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Detection of differences in soil carbon and nitrogen stocks between paired dairy and drystock pastures

A thesis

submitted in partial fulfilment

of the requirements for the degree

of

Master of Science in Earth Sciences

at

The University of Waikato

by

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2014
ABSTRACT

Soil is the largest terrestrial store of carbon (C) with some 2000 Pg to a depth of 1 m compared to 500 Pg in the atmosphere. Maximizing storage of C in soil is not only important for reducing atmospheric CO₂ concentrations but also for maintaining soil quality. Recent research has shown that land use management is a key factor in determining the storage of C in pastoral systems. Barnett et al. (2014, AEE 185:34-40) used a paired pit approach to sample 25 adjacent dairy and drystock pastures to a fixed depth of 0.6 m and showed that soils under drystock sites had about 8.6 t.ha⁻¹ more C in the top soil than adjacent dairy sites (P<0.05). However, there was no significant difference between land uses when C was accumulated to 0.6 m.

The main objective of this research was to test a potentially more accurate method for estimating differences in C stocks between sites sampled by Barnett et al. (2014), with a second objective being to better understand the effect of dairy and drystock grazed pastures on soil C and N stocks. A third objective was to investigate the effect of dairy and drystock managed pastures on earthworm abundance and biomass.

A synthesis of recent literature showed that measuring differences in soil C stocks is difficult, given the high variability of soil C over small spatial scales. However, careful consideration to sampling methodology and statistical analysis can greatly improve the detection of differences in soil C stocks.

Twenty three paired dairy and drystock sites were sampled to a depth of 0.6 m by taking 5 soil cores from each of two plots (5x5 m) within a paddock of each land use and soil C/N and soil mass were determined. Seventeen of the paired dairy and drystock farms were sampled from 3 points in each paddock between August and November 2013 for earthworms. Samples were sorted and earthworms were classified to species level.

To a depth of ~60 cm (C stocks adjusted for equivalent soil mass), drystock sites had 1.6 t ha⁻¹ more than dairy sites but this was not significant. However, when soil layers were analysed separately, drystock sites contained more C (4.1 ± 2.1 t C ha⁻¹) in the top 10 cm (P=0.06) and dairy farms had significantly more C (3.7 ± 1.7 t C ha⁻¹) in the 25-60 cm layer (P=0.04). The difference in the relative distribution of soil C in dairy and drystock sites may be due to the greater size and concentration of dairy urine patches which can solubilise C in the top-soil and redeposit dissolved C lower in the profile.

When comparing whole-profile C stocks between dairy and drystock sites, the two-plot coring approach would have been able to detect a true difference of 9.3 t C ha⁻¹, had it occurred, compared to 13.6 t C ha⁻¹ for the pit approach (P<0.05). For the purpose of providing information for future sampling, power analysis was also conducted and revealed that with 23 paired sites, the pit approach could detect a significant difference (P<0.05) of 16 t C ha⁻¹ with 66% certainty. In
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contrast, the coring approach could detect the same difference of 16 t C ha\textsuperscript{-1} with 90\% certainty. These results supported the literature synthesis which demonstrated that sampling methodologies that include spatial variability of soil C can greatly improve the detection of differences. Furthermore, the coring approach reduced cost and increased efficiency compared to the single-pit approach.

Earthworm abundance and biomass were not significantly different between dairy and drystock farms despite the significantly higher grazing intensity and top soil bulk density of dairy sites. Total earthworm abundance and biomass averaged 193 ± 30 ind m\textsuperscript{-2} and 77 ± 12 g m\textsuperscript{-2} for dairy farms compared to 188 ± 26 ind m\textsuperscript{-2} and 75 ± 13 g m\textsuperscript{-2} for drystock farms. These results suggested that for Allophanic Soils in the Waikato Region, the effects of varying grazing management on earthworm abundance and biomass is negligible.
ACKNOWLEDGEMENTS

I would like to extend a sincere thank you to the following people and organisations:

Professor Louis Schipper, my chief supervisor for all his help and for being so approachable. Thank you for your constructive comments and discussions which have helped grow and develop me as a scientist. I have learned to think out-side of the box and explore those “big picture” ideas.

Thank you to Paul Mudge and Scott Fraser from Landcare Research who provided operational support and feedback on experimental design at the initial stages of the project. Dr Ray Littler, for his assistance with statistical analysis; your contribution was greatly valued.

A big thank you to Nicole Schon and Ross Gray from AgResearch who carried out identification of earthworms and conducted some of the initial data analysis.

Jack Pronger, for assistance with field work and for keeping me entertained while meticulously searching for earthworms. Thanks to all others who assisted me in the field, you know who you are. To Janine Ryburn, for assistance with lab-related work; your help was much appreciated. A big thank you is also due to the other Msc and Phd students who have made my experience of writing a thesis an enjoyable and memorable one.

I would like to acknowledge the many farmers I approached for allowing me access to field sites and assisting with site information. Most farmers could not have been more approachable and helpful.

Dairy NZ and for financial assistance and the following scholarships which I was honoured to receive:

- University of Waikato Masters Fees Scholarship
- University of Waikato Masters Research Scholarship

This financial assistance has been greatly appreciated.

My family, for your constant love and support through my years of study. Matt and Paul, thanks in particular for providing me with many welcome distractions by way of my fly rod and hiking boots. All my friends who have encouraged me and supported me along the way, thank you.

Finally, I would like to thank God who has given me the strength and ability to complete this work.
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<th>Description</th>
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<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>CMS</td>
<td>Carbon monitoring system</td>
</tr>
<tr>
<td>C-POM</td>
<td>Coarse particulate organic matter</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>ES</td>
<td>Effect size</td>
</tr>
<tr>
<td>ESM</td>
<td>Equivalent soil mass</td>
</tr>
<tr>
<td>IPCC</td>
<td>Intergovernmental Panel on Climate Change</td>
</tr>
<tr>
<td>i-POM</td>
<td>intra-aggregate particulate organic matter</td>
</tr>
<tr>
<td>LSD</td>
<td>Least significant difference</td>
</tr>
<tr>
<td>MDD</td>
<td>Minimum detectable difference</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NPP</td>
<td>Net primary productivity</td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>Variance</td>
</tr>
<tr>
<td>Pg</td>
<td>Petagram ($10^{15}$ g)</td>
</tr>
<tr>
<td>SOC</td>
<td>Soil organic carbon</td>
</tr>
<tr>
<td>SOM</td>
<td>Soil organic matter</td>
</tr>
<tr>
<td>UNFCCC</td>
<td>United Nations Framework Convention on Climate Change</td>
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CHAPTER ONE

Introduction

1.1 Background

The amount of carbon (C) stored in soil is equivalent to the amount stored in both the atmosphere and terrestrial vegetation (Conant et al., 2003). Soil plays a fundamental role in the global C cycle with the upper 1 m containing 1500-2000 Pg \((10^{15} \text{ g})\) of organic C (Don et al., 2007; McSherry and Ritchie, 2013). The global soil C sink is highly dependent on the fragile balance between C input and C mineralization (Don et al., 2007). Human induced changes to this dynamic balance have resulted in large transfers of C from the soil to the atmosphere (Conant et al., 2003). There is much concern that changing land use and poor land use management could result in increased atmospheric CO\(_2\) concentrations, thus enhancing global warming (Han et al., 2010). For example, Fernández-Ugalde et al. (2013) detected C losses of about 0.6 g kg\(^{-1}\) year\(^{-1}\) across 2000 sites in England and Wales which equated to 8% of the UK emissions in 1990. Soil C is also an essential component of soil quality as it improves soil structure, nutrient cycling and soil moisture holding capacity (Han et al., 2010).

About 40% of earth’s land is grassland with 25% being grazed under a range of grazing intensities and management practices (McSherry and Ritchie, 2013). Studies have shown that increased grazing can increase soil C stocks (Li et al., 2007), decrease soil C stocks (Golluscio et al., 2009) or have no effect on soil C stocks (Abril and Butcher, 2001). McSherry and Ritchie (2013) carried out a meta-analysis of studies focusing on the effect of grazing on soil C stocks. Six variables, including; soil texture, precipitation, grass type, study duration, grazing intensity and sampling depth explained 85% of the variation in SOC
Introduction

stocks. Such evidence suggests that the effect of grazing on SOC stocks was highly context specific. The study by McSherry and Ritchie (2013) clearly demonstrated that further investigation on the effect of grassland management on soil C stocks is needed. This is particularly important in New Zealand because of the large area of grazed pastures and increasing intensity of their use.

Many studies have recognised the importance of increasing the sequestration of C into soil as a means of reducing atmospheric CO$_2$ concentrations and as a result, the United Nations Framework Convention on Climate Change (UNFCCC) made it mandatory to report greenhouse gas removals and emissions for soil C pools at the national scale (Fernández-Ugalde et al., 2013; Han et al., 2010; Hewitt et al., 2012; Six et al., 2000; Smith et al., 2008). In New Zealand, the Soil Carbon Monitoring System (CMS) was developed as a national inventory for soil C stocks (Scott et al., 2002; Tate et al., 2005).

The implementation of carbon-trading schemes has seen the scope narrow from national inventories to farm scale inventories where the direct measurement of soil C stocks is required (Singh et al., 2013). For example, the Waikato Regional Council is developing a regional carbon strategy which involves converting marginal land to forestry or natural bush, thus enhancing C sequestration into soils and biomass at a regional scale. With such legislation, comes the need to accurately and efficiently quantify C stocks at the farm scale. However, soil C stocks are highly variable at the farm scale and changes in C stocks (through time and space) are relatively small compared to the total amount of C stored in the soil (Allen et al., 2010; Chan et al., 2009). At the paddock scale, the coefficient of variation of C stocks for pasture systems can range from 8% (Giltrap and Hewitt, 2004) to as high as 40% (Heckman et al., 2009; Ozgoz et al., 2011) depending on the landscape and management.

Detecting changes in C stocks associated with changes in land use and/or management is dependent on statistical power which is the probability of detecting a difference between treatments, if a difference does in fact occur
Introduction

(VandenBygaart et al., 2007). Power analysis determines the number of samples required to quantify a specified difference (minimum detectable difference) between treatments (Kravchenko and Robertson, 2011; Singh et al., 2013; VandenBygaart et al., 2007). The power to detect small changes in soil C stocks is dependent on the sampling design and/or the number of samples taken (Allen et al., 2010; Kravchenko and Robertson, 2011; Vandenbygaart and Allen, 2011). Poor sampling design and/or a small sample size may increase the probability of committing type II error, that is when no difference is found between treatments when in fact a true difference does exist (Kravchenko and Robertson, 2011). Unfortunately, many C sequestration studies have failed to use power analysis and there is high chance that many of these studies have committed type II error (Kravchenko and Robertson, 2011; VandenBygaart and Angers, 2006).

The difficulty in detecting differences in C stocks is often confounded by differences in bulk density, either through time or between land uses. The Intergovernmental Panel on Climate Change (IPCC) have made it mandatory to calculate C stocks using the depth based approach where the C stock (t C ha\(^{-1}\)) is calculated as a product of depth (m), bulk density (t m\(^{-3}\)) and percent C (Gifford and Roderick, 2003). However, failing to take differences of soil mass into account can lead to false conclusions around the difference in C stocks (Ellert and Bettany, 1995; Gifford and Roderick, 2003). As a consequence, a number of calculations have been developed to compare C stocks by an equivalent soil mass (ESM) rather than to a fixed depth (Ellert and Bettany, 1995; Gifford and Roderick, 2003; Wendt and Hauser, 2013). For example, Wilson et al. (2010) compared C stocks between improved pasture and woodland down to 30 cm and found the depth based approach to give a non-significant difference of 21.8 t C ha\(^{-1}\) (P>0.05). However, recalculation of C stocks to an ESM gave a statistically significant difference of 29.5 t C ha\(^{-1}\) (P<0.05).

In New Zealand, at least 75% of the original forest cover was converted to native or exotic pasture systems (Hewitt et al., 2012). Grazed land occupies
11.1 million ha of New Zealand’s land with 48% on flat to gently rolling land (<15°) and 52% on hill country (>15°) (Schipper et al., 2010). A number of studies have focussed on the consequences of land use management / changes in land use on soil C stocks in New Zealand (Barnett et al., 2014; Hewitt et al., 2012; Schipper et al., 2007; Schipper et al., 2010; Tate et al., 1997). Schipper et al. (2007) re-sampled 31 soil profiles throughout New Zealand which had originally been sampled 17-30 years previously. Losses of C averaged 2.1 kg C m⁻² which equated to an average decline of 106 g C m⁻² yr⁻¹. Schipper et al. (2010) extended re-sampling to 83 profiles to determine whether C stocks were related to land use. On flat land, dairy grazed systems lost 0.73 ± 0.16 t C ha⁻¹ yr⁻¹ (0-30 cm) while drystock (beef and sheep) systems showed no significant change in C stocks. Schipper et al. (2014) further extended their initial resampling of 83 profiles to 144 profiles to better detect changes in total C stocks (0-0.9 m) over a period of 2-3 decades throughout New Zealand. Losses of C through time were constrained to Allophanic and Gley soils but grazing type (dairy vs. drystock) was no longer found to be a significant predictor of C loss.

Barnett et al. (2014) went on to test the hypothesis that drystock systems have less C than dairy systems by sampling 25 adjacent dairy and drystock farms in the Waikato. On average, in the top 60 cm, dairy systems had 173.1 ± 12.4 t C ha⁻¹ and drystock systems had 182.7 ± 15 t C ha⁻¹ and there was no significant difference. However, when only the A horizons were considered, there was significantly more C in the drystock systems (8.6 t C ha⁻¹) compared to the dairy systems. Both Schipper et al. (2014) and Barnett et al. (2014) used the pit approach to quantify soil C stocks and were thus likely constrained in their ability to detect small changes or differences in soil C stocks.

The rationale behind this thesis was that detecting smaller differences in C stocks between adjacent dairy and drystock paddocks (Barnett et al., 2014) was possible by using a more powerful sampling strategy. A replicated coring approach that took into account the inherent spatial variability in C stocks would be able to detect smaller differences in C stocks between land uses.
1.2 Aims and objectives

The overall aim of this research was to improve our understanding on the effects of dairy and drystock managed land on soil C and N stocks by using an improved sampling approach.

The following objectives were set:

1. To test the findings of Barnett et al. (2014) and determine whether there was a statistically significant difference in C and N stocks between adjacent dairy and drystock farms.

2. To test a potentially more powerful and efficient method for detecting differences in C stocks through time and between land uses.

3. Determine the effect of dairy and drystock managed farms on earthworm biomass and abundance.

1.3 Thesis layout

Chapter two reviews the literature on the factors affecting the storage of C in pasture soils and focuses specifically on the range of experimental designs and sampling methods which are used to measure and quantify C stocks.

Chapter three is the focus of this thesis and presents results from a study on the detection of differences in C and N stocks between adjacent dairy and drystock farms. Chapter three has been written in the form of a scientific paper for subsequent submission to a peer reviewed journal. Additional methods and raw data for this study are found in the appendices.
Chapter four presents results from a study on the impact of dairy and drystock managed farms on earthworm abundance and biomass.

Chapter five provides a summary, conclusions and recommendations for future research.
CHAPTER TWO

Literature review

2.1 Purpose and structure of this literature review

This literature review provides an overview of the effect of land use on soil C and N stocks with a particular focus on the measurement of soil C and N stocks in pastoral systems. In section 2.2, the different forms of C and N are described and the importance of C:N ratio reviewed. The factors affecting the storage of soil C will be discussed (section 2.3) followed by a brief section on land use effects on soil C and N stocks (section 2.4). The different experimental designs used to detect changes in SOC (spatially and temporally) are outlined in section 2.5, including an examination of statistical power. Lastly, a review of studies looking at the calculation of SOC stocks is given in section 2.6.

2.2 Soil C and N

Soil organic matter (SOM) is important for a number of soil processes including soil fertility, erosion, soil structure and water retention/transmission (Blanco-Canqui and Lal, 2004). On a global scale, 1500-2000 Pg ($10^{15}$ g) of organic C is stored in the upper 1 m in the form of decomposed plant litter and residues (Don et al., 2007; McSherry and Ritchie, 2013). Soil organic matter is a mixture of living matter (e.g. roots and microorganisms), un-decomposed dead material and highly decomposed matter (McSherry and Ritchie, 2013). Soil organic matter contains about 55% soil C and 45% other essential elements (Blanco-Canqui and Lal, 2004). The accumulation or depletion of C in the profile is dependent on the dynamic balance between the inputs (e.g. photosynthesis, re-deposition of eroded C, organic matter imports) and the outputs (e.g. erosion, leaching, ecosystem respiration, product export) (Guo and Gifford, 2002). This balance is dependent on the interaction between biota (autotrophs and heterotrophs), environmental
factors (e.g. moisture and temperature) and land use effects (Feller and Beare, 1997; Post et al., 2001).

Soil organic C is partitioned into two main groups: the light fraction, also known as coarse particulate organic matter (C-POM) and the heavy fraction (Tan et al., 2007). The light fraction consists of particulate plant and animal residues which are not complexed with mineral particles. The heavy fraction consists of C which has been stabilized into organo-mineral complexes with clay and silt particles by soil fauna and microbes (Post et al., 2001; Tan et al., 2007). The input of C rich plant residues encourages the formation of microbial-derived binding agents which initiate the formation of macro-aggregates (Gregorich et al., 2006; Six et al., 2004). Contained within the macro-aggregates is coarse intra-aggregate particulate organic matter (coarse iPOM). The coarse iPOM is decomposed further into fine intra-particulate organic matter (fine iPOM) which becomes encrusted with minerals to form stabilised micro-aggregates within the macro-aggregates (Six et al., 2004). The turnover rate of the heavy fraction (micro-aggregates) is in the order of decades/centuries compared to a time scale of months to years for the light fraction (Post et al., 2001).

Most of the N (>95%) stored in soil is in an organic form, covalently bound to C. The remaining N is in inorganic forms, including ammonium, nitrate and nitrite (Schlesinger, 2009). A number of studies have demonstrated strong linkages between changes in C and N (Piñeiro et al., 2009a; Schipper et al., 2004). For example, Schipper et al. (2010) re-sampled 83 sites to determine the change in soil C and N stocks over time under a range of pastoral land uses and landscapes throughout New Zealand. They found a strong relationship ($R^2=0.79$) between the change in C and change in N stocks through time suggesting that changes in the storage of N was highly dependent on changes in C stocks. Similarly, Piñeiro et al. (2009a) demonstrated a strong relationship between changing C and N stocks ($R^2=0.9$) associated with different grazing regimes.

The C:N ratio gives an indication of how much more N can accumulate in a soil. Soils rarely have a C:N ratio of less than 10 because organic forms of N are
rapidly hydrolysed or mineralized to inorganic forms (Schipper et al., 2004). Schipper et al. (2004) studied 138 New Zealand soils and demonstrated that 5% of the soils could no longer hold any more N and 12% would have reached full capacity in the next 30 years. Low C:N ratios may cause lower immobilisation rates resulting in a greater proportion of applied N been lost through leaching pathways (Schipper et al., 2004).

2.3 The effect of mineralogy on the storage of C

A number of site and soil related factors influence the storage of SOC including soil fauna, roots, microorganisms, soil mineralogy, slope, aspect and climate (Allen et al., 2010; Six et al., 2004). Many studies have shown soil mineralogy to have the greatest effect on soil C storage (Allen et al., 2010; Don et al., 2007; Shukla et al., 2004). For example, Bayer et al. (2006) observed a positive relationship between the concentration of kaolinite and iron oxide and C concentrations for tropical and sub-tropical soils in Brazil. They suggested that minerals such as kaolinite are important for physically protecting and stabilising SOM. For tropical soils, the concentration of soil C is highest in micro-aggregates which have been stabilised by minerals and oxides (Six et al., 2004). Although clay content plays an important role in stabilizing SOM in soil, there is not definitive evidence to suggest that increased clay content always causes increased storage of C (Oades, 1988). This is because clay content also indirectly affects plant growth (and therefore C inputs) by influencing the chemical, biological and physical properties of a soil (Gregorich et al., 2006).

Soils developed from volcanic ash accumulate large quantities of C with average C stocks of 254 t C ha\(^{-1}\) to a depth of 1 m (Batjes, 2014; Krull et al., 2001). In New Zealand, Allophanic Soils have high C contents compared to most other soils with mean stocks of 128 t ha\(^{-1}\) to a depth of 20 cm (Percival et al., 2000). Allophane is thought to physically protect SOM because organic molecules bind with the inter-tubular spaces of the imogolite spherules (Boudot, 1992). However, Percival et al. (2000) found a poor correlation between allophane content and C
content and suggested the high C content of Allophanic soils can be attributed to the good physical conditions of the soil and high P content. The protection of C in Allophanic soils is not only due to the protection of C by Al-containing allophanic clays but also the formation of organo-metallic complexes between Al$^{3+}$ and organic molecules (Krull et al., 2001).

2.4 Effect of land management on C stocks of pasture soils

The impact of grazing on soil C and N stocks is a complex and controversial subject (Piñeiro et al., 2009a). Studies have shown highly intensive grazing systems to increase soil C (Li et al., 2007), decrease soil C (Golluscio et al., 2009) or have no effect on soil C stocks (Abril and Bucher, 2001). A number of hypotheses have been postulated to describe the effect of grazing on Soil C stocks (Abril and Bucher, 2001; Piñeiro et al., 2009b; Steffens et al., 2008). Grazing intensity can change soil C stocks indirectly by changing the N dynamics of a system or directly by changing the physical properties of the soil environment.

Some studies have suggested that grazing intensity affects soil C storage indirectly because of changes in soil N stocks. Piñeiro et al. (2009a) proposed the *N-loss hypothesis* which describes how increased grazing pressure increases losses of N through leaching pathways and volatilization (Piñeiro et al., 2009b). Total N stocks may be reduced which then limits the formation and storage of SOM in response to decreased primary productivity. The *N-loss hypothesis* assumes that grazing pressure increases N losses while external N inputs remain constant (Piñeiro et al., 2009b; Piñeiro et al., 2006). In contrast, the *root-N retention hypothesis* states that the higher N inputs associated with intensively grazed systems stimulates root production, thus increasing soil C stocks (Piñeiro et al., 2009b). For example, Conant et al. (2001) found that soil C increased in response to improved management of grasslands because N fertilization and irrigation were increased. Irrigation and fertilization were found to stimulate pasture production, thus increasing inputs of organic matter into the soil.
Higher grazing intensity may also alter soil C storage directly because organic matter inputs may be reduced as a consequence of lower pasture production (Abril and Bucher, 2001; Steffens et al., 2008). High stocking rates can cause severe soil compaction, leading to a deterioration of soil physical properties and reduced pasture growth (Steffens et al., 2009; Steffens et al., 2008). In addition to reduced organic matter inputs, increased soil compaction and disturbance can stimulate organic matter decomposition because soil aggregates are disturbed by mechanical stress (Six et al., 2004; Steffens et al., 2008). For example, Barnett et al. (2014) found drystock systems to have significantly more C in the A horizon compared to dairy systems and attributed the difference in part to the higher disturbance of dairy soils imposed by higher stocking rates.

The relative importance of direct vs. indirect mechanisms for determining the effect of grazing management on soil C stocks is dependent on a number of site related factors including soil type, vegetation and climate (McSherry and Ritchie, 2013). Schipper et al. (2010) sampled 83 sites in New Zealand to determine if temporal changes in soil C stocks had occurred for pastoral land uses. Over an average time period of 27 years, dairy farms were found to have lost significantly more C compared to drystock systems. Schipper et al. (2014) extended re-sampling of soil profiles to 148 sites to better balance the distribution of major soil orders and found that land use (dairy vs. drystock systems) was no longer a significant predictor of soil C loss. The difference in results obtained by Schipper et al. (2010) and Schipper et al. (2014) was related to a better distribution of major soil orders in the latter study. This supports the findings of McSherry and Ritchie (2013) that in addition to grazing management, a wide range of site specific factors are also important for determining C storage.

Since the effect of land management on soil C stocks may be confounded by a number site specific variables, it is essential that when comparing total C stocks from different land uses a) the site variables between respective land uses are constant and b) enough samples are taken to ensure sufficient power to pick up statistically significant differences (Kravchenko et al., 2006).
2.5 Detecting changes in soil C and N

Management practices and climate change can significantly alter the dynamic balance between the inputs and outputs, thus altering the storage of C in soil (Don et al., 2007; Guo and Gifford, 2002; McSherry and Ritchie, 2013). Detecting changes in soil C stocks is important since a small change in C stocks can result in significant changes to the global C cycle because a large percentage of C is stored in soil (Ostle et al., 2009). For example, Fernández-Ugalde et al. (2013) found mean C losses of approximately 0.6 g kg\(^{-1}\) year\(^{-1}\) across 2000 sites in Whales and England. When results were extrapolated to the entire United Kingdom, annual losses of C were estimated at 13 million tonnes or 8% of the UK emissions of CO\(_2\) in 1990 (Six et al., 2000).

The effect of land use and management on soil C stocks is an important part of the national greenhouse gas inventories which are mandatory under the Framework Convention on Climate Change (Guo and Gifford, 2002). However, detecting differences in C stocks between land uses is difficult because of the inherent spatial and temporal variability of soil C (Allen et al., 2010). In this section of the literature review, I aim to: (i) discuss the reasons for the high variability in soil C stocks (ii) review the sampling strategies described in the literature to quantify soil C and N stocks at the paddock scale and (iii) discuss the importance of using statistical power analysis when designing experiments to determine temporal or spatial changes in soil C.

2.5.1 Spatial variability of soil C in grazed grasslands

A range of factors affect the storage of C in soil (e.g. precipitation). These factors are highly variable and control stocks of C and N on a range of different spatial scales, including plant/pedon scales (mm-200 m), landscape scales (20 m-km) and regional scales (>km) (Allen et al., 2010; Heckman et al., 2009).
i. **Plant/pedon scale (cm-m)**

At the pedon scale, SOC heterogeneity is caused by plant community dynamics and vegetative patterns (VandenBygaart, 2006). Small differences in soil properties such as moisture content may drive differences in net primary productivity (NPP) and therefore inputs of C into the soil. The heterogeneity in soil C also affects the distribution of microbial populations which tend to congregate in areas already high in SOM which further increases soil C concentrations (Allen et al., 2010; Chan et al., 2009; VandenBygaart, 2006).

In grassland systems, SOM is supplied through plant material in the form of root exudates, litter drop and root death (Allen et al., 2010). A consequence of these inputs is that pastoral systems generally have more uniform above and below inputs of C than ecosystems with more heterogeneous plant distributions such as forests (VandenBygaart, 2006). Giltrap and Hewitt (2004) studied the spatial variability of several soil quality indicators over a number of different spatial scales in grazed systems. Significant differences (P<0.01) in total volumetric C and N were found when measurements were taken 100, 30 and 5 m apart from each other. Giltrap and Hewitt (2004) suggested that replicate soil samples should be taken at distances of at least 100 m apart to efficiently reduce error associated with the measurement of C and N. When samples were taken closer together, the potential for autocorrelation was elevated resulting in a false estimation of true field means (Giltrap and Hewitt, 2004).

ii. **Landscape Scale (m-km)**

At the landscape scale, soil C heterogeneity is influenced by pedogenic processes and site management, especially tillage (Allen et al., 2010; Chan et al., 2009; VandenBygaart, 2006). Land use has a considerable impact on the spatial variability of C stocks at the landscape scale because of the varying A horizon depths across the landscape (Heckman et al., 2009; VandenBygaart et al., 2007). Many studies have found the spatial variability of soil C concentrations to be higher in landscapes planted under forest compared to landscapes planted under
pasture because of the varying rooting depths under forests (Heckman et al., 2009; Hewitt et al., 1998). In an assessment of the lateral and vertical variability of pedologically distinct soils in Canada, VandenBygaart et al. (2007) found the standard deviation of mean C concentrations to be low at the soil surface and deeper in the profile. However, at intermediate depths, the standard deviations of mean soil C concentrations were high because of the spatial variability of A horizon depths. Furthermore, C concentrations were more variable below 20 cm for forest sites compared to pasture sites because of the greater spatial variability of tree root depth compared to pasture root depth. Giltrap and Hewitt (2004) carried out a number of statistical analysis on a dataset from Schipper and Sparling (2000) to determine soil and land use effects on soil variability. The coefficient of variation (CV) for the spatial distribution of soil C for pasture sites was 7.9% compared to 10.1% for sites under pine forest. Yu et al. (2011) demonstrated similar findings when measuring the spatial variability of Soil C under a range of land uses in China. Coefficients of variation for soil C were highest in forest land (64%-94%), followed by dry farmland (49%-58%), and lowest in paddy fields (29%-31%).

Table 2.1 provides a summary of 9 studies which measured the spatial distribution of soil C for a range of depths in forest, grassland and cropland systems. The CV’s for soil C ranged from 8-76% for grassland systems and 17-70% for forest ecosystems. Figure 2.1 demonstrates that C stocks are significantly more variable under forest systems compared pasture systems and has implications for how C stocks may be quantified under the respective land uses (see section 2.5.3). The standard deviation of C stocks also increases linearly with increasing mean C stocks and suggests that less replication may be required to detect changes in C stocks for shallower depths.
Figure 2.1. Relationship between mean C stock (t ha$^{-1}$) and estimated standard deviation in soil C stocks for pasture systems and forest ecosystems (predominantly boreal forests). Values are taken from Table 2.1. The equation for the linear regression for the forest sites is $y = 0.37x - 5.64$ ($R^2=0.75$) and for pasture sites, $y=0.098x + 43.1$ ($R^2=0.75$).
Table 2.1. Summary of the spatial variability of SOC under a range of different land uses and soil types

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Sampling strategy</th>
<th>Soil type</th>
<th>FS</th>
<th>Land management or pedological history</th>
<th>Depth</th>
<th>CV (%)</th>
<th>Mean (t C ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceddia et al.</td>
<td>South Eastern</td>
<td>Systematic grid design. There were a total of 89 sampling points</td>
<td>-</td>
<td>2.8</td>
<td>Permanent pasture dominated by Transvala grass (<em>Digitaria decumbens</em>)</td>
<td>0.1-0.2 m</td>
<td>60.9</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.2-0.3 m</td>
<td>77.5</td>
<td>10.1</td>
</tr>
<tr>
<td>Conant et al.</td>
<td>Washington</td>
<td>3 plots with 6 regularly aligned cores</td>
<td>Typic Haploths</td>
<td>*</td>
<td>Old growth forest dominated by Douglas fir (no disturbance for &gt;100 years)</td>
<td>0.3 m</td>
<td>57.7</td>
<td>73.6</td>
</tr>
<tr>
<td></td>
<td>Washington</td>
<td>3 plots with 6 regularly aligned cores</td>
<td>Typic Haploths</td>
<td>*</td>
<td>Second growth Douglas fir (39 years)</td>
<td>0.3 m</td>
<td>47</td>
<td>55.6</td>
</tr>
<tr>
<td></td>
<td>Tennessee</td>
<td>3 plots with 6 regularly aligned cores</td>
<td>Typic Paleudult</td>
<td>*</td>
<td>Conventional tillage (planted under maize)</td>
<td>0.3 m</td>
<td>9.4</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td>Tennessee</td>
<td>plots with 6 regularly aligned cores</td>
<td>Typic Hapludult</td>
<td>*</td>
<td>Mature mixed hardwood forest for &gt;50 years</td>
<td>0.3 m</td>
<td>13.3</td>
<td>29.7</td>
</tr>
<tr>
<td>Conen et al.</td>
<td>Perthshire UK</td>
<td>Stratified random design</td>
<td>Podsolic soil</td>
<td>0.85 ha</td>
<td>Planted in sitka spruce (<em>Picea sitchensis</em>) in 1981 (undisturbed)</td>
<td>A horizon depth</td>
<td>30</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Les Landes, France</td>
<td>Stratified sampling design</td>
<td>Sandy Podsol</td>
<td>9 ha</td>
<td>Mature maritime pine (<em>Pinus pinaster</em>)</td>
<td>0-1 m</td>
<td>70</td>
<td>69</td>
</tr>
</tbody>
</table>

*FS, field size
CV, coefficient of variation
Field size was not mentioned in study
<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Sampling strategy</th>
<th>Soil type</th>
<th>FS (ha)</th>
<th>Land management or pedological history</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conen et al. (2004)</strong></td>
<td>Northumberland, UK</td>
<td>Nested sampling approach</td>
<td>Peaty gley</td>
<td>578</td>
<td>Hard wood forest consisting of Sitka spruce (<em>Picea sitchensis</em>) and lodgepole pine (<em>Pinus contorta</em>)</td>
</tr>
<tr>
<td></td>
<td>Northumberland, UK</td>
<td>Nested sampling approach</td>
<td>Peaty gley</td>
<td>50</td>
<td>Hard wood forest consisting of Sitka spruce (<em>Picea sitchensis</em>) and lodgepole pine (<em>Pinus contorta</em>)</td>
</tr>
<tr>
<td></td>
<td>Perthshire, UK</td>
<td>Stratified random design</td>
<td>Podsol soil</td>
<td>0.85</td>
<td>Planted in Sitka spruce (<em>Picea sitchensis</em>) in 1981 (ploughed)</td>
</tr>
<tr>
<td><strong>Don et al. (2007)</strong></td>
<td>Kaltenborn, Germany</td>
<td>24x24m grids with 25 cores per ha.</td>
<td>Vertisols</td>
<td>6</td>
<td>Arable land until 1975. Converted to sheep grazed land</td>
</tr>
<tr>
<td><strong>Don et al. (2007)</strong></td>
<td>Mehrstedt, Germany</td>
<td>24x24m grids with 18 cores per ha.</td>
<td>Arenosols</td>
<td>17</td>
<td>Arable land until 1980. Converted to sheep grazed land</td>
</tr>
<tr>
<td><strong>Nyamadzawo et al. (2008)</strong></td>
<td>Ohio</td>
<td>Systematic grid design. Thirty core samples were taken from a 20x20 m grid</td>
<td>Udorthents</td>
<td>*</td>
<td>Reclaimed mine site which has been under continuous pasture since 1987</td>
</tr>
</tbody>
</table>

- Depth (m): 0-0.45, 0-0.05, 0.05-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5
- CV (%): 40, 213, 37, 213, 49, 97, 24, 16.4, 15, 10.9, 22, 15, 16, 9.6, 44, 4.8, 35, 3.6, 21, 28.7, 16, 23.8, 20, 19.5, 22, 16.04, 42, 10.1, 48, 7.4, 37, 17, 63, 17, 44, 50.6
<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Sampling strategy</th>
<th>Soil type</th>
<th>FS $^A$</th>
<th>Land management or pedological history</th>
<th>Depth</th>
<th>CV (%)</th>
<th>Mean (t C.ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozgoz et al. (2011)</td>
<td>Turkey</td>
<td>120x150 m grid divided into 30x30 m grids. A total of 60 samples were obtained</td>
<td>Typic Haplustoll</td>
<td>1.8</td>
<td>Native pasture</td>
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<tr>
<td>Schrumpf et al. (2011)</td>
<td>Laqueuille, France</td>
<td>100 cores were taken on a regular grid. Cores were taken 10-15 m apart</td>
<td>Andosol</td>
<td>*</td>
<td>Semi-natural grassland</td>
<td>0.05 m</td>
<td>15</td>
<td>30.2</td>
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<td></td>
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<td></td>
<td>0.01 m</td>
<td>10</td>
<td>64.6</td>
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<td></td>
<td></td>
<td></td>
<td>0.03 m</td>
<td>8</td>
<td>157.1</td>
</tr>
<tr>
<td>Schrumpf et al. (2011)</td>
<td>Bugac, Hungary</td>
<td>100 cores were taken on a regular grid. Cores were taken 10-15 m apart</td>
<td>Arenosol</td>
<td>*</td>
<td>Semi-natural grassland</td>
<td>0.05 m</td>
<td>17</td>
<td>28.7</td>
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<td></td>
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<td>0.01 m</td>
<td>16</td>
<td>52.6</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.03 m</td>
<td>17</td>
<td>92.3</td>
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<td></td>
<td></td>
<td></td>
<td>0.06 m</td>
<td>19</td>
<td>123.3</td>
</tr>
<tr>
<td>Schrumpf et al. (2011)</td>
<td>Easter Bush, UK</td>
<td>100 cores were taken on a regular grid. Cores were taken 10-15 m apart</td>
<td>Cambisol</td>
<td>*</td>
<td>Intensive permanent grassland</td>
<td>0.05 m</td>
<td>14</td>
<td>20.3</td>
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<tr>
<td></td>
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<td></td>
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<td>0.01 m</td>
<td>12</td>
<td>37.0</td>
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<td>0.03 m</td>
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<td>92.6</td>
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<td></td>
<td></td>
<td></td>
<td>0.06 m</td>
<td>12</td>
<td>122.8</td>
</tr>
<tr>
<td>Shukla et al. (2004)</td>
<td>Gross-Enzersdorf, Austria</td>
<td>60 soil samples were collected from a series of 50 m X 25 m grids</td>
<td>Chernozem</td>
<td>*</td>
<td>Continuous cropping: winter wheat, (Triticum aestivum), canola (Brassica napus), durum wheat and summer barley</td>
<td>0-0.15 m</td>
<td>17</td>
<td>21.4</td>
</tr>
<tr>
<td>Singh et al. (2013)</td>
<td>West New South Whales</td>
<td>100 m regular grid was placed over the field and 75 samplings were located within the grid</td>
<td>Red Chromosols and Red Kandosols</td>
<td>68ha</td>
<td>Continuous cropping from 1994-2007. Since 2007, the cropping practice has been zero tillage</td>
<td>0-0.3 m</td>
<td>23.2</td>
<td>53.1</td>
</tr>
</tbody>
</table>
The spatial variability of SOC is also strongly related to the time since a land use conversion has taken place. Heckman et al. (2009) set up a number of sampling regimes to assess the spatial variability of SOC in fields which had been converted from cropping to permanent pasture and from forestry to permanent pasture. The time since conversion from forestry/cropping to permanent pasture ranged from 2-48 years prior to the running of the experiment. The CV of soil C stocks for forest sites converted to pasture after 2 years were greater than 22%. In contrast, sites that had been converted from exotic forest to pasture at least 13 years prior to the experiment had CV’s ranging from 13-17%. Such differences in CV values imply that a more rigorous sampling regime may be required for newly established pastoral systems if errors are to be minimized at the paddock scale.

The variability of soil properties at the paddock scale has implications for the spatial variability of SOC stocks. A paddock with relatively uniform soil properties (and management) is likely to have low variability in SOC compared with a paddock with heterogeneous soil properties (Shukla et al., 2004). Soil type is strongly correlated with C content and soil properties such as clay content can vary significantly over relatively small spatial scales (Bayer et al., 2006). Soils with a higher clay content generally hold more nutrients and have a higher moisture retention, thus promoting greater plant growth (Allen et al., 2010). Furthermore, SOC is strongly adsorbed to clay which physically protects the SOC within macro- and micro-aggregates, thus reducing decomposition rates (Allen et al., 2010). Numerous studies have reported on the relationship between SOC concentrations and clay content (Don et al., 2007; Shukla et al., 2004; VandenBygaart et al., 2007). Shukla et al. (2004) measured the spatial variability of several soil properties in a flat field planted under a range of different crops. The CV for clay content was 14% and clay content was positively correlated (R²> 0.48) with TC and TN. VandenBygaart and Kay (2004) also demonstrated a positive correlation (R=0.62) between clay content and SOC for a series of cropped paddocks in Southern Ontario.
iii. **Regional Scale (>km)**

At the regional scale, it is the interaction between climate, topography, vegetation and soils that determine SOC stocks (Allen et al., 2010; VandenBygaart, 2006). Topography is of particular importance because slope affects a number of soil processes, including drainage and erosion. Downslope positions tend to be higher in SOC compared to upslope positions because C-rich material is transported downslope through erosive processes and re-deposited in low-lying regions. (Allen et al., 2010). Furthermore, the high moisture content of down slope positions acts as a driver for high above ground biomass production and therefore large inputs of C into the soil.

Climate affects SOC variability on a regional scale by driving differences in above ground biomass production and soil respiration. Soil organic carbon is likely to be higher in cool, wet climates compared to dry, warm climates (Davidson and Janssens, 2006). In the Canadian prairies, for example, the moisture gradient is largely responsible for the Great Group soils of the Chernozemic soil order. There is gradient from brown Chernozems to black Chernozems as SOC increases in response to increased rainfall (VandenBygaart and Angers, 2006).

### 2.5.2 Variability of soil C and N with depth

The Intergovernmental Panel on Climate Change (IPCC) recommend C stocks be measured to a depth of 0.3 m for C accounting purposes (Gifford and Roderick, 2003). However, in many soils, large quantities of C may be stored in subsoil horizons, especially in temperate regions. Some studies suggest that up to 60% of SOC is stored below 20 cm in the first metre of the profile (Don et al., 2007). The relationship between changes in SOC in the lower profile and factors relating to changing climate and land use management is poorly understood. However, even small increases or decreases in the subsoil C pool could have a significant impact on the global C balance as a whole (Don et al., 2007). Schipper et al. (2010) demonstrated that changes in soil C to 90 cm were 1.5 times larger than changes
in total C to 30 cm and mean changes in total N were about double the changes that were detected down to 30 cm. Thus, for monitoring purposes, it is essential that SOC stocks are measured to depths greater than the 30 cm recommended by the IPCC.

The boundaries between soil horizons are narrow zones where the rate of ecological transfers (e.g. the flow of C) change sharply (VandenBygaart et al., 2007). Much of the variability in SOC concentrations with depth can be attributed to the characteristics of the soil horizons (e.g. horizon permeability and parent material). Chan et al. (2009) measured the SOC down to 30 cm across 7 sites in Ontario, Canada and demonstrated a strong positive relationship between the variability of A horizon depth and variability of SOC stocks. They found that the A horizon explained 81% of the variation in total C stocks to 30 cm. In contrast, the SOC concentrations in the upper 10 cm explained only 6% of the variability in SOC stocks to 30 cm. Such results suggest that the A horizon depth plays a critical role in determining total C stocks. Therefore, a greater number of samples may be required to pick up small changes in SOC stocks (through time or space) if the variability of the A horizon depth is large (VandenBygaart et al., 2007).

2.5.3 Sampling designs to measure C stocks at the paddock scale

Along with the implementation of carbon-trading schemes or market-based instruments (MBI) has come the need for accurate estimations of SOC stocks at the farm scale (Singh et al., 2013). There are a wide range of sampling designs to estimate soil C stocks at the paddock scale (Table 2.2) and they fall into one of two categories: a design based or a model based approach to estimate C stocks (Allen et al., 2010). The designed based approach directly measures an area using a randomised sampling pattern to illuminate/reduce bias while the model based approach uses geostatistics and time series analysis (de Gruijter et al., 2006). Designed based experiments have the advantage of been unbiased but in their simplest form, spatial coverage may be minimal. In contrast, a model based approach optimises spatial coverage. However, this approach is not safeguarded against bias and analysis is often highly complex (Allen et al., 2010). For soil C
sequestration studies, scientists are usually interested in one or more of the following: (i) temporal changes in soil C at specified points, (ii) spatial variability in C stocks and the associated cycling processes, (iii) geographic data on variables such as soil properties and plant cover (Ellert et al., 2002). The sampling design that is employed depends on the question of interest and the desired statistical power (see section 2.5.4). Cost and efficiency are also important factors to consider. A stratified random sampling design for example may give a highly accurate estimate of the mean C stock in a paddock but such an approach is expensive because of the number of samples required.
Table 2.2. A summary of sampling designs to quantify C and N stocks at the paddock scale. Adapted from Allen et al. (2010) and de Gruijter et al. (2006).

<table>
<thead>
<tr>
<th>Design based approaches</th>
<th>Description</th>
<th>Ideal use</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Simple Random sampling</td>
<td>Points selected at random from a field</td>
<td>C monitoring⁰</td>
<td>Simple</td>
<td>High per-sample cost. Poor spatial coverage</td>
</tr>
<tr>
<td>2. Stratified random sampling</td>
<td>Points selected randomly from strata within a field</td>
<td>Land use studies. C monitoring</td>
<td>Efficient sampling is more representative</td>
<td>Poor stratification may lead to inefficiencies. Expensive due to large sample size</td>
</tr>
<tr>
<td>3. Two-stage random sampling</td>
<td>A number of points are randomly selected from randomly selected strata</td>
<td>Land use studies. C monitoring</td>
<td>Smaller spatial area to sample</td>
<td>Spatial clustering may lead to lower precision</td>
</tr>
<tr>
<td>4. Cluster sampling</td>
<td>A predefined set of locations are selected from a field. Points are located at a random distance and direction from the initial locations</td>
<td>Land use studies. C monitoring</td>
<td>Smaller spatial area to sample</td>
<td>Spatial clustering may lead to lower precision</td>
</tr>
<tr>
<td>5. Systematic random sampling</td>
<td>A grid location is chosen randomly from a field. Points are measured systematically within the grid</td>
<td>Chronosequence studies</td>
<td>High precision (Points can be relocated)</td>
<td>Lack of random repetition may lead to underestimated variance</td>
</tr>
<tr>
<td>Model based approaches</td>
<td>Spatial coverage of C modelled using statistical methods such as Kriging</td>
<td></td>
<td>Optimises spatial coverage</td>
<td>Model may contain bias. Complex analysis</td>
</tr>
</tbody>
</table>

⁰Ideal use but not exclusive
⁰⁰C monitoring refers to determining the mean C stock of a paddock
⁰⁰⁰Land use studies determine the effect of changing land use on the SOC stocks of a paddock
i. **Design based approaches to measuring C stocks**

Design based approaches estimate the sample mean and its uncertainty by carrying out probabilistic sampling (Singh et al., 2013). In most cases, designed based approaches are favoured over model based approaches because fewer observations are required and there is no need to test the validity of a model before conclusions are drawn. Simple random sampling (1, table 2.2) is a commonly used approach for comparing C stocks between land uses (Savadogo et al., 2007; Xu et al., 2011). For example, Hewitt et al. (2012) used simple random sampling for 21 paired pasture/forestry sites by collecting soil samples from 4 randomly selected locations in adjacent paddocks. However, because SOC stocks are so variable, a large number of samples may be required to accurately determine the paddock mean and increase the ability to detect real differences. Furthermore, randomly selecting samples from a field may inadvertently lead to a clustering effect which can lead to inaccurate estimates of the mean C stock (Allen et al., 2010). Stratified random sampling (2, table 2.2) reduces the clustering effect by dividing the field into strata and a predefined number of points are randomly selected from each strata (de Gruijter et al., 2006). Such a method ensures that there is a relatively uniform distribution of points throughout the field.

A major issue with comparative land use studies is the presence of confounding variables such as soil type which have an effect on SOC stocks over and above land management. Two stage random sampling (3, table 2.2) and cluster sampling (4, table 2.2) reduces confounding variables by only considering strata with similar physical characteristics (for e.g. slope, soil type) (Allen et al., 2010). Cui et al. (2005) used two stage sampling by randomly positioning five 1 m² plots within paired grazed and non-grazed paddocks and sampled 3 points within each plot. Individual cores can be bulked to increase efficiency and reduce cost but small scale spatial information may be lost (Cui et al., 2005). Two stage random sampling is often used in combination with a transect line where a series of cores is obtained at regular intervals. Giltrap and Hewitt (2004) quantified C stocks at
the paddock scale by taking a series of soil cores from five 5x5 m plots which were located 1 m apart along a 29 m long transect.

For temporal monitoring purposes, a systematic sampling approach (5, table 2.2) such as grid sampling is most suitable because points can be relocated and clustering of points is avoided (VandenBygaart et al., 2007). Ellert et al. (2002) proposed a sampling approach where 6 cores were evenly spaced along two transects in a 5x2 m plot. Electronic markers were placed at the initial sampling location and subsequent samples were taken at a distance of 1 m from the electronic marker. To account for the spatial variability in C stocks, VandenBygaart (2006) increased the size of the sampling grid originally proposed by Ellert et al. (2001) to 25 m² and sampled 16 points within the plot. Increasing the number of samples within the plot increases statistical power, allowing smaller differences to be detected through time (Kravchenko and Robertson, 2011). To further increase the power of detecting changes over time, it may be necessary to have replicate plots within the paddock of interest. However, replicate plots must be positioned on similar soils because the rate of C sequestration varies with properties such as moisture content and nutrient concentration (Ellert et al., 2002). Figure 2.2 provides a summary of the main differences between the sampling strategies discussed above.
2.5.4 Statistical power

Statistical power can be defined as the probability that a significant difference can be detected in a comparative test, if a difference does in fact occur (VandenBygaart et al., 2007). In most cases, the cost or time needed for analysis determines the number of samples taken which is often to the detriment of statistical power. A low sampling intensity may result in type II statistical error, that is when a difference between treatments is not detected when in fact there is a true difference (Kravchenko and Robertson, 2011; VandenBygaart et al., 2007). For example, Christopher et al. (2009) measured SOC stocks of 12 paired no-till (NT) vs. conventional tillage (CT) systems in the Midwestern United States and concluded that no significant difference in SOC stocks (0-30 cm) was observed for 7 of the 12 sites. In a further assessment of this paired NT vs. CT study, VandenBygaart (2009) concluded that there had been insufficient replication to determine whether or not any real difference existed between the two treatments, given the observed variability. Insufficient replication may result in a high standard error and an inability to detect treatment effects. The non-detection of important differences between treatments because of low sampling is a common
occurrence in land use studies and can be identified as a potential problem by applying a post-hoc power analysis (Kravchenko and Robertson, 2011).

Power analysis can be used to determine the number of samples that should be (or should have been) taken to detect a specified change in soil C, if indeed there is a difference that is considered to be important (Kravchenko and Robertson, 2011). Power is proportional to effect size (ES), sample size (n), variance (\( \sigma^2 \)) and significance level (Quinn and Keough, 2002). Kravchenko and Robertson (2011) demonstrated the importance of using power analysis to detect differences in soil C between different management regimes. For the Kravchenko and Robertson (2011) study, there was a 52% probability of detecting a 10% difference in total C between the CT and NT sites when 30 samples were collected from each paddock. However, below 30 cm, the probability of detecting a 10% difference between sites was less than 10%. The low probability of detecting a 10% difference below 30 cm was attributed to the high inherent variability of C stocks in deeper horizons. Further analysis demonstrated that the contribution of individual layers to the variability in C stocks for the whole profile was 20% for the surface horizon, 40% for the middle layer and 40% for the deep layer (Kravchenko and Robertson, 2011). The higher variability of SOC stocks in deeper soil layers may mask real changes that have occurred in the top layers when the whole profile is compared between land uses. Therefore, to avoid type II errors, conclusions should only be based on soil layers where significant differences have been detected.

Table 2.3 provides a summary of studies where C stocks have been measured at a range of scales (1-580 ha). For the majority of studies, the authors conducted a post hoc power analysis to determine the minimum detectable difference (MDD) for a given sample size. The MDD is the smallest significant difference that could have been detected, given the observed variance components. The increase in the MDD with increasing mean C stock (Fig. 2.3) suggests that the ability to pick up smaller differences in C stocks decreases as the mean C stock increases. This was consistent with a number of studies which were able to detect differences in C stocks between land uses to shallow depths but not to deeper depths due to
increasing variability added by C stocks lower in the profile (Barnett et al., 2014; Kravchenko and Robertson, 2011; Schipper and Sparling, 2011).

**Figure 2.3.** The relationship between MDD and mean C stocks for a range of studies (Table 2.3). Linear regression line was fitted to pasture sites and cropping sites only: \( y = 0.04 + 0.3 \) (\( R^2 = 0.81 \))
Table 2.3. The number of samples required to identify significant differences in SOC stocks at a given level of statistical power.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Vegetation</th>
<th>Management</th>
<th>Soil type</th>
<th>Field size</th>
<th>Depth (m)</th>
<th>Mean C (t C ha⁻¹)</th>
<th>SD ^a</th>
<th>Power analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>N^a</td>
<td>SP^a</td>
</tr>
<tr>
<td>* Carlow, Ireland</td>
<td>Cropland</td>
<td>No-till</td>
<td>Eutric</td>
<td>Cambisol</td>
<td>1-2 ha</td>
<td>0.0-0.5</td>
<td>9.7</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.1</td>
<td>19.9</td>
<td>4.1</td>
<td>100</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.3</td>
<td>60.3</td>
<td>8.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.6</td>
<td>74.1</td>
<td>14.2</td>
<td>100</td>
</tr>
<tr>
<td>Schrumpf et al. (2011)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N^a</td>
<td>SP^a</td>
</tr>
<tr>
<td>* Gebeesee, Germany</td>
<td>Cropland</td>
<td>Harvested and grubbed</td>
<td>Haplic</td>
<td>Phaeozem</td>
<td>1-2 ha</td>
<td>0.0-0.5</td>
<td>12.8</td>
<td>2.4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.1</td>
<td>27.6</td>
<td>4.7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.3</td>
<td>86.5</td>
<td>4.7</td>
<td>100</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.6</td>
<td>130.9</td>
<td>13.9</td>
<td>100</td>
</tr>
<tr>
<td>* Grignon, France</td>
<td>Cropland</td>
<td>Reduced tillage</td>
<td>Eutric</td>
<td>Cambisol</td>
<td>1-2 ha</td>
<td>0.0-0.5</td>
<td>13.6</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.1</td>
<td>27.9</td>
<td>3.2</td>
<td>100</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.3</td>
<td>82.4</td>
<td>8.1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.6</td>
<td>111.4</td>
<td>12.2</td>
<td>100</td>
</tr>
<tr>
<td>* Laqueuille France</td>
<td>Permanent pasture</td>
<td></td>
<td>Andosol</td>
<td></td>
<td>1-2 ha</td>
<td>0.0-0.5</td>
<td>30.3</td>
<td>4.6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.1</td>
<td>64.7</td>
<td>6.3</td>
<td>100</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.3</td>
<td>157.1</td>
<td>12.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.6</td>
<td>229.1</td>
<td>25.3</td>
<td>100</td>
</tr>
<tr>
<td>Schrumpf et al. (2011)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N^a</td>
<td>SP^a</td>
</tr>
<tr>
<td>* Bugac, Hungary</td>
<td>Permanent pasture</td>
<td></td>
<td>Arenosol</td>
<td></td>
<td>1-2 ha</td>
<td>0.0-0.5</td>
<td>28.7</td>
<td>4.9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.1</td>
<td>52.6</td>
<td>8.3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.3</td>
<td>92.3</td>
<td>15.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.6</td>
<td>123.3</td>
<td>23.3</td>
<td>100</td>
</tr>
<tr>
<td>* Easter Bush, UK</td>
<td>Permanent pasture</td>
<td></td>
<td>Cambisol</td>
<td></td>
<td>1-2 ha</td>
<td>0.0-0.5</td>
<td>20.3</td>
<td>2.9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.1</td>
<td>37.1</td>
<td>4.4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.3</td>
<td>92.6</td>
<td>10.1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0-0.6</td>
<td>122.8</td>
<td>14.7</td>
<td>100</td>
</tr>
<tr>
<td>Barnett et al. (2014)</td>
<td>+ Waikato, New Zealand</td>
<td>Permanent pasture</td>
<td>Dairy grazed</td>
<td></td>
<td></td>
<td>0-0.6</td>
<td>164.8</td>
<td>53.6</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Drystock grazed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N^a</td>
<td>SP^a</td>
</tr>
<tr>
<td>Steffens et al. (2008)</td>
<td>+ Inner Mongolia</td>
<td>Permanent pasture</td>
<td>Sheep grazed (0.5 U ha⁻¹ yr⁻¹)</td>
<td>Calcic</td>
<td>Chernozem</td>
<td>34 ha</td>
<td>0.04</td>
<td>4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sheep grazed (1.2 U ha⁻¹ yr⁻¹)</td>
<td>Calcic</td>
<td>Chernozem</td>
<td>24 ha</td>
<td>0.04</td>
<td>10.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Poussart et al. (2004)</td>
<td>+ Sudan</td>
<td>Permanent grassland</td>
<td></td>
<td>Dairy and drystock</td>
<td>-</td>
<td>0-0.2</td>
<td>4.1</td>
<td>2.2</td>
<td>90 0.9</td>
</tr>
<tr>
<td>Whitehead et al. (2010)</td>
<td>+ New Zealand</td>
<td>Permanent pasture</td>
<td>Dairy and drystock</td>
<td>-</td>
<td>0-0.6</td>
<td>60</td>
<td>0.9</td>
<td>14</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>N^a</td>
<td>SP^a</td>
</tr>
<tr>
<td>* Les Landes, France</td>
<td>Coniferous forest</td>
<td>Mature forest of maritime pine</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>0.4</td>
<td>69</td>
<td>48 0.9</td>
</tr>
<tr>
<td>Conen et al. (2004)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N^a</td>
<td>SP^a</td>
</tr>
<tr>
<td>* Perthshire, UK (undisturbed)</td>
<td>Coniferous forest</td>
<td>Planted with Sitka spruce</td>
<td></td>
<td>A horizon</td>
<td>0.85</td>
<td>A</td>
<td>98</td>
<td>29</td>
<td>100 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>N^a</td>
<td>SP^a</td>
</tr>
<tr>
<td>* Perthshire, UK (ploughed)</td>
<td>Coniferous forest</td>
<td>Ploughed and planted with sitka spruce</td>
<td></td>
<td>A horizon</td>
<td>0.85</td>
<td>A</td>
<td>97</td>
<td>47.5</td>
<td>100 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>N^a</td>
<td>SP^a</td>
</tr>
<tr>
<td>* Northumberland, UK (forest)</td>
<td>Coniferous forest</td>
<td>Sitka spruce and lodgepole pine</td>
<td></td>
<td></td>
<td>578</td>
<td>0.45</td>
<td>213</td>
<td>85.2</td>
<td>100 0.9</td>
</tr>
<tr>
<td>Heckman et al. (2009)</td>
<td>* Nebraska</td>
<td>Uncultivated grassland</td>
<td></td>
<td></td>
<td>-</td>
<td>0-0.2</td>
<td>224</td>
<td>9.7</td>
<td>2.8</td>
</tr>
</tbody>
</table>

^a SD, standard deviation; N, sample number; SP, statistical power (1-β); MDD, minimum detectable difference (t ha⁻¹)

^b Carbon stocks were compared between 25 paired dairy/drystock sites

^c Studies were carried out throughout New Zealand under a range of climates and soil types

^d Heckman et al. (2009) used power analysis to determine the number of sites that need to be sampled to pick up a significant difference in stocks over time for uncultivated grassland in the state of Nebraska

* Paired site study * cronosequence study
2.5.5 Other factors to consider

i. *Sampling fixed depth increments vs. genetic horizons*

An important consideration when designing any experiment to quantify C stocks is to decide whether to calculate C stocks for genetic horizons or for fixed depth increments. Many studies quantify C stocks for fixed depth increments as this allows for efficient sampling (Chan et al., 2009; Conant et al., 2003; VandenBygaart and Kay, 2004). Furthermore, quantifying C stocks for fixed depth increments (for e.g. 0-10 cm, 10-30 cm, 30-60 cm) is useful for comparative purposes and for subsequently applying equivalent soil mass calculations. However, fixed depth increments often span the boundary of two genetic horizons which may lead to higher measured variability in SOC stocks (VandenBygaart et al., 2007).

ii. *Bulking samples to increase efficiency*

Accurate measurement of SOC stocks always requires a balance between the number of samples required to detect a certain difference and cost of sampling and analysis. One method of reducing the cost of analysis while not reducing the number of samples is by bulking samples within micro-plots to attain an average SOC stock (VandenBygaart, 2006). Conant et al. (2003) measured the C content of soils under forest and cultivated sites in Tennessee and Washington using a replicated systematic grid design. Initial carbon stocks were measured ($T_1$) and then resampled some time later ($T_2$) to determine if a change in SOC stocks had occurred through time. As well as individually analysing samples (6 replicate cores) from $T_1$ and $T_2$, samples from $T_1$ and $T_2$ were also bulked to determine if an average change in C content could be detected. In most cases (31 of 36 plots in Tennessee and 34 of 36 plots in Washington), percent C for the bulked samples fell within the 95% confidence interval around the mean for the six replicate cores (Conant et al., 2003). The literature suggests that bulking samples within plots accurately represents the information gathered by individual cores, thereby reducing cost and increasing efficiency. However, the success and accuracy of
bulking individual cores is highly dependent on experimental design (e.g. the size of individual plots) (VandenBygaart et al., 2007). Furthermore, bulking samples results in the loss of valuable spatial information which is critical for developing geostatistical models.

2.6 Methods to calculate C and N stocks

Historically, C and N stocks have been calculated to a fixed depth. Such an approach is accurate for comparative purposes if differences in soil mass are minimal (i.e. no difference in bulking density through time or between compared soils). In the mid 1990’s, Ellert and Bettany (1995) proposed that nutrient stocks be calculated for an equivalent soil mass (ESM). A number of studies in the 2000’s confirmed the importance of applying ESM calculations when carrying out comparative land use studies (Ellert et al., 2001; Gifford and Roderick, 2003; McConkey et al., 2003; Piñeiro et al., 2009b; Sisti et al., 2004). Equivalent soil mass calculations are most important when soils are sampled to shallow depths (< 30 cm) or when bulk density differences between samples are large. There are a wide range of calculation approaches which can be applied to calculate nutrient stocks and there is need to determine when it is appropriate to apply the respective methods (Table 2.4) and these are discussed in the following sections.
Table 2.4. Summary of the different calculations used to measure soil nutrient stocks. In the text, each method is referred to by the number in brackets in the first column.

<table>
<thead>
<tr>
<th>Calculation method</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Sampling method</th>
<th>Ideal use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth based approach (1)</td>
<td>Stock calculated as a product of bulk density, soil depth and % C/N</td>
<td>Simple calculation</td>
<td>Doesn’t take bulk density differences (spatially or temporally) into account</td>
<td>Pit method</td>
<td>Where BD differences are &lt;5% For deep sampling</td>
</tr>
<tr>
<td>Original ESM method (Ellert and Bettany, 1995) (2)</td>
<td>Stocks are adjusted to an ESM using linear interpolation</td>
<td>Bulk density differences are taken into account</td>
<td>Can be difficult to apply ESM calculations if bulk density differences are very large. Calculations are complex</td>
<td>Soil cores</td>
<td>For shallow sampling</td>
</tr>
<tr>
<td>CMC approach (Gifford and Roderick, 2003) (3)</td>
<td>Stocks are adjusted to an ESM by fitting a cubic spline</td>
<td>More accurate than linear interpolation</td>
<td>Same as (2) and (3)</td>
<td>Same as (2) and (3)</td>
<td>Same as (2) and (3)</td>
</tr>
<tr>
<td>ESL approach (Solomon et al., 2002) (5)</td>
<td>The sampling depth is corrected based on bulk density differences</td>
<td>Simple to apply to single mass of soil</td>
<td>Corrections are not accurate for multi-layer assessments</td>
<td>Pit method</td>
<td>Soil cores</td>
</tr>
</tbody>
</table>

*ESL, equivalent soil layer; ESM, equivalent soil mass; CMC, cumulative mass coordinates
bIdeal but not exclusive
cThe original ESM method and CMC approach are equivalent calculations (see Appendix C)
* The ESM method by Wendt and Hauser (2013) is the same as the original ESM method and CMC approach except a cubic spline is fitted instead of a linear fit

2.6.1 Methods without mass corrections

The IPCC recommends that C stocks be expressed as a mass of organic C to a fixed depth (30 cm) (Gifford and Roderick, 2003). The problem with such an approach is that changes in bulk density (either spatially or temporally) are not taken into account. As a consequence, authors such as Ellert and Bettany (1995) recommended comparing C stocks based on an equivalent soil mass (ESM) where
soil masses are mathematically altered or an additional mass of soil is sampled to increase the mass of the overall sample.

In the majority of publications prior to 1970, the amount of SOC was simply expressed as a concentration (kg C Mg soil$^{-1}$). Although, SOC does increase with concentration, the storage of SOC is also dependent on soil bulk density and thickness (Ellert and Bettany, 1995). Comparing C stocks as a concentration when large bulk density differences occur can introduce significant error.

The fixed depth (FD) approach (1, table 2.4) calculates SOC stocks as a product of soil thickness (m), soil bulk density (t m$^{-3}$) and percent C (equation 3) (Ellert and Bettany, 1995; Lee et al., 2009; Toriyama et al., 2011). Many studies (e.g. Barnett et al. (2014), Schipper et al. (2007)) have used the FD approach in combination with a soil pit because horizons are easily identifiable. Once a pit is excavated to the desired depth, a bulk density core is taken from each horizon and a representative scraping is obtained for nutrient analysis. The FD approach may also be applied to soil cores where there is the added advantage of greater spatial coverage and increased statistical power (Kravchenko and Robertson, 2011).

Wendt and Hauser (2013) calculated the mass of soil by taking a series of cores which were bulked and applied the following equation:

$$M_{soil} = \frac{M_{sample(OD)}}{\pi \left(\frac{D}{2}\right)^2} \times 10000 \times n$$  \hspace{1cm} (2.1)

Where: $M_{soil}$ is the mass of the soil in t.ha$^{-1}$, $M_{sample(OD)}$ is the oven dry mass of soil (t), $\pi (D/2)^2$ is the cross-sectional area of the corer (m$^2$), $n$ is the number of cores taken and 10000 is a correction factor to convert m$^2$ to ha. Such a method is more efficient than excavating a pit and taking individual bulk density cores but compression associated with pushing the core into the soil can result in incorrect estimations of the soil mass per volume.

An advantage of using a pit along with the FD approach is that C and N stocks can be estimated for soils containing stones. Vadeboncoeur et al. (2012)
discussed how large systematic bias may be introduced when using soil corers because of the inability to sample below rocks. The quantitative pit method involves excavating a pit to a desired depth and directly measuring bulk density rather than taking bulk density cores (Whitehead et al., 2010). The volume of the pit is carefully measured and soil weighed to attain a measurement of mass/volume. The advantage of a quantitative pit method over the conventional soil pit is that bulk density cores are not required and an accurate mass of soil can be estimated even when large rocks may be present (Condron et al., 2012). However, the quantitative pit method is time consuming and statistical power may be compromised because of a lack of replication (Kravchenko and Robertson, 2011).

2.6.2 Equivalent soil mass corrections

Equivalent soil mass calculations adjust the total C stocks of different samples to a fixed mass of soil. Therefore, comparisons of total C stocks are made for a single mass of soil. This is in contrast to the fixed depth approach where total C stocks are compared by depth (e.g. to 60 cm) but not by an equal mass of soil (Ellert and Bettany, 1995; Gifford and Roderick, 2003). Toledo et al. (2013) compared calculations of total C down to 0.3 m using the FD approach and a number of ESM approaches for cultivated and pristine soils. The FD approach was found to underestimate the true difference in SOC between the different land uses because of the difference in soil mass. The pristine forest soils had bulk densities of ~0.79 t m\(^{-3}\) while the cultivated soils had bulk densities ranging from 0.9-1.2 t m\(^{-3}\). The lower bulk density of the forest soils meant that total C stocks were severely underestimated when calculated to a fixed depth and the difference in total C stocks between forest and cultivated sites was relatively small. Failing to apply ESM calculations for comparative land use studies can lead to false comparisons of total C data because different masses of soil are being compared. (Ellert and Bettany, 1995; Gifford and Roderick, 2003).
i. *Original ESM method and cumulative mass coordinates (CMC) approach*

Ellert and Bettany (1995) developed the initial ESM method (2, table 2.4) which in its simplest form involved calculating the additional soil thickness required for a lighter soil to reach a desired ESM. Bulk density values and C stocks were calculated separately for each genetic horizon and the heaviest soil was considered the ESM (Gifford and Roderick, 2003). The additional mass of soil required to increase the mass of the lighter soil to the ESM was calculated using the following equation:

\[
T_{\text{add}} = \frac{(M_{\text{equiv}} - M_{\text{soil}}) \cdot 0.001}{BD} \text{ ha} \cdot \text{m}^{-2} \quad (2.2)
\]

Where: \( T_{\text{add}} \) is the additional depth of soil (m) required to attain the ESM, \( M_{\text{equiv}} \) is the equivalent soil mass (t ha\(^{-1}\)) and \( BD \) is the subsurface bulk density in t m\(^{-3}\). The C stock of the equivalent mass of soil was attained by summing the initial C stock with the C stock of the additional subsurface layer. The original ESM method requires excavation of a pit since bulk density cores are required and sampling is carried out by horizon opposed to fixed depth increments (Gifford and Roderick, 2003). Furthermore, prior knowledge about the extent of bulk density differences is required before sampling is carried out. Ellert et al. (2001) modified their approach by taking soil cores and dividing the cores into fixed depths opposed to sampling by genetic horizons using pit. Such an approach eliminated the need for bulk density samples as the mass of soil could be estimated from the volume of the corer (Equation 2.1).

Gifford and Roderick (2003) introduced what they called the cumulative mass coordinates (CMC) approach (3, table 2.4) which builds on the methodology of Ellert et al. (2001). A core is sliced into depth increments and the mass of C (t ha\(^{-1}\)) is calculated for each layer using the depth based method (section 2.6.1). The cumulative soil mass was then plotted against the cumulative C stock and linear interpolation used to calculate the C stock corresponding to a specified ESM. Gifford and Roderick (2003) applied the following calculation:
\[ M_{OC(0-ref)} = M_{OC(0-a)} + \left( M_{ESM} - M_{soil(0-a)} \right) \times C_{OC(a-b)} \] (2.3)

Where: \( M_{OC(0-ref)} \) is the mass of C (t ha\(^{-1}\)) for the ESM, \( M_{OC(0-a)} \) is the mass of C above the deepest layer, \( M_{ESM} \) is the equivalent soil mass, \( M_{soil(0-a)} \) is the mass of soil above the deepest layer and \( C_{OC(a-b)} \) is the concentration of C in the deepest layer. Wendt and Hauser (2013) demonstrated that the ESM method (Ellert and Bettany, 1995; Ellert et al., 2001) and the CMC approach (Gifford and Roderick, 2003) are mathematically equivalent in that they both use linear interpolation to adjust the C stock to a specified ESM (see Appendix C for calculations). Linear interpolation can introduce error in that it implicitly assumes that the C concentration is constant within each layer. Wendt and Hauser (2013) fitted a cubic spline (e.g. Fig. 2.4) to their data to account for the fact that C stocks vary continually with depth.

Figure 2.4 shows total C (t ha\(^{-1}\)) plotted against soil mass (t ha\(^{-1}\)) for a paired dairy vs. drystock site (Barnett et al., 2014). The dairy site had a greater mass of soil to 60 cm (3700 t ha\(^{-1}\)) compared to the drystock site which had a soil mass of 3200 t ha\(^{-1}\)). A cubic spline fit (Wendt and Hauser, 2013) allows the total C stock of the dairy site to be adjusted to the mass of soil of the drystock site (dotted line).
Figure 2.4. A cubic spline fitted to data from Barnett et al. (2014) showing the correction of the dairy C stock to a ESM of 3200 t ha\(^{-1}\) (dotted line). The mass of soil of the dairy site to 60 cm was 3700 t ha\(^{-1}\) and the mass of soil of the drystock site was 3200 t ha\(^{-1}\). For an accurate comparison of the total C stock between the dairy/drystock sites, the soil C stocks must be adjusted to an ESM.

Table 2.5 summarises total C stocks that have been calculated to a fixed depth and to an ESM range for a range of land use comparison studies, using the methods of Gifford and Roderick (2003) and Ellert et al. (2002). Figure 2.6 shows the difference C stocks between land uses (calculated to a fixed depth) plotted against the difference in C stocks between land uses (calculated to an ESM). The points scatter around the 1:1 line which indicates that the fixed depth approach (1, table 2.4) can both underestimate and overestimate the differences in total C stocks between land uses. The application of ESM calculations is most important when differences in bulk density between land uses are significant. For example, in figure 2.5, the circled data points indicate 2 paired sites where differences in soil mass between land uses were between 300 and 600 t ha\(^{-1}\).
Figure 2.5. Difference in total stocks, calculated to a fixed depth vs. the difference in total C stocks, calculated to an ESM (bold numbers in table 2.5). Straight line is a 1:1 line and circled points indicate paired sites where the difference in soil mass was greater than 350 t ha$^{-1}$. 
<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Management</th>
<th>Method used</th>
<th>Profile depth (m)</th>
<th>Soil mass (t ha(^{-1}))</th>
<th>Mass diff (%) (^{\text{A}})</th>
<th>C mass (t ha(^{-1}))</th>
<th>P value</th>
<th>ESM (t ha(^{-2})) (^{\text{A}})</th>
<th>C mass (t ha(^{-2}))</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Barnett et al., 2014)</td>
<td>Waikato, New Zealand</td>
<td>Dairy grazed</td>
<td>Pit approach</td>
<td>0.6</td>
<td>3120 (^{\text{b}})</td>
<td>1.9 %</td>
<td>173 (12.4) (^{\text{b}})</td>
<td>0.24</td>
<td>*</td>
<td>171 (12.3)</td>
<td>0.25</td>
</tr>
<tr>
<td>Xu et al. (2011)</td>
<td>Northern China</td>
<td>Drystock grazed</td>
<td>Pit approach</td>
<td>0.6</td>
<td>3180</td>
<td></td>
<td>182.7 (15)</td>
<td></td>
<td>179.9 (14.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.6</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>Xu et al. (2011)</td>
<td>Northern China</td>
<td>Uncontrolled / free grazing grassland</td>
<td>60 cm diameter corer</td>
<td>0.5</td>
<td>7240</td>
<td>2.7 %</td>
<td>83.2</td>
<td>7000</td>
<td>88.65</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grassland enclosed, excluding grazing/mowing</td>
<td>60 cm diameter corer</td>
<td>0.5</td>
<td>7040</td>
<td>112.3</td>
<td>7000</td>
<td>111.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24.1</td>
<td>23.32</td>
<td></td>
</tr>
<tr>
<td>Xu et al. (2011)</td>
<td>Northern China</td>
<td>Grassland enclosed and mown in October</td>
<td>60 cm diameter corer</td>
<td>0.5</td>
<td>7010</td>
<td>3.1 %</td>
<td>107.6</td>
<td>7000</td>
<td>107.5</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grassland enclosed under controlled grazing</td>
<td>60 cm diameter corer</td>
<td>0.5</td>
<td>7240</td>
<td>134</td>
<td>7000</td>
<td>131</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26.4</td>
<td>23.5</td>
<td></td>
</tr>
</tbody>
</table>

\(^{\text{A}}\) ESM, equivalent soil mass; Mass (%), the difference in mass between the two land uses as a percentage of the land use with the lowest mass

\(^{\text{b}}\) Standard error of the mean

* Multiple paired sites were used for the study. A different ESM was used for each paired site
### Table 2.5. Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Management</th>
<th>Method used</th>
<th>Profile depth (m)</th>
<th>Soil mass (t ha⁻¹)</th>
<th>Mass diff (%)</th>
<th>C mass (t ha⁻¹)</th>
<th>P value</th>
<th>ESM (t ha⁻²)</th>
<th>ESM (t ha⁻²)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hewitt et al. (2012)</td>
<td>New Zealand</td>
<td>Low productivity grassland</td>
<td>Soil corer / quantitative pit method</td>
<td>0.3</td>
<td>2521</td>
<td>5.4%</td>
<td>97.3 (5.6)</td>
<td>&lt;0.01</td>
<td>*</td>
<td>87.3 (5.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forest planted pre-1990</td>
<td>Soil corer / quantitative pit method</td>
<td>0.3</td>
<td>2385</td>
<td>76.6 (5.4)</td>
<td>70.7 (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Difference</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20.7 (20.3)</td>
<td></td>
<td>16.7 (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hiltbrunner et al. (2012)</td>
<td>Switzerland</td>
<td>Bare steps formed by intensive trampling</td>
<td></td>
<td>0.25</td>
<td>2675</td>
<td>13%</td>
<td>60</td>
<td></td>
<td>2325</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slopes unaffected by trampling</td>
<td></td>
<td>0.25</td>
<td>2325</td>
<td>76</td>
<td></td>
<td></td>
<td>2325</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Difference</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td></td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toriyama et al. (2011)</td>
<td>Maribaya, Indonesia</td>
<td>Plantation containing A. mangium. C stock measured in 2001</td>
<td>Soil corer</td>
<td>0.3</td>
<td>2527</td>
<td>6.7%</td>
<td>66.1 (9.4)</td>
<td>&lt;0.05</td>
<td>*</td>
<td>66.1 (9.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plantation containing A. mangium. C stock measured in 2005</td>
<td>Soil corer</td>
<td>0.3</td>
<td>2357.5</td>
<td>70.7 (8)</td>
<td></td>
<td></td>
<td>74.9</td>
<td>74.9 (8.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Difference</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.6</td>
<td></td>
<td>8.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- P value not provided in study
- Soil mass refers to the average mass of soil down to a depth of 60 cm across 25 sites
- Mass difference (%), percent difference between the mass of soil to a specified depth
Table 2.5. Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Management</th>
<th>Method used</th>
<th>Profile depth (m)</th>
<th>Soil mass (t ha$^{-1}$)</th>
<th>Mass diff (%)</th>
<th>C mass (t ha$^{-1}$)</th>
<th>P value</th>
<th>ESM (t ha$^{-2}$)</th>
<th>C mass (t ha$^{-2}$)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toriyama et al. (2011)</td>
<td>Maribaya, Indonesia</td>
<td>Secondary forest containing S.wallichii. C stock measured in 2005</td>
<td>Soil corer</td>
<td>0.3</td>
<td>2766.5</td>
<td>18.5%</td>
<td>62.8 (7.5)</td>
<td>&gt;0.05</td>
<td>62.8 (7.5)</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Wilson et al. (2010)</td>
<td>NSW, Australia</td>
<td>Improved pasture</td>
<td>50 mm diameter corer</td>
<td>0.3</td>
<td>4095</td>
<td>13.3%</td>
<td>68.1</td>
<td>&gt;0.05</td>
<td>4180</td>
<td>68.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Woodland</td>
<td>50 mm diameter corer</td>
<td>0.3</td>
<td>3550</td>
<td>89.9</td>
<td>4180</td>
<td>98.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ernst and Siri-Prieto (2009)</td>
<td>North West Uruguay</td>
<td>Crop-pasture rotation with conventional tillage</td>
<td>Soil corer</td>
<td>0.18</td>
<td>2160</td>
<td>5.8%</td>
<td>50.6</td>
<td>2000</td>
<td>47.3</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Crop-pasture rotation with no tillage</td>
<td>Soil corer</td>
<td>0.18</td>
<td>2034</td>
<td>53.4</td>
<td>2000</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steffens et al. (2008)</td>
<td>Inner Mongolia</td>
<td>Heavily grazed with sheep/goats</td>
<td>Soil corer</td>
<td>0.04</td>
<td>436</td>
<td>8.6</td>
<td>377</td>
<td>6.4</td>
<td>377</td>
<td>9.6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ungrazed since 1999</td>
<td>Soil corer</td>
<td>0.04</td>
<td>512</td>
<td>14.8%</td>
<td>10.8</td>
<td>377</td>
<td>9.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Difference</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.8</td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>

* ESM calculations should be treated with caution as C stocks were only measured to 4 cm
ii. Equivalent soil layer (ESL) approach

The ESL approach (5, table 2.4) works by adjusting the depth of the sampled soil based on the ratio of the average bulk densities between the respective land uses (de Moraes et al., 1996; Solomon et al., 2002; Toriyama et al., 2011). The corrected depth is calculated as follows:

\[ Z_{\text{corrected}} = \left( \frac{BD_a}{BD_b} \right) \times Z_b \]  

(2.4)

Where: \( Z_{\text{corrected}} \) is the adjusted depth for the profile/layer, \( BD_a \) is the average bulk density of a profile/layer for land use a, \( BD_b \) is the average bulk density of the corresponding profile/layer for land use b, and \( Z_b \) is the depth of the soil profile for land use b. Toriyama et al. (2011) applied a range of calculation methods to estimate the C content of soils (down to 0.3 m) under citrus, tobacco and yerba mate. These authors reported that for all land uses, the ESL approach underestimated C stocks compared to other ESM calculations. The ESL method assumes that bulk density remains constant with depth and the method can only be applied to a single layer of soil.

2.6.3 Other considerations

i. Choosing an equivalent soil mass and reporting results by ESM

An ESM is the mass of the soil to which all respective samples must be adjusted. Many studies (Campbell et al., 1998; Ellert and Bettany, 1995; Henderson et al., 2004) considered the heaviest mass of soil as the ESM while others (McConkey et al., 2003; Piñeiro et al., 2009b) have considered the lightest soil as the ESM. Zan et al. (2001) used a different approach by calculating the ESM as the average of all soil masses down to a depth of 60 cm across all systems. The chosen ESM is not critical, so long as the same ESM is used across all treatments and through time (Wendt and Hauser, 2013). If the ESM is greater than the mass of an individual sample, it is critical that there is enough additional soil to attain the ESM to avoid extrapolation of C stocks. Extrapolation may lead to incorrect
estimation of C stocks because soil C is not measured directly. Wendt and Hauser (2013) suggested sampling to a fixed depth and deciding on an ESM once sampling is complete.

It is common practice to report the ESM layers to which the C stocks have been adjusted to (e.g. 0-1500, 1500-4000, 4000-6000 t soil ha\(^{-1}\)) (Wendt and Hauser, 2013). Ellert and Bettany (1995) reported C stocks to a ‘mass-equivalent depth’ which is the depth corresponding to each ESM layer. For example, the 0-1500 Mg soil ha\(^{-1}\) layer in figure 2.3 corresponds to a depth of ~15 cm. It is critical to report ESM layers, particularly for chronosequence studies where C stocks may need to be calculated to the same mass of soil at a later date (Wendt and Hauser, 2013).

**ii. Single vs. multiple layer assessments**

Multiple layer assessments require measurements of C stocks down the length of a profile. Equivalent soil mass calculations can only be applied where 2 or more depth layers have been sampled (e.g. 0-10 cm and 10-20 cm). Wendt and Hauser (2013) suggested that for monitoring purposes and to reduce cost, measuring C stocks for 2 depth increments may be sufficient. Gifford and Roderick (2003) also suggested using 2 depth increments as a means of quantifying C stocks as ESM calculations can still be applied and cost is greatly reduced. However, such an approach may lead to inaccurate measurements because C stocks are highly variable with depth. More research is required to determine an accurate and cost effective approach for calculating C stocks.
2.7 Summary and conclusions

The effect of grazing intensity on soil C stocks is poorly understood with some studies showing increased grazing intensity to increase C stocks (Li et al., 2007), decrease C stocks (Golluscio et al., 2009) or have no effect on C stocks (Abril and Bucher, 2001). The difference in results between studies could potentially be because of the different approaches used to calculate and compare C stocks in the respective studies. Management induced changes in C stocks can be difficult to detect because of the inherent spatial variability in site specific factors and soil properties. The storage of SOC in soils is reasonably well understood and the reader is referred to Six et al. (2004) and Six and Paustian (2014) for a comprehensive review on the topic.

The spatial variability in C stocks for pasture systems is low in comparison to native or exotic forests (Table 2.1) suggesting that a more rigorous sampling regime may be required when sampling nutrient stocks in forest systems. Furthermore, CV values vary with depth and are often greatest at horizon boundaries (Heckman et al., 2009). At the landscape scale, the variability in soil properties has a major impact on the variability in nutrient stocks. The spatial variability in C stocks for flat paddock with relatively uniform soil properties is likely to be low compared to a paddock with variable soil properties.

Despite lower spatial variability of pasture C stocks, detecting changes in SOC (temporally and spatially) is difficult because there still remains considerable spatial variability in SOC stocks. With high spatial variability comes the risk of failing to detect changes in SOC stocks when changes have in fact taken place (type II error) (Kravchenko et al., 2006). There are many cases in the literature where type II error may have been committed because power analysis was not applied and insufficient samples were taken to detect a treatment effect (Christopher et al., 2009; VandenBygaart, 2009). Statistical power is also strongly related to experimental design and there are a number of sampling regimes used to quantify C stocks at the paddock scale. For detecting land use effects on C stocks, simple random sampling is commonly used (Hewitt et al., 2012). However,
simple random sampling may fail to detect treatment effects because samples may be taken from a range of different soils. Stratified random sampling is also commonly used in the literature and involves randomly taking samples from predefined strata (e.g. soil type) (Allen et al., 2010). For monitoring purposes, a randomized grid design is most appropriate because points can be accurately re-sampled at a later date (Ellert et al., 2001). The sampling design required to detect treatment effects can be highly context specific and more research is required to determine when the respective sampling strategies should be applied.

The IPCC have recommended that C stocks be quantified to a depth of 30 cm using the depth based method (Gifford and Roderick, 2003). For shallow sampling (<30 cm) or when bulk density differences between samples are large, the depth based approach can give inaccurate estimations of C stocks (Ellert and Bettany, 1995). Many studies have suggested adjusting the C stocks of respective samples to an ESM to deal with differences in bulk density (Ellert and Bettany, 1995; Gifford and Roderick, 2003; Sisti et al., 2004). Although ESM calculations are widely used, they are often poorly understood and used incorrectly. There is a need for the IPCC to recommend a standard procedure for calculating nutrient stocks using ESM calculations.
CHAPTER THREE

Detection of differences in soil C and N stocks between paired dairy and drystock pastures

3.1 Abstract

Soil is the largest terrestrial store of carbon (C) with some 2000 Pg to a depth of 1 m compared to 500 Pg in the atmosphere. Maximizing storage of C in soil is not only important for reducing atmospheric CO₂ concentrations but also for maintaining soil quality. Recent research has shown that land use management is a key factor in determining the storage of C in pastoral systems. Barnett et al. (2014, AEE 185:34-40) used a paired pit approach to sample 25 adjacent dairy and drystock pastures to a fixed depth of 0.6 m and showed that drystock grazed soils had about 8.6 t ha⁻¹ more C in the top soil than adjacent dairy sites (P<0.05). However, there was no significant difference between land uses when C was accumulated to 0.6 m.

The main objective of this study was to test a potentially more accurate method for estimating differences in C stocks between sites sampled by Barnett et al. (2014). Twenty three of the paired dairy and drystock sites were sampled to a depth of 0.6 m by taking 5 soil cores from each of two plots (5x5 m) within a paddock of each land use and soil C/N and soil mass were determined.

To a depth of ~60 cm (C stocks adjusted for equivalent soil mass), drystock sites had 1.6 t ha⁻¹ more C than dairy sites but this was not significant. However, when soil layers were analysed separately, drystock sites contained more C (4.1 ± 2.1 t C ha⁻¹) in the top 10 cm (P=0.06) and dairy farms had significantly more C (3.7 ± 1.7 t C ha⁻¹) in the 25-60 cm layer (P=0.04). The difference in the relative distribution of soil C in dairy and drystock sites may be due to the greater size and concentration of dairy urine patches which can solubilise C in the top-soil and redeposit dissolved C lower in the profile.

When comparing whole-profile C stocks between dairy and drystock sites, the two-plot coring approach would have been able to detect a true difference of 9.3 t C ha⁻¹ had it occurred, compared to 13.6 t C ha⁻¹ for the pit approach (P<0.05). For the purpose of providing information for future sampling, power analysis was also conducted. With 23 paired sites, the pit approach could detect a significant difference (P<0.05) of 16 t C ha⁻¹ with 66% certainty while the coring approach could detect the same difference with 90% certainty.

These results suggested that a sampling methodology that included spatial variability of soil C using a replicated coring approach greatly improved the ability to detect differences. Furthermore, the coring approach reduced cost and increased efficiency compared to the single-pit approach. However, even the coring approach was constrained in its ability to pick up small (<5%) differences in C stocks and therefore further research is required to develop a method that can detect more subtle changes in C stocks.
3.2 Introduction

The soil plays a fundamental role in the global carbon (C) cycle, with some 1500-2000 Pg ($10^{15}$ g) of organic C stored in the upper 1 m in the form of decomposed plant litter, plant residues and stabilised organic matter (Don et al., 2007; McSherry and Ritchie, 2013). Soil can act as a net source of CO$_2$ or a net sink of CO$_2$, depending on the delicate balance between C inputs and outputs (Guo and Gifford, 2002). The equilibrium C stock in soils is dependent on environmental factors, including soil type and mean precipitation, and management related factors such as fertilizer use (Gifford and Roderick, 2003; McSherry and Ritchie, 2013).

Many studies have recognised the importance of increasing the sequestration of C into soil as a means of reducing atmospheric CO$_2$ concentrations (Han et al., 2010; Hewitt et al., 2012; Six et al., 2000). As a result, the United Nations Framework Convention on Climate Change (UNFCCC) made it mandatory to report greenhouse gas removals and emissions for soil C pools at the national scale (Fernández-Ugalde et al., 2013; Han et al., 2010; Hewitt et al., 2012; Six et al., 2000; Smith et al., 2008). Grassland soils in particular have potential to sequester significant amounts of C but this is highly dependent on management (McSherry and Ritchie, 2013). Managed grasslands occupy about 25% of the earth’s ice free land (McSherry and Ritchie, 2013) and in New Zealand, grazed grasslands account for 33% of the total land area (Ministry for the Environment, 2007).

The measured effects of grassland management on soil C stocks vary greatly, with some studies showing that increased grazing frequency can increase soil C stocks (Li et al., 2007), decrease soil C stocks (Golluscio et al., 2009) or have no effect on soil C stocks (Abril and Bucher, 2001). In New Zealand, grazing management practices vary with land use, specifically grazing by sheep/beef cattle (drystock) or dairy cows (dairy). Dairy systems occupy about 7% of New Zealand’s total land area while drystock systems (including hill country) make up 30% of the total land area (Ministry for the Environment, 2007). Dairy farms are generally more intensively managed compared to drystock farms, with higher stocking
Detection of differences in C and N stocks

rates, greater fertilizer and feed imports, higher product-export and heavier animals (Mackay, 2008).

Several studies have focussed on the effect of grassland management on soil C and N stocks in New Zealand (Barnett et al., 2014; Jackman, 1964; Schipper et al., 2007; Schipper et al., 2014; Schipper et al., 2010; Tate et al., 2005). Schipper et al. (2010) re-sampled 83 profiles throughout New Zealand to determine whether land use had an effect on soil C and N stocks. On average, over 27 years, they reported that dairy pastures lost $0.73 \pm 0.16 \, \text{t C ha}^{-1} \, \text{year}^{-1}$ and $0.057 \, \text{t N ha}^{-1} \, \text{year}^{-1}$ but there was no significant change in C or N stocks for drystock (sheep and beef) pastures on flat land. To test the hypothesis that dairy farms had lower C stocks compared to drystock farms, Barnett et al. (2014) sampled 25 adjacent dairy and drystock farms to 0.6 m depth and analysed samples for C and N. Dairy farms were found to contain an average of $173 \pm 12.4 \, \text{t C ha}^{-1}$ while drystock farms had $183 \pm 15 \, \text{t C ha}^{-1}$. This whole-profile (0-60 cm) difference was not significant but for the A horizon, drystock sites had $8.6 \, \text{t ha}^{-1}$ more C than dairy sites ($P<0.05$). Hypothesised causes for the difference in C stocks were that the higher stocking rates of dairy pastures increased organic matter (OM) mineralisation rates and decreased inputs of plant litter and residues. Furthermore, the deposition of more intense urine patches on dairy farms may drive higher solubilisation rates of organic matter with subsequent leaching (Lambie et al., 2012).

There is also evidence that soil order may be an important factor when determining the response of soil C and N stocks to changing land use. In a follow up study, Schipper et al. (2014) determined that grazing type (dairy vs. drystock) was no longer a significant predictor of soil C and N losses from New Zealand flat land. The change in interpretation from previous studies (Schipper et al., 2007; Schipper et al., 2010) was attributed to improved sampling of major soil orders across dairy and drystock sites. The distribution of soil orders across sites was important because some soil orders (e.g. Allophanic Soils) were more prone to losses of C and N. In previous sampling (Schipper et al., 2007; Schipper et al., 2010), dairy sites had a greater proportion of Allophanic and Gley Soils than drystock sites. The findings of Schipper et al. (2014) support the conclusions
made by McSherry and Ritchie (2013) that the effects of grazing management on soil C stocks are highly context specific depending on soil type, climate and vegetation.

Carbon and nitrogen are highly variable over relatively small spatial scales and measuring differences in stocks through time or through space is difficult (Chapter 2, section 2.5.2). Studies by Barnett et al. (2014) and Schipper et al. (2007, 2010, 2014) sampled sites using individual soil pits, which do not allow for the within site variability in C stocks to be determined. Accounting for the within site variability in C stocks allows for the detection of smaller significant differences between land uses (Allen et al., 2010). Consequently, there is need for a sampling method that includes within site variability in C and N stocks but is also cost effective and efficient. Taking into account the within paddock variability in C and N stocks increases statistical power to detect small changes in C stocks through time and space. A number of sampling protocols for monitoring and comparing C stocks have been proposed (Ellert et al., 2001; Ellert et al., 2002), however, many of these methods fail to detect differences in soil C stocks because there is often a lack of replication (Kravchenko and Robertson, 2011).

A study conducted by Giltrap and Hewitt (2004) on flat sites in the Waikato suggested that the variability in C stocks was relatively low over small distances (i.e. within 30 m) and higher over distances of 100 m or more. The study recommended that sampling should be spread throughout a paddock to measure the within paddock variability in C stocks. Previous sampling of 25 adjacent dairy and drystock sites in the Waikato (Barnett et al., 2014) indicated that a significant difference in C stocks occurred in the A horizon. However, the single-pit sampling strategy lacked power to detect significant differences below the A horizon because the within site variability in C stocks was not taken into account.
The first objective of this study was to test whether there was a true difference in C and N stocks between the dairy and drystock farms originally sampled by Barnett et al. (2014), by using a more powerful sampling strategy.

The second objective of this study was to determine the detectability of differences in C and N stocks between paired dairy and drystock sites using different sampling strategies. To determine the effectiveness of the respective sampling strategy for detecting differences, least significant differences (LSD) were calculated. For the purpose of providing information for future sampling, power analysis was conducted. The LSD addresses the question of “what difference could I have detected from my data?”, while power analysis addresses the question of “how many sites would I need to sample to detect a difference with some degree of certainty?”

3.3 Methods

3.3.1 Site description

The study was located in the Waikato region of New Zealand (Fig. 3.1) where annual rainfall ranges from 1116 to 1550 mm (Table 3.1). Soils were resampled from 23 adjacent dairy and drystock pastures following the study of Barnett et al. (2014). The assumption was made that land use management for all sites was similar to when the initial sampling was conducted 2 years previously.

We were able to resample 23 of the 25 sites (Table 3.1). The majority of sites (except sites 16 and 17) had undergone no change in management since the initial sampling 2-3 years previously. Paired sites were located on similar landscape units and soil type and had been under the respective farming systems for at least 10 years prior to the sampling by Barnett et al. (2014).

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1 Appendix 1 provides a full description of the laboratory methods used in this study.
Figure 3.1. The location of 25 adjacent dairy and drystock farms used in the study of Barnett et al. (2014). Closed symbols indicate the sites resampled in this study. Two sites were not resampled and are indicated by open symbols.
Table 3.1. Site information for the resampled dairy and drystock farms. A full description of sites is given in Barnett et al. (2014). GPS coordinates can be found in Appendix E.

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Date of resampling</th>
<th>Location</th>
<th>NZSC A</th>
<th>ST A</th>
<th>MAP B</th>
</tr>
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<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
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<tr>
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<td>Ando</td>
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</table>

* Sites were not resampled
A NZSC, soil order from New Zealand soil classification; ST, soil orders from US Soil Taxonomy. Ando, Andisol; Ult, Ultisol; Incep, Inceptisol
B MAP, mean annual precipitation. Data obtained from Niwa climate stations (http://cliflo.niwa.co.nz/pls/niwp/wgenf.genform1_proc) or Waikato Regional Council monitoring stations (http://www.waikatoregion.govt.nz/riverlevelsandrainfall/cgi-bin/hydwebserver.cgi/catchments/details?catchment=16)

3.3.2 Soil sampling

Sampling of soils occurred between August and November 2013. Each paired site was sampled on the same day to ensure there were no differences in weather conditions and site specific factors such as soil moisture content.
Within each paddock, the initial sampling point used by Barnett et al. (2014) was relocated using GPS coordinates and measurements to paddock boundaries. We were confident that we were able to relocate the pits with an accuracy of ± 5 m and often the previous pit location was obvious by eye. A 5x5 m plot was positioned at a distance of 2 m from the estimated pit location. A soil core was taken to ensure the soil within the 5x5 m plot matched the profile description of Barnett et al. (2014). An additional 5x5 m plot was established at a distance of 30 m from the first plot and in a random direction (Fig. 3.2). The criteria for the positioning of the plots were 1) the plots had to be positioned on the same landscape unit and on the same soil, and 2) plots were to be positioned at least 20 m from paddock boundaries or other areas where animal traffic was perceived to be high. These criteria were not always met and sites failing to meet these criteria were noted. For example, if the landscape was highly variable, plots may have been located on a different landscape unit but on the same soil type.

Figure 3.2. The general approach used to resample the paired sites. A 5x5 m plot was positioned ~2m from the estimated pit location. An additional 5x5 plot was positioned at a distance of 30 m (and in a random direction) from the first plot. For the majority of sites, all plots were located on the same soil type and landscape unit.
A soil corer with a 25.25 mm diameter tip was used to obtain soil samples and was carefully driven to a depth of 0.65 m using a wooden mallet. A total of 5 soil cores were taken randomly from each 5x5 m plot and carefully placed on a board (Fig. 3.3). The 5 soil cores were cut into 0-10, 10-25, 25-40, 40-60 and 60-65 cm depth increments and soil from each increment was bulked. Each sample was bagged, labelled and refrigerated at 4°C until further analysis was required.

Figure 3.3. An example of a 5x5 m plot. Within each 5x5 m plot, 5 soil cores were obtained to a depth of 0.65 m. The cores were cut into 0-10, 10-25, 25-40, 40-60 and 60-65 cm depth increments which were bulked by depth and subsequently placed into labelled bags.

3.3.3 Soil analysis

To prepare samples for analysis, field moist soil samples were air dried and passed through a 6 mm sieve to remove coarse roots and stones. The whole air-dried sample mass was weighed, passed through a 2 mm sieve and a representative sub sample was ground using an agate mortar and pestle. A subsample of the ground soils and 2 mm sieved soils were archived at the University of Waikato. The concentration of total C and N for all samples was determined using an Elementar (Isoprime 100) combustion analyser at the
University of Waikato. Concentrations of total C and N were expressed as a percentage to calculate the mass of C/N per unit area for each depth increment.

### 3.3.4 Data analysis

The air-dried mass of soil was converted to an oven-dry mass of soil using a moisture factor which was determined by drying a sub-sample at 105°C to a constant weight. The oven dry mass of soil per unit area (t ha⁻¹) for each soil layer was calculated:

\[
M_{\text{soil}} = \frac{M_{\text{sample(OD)}}}{\pi \left(\frac{D}{2}\right)^2 \times n} \times 10000
\]  

(3.1)

Where: \(M_{\text{soil}}\) was the mass of the soil per unit area in t ha⁻¹ to a specified depth, \(M_{\text{sample(OD)}}\) was the oven dry mass of collected soil (t), \(\pi (D/2)^2\) was the cross-sectional area of the corer (m²), \(n\) was the number of cores taken and 10 000 was a correction factor to convert m² to ha.

The total C stock (t ha⁻¹) for each depth increment was calculated:

\[
\text{Total C stock (t ha}^{-1}) = M_{\text{soil}} \times \%C_{\text{OD}}
\]  

(3.2)

Where: \(M_{\text{soil}}\) was the mass of the soil per unit area in t ha⁻¹ to a specified depth and \(\%C_{\text{OD}}\) was the oven dry concentration of C (% C).

To calculate the total N stocks for each increment, \(\%C_{\text{OD}}\) was substituted with \(\%N_{\text{OD}}\) in equation 3.2 which gives:

\[
\text{TN stock (t ha}^{-1}) = M_{\text{soil}} \times \%N_{\text{OD}}
\]  

(3.3)
Where: $M_{\text{soil}}$ was the mass of the soil per unit area in t ha$^{-1}$ and $\% N_{OD}$ was the oven dry concentration of N ($\% N$). Soil C/N stocks (t ha$^{-1}$) were calculated to a fixed depth of 60 cm for the respective plots at each site using equations 3.2 and 3.3.

Carbon/nitrogen stocks were adjusted to an equivalent soil mass (ESM) using a fitted cubic spline function in Microsoft Excel (Wendt and Hauser, 2013): (http://www.srs1software.com/SRS1CubicSplineForExcel.aspx). The lightest mass of soil from each paired site was considered as the ESM and the C/N stocks of all 4 plots at each paired site were adjusted to an ESM.

See Appendix A for a full description of the ESM calculations used in this study.

### 3.3.5 Statistical analysis

Data were analysed using analysis of variance (ANOVA) to test for significant differences in C and N stocks between paired dairy and drystock sites, with site and core sampling stations within farms as blocking factors, and land use as the treatment factor. Analyses were carried out separately for different soil layers and for the total soil profile from 0-0.6 m depth. A P value of less than 0.05 was considered a statistically significant result. Variance components and their standard errors were estimated using REML to facilitate power calculations.

Analysis of variance and analysis of variance components using REML was carried out using Genstat version 16 (VSN International Ltd.). Power analysis was conducted using Minitab 17 (Minitab Inc.).

Where error bounds are given, these represent ±1 SE, unless specified otherwise.

### 3.4 Results
Twenty-three adjacent dairy and drystock farms were resampled following the earlier work of Barnett et al. (2014). The soil orders sampled included Allophanic (15), Gley (5), Granular (2) and Brown (1) Soils. Total C and N stocks are reported to a fixed depth. However, to ensure that differences in bulk density between adjacent land uses were taken into account, C and N stocks were also calculated to an equivalent soil mass (ESM) for each paired site. Where reported, the difference in total C and N stocks for each site is for an equivalent soil mass (see Appendix D and F for raw data).

3.4.1 Dairy vs. drystock farms

3.4.1.1 Soil mass

The mass of soil (t ha\(^{-1}\)) was greater under dairy sites for all respective depths but the difference was only significantly different for the 0-10 cm soil layer (Table 3.2). The A horizon depth did not differ significantly between adjacent dairy and drystock sites.

<table>
<thead>
<tr>
<th>Soil depth</th>
<th>Dairy (t ha(^{-1}))</th>
<th>Drystock (t ha(^{-1}))</th>
<th>Difference (t ha(^{-1}))</th>
<th>SED</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.1 m</td>
<td>749 (32)</td>
<td>702 (27)</td>
<td>47</td>
<td>23.0</td>
<td>0.045</td>
</tr>
<tr>
<td>0.01-0.25 m</td>
<td>1244 (60)</td>
<td>1225 (66)</td>
<td>19</td>
<td>29.8</td>
<td>0.53</td>
</tr>
<tr>
<td>0.25-0.4 m</td>
<td>1276 (74)</td>
<td>1256 (84)</td>
<td>20</td>
<td>24.5</td>
<td>0.41</td>
</tr>
<tr>
<td>0.4-0.6 m</td>
<td>1799 (104)</td>
<td>1766 (114)</td>
<td>33</td>
<td>43.6</td>
<td>0.45</td>
</tr>
<tr>
<td>0-0.6</td>
<td>4998 (296)</td>
<td>4887 (327)</td>
<td>132</td>
<td>112</td>
<td>0.25</td>
</tr>
</tbody>
</table>

\(^{A}\) SED, Standard error of the difference between means
Standard error of the mean in parenthesis (n=23 paired sites)

3.4.1.2 Total C and N
Total C stocks to a depth of 60 cm ranged from 89 t C ha\(^{-1}\) (site 20) to 269 t C ha\(^{-1}\) (sites 12 and 17) (Fig. 3.4). Total C stocks were highest for the Allophanic Soils with a mean C stock of 178 t C ha\(^{-1}\) to 60 cm. Granular Soils had a mean C stock of 135 t C ha\(^{-1}\), followed by Gley Soils which had a mean C stock of 110 t C ha\(^{-1}\). Brown Soils were constrained to a single site (site 25) which had a mean C stock of 121 t C ha\(^{-1}\) to 60 cm depth.
Figure 3.4. Total C stocks (t C ha⁻¹) for the 23 paired dairy and drystock sites. Total C stocks were quantified to fixed depth increments of 0-10, 10-25, 25-40, 40-60 and 0-60 cm (not adjusted to an ESM). Columns to the left of each site number are dairy sites and columns to the right of each number are drystock sites.
The mean C stock of the dairy sites to 60 cm was 157.3 ± 10.8 t C ha\(^{-1}\) and 157.2 ± 10.5 t C ha\(^{-1}\) for the drystock sites with no significant difference between the two land use types (Table 3.3). There was a mean difference of 4.1 ± 2.1 t C ha\(^{-1}\) (P=0.06) for the 0-10 cm soil layer and a mean difference of 1.2 ± 2.4 t C ha\(^{-1}\) (P=0.6) for the 10-25 cm soil layer. Below 25 cm, dairy sites had more C than adjacent drystock sites, with a mean difference of 2.3 ± 1.1 t C ha\(^{-1}\) (P=0.04) for the 25-40 cm soil layer and 1.4 ± 0.7 t C ha\(^{-1}\) (P=0.07) for the 40-60 cm soil layer (Table 3.3).

Table 3.3. Mean C stocks for adjacent dairy and drystock sites for the respective soil layers. Mean C stocks (t C ha\(^{-1}\)) were calculated to a fixed depth while the difference in C stocks was calculated after adjustment for equivalent soil mass (see Appendix D and E)

<table>
<thead>
<tr>
<th>Land use</th>
<th>Depth</th>
<th>Mean C stock (t ha(^{-1}))</th>
<th>Difference (^{A}) (t ha(^{-1}))</th>
<th>SED (^{B})</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td>0-10 cm</td>
<td>60.0 (2.9)</td>
<td>-4.1</td>
<td>2.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Drystock</td>
<td></td>
<td>62.7 (3.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>10-25 cm</td>
<td>52.8 (4.0)</td>
<td>-1.2</td>
<td>2.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Drystock</td>
<td></td>
<td>53.4 (3.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>25-40 cm</td>
<td>24.4 (2.4)</td>
<td>2.3</td>
<td>1.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Drystock</td>
<td></td>
<td>22.4 (2.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>40-60 cm</td>
<td>20.1 (2.0)</td>
<td>1.4</td>
<td>0.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Drystock</td>
<td></td>
<td>18.7 (1.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>0-60 m</td>
<td>157.3 (10.8)</td>
<td>-1.6</td>
<td>4.5</td>
<td>0.72</td>
</tr>
<tr>
<td>Drystock</td>
<td></td>
<td>157.2 (10.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{A}\) Difference in total C stocks (dairy - drystock) were calculated using an equivalent soil mass (ESM) for each paired site (see appendix D and E)

\(^{B}\) SED, Standard error of the difference between means (n=23)
Total N stocks varied considerably across sites (Fig. 3.6), ranging from 8.7 t N ha\(^{-1}\) (site 20) to 23.3 t N ha\(^{-1}\) (site 17) and were strongly correlated to total C stocks (Fig 3.5). Allophanic Soils had the highest mean N stock (16.5 t ha\(^{-1}\)), followed by granular Soils (12.6 t ha\(^{-1}\)), and finally Gley Soils (11.2 t ha\(^{-1}\)). The relationship between soil order and total C and N stocks was not explored because of the imbalance in the distribution of major soil orders.

**Figure 3.5.** Relationship between total C and N stocks for dairy and drystock sites. Straight line is a linear regression line: \(y=0.07x + 4.02\) (\(R^2=0.87\)).
Figure 3.6. Total N stocks (t N ha\(^{-1}\)) for the 23 paired dairy and drystock sites. Total N stocks were quantified to fixed depth increments of 0-10, 10-25, 25-40, 40-60 and 0-60 cm (not adjusted to an ESM). Columns to the left of each site number are dairy sites and columns to the right of each number are drystock sites.
The difference in total N stocks between adjacent dairy and drystock farms followed the same trend as total C stocks. Drystock sites contained a greater quantity of total N for the 0-10 (P=0.3) and 10-25 cm layers (P=0.6) but lower stocks for the 25-40 (P=0.03) and 40-60 cm (P=0.04) layers compared to dairy sites. There was no significant difference in total N stocks to 60 cm between dairy and drystock sites (Table 3.4) but dairy sites had an average of 0.3 ± 0.5 t ha⁻¹ more N.

Table 3.4. Mean N stocks for adjacent dairy and drystock sites for different depths. Mean N stocks (t C ha⁻¹) were calculated to a fixed depth while the difference in N stocks was calculated using equivalent soil mass calculations (see Appendix D and E).

<table>
<thead>
<tr>
<th>Land use</th>
<th>Depth</th>
<th>N content (t ha⁻¹)</th>
<th>Difference (t ha⁻¹)</th>
<th>SED</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td>0-10 cm</td>
<td>5.8 (0.3)</td>
<td>-0.22</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Drystock</td>
<td></td>
<td>5.9 (0.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>10-25 cm</td>
<td>5.1 (0.3)</td>
<td>-0.13</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Drystock</td>
<td></td>
<td>4.9 (0.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>25-40 cm</td>
<td>2.4 (0.2)</td>
<td>0.25</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Drystock</td>
<td></td>
<td>2.2 (0.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>40-60 cm</td>
<td>2.0 (0.1)</td>
<td>0.15</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>Drystock</td>
<td></td>
<td>1.9 (0.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>0-60 cm</td>
<td>15.4 (0.8)</td>
<td>0.3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Drystock</td>
<td></td>
<td>14.9 (0.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Standard error of the mean in parenthesis

A Difference in total N stocks (dairy - dry stock) were calculated using an equivalent soil mass (ESM) for each paired site (see appendix D and E)

B SED, Standard error of the difference between means (n=23)

3.4.2 Pit approach vs. coring approach for measuring C and N stocks

When the initial pit sampling of paired dairy and drystock farms was undertaken (Barnett et al., 2014), total C and N stocks were compared by horizon. To compare the pit approach and coring approach, it was necessary to interpolate total C and N stocks to an equivalent soil mass for each paired site. Further interpolation was carried out on the pit data to determine the mass of soil to fixed
depth increments (0-10, 10-25, 25-40 and 40-60 cm) across all sites. Sites 2, 3, 4 and 16 were excluded from analysis as the precise location of the original pit in the respective paddocks was less certain.

### 3.4.2.1 Soil mass

The coring approach yielded a significantly lower soil mass than the pit approach for the 0-10, 10-25 and 25-40 cm soil layers (P<0.01, Fig. 3.7). The difference in estimated soil mass was especially large from 0-10 cm, where the average difference was 135.8 ± 108 t ha⁻¹ which represented a difference of 16% (P<0.01). The average difference in soil mass was 82.9 ± 16 t ha⁻¹ (7% difference) for the 10-25 cm depth increment and 76.9 ± 17 t ha⁻¹ (6% difference) for the 25-40 cm layer. For the 40-60 cm soil layer, the average difference in soil mass was 30.4 ± 31 t ha⁻¹ (2% difference) but this difference was not significant.

![Figure 3.7](image-url)

**Figure 3.7.** Average soil mass (± 1 standard error), estimated using the coring approach (plot 1) and the pit approach for a series of depth increments for dairy and drystock sites combined. Star symbols indicate a P value<0.01.
Although the pit method and coring method measured significantly different masses of soil for the 0-10, 10-25 and 25-40 cm soil layers, there was a strong correlation between the two methods (Fig. 3.8). Such a strong correlation suggests that the difference in soil mass was likely due to a systematic bias in sampling approach. For the 40-60 cm soil layer, the linear regression line closely matches the 1:1 line which suggested that the two methods estimated similar masses of soil.

![Figure 3.8](image_url)

**Figure 3.8.** Linear regressions of soil mass (t ha⁻¹), estimated using the pit method and the coring method. Dotted line is the 1:1 line. **A.** 0-10 cm depth increment; **B.** 10-25 cm depth increment; **C.** 25-40 cm depth increment; **D.** 40-60 cm depth increment.

### 3.4.2.2 Total C and N stocks

There was a strong correlation in total C stocks between plot 1 and the pit (Fig. 3.9 A). However, the linear regression line was above the 1:1 line suggesting that...
the coring method underestimated total C stocks or that the pit approach overestimated total C stocks. The correlation between the pit and plot 2 (Fig. 3.8 B) was similar to that of the pit and plot 1. This was despite the fact that plot 2 was positioned at least 30 m away from the pit compared to plot 1 which was positioned within 5 m of the pit.

Figure 3.9. A. Total C stocks (0-60 cm), estimated using the coring method (plot 1) vs. total C stocks, estimated using a single pit. Straight line is a linear regression ($y=0.99x+12.2$, $R^2 = 0.8$) and dotted line is a 1:1 line. B. Total C stocks (0-60 cm), estimated using the coring method (plot 2) vs. total C stocks, estimated using a single pit. Straight line is a linear regression ($y=1.01x+16.7$, $R^2 = 0.8$).
The average difference in C stocks between plot 1 and plot 2 for the dairy sites was $7.5 \pm 3.9 \, \text{t ha}^{-1}$ (P=0.06, Table 3.5). For the drystock sites, the average difference in C stocks between plot 1 and 2 was $7.3 \pm 4.6 \, \text{t ha}^{-1}$ (P=0.1). The difference in C stocks between plots was surprising, given that plots were positioned only 30 m apart and on the same soil type.

**Table 3.5.** Mean C stocks for plot 1 and 2 (measured using the coring method) and a single soil pit in dairy and drystock paddocks. Mean C stocks were calculated to an equivalent soil mass so that comparisons could be made between the pit and plot data. Therefore, mean C stocks were for a number of depths across sites, ranging from 50 and 60 cm (See Appendix D)

<table>
<thead>
<tr>
<th></th>
<th>Dairy</th>
<th></th>
<th>Drystock</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (t C ha$^{-1}$)</td>
<td>Diff (t C ha$^{-1}$)</td>
<td>P value</td>
<td>Mean (t C ha$^{-1}$)</td>
</tr>
<tr>
<td>Plot 1</td>
<td>158.1 (10.9)</td>
<td>7.4</td>
<td>0.06</td>
<td>158.8 (10.8)</td>
</tr>
<tr>
<td>Plot 2</td>
<td>150.7 (10.6)</td>
<td></td>
<td></td>
<td>151.5 (10.5)</td>
</tr>
<tr>
<td>Pit</td>
<td>164.8 (11.2)</td>
<td></td>
<td></td>
<td>174.7 (13.7)</td>
</tr>
</tbody>
</table>

Diff, Difference in mean C stocks (0-60 cm) between plot 1 and plot 2
Standard error of mean C stocks in parenthesis

As with the total C stocks, total N stocks of both plot 1 and plot 2 correlated well with the pit data (Fig. 3.10). However, the linear regression line was again above the 1:1 line, indicating a discrepancy in the measurement of N stocks between the coring method and the pit method. There was a significant difference of $1.01 \pm 0.37 \, \text{t N ha}^{-1}$ (P=0.01) between plot 1 and plot 2 for the dairy sites (Table 3.6). The difference in N stocks between plot 1 and plot 2 for the drystock sites was $0.5 \pm 0.6 \, \text{t N ha}^{-1}$ (P=0.14).
Detection of differences in C and N stocks

Figure 3.10. A. Total N stocks (0-60 cm), estimated using the coring method (plot 1) vs. total N stocks, estimated using a single pit. Straight line is a linear regression ($y=0.9x+4.1$, $R^2 = 0.66$) and dotted line is a 1:1 line. B. Total N stocks (0-60 cm), estimated using the coring method (plot 2) vs. total N stocks, estimated using a single pit. Straight line is a linear regression ($y=1.03x+2.9$, $R^2 = 0.72$).
Table 3.6. Mean N stocks for plot 1 and 2 (measured using the coring method) and a single soil pit in dairy and drystock paddocks. Mean N stocks were calculated to an equivalent soil mass so that comparisons could be made between the pit and plot data.

<table>
<thead>
<tr>
<th></th>
<th>Dairy</th>
<th>Drystock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (t C ha(^{-1}))</td>
<td>Diff (t C ha(^{-1}))</td>
</tr>
<tr>
<td>Plot 1</td>
<td>15.5 (0.9)</td>
<td>1.01</td>
</tr>
<tr>
<td>Plot 2</td>
<td>14.5 (0.8)</td>
<td>14.4 (0.7)</td>
</tr>
<tr>
<td>Pit</td>
<td>17.9 (0.9)</td>
<td>18.3 (1.1)</td>
</tr>
</tbody>
</table>

\(^{A}\) Diff, Difference in mean N stocks (0-60 cm) between plot 1 and plot 2
Standard error of mean N stocks in parenthesis

3.4.3 Ability to detect differences in total C and N stocks

In the context of this study, the least significant difference (LSD) is the smallest difference that could have been detected between dairy and drystock sites, had a difference occurred. For example, in the current study, I measured a non-significant (P=0.7) difference of 1.6 t C ha\(^{-1}\) from 0-60 cm between dairy and drystock farms. For a significant difference to have occurred (P<0.05), the measured difference would have had to be at least 9.3 t C ha\(^{-1}\) (LSD). Power analysis on the other hand determines what difference in C stocks could be detected between paired sites, should future sampling of paired sites occur. For example, to detect a whole profile difference in C stocks of 15 t C ha\(^{-1}\) with 80% certainty, 23 paired dairy and drystock sites would need to be sampled.

3.4.3.1 Least significant differences

The least significant difference (LSD) is the smallest significant difference (\(\alpha=0.05\)) that could have been detected between treatments for a given sampling design, had a difference occurred. That is, if the measured difference was less than the LSD, the measured difference would not be significant. Based on the measured variance components, the smallest significant difference in total C that could be detected for the 0-10 cm layer was only slightly higher than the measured difference (Table 3.7). However, for the 10-25 cm soil layer, the LSD was 5.1 t C ha\(^{-1}\) compared to a measured difference of 1.2 t C ha. The inability to
detect differences in the 10-25 cm layer was probably a result of the high variability of C in this layer associated with variable A horizon depths across sites. For the 25-40 and 40-60 cm layers, the measured differences were close to the LSD which was surprising given the fact that C stocks are usually highly variable at depth. From 0-60 cm (whole profile), the LSD was about 6 times higher than the measured difference. Therefore, a difference of 9.3 t C ha\(^{-1}\) would have had to be measured for this difference to be significant.

Table 3.7. Measured differences between dairy and drystock sites and least significant differences (LSD) of total C and N for the respective soil layers and whole profile (0-60 cm)

<table>
<thead>
<tr>
<th>Soil depth</th>
<th>C stocks</th>
<th>LSD</th>
<th>N stocks</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured difference (t ha(^{-1}))^ A</td>
<td>Measured difference (t ha(^{-1}))</td>
<td>Measured difference (t ha(^{-1}))</td>
<td>Measured difference (t ha(^{-1}))</td>
</tr>
<tr>
<td>0-10 cm</td>
<td>-4.1 (2.1)</td>
<td>4.4</td>
<td>-0.2 (0.2)</td>
<td>0.4</td>
</tr>
<tr>
<td>10-25 cm</td>
<td>-1.2 (2.4)</td>
<td>5.1</td>
<td>0.1 (0.3)</td>
<td>0.5</td>
</tr>
<tr>
<td>25-40 cm</td>
<td>2.3 (1.1)</td>
<td>2.28</td>
<td>0.2 (0.1)</td>
<td>0.2</td>
</tr>
<tr>
<td>40-60 cm</td>
<td>1.4 (0.7)</td>
<td>1.5</td>
<td>0.15 (0.1)</td>
<td>0.15</td>
</tr>
<tr>
<td>0-60</td>
<td>-1.6 (4.5)</td>
<td>9.3</td>
<td>0.3 (0.5)</td>
<td>1</td>
</tr>
</tbody>
</table>

Standard error of difference between mean C stocks in parenthesis

A The difference in C/N stocks was calculated as: dairy-drystock

B LSD, least significant difference at \(\alpha=0.05\)

If the measured difference is lower than the LSD, a post-hoc power analysis should be applied to determine if the lack of significance could be due to type II error. Type II error occurs when a significant difference between samples is not detected when in reality, there is a true difference between populations and that insufficient replicates were taken to determine a difference.

3.4.3.2 Power analysis of differences in total C stocks

Power analysis was conducted to determine the differences in C stocks that could be detected between dairy and drystock sites, should 23 randomly chosen paired sites be resampled in the future given the measured variability. It is important to note that the power analysis provided in this section is only applicable to dairy vs. drystocks sites, located on predominantly Allophanic soils in the Waikato Region.
and that measurements are made using the two-plot coring approach. Furthermore, the assumption is made that the measured standard deviation of the difference between mean C stocks reflects the true variability. Power analyses were conducted for the 0-25 cm, 25-40 cm and 0-60 cm (whole-profile) soil layers. Power analyses for the 0-25 and 25-40 cm soil layers can be found in Appendix B. For the 0-25 cm layer, a statistically significant difference of 10.8 t C ha\(^{-1}\) (\(\sigma=0.05\)) could be detected with 80% probability (Fig. B.1) if such a difference truly existed. A much smaller difference was detectable for the 25-60 cm layer where a statistically significant difference of 5 t C ha\(^{-1}\) could be detected with 80% certainty (Fig. B.3).

Overall, for the 0-60 cm increment of 23 paired sites, the probability (power) of detecting a significant difference of 1.6 t C ha\(^{-1}\) was only 6% given the between land use variability (Fig. 3.11). There would be an 80% chance of detecting a significant difference of 13.2 t C ha (\(\sigma=0.05\)) and a 90% chance of detecting a significant difference of 15.3 t C ha\(^{-1}\).

![Figure 3.11. Power curve of total C stocks to 60 cm for 23 paired dairy and drystock sites. Statistical power is the probability of detecting a significant difference (\(\alpha=0.05\)) with some degree of certainty. Difference (t C ha\(^{-1}\)) is the difference in C stocks that might be measured between adjacent dairy and drystock farms. Round symbols indicate the detectable difference for a statistical power of 70, 80 and 90%.](image-url)
Power analysis can also be used to estimate the number of replicates required to detect pre-specified differences between treatments, or in my study, a predefined difference in total C stocks between land uses. To measure a significant difference ($\alpha=0.05$) of 15 t C ha$^{-1}$ (0-60 cm) with 80% probability, 19 paired sites would be required (Fig 3.12). However, to detect a significant difference of 5 t C ha$^{-1}$ ($\alpha=0.05$) with 80% certainty, 149 paired dairy/drystock sites would be needed.

**Figure 3.12.** Power curves of total C stocks (0-60 cm) for 3 sample sizes (n=19, 39 and 149). Round symbols indicate the detectable difference in C stocks with 80% certainty for the respective sample sizes.

The number of sites required to detect a significant difference ($P<0.05$) of 5 t C ha$^{-1}$ and 10 t C ha$^{-1}$ increased considerably with increasing power (Fig. 3.13). For example, to detect a difference of 10 t C ha$^{-1}$ with 70% certainty, 31 paired sites would be required. However, to detect a difference of 10 t C ha$^{-1}$ with 90% certainty, 51 paired sites would be needed. Similarly, there is a 70% chance of detecting a difference of 5 t C ha$^{-1}$ with 118 paired sites but 199 paired sites would be required to detect a 5 t C ha$^{-1}$ difference with 90% certainty.
3.4.3.3 Detecting differences in total C using pit (Barnett et al., 2014) vs. coring method

Based on the variance components and with 23 paired sites, the LSD of the pit approach was 16.7 t C ha\(^{-1}\) from 0-60 cm (\(\alpha=0.05\)) (Table 3.8). However, when site 24 was removed from the analysis, the LSD of the pit approach was reduced to 13.6 t C ha\(^{-1}\), similar to that of the single-plot coring approach. The two-plot coring approach had a much lower LSD of 9.3 t C ha\(^{-1}\), 16\% less than the LSD of the single-plot coring approach. As well as having the ability to detect smaller differences in C stocks between dairy and drystock farms, the coring approach was considerably more efficient compared to the pit approach. The estimated time for a single person to collect samples from a paired site using the coring approach was about 5 hours compared to 1.5-2 days for when the pit method was used (Table 3.8).
Table 3.8. Least significant difference (LSD) between dairy and drystock sites for total C to 60 cm and the estimated time taken to sample a paired site using the respective methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Difference^A</th>
<th>SED^A</th>
<th>LSD^A</th>
<th>Time^A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single 5x5 m plot</td>
<td>-1.56</td>
<td>6.2</td>
<td>11</td>
<td>2 hrs.</td>
</tr>
<tr>
<td>Two 5x5 m plots</td>
<td>-1.63</td>
<td>4.5</td>
<td>9.3</td>
<td>5 hrs.</td>
</tr>
<tr>
<td>Single pit</td>
<td>-9.8</td>
<td>8.1</td>
<td>16.7</td>
<td>1.5-2 days</td>
</tr>
<tr>
<td>Single pit excluding site 24</td>
<td>-4.7</td>
<td>6.6</td>
<td>13.6</td>
<td></td>
</tr>
</tbody>
</table>

^A Difference, mean difference in C stocks between dairy and drystock pastures; SED, standard error of the difference between means; LSD, least significant difference (α=0.05); Time, the estimated time taken for 1 person to collect samples from a paired site using the respective methods.

Figure 3.14 shows the ability of the coring approach to detect difference in C stocks using different numbers of plots and sites. For example, had I sampled 23 sites using a single plot, a difference of 11 t C ha^-1 could have been detected. However, by adding an additional plot to each site, the LSD was reduced by 16% to 9.3 t C ha^-1. The relative decrease in the LSD becomes less as the number of plots increases. For example, increasing the number of plots from 4-5 (n=23) only reduces the LSD from 7.8 t C ha^-1 to 7.6 t C ha^-1.

Figure 3.14. The least significant difference (t C ha^-1) that could have been detected to 60 cm with varying numbers of plots and sites that could be sampled. Arrow represents the improvement in LSD in this study when going from 1 to 2 plots.
Detection of differences in C and N stocks

For the purpose of future sampling, power analysis was conducted on the pit data and the coring data (2 plots) to determine the probability (power) of detecting a 10% difference (P<0.05) in C stocks between 23 dairy and drystock sites. It is important to note that power analysis assumes that a given number of paired sites is randomly selected from a greater population of sites. In this study, the population of sites includes all paired dairy and drystock farms in the Waikato Region.

For whole-profile C stocks (0-60 cm), the pit approach could detect a 10% difference (~16 t C ha\(^{-1}\)) with 66% certainty (Fig. 3.15). However, there is a 90% chance that the two-plot coring approach would detect a 10% difference if 23 paired dairy/drystock sites were randomly selected and sampled. For the 0-25 cm layer, both the pit approach and coring approach have a relatively high chance of detecting a 10% (~11 t C ha\(^{-1}\)) difference. The pit approach could detect a 10% difference with 64% certainty while the two-plot coring approach could detect the same difference with 80% certainty. For the 25-60 cm depth increment, the coring approach is far more likely to detect a difference of 10% (~5 t C ha\(^{-1}\)) between paired dairy/drystock sites. The pit approach would have a 25% chance of detecting a 10% difference, while the coring approach could detect the same difference with 70% certainty.
Detection of differences in C and N stocks

Figure 3.15. The power of detecting a 10% difference in C stocks between 23 adjacent dairy and drystock paddocks using the coring approach and the pit approach (Barnett et al., 2014). For the 0-25 cm later, a 10% change represented ~11 t C ha\(^{-1}\) and for the 25-60 cm layer, a 10% change represented ~5 t C ha\(^{-1}\). Overall from 0-60, a 10% difference was equivalent to ~15 t C ha\(^{-1}\).

### 3.5 Discussion

#### 3.5.1 C and N stocks of dairy and drystock sites

In comparison to the study of Barnett et al. (2014), the current sampling strategy was more sensitive and efficient for determining total C and N stocks and differences in stocks between dairy and drystock systems. To a depth of 60 cm, the average C stock for dairy sites was 157.3 ± 10.8 t C ha\(^{-1}\) and 157.2 ± 10.5 t C ha\(^{-1}\) for drystock sites. Nitrogen stocks averaged 15.4 ± 0.8 t N ha\(^{-1}\) for dairy sites and 14.9 ± 0.8 t N ha\(^{-1}\) for drystock sites.

Previous sampling of the same sites measured greater average C stocks (0-60 cm) of 171.1 t C ha\(^{-1}\) for the dairy sites and 181.9 t C ha\(^{-1}\) for drystock sites (Barnett et al., 2014). The differences in measured C and N stocks between sampling approaches can be explained by the difference in calculated soil masses. Barnett et al. (2014) measured soil mass (t ha\(^{-1}\)) using a soil pit and by carving a series of
bulk density cores into each soil horizon down the length of a profile. In the current study, soil mass was measured directly using a single soil corer (chapter 2.6). The soil corer yielded significantly lower soil masses for the 0-10, 10-25 and 25-40 cm depth intervals, but there were no significant difference for the 40-60 cm layer (Fig. 3.7 and 3.8). The difference in soil mass between the two methods was especially large for the 0-10 cm depth interval. This large difference in soil mass was likely the result of an overestimation of the soil mass by the pit method because bulk density cores were placed beneath the rooting zone. The rooting zone has a lower bulk density compared to the soil below and failing to take the lower bulk density of the rooting zone into account would result in overestimated mass of soil at shallow depths. The overestimation of soil mass is promulgated into an overestimation of total C (Fig. 3.9) since total C (t ha\(^{-1}\)) is a function of soil mass (t ha\(^{-1}\)) and percent C. Although there were significant differences in soil mass for the 10-25 and 25-40 cm soil layers, these differences were small relative to the total mass of soil in each layer.

In New Zealand, C stocks have previously been determined using pit sampling (Tate et al., 1997). If the difference between coring and pit sampling holds true for other soil orders, it is possible that many of the farm scale surveys in New Zealand have systematically overestimated C stocks. This warrants further investigation, especially as new methods are developed to measure C stocks at the paddock scale. Quantifying C stocks using a recent method and comparing stocks to previous pit data may lead to false conclusions around losses or gains in soil C.

The mass of top soil in dairy sites was greater than drystock sites for the 0-10 cm depth interval by 47 t ha\(^{-1}\) which represented a 6% difference (P<0.05, Table 3.2). The difference in soil mass from 0-10 cm in dairy and drystock pastures was comparable to measurements made by Greenwood et al. (1998) who studied the effect of sheep stocking rates on soil physical properties in South Australia. Greenwood et al. (1998) found a mean difference in soil mass of 48 t ha\(^{-1}\) in the top 8 cm between un-grazed plots and heavily grazed plots with 20 sheep/ha. Given that the treading pressure of cows is double that of sheep (Schon et al., 2011b), it is perhaps not surprising that a significant difference in soil mass was
detected in the top 10 cm. There were no significant differences in soil mass between dairy and drystock grazed pastures below 10 cm and any differences were likely due to the spatial variability in bulk density as reflected by the large standard errors (Table 3.2). The detection of a significant difference in soil mass in the 0-10 cm layer but not in the sub-soil suggests that any impact of physical pressure by cows is constrained to the top soil.

Given that the topsoil of the dairy sites was compacted relative to the drystock sites, sampling to a fixed depth simply results in different dry masses of soil material being sampled (Gifford and Roderick, 2003). Recognising the dependence of the calculation of soil C stocks on soil thickness and bulk density (see chapter 2.6.2), many studies have compared C stocks by equivalent soil mass (ESM) (Ellert and Bettany, 1995; Ellert et al., 2002; Gifford and Roderick, 2003). Comparing C stocks by an ESM is most critical for shallow sampling because bulk density varies the most near the soil surface. In contrast to the 0-10 cm layer, the average soil mass of dairy sites for the 40-60 cm layer was only 1% greater than the average soil mass of soil for drystock sites. As a consequence, applying ESM calculations had no effect on the measured difference in C stocks between dairy and drystick farms for the 40-60 cm soil layer.

### 3.5.2 Differences in C stocks between dairy and drystock pastures

Drystock farms had an average of 4.1 ± 2.1 t ha\(^{-1}\) more C in the 0-10 cm layer compared to dairy farms (P=0.06, Table 3.3). This difference was similar to Barnett et al. (2014) who measured a difference of 8.6 ± 4.1 t C ha\(^{-1}\) (0-16 cm) for the same dairy/dry stock in the top 16 cm. The differences in C stocks between dairy and drystock sites detected in the current study were also comparable to measurements made by Ganjegunte et al. (2005) and Li et al. (2007) who demonstrated C stocks to be higher under light grazing than heavy grazing. Ganjegunte et al. (2005) measured a difference of 2.9 t C ha\(^{-1}\) (0-5 cm), and Li et al. (2007) found a difference of 7 t C ha\(^{-1}\) (0-20 cm). Other studies have shown the effect of grazing pressure on soil C stocks to be negligible (e.g. Abril and Bucher, 2001), although lack of significant differences in land use studies may be because
of insufficient sampling power to detect relatively small changes (Kravchenko and Robertson, 2011).

While drystock farms had comparatively higher C stocks from 0-10 and 10-25 cm (after being corrected to an ESM), dairy farms had more C from 25-40 cm and 40-60 cm. Although many studies have measured the effect of grazing on soil C stocks in the topsoil (Li et al., 2007; Steffens et al., 2008), few studies have measured C stocks below 30 cm. A likely reason for this is the effort and cost associated with deep soil sampling and that the effect of land use on soil C stocks is generally considered to be greatest in the top soil. However, land use management can influence the storage and distribution of soil C in the topsoil and subsurface layers. Conflicting results of comparisons between till and no-tillage on soil C stocks have often been attributed to differences in depth of sampling between treatments. For example, in a long-term tillage trial in Illinois, Olson et al. (2014) demonstrated an increase in soil C for the upper 5 cm of the no-tillage system but soil C was lost from the 5-75 cm layer.

The fact that dairy sites had more C below 25 cm was unexpected and more extensive research is required to determine the dynamics of sub-soil C storage with respect to land use. I can only speculate as to why the dairy sites contained less C in the top soil and more C in the subsoil. A possible explanation is related to the effective size and concentration of urine patches from animals on dairy and drystock farms. Urine patches from sheep and drystock cattle tend to be shallow and smaller compared to urine patches from dairy cows (Li et al., 2012). Lambie et al. (2012) found that the potential solubilisation of C from pasture top soils was 25-40% after the addition of cow urine. Solubilised C in the topsoil is likely to be leached and re-deposited in the lower profile. However, the study by Lambie et al. (2012) was carried out under laboratory conditions and the solubilisation of C under urine patches is likely to be less in a field situation. The solubilisation of C in the topsoil and the re-adsorption of dissolved organic carbon (DOC) to reactive mineral phases further down the profile is a potential mechanism by which soil C could be translocated from the top soil to the sub-soil (Kaiser and Guggenberger, 2000; Kalbitz et al., 2005). A second hypothesis is that the higher stocking rates
Detection of differences in C and N stocks of dairy sites enhanced the allocation of C to belowground biomass below 25 cm, however, this does not explain the loss of C from the top soil.

Greater than 95% of N stored in soil is in an organic form and is covalently bonded to soil C (Piñeiro et al., 2009a; Schipper et al., 2004). Figure 3.5 demonstrated a strong relationship ($R^2=0.87$) between total C stocks and total N stocks. Therefore, it is not surprising that drystock sites contained more N in the top 10 cm compared to dairy sites, although the difference was not significant. As with the total C stocks, dairy sites had significantly more N in the sub-surface soil layers (below 25 cm) compared to drystock sites.

Overall, in the top 60 cm, drystock sites contained $1.6 \pm 4.6$ t C ha$^{-1}$ more C than dairy sites but the difference was not significant. This finding confirmed previous sampling which showed drystock sites to contain $9.6 \pm 7.9$ t ha$^{-1}$ more C than dairy sites (Barnett et al., 2014). However, the results of Barnett et al. (2014) were heavily skewed by one site (site 24, Figure 3.9). When site 24 was removed from the data, drystock sites contained $4.3 \pm 6.2$ t ha$^{-1}$ more C than dairy sites. Both the current study and the study conducted by Barnett et al. (2014) were unable to detect significant differences in C stocks to 60 cm.

### 3.5.3 Detecting differences in C stocks between dairy and drystock pastures

A key objective of this study was to test a new approach for sampling soil C and N to depth that would allow detection of smaller differences between land uses compared to the pit method. To assess the effectiveness of the pit vs. coring approach for detecting differences in C stocks, the least significant differences (LSD) for the respective methods were compared. In the context of my study, the LSD was defined as the smallest significant ($\alpha=0.05$) difference in C stocks that could have been detected using the respective methods, had a difference occurred. For total C stocks, the LSD between dairy and drystock sites for the pit method and single-plot coring approach was $13.6$ t C ha$^{-1}$ (excluding site 24) and $11$ t C ha$^{-1}$ respectively. This similarity in LSD between the pit approach and plot 1 was somewhat surprising, given the fact that five replicate soil cores were taken from
Detection of differences in C and N stocks

each plot. However, when data from plots 1 and 2 were combined, the LSD declined to 9 t C ha⁻¹, allowing for the detection of smaller differences between land uses. Furthermore, the sampling time of the two-plot coring approach was considerably less than that of the pit approach.

The spatial variability of C stocks in grazed systems has been well studied (Conant et al., 2003; Giltrap and Hewitt, 2004). For example, Giltrap and Hewitt (2004) measured the spatial variability of soil C in Allophanic Soils over distances of 5, 30 and 100 m. They determined that taking samples 30 m apart did not fully account for the spatial variability in total volumetric C that occurred over distances of 100 m. However, in the current study, most paddocks were relatively small in comparison to those measured by Giltrap and Hewitt (2004) and therefore, 2 plots positioned 30 m apart likely provided sufficient spatial coverage. There was no explanation as to why the mean C stock and the LSD of plot 1 was so different to that of plot 2 (Tables 3.5 and 3.7). Clearly, the differences between plot 1 and plot 2 were not only due to natural variability in C stocks but also because of some unknown systematic error contribution. Nevertheless, when plots 1 and 2 were combined, the LSD between dairy and drystock sites was improved to 9 t C ha⁻¹. A number of studies have shown increased spatial replication improves the detectability of changes in soil C stocks at the paddock scale (Conant et al., 2003; Heckman et al., 2009). For example, Conant et al. (2003) sampled cultivated sites in Tennessee to determine the detectability of changes in C stocks over time. Analysis revealed that 5-9 micro-plots were required to detect a change of 0.5 t C year⁻¹, however, to detect a change of 0.25 t C year⁻¹, more than 20 micro-plots were needed.

For the purpose of providing information for future sampling, power analysis was applied to the pit data and coring data to determine the probability (power) of detecting a 10% difference in C stocks between 23 paired dairy/drystock sites. It is important to remember that power analysis assumes that the sample of 23 paired sites is randomly selected from a greater population of paired dairy/drystock sites. In the context of my study, the population of paired sites includes all paired dairy and drystock farms on flat land in the Waikato Region.
Power analysis revealed that with 23 paired sites, detecting whole-profile differences in C stocks of less than 10% is unrealistic given the high variability of C stocks, particularly at depth. For example, with 23 paired dairy/drystock sites, there is only a 6% chance that a significant whole-profile difference of 1.6 t C ha\textsuperscript{-1} (or 1% difference) could be detected using the coring method. The difficulty in detecting whole-profile changes in C stocks has been well documented (Conant et al., 2003; Kravchenko and Robertson, 2011; Syswerda et al., 2011). For example, Kravchenko and Robertson (2011) compared the C content of tillage vs. no tillage sites for a series of soil layers. They demonstrated that a 10% difference in C stocks could be detected with 50% certainty for the surface layers but for the 40-100 cm layer, the probability of detecting a 10% difference was less than 9%. Increasing variability of C stocks with depth makes the detection of whole-profile changes in soil C stocks difficult. However, the two-plot coring approach is far more likely to detect whole-profile differences in C stocks compared to the pit approach. Assuming that the measured variance components hold true and with 23 paired dairy/drystock sites, the coring approach would able detect a difference of 15 t C ha\textsuperscript{-1} or ~10% with 90% certainty. The pit approach on the other hand would only have a 66% chance of detecting a 10% difference (~15 t C ha\textsuperscript{-1}).

Power analysis was also conducted on the 0-25 cm and 25-60 cm layers to determine the relative power of the coring and pit approach to detect differences in C stocks during future samplings. Using the two-plot coring approach, a 10% difference in C stocks (11 t C ha\textsuperscript{-1}) for the 0-25 cm layer could be detected with 80% certainty (Fig. 3.15). The same 10% difference could be detected with 64% certainty if the pit approach was to be used. Many studies have demonstrated that relatively small changes in C stocks can be measured near the soil surface (Conant et al., 2001; Conen et al., 2004; Kravchenko and Robertson, 2011; Schrumpf et al., 2011). For example, at a no-tillage site in Ireland, Schrumpf et al. (2011) determined that with a 100 replicate samples and to 30 cm depth, a 2.8 t ha\textsuperscript{-1} change in C stocks could be detected through time. The inability of both the coring approach and pit approach to detect small differences in C stocks in the top soil is likely a result of the high variability in topsoil C stocks across dairy and drystock sites. A synthesis of the literature (chapter 2.5) demonstrated that a
number of environmental factors (e.g. soil type) and management factors (e.g. stocking rate) affect the storage of C in the top soil of grazed systems. Since management factors and soil edaphic factors varied so much across sites, it is not surprising that the detection of small differences in topsoil C stocks is unlikely.

Interestingly, for the 25-40 cm layer, a 10% (5 t ha\(^{-1}\)) difference could be detected with 70% certainty if the coring approach is used (Fig. 3.15). This finding was unexpected, given that the detectability of differences in C stocks is generally thought to diminish with depth (Kravchenko and Robertson, 2011). For example, power analysis of the pit data from Barnett et al. (2014) revealed that there was only a 25% chance of detecting a 10% difference in the 25-60 cm layer (Fig. 3.16). The detectable differences calculated from Barnett et al. (2014) are comparable to those calculated by Yang et al. (2008) who compared soil C stocks of 3 till vs. no-tillage sites in America and Canada. They determined that for the Canadian sites and with a power of 80%, a 12-15% difference could be detected in the 0-20 cm layer but for the 20-50 cm soil layers, a difference of only 24-36% could be detected.

It is important to note that the power analysis applied in this study is only directly applicable to paired/dairy and drystock sites, positioned on predominantly Allophanic Soils. This is because the relationship between sample size and statistical power is influenced by a number of site specific factors such as land use and soil type (Conant et al., 2003). Nevertheless, using replicated plots with bulked soil cores from each plot appears to greatly improve the detectability of changes in C stocks compared to the single pit approach. Detecting changes in C stocks, even at depth, is possible if careful consideration is given to sampling design and statistical analysis (Conant et al., 2003; Kravchenko and Robertson, 2011). Importantly, power analysis of the pit vs. coring approach demonstrated that there is a lower limit to the size of differences in C stocks that can be detected with soil sampling approaches.
3.6 Conclusions

The near significant difference (P=0.06) in C stocks for the 0-10 cm soil layer supported the findings of Barnett et al. (2014) that drystock sites had significantly more C in the A horizon compared to dairy sites. However, in the lower soil layers, (25-40 cm and 40-60 cm), dairy sites had more C than drystock sites. A possible explanation for higher C stocks lower down the soil profile under dairy sites is the effective size and concentration of dairy cow urine patches which can solubilise organic C (Lambie et al., 2012). Urine patches from sheep and drystock cattle tend to be shallow and smaller compared to urine patches from dairy cows (Li et al., 2012). The larger size and concentration of dairy cow urine patches relative to sheep/beef cattle could acts as a mechanism for transporting greater quantities of C from the topsoil to sub-soil horizons in dairy sites. However, further research is required to test these findings because the dynamics of C storage lower in the soil profile is poorly understood.

While differences in soil C were observed in different layers of the soil profile, for the top 60 cm there was no significant difference in C stocks between dairy and drystock sites. A synthesis of the literature demonstrated that detecting whole-profile differences in C stocks is difficult, mostly because of the high variability of soil C at depth (Kravchenko and Robertson, 2011; Syswerda et al., 2011). Compared to the pit approach (Barnett et al., 2014), the coring approach had the ability to detect smaller whole-profile differences in C stocks. As reflected by the relatively small LSD, the use of two plots (with 5 cores each) in each paddock improved the detectability of C stocks by accounting for more of the with-in paddock variability in soil C (Conant et al., 2003). In fact, the LSD for whole-profile C stocks was reduced by 16% when increasing the number of plots from one to two. However, there is little benefit in using more than 3 plots per paddock as the relative decrease in LSD becomes less with increasing numbers of plots.

Power analysis was conducted to determine the probability of detecting pre-defined differences in C stocks for the purpose of future sampling. The two-plot coring approach is far more likely to detect both whole-profile differences in C
Detection of differences in C and N stocks

stocks and changes that occur in the 0-25 and 25-60 cm layers. Furthermore, the coring approach was considerably more efficient and cost effective compared to the pit approach. However, soil pits provide important descriptive information which may not be provided by soil cores.

It is important to note that the power analysis applied in this study is only directly applicable to paired dairy/drystock sites, located on predominantly Allophanic Soils. However, power analysis revealed that careful sampling and statistical analysis is required to accurately determine changes in soil C storage. Moreover, compared to previous sampling (Barnett et al., 2014), the use of a relatively simple and cost effective coring approach greatly improved the detectability of differences in C stocks between dairy and drystock pastures.

Further research is required to determine how changes in C stocks can be accurately quantified for different land uses, particularly at depth where C stocks are highly variable.
CHAPTER FOUR

Earthworm abundance and biomass in adjacent dairy and drystock pastures

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Tim Norris and Louis Schipper developed the research objective and experimental design. Tim Norris with assistance from Jack Pronger (not in author list) carried out field sampling. Ross Gray and Nicole Schon carried out identification of earthworms and attribution of species to functional groups. Tim Norris conducted data analysis and statistical analysis. This chapter was written by Tim Norris and reviewed by Louis Schipper.
4.1 Abstract

Earthworms have potential to greatly enhance the incorporation of plant litter into the soil profile, potentially increasing the storage of C. Furthermore, earthworms are known to increase soil quality by improving soil structure and nutrient retention. The objective of this study was to compare earthworm abundance and biomass between adjacent dairy and drystock farms.

Seventeen adjacent dairy and drystock farms, located in the Waikato Region and on predominantly Allophanic Soils were sampled from 3 points in each paddock between August and November 2013. Samples were sorted for earthworms and classified. Total abundance and biomass was calculated and earthworms were also classed into 3 functional groups: the epigeic group (dominant at the soil surface), endogeic group (found throughout the top-soil) and anecic group (deep burrowing earthworms).

A previous study (Barnett et al., 2014, AEE 185:34-40) on the same sites found dairy farms to have significantly higher stocking rates compared to drystock farms. Despite the higher stocking rate and greater soil bulk density, there was no significant difference in total abundance or biomass. Total earthworm abundance and biomass averaged 193 ± 30 ind m\(^{-2}\) and 77 ± 12 g m\(^{-2}\) for dairy farms compared to 188 ± 26 ind m\(^{-2}\) and 75 ± 13 g m\(^{-2}\) for drystock farms.

These results suggested that for Allophanic soils in the Waikato Region, the effects of varying grazing management on earthworm abundance and biomass is negligible.
4.2 Introduction

Earthworms play a fundamental role in the incorporation of plant litter into the profile and the turnover and stabilisation of organic matter into aggregates (Blanco-Canqui and Lal, 2004; Six et al., 2004). The stabilisation of C in soils is largely mediated by soil fauna and microbes that form organo-mineral complexes which are low in mineralizable C (Post et al., 2001; Tan et al., 2004). Through cast formation and burrowing, earthworms increase the stabilized fraction of C in soil, thus enhancing the long term storage of C (Six et al., 2004). In agricultural systems, earthworms are also important for the maintenance of soil structure and retention of nutrients, both of which are essential for optimal plant growth (Schon et al., 2011a).

In New Zealand, 75% of the original native forest was cleared by 13th C Polynesians and 19th C European settlers to make way for exotic pasture (Hewitt et al., 2012). Such land use conversions displaced a significant proportion of New Zealand native earthworms and although there are over 200 native species, most are found exclusively in forests (AgResearch, 2011). Earthworm species in New Zealand grazed pasture soils are restricted to 3 dominant species: *Aporrectodea spp.*, *Lumbricus spp.*, and *Octolasion spp.*, all of which were introduced by European settlers (Schon et al., 2011a). Introduction of earthworms into pasture systems was found to increase plant growth by as much 113% because of the increased movement of water and fertilizer through the soil profile (Schon et al., 2011a).

Earthworms belong to one of three functional groups according to Bouché’s (1977) classification of earthworms. Functional categories include epigeic, endogeic and anecic species. Anecic species (body width 6-9 mm) excavate permanent burrows to depths of up to a few meters below ground (Felten and Emmerling, 2009; Schon et al., 2011c). Endogeic species (body width 2-6 mm) excavate semi-permanent burrows which are found predominantly in the top soil but also just below the A horizon (Felten and Emmerling, 2009). Both anecic and endogeic species feed on organic matter in the top soil and incorporate this into
lower parts of soil profile (Schon et al., 2011c). Anecic earthworms are particularly effective at mixing organic matter into the soil profile as they transfer organic matter deep below the soil surface. Epigeic earthworms (width 2-6 mm) do not form permanent burrows and feed on organic matter near/on the soil surface (AgResearch, 2011).

Earthworm abundance and biomass is strongly affected by food supply and management practices which have an impact on the amount and quality of organic matter returned to the soil (Curry et al., 2008). Some studies have found earthworm abundance and biomass to increase with increasing grazing intensity and fertilizer use (Muldowney et al., 2003). For example, Curry et al. (2008) demonstrated a significant positive relationship between N application rate and earthworm abundance while treading pressure had no effect on earthworm abundance. Mineral fertilizers have been found to increase litter quality and quantity, thus promoting higher earthworm abundance and biomass (Villenave et al., 2011). Furthermore, intensively managed pastures with high stocking rates contain large quantities of animal excreta (dung and urine) which are hot spots for organic matter and N inputs (Villenave et al., 2011).

High animal stocking rates have also been known to adversely affect earthworm abundance and biomass (Curry et al., 2008; Muldowney et al., 2003; Schon et al., 2012a; Villenave et al., 2011). The effects of stocking rate on earthworm numbers and biomass are twofold. Firstly, increased grazing and disturbance may drive a decrease in organic matter inputs and therefore cause changes in the detrital food chain. A number of studies have attributed losses of total C under intensively grazed systems to reduced inputs of plant material and increased mineralization of organic matter due to disturbance (Barnett et al., 2014; Ganjegunte et al., 2005; Li et al., 2007). For example, Barnett et al. (2014) measured total C and N stocks of 25 adjacent dairy and drystock sites in the Waikato and demonstrated that the more intensively managed dairy sites contained 8.6 ± 4.1 t ha⁻¹ less C than adjacent drystock sites. Secondly, increased grazing intensity may alter soil physical conditions, thus reducing habitable pore spaces for earthworms (Curry et al., 2008; Schon et al., 2012b). In addition to nutrient inputs and grazing pressure,
Earthworms in adjacent dairy and drystock pastures

earthworm abundance and biomass is also highly dependent on site specific factors such as soil type, climate and aspect (Schon et al., 2012a).

In New Zealand pastoral systems, stocking rates vary considerably, especially between dairy and drystock systems. Dairy farms are generally more intensively managed compared to drystock farms, with higher stocking rates, greater fertilizer and feed imports, higher product-export and heavier animals (Mackay, 2008). The effect of dairy vs. drystock managed pastures on earthworm populations is unclear, mainly because of the large variation in stocking rates and fertilizer use across sites. Previous studies exploring the effect of stocking rate on earthworm populations in New Zealand pastures (e.g. Schon et al., 2011a) have found increased stocking rate to adversely affect earthworm abundance and biomass. However, more research is required to reconcile this hypothesis.

The objective of this study was to quantify earthworm abundance and biomass in 17 adjacent dairy and drystock farms, located in the Waikato Region of New Zealand. More importantly, we were interested in how varying management practices, particularly stocking rate would influence the abundance and biomass of earthworms. This study was carried out on the same sites used to quantify differences in total C and N stocks between dairy and drystock farms (Chapter 3).

4.3 Methods

4.3.1 Site description

The study was conducted on 17 adjacent dairy and drystock farms in the Waikato Region of New Zealand (Table 1). The geographical distribution of sites ranged from Rangitoto (Southern Waikato) to Te Aroha (Eastern Waikato). Soils spanned four soil orders, the majority of which were Allophanic Soils. Annual rainfall across sites ranges from 1127 mm to 1550 mm.
Table 4.1. Site information for the 17 adjacent dairy and drystock farms

<table>
<thead>
<tr>
<th>Site number</th>
<th>Date of sampling</th>
<th>Location</th>
<th>NZSC A</th>
<th>STB</th>
<th>MAP C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>14/11/2013</td>
<td>Te Aroha</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1116</td>
</tr>
<tr>
<td>3</td>
<td>15/11/2013</td>
<td>Te Aroha</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1116</td>
</tr>
<tr>
<td>6</td>
<td>08/08/2013</td>
<td>Cambridge</td>
<td>Gley</td>
<td>Incep</td>
<td>1189</td>
</tr>
<tr>
<td>8</td>
<td>05/09/2013</td>
<td>Maihihi</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1550</td>
</tr>
<tr>
<td>12</td>
<td>17/10/2013</td>
<td>Puketotara</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1303</td>
</tr>
<tr>
<td>13</td>
<td>19/09/2013</td>
<td>Pirongia</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1303</td>
</tr>
<tr>
<td>14</td>
<td>09/08/2013</td>
<td>Te Miro</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1189</td>
</tr>
<tr>
<td>16</td>
<td>30/08/2013</td>
<td>Puakeuta</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1127</td>
</tr>
<tr>
<td>17</td>
<td>18/09/2013</td>
<td>Rangitoto</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1550</td>
</tr>
<tr>
<td>18</td>
<td>28/08/2013</td>
<td>Rotoorangi</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1127</td>
</tr>
<tr>
<td>19</td>
<td>14/08/2013</td>
<td>Tauwhare</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1189</td>
</tr>
<tr>
<td>21</td>
<td>13/11/2013</td>
<td>Tauwhare</td>
<td>Granular</td>
<td>Ando</td>
<td>1189</td>
</tr>
<tr>
<td>22</td>
<td>07/08/2013</td>
<td>Te Miro</td>
<td>Gley</td>
<td>Incep</td>
<td>1189</td>
</tr>
<tr>
<td>23</td>
<td>27/09/2013</td>
<td>Te Pahu</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1303</td>
</tr>
<tr>
<td>24</td>
<td>26/09/2013</td>
<td>Te Pahu</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1303</td>
</tr>
<tr>
<td>25</td>
<td>23/10/2013</td>
<td>Karamu</td>
<td>Brown</td>
<td>Ando</td>
<td>1303</td>
</tr>
<tr>
<td>26</td>
<td>22/10/2013</td>
<td>Tauwhare</td>
<td>Allophanic</td>
<td>Ando</td>
<td>1189</td>
</tr>
</tbody>
</table>

A NZSC, soil order from New Zealand soil classification; ST, soil orders from US Soil Taxonomy
B Ando, Andisol; Ult, Ultisol; Incep, Inceptisol
C MAP, mean annual precipitation. Data obtained from Niwa climate stations (http://cliflo.niwa.co.nz/pls/niwp/wgenf.genform1_proc) or Waikato Regional Council monitoring stations (http://www.waikatoregion.govt.nz/riverlevelsandrainfall/cgi-bin/hydwebserver.cgi/catchments/details?catchment=16)

The 17 adjacent sites had been under their respective land uses for a minimum of 10 years, although sites 16 and 17 had been cultivated the year prior to sampling. Grazing intensity and fertilizer management varied greatly amongst sites of the same land use. Barnett et al. (2014) calculated stocking rates for 16 dairy and 16 drystock farms, many of which were used in this study. The average stocking rate (calculated using relationships given in Coop (1965)) of dairy farms (± 1 SE) was 24 ± 0.8 SU ha⁻¹ and was significantly greater (P<0.01) than the stocking rate of drystock farms which was 14 ± 2 SU ha⁻¹. However, stocking rates for both land uses were highly variable, ranging from 14-27 SU ha⁻¹ for the dairy sites and 6-30 SU ha⁻¹ for the drystock sites.

To a depth of 40 cm, total C stocks varied considerably amongst sites with values ranging from 80 t C ha⁻¹ to 226 t C ha⁻¹ for the dairy sites and 79 t C ha⁻¹ to 231 t C ha⁻¹ for drystock sites (chapter 3.4.1). Total N stocks ranged from 8 t N ha⁻¹ to
20 t N ha\(^{-1}\) for dairy sites and 7 t N ha\(^{-1}\) to 18 t N ha\(^{-1}\) for drystock sites. The average difference (± 1 SE) in C and N stocks to 40 cm between dairy and drystock sites was 0.9 ± 5 t C ha\(^{-1}\) and 0.2 ± 0.6 t N ha\(^{-1}\) (Table 4.2). Bulk density was significantly greater (P<0.05) under dairy sites compared to drystock sites with an average difference of 0.04 ± 0.02 t m\(^{-3}\).

**Table 4.2.** Dry bulk density, total C (t ha\(^{-1}\)) and total N (t ha\(^{-1}\)) for dairy and drystock sites (see chapter 3.4)

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Dairy sites</th>
<th>Drystock sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BD (t m(^{-3}))</td>
<td>C (t ha(^{-1}))</td>
</tr>
<tr>
<td>0-10</td>
<td>0.71</td>
<td>60.4</td>
</tr>
<tr>
<td>10-25</td>
<td>0.78</td>
<td>58.2</td>
</tr>
<tr>
<td>25-40</td>
<td>0.78*</td>
<td>30.3*</td>
</tr>
<tr>
<td>0-40</td>
<td>0.76*</td>
<td>148.9</td>
</tr>
</tbody>
</table>

* Significant difference between dairy and drystock sites (P<0.05)

Values of C and N in the table are calculated to a fixed depth. The differences in means were calculated to an equivalent soil mass.

### 4.3.2 Sampling

Sampling of earthworms was carried out from August to November 2013. In each paddock, earthworms were sampled from 3 points, located ~15 m apart. The first sampling point (1) was positioned at the centre of a randomly positioned 5x5 m plot (Fig 4.1). A second sampling point (2) was positioned 30 m away and in a random direction from the initial plot. Sampling point 3 was positioned mid-way between points 1 and 2. All points were on the same soil type and were at least 20 m away from fence boundaries or areas of high animal traffic.
Earthworms in adjacent dairy and drystock pastures

Figure 4.1. The sampling strategy used to sample earthworms from adjacent dairy and drystock paddocks. A total of three points (red stars) were sampled from each paddock and were positioned 15 m apart.

At each sampling point, a hole measuring 20x20 cm wide x 20 cm deep was dug and all soil was placed on a plastic sheet (Fig 4.2.). Each mass of soil was hand sorted by crumbling the soil onto the plastic sheet and earthworms were collected. The root zone was torn apart and carefully sorted to ensure all visible earthworms were removed. Earthworms were placed in plastic bags containing topsoil and were stored at the University of Waikato at 4° C for later analysis. A total of 102 samples, across 17 pairs of dairy and drystock farms were collected and earthworms were counted, weighed and identified to species level.

Figure 4.2. At each sampling point, a hole measuring 20x20 cm wide and 40 cm deep was dug and the soil was placed on a plastic sheet. The mass of soil and root zone were carefully sorted for earthworms.
4.3.3 Data analysis

Earthworm abundance was calculated as the number of worms per unit area (m\(^2\)) and biomass was calculated as the wet weight of worms (g) per unit area (m\(^2\)). Total biomass and abundance were calculated as an average of the three sampling points from each paddock. Abundance and biomass were also calculated separately for the 3 dominant earthworm types in New Zealand pastures: Epigeic, endogeic and anecic functional groups (Schon et al., 2011a; Schon et al., 2012b).

Evidence for differences between the means of earthworm abundance and earthworm density between dairy and drystock sites were calculated using analysis of variance (ANOVA) with site as the blocking factor and land use as the treatment factor. A P value of less than 0.05 was considered statistically significant.

Error bounds are presented as the standard error (SE) of the mean or the SE of the difference between means, unless stated otherwise.

4.4 Results

Earthworm abundance was similar between dairy and drystock farms with no significant differences for any of the earthworm groups (Table 4.3). The most common species of worm was endogeic *Aporrectodea caliginosa*. Other species that were found included epigeic *Lumbricus rubellus*, endogeic *Octolasion cyaneum, Aporrectodea rosea, Aporrectodea trapezoides*, and anecic *Aporrectodea longa*. The abundance of anecic earthworms was low with 7.8 ± 3.4 ind m\(^{-2}\) in dairy sites and 12.3 ± 5.5 ind m\(^{-2}\) in drystock sites. Anecic earthworms were absent in over 80% of the paired sites sampled. Endogeic earthworm abundance was also relatively low with 145.1 ± 24.5 ind m\(^{-2}\) in dairy sites and 138.7 ± 20.2 ind m\(^{-2}\) in drystock sites, however, endogeic earthworms were found in over 90% of the sites. Epigeic earthworm abundance averaged over 35 ind m\(^{-2}\) in both dairy and drystock sites. Overall, the abundance of earthworms in dairy pastures was 192.6 ± 30 ind m\(^{-2}\) compared to 188.2 ± 26 ind m\(^{-2}\) for drystock pastures (Table
4.3). There was also a geographical trend in earthworm abundance with farms in the Te Aroha and Rotorangi areas (Table 4.1) containing the lowest number of earthworms.

| Table 4.3. Earthworm abundance (ind m\(^{-2}\)) for 3 major groups of earthworms and total earthworm abundance in 17 adjacent dairy and drystock farms |

<table>
<thead>
<tr>
<th></th>
<th>Dairy (ind m(^{-2}))</th>
<th>Drystock (ind m(^{-2}))</th>
<th>Difference (ind m(^{-2}))</th>
<th>SED(^A)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epigeic</td>
<td>39.7 (8.3)</td>
<td>37.3 (12.5)</td>
<td>2.5</td>
<td>12.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Endogeic</td>
<td>145.1 (24.5)</td>
<td>138.7 (20.2)</td>
<td>6.4</td>
<td>27.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Anecic</td>
<td>7.8 (3.4)</td>
<td>12.3 (5.5)</td>
<td>-4.4</td>
<td>6.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>192.6 (30.4)</td>
<td>188.2 (26)</td>
<td>4.4</td>
<td>33.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Standard error of the mean in parenthesis
\(^A\) SED, standard error of the difference between means

The biomass of epigeic earthworms in drystock sites was on average 9.9 ± 7.2 g m\(^{-2}\) greater than adjacent dairy sites (Table 4.4). Epigeic earthworms had a 9.9 g m\(^{-2}\) greater biomass in dairy sites compared to drystock sites but the difference was not significant. Anecic earthworms were absent from over 80% of the sites sampled and as a consequence average biomass was low. The average biomass of all earthworm species in dairy sites was 77.2 ± 11.7 g m\(^{-2}\) compared to 74.7 ± 12.5 g m\(^{-2}\) in drystock sites. The differences in total biomass and abundance between dairy and drystock sites were not significant at the 5% level.

| Table 4.4. Earthworm biomass (g wet wt. m\(^{-2}\)) for 3 major groups of earthworms and total earthworm abundance in 17 adjacent dairy and drystock farms |

<table>
<thead>
<tr>
<th></th>
<th>Dairy (g m(^{-2})) (^A)</th>
<th>Drystock (g m(^{-2}))</th>
<th>Difference (g m(^{-2}))</th>
<th>SED(^B)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epigeic</td>
<td>25.8 (6.2)</td>
<td>15.8 (5.1)</td>
<td>9.9</td>
<td>7.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Endogeic</td>
<td>41.8 (7.0)</td>
<td>39.9 (7.0)</td>
<td>1.8</td>
<td>8.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Anecic</td>
<td>9.7 (4.3)</td>
<td>19.0 (8.1)</td>
<td>-9.3</td>
<td>9.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Total</td>
<td>77.2 (11.7)</td>
<td>74.7 (12.5)</td>
<td>2.5 (15.8)</td>
<td>15.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Standard error of the mean in parenthesis
\(^A\) biomass is measured in g wet weight. m\(^{-2}\)
\(^B\) SED, standard error of the difference between means

Earthworms in adjacent dairy and drystock pastures

Figure 4.3. Average earthworm abundance and biomass for 17 paired dairy and drystock sites. Error bars are for the total earthworm abundance and biomass and represent ± 1 SE.

4.5 Discussion

4.5.1 Earthworm abundance and biomass

Total earthworm abundance was 193 ± 30 ind m⁻² for dairy sites and 188 ± 26 ind m⁻² for drystock sites. These values were low in comparison to other values measured throughout New Zealand. For example, at a dairy farm in the same region as the current study, Schon et al. (2011b) measured much higher earthworm abundances of 382 ind m⁻² for an Allophanic Soil and 435 ind m⁻² for a Gley Soil under stocking rates of 3 cows ha⁻¹. However, the study of Schon et al. (2011b) was conducted on a single farm and earthworm abundance is known to vary greatly over small distances in response to varying soil edaphic factors, management and climate (Schon et al., 2012a; Six et al., 2004). Our study measured earthworm abundance and biomass on a regional scale and earthworm abundance and biomass varied greatly between sites. For example, sites located in Te Aroha and Rotoorangi areas had earthworm abundances of less than 70 ind m⁻².
Earthworms in adjacent dairy and drystock pastures

2 while at Pirongia, one site had earthworm abundances of greater than 450 ind m\(^{-2}\). Schon et al. (2011a) also measured large variability in earthworm abundance and biomass while exploring the effect of increased fertilizer, pasture production and grazing intensity on earthworm populations. Abundance and biomass ranged from 291 ind m\(^{-2}\) to 1070 ind m\(^{-2}\) and 89 g m\(^{-2}\) to 398 g m\(^{-2}\) respectively.

Of the three main earthworm types, endogeic earthworms which were found throughout the top soil had the highest abundance and biomass. This was consistent with previous studies which have shown New Zealand pastures to be dominated by endogeic species (Schon et al., 2011a; Springett, 1992). Epigeic earthworms (surface dwellers) were present in about 85% of the farms and average abundance was 39 ind m\(^{-2}\) which is comparable to measurements made by Schon et al. (2011a) who measured an average epigeic abundance of 42 ind m\(^{-2}\) across 8 intensively managed dairy sites on Allophanic Soils.

The deep burrowing anecic earthworms (A.longa) were absent in over 80% of the sites sampled, resulting in low averages of abundance and biomass across sites. A number of studies have shown a sporadic distribution of these earthworms throughout New Zealand. Springett (1992) conducted a national survey of lumbricid earthworms by sampling 216 farms throughout New Zealand. The Anecic earthworm A.longa was found in only 28% of sites in the South Auckland region and 22% of sites in the southern North Island. Schon et al. (2011a) estimated that about 14% of exotic pastures in the Waikato contain A.longa. However, many regions have not been surveyed for earthworms in New Zealand and the percentage of high producing pastures containing A.longa could range anywhere from 27-41% (Schon et al., 2011a). Anecic earthworms enhance nutrient cycling by incorporating organic matter into the soil and improve soil structure by burrowing deep into the soil (Schon et al., 2014). Some studies have estimated that up to 6.5 million ha of New Zealand pastures would benefit from anecic earthworm introduction (Schon et al., 2011a; Schon et al., 2014). The low abundance and biomass of anecic earthworms in Waikato pastures (Tables 4.2 and 4.3) would suggest that many of these pastures would benefit from A.longa introductions.
4.5.2 Land use effects on earthworm abundance and biomass

There was no difference in either number or biomass between adjacent dairy and drystock sites (Tables 4.3 and 4.4). A number of other studies have attempted to determine the effect of intensification on earthworm abundance and biomass (Table 4.5), however, these studies generally use N application rate as an index of intensity. In grazing systems, the effects of intensification on earthworm abundance and biomass are not consistent with studies showing intensification to increase earthworm abundance (Curry et al., 2008; Muldowney et al., 2003), decrease earthworm abundance (Curry et al., 2008; Muldowney et al., 2003; Schon et al., 2012a; Villenave et al., 2011), or have no effect (Muldowney et al., 2003) (Table 4.5). The effect of intensification on earthworm populations is dependent on the balance between available food resources and the state of the physical environment (Schon et al., 2011b). Therefore, it may be that in some cases, intensification improves the habitat for earthworms (e.g. Curry et al., 2008) while in other cases, habitat is adversely affected (e.g. Schon et al., 2012a).
Table 4.5. The effect of increasing N fertilizer and stocking rate on total earthworm abundance and biomass from a range of studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Grazing intensity (SU ha(^{-1}))</th>
<th>Fertilizer N applied (^{A})</th>
<th>Estimated difference in abundance (^{B})</th>
<th>Estimated difference in biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Curry et al., 2008)</td>
<td>Tipperary Co., Ireland</td>
<td>14-21</td>
<td>80 &amp; 350 Kg N ha(^{-1})</td>
<td>33 ind m(^{-2}) more under higher grazing intensity</td>
<td>3.1 g m(^{-2}) more under higher stocking rate</td>
</tr>
<tr>
<td>(Curry et al., 2008)(^{A})</td>
<td>Meath Co., Ireland</td>
<td>7 &amp;10</td>
<td>100 and 225 Kg N ha(^{-1})</td>
<td>42 ind.m(^{-2}) more under higher intensity grazing</td>
<td>21 g m(^{-2}) higher under higher stocking rates(^{*})</td>
</tr>
<tr>
<td>(Muldowney et al., 2003)</td>
<td>Ireland</td>
<td>7-32</td>
<td>40-375 Kg N ha(^{-1})</td>
<td>No significant relationship between stocking rate and abundance</td>
<td>Significant positive relationship between stocking rate and biomass</td>
</tr>
<tr>
<td>(Schon et al., 2011a)</td>
<td>New Zealand</td>
<td>15 &amp; 24</td>
<td>Various</td>
<td>Epigeic = 61 ind m(^{-2})</td>
<td>Epigeic = 264 kg ha(^{-1})*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Endogeic = 71 ind m(^{-2})</td>
<td>Endogeic = 284 kg ha(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anecic = -87 in m(^{-2})</td>
<td>Anecic = -1089 kg ha(^{-1})*</td>
</tr>
<tr>
<td>(Schon et al., 2011b)</td>
<td>New Zealand</td>
<td>15 &amp; 25</td>
<td>170 Kg N ha year(^{-1})</td>
<td>95 ind m(^{-2}) higher under higher intensity grazing</td>
<td>12 g m(^{-2}) higher under lower stocking rates</td>
</tr>
<tr>
<td>This study</td>
<td>New Zealand</td>
<td>6-30 &amp; 14-27</td>
<td>Various</td>
<td>No significant relationship between land use and earthworm abundance</td>
<td>No significant relationship between land use and biomass</td>
</tr>
</tbody>
</table>

\(^{A}\) Stocking units estimated using Coop (1965)

\(^{B}\) Lower fertilizer application corresponds with lower stocking rate

\(^{C}\) negative numbers indicate sites where the higher grazing intensity plots had greater earthworm abundance

\(^{*}\) Significant relationship between stocking rate and earthworm biomass and/or abundance which may increase or decrease

The majority of studies that have investigated the effect of intensification on earthworm populations have done so using small plot trials or non-paired sites, making comparisons to the results from the current study difficult. In most studies, fertilizer application and/or stocking rate is carefully controlled between treatments (Curry et al., 2008; Schon et al., 2011b), while in the current study, management varied considerably across sites. For example, stocking rates varied from 14-27 SU ha\(^{-1}\) for the dairy sites and 6-30 SU ha\(^{-1}\) for the drystock sites (Barnett et al., 2014). Furthermore, due to time restraints, we were only able to quantify earthworm abundance and biomass from three points in each paddock which may have constrained the detectability of differences between sites. However, given that the differences in earthworm abundance and biomass were so
small between adjacent sites, it unlikely that increasing the replication of plots would have improved the detectability of differences.

4.6 Conclusions

The 17 adjacent and dairy and drystock sites provided an opportunity to test the hypothesis that increased grazing intensity of pastoral systems drives a decrease in earthworm abundance and biomass.

The physical environment of the topsoil was adversely affected by the higher stocking rates of dairy systems and was reflected in the significantly higher bulk density of the 0-10 cm soil layer in dairy farms. The higher bulk density in the top soil of dairy systems was a result of higher stocking rates (Barnett et al., 2014).

Total earthworm abundance and biomass was low in comparison to other earthworm surveys carried out in the Waikato (Schon et al., 2011a; Schon et al., 2011c). Furthermore, land use was found to have no significant effect on earthworm abundance and density which is in contrast to some New Zealand based studies (Schon et al., 2011a). The results from this paired land use study suggested that for Allophanic soils in the Waikato Region, the effects of varying grazing management on earthworm abundance and biomass is negligible.
CHAPTER FIVE

Summary and conclusions

5.1 Introduction

On a global scale, soil is a major C sink and small changes to this reservoir can potentially have a major impact on atmospheric CO₂ concentrations. Furthermore, soil C is essential for nutrient cycling, moisture retention and soil structure, all of which are essential for maintaining high soil quality.

A recent study on the effect of dairy and drystock grazed pastures on soil C stocks found that under flat land, dairy sites had significantly more C in the A horizon relative to drystock sites (Barnett et al., 2014). However, when the whole profile (0-60 cm) was compared between land uses, there was no significant difference in C stocks. The study of Barnett et al. (2014) used a single pit in each paddock to quantify C stocks and the pit approach did not take the with-in paddock variability in C stocks into account. Therefore, the power to detect whole-profile differences in C and N stocks between adjacent land uses was relatively low.

A review of the literature suggested that measuring changes in soil C stocks in pastoral systems is difficult, given the high spatial variability of C. However, detectable changes can be greatly reduced if careful consideration is given to sampling design and statistical analysis.

The overall aim of this thesis was to investigate the differences in C and N stocks of soil on flat land under different pastoral management in the Waikato Region, using an improved sampling strategy that was likely to be able to detect smaller differences. To achieve this aim, 23 paired dairy and drystock farms (Barnett et al., 2014) were resampled using a replicated coring approach and C and N stocks were calculated using equivalent soil mass calculations. Statistical power analysis was used to compare the effectiveness of the previously-used pit approach
Summary and conclusions

(Barnett et al., 2014) and coring approach for detecting differences in C stocks between adjacent land uses.

The following three sections will summarize the key findings in relation to the objectives of this study which were outlined in chapter 1.2. Section 5.5 provides recommendations for future research.

5.2 Differences in C and N stocks between paired dairy and drystock pastures

In the 0-10 cm soil layer, drystock sites had 4.1 ± 2.1 t ha⁻¹ more C than dairy sites (P=0.06). This finding supported Barnett et al. (2014) who demonstrated that for soils under flat land, drystock sites had significantly more C in the A horizon compared to dairy sites. A possible explanation for the lower C stocks in the top soil of dairy sites was related to grazing intensity. Dairy sites are far more intensively managed compared to drystock sites, with higher stocking rates, greater fertilizer and feed imports, higher product export and heavier animals (Mackay, 2008). The higher treading pressure of heavily grazed systems may stimulate organic matter decomposition through the disruption of soil aggregates by mechanical stress (Six et al., 2004; Steffens et al., 2008). Many studies have shown strong linkages between grazing intensity and losses of C from the topsoil (Ganjegunte et al., 2005; Steffens et al., 2009; Steffens et al., 2008).

Dairy sites had more C than drystock sites in the sub-soil. When the sub-soil layers were combined (25-60 cm), dairy sites had 3.7 ± 1.7 t ha⁻¹ more C in comparison to drystock sites (P=0.04). The fact that dairy sites had lower C stocks in topsoil but significantly higher C stocks in the sub-soil is in accordance with possible redistribution of C through the soil column. Lambie et al. (2012) demonstrated that under dairy urine patches, potential solubilisation of C from pasture top soils was as high as 40%, increasing its vulnerability to leaching to lower horizons. Kaiser and Guggenberger (2000) found solubilised C can be reabsorbed further in lower horizons. Therefore, a possible explanation for the redistribution of C under dairy sites was that C solubilisation in the top soil of
dairy sites was greater than that of drystock sites because of more intense urine patches. However, this mechanism is poorly understood and further research is required to better understand the dynamics of sub-soil C storage.

For the whole soil profile (0-60 cm), drystock sites had $1.6 \pm 4.5$ t ha$^{-1}$ more C than dairy sites but this was not a significant difference ($P=0.7$). The measured difference of 1.6 t ha$^{-1}$ was considerably smaller than that measured by Barnett et al. (2014) who measured a non-significant difference of 9.6 t C ha$^{-1}$. Measuring whole-profile differences in soil C stocks is challenging, given that soil C is highly variable, particularly at depth (Kravchenko and Robertson, 2011; Syswerda et al., 2011). Therefore, it is not surprising that a difference of 1.6 t C ha$^{-1}$ could not be shown as significant.

Drystock sites had a higher quantity of soil N in the topsoil but dairy sites had greater N stocks in the lower profile. Nitrogen exists in a number of different forms in soil but most N is locked up in an organic form. In fact greater than 95% of N stored in soil is in an organic form, covalently bonded to C (Schlesinger, 2009). Therefore, it was somewhat expected that total N followed a similar trend to total C.

5.3 Ability to detect differences in C and N stocks between adjacent dairy and drystock pastures

The second objective of this thesis was to compare the pit approach (Barnett et al., 2014) and a replicated coring approach for their ability to detect differences in C stocks at the paddock scale. Least significant differences (LSD) were used to determine the smallest significant difference that could have been detected given the sampling strategy used and the measured variability. For the purpose of providing guidance for future sampling, power analysis was conducted to determine the number of paired sites required to detect a significant difference ($P<0.05$) with some degree of certainty (for e.g. 80%).
Summary and conclusions

From 0-60 cm, the smallest significant difference (P<0.05) that could have been detected (LSD) in this study when using the two-plot coring approach was 9.3 t C ha\(^{-1}\). In contrast, the LSD calculated for the paired pit approach of Barnett et al. (2014) was 18% greater than the coring approach tested here. Additionally, the two-plot coring approach was more efficient and cost effective compared to the pit approach, although the pit approach provides important additional descriptive information of the soil profile. Although C stocks are highly variable, this study reiterates that careful consideration to experimental design and statistical analysis can greatly improve the detectability of changes in C stocks.

Detecting whole profile differences in C stocks is difficult, given the high variability of C stocks. In many studies, researchers have interpreted an absence of a significant difference in C stocks to infer that there is no real difference (Christopher et al., 2009). An experimental design with low power may result in a researcher committing type II error (Kravchenko and Robertson, 2011; VandenBygaart et al., 2007). Power analysis for the coring data revealed that a significant whole-profile (0-60 cm) change of 10% change could be detected with a high degree certainty (~90%) should 23 paired sites be resampled in the future.

Power analysis from this study confirms conclusions made by Kravchenko and Robertson (2011) that post-hoc power analyses are essential, especially when concerning important policy decisions. Furthermore, results from this study confirm that the International Panel on Climate Change (IPCC) need to reconsider protocol as to how C stocks are quantified, especially given the unpredictable nature of C stocks in the sub-soil.

5.4 Earthworm abundance and biomass in adjacent dairy and drystock pastures

In pastoral systems, earthworms play an important role in incorporating plant litter into the soil profile and stabilising organic matter into aggregates, thus increasing the sequestration of C in soil (Blanco-Canqui and Lal, 2004; Six et al., 2004). The objective of this study was to test the hypothesis of whether increased
management intensity of pastoral systems drives a decrease in earthworm abundance and biomass.

There were no significant differences in earthworm abundance or biomass across the 17 dairy and drystock farms, despite the fact that dairy sites had significantly higher stocking rates (Barnett et al., 2014) and top soil bulk density (chapter 3.4.1).

In addition to earthworm biomass and abundance, the findings of this study demonstrated that of the three functional groups, endogeic earthworms were most common which confirms other New Zealand based studies (Schon et al., 2011a; Springett, 1992). In light of previous research (Schon et al., 2011a), the results from my study also demonstrated that many pastures in the Waikato could benefit from the introduction of the deep burrowing anecic earthworms. Introduction of anecic earthworms could help enhance sequestration of C by incorporating litter deep into the profile while simultaneously improving soil structure (Schon et al., 2011a)

### 5.5 Future Research

One of the main questions that arose from this thesis was: what factors are important for determining the storage of C at depth? Some studies have suggested that on a global scale, more than 60% of soil C is stored below 20 cm and therefore determining the factors that drive sub-soil C storage is essential for increasing soil C sequestration (Don et al., 2007). It was clear from my study that land use had a significant effect on the amount of C stored at depth. I hypothesised that the difference may be related to the greater effective size and concentration of dairy urine patches which could drive higher solubilisation rates and subsequent re-deposition lower in the profile. Although potential solubilisation of soil C and has been carried out in laboratory conditions (Lambie et al., 2012), this phenomenon has not been tested under field conditions.
In light of recent research (Schipper et al., 2014) which demonstrated that some soil orders (e.g. Allophaic Soils) were more prone to losses of C than others (e.g. Brown Soils), it would be interesting to extend the number of sites to include a broader range of soil orders. In the current study, 16 of the 23 resampled sites were under Allophanic Soils. Is the effect of land management (i.e. dairy vs. drystock) different for different soil orders? What soil related factors may drive differences with respect to the effect of land use on the storage of C?

The scope of this study could be narrowed further improve our understanding of how management of pastoral systems affects the partitioning of C in soil. This is important because soil C is partitioned into two major pools known as the light fraction (coarse particulate organic matter) and the heavy fraction (Six et al., 2004) with the turnover rate of the latter being in the order of decades/centuries compared to months/years for the light fraction. Grazing pressure has long been known to adversely impact the formation of macro-aggregates which are essential for the long term storage of C in the form of micro-aggregates. Fractionation of soil samples would determine the relative size of the various pools of C in dairy and drystock systems. This would give the researcher an improved understanding of what factors are important for driving storage of C in different pastoral systems.

An important part of my study was determining whether a simple replicated coring approach could improve the detectability of differences in C stocks compared to the pit approach. Although the coring approach improved the detectability of differences, there is still need for an efficient and cost effective method which can detect small (<5%) changes in whole-profile C stocks. This is particularly important for the implementation of C schemes which require accurate monitoring of C stocks at the paddock/farm scale. An advantage of the pit approach is that detailed descriptive information can be obtained from the soil profile. Is it possible for a pedologist to obtain descriptive information from soil cores? It would be interesting to compare a profile description attained from a soil pit to a description based on soil cores.
REFERENCES


AgResearch. (2011) Pasture Earthworms. AgResearch, Christchurch.


References


Determination of soil mass

Soil samples from each soil layer were air dried and passed through a 6 mm sieve to remove course roots and stones. Soil samples were weighed and the moisture content was determined to convert the mass of soil to an oven dry mass. To determine the moisture content of each sample, a subsample of soil (approximately 3 g) was weighed and placed in an oven at 105° C for 48 hours. Samples were subsequently placed in a desiccator and re-weighed. The moisture content was calculated using the following formula:

\[ MF = \frac{M_{AD} - M_t}{M_{OD} - M_t} \]  \hspace{1cm} (A.1)

Where: MF was the moisture factor, \( M_{AD} \) was the air dried mass of soil (g), \( M_t \) was the mass the aluminium tray (g), and \( M_{OD} \) was the oven dry mass of soil (g).

The mass of soil per unit area (t ha\(^{-1}\)) for each depth increment was calculated using the method of Wendt and Hauser (2013):

\[ M_{soil} = \frac{M_{sample(OD)}}{\pi \left( \frac{D}{2} \right)^2} \times n \times 10000 \]  \hspace{1cm} (A.2)

Where: \( M_{soil} \) was the mass of the soil per unit area in t.ha\(^{-1}\), \( M_{sample(OD)} \) was the oven dry mass of soil (t), \( \pi(D/2)^2 \) was the cross-sectional area of the corer, \( n \) was the number of cores taken and 10 000 is a correction factor to convert m\(^2\) to ha.
Correction of soil mass for stones

The percentage of stones for each depth soil layer was calculated using the method of Hewitt et al. (2012):\

\[
\%_{\text{stones}} = \frac{M_s}{M_s + M_{6\text{mm}}} 
\]

(A.3)

Where: \(\%_{\text{stones}}\) was the percentage of stones in each soil layer, \(M_s\) was the mass of stones > 6 mm, and \(M_{6\text{mm}}\) was the oven dry mass of soil passed through a 6 mm sieve.

The final mass of soil for each soil layer was calculated as:

\[
M_{sc} = M_{\text{soil}} - (M_{\text{soil}} \times \%_{\text{stones}}) 
\]

(A.4)

Where: \(M_{sc}\) was the corrected mass of soil per unit area, excluding stones (t ha\(^{-1}\)), \(M_{\text{soil}}\) was the mass of the fine fraction (< 6 mm) per unit area and \(\%_{\text{stones}}\) was the percentage of stones in each depth increment.

Determination of total C and N stocks

The total C and N stock for each soil layer was calculated using the mass of soil per unit area, excluding stones (equation A.4), \(\%C\) or \(\%N\) of the air dried sample and a moisture factor to convert the air dried \(\%C\) or \(\%N\) to an oven dried value. The following formula was used to calculate the C stock for each depth increment:

\[
TC = M_{sc} \times \%_{\text{C}_{\text{AD}}} \times MF 
\]

(A.5)

Where: \(M_{sc}\) was the mass of soil per unit area, excluding stones (t ha\(^{-1}\)), \(\%_{\text{C}_{\text{AD}}}\) was the percent C of the air dried sample and MF was the moisture factor.
To calculate total N for each depth increment, the $\%C_{AD}$ in equation A.5 was substituted with $\%N_{AD}$:

$$TC = M_{sc} \times \%N_{AD} \times MF$$  \hspace{1cm} (A.6)

Where: $M_{sc}$ was the mass of soil per unit area, excluding stones (t ha$^{-1}$), $\%N_{AD}$ was the percent N of the air dried sample and MF is a moisture factor.

**Equivalent soil mass calculations**

To account for the fact that dairy and drystock paddocks had a different mass of soil to a fixed depth, Soil C and N stocks were corrected to an equivalent soil mass for each paired site (Fig. A.1).

![Figure A.1](image)

**Figure A.1.** To a fixed depth of 60 cm, the mass of soil varied between dairy and drystock sites and between plots in the same paddock. As an example, the red line represents the depth of soil required to attain an ESM of 6000 t ha$^{-1}$. Equivalent soil mass calculations adjust C/N stocks to an equal mass of soil which in this case is 6000 t ha$^{-1}$. Photographs of profiles were obtained from Barnett et al. (2014).
Sampling of soils was carried out to a depth of 65 cm and total C and N stocks were calculated to a fixed depth of 60 cm. The mass of soil to 60 cm was compared between the 4 plots of each paired site (figure A.2) and the lowest soil mass was considered as the ESM. For example, in figure A.2, the lowest mass of soil to 60 cm for the paired site was 3000 t ha\(^{-1}\) and was considered as the ESM for that particular site.

**Figure A.2.** An example of a paired dairy/drystock site and the mass of soil to 60 cm (t ha\(^{-1}\)) for each plot. The lightest mass of soil (plot 1 in the drystock paddock) was chosen as the ESM.

For each plot, total C/N was plotted against soil mass (figure A.3) and a cubic spline function was fitted using Microsoft Excel and a free add on from SRS Software, LLC (http://www.srs1software.com/SRS1CubicSplineForExcel.aspx). The soil mass for each plot was adjusted to the ESM and the C/N stock was adjusted accordingly. For example, in figure A.3, the mass of soil is lowered from 3000 t ha\(^{-1}\) (blue line) to 2750 t ha\(^{-1}\) (red line) and the dotted line indicates the interpolated C stock.
Figure A.3. An example of the cubic spline function which was fitted to the data to interpolate C stocks to an ESM. In this case, the mass of soil was reduced from 3000 t ha$^{-1}$ (blue line) to 2750 t ha$^{-1}$ (red line). The mass of C (t C ha$^{-1}$) was adjusted accordingly using interpolation (dotted line).
APPENDIX B

Further Power analyses

Figure B.1. Power curve of total C stocks to 25 cm for 23 paired dairy and drystock sites. Difference (t C ha$^{-1}$) is the difference in C stocks between adjacent dairy and drystock farms. Round symbols indicate the detectable difference for statistical power of 0.7, 0.8 and 0.9 ($\alpha$=0.05).

Figure B.2. Power curves of total C stocks (0-25 cm) for 3 sample sizes (n=13, 27 and 101). Round symbols indicate the detectable difference in C stocks with 80% certainty for the respective sample sizes ($\alpha$=0.05).
Figure B.3. Power curve of total C stocks from 25-60 cm for 23 paired dairy and drystock sites. Difference (t C ha\(^{-1}\)) is the difference in C stocks between adjacent dairy and drystock farms. Round symbols indicate the detectable difference for statistical power of 0.7, 0.8 and 0.9 (\(\alpha=0.05\)).

Figure B.4. Power curves of total C stocks (25-60 cm) for 3 sample sizes (n=13, 27 and 101). Round symbols indicate the detectable difference in C stocks with 80% certainty for the respective sample sizes (\(\alpha=0.05\)).
Figure B.5. The least significant difference that could have been detected (0-60 cm) between dairy and drystock farms with varying numbers of plots and sites.
**APPENDIX C**

*Equivalent soil mass calculations*

In the literature, a number of calculations have been applied to adjust C stocks to an equivalent soil mass (ESM). The most common approach is to use linear interpolation to adjust C stocks. Many ESM calculations which use linear interpolation appear to be highly complex but are in fact mathematically equivalent. The following section provides an illustrative example of how 3 commonly used ESM calculations (Ellert and Bettany, 1995; Gifford and Roderick, 2003; Sisti et al., 2004) are all mathematically equivalent. The conventions used in Fig. C.1. are used in all the following calculations.

![Figure C.1](image-url)  
*Figure C.1.* An example of cumulative soil mass (t ha\(^{-1}\)) plotted against cumulative C stock (t C ha\(^{-1}\)). In this example, linear interpolation is used to interpolate between points. The red line indicates the equivalent soil mass (Ref), *a* represents the mass of C from 0-2200 t ha\(^{-1}\) of soil, and *b* represents the mass of C from 0-3550 t ha\(^{-1}\) of soil.
For the purpose of the following calculations, M denotes the mass of soil (t ha\(^{-1}\)) and C represents the mass of C (t C ha\(^{-1}\)).

**Cumulative mass co-ordinates (CMC) approach (Gifford and Roderick, 2003)**

\[
C_{0..\text{ref}} = C_{0..a} + \left( \frac{M_{0..\text{ref}} - M_{0..a}}{M_{0..b} - M_{0..a}} \right) \times \left( C_{0..b} - C_{0..a} \right)
\]  
(C.1)

Equation 1 can be simplified to:

\[
C_{0..\text{ref}} = C_{0..a} + \left( \frac{M_{a..\text{ref}}}{M_{a..b}} \right) \times \left( C_{a..b} \right)
\]

**Linear interpolation by Sisti et al. (2004)**

\[
C_{0..\text{ref}} = C_{0..a} + \left[ M_{a..b} - (M_{a..b} - M_{a..\text{ref}}) \right] \times \left( \frac{C_{a..b}}{M_{a..b}} \right)
\]  
(C.2)

\[
C_{0..\text{ref}} = C_{0..a} + M_{a..\text{ref}} \times \left( \frac{C_{a..b}}{M_{a..b}} \right)
\]

\[
C_{0..\text{ref}} = C_{0..a} + \left( \frac{M_{a..\text{ref}}}{M_{a..b}} \right) \times \left( C_{a..b} \right)
\]

**Original ESM Method (Ellert and Bettany, 1995)**

\[
T = \frac{M_{0..\text{ref}} - M_{0..a}}{BD}
\]  
(C.3)

Where:

T is the additional thickness required to attain the equivalent soil mass, M is the mass of soil (t m\(^{-2}\)) and BD is bulk density (t m\(^{-3}\)).

Equation C.3. can also be written as the mass of soil required to attain the equivalent soil mass:
\[ M_{a-ref} = M_{a-b} - (M_{0-b} - M_{0-ref}) \]  \hspace{1cm} (C.4)

Equation C.4 can be expanded further to calculate the C stock within the equivalent soil mass which yields the same equation as Sisti et al. (2004):

\[
C_{0-ref} = C_{0-a} + \left[ M_{a-b} - M_{0-ref} \right] \times \left( \frac{C_{a-b}}{M_{a-b}} \right)
\]

\[
= \quad C_{0-ref} = C_{0-a} + \left( \frac{M_{a-ref}}{M_{a-b}} \right) \times (C_{a-b})
\]
## APPENDIX D

Whole-profile equivalent soil masses and equivalent soil depths

Table D.1. Whole-profile soil masses for all respective plots and sites. The equivalent soil mass is the mass of soil to which all plots were adjusted to at a given site. The mass equivalent depth is the soil depth which corresponds to the equivalent soil mass.

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<th>plot number</th>
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## Table D.1. Raw data for total C and N calculations. Abbreviations are: AD is air dried soil, OD is oven dried soil

| Site ID | Land use | plot n.o | GPS coordinates | depth | AD soil mass (t ha⁻¹) | MF | OD soil mass (t ha⁻¹) | %C (OD) | %N (OD) | C stock (t ha⁻¹) | N stock (t ha⁻¹) |
|---------|----------|----------|-----------------|-------|----------------------|----|----------------------|---------|---------|----------------|----------------|}
<p>| 2       | Dairy    | 1        | 37 30 49.141 S  | 0-10 cm | 920.9                | 1.17 | 788.1                | 7.53    | 0.75    | 59.3           | 5.9            |
| 2       | Dairy    | 1        | 37 30 49.141 S  | 10-25 cm | 1407.6               | 1.26 | 1114.1               | 4.74    | 0.48    | 52.8           | 5.4            |
| 2       | Dairy    | 1        | 37 30 49.141 S  | 25-40 cm | 1464.0               | 1.25 | 1170.5               | 2.28    | 0.24    | 26.7           | 2.8            |
| 2       | Dairy    | 1        | 37 30 49.141 S  | 40-60 cm | 2383.7               | 1.22 | 1953.8               | 0.73    | 0.08    | 14.2           | 1.6            |
| 2       | Dairy    | 1        | 37 30 49.141 S  | 0-10 cm  | 934.1                | 1.17 | 799.9                | 6.87    | 0.66    | 55.0           | 5.3            |
| 2       | Dairy    | 1        | 37 30 49.141 S  | 10-25 cm | 1481.1               | 1.23 | 1202.2               | 4.11    | 0.38    | 49.4           | 4.6            |
| 2       | Dairy    | 1        | 37 30 49.141 S  | 25-40 cm | 1363.1               | 1.22 | 1344.7               | 1.84    | 0.18    | 24.8           | 2.5            |
| 2       | Dairy    | 1        | 37 30 49.141 S  | 40-60 cm | 2549.4               | 1.22 | 2096.4               | 0.86    | 0.09    | 17.9           | 1.8            |
| 2       | Drystock | 1        | 37 30 53.011 S  | 0-10 cm  | 900.5                | 1.19 | 755.3                | 7.14    | 0.77    | 53.9           | 5.8            |
| 2       | Drystock | 1        | 37 30 53.011 S  | 10-25 cm | 1444.9               | 1.28 | 1127.1               | 4.14    | 0.44    | 46.7           | 5.0            |
| 2       | Drystock | 1        | 37 30 53.011 S  | 25-40 cm | 1551.9               | 1.26 | 1234.3               | 1.37    | 0.15    | 16.9           | 1.9            |
| 2       | Drystock | 1        | 37 30 53.011 S  | 40-60 cm | 2357.9               | 1.19 | 1980.7               | 0.51    | 0.06    | 10.0           | 1.1            |
| 2       | Drystock | 1        | 37 30 53.011 S  | 0-10 cm  | 861.0                | 1.22 | 708.4                | 9.10    | 0.95    | 64.4           | 6.7            |
| 2       | Drystock | 1        | 37 30 53.011 S  | 10-25 cm | 1407.3               | 1.28 | 1095.6               | 4.61    | 0.46    | 50.5           | 5.0            |
| 2       | Drystock | 1        | 37 30 53.011 S  | 25-40 cm | 1480.8               | 1.28 | 1156.0               | 1.56    | 0.16    | 18.0           | 1.9            |
| 2       | Drystock | 1        | 37 30 53.011 S  | 40-60 cm | 2275.9               | 1.29 | 1761.1               | 0.72    | 0.08    | 12.6           | 1.3            |
| 3       | Dairy    | 1        | 37 31 08.586 S  | 0-10 cm  | 920.9                | 1.10 | 840.5                | 7.21    | 0.75    | 60.6           | 6.3            |
| 3       | Dairy    | 1        | 37 31 08.586 S  | 10-25 cm | 1407.6               | 1.13 | 1243.4               | 4.06    | 0.41    | 50.5           | 5.1            |
| 3       | Dairy    | 1        | 37 31 08.586 S  | 25-40 cm | 1464.0               | 1.12 | 1311.7               | 1.13    | 0.12    | 14.8           | 1.5            |
| 3       | Dairy    | 1        | 37 31 08.586 S  | 40-60 cm | 2383.7               | 1.12 | 2124.1               | 0.54    | 0.06    | 11.5           | 1.3            |
| 3       | Dairy    | 1        | 37 31 08.586 S  | 0-10 cm  | 934.1                | 1.09 | 856.7                | 7.80    | 0.80    | 66.8           | 6.9            |
| 3       | Dairy    | 1        | 37 31 08.586 S  | 10-25 cm | 1481.1               | 1.12 | 1322.3               | 4.45    | 0.42    | 58.8           | 5.6            |
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APPENDIX F

Digital appendices

The attached CD-ROM includes further information relevant to this research. The disk contains:

- Photographs of the 23 adjacent dairy and drystock farms
- Additional farm information
- Raw data including equivalent soil mass calculations