Soil carbon loss under pasture and pine:

Responses to urine addition

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Abstract

There have been reports of losses of soil carbon (C) in New Zealand pastures under dairy grazing. Acceleration of mineralisation or leaching of soil C following urine deposition may decrease soil C. However, little research of the effect of cow urine on soil C has been conducted. The overall objective of this thesis was to investigate the potential for dairy cow urine to solubilise soil C, which can then be lost through mineralisation or leaching. Soils from both grazed pasture and *Pinus radiata* plantations (termed pasture and pine soils) were investigated.

The concentration, composition, and bioavailability of urine-C were investigated. Cow urine contained 7.5±1.2 g C L^{-1}, with a C:N ratio of 2:1. About 45% of the C content of cow urine was attributed to hippuric acid, the other major contributors were urea, an unidentified amide and phenaceturic acid. On incubation at 25°C, up to 25% of urine-C was degraded in 28 days, demonstrating that cow urine is biodegradable and could potentially act to prime C mineralisation in soils.

Solubilisation of soil C (0-20 cm soil depth) following urine application was tested by measuring adsorption of urine-C and subsequent desorption of soil C in air-dried pine and pasture soils. While adsorption was low at 3% of urine-C, the solubilisation of soil C by urine ranged between 11-28% of soil C concentration for 5 different soils, however, solubilisation was likely overestimated due to the use of air-dried soils. Soil C solubilisation was also measured in field moist soils applied with artificial urine, and was less than that reported from air-dried soils.

Priming of soil C mineralisation, solubilisation, and extracellular enzyme activity were measured using moist repacked pine and pasture soil cores

(0-5 cm soil depth) treated with cow urine or radio-labelled artificial urine. Positive priming of soil C mineralisation, where more carbon dioxide (CO_2) was produced than C added, was measured following application of cow urine in both pine and pasture soils. The pasture soils lost 5.1±0.9%, and the pine soils 4.0±0.1%, of soil C concentration as CO_2 during a 84 day incubation. Positive priming was attributed to increased microbial and urease activity and accelerated soil C mineralisation in urine treated soils. The remaining extracellular enzyme activities assayed were unlikely to have contributed to soil C priming. In contrast to the positive priming measured following cow urine application, treatment of soil with artificial urine resulted in less CO_2 produced than C added – or negative priming. Increased soil pH following urine application may have played an important role in increasing C mineralisation as water soluble C increased with increasing soil pH. Therefore, cow urine can cause priming of soil C mineralisation and lead to a loss of soil C. However, artificial urine may not adequately model cow urine with respect to C cycling.

Soil C solubilisation by urine and subsequent leaching from undisturbed pasture soil (0-5 cm soil depth) was assessed by applying $\delta^{13}C$ enriched urine. Leaching resulted in a loss of 0.45±0.03% of soil C concentration, which was 10 times greater than the loss of soil C in the water control treatment (0.048±0.001%). The leaching of soil C was small compared to the 5% loss of soil C by priming in the repacked core experiment. Soil solubilisation in the undisturbed cores was less than both repacked cores and air-dried soils, demonstrating that soil C solubilisation increases with increasing soil disturbance. The acid neutralising capacity (ANC) forcing potential of cow urine was 11.8 meq L^{-1}, which was more than 20 times greater than sodium nitrate fertiliser application (30 kg N ha^{-1} $year^{-1}$), although the nitrogen loading rates of the urine were higher than the fertiliser . ANC forcing has been linked to increasing soil pH and dissolved organic C leaching, and may have been a factor in soil C solubilisation under urine patches. Upon assessing water stable aggregates after urine

application, disaggregation of soil was not a major factor in soil C solubilisation.

The key conclusions were that:

- there was strong evidence to support the hypothesis that urine deposition led to soil C solubilisation and priming of soil C mineralisation, that could have contributed to the reported declines in soil C in dairy pastures;
- priming of soil C decomposition was 10 times greater than leaching and may be the predominant mechanism of soil C loss following urine deposition; and
- urine deposition resulted in greater losses of soil C from pasture soils than pine soils, in contrast to expectations that soil C in pasture soils would have been acclimatised to urine application.

The work in this thesis was laboratory based and requires further testing under field conditions. Further work is also needed to establish the mechanisms of soil C solubilisation and the role of solubilised soil C in priming of soil C mineralisation.

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training, I'm afraid I really needed it, and resisted for as long as possible, but I now find the knowledge I've gained on Genstat to be invaluable on current research projects. Thanks to Alec Mackay and Graham Shepherd for your enthusiasm for my project whenever I saw you, I found it really heartening with so little interest within my own organisation, with the exceptions of those listed above. Thanks for various co-workers who asked occasionally how the work was going, only to be greeted with the response "it's never going to end!"

While I've learnt a huge amount about the scientific process from my various advisors, in particular Louis, I've also learnt that doing a part-time PhD, remote from supervisors, while away on field work for substantial chunks of the year, is NOT a good idea. Hindsight is a wonderful thing, but now I can look to the future.

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List of Acronyms

C/TC	Carbon/total carbon
N/TN	Nitrogen/total nitrogen
C:N	Carbon to nitrogen ratio
CU	Cow urine
AU	Artificial urine
WSC	Water soluble carbon
CWSC	Cold water soluble carbon
HWSC	Hot water soluble carbon
DOC	Dissolved organic carbon
OC	Organic carbon
TOC	Total organic carbon
TIC	Total inorganic carbon
CO_2/CO_2-C	Carbon dioxide/carbon dioxide carbon
SOM	Soil organic matter
DOM	Dissolved organic matter
SOC	Soil organic carbon
MBC	Microbial biomass carbon
M	Molarity of solution
EC	Electrical conductivity
pH	-log H^+

ANC	Acid neutralising capacity
S&B	Sheep-and-beef grazed soil
GC-MS	Gas chromatography-mass spectrometry
ANOVA	Analysis of variance
r^2	Coefficient of determination
P	Probability test statistic
n	Number of samples

Chapter 1

Introduction

1.1 The effect of land use change and intensification on soil organic matter

Soil organic matter (SOM) is important for maintaining soil quality and supporting agricultural production (Sparling *et al.* 2003; 2006). SOM influences many soil processes including water retention, aggregate stability, compaction resistance, ion exchange, and nutrient supply (Dubbin & Fancett 2001; Lal 2004; Haynes 2005). In addition, SOM is a large carbon (C) reservoir, and sequestration of C within the soil pool can mitigate the effects of increasing atmospheric carbon dioxide (CO_2) concentrations (Lal 2004).

Previously, it was thought that soil C in New Zealand pastures was at steady state (Tate *et al.* 1997). However, recent research indicated that SOM had declined under intensively grazed pastures, predominantly those used for dairying (Schipper *et al.* 2007; 2010). Mean profile losses of 2.1 kg C m^{-2} in the 17-30 years before 2007 were measured in pasture soils (Schipper *et al.* 2007). Losses of C were greatest under dairy systems while there was no change in soil C in dry stock systems (Schipper *et al.* 2010). The reasons for the reported losses in soil C were not known.

New Zealand pastures are undergoing intensification, particularly in the dairy sector, and there has been considerable land use change from forestry to dairy grazing (Parliamentary Commissioner for the Environment 2004; MacLeod & Moller 2006). Between 1994 and 2003, stocking rates increased by 19% and the amount of land used for dairy production by 12% (Parliamentary Commissioner for the Environment 2004). In contrast, the amount of land used for sheep and beef grazing and plantation

forestry has declined (Ministry for the Environment 2007). Conversion of forestry to pasture can increase or decrease soil C stocks depending upon post conversion management (Scott *et al.* 1999; Murty *et al.* 2002, Grünzweig *et al.* 2004; Oliver *et al.* 2004; Harms *et al.* 2005).

1.2 Can dairy cow urine decrease soil carbon?

Increasing the grazing intensity of cows will also increase the amount of urine returned to the soil surface (Pleasants *et al.* 2007; Moir *et al.* 2011). Cow urine deposition causes localised points of increased soil pH (Doak 1952; Bristow *et al.* 1992) and may lead to soil C solubilisation (Jackman 1960; Lovell & Jarvis 1996; Curtin *et al.* 1998; Shand *et al.* 2000). Urine application can also cause priming of the mineralisation of soil C (Lovell & Jarvis 1996; Kool *et al.* 2006; Uchida *et al.* 2011). Priming occurs when the addition of a C source increases soil C mineralisation (Kuzyakov *et al.* 2000). The contribution of cow urine to nutrient cycling has mostly focused on nitrogen (N; e.g. Ball *et al.* 1979; Lockyer 1984; Lovell & Jarvis 1996), and there is little research on the effect of cow urine on soil C.

1.3 Thesis objectives

The overall objective of this thesis was to establish if dairy cow urine application can cause losses of soil C by dissolution followed by mineralisation or leaching in soils collected from grazed pasture and *Pinus radiata* plantation (known as pasture and pine soils).

The specific objectives were to:

- determine the C content, composition and bioavailability of dairy cow urine (Chapter 3 and 7);
- determine if cow urine causes solubilisation and leaching of soil C from pine and pasture soils (Chapters 5, 6 and 7), *and to test the hypothesis that soil C solubilisation would be greater in pine soils than pasture soils;* and

- determine if cow urine can cause priming of soil C in pine and pasture soils (Chapter 6), *and to test the hypothesis that priming would be greater in pine soils than pasture soils due to previous acclimation in the pasture soils.*

1.4 Thesis structure

Chapter 1 contains background and objectives of the thesis, Chapter 2 is a literature review, and Chapters 3-7 present the results of experimental work. Chapters 5 and 7 have been published in Soil Research and copies of the published papers have been included in Appendix 3. Chapter 8 provides an overview of the thesis, discussing the findings from the experimental work and summarising the overall conclusions. I have undertaken the work conducted in this thesis with the guidance of my supervisors, who are recognised as co-authors on chapters presented as papers.

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Chapter 2

Fate and effects of dairy cow urine in soil

2.1 Introduction

Soil organic matter (SOM) is a vital component in soil fertility, sustainability and productivity through its contribution to cation exchange capacity, nutrient pools, stabilisation of structure, aggregate formation, and the improvement of aeration, water-holding capacity, and permeability (Jackman 1960; Lynch & Bragg 1985; Stevenson & Cole 1999; Johnston *et al.* 2009). SOM also plays an important role in greenhouse gas cycling, both emitting and sequestering atmospheric carbon dioxide (CO_2; Schlesinger & Andrews 2000; Johnston *et al.* 2009).

A large portion of New Zealand's economy is derived from agriculture, with the dairy industry contributing export earnings of $10.6 billion in 2010 (Ministry of Agriculture and Forestry 2011). The expansion and intensification of our dairy sector led to an increase of 24% in the number of dairy cows nationally between 1996 and 2006 (Parliamentary Commissioner for the Environment 2004; Ministry for the Environment 2007). The demand for land and high profits within the dairy industry have contributed to substantial conversion of less intensively used land, mainly dry stock farming and plantation forestry, to dairying (Ministry of Agriculture and Forestry 2009; Ministry for the Environment 2007).

Previously, C stocks in grazed New Zealand pastures were considered to be at steady state (Jackman 1964; Haynes & Francis 1993; Tate *et al.* 1997). However, recent research has measured a decline in soil C under intensively grazed pastures (Schipper *et al.* 2007; 2010), the mechanisms of which have yet to be clarified. The effect of urine on soil C has received

little investigation although cow urine has the potential to cause losses of soil C in two ways:

1. Rapidly increasing soil pH leading to soil C dissolution (Jackman 1960; Monaghan & Barraclough 1993; Lovell & Jarvis 1996); and
2. Enhancement of microbial activity and SOM mineralisation (Lovell & Jarvis 1996; Kool *et al.* 2006).

This review discusses the findings of the literature with respect to the fate of urine-C in soils and the effects of urine on soil C. First, the nature of SOM, C cycling, and urine composition have been outlined.

2.2 Soil organic matter

2.2.1 Organic matter structure

The structure of SOM can be described using several fractionation techniques (Schnitzer 1991). Generally chemical or physical fractionations are used to look at structure of SOM, comparisons of the methodologies used for these fractionations have been assessed elsewhere (Baldock *et al.* 1992; Swift 1996; Christensen 2001).

Chemical fractionation separates SOM into two pools, humic and non-humic substances, which are not isolated from one another but, interact between themselves and other soil components to form a complex matrix (Stevenson & Cole 1999). Non-humic substances are still chemically identifiable (e.g. lipids), whereas humic substances no longer show any resemblance to identifiable structures (Saiz-Jimenez 1996). Humic substances are produced by the breakdown of many compounds, including lignin, lignin-derived phenolics, non-lignin derived phenolics, and sugars, to form humic and fulvic acids (Stevenson & Cole 1999). Humic and fulvic acids make up as much as 80% of SOM, and are recalcitrant compared with non-humic substances (Schnitzer 1991; Stevenson 1994; Stevenson & Cole 1999). Non-humic substances comprise lipids (1–6% of SOM), carbohydrates (5–25% of SOM), proteins/peptides and amino acids

(9–16% of SOM; Schnitzer 1991; Stevenson 1994; Stevenson & Cole 1999).

Recently, pyrolysis gas chromatography-mass spectrometry has been used to assess the composition of C associated with three soil class sizes. Grandy and Neff (2008) found that particulate organic matter is mostly (70%) lignin, with only a small amount of polysaccharides and proteins present. The 38-53 µm and <38 µm size classes, on the other hand, contained mostly polysaccharides (30-52%) and proteins (10-12%), but contained very little lignin (2-6%; Grandy & Neff 2008).

2.2.2 The global carbon cycle

The C cycle consists of five main pools that interact continuously (Janzen 2004; Lal 2004; Figure 2.1). The dynamics between each compartment have been altered by humans, mostly by land-use change and fossil fuel consumption (Janzen 2004).

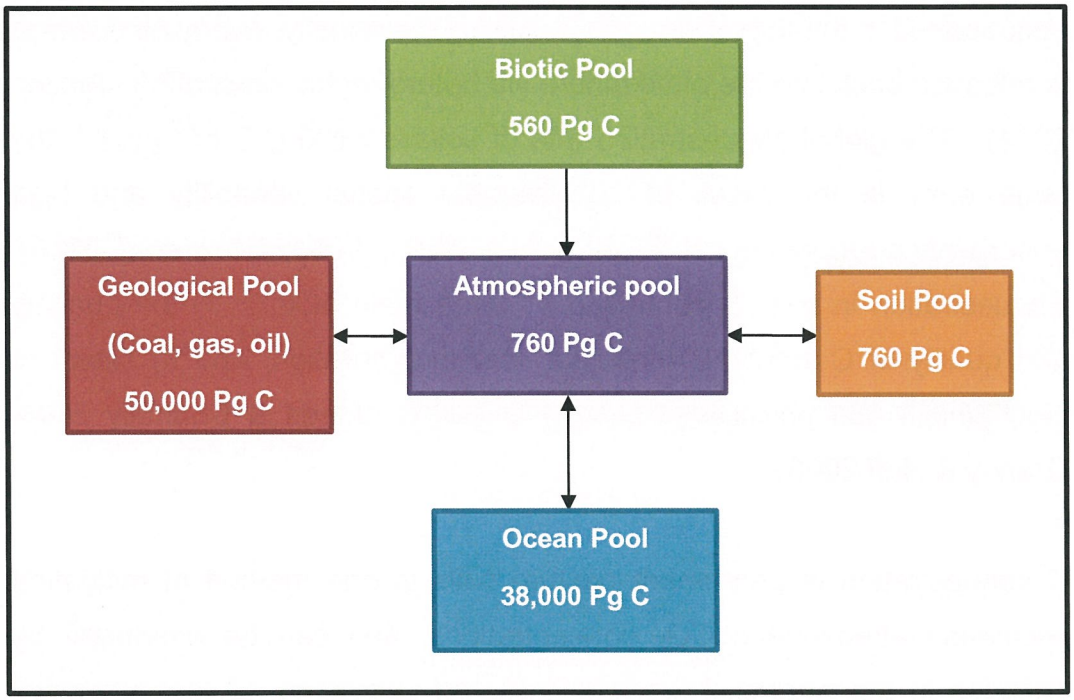

Figure 2.1: Global carbon stocks (Lal 2004).

Over the past 400 years there has been significant conversion of native grasslands and forests to agricultural use, with agriculture now covering as much as 30% of the Earth's land surface (Janzen 2004). CO_2 emissions are usually generated from deforestation, biomass burning, agricultural intensification, wetland drainage and cultivation, agricultural land abandonment, and wood harvesting (Lal 2004). Since the beginning of the industrial revolution, land-use change and cultivation have been estimated to have contributed ~136 Pg C to atmospheric CO_2, and depletion of SOM may be responsible for nearly 60% of this (Lal 2004). The terrestrial C pool is relatively large, so even a small change in its size may cause significant alteration to atmospheric CO_2 concentrations (Paul & Polglase 2004; Mackay 2008). A 5% decrease in soil C could increase atmospheric CO_2 by 16% (Mackay 2008).

While soils are often a source of CO_2 emissions to the atmosphere, they can also act as a sink: C sequestration has been defined by Jones and Donnelly (2004) as "the process of removing CO_2 from the atmosphere and storing it in C pools of varying lifetime" (pg. 424). About 120 Pg. C are sequestered in the terrestrial pool (Soil+Biotic) annually, nearly all of which is released back into the atmosphere via heterotrophic respiration (Janzen 2004). The global sequestration rate of soils is 5±30 g C m^{-2} $year^{-1}$; the large error is the result of considerable spatial variability and high uncertainty surrounding soil C stock measurement (Soussana *et al.* 2010). Sequestration in soils is controlled by three main factors: 1) the quantity and quality of C inputs, 2) depth of litter incorporation, and 3) extent of biochemical and physical protection of soil C (Jones & Donnelly 2004; Grandy & Neff 2008).

C sequestration in permanent pasture soils is one method of mitigating escalating atmospheric CO_2 concentrations, and can be enhanced by changes in management, particularly 1) no cultivation, 2) less intensive use, 3) changes to nitrogen (N) fertiliser application rates, and 4) increasing legume populations (Conant *et al.* 2001; Soussana *et al.* 2010). While Soussana *et al.* (2010) suggest that decreasing N fertiliser

increases soil C sequestration, Conant *et al.* (2001) reported fertilisation can increase soil C concentrations. The accumulation of C can be reversed by cultivation, which disrupts aggregates releasing previously unavailable C (Lal 2005). Droughts can also cause reversion of C sequestration, for example, following a drought in Europe in 2003, CO_2 emissions increased from soils by 0.5 Gt C $year^{-1}$ (Ciais *et al.* 2005). Deposition of dairy cow urine may also reverse C sequestration, as the deposition of cow urine can increase CO_2 fluxes from soils (Lovell & Jarvis 1996; Kool *et al.* 2006; Uchida *et al.* 2011; Section 2.5.1; 2.6.3.3).

2.3 Soil carbon in grazed pasture

Contrary to previous findings that soil C stocks in New Zealand pastures were at a steady state (Jackman 1964; Haynes & Francis 1993; Tate *et al.* 1997), recent research has measured a decrease in soil C under flat, intensively grazed pastures. Schipper *et al.* (2007) resampled 31 soil profiles, to 1 metre, following long-term (17-30 years) pastoral grazing. C contents of the profiles were compared with archived samples from the same profiles, showing a mean loss of 21 Mg C ha^{-1}, with a rate of loss of 1.06 Mg C ha^{-1} yr^{-1} (Schipper *et al.* 2007). Schipper *et al.* (2007) suggested that the losses in soil C may have been contributed to by leaching, erosion and/or accelerated mineralisation of SOM. Further research into losses of soil C from flat dairy-grazed pastures found a decrease in soil C of 0.73±0.16 Mg C ha^{-1} $year^{-1}$ in the top 30 cm of the profile (Schipper *et al.* 2010).

Declining soil C stocks under pasture, as measured in New Zealand, is not an isolated case. Despite increasing soil organic C (SOC) contents between 1960 and 1990, 74% of agricultural soils in Belgium exhibited a subsequent loss of SOC between 1990 and 2000 (Lettens *et al.* 2005a). Grazed pastures lost 5 t C ha^{-1} in the top 30 cm, and 6% of SOC down to 1 metre (Lettens *et al.* 2005a, b). Restriction of the application of manure to land led to decreasing C inputs and may ultimately have led to a loss in soil C (Lettens *et al.* 2005b).

Bellamy *et al.* (2005) reported losses of C from English and Welsh soils between 1978 and 2003, with a mean rate of loss of 0.6% $year^{-1}$. The extent of soil C loss increased with increasing soil C content, with losses of 2% $year^{-1}$ for soils with C contents above 100 g kg^{-1} (Bellamy *et al.* 2005). When no correlation was found to land use, rainfall, or soil texture, Bellamy *et al.* concluded that the losses of soil C were partially driven by climate change. However, Smith *et al.* (2007a) question the accuracy of SOC contents based on bulk densities estimated by SOC contents, such as those reported by Bellamy *et al.* (2005), arguing that a small change in bulk density can led to large apparent changes in soil C, particularly in organic soils, which showed the greatest losses of soil C in Bellamy *et al.* (2005). Smith *et al.* (2007a) suggest that only 10–20% of the soil C loss in England and Wales was attributable to climate change and that, as in Belgium, the remaining loss of soil C was more likely the result of decreased manure inputs.

Losses of C from New Zealand's hill country soils have also been reported (Lambert *et al.* 2000). However, more recent research found an increase in soil C in 15 sheep-and-beef-grazed hill country sites around the North Island of New Zealand (Schipper *et al.* 2010). Hill-country soils gained an average of 0.52±0.18 Mg C ha^{-1} yr^{-1} in the top 30 cm of the profile (Schipper *et al.* 2010). Increases of soil C in these hill-country soils were mostly attributed to increased soil C cycling due to pasture inputs, but deposition of soil onto eroded sites could also have contributed to reported gains (Roger Parfitt, pers.comm.).

With appropriate management, grazing of pasture can lead to increases in soil C content by increasing root exudation, litter degradation, and litter incorporation into the soil (Schuman *et al.* 1999; Conant *et al.* 2001; Ganjegunte *et al.* 2005). Increased soil C in grazed pastures has been linked to enhanced root turnover, which in turn can be aided by increased microbial biomass and improved pasture species (Williams & Haynes 1990; Frank *et al.* 1995; Grünzweig *et al.* 2004; Liebig *et al.* 2006). On the other hand, grazing can also lead to increased shoot turnover (2–12%),

decreasing root turnover and belowground C inputs and, possibly decreasing soil C (Holland & Detling 1990; Schuman *et al.* 1999)

Grazing intensity has been reported to both increase and decrease soil C. Lightly grazed soils can contain more SOC in the top 5 cm of the profile (13.8 Mg ha^{-1}, 0–5 cm depth) than heavily grazed pasture soils (10.9 Mg ha^{-1}) and exclusion zones (10.8 Mg ha^{-1}; Ganjegunte *et al.* 2005). Liebig *et al.* (2006), however, measured greater SOC (5–10 cm soil depth) in heavily grazed soils than in moderately grazed soils. Frank *et al.* (1995) also found that heavier grazed and exclusion zone soils had more soil C (7.2–7.4 Mg ha^{-1}) in the top 30.4 cm than lightly grazed soils (6.4 Mg ha^{-1}). Although Manley *et al.* (1995) found that exclusion plots contained less SOC than grazed plots. Comparing reports such as those cited above has many difficulties due to the range of soils tested, the different soil depths assessed, and differing definitions of grazing intensities – what may be described as "lightly" grazed by one author may be "moderately" grazed by another.

Haynes and Williams (1999) report increased SOC and water soluble C under stock camping zones, which was likely a result of increased excrement input in these areas. Carran and Theobald (2000) also found that excretal returns increased soil C by 20% in grazed compared to ungrazed portions of the same paddocks.

One of the potential mechanisms for the losses of soil C reported by Schipper *et al.* (2010) is urine deposition. Urine deposition leads to a rapid increase in soil pH (Haynes & Williams 1992) which can lead to the solubilisation of soil C and the potentially subsequent loss through either mineralisation or leaching.

2.3.1 *Soil carbon after land-use change from plantation forestry to pasture*

Plantation forestry covered 1.76 million hectares of New Zealand's land surface at 1 April 2008 (Ministry of Agriculture and Forestry 2009). With the current demand for land from the dairy industry there has been less forestry development in New Zealand compared to mid-1990s (Ministry for the Environment 2007). Some pine plantations have even been converted to dairying before tree maturity to take advantage of high dairy profits (Ministry for the Environment 2007). Approximately 15,600 hectares of plantation forest had been converted to grazing in the year ending 31 March 2008 (Ministry of Agriculture and Forestry 2009).

For conversion of forests to grazing, deforestation has to occur and, similar to the effects for grazing intensity, deforestation has been shown to cause both increases and decreases in soil C. In New Zealand, Oliver *et al.* (2004) measured losses of 3.1 Mg ha^{-1} in the top 10 cm of mineral soil following forest harvest and an increase of 22% in soil C between 50 and 100 cm depth. In this case, mixing of the top soil with subsoil during harvesting led to dilution of soil C in the topsoil (Oliver *et al.* 2004). In contrast, Harms *et al.* (2005) reported losses of 8% of soil C in the top 30 cm, and about 5.4% in the top metre of the profile following deforestation. Soil C losses increased with increasing soil C content, and were found to be greater in sandy soils than silty soils (Harms *et al.* 2005). Disruption of soil aggregates during forest harvest can contribute to losses of soil C by releasing previously unavailable C, which is then rapidly mineralised (Janzen 2004; García-Oliva *et al.* 2006).

While the deforestation stage of land conversion to grazing can lead to a decrease in soil C, this may be short term losses from disturbance. Grünzweig *et al.* (2004) measured an initial decrease in soil C (8.3 kg m^{-2}) over 20 years after conversion to grazing, but after 60 years soil C stocks had increased by 2.1 kg m^{-2}. Murty *et al.* (2002) undertook a meta-analysis of soil C change following conversion from forestry to grazing.

Although, they found soil C changes in the range of –50% to +160%; the analysis found no net increase or decrease in global soil C contents following conversion (Murty *et al.* 2002).

Newly converted pine plantation soils may be more susceptible to soil C solubilisation under urine patches than long-term grazed pastures. As these soils have not previously been exposed to urine deposition, the rapid increase in soil pH following urine application may be less buffered than long-term grazed soils and led to greater soil C solubilisation. No research on the effect of urine on newly converted soils could be located.

2.4 Potential for cow urine to decrease carbon in pasture soils

As previously stated, cow urine has the potential to decrease soil C by increasing the amount of soil C in solution, and therefore the amount of soil C available for mineralisation and leaching. In the next few sections the following are reviewed; the distribution and composition of cow urine, and the fate of urine-C in soils.

2.4.1 Spatial distribution

Cattle intake large amounts of nutrients in their feedstock, of which 65–90% are returned to the pasture in excrement distributed in concentrated patches (Haynes & Williams 1993; Shand *et al.* 2000). The size of a cattle urine patch ranges from 0.34 to 0.42 m^2 and can reach to a depth of 40 cm down the soil profile (Williams & Haynes 1994; Moir *et al.* 2011). The proportion of the pasture surface that is covered each year with excreta was reported to be within 20-40% (Saunders 1984; Moir *et al.* 2011), although the area of pasture affected increases with increasing grazing pressure and can be concentrated in stock camping areas (Saunders 1984; Haynes & Williams 1999). A single grazing event has been estimated to cover 4-9% of a paddock's surface with urine (Richards & Wolton 1976a; Moir *et al.* 2011). Although, with increasing stocking rates,

there is an increase in the area of soil affected by urine deposition (Moir *et al*. 2011).

Dairy cows produce greater volumes of urine than other livestock (Bilotta *et al*. 2007); cattle generally urinate between 10 and 12 times per day, with each urination producing 1.6 to 2.2 litres of urine (Hardison *et al*. 1956; Haynes & Williams 1993). Doak (1952) and Hogg (1981) suggest that a single urination may be equivalent to 5–11 mm of rainfall in a small area of pasture. Church (1976b) and Somda *et al*. (1997) report the volume of urine is dependent on water and feed intake, climatic conditions, animal activity, and kidney function. Although, the volume of urine produced can vary between animals, even under the same experimental conditions (Betteridge *et al*. 1986; Jarvis *et al*. 1995).

The large nutrient return within a urine patch often exceeds plant requirements (Williams & Haynes 1990). Urine deposition may double or triple pasture production, however clover production is impeded by urine (Ledgard *et al*. 1982; Saunders 1984; Williams & Haynes 1994; Silva *et al*. 2005).

2.4.2 Urine composition

Substantial research has been undertaken to determine the nature of urine-N and its role in the soil N cycle (Doak 1952; Ball *et al*. 1979; Stillwell & Woodmansee 1981; Bristow *et al*. 1992). The major component in urine-N is urea, making up between 50 and 94% of urine-N content, followed by allantoin (2–12% of urine-N) and hippuric acid (2–6% of urine-N; Doak 1952; Bristow *et al*. 1992). Other N bearing compounds isolated in urine include creatine, creatinine, uric acid, ammonium, and amino acids (Doak 1952; Bristow *et al*. 1992). The average N concentration in a urine patch is 1000 kg N ha^{-1} (Haynes & Williams 1993).

Other cations and anions studied in cow urine include;

- Potassium

  - 4.2 g L^{-1} (Richards & Wolton 1976b)
  - 10.4 g L^{-1} (Orwin *et al.* 2010)
  - 33-76% of daily intake is excreted in urine (Betteridge *et al.* 1986)
  - Contributes 60-70% to total urine cation content (Haynes & Williams 1993)
- Sodium
  - 1.2 g L^{-1} (Richards & Wolton 1976b)
- Magnesium
  - 12% of intake is excreted in urine (Hutton *et al.* 1965)
- Chloride
  - 4.7 g L^{-1} (Monaghan & Barraclough 1992)
- Sulphur
  - 50±2.6 mg day^{-1} (Bird 1972)
- Phosphorus
  - Only small amounts in urine, as the vast majority is excreted in dung (Hutton *et al.* 1967; Betteridge *et al.* 1986; Barrow 1987; Powell *et al.* 1998).

The electrical conductivity of urine has been reported between 12.3 and 21.3 mS cm^{-1} (Lantinga *et al.* 1987), and urine pH between 7.3 and 8.6 (Suemitsu *et al.* 1970; Richards & Wolton 1976b). The pH of urine is attributed to potassium and sodium concentrations (Hutton *et al.* 1967; Haynes & Williams 1992).

Further, other compounds and groups of compounds have been studied in ovine/bovine urine (Table 2.1). While some of these components are C bearing, a full analysis of the C composition and the relative proportions of C bearing compounds in cow urine has not been undertaken. Compounds such as hippuric acid, phenylacetic acid, glycine, proteins, amino acids, and carbohydrates are likely to contribute to urine-C (Martin 1973).

Table 2.1: Compounds and compound groups isolated in ovine or bovine urine.

Compound/Compound Group	Reference
N and N bearing compounds	Doak 1952; Martin 1970; Bristow *et al.* 1992
Aromatic compounds	Wayne 1928; Suemitsu *et al.* 1968; Martin 1969, 1970; Suemitsu *et al.* 1970; Martin 1973; Kruela *et al.* 1978; Martin 1982b, 1982c; Martin *et al.* 1983; Pagella *et al.* 1997
Steroids and lipids	Suemitsu *et al.* 1968; Martin 1982a
Fatty acids	Hradecký 1986
Volatile compounds	Bassette *et al.* 1966
Amino acids	Bathurst 1952; Doak 1952; Bristow *et al.* 1992
Phenols	Lane & Fraser 1999
Lactic, pyruvic and acetic acids	Ewaschuk *et al.* 2004
Ascorbic acid	Knight *et al.* 1941
Pseudouridine	Shingfield & Offer 1999
Methylmalonic acid	Elliott *et al.* 1979
Other nutrients	Richards & Wolton 1976b; Haynes & Williams 1992

In a grazed system, a large proportion of above-ground C is consumed by grazing livestock, 80% of ingested C is lost by respiration, some of the remainder is used for milk production, animal growth and maintenance, methane production and the remainder is returned to the soil in excrement (Dean *et al.* 1975; Moe & Tyrell 1979; Ball & Ryden 1984; Soussana *et al.* 2010). The metabolism of C in cattle occurs through fermentation of feed

to volatile fatty acids, which are then absorbed by the gastro-intestinal tract and excreted (Church 1976a). Tracer techniques found that following the intake of ^{14}C labelled feed, the ^{14}C content of urine peaked within 4–5 hours of ingestion, and rapidly decreased over the next 30 hours (Yadava *et al.* 1964).

The concentration of C in urine has been reported to range widely e.g.1.9 g L^{-1} (Uchida *et al.* 2011) and 200.3 g L^{-1} (van Groenigen *et al.* 2005). There are substantial differences between these urine-C contents, indicating that there may be large variability in the C concentration of cow urine.

2.4.3 Artificial urine

The bulk of cow urine research has utilised artificial urine. The main reasons for artificial urine use include constant composition and concentration of urine components and avoidance of expensive and time-consuming feeding trials to alter urine composition (Kool *et al.* 2006).

The main constituents of artificial urine usually include:

- potassium bicarbonate;
- potassium bromide;
- potassium chloride;
- potassium sulphate;
- urea; and
- glycine.

These compounds often occur in different combinations and concentrations depending on the purpose of the research (e.g. Holland & During 1977; Stillwell & Woodmansee 1981; Fraser *et al.* 1994; Clough *et al.* 1996; Shand *et al.* 2000; de Klein *et al.* 2003).

Of the studies utilising artificial urines, few have assessed the performance of artificial urine in comparison with real cow urine. Kool *et al.* (2006) and Lovell and Jarvis (1996) measured greater soil respiration fluxes following the application of cow urine than artificial urine. Kool *et al.* (2006) suggest that lower organic C contents in the artificial urine may impede respiration and also cause N immobilisation. Also, urea in urine degrades faster and to a greater extent than urea-only solutions (Sherlock & Goh 1984). The reduced degradability of urea in solution compared with urine-urea may impede respiration, resulting in the lower respiration fluxes measured by both Kool *et al.* (2006) and Lovell and Jarvis (1996).

Not only may artificial urine result in lower respiration fluxes than cow urine, it can also lead to smaller increases in soil pH than cow urine (Somda *et al.* 1997). Although, urea + hippuric acid solutions caused a greater increase in soil pH than urea alone solutions (Whitehead *et al.* 1989).

2.5 Fate of urine derived carbon in soils

The fate of urine-C in soils is potentially influenced by mineralisation (2.5.1), leaching (2.5.2) and adsorption (2.5.3).

2.5.1 Mineralisation

The application of urine and artificial urine to soils generally leads to increased CO_2 fluxes compared to control treatments (e.g. Lovell & Jarvis 1996; Kelliher *et al.* 2005b; Kool *et al.* 2006). Lovell and Jarvis (1996) reported a doubling of CO_2 evolution following urine deposition, to rates as high as 30.8 mg CO_2 m^{-2} min^{-1}. In this case, hydrolysis of urea was considered to be the main source of increased CO_2 emissions (Lovell & Jarvis 1996).

Initially, urea applied to soil is degraded by urease to ammonium, which then undergoes nitrification to nitrate (Doak 1952; Black 1992; Haynes &

Williams 1993; Powell *et al.* 1998). Urea hydrolysis in soil can be rapid; for example, ^{13}C labelling techniques measured complete hydrolysis of urea in the first 24-48 hours after application (Bol *et al.* 2004; Petersen *et al.* 2004). Nitrification may occur between 14 and 29 days after urine deposition (Williams & Haynes 1994).

While the hydrolysis of urea in soils has been well studied (Smith *et al.* 2007b), there is no published information on the degradation of other C bearing compounds in urine. The application of urine to soils has also been reported by some authors to cause priming of soil C (Lovell & Jarvis 1996; Kool *et al.* 2006; Uchida *et al.* 2011; Section 2.6.3.3).

2.5.2 Leaching

Several authors have examined leaching of soil C under grazed pastures. Shepherd *et al.* (2010) investigated the addition of C rich additives to reduce N leaching from urine patches in pastures. They report total C leaching from cattle urine patches was 4 g C m^{-2} over 74 days or 20 g C m^{-2} $year^{-1}$ (Shepherd *et al.* 2010). However, Shepherd *et al.* (2010) did not have a water control treatment, so the effect of urine on soil C leaching could not be determined.

Parfitt *et al.* (2009) measured dissolved organic C (DOC) losses from sheep grazed hill country in New Zealand ranging from 12–23 g C m^{-2} $year^{-1}$. McTiernan *et al.* (2001) reported losses of DOC from cattle grazed pastures in the range of 4.2 and 11.8 g C m^{-2} over a period two months, or 25–71 g C m^{-2} $year^{-1}$. Although the DOC losses reported by Parfitt *et al.* (2009) were lower than other authors, this is likely due to the differences in the volume of urination between cattle and sheep (Haynes & Williams 1993). Also, Williams and Haynes (1994) found that N leaching was less from the same soil grazed with sheep than cattle.

Ghani *et al.* (2010) measured DOC leaching from 6 soils over 25 weeks, the leaching ranged between 0.5 and 2.6 g C m^{-2}, or 28-169 g C m^{-2} $year^{-}$

[1]. Leaching from pasture soils was less from allophanic soils than gley soils (Ghani *et al.* 2010). Ghani *et al.* (2010) collected soils that would not have been affected by recent addition of cattle urine, and yet these soils compared well with leaching of soil C under grazed pastures (McTiernan *et al.* 2001; Shepherd *et al.* 2010).

While research suggests macropore flow has the greatest influence on the leaching of urine (Williams *et al.* 1990; Williams & Haynes 1994; Jarvis *et al.* 1995; Pakrou & Dillon 1995), the depth to which urine can be measured in the soil profile varies through the literature (Table 2.2) and was likely to be controlled by the soil tested and target compound investigated.

Table 2.2: Depth of leaching and proportion of compound measured in urine studies.

Depth (cm)	Compound	% of added	Reference
5	^{15}N	55-66	Williams & Haynes 1994
15	Potassium	60-80	Williams *et al.* 1990
20	KBr-pyr**	17	Monaghan *et al.* 1999
30	^{15}N	17*	Pakrou & Dillon 1995
45	^{15}N	2*	Pakrou & Dillon 1995

*Irrigated pasture

** Potassium bromide-pyranine dye solution

2.5.3 Adsorption

Sorption, the main process by which soils retain and control a compound's mobility, is made up of two parts – adsorption and absorption. Adsorption is a rapid process by which molecules in a solution or gas are attracted onto the surface of another substance, for example soil (Ferrante 1996). Adsorption can be through both physical and chemical mechanisms (Jardine *et al.* 1989). Absorption, on the other hand, is slow process where the molecules enter the structure of the other substance (Ferrante 1996). The dominant process by which dissolved organic C is retained on

soil surfaces is adsorption rather than absorption (Nodvin *et al.* 1986; Jardine *et al.* 1989; Kaiser & Guggenberger 2000; Kothawala *et al.* 2008).

The main soil components responsible for adsorption of DOC are iron oxides, aluminium oxides, clays, and cations (Greenland 1971; Jardine *et al.* 1989; Bouwman 1990; Gu *et al.* 1994; Guggenberger *et al.* 1998; Riffaldi *et al.* 1998; Kaiser & Zech 2000). Calcium, magnesium and sodium can enhance the adsorption of charged organic compounds to activated C by cation bridging; but not of non-ionised organic compounds (Randtke & Jepsen 1982; Guggenberger & Zech 1993). The cations act to neutralise the electrostatic forces between the two negatively charged organic compounds (Randtke & Jepsen 1982).

The adsorption of DOC to soils has been widely investigated (e.g. Jardine *et al.* 1989; Guggenberger *et al.* 1998; Kaiser & Zech 1998; 2000). Nodvin *et al.* (1986) suggested that DOC adsorption may be finite as DOC adsorption decreased with increasing DOC concentration. Guggenberger and Zech (1992) investigated the retention of organic carbon in forest soils. They found that the addition of a DOC solution led to a decrease in soil C in the topsoils of some profiles. In the same profiles where DOC application led to a loss of soil C, adsorption of DOC was measured in the lower part of the profile. Therefore, soil C lost from topsoils, under these conditions, may not necessarily be lost from the profile, due to retention at lower depths.

While the adsorption of urine-C has not been investigated, and sorption of urea and other urine constituents has been reported. Williams and Haynes (1990; 1994) suggest that the infiltration of urine down the soil profile will be too rapid for adsorption to occur, however, adsorption of potassium from artificial urine to pasture soil has been measured (Early *et al.* 1998). Holland and During (1977) found significant adsorption of chloride and nitrate to allophanic soil following artificial urine application. Urea may also be sorbed on soils (Broadbent *et al.* 1958; Overrein & Moe 1967).

The adsorption of DOC to soils can be impeded by native SOM, which may block binding sites on colloids and therefore reduce the amount of sites available for DOC adsorption (Jardine *et al.* 1989; Kaiser & Zech 1998). Kaiser and Zech (2000), however, found that the native SOM in the soils they tested (1–2 g kg^{-1} SOC content) did not impede DOC adsorption. Sulphate has also been reported to compete with DOC for adsorption sites in soils (Guggenberger & Zech 1993). Cow urine contains sulphate (Bird 1972) and may decrease urine-C adsorption in urine patches, although this has not yet been tested.

2.6 Effects of urine on soil carbon

The potential effects of urine on soil C cycling can be divided into three main sections, 1) urine scorch (2.6.1), 2) alteration of soil pH (2.6.2), and 3) alteration of soil microbiology (2.6.3).

2.6.1 Urine scorch

The high ionic strength of urine and ammonium production following urea hydrolysis, can lead to root scorch (Haynes & Williams, 1993; Monaghan & Barraclough 1993; Shand *et al.* 2002). Root scorch is most common in dry conditions, when plants are near wilting point, and the osmotic pressure exerted by urine can lead to the death of plant roots, particularly clover (Doak 1954).

Root death may immediately release DOC into soil solution, and over the long term impede below-ground C inputs from root turnover (Monaghan & Barraclough 1993; Shand *et al.* 2002). Urine also reduces grass recovery by killing the seed bank and inhibiting seedling growth (Doak 1954). Cow urine contains a root inhibitor that kills the growing tip of roots, the inhibitor is then either rapidly degraded or altered to a root stimulant, and may be an "inhibitor-auxin complex" incorporating "heteroauxin" present in urine (Doak 1954).

Veena and Narendranath (1993) also report that cow urine has an inhibitory effect of the germination of seeds, the urine from pregnant cows significantly decreased wheat seed germination, although the urine from non-pregnant cows did not. Nirmala *et al.* (2008) investigated the effect of estrogen and progesterone on wheat and green gram seed germination, and found that these compounds were not responsible for the inhibitory effect of cow urine found by Veena and Narendranath (1993). Cow urine has also been shown to inhibit fungal growth on the roots of some plants, such as *Fuscarium solani* and *Sclerontinua sclerotiorum* (Basak *et al* 2002a; 2002b).

2.6.2 Alteration of soil pH

As urea degrades it consumes hydrogen ions during the formation of ammonium (Doak 1952; Stillwell & Woodmansee 1981), which leads to localised points of increased soil pH (Doak 1952; Bristow *et al.* 1992; Haynes & Williams 1992). While urea hydrolysis is thought to be the main cause of increased soil pH under urine patches, Somda *et al.* (1997) suggest that the high pH of urine may also contribute directly to an increase in soil pH. Stillwell and Woodmansee (1981) reported stratification of soil pH under a urine patch, whereby the pH of the top 15 cm of soil increased in pH but no increase was measured below this depth.

Increases in soil pH can lead to decreases in soil C in two ways, dissolution/desorption of soil C (Section 2.6.2.1), and disaggregation (2.6.2.2), followed by leaching or mineralisation of soil C. Increases in soil pH may not solely attributed to urea hydrolysis (Section 2.5.1), but may also result from acid neutralising capacity forcing (Section 2.6.2.3).

2.6.2.1 Soil carbon dissolution or desorption

Desorption is the opposite process to sorption, where molecules become unattached from the outside of another substance (e.g. solid) and move

back into solution or gas phases (Limousin *et al.* 2007). Dissolution is the process where by a material is dissolved in a liquid.

The rapid increase in soil pH following urine deposition can lead to the dissolution of SOM (Jackman 1960; Monaghan & Barraclough 1993; Lovell & Jarvis 1996). For example, Shand *et al.* (2000) reported a greater than 20 fold increase in DOC after the addition of synthetic sheep urine to soil, of up to 4 g L^{-1}. DOC:dissolved organic N ratios indicated that the majority of the DOC increase was SOM derived and the urine itself contributed little (Shand *et al.* 2000). The increase in soil DOC following urine deposition may be long lived, Wachendorf *et al.* (2008) reported that soil DOC contents peaked 121 days after urine application. Uchida *et al.* (2011) also measured an increase in soil water soluble C following the application of urine to soil. Water soluble C increased by 600 µg g^{-1} over the control treatments at 11° and 23°C, but was less in the urine treatment than the control by about 400 µg g^{-1} at 19°C. Orwin *et al.* (2010) also measured an increase in WSC contents of between 750 and 1070 µg g^{-1} after urine application.

The concentration of salts in soil solution can influence the amount and quality of organic matter desorbing from aggregates into solution (Reemtsma *et al.* 1999). The ionic strength of the soil solution under urine patches can be increased from 4–6 mM to 24–61 mM in the top 2.5 cm of the soil profile (Haynes & Williams 1992). Orwin *et al.* (2010) also report an increase in soil electrical conductivity following urine deposition in the range of 1000-1600 µS cm^{-1}, and the electrical conductivity remained elevated for at least 44 days. High concentrations of soluble salts derived from animal excrement are also found in stock camping areas (Haynes & Williams 1999). It is possible that the salt contents of urine could result in the desorption of soil C into solution.

2.6.2.2 Disaggregation

Aggregate disruption can cause the release of previously unavailable C, leading to an acceleration of C mineralisation (Gregorich *et al.* 1989; Chandra *et al.* 2002). Uchida *et al.* (2008) reported the breakdown of aggregates in the 0–2 mm size class of the Temuka silt loam following urine deposition. The rapid increase in soil pH, along with the high salt load from urine deposition, may have led to dispersal of soil aggregates (Uchida *et al.* 2008). Uchida *et al.* (2008) also suggest that the high surface area to volume of the smaller aggregates (0-2 mm) may have made them more susceptible to the effects of urine.

Urine contains sodium (Richards & Wolton 1976b), and while urine has not been tested for its ability to disrupt aggregates, sodium rich effluents have. Assefa *et al.* (2004) reported that four annual applications of pig manure increased the sodium adsorption ratio of the soil by 148-354% and decreased the mean aggregate size by 30–40%. Meneer *et al.* (2001) found that there may have been compromised soil structure following the application of high sodium effluents, but this was somewhat mitigated by macropore flow. On the other hand, Cameron *et al.* (2003) found an increase in wet aggregate stability following the application of high sodium dairy factory effluent to soils. The organic matter in the effluent increased wet aggregate stability and may have mitigated the effect of reduced hydraulic conductivity also measured by Cameron *et al.* (2003).

2.6.2.3 Acid neutralising capacity forcing

Acid neutralising capacity (ANC) forcing is a measure of the impact of fertiliser on soil pH, whereby the balance of cations and anions in leachate from the soil determines the potential of a fertiliser to change the soil pH (Evans *et al.* 2008). Evans *et al.* (2008) found with increasing ANC forcing there was a concurrent increase in DOC losses from a range of soils following the application of N fertilisers.

Evans *et al.* (2008) found that sodium nitrate fertilisers increased soil pH; cations in the urine displaced hydrogen ions into solution, and reduced the proportion of acid saturation and therefore increased soil pH. The application of ammonium salt fertilisers was generally found to decrease soil pH as ammonium in soils is rapidly converted to nitrate, producing hydrogen ions (Evans *et al.* 2008).

Urine contains large amounts of both cations and anions and although the predominant cause of soil pH increase following urine application is due to urea hydrolysis (Section 2.6.2), ANC forcing may also contribute to pH increases. The ANC forcing potential of urine or urea has not yet been assessed.

2.6.3 Alteration of soil microbiology

Urine deposition has many effects on soil microbial populations. In particular, influencing microbial activity, biomass, community structure (2.6.3.1), enzymes (2.6.3.2) and causing priming of soil C mineralisation (2.6.3.3).

2.6.3.1 Microbial activity, biomass, and community structure

While the activity of microbes usually increases with urine application (e.g. Lovell & Jarvis, 1996; Williams *et al.* 2000; Nunan *et al.* 2006; Rooney *et al.* 2006), it may also decrease (Williams *et al.* 2000). Williams *et al.* (2000) measured the microbial activity in two soils treated with synthetic sheep urine and found that activity increased in one soil by 32%, while in the other, soil activity decreased by 37%. Increases in microbial activity following urine deposition do not necessarily coincide with increases in microbial biomass (Williams *et al.* 2000; Nunan *et al.* 2006; Rooney *et al.* 2006). In fact, Lovell and Jarvis (1996) reported a decline of 11% in microbial biomass C after 14 days incubation with cow urine.

Immobilisation of added dissolved organic C in microbial populations has

been shown to occur in aquatic systems as well as forest soils (Brugger *et al.* 2001; Devi & Yadava 2009). Carran *et al.* (1982) found no immobilisation of urine-N, however, Williams and Haynes (1994) reported that immobilisation and subsequent mineralisation of urine-N may have occurred. Immobilisation of C is generally measured as an increase in microbial biomass C, however, Lovell and Jarvis (1996) measured a decrease in soil biomass C in urine treated soil. C immobilisation would likely occur in C limited soils, whereas urine patches are not C limited, due to carbon added in the urine and soil C solubilisation (Monaghan & Barraclough 1993).

The application of artificial urine can also lead to changes in microbial community structure in pastoral soils (Williams *et al.* 2000; Rooney *et al.* 2006). Iyyemperumal and Shi (2007) suggest that changes in soil pH, like those under urine patches, can also alter soil community structure and enzyme activities.

2.6.3.2 Soil enzymes

Soils contain microbes and extracellular enzymes that are vital for the cycling of nutrients (Skujiņš 1976; Allison & Vitousek 2005). Extracellular enzymes can be excreted from both living and dead cells, and once excreted they may be either free or bound to soil, bound enzymes are usually sorbed to SOM or clays (Skujiņš 1976; Burns 1982).

To determine the maximum activity of a target enzyme, soil enzyme activities are assayed under ideal conditions; and are determined by the rate of degradation of a substrate (Iyyemperumal & Shi 2008). The total activity of an enzyme can be contributed to by enzymes that are bound and free (Skujiņš 1976; Burns 1982). When measuring extra-cellular enzyme activity both free (in solution) and bound enzymes are measured.

The application of various effluents has been reported to increase and decrease the activity of a range of extracellular enzymes (Table 2.3). Urea is a major component of urine and is hydrolysed in soils by the enzyme urease (Lloyd & Sheaffe 1973; Zantua & Bremner 1976).

Table 2.3: Soil enzyme activity response to the land application of a range of effluents.

Effluent	Enzyme activity	Source
Piggery effluent	↑cellobiohydrolase ↑β-glucosidase ↑β-glucosaminidase ↑protease	Iyyemperumal & Shi 2008
Poultry litter	↑β-glucosidase ↑β-glucosaminidase ↑α-galactosidase	Acosta-Martínez & Harmel 2006
Tertiary treated municipal effluent	↑invertase ↓phosphatase ↕*sulphatase activity	Schipper *et al.* 1996
Straw manure	↑acid phosphatase ↑alkaline phosphatase ↑arlysulphatase ↑β-glucosidase ↑urease ↑amidase	Dick *et al.* 1988
Dairy shed effluent	↑protease ↑urease	Zaman *et al.* 2002
Stock camping and effluent application	↑arginine ammonification ↑protease ↑histidase ↑urease ↑acid phosphatase ↑arlysulphatase	Haynes & Williams 1999

*↕ = no change

Increased urease activity does not always result from urea application, but will often increase following the addition of compounds containing easily degradable C (Lloyd & Sheaffe 1973; Zantua & Bremner 1976).

Dehydrogenase is an intra-cellular enzyme that can be used as an indicator of microbial activity (Stevenson 1959; Amato & Ladd 1988; Garcia *et al.* 1997; Ciardi 1998), and increased dehydrogenase activity under urine patches has been reported. Orwin *et al.* (2010) found increased dehydrogenase activity following the application of bovine urine to soils at two different moisture contents, dehydrogenase activity was increased on day 1 of the incubation, but by day 3 had declined to background levels.

2.6.3.3 Priming of soil carbon

Priming is defined as the negative or positive changes in the rate of SOM mineralisation following the addition of relatively small amounts of C (Jenkinson 1963; Kuzyakov *et al.* 2000; Fontaine *et al.* 2003; Mondini *et al.* 2006), and is therefore driven by microbial dynamics (Figure 2.2). Positive priming is generally associated with an increase in microbial activity or biomass leading to an increase in the turnover of SOM (Kuzyakov *et al.* 2000).

Enhanced enzyme production due to the greater amounts of easily degradable C in soil may also lead to soil C priming (Kuzyakov *et al.* 2000; Fontaine *et al.* 2004a; Hamer & Marschner 2005). Increased death and turnover of the microbial biomass following C addition can be mistaken for soil C priming and is known as "apparent" priming (Dalenberg & Jager 1989; Wu *et al.* 1993; Kuzyakov *et al.* 2000; Kuzyakov & Bol 2006).

Blagodatskaya and Kuzyakov (2008) suggest that priming of soil C may be dependent upon the amount of C source added in relation to the amount of microbial biomass C (MBC) in the soil. They suggest that priming has a positive linear relationship with the amount of C added, if less than 15% of

MBC (Blagodatskaya & Kuzyakov 2008). Further, additions of C greater than 50% of MBC will lead to an exponential decrease of priming as C addition increases, and that C additions over 200% of MBC will exhibit either no or negative priming (Blagodatskaya & Kuzyakov 2008).

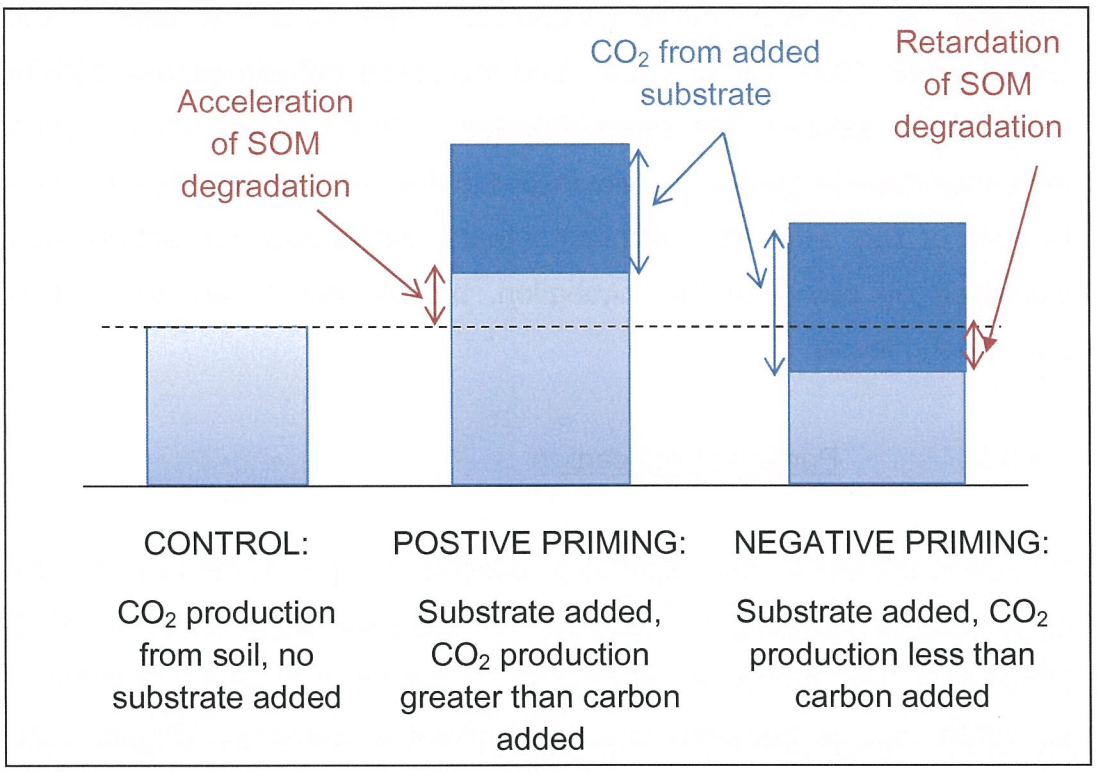

Figure 2.2: Positive and negative soil organic matter priming (Kuzyakov *et al.* 2000).

Negative priming occurs when CO_2-C fluxes account for less than the amount of C added to the soil (Figure 2.2; Kuzyakov *et al.* 2000). Negative priming is caused by immobilisation of the added C source before it can be degraded (Kuzyakov *et al.* 2000). Kuzyakov & Bol (2006) also suggest that negative priming following the addition of large amounts of C can be the result of the preferential mineralisation of the added C over SOM, therefore leading to a short-term decrease in the degradation of SOM. This initial negative priming can be followed by positive priming once the microbes return to degrading SOM (Kuzyakov & Bol 2006; Smith *et al.* 2007b), but the length of time that CO_2-C is assessed for after C addition may dictate whether negative or positive priming is measured.

Priming does not necessarily result in a loss of soil C; in fact incomplete mineralisation of the added C source can be greater than the soil C lost by priming, resulting in a net increase in soil C (Dalenberg & Jager 1989; Hamer & Marschner 2005; Mondini *et al.* 2006; Smith *et al.* 2007b).

Fontaine *et al.* (2003) suggest that there are two kinds of microbes in soils, r- and k-strategists. r-strategists respond rapidly to C source addition, but die or become dormant after the source has been consumed. r-strategists may not contribute to the mineralisation of SOM (De Nobili *et al.* 2001; Fontaine *et al.* 2003; Kelliher *et al.* 2005a; Mondini *et al.* 2006), but this has yet to be proven (Blagodatskaya & Kuzyakov 2008). k-strategists degrade SOM and remain continuously active, but may not be capable of the rapid growth required to compete with r-strategists for fresh C inputs (Fontaine *et al.* 2003). However, if r-strategists cannot degrade SOM, k-strategists must have at least some ability to compete for substrate in priming events where SOM cycling is accelerated. Therefore priming may be a result of competition between the two groups of microbes for C source (Fontaine *et al.* 2003; 2004b).

Positive priming has been reported following the application of cow urine to soils (Lovell & Jarvis 1996; Kool *et al.* 2006). Uchida *et al.* (2011) measured priming of soil C at three different temperatures, 0.4, 0.4 and 0.6 mg C g^{-1} oven dried soil was lost at 11, 19 and 23°C. Enhanced microbial activity from the application of degradable C in the urine, as well as soil C dissolved after urine application, may have been the source of priming (Lovell & Jarvis 1996; Kool *et al.* 2006). Clough *et al.* (2003) reported about 1.7 mg C g^{-1} of priming over 60 days following the application of artificial urine.

Priming is influenced by the amount of C added, both positively and negatively. Wu *et al.* (1993) found that after a large addition of glucose there was a positive priming effect, but after a small glucose application there was no priming. While the addition of small amounts of C can lead to immobilisation by microbes, inputs of large quantities of C allow enough

for both immobilisation and priming to occur (Wu *et al.* 1993). Mondini *et al.* (2006), on the other hand, found that following the addition of larger amounts of glucose there was lower CO_2 production than when small amounts of glucose were added. The complexity and the composition of the substrate may also influence priming, for example Mondini *et al.* (2006) found CO_2 production was greater following the application of complex (carbohydrate mixture) than simpler C (glucose).

Priming is not isolated to surface soil layers, and has been shown to occur in deep soils. Fontaine *et al.* (2007) reported that cellulose addition to deep soil layers lead to the degradation of old soil C.

Several authors suggest increased soil enzyme activity may also lead to priming (e.g. Jenkinson 1963; Asmar *et al.* 1994; Degens & Sparling 1996). Wu *et al.* (1993) proposed that the death of microbes during a priming event may increase extracellular enzyme activity following lysis of the cells. However, the role of enzymes in priming events following cow urine deposition has yet to be investigated.

2.7 Summary and conclusions

SOM is an important component in soil fertility, greenhouse gas exchange, and C storage. Soil C contents have declined under flat, intensively grazed pastures in New Zealand, although the mechanisms of these losses are unclear. Urine deposition may be a factor in soil C losses under dairy pastures by increasing soil C solubilisation and its subsequent loss by leaching or mineralisation. Conversion of pine plantations to dairy farming has been common in recent time in New Zealand, and the newly converted pasture soils may be more susceptible to soil C loss following urine deposition.

The majority of nutrient intake by cows is excreted, causing isolated patches of excess nutrients across paddocks. Although many aspects of urine have been studied, there are few published reports on the C content

and constituents of cow urine. Many authors substitute artificial urine for cow urine in studying N cycling, however, there are few instances in the literature where the impacts of artificial urine on soil have been directly compared to the impacts of cow urine. Further work assessing how well artificial urine mimics the effects of cow urine is required.

There are three main soil processes that may determine the fate of urine-C in soil: 1) mineralisation, 2) leaching, and 3) adsorption. The application of urine to soils generally leads to a rapid increase in soil CO_2 fluxes, indicating the mineralisation of urine-C and possibly soil-derived C. Several authors have reported leaching of soil C under grazed and ungrazed pastures, but leaching is likely to be influenced by adsorption of urine-C. Urea, cations and anions, and DOC have been reported to be subject to adsorption, however no assessment of the potential for urine-C to be adsorbed to soil could be found.

Urine scorch, alteration of soil pH, and modification of microbial populations are the main potential effects of urine on soil C cycling. Urine scorch can lead to both short- and long-term DOC losses, and urine may also inhibit the recovery of pasture under a urine patch, leading to decreased C inputs.

Increased soil pH under urine patches can lead to a decrease in soil C by dissolution/desorption of soil C or disaggregation. These factors can also be contributed to by the high salt load of the urine. The rapid increase in soil pH following urine deposition may not be solely attributable to urea hydrolysis, and may also be aided by the high pH of the urine itself and ANC forcing, although further investigation is required.

Urine deposition generally leads to increased soil microbial activity, but may also decrease microbial biomass and alter the microbial community structure. Increases in soil activity have been reported to lead to accelerated degradation of SOM, or positive priming. Increases in

extracellular enzyme activity following urine deposition may also contribute to positive priming of soil C, but as of yet this has not been investigated.

This literature review found many gaps in the knowledge about the fate of urine-C in soils, and the effects of urine on soil C cycling. This highlighted the necessity to undertake the:

- investigation of the content, composition and bioavailability of urine-C (Chapter 3);
- determination of urine-C adsorption to soils and the potential solubilisation of urine in pine and pasture soils (Chapters 4 & 5);
- examination of priming in repacked soil cores from pine and pasture soil following urine application (Chapters 4 & 6); and
- quantification of leaching from soil C from undisturbed cores applied with urine (Chapters 4 & 7).

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Chapter 3

Composition and bioavailability of carbon in dairy cow urine

3.1 Introduction

New Zealand's dairy industry is currently undergoing agricultural intensification, with dairy cow stocking rates increasing by 19% between 1994 and 2002 (Parliamentary Commissioner for the Environment 2004; MacLeod & Moller 2006). With increasing stocking rates, there is an increase in urine return to the soil surface (Pleasants *et al.* 2007; Moir *et al.* 2011). Urine deposition leads to localised increases in soil pH and may lead to dissolution of soil carbon (C; Haynes & Williams 1992; Lovell & Jarvis 1996; Shand *et al.* 2002). Urine deposition may also contribute to recently reported losses of soil C from dairy systems (Schipper *et al.* 2007; 2010).

Past studies of cow urine have identified many constituents, while some of these previously isolated compounds contain C, a comprehensive analysis of C bearing compounds and their relative proportions had not previously been undertaken.

Due to the variability and complexity of real urine, artificial urine is commonly used to determine the effects of urine on soils (e.g. Holland & During 1977; Fraser *et al.* 1994; Shand *et al.* 2000; Clough & Kelliher 2005). Although the use of artificial urine is common in studies on nutrient cycling, few authors have assessed if artificial urine adequately represents real urine. Lovell and Jarvis (1996) and Kool *et al.* (2006) reported the application of artificial urine led to lower carbon dioxide (CO_2) emissions

than cow urine. Somda *et al.* (1997) also measured lower soil pH in artificial urine treated soil than soil applied with real urine.

Priming of the decomposition of soil C following urine application in pastoral soils has been reported (Lovell & Jarvis 1996; Kool *et al.* 2006; Uchida *et al.* 2011). Priming occurs when an easily degradable C source is added to a soil leading to acceleration of soil C mineralisation and, potentially, decreasing soil C (Jenkinson 1963; Kuzyakov *et al.* 2000; Fontaine *et al.* 2003; Mondini *et al.* 2006). The priming potential of cow urine would likely be dependent on its bioavailability to microbes. Bioavailability of a compound is commonly assessed by determining the loss of the target compound, or the increase in CO_2 production above a control over time (McDowell *et al.* 2006; Andreasson *et al.* 2009).

The main objective of this chapter was to determine the C content, composition, and bioavailability of cow urine. The content and composition of urine-C were determined using wet chemistry and pyrolysis gas chromatography-mass spectrometry (GC-MS). The bioavailability of urine-C was assessed by measuring urine total organic C (TOC) contents and cumulative carbon dioxide (CO_2-C) fluxes over a 28-day incubation. The effect of the addition of soil microbes on urine-C degradation was also investigated.

3.2 Materials and methods

3.2.1 Carbon content and composition

Urine from 10 Friesian dairy cows was collected (Dairy 3; Massey University, Palmerston North, New Zealand) in October 2006 during afternoon milking, following grazing of ryegrass-clover pasture (T Smith, pers.comm.). The cow urine was filtered (0.45 µm) and analysed immediately for total, inorganic, and organic C contents (Win High TOC II, Elementar Analysensystems GmbH, Hanau, Germany), and total N

content by combustion furnace (LECO FP-2000 CNS analyser; LECO Corp., St Joseph, MI, USA).

Urine-urea was extracted with 10% tricholoracetic acid, then digested with sulphuric-orthophosphoric acid solution, 2, 3-butane-dione-2-oxime and thiosemicarbazide for 30 minutes to develop a colour reaction. Urea was then quantified spectrophotometrically at 520 nm (Marsh *et al.* 1965).

Carbohydrates were measured following the incubation of urine at room temperature with a 5% phenol solution and sulphuric acid for 10 minutes, then for a further 15 minutes at 25°C (Dubois *et al.* 1956). Total carbohydrates were determined against glucose standards at 485 nm and reported in glucose-C equivalents.

For the determination of total phenolics contents, urine was incubated at room temperature for 90 minutes, in the dark with Folin & Ciocalteu's reagent and 1.9 *M* sodium carbonate solution (Blum *et al.* 1994). Total phenolics were measured spectrophotometrically against tannic acid standards at 700 nm.

Electrical conductivity (EC) was measured against a 2 *M* potassium chloride standard and pH was measured using a glass electrode (Blakemore *et al.* 1987).

The remainder of each urine sample was freeze dried at about –50°C at a vacuum of 0.5 kPa for 24–48 hours (Modulyod Freeze Dryer, Thermosavant, BioLab Limited, Auckland, New Zealand). Eight urine samples were analysed for C bearing compounds using pyrolysis GC-MS (GNS Science, Lower Hutt, New Zealand). An internal standard containing trans-cinnamic acid, 16-hydroxyhexadecanoic acid and Et-vanillin were added to each sample before silylation with N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TCMS) in 100 µL vials within closed 2 mL vials for 30 minutes at 80°C. One µL of silylated solution was injected into a GCMS (Agilent 7890 GC

and 5975C MSD) on 5% polar column with a temperature ramping programme. Compounds were identified using NIST08 spectral library in AMDIS deconvolution software. The relative quantities of the compounds (mg compound mg urine^{-1}) were calculated using the response factor of hippuric acid against an ethyl vanillin internal standard. The relative abundance of each isolated compound as a proportion of the total compounds isolated is presented. Compounds with a relative abundance greater than 5% were presented separately from the remaining compounds. Compounds that contributed less than 5% of the relative abundance were grouped into the following groups: alcohols, carbohydrates, carboxylic acids, tricarboxylic acids, and hydroxy acids.

3.2.2 *Bioavailability of urine carbon*

Three cow urine samples were collected and frozen to –20°C within 1 hour of collection (Dairy 3 Farm, Massey University, Palmerston North, New Zealand):

1. Urine 1: Urine from 5 cows combined (September 2007; pH 8.3; TOC: 13.8 g L^{-1})
2. Urine 2: Urine from 3 cows combined (July 2007; pH 8.2; TOC: 17.5 g L^{-1})
3. Urine 3: 20 mL of each of the 10 urines collected for C content and composition work combined (October 2006; Table 3.1).

Three replicates of each urine (25 mL) were measured into glass jars (50 mL), then placed into an Agee jar (1.8 L), sealed and incubated at 25±1°C in the dark for 28 days. A 1-mL subsample of each urine sample was taken on days 0, 1, 4, 14, and 28 of the incubation, filtered to 0.45 µm and analysed for TOC and inorganic C content (Win High TOC II, Elementar Analysensystems GmbH, Hanau, Germany). A second set of three replicates for each urine were also treated the same as above, except for the addition of a microbial inoculant. Five g of wet soil (Foxton black sand) was mixed with 25 mL of Milli-Q water for 30 minutes on a magnetic stirrer and left to equilibrate unstirred at 4°C overnight. The unfiltered soil slurry

was added (2 mL litre^{-1}) to the urine and water as microbial inoculant (Boyer & Groffman 1996).

CO_2 fluxes from the urine were measured by sampling the headspace gas (25 mL) on days 1, 4, 7, 14, and 28 of the incubation. The headspace CO_2-C concentration was quantified using a gas chromatograph equipped with a flame ionisation detector after conversion of CO_2 to methane by a methanizer (Shimadzu 2010, Kyoto, Japan). After gas sampling, the incubation jars were flushed for 30 minutes before being returned to the incubation. The rate of gas evolution was calculated using Ideal Gas Law, and cumulative CO_2-C fluxes were determined using linear interpolation for incubation days between gas samplings.

As urine-C is degraded, the CO_2 produced will either be released in gas form or held dissolved in the urine. The retention of CO_2 in solution increases above a pH of 6.5 (Sparling & West 1990). Retention of dissolved CO_2-C would lead to an underestimation of CO_2-C fluxes and urine-C biodegradability. Therefore, accumulation of dissolved CO_2-C in the urine was determined by measuring the inorganic C content of the urine (Gamble 1922). Cumulative CO_2-C measured in the headspace was corrected for the accumulation of inorganic C in urine over the incubation period.

Statistical analyses were undertaken using ANOVA (Genstat 12), and differences between treatments were determined using Student-Newman-Kuels testing and were considered significant if $P < 0.05$. Unpaired, two-sample t-tests were used to determine if there was a difference between methods of C loss measurement (TOC or corrected CO_2-C fluxes) and also if there was a significant effect of soil microbe inoculation on urine-C degradation.

3.3 Results

Data are presented as a mean with standard error, unless otherwise stated.

3.3.1 Carbon content and composition

The total C content of the urine samples was 7.5±1.2 g L^{-1}, of which about 91% was organic C, and the C:N ratio was 2.1±0.1 (Table 3.1). Approximately 21% of the urine-C was urea, carbohydrates, phenolics and inorganic C as measured by wet chemistry (Table 3.1). The EC and pH of the urine was 24.5±2.4 mS cm^{-1} and 8.13±0.04, respectively.

Table 3.1: Total carbon (TC), total inorganic carbon (TIC), total organic carbon (TOC), total nitrogen (TN), carbon to nitrogen ratio, urea-C, carbohydrates-C (Carb-C) and phenolics-C (Phen-C) contents in cow urine. Numbers in brackets represent standard error (n = 10).

TC g L^{-1}	TIC g L^{-1}	TOC g L^{-1}	TN g L^{-1}	C:N	Urea-C g L^{-1}	Carb-C g L^{-1}	Phen-C g L^{-1}
7.5	0.65	6.8	3.5	2.1	0.57	0.11	0.19
(1.2)	(0.11)	(1.1)	(0.5)	(0.1)	(0.09)	(0.04)	(0.01)

The dominant compound identified by GC-MS was hippuric acid, making up almost half (~45%) of the isolated compounds (Figure 3.1; Appendix 1). Alcohols and carbohydrates were the largest groups of compounds recovered (Figure 3.1). Pseudouridine did not fit into the designated compound groups. Glycine was the only amino acid isolated in the urine, and due to the small amounts of it recovered, amino acid was not one of the designated compound groups.

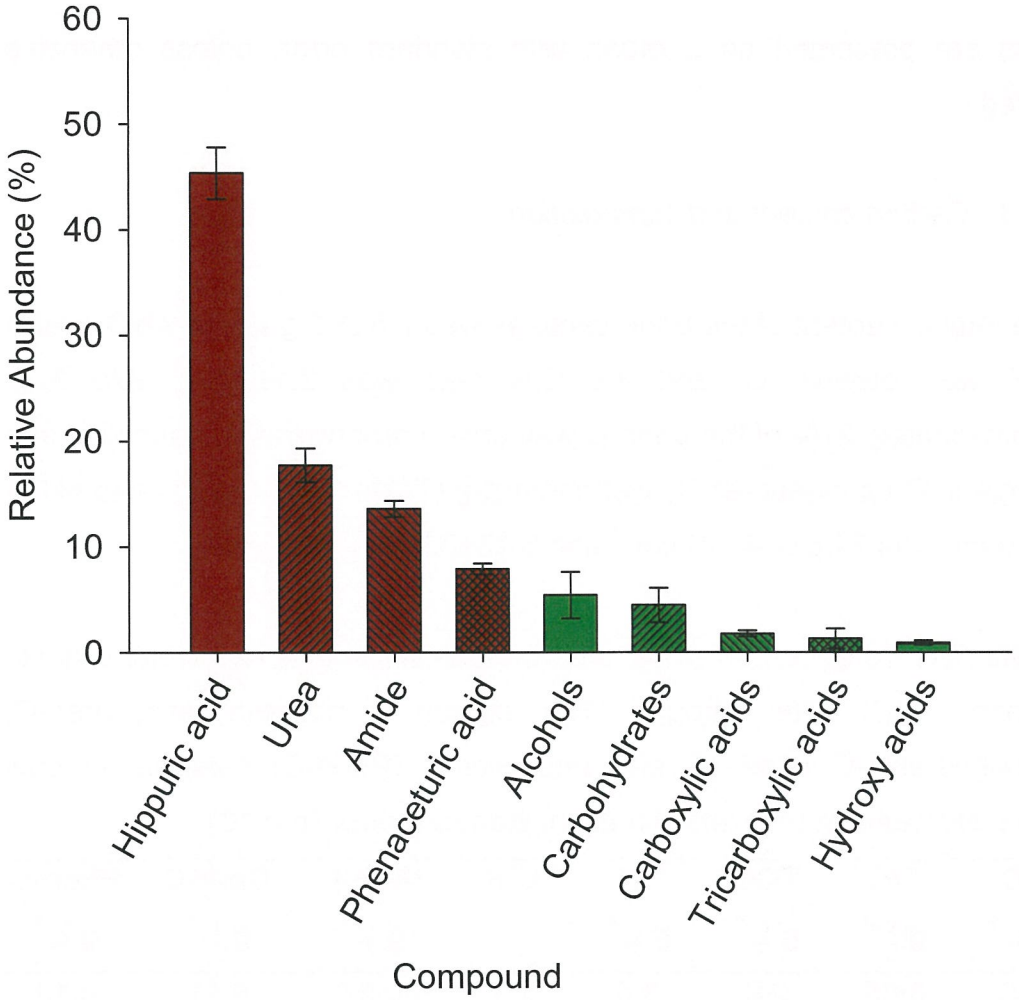

Figure 3.1: Relative abundance of carbon bearing compounds and groups of compounds in cow urine by pyrolysis GC-MS. Error bars represent standard error (n = 8).

3.3.2 *Bioavailability of urine carbon*

After 28 days of incubation, the TOC content of cow urine decreased by between 13 and 25%, while cumulative CO_2-C accounted for 7–14% of the initial urine-C content (Figure 3.2). There was no effect of inoculation with soil microbes on urine-C degradability (Table 3.2).

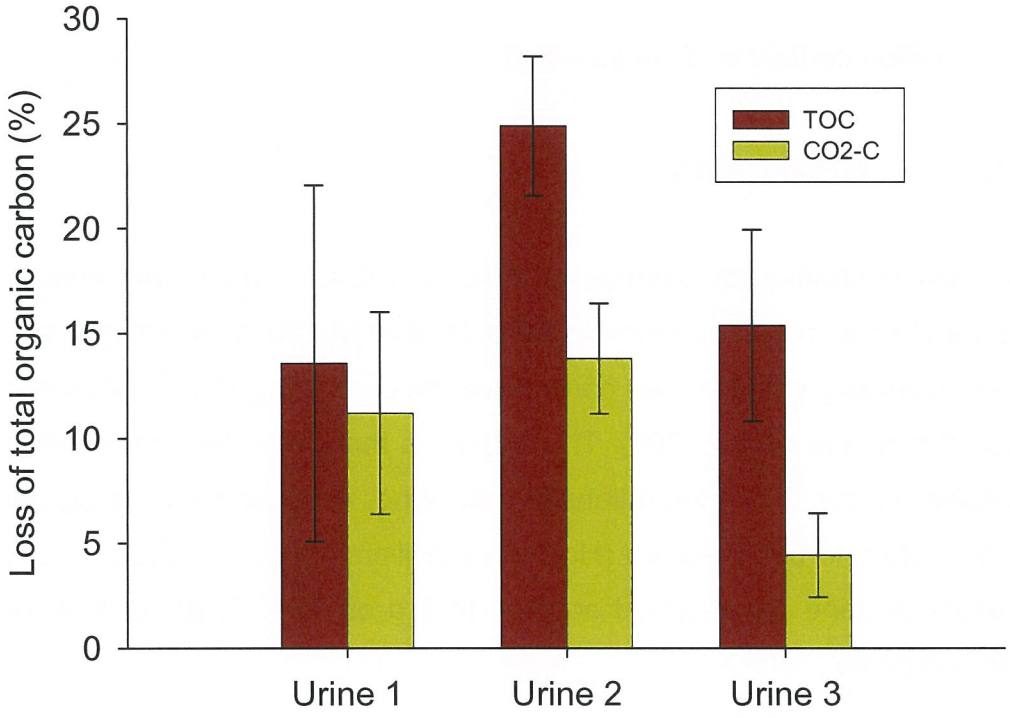

Figure 3.2: Biodegradability of cow urine-C as determined by total organic C (TOC) concentration and cumulative carbon dioxide (CO_2-C) fluxes after 28 day (25°C) incubation. Error bars represent standard error (n = 3).

Table 3.2: Total organic C loss in urine incubated for 28 days (25°C) with and without soil microbe inoculation. Numbers in brackets represent standard error (n = 9), values with different letters were significantly different ($P < 0.05$).

Treatment	TOC loss (%)
Inoculated	16.7 (2.7)[a]
Non-inoculated	18.5 (2.3)[a]

3.4 Discussion and conclusions

3.4.1 Carbon content and composition

3.4.1.1 Wet chemistry

Cow urine contained an average of 7.5±1.2 g C L^{-1}, which was greater than the C concentrations reported by Uchida *et al.* (2011) of 1.9 g C L^{-1}, and considerably smaller than concentrations of 200.3 g C L^{-1} measured by van Groenigen *et al.* (2005). This suggests there may be considerable variability in urine-C concentrations in cow urine. Assuming an average of 1.9 litres of urine per urination (Haynes & Williams 1993), at 7.5 g C L^{-1}, the average urine patch would contain 14.3 g of urine-C, about 90% of which is organic.

Urine-N contents (3.5±0.5 g N L^{-1}) were within the range reported in New Zealand cattle urine by Doak (1952) of 2.5–8.3 g N L^{-1}, but were lower than N content reported by Bristow *et al.* (1992) from British cattle urine, of 6.9–21.6 g N L^{-1}. Urea has been identified as the major N compound contributing to urine-N (Doak 1952; Bristow *et al.* 1992) but was found in the present study to contribute less than 20% of the total C content (Table 3.1; Figure 3.1). Kishan *et al.* (1989) measured C:N ratios of 0.2-0.6 depending upon diet and length of fasting time, which were considerably lower than the C:N ratio measured here of 2.1.

The EC and pH of the urine measured were comparable to previously published data (Suemitsu *et al.* 1970; Richards & Wolton 1976; Lantinga *et al.* 1987).

3.4.1.2 Pyrolysis GC-MS

Hippuric acid was the major C bearing compound isolated from cow urine. Benzoic acid derivatives, such as hippuric acid, in urine result from the intake of lignocellulose (Pagella *et al.* 1997). Hippuric acid has been

reported in cattle urine in concentrations of 6-12 g L^{-1} (Kruela *et al.* 1978; Bristow *et al.* 1992; Bertram *et al.* 2009). An unidentified amide contributed nearly 15% of total compounds isolated in these cow urines, and further work is needed to determine more information about this compound.

Phenaceturic acid, a glycine conjugate of phenylacetic acid, is one of the predominant aromatic acids found in the urine of ruminants (Figure 3.1; Martin 1982). We measured phenylacetic acid as a minor contributor to urine-C, although Suemitsu *et al.* (1968; 1970) had found phenylacetic acid in greater abundance. The small amounts of glycine and phenylacetic acid in our work suggest that these compounds were preferentially excreted as phenaceturic acid (Martin 1973).

Alcohols were the largest group of compounds isolated, making up 5%, which was mostly methanol. Methanol results from the fermentation of pectin in the rumen (Vantcheva *et al.* 1970). The main carbohydrates isolated were glucose and maltose, which are non-structural carbohydrates found in cattle forage foods (Butler & Bailey 1973). Adult animals typically excrete small amounts of carbohydrates due to a high capacity for their removal by kidneys (Church 1976). The degradation of carbohydrates in the rumen results in the production of lactic acid and volatile fatty acids (Church 1976), although these were also found in minor amounts due to re-adsorption (Barcroft *et al.* 1944; Sutton *et al.* 1963). Carboxylic acids were predominantly made up of carbohydrate oxidation products (Church 1976; Beyer & Walter 1996), of which, oxalic acid had been isolated previously in the urine of ruminants by Elliott *et al.* (1979). Isocitric acid was the main tricarboxylic acid and is part of the citric acid cycle (Beyer & Walter 1996), and had not previously been reported in cattle urine. The largest contributor to the hydroxyl group was 3-hydroxyphenylacetic acid, which had not previously been isolated in cow urine.

Pseudouridine, an essential transfer and ribosomal RNA nucleoside, was recovered in half the urine samples analysed and had been isolated in

cow urine previously (Shingfield & Offer 1999). Several amino acids have been reported in cow urine, in particular glycine and taurine (Bathurst 1952; Bristow *et al.* 1992). Glycine was found in minor amounts as discussed earlier, but taurine was not isolated in any of our samples. Phenolic compounds such as p-cresol, catechol, phenol and 4-methyl-cathecol have been found in sheep urine (Martin *et al.* 1983), but were not isolated in the cow urine analysed here.

Commonly used artificial urines may not adequately model the C composition or C:N ratio of cow urine. Holland and During (1977) developed an artificial urine which many subsequent researchers have used as the basis for their own artificial urine. Holland and During's artificial urine contained 10.7 g N L^{-1}, with an estimated C content of 8.2 g C L^{-1}, while the C content is approximately what was measured in cow urine (Table 3.1) the C:N ratio is considerably lower (0.8), indicating an excess of nitrogen. Hippuric acid was the major contributor to urine C, yet is usually used in small amounts or not at all in artificial urines, as urea is the dominant compound contributing to urine N (Kool *et al.* 2006). Artificial urine should closely mimic the composition of cow urine to model adequately the impacts of the application of cow urine on both soil C and N cycling. As conclusions based on artificial urines containing incorrect C composition or C:N ratios may be erroneous, further work is needed to compare artificial and cow urine in soils, in order to clarify whether artificial urine sufficiently represents cow urine.

3.4.2 Bioavailability of urine carbon

Between 7 and 25% of urine-C was degraded over 28 days. We found no other published literature investigating the bioavailability of urine-C. There was greater urine-C degradation measured by TOC loss than by cumulative CO_2-C fluxes ($P < 0.01$), which was also reported by Andreasson *et al.* (2009). Two pathways of inorganic C consumption were not measured in this incubation: bacterial uptake (Belser 1984; Berounsky & Nixon 1990; Udert *et al.* 2003; Lalonde *et al.* 2007) and degassing of

dissolved CO_2 during the ventilation of the samples over the incubation period. These factors may have compromised the accuracy of the corrected CO_2-C fluxes from urine measured here.

Priming of soil C decomposition has been reported following treatment of soil with cow urine (Lovell & Jarvis 1996; Kool *et al.* 2006; Uchida *et al.* 2011). In a urine patch, there are two potential C sources that can be used for priming, urine-C and soil C solubilised following urine deposition. The bioavailability of urine-C indicates that urine-C may act as a priming agent in soils, although further work is needed to assess the bioavailability of soil C solubilised by urine.

In conclusion, the total C content of cow urine from animals grazing on ryegrass-clover pasture was 7.5±1.2 g L^{-1}. A urine patch may contain up to 14.3 g urine-C, with a C:N ratio of 2.1±0.1. Wet chemistry showed that urea, carbohydrates, phenolics and inorganic C contributed about 20% of the total C content of the urine samples. Pyrolysis GC-MS found hippuric acid, urea, an unidentified amide, and phenaceturic acid were the dominant C compounds isolated in cow urine. Between 7 and 25% of the urine-C was degraded over 28 days at 25°C. Inoculation of urine with soil microbes did not enhance urine-C degradation.

Our results have important implications with respect to C cycling under urine patches. Firstly, some artificial urines used in studies investigating the effect of cow urine on nutrient cycling may not adequately model cow urine with respect to C composition and C:N ratio. Secondly, cow urine has the potential to cause priming of soil C by contributing degradable organic C.

3.6 References

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Chapter 4

Fate of urine carbon in soil from three land uses

4.1 Introduction

The dairy industry in New Zealand has been intensifying, increasing in not only the amount of land used in this sector, but also stocking rates (Parliamentary Commissioner for the Environment 2004; MacLeod & Moller 2006). Soil carbon (C) under intensively grazed systems may be decreasing, although the causes for the measured soil C loss have not been fully established (Schipper *et al.* 2007; 2010). Cow urine has the potential to accelerate soil C cycling by increasing soil C in solution in two ways:

- First, high salt solutions have been shown to increase dissolution of soil C (Sollins *et al.* 1996). While cow urine has a high electrical conductivity (24.5±2.4 mS cm^{-1}; Chapter 3), and can contain large quantities of sodium and potassium (Haynes & Williams 1992), it has not yet been established that the electrical conductivity of urine is a factor in soil C dissolution.
- Second, the application of urine to soils causes increased soil pH (Doak 1952; Bristow *et al.* 1992; Haynes & Williams 1992; Curtin *et al.* 1998). The rise in soil pH is attributed to the hydrolysis of urea to ammonium consuming hydrogen ions (Doak 1952; Jackman 1960). Increased soil pH has been reported to cause soil C dissolution (Jackman 1960; Monaghan & Barraclough 1993; Lovell & Jarvis 1996).

The majority of urine research has focused on soil nitrogen (N) cycling and trace gas production (e.g. Ball *et al.* 1979; Clough *et al.* 1996; Clough *et al.* 2003; Clough & Kelliher 2005; Kool *et al.* 2006), and there has been minimal research on the effect of cow urine on soil C cycling. Research into the effects of urine on soils has often used artificial urine to decrease variability and clarify results (e.g. Holland & During 1977; Fraser *et al.* 1994; Clough *et al.* 1996; Shand *et al.* 2000; de Klein *et al.* 2003; Clough & Kelliher 2005). However, it has been shown that commonly used artificial urines may not adequately represent the C content, C composition or the C:N ratio of cow urine (Chapter 3). Also, Lovell and Jarvis (1996) and Kool *et al.* (2006) found artificial urine treated soils had lower carbon dioxide (CO_2-C) fluxes than soils treated with cow urine. Likewise, Somda *et al.* (1997) found that soil pH was greater in soils applied with real urine than artificial urine.

There were two main objectives of the work undertaken in this chapter. Firstly, to determine the fate of cow urine-C in soils using a C balance, and secondly, to assess urine pH, electrical conductivity and urea content in the enhancement of soil C cycling. A comparison of artificial and cow urine was also undertaken. Soils from under three land uses (dairy-grazed pasture, sheep-and-beef-grazed pasture, *Pinus radiata* plantation) were assessed to determine if soil C cycling under urine patches was influenced by land use intensity.

4.2 Materials and methods

4.2.1 Soil

Foxton black sand (Typic Sandy Brown Soil; Hewitt 1998; Ultic Udipsamment Sandy; Soil Survey Staff 1999) was collected on a Hokio Beach farm in October 2006, from a 15-year-old *Pinus radiata* stand (1790825E 5503085N, NZTM), a sheep-and-beef-grazed paddock

(1786986E 5502778N), and a dairy-grazed paddock (1789340E 5502778N). Each of the sites was on the same age of sand dune (Hugh Wilde, pers. comm.) and, where possible, was matched for aspect and slope.

Three 30-m transects were established across the slope of each site. Core samples (25.4 mm diameter) were taken to 50 mm depth at every metre along each transect and bulked to give three replicates for each land use. Each bulked sample was sieved at field moisture to <2 mm. Every 10 metres along the transect, a 50-mm-deep soil core (56 mm diameter) was taken for bulk density measurement (g cm^{-3}), whereby the dry weight of the soil (105°C) was divided by the volume of soil.

The water-holding capacity of the bulked soil samples was measured according to Harding and Ross (1964). Briefly, a 30-g sample of field moist soil was weighed into a stoppered funnel, sufficient water was added to the funnel to submerge the soil, and the soil was left to soak overnight. The following day, the stopper was removed from the funnel and the soil was left to drain for 3 hours. The soil was then considered to be at field capacity and the moisture content measured gravimetrically by oven drying at 105°C (Harding & Ross 1964). Each of the soils was adjusted to 60% of their water-holding capacity (w/w%); dairy 43%, sheep and beef 50%, and pine soil 49%. The adjusted soils were pre-incubated for 5 days before being repacked at field bulk density (0.7 g cm^{-3}) into 50-mm diameter plastic liners, to a depth of 50 mm. A filter paper and 280-µm mesh were attached to the bottom of each of the liners to retain fine particulates.

4.2.2 Cow urine

Dairy cow urine (5 L) was collected from several (~5) cows on 9 October 2006 before and during afternoon milking (Dairy 3, Massey University, Palmerston North, New Zealand). Cows had been grazing ryegrass-clover pasture until milking. Total C and N of the urine were measured by

combustion furnace (LECO FP-2000 CNS analyser LECO Corp. St Joseph, MI, USA) on the day of collection. Urea was measured by acid digestion for 30 minutes in boiling water, following the extraction of urea from urine using 10% trichloroacetic acid solution. The urea content was measured spectrophotometrically against urea standards at 520 nm (Marsh *et al.* 1965). The electrical conductivity (EC) of the urine was measured against a 2 *M* potassium chloride standard and the pH was measured using a glass electrode (Blakemore *et al.* 1987).

4.2.3 Treatments

Five solutions were applied to the soil cores to investigate how the chemistry of cow urine might influence soil C cycling:

1. **No urea,** solution had the same pH and EC as cow urine but no urea was added
   - 2.937 g L^{-1} potassium chloride (Labserv, BioLab, Auckland, New Zealand) dissolved in Milli-Q water and adjusted to a pH of 8.1 by adding sodium hydroxide solution.
2. **Urea,** solution had the same urea content, pH and EC as cow urine
   - 2.82 g L^{-1} urea (Aldrich, Sigma-Aldrich, Auckland, New Zealand) and 2.937 g L^{-1} potassium chloride (Labserv, BioLab, Auckland, New Zealand) were dissolved in Milli-Q water and adjusted to a pH of 8.1.
3. **Artificial urine,** solution had the same total C, total N, urea content, pH and EC as cow urine, adapted from Shand *et al.* (2000).
   - 23.1 g L^{-1} potassium carbonate (AnalaR, BDH, Poole, England), 2.82 g L^{-1} urea (Aldrich, Sigma-Aldrich, Auckland, New Zealand), 11.38 g L^{-1} glycine (AnalaR, BDH, Poole, England), 9.104 g L^{-1} glucose (AnalaR, BDH, Poole, England) and 3.401 g L^{-1} of potassium chloride (Labserv, BioLab, Auckland, New Zealand).
4. **Cow urine,** dairy cow urine
5. **Control,** Milli-Q water

Each solution was applied to repacked soil cores from each land use (in triplicate) resulting in 15 treatments (Table 4.1).

Table 4.1: Land uses sampled for soil, solutions applied, and treatment labels for Foxton soil incubation experiment.

Soil	Solution applied	Treatment Labels
Dairy	Water	Dairy+Water
S&B*	Water	S&B+Water
Pine	Water	Pine+Water
Dairy	No urea	Dairy+No urea
S&B	No urea	S&B+No urea
Pine	No urea	Pine+No urea
Dairy	Urea	Dairy+Urea
S&B	Urea	S&B+Urea
Pine	Urea	Pine+Urea
Dairy	Artificial urine	Dairy+AU
S&B	Artificial urine	S&B+AU
Pine	Artificial urine	Pine+AU
Dairy	Cow urine	Dairy+CU
S&B	Cow urine	S&B+CU
Pine	Cow urine	Pine+CU

*Sheep-and-beef-grazed soil

The cow and artificial urines were applied at a rate of 1000 kg N ha^{-1}, the average concentration of a urine patch (Haynes & Williams 1993), which equated to 56 mL applied to each core. The same volumes of the other solutions were added in the remaining treatments. The cores were left to drain for 1 hour, and the leachate was captured for analysis. The treated cores were placed into Agee jars (1 L), sealed with lids that had a septa inserted, and incubated for 14 days at 25±1°C in the dark.

4.2.4 Leachate analysis

Leachates from cores were analysed for total C (High TOC II, Elementar Analysensystems GmbH, Hanau, Germany) and total N (LECO FP-2000 CNS analyser LECO Corp. St Joseph, MI, USA). Carbohydrate concentrations were determined following incubation of leachate with 5% phenol solution and sulphuric acid for 1 hour and quantified spectrophotometrically against glucose standards (Dubois *et al.* 1956). Phenolics were determined by incubating leachate with Folin & Ciocalteu's reagent for 90 minutes at room temperature and quantified spectrophotometrically against tannic acid standards (Blum *et al.* 1992). Leachate pH and EC were measured as previously described (4.2.2).

4.2.5 Soil analysis

Soil total C and total N were measured by combustion furnace (LECO FP-2000 CNS analyser LECO Corp. St Joseph, MI, USA). Cold water soluble C (CWSC) was measured as an indicator of the amount of labile organic matter available for microbial mineralisation (Sparling *et al.* 1998). CWSC was determined by shaking 3 grams (oven dry equivalent) of wet soil with 30 mL of Milli-Q water at 50 rpm for 20 minutes (20°C), centrifuged at 2500 rpm for 20 minutes before filtering through GF/F filter papers (Labserv, BioLab, New Zealand) under suction (Ghani *et al.* 2003). Hot water soluble C (HWSC) was extracted subsequently after CWSC extraction. Thirty mL of water was added to the soil, and the slurry was incubated for 16 hours at 80°C, before centrifugation, filtration and analysis as for CWSC. The CWSC extracts were analysed for carbohydrates (Dubois *et al.* 1956) and phenolics (Blum *et al.* 1992) as described in 4.2.4. Soil microbial biomass (MBC) was measured before and after incubation, using fumigation-extraction with an extraction efficiency factor of 0.41 (Vance *et al.* 1987). Soil EC and pH were measured on 5 g of air dried soil mixed with 25 mL of Milli-Q water, left to equilibrate overnight (Blakemore *et al.* 1987). Analyses were compared pre- and post-incubation.

4.2.6 Soil respiration

Carbon dioxide (CO_2-C) production was measured from the repacked cores by taking a headspace gas sample after a 24 hour trapping period. A needle attached to a 60-mL syringe was inserted through the septum in the incubation jar's lid and 25 mL of headspace removed. The headspace gas was then transferred into an evacuated 12-mL vial (Labco Limited, Buckinghamshire, United Kingdom). Gas samples were taken on days 1, 4, 7, and 14 of the incubation and analysed for CO_2-C by flame ionisation after conversion of CO_2 to methane (GC 2010, Shimadzu, Kyoto, Japan). Jars were evacuated before and after the trapping period for 30 minutes. CO_2-C fluxes were calculated using Ideal Gas Law, equation 1:

$$n = \frac{(PV/RT)*12.011}{DW_{soil} * t} \quad (1)$$

Where:

- n = CO_2-C production (μg CO_2-C g^{-1} $hour^{-1}$)
- P = absolute pressure of the gas (kPa)
- V = volume of headspace (L)
- R = universal gas constant (8.314472 J K^{-1} $mole^{-1}$)
- T = temperature (°Kelvins)
- DW_{soil} = weight of oven dry soil at 105°C (g)
- 12.011 = molecular weight of C (g mol^{-1})
- t = gas trapping period (hours).

Cumulative CO_2-C (μg CO_2-C g^{-1}) over the 14-day incubation was calculated by linear interpolation for the days between measurements using Equation 2:

$$\text{Cum } CO_2\text{-C} = F_1 + [((F_1+F_4)/T)*T\text{-}2] + F_4 + [((F_4+F_7)/T)*T\text{-}2] + F_7+[((F_7+F_{14})/T)*T\text{-}2] + F_{14} \quad (2)$$

Where:

- F_1 = CO_2-C flux on day 1 of the incubation (µg CO_2-C g^{-1} day^{-1})
- F_4 = CO_2-C flux on day 4 of the incubation (µg CO_2-C mL^{-1} day^{-1})
- F_7 = CO_2-C flux on day 7 of the incubation (µg CO_2-C mL^{-1} day^{-1})
- F_{14} = CO_2-C flux on day 14 of the incubation (µg CO_2-C mL^{-1} day^{-1})
- T = incubation time (days)

4.2.7 Carbon balance

A C balance was used to determine the fate of C added in the Solutions within the soil. The amount of Solution-C retained was calculated using Equation 3.

Retained-C = Solution-C - Leachate-C (3)

Where:

- Retained-C = the amount of added C retained in the soil (µg g^{-1})
- Solution-C = C concentration of the Solution added (µg g^{-1})
- Leachate-C = C concentration of the leachate from the repacked cores after solution application (µg g^{-1})

The amount of Solution-C that was recovered in different C fractions was calculated using equation 4.

Recovered-C = Solution-C – (Leachate-C + Cum CO_2-C + CWSC) (4)

Where:

- Recovered-C = the amount of added C recovered (µg g^{-1})
- Solution-C (µg g^{-1}), Leachate C (µg g^{-1}), Cum CO_2-C (µg g^{-1}), and CWSC (µg g^{-1}), had been corrected for the water control.

The C balance measured bulk changes in C, but did not distinguish between C derived from soil or added Solution-C in the fractions measured.

4.2.8 Statistical analysis

All statistical analyses were undertaken using Genstat 12. Differences between treatments were determined by ANOVA and were reported as significant when $P < 0.05$. Differences between soils within a treatment and within each soil for treatment effects were determined using Student-Newman-Kuels testing ($P < 0.05$). To remove bias from Student-Newman-Kuels testing, treatments with large variability were analysed separately from treatments with small variability, and were therefore grouped with respect to the solution added for analysis.

Student-Newman-Kuels analysis of leachate total C and pH data was grouped by solution as bracketed (water, no urea, urea) and (cow and artificial urine). Leachate total N data were grouped as follows: (water, no urea) and (urea, cow and artificial urine) and leachate EC data; (water) and (no urea, urea, artificial and cow urine). For testing of treatment differences in leachate carbohydrate, the treatments were grouped as follows: (cow urine), (artificial urine) and (water, no urea and urea). Leachate phenolics data had similar levels of variability in all treatments and did not require segregation into groups.

Soil microbial biomass C, HWSC, CWSC, and soil pH did not require grouping according to treatment variability for Student-Newman-Kuels analysis. Cold water soluble carbohydrates and phenolics were grouped for testing as follows: (Pre-treatment, water, no urea and urea) and (artificial and cow urine). Soil EC was separated into (Pre-treatment, water) and (no urea, urea, artificial and cow urine) groups of treatments.

Cumulative CO_2-C data were transformed logarithmically (log 10) before statistical analysis, for Student-Newman-Kuels testing the treatments were

grouped as bracketed: (water, no urea, urea) and (artificial and cow urine). Treatment grouping of percentage of Retained-C respired, percentage of Recovered-C, Recovered-C and Solution-C minus Recovered-C data was not required. Cubic regression analysis to test the correlation between soil pH and CWSC was undertaken in Sigma Plot 8. Soil C change (Table 4.3) was tested for significance from zero using a one-sample, two sided t-test with a confidence interval of 95% (Genstat 12).

4.3 Results

The C content of the dairy cow urine collected was 9.6 g L^{-1} (Table 4.2), which is slightly greater than the urine-C content measured previously of 7.5 g L^{-1} (Chapter 3). The pH, EC, urea, total C, and total N contents of the Treatment Solutions, where appropriate, were manipulated to be the same as the cow urine (Table 4.2; Section 4.2.3).

Table 4.2: Total carbon, total nitrogen, urea content, pH and electrical conductivity of cow urine.

Chemical Properties	Urine
Total carbon (g L^{-1})	9.6
Total nitrogen (g L^{-1})	3.4
Urea (g L^{-1})	2.8
pH	8.1
Electrical conductivity (mS/cm)	23.9

4.3.1 Leachate

The leachate C and N concentrations were greater ($P < 0.001$) in the artificial and cow urine treatments than the remaining treatments (Figure 4.1; 4.2).

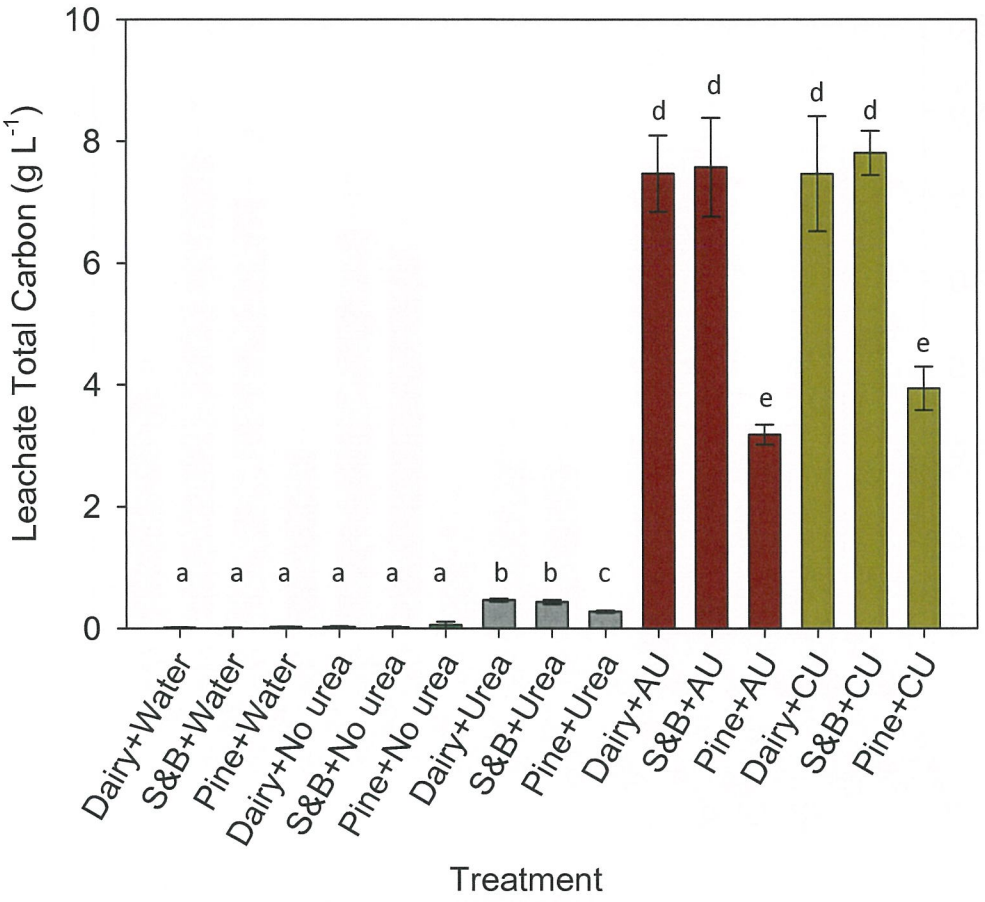

Figure 4.1: Total carbon concentration in leachate from dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils after application of water, no urea, urea, artificial urine (AU), and cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.001$).

In the cow and artificial urine treatments, the leachate from the pine soil had lower ($P < 0.001$) concentrations of total C and N than the leachates from the sheep-and-beef- or dairy-grazed soils (Figure 4.1; 4.2). For example, about 20% of the C added in the cow urine was retained in the two grazed soils, whereas about 65% was held in the pine soil.

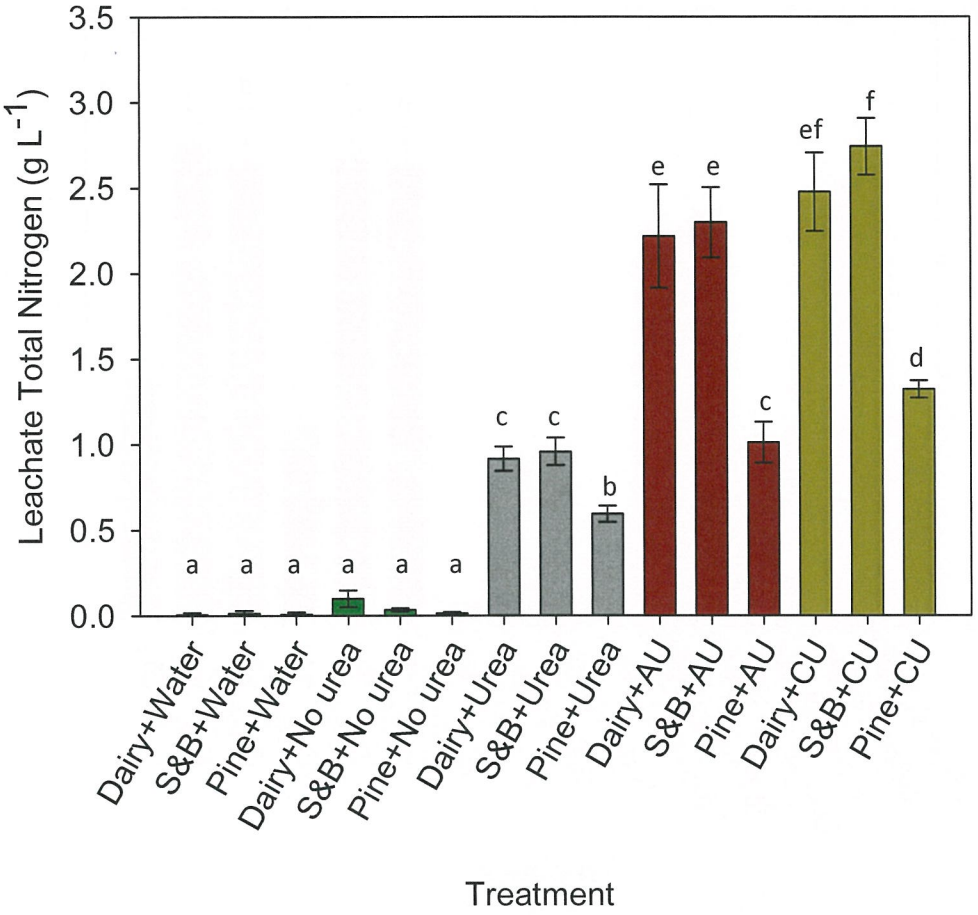

Figure 4.2: Total nitrogen concentration in leachate from dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils after application of water, no urea, urea, artificial urine (AU), and cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.001$).

Leachate carbohydrate concentration was greater ($P < 0.001$) in the artificial urine treatment, which contained large amounts of glucose, than in the other treatments (Figure 4.3). In the artificial urine treatment, the carbohydrate concentration in the leachates was lower ($P < 0.01$) from the pine soil (50% of artificial urine content) than from the grazed soils (Dairy = ~85%, S&B = 70%). The pine soil also showed capacity for removing carbohydrates from the cow urine, where 95% of the 300 mg L^{-1} of carbohydrates added were held in the soil.

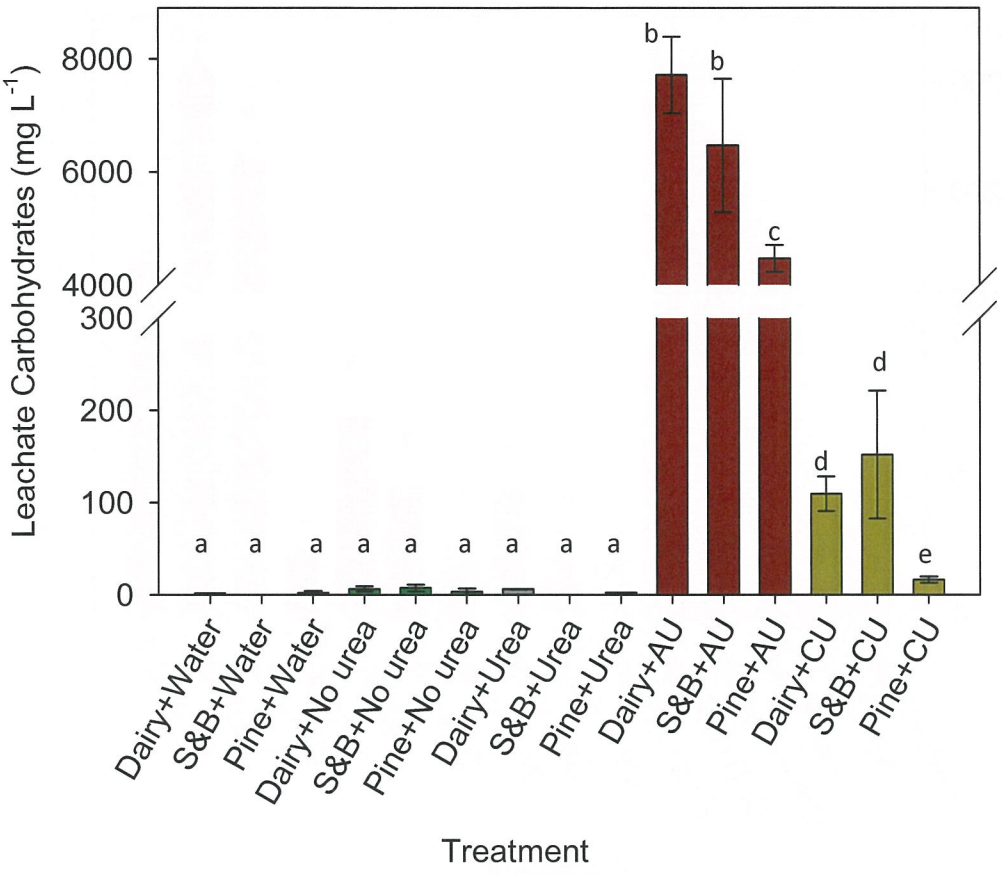

Figure 4.3: Total carbohydrate concentration in leachate from dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils after application of water, no urea, urea, artificial urine (AU), and cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different (CU $P < 0.05$; AU $P < 0.01$).

Leachate phenolic concentrations were the same in the artificial urine, no urea, urea and water treatments, but were greater ($P < 0.001$) in the cow urine treatments (Figure 4.4). As for leachate carbohydrates, the leachate from the pine soil in the cow urine treatment contained significantly less phenolics ($P < 0.001$; Figure 4.4) than the grazed soils.

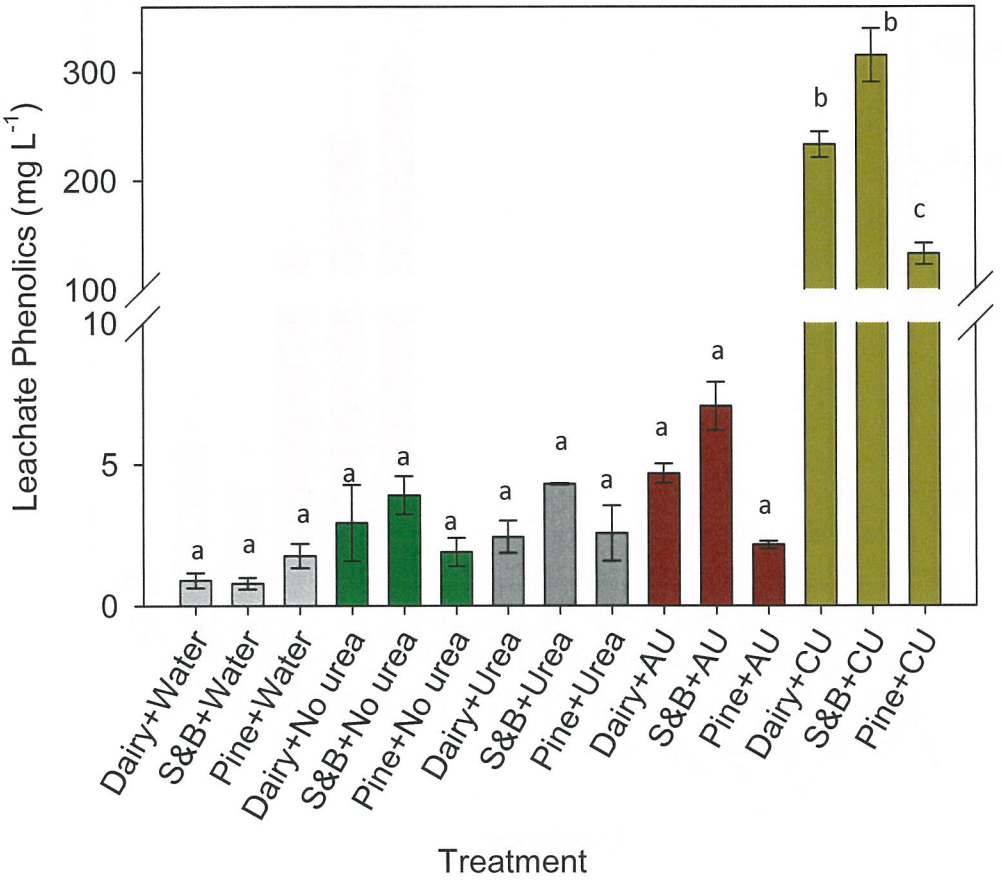

Figure 4.4: Total phenolic concentration in leachate from dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils after application of water, no urea, urea, artificial urine (AU), and cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.001$).

The pH of the leachates from the artificial and cow urine treatments was greater ($P < 0.001$) than the water, no urea, and urea treatments (Figure 4.5). In the cow urine treatment, the pH of the leachate from the pine soils ($P < 0.001$) was less than the grazed soils (Figure 4.5). The EC of the leachate in all treatments, except the water control, was less ($P < 0.05$) in the pine soil than the grazed soils (Figure 4.6).

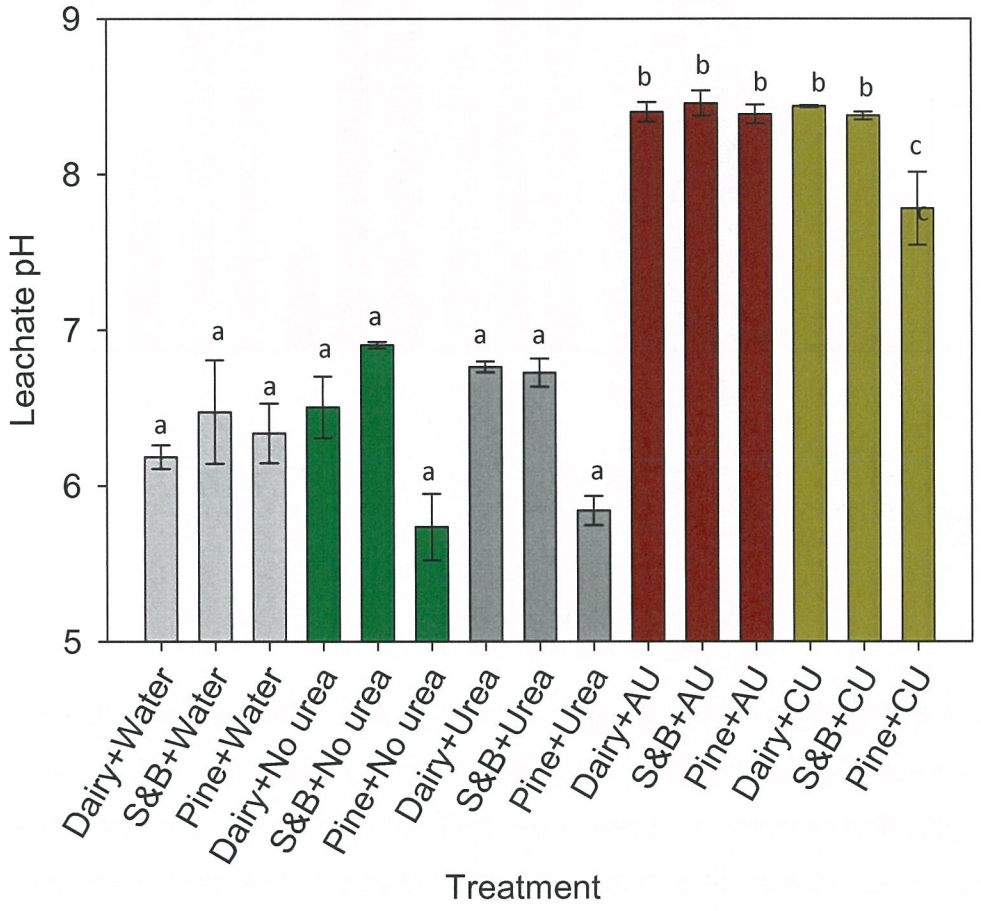

Figure 4.5: Leachate pH from dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils after application of water, no urea, urea, artificial urine (AU), and cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.001$).

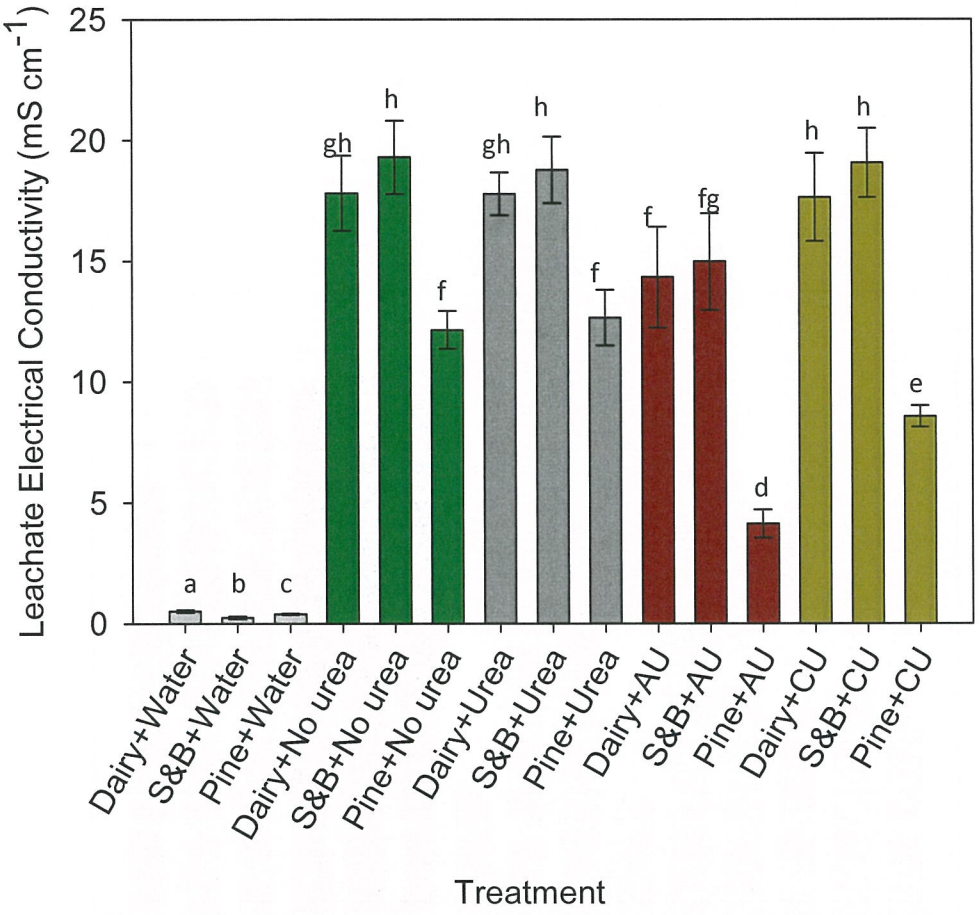

Figure 4.6: Electrical conductivity of leachate from dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils after application of water, no urea, urea, artificial urine (AU), and cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different (Water: $P < 0.001$; no urea, Urea, AU and CU: $P < 0.05$).

4.3.2 Soil Chemistry

Soil total C, total N, and microbial biomass showed no measureable change in any of the treatments compared with pre-treatment contents (data not shown).

Hot water soluble C (HWSC) in the no urea and water treatments was not significantly different from the pre-treatment contents, but the cow and artificial urine treatments had greater HWSC ($P < 0.001$) than the pre-treatment soils (Figure 4.7). The HWSC content of the sheep-and-beef-grazed soil was less ($P < 0.001$) than the pine and dairy-grazed soils in the artificial and cow urine treatments (Figure 4.7).

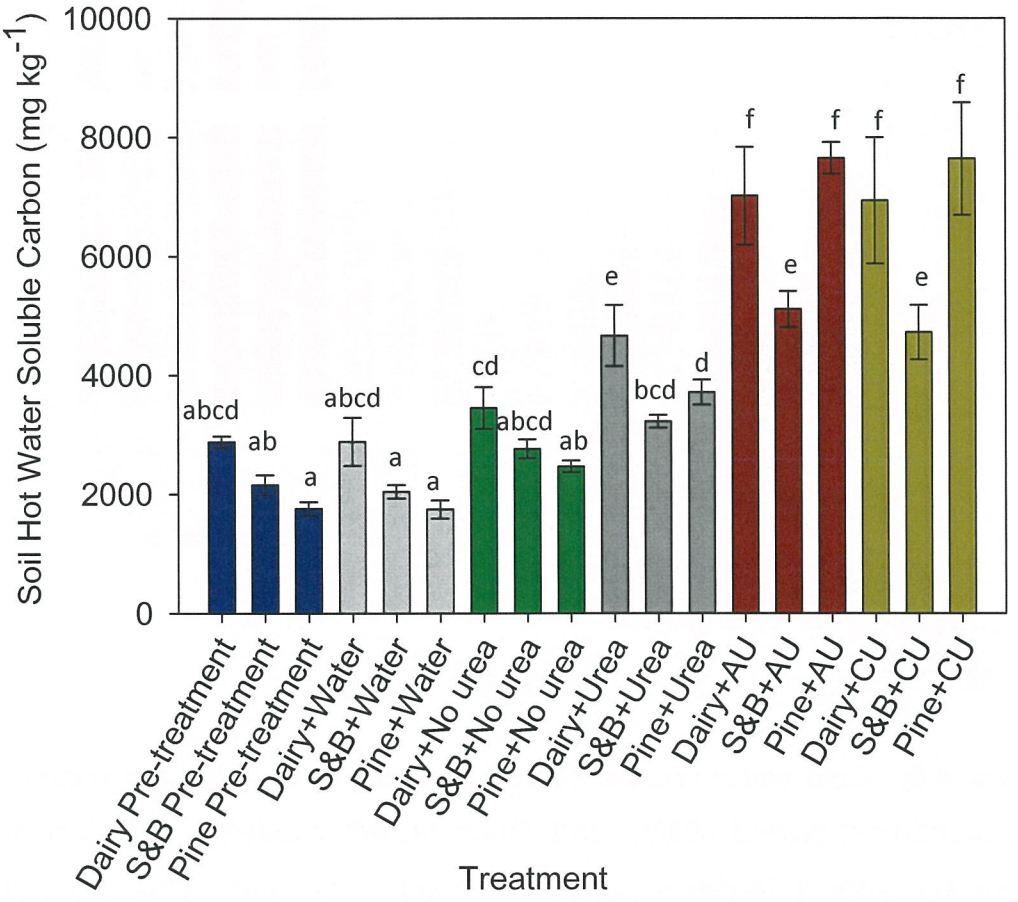

Figure 4.7: Hot water soluble carbon content in dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils before and after a 14-day incubation with water, no urea, urea, artificial urine (AU), cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.001$).

There was no difference in cold water soluble C (CWSC) contents between the pre-treatment, water, no urea, and urea treatments, but there was greater CWSC ($P < 0.001$) in the artificial and cow urine treatments compared with the other treatments (Figure 4.8). The CWSC content of

the artificial and cow urine treatments were not difference from each other (Figure 4.8).

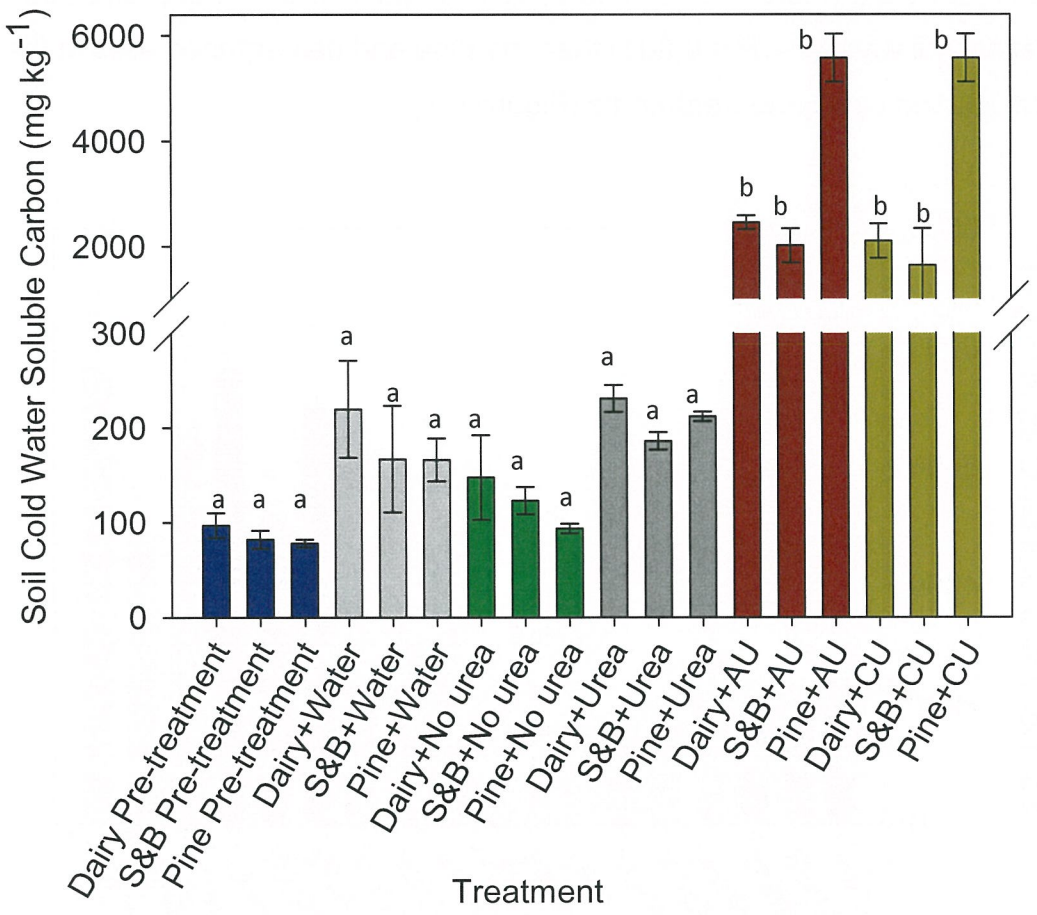

Figure 4.8: Cold water soluble carbon contents in dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils before and after a 14-day incubation with water, no urea, urea, artificial urine (AU), cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.001$).

Cold water soluble carbohydrates were greater ($P < 0.001$) in the cow and artificial urine treatments, but there was no difference between cold water soluble carbohydrates in the pre-treatment, water, no urea and urea treatments (Figure 4.9). Cold water soluble phenolics were not detectable in pre-treatment soils, but were present in all treatments after the incubation (Figure 4.10). The greatest cold water soluble phenolic

concentrations were observed in the artificial and cow urine treatments, which showed no difference from each other between the land uses.

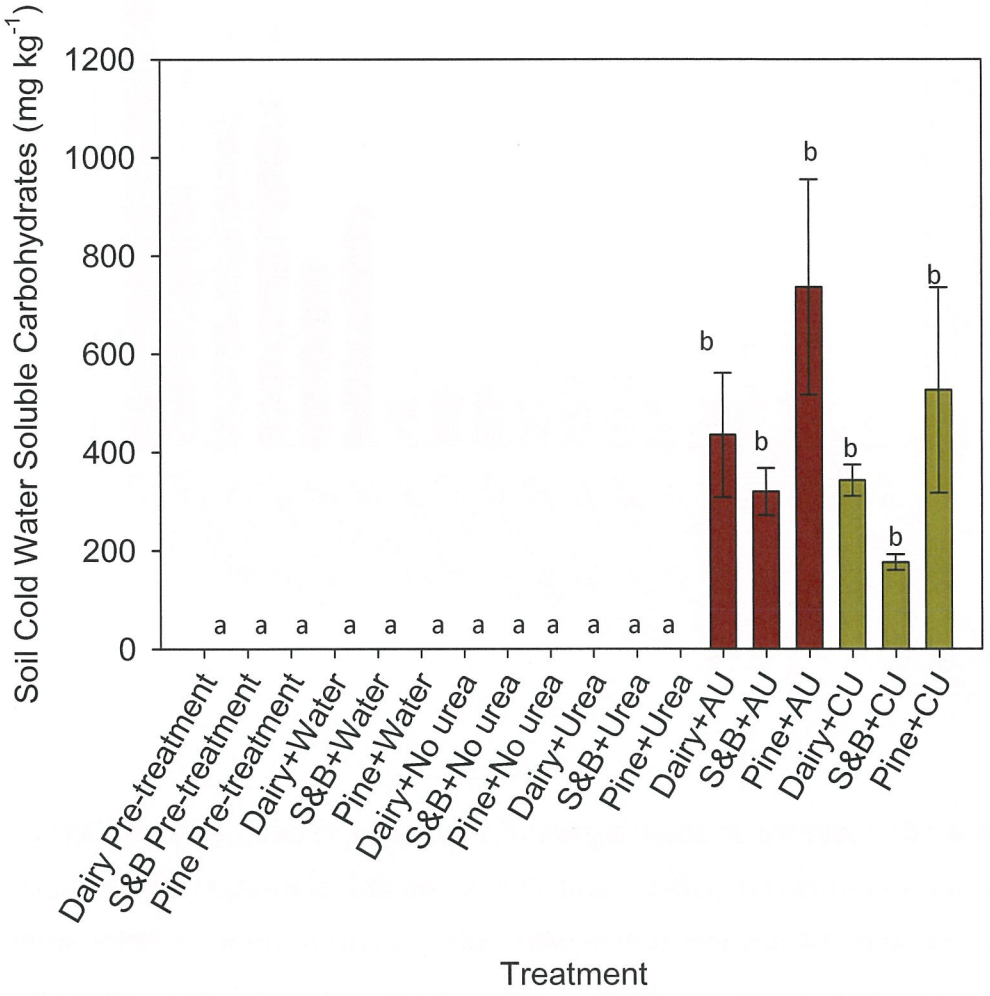

Figure 4.9: Cold water soluble carbohydrate contents in dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils before and after a 14-day incubation with water, no urea, urea, artificial urine (AU), cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.001$).

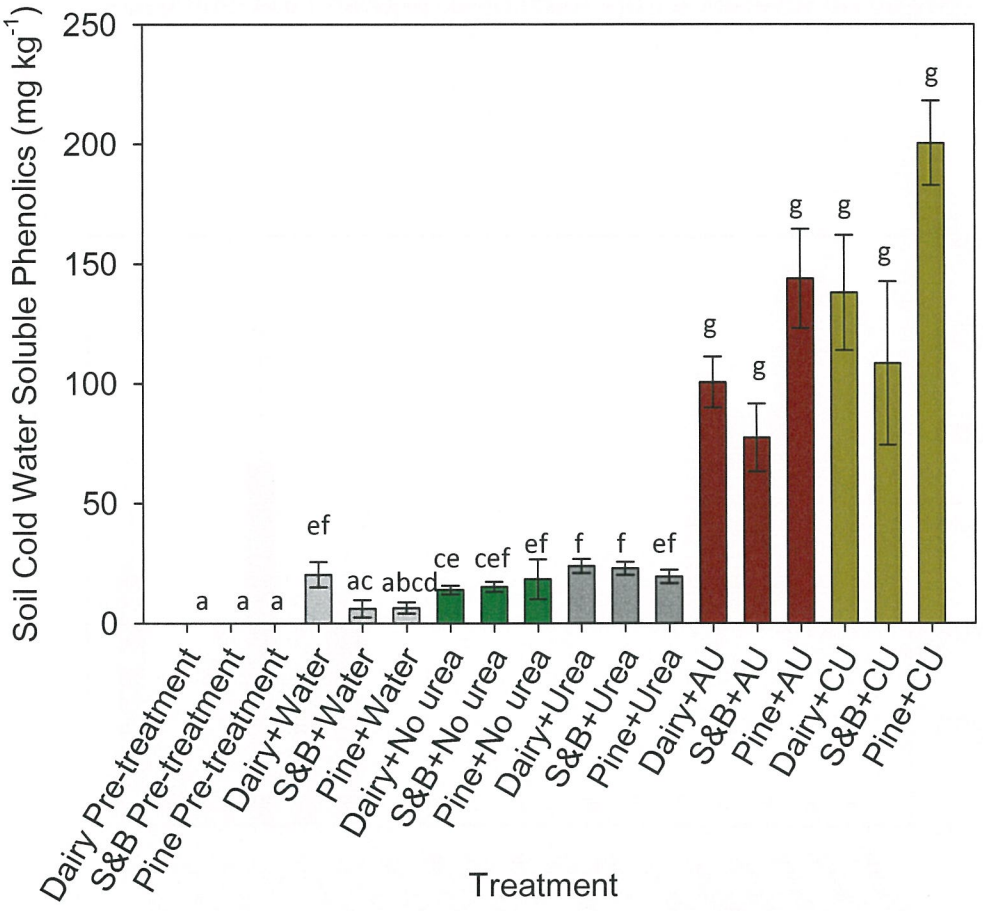

Figure 4.10: Cold water soluble phenolic contents in dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils before and after 14-day incubation with water, no urea, urea, artificial urine (AU), cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.001$).

Soil pH increased compared to pre-treatment measurements in the urea, water, cow and artificial urine treatments ($P < 0.01$), but not in the no urea treatment (Figure 4.11). The greatest increases in soil pH compared with pre-treatment soils were measured in the artificial and cow urine treatments ($P < 0.01$), in particular the Pine+AU treatment had an increase of 2.8 pH units (Figure 4.11). There was a strong positive correlation between soil pH and CWSC (Figure 4.12; $P < 0.001$; $r^2 = 0.74$).

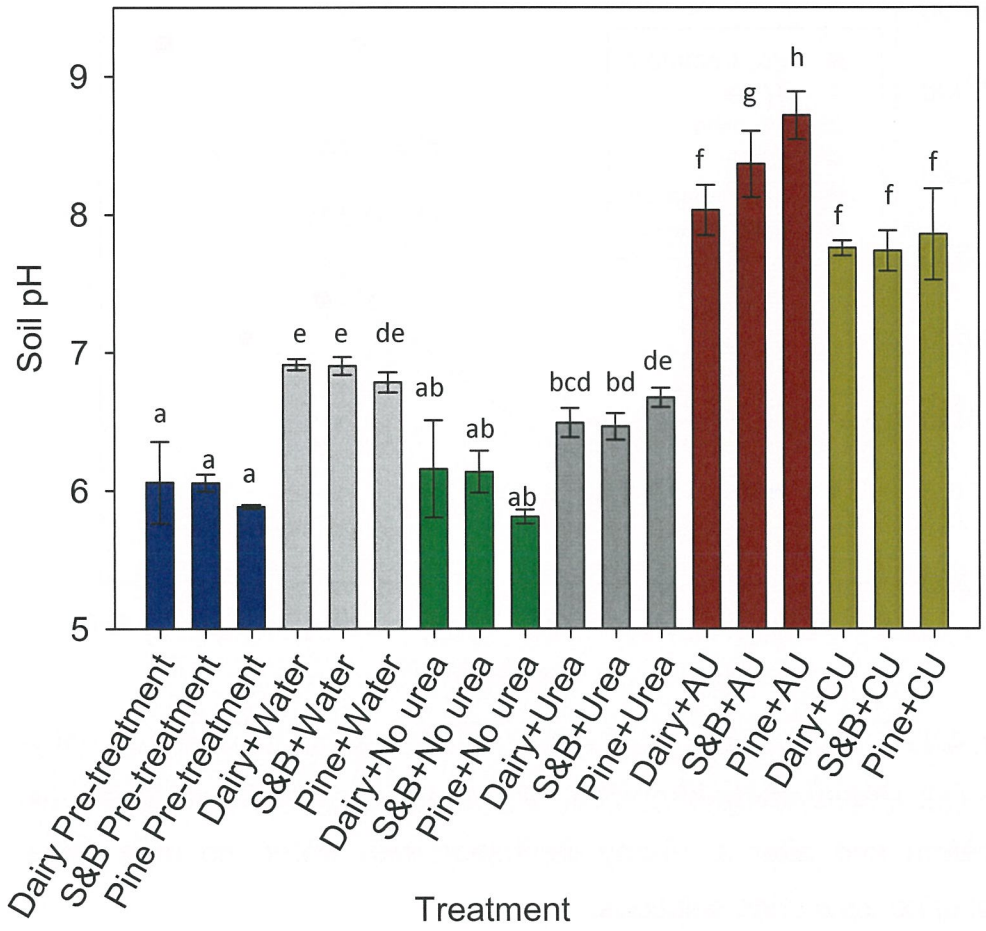

Figure 4.11: Soil pH in dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils before and after a 14-day incubation with water, no urea, urea, artificial urine (AU), cow urine (CU) solutions. Error bars represent standard deviation; bars with different letters were significantly different ($P < 0.01$).

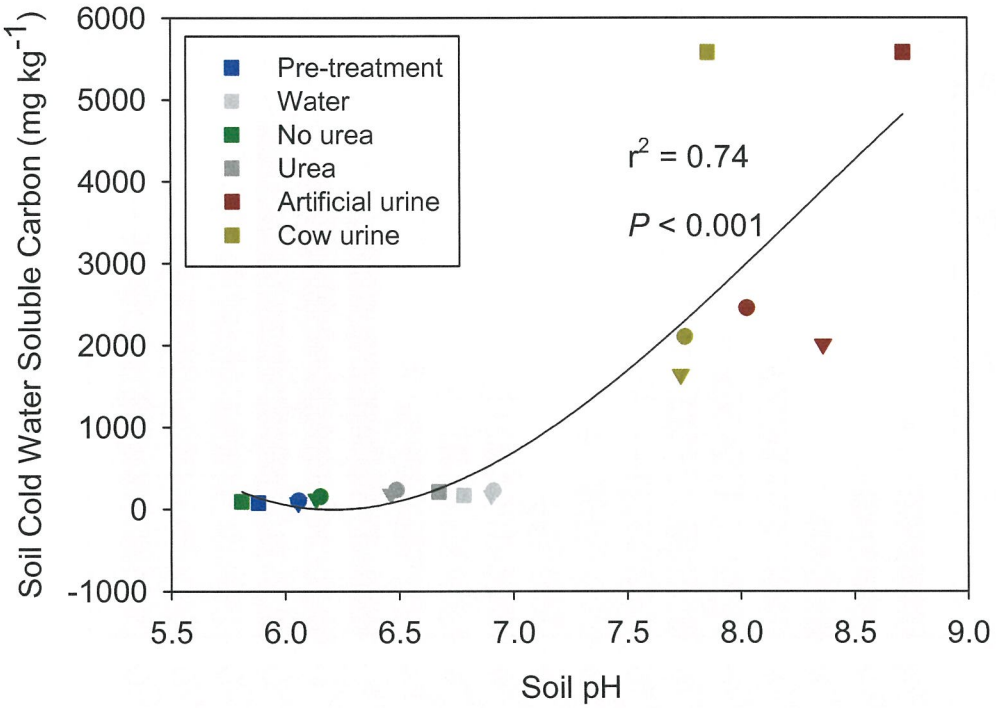

Figure 4.12: Soil pH and cold water soluble carbon contents in dairy-grazed (●), sheep-and-beef-grazed (▼), and *Pinus radiata* plantation (■) soils before and after a 14-day incubation with water, no urea, urea, artificial urine, cow urine solutions.

Compared with pre-treatment levels, soil EC increased in all treatments, with the exception of the water control (Figure 4.13; $P < 0.001$). There were no differences in EC between no urea, urea, or either urine treatment at the end of the incubation.

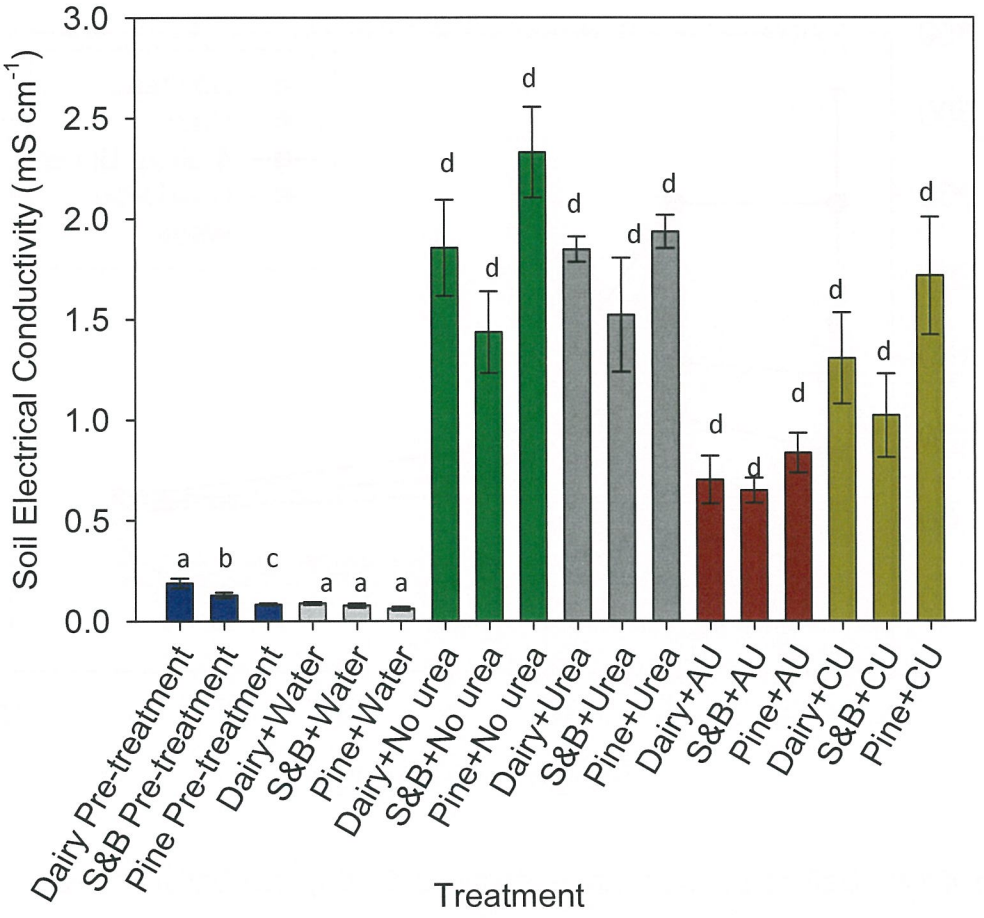

Figure 4.13: Soil electrical conductivity in dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* (Pine) plantation soils before and after a 14-day incubation with water, no urea, urea, artificial urine (AU), cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.001$).

4.3.3 Carbon balance

4.3.3.1 Soil Respiration

CO_2-C fluxes were greatest in the artificial and cow urine treatments and the rate of CO_2-C production in all treatments peaked in the first 4 days of the incubation (Figure 4.14).

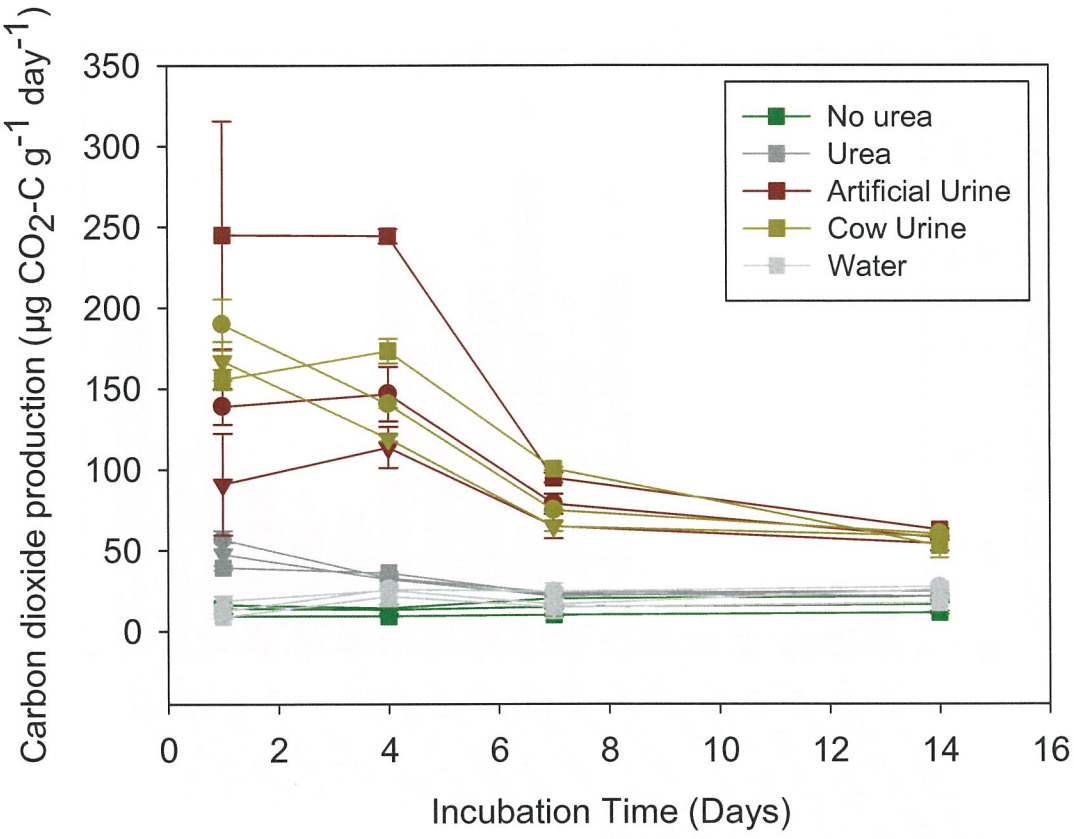

Figure 4.14: Soil respiration fluxes during a 14-day incubation of dairy-grazed (Dairy;●), sheep-and-beef-grazed (S&B;▼), and *Pinus radiata* plantation (Pine;■) soils after application of water, no urea, urea, artificial urine (AU), cow urine (CU) solutions. Error bars represent one standard deviation.

Cumulative CO_2-C was greatest ($P < 0.01$) in the artificial and urine treatments, which were not different from one another (Figure 4.15). The proportion of Retained-C respired was greatest ($P < 0.001$) in the urea treatment (Figure 4.16). Cumulative respiration accounted for between 12 and 17% of the Retained-C in the soil in the cow and artificial urine treatments (Figure 4.16).

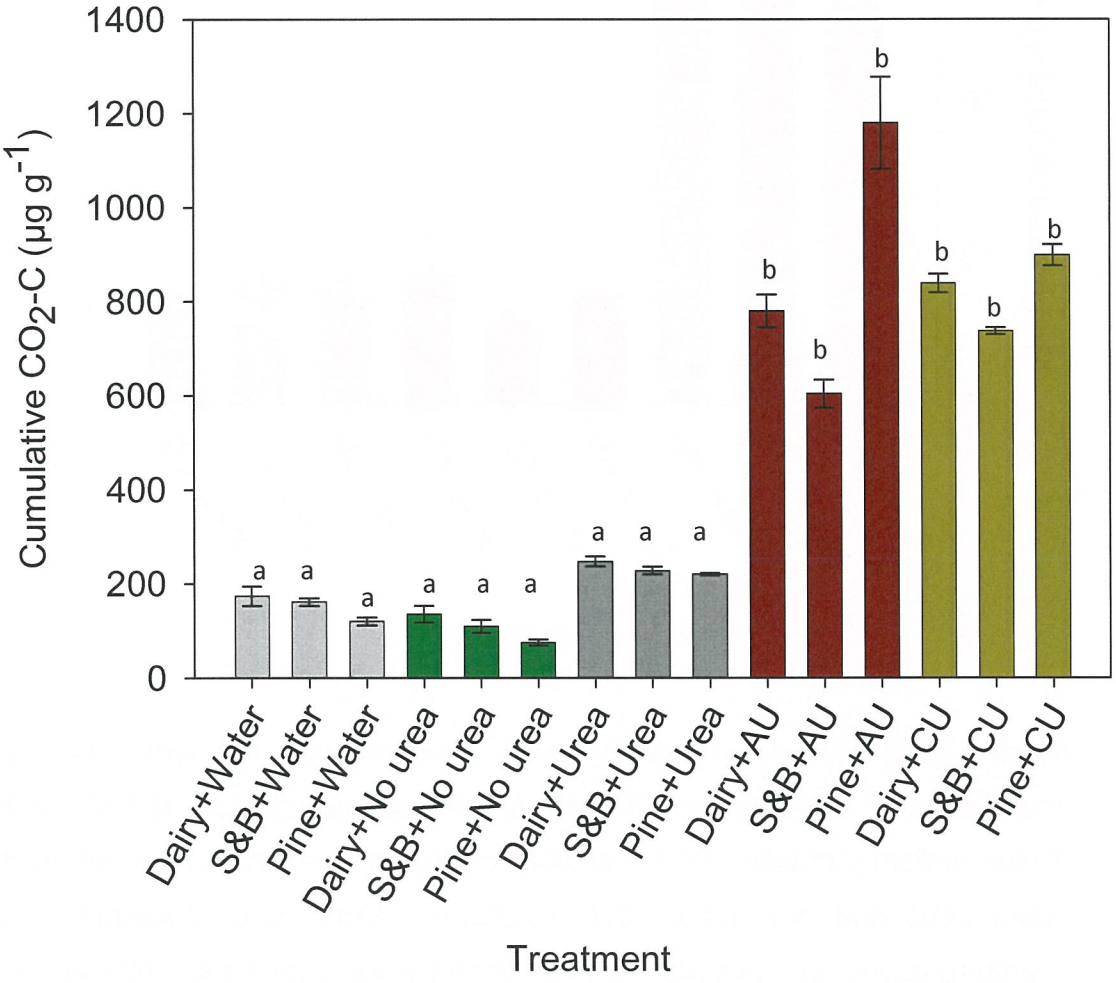

Figure 4.15: Cumulative CO_2-C from dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils in a 14-day incubation following application of water, no urea, urea, artificial urine (AU), cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.01$).

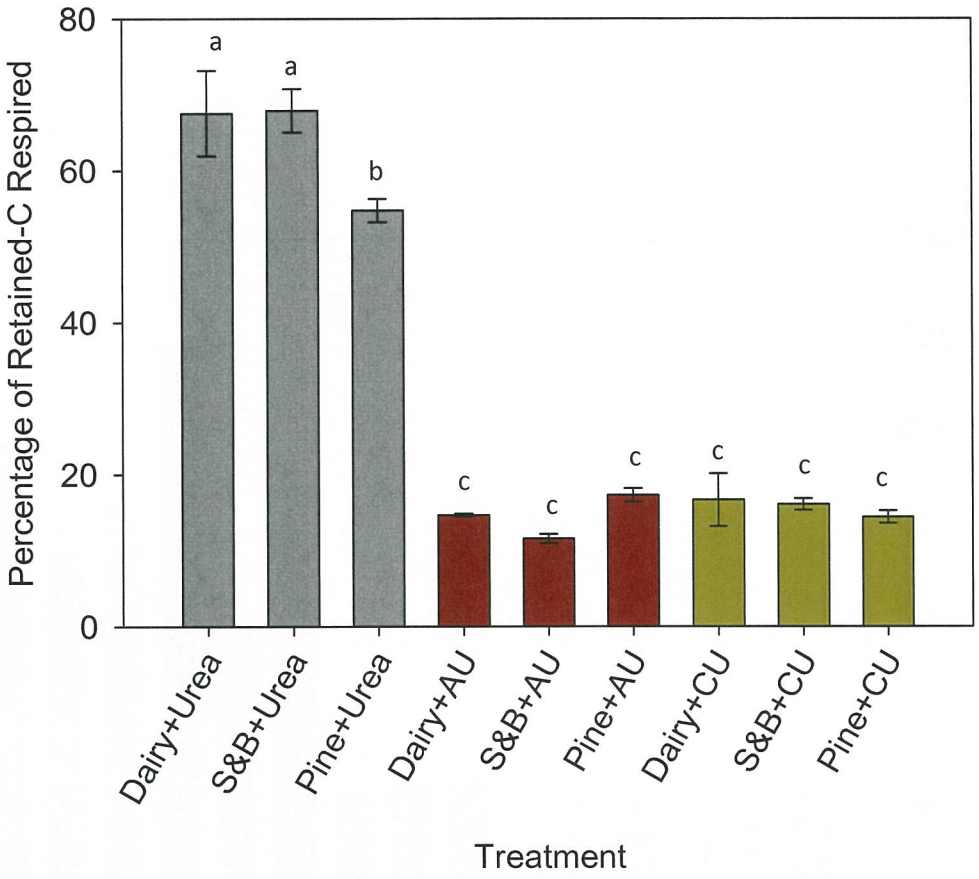

Figure 4.16: Percentage of Retained-C that was respired over a 14-day incubation of dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils following application of urea, artificial urine (AU) and cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.001$).

4.3.3.2 Recovered-C

Recovered-C was the amount of Solution-C recovered in the leachate, cumulative CO_2-C, and soil CWSC after the incubation. Recovered-C was significantly different from zero for all the soils in the urea, artificial and cow urine treatments (Table 4.3).

Table 4.3: Solution-C, Leachate-C, Cumulative CO_2-C, Soil cold water soluble C (CWSC), Recovered-C and Soil C change after a 14-day incubation of dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* plantation (Pine) soils with water, no urea, urea, artificial urine (AU), cow urine (CU) solutions. Numbers in brackets represent one standard deviation. Values within the same columns with different letters were significantly different ($P < 0.001$). Numbers with stars were significantly different from zero (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Recovered-C and Soil C change values have been corrected for the water control.

Treatment	Solution-C (a) (μg C g^{-1})	Leachate-C (μg C g^{-1})	Cumulative CO_2-C (μg C g^{-1})	CWSC (μg C g^{-1})	Recovered-C (b) (μg C g^{-1})	Soil C change (a)-(b) (μg C g^{-1})
Dairy+Water	0 (0)	6 (1)	174 (20)	220 (51)	-	-
S&B+Water	0 (0)	5 (1)	162 (8)	167 (56)	-	-
Pine+Water	0 (0)	9(1)	120 (8)	166 (23)	-	-
Dairy+No urea	1 (1)	9 (3)	136 (18)	148 (45)	-101 (24)^{a*}	-100 (24)^{a*}
S&B+No urea	1 (1)	9 (5)	110 (13)	123 (14)	-87 (62)^{a}	-85 (62)^{a}
Pine+No urea	1 (1)	9 (1)	75 (7)	94 (5)	-96 (50)^{a}	-94 (50)^{a}
Dairy+Urea	478 (10)	110 (24)	248 (10)	231 (14)	195 (55)^{a*}	283 (57)^{a*}
S&B+Urea	478 (10)	142 (26)	228 (8)	186 (9)	227 (77)^{a*}	257 (77)^{a*}
Pine+Urea	478 (10)	75 (8)	221 (2)	212 (5)	221 (19)^{a***}	263 (19)^{a**}
Dairy+AU	7543 (118)	2228 (182)	780 (35)	2449 (130)	5063 (94)^{b***}	2480 (94)^{b***}
S&B+AU	7543 (118)	2321 (262)	604 (30)	2008 (325)	4604 (423)^{b***}	2939 (423)^{b**}
Pine+AU	7543 (118)	734 (286)	1179 (98)	6053 (87)	7785 (35)^{c*}	-242 (35)^{a}
Dairy+CU	7561 (24)	2396 (955)	838 (20)	2097 (330)	4938 (773)^{b**}	2623 (773)^{b*}
S&B+CU	7561 (24)	3039 (155)	737 (7)	1638 (699)	5085 (672)^{b*}	2476 (672)^{b*}
Pine+CU	7561 (24)	1325 (239)	898 (23)	5575 (455)	7221 (178)^{c***}	340 (178)^{a}

Recovered-C was greater ($P < 0.001$) in the artificial and cow urine treatments (Table 4.3). The percentage of Recovered-C was also greater ($P < 0.01$) in the pine soil than the grazed soils in the artificial and cow urine treatments (Figure 4.17).

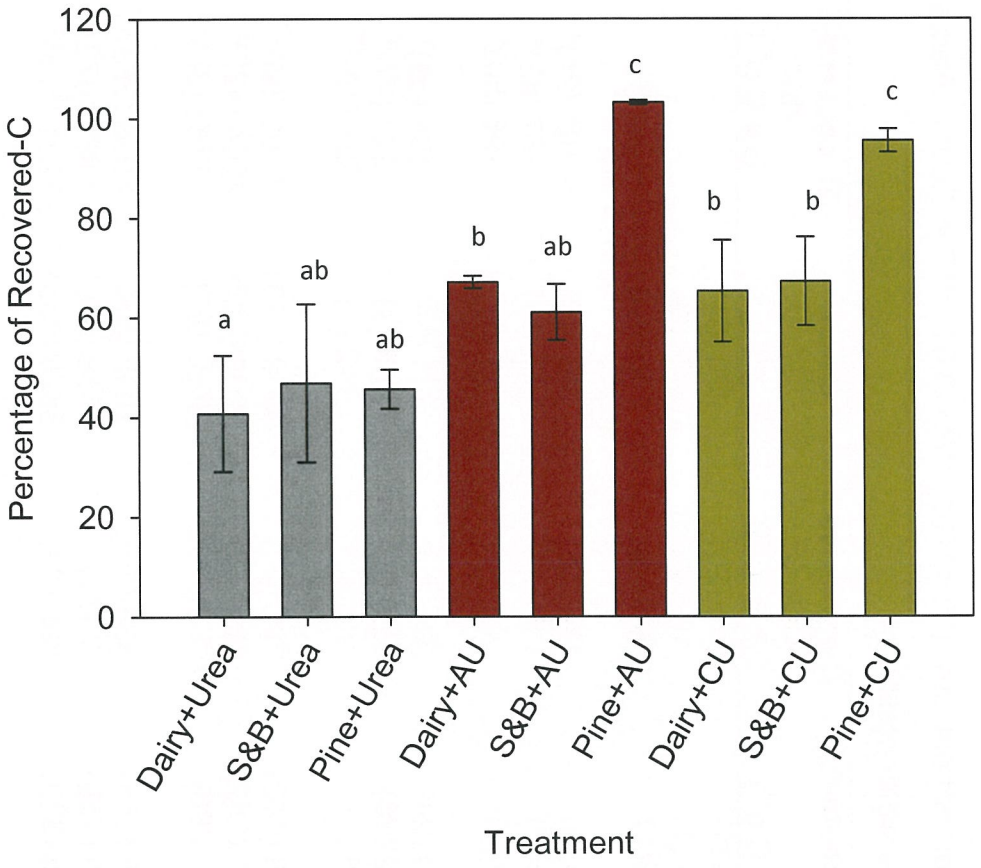

Figure 4.17: Percentage of Recovered-C in dairy-grazed (Dairy), sheep-and-beef-grazed (S&B), and *Pinus radiata* (Pine) soils applied with urea, artificial urine (AU) and cow urine (CU) solutions. Error bars represent one standard deviation; bars with different letters were significantly different ($P < 0.01$).

Soil C change (Solution-C minus Recovered-C) was either positive or negative. A positive C change indicated some of Solution-C in the soil was not extractable with cold water. A negative difference indicated a loss of soil C during the incubation with the added Solutions. The Dairy+No urea treatment was the only treatment to exhibit a significant ($P < 0.05$)

loss of soil C. Soil C increased ($P < 0.05$, $P < 0.01$) in all soils following treatment with urea solution. There was also an increase in soil C in the two grazed soils in both the artificial and cow urine treatments compared with the pine soils, but the change in C content of the pine soils was not significantly different to zero.

4.4 Discussion

A 14-day incubation was undertaken, with soil collected from 3 land uses, to investigate the fate of urine-C in soils and the effects of pH, EC, urea, artificial and cow urine solutions on soil C cycling.

4.4.1 Fate of urine carbon in soil

The fate of urine-C was influenced by leaching, sorption, and mineralisation.

There was greater leaching of Solution-C in the grazed soils (sheep-and-beef- and dairy-grazed soils) than the pine soil in the urea, artificial and cow urine treatments. Of the ~7550 μg g^{-1} of added C in the urine treatments, the C content of the leachate from the grazed soils was between 2000 and 3100 μg g^{-1}. Total C leaching under urine patches has been reported to be 20 g C m^{-2} $year^{-1}$ (Shepherd *et al.* 2010). The leaching of total C from the repacked cores in this chapter was 0.07-0.11 g C m^{-2} after a single urine application. The difference in leaching results between these two pieces of work was most likely due to preferential flow in the large undisturbed cores used by Shepherd *et al.* (2010). The repacked cores used in my work were designed to reduce heterogeneity between the cores, and would have been unlikely to exhibit macropore flow (Smith *et al.* 1985; Ghuman & Lal 1987). The undisturbed cores were also to a much greater depth (50 cm) than used in my work, and would have contained a greater amount of soil C, therefore there was more soil C available for leaching in the work presented by Shepherd *et al.* (2010).

Leachate analysis showed a greater retention of C, N, carbohydrates, and phenolics from added cow and artificial urine in the pine soils compared with the grazed soils. While the retention of Solution-C in the pine soils did not correspond to an increase in hot water soluble C (HWSC) the pine soils did have a greater cold water soluble C (CWSC) content. A greater recovery of added urine-C was also measured in the pine soils, where nearly 100% of the added urine-C was accounted for in respiration, CWSC and leachate C. Between 30-40% of the Retained-C in the grazed soils applied with urine was not extractable with cold water. Therefore, urine-C may have been irreversibly sorbed, possibly reducing its availability in the grazed soils. Sorption can influence a compound's distribution in soil and its availability for mineralisation and leaching (Scow *et al.* 1993). Also, an increase in soil C after both artificial and cow urine application was measured in the grazed soils, but not in the pine soil. No previous research on the sorption behaviour of urine-C has been undertaken, and further investigation is required.

The bioavailability of urine-C was estimated using the cumulative CO_2 fluxes during the incubation, however this approach assumes that all of the CO_2-C was derived from the added urine. Over the 14-day incubation, 12–17% of the cow urine-C was degraded. Although, the biodegradability of urine-C was likely overestimated due to the contribution of soil-C to CO_2-C fluxes, the data compared well with previous research showing the bioavailability of urine-C ranged between 7 and 25% over 28 days (Chapter 3).

4.4.2 Effect of urine on soil carbon cycling

Urine can increase the amount of soil C available for mineralisation either by acting as a salt solution or by rapidly increasing soil pH. The no urea treatment had no influence on CO_2 fluxes or CWSC compared with water controls. Of all the added Solutions, the no urea treatment was the only treatment that did not lead to an increase in soil pH. Therefore, as the no urea treatment had the same pH and EC as the cow urine, solution pH

and EC were not controlling factors in raising soil pH and were unlikely to cause soil C dissolution.

The rapid increases in soil pH following cow urine application were thought to be due to hydrolysis of urea (Doak 1952; Jackman 1960). However, the urea treatment only resulted in a small increase in soil pH (Figure 4.11) compared with the artificial and cow urine treatments. The rapid increase in soil pH is usually followed by a decrease in soil pH, as the ammonium produced from urea hydrolysis undergoes nitrification (Black 1992; Haynes & Williams, 1993; Condon *et al.* 2004). It was not possible to determine whether the soil pH was still increasing or was decreasing when measured. However, pure urea solutions degrade slower and to a lesser extent than urea applied in urine (Doak 1952; Sherlock & Goh 1984), and it is possible that the soil pH in the urea treatment would have continued to increase had the incubation continued. The inclusion of hippuric acid in urea solutions has been shown to increase soil pH above that of urea alone solutions (Whitehead *et al.* 1989). Hippuric acid in the cow urine may have aided the increase in soil pH, however, hippuric acid was not included in the artificial urine and would only have been a factor in raising the soil pH in the artificial urine treatment if the glycine was converted to hippuric acid in the soil.

Increases in soil pH can lead to soil organic matter dissolution (Haynes & Williams 1992; Lovell & Jarvis 1996; Curtin *et al.* 1998; Shand *et al.* 2000). Dissolution of soil C may explain the positive correlation between post-incubation soil pH and CWSC. However, a portion of the CWSC would have been derived from added Solution-C. Due to the inability to distinguish soil C from added Solution-C, quantification of soil C dissolution due to pH increases could not be determined.

Priming of soil C was not measured in any of the treatments. Priming may have been hidden by the inability to distinguish CO_2-C fluxes derived from soil C and Solution-C. It may also have been inhibited by adsorption of Solution-C in the grazed soils. Cow urine was the only Solution to contain

phenolics; however, all the treatments contained water soluble phenolics at the termination of the incubation. SOM contains phenolics (Martens 2002) and their presence in water soluble form after the incubation infers that SOM may have been degraded during the incubation. Greater cold water soluble phenolics in the cow urine treatment may be due to incomplete degradation of urine derived phenolics, particularly in the pine soil, which retained greater proportions of the added phenolics.

4.4.3 Artificial urine

The artificial urine had the same cumulative CO_2-C as the cow urine treatment for all land uses (Figure 4.15; $P < 0.001$), which is contrary to Lovell and Jarvis (1996) and Kool *et al.* (2006), who found a lesser carbon dioxide flux from artificial urine than cow urine. The proportion of Retained-C that was respired (Figure 4.17) was the same in both urine treatments, but was significantly greater in the urea treatment. It was possible that the lesser amount of C added in the urea treatment meant the microbial population was able to use a greater proportion of the added C over the incubation period, but were not able to mineralise as much of the urine-C due to the much greater amounts of C added.

There was no difference in cumulative CO_2-C for the water, no urea, and urea treatments, but the cow and artificial urine treatments had greater respiration. It is therefore apparent that urea was not a major controlling factor in CO_2 evolution following cow urine application. This is an important finding for N cycling research, given that urea solutions are often used instead of a more comprehensive artificial urine or cow urine.

4.5 Summary and conclusions

The objective of this experiment was to determine the fate of cow urine-C and the effects of urine on soil C. Soil from three land uses (dairy-grazed, sheep-and-beef-grazed, and pine plantation) were compared and the

effects of pH, EC, urea content, artificial urine and cow urine on C cycling were investigated.

The main findings were:

- the pine soil retained more urine-C and adsorption may have impeded bioavailability in the grazed soils
- the pH or EC of the solutions did not increase soil pH;
- urea was not solely responsible for increases in soil pH and CO_2-C evolution under urine patches;
- although no priming was measured, it could not ruled out due to the inability to separate soil C from urine-C.

For the most part, the sheep-and-beef-grazed and the dairy-grazed soils responded similarly to the treatment Solutions. Therefore, it is recommended that, due to the similarities between the two grazed land uses, and the difficulty in locating one soil under three different land uses, future work use only paired grazed pasture and pine sites.

The results presented here indicated several aspects that require further investigation. Firstly, sorption may influence the fate and availability of urine-C in soil. Secondly, the mass balance approach used here did not adequately elucidate if the application of cow urine led to dissolution or priming of soil C. Further work using radio-labelled techniques may aid clarification.

4.6 References

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Chapter 5

Research paper

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Title: Solubilisation of soil carbon following treatment with cow urine under laboratory conditions

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5.1 Abstract

There have been reported losses of soil C under intensively grazed pastures and soil C solubilisation following cow urine deposition was identified as a possible mechanism. We measured potential soil C solubilisation in pasture and plantation pine soils following treatment with cow urine. Soils from 5 paired pasture and pine sites were collected. Adsorption of urine-C and desorption of soil C was determined by shaking air-dried soil with cow urine for 4 hours at 4°C, decanting the urine and then extracting the soil with water. Soil C solubilisation was the difference between adsorption of urine-C and desorption of soil C. Solubilisation of soil C in the pine soils including the organic layers was 21.6±2.6 mg g^{-1} (10.5±1.1% of soil C concentration), in the pine soils excluding the organic layers was 7.5±2.2 mg g^{-1} (18.7±5.8%), and 12.4±5.3 mg g^{-1} (27.8±7.3%) was solubilised in the pasture soils. There was no significant difference with respect to soil C solubilisation between the pine (with and without organic layers) and pasture soils. Soil C lower in the profile may be as susceptible to solubilisation as top soils. Adsorption of urine-C was minimal. Solubilisation of soil C under urine patches may contribute to losses of soil C under intensively grazed pastures and this hypothesis would benefit from further testing under field conditions.

Keywords: Adsorption; desorption; carbon; pasture; *Pinus radiata* plantation

5.2 Introduction

Soil organic matter is an integral part of soil quality, influencing water and nutrient retention, aggregate stability, and compaction resistance (Lal 2004; Haynes 2005). Soil carbon (C) is an integral component of soil organic matter and its maintenance is a key factor for sustainable land use (Lal 2004; Haynes 2005). In recent years, intensification of New Zealand's dairy pastures has led to increased nitrogen inputs and cow stocking rates (MacLeod & Moller 2006; Ministry for the Environment 2007), also a decline in soil C has been measured in some dairy pastures (Schipper *et al.* 2007, 2010). The losses of soil C averaged 0.73±0.16 Mg C ha^{-1} $year^{-1}$ in the top 30 cm of dairy pasture (Schipper *et al.* 2010) and the cause of the C losses was unclear. In contrast, the soil C content of dry stock pastures had not changed over the same time period (Schipper *et al.* 2010).

A key difference between dry stock and dairy farming is greater deposition of urine from dairy cows in comparison to both sheep and dry stock cattle (Bilotta *et al.* 2007; Haynes & Williams 1993). Urine is alkaline and has a high salt content (Lantinga *et al.* 1987; Haynes & Williams 1993), both of which may increase the solubility of soil C (Reemtsma *et al.* 1999). Also, urea hydrolysis in urine patches leads to localised increases in soil pH and solubilisation of soil C (Jackman 1960; Lovell & Jarvis 1996; Shand *et al.* 2002). Soil C solubilisation is the process whereby soil C is dissolved into solution, and may enhance soil C decomposition.

Adsorption occurs when molecules from a solution are attracted onto another substance, for example soil; desorption is the opposite process (Ferrante 1996). Adsorption may determine the fate of urine-C in soils, while urea and dissolved organic C (DOC) have both been shown to adsorb to soil (e.g., Broadbent *et al.* 1958; Overrein & Moe 1967; Jardine *et al.* 1989; Liang *et al.* 1996; Kaiser *et al.* 2000; Kalbitz *et al.* 2005). Adsorption is the major process in the retention of dissolved organic C in soils (Nodvin *et al.* 1986; Jardine *et al.* 1989; Kaiser & Guggenberger

2000; Kothawala *et al.* 2008), although the adsorption of urine-C had yet to be tested. Clays have an important role in the storage of organic matter (Torn *et al.* 1997), and can play a key role in the adsorption of DOC (Jardine *et al.* 1989; Kaiser & Zech 2000). While clay minerals may adsorb DOC, Uchida *et al.* (2008) suggest that urine deposition may lead to soil disaggregation, which could release soil C not previously available (Gregorich *et al.* 1989; Chandra *et al.* 2002). The influence of soil clay content on urine-C adsorption and soil C solubilisation had not previously been studied.

Recently, there has been considerable conversion of forestry to intensive grazing in New Zealand, with an estimated 15 600 hectares undergoing conversion in the year ending March 2008 (Ministry of Agriculture and Forestry 2009). The response of recently converted pine-to-pasture soils to urine deposition had not yet been examined. We hypothesise that pine soils would undergo greater soil C solubilisation following urine deposition than pasture soils, as pasture soils would have come into contact with urine before and soil C readily soluble by urine would have already been removed.

There were three objectives of this work, firstly to determine the potential solubilisation of soil C following treatment with cow urine. Secondly, to examine if soil C from plantation forestry is more susceptible to solubilisation by urine than soil C from long term grazed pasture soils. Thirdly, we assessed the role of soil clay content on urine-C adsorption and soil C solubilisation.

5.3 Materials and methods

5.3.1 Soils

Five different soils from the North Island, New Zealand, were collected from paired *Pinus radiata* plantations and adjacent grazed pastures (Table 5.1, 5.2). The trees on the pine sites were greater than 20 years old and

closed canopy had been reached. Pastures had been grazed for at least 10 years.

A 30-m transect was laid randomly across each sampling site, and a 2.5-cm diameter core was taken every 2 m along each transect and bulked for each sampling depth. The mineral soil was collected in increments of 0–50 mm, 50–100 mm, and 100–200 mm from the top of the mineral soil profile. The organic layers from the pine soils (litter and humus (FH)) were retained as separate samples. All soil samples were air-dried at 35°C (±1°C), sieved to 2 mm (with the exception of the litter layer), and any roots or stones removed. A 5.6-cm diameter core was taken for bulk density at 10 metres along the transect. The bulk density of the soil was calculated by dividing the oven dry weight of the soil by the volume collected.

The soil layers were analysed for total C and nitrogen (N) (LECO FP-2000 CNS Analyser, LECO Corp., St Joseph, MI, USA), and water soluble C (Ghani *et al.* 2003). Soil pH was measured in a 1:2.5 soil-to-water slurry, and soil electrical conductivity was measured against a 2 *M* potassium chloride standard (Blakemore *et al.* 1987). The clay content of each soil layer was determined using the pipette method, following dispersion and sieving (Claydon 1989).

5.3.2 Urine

Urine was collected from several (c. 5) grass-fed Friesian cows waiting for 3 pm milking in September 2007 (Dairy 1, Massey University, Palmerton North, New Zealand) and bulked as collected. The total C content of the urine was measured immediately after collection (Win High TOC II; Elementar Analysensysteme GMbH, Hanau, Germany) and the pH of the urine was measured using a glass electrode. The remaining urine was frozen at –20°C until required.

Table 5.1: Location, map position, name and classification (Hewitt 1998) of soils collected from pine and pasture land uses

Location	Map Position (NZTM)	Soil Name	New Zealand Soil Classification
Maramarua	1800035E 5867423N	Maramarua clay loam	Typic Yellow Ultic Soil
Turangi	1841564E 5666788N	Rangipo sandy loam	Podzolic Orthic Pumice Soil
Waitarere Beach	1787675E 5510506N	Motuiti brown sand	Typic Sandy Brown Soil
Palmerston North	1820997E 5524533N	Tokomaru silt loam	Argillic-fragic Perch-gley Pallic Soil
Ngaumu	1844225E 5452840N	Ngaumu silt loam	Mottled Orthic Brown Soil

Table 5.2: Total N, total C, water soluble C (WSC), clay content, pH and bulk density of soil layers collected from five paired pine and pasture sites. All analyses were undertaken using one subsample from each layer, with the exception of WSC, which was extracted in triplicate for each layer.

Soil Layer	Soil Name	Total N (%)		Total C (%)		WSC (mg kg^{-1})		Clay content (%)		pH		Bulk density g cm^{-3}	
		Pine	*Past*	*Pine*	*Past*	*Pine*	*Past*	*Pine*	*Past*	*Pine*	*Past*	*Pine*	*Past*
Litter	Maramarua	0.6	-	52.5	-	3280	-	-	-	-	-	-	-
	Rangipo	0.6	-	57.0	-	3100	-	-	-	-	-	-	-
	Motuiti	0.8	-	53.7	-	1910	-	-	-	-	-	-	-
	Tokomaru	0.8	-	53.4	-	1450	-	-	-	-	-	-	-
	Ngaumu	0.8	-	53.3	-	2810	-	-	-	-	-	-	-
FH*	Maramarua	1.1	-	27.6	-	560	-	-	-	4.9	-	-	-
	Rangipo	1.7	-	40.5	-	460	-	-	-	4.5	-	-	-
	Motuiti	1.5	-	48.8	-	5340	-	-	-	4.5	-	-	-
	Tokomaru	1.6	-	40.3	-	3110	-	-	-	4.8	-	-	-
	Ngaumu	1.6	-	38.4	-	1890	-	-	-	4.5	-	-	-

0–50 mm	Maramarua	0.2	0.4	5.9	5.2	430	140	31	25	4.8	6.1	0.89	0.89
	Rangipo	0.3	0.7	5.1	10.2	70	60	6	6	5.4	5.9	0.60	0.47
	Motuiti	0.3	0.5	5.2	5.1	380	70	5	9	4.5	5.4	0.84	1.02
	Tokomaru	0.4	0.5	5.8	4.8	250	140	17	23	5.0	6.5	0.79	0.74
	Ngaumu	0.4	0.4	6.7	5.1	210	300	22	26	4.8	6.3	0.63	0.74
50–100 mm	Maramarua	0.2	0.3	3.7	3.5	280	240	36	28	4.7	6	1.19	1.16
	Rangipo	0.3	0.5	4.6	6.9	30	60	8	6	5.5	6.1	0.72	0.91
	Motuiti	0.2	0.3	2.9	2.8	190	60	5	9	4.9	5.5	1.23	1.23
	Tokomaru	0.3	0.3	3	3.4	160	110	20	22	4.9	6.1	1.16	1.11
	Ngaumu	0.3	0.4	4.8	4.2	140	240	27	24	4.9	6.4	0.94	0.99
100–200 mm	Maramarua	0.1	0.1	1.5	1.6	200	330	37	33	4.8	5.4	1.22	0.66
	Rangipo	0.2	0.3	3.2	4.4	10	70	7	7	6.1	6.1	0.83	0.85
	Motuiti	0.1	0.1	1.5	1.6	130	40	4	9	5.1	5.7	0.99	1.33
	Tokomaru	0.2	0.3	2.1	2.1	130	90	23	22	5	5.7	1.16	1.13
	Ngaumu	0.2	0.2	3	2.8	110	260	27	24	5.2	6.6	1.15	1.15

* Fresh humus

5.3.3 *Adsorption and desorption*

To determine the amount of soil C solubilised following mixing with cow urine, both adsorption of urine-C and desorption of soil C were measured. The 40 soil samples, in triplicate, were shaken with urine or water at 4°C to inhibit microbial activity (Liang *et al.* 1996). Air-dry soil (2.5 g) was shaken with urine (0.025 L) or water (0.025 L) as a control, at 50 rpm for 4 hours. A pilot study determined a shaking time of 4 hours (4°C) was required for the soil C and urine-C concentrations to reach equilibrium as determined by a constant C concentration measured in the liquid phase following centrifugation and filtration (Figure 5.1). The soil:urine slurry was then centrifuged for 20 minutes at 2500 rpm and the supernatant filtered through GF/F filter papers (0.45 µm) under suction (Labserv, BioLab Limited, Auckland, New Zealand). The supernatant was analysed for total C (Win High TOC II, Elementar Analysensysteme GMbH, Hanau, Germany). Adsorption of urine-C (mg C g^{-1}) was calculated using equation 1:

$$\text{Adsorption} = \frac{(\alpha - \beta) * V}{DW} \quad (1)$$

Where:

- α = C concentration (mg L^{-1}) in urine
- β = C concentration (mg L^{-1}) in urine following shaking with soil
- V = Volume of urine (0.025 L)
- DW = dry weight of the soil at 105°C (g)

The mean adsorption (mg C g^{-1}) of urine-C on each soil sample was corrected for the mean adsorption of the water controls. A positive number indicated C had been removed from the urine and net adsorption had occurred. A negative number indicated that the C concentration of the urine had increased during shaking due to net desorption of soil C.

Total adsorption of urine-C was calculated for each soil by summing the amount of urine-C adsorbed (mg C) from each layer (water corrected) divided by the sum weight of soil shaken with urine. Total adsorption for each soil in each land use (n = 5) was then averaged for the pine soils including organic layers (Total Pine Litter–200 mm), pine soils excluding the organic layers (Total Pine 0–200 mm) and the pasture soils (Total Pasture 0–200 mm).

After measurement of urine-C adsorption, the soil was refrigerated overnight and then extracted three times with water (2.5 g soil:25 mL water) for 4 hours (4°C). The C content of the supernatant was measured following centrifugation and filtration as for urine-C adsorption measurement, and the extracted C (mg g^{-1}) was calculated using Equation 2:

$$\text{Extraction} = \frac{(\epsilon - \lambda)^{*}V}{DW} \qquad (2)$$

Where:

- ϵ = C concentration (mg L^{-1}) in water after shaking with soil that had been previously shaken with urine
- λ = C concentration (mg L^{-1}) in water
- V = volume of water (0.025 L)
- DW = dry weight of soil 105°C (g)

Desorption (mg C g^{-1}) was calculated as the sum of the three water extractions (Equation 2). Mean desorption of soil C previously shaken with urine was corrected by the mean desorption of soil C previously shaken with water only.

Total desorption was calculated for each soil profile by summing desorption (water corrected) of soil C (mg C) from each layer divided by the sum weight of the soil shaken with urine. Total desorption was averaged within each land use (n = 5), for the pine soils with organic

layers included (Total Pine Litter–200 mm), pine soils without the organic layers (Total Pine 0–200 mm) and the pasture soils (Total Pasture 0–200 mm).

5.3.4 Soil C solubilisation

The potential solubilisation of soil C (mg g^{-1} soil) for each soil layer was calculated by subtracting the amount of urine-C that was adsorbed (water corrected) from the amount of soil C that was desorbed (water corrected); a positive result indicated that soil C had been released into solution. Solubilisation of soil C as a percentage of the soil's initial total C concentration was also calculated.

Total soil C solubilisation in each soil profile was calculated by subtracting adsorption (water corrected) of urine-C (mg C) from desorption (water corrected) of soil C (mg C) for each layer, then summing the mass solubilisation of soil C and dividing by the summed weight of soil shaken. The average solubilisation of soil C from each land use (n = 5) was calculated for the pasture soils and for the pine soils with and without the organic layers. Total soil C solubilisation was calculated as a percentage of the initial soil C content for each soil averaged within each land use, and also presented for the pine soils with and without the inclusion of the organic layers.

5.3.5 Statistical Analyses

All statistical analyses were undertaken in Genstat 12. Adsorption, desorption and soil C solubilisation were considered to have occurred for each soil layer if significantly different from zero ($P < 0.05$) as tested by one sample, two tailed, T-tests of the water-corrected means for each soil layer collected.

To determine if there were differences in adsorption of urine-C, desorption of soil C and soil C solubilisation between the pine, with and without

organic layers, and pasture soils, the data sets were assessed using ANOVA with Student-Newman-Kuels analysis. There were considered to be differences between the pine and pasture soils if $P < 0.05$.

Regression analysis was used to test correlations between adsorption, desorption, and soil C solubilisation to soil depth. The organic layers from the pine soils were designated nominal depth titles: –100 mm for the litter layers, and –50 mm for the FH layers. Regression analysis was also used to determine correlations between soil characteristics (soil C concentration and soil clay content) and adsorption, desorption, and soil C solubilisation.

5.4 Results

All data are presented as means with standard errors, unless otherwise stated. The organic C content of the cow urine was 14813±704 mg L^{-1}, the inorganic C content was 1100±49 mg L^{-1}, and the urine had a pH of 8.3. The kinetics pilot study found that 4 hours shaking time was sufficient for this experiment (Figure 5.1).

The water controls solubilised small amounts of soil C during the adsorption and desorption phases for all the soil layers (n = 40). A small amount of soil C (1.1±0.2 mg C g^{-1}) was extracted during the adsorption phase, and a further 2.5±0.6 mg C g^{-1} was extracted during the desorption phase. Total solubilisation of soil C was 3.6±0.4 mg C g^{-1} in the water controls, which was used to correct solubilisation in the urine treatments.

Adsorption of urine-C was measured in the litter, FH, and pine 50–100 mm layers, but there was no adsorption on any of the pasture soil layers (Table 5.3). There was also no difference in total adsorption between the pine and pasture soils, regardless of whether organic layers were included in the pine soils. The mean C concentration of the urine was 16438±659 mg C L^{-1} (n = 19). The mean adsorption of urine-C for all of the soil layers sampled (n = 40) was 2.9±0.4% of urine-C content. Urine-C adsorption

showed a weak positive relationship to soil C content ($r^2 = 0.11$; $P < 0.05$), but was not correlated to depth or soil clay content.

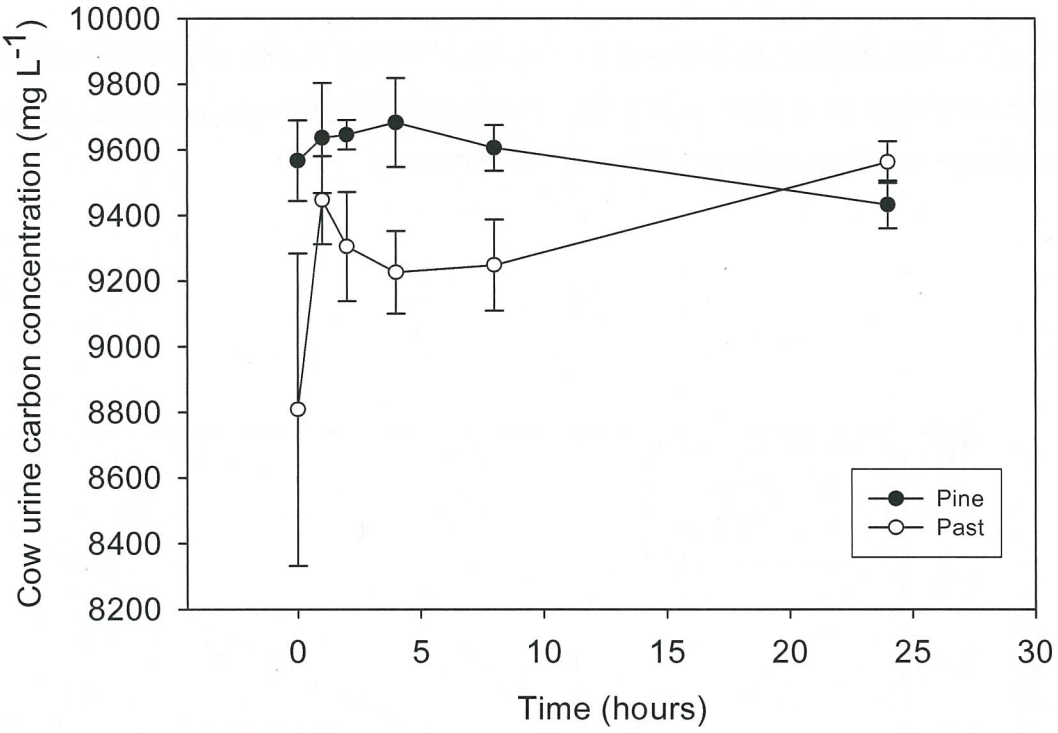

Figure 5.1: Carbon concentration of urine shaken with Maramarua pine and pasture soil over a 24 hour period. Error bars represent standard error.

Following shaking with urine, desorption of soil C occurred in every soil layer tested (Table 5.3). There was greater total desorption ($P < 0.05$) of soil C in the pine soils when organic layers were included, than the pine soils excluding the organic layers or the pasture soils (Table 5.3). Desorption of soil C decreased with increasing depth ($r^2 = 0.52$; $P < 0.001$), and increased with increasing soil C concentration ($r^2 = 0.67$; $P < 0.001$). There was no correlation between soil clay content and soil C desorption.

The potential solubilisation of soil C was estimated by subtracting the mass of soil C desorbed from the mass of urine-C adsorbed. All the soil

layers exhibited significant solubilisation of soil C, except the pine 100–200 mm layer (Table 5.3). Total solubilisation was not significantly different between the pine soils (with and without the organic layers) and the pasture soils (Table 5.3).

Soil C solubilisation decreased ($r^2 = 0.48$; $P < 0.001$) with depth and soil C concentration ($r^2 = 0.61$; $P < 0.001$; Figure 5.2). There was no relationship between soil clay content and soil C solubilisation.

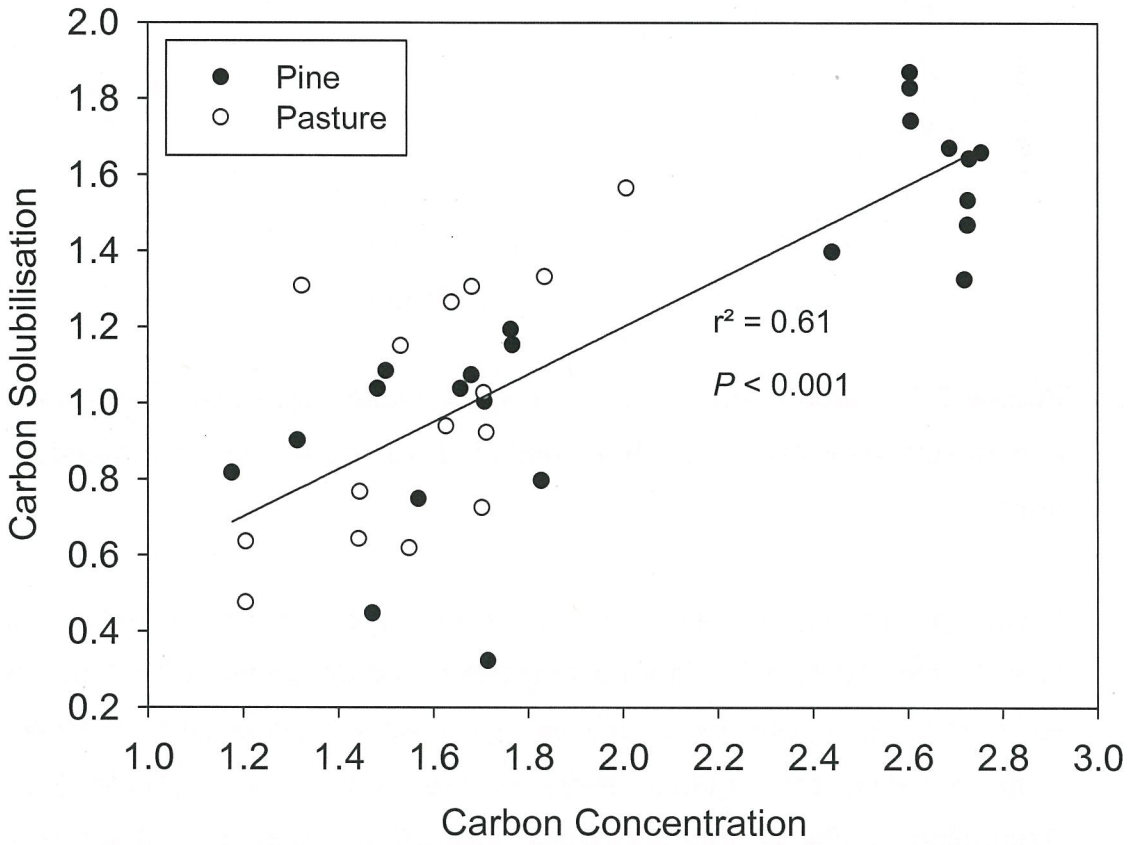

Figure 5.2: Correlation between the soil C concentration (log_{10}) and solubilisation (log_{10}) for five pine and pasture soils to a depth of 200 mm (n = 40).

Table 5.3: Adsorption of urine-C, desorption of soil C, soil C solubilisation for soil layers shaken for 4 hours (4°C) with cow urine. All data have been corrected for water controls. Numbers in brackets represent standard error (n = 5). Values with a star were significantly different to zero, and values in each column with different letters were significantly different ($P < 0.05$)

Soil Layer	Adsorption (mg C g^{-1})	Desorption (mg C g^{-1})	Soil C solubilisation (mg C g^{-1})	Soil C solubilisation (%)
Litter	5.5 (1.5)*	40.3 (5.8)*	34.8 (4.6)*	6.4 (0.8)*
Fresh humus	8.5 (2.0)*	62.1 (7.6)*	53.6 (8.6)*	13.5 (1.9)*
Pine 0–50 mm	4.8 (2.6)	14.4 (1.9)*	9.6 (2.5)*	16.8 (4.4)*
Pine 50–100 mm	3.9 (0.7)*	11.7 (1.9)*	7.8 (2.3)*	19.8 (6.0)*
Pine 100–200 mm	4.8 (1.8)	9.9 (1.9)*	5.1 (2.7)	20.9 (13.0)
Total: Pine Litter-200 mm	**5.4 (1.3)*[a]**	**27.1 (2.8)*[a]**	**21.6 (2.6)*[a]**	**10.5 (1.1)*[a]**
Total: Pine 0–200 mm	**4.4 (1.5)*[a]**	**12.0 (1.8)*[b]**	**7.5 (2.2)*[a]**	**18.7 (5.8)*[a]**
Pasture 0–50 mm	0.9 (1.9)	17.2 (4.7)*	16.2 (5.7)*	25.1 (6.0)*
Pasture 50–100 mm	3.0 (1.3)	13.4 (2.3)*	10.5 (3.3)*	24.1 (5.4)*
Pasture 100–200 mm	1.5 (2.6)	11.9 (2.4)*	10.4 (3.7)*	40.1 (14.4)*
Total: Pasture 0–200 mm	**1.9 (1.8)[a]**	**14.2 (4.0)*[b]**	**12.4 (5.3)*[a]**	**27.8 (7.3)*[a]**

All the soil layers, except the pine 100–200 mm layer, lost a significant proportion of their soil C to solubilisation following shaking with urine (Table 5.3). The total proportion of soil C solubilised was not significantly different from the pine soils, with or without the inclusion of the organic layers, and the pasture soils (Table 5.3). The proportion of soil C solubilisation increased with increasing depth (r^2 = 0.17; P < 0.01; Figure 5.3). The proportion of soil C solubilised decreased with increasing soil C concentration (r^2 = 0.13; P < 0.05). There was no relationship between the proportion of soil C solubilised and soil clay content.

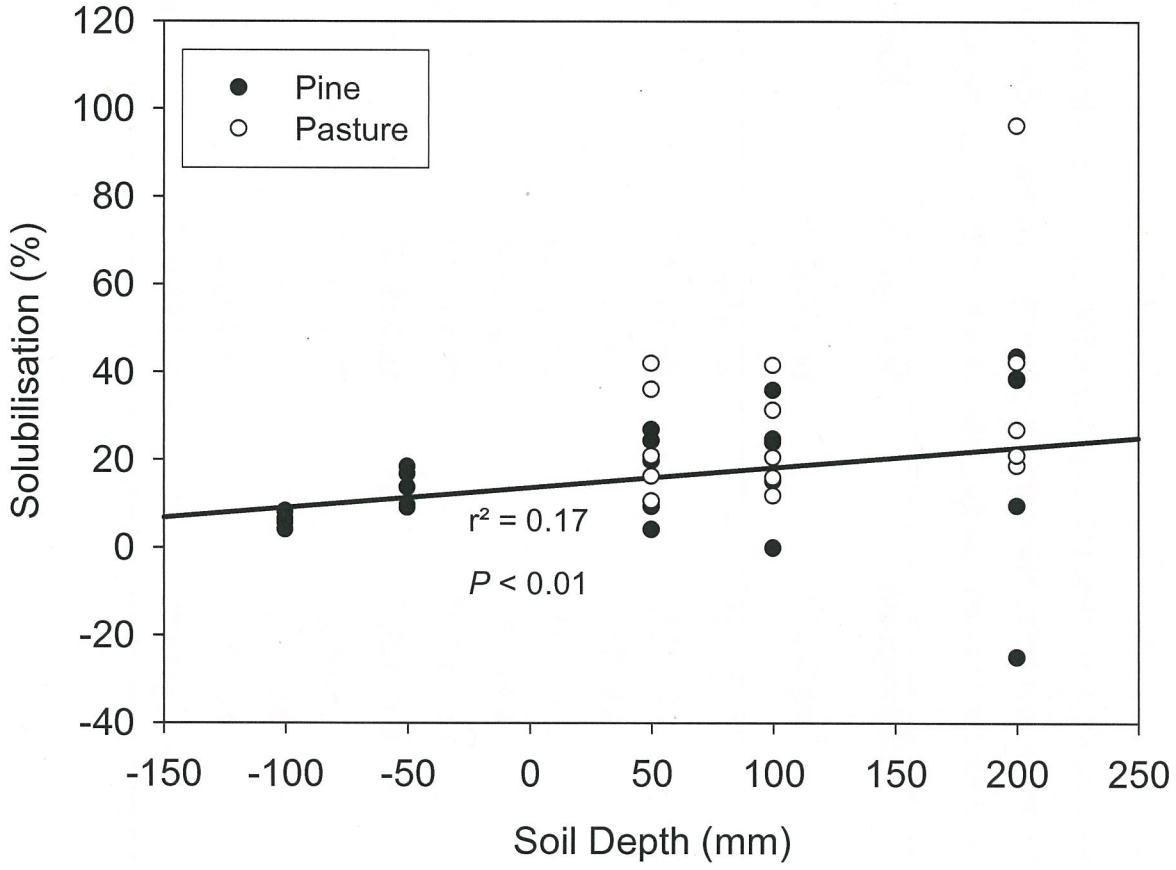

Figure 5.3: Proportion of soil C solubilisation down the profile from five paired pine and pasture soils to 200 mm. Litter and FH layers in the pine soils were designated –100 and –50 mm titles respectively (n = 40).

5.5 Discussion

Little is known about the fate of urine-C in soils. However, the small amount of adsorption measured here (about 3% of the urine's C concentration) indicated urine-C remained predominantly in solution. There are several mechanisms that can contribute to DOC adsorption in soil including binding to iron and aluminium oxides (Jardine *et al.* 1989; Kaiser & Zech 2000), cation bridging (Greenland 1971; Randtke & Jepsen 1982) and retention by clays (Jardine *et al.* 1989; Riffaldi *et al.* 1998; Kaiser & Zech 2000).

While adsorption of DOC can be impeded by native soil organic matter (Jardine *et al.* 1989; Kaiser & Zech 1998), in this study there was a weak correlation between urine-C adsorption and soil C content (data not shown). There was no correlation between urine-C adsorption and clay content, despite there being a wide range of clay contents (Table 5.2) in the soils tested (results not shown). Adsorption of urine-C to clay may have been impeded by the high pH of the soil following treatment with cow urine. For example, Specht *et al.* (2000) reported reduced adsorption of organic material on clays with increasing pH. The composition of urine-C may also have inhibited the adsorption to clays. Rennert and Mansfeldt (2003) found the composition of DOC was more important in determining adsorption to clay and silt fractions than the soil mineral composition. Specht *et al.* (2000) also found large molecules in organic material were adsorbed whereas smaller compounds with aromatic or carboxylic functional groups were not. The carboxylic acid hippuric acid, makes up nearly 50% of urine-C (Chapter 3), and may have resulted in lower overall adsorption.

Soil C desorption was measured in all soil layers following shaking with cow urine. Total desorption of soil C was greater ($P < 0.05$) from the pine soils including the organic layers, than the pine soils without the organic layers and the pasture soils. When the organic layers were not included, the total desorption of soil C was the same in the pine and pasture soils.

The mass of soil C desorbed was much greater than the mass of urine-C adsorbed, therefore the soils exhibited substantial solubilisation of soil C in nearly all the soil

layers tested. There was, however, no relationship between clay content and soil C solubilisation, indicating that disaggregation may not have occurred in our experiment, but further testing is necessary to confirm this. The solubilisation of soil C in the water controls (3.6±0.4 mg g^{-1}) was relatively insignificant compared to the urine treatments. The solubilisation of soil C in our experiment was a worst case scenario, and the soil C solubilisation measured here is likely to be greater than would occur in the field. Air-drying soils and shaking during experimentation can increase the amount of DOC released from soils (Kaiser *et al.* 2001; Limousin *et al.* 2007), and may have increased soil C extraction from the soils tested here. However, the effects of air-drying and shaking with water did not lead to a substantial release of soil C, as shown in the water controls.

Adsorption may have been overestimated due to a loss of urine-C by bicarbonate degassing of inorganic C added in the urine. Although bicarbonate can be converted to carbon dioxide (CO_2) by chemical reactions in acidic soils (Zabowski & Sletten 1991), the addition of urine to soils rapidly increases soil pH and can cause an acidic soil to become temporarily alkaline (Haynes & Williams 1992). There was a decrease of 13% of inorganic C following shaking with soil (Data not shown; $P < 0.05$). As the pH of the solutions or soil was not measured during the experiment, we cannot confirm whether the loss of inorganic C was due to adsorption or degassing of bicarbonate to CO_2. The inorganic C content of the urine was on average 7% of the total C content, the effect of the decrease of inorganic C on the total C concentration in the estimation of urine-C adsorption in the adsorption experiment was minimal (0.9%of total C). Adsorption may also have been overestimated due to microbial immobilisation of the urine-C, however, no published literature assessing microbial immobilisation of urine-C, or added dissolved organic C, could be located. We did not measure microbial biomass C as a part of this experiment, but future work would assessing the fate of urine-C in soils would benefit from investigating microbial immobilisation.

There was no significant soil C solubilisation in the pine 100–200 mm layer following treatment with urine. Jandel and Sollins (1997) measured a decrease in water soluble C in a forest soil at the same soil depth. While the reasons for the decrease in solubilisation at this depth in forest soil is not known, it is possible that

compositional changes in soil C down the profile (Beyer *et al.* 1993; Kogel-Knabner *et al.* 1991) may lead to soil C that is more resistant to solubilisation in this soil layer.

Our original hypothesis was that less soil C would be solubilised in the pasture soils than the pine soils because the soil C that was easily extracted by urine in pasture soils would already have been lost due to previous urine deposition. However, there was no significant difference in total solubilisation between the pine and pasture soils. This suggests that although the pasture soils would have been exposed to urine previously, the amount of soil C extracted by urine may have been replenished with time, so that soil C remained susceptible to further solubilisation by subsequent urine deposition.

The proportion of soil C solubilisation increased with depth, suggesting that soil C throughout the profile was susceptible to dissolution following urine deposition. Cow urine can travel by macropore flow to as deep as 400 mm in the profile (Williams & Haynes 1994) and 15–25% of applied urine has reached greater than 150 mm below the soil surface (Haynes & Williams 1992), with a mean of 17% below 200 mm soil depth (Monaghan *et al.* 1999). In some soils, therefore, soil C lower in the profile is likely to be in direct contact with urine transported by macropore flow. There is some evidence that soil C below 200 mm in the profile is susceptible to mineralisation once released into solution (Schimel *et al.* 2011) and losses of soil C have been reported below 300 mm in the profile under intensive dairy management (Schipper *et al.* 2007, 2010). Cow urine may have contributed to this solubilisation.

Two main factors may regulate soil C solubilisation following urine deposition: rapid increases in soil pH (Jackman 1960; Haynes & Williams 1992; Lovell & Jarvis 1996; Curtin *et al.* 1998; Shand *et al.* 2002; Shand & Coutts 2006), and aggregate disruption (Uchida *et al.* 2008), which releases previously protected soil C (Gregorich *et al.* 1989; Chandra *et al.* 2002). The relative contribution of these mechanisms to soil C solubilisation requires further research.

The fate of dissolved soil C released after cow urine deposition is unclear. The released C may be re-adsorbed lower in the profile, leached, or mineralised by microbes. Guggenberger and Zech (1992) found that although there was significant

desorption of soil C following the application of DOC to topsoils, DOC was adsorbed in the B horizons. The adsorption capacity of layers lower in the profile would have to be large to adsorb the amounts of soil C released from upper horizons measured here, particularly in the pine soils. Further research is needed to determine if soil C released in the upper soil horizons following urine deposition is adsorbed lower in the profile.

The release of previously inaccessible soil C by solubilisation following urine deposition may enhance mineralisation rates (Chandra *et al.* 2002). Solubilisation could release soil C with a greater degradability and could potentially lead to priming of soil C lower in the profile. Priming occurs when a C source is added to a soil which enhances the mineralisation of soil C. However, the re-adsorption of solubilised soil C lower in the profile may decrease the pools of soil C available for mineralisation (Scow *et al.* 1993). Leaching of soil C from grazed pastures has been previously reported (Parfitt *et al.* 2009, Shepherd *et al.* 2010), but the extent to which solubilisation of organic matter in urine patches contributed to these leaching losses was not determined.

5.5 Conclusions

Solubilisation of soil C occurred in nearly all the soil layers investigated after mixing with urine. Soil C solubilisation and the proportion of soil C solubilisation were not significantly different between the pine and pasture soils, whether the organic layers were included in the pine soils or not. The proportion of soil C solubilisation increased with depth, indicating that soil C at depth may still be susceptible to mobilisation following urine deposition. Clay content was not a factor in either urine-C adsorption or soil C solubilisation in the soil layers examined. While the fate of solubilised soil C is not clear, it is likely to be readily available for mineralisation.

While this laboratory experiment requires testing in the field, there are important implications for the sustainability of farming the pasture soils investigated here. Soil C in both newly converted and long-term pasture soils may be solubilised following urine deposition and subsequently either leached or mineralised. Unless the losses of soil C are matched by C inputs from root turnover, litter or dung, an overall loss of

soil C in grazed soils is likely. Therefore, dissolution of soil C following urine deposition could partly explain the soil C losses reported by Schipper *et al.* (2007, 2010). Further work is required to determine if soil C solubilisation following urine deposition occurs under field conditions, and to establish the mechanisms of any soil C loss following cow urine application.

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Chapter 6

Research paper

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Title: Priming of carbon in grazed pasture and *Pinus radiata* plantation soils following treatment with cow and artificial urine.

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6.1 Abstract

Declines in soil carbon (C) have been reported in New Zealand dairy pastures, and may be attributed to increased cow urine application. Concurrently, there has been substantial conversion from plantation forestry to grazing, and the impact of cow urine on soil C cycling in converted soils is unknown. The main objective of our work was to quantify priming of soil C in pine and pastures soils following urine deposition. Further, we assessed soil C solubilisation and extracellular enzyme activity as contributors to priming events. We also investigated artificial urine as a model for cow urine. Cow urine, water, ^{14}C urea and ^{14}C glucose artificial urines were applied to repacked soil cores and incubated at 25°C for 84 days. Soil C, water soluble C (WSC), pH, carbon dioxide (CO_2) production, and enzyme activities (dehydrogenase, β-glucosidase, cellobiohydrolase, urease and protease) were measured during the incubation. Positive priming was measured in the pasture and pine soils treated with cow urine. Losses of soil C were greater ($P < 0.01$) from pasture (4.2±1.3 mg C g^{-1}; 5.1±0.9% of soil C concentration) than pine soil (2.0±0.1 mg C g^{-1}; 4.0±0.1%). Positive priming was attributed to increased microbial and urease activity in urine treated soils. Both soils treated with artificial urine exhibited negative priming. Soil pH increased in both soils following cow urine application and was positively correlated with WSC ($P < 0.001$, $r^2 = 0.68$). Soil C solubilisation was measured in the artificial urine treatments, and was greatest in the pine soil ($P < 0.05$). In conclusion, cow urine can cause a decrease in soil C by positively priming soil C decomposition in pine and pastures soils. The pasture soil was more susceptible to soil C loss by priming than the pine soil. Cow urine was not adequately modelled by artificial urine with respect to priming. While priming of soil C mineralisation following cow urine application was demonstrated under laboratory conditions, further confirmation under field conditions is needed.

Keywords: Priming, carbon, enzymes, respiration, radio-labelled

6.2 Introduction

The dairy industry in New Zealand has been undergoing intensification, increasing in both the amount of land under dairying and stocking rates (Parliamentary Commissioner for the Environment 2004; MacLeod & Moller 2006). There has also been substantial land use change from *Pinus radiata* forestry to grazing, with an estimated 15,600 hectares converted in the year ending March 2008 (Ministry of Agriculture & Forestry 2009). Declines in soil C under flat intensively grazed pastures have been reported (Schipper *et al.* 2007; 2010), with an average loss of 106 g C m^{-2} $year^{-1}$ in the top 1 metre of soil (Schipper *et al.* 2007). Although Schipper *et al.* (2007) did not investigate the mechanisms for the measured losses of soil C, urine deposition may cause priming of soil C decomposition and could have contributed. Urine deposition has been reported to cause positive priming of soil C in pastoral soils (Uchida *et al.* 2011; Lovell & Jarvis 1996; Clough *et al.* 2003; Kool *et al.* 2006). Positive priming is measured as more carbon dioxide (CO_2-C) produced from a soil than can be attributed to added C (Kuzyakov *et al.* 2000).

Cow urine deposition causes rapid increases in soil pH (Doak 1952; Bristow *et al.* 1992; Haynes & Williams 1992) leading to the solubilisation of soil C (Haynes & Williams 1992; Lovell & Jarvis 1996; Curtin *et al.* 1998; Shand *et al.* 2000). The dissolved soil C and urine-C under urine patches may be C sources for positive priming (Lovell & Jarvis 1996; Kool *et al.* 2006). Positive priming may also be caused by enhanced microbial activity or extracellular enzyme activity (Asmar *et al.* 1994; Degens & Sparling 1996; Lovell & Jarvis 1996). No published literature could be located investigating the extracellular enzyme activity under urine patches. Dehydrogenase is an intracellular enzyme that can be used to infer microbial activity (Amato & Ladd 1988; Garcia *et al.* 1997), and dehydrogenase activity has been reported to increase in urine treated soil (Orwin *et al.* 2010).

Studies on the effects of urine on soil nutrient cycling commonly use artificial urine to overcome the variable characteristics of urine (e.g. Fraser *et al.* 1994; Clough & Kelliher 2005; Kool *et al.* 2006; Lucas & Jones 2006). Few authors have assessed artificial urine as a substitute for cow urine with respect to C cycling, however, Lovell and Jarvis (1996) and Kool *et al.* (2006) reported greater CO_2-C evolution from cow urine than artificial urine in pastoral soils.

The primary objective of our study was to investigate whether urine deposition could cause positive priming of soil C in pine and pasture soils. We also examined extracellular enzyme activity and soil C solubilisation as contributors to soil C priming. A further objective was to evaluate artificial urine as a model of cow urine with respect to soil C cycling.

6.3 Materials and methods

Rangipo sandy loam (Podzolic Orthic Pumice Soil; Hewitt 1998; Typic Udivitrand; Soil Science Staff 2011) was collected to a depth of 5 cm from grazed pasture (1841575E 5666813N, NZTM) and *Pinus radiata* plantations (1841409E 5666694N) near Turangi, North Island, New Zealand in January 2008. The top 5 cm of the soil was collected as the majority of soil microbial biomass, and therefore the capacity for urine decomposition, is located in the top 5 cm of the soil (Murphy *et al.* 1998). The pasture was grazed by cattle and sheep at a rate of 5–6 stock units per hectare for at least 10 years (Paul Winterburn, Pers. Comm.). The pine trees were 21 years of age and were planted into scrubland (Graham Hardesty, Pers. Comm.). From here on, soil collected from grazed pasture or *P.radiata* plantation will be referred to as pasture and pine soil.

The soils were sieved at field moisture (2 mm) and adjusted to 60% water holding capacity (Harding & Ross 1964). The soil was repacked at field bulk density (Table 6.1) in core liners (5 cm diameter; 5 cm height) and pre-incubated for 5 days (25°C). Moisture content of the soil was

maintained by monitoring the weight of the samples and adding Milli-Q water when necessary.

Four treatments were applied to each soil:

- Cow urine (urine-pine, urine-pasture)
- Artificial urine labelled with ^{14}C urea (^{14}C urea-pine, ^{14}C urea-pasture)
- Artificial urine labelled with ^{14}C glucose (^{14}C glucose-pine, ^{14}C glucose-pasture)
- Water (water-pine and water-pasture).

Dairy cow urine was collected from cows (*c.* 5) during milking, after grazing ryegrass-clover pasture, in September 2007 (Dairy 1 Farm; Massey University, Palmerston North, New Zealand). The cow urine was mixed as collected and subsamples analysed for total C and nitrogen (N) (LECO FP-2000 CNS Analyser, LECO Corp., St Joseph, MI, USA), pH and electrical conductivity immediately after collection (Table 6.1). The remaining urine was frozen (–20°C) until required.

Table 6.1: Characteristics of cow urine and Rangipo sandy loam (0–5 cm; oven dry at 105°C) collected from pine and pasture land uses.

Characteristic	Urine	Pine	Pasture
Bulk density (g cm^{-3})	-	0.60	0.47
Total C (%)	1.51	4.9	8.3
Total N (%)	0.64	0.3	0.6
pH	8.3	5.5	5.8
Electrical conductivity (mS cm^{-1})	28.4	-	-

Radio-labelled artificial urine was used to separate urine-derived and soil-derived CO_2-C. The artificial urine had the same total C, total N, pH and electrical conductivity as cow urine (Table 6.1). Two artificial urines were prepared, each containing 15.5 g L^{-1} urea (Univar, BioLab Limited, Auckland, New Zealand) and 28.0 g L^{-1} glucose (AnalaR, BDH chemicals,

Poole, England). The electrical conductivity of the artificial urines was adjusted to 28.4 mS cm^{-1}, with potassium chloride, and a pH of 8.3 with sodium hydroxide. One artificial urine was labelled with ^{14}C-urea (7080 Bq L^{-1}), and the other with ^{14}C-glucose (3199 Bq L^{-1}). The amount of C added during labelling of the urine was negligible at 0.03% of the C content.

Cow and artificial urine was applied to soil cores at a rate of 1000 kg N ha^{-1} which is the average N concentration in a urine patch (Haynes & Williams 1993). This equated to the addition of 8 mg C g^{-1} in the pine soil and 10 mg C g^{-1} in the pasture soil. The same volume of Milli-Q water was applied in the control treatments. The cores were drained for 1 hour after solution application and all of the leachate was collected, after which time no further drainage was detected. The leachate was analysed for total C (High TOC II, Elementar Analysensysteme GmbH, Hanau, Germany) and ^{14}C (Tri-Carb 2910-TR, Perkin Elmer, Downers Grove, Illinois). Scintillation counting was undertaken using an automatic quench correction, with an efficiency of 97-98%. The cores were placed into 1.8 litre preserving jars and incubated (25°C) in the dark for 84 days.

The experimental design used here established ideal conditions for priming to enable us to test whether cow urine application could prime soil C in pine and pasture soils. There were two limitations to our experimental design; firstly, we were unable to obtain radio-labelled hippuric acid, which would have been a more preferable C compound in the artificial urine instead of glucose (Kool *et al.* 2006). Secondly, application of solutions to the cores may have displaced soil water but we assumed that all of the leachate from the cores was derived from applied solution.

6.3.1 Respiration

CO_2-C produced during the incubation was continuously trapped using 2 *M* sodium hydroxide traps, CO_2-C was quantified by back titration of trapping solutions with 0.1 *M* hydrochloric acid against a phenolphthalein indicator following the precipitation of carbonates with excess barium

chloride solution (Saggar *et al.* 1999). The trapping solutions from the artificial urine treatments were also assessed for ^{14}C using scintillation counting. Trapping solutions were refreshed on days 1, 2, 4, 7, 14, 21, 28, 42, 63, and 84 during the incubation, and the CO_2-C flux rate determined. Cumulative CO_2-C was calculated by summing CO_2-C per gram of dry soil from each sampling day during the incubation.

6.3.2 Biochemical analyses

Three cores from each treatment were harvested on Days 0, 2, 7, 14, 28, 42, 63 and 84 for analysis. Total C was measured by combustion furnace (LECO FP-2000 CNS Analyser, LECO Corp., St Joseph, MI, USA). ^{14}C in the soil was determined in artificial urine treatments by CO_2-C trapping during acid digestion, followed by precipitation of carbonates using barium chloride and back titration against a phenolphthalein indicator with hydrochloric acid (Sparling *et al.* 1991). Water soluble C (WSC) was determined by shaking wet soil (3 grams, 105°C oven dry equivalent) with 30 mLs of Milli-Q water for 20 minutes at 50 rpm, centrifuging for 20 minutes at 2500 rpm and filtering the solution under suction through GF/F filter papers (Ghani *et al.* 2003). ^{14}C of the WSC extracts from the artificial urine was also quantified to determine soil C solubilisation. A sub-sample of soil from each replicate core was air dried at 35°C and analysed for pH in a 2.5:1 water to soil slurry (Blakemore *et al.* 1987).

6.3.3 Enzyme assays

Enzyme activities were assayed in cow urine and water treatments, but not artificial urine treatments due to equipment limitations for analysis of radioactive samples.

Dehydrogenase activity is often used as an indicator of microbial activity (Skujiņš 2008) and was assayed in triplicate for each core replicate (n = 9), apart for days 63 and 84 of the incubation, where substrate limitation reduced analysis capacity (n = 3). Five g of wet soil was mixed with 1 mL

of 3% 2,3,5-triphenyltetrazolium chloride and 2.5 mLs of 0.5 *M* Tris buffer (pH 7.6), and incubated for 24 hours at 37°C (Ross 1971; Tabatabai *et al.* 1994). Triphenyl formazan was extracted with methanol, and quantified using spectrophotometry (Tabatabai *et al.* 1994).

β-glucosidase and cellobiohydrolase were chosen for their important roles within the C cycle (Eivazi & Tabatabai 1990; Turner *et al.* 2002; Verchot & Borelli 2005). *β*-glucosidase activity was assayed in triplicate (n = 9) on each incubation replicate, using 1 g of wet soil to which 4 mLs of 0.05 *M* buffer was added with 1 mL of 0.025 *M* ρ-nitrophenyl-*β*-D-glucoside substrate (Tabatabai *et al.* 1994; Turner *et al.* 2002). The mixture was incubated for 1 hour at 37°C, the activity was stopped by the addition of 1 mL of 0.5 *M* calcium chloride and 4 mLs of 0.2 *M* Tris buffer, the solution was then gravity filtered through No.2 filters (Labserv, BioLab Limited, Auckland, New Zealand). The resulting solution was measured spectrophotometrically for ρ-nitrophenol (Tabatabai *et al.* 1994; Turner *et al.* 2002).

Cellobiohydrolase activity was measured, on one replicate per incubation replicate (n = 3), 1.5 g of soil was mixed with 10 mLs of 0.05 *M* sodium acetate buffer, 0.5 mL of the slurry was taken in triplicate and incubated (25°C) with 0.5 mLs of 0.002 *M* ρNP-*β*-D-cellobioside for 2 hours. ρ-nitrophenol was production was then measured spectrophotometrically (adapted from Verchot & Borelli 2005). Cellobiohydrolase was assayed until Day 28 of the incubation due to substrate constraints.

Urea is the predominant N component in urine, and urease plays a vital role in the degradation of urea in soils (Tabatabai *et al.* 1994). Urease activity was measured in triplicate on each soil sample by incubating 2.5 g of wet soil with 2.5 mLs of 1% urea solution for 5 hours at 37°C (Tabatabai *et al.* 1994). At the termination of the incubation period, the remaining urea was extracted using 2 *M* potassium chloride-phenylmercuric acetate solution. The extractant solution was analysed for urea by acid digestion in boiling water for 30 minutes (Marsh *et al.* 1965). Urea concentration was

then determined spectrophotometrically against urea standards in potassium chloride-phenylmercuric acetate solution (Marsh *et al.* 1965).

Protease is part of a chain of enzymes that degrade polypeptidic compounds (Pascual *et al.* 1998). Protease activity was assayed, in triplicate, on each soil replicate (n = 9); 4 g of wet soil was shaken with 10 mLs of 1% sodium casineate in 0.1 *M* Tris buffer for 1 hour at 50°C (Ladd & Butler 1972; Burton & McGill 1992). Trichloroacetic acid was then added, and the mixture filtered. The filtrate was measured for phenol concentration against L-tyrosine standards after addition of Folin reagent (adapted from Blum *et al.* 1992).

6.3.4 Data analysis

A mass balance was used to determine whether priming had occurred in soils applied with cow and artificial urine. The amount of urine-C initially retained in each soil core (mg C g^{-1}) was calculated using Equation 1:

$$U_{retained} = \frac{(U_{added} \times V_{added}) - (U_{leachate} \times V_{leachate})}{W_s} \quad (1)$$

Where:

- U_{added} = C concentration of the solution added (mg C L^{-1})
- V_{added} = amount of urine added (0.0305 L)
- $U_{leachate}$ = C concentration in the core leachate (mg C L^{-1})
- $V_{leachate}$ = volume of the leachate (L)
- W_s = dry weight of soil (g).

Cumulative CO_2-C was calculated by arranging the data into three blocks within each treatment. Each block contained the respiration rate from one of three replicates from every sampling day i.e. Block 1 = replicate 1 from every sampling day, Block 2 = replicate 2 from every sampling day, and Block 3 = replicate 3 from every sampling day. Cumulative respiration was

calculated by summing the amount of CO_2-C g^{-1} measured on each of the sampling days in each data block. The amount of soil C primed in cow and artificial urine treatments (mg C g^{-1}) was calculated using (Equation 2):

$$\text{Priming} = CO_2\text{-}C_{treatment} - CO_2\text{-}C_{control} - U_{retained} \quad (2)$$

Where:

- CO_2-$C_{treatment}$ = cumulative respiration from a treatment (mg CO_2-C g^{-1})
- CO_2-$C_{control}$ = cumulative respiration from the water treatment on the same soil (mg CO_2-C g^{-1})
- $U_{retained}$ = amount of C retained after urine application (mg C g^{-1}; Equation 1).

^{14}C was also used to determine priming in the pine and pasture soils treated with artificial urine. To establish the extent to which the two C compounds in the artificial urine contributed to the total respiration, the respiration evolved from the ^{14}C urea and ^{14}C glucose was summed within each soil. The proportion of ^{14}C activity in the respiration (f) was determined for each replicate within each artificial urine treatment by dividing the total amount of activity emitted in the respiration over the term of the incubation (Bq) by the activity of the artificial urine that was retained in the soil (Bq). This assumes the proportion of ^{14}C in the soil is the same as the proportion of ^{14}C in CO_2 fluxes. The cumulative respiration (mg CO_2-C g^{-1}) evolved from each replicate was then multiplied by the f ratio to determine the amount of the total respiration that was derived from the radiolabelled compound, either urea or glucose. For each land use, the mean respiration evolved from the ^{14}C urea was added to the mean respiration evolved from ^{14}C glucose. The total was then subtracted from the total cumulative respiration from each soil (pine or pasture; n = 6), and further corrected for the water controls to calculate priming.

Linear regression analysis was used to; 1) determine if there was a correlation between respiration (log_{10}) and dehydrogenase (log_{10}) or

urease (log_{10}) in the urine and water treatments, and 2) investigate if there was a correlation between WSC and respiration fluxes evolved through the incubation.

Soil C solubilisation was also calculated in the artificial urine treatments. The proportion of ^{14}C activity in WSC (ψ) was calculated by dividing the activity of the WSC (Bq) by the activity of the artificial urine retained in the soil (Bq). WSC for each day was then multiplied by ψ to determine the amount of artificial urine present in the WSC, this was then subtracted from the total water corrected WSC to determine the amount of soil C in the WSC.

6.4 Results

6.4.1 Respiration

Respiration rates peaked in the cow and artificial urine treatments in the first 7 days of incubation, returning to control levels by Day 63 (Figure 6.1). The greatest respiration fluxes were measured in the urine-pasture treatment ($P < 0.001$), which were greater than the urine-pine treatment until Day 63 of the incubation. Total respiration fluxes from the ^{14}C glucose-pine and ^{14}C glucose-pasture soils were the same as each other through much of the incubation; this was also the case for the ^{14}C urea-pine and ^{14}C urea-pasture treatments.

Positive priming was measured in both pine and pasture soils applied with cow urine (Table 6.2); however, negative priming was measured in the artificial urine treatments (Table 6.2; 6.3). The pine soil lost 4.0±0.1% of its soil C concentration, and the pasture soil 5.1±0.9% in the cow urine treatment. Scintillation counting efficiency ranged between 93-95%, but the recovery of the added ^{14}C label at the end of the incubation from each treatment was 60-70%.

6.4.2 *Biochemistry*

Soil total C did not change during the incubation (data not shown), and was not sensitive enough to detect the losses in soil C measured in the urine-pine and urine-pasture treatments. Solubilisation of soil C occurred in the artificial urine treatments, in both the pine and pastures soils (Figure 6.2), which was greater in the pine soil than the pasture soil ($P < 0.05$).

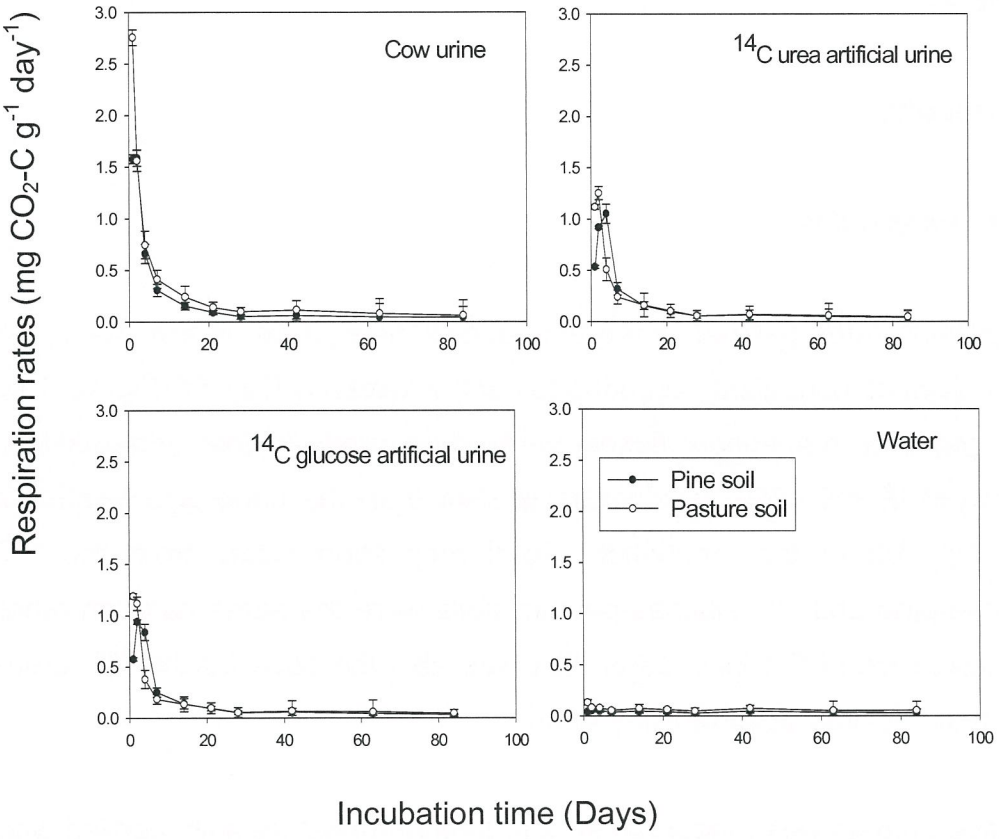

Figure 6.1: Respiration rates (mg CO_2-C g^{-1} day^{-1}) for pine and pasture soils treated with cow urine, ^{14}C urea artificial urine, ^{14}C glucose artificial urine and water over an 84 day incubation. Error bars represent standard error.

Table 6.2: (a) Cumulative C respired over an 84 day incubation and (b) urine-C retained in both pine and pasture soils applied with cow urine, ^{14}C urea artificial urine, ^{14}C glucose artificial urine and water. (c) Priming of soil C in the urine and artificial urine treatments in the pine and pasture soils corrected for the water controls. Units are mg g^{-1} of dry soil for all columns. Values in brackets represent standard error (n = 3), values within paired pine and pasture columns with different letters were significantly different.

Treatment	**(a) Cumulative C respired**		**(b) Urine-C retained**		**(c) Priming (a-b-Water control)**	
	Pine	*Pasture*	*Pine*	*Pasture*	*Pine*	*Pasture*
Cow urine	10.3 (0.3)^{a}	15.1 (1.0)^{b}	5.2 (0.3)^{a}	5.7 (0.2)^{b}	2.0 (0.1)^{a}	4.2 (0.7)^{b}
^{14}C urea	9.5 (0.4)^{a}	9.7 (0.2)^{a}	7.6 (0.2)^{c}	7.0 (0.1)^{d}	-1.3 (0.2)^{c}	-2.5 (0.2)^{cd}
^{14}C glucose	8.4 (0.1)^{a}	8.8 (0.5)^{a}	7.1 (0.2)^{d}	6.8 (0.1)^{d}	-1.8 (0.2)^{cd}	-3.1 (0.6)^{d}
Water	3.1 (0.1)^{c}	5.2 (0.1)^{d}	0.0 (0.0)^{e}	0.0 (0.0)^{e}		
P values	*P < 0.001*		*P < 0.05*		*P < 0.01*	

Table 6.3: (a) Cumulative C respired (urine and soil), (b) cumulative artificial urine-C respired, (c) cumulative C respired from the water controls, and (d) the amount of soil C primed in pine and pasture soils following the application of ^{14}C labelled artificial urine. Units are mg C g^{-1} of dry weight soil for all columns. Values in brackets represent standard error.

Soil	(a) Cumulative C respired (n = 6)	(b) Cumulative artificial urine-C respired* (n = 6)	(c) Cumulative C respired from water controls (n = 3)	(d) Priming (a-b-c) (n = 3)
Pine	9.0 (0.3)	9.0 (0.3)	3.1 (0.1)	-3.2 (0.5)
Pasture	9.2 (0.3)	7.2 (0.4)	5.2 (0.1)	-3.1 (0.5)

*Summation of respiration derived from ^{14}C urea and ^{14}C glucose in the artificial urine treatments calculated using the proportion of 14C activity in the total cumulative respiration or f (Section 6.3.4).

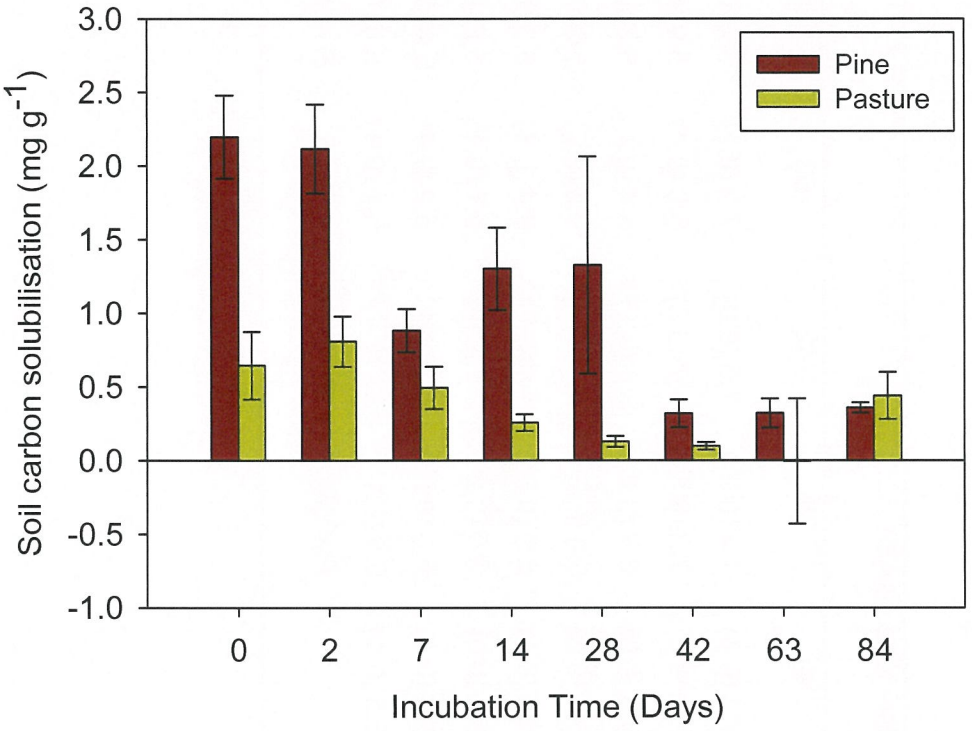

Figure 6.2: Solubilisation of soil carbon from Rangipo sandy loam (pine and pasture) treated with ^{14}C labelled artificial urine. Error bars represent standard error.

Soil pH increased ($P < 0.001$) immediately after cow and artificial urine application in both pine and pasture soils compared to water controls (Table 6.4). Soil pH in the urine-pine treatment was generally greater than all other treatments until the end of the incubation. WSC was positively correlated with soil (Figure 6.3; $P < 0.001$; $r^2 = 0.68$). Above a soil pH of 6.8, WSC increased dramatically indicating that soil C became more soluble at higher pH levels.

Table 6.4: Soil pH before and during an 84 day incubation of pine and pasture soil with cow urine, ^{14}C urea artificial urine, ^{14}C glucose artificial urine and water. Values in brackets represent standard error. Values with different letters in each column were significantly different.

Treatment		**Incubation time (days)**							
Soil pH	Pre-inc	0	2	7	14	28	42	63	84
Urine-pine	-	7.2 (0.0)^{a}	7.4 (0.1)^{a}	7.3 (0.0)^{a}	7.4 (0.4)	7.4 (0.2)^{a}	7.5 (0.0)^{a}	7.2 (0.2)	7.4 (0.0)^{a}
Urine-pasture	-	6.9 (0.0)^{b}	7.0 (0.1)^{b}	6.9 (0.2)^{b}	7.1 (0.3)	7.3 (0.1)^{a}	6.7 (0.3)^{b}	6.6 (0.4)	5.9 (0.2)^{c}
^{14}C urea-pine	-	6.8 (0.0)^{b}	6.8 (0.0)^{bcd}	6.8 (0.0)^{b}	6.9 (0.0)	6.7 (0.0)^{b}	6.6 (0.1)^{bc}	6.4 (0.1)	6.3 (0.1)^{b}
^{14}C urea-pasture	-	6.8 (0.0)^{b}	6.4 (0.0)^{e}	6.3 (0.1)^{c}	6.1 (0.0)	5.8 (0.0)^{cd}	5.7 (0.0)^{d}	5.5 (0.0)	5.6 (0.2)^{cd}
^{14}C glucose-pine	-	6.7 (0.0)^{bc}	6.8 (0.0)^{bc}	6.8 (0.0)^{b}	6.8 (0.1)	6.9 (0.3)^{b}	6.5 (0.0)^{bc}	6.4 (0.4)	6.3 (0.1)^{b}
^{14}C glucose-pasture	-	6.6 (0.0)^{c}	6.6 (0.0)^{ce}	6.4 (0.0)^{c}	6.2 (0.0)	6.0 (0.0)^{c}	5.7 (0.1)^{cd}	5.7 (0.1)	5.2 (0.0)^{e}
Water-pine	5.5 (0.1)	5.8 (0.0)^{d}	5.6 (0.1)^{f}	5.6 (0.1)^{d}	5.6 (0.1)	5.3 (0.0)^{e}	5.5 (0.2)^{d}	5.2 (0.1)	5.4 (0.1)^{de}
Water-pasture	5.8 (0.1)	6.0 (0.0)^{e}	6.0 (0.1)^{g}	6.1 (0.1)^{c}	5.8 (0.1)	5.5 (0.1)^{de}	5.9 (0.4)^{bcd}	5.1 (0.1)	5.4 (0.1)^{e}
		P < 0.001	*P < 0.001*	*P < 0.001*		*P < 0.001*	*P < 0.05*		*P < 0.001*

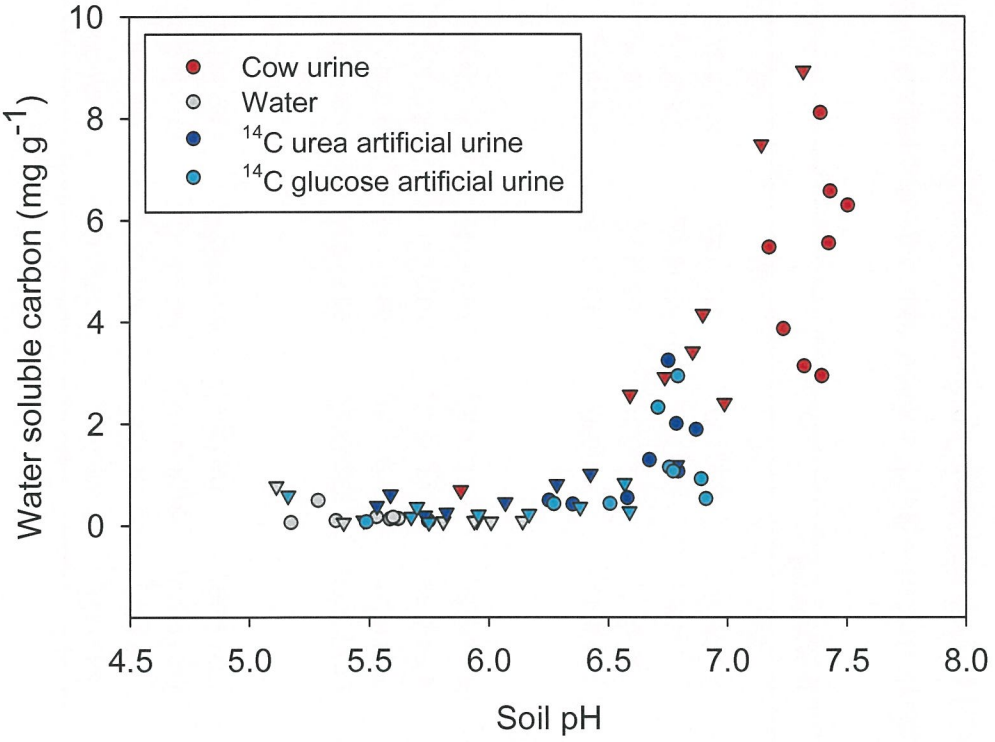

Figure 6.3: Soil pH and water soluble carbon concentration in pine and pasture soils before and during an incubation for 84 day incubation with cow urine, ^{14}C urea artificial urine, ^{14}C glucose artificial urine and water (n = 72).

Dehydrogenase activity increased rapidly (Day 0; $P < 0.001$; Table 6.4) above water controls after cow urine application in both soils, and remained elevated for the first 7 days of the incubation. Dehydrogenase and respiration activities were positively correlated ($P < 0.05$; $r^2 = 0.43$; Figure 6.4). In the urine treatment, the pasture soil had greater dehydrogenase activity than the pine soil for the first 7 days of the incubation (Table 6.5).

Table 6.5: Dehydrogenase, urease, and protease activity (μg g^{-1} hr^{-1}) before and during an incubation of pine and pasture soil with cow urine or water for 84 days. Values in brackets represent standard error (n = 3–9), values with different letters in each column, within each enzyme assay, were significantly different ($P < 0.05$).

Treatment	**Sampling Day**								
Dehydrogenase	Pre-inc*	0	2	7	14	28	42	63	84
Urine-pine	-	9.8 (1.6)^{a}	5.1 (0.8)^{a}	3.2 (0.4)^{a}	6.2 (0.9)^{a}	5.3 (1.2)^{a}	3.9 (1.2)^{a}	2.1 (0.2)^{a}	2.5 (0.7)^{a}
Urine-pasture	-	42.5 (1.4)^{b}	17.3 (2.1)^{b}	8.4 (0.6)^{b}	8.7 (1.8)^{a}	7.1 (1.4)^{a}	4.5 (1.4)^{a}	2.2 (0.1)^{a}	1.5 (0.1)^{b}
Water-pine	1.1 (0.1)^{a}	0.8 (0.2)^{c}	0.6 (0.3)^{a}	0.3 (0.1)^{c}	1.3 (0.1)^{a}	0.8 (0.0)^{a}	1.2 (0.1)^{a}	1.7 (0.5)^{a}	1.4 (0.1)^{b}
Water-pasture	3.4 (0.5)^{b}	5.9 (0.5)^{d}	2.7 (0.4)^{a}	3.3 (0.5)^{a}	3.7 (0.5)^{a}	3.3 (0.6)^{a}	1.2 (0.3)^{a}	1.4 (0.0)^{a}	2.1 (0.5)^{ab}
Urease									
Urine-pine	-	104.5 (1.9)^{a}	103.1 (4.3)^{a}	69.8 (3.3)^{a}	81.6 (9.3)^{a}	67.0 (4.9)^{a}	68.2 (2.5)^{a}	91.6 (11.4)^{a}	77.6 (7.8)^{a}
Urine-pasture	-	115.5 (2.6)^{a}	106.4 (5.5)^{a}	80.6 (2.0)^{a}	69.8 (6.6)^{a}	74.2 (2.4)^{a}	103.0 (10.1)^{a}	95.7 (10.7)^{a}	113.9 (3.2)^{a}
Water-pine	39.8 (2.4)^{a}	51.0 (5.6)^{a}	46.9 (2.6)^{a}	50.7 (1.9)^{a}	59.2 (2.1)^{a}	56.5 (1.5)^{a}	50.9 (1.4)^{a}	47.3 (1.2)^{a}	46.2 (1.3)^{a}
Water-pasture	52.4 (2.2)^{b}	53.1 (2.0)^{a}	53.4 (2.1)^{a}	55.3 (1.1)^{a}	71.7 (10.0)^{a}	63.9 (3.7)^{a}	52.9 (2.2)^{a}	71.9 (12.1)^{a}	65.7 (6.6)^{a}
Protease									
Urine-pine	-	1714 (45)^{a}	1998 (132)^{a}	969 (113)^{a}	1291 (276)^{a}	1476 (306)^{a}	2275 (424)^{a}	1585 (306)^{a}	604 (108)^{a}
Urine-pasture	-	2542 (334)^{a}	2994 (212)^{b}	1446 (145)^{a}	1750 (273)^{a}	3424 (1017)^{a}	2085 (153)^{a}	1319 (120)^{a}	1121 (93)^{b}
Water-pine	1209 (41)^{a}	1490 (74)^{a}	1973 (132)^{a}	766 (88)^{a}	575 (57)^{a}	735 (50)^{a}	491 (50)^{a}	757 (76)^{a}	482 (62)^{a}
Water-pasture	1662 (66)^{b}	1813 (71)^{a}	1116 (117)^{c}	842 (35)^{a}	849 (31)^{a}	827 (34)^{a}	1732 (34)^{a}	1145 (61)^{a}	1200 (57)^{b}

*Pre-inc = pre-incubation

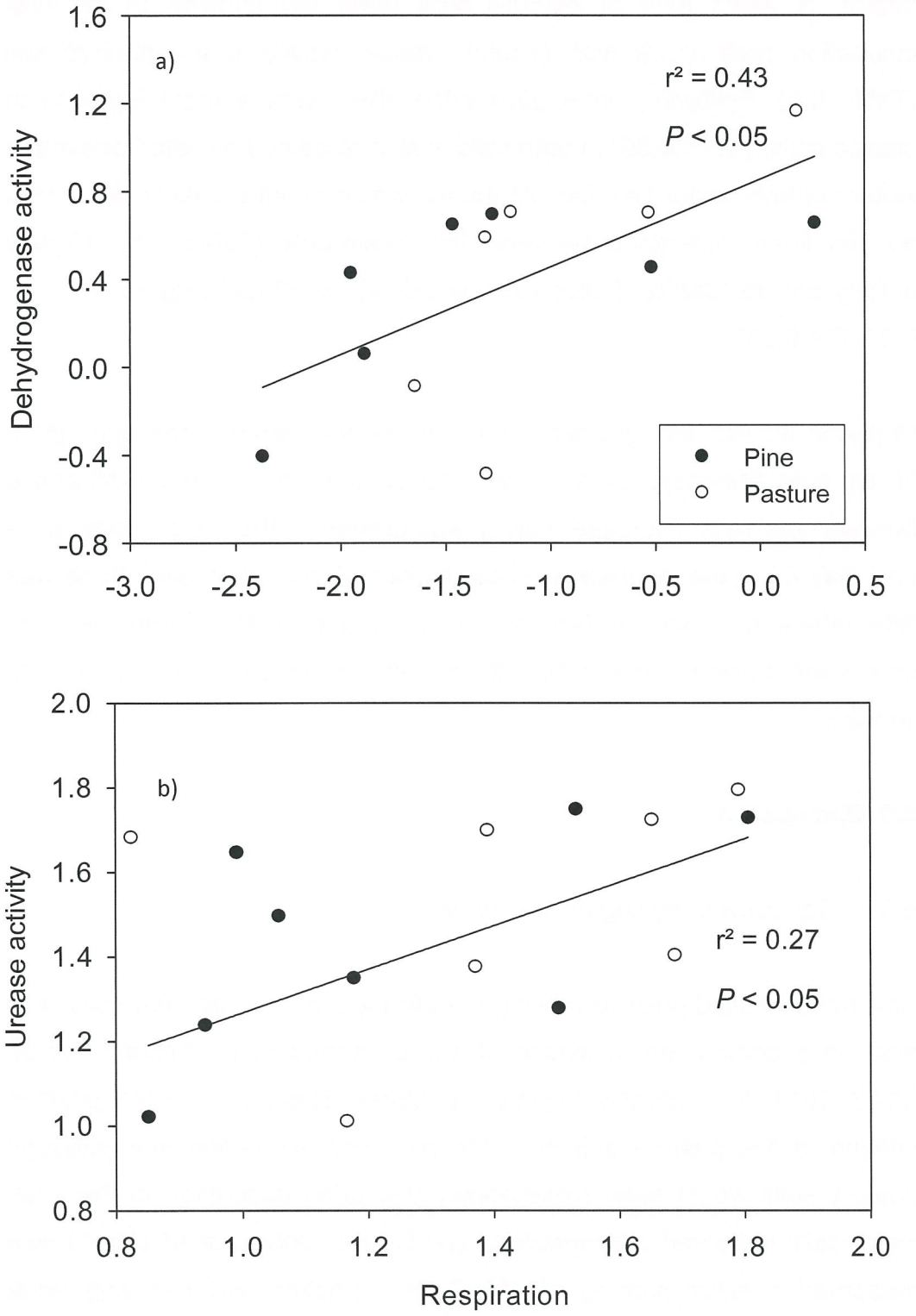

Figure 6.4: a) Dehydrogenase activity (log_{10}) and respiration rate (log_{10}), b) urease activity (log_{10}) and respiration rate (log_{10}) for pine and pasture soils treated with cow urine over 84 days. Data have been corrected for water controls.

Cow urine application did not increase β-glucosidase or cellobiohydrolase activity in either pine or pasture soils (data not shown). In the pre-incubation soils, there was greater urease activity in the pasture soil (Table 6.5). Following urine application, there was a rapid increase in urease activity ($P < 0.05$) in both soils, which remained elevated above the water controls under Day 84. At the individual treatment level, there was no significant difference between the treatments (Table 6.5). Urease activity and respiration fluxes were positively correlated (Figure 6.4; $r^2 = 0.27$; $P < 0.05$).

Protease activity was greater in the pasture soil before urine application (Table 6.5). Following urine or water application, there was no difference between the soils. Protease activity was greater in the urine treated soils until Day 63 of the incubation. At the individual treatment level, there was little difference between the treatments (Figure 6.4). There was no correlation between respiration fluxes and protease activity (data not shown).

6.5 Discussion

6.5.1 Soil carbon priming by cow urine

Cow urine caused positive priming in both the pine and pasture soils, and may have contributed to losses of soil C measured by Schipper *et al.* (2007; 2011) from intensively grazed pastures. There was greater positive priming in the pasture soil than the pine soil, indicating that although pasture soils would have experienced cow urine deposition in the past, these soils remained susceptible to soil C loss. Uchida *et al.* (2011) also measured positive priming of soil C in a pasture soil following urine application, they measured a loss of 0.4 mg C g^{-1} (or about 3%) of soil C in the Kerikeri friable clay loam (0-50 mm). This is similar to the priming of soil C reported in this paper, where the pasture soil lost about 5% of its soil C concentration due to priming. Even though Uchida *et al.* (2011)

added a lower amount of urine-C (2 mg C g^{-1}) compared to 5-8 mg C g^{-1} added to the soil cores in our work, the results were comparable.

CO_2 emissions from the urine treatments peaked in the first 24 hours after urine application, which agrees well with previously reported work (Clough & Kelliher 2005; Kool *et al.* 2006). Kool *et al.* (2006) found that CO_2 fluxes in urine treated soils returned to background levels in 5-10 days, whereas our urine treatments had elevated CO_2 rates until day 63 of the incubation. The soils used by Kool *et al.* (2006) contained about 34 mg C g^{-1} and the greater C contents of the Rangipo sandy loam may have been able to sustain a longer period of elevated CO_2 evolution.

Urine addition increased soil microbial activity, extracellular activities and soil C mineralisation. Other authors have also reported increased microbial activity in urine treated soils during priming events (Lovell & Jarvis 1996; Clough & Kelliher 2005; Kool *et al.* 2006). Urease and protease activity increased following urine application, but no previously published literature could be located assessing the influence of cow urine on extracellular enzyme activity. However, Haynes and Williams (1999) measured increased protease and urease activity in stock camping soils also applied with effluent. Increased urease and protease activity has also been reported in soils affected with dairy shed effluent (Zaman *et al.* 1999; 2002). Urease activity was positively correlated to soil respiration fluxes and may have contributed to priming in both pine and pastures soils. β-glucosidase or cellobiohydrolase activity was not increased by urine application and was unlikely to have contributed to soil priming. Priming of soil C has also been suggested to result from the death and degradation of microbes after the C source has been exhausted (Dalenberg & Jager 1989), the extent to which this contributed to the priming in our work was not determined.

6.5.2 Soil carbon priming by artificial urine

Negative priming was measured in the artificial urine treatments in both the pine and pasture soils. Negative priming is the retardation of soil C cycling due to the addition of a C source (Kuzyakov *et al.* 2000). The addition of a highly "utilisable" substrate can cause the microbes that degrade soil C switching to mineralising the added substrate, therefore mineralisation of soil C is reduced (Kuzyakov & Bol 2006). As urine-C contains about 13±2% easily degradable C (Chapter 7) it is possible that this was an insufficient amount of degradable C to lead to negative priming – but enough to initiate positive priming.

Both non-labelled and radio-labelled techniques measured negative priming in the artificial urine treatments, however only 60-70% of the added radio-label was recovered at the end of the incubation. Therefore, conclusions drawn from the artificial urine treatments require caution. The discrepancy in the recovery of radio-label was likely due to either incomplete trapping of gas fluxes or incomplete measurement of the various forms of C in the soil. The trapping of C bearing gases other than carbon dioxide, such as methane or ethylene, was not undertaken within this experiment. Although these gases are generally produced in small amounts from soil (Considine *et al.* 1977; Lovell & Jarvis 1996; Zechmeister-Boltenstern & Smith 1998) they may have contributed to the unrecovered ^{14}C in our work. Allophane protects organic matter from degradation by forming stable complexes (Parfitt 2009) and has also been shown to retard the degradation of ^{14}C glucose in soils (Zunino *et al.* 1982; Saggar *et al.* 1994). However, the Rangipo sandy loam contains very low amounts (less than 1.1% in the top 5 cm) of allophane (National Soils Database; Landcare Research) and would have had minimal effect on the recovery of the added radio-label, and only if the complexes formed were not acid oxidisable. The soil used in our work did not contain carbonate, eliminating the possibility of $^{14}CO_2$-C being lost to carbonates (Stevenson & Cole 1999). Chromic acid digestion, such as that used in this experiment to determine the amount of ^{14}C in the soil, is only capable of digesting

easily degradable organic C (Shulte & Hoskins 2011). It was possible that the ^{14}C labelled urine formed non-oxidisable compounds during the incubation, and therefore the chromic acid digestion may not have been accounted for all of ^{14}C label in the soil. Due to the uncertainty in the accuracy of the ^{14}C evolved as CO_2-C in the artificial urine, this technique could not be used to confirm the occurrence of positive or negative priming in soil artificial urine treatments. However, negative priming was measured using mass balance calculations (Table 6.2).

The artificial urine used was not an adequate representative of cow urine, despite having the same total C, total N, pH and electrical conductivity. Respiration rates and cumulative C respired were greater in the cow urine treatments compared to the artificial urine treated soils, and negative priming was measured in the artificial urine treatments. A lower respiration flux from artificial urine than cow urine treated soils has been reported previously (Lovell & Jarvis 1996; Kool *et al.* 2006). Differences in respiration rates between cow and artificial urine treatments were most likely due to C compositional differences. The artificial urines contained only urea and glucose, whereas the cow urine contains a more complex mixture of C compounds including hippuric acid (Chapter 3). Caution is advised when using artificial urine to model the role of cow urine in the soil C cycle or processes dependent on WSC such as denitrification.

6.5.3 Soil carbon solubilisation

Soil C solubilised under urine patches adds to the already present pool of dissolved organic C (DOC) in a soil. To distinguish between the DOC pool before urine deposition and soil C dissolved following urine deposition, the latter has been termed solubilised soil C. We measured soil C solubilisation in both the pine and pasture soils following the application of radio-labelled artificial urine (Figure 6.2), which was greater in the pine soil than the pasture soil. Therefore, the potential for soil C leaching to occur under urine patches may be greater under soils recently converted from pine plantations to grazing.

The potential maximum solubilisation of soil C by cow urine was 10.1 mg C g^{-1} in the pine soil and 36.7 mg C g^{-1} in the pasture soil (Chapter 5). This potential solubilisation was greater than the solubilisation measured in the artificial urine treatment and was likely an effect of air drying and sieving of the soils in the previous experiment (Chapter 5). Uchida *et al.* (2011) measured an increase in WSC content of 0.8 mg C g^{-1} in soil following cow urine application, but it is not clear from their work how much of the WSC originated from the soil rather than the urine.

The greatest increase in soil C solubilisation occurred at the start of the incubation period when there was the greatest increase in soil pH. Rapid increases in soil pH under urine patches have been reported to cause soil C solubilisation (Haynes & Williams 1992; Lovell & Jarvis 1996; Shand *et al.* 2002). In our treatments, there was a sharp increase in WSC content in soils above a soil pH of 6.8. Although soil C solubilisation could be quantified in the artificial urine treatments, the artificial urine used in our work did not mimic cow urine and therefore the amount of soil C solubilisation in the cow urine treatments was not inferred from the artificial urine treatments.

While some of the soil C solubilised may have been mineralised, it may also have been subject to sorption or leaching processes. Sorption of urine-C was low (~3%; Chapter 5) but the adsorption of soil C solubilised under urine patches has not been assessed. Several authors have shown that naturally occurring DOC is susceptible to sorption on soils (e.g. Jardine *et al.* 1989; Guggenberger *et al.* 1998; Kaizer & Zech 2000). DOC leaching of soil C under grazed pastures ranges from 12-169 g C m^{-2} $year^{-1}$ (McTiernan *et al.* 2001; Parfitt *et al.* 2009; Ghani *et al.* 2010; Shepherd *et al.* 2010). The solubilisation of soil C would not only influence the C cycle, but would also affect nitrogen (N) cycling. Both nitrification and denitrification is increased in urine patches due to the increase in both N and C in urine patches (Koops *et al.* 1997; Ambus *et al.* 2007; Carter 2007). However, there is also some evidence that other urine-borne compounds (benzoic and hippuric acid) inhibit denitrification in urine

patches (van Groenigen *et al.* 2006). Overall, the fate of solubilised soil C in soil following urine deposition requires further investigation.

Increases in soil pH following urine application have been assumed to be due to consumption of hydrogen ions during urea hydrolysis to ammonium (Doak 1952; Stillwell & Woodmansee 1981). The soil pH in the artificial urine treatments was generally less than the cow urine treatments, even though the artificial urine contained 15.5 g L^{-1} of urea, and the cow urine about 3 g L^{-1}. Therefore, urea hydrolysis was not the only factor involved in increasing soil pH in the urine treatments. Acid neutralising capacity (ANC) forcing following the application of sodium nitrate fertiliser can raise soil pH, by substitution of urine-borne cations for hydrogen ions decreasing the proportion of acid saturation and increasing soil pH (Evans *et al.* 2008). ANC forcing by urine has not been investigated, but may contribute to the increase in soil pH and subsequent soil C solubilisation in the cow urine treatments.

6.6 Conclusions

Application of cow urine caused positive priming in both pine and pasture soils. Both soils lost ~2-4 mg C g^{-1} (4-5% of soil C concentration) as CO_2-C after a single application of cow urine. The positive priming was attributed to enhanced soil C cycling due to increased microbial and urease activity. The other extracellular enzymes assayed were unlikely to have contributed to priming as they were not correlated to increased respiration fluxes. Soil C solubilisation under urine patches may also have contributed to positive priming events, although further investigation is required into the role of solubilisation in soil C cycling under urine patches. The pasture soil exhibited a faster and greater respiration response following urine application, and lost a greater amount of soil C, than the pine soil. More work is required to determine the mechanisms for soil C priming following cow urine deposition, particularly in field environments. The artificial urine used in this experiment was not an adequate model of cow urine, particularly with respect to priming.

There are important implications for our work as positive priming of soil C may be one of the causes responsible for the decline in soil C under flat, dairy grazed pastures as reported by Schipper *et al.* (2010). Positive priming of soil C may also lead to declines in soil C following conversion of pine plantations to grazing. Soil C solubilisation was greater in the pine soils, inferring that leaching losses of C may be greater from converted pine soils than from long-term grazed pasture soils.

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Chapter 7

Regular paper

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Title: Carbon leaching from undisturbed soil cores treated with dairy cow urine

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7.1 Abstract

Losses of soil carbon (C) from intensively grazed pastures have been reported, possibly due to solubilisation of soil C under urine patches leading to priming or leaching. We investigated the solubilisation and bioavailability of soil C in undisturbed soils treated with cow urine. We also investigated the contribution of acid neutralising capacity (ANC) forcing and aggregate disruption as mechanisms of soil C solubilisation. Undisturbed soil cores (Rangipo sandy loam; 10 cm diameter, 5 cm deep) were treated with urine or water, incubated for 6 hours and then leached with water. Urine was obtained from cows fed on a C4 plant enriched diet allowing for the differentiation between soil C and urine-C. Urine deposition increased total C and DOC leaching by 8 g C m^{-2} compared to leachate from water controls (WLeachate). $\delta^{13}C$ analysis determined that 28.1±0.9% of the C in the leachate from urine treated cores (ULeachate) was soil C. The ANC forcing of cow urine was 11.8 meq L^{-1} and may have contributed to enhancing soil C leaching. There was no significant aggregate disruption following urine application and was therefore unlikely to have contributed to solubilisation of C. The bioavailability of ULeachate was 4 times greater than that of both cow urine and WLeachate. It is possible that ULeachate may lead to priming of soil C lower in the profile. More investigation is required under field conditions to determine long term contribution of urine deposition to DOC leaching and the fate of solubilised C in pastoral soils.

7.2 Introduction

Solubilisation and subsequent leaching of soil C under urine patches may be an important contributor in the losses of soil C measured by Schipper *et al.* (2007; 2010) from intensively grazed dairy soils. Cow urine has been shown to solubilise large quantities of soil C in air dried and sieved soils (Chapter 5) and the application of radio-labelled artificial urine led to soil C solubilisation in field-moist repacked cores (Chapter 6). Soil C solubilisation appeared to be enhanced by the air drying and sieving of soils. While these two previous studies demonstrated in principle that cow urine can cause soil C solubilisation in soil, the extent to which soil disturbance contributed to C release needed to be addressed. To overcome the potential effect of soil preparation on soil C solubilisation by cow urine, a further experiment was established to determine if soil C solubilisation occurs in undisturbed soil cores following treatment with urine.

While the solubilisation of soil C has been reported following urine application (e.g. Chapter 5; Chapter 6; Monaghan & Barraclough 1993; Lovell & Jarvis 1996; Shand *et al.* 2000), the mechanisms of solubilisation have not yet been fully investigated. Soil C solubilisation under urine patches was thought to be due to the rapid increase in soil pH as urea was hydrolysed (Doak 1952; Stillwell & Woodmansee 1981). However, previous experimentation (Chapter 4; 6) found that urea was not solely responsible for increases in soil pH following treatment with urine. Acid neutralising capacity (ANC) forcing may also increase soil pH by altering the cation balance (Evans *et al.* 2008). The application of cations in urine (Hutton *et al.* 1965; Richards & Wolton 1976; Haynes & Williams 1993) could displace hydrogen ions from "exchangeable acid cation" sites (pg. 365) into solution, decreasing the proportion of acid saturation and increasing soil pH (Brady & Weil 2008). Evans *et al.* (2008) reported a positive correlation between ANC forcing and DOC losses from soils

following application of nitrogen fertilisers. ANC forcing of soil treated with urine had not previously been investigated.

The rapid increase in soil pH under urine patches may also lead to the disruption of soil aggregates. Uchida *et al.* (2008) reported disruption of 0-2000 µm sized aggregates following urine deposition. Aggregate disruption can release previously unavailable soil C into solution, which can then be rapidly mineralised (Gregorich *et al.* 1989; Chandra *et al.* 2002). It is possible that aggregate disruption by urine may have contributed to the solubilisation of soil C measured previously but required direct measurement.

There are two main mechanisms for soil C loss from the profile following solubilisation; 1) mineralisation, and 2) leaching. Enhanced mineralisation, or priming, of soil C degradation, in both pine and pasture soils following treatment with urine has been measured previously (Chapter 6; Lovell & Jarvis 1996; Kool *et al.* 2006). Priming occurs when the mineralisation of soil C is accelerated following the addition of a small amount of degradable C. The potential of urine deposition to lead to priming is dependent on the bioavailability of both the urine-C and soil-C solubilised after urine application. Bioavailability is commonly assessed by measuring the degradability of a target compound in controlled laboratory conditions (McDowell *et al.* 2006; Andreasson *et al.* 2009). The bioavailability of C in cow urine was measured to be between 17 and 25% over 28 days (Chapter 3), however, the bioavailability of leachate from urine treated soils had not been previously assessed.

Leaching of DOC under pastoral soils has been reported by several authors. McTiernan *et al.* (2009) reported DOC leaching losses from pasture over a two month period under cattle grazing of between 4 and 12 g C m^{-2}. Shepherd *et al.* (2010) also reported DOC leaching of 4 g C m^{-2} over the course of 74 days. Ghani *et al.* (2010) measured DOC leaching

under pasture soils that had not been recently grazed, and report DOC losses of 0.5-3 g C m^{-2} over 25 weeks. When comparing DOC losses from the ungrazed with grazed pasture soil there is some indication that grazing by cattle may increase soil C solubility.

One of the major difficulties in determining the influence of cow urine on soil C is separating urine and soil derived C in leachates. Previously, radio-labelled techniques were somewhat useful in the quantification of priming and solubilisation of soil C (Chapter 6). However, the use of ^{14}C label required application of artificial urine, and some doubt remains as to the accuracy of using artificial urine to represent cow urine (Chapter 6). Therefore, urine naturally labelled with ^{13}C was chosen for the leaching component of this work to differentiate between soil and urine C sources.

The majority of New Zealand's pastures are dominated by C_3 ryegrass rather than C_4 plants (maize or palm kernel), which are often used as cattle feed when required (Clark *et al.* 2007). C_4 plants use phosphoenol-pyruvate carboxylase (PEPcase) to fix CO_2 during photosynthesis, which is more efficient at fixing CO_2 (Griffiths 2006). PEPcase is also less discriminant against the heavier ^{13}C isotope than the CO_2 fixing enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) in C_3 plants (Griffiths 2006). This difference in uptake of ^{13}C by C_3 and C_4 plants results in less depletion of ^{13}C in C_4 plants compared to C_3 plants (Griffiths 2006). Cows being fed on a predominately C_4 plant diet will therefore have urine that is less depleted in ^{13}C than the organic matter in soils derived from the turnover of the C_3 plants which make up the majority of pastoral grasses.

Urine was collected from cows fed on a C_4 plant enriched diet and applied to undisturbed cores, the cores were leached with water and the soil and leachate analysed. The undisturbed cores were collected from C_3 plant dominated pasture. The main objective of this work was to quantify soil C

solubilisation and leaching following urine application in undisturbed soil cores. Further, we assessed the bioavailability of C leached from urine patches. Lastly, we determined the contribution of ANC forcing and aggregate disruption to soil C solubilisation.

7.3 Materials and methods

7.3.1 Urine

Ten litres of dairy cow urine was collected from several cows, fed on a high maize diet on 24 April, 2010 (10–12 kg dry weight maize silage + 4 kg dry weight palm kernel; R & S Shearer, Drury, New Zealand). The urine was frozen at –20°C until required. The total C content of the cow urine was 9.1 g C L^{-1}, and the organic C content was 8.8 g C L^{-1}.

7.3.2 Soil

Thirty undisturbed cores (10 cm diameter), to a depth of 5 cm, were taken from Rangipo sandy loam (Podzolic Orthic Pumice Soil; Hewitt 1998; Typic Udivitrand; Soil Survey Staff 2011) under pasture. The top 5 cm of the profile was chosen for this work due to the shallow rooting nature of the grass sward and to be able to directly compare with soil C losses from priming (Chapter 6). Cattle had grazed the paddock two days before collection, at a rate of 5–6 stock units per hectare (Robert Holland, Rangipo Farm Manager, Department of Corrections, Turangi, New Zealand, pers. comm.). Two transects were placed across the paddock, and fifteen cores taken 2 metres apart on each transect line.

Saturated hydraulic conductivity of the cores was measured to determine the rate at which the water should be added during the leaching phase of

the experiment, to avoid ponding of water on the core surface and reduce the possibility of macropore flow. Ten of the 30 cores collected were randomly selected for saturated hydraulic conductivity measurement as determined by Klute & Dirksen (1986). Fifteen of the remaining cores were adjusted to field capacity (–10 kPa). The grass on the top of the cores was trimmed to approximately 2 cm above the soil surface and left overnight (20°C) until urine application. The remaining soil cores were used for the pilot study.

7.3.3 Soil pH pilot study

A pilot study was initiated to determine the time required for soil pH to change after urine application. The pilot experiment consisted of 2 treatments, water and urine. The soil was sieved (5 mm) at field moisture content and 20 g of wet soil, in triplicate, was used for each incubation time and for each treatment.

Urine was applied at rate equivalent to 1000 kg N ha^{-1} (Haynes & Williams 1993), or 16.8 mLs of urine, the same volume of water was added to the controls. Soil pH was measured at 0, ½ hour, 1 hour, 1 ½ hours, 2 hours, 4 hours, 6 hours, 8 hours and 16 hours after urine application. At each time, 100-mL of Milli-Q water was added to the urine- and water-treated soils and periodically shaken over an hour and the pH was measured with a glass electrode (Rayment & Higginson 1992).

7.3.4 Core leaching

Ten undisturbed cores were irrigated with 105 mL of urine (equivalent to 500 kg N ha^{-1}), a further 5 cores were irrigated with the same volume of Milli-Q water. The urine or water was applied with a dropper over 30 minutes to minimise macropore flow, ponding and disruption of the soil

surface. After 6 hours, each core was put in a 110 mm ceramic Buchner funnel and placed under –10 kPa suction to avoid saturation of the soil at the base of the core and to simulate suction that would occur in the field under natural conditions (Phillips & Burton 2005). Milli-Q water (500 mL, approximately 2 pore volumes) was then applied to the top of each core, at a rate less than the cores saturated hydraulic conductivity (599 mm hr^{-1}). The leachate from the cores treated with water (WLeachate) and the cores treated with urine (ULeachate) were collected in Buchner flasks and weighed to obtain the volume of leachate drained from each core.

7.3.5 Soil carbon solubilisation

To determine the amount of soil C present in the ULeachate, freeze dried subsamples of the urine (n = 3), WLeachate (n = 5) and ULeachate (n = 10) were analysed for $\delta^{13}C$ using isotope ratio mass spectrometry (National Isotope Centre, GNS Science, Lower Hutt, New Zealand). $\delta^{13}C$ of the soil organic matter (n = 3) was also determined.

The percentage of soil C present in ULeachate (Dissolved soil C) was determined using a mixing model (Fry 2006; Equation 1).

$$\text{Dissolved soil C} = 100 - \frac{\delta_{\text{ULeachate}} - \delta_{\text{Soil}}}{\delta_{\text{Urine}} - \delta_{\text{Soil}}} \quad (1)$$

Where:

- $\delta_{\text{ULeachate}}$ was $\delta^{13}C$ in the leachate from undisturbed cores treated with cow urine
- δ_{Soil} was $\delta^{13}C$ of the soil
- δ_{Urine} was $\delta^{13}C$ in the cow urine.

All of the leachates and three subsamples of the added urine and water were filtered to 0.45 µm (Labserv, BioLab, Auckland, New Zealand) and analysed for total, inorganic and organic C contents (High TOC II, Elementar Analysensystem GmbH, Hanau, Germany).

The Rangipo sandy loam has a C content of 10.2%, with an average oven dry (105°C) weight of 285 g soil core^{-1}, therefore each core contained 29 g of soil C.

7.3.6 Acid neutralising capacity forcing

Acid neutralising capacity (ANC) forcing of the urine was measured by assessing the difference in cation and anion content in the WLeachate and ULeachate. Subsamples (100 mL) of each leachate was analysed for sodium, calcium, potassium, chloride, magnesium, sulphate, ammonium, and nitrate content (NZ Labs Ltd, Hamilton, New Zealand).

The concentration of X_{ion} in the leachates was multiplied by the inverse of the milli-equivalent weight of X_{ion} to convert them from mg L^{-1} to meq L^{-1}. To calculate ANC of the urine, the concentration of X_{ion} (meq L^{-1}) in the WLeachate was subtracted from the concentration of X_{ion} (meq L^{-1}) in ULeachate and equation 2 applied to the resulting data (Evans *et al.* 2008):

$$ANC = \Delta NH_4^+ + \Delta Na^+ + \Delta Ca^{2+} + \Delta K^+ + \Delta Mg^{2+} - \Delta NO_3^- - \Delta SO_4^{2-} - \Delta Cl^- \quad (2)$$

If the resulting total was positive then there was an increase in ANC and pH and a negative number indicated the reverse (Evans *et al.* 2008).

7.3.7 Leachate bioavailability

The bioavailability of the leachates from the cores was determined by measuring the degradation of organic C (OC) in the core leachates. One 25 mL sample from each leachate (ULeachate (n = 10) and WLeachate (n = 5)), and three subsamples of the applied urine were placed into 50-mL glass jars. The glass jars were then placed into 1.8 litre Agee jars, sealed and incubated at 25°C for 28 days. The jars were ventilated in a fume cupboard for 30 minutes every 2-3 days. Following the incubation, samples were filtered to 0.45 µm (Labserv, BioLab, Auckland, New Zealand) and analysed for OC contents (High TOC II, Elementar Analysensystem GmbH, Hanau, Germany). Bioavailability was also determined as the amount of OC degraded in 28 days as a proportion of the initial OC content of the leachate or urine.

7.3.8 Soil water stable aggregates and pH

Each core was removed intact from the liner and cut into quarters from the top down. Two opposite quarters were used for the measurement of water stable aggregates (see below) and the remaining quarters were sieved through a 4.75 mm mesh and a subsample was taken for measurement of soil pH and moisture content. Soil pH was determined on wet soils in water, 5 grams of soil was mixed with 25 mL of water, and pH was measured as described in Section 7.2.3.

Water stable aggregates were measured to determine if urine application led to the disruption of soil aggregates, using an adaptation of the method of Gradwell and Birrell (1979). The soil was air dried at room temperature for 16 hours until it was dry enough to go through an 8 mm sieve and not smear or disrupt aggregates (53–76% moisture). The samples were not

air-dried completely as is usual for water stable aggregate analysis as air drying can lead to strengthening of aggregate bonds and an inaccurate measure of water stable aggregate content and distribution (Kemper & Rosenau 1986; Haynes & Swift 1990). Soil (100 g) was placed in a 20 cm diameter sieve pan and 500 mL of deionised water added. This volume of water was found to give the best swirling movement of aggregates in the pan. The pan was placed in an orbital shaker at 50 rpm for 30 minutes. Thirty minutes was found to be the best shaking time for this method as any time above this led to a breakdown of macro-aggregates. Following the gentle agitation in the orbital shaker, the soil was then wet sieved in a large bucket containing a nest of 6 sieves with mesh sizes of 2000 µm, 1000 µm, 500 µm, 250 µm, 125 µm, and 63 µm. The samples were manually sieved, lifting and dropping approximately 50 mm at a rate of 120 rpm for 1 minute. Each sieve was washed into an aluminium pot and oven dried at 105°C. The aggregates were weighed in each size class.

7.3.9 Statistical analyses

Statistical analyses were conducted using Genstat 12 and were considered to be significant if $P < 0.05$. ANOVA with Student-Newman-Kuels testing was used to determine if there was a significant difference between the $\delta^{13}C$, total C, inorganic C and organic C, each cation and each anion of the urine, ULeachate and WLeachate. ANOVA with Student-Newman-Kuels testing was also used to determine if there was a difference between the degradation of OC in the urine, ULeachate and WLeachate and to assess if urine deposition led to a decrease in water stable aggregates. The amount of soil C lost through leaching was tested for difference from zero using a one sample, two-sided t-test.

7.4 Results

All results are presented as a mean with standard error, unless otherwise stated.

7.4.1 Soil pH pilot study

The purpose of this pilot study was to determine the time needed for the urine to have the maximum effect on soil pH. The pH of the urine treated soil peaked 6 hours following treatment with urine (Figure 7.1). After that time, the pH of the urine treated soils levelled off.

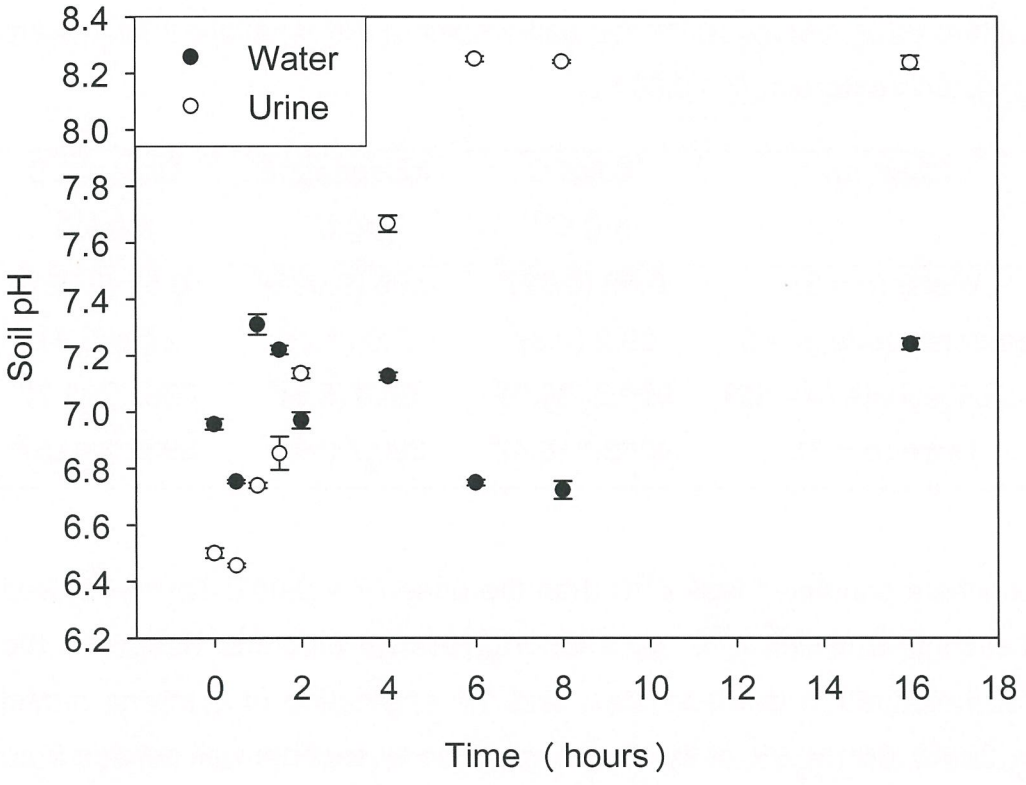

Figure 7.1: pH of soil treated with water or urine over 16 hours. Error bars represent standard error (n = 3).

7.4.2 Soil carbon leaching

The volume of leachate from the cores was 488±3 mL (n = 15), and there was no difference between the volume of leachate from the urine and water treatments. The total, inorganic, and OC contents of the treated water and WLeachate were not significantly different (Table 7.1). The cow urine contained more total C and OC ($P < 0.001$) than ULeachate, but ULeachate contained more inorganic C than the urine (Table 7.1). The urine and ULeachate had greater total, inorganic and OC contents ($P < 0.001$) than the water and WLeachate.

Table 7.1: Total, inorganic and organic carbon concentration in urine and water applied to and leachates from undisturbed cores following application with water or cow urine. Numbers in brackets represent standard error. Values within the same column with a different letter were significantly different ($P < 0.001$).

Solution	Total C mg L^{-1}	Inorganic C mg L^{-1}	Organic C mg L^{-1}
Water (n = 3)	0.63 (0.02)^{a}	0.06 (0.001)^{a}	0.57 (0.02)^{a}
Water leachate (n = 5)	29.2 (2.3)^{a}	2.0 (1.2)^{a}	27.2 (2.5)^{a}
Urine leachate (n = 10)	986.3 (38.9)^{b}	98.3 (3.8)^{b}	888.0 (35.7)^{b}
Urine (n = 3)	9262.0 (0.4)^{c}	329.7 (0.0)^{c}	8932.2 (0.5)^{c}

ULeachate contained less $\delta^{13}C$ than the urine ($P < 0.001$; Table 7.2) and WLeachate and soil $\delta^{13}C$ were not significantly different. Based on the $\delta^{13}C$ measured in the leachates, and the application of a mixing model (Fry 2006), 28.1±0.9% of the C present in the ULeachate was derived from soil C. The ULeachate contained 0.48±0.02 g of total C, so that 0.14±0.01 g of that was soil C. Therefore, the soil C solubilised during a single urine application equated to 0.45±0.03% of the soil C in the core. Although the amount of soil C leached was small, it was significantly different to zero (*P*

< 0.001). Treatment with urine led to an increase of total C solubilisation of 0.12±0.01 g C (8 g C m^{-2}). The urine contained 97% organic C, and the increase in DOC leaching was similar to that of the total C leaching of 0.11±0.00 g C (8.g C m^{-2}). As 28.1% of the C in the ULeachate was soil derived, the other 71.9% was assumed to be urine derived. The amount of urine-C retained by the soil was 64.5±1.3% (0.63±0.01 g C) of that added, and therefore the amount of urine-C retained was nearly 5 times what was leached.

The application of water to the undisturbed cores led to a small amount of soil C leaching, (0.01±0.00 g soil C; 1.0 g C m^{-2}) 0.048±0.001% of the soil C content was solubilised in the water treatment, which was 10 times less than the amount of soil C solubilised in the urine treatment.

Table 7.2: $\delta^{13}C$ of cow urine, leachate from urine treated soil (ULeachate), leachate from water treated soil (WLeachate) and soil. Numbers in brackets represent standard error. Values with different letters were significantly different ($P < 0.001$).

Sample	$\delta^{13}C$ (‰)
Urine (n = 3)	-20.7 (0.1)[a]
ULeachate (n = 10)	-22.7 (0.2)[b]
WLeachate (n = 5)	-28.3 (1.0)[c]
Soil (n = 3)	-27.8 (0.0)[c]

7.4.3 Degradation of leachate carbon

The proportion of OC degraded in the WLeachate and urine was the same ($P < 0.001$), with 12–13% of OC degraded over 28 days (Figure 7.2). The proportion of C degraded in the ULeachate was considerably greater ($P <$

0.001) than both the WLeachate and urine, with half of the organic C degraded in 28 days (Figure 7.2).

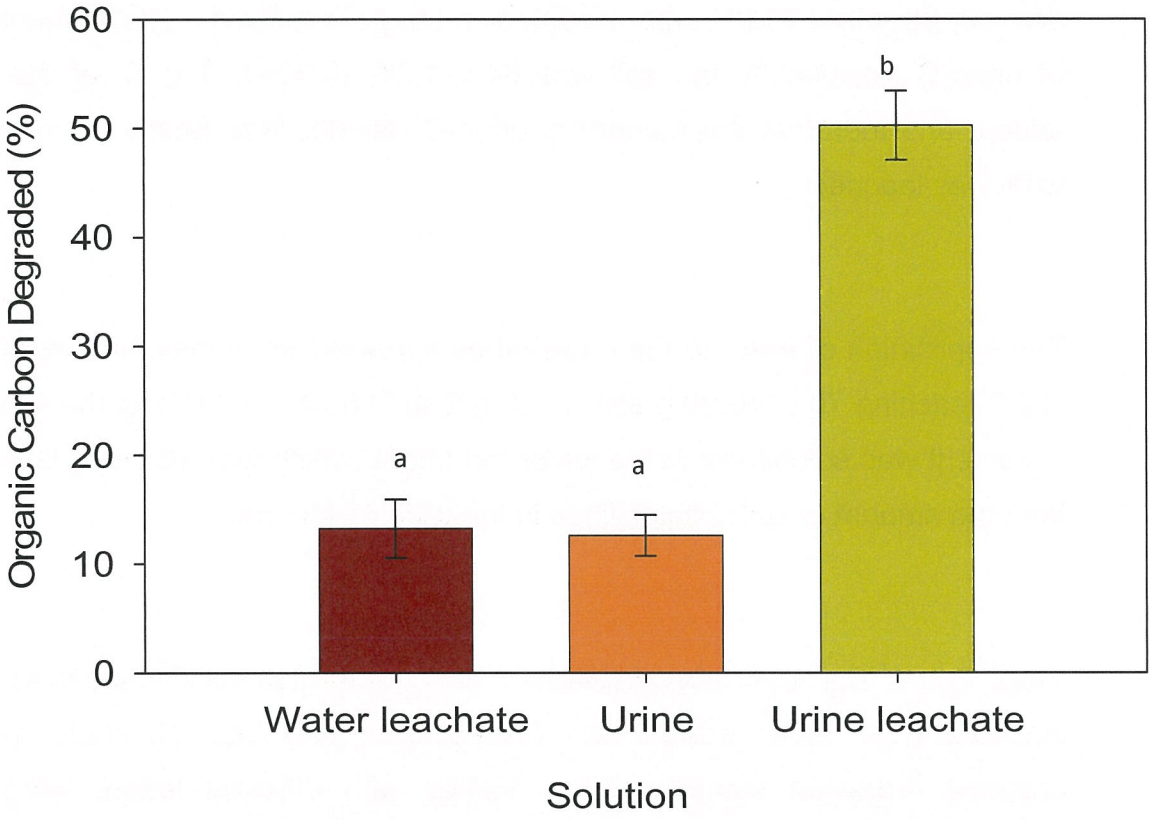

Figure 7.2: The proportion of total organic C degraded in 28 days in the leachate from water treated soil cores (n = 5), cow urine (n = 3) and leachate from cow urine treated soil cores (n = 10). Error bars represent standard error; bars with different letters were significantly different ($P < 0.001$).

7.4.4 Acid neutralising capacity forcing, water stable aggregates, and soil pH

The cation and anion concentrations in the ULeachate and WLeachate were generally less than the concentration in the urine, with the exception of nitrate, which showed no change, and ammonium which increased (Table 7.3).

Table 7.3: Cation and anion concentrations in the leachate collected from water treated soil cores (WLeachate; n = 5), cow urine (n = 3) and leachate collected from urine treated soil cores (ULeachate; n = 10) and the percentage difference between cow urine and ULeachate. Numbers in brackets represent standard error. Values with a different letter in each row were significantly different ($P < 0.01$).

Cation/Anion	WLeachate mg L^{-1}	Urine mg L^{-1}	ULeachate mg L^{-1}	Change %
NH_4^+	0.1 (0.0)^a	90.2 (1.3)^b	174.2 (7.4)^c	↑193
Na^{2+}	3.9 (0.4)^a	291.8 (6.6)^b	43.2 (1.9)^c	↓85
Ca^{2+}	2.3 (0.3)^a	28.2 (0.7)^b	15.3 (1.8)^c	↓46
K^+	3.9 (0.4)^a	53.4 (0.2)^b	3.4 (0.1)^a	↓94
Mg^{2+}	0.4 (0.1)^a	73.0 (0.2)^b	4.4 (0.5)^c	↓94
NO_3^-	0.1 (0.1)^b	0.0 (0.0)^a	0.0 (0.0)^{ab}	nc*
SO_4^{2-}	2.5 (0.5)^a	228.1 (0.9)^b	30.0 (1.2)^c	↓87
Cl^-	2.8 (0.4)^a	16.6 (0.0)^b	2.3 (0.1)^a	↓86

*nc = no change

Cow urine had an acid neutralising capacity (ANC) forcing of 11.8±0.6 meq L^{-1} in this soil. The amount of water stable aggregates for all class sizes in both the water and urine treatments were the same (Figure 7.3). The pH of the soil increased to 7.3±0.1 in the urine treatment, and was significantly greater ($P < 0.001$) than the water treatment, which had a soil pH of 6.7±0.1.

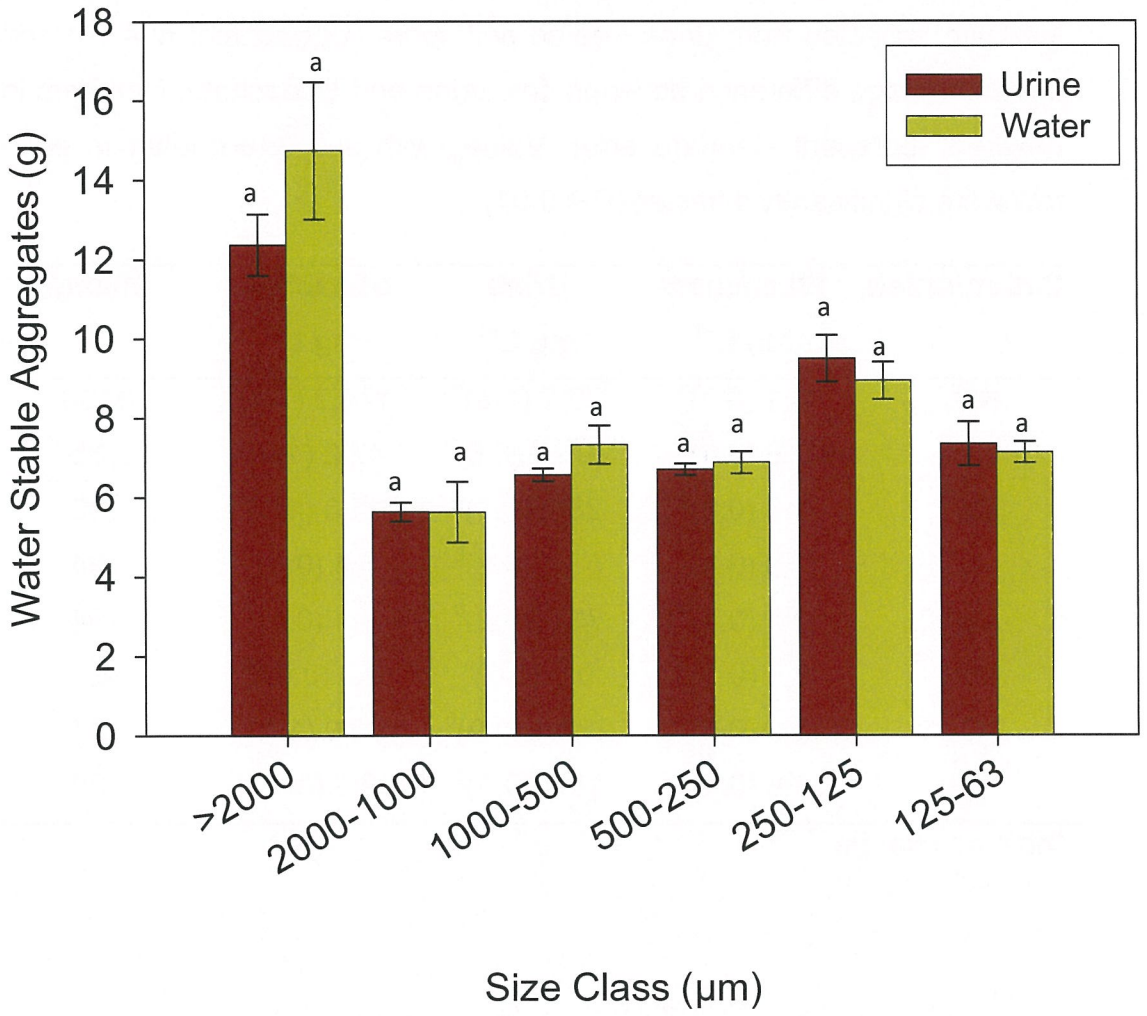

Figure 7.3: Soil water stable aggregates following application with water or urine to undisturbed cores in six aggregate size classes. Error bars represent standard error, water n = 5, urine n = 10; bars with different letters within each class size were significantly different (P <0.05).

7.5 Discussion and conclusions

7.5.1 *Soil carbon leaching*

The main objective of our work was to determine if cow urine deposition increased the solubility and leaching of soil C in undisturbed cores.

Secondly, the decomposition of organic C (OC) in the leachate from water (WLeachate) and urine treated cores (ULeachate), and cow urine, was investigated to determine their bioavailability. Lastly, ANC forcing and disruption of aggregates by urine application were investigated to determine whether these factors contributed to soil C solubilisation.

The potential solubilisation of soil C from air-dried Rangipo sandy loam pasture soil (0-5 cm) was 863 g C m^{-2} (Chapter 5). This experiment represented a worst case scenario for soil C solubilisation under urine patches, as the soil was air dried, sieved and shaken with urine under laboratory conditions. The amount of soil total C lost from urine application in the current experiment, 8 g C m^{-2}, was considerably less than this potential loss. Soil C solubilisation was also previously measured following the addition of ^{14}C labelled artificial urine (Chapter 6). Over a 84 day incubation, the maximum amount of soil C in water soluble C extraction was 19 g C m^{-2}. The artificial urine was applied to wet sieved, repacked cores, and it's possible that this disturbance of the soil increased soil C solubilisation by urine.

Stable isotope analysis of the urine leachate demonstrated that there was ten times more solubilisation of soil C following urine application than water application. As there was little inorganic C in the leachate, DOC solubilisation in our soil was not different from total C leached at 8 g C m^{-2}. This compared well with DOC leaching measured by McTiernan *et al.* (2001), who measured 4-12 g C m^{-2} of DOC leaching from grazed pasture over two months. Parfitt *et al.* (2009) measured DOC leaching from high country soils in New Zealand at a rate of 12-23 g C m^{-2} $year^{-1}$, which if assessed for a two month time period would equate to 2-4 g C m^{-2}, which is less than reported by McTiernan *et al.* (2001). The leaching measured in our work was likely to be an underestimation of what occurs under field conditions, due to the shallow depth of soil tested and single application of urine. Although C leached from shallow cores may be subsequently

adsorbed lower in the profile, and further investigation using deeper cores is required.

DOC leaching from water treated cores was less than urine treated cores, and compared well with the leaching reported by Ghani *et al.* (2010). DOC leaching from ungrazed pasture soil (i.e. soil had not recently been exposed to cow urine) had been measured by Ghani *et al.* (2010), who reported 0.5-3.0 g C m^{-2} of soil DOC leaching from the top 25 cm of the profile over a period of 25 weeks. The DOC leaching in our water treatment was 1.0 g C m^{-2}, which compares well to the results of Ghani *et al.* (2010), although our result is only after a single application of water on a shallow core. In comparison, DOC leached under urine patches was 3–18 times greater than ungrazed pasture DOC leaching (Ghani *et al.* 2010).

7.5.2 Acid neutralising forcing capacity and water stable aggregates

Acid neutralising capacity (ANC) forcing has been linked to increased soil pH (Evans *et al.* 2008) and may also be partially responsible for increasing soil pH under urine patches. The ANC forcing capacity of urine was attributed to the large displacement of ammonium ions from the soil into solution by urine, possibly by urine derived potassium, magnesium, and calcium (Brady & Weil 2008). Degradation of urea applied in the urine may have also contributed to ammonium that was measured in the leachate. Cow urine had ANC forcing of 11.8 meq L^{-1}, compared to the greatest ANC forcing measured by Evans *et al.* (2008) of 0.5 meq L^{-1} following the application of sodium nitrate fertiliser (3 g N m^{-2} $year^{-1}$). Cow urine has a greater potential to increase soil pH than the fertilisers tested by Evans *et al.* (2008), and may be a factor in raising the soil pH under urine patches soil C dissolution. To be able to determine the relationship between DOC leaching and ANC forcing under urine patches further testing is required with a larger range of soils.

There was no difference in soil water stable aggregates (WSA) between the water and urine treatments, therefore the disruption of soil aggregates was unlikely to have been a major cause of soil C solubilisation in our soil following urine deposition. In contrast, Uchida *et al.* (2008) found an increase in the instability of soil aggregates in the 0-2000 µm size class following urine application. The soil that Uchida *et al.* used was the Temuka silt loam, which is a gley soil with impeded drainage (National Soils Database, Landcare Research). Therefore, the urine may have had a greater residence time in the Temuka soil than the free draining Rangipo soil, and therefore had a greater effect due to longer contact time with the aggregates.

The stability of aggregates can also be increased by the presence of perennial ryegrass roots (Reid & Goss 1981) and water stable aggregates increase with increasing organic matter (Chaney & Swift 1984). It is possible that the increased root presence and high organic matter content of our soil reduced the effect of urine on aggregate disruption, although further testing is necessary to determine their role in aggregate stability under urine patches.

7.5.3 Leachate Bioavailability

Urine deposition not only led to solubilisation of soil C, but the leachate was more bioavailable than cow urine. About 50±3% of the OC in the ULeachate was degraded over 28 days, while only 13±2% of the urine-OC was degraded. The degradation of urine-C compared well with results of previous work where 7-25% of the urine-C was degraded in 28 days (Chapter 3). Because of the greater bioavailability of the ULeachate-C, it may act as a source of more degradable C for priming lower in the profile. Priming of soil C has been demonstrated in the Rangipo soil previously following the application of cow urine (Chapter 6), whether ULeachate

from the top 5 cm measured in this chapter could lead to priming lower in the profile requires further investigation.

There are two potential reasons for the greater bioavailability of C in the ULeachate; 1) there may have been preferential retention of the more recalcitrant urine compounds in the soil, and/or 2) preferential loss of soil C with greater degradability subsequent to urine deposition. To date, there are no published reports of preferential retention of urine derived C compounds, in fact, adsorption of urine-C was found to be minimal, with only 2.9 ±0.4% of urine-C content being adsorbed (Chapter 5). However, preferential adsorption to soils of more recalcitrant or larger compounds from dissolved organic matter has been reported (e.g. Liang *et al.* 1996; Guggenberger *et al.* 1998; Guo & Chorover 2003; Kalbitz *et al.* 2005).

The soil retained 64.5±1.3% of the added total C of the urine, and leached a small amount of soil C, the fate of the large amount of retained urine-C would presumably be influenced by mineralisation and sorption. The bioavailability of ULeachate was significantly greater than that of the urine, although further testing as to the bioavailability of the urine remaining in the soil is required. The urine may also lead to further soil C losses by priming, which has been shown to decrease soil C concentrations by 5.1±0.9% in this soil.

In conclusion, a single urine deposition could increase soil carbon leaching from undisturbed soil cores by up to 10 times that of water treated soil, although only 0.5% of soil C was leached. It is still unclear if urine deposition may have contributed to the losses of soil C measured by Schipper *et al.* (2007; 2010). Further investigation is required to determine whether DOC leaching is increased with the frequency of urine deposition or after repeated application of urine. The time between urine deposition and rainfall events may also influence the extent of leaching and requires

further assessment. Aggregate disruption was not a major mechanism of soil C loss following urine deposition in this soil, but ANC forcing may have contributed to increases in soil pH leading to soil C solubilisation. The bioavailability of ULeachate was significantly greater than urine and may contribute to priming of soil C lower in the profile. The fate of solubilised C under urine patches requires further testing.

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Chapter 8

Summary and discussion

8.1 Introduction

This chapter presents the objectives, and a summary of the main findings of the research conducted in my thesis, also suggestions of future work. Soils used for experimentation were sampled from under grazed pasture and *Pinus radiata* plantations, and from here on, the soils will be termed pasture and pine soils.

8.2 Objectives of the thesis

Schipper *et al.* (2007; 2010) measured losses of soil carbon (C) from intensively grazed pastures in New Zealand. A potential contributor to these losses was soil C solubilisation under urine patches. The overall goal of my thesis was to determine if dairy cow urine can decrease soil C by solubilisation and subsequent mineralisation or leaching. Several specific objectives were to:

- determine the C content, C composition and bioavailability of dairy cow urine (Chapter 3 and 7);
- determine if cow urine causes dissolution and leaching of soil C from pine and pasture soils (Chapters 5, 6 and 7), *and to test the hypothesis that soil C solubilisation would be greater in pine soils than pasture soils,* and
- determine if cow urine can cause priming of soil C in pine and pasture soils (Chapter 6), *and to test the hypothesis that priming*

would be greater in pine soils than pasture soils due to previous acclimation in the pasture soils.

8.3 Summary of main findings

8.3.1 Chapter 3: Carbon composition and bioavailability of dairy cow urine

A pilot experiment was initiated to determine the C concentration and C composition in cow urine as little information was found in the published literature. The C composition of the cow urine was determined using wet chemistry and pyrolysis gas chromatography-mass spectrometry (GC-MS). The bioavailability of the urine-C was also investigated to determine if urine-C may act as a priming agent for soil C. Bioavailability was determined by measuring the amount of urine-C degraded over 28 days (25°C).

The mean C concentration in cow urine (n = 10) was 7.5±1.2 g L^{-1}, of which urea, carbohydrates, phenolics, and inorganic C made up about 20%. Pyrolysis GC-MS showed that hippuric acid contributed nearly half of the urine-C, followed by urea, an unidentified amide, and phenaceturic acid. The dairy cow urine had a C:N ratio of 2.1.

Between 7 and 25% of the urine-C was degraded over 28 days, and the addition of soil microbes to the urine did not lead to an increase in urine-C degradation. The degradation of urine-C may determine priming of soil C in urine patches, and priming has been reported by several authors following urine application to soil (Lovell & Jarvis 1996; Kool *et al.* 2006; Uchida *et al.* 2011). There are two possible priming agents under a urine patch, the C added in the urine and the soil C dissolved following urine

deposition (Lovell & Jarvis 1996; Uchida *et al.* 2011). Given the relatively rapid degradation of up to 25% of urine-C it was possible that urine-C could act as a priming agent when applied to soil.

There were several implications from the work conducted in Chapter 3. The range of compounds isolated in the cow urine, and it's C content and C:N ratio suggested that commonly used artificial urines may not adequately represent cow urine. Therefore, conclusions made from studies using artificial urines with respect to C or nitrogen (N) cycling may potentially be misleading. The bioavailability of urine-C indicated that urine derived C may act as a priming agent in soils, possibly leading to a decrease in soil C under urine patches.

8.3.2 Chapter 4: Fate of urine carbon in soil from three land uses

The objectives of the pilot study conducted in Chapter 4 were to:

- investigate the fate of urine-C in soils from three land uses; dairy-grazed pasture, sheep-and-beef-grazed pasture and *Pinus radiata* plantation soils;
- assess the chemical characteristics of urine that may enhance soil C cycling (pH, electrical conductivity, and urea content); and
- compare the effects of artificial urine and cow urine on soil C cycling.

Soil (Foxton black sand; Inceptisol) was collected from dairy-grazed pasture, sheep-and-beef-grazed pasture and a *Pinus radiata* plantation. The soils were sieved at field moisture (2 mm) and adjusted to 60% of water holding capacity before being repacked into core liners. To differentiate the aspects of cow urine that affected soil attributes the following solutions were applied to the soil cores:

1. No urea: same pH and electrical conductivity as cow urine
2. Urea: same pH, electrical conductivity and urea content as cow urine
3. Artificial urine: same pH, electrical conductivity, urea content, C and N content as cow urine
4. Cow urine
5. Water control.

The pine soil initially retained more urine-C than the grazed soils, but there was greater recovery of urine-C from the pine soils, therefore adsorption may have reduced availability of urine-C in the pasture soils. Respiration fluxes from the treated soils were assumed to be solely derived from the added Solution-C. Between 12 and 17% of the urine-C was degraded in the soils over the 14 day incubation, which compared well with the measurements of the bioavailability of urine-C in Chapter 3.

There was a large increase in soil pH following the application of cow and artificial urine. However, the addition of urea solution did not increase soil pH to the same extent as the urine treatments. This was an important finding, as the general consensus in the literature is that urea hydrolysis is the main cause of soil pH increase in urine patches, but there was some other factor that contributed to increased pH in urine treatments. There was a positive correlation between soil pH and the water soluble C content of the soil.

There was no difference in respiration fluxes between the cow and artificial urine treatments for any of the soils. There was also no priming measured in this incubation study using a mass balance approach. However, it was not possible to distinguish between urine-C and soil C in the leachates, soil water soluble C extracts or respiration fluxes and therefore priming may have occurred but was not measured. The urea treatments also had significantly lower ($P < 0.01$) cumulative CO_2-C fluxes than the urine

treatments for all soils tested. Therefore, urea degradation was not the only contributor to CO_2-C fluxes in urine treatments.

Overall, the pine soil had responded differently to urine addition than the two grazed pasture soils. There were few differences in the response to urine addition between the dairy-grazed and sheep-and-beef-grazed soils in the soil chemical characteristics measured in Chapter 4. Therefore further work was restricted to comparisons between grazed pasture and *Pinus radiata* plantation soils.

The pilot study conducted in Chapter 4 highlighted two main areas that required further investigation: 1) sorption of urine-C may have influenced its availability for mineralisation and, 2) as urine derived CO_2 fluxes could not be differentiated from CO_2 fluxes evolved from the soil, priming of soil C could not be ruled out and further work to determine priming required the use of radio-labelled C. Adsorption of urine-C had not previously been investigated, and neither had the priming potential of urine in pine soils.

8.3.3 Chapter 5: Solubilisation of soil carbon following treatment with cow urine under laboratory conditions

An experiment was undertaken to determine the potential maximum soil C solubilisation following treatment with cow urine and to investigate if adsorption of urine-C to soil may impede the mineralisation of urine-C in soils. The responses of soils under pine and pasture were compared to determine whether soils from long-term grazing sites were less susceptible to soil C dissolution than pine soils. The soils collected also represented a range of clay contents to investigate the influence of clays on urine-C adsorption and soil C dissolution.

Soil samples were collected from five depths (litter, FH, 0-50 mm, 50-100 mm and 100-200 mm) at five paired pine and pasture sites. The soils sampled were the Maramarua clay loam (Hapludult), Rangipo sandy loam (Udivitrand), Motuiti brown sand (Udipsamment), Tokomaru silt loam (Fragiaqualf), Ngaumu silt loam (Dystrustept). Each soil layer was air dried and sieved (2 mm).

To measure adsorption of urine-C to soil, each soil layer was shaken with urine, or water as a control, for 4 hours at 4°C. All experimentation was undertaken at 4°C to minimise microbial mineralisation of C in solution (Liang *et al.* 1996). The slurries were centrifuged and filtered for C analysis and the adsorption of urine-C was calculated. The soil was then extracted with water three times, and soil C desorption and solubilisation was calculated from the C content of the water extracts. Adsorption of urine-C and desorption of soil C were corrected for the water controls.

Adsorption of urine-C to soil was minimal, occurring in some of the layers in the pine soils, but not to any of the layers in the pasture soils. Adsorption of urine-C on all of the layers tested was about 3% of the urine-C content. Therefore, adsorption by soil was unlikely to greatly reduce the availability of urine-C for mineralisation.

Each soil layer exhibited significant desorption of soil C following treatment with urine. Between 9 and 62 mg C g^{-1} of soil C was extracted by cow urine. Soil C solubilisation was significantly different to zero in all soil layers, with the exception of the Pine 100-200 mm layer. The pine soils, inclusive of the litter layers, lost 11% of their C concentration following treatment with cow urine. Excluding the litter layers, the pine soils lost 19%, while the pasture soils lost 28% of their total C concentration. There was large variability between the soils tested, and no significant difference in soil C solubilisation between the pine and pasture soils. It was inferred

that although the pasture soils would have been exposed to urine previously, they remained just as susceptible to soil C solubilisation as the pine soils which had not been previously exposed to urine. The proportion of soil C dissolution increased slightly with increasing soil depth, therefore soil C at depth (100-200 mm) was as susceptible to dissolution by urine treatment as topsoils.

This experiment demonstrated substantial solubilisation of soil C by cow urine, the solubilised soil C would potentially be available for mineralisation or leaching. Soil preparation (air-drying and sieving) likely enhanced soil C solubilisation compared to undisturbed soils, the effect of drying of soils in the field on soil C solubilisation is not known. Further work was required to determine if soil C solubilisation occurs under more natural experimental conditions following treatment with cow urine.

8.3.4 Chapter 6: Priming of carbon in grazed pasture and Pinus radiata plantation soils following application of cow and artificial urine

The primary objective of the work I conducted in Chapter 6 was to determine if cow urine deposition could prime soil C in both pine and pasture soil. A further objective was to determine whether extracellular enzymes and soil C solubilisation contributed to priming events under urine patches. Given that Chapter 4 indicated that urine did not cause priming using a mass balance, this experiment was conducted using radio-labelled urine to be able to differentiate soil C from urine-C. Artificial urine was also assessed for its ability to model cow urine with respect to soil C cycling.

Rangipo sandy loam was collected from paired pine and pasture sites, sieved at field moisture (2 mm), adjusted to 60% of water holding capacity, and repacked into core liners. The soil cores were treated with artificial

urine (labelled with ^{14}C urea or ^{14}C glucose), cow urine, or water, and incubated for 84 days at 25°C.

Respiration fluxes were monitored in all treatments; extracellular enzyme activities were measured throughout the incubation in the cow urine and water treatments but not in the artificial urine treatments. Dehydrogenase activity was monitored as a measure of soil microbial activity, and the extracellular enzymes (β-glucosidase, cellobiohydrolase, urease, and protease) were assayed to determine their role in priming events. Water soluble C and soil pH were measured before and after the incubation in all treatments.

Previous studies had shown that cow urine could lead to soil C priming in pasture soils (Lovell & Jarvis 1996; Kool *et al.* 2006; Uchida *et al.* 2011). However, no previous work could be found investigating if cow urine deposition could prime mineralisation of soil C in pine soils. Positive priming, more C lost by soil respiration than added to the soil in cow urine, occurred in both the pine and pasture soils. Following treatment with cow urine there was a greater loss ($P < 0.01$) of soil C from the pasture soil (4.2±1.3 mg C g^{-1}, 5.1±0.9% of soil C concentration) than the pine soil (2.0±0.1 mg C g^{-1}, 4.0±0.1%). However, the artificial urine treatments exhibited negative priming (less C lost by soil respiration than C added in the artificial urine) and had significantly lower respiration fluxes than the cow urine treatments.

Dehydrogenase, urease and protease activity increased in urine treated soils above the water controls. However, only urease and dehydrogenase activities were positively correlated with soil respiration fluxes. Therefore, priming in our soil was attributed to increased soil microbial and urease activity, leading to enhanced mineralisation of soil C. The remaining extracellular enzymes assayed were unlikely to have contributed to

priming, as there activity did not increase due to urine application. However, only a fraction of the extracellular enzymes in soils was assayed in this work, and it is possible that other enzymes may contribute to priming events under urine patches. The contribution of microbial death and subsequent decomposition to the priming event was not investigated and requires addressing.

The pine and pasture soils also exhibited soil C solubilisation in the artificial urine treatments. Up to 2.2 mg C g^{-1} of soil C (4% of soil C concentration) was solubilised in the pine soil, and 0.8 mg C g^{-1} (0.8%) in the pasture soil. However, solubilisation of soil C did not lead to priming in the artificial urine treatments, implying that the artificial urine was preferentially degraded rather than the soil C that was solubilised. Solubilisation of soil C in the cow urine treatments could not be measured due to inability to distinguish between cow urine derived C and soil C.

The overall conclusion from Chapter 6 was that priming of soil C mineralisation following urine deposition could have contributed to the soil C losses measured by Schipper *et al.* (2007; 2010) under intensively grazed pasture soils. Cow urine primed C mineralisation in pine soil, although to a lesser extent than in pasture soil ($P < 0.01$). There was greater dissolution of C in the pine soil, which did not contribute to greater priming, and the soil C solubilised may not have been as degradable as that of the artificial urine. As this experiment was conducted using repacked cores, further investigation of soil C solubilisation using undisturbed soil was needed.

8.3.5 *Chapter 7: Carbon leaching from undisturbed soil cores treated with dairy cow urine*

The main objective of Chapter 7 was to determine the extent of soil C solubilisation and subsequent leaching in undisturbed soil cores following treatment with cow urine. A second objective was to determine if disruption of soil aggregates or acid neutralising capacity (ANC) forcing were mechanisms of soil C solubilisation. To assess the potential of solubilised soil C to cause priming of soil C lower in the profile, the bioavailability of the leachates from cores was investigated by measuring the degradation of leachate-C over 28 days (25°C).

Solubilisation of soil C following urine inputs was shown to occur in Chapters 5 and 6, but these studies were conducted on disturbed soils which had been air-dried and/or sieved. A further complication of the previous experiments was differentiating between soil C and urine derived C. In the experiment described in Chapter 7, urine from cows fed on a C4 plant enriched diet was applied to the soil cores and $\delta^{13}C$ was used to distinguish between soil C and urine-C.

Undisturbed soil cores of Rangipo sandy loam were taken (10 cm diameter, 5 cm depth) from grazed pasture. The cores were adjusted to field capacity and treated with cow urine (n = 10) that was less depleted in $\delta^{13}C$ relative to the organic matter of the soil. Water was applied to a further 5 cores as controls. The cores were left for 6 hours, as predetermined by a pilot study to be sufficient time for the urine application to lead to the maximum soil pH. All of the cores were then leached with 500 mL of water.

A mixing model was used to determine the amount of soil C in the leachate $\delta^{13}C$ signatures. The leachate-C from the urine cores (ULeachate) contained 28.1±0.9% soil C, and the remaining C in the leachate was assumed to be urine-C. The soil C leached equated to 0.45±0.03% of the soil C concentration leached following urine application. While soil C leaching following urine application was small, it was 10 times the amount of soil C leached (0.048±0.001%) from the water treated cores. About 65±1% (0.63±0.01 g C) of the total C added in the urine was retained by the soil. While there was a small amount of soil C leached (0.14±0.01 g C), there may have been an overall increase in soil C due to the retention of urine-C. While, an overall increase in soil C was indicated in this work, previous work measured a loss of 5.1±0.9% of soil C concentration due to priming of soil C mineralisation following treatment with urine (Chapter 6). Therefore,

Soil pH increased by 0.6 following treatment with cow urine, and was significantly ($P < 0.001$) greater than the water control. The amount of water stable aggregates did not decrease following treatment with urine, and disruption of aggregates was unlikely to have been a major factor in soil C loss from this soil. Urine had a high ANC forcing potential, at 11.8 meq L^{-1} and may have contributed to increases in soil pH, which then ultimately may have enhanced soil C dissolution.

The bioavailability of C in the ULeachate was greater ($P < 0.001$) than that of the urine, as nearly 50% of ULeachate-C, and 13% of the urine-C, was degraded within 28 days. This suggests that there was either preferential retention of less degradable urine-C in the soil or preferential dissolution of more degradable soil C into solution following application of urine to undisturbed cores. The much greater bioavailability of the ULeachate suggests that ULeachate descending through the profile may lead to priming of soil C at depth.

The main conclusions from Chapter 7 were that the application of cow urine caused solubilisation and leaching of soil C. While the leaching of soil C by urine was small (0.5% of soil C concentrations), it was considerably more than would occur following rainfall alone (0.05% of soil C concentration). Soil C deeper than 5 cm in the profile may also contribute to soil C leaching under urine patches (Chapter 5). Therefore, had deeper cores been used in this experiment it was likely that leaching may have occurred lower in the profile consequently current work may have underestimated total C solubilisation and leaching of soil C. However, the fate of solubilised C is not clear as soil C that was leached from the top 5 cm may have been retained lower in the profile or been decomposed. Aggregate disruption was unlikely to have contributed to soil C solubilisation but ANC forcing may have contributed to increasing soil pH, and enhanced soil C solubilisation. Following the movement of urine through the soil, the bioavailability of the urine was enhanced, and could contribute to priming of soil C mineralisation below 5 cm soil depth.

8.4 Discussion

The purpose of my thesis was to investigate if dairy cow urine deposition could lead to a decrease in soil C in pine and pasture soils. The overall conclusion based on the series of experiments was that urine deposition leads to the solubilisation of soil C in the top 5 cm of the profile. The released soil C may then be either mineralised, lead to priming, or may be leached from the top soil (Figure 8.1). Also, priming may be a greater mechanism of soil C loss than leaching.

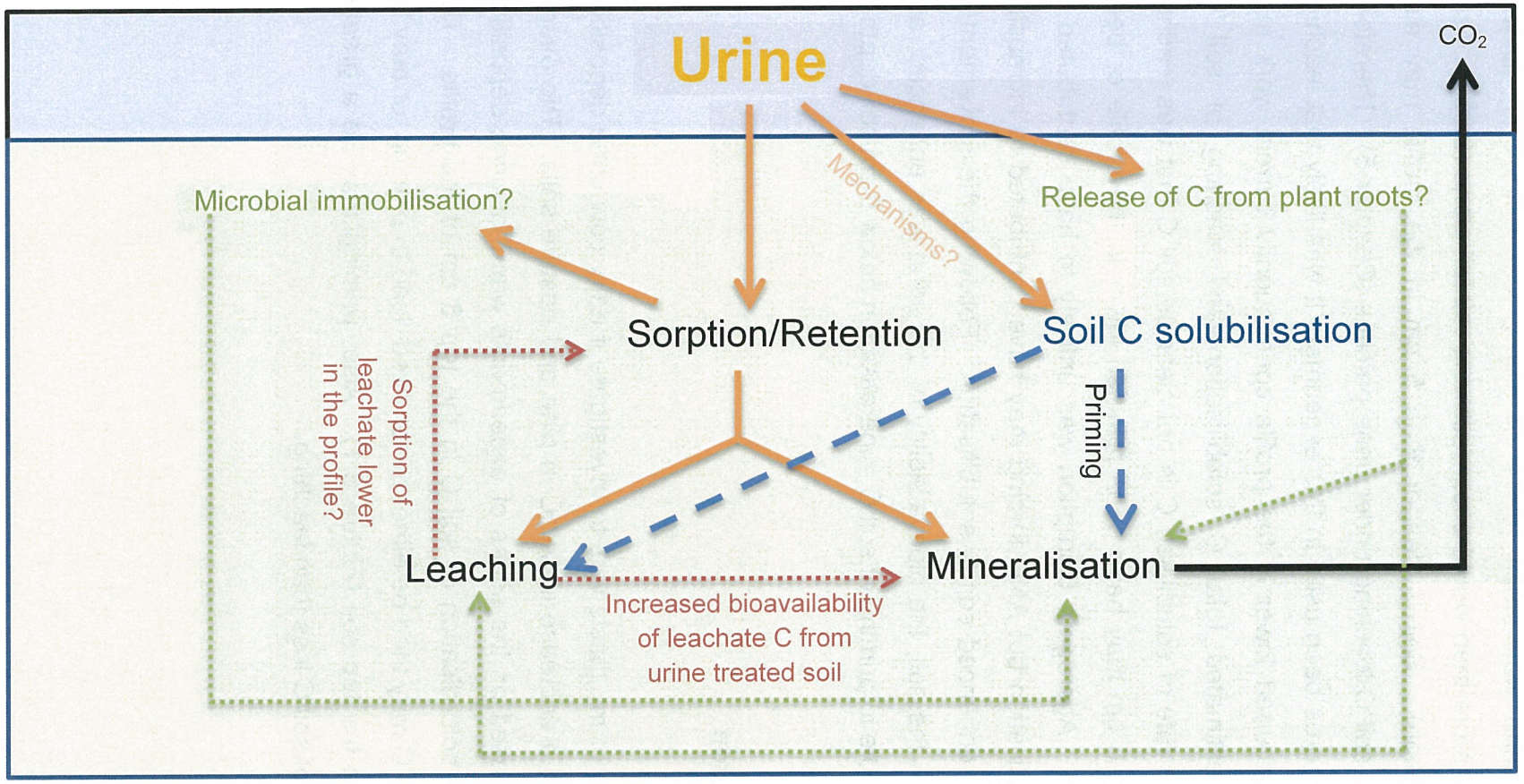

Figure 8.1: Urine carbon cycle for top 5 cm of pasture soil.

8.4.1 Soil carbon solubilisation and leaching

Previous authors have measured increases in dissolved organic C following urine deposition (Shand *et al.* 2000; Uchida *et al.* 2011), however they did not differentiate between soil derived and urine derived C. Using mass balance of radio-labelled ^{14}C and $\delta^{13}C$ enriched urine, the quantification of soil C dissolution was undertaken, and showed that urine deposition led to soil C solubilisation in both pine and pasture soils (Chapter 6; 7). Soil C solubilisation in cow urine patches would be susceptible to leaching processes (Figure 8.1). Although the amount of soil C leached was small, it was 10 times greater than soil C leached from the water controls, and significantly greater than zero ($P < 0.001$). However, the amount of urine-C retained was about 5 times greater than the amount of soil C lost to leaching, which would indicate a potential increase in soil C if the amount of urine-C degraded (~15%; Chapter 4) was less than the amount of soil C primed (5% of soil C concentration). There are some indications that urine deposition can lead to disruption of plant roots (e.g. Doak 1954), and the extent to which carbon released from plant roots contributed to soil C leaching is not known.

The extent of soil C solubilisation was dependent on soil preparation as demonstrated in the Rangipo sandy loam (0-5 cm) soil in Chapters 5, 6 and 7 following different extents of soil preparation. The potential solubilisation of soil C was 35% of soil C concentration in air-dried, sieved soil (Chapter 5). Solubilisation in wet, sieved and repacked soils (Chapter 6) was 0.8% of soil C concentration, and 0.45±0.03% of soil C concentration was solubilised in undisturbed cores (Chapter 7). The largest losses of soil C were found from air-dried and sieved soils, which does not represent the majority of in situ conditions. However, soil can experience drought and disturbance in the field, and the extent of soil C solubilisation under these circumstances has yet to be investigated.

The main mechanism of soil C solubilisation is thought to be the rapid increase in soil pH (Doak 1952; Bristow *et al.* 1992; Haynes & Williams 1992). The increase in soil pH after urine deposition has generally been attributed to the degradation of urine derived urea to ammonium, consuming hydrogen ions. However, urea hydrolysis was not solely responsible for the increase in soil pH (Chapter 4), and it was possible that the cations/anions added in cow urine also increased soil pH. Evans *et al.* (2008) reported a positive correlation between acid neutralising capacity (ANC) forcing, soil pH, and dissolved organic C leaching in soils applied with nitrate based fertilisers. The ANC forcing potential of urine was greater than that of the fertilisers investigated by Evans *et al.* (2008), and ANC forcing may also have raised soil pH and contributed to soil C solubilisation (Chapter 7).

The amount of soil C dissolution was influenced by land use, although the results between the chapters in my thesis were not consistent. The potential dissolution of soil C following treatment with urine (Chapter 5) was the same in the pine and pasture soils. However, in Chapter 6 I measured greater solubilisation of soil C in the pine soil in comparison to the pasture soil treated with artificial urine. Land use treatment differences may have been undetectable in Chapter 5 due to variability, whereas experimentation in Chapter 6 had much less variability, likely as a result of testing with one soil instead of many, as in Chapter 5.

8.4.2 Priming of soil carbon

Urine deposition has previously been reported to cause priming of soil C in grazed pasture soil (Lovell & Jarvis 1996; Kool *et al.* 2006; Uchida *et al.* 2011), but no published literature addressing priming by urine in pine soils could be located. Priming in both pine and pasture soils treated with cow urine was measured in Chapter 6 and loses were greater in the pasture

soil than the pine soil (Figure 8.1). This nullified the hypothesis that pine soils would be more susceptible to soil C loss following urine deposition. This was an unexpected result as pasture soils would have been exposed to urine previously, presumably removing the soil C susceptible to solubilisation and subsequent priming.

There are two possible priming agents under urine patches, C added in the urine itself or soil C solubilised following urine deposition (Figure 8.1). The bioavailability of urine was significantly ($P < 0.001$) less than that of leachate from urine treated pasture soil, which suggests there may have been retention of less degradable urine-C in the soil, or preferential solubilisation of more degradable soil C. Further investigation is required to determine if the priming was a result of the death and turnover of soil microbial biomass as well as increased soil microbial activity.

8.4.3 Artificial urine

Artificial urines may not be an adequate substitute for real urine, despite their common use in the study of N cycling. Few authors have assessed the differences in effects of real and artificial urines on soil nutrient cycling (e.g. Lovell & Jarvis 1996; Kool *et al.* 2006). Hippuric acid was found to be the main component of cow urine (Chapter 3), but is usually included in artificial urines as only a minor component, if at all. Treatment of soil with artificial urine led to negative priming (Chapter 6), whereas cow urine caused positive priming of soil C. This was in agreement with Lovell and Jarvis (1996) and Kool *et al.* (2006) who reported greater CO_2 fluxes from soils treated with cow urine than soils applied with artificial urine. Caution is recommended when using artificial urine to model cow urine in soil C cycling, and further investigation into the composition of artificial urines that could be best used for C cycling research is suggested.

8.5 Limitations of work

I recognise that there are several limitations to the work presented in my thesis. Particularly the laboratory based nature of my work, as this was a relatively untouched area of research; some ground work had to be established. The potential contributors to soil C loss under urine patches have not been assessed in conjunction with one another. While soil C solubilisation was measured during the priming (Chapter 6) and leaching (Chapter 7) experiments, priming and leaching were not measured simultaneously.

For much of the work conducted in this thesis, I have used a comparison of paired pine and pasture soils, with the assumption that the pasture soils would have been less susceptible to soil C loss following urine deposition due to pre-conditioning. While, the paired soils were the same, the different land-uses that they were under would have influenced the quality and quantity of the organic matter present (Guggenberger *et al.* 1994). While previous urine application may have led to pre-conditioning of the pasture soil, it is possible that the compositional differences in the organic matter between the soils may also explain greater losses in the pasture than pine soil.

To decrease experimental variation, the soils collected were often sieved or dried depending upon the purpose of the experiment. Soil preparation, as discussed in Section 8.4.1, has potentially led to overestimation of soil C solubilisation in Chapters 5 and 6. The effect that the soil preparation has on other aspects of soil C cycling is not known, soil priming may also have been enhanced due to repacking and moisture adjustment of the soil (Chapter 6).

The majority of my experiments used only the top 5 cm of the profile, while this shallow layer of soil was most likely to undergo exposure to urine in the paddock, the results of my work were usually qualified by the fact that what occurs below 5 cm is still undetermined for most of the experiments.

While I have shown that urine deposition may lead to a loss of soil C by leaching and/or priming, this loss may not be significant in relation to the amount of C added in the urine or dung deposits. As shown in Chapter 7, the amount of soil C leached (0.14 g C) was less than the amount of urine C retained by the soil (0.65 g C). Whether the retained C was subsequently degraded during the experiment was not measured and the rapid increase in microbial activity following urine deposition (Chapter 6) may have led to at least partial degradation of the retained urine-C.

One particular methodological change that I would make, in retrospect, would be the use of radioisotopes in the priming experiment (Chapter 6). There was a substantial amount of radioactivity that was not recovered at the end of the experiment, and therefore negative priming in the artificial urine treatments was not supported using radio-labelled isotopes. In much of the literature, the recovery of an added isotope is assessed directly following its addition (e.g. Saggar *et al.* 1994) rather than at the end of the experiment. The recovery of the isotopes extracted directly after addition would therefore not be influenced by soil processes during an incubation following the assessment of the recovery. The use of isotopes also inhibited the analyses that could be conducted in the artificial urine treatments, as the isotope facility that I had access to contained somewhat limited equipment. In particular, no assessment of enzyme activity in artificial urine applied soils could be undertaken, which would have been an interesting comparison to the cow urine treatment.

8.6 Future directions of research

The work conducted in this thesis has identified several gaps in the current knowledge of the effect of cow urine on soil C cycling. As all the work was conducted within the laboratory, the next step would be to scale up experimentation, initially still in the laboratory, but eventually to field scale.

Further work is required to determine the mechanisms of soil pH increase following urine deposition. Although urea degradation is likely to contribute to the majority of soil pH increases under urine patches, further investigation into the effect of cation and anion additions is required to determine their role in changes in soil pH through ANC forcing. The soil enzyme urease is responsible for the degradation of urea to ammonium. By inhibiting urease with catechol (Bremner & Douglas 1971) and assessing soil pH change following urine application it may be possible to confirm which other factors in cow urine lead to increases in soil pH. Further, a matrix of solutions containing a range of concentrations of urea, sodium, potassium, calcium, chloride, ammonium salt, magnesium and sulphate could be used to identify the possible cations or anions, or their ratios to each other and urea, that may be responsible for increasing soil pH in urine patches.

A further laboratory experiment would investigate soil solubilisation down through the profile, as well as investigating whether urine or leachate under urine patches can lead to priming of soil C cycling deeper in the profile. This would be a more whole system approach, whereby soil C solubilisation, leaching, and CO_2 evolution would be assessed down the profile in 10 cm increments to a depth of about 50 cm. For this experiment, I would recommend the use of $\delta^{13}C$ enriched urine to differentiate between soil C and urine-C. Although initially, the C composition of urine from cows grazing on a C4 diet would need to be

compared to that of urine from a C3 diet to determine the extent of the different diets on the C composition of the urine.

Eventually, the effect of urine on soil C cycling should be assessed at the field scale. Ideally this would involve the use of large outdoor barrel lysimeters, but as access to such expensive equipment is improbable, a less ideal situation would involve the installation of suction lysimeters to different depths down to 1 metre soil depth in a grazed pasture. Soil respiration and leaching could then be monitored between urine deposition events. Again $\delta^{13}C$ enriched urine would likely be used to separate soil C and urine-C.

Pasture soils appeared to be more susceptible to priming by cow urine than pine soils. To test the influence of the length of time under pasture grazing on priming, I would collect undisturbed soil cores from a pine-to-pasture chronosequence and determine priming of soil C and water soluble C in these cores following cow urine application. I would use a mass balance approach and also determine changes in buffering capacity with respect to soil pH as the length of time under pasture increases.

8.7 Conclusions

My thesis investigated the influence of dairy cow urine on soil C cycling in pine and pasture soils, and has resulted in several important findings.

1. Soil C could be solubilised and lost by leaching or priming under urine patches. There was also greater solubilisation of in the pine soil than the pasture soil.
2. Soil C solubilised in the top 5 cm of the soil following urine addition was available for leaching, 0.5% of the soil's C concentration was leached, compared to the 5% loss due to priming of soil C

mineralisation. Therefore, priming of soil C may be the dominant pathway of soil C loss under urine patches.

3. Soil C loss was greater in the pasture soil than the pine soil to priming. Priming of soil C under urine patches was attributed to increased microbial and urease activity. Further investigation of contribution of microbial death and turnover to the extra CO_2 evolution is needed.

The identification of the major C components of cow urine was an important step in understanding the urine itself. The major component of cow urine was hippuric acid, which although has been the focus of some research in the reduction of nitrous oxide fluxes from urine patches, was not always considered a major component of urine. Hippuric acid, if included in artificial urines, is added in small amounts, so that artificial urines may not adequately model cow urine in studies of soil C or N cycling.

Although soil C solubilisation was measured in pine and pasture soils applied with artificial urine, there was negative priming in both soils. Therefore the relationship between soil C solubilisation and priming requires further investigation, and may be related to the bioavailability of the solubilised soil C. In particular, leachate from urine treated cores was significantly ($P < 0.001$) more bioavailable than the urine itself and could promote priming lower in the profile, but the reasons for this are not known. Further research is required to determine if the small but significant amount of soil C leached from the top soil is held or degraded lower in the profile.

While solubilisation of soil C was quantified, the mechanisms that contribute to solubilisation have not yet been established. An increase in soil pH correlated well with soil C solubilisation (Chapters 4; 6), but the causes of the increase in pH following urine deposition require further investigation. The pH of the urine had no effect on soil pH and urea

degradation was not the sole driver of increasing soil pH, but acid neutralising capacity (ANC) forcing may have been a factor in the increase in soil pH. Soil C solubilisation was not due to disaggregation in the soil tested, and the source of the solubilised soil C has not yet been identified.

A preliminary estimate of the net effect of urine deposition can tentatively be made using the results of this thesis. If I assume that leaching and priming would occur simultaneously, then soil C leaching (0.4 mg g^{-1}) and priming of soil C (4.2 mg g^{-1}) would lead to a loss of 4.6 mg g^{-1}. About 1.8 mg g^{-1} of urine-C was retained in the soil following urine deposition, so there would be a net loss of soil C of 2.8 mg g^{-1} (0.65 Mg C ha^{-1}) from the top 5 cm of the pasture soil that I used for this work. Schipper *et al.* (2011) measured losses of 0.73 Mg C ha^{-1} $year^{-1}$ from the top 30 cm under dairy grazing. Urine deposition may have contributed to these soil C losses reported. There are many assumptions in this calculation, including that all of the soils in New Zealand would lose about the same amount of the Rangipo sandy loam, and that losses of soil C under urine patches would not be balanced by dung or litter inputs. Pine plantations recently converted to pasture grazing may also be susceptible to loss of soil C through priming under urine patches.

8.8 References

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Appendices

Appendix 1: Concentration of carbon compounds isolated from cow urine by pyrolysis GC-MS (µg compound mg urine^{-1}) and their relative abundance. Compounds greater than 5% of the total recovered are in bold. Letters after compound names refer to the group the compound was assigned to if under 5% of the total. [a]Alcohols. [b]Carbohydrates. [c]Carboxylic acids. [d]Tricarboxylic acids. [e]Hydroxy acids.

Compound	Mean (µg mg^{-1})	Std error	Mean (%)	Std error
Hippuric acid	**77.95**	**16.79**	**45.38**	**2.44**
Urea	**53.27**	**8.95**	**17.72**	**1.60**
Amide	**24.50**	**6.25**	**13.60**	**0.95**
Phenaceturic acid	**12.68**	**2.22**	**7.90**	**1.70**
Methanol[a]	7.73	4.77	4.19	2.33
Lactose[b]	7.55	4.41	2.89	1.27
Isocitric acid[d]	0.90	0.65	1.09	0.98
Creatinine[a]	1.65	0.53	0.88	0.20
Oxalic acid[c]	1.47	0.48	0.77	0.14
Maltose[b]	1.89	1.33	0.62	0.39

4-O-α-D-galactopyranosyl]-D-glucose[b]	0.93	0.32	0.59	0.19
Aminomalonic acid[c]	0.99	0.48	0.43	0.13
3-hydroxyphenylacetic acid[e]	0.68	0.15	0.43	0.07
D-glucuronic acid[c]	0.64	0.21	0.34	0.05
D-galactofuranose[b]	0.56	0.31	0.26	0.09
2-ethyl-3-hydroxypropionic acid[e]	0.40	0.11	0.23	0.06
Glycine	0.33	0.12	0.00	0.00
Arabinitol[a]	0.23	0.10	0.13	0.07
Phenylacetic acid[c]	0.22	0.06	0.14	0.03
Erythronic acid[e]	0.17	0.04	0.13	0.06
Tricarballylic acid[d]	0.16	0.06	0.13	0.06
4-phenyl-4-piperidinecarboxylic acid[c]	0.26	0.12	0.10	0.03
3,4-dihydroxybenzyl alcohol[a]	0.15	0.07	0.09	0.04
Aconitic acid[d]	0.13	0.06	0.08	0.05
Pseudouridine	0.14	0.07	0.07	0.03
2,4-hexadien-1,6-diol[a]	0.10	0.04	0.07	0.03
Lactic acid[e]	0.08	0.04	0.07	0.03

D-gluconic acid[e]	0.07	0.02	0.05	0.02
Erythritol[a]	0.06	0.03	0.06	0.03
3-hydroxyphenylpropionic acid[e]	0.08	0.03	0.05	0.02
Glucose[b]	0.17	0.10	0.12	0.08
Total compounds	176.24	40.15	-	-

Appendix 2: Adsorption of urine-C, desorption of soil C, soil C solubilisation for all soil layers shaken with urine (water control corrected). Numbers in brackets represent standard error.

Soil layer	Soil	Adsorption (mg C g^{-1})	Desorption (mg C g^{-1})	Soil C solubilisation (mg C g^{-1})	Soil C solubilisation (%)
Litter	Maramarua	2.6 (1.0)	23.7 (1.3)	21.1	4.0
	Rangipo	7.7 (8.8)	53.2 (6.7)	45.5	8.0
	Motuiti	9.8 (4.6)	53.7 (9.7)	43.9	8.2
	Tokomaru	1.8 (4.9)	36.0 (1.9)	34.1	6.4
	Ngaumu	5.8 (4.0)	35.1 (0.7)	29.4	5.5
FH	Maramarua	9.0 (0.7)	33.9 (1.1)	24.9	9.0
	Rangipo	10.5 (7.3)	65.6 (3.0)	55.1	13.6
	Motuiti	14.1 (2.5)	41.8 (0.7)	27.6	5.7
	Tokomaru	2.1 (1.5)	76.0 (1.3)	73.9	18.3
	Ngaumu	6.7 (0.5)	74.0 (4.2)	67.3	16.7
Pine 0–50 mm	Maramarua	–3.7 (9.5)	10.5 (0.5)	14.2	24.2
	Rangipo	7.2 (2.7)	17.3 (0.7)	10.1	19.7
	Motuiti	7.1 (1.2)	9.2 (1.9)	2.1	4.0
	Tokomaru	1.9 (1.2)	17.4 (0.6)	15.6	26.8
	Ngaumu	11.1 (1.7)	17.4 (0.3)	6.2	9.3

Pine 50–100 mm	Maramarua	3.2 (0.9)	8.8 (0.1)	5.6	15.1
	Rangipo	3.6 (3.3)	14.5 (0.7)	10.9	23.9
	Motuiti	6.2 (1.7)	6.2 (1.2)	–0.1	–0.2
	Tokomaru	1.8 (1.2)	12.6 (0.3)	10.9	35.7
	Ngaumu	4.5 (2.1)	16.3 (0.7)	11.8	24.6
Pine 100–200 mm	Maramarua	1.2 (0.3)	7.7 (0.2)	6.5	43.4
	Rangipo	1.4 (1.9)	13.5 (0.1)	12.1	38.2
	Motuiti	7.3 (2.5)	3.5 (0.6)	–3.8	–25.1
	Tokomaru	3.6 (3.7)	11.6 (0.6)	8.0	38.5
	Ngaumu	10.4 (2.9)	13.2 (0.5)	2.8	9.4
Pasture 0–50 mm	Maramarua	1.7 (0.3)	10.1 (0.2)	8.3	16.2
	Rangipo	–0.9 (1.7)	35.8 (1.1)	36.7	36.0
	Motuiti	6.0 (0.8)	11.3 (0.3)	5.3	10.5
	Tokomaru	–5.3 (1.0)	14.9 (0.1)	20.2	41.9
	Ngaumu	3.2 (4.0)	13.8 (0.6)	10.6	20.8
Pasture 50–100 mm	Maramarua	4.8 (0.8)	9.0 (0.3)	4.1	11.7
	Rangipo	0.7 (1.2)	22.1 (0.3)	21.4	31.2
	Motuiti	5.9 (3.8)	10.3 (0.1)	4.4	15.8
	Tokomaru	–0.8 (3.9)	13.4 (0.2)	14.1	41.5
	Ngaumu	4.4 (2.1)	13.1 (0.4)	8.7	20.4

Pasture 100–200 mm	Maramarua	4.9 (0.4)	7.9 (0.1)	3.0	18.6
	Rangipo	2.8 (2.4)	21.2 (0.2)	18.4	42.1
	Motuiti	3.6 (2.5)	7.9 (0.2)	4.3	26.8
	Tokomaru	–8.8 (2.0)	11.4 (0.1)	20.3	96.1
	Ngaumu	5.1 (1.9)	11.0 (0.3)	5.8	20.9

Appendix 3: Published papers at September 2012.

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Solubilisation of soil carbon following treatment with cow urine under laboratory conditions

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Abstract. There have been reported losses of soil carbon (C) under intensively grazed pastures, and soil C solubilisation following cow urine deposition was identified as a possible mechanism. We measured potential soil C solubilisation in pasture and plantation pine soils following treatment of soil with cow urine. Soils from five paired pasture and pine sites were collected. Adsorption of urine-C and desorption of soil C was determined by shaking air-dried soil with cow urine for 4 h at 4°C, decanting the urine, and then extracting the soil with water. Soil C solubilisation was the difference between adsorption of urine-C and desorption of soil C. Solubilisation of soil C in the pine soils including the organic layers was 21.6 ± 2.6 mg/g (10.5 ± 1.1% of soil C concentration), in the pine soils excluding the organic layers 7.5 ± 2.2 mg/g (18.7 ± 5.8%), and in the pasture soils 12.4 ± 5.3 mg/g (27.8 ± 7.3%). There was no significant difference with respect to soil C solubilisation between the pine (with and without organic layers) and pasture soils. Soil C lower in the profile may be as susceptible to solubilisation as C in topsoils. Adsorption of urine-C was minimal. Solubilisation of soil C under urine patches may contribute to losses of soil C under intensively grazed pastures, and this hypothesis would benefit from further testing under field conditions.

Additional keywords: adsorption, desorption, carbon, pasture, *Pinus radiata* plantation.

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Introduction

Soil organic matter is an integral part of soil quality, influencing water and nutrient retention, aggregate stability, and compaction resistance (Lal 2004; Haynes 2005). Soil carbon (C) is a key component of soil organic matter, and as such, its maintenance is a key factor for sustainable land use (Lal 2004; Haynes 2005). In recent years, intensification of New Zealand's dairy pastures has led to increased nitrogen (N) inputs and cow stocking rates (MacLeod and Moller 2006; Ministry for the Environment 2007), and a decline in soil C has been measured in some dairy pastures (Schipper *et al.* 2007, 2010). The losses of soil C averaged 0.73 ± 0.16 Mg C/ha.year in the top 30 cm of dairy pasture (Schipper *et al.* 2010) and the cause of the C losses was unclear. In contrast, the soil C content of dry stock pastures had not changed over the same period (Schipper *et al.* 2010).

A key difference between dry stock and dairy farming is greater deposition of urine from dairy cows compared with both sheep and dry stock cattle (Haynes and Williams 1993; Bilotta *et al.* 2007). Urine is alkaline and has a high salt content (Lantinga *et al.* 1987; Haynes and Williams 1993), both of which may increase the solubility of soil C (Reemtsma *et al.* 1999). Also, urea degradation in urine patches leads to localised increases in soil pH and solubilisation of soil C (Jackman 1960; Lovell and Jarvis 1996; Shand *et al.* 2002), which may enhance soil C decomposition.

Adsorption may determine the fate of C in soils, and while urea and dissolved organic C (DOC) have both been shown to adsorb to soil (e.g. Broadbent *et al.* 1958; Overrein and Moe 1967; Jardine *et al.* 1989; Liang *et al.* 1996; Kaiser *et al.* 2000; Kalbitz *et al.* 2005), the adsorption of urine-C has yet to be tested. Clays have an important role in the storage of organic matter (Torn *et al.* 1997), and can play a key role in the adsorption of DOC (Jardine *et al.* 1989; Kaiser and Zech 2000). While clay minerals may adsorb DOC, Uchida *et al.* (2008) suggest that urine deposition may lead to soil disaggregation, which could release soil C not previously available (Gregorich *et al.* 1989; Chandra *et al.* 2002). The influence of soil clay content on urine-C adsorption and soil C solubilisation had not been investigated before the present study.

Recently, there has been considerable conversion of forestry to intensive grazing in New Zealand, with an estimated 15 600 ha undergoing conversion in the year ending March 2008 (Ministry of Agriculture and Forestry 2009). The response of recently converted pine-to-pasture soils to urine deposition has not yet been examined. We hypothesise that pine soils will undergo greater soil C solubilisation following urine deposition than pasture soils, as pasture soils would have come into contact with urine previously, and soil C readily soluble by urine would have already been removed.

There were three objectives of this work: (*i*) to determine the potential solubilisation of soil C following treatment with cow urine; (*ii*) to examine whether soil C from plantation forestry is more susceptible to solubilisation by urine than soil C from long-term, grazed pasture soils; (*iii*) to assess the role of soil clay content on urine-C adsorption and soil C solubilisation.

Materials and methods

Soils

Five different soils from the North Island, New Zealand, were collected from paired *Pinus radiata* plantations and adjacent grazed pastures (Tables 1, 2). The trees on the pine sites were >20 years old and closed canopy had been reached; pastures had been grazed for ≥10 years.

A 30-m transect was laid randomly across each sampling site, and a 2.5-cm-diameter core was taken every 2 m along each transect and bulked for each sampling depth. The mineral soil was collected in depth increments 0–50, 50–100, and 100–200 mm from the top of the mineral soil profile. The organic layers from the pine soils (litter; fresh humus, FH) were retained as separate samples. All soil samples were air-dried at 35°C (±1°C), sieved to 2 mm (with the exception of the litter layer), and any roots or stones removed. A 5.6-cm-diameter core was taken for bulk density determination at 10 m along the transect. The bulk density of the soil was calculated by dividing the oven-dry weight of the soil by the volume collected.

The soil layers were analysed for total C and N (LECO FP-2000 CNS Analyzer, LECO Corp., St Joseph, MI, USA), and water-soluble C (Ghani *et al.* 2003). Soil pH was measured in a 1 : 2.5 soil-to-water slurry, and soil electrical conductivity was measured against a 2 M potassium chloride standard (Blakemore *et al.* 1987). The clay content of each soil layer was determined using the pipette method, following dispersion and sieving (Claydon 1989).

Urine

Urine was collected from several grass-fed Friesian cows waiting for 3 p.m. milking in September 2007 (Dairy 1,

Table 1. Location, map position, name, and classification of soils collected from pine and pasture land uses

Location	Map position (NZTM)	Soil name	New Zealand soil classification (Hewitt 1998)
Maramarua	1800035′E, 5867423′N	Maramarua clay loam	Typic Yellow Ultic Soil
Turangi	1841564′E, 5666788′N	Rangipo sandy loam	Podzolic Orthic Pumice Soil
Waitarere Beach	1787675′E, 5510506′N	Motuiti brown sand	Typic Sandy Brown Soil
Palmerston North	1820997′E, 5524533′N	Tokomaru silt loam	Argillic-fragic Perch-gley Pallic Soil
Ngaumu	1844225′E, 5452840′N	Ngaumu silt loam	Mottled Orthic Brown Soil

Table 2. Total N, total C, water-soluble C (WSC), clay content, pH, and bulk density (BD) of soil layers collected from five paired pine and pasture sites

All analyses were undertaken using one subsample from each layer, with the exception of WSC, which was extracted in triplicate for each layer

Soil layer	Soil name	Total N (%)		Total C (%)		WSC (mg/kg)		Clay content (%)		pH		BD (g/cm^3)	
		Pine	Pasture	Pine	Pasture	Pine	Pasture	Pine	Pasture	Pine	Pasture	Pine	Pasture
Litter	Maramarua	0.6	–	52.5	–	3280	–	–	–	–	–	–	–
	Rangipo	0.6	–	57.0	–	3100	–	–	–	–	–	–	–
	Motuiti	0.8	–	53.7	–	1910	–	–	–	–	–	–	–
	Tokomaru	0.8	–	53.4	–	1450	–	–	–	–	–	–	–
	Ngaumu	0.8	–	53.3	–	2810	–	–	–	–	–	–	–
Fresh humus	Maramarua	1.1	–	27.6	–	560	–	–	–	4.9	–	–	–
	Rangipo	1.7	–	40.5	–	460	–	–	–	4.5	–	–	–
	Motuiti	1.5	–	48.8	–	5340	–	–	–	4.5	–	–	–
	Tokomaru	1.6	–	40.3	–	3110	–	–	–	4.8	–	–	–
	Ngaumu	1.6	–	38.4	–	1890	–	–	–	4.5	–	–	–
0–50 mm	Maramarua	0.2	0.4	5.9	5.2	430	140	31	25	4.8	6.1	0.89	0.89
	Rangipo	0.3	0.7	5.1	10.2	70	60	6	6	5.4	5.9	0.60	0.47
	Motuiti	0.3	0.5	5.2	5.1	380	70	5	9	4.5	5.4	0.84	1.02
	Tokomaru	0.4	0.5	5.8	4.8	250	140	17	23	5.0	6.5	0.79	0.74
	Ngaumu	0.4	0.4	6.7	5.1	210	300	22	26	4.8	6.3	0.63	0.74
50–100 mm	Maramarua	0.2	0.3	3.7	3.5	280	240	36	28	4.7	6	1.19	1.16
	Rangipo	0.3	0.5	4.6	6.9	30	60	8	6	5.5	6.1	0.72	0.91
	Motuiti	0.2	0.3	2.9	2.8	190	60	5	9	4.9	5.5	1.23	1.23
	Tokomaru	0.3	0.3	3	3.4	160	110	20	22	4.9	6.1	1.16	1.11
	Ngaumu	0.3	0.4	4.8	4.2	140	240	27	24	4.9	6.4	0.94	0.99
100–200 mm	Maramarua	0.1	0.1	1.5	1.6	200	330	37	33	4.8	5.4	1.22	0.66
	Rangipo	0.2	0.3	3.2	4.4	10	70	7	7	6.1	6.1	0.83	0.85
	Motuiti	0.1	0.1	1.5	1.6	130	40	4	9	5.1	5.7	0.99	1.33
	Tokomaru	0.2	0.3	2.1	2.1	130	90	23	22	5	5.7	1.16	1.13
	Ngaumu	0.2	0.2	3	2.8	110	260	27	24	5.2	6.6	1.15	1.15

Massey University, Palmerton North, New Zealand) and bulked as collected. The total C content of the urine was measured immediately after collection (Win High TOC II; Elementar Analysensysteme GMbH, Hanau, Germany), and the pH of the urine was measured using a glass electrode. The remaining urine was frozen at −20°C until required.

Adsorption and desorption

To determine the amount of soil C solubilised following mixing with cow urine, both adsorption of urine-C and desorption of soil C were measured. The 40 soil samples were tested in triplicate, and shaken with urine or water at 4°C to inhibit microbial activity (Liang *et al.* 1996). Air-dry soil (2.5 g) was shaken with urine (0.025 L) or water (0.025 L) as a control, at 50 rpm for 4 h. A pilot study determined that a shaking time of 4 h (4°C) was required for the soil C and urine-C concentrations to reach equilibrium as determined by a constant C concentration measured in liquid phase following centrifugation and filtration (Fig. 1). The soil : urine slurry was then centrifuged for 20 min at 2500 rpm and the supernatant filtered to 0.45 µm under suction (Labserv, BioLab Ltd, Auckland, New Zealand). The supernatant was analysed for total C (Win High TOC II, Elementar Analysensysteme GMbH, Hanau, Germany). Adsorption of urine-C (mg C/g) was calculated using Eqn 1:

$$\text{Adsorption} = \frac{(\alpha - \beta)*V}{DW} \quad (1)$$

where α is C concentration (mg/L) in urine, β is C concentration (mg/L) in urine following shaking with soil, V is volume of urine (0.025 L), and DW is dry weight of the soil at 105°C (g).

The mean adsorption (mg C/g) of urine-C on each soil sample was corrected for the mean adsorption of the water controls. A positive number indicated that C had been removed from the urine and net adsorption had occurred. A negative number indicated that the C concentration of the urine had increased during shaking due to net desorption of soil C.

Total adsorption of urine-C was calculated for each soil by summing the amount of urine-C adsorbed (mg C) from each layer (water-corrected) divided by the sum weight of soil shaken with urine. Total adsorption for each soil in each land use ($n = 5$) was then averaged for the pine soils including organic layers (total pine litter–200 mm), pine soils excluding the organic layers (total pine 0–200 mm), and the pasture soils (total pasture 0–200 mm).

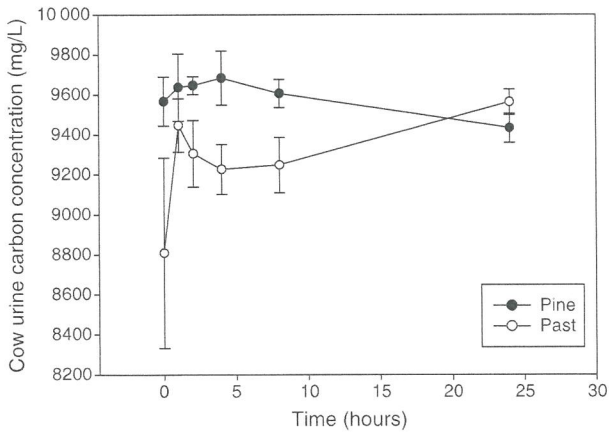

Fig. 1. Carbon concentration of urine shaken with Maramarua pine and pasture soil over a 24-h period. Error bars are ±1 standard error.

After measurement of urine-C adsorption, the soil was refrigerated overnight and then extracted three times with water (2.5 g soil : 25 mL water) for 4 h at 4°C. The C content of the supernatant was measured following centrifugation and filtration as for urine-C adsorption measurement, and the extracted C (mg/g) was calculated using Eqn 2:

$$\text{Extraction} = \frac{(\varepsilon - \lambda)*V}{DW} \quad (2)$$

where ε is C concentration (mg/L) in water after shaking with soil that had been previously shaken with urine, λ is C concentration (mg/L) in water, V is volume of water (0.025 L), and DW is dry weight of soil at 105°C.

Desorption (mg C/g) was calculated as the sum of the three water extractions (Eqn 2). Mean desorption of soil C previously shaken with urine was corrected by the mean desorption of soil C previously shaken with water only.

Total desorption was calculated for each soil profile by summing desorption (water-corrected) of soil C (mg C) from each layer divided by the sum weight of the soil shaken with urine. Total desorption was averaged within each land use ($n = 5$), for the pine soils with organic layers included (total pine litter–200 mm), pine soils without the organic layers (total pine 0–200 mm), and the pasture soils (total pasture 0–200 mm).

Soil C solubilisation

The potential solubilisation of soil C (mg/g soil) for each soil layer was calculated by subtracting the amount of urine-C that was adsorbed (water-corrected) from the amount of soil C that was desorbed (water-corrected); a positive result indicated that soil C had been released into solution. Solubilisation of soil C as a percentage of the soil's initial total C content was also calculated.

Total soil C solubilisation from each soil profile was calculated by subtracting adsorption (water-corrected) of urine-C (mg C) from desorption (water-corrected) of soil C (mg C) for each layer, then summing the mass solubilisation of soil C and dividing by the sum weight of soil shaken. The average solubilisation of soil C from each land use ($n = 5$) was calculated for the pasture soils and for the pine soils with and without the organic layers. Total soil C solubilisation was calculated as a percentage of the initial soil C content for each soil averaged within each land use, and also presented for the pine soils with and without the inclusion of the organic layers.

Statistical analyses

All statistical analyses were undertaken in GENSTAT 12. Adsorption, desorption, and soil C solubilisation were considered to have occurred for each soil layer if significantly different from zero ($P < 0.05$) as tested by one-sample, two-tailed, *t*-tests of the water-corrected means for each soil layer collected.

To determine if there were differences in adsorption of urine-C, desorption of soil C, and soil C solubilisation between the pine, with and without organic layers, and pasture soils, the datasets were assessed using ANOVA with Student–Newman–Kuels analysis. There were considered to be differences between the pine and pasture soils if $P < 0.05$.

Regression analysis was used to test correlations between adsorption, desorption, and soil C solubilisation to soil depth. The organic layers from the pine soils were designated nominal depth titles: –100 mm for the litter layers, and –50 mm for the FH layers. Regression analysis was also used to determine correlations between soil characteristics (soil C concentration and soil clay content) and adsorption, desorption, and soil C solubilisation.

Results

All data are presented as means with standard errors, unless otherwise stated. The organic C content of the cow urine was 14813 ± 704 mg/L, the inorganic C content was 1100 ± 49 mg/L, and the urine had a pH of 8.3. The kinetics pilot study found that 4 h shaking time was sufficient for this experiment (Fig. 1).

The water controls solubilised small amounts of soil C during the adsorption and desorption phases for all of the soil layers ($n = 40$). A small amount of soil C (1.1 ± 0.2 mg C/g) was extracted during the adsorption phase, and a further 2.5 ± 0.6 mg C/g was extracted during the desorption phase. Total solubilisation of soil C was 3.6 ± 0.4 mg C/g soil C in the water controls, which was used to correct solubilisation in the urine treatments.

Adsorption of urine-C was measured in the litter, FH, and pine 50–100 mm layers, but there was no adsorption to any of the pasture soil layers (Table 3). There was also no difference in total adsorption between the pine and pasture soils, regardless of whether organic layers were included in the pine soils. The mean C content of the urine was 16.4 ± 2.9 g C/L ($n = 19$). The mean adsorption of urine-C for all of the soil layers sampled ($n = 40$) was 2.9 ± 0.4% of urine-C content. Urine-C adsorption showed a weak positive relationship to soil C content ($r^2 = 0.11$, $P < 0.05$), but was not correlated with depth or soil clay content.

Following shaking with urine, desorption of soil C occurred in every soil layer tested (Table 3). There was greater total desorption ($P < 0.05$) of soil C in the pine soils when organic layers were included than in the pine soils excluding the organic layers or the pasture soils (Table 3). Desorption of soil C decreased with increasing depth ($r^2 = 0.52$, $P < 0.001$), and increased with increasing soil C concentration ($r^2 = 0.67$, $P < 0.001$). There was no correlation between soil clay content and soil C desorption.

The potential solubilisation of soil C was estimated by subtracting the mass of soil C desorbed from the mass of urine-C adsorbed. All of the soil layers exhibited significant solubilisation of soil C, except the pine 100–200 mm layer (Table 3). Total solubilisation was not significantly different between the pine soils (with and without the organic layers) and the pasture soils (Table 3). Soil C solubilisation decreased ($r^2 = 0.48$, $P < 0.001$) with depth and soil C concentration ($r^2 = 0.61$, $P < 0.001$; Fig. 2). There was no relationship between soil clay content and soil C solubilisation.

All of the soil layers, except the pine 100–200 mm layer, lost a significant proportion of their soil C to solubilisation following shaking with urine (Table 3). The total proportion of soil C solubilised was not significantly different for the pine soils, with or without the inclusion of the organic layers, and the pasture soils (Table 3). The proportion of soil C solubilisation increased with increasing depth ($r^2 = 0.17$, $P < 0.01$; Fig. 3). The proportion of soil C solubilised decreased with increasing soil C concentration ($r^2 = 0.13$, $P < 0.05$). There was no relationship between the proportion of soil C solubilised and soil clay content.

Discussion

Little is known about the fate of urine-C in soils. However, the small amount of adsorption measured here (~3% of the urine's C concentration) indicated that urine-C remained predominantly in solution or in a readily exchangeable form. There are several mechanisms that can contribute to DOC adsorption in soil, including binding to iron and aluminium oxides (Jardine *et al.* 1989; Kaiser and Zech 2000), cation bridging (Greenland 1971; Randtke and Jepsen 1982), and retention by clays (Jardine *et al.* 1989; Riffaldi *et al.* 1998;

Table 3. Adsorption of urine-C, desorption of soil C, and soil C solubilisation for soil layers shaken with cow urine
All data have been corrected for water controls; standard errors are in parentheses ($n = 5$). Values with an asterisk are significantly different from zero ($P < 0.05$). Values in each column followed by the same letter are not significantly different ($P > 0.05$)

Soil layer	Adsorption (mg C/g)	Desorption (mg C/g)	Soil C solubilisation (mg C/g)	Soil C solubilisation (%)
Litter	5.5 (1.5)*	40.3 (5.8)*	34.8 (4.6)*	6.4 (0.8)*
Fresh humus	8.5 (2.0)*	62.1 (7.6)*	53.6 (8.6)*	13.5 (1.9)*
Pine 0–50 mm	4.8 (2.6)	14.4 (1.9)*	9.6 (2.5)*	16.8 (4.4)*
Pine 50–100 mm	3.9 (0.7)*	11.7 (1.9)*	7.8 (2.3)*	19.8 (6.0)*
Pine 100–200 mm	4.8 (1.8)	9.9 (1.9)*	5.1 (2.7)	20.9 (13.0)
Total pine litter–200 mm	**5.4 (1.3)*a**	**27.1 (2.8)*a**	**21.6 (2.6)*a**	**10.5 (1.1)*a**
Total pine 0–200 mm	**4.4 (1.5)*a**	**12.0 (1.8)*b**	**7.5 (2.2)*a**	**18.7 (5.8)*a**
Pasture 0–50 mm	0.9 (1.9)	17.2 (4.7)*	16.2 (5.7)*	25.1 (6.0)*
Pasture 50–100 mm	3.0 (1.3)	13.4 (2.3)*	10.5 (3.3)*	24.1 (5.4)*
Pasture 100–200 mm	1.5 (2.6)	11.9 (2.4)*	10.4 (3.7)*	40.1 (14.4)*
Total pasture 0–200 mm	**1.9 (1.8)a**	**14.2 (4.0)*b**	**12.4 (5.3)*a**	**27.8 (7.3)*a**

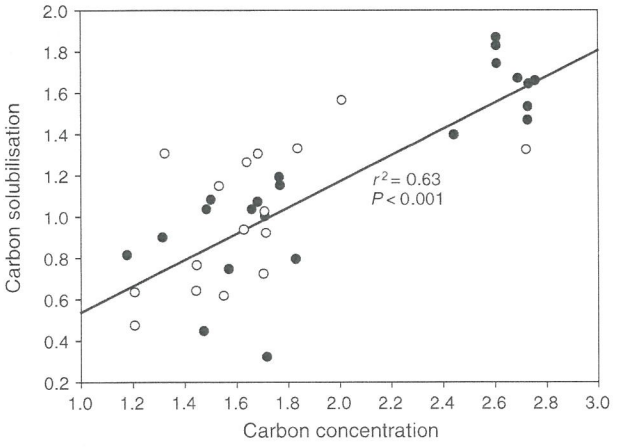

Fig. 2. Correlation between soil C concentration (mg/g) and solubilisation for five pine (●) and pasture (○) soils to a depth of 200 mm ($n = 40$).

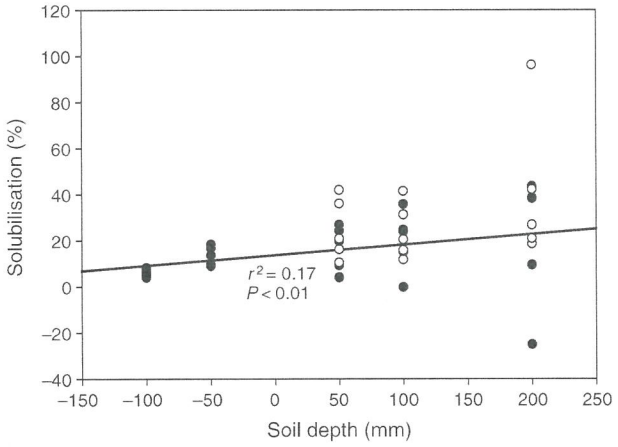

Fig. 3. Proportion of soil C solubilisation down the profile from five paired pine (●) and pasture (○) soils to 200 mm. Litter and FH layers in the pine soils were designated −100 and −50 mm titles ($n = 40$).

Kaiser and Zech 2000). While adsorption of DOC can be impeded by native soil organic matter (Jardine *et al.* 1989; Kaiser and Zech 1998), in this study there was a weak correlation between urine-C adsorption and soil C content (data not shown). There was no correlation between urine-C adsorption and clay content, despite there being a wide range of clay contents (Table 2) in the soils tested (results not shown). Adsorption of urine-C to clay may have been impeded by the high pH of the soil following treatment with cow urine. For example, Specht *et al.* (2000) reported reduced adsorption of organic material on clays with increasing pH. The composition of urine-C may also have inhibited the adsorption to clays. Rennert and Mansfeldt (2003) found the composition of DOC was more important in determining adsorption to clay and silt fractions than was the soil mineral composition. Specht *et al.* (2000) also found that large molecules in organic material were adsorbed, whereas smaller compounds with aromatic or carboxylic functional groups were not. The carboxylic acid hippuric acid makes up nearly 50% of urine-C (Lambie 2011), and may have resulted in lower overall adsorption.

Soil C desorption was measured in all soil layers following shaking with cow urine. Total desorption of soil C was greater ($P < 0.05$) from the pine soils including the organic layers than from the pine soils without the organic layers and the pasture soils. When the organic layers were not included, total desorption of soil C was the same in the pine and pasture soils.

The mass of soil C desorbed was much greater than the mass of urine-C adsorbed; therefore, the soils exhibited substantial solubilisation of soil C in nearly all of the soil layers tested. There was, however, no relationship between clay content and soil C solubilisation, indicating that disaggregation may not have occurred in our experiment, but further testing is necessary to confirm this. The solubilisation of soil C in the water controls (3.6 ± 0.4 mg/g) was relatively insignificant compared with the urine treatments. The solubilisation of soil C in our experiment was a worst-case scenario, and the soil C solubilisation measured here is likely to be greater than would occur in the field. Air-drying of soils and shaking during experimentation can increase the amount of DOC released from soils (Kaiser *et al.* 2001; Limousin *et al.* 2007), and may have increased soil C extraction from the soils tested here. However, the effects of air-drying and shaking with water did not lead to a substantial release of soil C, as shown in the water controls.

Adsorption may have been overestimated due to a loss of urine-C by bicarbonate degassing. Although bicarbonate can be converted to CO_2 by chemical reactions in acidic soils (Zabowski and Sletten 1991), the addition of urine to soils rapidly increases soil pH and can cause an acidic soil to become temporarily alkaline (Haynes and Williams 1992). There was a decrease of 13% of inorganic C following shaking with soil (data not shown, $P < 0.05$). As the pH of the solutions or soil was not measured during the experiment, we cannot confirm whether the loss of inorganic C was due to adsorption or degassing of bicarbonate to CO_2. Since the inorganic C content of the urine was 7% of the total C content, the effect of the decrease in inorganic C on the total C concentration in the estimation of urine-C adsorption was minimal (0.9% of total C).

There was no significant soil C solubilisation in the pine 100–200 mm layer following treatment with urine. Jandl and Sollins (1997) measured a decrease in water-soluble C in a forest soil at the same soil depth. While the reasons for the decrease in solubilisation at this depth in forest soil are not known, it is possible that compositional changes in soil C down the profile (Kögel-Knabner *et al.* 1991; Beyer *et al.* 1993) may lead to soil C that is more resistant to solubilisation in this soil layer.

Our original hypothesis was that less soil C would be solubilised in the pasture soils than the pine soils because the soil C that was easily extracted by urine in pasture soils would already have been lost due to previous urine deposition. However, there was no significant difference in total solubilisation between the pine and pasture soils. This suggests that although the pasture soils would have been exposed to urine previously, the amount of soil C extracted by urine may have been replenished with time, so that soil C remained susceptible to further solubilisation by subsequent urine deposition.

The proportion of soil C solubilisation increased with depth, suggesting that soil C throughout the profile was susceptible to dissolution following urine deposition. Cow urine can travel by macropore flow to as deep as 400 mm in the profile (Williams and Haynes 1994), and 15–25% of applied urine has reached further than 150 mm below the soil surface (Haynes and Williams 1992), with a mean of 17% below 200 mm soil depth (Monaghan *et al.* 1999). In some soils, therefore, soil C lower in the profile is likely to be in direct contact with urine transported by macropore flow. There is some evidence that soil C below 200 mm in the profile is susceptible to mineralisation once released into solution (Schimel *et al.* 2011), and losses of soil C have been reported below 300 mm in the profile under intensive dairy management (Schipper *et al.* 2007, 2010). Cow urine may have contributed to this solubilisation.

Two main factors may regulate soil C solubilisation following urine deposition: rapid increases in soil pH (Jackman 1960; Haynes and Williams 1992; Lovell and Jarvis 1996; Curtin *et al.* 1998; Shand *et al.* 2002; Shand and Coutts 2006); and aggregate disruption (Uchida *et al.* 2008), which releases previously protected soil C (Gregorich *et al.* 1989; Chandra *et al.* 2002). The relative contribution of these mechanisms to soil C solubilisation will need further research.

The fate of dissolved soil C released after cow urine deposition is unclear. The released C may be re-adsorbed lower in the profile, leached, or mineralised by microbes. Guggenberger and Zech (1992) found that, although there was significant desorption of soil C following the application of DOC to topsoils, DOC was adsorbed in the B horizons. The adsorption capacity of layers lower in the profile would have to be large to adsorb the amounts of soil C released from upper horizons measured here, particularly in the pine soils. Further research is needed to determine if soil C released in the upper soil horizons following urine deposition is adsorbed lower in the profile.

The release of previously inaccessible soil C by solubilisation following urine deposition may enhance mineralisation rates (Chandra *et al.* 2002). Solubilisation could release soil C with a greater degradability and could potentially lead to priming of soil C lower in the profile. Priming occurs when a C source is added to a soil which enhances the mineralisation of soil C. However, the re-adsorption of solubilised soil C lower in the profile may decrease the pools of soil C available for mineralisation (Scow *et al.* 1993). Leaching of soil C from grazed pastures has been previously reported Ghani *et al.* (2007, 2010), but the extent to which solubilisation of organic matter in urine patches contributed to these leaching losses was not determined.

Conclusions

Solubilisation of soil C occurred in nearly all of the soil layers investigated after mixing with urine. Soil C solubilisation and the proportion of soil C solubilisation were not significantly different between the pine and pasture soils, whether or not the organic layers were included in the pine soils. The proportion of soil C solubilisation increased with depth, indicating that soil C at depth may still be susceptible to mobilisation following urine deposition. Clay content was not a factor in either urine-C adsorption or soil C solubilisation in the soil layers examined. While the fate of solubilised soil C is not clear, it is likely to be readily available for mineralisation.

While this laboratory experiment requires testing in the field, there are important implications for the sustainability of farming the pasture soils investigated here. Soil C in both newly converted and long-term pasture soils may be solubilised following urine deposition, and subsequently either leached or mineralised. Unless the losses of soil C are matched by C inputs from root turnover, litter, or dung input, an overall loss of soil C in grazed soils is likely. Therefore, dissolution of soil C following urine deposition could partly explain the soil C losses reported by Schipper *et al.* (2007, 2010). Further work is required to determine if soil C solubilisation following urine deposition occurs under field conditions, and to establish the mechanisms of any soil C loss following cow urine application.

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Carbon leaching from undisturbed soil cores treated with dairy cow urine

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Abstract. Solubilisation of soil carbon (C) under cow urine patches may lead to losses of soil C by priming or leaching. We investigated the solubilisation and bioavailability of soil C in undisturbed pasture soil treated with urine. We also studied the contribution of acid-neutralising capacity (ANC) forcing and aggregate disruption as mechanisms of soil C solubilisation. Undisturbed soil cores (0–5 cm; Typic Udivitrand) were treated with water or δ^{13}C-enriched urine and subsequently leached. Urine deposition increased total C and dissolved organic C leaching by 8 g C m^{-2} compared with water. Soil C contributed 28.1 ± 0.9% of the C in the leachate from urine-treated cores (ULeachate). ANC forcing of urine was 11.8 meq L^{-1} and may have contributed to soil C leaching, but aggregate disruption was unlikely to have contributed. The bioavailability of organic C in ULeachate was four times greater than in both cow urine and water leachate. It is possible that ULeachate may lead to priming of soil C decomposition lower in the profile. Further testing under field conditions would determine the long-term contribution of urine deposition to dissolved organic C leaching and the fate of solubilised C in pastoral soils.

Additional keywords: acid neutralising capacity forcing, Carbon-13, cow urine, leaching, pH.

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Introduction

Solubilisation and subsequent leaching of soil carbon (C) under urine patches may be an important contributor to the losses of soil C from intensively grazed dairy soils, as reported by Schipper *et al.* (2007, 2010). Cow urine has been shown to solubilise large quantities of soil C in air-dried and sieved soils (Lambie *et al.* 2012); however, the extent to which soil disturbance contributed to C release was not determined. To overcome the potential effect of soil preparation on soil C solubilisation by cow urine, we investigated soil C solubilisation in undisturbed soil cores following urine application.

While the solubilisation of soil C has been reported following urine application (e.g. Monaghan and Barraclough 1993; Lovell and Jarvis 1996; Shand *et al.* 2000; Lambie 2011), the mechanisms of solubilisation have not been fully investigated. Soil C solubilisation under urine patches was thought to be due to the rapid increase in soil pH as urea was hydrolysed (Doak 1952; Stillwell and Woodmansee 1981). However, hydrolysis of urea is not solely responsible for increases in soil pH following treatment with urine (Lambie 2011). Acid-neutralising capacity (ANC) forcing may also increase soil pH by altering the cation balance (Evans *et al.* 2008). The application of cations in urine (Hutton *et al.* 1965; Richards and Wolton 1976; Haynes and Williams 1993) could displace hydrogen ions from soil cation ion exchange sites into solution, decreasing the proportion of acid saturation and increasing soil pH (Brady and Weil 2008). Evans *et al.* (2008) reported a positive correlation between ANC forcing and dissolved organic C (DOC) losses from soils following application of nitrogen fertilisers, but ANC forcing potential of cow urine has not been investigated.

Rapid increase in soil pH under urine patches may lead to the disruption of soil aggregates; for example, Uchida *et al.* (2008) reported disruption of <2000 µm sized aggregates following urine deposition. Aggregate disruption can release previously unavailable soil C into solution, which can then be rapidly mineralised (Gregorich *et al.* 1989; Chandra *et al.* 2002). Direct measurement has not been used previously to determine the contribution of aggregate disruption by urine to solubilisation of soil C.

Soil C can be lost from the profile following solubilisation by priming and/or leaching. Priming of soil C in both pine and pasture soils following treatment with urine has been measured (Lovell and Jarvis 1996; Kool *et al.* 2006; Lambie 2011). Priming occurs when the mineralisation of soil C is accelerated following the addition of a small amount of degradable C. The potential of urine deposition to lead to priming is dependent on the bioavailability of both the urine-C and soil-C solubilised after urine application, commonly assessed by measuring the degradability of a target compound

in controlled laboratory conditions (McDowell *et al.* 2006; Andreasson *et al.* 2009). The bioavailability of the leachate from soil treated with urine has not been investigated, but the bioavailability of organic C in cow urine was 17–25% over 28 days (Lambie 2011).

The majority of New Zealand's pastures are dominated by C_3 ryegrass rather than C_4 plants (maize or palm kernel), which are used as cattle feed when required (Clark *et al.* 2007). Due to preferential uptake of $^{13}CO_2$ in C_4 plants (Griffiths 2006), cows being fed on a predominately C_4 plant diet will have urine that is less depleted in ^{13}C than the organic matter in soils derived from the turnover of the C_3 pastoral plants. We applied naturally $\delta^{13}C$-enriched cow urine to undisturbed cores collected from pasture dominated by C_3 plants and subsequently leached with water. The main objective of this work was to quantify soil C solubilisation and leaching following urine application in undisturbed soil cores. Further, we assessed the microbial bioavailability of C leached from urine patches and determined the contribution of ANC forcing and aggregate disruption to soil C solubilisation.

Materials and methods

Urine

Ten litres of dairy cow urine was collected from several cows during afternoon milking on the 24 April 2010. The cows had been fed on a high maize diet for several weeks during a drought period (10–12 kg dry weight maize silage + 4 kg dry weight palm kernel; R & S Shearer, Drury, New Zealand). The urine was frozen at −20°C until required. The total C content of the cow urine was 9.1 g C L^{-1}, and the organic C content was 8.8 g C L^{-1}. The urine was frozen at −20°C until required. The total C content of the cow urine was 9.1 g C L^{-1}, and the organic C content was 8.8 g C L^{-1}.

Soil

Thirty undisturbed cores (10 cm diameter), to a depth of 5 cm, were taken in stainless steel liners from Rangipo sandy loam (Podzolic Orthic Pumice Soil, Hewitt 1998; Typic Udivitrand, Soil Survey Staff 1999) under pasture. Some soil characteristics are presented in Table 1. Cattle had grazed the 2.75-ha paddock until two days before collection, at a rate of 5–6 stock units ha^{-1} (Robert Holland, Rangipo Farm Manager, Department of Corrections, Turangi, New Zealand, pers. comm.). Two parallel transects were placed ~2 m apart across the paddock, and 15 cores were taken 2 m apart on each transect line.

Saturated hydraulic conductivity of the cores was measured to determine the rate at which the water should be added during the leaching phase of the experiment to avoid ponding of water on the core surface and reduce the possibility of macropore flow. Ten of the 30 cores collected were randomly selected for saturated hydraulic conductivity measurement as determined by Klute and Dirkson (1986). Fifteen of the remaining cores were adjusted to field capacity by slowly saturating the cores from the base, and draining on 0.5-bar ceramic plates (Soilmoisture Equipment Corp., Santa Barbara, USA) to −10 kPa. The grass on the top of the cores was trimmed to ~2 cm above the soil surface and the soil cores left overnight at 20°C until urine application. The soil in the remaining cores ($n = 5$) was removed from the liners and used for the pilot study (see below).

Table 1. Characteristics of the Rangipo sandy loam at 0–5 cm

Total N (%)	Total C (%)	WSC (mg kg^{-1})	pH	Bulk density (g cm^{-3})	Sand (%)	Silt (%)	Clay (%)
0.7	10.2	60	6.1	0.89	64	30	6

Soil pH pilot study

A pilot study was conducted to determine the time required for soil pH to change after urine application. The pilot experiment consisted of two treatments—water and urine. The soil was sieved (5 mm) at field moisture content, and 20 g of wet soil, in triplicate, was used at each measurement time and for each treatment.

Urine was applied at rate equivalent to 1000 kg N ha^{-1} (Haynes and Williams 1993), or 16.8 mL of urine; the same volume of water was added to the controls. Soil pH was measured at 0, 0.5, 1, 1.5, 2, 4, 6, 8, and 16 h after urine application. On each occasion, 100 mL of ultrapure water was added to the urine- and water-treated soils and periodically shaken over 1 h and the pH was measured with a glass electrode (Rayment and Higginson 1992).

Core leaching

Ten undisturbed cores were irrigated with 105 mL of urine (equivalent to 500 kg N ha^{-1}); a further five cores were irrigated with the same volume of ultrapure water. The urine was applied at 500 kg N ha^{-1} to inhibit leachate evolution before water was applied to the cores in the leaching phase of the experiment. The urine or water was applied with a dropper over 30 min to minimise macropore flow, ponding, and disruption of the soil surface. After 6 h, each core was put in a 110-mm ceramic Buchner funnel and placed under −10 kPa suction to avoid saturation of the soil at the base of the core and to simulate suction that would occur in the field under natural conditions (Phillips and Burton 2005). Milli-Q water (500 mL, ~2 pore volumes) was then applied to the top of each core, at a rate less than the core's saturated hydraulic conductivity (599 mm h^{-1}). The leachate from the cores treated with water (WLeachate) and the cores treated with urine (ULeachate) was collected in Buchner flasks and weighed to obtain the volume of leachate drained from each core.

Soil carbon solubilisation

To determine the amount of soil C present in the ULeachate, freeze-dried subsamples of the urine ($n = 3$), WLeachate ($n = 5$), and ULeachate ($n = 10$) were analysed for $\delta^{13}C$ using isotope ratio mass spectrometry (National Isotope Centre, GNS Science, Lower Hutt, New Zealand). The $\delta^{13}C$ of the soil organic matter ($n = 3$) was also determined.

The percentage of soil C present in ULeachate (dissolved soil C) was determined using a mixing model (Fry 2006):

$$\text{Dissolved soil C} = 100 - [(\delta_{\text{ULeachate}} - \delta_{\text{Soil}})/(\delta_{\text{Urine}} - \delta_{\text{Soil}})] \quad (1)$$

where $\delta_{ULeachate}$ is $\delta^{13}C$ in the leachate from undisturbed cores treated with cow urine, δ_{Soil} is $\delta^{13}C$ of the soil, and δ_{Urine} is $\delta^{13}C$ in the cow urine.

All of the leachates and three subsamples of the added urine and water were filtered to 0.45 μm (Labserv, BioLab, Auckland, New Zealand) and analysed for total, inorganic, and organic C contents (High TOC II, Elementar Analysensysteme GmbH, Hanau, Germany).

The Rangipo sandy loam has a C content of 10.2%, with an average oven-dry (105°C) weight of 285 g soil core $^{-1}$; therefore, each core contained an average of 29 g soil C.

Acid-neutralising capacity forcing

The ANC forcing of the urine was measured by assessing the difference in cation and anion contents in the WLeachate and ULeachate. Subsamples (100 mL) of each leachate were analysed for sodium, calcium, potassium, chloride, magnesium, sulfate, ammonium, and nitrate content (NZ Laboratories Ltd, Hamilton, New Zealand).

The concentration of X_{ion} in the leachates was multiplied by the inverse of the milli-equivalent weight of X_{ion} to convert them from mg L^{-1} to meq L^{-1}. To calculate the ANC of the urine, the concentration of X_{ion} (meq L^{-1}) in the WLeachate was subtracted from the concentration of X_{ion} (meq L^{-1}) in ULeachate and Eqn 2 was applied to the resulting data (Evans *et al.* 2008):

$$ANC = \Delta NH_4^+ + \Delta Na^+ + \Delta Ca^{2+} + \Delta K^+ + \Delta Mg^{2+} - \Delta NO_3^- - \Delta SO_4^{2-} - \Delta Cl^- \quad (2)$$

If the resulting total was positive there was an increase in ANC and pH; a negative number indicated the reverse (Evans *et al.* 2008).

Leachate bioavailability

Bioavailability of the leachates from the cores was determined by measuring the microbial degradation of organic C (OC) in the core leachates. One 25-mL sample from each leachate (ULeachate, $n = 10$; and WLeachate, $n = 5$) and three subsamples of the applied urine were placed into 50-mL glass jars. The glass jars were then placed into 1.8-L Agee jars, sealed, and incubated at 25°C for 28 days. The jars were ventilated in a fume cupboard for 30 min every 2–3 days. Following the incubation, samples were filtered to 0.45 μm (Labserv, BioLab, Auckland, New Zealand) and analysed for OC contents (High TOC II, Elementar Analysensysteme GmbH, Hanau, Germany). Bioavailability was also determined as the amount of OC degraded in 28 days as a proportion of the initial OC content of the leachate or urine.

Soil water-stable aggregates and pH

Each core was removed intact from the liner and cut into quarters from the top down. Two opposite quarters were used for the measurement of water-stable aggregates (see below) and the remaining quarters were sieved through a 4.75-mm mesh and a subsample was taken for measurement of soil pH and moisture content. Soil pH was determined on wet soils in water, 5 g of soil was mixed with 25 mL of water, and pH was measured as described previously.

Water-stable aggregates were measured to determine whether urine application led to the disruption of soil aggregates, using an adaptation of the method of Gradwell and Birrell (1979). The soil was air-dried at room temperature for 16 h until it was dry enough to go through an 8-mm sieve and not smear or disrupt aggregates (53–76% moisture). The samples were not air-dried completely, as is usual for water-stable aggregate analysis, as air drying can lead to strengthening of aggregate bonds and an inaccurate measure of water-stable aggregate content and distribution (Kemper and Rosenau 1986; Haynes and Swift 1990). Soil (100 g) was placed in a 20-cm-diameter sieve pan and 500 mL of deionised water added. This volume of water was found to give the best swirling movement of aggregates in the pan. The pan was placed in an orbital shaker at 50 rpm for 30 min (the optimum shaking time for this method, as any further time led to a breakdown of macro-aggregates). Following gentle agitation in the orbital shaker, the soil was then wet-sieved in a large bucket containing a nest of six sieves with mesh sizes of 2000, 1000, 500, 250, 125, and 63 μm. The samples were manually sieved, lifting and dropping ~50 mm at a rate of 120 rpm for 1 min. Each sieve was washed into aluminium pots and oven-dried at 105°C. The aggregates were weighed in each size class.

Statistical analyses

Statistical analyses were conducted using GENSTAT 12 and were considered to be significant if $P < 0.05$. ANOVA with Student–Newman–Kuels testing was used to determine whether there was a significant difference in the $\delta^{13}C$, total C, inorganic C, OC, each cation, and each anion between the urine, ULeachate, and WLeachate. ANOVA with Student–Newman–Kuels testing was also used to determine if there was a difference in the degradation of OC between the urine, ULeachate, and WLeachate and to assess whether urine deposition led to a decrease in water-stable aggregates. The amount of soil C lost through leaching was tested for difference from zero using a one-sample, two-sided *t*-test. All results are presented as a mean with standard error, unless otherwise stated.

Results

Soil pH pilot study

The pH of the soil peaked (pH 8.3) at 6 h after treatment with urine (Fig. 1). After that time, the pH of the urine-treated soils levelled off. The pH of water treatments was clustered between 6.7 and 7.3 and was quite variable over time. Therefore, the soil cores were incubated for 6 h before leaching with water, as this was when maximum soil C solubilisation may have occurred.

Soil carbon leaching

The volume of leachate from the cores was 488 ± 3 mL ($n = 15$), and there was no difference in the volume of leachate between the urine and water treatments. The total C, inorganic C, and OC contents of the treated water and WLeachate were not significantly different (Table 2). The cow urine contained more total C, OC, and inorganic C ($P < 0.001$) than ULeachate (Table 2). The urine and ULeachate had greater

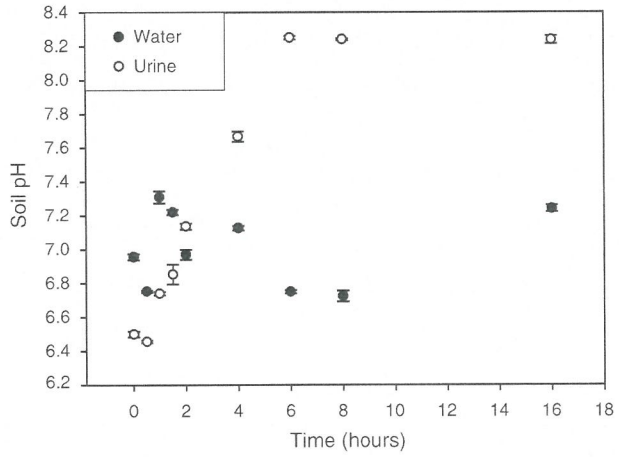

Fig. 1. The pH of soil treated with water or urine over 16 h. Error bars represent 1 standard error ($n = 3$). ○, Urine-treated soil; ●, water-treated soil.

total C, inorganic C, and OC contents ($P < 0.001$) than the water and WLeachate.

The $\delta^{13}C$ of the ULeachate was more negative than that of the urine ($P < 0.001$; Table 3); WLeachate $\delta^{13}C$ and soil $\delta^{13}C$ were not significantly different. A mixing model estimated $28.1 \pm 0.9\%$ of the C present in the ULeachate was derived from soil C (Eqn 1). ULeachate contained 0.481 ± 0.018 g of total C, including both urine and soil C. Therefore, using the mixing model prediction of soil C in ULeachate, the amount of soil-derived total C present in the ULeachate was 0.135 ± 0.005 g. Each soil core contained 29 g C, and so $0.466 \pm 0.018\%$ of the soil's C concentration was lost by leaching after treatment with urine. Although this amount of soil C leaching was small, it was significantly different from zero ($P < 0.001$).

The application of water to the undisturbed cores led to a small amount of soil C leaching, i.e. 0.014 ± 0.001 g soil C or $1\ g\ C\ m^{-2}$, which equated to $0.048 \pm 0.003\%$ of the soil C concentration being solubilised in the water treatment. Leaching in the soil treatment was 10 times less than the amount of soil C solubilised in the urine treatment.

When taking into account the small effect of the addition of water in the control treatment, the amount of soil C in the ULeachate was 0.121 ± 0.005 g total C or $8 \pm 1\ g\ C\ m^{-2}$, and equated to $0.417 \pm 0.018\%$ of the soil C concentration. As 97% of the urine C was organic, the amount of DOC leached in the urine treatments was similar to the total C leached, 0.117 ± 0.005 g C ($8 \pm 0\ g\ C\ m^{-2}$).

As $28.1 \pm 0.9\%$ of the C in the ULeachate was soil-derived, the other $71.9 \pm 0.9\%$ was assumed to be urine-derived. The amount of urine C retained by the soil was $64.5 \pm 1.3\%$ (0.627 ± 0.013 g C) of that added, and therefore the amount of urine C retained was nearly five times what was leached.

Bioavailability of leachate carbon

The proportion of OC degraded in the WLeachate and urine was the same, with 12–13% of OC degraded over 28 days (Fig. 2). The proportion of C degraded in the ULeachate was considerably greater ($P < 0.001$) than in both the WLeachate and urine, with half of the organic C degraded in 28 days (Fig. 2).

Acid-neutralising capacity forcing, water-stable aggregates, and soil pH

The cation and anion concentrations in the ULeachate and WLeachate were generally less than the concentrations in the urine, with the exception of nitrate, which showed no change, and ammonium which increased (Table 4). Cow urine had an ANC forcing of $11.8 \pm 0.6\ meq\ L^{-1}$ in this soil.

Water-stable aggregates for all class sizes in both the water and urine treatments were the same (Fig. 3). The pH of the soil increased to $7.3. \pm 0.1$ in the urine treatment, and was significantly greater ($P < 0.001$) than in the water treatment, which had a soil pH of 6.7 ± 0.1.

Discussion and conclusions

Soil carbon leaching

Stable isotope analysis of the urine leachate demonstrated that there was 10 times more solubilisation of soil C following urine application than water application. As there was little inorganic C in the leachate, DOC solubilisation in our soil was not different from total C leached at $8\ g\ C\ m^{-2}$. This compared well with measurements of DOC leaching under grazed systems. For example, McTiernan *et al.* (2001) and Shepherd *et al.* (2010) measured $4–12\ g\ C\ m^{-2}$ of DOC leaching over 60–74 days. Parfitt *et al.* (2009) measured DOC leaching from North Island hill country soils in New Zealand at rates of $12–23\ g\ C\ m^{-2}\ year^{-1}$, which if assessed for a 2-month period would equate to $2–4\ g\ C\ m^{-2}$; this is less than reported by McTiernan *et al.* (2001). The leaching measured in our work was likely to be an under-estimation of what occurs under field conditions, due to the shallow depth of soil tested and the single application of urine. Although C

Table 2. Total, inorganic, and organic carbon concentration in urine and water applied to, and leachates from, undisturbed cores following application with water or cow urine

Values in parentheses represent 1 standard error. Within columns, values followed by a different letter are significantly different at $P < 0.001$

Solution	Total C		Inorganic C		Organic C	
	($mg\ L^{-1}$)	($g\ m^{-2}$)	($mg\ L^{-1}$)	($g\ m^{-2}$)	($mg\ L^{-1}$)	($g\ m^{-2}$)
Water ($n = 3$)	0.6 (0.0)a	0.0 (0.0)a	0.1 (0.0)a	0.0 (0.0)a	0.6 (0.0)a	0.0 (0.0)a
Water leachate ($n = 5$)	29.2 (2.3)a	1.0 (0.1)a	2.0 (1.2)a	0.1 (0.0)a	27.2 (2.5)a	1.0 (0.1)a
Urine leachate ($n = 10$)	986.3 (38.9)b	31.6 (1.7)b	98.3 (3.8)b	3.2 (0.2)b	888.0 (35.7)b	28.4 (1.6)b
Urine ($n = 3$)	9262.0 (0.4)c	63.9 (2.3)c	329.7 (0.0)c	2.3 (0.1)c	8932.2 (0.5)c	61.6 (2.3)c

Table 3. $\delta^{13}C$ of cow urine, leachate from urine treated soil (ULeachate) and water treated soil (WLeachate) and soil

Values in parentheses represent 1 standard error. Means followed by a different letter are significantly different at $P < 0.001$

Sample	$\delta^{13}C$ (‰)
Urine ($n = 3$)	−20.7 (0.1)a
ULeachate ($n = 10$)	−22.7 (0.1)b
WLeachate ($n = 5$)	−28.3 (0.4)c
Soil ($n = 3$)	−27.8 (0.0)c

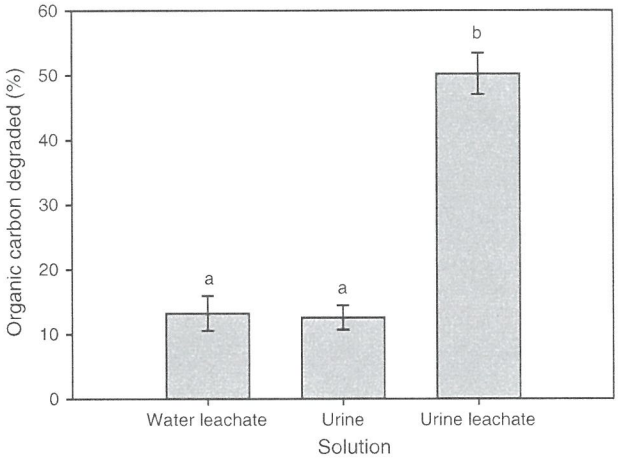

Fig. 2. Proportion of total organic carbon degraded in 28 days in the leachate from water-treated soil cores ($n = 5$), cow urine ($n = 3$), and leachate from soil cores treated with cow urine ($n = 10$). Error bars represent 1 standard error; bars with a different letter are significantly different ($P < 0.001$).

Table 4. Cation and anion concentrations (mg L^{-1}) in the leachate collected from water-treated soil cores (WLeachate; $n = 5$), cow urine ($n = 3$), and leachate collected from urine-treated soil cores (ULeachate; $n = 10$) and the percentage difference between cow urine and ULeachate

Values in parentheses represent 1 standard error. n.c., No change. Means followed by a different letter are significantly different at $P < 0.01$

Cation/anion	WLeachate	Urine	ULeachate	Change (%)
NH_4^+	0.1 (0.0)a	90.2 (1.3)b	174.2 (7.4)c	↑193
Na^+	3.9 (0.4)a	291.8 (6.6)b	43.2 (1.9)c	↓85
Ca^{2+}	2.3 (0.3)a	28.2 (0.7)b	15.3 (1.8)c	↓46
K^+	3.9 (0.4)a	53.4 (0.2)b	3.4 (0.1)a	↓94
Mg^{2+}	0.4 (0.1)a	73.0 (0.2)b	4.4 (0.5)c	↓94
NO_3^-	0.1 (0.1)a	0.0 (0.0)a	0.0 (0.0)a	n.c.
SO_4^{2-}	2.5 (0.5)a	228.1 (0.9)b	30.0 (1.2)c	↓87
Cl^-	2.8 (0.4)a	16.6 (0.0)b	2.3 (0.1)a	↓86

leached from shallow cores may be subsequently adsorbed lower in the profile, further investigation using deeper cores is required.

The potential solubilisation of soil C from air-dried Rangipo sandy loam pasture soil (0–5 cm) was 863 g C m^{-2} (Lambie *et al.* 2012). That experiment represented a worst-case scenario for soil C solubilisation under urine patches, as the soil was air-dried, sieved, and shaken with urine. The amount of soil total C lost from urine application in the current experiment, 8 g C m^{-2}, was considerably less than this potential loss.

Leaching of DOC from water-treated cores was less than from urine-treated cores, and compared well with the leaching reported by Ghani *et al.* (2010). Those authors measured DOC leaching from ungrazed pasture soil (i.e. soil that had not recently been exposed to cow urine) and reported 0.5–3.0 g C m^{-2} of soil DOC leaching from the top 25 cm of the profile over 25 weeks. The DOC leaching in our water treatment was 1.0 g C m^{-2}, which compares well with the results of Ghani *et al.* (2010), although our result occurred after only a single application of water on a shallow core. By comparison, DOC leached under urine patches was 3–18 times greater than DOC leaching under ungrazed pasture (Ghani *et al.* 2010).

Acid-neutralising forcing capacity, soil pH, and water-stable aggregates

ANC forcing has been linked to increased soil pH (Evans *et al.* 2008) and may also be partially responsible for increasing soil pH under urine patches. The ANC forcing capacity of urine was attributed to the large displacement of ammonium ions from the soil into solution by urine, possibly by urine-derived potassium, magnesium, and calcium (Brady and Weil 2008). Degradation of urea applied in the urine may have also contributed to ammonium that was measured in the leachate. Cow urine had ANC forcing of 11.8 meq L^{-1}, compared with the greatest ANC forcing measured by Evans *et al.* (2008) of 0.5 meq L^{-1} following the application of sodium nitrate fertiliser (3 g N m^{-2} $year^{-1}$). Cow urine has a greater potential to increase soil pH than the fertilisers tested by Evans *et al.* (2008), and soil C dissolution under urine patches may be greater than that of $NaCO_3$ fertiliser. To be able to determine the relationship between DOC leaching and ANC forcing under urine patches, further testing is required with a larger range of soils.

The pH of the urine-treated soils increased by ~1 unit, which is within the range of increases in soil pH of 0.5–1.5 following urine application (Stillwell and Woodmansee 1981; Haynes and Williams 1992). Filep *et al.* (2003) found that DOC content of a soil increased exponentially with increased soil pH. Therefore, increases in soil pH could potentially increase availability for leaching or mineralisation of DOC under urine patches.

There was no difference in soil water-stable aggregates between the water and urine treatments; therefore, the disruption of soil aggregates was unlikely to have been a major cause of soil C solubilisation in our soil following urine deposition. In contrast, Uchida *et al.* (2008) found an increase in the instability of soil aggregates in the <2000 µm size class following urine application, using Temuka silt loam, which is a gley soil with impeded drainage (National Soils Database, Landcare Research, NZ). The urine may have had a greater residence time in the Temuka soil than the free-draining Rangipo soil, and therefore had a greater effect due to longer contact time with the aggregates. However, this would be dependent on the extent of preferential flow, which can decrease residence time (Clough *et al.* 1998). Aggregation in soils has been positively correlated with sesquioxide content

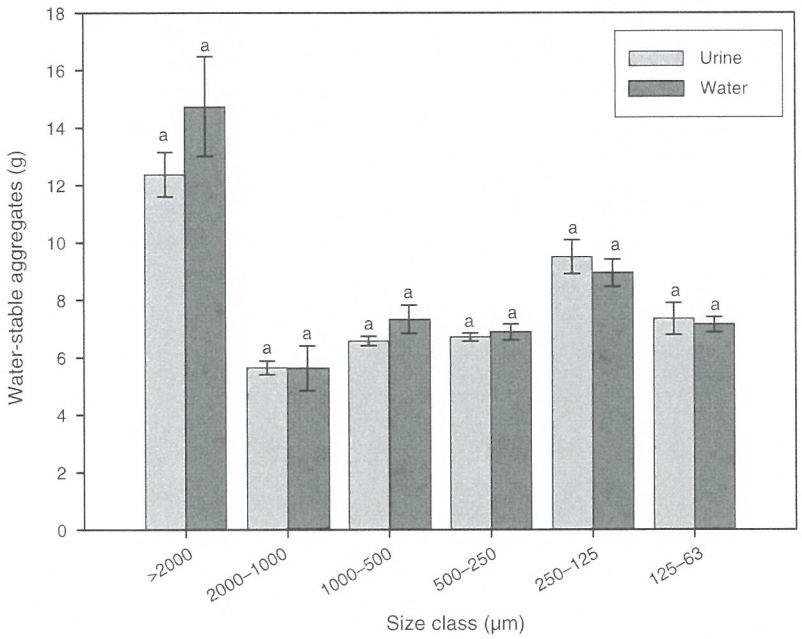

Fig. 3. Soil water-stable aggregates following application with water or urine to undisturbed cores in six aggregate size classes. Solid bars are water treatments, and cross-hatched bars are urine treatments. Error bars represent one standard error, water $n = 5$, urine $n = 10$; bars with the same letters within each class size were not significantly different.

(Barthès *et al.* 2008), which was ~35% of the Temuka soil, whereas the Rangipo soil contained very little of these compounds (National Soils Database, Landcare Research, NZ). The presence of sesquioxides has also been positively correlated with organic matter stability (Barthès *et al.* 2008). It is not known whether urine is capable of disrupting bonds between organic matter and sesquioxides leading to the release of soil C into solution. Further research addressing the influence of soil texture and mineralogy on soil C leaching under urine patches is needed.

Leachate bioavailability

Urine deposition not only led to solubilisation of soil C, but the leachate was more microbially bioavailable than cow urine. About $50 \pm 3\%$ of the OC in the ULeachate was degraded over 28 days, while only $13 \pm 2\%$ of the urine OC was degraded. The degradation of urine C compared well with results of previous work in which 7–25% of the urine C was degraded in 28 days (Lambie 2011). Because of the greater bioavailability of the ULeachate C, it may act as a source of more degradable C for priming lower in the profile. Priming of soil C has been previously demonstrated in the Rangipo soil following the application of cow urine (Lambie 2011); whether ULeachate from the top 5 cm measured in the current study could lead to priming lower in the profile requires further investigation.

There are several potential reasons for the greater bioavailability of C in the ULeachate including: (*1*) preferential retention of the more recalcitrant urine compounds in the soil; (*2*) preferential loss of soil C with greater degradability subsequent to urine deposition; and (*3*) alteration of more recalcitrant urine compounds to more degradable forms during leaching. To date, there are no published reports of preferential retention of urine-derived C compounds; in fact, adsorption of urine C was found to be minimal, with only $2.9 \pm 0.4\%$ of urine C content being adsorbed (Lambie *et al.* 2012). However, preferential adsorption to soils of more recalcitrant or larger compounds from dissolved organic matter has been reported (e.g. Liang *et al.* 1996; Guggenberger *et al.* 1998; Guo and Chorover 2003; Kalbitz *et al.* 2005).

The soil retained $64.5 \pm 1.3\%$ of the added total C of the urine, and leached a small amount of soil C. It is likely that large amounts of retained urine C would be influenced by mineralisation and sorption. The bioavailability of ULeachate was significantly greater than that of the urine, although further testing of the bioavailability of the urine remaining in the soil is required. The urine may also lead to further soil C losses by priming, which has been shown to decrease soil C concentrations by $5.1 \pm 0.9\%$ in this soil (Lambie 2011).

In conclusion, a single urine deposition could increase soil C leaching from undisturbed soil cores by up to 10 times that of water-treated soil, although only $0.42 \pm 0.02\%$ of soil C was leached. However, any losses of soil C through leaching may be mitigated by retention of urine-derived C into soil pools. It is still unclear whether urine deposition may have contributed to the losses of soil C measured by Schipper *et al.* (2007, 2010). Further investigation is required to determine whether DOC leaching is increased with the frequency of urine deposition or after repeated application of urine. Aggregate disruption was not a major mechanism of soil C loss following urine deposition in

this soil, but ANC forcing may have contributed to increases in soil pH leading to soil C solubilisation. The microbial bioavailability of OC in ULeachate was significantly greater than in urine and might contribute to the priming of soil C lower in the profile. The fate of solubilised C under urine patches requires further testing, particularly under field conditions.

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