Influence of shelterbelts on nitrous oxide emissions and denitrification enzyme activity in Manawatu pasture soils, New Zealand

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Abstract

Nitrification and denitrification are the important soil biological nitrogen (N) transformation processes that are major contributors to nitrous oxide (N_2O) emissions. In temperate pastoral soils, denitrification by microbial activity is the major producer of N_2O emissions. One of the potential mitigation options that could reduce greenhouse gas (GHG) emissions from pastoral systems is the use of shelterbelts, as these sequester carbon (C) in soil and biomass. Yet shelterbelts could also alter the N cycle and, therefore, prior to widely proposing the establishment of shelterbelts in New Zealand (NZ) pastoral systems to mitigate GHG emissions, their influence on soil N_2O emissions needs to be investigated.

The objective of this study was to investigate whether shelterbelt establishment in animal grazed pastures provides an additional benefit of N_2O emission reduction in the Manawatu Region, NZ. To achieve these objectives, one laboratory incubation to measure denitrification enzyme activity (DEA) in soil samples taken from 5 paired sites in the Manawatu Region. The study farms were dairy farm (MF) - Massey University Dairy 4, dairy farm (SD) - Stewart Dairy Land, Ashurst, dairy farm (TF) - Table flat farm, Apiti, dairy farm (GO) - Glen Oroua, Rongotea and sheep and beef farm (TP) - Tuapaka Farm, Massey University. Field trials of N_2O measurements were conducted in two of these paired sites (MF and SD) during winter/autumn 2020. Soil physico-chemical characteristics were also assessed. At each farm, a control paddock (without shelterbelt) and a treatment paddock (with shelterbelt) were identified. Soils were taken at 0-10 cm depth from six distances (1, 5, 10, 20, 40, and 80 m) from the shelterbelt and from the roadside boundary of the paddock. Like DEA, field N_2O emission measurements were also carried out in two out of the five farms at the same above-mentioned distances.

The results indicated that, there was a significant effect of shelterbelt on soil pH in all but one site, yet the trend was not consistent among sites. At three sites, a significantly higher soil water content was observed in soils under shelterbelt's influence. In general, a higher NO_3^--N content accumulated at the shorter distance from the shelterbelt (1, 5, and 10 m) compared with farther away from trees (20, 40, and 80 m), while a lower soil NH_4^+-N content was found closer to the trees. No significant influence of shelterbelt on DEA was detected except for one study site. Nitrous oxide emission was positively related to high NO_3^--N content. Out of the two study farms in which N_2O emissions were measured, the effect of the shelterbelt was only detected in one of them, with a significantly higher N_2O emission from non-shelterbelt soils than from shelterbelt soils. The two experiments of DEA and field N_2O

emission have indicated that soil pH and NO_3^--N content are the main soil factors influencing denitrification and N_2O emission in this study soils. By reducing NO_3^--N content and modifying the pH value, especially in the close vicinity of trees, shelterbelts could reduce N loss by suppressing denitrification transformation processes in soils.

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Chapter 1 Introduction

Several natural greenhouse gases (GHGs) such as water vapor, carbon dioxide (CO_2), ozone (O_3), methane (CH_4) and nitrous oxide (N_2O) trap heat and cause global warming. Apart from these naturally occurring gases, other GHGs such as hydrofluorocarbon, perfluorocarbons and sulfur hexafluorides, resulting from anthropogenic activities, are also contributors to global warming and climate change problems (IPCC, 1996).

Among these GHGs, CO_2, CH_4, and N_2O are the three major gases emitted to the atmosphere (IPCC, 1996). According to IPCC (2014), report agriculture, land use, and land use change and forestry (LULUCF) accounts for 24 % of global GHGs emissions. The intensification of land use and agricultural activities are the principal causes of the increase in CO_2, CH_4 and N_2O emissions (Waggoner, 1994).

Intensification of agricultural activities lead to increased use of nitrogen (N) fertilizers to sustain agricultural productivity. Despite being a major nutrient in grazed systems, N is also a key contributor to environmental pollution when its amount exceeds the plant and soil microorganisms' requirements (Saggar et al., 2005). Nitrous oxide is a powerful GHG with a global warming potential (GWP) of 265, which means its GWP is 265 times larger than that of CO_2 over a 100-year period (IPCC, 2014). Biological N transformation processes in agricultural soils, lead to N_2O emissions (Butterbach-Bahl et al., 2013).

Nitrification is regarded as the oxidation of ammonium to nitrate ions, carried out by autotrophs and heterotrophs microorganisms from which gaseous nitrogen compounds (NO, N_2O and N_2) are produced. Denitrification is one of such processes emitting N_2O in agricultural soils. Denitrification is microbial reduction of nitrate (NO_3^-), and nitrite (NO_2^-) to different gaseous oxides of N including N_2O. Various soil physio-chemical and biological properties control N_2O production from soils (Szajdak et al., 2005; Venterea and Rolston, 2002).

In New Zealand pasture soils, denitrification is a major source of N_2O (Saggar et al., 2009), and, globally, the process produces approximately 60% of total N_2O emissions to the atmosphere (Mosier et al., 1998; Kroeze et al., 1999). Measuring denitrification enzyme activity is a proxy to estimate denitrification potential of a soil. Denitrification enzyme activity (DEA) could be influenced by physical, chemical, biological soil factors, environmental factors, and other management conditions in soil (Albrecht and Kandji, 2003).

There are many management options that can be implemented to mitigate GHG emissions from agricultural land (Smith et al., 2013). One of the effective mitigation options for reducing agricultural GHG emission is agroforestry. It is the establishment or retaining of tree species (woody plants) in agricultural land. Agroforestry is the combination of either crop cultivation or livestock farming and tree species in the same land, including the incorporation of shelter/timberbelts in pastoral land (Smith et al., 2008).

Agroforestry has been advocated as a management practice for balancing non-CO_2-GHG emissions due to their ability to sequester atmospheric C in the soil, and thus contribute as a carbon dioxide removal (CDR) option (Clary, 2012; Breuer et al., 2006; Mutuo et al., 2005; Schoeneberger et al., 2012). Although it is evident that planting trees help sequester soil C and reducing CO_2 emissions, the effect of trees on CH_4 or N_2O emissions from soil is still largely unknown (Albrecht and Kandji, 2003). Although since last many decades denitrification is being measured in different agricultural land uses, however, the detail information of denitrification in soils under shelterbelt is lacking (Szajdak and Gaca, 2010).

According to Meinzer et al. (2001) and Curiel Yuste et al. (2007), tree species have an indirect influence on GHGs' released through the creation of a different microclimate of the surrounding area and controlling mineralizable C and N contents in soils. Woody plants could affect soil temperature and moisture, which decreases soil microbial processes' and thus the release of GHGs (Evers et al., 2010).

It has been found that increasing soil wetness by the shading effect of trees may favour soil N_2O emission via denitrification (Green et al., 2003; Vargas et al., 2012; Butterbach-Bahl et al., 2013). Yet, the water requirements of the trees might lower the water table and contribute to soil aeration. Given that only limited studies have investigated the influence of trees on N_2O emissions from pastoral lands under grazing conditions, there is an urgent need to study the influence of woody species on N_2O production of grazed soils.

The aim of the present study is to investigate the effect of shelterbelts as a mitigation tool to reduce N_2O emissions from pasture soils in Manawatu Region, New Zealand. To achieve these objectives, one laboratory incubation experiment of five study farms and two field trials were conducted in autumn to evaluate the influence of shelterbelt on (i) DEA, and (ii) N_2O emissions soils in pasture soils.

This research involves comparing the effect of shelterbelt (treatment) and non-shelterbelt (control) together with different soil sampling distances from trees. The research questions in

this study were (i) how does shelterbelt affect soil physicochemical properties and DEA? (ii) would there be less N_2O emission in shelterbelt plot compared with non-shelterbelt one. This study is based on the hypothesis that shelterbelt trees can take up excess nitrate, and soil water content from the grazed pastures, thus, DEA and N_2O emissions will be lower in the pasture soils under shelterbelt as compared to pasture soils with no shelterbelt.

Thesis Structure

The thesis comprises six chapters, outlined as follows:

Chapter 1 provides a brief introduction about global GHG emissions and methods of mitigation. This chapter also, describes objectives, hypotheses and major goals of the study followed by an outline of the thesis.

Chapter 2 reviews the relevant research literature and summarizes the studies on N transformation in agricultural soils, as well as the effects of shelterbelts on soil properties and GHG emissions. Moreover, it states the methodologies and main factors influencing the denitrification potential, and nitrous oxide emissions.

Chapter 3 describes the details of the study area or experimental sites, along with the procedure for conducting the field work (soil and gas sampling), and the laboratory incubation studies. In addition, it also outlines the laboratory analytical procedures, data calculation, and statistical analysis.

Chapter 4 covers the results of the thesis and provides a discussion with supporting references. The data is presented in two sections. The first section presents the comparison of the shelterbelt and non-shelterbelt on DEA in five study-sites, including soil physio-chemical properties. The second section is the effect of shelterbelts on field N_2O flux and soil properties in two out of the five farms investigated. Finally, the chapter compares and explores the findings from similar studies.

Chapter 5 provides the snapshot of the whole study, examining the implications of the effect of shelterbelts on the N transformations (DEA and N_2O flux) from dairy farms. Also, it highlights the applicable outcomes of this study, together with the conclusions and suggestions for further research on this topic.

Chapter 2 Literature Review

Introduction

Nitrous oxide (N_2O) a potent greenhouse gas is produced by natural and anthropogenic sources. Soils under vegetation, oceans and the atmosphere are the natural sources of N_2O emissions while the anthropogenic sources include agriculture, industrial processes, energy consumption, and waste management. In general, nitrification and denitrification are the major sources of N2O emissions in agricultural lands (Maag and Vinther, 1996, Mosier et al., 2006; Butterbach-Bahl et al., 2013).

According to Saggar et al. (2009), in temperate grassland soils, the production of N_2O occurs mostly through denitrification rather than nitrification. Pastoral soils make for 46–52% of the global anthropogenic N_2O emissions (IPCC, 2007). For example, 80% of New Zealand (NZ) land use correspond to grasslands (MfE, 2007), and thus the contribution to NZ's N_2O production is important and associated with the regular application of inorganic fertilisers as N inputs, the biological fixation of atmospheric N_2, and the deposition of N as excreta and urine by grazing animals, or usage of farm dairy effluent (de Klein et al. 2003). The purpose of literature is to explore the possible effects shelterbelt planting on soil properties, microclimate and how do they influence on nitrous oxide emission and denitrification activity from pastoral soils.

2.1 New Zealand's GHG emissions in agricultural soils

There are various sources of global GHG emissions. The atmospheric emissions originate from industries, transport, waste management, agriculture, forestry, and other land (AFOLU) uses. Among these, around 75% of total emissions are associated with CO_2 from fossil fuels combustion/industrial processes and AFOLU. Out of total global anthropogenic GHG emissions, methane (CH_4) emissions account for 16%, N_2O 6%, whereas fluorinated gases 2% (IPCC, 2010).

In comparison with other developed countries, GHG emission profile in NZ is unique. Generally, the industry sector is the main contributor of GHG emissions in advanced countries as opposed to NZ, where the main source of GHG emissions is the agricultural sector. About half (48%) of the NZ's total GHG emissions come from agriculture (Ministry for the Environment, 2019).

New Zealand is one of the countries that has adopted the Paris Agreement, which is a global agreement for GHG adaptation and mitigation. Accordingly, NZ commits to reduce "the annual GHG emissions 30% below 2005 levels in 2030, it means 11% below 1990 levels" (Government of NZ, 2015). In order to meet the targets of this agreement, the global CH_4 and N_2O emissions from livestock production must be turned down (Wollenberg et.al, 2016).

2.2 Sources of N_2O emissions

Agriculture releases atmospheric N_2O directly and indirectly. Causes of direct emissions are application of inorganic nitrogen fertilizer and animal manure to agricultural soils, which has a 42% contribution while runoff and leaching process are indirect sources of N_2O emission (25%) (IPCC, 2007).

New Zealand's main type of agriculture is livestock farming, and animals graze on the farm in all seasons. Grazed animals in NZ only a small proportion of nutrients taken up while grazing is converted into milk/or meat products. Most of the nutrients, including N exuded as excreta (urine and dung) (Bolan et al., 2004). The discharged N has potential to cause environmental contamination by a consequence of NO_3^- leaching and GHG (e.g., N_2O) emissions. Especially, in autumn and springtime in NZ, high rates of nitrate leaching and N_2O emission can occur due to the high precipitation which favours soil moisture while temperatures are not too cold to halt microbial activity (Luo et al., 2000; de Klein et al.,2001). According to MfE 2020, agricultural soils are the major sources of the national N_2O emission (92.5 percent), mainly by deposited urine and dung from grazing animals. Overall, N_2O emission accounts for 10.2 % of gross emissions in 2019 and majority of this comes from agriculture.

Both biological and non-biological processes are responsible for N_2O emissions from the soil. (Müller, 1995). Major processes forming N_2O emission include: (1) nitrification, (2) denitrification (3) dissimilatory nitrate reduction to ammonium (DNRA), (4) respiratory denitrification, (5) non-respiratory N_2O production and (6) nitrite-induced (chemo) denitrification (Tiedje, 1988). Under O_2 limited condition (anaerobic), denitrification is a main N_2O producing mechanism in soils (Šimek et.al., 2002).

Figure 2.1 Nitrogen cycle (adapted from Singh et al., 2019).

Owing to their radiative or chemical impacts on the atmosphere, many studies focus on gaseous N, such as ammonia (NH_3), nitrous oxide (N_2O) and nitric oxide. In the naturally occurring N-cycle, nitric oxide and N_2O are intermediate products from the denitrification process, and some may release to the atmosphere before changing to N_2. On the other hand, denitrification takes N_2 back to the atmosphere and finishes the N-cycle (Fig. 2.1 and 2.2). Although denitrification is the primary source of N_2O emissions, nitrification by nitrifying bacteria may also contribute to N_2O emissions at low oxygen level in soils (Bolan et al., 2004).

Figure 2.2 Schematic representation of nitrogen transformations in legume-based pastures (Adapted from Saggar et al., 2013).

2.3 Mitigation options for agricultural GHG emissions

A wide range of mitigation options exists for reducing soil GHG emissions from various agricultural systems. This includes cropland management, grazing land management, N fertilizer management, growing cover crops, and replacing summer fallow with animal fodder (Singh et al., 2019).

The common alleviation methods for agricultural emissions are mostly focussed on soil management practices and resource improvement methods. For example, fertilizer application method, improving the efficiency pasture/grazing systems, and zero/minimum tillage, increasing soil C sequestration, planting shelterbelts/tree, and efficient farm waste management (Beach et al., 2015; Moran et al., 2011; Whittle et al., 2013).

Some of the evidence is showing that farm/livestock management, the sustainable use of fertile soils, and the restoration of degraded lands could be valid mitigation options for reducing agricultural GHG emissions. Cropland management includes nutrient and crop residue management, the conversion to an agroforestry system with the use of shelterbelt. In this review, the use of shelterbelts, and specifically types, usage, and their effects on soil processes and GHG emissions will be discussed.

2.3.1 Shelterbelt and its uses

Agroforestry systems are approved by the Intergovernmental Panel on Climate Change (IPCC) as a sustainable mitigation option for reducing GHG emissions from agricultural systems (Schoeneberger, 2009). One of the agroforestry practices is the use of shelterbelts or windbreaks, in which trees are generally planted in linear rows across the farmyard.

Shelterbelt planting is multifunctional in agriculture. “as a windbreak/shelterbelt method it has countless advantages and usage; cooperation of shelterbelt planting in sheep and beef farm or dairy farm has been more popular in NZ farm” (Hawke et al., 1999). The use of shelterbelts in NZ is for controlling of wind speed and protect soils, plants, and livestock as a primary purpose. The blustery wind results in physiological stress in crops and livestock (Brandle et al., 2004; Mize et al., 2008).

For the shelterbelt to be effective, it is crucial that it has the appropriate arrangement and types of tree species. A range of shelterbelt types can meet the needs of the growers. Nevertheless, weather conditions, free space, budget, and growers’ preferences will determine the final design. Types of shelterbelts include dense shelter, porous/semi-permeable shelter, hybrid shelter, and overhead canopy shelter (Waikato regional council, 2002). In addition, there can be differences in the arrangements of the tree species in the shelterbelt. The common practiced shelterbelt types are the following (Taranaki Regional Council):

1. Single row with single species, interplanted with rapid growth species

2. Double row with low growth species on the side of wind (windward) and taller species leeward in the direction of wind blowing

3. Double row with slow-growing permanent shelter species windward and faster-growing

timber species leeward.

In agricultural system shelterbelts comprised of trees or shrubs with different designs (linear or patchy) have an important influence on living organisms and their surroundings. Shelterbelts can form "biogeochemical barriers" due to their effects on solar radiation, nutrient/water cycling and microclimatic conditions of sheltered field. Additionally, windbreaks can inhibit the disperse of harmful chemicals and restrict the movement of inorganic compounds from agricultural lands (Szajdak et .al, 2018).

Reports suggest that trees can sequester mainly atmospheric CO_2 in soils because tree biomass can add higher amount of C inputs in soils, and there is less soil disturbance by management practices compared with crops cultivation (Oelbermann et al. 2004; Young 1997). When talking about GHGs other than CO_2, shelterbelts can also influence N_2O emissions in soils. The N taken up by the trees is retained in the tree biomass, and most is returned to the soil through litterfall (Allen et al., 2004). This process can facilitate the N cycling, reduce the application of nitrogenous fertilizers nearby the trees, which will lead to alleviate N_2O emissions from inorganic fertilizers (Amadi et al., 2016).

One of the advantages of using shelterbelts in agriculture includes reducing GHG emissions, particularly the release of CO_2 from soils. Woody plants can directly impact on CO_2 emissions by changing the plant root respiration and root mycorrhizal fungi from agricultural landscape (Boone et al., 1998). In addition, they can indirectly influence GHG emissions by controlling plant available N content in soil and modifying soil temperature and water content with the surround environment (Meinzer et al., 2001; Curiel Yuste et al., 2007).

Temperature is one of the key factors controlling microbial activity in soil. The degradation of organic detritus by soil microbes is delayed at low soil temperature, which subsequently slows down the production of GHGs (Evers et al., 2010). In addition, in shaded places under shelterbelts, a reduced evaporation from soil surface is occurs, which avoids desiccation (Green et al., 2003). Increased soil-water content stimulates soil N_2O emissions by denitrification when this depletes O_2 below 1% (Vargas et al., 2012; Butterbach-Bahl et al., 2013) and promote CH_4 emissions if redox conditions are very reduced (Abdalla et al., 2009).

Some studies indicated that more lignified plant residues from trees could improve N immobilization by microbes and, therefore, minimize NO_3^- availability, which may in turn reduce N_2O emissions (Dougherty et al., 2009; Evers et al., 2010; Bergeron et al., 2011).

Most of the research have tested the advantages of tree plants on GHG emissions as a mitigation tool in in cropped fields (e.g., Peichl et al., 2006). It is significant that cooperation of trees in agricultural landscapes has much potential to mitigate the considerable amount of GHG emissions (Evers et al., 2010).

Numerous studies support the positive effects of planting sheltered trees in agricultural landscape on C sequestration (Nair et al., 2009). However, knowledge on the effect of shelterbelt planting on soil N_2O emission from grazed pastoral soils is lacking – most of the related studies are implemented without animals grazing. There is more need for robust information on the influences of tree species on gaseous N losses from the grassland soils.

2.4 Importance of Denitrification in Agriculture

Denitrification is the final step of N cycle in which NO_3^- is reduced to N_2. This NO_3^- reduction is essentially performed by denitrifies, commonly found in all soils. Generally, the respiratory denitrifying microbes are facultative anaerobes, so, they gain the required energy from NO_3^- reduction mechanism especially in anoxic conditions (Tiedje, 1988). *Pseudomonas*, *Bacillus*, *Alculigenes* and *Flavobacterium* are regarded as the major denitrifiers in soil (Payne, 1981, Tiedje, 1988).

The mechanism of NO_3^- reduction in denitrification stated by Payne (1981) and Firestone (1982), is represented in the following equation (2.1):

$$NO_3^- \Rightarrow NO_2^- \Rightarrow NO \Rightarrow N_2O \Rightarrow N_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.1)$$

Although denitrification leads to loses of essential plant nutrient such as N, complete denitrification could be benign for environment as it reduces excess NO_3^- to harmless N_2. Denitrification uses NO_3^-, which could leach into adjacent waterways; rivers or lakes, this would be an advantage for the environment denitrification process, the process could be of great ecological service to eliminate NO_3^- from drainage waters (Kaushik et al., 1981) and it is already used in wastewater treatment plants to eliminate N from wastewaters. But it is when incomplete denitrification occurs that N_2O is produced thus becoming an environmental issue (Saggar, 2013).

2.4.1 Factors influencing denitrification and nitrous oxide production

Complex interactions among soil physical, chemical, and biological conditions are involved in controlling denitrification activity (Skiba and Ball, 2002; Koponen et al., 2006; Saggar et al., 2013).

Soil properties, management practices, environmental factors and interaction among the factors influence denitrification potential, the rates and loss of nitrogen products (N, N_2O and NO) from the soil (Albrecht and Kandji, 2003). Amundson and Davidson (1990) stated that (i) the presence denitrifiers and energy source such as C-compounds, (ii) anoxic conditions, and (iii) electron acceptors are the basic needs for the microbial denitrification. There are two main categories of factors that affect denitrification in soil; proximal and distal regulators (Klein et al., 2002; Wallenstein et al., 2006) (Figure 2.3).

Proximal regulators have direct effect on changes of denitrification rate such as NO_3^- concentrations, C availability, O_2 concentration and temperature (Drury et al., 2008; Saggar et al., 2009; Wallenstein et al., 2006). Rainfall, continuing cultivation methods, soil factors (pH, texture, pore size, soil water content (SWC)) are distal regulators which influence on proximate stimulators to a greater extent because they influence on denitrifying communities over a wider scale than proximal (Luo, 1996). The major factors (distal and proximal) which are stated by Saggar et.al (2013) are examined in this literature review.

Soil texture, soil moisture level, pH and denitrifier community are prominent influencing factors of denitrification (Cuhel et al., 2010; Miller et al., 2009 and Mørkved et al., 2007). Therefore, in this study, choosing the soil regulators which control DEA and N_2O emission, we decided to focus on those prominent factors (soil pH, SWC) and one proximal regulator (soil NO_3^- concentrations) due to the short time frame for the study.

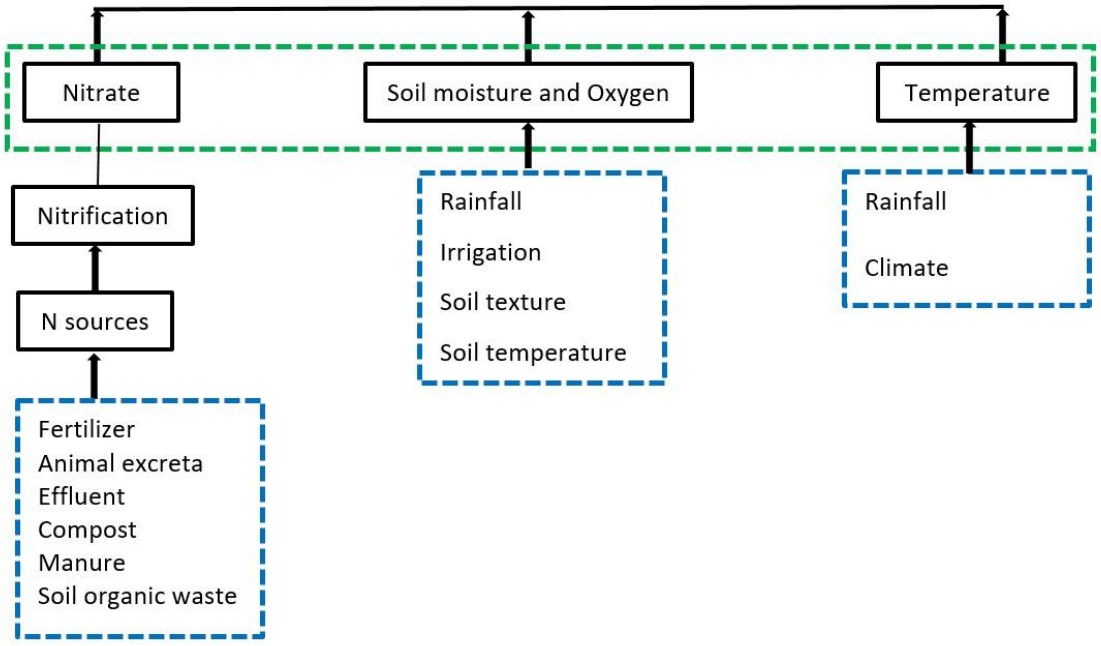


Figure 2.3 Factors affecting denitrification in soils (green shaded: Proximal factors; blue shaded: Distal factors). Adapted from Tiedje (1988); Groffman (1991); and Saggar et.al (2013).

Several studies at both laboratory and field scale identified the relationship between soil and environmental factors and denitrification process (Knowles, 1982; Davidson and Swank, 1986; Luo et al., 2000). Introduction of woody species to agricultural soils can reduce soil N_2O emission (Baah-Acheamfour et al., 2016). Trees could influence soil temperature and moisture status, which in turn, decreases soil microbial processes' and subsequently, the release of soil GHGs emission (Evers et al., 2010). It has been found that increasing soil water content by the shading effect of trees may favour soil N_2O emission via denitrification (Green et al., 2003; Vargas et al., 2012; Butterbach-Bahl et al., 2013).

(i) Soil mineral nitrogen

Soil N, in particular, NO_3^- is a main electron source of denitrifying bacteria, the availability of nitrogen (NO_3^-) in the soil solution is the principal necessity source for dentification. Therefore, the presence of NO_3^- is one of the major factors which controls denitrification in soil

There are various sources of N to soils; application of inorganic nitrogenous fertilizers, farm waste or slurry and biological N fixation (Seitzinger et al., 2006). The greater amount of NO_3^- can be observed in the urine patches in grazed pasture lands which may enhance N_2O

production. One study reported that in grazed pasture soils, urine patches contribute 8% of the annual N_2O emission during a day (Williams et al, 1999).

However, other factors also determine the availability of NO_3^- in soil. For example, mineralisation and nitrification rates, the amount of N absorbed by plants, immobilisation by soil microorganisms and NO_3^- losses by leaching and diffusion (Tiedje, 1988; Zaman et al., 2007). It has been stated that NO_3^- concentrations may influence on the gaseous products (N_2O) from denitrification due to limiting the reduction of N_2O to N_2 (Blackmer & Bremner, 1978). Thus, N_2 is the influencing product at low NO_3^-- N concentrations while at high NO_3^--N conditions, N_2O is dominant (Arah and Smith, 1990).

Therefore, the accessibility of mineral nitrogen plays a principal role to initiate denitrification process (Bolan et al., 2004b). As denitrifiers are facultative anaerobes, when O_2 availability is limited, NO_3^- becomes a dominant reductant (Aulakh et al., 1984). If other main factors; soil moisture and readily C are enormous, the supplement of NO_3^- can enhance soil denitrification rates.

Woody species have the characteristics of being deep-rooted. Deep root systems can absorb residual NO_3^- and prevent denitrification and also reduce NO_3^- leaching and associated indirect N_2O emission (Amadi et al., 2016). Studies showed that, if decomposition of the organic matter with a C:N ratio of 30, mineral N will tend to be immobilized by soil microorganisms (Brady and Weil, 2002). This points out that forest litter has ability to raise biological immobilization of N and decrease N availability, as needed for the production N_2O (Bergeron et al., 2011; Dougherty et al., 2009; Evers et al., 2010). Therefore, regarding the lower NO_3^- availability under shelterbelt soils, it could be assumed that denitrification potential and N_2O emissions from those soils will be lower compared to non-shelterbelt soils.

Besides NO_3^- concentration, the accessibility of available C is also another major energy source of denitrifying bacteria to perform the reduction of the added NO_3^- (Burford and Bremner, 1975; Delwiche, 1981). Carbon compounds are essential for the cell growth of microbes, so, they are vital and control the rate of denitrification process (Burford and Bremner, 1975, Reddy et al., 1982, Robertson and Tiedje, 1984). By seeing the equation (3.2), it is obvious that in the heterotrophic denitrification, 5 mol of glucose (30 mol of C) is utilized for the denitrification of 24 mol NO_3^- (Burford and Bremner, 1975; Delwiche, 1981).

$$5C_6H_{12}O_6 + 24NO_3^- = 12N_2 + 30CO_2 + 18H_2O + 24OH^- \quad \text{(3.2)}$$

Therefore, readily available C can limit the rate of heterotrophic denitrification in agricultural soils. Subsequently, other factors which control the rate of C mineralisation in soils (e.g., temperature, drying–wetting, cultivation practices such as tillage, lime application, application of stubbles and farm manure and usage of organic or inorganic fertiliser, root excreta) can play a major role in determining the rate of denitrification in agricultural soils (Saggar et al., 2013).

In the soils which have much plant residues (cut or damage), the high rates of denitrification are observed. Some of the readily available C can leach to the soils through the remaining roots (Beck and Christensen, 1987; Robertson et al., 1987). Therefore, in the winter grazing time, available C increases in the root region which may subsequently boost DEA, particularly when the soil has high soil water level. These are some of the reasons why labile C availability to soil microorganism becomes the essential factor in denitrification process (Myrold and Tiedje, 1985). Although it is commonly assumed that the increased amount of labile C can reduce the ratio the ratio of N_2O:N_2 (Arah and Smith, 1990; Dendooven et al., 1998; Smith and Tiedje, 1979), the effect of C may vary with soil condition. For example, the impact of readily available C on the production and emission of N_2O and N_2 from soils can depend on the presence of NO_3^- concentration and aeration status (water-filled pore-space; WFPS) (Tiedje, 1988; Weier et al., 1993).

Trees have the potential to increase the capacity of soil to capture and store atmospheric CO_2 (Guo and Gifford 2002; Paul et al. 2003; Mutuo et al. 2005). One of the agroforestry systems, shelterbelts, may sustain or maximize the soil organic carbon (SOC), in terms of root turnover and the long-term addition of plant debris to the soil (Amadi et al., 2016). Many studies have proved the larger SOC content in soils with trees than cropped fields (Dhillon and Van Rees, 2017). The possible reason for increasing SOC in soil under shelterbelts could be the continuous inputs of litter from trees and the capturing of organic matter-rich sediments deposited by wind (Sauer et al., 2007; Amadi et al., 2016).

This addition of plant residues increases denitrification especially when the soil has high soil moisture and adequate NO_3^- concentration (Aulakh et al., 1991). Therefore, Denitrification may dominate in soils rich in organic matter and hence N_2O production (Clough, 1994). That is why the access of available C to microorganisms becomes a controlling factor for denitrification, especially under field conditions.

Under anoxic condition of fertilised soils, denitrification final product ratio (N_2O:N_2) is mainly controlled by NO_3^- concentration whereas the amount of readily available C is responsible for controlling the rate of denitrification (Tiedje, 1988; Weier et al., 1993). The possible effect of labile C and NO_3^- ratio could be influencing on enzyme activity and diffusion status of NO_3^-into denitrifying communities (Swerts et al., 1996; Weymann et al., 2010).

(ii) Degree of soil acidity or alkalinity

Soil pH is one of the major distal regulators for N_2 and N_2O production by microbial process in soil. In general, most denitrifiers are more likely to grow at neutral pH value (pH 6-8). Acidic condition (lower than pH 7) is not an ideal condition for denitrification process (Bremner and Shaw, 1958; Fageria and Baligar, 2008; Liu et al., 2010). Many studies proved that N_2O: N_2 ratio is raised when the soil is in low pH condition (Koskinen and Keeney, 1982; Struwe and Kjøller, 1994); thus, N_2O seems to be a dominant product in those soils (Christensen, 1985; Parkin et al., 1985).

The extensive studies on the relationship between pH and denitrification, revealed that lower denitrification rate normally occurs in acidic soils rather than neutral or alkali soils (Bremner and Shaw, 1958; Bryan, 1981; Cooper, 2002; Nagele and Conrad, 1990;). Acid soils can diminish denitrification and increase N_2O production. Application of N forming fertilizers for many years result in acid soils and subsequently enhance N_2O emission (Guo et al., 2010). It is generally accepted that pH value from 6 to 8 is favourable condition for denitrifier communities.

One research in the grazed pasture of Australia revealed that the scattered trees have some effects on soil spatial patterns on-the-spot. It has been observed that the higher soil pH is found “inside” of eucalypts trees canopy compared with “outside” ones (Wilson, 2002; Graham et al., 2004; Wilson et al., 2007). However, the effect of trees on microclimate, resources availability and soil properties are a heterogenous complex process.

(iii) Soil moisture and oxygen

Soil water content (SWC) may affect denitrification directly or indirectly in terms of reducing O_2 diffusion in water filled pore spaces, producing available forms of C and N, providing favourable conditions such as substrates and products for growing microbial population and processes (Aulakh et al., 1992). Denitrification is anaerobic process, therefore, O_2 availability is one of the principal controlling factors which can hinder DEA in the soil (Knowles, 1982; Lloyd, 1993).

Numerous research has proved that high denitrification rate is found in high SWC soils (Bolan et al., 2004b; de Klein and Van Logtestijn, 1994; Ledgard et al., 199). However, SWC level and N_2O emission can be increased by rainfall pattern, irrigation, and grazing events.

The presence of O_2 can be varied with different soil types. Generally, soil anoxic microsites can occur in fine soils at lower SWC level compared with in coarse-textured soils (Groffman and Tiedje, 1991; Parton et al., 1996). Likewise, the O_2 availability in the root zone is mainly dependent on the presence of soil moisture and respiration process by plant roots and soil microorganisms (Tiedje, 1988). Additionally, soil properties which control O_2 diffusion or usage impact on the aeration status of soil and consequently, DEA. Porosity (number and sizes of pores) in the soil is the major influencing characteristics for O_2 diffusion (Aulakh et al., 1992).

Camping of animals cause soil compaction which increases anaerobic conditions and denitrification (Oenema et al., 1997). In cooler region, soils have high SWC in late-autumn or winter season because of high rainfall as well as at this time, soil compaction by animals grazing and low pasture N absorption caused by low soil temperature. These conditions are favoured to maximize denitrification process (Bhandral et al., 2007b; de Klein et al., 2006; Saggar et al., 2004).

The covering of woody plants makes minor changes in the microclimate beneath and surrounding environment because of blocking precipitation and shading. Both of which regulates the amount of available SWC (Johnsen 1962; Collings 1966; Young and Evans, 1987; Belsky et al., 1989). Rainfall can influence SWC, nutrient solubility, lower O_2 availability which increase denitrification rate (Ellis et al., 1998). The maximum rate of denitrification is found after the rainfall (de Klein et al., 1999; Velthof et al., 1996).

Investigations of effects of rainfall on denitrification reported that increasing denitrification activity and rates and N_2O emissions after precipitation have been related with allowing the

supply substrates into contact with soil denitrifying micro-organisms and minimizing the availability of O_2 levels in soil (Ellis et al., 1998; Luo et al., 2000; Van Kessel et al., 1993). Other researchers also supported that the highest N_2O emissions or denitrification rates is generally observed subsequent rainfall or irrigation days in temperate grassland soils (Luo et al., 2000; Saggar et al., 2004a; Velthof et al., 1996).

It is also suggested that topography also one of the major influential factors on the effect of rainfall on denitrification and N_2O emission rates. One study stated that there is a positive relationship between the intensity of rainfall and emission rates from the bottom of slope topography ($R^2 = 0.73$) compared with a flat area ($R^2 = 0.57$) (Corré et al. 1990).

(iv) Soil temperature

Soil temperature can directly influence denitrification by enhancing or declining denitrifier activity in soils. Temperature is one of the major regulators which causes temporal variation in denitrification (Ryden, 1983). Knowles (1982) reported that denitrification process can take place over a broad soil temperature starting from sub-zero to 75°C. In general, the lowest soil temperature for denitrification process, is related to the presence of free water whilst the maximum temperature could probably limit denitrifier activity and substrate supply; NO_3^- availability in the soil. The fluctuations of temperature can impact on the availability of substrate availability and which can control the performances of specific enzymes in denitrification. However, temperature changes cannot influence on growth and community structure of denitrifying microbes (Stres et al., 2008).

Apart from the direct influences of temperature on denitrification process, indirect effects are altering gas solubility, and gas diffusion (Craswell, 1978), together with controlling substrate supply, inhibiting the presence of O_2, NO_3^- and labile C in soil which are important regulators for denitrification process. The high N_2O emission in winter and denitrification loss is associated with increasing water filled pore spaces (greater than 0.60), increasing soil NO_3^--N value and low N absorption by plants. Several investigations on grassland soils in NZ have stated greater number of losses by denitrification in winter as the temperature is 10 ^oC (Luo et al., 2000; Ruz- Jerez et al., 1994).

Similarly, high soil temperature can enhance denitrification process, but the critical soil temperature is 30 ^oC. Increasing temperature from critical value corrupts the enzymes activity

and result in decreasing denitrification (Bolan et al, 2004b). In general, the process can adjust the soil temperature between 5 – 25 ºC (Malhi et al., 1990; Powlson et al., 1988).

Under woody canopy, lower soil temperature can generally observe as the effect of plants debris build-up and shading than intercanopy patches (Everett and Sharrow, 1985; Belsky et al., 1989; Pierson and Wight, 1991). Besides, tree canopies can change soil moisture directly via interception and indirectly controlling on soil evaporation rates. Loss of SWC by soil evaporation which in turn minimizes the amount of plant available water in soils (Breshears et al., 1998). On the other hand, depending on the seasonal changes (e.g fall and spring) this relationship changes like a warmer temperature have found in canopy patches. This reverse relation comes from mixed results of lower sun angle and insulation by litter (Breshears et al., 1997).

There is a still controversy about the effect of temperature on N_2O production. Some researchers have suggested that the greater amount of N_2O is produced at low temperatures and the N_2O:N_2 ratio is high (Keeney et al., 1979; Avalakki et al., 1995) as "the activation energy of N_2O reduction is higher than the activation energy of N_2O production" (Holtan-Hartwig et al., 2002, p.8). Whereas some studies have resulted that a lower N_2O:N_2 ratio is found associated with increasing soil temperature (Bailey, 1976; McKenney et al., 1984; Maag and Vinther, 1996). Moreover, Focht, 1974 and Rudaz et al., 1999 discussed that there is a no correlation between denitrification rate and soil temperature. It can be clearly seen that the mystery effects of temperature observed in those studies seems that temperature may regulate denitrification with interacting ways but many of this effect have still not been fully explained (Saggar et al., 2013).

(v) Management practices

Application of heavy machines for cultivation causes low soil O_2 availability because of soil compaction, thus, increase denitrification rate (Bakken et al., 1987). Other practices can also enhance denitrification activity by limiting O_2 content. These involve the addition of farmyard manures (Guenzi et al., 1978) and plant residues or organic matter (Aulakh and Rennie, 1987) reduced O_2 concentration due to the respiration of aerobic bacteria.

Agricultural practices can govern the presence and available forms of N in soils. For example, crop varieties, fertilizer and farm waste management can impact on N availability. Similarly, different products of applied fertilizer, N application rate, method and timing could

determine the period of N availability and the forms of N present in soils. Finally, farm waste management such as incorporation or burning of crop residues, is also of the factors determining N supply in soils because the uptake amount of nitrogen nutrient is different depending on different crop types (Bouwman et al., 2002).

Regards with tillage methods, higher rates of denitrification are mostly occurred in soils which has no tillage rather than ploughed soils (Aulakh et al., 1985b; Staley et al., 1990). It is speculated that this related to increasing soil organic matter and the presence of labile C in the topsoil (Aulakh et al., 1992). Doran, 1980 revealed that zero tillage method is a favourable condition for denitrifying bacteria.

As NO_3^- and labile C are the principal substrate of denitrifying microsites, the practices which increase or decrease those substrates could result in limiting denitrification rates. Effluent irrigation and biochar application for increasing the presence of soil C can reduce N_2O:N_2 ratio, however, not all biochar can be lower N_2O emission (Clough et al., 2010; Saggar et al., 2011). Reducing NO_3^- concentration is a very basic approach to control denitrification rate in grazed pasture soils (Saggar et.al, 2013).

Therefore, minimizing animals grazing events during autumn/winter period (Ledgard et al., 2006; Luo et al., 2006), the addition of salts to animal feed and feeding low protein silage such as maize silage (Ledgard et al., 2007; Mulligan et al., 2004), usage of nitrification and/or urease inhibitors (Parkin and Hatfield, 2010; Saggar et al., 2008; Zaman et al., 2009) and incorporating deep rooted perennial pasture species for reducing NO_3^- availability by Dear et al., 2009; Saggar et al., 2013, are the possible on-farm management practices to control denitrification mechanism, particularly, in temperate grassland soils.

The highest number of N_2O emissions are found in dairy-grazed pastures (10–12 kg N_2O-N ha^{-1} $year^{-1}$), followed by sheep grazed pastures, (4–6 kg N_2O-N ha^{-1} $year^{-1}$). The lowest number (1–2 kg N_2O-N ha^{-1} $year^{-1}$) was observed in woody trees, shrubland and non-grazing soils (Saggar et al., 2008). It indicated that the effect of woody trees on soil properties has also influence on N_2O fluxes. Butterbach-Bahl et al., (2002) reported that the distance from tree stems has significantly fluence N_2O emissions, highest emission has been found the areas which is closed to tree stems than the intermediate stem areas.

One research revealed that the lowest nitrous oxide emission was detected from soils with young shelterbelt (19.2 µg $N_2O \cdot m^{-2} \cdot hr^{-1}$) while the elevated amount was observed from cultivated field which is adjacent to young shelterbelt areas (351.0 µg $N_2O \cdot m^{-2} \cdot hr^{-1}$). It is

clearly shown that shelterbelt has a positive on the reduction of nitrous oxide emission from soils (Szajdak et al., 2018). Similarly, Kesik et al., (2005), also revealed that the N_2O emissions from forest soils are roughly ten times lower compared to the cultivated soils. It has stated that there is a correlation between the age of shelterbelt and N_2O emissions. Thus, establishing new shelterbelt is an important tool in agricultural landscape both improving soil physical, chemical, and biochemical properties and controlling GHG fluxes (Szajdak et al., 2018).

2.5 Approaches for denitrification measurement

There are various measurement techniques available to quantify denitrification and nitrous oxide depending upon the research objectives or resource availability. Denitrification measurement approaches include acetylene(C_2H_2) inhibition method (AI technique), 15^N tracer technique, direct N_2 quantification, isotope method, micrometeorological and modelling approaches. in AI technique N_2O is analysed by gas chromatography with electron capture detector (Kaspar and Tiedje, 1980) and isotopic method such as 15^N which determines gases by isotope-ratio mass spectrometry (Mosier and Klemedtsson, 1994) are the two most used methods to determine denitrification rate from the calculations of and N_2O loss.

In direct quantification of N_2 airtight systems with He or Ar (Butterbach-Bahl et al., 2002) or a closed system with regular headspace sampling is included. However, in contrast with AI approach, the other techniques are expensive and complicated in use, therefore, great technical expertise could be essential to manage the system properly. This literature will emphasize on acetylene incubation approach.

Over the last few decades, direct gas measurement methods have been developed (Tieje et al., 1989; Myrold, 1991). The methods include:

• The use of acetylene (C_2H_2) to inhibit N_2O reduction to N_2 in order that all denitrification N loss will be measured in the form of N_2O; this method is used in conjunction with gas chromatography with a N_2O detection system. This is the one of most widely used methods in denitrification studies.

• A micrometeorological approach is a conceptually ideal method to measure trace gases emissions over large ecologically uniform areas, and the technique can reduce the problems of spatial variability that limit the accuracy of other technologies when sampling.

• The use of labelled isotopes, such as ^{15}N-labelled fertiliser, is relatively common as a technique to measure denitrification. The ^{15}N-labelled gases are then determined by mass spectrometry that allows quantification of the N_2 that is solely produced from denitrification.

The potential for denitrification can be assessed using a short-term DEA assay (Luo et al., 1996). The short-term denitrification enzyme assay is usually conducted under anaerobic conditions with no limiting substrates (i.e., with an excess of NO_3^- and available carbon) and under these conditions the result can be obtained in a few hours (Luo et al., 1996). This assay can be used to compare denitrification rates among different materials. It has also been used to provide a credible estimate of actual field denitrification rates (Tieje et al., 1989). I have used the same technique in this study to measure DEA in field moist soils.

2.6 Manual chamber technique of measuring in field nitrous oxide emission

There are several techniques and methods to measure the rate of N_2O exchange from the soil surface to the atmosphere. These involve the easy and commonly used static chambers (open and closed) method and the expensive and complex micrometeorological methods. Several authors have reviewed and discussed about the pros and cons of these different approaches (Pattey et al., 2007; Denmead 2008; Saggar et al., 2009). In this literature, only usage of manual chamber for field N_2O emission is descried in brief.

Generally, the chambers need to be installed 10 cm above the ground firmly attached to the soil during the measurement period. It is required to install static chambers some time prior to gas measurement, normally, 24 hr in order to exclude the effects of initial flush in soil respiration and soil physical disturbance on ebullition of soil gases. According to the previous studies, there is no shading effect of chamber on pasture growth (Saggar et al. 2004, 2007). At least weekly fluxes measurements are strongly suggested if the required resources are available (Parkin, 2008).

Gas fluxes are generally measured by collecting a gas sample from the chamber headspace and, after that, the fluxes are determined in the laboratory. During the sampling time, gas samples should be taking out at the exact time intervals. For flux calculation, gas samples should be collected from at least 3-time intervals such as time 0, and two equally spaced points (e.g., 0, 30, 60 min). The stainless steel or other type chambers such as PVC chamber ring with a lid placed in the soil by covering an area of 0.2 m^2 (Fig. 2.4) (Klemedtsson et al., 1997).

Figure 2.4 An example of the apparatus involved in closed chamber method (adapted from Jämsén et.al, 2015).

As chambers have a simple design and are easy to apply, they are useful under most climatic and site conditions. In addition, the chamber can detect even low fluxes and support the needed information related to spatial variability. When the fluxes are measured by chamber method, it should be conducted under field conditions with accurate procedure (Clayton et al., 1994).

2.6.1 Installation of closed chambers

There are numerous methods and approaches to determine the rate of soil surface atmosphere exchange of N_2O. These include the simple and widely used enclosure methods (static chambers), and diffuse source micrometeorological methods with various degrees of complexity. These latter methods, despite being generally expensive, have the advantage of providing continual measurement and achieving spatial integration of fluxes (Pattey et al. 2007; Denmead 2008; Saggar et al. 2009).

Chambers are the most common, easy to use and economical approach to measure gas fluxes from the soil surface, enabling the accumulation of gases of interest in a known volume. The

size of the chamber can show a great range, from less than 1 m^3 to greater than 150 m^3. The chamber is placed over the soil surface and enclosed for a period of time, during which, samples are collected and analysed to determine the change in N_2O concentration. The advantage of chambers is that they can be deployed easily and ability to use without extremely accurate or rapid analytical techniques.

2.7 In-field measurements of nitrous oxide emission

As many factors and reactions which release and absorb N_2O are involved, the fluxes are episodic and show broad temporal and spatial dissimilarities. The production and process of N_2O fluxes are controlled by a wide variety of regulators, such as temperature, soil pH, SWC and organic carbon. Therefore, it is demanding to capture the accurate measurement of wide-ranging N_2O emissions (Dala et al., 2003; Farquharson and Baldock, 2008).

However, the method has some distractions such as altering the immediate environment of soil gas exchange in the sample site and preventing gas diffusion to outside (Christensen, 1983). Chamber method has two designs; closed and open and each design has their special benefits and objectives. In the open chamber design, emitted N_2O is taken by molecular sieve entrapment (Ryden et al., 1979) while N_2O diffusion can be collected by syringe from the closed chambers (Webster and Dowdell, 1982). N_2O flux can be measured by gas chromatography after the gas samples collection.

2.8 Conclusions

According to the various literature, it is difficult to quantify N_2O emissions in terrestrial systems because the emission can vary with spatial and temporal variability. Therefore, large scales of N_2O fluxes measurement should extrapolate to extend the knowledge of the soil and the environmental factors that monitoring the emission rate (Matson et al., 1989). Introduction of trees into the agricultural systems decreased soil derived N_2O emissions (Evers et al. 2010).

Additionally, the proximity to stem of trees has a possible influence on N_2O emissions, revealed by the study of Butterbach-Bahl et al. (2002). However, the studies about the detailed influences of shelterbelt on CO_2, CH_4 and N_2O emissions from cultivated soils are still required, especially, at different distances from the tree (Butterbach-Bahl et al., 2002; Chukwudi et al., 2013). The subsequent chapters of this thesis describe the experiments

conducted with the aim of exploring the influence of trees at varying distances on soil DEA and N_2O emissions in the pasture soils collected from various dairy farms in NZ.

Chapter 3 Materials and Methods

3.1 Study site

This project was conducted to focus on measurement of DEA which is indicated by the emission of nitrous oxide (N_2O), the end product, from different pastoral lands. The study areas were:

1. Dairy farm (MF) - Massey University Dairy 4, Palmerston North
2. Dairy farm (SD) – Stewart Dairy Land, Ashurst, Manawatu
3. Dairy farm (TF) – Table flat farm, Apiti, Manawatu
4. Dairy farm (GO) - Glen Oroua, Rongotea, Manawatu
5. Sheep and Beef farm (TP) – Tuapaka Farm, Massey University, Palmerston North

For each farm DEA was determined while field N_2O emission was collected from two farms: site 1 and site 2. In considering the shelterbelts' effect, shelterbelts which ran both north-south and west-east comprised the study area for the effects of shade and shelter. For each farm, the soils from paired paddocks, with and without shelterbelts, were collected and analysed for DEA, , and other soil properties, in order to compare the effects of shelterbelts on DEA.

3.1.1 Study sites' description

We selected five sites for this study with slight variation in location and soil type in Manawatu region of NZ (Figure 3.1). All the selected sites are dairy farms except for one sheep and beef farm. The detailed site description is provided in Table 3.1. Dairy 4 (MF) is located adjacent to the Massey University campus, Tennent Drive (SH57) approximately 5 km from Palmerston North City.

The soil type is predominantly Tokomaru silt loam and subsoil tend to be compact clay loam. As this soil type has poor natural drainage, it dries out in summer. The farm has moderate natural fertility, and it is all artificially drained. The soil type at Apiti site is Kopua stony silt loam soil. This soil is suitable for both the grazing of beef cattle and for intensive sheep farming (Cowie, and Rijkse, 1977). Glen Oroua site has Concretionary sandy loam soil. Soil type in Tuapaka farm is Ohakea silt loam. These soil types are categorized as brown soils on the New Zealand soil classification system (Hewitt, 2010). The soil type in Stewart Dairy Land (SD) is sedimentary recent Pallic soil (Hewitt, 2010). The soil is clayey on the farm

and mainly poorly drained. The soils on the farm are susceptible to pugging during wet soil conditions (Cowie, and Rijkse, 1977).

Only site 1 and 2 are used for field N_2O flux experiment. On all sites, the pasture is mostly perennial ryegrass and white clover species.

Table 3.1 Descriptions of soil samples used for denitrification enzyme activity experiment and field nitrous oxide flux experiment

Site	Location	Farm Code	Latitude and Longitude	Land Use	Soil Type	Type of tree in shelterbelt
1	Palmerston North	MF	-40.397403, 175.613027	Dairy Farm	Tokomaru silt loam	Radiata pine
2	Ashurst	SD	-40.2795, 175.7053	Dairy Farm	Pallic soil	Radiata pine
3	Apiti	TF	-40.338301, 175.642015	Dairy Farm	Kopua stony silt loam	Radiata pine
4	Glen Oroua	GO	-40.283502, 175.39907	Dairy Farm	Concretionary sandy loam soil	Macrocarpa
5	Palmerston North	TP	-40.255043, 176.003773	Sheep and Beef Farm	Ohakea silt loam	Radiata pine

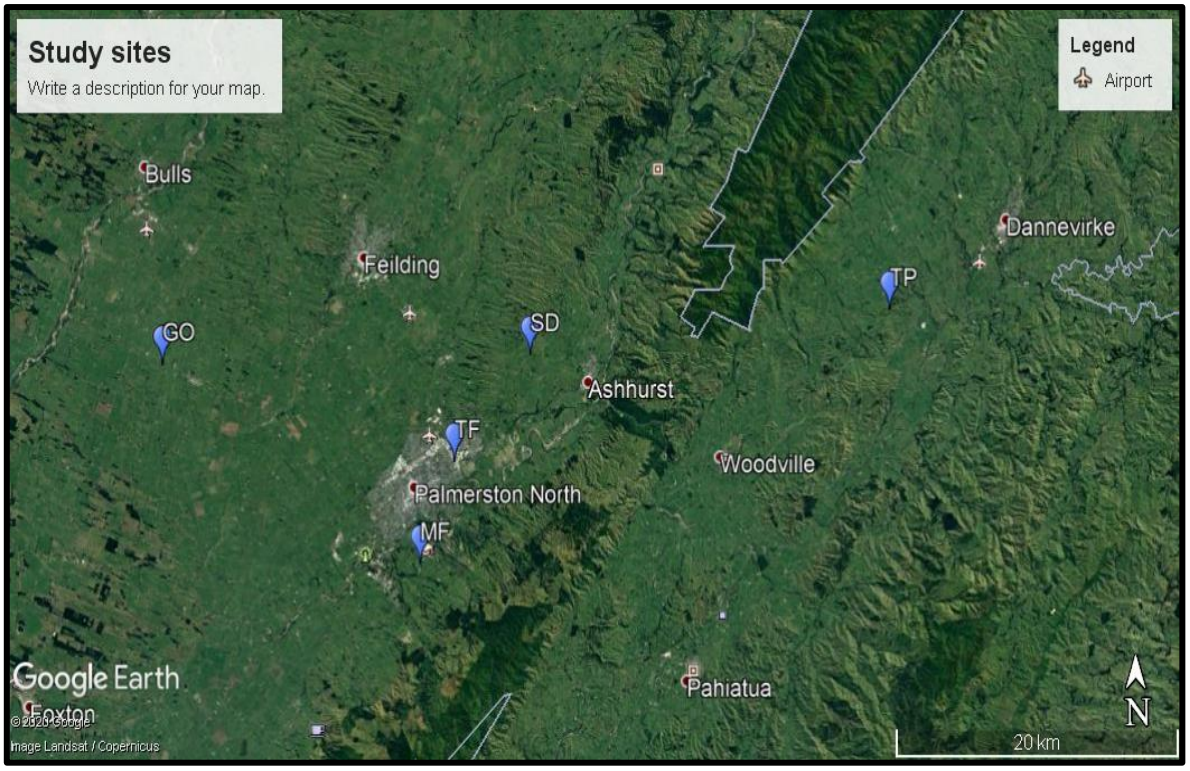


Figure 3.1 Map of study sites for denitrification enzyme activity measurement.

3.2 Collection of soil samples

In each farm, there were two paddocks from which the soils were collected, the control paddock (with no shelterbelt) and the paddock with the shelterbelt. Each paddock was divided into sampling transects by drawing imaginary line starting from the beginning of the paddock moving to 25, 50, and 75 m (Figure 3.2). Soil samples were collected either from the fence or boundary of the shelterbelt from the control and the shelterbelt paddocks respectively. In both the paddocks, soil samples were taken from six distances (1, 5, 10, 20, 40, and 80 m) from the shelterbelt and from the roadside boundary of paddock without the shelterbelt from the three transects. Soil samples (0–10 cm) were collected from three locations from each distance by composite soil sampling, sieved (2 mm) and stored at 4°C until DEA analysis and soil physio-chemical measurements.

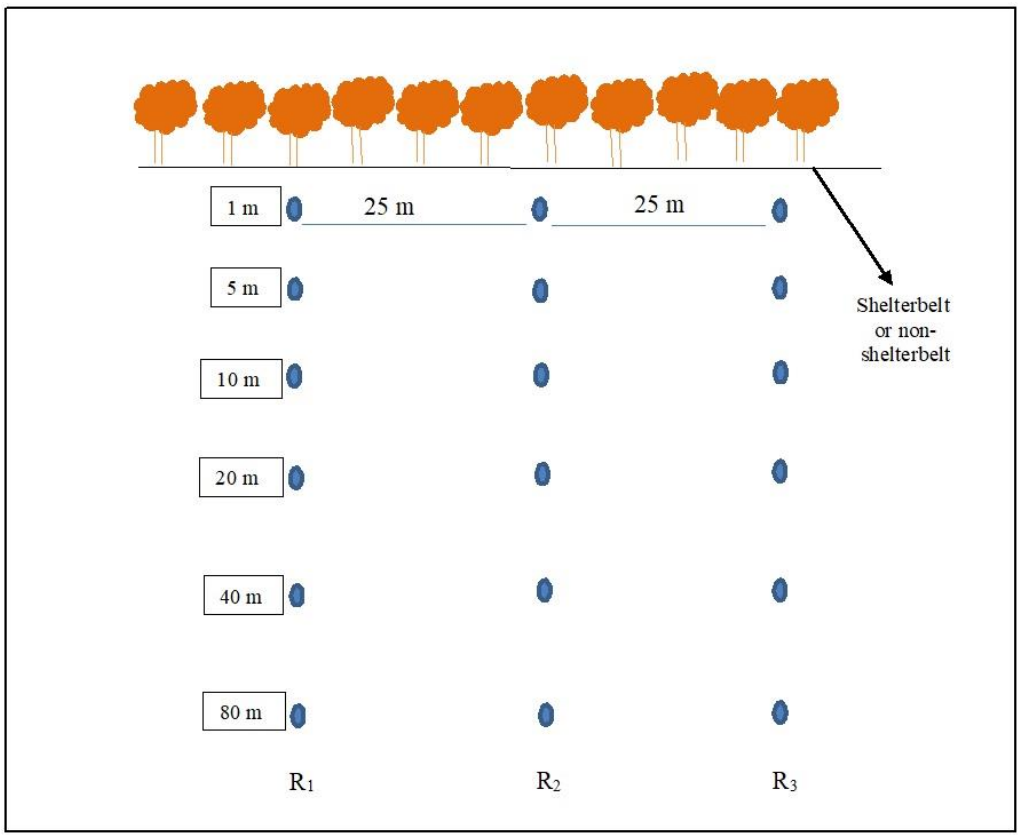


Figure 3.2 Illustration of treatments and soil sample collection from shelterbelt and without shelterbelt areas (R_1, R_2 and R_3 represent three sampling positions of each paddock).

3.3 Treatments

The main treatment effect is the presence or absence of shelterbelt on the paddock. In the paddocks with shelterbelt, a further treatment effect was the distance of sampling from the trees. The three sampling positions in the paddock 25 m, 50 m, and 75 m from the side fence of the paddock were served as the replicates of the treatments. Therefore, in each paddock, 18 samples (6 distances x 3 replications) were tested for DEA and N_2O flux and some soil physico-chemical properties. On each farm, there were 18 x 2 = 36 samples taken in total.

3.4 Soil physico-chemical measurement

Soil physico-chemical properties: soil moisture, (soil temperature), soil pH, mineral N (NO_3^- and NH_4^+) which can affect DEA rate and (N_2O) emission were analyzed for each treatment (Figure 3.3). Soil moisture was measured by using gravimetric method, by determining the weight loss from field moist soil by overnight oven-dry soil at 105^{o}C.

The following equation is used for computing gravimetric soil water content based on the weight of water present in soil and calculated as:

$$\text{SWC} = \left(\frac{\text{Weight of water (g)}}{\text{Weight of dry soil (g)}}\right) \times 100 \qquad \text{(Eq 3.1)}$$

Soil pH was analyzed by making 1:2.5 ratio of soil to water with a portable pH meter (Blakemore, 1987). For mineral N measurement, 5 g of soil was shaken with 25 ml of 2 M KCl on orbital shaker for 1 h. After that, the soil solution was centrifuged for 3 min at 5000 rpm and Whatman No.42 filter paper was used to filter the suspension. The extracts were determined with Autoanaylzer for NO_3-N and NH_4^+-N (Kamphake et.al, 1967).

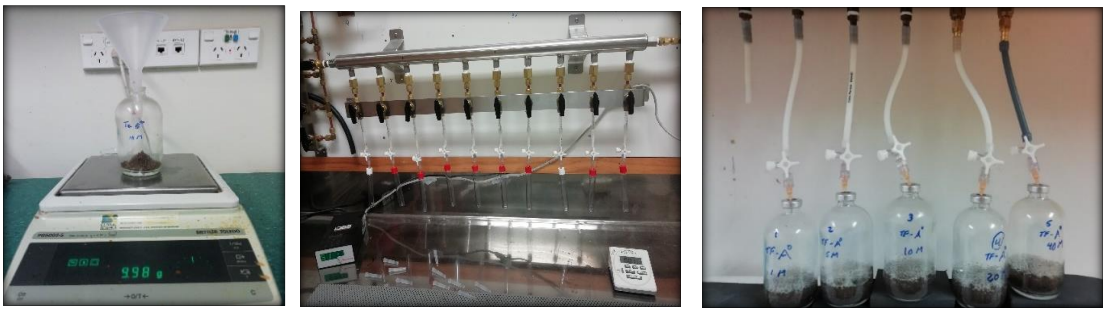
Figure 3.3 Soil sample collection and preparation for soil analysis: (a) soil taken from each distance; (b) soil drying at room temperature; (c) soil grinding and (d) soil sieving with 2mm diameter sieve.

3.5 Measurement of denitrification enzyme (DEA) activity in soil (Laboratory short incubation experiment)

The denitrification assay involved anaerobic incubation of soil samples in a nutrient solution containing sources of NO_3^--N(KNO_3) and carbon (glucose), and 10 ppm chloramphenicol to inhibit microbial growth and de novo synthesis of new enzymes (Figure 3.4). In this study, 250 µg glucose per g soil, 50 µg potassium nitrate (KNO_3) (Drury et al., 2008) and 10 ppm chloramphenicol dissolved and volume made-up with deionized water to 1 L DEA solution. The solution was kept in refrigerator before using in the DEA assays.

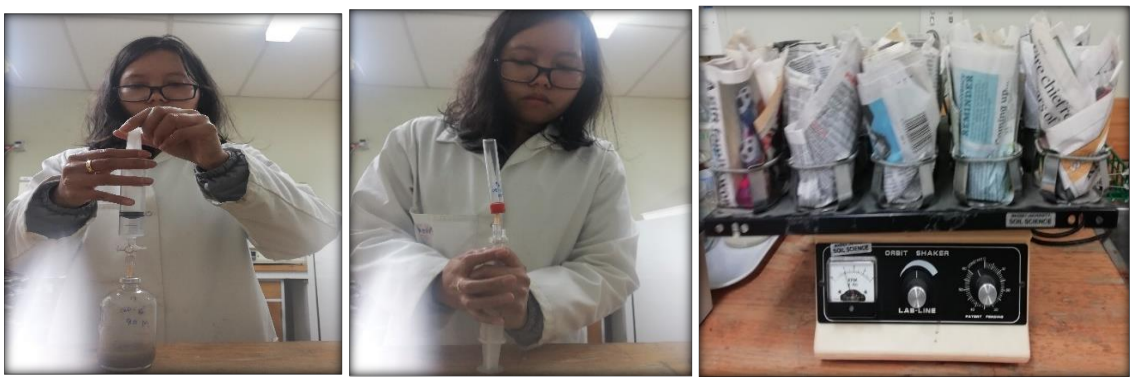
Incubations were conducted in 125 ml Wheaton serum bottles (Sigma-Aldrich®) as used in various incubation studies by McMillan et al (2014). Field fresh soil samples in three replicates (10 g dry equivalent, sieved <2 mm) were placed in the flasks to measure DEA (Figure 3.4 a). Next, 25 ml of nutrient solution was added to the flasks fitted with suba-seals and aluminium crimps and then evacuated. The serum bottles and glass vials were evacuated

using vacuum pump at the Manaaki Whenua - Landcare Research laboratory, Palmerston North (Figure 3.4 b and c). After removing the air from the serum bottles, 180 ml of N_2 was inserted to comprise the headspace. Then, 13 ml of N_2 was exchanged with purified acetylene to inhibit the reduction of N_2O to N_2 and sample collection was started for time 0. Two blank samples were also prepared with the same procedure without soil. Gas samples: 25 ml (10 ml sample + diluted with15 ml N_2) was collected at 0, 2, 4, and 6 hrs from the start of incubation (Rivas, 2014; Jha et.al, 2017) and transferred into pre-evacuated 12 ml gas vials (see in Figure 3.5).



(a) (b) (c)

Figure 3.4. Processes of Laboratory DEA analysis (a) soil weighing, (b) evacuation of gas vials for 2 minutes and (c) Evacuation of Erlenmeyer flasks containing DEA solution and soil for 7 minutes at Landcare Research Laboratory, Palmerston North.



(a) (b)

Figure 3.5 Illustration of sample collection for DEA analysis: (a) taking sample from each flask and transferring to evacuated gas vials, (b) Keeping Erlenmeyer flasks on rotary shaker at 1000 rpm in the incubation room.

After sample collection, 10 ml of N_2 is refilled to serum bottles to sustain the headspace for each sampling time. The samples were incubated in the dark at 25°C keeping on an orbit rotary shaker (Figure 3.5).

3.5.1 Calculations of DEA

The N_2O production rate in the serum bottles were determined by the slope of the linear regression of the N_2O in samples over the incubated time (0–6 hrs). The measured volumetric N_2O concentrations were used to calculate the amount of N_2O contained in the water and gas phases as follows:

$$N_2O \text{ flux} = \frac{N \times DF \times (V_g + V_l \times \alpha)}{W} \quad \text{(Eq 3.2)}$$

where: N_2O flux = rate of N_2O produced (µL-N_2O kg^{-1}soil hr^{-1}) during the incubation period; N = N_2O production rate in each flask (µL-N_2O L^{-1} gas hr^{-1}); DF (Dilution factor) = (5ml sample+ 20 ml N_2 gas)/(5-ml sample) = 25/5 = 5 at all the sampling times ; V_g = volume (L) of gas phase in the incubation flasks; V_l = volume (L) of liquid phase (soil + water) in the incubation flasks; α (the Bunsen absorption coefficient) = 0.544 (Groffman et al., 1999); W= weight of soil (kg).

$$DEA = N_2O \text{ flux} \times \rho \quad \text{(Eq 3.3)}$$

where: DEA is the denitrification enzyme activity (µg N_2O-N kg^{-1} soil hr^{-1}); N_2O flux = rate of N_2O produced (µL N_2O kg^{-1} soil hr^{-1}) during the incubation period; ρ = the density of N_2O-N (1.145 µg N_2O-N μL^{-1}) at normal temperature (25°C) and pressure (1 atm).

3.6. In-field measurements of nitrous oxide

Field N_2O flux meauserment was studied in two different dairy farms from Palmerston North city, NZ (Figures 3.6 and 3.7). The study areas are:

1. Dairy farm - Massey University Dairy farm 4, Manawatu
2. Dairy farm – Stewart Dairy Land, Ashhurst, Manawatu

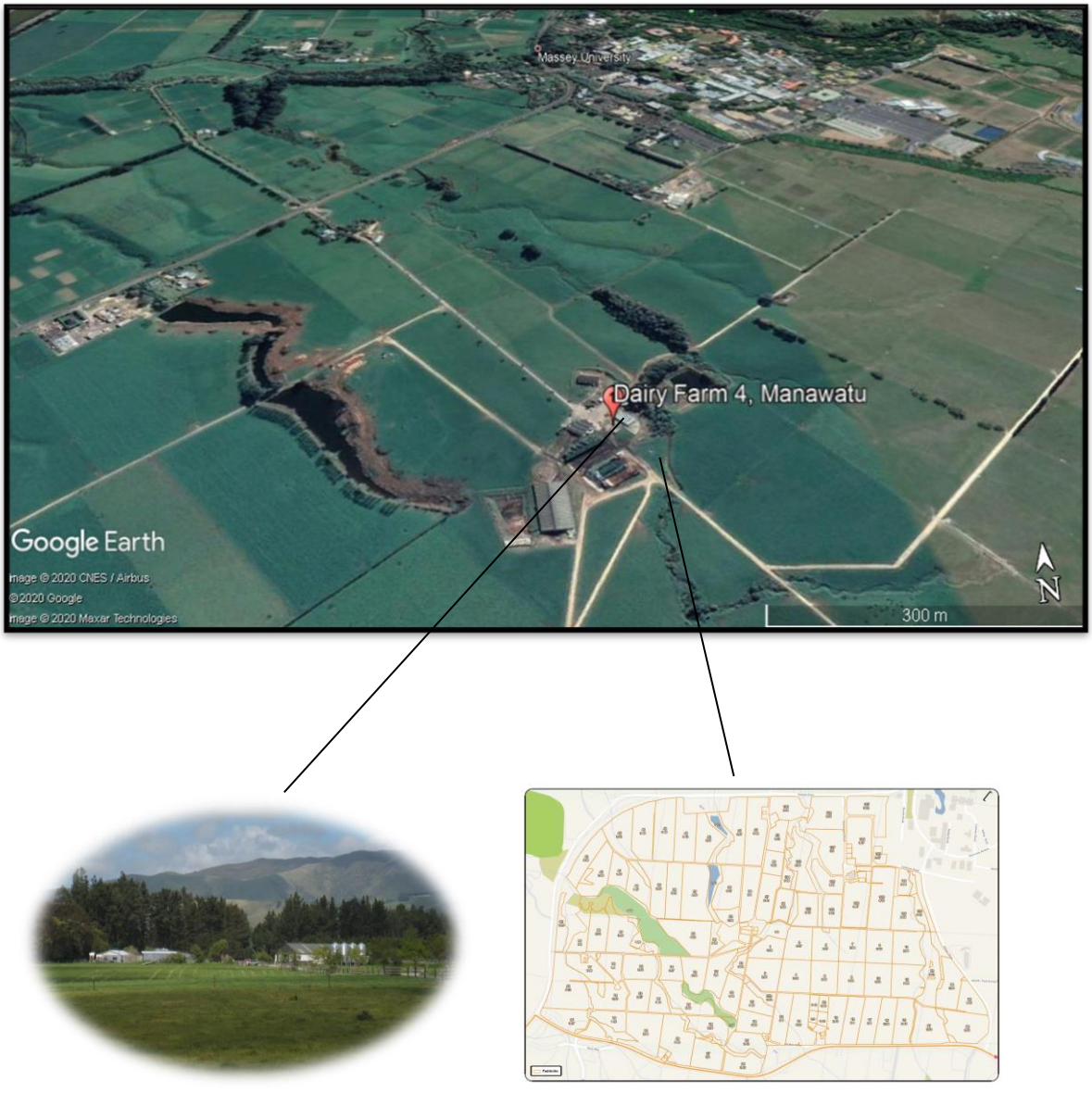


Figure 3.6 Showing the location and map of field study site (1): Dairy farm (2), Manawatu.

Figure 3.7 Map showing the field study site (2): Stewart dairy land, Ashurst, Manawatu.

3.6.1 Installation of chambers in study sites

In both shelterbelt and non-shelterbelt areas, chambers were put in place at each distance (1, 5, 10, 20, 40 and 8m) for field N_2O measurement (see in Figure 3.8). There were 18 chambers for each plot. These chambers and the procedures for their installation and collection of gas samples are described by Saggar et al. (2004).

Since the plots were not fenced, during the grazing event, chambers were removed to avoid being damaged by the cattle and were reinstalled in the same area (but not in same positions)

and with the same layout. The measurements were made during the cows grazing the winter and early spring period in Aug- Oct 2020.

(a)

(b)

(c)

Figure 3.8 Preparation and steps in field nitrous oxide flux experiment (a): marking each distance from shelterbelt and non-shelterbelt, (b): putting chamber into the soil and (c) soil sample collection from each treatment.

3.6.2 Gas sampling

On each sampling day, the chamber was closed with a lid for 1 hr (Figure 3.9), and air sample above the soil surface was extracted through a three-way tap on the chamber lid, with a 60-ml syringe (Figure 3.9a). A 50-ml gas sample was taken from each chamber at 0 min (T_0), 30 min (T_{30}) and 60 min (T_{60}) twice a week. A 25-ml subsample of the 50-ml gas samples was injected into an evacuated 12-ml exetainer within 1 hr of gas sampling. The gas sample in the exetainer was then stored until GC gas analysis was undertaken.

The samples for field N_2O emission were collected for 4 weeks, started August in dairy farm 4 and Stewart farm in October 2020. In total the gas samples for N_2O measurement in field conditions were taken at 6 time points in site-1 and 8 time points in site-2. Chamber air temperature (0–10 cm) was also recorded using a 10 cm probe digital thermometer (Figure 3.9b) as well as topsoils (0- 10 cm) were also taken from each distance and kept in chiller room for soil-physiochemical analysis. Soil pH, mineral-N and SWC were analysed and compared for each treatment.

(a)

(b)

Figure 3.9 Illustration of field nitrous oxide measurement; (a): gas sample collection from closed chamber, (b): temperature gauging in each treatment.

3.7 Gas Chromatograph Analysis

Gas chromatograph (GC) with an electron-capture detector (ECD) is the most widely used analytical method for measuring N_2O. The low cost of GC methods is one of their main advantages and makes them ideal for analysis compared to other analytical techniques. Gas Chromatograph was used for simultaneously analysing N_2O, CH_4 and CO_2. Further methodology details are given by Hedley et al. (2006).

Prior to analyse field N_2O and DEA samples in Gas Chromatograph (GC), N_2O standards are prepared and analysed. The standards are 100 ppm, 25 ppm, 12.5 ppm, 6.25 ppm, 3.125 ppm, 1.5625 ppm, 0.78125 ppm, 0.39063 ppm and 0.19531 ppm N_2O. There are 9 N_2O standards for denitrification analysis whereas 7 standards for field N_2O flux starting from 25 ppm N_2O. In addition, N_2 is also used as blank sample (0 ppm). After running the standards, 3ml of each sample is taken by 5 ml sample syringe and injected into GC machine. Before taking the actual sample, the syringe was flushed with about 2 to 3 ml of sample. The machine took about 5 minutes for analysing each sample.

The increase in N_2O concentration within the chamber headspace, for the gas samples collected at the different points of the sampling period was generally linear (R^2 >0.90). Therefore, the hourly N_2O fluxes (in mg N_2O-N m^{-2} hr^{-1}) were calculated using linear regression and the ideal gas law according to Mosier and Mack (1980) Eq. 3.3 (Saggar et al., 2010):

$$N_2O\ flux = \frac{\delta N_2O}{\delta T} * \frac{M}{Vm} * \frac{V}{A} \qquad \text{Eq. 3.3}$$

where, δN_2O is the increase in head space N_2O over time (mL L^{-1}); dT is the enclosure period (hrs); M is the molar weight of N in N_2O (g mol^{-1}); Vm is the molar volume of gas at the sampling temperature (L mol^{-1}); V is the headspace volume (m^3); and A is the area covered (m^2).

3.8 Rainfall and soil temperature

For the investigation of field nitrous emission from dairy pastoral farms, the daily gas flux was collected from two sites, MF site in August while SD site in October. In August, the temperature ranged from low of 5°C (41°F) and an average high of 13.5°C (56.3°F). The rain falls for 13 days and the total precipitation is up to 76.9 mm (3.03"). The average temperature

varied from a maximum of 16.7°C (62.1°F) to a minimum of 8.1°C (46.6°F) in October. The most rainfall occurs in October, rain days account for 11.8 days and precipitates 96.4mm (3.8") of precipitation (Figure 3.10 a) (adapted from weather-atlas.com).

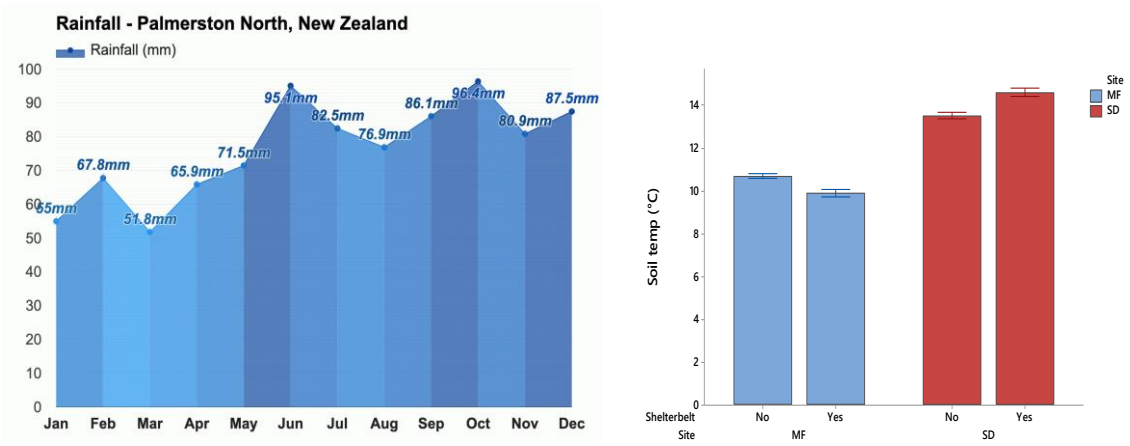


Figure 3.10 (a) the average monthly rainfall (mm) of Manawatu and (b) average soil temperature (°C) of MF and SD site.

Among the study sites, SD had the highest mean soil temperature during the gas sample collection. The shelterbelt paddock (SB) of site SD had the highest soil temperature (14.6 °C) and 13.5 °C in the without shelterbelt soil (NSB). The lowest soil temperature (11 °C) was observed in day-4 sampling. However, in the MF site, the soil temperature of both SB and NSB paddocks was not much different, ranging from 10 to 10.7 °C (Figure 3.10b).

3.9 Statistical analysis

Statistical analyses for the effect of shelterbelt on DEA, N_2O emission, soil chemical properties, were determined by analysis of variance (ANOVA) test using Minitab 18.1 software. To test significant differences among treatments, two-way ANOVA combined with Fisher Pairwise Comparisons method at 95% confidence level was applied in order to compare individual treatments from two main factors (shelterbelt and non-shelterbelt). Correlation analysis by Minitab and principal Component Analysis (PCA) by R 4.05 were used to detect the combinations of soil physicochemical properties which are likely to provide the maximum influences between individual sites.

Chapter 4 Results

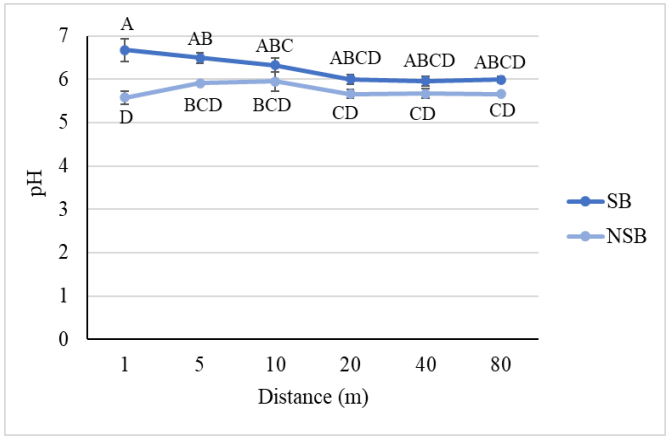
Experiment 1. Laboratory incubation to study the influence of shelterbelts on denitrification enzyme activity (DEA) in grazed dairy pasture soils

4.1 Soil properties influenced by the presence of shelterbelt on grazed pastures

4.1.1 Soil pH

Soil pH values in the studied farms (including both the shelterbelt, (SB) and non-shelterbelt, (NSB) paddocks) ranged from 4.9 to 6.7 (Table S1 to S6). The effects of (i) presence of shelterbelt (S), (ii) distance of sampling from shelterbelt (D), and (iii) the interaction effect of S and D were evaluated using a two-way analysis of variance (ANOVA) test (Table S10).

At site GO, there was no significant effect of either S or D or the interaction of S and D. At sites MF, TP, and SD, there was significant effect of S on soil pH, however, there was contrasting effect of S on soil pH in each of the three sites. While pH values were significantly larger in the SB plots (6.2) than the NSB plots (5.7) at site MF, the contrary was observed at the other two sites (TP: (SB) 5.7 vs. (NSB) 6.4; SD: (SB) 5.5 vs. (NSB) 6.0). At site TF, there was only significant influence of D on soil pH and the smallest soil pH (5.5) was observed at 1m distance from tree. At sites MF and TP, these differences in pH among SB and NSB plots were more pronounced in soils closer to the shelterbelt. The interaction of both S and D had significant effect on the soil pH only in site SD.



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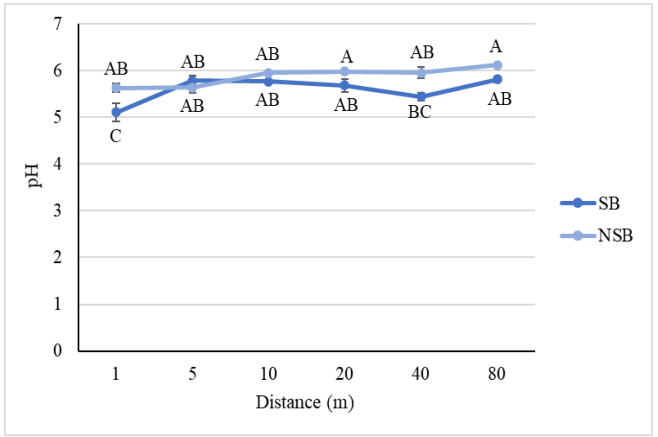

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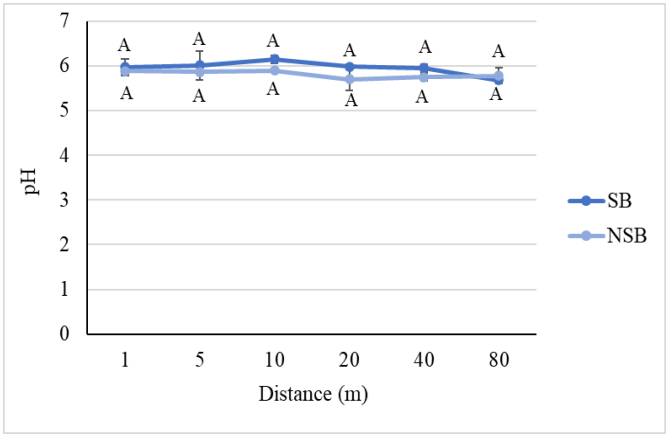

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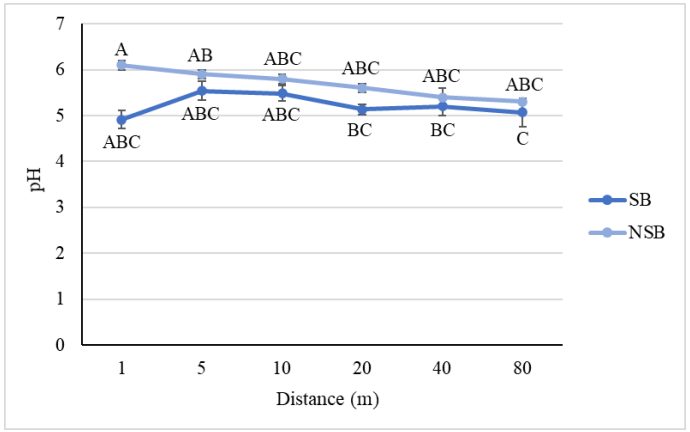

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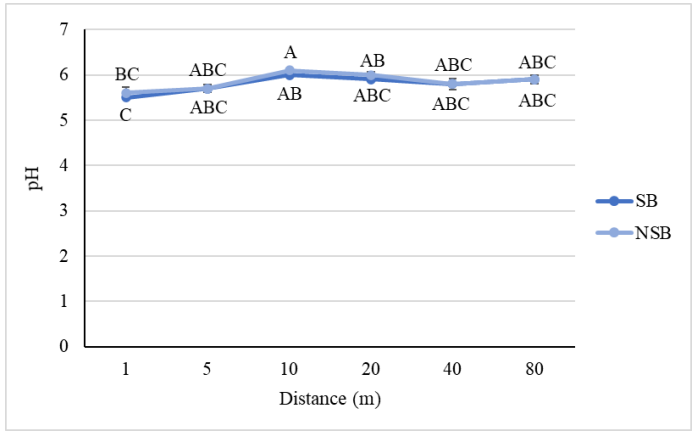
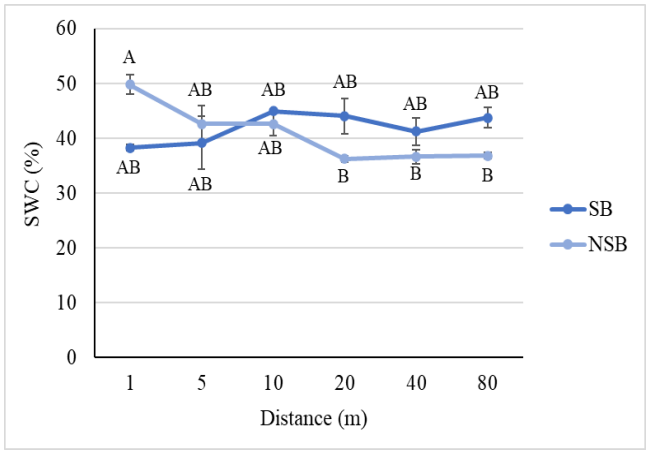


(TF)

Figure 4.1 Comparison of the effect of shelterbelt on soil pH of five dairy pasture farms under six distances: MF = Palmerston North dairy farm; (b) SD = Ashhurst dairy farm; (c) GO = Glen Oroua dairy farm; (d) TP = Apiti dairy farm and (e) TF = Palmerston North sheep and beef farm. Each point represents mean (n=3) ± Standard error of mean. Letter values denote two-way analysis of variance test. Same letters represent no significant different between shelterbelt and non-shelterbelt paddocks at the same sampling distance. SB = shelterbelt; NSB = non-shelterbelt.

4.1.2 Soil water content (SWC)

Among all the 5 sites (including both SB and NSB paddocks), gravimetric soil water content (SWC) ranged from 33.1 to 48.2 %. The two-way ANOVA (Table S10) indicated that SWC was significantly influenced by the presence of shelterbelt (S) at sites TF and SD whereas the effect of distance of sampling from shelterbelt (D) was not significant. The interaction of both S and D was significant at MF and SD sites. At 1 m sampling distance, SWC was significantly higher in the NSB plot as compared to the SB plot in the MF and TP sites (Fig. 4.2).



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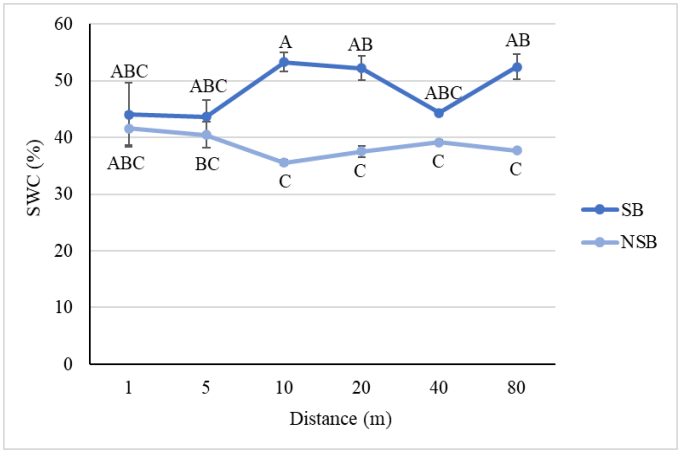
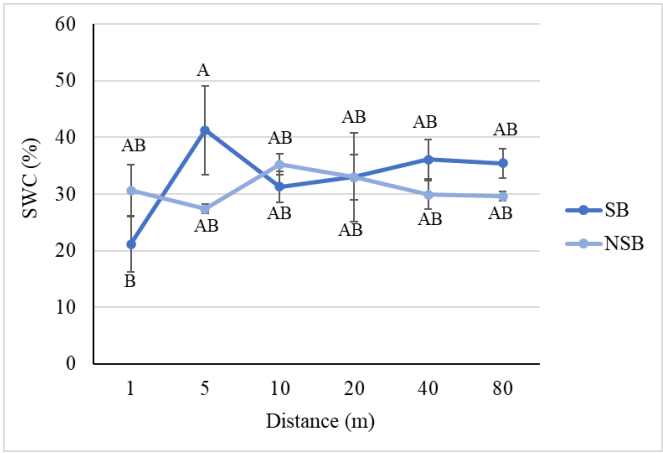


(SD)



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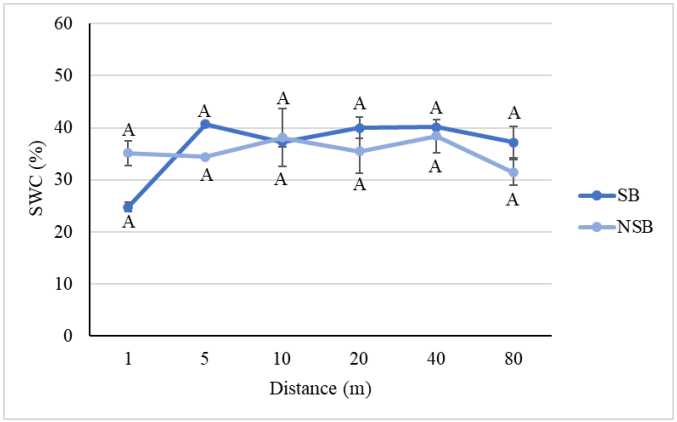

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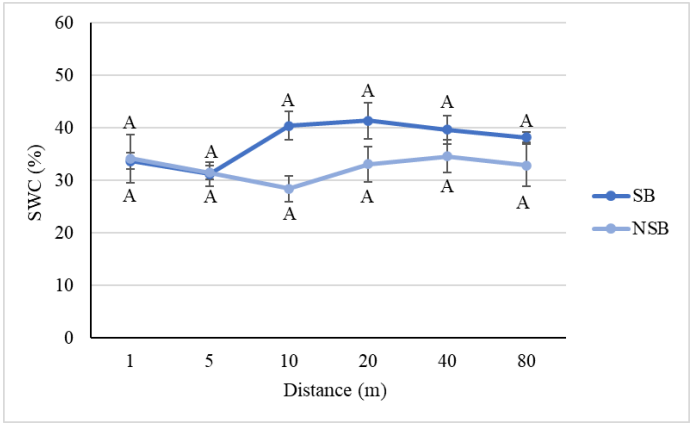


(TF)

Figure 4.2 Comparison of the effect of shelterbelt on soil water content (SWC) from five study sites (n=3 ± Standard error of mean): (a) MF = Palmerston North dairy farm; (b) SD = Ashhurst dairy farm; (c) GO = Glen Oroua dairy farm; (d) TP = Apiti dairy farm and (e) TF = Palmerston North sheep and beef farm. Each point represents mean (n=3) ± Standard error of mean. Letter values denote two-way analysis of variance test. Same letters represent no significant different between shelterbelt and non-shelterbelt paddocks at the same sampling distance. SB = shelterbelt; NSB = non-shelterbelt.

4.2. Soil mineral nitrogen content nitrate (NO_3^-) and ammonium (NH_4^+)

4.2.1 Soil nitrate-N content (NO_3^--N)

Soil NO_3^--N values in the study farms from both SB and NSB paddocks varied from 95.4 to 339.0 μg g^{-1} soil. There was a contrasting effect of shelterbelts on soil NO_3^--N at the different study sites. The two-way ANOVA (Table S10) showed that NO_3^--N content was significantly affected by the presence of shelterbelt (S) at sites MF and TP whereas the effect of distance of sampling from shelterbelt (D) was significant at sites SD, GO and TP. The interaction of both S and D was only significant at TF site (Fig 4.3). At 10 m sampling distance, NO_3^--N was significantly higher in the SB plot as compared to the NSB plot in the TF site.

4.2.2 Soil ammonium-N content (NH_4^+-N)

The interaction effect of S and D on NH_4^+-N was only significant at SD and TF sites (Fig 4.4.b and 4.4.e). At TF site, a pattern opposed to that of NO_3^--N concentration was observed here, where NH_4^+-N of NSB paddocks was significantly smaller than that of SB soils. There was no significant influence of S on NH_4^+-N concentration in MF, GO and TP sites. Overall, three study sites of NSB soils had the larger NH_4^+-N value rather than those of SB soils (Fig 4.4).

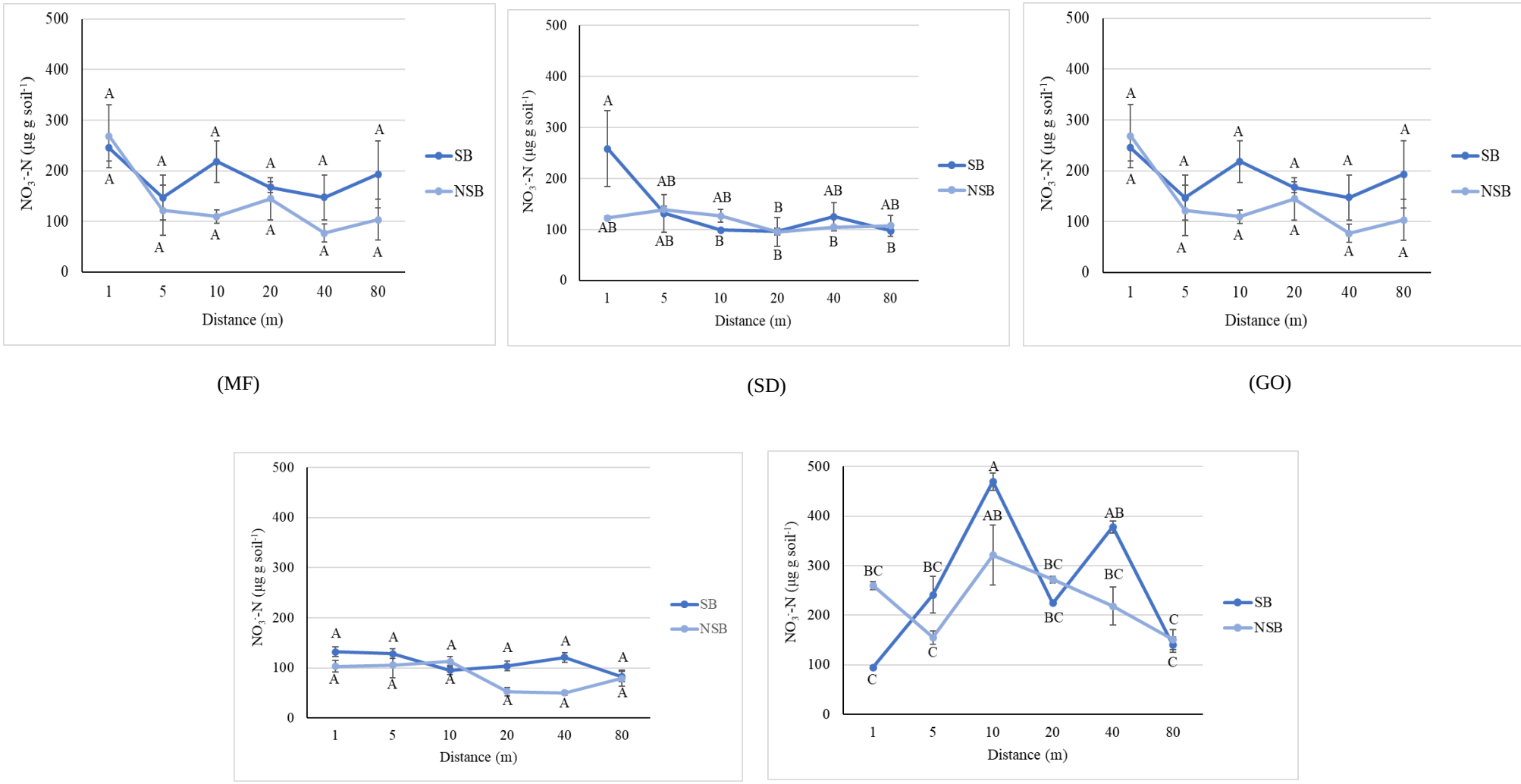
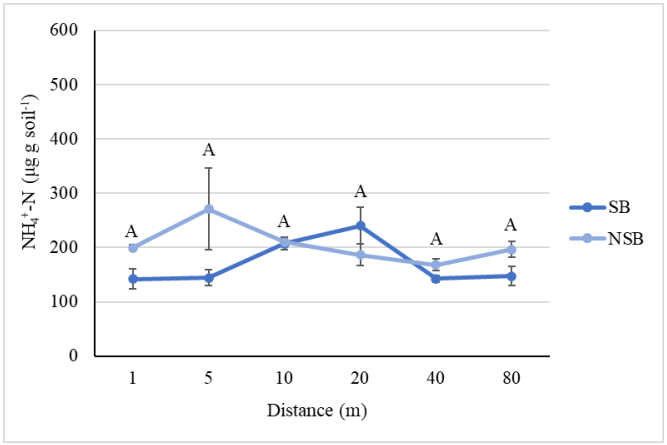


Figure 4.3 Comparison of the effect of shelterbelt on soil nitrate-nitrogen concentration (NO_3^--N) from five study sites (n=3 ± Standard error of mean): (a) MF = Palmerston North dairy farm; (b) SD = Ashhurst dairy farm; (c) GO = Glen Oroua dairy farm; (d) TP = Apiti dairy farm and (e) TF = Palmerston North sheep and beef farm. Each point represents mean (n=3) ± Standard error of mean. Letter values denote two-way analysis of variance test. Same letters represent no significant different between shelterbelt and non-shelterbelt paddocks at the same sampling distance. SB = shelterbelt; NSB = non-shelterbelt.



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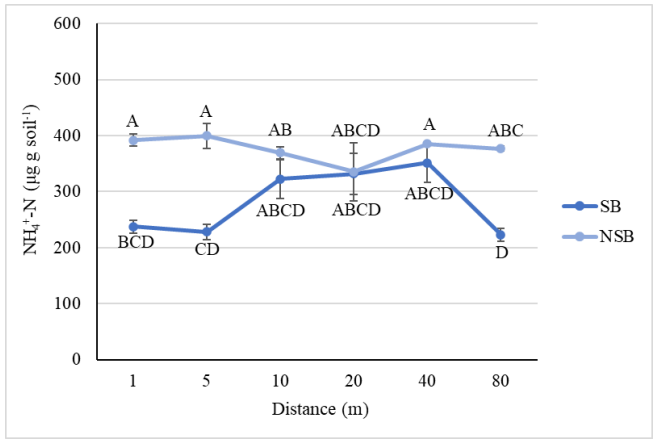

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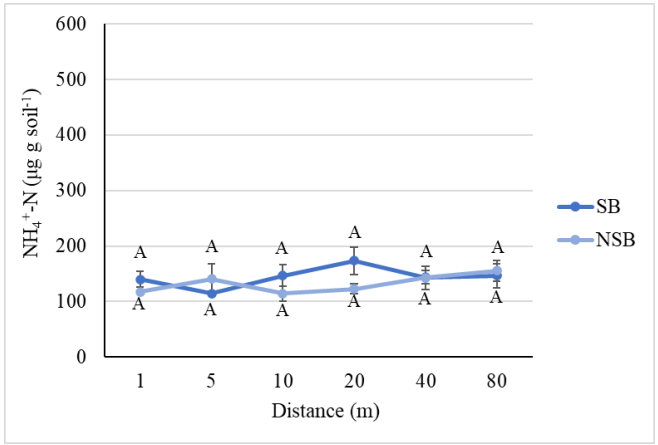

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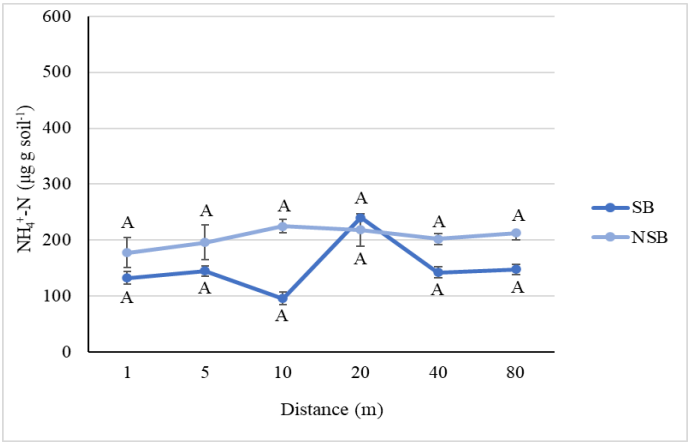

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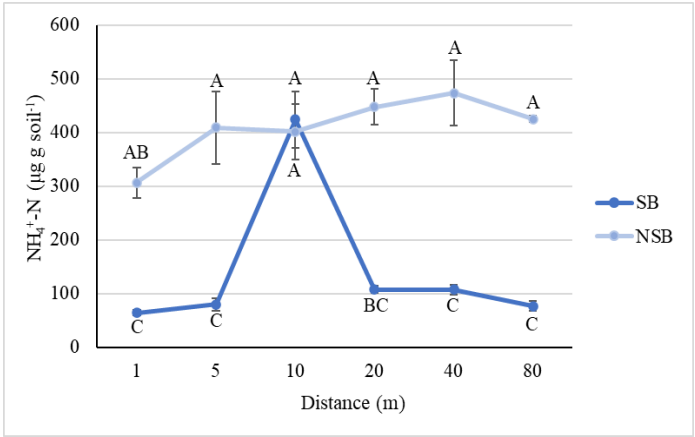

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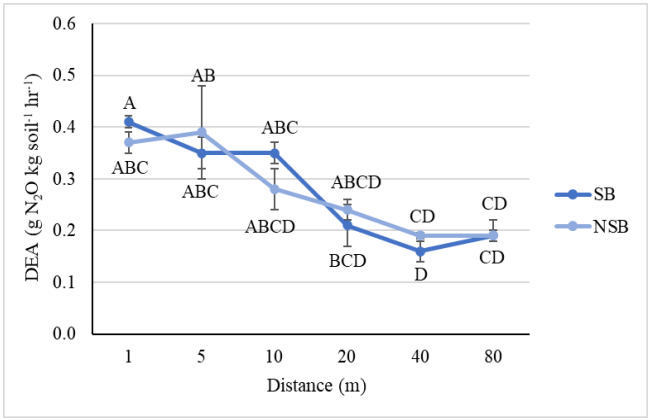
Figure 4.4 Comparison of the effect of shelterbelt on soil ammonium-nitrogen concentration (NH_4^+-N) from five study sites (n=3 ± Standard error of mean): (a) MF = Palmerston North dairy farm; (b) SD = Ashhurst dairy farm; (c) GO = Glen Oroua dairy farm; (d) TP = Apiti dairy farm and (e) TF = Palmerston North sheep and beef farm. Each point represents mean (n=3) ± Standard error of mean. Letter values denote two-way analysis of variance test. Same letters represent no significant different between shelterbelt and non-shelterbelt paddocks at the same sampling distance. SB = shelterbelt; NSB = non-shelterbelt.

4.3 Denitrification enzyme activity (DEA)

The presence of shelterbelt did not have a clear influence on soil DEA at the tested sites. Only at SD site, DEA was greater in the NSB plot than the SB plot. There was a significant of effect D on DEA at sites MF and TF. At site MF, DEA was significantly larger in samples closer to tree than sampled further from trees. The interaction of both S and D was only significant at TF site. In TF site at the sampling distances of 10 and 40 m DEA was significantly greater in the SB plot than the NSB plot (Fig 4.5).



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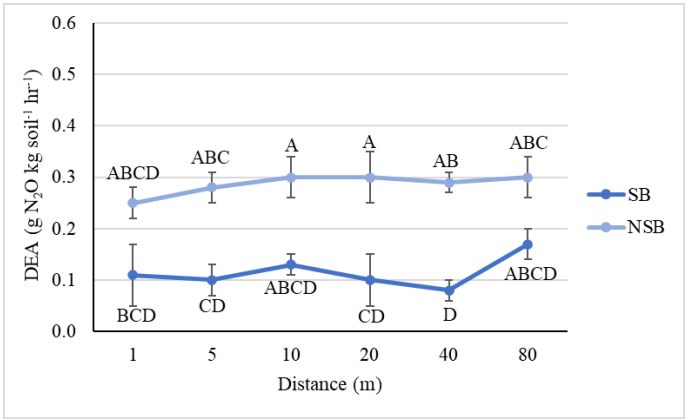

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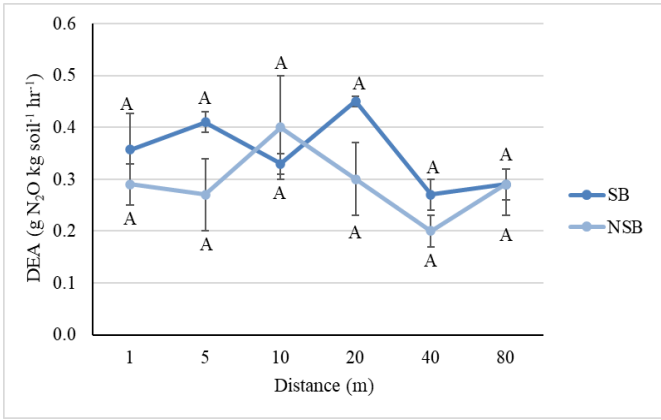

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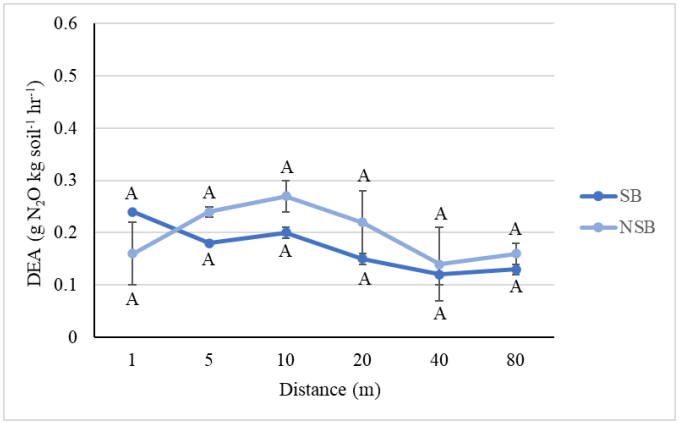

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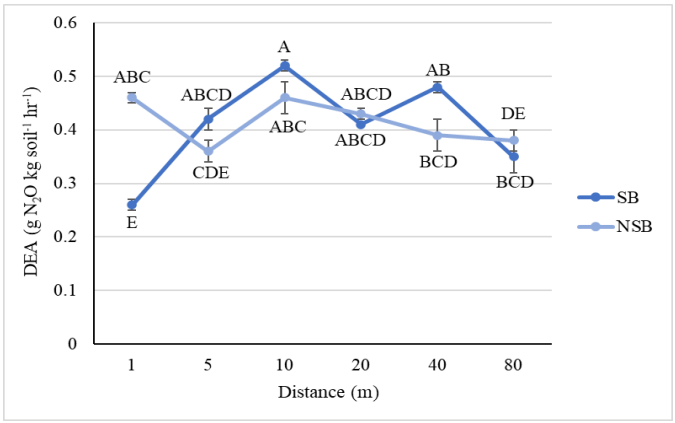
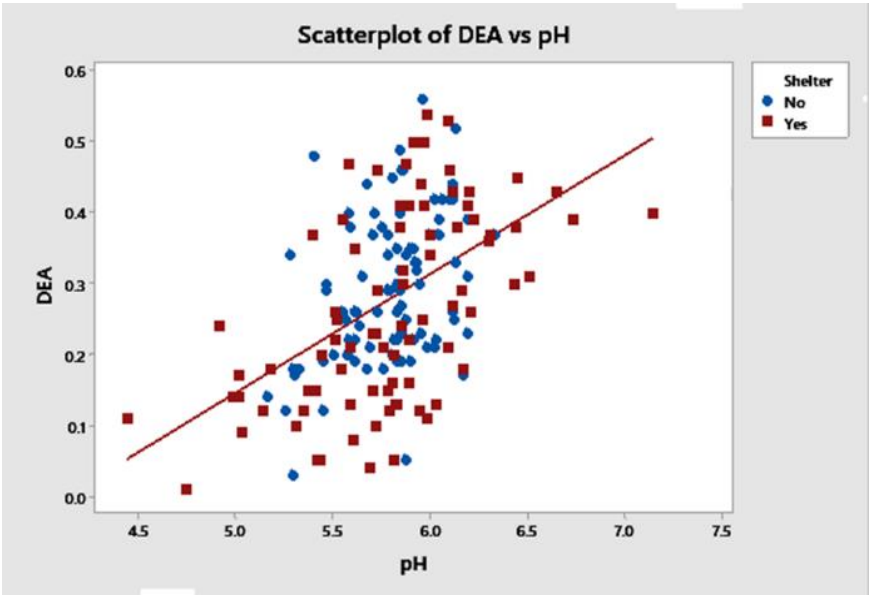


(TF)

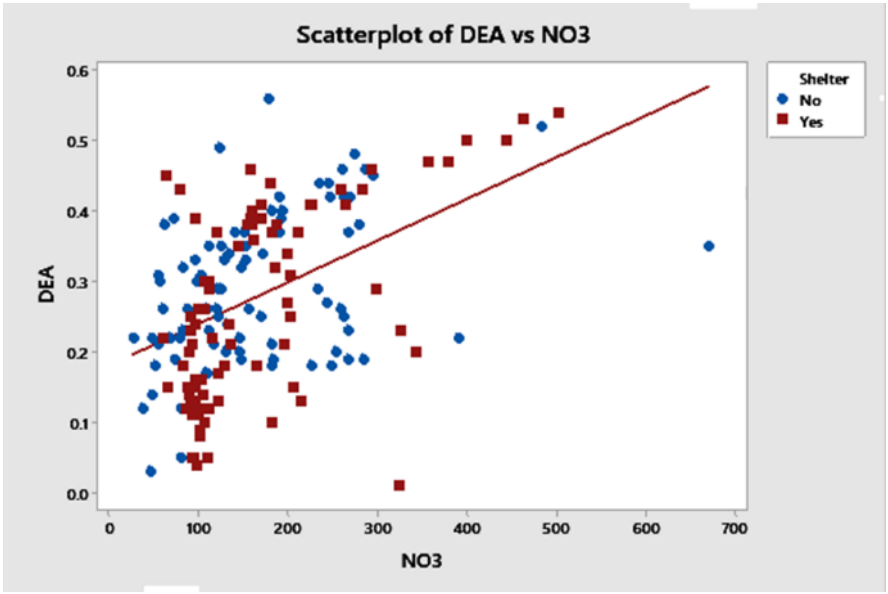
Figure 4.5 Comparison of the effect of shelterbelt on denitrification enzyme activity (DEA) produced from shelterbelt (SB) and non-shelterbelt (NSB) of soil samples by acetylene incubation method; data collected from (0-6 hrs) (n=3 ± Standard error of mean): (a) MF = Palmerston North dairy farm; (b) SD = Ashhurst dairy farm; (c) GO = Glen Oroua dairy farm; (d) TP = Apiti dairy farm and (e) TF = Palmerston North sheep and beef farm. Each point represents mean (n=3) ± Standard error of mean. Letter values denote two-way analysis of variance test. Same letters represent no significant different between shelterbelt and non-shelterbelt paddocks at the same sampling distance. SB = shelterbelt; NSB = non-shelterbelt.

4.4 Correlation of soil properties and denitrification enzyme activity (DEA)

Correlation analysis between soil properties and DEA showed (Figs. 4.6 a and b) significant ($P< 0.05$ and $R^2 = 0.5$) positive relation between pH, soil nitrate concentration, and DEA. This indicates that if pH and soil NO_3^--N content increase, the amount of N_2O emission from DEA tends to be increased.



$R^2 = 0.5$
P-value < 0.001



$R^2 = 0.5$
P-value < 0.001

Figure 4.6 (a) Scatterplots between relevant properties ([a] pH, and [b] soil nitrate co) and soil denitrification enzyme activity (DEA) (in all cases $P \leq 0.05$). Pearson coefficients of correlation (R^2) and *p*-values are displayed.

Experiment 2. Influence of shelterbelts on nitrous oxide flux from pastoral lands (field experiment)

The results of the laboratory incubation experiment showed that the presence of a shelterbelt only had significant effect on denitrification activity at the one of the five tested sites. In this experiment it was planned to test two contrasting farms (MF and SD) for in-field N_2O emissions and if shelterbelt influenced those emissions. The experiment was planned to test the hypothesis that there would be more N_2O emissions from NSB plot than the SB plots.

4.5 Soil properties at the time of gas sampling

There was significant effect of presence of S and sampling D on soil pH. However, these differences were different at the two sites (Table S12 and S13). At MF site, soil pH was higher in the SB plot (6.7) and also in the samples collected from closer to the trees/fence (6.5 to 6.7) as compared to the samples taken further away (around 6) (Table S6 and S8). In case of SD site, soil pH was higher in the NSB plot, and soil pH was not significantly different at various sampling distances apart from 1m, where soil pH was significantly lower than the other distances (Table S7 and S9).

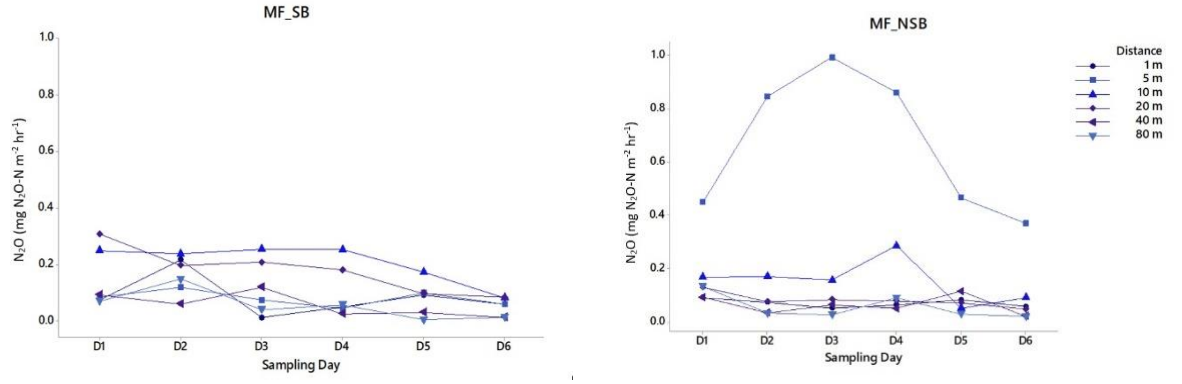
In the two sampling sites there were contrasting differences in the SWC at the time of gas sampling. At the MF site, there was no significant difference in the SWC in the SB and NSB plot, and the sampling points closer to shelterbelt relatively had greater SWC than the sampling points further away from trees (Table S6 and S8). In SD site there was significantly higher SWC (49%) in the SB plot than the NSB plot (36%). However, there was no significant difference in the SWC measured at various sampling locations in paddock at SD site (Table S7 and S9).

There was no difference in the NO_3^-- N and NH_4^+-N contents in the MF site among the SB and NSB plot, also there was no significant difference in the NO_3^--N and NH_4^+-N contents at the various sampling distances (Table S6 and S8). In contrast to the MF site, at the SD site, NO_3^--N content was significantly larger in the SB (190 to 220 μg g^{-1} soil) plot than the NSB (73 to 85 μg g^{-1} soil); also, there was greater soil NO_3^--N content (218 μg g^{-1} soil) at the sampling points closer to trees than the distances going further. However, NH_4^+-N content in NSB plot (290 μg g^{-1} soil) was significantly larger than that SB plot (200 μg g^{-1} soil) in site SD (Table S7 and S9).

4.6 Nitrous oxide emissions in two farms

In general, two-way ANOVA indicated that, at the MF site, there was significant effect of S, D and interaction of S and D on N_2O emissions. At the SD site, there was no significant effect of shelterbelt on soil N_2O emissions.

When considering the daily N_2O emissions from each site in the SB and NSB plots (Figure 4.7 (a)) it was apparent that in SB pot N_2O emissions increased during the first two sampling days. However, after day 3, the flux gradually decreased in both SB and NSB plots at the MF site. The amount of daily N_2O emission varied from 0.02 to 0.99 mg N_2O-N m^{-2} hr^{-1} in NSB plot and 0.01 to 0.31 mg N_2O-N m^{-2} hr^{-1} in SB plot during the 4 weeks of gas sampling.

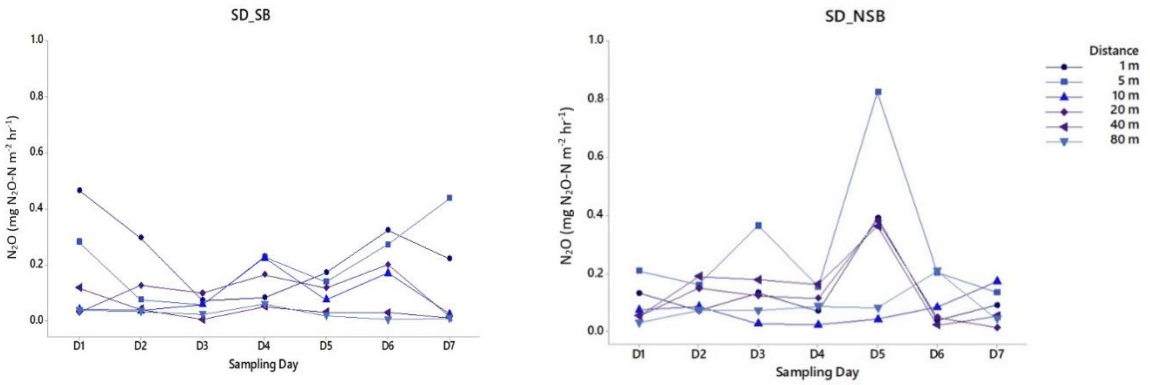


(a) (b)

Figure 4.7 Daily N_2O emission (mg N_2O-N m^{-2} hr^{-1}) produced from MF site (a) MF_SB (Palmerston North dairy farm shelterbelt) and (b) MF_NSB (Palmerston North dairy farm non-shelterbelt).

At MF site, in the SB plot the greatest daily emission was observed at 10 m distance from the SB on all sampling days except on day 1 and the smallest N_2O emissions at the sampling distances of 40 and 80 m (0.01 mg N_2O-N m^{-2} hr^{-1}) on the final day of sampling (Figure 4.7a).

N_2O emission at 5 m from the edge of the fence was 3-times higher than that of the rest of distances, on average, with values always above 0.90 mg N_2O-N m^{-2} hr^{-1}. Also, at this distance, there was a clear peak in N_2O emissions on day 3, with a sharp decrease to initial values thereafter. The N_2O emissions at the rest of distances were small, and below 0.30 mg N_2O-N m^{-2} hr^{-1}.



(a) (b)

Figure 4.8 Daily N_2O emission (mg N_2O-N m^{-2} hr^{-1}) in in SD site. (a) SD_SB and (b) SD_NSB. SD = Ashurst dairy farm. SB = shelterbelt; NSB = non-shelterbelt.

At the SD sites, there was, in general, a big fluctuation in daily N_2O emission at all the sampling distances in the SB plot. The emissions at all sampling distances ranged from 0.01 to 0.46 mg N_2O-N m^{-2} hr^{-1}in the SB plot. On all the sampling days except on day 3 in the SB plot the highest N_2O emissions were observed at sampling distances of 1 and 5 m, and the N_2O emissions decreased as the sampling distances increased from 20 m onwards. On the 7th day of gas sampling, at all the distances 20m and further from trees, N_2O emission were the least as compared to all the other sampling days (Figure 4.8a).

In NSB plots, at all sampling distances, N_2O emission gradually increased with sampling days (Fig.4.8b). We observed the greatest N_2O emission on D5 at 5 m sampling distance from the fence (0.83 mg N_2O-N m^{-2} hr^{-1}). The least N_2O emission (0.01 mg N_2O-N m^{-2} hr^{-1}) was measured at 20 m distance on the last day of sampling. Similar to MF site, NSB soils produce a higher flux than SB soils. In NSB plot, on average, the emission at 5 m distance was 2-times larger value always above 0.80 mg N_2O-N m^{-2} hr^{-1} than the rest of distances, the values were always below (0.40 mg N_2O-N m^{-2} hr^{-1}).

Table 4.1 Average of cumulative nitrous oxide emission (mg N_2O-N m^{-2}) over four weeks for two study farms. Letter values denote two-way analysis of variance test. Same letters represent no significant different between shelterbelt and non-shelterbelt paddocks at the same sampling distance.

Sampling distance	MF Site	SD Site
Shelterbelt		
1 m	2.4^{CDE}	6.7^{AB}
5 m	2.2^{CDE}	5.4^{ABC}
10 m	5.8^{B}	2.7^{CD}
20 m	4.8^{BC}	3.4^{BCD}
40 m	1.5^{E}	1.1^{D}
80 m	1.6^{DE}	0.7^{D}
Non-Shelterbelt		
1 m	1.9^{DE}	3.9^{BCD}
5 m	18.7^{A}	8.9^{A}
10 m	4.3^{BCD}	1.9^{CD}
20 m	2.4^{CDE}	4.0^{BCD}
40 m	1.8^{DE}	4.4^{BCD}
80 m	1.5^{E}	2.7^{CD}

MF = Palmerston North dairy farm; SD = Ashhurst dairy farm

The two-way ANOVA of cumulative N_2O (Table 4.1) showed that, at the MF site, there was significant difference of shelterbelt on cumulative N_2O emissions. The highest emission was observed at 5 m distance of NSB plot, which is significantly higher than all distance treatments from SB plot. At the SD site, there was no significant effect of shelterbelt on soil cumulative N_2O emissions.

4.7 Principal Component Analysis

The projection of variables on the factor plane (Fig. 4.9) revealed the strength of variance and relationship among soil variables with ordination axes. The principal component analysis (PCA) between soil physicochemical properties, soil temperature, distances of sampling, and field N_2O flux at two sampling sites generated six principal components (PCs).

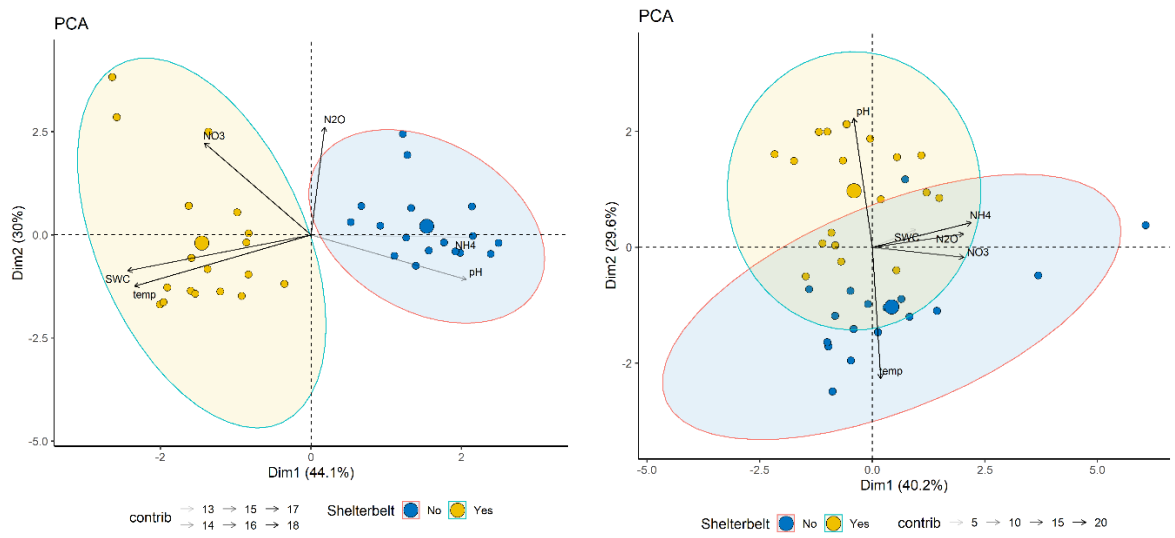


Figure 4.9 PCA biplot of two sampling sites as influenced by shelterbelt: SD and MF site. SD = Ashurst dairy farm, MF = Palmerston North dairy farm.

The PCA indicates that the first two components or Dim.1 and 2 explained the largest variation 44 % and 30 % in site SD whereas 40 % and 30 % in site MF, respectively. Therefore, to illustrate the data variation, only the first two PCs were considered because the subsequent PCs did not show a significant contribution.

A PCA biplot (Fig. 4.9a) typically graphs the projection of the first two components. In site SD, Dim.1 had large positive associations with soil pH, N_2O and NH_4^+ while the second component had only positive associations with soil NO_3^-. The other soil parameters had a large negative relation with Dim.1 and Dim.2. As Dim.1 increased soil pH and soil NH_4^+ content increased. This suggests that all these soil criteria varied together. In other words, if one parameter tends to increase, the other characteristics tend to rise as well. The grouping analysis denoted the two distinct groups of shelterbelt and non-shelterbelt in site SD. The

group corresponding to the non-shelterbelt (No) was associated with positive Dim.1 while shelterbelt (Yes) plotted in negative Dim.1 score.

On the other hand, in site MF, Dim.1 was driven by all measured parameters (soil N_2O, SWC, NO_3^- and NH_4^+) except soil pH where Dim.2 had a positive relation with soil pH (Fig 4.9.b). When Dim.1 increased, soil N_2O, SWC, NO_3^- and NH_4^+ increased. Although the grouping by PCA of shelterbelt (SB) and non-shelterbelt (NSB) was not evident, it could be assumed that NSB mostly corresponds to positive Dim.1.

Chapter 5 Discussion

5.1 Influence of planting shelterbelts on soil properties in a grazed pasture

Planting shelterbelts on agricultural soils is becoming a more common practice to protect soil and animals from strong wind and other harsh environmental conditions. Along with sequestering atmospheric CO_2, in tree biomass, shelterbelts are also effective in adding organic carbon to soil, conserving soil moisture, increasing soil pH, and provide addition nutrients for plant growth (Graham et al., 2005; Mayrinck et al., 2019). Soil pH is one of the major distal regulators for N_2 and N_2O production by microbial process in soil (Bremner and Shaw, 1958). In our study, pH values were significantly larger in the SB plots than the NSB plots at only one site (MF). One research in the grazed pasture of Australia revealed that the scattered trees have some effects on soil spatial patterns on the spot. It has been observed that the higher soil pH is found "inside" of the eucalyptus trees' canopy compared with the "outside" ones (Wilson 2002; Graham et al. 2005).

On the other hand, the fact that in site SD and TP, soil pH of shelterbelt was lower than that of non-shelterbelt paddocks is consistent with the results obtained by Graham et al. (2005) stated that lower soil pH was found at the proximity to the eucalypt trees. He suggested that this different pattern of soil pH value at trees plots might be explained by different soil type or soil parent materials of each site. Similarly, in the current study, the response of soil pH under shelterbelt is different for various study sites due to variability in soil type and parent materials (Table 3.1).

Pine needles are acidic in nature and when fall on ground, they make the soil acidic, that might be the reason for more acidic soil closer to the tree as compared to further away like the results obtained at SD and TP sites. Observed differences in the pH value of different shelterbelt plots compared with their pairs without shelterbelts in our study might be related, not only to the acidifying effect of pine trees, but also to the effect of trees on microclimate, resource availability, and soil properties are a heterogenous complex process (Gonzalez-Polo et al., 2019).

In this study at one site (MF) the decrease of pH was more pronounced with distance from the tree on the shelterbelt sites. Studies stated that soil pH, C, and P contents were shown to decrease with increasing distance from most of the trees studied in Australia (Ryan and McGarity,1983 and Wilson 2002) and overseas despite the soils having different management practices (Kellman 1979; Rhoades 1996; Bochet et al. 1999; Dean et al. 1999). This

systematic pattern might be a common feature of soils with trees in a range of environments. Therefore, the individual tree might have the potential for regulating soil properties, and particularly for soil acidity. Not only the shelterbelt trees but trees planted in random on farms also have the ability to improve soil quality, and especially soil acidity, in the grazed landscapes of northern NSW, Australia (Graham et al., 2005).

The higher SWC in SB plots compared with NSB plots could be explained by the fact that trees have a very close association with the soil they grow in. The soil surrounding the trees not only provide anchorage to tree roots, also it acts like a storehouse of water and other nutrients required by the trees for their growth. The effect of shading and reducing the wind speed of trees could minimize the evaporation and infiltration process of soils by increasing mulch layers deposited by litter from the trees and consequently improve soil structure. These are the major beneficial effects of trees which can facilitate the conservation of soil water in cropland (Young, 1997; Brandle et al., 2004; Campi et al., 2009).

The shading effect trees could increase soil moisture content by lowering surface evaporation from the soil and air movement (Green et al., 2003). The covering of woody plants makes minor changes in the microclimate beneath and the surrounding environment because of blocking precipitation and shading, both of which regulate the amount of available SWC (Young and Evans, 1987; Belsky et al., 1989; Breshears et al., 1997).

Regarding the effect of shelterbelt on soil moisture conservation, the paddock with trees on them could preserve more SWC than the paddock without shelterbelt, and this result is more prominent on the SD site. Similar results have been observed in soils in various other cropping systems where higher SWC has been observed in samples close to shelterbelt (Miller and Pallardy, 2001; Liversley et al., 2004; Shen et al., 2014).

The soil water content at four of the five study sites were lower in soils closer to shelterbelt and, with increasing distance from the trees SWC increased in those soils. This might be because of different distances from shelterbelt which can influence the capacity of trees for taking up soil water and NO_3^- content by competing with crop plants. Trees take up water from adjacent cropland which could reduce SWCs and, consequently, higher NO_3^- accumulation at the short distance from the shelterbelt (Qiao et al., 2016). These results are in line with Okorio (2000), who claimed that SWCs were lower in the soils in proximity with the shelterbelt rather than longer distances, also due to lack of moisture or shading effect crop productivity decreased in the area of the farm closer to the shelterbelt.

Owing to different soil origin, geographical location, and the management practices like fertilizer application and grazing period followed by the five dairy-grazed sites, the collected soils have shown a large variation in the physicochemical properties. The variations of NO_3^--N and NH_4^+-N concentration at five grazed pasture soils might generally be due to the background of N inputs from inorganic N fertilizer application and/or effluent irrigation, or animals' excreta (Jha, 2015).

In general, a higher content of NO_3^--N was accumulated at the shorter distance from the shelterbelt (1, 5 and 10 m) compared to farther away from trees (20, 40 and 80 m). Qiao et al., (2016) where they reported more NO_3^--N (470–600 kg $ha^{-1)}$ at close distances from trees of 2 and 14 m while 100–290 kg ha^{-1} was found at a longer distance from the shelterbelt (29 and 42 m).

The higher amount of residual nitrogen (N) closer to the shelterbelt could possibly be due to the shading of the pasture, its poor growth by the trees that reduced N uptake, mineralization of soil organic matter, and accumulation of litter and increased the lateral movement of nitrate. These results indicate that distance can influence the capacity of trees for taking up soil water and NO_3^--N content by competing with crop plants. This suggests that trees can absorb a generous amount of nutrients which might bring about lower nutrient levels in the soils at a closer distance rather than longer ones from the trees (Qiao et al., 2016).

Th smaller NH_4^+-N amount in SB soils of three study compared to NSB plots could be assumed that trees have been shown to reduce NH_4^+-N concentration in soils under shelterbelts. Ryszkowski and Kędziora (2007) who also revealed that shelterbelts soils had ability to reduce soil NH_4^+-N concentration in both soils in wintertime while increase the concentration in summertime. The reason for these different results in NH_4^+-N value might be due to variation in environmental factors, such as temperature and moisture at the sampling time. The lack of clear influences of shelterbelt on the soil properties studied might be because of a high spatial heterogeneity of trees, prior land use and different soil types (Gonzalez-Polo et al., 2019).

5.2 Denitrification enzyme activity in pasture soils influenced by the presence of shelterbelt

Denitrification is the major pathway of N losses in the form of N_2O in NZ pastoral soils and (Luo et al., 1999; Saggar, 2004 and 2007). Soil properties such as pH, SWC, availability of mineral N, soil temperature, and available soil carbon are the well-known factors affecting the N losses from the soil through denitrification (Groffman et al., 1987).

In this study, the correlation analysis between soil physicochemical properties and DEA suggests that soil pH and NO_3^--N content have a positive influence on the soil denitrification activity of the studied soils. This indicates that if pH and soil NO_3^--N content increase, the amount of N_2O emission from DEA tends to be increased.

In this study at some sites (MF and TF), it was observed soils closer to trees, had higher pH and NO_3^--N content, which potentially make them hotspots for higher DEA, however lack of SWC or anaerobic condition closer to the trees might prevent complete denitrification. It is generally accepted that a pH value from 6 to 8 is a favorable condition for denitrifier populations. The possible reason could be that there is a low chance of the N_2O reductase activity occurring at a low pH and, thus, these soils might be deficient in the active denitrifiers to perform the denitrification process, even in the presence of oxygen (Cuhel and Šimek, 2011; Hansen et al., 2014; Šimek and Cooper, 2002).

It is known that the higher level of NO_3^--N in soil inhibits the denitrification process and consequently causes a higher ratio of N_2O and N_2 by restricting Nos enzyme activity, which is responsible for the transformation of N_2O to N_2 (Scholefield et al., 1997; Stevens and Laughlin, 1998). Similarly, the accessibility of mineral N plays a principal role to initiate the denitrification process (Bolan et al., 2004b). As denitrifiers are facultative anaerobes, when O_2 availability is limited, NO_3^- becomes a dominant reductant (Aulakh et al., 1984). Lack of threshold SWC might prevent reduction of available NO_3 and further steps in denitrification. Therefore, the stated hypothesis to observe lower DEA in plots with planted trees proved only correct for one site (SD) in which SB paddocks had lower pH and lower NO_3^--N concentration than NSB plot.

5.3 Influence of shelterbelt and soil properties on field N_2O emissions in grazed pastures

Grazed temperate pastures in NZ are the major source of N_2O emissions, due to animal excretal deposition and continuous wet weather leading to high N_2O emissions from pasture soils. Plantation of trees on pastures is suggested as one of the mitigation options to combat higher N_2O emissions from pastures as trees can regulate soil pH, their roots will act as additional C source to fuel the microbial activities in soil and will also help absorb excess N and water present in soil, especially in the close vicinity of trees (Qiao et al., 2016). Shading and reduction in wind speed can reduce evaporation, and infiltration can be increased by the presence of mulch layers created by litter from the trees which, together with the tree roots, can improve soil structure (Torquebiau and Kwesiga, 1996; Young, 1997; Brandle et al., 2004).

Among the two studied sites, the hypothesis of this study was true only for the MF site where NSB paddock emitted more N_2O emission as compared to SB plot. At SD site there was no significance effect of shelterbelt on field N_2O emissions. One of the reasons for higher N_2O emission in the NSB plots in the MF site as compared to SB plots could be slightly higher mineral N contents in the NSB plot as compared to the SB plot at the MF site. Especially at 5 m distance from the fence in the NSB plot it was observed to have very high amount of NH_4^+-N content than the rest of the sampling distances.

The high mineral N content is directly related to high N_2O emissions. Studies stated that soil N_2O emissions will be higher when the soils had high NO_3^--N concentration (Carmo et al. 2005, Ruser et al. 2006, Zanatta et al. 2010). In this case since the SWC is less than 50% in the sampled soils, nitrification might be the main source of N_2O emission in this site. The study by Tan et al., 2018 also indicated that nitrification rates significantly increase with decreasing SWC. At the SD site, during the 4 weeks of gas sampling there were two grazing events which might have overshadowed the shelterbelt effect on soil N_2O emission at this site. Moreover, the sampling duration was not long enough to capture the effect of shelterbelt post grazing on soil N_2O.

5.4 Influence of shelterbelt on field nitrous oxide emissions

Shelterbelt plantation can indirectly influence GHG emissions by controlling plant-available N content in soil and modifying soil temperature and water content with the surrounding environment (Meinzer et al., 2001; Curiel Yuste et al., 2007). As expected, the N_2O emissions in these measurements were episodic and showed temporal changes and spatial dissimilarities among the SB and NSB plots.

Soil temperature is one of the major factors that controls N_2O and NO emissions. Studies have shown that soil temperature and N_2O and NO emissions are positively correlated increased in most temperate forest soils (Schindlbacher et al., 2004; Pilegaard et al., 2006; Schaufler et al., 2010). In this study, the shelterbelt paddock of SD site had a higher soil temperature than the MF site, which might be one of the reasons for observing an increased N_2O emission in the shortest distance of SD site from trees. This is due to the rates of enzymatic processes generally increasing exponentially with temperature, as long as other factors (e.g., substrate or moisture availability) are not limiting (Meixner and Yang, 2006). Surprisingly, in both non-shelterbelts of sites SD and MF, a distance of 5 m produced the maximum N_2O emission throughout the sampling days.

Woody species have the characteristics of being deep-rooted. Deep-root systems can absorb residual NO_3^- and prevent denitrification and also reduce NO_3^-leaching and associated indirect N_2O emission (Amadi et al., 2016). Studies showed that if the decomposition of the organic matter with a C: N ratio of 30, mineral N will tend to be immobilized by soil microorganisms (Brady and Weil, 2002). This points out that forest litter can raise biological immobilization of N and decrease N availability, as needed to produce N_2O (Bergeron et al., 2011; Dougherty et al., 2009; Evers et al., 2010).

In this field N_2O flux study, it is seen that high N_2O emission is generally produced from the treatments which had high NO_3^- concentration in both sites. High NO_3^- availability usually inhibits or retards N_2O reduction, also resulting in relatively high N_2O emission (Blackmer and Bremner 1978; Schlegel 1992; Van Cleemput 1998).

One of the possibilities of higher N_2O emissions in closer distance of SB plots at both the SD and MF sites, and especially at 5 m distance from the shelterbelt at the MF site could be grazing effect of animals/ animals taking shelter from rain, sun, or wind and addition of excreta as source of nitrous oxide emission. During the 4 weeks of N_2O sampling period in this study at the SD site, there were two occasions of grazing events, although we did not

observe any immediate influence of grazing on our field N_2O measurements. Effect of animal grazing on N_2O emission is well documented in literature (Saggar et al. 2008)

This result is in accordance with another study by Saggar et al. (2008) who stated that the highest number of N_2O emissions was found in dairy-grazed pastures (10–12 kg N_2O–N ha^{-1} $year^{-1}$), followed by sheep-grazed pastures, (4–6 kg N_2O-N ha^{-1} $year^{-1}$). The lowest number (1–2 kg N_2O–N ha^{-1} $year^{-1}$) was observed in woody trees, shrubland, and non-grazing soils. It indicated that the effect of woody trees on soil properties has also had an influence on N_2O fluxes (Saggar et al., 2008). Butterbach-Bahl et al., (2002) reported that the distance from tree stems has significantly influenced N_2O emissions; the highest emission has been found in the areas which are closer to tree stems than the intermediate stem areas.

Chapter 6 Conclusion

The conclusions from the observations in this study are listed below.

(i) Soil properties as influenced by planting shelterbelts on pasture soils.

Except from one site, there was a significant effect of shelterbelt on soil pH. However, there was no common trend, causing either an increase or a decrease in pH. The effect of shelterbelt on soil water storge was prominent in three sites, in which, SWC of shelterbelt paddocks was significantly greater than that of non-shelterbelt soils.

There was a contrasting effect of shelterbelts on soil NO_3^--N and NH_4^{+-}N concentration at the different study sites. In general, a higher NO_3^--N content accumulated at the shorter distance from the shelterbelt (1m, 5 m, and 10 m) compared with farther away from trees (20, 40, and 80 m). At the same time lower NH_4^{+-}N content was found in soil closer to trees.

(ii) Effect of shelterbelt on soil denitrification enzyme activity.

Out of the five studied sites presence of shelterbelt significantly decreased DEA in one of the pasture soils. The correlation analysis suggested that DEA in the tested soil had moderate linear relationship between soil pH and NO_3^--N concentration.

(iii) Effect of shelterbelt on in filed N_2O emissions.

Field N_2O measurements were conducted only at MF and SD sites. Higher N_2O emission was positively related to high NO_3^--N content in both sites. At the MF site there was a significantly higher N_2O emission from the NSB plot than the SB plot. At the MF site there was very high N_2O emission in the NSB plot during the 4 weeks on measurement period, which was attributed to the large mineral N content at 5 m distance.

The two experiments of DEA and field N_2O emission suggest that planting shelterbelts especially pine on pasture soils can potentially decrease soil pH and NO_3^--N contents in pasture soils. Therefore, shelterbelts can indirectly reduce incomplete denitrification and thus reduce overall N_2O emissions in a site-specific way. The results presented in this study are from selected few farms and limited parameters for measuring soil DEA and N_2O emission. Therefore, for further studies, there is a need to collect robust information from other sites, at various locations, to measure soil C and nitrification potential and ensure that seasonal variations in N_2O emission under shelterbelts are measured.

Supplementary Information

Table S1. The analysed physicochemical properties of soil collected (0-10 cm depth) from paddocks with and without shelterbelt paddocks of MF site under different distances. Data are mean (n=3) ± standard errors.

	Gravimetric SWC (%)		pH (1:2.5, soil: water ratio)		NO_3^--N ($\mu g\ g^{-1}$ soil)		NH_4^+-N ($\mu g\ g^{-1}$ soil)	
Distance from shelterbelt/fence	SB	NSB	SB	NSB	SB	NSB	SB	NSB
1M	38.3 ± 0.6	49.8 ± 1.7	6.7 ± 0.3	5.6 ± 0.15	110.3 ± 24.8	210.1 ± 34.4	141.7 ± 18.4	199.7 ± 5.1
5M	39.2 ± 4.8	42.6 ± 3.3	6.5 ± 0.1	5.9 ± 0.03	127.1 ± 15.0	339.0 ± 16.5	144.5 ± 15.0	270.8 ± 34.9
10M	44.9 ± 0.3	42.6 ± 2.0	6.3 ± 0.2	5.9 ± 0.22	189.2 ± 5.9	265.3 ± 1.5	206.9 ± 11.7	210.3 ± 7.9
20M	44.1 ± 3.2	36.2 ± 0.5	6.0 ± 0.1	5.7 ± 0.09	173.6 ± 34.3	251.3 ± 4.7	240.3 ± 33.8	186.6 ± 19.8
40M	41.3 ± 2.5	36.7 ± 1.3	6.0 ± 0.1	5.7 ± 0.11	120.8 ± 22.0	219.7 ± 37.8	142.2 ± 5.6	168.3 ± 11.0
80M	43.7 ± 1.8	36.8 ± 0.6	6.0 ± 0.1	5.7 ± 0.02	106.0 ± 14.0	229.9 ± 30.1	147.4 ± 18.0	196.5 ± 14.3

SWC = soil water content; NO_3^--N = nitrate-nitrogen; NH_4^+-N = ammonium-nitrogen; MF = Palmerston North dairy farm; SB = shelterbelt; NSB = non-shelterbelt

Table S2. The analysed physicochemical properties of soils collected (0-10 cm) from with and without shelterbelt paddocks of SD site under different distances. Data are mean (n=3) ± standard errors.

	Gravimetric SWC (%)		pH (1:2.5, soil: water ratio)		NO_3^--N (μg g^{-1} soil)		NH_4^+-N (μg g^{-1} soil)	
Distance from shelterbelt/fence	SB	NSB	SB	NSB	SB	NSB	SB	NSB
1M	44.0 ± 5.6	41.6 ± 2.9	5.1 ± 0.20	5.6 ± 0.08	258.3 ± 34.3	122.6 ± 3.0	141.7 ± 11.3	237.4 ± 11.3
5M	43.7 ± 2.9	40.5 ± 2.2	5.8 ± 0.09	5.6 ± 0.11	131.6 ± 37.0	138.9 ± 7.0	144.5 ± 21.4	228.1 ± 22.4
10M	53.3 ± 1.7	35.6 ± 0.4	5.8 ± 0.03	6.0 ± 0.04	98.7 ± 3.2	126.5 ± 12.8	206.9 ± 34.5	322.4 ± 10.8
20M	52.2 ± 2.1	37.5 ± 0.1	5.7 ± 0.13	6.0 ± 0.03	95.9 ± 6.2	95.4 ± 28.5	240.3 ± 37.1	331.5 ± 51.7
40M	44.3 ± 0.5	39.2 ± 0.4	5.5 ± 0.08	6.0 ± 0.12	125.2 ± 27.8	104.3 ± 2.0	142.2 ± 34.9	351.5 ± 2.0
80M	52.5 ± 2.2	37.6 ± 0.1	5.8 ± 0.07	6.1 ± 0.01	97.4 ± 4.8	107.1 ± 20.5	147.4 ± 12.0	223.0 ± 4.3

SWC = soil water content; NO_3^--N = nitrate-nitrogen; NH_4^+-N = ammonium-nitrogen; SD = Ashhurst dairy farm; SB = shelterbelt; NSB = non-shelterbelt

Table S3. The analysed physicochemical properties of soils collected (0-10 cm depth) from GO site of shelterbelt and without shelterbelt paddocks under different distances. Data are mean (n=3) ± standard errors.

	Gravimetric SWC (%)		pH (1:2.5, soil: water ratio)		NO_3^--N (μg g^{-1} soil)		NH_4^+-N (μg g^{-1} soil)	
Distance from shelterbelt/fence	SB	NSB	SB	NSB	SB	NSB	SB	NSB
1M	21.2 ± 5.0	30.7 ± 4.6	6.0 ± 0.19	5.9 ± 0.06	245.4 ± 25.9	268.6 ± 62.4	140.1 ± 14.8	117.3 ± 2.6
5M	41.3 ± 7.8	27.4 ± 0.8	6.0 ± 0.32	5.9 ± 0.03	147.1 ± 44.1	121.6 ± 49.7	114.2 ± 3.9	140.4 ± 26.8
10M	31.3 ± 2.7	35.2 ± 1.8	6.2 ± 0.09	5.9 ± 0.05	218.5 ± 41.0	109.9 ± 13.2	146.5 ± 19.2	114.4 ± 13.3
20M	33.0 ± 3.2	33.0 ± 4.0	6.0 ± 0.08	5.7 ± 0.26	167.6 ± 11.1	144.5 ± 41.8	173.6 ± 24.8	123.0 ± 8.4
40M	36.2 ± 3.5	30.0 ± 2.5	6.0 ± 0.09	5.8 ± 0.09	147.4 ± 44.3	76.9 ± 18.4	143.6 ± 25.4	142.5 ± 20.6
80M	35.4 ± 2.5	29.6 ± 0.8	5.7 ± 0.04	5.8 ± 0.17	193.1 ± 66.4	102.9 ± 40.4	121.6 ± 3.7	155.2 ± 18.9

SWC = soil water content; NO_3^--N = nitrate-nitrogen; NH_4^+-N = ammonium-nitrogen; GO = Glen Oroua dairy farm; SB = shelterbelt; NSB = non-shelterbelt

Table S4. The physicochemical properties of soils collected (0-10 cm depth) from shelterbelt and non-shelterbelt paddocks of TP site under different distances. Data are mean (n=3) ± standard errors.

	Gravimetric SWC (%)		pH (1:2.5, soil: water ratio)		NO_3^--N ($\mu g\ g^{-1}$ soil)		NH_4^+-N ($\mu g\ g^{-1}$ soil)	
Distance from shelterbelt/fence	SB	NSB	SB	NSB	SB	NSB	SB	NSB
1M	24.8 ± 0.9	35.1 ± 2.3	4.9 ± 0.2	6.1 ± 0.09	132.3 ± 9.5	103.2 ± 12	177.4 ± 11.5	199.0 ± 26.7
5M	40.7 ± 0.1	34.4 ± 0.3	5.5 ± 0.2	5.9 ± 0.17	127.9 ± 9.6	105.3 ± 25	196.0 ± 9.6	213.2 ± 30.4
10M	37.3 ± 0.9	38.1 ± 5.5	5.5 ± 0.2	5.8 ± 0.09	95.5 ± 9.5	112.5 ± 10.8	224.6 ± 11.5	139.8 ± 11.9
20M	40.0 ± 2.1	35.5 ± 4.3	5.1 ± 0.1	5.6 ± 0.11	103.8 ± 10.0	52.5 ± 8.8	217.9 ± 6.1	151.8 ± 29.3
40M	40.1 ± 0.1	38.4 ± 3.2	5.2 ± 0.2	5.4 ± 0.17	120.8 ± 9.6	50.4 ± 4.6	201.7 ± 9.6	139.2 ± 10.0
80M	37.2 ± 3.0	31.4 ± 2.4	5.1 ± 0.3	5.3 ± 0.02	82.6 ± 10.4	79.4 ± 16.5	212.8 ± 9.4	151.5 ± 12.5

SWC = soil water content; NO_3^--N = nitrate-nitrogen; NH_4^+-N = ammonium-nitrogen; TP = Apiti dairy farm; SB = shelterbelt; NSB = non-shelterbelt

Table S5. The analysed physicochemical properties of soils collected (0-10 cm depth) from shelterbelt and non-shelterbelt paddocks of TF site under different distances. Data are mean (n=3) ± standard errors.

	Gravimetric SWC (%)		pH (1:2.5, soil: water ratio)		NO_3^--N ($\mu g\ g^{-1}$ soil)		NH_4^+-N ($\mu g\ g^{-1}$ soil)	
Distance from shelterbelt/fence	SB	NSB	SB	NSB	SB	NSB	SB	NSB
1M	33.7 ± 1.5	34.2 ± 4.58	5.5 ± 0.01	5.6 ± 0.13	94.3 ± 4.6	259.6 ± 8.4	64.0 ± 5.4	306.3 ± 28.1
5M	31.2 ± 2.30	31.5 ± 1.33	5.7 ± 0.08	5.7 ± 0.08	241.2 ± 37.4	155.0 ± 13.8	79.5 ± 11.8	408.7 ± 66.8
10M	40.4 ± 2.78	28.4 ± 2.49	6.0 ± 0.05	6.1 ± 0.02	469.4 ± 17.2	321.3 ± 61.0	424.2 ± 52.6	401.3 ± 52.0
20M	41.2 ± 3.42	33.1 ± 3.32	5.9 ± 0.04	6.0 ± 0.07	224.1 ± 1.1	271.8 ± 6.8	107.7 ± 7.2	447.4 ± 32.9
40M	39.7 ± 2.70	34.6 ± 3.13	5.8 ± 0.12	5.8 ± 0.05	377.9 ± 12.0	218.4 ± 38.7	107.1 ± 8.8	473.4 ± 60.6
80M	48.2 ± 1.00	32.9 ± 3.96	5.9 ± 0.10	5.9 ± 0.10	140.2 ± 15.3	150.7 ± 19.6	76.6 ± 9.7	425.2 ± 5.6

SWC = soil water content; NO_3^--N = nitrate-nitrogen; NH_4^+-N = ammonium-nitrogen; TF = Palmerston north sheep and beef farm; SB = shelterbelt; NSB = non-shelterbelt

Table S6. The physicochemical properties of soil collected (0-10 cm depth) paddocks of MF site with and without shelterbelts under different distances at the first sampling day. Data are mean (n=3) ± standard errors.

	Gravimetric SWC (%)		pH (1:2.5, soil: water ratio)		NO_3^--N ($\mu g\ g^{-1}$ soil)		NH_4^+-N ($\mu g\ g^{-1}$ soil)	
Distance from shelterbelt/fence	SB	NSB	SB	NSB	SB	NSB	SB	NSB
1M	38.3 ± 0.6	49.8 ± 1.7	6.8 ± 0.3	5.6 ± 0.15	110.3 ± 24.8	210.1 ± 34.4	141.7 ± 18.4	199.7 ± 5.1
5M	39.2 ± 4.8	42.6 ± 3.3	6.5 ± 0.1	5.9 ± 0.03	127.1 ± 15.0	339.0 ± 16.5	144.5 ± 15.0	270.8 ± 34.9
10M	44.9 ± 0.3	42.6 ± 2.0	6.3 ± 0.2	5.9 ± 0.22	189.2 ± 5.9	265.3 ± 1.5	206.9 ± 11.7	210.3 ± 7.9
20M	44.1 ± 3.2	36.2 ± 0.5	6.0 ± 0.1	5.7 ± 0.09	173.6 ± 34.3	251.3 ± 4.7	240.3 ± 33.8	186.6 ± 19.8
40M	41.3 ± 2.5	36.7 ± 1.3	6.0 ± 0.1	5.7 ± 0.11	120.8 ± 22.0	219.7 ± 37.8	142.2 ± 5.6	168.3 ± 11.0
80M	43.7 ± 1.8	36.8 ± 0.6	6.0 ± 0.1	5.7 ± 0.02	106.0 ± 14.0	229.9 ± 30.1	147.4 ± 18.0	196.5 ± 14.3

SWC = soil water content; NO_3^--N = nitrate-nitrogen; NH_4^+-N = ammonium-nitrogen; MF = Palmerston North dairy farm; SB = shelterbelt; NSB = non-shelterbelt

Table S7. The analysed physicochemical properties of soil collected (0-10 cm depth) from paddocks of SD site with and without shelterbelts under different distances at the first sampling day. Data are mean (n=3) ± standard errors.

	Gravimetric SWC (%)		pH (1:2.5, soil: water ratio)		NO_3^--N (μg g^{-1} soil)		NH_4^+-N (μg g^{-1} soil)	
Distance from shelterbelt/fence	SB	NSB	SB	NSB	SB	NSB	SB	NSB
1M	44.0 ± 5.6	41.6 ± 2.9	5.1 ± 0.20	5.6 ± 0.08	258.3 ± 34.3	122.6 ± 3.0	141.7 ± 11,3	237.4 ± 11.3
5M	43.7 ± 2.9	40.5 ± 2.2	5.8 ± 0.09	5.6 ± 0.11	131.6 ± 37.0	138.9 ± 7.0	144.5 ± 21.4	228.1 ± 22.4
10M	53.3 ± 1.7	35.6 ± 0.4	5.8 ± 0.03	6.0 ± 0.04	98.7 ± 3.26	126.5 ± 12.8	206.9 ± 34.5	322.4 ± 10.8
20M	52.2 ± 2.1	37.5 ± 0.1	5.7 ± 0.13	6.0 ± 0.03	95.9 ± 6.2	95.4 ± 28.5	240.3 ± 37.1	331.5 ± 51.7
40M	44.3 ± 0.5	39.2 ± 0.4	5.5 ± 0.08	6.0 ± 0.12	125.2 ± 27.8	104.3 ± 2.0	142.2 ± 34.9	351.5 ± 2.0
80M	52.5 ± 2.2	37.6 ± 0.1	5.8 ± 0.07	6.1 ± 0.01	97.4 ± 4.8	107.1 ± 20.5	147.4 ± 12.0	223.0 ± 4.3

SWC = soil water content; NO_3^--N = nitrate-nitrogen; NH_4^+-N = ammonium-nitrogen; SD = Ashhurst dairy farm; SB = shelterbelt; NSB = non-shelterbelt

Table S8. The analysed physicochemical properties of soil collected (0-10 cm depth) from paddocks of MF site with and without shelterbelts under different distances at the final sampling day. Data are mean (n=3) ± standard errors.

	Gravimetric SWC (%)		pH (1:2.5, soil: water ratio)		NO_3^--N ($\mu g\ g^{-1}$ soil)		NH_4^+-N ($\mu g\ g^{-1}$ soil)	
Distance from shelterbelt/fence	SB	NSB	SB	NSB	SB	NSB	SB	NSB
1M	38.4 ± 0.2	50.8 ± 1.9	6.7 ± 0.2	5.5 ± 0.2	175.5 ± 46.6	179.2 ± 43.0	276.4 ± 20.6	260.6 ± 7.1
5M	38.4 ± 5.3	46.0 ± 1.1	6.6 ± 0.1	5.8 ± 0.2	224.0 ± 15.1	519.0 ± 19.3	280.6 ± 23.6	339.2 ± 30.0
10M	49.1 ± 0.4	47.7 ± 3.9	6.3 ± 0.1	5.9 ± 0.3	246.1 ± 34.8	204.8 ± 45.0	315.3 ± 19.5	295.0 ± 9.8
20M	47.2 ± 3.2	40.0 ± 0.4	6.3 ± 0.1	5.6± 0.1	148.8 ± 27.5	157.2 ± 10.8	342.2 ± 7.9	261.6 ± 3.2
40M	41.3 ± 2.5	37.3 ± 1.5	6.0 ± 0.3	5.6 ± 0.1	310.0 ± 19.8	108.1 ± 9.8	274.0 ± 16.3	248.3 ± 17.8
80M	47.6 ± 0.5	36.7 ± 0.8	6.0 ± 0.1	5.7 ± 0.1	144.7 ± 10.2	292.0 ± 16.2	281.5 ± 6.7	247.7 ± 1.2

SWC = soil water content; NO_3^--N = nitrate-nitrogen; NH_4^+-N = ammonium-nitrogen; MF = Palmerston North dairy farm; SB = shelterbelt; NSB = non-shelterbelt

Table S9. The analysed physicochemical properties of soil collected (0-10 cm depth) from paddocks of SD site with and without shelterbelts under different distances at the final sampling day. Data are mean (n=3) ± standard errors.

	Gravimetric SWC (%)		pH (1:2.5, soil: water ratio)		NO_3^--N ($\mu g\ g^{-1}$ soil)		NH_4^+-N ($\mu g\ g^{-1}$ soil)	
Distance from shelterbelt/fence	SB	NSB	SB	NSB	SB	NSB	SB	NSB
1M	53.7 ± 8.2	39.5 ± 0.3	5.7 ± 0.33	5.8 ± 0.12	179.7 ± 49.3	64.1 ± 10.6	173.8 ± 31.2	159.1 ± 1.9
5M	49.4 ± 0.8	36.4 ± 1.9	5.9 ± 0.21	6.0 ± 0.03	250.7 ± 13.1	82.1 ± 8.2	175.2 ± 23.4	152.5 ± 5.6
10M	50.1 ± 2.3	36.9 ± 0.3	5.8 ± 0.05	6.3 ± 0.04	79.7 ± 7.0	60.5 ± 11.1	174.3 ± 23.6	159.3 ± 3.0
20M	47.2 ± 2.1	35.3 ± 0.6	5.8 ± 0.06	6.2 ± 0.15	81.1 ± 2.7	52.0 ± 4.4	158.9 ± 10.9	154.8 ± 4.3
40M	47.6 ± 2.1	38.7 ± 1.2	5.8 ± 0.06	6.2 ± 0.23	77.5 ± 8.9	66.8 ± 9.7	154.5 ± 9.2	186.2 ± 19.4
80M	45.8 ± 2.5	37.6 ± 2.1	5.8 ± 0.01	6.4 ± 0.28	82.2 ± 2.9	48.7 ± 3.3	164.3 ± 1.5	202.3 ± 39.4

SWC = soil water content; NO_3^--N = nitrate-nitrogen; NH_4^+-N = ammonium-nitrogen; SD = Ashhurst dairy farm; SB = shelterbelt; NSB = non-shelterbelt

Table S10. Two-way ANOVA *P*-values of soil properties in paddocks with and without shelterbelts for five dairy pasture farms.

Site	Source	pH	SWC (%)	NO_3^--N (μg g^{-1} soil)	NH_4^+-N (μg g^{-1} soil)
MF	Shelterbelt	<.001	0.414	0.001	0.034
	Distance	0.018	0.196	0.586	0.168
	Shelter*Distance	0.069	0.003	0.803	0.058
SD	Shelterbelt	<.001	<.001	0.267	<.001
	Distance	<.001	0.623	0.029	0.171
	Shelter*Distance	0.028	0.011	0.083	0.011
GO	Shelterbelt	0.114	0.371	0.074	0.447
	Distance	0.606	0.320	0.051	0.797
	Shelter*Distance	0.815	0.081	0.703	0.157
TP	Shelterbelt	<.001	0.543	0.012	0.004
	Distance	0.050	0.165	0.027	0.770
	Shelter*Distance	0.267	0.286	0.090	0.119
TF	Shelterbelt	0.344	0.008	0.145	<.001
	Distance	<.001	0.382	<.001	<.001
	Shelter*Distance	0.931	0.289	<.001	<.001

SWC = soil water content; NO_3^--N = nitrate-nitrogen; NH_4^+-N = ammonium-nitrogen; MF = Palmerston North dairy farm; SD = Ashhurst dairy farm; GO = Glen Oroua dairy farm; TP = Apiti dairy farm; TF = Palmerston north sheep and beef farm.

Table S11. Two-way ANOVA *P*-values of denitrification enzyme activity (DEA) produced from paddocks of shelterbelt and without shelterbelts in five study sites.

Source	MF	SD	GO	TP	TF
Shelterbelt	0.958	<.001	0.099	0.543	0.573
Distance	<.001	0.749	0.166	0.165	<.001
Shelter*Distance	0.626	0.843	0.370	0.286	<.001

SWC = soil water content; MF = Palmerston North dairy farm; SD = Ashurst dairy farm; GO = Glen Oroua dairy farm; TP = Apiti dairy farm; TF = Palmerston north sheep and beef farm

Table S12. Two-way ANOVA *P*-values of soil properties in study sites at the first sampling day of field N2O flux study

Site	Source	pH	SWC (%)	NO_3^--N ($\mu g\ g^{-1}$ soil)	NH_4^+-N ($\mu g\ g^{-1}$ soil)
MF	Shelterbelt	<.001	0.41	0.001	0.03
	Distance	0.02	0.20	0.59	0.17
	Shelterbelt* Distance	0.07	0.003	0.80	0.06
SD	Shelterbelt	<.001	<.001	0.27	<.001
	Distance	<.001	0.62	0.03	0.17
	Shelterbelt* Distance	0.03	0.08	0.08	0.01

SWC = soil water content; MF = Palmerston North dairy farm; SD = Ashurst dairy farm

Table S13. Two-way ANOVA *P*-values of soil properties in study sites at the final sampling day of field N2O flux study

Site	Source	pH	SWC (%)	NO_3^--N ($\mu g\ g^{-1}$ soil)	NH_4^+-N ($\mu g\ g^{-1}$ soil)
MF	Shelterbelt	<.001	0.20	0.74	0.05
	Distance	0.05	0.11	0.42	0.01
	Shelterbelt* Distance	0.09	<.001	0.17	0.01
SD	Shelterbelt	0.001	<.001	<.001	0.83
	Distance	0.26	0.55	<.001	0.79
	Shelterbelt* Distance	0.54	0.89	<.001	0.38

SWC = soil water content; MF = Palmerston North dairy farm; SD = Ashurst dairy farm

Chapter 7 Reference

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