completed, three replicates were destructively harvested on 11 September 2012. A subsample of soil was taken and refrigerated until analysis. Field moist soil (10 ± 0.05 g) was weighed in duplicate into a 70 mL plastic vial to which 25 mL of deionised water was added. This was stirred and left to stabilise overnight. Soil pH was read on a pH meter (S20 SevenEasy™ pH; Mettler-Toledo, Switzerland) following calibration with pH 4 and pH 7 buffer solutions. Soil pH data were transformed into hydrogen ion (H^+) concentration via the equation pH = -log[H^+] to calculate means, this was then transformed back to pH values. Error bars were also calculated in this way, the standard error of the mean (SEM) (SEM = standard deviation/√(n)) of the H^+ was added and subtracted from the mean to give the standard error range, which was converted back to the pH scale and difference between this and the mean gave values used for the error bars.

3.3.9 Plant harvest

Prior to the experiment commencing, on 7 December 2011, a manuka and a kanuka plant were destructively harvested to determine the approximate initial size of the plants. These were oven dried at 70 °C for 48 hours to give dry weight. During the destructive harvest on 11 September 2012, plant shoot and root material were harvested, placed into paper bags and oven dried at 70 °C for 48 hours to give dry weight.

3.3.10 Statistical analysis

Method testing data (for Figure 3.3 and Figure 3.4) and preliminary N_2O flux data were analysed for significance using one-way analysis of variance (ANOVA). Method testing data for Figure 3.5 were analysed for significance using a two-way ANOVA with time and standard injected as factors. Soil water NO_3^- and NH_4^+ data were log transformed to give normal distribution then analysed for significance using a repeated measures ANOVA. For soil pH, H^+ data were analysed for significance using a one-way ANOVA. Estimated soil moisture content data were also analysed for significance using a repeated measures ANOVA. Where data were significant, Fisher's protected least significant difference (LSD) test ($P<0.05$) was the *post hoc* test used to identify differences between treatment means. All statistical analyses were carried out in Genstat 14.1 (VSN International Ltd., UK).

3.4 Results

3.4.1 N_2O Fluxes – Preliminary Trial

Though not statistically significant, due to low replication (n = 3) ($F_{2,6}=1.69; P=0.261$), preliminary experiments indicated a trend showing reduced N_2O fluxes from soil planted with manuka (Figure 3.10).

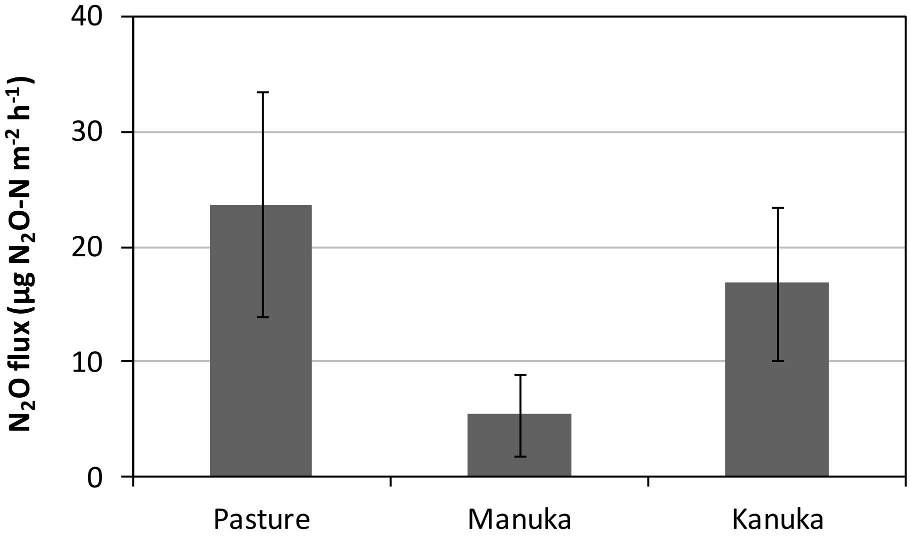

Figure 3.10 Preliminary nitrous oxide fluxes (µg N_2O-N m^{-2} h^{-1}) beneath plant species: pasture (P), manuka (M), kanuka (K) following application of ~200 kg N ha^{-1} dairy shed effluent. Error bars are standard error of the mean (n = 3).

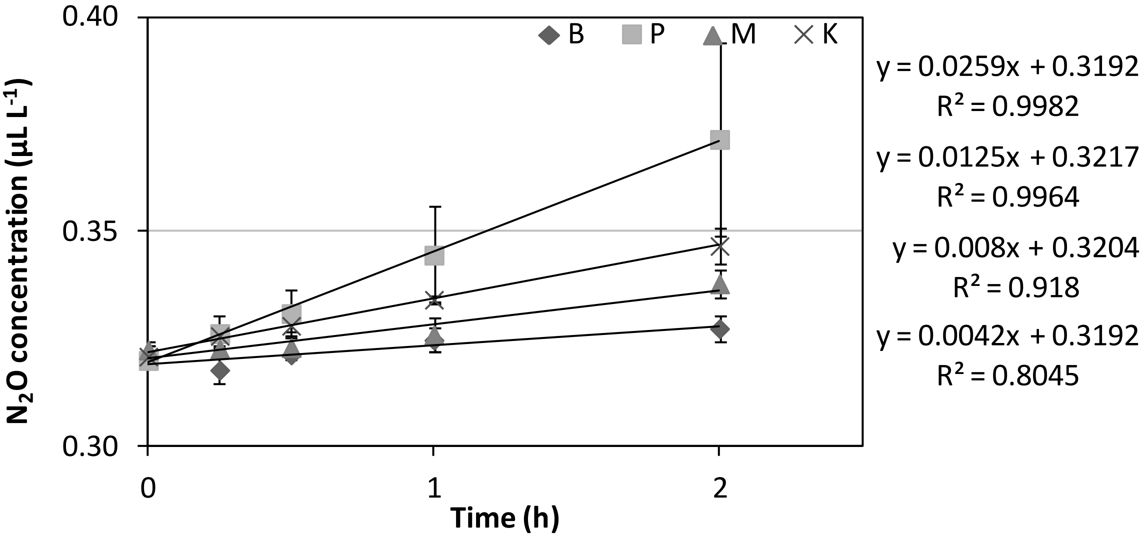

Figure 3.11 Preliminary N_2O concentrations (µL L^{-1}) for blank (B), pasture (P), manuka (M), and kanuka (K) species following application of ~200 kg N ha^{-1} dairy shed effluent. Error bars are standard error of the mean (n = 3).

3.4.2 N_2O Fluxes

Examination of raw N_2O concentration data showed high variability and a lack of a linear relationship with the time the samples were taken following chamber closure (Figure 3.12). A large proportion of R^2 values from a linear regression were low (39% were ≤ 0.3) (Figure 3.13). Thus it was not possible to obtain an accurate value for the N_2O fluxes.

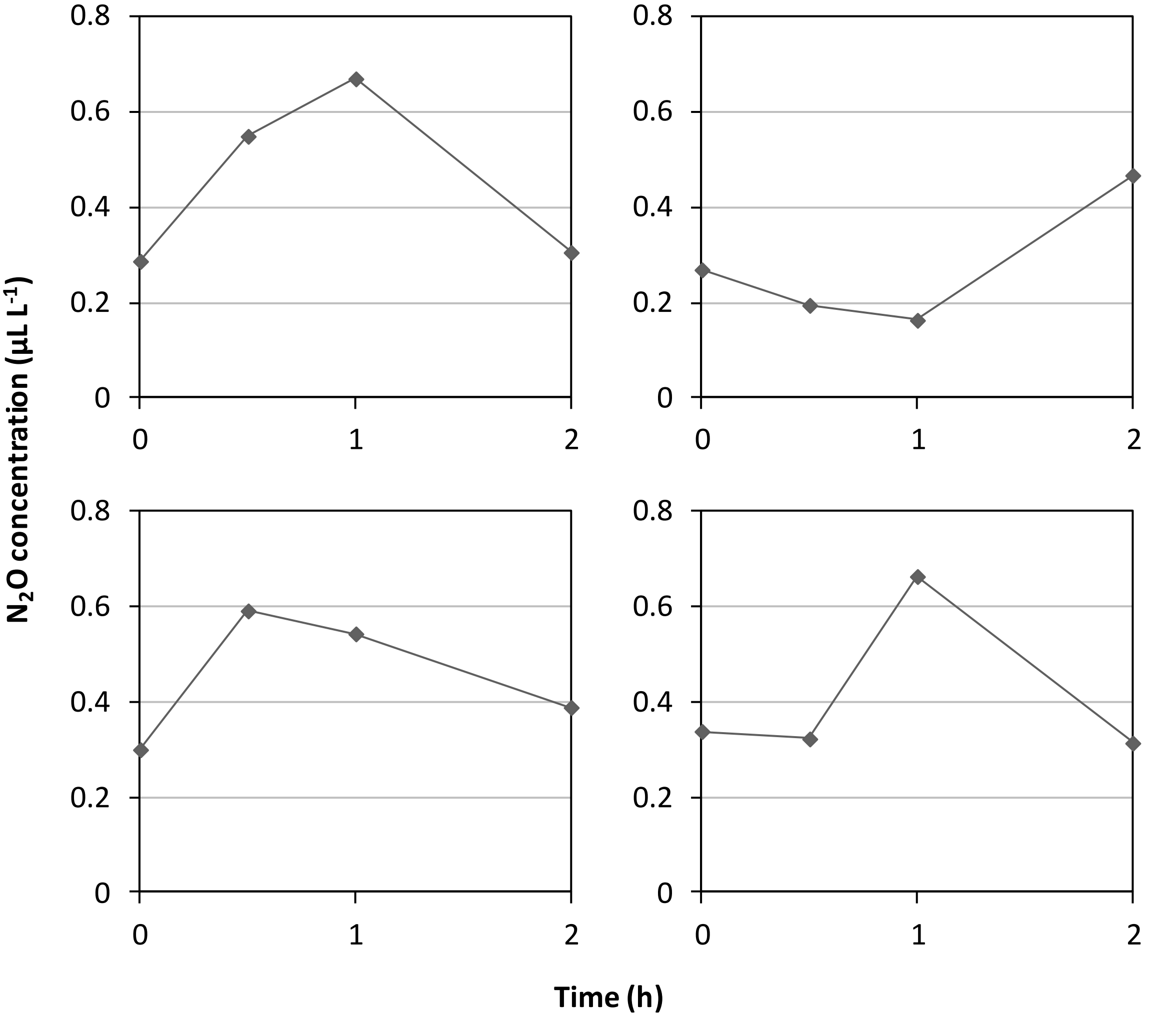

Figure 3.12 Examples of the results obtained from the nitrous oxide (N_2O) concentrations (µL L^{-1}) in samples taken after the chamber had been on for 0, 0.5, 1 and 2 h.

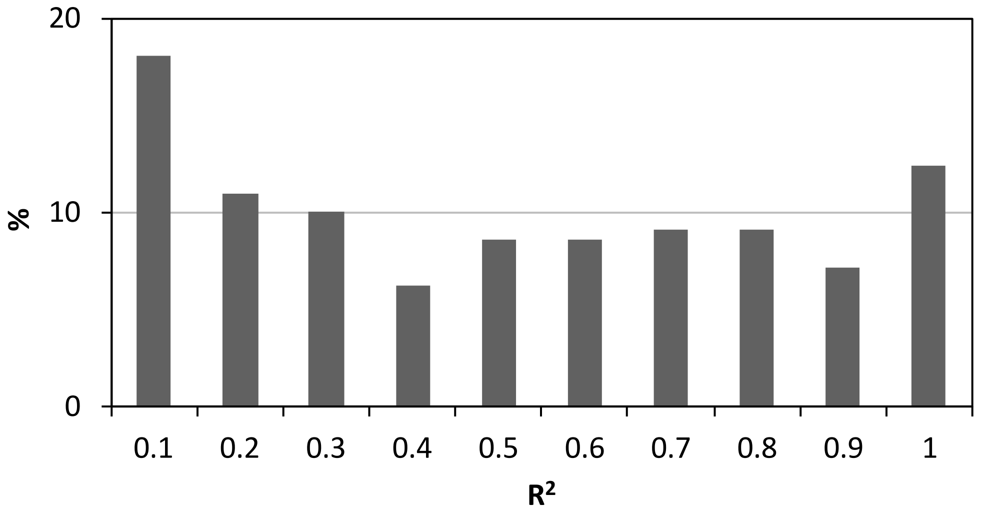

Figure 3.13 Percentage (%) distribution of R^2 values obtained by linear regression of N_2O concentrations against time sample taken.

3.4.3 Soil water NO_3^- and NH_4^+

Soil water NO_3^- levels were 99% higher in pots treated with effluent compared with control pots ($F_{1,18}=78.72$;$P<0.001$). Pasture NO_3^- levels were 88-91% lower than manuka and kanuka species ($F_{2,18}=4.34$;$P=0.029$) (Figure 3.14). A significant time ($F_{2,33}=7.31$;$P=0.003$), and time*treatment interaction ($F_{2,18}=25.45$;$P<0.001$) were also observed.

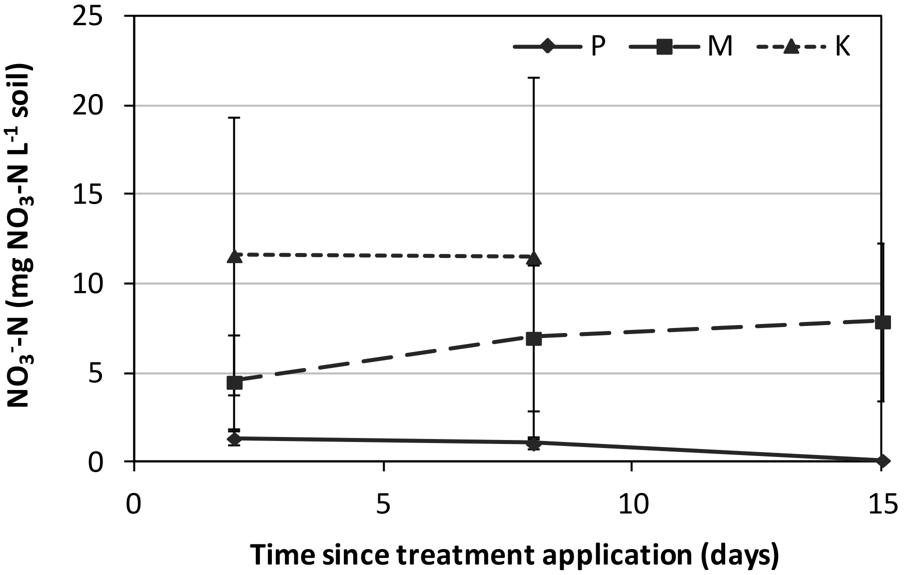

Figure 3.14 Relationship between nitrate (NO_3^-) concentration (mg NO_3^--N L^{-1} soil) extracted in soil water and time since treatment application, for pasture (P), manuka (M), and kanuka (K) species treated with effluent. Error bars are standard error of the mean.

Soil water NH_4^+ levels were 96% higher in pots treated with effluent compared with control pots ($F_{1,17}=9.19$;$P=0.008$). A significant treatment*species interaction ($F_{2,17}=5.07$;$P<0.019$) was observed. NH_4^+ levels decreased with time with a 97% decrease in levels by 8 days ($F_{2,22}=64.79$;$P<0.001$), this effect was particularly observed in the effluent treatment. The interaction between time*treatment was also significant ($F_{2,22}=28.20$;$P<0.001$). No species effect was observed ($F_{2,17}=1.58$;$P=0.236$).

3.4.4 Soil pH

Soil pH was significantly different among species ($F_{2,12}=9.69$;$P=0.003$). Pasture pH was on average 4-5% higher than that of manuka and kanuka. There was no difference in pH between treatments ($F_{1,12}=3.41$;$P=0.090$) (Figure 3.15).

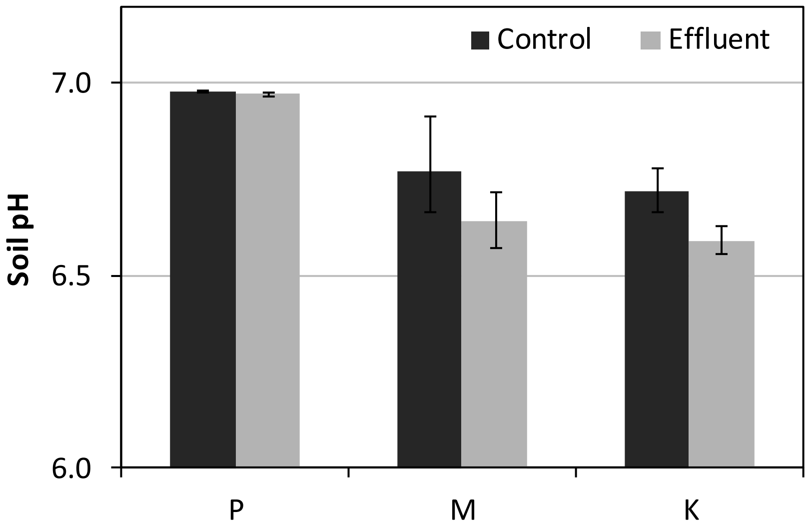

Figure 3.15 Soil pH under the control and effluent treatments and species pasture (P), manuka (M) and kanuka (K). Error bars are standard error of the mean (n = 3). See section 3.3.8 for a description of how average pH and standard errors were calculated.

3.4.5 Estimated moisture content

Significant differences in estimated moisture content (%) were observed between treatment ($F_{1,20}$=21.26;*P*<0.001), species ($F_{1,20}$=8.17;*P*=0.003), and the interaction between treatment and species ($F_{1,20}$=4.39;*P*=0.026) (Figure 3.16). When averaged across all species and times, pots treated with effluent were 8% drier than control pots (moisture contents were 29% and 37%, respectively). Similarly when averaged across both treatments and all times, kanuka was 7.6% drier than both the pasture and manuka plants (moisture contents were 27.9%, 35.4% and 35.5% respectively). Moisture contents were also different across time ($F_{1,48}$=6.19;*P*=0.004), and interactions between time*treatment ($F_{1,48}$=8.42;*P*<0.001), time*species ($F_{1,48}$=3.64;*P*=0.012), and time*treatment*species ($F_{1,48}$=5.48;*P*=0.001) were also found. In general, moisture content tended to decrease with time with a 2.6% decrease from day 1 to day 14.

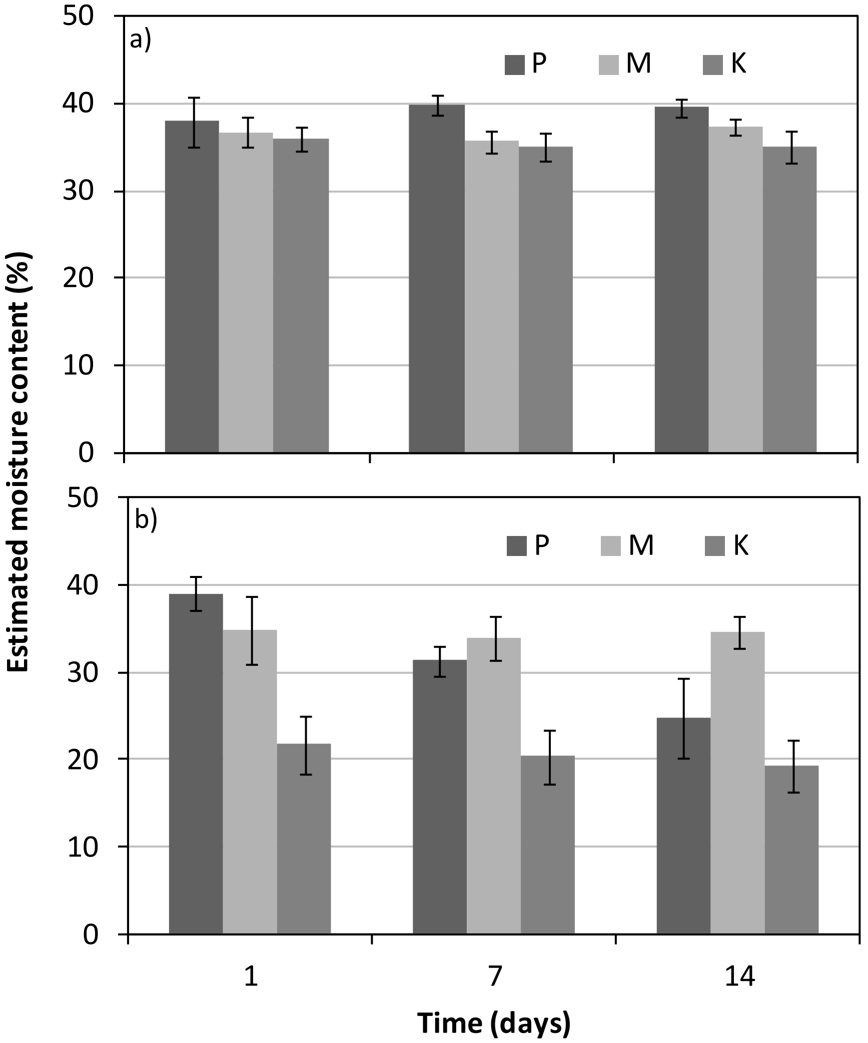

Figure 3.16 Estimated moisture content (%) in pots at 1, 7 and 14 days after treatment application for pasture (P), manuka (M) and kanuka (K) species treated with a) control and b) effluent. Error bars are standard error of the mean (n = 5).

3.4.6 Plant harvest

Manuka and kanuka plants used in this trial increased in dry weight by 9.6 g and 25.9 g, respectively over 9 months (Table 3.2). Shoot dry weights were significantly different between species ($F_{1,8}$=38.10;P<0.001), but not treatment ($F_{1,8}$=3.00;P=0.121). Kanuka had a 64% greater yield than manuka. No difference in root dry weights of the final plants was observed between treatment ($F_{1,8}$=0.15;P=0.709) or species ($F_{1,8}$=0.32;P=0.588).

Table 3.2 Initial and final manuka and kanuka dry weight yields (g) ± standard error of the mean.

	Treatment	Manuka		Kanuka	
		Shoot	**Root**	**Shoot**	**Root**
Initial (12 December 2011)		7.6	7.0	2.4	0.7
Final (11 September 2012)	**Control**	17.0 ± 2.4	18.5 ± 4.4	25.3 ± 0.2	12.6 ± 2.6
	Effluent	17.3 ± 2.2	15.7 ± 3.8	31.2 ± 1.5	17.9 ± 1.6

3.5 Discussion

3.5.1 N_2O method testing and implications

Method testing began first with empty buckets injected with 2 µL L^{-1} N_2O standard, measurements were taken after 15 and 30 minutes (Figure 3.3). The result of no significant difference in N_2O levels after each of these times was expected, showing that after 30 minutes N_2O was not lost from the chamber. The levels of around 2.2 µL L^{-1} were close to what was expected with the amount of standard injected plus ambient levels with only 0.1 µL L^{-1} N_2O unaccounted for, assuming ambient levels of around 0.33 µL L^{-1} N_2O.

The next set of method testing (Figure 3.4) was carried out over a longer period of time (up to 5 hours) to determine whether over a longer period of time N_2O was lost from the system via dissolution in the water at the bottom of the chamber or through leaks in the plastic. Data ranged from 2.74 to 2.80 µL L^{-1} N_2O within the 5 hours. This consistency of values indicated that any leaks or dissolution of N_2O from the system were negligible. However N_2O concentrations of around 2.8 µL L^{-1} N_2O were not expected following injection of up to 1 µL L^{-1} N_2O standard, compared with the 2.2 µL L^{-1} N_2O obtained following injection of 2 µL L^{-1} N_2O standard.

The third method testing (Figure 3.5) was conducted to confirm that there was no N_2O being produced from the chamber, due to the unusually high values found in the previous method testing results (Figure 3.4). A blank containing ambient air from the lab was injected into the chambers as well as N_2O standard up to 0.5 µL L^{-1} N_2O. The values obtained of 0.39 and 0.88 µL L^{-1} N_2O for the blank, and 0.5 µL L^{-1} N_2O injected standard, respectively were similar to expectations if the 0.5 µL L^{-1} N_2O standard was added to the ambient levels of 0.39 this would give 0.89 µL L^{-1} N_2O. These results indicated that the chambers were not emitting N_2O. Again consistency was shown between the 15, 30 and 60 minute samples, reinforcing the observation that there were no leaks from the system.

Method testing studies showed the chambers had no significant leaks, as illustrated by the consistent measurement of N_2O concentrations over a period of 5 hours. Also measurement of blank buckets showed no interference of the buckets with N_2O levels measured. Some key findings from this method testing included: temperatures could be monitored in the greenhouse to accurately determine its effects on the production of N_2O and in order to measure a N_2O flux it may be necessary to supply some nitrogen to the system in the form of fertiliser, effluent or urine.

3.5.2 N_2O fluxes

A preliminary trial using ~200 kg N ha^{-1} dairy shed effluent as a N source was carried out. This showed promising trends towards reduced N_2O emissions under manuka plant species. Although method testing was successful and preliminary results appeared promising the full-scale trial was unsuccessful. Following examination of raw data it was obvious that the data did not fit into the model described in the methods therefore no fluxes were calculated. Unlike in the preliminary study (Figure 3.11) N_2O concentrations over time often did not show a linear relationship, examples of this are illustrated in Figure 3.12. Fluxes could have been calculated but any flux values obtained would not have been reliable.

The N_2O concentrations in the current study suggested negative N_2O fluxes were observed. It is possible that some of these occurred as a result of random leaks of gas from the system, in other cases N_2O consumption may have occurred. Negative fluxes of N_2O have been reported and it has been suggested that these occur too frequently to be dismissed as experimental error (Chapuis-Lardy *et al.*, 2007). However, most studies show net N_2O production to occur. The ratio between N_2O production and N_2O reduction to N_2 determines whether a soil acts as a sink for N_2O. This is regulated by the relative activities of nitric oxide reductase (NIR) and N_2O reductase (NOR). If NOR activity increases relative to NIR activity, the N_2:N_2O ratio increases resulting in gross N_2O consumption. NOR activity is affected by several factors and net N_2O consumption in pastoral soils has sometimes been measured under high soil water content and low soil N availability but it has not yet been studied systematically. However, in grazed pastoral soils where N availability is high due to either excreta N and/or N fertilizer applications, net N_2O consumption is unlikely to exceed N_2O production (de Klein *et al.*, 2008). Thus as effluent was a N source for one of the treatments in the current study and despite this, negative fluxes were still observed implies that measurement of N_2O fluxes was unsuccessful. A possible explanation for this is the high volume to soil surface area ratio (0.17 m^3:0.02 m^2) of the chambers used, meaning that they were not sensitive enough to detect N_2O fluxes in this study. Future improvements could include: the development of a more sensitive N_2O measurement chamber compatible with a pot trial; or a repeat of the experiment with the same chambers adapted to have a reduced volume (e.g. some of the chamber volume taken up with polystyrene). One of the negative aspects of the pot trial was that the N_2O measurement chamber was designed to enclose the whole plant-pot system. Alternatively field experiments could be carried out using an existing, reputable method to test the effect manuka and kanuka have on N_2O emissions from the soil beneath them. As the current experiment was carried out in a greenhouse, results may not necessarily have represented what could occur in the field, thus a field experiment would likely provide a more realistic set of results.

3.5.3 Soil and plant measurements

Soil moisture was different between species with both manuka and kanuka pots drying more quickly than that of pasture. If the experiment was to be repeated, controlling this parameter could be of use. This could help determine whether any differences in N_2O emissions were due to changes in soil aeration or moisture status, or due to manuka or kanuka species having an antimicrobial effect on the N cycle or inhibiting nitrification. The soil moisture difference also had implications for the rhizon sampling of the soil water. Due to their higher moisture content, soil water samples were much more easily obtained from the pasture pots than the manuka and kanuka plots.

Plant harvest data indicated that under the greenhouse conditions of the experiment, kanuka growth by far surpassed that of the manuka plants.

3.6 Conclusion

Method testing was successful as it showed chambers to have negligible leakage of gas. A preliminary trial showed promising but non-significant results which indicated that manuka plants showed reduced N_2O fluxes. However the full-scale experiment was unsuccessful as N_2O fluxes were not able to be measured. This was attributed to the high volume to soil surface area of the chambers used, meaning that they were not sensitive enough to detect N_2O fluxes in this study. Thus it is suggested that the study be repeated either using a different chamber design, or with the same chambers but reduce the volume inside them e.g. using space fillers such as polystyrene. Alternatively a field trial would be more representative of what would occur on farm and methods have already been developed to successfully measure N_2O emissions in field trials. Other implications included the control of moisture content in future trials as this was found to vary significantly with pasture having a higher moisture content than either manuka or kanuka species.

Chapter 4

Nitrous Oxide Field Experiment

4.1 Introduction

The greenhouse experiment revealed that there may be some effect of manuka and kanuka on N_2O fluxes from soil, but the method that was developed to measure this was insufficient to accurately measure N_2O fluxes in the greenhouse. For this reason, a well established closed-chamber method for field measurements was used in this study. Field trials can be more applicable to what is likely to occur on farm, thus a field experiment seemed an appropriate next step, particularly because the chambers used had been designed for field experiments.

One of the challenges in this experiment was to find manuka and kanuka plants of a similar age on a similar soil type, without an excess of other species. Manuka is not a native plant in the Canterbury region. However, kanuka is relatively abundant. The Lincoln University Dairy Farm has planted stands of native species, including kanuka, in the paddock corners. Full weed control has been undertaken with the objective of keeping the sites weed free for the first two seasons. This provides the possibility to conduct a replicated experiment with kanuka trees of a similar age (5 years) compared to bare soil as a control. This site was chosen for the study because of its close proximity to Lincoln University campus, and its easy accessibility.

4.2 Aim

To determine using an established method whether the planting of kanuka was likely to reduce emissions of nitrous oxide from soil compared with bare soil in the field, particularly when treated with dairy shed effluent.

4.3 Methods

4.3.1 Site details

The experiment was carried out in the native plantation in a corner of the Lincoln University Dairy Farm (LUDF), Canterbury, New Zealand (43°38’38.00”S, 172°26’2.00”E) (Figure 4.1). The native plantation is triangular in shape with drainage ditches running along two of the three sides, and cattle pasture adjacent to the third side. It contained a range of native plants, including *Kunzea* *ericoides* (kanuka). See Appendix C for a list of natives planted on LUDF. Prior to establishing the plants, sites were sprayed, ripped, cultivated and rolled. Both machine and hand planting was employed to establish the plants. They were planted in September and October 2008, with slow release fertiliser at the time of planting to promote establishment and early growth (Figure 4.2). Combi-guard sleeves, wool mats and stakes were installed to provide wild life protection and easy maintenance when spray release work was undertaken.

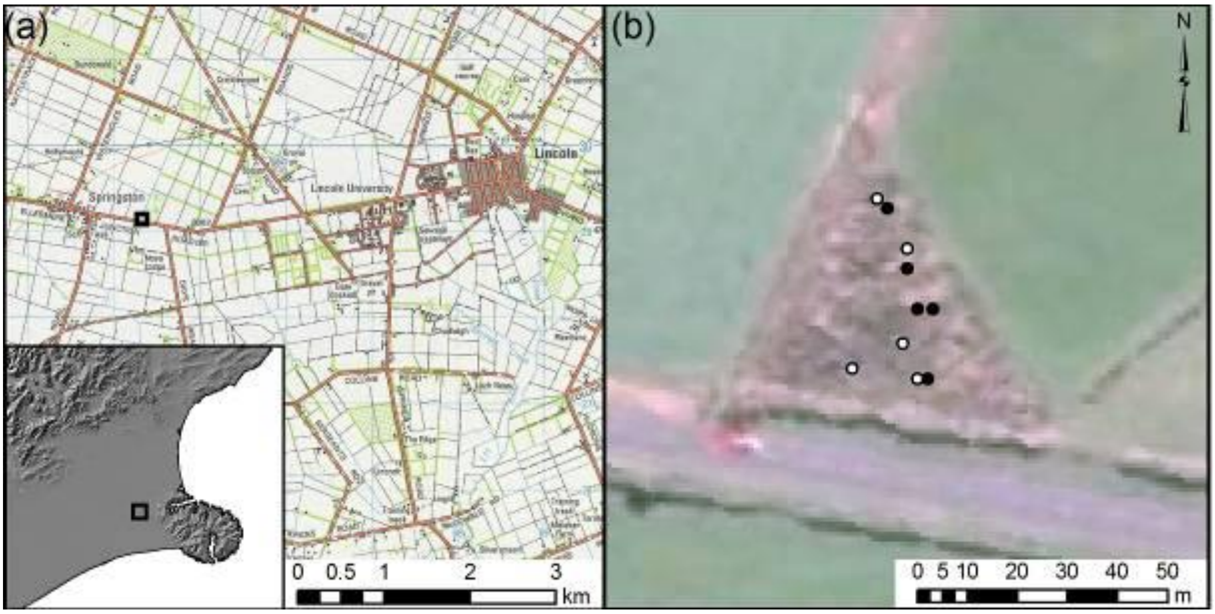

Figure 4.1 a) Map showing field site location, Lincoln University Dairy Farm. b) Map of the field site showing plots: control (bare soil) plots (○), and kanuka plots (●).

Figure 4.2 Native plantation field site (Lincoln University Dairy Farm) at various stages of its development.

4.3.2 Experimental setup and design

Five kanuka trees and five areas of bare ground (control) were selected based on ease of access to the ground beneath and were relatively spaced out within the native plantation (Figure 4.1). Each of these locations consisted of four plots, two from which gas samples were taken and two which received the same treatment, but were used for destructive soil sampling throughout the study (Figure 4.3 and Figure 4.4). Stainless steel gas rings of a diameter 0.485 m were installed to a depth of at least 5 cm and the headspace heights were measured with a ruler. Treatments were dairy shed effluent (~50 kg N ha^{-1} equal to 2 L) and water (2 L) (as a control) and were applied to both the gas rings and soil sampling rings on 7 August 2012. The dairy shed effluent was sourced from the same batch and calculated in the same manner as in Chapter 3 (section 3.3.4).

Figure 4.3 Bare soil sites with (left) and without (right) gas collection chambers.

Figure 4.4 Kanuka sites with (left) and without (right) gas collection chambers.

4.3.3 Gas sampling - collection and analysis

A closed-chamber method of N_2O collection was once again used. Samples of gas were taken on the day prior to treatment application 6 August 2012, the afternoon following treatment application 7 August 2012 and then again on 8, 9, 15, 22, and 29 August 2012. Gas sampling was carried out around 1.30 pm on most occasions, but within the range of 12.30 to 2.30 pm. Chambers were constructed of a metal cylinder, insulated with polystyrene foam to avoid heating of the atmosphere in the chamber during sampling. A metal ring was inserted into the soil; around the perimeter of this a U-shaped water trough was fitted. Chambers fitted inside this water trough to provide an air-tight seal. The headspace was on average 0.14 m above a soil surface area of 0.18 m^2. Chambers had two holes in the top, one with a cut down 30 mL plastic vial with a screw lid, the other a rubber septum in which to insert the sampling needle and valve. The sampling needle consisted of a Baxter three-way large bore stopcock with rotating male luer lock adaptor, a 1.6 by 40 mm BD Microlance^{TM} (white), and a 0.8 by 0.38 mm BD PrecisionGlide^{TM} (green) needle joined with a male luer couple (Figure 4.5).

Figure 4.5 Field gas collection chamber setup.

Samples were collected by placing a 6 mL Exetainer® with a septum in the lid onto the green needle (Figure 4.6). A syringe attached to a three-way stopcock and green needle was also inserted into the septum of the Exetainer® (Figure 4.7). A 20 mL sample of gas was drawn out of the chamber and was discarded to flush the ambient air out of the Exetainer®. Next 20 mL was drawn out and pumped 3 times to mix before the 20 mL sample was drawn out, the valve to the chamber closed and 15 mL of the sample was pumped into the Exetainer®. Chemical analysis of N_2O and calculation of N_2O flux were carried out in the same manner as in Chapter 3 (sections 3.3.2 and 3.3.4). Cumulative flux values over the experimental period were calculated by converting the hourly N_2O fluxes into daily

N_2O fluxes by multiplication by 24. Integration was then used to give the total or cumulative flux value for each treatment combination.

Figure 4.6 Collection of gas samples.

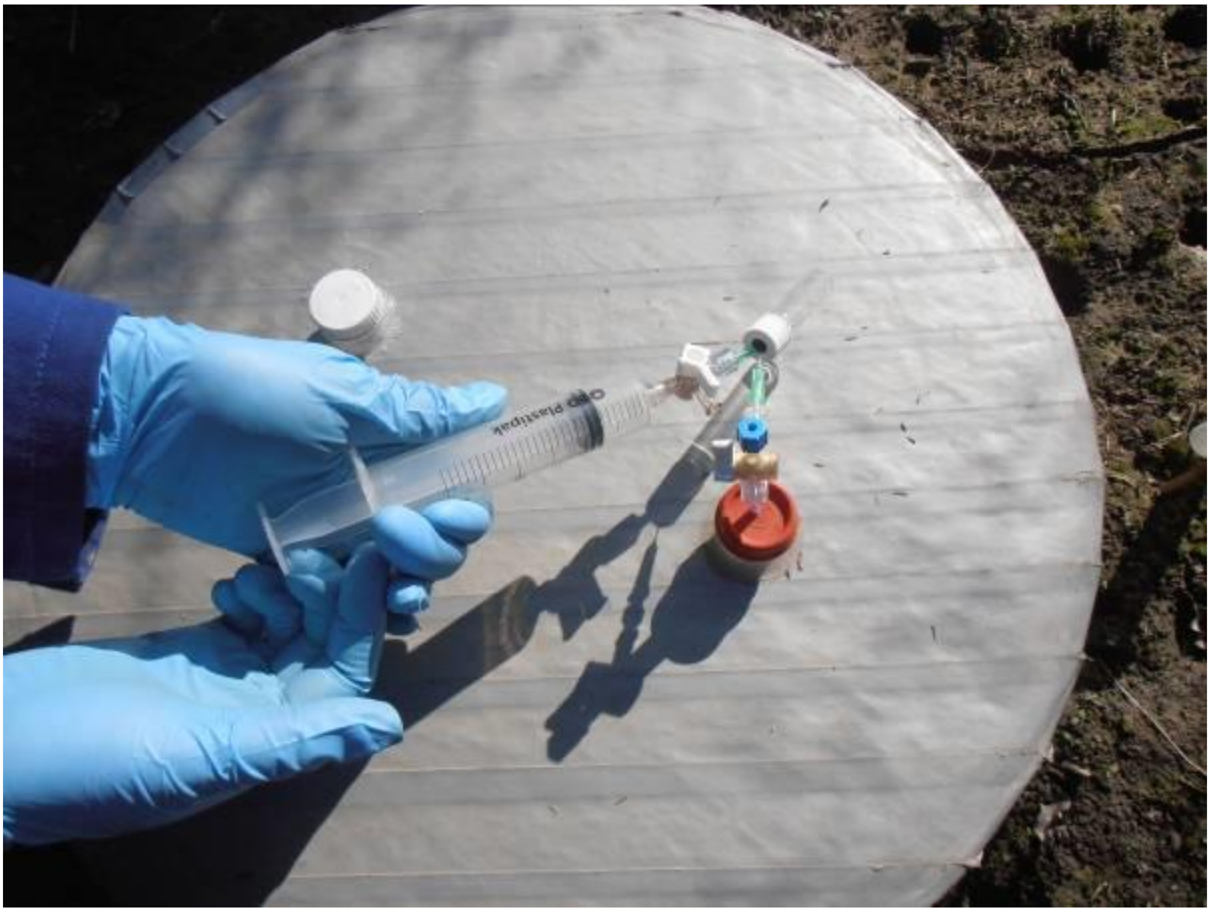

Figure 4.7 Gas sampling syringe and needle setup.

4.3.4 Soil collection and analysis

Soil samples were collected using a 7.5 cm soil corer and placed into re-sealable plastic bags. These were collected in the morning on 6, 8, 9, 15, 22, and 29 August 2012. Samples were taken back to the lab where they were weighed out for analysis.

Soil pH

Field moist soil (10 ± 0.05 g) was weighed into a 70 mL plastic vial to which 25 mL of deionised water was added. This was stirred and left to stabilise overnight. Soil pH was read on a pH meter (S20 SevenEasy^{TM} pH; Mettler-Toledo, Switzerland) following calibration with pH 4 and pH 7 buffer solutions. Soil pH data were transformed to calculate averages and standard errors in the same manner as in Chapter 3 (3.3.8).

Soil gravimetric moisture content

Approximately 10-20 g of field moist soil was accurately weighed into a metal dish and dried in an oven at 105 ^{o}C for 24 hours. Lids were fitted on the metal containers and they were placed in a desiccator until cool. Samples were reweighed once cool. Moisture content was determined by the equation: Moisture content = (moist weight – dry weight * 100)/dry weight.

Soil NH_4^+ and NO_3^- content

Field moist soil (4 g) was weighed into a 50 mL centrifuge tube, to which 40 mL of 2M KCl was added. These were shaken for 1 hour on an end over end shaker, then centrifuged at 2000 rpm for 10 minutes and filtered through MicroScience MS 5AS 110 mm Ashless filter paper. Samples were frozen until they were able to be analysed by Flow Injection Analyser (FIA) (FOSS FIAstar 5000 triple channel with SoFIA software version 1.30; Foss Tecator, Hoganas, Sweden). Further details of this methodology can be found in *Nitrate/nitrite in soils by Cd reduction: Method Abstract* (O.I. Analytical) and *TKN by Gas Diffusion and FIA: Method Abstract* (O.I. Analytical).

4.3.5 Statistical analysis

N_2O flux data were log transformed to give normal distribution and analysed for significance using repeated measures analysis of variance (ANOVA) using species, treatment and time as factors. For soil pH, H^+ data were analysed for significance using a repeated measures ANOVA where species, treatment and time were factors. Soil moisture content, NO_3^- and NH_4^+ data were also analysed for significance using repeated measures ANOVA. Where data were significant, the *post hoc* test used to identify differences among means was Fischer's protected least significant differences (LSD) ($P<0.05$). All statistical analyses were carried out in Genstat 14.1 (VSN International Ltd., UK).

4.4 Results

4.4.1 N_2O flux

N_2O flux data (µg N_2O-N m^{-2} h^{-1}) showed the control (bare soil) plus effluent plots emitted significantly (Table 4.1) higher amounts of N_2O (cumulative flux of 65 mg N_2O-N m^{-2}) compared with the kanuka plots with (13 mg N_2O-N m^{-2}) and without effluent (9 mg N_2O-N m^{-2}) and the control plots without effluent (15 mg N_2O-N m^{-2}) (Figure 4.8).

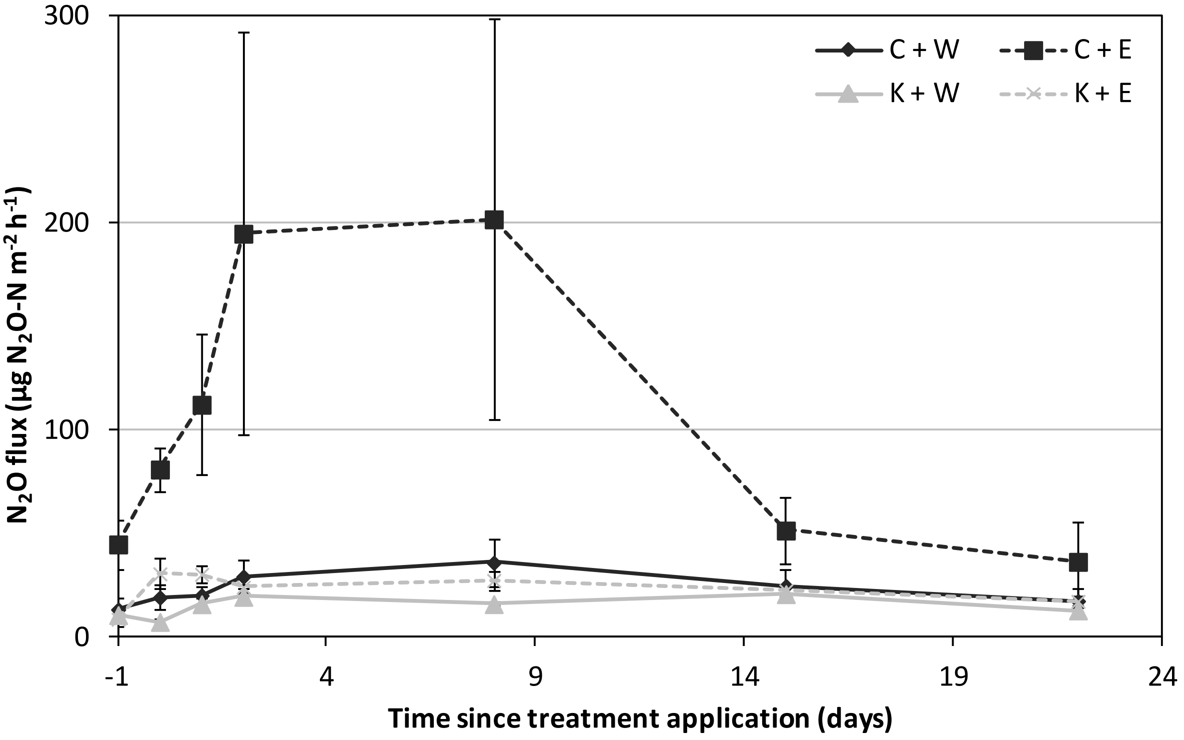

Figure 4.8 Nitrous oxide (N_2O) fluxes (µg N_2O-N m^{-2} h^{-1}) for control (bare soil) (C) plots and kanuka (K) plots treated with either water (W) or effluent (E). Error bars are standard error of the mean (n = 5).

Table 4.1 Statistical analysis output of significant N_2O flux data showing degrees of freedom (d.f.) for the number of levels (k) and error d.f., and the variance ratio (F), and *P*-values (probability of null hypothesis).

	d.f. (k, error)	F	*P*
Treatment	1,16	19.10	<0.001
Species	1,16	12.90	0.002
Treatment*Species	1,16	5.77	0.029
Time	6,95	7.27	<0.001

4.4.2 Soil data

Soil pH data were significantly different between species ($F_{1,16}$=13.48;*P*=0.002) and across time ($F_{5,80}$=3.35;*P*=0.009), but no significant difference in pH was observed between treatments. Control plot soil pH was higher than that of kanuka plots with mean values of 5.8 and 5.5, respectively (Figure 4.9). Soil pH was also significantly lower on day -1 prior to treatment application, compared with all other days.

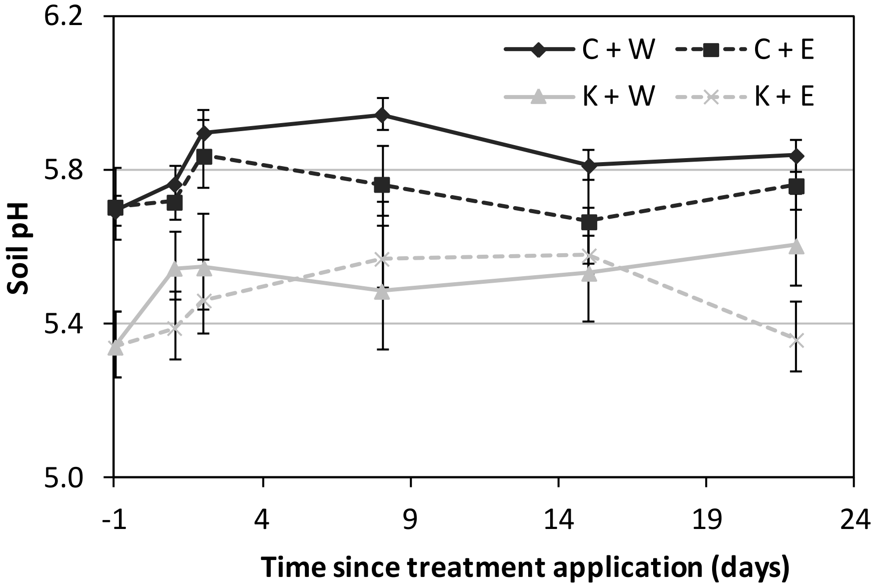

Figure 4.9 Soil pH of field moist soil with time for control (bare soil) (C) plots and kanuka (K) plots treated with either water (W) or effluent (E). Error bars are standard error of the mean (n = 5). See section 3.3.8 for a description of how average pH and standard errors were calculated.

Soil gravimetric moisture content (%) was significantly different ($F_{1,16}$=6.31;*P*=0.023) between species and across time ($F_{5,79}$=9.35;*P*<0.001), but not between treatments (Figure 4.10). Kanuka plots tended to have lower moisture contents than the control plots with average values of 30% and 33%, respectively.

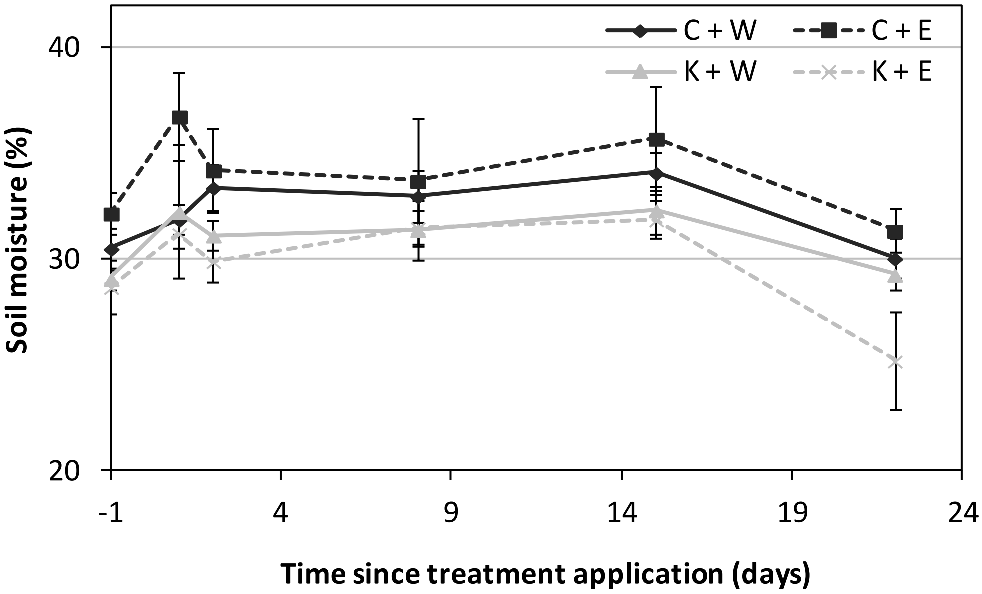

Figure 4.10 Soil gravimetric moisture content (%) of field moist soil with time for control (bare soil) (C) plots and kanuka (K) plots treated with either water (W) or effluent (E). Error bars are standard error of the mean (n = 5).

Soil nitrate (NO_3^-) levels (µg g^{-1} soil) were significantly different between species ($F_{1,16}$=14.26;*P*=0.002) and across time ($F_{5,80}$=5.03;*P*=0.009) with a significant interaction between species and time ($F_{5,80}$=3.38;*P*=0.039). This was apparent both before treatment application and afterwards. NO_3^- levels in the soil were higher under the kanuka than in the bare soil control plots with mean values of 17.1 and 3.3 µg g^{-1} soil, respectively for the duration of the experiment (Figure 4.11).

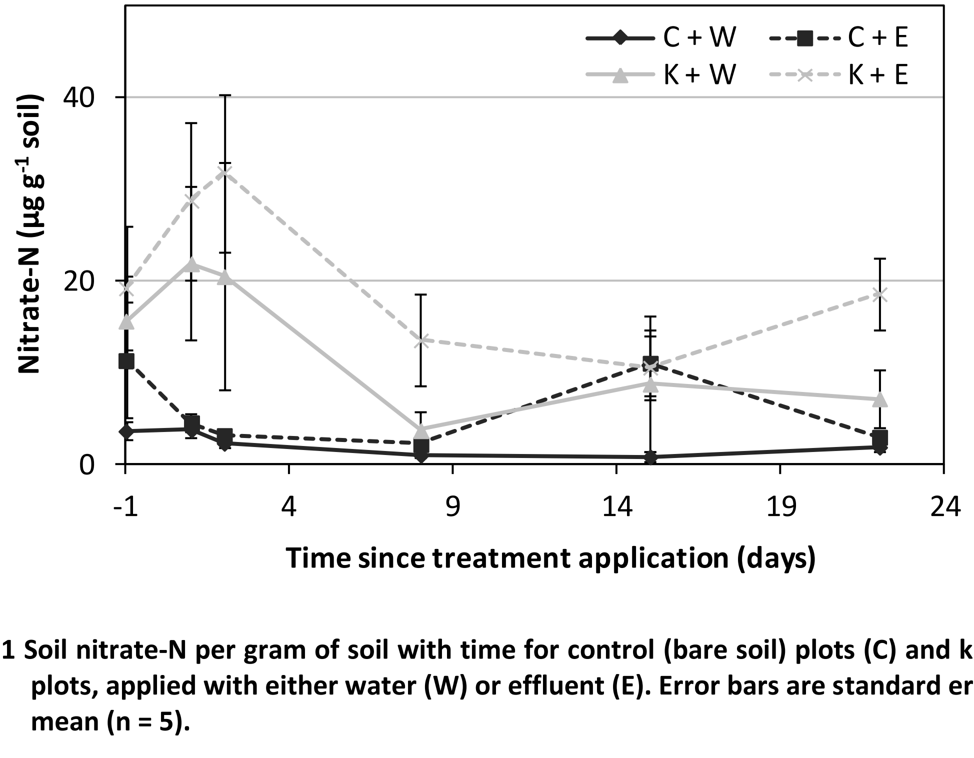

Figure 4.11 Soil nitrate-N per gram of soil with time for control (bare soil) plots (C) and kanuka (K) plots, applied with either water (W) or effluent (E). Error bars are standard error of the mean (n = 5).

Soil ammonium levels (NH_4^+) (µg g^{-1} soil) were significantly different between treatments ($F_{1,16}$=19.50;*P*=<0.001) and time ($F_{5,80}$=7.49;*P*=0.001) and there was a significant interaction between treatment and time ($F_{5,80}$=3.61;*P*=0.031) but not between species (Figure 4.12). NH_4^+ levels were on average 4.0 and 8.4 µg g^{-1} soil for the water and effluent treatments respectively.

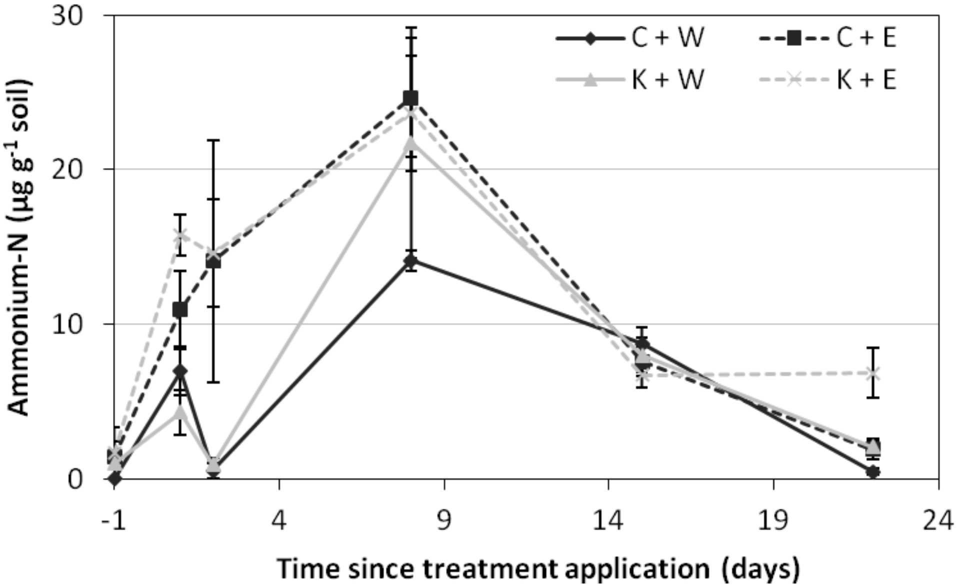

Figure 4.12 Soil ammonium-N per gram of soil with time for control (bare soil) plots (C) and kanuka (K) plots, applied with either water (W) or effluent (E). Error bars are standard error of the mean (n = 5).

4.5 Discussion

4.5.1 N_2O fluxes

N_2O fluxes were significantly higher from control plots treated with effluent compared with kanuka plots with effluent. N_2O fluxes from kanuka plots treated with effluent were slightly higher than those of kanuka plots which did not receive effluent and were comparable to fluxes of the control treatment without effluent. This provides some evidence for reduced N_2O emissions beneath kanuka plants. Of course the trial should be repeated in order to confirm the findings of this study. If confirmed by a repeated study, the benefits of reduced N_2O emissions beneath kanuka plants include: reduced environmental impacts of dairy farming if these are planted on farms, particularly if a dense kanuka stand was used for effluent disposal, in the future this could have implications in terms of the Emissions Trading Scheme; the provision of an incentive to increase the planting of native vegetation on New Zealand dairy farms, which in turn could help to increase biodiversity of native wildlife; as well as improved public perception of dairy farming on dairy farms planted with native bush. It appears that this study is the first to examine the influence of manuka and kanuka plants on the production of N_2O from the soil and thus no literature has been found to compare the results of this study to.

4.5.2 Soil measurements

Higher NO_3^- levels below kanuka plants could be a result of increased NO_3^- retention by the soil which would reduce NO_3^- losses. Another explanation for this could be increased nitrification, if this is the case this would result in increased N_2O losses. ^{15}N tracer studies could determine mechanisms, highly important to understand which one of these is going on. Despite being higher levels of NO_3^- substrate in the soil beneath kanuka trees, reduced N_2O was observed, which indicated a possible inhibition of denitrification. NO_3^- concentration has been described to influence the rate of denitrification, it has also been described to influence the $N_2O:N_2$ ratio, so that at high NO_3^- concentrations N_2O production is more predominant (Bolan *et al.*, 2004). This does not appear to have happened in the current study.

Soil pH tended to be lower in soil from kanuka plots than control plots. Though the pH range for denitrification is broad, in acidic conditions denitrification tends to slow down and the proportion of N_2O increases as pH decreases (Bolan *et al.*, 2004). Reduced denitrification under kanuka due to lower pH could explain the reduced N_2O emissions, however as the pH difference was less than 1 pH unit (pH 5.3 to 5.9) and thus not extremely different, such a significant reduction in N_2O emission is unlikely to be attributed to differences in pH alone. Similarly Simek *et al.*, (2002) found no simple relationship between denitrifying enzyme activity (DEA) and soil pH as high DEA was found both in acid and alkaline soils. Soil denitfiers appeared to be adapted to their natural soil pH.

Soil gravimetric moisture content tended to be lower in soil beneath kanuka trees. This could explain the reduced N_2O emissions found beneath kanuka in this study as soil aeration (and hence moisture status) affect both nitrification and denitrification. Aerobic conditions decrease the activity of N_2O-reductase in denitrification (Bakken & Dorsch, 2007) and under increased moisture conditions (such as what was observed in the control plots) denitrifiers switch from using O_2 to NO_3^- as a terminal electron acceptor (Sherlock, 1992). The no difference in soil moisture content between treatments was expected because the same volume of water and effluent were applied the plots during treatment application.

4.6 Conclusion

N_2O fluxes were significantly higher from control plots treated with effluent compared with kanuka plots with effluent. This provides some evidence for reduced N_2O emissions beneath kanuka plants. This study should be repeated in the future in order to confirm these findings. ^{15}N tracer studies would also be useful to tell whether the N_2O emitted is produced by nitrification or denitrification. Also higher soil NO_3^- levels were found beneath kanuka plants than control plots though these did not result in increased N_2O emissions, instead emissions were decreased. It is unclear at this stage whether this was a result of increased NO_3^- retention, inhibition of denitrification or increased nitrification. Kanuka stands could be planted on many areas on dairy farms including effluent disposal blocks. This line of research has implications for reduced environmental impacts of dairy farms in NZ, increased biodiversity, and increased public perception of dairy farms.

Chapter 5

Antimicrobial Experiment

5.1 Introduction

The inhibition of N_2O emissions from soils by kanuka may be due to a change in microbial activity or survival in the soil. Most soil microbes are not easily cultured in the laboratory (Pham & Kim, 2012; Stewart, 2012). Therefore a variety of *Escherichia coli* (commonly found in soil), which could be quantified in the soil over time, was added. *E. coli* is also found in animal excreta (Ingerson-Mahar & Reid, 2011) and thus is likely to be found in New Zealand dairy systems via effluent deposition or application to land.

5.2 Aim

To determine whether manuka and kanuka demonstrate antimicrobial properties in the surrounding soil.

5.3 Methods

5.3.1 Experimental set up and design

Pots were set up using the same method as described at the beginning of Chapter 3 (section 3.3.4). These were arranged as a stratified design in the greenhouse (Figure 5.1) with nine replicates of each species. Three of each species were chosen at random to be destructively harvested on days: 1, 3, and 7.

Figure 5.1 Experimental setup of pots in the greenhouse on 17 January 2012.

5.3.2 *E. coli* enumeration

The surface of the pots were spiked with 1 x 10^{-9} *Escherichia coli*, after which, one pore volume of water was applied at the rate of 15 mm hr^{-1}, subsamples of the *E. coli* were taken for enumeration. After 24 hours, three replicates of each plant were destructively harvested and a sample of the rhizosphere soil was taken by shaking soil off the roots, this was well homogenised. A 10 g subsample of each soil was added to 90 mL of phosphate buffered saline (PBS) and shaken for 1 hour at 200 rpm, room temperature. This was then diluted as required into 9 mL PBS. 1 mL of each dilution was inoculated into 5 replicate lauryl tryptose broths (Difco) with inverted glass tubes for glass collection and incubated at 35 ^{o}C ± 0.5 ^{o}C for 24 h. A 1 µL loopful of culture from positive tubes showing gas production was then transferred to EC-broth + MUG (Difco) and incubated at 44 ^{o}C ± 0.2 ^{o}C for 24 h. Negative lauryl tryptose tubes were incubated for a further 24 h and then transferred into EC + MUG broth if positive after 48 h. After 24 h those EC + MUG tubes showing gas production and that fluoresced blue/purple under UV light (366 nm) were scored positive for *E. coli* (Figure 5.2). Harvest and subsequent enumeration was repeated 3 days and 7 days after *E. coli* application.

Figure 5.2 UV light used to score EC + MUG vials negative (far left) or positive (four to the right) for *E. coli*.

5.3.3 Maintenance of pots

Prior to spiking with *E. coli,* pots were watered daily. On commencement of study, following *E. coli* application and irrigation, saucers were placed underneath each pot and plants were watered via the trays in order to attempt to avoid bypass flow, and subsequent leaching of *E. coli.*

5.3.4 pH

Soil pH of samples was determined for a 4 g subsample of fresh soil to which 10 mL of deionised water was added, soil was dispersed thoroughly with a glass rod, vials were capped and left over night. Soil pH was read on a pH meter (S20 SevenEasy^{TM} pH; Mettler-Toledo, Switzerland) following calibration with pH 4 and pH 7 buffer solutions. Soil pH data were transformed to calculate averages and standard errors in the same manner as in Chapter 3 (3.3.8).

5.3.5 Moisture content

Moisture content of soil samples was determined by the difference between the weights of a field moist 5 g subsample and its dry weight after being oven dried at 105 ^{o}C for 24 h. A moisture factor was calculated by: (H_2O/Dry weight) + 1 = Moisture factor.

5.3.6 Statistical analysis

E. coli enumeration data were log transformed and analysed for significance using a two-way analysis of variance (ANOVA) with species and day as factors. Soil moisture content and soil pH (H^+) data were analysed for significance using a two-way ANOVA with species and day as factors. Where data were significant, the *post hoc* test used to identify differences among means was Fischer's protected least significant differences (LSD) ($P<0.05$). All statistical analyses were carried out in Genstat 14.1 (VSN International Ltd., UK).

5.4 Results

5.4.1 *E. coli* enumeration

E. coli enumeration results showed a significant difference between species ($F_{2,18}=3.76; P=0.043$) and time ($F_{2,18}=7.50; P=0.004$). No difference in the number of colony forming units (cfu) between species was observed 1 day after the application of the *E. coli* with an average value of 71 000 cfu g^{-1}. However, after 3 days the levels of *E. coli* in the manuka and kanuka soils decreased to an average of 37 000 cfu g^{-1} compared with 87 000 cfu g^{-1} for the pasture. After 7 days the *E. coli* levels in the manuka and kanuka soil had dropped off even more to an average of 8 000 cfu g^{-1} for manuka and kanuka compared with 65 000 cfu g^{-1} for the soil under pasture (Figure 5.3).

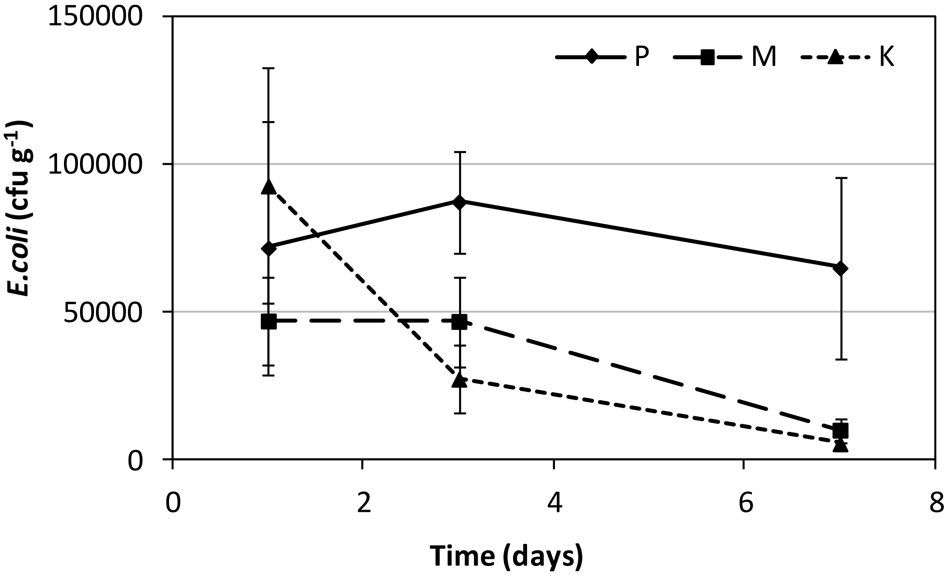

Figure 5.3 Survival of *E. coli* under three contrasting plant species: pasture (P), manuka (M), and kanuka (K) as measured 1, 3 and 7 days after application of *E. coli* to the surface of the soil. Error bars are standard error of the mean (n = 3).

5.4.2 Soil pH

Soil pH was significantly different among species ($F_{2,18}$=4.56;*P*=0.025). Pasture (pH 6.6) had a higher average pH than kanuka (pH 6.4) (Figure 5.4). Soil pH was also significantly different across time ($F_{2,18}$=11.16;*P*<0.001) with lower average pH on day 1 (pH 6.4) compared with days 3 and 7 (pH 6.6 and 6.5, respectively).

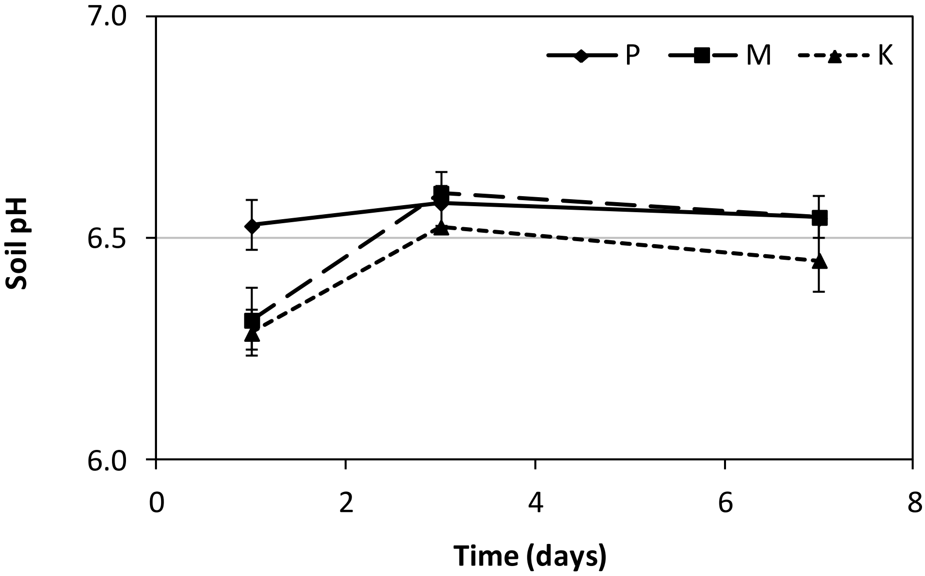

Figure 5.4 Soil pH of soil from beneath pasture (P), manuka (M), and kanuka (K) plant species. Error bars are standard error of the mean (n = 3). See section 3.3.8 for a description of how average pH and standard errors were calculated.

5.4.3 Soil moisture content

Soil moisture content (%) was significantly different among species ($F_{2,18}$=18.53;*P*<0.001). Average pasture soil moisture content was higher than manuka and kanuka species, at 47%, compared with 40% and 37%, respectively (Figure 5.5).

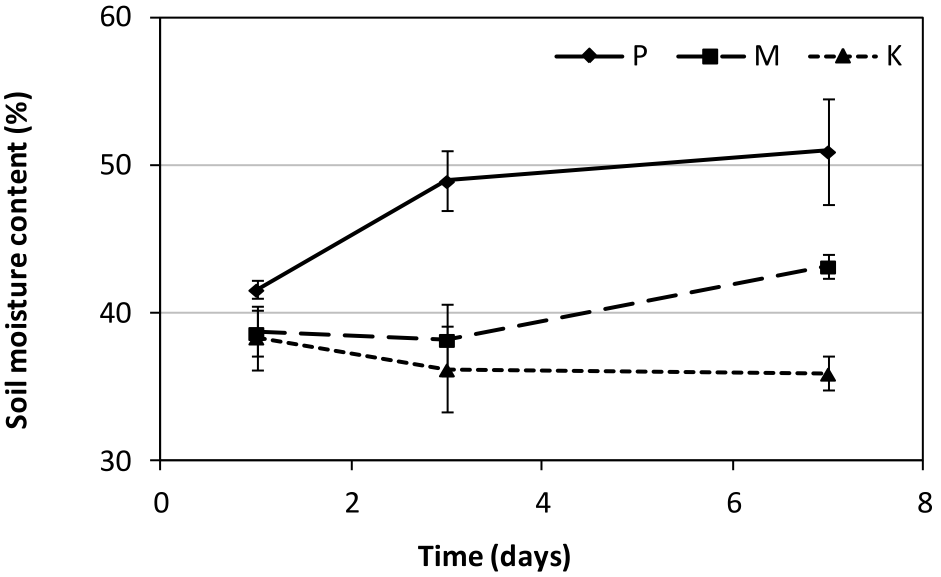

Figure 5.5 Soil moisture content (%) of soil from beneath pasture (P), manuka (M), and kanuka (K) plant species. Error bars are standard error of the mean (n = 3).

5.5 Discussion

The significant drop off in *E. coli* cfu present in the soil beneath kanuka and manuka plants after 3 and 7 days has huge implications for the use of these plants for remediation of contaminated sites. For example the application of dairy shed effluent to land has many negative implications including the addition of pathogenic microbes to soil. If dairy shed effluent were applied to land planted in manuka or kanuka plants, the results from this study indicate the potential ability of these plants to rapidly reduce the numbers of these pathogenic microbes in these systems. If planted adjacent to waterways, these plants could also help to decrease the pathogen contamination of water. A study conducted by Prosser (2011) showed that the presence of manuka water extracts significantly reduced the growth of five bacterial strains (*Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli*, *Campylobacter jejuni* and *Clostridium perfringins*), though *in-situ* effects of antimicrobial ability were not observed. However this has been demonstrated in the current study.

The observed *in-situ* antimicrobial effects of manuka and kanuka plants on the soil in this study are consistent with an inhibitory effect and may have implications for the N cycle and thus the production of N_2O. This is because processes such as nitrification and denitrification are biologically driven processes in the soil. As illustrated in Figure 1.4, there are many potential sites in the N cycle in agricultural soils which antimicrobial compounds could inhibit. It is also possible that these soil microbes involved in the N cycle may respond differently to manuka and kanuka than that of *E. coli* used in this study.

Despite pots sitting in saucers of water in an attempt to maintain and regulate moisture content in the pots, soil in pots planted with pasture tended to have a higher moisture content than manuka and kanuka plants. This could explain the difference in microbial survival beneath the different plant species, however the 10% difference in moisture content between pasture and kanuka pots may not have been large enough to explain such a reduction in *E. coli* cfu.

Limitations of the current study include that it only examined one strain of *E. coli* and in order to provide more useful results relating to land application of effluent, more strains and species of bacterial pathogens should be used. Future studies should look at repeating this in order to confirm these findings, and conduct a field study to determine whether these observations still apply in the field or in different soil types. In terms of the antimicrobial effect having an influence on the N cycle, future studies could look specifically at manuka and kanuka influence on nitrifying and denitrifying bacteria in the soil.

5.6 Conclusion

A decrease in *E. coli* present in the soil beneath manuka and kanuka plants, compared with pasture, was observed 3 and 7 days after the *E. coli* was applied to the soil. This may have been attributed to the higher moisture content in the pasture pots. However, another likely explanation is that the manuka and kanuka had an *in-situ* antimicrobial influence on the *E. coli* added. The study should be repeated to confirm the results, however these findings could have implications for reduced soil and water pathogen contamination on dairy farms. It is also possible that these plants may influence the N cycle, which is biologically driven by soil microbes. Future studies could look at a wider range of bacteria, including nitrifying and denitrifying bacteria to better understand whether antimicrobial inhibition of the N cycle is occurring in the soil beneath these plants.

Chapter 6

General Discussion and Conclusion

6.1 Nitrous oxide

Similar trends between results of the preliminary greenhouse study and field trial were observed. Both indicated that manuka and kanuka plants may reduce N_2O fluxes from the soil. However the limitations of these studies include that N_2O fluxes were only tested on one soil type and under one set of climate conditions. Future studies could examine N_2O fluxes on a range of different soil types and climate conditions beneath manuka and kanuka plants.

6.1.1 Soil nitrate and ammonium levels

Soil nitrate levels following treatment of dairy shed effluent in both the greenhouse experiment and the field experiment were higher under the kanuka. In the greenhouse experiment soil water NO_3^- concentrations under pasture were 88-91% lower than manuka and kanuka levels. This was reinforced by the field study where NO_3^- concentrations extracted from the soil were 81% lower for the bare soil plots than the kanuka plots. As described in Chapter 4 (section 4.5.2) the cause of this increased NO_3^- beneath kanuka is unclear. Possibilities include: increased NO_3^- retention which would reduce NO_3^- leaching allowing more NO_3^- to be taken up by the plant. This could be combined with inhibition of denitrifying bacteria which would reduce N_2O emissions. This was observed in the field trial so could be the mechanism which is going on here. Alternatively, increased NO_3^- could occur due to increased nitrification which would likely result in increased N_2O emissions and have negative environmental effects, but this did not occur in this study.

In both the greenhouse and field study ammonium levels were significantly different between treatments, however in both studies no differences were observed between plant species.

6.1.2 Soil moisture

Soil planted with kanuka tended to have lower moisture content than other plant species. This was first observed in the greenhouse study where kanuka pots had a soil moisture content 7.6% lower than that of pasture and manuka pots. This trend was reinforced by the field study where kanuka plots had a 3% lower moisture content than bare soil plots. The antimicrobial study also measured moisture content and a 10% reduction in soil moisture content was observed between the pasture pots and the pots planted with kanuka. A reduced moisture content beneath kanuka plants would reduce the amount of NO_3^- lost via leaching and in doing so could explain the higher NO_3^- levels observed beneath these plants. Reduced moisture content itself also has implications for N_2O production in that it could increase the amount of partial denitrification which occurs, it also allows more N_2O to be diffused from soil (Bolan *et al.*, 2004; de Klein *et al.*, 2008). At the same time, depending on how aerobic the soils are, denitrification (which occurs under anaerobic conditions) may be inhibited.

6.1.3 Soil pH

Soil pH tended to be lower beneath the kanuka plants than that of pasture or bare soil. Soil pH values in the greenhouse study were 5% higher for the pasture pots than manuka and kanuka pots. The field study reinforced this as bare soil plots had a pH 5% higher than kanuka plots. In the antimicrobial experiment, a similar trend was shown with a 3% higher soil pH in pasture pots than kanuka pots. Soil pH has been described to affect both the rate of nitrification and denitrification (de Klein *et al.*, 2008). Though it is unlikely that these pH changes of <<1 pH unit would have any huge implications in terms of reduced N_2O production. Similarly as described in Chapter 4 Simek *et al.*, (2002) found no simple relationship between denitrifying enzyme activity (DEA) and soil pH as high DEA was found both in acid and alkaline soils. Soil denitfiers appeared to be adapted to their natural soil pH.

6.2 Implications

6.2.1 Possible use on NZ dairy farms

Manuka and kanuka could be strategically incorporated into dairy farms in New Zealand as shelter belts, riparian margins, or in unused corners of paddocks out of the reach of centre pivot irrigation. Another option is to have a block of the farm planted in manuka and kanuka onto which dairy shed effluent is applied (Figure 6.1).

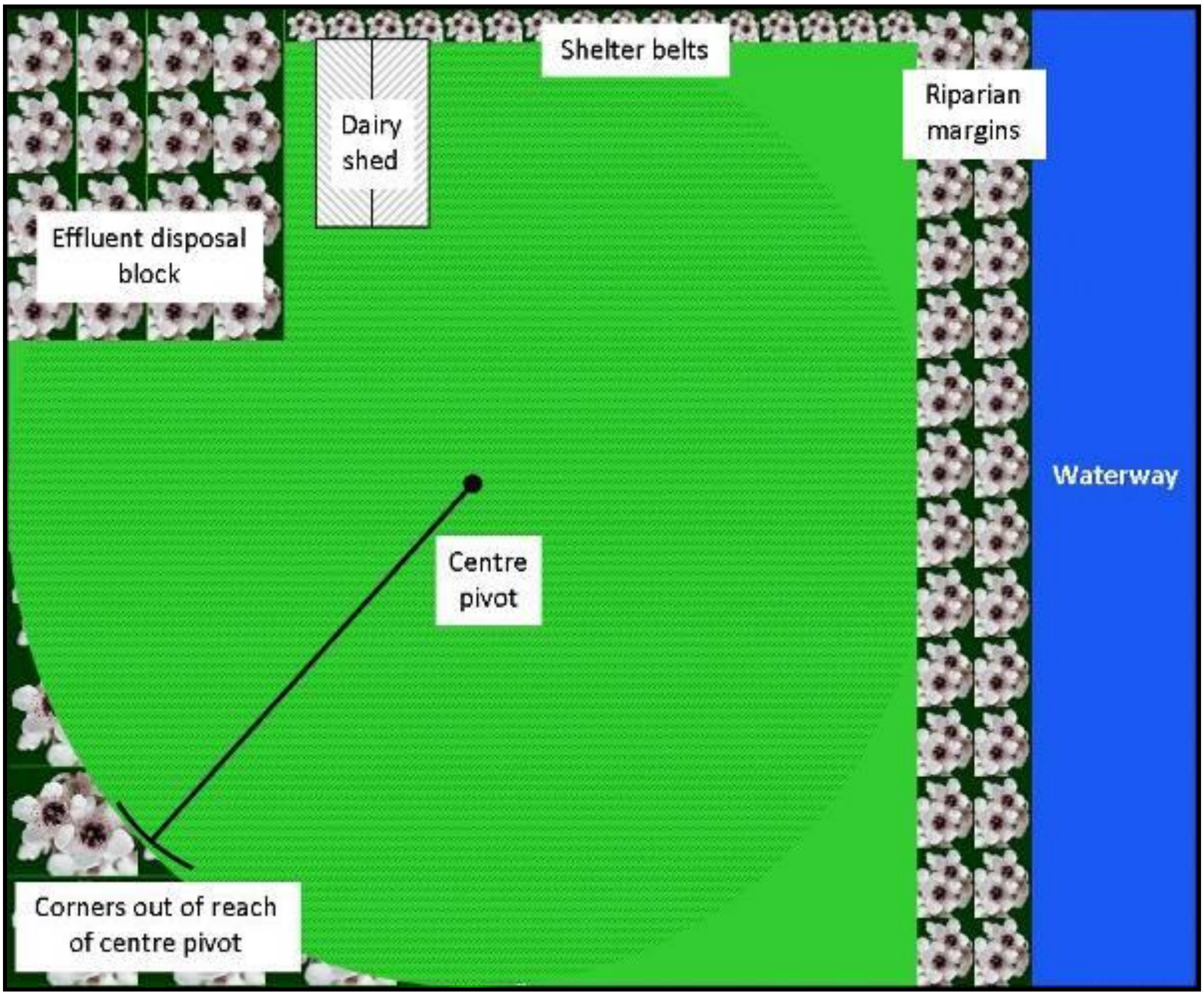

Figure 6.1 Schematic showing potential planting locations of manuka or kanuka on a New Zealand dairy farm (green = pasture).

Effluent disposal blocks

Dairy shed effluent may contain a variety of pathogenic microorganisms, including bacteria, protozoa and viruses. This may penetrate the surface soil layers and contaminate groundwater or runoff to surface waters (Jiang *et al.*, 2008). It is important to ensure that dairy shed effluent irrigated onto land does not cause adverse effects on the wider environment. In their study Jiang *et al.* (2008) correlated bacterial concentration in leachate with volumetric water content and water potential, and sometimes drainage rate. Jiang *et al.* (2010) found that topsoils with higher clay content and soils under flood irrigation were at a high risk of bacteria leaching through preferential flow paths. In a study to determine the fate of N from dairy shed effluent applied to land at rates of 300 or 600 kg N ha^{-1} y^{-1}, Cameron *et al.* (2002) found that 3.7-51.4% was taken up by the pasture, 0.2-0.3% was leached, 8.4-12.2% was lost by denitrification, 51.6-63.6% was recovered in the soil organic N, and <1% was lost by volatilisation.

Disposal of effluent onto blocks planted in manuka and kanuka which have a dense, intricate root system could allow for increased uptake of nitrogen before it is leached. If field antimicrobial effects of manuka and kanuka are found, planting these onto effluent disposal blocks have the potential to kill off or reduce the numbers of faecal bacteria from the effluent. In support of this, Prosser (2011) found that manuka water extracts significantly reduced the growth of five pathogenic bacterial strains potentially found in biosolids which are likely to be similar to that of dairy shed effluent.

6.2.2 Implications for strategic planting of manuka and kanuka on NZ dairy farms

When attempting to incorporate manuka and kanuka onto New Zealand dairy farms, implications to consider include: the status of the soil of the dairy farm including nutrient levels and soil physical characteristics such as soil compaction; whether manuka and kanuka can cope in the high nitrogen environments found on dairy farms; how they cope under high moisture environments near waterways or under irrigation; and whether they can establish.

Soil status

Manuka and kanuka both form ectomycorrhizal interactions and share many ectomycorrhizal fungal species (McKenzie *et al.*, 2006). These have the main role of improved phosphorus uptake which allows for rapid growth and exploitation of available light. The level of infection is correlated with the available phosphorus and growth conditions (Stephens *et al.*, 2005). Kanuka stands were found to be of a lower overall elevation than manuka stands and had higher levels of soil available P (Davis & Smaill, 2009). Manuka was found to be more tolerant of low fertility soils, whereas kanuka was more successful on fertile soils (Whitehead *et al.*, 2004). Thus through their ectomycorrhizal interactions and varying tolerance of fertility, between the two species, at least one should be able to grow well on a selected dairy farm.

Soil compaction can be a common problem for restoration on land which has been grazed. In a study by Bassett (2003) manuka seedlings were shown to have improved establishment at an intermediate compaction level, which showed a degree of compaction tolerance. Root length and shoot height decreased with increasing compaction. Manuka seedling root length was reduced to ≤ 37% of its maximum at 1.3 MPa (Bassett, 2003). This provides some evidence that compaction, such as what may occur on dairy farms, is unlikely to impede establishment of manuka.

High nitrogen environments

Manuka stands are often found on low fertile sites, whereas kanuka tends to be found on more fertile sites (Whitehead *et al.*, 2004). Thus it is difficult to determine whether these plant species will do well when strategically planted on dairy farms. In natural systems, on high fertility sites manuka and kanuka are often succeeded by taller forest species (Stephens *et al.*, 2005). For this reason weed control may be necessary at least for the first year. Further research should determine the tolerance of manuka and kanuka to high nitrogen levels similar to what may be found on a typical dairy farm. Though the statement that kanuka tends to be found on more fertile sites suggests that it may be more able to grow on a dairy farm.

Moisture

Manuka is often found to be the dominant species on soil that is too wet, dry or infertile for other forest species (Stephens *et al.*, 2005). Kanuka does not tolerate the poorly drained sites which manuka can be found on (Wardle, 2002; Wardle, 2011). It only forms a self perpetuating community in the driest parts of the country such as Central Otago and parts of Marlborough and is very tolerant of drought. Elsewhere, where rainfall is higher, kanuka has a major role as a coloniser of new ground or short vegetation following destruction of forest by fire, erosion or other agents (Wardle, 2011). On dry sites manuka growth tends to be stunted (Wardle, 2002).

Establishment

In general, manuka flowers from October to February. This can occur even when plants are only a few centimetres high. The flowers develop in the leaf axils, are normally 6-12 mm in size and are pure white or slightly tinged with pink. Following pollination a hard woody capsule develops which is about 7 mm in diameter and divided into five segments. These capsules often remain closed for a number of years. When they open, usually as a result of fire or during hot dry weather, numerous minute seeds are released from each of the five segments (Wardle, 2011). Manuka establishes readily on exposed soil ground by direct seeding (Bergin *et al.*, 1995; Wardle, 2011). This can be accomplished by scattering ripe manuka seed capsules over the ground, or by laying a mulch of cut manuka containing ripe or near ripe seed capsules. Germination usually occurs within about 10 days. Cuttings taken from March to May can also be used, though it is usually necessary to apply a hormone treatment (Wardle, 2011). The abundant flowering of manuka in young trees, copious production of fine seeds which are easily dispersed by wind aid its rapid establishment on disturbed sites (Whitehead *et al.*, 2004; Ross *et al.*, 2009).

Different chemotypes of manuka have been identified, each containing different compositions of the chemical constituents: triketones and monoterpenes. This is likely to have implications for choosing an appropriate seed source for manuka to establish on a dairy farm, so should be taken into account, especially in terms of antimicrobial compounds.

Kanuka also produces flowers while still quite young. The flowers are small, 3-6 mm in diameter, white, sometimes with a yellowish tinge. They occur either singly or in clusters of up to five individuals with each suspended on its own short flower stalk about 5 mm long. Flowering occurs in the summer and the seed capsules ripen the following autumn, from March to May. The seed capsules are small, 2-4 mm in diameter, and easily crushed by hand. The seeds are very small and are produced in large quantities and therefore well suited to wind dispersal. Kanuka is easily propagated using seed, with germination usually occurring within two weeks. When necessary, kanuka can be propagated from semi-hardwood cuttings preferably taken during March and April, but it is best to treat with hormones and success rates may be variable (Wardle, 2011). Kanuka is a strong light demander which will not regenerate either in the deep shade of other forest species or even under its own parent canopy (Wardle, 2011). Thus weed control is likely to be necessary prior to its establishment.

6.3 Conclusion

The closed-chamber method developed for measurement of N_2O fluxes from pots in the greenhouse showed promise following method testing, however, N_2O fluxes were not able to be obtained from the full-scale trial. This indicated that in order for successful measurements the method would need to be refined and the chamber volume to soil surface area ratio reduced to give greater sensitivity. The preliminary trial indicated that manuka plants showed reduced N_2O fluxes.

Field N_2O fluxes were significantly higher from control plots treated with effluent compared with kanuka plots with effluent. Higher soil NO_3^- levels were found beneath kanuka plants than control plots though these did not result in increased N_2O emissions, instead they were decreased. It was unclear whether this was a result of increased NO_3^- retention, inhibition of denitrification or increased nitrification. This line of research has implications for reduced environmental impacts of dairy farms in NZ, increased biodiversity, and increased public perception of dairy farms.

A decrease in *E. coli* present in the soil beneath manuka and kanuka plants, compared with pasture, was observed 3 and 7 days after the *E. coli* was applied to the soil. This may have been attributed to the higher moisture content in the pasture pots. However, another likely explanation is that the manuka and kanuka had an *in-situ* antimicrobial influence on the *E. coli* added. These findings could have implications for reduced soil and water pathogen contamination on dairy farms. It is also possible that these plants may influence the N cycle, which is biologically driven by soil microbes.

Manuka and kanuka could be strategically incorporated into NZ dairy farms on effluent disposal blocks, as riparian margins, as shelter belts or in the corners of paddocks out of reach of centre pivot irrigation. Implications to consider include the status of the soil including fertility and compaction; moisture status or drainage; and ability to establish.

All of the experiments reported here should be repeated to confirm findings. Future studies could include ^{15}N tracer studies to determine whether the N_2O emitted is produced by nitrification or denitrification. These studies could also help to explain the higher NO_3^- found beneath kanuka trees compared with bare soil. In antimicrobial experiments, a wider range of bacteria, including nitrifying and denitrifying bacteria could be examined to better understand whether antimicrobial inhibition of the N cycle is occurring in the soil beneath manuka and kanuka plants.

References

Adams, C. J., Manley-Harris, M., & Molan, P. C. (2009). The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydrate Research, 344*(8), 1050-1053. doi:10.1016/j.carres.2009.03.020

Aeschlimann, U., Nosberger, J., Edwards, P. J., Schneider, M. K., Richter, M., & Blum, H. (2005). Responses of net ecosystem CO_2 exchange in managed grassland to long-term CO_2 enrichment, N fertilization and plant species. *Plant Cell and Environment, 28*(7), 823-833. doi:10.1111/j.1365-3040.2005.01331.x

Aldridge, R., & Jackson, R. J. (1968). Interception of rainfall by manuka (*Leptospermum scoparium*) at Taita, New Zealand. *New Zealand Journal of Science, 11*(2), 301-317.

Allen, K. L., Molan, P. C., & Reid, G. M. (1991). A survey of the antimicrobial activity of some New Zealand honeys. *Journal of Pharmacy and Pharmacology, 43*, 817-822.

Ambus, P., Petersen, S. O., & Soussana, J. F. (2007). Short-term carbon and nitrogen cycling in urine patches assessed by combined carbon-13 and nitrogen-15 labelling. *Agriculture Ecosystems & Environment, 121*(1-2), 84-92. doi:10.1016/j.agee.2006.12.007

Azam, F., Muller, C., Weiske, A., Benckiser, G., & Ottow, J. C. G. (2002). Nitrification and denitrification as sources of atmospheric nitrous oxide - role of oxidizable carbon and applied nitrogen. *Biology and Fertility of Soils, 35*(1), 54-61. doi:10.1007/s00374-001-0441-5

Badet, C., & Quero, F. (2011). The in vitro effect of manuka honeys on growth and adherence of oral bacteria. *Anaerobe, 17*(1), 19-22. doi:10.1016/j.anaerobe.2010.12.007

Baggs, E. M. (2008). A review of stable isotope techniques for N_2O source partitioning in soils: recent progress, remaining challenges and future considerations. *Rapid Communications in Mass Spectrometry, 22*(11), 1664-1672. doi:10.1002/rcm.3456

Bakken, L., & Dorsch, P., & H. Bothe, S. J. Ferguson & W. E. Newton. (2007). *Nitrous oxide emission and global changes: modelling approaches* [Biology of the Nitrogen cycle]. Amsterdam; Boston: Elsevier.

Bassett, I. E. (2003). *Cabbage tree and Manuka seedling responses to soil compaction: Implications for ecological restoration.* The University of Auckland, Auckland, NZ.

Bergin, D. O., Kimberley, M. O., & Marden, M. (1995). Protective value of regenerating tea tree stands on erosion-prone hill country, East Coast, North Island, New Zealand. *New Zealand Journal of Forestry Science, 25*(1), 3-19.

Bhandral, R., Bolan, N. S., & Saggar, S. (2010). Nitrous oxide emission from farm dairy effluent application in grazed grassland. *Journal of Soil Science and Plant Nutrition, 10*(1), 22-34.

Bolan, N. S., Saggar, S., Luo, J. F., Bhandral, R., & Singh, J. (2004). Gaseous emissions of nitrogen from grazed pastures: Processes, measurements and modelling, environmental implications, and mitigation. In D. L. Sparks (Ed.), *Advances in Agronomy,* (Vol. 84, pp. 37-120). doi:10.1016/s0065-2113(04)84002-1

Boquel, G., & Suavin, L. (1972). Inhibition of nitrification by aqueous extracts of litter of *Tectona grandis* and *Melaleuca leucadendron*. *Revue d'Ecologie et de Biologie du Sol, 9*(4), 641-654.

Bremner, J. M. (1997). Sources of nitrous oxide in soils. *Nutrient Cycling in Agroecosystems, 49*(1-3), 7-16. doi:10.1023/a:1009798022569

Brooker, S. G., & Cooper, R. C. (1961). New Zealand medicinal plants. *Economic Botany, 15*(1), 1-10. doi:10.1007/bf02906757

Burke, C. R., & Verkerk, G. A. (2010). The development of reproductive management practices in New Zealand: what will the future hold in a consumer-focused, environmentally-conscious, export-driven marketplace? In M. C. Lucy, J. L. Pate, M. F. Smith & T. E. Spencer (Eds.), *Reproduction in Domestic Ruminants Vii* (Vol. 67, pp. 341-355). Loughborough: Nottingham University Press.

Cameron, K. C., Di, H. J., Reijnen, B. P. A., Li, Z., Russell, J. M., & Barnett, J. W. (2002). Fate of nitrogen in dairy factory effluent irrigated onto land. *New Zealand Journal of Agricultural Research, 45*(3), 207-216.

Chapuis-Lardy, L., Wrage, N., Metay, A., Chotte, J.-L., & Bernoux, M. (2007). Soils, a sink for N_2O? A review. *Global Change Biology, 13*(1), 1-17. doi:10.1111/j.1365-2486.2006.01280.x

Christoph, F., Kubeczka, K. H., & Stahl-Biskup, E. (1999). The composition of commercial manuka oils from New Zealand. *Journal of Essential Oil Research, 11*(6), 705-710.

Clough, T. J., Bertram, J. E., Ray, J. L., Condron, L. M., O'Callaghan, M., Sherlock, R. R., & Wells, N. S. (2010). Unweathered wood biochar impact on nitrous oxide emissions from a bovine-urine-amended pasture soil. *Soil Science Society of America Journal, 74*(3), 852-860. doi:10.2136/sssaj2009.0185

Clough, T. J., & Kelliher, F. M. (2005). Dairy farm effluent effects on urine patch nitrous oxide and carbon dioxide emissions. *Journal of Environmental Quality, 34*(3), 979-986. doi:10.2134/jeq2004.0360

Clough, T. J., Sherlock, R. R., & Kelliher, F. M. (2003). Can liming mitigate N_2O fluxes from a urine-amended soil? *Soil Research, 41*(3), 439-457. doi:10.1071/SR02079

Collins, H. P., Alva, A. K., Streubel, J. D., Fransen, S. F., Frear, C., Chen, S., Kruger, C., & Granatstein, D. (2011). Greenhouse gas emissions from an irrigated silt loam soil amended with anaerobically digested dairy manure. *Soil Science Society of America Journal, 75*(6), 2206-2216. doi:10.2136/sssaj2010.0360

Davis, M., & Smaill, S. (2009). Mycorrhizal colonisation of exotic conifers in kanuka and manuka shrublands. *New Zealand Journal of Ecology, 33*(2), 147-155.

de Klein, C. A. M., Pinares-Patino, C., & Waghorn, G. C. (2008). Greenhouse gas emissions. In R. W. McDowell (Ed.), *Environmental impacts of pasture-based farming* (pp. 1-32)

de Klein, C. A. M., Sherlock, R. R., Cameron, K. C., & van der Weerden, T. J. (2001). Nitrous oxide emissions from agricultural soils in New Zealand - a review of current knowledge and directions for future research. *Journal of the Royal Society of New Zealand, 31*(3), 543-574.

DeLuca, T. H., MacKenzie, M. D., & Gundale, M. J. (2009). Biochar effects on soil nutrient transformations. In J. Lehmann & S. Joseph (Eds.), *Biochar for environmental management: science and technology*. London; Sterling, VA: Earthscan.

Di, H. J., & Cameron, K. C. (2000). Calculating nitrogen leaching losses and critical nitrogen application rates in dairy pasture systems using a semi-empirical model. *New Zealand Journal of Agricultural Research, 43*(1), 139-147.

Di, H. J., & Cameron, K. C. (2002). Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutrient Cycling in Agroecosystems, 64*(3), 237-256. doi:10.1023/a:1021471531188

Di, H. J., & Cameron, K. C. (2012). How does the application of different nitrification inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in grazed pastures? *Soil Use and Management, 28*(1), 54-61. doi:10.1111/j.1475-2743.2011.00373.x

Douglas, M., Anderson, R., van Klink, J., Perry, N., & Smallfield, B. (2001). *Defining North Island manuka chemotype resources* (447): New Zealand Institute for Crop and Food Research Limited.

Gopalakrishnan, S., Watanabe, T., Pearse, S. J., Ito, O., Hossain, Z., & Subbarao, G. V. (2009). Biological nitrification inhibition by *Brachiaria humidicola* roots varies with soil type and inhibits nitrifying bacteria, but not other major soil microorganisms. *Soil Science and Plant Nutrition, 55*(5), 725-733. doi:10.1111/j.1747-0765.2009.00398.x

Haile, W., Mala, T., Osotsapar, Y., & Verasan, V. (2006). Screening of Ethiopian traditional medicinal herbs for their nitrification inhibiting ability. *Kasetsart Journal, Natural Sciences, 40*(1), 74-82.

Howarth, R. W. (1988). Nutrient limitation of net primary production in marine ecosystems. *Annual Review of Ecology and Systematics, 19*, 89-110. doi:10.1146/annurev.ecolsys.19.1.89

Hutchinson, G. L., & Mosier, A. R. (1981). Improved soil cover method for field measurement of nitrous-oxide fluxes. *Soil Science Society of America Journal, 45*(2), 311-316.

Ingerson-Mahar, M., & Reid, A. (2011). *E. coli:* good, bad, & deadly. *American Academy of Microbiology*.

IPCC. (2007a). Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In Core Writing Team, R. K. Pachauri & A. Reisinger (Eds.). Geneva, Switzerland

IPCC. (2007b). Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor & H. L. Miller (Eds.). Cambridge, UK; New York, NY, USA: Cambridge University Press.

Jay, M. (2005). Remnants of the Waikato: native forest survival in a production landscape. *New Zealand Geographer, 61*(1), 14-28.

Jeong, E.-Y., Jeon, J.-H., Kim, H.-W., Kim, M.-G., & Lee, H.-S. (2009). Antimicrobial activity of leptospermone and its derivatives against human intestinal bacteria. *Food Chemistry, 115*, 1401-1404.

Jervis-Bardy, J., Foreman, A., Bray, S., Tan, L., & Wormald, P. (2011). Methylglyoxal-infused honey mimics the anti-*Staphylococcus aureus* biofilm activity of manuka honey: potential implication in chronic rhinosinusitis. *Laryngoscope, 121*(5), 1104-1107. doi:10.1002/lary.21717

Jezierska-Tys, S., & Frqc, M. (2007). CO_2, N_2O and NH_3 emissions from two different type of soils as affected by applications of dairy sewage sludge. *International Agrophysics, 21*(4), 323-328.

Jiang, S., Buchan, G. D., Noonan, M. J., Smith, N., Pang, L., & Close, M. (2008). Bacterial leaching from dairy shed effluent applied to a fine sandy loam under irrigated pasture. *Australian Journal of Soil Research, 46*(6-7), 552-564. doi:10.1071/sr07216

Jiang, S., Pang, L., Buchan, G. D., Simunek, J., Noonan, M. J., & Close, M. E. (2010). Modeling water flow and bacterial transport in undisturbed lysimeters under irrigations of dairy shed effluent and water using HYDRUS-1D. *Water Research, 44*(4), 1050-1061. doi:10.1016/j.watres.2009.08.039

Kammann, C., Ratering, S., Eckhard, C., & Muller, C. (2012). Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, and methane) fluxes from soils. *Journal of Environmental Quality, 41*(4), 1052-1066. doi:10.2134/jeq2011.0132

Kelly, K. B., Phillips, F. A., & Baigent, R. (2008). Impact of dicyandiamide application on nitrous oxide emissions from urine patches in northern Victoria, Australia. *Australian Journal of Experimental Agriculture, 48*(1-2), 156-159. doi:10.1071/ea07251

Kim, C. H., & Hollocher, T. C. (1983). N-15 tracer studies on the reduction of nitrite by the purified dissimilatory nitrite reductase of *Pseudomonas aeruginosa* - evidence for direct production of N_2O without free NO as an intermediate. *Journal of Biological Chemistry, 258*(8), 4861-4863.

Knowles, O. A., Robinson, B. H., Contangelo, A., & Clucas, L. (2011). Biochar for the mitigation of nitrate leaching from soil amended with biosolids. *Science of the Total Environment, 409*(17), 3206-3210. doi:10.1016/j.scitotenv.2011.05.011

Li, K., Gong, Y., Song, W., Lv, J., Chang, Y., Hu, Y., Tian, C., Christie, P., & Liu, X. (2012). No significant nitrous oxide emissions during spring thaw under grazing and nitrogen addition in an alpine grassland. *Global Change Biology, 18*(8), 2546-2554.

Luo, J., de Klein, C., Shepherd, M., & Ledgard, S. (2010). Effect of nitrification inhibitor on nitrous oxide emissions in pasture soils. *Proceedings of the 19th World Congress of Soil Science: Soil solutions for a changing world, Brisbane, Australia, 1-6 August 2010. Congress Symposium 4: Greenhouse gases from soils*, 59-62.

Luo, J., Saggar, S., Bhandral, R., Bolan, N., Ledgard, S., Lindsey, S., & Sun, W. (2008). Effects of irrigating dairy-grazed grassland with farm dairy effluent on nitrous oxide emissions. *Plant and Soil, 309*(1-2), 119-130. doi:10.1007/s11104-008-9550-3

Maddocks-Jennings, W., Wilkinson, J. M., Shillington, D., & Cavanagh, H. (2005). A fresh look at manuka and kanuka essential oils from New Zealand. *International Journal of Aromatherapy, 15*(3), 141-146. doi:10.1016/j.ijat.2005.07.003

Mavric, E., Wittmann, S., Barth, G., & Henle, T. (2008). Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. *Molecular Nutrition & Food Research, 52*(4), 483-489. doi:10.1002/mnfr.200700282

McKenzie, E. H. C., Johnston, P. R., & Buchanan, P. K. (2006). Checklist of fungi on teatree (*Kunzea* and *Leptospermum* species) in New Zealand. *New Zealand Journal of Botany, 44*(3), 293-335.

McLaren, R. G., & Cameron, K. C. (1996). Soil, plant and fertiliser nitrogen. In R. G. McLaren & K. C. Cameron (Eds.), *Soil Science: Sustainable production and environmental protection* (pp. 192-207). Auckland, N.Z.: Oxford University Press.

Meurk, C. D., & Hall, G. M. J. (2006). Options for enhancing forest biodiversity across New Zealand's managed landscapes based on ecosystem modelling and spatial design. *New Zealand Journal of Ecology, 30*(1), 131-146.

Ministry for the Environment. (2011, 16 December). Retrieved 26 April, 2012, from http://www.climatechange.govt.nz/emissions-trading-scheme/participating/agriculture/

Ministry for the Environment. (2012). *New Zealand's Greenhouse Gas Inventory 1990-2010*. Retrieved16 April, 2012,from http://www.mfe.govt.nz/publications/climate/greenhouse-gas-inventory-2010/index.html

Mosier, A., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S., & van Cleemput, O. (1998). Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle - OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. *Nutrient Cycling in Agroecosystems, 52*(2-3), 225-248. doi:10.1023/a:1009740530221

Muller, C. (1995). *Nitrous oxide emission from intensive grassland in Canterbury, New Zealand*. Lincoln University, Christchurch, NZ.

Nazra, A. (2011). *Understanding the spread of riparian restoration in the Te Waihora/Lake Ellesmere catchment*. Lincoln University, Christchurch, NZ.

Norton, D. A., & Miller, C. J. (2000). Some issues and options for the conservation of native biodiversity in rural New Zealand. *Ecological Management & Restoration, 1*(1), 26-34. doi:10.1046/j.1442-8903.2000.00005.x

O.I. Analytical. *Nitrate/nitrite in soils by Cd reduction: Method Abstract*. Retrieved October 25, 2012,from http://www.oico.com/default.aspx

O.I. Analytical. *TKN by Gas Diffusion and FIA: Method Abstract*. Retrieved October 25, 2012,from http://www.oico.com/default.aspx

Pereira, J., Fangueiro, D., Chadwick, D. R., Misselbrook, T. H., Coutinho, J., & Trindade, H. (2010). Effect of cattle slurry pre-treatment by separation and addition of nitrification inhibitors on gaseous emissions and N dynamics: A laboratory study. *Chemosphere, 79*(6), 620-627. doi:10.1016/j.chemosphere.2010.02.029

Pham, V. H. T., & Kim, J. (2012). Cultivation of unculturable soil bacteria. *Trends in Biotechnology, 30*(9), 475-484. doi:10.1016/j.tibtech.2012.05.007

Pinares-Patino, C. S., Waghorn, G. C., Hegarty, R. S., & Hoskin, S. O. (2009). Effects of intensification of pastoral farming on greenhouse gas emissions in New Zealand. *New Zealand Veterinary Journal, 57*(5), 252-261.

Porter, N. G., & Wilkins, A. L. (1998). Chemical, physical and antimicrobial properties of essential oils of *Leptospermum scoparium* and *Kunzea ericoides*. *Phytochemistry, 50*(3), 407-415. doi:10.1016/s0031-9422(98)00548-2

Prosser, J. (2011). *Manuka (Leptospermum scoparium) as a remediation species for biosolids amended land*. Massey University, New Zealand.

Ross, D. J., Scott, N. A., Lambie, S. M., Trotter, C. M., Rodda, N. J., & Townsend, J. A. (2009). Nitrogen and carbon cycling in a New Zealand pumice soil under a manuka (*Leptospermum scoparium*) and kanuka (*Kunzea ericoides*) shrubland. *Australian Journal of Soil Research, 47*(7), 725-736. doi:10.1071/sr08261

Russell, K. M., Molan, P. C., Wilkins, A. L., & Holland, P. T. (1990). Identification of some antibacterial constituents of New Zealand manuka honey. *Journal of Agricultural and Food Chemistry, 38*(1), 10-13. doi:10.1021/jf00091a002

Schnitzler, P., Wiesenhofer, K., & Reichling, J. (2008). Comparative study on the cytotoxicity of different Myrtaceae essential oils on cultured Vero and RC-37 cells. *Pharmazie, 63*(11), 830-835. doi:10.1691/ph.2008.8597

Scott, N. A., White, J. D., Townsend, J. A., Whitehead, D., Leathwick, J. R., Hall, G. M. J., Marden, M., Rogers, G. N. D., Watson, A. J., & Whaley, P. T. (2000). Carbon and nitrogen distribution and accumulation in a New Zealand scrubland ecosystem. *Canadian Journal of Forest Research, 30*, 1246-1255.

Sherlock, O., Dolan, A., Athman, R., Power, A., Gethin, G., Cowman, S., & Humphreys, H. (2010). Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complementary and Alternative Medicine, 10*(47).

Sherlock, R. R. (1992). Nitrous oxide sources and sinks: a New Zealand perspective. In W. Williams (Ed.), *Global change: impacts on agriculture and forestry, proceedings of the conference held at Science House, Wellington, 13-14 August, 1991*. Wellington, NZ: The Royal Society of New Zealand.

Simek, M., Jisova, L., & Hopkins, D. W. (2002). What is the so-called optimum pH for denitrification in soil? *Soil Biology & Biochemistry, 34*(9), 1227-1234. doi:10.1016/s0038-0717(02)00059-7

Smith, L. C., de Klein, C. A. M., & Catto, W. D. (2008). Effect of dicyandiamide applied in a granular form on nitrous oxide emissions from a grazed dairy pasture in Southland, New Zealand. *New Zealand Journal of Agricultural Research, 51*(4), 387-396.

Statistics New Zealand. (2011, 22 December). *Infoshare.* Retrieved 13 April, 2012,from http://www.stats.govt.nz/infoshare/ViewTable.aspx?pxID=f1307a0f-bf2b-4bbd-9146-54eceadff182

Stephens, J. M., Schlothauer, R. C., Morris, B. D., Yang, D., Fearnley, L., Greenwood, D. R., & Loomes, K. M. (2010). Phenolic compounds and methylglyoxal in some New Zealand manuka and kanuka honeys. *Food Chemistry, 120*(1), 78-86. doi:10.1016/j.foodchem.2009.09.074

Stephens, J. M. C., Molan, P. C., & Clarkson, B. D. (2005). A review of *Leptospermum scoparium* (Myrtaceae) in New Zealand. *New Zealand Journal of Botany, 43*(2), 431-449.

Stewart, E. J. (2012). Growing Unculturable Bacteria. *Journal of Bacteriology, 194*(16), 4151-4160. doi:10.1128/jb.00345-12

Sykes, W. R. (2002). *Poisonous Plants in New Zealand - Introduction*. Retrieved 14 June, 2012,from http://www.landcareresearch.co.nz/publications/infosheets/poisonplants/poisplants_intro.asp

Uchida, Y. (2010). *The effects of substrate, temperature and soil fertility on respiration and N_2O production in pastoral soils*. Lincoln University.

van Klink, J. W., Brophy, J. J., Perry, N. B., & Weavers, R. T. (1999). beta-triketones from Myrtaceae: isoleptospermone from *Leptospermum scoparium* and papuanone from *Corymbia dallachiana*. *Journal of Natural Products, 62*(3), 487-489. doi:10.1021/np980350n van Zwieten, L., Singh, B., Joseph, S., Kimber, S., Cowie, A. L., & Chan, K. Y. (2009). Biochar and emissions of non-CO_2 greenhouse gases from soil. In J. Lehmann & S. Joseph (Eds.), *Biochar for environmental management: science and technology*. London; Sterling, VA: Earthscan.

Visavadia, B. G., Honeysett, J., & Danford, M. H. (2008). Manuka honey dressing: An effective treatment for chronic wound infections. *British Journal of Oral and Maxillofacial Surgery, 46*, 55-56.

Walls, G. (2009). *Te Aka Kakariki: Greenway Canterbury, Independent report to justify a funding application*. Christchurch: Taramoa Limited.

Ward, B. B., Courtney, K. J., & Langenheim, J. H. (1997). Inhibition of *Nitrosomonas europaea* by monoterpenes from coastal redwood (*Sequoia sempervirens*) in whole-cell studies. *Journal of Chemical Ecology, 23*(11), 2583-2598. doi:10.1023/B:JOEC.0000006668.48855.b7

Wardle, J. (2011). *Wardle's Native Trees of New Zealand and their story*. Wellington: New Zealand Farm Forestry Association.

Wardle, P. (2002). *Vegetation of New Zealand*. Caldwell, N. J.: The Blackburn Press.

Watson, A., & O'Loughlin, C. (1985). Morphology, strength, and biomass of manuka roots and their influence on slope stability. *New Zealand Journal of Forestry Science, 15*, 337-348.

Weston, R. J., Brocklebank, L. K., & Lu, Y. R. (2000). Identification and quantitative levels of antibacterial components of some New Zealand honeys. *Food Chemistry, 70*(4), 427-435. doi:10.1016/s0308-8146(00)00127-8

Whitehead, D., Walcroft, A. S., Scott, N. A., Townsend, J. A., Trotter, C. M., & Rogers, G. N. D. (2004). Characteristics of photosynthesis and stomatal conductance in the shrubland species manuka (*Leptospermum scoparium*) and kanuka (*Kunzea ericoides*) for the estimation of annual canopy carbon uptake. *Tree Physiology, 24*(7), 795-804.

Wilson, G. A. (1992). A survey on attitudes of landholders to native forest on farmland. *Journal of Environmental Management, 34*(2), 117-136. doi:10.1016/s0301-4797(06)80018-6

Wrage, N., van Groenigen, J. W., Oenema, O., & Baggs, E. M. (2005). A novel dual-isotope labelling method for distinguishing between soil sources of N_2O. *Rapid Communications in Mass Spectrometry, 19*(22), 3298-3306. doi:10.1002/rcm.2191

Zakir, H. A. K. M., Subbarao, G. V., Pearse, S. J., Gopalakrishnan, S., Ito, O., Ishikawa, T., Kawano, N., Nakahara, K., Yoshihashi, T., Ono, H., & Yoshida, M. (2008). Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (*Sorghum bicolor*). *New Phytologist, 180*(2), 442-451. doi:10.1111/j.1469-8137.2008.02576.x

Zaman, M., Nguyen, M. L., Blennerhassett, J. D., & Quin, B. F. (2008). Reducing NH_3, N_2O and NO_3^--N losses from a pasture soil with urease or nitrification inhibitors and elemental S-amended nitrogenous fertilizers. *Biology and Fertility of Soils, 44*(5), 693-705. doi:10.1007/s00374-007-0252-4

Appendix A

Further benefits of manuka and kanuka on farms

A.1 Nitrate leaching

Nitrate (NO_3^-) leaching occurs when there is an accumulation of nitrate in the soil profile that coincides with or is followed by a period of high drainage (Di & Cameron, 2002). The leaching of NO_3^- and the contamination of ground and surface waters are a major concern and intensive agriculture is considered to be a major contributor. High concentrations of NO_3^- in drinking water can be harmful to human health. For this reason the New Zealand Ministry of Health have established a drinking water standard of 11.3 mg NO_3-N L^{-1} (Di & Cameron, 2000). Contamination of surface waters by leached NO_3^- causes eutrophication (a process by which high nutrient levels cause algal blooms on the surface of water ways which consume oxygen causing other aquatic life to die) (Howarth, 1988).

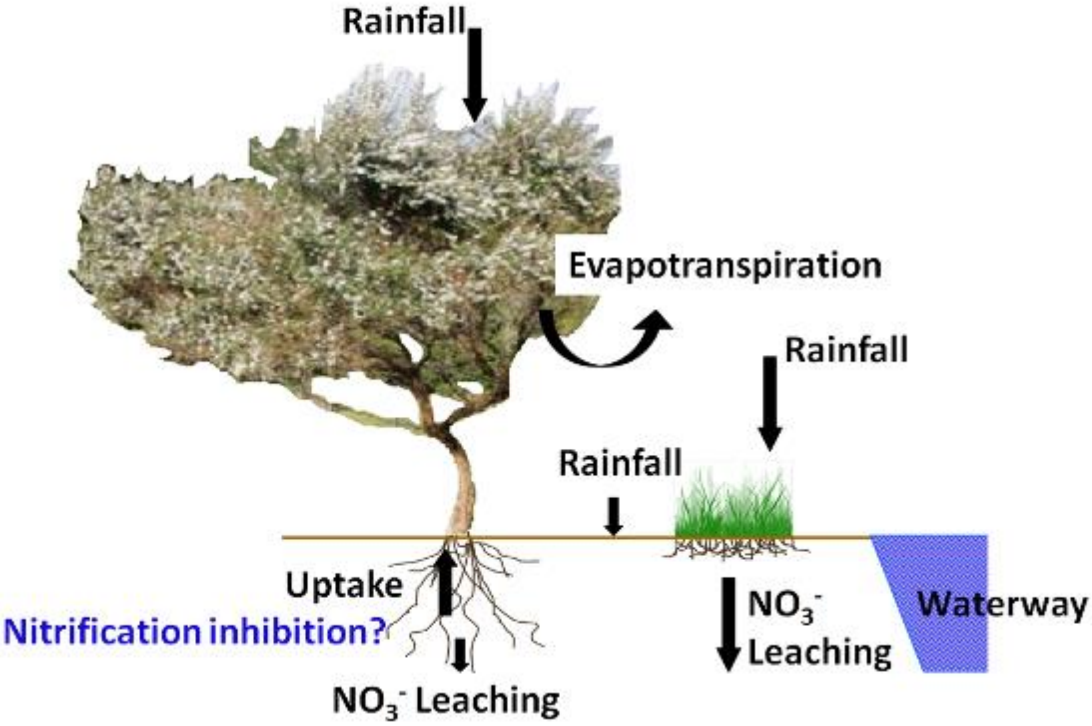

Figure A 1 The potential benefits effects of manuka and kanuka on nitrate leaching.

If antimicrobial effects are found in the field, potential benefits of strategically incorporating manuka or kanuka onto dairy farms include reduced nitrification through inhibition of the microbes involved.

This could prevent much of the soil nitrogen to be present as NO_3^- and thus less able to be leached (Figure A 1).

Planting manuka around waterways or on blocks where high nitrogen containing dairy shed effluent is applied could allow uptake of N through manuka's intricate root system (Watson & O'Loughlin, 1985) before it is able to reach the waterways or be leached. Another possible benefit of this shallow, intricate root system is that it could reduce water movement through the soil through increased removal of moisture in the surface layers (Prosser, 2011). Reduced drainage of water through this mechanism would decrease the amount of NO_3^- lost.

Another benefit of manuka and kanuka, being shrub species, is that when grown together they form a canopy or dense thicket (Wardle, 2011). This reduces the intensity of the rainfall experienced beneath the canopy. Decreased infiltration, and thus reduced drainage, can subsequently reduce the amount of NO_3^- leached. Aldridge and Jackson (1968) measured the net rainfall beneath a 4.5 m tall stand of manuka. Rainfalls less than 1.27 mm gave no measurable rainfall beneath the canopy. Whereas when rainfall events were greater than 6.35 mm, 40-50% of the gross rainfall reached the ground as throughfall. Increased storm size, increased the proportion of the gross rainfall which reached the ground as stemflow, which contributed almost half of the net rainfall from storms greater than 38.1 mm. Over a 10 month period, interception loss by the manuka canopy amounted to 39% of the gross rainfall.

A.2 Carbon sequestration and soil stabilisation

There has been interest in the potential for managing manuka plantations for wood production (Bergin *et al.*, 1995). Native scrubland such as manuka and kanuka have been suggested to accumulate as much carbon (C) in biomass as New Zealand exotic forestry plantations which are used to offset a portion of the country's GHG emissions (Scott *et al.*, 2000). Reversion of land to manuka scrubland may be a useful C sink for New Zealand, and an economic offset for fossil-fuel emissions through the sequestration of C (Scott *et al.*, 2000). Whitehead *et al.* (2004) carried out a study to estimate the annual C uptake of manuka and kanuka shrubland species. It was found that, over the course of a year, 76% of incident irradiance (400-700 nm) was absorbed by the canopy, annual net photosynthesis per unit ground area was equivalent to 1.97 kg C m^{-2} y^{-1} and respiration loss from leaves at night 0.45 kg C m^{-2} y^{-1}. In comparison, a study by Aeschlimann *et al.* (2005) showed annual net photosynthesis for perennial ryegrass (*Lolium perenne*) to be much lower at 1.06 kg C m^{-2} y^{-1} and respiration losses to be 0.34 kg C m^{-2} y^{-1}. These findings reinforce the promise of manuka and kanuka to be used as C sinks to offset emissions of GHGs.

As well as possible benefits in terms of N cycling which are yet to be tested, if manuka or kanuka were incorporated into farm systems it may be possible to collect C credits in terms of the Emissions Trading Scheme as manuka has been shown to be a method of carbon sequestration.

A.3 Honey

Manuka and kanuka honeys from New Zealand both exhibit non-peroxide antibacterial activity. For manuka honey, the antibacterial activity was almost entirely due to its non-peroxide components (Allen *et al.*, 1991). Manuka honey is marketed around the world for its antimicrobial properties. Polyphenolic acids and flavonoids have been identified as bioactive components in manuka honey. The "Unique Manuka Factor" (UMF) is a term used to describe the potency of the antibacterial activity of manuka honey. It is measured in the laboratory and compared with a standard antiseptic (phenol) to prove its potency (Badet & Quero, 2011).

The presence of methylglyoxal (MGO) in manuka honey contributes to its uniqueness (Sherlock *et al.*, 2010) and has been suggested as the principal component responsible for manuka honey's beneficial effect (Stephens *et al.*, 2010). The pronounced antibacterial activity of New Zealand manuka honey directly originates from MGO. The minimum concentration of MGO needed for inhibition of bacterial growth was 1.1 mM for both *Escherichia coli* and *Staphylococcus aureus.* The manuka honey when diluted to 15-30% exhibited antibacterial activity which corresponded to MGO concentrations of 1.1 to 1.8 mM (Mavric *et al.*, 2008). In contrast to this, Jervis-Bardy *et al.* (2011) found MGO to be only partially responsible for the anti-biofilm activity of manuka honey. Honey solutions containing 0.53 mg MGO mL^{-1} or greater demonstrated anti-biofilm activity. Equivalent activity was achieved with ≥ 1.05 mg mL^{-1} MGO solution (Jervis-Bardy *et al.*, 2011). The source of MGO, though not well understood, has been thought to be the non-enzymatic conversion of dihydroxyacetone from the nectar of the manuka flower (Adams *et al.*, 2009). Methyl 3,4,5-trimethoxybenzoate and 3,4,5-trimethoxybenzoic acid, syringic acid and methyl syringate are other compounds found in manuka honey which were found to possess significant antibacterial activity (Russell *et al.*, 1990). Similarly, methyl syringate was a floral marker and constituted more than 70% w/w of the phenolic fraction in a study by Weston *et al.* (2000). The authors stated that phenolic components of manuka honey contributed to, but did not account for all the observed non-peroxide antibacterial activity of manuka honey.

It is thought that the components responsible for the antibacterial activity of extracted oil are not the same as those for honey (Weston *et al.*, 2000; Stephens *et al.*, 2005). Leptospermone is an antibacterial β-triketone found in manuka essential oils, but was not detected in manuka honey. It was suggested that this was due to leptospermone not being soluble in water and therefore it was unlikely to be present in the nectar. Leptospermone tends to occur characteristically in the leaves and twigs of manuka (Weston *et al.*, 2000).

Due to its antimicrobial properties, manuka honey (particularly high UMF) sells at a premium. It is possible that if manuka was to be incorporated onto dairy farms that this environmental incentive may be economically viable to farmers if the production of manuka honey was possible.

Appendix B

B.1 Method testing calculation of standards

To test the reliability of the N_2O collection vessels, a standard of N_2O was injected into three chambers according to the following calculation in order to produce a sample with a N_2O concentration of approximately 2 µL L^{-1}.

$C_1V_1 = C_2V_2$ C_1= 100 µL L^{-1} standard
V_1= 400 mL
V_2= 20000 mL chamber
C_2= (100*400)/20000 = 2 µL L^{-1}

B.2 Method testing to determine best time of day for sampling

Measurements of N_2O taken in the greenhouse after 15 minutes under the chamber showed no significant difference between species at any of the different times of the day and no significant difference ($F_{2,36}$=2.15;*P*=0.131) in N_2O levels at the different times of the day with an average of 0.326 µL L^{-1} N_2O (Figure B 1). This indicated that time of day measurements are taken should not influence experimental results. Calculated fluxes could take into account temperature.

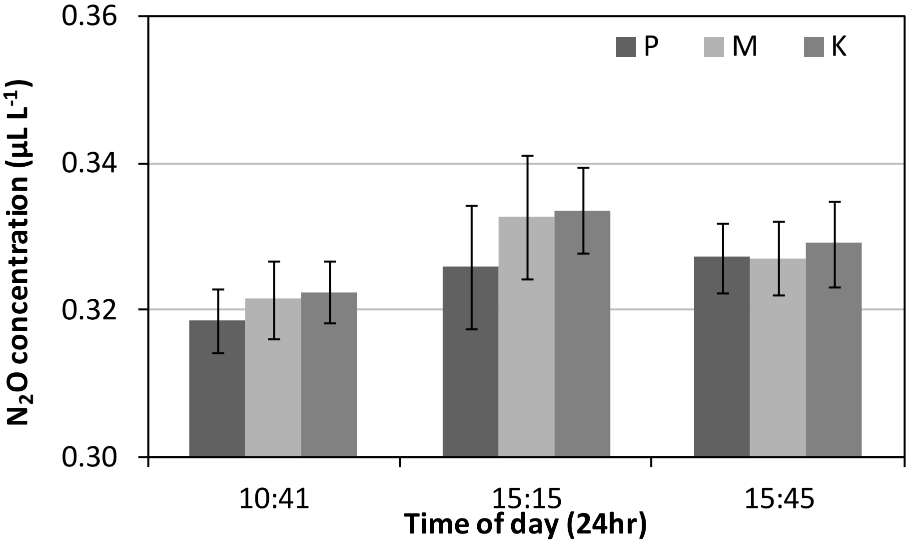

Figure B 1 Nitrous oxide (N_2O) production for different species: pasture (P), manuka (M), and kanuka (K) after 15 minutes and three different times of the day 10.41 am, 3.15 pm and 3.45 pm. Error bars are standard error of the mean (n = 5).

Appendix C

Field trial

C.1 Native species planted on the Lincoln University Dairy Farm

Family	Scientific name	Botanical reference	Common name
Araliaceae	*Pseudopanax arboreus*	(L.f.) Allan, (Murray) Philipson	Five Finger Whauwhaupaku Puahou
	Pseudopanax crassifolius	(A.Cunn.) K.Koch	Horoeka Lancewood
	Pseudopanax ferox	Kirk	Fierce lancewood Horoeka Toothed lancewood
Asparagaceae	*Cordyline australis*	(Forst. f.) Endl., Hook. f.	Cabbage tree Ti kouka
Asteraceae	*Ozothamnus vauvilliersii (Cassinia vauvilliersii)*	Hombr. et Jacquinot ex Decne	Mountain Tauhinu Mountain Cottonwood
	Olearia fragrantissima	Petrie	Fragrant tree daisy
	Olearia paniculata	(J.R.Forst. et G.Forst.) Druce	Akiraho
Cyperaceae	*Carex secta*	Boott (1853)	Makuru Pukio Sedge
	Isolepis nodosa (*Ficinia nodosa*)	(Rottb.) Goetgh. et al.	Wiwi Knobby club rush Ethel sedge
Fabaceae	*Carmichaelia australis*	R. Br	New Zealand Broom Makaka
	Sophora microphylla	Aiton	Kowhai Weeping kowhai Small-leaved kowhai
Griseliniaceae	*Griselinia littoralis*	Raoul	Kapuka New Zealand Broadleaf
Iridaceae	*Libertia ixioides*	(Forster fil.) Sprengel	New Zealand Iris
Malvaceae	*Hoheria angustifolia*	Raoul	Narrow-leaved lacebark Narrow-leaved Houhere
	Plagianthus regius	(Poit.) Hochr. (1907)	Lowland ribbonwood Manatu Ribbonwood
	Plagianthus divaricatus	J.R.Forst. et G.Forst	Salt marsh ribbonwood Marsh ribbonwood Makaka
Myrsinaceae	*Myrsine divaricata*	A.Cunn.	Weeping matipo Weeping mapou
Myrtaceae	*Kunzea ericoides*	(A. Rich.) Joy	Kanuka

		Thomps.	White tea-tree Burgan
Pittosporaceae	*Pittosporum eugenioides*	A.Cunn.	Lemonwood Tarata
	Pittosporum tenuifolium	Gaertn.	Kohukohu Kohuhu Black matipo
Plantaginaceae	*Hebe salicifolia*	(G. Forst.) Pennell	Koromiko Willow-leaf hebe
Poaceae	*Anemanthele lessoniana*	(Steud.) Veldk	Gossamer grass New Zealand wind grass Windgrass
	Chionochloa rubra	(Zotov, 1963)	Red tussock
	Cortaderia richardii	(Endl.) Zotov	Toetoe
Podocarpaceae	*Podocarpus totara*	G.Benn. ex D.Don	Totara
Polygonaceae	*Muehlenbeckia astonii*	Petrie	Shrubby tororaro Wiggy-wig bush
	Muehlenbeckia complexa	(A.Cunn) Meisn.	Maidenhair vine Creeping wire vine Lacy wire vine Angel vine Mattress vine Mattress wire weed Necklace vine Wire vine
Rubiaceae	*Coprosma crassifolia*		Thick leaved mikimiki
	Coprosma propinqua	A. Cunn.	Mingimingi
	Coprosma propinqua hbrid		Mikimiki
	Coprosma rigida	Cheeseman	Mikimiki
	Coprosma robusta	Raoul	Karamu
	Coprosma rubra	Petrie	Mikimiki
	Coprosma virescens	Petrie	Mikimiki
	Coprosma wallii	Petrie	Mikimiki
Sapindaceae	*Dodonaea viscosa*	Jacq.	Hopbush Akeake
Verbenaceae	*Teucridium parvifolium*	Hook.f.	Teucridium
Xanthorrhoeaceae	*Phormium tenax*	J.R.Forst et G.Forst.	Flax Harakeke Korari New Zealand Flax New Zealand Hemp
	Phormium cookianum	Le Jol.	Mountain flax Wharariki