Plant derived nitrous oxide emissions from intensively grazed dairy pastures

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The Intergovernmental Panel on Climate Change (IPCC) includes above- and below-ground residues of all non-N and N-fixing crops in its definition of crop residues. Residues from pastures and from perennial forage crops are only accounted for during pasture renewal. The IPCC also confirms that the nitrogen (N) contained in crop residues in arable systems can contribute significantly to N cycling and be a significant source of nitrous oxide (N_2O) emissions. Despite the fact that 70% of the world's agricultural area and 90% of New Zealand's total farm area are pastoral systems, the current IPCC methodology does not consider the potential contribution of pasture residues outside of the renewal period with respect to N_2O emissions. Nitrous oxide is an obligate intermediate in the denitrification process and a by-product of nitrification. These microbial processes cause N_2O to be released from soil into the troposphere. Rates of N_2O emission and microbial pathways for production are dependent, amongst other factors, on soil water content and inorganic N in the soil.

Therefore, the questions posed here were: Do pasture residues (collectively called 'litter') occur in significant quantities during grazing? And what is the role of herbage embodied-N with respect to N_2O emissions? The overall objective of the research was to quantify the contribution of such plant-derived N_2O emissions in intensively grazed dairy pastures to New Zealand's agricultural greenhouse gas emissions inventory.

Experiment 1 (Chapter 4), was a field survey performed at Lincoln University Dairy Farm (LUDF), to quantify grazing-induced litter-fall i.e. the fraction of freshly harvested but un-ingested litter dropped by dairy cattle while grazing. Each paddock at the LUDF was grazed 12 times annually. This research showed, for the first time, that the rate of fresh litter-fall equated to 53 ± 24 kg DM ha^{-1} per grazing event in an intensively grazed dairy pasture and was equal to 4% of the apparent dry matter consumption of the dairy cattle.

Annually, fresh and senesced litter equated to N application rates of 15.9 kg N ha^{-1} y^{-1} and 3.5 kg N ha^{-1} y^{-1}, respectively. The aforementioned quantities of litter-fall formed the rationale for further experiments.

Experiment 2 (Chapter 5), a field study conducted in two parts (A and B), examined the effect of simulated animal treading on herbage decomposition and its implications on N_2O emissions. Presence or absence of herbage did not affect the N_2O emissions with N_2O emissions increasing regardless of the herbage presence. Soil NO_3^- levels declined due to treading, presumably due to induced anaerobic conditions and denitrification. The results were confirmed using a ^{15}N technique (part B) which showed that a major fraction of the N_2O emitted under herbage-trodden pasture originated from the soil inorganic N pool. However, the ^{15}N enrichment of the inorganic N pool also showed that the size of the soil inorganic-N pool was diluted due to N being released from either the herbage or the soil organic matter pools as a consequence of treading.

Experiment 3 (Chapter 6) investigated the effect of incorporating litter of the dominant New Zealand pasture species (clover and ryegrass) and a pasture supplement (maize) with soil, at two soil water contents (54 and 86% water-filled pore space (WFPS)), incubated at 20^oC. At field capacity (86% WFPS), the emission factor (EF) of N_2O equated to 2–3% of the litter-N with no differences due to litter species, while at 54% WFPS, the EF was significantly less with 1.7% > 0.7% = 0.5% for clover, ryegrass and maize, respectively. The decomposition rates were also similar at 86% WFPS. The differences in N_2O emissions were attributed to the biochemical properties of the species' litter, especially cellulose concentrations and their differing C: N ratios.

To further investigate the role of biochemical composition, specifically the C: N ratio of the plant litter to contribute to N_2O emissions, increasing amounts of cellulose were mixed with a constant mass of clover litter and incorporated into a pastoral soil (Experiment 4; Chapter 7). Increasing the C: N ratio via cellulose addition enhanced N_2O emissions, indicating that the incorporated cellulose acted as a labile C source favouring denitrification. Higher N_2O emissions from the highest C: N ratio treatments showed that the biochemical availability of C played a critical role in litter-derived N_2O emissions. Therefore higher emissions observed from the clover litter incorporated in Experiment 3 were most likely due to the labile forms of C embodied in the clover leaf tissues and not just attributable to the amount of N in the litter.

In Experiment 5 (Chapter 8), ^{15}N-labelled ryegrass was placed on the surface of a pastoral soil in litterbags at the rate of 213 kg N ha^{-1} (simulating litter-fall) and N_2O and CO_2 emissions were measured. This current study is the first to report soil N dynamics and N_2O

emissions using ^{15}N-labelled pasture litter placed *in situ*. Approximately 70% of the N_2O originated from the litter when surface-applied. Emissions of N_2O likely resulted from ammonification followed by a coupling of nitrification and denitrification during litter decomposition on the soil surface. The litter contributed to both the ^{15}N enrichment of soil NO_3^- and N_2O emissions which originated from litter-N. The ^{15}N enrichment of the soil NO_3^- pool showed that litter-N enhanced the soil inorganic N pool, verifying the conclusions drawn in Experiment 2 (part B), where *in situ* treading of herbage led to an increase in the soil inorganic N pool as evidenced by the decrease in ^{15}N enrichment of the NO_3^- pool. The EF of the *in situ* placed litter was 0.9%; similar to the IPCC default EF value of 1%.

This suite of experiments has shown that the contribution(s) of herbage-N to N cycling and N_2O emissions are significant, yet, not considered within the current IPCC methodology. If the litter-fall data is extrapolated using the various N contents and EFs measured in this thesis, litter-fall accounts for 4.5–10.9% of the total N_2O-N emitted due to dairy cattle. This thesis has also shown that 4% of the total pasture on-offer can be lost as litter-fall resulting in lower dry matter intake (DMI) of dairy cattle. If this is worked through the inventory calculations, the DMI remains unaffected. However, including the litter-fall-derived N_2O emissions in inventory calculations provides a more accurate and refined accounting of the N_2O-N released from grazed pasture N cycling. Before solid recommendations can be made to alter the IPCC inventory methodologies, further data on the effects of different grazing managements, animal and pasture species, and climate are needed.

Keywords: Litter-fall, nitrous oxide, carbon dioxide, pasture, litter, animal treading, surface decomposition, clover, ryegrass, maize, IPCC methodology

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Chapter 1
Introduction

Pastoral agriculture in New Zealand continues to intensify. Between 1989 and 2009, the dairy cattle numbers in New Zealand increased from 3.3 million to 6.2 million (Statistics New Zealand 2011). Grazing animals increase nutrient cycling and nitrous oxide (N_2O) emissions. Numerous studies have helped to quantify greenhouse (GHG) emissions resulting from cattle urine and excreta deposition in New Zealand pastures. However, the role of pasture litter, which contains both carbon (C) and nitrogen (N), has not been studied with respect to N_2O emissions.

Close observation of the grazing behaviour of dairy cattle shows that a portion of the harvested pasture vegetation remains unconsumed and falls from the animal's mouth during grazing. This results in the formation of pasture plant litter. The process of pasture plant litter production by this mechanism is hereafter termed 'litter-fall'. Plant litter may consist of fresh and senesced herbage. After grazing, this herbage remains where it falls until it decomposes. The review of literature (Chapter 2) that follows shows that only three studies have attempted to quantify litter-fall. Moreover, the contribution of litter decomposition and animal treading to N_2O emissions in pastoral conditions has not been investigated in detail. Litter-fall rates have not been quantified for intensive dairying operations and they are NOT considered in the Intergovernmental Panel on Climate Change (IPCC) best practice guidelines as a source of N_2O; moreover, the fate of this litter formed from litter-fall is unknown. Hence, the rationale for experiment 1 (Chapter 4) was to quantify litter-fall in pastures grazed by dairy cattle and determine if relationships existed with pasture management factors (Figure 1.1).

Litter may either be partially incorporated into the soil via anthropogenic activities such as animal treading/vehicular traffic or it may sit on the soil surface. In both cases, various biotic and abiotic factors aid in the decomposition of litter and this can potentially contribute to soil N and C cycling pools.

Results from experiment 1 (Chapter 4) were 'unique' – with significant quantities of litter-fall measured, that may potentially contribute to anthropogenic N_2O emissions. Hence the fate of this litter-fall was further investigated in two studies that examined partial (Chapters 5 and 8) or complete (Chapters 6 and 7) litter incorporation. The potential for litter-fall to contribute to N_2O emissions due to partial incorporation from animal treading was studied (Chapter 5, field study), and the fate of surface-applied, fresh ryegrass litter was also examined for N_2O emissions (Chapter 8, field study). For Chapter 5 it was observed that during simulated animal treading, the plant litter was only partially incorporated into the soil

which may not allow complete decomposition of the litter. Moreover, if the soil water content was not maintained at field capacity, treading damage to herbage was minimal, also indicating the significance of soil water content. This led to the lab study (Chapter 6), where litter from three dominant pasture species was thoroughly incorporated in soil at two moisture levels and N_2O emissions measured. Contrasting soil water contents were selected so as to simulate *in situ* conditions and evaluate litter decomposition and N_2O emissions. The results revealed that the emissions were governed by the biochemical composition, especially cellulose, of the litter. Based on the results of Chapter 6, Chapter 7 dealt with incorporating litter and cellulose in various proportions and its response to N_2O emissions. Figure 1.1 shows a flowchart of the experiments with the associated rationale.

The main objectives of the thesis were to:

1. Quantify pre- and post-grazing litter-fall under field conditions (Chapter 4),
2. Evaluate the effect of animal treading and its contribution to inducing plant-derived GHG emissions in pastures (Chapter 5),
3. Examine how common pasture litter materials (clover, ryegrass and maize) affect N_2O emissions and determine their decomposition rates at different moisture levels (Chapters 6 and 7),
4. Determine the N_2O emissions of incorporated clover residues with varying cellulose concentrations (Chapters 6 and 7), and
5. Determine the effect of surface-placed, fresh plant litter on N_2O emissions under field conditions (Chapter 8).

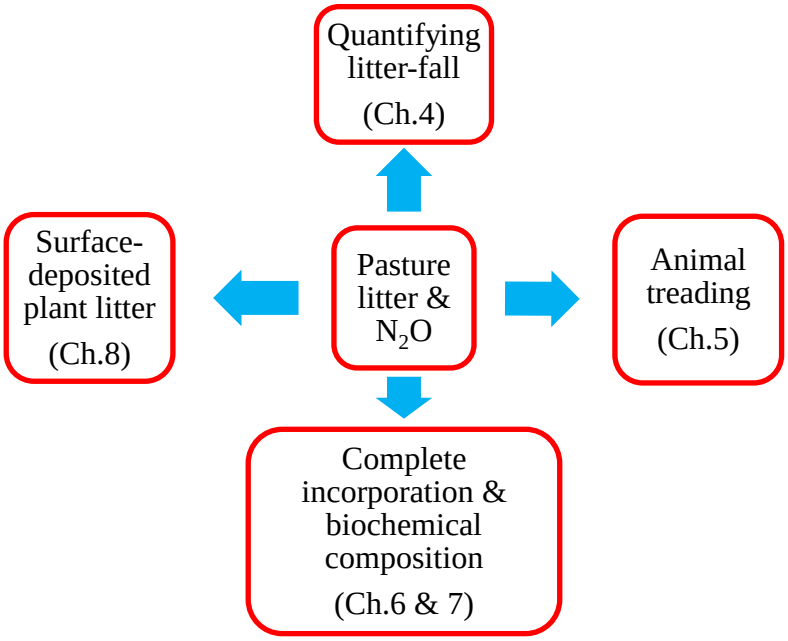

Figure 1.1 Interrelationships of plant litter to different experiments.

Chapter 2
Literature review

2.1 Introduction

Grasslands are defined as terrestrial ecosystems consisting of plant communities, in which grasses and/or legumes, including harvested forages, make up the dominant vegetation (Barnes and Nelson 2003). About 3.5 billion ha, representing 26% of the world land area, and 70% of the world agricultural area, are under pasture and fodder crops (FAOSTAT 2000). Pastures exist under diverse topography and management. Hence annual pasture production can vary from 1000 kg DM ha^{-1} in arid regions to ~20,000 kg DM ha^{-1} in warm, fertile temperate climatic zones (McDowell 2008).

Introduced pasture species, relatively high stocking rates, and grazing management are the key drivers which regulate C and N cycling in anthropogenic grassland ecosystems via plant litter returns; excreta deposition and management practices such as pasture renewal. Pasture plant litter is a generic term. It has three main components that are in continuous transition viz. ungrazed mature vegetation attached to a plant as standing residue, herbage detached from the plant and lying on the soil surface, and decomposing residues in the soil (Molinar *et al.* 2001). This thesis, however will define pasture plant litter as the harvested but unconsumed pasture vegetation which falls from the animal's mouth during grazing. This harvested but unconsumed herbage is collectively termed litter and the process of its creation, litter-fall. Plant litter deposits created from litter-fall may stay on the soil surface and start to decompose, contributing to previous litter already decomposing in the soil; and/or get partially or completely incorporated in the soil due to animal treading and then decompose further (Hutchinson and King 1989; Molinar *et al.* 2001). Decomposition of litter, either on the soil surface or incorporated in soil, leads to the mineralisation/immobilisation of nutrients contained in the litter and hence these are returned back to the soil. These nutrients may then be further transformed in the case of N, and potentially be lost from the grazed pasture as discussed below (Section 2.2.2).

Greenhouse gases such as nitrous oxide (N_2O) and carbon dioxide (CO_2) are atmospheric trace gases that absorb infra-red radiation reflected from the Earth's surface, thus trapping radiated heat and contributing to increases in global temperatures. The mean global surface temperature has increased by 0.74 ± 0.18°C between 1905 and 2005 (Trenberth *et al.* 2007). The rate of warming over the last 50 years is almost double that over the last 100 years

(0.13 ± 0.03°C vs. 0.07 ± 0.02°C per decade, respectively) with 2005 one of the warmest years on record.

The greenhouse gas – N_2O is a precursor to compounds involved in stratospheric ozone depletion (Crutzen 1981) and has a global warming potential (GWP) of 298 over a 100 year timeframe (Forster *et al.* 2007). Its atmospheric concentration is increasing linearly (0.26% y^{-1}), rising from 270 nL L^{-1} in 1750 to 319 nL L^{-1} in 2005 (Forster *et al.* 2007). Agriculture is the largest source of N_2O accounting for about 60% of the total global anthropogenic N_2O emissions (Kroeze *et al.* 1999; Mosier *et al.* 1998). Among other anthropogenic ozone-depleting-substances, N_2O is currently rated as having the highest ozone depleting potential (Ravishankara *et al.* 2009).

Approximately 90% of New Zealand's total farm area is considered to be a pastoral ecosystem (Statistics New Zealand 2003) and the agricultural sector accounted for 46.6% of New Zealand's GHG inventory in 2008 (Ministry for the Environment 2011). New Zealand agriculture is dominated by grazed pasture systems. Such systems have been identified as significant sources of N_2O (Flessa *et al.* 1996; Oenema *et al.* 2005). Nitrous oxide accounted for 16% of New Zealand's GHG inventory in 2008; an increase of 21.8% since 1990 (Ministry for the Environment 2011) i.e. approximately 85% of the total anthropogenic N_2O emissions come from grazed pastures (Cameron *et al*. 2000).

The Intergovernmental Panel on Climate Change (IPCC) best practice guidelines account for N_2O emissions occurring from livestock and manure management, fertiliser additions, and cropping residues[*] but they do NOT account for the possibility of litter-fall, or supplementary feed litter contributing to anthropogenic N_2O emissions (IPCC 2006). The only mention the IPCC guidelines make with respect to pasture, is when considering the potential for emissions as a result of pasture renovation.

The following literature review describes the rates and pathways for N cycling in grazed pastures, possible effects of pasture plant litter and identifies the dearth of knowledge with respect to plant litter and N_2O emissions.

[*] Cropping residues in this thesis will be defined as the materials remaining in the field after a crop has been harvested in agricultural and horticultural systems. These residues include stalks, stubbles, stems, leaves and seed pods.

2.2 N cycling in grazed pastures

Nitrogen (N) is an essential element for plant functions. It is a primary constituent of basic amino acids (the building blocks of proteins such as enzymes) and nucleic acids (Kapal 2008). Nitrogen is primarily obtained from the atmosphere, through N fixation by legumes and microbially-mediated mineralisation processes in the soil (Cameron 1992).

Nitrogen transformations occur through a variety of processes within the soil/plant/atmosphere system which is generally referred to as the 'nitrogen cycle'. There is a high abundance of N in pasture soils but not all forms are plant available. In addition to the N_2 in the soil atmosphere, the three main forms of N in the soil are: (i) organic compounds contained in plant matter, soil organisms and humus; (ii) ammonium (NH_4^+–N) ions fixed to clay minerals; and (iii) mineral N i.e. NH_4^+–N, NO_3^-–N (nitrate); and NO_2^-–N (nitrite)) in the soil solution (McLaren and Cameron 1996). Mineralisation and immobilisation are the biological transformations of soil N which determine the abundance of mineral N in the soil.

Soil-N is also subject to mineralisation and immobilisation processes. Nitrogen mineralisation is the conversion of organic N contained in soil organic matter (SOM), plant matter or microbial biomass, into more available forms of mineral N. In the case of NO_3^-–N, it is more susceptible to leaching from the soil profile, if not assimilated into microbial tissue, or taken up by vegetation (Stevenson 1982). Mineralisation thus governs the plant available N supply (Monaghan and Barraclough 1997).

Nitrogen immobilisation is the process of assimilation of mineral forms of N into the microbial biomass thereby rendering it unavailable for plant uptake (Whitehead 1995). Nitrogen is immobilised when conditions favour microbial growth. In a steady state, these two processes are balanced.

Factors affecting the rate of mineralisation and immobilisation are substrate quality (C: N ratio); environmental parameters (such as soil aeration, soil temperature and moisture), fertiliser application, soil texture and soil pH (Haynes 1986). Soil rewetting and drying cycles, freezing and thawing are also of particular importance as they cause a "flush" of microbial activity, and consequently N mineralisation. Cultivation affects may also affect the degree of N immobilisation in the soil (Jarvis *et al.* 1996).

Various N transformation processes occurs as a result of natural/anthropogenic N inputs. Inputs of N in pastures can occur as a result of biological nitrogen fixation (BNF), atmospheric deposition, fertiliser, slurry applications and animal excreta (animal urine and dung deposition). Other processes assist to transform N within the pasture soil, such as nitrification, denitrification, volatilisation and leaching. These processes are discussed further below.

2.2.1 N inputs

2.2.1.1 BNF and atmospheric deposition

There are several N-fixing organisms which are symbionts and enzymatically convert atmospheric dinitrogen (N_2) to ammonia (NH_3) (Esser *et al.* 2011). This process is called biological nitrogen fixation (BNF) and amounts to about 130 Tg y^{-1} globally (Galloway *et al.* 2004). Traditionally, legumes are the basis of New Zealand's pastoral system. Legumes such as clover (*Trifolium repens* L.) have special root nodules created by N_2-fixing bacteria (*Rhizobium* sp.) that facilitate BNF. The rate of BNF by clover ranges from 100 to 350 kg N ha^{-1} y^{-1} depending on the pastoral dominance of clover, clover growth rate, soil fertility, soil moisture and temperature (Menneer *et al.* 2004). Ledgard (2001) estimated that BNF from clover-grass pastures ranged from 20–270 kg N ha^{-1} y^{-1}. In white clover-based pastures, ideally 25−35% of the total pasture is sown with clover in order to ensure adequate rates of N fixation (Ledgard *et al.* 1990; White and Hodgson 1999).

A small input of reactive N into the biosphere, equal to about 2–5% of the global BNF inputs, comes from the oxidation of N_2 by lightning (Lelieveld and Dentener 2000). Atmospheric N compounds cycle to the land and water through atmospheric deposition. Wet deposition, predominantly rain and snow, carries NH_4^+ and NO_3^- while dry deposition involves complex interactions between airborne N compounds and plant, water, soil, rock, or building surfaces (Churkina *et al.* 2007). Wet deposition via rainfall onto New Zealand soils ranges from 1–5 kg N ha^{-1} y^{-1} while dry deposition at 14 sites in the Waikato and Manawatu regions, adjacent to dairy farms ranged from 5–10 kg N ha^{-1} y^{-1} (Parfitt *et al.* 2006).

2.2.1.2 Typical fertiliser rates in dairy pastures

Fertiliser application has greatly increased pasture production in New Zealand over the last few decades (Christie and Grundy 2008). Typical fertiliser rates in New Zealand and Australian clover-ryegrass pastures are 100–150 kg N ha^{-1} y^{-1}. Pasture growth responds almost linearly up to 200–400 kg N ha^{-1} y^{-1} (Whitehead 2000). Commonly used fertilisers in New Zealand pastures include urea (46% N), ammonium sulphate (AS, 21% N) and diammonium phosphate (DAP, 18% N). Emissions of N_2O from fertiliser depend on fertiliser type and soil conditions, e.g. in wet conditions, NO_3^- fertilisers cause higher emissions than urea or NH_4^+-based fertilisers, while the converse is true in warm and dry conditions (Smith *et al.* 1997). The choice of fertiliser depends mainly on its cost but optimised application strategies aim to minimise losses via NH_3 volatilisation, denitrification and leaching, and maximise plant N uptake. Approximately 0.30 M t of fertiliser N is applied to New Zealand pastoral soils on a yearly basis (Saggar *et al.* 2004).

2.2.1.3 Effluents

Dairy farm effluents (DFE), comprising animal excreta, urine and wash-down water (Clough and Kelliher 2005), are generated when the milking sheds and holding yards in a dairy farm are cleaned with high-pressure hoses, at the rate of approximately 50 L effluent cow^{-1} d^{-1} (Saggar *et al.* 2004). The predominant constituents of the DFE are urine and dung. The composition of DFE varies according to animal numbers, feed quality, and volume of the wash-down water. Effluents contain valuable nutrients (Longhurst *et al.* 2000) and hence are applied onto land to improve soil fertility (Cameron *et al.* 1997). It is estimated that about 70 million m^3 of DFE are generated from dairy farms in New Zealand annually (Saggar *et al.* 2004). Studies report that dairy and piggery effluents in New Zealand can annually supply N, P and K equivalent to 17,500 t of urea, 12,500 t of single super-phosphate, and 28,300 t of potassium chloride, respectively (Bolan *et al.* 2003; Roberts *et al.* 1992).

2.2.1.4 Urine and dung

Grazing livestock returns N to the soil via the excretion of both dung and urine. The amount depends on the type of animal, the type of herbage consumed and its N content, and total dry matter intake (Whitehead 1986). Moir *et al.* (2010) reported that approximately a quarter of a grazed paddock may be covered by urine patches on an annual basis. In an intensively grazed pasture, it was calculated that more than half of the consumed N was excreted as urine (Haynes and Williams 1993). The area over which a typical urination event occurs may receive the equivalent of 1000 kg N ha^{-1} (Ball and Ryden 1984; Cameron 1993), and cover an area of between 0.16–0.49 m^2 (Haynes and Williams 1993). Such a high loading rate of N within a urine patch may potentially lead to large N losses from the grazed system via N leaching, denitrification, and NH_3 volatilisation (Ball *et al.* 1979). Inputs of N from animal excreta to New Zealand pastoral soils can be about 1.5 M t annually (Saggar *et al.* 2004).

2.2.1.5 Pasture litter inputs

During grazing, animals consume a large proportion of the herbage on-offer, but the remainder (post-grazing residuals) along with all dead root material ultimately undergoes decomposition *in situ* which returns N back to the soil (Whitehead 2000). Nitrogen returns via pasture litter decomposition can be a dominant source of N for new herbage growth if there is little N input from anthropogenic sources. These N returns depend on the N content of the litter which is related to the rates and types of N inputs. Parsons (1988) indicated that the amount of herbage decomposing *in situ* each year was broadly similar to the amount

consumed by grazing animals or harvested for hay or silage in an intensively managed pasture.

Senesced litter typically has a C: N ratio of >20: 1 (Whitehead 2000). Depending on the intensity of management, the total amount of herbage material decomposing *in situ* may range from 1000 to 10,000 kg DM ha^{-1} y^{-1} (Whitehead 2000). However, in most intensively managed pastures, unlike residues from cropping systems, litter deposition rates have rarely been measured, let alone been considered an N source, hence, no studies have been performed to study N inputs from pasture litter and residual herbage incorporation. Pasture residues when ploughed-in can cause N mineralisation, e.g. Vinten *et al.* (2002) reported N mineralisation rates of 87 kg N ha^{-1}, one year after ploughing clover-ryegrass swards in a ley cropping system while Eriksen (2000) found 90–100 kg N ha^{-1} in a sandy loam soil after ryegrass leys were ploughed. However, there is a dearth of studies measuring C and N cycling from pasture litter and actual litter-fall rates in pasture systems grazed by dairy cattle.

Residues in cropping systems[†] are a significant N source and can influence soil quality, N cycling and microbial processes (Vigil and Kissel 1991). The literature review on the contribution of crop residues to N cycling, however, is ambiguous because N in the crop residues is supposedly more protected than that of fertilisers and hence N losses from leaching/volatilisation may not always occur from crop residues (Whitehead 2000). Legume crop residues, when incorporated into soil decompose rapidly and release mineral N and organic C into the soil (Aulakh *et al.* 1991b); this mineral N may potentially be lost into the atmosphere via various pathways (Section 2.2.2). Conversely, high C: N ratio crop residues can promote immobilisation of the available N in the soil organic matter (Delgado *et al.* 2010; Delgado *et al.* 2004). Smit *et al.* (1995) reported that sugar beet residues after harvest could contain 100–160 kg N ha^{-1}, *Brassica* residues >200 kg N ha^{-1} (Fink *et al.* 1999), and field pea residues, 30–100 kg N ha^{-1} (Evans *et al.* 1991). Vigil and Kissel (1991) reported that applying residues with a low C: N ratio encouraged N mineralisation, but applying residues with higher C: N ratios increased N immobilisation. Dendooven *et al.* (1996) indicated that studies using crop residues in field conditions are fewer due to the difficulty in assessing C and N availability in these complex C and N sources i.e. crop residues (Beauchamp *et al.* 1989). No study has so far reported pasture litter decomposition *in situ* and associated N cycling fluxes.

[†] Cropping residues in this thesis will be defined as the materials remaining in the field after a crop has been harvested in agricultural and horticultural systems. These residues include stalks, stubbles, stems, leaves and seed pods.

2.2.1.6 Conclusion (N inputs)

It is clear that numerous studies in pastures have focused on animal urine, dung and fertiliser N inputs but no studies have been performed with respect to litter-fall. This might have been due to the lack of recognition of the concept of litter-fall. The literature review shows that crop residues can make significant contributions to N inputs. The nutrient composition of pasture litter is comparable to that of cropping residues, hence, pasture litter decomposition may potentially contribute to N and C cycling and its significance needs to be determined.

2.2.2 N transformations and losses

2.2.2.1 Nitrate Leaching

Nitrogen is leached mainly as NO_3^-, largely because NO_3^- ions are highly soluble and are not retained by the soil's exchange complex (Hillel 1998). Nitrate leaching occurs when there is an accumulation of NO_3^- in the soil profile that coincides with, or is followed by a period of high drainage (Di and Cameron 2002a). Factors affecting intensities of leaching are rates of rainfall and irrigation, evaporation, soil type, and plant cover (Di and Cameron 2002a; Haynes 1986). Excessive fertigation, DFE application and animal urine excretion can therefore have a major impact on NO_3^- leaching from grazed pastures. This can have deleterious impacts on the environment causing eutrophication in water bodies and groundwater contamination.

2.2.2.2 Ammonia volatilisation and urea hydrolysis

Urea in fertiliser and urinary-N undergoes rapid hydrolysis to NH_4^+–N (Equation 2.1) and may be completely hydrolysed within 2 d (Holland and During 1977; Sherlock and Goh 1984). The optimum conditions for urea hydrolysis are a pH >6.5 and elevated soil temperatures (Jarvis and Pain 1990).

$$\underset{\textbf{Urea}}{CO(NH_2)_2} + 2H_2O \xrightarrow{urease} NH_4^+ + NH_3 + HCO_3^-$$

Equation 2.1

Urease is an enzyme ubiquitous in pastoral environment that catalyses the hydrolysis of urea. Urease enzyme activity generally increases with increasing temperature (Moyo *et al.* 1989). A fraction of the NH_4^+ is also converted to ammonia (NH_3) (Equation 2.2).

The process of NH_3 loss to the atmosphere is termed ammonia volatilisation. This is a physico-chemical process requiring an alkaline pH unlike urea hydrolysis which is a biochemical reaction (Sherlock *et al.* 2002).

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

Equation 2.2

In pastures, NH_3 volatilisation occurs from urine patches because of the presence of both high pH and NH_4^+ (Haynes and Williams 1993); the production of NH_3 increases with increasing pH. The proportion of total urinary-N volatilised as NH_3–N can range from 3 to 52% (Petersen *et al.* 1998). Factors governing NH_3 volatilisation are soil pH, cation exchange capacity (CEC), soil moisture and soil temperature and relative concentrations of NH_3 and NH_4^+ in the soil solution (Haynes and Sherlock 1986).

2.2.2.3 Nitrification

Nitrification is the biological oxidation of NH_4^+ to NO_3^- which occurs in two steps (Figure 2.1). The first step is the oxidation of NH_3 to NO_2^- by ammonia-oxidising bacteria (AOB, mainly *Nitrosomonas* sp. and *Nitrosococcus* sp.), and ammonia-oxidising archaea (AOA, *Nitrosopumilus maritimus*) (Leininger *et al.* 2006; Prosser and Nicol 2008) and certain fungi (Laughlin *et al.* 2008). The second step is the oxidation of NO_2^- to NO_3^- by nitrite-oxidising bacteria such as *Nitrobacter* sp. (Figure 2.1; Equation 2.3). Both these groups of bacteria are autotrophic, obligatory aerobic members of the gram-negative bacterial phylum Proteobacteria (Kowalchuk and Stephen 2001).

$$\underset{\text{Ammonia}}{NH_3} + O_2 + 2H^+ + 2e^- \longrightarrow \underset{\text{Hydroxylamine}}{NH_2OH} + H_2O \longrightarrow \underset{\text{Nitrite}}{NO_2^-} + 5H^+ + 4e^-$$

$$\underset{\text{Nitrite}}{NO_2^-} + H_2O \longrightarrow \underset{\text{Nitrate}}{NO_3^-} + 2H^+ + 2e^-$$

Equation 2.3

Nitrification produces H^+ ions which results in a decrease in the soil pH. Nitrous oxide is produced during nitrification as a by-product (Figure 2.1). In pastures, following a urine deposition event, nitrification is enhanced due to the presence of substrate and moisture.

Both nitrification steps are highly dependent on the pH; both *Nitrosomonas* and *Nitrobacter* sp. are inhibited by high concentrations of NO_2^- at a pH $\leq$ 7.5 (Hunik *et al.* 1993) while *Nitrobacter* sp. can be inhibited by NO_3^- at a pH range of 6.5–7.5 and by NH_4^+ at pH 6.5 (Hunik *et al.* 1993).

Soil water content and aeration affect the rate of nitrification and subsequent N_2O production from nitrification. The rate of N_2O production is normally low at < 40% WFPS (water-filled pore space) but increases rapidly up to 55–65% WFPS. Above 60–70% WFPS, soil aeration is reduced (limited oxygen diffusion) and denitrification may dominate N_2O production at >70% WFPS (Dalal *et al.* 2003).

Another factor affecting the rate of nitrification is temperature with an optimum range of 25–35^{o}C (Whitehead 1995) but indigenous nitrifier species may adapt rapidly to local conditions (Malhi and McGill 1982).

Figure 2.1 Transformations of mineral N in soil (Wrage *et al.* 2001)

2.2.2.4 Denitrification

Denitrification is the dissimilatory reduction of NO_3^- to NO_2^- and its sequential reduction via nitric oxide (NO) and N_2O to N_2 (Figure 2.1). Reductase enzymes occur at each step of the entire process (Equation 2.4).

$$\underset{\text{Nitrate}}{NO_3^-} \xrightarrow{\textit{nitrate reductase}} \underset{\text{Nitrite}}{NO_2^-} \xrightarrow{\textit{nitrite reductase}} \underset{\text{Nitric oxide}}{NO} \xrightarrow{\textit{nitric oxide reductase}} \underset{\text{Nitrous oxide}}{N_2O} \xrightarrow{\textit{nitrous oxide reductase}} \underset{\text{Dinitrogen}}{N_2}$$

Equation 2.4

Denitrifiers are facultative, anaerobic, usually gram-negative bacteria of the phylum Proteobacteria (Philippot *et al.* 2007) having the ability to utilise NO_3^- and NO_2^- as electron acceptors when oxygen is unavailable (Firestone 1982). Fungi may also play a denitrifying role in grassland soils (Laughlin and Stevens 2002). Microbial denitrification occurs

predominantly in wet soils, but may also occur in anaerobic microsites in drier soils (Muller 2003; Whitehead 1995).

Denitrification rates and the rates of production of denitrification products (e.g. N_2O: N_2) are affected by various factors including oxygen diffusion; soil moisture which affects gas diffusion, temperature, pH and availability of C and NO_3^- substrates (Weier *et al.* 1993).

Soil water content is one of the most important factors regulating denitrification because of its influence on terminal electron acceptor (TEA) supply (O_2, NO_3^-, NO_2^-, N_2O) (Firestone and Davidson 1989). Denitrifiers prefer O_2 over other TEAs because its reduction yields more energy (Fazzolari *et al.* 1998). Denitrification increases with increasing WFPS (>60% WFPS), reaching a maximum at 95% WFPS (Drury *et al.* 2003). Increasing WFPS up to a value of 95% also decreases the proportion of gaseous N lost as N_2O (i.e. reduces the N_2O: (N_2O+N_2) ratio) (Bateman and Baggs 2005; Weier *et al.* 1993). The decrease in this ratio occurs through two mechanisms. First, N_2O-reductase activity is inhibited at lower WFPS due to increased O_2 availability, making N_2O the principle product of denitrification (Weier *et al.* 1993). Second, the rate of diffusion of N_2O from the site of denitrification is decreased at higher WFPS (Peterson and Anderson 1996), thereby increasing the opportunity for N_2O to be reduced to N_2 (Weier *et al.* 1993).

Denitrifiers are heterotrophs therefore C substrate availability is another important factor governing denitrification (Knowles 1982). Increased C availability increases denitrification directly by increasing the energy and electron supply to denitrifiers, and indirectly through enhanced microbial growth and metabolism thereby stimulating high O_2 consumption (Beauchamp *et al.* 1989; Gillam *et al.* 2008). Denitrification rates vary with the type of C substrate from simple compounds such as glucose to complex crop residues (Aulakh *et al.* 1991a; Weier *et al.* 1993).

Denitrification rates and N_2O emissions increase with increasing soil NO_3^- concentrations because NO_3^- is used preferentially to N_2O as a TEA under anaerobic conditions (Firestone 1982). This is only true when other factors (O_2 diffusion and C availability) are optimised and NO_3^- may not become limiting until after the addition of a C source (Weier *et al.* 1993).

The optimal rate of both nitrification and denitrification occur at a pH range of 7–8 (Haynes 1986). Salinity usually inhibits both processes. Rates of denitrification are highest at 60–70°C (Bremner and Shaw 1958; Keeney *et al.* 1979), although non-biological reactions (such as chemodenitrification) probably contribute at these high temperatures (Firestone 1982). In terms of pH, denitrification yields relatively more N_2O at lower soil pH, however,

the overall rate of production and emission of N_2O and N_2 is relatively higher in neutral to slightly alkaline than acidic soils (Simek *et al.* 2002).

2.2.2.5 Other N_2O production pathways in pasture ecosystems

Emissions of N_2O are mainly governed by nitrification and denitrification, although other pathways such as nitrifier-denitrification, DNRA (dissimilatory nitrate reduction to ammonium) and chemodenitrification cannot be ignored (Anderson *et al.* 1993; Chalk and Smith 1983; Papen *et al.* 1989). However, the latter processes may contribute only partly to the total N_2O emissions in pastoral systems (Bowden 1986; Kaplan and Wofsy 1985).

Nitrifier-denitrification is the nitrification pathway governed by autotrophic nitrifiers (Figure 2.1) in which NH_3 is oxidised to NO_2^- followed by the reduction of NO_2^- to NO, N_2O and N_2 (Ferguson *et al.* 2007). It is carried out by AOB such as *Nitrosomonas* under O_2 depleted conditions. This process was previously thought to be of minor importance in soils (Dalal *et al.* 2003) but recent studies (Kool *et al.* 2011; Wrage *et al.* 2005) have shown that significant N_2O emissions from nitrifier-denitrification can occur, especially under moisture conditions that are sub-optimal for heterotrophic NO_3^- denitrification (Kool *et al.* 2011).

Dissimilatory nitrate reduction to ammonium (DNRA) also called nitrate ammonification, is the process where NO_3^- is reduced directly to NH_4^+ (Kelso *et al.* 1997; Mohan *et al.* 2004). Previously DNRA was only thought to be favoured in intensively reduced, C-rich environments (Tiedje 1988). Recent studies show that it is not restricted to reduced environments; it can also occur in rice paddies (Yin *et al.* 2002), calcareous agricultural soils (Wan *et al.* 2009) and in temperate arable soils where it was shown to be limited by low-molecular weight C source (Schmidt *et al.* 2011).

Chemodenitrification is the term used to describe NO_x emissions resulting from chemical reactions between NO_2^- and soil organic matter. Haynes and Sherlock (1986) estimated NO_x losses from chemodenitrification to be in the order of < 1 kg N ha^{-1} y^{-1} and hence not environmentally significant.

2.2.3 Emissions of N_2O in pastures

Nitrogen cycling in livestock farming systems is often considered ‘leaky’ because of the many potential routes for the loss of N (Hatch *et al.* 2004; Jarvis and Pain 1997). Only 5−45% of the plant protein consumed by the animals is transformed into animal proteins, depending on animal age, animal species and management (Oenema *et al.* 2008). The remainder is excreted as urine and dung and is subject to subsequent N transformations and possible loss from the ecosystem as N_2O, N_2, NH_3, NO_3^- and dissolved organic N. Several

studies have focussed on factors affecting N_2O emissions from animal excreta (Clough *et al.* 1998; Sherlock and Goh 1983; Sherlock *et al.* 2002) and possible mitigation strategies (De Klein *et al.* 2003; Di and Cameron 2002a; b; Di *et al.* 2007); however the mitigation strategies are outside the scope of this thesis.

Figure 2.2 Schematic diagram of the N cycle and N_2O emission pathways in a grazed system. Dotted lines are pathways resulting in indirect N_2O losses from grazing systems (McDowell 2008).

Intensively grazed pasture ecosystems contribute significantly to N_2O emissions (Oenema *et al.* 2005) due to regular N inputs in the form of fertiliser and excreta-N (urine and dung) deposited by grazing animals (De Klein *et al.* 2003).

Annual emissions of N_2O from animal production systems and animal waste have been estimated at 2.7 Tg N [range 0.7–4.2; (Mosier *et al.* 1998)]. Annual N_2O emissions from dairy pasture soils in New Zealand and Australia range from 6 to 12 kg N_2O-N ha^{-1} y^{-1} (Dalal *et al.* 2003; Luo *et al.* 2008b; Saggar *et al.* 2008). Emissions of N_2O from pastures vary depending on various factors such as rainfall and management. The proportion of urinary-N emitted as N_2O varies widely, between 0.1% (Di *et al.* 2007) and 13.3% (Kool *et al.* 2006). Luo *et al.* (2008a) reported seasonal effects from urine application to soils at 1000 kg N ha^{-1} with an EF (i.e. the proportion N_2O-N emitted per unit of N applied) of 0.02–1.52% and concluded that N_2O emissions were reduced under wet conditions. When bovine urine was applied at 500 kg N ha^{-1} to a grazed pasture soil, N_2O emissions accounted for 0.3–3.9% of the applied N when the water-filled pore space (WFPS) of the soil varied from 40–87% respectively (Uchida *et al.* 2008). Clough *et al.* (2004) showed a strong exponential relationship ($r^2 = 0.8$) between the N_2O emissions from urine (500 kg N ha^{-1}) and the soil's WFPS. When synthetic urine was applied at 1000 kg N ha^{-1} to different soil types, N_2O emissions were 0.8–1.9% of the N applied from silt loam and clay soils, respectively (Clough *et al.* 1998).

Higher N_2O emissions can also occur as a result of pasture renovation (Davies *et al.* 2001; Mori and Hojito 2007) due to an increase in soil mineral N content, following the mineralisation of pasture root and shoot material once it is incorporated into the soil (Luo *et al.* 2010). Velthof *et al.* (2010) found higher N_2O emissions following pasture renovation in spring than in autumn and suggested that the lower emissions in autumn were probably due to a lack of soil mineral N as a result of enhanced NO_3^- leaching and denitrification under wet conditions.

Volatilisation of NH_3 from agricultural systems is a major source of NH_3 emissions that accounts for 10–30% of fertiliser and excreta N (Bouwman *et al.* 2002). Typically 10−25% of the N in urea can be lost as NH_3 (Harrison and Webb 2001) but may be as high as 52% (Petersen *et al.* 1998). The emitted NH_3 can be redeposited back to land or water and contribute to indirect N_2O emissions. The IPCC stipulates a default EF of 1% for these indirect N_2O emissions (Mosier *et al.* 1998).

As noted earlier, N can also be lost via leaching down the soil profile. The amounts of NO_3^- leaching/runoff from grazed pastures have been quantified (Di and Cameron 2002a) but the fraction of this leached N that is converted to N_2O is not reported widely. Clough *et al.* (2000) reported that the fate of N_2O in the soil profile is unknown and it can potentially be further denitrified in the subsoil. This is the reason for the wider range of EF of 0.05 to 2.5% for the leached N (IPCC 2006).

Emissions of N_2O in pastures are enhanced following the application of fertilisers, animal manures, effluents and slurries and they vary due to type of application and soil type, e.g. EF for effluents can range from 0.03 to 4.93% (Bhandral *et al.* 2007a; Luo *et al.* 2008a). Approximately 2–4% of the N in NH_4^+ fertilisers and 5–12% of the N in NO_3^- fertilisers are emitted as N_2O when applied to wet soils (Stevens and Laughlin 1997; Velthof *et al.* 1997). Emissions of N_2O were higher when slurry and inorganic fertiliser were applied together compared to separate applications (McTaggart *et al.* 1997).

It is clear that rates of N_2O emissions from pastures can vary due to soil type and rainfall, and rate and the form of N inputs, and seasonally. These emissions therefore have been divided into direct and indirect emissions by the IPCC best practice guidelines.

2.2.4 IPCC methodology

The IPCC guidelines divide anthropogenic N_2O emissions into direct and indirect pathways. *Direct* N_2O emissions account for the emissions directly from the soils where the N input occurs while *indirect* N_2O emissions result from leaching and runoff of N (mainly as NO_3^-) from managed soils; and NH_3 and NO_x volatilisation from managed soils, fossil fuel combustion and biomass burning, and the subsequent redeposition of these gases and/or salts.

The N sources which are included in the IPCC methodology (IPCC 2006) for estimating *direct* N_2O emissions read as:

- synthetic N fertilisers;
- organic N applied as fertiliser (e.g., animal manure, compost, sewage sludge, rendering waste);
- urine and dung N deposited on pasture, range and paddock by grazing animals;
- N in crop residues (above-ground and below-ground), including from N-fixing crops (via BNF) and from forages during pasture renewal (The N residue from perennial forage crops is only accounted for during periodic pasture renewal, i.e. not necessarily on an annual basis as is the case with annual crops);
- N mineralisation associated with loss of soil organic matter resulting from change of land use or management of mineral soils; and
- drainage/management of organic soils (i.e., Histosols).

Emissions of N_2O associated with BNF are substantially lower and are comparable to background N_2O emissions from agricultural crops (Rochette and Janzen 2005). Hence these emissions are not included in the recent (2006) IPCC guidelines.

New Zealand's pastoral agriculture is dominated by year round grazing of clover-ryegrass pastures. As a result, the IPCC inventory methodology identifies animal excreta to be the single largest source of N_2O in New Zealand (De Klein *et al.* 2001). The IPCC methodology estimates N_2O emissions based on emission factors (EF, i.e. the proportion N_2O-N emitted per unit of N applied) for all anthropogenic N inputs to the system. The IPCC has stipulated an EF range of 0.5–3.0% for animal excreta. However, following extensive field monitoring at representative sites across the country, New Zealand has adopted a country-specific EF value of 1% for animal excreta (IPCC 2001).

The IPCC methodology therefore accounts for the N losses from fertiliser, manures, crop residues, and N released via mineralisation associated with soil organic matter losses (mineralised N) when assessing direct and indirect emissions of N_2O-N (Eggleston *et al.* 2006). The IPCC suggests a default EF of 1% with an uncertainty range of 0.3–3.0% for all

anthropogenic N additions (mineral fertilisers, organic amendments and crop residues) as direct N_2O emissions (IPCC 2006). This methodology also assumes that 30% of the fertiliser N from these sources is leached and/or lost in runoff of water to streams and rivers, and 0.75% of this N is indirectly emitted as N_2O-N beyond the original site of the N additions (De Klein *et al.* 2006a; Eggleston *et al.* 2006). It further assumes that 10% of the fertiliser and manure N applied to agricultural fields is lost through NH_3–N volatilisation and NO_x–N emissions, and about 1.0% of this N is later emitted as N_2O-N.

The IPCC guidelines account for direct N_2O emissions from both above ground and below ground crop residues[‡]. However, the IPCC Good Practice Guidelines state, "The nitrogen residue from perennial forage crops is only accounted for during periodic pasture renewal, i.e. not necessarily on an annual basis as is the case with annual crops" (IPCC 2006). Despite the IPCC noting "Care should also be taken to ensure that the emission estimates developed through the use of models or measurements account for all anthropogenic N_2O emissions" (IPCC 2006), the IPCC methodology does not account for N_2O emissions from the decomposition of *pasture* residues created as a result of litter-fall. It is also clear that the IPCC methodology does NOT differentiate between crop residues and pasture residues. Abiven and Recous (2007) investigated the decomposition kinetics of surface-placed crop residues that included rice, soybean, sorghum and wheat (0.4–2.0% N range) and concluded that residues with lowest C: N ratio increased net N mineralisation. Ambus and Jensen (1997) measured N mineralisation and denitrification rates from barley (44% C, 0.9% N) and pea residues (45% C, 2.1% N). Over 60 d, 42 and 63 mg N kg^{-1} soil was immobilised, respectively, and relatively higher N_2O emissions were recorded from pea residues in the first 3 d of incubation. Delgado *et al.* (2010) concluded that, with respect to crop residues, the IPCC methodology should be re-evaluated to determine if the direct and indirect N_2O-N emission coefficients needed to be lowered to reflect lower emissions from high C: N crop residue N inputs. Relatively high spatial variations have been reported in EFs of N_2O depending on the type of crops used, e.g. Velthof *et al.* (2002) reported EFs from vegetable crop residues in the range of 0.13–14.6% while it was 0.1–1.0% for layers of grass mulch (ryegrass and fescue) and alfalfa (Larsson *et al.* 1998).

The N contents of senesced ryegrass herbage with nil fertiliser and high fertiliser were reported to be 1.1 and 2.5%, respectively (Whitehead 1995) while corresponding values for ryegrass roots were 1.1 and 1.6% (Whitehead 1970). Nitrogen contents of white clover roots and herbage were 2.7% (Whitehead 1995) and 3.8% (Whitehead 1970), respectively.

[‡] Cropping residues in this thesis are defined as the materials remaining in the field after a crop has been harvested in agricultural and horticultural systems. These residues include stalks, stubbles, stems, leaves and seed pods.

Decomposition of roots is also important as it has also been reported that root mortality is increased followed by heavy defoliation by grazing animals (Eason and Newman 1990; Evans 1973). It is clear that the N and C contents of both above- and below-ground pasture residues is similar to crop residues and hence has the potential to contribute to N cycling and N_2O emissions. However, this is NOT accounted for in the IPCC guidelines.

Another common scenario in pastoral systems is soil compaction of urine patches resulting from animal treading which has been shown to significantly elevate N_2O emissions (Bhandral *et al.* 2007b; Thomas *et al.* 2008; Van Groenigen *et al.* 2005). This topic is discussed further in Section 2.5. These elevated emissions resulting from the combined effects of compaction and animal excreta are also not specifically addressed in the IPCC best practice guidelines.

2.2.5 Background N_2O emissions

The current approach for estimating an EF is based on the difference in cumulative emissions from treated plots and non-treated 'background' control plots (Bouwman 1996). At steady state, the fraction of the total N_2O that is emitted from the soil-plant system is termed the 'background' N_2O emission implying a "natural" origin (Bouwman 1996). However, the IPCC guidelines (IPCC 2006) dismiss this concept stating they are "...not 'natural' emissions but are mostly due to contributions of N from crop residue. These emissions are anthropogenic and accounted for in the IPCC methodology" and are set at a default value of 1.0 kg N_2O-N ha^{-1} y^{-1} (IPCC 2006). In some recent studies these emissions have been attributed to anthropogenic effects such as soil cultivation (Van Beek *et al.* 2011), nitrification of native soil N following rainfall events (Rafique *et al.* 2011) and/or the 'carryover' effect of previously deposited animal urine/excreta. A study by Van Beek *et al.* (2011) attributed 10−22% of the cumulative emissions to background emissions from grazed peat pasture soils while it was 61% in the study of Rafique *et al.* (2011) from a grazed grassland. Petersen *et al.* (2006) reported annual background emissions of 1.4 ± 0.3 kg N_2O-N ha^{-1} y^{-1} over a 12-month period from dairy crop rotations in European countries. Losses of N_2O from unfertilised clover-ryegrass pasture equalled 6 kg N ha^{-1} y^{-1} in an Australian study (Eckard *et al.* 2003) while Ruz-Jerez *et al.* (1994) and Ledgard *et al.* (1999) reported total denitrification losses of 3.4 and 3–7 kg N ha^{-1} y^{-1} from unfertilised plots, respectively, in New Zealand pastures. A silt loam beneath a mixed herb ley sward at Lincoln, New Zealand, yielded an estimate of 0.2 kg N_2O-N ha^{-1} y^{-1} (Van der Weerden *et al.* 1999; 2000). A grass sward on a loamy sand near Giessen, Germany, produced an average 0.2 kg N_2O-N ha^{-1} y^{-1} (Kammann *et al.* 1998) while Galbally *et al.* (2010) reported an average of 0.4 kg N_2O-N ha^{-1} y^{-1}. It is clear that significant

background emissions can occur from grazed pastures and these are attributed to carryover effects of fertilisers with no clear consideration given to the background emissions resulting specifically from surface decomposition of pasture litter or pasture trodden into the soil.

2.3 Litter-fall quantities in field conditions

Whitehead (2000) states that quantifying the herbage material from grasslands and its decomposition *in situ*, is difficult partly because some of the herbage is consumed during the senescent stage by soil fauna and partly because of wide spatial variability in decomposition rates due to temperature, rainfall and management. Defoliation by grazing animals can affect the size of plants or plant parts, plant biodiversity, plant density and consequently, N_2 fixation and photosynthesis (Barger *et al.* 2004; Campanella and Bisigato 2010; Whitehead 2000). It is also controlled by the foraging behaviour and management of the cattle e.g. selective grazing by the animal (of certain plants or plant parts) and the frequency, timing and intensity of grazing (Menneer *et al.* 2004).

Naeth *et al.* (1991) appears to be the first study to have measured pasture litter-fall rates following full season grazing events finding that season and grazing intensity significantly affected the amounts of litter; it decreased with increasing grazing intensity. In another study (Mapfumo *et al.* 2002), litter C and N pools were investigated with litter-fall also decreasing with increasing grazing intensity. Lodge *et al.* (2006) found that Merino wethers produced a mean 111 kg DM ha^{-1} over 4 to 6 week of continuous grazing on forage grasses in New South Wales, Australia. In these three studies discussed above, litter-fall decreased with increasing stocking rates because higher stocking rate led to higher removal of green herbage. Litter-fall in these studies was not measured following each grazing event, so the numbers reported were cumulative values. In these studies, the species under investigation were prairie forage grasses and the grazing animals were sheep. Campanella and Bisigato (2010) reported litter-fall rates of 60−160 kg DM ha^{-1} y^{-1}, collected on a monthly basis using litter traps from arid, extensive rangelands that adopted set-stocked sheep grazing (0.11–0.14 sheep ha^{-1}) and were dominated by forage and perennial grasses such as *Larrea divaricata*, *Chuquiraga hystrix*, *Stipa tenuis* and *Poa ligularis*. Carrera *et al.* (2008) reported litter-fall rates of 260–310 kg DM ha^{-1} y^{-1} from similar rangeland systems including shrubs (*Larrea* spp. and *Stipa* spp.) grazed by sheep (0.14 sheep ha^{-1}). In these studies, either the litter-fall rate was not measured following each grazing event (the rates reported are cumulative values) or the animals were set-stocked over time. It might be expected that litter might have decomposed prior to measuring litter-fall rates in these studies, hence the lower rates. Lodge *et al.* (2006) has indicated that litter-fall has the potential to contribute to N cycling. However, no consideration has been given to N_2O

emissions resulting from pasture litter decomposition. Clearly, no study has so far attempted to quantify litter-fall in dairy pasture cattle and define potential N_2O emissions due to pasture litter decomposition.

Figure 2.3 Fresh herbage being dropped during grazing by animals.

2.4 Plant litter and N_2O emissions

Litter-fall may stay on the pasture soil surface and decompose, contributing to previous litter already decomposing in the soil; and/or get partially or completely incorporated in the soil due to animal treading and decompose further; while the standing senesced material may move downwards via abscission, lodging, and animal treading (Hutchinson and King 1989; Molinar *et al.* 2001). Plant litter in pastures contributes significantly to nutrient cycling (Hoorens *et al.* 2003) since it is continuously being deposited due to the abscission, lodging and trampling of above-ground plant parts (Lodge *et al.* 2006). The decomposition of the above- and below-ground litter supposedly causes the mineralisation of C and N (Molinar *et al.* 2001). A few studies have examined N_2O emissions from pasture litter under arable conditions. For example, McKenney *et al.* (1993), in a lab study, determined the effect of anaerobic periods on NO + N_2O emissions from annual ryegrass and red clover and found 25.1 and 47.1 mg N kg^{-1} soil after a 5 d incubation at 20^{o}C, respectively. Gillam *et al.* (2008) examined the effects of red clover and barley straw as a C source following NO_3^- addition to an arable soil while Larsson *et al.* (1998) determined N_2O losses following the application of mulches to the soil surface that comprised of low- and high-N grasses, and alfalfa, and found N_2O losses in the range of 0.1–1.0% of the N loading. Kaiser *et al.* (1998b) reported higher and prolonged N_2O emissions after the incorporation of ryegrass and red clover residues in a barley cropping system due to an increase in soil available N resulting from the mineralisation of the forage residues, but these emissions were not influenced by the C: N ratio of the residues. Baggs *et al.* (1996) incorporated ryegrass and clover residues by rotary tillage and

recorded short-lived N_2O emissions ranging from 14–23 g N_2O-N ha^{-1} d^{-1} that correlated with rises in soil temperature. However, these studies were performed in arable systems and no studies have examined pasture litter and associated N_2O emissions. To date, litter-fall quantities have NOT been quantified from single grazing events, using dairy cattle in clover-ryegrass pastures that dominate in humid temperate countries such as New Zealand and Australia.

In cropping systems, residues have been shown to affect N_2O emissions (Bouwman 1996) by supplying easily mineralisable N and C, which can enhance denitrifier activity and, thereby, N_2O emissions from both soil mineral N and crop residue N (Paul and Beauchamp 1989; Velthof *et al.* 2002). According to crop species, residues differ in biochemical composition in terms of total C and N (Constantinides and Fownes 1994; Trinsoutrot *et al.* 2000a), cellulose, lignin (Melillo *et al.* 1982) and soluble polyphenol contents (Palm and Sanchez 1991). Differences in the biochemical composition of plant residues can influence litter decomposition kinetics and ultimately affect N mineralisation and immobilisation rates (Aulakh *et al.* 2000; Baggs *et al.* 2000; Hadas *et al.* 2004; Palm and Rowland 1997).

Litter decomposition in soil is a complex process which generally reflects soil respiration rates. Soil respiration in pasture ecosystems is a function of root respiration, rhizosphere respiration, soil organic matter (SOM) oxidation and litter decomposition (Luo and Zhou 2006). Microbial decomposition of litter and SOM oxidation are major contributors to soil respiration (Coleman *et al.* 2004). Nutrient cycling via litter decomposition is a major process in temperate pasture ecosystems, with about 70% of net primary production being associated with the activity of microbial decomposers in the litter and soil (Hutchinson and King 1982; Whitehead 1995).

Mineralisation of incorporated plant litter is mainly governed by various factors such as litter treatment characteristics such as plant species; rate, form, placement and biochemical composition of the litter (Aulakh *et al.* 1991a; Loecke and Robertson 2009); soil temperature (Devevre and Horwath 2000) and soil water content. It is well recognised that agricultural cropping residues can contribute to N_2O emissions (Huang *et al.* 2004; Mori *et al.* 2005; Potthoff *et al.* 2005). However, emissions of N_2O differ significantly due to litter characteristics, as detailed below.

2.4.1 Biochemical composition

Biochemical composition has been shown to be one of the most crucial governors of N_2O emissions during decomposition of plant litter in cropping systems (Palm and Rowland 1997; Toma and Hatano 2007). Several studies have reported that emissions of N_2O were

negatively correlated with the C: N content of the residues (Baggs *et al.* 2000; Kaiser *et al.* 1998b). Aulakh *et al*. (1991a) incorporated crop residues of varying C: N ratio (8–82) and incubated these with soil at 25^{o}C for 35 d. They observed short-lived N_2O fluxes in the initial period (8 d) that accounted for 94% of total emissions from hairy vetch. Aulakh *et al*. (1991a) concluded that denitrification losses as well as intensity of denitrification in the initial 8 d period were related to the C: N ratio of the crop residues. Breland (1994a) conducted a study on mineralisation and denitrification from clover shoots and indicated that both the readily decomposable and the recalcitrant fraction of organic C from the clover shoots decomposed at faster rates than those of SOM. The study of Breland (1994a) found that 38–56% of the clover-N (and 57–69% C) was mineralised during the initial phase (52 d) of rapid mineralisation. Studies such as Baggs *et al.* (2000) and Huang *et al.* (2004) demonstrated that high N_2O and CO_2 emissions corresponded to relatively low C: N ratios of incorporated crop residues (such as lettuce, rapeseed, potato, maize, wheat and sugarcane) and that the N mineralisation rates of the residues were dependent on their C: N ratios (Aulakh *et al.* 1991a; Eichner 1990). De Neergaard *et al.* (2002) found that net N mineralisation of white clover and perennial ryegrass was correlated with the C: N ratio and N content of litter and inversely proportional to lignin content when incubated at 9^{o}C for 94 d.

2.4.1.1 Litter carbon

Studies show that when easily decomposable C and N sources are added to soil, the microbial biomass switches from the more recalcitrant SOM to more readily available C and N sources (Cheng 1996; Gentile *et al.* 2008; Soon and Arshad 2002; Sparling *et al.* 1982). Soil C and litter-bound C act as substrate for most microbially mediated processes, particularly N mineralisation, N losses (mainly denitrification) and SOM decomposition. Quality of C in this context is particularly important as it constrains the supply of energy for enzyme production and growth (Fontaine *et al.* 2003). Toma and Hatano (2007) investigated N_2O emissions using low C: N ratio crops like onion leaf (C: N, 11.6) and soybean plant parts (C: N, 14.5) concluding that the decomposition process actually mineralises the C and N from the plant residues and thus enhances the N_2O and CO_2 emissions. Other studies (Gunnarsson and Marstorp 2002; Trinsoutrot *et al.* 2000b) have shown the concept of 'sequential degradation' where over the initial few days of litter decomposition in soil, soluble carbohydrates are decomposed together with the most readily degradable N-rich components. After this time, proteins, and to a large extent the non-cellulose structural carbohydrates such as pectic substances, hemicellulose constituents and cellulose, are sequentially degraded (Gunnarsson and Marstorp 2002; Henriksen and Breland 1999; Martin and Haider 1986).

Litter-bound C may be present as glucose, cellulose, hemicellulose and/or lignin; the proportions of which vary according to plant species. Cellulose is almost an intermediate in terms of its microbial degradation potential with glucose and lignin at the extremes. Cellulose ($C_6H_{10}O_5$), an unbranched, β–(1,4)-linked, linear polymer of glucose, is a carbohydrate synthesised by plants and the most abundant organic polymer in nature (Figure 2.4). Biodegradation of cellulose requires a distinct set of extracellular enzymes viz. cellulase, cellobiohydrolase and β–glucosidase, which hydrolyse the β–1,4 bonds of cellulose to glucose for further energy generation processes (Clark 1997; Geisseler and Horwath 2011). Activity of these extracellular enzymes is believed to be the rate-limiting step in the decomposition of recalcitrant forms of C (Schimel and Weintraub 2003). Cellulolytic microorganisms, mainly fungi (*Penicillium* sp., *Aspergillus* sp.) and some bacteria (*Cellulomonas* sp., *Streptomyces* sp., *Pseudomonas* sp.), aid in the extracellular cleavage of cellulose. The biochemistry of cellulose, so far, has been studied in great detail but its interaction with various parameters in nature is still unexplored. With respect to N_2O emissions, recalcitrant compounds such as cellulose and lignin generally decrease the emissions and N turnover because the decomposers and denitrifiers both prefer an easily degradable C source for microbial growth and activity (Dendooven *et al.* 1996; Goek and Ottow 1988; Mengel and Schmeer 1985). Lygnolytic and cellulolytic microbes also require an easily available C source other than lignin and cellulose, at least in the initial growth phase (Swift *et al.* 1979).

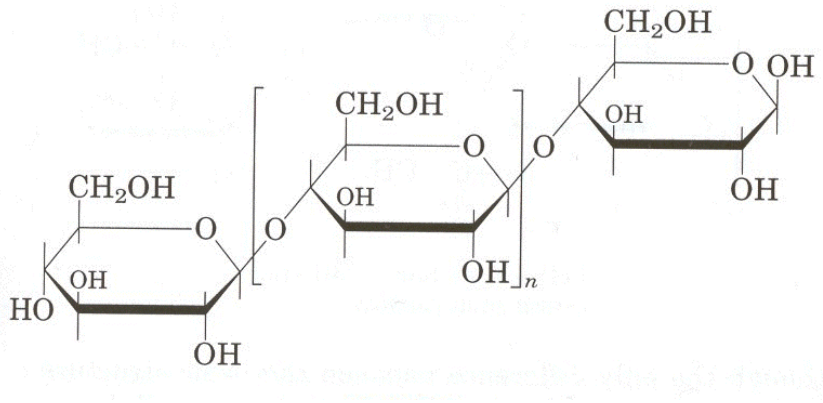

Figure 2.4 Polymeric structure of cellulose molecule.

2.4.2 Particle size and placement of litter

Litter decomposition is influenced by depth of incorporation in soil, rate and time of residue incorporation and form of residues (Ambus and Jensen 1997; Velthof *et al.* 2002). Ground or finely chopped residues are more susceptible to microbial interaction than intact plant parts due to improved soil-residue contact and increased surface area (Angers and

Recous 1997) and a decreased lignified barrier tissue (Summerell and Burgess 1989). However, ground particles may also be protected against decomposition through physical protection by clay and other particles (Breland 1994a; Stickler and Frederick 1959). Ambus and Jensen (1997) investigated the effect of particle size on N dynamics and found that short-term N immobilisation was higher for ground residues than coarse residue incorporation of barley and pea. Fine residues also caused more denitrification than coarse residues, but only on a short-term basis. Loecke and Robertson (2009) reported similar results with higher N_2O emissions from finer particles of red clover residues.

In terms of residue placement, Aulakh *et al.* (1991a) reported significantly higher N mineralisation rates and N_2O emissions from an incorporated residue treatment, while Abiven and Recous (2007) reported that surface-placed residues had slightly higher net N mineralisation rates. They did not find significant effects of residue placement on C mineralisation kinetics. Breland (1994a) reported that layered red clover litter decomposed faster than uniformly incorporated litter because the microbial biomass was 'protected' against mineralisation in the latter case.

2.4.3 Soil moisture content

As mentioned earlier, N_2O emissions occur via nitrification in the range of 45–60% WFPS while at higher soil moisture, N_2O emissions mainly occur due to denitrification (Dalal *et al.* 2003). Aulakh *et al.* (1991b) reported substantial litter-N immobilisation, from crop residues with C: N ratios ranging from 8–82, at 60% WFPS, whereas, at 90% WFPS significant denitrification losses occurred. Shelton *et al.* (2000) found a linear relationship between denitrification and soil water content with 60% WFPS as a threshold for denitrification during decomposition of surface-placed hairy vetch residues. Gillam *et al.* (2008) showed higher N_2O emissions from wetter soils due to reduced aeration. Potthoff *et al.* (2005) reported that aerobic decomposition of crop residues decreased O_2 availability due to microbial respiration, thus favouring denitrification at higher soil water contents.

2.4.4 Conclusion (plant litter)

It is clear that studies have examined N_2O emissions resulting from arable crop residues (Aulakh *et al.* 1991b; Aulakh *et al.* 2001; Baggs *et al.* 2000), however, there is a dearth of information with respect to the effects of perennial pasture species and supplementary feed litters on N_2O emissions in grazed systems. The few studies that have examined potential N_2O emissions from pasture litter (*Trifolium* sp., *Lolium* sp., *Vicia* sp., *Zea* sp., *Hordeum* sp.) have done so under arable/ley conditions (Gillam *et al.* 2008; Larsson *et al.* 1998; McKenney *et al.*

1993). Nowhere, however, does there appear to be any work that has focused directly on grazed pasture litter and their associated direct N_2O emission factors and thus the potential for such emissions to contribute to the grazed grassland N_2O inventory is unknown.

2.5 Animal treading and N_2O emissions

Intensification of livestock-based agriculture has become a major global phenomenon. However, it can lead to detrimental environmental consequences such deforestation, overgrazing, soil degradation, soil compaction, soil erosion, and water pollution (Steinfeld *et al.* 2006). Approximately 20% of the world's pastures and rangelands are considered degraded through overgrazing and compaction (Steinfeld *et al.* 2006). The magnitude of compaction depends on the stocking rate, soil type, moisture content and animal species (Naeth *et al.* 1990; Warren *et al.* 1986), e.g. grazing cattle and sheep can exert static pressures of 160–192 and 83 kPa on the soil, respectively (Di *et al.* 2001; Willatt and Pullar 1983) which is comparable to the compaction caused by vehicular traffic. Soil compaction by animal treading can therefore, have severe impacts on soil physical conditions and pasture production. Moreover, N_2O emissions from the dung and urine patches are exacerbated due to reduced soil aeration occurring as a result of animal treading-induced soil compaction (De Klein *et al.* 2006b). Animal treading has received sparse attention with respect to soil N_2O emissions (Oenema *et al.* 1997).

2.5.1 Animal treading

Poaching/puddling occurs under saturated soil conditions when animal treading induces the soil to become slurry-like under very wet conditions (usually in winter-grazed management systems); and pugging occurs in wet, soft soil causing deep hoof imprints and is often associated with considerable pasture damage (Drewry *et al.* 2008). Both of these processes cause soil compaction and pasture damage which disrupts the ongoing biological processes in the soil. The extent of compaction and damage depends on the soil wetness, animal species and age, vegetation cover, stocking rate and management conditions such as rotational grazing or set grazing (i.e. grazing intensity) (Bilotta *et al.* 2007; Drewry *et al.* 2008).

2.5.2 Effect on soil physical conditions

The depth of soil compaction due to treading varies from 2–12 cm below the soil surface depending on the above factors (Butler and Adams 1990; Scholefield and Hall 1985). This creates an increased soil bulk density layer and decreased pore space, restricting the

movement of water and air through the soil (Greenwood and McKenzie 2001), leading to water logging and decreased aeration within the compacted zone. Severe treading damage can have long-lasting effects (>18 months) on physical soil characteristics such as hydraulic conductivity, aggregate size, total porosity, pore size distribution, and bulk density (Singleton *et al.* 2000). Increased bulk density, as a result of treading can result in decreased infiltration rates (Mulholland and Fullen 1991) and hydraulic conductivities of soil (Greenwood *et al.* 1997). Daniel *et al.* (2002) reported increased resistance to penetration and bulk density values, but only in 0–10 cm from the soil surface, due to long-term (10 yr) livestock grazing. Grazing at high stocking rates reduced surface soil macroporosity by 10–40%, although it reached 60% under wetter conditions (Drewry *et al.* 2008; Singleton *et al.* 2000).

Treading damage is exacerbated in wetter soils (McDowell 2008; Mullen *et al.* 1974) since wet soil is more readily compacted than dry soil (Di *et al.* 2001) however, Schofield and Hall (1985) reported that treading damage was independent of soil water content over a wider range. For soils with higher clay contents, treading damage can occur even at relatively low soil moisture contents because of the plastic nature of clay soils.

2.5.3 Effect on pasture growth

Animal grazing and treading, particularly in wet soil conditions, can affect pasture yields directly through leaf burial in mud, crushing, bruising, and decreased dry matter production (Hamilton and Horne 1988; Ledgard *et al.* 1996; Nie *et al.* 2001). Research on pasture growth impedance due to animal treading was first performed by Edmond (1963) who later on, tested the effects of treading using different animals, pasture species and soil moisture levels on pasture growth. However, this research only explored the effects on pasture growth.

Shearing movement of the hooves of grazing animals can directly cause plant damage via crushing, bruising and burial and alter the rhizosphere by compaction. Indirect effects include impeded root growth. This can decrease soil biodiversity and vegetative cover making the soil prone to erosion (Bilotta *et al.* 2007). Severe treading damage can decrease pasture plant growth significantly (Drewry *et al.* 2001; Drewry and Paton 2005; Menneer *et al.* 2005c) and may also alter species and herbage composition (Haynes and Williams 1993). Ledgard *et al.* (1996) reported a 20–80% decrease in pasture production from a single treading event during winter (relatively wetter conditions) on a silt loam soil while values of 40–42% were reported under medium to heavy animal treading (Nie *et al.* 2001). Annual herbage production decreased by 16% and 34% under moderate (450 cows ha^{-1} for 1.5 h on

15 m^2 plots) and severe (450 cows ha^{-1} for 2.5 h) animal treading, respectively, for a clover-ryegrass pasture (Menneer *et al.* 2005b).

2.5.4 Effect of animal treading on the N cycle and N_2O emissions

Van Groenigen *et al.* (2005) noted a dearth of knowledge with respect to animal treading and associated N_2O emissions *in situ*. As stated above, animal treading disrupts soil physical conditions and soil microbial processes. Studies have shown that animal treading can affect BNF, denitrification rates and N_2O emissions (Menneer *et al.* 2005a; b; Oenema *et al.* 1997; Simek *et al.* 2006; Thomas *et al.* 2004; Thomas *et al.* 2008) resulting from urine and excreta deposition. Earlier studies (Carran *et al.* 1995; Luo *et al.* 1999; Ruz-Jerez *et al.* 1994) have speculated that higher denitrification rates and N_2O emissions, under field conditions are observed soon after grazing. The reasons suggested include increased soil NO_3^-–N concentrations due to limited plant N uptake after defoliation and also resulting greater N substrates due to excreta and urine deposition. Thomas *et al.* (2004) observed higher denitrification rates and higher cumulative N_2O emissions (14.9 kg N ha^{-1}) after 90 d from intensively tilled soil receiving urine and trodden at >field capacity. Ball *et al.* (1999) associated increased N_2O emissions from compacted soils (using farm vehicles) due to increased soil wetness and resulting reduced diffusivity. Moreover, heavier compaction gave a greater N_2O emission response. Thomas *et al.* (2008) and Bhandral *et al.* (2007b) reported two- to seven-fold increases in N_2O emissions following treading and urine application in New Zealand pastoral soils during spring and early summer. Also, soil tillage without compaction did not affect N_2O emissions. Relatively higher N_2O emissions are expected from more severe animal treading, however this was not true in the study of Simek *et al.* (2006) who reported lower N_2O emissions from severely-trodden plots than moderately trodden plots. They suggested that conditions in severely-trodden plots were more conducive to produce N_2 rather than N_2O. Most of these studies dealt with emissions resulting from a combination of soil compaction (animals and/or vehicular traffic) and excretal depositions. Moreover, emissions of N_2O did not differ between trodden and untrodden plots that were devoid of urine application (Thomas *et al.* 2008; Van Groenigen *et al.* 2005).

Menneer *et al.* (2005a; b) reported the first studies that showed the impacts of animal treading on N_2 fixation and N_2O emissions in the absence of animal excreta. Menneer *et al.* (2005a) reported reductions of 13% and 53% in annual N_2 fixation under moderate and severe treading, respectively, devoid of the influence of animal urine. The decrease in N_2 fixation was due mainly to substantial losses of annual clover DM production that occurred under moderate and severe treading (9% and 52%, respectively). Menneer *et al.* (2005b) measured

3–6 fold higher N_2O emissions at 8 d after severe treading compared to nil-treading. They reasoned the higher emissions were due to the fact that treading caused a compaction of soil thereby reducing soil aeration causing higher denitrification. Moreover, due to treading, plant growth was reduced which increased soil NH_4^+–N and NO_3^-–N concentrations (due to reduced N uptake) thus contributing to denitrification.

2.5.5 Conclusion (animal treading)

The above studies show that higher N_2O emissions occur due to soil compaction from animal treading and are elevated under the combined effect of animal excretion and high soil water content. However, none of the studies considered the possibility that animal treading may increase N_2O emissions due to enhanced plant damage and subsequent release of plant-derived N and C into the soil.

2.6 Summary

About 3.5 billion ha of the total world area, representing 26% of the world's land area and 70% of the world's agricultural area are under pasture and fodder crops. Pasture plant species, farm animals and anthropogenic activities are the key drivers which regulate C and N cycling in grassland ecosystems via plant litter returns; urine and faeces deposition and, management practices. Pastures are recognised sources of significant GHG emissions.

Greenhouse gases, such as N_2O are atmospheric trace gases that absorb infra-red radiation reflected from the Earth's surface, thus trapping radiated heat and contributing to increases in global temperatures. The GHG – N_2O is a precursor to compounds involved in stratospheric ozone depletion and has a global warming potential of 298 times over a 100 year timeframe.

Approximately 90% of the total New Zealand farm area is considered as a pastoral ecosystem and the agricultural sector accounted for 46.6% of New Zealand's GHG inventory in 2008. New Zealand agriculture is dominated by grazed pastures and they are an important source of N_2O. Nitrous oxide contributed 16% to New Zealand's GHG inventory in 2008 which has increased by 21.8% from 1990 levels.

Inputs of N in pastures generally occur from biological nitrogen fixation (BNF) and atmospheric deposition; fertiliser, manure and effluent applications and animal excreta (animal urine and dung deposition) while N losses occur due to nitrification, denitrification, volatilisation and leaching. In New Zealand pastoral soils, N inputs result from about 1.5 M t of N originating from animal excreta, 1.1 M t of N through BNF, 0.30 M t of fertiliser N and about 0.01–0.015 M t of atmospheric N deposition.

Close observation of the grazing behaviour of dairy cattle reveals that harvested but un-ingested litter falls during grazing. After grazing finishes, this litter remains where it falls. Litter-fall rates have so far not been quantified. Litter-fall is NOT accounted for as an N_2O source in the IPCC best practice guidelines; moreover, the fate of this unconsumed litter is unknown.

Decomposition of litter is mainly governed by its biochemical composition while other factors such as litter treatment characteristics (plant species; rate, form, placement) and; soil temperature and soil water content, are also equally important. It is well recognised that agricultural cropping residues can contribute to N_2O emissions but the contribution of pasture residues to N_2O emissions have been studied sparsely and only under arable conditions.

The IPCC methodology estimates N_2O emissions using human-induced net N additions to soils (e.g. synthetic or organic fertilisers, deposited manure, crop residues, sewage sludge), or from mineralisation of N in soil organic matter following drainage/management of organic soils, or cultivation/land-use change on mineral soils. The residues from forages by the IPCC are only accounted for during pasture renewal. The literature review demonstrates that *pastures* are significant N_2O sources mainly resulting from fertiliser inputs and animal excrements. Emissions of N_2O resulting from litter-fall-, or supplementary feed litter-decomposition and the emissions resulting from animal treading not influenced by excretal depositions, are not accounted for in the IPCC methodology for calculating N_2O inventories and hence require further investigation.

Chapter 3
Generic materials and methods

3.1 Herbage collection and analyses

For the laboratory incubation experiments 3 and 4 (Chapters 6 and 7), fresh leaves of white clover (*Trifolium repens* L.), ryegrass (*Lolium perenne* L.) and maize (*Zea mays* L.) were collected and dried in an oven at 65°C for 48 h. These plant materials were then finely ground (200 μm) in a ball mill and their chemical characteristics (total N, total C, cellulose, hemicellulose, lignin and C: N ratios) were determined using standard procedures (Rowland and Roberts 1994; Section 3.1.1).

Total C was determined by Dumas combustion and total N was determined by near infrared reflectance spectroscopy (NIR) which was calibrated using Dumas combustion.

3.1.1 Biochemical components

For herbage analysis, three different fractions were analysed viz. NDF (neutral detergent fibre), ADF (acid detergent fibre) and lignin. The NDF fraction is the sum of hemicellulose, cellulose and lignin while ADF is the sum of cellulose and lignin fractions only. The individual components were computed by taking the differences between NDF, ADF and lignin fractions.

3.1.1.1 Acid detergent fibre

A sub-sample of the dried and ground herbage (0.5 g, W_1) was weighed into a 250 mL conical flask to which 100 mL CTAB reagent (50 g cetyltrimethyl ammonium bromide in 5.0 L, 0.5 M H_2SO_4) and three drops of octan-2-ol (antifoaming agent) were added, the whole solution was then simmered on a hot plate for 1 h. This was filtered hot through an ignited and pre-weighed porous no. 2 sinter (W_2) under gentle suction. The residue was washed three times using boiling deionised (DI) water (50 mL aliquots) and then using acetone until no more colour from the residues was removed. The residue was sucked dry and the sinter was further dried for 2 h at 105^{o}C, cooled in a desiccator and weighed (W_3). Percentage ADF was calculated using Equation 3.1 below.

$$\%\ \mathrm{ADF} = \frac{W_3 - W_2}{W_1} \times \frac{100}{1}$$

Equation 3.1

3.1.1.2 Lignin

Following on from the procedure above, the cooled sinter, after recording the weight (W_3), was half-filled with 72% H_2SO_4, and stirred with a glass rod to make a smooth paste. The acid was allowed to drain. This was repeated again. After 3 h, the acid was filtered using a Buchner apparatus, washed three times with hot water and once with acetone, followed by oven-drying at 105^{o}C for 2 h (W_4) and then charred at 500^{o}C for 2 h to determine the ash content (W_a). Percentage lignin was calculated using Equation 3.2 below.

$$\% \text{ lignin} = \frac{W_4 - W_a}{W_1} \text{x } \frac{100}{1}$$

Equation 3.2

3.1.1.3 Neutral detergent fibre

A sub-sample of the dried and ground herbage (1.0 g, W_1) was weighed in a beaker to which 50 mL of the neutral detergent solution (95 g EDTA, 135 g ammonium pentaborate and 150g sodium lauryl sulphate in 5.0 L water) was added. The beakers where placed on a hot plate and connected to a condenser, and refluxed for 1 h. The contents were transferred into preconditioned Gooch crucibles (oven-dried at 105^{o}C for 2 h and cooled in a dessicator) and the aliquot was drained using a Buchner apparatus followed by rinsing 3 times with hot DI water and once with acetone. The contents of the crucibles were oven-dried at 105^{o}C for 12 h, cooled in a dessicator and weighed (W_2) followed by charring at 500^{o}C for 2 h to determine the ash content (W_a). Percentage NDF was calculated using Equation 3.3 below.

$$\% \text{ NDF} = \frac{W_2 - W_a}{W_1} \times \frac{100}{1}$$

Equation 3.3

3.2 Gravimetric soil water content

A sub-sample of field moist soil (10 g) was weighed into a container of known weight and dried in an oven for 24 h at 105^{o}C. Then it was cooled in a dessicator and reweighed. The gravimetric soil water content (θ_g) was calculated using Equation 3.4 (Blakemore *et al.* 1987). The volumetric soil water content (θ_v) was obtained by multiplying θ_g by the soil bulk density. Soil water content was expressed as water-filled pore space [WFPS, (θ_g/porosity)] in Chapters 6 and 7 where the soil porosity = [1 – (bulk density/2.65)] and 2.65 Mg m^{-3} was the assumed soil particle density.

$$\theta_g = \frac{M_m - M_d}{M_d}$$

Equation 3.4

where;

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

M_m = mass of field moist soil (g)

M_d = mass of oven dry soil (g)

3.3 Soil surface pH measurement *in situ*

Soil surface pH for the field experiments was measured using a Hanna HI 9025C portable pH meter fitted with a soil surface probe (Broadley-James Corporation, Irvine, CA, USA), after moistening the soil surface with a drop of DI water. The pH meter was calibrated using buffer solutions ranging from 4.0–7.0 (source standards) prior to each measurement (Blakemore *et al.* 1987).

3.4 Soil bulk density

Soil bulk densities were determined in the animal treading experiments (Chapter 5). Soil was collected using a soil core (0.09 m diameter × 0.12 m deep) followed by drying at 105^{o}C for 48 h to determine its gravimetric moisture content prior to calculating the bulk density. Soil bulk density measurements were performed at depths of 0–3, 3–6, 6–12, and 0−12 cm.

3.5 Anaerobically mineralisable N

The anaerobically mineralisable N (AMN, expressed in μg g^{-1}) was measured using near infrared reflectance spectroscopy (NIR). Calibration was performed with soil that had been anaerobically incubated at 40^{o}C for 7 d, followed by extraction with 2M KCl for 15 min and analysed using Berthelot colorimetry (Hinds and Lowe 1980; Keeney and Bremner 1966).

3.6 Inorganic N

Inorganic N extractions were performed using 2 M KCl with a 1: 10 ratio (soil: KCl) on an end-over-end shaker for 1 h followed by centrifuging the extractant at 2000 rev min^{-1} (480*g*) for 10 min, and filtering (Whatman No. 41) into 30 mL plastic containers before storage at 4^{o}C. Analyses of ammonium (NH_4^+–N), nitrate (NO_3^-–N) and nitrite (NO_2^-–N)

were performed on an Alpkem FS3000 twin channel Flow Injection Analyser (FIA) (Alpkem, College Station, TX, USA). The diffusion technique of Brooks *et al.* (1989) was employed for determining the ^{15}N enrichment of the inorganic N detailed in Section 3.8.2. The concentration of inorganic N was calculated using Equation 3.5.

$$N_s = \frac{(N_e \times V)}{M_d}$$

Equation 3.5

where;

N_s = inorganic N content (µg g^{-1} dry soil)

N_e = inorganic N concentration of sampled extract (µg mL^{-1})

V = volume of solution (KCl + soil moisture) (mL)

M_d = mass of oven dry soil (g)

3.7 Microbial biomass C

Treated samples for the second laboratory experiment (Chapter 7) were analysed for microbial biomass C (MBC) using the chloroform fumigation extraction (CFE) method (Vance *et al.* 1987) as detailed below.

3.7.1 Purification of chloroform

Commercially available chloroform (Analar grade) contains ethanol, a stabiliser (Jenkinson *et al.* 2004). To remove the ethanol, chloroform (200 mL) was shaken with 5% H_2SO_4 (400 mL) in a separating funnel. The lower layer, containing acid, was discarded and the process repeated two more times followed by rinsing with 400 mL of DI water. The purified chloroform was stored at 5^oC, after adding 10 g anhydrous Na_2SO_4, (to remove the traces of water) until required for analysis. A further addition of 5 g anhydrous Na_2SO_4 may be required if it had not clumped; clumping indicating removal of water.

3.7.2 Extraction

Duplicate soil sub-samples (5 g) were used. One fumigated (as described below) and the other non-fumigated was extracted with 20 mL, 0.5 M K_2SO_4 (1: 4, soil: extractant ratio) for 2 h on an end-over-end shaker at 15 rev min^{-1}. The extractant was centrifuged at 750*g* for 10 minutes and filtered (Whatman No. 41) into 30 mL plastic containers before storage at 4^oC until analysis.

3.7.3 Fumigation

The samples to be fumigated were placed in a vacuum chamber. A beaker containing ~25 mL of purified chloroform and three boiling chips were placed at the bottom of the vacuum chamber. After sealing, evacuation of the vacuum chamber commenced until the chloroform started to boil whereupon evacuation ceased. The vacuum chamber was then covered and the samples were allowed to fumigate in the dark for 24 h. Before starting the extraction of the fumigated samples, the vacuum chamber was flushed (2–3 times) with fresh air to remove any remaining chloroform vapour.

3.7.4 Calculation of MBC

Extracts from both fumigated and non-fumigated samples were analysed for total organic carbon (TOC) using a Shimadzu Total Organic Carbon Analyser TOC 5000A (Shimadzu Oceania Pty Ltd, Sydney, Australia) fitted with a Shimadzu ASI-5000A autosampler. Microbial biomass C was calculated as the difference between the values for fumigated and non-fumigated samples and the difference was divided by the k_{EC} value of 0.45 (Jenkinson *et al.* 2004). This value is considered a constant and accounts for the efficiency of the soil microbial biomass extraction. The difference in TOC values between the fumigated and non-fumigated samples is assumed to be due to the release of organic C from the lysed microbial cells (Jenkinson 1976). Equation 3.6 was used to calculate the concentration of MBC in the filtered samples:

$$\mathrm{MBC} = \left[\frac{(\mathrm{TOC_F} \times \mathrm{V_F})}{\mathrm{M_{Fd}}} - \frac{(\mathrm{TOC_{NF}} \times \mathrm{V_{NF}})}{\mathrm{M_{NFd}}}\right] / 0.45$$

Equation 3.6

where;

MBC = microbial biomass carbon (µg g^{-1} dry soil)

TOC_F = total organic carbon concentration of extract after fumigation (µg mL^{-1})

V_F = volume of solution for fumigated samples (K_2SO_4 + soil moisture) (mL)

M_{Fd} = mass of oven dry soil for fumigation (g)

TOC_{NF} = total organic carbon concentration of non-fumigated extract (µg mL^{-1})

V_{NF} = volume of solution for non-fumigated samples (K_2SO_4 + soil moisture) (mL)

M_{NFd} = mass of oven dry soil for non-fumigated samples (g)

3.8 Microbial biomass N using stable isotope – ^{15}N

Measurement of the isotopic enrichment of ^{15}N in the microbial biomass ($MB^{15}N$) was performed in Chapter 8 for the soil samples collected at different depths using methods of Templer *et al.* (2003). Prior to isotopic analysis, inorganic N (NH_4^+–N and NO_3^-–N) concentrations of the MBC extracts were determined using FIA (Section 3.5). Further analysis was performed in two phases viz. alkaline persulphate oxidation, which converted the total N in the samples to the NO_3^- form, for determining the total N in the microbial biomass extracts (Cabrera and Beare 1993); and secondly, the ^{15}N diffusion technique (Brooks *et al.* 1989) to measure ^{15}N in the microbial biomass using the extracts obtained from phase 1.

3.8.1 Alkaline persulphate oxidation

The extracts obtained from the MBC procedure above (Section 3.7) were used for further analysis. One litre of oxidising solution was prepared by dissolving 50 g $K_2S_2O_8$, 30 g H_3BO_4 and 7.5 g NaOH in a volumetric flask. Digestion of the extracts was done using soil extract: oxidising agent in 1: 1 ratio and autoclaved at 120^{o}C for 1 h. The resultant extracts were cooled and analysed for total dissolved N (TDN).

3.8.2 Microbial biomass – ^{15}N measurement

The diffusion technique of Brooks *et al.* (1989) was employed for determining the ^{15}N enrichment of the microbial biomass. Discs of Whatman GF/D filter paper (7 mm diameter) were cut using a paper punch and suspended on stainless steel wire placed inside a 120 mL vial. Immediately before commencing, 10 µL of 2.5 M $KHSO_4$ was pipetted on to each disc. Sufficient extract (obtained in Section 3.7.2) to give a total of 50–100 µg N in the solution, was placed in the vial. After adding 0.2 g MgO and 4 mm glass beads, the vial was immediately sealed and left for 6 d. The wire along with the filter paper disc was then removed and dried overnight at 40–50^{o}C; and transferred to tin capsules for analysis by Continuous Flow Isotope Ratio Mass Spectrometer (IRMS) (PDZ Europa Ltd, Crewe, UK) (Section 3.12).

$$MB^{15}N = \frac{(TN_F \times Atom\%_F)}{100} - \frac{(TN_{NF} \times Atom\%_{NF})}{100}$$

Equation 3.7

where;

$MB^{15}N$ = microbial biomass ^{15}N (µg ^{15}N g^{-1} dry soil)

TN_F = total dissolved N of fumigated soil (µg N g^{-1} dry soil)

$Atom\%_F$ = atom% of the total dissolved N of the fumigated soil (atom%)

TN_{NF} = total dissolved N of the non-fumigated soil (µg N g^{-1} dry soil)

$Atom\%_{NF}$ = atom% of the total dissolved N of the non-fumigated soil (atom%)

3.9 Water soluble C

For Chapter 5 (field study), soil samples were analysed for water soluble C (WSC) by extracting with DI water (1: 10, soil: water ratio) on an end-over-end shaker at 30 rev min^{-1} for 30 min followed by centrifuging the extractant at 3500 rev min^{-1} for 20 min, and filtering (through 0.45 µm cellulose nitrate membrane filter) into 30 mL plastic containers before storage at 4^{o}C until analysis (Ghani *et al.* 2003). The filtered samples were analysed for WSC using a Shimadzu Total Organic Carbon Analyser TOC 5000A (Shimadzu Oceania Pty Ltd, Sydney, Australia) fitted with a Shimadzu ASI-5000A autosampler. Equation 3.8 was used to calculate the concentration water soluble carbon in the filtered samples.

$$\mathrm{WSC} = \frac{(\mathrm{TOC} \times \mathrm{V})}{\mathrm{M_d}}$$

Equation 3.8

where;

WSC = water soluble carbon (µg g^{-1} dry soil)

TOC = total organic carbon concentration of extract (µg mL^{-1})

V_F = volume of solution (DI water + soil moisture) (mL)

M_d = mass of oven dry soil (g)

3.10 Headspace gas sampling and analyses

The closed-chamber technique (De Klein *et al.* 2003) was used for all N_2O and CO_2 field measurements (Chapters 5 and 8). The details of the headspace chambers are provided in the later chapters. In Chapters 6 and 7 (laboratory studies), an infrared gas analyser (IRGA) was used to measure instantaneous CO_2 fluxes from the soil cores. At each gas sampling event, a portable soil respiration chamber (SRC) was placed directly onto each PVC (polyvinyl chloride) container that was connected to an infrared gas analyser (SRC–1 and EGM–3, PP Systems, Hitchin, UK; Figure 6.2) and the emissions were determined over a 2 min period. Variable climatic conditions in the field studies limited the use of IRGA and interpretation of data in field conditions, hence for the field studies, CO_2 emissions were determined using gas chromatographic analyses.

Common to all experiments were gas sample vial sizes and analyses. For Chapters 6, 7 and 8, a gas-tight, screw-on PVC lid containing a rubber septum was attached to the top of the

PVC containers (internal diameter 8.0 cm, 4.5 cm depth, total height 10 cm; part no. D05880, iPlex pipelines, New Zealand). For the field experiment (Chapter 5), steel ring bases were pre-installed to a soil depth of 10 cm. These were covered with insulated, steel chambers (45 cm diameter, 12 cm height) which contained a rubber gas sampling septum.

At each gas sampling event, gas samples (8 mL) were collected at 0, 10 and 20 min for the laboratory experiments (Chapters 6 and 7) and 0, 30, and 60 min for the field studies (Chapters 5 and 8), after lid closure using a hypodermic needle, attached to a 20 mL glass syringe via a three-way tap, and placed in 6 mL Exetainer® vials (Labco Ltd, High Wycombe, UK); the over pressurisation prevented ambient air diffusing into the Exetainer®. The gas vials were reduced to ambient atmospheric pressure immediately prior to analysis using a double-ended needle to release the extra pressure into a beaker of water. Samples of ambient air were also collected at each experimental location before commencing headspace gas sampling.

For N_2O–^{15}N gas sample (15 mL) collection in the field studies (Chapters 5 and 8), the headspace chamber was kept closed for another 3 h after gas collection for N_2O-N prior to taking a 15 mL sample. Gas samples were transferred to pre-evacuated, 12 mL Exetainers®, the over pressurisation prevented ambient air diffusing into the Exetainer®. Analyses were carried out as in Section 3.12.3.

3.11 Gas chromatographic analysis

The gas samples were analysed on an automated SRI 8610 gas chromatograph (GC) (SRI Instruments, Torrance, CA, USA) interfaced with a Gilson 222XL liquid autosampler, configured as in Clough *et al.* (1998) and similar to the configuration used by Mosier and Mack (1980). To enable gas analyses a purpose-built double concentric injection needle replaced the liquid sample sipper on the autosampler, allowing rapid purging of the gas sample for injection. A 1 m long pre-column preceded a 6 m long analytical column, both 3 mm OD stainless steel packed with Haysep Q. An automated 10-port gas-sampling valve on the GC sent the oxygen-free N_2 carrier gas (40 mL min^{-1}) through both the pre-column and analytical column in series (in inject mode) or back-flushed the pre-column. At the posterior end of the analytical column a 4-port gas-sampling valve was synchronised to send the gas stream to the detector. The electron capture detector (ECD) was also supplied with a 10% CH_4/Ar – 'makeup gas' to enhance the detector's response to N_2O and to effectively eliminate any complications due to varying CO_2 levels in the stored headspace samples (Zheng *et al.* 2008). The 'makeup gas' flow was regulated at 7 mL min^{-1}.

3.11.1 Nitrous oxide

Nitrous oxide concentrations were analysed using a ^{63}Ni electron capture detector (ECD) at 310°C. Each batch of N_2O samples was preceded by a series of standards (1.2, 2.5, 5.8 and 9 µL L^{-1}) and interspersed with 1.2 µL L^{-1} reference standards. Nitrous oxide flux determination was performed by using two concentration values on each occasion and one of those values at time 0 was the mean of three background concentrations. By employing this method of flux calculation, the departure of most of the flux values from linearity was within the analytical uncertainty (2–5%) of the gas chromatograph. Emissions were calculated using Equation 3.9:

$$F_{N2O} = \frac{\left[(C_1 - C_0) \times V_h \times C_L \times P \times MW_{N2O-N} \times C_{\mu g})\right]}{(R \times T \times t \times A_s)}$$

Equation 3.9

where;

F_{N2O} = N_2O flux (µg N_2O-N m^{-2} h^{-1})

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

C_0 = background N_2O concentration at time 0 (µL L^{-1}; n = 3)

V_h = headspace volume (L)

C_L = conversion factor for µL to L [0.000001 L μL^{-1}]

P = atmospheric pressure [1 atm]

MW_{N2O-N} = molecular weight of N_2O-N [28.01 g mol^{-1}]

$C_{\mu g}$ = conversion factor for g to µg [10^6 µg g^{-1}]

R = Universal gas constant [0.0821 L atm K^{-1} mol^{-1}]

T = temperature in Kelvin (K)

t = sampling time (h)

A_s = soil surface area (m^2)

3.11.2 Carbon dioxide

The gas sample was separated on the GC column and the CO_2 was subsequently converted to CH_4 in a methaniser by a Ni catalyst at 380°C and measured with a flame ionisation detector (FID). Preceding each batch of samples a series of CO_2 standards (0.2, 0.5, 1, 2 and 3% v v^{-1}) were analysed, and used to create a standard curve. Interspersed with the samples were 1% reference standards. Fluxes of CO_2 were determined in a similar fashion to N_2O emissions using Equation 3.10:

$$F_{CO2} = \frac{\left[(C_1 - C_0) \times V_h \times C_L \times P \times MW_{CO2-C} \times C_{\mu g})\right]}{(R \times T \times t \times A_s)}$$

Equation 3.10

The terms used here are same as in Equation 3.9 except for the following.

F_{CO2} = CO_2 flux (μg CO_2-C m^{-2} h^{-1})

MW_{CO2-C} = molecular weight of CO_2-C [12.0107 g mol^{-1}]

3.12 Isotopic ^{15}N enrichment of plant material

3.12.1 Isotope enrichment

Ammonium sulphate ($(NH_4)_2SO_4$), enriched with ^{15}N (10.4 atom%; Isotec Inc., Matheson, USA) was added to ryegrass plants (Chapter 8) to achieve a final ^{15}N enrichment of 5.4 atom% in the growing herbage. All isotopically labelled herbage samples and ^{15}N enriched soil samples were analysed (Section 3.12.2) on a Continuous Flow Isotope Ratio Mass Spectrometer (IRMS) (PDZ Europa Ltd, Crewe, UK).

3.12.2 Soil and herbage analyses for ^{15}N enrichment

Ground (< 200 μm) and dried samples (either soil or plant) were weighed into tin capsules, sealed and loaded into the autosampler of the PDZ Europa (Crewe, UK) GSL elemental analyser. A reference material was also weighed out in the same manner to match the composition and abundance of the samples. The samples were combusted in the presence of oxygen to convert the N in the material to a NO_x mixture of gas. The resultant NO_x species are reduced to N_2 by passing through a packed copper column at 600^{o}C before being resolved on a gas chromatograph packed column and passed into the PDZ Europa 20-20 IRMS, where the ion beams of the N_2 species 28, 29 and 30, were measured and the blank corrected ratios of 29/28 and 30/28 were used to determine ^{15}N enrichment. Both references and samples were analysed in a batch process, whereby a number of samples are bracketed between references. The samples were measured with a duplication rate of 1 in 8.The working reference material used during analysis of all samples was EM-WHEAT ($\delta^{15}N_{air}$ = 1.66 ‰; Elemental Microanalysis Ltd, Devon, UK) which was also used to run dummy samples in order to check precision and accuracy. This working reference standard (EM-WHEAT) had been normalised against the international reference material IAEA-N-1 (Ammonium Sulphate, $\delta^{15}N_{air}$ = 0.4 ‰).

3.12.3 IRMS analyses of N_2O

Prior to analysis, the 12 mL Exetainers® were reduced to ambient pressure using a double-ended needle to release the extra pressure into a beaker of water (Section 3.10). The gas samples were automatically injected into the TGII trace gas system using a similar double concentric needle to that described earlier (Section 3.11) and the N_2O in the gas sample was concentrated by cryo-trapping and focusing. The gas was then transferred in the He carrier flow to the IRMS where the reduction column was bypassed and ion currents at *m/z* 44, 45, and 46 was integrated separately at an electron current of 300 μA. Total ion beams for each *m/z* were calculated relative to the blank signal obtained from leading and trailing zero observation periods before and after peak collection. The ion currents were compared to relative *m/z* ratios followed by calculation of the ^{15}N enrichment (Stevens *et al.* 1993). Each run was bracketed by N_2O standards (35 μL L^{-1}) and interspersed with 2 standards after every 9 samples analysed.

3.13 Statistical analysis

Statistical analysis of all data was performed using Minitab® (version 15.1; © 2006, Minitab Inc.). Gas emission data for each sampling was tested for skewness using the Anderson-Darling test and if required, the data were log-transformed to ensure normality. Analysis of variance (ANOVA) was performed with 95% confidence limits (P <0.05) to indicate the level of significance. Statistical significance was indicated using standard deviation (sd) or least significant differences (lsd). Pearson's correlation coefficient was used to test the relation(s) between various data in Chapter 4. Graphs were designed using SigmaPlot® (version 11.2.0.5; © 2008, SPSS Inc.).

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Chapter 4
Quantification of pre- and post-grazing litter-fall

4.1 Introduction

New Zealand's exports are dominated by dairy products from pastoral systems (Holmes *et al.* 2007). Until 2007, there were about 13,860 dairy farms in New Zealand with 3.4 million cows managed on 1.3 million ha, producing about 11,000 million litres of milk per annum while consuming 12 million tonnes of pasture dry matter (Holmes *et al.* 2007). Between 1989 and 2009, New Zealand's dairy cattle numbers have increased from 3.3 million to approximately 6.2 million (Statistics New Zealand 2011). The strength of New Zealand's pasture industries is based on the efficient utilisation of ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pasture, grazed *in situ*; pasture management strategies are planned to balance the seasonal variations in pasture supply and demand. New Zealand dairy farmers generally follow rotational grazing practices. Thus animals are offered a fresh area of pasture at regular intervals (i.e. rotation lengths). After every grazing event, each area has a period when it is not grazed. This allows simplified grazing control, conservation of pasture and minimises pasture wastage.

The rationale for this study came from observing dairy cows failing to ingest all their harvested pasture. While grazing, some freshly harvested herbage was seen to fall from the animal's mouth on to the soil surface (Figure 4.1). Hoof movement may also cause shearing-off of herbage, further adding to litter. These two pools of harvested but unconsumed herbage are collectively termed 'litter' and the process of its creation, 'litter-fall'. Previous studies have shown that plant leaf materials can contribute to N_2O emissions when in contact with soil but none of them have considered litter-fall effects on N_2O emissions in intensively grazing dairy pasture systems. From the literature review (Chapter 2), it is apparent that no study has so far attempted to quantify litter-fall resulting from single grazing event(s) with respect to grazing dairy cattle. Hence, the rationale for the present study was to quantify litter-fall during dairy cattle grazing events.

Figure 4.1 Dairy cow in the process of dropping freshly harvested pasture (litter) while grazing. The highlighted part shows litter just before landing onto the soil surface.

4.2 Materials and methods

4.2.1 Site description

A field survey was conducted on the Lincoln University Dairy Farm (LUDF), located near Lincoln, New Zealand (43°38'41" S, 172°26'31" E). The LUDF utilises a rotational grazing system based on white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) pasture, grazed by Friesian cows, with an effective grazing area of 159 ha split into 21 paddocks, producing 1630 kg milksolids ha^{-1} y^{-1} (further details in Table 4.1). The quantity of pre-grazing and post-grazing herbage as well as the litter-fall rate during grazing was measured on 30 occasions (as described in Section 4.2.2) during the 315-d-long milking season between December 2010 and April 2011. The period between grazing events, a grazing interval, ranged from 21 d (14 Sept 2010 – 1 March 2011) to 30 d (1 March – 31 May 2011). The cows grazed for 24 h in each fenced paddock at an average stocking density of 84 cows ha^{-1}. For context, a mean daily herbage dry matter intake rate (DMI, kg DM cow^{-1} d^{-1}) was determined using the farm's weekly measurements of pre- and post-grazing pasture herbage DM, the area grazed and the number of cows.

Table 4.1 Mean climatic parameters and management factors at the Lincoln University Dairy Farm during 2010–2011.

Climate parameters	
Maximum temperature	32^{o}C
Minimum temperature	4^{o}C
Days of screen frost	36 d
Mean average bright sunshine	2040 hours
Rainfall	666 mm
Evapotranspiration	870 mm

Management factors	
Irrigation per annum	450 mm
Urea fertiliser application	250 kg N ha^{-1} y^{-1} in split dressings of 50 kg N ha^{-1}
Effluent application	100 kg N ha^{-1}
Lime application	400 kg ha^{-1}
Stocking rate	4.1–4.4 cows ha^{-1}
Average daily dry matter intake*	~16 kg DM cow^{-1} d^{-1}

*Daily DMI was calculated by using the LUDF's farm data measured by a rising plate meter.

4.2.2 Litter collection procedure

Before grazing, litter was collected to determine if any remained from the previous grazing event. Three 1 m x 1 m quadrats were randomly placed in the paddock to be grazed, avoiding excreta patches. These areas were sampled using a vacuum pump (STIHL BG 75, Virginia, VA, USA). Afterwards, the standing herbage was trimmed with electric clippers (Oster Shearmaster®, USA) to a height of 0.5–1.0 cm. Post-grazing (POG), the procedure was repeated in five 1 x 1 m plots (Figure 4.2a). The litter collected using the vacuum pump was sieved (1 mm) to enable the separation and removal of dust, seeds and stones. By colour, the total POG litter-fall (POG_{Tot}) was segregated (Figure 4.2b) into fresh (green, POG_F) and senesced (brown, POG_S) material. The POG_F fraction was further segregated into ryegrass and clover. After litter collection, the remaining unharvested herbage (i.e. post-grazing residuals) was trimmed to a height of 0.5–1.0 cm (Figure 4.2c). The litter and standing herbage samples were dried at 65^{o}C and analysed for total C and N contents using standard procedures [Section 3.1; (Rowland and Roberts 1994)]. Dry matter and litter data were analysed using an analysis of variance (ANOVA) approach with two factors (paddocks and dates, where the date factor was nested within the paddock factor). Since these analyses

detected significant temporal differences (date effects) in addition to the paddock effects, each plot datum (combination of paddock and date) was used as an individual sample for calculating statistics.

Statistical analyses of dry matter and litter data were performed using the statistical software Minitab® (version 15.0). Statistical significance for all data was indicated using standard deviation (sd).

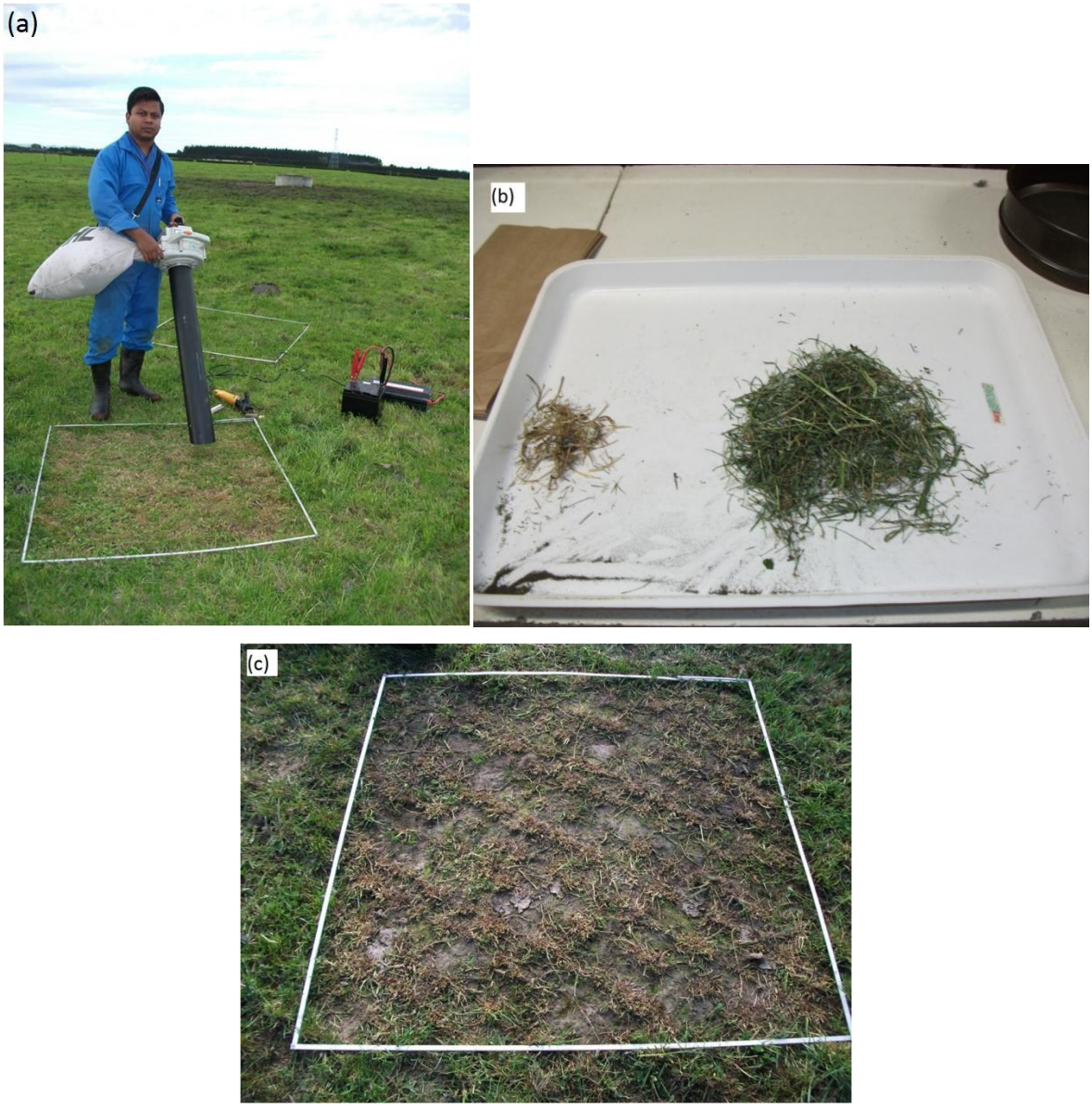

Figure 4.2 Litter-fall collection and litter separation, (a) litter-fall collection using a vacuum pump from a single 1 x 1 m quadrat following a grazing event, (b) The vacuumed litter separated to fresh (green) and senesced (brown) litter from the same quadrat, and (c) the same quadrat after clipping the remaining herbage to estimate post-grazing residual DM.

4.3 Results

Using the LUDF's weekly farm data [measured with a rising plate meter (F200, Farmworks Ltd., Fielding)] over the 315-d milking season, the mean daily DMI of the cattle was calculated to increase from 6 kg DM cow^{-1} in August, 2010, when the calves were born, up to 18 kg DM cow^{-1} in November and December, declining thereafter to 10 kg DM cow^{-1} by the end of May, 2011, when milking ceased (Figure 4.3b).

Pasture DM on-offer (pre-grazing) ranged from 1405 to 4086 kg DM ha^{-1} (Figure 4.3a) with no significant differences over time, with a mean pre-grazing DM value of 2516 ± 636 kg DM ha^{-1} (± sd, n = 90). The mixed pasture had C and N contents of 396 ± 29 and 28 ± 7 mg g^{-1}, respectively. Pre-grazing, no litter was recovered from the pasture surface at any time.

Post-grazing the pasture DM residuals ranged from 647 to 1849 kg DM ha^{-1} (Figure 4.3a) with no significant differences over time, and a mean value of 1167 ± 265 kg DM ha^{-1} (± sd, n = 150). Carbon and N contents of this post-grazing DM were 320 ± 72 and 20 ± 4 mg g^{-1}, respectively. Subtracting post-grazing residual DM from the pre-grazing dry matter gave an average apparent consumption of 1349 kg DM ha^{-1} equal to 53% of the pasture on offer pre-grazing. Post-grazing, the fresh litter-fall (POG_F) had a ryegrass: clover ratio of 9: 1 by dry mass. The POG_F litter-fall rate averaged 53 ± 24 kg DM ha^{-1} per grazing event with no differences due to sampling time (Figure 4.3a), and had average C and N contents of 398 ± 14 and 25 ± 5 mg g^{-1}, respectively. There were 12 grazing events per annum, thus the POG_F, N application rate equated to 1.3 ± 0.7 kg N ha^{-1} per grazing event, or 15.9 kg N ha^{-1} y^{-1}. The post-grazing senesced litter-fall (POG_S) averaged 19 ± 18 kg DM ha^{-1} per grazing event with mean C and N contents of 397 ± 16 and 15 ± 3 mg g^{-1}, respectively, and on an annual basis, POG_S litter deposited 3.5 kg N ha^{-1} y^{-1}.

Post-grazing, litter-fall did not correlate with either DMI, or pre- and post-grazing levels of DM. On average, the litter-fall rate was 4 and 5% of the cattle dry matter intake rate on a fresh or fresh + senesced basis, respectively. The actual DM consumption by the dairy cows accounted for 49% of the herbage on-offer, when litter-fall was accounted for in the calculations. This indicated that 4% of the DMI was lost as litter-fall.

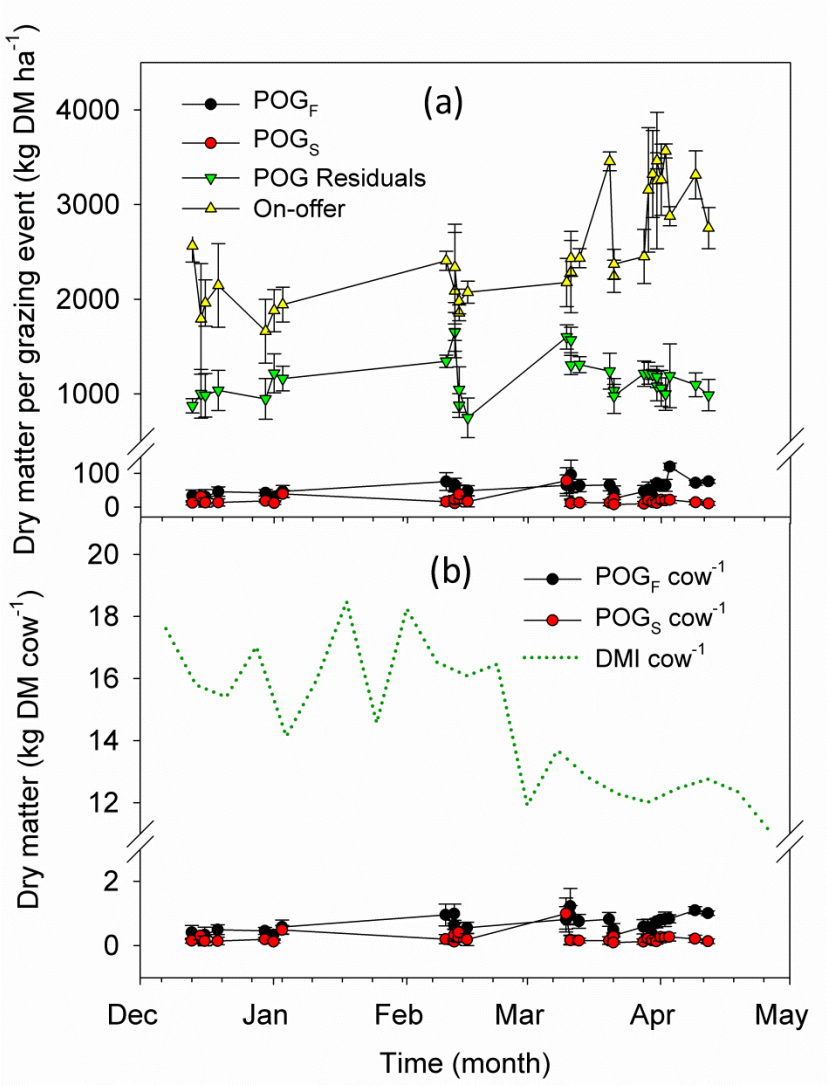

Figure 4.3 Herbage on-offer and litter-fall rates for 30 grazing events over an annual lactation period, (a) herbage dry matter and litter-fall per grazing event, (b) DMI and litter-fall on an individual cow basis. POG_F and POG_S are the post-grazing-fresh (green) and -senesced (brown) litter, respectively. POG Res is the residual dry matter remaining after grazing, and 'on-offer' is the dry matter present at start of grazing. DMI denotes the calculated daily dry matter intake of a cow. Data are mean ± sd.

4.4 Discussion

The lack of any differences, over time, in either the DM on-offer or post-grazing DM residuals demonstrated that the aims of the LUDF rotational grazing management were achieved. Prior to grazing, there was no litter on the soil surface. Thus, litter-fall from the previous grazing event(s) had decomposed, releasing C and N to the soil (Sanaullah *et al.* 2010). This study has shown, for the first time, that in intensively grazed dairy pastures significant litter-fall occurs. In this study it accounted for 4% of what was the apparent DM consumption of the cows. While DM on offer did not affect litter-fall rates in the grazing system studied here, it is possible that grazing management, animal species and pasture dynamics may affect litter-fall rates. For example, a study of grazing cattle (Soder *et al.* 2009) found DM per bite differed with sward structure. It may be that bite size could also affect litter-fall rates. While the litter-fall rate did not vary with cow DMI, on average, it was equivalent to 4 and 5% of a cow's apparent DMI on a fresh and fresh + senesced basis, respectively. The examination of animal species and sward structure effects and interactions on litter-fall are the outside the scope of this study but potential influences on litter-fall clearly warrant further study.

Naeth *et al.* (1991) measured litter-fall following full season grazing events (ranging from 1–5 months) by beef cattle in prairie- and fescue-grasslands (dominated by *Fescuta, Agropyron* and *Stipa* sp.) in Canada, and found that season and grazing intensity significantly affected the amounts of litter; it decreased with increasing grazing intensity. Mapfumo *et al.* (2002) investigated litter C and N pools after beef heifers grazed perennial pastures for 1–3 times under light and heavy grazing intensities which included smooth bromegrass (*Bromus inermis* L.), meadow bromegrass (*Bromus riparius* Rhem.), and the annual grass, winter triticale (*Triticosecale* W.). In these studies litter C and N pools decreased with increasing grazing intensity; however, the grazing density was relatively high (270 beef heifers ha^{-1}) when compared to the present study (84 dairy cows ha^{-1}). Lodge *et al.* (2006) manually collected litter after full season grazing events (4–6 weeks continuous grazing) of Merino wethers which declined seasonally and ranged from 32 to 66 kg DM ha^{-1} at light and heavy grazing intensities using forage grasses in New South Wales, Australia. They recorded the highest value of 508 kg DM ha^{-1} for Manilla grass which is comparable to the annual litter-fall value of 864 kg DM ha^{-1} y^{-1} measured in this study.

Campanella and Bisigato (2010) reported litter-fall rates of 60–160 kg ha^{-1} y^{-1}, collected on a monthly basis using litter traps from arid, extensive rangelands that adopted set-stocked sheep grazing (0.11–0.14 sheep ha^{-1}) and were dominated by forage and perennial grasses such as *Larrea divaricata*, *Chuquiraga hystrix*, *Stipa tenuis* and *Poa ligularis*. Carrera

et al. (2008) reported litter-fall rates of 260–310 kg ha^{-1} y^{-1} from similar rangeland systems including shrubs (*Larrea* spp. and *Stipa* spp.) grazed by sheep (0.14 sheep ha^{-1}).

In these last two studies discussed above, annual litter-fall values were relatively lower compared to the present study which decreased with increasing stocking rates with stated reasons being that higher stocking rates led to higher removal of green herbage. In both studies, either the litter-fall rate was not measured following each grazing event (the rates reported are cumulative values) or the animals were set-stocked over time. It is expected that some litter must have decomposed already, hence the lower litter-fall rates.

In all the studies reported above, the plant species on-offer were prairie/forage grasses/shrubs which are managed differently and which have different morphological and growth characteristics when compared to clover-ryegrass pastures. Furthermore, the grazing animals in the past studies were not dairy cattle (beef heifer/sheep). It is recognised that litter-fall has the potential to contribute to nutrient cycling but no consideration has been given to N_2O emissions resulting from pasture litter decomposition in any previous studies.

Typical fertiliser rates in New Zealand and Australian clover-ryegrass pastures are 100−150 kg N ha^{-1} y^{-1} in split applications of 25–50 kg N ha^{-1}. The annual N application from litter-fall in this study was ~16 kg N ha^{-1} y^{-1} which may not be as high as a fertiliser application but is comparable to a split application rate.

Forster *et al.* (2007) note that the nitrous oxide (N_2O) molecule is a potent greenhouse gas (GHG). It is currently also the dominant stratospheric ozone depleting substance (Ravishankara *et al.* 2009) . Intensively managed agricultural soils are a significant anthropogenic global source of N_2O with grazed pasture a major component of this, via ruminant excreta and fertiliser (De Klein *et al.* 2003; Mosier *et al.* 1998; Oenema *et al.* 1997). The current IPCC inventory guidelines includes N from crop residues (aboveground and belowground), N from BNF and from residues that occur during pasture or forage crop renewal (IPCC 2006). It is clear that the IPCC methodology does NOT account for the potential emission of N_2O from the decomposition of pasture plant litter (i.e. resulting from litter-fall). Presumably this omission has occurred due to a lack of recognition of the concept of litter-fall and its potential contribution to N_2O emissions.

The total C and N contents of the litter collected in this study are, however, comparable to some cropping residues that are usually incorporated in soil to improve soil quality and fertility (not stubbles e.g. wheat and maize); litter therefore is highly likely to contribute to C and N cycling in pastures and hence there is a need to examine it further with respect to litter decomposition rates and N_2O production.

4.5 Conclusion

Some herbage harvested by dairy cattle is not ingested but ends up on the soil surface in the form of litter-fall. For the first time, this study has quantified litter-fall in intensively grazed dairy pastures. On average, litter-fall rate equated to 4 and 5% of the cattle dry matter intake rate on a fresh or fresh + senesced basis, respectively. Litter-fall was not related to dry matter intake of animals or post-grazing residuals during the measurement period of April 2010–2011. The N content of the post-grazing fresh litter was 25 mg g^{-1} and this at 12 grazing events per paddock per year, equated to an N application rate of 15.9 kg N ha^{-1} y^{-1}, comparable in magnitude to a typical fertiliser application. It is hypothesised that during its decomposition it may contribute to N cycling and N_2O emissions. The results of this study warrant further investigation of the fate of litter-fall under field conditions and the potential to contribute to N_2O emissions.

Chapter 5
Plant-derived N_2O emissions resulting from animal treading in pastures

5.1 Introduction

Intensively managed grazed pastures are significant sources of N_2O due to regular anthropogenic N inputs (fertiliser and BNF), animal excreta and soil compaction resulting from animal treading (Oenema *et al.* 1997). Emissions are exacerbated under wet and saturated soil conditions (De Klein *et al.* 2006b; Luo *et al.* 2008a). Animal treading can create anaerobic soil conditions as a result of compaction, often with animal excreta present which provides abundant C and N substrates. However, studies on emissions involving animal treading in the absence of animal excreta or fertilisers are scarce (Menneer *et al.* 2005b). Menneer *et al.* (2005b) showed that animal treading, in the absence of animal urine and dung, produced 3–6 fold higher N_2O emissions 8 d after severe treading compared to nil-treading. They reasoned the higher emissions were due to treading causing compaction of soil and thereby reducing soil aeration which resulted in higher denitrification rates. Moreover, they reasoned that due to treading, plant growth was reduced which increased NH_4^+–N and NO_3^-−N availability (due to reduced N uptake) for soil microorganisms responsible for N_2O production. However, consideration was not given to the possibility that N embodied in pasture leaf tissues was released into the soil to contribute to N_2O emissions and/or inorganic-N pools. No previous studies have reported N_2O emissions as a result of animal treading releasing N embodied in the herbage.

Results from Chapter 4 revealed that intensively grazed dairy cattle can produce significant amounts of litter-fall. During and after grazing events, this litter along with the unharvested herbage, could potentially become incorporated into the soil due to animal treading, especially under winter conditions when saturated soil conditions prevail. This partially incorporated herbage may decompose and contribute to N_2O emissions. The following experiment was designed to examine the effect of animal treading on N_2O emissions and potential herbage effects.

It was hypothesised that trodden herbage would act as a source of C and N for the microorganisms and contribute to N_2O emissions.

5.2 Materials and methods

5.2.1 Experimental site and preparation

Two experimental sites were located on two separate paddocks at the Lincoln University Dairy Farm (LUDF) (43°38.59'S, 172°26.21'E; elevation 13 m). The soil at both sites was a strongly gleyed, Temuka clay loam [Typic Orthic Gley; (Hewitt 1998)] with impeded subsoil drainage. The pasture species were perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) which were grazed regularly by dairy cows (Section 4.2.1, Table 4.1). To avoid antecedent effects of grazing animals, an area of pasture (approximately 15 m × 20 m) was surrounded by an electric fence six months prior to the start of the experiment. Part A (Experiment 1) was performed at paddock 1 in July-October, 2010. This paddock was sown in February, 2007 with a mixture of 'Arrow' and 'Alto' perennial ryegrass cultivars. Part B (Experiment 2) was performed at paddock 2 in April-May, 2011 which was sown in March, 2003 with 'Bealey' perennial ryegrass and managed in an identical fashion to paddock 1. The stable isotope technique (^{15}N-enriched fertiliser) was used in Part B of this experiment to better understand the source(s) of the N_2O emissions i.e. to separate soil-derived- and herbage-derived N_2O emissions.

5.2.2 Experimental design and treatments

5.2.2.1 Part A (no fertiliser addition)

The experimental design was a randomised block design with 4 treatments; two levels of herbage (present/absent i.e. H1/H0) and two levels of treading (present/absent i.e. T1/T0), each replicated 5 times (Figure 5.1). The treatment combination – H1T0 was the control for part A. For soil gas emission measurements, headspace chamber bases (45 cm diameter, stainless steel), which protruded 12 cm into the soil, were installed, in such a way that each sub-plot was 1 m apart and 1 m away from the fence. These chamber bases contained an annular water-filled trough which provided a seal when chambers were placed in position when, gas measurements were taken. During gas sampling events, stainless steel chambers (insulated with polystyrene foam; 45 cm diameter, 10 cm high) created a 19.1 L headspace when placed on the bases. The chamber was placed on the annular water-filled trough, creating a gas-tight seal. Further non-chamber areas were set-up and treated in an identical fashion but used for soil sampling and pH measurements. The gas chambers were of the same dimensions in both parts A and B.

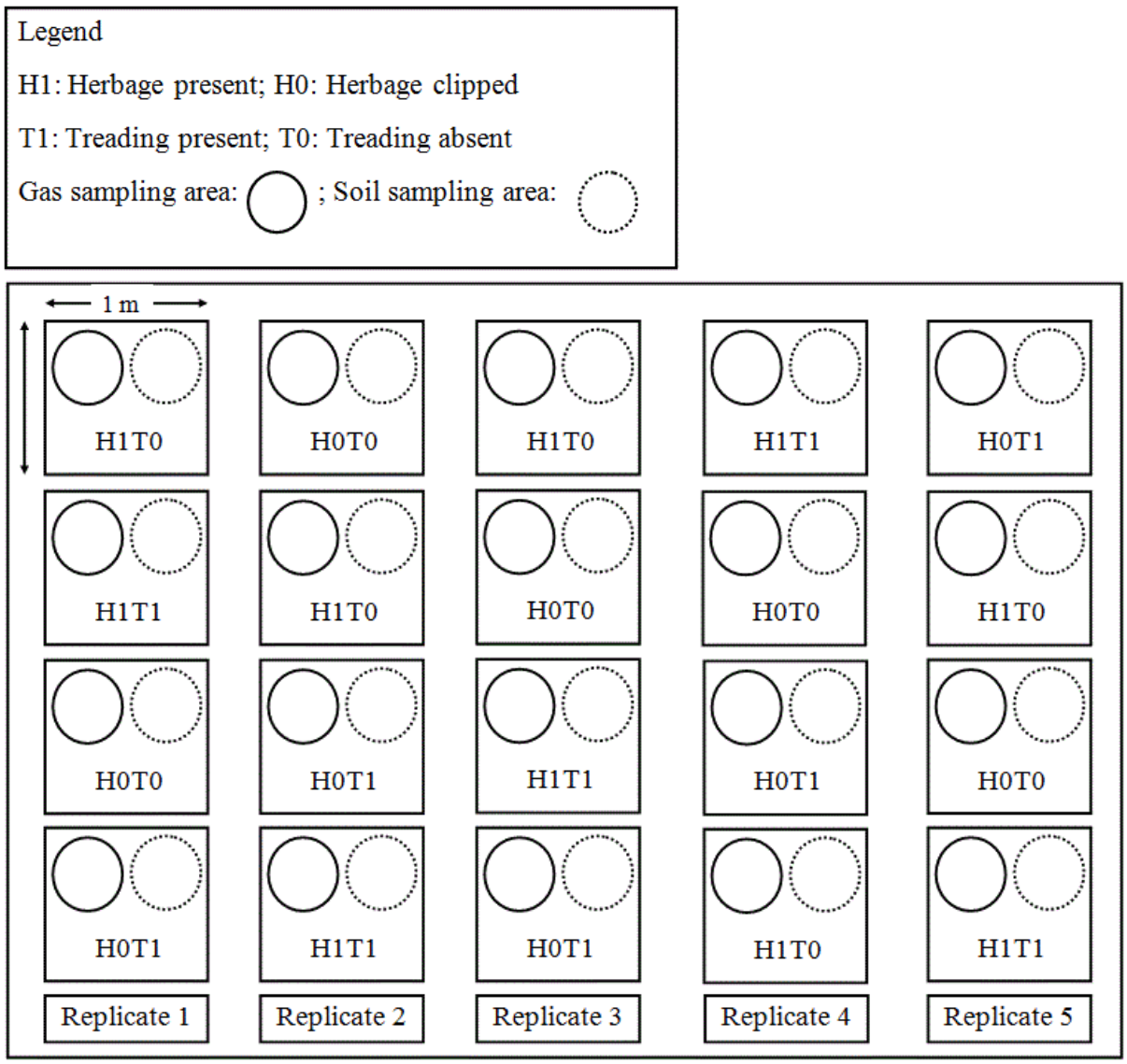

Figure 5.1 Field layout of paddock 1 for part A showing the position of the gas chambers and soil sampling plots for the treading and herbage treated plots.

5.2.2.2 Part B (^{15}N-labelled fertiliser addition)

Part B was also a randomised block design with the same treatments as in part A replicated 5 times (Figure 5.2). Each subplot received 50 kg N ha^{-1} in the form of ^{15}N-double-labelled ammonium nitrate [($^{15}NH_4{}^{15}NO_3$), Section 5.2.3.2 below] except an additional 'control' plot was included where no fertiliser or treading were imposed. Other parameters such as headspace volume, soil type and pasture species were identical to part A.

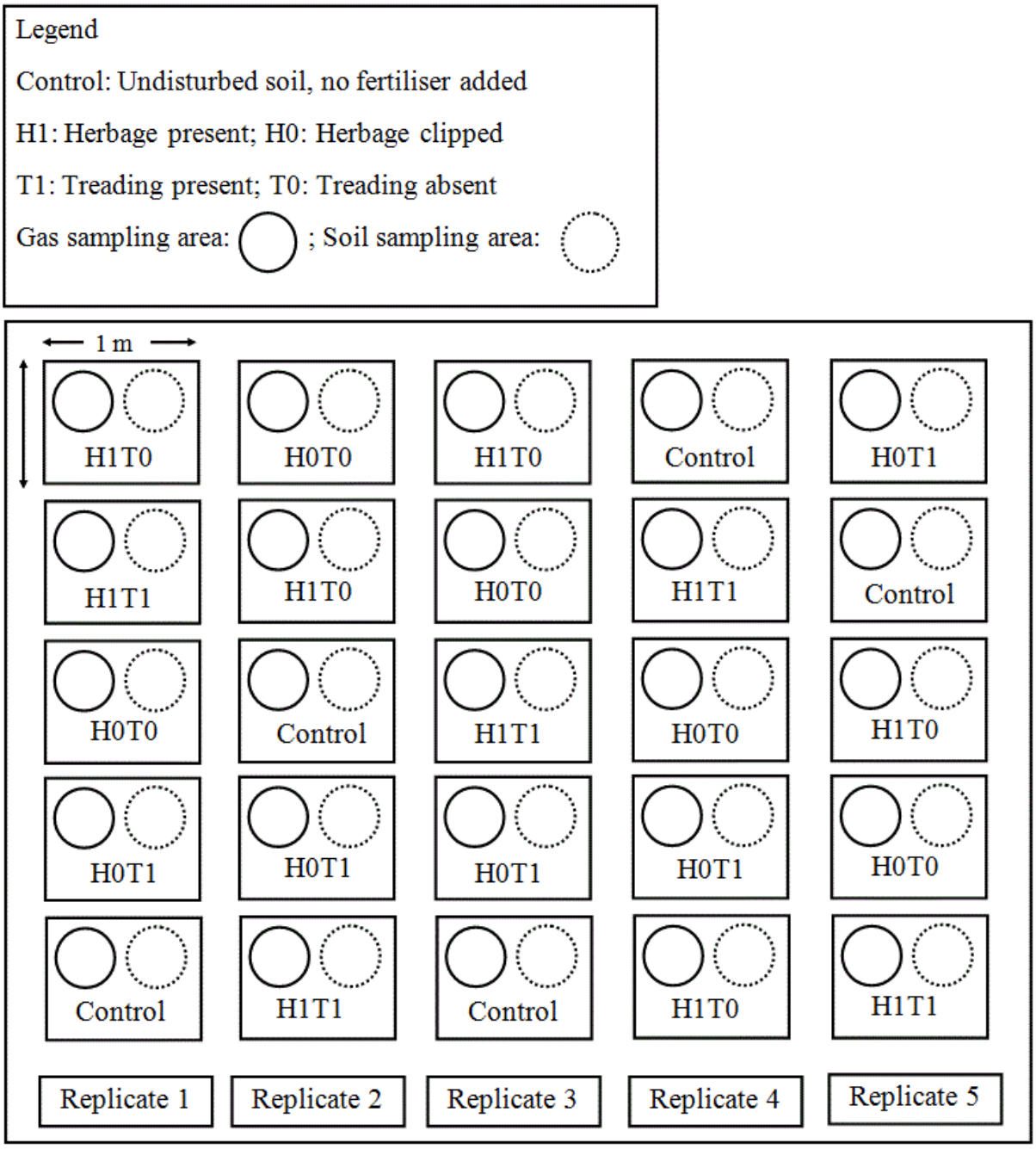

Figure 5.2 Field layout of paddock 2 for part B showing the position of the gas chambers and soil sampling plots for the treading and herbage treated plots.

5.2.3 Treatment application

For both parts A and B, prior to the start of the experiments, the paddocks were mown to a herbage height of 10 cm and then left undisturbed for 1 month. Prior to imposing the treatments paddock 1 had 1584 ± 110 kg DM ha^{-1} (n = 10) on-offer. At paddock 2, corresponding values were 1636 ± 335 kg DM ha^{-1} (n = 10). This herbage height simulated post-grazing residual DM in pastures which ranged from 700 to as high as 1600 kg DM ha^{-1} at the LUDF during 2010–2011 (Chapter 4).

5.2.3.1 Part A (no fertiliser addition)

For the H0 treatment, the herbage within the subplots was clipped using electric clippers (Oster Shearmaster®, USA) to a height of 0.5–1.0 cm (Figure 5.3a) immediately prior to imposing the treading treatments and then dried at 65°C for 48 h (to determine kg DM ha^{-1}), ground and analysed for total C and N contents using standard procedures [(Rowland and Roberts 1994); Section 3.1].

For imposing the treading treatment (T1), a mechanical hoof [hoof print area 90 cm^2; (Di *et al.* 2001)] was used that mimicked the hoof of an adult (2 year old) Friesian cow (weighing 450 kg), delivering a pressure of 220 kPa on the soil surface. The T1 treatment received 60 hoof-treads (377 hoofs m^{-2}) within the soil surface of each subplot that equated to 339.6% of the total area (Figure 5.3b).

5.2.3.2 Part B (^{15}N-labelled fertiliser addition)

At paddock 2, immediately prior to imposing treading treatment, ^{15}N-labelled $^{15}NH_4{}^{15}NO_3$ enriched with 10 atom% ^{15}N, at an N rate of 50 kg N ha^{-1} in a water solution at 300 mL $subplot^{-1}$, was added to all subplots except the control treatments (Figure 5.2). The herbage and treading treatments were then imposed in a similar fashion as part A. Additional control plots were introduced which did not receive fertiliser-N or treading treatments.

5.2.4 Soil-, herbage- and gas-sampling and micrometeorological measurements

General soil properties were tested by a commercial laboratory (Hill Laboratories, Hamilton, New Zealand). Thirty soil cores were taken from each paddock (1 and 2), prior to the start of each experiment. These were collected at a depth of 0 to 7.5 cm from the experimental site, bulked, and submitted for individual analysis (n = 1). For part A, soil analyses for inorganic N and WSC were performed on 11 occasions (days 1, 2, 3, 4, 6, 11, 14, 17, 28, 38 and 47) after treatment application while for part B these analyses were also performed on 11 occasions (days 1, 2, 3, 4, 6, 9, 12, 13, 16, 23 and 30) after treatment

application. For part B, enrichment of ^{15}N in soil inorganic N pools was determined using the diffusion technique of Brooks *et al*. (1989) (Section 3.8.2) for days 1 to 4 after treatment application (depending on the soil inorganic N concentrations and availability of KCl extracts).

Field-moist soil samples were taken in duplicate, using a soil corer (7.5 cm deep × 2.5 cm diameter), from each treated subplot (4 replicates treatment^{-1}) and analysed for inorganic N, ^{15}N enrichment and WSC (Sections 3.6, 3.8.2 and 3.9, respectively). Surface soil pH was determined on all gas sampling occasions (Section 3.3). Soil bulk densities (5 replicates) were determined (Section 3.4) before and after imposing the treading treatments. Soil temperature, air temperature and rainfall data were attained from a meteorological station, 1 km away from Lincoln University. The harvested herbage from the 'herbage clipping' treatment was dried at 65^{o}C and analysed for total C and N contents using standard procedures [Section 3.1; (Rowland and Roberts 1994)].

Soil N_2O sampling was performed on 17 occasions for both parts A and B over experimental periods of 47 and 30 d, respectively. On each gas sampling event, 10 mL gas samples were manually drawn using glass syringes fitted with three-way taps and compressed into 6 mL Exetainer® vials (Labco Ltd, High Wycombe, UK) at 0, 30, and 60 min, after positioning the headspace cover. The gas samples were analysed, within 48 h, for N_2O and CO_2 using gas chromatography (Sections 3.10 and 3.11). For part B, three hours after gas sampling, a further 15 mL headspace gas sample was drawn into 12 mL Exetainer® vials and equilibrated to atmospheric pressure, before analysis for N_2O-^{15}N enrichment using IRMS (Sections 3.10 and 3.12.3, respectively).

5.2.5 Statistical analysis

Gas emission data on each gas sampling occasion and the cumulative emissions were tested for normality using the Anderson-Darling test and skewed data was log transformed [ln(flux+1)] (Press *et al.* 1989). Analysis of variance (ANOVA) was performed with 95% confidence limits (P <0.05) to indicate the level of significance. Treatment differences were calculated using Tukey's test. Statistical analyses were performed using Minitab (version 15.1; © 2006, Minitab Inc.).

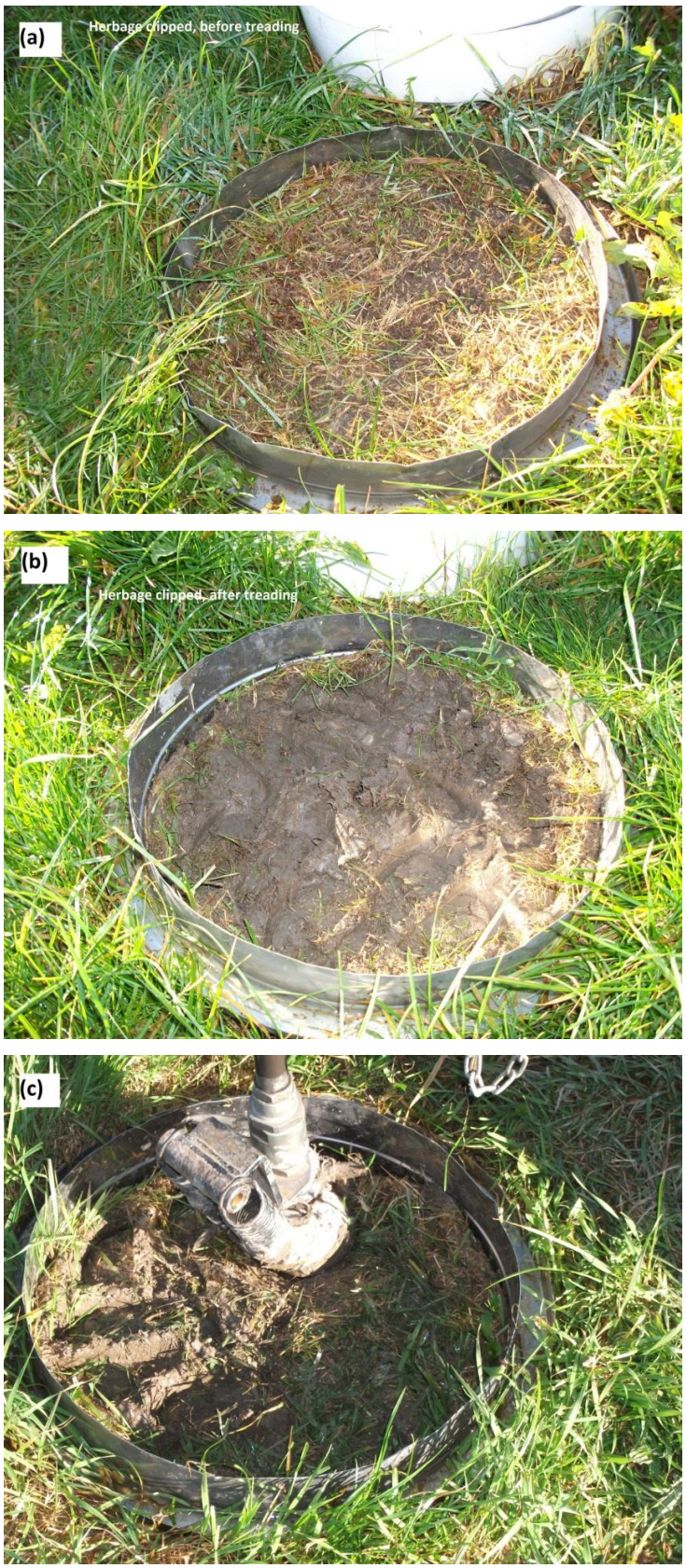

Figure 5.3 Photograph showing (a) the 'herbage clipped-treading present' (H0T1) treatment after herbage clipping, (b) after the H0T1 treatment had received the treading treatment, and, (c) the treading treatment being imposed in the 'herbage present-treading present' (H1T1) treatment using the mechanical hoof.

5.3 Results

5.3.1 Part A – no fertiliser addition

5.3.1.1 Soil properties and meteorological data

Chemical properties of the soil at the experimental site (paddock 1) are shown in Table 5.1.

Table 5.1 Chemical properties of the soils used during the study at paddock 1.

Soil properties	Paddock 1 (July, 2010)
pH (1: 2)	6.0
Total C (g kg^{-1})	39.0
Total N (g kg^{-1})	4.1
Anaerobically mineralisable N (µg g^{-1})	162
Available N (kg ha^{-1})	198
Olsen P (mg kg^{-1})	25
Potassium ($cmol_c$ kg^{-1})	0.58
Calcium ($cmol_c$ kg^{-1})	10.9
Magnesium ($cmol_c$ kg^{-1})	2.81
Sodium ($cmol_c$ kg^{-1})	0.29
Cation exchange capacity ($cmol_c$ kg^{-1})	20
Total base saturation (%)	73

During the experimental period (47 d) at paddock 1, day 38 recorded the highest rainfall of 23.3 mm, followed by day 8 and day 21 with 3.7 and 7.1 mm, respectively. The average daily air temperature ranged from 1.2 to 14.7°C and the average daily soil temperature ranged from 2.4 to 9.3°C over the same period (Figure 5.4).

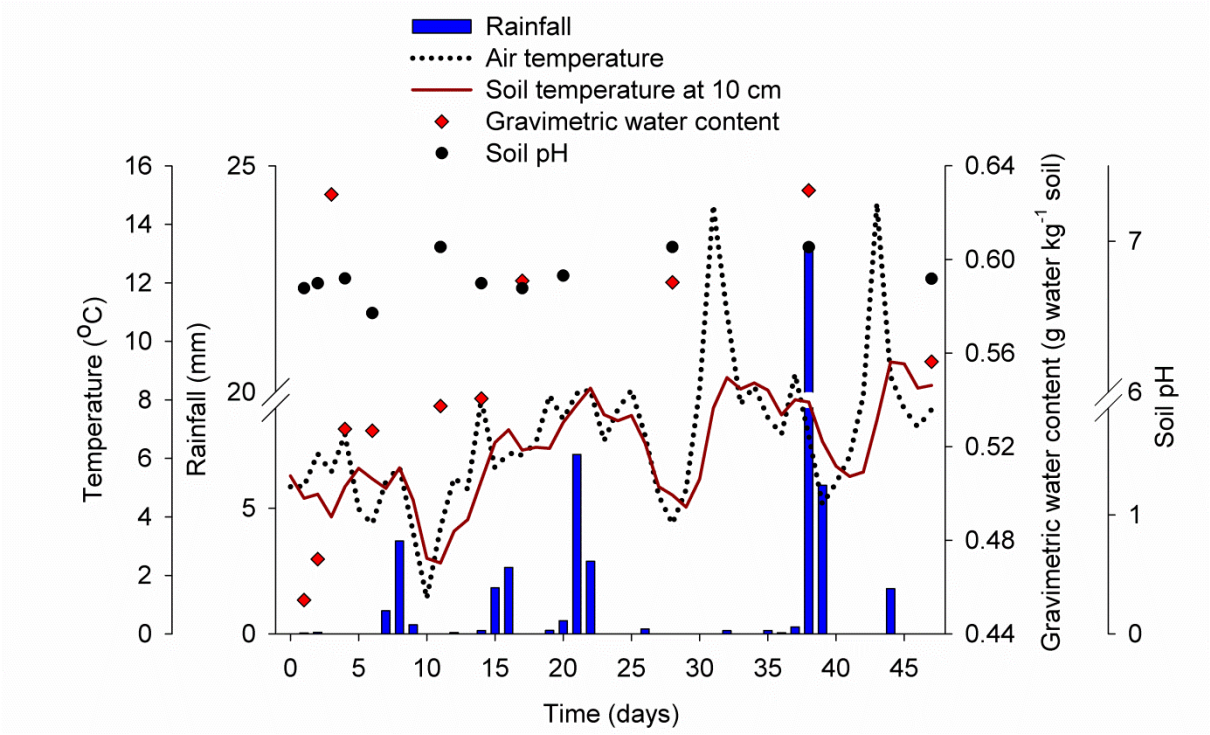

Figure 5.4 Meteorological data during the experimental period – 1 July to 17 August, 2010, at paddock 1 for Part A. Rainfall, air temperature and soil temperature are daily average values. Gravimetric water content was measured at 7.5 cm soil depth.

5.3.1.2 Herbage on-offer and regrowth

Nitrogen and C contents of the herbage that was clipped from the H0 plots at the start of the experiment contained 37 ± 5 mg g^{-1} and 388 ± 9 mg g^{-1} (n = 10), respectively. Three months after treading treatment, herbage regrowth from the trodden plots was significantly lower (P <0.05; n = 5) than the non-trodden plots with 1333 ± 273 and 2257 ± 135 kg DM ha^{-1}, respectively. Corresponding values for the nil-herbage treatment (H0) after 3 months averaged 808 ± 144 and 937 ± 202 kg DM ha^{-1} and did not differ significantly.

5.3.1.3 Soil pH, soil bulk density and soil gravimetric water content

Treatments had no significant effect on soil pH throughout the experimental period and average pH values ranged from 6.4–7.2 units (n = 20 on each occasion).

Gravimetric soil water content (θ_g) was significantly affected by the treatments until day 6 with higher values (P <0.05) in the H0T0 and H1T1 treatments ranging from 0.59−0.72 g water g^{-1} dry soil and lower values (0.43–0.55 g g^{-1}) for H0T1 treatment over the same period. By assuming an average bulk density of 0.87 Mg m^{-3}, corresponding values of volumetric soil water content for H0T0 and H1T1 treatments ranged from 0.51–0.63 m^3 water m^{-3} soil and 0.37–0.48 m^3 m^{-3} for the H0T1 treatment.

No significant treatment differences occurred in soil bulk density values which, averaged over all treatments were, 0.74 ± 0.14, 1.0 ± 0.10, 1.07 ± 0.05, and 0.97 ± 0.07 Mg m^{-3} (mean ± sd; n = 12) in the 0–3, 3–6, 6–12, and 0–12 cm depths, respectively.

5.3.1.4 Soil inorganic N and WSC

Soil NH_4^+–N concentrations were significantly higher in the control (H1T0) than the other treatments until day 6 and from day 11 onwards, concentrations did not differ due to treatments until day 47 (Figure 5.5a). On day 1, NH_4^+–N concentrations were 14.8 ± 1.9 = 13.5 ± 1.7 > 11.4 ± 0.8 = 9.8 ± 2.2 µg g^{-1} dry soil (P <0.05; mean ± sd; n = 4) for the H1T0, H1T1, H0T1 and H0T0 treatments, respectively. Concentrations from both treading treatments did increase on day 2 but did not differ from the control treatment due to high variability. During days 1 to 6, a significant herbage effect was observed on days 1, 3 and 4 with higher values in the absence of herbage. During the same period, the treading effect became significant on days 2, 4 and 6 with higher concentrations from non-trodden treatments. Maximum concentrations were observed on day 3 with minimum and maximum values of 8.5 ± 1.3 and 41.2 ± 27.9 µg g^{-1} in the H0T0 and H1T0 treatments, respectively.

Soil NO_3^-–N concentrations were higher from the control treatment on most soil sampling occasions (Figure 5.5b). The treading treatment lowered (P <0.001) soil NO_3^-–N concentrations over the entire experiment irrespective of the presence or absence of herbage. Significant herbage and interaction effects were observed on days 38 and 47 with higher NO_3^-−N concentrations recorded in the presence of herbage. Average soil NO_3^-–N concentrations over the experimental period for the H0T1 and H1T1 treatments were 3.5 and 3.5 µg g^{-1} soil, respectively, while in the H0T0 and H1T0 treatments, the respective values were 9.2 and 10.0 µg g^{-1} soil (Figure 5.5b).

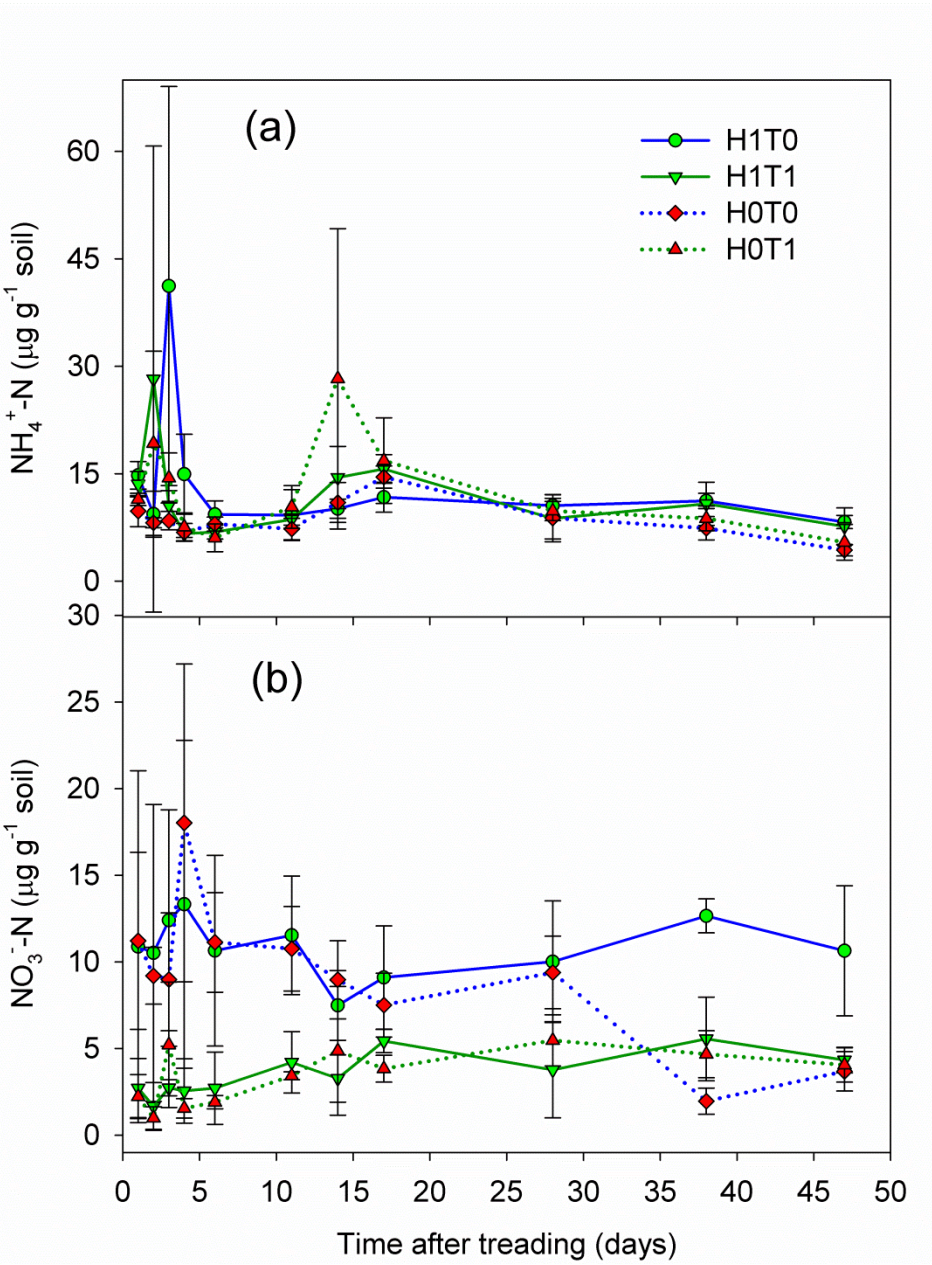

Figure 5.5 Inorganic N concentrations after treading at day zero for part A. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 5.2.2.1 and Figure 5.1).

Concentrations of water soluble C were significantly higher (P <0.001) on days 2, 4, 6 and 14 (Figure 5.6) in the H0T1 treatment while concentrations in the other treatments did not differ. Maximum concentrations from the H0T1 treatment were observed on day 6 and they averaged 390 ± 10, 236 ± 22, 288 ± 19 and 271 ± 19 µg g^{-1}, in the H0T1, H1T0, H1T1 and H0T0 treatments, respectively.

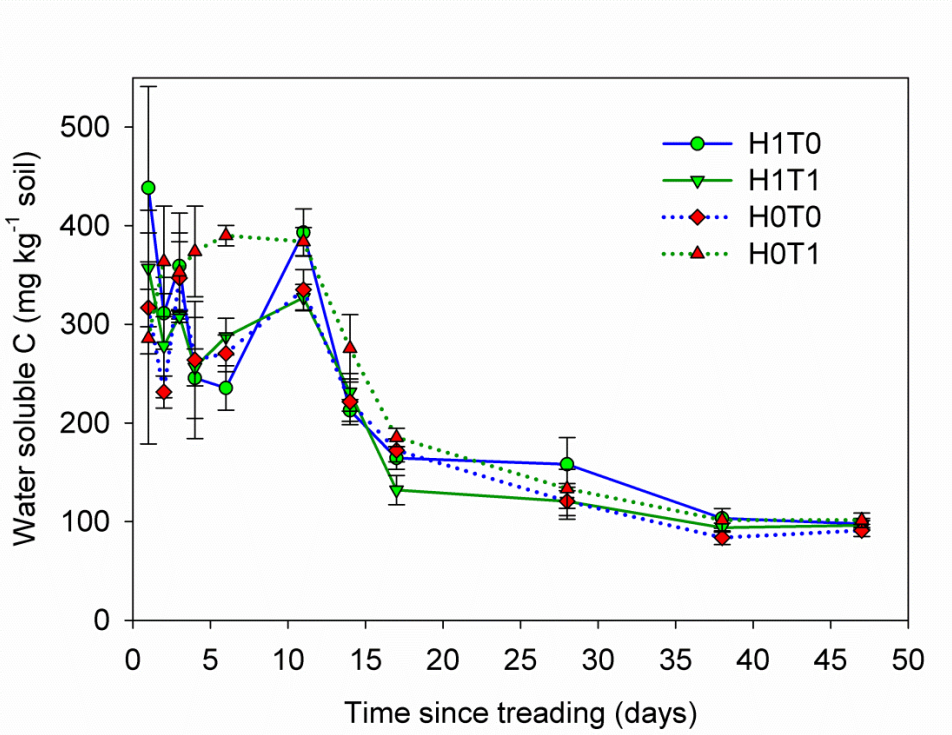

Figure 5.6 Concentrations of WSC after treading at day zero for part A. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 5.2.2.1 and Figure 5.1).

5.3.1.5 Nitrous oxide emissions

The emission data did not follow a normal distribution and were log transformed prior to statistical analyses (Section 5.2.5). On day 1.1 (27 h after treatment application), N_2O emissions did not differ with treatments (Figure 5.7a). From days 1.9–14.1, treading caused higher N_2O emissions (P <0.05) than the control (Figure 5.7a). The highest N_2O emissions were observed at 2.2 d and averaged 98 ± 68 = 46 ± 37 > 5 ± 3 = 5 ± 12 µg N_2O-N m^{-2} h^{-1} (± sd, P <0.05) for the H0T1, H1T1, H0T0 and H1T0 treatments, respectively. From days 17.1–47.1, no treatment differences occurred in N_2O emissions (Figure 5.7a). Emissions of N_2O integrated over 47 d averaged 16.7 ± 11.1 = 9.3 ± 5.5 > 5.5 ± 4.8 > 2.2 ± 1.1 mg N_2O-N m^{-2} for the H0T1, H1T1, H0T0 and H1T0 treatments, respectively. As treatment differences occurred only till day 14.1, on average, this period accounted for 33−75% of the total N_2O emissions.

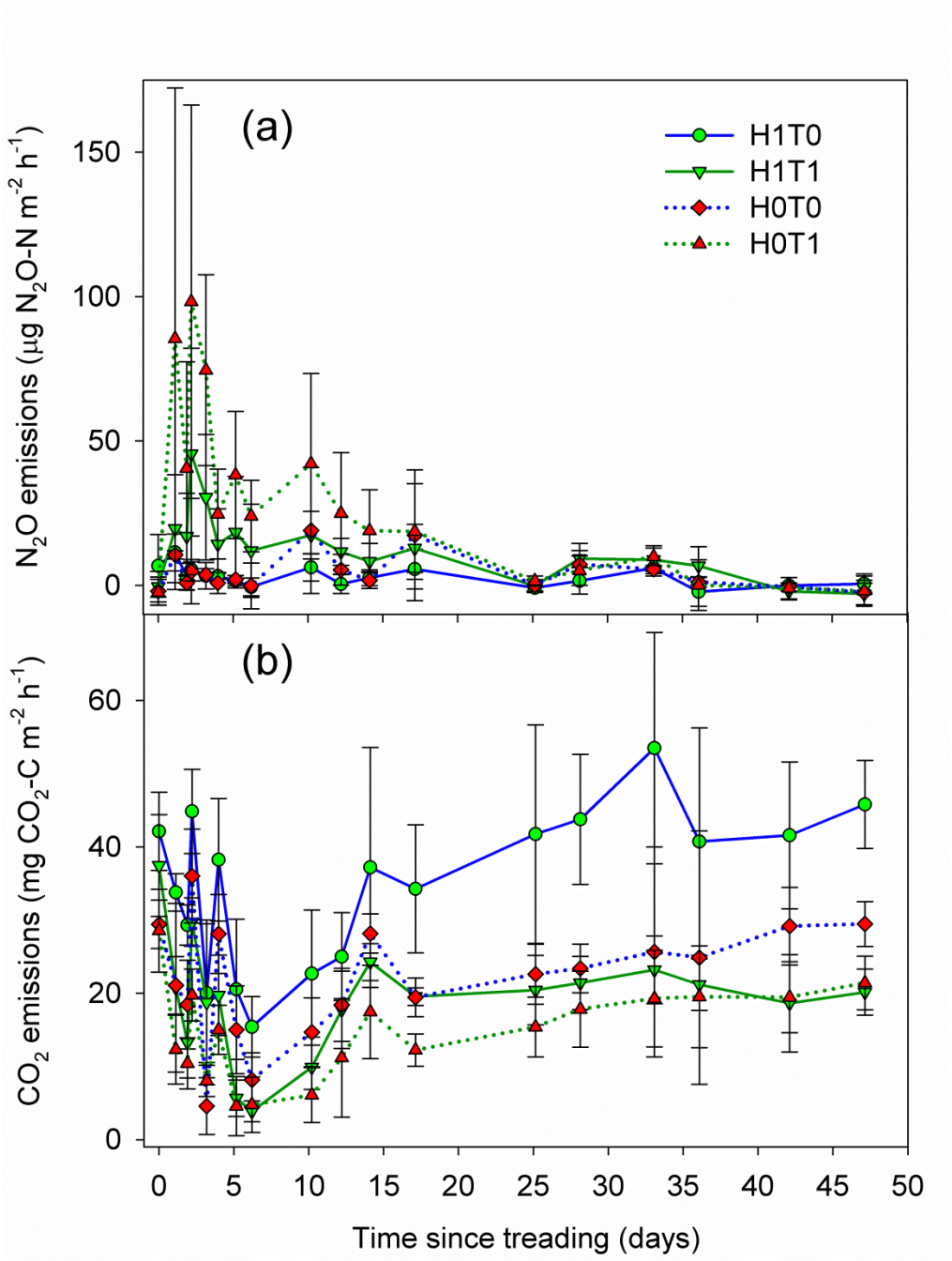

Figure 5.7 Emissions of (a) N_2O, and (b) CO_2, after treading at day zero for part A. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 5.2.2.1 and Figure 5.1).

5.3.1.6 Carbon dioxide emissions

Emissions of CO_2 were higher (P <0.001) from the control treatments (H1T0) than the other treatments at almost all gas samplings until day 47 while lower emissions throughout this period were recorded from the H0T1 treatment (Figure 5.7b). Overall, treading caused lower CO_2 emissions. Emissions of CO_2 on day 1 averaged 20.8 ± 11.5, 33.8 ± 2.6, 12.3 ± 4.7 and 21.1 ± 3.9 mg CO_2-C m^{-2} h^{-1} (± sd, P <0.05) for the H1T1, H1T0, H0T1 and H0T0 treatments, respectively. Average emissions by day 47 were 20.1 ± 3.1, 45.8 ± 6.0, 21.4 ± 3.7

and 29.5 ± 3.5 mg CO_2-C m^{-2} h^{-1} for the H1T1, H1T0, H0T1 and H0T0 treatments, respectively. Cumulative CO_2 emissions over 47 d were significantly lower from the trodden plots than non-trodden plots and equated to 20.6 ± 2.0, 16.7 ± 3.4 and 24.8 ± 1.6 g CO_2-C m^{-2} from the H1T1, H0T1 and H0T0 treatments respectively, with the highest emissions from the control with values of 41.2 ± 6.7 g CO_2-C m^{-2} over the same period.

5.3.2 Part B – ^{15}N-labelled fertiliser addition

5.3.2.1 Soil properties and meteorological data

Chemical properties of the soil at the experimental site (paddock 2) are shown in Table 5.2. During the experimental period at paddock 2, day 30 recorded the highest rainfall of 15.8 mm, followed by day 9 and day 10 with 7.0 and 14.8 mm, respectively. The average daily air temperature ranged from 5.3 to 17.6^{o}C and the soil temperature ranged from 7.9 to 14.5^{o}C over the same period (Figure 5.8). Soil water contents fluctuated naturally at both paddocks following rainfall events.

Table 5.2 Chemical properties of the soil used during the study at paddock 2.

Soil properties	Paddock 2 (April, 2011)
pH (1: 2)	5.9
Total C (g kg^{-1})	56.0
Total N (g kg^{-1})	5.5
Anaerobically mineralisable N (µg g^{-1})	256
Available N (kg ha^{-1})	287
Olsen P (mg kg^{-1})	62
Potassium (cmol$_c$ kg^{-1})	0.94
Calcium (cmol$_c$ kg^{-1})	16.1
Magnesium (cmol$_c$ kg^{-1})	3.67
Sodium (cmol$_c$ kg^{-1})	0.51
Cation exchange capacity (cmol$_c$ kg^{-1})	29
Total base saturation (%)	73

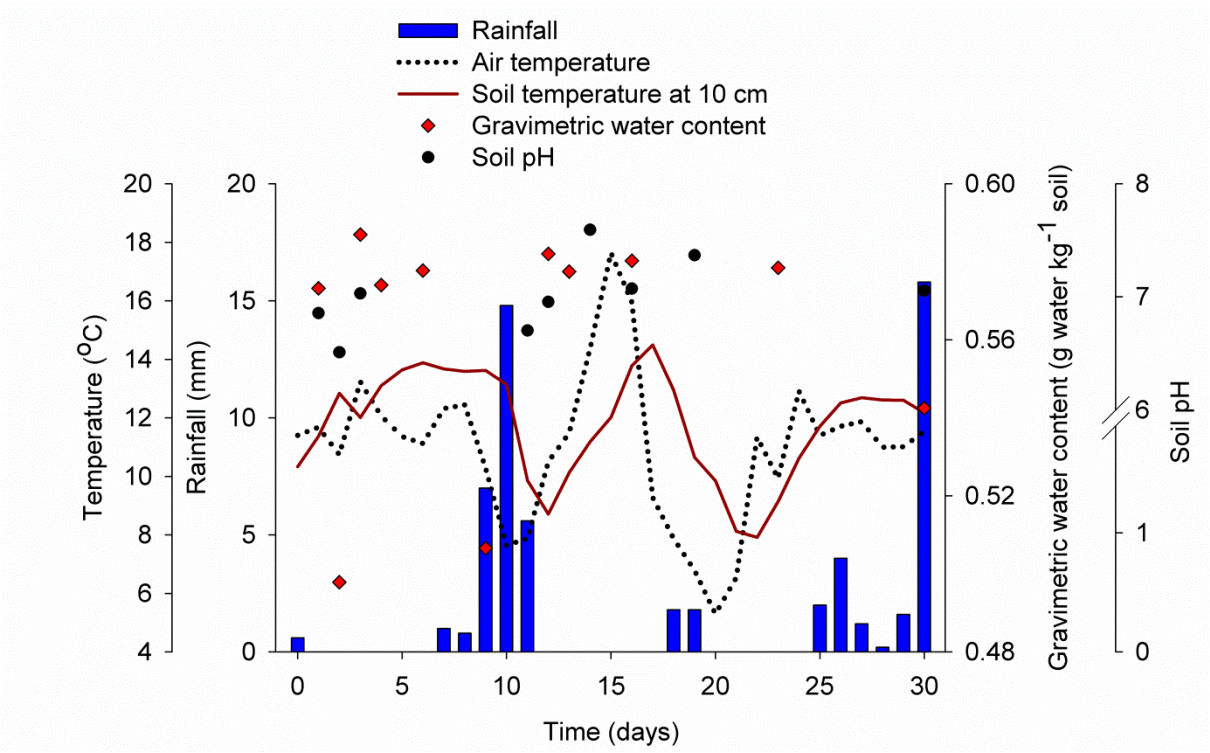

Figure 5.8 Meteorological data during the experimental period – 8 April to 8 May, 2011, at paddock 2 for Part B. Rainfall, air temperature and soil temperature are daily average values. Gravimetric water content was measured at 7.5 cm soil depth.

5.3.2.2 Herbage on-offer and regrowth

Herbage present in the subplots before imposing the treading treatment had 1636 ± 335 kg DM ha^{-1} with N and C contents and a ^{15}N enrichment of 33 ± 7 and 402 ± 6 g kg^{-1}, and 0.367 ± 0.02 atom%, respectively (n = 10). Three months after the treading treatment, herbage regrowth from the trodden plots was significantly lower (P <0.05; n = 5) than the non-trodden plots with 1807 ± 379 and 2608 ± 341 kg DM ha^{-1}, respectively. Corresponding values for the nil-herbage treatment (H0) after 3 months averaged 1080 ± 261 and 990 ± 218 kg DM ha^{-1} but they did not differ from each other. Herbage regrowth (after 3 months) from the treated plots had N and C contents of 36 ± 5 and 388 ± 10 g kg^{-1}, respectively, while the ^{15}N enrichment of the herbage of the subplots that had received ^{15}N fertiliser was 1.57 ± 0.25 atom% while the ^{15}N enrichment of the control remained 0.367 ± 0.01 atom%.

5.3.2.3 Soil pH, soil bulk density and soil gravimetric water content

Treatments had no significant effect on soil pH throughout the experimental period and ranged from 6.1–7.9 units (n = 20 on each occasion).

Bulk density values did not differ due to treatments at any depth and averaged 0.69 ± 0.23, 0.99 ± 0.11, 1.05 ± 0.07, and 0.91 ± 0.14 Mg m^{-3} (n = 12) in the 0–3, 3−6, 6–12, and 0–12 cm depths, respectively.

Mean gravimetric soil water content (θ_g) from the H1T0 treatment equalled 0.53 g g^{-1} soil (θ_g = 0.45 m^3 m^{-3}) and was higher (P <0.05) than in the H1T1 treatment (θ_g =0.44 g g^{-1}; θ_v = 0.37 m^3 m^{-3}) on days 2, 9, 12 and 30 due to rainfall. On the remaining days, values did not differ with treatment ranging from 0.54 to 0.60 g g^{-1} which is equivalent to θ_v of 0.45−0.50 m^3 m^{-3}.

5.3.2.4 Soil inorganic N and WSC

Concentrations of soil NH_4^+–N were higher (P <0.05) in the H1T0 treatment than in the H0T0 treatment on most days except days 3 and 4 (Figure 5.9a). By day 30, NH_4^+ concentrations had increased in the H1T0 treatment (i.e. 1.6 times its value on day 1) but did not differ statistically from other treatments. Analysis of ^{15}N enrichment for NH_4^+ was not performed as the soil extract concentrations and extract volumes were not sufficient.

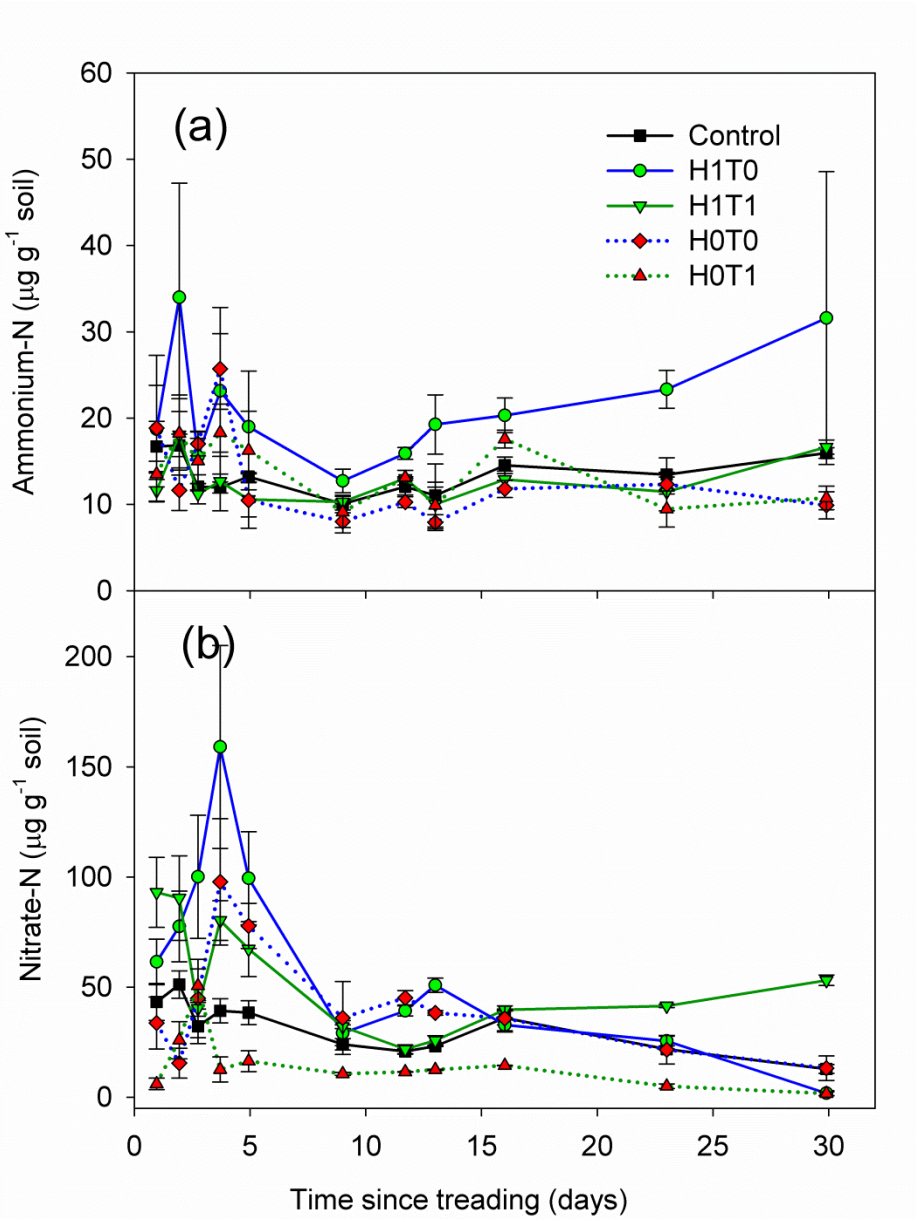

Figure 5.9 Inorganic N concentrations after treading at day zero for part B. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 5.2.2.2 and Figure 5.2).

Concentrations of soil NO_3^-–N were lowest in the H0T1 treatment on almost all days except days 2, 3 and 9 when concentrations did not differ due to treatments (Figure 5.9b). From day 16 onwards, concentration differences were only significant in the H0T1 and H1T1 treatments with lower concentrations in the H0T1 treatment. By day 30, concentrations in the H1T1 treatment were significantly higher than the control and H0T0 treatment, which were higher than the H1T0 and H0T1 treatments and equated to 53 ± 2 > 13 ± 6 = 13 ± 2 > 2 ± 1 = 2 ± 1 µg g^{-1}, respectively. The ^{15}N enrichment of the NO_3^- –N was lower (P <0.001) in the H0T1 treatment on days 1, 2 and 4 (Table 5.3).

Table 5.3 Enrichment of ^{15}N in soil NO_3^- in treatments over time.

Treatment combination	Enrichment of ^{15}N-NO_3^- (atom%)			
	Day 1	Day 2	Day 3	Day 4
Control	0.38 ± 0.03^{c}	0.38 ± 0.00^{c}	0.36 ± 0.01^{b}	0.37 ± 0.03^{d}
H0T0	2.5 ± 1.5^{a}	1.9 ± 1.0^{ab}	2.5 ± 1.6^{a}	4.0 ± 0.8^{a}
H0T1	1.0 ± 0.3^{b}	1.7 ± 0.6^{b}	2.2 ± 0.2^{a}	1.2 ± 0.6^{c}
H1T0	3.3 ± 0.5^{a}	2.3 ± 0.8^{ab}	1.5 ± 0.4^{a}	2.1 ± 0.8^{b}
H1T1	4.3 ± 0.2^{a}	3.4 ± 0.2^{a}	2.1 ± 0.7^{a}	3.4 ± 0.1^{a}

Significant differences (P <0.05) are shown by different letters in the same column using Tukey's test. Data are mean ± sd.

Water soluble C concentrations in the H0T1 treatment was higher (P <0.001) than the concentrations in the other treatments until day 12 and they did not differ until day 30 (Figure 5.10). However, at day 3, concentration from the control treatment was significantly higher than the other treatments.

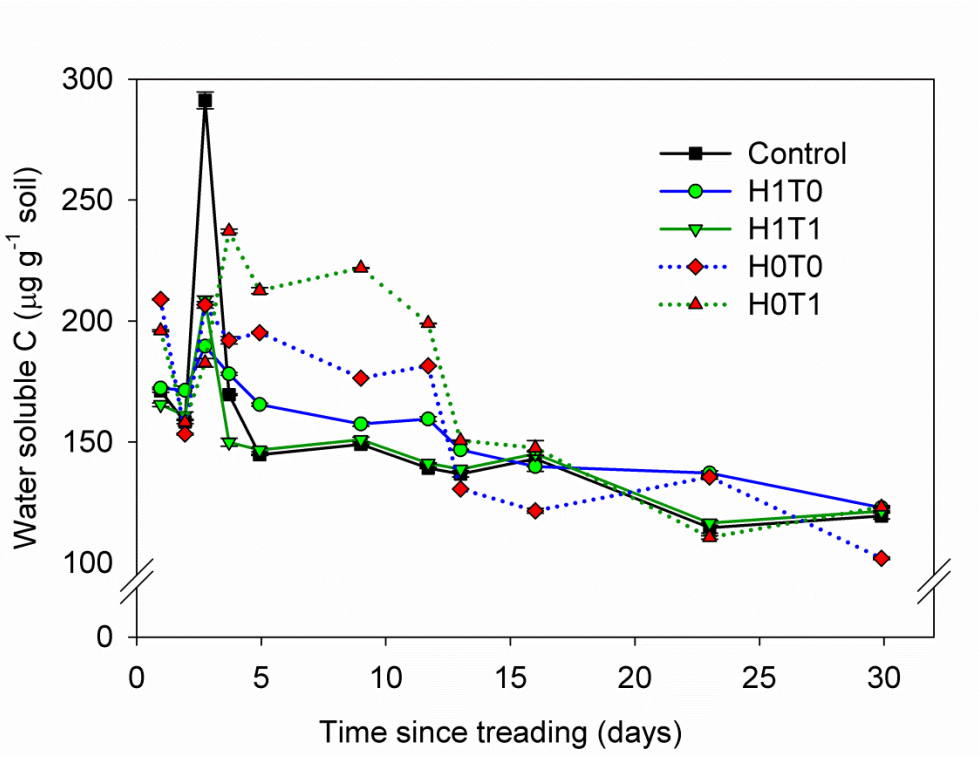

Figure 5.10 Water soluble C concentrations after treading at day zero for part B. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 5.2.2.2 and Figure 5.2).

5.3.2.5 Nitrous oxide emissions and ^{15}N enrichment

The emission data did not follow a normal distribution and were log transformed prior to statistical analyses (Section 5.2.5). Emissions of N_2O from the control were lower (P <0.05) than the other treatments throughout the experimental period. Treading treatments decreased (P < 0.05) the N_2O emissions at days 0.8, 10.9 and 11.7 d while the presence of herbage decreased the emissions on days 1.0 and 11.7 (Figure 5.11a). The herbage treatment did not affect N_2O emissions on any other day over the experimental period. Emissions at 0.8 d averaged 5 ± 4, 14 ± 3, 11 ± 5, 13 ± 4, and 0.05 ± 0.05 mg N_2O-N m^{-2} h^{-1} in the H0T0, H0T1, H1T0, H1T1 and control treatments, respectively. Cumulative N_2O emissions over 30 d were significantly lower from the control treatment but the values from other treatments did not differ and averaged 1.9 ± 0.6, 2.0 ± 1.3, 1.6 ± 0.5, 1.6 ± 0.8, and 0.5 ± 0.2 g N_2O-N m^{-2} in the H0T0, H0T1, H1T0, H1T1 and control treatments, respectively.

Enrichment of N_2O-^{15}N remained significantly higher in the H0T0 treatment than all or mostly the H0T1 treatments (Figure 5.11b) during day 5 to day 30 with an average of 4.2 atom% over this period. Enrichment of N_2O-^{15}N from the control was at natural abundance throughout the experimental period ranging from 0.36 to 0.38 atom% and was lower than all the treatments over the entire experimental period. Mean ^{15}N enrichment of the N_2O over the experimental period equated to 5.3, 4.0, 4.3, 4.0 and 0.37 atom% in the H0T0, H0T1, H1T0, H1T1 and control treatments, respectively.

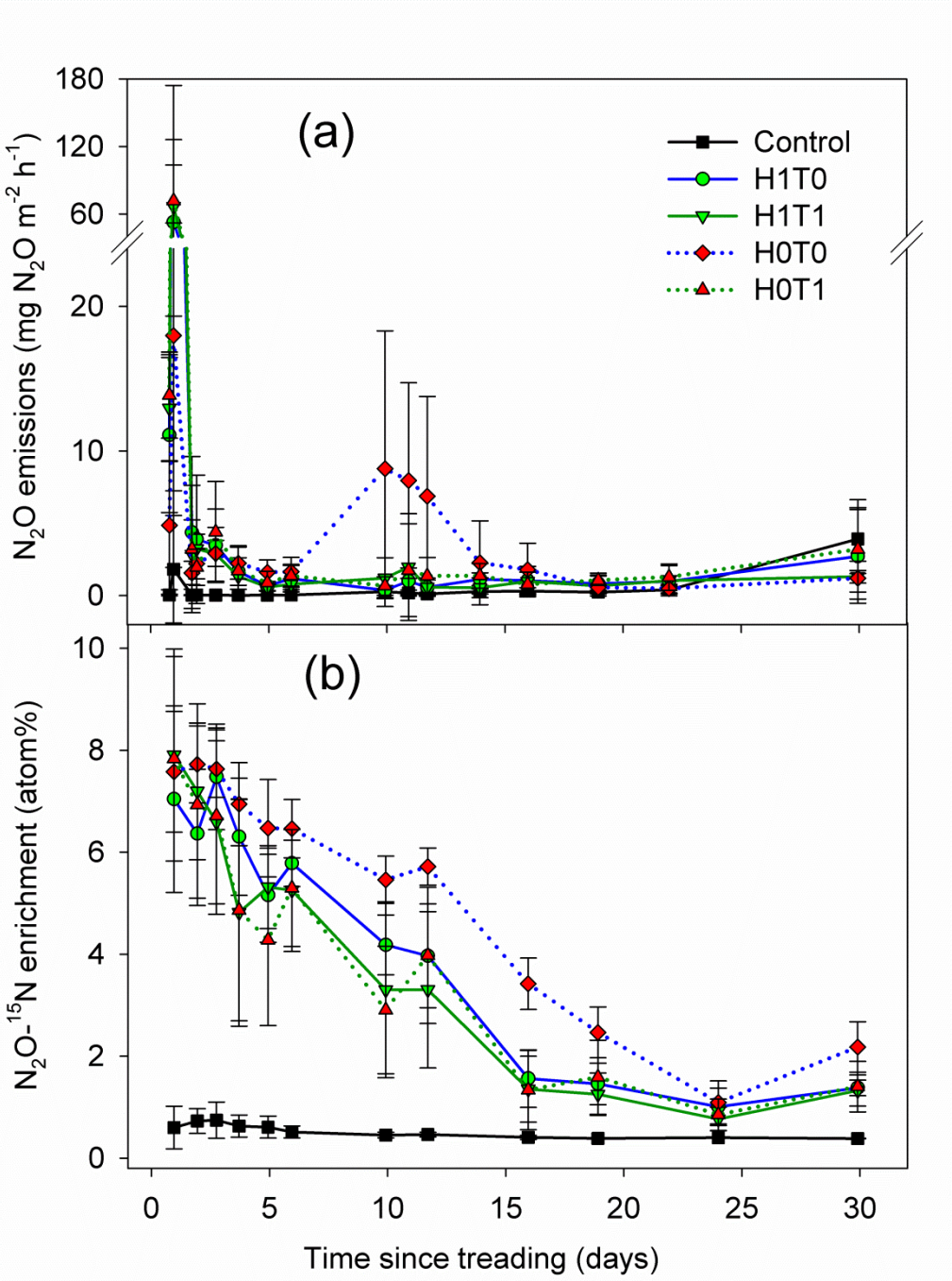

Figure 5.11 Emissions of (a) N_2O, (b) ^{15}N enrichment of N_2O, after treading at day zero for part B. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 5.2.2.2 and Figure 5.2).

5.3.2.6 Carbon dioxide emissions

Emissions of CO_2 did not differ due to treatments on most of the days except on days 1.8 and 5.9 when the treading treatment suppressed the CO_2 emissions (Figure 5.12). The emissions peaked at day 1.0, averaging 366 ± 396, 285 ± 346, 694 ± 502, 491 ± 560, and 296 ± 355 mg CO_2 m^{-2} h^{-1} in the H0T0, H0T1, H1T0, H1T1 and control treatments, respectively. Cumulative CO_2 emissions over 30 d averaged 61 ± 4, 44 ± 12, 76 ± 21, 70 ± 14, and 65 ± 10 g CO_2 m^{-2} in the H0T0, H0T1, H1T0, H1T1 and control treatments, respectively with the lowest emissions in the H0T1 treatment.

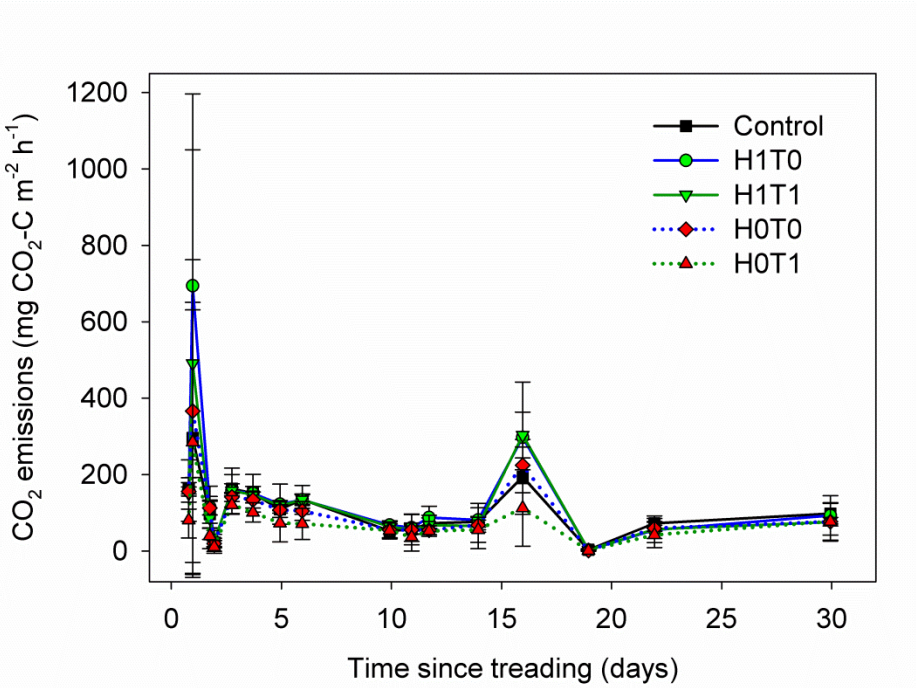

Figure 5.12 Emissions of CO_2 after treading at day zero for part B. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 5.2.2.2 and Figure 5.2).

5.4 Discussion

Treading stimulated N_2O emissions irrespective of the presence or absence of herbage until 14 d (part A). Treading might have created partially anaerobic conditions in addition to the mineralisation of soil-N, due to disturbance of the upper soil layer (0–12 cm), and ultimately, transformation of the mineralised soil NO_3^- to N_2O. This theory is supported by the depleted soil NO_3^- levels under treading (parts A and B), while the ^{15}N enrichment of the NO_3^- (part B) showed that treading resulted in a release of soil-N and/or herbage-N that contributed significantly to the N_2O emissions. The suppression of the CO_2 emissions due to treading (parts A and B) indicated that anaerobic conditions prevailed in the trodden plots thereby increasing the chances of denitrification contributing to N_2O emissions.

5.4.1 Nitrous oxide and CO_2 emissions

Many studies have shown elevated N_2O emissions soon after grazing events in field conditions (Bhandral *et al.* 2007b; Carran *et al.* 1995; Luo *et al.* 1999; Ruz-Jerez *et al.* 1994). However, the emissions are generally attributed to animal excreta or N application. Other factors that may contribute are increased soil NO_3^-–N due to reduced N uptake after defoliation, increased soil C from excreta deposition and reduced soil aeration due to treading (Menneer *et al.* 2005b). Menneer *et al.* (2005b) measured N_2O emissions following treading in the absence of animal excreta or N additions. Moderate to severe treading was imposed by walking dairy cattle at 4.5 cows 100 m^{-2} for 1.5 and 2.5 h, respectively and N_2O emissions were measured over 28 d. They observed 3–6 fold higher emissions for 21 d after treading with a maximum of 52 g N_2O-N ha^{-1} d^{-1} on day 8 while in the current study (part A), emissions due to treading treatments were significant (10–20 fold higher) till 14 d with maximum values ranging from 2.2–39.9 g N_2O-N ha^{-1} d^{-1} (P >0.05) from the herbage and nil-herbage, respectively, 2.2 d after imposing treading. The emissions from the treatments were higher than the control treatment throughout the experimental period (part A) in the current study. The N_2O emission data are comparable to the study of Menneer *et al.* (2005b).

Menneer *et al.* (2005b) observed elevated soil inorganic N concentration (10–16 mg NO_3^-–N kg^{-1} soil) in the first 3 d after treading. In Menneer *et al.* (2005b)'s study, NO_3^-–N concentrations were comparable and higher in the first 6 d ranging from 8.9–18.0 mg kg^{-1} soil from the non-trodden and trodden plots, respectively. However, treading treatments in the current experiment, lowered NO_3^- concentrations during this period when compared to non-trodden treatments in contrast to the Menneer *et al.* (2005b) study where higher NO_3^- concentrations were recorded due to treading. Menneer *et al.* (2005b) indicated that severe treading increased inorganic N concentrations due to increased soil-N mineralisation activated by the disturbance of soil as well as burial of organic matter during treading. Bhandral *et al.* (2007b) performed a simulated treading trial on New Zealand pasture soil using various N sources and observed that N_2O emissions during the initial period did not directly result from N addition rather from the mineralisation of soil N and the N-induced solubilisation of soil C (Williams *et al.* 1999). The current study also showed higher WSC concentrations in the H0T1 treatment supporting the findings of Bhandral *et al.* (2007b) and Williams *et al.* (1999). In this current study (part B), treading was shown to dilute the NO_3^--^{15}N pool presumably due to the release of soil-N and/or herbage-N.

In the current study higher levels of NH_4^+ were observed in the absence of herbage treatment. MacDuff and Jackson (1992) showed that defoliation of Italian ryegrass (*Lolium multiflorum* L.) and white clover in hydroponic culture increased root-efflux of NH_4^+– and

NO_3^-–N. Bardgett *et al.* (1998) suggested that defoliation causes mineralisation of soil N and C allocation which in turn may reduce regrowth but improve subsequent herbage quality. Mikola *et al.* (2001) also found that defoliation of perennial ryegrass and white clover in New Zealand pastures led to an increase in the belowground nutrient pool of C and N for faster regrowth of shoots. Laboratory experiments on grasses found that clipping led to an accumulation of assimilates (inorganic N and water soluble C) in the roots and rhizosphere and increased root respiration and exudation (Bokhari and Singh 1974; Dyer and Bokhari 1976). In the current study (parts A and B), the elevated NH_4^+ concentrations in the H0T1 treatment indicated that the treatments evidently created a combined effect of mineralisation of soil-N and/or residual plant-N and C due to defoliation.

Thus the N_2O emissions from the nil-herbage-trodden (H0T1) treatment were higher, firstly because of anaerobic conditions due to a higher soil water content and treading; and secondly, because defoliation along with treading might have caused the mineralisation of inorganic N (due to soil disturbance and burial of residual herbage in the soil during treading) thus providing substrate for denitrification.

In part B, the emissions trend was different with treading or presence of herbage decreasing N_2O emissions, but only on the days following rainfall events (days 0, 10 and 11). Adding fertiliser-N might have created an artefact by priming the microbial community; however this was not investigated in this experiment. Treading, on average, decreased the ^{15}N enrichment of the N_2O compared to the non-trodden treatment (4.0 vs.4.8 atom%). This has a two-fold explanation; firstly, most of the N_2O might have been converted further to N_2 (Simek *et al.* 2006) over the entire experimental period except after periods of rainfall i.e. the denitrification process was pushed towards completion in the presence of NO_3^- and WSC and second, because of lower $^{15}NO_3^-$ in the H0T1 treatment, the residual plant-N (stubbles and/or roots) also might have contributed to the soil-N pool and consequently lower ^{15}N enrichment in N_2O emissions. Ruser *et al.* (2006) reported conversion of N_2O to N_2 in compacted treatments at soil WFPS ≥90%. Ruser *et al.* (2006) also observed that CO_2 emissions were suppressed due to soil compaction similar to the present study (both parts A and B) thus indicating anaerobic conditions that are conducive for denitrification. Treading in the H1T1 treatment might not have been sufficient to cause herbage decomposition and subsequent N release because abundant herbage might have protected the soil against treading damage (Climo and Richardson 1984) and hence the reported soil $^{15}NO_3^-$ values were similar to the H0T0 treatment in the absence of treading (3.4 vs.4.0 atom%, P >0.05).

Part A of the study indicated that antecedent soil NO_3^- was a key precursor to N_2O formation after treading since NO_3^- concentrations decreased in the treading treatments while

part B, using a ^{15}N technique, proved the $^{15}NO_3^-$ pool was converted to N_2O-^{15}N but additionally the $^{15}NO_3^-$ pool was also being diluted by the natural abundance ^{14}N originating from soil organic matter and/or residual plant-N. Moreover, in part B, compaction due to treading may have enhanced the denitrification of N_2O to N_2 because soil compaction reduces the soil gas diffusivity thereby increasing the residence time of N_2O in the soil pores (Simek *et al.* 2006; Yamulki and Jarvis 2002).

Soil moisture has been shown to be an important factor influencing soil compaction processes; increasing soil moisture reduces the internal soil strength (Hamza and Anderson 2005), and the ability of the soil to resist the pressure exerted by animal hooves (Bilotta *et al.* 2007). In the present study, N_2O emissions from the treatments (H0T0 and H1T1) with higher water contents (90–100% WFPS) had relatively lower N_2O emissions compared to the treatment (H0T1) having lower soil water content (66–84% WFPS). Reported threshold values of soil WFPS causing denitrification differ according to soil type, but for soils similar to those used in the current study (silt loams), threshold soil WFPS ranging from 50 to 74% have been reported (Nelson and Terry 1996; Sexstone *et al.* 1988).

Several studies in the recent past have focussed on N_2O emissions due to treading; however, in these studies, treading was either imposed using farm traffic (Bell *et al.* 2011; Van Groenigen *et al.* 2005) or in the presence of N sources such as fertilisers, urine or slurry (Bhandral *et al.* 2007b). The cumulative emissions from the trodden plots in the present study (Part A, 0.7–1.3 kg N_2O-N ha^{-1} y^{-1}) were only comparable to the control plots in these prior studies, e.g. Bhandral *et al.* (2007b) observed 4.5–10.4 kg N_2O-N ha^{-1} y^{-1} from controls of non-trodden and trodden plots, respectively. However, compaction in their study (using farm vehicle) was 3 times higher than the present study (220 vs. 632 kPa).

5.4.2 Treatment effects on herbage yields

Reduced herbage yield from the trodden plots was in accordance with past studies which showed adverse effects of treading on shoot and root yields (Cook *et al.* 1996; Nadian *et al.* 1996), legume production (Edmond 1958; 1962) and pastoral growth and plant morphological development (Drewry *et al.* 2001; 2008; Menneer *et al.* 2005c). Brown and Evans (1973) reported 63% reduction in herbage yields from high stocking rates in wet soil conditions while the present study had an average reduction of 59% in herbage yield. Vegetation cover offers protection to soil damage from animal treading, however, the degree of protection depends on the quality and quantity of herbage (Climo and Richardson 1984). Studies have shown that perennial ryegrass was found to be the most tolerant to heavy treading treatments when compared to 10 pasture species at five treading rates (Brown and

Evans 1973; Edmond 1964). Age of a pasture (i.e. time since sowing/renovating) may also play an important role in protection against treading damage. Well established plants have deeper roots and lower quality herbage which may offer better protection against soil damage due to treading. Moreover, treading damage is often confined only in the top 2–12 cm [(Scholefield and Hall 1985); (Section 2.5.2)]. Paddock 1 of this study was a recently sown and renovated pasture (4 yr old pasture), evidently more prone to treading, while paddock 2 was a well-established 8 yr old pasture which also helps explain the non-significant differences in the N_2O emissions in the treatments. Treading, therefore, in the presence of a substantial herbage mass or turf may meet a 'cushion' effect that protects the soil from damage. In the current study, herbage in the H1T1 treatment (both parts A and B) was damaged due to treading but not detached from the plant and hence was only partially incorporated in soil allowing only limited decomposition of the plant shoots. This also might have decreased the vigour of regrowth of the plots receiving treading. Moreover, the average temperature (8^oC) during the experimental period at both paddocks was relatively low, which again, was not conducive for decomposition. This may explain the lower and non-significant N_2O emissions from the herbage treatment when trodden.

5.4.3 Treatment effects on soil properties

Soils with higher clay contents can behave in a plastic manner even at lower soil moisture contents which makes them particularly susceptible to treading damage. The current study (both parts A and B) used a clay loam soil but presence of vegetation, particularly ryegrass herbage [(Edmond 1964); Section 5.4.2] must have protected it from compaction. Scholefield and Hall (1985) found that over a wide range of water contents, soil deformation due to treading was independent of soil water content. In the present study, treading damage was significantly higher even at lower soil water contents, especially in the absence of herbage, when observed visually. Soil type may also play a major role in resistance to damage (Patto *et al.* 1978). Soil bulk density in the present study (both parts A and B) was not affected due to treading at both paddocks which was in accordance with findings of Menneer *et al*. (2005b). The amount of soil damage due to treading also depends on the hoof pressure of the animal. The cow hoof used in this study delivered a hoof pressure of about 220 kPa (Di *et al.* 2001). However, in reality hoof pressures vary and will depend on factors including the type and size of the animal, and whether the animal is moving or stationary. Cattle hoof pressures of up to 300 to 400 kPa have been reported for walking cows (Scholefield and Hall 1985). Moreover, the frequency of the same spot receiving a hoof impact may also play an important role.

5.5 Conclusion

Maximum N_2O emissions occurred 2 d after imposing simulated animal treading to nil-herbage plots (part A). Presence or absence of herbage did not affect the N_2O emissions due to treading. In the 47 d experimental period (part A), emissions were significantly higher from the trodden plots till day 14 and contributed 33–75% of the cumulative emissions ranging from 9–17 mg N_2O-N m^{-2}. These higher emissions also corresponded with lower NO_3^-–N concentrations. Emissions of N_2O from the nil-herbage-trodden (H0T1) treatment were higher, firstly because of anaerobic conditions due to high soil water contents and treading, and secondly, because defoliation along with treading might have caused the mineralisation of inorganic N (due to soil disturbance and burial of residual plant-N in the soil during treading) thus providing substrate for denitrification. These results were confirmed using a ^{15}N technique (part B) which showed that a major fraction of the emitted N_2O originated from soil-N and/or from plant-N decomposition, in the treading treatments. Further studies should be performed using ^{15}N-labelled herbage and using live animals, to further delineate the contribution of herbage-N and/or soil-N to N_2O emissions as a consequence of treading.

Chapter 6
Contribution of pasture plant litter to N_2O and CO_2 emissions

6.1 Introduction

Intensively grazed pasture ecosystems contribute significantly to N_2O emissions (Oenema *et al.* 2005) due to regular N inputs of fertiliser and excreta-N (urine and dung) deposited by grazing animals (De Klein *et al.* 2003). It is also recognised that the application of crop residues[§] to soil can produce N_2O emissions (Huang *et al.* 2004; Mori *et al.* 2005). These emissions are generally proportional to the biochemical composition, rate and/or placement of the crop residues (Aulakh *et al.* 2000; Baggs *et al.* 2000).

Results from Chapter 4 revealed that a significant quantity of litter is formed due to litter-fall in intensively managed pastures grazed by dairy cattle but the fate of this litter has not been explored. From visual observation, this litter may stay on the soil surface and decompose or it may get partially and/completely incorporated due to animal treading. The effect of partial incorporation via treading was explored in Chapter 5. The effect of complete incorporation of pasture litter with respect to N_2O emissions has not been studied.

In a grazed pasture system, litter sources include the pasture species grazed *in situ* and also litter that might occur during the feeding out of supplements. According to a decision support system for organic residues (Palm *et al.* 2001), clover and ryegrass are classified as high quality – class I (high N, >2.5% N; low lignin, <15% lignin; <4% polyphenols) while maize is classified as being of medium quality, class II (i.e. <2.5% N; <15% lignin; >4% polyphenols). Clover and ryegrass litters were selected based on their C: N ratio and their dominance as pasture species. Maize, a species with a comparatively higher C: N ratio was also selected because it is commonly used as a feed supplement for grazing ruminants, where it is fed out onto the pasture surface.

As noted earlier (Section 2.4), direct N_2O emissions from pasture litter incorporation have not been quantified. The emission patterns from pasture litters are expected to differ from cropping residues because of differences in the biochemical composition, management conditions and naturally fluctuating moisture regimes. The following laboratory experiment was therefore performed to measure direct N_2O emissions in response to litter (clover,

[§] Cropping residues in this thesis will be defined as the materials remaining in the field after a crop has been harvested in agricultural and horticultural systems. These residues include stalks, stubbles, stems, leaves and seed pods.

ryegrass and maize) incorporation with soil, incubated at two different levels of soil moisture. Emissions of CO_2 were also measured in this study because they provide an indication of the relative litter decomposition rates as a function of soil microbial respiration.

The soil moisture content affects microbially mediated N dynamics, with denitrification occurring in anaerobic sites while nitrification requires aerobic conditions (Bateman and Baggs 2005). Therefore the soil moisture treatments chosen for this study were 86% water-filled pore space (WFPS) (θ_v = 0.63 m^3 m^{-3}) as field-capacity and another contrasting soil water content of 54% WFPS (θ_v = 0.40 m^3 m^{-3}) which indicates the potential soil moisture deficit during summer when rainfall is less than evaporation as also observed in the field study in Chapter 5 where soil water contents (θ_v) ranged from 0.37–0.63 m^3 m^{-3} across various treatments. These two soil moisture contents were also selected on the basis that denitrification and nitrification dominate at high and low WFPS, respectively (Section 2.4.3).

The objective of this study was to evaluate N_2O emissions from the decomposition of litter from dominant pasture and supplementary feed species and determine the effect of *complete* incorporation of litter on litter decomposition under controlled conditions, and extrapolate the results using litter-fall rates measured in Chapter 4.

The hypotheses tested were,

1) clover would have higher N_2O emissions than ryegrass followed by maize because of the differences in the C: N ratios and biochemical compositions.

2) higher emissions of both N_2O and CO_2 would occur from the 86% WFPS treatment as a result of anaerobic conditions (and in the absence of soil compaction (Chapter 5)) and enhanced litter decomposition.

6.2 Materials and Methods

6.2.1 Experimental design and treatments

The experimental design was a 2 × 4 factorial randomised block design. Factors included litter amendments (clover, ryegrass, maize and a control) and two soil water contents (54% WFPS, sub-field capacity and 86% WFPS, field capacity), replicated 5 times, thereby giving a total of 40 soil cores (4 treatments × 2 soil water contents × 5 replications). Each replicate consisted of a PVC container (Section 3.8) randomly allocated within blocks in an incubator.

6.2.2 Soil preparation and treatment procedure

A poorly drained Temuka silt loam soil [Fluvaquentic Endoaquept, (Hewitt 1998)] was collected from a clover-ryegrass grazed pasture (0–10 cm depth) near Lincoln, New

Zealand (43°38.70'S, 172°28.62'E, 8 m above sea level) in autumn (April), 2008. The key properties of the soil are described in Table 6.1. The collected soil was sieved (4 mm) within 48 h of collection, and any obvious organic material was removed. The sampled (field moist) soil had gravimetric water content, volumetric water content, bulk density, and porosity values of 0.36 kg water kg^{-1} soil, 0.25 m^3 water m^{-3} soil, 0.69 Mg m^{-3} and 0.74 m^3 pores m^{-3} soil, respectively (Section 3.2). The soil had 1.0 mg NH_4^+–N kg^{-1} soil and 98.0 mg NO_3^-–N kg^{-1} soil. The sieved soil was then stored at its field moisture content in sealed plastic bags at 4°C before being packed into PVC containers. To obtain the plant litter, fresh leaves of clover, ryegrass and maize were dried (65°C) and ground (<200 μm). The chemical characteristics of the litter are presented in Table 6.2.

Table 6.1 Chemical properties of the Temuka silt loam soil.

Soil properties	April, 2008
pH (1: 2)	5.8
Total C (g kg^{-1})	90.0
Total N (g kg^{-1})	9.0
Anaerobically mineralisable N (μg g^{-1})	327
Available N (kg ha^{-1})	444
Olsen P (mg kg^{-1})	63
Potassium ($cmol_c$ kg^{-1})	0.57
Calcium ($cmol_c$ kg^{-1})	20.6
Magnesium ($cmol_c$ kg^{-1})	3.93
Sodium ($cmol_c$ kg^{-1})	0.53
Cation exchange capacity ($cmol_c$ kg^{-1})	35
Total base saturation (%)	74

The plant litter was applied on a mass basis, with 5 g of litter added to 165 g dry soil (3% by weight). These unrealistically high application rates were used to quantify a detectable response of the gas emissions and also to compare the results with that of Kelliher *et al*. (2007) who used a similar litter application rate. Equal amounts of the litter were applied to ensure that the soil matrix was similar and that constant rates of C were applied across the plant residue treatments (13 g C kg^{-1} soil) since C can also affect denitrification rates.This litter was thoroughly mixed with the field moist, sieved soil and then immediately packed to a depth of 4.5 cm in the PVC containers (internal diameter 8 cm, height 10 cm). The base of each PVC container was covered by fine nylon mesh to prevent loss of soil material. Since the

plant litter was incorporated on a mass basis, the rate of litter-C added was constant between litter species. However, since C: N ratios differed between litter species, the mass of N added differed, with N rates equal to 1.5, 1.0 and 0.6 g N kg^{-1} soil for clover, ryegrass and maize, respectively. Deionised water was added to obtain either 54% WFPS (θ_v = 0.40 m^3 m^{-3}) or 86% WFPS (θ_v = 0.63 m^3 m^{-3}). The soil water contents were then maintained by daily misting the soil surface to a pre-determined mass. Soil samples were incubated at 20^oC for 42 d except for brief periods (30 min) during gas measurements.

Table 6.2 Chemical properties of the plant species' litter that were incorporated into the soil.

Plant material	Lignin	Hemi-cellulose	Cellulose	Total N	Total C	C: N ratio	Class[a]
			(g kg^{-1})				
Clover	23[b]	83	203	50	439	8.8	I
Ryegrass	19	153	400	34	418	12.3	I
Maize	19	215	449	20	409	20.6	II

[a]According to a decision support system for organic residues (Palm *et al.* 2001). [b]Values are the mean of 3 replicates; sd <0.01 are not shown.

6.2.3 Gas sampling

The N_2O emissions were determined on 10 occasions over the first 14 d of the experiment using the closed-chamber technique (Section 3.10). Gas samples were collected at 0, 10 and 20 min on each occasion. The gas samples were analysed, within 48 h, for N_2O using gas chromatography (Section 3.11.1).

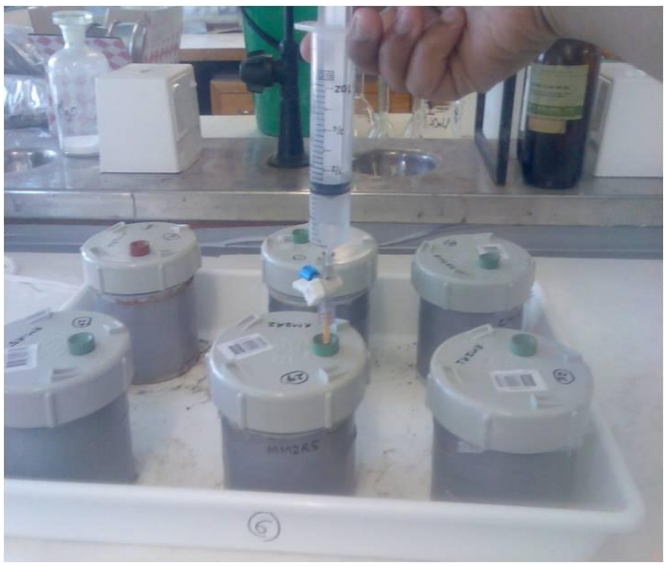

Figure 6.1 Gas collection from litter-treated PVC containers.

Soil CO_2 emissions were measured on 24 occasions over the 42 d duration of the experiment from the same soil cores 10 min after the N_2O measurements. At each gas sampling event, a portable soil respiration chamber (SRC) was placed directly onto each PVC container that was connected to an infrared gas analyser (SRC–1 and EGM–3, PP Systems, Hitchin, UK; Section 3.10, Figure 6.2) and the emissions were determined over a 2 min period.

Figure 6.2 Portable chamber with an infrared gas analyser.

6.2.4 Data analysis

Gas emission data on each gas sampling occasion and the cumulative emissions were tested for normality using the Anderson-Darling test and skewed data was log transformed [ln(flux+1)] (Press *et al.* 1989). Analysis of variance was used to determine if differences between treatments occurred and significant differences between treatments were compared using Tukey's test. The associated errors are expressed as ± sd. All data were analysed using the statistical software Minitab (version 15.1; © 2006, Minitab Inc.). Emission factors (expressed as a percentage of the N or C applied) were calculated by determining the cumulative mass of N or C emitted (as N_2O-N or CO_2-C, respectively), subtracting the integrated control values, and dividing the difference by the mass of N or C applied to the soil in the form of leaf litter. The mean treatment responses were expressed as differences with respect to the unamended soil (control). To quantify the temporal response of the CO_2 emissions to litter application and their decline over time (*t*, hours after litter application), a model (as used by Kelliher *et al.* 2007) was fitted to the data using Equation 6.1.

$$F_{CO_2}(t) = a + br^t$$

Equation 6.1

where; F_{CO_2} = CO_2 flux (mg CO_2 kg^{-1} soil h^{-1})

a = an asymptote which provides an estimate of the 'C priming effect', if any

b = a scaling factor

r = the response parameter that indicates the rate of decline in CO_2 emissions

t = time since litter incorporation (h)

The *r* value was also expressed as K = –ln(*r*), which shows the relative decomposition rate of the litter. The K value also enabled a comparison with the results of Kelliher *et al.* (2007). The cumulative N_2O emissions were converted to CO_2-eq kg^{-1} soil by multiplying by 298, the global warming potential (GWP) of N_2O, (Forster *et al.* 2007) thus enabling comparisons with the cumulative CO_2 emissions.

6.3 Results

6.3.1 N_2O emissions

Maximum N_2O emissions occurred 0.5 d after incorporation of plant litter irrespective of soil water content (Figure 6.3). At 54% WFPS, respective maximum N_2O emissions for the control, clover, ryegrass and maize treatments were 4 ± 3, 1531 ± 111, 786 ± 66 and 303 ± 43 μg N_2O kg^{-1} h^{-1} (± sd, n = 5) while at 86% WFPS, they were 4 ± 2, 2430 ± 181, 1439 ± 185 and 1008 ± 136 μg N_2O kg^{-1} h^{-1} (Figure 6.3a,b). These maximum emissions differed (P <0.001) due to plant species with the clover treatment having the highest N_2O emission at both soil water contents (while the controls did not differ between the soil water contents). The N_2O emissions, when averaged over all plant species at 0.5 d were higher (P <0.05) at 86% WFPS than at 54% WFPS. By day 1.0 in the maize and ryegrass treatments a 10-fold decrease in N_2O emissions had occurred in the 54% WFPS treatment and ~1.5-fold decrease in the 86% WFPS treatment (Figure 6.3a, b). By day 1.0, N_2O emissions in the clover treatment had also declined but only by a factor of 2 when compared to their earlier maximum in both WFPS treatments. On day 1, N_2O emissions from all the treatments were higher (P <0.001) at 86% WFPS than at 54% WFPS. The clover treatment had significantly higher N_2O emissions until day 10 regardless of soil water content. By day 12, the N_2O emissions in the litter amended treatments at 54% WFPS did not differ from the control (P >0.05) but they remained higher (P <0.001) at 86% WFPS until day 14.

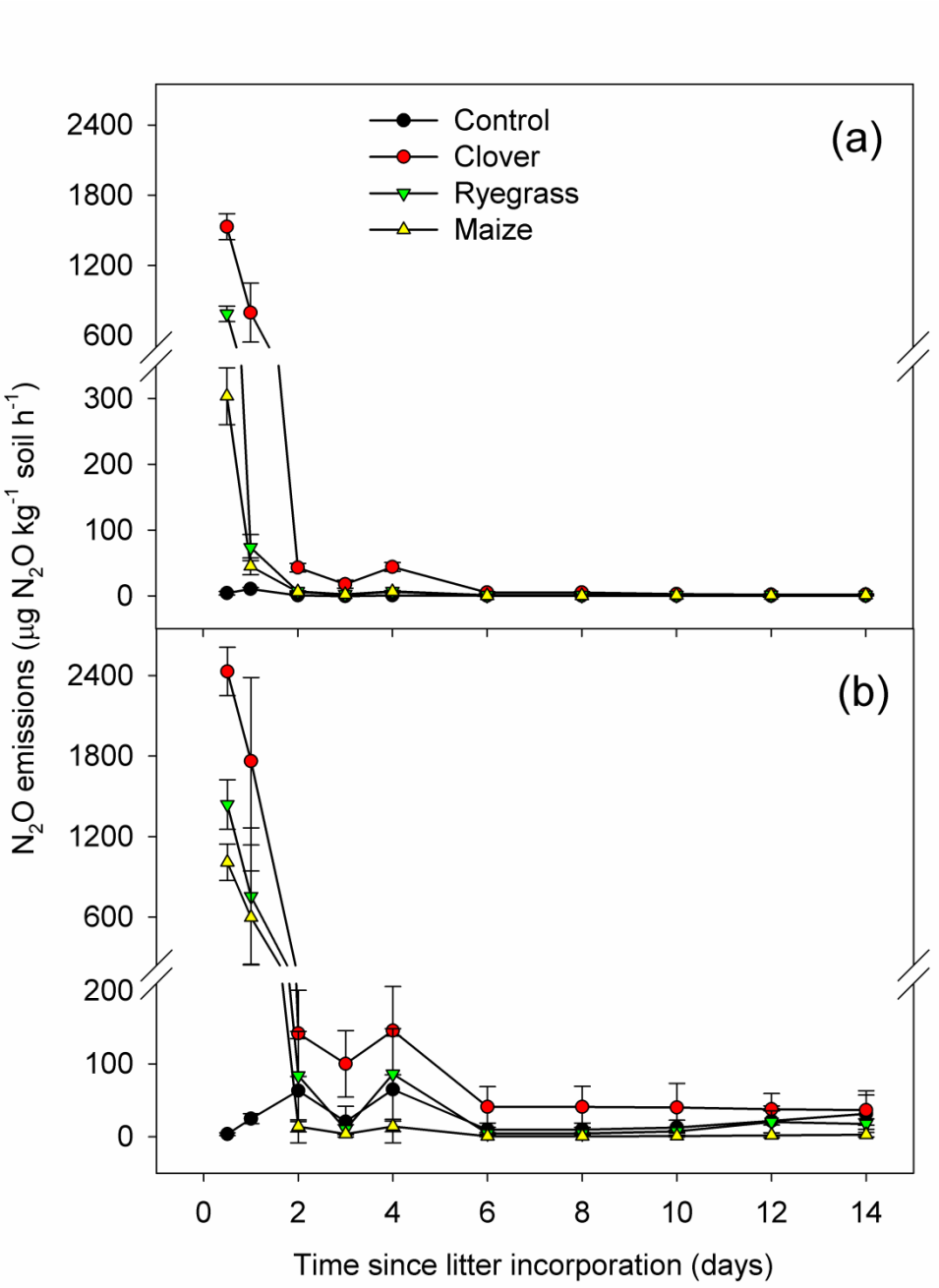

Figure 6.3 Soil N_2O emissions at (a) 54% WFPS and, (b) 86% WFPS, during incubation after incorporation of plant litter; dried, ground shoots of clover, ryegrass or maize. Data are mean ± sd (*n* = 5).

The cumulative N_2O emissions (over 14 d) from the control, clover, ryegrass and maize treatments averaged 0.2 ± 0.1, 25.3 ± 2.6, 7.6 ± 0.7 and 3.3 ± 0.5 mg N_2O-N kg^{-1} soil respectively, at 54% WFPS, while at 86% WFPS the corresponding values were 5.3 ± 4.9, 43.4 ± 10.8, 32.5 ± 5.1 and 19.3 ± 4.8 mg N_2O-N kg^{-1} soil, respectively. Approximately 92−95% of the total cumulative N_2O under both soil water contents was emitted within 2 d of plant litter incorporation.

The N_2O emission factor (EF_{N_2O}, calculated as a % of N applied and corrected for the control) at 54% WFPS, for clover, was significantly higher (P <0.001) than for the ryegrass and maize treatments. Further, at 54% WFPS, the ryegrass EF_{N_2O} was higher (P <0.05) than for maize (Table 6.3). At 86% WFPS, EF_{N_2O} did not differ (P >0.05) with litter species.

Table 6.3 Nitrous oxide emission factor (EF_{N_2O}) at 54% and 86% WFPS, as a % of N applied, over 14 d after incorporation of plant litter into soil samples.

Plant species	g N kg^{-1} soil	EF_{N_2O} (%)	
		54% WFPS	86% WFPS
Clover	1.5	1.7 ± 0.2^{a}	2.9 ± 0.7^{d}
Ryegrass	1.0	0.7 ± 0.1^{b}	3.1 ± 0.5^{d}
Maize	0.6	0.5 ± 0.1^{c}	2.3 ± 0.8^{d}

The data are mean ± sd (n = 5). Significant differences are indicated by different letters in the same column or row (P <0.05)

6.3.2 CO_2 emissions

After 0.5 d, at 54% WFPS, average CO_2 emissions for the control, clover, ryegrass and maize treatments were 2.8 ± 1.7, 37.7 ± 6.4, 71.3 ± 12.2 and 71.2 ± 13.1 mg CO_2 kg^{-1} h^{-1}, (± sd) respectively, while at 86% WFPS, the corresponding values were 3.8 ± 1.1, 39.9 ± 5.4, 68.6 ± 16.0 and 55.5 ± 14.6 mg CO_2 kg^{-1} h^{-1}, respectively (Figure 6.4). For ryegrass and maize, at both soil water contents, maximum CO_2 emissions occurred at 0.5 d and gradually decreased over time (Figure 6.4b,c). In contrast, the CO_2 emissions from the clover treatment increased (P <0.001) by a factor of 3 at 0.9 d at 54% WFPS and by a factor of 2 at 86% WFPS (compared to 0.5 d) and then decreased rapidly. From 4.7–17.8 d, the daily CO_2 emissions from the litter treated soils were higher (P <0.05) than the control at any given time but they did not differ due to litter species or WFPS treatment. From days 0.5–2.7, the clover treatment had higher CO_2 emissions irrespective of the soil water content (P <0.05). Soil water content had no significant effect on CO_2 emissions between 0.5–6.7 d but it did induce a significant effect from 12.9–37.9 d when higher emissions from the 86% WFPS treatment occurred. By day 42, soil water content and litter species had no effect on the CO_2 emissions.

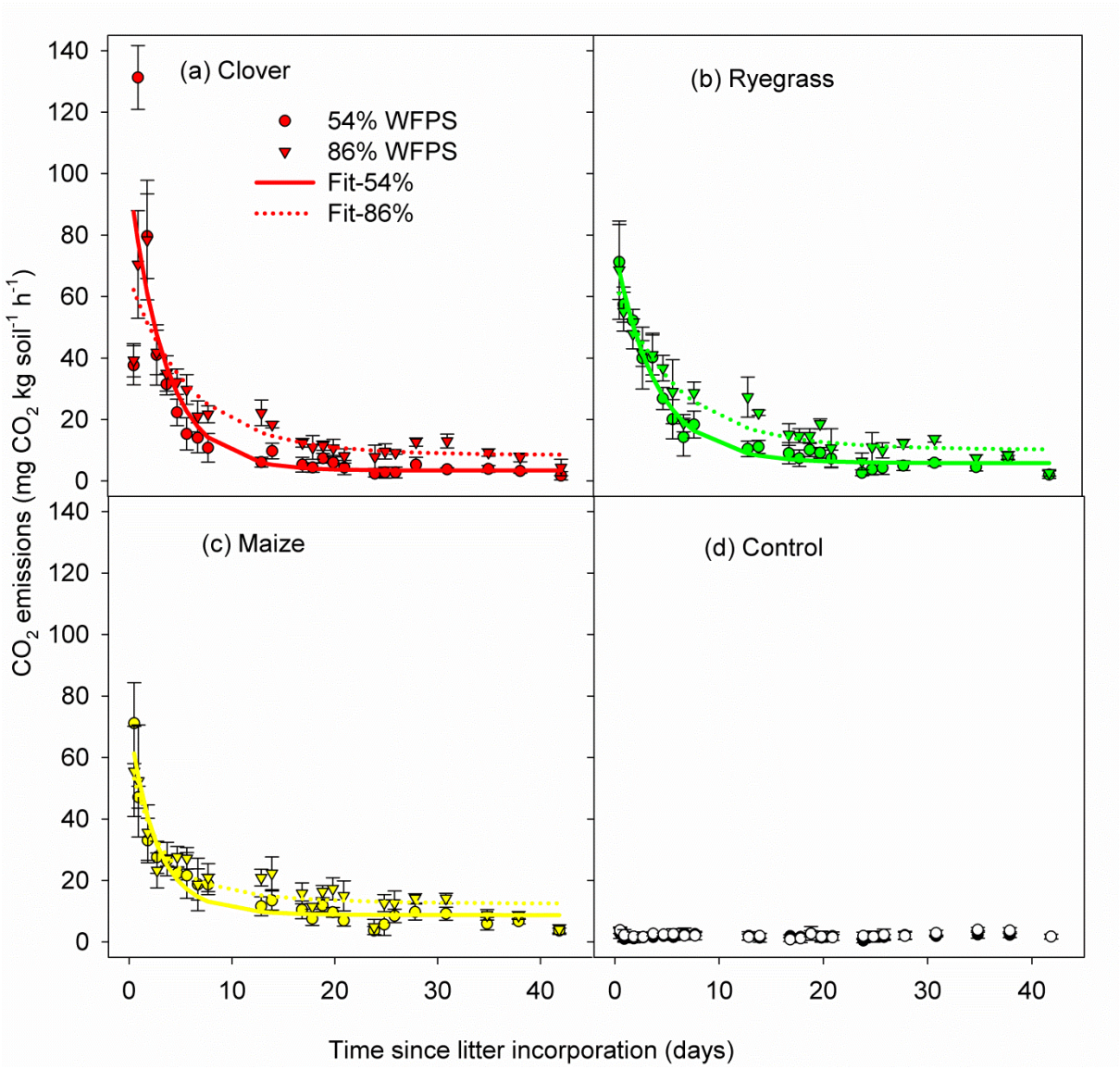

Figure 6.4 Soil CO_2 emissions 54% and 86% WFPS, during incubation after incorporation of plant litter; dried, ground shoots of (a) clover, (b) ryegrass or (c) maize and (d) control. Also shown is the time-response model, $F_{CO_2}(t) = a + br^t$, where *a*, *b* and *r* are parameters fitted to the CO_2 emissions over time (*t*). Data are mean ± sd (*n* = 5). Values of the parameters are reported in Table 6.6 but expressed as μg CO_2 kg^{-1} s^{-1}.

Cumulative CO_2 emissions over time (42 d) at 54% WFPS for the control, clover, ryegrass and maize treatments were 0.6 ± 0.1, 3.0 ± 0.2, 3.3 ± 0.3, and 3.3 ± 0.3 g CO_2-C kg^{-1} soil, respectively, while at 86% WFPS the corresponding values were 0.6 ± 0.1, 4.7 ± 0.4, 4.9 ± 0.3, 4.4 ± 0.5 g CO_2-C kg^{-1} soil, respectively. Cumulative emissions at 86% WFPS were higher than at 54% WFPS (P <0.05).

The EF_{CO_2} values were higher at 86% WFPS (P <0.001) than at 54% WFPS (Table 6.4). At 54% WFPS, the EF_{CO_2} of clover was lower than the EF_{CO_2} of ryegrass and maize

(P <0.05), while at 86% WFPS there were no significant differences between litter species (Table 6.4). When cumulative N_2O and CO_2 emissions were considered on a CO_2-eq basis, over the first 14 d of the experiment, the N_2O emissions contributed 18–59% of the total emissions from the litter treatments at 54% WFPS and 52–67% at 86% WFPS (Table 6.5).

Table 6.4 Carbon dioxide emission factor (EF_{CO_2}) at 54% and 86% WFPS, as a % of C applied, over 42 d after incorporation of plant litter into soil samples.

Plant species	g C kg^{-1} soil	EF_{CO_2} (%)	
		54% WFPS	86% WFPS
Clover	13.3	18.9 ± 1.7^{b}	30.4 ± 2.8^{c}
Ryegrass	12.7	21.9 ± 2.3^{a}	33.9 ± 2.5^{c}
Maize	12.4	22.7 ± 2.3^{a}	30.7 ± 4.0^{c}

The data are mean ± sd (n = 5). Significant differences are indicated by different letters in the same column or row (P <0.05).

Table 6.5 Fourteen day cumulative N_2O (in CO_2-eq), cumulative CO_2 and total emissions at 54% and 86% WFPS. The data are mean ± standard error (n = 5).

Plant species	Cumulative N_2O emission (CO_2-eq)	Cumulative CO_2 emission	Total emission[a] (CO_2+N_2O) (CO_2-eq)	Contribution of N_2O to total emission (%)
		(g kg^{-1} soil)		
		54% WFPS		
Clover	11.8 ± 0.5	8.3 ± 0.2	20.1 ± 0.7	58.9
Ryegrass	3.6 ± 0.1	8.0 ± 0.3	11.6 ± 0.4	30.8
Maize	1.6 ± 0.1	7.0 ± 0.3	8.6 ± 0.4	18.3
Control	0.1 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	13.7
		86% WFPS		
Clover	20.3 ± 2.3	10.3 ± 0.4	30.6	66.5
Ryegrass	15.2 ± 1.1	10.6 ± 0.3	25.8	58.9
Maize	9.0 ± 0.1	8.2 ± 0.5	17.2	52.4
Control	2.5 ± 1.0	0.7 ± 0.1	3.1	79.0

[a]Total emission (CO_2-eq) is the sum of the cumulative N_2O emission (CO_2-eq) and cumulative CO_2 emissions. After 42 d, the cumulative CO_2 emissions for clover, ryegrass, maize and control at 54% WFPS were 11.1 ± 0.4, 12.1 ± 0.5, 12.2 ± 0.5 and 1.9 ± 0.2 g CO_2 kg^{-1} soil, respectively. At 86% WFPS, the corresponding cumulative CO_2 emissions were 17.1 ± 0.7, 18.1 ± 0.5, 16.2 ± 0.8 and 2.3 ± 0.2 g CO_2 kg^{-1} soil).

For the first 3 d after clover, ryegrass and maize litters had been incorporated at 54% WFPS, the CO_2 emissions declined at broadly similar rates (P <0.05) according to the term K = –ln(r) in the model fitted to the data (Figure 6.4, Table 6.6). Proportionally, a significantly greater value of K meant that the corresponding CO_2 emissions declined more rapidly after incorporation of the maize litter. All model parameters *a*, *b* and *r* had significantly higher values for the treated soils than the controls at both WFPS treatments. At 54% WFPS, the asymptote parameter *a* for clover > ryegrass > maize (P <0.001) and clover > ryegrass = maize for the scaling parameter *b*. However, the litter treatment effects at 86% WFPS were statistically indistinguishable (Table 6.6).

Table 6.6 The decline of soil CO_2 emissions over time after litter amendment (*t*, hours) determined using a model written as $F_{CO_2}(t) = a + br^t$, where parameter *a* is an asymptote, parameter *b* is a scaling factor and *r* is the rate of decline of the CO_2 emissions.

Plant species	*a*	*b*	*r*
	54% WFPS		
Clover	0.96 ± 0.24^{d}	26.96 ± 2.90^{g}	0.988 ± 0.001^{i}
Ryegrass	1.62 ± 0.25^{e}	19.78 ± 2.63^{h}	0.990 ± 0.002^{i}
Maize	2.43 ± 0.16^{f}	17.79 ± 3.06^{h}	0.984 ± 0.005^{i}
	86% WFPS		
Clover	2.34 ± 0.33^{x}	16.26 ± 2.61^{y}	0.993 ± 0.002^{z}
Ryegrass	2.80 ± 0.89^{x}	15.42 ± 2.00^{y}	0.993 ± 0.002^{z}
Maize	3.44 ± 0.85^{x}	13.44 ± 6.28^{y}	0.988 ± 0.007^{z}

The data are mean ± sd (n = 5). Significant differences are indicated by different letters in the same column or row (P <0.05). Values reported here are expressed as μg CO_2 kg^{-1} s^{-1} for comparison with that of Kelliher *et al*. (2007).

6.4 Discussion

The relatively high emissions of N_2O immediately after litter incorporation (which had been ground to a fine powder) is in accordance with previous studies [(Aulakh *et al.* 1991b) – 10 d after legume residue incorporation; (McCarty and Bremner 1992) – 3 d after maize and soybean incorporation], indicating rapid mineralisation of plant litter-N and subsequent utilisation by nitrifiers and/or denitrifiers (Baggs *et al.* 1996; Kaiser *et al.* 1998a). The very low N_2O emissions in the control at both soil water contents demonstrated that disturbance due to repacking and rewetting was not responsible for the elevated N_2O production following plant litter incorporation. Smith and Tiedje (1979b), studying the effect of reduced aeration in four different soils, postulated that the maximum activity of denitrifiers occurs within 4–8 h of N substrate addition under anaerobic conditions and in the presence of available C. The maximum N_2O emissions observed at 0.5 d in the present study suggests that pre-existing enzymes had been activated and enhanced by the addition of the readily available C and N sources added to the soil in the form of litter. Such activity is also in accordance with previous studies (Aulakh *et al.* 1991b; Baggs *et al.* 2000), where short-lived N_2O emissions followed the incorporation of arable and horticultural crop residues to soils, but these short-lived emissions occurred over a range of 8–14 d as opposed to 2 d in the present study. Aulakh *et al.* (1991a) incorporated (and/or surface-placed) cereal and legume crop residues (dried and

chopped to 5–7 mm) in soil at 2.5 g residue kg^{-1} and incubated these at 25°C for 35 d at 90% WFPS and recorded cumulative emissions of 50 and 46 mg N kg^{-1}, from hairy vetch and soybean, respectively. These results are comparable to the clover litter applied in the current study (43 mg N_2O-N kg^{-1}) at 86% WFPS. The prolonged occurrence of the maximum N_2O emission observed by Aulakh *et al.* (1991a) at 8 d was probably due to differences in species, particle size, placement of the crop residues and incubation temperature. The relatively higher N_2O emissions that occurred in the current study, at day 2, when compared to other studies may have also been due to higher soil C and N contents in the sampled soil. Moreover, the native soil microbes were probably adapted to regular litter-fall but the litter application was comparatively large, hence the higher emissions. Ambus and Jensen (1997) measured N mineralisation and denitrification rates from barley (44% C, 0.9% N) and pea residues (45% C, 2.1% N) incorporated into soil at the rate of 3.5 g DM kg^{-1} soil. Over 60 d, 42 and 63 mg N kg^{-1} soil was immobilised and relatively higher N_2O emissions were recorded from pea residues in the first 3 d of incubation, with maximum emission rates similar to those of the current study.

The addition of litter to a soil can stimulate higher oxygen consumption both directly, by increasing C availability to denitrifiers, and indirectly by enhancing microbial growth and metabolism (Beauchamp *et al.* 1989; Gillam *et al.* 2008). Thus in the present study, the higher initial N_2O emissions suggest that anaerobic conditions may have been created in the soil due to litter addition. Further, there were higher CO_2 emissions at 86% WFPS.

In this study, the N_2O emissions were highest from the clover litter which had the lowest C: N ratio. This corresponded with its biochemical composition i.e. relatively lower cellulose (203 g kg^{-1}), hemicellulose (83 g kg^{-1}), and C: N ratio (8.8). These properties indicate a relatively more labile source of C when compared with the ryegrass and maize litters. When easily decomposable C and N sources are added to soil, the microbial biomass can switch from more recalcitrant SOM to more readily available C and N sources (Cheng 1996; Sparling *et al.* 1982). A study by Breland (1994b) on clover shoots suggested that the organic C from the clover shoots decayed at faster rates than those of SOM when incubated at 15°C because clover shoots are more readily decomposable than SOM.

The immediately enhanced, yet rapidly decaying N_2O emissions may also have occurred as a result of the destruction of the cell walls (lignified tissues), as a result of plant litter grinding, and this may have increased the availability of N to the soil microorganisms. The thorough mixing of the litter with soil may also have contributed to the early occurrence of peak N_2O emissions since the mixing could have activated aerobic decomposition resulting in enhanced mineralisation of the organic C which in turn may have further favoured N_2O

production. Decomposition also may have enhanced O_2 consumption and this would increase anaerobic conditions and denitrification.

The higher N_2O emissions recorded from the higher soil water content strongly suggest that the emissions originated predominantly via denitrification although nitrification and nitrifier-denitrification cannot be ruled out (Russow *et al.* 2009). Higher N_2O emissions from the 86% WFPS (θ_v = 0.63 m^3 m^{-3}) treatment reflected the reduced soil aeration at field capacity of the sampled soil since a greater proportion of the pore space was water-filled than at 54% WFPS (θ_v = 0.40 m^3 m^{-3}), thereby enhancing the potential for denitrification. Watt and Burgham (1992) reported similar field capacity values (θ_v = 0.54 m^3 m^{-3} for Wakanui deep silt loam and θ_v = 0.59 m^3 m^{-3} for Temuka clay loam) near Lincoln area, Canterbury, indicating higher water holding capacity of the sampled soil in the current study and hence more anaerobic conditions. Bateman and Baggs (2005) concluded that in the 35–60% WFPS range, nitrification produced N_2O while at a higher WFPS, denitrification dominated. The present findings were in accordance with other studies (Gillam *et al.* 2008; Potthoff *et al.* 2005) which showed higher N_2O and CO_2 emissions from wetter soils. In contrast, Aulakh *et al.* (1991a) found significantly lower CO_2 emissions at 90% WFPS than 60% WFPS in the first 8 d, arguing lower microbial activity due to restricted aeration and diffusion as the probable reason. The differences between the current study and that of Aulakh *et al.* (1991a) may have been due to differences in litter type, soil type, soil sieving and repacking procedures, all of which could have resulted in differences in aeration and O_2 diffusion. These are not simply proportional to WFPS because of different soil pore size distributions (Farquharson and Baldock 2008).

The clover treatment received more labile C compared to the ryegrass and maize treatments and this was mineralised earlier as indicated by the higher CO_2 emissions measured, while the lower but consistent CO_2 emissions over an extended period of 42 d evidently indicated the mineralisation of the recalcitrant pools (from all the litter treatments). Cellulose, hemicellulose and lignin (in increasing order of recalcitrance) are considered to be recalcitrant forms of C and these fractions were comparatively lower in clover. The initial stages of plant residue decomposition are characterised by the mineralisation of labile C, leaving the recalcitrant components intact; the recalcitrant components such as cellulose and lignin are mineralised later (Kogel-Knabner 1993). Trinsoutrot *et al.* (2000a) and Gunnarsson and Marstorp (2002) showed that during the first few days of litter decomposition in soil, soluble carbohydrates are decomposed along with the most readily degradable N-rich components. After this, proteins followed by hemicellulose and cellulose, are sequentially degraded (Gunnarsson and Marstorp 2002; Henriksen and Breland 1999; Martin and Haider

1986). If the incubation in the current study had lasted longer, other slower phases of decomposition may have taken place.

The response of soil microbial respiration to ryegrass litter and urea has also been reported by Kelliher *et al.* (2007). The soil used by Kelliher *et al.* (2007) was sampled from the same farm as the current study, but from a depth of 0–5 cm while 0–10 cm was the depth sampled in the current study. Kelliher *et al.* (2007) applied ryegrass (dried and ground) at the same rate of ~30 g litter kg^{-1} soil at 70% WFPS, incubated at 20^{o}C for 24 d. The results were comparable to this current study. At 1.5 h after ryegrass litter application Kelliher *et al.* (2007) found, the CO_2 emissions averaged 25.0 ± 1.7 μg CO_2 kg^{-1} s^{-1} while 12 h after ryegrass litter incorporation in the current study, CO_2 emissions were 19.8 ± 1.5 and 19.1 ± 2.0 μg CO_2 kg^{-1} s^{-1} at 54% and 86% WFPS, respectively. Over 24 d, for ryegrass, cumulative CO_2 emissions were 12.7 ± 0.4 g CO_2 kg^{-1} for Kelliher *et al.* (2007) and 9.9 ± 0.4 and 14.0 ± 0.4 g CO_2 kg^{-1} at 54% and 86% WFPS, respectively, in this study. Thus, the combination of results from the two studies suggests that ryegrass decomposition rates were proportional to the WFPS.

The K value of 0.007 (which shows the relative decomposition rate of the litter) for the clover and ryegrass treatments at 86% WFPS, was equal to that of Kelliher *et al.* (2007) at 70% WFPS, but in their study, the K value was applied to incubation days 2 to 24, while in the current study, it applied to days 0–42. Kelliher *et al.* (2007) used a multi-component time-response model and for days 0–2, K was 0.019. While in the current study, the K values indicated that clover and ryegrass had similar decomposition rates, at 54% and at 86% WFPS, the asymptote values were higher for ryegrass at both WFPS treatments. Furthermore, like ryegrass, clover had decomposition rates that were proportional to the WFPS.

The parameter *r* which is also an indirect measure of the so called r-strategist activity of rapid catabolism of the fresh organic matter in soil (Fontaine *et al.* 2003); was the same for all the litter treatments at 86% WFPS thus indicating a uniform trend of decomposition of the added litter. At 86% WFPS, parameter *a* values were significantly higher than the control but not different among the litter species indicating that there was evidently a positive 'C priming effect' when compared to the controls. However, the 'priming effect' did not differ due to the plant species. This is an important indication that *in situ* residue decomposition occurs at similar rates (regardless of the pasture species) under considerably wet soil conditions while at drier water contents decomposition rates can vary (in phases and/or related to litter type).

The IPCC (2001) best practice guidelines for inventory building stipulate an EF_{N_2O} range of 0.5–3.0% for animal excreta in grazed pastures. The EF_{N_2O} range for clover at both WFPS levels (1.7–2.9%) and for maize and ryegrass at 86% WFPS was high (2.3–3.1%) when compared to New Zealand's country specific EF_{N_2O} value of 1.0% for total excreta (MAF 2007). However, the current study was conducted under optimal conditions; incubation at 20^{o}C, high soil water content and relatively high rates of C and N input via the plant litter. This study demonstrates, however, the potential for litter deposition to be a component of grazed pasture N_2O inventories. To extend this work and assess the practical implications of the current study, *in situ* experiments with litter are required in conjunction with further measures of litter deposition rates. These *in situ* studies should also incorporate ^{15}N methodologies to establish the potential for N priming as a consequence of litter deposition and to provide a direct measure of the litter's contribution to the N_2O emissions under varying conditions.

6.4.1 Extrapolation of N_2O emissions with respect to litter-fall

Litter resulting from litter-fall during grazing event(s) contains N (Chapter 4) and hence its decomposition may contribute to C and N cycling in soil (Sanaullah *et al.* 2010). Under the right conditions, some of this N applied to soils transforms to N_2O and is emitted to the atmosphere according to the above results of the incubation study. The emission factor (EF) of N_2O varied with soil water content, being significantly greater under very wet conditions (Table 6.3). Annually, the Lincoln University Dairy Farm (LUDF) receives ~1100 mm in the form of irrigation (~450 mm) and rainfall (~650 mm) and the soil being a Temuka silt loam, it can be estimated that the soil should be at field capacity 80% of the time. Also, at the LUDF, litter comprised 90% ryegrass and 10% clover (Chapter 4). Using the pastoral dominance of ryegrass and clover, their EFs (Table 6.3), and the soil water contents, a weighted mean EF from the current incubation experiment would be 1.3%. This is similar to the IPCC default value of 1%, with a quoted uncertainty range of 0.03–3%, for anthropogenic N applications to soils including fertiliser, organic amendments and crop residues (IPCC 2006). Combining fresh and senesced litter rates of 19.4 kg N ha^{-1} y^{-1} from the field survey and 1.3% EF from the incubation experiment, and converting units, estimated N_2O emissions attributable to litter-fall would be 0.4 kg N_2O ha^{-1} y^{-1} (see Appendix, Table A). This also assumes that the EF from the incubation is not litter rate dependent.

This extrapolation shows that intensive grazing by dairy cattle, which produce litter-fall, has the potential to contribute to N_2O emissions. For context, this estimate of 0.4 kg N_2O

$ha^{-1}\ y^{-1}$ will be compared to annual N_2O emissions from unfertilised soils beneath grass. Bouwman's seminal review (1996) included 11 studies, but unlike Bouwman (1996), who evidently considered timothy (*Phleum pratense* L.) a weed, two of his reported studies where timothy was the vegetation, have been included because it is a common grass species in pastures throughout the north-eastern USA. Since this review, there have been three additional studies with unfertilised soils beneath grass. For a silt loam beneath a mixed herb ley sward at Lincoln, New Zealand, integration and extrapolation yielded an estimate of 0.4 kg $N_2O\ ha^{-1}\ y^{-1}$ (Van der Weerden *et al.* 1999; 2000). On a loamy sand beneath 12 species of grass, two legumes and 15 herbs located near Giessen, Germany, the N_2O emissions averaged 0.3 kg $N_2O\ ha^{-1}\ y^{-1}$ (Kammann *et al.* 1998). While for a sandy loam beneath annual ryegrass and clover located near Wagga Wagga, Australia, the N_2O emissions averaged 0.6 kg $N_2O\ ha^{-1}\ y^{-1}$ (Galbally *et al.* 2010). On average, from these 14 studies, the N_2O emissions were 1.4 ± 1.9 kg $N_2O\ ha^{-1}\ y^{-1}$ and the median was 0.6 kg $N_2O\ ha^{-1}\ y^{-1}$.

Bouwman (1996) suggested the N_2O emissions from unfertilised soils were "background" emissions, implying a "natural" origin. However, the IPCC guidelines (IPCC 2006) dismiss his concept stating they are "...not 'natural' emissions but are mostly due to contributions of N from crop residue. These emissions are anthropogenic and accounted for in the IPCC methodology". In terms of intensive pasture grazing, the N embodied in the litter may have been derived from the soil mineralised N, fertiliser or excreta inputs. A discussion on the contribution of litter-fall to anthropogenic N_2O emissions is made in the concluding chapter (Chapter 9).

6.5 Conclusion

The soil microbial community responded rapidly and significantly to the incorporation of dried, ground plant litter with maximum N_2O and CO_2 emissions obtained within 0.5 d, and N_2O emissions were virtually complete within 2 d. At field capacity (86% WFPS), EF_{N_2O} equated to 2–3% of the incorporated N with no significant litter species differences. At 54% WFPS, EF_{N_2O} was significantly less with 1.7% > 0.7% = 0.5% for clover, ryegrass and maize, respectively; these differences being attributed to the biochemical properties of the species' litter including their differing C: N ratios. At 86% the EF_{CO_2} was greater than at 54% WFPS, mean 32% and 21%, respectively, with no significant differences due to litter amendments but the litter incorporation effect lasted 38 d. A time-response model fitted to the CO_2 emissions showed no significant differences in the estimated treatment response decay rate at 86% WFPS, but at 54% WFPS the estimated asymptotic emissions were in the order maize >

ryegrass > clover. Expressing N_2O emissions on a CO_2-eq basis, the N_2O emissions contributed half of the total CO_2-eq emissions for clover, about 0.5 and 0.3 for ryegrass and 0.3 and 0.1 for maize for the 86% and 54% WFPS treatments, respectively. The significant contribution of N_2O emissions, especially for clover and ryegrass, warrants further study under *in situ* pasture conditions. Combining EF_{N_2O} and the field survey of litter-fall data, it is estimated that N_2O emissions attributable to litter-fall could be 0.4 kg N_2O ha^{-1} y^{-1}.

Chapter 7
Nitrous oxide emissions following clover litter incorporation at varying levels of cellulose incorporation

7.1 Introduction

Recalcitrant forms of carbon (C) such as cellulose may have determinant effects on soil C and nitrogen (N) dynamics in terms of N_2O emissions (Section 2.4.1.1). Studies examining N_2O emissions from plant residues have concluded that the emissions are proportional to the biochemical composition; mainly the C: N ratio of the residues (Section 2.4.1). Results from Chapter 6 showed that N_2O emissions were higher from clover litter incorporation due to a lower C: N ratio (i.e. a higher N rate) and lower cellulose and hemicellulose contents. Emissions of N_2O from ryegrass and maize were relatively lower because of lower N contents and higher concentrations of cellulose which is a relatively recalcitrant form of C. The question that arises from the study in Chapter 6 is whether or not lower cellulose also affected the N_2O emissions in addition to an N rate effect. The literature review (Section 2.4.1.1) shows that lignin can take a relatively long time (>10 yr) to fully decompose and that cellulose is intermediary between glucose and lignin with respect to decomposition. Hence, cellulose was chosen to manipulate the C: N ratio of clover litter under this study.

The objective of this experiment was to evaluate N_2O and CO_2 emissions following the incorporation of clover litter with varying levels of cellulose. To study the litter-N interactions with the sampled pastoral soil, clover litter was selected for incorporation along with analar grade cellulose powder at a relatively higher soil water content of 86% WFPS.

It was hypothesised that increasing cellulose rates would cause lower N_2O emissions since the microorganisms responsible for N_2O emissions would not have access to a labile C source except to soil C and hence cause lower N_2O emissions in the absence of an additional C substrate.

7.2 Materials and Methods

7.2.1 Experimental design and treatments

The experimental design was a randomised block design with four cellulose-litter treatments and a control that received no litter or cellulose. Treatments included a 'clover only' treatment that consisted solely of litter and did not receive any cellulose; a 'C: N 20' treatment that received the same amount of clover litter (dry weight basis) along with an amount of cellulose that brought the C: N ratio to 20. The treatments 'C: N 30' and 'C: N 40' were prepared with different cellulose rates to give C: N ratios of 30 and 40. The treatments were replicated 5 times giving a total of 25 cores (5 treatments × 5 replications) for gas emission measurement. Each replicate consisted of a PVC container (as used in Chapter 6; Section 3.10) randomly allocated within blocks in an incubator maintained at 20^{o}C.

7.2.2 Soil preparation and treatment procedure

A poorly drained Temuka silt loam soil [Fluvaquentic Endoaquept, (Hewitt 1998)] was collected from a clover-ryegrass pasture (0–10 cm depth) near Lincoln, New Zealand (43^{o}38.70'S, 172^{o}28.62'E, 8 m above sea level) in summer, 2009. The key properties of the soil are described in Table 7.1. The collected soil was sieved (4 mm) within 48 h of collection to remove any obvious organic material. The sampled (field moist) soil had gravimetric water content, volumetric water content, bulk density, and porosity values of 0.31 kg water kg^{-1} soil, 0.23 m^{3} water m^{-3} soil, 0.74 Mg m^{-3} and 0.72 m^{3} pores m^{-3} soil, respectively (Section 3.2). The sieved soil was then stored at its field moisture content, at 4^{o}C, before being packed into PVC containers. To obtain the plant litter, fresh clover leaves were dried (65^{o}C) and ground (<200 μm) before being incorporated into the soil samples.

Table 7.1 Chemical properties of the Temuka silt loam soil used during the study.

Soil properties	November, 2009
pH (1: 2)	5.7
Total C (g kg^{-1})	64.0
Total N (g kg^{-1})	6.0
Anaerobically mineralisable N (µg g^{-1})	218
Olsen P (mg kg^{-1})	48
Potassium ($cmol_c$ kg^{-1})	0.76
Calcium ($cmol_c$ kg^{-1})	14.9
Magnesium ($cmol_c$ kg^{-1})	3.31
Sodium ($cmol_c$ kg^{-1})	0.34
Cation exchange capacity ($cmol_c$ kg^{-1})	27
Total base saturation (%)	73

The clover litter had a C: N ratio of 9. In treatments receiving cellulose, appropriate proportions, were mixed with clover litter (5 g) on a plastic sheet to obtain 'cellulose-litter' mixtures with C: N ratios of 20, 30 and 40 which represented, 'C: N 20', C: N 30' and 'C: N 40' treatments, respectively. The 'cellulose-litter' mixtures were thoroughly mixed with 175 g dry soil before packing into PVC containers. Pre-mixing prevented possible effects (such as localized N rich microsites), on microbial degradation arising due to differences in soil-residue contact (Abiven and Recous 2007; Loecke and Robertson 2009). The cellulose-litter-soil mixture was packed into PVC containers to a depth of 4.5 cm (internal diameter 8.0 cm, height 10 cm). The base of the containers was covered by fine nylon mesh to prevent loss of soil material. Deionised water was added to the treatments to attain field capacity (86% WFPS). This WFPS provided a comparison with Chapter 6. The soil water content was maintained by daily misting the soil surface to a pre-determined mass. Soil cores were incubated at 20^{o}C for 145 d.

7.2.3 Soil analysis and gas sampling

Soil cores were destructively analysed on days 0, 9, 14, 21, 54 and 145 after treatment application for determining inorganic N and microbial biomass C using methods described in Sections 3.6 and 3.7, respectively.

The N_2O emissions were determined on 25 occasions over the first 42 d of the experiment using the closed-chamber technique (Section 3.10). Gas samples were collected at 0, 10 and 20 min on each occasion.. The gas samples were analysed, within 48 h, for N_2O

using gas chromatography (Sections 3.10 and 3.11) and N_2O fluxes were calculated using Equation 3.9. The cumulative N_2O emissions were converted to CO_2-eq kg^{-1} soil by multiplying them by 298, the global warming potential (GWP) of N_2O, (Forster *et al.* 2007) thus enabling comparisons with the cumulative CO_2 emissions.

Soil CO_2 emissions were measured from the same soil containers, approximately 10 min after the N_2O measurements, on 47 occasions over the experimental period of 145 d (Section 3.11). For each CO_2 sampling event, a portable soil respiration chamber (SRC), connected to an infrared gas analyser (SRC-1 and EGM-3, PP Systems, Hitchin, UK; Figure 6.2), was placed directly onto the PVC container and the emissions were determined over a 2 min period.

Emission factors (expressed as a percentage of the N or C applied) were calculated by determining the cumulative mass of N or C emitted (as N_2O-N or CO_2-C, respectively), subtracting the integrated control values, and dividing the difference by the mass of N or C applied to the soil in the form of leaf litter. The mean treatment responses were expressed as differences with respect to the unamended soil (control).

7.2.4 Data analysis

Statistical analysis of all data was performed using Minitab® (version 15.1; © 2006, Minitab Inc.). Gas emission data for each sampling was tested for skewness using the Anderson-Darling test and if required, the data were log-transformed to ensure normality. Analysis of variance (ANOVA) was performed to determine if differences between means occurred. If they did, significantly different treatments were found using Tukey's test. Statistical variation is indicated using standard deviation (sd) or least significant differences (lsd). Calculation of the emission factors and conversion of cumulative N_2O emissions to CO_2-eq kg^{-1} soil were calculated using same method used in Section 6.2.4.

7.3 Results

7.3.1 Soil inorganic N

Soil NH_4^+–N concentrations increased in all treatments immediately after treatment application (Figure 7.1a). On day 0, NH_4^+–N concentrations from the treatments were significantly higher than the control with 'clover only' = 'C: N 20', while the 'C: N 30' and 'C: N 40' treatments showed higher concentrations compared to the other treatments. Concentrations increased further in the 'clover only' and control treatments at day 9. However, at this time NH_4^+–N concentrations in the other treatments had declined. From day 9 onwards, NH_4^+–N concentrations continued to decline and from days 21 to 145, they

did not differ from the control in any treatment. Concentrations recorded on day 9 were 221.0 ± 38.4 > 101.3 ± 17.0 = 100.6 ± 20.9 = 97.1 ± 18.2 > 58.0 ± 16.3 µg N g^{-1} dry soil from 'clover only' > 'C: N 20' = 'C: N 30' = 'C: N 40' > control.

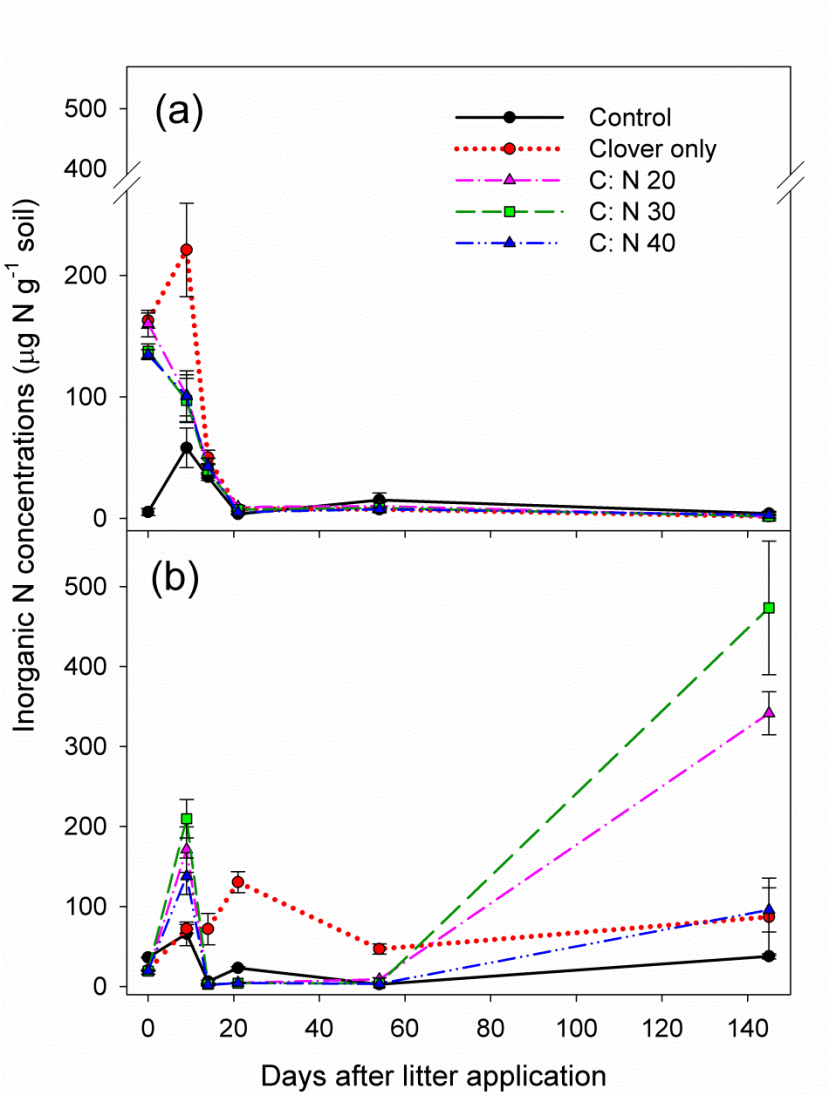

Figure 7.1 Soil inorganic N concentrations of (a) NH_4^+–N, and (b) NO_3^-–N, after a 145 d incubation following incorporation of clover litter with varying levels of cellulose. Data are mean ± sd (*n* = 5).

By day 9, NO_3^-–N concentrations in the cellulose-amended treatments ('C: N 20' to 'C: N 40') increased and were higher than the 'clover only' and control treatments (Figure 7.1b). After this they declined rapidly before they increased again at day 54, with concentrations at day 145 significantly higher in the 'C: N 20' and 'C: N 30' treatments. However, in the 'clover only' treatment, NO_3^-–N concentrations were significantly higher on days 14, 21 and 54 but they then returned to levels observed in the controls thereafter.

7.3.2 Microbial biomass C

On day 0, microbial biomass C (MBC) did not differ with treatment but then MBC concentrations were higher in the non-control treatments on days 9, 21 and 54 (P <0.05). A steep decline in MBC was observed on day 21 with no differences among treatments (Figure 7.2). Concentrations had increased by day 54 but did not differ from the controls on 145 d except 'C: N 40' treatment which was higher (P <0.001) from other treatments with recorded concentration of 4216 ± 125 µg C g^{-1} dry soil.

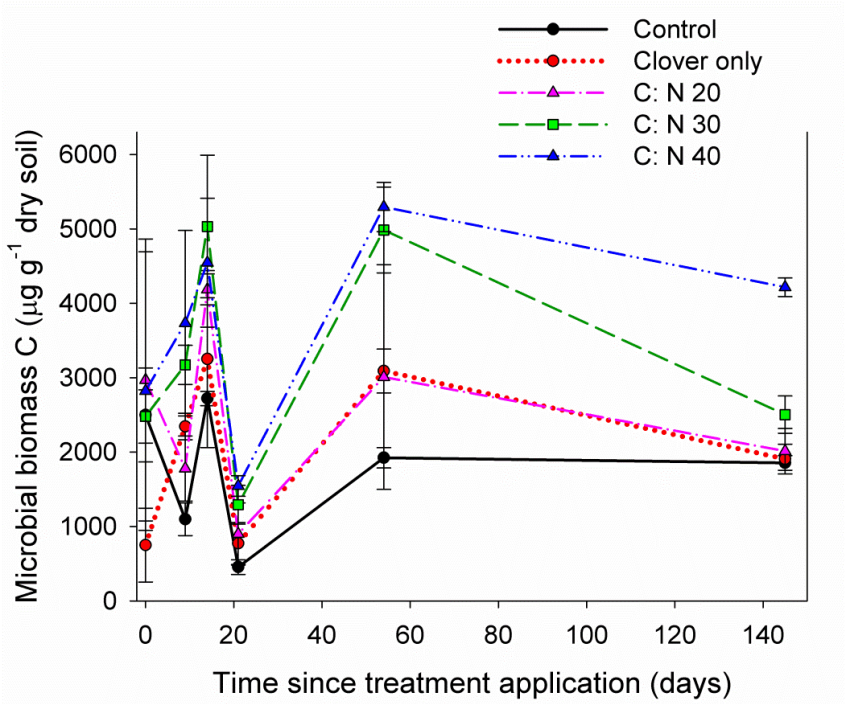

Figure 7.2 Soil microbial biomass C concentrations after a 145 d incubation following incorporation of clover litter with varying levels of cellulose. Data are mean ± sd (n = 5).

7.3.3 N_2O emissions

Maximum N_2O emissions were recorded 8 h after treatment incorporation, with N_2O emissions increasing with C: N ratio at this time and differing with treatment (P <0.05, Figure 7.3). After 8 h, the maximum emissions were 548 ± 81, 352 ± 104, 164 ± 61, 78 ± 25 and 28 ± 20 µg N_2O kg^{-1} h^{-1} (± sd, n = 5) for the 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control, respectively. After 11 h (0.8 d), N_2O emissions had decreased significantly and although emissions from the 'C: N 40' and 'C: N 30' treatments were higher than in the 'C: N 20' and 'clover only' treatments which were in turn higher than those of the control (Figure 7.3) these emissions had decreased by a factor of ~2, 6, 4 and 2, respectively, when compared to their earlier maxima (i.e. at 8 h). Emissions of N_2O then gradually declined

over time and had returned to levels found in the control by 12.4 d in all treatments. After this time, until day 42, the N_2O emissions from the treatments did not differ from the controls.

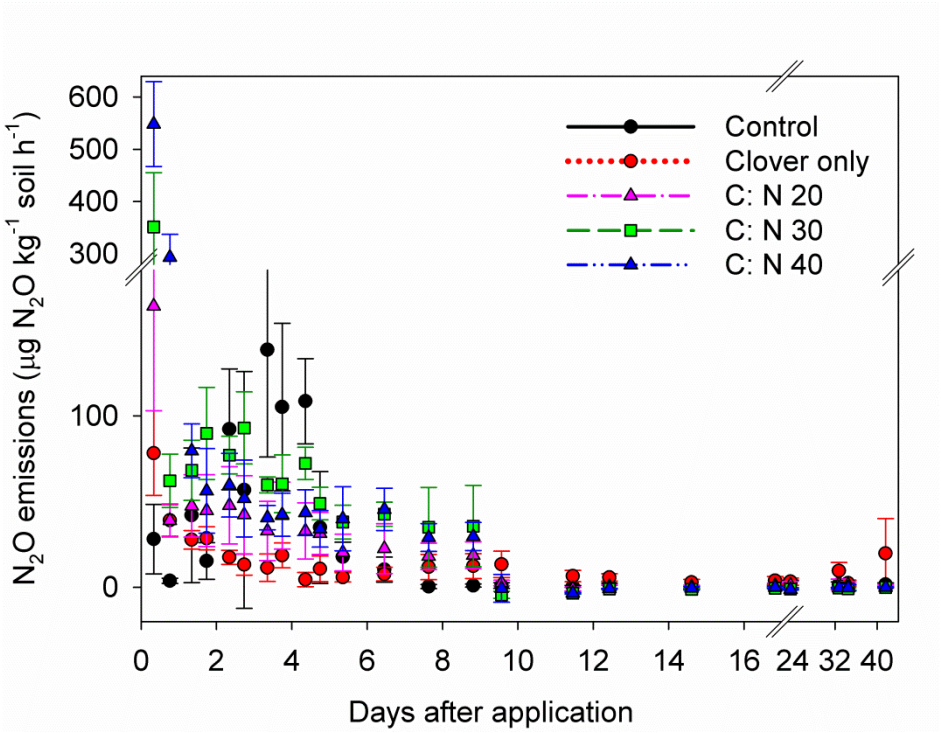

Figure 7.3 Soil N_2O emissions at 86% WFPS, during a 42 d incubation after incorporation of plant litter; dried, ground shoots of clover and/or increasing proportions of cellulose. Data are mean ± sd (*n* = 5).

Nitrous oxide emissions from the control did not remain constant but increased (P <0.05) at 1.3, 2.3 and 3.4 d when compared to the other treatments (Figure 7.3). At 1.3 d this represented a 10-fold increase in the control while the emissions from the other treatments decreased. Emissions from the control peaked at 3.4 d and decreased thereafter.

Cumulative N_2O emissions from the 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control were 14.3 ± 0.5, 12.4 ± 1.2, 8.0 ± 0.6, 8.7 ± 1.2 and 9.1 ± 0.9 mg N_2O kg^{-1} soil, respectively (Figure 7.4) with differences between treatments as follows: control = 'clover only' = 'C: N 20' < 'C: N 30' < 'C: N 40' (P <0.05). While the cumulative N_2O emissions from the control treatment did not differ from the 'clover only' and 'C: N 20' treatments, the time courses of these three treatments differed significantly with 90% of the cumulative emissions occurring within ~7, 38 and 9 d, respectively. Corresponding N_2O emissions from the 'C: N 30' and 'C: N 40' treatments had evolved 90% of the total N_2O emissions by ~9 d (Figure 7.4).

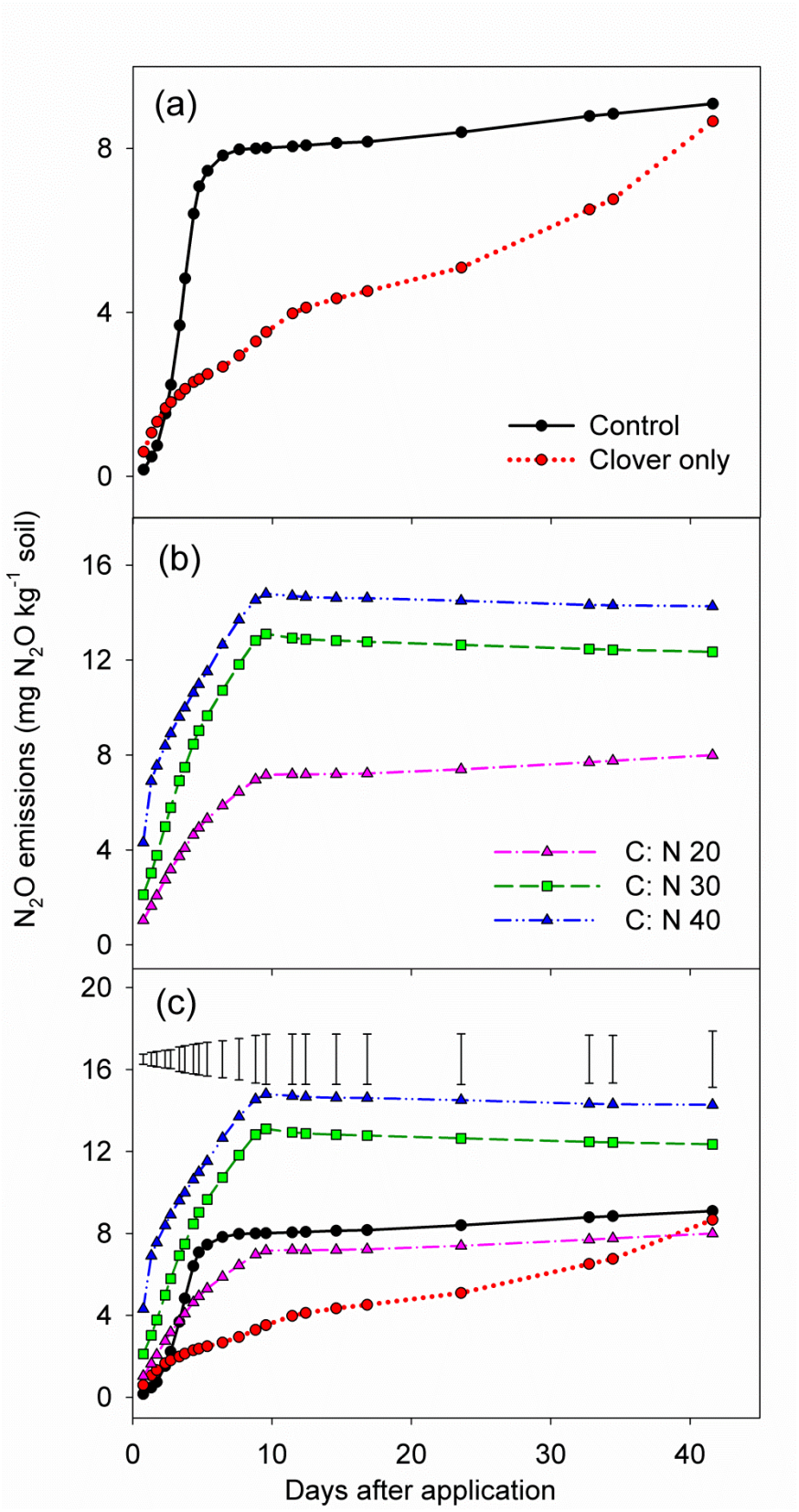

Figure 7.4 Cumulative N_2O emissions from (a) 'control' and 'clover only' treatments; (b) 'C: N 20', 'C: N 30' and 'C: N 40' treatments during a 42 d incubation; (c) error bars are least significant differences of mean (at 5% level of significance).

The EF_{N_2O} values (Figure 7.5) for the 'C: N 40', 'C: N 30', 'C: N 20' and 'clover only' were 0.23 ± 0.02, 0.14 ± 0.05, –0.05 ± 0.03 and –0.02 ± 0.05, respectively, with the EF_{N_2O} of 'C: N 40' significantly higher (P <0.001) than the other treatments.

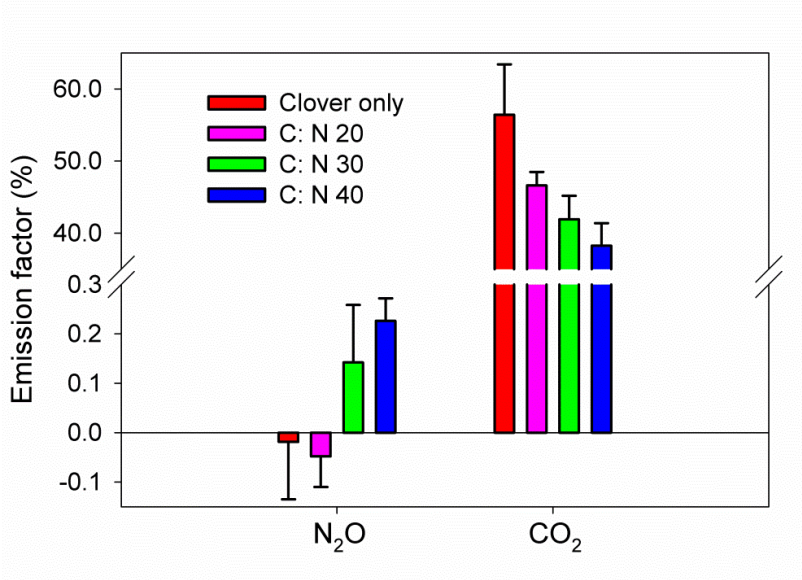

Figure 7.5 Emission factor (%) of N_2O and CO_2 after a 145 d incubation following incorporation of clover litter with varying levels of cellulose. Data are mean ± sd.

7.3.4 CO_2 emissions

Four hours after treatment application the CO_2 emissions were 43.3 ± 3.2 < 62.8 ± 8.7 = 54.2 ± 17.4 = 42.0 ± 8.3 > 6.7 ± 1 mg CO_2 kg^{-1} h^{-1} (± sd, n = 5) from the 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control treatments, respectively (Figure 7.6). The maximum CO_2 emissions occurred after 1.4 d with emissions of 86.2 ± 5.4 < 90.2 ± 6.6 > 80.7 ± 20.5 > 64.9 ± 14.3 > 4.9 ± 2.8 mg CO_2 kg^{-1} h^{-1} from the 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control treatments, respectively. The CO_2 emissions then declined steadily until 10.3–11.2 d when a further increase in CO_2 emissions was observed in all the treatments (Figure 7.6) but this increase was relatively small in the 'clover only' treatment. Furthermore, the 'secondary peak' in those treatments with cellulose additions was dependant on the rate of cellulose applied (i.e. 'clover only' < 'C: N 20' < 'C: N 30' = 'C: N 40'). The 'secondary peak' emissions were 60.3 ± 2.7, 58.1 ± 3.3, 52.1 ± 5.4, 27.5 ± 4.5 and 4.8 ± 0.5 mg CO_2 kg^{-1} h^{-1} from 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control, respectively which accounted for 3–4% of the total CO_2 emissions integrated over the 145 d. The emissions from the 'secondary peak' were significantly higher (P <0.05) from the non-control treatments but they did not differ between cellulose treatments, with the 'clover

only' treatment intermediate between these treatments. At 145 d, except 'C: N 40' treatment, the emissions from the other treatments had reached control levels.

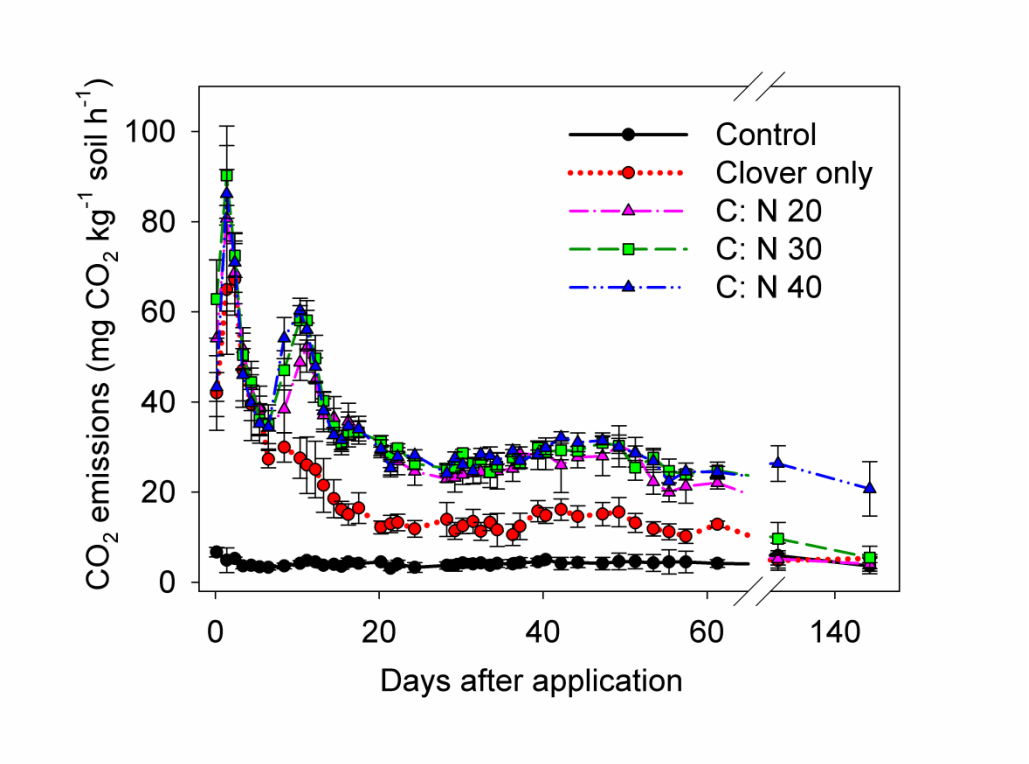

Figure 7.6 Soil CO_2 emissions from 'clover only', 'C: N 20', 'C: N 30' and 'C: N 40' treatments and controls during a 145 d incubation. Data are mean ± sd (*n* = 5).

A significant 'C dose effect' (P <0.05) was observed when CO_2 emissions were integrated over the entire incubation period, with the 'clover only' and 'C: N 20' treatments equalling the control treatment levels at 112 d followed by the 'C: N 30' treatment at 145 d. The cumulative CO_2 emissions over 145 d averaged 98.5 ± 3.0, 83.8 ± 2.3, 66.4 ± 0.9, 42.0 ± 1.4 and 16.6 ± 2.5 g CO_2 kg^{-1} soil from the 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control, respectively and were significantly different from one another. Over the period 42 or 145 d, CO_2 emissions from the controls averaged 4 and 17 g kg^{-1} soil and the 'C dose effect' was 0.15 and 0.38 g CO_2 g^{-1} C, respectively. Over 42 d, on a CO_2-equivalent basis, CO_2 emissions were ~90% of 'CO_2 + N_2O' emissions following clover and cellulose incorporation into the soil (Table 7.2). The EF_{CO_2} values were 38.3 ± 1.4, 41.9 ± 1.5, 46.6 ± 0.8 and 56.4 ± 3.1 in the order of 'C: N 40' = 'C: N 30' > 'C: N 20' << 'clover only', respectively (P <0.05).

Table 7.2 Cumulative N_2O (in CO_2-eq), cumulative CO_2 and total emissions at 86% WFPS integrated over 42 d. The data are mean ± standard error (*n* = 5).

Treatment	Cumulative N_2O emission (CO_2-eq)	Cumulative CO_2 emission ($g\ kg^{-1}$ soil)	Total emission[a] (CO_2+N_2O) (CO_2-eq)	Contribution of N_2O to total emission (%)
C: N 40	4.3 ± 0.1	36.3 ± 0.6	40.6 ± 0.7	10.6
C: N 30	3.7 ± 0.4	36.6 ± 0.3	40.3 ± 0.7	9.2
C: N 20	2.4 ± 0.2	34.3 ± 1.0	36.7 ± 1.2	6.5
Clover only	2.6 ± 0.4	21.3 ± 1.0	23.9 ± 1.4	10.9
Control	2.7 ± 0.3	4.2 ± 0.2	6.9 ± 0.5	39.1

[a]Total emission (CO_2-eq) is the sum of the cumulative N_2O emission (CO_2-eq) and cumulative CO_2 emissions. After 145 d, the cumulative values for 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control were 98.5 ± 3.0, 83.8 ± 2.3, 66.4 ± 0.9, 42.0 ± 1.4 and 16.6 ± 2.5 g CO_2 kg^{-1} soil.

7.4 Discussion

7.4.1 N_2O emissions

The incorporated cellulose acted as a more labile C source apparently favouring denitrification since higher N_2O emissions were observed from the highest C: N ratio treatments, hence the hypothesis was rejected.

Relatively higher inorganic N concentrations on day 9, a decrease in N_2O emissions after 12.4 d, and an increase in MBC concentrations during day 9 to day 14, showed that microbial activity was maximum during the initial period (0–14 d) of incubation. Increases in NH_4^+–N concentrations from day 0 in all but the control and in the 'clover only' treatment at day 9 indicated ammonification of the plant litter (Wei *et al.* 2011). The subsequent decline in NH_4^+ was due either to nitrification of NH_4^+ or immobilisation. The increase in NO_3^- indicated that nitrification occurred. The rapid decline in NO_3^- and lower levels until day 54 in the cellulose-added treatments demonstrate a period of NO_3^-–N consumption which given the additional C source was probably the result of immobilisation, since NO_3^- concentrations in 'clover only' remained higher at that time. The N_2O concentrations were also higher while NO_3^- was declining. Cumulative N_2O increased with increasing C: N ratio which indicated denitrification of NO_3^-, originating from plant litter which was the source of the N_2O.

Kuzyakov *et al.* (2000) suggest that when an organic substrate has a C: N ratio >16, N immobilisation occurs as a result of micro organisms using the available N for their own growth in cells and tissues. Such a relation was not found in this study, since in the treatments with C: N >16, NO_3^-–N increased 10-fold during 0–9 d indicating N mineralisation which corresponded well with higher N_2O emissions. However, a part of this mineralisation from the treatments can be attributed to a 'disturbance effect' (caused due to soil sieving) since a large increase in NH_4^+–N and NO_3^-–N levels was also observed in the control during the same time period. Presumably the soil disturbance effect was enhanced in the control treatment because the soil was subject to mechanical sieving which might have exposed the N in the soil aggregates, however, this might not have happened in the control treatment in Chapter 6 because the sampled soil was manually sieved. Nevertheless, clover litter caused an increase in NH_4^+–N and NO_3^-–N concentrations. Goek and Ottow (1988) applied oat straw and cellulose at two rates (0.5 and 1.0%) and found that denitrification losses were similar for both of their treatments but suppressed after straw and cellulose incorporation. Their treatment also increased CO_2 production and NH_4^+ immobilisation. Mengel and Schmeer (1985) also observed that N_2O emissions decreased after incorporating straw, cellulose or lignin to soil because of N immobilisation under the influence of the added substrates. Immobilisation may also have occurred in the present study but N_2O emissions were enhanced with increasing rates of cellulose in this study as opposed to the aforementioned studies.

Cellulose occurs naturally in plant tissues and forms the basis of plant cell walls. The extracellular enzymes have to catabolise/cleave the bound cellulose to simpler sub-units i.e. glucose, for use in energy generation processes (Clark 1997). In the present study, cellulose was applied in a pure, uncleaved form, which potentially allowed the microorganisms to use it as a readily available source similar to glucose.

Cellulolytic microorganisms' activity is enhanced under anaerobic conditions (Clark 1997). In this study, incorporating abundant cellulose-C and litter may have aided in creating anaerobic conditions by blocking soil pores and reducing the O_2 diffusion rate. The high soil moisture content (86% WFPS) would also have produced conditions that were conducive for the cellulolytic organisms. This would produce higher CO_2 emissions due to enhanced decomposition of the incorporated cellulose; this is in fact what was observed (Figure 7.6). Higher CO_2 emissions in turn, may have contributed to the generation of anaerobic conditions within the microsites, thus further stimulating denitrifying activity, and as a consequence, this could have contributed to the higher N_2O emissions. Moreover, under partially anaerobic conditions and in the presence of labile C substrate, the denitrification process could be

pushed towards completion and more N_2 may have been produced from the treatments rather than just N_2O. This may explain the decline in cumulative N_2O emissions with increasing cellulose (Figure 7.4c).The control treatment had less labile C and the higher N_2O emitted until 3.4 d from the control may have been a consequence of incomplete denitrification

The presence of available C can increase denitrification, directly, by increasing energy and electron supply to the denitrifiers, and indirectly, by enhancing microbial growth and metabolism, thereby stimulating higher O_2 consumption (Beauchamp *et al.* 1989; Gillam *et al.* 2008) which creates anaerobic conditions. The current results cannot delineate the N_2O production mechanism but given the soil moisture content and the fact that cellulose was being utilised as evidenced by higher CO_2 in the treatments, and that the N_2O emissions were lower than the control during 2.3–4.3 d; it is likely that the C substrate further enhanced denitrification and permitted the further reduction of N_2O to N_2 (Firestone and Tiedje 1979).

7.4.2 CO_2 emissions

The CO_2 emissions curve peaked twice during the course of incubation presumably due to 'sequential degradation' (Gunnarsson *et al.* 2008) and 'preferential substrate utilisation' (Kuzyakov and Bol 2006; Sparling *et al.* 1982).

Previous studies have shown that during the first few days of litter decomposition in soil, soluble carbohydrates are decomposed along with the most readily degradable N-rich components. After this, proteins and cellulose are sequentially degraded followed by lignin (Gunnarsson and Marstorp 2002; Henriksen and Breland 1999; Martin and Haider 1986; Trinsoutrot *et al.* 2000a). The first CO_2 peak (at 1.4 d) was presumably due to the decomposition of the most labile C pools supplied from the litter and mineralisation of the native soil C. This trend in N_2O emissions was similar to the previous lab experiment (Chapter 6) using plant litter only. The second (smaller) peak was evidently due to the decomposition of cellulose since the emissions followed a linear C dose effect. This was also apparent from the increase in MBC concentrations from days 9 and 14 possibly due to an increase in cellulolytic microorganisms. The higher emissions from the 'C: N 40' treatment also coincided with higher MBC concentrations at the end of the incubation period indicating the availability of cellulose for the cellulolytic microbes. The protracted and continuous CO_2 emissions (greater than the control) over time suggest that these emissions were from the recalcitrant pools such as hemicellulose and lignin. The above results are similar to Dalenberg and Jager (1989) who also observed two peaks in the CO_2 curve after addition of cellulose (as pulverised filter paper (Whatman® 1)), wheat straw or sewage sludge. The explanation for the two peaks was the same in their study however their first peak was smaller than the second

and they attributed it to the faster decomposition of cellulose. There is no obvious explanation for this discrepancy other than it may reflect differences in soils or treatment procedures.

The higher CO_2 emissions from the treatments also suggest that the soil microbial biomass may have switched from the recalcitrant SOM to the incorporated and more readily available substrates (Cheng 1996; Sparling *et al.* 1982). These higher emissions can be accounted for by *r*-strategists; the microorganisms specialised in rapid catabolism of the fresh organic matter in soil (Fontaine *et al.* 2003), i.e. litter and cellulose in this case. When the most easily utilisable substrate is almost completely consumed, the activated microorganisms target the remaining substrates with the highest utilisability and use these until there is no substrate, otherwise known as 'preferential substrate utilisation' (Kuzyakov and Bol 2006).

Naturally occurring lignin present in the plant tissues physically protects, and therefore retards the catabolism of the bound cellulose but absence of lignin may aid in faster decomposition of cellulose (Fuller and Norman 1943; Swift *et al.* 1979). Grinding of the plant litter in the present study may have exposed the labile forms due destruction of the lignified barrier tissue (Ambus and Jensen 1997). Hence, the grinding process, evidently promoted cellulose decomposition in the presence of very low and destroyed lignin content due to grinding.

Available N can also stimulate decomposition rates by increasing the activity of enzymes responsible for carbohydrate hydrolysis (Carreiro *et al.* 2000; Geisseler and Horwath 2011). The mineralisation of N from all the 'treatments' during 0–9 d of destructive sampling, suggest that the labile-N originating from the plant litter in the present study, may have stimulated the cellulose decomposition and hence caused higher CO_2 generation with an additive effect on N_2O generation.

Nicolardot *et al.* (1994) found that cellulose can be rapidly decomposed under optimal conditions but its decomposition is roughly 3–5 times slower than that of glucose. Nicolardot *et al.* (1994) also showed a rate constant of 0.2–0.3 d^{-1} at 28^{o}C for cellulose decomposition i.e. cellulose was virtually completely decomposed in 15–25 d. In the current study, the secondary peak of CO_2 emissions, which is assumed to be the result of cellulose decomposition, was nearly complete in ~15 d which is accordance with Nicolardot *et al.* (1994).

Geisseler and Horwath (2011) incorporated 2, 4 or 6 g cellulose kg^{-1} soil along with $(NH_4)_2SO_4$ to obtain C: N ratios of 10 and 40 and incubated soils at 22^{o}C for 30 d. They observed EF_{CO_2} values of 34–40% and cumulative emission of 1.6 g CO_2-C kg^{-1} soil (from the 6 g cellulose kg^{-1} soil treatment). In the present study, the EF_{CO_2} range was 38–56% and the cumulative emissions were 4.2–6.8 g CO_2-C kg^{-1} when integrated over 27 d which is only

2−4 times higher than that observed by Geisseler and Horwath (2011), in addition to the fact that the present study included a much higher (i.e. about 6.3–17.2 times, 38–103 g cellulose kg^{-1} soil) cellulose incorporation rate. They also found enhanced cellulase activity under the influence of added N. Fontaine *et al.* (2004) applied 0.5 g cellulose-C kg^{-1} soil and incubated at 28^{o}C. In their study, after 70 d, cumulative CO_2 emissions accounted for 0.9 g CO_2-C kg^{-1} from cellulose-amended soil. When compared to their study, nearly 7 times less CO_2-C was evolved kg^{-1} soil in the present study. This difference was apparently due to the fact that they also amended their soils with cellulase enzyme amendments and hence better and quicker cellulose degradation was observed. Gillam *et al.* (2008) observed 0.54, 0.26 and 0.15 g CO_2-C g^{-1} C from glucose, red clover and barley straw, respectively while we got a C dose effect of 0.38 g CO_2 g^{-1} C in the present study.

7.5 Conclusion

Over 42 d, cumulative N_2O emissions from the controls were statistically indistinguishable from the 'clover only' and 'C: N 20' treatments. However, time courses of the N_2O emissions differed significantly; 90% of the total emissions were complete in ~7, 38 and 9 d for controls, 'clover only' and 'C: N 20' treatments, respectively. Corresponding cumulative N_2O emissions from the 'C: N 30' and 'C: N 40' treatments were nearly 50% greater and 90% of the total N_2O emissions were completed in ~9 d. Thus, clover incorporation produced the most rapid N_2O emissions response and increasing the C: N ratio using cellulose to ≥ 40 significantly enhanced the rate of N_2O emissions and total emissions, when compared to the response to clover incorporation.

Chapter 8
Determine the effect of surface-placed, fresh plant litter on N_2O emissions in field conditions

8.1 Introduction

Chapter 4 of this thesis showed that significant litter-fall occurs during a grazing event (Pal *et al.* 2012). However, the *in situ* contribution of this litter to N_2O emissions is unknown. Litter may either sit on the soil surface and decompose or it may become partially incorporated into soil via animal treading. Surface decomposition of pasture litter in conjunction with N_2O emissions has not been researched in pastoral soils. A few studies (Brunetto *et al.* 2011; Larsson *et al.* 1998) have investigated surface decomposition using pasture litter as a mulch in arable systems; De Ruijter *et al.* (2010) measured NH_3 emissions from surface decomposition of 'pasture topping', but no studies have reported N_2O emissions in pastoral systems resulting from pasture litter.

Previously, in Chapter 6, the complete incorporation of litter species dominant in New Zealand pastures was investigated. The N_2O emissions increased with lower C: N ratios and labile biochemical components. However, the experiment was performed under controlled conditions and the litter was ground and thoroughly incorporated into soil. Litter, in field conditions is not incorporated to this extent and certainly not ground. Also, temperature and soil water contents fluctuate under field conditions. Studies investigating pasture litter decomposition in pastoral conditions are also scarce (Kuzyakov *et al.* 1999; Vinten *et al.* 2002). Brunetto *et al.* (2011) investigated the fate of common New Zealand pasture species (clover, *Trifolium repens* L. and ryegrass, *Lolium perenne* L.) by placing litterbags on the soil surface, and used a ^{15}N technique to show that the grasses decomposed relatively rapidly, however, N_2O emissions were not measured in their study and this study was performed in vineyards. Pasture management and soil nutrient parameters are considerably different to conditions found in vineyards.

Since no study has examined the fate of pasture litter with respect to N_2O emissions under field conditions the following experiment was conducted to quantify N_2O emissions resulting from surface-placed litter of ryegrass shoots in order to investigate the contribution of such litter, to soil N cycling and N_2O emissions.

The study performed here includes defoliation treatments in order to simulate grazing. Studies such as Bardgett *et al.* (1998) and Mikola *et al.* (2001) have shown that defoliation of

pasture plant species can also influence N and C cycling. Macduff and Jackson (1992) showed that defoliation of Italian ryegrass (*L. multiflorum*) and white clover in hydroponic culture increased root-efflux of NH_4^+– and NO_3^-–N. Root decomposition may also contribute to N_2O emissions due to effects on N cycling (Smith and Tiedje 1979a). To differentiate any artefact of defoliation that could contribute to N_2O emissions, treatments in this experiment also included defoliation and root removal. The choice of ryegrass was based on it being the dominant pasture species. The hypothesis tested was:
Surface decomposition of litter will result in N_2O emissions over and above those found in non-litter affected soil.

8.2 Materials and methods

8.2.1 Experimental site and soil preparation

The field site was located at a pasture site of Lincoln University (43°38.50'S, 172°27.17'E; elevation above sea level 10 m). The soil was a Temuka clay loam [Typic Orthic Gley; (Hewitt 1998)]. Pasture species included perennial ryegrass and white clover grazed regularly by sheep. To avoid antecedent effects of animal grazing, the experimental site (approximately 15 m x 20 m) was surrounded by an electric fence one year prior to the start of the experiment. The experiment was conducted in summer, 2011 (January-February). To maintain typical fresh pasture growth, the fenced-off area was mown to a 5 cm height at 30 d intervals with all the mown herbage removed (Figure 8.1). For ease of gas sampling during the experimental period, the pathways surrounding the gas chambers were mown to ground level as shown in Figure 8.1.

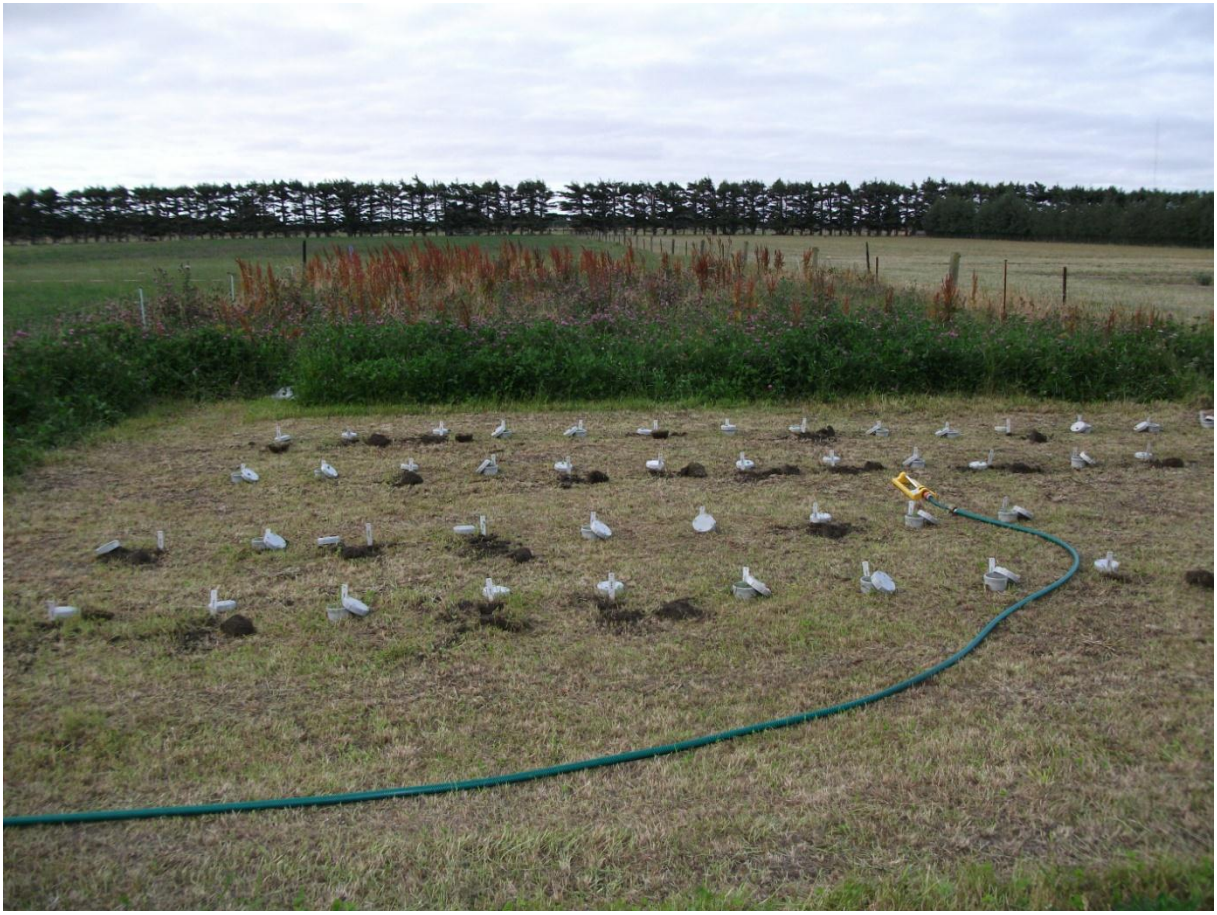

Figure 8.1 View of the experimental site with treatments in preparation.

Ryegrass seeds were sown in washed sand with five seeds per pot (14 cm diameter, 10 cm height) and fed with 20 mL, 1x Hoagland's solution (Hoagland and Arnon 1950) everyday for 3 months in a glasshouse (Figure 8.2). The plants were then enriched with a solution of ^{15}N enriched, ammonium sulphate [$(NH_4)_2SO_4$); 10.4 atom%; Isotec Inc., Matheson, USA] at the rate of 50 kg N ha^{-1} (i.e. at 15 mL pot^{-1}; 77 mg N pot^{-1}) in two split applications over a 15 d period (Section 3.12.1). Shoots of the ryegrass plants were cut at ground level and chopped into 1 cm pieces using scissors on the day of litter treatment application at the field site. Roots of these plants were also washed thoroughly removing any sand and debris, and also chopped into 1 cm pieces and inserted into litterbags as explained below. Subsamples of the litter (shoots and roots) were dried at 65^{o}C for 48 h and analysed for hemi-cellulose, cellulose, lignin, total C, total N (Section 3.1) and ^{15}N enrichment (Section 3.12.2) and the results are shown in Table 8.3.

Figure 8.2 Ryegrass seedlings in pot cultures in the glasshouse prior to shoot and root harvest for litter treatment application.

8.2.2 Experimental design and treatment application

The experimental design was a randomised block design with 6 treatments (Figure 8.3, Table 8.1) each replicated 5 times. The fresh shoots or roots, as applicable (Section 8.2.1), were inserted into circular litterbags (1 mm mesh size, 7 cm diameter; Figure 8.4) made of fibreglass mesh and stapled at the ends to avoid loss of litter and then pinned to the soil surface. The mesh size and material were selected to allow optimum access to soil invertebrates and had been previously used (Hobbie and Vitousek 2000). Soil PVC containers (internal diameter 8 cm, height 10 cm) were installed at the field site to a depth of 5 cm. The litterbags were then placed on the soil surface (or buried, as applicable) within the PVC containers' surface area. Further, identical treatments, replicated 3 times, were also installed to allow destructive sampling of soil at days 66 and 139 after treatment application.

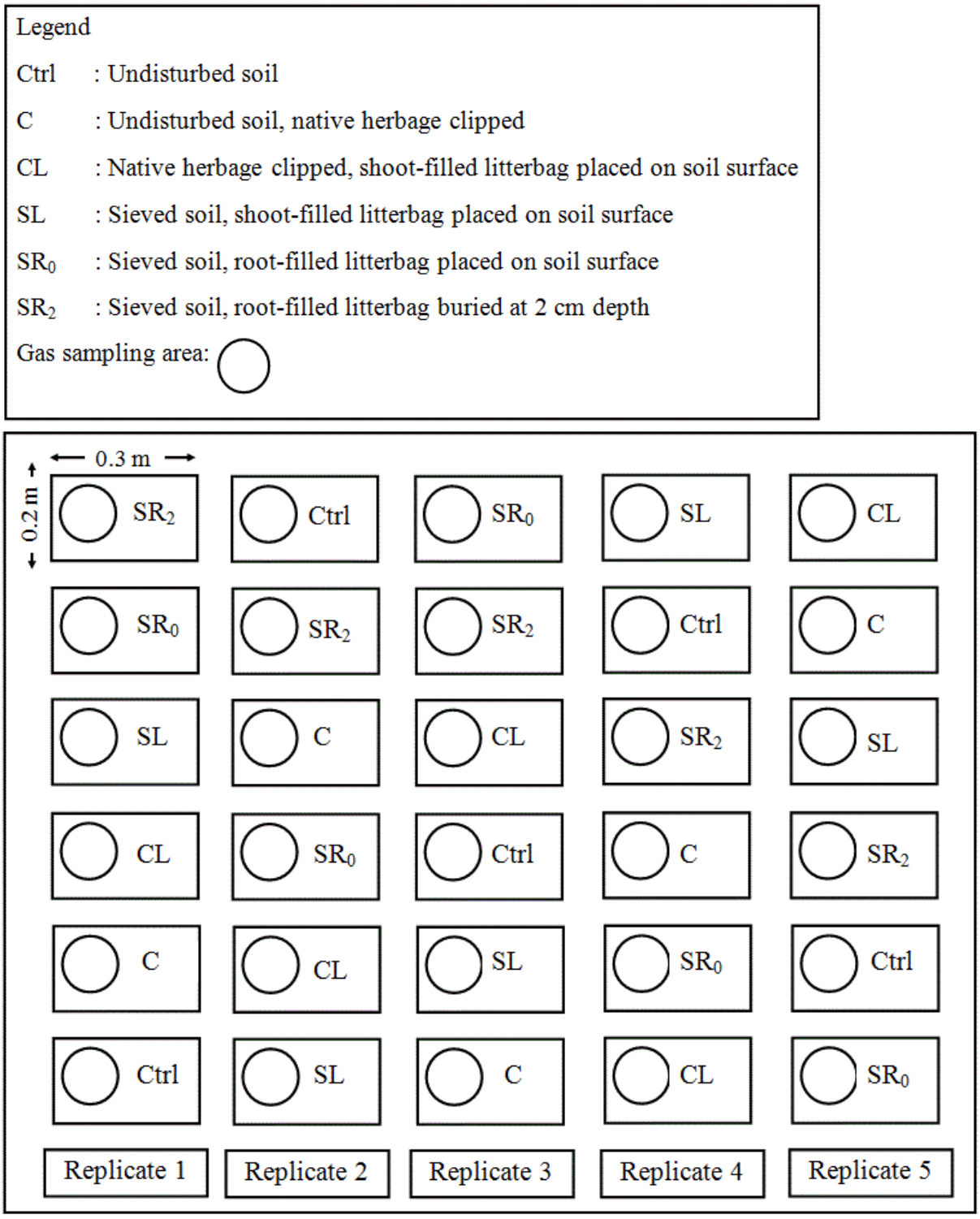

Figure 8.3 Field layout of the experimental site showing the position of the gas chambers. Treatment abbreviations are discussed in the text (Section 8.2.2 and Table 8.1).

Figure 8.4 Litterbags made from ryegrass shoots (left) and roots (right).

Treatment 1 was the 'true' control where neither the pasture was clipped nor ^{15}N shoots/roots were added into the litterbags. Hence, for the 'control' the pasture within the PVC cores was 5 cm high at the time of treatment application. For treatment 'C', the pasture was clipped to ground level but no shoot/root material was added into the litterbag. This treatment simulated field conditions immediately after a grazing event devoid of litter being deposited onto the soil surface. For treatment 'CL', the pasture was clipped to ground level and the ^{15}N-enriched, freshly-chopped ryegrass shoot litter (51.7 mg g^{-1}, total N; 5.35 atom%; 412 g DM m^{-2}), inside the litterbags, was placed on the surface of the soil inside the PVC cores. This equated to a rate of 9 g bag^{-1} i.e. 1.8 kg fresh shoots m^{-2} (resulting in an N application rate of 213 kg N ha^{-1}). This 'shoots only' treatment simulated a situation where litter falls onto an area of harvested pasture. However, the application rate of the litter was about 4 times the rate of litter-fall per grazing event previously measured [Chapter 4; (Pal *et al.* 2012)]. This rate was used to ensure that N_2O gas emissions were above the detection limit of the gas chromatograph (GC). For treatment 'SL', the native soil was dug out to a depth of 5 cm (Figure 8.5) and then sieved to remove roots (4 mm sieve size) which might potentially have contributed to N_2O emissions via root decomposition (Smith and Tiedje 1979a). This soil was then packed, to a previously determined field bulk density (1.08 ± 0.03 Mg m^{-3}), back into the PVC containers to the same initial depth of 5 cm. Litterbags, were then placed on the soil surface of the repacked soil cores for the SL treatment. The PVC containers were covered by a fine mesh at the base to avoid migration of soil material as a result of irrigation.

Since pasture was clipped to ground level in the 'C' and 'CL' treatments, it was considered possible that N cycling could be enhanced as a result of root decomposition and thus N_2O emissions might be enhanced, hence, root treatments were also introduced to evaluate the net effect of root decomposition on N_2O emissions in the absence of litter. For

treatments SR_0 and SR_2, soil was sieved in a similar manner as for the 'SL' treatment, and litterbags containing roots (5 g bag^{-1} i.e. 1.0 kg fresh roots m^{-2} (6.4 mg g^{-1}, total N; 3.99 atom%; 229 g DM m^{-2}), that resulted in an N application rate of 15 kg N ha^{-1}; were placed on the soil surface or buried to a depth of 2 cm, respectively.

Table 8.1 Treatment details used in the present study.

Treatment notation	Pasture clipping	Litter addition	
		Shoots	Roots
Control	× (pasture–5cm high)	×	×
C	✓	×	×
CL	✓	✓ (chopped–1cm)	×
SL	× (sieved soil–4mm)	✓	×
SR_0	× (sieved soil)	×	✓- surface applied
SR_2	× (sieved soil)	×	✓ - buried (2cm)

× denotes absence of pasture clipping and/or litter addition.
✓ denotes presence of pasture clipping and/or litter addition.

Figure 8.5 The sieved soil treatment in preparation at the field site.

8.2.3 Soil-, herbage- and gas-sampling and micrometeorological measurements

General soil properties at the field site were determined using a commercial laboratory; Hill Laboratories, Hamilton, New Zealand. Thirty soil cores (2.5 cm diameter, 7.5 cm deep) from the experimental site were collected, bulked, and submitted for analysis (n = 1).

Destructive analysis of the soil within the PVC cores was performed on 3 occasions (day 0 – 21st January, day 66 – 27th March and day 139 – 8th June) after treatment application. Field-moist soil cores were collected using a soil corer (7.5 cm deep × 2.5 cm diameter) from within each PVC core (3 replicates per treatment), sub-divided to depths of 0–2, 2–4 and 4−6 cm, and analysed for inorganic N, microbial biomass nitrogen (MBN) and WSC (Sections 3.6, 3.8 and 3.9, respectively). In the sieved soil treatments (SL, SR_0 and SR_2), the sieved soil was not deep enough to be collected at 4–6 cm; hence it was only collected at the first two depths. The soil extracts taken for MBN analysis were further analysed for ^{15}N enrichment of the microbial biomass [(MB-^{15}N); Section 3.8.2] using the method of Templer *et al.* (2003) and calculated as follows (Equation 8.1).

$$MB^{15}N = \frac{(TN_F \times Atom\%_F)}{100} - \frac{(TN_{NF} \times Atom\%_{NF})}{100}$$

Equation 8.1

where;

$MB^{15}N$ = microbial biomass ^{15}N (μg ^{15}N g^{-1} dry soil)

TN_F = total dissolved N of fumigated soil (μg N g^{-1} dry soil)

$Atom\%_F$ = atom% of the total dissolved N of the fumigated soil (atom%)

TN_{NF} = total dissolved N of the non-fumigated soil (μg N g^{-1} dry soil)

$Atom\%_{NF}$ = atom% of the total dissolved N of the non-fumigated soil (atom%)

Soil temperature (107-L; Campbell Scientific, USA) and soil water content (CS616-L; Campbell Scientific, USA) were monitored using sensory probes inserted in the soil at 2.5 and 5.0 cm depths. Air temperature and rainfall data were monitored on a daily basis with data logged accordingly (CR23X; Campbell Scientific, Logan, Utah, USA).

Gas sampling for N_2O and CO_2 emissions was performed on 16 occasions from 21 January (day 1) to 10 March, 2011 (day 49) when the emissions had reached control levels. On each gas sampling occasion, 10 mL gas samples were manually drawn, using glass syringes fitted with three-way taps, and compressed into 6 mL Exetainer® vials (Labco Ltd,

High Wycombe, UK) at 0, 30, and 60 min, after positioning the headspace cover. The gas samples were analysed, within 48 h, for N_2O and CO_2 using gas chromatography (Section 3.11, Equation 3.9). Three hours after gas sampling, a further 15 mL headspace gas sample was drawn and put into 12 mL Exetainer® vials. These samples were equilibrated to atmospheric pressure immediately before analysis for N_2O-^{15}N enrichment using IRMS (Section 3.12.3). Emission factors (expressed as a percentage of the N applied) were calculated by determining the cumulative mass of N_2O-N emitted, subtracting the integrated control values, and dividing the difference by the mass of N applied to the soil in the form of either shoots or roots.

8.2.4 Statistical analysis

Gas emission data on each gas sampling occasion and the cumulative emissions were tested for normality using the Anderson-Darling test and if the data was skewed then it was log transformed [ln(flux+1)] to attain normality (Press *et al.* 1989). The statistical software Minitab (version 15.1; © 2006, Minitab Inc.) was used to perform the analysis of variance (ANOVA) on the emission data to determine if treatment means were equal. Treatment differences were tested using Tukey's test. All data presented here are mean ± sd.

8.3 Results

8.3.1 Meteorological measurements and soil and herbage characteristics

Volumetric water content (θ_v) of the soil ranged from 0.20 to 0.46 m^3 water m^{-3} soil at 5 cm soil depth and fluctuated with irrigation and rainfall. The highest daily rainfall event recorded during the experimental period was 32.3 mm (Figure 8.6). The average daily soil temperature (5 cm depth) ranged from 5.1 to 27.9°C, following trends in the average daily air temperature which ranged from 5.0 to 34.5°C (Figure 8.6). General properties of the soil at the field site are shown in Table 8.2.

Table 8.2 Chemical properties of the soil at the field site.

Soil properties	Lincoln university (Jan, 2011)
pH (1: 2)	6.0
Total C (g kg^{-1})	31
Total N (g kg^{-1})	2.8
Anaerobically mineralisable N (µg g^{-1})	61
Available N (kg ha^{-1})	89
Olsen P (mg L^{-1})	13
Potassium ($cmol_c$ kg^{-1})	0.34
Calcium ($cmol_c$ kg^{-1})	6.9
Magnesium ($cmol_c$ kg^{-1})	1.19
Sodium ($cmol_c$ kg^{-1})	0.18
Cation exchange capacity ($cmol_c$ kg^{-1})	14
Total base saturation (%)	60

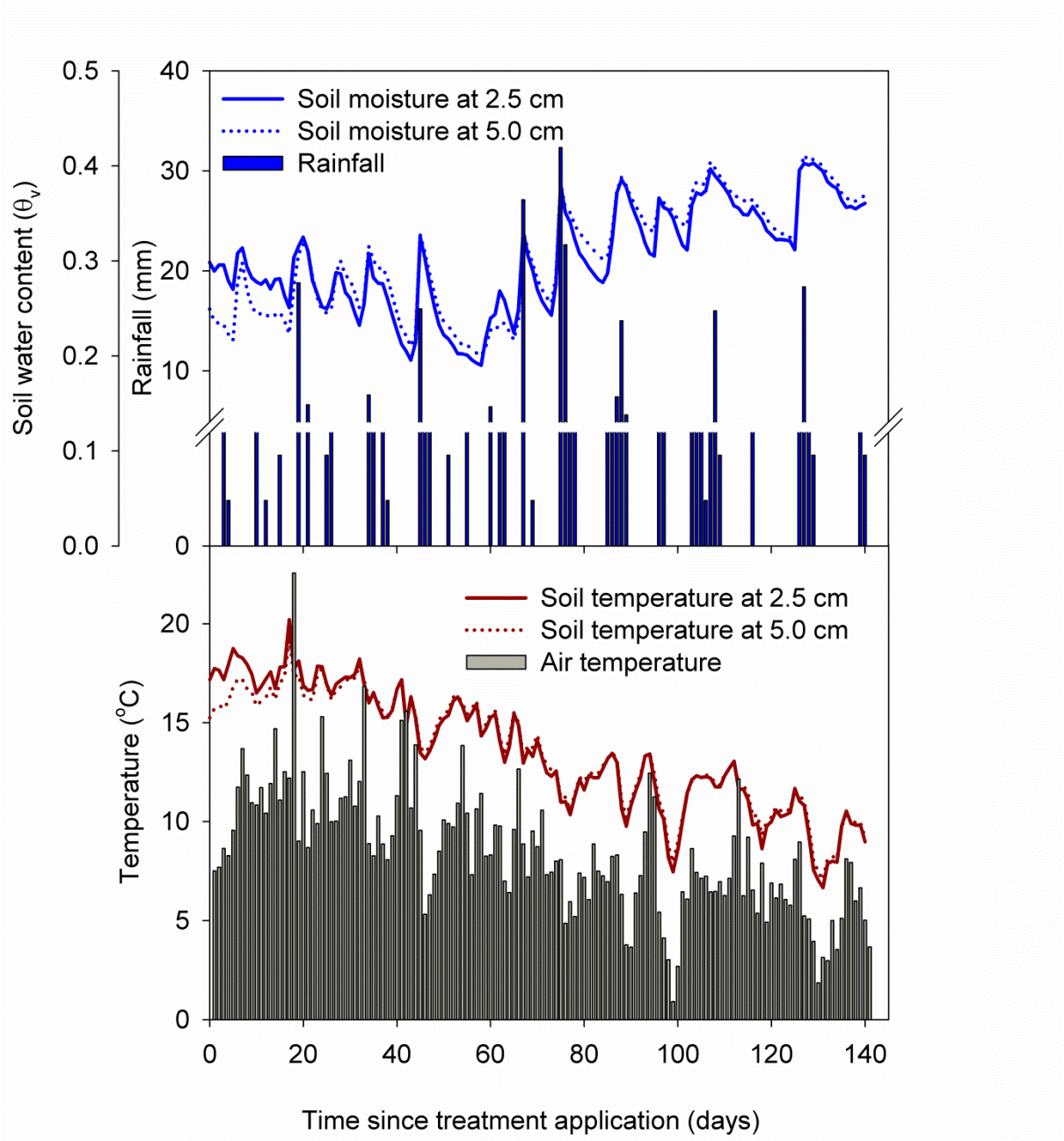

Figure 8.6 Meteorological data during the experimental period (20 January – 9 June, 2011) at the field site.

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Ryegrass shoots that were placed into the litterbags had 408 mg C g^{-1}, 51.7 mg N g^{-1}, 191 mg g^{-1} hemi-cellulose, 398 mg g^{-1} cellulose, 26.3 mg g^{-1} lignin and a C: N ratio of 8 while the ryegrass root material had 237 mg C g^{-1}, 6.4 mg N g^{-1}, 188 mg g^{-1} hemi-cellulose, 318 mg g^{-1} cellulose, 94.1 mg g^{-1} lignin and a C: N ratio of 37: 1, respectively. The pasture at the field site contained 410 mg C g^{-1}, 28.5 mg N g^{-1}, 183 mg g^{-1} hemi-cellulose, 387 mg g^{-1} cellulose, 23.2 mg g^{-1} lignin and a C: N ratio of 14. Enrichments of ^{15}N in the ryegrass shoots, roots and pasture on the day of treatment application were 5.35 ± 0.01, 3.99 ± 0.06, and 0.3668 ± 0.02 atom%, respectively. Over time, N contents of the shoot material decreased by 3-fold and 2-fold of its value on day 0 in the 'CL' and 'SL' treatments, respectively (Table 8.3). However, the N contents from the respective treatments did not change between days 66 to 139. The N content of the root treatments had doubled by day 66 when compared to day 0 values but values at day 66 did not differ from day 139 for the root treatments. Compared to day 0 values, the C content of the shoot material decreased significantly (P <0.05) over time in the shoot treatments but it did not differ in the root treatments on any day of destructive sampling. The fastest decline in ^{15}N enrichment of the shoot material occurred in the 'CL' treatment (Table 8.3) and on day 66 it was half its original value on day 0, and by day 139, it had decreased to 0.90 atom%. The values decreased by a factor of 3 in the root treatments by day 139 when compared to values at day 0.

Dry matter loss of the shoot material from the litterbags showed that 82 ± 7 and 46 ± 11% was lost from the 'CL' and 'SL' treatments by day 66, respectively. Litter mass in these treatments at day 139 was 0.7 ± 0.5 g bag^{-1} and 1.2 ± 0.1 g bag^{-1}, respectively. Corresponding values for roots in the 'SR_0' and 'SR_2' treatments were 50 ± 8 and 57 ± 5% at day 66 which increased to be 53–84%, respectively, at day 139.

Table 8.3 Characteristics of the residual litter in the litterbags over time.

Treatment	^{15}N (atom%)	C content (mg g^{-1})	N content (mg g^{-1})
	Day 0		
Shoot litter	5.35 ± 0.01^{a}*	408 ± 20.2^{a}	51.7 ± 0.6^{a}
Root litter	3.99 ± 0.06^{A}	237 ± 11.1^{A}	6.4 ± 1.5^{A}
Existing pasture	0.367 ± 0.02	410 ± 15.0	28.5 ± 1.7
	Day 66		
Control(s)†	0.36 ± 0.00	376 ± 23.7	21.9 ± 2.6
CL	2.13 ± 0.30^{b}	286 ± 21.2^{b}	16.7 ± 0.9^{b}
SL	4.37 ± 0.33^{c}	360 ± 16.4^{c}	27.1 ± 1.2^{c}
SR_0	1.92 ± 0.11^{B}	245 ± 16.9^{A}	12.7 ± 2.2^{B}
SR_2	1.77 ± 0.20^{C}	276 ± 10.7^{B}	11.2 ± 0.4^{C}
	Day 139		
Control(s)	0.37 ± 0.00	383 ± 25.9	21.9 ± 1.8
CL	0.90 ± 0.38^{d}	282 ± 31.6^{b}	14.4 ± 3.2^{b}
SL	3.47 ± 1.00^{e}	326 ± 56.4^{d}	25.1 ± 4.1^{c}
SR_0	1.27 ± 0.39^{D}	255 ± 15.4^{A}	12.4 ± 2.2^{B}
SR_2	1.14 ± 0.11^{E}	246 ± 19.6^{A}	12.9 ± 0.9^{D}

*Data are mean ± sd (n = 3).

†Reported values are calculated from the chemical analysis of regrown pasture from the control and 'C' treatments.

NB: Significant differences (P <0.05) over time (days 0, 66 and 139) between the shoot treatments ('CL' and 'SL') are shown vertically by lower case superscripts and the root treatments ('SR_0' and 'SR_2') in upper case superscripts. Control treatments are not included in the statistical analyses and comparisons are made against day 0 shoot and root variable values. Treatment abbreviations are discussed in the text (Section 8.2.2 and Table 8.1).

8.3.2 Soil analyses

8.3.2.1 Inorganic N and MBN

Soil NH_4^+–N concentrations increased over time (P <0.05) at all depths and on both days 66 and 139 in the 'control' and 'C' treatments (Table 8.4). In the 'CL' and 'SL' treatments NH_4^+–N concentrations were elevated in all depths at day 139 (P <0.05; Table 8.4). In the treatments receiving root material, NH_4^+–N concentrations fluctuated but had generally increased by day 139. There were no treatment effects on concentrations of NH_4^+–N at day 0. On days 66 and 139, the 'control', 'C' and 'CL' treatments generally had higher NH_4^+–N concentrations than the other treatments (Table 8.4). After day 0, concentrations of NH_4^+–N did not differ with depth on day 66 but decreased with increasing soil depth averaging 17.5 and 12.2 mg kg^{-1} soil at 0–2 and 2–4 cm, respectively on day 139. A significant interaction effect (P <0.001) of treatment with depth was observed at both days 66 and 139 where higher concentrations occurred at lower soil depth in all treatments except in the 'SR_2' treatment which had higher concentration at a lower depth on day 66. Enrichment of ^{15}N for NH_4^+ is not reported here because the volume of soil extract was insufficient.

Table 8.4 Soil NH_4^+–N at 0–2, 2–4 and 4–6 cm from control, shoot and root treatments over time following surface application of ryegrass litter.

NH_4^+–N (mg kg^{-1})			
0–2 cm			
	Day 0	Day 66	Day 139
Control	6.5 ± 0.8^{aA}	9.8 ± 0.7^{aB}	26.1 ± 5.4^{aC}
C	6.5 ± 0.8^{aA}	9.9 ± 0.5^{aB}	21.5 ± 5.5^{abC}
CL	6.5 ± 0.8^{aA}	7.3 ± 1.4^{abA}	19.9 ± 1.9^{abB}
SL	6.5 ± 0.8^{aA}	5.2 ± 0.6^{bcA}	10.9 ± 0.2^{cB}
SR_0	6.5 ± 0.8^{aB}	4.4 ± 1.0^{cA}	11.8 ± 1.0^{cC}
SR_2	6.5 ± 0.8^{aB}	6.0 ± 1.1^{bcA}	15.1 ± 1.6^{bcC}
2–4 cm			
Control	5.2 ± 1.2^{aB}	7.2 ± 1.2^{abA}	15.5 ± 1.7^{aC}
C	5.2 ± 1.2^{aA}	7.8 ± 1.3^{abB}	12.8 ± 2.7^{abC}
CL	5.2 ± 1.2^{aA}	5.3 ± 1.3^{bA}	12.4 ± 0.6^{abB}
SL	5.2 ± 1.2^{aA}	5.4 ± 0.2^{bA}	11.0 ± 1.1^{abB}
SR_0	5.2 ± 1.2^{aA}	4.5 ± 0.6^{bA}	11.9 ± 2.1^{abB}
SR_2	5.2 ± 1.2^{aA}	11.0 ± 2.9^{aC}	9.8 ± 0.7^{bB}
4–6 cm			
Control	4.7 ± 0.2^{aA}	6.7 ± 0.2^{aB}	12.5 ± 2.8^{aC}
C	4.7 ± 0.2^{aA}	6.5 ± 0.6^{aB}	10.9 ± 0.8^{aC}
CL	4.7 ± 0.2^{aA}	5.6 ± 1.1^{aA}	20.7 ± 11.6^{aB}
SL	4.7 ± 0.2^{a}	-n.a-	-n.a-
SR_0	4.7 ± 0.2^{a}	-n.a-	-n.a-
SR_2	4.7 ± 0.2^{a}	-n.a-	-n.a-

Significant differences between treatments (vertical comparison) for any given depth are shown by lower case superscripts (Tukey's test; $P < 0.05$). Significant differences due to time (across rows) are shown by upper case superscripts (Tukey's test; $P < 0.05$). Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 8.2.2 and Table 8.1).

Concentrations of soil NO_3^-–N had increased at all depths in the control by day 66 (P <0.05; Table 8.5) and then by day 139, had declined to levels less than those initially present at day 0. Soil NO_3^-–N had also increased in the 'C' treatment by day 66 but concentrations had also decreased by day 139, returning to similar values observed at day 0. In the remaining treatments, soil NO_3^-–N concentrations again increased by day 66 generally following the trend observed in the 'C' treatment. At day 66, soil NO_3^-–N was lower in the treatments receiving root litter (SR_0 and SR_2) at 0–2 cm, with no differences due to treatment at 2–4 cm depth while at 4–6 cm depth, NO_3^-–N was higher in the 'CL' treatment at day 66. On day 139, soil NO_3^-–N was higher in the SR_2 treatment at both 0–2 and 2–4 cm depths (Table 8.5). A significant interaction effect (P <0.001) of treatment with depth was observed on day 66 where higher concentrations occurred at lower soil depth in all the treatments except the 'SR_2' treatment.

Table 8.5 Soil NO_3^-–N at 0–2, 2–4 and 4–6 cm from control, shoot and root treatments over time following surface application of ryegrass litter.

NO_3^-–N (mg kg^{-1})			
0–2 cm			
	Day 0	Day 66	Day 139
Control	5.2 ± 2.1^{aB}	53.2 ± 8.1^{aC}	0.9 ± 0.1^{cA}
C	5.2 ± 2.1^{aA}	55.1 ± 21.3^{aB}	2.6 ± 1.5^{bcA}
CL	5.2 ± 2.1^{aB}	63.3 ± 15.2^{aC}	1.9 ± 0.5^{bcA}
SL	5.2 ± 2.1^{aA}	47.5 ± 9.0^{aB}	3.6 ± 0.7^{bA}
SR_0	5.2 ± 2.1^{aB}	23.2 ± 0.3^{bC}	2.2 ± 0.7^{bcA}
SR_2	5.2 ± 2.1^{aA}	11.2 ± 2.9^{cB}	14.9 ± 4.8^{aB}
2–4 cm			
Control	3.0 ± 1.1^{aB}	26.0 ± 5.0^{aC}	0.8 ± 0.0^{bA}
C	3.0 ± 1.1^{aA}	21.6 ± 7.6^{aB}	1.3 ± 0.8^{bA}
CL	3.0 ± 1.1^{aB}	43.9 ± 21.8^{aC}	1.2 ± 0.3^{bA}
SL	3.0 ± 1.1^{aA}	31.5 ± 3.1^{aB}	2.1 ± 0.4^{bA}
SR_0	3.0 ± 1.1^{aA}	25.3 ± 3.9^{aB}	1.8 ± 0.3^{bA}
SR_2	3.0 ± 1.1^{aA}	23.7 ± 3.1^{aB}	14.5 ± 6.1^{aB}
4–6 cm			
Control	3.0 ± 1.2^{aB}	20.0 ± 3.2^{bC}	1.0 ± 0.2^{aA}
C	3.0 ± 1.2^{aA}	22.2 ± 6.0^{bB}	1.3 ± 0.5^{aA}
CL	3.0 ± 1.2^{aA}	43.7 ± 10.0^{aB}	2.2 ± 1.4^{aA}
SL	3.0 ± 1.2^{a}	-n.a-	-n.a-
SR_0	3.0 ± 1.2^{a}	-n.a-	-n.a-
SR_2	3.0 ± 1.2^{a}	-n.a-	-n.a-

Significant differences between treatments (vertical comparison) for any given depth are shown by lower case superscripts (Tukey's test; $P < 0.05$). Significant differences due to time (across rows) are shown by upper case superscripts (Tukey's test; $P < 0.05$). Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 8.2.2 and Table 8.1).

On day 66, ^{15}N enrichment of the soil NO_3^-–N in the shoot treatments was higher than in the root treatments while ^{15}N enrichment of the NO_3^-–N in all treatments (both shoot and root treatments) was significantly higher (P <0.001) than that of the control at 0–2 cm depth (Figure 8.7). At 2–4 and 4–6 cm, both shoot treatments were significantly higher than the control but root treatments did not differ from the control (Figure 8.7). Values for the 'CL', 'SL' and 'SR_0' treatments were also higher (P <0.01) at 0–2 cm than at 2–4 cm depth (Figure 8.7).

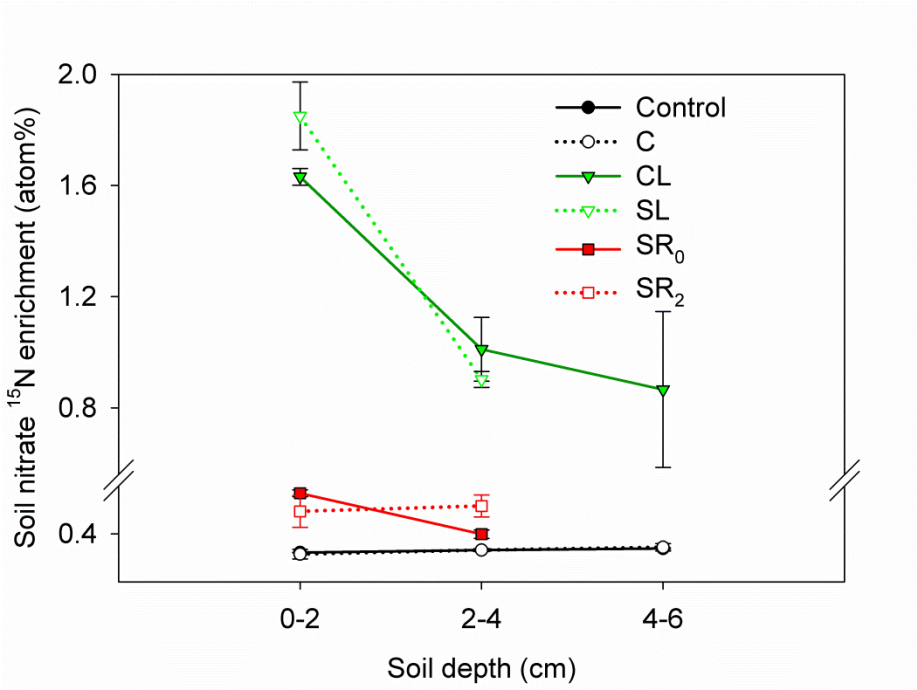

Figure 8.7 Enrichment of soil NO_3^-–^{15}N on day 66 after treatment application. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 8.2.2 and Table 8.1).

Microbial biomass N (MBN) did not differ with treatment on days 66 and 139. However, the overall treatment mean at 0–2 cm depth on day 66 (1.41 mg kg^{-1} soil) was higher (P <0.05) than the overall treatment mean (0.77 mg kg^{-1}) at day 139. Microbial biomass-^{15}N enrichment in the shoot treatments was significantly higher (P <0.05) than the remaining treatments at 0–2 cm depth on day 66 (Figure 8.7) and accounted for 0.04 ± 0.02 and 0.04 ± 0.01µg ^{15}N g^{-1} for the 'CL' and 'SL' treatments, respectively. Corresponding values of total MBN on day 66 were 1.7 ± 0.6 and 1.3 ± 0.6 mg kg^{-1}, respectively. Thus, 2.4 and 3.1% of the total MBN on day 66 had come from the shoot litter in the 'CL' and 'SL' treatments, respectively. Microbial biomass-^{15}N did not differ due to root treatment at any depth, averaging 0.003 µg ^{15}N g^{-1} soil which accounted for 0.3% of the total MBN.

8.3.2.2 Water soluble C

On days 66 and 139, concentrations of water soluble C (WSC) at 0–2 and 2–4 cm depths from the control treatment did not differ from the shoot litter treatments (CL and SL) but both were higher (P <0.05) than in the root treatments (SR_0 and SR_2). On average, WSC concentrations in the shoot and root treatments on day 66 were 111.5 ± 12.2 and 86.4 ± 8.3 mg kg^{-1} soil), respectively which then decreased significantly over time with corresponding values on day 139 of 84.6 ± 27.0 and 55.8 ± 15.7 mg kg^{-1}, respectively (Figure 8.8). A significant 'depth effect' was observed in the shoot treatments on day 66 (P <0.05) with higher concentrations at 0–2 cm (111.5 ± 12.2 mg kg^{-1}) than at 2–4 cm (87.7 ± 6.6 mg kg^{-1}).

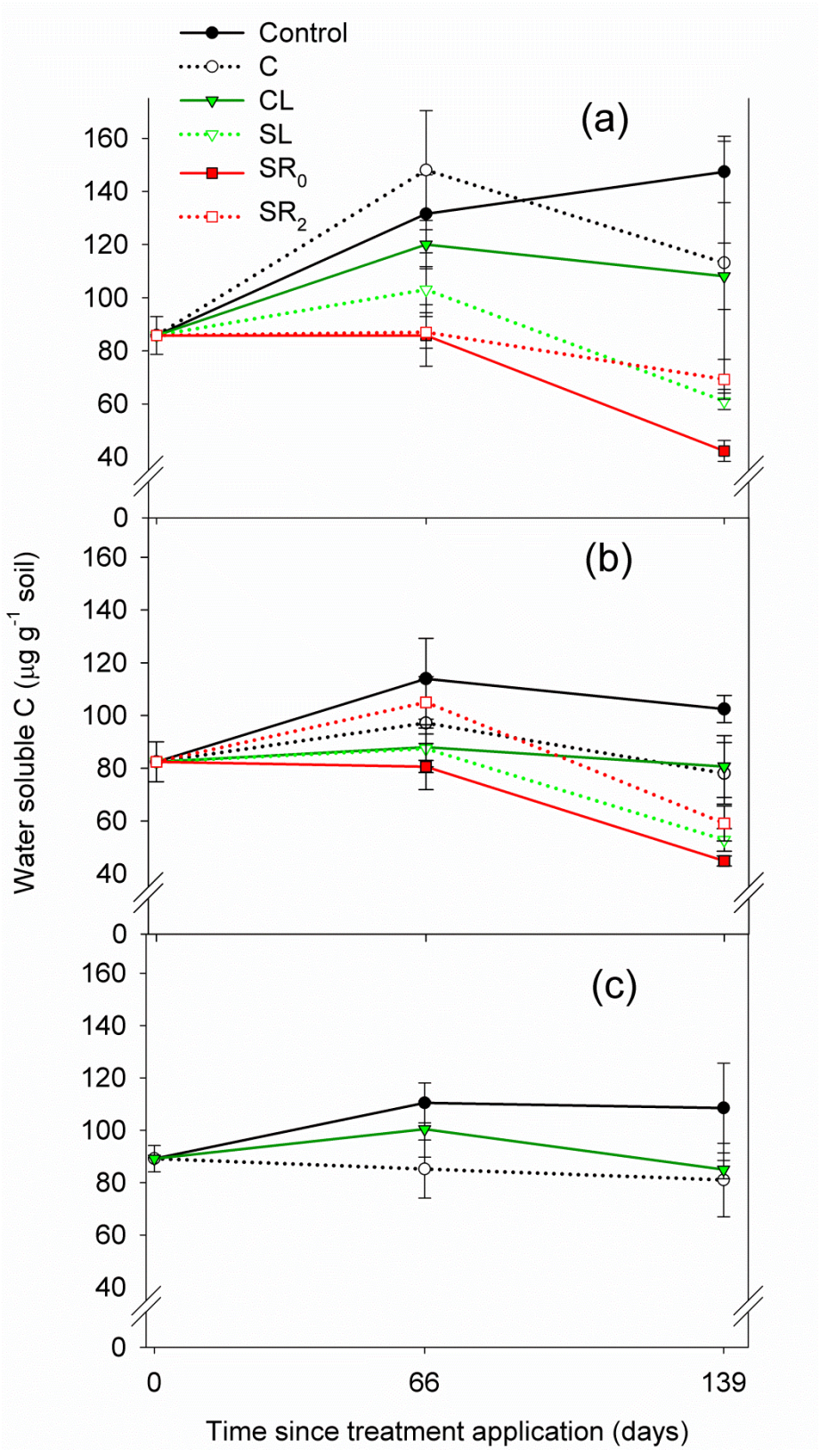

Figure 8.8 Water soluble C at (a) 0–2 cm, (b) 2–4 cm, and (c) 4–6 cm, over time following surface application of ryegrass shoots and roots. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 8.2.2 and Table 8.1).

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8.3.3 Nitrous oxide emissions

Emissions of N_2O did not follow a normal distribution and hence were transformed [ln(flux+1); Section 8.2.4]. Statistical differences were calculated on log transformed data and were then back transformed for reporting below. On day 1 (17 h after treatment application), N_2O emissions from all treatments did not differ from the 'control' and were 21 ± 6, 28 ± 11, 30 ± 11, 35 ± 20, 36 ± 15 and 38 ± 25 µg N_2O-N m^{-2} h^{-1} (± sd, P >0.05) for the control, 'C', 'CL', 'SL', 'SR_0' and 'SR_2' treatments, respectively (Figure 8.9a). Emissions were significantly lower from the control than in other treatments until day 4. Emissions of N_2O from the 'CL' treatment increased on days 5, 6 and 9 with maximum emissions occurring on day 6, significantly higher (P <0.001) than in the other treatments. Emissions of N_2O on day 6 were 2992 ± 2184, 774 ± 882, 177 ± 107, 168 ± 82, 111 ± 95 and 103 ± 76 µg N_2O-N m^{-2} h^{-1} from the 'CL', 'SL', 'C', 'SR_0', 'SR_2' and 'control' treatments, respectively (Figure 8.9a). From day 5, N_2O emissions from all treatments were significantly higher than from the control until day 16 and did not differ thereafter until day 49. Cumulative N_2O emissions integrated over 49 d were 26 ± 12, 27 ± 17, 213 ± 47, 82 ± 34, 46 ± 13 and 78 ± 28 mg N_2O-N m^{-2} for the 'control', 'C', 'CL', 'SL', 'SR_0' and 'SR_2' treatments, respectively. Approximately 37–71% and 18–27% of the total emissions occurred during the 4–10 d period of maximum activity from the shoot and root treatments, respectively. The emission factor (EF) for N_2O from the 'CL' treatment was significantly higher than in the 'SL' treatment (0.9 ± 0.2 > 0.3 ± 0.2%) over the 49 d period. The EF of the SR_2 treatment (3.6 ± 1.9%) was higher (P <0.001) than the other treatments while the 'SR_0' treatment was lower (1.4 ± 0.9%) than the 'SR_2' treatment.

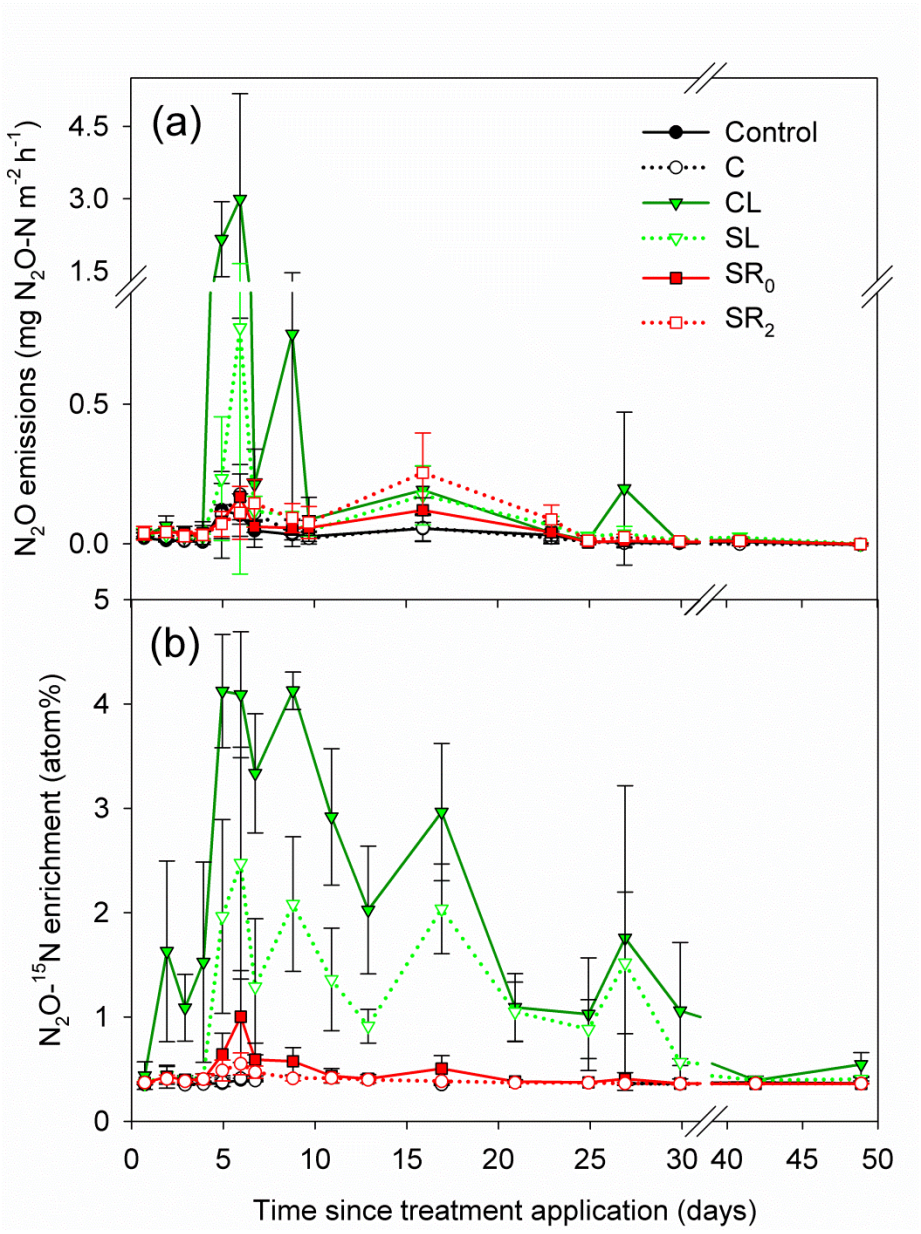

Figure 8.9 Emissions of (a) N_2O, and (b) N_2O-^{15}N enrichment, over time following surface application of ryegrass shoots and roots. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 8.2.2 and Table 8.1).

The ^{15}N enrichment of the N_2O-N from the 'CL' treatment was higher (P <0.001) than in the controls from day 2 through to day 49 (and day 5 to day 25 for the 'SL' treatment; Figure 8.9b). Maximum ^{15}N enrichment of the N_2O emission occurred on day 9 for the 'CL' treatment and day 6 for the remaining treatments and these values were 0.37 ± 0.02, 0.37 ± 0.01, 4.13 ± 0.18, 2.47 ± 1.11, 1.00 ± 0.44 and 0.55 ± 0.10 atom% in the 'control', 'C', 'CL', 'SL', 'SR_0', and 'SR_2' treatments, respectively. Mean atom% values for the 'control', 'C', 'CL', 'SL', 'SR_0', and 'SR_2' treatments over 49 d were 0.367, 0.367, 2.01, 1.09, 0.47 and 0.40, respectively.

8.3.4 Carbon dioxide emissions

Emissions of CO_2 did not differ significantly with treatments. Emissions from all treatments initially peaked during days 6 and 7 which also coincided with the peak N_2O emissions (Figure 8.10). However, maximum CO_2 emissions were recorded at 49 d with values of 217 ± 72, 123 ± 48, 163 ± 58, 156 ± 50, 174 ± 72 and 206 ± 44 mg CO_2-C m^{-2} h^{-1} (mean ± sd) for the 'control', 'C', 'CL', 'SL', 'SR_0' and 'SR_2' treatments, respectively, with no differences due to treatments (P = 0.174). Cumulative CO_2 emissions over 49 d equated to 106.9 ± 23.8, 69.7 ± 7.4, 96.0 ± 9.5, 95.4 ± 15.1, 87.8 ± 14.9 and 98.4 ± 12.0 g CO_2-C m^{-2} for the 'control', 'C', 'CL', 'SL', 'SR_0' and 'SR_2' treatments, respectively.

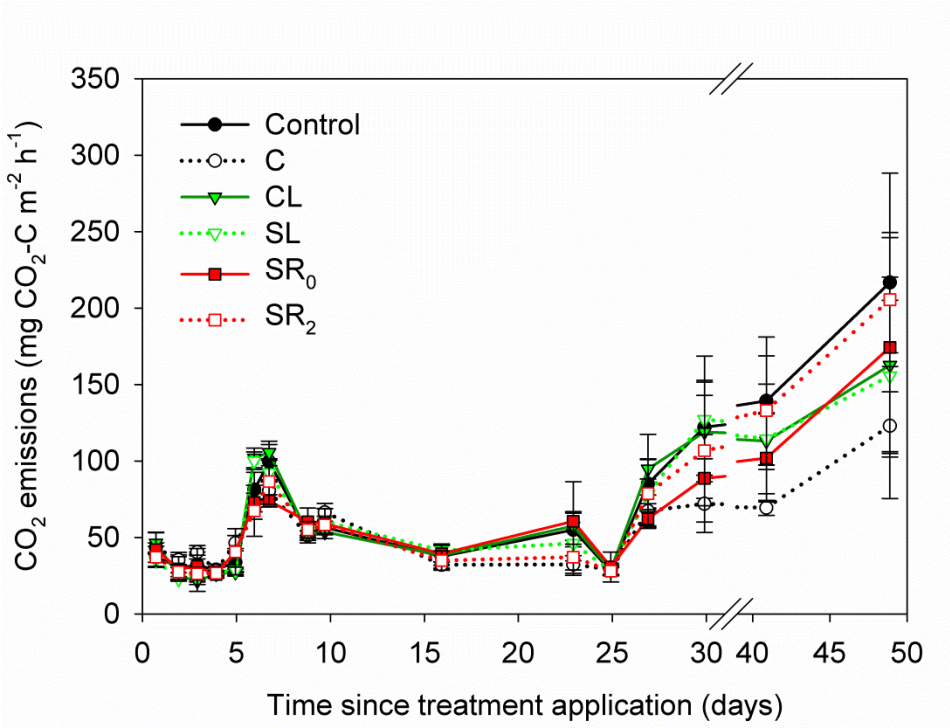

Figure 8.10 Emissions of CO_2 over time following surface application of ryegrass shoots and roots. Data are mean ± sd. Treatment abbreviations are discussed in the text (Section 8.2.2 and Table 8.1).

8.4 Discussion

8.4.1 Nitrous oxide emissions

Surface decomposition of ryegrass shoots and roots stimulated higher N_2O emissions than in the control. These emissions did not become significant until day 5 and this delay may have been due to increases in the microbial growth and biomass, temporarily immobilising soil mineral N (Larsson *et al.* 1998; McKenney *et al.* 1995) or the time required for mineralisation. Perennial ryegrass has a relatively high soluble carbohydrate content (Aldrich 1984), a lower C: N ratio and higher enzymatic activity during decomposition (Dilly *et al.*

2007) and this, along with rainfall on day 2, might have accelerated the process of soil-N and litter-N mineralisation and subsequent N_2O emissions (Larsson *et al.* 1998). The ^{15}N enrichment of the N_2O emissions demonstrates that ryegrass litter was the dominant source of N_2O in the 'CL' treatment with approximately 70% of the N_2O originating from litter during peak N_2O emissions on days 5 to 10 while in the 'SL' treatment the value was reduced to 40−50%.

Generally, any plant residue – surface-placed or incorporated into the soil, primarily undergoes ammonification i.e. the process where mineralisation of plant proteins occurs, forming NH_4^+ via microbial decomposition. This NH_4^+ can further be transformed to NO_3^- via nitrification (Flessa *et al.* 2002) or it can be volatilised to NH_3 into the atmosphere (De Ruijter *et al.* 2010). The soil pH at the field site makes the possibility of the latter process unlikely (Table 8.2). Nitrous oxide is an intermediate product of both nitrification and denitrification, and the N_2O emissions in this study may have resulted from a coupling of these processes during litter decomposition on the soil surface. The ^{15}N enrichment of the soil NO_3^- was still elevated at ca.1.8 atom% at day 66. This demonstrates that litter-^{15}N was transformed and available as inorganic-N for denitrifiers. And, although not measured directly, this NO_3^-–^{15}N would have originated from litter derived NH_4^+–^{15}N. Determining the exact microbial process responsible for N_2O emissions from the decomposing litter requires more detailed study and was not the aim of the current study. Nevertheless, the mechanism may be speculated upon. Soil water content (46–52% WFPS) during the period of maximum emissions (5–10 d) in this study, was not ideal for denitrification, however, studies have shown that aerobic denitrification can occur in soils with WFPS values as low as 20% (Bateman and Baggs 2005) and that N_2O emissions may also occur from anaerobic micro-sites within soil aggregates over a wide range of soil WFPS (Ambus and Christensen 1994; Novosad and Kay 2007).

Flessa *et al.* (2002) measured N_2O emissions from surface-placed fresh grass leaves (*Poa pratensis* L.) at the rate of 9.2 g N m^{-2} and concluded that the emissions occurred due to a coupling of nitrification and denitrification of litter-derived N. The present study is the first study that reports soil N dynamics and N_2O emissions using a ^{15}N technique with pasture litter *in situ*. The ^{15}N enrichment of N_2O in this study proved that N_2O emissions originated from litter derived N. The soil used in this study was a fertile pasture soil with high available N (Table 8.2) which was demonstrated by the presence of higher NH_4^+ and NO_3^- in the control treatment. Soil NO_3^- concentrations in the shoot treatments did not differ from the control treatment on days 66 or 139 but ^{15}N analysis of soil NO_3^- showed that it was ^{15}N-enriched as a result of litter treatment and that N mineralisation of plant litter significantly

contributed to the soil NO_3^- pool and hence could be used as a substrate for N_2O emissions although by day 66, N_2O emissions had decreased.

8.4.2 Emission factors

Flessa *et al.* (2002) found cumulative N_2O emissions of 25 mg m^{-2} and an EF of 0.3% over 50 d from surface-placed grass leaves applied at a rate of 9.2 g N m^{-2}. Larsson *et al.* (1998) applied ryegrass onto soil surface at the rate of 34.8 g N m^{-2} and incubated over a 92 d period but emissions of N_2O did not differ from a control soil and the reported EF was only 0.1%. Aulakh *et al.* (1991a) used dried and chopped, surface-placed residues of hairy vetch (C: N ratio of 8, similar to the ryegrass shoots used here) and corn (C: N, 39; similar to root treatments in this study) showing that cumulative N_2O emissions were 6.1 and 3.2 mg N kg^{-1} soil at 90% WFPS at 25^{o}C for 35 d. In the present study, cumulative emissions from the shoot litter treatments were almost 3–10 times higher compared to that of Flessa *et al.* (2002) and Aulakh *et al.* (1991a); however, the rate of N applied was almost double (21.3 g N m^{-2}) in the present study. Moreover, in the study of Flessa *et al.* (2002), the emissions commenced a week after treatment application which was similar (5 d) to the present study but they observed elevated emissions over 28 d as opposed to 10 d in this study. Aulakh *et al.* (1991a) and Pal *et al.* (2012) also observed short-lived, elevated N_2O emissions after 4 and 8 d from pasture and crop residue incorporation, respectively, similar to the present study.

Emission factors in this current study, for the shoot litter treatments ranged from 0.3 to 0.9% which is broadly similar to the above studies after considering the factors such as N content of litter applied, soil type and climate. Soil water content (θ_v) in the current study ranged from 0.22 to 0.40 m^3 m^{-3} that equates to a mean value of 0.31 m^3 m^{-3}. Chapter 6 in this thesis reported EF values of 0.7 and 1.7 % for clover and ryegrass (Table 6.3; mean 1.2%) at 0.40 m^3 m^{-3} (54% WFPS). On this basis, both the lab study and the current study had similar results for the EF values (1.2% vs. 0.9 ± 0.2%). The EF of 0.9 ± 0.2% from the 'CL' treatment was also close to the default EF (of 1%) stipulated by IPCC best practice guidelines for crop residues; it does not account for pasture residues.

8.4.3 Biochemical composition

The 'SL' treatment had relatively lower N_2O emissions compared to the 'CL' treatment (EF of 0.3 vs. 0.9%), in addition to the average ^{15}N enrichment of N_2O being half that of the 'CL' treatment (1.09 vs. 2.01 atom%). This difference might be because a greater proportion of the emitted N_2O in the 'SL' treatment originated from mineralised soil-N rather than litter-N. Soil sieving and repacking into the PVC cores and the consequent disturbance of the

microbial biomass in the surface layers (0–10 cm) may also have reduced the microbial activity thereby causing lower N_2O emissions. The relatively lower N_2O emissions in the 'C' treatment compared to the litter treatments showed that potential artefacts as a result of defoliation did not significantly enhance N_2O emissions. Thus, any release of NH_4^+ and NO_3^- from defoliated plants did not result in significant N_2O emissions. In the root treatments, where N_2O emissions were recorded over a more protracted period, most of the N_2O came from the mineralisation of the soil-N as indicated by the lower ^{15}N enrichment of N_2O from the root treatments.

Further, the root treatments did not produce an 'immediate' short-lived, N_2O effect as in the shoot treatments; rather they showed a protracted response probably because of their recalcitrant biochemical composition which included a higher C: N ratio and lignin content [Section 8.3.1; (Aulakh *et al.* 1991a; Huang *et al.* 2004)]. Studies have shown that recalcitrant materials such as stubbles and roots (Cusack *et al.* 2009) with high C: N ratios, can take relatively long periods to mineralise (Gentile *et al.* 2008; Kuzyakov *et al.* 1999). The highest EF (3.6%) in the 'SR_2' treatment showed that a delayed yet continuous mineralisation of the recalcitrant root material (94 mg g^{-1} vs. 26 mg g^{-1} lignin in shoot material) occurred and this might have been enhanced due to its incorporation at 2 cm.

Aulakh *et al.* (1991a) concluded that differences in the N_2O emissions from plant materials were due to differences in the C: N contents of the plant residues used. They also indicated that incorporation or surface placement of wide-C: N-ratio residues could cause a significant immobilisation of mineral N for several weeks, and this was possibly the reason for lower inorganic N concentrations in the root treatments in this study. Dilly *et al.* (2007) compared decomposition rates of wheat (*Triticum* sp.), rye (*Secale* sp.) and ryegrass and found that ryegrass decomposed relatively rapidly (maximum in first 2 months) compared to the other grasses because of higher ammonification rates and a low C: N ratio. The direct relation of C: N ratio on N_2O emissions has been documented widely (Baggs *et al.* 2003; Velthof *et al.* 2002) and therefore plant species under investigation can be a governing factor for N_2O emissions e.g. EF for horticultural crop residues was 14.6% as reported by Velthof *et al.* (2002).

8.4.4 Carbon-, nitrogen- and mass-loss of the litter

Mass loss and N content of the shoot litter material showed that litter-N mass was already depleted due to microbial utilisation by day 66. In the present study the increasing soil NH_4^+ concentrations over time appear to have come from the soil-N pool since this effect was also seen in the control. Geisseler and Horwath (2011) also found higher NH_4^+ concentrations

from a control treatment, 60 d after surface application of an oats-legume cover crop (C: N, 30) at different moisture levels and indicated that lower NH_4^+ concentrations from the cover crop was due to N immobilisation in the initial 5 d.

Brunetto *et al.* (2011) tracked the decomposition of ^{15}N-labelled ryegrass and clover in vineyards over 16 weeks and found 45% mass loss after 56 d of incubation when using surface-placed litterbags. Mass loss in the present study on day 66 from the 'SL' treatment was similar (46%) to Brunetto *et al.* (2011) however the values were almost double (82%) for the 'CL' treatment on the same day. This might be because in the 'CL' treatment, presence of undisturbed plant roots and associated microbes might have aided in decomposition of the added litter. However, in the present study, soil analyses was not performed during the initial period (10–20 d after treatment), when the microbial activity was assumed to be maximum.

Tutua *et al.* (2002) surface-placed a mixture of ryegrass and clover in a New Zealand apple orchard and found that about 60% of the initial mass was lost after 90 d from the placement of litterbags; the faster rate of decomposition was attributed to irrigation and soil burial of the litterbags. The 'SR_0' treatment had similar values (50% at day 66) compared to the results of Tutua *et al*. (2002) but the value of 53% on day 139 was presumably due to the recalcitrant biochemical composition of the root material (Glasener *et al.* 2002; Wang *et al.* 2010). Tutua *et al.* (2002) reported higher decomposition rates from the buried litterbags than the surface-placed litterbags. Mass loss from the 'SR_2' and 'SR_0' treatments in this study were similar by day 66 but it increased significantly to 84% by day139 – the maximum loss compared to all the treatments thus showing the effect of incorporation, similar to the results of Tutua *et al.* (2002). However, the observed increase in the litter mass of the shoot treatments at day 139 in the current might be an artefact due to new herbage growing through the mesh of the litterbags in the 'CL' and 'SL' treatments.

Recent studies using litterbags (Sanaullah *et al.* 2010; Wang *et al.* 2010) have reported changes in the C and N composition of the litter over time. Wang *et al.* (2010) investigated the decomposition of dried leaf litter of forest tree species in litterbags and found that the C content of the litter decreased over 12 months while the N content increased over the same period while Sanaullah *et al.* (2010) reported reductions in the C and N contents of leaf litter of ryegrass over 11 months. Kriauciuniene *et al.* (2008) reported similar results from root and shoots of rape (*Brassica napus* L.), winter wheat (*Triticum aestivum* L.) and red clover (*Trifolium pratense* L.) with maximum reduction during 33–63 weeks. The present study showed reductions in C and N contents from both shoot and root treatments with an exception that the N content of root material increased over time, similar to Wang *et al.* (2010). This may have been due to N immobilisation as reported by Wang *et al.* (2010).

Unlike the current study, others have found that surface application of plant material increases the CO_2 emissions (Geisseler and Horwath 2011). However, in this current study the CO_2 emissions peaked at days 6 and 7 irrespectively of the soil temperature and soil water contents, which coincided with peak N_2O emissions. This may have been due to higher microbial activity in the initial phase of treatment application, when soil analyses were not performed. Flessa *et al*. (2002) reported elevated CO_2 emissions immediately after grass mulch application and concluded that CO_2 was emitted from the combination of soil and plant respiration and activity of decomposer organisms.

8.5 Conclusion

Surface decomposition of ryegrass shoots and roots stimulated N_2O emissions with maximum emissions 5–10 d after treatment application with emissions continuing for a period of 49 d. The ^{15}N analyses showed that litter-N made a significant contribution to the N_2O flux and approximately 70% of the total N_2O in the 'CL' treatment and 40–50% in the 'SL' treatment originated from the litter in the surface-placed shoot litter ('CL' and 'SL') treatments. The elevated emissions in the shoot litter treatments are attributed to the lower C: N ratio and rich biochemical composition of the ryegrass. The N_2O emissions most likely resulted from a coupling of nitrification and denitrification of the litter-derived-N. An emission factor of 0.9 ± 0.2% was calculated for the 'shoot only' (CL) treatment which is similar to the EF values in the lab study (Chapter 6) and to the default EF of 1% stipulated by the IPCC for crop residues. This treatment (CL) also most closely resembles the effect of *in situ* litter-fall onto grazed pasture. The implications of a 0.9% EF for litter-fall are discussed in Chapter 9. Litter treatments did not affect CO_2 emissions. Dry matter loss from the litterbags ranged from 46–82% for the shoot treatments and 50–57% for the root treatments. The C and N contents of the litter from litterbags decreased over time. Investigation using pasture litter of varying biochemical composition is warranted to further consider the impacts of litter rates and the microbial processes responsible for the N_2O emissions observed following litter deposition onto the soil surface.

Chapter 9
Conclusions and recommendations

9.1 Introduction

The Intergovernmental Panel on Climate Change (IPCC) confirms that crop residues[**] in arable systems can contribute significantly to C and N cycling (Section 2.2.4) and also cause significant N_2O emissions – a potent greenhouse gas (Section 2.2.2). A national, annual emissions inventory compiled according to the IPCC Guidelines accounts for direct N_2O emissions from crop residues both above and below ground (IPCC 2006). The guidelines state, "The nitrogen residue from perennial forage crops is only accounted for during periodic pasture renewal, i.e. not necessarily on an annual basis as is the case with annual crops" (IPCC 2006). Despite the fact that 70% of the world agricultural area (FAOSTAT 2000) and 90% of New Zealand's total farm area is considered to be pastoral ecosystem (Statistics New Zealand 2003), the IPCC does not consider the potential contribution of pasture residues, if any, to be significant with respect to N_2O emissions. Therefore, the questions posed in this thesis were firstly: Do pasture residues (collectively called 'litter') occur in significant quantities during grazing? And secondly, what is the role of herbage embodied-N with respect to N_2O emissions? The overall objective of the research was to quantify the contribution of plant-derived N_2O emissions in intensively grazed dairy pastures to New Zealand's agricultural greenhouse gas inventory.

This chapter summarises the key results of the experiments performed (Section 9.2) and discusses the implications of the research findings (Section 9.3).

9.2 Overall summary

9.2.1 Litter-fall: significant quantities in intensively grazed dairy pastures

The rationale for this study came from observing grazing dairy cattle dropping freshly harvested plant material onto the soil surface, hereafter called litter-fall. For the first time, this study (Chapter 4) quantified litter-fall in intensively grazed dairy pastures. During grazing the fresh litter-fall rate was 53 ± 24 kg DM ha^{-1} per grazing event. This equated to an annual N application rate of 15.9 kg N ha^{-1} y^{-1} and 3.5 kg N ha^{-1} y^{-1} for fresh and senesced litter, respectively. The amount of N contained in the annual litter-fall rate was comparable in

[**] Crop residues in this thesis is defined as the materials remaining in the field after a crop has been harvested in agricultural and horticultural systems. These residues include stalks, stubbles, stems, leaves and seed pods.

magnitude to a typical fertiliser application rate (~25 kg N ha^{-1}). Litter-fall accounted for 4% of the apparent dry matter consumption of the dairy cattle. This has implications for dry matter budgeting i.e. budgets not accounting for litter-fall may overestimate DMI of the animals. Since this litter contained N, it was hypothesised that during its decomposition it could contribute to N cycling and N_2O emissions. The litter-fall rates measured also raised further questions.

- Would grazing management change litter-fall rates? For example, pasture utilisation, stocking rate, pasture and animal species, climate, grazing intensity, could all potentially affect litter-fall.
- Could litter-fall at the above measured rates contribute to N_2O emissions?
- If so, are N_2O emissions affected by the biochemical composition of the litter?
- If litter-fall contributes to N_2O emissions, are there implications for the IPCC methodologies or assumptions?

The answers to some of these questions were obtained in this current work.

9.2.2 Animal treading increases N_2O emissions irrespective of the presence of herbage

Approximately 20% of the world's pastures and rangelands are considered degraded through overgrazing and compaction (Steinfeld *et al.* 2006). The magnitude of compaction depends on the stocking rate, soil type, moisture content and animal species (Naeth *et al.* 1990; Warren *et al.* 1986). Chapter 5 investigated the effect of partial incorporation of pasture herbage due to animal treading on N_2O emissions. Results showed that:

- Treading lowered soil NO_3^-–N concentrations and increased N_2O emissions, irrespective of the presence or absence of herbage indicating utilisation of NO_3^- by N_2O producing microorganisms. In Chapter 5 (part B), treading diluted the ^{15}N enrichment of the soil NO_3^- pool presumably due to the release of unlabelled soil-N and/or herbage-N.
- The suppression of the CO_2 emissions due to treading (parts A and B) indicated an enhancement of anaerobic conditions in the trodden plots thereby increasing the chances of denitrification contributing to N_2O emissions.
- To further understand the effect of herbage treading on N_2O emissions given the results of Chapter 5 and other work (Chapter 8) in this thesis, ^{15}N labelled herbage should be used in further studies to determine the extent of the increase in the soil inorganic N pool following treading.

9.2.3 Pasture litter – a significant, anthropogenic N_2O source

Studies have shown that N_2O emissions occur from incorporation of crop residues into soil (Section 2.4) and the magnitude of these emissions has been shown to depend on the biochemical composition, rate and placement of litter into the soil (Section 2.4). Chapter 4 showed that litter-fall was significant in pasture. Chapter 6 was a laboratory study to quantify N_2O emissions using two pasture species and a winter supplement that dominate in New Zealand. Ground shoots of clover, ryegrass and maize were incorporated into soil. Results showed that:

- Maximum N_2O emissions occurred relatively rapidly (0.5 d) after litter incorporation indicating rapid mineralisation of plant litter-N and subsequent utilisation by nitrifiers and/or denitrifiers.
- Emission factors (EF) for N_2O equated to 2–3% of the incorporated N at 86% WFPS while at 54% WFPS, EF was significantly less with 1.7% > 0.7% = 0.5% for clover, ryegrass and maize, respectively; these differences between species were attributed to the biochemical properties of the litter species including their differing C: N ratios.
- Cumulative emissions from these, albeit unrealistically high rates of litter-N incorporation ranged from 63–209 kg N_2O-N ha^{-1} y^{-1} at 54% WFPS and 269–359 kg N_2O-N ha^{-1} y^{-1} at 86% WFPS, respectively. The significant N_2O emissions, especially for clover and ryegrass, warranted further study (Chapters 7 and 8). Combining the EF results and the *in situ* litter-fall data, it was estimated that N_2O emissions, attributable to litter-fall alone, could be 0.4 kg N_2O ha^{-1} y^{-1}, which is similar to the reported values of 'background' emissions from grazed pasture soils (Section 2.2.5). But unlike these so called 'background' emissions, these litter-fall-derived N_2O emissions are clearly anthropogenic and therefore should be acknowledged and accounted for in the IPCC methodology.

9.2.4 Biochemical composition of litter: effect of cellulose on N_2O emissions

Results from Chapter 6 showed that the biochemical composition of the litter could determine the N_2O emissions. Clover had a lower C: N ratio (of 9) and lower cellulose and hemicellulose contents. To investigate the role of the C: N ratio with respect to N_2O emissions, increasing amounts of cellulose were incorporated with a constant amount of clover litter (in Chapter 7) since it was rationalised that the lower C: N ratio of the clover litter in Chapter 6 may have meant a lack of a C substrate for denitrifiers to complete the reduction of N_2O to N_2. Results showed that:

- Clover incorporation into soil rapidly produced N_2O emissions and adding increasing quantities of cellulose significantly enhanced those N_2O emissions, indicating that the incorporated cellulose acted as a labile C source for the heterotrophic organisms responsible for denitrification.
- Increases in soil inorganic N concentrations from the treatments indicated that ammonification of the plant litter occurred followed by nitrification of the NH_4^+ to NO_3^- and its further consumption in the presence of the added C substrate i.e. cellulose.
- Over 42 d, 50–90% of the total N_2O was emitted in ~9 d from the cellulose-amended treatments. An important implication from this study was that higher C: N ratios did not necessarily mean that the material was recalcitrant with respect to decomposition and N_2O emissions; rather it was the biochemical composition, relative concentrations and the form of the recalcitrant compounds in the plant litter which played key roles.
- Emissions of N_2O increased with increasing C: N ratio of the litter and cellulose combinations.
- Further investigation should be performed using ^{15}N-labelled plant materials to further establish associated N_2O emissions and N cycling during their decomposition.

9.2.5 Surface-placed litter stimulates N_2O emissions

In Chapter 8, ^{15}N-labelled ryegrass was placed on the surface of a pastoral soil in litterbags at an unrealistically high rate of 213 kg N ha^{-1} and N_2O and CO_2 emissions were measured. This study is the first study that reports soil N dynamics and N_2O emissions using a ^{15}N technique with pasture litter *in situ*. Results showed that:

- Approximately 70% of the N_2O came from the shoots with peak N_2O emissions 5 to 10 d after litter placement.
- Emissions of N_2O may have resulted from ammonification followed by a coupling of nitrification and denitrification during litter decomposition on the soil surface. The ^{15}N enrichment of soil inorganic N (NO_3^-) and the evolved N_2O demonstrated that the emissions originated from litter-N. These processes supported the hypotheses established in Chapters 6 and 7.
- The EF of the *in situ* placed litter was 0.9%. This result in conjunction with the litter-fall rates measured (Chapter 4) has implications for background N_2O emissions and this is discussed further below.

9.3 Implications of this research

9.3.1 Introduction

Figure 9.1 summarises the various facets of research that comprise this thesis. The frequency and timing of pasture grazing by livestock is determined primarily by DM on-offer and this is a function of management practices, climate and pasture species. The duration and intensity of grazing depends on the required degree of pasture utilisation.

Grazing induces litter-fall (Chapter 4) and the associated animal treading affects both soil and pasture characteristics (Chapters 5 and 8). Decomposition of litter-fall on the soil surface results in N release from the harvested but un-ingested litter and this contributes to the soil inorganic N pool (Chapter 8). The rate and total contribution of litter-fall to the soil inorganic N pool will depend on pasture species and the biochemical composition of the litter. It is highly likely that the stocking rate, ruminant species and pasture utilisation rates will also affect litter-fall but further work is required to assess this. The N input from litter-fall to the soil inorganic N pool then has the potential to contribute to N_2O emissions. The exact mechanisms for these N_2O emissions are unknown but coupled nitrification-denitrification is highly probable. Competition for this soil inorganic N will occur from soil microorganisms and plant N uptake if grazing animals have left the pasture in a stage that is suitable for rapid regrowth.

Grazing causes animal treading of the pasture and soil, with the degree of physical damage dependent on pasture cover and species and on the nature of the grazing animal (i.e. sheep or cattle). It would be expected that lower C: N ratio species such as clover, would be more susceptible to treading damage. One of the experiments (Chapter 5) in this thesis showed that herbage presence had no effect on net soil inorganic N concentrations with depletion in NO_3^-–N while N_2O-N emissions increased. However, repeating this experiment with prior ^{15}N labelling of the soil NO_3^- pool showed that there was a flux of N being added to the soil. This source was unidentified, but could have been from damaged pasture shoots or roots and/or soil organic matter exposed as result of treading. Any contribution of herbage damaged by treading, to the soil inorganic N pool will again depend on biochemical composition, competition for N released, the degree of soil physical damage and other parameters such as soil type and soil water content. Further studies need to determine the source and rate of N inputs to the soil inorganic N pool following treading.

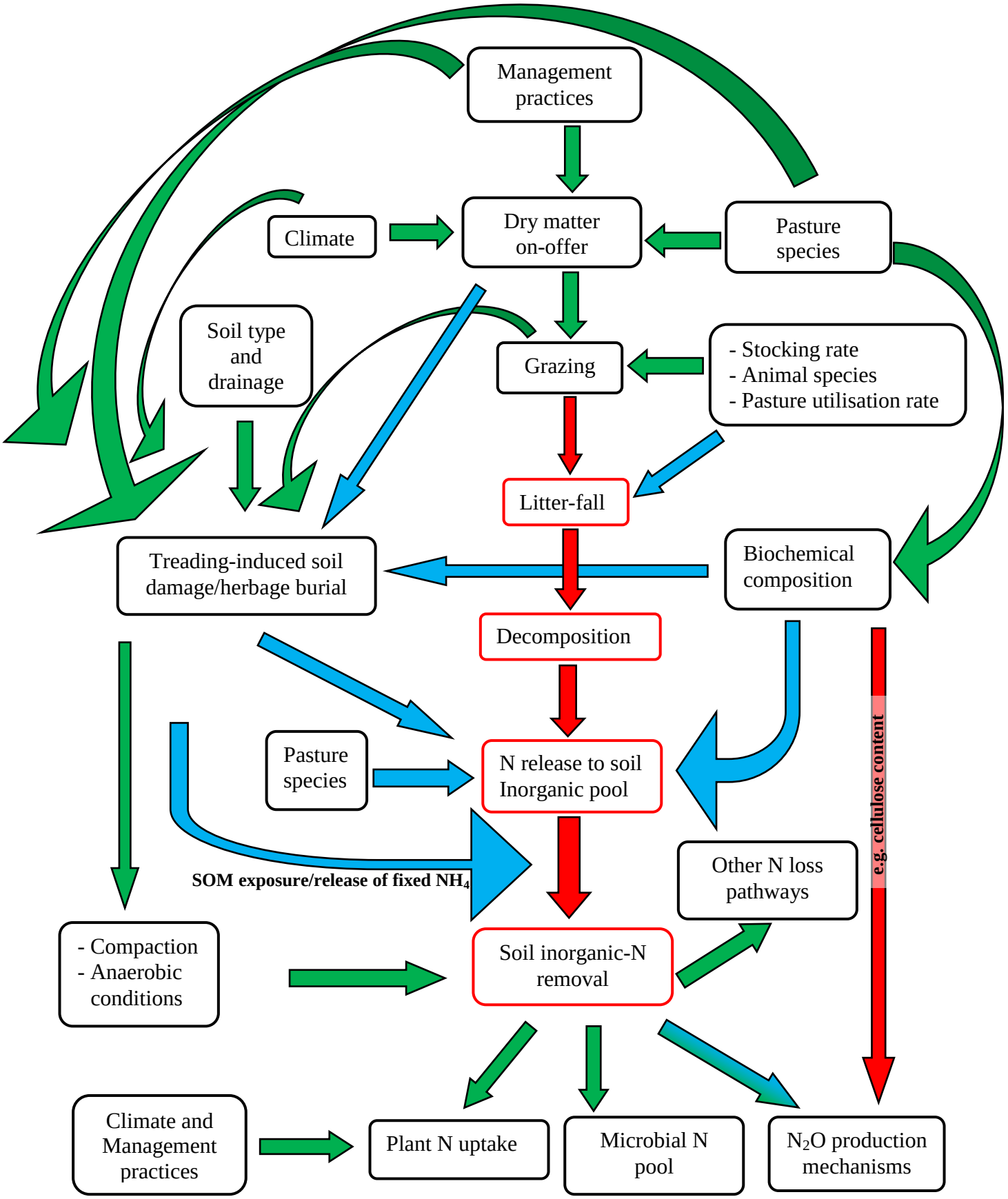

Figure 9.1 Summary of this thesis, key findings and raised questions

NB: Recognised pathways, thesis-oriented research and pathways requiring further research are shown in green, red and blue, respectively.

9.3.2 Inventory implications

Nitrous oxide emissions are expressed as an emission factor (EF) that is calculated by determining the cumulative mass of N emitted (as N_2O-N) over a period, subtracting the cumulative emissions from non-treated 'background' control plots, and dividing the difference by the mass of N applied to the soil (in the form of residues, fertiliser or excreta). The IPCC methodology states that "...The so-called 'background' emissions...are anthropogenic and are accounted for in the IPCC methodology". These emissions are assumed to mainly originate from the contribution of N in crop residues (IPCC definition) and/or antecedent effects of animal excreta and fertiliser. The IPCC also assumes that the emissions from managed and unmanaged lands are equal and are set to 1 kg N_2O-N ha^{-1} y^{-1} under zero fertiliser N addition based on estimates of Bouwman (1996) while Kelliher *et al.* (2010) reported a value of 0.3 kg N_2O-N ha^{-1} y^{-1} for background emissions for New Zealand pastures. In the absence of country specific data for background emissions for New Zealand, there has been discussion on ways to include background emissions.

9.3.2.1 Relative magnitude of litter-fall emissions at the urine patch scale

Fresh litter-fall at the rate of 53 kg DM ha^{-1} d^{-1} on a headspace chamber area of 0.16 m^2 yielded 0.39 mg N_2O-N $chamber^{-1}$ over 7 d (mean of peak emissions during 4–10 d). Assuming an EF of 0.9% and dry matter N content of 51.7 mg N g^{-1}, this equates to a daily flux of 0.35 mg N_2O-N m^{-2} d^{-1}. Studies have reported daily N_2O fluxes from urine application in the range of 7–8 mg N_2O-N m^{-2} d^{-1}. For example, De Klein *et al.* (2003) applied cow urine (at 655 kg N ha^{-1}) to a silt loam pasture soil in New Zealand and recorded peak N_2O fluxes of 7.2 mg N_2O-N m^{-2} d^{-1} while Clough *et al.* (1998) recorded a peak N_2O flux of ~8 mg N_2O-N m^{-2} d^{-1} when urine was applied at the rate of 1000 kg N ha^{-1}. Thus the litter-fall-derived daily N_2O flux is approximately 4.4–4.9% of a daily urine-derived flux. Thus, compared to the emissions from urine, litter-fall-derived emissions are small in magnitude, but they are likely to occur daily for about 10 d after litter-fall.

9.3.2.2 Relative magnitude of litter-fall emissions at the national scale

New Zealand's total (direct and indirect) N_2O inventory from agricultural soils was reported to be 19.5 Gg N_2O-N y^{-1} in 2009 (Ministry for the Environment 2011). The dairy industry has 6.2 million dairy cattle (Statistics New Zealand 2011) and is responsible for 4.67 Gg N_2O-N y^{-1} (direct emissions) or 32% of the total, direct emissions from agricultural soils. Approximately 0.6 kg DM cow^{-1} is dropped as litter-fall per grazing event i.e. per day (Chapter 4). Assuming 6.2 million cows graze for 365 d on pasture with an N content of 51.7 mg N g^{-1} and litter-fall has an EF of 0.9%, then 0.63 Gg N_2O-N y^{-1} will be emitted from

litter-fall annually. As a proportion of the total N_2O-N (5.780 Gg N_2O-N y^{-1}) emitted from dairy cattle in 2009 (4.668 Gg N_2O-N y^{-1} directly; 1.112 Gg N_2O-N y^{-1} indirectly), emissions from litter-fall equates to 10.9%. This simple calculation uses the pasture N content of 51.7 mg N g^{-1} based on the fertilised litter used in Chapter 8. Using a pasture N content value of 37 mg N g^{-1} and an EF of 1% adopted by the national N_2O inventory calculations (CSIRO 2007), this value equates to 8.7%. However, in the field survey (Chapter 4), litter N content was roughly half this value i.e. 25 mg N g^{-1}. Furthermore, the calculation assumes that cattle grazed pasture for 365 d. In reality, this is more likely to be about 315 d, based on the LUDF's management. During the remaining period, dairy cows may either graze forage crops or be fed supplements. Revising the litter-fall's contribution based on a shorter grazing period (315 d) and lower N content (25 mg N g^{-1}) gives 0.26 Gg N_2O-N y^{-1} which equates to 4.5% of the total dairy cattle emissions.

National N_2O inventory calculations for direct and indirect N_2O emissions derived from dairy cattle is determined as a back calculation of an animal's annual production rate and weight gain. Production data are utilised for the inventory and annual weight gain estimated in order to estimate the animal's energy requirement. This requirement is then converted into a DMI based on the energy content of the pasture (CSIRO 2007). This thesis has shown that 4% of the total pasture on-offer is actually lost as litter-fall. However, dropping freshly harvested pasture would not affect the inventory determination of DMI for dairy cattle but by neglecting the contribution of litter-fall-derived N_2O emissions, there would be a systematic error of 4% (that is NOT an uncertainty using a litter-fall-N application rate of 15.9 kg N ha^{-1} y^{-1}) in New Zealand's inventory of agricultural soils N_2O emissions; this has been identified by this research. Thus, including the effect(s) of litter-fall in inventory calculation provides a more accurate and refined accounting of the N_2O-N released from grazed pasture N cycling. However, the above implications are based on one field survey of litter-fall (Chapter 4) and one *in situ* measurement of N_2O-N emissions (Chapter 8). Before solid recommendations can be made to alter IPCC inventory methodologies, further data on the effects of different grazing managements, animal and pasture species, and climate are needed (Figure 9.1).

9.3.3 Implications for methodology of N_2O flux measurement

Currently N_2O fluxes are commonly measured using static chambers deployed after the application of a fertiliser or excreta treatment (Figure 9.2a). The measurements are typically made after fencing-off an area of pasture >1 month prior to application of the treatment (urine/fertiliser), to prevent antecedent effects of urine/dung deposition. The untreated plots (control) do not receive the treatment and any emissions of N_2O from the

control plots are assumed to be arising from the antecedent effects of N cycling. The litter-fall deposited onto the surface prior to fencing off the experimental area will have decomposed and N_2O emissions may be complete (Chapters 5 and 8). However, contributions to the soil inorganic N pool may still exist after 30 d (Chapter 8). Thus, the daily fluxes from the control will be equal to background N_2O emissions [0.08 mg N_2O-N m^{-2} d^{-1}; (Kelliher *et al.* 2007)] which are then deducted from the treatment fluxes. Figure 9.2a shows that the 'net' emissions from the urine treatment would be 7.92 mg N_2O-N m^{-2} d^{-1} after this deduction using the existing N_2O flux measurement method.

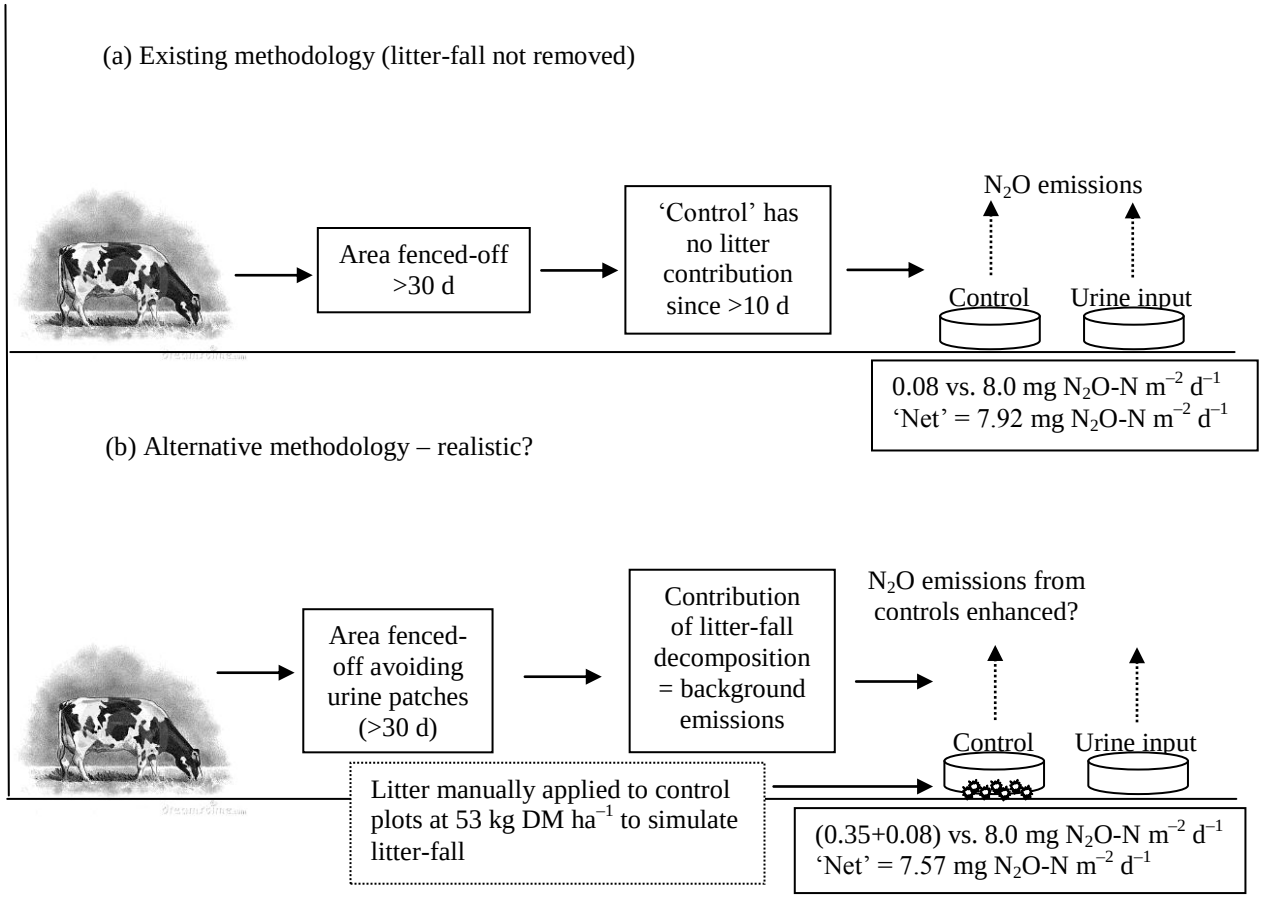

Figure 9.2 Existing and alternative methodologies for N_2O flux measurement

An alternative methodology for flux measurement is proposed. Following grazing, if an area of pasture devoid of excreta patches is fenced-off >1 month prior to treatment application onto 'treatment' plots (except the control plots), would the measured and calculated emissions be the same or different from those deduced using existing methodology?

I propose that the emissions would be different because of the decomposing litter-fall on the soil surface. This is explained as follows. After a single grazing event, approximately 3% of the grazed area has urine deposited on it (Moir *et al.* 2011; Williams and Haynes 1994). This means that 97% of the grazed area was not subject to urine deposition. On this basis, for Figure 9.2b, it can be argued that there would be a 97% chance that litter would fall onto a so called control area and only a 3% chance it would fall onto an area to which urine has been applied during the grazing event. In Figure 9.2b, the emissions from the control plots would be 0.35 mg N_2O-N m^{-2} d^{-1} (from litter-fall, Section 9.3.2.1) plus the daily background flux of 0.08 mg N_2O-N m^{-2} d^{-1} giving 0.43 mg N_2O-N m^{-2} d^{-1}. Emissions of N_2O from the urine-treated plots would be the same i.e. 8.0 mg N_2O-N m^{-2} d^{-1} giving net emissions of 7.57 mg N_2O-N m^{-2} d^{-1}. Thus, it can be argued that N_2O emissions from the control in Figure 9.2b have been correctly estimated by including the contribution of litter-fall-derived N_2O emissions, but this should not be done for the urine patch because of the very small chance of litter falling onto such areas. Thus, including a litter-fall effect would increase N_2O emissions from the controls, and hence this alternative methodology would yield a smaller N_2O emission factor (0.96%) than the existing methodology (1%).

Are potential N_2O emissions from litter-fall already captured elsewhere in the IPCC guidelines? In terms of intensive pasture grazing and litter-fall, the pasture's grass N content may have derived from fertiliser or biological N fixation (BNF), since biologically fixed N can ultimately be used by non-legumes. The IPCC guidelines recognise that the actual BNF process does not result in significant N_2O emissions. However, the guidelines do not consider the fate of symbiotically fixed N in grazed pastures cycling via litter-fall prior to any pasture renewal operation. Legumes grown specifically for their ability to fix N in anthropogenic farming systems are designated as an anthropogenic activity. If the N in the litter-fall is derived from fertiliser N or anthropogenic BNF, as is the case in intensive anthropogenic systems, then any additional N_2O emission resulting from litter-fall containing these N sources should strictly be included in inventory calculations.

The current research concludes that potential contribution(s) of litter-N cycling to N_2O emissions are significant, yet, not considered by the IPCC and hence, the IPCC methodology

should be re-evaluated. However, in order to do this, there is a need to further understand the influence of grazing management on litter-fall fate and quantities.

9.4 Conclusions

It can be concluded that:

- Pasture litter is a complex source of C and N. The mineralisation (i.e. ammonification) of C and N from litter is a slower process compared to fertiliser- or urine-N and it is dependent on the litter's biochemical composition, soil type and climatic parameters, mainly soil water content and soil temperature.
- Significant quantities of litter are produced in pastures following grazing events and this has the potential to contribute to N_2O emissions: Previously unrecognised and not accounted for in the IPCC best practice guidelines.
- Presently, there is no EF of N_2O for pasture residues. The emission factor for pasture residues *in situ* was determined to be 0.9% but should be further evaluated under different pastoral management.
- Peak N_2O emissions following pasture litter incorporation, surface decomposition and animal treading are generally short-lived and range from 4–10 d after application.
- The effect of pasture litter on the whole greenhouse gas budget should be investigated in the future studies.

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Appendix

This section compares N application resulting from litter-fall in Appendix 1 and the publications from this thesis are shown in Appendices 2 and 3.

Appendix 1.

The IPCC suggests a default emissions factor of 1% (i.e. 0.01) with an uncertainty range of 0.003−0.03 for anthropogenic additions (mineral fertilisers, organic amendments and crop residues). In Chapter 6 (experiment 3) when clover-ryegrass was incorporated into soil, an EF of 3% was recorded at an application rate of 313 kg N ha^{-1}. To estimate annual N_2O emissions from the fresh litter, the annual litter-fall rate of 15.9 kg N ha^{-1} y^{-1} was multiplied by the EF of 3% and the N_2O molecular ratio (44/28). The direct N_2O emissions from the fresh grazing-induced litter-fall were 0.75 kg N_2O ha^{-1} y^{-1}. When the default EF of 1% was used for the above calculations, N_2O emissions were 0.25 kg N_2O ha^{-1} y^{-1}. The corresponding values for the senesced litter were 0.16 and 0.05 kg N_2O ha^{-1} y^{-1} using an EF of 3 and 1%, respectively (Table A).

Based on measurements made at four sites in New Zealand, for lowland soils grazed by dairy cattle, mean and median values for background N_2O emissions were 1.0 and 0.6 kg N ha^{-1} y^{-1}, respectively (Kelliher *et al.* 2010) which had corresponding background N_2O emissions of 1.6 and 0.9 kg N_2O ha^{-1} y^{-1}, respectively. Comparing these mean and median values with estimated annual N_2O emissions in this study, N_2O emissions from green litter-fall were equivalent to 48 and 80% of the annual background N_2O emissions while using 1% as the EF, the corresponding contributions were 16 and 27%, respectively (Tables A and B for a N and C budget). The potential contribution of the green litter to background N_2O emissions can remain unaccounted for in the definition of crop residues of the IPCC best practice guidelines.

Table A. Compilation of the N budget from litter-fall and comparison of estimated values with published studies. Data are mean ± sd.

Component of calculation	Fresh litter-fall (POG_F)	Senesced litter-fall (POG_S)
Each grazing event (kg DM ha^{-1})	53.0 ± 24.4	19.4 ± 17.6
N content (%)	2.5 ± 0.5	1.5 ± 0.3
N deposition per grazing event (kg N ha^{-1})	1.3 ± 0.7	0.3± 0.2
Annual litter deposition (kg N ha^{-1} y^{-1})	15.9	3.5
Mean DMI (kg DM cow^{-1})	12.3 ± 4.8	-
Mean litter-fall (kg DM cow^{-1})	0.6 ± 0.3	-
Direct N_2O emissions from POG_F annually, EF 3% and 1% (kg N_2O ha^{-1} y^{-1})	0.75, 0.25	0.16, 0.05
Percent contribution to background N_2O, mean = 1.6 kg N_2O ha^{-1} y^{-1}, EF 3% and 1%	48, 16	25, 3
Percent contribution to background N_2O, median = 0.9 kg N_2O ha^{-1} y^{-1}, EF 3% and 1%	80, 27	17, 5

Table B. Compilation of the C budget from litter-fall and comparison of estimated values with published studies. Data are mean ± sd.

Component of calculation	Fresh litter-fall (POG_F)	Senesced litter-fall (POG_S)
Each grazing event (kg DM ha^{-1})	53.0 ± 24.4	19.4 ± 17.6
C content (%)	39.8 ± 1.4	39.7 ± 1.6
C deposition per grazing event (kg C ha^{-1})	21	7.7
Annual litter deposition (kg C ha^{-1} y^{-1})	253	92.4
Direct CO_2 emissions from POG_F annually, EF 32% (kg CO_2 ha^{-1} y^{-1})	296.8	108.4
CO_2-eq of N_2O emissions at 86% WFPS (kg CO_2-eq ha^{-1} y^{-1})	223.5	223.5
Total annual CO_2 emissions (kg CO_2 ha^{-1} y^{-1})	520.3	331.9
Percent contribution of N_2O to total CO_2 budget (%)	43	67

Appendix 2.

Pal P, Clough TJ, Kelliher FM, Van Koten C, Sherlock RR (2012) Intensive cattle grazing affects pasture litter-fall – an unrecognised nitrous oxide source. *Journal of Environmental Quality* **41**, 444–448. doi: 10.2134/jeq2011.0277

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Appendix 3.

Pal P, Clough TJ, Kelliher FM, Sherlock RR (2010) N_2O and CO_2 emissions following clover and cellulose incorporation into a New Zealand pastoral soil. In 'Proceedings of the 19th World Congress of Soil Science; Soil Solutions for a Changing World'. (Eds RJ Gilkes, N Prakongkep) pp. 154–157. (IUSS: Brisbane, Australia)

N_2O and CO_2 emissions following clover and cellulose incorporation into a New Zealand pastoral soil

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Abstract
Clover (*Trifolium repens* L.) and clover + different proportions of cellulose were incorporated into soil and the nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions measured. Ground, dried clover shoots and cellulose were mixed to carbon: nitrogen (C: N) ratios of ~9 ('clover only'), 20, 30 and 40. Soil samples were incubated at water–filled pore space (WFPS) of 86% and 20°C. Over 42 d, N_2O emissions from the controls averaged 9 mg/kg soil (6 g total N/kg soil), indistinguishable from the 'clover only' (1.5 g N incorporated/kg soil) and 'C: N 20' treatments. Corresponding N_2O emissions from the 'C: N 30' and 'C: N 40' treatments averaged nearly 50% greater (P <0.05) and these two treatment effects were indistinguishable. Over 42 d, CO_2 emissions from the controls averaged 4 g/kg soil. There was a linear C (incorporation rate) 'dose effect' on CO_2 emissions (0.15 g CO_2/g C, R^2 = 0.80) with no difference between clover and clover + cellulose. Over 145 d, CO_2 emissions from the controls averaged 17 g/kg soil and the C 'dose effect' was 0.38 g CO_2/g C (R^2 = 0.98). Incorporating different plant materials into soil affected the N_2O and CO_2 emissions differently.

Key Words
'Dose effect', plant litter, C: N ratio, decomposition, nitrous oxide, carbon dioxide.

Introduction
Plant litter is a complex C and N source, so its biochemical composition may affect mineralization rate. For example, plant litter of a lower C: N ratio may be more susceptible to decomposition and mineralization (Pal *et al*. 2010). Plant litter mainly contains soluble carbohydrates, cellulose, hemicellulose and lignin (in increasing order of recalcitrance) (Melillo *et al*. 1982), as well as N. Cellulose ($C_6H_{10}O_5$), an unbranched, β–(1,4)–linked, linear polymer of glucose, is a carbohydrate synthesized by plants and the most abundant organic polymer. Biodegradation of cellulose requires a distinct set of extracellular enzymes viz. cellulase, cellobiohydrolase and β–glucosidase, which act synergistically to hydrolyse the β–1,4 bonds of cellulose to glucose for further energy generation processes (Clarke 1997). Fungi including *Penicillium sp.* and *Aspergillus sp.* and bacteria such as *Streptomyces sp.* and *Pseudomonas sp.* aid in the extracellular cleavage of cellulose. The effect of cellulose in plant litter on N_2O emissions has received little attention. A potent greenhouse gas, N_2O has a global warming potential of 298 over 100 years and it is a precursor molecule involved in stratospheric ozone depletion (Forster *et al*. 2007). This paper reports the results of an experiment to measure N_2O and CO_2 emissions following clover and cellulose incorporation into soil sampled beneath pasture grazed by dairy cattle near Lincoln, New Zealand.

Methods
Litter incorporation and measurements
Temuka silt loam soil was sampled at a grazed pasture site (0–10 cm) and sieved to ≤ 4 mm. Dried, ground clover shoots and cellulose (in different proportions) were incorporated into the soil to achieve C: N ratios of ~9, 20, 30 and 40 representing 'clover only', 'C: N 20', C: N 30' and C: N 40' treatments, respectively. After treatment, soil was packed into PVC containers (internal diameter 8.0 cm, total height 10 cm) to a depth of 4.5 cm with the bottom covered by fine nylon mesh. The soil was incubated at 86% water filled pore space (WFPS) and 20°C for 145 d. Emissions of N_2O and CO_2 were measured using a chamber technique with gas chromatography and infrared gas analysis, respectively.

Results and Discussion
Soil and litter properties
The θ_g, θ_v, ρ_b, and ϕ were 0.31 kg water/kg dry soil, 0.23 m^3 water/m^3 dry soil, 736 kg soil/m^3 soil and 0.72

m^3 pores/m^3 soil. The pH was 5.7, and there was 64 g/kg total C and 6 g/kg total N. The clover shoots had 51 g N/kg, 430 g C/kg, a C: N ratio of 8.5, 155 g cellulose/kg, 37 g hemicellulose/kg, and 23 g lignin/kg.

N_2O emissions

The maximum N_2O emissions were 8 h after treatment at 152.3 ± 10.1, 97.6 ± 12.9, 45.6 ± 7.6 and 21.7 ± 3.1 ng N_2O/kg soil/s (mean ± standard error of the mean, $n = 5$) for the 'C: N 40', 'C: N 30', 'C: N 20' and 'clover only' treatments, respectively ($P < 0.05$, Figure 1). Eleven hours later, N_2O emissions from the 'C: N 40' and 'C: N 30' treatments remained significantly greater than the 'C: N 20' and 'clover only' treatments, in turn greater than the controls. Over 42 d, when N_2O emissions measurements ceased, the cumulative emissions from the 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control were 14.3 ± 0.5, 12.4 ± 1.2, 8.0 ± 0.6, 8.7 ± 1.2 and 9.1 ± 0.9 mg N_2O/kg soil, respectively. These emissions were in the order of control = 'clover only' = 'C: N 20' < 'C: N 30' < 'C: N 40' ($P < 0.05$). While, unexpectedly, cumulative N_2O emissions from the controls were indistinguishable from the 'clover only' and 'C: N 20' treatments, the time courses differed significantly with 90% of the corresponding totals completed in ~38, 7 and 9 d. Corresponding N_2O emissions from the 'C: N 30' and 'C: N 40' treatments included 90% of the total completed in ~9 d. Thus, clover incorporation produced the most rapid N_2O emissions' response and adding the largest quantities of cellulose significantly enhanced the N_2O emissions response to clover incorporation.

The dry, ground clover and cellulose incorporated into the soil evidently blocked the soil pores, reducing oxygen diffusion rate and contributing to the attainment of anaerobic conditions. Higher N_2O emissions would be expected under more anaerobic conditions. The presence of available C can increase denitrification, directly, by increasing energy and electron supply to the denitrifiers, and indirectly, by enhanced microbial growth and metabolism, thereby stimulating higher O_2 consumption (Beauchamp *et al*. 1989; Gillam *et al*. 2008). The current results cannot delineate the N_2O production mechanism but given the soil moisture content and the fact that cellulose was being utilised, and that the N_2O emissions were lower than the control during 2.3–4.3 d; it is likely that the C substrate further enhanced denitrification and permitted the further reduction of N_2O to N_2 (Firestone and Tiedje 1979).

CO_2 emissions

Four hours after treatment the CO_2 emissions were 12.0 ± 0.4 < 17.5 ± 1.1 = 15.0 ± 2.2 = 11.7 ± 1.0 > 1.9 ± 0.1 μg CO_2/kg soil/s from the 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control, respectively (Figure 2). The maximum CO_2 emissions occurred at 1.4 d with 23.9 ± 0.04 > 25.1 ± 0.05 > 22.4 ± 0.17 > 18.0 ± 0.12 > 1.4 ± 0.01 μg CO_2/kg/s from 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control, respectively. The relatively low CO_2 emissions from controls suggest disturbance was not responsible for the higher emissions of treated soil; rather, the soil microbial biomass may have switched from the recalcitrant soil organic matter to the incorporated substrate (Sparling *et al.* 1982; Cheng 1996). Moreover, these higher emissions can be accounted for the so called r–strategist activity of rapid catabolism of the fresh organic matter in soil (Fontaine *et al*. 2003).

The CO_2 emissions steadily declined after 1.4 d but at 10.3–11.1 d, a further increase in CO_2 emissions was observed (Figure 2) but this increase was very minor in the 'clover only' treatment. Furthermore the 'secondary peak' in those treatments with cellulose additions was dependant on the rate of cellulose applied (i.e. 'clover only' < 'C: N 20' < 'C: N 30' = 'C: N 40') which indicated cellulose utilisation as an energy source. The 'C: N 30' and 'C: N 40' emissions did not significantly differ at 1.4 d probably because of the abundant amount of added cellulose already present in the soil. A significant 'C dose effect' was observed over the entire incubation period since the 'clover only' and 'C: N 20' treatments reached the control levels at 112.2 d followed by 'C: N 30' at 145.3 d. The cumulative CO_2 emissions over 145 d averaged 98.5 ± 3.0, 83.8 ± 2.3, 66.4 ± 0.9, 42.0 ± 1.4 and 16.6 ± 2.5 g CO_2/kg soil from 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control, respectively and were significantly different from one another. There was a linear 'C dose effect' (incorporation rate) on CO_2 emissions with no difference between the incorporation of clover and clover + cellulose into the soil (data not shown). Over 42 and 145 d, CO_2 emissions from the controls averaged 4 and 17 g/kg soil and the 'C dose effect' was 0.15 and 0.38 g CO_2/g C, respectively. Over 42 d, on a CO_2–equivalent basis, CO_2 emissions were ~90% of 'CO_2 + N_2O' emissions following clover and cellulose incorporation into the soil.

Cellulose occurs naturally in plant tissues and forms the basis of plant cell walls. It requires more energy to catabolise/cleave the bound cellulose for use in energy generation processes. Moreover, lignin present in the

plant tissues physically protects, and therefore retards the catabolism of the bound cellulose but in the absence of lignin; it may aid the cellulose to decompose faster (Swift *et al*. 1979). We incorporated pure cellulose powder directly in the soil which although may be a recalcitrant form of C, was labile enough for the microbes as an energy source (as it was not required to be cleaved before use). Moreover, N availability can stimulate the decomposition rates (Carreiro *et al*. 2000; Geisseler and Horwath 2009). The labile–N originating from the plant litter in the present study may have stimulated the cellulose decomposition and hence caused higher CO_2 generation with an additive effect with N_2O generation.

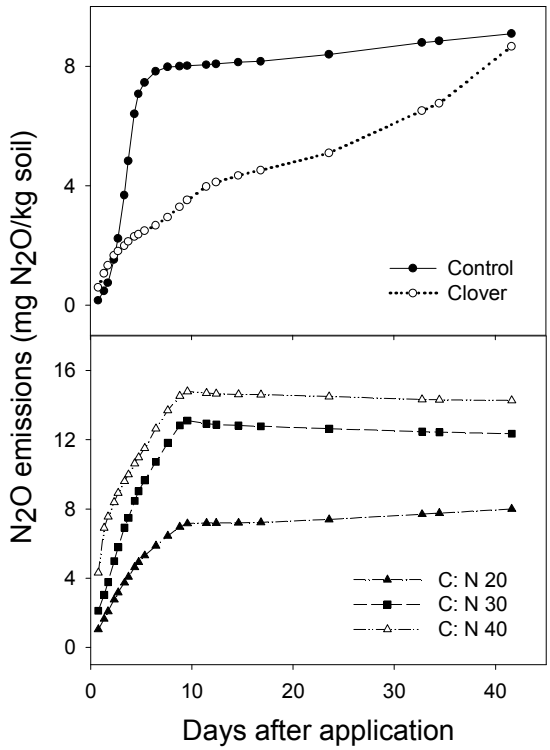

Figure 1. Cumulative N_2O emissions from 'clover only', 'C: N 20', 'C: N 30' and 'C: N 40' treatments and controls during incubation (see Methods for details).

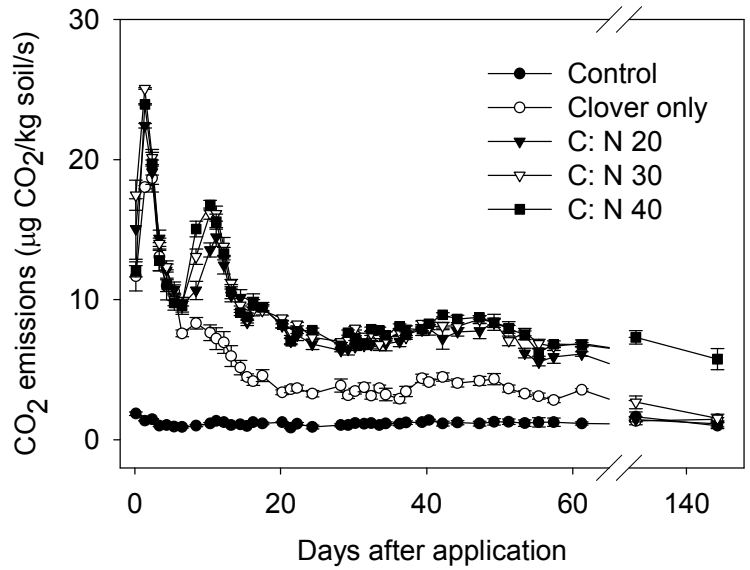

Figure 2. Soil CO_2 emissions from 'clover only', 'C: N 20', 'C: N 30' and 'C: N 40' treatments and controls during incubation. Data are means ± SE (n = 5) (see Methods for details).

Cellulolytic microorganisms thrive well and are enhanced in anaerobic conditions (Clarke 1997). In the present study, the high moisture content of the soil (86% WFPS) could also have produced conditions that were conducive for the cellulolytic organisms thus causing higher CO_2 emissions due to better decomposition of the incorporated cellulose.

Conclusion

Over 42 d, unexpectedly, N_2O emissions from the controls were indistinguishable from the 'clover only' and 'C: N 20' treatments. However, time courses of the N_2O emissions differed significantly; 90% of the total was completed in ~38, 7 and 9 d for controls, 'clover only' and 'C: N 20' treatments, respectively. Corresponding N_2O emissions from the 'C: N 30' and 'C: N 40' treatments averaged nearly 50% greater and 90% of the total was completed in ~9 d. Thus, clover incorporation produced the most rapid N_2O emissions response and adding the largest quantities of cellulose significantly enhanced the N_2O emissions response to clover incorporation. There was a linear C dose effect on CO_2 emissions with no difference between the incorporation of clover and clover + cellulose into the soil. Over 42 and 145 d, CO_2 emissions from the controls averaged 4 and 17 g/kg soil and the 'C dose effect' (incorporation rate) was 0.15 and 0.38 g CO_2/g C, respectively. Over 42 d, on a CO_2–equivalent basis, N_2O emissions were ~10% of 'N_2O + CO_2' emissions following clover and cellulose incorporation into the soil.

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