Changes in δ^{15}N in pastoral soils under varying management intensity

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Abstract

In New Zealand, nitrogen (N) inputs and losses have increased dramatically following establishment (and subsequent intensification) of European style pastoral agriculture from the 1800s. An indicator that could identify soils which are most vulnerable to N loss would be useful to help target management practices aimed at reducing unwanted N losses. The natural abundance of ^{15}N relative to ^{14}N ($\delta^{15}N$) in soils is one potential indicator of N loss, because during most N transformations in soils, ^{14}N is preferentially processed and lost (e.g. via ammonia volatilisation, denitrification or nitrate leaching). Therefore the overarching hypothesis for this thesis was that pastoral soils under intensive management regimes (with high N inputs, cycling and losses) would become progressively enriched with ^{15}N relative to soils under less intensive management. This hypothesis was tested by measuring $\delta^{15}N$ in soils from four forest-to-pasture chronosequences, and in archived soils from six long-term (4–57 year) grazed field trials with different fertiliser or irrigation regimes.

Three of the forest-to-pasture chronosequences were on pumice soils where pine forests had been converted to dairy pastures. The fourth chronosequence was on a podzol soil in Northland, where native scrub had been converted to sheep grazed pastures. Surface soil $\delta^{15}N$ on the pumice soil increased significantly from pine forests (2 ‰) to long-term pastures (4.1 ‰). In contrast, there was no clear relationship between pasture age and soil $\delta^{15}N$ for the chronosequence on the podzol soil. The Northland soil displayed extreme podzolization and weathering, and had been previously disturbed by gum diggers, implying results could be relatively unique to this soil.

The two longest field trials were at Winchmore on the Canterbury plains, where different rates of superphosphate and irrigation had been applied for ~50 years. Soil $\delta^{15}N$ increased more in treatments receiving higher rates of superphosphate or more frequent irrigation, and there were significant positive correlations between the average rate of change in soil $\delta^{15}N$, and total pasture production, clover production (thus N fixation) and calculated N losses.

Soil $\delta^{15}N$ was also measured in archived soils from three long-term (15–25 year) superphosphate trials on North Island hill country. In these trials there were no consistent differences in soil $\delta^{15}N$ between treatments. The shorter duration of the hill country trials (compared to the Winchmore trials), combined with smaller differences in pasture production between treatments and higher variability due to complex topography, may have contributed to the lack of observed differences between treatments. Indeed, slope and aspect did have a significant influence on soil $\delta^{15}N$ with higher values on sheltered east facing slopes, and on easy slopes than steep slopes.

The final trial studied was a N fertiliser trial, in which N rates ranging from 0 to 750 kg ha^{-1} y^{-1} were applied to hill country pastures over a 4 year period. Soil $\delta^{15}N$ increased significantly with time in treatments receiving $\geq$100 kg N ha^{-1} y^{-1}, and the increase was more rapid as N rate increased. There was also a positive correlation between the rate of change in soil $\delta^{15}N$ and nitrate leaching ($p<0.001$).

In general, results from this thesis showed that soil $\delta^{15}N$ under intensively managed pastures (i.e. those receiving higher rates of fertiliser or irrigation) was higher than under less intensively managed pastures. It was concluded that higher soil $\delta^{15}N$ in the more intensively managed pastures was most likely due to the influence that fertiliser or irrigation had on pasture production, N fixation by clover, and the flow on effects this had on animal stocking rates and N cycling and isotope fractionating loss processes. However, results from the hill country superphosphate trials and the chronosequence on the podzol soil, demonstrated that other factors (such as slope and aspect) can overwhelm or suppress the expected increases in $\delta^{15}N$ in some situations. Therefore soil $\delta^{15}N$ will probably be most accurate as an indicator of long-term management intensity, and management induced N losses, at sites with the same (or similar) topography, soils and climate.

Average rates of change in soil $\delta^{15}N$ over the duration of the trials investigated in this thesis, ranged from –0.007 ‰ y^{-1} to 0.35 ‰ y^{-1}, with rates of change being <0.1 ‰ y^{-1} in all trials except the N rate trial (where N inputs were very high).

This suggests that under ‘typical’ pastoral management regimes, any changes in bulk soil δ^{15}N will probably only be detectable at decadal time scales.

A preliminary indicator of past management intensity and N inputs and losses was proposed for New Zealand soils, based on surface soil δ^{15}N values. (1) Soils with δ^{15}N values <3 ‰ will be from extensively managed pastures, forests, or other natural ecosystems with low N inputs and losses. (2) Soils with intermediate δ^{15}N values (3–5 ‰) will be from sites which have been under moderate management intensity, with moderate N inputs and losses. (3) Soils with δ^{15}N values >5 ‰ will be from sites which have been intensively managed for a number of decades and subject to high N inputs and losses (e.g. N inputs >100 kg ha^{-1} y^{-1}). More research will be required to further test the usefulness of this indicator. Key areas for future research include: gaining a better understanding of the effect of topography and soil type on soil δ^{15}N, and making more direct measurements of the isotopic composition of different N inputs and outputs to more clearly identify mechanisms driving changes in soil δ^{15}N in pastoral systems.

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1 Introduction

1.1 Background

Nitrogen (N) is a key element required for the growth of plants and animals, because it is an important component of proteins and nucleic acids (Whitehead, 1995; Nieder and Benbi, 2008). In many natural ecosystems and the majority of agricultural ecosystems, N is the most limiting factor for plant growth (Vitousek *et al.*, 1997). To alleviate the natural N limitation on pasture and crop production, humans have intervened in the N cycle. Initially intervention was through the integration of legumes into agricultural systems, because many legumes have symbiotic relationships with N-fixing bacteria (Whitehead, 1995). Another significant change came after the development of the Haber-Bosch process – an industrial process where extreme pressure and heat are used to convert N_2 to ammonia (NH_3), from which fertiliser can be made. The widespread cultivation of legumes and use of nitrogen fertiliser has greatly increased the amount of food produced in the world, which has been important to feed a rapidly growing population. For example, Smil (2002) estimated that around 40% of the protein consumed by humans was produced with N derived from the Haber-Bosch process.

The large increase in N inputs facilitated by humans has had negative environmental consequences. At a global scale, N fixation from the atmosphere is now greater than returns to the atmosphere via denitrification, which means reactive N is accumulating in the environment (Galloway *et al.*, 2003). Excess reactive N causes problems in both terrestrial and aquatic environments; contributing to soil and water acidification, loss of biodiversity, eutrophication, atmospheric smog, production of tropospheric ozone, and nitrous oxide, which is a potent greenhouse gas (Vitousek *et al.*, 1997; Galloway *et al.*, 2003; Gruber and Galloway, 2008; Robertson and Vitousek, 2009).

New Zealand has followed the global trend of increased N inputs since settlement of Europeans from the 1800s. Parfitt *et al.* (2008) estimated total N inputs to New

Zealand were ~133 Gg in 1860, compared to 1023 Gg in 2001, and inputs were projected to increase to between 1171 Gg and 3565 Gg by 2050, depending on future policies. The large increase in N input from 1860 was primarily due to clearance of large tracts of native vegetation (mainly forest), and sowing of grass/legume pastures, where legume growth (thus N fixation) was promoted by the addition of superphosphate and lime, and in some regions irrigation (MacLeod and Moller, 2006). Other large changes have been a six fold increase in N fertiliser imports, and more than doubling of N imports in supplementary feed (e.g. grain or palm kernel extract) between 1990 and 2010 (Parfitt *et al.*, in press-b). Most N inputs to the land surface in New Zealand are from N fixation and N fertilisers and atmospheric N deposition is generally low (<5 kg ha^{-1} y^{-1}). Low atmospheric deposition in New Zealand is due to comparatively little industry, few housed livestock operations, and because New Zealand is a long narrow country surrounded by ocean, much of the N emitted is blown out to sea (not re-deposited) (Parfitt *et al.*, 2006).

Increased N inputs from N_2 fixation, fertiliser and supplementary feed, has led to large increases in pasture and animal production (Lambert and Clark, 1986; Clark *et al.*, 2007), but also increased N losses (e.g. nitrate leaching and N_2O emissions) (Ledgard *et al.*, 1999; Ledgard, 2001; Monaghan *et al.*, 2005). New Zealand's pastoral soils have also become progressively enriched with N via immobilisation in organic matter, and soil C:N ratios have declined (Walker *et al.*, 1959; Lambert *et al.*, 2000; Sparling and Schipper, 2004). Schipper *et al.* (2004) argued that soils cannot continue to store N indefinitely, and that soil organic matter in many of New Zealand's pastoral soils would likely become 'saturated' with N within 50 years. In support of this hypothesis, Schipper and Sparling (2011) demonstrated that net N immobilisation rates were highest in soils with high C:N ratios, and declined as C:N ratios declined. If N immobilisation rates decline, the risk of N loss increases, particularly if N inputs remain the same. There is particular concern about increasing N_2O emissions, due to implications on climate change and New Zealand's greenhouse gas budget, while increased N leaching losses have had detrimental impacts on ground and surface water quality (Parliamentary Commissioner for the Environment, 2004).

Concern over increased N losses from pastoral agriculture has resulted in considerable research into new farming practices that can reduce N losses (e.g. nitrification inhibitors and stand-off pads, de Klein and Eckard, 2008), and recently there has also been a move towards regulating farming practices in New Zealand, particularly in sensitive catchments with high conservation or recreational values (e.g. Lake Taupo). Direct measurement of N losses (i.e. via leaching or gaseous pathways) is labour intensive and expensive, and can only realistically be carried out at a limited number of sites for relatively short time periods (usually <5 years). Therefore a simple indicator which could provide information on long-term N inputs and losses under different land uses or management regimes would be useful to help target mitigation practices.

1.2 Natural abundance nitrogen isotopes

The natural abundance of stable nitrogen isotopes (the ratio of ^{15}N to ^{14}N in relation to atmospheric N_2 (δ^{15}N)) have been widely used to study N dynamics in both plants and soils, and can provide an integrated measure of N cycle processes (Högberg, 1997; Robinson, 2001). This occurs because the δ^{15}N value of a system is determined by the mass and isotopic signature of N inputs and N losses, and the isotopic signature of N losses in particular can be strongly influenced by isotopic fractionation (the separation of isotopes in physical, chemical or biological processes). Isotopic fractionation occurs because the slight difference in mass between ^{14}N and ^{15}N means the two isotopes behave differently in many processes. The heavy isotope (^{15}N) forms stronger chemical bonds, which means the light isotope (^{14}N) generally reacts faster (Shearer and Kohl, 1986; Högberg, 1997). Therefore, during most N transformation processes in soils (e.g. nitrification, denitrification and volatilisation), ^{14}N accumulates in products (e.g. NO_3^-, NH_3, N_2, N_2O). These products tend to be preferentially lost from the soil, so that the remaining substrate (e.g. NH_4^+ or organic N) retained in the soil becomes enriched with ^{15}N (provided that not all of the substrate is converted to product, in which case there could be no net fractionation, Högberg, 1997; Robinson, 2001). Changes in soil δ^{15}N occur slowly during the weathering of undisturbed soils over millennia (Vitousek *et al.*, 1989; Brenner *et al.*, 2001), but can be greatly accelerated with disturbance (Pardo *et al.*, 2002) or anthropogenic N inputs (Högberg, 1991; Watzka *et al.*, 2006).

The main N inputs to pastoral systems in New Zealand are N fertiliser (mostly urea) and N fixation (in association with legumes), which both have δ^{15}N values of ~0 ‰. Therefore changes in soil δ^{15}N will be driven by the balance between N immobilisation in the soil and fractionating N losses (e.g. ammonia volatilisation and denitrification). In soils subject to high N inputs, cycling and loss, there will be ample opportunity for a large amount of isotopic fractionation to occur, and therefore such soils are likely to be enriched with ^{15}N relative to soils with lower N inputs, cycling and loss (Watzka *et al.*, 2006; Stevenson *et al.*, 2010). Soils contain a large pool of slowly cycling N (in organic form), and consequently changes in bulk soil δ^{15}N will occur relatively slowly, but will also be preserved in the soil for a long-time (Johannisson and Högberg, 1994). Therefore soil δ^{15}N may be a simple indicator of the long-term balance between N inputs and losses in pastoral soils. However, little is known about rates of change in soil δ^{15}N in agricultural systems, and the influence of different management regimes on rates of change. Therefore a logical first step in evaluating the suitability of δ^{15}N as an indicator of N losses would be to determine how soil δ^{15}N changes with time, and identify the broad drivers of any changes.

1.3 Hypotheses

In this thesis the overarching hypothesis was that pastoral soils under intensive management regimes (with high N inputs, cycling and losses) would become progressively enriched with ^{15}N relative to soils under less intensive management. Here an increase in 'management intensity' is defined as increased above-ground biomass production and associated increases in grazing intensity, driven by greater external inputs such as fertiliser or irrigation. More specific hypotheses are listed on the next page.

Because soil δ^{15}N was expected to change relatively slowly (i.e. ~decadal time scales), it was not practical to conduct suitable experimental work within a 3 year PhD. Therefore for this thesis, soil δ^{15}N was measured in samples taken from chronosequences (space for time substitution), and also in archived soils taken from a number of long-term grazed field trials in New Zealand. The datasets developed represent periods ranging from ~5 to ~50 years.

Specific hypotheses were that:

1. ***Soil δ^{15}N will increase with time following conversion of forest or scrub to pasture, due to increased N inputs and isotope fractionating N losses.***

This hypothesis was tested by analysing soils from four forest-to-pasture chronosequences. This question is relevant because most of New Zealand's pastoral land was converted from native forest between 1850 and 1950, and from around 2000 exotic production forest was also converted to pasture as commodity prices changed (particularly in favour of dairy farming).

2. ***Soil δ^{15}N will increase more rapidly in more intensively managed pastures receiving higher rates of superphosphate fertiliser, nitrogen fertiliser or more frequent irrigation.***

This hypothesis was tested by analysing archived soils taken from long-term (4–60 year) field trials receiving different rates of superphosphate fertiliser, nitrogen fertiliser or irrigation. A more rapid increase in δ^{15}N in treatments receiving more fertiliser or irrigation was hypothesised to occur because fertiliser and irrigation will increase pasture production, grazing intensity, and N inputs, cycling and losses, and therefore opportunities for isotope fractionating N losses will also increase.

3. ***Soil δ^{15}N will be a useful indicator of long-term N inputs, cycling and losses from pastoral systems.***

This hypothesis was evaluated in the conclusions chapter by considering all the results from this thesis, and other similar studies published in the literature.

While the focus of the thesis is on nitrogen isotopes, δ^{15}N cannot be studied in isolation, and changes in δ^{15}N were also related to changes in total soil C and N. In some cases C and N data had been published previously (or submitted for publication), while in part of Chapter 3, and in Chapter 6, C and N data are reported for the first time.

1.4 Thesis outline

Chapter 1 (this chapter) provides some general background on nitrogen, and nitrogen isotopes, the key hypotheses, and an outline of the thesis.

Chapter 2 is a review of the literature, and presents some theory behind the use of nitrogen isotopes in ecological studies, summarises the influence of natural factors (e.g. topography) on soil δ^{15}N, and then focuses on the influence of land use and management on soil δ^{15}N, with an emphasis on pastoral systems.

Chapters 3–6 present and discuss the research results from this thesis, and are written as stand-alone chapters in manuscript format. The order of chapters 3–6 are not necessarily in the order the work was conducted, but are ordered to represent the general development of pastures in New Zealand; conversion of forests to pasture, superphosphate fertiliser application (to flat land), development of hill country, and then N fertiliser application to land.

> Chapter 3 reports soil C, N and δ^{15}N data from four chronosequences where either pine forest or native scrub had been converted to intensive pastures.
>
> Chapter 4 describes how soil δ^{15}N changed during ~50 years in grazed irrigation and superphosphate trials on the Canterbury Plains.
>
> Chapter 5 presents soil δ^{15}N data from three 15–25 year superphosphate trials on North Island hill country, and explores the influence of slope and aspect on δ^{15}N in addition to the effect of superphosphate.
>
> Chapter 6 reports on changes in soil C, N and δ^{15}N during a four year N fertiliser trial, where N rates ranged from 0–750 kg N ha^{-1} y^{-1}, and N leaching losses were also measured.

Because chapters 3–6 are written in manuscript form, there is some repetition of introductory materials, and methods. To avoid repetition all references are in one list at the end of the thesis. Before each of these chapters is a statement of the contribution of each of the listed authors.

Chapter 7 provides a summary and conclusions, and evaluates how the hypotheses listed above (section 1.3) were supported (or not supported) by the results in this thesis. A number of recommendations for future research are also provided.

Appendix A contains a published journal paper (Stevenson *et al.*, 2010), on which Paul Mudge was a co-author. This paper was produced during the mid-stages of this PhD, and reports δ^{15}N values from soils under a range of land uses in New Zealand. The paper is not directly part of work conducted for this PhD, but was directly aligned and is therefore provided for context. Paul Mudge supplied the lead author (Stevenson) with a literature review on soil δ^{15}N under different land uses, and contributed to interpretation of results and writing of the manuscript.

2 Literature review

2.1 Introduction

Chapter 1 provided the general background information relevant to this literature review, such as: an overview of how humans have intervened in the nitrogen (N) cycle through cultivation of legumes and creation of artificial fertilisers, and how this has been important to help feed a rapidly growing population, but has also had negative environmental consequences. The introductory chapter also briefly summarised how settlement of Europeans in New Zealand from the 1800s, and establishment of European style pastoral agriculture greatly increased N inputs, soil N, and N losses to the wider environment. An overview of how the natural abundance of nitrogen isotopes (^{14}N and ^{15}N) can be used to help understand N cycle processes, and potentially be an indicator of past N cycle processes in pastoral soils was also provided.

2.1.1 Purpose and structure of this literature review

The main purpose of this review is to provide a synthesis of the effect that different land uses and land management regimes have on the natural abundance of stable N isotopes (^{14}N and ^{15}N) in soil. However, the review will begin with a section outlining what isotopes are, terminology associated with isotopes, and theory behind the use of N isotopes in ecological studies. The influence that natural factors other than land use (e.g. climate, soil type, topography) have on N isotope ratios will then be summarised, because understanding the effect of these factors is important when interpreting soil N isotope signatures in respect to land use. Attention will then turn to that effect land use and management has on N isotopes, with a focus on grazed grasslands, which are an important land use globally, and in New Zealand.

2.2 Nitrogen isotopes

2.2.1 *What are isotopes?*

An isotope is the name given to one of two or more atoms of an element, that have the same number of protons but which contain different numbers of neutrons (Robinson, 2001). There are two types of isotopes, 'stable isotopes' which do not undergo radioactive decay and 'unstable isotopes' which are radioactive and do decay (Fry, 2006). This thesis focuses on the natural abundance of the two stable isotopes of nitrogen; ^{14}N and ^{15}N.

Atoms of ^{14}N contain seven neutrons and seven protons, while ^{15}N atoms contain eight neutrons and seven protons. The extra neutron in ^{15}N means that it has a slightly greater mass than ^{14}N. This difference in mass makes the two isotopes behave in a slightly different way in some physical, chemical and biological processes. This small difference in behaviour is part of the basis for natural abundance isotope studies and is explained further in section 2.2.3.

2.2.2 *Terminology and notation*

The atmosphere is 78 % N, and this is the largest (Nieder and Benbi, 2008), and most well mixed pool of N in the world (Mariotti, 1983). Atmospheric N is comprised of 99.6337 % ^{14}N, with the remaining 0.3663 % being ^{15}N (Mariotti, 1983). Expressing isotope abundance on an absolute percentage basis is termed atom%. The isotopic composition of other natural nitrogenous materials can differ from the atmospheric composition, with differences smaller than 0.0004 atom% being important (Handley and Raven, 1992). These very small differences make recording and communicating differences in atom% difficult. Therefore the natural abundance of ^{15}N is usually expressed in delta notation (δ) as parts per thousand (‰) using the following equation (after Coplen, 2011).

$$\delta^{15}N = R(^{15}N/^{14}N)_{sample} - R(^{15}N/^{14}N)_{air} - 1$$

where $R(^{15}N/^{14}N)_{sample}$ is the ratio of $^{15}N/^{14}N$ in the sample, and $R(^{15}N/^{14}N)_{air}$ is the ratio of $^{15}N/^{14}N$ in atmospheric N_2 (the international standard).

By definition $\delta^{15}N$ in the atmosphere is zero and therefore positive $\delta^{15}N$ values indicate a sample has more ^{15}N than the atmosphere and negative values less ^{15}N than the atmosphere. Most naturally occurring substances have $\delta^{15}N$ values between –30 ‰ and 30 ‰ (Robinson, 2001).

2.2.3 Isotopic fractionation

The reason why different nitrogenous materials have different isotopic compositions is due to isotopic fractionation. Isotopic fractionation is a term used to describe the separation of isotopes during physical, chemical or biochemical processes. Isotopic fractionation occurs predominantly due to the difference in mass between isotopes. Heavy isotopes form stronger chemical bonds than light isotopes, and therefore more energy is required to form and break bonds between heavy isotopes than light isotopes (Robinson, 2001).

There are two main mechanisms that cause isotopic fractionation; equilibrium and kinetic isotope effects (Shearer and Kohl, 1986; Högberg, 1997). In equilibrium exchange reactions heavy isotopes accumulate where bonds are strongest, such as in liquids rather than gases and in molecules with larger mass or higher oxidation state (Fry, 2006; Sulzman, 2007). For example, in the equilibrium reaction $NH_4^+ \leftrightarrow NH_3$ in solution, ^{15}N tends to accumulate in NH_4^+ due to its greater mass. Equilibrium isotope effects are temperature dependent, with greater fractionation occurring at lower temperatures (Sulzman, 2007).

Kinetic isotope effects occur in unidirectional reactions, and are caused because light isotopes react faster than heavy isotopes (Shearer and Kohl, 1986; Robinson, 2001; Sulzman, 2007). The faster reaction of light isotopes means that during most reactions or processes, products tend to become depleted in the heavy isotope, while remaining substrate becomes enriched (Sulzman, 2007). It is important to note, that isotopic fractionation can only occur when reactions are incomplete, because if all atoms in a substrate are converted to product, the isotopic composition must be the same as the substrate (Högberg, 1997; Sulzman, 2007). Within any system, the overall $\delta^{15}N$ cannot change unless N enters or leaves the system, and the N entering or leaving must have a different isotopic signature from the total N in the system (Handley and Raven, 1992; Högberg, 1997; Robinson, 2001).

The degree of isotopic fractionation (α) for a given process (or series of processes) is calculated as $R(^{15}N/^{14}N)_{product} / R(^{15}N/^{14}N)_{substrate}$, and can also be expressed on the δ scale as parts per thousand (‰) simply as ε = α – 1 (Coplen, 2011). Table 2.1 shows a range of isotopic fractionation factors for some of the processes of the nitrogen cycle as collated by Robinson (2001). A summary of factors affecting each of the fractionation processes presented in Table 2.1, is given in reviews by Shearer and Kohl (1986) and Högberg (1997), and the reader is referred to these reviews for further details.

Table 2.1 Range of isotope fractionation factors for some of the key processes of the nitrogen cycle as collated by Robinson (2001).

N cycle processes	**ε (‰)**
N_2 fixation via nitrogenase	0–6
NH_3 volatilisation	40–60
N_2O and NO production during NH_4^+ oxidation (nitrification)	35–60
N_2O and N_2 production during NO_3^- reduction (denitrification)	28–33
NO_3^- assimilation into organic N by plants	0–19
NH_4^+ assimilation into organic N by plants	9–18
NO_3^- or organic N assimilation by microbes	13
NH_4^+ assimilation by microbes	14–20
NH_4^+ production from organic matter decomposition (ammonification)	0–5
NO_3^- production during nitrification	15–35
Organic N assimilation by animals (deamination and transamination)	1–6

2.2.4 How are stable N isotopes used?

There are two main ways in which stable N isotopes are used in ecological studies: (1) tracer studies involving the addition of material artificially enriched (or occasionally depleted) in ^{15}N, and (2) measurements of natural isotope abundance. In ^{15}N enriched tracer studies, a known quantity of material enriched in ^{15}N is added to a system. The movement, turnover or distribution of the added N is calculated from the isotopic composition of different nitrogenous materials sampled from within the system after a period of time. The expense of artificially enriched ^{15}N usually limits this approach to small plot or at most paddock scale experimental work (Bedard-Haughn *et al.*, 2003). Artificial ^{15}N tracer studies are not the focus of this thesis and will not be discussed further.

Early research on natural N isotope abundance was mainly directed towards using the natural δ^{15}N signature from bacterial N_2 fixation and N fertilisers, to assess the contribution of these N sources to plant N uptake (e.g. Meints *et al.*, 1975). The theory behind this approach was that most synthetic N fertilisers, and N_2 derived from bacterial fixation, have δ^{15}N values of about 0 ‰ (Shearer and Kohl, 1986; Bateman and Kelly, 2007), while δ^{15}N of total soil N is usually much higher (e.g. Shearer *et al.*, 1978). Mixing models were used to determine the relative contribution of N from the different sources (Robinson, 2001). While successful in some situations, the N isotope signature is often not a conservative tracer (due to isotopic fractionation), which combined with a large amount of spatial variability can limit this approach (Högberg, 1997; Robinson, 2001).

Recognition that isotopic fractionation could confound studies seeking to use natural abundance N isotopes as tracers, led researchers to re-think how natural abundance N isotopes could be used. It is now widely accepted that δ^{15}N is a valuable integrator of the many complex N fractionation and mixing processes that occur within natural systems (Robinson, 2001; Evans, 2007). The δ^{15}N signature of soil can reflect the degree and type of nitrogen cycle processes that have occurred at a given site. For example, soils from lower lying and wetter portions of the landscape often have higher δ^{15}N values than well drained areas, due to greater denitrification, which causes large isotopic fractionation (Bedard-Haughn *et al.*, 2003).

2.3 Relationship between plant and soil δ^{15}N

The focus of this review is on N isotopes in soil. However, numerous studies have also reported δ^{15}N values of plant material (more studies than soil studies), and some of these studies have been included where relevant. This section provides a brief summary of the relationship between plant and soil δ^{15}N. In general there is a positive correlation between plant and soil δ^{15}N, with r^2 values >0.5 commonly reported for samples taken from non N fixing plants at the same site (Johannisson and Högberg, 1994; Watzka *et al.*, 2006; Kriszan *et al.*, 2009; Xu *et al.*, 2010). The relationship between soil and plant δ^{15}N values is not 1:1, with values of plants almost always lower than the underlying soil (Sutherland *et al.*, 1991; Sutherland *et al.*, 1993; Evans and Belnap, 1999; Watzka *et al.*, 2006; Kriszan *et al.*, 2009). Lower δ^{15}N values of plants than soils, is generally attributed to plants taking up N depleted in ^{15}N relative to the bulk soil. This can occur for a number of reasons, such as (1) fractionation against ^{15}N during mineralisation of organic N and then subsequent uptake of inorganic N depleted in ^{15}N by plants (Högberg, 1997; Kriszan *et al.*, 2009), (2) preferential passage of ^{15}N depleted N from soil to the plant via mycorrhizal fungi (Hobbie and Ouimette, 2009; Wallander *et al.*, 2009), and (3) plant roots can also directly discriminate against ^{15}N during uptake (Högberg, 1997).

Plant δ^{15}N values are much more variable than soil δ^{15}N values. For example, Sutherland *et al.* (1993) found that plant δ^{15}N values were consistently 6–7 times more variable than soils at the same site. This is because plants generally access the mineral pool of N, which is far more isotopically variable (spatially and temporally) than total soil N (Sutherland *et al.*, 1993; Handley *et al.*, 1999). Johannisson and Högberg (1994) suggested that plant δ^{15}N may be a more sensitive indicator of changes in N cycling over short time periods (years–decades) than soil δ^{15}N, because the large background pool of un-reactive soil N tends to mask changes occurring in the smaller active N pools. However, Handley *et al.* (1999, p.187), suggested that whole soil δ^{15}N "integrates more of the influences on ecosystem N cycling than does foliar δ^{15}N".

2.4 The influence of natural factors (other than land use) on δ^{15}N

2.4.1 Climate

A large amount of research has been carried out to try and understand the influence that climate has on plant and soil δ^{15}N. In one of the earlier studies, Martinelli *et al.* (1999) found that soil δ^{15}N values were ~8 ‰ higher in tropical forests compared to temperate forests, while the difference in foliage δ^{15}N was 6.5 ‰. This large difference was attributed to "tropical forests having a more open nitrogen cycle, with greater losses via isotope fractionating pathways, suggesting that N is in relative excess in many moist tropical forests" (Martinelli *et al.*, 1999, p.47). Sites with high N fertility in the tropics had significantly higher δ^{15}N values than sites with low N fertility, which supported the hypothesis that N availability (or excess) was driving ^{15}N enrichment. A number of studies have investigated changes in δ^{15}N along rainfall gradients, with both plant and soil δ^{15}N values generally decreasing with increasing mean annual precipitation (Shearer *et al.*, 1978; Austin and Vitousek, 1998; Austin *et al.*, 1999; Handley *et al.*, 1999; Schuur and Matson, 2001; Swap *et al.*, 2004; Cheng *et al.*, 2010). In a synthesis of soil and plant δ^{15}N data from a number of studies conducted around the world, Amundson *et al.* (2003) found that as mean annual precipitation (MAP) increased, δ^{15}N values decreased, while when mean annual temperature (MAT) increased so too did δ^{15}N. Amundson *et al.* (2003) attributed these trends to greater losses of ^{15}N-depleted N in warmer and drier environments (due to a more 'open N cycle'), but concluded that understanding of the mechanisms behind these trends was still in its infancy. Craine *et al.* (2009) carried out a more recent synthesis of 11,000 plant samples from throughout the world, and found the same general patterns as Amundson *et al.* (2003), but also that the presence (and type) of mycorrhizal associations had a significant influence on plant δ^{15}N. Therefore, it is likely that climate may not directly drive δ^{15}N trends, but rather a combination of factors such as climate and soil type/age affects vegetation assemblages and mycorrhizal associations, which influences N cycling (Hobbie and Ouimette, 2009).

2.4.2 Soil age

Brenner *et al.* (2001) measured percent N and δ^{15}N along a 3–3000 year chronosequence in an annual grassland in California. They found a weak trend of increasing δ^{15}N as soil age increased (particularly at the oldest site), with the trend being strongest below 200 mm depth. Mean δ^{15}N (to 1 m depth) at the youngest site was 3.5 ‰ compared to 6.1 ‰ at the oldest site. Brenner *et al.* (2001) suggested that the increase in δ^{15}N with soil age was due to proportionally greater inorganic N losses (mainly NO_3^-) compared to organic N losses, and that the inorganic N would have been more depleted in δ^{15}N than organic N. Greater NO_3^- availability and losses from the older soils was suggested to be due to a switch from N to P limitation.

Similar increases in δ^{15}N as soils get older have been observed in Hawaii. Vitousek *et al*. (1989) took soil and tree foliage samples from two sites: one with a substrate age of 197 years and the other with substrate age of ~67,000 years. Average soil δ^{15}N (0–150 mm) was –1.4 ‰ at the young site compared to 3.65 ‰ at the old site. Foliar δ^{15}N values of a native non N fixing tree (*Metrosideros polymorpha*) were –5.9 ‰ and –0.9 ‰ at the young and old site respectively. Tree foliage (*Metrosideros sp.*) was also sampled along a more complete chronosequence with ages of 28, 197, ~2,000 and > 60,000 years. Average δ^{15}N values of the foliage, increasing in order from youngest to oldest sites were, –4.7, –5.9, –2.2 and –0.95 ‰.

Martinelli *et al*. (1999) carried out a similar study in Hawaii on a longer chronosequence, with substrate ages of 300, 2100, 20,000, 150,000, 1.4 million, and 4.1 million years. Surface soil δ^{15}N values were lowest in the youngest two sites (–2 ‰), and then increased in the 20,000 year old site (~1.75 ‰), after which values decreased to around 0 ‰ at the three oldest sites. Deeper soil and foliage δ^{15}N values showed the same trend, but the differences between the youngest site and the 20,000 year old site were greater (e.g. ~5 ‰ for soils and 9 ‰ for foliage). Martinelli *et al*. (1999) explained that the youngest site was very N limited, which would have led to a closed nitrogen cycle, with few N losses and therefore little scope for isotopic fractionation. By 20,000 years N was not the only factor limiting production and at the oldest site P was the most limiting factor, with high rates of N cycling and N loss, providing ideal conditions for isotopic fractionation and δ^{15}N enrichment.

These studies clearly demonstrate that soil age should be considered when interpreting differences in δ^{15}N between soils.

2.4.3 Parent material and soil type

Parent material and soil type can influence soil δ^{15}N directly via the δ^{15}N values of the raw materials of the soil and indirectly because parent material and soil type influence the chemical and physical properties of the soil, which in turn influences plant growth, species composition and N cycling processes.

In a review, Martinelli *et al*. (1999) discovered that foliage from trees growing on N poor, white sand soils in the tropics were significantly depleted in δ^{15}N (~7.8 ‰ lower on average) compared to trees growing on other soil types. The low N status of the white sand soils probably meant that N cycling would have been slow (relatively closed cycle), so there would have been little isotopic fractionation and N loss. However, the reason why these soils were so severely N limited was not discussed, but is likely related to the vegetation assemblages which are adapted to growing on these sandy soils.

Ledgard *et al*. (1984) measured δ^{15}N of pasture soils and parent materials underlying these soils in south-eastern Australia. Basalt had δ^{15}N values of 1.8 ‰, granite 5.3 ‰ and sedimentary rocks 3.9 ‰. There was no relationship between soil δ^{15}N and parent material. This was suggested to indicate that processes associated with the accumulation and transformation of organic N, were the main drivers of surface soil δ^{15}N (Ledgard *et al.*, 1984). δ^{15}N values of the parent materials were much lower (1.8–5.3 ‰) than δ^{15}N of soil from 500–600 mm depth (7.2–10.0 ‰). Ledgard *et al*. (1984) suggested that this indicated that the process of soil formation had caused δ^{15}N enrichment relative to the parent material.

Silver *et al*. (2000) found significantly higher δ^{15}N values (1.5 ‰ higher) in clay soils than sandy soils in a lowland Brazilian forest. Clay soils also had higher rates of nitrification and denitrification enzyme activity than the sandy soils. This suggests that greater isotope fractionating N losses via denitrification and possibly N leaching were responsible for the higher δ^{15}N values of the clay soil (Silver *et al.*, 2000). The textural differences at the site were thought to be a reflection of the underlying sedimentary rock, which was tilted, and had alternating beds of sand and clay sediments. Therefore the difference in δ^{15}N was probably due solely to soil properties, rather than the combination topographic position and soil properties which commonly occurs.

Fractionating individual soils into different particle size classes has shown that the finer fraction (e.g. clays) are usually considerably more enriched compared to the coarser fraction (e.g. silts and sands) (Ledgard *et al.*, 1984; Tiessen *et al.*, 1984; Gerzabek *et al.*, 2001; Quideau *et al.*, 2003; Lobe *et al.*, 2005).

2.4.4 Landscape position and topography

Bedard-Haughn *et al.* (2003) reviewed the literature for landscape patterns of $\delta^{15}N$, and developed a conceptual model to explain the influence that landscape position has on soil $\delta^{15}N$. In general, higher elevation and convex portions of the landscape had lower $\delta^{15}N$ than low lying areas such as footslopes and riparian zones. The most common explanation for this pattern is that denitrification is higher in lower lying areas, due to the accumulation of water creating anaerobic conditions (Sutherland *et al.*, 1993; Bedard-Haughn *et al.*, 2003; Biswas *et al.*, 2008). Denitrification can cause large isotopic fractionation (see Table 2.1), provided all N is not denitrified. In addition, Amundson *et al.* (2003) explained that convex portions of the landscape tend to lose soil via erosion, and demonstrated that as slope increased, soil $\delta^{15}N$ values decreased. This trend was attributed to higher erosion and thus lower soil residence time on steeper slopes (and thus less time for fractionation and N losses to occur), and therefore soil $\delta^{15}N$ values on steep slopes would be similar to N inputs (which are usually depleted in ^{15}N compared to soil). Erosion also tends to preferentially remove finer soil particles which are usually enriched in ^{15}N relative to bulk soil (Ledgard *et al.*, 1984; Tiessen *et al.*, 1984; Gerzabek *et al.*, 2001; Fox and Papanicolaou, 2007), which could also contribute to lower $\delta^{15}N$ on steep slopes. Another simple explanation (not explored by Amundson) for lower $\delta^{15}N$ on steeper slopes is that productivity is usually lower (also less N fixation) and therefore N cycling will be lower which provides less opportunities for isotope fractionating N losses.

Sutherland *et al.* (1993) conducted an intensive study in a wheat field in Saskatchewan. They measured $\delta^{15}N$ in 144 soil samples and 144 samples from ears of grain collected from within an area 110 m x 110 m. Soil $\delta^{15}N$ in the 110 m x 110 m sampling area ranged from 6.2 to 10.3 ‰ with a mean of 8.12 ‰. Mean $\delta^{15}N$ of the grain was lower (6.13 ‰) but the range in values was greater (1.6 to 24.4 ‰). Elevation within the sampling area differed by 1.8 m, and $\delta^{15}N$ values were generally higher for both soil and plants in the low lying areas. Elevation explained 26 % of the variation in soil δ^{15}N and 31 % of the variation in plant δ^{15}N. Elevation also had a significant effect on other soil properties and processes, with higher volumetric soil moisture contents, more fine particles (silts and clays) and higher rates of denitrification in the lower areas. Sutherland *et al.* (1993) also took 144 soil samples from a smaller 11 m x 11 m subplot within the larger plot. δ^{15}N values ranged from 7.1 to 9.1 ‰ and showed a random pattern with no significant relationships between δ^{15}N and elevation (which varied by 0.3 m), or other soil variables.

Biswas *et al.* (2008) carried out another intensive paddock scale study in two undulating fields in Saskatchewan (fields had been cultivated for over 50 years). A 384 m sampling transect was laid out at each site with soil samples taken to a depth of 150 mm every 3 m (total of 128 samples per transect). Using basic statistics, they found a significant negative correlation between δ^{15}N and elevation (i.e. highest δ^{15}N values in depressions) for the first field, but there was no significant correlation in the second field. However, using Wavelet Analysis they revealed that there was a "location dependent relationship between δ^{15}N and elevation", with highest δ^{15}N values generally occurring in depressions. In the first field, the difference in δ^{15}N between the two highest knolls and two hollows was about 3 ‰.

Karamanos *et al.* (1981) found that within one field δ^{15}N was higher in samples taken from lower slopes than upper slopes and this difference occurred at all depths (4 depth increments) down to a depth of 80 cm. Averaged over the 4 sampling depths δ^{15}N was 4.75 ‰ higher from the lower slope samples than the upper slope samples.

2.4.5 Depth in the soil profile

In general, δ^{15}N increases with soil depth in both natural and modified ecosystems (Ledgard *et al.*, 1984; Högberg, 1997; Hobbie and Ouimette, 2009). There are a number of mechanisms which contribute to this pattern, although mechanisms are not fully understood.

One mechanism thought to contribute to the increase in $\delta^{15}N$ with depth, is that further down the profile organic matter is generally older and has undergone more biological transformations (i.e. more N cycling) and therefore has had more opportunities for isotopic fractionation and N loss (Brenner *et al.*, 2001; Hobbie and Ouimette, 2009). However, Hobbie and Ouimette (2009) explain that the enrichment factors observed in relation to decomposition cannot fully account for the increase in $\delta^{15}N$ with depth in many soil profiles. A second mechanism is that plants generally take up N which is slightly depleted in ^{15}N relative to the bulk soil (due to isotopic fractionation during mineralisation and plant uptake). Therefore plant material is depleted in ^{15}N relative to bulk soil N, and this material is returned to the soil surface via litter (Högberg, 1997). Mycorrhizal fungi have been shown to accentuate this pattern by strongly fractionating against ^{15}N when N is transferred from fungi to plants (Hobbie and Ouimette, 2009; Wallander *et al.*, 2009). Therefore, ^{15}N enriched N accumulates in fungi at depth, while N depleted in ^{15}N accumulates at the soil surface via litter fall. Fungal type is important, with the average $\delta^{15}N$ enrichment between surface soil and soil at ~500 mm depth, being twice as high in ectomycorrhizal systems compared to arbuscular mycorrhizal systems (Hobbie and Ouimette, 2009).

In some soils (particularly those with high N availability and/or dominated by arbuscular mycorrhizal vegetation) maximum $\delta^{15}N$ can occur at intermediate depth, which may be due to ^{15}N enrichment of residual nitrate during denitrification, and then immobilisation of this enriched nitrate in soil organic matter (Hobbie and Ouimette, 2009).

2.4.6 *Summary of effect of natural factors on $\delta^{15}N$*

This section has shown that climate, soil age, parent material and even subtle differences in topography can have a large influence on soil $\delta^{15}N$ values. In general soil and plant $\delta^{15}N$ values decrease with increasing MAP, and increase with increasing MAT (Amundson *et al.*, 2003). The influence of climate is thought to be a key driver of the on average ~8 ‰ higher $\delta^{15}N$ values in tropical forest soils compared to temperate forest soils (Martinelli *et al.*, 1999). Landscape position within the same field has been shown to affect soil $\delta^{15}N$ by as much as 4.75 ‰ (Karamanos *et al.*, 1981). Soils differing in age by about 20,000 years had

δ^{15}N values which differed by 5–6 ‰ (Martinelli *et al.*, 1999). Soil parent material has been shown to have no significant effect (Ledgard *et al.*, 1984), or a large effect (~7.8 ‰) on soil δ^{15}N (Martinelli *et al.*, 1999). In both natural and managed ecosystems, δ^{15}N generally increases with depth in the soil profile, with differences >5 ‰ between the surface soil and subsoil being relatively common (Hobbie and Ouimette, 2009).

The potentially large influence of 'natural factors' on soil δ^{15}N, demonstrates that when investigating the effect of land use and management on soil δ^{15}N, 'natural factors' must be considered during development of research questions, sampling strategies, and during data interpretation.

2.5 The effect of land use and management on soil δ^{15}N

There are numerous land uses and management practices which can influence plant and soil δ^{15}N. This section will begin by looking at broad patterns of soil δ^{15}N under different land uses, and will then delve into more detail on some specific management practices, with particular emphasis on practices relevant to pastoral systems (e.g. grazing and fertiliser application).

2.5.1 General patterns of soil δ^{15}N under different land uses

One of the earlier surveys of soil δ^{15}N under a number of different land uses was carried out by Shearer *et al.* (1978), who analysed 124 surface soil samples (0–150 mm) from 20 states in the U.S.A. They found that δ^{15}N ranged from ~2 to ~15 ‰ with a mean of 9.2 ‰, but there was no clear difference between samples taken from under cropland, pasture, native herbs or forest (Table 2.2). In contrast, a recent analysis of 210 surface soils (0–100 mm) from throughout New Zealand, revealed clear differences in δ^{15}N between land uses; with native forests having the lowest values (2.1 ‰), followed by exotic plantation forests (2.8 ‰), then pastures grazed by sheep and/or beef cattle (3.8 ‰), dairy pastures (5.4 ‰) and finally cropping soils (6.2 ‰) (Stevenson *et al.*, 2010). Differences in δ^{15}N between land uses were attributed to differences in management intensity and isotope fractionating N loss processes (e.g. ammonia volatilisation and nitrification followed by denitrification or nitrate leaching), which typically increase from indigenous forests through to cropped land (Stevenson *et al.*, 2010). The average values for pastoral soils was 4.6 ‰, which is higher than the mean value of 3.2 ‰ (range –1.1 to 6.8 ‰) reported by Steele and Wilson (1981), for 61 surface soil samples (0–75 mm) from improved pastures throughout New Zealand. This difference may simply be due to a different distribution of sample sites, or the slightly shallower sampling depth of Steele and Wilson, but could have also been caused by ^{15}N enrichment with time (the Stevenson *et al.* samples were taken 20–30 years after those of Steele and Wilson).

A number of studies have looked at soil δ^{15}N under forest and cropland, and a few studies have compared forests and grasslands/pastures, or grasslands and cultivated land. Surface soil δ^{15}N values from a number of studies are summarised in (Table 2.2). In general, soil δ^{15}N values were higher under cropping land than forests (e.g. Lemenih *et al.*, 2005; Awiti *et al.*, 2008; Llorente *et al.*, 2010; Stevenson *et al.*, 2010). Some studies found higher soil δ^{15}N in grasslands than forests (Pocewicz *et al.*, 2007; Stevenson *et al.*, 2010), some no difference (Piccolo *et al.*, 1996; Eshetu and Högberg, 2000), and some lower δ^{15}N under grasslands (Piccolo *et al.*, 1994a; Piccolo *et al.*, 1996). There was either no difference in soil δ^{15}N between grasslands and cultivated sites (Karamanos *et al.*, 1981; Tiessen *et al.*, 1984), or cultivated sites had higher δ^{15}N (Selles *et al.*, 1986; Stevenson *et al.*, 2010).

The tendency for cropped soils to have higher δ^{15}N than forests or grasslands could be because cultivation generally causes a loss of soil organic matter (including organic N), and ^{14}N is preferentially processed during mineralisation, and is therefore more likely to be lost from the soil (e.g. by leaching or denitrification). Fertilisation of cropping soils with organic (i.e. manure) or synthetic N fertilisers could also contribute to ^{15}N enrichment. Organic fertilisers are usually enriched with ^{15}N relative to the soil (Watzka *et al.*, 2006; Bateman and Kelly, 2007), and could therefore directly cause an increase in δ^{15}N, while the added N in organic or synthetic fertiliser would cause increased N cycling and losses, and thus opportunities for isotopic fractionation. The effect of fertiliser on soil δ^{15}N is discussed further in section 2.5.3. Cultivation of legumes would also increase N inputs via symbiotic N fixation, and potentially isotope fractionating N losses (thus ^{15}N enrichment of the soil), although Gubsch *et al*. (2011) found that soil δ^{15}N decreased significantly more in plots with legumes relative to plots without legumes, presumably due to immobilisation of fixed N (with low δ^{15}N) in the soil. The harvest and export of plant biomass could contribute to ^{15}N enrichment of soil because plants almost always have lower δ^{15}N than underlying soils (see section 2.3). Higher soil δ^{15}N in some pastures compared to forests (Pocewicz *et al.*, 2007; Stevenson *et al.*, 2010) was attributed to increased N inputs and isotopic fractionating N losses, while decreases in some pastures were attributed to accumulation of fixed N with low δ^{15}N values (Piccolo *et al.*, 1996).

Table 2.2 Summary of site characteristics and soil $\delta^{15}N$ values under different land uses from a number of studies in the literature. Soil $\delta^{15}N$ values within a study which are followed by a different lower case letter were reported to be significantly different by the original authors. Note: some $\delta^{15}N$ values are approximate because they were estimated from graphs.

Reference	Country	Soil	MAP (mm)	MAT (°C)	Horizon or sample depth (mm)	Land use	Number of sites	Soil $\delta^{15}N$	
Stephenson *et al.* (2010)	New Zealand	Various	na	na	0-100	Native forest	40	2.1	a
						Exotic plantation forest	30	2.8	a
						Sheep & beef cattle pasture	50	3.8	b
						Dairy cattle pasture	50	5.4	c
						Cropping	40	6.2	d
Shearer *et al.* (1978)	USA	Various	na	na	0-150	Forest	13	8.48	a
						Native herbs	19	9.81	a
						Pasture	15	8.36	a
						Cropping	57	9.81	a
Llorente *et al.* (2010)	Spain	Xerepts	<420	12.3	0-100	Native forest	12	1.9	a
						Cultivated (barley)	12	4.5	b
Awiti *et al.* (2008)	Kenya	Clay texture	~2000	~20	0-200	Native forest	50	5.9	a
						Cropland, cultivated for 17-60 years	50	6.8	b
Lemenih *et al.* (2005)	Ethiopia	Humic Haplustands	1200	20	0-200	Native forest	1	7.1	a
						Cropland, cultivated for 7-53 years (maize, sorghum)	5	8.84	b
Lemma & Olsson (2006)	Ethiopia	Humic Nitosols (FAO)	1517	19.4	0-200	Native forest	1	7	a
						Cultivated land	1	8.3	a
Eshetu & Högberg (2000)	Ethiopia	NR	1244	19	0-200	Native forests	2	6.2	a
						Pasture	2	7.2	a
						Cultivated land	3	7.3	a
Lobe *et al.* (2005)	South Africa	NR	516-625	13.8-16.6	0-200	Savannah grazed by domestic cattle or sheep	3	~7.8	a
						Cultivated for 2-98 years (wheat, maize)	24	~9	a

NR not reported

Table continued on next page.

Table 2.2 continued.

Reference	Country	Soil	MAP (mm)	MAT (°C)	Horizon or sample depth (mm)	Land use and management	Number of sites	Soil $\delta^{15}N$	
Karamanos *et al.* (1981)	Canada	Various	na	na	Surface	Native pasture (prairie)	11	9.3	a
						Cultivated prairie	38	8.4	a
Selles *et al.* (1986)	Canada	Typic Agriborolls	NR	NR	A horizon	Native prairie	1	7.9	a
						Cultivated prairie (30 years)	1	8.8	b
Tissen *et al.* (1984)	Canada	Typic Cryoborolls	NR	NR	A horizon	Native prairie	2	~10.7	a
						Cultivated prairie (60-90 years)	3	~9.5	a
Pocewicz *et al.* (2007)	USA	Mollisols, Inceptisols, Alfisols	~630	NR	50-100	Native forest	9	~3.3	a
						Never cultivated or fertilised grassland	3	3.8	a
						Grassland, previously cultivated and fertilised	3	5.3	b
						Fertilised crop or grassland	3	5.1	b
Norra *et al.* (2005)	Germany	NR	NR	NR	0-20	Forest	7	0.8	NR
						Agriculture	18	3.7	NR
Piccolo *et al.* (1994a)	Brazil	Tropudults	2200	~22	0-200	Native forest	2	11.8	a
						Pasture (20 years old)	2	10.2	b
Choi *et al.* (2005)	USA	Psammentic Hapludult	NR	NR	0-200	Non irrigated loblolly pine forest	1	2.8	a
						Irrigated loblolly pine forest	1	2.2	a
Boutton & Liao (2010)	USA	Typic and Pachic Argiustolls	715	22.4	0-150	Grazed grassland	3	7.5	a
						~100 year old woodland. Previously grassland	3	6	b

NR not reported

2.5.2 *The influence of grazing on soil δ^{15}N*

Grazing can influence soil (and plant) δ^{15}N in numerous direct and indirect ways. The following section (2.5.2.1) discusses the mechanisms by which grazing can influence soil δ^{15}N. This will be followed by a section (2.5.2.2) summarising and discussing results from a number of grazing trials where soil δ^{15}N was measured.

2.5.2.1 *Ways that grazing can affect soil δ^{15}N*

Soil δ^{15}N in grazed systems (as for any ecosystem) is ultimately determined by the balance between the mass and isotopic composition of N inputs, and N losses. Fig. 2.1 shows an annual N balance for a 'typical' New Zealand dairy farm, along with the isotopic signature of the different N fluxes and pools estimated from the literature. The main N inputs to grazed systems are N fixation (usually in association with legumes), atmospheric deposition, fertiliser, and in some cases imported feed (Fig. 2.1). Atmospheric deposition, N fixation and synthetic fertiliser N generally have δ^{15}N values similar to the atmosphere (~0 ‰), while organic fertilisers and imported feed can have higher values. Mammalian herbivore tissues (e.g. meat, blood), fibres (e.g. wool, hair) and milk are generally enriched with ^{15}N relative to diet and N inputs (Steele and Daniel, 1978; Sponheimer *et al.*, 2003; Cheng *et al.*, 2011; Wittmer *et al.*, 2011), and therefore net export of any of these products from a system will cause ^{15}N depletion of remaining ecosystem components. However, product export is often a relatively small component of total N losses from grassland systems (particularly natural systems or those producing meat and fibre), and therefore other N loss pathways are also important for the ^{15}N and total N balance (Fig. 2.1).

In grazed grasslands, a large portion (60–90%) of ingested N is cycled back to the ecosystem via dung and urine (Fig. 2.1) (Castillo *et al.*, 2000; Di and Cameron, 2002; Powell *et al.*, 2010). This high rate of internal N cycling through grazing ruminants provides numerous opportunities for isotopic fractionation, and potentially ^{15}N enrichment of soil, because a large proportion of the N lost from grazed grasslands is lost from patches of animal excreta (particularly urine) (Haynes and Williams, 1993; Ledgard, 2001; Di and Cameron, 2002; Saggar *et al.*, 2004; Di and Cameron, 2007). High N losses from animal excreta occurs because grazers forage over a large area, but deposition of dung and urine occurs

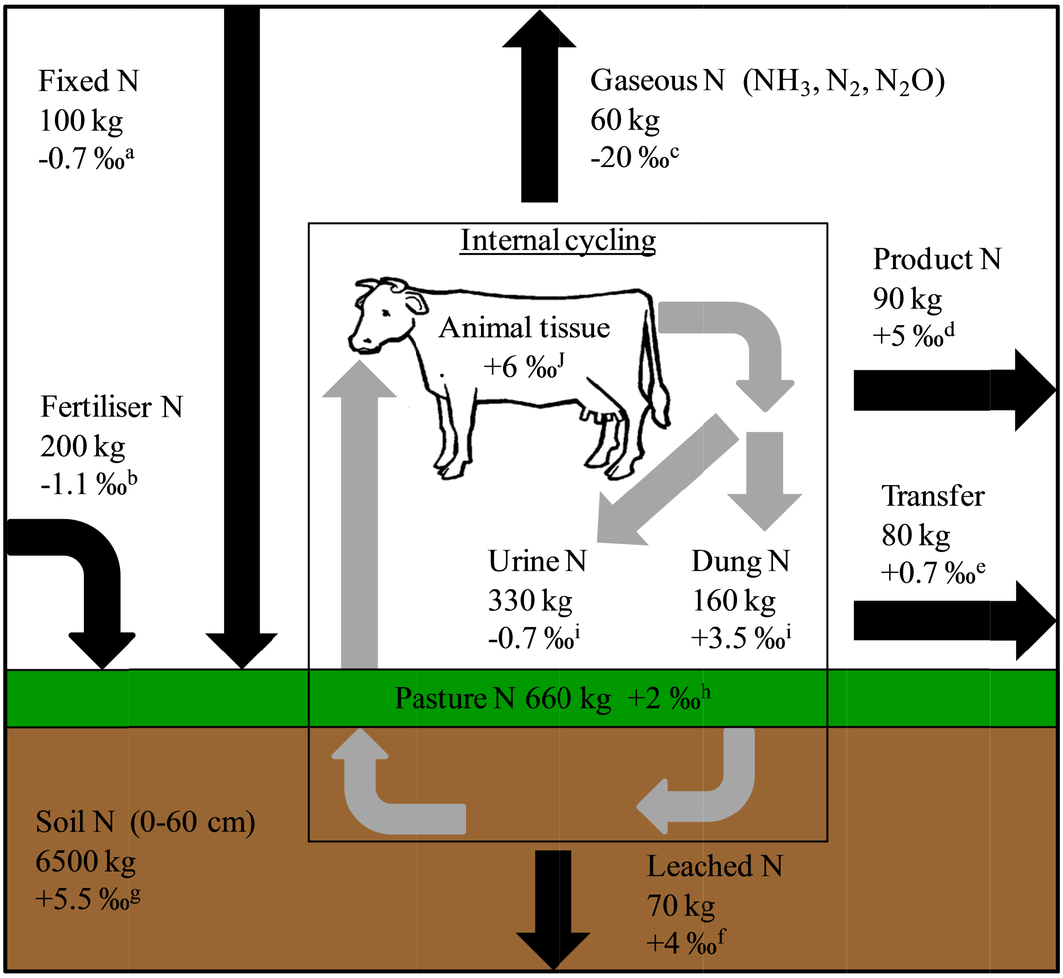

Fig. 2.1. Average annual nitrogen fluxes (kg ha^{-1}) in a New Zealand dairy farm system (simplified from Ledgard *et al.*, 2001). Also shown are δ^{15}N values for the different N fluxes and pools estimated from the literature (see footnotes below). The soil (or ecosystem) δ^{15}N value at the end of a given period of time can be determined using the following equation: $\delta^{15}N_{end} = (((N_{soil\_start} * \delta^{15}N_{soil\_start}) + (N_{fix} * \delta^{15}N_{fix}) + (N_{fert} * \delta^{15}N_{fert})) - ((N_{prod} * \delta^{15}N_{prod}) + (N_{leach} * \delta^{15}N_{leach}) + (N_{gas} * \delta^{15}N_{gas}))) / ((N_{start} + N_{fix} + N_{fert}) - (N_{prod} + N_{leach} + N_{gas}))$. The difference in δ^{15}N between N pools or fluxes is caused by isotopic fractionation, and the range of measured fractionation factors for a number of N cycle processes are presented in Table 2.1.

[a] Average value from four studies using white clover grown hydroponically (Ledgard, 1989).
[b] Urea fertiliser (Stevenson *et al.*, 2010; Clough *et al.*, 2011).
[c] Frank *et al.* (2004) and Houlton and Bai (2009).
[d] Based on Steele *et al.* (1978) and Cheng *et al.* (2011), milk is enriched by 3 ‰ relative to pasture (product export from dairy farms is mostly milk, with a small amount of meat).
[e] Weighted average assuming 66% of excreted N is in urine and 33% in dung.
[f] Based on nitrate in stream water draining predominantly pastoral areas in New Zealand (Clough *et al.*, 2011; N Wells pers. comm. July 2012). Nitrate in stream water may have had higher δ^{15}N than when leaving the soil profile due to ^{15}N enrichment in the vadoze zone and groundwater via denitrification.
[g] Stevenson *et al.* (2010).
[h] Pasture δ^{15}N 3.5 ‰ lower than soil δ^{15}N, based on Chapter 4 and P. Mudge unpublished data.
[i] Urine δ^{15}N 2.7 ‰ lower than pasture δ^{15}N and dung δ^{15}N 1.5 ‰ higher than pasture δ^{15}N, based on Steele *et al.* (1978) and Cheng *et al.* (2011).
[J] Based on Steele *et al.* (1978) where blood was ~4 ‰ higher than pasture.

in small patches where N loading rates are very high (e.g. up to 1000 kg ha^{-1} for cattle urine patches, Haynes and Williams, 1993). Furthermore, dung and urine patches are not spread uniformly across an ecosystem, with larger concentrations in campsites where animals rest, and in managed systems excreta can also be transferred from grazed areas to raceways, milking sheds or housing facilities (Haynes and Williams, 1993). Because plants are typically depleted in ^{15}N relative to soils (see section 2.3), harvest from within the grazed area and deposition elsewhere could gradually lead to ^{15}N enrichment of soil N in the grazed area. It might therefore be expected that campsite soils would have lower δ^{15}N due to large inputs of excreta depleted in ^{15}N relative to the soil. However, Hawke (2001) found higher soil δ^{15}N in sheep campsites, which was attributed to high N loading rates at campsites leading to high isotope fractionating N losses, which must have offset any dilution effect from deposition of excreta with low δ^{15}N.

Isotopic fractionation during digestion and excretion processes means that dung is enriched in ^{15}N relative to diet, while urine is depleted in ^{15}N (Steele and Daniel, 1978; Cheng *et al.*, 2011) (Fig. 2.1). Generally more N is partitioned to urine than dung, and urinary N is more readily lost via leaching and gaseous pathways than dung N (Haynes and Williams, 1993; Castillo *et al.*, 2000; Ledgard, 2001; Monaghan *et al.*, 2007). Therefore preferential loss of large amounts of urinary N relative to dung N could facilitate enrichment of soil N, and this effect could be further accentuated because not all urinary N would be lost, and the N retained in the soil would be enriched relative to the N lost (Frank *et al.*, 2004). In general it appears that leached N is slightly depleted in ^{15}N relative to the soil (Houlton and Bai, 2009; Billy *et al.*, 2010; Clough *et al.*, 2011; Koba *et al.*, 2012), but there have been few measurements, particularly in grazed pastoral systems. Measuring δ^{15}N in leachate from pastoral systems, and particularly leached N associated with urine patches is an area which warrants further research.

Excreta returns combined with the physical action of grazing and animal treading can also affect pasture species composition and soil properties (Menneer *et al.*, 2004a), which could in turn influence soil δ^{15}N. For example, grazing during wet periods can lead to soil pugging (deformation of soil by animal hooves), which has been shown to increase denitrification (Menneer *et al.*, 2005). Denitrification can lead to strong isotopic fractionation (Robinson, 2001), and therefore pugging could lead to an increase in soil δ^{15}N. Grazing can also influence legume abundance and N fixation, and therefore the magnitude of N inputs to a system (Menneer *et al.*, 2004a).

2.5.2.2 Summary and discussion of past grazing studies

The previous section explored the different ways in which grazing could affect soil δ^{15}N. This section will seek to determine the 'net effect' of all these different processes, by reviewing a number of studies where soil δ^{15}N has been measured from under various grazing regimes.

Reported soil δ^{15}N values under different grazing regimes vary between studies, and are summarised in Table 2.3. Frank and Evans (1997) measured soil and plant δ^{15}N from grazing exclosures (grazers excluded for 32–36 years) and adjacent grazed areas (mainly grazed by Elk and Bison), at six locations within Yellowstone National Park. Averaged across all sites, surface soil (0–20 cm) δ^{15}N was significantly higher in the grazed, compared to the ungrazed sites. In grazed areas, ^{15}N enrichment was positively correlated with grazing intensity (estimated from dung abundance), and soil δ^{15}N under dung and urine patches was significantly higher than adjacent areas without dung or urine (Frank and Evans, 1997). Grazing had the opposite effect on plant δ^{15}N, with grazed plots having lower plant δ^{15}N values than ungrazed plots (Frank and Evans, 1997). In a later study at Yellowstone, Frank *et al.* (2004) demonstrated that volatilisation of isotopically light N from urine patches was likely one of the key mechanisms driving enrichment of soil δ^{15}N in grazed plots, and that adsorption of volatilised NH_3 (from urine patches) through the stomata and the cuticle caused the lower δ^{15}N of plants. Grazing intensity has also been shown to increase soil δ^{15}N in other studies. Coetsee *et al.* (2011) found marginally ($p=0.06$) higher soil δ^{15}N in intensively grazed lawns, compared to adjacent tall grass areas in an African Savannah, and Hawke (2001) found that δ^{15}N in soils at sheep camps was significantly higher than non camps and attributed this to greater volatilisation, N_2O emissions and N leaching from the campsite areas due to high deposition of dung and urine. In a broad survey of surface soils throughout New Zealand, Stevenson *et al.* (2010) found significantly higher δ^{15}N under dairy pastures than drystock (sheep and beef) pastures. Dairy pastures were typically grazed more intensively than drystock pastures and had higher N inputs and losses (Stevenson *et al.*, 2010).

In contrast to the previous studies, a number of studies have found no difference in surface soil δ^{15}N under different grazing regimes (Ledgard *et al.*, 1984; Neilson *et al.*, 1998; Cook, 2001; Han *et al.*, 2008; Xu *et al.*, 2010). The lack of difference in soil δ^{15}N may have been due to the relatively short duration of some studies (see Table 2.3), and that any small changes could easily be masked by a large background pool of soil N (Johannisson and Högberg, 1994; Xu *et al.*, 2010; Gubsch *et al.*, 2011). In the study of Ledgard *et al.* (1984), the lack of difference in δ^{15}N was attributed to an accumulation of fixed N (with a δ value of ~0 ‰) in the improved pasture soils, which must have been balanced by enrichment due to fractionating N losses.

Some studies have also reported lower δ^{15}N in surface soils (Golluscio *et al.*, 2009; Xu *et al.*, 2010) and plants (Frank and Evans, 1997; Han *et al.*, 2008) of more intensively grazed treatments, relative to ungrazed or more extensively grazed treatments. Assimilation of volatilised N depleted in ^{15}N is the most likely explanation for the depleted ^{15}N in plants (Frank *et al.*, 2004), and may have also contributed to the lower δ^{15}N of surface soils in the studies of Golluscio *et al.* (2009) and Xu *et al.* (2010). However, Golluscio *et al.* (2009) speculated that lower δ^{15}N in grazed treatments may have been due to lower N mineralisation (though no measurements were made), or that grazing altered biotic interactions between plants and microbes which affected the isotopic signature of litter inputs. Golluscio *et al.* (2009) measured a net decrease in soil N in the most intensively grazed treatment, and therefore accumulation of fixed N is unlikely to have contributed to the lower δ^{15}N.

This review has shown that differences in grazing intensity can have varied impacts on soil δ^{15}N, with some studies showing increases with higher grazing intensities, some no change, and others a decrease in δ^{15}N. Observed increases in soil δ^{15}N at more intensively grazed sites appeared to be driven by greater N inputs, cycling and losses, with dung and urine patches being an important part of the enrichment process (Frank and Evans, 1997; Hawke, 2001; Frank *et al.*, 2004). No change in soil $\delta^{15}N$ under increasing grazing intensity could simply be because changes are not large enough to detect against a large background pool of soil N (Johannisson and Högberg, 1994; Xu *et al.*, 2010), or that increases in fractionating N losses are balanced by accumulation of fixed N with low delta values (Ledgard *et al.*, 1984). No clear mechanism has been identified for the decrease in surface soil $\delta^{15}N$ at higher grazing intensities in some studies (Golluscio *et al.*, 2009; Xu *et al.*, 2010), but accumulation of fixed N and/or greater deposition of ^{15}N depleted NH_3 from urine patches are possible explanations. There are also bound to be many more complex processes occurring, such as interactions between grazers, plants and the microbial community which we do not yet fully understand.

The majority of studies cited above were in natural ecosystems or extensively managed pastures, in cold dry environments (i.e. MAP <800 mm) (Table 2.3). Further research on more intensively managed grasslands in warmer, wetter regions is required. It is likely that changes in soil $\delta^{15}N$ will be more rapid in more intensively managed systems, due to higher N inputs, cycling and losses, and therefore more opportunity for isotopic fractionation. Another gap in knowledge is that no studies have tracked how soil $\delta^{15}N$ changes with time under different grazing regimes.

Table 2.3 Summary of site characteristics and soil $\delta^{15}N$ for a number of grazing studies reported in the literature. Soil $\delta^{15}N$ values within a study which are followed by a different lower case letter were reported to be significantly different by the original authors. Note: some values are approximate because they were estimated from graphs.

Reference	Country	Soil type	MAP (mm)	MAT (°C)	Depth (mm)	Grazing regime	Grazing sp.	Duration (yrs)	Soil $\delta^{15}N$	
Stevenson *et al.* (2010)	New Zealand	Various	na	na	0-100	Drystock pastures	Sheep & cattle	na	3.8	a
						Dairy pastures	Cattle	na	5.4	b
Hawke (2001)	New Zealand	Orthic Recent Soils	NR	NR	0-150	Non-campsite area	Sheep & cattle	na	5.4	a
						Campsite	Sheep & cattle	na	6.5	b
Neilson *et al.* (1998)	Scotland	Gley	1100	NR	0-150	Ungrazed	na	4	~4	a
						Grazed and fertilised (140 kg N ha^{-1} y^{-1})	Sheep	4^{a}	~4	a
Han *et al.* (2008)	China	Light Brown Chernozem	350-450	1	0-100	Low grazing intensity	Dairy cows	>30	4.6	a
						Moderate grazing intensity	Dairy cows	>30	4.68	a
						High grazing intensity	Dairy cows	>30	5.09	a
Golluscio *et al.* (2009)	Argentina	Petrocalcic Calciorthids	150	~4.5	0-50	Ungrazed	Sheep	6-50	~3.2	a
						Moderate grazing (0.1-0.3 sheep ha^{-1})	Sheep	~50-100	~2.65	b
						High grazing intensity	Sheep	~50-100	~2.4	b
Frank & Evans (1997)	USA	Formed from glacial till	379	4.6	0-20	Ungrazed	na	32-36	Lower	a
						Grazed	Elk and bison	>100	Higher	b
Frank *et al.* (2004)	USA	Formed from glacial till	379	4.6	0-20	Non urine patch	na	na	6.1	a
						Urine patch	na	11 days	6.9	b
Ledgard *et al.* (1984)	Australia	Various	na	na	0-50	Native pasture	NR		NR	a
						Improved pastureb	NR	25-55	NR	a
Cook *et al.* (2001)	Australia	Calcareous red loams	656	NR	0-100	Ungrazed	na	26	5.5	a
						Grazed	Cattle	~100	5.31	a
Coetsee *et al.* (2011)	South Africa	NR	600-800	NR	0-50	Tall grass areas	Various	na	5.8	a^{b}
						Intensively grazed 'lawn' areas	Various	na	6.6	b^{b}

NR not reported

Table continued on next page

Table 2.3 continued.

Reference	Country	Soil type	MAP (mm)	MAT (°C)	Depth (mm)	Grazing regime	Grazing sp.	Duration (yrs)	Soil $\delta^{15}N$	
Xu *et al.* (2010)	China	Coarse textured (73% sand, 15% silt, 9% sand)	350	-4	0-100	Ungrazed	na	17	5.2	a
						1.33 sheep ha^{-1}	Sheep	17	~5.5	a
						2.67 sheep ha^{-1}	Sheep	17	~5.5	a
						4 sheep ha^{-1}	Sheep	17	~5.4	a
						5.66 sheep ha^{-1}	Sheep	17	5.96	a
					0-20	Ungrazed	na	17	2.92	a
						1.33 sheep ha^{-1}	Sheep	17	~1.9	ab
						2.67 sheep ha^{-1}	Sheep	17	~1.8	ab
						4 sheep ha^{-1}	Sheep	17	~1.6	ab
						5.66 sheep ha^{-1}	Sheep	17	1.44	b

[a] Essentially a continuation of prior management which would have probably been occurring for many more than 4 years

[b] Improved by growing Trifolium Subterranean

[c] p was only 0.06

2.5.3 Fertiliser application

The effect application of nitrogenous fertiliser has on soil δ^{15}N, depends on the δ^{15}N value of the fertiliser, subsequent isotope fractionating processes and the isotopic signature of N lost from the system. Plant δ^{15}N is affected in the same way, but can also be affected by isotopic fractionation during plant uptake (Shearer and Kohl, 1986; Högberg, 1997), and plants only access N from a small temporally dynamic pool of soil N (Högberg *et al.*, 1995), which can be derived from different depths in the soil profile. Application of non-nitrogenous fertilisers could affect soil and plant δ^{15}N values by affecting plant growth, microbial activity (particularly N fixation) and thus N cycling.

2.5.3.1 Nitrogenous fertilisers (including manure)

Isotopic signature of nitrogenous fertilisers

Bateman and Kelly (2007) carried out an analysis of ^{15}N abundance of synthetic N fertilisers and fertilisers that were permitted in organic agriculture in the UK, and combined this with other published data to give a total of 153 δ^{15}N values for synthetic fertilisers and 83 δ^{15}N values for organic fertilisers. Mean δ^{15}N of the synthetic fertilisers was 0.2 ‰, with 80 % of samples having values between –2 and 2 ‰, and 98.5 % having values less than 4 ‰. Mean δ^{15}N of the fertilisers permitted in organic agriculture was much higher at 8.6 ‰, and there was a much greater range of δ^{15}N values (0.6–36.7 ‰). The majority of organic fertiliser samples were animal manures, with some fish, seaweed and animal based fertilisers (e.g. blood and bone) also analysed. Delta values of ~0 ‰ for most synthetic fertilisers, occurs because they are made from atmospheric N_2, which by definition has a δ value of 0 ‰. Elevated δ^{15}N in manures and slurries is primarily caused by large fractionating N losses, via ammonia volatilisation during storage prior to application (Bateman and Kelly, 2007). High δ^{15}N could also occur if the manure is largely comprised of dung, because fractionation during digestion and excretion processes means that dung is enriched with ^{15}N relative to diet, while urine is depleted in ^{15}N (Steele and Daniel, 1978; Cheng *et al.*, 2011).

Influence of N fertilisers on plant and soil δ^{15}N

There has been a large amount of research into the effect that nitrogenous fertilisers have on plant isotopic composition, but fewer studies have reported the effect on soil δ^{15}N. The most consistent N fertilisation effect observed in the literature, was that application of organic fertilisers (usually manures or slurries) almost always caused an increase in soil and plant δ^{15}N relative to plots receiving no fertiliser or synthetic N fertiliser (Choi *et al.*, 2003; Bol *et al.*, 2005; Choi *et al.*, 2006; Watzka *et al.*, 2006; Senbayram *et al.*, 2008; Kriszan *et al.*, 2009). This has largely been attributed to the δ^{15}N values of plants and soils reflecting the elevated δ^{15}N values of the organic fertilisers applied, but also through further enrichment from fractionating N losses (Watzka *et al.*, 2006; Kriszan *et al.*, 2009).

While application of organic nitrogenous fertilisers tends to have a reasonably consistent effect on soil and plant δ^{15}N (causing an increase), the effect of synthetic N fertilisers is much more varied. Kohl *et al.* (1973) found that as application rates of synthetic N fertiliser increased from 0 to 448 kg N ha^{-1} y^{-1}, δ^{15}N of corn leaves and grain decreased from 3–4 ‰ to near 0 ‰. The same trend was observed in wheat samples from five different trial sites where N fertiliser was applied at rates of 0–168 kg N ha^{-1} y^{-1} (Shearer and Legg, 1975), and more recent studies have found similar results (Choi *et al.*, 2006; Serret *et al.*, 2008). These trends were attributed to an increasing proportion of fertiliser N (δ^{15}N ~0 ‰) taken up by plants, as N fertilisation rates increased (Kohl *et al.*, 1973).

However, a decrease in plant δ^{15}N with increasing synthetic N fertiliser application rate does not occur in all situations. Meints *et al.* (1975) sampled corn leaves from plots fertilised with ammonium nitrate for 8 and 20 years, and found that there was a significant decrease in δ^{15}N in corn leaves as N application rates increased between 0–134 kg N ha^{-1} y^{-1}, but a significant increase in δ^{15}N when application increased from 134–269 kg N ha^{-1} y^{-1}. Similar results were found in two ~20 year forest fertilisation experiments in Sweden (Högberg, 1990, 1991; Högberg *et al.*, 1992). At low rates of N fertilisation (~30 kg N ha^{-1} y^{-1}), there was a decrease in plant (grass or pine needle) δ^{15}N values, while at high N applications (~70 and ~100 kg N ha^{-1} y^{-1}) there was an increase in δ^{15}N. The decrease in plant δ^{15}N at low N rates and increase at high rates observed by Meints *et al.* and Högberg *et al.*, was probably because at low N rates, most of the N was retained in the system (Högberg and Johannisson, 1993), and therefore plant $\delta^{15}N$ decreased to values more similar to the N fertiliser, while at higher application rates, N cycling would have been higher and fractionating N losses greater, resulting in ^{15}N enrichment (Högberg and Johannisson, 1993). This hypothesis was supported by the greater $\delta^{15}N$ values in plots fertilised with urea compared with ammonium nitrate (urea plot $\delta^{15}N$ was ~3 times higher than ammonium nitrate plots) in the study of Högberg (1990, 1991). Soil nitrate concentrations in the urea and ammonium nitrate treatments were similar, and because half of the ammonium nitrate fertiliser was in nitrate form, it was argued that there must have been more nitrification (and thus potential for isotopic fractionation) in the urea plots (Högberg, 1990). There was also the possibility of volatilisation from the urea fertiliser which can cause strong isotopic fractionation (see Table 2.1).

Soil $\delta^{15}N$ was also measured in some of the studies reported in the previous two paragraphs (Kohl *et al.*, 1973; Meints *et al.*, 1975; Choi *et al.*, 2006), and in these studies there was no significant difference between treatments. Other studies have also reported no difference in soil $\delta^{15}N$ between control plots and plots fertilised with synthetic N fertiliser (Shearer *et al.*, 1978; Bol *et al.*, 2005; Kriszan *et al.*, 2009). This appears somewhat surprising, given that immobilisation of synthetic fertiliser N ($\delta^{15}N$ ~0 ‰) should lead to a decrease in soil $\delta^{15}N$, but only a few studies have reported decreases in soil $\delta^{15}N$ following application of synthetic N fertiliser (Gerzabek *et al.*, 2001; Fang *et al.*, 2011). One reason why no significant change in soil $\delta^{15}N$ has been reported for many studies, could simply be because any differences were not large enough to detect against a large background pool of stable soil N (which would be particularly likely for short term trials) (Meints *et al.*, 1975; Johannisson and Högberg, 1994). However, another equally likely scenario is that isotopic fractionation and preferential loss of ^{14}N, balanced any effect caused by immobilisation of fertiliser N with low $\delta^{15}N$ (Watzka *et al.*, 2006; Senbayram *et al.*, 2008; Kriszan *et al.*, 2009). Elevated isotope fractionating N losses was suggested as the most likely cause for increases in $\delta^{15}N$ in some soils fertilised with synthetic N (Johannisson and Högberg, 1994; Watzka *et al.*, 2006; Senbayram *et al.*, 2008). Indeed, Watzka *et al.* (2006) found a significant positive correlation between soil $\delta^{15}N$ and N fertiliser inputs and also ‘excess N’, which was defined as N inputs minus N exported in harvested biomass in cut grassland.

Högberg and Johannisson (1993) found a positive correlation between increases in pine needle δ^{15}N over 20 years and the percentage of added fertiliser N lost from a whole forest ecosystem (plants and soils) during the same time period. Further, Johannisson and Högberg (1994) found a positive correlation between historic N fertiliser inputs and soil (mor layer) and plant δ^{15}N, 10 years after N fertilisation ceased in another forest fertilisation experiment.

2.5.3.2 Non-nitrogenous fertilisers

To date it appears that no studies have investigated the effect of non-nitrogenous fertilisers on soil δ^{15}N in agricultural systems. There has been some research into this in other ecosystems, although the focus has been mainly on plant foliage.

In wetland and mangrove ecosystems plant foliage (of some species) can be very depleted in ^{15}N when P is limiting, compared to when P is not limiting (up to ~12 ‰ difference) (McKee *et al.*, 2002; Clarkson *et al.*, 2005; Inglett and Reddy, 2006; Inglett *et al.*, 2007). This is thought to occur because when P is limiting, N is in relative abundance and therefore plants tend to fractionate against the heavy isotope during uptake, while when N is less abundant all available N is assimilated and therefore there is no net fractionation (Evans, 2001; Inglett *et al.*, 2007). Isotopic fractionation during plant uptake will not necessarily correspond to changes in soil δ^{15}N, because most of the plant N will be returned to the soil at a later date (particularly in natural ecosystems). However, Inglett *et al.* (2007) found a strong positive correlation between δ^{15}N and total P in soils (peat) and litter along a P enrichment gradient in the everglades wetland. Inglett *et al.* (2007) suggested that P may have enhanced microbial activity (particularly mineralisation and denitrification), which could have led to loss isotopically light N from the system. However, in a controlled replicated fertilisation experiment at the same site, different P application rates did not have any effect on soil δ^{15}N after five years (Inglett *et al.*, 2007).

In a long-term forest fertilisation experiment in Sweden, P, and K plus other micronutrients, had no significant influence on δ^{15}N of spruce needles (Högberg *et al.*, 1992). There was a general tendency (non-significant) for plots receiving P and high rates of N to be enriched with ^{15}N relative to plots not receiving P (Högberg *et al.*, 1992). In a hydroponic glasshouse experiment, Ledgard (1989) found that rate of P addition had no effect on $\delta^{15}N$ in roots, shoots or whole white clover plants. When Mo was limiting, plants had more negative $\delta^{15}N$ (–0.62 vs –0.39 ‰) and this effect was stronger at low P status. The reason for this effect was not known, but was presumably related to the importance of Mo in the N fixation process. These results suggest that micronutrients have the potential to exert a small effect on the $\delta^{15}N$ signature of fixed N, which could have flow on effects to other ecosystem components.

Based on this review of the literature, it appears that the largest effect of non-nitrogenous fertilisers on soil $\delta^{15}N$ in pasture systems will be due to the influence of fertiliser on N_2 fixation, pasture production, animal stocking rates and the flow on effect to N cycling and loss processes. However, there is very little data on this topic, and it would be an interesting avenue for further research.

2.5.4 Change in soil $\delta^{15}N$ with time, and influence of land use and management on rates of change

Chronosequence studies spanning hundreds to millions of years (discussed in section 2.4.2) have demonstrated that in general $\delta^{15}N$ increases slowly as soils get older (Vitousek *et al.*, 1989; Martinelli *et al.*, 1999; Brenner *et al.*, 2001). Studies have also shown that land use and management influences soil $\delta^{15}N$ (Watzka *et al.*, 2006; Stevenson *et al.*, 2010). This suggests that superimposed on top of any background rate of change in soil $\delta^{15}N$, is the shorter term influence of land use. Probably the best way to determine how soil $\delta^{15}N$ changes through time in response to changes in landuse is to analyse archived soils (e.g. Billings and Richter, 2006). These studies are very valuable, but are limited by the number of sites where archived soil samples have been kept, and landuse or management changes are well documented. Another approach is to sample from chronosequences, where space is substituted for time (Walker *et al.*, 2010). This section will focus on how soil $\delta^{15}N$ changes during time periods ranging from years to decades, and how landuse and management can influence the rate of change.

Table 2.4 provides a summary of rates of change in soil $\delta^{15}N$ in studies where archived soils were analysed. The longest study was carried out by Bol *et al.*

(2005) who analysed archived soils (eight samplings) spanning a period of 73 years, from the Askov long-term experiments in Denmark (Table 2.4). There were three treatments, unfertilised, mineral N fertiliser and animal manure. In the unfertilised and mineral N treatments there was a trend of decreasing soil δ^{15}N with time, while δ^{15}N in the animal manure treatment first increased, then decreased and then increased again. The inconsistent trend in the manure treatment was suggested to be due to changes in the isotopic composition of the manure, as a result of different proportions of dung and urine. The rate of change in soil δ^{15}N, (calculated from the first and last sample in each treatment) ranged between –0.012 ‰ y^{-1} in the unfertilised treatment to 0.003 ‰ y^{-1} in the manure treatment (Table 2.4). It was not reported whether any of the changes in δ^{15}N were significant (probably because the soils were bulked by treatment, which would have limited statistical analyses).

Gerzabek *et al.* (1999) and Gerzabek *et al.* (2001) analysed soils from a long-term (42 year) small plot experiment designed to investigate the effect of organic manures and N fertiliser on C and N dynamics in an arable cropping system. Soil δ^{15}N values decreased at rates ranging from about –0.023 to –0.077 ‰ y^{-1} in plots receiving peat, sewage sludge, green manure, and calcium nitrate additions (Table 2.4). These decreases were attributed to the added N in these amendments having lower δ^{15}N values than the soil. In contrast there was no significant change with time in a bare fallow plot, and plots receiving no N, or animal manure. In another study, Senbayram *et al.* (2008) analysed archived soil samples taken in 1966 and 2000 from the Broadbalk Wheat Experiment at Rothamsted, UK. Soil δ^{15}N increased in all treatments over this time period, with increases ranging from 0.02 to 0.074 ‰ y^{-1}. However, N fertiliser rate appeared to have no effect on the rate of change. In a much shorter 4 year experiment, Gubsch *et al.* (2011) measured significant decreases in surface soil (0–50 mm) δ^{15}N in grassland plots both with and without legumes. However, the decrease in δ^{15}N was much greater in plots containing legumes (–0.125 ‰ y^{-1}), than plots without legumes (–0.044 ‰ y^{-1}), which was attributed to accumulation of ^{15}N depleted N derived from N fixation by legumes. Interestingly, for the 50–250 mm depth, soil δ^{15}N in plots without legumes increased by about 0.033 ‰ y^{-1}, while there was no change in this depth increment in plots with legumes. The increase in δ^{15}N in the plots without legumes was attributed to loss of ^{15}N depleted N, while in the legume plots any loss of ^{15}N depleted N must have been offset by accumulation of ^{15}N depleted N derived from N fixation.

Changes in soil δ^{15}N with time have also been assessed in forest ecosystems. Billings and Richter (2006) measured archived soils taken over a period of 35 years, from a site where a pine forest had been planted on a former cotton field. Six years after the forest had been planted δ^{15}N values of the mineral soil at four depths (0–600 mm) were relatively similar, ranging from 1.5 to 3.3 ‰ (Table 2.4). The similarity of values was attributed to constant mixing (via cultivation) of at least the surface 200 mm when cotton was being grown. During the next 35 years, the characteristic increase in δ^{15}N with depth observed in most forest soils developed. There was no significant change in the δ^{15}N value of the surface soil, but δ^{15}N values for the other three depths increased significantly, with the greatest increase of ~0.19 ‰ y^{-1} occurring for the 350–600 mm depth increment (Billings and Richter, 2006). The rapid increase in δ^{15}N values at depth was mainly attributed to heterotrophic organisms (particularly ectomycorrhizal fungi) preferentially excreting ^{15}N depleted N, which was taken up by tree roots (Billings and Richter, 2006). The ^{15}N enrichment at depth in this soil was probably accentuated by the transfer of a large amount of N (820 kg ha^{-1}) from the mineral soil to above ground biomass (living trees and litter) (Billings and Richter, 2006). The lack of change in δ^{15}N of the surface soil was attributed to ^{15}N depleted litter falling on the soil surface, and there was little incorporation of this litter into deeper soil horizons (determined from C isotope analysis) (Billings and Richter, 2006). In another forest study, Pardo *et al.* (2002) measured rapid increases in δ^{15}N in organic soil horizons for the first three years following forest clear cutting, and then between 3 and 15 years after clear cutting δ^{15}N values decreased back to values similar to what they were prior to clear cutting (Table 2.4). The rapid increase in δ^{15}N immediately following clear cutting coincided with a rapid increase in stream water nitrate concentrations, and Pardo *et al.* (2002) concluded that increased nitrification and loss of ^{15}N depleted nitrate probably caused the increase in organic soil δ^{15}N. There was no temporal change in mineral soil δ^{15}N.

Plant foliage has also been analysed through time in a number of forest fertilisation experiments (Högberg, 1991; Högberg and Johannisson, 1993; Högberg *et al.*, 1995; Johannisson, 1996; Högberg *et al.*, 2011). Rates of change were generally greater than those reported for mineral soils, with increases of up to ~0.45 ‰ y^{-1} in treatments receiving high rates of urea (Högberg, 1991; Högberg and Johannisson, 1993), and decreases of up to ~0.45 ‰ y^{-1} when fertilisation ceased (Högberg *et al.*, 2011). More rapid changes in foliage samples than soil samples was probably because plants only assimilate N from the actively cycling N pool (which would be readily affected by land management), while bulk soil samples have a large background pool of soil N which would have been present before treatments commenced (Johannisson and Högberg, 1994). Approximate rates of change in soil δ^{15}N can also be calculated for the chronosequence studies in Table 2.2. Rates of change between the endpoints of the chronosequences were relatively slow ranging between about –0.015 ‰ and 0.04 ‰ (Selles *et al.*, 1986; Piccolo *et al.*, 1994a; Lemenih *et al.*, 2005; Boutton and Liao, 2010).

As for most other topics in this literature review, changes in soil δ^{15}N with time varied considerably between (and within) studies, with some studies reporting increases in soil δ^{15}N with time, some no change and others decreases (Table 2.4). In the studies where mineral soils were analysed, long-term rates of change in δ^{15}N were relatively slow, ranging from –0.13 ‰ y^{-1} to 0.19 ‰ y^{-1}. Therefore in most field situations changes in soil δ^{15}N will probably only be detectable after a few years (although see discussion in paragraph below).

Changes in soil δ^{15}N on monthly time scales

Three studies carried out in Scotland have reported large changes in soil δ^{15}N at monthly time scales. In two upland pastures, Neilson *et al.* (1998) found that whole soil δ^{15}N increased by an average of 2.5 ‰ between April and August and then decreased by ~1 ‰ by October. In an old abandoned agricultural field, (Handley and Scrimgeour, 1997) found that soil δ^{15}N increased significantly by about 1 ‰ between June and October. Handley *et al.,* (1999) cited the previous two studies and also reported on unpublished data (from the same sites), explaining that the increase in δ^{15}N during the growing season was associated with a decrease in soil moisture content, and that this trend was the same as that observed at the global scale, where δ^{15}N increased with decreasing MAP. Denitrification was argued to be the most likely cause of these rapid changes in soil δ^{15}N, with Neilson *et al.* (1998) calculating that denitrification rates of 2.6 kg ha^{-1} day^{-1}, and a δ^{15}N value for denitrified N of –29 ‰ could account for this change. However, denitrification rates of 2.6 kg ha^{-1} day^{-1} for 4 months represents a total loss of about 300 kg N ha^{-1}, which is very high, considering that in a review, Barton *et al.* (1999) found that annual denitrification rates ranged from 0–239 kg N ha^{-1} (in both natural and agricultural systems). In the third study, Marriott *et al.* (1997) measured decreases of 1 ‰ during four weeks in a grassland soil, and suggested that this may have been due to accumulation of N fixed in association with legumes (with δ^{15}N of ~0 ‰). However, Marriott *et al.* (1997) calculated that N fixation and accumulation in the soil would have had to be 70 kg ha^{-1} d^{-1} (~2000 kg over 4 weeks) to have this effect. Such high rates of N fixation are very unlikely, considering that annual fixation rates in pastures rarely exceed 300 kg ha^{-1}, with the reported upper range being ~700 kg ha^{-1} (Ledgard, 2001). Further work is required to determine if temporal variation in soil δ^{15}N (on a monthly time scale) is a common occurrence, and what could cause such large and rapid changes if they do indeed occur.

Table 2.4 Rates of change in soil δ^{15}N with time, in studies where archived soils taken at different dates were analysed. Many of the values were read off graphs so will be approximate. Rates of change were calculated from δ^{15}N values at the first and last sampling, and therefore rates of change may have been different between other sampling dates.

Reference	Country	Soil type	Horizon or sampling depth (mm)	Landuse	Treatment	Time between first and last measurement (years)	Initial δ^{15}N (‰)	Final δ^{15}N (‰)	Change in δ^{15}N (‰)	Significant change?	Rate of change (‰, y^{-1})
Bol *et al.* (2005)	Denmark	Typic Hapludalf	0-200	Cropping	Unfertilised	57	5.5	4.8	-0.7	NR	-0.012
					Mineral fertiliser	73	5.6	5.4	-0.2	NR	-0.003
					Animal manure	73	6	6.2	0.2	NR	0.003
Gerzabek *et al.* (2001)	Sweden	Eutric Cambisol	0-200	Cropping	Bare fallow	42	8.65[a]	8.7	0.05	No	0.001
					No N	42	8.65[a]	8.5	-0.15	No	-0.004
					Calcium nitrate	42	8.65[a]	7.7	-0.95	**Yes**	**-0.023**
					Green manure	42	8.65[a]	7.3	-1.35	**Yes**	**-0.032**
					Animal manure	42	8.65[a]	8.7	0.05	No	0.001
					Sewage sludge	42	8.65[a]	7.2	-1.45	**Yes**	**-0.035**
					Peat	42	8.65[a]	5.4	-3.25	**Yes**	**-0.077**
Senbayram *et al.* (2008)	England	NR	NR	Cropping (wheat)	0 kg ha^{-1} N fert	34	2.1	3.6	1.5	NR	0.044
					48 kg ha^{-1} N fert	34	2.1	4.6	2.5	NR	0.074
					96 kg ha^{-1} N fert	34	2.7	4.3	1.6	NR	0.047
					144 kg ha^{-1} N fert	34	1.9	3.6	1.7	NR	0.050
					192 kg ha^{-1} N fert	34	3.3	4	0.7	NR	0.021
					Manure (200 kg N ha^{-1})	34	5.2	6.3	1.1	NR	0.032
Gubsch *et al.* (2011)	Germany	Eutric Fluvisol	0-50	Grassland, ungrazed	Plots with legumes	4			-0.5	**Yes**	**-0.125**
					Plots without legumes	4			-0.175	**Yes**	**-0.044**

NR not reported

[a] Specific values for each treatment at the start of the trial were not reported, only the range of 8.4–8.9 ‰. Therefore the average of these two values was taken for the calculated rate of change presented here.

Table 2.4 continued.

Reference	Country	Soil type	Horizon or sampling depth (mm)	Landuse	Treatment	Time between first and last measurement (years)	Initial δ^{15}N (‰)	Final δ^{15}N (‰)	Change in δ^{15}N (‰)	Significant change?	Rate of change (‰, y^{-1})
Billings & Richter (2006)	U.S.A	Ultisol	0-75	Pine forest	Na	35	2.5	3	0.5	No	0.014
			75-150			35	3.3	5	1.7	**Yes**	**0.049**
			150-350			35	3.2	7.2	4	**Yes**	**0.114**
			350-600			35	1.5	8	6.5	**Yes**	**0.186**
Pardo *et al.* (2002)	U.S.A	Haplorthods	Oie horizon	Mixed forest	First 3 years after clear cutting	3	0	1.3	1.3	**Yes**	**0.433**
			Oa horizon			3	3.2	4.1	0.9	**Yes**	**0.300**
			Bs horizon			3	7.4	7	-0.4	No	-0.133
			C horzon			3	7.75	7.3	-0.45	No	-0.150
			Oie horizon	Mixed forest	Years 3-15 after clear cutting	12	1.3	0.04	-1.26	**Yes**	**-0.105**
			Oa horizon			12	4.1	3.2	-0.9	**Yes**	**-0.075**
			Bs horizon			12	7	7.25	0.25	No	0.021
			C horzon			12	7.3	NR	NR	NR	NR

2.6 Summary and conclusions

This review has discussed how natural abundance nitrogen isotopes (δ^{15}N) are used in ecological studies, how natural factors such as climate and topography can affect soil δ^{15}N, and also how differences in land use and management can influence soil δ^{15}N. The key findings and conclusions of the review are summarised below.

2.6.1 The effect of natural factors on soil δ^{15}N

Natural factors such as climate, soil age, topography, and soil type or parent material can have a large influence on soil δ^{15}N. For example, climate has been shown to affect soil δ^{15}N by up to ~13 ‰ (Amundson *et al.*, 2003), landscape position by ~4.75 ‰ (Karamanos *et al.*, 1981), and soil age by 5–6 ‰ (Martinelli *et al.*, 1999). Care must therefore be taken when designing sampling strategies or interpreting data, to ensure that any observed differences are in fact due to landuse or management and not natural factors (if the effect of land use or management on soil δ^{15}N is indeed the research question).

2.6.2 The effect of land use and management on soil δ^{15}N

The effect of landuse and management on soil δ^{15}N varied considerably, as shown in Table 2.5. There were however some relatively consistent trends in the literature. In general forests and natural grasslands had lower soil δ^{15}N than agricultural systems (particularly cropping sites). The higher soil δ^{15}N in cropping systems was most likely due to preferential loss of ^{14}N as soil organic matter levels declined, and/or because of increased isotope fractionating N losses as a result of increased N inputs via fertiliser or cultivation of legumes. Application of organic fertilisers such as manure could have also directly contributed to ^{15}N enrichment of the soil. Higher soil δ^{15}N in some pastures than forests was attributed to greater N inputs and isotope fractionating N losses, while the lower soil δ^{15}N in some pastures was attributed to accumulation of fixed N with low δ^{15}N values.

Table 2.5 Summary of the effect of changes in land use and management on soil δ^{15}N. Actual δ^{15}N values from individual studies are presented in Tables 2.2–2.4, and are discussed in section 2.5. The symbols '–', '=' and '+' represent a decrease, no change and increase in soil δ^{15}N respectively. Underlined symbols within a row with more than one symbol indicate that that effect was more common.

Land use change, or management practice	Effect on soil δ^{15}N
Conversion of forest or natural grassland to cropping	$= \underline{+}$
Conversion of forest to pasture	– = +
Conversion (or reversion) of agricultural land to forest	– +
Manure application	$= \underline{+}$
Mineral N fertiliser application	– = +
Non nitrogenous fertiliser application (e.g. P, K, S)	=
Increased grazing intensity	$- \underline{=} \underline{+}$
Sowing legumes in pastures (excl forest-to-pasture conversions)	– =
Irrigation	=
Increased cutting frequency in grassland [a]	=

[a] From Watzka *et al.* (2006), cutting frequency was not discussed in above review.

Grazing

There are numerous ways in which grazing can affect soil δ^{15}N, but the deposition of animal excreta (particularly urine) in concentrated patches probably has the largest influence. The very high N loading rates in urine patches means that these areas are hotspots for N losses via leaching or gaseous pathways, with gaseous N losses in particular strongly fractionating against ^{15}N. The 'net effect' of grazing varied considerably between studies, with some studies showing increases in soil δ^{15}N with higher grazing intensities, some no change, and others a decrease in δ^{15}N. Most of the grazing studies reviewed were in natural ecosystems or extensively managed pastures, in cold dry environments (Table 2.3). Further research on more intensively managed grasslands in warmer, wetter regions is required. In more intensively managed pastoral systems increased grazing intensity will generally be associated with a suite of other management changes, such as increased pasture production as a result of fertilisation, irrigation, and sowing of more productive pasture species, and in some cases supplementary feed will be imported. Changes in soil δ^{15}N will probably be more rapid in these more intensively managed pastoral systems, due to higher N inputs, cycling and losses, providing more opportunity for isotopic fractionation. This hypothesis needs to be tested further.

Fertilisers

Probably the most consistent of all the management effects observed, was that δ^{15}N values of soils and plants from plots receiving organic fertilisers (e.g. manure) were almost always significantly higher than control plots, or plots receiving synthetic N fertiliser. This effect was largely attributed to the elevated δ^{15}N values of most organic fertilisers. In some cases application of synthetic N fertiliser (with δ^{15}N of ~0 ‰) resulted in enrichment of soils with ^{15}N, while often there was no effect, and in a few cases there was a decrease in δ^{15}N. No change, or increases in soil (or plant) δ^{15}N at sites where synthetic N fertiliser was applied, indicates that isotopic fractionation must have occurred and that ^{14}N must have been preferentially lost (e.g. via volatilisation). This assertion is supported by the strong positive correlation between soil (or plant) δ^{15}N and N fertiliser loading rate or N losses observed in some studies. There is very little data on the effect of non nitrogenous fertilisers (e.g. phosphorus, sulphur) on soil δ^{15}N, but it appears that any effect in pastoral systems will be due to the influence of fertiliser on N_2 fixation, pasture production, animal stocking rates and the flow on effect to N cycle processes. This hypothesis remains to be tested.

Rates of change in soil $\delta^{15}N$

A limited number of studies have tracked how soil δ^{15}N changed with time in cropping or forest ecosystems (by analysing archived soils). In these studies there were few consistent trends; with some studies or treatments showing increases in soil δ^{15}N with time, some no change, and others decreases (Table 2.4). Long-term rates of change in mineral soil δ^{15}N were relatively slow, ranging from –0.13 ‰ y^{-1} to 0.19 ‰ y^{-1}, and therefore changes will generally only be detectable at annual to decadal time scales. No studies have measured long-term changes in soil δ^{15}N in grazed pastoral systems, and this is an avenue for further research. Tracking changes in soil δ^{15}N with time will improve our understanding of the sensitivity of soil δ^{15}N to differences in pastoral management regimes. This is important because it will constrain what time scale changes in soil δ^{15}N might be expected to be detected, and therefore the suitability of soil δ^{15}N as an indicator of past land use and management.

3 Changes in soil C, N and δ^{15}N along four forest to pasture chronosequences in New Zealand

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The contribution of authors was as follows:

> Paul Mudge, Louis Schipper, Riki Lewis, and Megan Balks (not on author list) decided on the experimental design for the three chronosequences on the pumice soil. Paul Mudge, Riki Lewis, and Djuro Paripovic (not on author list) located suitable field sites, gathered historical information from farmers and sampled the sites. Paul Mudge carried out data analysis and wrote the manuscript which was primarily reviewed by Louis Schipper. Troy Baisden provided additional comments on the manuscript and some technical expertise around measurement of the full profile samples. Anwar Ghani acted as an advisor. Archived soils from the Northland chronosequence on the podzol soil were sampled by Jenni Edwards as part of a PhD which was completed in 1997.

Abstract

Changes in total soil carbon (C), nitrogen (N) and natural abundance nitrogen isotopes (δ^{15}N) were measured along four forest-to-pasture chronosequences in New Zealand. The primary objective of the study was to test the hypothesis that surface soil δ^{15}N would increase with time following conversion of forest to pasture, due to increased N inputs and isotope fractionating N losses. Three chronosequences were on pumice soils in the Central North Island, where exotic pine forests had been converted to intensive dairy pastures 2–11 years prior to sampling, and samples were also taken from remaining pine forests and long-term pastures (40–80 years old). The fourth chronosequence was on a podzol soil in Northland, where native scrub had been converted to sheep grazed pastures over a 32 year period. Results from the three chronosequences on the pumice soil supported our hypothesis, with linear regression revealing significant ($p<0.001$) positive correlations between log-transformed pasture age ($\log_{10}$[pasture age +1]) and soil δ^{15}N, total N, total C and a negative correlation with C:N ratio. There was also a significant positive correlation ($p<0.001$) between δ^{15}N and total N, and a negative correlation with C:N ratio when C:N <13.6. This suggested that as soils became more N 'saturated', isotope fractionating N loss processes increased. Surface soil δ^{15}N in the pine forests was significantly lower than subsoil δ^{15}N, but there was no significant difference between horizons in the long-term pastures, due to ^{15}N enrichment of the surface soil. In contrast to the results from the chronosequences on the pumice soil, results from the Northland chronosequence on the podzol soil did not support our hypothesis, with no detectable relationship between pasture age and soil δ^{15}N, despite a significant increase in total soil N and a significant decrease in C:N ratio. The Northland soil displayed extreme podzolization and weathering, and had been previously disturbed by gum diggers, implying results could be relatively unique to this soil. Over all four chronosequences, total N and C:N ratio showed the strongest and most reliable relationship with pasture age. However, δ^{15}N still provided useful information. For example, the increase in δ^{15}N in the three chronosequences on the pumice soil indicated that a large amount of N must have been lost from the soils, in addition to the N immobilised. The combination of total soil N, C:N and δ^{15}N will provide the most information on long-term N dynamics at a particular site.

***Keywords*:** natural abundance, nitrogen isotopes, deforestation, land use change

3.1 Introduction

The understanding of soil properties and ecosystem development has been advanced by studying chronosequences, which are defined as *"a set of sites formed from the same parent material or substrate that differ in the time since they were formed"* (Walker *et al.*, 2010). Chronosequence studies are also referred to as space-for-time substitutions, and allow insight into how ecosystem properties change over time periods which are longer than would be possible via direct measurement (i.e. millennia, Vitousek *et al.*, 1989) or studies over shorter time periods (i.e. decades) where samples have not been taken through time (Lemenih *et al.*, 2005). Many decadal scale chronosequence studies have investigated the effect that changes in land use, such as conversion of forest to agricultural land (Piccolo *et al.*, 1996; Awiti *et al.*, 2008) or reversion of agricultural land to natural ecosystems (Compton *et al.*, 2007; Wang *et al.*, 2007), have on soil properties. Soil carbon (C) and nitrogen (N) are commonly measured in chronosequence studies, due to the fundamental importance of soil organic matter in ecosystem processes such as nutrient cycling. The natural abundance of C and N isotopes in soil and vegetation have also been shown to be a useful tool in chronosequence studies, with nitrogen isotopes (δ^{15}N) in particular providing useful information on N cycling and loss processes (Piccolo *et al.*, 1996; Martinelli *et al.*, 1999; Brenner *et al.*, 2001; Lemenih *et al.*, 2005; Compton *et al.*, 2007).

Prior to European settlement, New Zealand was predominantly covered by native forest or scrub, with forest clearance for pastoral agriculture commencing in earnest around 1850 and continuing until around 1980, although much of the currently productive land was cleared by 1920 (MacLeod and Moller, 2006). Currently, 39 % of New Zealand's land area is in pastoral agriculture, 9 % in exotic forest/scrub and 50 % under native land cover (which is primarily forest in upland areas) (MFE, 2009). Despite the large scale, and rapid conversion of forest to pasture in New Zealand, few studies have measured how soil properties changed during the conversion process (except see, Walker *et al.*, 1959; Jackman, 1964a, b; Edwards, 1997). Jackman (1964a, b) and Walker *et al.* (1959), conducted chronosequence studies spanning 19–66 years, and found that soil C and N generally increased when native scrub or poor producing pasture was converted to high producing pasture. Increases in C and N were rapid during the first few years following conversion, and then slowed as a new equilibrium was approached. The C:N ratio tended to decrease with time under pasture, indicating that the soils were becoming saturated with nitrogen, therefore increasing the likelihood of N losses via leaching and gaseous pathways (Walker *et al.*, 1959; Schipper *et al.*, 2004; Schipper and Sparling, 2011).

New Zealand's history of land-use change has been associated with various transitions between native and exotic forest and pasture. The clearing of native vegetation for pasture slowed or stopped by ~1980, but economic reforms and spikes in log prices led to conversions of large areas of pasture to exotic forest (mainly *Pinus radiata*) during the 1990s. A recent downturn in returns from plantation forestry, and good returns from dairy farming, has led to conversion of large areas of exotic forest (mainly *Pinus radiata*) to dairy pastures, during the decade leading up to 2008 when article 3.3 of the Kyoto Protocol took effect. This provided an opportunity to further improve our understanding of how soil C and N changed during the first few years following forest clearance. There was particular interest in soil C dynamics because of the implications of forest conversion on New Zealand's greenhouse gas obligations under the Kyoto protocol, and replacement of forests with pasture was expected to significantly increase N leaching losses, with implications on water quality (Hamilton, 2005). With a focus mainly on estimating soil C changes associated with afforestation of pasture, a number of number of studies have compared soil properties under pasture and plantation forest sites in New Zealand (Giddens *et al.*, 1997; Davis and Condron, 2002; Sparling and Schipper, 2004; Tate *et al.*, 2005; Baisden *et al.*, 2006), but only one chronosequence study has been published where forest had been converted to pasture (Hedley *et al.*, 2009).

Globally, few studies have looked at N isotopes along forest to pasture chronosequences, and no such studies have been carried out in New Zealand. However, Stevenson *et al.* (2010) measured natural abundance nitrogen isotopes in surface soils from throughout New Zealand, and found a clear separation between land uses, with lower δ^{15}N under forests (native and exotic) than under pastoral agriculture or cropping land. Lower δ^{15}N in soils under forests than pasture was attributed to differences in land use intensity, with higher N inputs, cycling and isotope fractionating N losses (e.g. volatilisation) from the pasture sites. However, the sites were not paired, and therefore it was not possible to be certain whether differences in δ^{15}N were specifically driven by land use, or if the location of the different land uses had an influence. For example, dairy farms would have probably tended to be on better quality land (i.e. flatter) than forest sites.

Based on the work of Stevenson *et al.* (2010), we hypothesised that surface soil δ^{15}N values would increase with time following conversion of forest to pasture, due to increased N inputs and isotope fractionating N losses. The primary objective of this study was to test this hypothesis by analysing soils taken from four chronosequences where forest or scrub had been converted to pasture. Three of the chronosequences were on pumice soils in the Central North Island, where exotic pine forests had been converted to intensive dairy pastures. Thanks to the foresight of Edwards (1997) to archive soils, we were also able analyse soils collected from a chronosequence on a podzol soil in Northland, where native scrub had been converted to pastures grazed by sheep. We tested the hypothesis that surface soil δ^{15}N values would increase with time under pasture, in two ways: 1) an increase in absolute δ^{15}N values, and 2), a relative increase in surface soil δ^{15}N values compared to a baseline estimated using subsoil δ^{15}N values.

3.2 Methods

3.2.1 Site descriptions and soil sampling

3.2.1.1 Pumice soil chronosequences

The three pumice soil chronosequences were located in the central North Island, near the settlements of either Tokoroa, Atiamuri or Wairakei (Fig. 3.1). All three chronosequences were on the Taupo Pumice soil (Immature Orthic Pumice Soil, Hewitt, 1998), formed on the non-welded Taupo ignimbrite (of rhyolitic composition) deposited as a pyroclastic flow around 232 AD (Hogg *et al.*, 2012). This soil is classified as a Typic Udivitrand according to USDA Soil Taxonomy (Soil Survey Staff, 2010). The soil profiles at the sampling sites typically had three horizons within 600 mm of the soil surface: an Ap horizon (~0–150 mm), a Bw horizon (~150–300 mm), and a Cu horizon (~300–600+ mm).

The Atiamuri and Wairakei study areas were flat or very gently sloping, while at Tokoroa the topography was more varied, but all samples were taken from a remnant terrace landform which was virtually flat. The Tokoroa and Wairakei chronosequences were 60 km apart, and the Atiamuri chronosequence was in-between (Fig. 3.1). At Atiamuri all sampling sites were within 2 km of each other, while at Tokoroa, sites were within 3.5 km of each other and at Wairakei sites were within ~10 km of each other (although most sites were within 5 km). Annual rainfall and temperature was similar between the three sites (Table 3.1).

At all three chronosequences, second or third rotation pine forest (*Pinus radiata*) had been converted to intensive pastoral dairy farms 2–11 years prior to sampling which occurred in March–July 2010. Prior to land preparation for pasture, mature forests were harvested, while immature trees were simply pulled or pushed out with diggers or bulldozers. Pine tree debris were deposited in windrows or slash heaps, most of which were still present at the time of sampling, although at Atiamuri and Wairakei some had been removed (for fuel), burned or buried. Where windrows and slash heaps were no longer present, their location was determined from older aerial photographs and talking to the respective farmers. These disturbed locations were also identifiable on the ground due to the abundance of woody debris and/or charcoal and were avoided during sampling. Following removal of the pine trees at Tokoroa and Atiamuri, the soil was disc-cultivated, harrowed, rolled and then seeded. At Wairakei, heavy duty mulchers were first used to break up remaining wood on or in the surface soil (excluding that deposited in windrows and slash heaps), and then the land was harrowed, rolled and seeded.

At each of the three chronosequences, samples were also taken from remaining mature pine forests and long-term dairy pastures (>40 years under pasture). A long-term sheep and beef pasture was also sampled at Tokoroa, and at Wairakei a dairy runoff (used to make silage, and graze dairy cows during winter) was sampled (Table 3.1). The long-term pasture sites had not previously been in pine forest, but it was assumed that soil properties under pine and the native forest (from which the long-term pasture sites were originally established) would have been similar (Sparling and Schipper, 2004; Stevenson *et al.*, 2010).

Management on all dairy farms was similar, with stocking rates of 2.5–2.9 cows ha^{-1} and milksolids (milk fat + milk protein) production of 800–1100 kg ha^{-1} y^{-1}. On the recently converted farms, N fertiliser inputs were about 200–300 kg ha^{-1} y^{-1} for the first two years, after which rates were 150–200 kg ha^{-1} y^{-1}, which was similar to rates on the long-term dairy pastures. The long-term sheep and beef pasture at Tokoroa received about 40 kg N ha^{-1} y^{-1}, and the dairy runoff at Wairakei 120 kg N ha^{-1} y^{-1}. The recently converted sites received large inputs of lime (2–3.5 t ha^{-1}), and superphosphate (~2 t ha^{-1}) or diammonium phosphate (800 kg ha^{-1}) during the first two years following pasture establishment, to raise soil pH and Olsen P. Subsequently all pastures received about 500 kg superphosphate (or other fertiliser with equivalent P and S) to maintain soil P and S, and other nutrients and trace elements (e.g. K, Co, Se) were also applied as required. Pastures at all sampling sites were predominantly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). Clover content in the recently converted pastures appeared to be higher than in the long-term pastures (although this was not specifically quantified).

At each of the sites identified in Table 3.1, three 50 m transects were laid out. For the Wairakei and Atiamuri pasture sites, the three transects were generally located in separate paddocks, while at Tokoroa there was a limited number of suitable paddocks and therefore all three transects were usually located within the same paddock. The three transects in the pine forest were distributed over a similar area to transects at the pasture sites (i.e. at all sites the three transects were within 200–300 m of each other). For each transect, 20 surface soil cores (0–75 mm depth x 22 mm diameter) were taken at pre-determined random intervals and bulked into one sample. In addition, at seven of the points along each transect, 25 mm diameter cores were taken to 600 mm depth with the aid of a wooden maul. Each individual 600 mm core was split by horizon (with horizon depth recorded) and the seven cores bulked by horizon to give one sample per horizon per transect. Random sampling intervals along the transects were used to avoid any potential for set sample spacing to coincide with pine tree rows. Pits were dug at 25 m along each transect and three bulk density samples were taken from each horizon (in each pit). Bulk density cores were 60 mm in diameter and 50 mm deep.

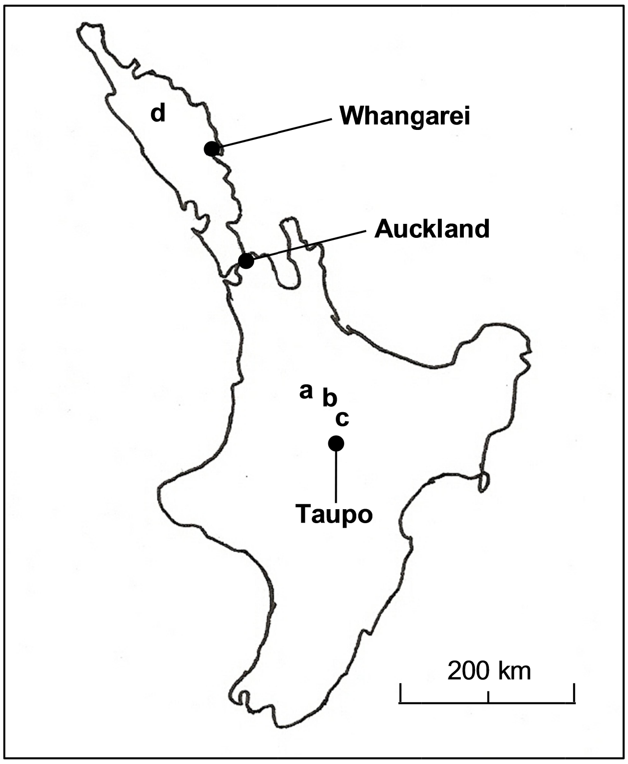

Fig. 3.1. Location of the four chronosequences investigated in this study, in relation to North Island New Zealand. The three chronosequences on the pumice soil were located near (a) Tokoroa, (b) Atiamuri and (c) Wairakei in the Central North Island, while the chronosequence on the podzol soil (d) was located in Northland near Kaikohe.

3.2.1.2 Podzol soil chronosequence

The fourth chronosequence was located at the old Kaikohe Research Station in Northland (Fig. 3.1), where native scrub had been successively cleared for pasture commencing in the late 1950s (Edwards, 1997). The site was originally covered with kauri forest (*Agathis australis*), but this was felled for timber between about 1850 and 1900, and was replaced with regenerating scrub dominated by stunted manuka (*Leptospermum scoparium*) (Esler and Rumball, 1975; Edwards, 1997). Relatively frequent fires meant that this scrub vegetation was maintained rather than kauri forest re-generating (Esler and Rumball, 1975). Most of the research station had been dug over by gum diggers between ~1850 and 1950, and therefore soil profiles at all sites may have been disturbed to some degree. Gum diggers were searching for fossilized kauri gum, a resin exuded by trees (in the original kauri forest) as protection against insect attack, and used for making varnish and linoleum. Topography was rolling and samples were taken from sites with slopes ranging from 4° to 14°. Pastures were grazed by sheep, and management was relatively intensive with stocking rates of about 16 stock units per ha^{-1} (with a stock unit defined here as one 55 kg ewe rearing a single lamb and consuming about 550 kg DM per year).

The soil sampled at the site was the Wharekohe podzol (with pan), a strongly weathered soil, classified (by Edwards, 1997) as a densipan podzol using the New Zealand Soil Classification, or a Ultisol (Typic albaquult) using USDA soil Taxonomy (Soil Survey Staff, 2010). The pale grey A horizon was on average about 150 mm deep, the dense bleached quartz and silica rich E horizon 100 mm thick, and the clay rich B horizon continued to at least 30 cm below the E horizon (Edwards, 1997). The presence of the pan in the E horizon combined with the dense clay rich B horizon meant drainage was poor, and the A horizon became waterlogged during the winter months (Edwards, 1997).

Soils were sampled in 1990 by Edwards (1997) as part of a PhD project investigating P dynamics, and had been archived air dry at AgResearch. Samples were available from five pasture ages ranging from 0–32 years (Table 3.1), with each pasture age (except one) replicated twice (i.e. two separate paddocks were sampled). In each paddock, samples were analysed from three pits dug on the sides of a 10 x 10 m square (located in an area where animals did not camp). Pits were dug to a depth of about 600 mm and samples taken from six depth increments (0–30 mm, 30–75 mm, 75 mm to the E horizon, the E horizon, 100 mm below the E horizon and 200–300 mm below the E horizon). Bulk density was determined from three intact soil cores (48 mm diameter by 23 mm deep) taken from each depth increment in each pit. To remain consistent with the pine-pasture chronosequences (and other archived samples analysed in this thesis) and to reduce analysis costs, the 0–30 mm and 30–70 mm samples were combined (using a weighted average based on bulk density) prior to grinding and analysis.

3.2.2 Sample preparation and analysis

Soil samples were air dried, sieved through a 2 mm sieve, and any wood or pumice that would not pass through the sieve was discarded. Sub-samples were obtained by passing each sample through a riffle, which split the sample in half until about 10 g was obtained. Each subsample was then fine ground with an agate mortar and pestle, and analysed for total carbon and nitrogen using a Leco FP2000 analyser (TruSpec, St Joseph, Mississippi). All 0–75 mm samples were analysed for δ^{15}N using a Europa Scientific 20-20 Stable Isotope Analyser at the University of Waikato Stable Isotope Unit. Profile samples from one forest and one long-term pasture site at each of the four chronosequences were analysed for δ^{15}N using an Isoprime mass spectrometer (Elementar), coupled to a Eurovector EA modified with large capacity carousel and combustion furnace at GNS Science. Profile samples were run on the Isoprime at GNS because this instrument provided enhanced sensitivity on samples with lower N contents in the subsoil horizons. The estimated repeatability of δ^{15}N measurement was 0.1 ‰ for surface soil samples, and was inversely related to %N for subsoil samples, reaching ~0.2 ‰ at 0.1 % N and ~0.3 ‰ for samples measured with the lowest N content. Ten samples were run in both laboratories, and confirmed that results were comparable within 0.1 ‰.

Removal of pumice and wood fragments from soils (during sieving) for the chronosequences on the pumice soil meant that only the fine earth fraction (FEF) of soil was analysed for C and N (and δ^{15}N). Therefore the bulk density of the FEF was required to express C and N on an area basis. Bulk density of the FEF was determined by sieving the whole bulk density cores (after drying and weighing) through a 2 mm sieve, then re-drying the FEF and dividing the weight of soil in the FEF by the total volume of the bulk density core. Bulk density measurements were not specifically made for the 0–75 mm depth and therefore soil %C and %N data for this depth increment were converted to an area basis using bulk density measurements from the A horizon.

3.2.3 Statistical analysis

3.2.3.1 Pumice soil chronosequences

For the three chronosequences on the pumice soil, data from the three replicate transects at each site (pasture age) were averaged prior to analysis. This conservative approach was taken because samples from the three transects at the same site were essentially pseudoreplicates (particularly where transects were located in the same paddock). Transects in separate paddocks could have potentially been treated as separate replicate samples because management would have differed slightly between paddocks. However it was decided not to take this approach and instead standard errors are presented for each point in Fig. 3.2 to show the variability between transects at each site. Data were plotted against the number of years the sites had been in pasture, but due to the large spread and uneven distribution of pasture ages (i.e. 0–11 years for recent conversions and 40–80 years for long-term pastures) the time data was log-transformed. The transformation $\log_{10} +1$ was used to account for the pine forest sites where the pasture age was 0. Data were also analysed with the long-term pasture sites excluded to better understand changes soon after the conversion process. In this case data did not require log-transformation. For full profile samples, the significance of differences between depths within the forests or pastures, and between the forests and pastures for each depth, were determined using analysis of variance (in Genstat v 13).

3.2.3.2 Podzol soil chronosequence

For the chronosequence on the podzol soil, some of the data from the two paddocks of the same age were quite different, suggesting that site specific factors were important. Therefore, for analysis, the three replicate samples from within a paddock were averaged, and the two different paddocks for each age treated as separate replicates. This data was plotted against years since conversion, and due to the different pasture ages being more evenly spread than in the pumice soil chronosequences, pasture age was not log-transformed. Profiles from the scrub and long-term pasture sites were analysed in the same way as the pumie soil chronosequences. Unfortunately it was not possible to find bulk density or horizon depth data for four of the sites reported by Edwards (1997). Therefore when data for these sites was expressed on an area basis, the bulk density, and horizon depths (for the 75 mm to E horizon and E horizons) were estimated as the average of the other site of the same age.

Table 3.1 Land use and years since conversion from forest to pasture for the four chronosequences used in this study. Also shown is mean annual precipitation (MAP) and mean annual air temperature (MAT) for each chronosequence. At the three chronosequences on the pumice soil, soils were sampled from three 50 m transects for each pasture age, with transects generally located in three separate paddocks. For the Northland chronosequence on the podzol soil, samples were taken from six pits for each pasture age (three pits in each of two paddocks) except for the 25 year old pasture where there was only one paddock.

Chronosequence	MAP[a] (mm)	MAT[a] (°C)	Land use	Pasture age (yr)
Atiamuri	1450	12.3	Pine forest	0
			Dairy pasture	2
			Dairy pasture	5
			Dairy pasture	11
			Long-term dairy pasture	80
Wairakei	1200	12	Pine forest	0
			Dairy pasture	2.5
			Dairy pasture	3
			Dairy pasture	3.5
			Dairy pasture	4
			Dairy Pasture	4.5
			Long-term dairy runoff	43
			Long-term dairy pasture	43
			Long-term dairy pasture	60
Tokoroa	1520	12.4	Pine forest	0
			Dairy pasture	1.5[b]
			Dairy pasture	2
			Dairy pasture	3
			Dairy pasture	5
			Long-term dairy pasture	40
			Long-term sheep and beef pasture	50
Northland	1590	14.7	Native scrub	0
			Sheep pasture	8
			Sheep pasture	23
			Sheep pasture	25
			Sheep pasture	30
			Sheep pasture	32

[a] For the three pumice soil chronosequences MAP and MAT data (1980–2009) were obtained from the NIWA Virtual Climate Station database (Tait *et al.*, 2006), while data from the Northland chronosequence were measured data (1980–2009) from a meteorological station located on the farm.

[b] This site was in a crop of swedes for six months prior to establishment of pasture.

3.3 Results

3.3.1 Pumice soil chronosequences

The clearest trend for the pumice soil chronosequences, was higher total N and δ^{15}N, and lower C:N ratio in the long-term pasture soils compared to the pine forests or recently converted soils (Fig. 3.2). For example, average δ^{15}N, %N and C:N ratio in the forest sites were 2, 0.47 and 17 respectively, compared to 4.3, 0.86 and 10.7 in the long-term pasture sites. For the three individual chronosequences there was a significant positive correlation between log-transformed pasture age ($\log_{10}$[pasture age +1]) and soil δ^{15}N, total N, total C and a negative correlation with C:N ratio (Table 3.2). One exception was the Wairakei chronosequence where there was no significant correlation between pasture age and soil C (Table 3.2).

Values for soil C, N and δ^{15}N were very similar between the three chronosequences, with overlap between values from sites in pine forests, recent conversions and long-term pastures. Due to this similarity, and the fact that all chronosequences were on the same soil type, we decided to combine the data and analyse together as a single set. Regressions from this combined analysis were all highly significant ($p<0.001$) (Fig. 3.2, Table 3.2). The combined data were also analysed with the long-term pasture sites excluded, which revealed that there was still a significant positive correlation ($p<0.05$) between pasture age (not log-transformed in this case) and C, N and δ^{15}N. Based on the model fitted to all the data from the three chronosequences combined (Table 3.2), total N in the 0–75 mm depth increased by 165 kg ha^{-1} y^{-1} during the first 10 years following pasture establishment, with the rate slowing to 26 ha^{-1} y^{-1} between 10 and 50 years. Soil C showed a similar trend, increasing by 908 kg ha^{-1} y^{-1} during the first 10 years, with the rate slowing to 145 kg ha^{-1} y^{-1} between 10 and 50 years.

Plotting data from all individual transects, revealed a significant ($p<0.001$) positive correlation between soil δ^{15}N values and soil %N (Fig. 3.3a). Soil δ^{15}N and C:N ratio data could be fitted with the ‘broken stick’ model, with a significant negative correlation between δ^{15}N and C:N when C:N was <13.6, but no significant relationship when C:N was >13.6 (Fig. 3.3b).

For the full profiles in the pine forests, %N decreased and δ^{15}N increased significantly from the A to the B horizon, but there were no significant changes between the B and C horizons (Fig. 3.4a, b). In the long-term pasture sites, both %N and δ^{15}N were significantly higher in the A horizon than in the forest sites, and %N decreased with depth like in the forest sites. However, there was no significant change in δ^{15}N with depth in the long-term pastures (Fig. 3.4a). At all three chronosequences, total mass of N to 600 mm depth, was significantly higher in the long-term pastures, and average δ^{15}N (weighted by mass of N in each horizon) was significantly higher in the pasture profiles compared to the forest profiles at two out of three chronosequences. This suggests that the higher total N and δ^{15}N in the surface soils of the long-term pastures was not simply due to a difference in N or ^{15}N distribution within the profile (i.e. Billings and Richter, 2006; Högberg *et al.*, 2011). C and N data for the full profile (for all sites) will be reported in (Lewis *et al.*, in prep).

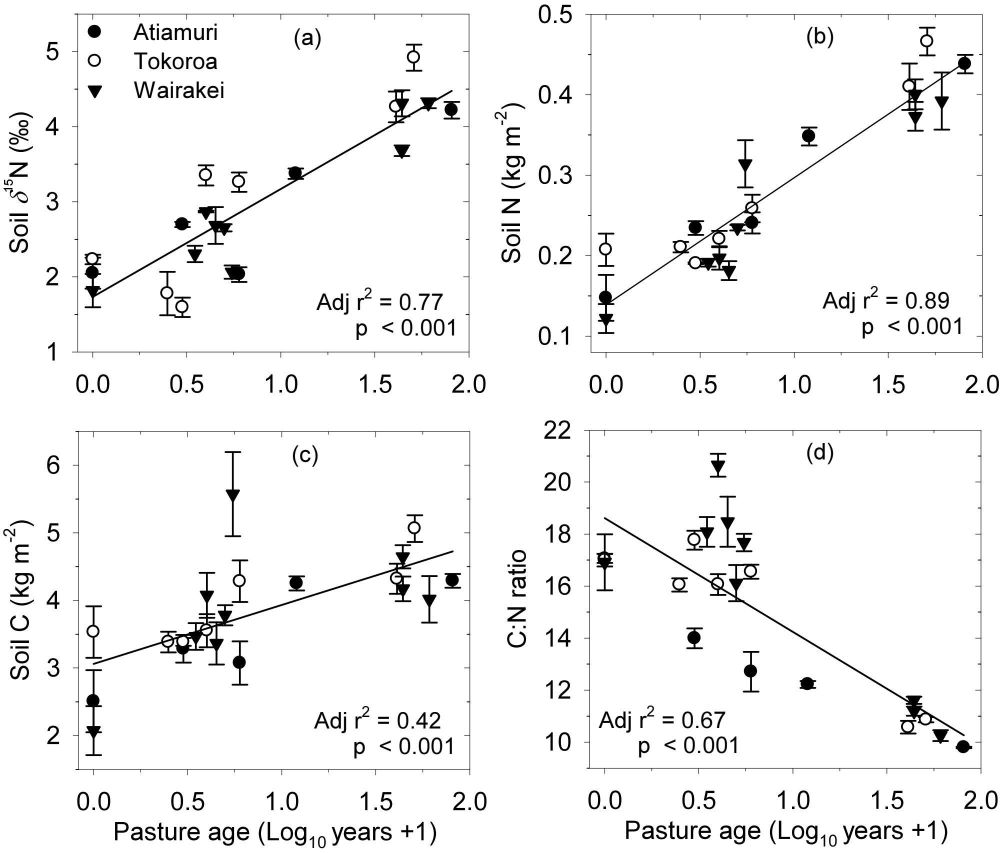

Fig. 3.2. Semi-log plot showing the relationship between time under pasture and surface soil (0–75 mm) δ^{15}N (a), total N (b), total C (c), and C:N ratio (d) for three forest-to-pasture chronosequences on pumice soils in the Central North Island. Each point is the average of three replicate transects (20 bulked cores per transect) at each pasture age. Error bars are ± 1 SE. Table 3.2 shows further details for the regression lines and also for regression lines fitted for each of the three chronosequences individually.

Table 3.2 Regression statistics for the relationship between time under pasture ($\log_{10}$[pasture age +1]) and surface soil (0–75 mm) δ^{15}N, total C, total N, and C:N ratio for the Atiamuri, Tokoroa and Wairakei chronosequences, and all three chronosequences combined. Values in brackets are standard errors.

Variable	Chronosequence	Slope	Slope p	Intercept	Intercept p	Adjusted r^2
δ^{15}N	Atiamuri	1.16 (0.35)	0.044	1.89 (0.37)	0.014	0.72
	Tokoroa	1.75 (0.41)	0.008	1.66 (0.41)	0.01	0.74
	Wairakei	1.42 (0.20)	<0.001	1.66 (0.21)	<0.001	0.87
	Combined	1.44 (0.17)	<0.001	1.73 (0.18)	<0.001	0.77
N (kg m^{-2})	Atiamuri	0.15 (0.02)	0.003	0.15 (0.02)	0.004	0.95
	Tokoroa	0.17 (0.02)	0.001	0.15 (0.02)	0.002	0.88
	Wairakei	0.16 (0.02)	<0.001	0.12 (0.02)	0.001	0.88
	Combined	0.16 (0.01)	<0.001	0.14 (0.01)	<0.001	0.89
C (kg m^{-2})	Atiamuri	0.96 (0.29)	0.045	2.67 (0.31)	0.003	0.72
	Tokoroa	0.88 (0.22)	0.011	3.23 (0.22)	<0.001	0.71
	Wairakei	0.82 (0.50)	0.143	3.15 (0.54)	<0.001	0.18
	Combined	0.87 (0.22)	<0.001	3.06 (0.23)	<0.001	0.42
C:N	Atiamuri	-3.61 (0.53)	0.006	16.22 (0.56)	<0.001	0.92
	Tokoroa	-4.33 (0.79)	0.003	18.43 (0.78)	<0.001	0.83
	Wairakei	-5.18 (1.17)	0.003	20.44 (1.27)	<0.001	0.70
	Combined	-4.37 (0.68)	<0.001	18.61 (0.72)	<0.001	0.67

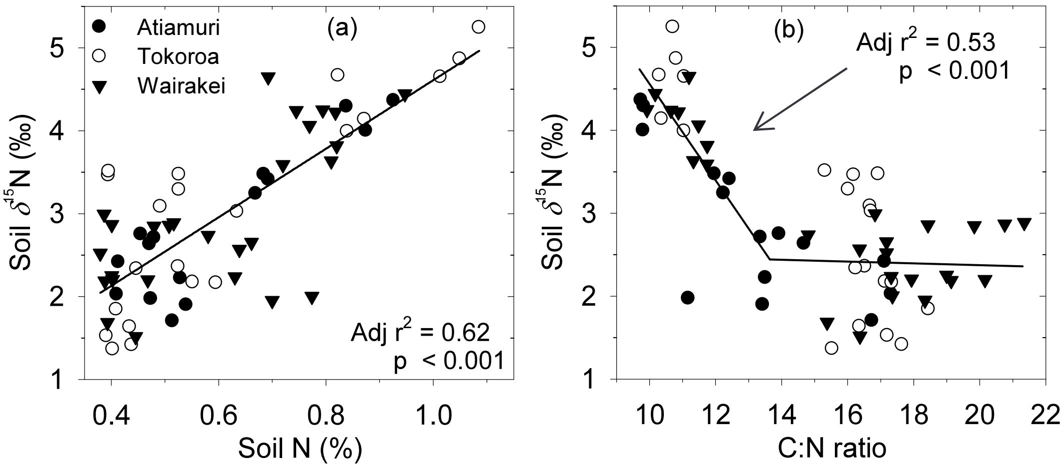

Fig. 3.3. Relationship between (a) soil δ^{15}N and total N and (b) δ^{15}N and C:N ratio, using data from the three chronosequences on the pumice soil. The line in panel (a) is a simple linear regression line, while data in panel (b) is fitted with the 'broken stick' model in Genstat. Each point is a value from an individual transect (20 bulked cores per transect).

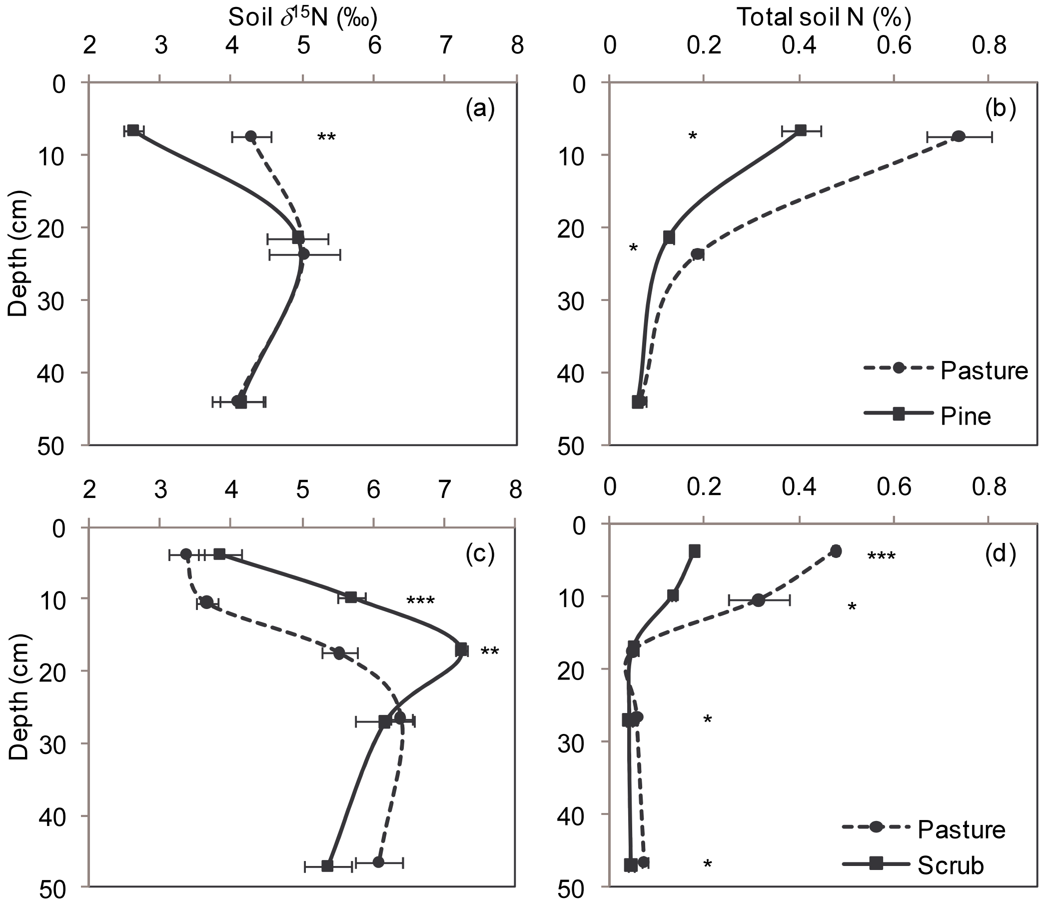

Fig. 3.4. Average (a) δ^{15}N and (b) total N in soil profiles under pine forests and long-term (>40 year) pastures at three forest-to-pasture chronosequences on pumice soils in the Central North Island. Also shown is average soil (c) δ^{15}N and (d) total N for the soil profile of one scrub site and one 30 year pasture site at a scrub-to-pasture chronosequence on a podzol soil in Northland. Symbols are located at the midpoint of each sampling depth. Error bars are ± 1 SE, and significant differences within the same horizon are denoted with asterisks (*P<0.05, ** P<0.01, ***P<0.001).

3.3.2 Podzol soil chronosequence

Unlike the three chronosequences on the pumice soil where there was a clear difference in soil δ^{15}N between forests and long-term pasture sites, there was no clear relationship between surface soil δ^{15}N and pasture age in the Northland chronosequence (Fig. 3.5a). In general, the three replicate samples from within the same paddock had similar values, but samples from other paddocks with the same (or similar ages) were up to ~2 ‰ different. An exception was the forest sites, where δ^{15}N of the three samples taken from the edges of the same 10 x 10 m area were up to 2.8 ‰ different (Fig. 3.5a). There was also no relationship between pasture age and soil C, with values appearing to be randomly distributed across the different pasture ages in a similar manner to δ^{15}N (Fig. 3.5b). As for δ^{15}N, sites from the same paddock tended to have similar C values, and samples from the forest sites were the most variable. In contrast to soil C and δ^{15}N, soil N increased significantly ($p<0.001$) with pasture age, with most of the increase in N occurring in the first few years following pasture establishment (Fig. 3.5c). Based on the regression line in (Fig. 3.5c), N increased by 108 kg ha^{-1} y^{-1} during the first 10 years, 53 kg ha^{-1} y^{-1} between 10 and 20 years and then there was no change between 20 and 30 years. The C:N ratio decreased significantly ($p<0.001$) as pasture age increased, from about 28 in the forest sites to about 12 in pastures older than 20 years (Fig. 3.5d). The C:N ratio showed the inverse trend to soil N, decreasing rapidly at first, after which the rate of change slowed (Fig. 3.5b). Carbon and nitrogen were also measured in all samples taken from 75 mm to the E horizon (~150 mm). Pasture age did not influence soil C or N for this depth increment, but there was a significant decrease ($p<0.01$) in the C:N ratio as time under pasture increased (data not shown).

For the 0–75 mm depth data, there was a tendency for sites which had high soil C to have low δ^{15}N, and there was a significant negative correlation ($p<0.001$, Adj $r^2=0.55$) between δ^{15}N and %C (data not shown). There was no significant correlation between δ^{15}N and total N or the C:N ratio, but there was a significant positive correlation between soil C and N ($p<0.001$, Adj $r^2=0.32$). The correlation between soil C and N helps explain why the relationship between C:N ratio and pasture age (Fig. 3.5d) was stronger than for C or N alone.

Analysis of the full profile at one scrub and one long-term pasture site revealed that %N decreased significantly to the E horizon and then stayed constant (Fig. 3.4d). At the scrub site, δ^{15}N increased with depth to the E horizon and then decreased, while in the pasture site δ^{15}N increased rapidly to the E horizon, and then increased slightly in the lower two horizons (Fig. 3.4c). Soil %N in the A horizon was higher in the pasture site, but δ^{15}N in the 75 mm to E horizon, and E horizon was highest in the scrub site.

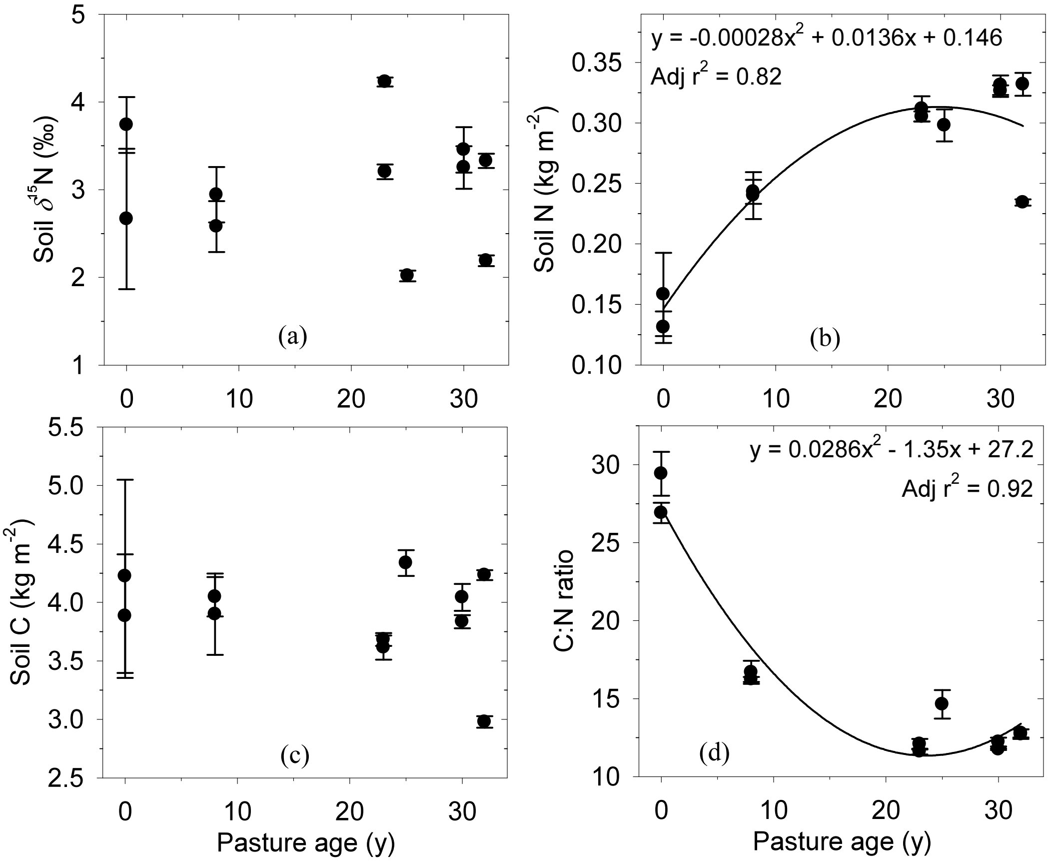

Fig. 3.5. Plots showing the relationship between time under pasture and surface soil (0–75 mm) δ^{15}N (a), total N (b), total C (c), and C:N ratio (d) for a native scrub to pasture chronosequence on a podzol soil in Northland. Each point is the average of samples from three replicate pits in a single paddock. Error bars are ± 1 SE. Regression lines in panels b and d are second order polynomials.

3.4 Discussion

Based on Stevenson *et al.* (2010), we hypothesised that surface soil δ^{15}N would increase with time following conversion of exotic plantation pine forest or native forest/scrub to pasture. Data from the three chronosequences on pumice soils supported this hypothesis, with a clear increase in soil δ^{15}N between the pine forests and the long-term pastures (Fig. 3.2a). These results suggested that the differences observed by Stevenson *et al.* (2010), were indeed driven by differences in land use, since soil and climatic conditions were the same (or very similar) between pine and pasture sites in the current study. However, results from the Northland chronosequence on the podzol soil did not support our hypothesis, with no relationship between pasture age and soil δ^{15}N (Fig. 3.5a). Causes for the patterns observed in the chronosequences on the two soil types will be explored below.

Varied results have been reported for soil δ^{15}N in other land use change chronosequence studies. In two (of the few) forest-to-pasture chronosequence studies where nitrogen isotopes were measured, Piccolo *et al.* (1994a) and Piccolo *et al.* (1996) found either no difference, or lower surface soil δ^{15}N in pasture sites compared to forest sites, in the Brazilian Amazon. At the sites where pasture soils had lower δ^{15}N than forest soils, δ^{15}N tended to be lowest in older pastures, which was opposite to what was observed in the three chronosequences on the pumice soil (Fig. 3.2a). The lower δ^{15}N in some pasture sites reported by Piccolo *et al.* (1994a; 1996) was suggested to be due to increased N inputs from N_2 fixation (by free living bacteria, since legumes were not sown), and N derived via fixation typically has δ^{15}N values of ~0 ‰, which was much lower than the surface soils (~7–12 ‰). In addition, no fertiliser was applied to the pastures, and both N mineralisation and nitrification rates were higher in the forest soils (Piccolo *et al.*, 1994a, b; Piccolo *et al.*, 1996), which suggests that N cycling was tighter in the pastures, and isotope fractionating N losses (e.g. denitrification) may have also been lower. We did not measure N mineralisation the current study, but Stevenson *et al.* (2010) found that N mineralisation rates were twice as high in long-term pastures compared to native or exotic forests in New Zealand.

Increases in soil δ^{15}N similar to the chronosequences on the pumice soil, have been reported when forests have been converted to cropland (Lemenih *et al.*, 2005; Lemma and Olsson, 2006; Awiti *et al.*, 2008; Llorente *et al.*, 2010). In these studies the increase in δ^{15}N was mirrored by a decrease in total N (and C), which suggests that conversion led to increased N losses, and the N lost was depleted in ^{15}N. During the conversion of agricultural land to forest, Compton *et al.* (2007) and Billings and Richter (2006) also found that soil δ^{15}N increased with time, although this was largely attributed to re-distribution of N (depleted in ^{15}N), from the mineral soil to above ground plant biomass, facilitated by mycorrhizal fungi which can cause strong isotopic fractionation (Hobbie and Ouimette, 2009). In contrast, Boutton and Liao (2010) and Wang *et al.* (2007) found that soil δ^{15}N decreased in chronosequences where agricultural land had been allowed to revert to forest or scrub. Boutton and Liao (2010) attributed the decrease in soil δ^{15}N to an increase in soil N which was largely derived via N_2 fixation, and hence had a delta value of ~0 ‰.

3.4.1 Pumice soil chronosequences

The increase in both δ^{15}N and total N with pasture age in the three chronosequences on the pumice soil differed compared to most other land use change chronosequence studies, where soil N and δ^{15}N often had opposite trends (see above). The main difference between the pumice soil chronosequences, and the previously mentioned studies, was the high N loading rate to the pastures. Total N input to the forest sites would have been <10 kg ha^{-1} y^{-1} mainly from atmospheric deposition (Parfitt *et al.*, 2006; Stevenson *et al.*, 2010), while inputs to the pasture soils would have probably been in excess of 200 kg ha^{-1} y^{-1}, via inputs from N fertiliser and N_2 fixation associated with clover. Total N in the 0–75 mm depth increased significantly with time under pasture (55 kg N ha^{-1} y^{-1} during the first 50 years), indicating that a portion of the added/fixed N was immobilised in the soil, although a large amount of N must have also been lost. Fixed N and fertiliser N generally have δ^{15}N values of ~0 ‰ (Högberg, 1997; Bateman and Kelly, 2007), and therefore N immobilisation should have led to a decrease in soil δ^{15}N (Piccolo *et al.*, 1996; Boutton and Liao, 2010; Gubsch *et al.*, 2011). The increase in soil δ^{15}N despite N immobilisation in the soil, therefore indicates that isotope fractionating N loss processes (e.g. volatilisation and nitrification followed by nitrate leaching or denitrification) must have occurred, leading to a loss of ^{15}N depleted N. It is well established in New Zealand pastoral systems that isotope fractionating N losses such as volatilisation and nitrification followed by nitrate leaching or denitrification, increase as N inputs increase (Ledgard *et al.*, 1999; Monaghan *et al.*, 2005), and that N losses are much higher under pasture than forest (Di and Cameron, 2002; Menneer *et al.*, 2004b; Stevenson *et al.*, 2010).

A simple mass balance calculation, based on a total N input of 200 kg ha^{-1} y^{-1} (with a δ^{15}N value of 0 ‰) and a net N immobilisation rate of 55 kg ha^{-1} y^{-1} during 50 years of pasture development (calculated from the regression equation from Fig. 3.2b, Table 3.2), revealed that the net δ^{15}N of N immobilised in the surface soil must have been about 5.7 ‰, and net δ^{15}N of N lost –2.1 ‰. These values were similar to those calculated for immobilised and lost N in two long-term (~50 year) superphosphate and irrigation trials at Winchmore in Canterbury, where archived soils taken through time were analysed for δ^{15}N (see Chapter 4). The average rate of change in surface soil δ^{15}N between the pine forests and 50 year old pastures was 0.05 ‰ (calculated from the regression equation from Fig. 3.2a, Table 3.2), which was also of a similar magnitude to the average rate of change (~0.03 ‰) reported in the more intensive treatments of the two Winchmore trials (see Chapter 4, Fig. 4.2).

Average soil δ^{15}N (0–75 mm depth) under the pine forests and long-term dairy pastures was 2 ‰ and 4.1 ‰ respectively. These values were lower than the average reported by Stevenson *et al.* (2010) for 30 plantation forest sites (2.8 ‰) and 50 dairy farm sites (5.4 ‰) located throughout New Zealand. Lower δ^{15}N values in the current study under both pine forest and long-term dairy pasture may have been due to the pumice soil being young (~1800 years) relative to many other soils in New Zealand. Soil δ^{15}N tends to increase with soil age (Vitousek *et al.*, 1989; Martinelli *et al.*, 1999; Brenner *et al.*, 2001) and therefore there would have been less time for ^{15}N enrichment to have occurred in the pumice soils. In addition, the long-term pasture sites in the current study had probably been in pasture for a shorter time than many of the sites reported by Stevenson *et al.* (2010), because land clearance in the Central North Island, would have on average occurred later than in many other parts of New Zealand. Some of the pine forests reported by Stevenson *et al.* (2010) were also planted on sites which had previously been in pasture, and therefore δ^{15}N may have become elevated prior to establishment of the trees. As far as we can determine, none of the pine forest sites in the current study were previously in pasture. The similarity of δ^{15}N from the long-term dairy pastures and the long-term sheep and beef pasture at Tokoroa and the dairy runoff at Wairakei, was not consistent with Stevenson *et al.* (2010), where average δ^{15}N was higher in dairy soils. However, this was probably because both of the drystock pastures were intensively managed like the dairy pastures.

3.4.1.1 Changes in soil C and N, and correlations with δ^{15}N

The increase in total C and N from the pine forests to the long-term pastures for the 0–75 mm samples (Fig. 3.2b, c), was consistent with results from the full profiles (Lewis *et al.*, in prep). Average accumulation rates for C and N during the first 10 years following conversion were 908 kg C ha^{-1} y^{-1} and 165 kg N ha^{-1} y^{-1}, which were within the range of values reported from other similar studies in New Zealand (Walker *et al.*, 1959; Jackman, 1964a, b; Hedley *et al.*, 2009; Schipper and Sparling, 2011). The decline in C:N ratio from forests or recently converted sites, to long-term pastures was also consistent with previous studies (Sparling and Schipper, 2004; Hedley *et al.*, 2009). Relatively high rates of N accumulation were probably due to high N inputs to the pasture systems from N fixation and N fertiliser, which coupled with the initially high C:N ratios of the forest soils, would have provided the ideal environment for net N immobilisation. However, caution is required when drawing conclusions about changes in total C and N stocks when only surface soils are sampled, because changes often occur throughout the profile (Schipper and Sparling, 2011).

The positive correlation between δ^{15}N and total N and the negative correlation with C:N (Fig. 3.3a, b) suggested that as soils became more 'saturated' with N, isotope fractionating N loss processes increased. The lack of any clear relationship between δ^{15}N and the C:N ratio when C:N was >13.6 (Fig. 3.3b), was probably because at higher C:N ratios isotope fractionating N loss processes would have been lower, due to N immobilisation in the soil. Alternatively N losses in soils with high C:N ratios could have been quite high, but the higher C:N ratios would have still led to a large amount of N immobilisation, and since fertiliser and fixed N generally have δ^{15}N values of ~0 ‰, immobilisation would tend to balance out ^{15}N enrichment due to isotope fractionating N losses. In a broader survey of New Zealand soils, Stevenson *et al.* (2010) also found a significant negative correlation between δ^{15}N and C:N, which suggests that the combination of total N, C:N ratio and δ^{15}N, could provide more information on the 'N status' or history of a system, than if only one of these variables were measured. For example, if a soil had high total N (relative to the soils capacity to store N), high δ^{15}N, and low C:N ratio, it would have probably been subject to high N inputs and losses (i.e. long-term pastures in the current study). A soil with low total N and low C:N ratio, but with high δ^{15}N, has likely lost organic N (i.e. soil from a long-term cultivated site, Stevenson *et al.*, 2010). In contrast, if a soil had low N, low δ^{15}N and high C:N ratio, it is likely from a natural ecosystem or an extensively managed pasture, with low N inputs and losses. These hypotheses need to be further explored.

3.4.1.2 Full soil profiles

The significant difference in A horizon δ^{15}N between the forest and pasture sites, but no difference for the two subsoil horizons (Fig. 3.4a), was consistent with other studies (Piccolo *et al.*, 1994a; Lemma and Olsson, 2006; Llorente *et al.*, 2010), and was presumably because processes affecting δ^{15}N (e.g. volatilisation and nitrification) were higher in the surface horizon (Piccolo *et al.*, 1994a).

Previous studies have shown, that in general, %N decreases and δ^{15}N increases with soil depth in both forest and pasture ecosystems (Steele and Wilson, 1981; Ledgard *et al.*, 1984; Piccolo *et al.*, 1996; Hobbie and Ouimette, 2009). The increase in δ^{15}N with depth is thought to occur because organic N at depth is older and more processed and therefore has had more opportunities for isotope fractionating N losses, and also because plants (particularly those associated with ectomycorrhizal fungi) tend to take up N which is depleted in ^{15}N relative to bulk soil N, and thus litter incorporated into the surface soil has low δ^{15}N (Hobbie and Ouimette, 2009). The trend of decreasing %N and increasing δ^{15}N with depth in the soil profiles under the pine forests (Fig. 3.4a, b), was therefore consistent with most other studies, but the lack of change in δ^{15}N with depth in the long-term pastures was not. However, a few other studies have also found little difference between topsoil and subsoil δ^{15}N in modified ecosystems, compared to more ‘natural’ systems where there was the typical increase in δ^{15}N with depth. For example, Eshetu and Högberg (2000), and Lemma and Olsson (2006) found a greater increase in soil δ^{15}N with depth under forests than under pastures or cropped land, and in a forest ecosystem Högberg *et al.* (1996) found that δ^{15}N in plots fertilized with high rates of N decreased with depth (from the top of the litter layer to 50 mm depth in the mineral soil), while in control plots which did not receive N there was the typical increase in δ^{15}N with depth (Table 3.3). In contrast, Piccolo *et al.* (1994a) found the opposite trend, with the surface soil of pastures being more depleted in ^{15}N relative to subsoils than in forests (Table 3.3). This was probably because of immobilisation of fixed N (with low δ^{15}N) in the surface soils of pastures.

These results suggest that differences between topsoil and subsoil δ^{15}N may be a useful indicator of past land management. Undisturbed or extensively managed sites with low N inputs and losses will likely show the typical increase in δ^{15}N with depth, while at more intensively managed sites there may be no change with depth, or even decreases in δ^{15}N. One advantage of using the difference between topsoil and subsoil δ^{15}N, is that it may be more applicable for comparing across soils which have different ‘baseline’ δ^{15}N values. Subsoil δ^{15}N in general appears to be less affected by landuse than surface soil δ^{15}N (except see Billings and Richter, 2006), and therefore the difference between the topsoil and subsoil could reflect the impact of management irrespective of the soils initial δ^{15}N value. An example of this can be seen in Table 3.3, where differences in mineral soil δ^{15}N between studies varied by up to 9 ‰, but differences between surface soil and subsoil was relatively consistent, ranging from 0–2.5 ‰, depending on land use. Further research on multiple soil types under similar management will be required to test this hypothesis.

The much higher δ^{15}N values (~7–13 ‰) reported by Eshetu and Högberg (2000), Lemma and Olsson (2006) and Piccolo *et al.* (1994a), than in the current study (~2–5 ‰) (Table 3.3), were presumably because the soils were older (thus had more time to become enriched) and were also from the tropics where N cycling tends to be more open, thus providing more opportunity for isotope fractionating N losses (Martinelli *et al.*, 1999).

Table 3.3 Surface soil and subsoil δ^{15}N, and the difference between the two depths under different land uses or treatments. For the first four studies, surface soil was from ~0–200 mm depth and subsoil from ~300–600 mm depth. In the last study (Högberg *et al.*, 1996) , the 'surface soil' was the top layer of litter and the 'subsurface soil' the top 50 mm of the mineral soil. Note: δ^{15}N values other than in the current study are approximate because they were read off graphs.

Land use or treatment	Surface soil δ^{15}N (‰)	Subsoil δ^{15}N (‰)	Surface soil - subsoil δ^{15}N (‰)	Reference
Plantation pine forest	2.63	4.14	-1.51	Current study
Pasture	4.29	4.09	0.2	
Native forest	7	9	-2	Eshetu and Högberg (2000)
Pasture	7.2	6.6	0.6	
Cultivated land	7.3	7.7	-0.4	
Native forest	7	9.2	-2.2	Lemma and Olsson (2006)
Cultivated land	8.3	9.0	-0.7	
Native forest	11.8[a]	12.6	-0.9	Piccolo *et al.* (1994)
Pasture	10.2	12.5	-2.3	
Un-fertilised forest plots	0.3	5.1	-4.8	Högberg *et al.* (1996)
Fertilised forest plots (urea)	3.1	3.5	-0.4	

[a] Oe and Oa layers were included in the surface mineral soil samples from the forests in this study.

3.4.2 *Podzol soil chronosequence*

While results from the chronosequences on the pumice soil supported the hypothesis that soil δ^{15}N would increase with time under pasture (Fig. 3.2a), there was no significant relationship between pasture age and surface soil δ^{15}N for the chronosequence on the podzol soil in Northland (Fig. 3.5a). This was somewhat surprising, particularly when there was a clear increase in total N (Fig. 3.5b) and a decrease in C:N ratio (Fig. 3.5d), similar to what was observed for the pumice soil chronosequences (Fig. 3.2b, d). There was also no relationship between pasture age and total C (Fig. 3.2c). The similarity of soil δ^{15}N and C data from within the same paddock (Fig. 3.5a, c), suggested that site specific factors were important, and the significant negative correlation between soil δ^{15}N and soil C, indicates that the same factors likely influenced both variables. Slope has been shown to influence soil δ^{15}N (Amundson *et al.*, 2003; Chapter 5), and topography at the Northland chronosequence was rolling, with samples taken from slopes ranging from 4° to 14°. However, there was no relationship between slope and δ^{15}N (or any of the other variables), which suggests that slope was not responsible for the variability in δ^{15}N (and C).

It is unclear exactly why surface soil δ^{15}N did not appear to be affected by pasture age, or why there was so much variability between sites. However, the variability within the undeveloped scrub sites (Fig. 3.5a), suggests that the differences between pasture sites were likely present prior to pasture establishment. One simple explanation for this variability, is that disturbance of the soil profile by gum diggers differed between sites, and sites with higher surface soil δ^{15}N were in areas where more subsoil (with high δ^{15}N, Fig. 3.4c) had been mixed into the surface horizon. Differences in profile mixing could have also occurred during cultivation when land was prepared for pasture (Edwards, 1997). High variability in surface soil δ^{15}N, may have also occurred because the podzol soil was old and weathered, and therefore even small differences in N cycling (due to differences in vegetation or soil properties) over long time periods could have led to large differences in δ^{15}N. For example, the soil was poorly drained, and therefore N losses via denitrification were probably high. Sites with low δ^{15}N may have been in areas where denitrification consumed all available nitrate, and therefore the strong isotope effect of denitrification was not expressed, while at sites with high δ^{15}N, complete denitrification may not have occurred, leading to enrichment of soil with ^{15}N (Houlton *et al.*, 2006). Furthermore, the location of large kauri trees in the previous forest would have also influenced long-term N cycling (Silvester, 2000), and Weber *et al.* (2008) found that δ^{15}N in surface soil increased significantly with distance from large trees in a mature pine forest (median δ^{15}N increased from 1.5 ‰ near the trunk to 2.8 ‰ beyond the canopy). This pattern was attributed to greater deposition of needles near the large trees, and needles were depleted in ^{15}N relative to the soil. This same pattern may have been present under the original kauri forest at the Northland chronosequence, and could have contributed to the variability in δ^{15}N between sampling sites. It is important to emphasise that the Wharekohe podzol is an extreme soil (strongly weathered), largely due to its formation under kauri forest, and therefore results could be relatively unique to this soil.

3.4.2.1 Changes in soil C and N

There was no change in soil C as pasture age increased in the Northland chronosequence, but N accumulated at about 110 kg ha^{-1} y^{-1} during the first 10 years following pasture establishment. This rate of N accumulation was lower than in the chronosequences on the pumice soil, possibly due to lower N inputs via fertiliser. However, Rumball (1979) measured N fixation by clover of 380 kg ha^{-1} y^{-1} in pastures at the Kaikohe Research Station, which suggests that there would have been ample N available for the observed rates of accumulation. The increase in N was confined to the top 75 mm of soil, as observed by Jackman (1964b) and Walker *et al.* (1959), but the C:N ratio in the 75 mm to E horizon samples declined with pasture age in a similar manner to the surface soil. Therefore, for the Northland chronosequence, total N and C:N ratio were a better indicator of time since conversion to pasture than soil C and δ^{15}N.

3.4.2.2 Full soil profiles

The general decrease in %N and increase in δ^{15}N with depth for both the undeveloped scrub site and the 30 year pasture site (Fig. 3.4c, d), was similar to profiles in chronosequences on the pumice soil (Fig. 3.4a, b). However, δ^{15}N values in the subsoil horizons were about 1–2 ‰ higher than for similar soil depths in the chronosequences on pumice soils (Fig. 3.4a). Higher δ^{15}N in lower horizons of the Northland chronosequence may have been because the soils were older (and hence had more time to become enriched, Piccolo *et al.*, 1994a), and drainage was also much poorer than the pumice soils and therefore denitrification (which can cause strong isotopic fractionation) may have been higher. The higher N content in the A horizon of the pasture site was very likely due to time under pasture, since this matches with the strong trend for the 0–75 mm samples, which were analysed for all 11 sites (Fig. 3.5b). However, the higher δ^{15}N in the A and E horizons of the scrub site may have just been a ‘site effect’, possibly related to different disturbance histories (see above). This suggestion is made because pasture age appeared to have no effect on soil δ^{15}N in the 0–75 mm samples (Fig. 3.5a).

3.5 Conclusion

The key hypothesis tested in this study was that soil δ^{15}N would increase with time following clearance of forest or scrub and conversion to pasture. Results from the three chronosequences on pumice soils in the Central North Island provided strong support for this hypothesis, with a clear increase in δ^{15}N in surface (0–75 mm) soils between pine forests (2 ‰) and long-term pastures (4.1 ‰). There was also a significant increase in total N and C, and a decrease in the C:N ratio in the surface soils. Analysis of full soil profiles under long-term pasture, and remaining forest at each of the three chronosequences, revealed a significant increase in δ^{15}N between the A horizon and subsoils of the pine forests, but no significant differences between depth increments in the long-term pastures (due to ^{15}N enrichment of the A horizon). These profile results suggested differences in δ^{15}N between the surface soil and subsoil may be a useful indicator of past land management, with the added advantage of potentially being more applicable for comparing across soil types with different 'baseline' δ^{15}N values. This hypothesis needs to be tested further.

In contrast to results from the chronosequences on the pumice soil, there was no clear relationship between pasture age and soil δ^{15}N for the native scrub-to-pasture chronosequence on the more extreme podzol soil in Northland, where site specific factors (such as previous disturbance by gum diggers) appeared to have had more of an impact on soil δ^{15}N than pasture age. Interestingly, total N increased significantly and C:N ratio decreased significantly with increasing pasture age, which was similar to what was observed for pumice soil chronosequences. Therefore, over all four chronosequences, total N and C:N ratio were the most reliable indicators of pasture age. However, δ^{15}N still provided useful additional information, with the increase δ^{15}N in the pumice soil chronosequences in particular indicating that a large amount of N must have been lost via isotope fractionating pathways (e.g. ammonia volatilisation), otherwise the net immobilisation of fixed or fertiliser N (with δ^{15}N values of ~0 ‰), in the soil would have resulted in a decrease in soil δ^{15}N. The combination of total soil N, C:N and δ^{15}N will provide the most information on long-term N dynamics at a particular site.

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4 Changes in soil δ^{15}N during multiple decades under pastures receiving differing amounts of superphosphate fertiliser and irrigation

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The contribution of authors was as follows:

> The two field trials were initiated and maintained by staff at AgResearch in Christchurch. Paul Mudge obtained archived soils from the trials and analysed them for natural abundance nitrogen isotopes. Paul Mudge collated field information on the two trials, and carried out data analysis with statistical support from Martin Upsdell. Paul Mudge wrote the manuscript which was primarily reviewed by Louis Schipper. Anwar Ghani and Troy Baisden also read the manuscript and provided suggestions for improvement.

Abstract

Intensification of pastoral agriculture has led to increased nitrogen (N) losses to the wider environment. This in turn has led to an emphasis on improving our understanding of soil N dynamics, and development of management practices which mitigate N losses. The natural abundance of the stable isotope ^{15}N relative to ^{14}N ($\delta^{15}N$) in soils can provide an integrated measure of past N cycle processes, and in particular soil $\delta^{15}N$ can reflect past N losses, because during most N transformation processes ^{14}N is preferentially lost. We therefore hypothesised that pastoral soils under intensive management regimes (with high N inputs, cycling and loss) would become progressively enriched with ^{15}N relative to soils under less intensive management. To test this hypothesis we analysed archived surface soil (0–75 mm) samples from two long-term grazed field trials in New Zealand. The trials were located at Winchmore on the Canterbury plains and different rates of irrigation and superphosphate fertiliser had been applied for ~50 years. In all treatments except one (a treatment receiving no fertiliser), $\delta^{15}N$ increased with time and the increase was greater in treatments receiving more superphosphate or irrigation (average increases ranged from ~0.015 to 0.034 ‰ per year). Pasture production and grazing intensity also increased with increasing fertiliser rate and irrigation frequency, and we found positive correlations between the average rate of change in soil $\delta^{15}N$, and average total pasture production ($r^2=0.77$, $p=0.02$), clover production ($r^2=0.95$, $p<0.001$) and calculated N losses ($r^2=0.98$, $p<0.001$) over ~50 years. These results provide strong support for our hypothesis that soil $\delta^{15}N$ is related to past management intensity and N cycling and loss processes. We suggest that most of the difference in $\delta^{15}N$ observed between treatments due to the influence that fertiliser and irrigation had on pasture production, and the flow on effects this had on isotope fractionating N loss processes. Soil $\delta^{15}N$ could therefore be a useful indicator of past management intensity and N cycling and loss from pastoral systems.

Keywords: *Isotope, natural abundance, grassland, grazed*

4.1 Introduction

Globally, livestock production systems have intensified to meet the food demands of a growing population, consuming an increasing proportion of animal based products (Steinfeld *et al.*, 2006). New Zealand has followed this global trend, with pastoral agriculture (the dominant form of agriculture in New Zealand) continuing to intensify since forest clearance 50–150 years ago (MacLeod and Moller, 2006). Intensification of New Zealand's pastoral systems has largely been made possible via the use of large amounts of superphosphate fertiliser which lifted the naturally low soil phosphorus (P) and sulphur (S) levels, and indirectly increased soil nitrogen (N) by promoting clover growth and thus enhancing N_2 fixation. In drier eastern regions, like the Canterbury plains irrigation has become common, and in recent decades N fertiliser use has increased considerably (Parfitt *et al.*, 2008). Intensification has led to increased N losses to the wider environment (air and water) and there is particular concern about increased N losses to waterways in sensitive and iconic catchments (e.g. Lake Taupo).

Concern over increasing N losses from pastoral agriculture in New Zealand (and internationally) has resulted in considerable research into new technologies and farming practices that can reduce N losses (e.g. nitrification inhibitors and stand-off pads, de Klein and Eckard, 2008). Research has largely focussed on reducing nitrate leaching and nitrous oxide emissions, with most studies being short term (<5 years) (e.g. Di *et al.*, 2009; de Klein *et al.*, 2011). Further research on soil N dynamics over longer time periods (i.e. decades) would help improve our fundamental understanding of N cycling in pastoral systems, which could be useful for the development of management practices and regulations aimed at reducing unwanted N losses (Richter *et al.*, 2007). The natural abundance of the stable isotope ^{15}N relative to ^{14}N ($\delta^{15}N$) in soils is considered to provide an integrated measure of past N cycle processes, and in particular soil $\delta^{15}N$ can reflect past N losses, because during most N loss processes (e.g. ammonia volatilisation and nitrification followed by denitrification or N leaching) ^{14}N is preferentially lost (Högberg, 1997; Robinson, 2001). Preferential loss of ^{14}N occurs because it is lighter than ^{15}N and reacts faster, and therefore in incomplete reactions, products (e.g. NO_3^-, NH_3, N_2 and N_2O) typically lost from soils are depleted in ^{15}N, while remaining substrate (e.g. NH_4^+ and organic N) becomes enriched.

Extensive research in forest ecosystems, has revealed that soils (or vegetation) at sites with high N inputs, cycling and losses are generally enriched with ^{15}N relative to sites with lower N inputs, cycling and losses (Högberg and Johannisson, 1993; Johannisson and Högberg, 1994; Högberg *et al.*, 1995; Martinelli *et al.*, 1999; Pardo *et al.*, 2006; Fang *et al.*, 2011). This has led to $\delta^{15}N$ being considered a useful indicator of N saturation or N leakiness in forest ecosystems (Högberg, 1990; Högberg *et al.*, 1996; Pardo *et al.*, 2006; Fang *et al.*, 2011). Less research has been conducted in grassland ecosystems, but similar results have been obtained, with soil $\delta^{15}N$ shown to be elevated at sites with high N inputs, cycling and losses (Frank and Evans, 1997; Watzka *et al.*, 2006; Kahmen *et al.*, 2008).

Previous work in New Zealand (Stevenson *et al.*, 2010) revealed significant differences in surface soil $\delta^{15}N$ between land uses; with indigenous forests having the lowest values (2.1 ‰), followed by exotic plantation forests (2.8 ‰), then pastures grazed by sheep and/or beef cattle (3.8 ‰), dairy pastures (5.4 ‰) and finally cropping soils (6.2 ‰). Stevenson *et al.* (2010) attributed differences in $\delta^{15}N$ between land uses to differences in management intensity and isotope fractionating N loss processes (e.g. ammonia volatilisation and nitrification followed by denitrification or nitrate leaching), which typically increase from indigenous forests through to cropped land. Elevated $\delta^{15}N$ in soils under dairy pastures compared to pastures grazed by sheep and beef cattle was likely related to higher grazing intensity (as a result of higher pasture production). Internationally there is little consensus on the influence of grazing intensity on soil $\delta^{15}N$, with some studies reporting increases in $\delta^{15}N$ with increasing grazing intensity (Frank and Evans, 1997; Hawke, 2001; Coetsee *et al.*, 2011), some no change (Neilson *et al.*, 1998; Han *et al.*, 2008; Wittmer *et al.*, 2011), and some decreases (Golluscio *et al.*, 2009; Xu *et al.*, 2010). Elevated soil $\delta^{15}N$ at more intensively grazed sites appears to be driven by greater N inputs, cycling and losses, with fractionating N losses from dung and urine patches being an important part of the enrichment process (Frank and Evans, 1997; Hawke, 2001;

Frank *et al.*, 2004; Stevenson *et al.*, 2010). No change in soil δ^{15}N under increasing grazing intensity could simply be because any changes are not large enough to detect against a large background pool of soil N (Johannisson and Högberg, 1994; Xu *et al.*, 2010), or that increases in fractionating N losses are balanced by accumulation of fixed N with low delta values (Ledgard *et al.*, 1984). No clear mechanism has been identified for the decrease in surface soil δ^{15}N at higher grazing intensities observed in some studies (Golluscio *et al.*, 2009; Xu *et al.*, 2010).

Most studies investigating soil δ^{15}N in grazed grasslands have taken samples at one point in time, with no studies tracking how δ^{15}N changes over multiple decades. Following evidence for systematic patterns of soil δ^{15}N across land uses in New Zealand (Stevenson *et al.*, 2010), we identified the potential for soil δ^{15}N to serve as an integrator of past N cycling in New Zealand-style pastoral systems where N inputs from industrial and urban sources via atmospheric deposition are negligible, and N fertiliser application is generally low and in the form of urea with an isotopic composition close to N fixation (~0 ‰). The objective of the current study was to improve our understanding of how soil δ^{15}N changes with time in pastoral systems, and determine if soil δ^{15}N could be related to past management intensity and N inputs, cycling and loss (here we define an increase in 'intensity' as increased above-ground biomass production and associated increases in grazing intensity, driven by greater external inputs such as fertiliser or irrigation). To achieve this objective, we analysed archived surface soil (0–75 mm) samples collected ~annually from two long-term grazed (by sheep) field trials in New Zealand, which had received different rates of superphosphate fertiliser or irrigation for ~50 years. Different rates of fertiliser or irrigation led to differences in total pasture production, clover production (thus N inputs via N_2 fixation), grazing intensity and calculated N losses, which allowed us to evaluate the hypothesis that pastoral soils under intensive management regimes (with high N inputs, cycling and loss) would become progressively enriched with ^{15}N relative to soils under less intensive management.

4.2 Methods

4.2.1 General site information

4.2.1.1 Location, soils and climate

The Winchmore Irrigation Research Station is located on the Canterbury Plains, South Island, New Zealand (43°47'S, 171°47'E). The soil at the site is the Lismore stony silt loam, classified as an Orthic Brown Soil (New Zealand Soil Classification) or Udic ustochrept (Soil Taxonomy) (McDowell and Rowley, 2008). The soil is well drained and formed on moderately weathered greywacke loess over gravels (Nguyen *et al.*, 1989). Between 1952 and 2009, mean annual rainfall and temperature were 733 mm and 11°C respectively. Rainfall was evenly distributed throughout the year, but water deficits often occurred during summer and autumn due to high evapo-transpiration losses (White *et al.*, 2008). The irrigation and fertiliser trials were located about 500 m apart and were completely independent of each other, although design and management was similar for each trial.

4.2.1.2 Irrigation system construction

Both trials were flood irrigated using the border dyke method, with water derived from the Rangitata River (Fitzgerald, 1970). Border strips were about 9 m wide by 100 m long and were individually fenced. The irrigation system was constructed in 1948 (irrigation trial) and 1949 (fertiliser trial), which involved ploughing the area to a depth of 175–200 mm, discing and then using a grader to move soil from near the edges of the border strips to create earth dykes 200–300 mm high between strips (Saville *et al.*, 1997). Soil was then moved from the centre of the strip to the edges to replace soil used to create the border. Overall, topsoil depth in the strips was reduced by 20–30 mm due to construction of the borders (Saville *et al.*, 1997). Following border construction, both trials were sown with a winter cereal greenfeed crop, then ploughed again in spring, left fallow over summer and sown to permanent pasture in autumn (in 1949 for the irrigation trial and in 1950 for the fertiliser trial).

4.2.1.3 Irrigation

Irrigation was scheduled based on gravimetric soil moisture content (0–100 mm depth), which was determined on soil cores prior to 1997 and then subsequently by time domain reflectometry (Srinivasan and McDowell, 2009). Irrigation occurred when soil moisture content fell below the trigger point for the respective treatments (Table 4.1), and about 100 mm of water was applied over a 50 minute period (Srinivasan and McDowell, 2009).

4.2.1.4 Pastures and grazing

Pasture species sown at the commencement of the trials were perennial ryegrass (*Lolium perenne*), short rotation ryegrass (*Lolium multiflorum*), cocksfoot (*Dactylis glomerata*), timothy (*Phleum pretense*), white clover (*Trifolium repens*), red clover (*Trifolium. pretense*) and subterranean clover (*Trifolium subterraneum*) (Rickard, 1968; Rickard and McBride, 1986). Species composition changed through time and was influenced by treatment. For example, weeds (defined here simply as anything that is not a grass or legume) and non sown grass species (e.g. *Agrostis capillaries*) became more common in the unfertilised and unirrigated treatments, and subterranean clover was the dominant clover in the unirrigated treatment, compared to white clover in all irrigated treatments (Rickard and McBride, 1986; Nguyen *et al.*, 1989).

Both trials were rotationally grazed by sheep, with a separate flock allocated to each treatment to avoid nutrient transfer via dung and urine. Grazing intensity varied depending on pasture production and was adjusted to achieve even and adequate control of growth in each treatment (Rickard and McBride, 1987). The trials were typically grazed by hoggets between 12 September and 1 February and then lambs until 10 May. However, the dryland treatment of the irrigation trial was usually ungrazed from 1 February until 10 April because of low pasture growth rates during this period (R. Moss Pers. Com. 2010). The trials were not grazed during winter (Rickard and McBride, 1987; McDowell and Rowley, 2008).

Table 4.1 provides a summary of trial dates, fertiliser and irrigation regimes and grazing intensity for the two trials. Further management details specific to the respective trials are described in the section 4.2.2.

Table 4.1 Summary of the two different trials used in this study. See section 4.2.2 for full site descriptions and explanation of management for each trial.

Trial	Trial dates	Treatment abbreviation	Fertiliser regime kg ha^{-1} y^{-1} single superphosphate	Irrigation regime[a]	Grazing intensity Stock units ha^{-1} [b]
Irrigation	1958–2002	Dryland	250	Not irrigated	9
		Irr10%	250	10 %	14
		Irr20%	250	20 %	16
Fertiliser	1952–2009	Fertcon	0	15 %	7
		Fertres	376–0–250[c]	15 %	14
		Fert376	376	15 %	16

[a] Irrigated when gravimetric moisture content of surface soil (0–100mm) fell below reported level.

[b] One stock unit is equivalent to one 55 kg ewe rearing a single lamb and consuming about 520 kg of good quality pasture dry matter per year (Statistics New Zealand, n.d.-b). Stocking rate was calculated using the stock unit definition above and mean annual pasture production (see Table 4.2) and assuming that 70 % of pasture grown was consumed by sheep.

[c] 376 kg ha^{-1} y^{-1} superphosphate from 1952–1958, no fertiliser from 1958-1980, then 250 kg ha^{-1} y^{-1} superphosphate or rock phosphate and elemental sulphur to achieve the same P and S application rates as 250 kg superphosphate from 1980–2009.

4.2.2 Details specific to each of the two trials

4.2.2.1 Superphosphate fertiliser trial

During the establishment phase of the fertiliser trial (1948–1952), 5000 kg ha^{-1} of lime and 376 kg ha^{-1} of superphosphate was applied to all border strips (Nguyen *et al.*, 1989). The fertiliser trial was initiated in 1952 on a two year old pasture which was ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) dominant (Rickard and McBride, 1987). There were four replicates of five treatments arranged in a randomised block design on 20 border strips. Samples from three treatments were analysed for this study: Fertcon (no fertiliser since 1952), Fert376 (376 kg superphosphate ha^{-1} y^{-1}) and a more complicated residual treatment (Fertres), where 376 kg superphosphate ha^{-1} y^{-1} was applied between 1952 and 1957, no fertiliser from 1958 to 1979 and in 1980 two border strips received 860 kg ha^{-1} of superphosphate and the other two strips received 750 kg ha^{-1} of a rock phosphate (Sechura) and gypsum ($CaSO_4$) mix. From 1981 to 2009 two strips in the Fertres treatment received 250 kg ha^{-1} y^{-1} of superphosphate while the other two strips received 170 kg ha^{-1} y^{-1} of rock phosphate and 27 kg of elemental sulphur. Despite the differences in fertiliser form after 1980 on the Fertres treatment, total P and S inputs were the same on all four replicates.

In 1972 a further 4400 kg ha^{-1} of lime was applied to all treatments (Rickard and McBride, 1987). In August 1980, border strips in the Fertres treatment were deliberately heavily trampled by a flock of sheep to break up the pasture (which contained large proportions of moss, weeds and weed grasses) and then all strips were direct drilled with ryegrass and white clover seed (Rickard and McBride, 1987). All treatments were irrigated when gravimetric soil moisture content in the top 100 mm was 15 %, and on average the trial was irrigated 4.3 times per year (McDowell and Rowley, 2008).

Due to restricted area during construction of the border dyke system, one replicate border strip of the Fertcon treatment (strip 20) was much smaller than the others (just over half the size). The smaller size meant that this strip received less irrigation, tended to be grazed harder and grew more drymatter than other strips in the Fertcon treatment (R. Moss pers. comm. 2010). The $\delta^{15}N$ values from strip 20 were always higher (~0.75 ‰ higher) than the other three strips in the Fertcon treatment which all had similar values. Indeed, values for strips within the same treatment for both the fertiliser and irrigation trials were very similar. This suggests that something during the construction of border strip 20 and/or subsequent management differences due to its smaller size affected soil $\delta^{15}N$. Therefore strip 20 was excluded from isotopic analysis.

4.2.2.2 Irrigation trial

An initial irrigation trial was established in 1949, with six treatments allocated to 24 border strips in a randomized block design (Rickard, 1972). The treatments were (1) no irrigation, (2) weekly irrigation, (3) fortnightly irrigation, (4) three weekly irrigation, (5) six weekly irrigation and (6) three weekly irrigation but only in alternate years. In 1954 it was recognised that irrigating weekly or every two weeks was too frequent, and therefore from 1954 to 1957 the weekly and fortnightly treatments were changed and were irrigated when gravimetric soil moisture (0–100 mm) declined below 20 % and 10 % respectively (Rickard, 1972). The initial trial with six treatments ceased in 1957, and the entire area was cultivated using a rotary hoe and grubber in mid-November, and pasture was re-sown in March 1958.

The long-term irrigation trial reported on in this study commenced in 1958 with five treatments (four irrigation treatments and one dryland treatment) applied to the 24 border strips. There were five replicates of the irrigation treatments and four of the dryland treatment. Dryland replicates were applied to border strips that had previously been in the dryland treatment, while the irrigated treatments were randomly allocated to the previously irrigated strips. Samples were analysed from the dryland treatment (Dryland) and treatments irrigated when gravimetric soil moisture (0–100 mm) was 10% (Irr10%) and 20 % (Irr20%) respectively. For the Lismore soil, 10 % and 20 % gravimetric soil moisture are about wilting point and 50 % of plant available water respectively (Rickard and McBride, 1986). On average the Irr10% treatment was irrigated about 2.6 times per year while the Irr20% treatment was irrigated about 7.7 times per year (Rickard and McBride, 1986; Srinivasan and McDowell, 2009). All treatments received 250 kg ha^{-1} of superphosphate annually, and lime was applied in 1948 (5000 kg ha^{-1}), 1953 (1900 kg ha^{-1}) and 1965 (4100 kg ha^{-1}) (Rickard, 1972; Rickard and McBride, 1986). The last available soil sample from the irrigation trial was taken in 2002. For the irrigation trial, samples from only four of the five replicates of the Irr10% and Irr20% treatments were analysed, to match with the four replicates of the Dryland treatment.

4.2.3 Soil and pasture sampling

Replicate soil samples from individual border strips were available from 1958 – 2009 for the fertiliser trial and 1959–2002 for the irrigation trial. Soil samples were generally collected seasonally, with 15 cores (25 mm diameter x 75 mm deep) taken in a zig-zag pattern down the length of the border strips. Samples were taken no closer than 1.5 m from the centre of the borders (R. Moss Pers. Com. 2010). The 15 cores from the same strip were bulked, air dried, passed through a 2 mm sieve and then archived at room temperature. Samples taken during winter (usually July), at approximately four year intervals were selected for analysis in this study.

Above ground pasture production has been measured for the duration of both trials using the double trim exclusion cage technique, and botanical composition of the pastures has also been determined (for more details see Schipper *et al.*, in press). Archived pasture samples were not available, but a one off sampling from the fertiliser trial was undertaken in May 2011 to get an indication of the δ^{15}N of above ground plant material (the irrigation trial had finished so no pasture samples could be taken). Subsamples were taken from two temporary grazing exclusion cages in each border strip (the same cages used to measure annual pasture production), and dissected into legumes, grasses and weeds. These samples were dried at 60 °C, weighed to determine the proportion each component made up of total pasture on a dry matter basis, and then fine ground and analysed for δ^{15}N.

4.2.4 *Sample preparation and analysis*

A representative subsample of the archived soil samples was obtained by passing the whole sample through a stainless steel riffle (which split the sample) until the desired subsample size was reached (about 5 g). Any obvious plant material was then removed with tweezers before fine grinding using an agate mortar and pestle. Samples were analysed for N isotopes using a Europa Scientific 20-20 Stable Isotope Analyser at the University of Waikato Stable Isotope Unit. The standard deviation of nine repeat measurements of a soil sample from the fertiliser trial was 0.02 ‰, and overall reproducibility of laboratory measurements is ~0.2 ‰. The natural abundance of ^{15}N is expressed in delta notation (δ) as parts per thousand (‰) using the following equation (after Coplen, 2011):

$$\delta^{15}N = R(^{15}N/^{14}N)_{sample} - R(^{15}N/^{14}N)_{air} - 1$$

where R $(^{15}N/^{14}N)_{sample}$ is the ratio of $^{15}N/^{14}N$ in the sample and $R(^{15}N/^{14}N)_{air}$ is the ratio of $^{15}N/^{14}N$ in atmospheric N_2 (the international standard).

4.2.5 *Statistical analysis*

Time series δ^{15}N data from the two trials was analysed using a Bayesian smoothing program (Upsdell, 1994) to determine if there were temporal changes in δ^{15}N, and whether δ^{15}N differed between treatments. The time series were modelled as doubly integrated wiener processes, which corresponds to fitting cubic splines. Terms for Date*Treatment were used to estimate different Treatment time series. The individual paddock time series were modelled with terms Plot/Date, which accounts for the correlations induced by measuring the same plot repeatedly. A random effect for the effect of the day of sampling was also added (DayEffect). The block term was found to be negligible in a preliminary model which included it and so was left out of the model. The full model fitted for the two trials was Y = Date*Treatment + Plot/Date + DayEffect, where the operators have the same meaning as in Genstat model statements. Using a mixed model smoother allows us to extract estimates for the Date curves for each of the treatments after allowing for the effects of the nuisance variables Plot and DayEffect. Standard Analysis of Variance in GenStat v 13 was used to analyse the 2011 pasture data from the fertiliser trial, and simple linear regression in GenStat v 13 was used to explore the relationship between the average rate of change in soil δ^{15}N with time and pasture production, clover production and N losses.

4.3 Results

4.3.1 Pasture production and N balances

To understand plant and soil N isotope systematics, we compiled the best available information on N dynamics for the Winchmore trials. Fertiliser application and irrigation significantly increased average annual pasture production, with production in the Fertcon treatment being only 41 % of that in the Fert376 treatment and the Dryland treatment produced only 57 % of the pasture in the Irr20% treatment (Table 4.2). The increase in total pasture production with increasing P application or irrigation frequency would have been directly influenced by reduced P deficiency or moisture limitation, but also indirectly via reduced N limitation, because clover production (thus N_2 fixation) was enhanced by P application and irrigation. Nitrogen fixation was calculated from clover production (see footnote Table 4.2) and was much lower in the Fertcon treatment (30 kg N ha^{-1} y^{-1}) than in the other treatments (116–159 kg N ha^{-1} y^{-1}) which all received P fertiliser (Schipper *et al.*, in press, Table 4.2). N immobilisation rate in the surface soil ranged from 11 to 30 kg N ha^{-1} y^{-1} and was lowest in the Irr20% treatment and highest in the Fertres treatment (Schipper *et al.*, in press, Table 4.2). Calculation of a simple N budget (N fixation – immobilisation) indicated that total N losses from the top 75 mm of soil were considerably higher in treatments receiving higher rates of fertiliser or irrigation, ranging from 9 kg N ha^{-1} y^{-1} in the Fertcon treatment to 148 kg N ha^{-1} y^{-1} in the Irr20% treatment (Table 4.2). Loss pathways would have included gaseous emissions (i.e. ammonia volatilisation and dentrification), N leaching, export in product (meat and wool) and transfer of N in dung and urine to plot margins or races outside the plot area.

Table 4.2 Average annual above-ground total and clover dry matter (DM) production, along with calculated N fixation, N immobilisation and N loss for treatments in the irrigation and fertiliser trials. Pasture production, N fixation and N immobilisation values are from Schipper *et al.* (in press).

Treatment	Total pasture production	Clover production	N fixation [a]	N immobilisation	N loss from top 0–75 mm of soil [b]	Percentage of fixed N immobilised in the top 75 mm
	t DM ha^{-1}	t DM ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	%
Fertcon	4.99	0.49	30	21	9	70
Fertres	10.03	2.10	129	30	99	23
Fert376	12.18	2.47	151	27	124	18
Dryland	6.90	1.93	116	18	98	15
Irr10%	10.08	2.41	147	16	131	11
Irr20%	12.05	2.65	159	11	148	7

[a] Calculated from clover DM production using the following equation:

$N_{fixation}$ = clover DM yield x clover %N x %N fixed x whole plant conversion factor (1.7). Clover %N was assumed to be 5 % (Ledgard *et al.*, 1996), the %N fixed 72 % (Ledgard, 2001), and the whole plant conversion factor of 1.7 was used to account for N fixed in stolons and roots (Jørgensen and Ledgard, 1997).

[b] Calculated as fixation – immobilisation

4.3.2 Changes in soil δ^{15}N

Soil δ^{15}N was similar (~3.4 ‰) in all three treatments of the Winchmore fertiliser trial in 1958 (six years after the trial commenced), and began to diverge soon after (Fig. 4.1a). The δ^{15}N values in the Fert376 treatment increased gradually with time and by 2009 was 4.56 ‰. In the Fertcon treatment δ^{15}N declined to ~2.8 ‰ by 1990, and then increased slightly (though not significantly). The δ^{15}N values in the Fertres treatment was intermediate between the other two treatments, staying constant until around 1980 and then increasing rapidly to be similar to Fert376 by 2009 (Fig. 4.1a). The increase coincided with re-commencement of fertiliser application which increased pasture production. Pasture production in the Fertres treatment had declined gradually after 1958 when fertiliser application stopped, but after fertilisation recommenced in 1980 pasture production in the Fertres and Fert376 treatments was very similar (data not shown). At the start of the Winchmore irrigation trial, δ^{15}N was ~3.2 ‰ in all treatments and there was no significant change in δ^{15}N with time for the about first 10–15 years in any of the treatments. The δ^{15}N values then diverged over time, increasing more in the Irr20% treatment than the Irr10% treatment which in turn increased more than the Dryland treatment (Fig. 4.1b). By 2002, δ^{15}N had increased to 3.87, 4.36 and 4.66 ‰ in the Dryland, Irr10% and Irr20% treatments respectively (Fig. 4.1b).

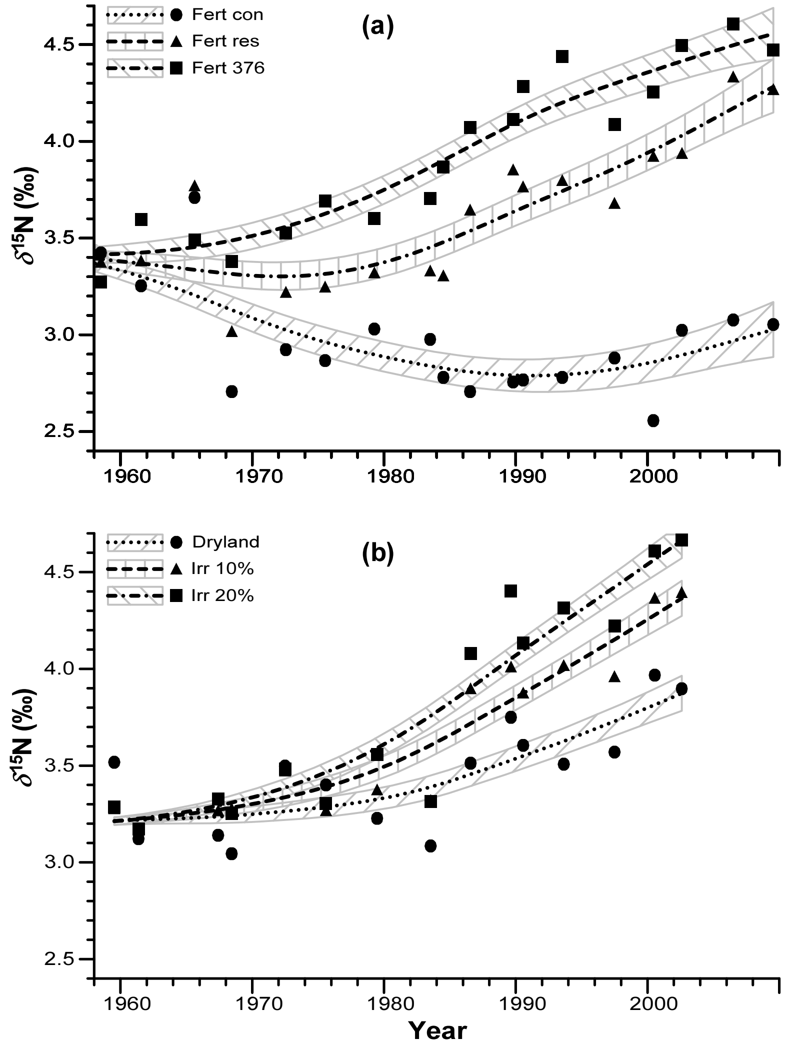

Fig. 4.1 Change in soil δ^{15}N with time in the Winchmore (a) fertiliser and (b) irrigation trials. Symbols represent treatment means, and dotted lines are predicted treatment means using a Bayesian smoother (Upsdell, 1994). Hatched bands are 95 % least significant intervals and treatments are significantly different ($p<0.05$) if bands do not overlap.

4.3.3 *δ^{15}N of pasture samples taken in 2011*

Pasture samples taken in 2011 (from the fertiliser trial) all had lower δ^{15}N values than the soils they were growing on (Fig. 4.2) (the irrigation trial had finished by 2011, so no samples were available). Clover δ^{15}N was significantly lower than grasses and weeds, and there was no difference in clover δ^{15}N between treatments (average –1.18 ‰). This suggests that the majority of clover N was derived from fixation because soil δ^{15}N differed between treatments (Fig. 4.1a). Average δ^{15}N of grasses and weeds (non N_2 fixing) was significantly lower in the Fertcon treatment (–0.45 ‰) than in the Fertres and Fert376 treatments (1.16 ‰) (Fig. 4.2), consistent with lower soil δ^{15}N in the Fertcon treatment (Fig. 4.1a). The weighted average δ^{15}N of all pasture components was also significantly lower in the Fertcon treatment than the other two treatments (Fig. 4.2). The difference between grass and 2009 soil δ^{15}N was about –3.3 ‰, and did not differ between treatments.

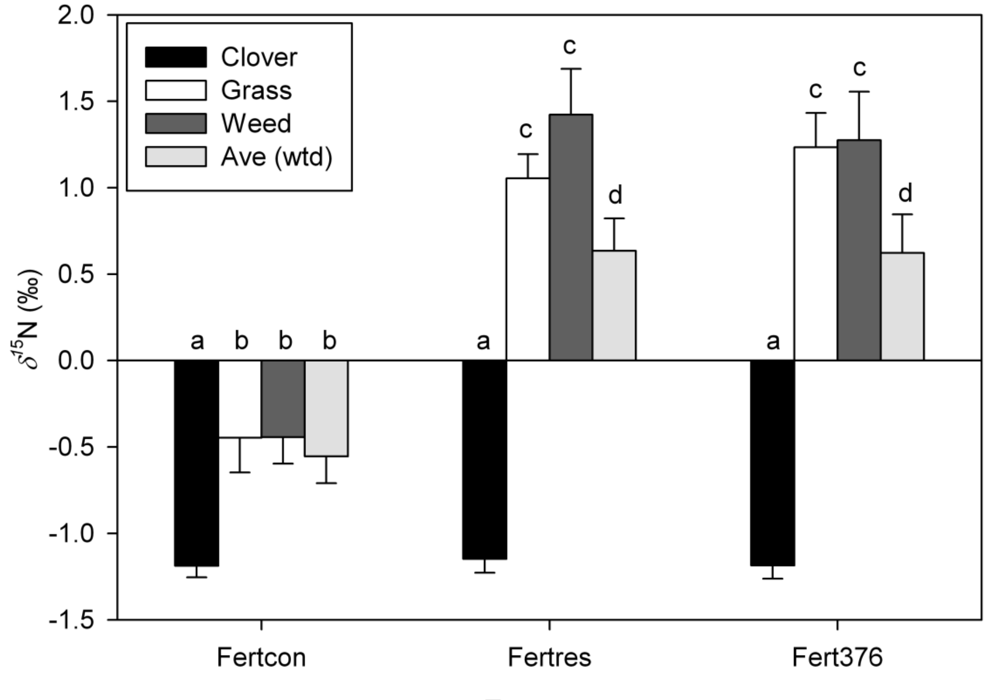

Fig. 4.2 δ^{15}N of clover, grasses, weeds and a weighted average (wtd) of all pasture components in the three treatments of the fertiliser trial. Samples were taken in May 2011. Note: a 'weed' here is classified as anything that is not a grass or legume. Bars with the same letter above are not significantly different (p>0.05). Error bars are ± 1 standard error.

4.3.4 Relationship between soil $\delta^{15}N$, pasture production and N losses

Using data from both the irrigation and fertiliser trials, there was a significant positive correlation between the average rate of change in δ^{15}N per year (or final δ^{15}N, not shown) and mean annual pasture production, clover production, and calculated N losses (Fig. 4.3). Since stocking rate was matched to pasture production, the relationship between change in δ^{15}N and stocking rate, would probably be very similar to the relationship between change in δ^{15}N and pasture production (i.e. Fig. 4.3a). Similarly, the relationship between change in δ^{15}N and clover production (Fig. 4.3b), would probably be similar to the relationship with N_2 fixation (the main N input to these pasture systems), since N fixation is positively correlated with clover production (Ledgard *et al.*, 1996).

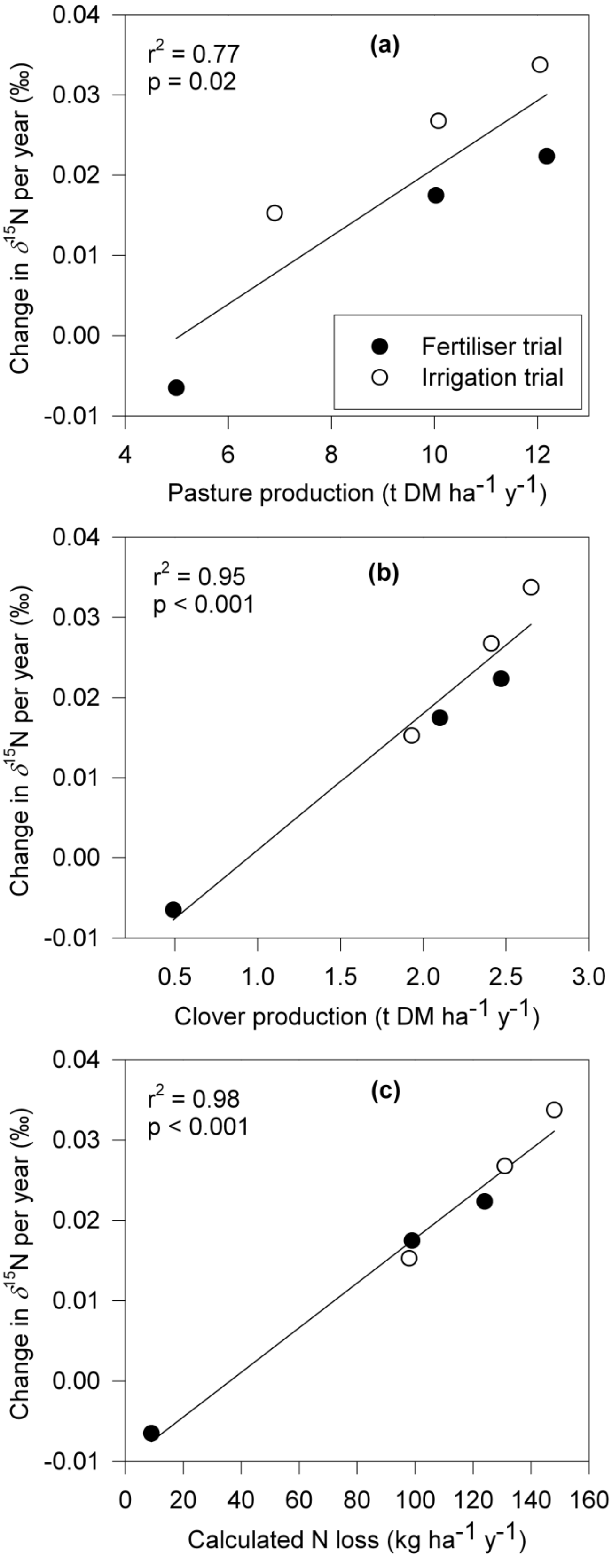

Fig. 4.3 Relationship between the average rate of change in δ^{15}N per year in the Winchmore fertiliser and irrigation trials and (a) mean annual pasture production, (b) clover production and (c) calculated N losses from the top 0-75 mm of soil (N fixation – N immobilisation). The regressions were similar if δ^{15}N values from the end of the two trials were used. Plotting as a 'rate of change' normalizes for the different lengths of the two trials.

4.4 Discussion

Results from the Winchmore irrigation and fertiliser trials supported our hypothesis that pastoral soils under more intensive management regimes (i.e. those receiving high external inputs of fertiliser or irrigation) would become progressively enriched with ^{15}N relative to soils under less intensive management (Fig. 4.1a, b). These results are also in agreement with Stevenson *et al.* (2010) who found higher soil δ^{15}N under intensively grazed dairy pastures than in more extensively grazed sheep and beef pastures in New Zealand. Elevated δ^{15}N in treatments receiving higher rates of fertiliser or irrigation was most likely related to increased clover and total pasture production, which led to increased grazing intensity and greater N inputs, cycling and losses (Table 4.2).

4.4.1 Comparison between actual and theoretical δ^{15}N values

Knowing the approximate amount and isotopic value of N inputs to the treatments, combined with the amount of N immobilised in the soil (and initial and final soil δ^{15}N), allowed us to calculate theoretical soil δ^{15}N values and compare these to actual values, from which we could infer differences in N cycling and loss between treatments. Biological N_2 fixation (calculated from clover abundance) ranged from 30 kg ha^{-1} y^{-1} in the Fertcon treatment to 159 kg ha^{-1} y^{-1} in the Irr20% treatment. N deposition in New Zealand is generally low (<5 kg ha^{-1} y^{-1}, Parfitt *et al.*, 2008), and therefore fixed N_2 with a δ value of about –1.18 ‰ (see Fig. 4.2), would have been the major N input to these pasture systems. All treatments accumulated N in the top 75 mm of soil (Table 4.2), and therefore it might have been expected that δ^{15}N would have declined to a value more similar to fixed N. In theory, if no isotope fractionation had occurred during N loss (i.e. immobilised N had a δ value of –1.18 ‰), all treatments would have had values of between 1.7 and 2.5 ‰ at the last sampling (Table 4.3). However, δ^{15}N increased in all treatments except the Fertcon treatment. For δ^{15}N to increase, ^{14}N must have been preferentially lost from the top 75 mm of soil and heavier ^{15}N retained, and this effect must have been greater in the more intensive treatments. This assertion is supported by calculated δ^{15}N values for immobilised N, which increased with increasing fertiliser application rates and irrigation frequency (see Table 4.3). The small decline in soil δ^{15}N in the Fertcon treatment was probably because a large proportion of fixed N (with a δ^{15}N value of about –1.18 ‰) was immobilised in the soil, and this caused a greater dilution effect than enrichment caused by isotope fractionating N losses (Ledgard *et al.*, 1984; Gubsch *et al.*, 2011). In the other treatments, enrichment due to isotope fractionating N losses must have been greater than any dilution effect.

Calculated δ^{15}N values for N lost from the surface soil were all negative, with the most negative value in the Fertcon treatment, which also had the lowest δ^{15}N value for immobilised N (Table 4.3). It might seem logical that the treatment losing the N most depleted in ^{15}N, would conversely have immobilised N most enriched with ^{15}N. However, this was not the case, probably because as illustrated in Fig. 4.4, the proportion of substrate consumed (in this case the proportion of fixed N lost) also has a large influence on the δ^{15}N of remaining substrate (in this case immobilised N). It is interesting that the relationship between the proportion of fixed N lost and the δ^{15}N values of immobilised N and lost N, show the same general pattern as the theoretical relationship for a single isotope fractionating process in a closed system (Fig. 4.4). This similarity is somewhat surprising, given that the data from the current study was calculated from field scale measurements, made across multiple independent treatments. This suggests that at least in some instances, isotope fractionation kinetics that apply to single processes (in closed systems) may also be applicable at ecosystem scales, where various N fractionation and mixing processes are operating. However, the model and calculations underlying Fig. 4.4 are probably an oversimplification of the system, and future studies need to directly measure δ^{15}N of N inputs and outputs to provide confidence that the tends observed in the current study are indeed correct.

Table 4.3 Actual and theoretical δ^{15}N values at the end of the fertiliser and irrigation trials. $\delta^{15}N_{end\_actual}$ is the end point of the curves of the respective treatments in Fig. 4.1a and b, $\delta^{15}N_{end\_theory}$ is theoretical δ^{15}N if all immobilised N had a δ value of –1.18 ‰ (i.e. the same as fixed N), $\delta^{15}N_{immob\_N}$ is the net δ value of immobilised N required to give the observed values at the end of the trials ($\delta^{15}N_{end\_actual}$), and $\delta^{15}N_{lost}$ is the calculated δ value of N lost from the system. Equations for the calculations are given below the table.

Treatment	$\delta^{15}N_{end\_actual}$	$\delta^{15}N_{end\_theory}$	$\delta^{15}N_{actual–theory}$	$\delta^{15}N_{immob\_N}$	$\delta^{15}N_{lost}$
Fertcon	3.03	2.04	0.99	2.22	-9.17
Fertres	4.28	1.76	2.52	5.89	-3.33
Fert376	4.56	1.88	2.67	6.83	-2.91
Dryland	3.87	2.37	1.50	6.64	-2.58
Irr10%	4.36	2.43	1.93	9.71	-2.53
Irr20%	4.66	2.61	2.05	13.81	-2.34

$$\delta^{15}N_{end\_theory} = ((N_{start} * \delta^{15}N_{start}) + (N_{immob} * \delta^{15}N_{immob\_N})) / N_{end}$$

$$\delta^{15}N_{immob\_N} = ((N_{end} * \delta^{15}N_{end\_actual}) - (N_{start} * \delta^{15}N_{start})) / N_{immob}$$

$$\delta^{15}N_{lost} = ((N_{fix} * \delta^{15}N_{fix}) - (N_{immob} * \delta^{15}N_{immob})) / N_{lost}$$

where N_{start} and $\delta^{15}N_{start}$ refer to the mass of soil N and δ^{15}N values at the start of the trials, N_{end} and $\delta^{15}N_{end}$ the mass of soil N and δ^{15}N values at the end of the trials, N_{immob} and $\delta^{15}N_{immob\_N}$ the mass of N and δ value of N immobilised during the trials, N_{fix} and $\delta^{15}N_{fix}$ the mass of N and δ value of N fixed during the trials, and N_{lost} and $\delta^{15}N_{lost}$ the mass of N and δ value of N lost during the trials.

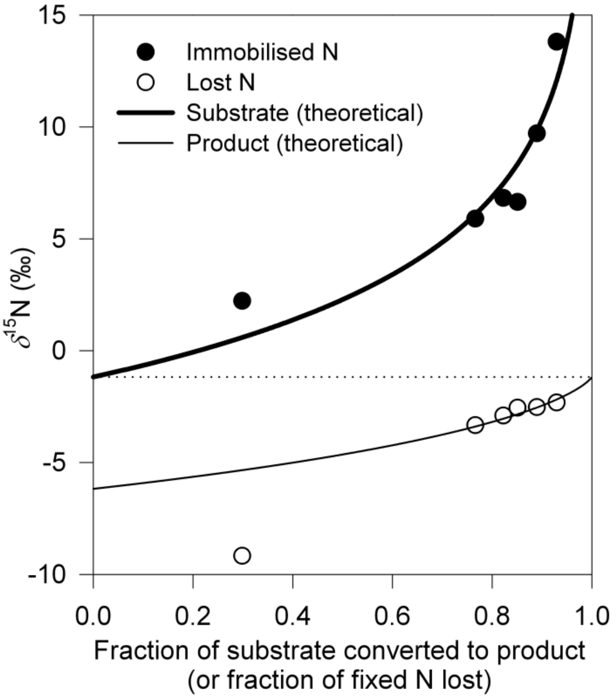

Fig. 4.4 Theoretical δ^{15}N values for cumulative product and remaining substrate in a closed system for a process with an isotope fractionation factor of 5 (redrawn from Robinson, 2001). Also shown are the calculated values for immobilised N (substrate) and N lost (cumulative product) for the Winchmore irrigation and fertiliser trials. 'Lost N' is simply defined here as fixed N – N immobilised in the top 75 mm of soil. The horizontal dotted line represents the isotopic signature of N input (fixed N) which was –1.18 ‰ (i.e. the delta value of clover, see Fig. 4.2).

4.4.2 Key processes influencing soil $\delta^{15}N$

There are a number of processes which could have influenced soil δ^{15}N in the different treatments, but we suggest that processes related to differences in clover and total pasture production and the flow on effects to stocking rates and N cycling and loss processes will have had the biggest effect. This assertion is supported by the significant positive correlations between the rate of change in δ^{15}N per year and total pasture production, clover production (thus N inputs) and calculated N losses (Fig. 4.3). The relationships between clover production (thus N inputs) and calculated N losses, were qualitatively similar to relationships observed in other grasslands (Watzka *et al.*, 2006; Stevenson *et al.*, 2010) and also forest ecosystems (Högberg and Johannisson, 1993; Johannisson and Högberg, 1994), but correlations with total pasture production have not been reported in other studies, although elevated δ^{15}N at sites with higher grazing intensities has been observed (Frank and Evans, 1997; Hawke, 2001; Stevenson *et al.*, 2010; Coetsee *et al.*, 2011). The increase in calculated N losses (in both trials) with increasing pasture production and stocking rates (Table 4.2), is consistent with other studies where N losses have been directly measured (e.g. Ledgard *et al.*, 1999; Monaghan *et al.*, 2005). In grazed systems, one of the key areas of N loss is from patches of animal excreta (dung and urine) where isotope fractionating N loss processes (e.g. ammonia volatilisation, and nitrification followed by denitrification or N leaching) can be high (Ledgard, 2001; Di and Cameron, 2002; Frank *et al.*, 2004; Saggar *et al.*, 2004). In a natural grassland, Frank and Evans (1997) found that soil δ^{15}N was higher at grazed than ungrazed sites, and at the grazed sites ^{15}N enrichment was positively correlated with grazing intensity (determined from dung abundance). Soil δ^{15}N under dung and urine patches was significantly higher than adjacent areas without dung or urine. Subsequent research (Frank *et al.*, 2004) revealed that preferential loss of ^{14}N via volatilisation from urine patches was one of the key mechanisms driving the ^{15}N enrichment of soils in this natural system. Similarly, Hawke (2001) found that soil δ^{15}N was elevated at stock campsites (where N inputs via dung and urine are high) compared to non-campsite areas, and Coetsee *et al.* (2011) found marginally higher soil δ^{15}N in intensively grazed lawns, compared to adjacent tall grass areas in an African Savannah. At Winchmore, higher clover and total pasture production in treatments receiving more fertiliser or more frequent irrigation, led to higher grazing intensities, which in turn would have led to increased excreta deposition. Therefore the differences in excreta deposition and associated isotope fractionating N losses, was likely a key driver of the divergence in δ^{15}N between treatments with time at Winchmore.

4.4.3 Other factors possibly influencing soil $\delta^{15}N$

Differences in pasture production and stocking rates would have also influenced N cycling and loss pathways other than those associated with excreta deposition. Higher stocking rates in the fertilised and irrigated treatments would have led to greater N export in products (meat and wool). For example, the OVERSEER® nutrient budgeting model (Wheeler *et al.*, 2006), developed for New Zealand pastures, predicted that annual N removal in product would have been about 6 kg ha^{-1} in the Fertcon treatment compared to14 kg ha^{-1} in the Fert376 and Irr20% treatments (the other treatments had intermediate values). Animal tissue is usually enriched with ^{15}N relative to diet (Steele and Daniel, 1978; Wittmer *et al.*, 2011), and therefore N export in product would have been expected to cause depletion of remaining ecosystem components, and depletion should have been greater in treatments with higher pasture production and stocking rates. However, the reverse occurred and therefore other isotope fractionating loss processes (e.g. volatilisation, and nitrification followed by N leaching or denitrification) must have been greater than any depletion caused by product export (probably because only a relatively small amount of N was exported in product, and the fractionation factor between forage and grazer tissue is relatively small (~2–5‰, Sponheimer *et al.*, 2003; Murphy and Bowman, 2006; Wittmer *et al.*, 2011)).

Different rates of fertiliser or irrigation could have also influenced soil δ^{15}N in other ways. In the fertiliser trial, addition of phosphorus (P) and sulphur (S) in superphosphate may have directly increased microbial activity and rates of N cycling (and thus opportunities for isotopic fractionation) in the Fert376 and Fertres treatments relative to the Fertcon treatment (Högberg *et al.*, 1992; Inglett *et al.*, 2007). Research in wetland and mangrove ecosystems has shown that plant foliage can be very depleted in ^{15}N when P is limiting, compared to when P is not limiting (McKee *et al.*, 2002; Clarkson *et al.*, 2005; Inglett *et al.*, 2007), which matches with the lower δ^{15}N of plants and soils of the Fertcon treatment relative to the fertilised treatments (Fig. 4.1a & Fig. 4.2). Lower foliage δ^{15}N when P is limiting is thought to occur because when P is limiting, N is in relative abundance and therefore plants tend to fractionate against the heavy isotope during uptake, while when N is less abundant all available N is assimilated and therefore there is no net fractionation (Evans, 2001; Inglett *et al.*, 2007). However, it is unlikely that this process would have caused the lower soil δ^{15}N in the Fertcon treatment. First, P or N limitation is seldom as extreme in agricultural systems as in natural ecosystems and therefore any effect is likely to be smaller. And second, even if there was isotopic fractionation during plant uptake, this will not necessarily correspond to net changes in soil δ^{15}N because most of the plant N will be returned to the soil at a later date. Therefore as mentioned earlier, the most likely cause for the slight decrease in δ^{15}N in the Fertcon treatment, was immobilisation of fixed N with low delta values. Ledgard (1989) found that rate of P addition had no effect on δ^{15}N of white clover (*Trifolium repens*) shoots or roots, which is consistent with what we observed (see Fig. 4.2), and suggests that differential applications of P in the fertiliser trial is unlikely to have affected the δ^{15}N value of fixed N (the main N input to these pastures).

In the irrigation trial, more frequent irrigation probably increased N mineralisation rates (particularly during the dry summer months), which would have likely contributed to the increased N losses from the system (Table 4.2). This suggestion is supported by the lower total N observed in the Irr20% treatment (see Schipper *et al.*, in press). Mineralisation itself causes virtually no isotopic fractionation (Högberg, 1997), but higher mineralisation rates would provide a larger pool of NH_4^+ for further transformation and loss processes (e.g. nitrification, then denitrification or leaching), which could cause further fractionation. Irrigation could have also directly influenced denitrification, with the anaerobic conditions required for denitrification potentially occurring more often in the more frequently irrigated treatments (Phillips *et al.*, 2007). However, the soil was well drained, and therefore prolonged periods of saturation are unlikely to have occurred and so any effect would have probably been small. Differences in irrigation frequency could have also slightly influenced the δ^{15}N of fixed N. In a hydroponic glasshouse experiment Ledgard (1989) found that white clover plants under water stress had slightly lower δ^{15}N than those not under stress (–0.63 vs –0.38 ‰

respectively). Therefore the fixed N in the Dryland treatment may have been slightly depleted in ^{15}N relative to the irrigated treatments, which is consistent with the lower soil δ^{15}N observed in the Dryland treatment (Fig. 4.1b).

4.4.4 Implications

The differences in δ^{15}N between treatments in this study appear to mirror differences in the intensity of pastoral agriculture, with differences most likely caused by differences in the amount of N being lost via isotope fractionating pathways (e.g. ammonia volatilisation). We have studied two specific and unique long-term experiments, but the results should be generalizable to a wide range of pastoral ecosystems, with soil δ^{15}N potentially providing a simple integrated measure of past land use intensity and N cycling and loss processes. It is worth noting that the absolute difference in δ^{15}N between treatments in both trials was relatively small (between ~0.3 and 1.5 ‰), and under the management (and climatic) regimes of the two trials, differences would only be detectable at decadal time scales. The trials in this study were exclusively grazed by sheep and no N fertiliser was applied. Many pastoral systems in New Zealand (and internationally) are now much more intensive than these two trials, with N fertiliser application rates of 100–200 kg ha^{-1} y^{-1} being relatively common (particularly on dairy farms). Total N input, cycling and loss would almost certainly be higher in more intensive systems and we suggest that changes in soil δ^{15}N will occur faster than what we observed in the current study. Further research is required at sites where N fertiliser is applied and where cattle are grazed.

4.5 Conclusion

Results from the two trials supported our hypothesis, with soils in the more intensively managed treatments (i.e. those receiving higher superphosphate fertiliser applications or more frequent irrigation) becoming progressively enriched with ^{15}N relative to soils in less intensively managed treatments. Pasture production and grazing intensity also increased with increasing fertiliser rate and irrigation frequency, and we found positive correlations between the average rate of change in soil δ^{15}N (or final δ^{15}N), and pasture production, clover production (thus N inputs via N_2 fixation) and calculated N losses (Fig. 4.3). We suggest that most of the difference in δ^{15}N observed between treatments was due to the influence that superphosphate and irrigation had on clover and total pasture production, and the flow on effects this had on N cycling and isotope fractionating loss processes, and particularly those associated with animal excreta deposition (e.g. ammonia volatilisation).

We see no reason why the general trends observed in the current study will not be applicable to other grazed grasslands, and indeed our results are in agreement with other studies (e.g. Frank and Evans, 1997; Stevenson *et al.*, 2010). We therefore conclude that soil δ^{15}N shows potential to be a simple indicator of past management intensity, and N cycling and loss from pastoral systems. Similar studies could be easily carried out at other long-term research sites (ideally where archived soils are stored and N fluxes have been measured), and would be a simple way to further our understanding of soil δ^{15}N and N cycle processes in relation to different management regimes and site conditions (e.g. climate and topography). Future studies should endeavor to sample soils to greater depth than the current study, and directly measure the isotopic signature of N inputs and losses from pasture systems, to help improve understanding of mechanisms driving changes in soil δ^{15}N.

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5 Changes in soil δ^{15}N in three hill country pastures receiving differing amounts of superphosphate fertiliser for 15–25 years

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The contribution of authors was as follows:

> The three field trials investigated in this study were initiated and maintained by staff at AgResearch. Paul Mudge and Louis Schipper obtained archived soils from the trials and analysed them for carbon, nitrogen and natural abundance nitrogen isotopes. Paul Mudge collated field information on the three trials, and carried out data analysis with statistical support from Martin Upsdell. Paul Mudge wrote the manuscript which was primarily reviewed by Louis Schipper. Anwar Ghani and Troy Baisden also read the manuscript and provided suggestions for improvement.

Abstract

The natural abundance of the stable nitrogen isotope ^{15}N relative to ^{14}N ($\delta^{15}N$) in soil can provide an integrated measure of past nitrogen (N) cycle processes. Recent research on irrigated flatland pastures in New Zealand revealed that soil $\delta^{15}N$ increased faster in treatments receiving higher rates of superphosphate fertiliser, which was attributed to the influence that fertiliser had on pasture production and N fixation by clover, and the flow on effects this had on N cycling and isotope fractionating loss processes. In this study, we were interested to determine whether soil $\delta^{15}N$ showed similar responses to superphosphate in more topographically complex hill country pastures. Soil $\delta^{15}N$ was measured in archived soils from three long-term (15–25 year) superphosphate trials located in North Island hill country. Results revealed no consistent effect of superphosphate on soil $\delta^{15}N$ in any of the three trials. The lack of effect may have been due to the shorter duration of the trials, smaller differences in pasture production between treatments, or the higher variability of the complex topography. Indeed, soil $\delta^{15}N$ was higher (0.5 ‰ higher) on sheltered east facing slopes than northwest slopes, and easy slopes had higher $\delta^{15}N$ (~0.37 ‰ higher) than steep slopes. The lack of differences observed between treatments, combined with the significant influence of slope and aspect will complicate the use of $\delta^{15}N$ as an indicator of past management intensity and management induced N losses in hill country pastures. Soil $\delta^{15}N$ may however be a useful tool for improving our understanding of N cycling and loss processes occurring on different slopes and aspects in hill country pastures, and this warrants further research.

Keywords: *Isotope, Natural abundance, grassland, topography, slope, aspect*

5.1 Introduction

The natural abundance of stable nitrogen isotopes (^{15}N:^{14}N (δ^{15}N)) in plants and soils have been shown to provide useful information on N cycling and loss processes (Högberg, 1997). One common finding in both forest and agricultural ecosystems is that plants and soils at sites with high N inputs and losses are often enriched with ^{15}N (Chapter 3; Chapter 4; Chapter 6; Johannisson and Högberg, 1994; Högberg *et al.*, 1996; Watzka *et al.*, 2006; Stevenson *et al.*, 2010). Enrichment of soils with ^{15}N occurs due to preferential processing and subsequent loss of ^{14}N during most N transformation processes in soil (e.g. ammonia volatilisation, nitrification, denitrification and mineralisation). This isotopic fractionation occurs because ^{14}N is lighter and tends to react faster than the heavier ^{15}N isotope.

Results from two long-term (~50 year) field trials on flatland pastures in New Zealand (Chapter 4) revealed that soil δ^{15}N was influenced by superphosphate fertiliser and irrigation, with δ^{15}N increasing more with increasing rates of superphosphate or irrigation frequency. It was concluded that most of the difference in soil δ^{15}N between treatments was due to the influence that P fertiliser and irrigation had on pasture production and N fixation by clover, and the flow on effects this had on N cycling and isotope fractionating loss processes (e.g. ammonia volatilisation). Results from Chapter 4 were also in agreement with Stevenson *et al.* (2010) who concluded that δ^{15}N in New Zealand soils increased with increasing management intensity (i.e. δ^{15}N in native forests < exotic plantation forests < sheep/beef pastures < dairy pastures < cropping systems) as a result of greater N inputs and isotope fractionating N losses. These results suggested that soil δ^{15}N might be a useful indicator of past management intensity and N inputs and losses from New Zealand pasture systems.

The Winchmore superphosphate and irrigation trials were located on flat land, and most of the sites reported by Stevenson *et al.* (2010) were also on flat or rolling land. However, much of New Zealand's pastoral land is located in hill country. Hill country pastures are typically more extensively grazed than flatland pastures, and topography is more complex, with large variation of both slope and aspect. Furthermore, stock camping behaviour and reallocation of nutrients is more extreme in hill country than flat land (Saggar *et al.*, 1990; Haynes and Williams, 1993). In this study, we were interested to determine whether soil δ^{15}N in topographically complex hill country pastures, increased in response to superphosphate application as was observed on flatland irrigated pastures at Winchmore. We analysed archived soils from three long-term (15–25 year) superphosphate trials located on North Island hill County, at the Ballantrae, Whatawhata and Te Kuiti research stations (Fig. 5.1).

5.2 Methods

5.2.1 Site descriptions and soil sampling

Fig. 5.1 shows the location of the three trials from which the archived soil samples were taken from. A summary of trial dates, fertiliser regimes and grazing intensity for each trial is shown in Table 5.1, with further details provided in the following sections.

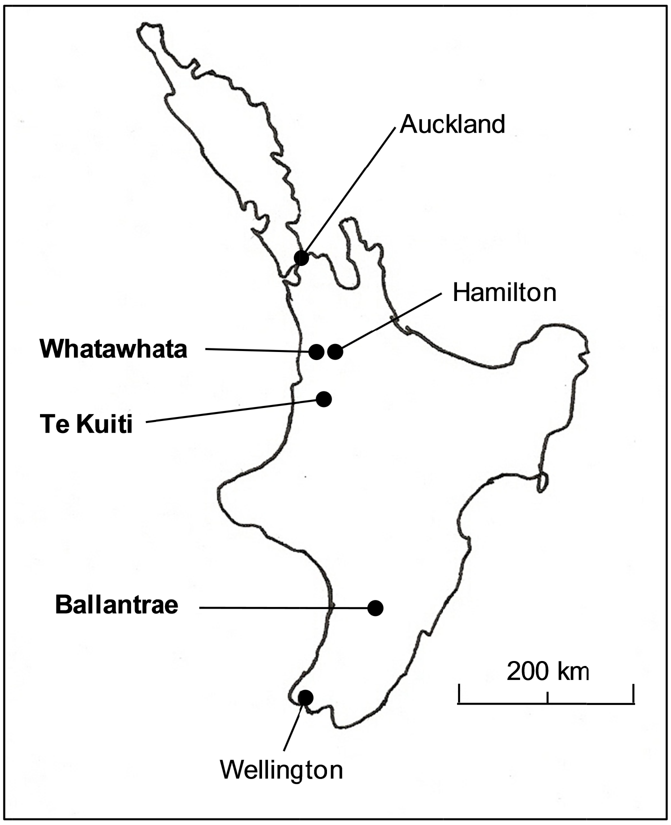

Fig. 5.1. Location of the three long-term superphosphate field trials (Whatawhata, Te Kuiti and Ballantrae), in relation to North Island New Zealand.

Table 5.1 Summary of trial dates fertiliser regimes and grazing intensity of the three long-term field trials used in this study. See text for full site descriptions and explanation of management for each trial.

Trial	Trial dates	Treatment abbreviation	Fertiliser regime kg ha^{-1} y^{-1} single superphosphate	Grazing intensity Stock units ha^{-1} [a]
Ballantrae	1975–1992	BloP	125	10.3[b]
		BloPnoP	125–0[c]	8.3[b]
		BhiP	585–375[d]	14.8[b]
		BhiPnoP	585–0[e]	13.8[b]
Whatawhata	1980–2006	WhiP	1075[f]	12[h]
		WnoP	110–0[g]	18[h]
Te Kuiti	1983–1998	TnoP	0	11[i]
		ThiP	250	15[i]

[a] One stock unit is equivalent to one 55 kg ewe rearing a single lamb and consuming about 520 kg of good quality pasture dry matter per year (Statistics New Zealand, n.d.-b).

[b] Stocking rates from Lambert *et al.*, (1996).

[c] 125 kg ha^{-1} y^{-1} superphosphate from 1975–1980 and then no fertiliser thereafter.

[d] 585 kg ha^{-1} y^{-1} superphosphate from 1975–1980 and then 375 kg ha^{-1} y^{-1} thereafter.

[e] 585 kg ha^{-1} y^{-1} superphosphate from 1975–1980 and then no fertiliser thereafter.

[f] After 1989 triple superphosphate was applied at a rate to achieve the same P loading as 1075 kg ha^{-1} of single superphosphate.

[g] 110 kg ha^{-1} y^{-1} superphosphate from 1980-1984 and then no fertiliser thereafter.

[h] Stocking rates from Schipper *et al.* (2011).

[i] Stocking rates from O'Connor and Smart, (1990).

5.2.2 Ballantrae long-term superphosphate fertiliser trial

5.2.2.1 Location, soils and climate

Ballantrae Research Station is a hill country sheep and beef farm located, in southern Hawke’s Bay, North Island New Zealand (40°19’S, 175°50’E). Soils at the site are mainly formed on tertiary sandstone, siltstone and mudstone and are classified as Brown and Pallic soils (New Zealand Soil Classification) or Andic Distrochrepts, Typic Distrochrepts and Typic Eutrochrepts (Soil Taxonomy) (Lambert *et al.*, 2000). Altitude in the study area ranged from about 250–350 m above sea level, and mean annual rainfall and temperature are 1270 mm and 12.8°C respectively (Lambert *et al.*, 2000). The site is characterised by high wind speeds (particularly the prevailing wind from the northwest) with the average between 1971 and 1981 being 28 km hr^{-1} (Lambert *et al.*, 1983).

5.2.2.2 Site history and trial design

The Ballantrae Research Station was cleared from native forest and pastures sown in the early 1900s. Management was relatively extensive prior to the 1970s with stocking rates of ~7 stock units ha^{-1}, and as far as is known no fertiliser had been applied to the trial area. In 1975, a long-term superphosphate fertiliser and grazing trial was established on four farmlets at Ballantrae. The four farmlets were located adjacent to each other, were 7–14 ha in size, and had 4–5 paddocks with a similar balance of slope and aspect (Lambert *et al.*, 2000). In 1973, soil phosphorus levels were very low (Olsen P 4 µg g^{-1}), and in 1973 and 1974, single superphosphate was applied to the whole trial area at a rate of 250 kg ha^{-1} and Olsen P levels rose to 7 µg g^{-1} in 1975 (Lambert *et al.*, 1983, 1986). In June 1974 the area was close-grazed, then oversown with a mixture of clover (*Trifolium repens, T. Pratense* and *T. Subterraneum*) and lotus (*Lotus pedunculatus*) seed. At the commencement of the trial in 1975, pastures were dominated low fertility grasses, such as browntop (*Agrostis capillaries*) and sweet vernal (*Anthroxanthum odoratum*) and flatweeds such as catsear (*Hypochaeris radicata*). Legumes contributed only 2% to annual pasture production (Lambert *et al.*, 2000).

Between 1975 and 1980 two of the four farmlets received on average 585 kg ha^{-1} y^{-1} of superphosphate and then from 1980 to 1992, one of these farmlets received 375 kg ha^{-1} y^{-1} of superphosphate (BhiP), while no fertiliser was applied to the other farmlet (BhiPnoP). The other two farmlets both received on average 125 kg ha^{-1} of superphosphate annually from 1975 to 1980 and then from 1981 to 1992 one farmlet continued to receive 125 kg ha^{-1} y^{-1} of superphosphate (BloP) and the other no fertiliser (BloPnoP). In 1975, 1250 kg ha^{-1} of lime was applied to both the BhiP and BhiPnoP farmlets, with a further 2500 kg ha^{-1} applied in 1979 (Lambert *et al.*, 2000). The trial area was grazed almost exclusively by sheep for the duration of the trial. Average stocking rates between 1975 and 1993 were 8.3 10.3, 13.8 and 14.8 SU ha^{-1} for the BloPnoP, BloP, BhiPnoP and BhiP treatments respectively (Lambert *et al.*, 1996).

5.2.2.3 Soil sampling

Samples analysed for this study were taken from medium slopes (13–25°) on northwest and easterly aspects. Each farmlet had two sampling sites per aspect, except for the BloPloP treatment where there was only one sampling site for the east aspect. Each site was sampled annually during winter by taking six soil cores (0–75 mm depth and 25 mm diameter), bulking these samples and then air drying and archiving.

5.2.3 Whatawhata Hill Country Research Station

5.2.3.1 Location, soils and climate

The Whatawhata Research Station is a hill country sheep and beef farm located 22 km west of Hamilton, North Island, New Zealand (37° 48' S, 175° 05' E). Easy slopes of the study area have Allophanic and Granular soils (New Zealand Soil Classification) or Ultic Hapludands and Typic Haplohumults (Soil Taxonomy), while steep slopes have Ultic soils (New Zealand Soil Classification) or Typic Haplohumults (Soil Taxonomy) (Schipper *et al.*, 2011). Elevation in the study area ranged from 80–150 m above sea level, and mean annual precipitation and temperature were about 1630 mm and 13°C respectively (Schipper *et al.*, 2011).

5.2.3.2 Site history and trial design

The farm was converted from native forest to pasture around 1920 and the fertiliser trial commenced in 1980. For at least twelve years prior to the start of the trial, about 385 kg ha^{-1} of single superphosphate was applied annually (Schipper *et al.*, 2011). The trial was on a 14.2 ha area of the farm, which was subdivided into 20 paddocks. Each paddock had a north westerly aspect and two slope classes (easy, 10–20° and steep, 30–40°). In 1980, five single superphosphate application rates ranging from 110 to 1075 kg ha^{-1} y^{-1} were applied to four replicate paddocks for each treatment. From 1985, fertiliser was withheld from two replicate paddocks of each treatment to assess the residual effects of superphosphate. These treatments continued to 2006, although after 1989 single superphosphate was replaced with triple superphosphate, but with the same P loading. Soils analysed for this study were from the two paddocks which received 110 kg ha^{-1} y^{-1} of single superphosphate from 1980 to 1984 and then no fertiliser thereafter (WnoP), and the two paddocks that received continuous P inputs equivalent to 1075 kg ha^{-1} y^{-1} of single superphosphate from 1980 to 2006 (WhiP). Soils from both easy and steep slopes were analysed (separately). The trial was grazed by sheep with a stocking rate of ~12 stock units ha^{-1} on the WnoP sites and ~18 on the WhiP sites (Schipper *et al.*, 2011).

5.2.3.3 Soil sampling

Between 1983 and 2006 separate soil samples from steep and easy slopes in each paddock were collected ~annually, air dried, passed through a 2 mm sieve and then archived. Prior to 1993 cores were taken to 70 mm depth and sectioned into 0–30 mm and 30–70 mm depth increments in the field (Schipper *et al.*, 2011). The two depth increments were analysed separately and then a weighted average was used to calculate the final C, N and $\delta^{15}N$ values for the 0–70 mm depth. From 1993 onwards cores were taken to 75 mm depth and were not divided. We believe the small difference in sampling depth (70 mm cf 75 mm) will have negligible impact on results due to the well mixed nature of the topsoil.

5.2.4 Te Kuiti Hill Country Research Station

The Te Kuiti superphosphate trial was established in 1983. For 10 years prior to commencement of the trial, the area had received 250 kg ha^{-1} of superphosphate annually (Roach *et al.*, 1996). The trial comprised two farmlets with six replicate paddocks each. After 1983, one farmlet continued to receive superphosphate at 250 kg ha^{-1} y^{-1} (ThiP) while another farmlet received no fertiliser (TnoP).

Pastures were predominantly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and the trial was rotationally grazed by breeding ewes (Roach *et al.*, 1996). Between 1983 and 1987 stocking rate was the same on both farmlets (14 SU ha^{-1}) with differences in pasture production (as a result of differences in fertiliser application) being manifest in differences in per animal performance (Gillingham and Richardson, 1990). From 1988 until the end of the trial in 1998 the TnoP farmlet was stocked at 11 SU ha^{-1} and the ThiP farmlet at 15 SU ha^{-1}. Archived soil samples (0–75 mm depth) were only available from a final sampling of the trial in 1998 (Ghani *et al.*, 2003). Samples were taken from all six paddocks of the TnoP and ThiP farmlets and from both easy (10–20°) and steep (25–35°) slopes.

5.2.5 *Sample preparation and analysis*

A representative subsample of the archived samples was obtained by passing the whole sample through a stainless steel riffle (which split the sample) until the desired subsample size was reached (about 5 g). Any obvious plant material was then removed with tweezers before fine grinding using an agate mortar and pestle. Samples were analysed for N isotopes using a Europa Scientific 20-20 Stable Isotope Analyser at the University of Waikato Stable Isotope Unit. The overall reproducibility of laboratory measurements was ~0.2 ‰. The natural abundance of ^{15}N is expressed in delta notation (δ) using the following equation (after Coplen, 2011):

$$\delta^{15}N = R(^{15}N/^{14}N)_{sample} - R(^{15}N/^{14}N)_{air} - 1$$

where R $(^{15}N/^{14}N)_{sample}$ is the ratio of $^{15}N/^{14}N$ in the sample, and $R(^{15}N/^{14}N)_{air}$ is the ratio of $^{15}N/^{14}N$ in atmospheric N_2 (the international standard).

5.2.6 *Statistical analysis*

Time series $\delta^{15}N$ data from the Whatawhata and Ballantrae trials was analysed using a Bayesian smoothing program (Upsdell, 1994) to determine if there were temporal changes in $\delta^{15}N$, and whether $\delta^{15}N$ differed between treatments, aspects and slopes. The time series from the two trials were modelled as doubly integrated wiener processes, which corresponds to fitting cubic splines. Terms for Date*Treatment were used to estimate different Treatment time series. The individual paddock time series were modelled with terms Plot/Date, which accounts for the correlations induced by measuring the same plot repeatedly. A random effect for the effect of the day of sampling was also added (DayEffect). The full model fitted for the Whatawhata and Ballantrae trials was: Y = Date * Treatment + (Aspect or Slope)*Date + Plot / Date + DayEffect. An initial model showed that there was no interaction between treatment and aspect or slope, and therefore no interaction term was included in the model. Using a mixed model smoother allows us to extract estimates for the Date curves for each of the treatments after allowing for the effects of the nuisance variables Plot and DayEffect. Because there was only one sampling site for the east aspect of the BloPloP treatment in the Ballantrae trial, the design was not balanced and therefore we adjusted for this using partial residual plots. Standard Analysis of Variance in GenStat v 13 was used to analyse the one off soil sampling from the Te Kuiti trial.

5.3 Results

5.3.1 Pasture production

A summary of pasture production data from the three hill country superphosphate trials has been compiled and is presented in Table 5.2. Pasture data was not nearly as complete or comprehensive as data from the Winchmore superphosphate trial in Chapter 4, with a number of years when data was not collected. Across the three trials, total pasture production was 20–40 % lower in unfertilised treatments compared to treatments receiving superphosphate (Table 5.2). At Whatawhata legume production was 20–40 % lower in the unfertilised treatments compared to fertilised treatments, while at Te Kuiti and Ballantrae legume production in the unfertilised treatments was 60–70 % lower than fertilised treatments (although at both Te Kuiti and Ballantrae legume data was only for two years) (Table 5.2). The legume production data reported by Lambert *et al.* (1996) for Ballantrae, was much lower than values for Whatawhata and Te Kuiti (Table 5.2). However, Lambert *et al*. (1986) reported legume data at Ballantrae for the period 1975–1981, where average legume DM production was 900 kg ha^{-1} y^{-1} and 1270 kg ha^{-1} y^{-1} for BloP and BhiP respectively, which was similar to values for Whatawhata and Te Kuiti (Table 5.2). This difference in legume production may have been because one study (Lambert *et al.*, 1986) was during the first few years of the trials (and measurements were made across all slopes), while the other study (Lambert *et al.*, 1996) was ~15 years later, and samples were only taken from moderate slopes (see footnote Table 5.2).

Table 5.2 Summary of total dry matter (DM) and legume DM production for the Whatawhata, Te Kuiti and Ballantrae superphosphate trials. Actual rates of superphosphate applied to each treatment are shown in Table 5.1.

Trial	Period over which pasture data collected	Slope category and range slopes	Treatment abbreviation	Total DM production (kg ha^{-1} y^{-1})	Legume DM production (kg ha^{-1} y^{-1})	References
Whatawhata	1984–2002	Easy 10–20°	WnoP	10,100	990	Gillingham & Richardson (1990), and AgResearch unpublished data
			WhiP	14,300	1250	
		Steep 30–40°	WnoP	6700	710	
			WhiP	8400	1140	
Te Kuiti	1992 & 1993	Easy 10–20°	TnoP	7000	570	Roach *et al.* (1996)
			ThiP	10,000	1400	
		Steep 25–35°	TnoP	3200	270	
			ThiP	4900	850	
Ballantrae	1976–1987 [a]	1–>25° [b]	BloPnoP	7400	130	Total DM from Lambert *et al.* (1990), Legume DM from Lambert *et al.* (1996)
			BloP	8400	270	
			BhiPnoP	8800	180	
			BhiP	12,900	390	

[a] Legume production data only for 1992 and 1993, and only from moderate slopes (~20°).

[b] Total pasture production data for Ballantrae is the average from three slope categories spanning slopes from 1 to >25°. The soil samples analysed for δ^{15}N at Ballantrae were from sites on moderate slopes (13–25°).

5.3.2 Soil δ^{15}N

5.3.2.1 Ballantrae

At Ballantrae average soil δ^{15}N was similar in all treatments at the start of the trial, ranging from about 2.2–2.6 ‰ (Fig. 5.2a). Only δ^{15}N in the BhiPnoP treatment increased significantly with time, and at the end of the trial δ^{15}N in this treatment was higher than the BhiP and BloP treatments, but not the BloPnoP treatment (Fig. 5.2a). If soil δ^{15}N was driven by fertiliser application rates (and the flow on effects to pasture production etc), the BhiP treatment would have been expected to have the highest δ^{15}N and the BloPnoP treatment the lowest δ^{15}N, which was not the case. Aspect had a significant influence on δ^{15}N, with values from easterly facing slopes being on average 0.5 ‰ higher than northwest slopes (Fig. 5.2b, Table 5.3). The aspect effect was present from the start of the trial and the magnitude did not change with time (Fig. 5.2b).

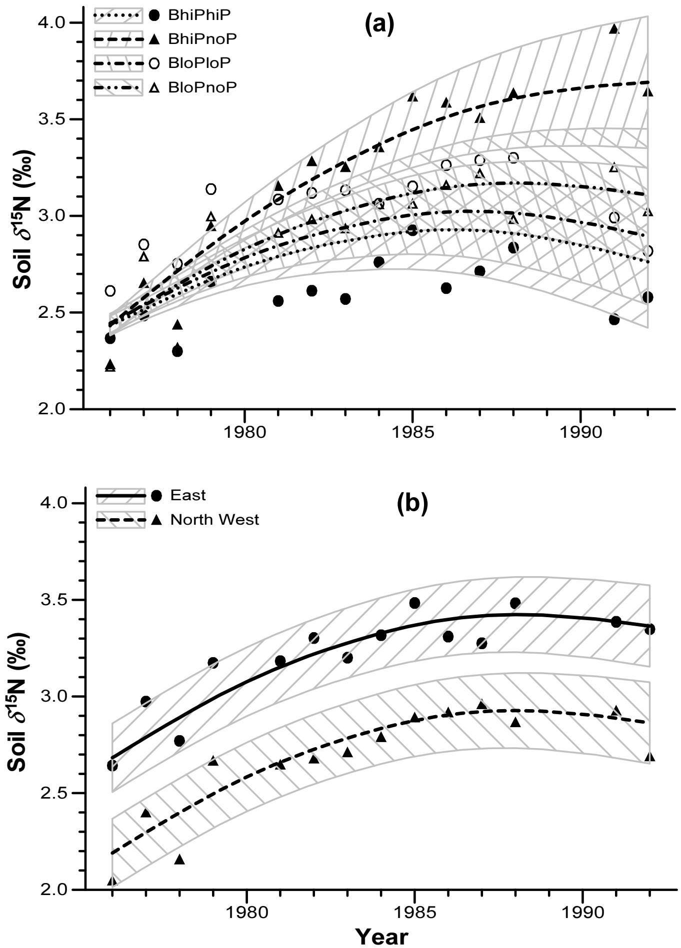

Fig. 5.2. Average soil δ^{15}N in (a) the four treatments of the Ballantrae superphosphate trial (averaged across aspects), and (b) the two different aspects (averaged across treatments). Hatched bands are 95% least significant intervals, with treatments or aspects significantly different ($p<0.05$) if bands do not overlap.

Table 5.3 Soil δ^{15}N values for the different treatment/aspect combinations in the Ballantrae superphosphate trial. Values are the average from two replicate paddocks for each aspect. Actual superphosphate loading rates for the different treatments are shown in Table 5.1.

Year	BhiP		BhiPnoP		BloP		BloPnoP	
	E	NW	E	NW	E	NW	E	NW
1976	2.64	2.10	2.73	1.74	3.11	2.24	2.33	2.11
1977	2.63	2.34	3.09	2.21	3.39	2.46	2.99	2.59
1978	2.20	2.40	2.96	1.92	3.20	2.41	2.61	2.05
1979	2.79	2.53	3.43	2.46	3.58	2.79	3.11	2.88
1981	2.65	2.47	3.64	2.66	3.70	2.66	3.02	2.80
1982	2.82	2.41	3.85	2.72	3.65	2.73	3.11	2.86
1983	2.80	2.33	3.67	2.84	3.58	2.78	2.99	2.89
1984	3.09	2.42	3.86	2.85	3.34	2.80	3.03	3.09
1985	3.10	2.74	4.08	3.15	3.67	2.77	3.23	2.89
1986	2.63	2.50	4.28	3.11	ND	3.01	3.15	3.04
1987	2.68	2.61	4.10	3.09	ND	3.04	3.19	3.11
1988	3.06	2.61	4.15	3.12	3.73	2.96	3.18	2.78
1991	2.10	2.52	4.79	3.43	ND	2.74	3.47	3.01
1992	2.79	2.37	3.98	3.30	3.21	2.49	3.45	2.60
Average	**2.77**	**2.46**	**3.68**	**2.76**	**3.47**	**2.71**	**3.05**	**2.77**

ND not determined.

5.3.2.2 Whatawhata and Te Kuiti

At the Whatawhata trial there was no difference in δ^{15}N between treatments (on both steep and easy slopes) despite the very large difference in superphosphate application rates (Fig. 5.3a). Averaged across treatments, δ^{15}N of soils from the steep slopes was significantly lower (average 0.4 ‰ lower) than easy slopes (Fig. 5.3b, Table 5.4). The slope effect was present at the start of the trial and did not change significantly during the trial. There was a significant ($p<0.05$) increase in soil δ^{15}N between the start and end of the trial, on both steep and easy slopes (Fig. 5.3b). At Te Kuiti, there was no difference in soil δ^{15}N between the two fertiliser treatments. However, like at Whatawhata δ^{15}N was significantly lower on the steep slopes (3.58 ‰) than easy slopes (3.92 ‰) (Fig. 5.3b, Table 5.4).

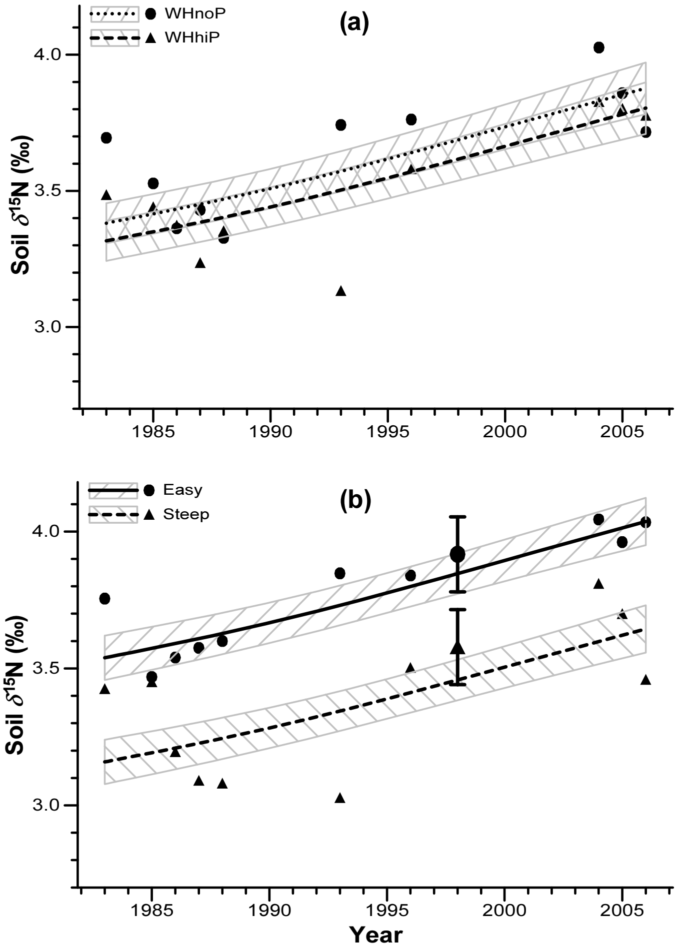

Fig. 5.3. Average soil δ^{15}N in (a) the two treatments of the Whatawhata superphosphate trial (averaged across slopes), and (b) the two different slopes (averaged across treatments). Also shown in (b) are the average values for each slope in the Te Kuiti superphosphate trial from the sampling in 1998 (larger symbols with conventional error bars). Hatched bands for the Whatawhata data are 95% least significant intervals, with treatments or slopes significantly different ($p<0.05$) if bands do not overlap. Error bars for the Te Kuiti data are 95% least significant differences (which do not overlap and so the two slopes are significantly different).

Table 5.4 Soil δ^{15}N values for the different treatment/slope combinations in the Whatawhata and Te Kuiti superphosphate trials. At Whatawhata values are the average from two replicate paddocks for each slope, while at Te Kuiti values are the average of six replicate paddocks for each slope. Steep and easy slope sites were located in the same paddocks. Actual superphosphate loading rates for the different treatments are shown in Table 5.1.

Whatawhata	WnoP		WhiP	
Year	Easy	Steep	Easy	Steep
1983	3.78	3.61	3.73	3.24
1985	3.51	3.54	3.41	3.36
1986	3.46	3.26	3.61	3.13
1987	3.73	3.12	3.41	3.06
1988	3.61	3.05	3.59	3.12
1993	3.96	3.52	3.73	2.53
1996	3.98	3.55	3.70	3.45
2004	4.19	3.87	3.90	3.75
2005	3.89	3.83	4.04	3.57
2006	3.98	3.45	4.09	3.46
Average	**3.81**	**3.47**	**3.74**	**3.27**

Te Kuiti	TnoP		ThiP	
1998	3.78	3.48	4.05	3.67

5.4 Discussion

5.4.1 *Treatment effects on soil δ^{15}N*

Based on results from the Winchmore superphosphate trial (and irrigation trial) in Chapter 4, it was expected that in the current study, soil δ^{15}N would have been higher in the treatments receiving higher rates of superphosphate. Superphosphate was expected to affect soil δ^{15}N by increasing pasture production, N fixation by clover, grazing intensity and isotope fractionating loss processes (e.g. ammonia volatilisation). However, no consistent differences in soil δ^{15}N developed between treatments in any of the three hill country trials, despite the expected differences in pasture production and grazing intensity (Table 5.1 and Table 5.2). A number of studies in the literature have also reported no difference in soil δ^{15}N between different grazing regimes (Ledgard *et al.*, 1984; Neilson *et al.*, 1998; Cook, 2001; Han *et al.*, 2008; Xu *et al.*, 2010). One likely cause for the lack of differences observed between treatments in the current trials (and other published studies) is that any differences were simply too small to detect against a large background pool of stable soil N (Johannisson and Högberg, 1994; Xu *et al.*, 2010).

The least significant intervals in Fig. 2.1a and Fig. 5.3a, indicate the size of detectable differences in δ^{15}N between treatments, given the designs and sampling regimes used. At the end of the trials, a difference of about 0.2 ‰ between treatments would have been detectable at Whatawhata, and a difference of 0.4 ‰ Te Kuiti. Therefore the observed differences of ~0.06 ‰ and 0.23 ‰ between the no P fertiliser and high P fertiliser treatments in the Whatawhata and Te Kuiti trials respectively were far from significant. Variation was much greater at Ballantrae and differences between treatments would have needed to be >0.70 ‰ to be detectable at the end of the trial. Therefore the differences between three treatments at Ballantrae were far from significant, but the BhiPnoP treatment had significantly higher δ^{15}N than two of the other treatments (Fig. 5.2a). It is unclear why δ^{15}N increased significantly in the BhiPnoP treatment but not the other treatments, and why the BhiPhiP treatment had the lowest δ^{15}N (Fig. 5.2a). We would have expected the two ‘high P’ treatments to be the most similar, and higher than the two ‘low P’ treatments. One possible explanation for this unexpected ordering of the Ballantrae treatments was that the different farmlets were not replicated, and paddocks were not randomly allocated to the respective farmlets. Therefore site specific factors related each farmlet may have been greater than any treatment effect.

Another contributing factor for the lack of differences observed between treatments in the hill country trials, may have been that trial duration too short for differences to become apparent (i.e. much shorter than the Winchmore trials, 15–25 cf ~50 years). Differences in pasture production and stocking rates between treatments were also not quite as extreme in the hill country trials, as at Winchmore (see Table 5.1 and Table 5.2), meaning differences in N inputs and total N cycling would have been lower, therefore providing less opportunity for differences in isotope fractionating N losses. In addition, the complex terrain in the hill country trials likely introduced more variability between sampling sites, because subtle differences in topography and aspect could have influenced, pasture production, soil properties, stock behaviour and therefore soil δ^{15}N (see discussion in section 5.4.2).

Differences in erosion between treatments could have also contributed to the absence of differences in δ^{15}N. In the low or nil superphosphate treatments, there was usually a thick layer of thatch on the soil surface, while in the high fertility treatments this thatch layer was largely absent. Therefore erosion of clay and silt size particles enriched with ^{15}N (Ledgard *et al.*, 1984; Fox and Papanicolaou, 2007), may have been higher from the high fertility treatments, and this could have offset any ^{15}N enrichment due to greater management intensity. Measurements of erosion rates and the δ^{15}N value of eroded sediment would be required to test this hypothesis.

Higher legume production in the treatments receiving more superphosphate (Table 5.2) would have increased N fixation. Therefore these treatments might have been expected to have greater accumulation of soil N (depleted in ^{15}N, because fixed N generally has delta values of ~0 ‰), and this could have offset any increase in isotope fractionating N losses due to increased N inputs and grazing intensity (Ledgard *et al.*, 1984). However, for the soils analysed at Ballantrae, Whatawhata and Te Kuiti, there were no differences in total nitrogen between treatments, and total nitrogen did not change significantly with time. Therefore it seems unlikely that greater accumulation of fixed N with low delta values in the high P treatments would have contributed to the lack of observed differences between treatments. However, it is possible that older soil N was preferentially lost and was replaced by more recently fixed N with lower delta values (Kriszan *et al.*, 2009), and that this effect was greater in the high P treatments (see Lambert *et al.*, 2000).

5.4.2 *Effect of slope and aspect on soil δ^{15}N*

While no consistent differences were observed between treatments, slope and aspect did have a consistent effect. At Ballantrae δ^{15}N was higher (by 0.5 ‰) at sites with east aspects than north west aspects, and at Whatawhata and Te Kuiti easy slopes had higher δ^{15}N than steep slopes (0.4 ‰ and 0.34 ‰ higher for Whatawhata and Te Kuiti respectively).

5.4.2.1 Aspect

The aspect effect at Ballantrae was present from the start of the trial and the magnitude did not change with time (Fig. 5.2b).This suggests that it was probably a relic feature which developed under the previous farm management regime or potentially under native forest prior to land clearance for pasture. At Ballantrae there was a strong prevailing wind from the NW, while E slopes were more sheltered. The differences in wind velocity between the two aspects may have influenced both the productivity and species composition of native vegetation prior to clearance, which could have had flow on effects to N cycling and loss processes. If this was the case it would have occurred for hundreds of years, which is ample time of the relatively small differences in δ^{15}N to have developed. Another possible cause for the aspect effect, was that the strong prevailing wind from the NW caused sheep to spend more time on sheltered E facing slopes, which resulted in greater dung and urine deposition and a trend for higher fertility on E slopes (Lambert and Roberts, 1978; Lambert *et al.*, 1983; Saggar *et al.*, 1990). Higher rates of excreta deposition on the E slopes could have facilitated greater isotope fractionating N losses and thus the ^{15}N enrichment we observed (Frank and Evans, 1997; Hawke, 2001; Frank *et al.*, 2004). Pasture production on E and NW slopes at Ballantrae was virtually the same (Lambert *et al.*, 1983).

5.4.2.2 Slope

There are a number of possible explanations for the lower δ^{15}N on the steep slopes compared to easy slopes at Whatawhata and Te Kuiti. As for aspect at Ballantrae, the slope effect was present at Whatawhata at the start of the trial, which suggested that the difference between slopes developed under the previous farm management or under the native forest prior to land clearance. Total pasture, and clover production on the steep slopes were about 20–50% lower than on easy slopes at Whatawhata and Te Kuiti (Table 5.2). Lower total pasture and clover production on steep slopes would have led to a reduction in total N inputs, cycling and losses, providing less opportunity for isotopic fractionation. Lower pasture production would have also led to lower inputs of dung and urine on steep slopes, and this would have been accentuated further because in hill country there is a net transfer of excreta from steep slopes to flatter areas where animals tend to camp (Saggar *et al.*, 1990; Williams and Haynes, 1990). Because plants are depleted in

^{15}N relative to soil, net transport in excreta from steep to easy slopes, should lead to a decrease in soil δ^{15}N on the steep slopes. Further, higher excreta deposition and resulting increases in N losses, on the easy slopes would have likely contributed to the higher soil δ^{15}N on easy slopes (Frank and Evans, 1997; Hawke, 2001; Frank *et al.*, 2004). Recently, Hoogendoorn and Devantier (2011) demonstrated that N leaching losses were significantly higher on easy slopes relative to steep slopes in hill country pastures. Productivity of the native forest prior to clearance for pasture would have also likely been higher on easy slopes and therefore N cycling and isotope fractionating loss processes may have been greater than on the steep slopes.

Another possible cause for the lower δ^{15}N on the steep slopes was that erosion would have been higher. Amundson *et al.* (2003) found that as slope increased, soil δ^{15}N values decreased. They argued that greater erosion on steeper slopes would decrease soil residence time (and thus time for isotopic fractionation and N losses to occur), and therefore soil δ^{15}N values would be more similar to atmospheric N inputs (δ^{15}N of ~0 ‰) compared to soils on easier slopes where residence time was longer. Erosion also tends to preferentially remove finer soil particles which are usually enriched in ^{15}N relative to bulk soil (Ledgard *et al.*, 1984; Tiessen *et al.*, 1984; Gerzabek *et al.*, 2001; Fox and Papanicolaou, 2007), which could have contributed to lower δ^{15}N on steep slopes. Further research is required to more fully understand the influence of slope and aspect on soil δ^{15}N.

5.5 Conclusion

In contrast to the results from the two long-term field trials at Winchmore (see Chapter 4), there were no consistent differences in soil δ^{15}N between superphosphate fertiliser treatments in the three North Island hill country trials. It is unclear exactly why differences were not observed in the hill country trials, but it may have been related to the shorter duration of the trials, smaller differences in pasture production between treatments, differences in erosion between treatments (due to differences in thatch build-up), or higher variability due to complex topography. Indeed, slope and aspect did have a significant influence on soil δ^{15}N with higher values on sheltered east facing slopes at Ballantrae and on easy slopes than steep slopes at Whatawhata and Te Kuiti.

Soil δ^{15}N could therefore be a useful indicator of past management intensity and N losses from flat pastoral sites (see Chapter 4), but the lack of differences observed between treatments, combined with the significant influence of slope and aspect in the hill country trials will complicate the use of δ^{15}N as an indicator of past management intensity and management induced N losses in hill country areas. Soil δ^{15}N may however be a useful tool for improving our understanding of N cycling and loss processes occurring on different slopes and aspects in hill country pastures, and this warrants further research.

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6 Influence of nitrogen fertiliser loading rate on soil C, N and δ^{15}N in a sheep grazed hill country pasture

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The contribution of authors was as follows:

> The field trial investigated in this study was initiated and maintained by staff at AgResearch in Palmerston North (e.g. B Devantier and C Hoogendoorn). Paul Mudge and Louis Schipper obtained archived soils from the trial and analysed them for natural abundance nitrogen isotopes. Coby Hoogendoorn provided data on pasture production, N leaching, and total carbon and nitrogen. Paul Mudge carried out data analysis with statistical support from Martin Upsdell. Paul Mudge wrote the manuscript which was primarily reviewed by Louis Schipper. Anwar Ghani, Troy Baisden and Coby Hoogendoorn also read the manuscript and provided suggestions for improvement.

Abstract

Nitrogen (N) fertiliser has been shown to increase pasture and animal production, but less is known about the impacts of N fertilisation on soil carbon (C) and N, and there is growing concern about increased N losses to the wider environment. The objective of this study was to determine whether different rates of mineral N fertiliser (urea) affected soil C, N and natural abundance nitrogen isotopes (δ^{15}N). To achieve this we analysed archived soils (0–75 mm and 75–150 mm depth) collected annually from a sheep grazed hill country field trial in New Zealand which had received N fertiliser applications ranging from 0 to 750 kg N ha^{-1} y^{-1} for 4 years. The clearest trend in the data was a significant increase in δ^{15}N with time in treatments receiving $\geq$100 kg N ha^{-1} y^{-1}, with the increase being more rapid (up to 0.35 ‰ y^{-1}) as N application rate increased. Given that N inputs from fertiliser were ~0 ‰, we concluded that the observed increases in δ^{15}N were driven by increasing isotope fractionating N losses as N rate increased. This conclusion was supported by a strong positive correlation between the rate of change in soil δ^{15}N and average nitrate leaching ($p<0.001$, $r^2=0.6$). In contrast, there was no significant relationship between sampling date or N rate, and total Kjeldahl nitrogen. This lack of N retention was consistent with high leaching losses measured at the same site. Both C and the C:N ratio showed different trends in the two depths. In the 0–75 mm depth, C decreased by 0.11 % y^{-1} (~800 kg ha^{-1} y^{-1}) in the 0N treatment and 0.053 % y^{-1} (~380 kg ha^{-1} y^{-1}) in the 100N treatment, but there was no significant change in C at higher N rates. For the 75–150 mm depth there was a trend of increasing C in all treatments (increases of 0.052–0.082 % y^{-1} or 380–600 kg ha^{-1} y^{-1}), but the increases in the 0N and 750N treatments were not significant. There was no change in C:N with time in the 0–75 mm depth, but in the 75–150 mm depth C:N increased with time in all treatments. These results indicate that δ^{15}N is more sensitive to N inputs and losses than changes in total soil N or the C:N ratio, and therefore soil δ^{15}N could be a useful indicator of past N cycling and loss in pastoral systems.

Keywords: *Isotope, natural abundance, grassland*

6.1 Introduction

The rapidly increasing global population has led to increased demand for food, which has largely been met by increased use of synthetic nitrogen (N) fertiliser. For example, Smil (2002) estimated that around 40% of the protein consumed by humans was produced with N fertiliser derived from the Haber-Bosch process. While N fertiliser has been shown to increase crop (Tilman *et al.*, 2002), pasture and animal production (Lambert and Clark, 1986; Clark *et al.*, 2007), less is known about the impacts of N fertilisation on soil carbon (C) and nitrogen (N), and there is growing concern about increased N losses to the wider environment as a result of increased N fertiliser use (Ledgard *et al.*, 1999; Galloway *et al.*, 2003; Monaghan *et al.*, 2005).

The influence of N fertiliser on soil C and N has been somewhat controversial, with Khan *et al.* (2007) and Mulvaney *et al.* (2009) arguing that mineral N fertilisation in cropping systems led to a decrease in soil C and N. However, a number of other researchers (Reid, 2008; David *et al.*, 2010; Powlson *et al.*, 2010), disagreed with how Khan *et al.* and Mulvaney *et al.* interpreted the results of their studies. Subsequently, Ladha *et al.* (2011) conducted a comprehensive meta analysis on 135 long-term cereal based cropping trials and found that soil C and N generally decreased with time in both treatments fertilised with mineral N and those not fertilised, but the decrease in C and N was significantly greater in the unfertilised treatments (i.e. C and N declined faster when N was not applied). Less work has been conducted on the influence of mineral N fertiliser on total C and N in grassland soils, particularly under grazed pastures (Watson *et al.*, 2007).

In New Zealand, pastoral agriculture is the dominant land use, and is comprised of intensively managed flatland pastures, less intensively managed hill pastures and a smaller area of extensively managed tussock grassland. Sheep and beef production systems occupy the largest area of pastoral land (80% or 8.5 million ha, Statistics New Zealand, n.d.-a), and are predominantly located on hill country (i.e. slopes>15 degrees). Prior to 1950 production on hill country land was constrained by low soil fertility, but the advent of aerial top dressing in the late 1940s alleviated this constraint to some degree (MacLeod and Moller, 2006). Initially superphosphate was the main fertiliser applied, which increased pasture production due to the direct addition of phosphorus (P) and sulphur (S), and also indirectly by increasing nitrogen (N) fixation by legumes. Despite increased N fixation as a result of superphosphate application, pasture growth on hill country farms is often severely N limited, particularly in early spring (Ball *et al.*, 1982). Recognition of this N limitation, combined with favourable product prices led to a large increase in N fertiliser use on hill country farms from around the mid 1990s. For example, between 1996 and 2007 urea fertiliser use on sheep and beef farms increased 12 fold, from 6488 to 84,062 tonnes per year (Statistics New Zealand, n.d.-a).

In New Zealand, total soil C and N was shown to increase rapidly following initial conversion of indigenous vegetation to pasture, with accumulation rates slowing after a few decades when it appears a new steady state was being approached (Jackman, 1964a, b; Schipper and Sparling, 2011). However, Schipper *et al.* (2010) found large losses of C and N in soils under established dairy pastures on flat land, no change in soils under drystock (i.e. sheep, beef and deer) pastures on flat land, and increases in C and N in hill country pastures (grazed by sheep and beef cattle). The causes of the differences between land uses observed by Schipper *et al.* (2010) are not clear, but losses of soil C and N under dairy pastures may have been related to greater N fertiliser inputs and subsequent cycling through urine patches (Lambie *et al.*, 2012), while increases in C and N in hill country soils may have been due to recovery of organic matter following erosion during forest clearance. As pastures develop, soil N generally increases proportionally more than C and therefore the C:N ratio declines (Lambert *et al.*, 2000; Sparling and Schipper, 2002; Schipper *et al.*, 2011). As C:N decreases, the soils ability to store nitrogen is reduced (provided C is not increasing), and therefore N losses to the wider environment are likely to increase (Schipper *et al.*, 2004). C:N ratios in soils under hill country pastures are generally higher than in more intensively managed flatland soils, and therefore N storage capacity may be higher (Lambert *et al.*, 2003). Application of N fertiliser would be expected to accelerate the decline in C:N ratio and increase N losses. New Zealand has had a number of long-term grazed field trials investigating the effect of phosphorus fertiliser on pasture production and soil properties (Dodd and Ledgard, 1999; Lambert *et al.*, 2000; Schipper *et al.*, 2011; Schipper *et al.*, in press), but there have been no such long-term trials investigating the effect of N fertiliser.

While changes in total N can give an indication of whether a soil is gaining or losing N, total N provides little information on N cycling and loss processes and it can be difficult to detect changes due to high spatial variability and a large background pool of stable N (Watson *et al.*, 2007). The natural abundance of the stable nitrogen isotope ^{15}N relative to ^{14}N (δ^{15}N) in soil can provide an integrated measure of past N cycle processes occurring at a particular site (Robinson, 2001). This occurs because during most N transformation processes the lighter ^{14}N isotope accumulates in products (e.g. NO_3^-, NH_3, N_2, N_2O), which tend to be preferentially lost from the ecosystem, and therefore substrate (e.g. NH_4^+, organic N) retained in the soil becomes enriched with ^{15}N. Studies in both forest and grassland ecosystems have found that soil δ^{15}N can be positively correlated with N inputs (Högberg, 1990; Watzka *et al.*, 2006), calculated N losses (Johannisson and Högberg, 1994; Chapter 4) and rates of mineralisation and nitrification (Templer *et al.*, 2007; Kahmen *et al.*, 2008). However, no studies have directly related soil δ^{15}N to measured N losses in grazed pasture systems.

The objective of the current study was to determine if different rates of mineral N fertiliser (urea) affected soil C, N and δ^{15}N, and whether these variables could be related to N inputs, pasture production, grazing intensity and N leaching losses. To achieve this we analysed archived soils collected annually from a sheep grazed hill country pasture in New Zealand which had received annual N fertiliser applications of 0, 100, 200, 300, 400, 500 and 750 kg N ha^{-1} y^{-1} for 4 years. We were able to explore the relationship between soil δ^{15}N and pasture production, grazing intensity and N leaching because these variables were also measured at the same site (Hoogendoorn *et al.*, in prep). It is important to note that N fertiliser rates >200 kg ha^{-1} y^{-1} are uncommon in New Zealand pastures (particularly in hill country). Therefore the higher N rates used in this experiment provided an opportunity to examine the effects of an ‘extreme’ intensification scenario, on C and N mass balance as well as N isotopes.

6.2 Methods

6.2.1 Site description

The study was conducted at the AgResearch Ballantrae hill country sheep and beef research station, located in southern Hawke's Bay, North Island New Zealand (40°19'S, 175°50'E). The trial area was at an elevation of between 200 and 250 m and slopes ranged from 12 to 25°, with a predominantly south-south west aspect. The soil at the site was formed on sedimentary mudstone parent material, and was classified as a mottled argillic pallic soil (Hewitt, 1998). Mean annual rainfall was about 1270 mm (Lambert *et al.*, 2000).

Pastures at the site were sown in the early 1900s following clearance of indigenous forest. Prior to 1984 management was relatively extensive (~7 stock units (SU) ha^{-1}), but after 1984 superphosphate fertiliser application led to an increase in management intensity with the stocking rate between 1984 and 2004 being about 14 SU ha^{-1} (with a SU defined here as being equivalent to one 55 kg ewe rearing a single lamb and consuming about 550 kg pasture dry matter (DM) per year). The trial area was historically grazed by a mixture of sheep and beef cattle, although between 1994 and the start of the trial in 2004, only young cattle were grazed. At the start of the trial pastures were comprised of about 50% grasses responsive to high fertility (e.g. *Lolium perenne*), 40% low fertility tolerant grasses (e.g. *Agrostis tenuis*), 5% legumes (mainly *Trifolium repens*) and 5% weeds (Hoogendoorn *et al.*, in prep). As the trial progressed, the proportion of high fertility grasses increased in all treatments, and legume abundance declined to below 5% in all treatments after the first 6 months (Hoogendoorn *et al.*, in prep). The decline in legume abundance may have been due to an infestation of clover root weevil (*Sitona Lepidus*).

6.2.2 Trial design and grazing management

In August 2004 the trial area was fenced into 17 paddocks (0.2 to 0.4 ha each), which were grouped into three different blocks. The trial had a randomized incomplete block design, with seven N fertiliser rates (0, 100, 200, 300, 400, 500 and 750 kg N ha^{-1} y^{-1}) allocated randomly within the three blocks. Paddocks receiving 0, 200 and 400 kg N ha^{-1} y^{-1} were replicated three times (i.e. one paddock in each block), while the other treatments were replicated twice. N was applied as urea, in split dressings throughout the year (Table 6.1). N fertilisation commenced in September 2004 and finished in May 2008. During the trial, paddocks were rotationally grazed year round by female sheep (non lactating). The different N application rates led to differences in pasture production, and therefore animal numbers were adjusted for each paddock and for each grazing event so that grazing residuals and pasture utilisation was the same for all treatments.

Table 6.1 Schedule of annual nitrogen fertiliser timing and rates (kg N ha^{-1}). Table modified from Hoogendoorn *et al.* (in prep).

Treatment	September	November	January	March	May	July
0N						
100N				50		50
200N	50			50	50	50
300N	50	50	50	50	50	50
400N	67	67	67	67	67	67
500N	80	83	83	83	83	83
750N	125	125	125	125	125	125

6.2.3 *Soil sampling and analysis*

Soil samples (0–75 mm and 75–150 mm depth) were taken annually during winter, with the first sampling occurring in early September 2004 (which was prior to the first N application), and the last sampling in July 2008. Two cores were taken adjacent to nine lysimeters which were installed in each of the paddocks to measure N leaching (Hoogendoorn *et al.*, in prep). The lysimeters were spread evenly across 12–24° slopes in each paddock. A total of 18 cores were taken in each paddock and bulked by depth. Samples were air dried and passed through a 2 mm sieve before being archived. When the study was complete subsamples from the archived samples were taken and ground with an agate mortar and pestle, and then analysed for total Kjeldahl nitrogen (TKN), total carbon (C) via dry combustion in a LECO furnace, and natural abundance nitrogen isotopes (δ^{15}N) with a Europa Scientific 20-20 Stable Isotope Analyser. δ^{15}N is expressed as parts per thousand relative to atmospheric N_2 (the international standard) using the following equation (after Coplen, 2011):

$\delta^{15}N = R(^{15}N/^{14}N)_{sample} - R(^{15}N/^{14}N)_{air} - 1$

where R $(^{15}N/^{14}N)_{sample}$ is the ratio of $^{15}N/^{14}N$ in the sample, and $R(^{15}N/^{14}N)_{air}$ is the ratio of $^{15}N/^{14}N$ in atmospheric N_2.

6.2.4 Pasture production and N leaching measurements

Above ground pasture mass in each paddock was calculated prior to and after each grazing event using a rising plate meter. Net annual herbage accumulation was calculated as the difference between the post-grazing pasture mass and the pre-grazing pasture mass summed over all the grazing events for a given year. The rising plate meter was calibrated (via manual herbage cuts) every two months on both pre and post grazing pastures.

Mineral N leaching was measured using in situ PVC barrel lysimeters (150 mm diameter x 300 mm deep) containing intact soil cores taken from the field site in a separate study (Hoogendoorn *et al.*, in prep). There were 9 lysimeters in each paddock, spread evenly in 2° slope increments between 12 and 24°. Drainage from each lysimeter was collected in a 4.5 L plastic container placed in the ground 1–2 m distant from the lysimeter, on the downhill side. The lysimeters were installed in November 2004 and the area was left to settle until March 2005. At the end of March, clean leachate collection vessels were installed under each lysimeter. Drainage volume and a subsample of leachate from each lysimeter were measured each month or after 100 mm of rainfall, whichever occurred first, for three years until the end March 2008. For further details of lysimeter installation and sampling regime see Hoogendoorn *et al.* (in prep).

6.2.5 Statistical analysis

The data were primarily analysed as a time series using a Bayesian smoothing program (Upsdell, 1994). The sampling date and nitrogen rate data were modelled as doubly integrated wiener processes, which corresponds to fitting cubic splines. Terms paddock / sampling date were added to account for the correlations induced by measuring the same paddock repeatedly. The block term was found to be negligible in a preliminary model which included it and so was left out of the model. The first soil sampling in September 2004 was prior to the first N application, so there could have been no effect of treatment for these samples. Therefore in the model, treatments were forced to be the same at the first sampling, so that any differences present were attributed to individual paddock effects rather than treatment effects. The final fitted model was Y = (sampling date * depth) / N rate + paddock / sampling date, where the operators have the same meaning as in Genstat model statements. Data was also analysed when values from both depths had been averaged (with the depth term removed) to determine what results would have looked like if only one 0–150 mm core had been taken. The Akaike information criterion and the Bayesian information criterion were used to determine which type of model fitted the data best. In almost all cases a linear fit (in the sampling date direction) was better than other model options, and in no case was a linear fit significantly worse than other models. Therefore a linear model was used for all analyses. In addition, the rate of change in soil properties for each individual paddock was determined using linear regression, and these rates of change were then plotted against average pasture production, stocking rates and nitrate leaching to determine if there was a relationship between the rate of change in soil properties and these variables. Simple linear regression in Genstat v 13 was also used to investigate the relationship between actual soil properties (rather than the rate of change) from individual sampling dates, and pasture production, stocking rates and nitrate leaching. Data on pasture production, stocking rates and nitrate leaching were drawn from Hoogendorn *et al.* (in prep).

Soil bulk density was not measured through time, but a one off sampling in the 0N, 100N and 500N treatments was carried out in 2006, which showed that there were no differences between treatments (Letica, 2012). Schipper *et al.* (2011) also found no difference in bulk density under pastures grazed by sheep at different intensities for more than 20 years (also in hill country). This suggests that if converted to an area basis, trends in the current study would be the same as for the %C and %N data. In the results section some of the %C and %N data is also expressed on an area basis (using a bulk density of 0.97, Letica, 2012) to allow easier comparison with other studies.

6.3 Results

6.3.1 Pasture production, grazing intensity and N leaching

Data on pasture production, grazing intensity and N leaching will be presented and discussed in detail in Hoogendoorn *et al.* (in prep). However, to provide context for the soils data presented here, data are briefly summarised below. In general, as N rate increased so too did pasture production, stocking rates and N leaching (Table 6.2). Leaching losses were higher than some other studies in hill country (Sakadevan *et al.*, 1993; Bowatte *et al.*, 2006) but were similar to losses reported by Parfitt *et al.* (2009). Causes for the high N leaching losses will be further discussed in Hoogendoorn *et al.* (in prep), but may have been related to the shallow nature of the lysimeters (300 mm), as well as the more intensive grazing management and the higher stocking rates than that present in the studies of Sakadevan *et al.* (1993) and Bowatte *et al.* (2006). In any case, the relative difference in N leaching between treatments provides a good indication of the relative effect of N fertiliser. On average about 70–80% of the leached mineral N was in the form of nitrate, with the remainder being ammonium (Table 6.2, Hoogendoorn *et al.*, in prep).

Table 6.2 Average annual pasture dry matter (DM) production, stocking rates and N leaching during the three years from March 2005 to March 2008. Data from Hoogendoorn *et al.* (in prep).

Treatment	Pasture production (t DM ha^{-1} y^{-1})	Stocking rate (SU ha^{-1}) [a]	Nitrate leaching (kg N ha^{-1} y^{-1})	Ammonium leaching (kg N ha^{-1} y^{-1})	Total mineral N leaching (kg N ha^{-1} y^{-1})
0N	10.00	22	58	26	85
100N	11.48	22	74	34	108
200N	11.31	23	91	36	127
300N	12.12	26	122	51	172
400N	12.86	25	184	41	225
500N	12.92	26	273	71	343
750N	13.30	27	301	107	407

[a] Here a stock unit (SU) is defined as one 55 kg ewe rearing a single lamb and consuming about 550 kg DM per year.

6.3.2 Influence of sampling depth on soil properties

The clearest trend observed in the soils data was a significant difference in C and N concentrations between the two depth increments, irrespective of treatment (see supplementary data, Figs. S6.1 & S6.2). Averaged across treatments, mean C in the 0–75 mm depth was 5.2% compared to 3.3% in the 75–150 mm depth, and mean TKN was 0.43% and 0.29% in the two depths respectively. In 2004–2006, the C:N ratio was lower in the 75–150 mm depth than the 0–75 mm depth, but there was no significant difference between the two depths in 2007 and 2008 (see Fig. S6.3). When soil samples for δ^{15}N analysis were obtained, samples for the 75–150 mm depth for 2004–2006 had been discarded. There was no significant difference in δ^{15}N between the two depth increments in 2007 and 2008, and subsequent data analysis for δ^{15}N was only conducted for the 0–75 mm depth samples.

6.3.3 Influence of N rate on soil properties

Of the soil properties analysed, δ^{15}N was influenced most by N rate. There was no change in δ^{15}N with time in the 0N treatment, but δ^{15}N increased significantly with time in treatments receiving $\geq$100 kg N ha^{-1} y^{-1}, with the increase being more rapid as N rate increased (Fig. 6.1). Increases in δ^{15}N ranged between a non-significant increase of 0.05 ‰ y^{-1} in the 0N treatment to a significant increase of 0.35 ‰ y^{-1} in the 750N treatment. In contrast, there was no change in TKN with time in any of the treatments for either of the two depths (Fig. 6.2a). Both C and the C:N ratio showed different trends in the two depths. In the 0–75 mm depth, C decreased by 0.11 % per year (~800 kg ha^{-1} y^{-1}) in the 0N treatment and 0.053 % per year (~380 kg ha^{-1} y^{-1}) in the 100N treatment, but there was no significant change in C at higher N rates (Fig. 6.2b). For the 75–150 mm samples there was an increase in C with time in all treatments (increases of 0.052–0.082 % per year or 380–600 kg ha^{-1} y^{-1}), but the increases in the 0N and 750N treatments were not significantly different from zero (Fig. 6.2b). If both depths were averaged (which would have been the case if only one 0–150 mm core had been taken) there was no significant change in C for any of the treatments (data not shown). There was no change in C:N with time in the 0–75 mm depth, but in the 75–150 mm depth C:N ratio increased with time in all treatments by about 0.4 units per year (Fig. 6.2c). When both depths were averaged, there was a significant increase in C:N with time in all treatments (data not shown).

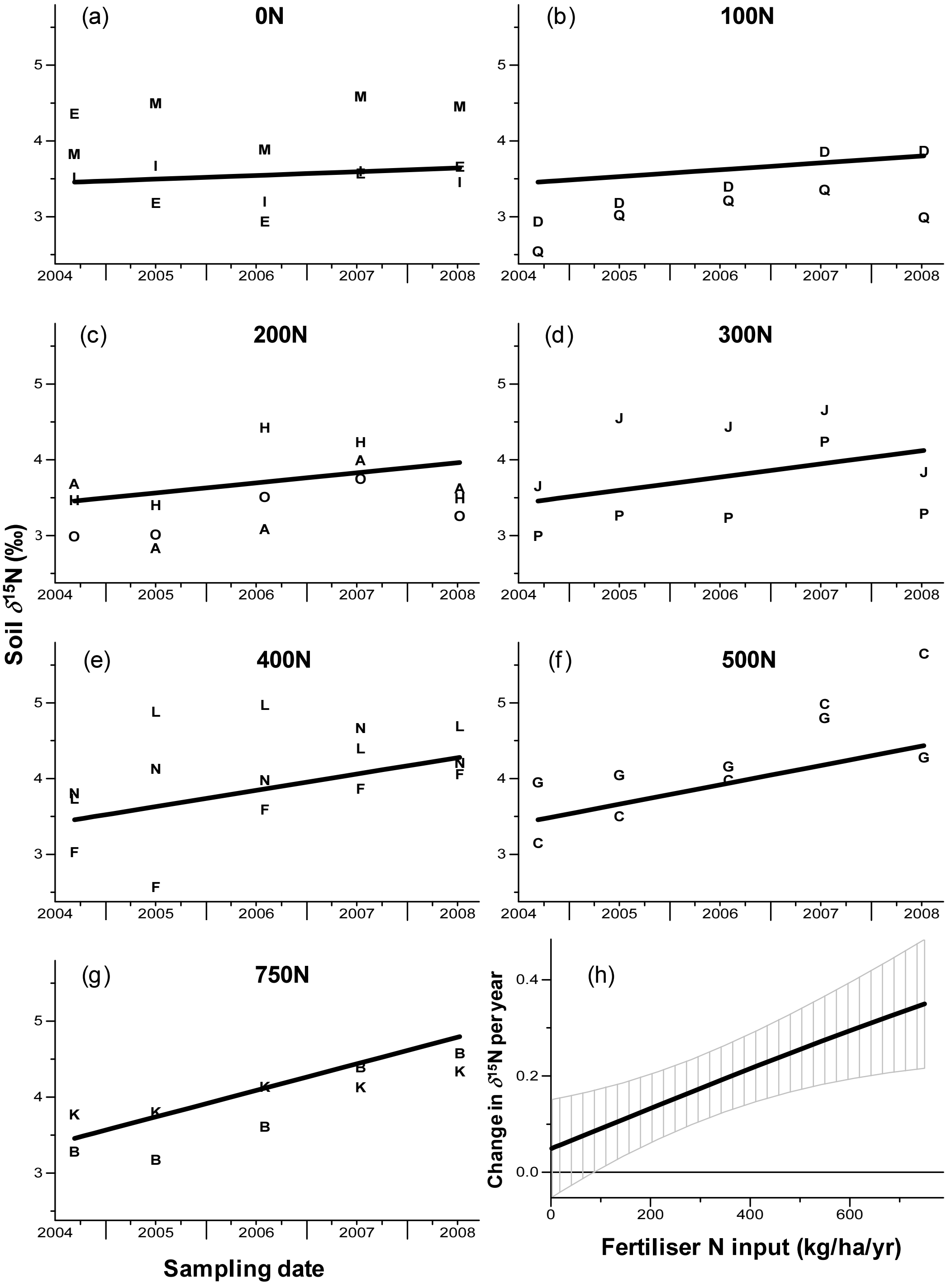

Fig. 6.1 Change in soil δ^{15}N with time in the different N fertiliser treatments (0–75 mm depth). Panels a–g show data for individual plots (denoted by different letters) and a linear regression line fitted to the data using a Bayesian smoother (Upsdell, 1994), which fits the line taking into account all the data, not just the data from an individual treatment. Note: all treatments were forced to be the same in 2004, because N applications did not commence until after the first soil sampling (see section 6.2.5 for more details). Panel h shows the average rate of change in δ^{15}N for different N rates with 95% confidence intervals (based on all the data in panels a–g). This shows that for N rates greater than about 100 kg ha^{-1} y^{-1} there was a significant increase in δ^{15}N with time.

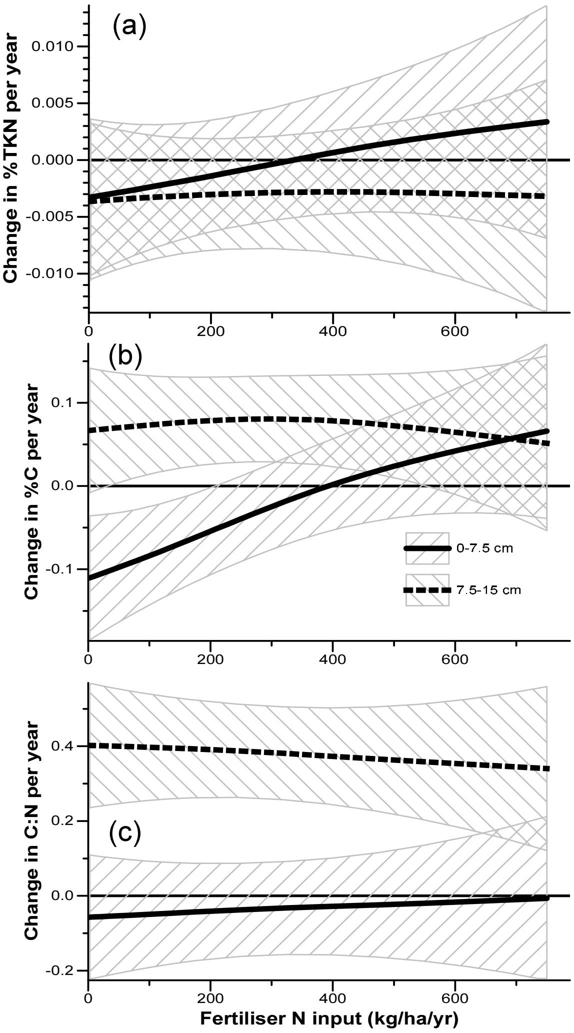

Fig. 6.2 Average rates of change per year for (a) %C, (b) total Kjehldahl Nitrogen (%TKN) and (c) C:N ratio, for the different N fertiliser rates and sampling depths. The lines for each depth are the best fit across all the data, with 95% confidence intervals and are fitted with a Bayesian smoother (Upsdell, 1994). If the confidence intervals do not include zero the rate of change for that N rate is significantly different from zero. Graphs showing the raw data and regressions for each treatment can be seen in supplementary material (Figs S6.1–S6.3).

6.3.4 Relationship between soil properties and N leaching etc.

The change in soil δ^{15}N in each paddock (determined using linear regression) was strongly correlated with average nitrate leaching ($p<0.001$, $r^2=0.6$) for each paddock, and was weakly correlated with average pasture production ($p=0.037$, $r^2=0.26$) (Fig. 6.3). Similar significant positive correlations between δ^{15}N and pasture production ($p=0.023$, $r^2=0.3$), and nitrate leaching ($p=0.001$, $r^2=0.5$) were also observed if δ^{15}N values from 2008 were used rather than the rate of change in δ^{15}N (data not shown). The relationship between δ^{15}N and N leaching was also very similar if total mineral N leaching (NO_3^- + NH_4^+) was used in the regression rather than just NO_3^- (data not shown). There was no significant correlation between $\delta^{15}N$ and stocking rate, despite stocking rates being correlated with DM production (r^2=0.68). There were no significant correlations between C, TKN or C:N and pasture production, stocking rate or nitrate leaching, except for a weak positive correlation between the average rate of change in C and nitrate leaching in the 75–150 mm depth (p=0.031, r^2=0.27) (data not shown).

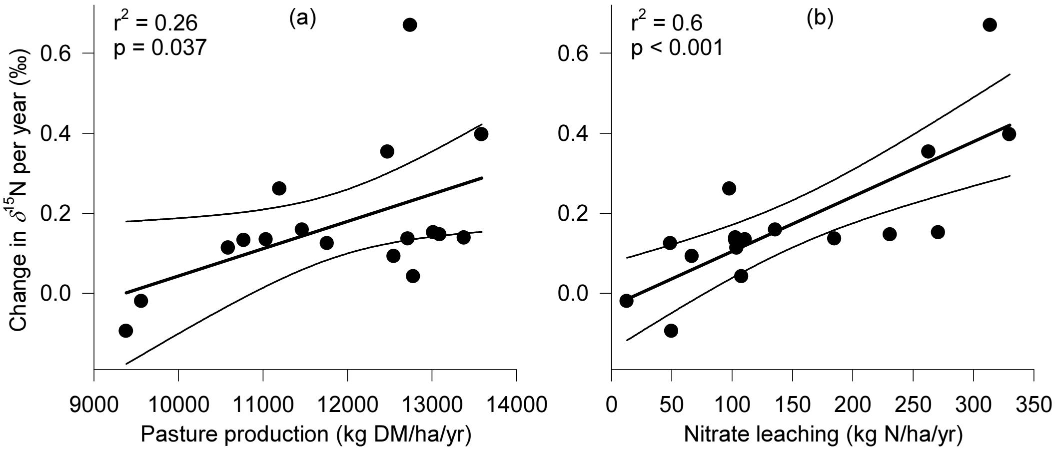

Fig. 6.3 Relationship between the average rate of change in $\delta^{15}N$ (determined via linear regression), and average (a) pasture production, and (b) nitrate leaching for each paddock. The line in each graph is a linear regression line with 95% confidence intervals.

6.4 Discussion

6.4.1 Nitrogen isotopes

Although N fertilisation (and associated changes in pasture production, stocking rates and N leaching) had no effect on total soil Kjeldahl nitrogen (TKN), it did influence the isotopic composition of soil N, with $\delta^{15}N$ increasing significantly with time in treatments receiving $\geq$100 kg N ha^{-1} y^{-1}, and increases were greater at higher N rates (Fig. 6.1). The increase in soil $\delta^{15}N$ with increasing mineral fertiliser N inputs is consistent with Watzka *et al.* (2006) and Johannisson and Högberg (1994), but other studies have found no change (Shearer *et al.*, 1978; Kriszan *et al.*, 2009) or decreases in soil $\delta^{15}N$ with increasing rates of mineral N fertiliser (Gerzabek *et al.*, 2001; Fang *et al.*, 2011). Decreases in soil $\delta^{15}N$ following application of mineral N fertiliser has been attributed to immobilisation of fertiliser N (generally with a delta value of ~0 ‰, Bateman and Kelly, 2007) in the soil causing dilution of the pool of total soil N (which usually has delta values of 3–10 ‰). It was not possible to directly measure the δ^{15}N of added N in the current trial (because no fertiliser was retained), but the same type of N fertiliser (urea) from the same source, was applied to another trial at Ballantrae and had a δ^{15}N value of –1.1 ‰ (Stevenson *et al.*, 2010). Therefore direct substitution of fertiliser N with soil N (remembering that there was no net change in soil N) would have caused a decrease in soil δ^{15}N, not an increase as was observed. For example, the top 75 mm of soil at Ballantrae contained about 3000 kg N ha^{-1}, which is the same as cumulative N fertiliser inputs to the 750N treatment over the four year study. Therefore if the added fertiliser N in the 750N treatment had mixed fully with N in the 0–75 mm depth, and there was no isotope fractionation during N loss, δ^{15}N at the end of the study would have been 1.2 ‰. Clearly this is much lower than the δ^{15}N values of ~4.5 ‰ measured in the 750N treatment at the end of the study (Fig. 6.1g), and indicates that although there was no net change in total soil N, ^{14}N must have been preferentially lost and ^{15}N retained.

There are a number of ways in which application of mineral N fertiliser could lead to an increase in soil δ^{15}N. One of the main N loss pathways in grazed grasslands is via nitrate leaching (Ledgard *et al.*, 1999; Di and Cameron, 2002; Monaghan *et al.*, 2005). At the site of this study, measured nitrate leaching increased significantly with increasing N application rates (Hoogendoorn *et al.*, in prep) and this likely contributed to the increase in soil δ^{15}N. The actual mass flow process of nitrate leaching does not directly cause isotopic fractionation (Högberg, 1997), but during mineralisation and nitrification (which occur prior to nitrate leaching) ^{14}N is preferentially processed (Robinson, 2001). Therefore the pool of soil nitrate is often depleted in ^{15}N, and since nitrate is very mobile it tends to be lost via leaching, while ^{15}N enriched ammonium (or organic N) is more likely to be retained in the soil (Handley and Raven, 1992). The significant positive correlation between nitrate leaching and the rate of change in δ^{15}N (Fig. 6.3) provides support for the suggestion that nitrate leaching contributed to the ^{15}N enrichment in treatments receiving higher rates of N. However, nitrate leaching was likely correlated with gaseous N losses and indeed at the same site Hoogendoorn *et al.* (2008) found that N_2O emissions also increased with N rate, and therefore gaseous N losses may have also contributed to ^{15}N enrichment (see below for more details). Because this was a retrospective study, it was not possible to measure the isotopic composition of leached N. Future studies should endeavour to measure δ^{15}N of leached N, particularly since Houlton and Bai (2009) demonstrated that the overall process of N leaching caused very little isotopic fractionation in unmanaged terrestrial ecosystems.

Mineral N leaching losses were on average 48% of added N (Hoogendoorn *et al.*, in prep), and since there was no net change in soil N, the other added N must have been lost via other pathways (i.e. volatilisation, denitrification, product export). Fertiliser was applied in the form of urea and therefore would have been susceptible to ammonia volatilisation, which can cause strong isotopic fractionation (Robinson, 2001; Frank *et al.*, 2004). Up to ~50 % of urea fertiliser N can be lost via ammonia volatilisation, with losses being greatest under warm, dry and windy conditions (Bolan *et al.*, 2004). At N rates $\geq$300 kg ha^{-1} y^{-1}, N was applied every two months (Table 6.1) and therefore volatilisation was probably proportionally higher from these treatments because more N was applied during warmer drier conditions. Increased pasture production as a result of N fertiliser, would have also meant that more N was ingested by sheep and then excreted in dung and urine, with urine patches in particular being hot spots for ammonia volatilisation (Bolan *et al.*, 2004; Saggar *et al.*, 2004). The trial site is characterised by consistently high windspeeds (Lambert *et al.*, 1983), which would have also facilitated high rates of volatilisation from fertiliser and sheep excreta. Other gaseous N losses such as denitrification and N_2O emissions also cause strong isotopic fractionation (Högberg, 1997; Robinson, 2001), and measured N_2O emissions at Ballantrae (both within and outside urine patches) increased with N application rate (Hoogendoorn *et al.*, 2008). Denitrification also tends to increase with N rate (Watson and Mills, 1998; Barton *et al.*, 1999; Bolan *et al.*, 2004), but was not measured at this site. The comparatively small amount of N exported in animal product (meat and wool) would have had minimal impact on soil δ^{15}N.

The positive correlations observed between soil δ^{15}N and N inputs (Fig. 6.1h), pasture production and N leaching losses (Fig. 6.3), were similar to some previous grassland studies. For example, Watzka *et al.* (2006) found that soil δ^{15}N was positively correlated with N fertiliser inputs and also ‘excess N’, which was defined as N inputs minus N exported in harvested biomass. Results from Chapter 4 showed that soil δ^{15}N was correlated with long-term average pasture production, calculated nitrogen fixation and N losses, and Frank and Evans (1997) found a positive correlation between ^{15}N enrichment in the soil and dung abundance (used as an indicator grazing intensity). Stevenson *et al.* (2010) qualitatively related soil δ^{15}N to management intensity and N inputs and losses in grazed pastures. However, the current study is the first that we are aware of that has found a direct correlation between measured N losses and δ^{15}N in grassland soils. Future studies should seek to relate soil δ^{15}N to more complete N budgets (i.e. quantify N loss pathways in addition to N leaching) and also directly measure the isotopic composition of N inputs and outputs (e.g. Frank *et al.*, 2004). This will help to identify more clearly the processes driving changes in soil δ^{15}N in grazed grassland systems.

The rate of change in δ^{15}N in the current study ranged between 0.05 and 0.35 ‰ y^{-1} (Fig. 6.1h), which is an order of magnitude higher than rates observed in long-term (~50 year) superphosphate and irrigation trials in New Zealand, where average rates of change ranged from –0.007 to 0.03 ‰ y^{-1}, and where differences between treatments were only detectable on decadal time scales (Chapter 4). Changes in δ^{15}N with time in other multiyear field trials ranged from decreases of 0.13 ‰ y^{-1} to increases of 0.18 ‰ y^{-1} (Gerzabek *et al.*, 2001; Bol *et al.*, 2005; Billings and Richter, 2006; Gubsch *et al.*, 2011), which are all lower than the increases of 0.22, 0.26 and 0.35 ‰ y^{-1} in the 400N, 500N and 750N treatments respectively (Fig. 6.1h). The high rates of change in δ^{15}N in the current study were probably related to the high N inputs, which would have facilitated high rates of N cycling and isotope fractionating N losses. It is important to note that all the N rates used in this study (except perhaps the 100N treatment) were considerably higher than rates currently used on commercial hill country farms, and so results must be interpreted with caution. Despite this, such high rates of N could give an indication of the cumulative effects of lower N rates applied over a longer time period (i.e. 750 kg ha^{-1} y^{-1} for 4 years is the same total N loading as 200 kg ha^{-1} y^{-1} for 15 years, although N losses will likely be less with lower annual N rates due to better N use efficiency, Hoogendoorn *et al.* in prep). Therefore under typical farm management practices changes in soil δ^{15}N will probably only be detectable on decadal time scales, as concluded in Chapter 4.

6.4.2 Carbon and nitrogen

Despite the relatively short duration of this study, the very high rates of N application and associated changes in pasture production and stocking rates would have provided the ideal situation for differences in C and N between treatments to have developed. Increasing rates of fertiliser would not only have increased N inputs to the soil, but also C inputs due to an increase in pasture production (Saggar *et al.*, 1997; Hoogendoorn *et al.*, in prep). However, when the full 0–150 mm depth of soil was considered there were no significant differences in C, N or C:N ratio between treatments. This indicates that overall, outputs of C and N must have matched inputs. Indeed, measured mineral N leaching losses increased significantly with increasing N rate (see Table 6.2). Nitrous oxide emissions also increased with N application rate (Hoogendoorn *et al.*, 2008), and it is likely rates of denitrification and ammonia volatilisation also increased (Barton *et al.*, 1999). The increase in pasture production (with increasing N rate) would have increased outputs of N in product (meat and wool), although the increase would have been relatively small compared to other N loss pathways. For example, the OVERSEER® nutrient budgeting model (developed for New Zealand pasture systems) predicts that about 12 kg N ha^{-1} would have been exported in product from the 0N treatment and 18 kg N ha^{-1} from the 750N treatment. Some N (and C) may have also been immobilised in the soil below 150 mm, in which case it would have not been measured in the soil or leachate. The major pathway for increased C loss was most likely via respiration, which tends to scale closely with gross primary production on an annual basis (Baldocchi, 2008).

The lack of difference in soil C and N between treatments is consistent with a number of other studies. In a small plot study on the same farm as the current trial, Parfitt *et al.* (in press-a) found no difference in soil C and N (0–100 mm depth) between a treatment which had received no N fertiliser and a treatment which had received 300 kg N ha^{-1} y^{-1} for 5 years. The C:N ratio however, was significantly lower in the N fertilised plots. In a similar small plot study, Dodd and Mackay (2011) also found no difference in soil C or N (0–75 mm depth) after 4 years of either no N application or N application of 400 kg N ha^{-1} y^{-1}. Studies of long-term superphosphate trials in New Zealand have also revealed no differences in soil C (0–75 mm depth) between treatments, despite large differences between above ground pasture production and stocking rates (Lambert *et al.*, 2000; Ghani *et al.*, 2003; Schipper *et al.*, 2011; Schipper *et al.*, in press). In two of these superphosphate trials (Lambert *et al.*, 2000; Schipper *et al.*, in press) soil N increased more in treatments receiving higher rates of superphosphate, and this was attributed to greater clover production leading to higher N inputs via N_2 fixation. A number of international studies have also found that the rate of C and N accumulation in grassland soils was not related to fertiliser N input (Clement and Williams, 1967; Hassink and Neeteson, 1991; Hassink, 1994; Watzka *et al.*, 2006; Kriszan *et al.*, 2009). Although like Parfitt *et al.* (in press-a), Hassink and Neeteson (1991) found significantly lower C:N ratios in plots fertilised with high rates of N (250–700 kg N ha^{-1} y^{-1}) than in unfertilised plots. In contrast to the previous studies, Watson *et al.* (2007) found a positive correlation between N inputs and soil C and N accumulation during an 11 year study in Ireland. The C:N ratio also decreased with increasing N rate. In the study of Watson *et al.* (2007), C and N accumulated rapidly in all treatments (~100–150 kg N ha^{-1} y^{-1} and ~1100–1450 kg C ha^{-1} y^{-1}), presumably due to cultivation and reseeding at the start of the trial and a change in management from a cut grassland to a grazed grassland, causing soil C and N to increase towards a new higher equilibrium. In the current trial, the soil had been under grazed pasture for ~100 years and may have been approaching an equilibrium C and N content (Jackman, 1964a; Hassink and Neeteson, 1991; Schipper and Sparling, 2011), and therefore the soil may not have had much capacity to store additional C and N, despite N fertilisation increasing inputs. The majority of studies therefore indicate that the rate of mineral N fertilisation has limited impact on C and N immobilisation rates in pasture soils, and therefore increasing N rates must lead to increased C and N losses, in step with inputs. Clearly, more work is required on the fate of applied fertiliser N, and long-term net C and N immobilisation rates under permanent pastures.

While there were no differences in C between treatments, or changes in C with time when the full sampling depth was considered, there were significant changes within the two depth increments (Fig. 6.1). Other studies have also found different trends in C (and N) in different depth increments, with a common occurrence being that a significant difference is observed between treatments (or sampling times) for the surface soil but no significant difference when the full profile is considered, due to opposite trends and/or greater variability lower in the profile (Watson *et al.*, 2007; Kravchenko and Robertson, 2011; Schipper and Sparling, 2011). Therefore analysis of different depths/horizons is important to more fully understand C and N dynamics at a particular site. The significant decrease in C with time in the 0–75 mm samples of treatments receiving <200 kg N ha^{-1} y^{-1} (Fig. 6.1b), was similar to Lambert *et al.* (2000) who measured decreases of about 200 kg C ha^{-1} y^{-1} in archived surface soil samples (0–75 mm) taken on the same farm (from farmlets receiving no or very little N fertiliser) between 1972 and 1987. In a small plot N trial on the same farm, Parfitt *et al.* (2010) reported a significant decline in C and N (in all treatments) within just one year. This was attributed to an infestation of porina moth caterpillars (*Wiseana spp.*) which severely reduced pasture production and presumably C inputs, and at the same time probably increased respiratory losses. C had recovered to pre-caterpillar infestation levels after 2 years (Parfitt *et al.*, 2010). In another hill country trial, Schipper *et al.* (2011) found that C and N in the surface soil (0–75 mm) increased during the first 6 years and then subsequently decreased, with the decrease primarily attributed to a series of dry summers. The current study and the studies of Parfitt *et al.* (2010), Schipper *et al.* (2011) and Lambert *et al.* (2000) therefore indicate that surface soil C (and N) under hill country pastures in New Zealand can be temporally dynamic, increasing or decreasing quite markedly during relatively short time periods.

Most studies of pastoral soils have found that the C:N ratio declines with time as pastures develop (Hassink, 1994; Lambert *et al.*, 2000; Sparling and Schipper, 2004; Schipper and Sparling, 2011), and that the C:N ratio is often lower in soils receiving higher rates of N fertiliser (Hassink and Neeteson, 1991; Watson *et al.*, 2007; Parfitt *et al.*, in press-a). Therefore the lack of a relationship between C:N and N fertiliser input, and the significant increase in C:N ratio with time in the 75–150 mm depth of all treatments in the current study was the opposite to what we expected. This increase in C:N ratio was largely driven by an increase in soil C

(Fig. 6.2b) combined with a slight decrease in TKN (Fig. 6.2a) in the lower depth. It is unclear why C tended to increase with time in the lower depth (in all treatments).

6.5 Conclusions

The main objective of this study was to determine if different rates of mineral N fertiliser (urea) influenced soil C, N and δ^{15}N in a sheep grazed hill country pasture. Despite the high rates of N fertiliser input (0–750 kg N ha^{-1} y^{-1}), there were no significant differences in soil C and N between treatments or changes with time in the top 0–150 mm of soil. The C:N ratio was also unaffected by N rate, but increased with time in all treatments, which is opposite to what typically occurs in pastoral soils. The lack of N retention in the soil is consistent with the high mineral N leaching losses reported by Hoogendoorn *et al.* (in prep) at the same site. While N fertilisation had no effect on the amount of soil N, it did alter the isotopic composition of soil N, with δ^{15}N increasing significantly with time in treatments receiving $\geq$100 kg N ha^{-1} y^{-1}. The increase in δ^{15}N cannot be directly due to the application of fertiliser N because applied N would have had lower δ^{15}N values than the soil. Therefore greater isotope fractionating N losses (e.g. nitrous oxide emissions, ammonia volatilisation and nitrate leaching) at higher N rates must have caused the increase in δ^{15}N. This assertion was supported by the significant positive correlation between the rate of change in δ^{15}N and nitrate leaching. These results suggest that δ^{15}N is more sensitive to N inputs and losses than changes in total soil N or the C:N ratio, and therefore soil δ^{15}N could be a useful indicator of past N cycling and loss in pastoral systems.

Acknowledgements

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6.6 Supplementary figures

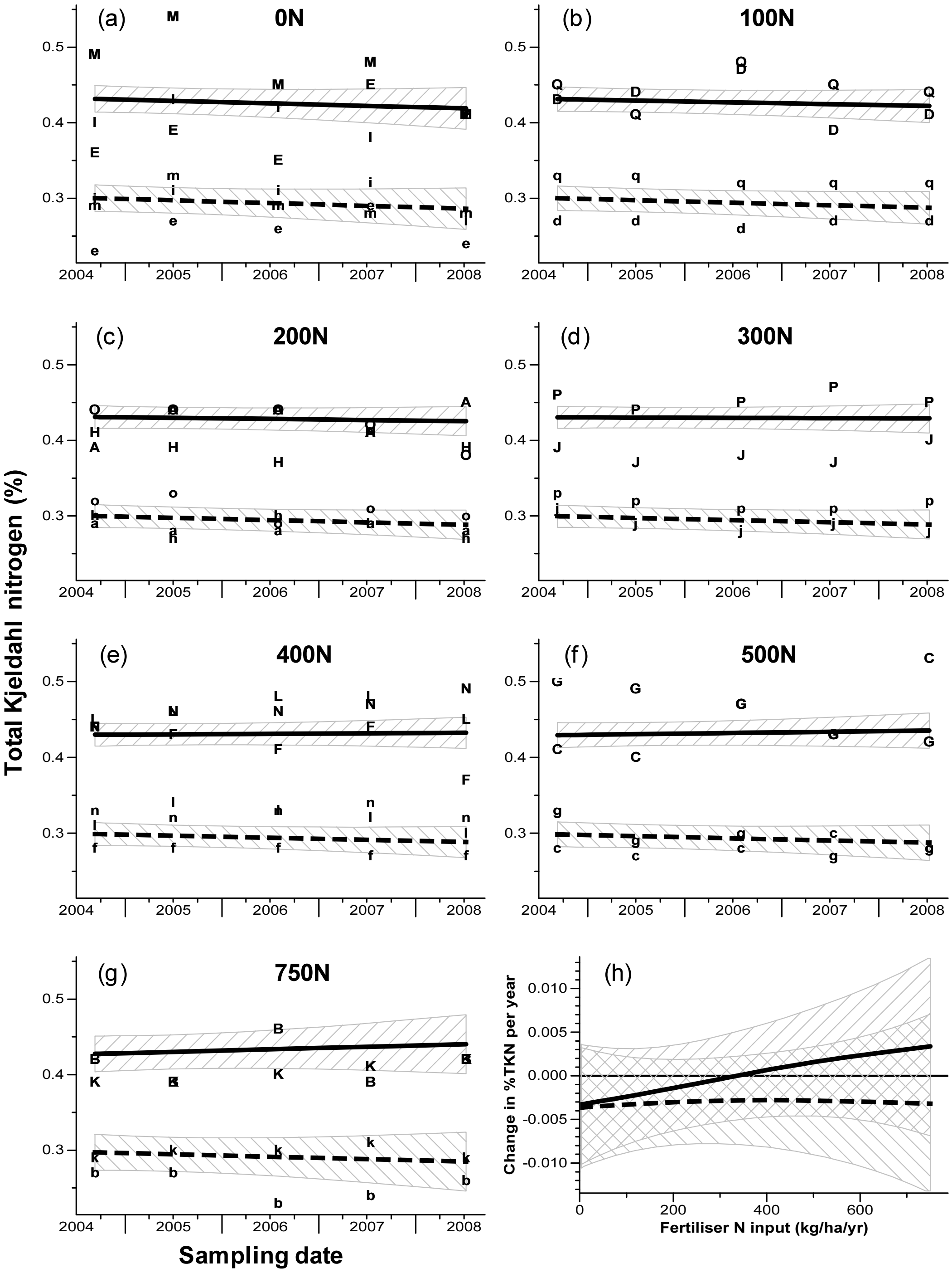

Fig. S6.1. Panels a–g show total Kjeldahl nitrogen (TKN) data for soils from individual paddocks in the different treatments. Upper case letters are for the 0–75 mm depth and lower case letters for the 75–150 mm depth. Lines are linear regression lines fitted to the data using a bayesian smoother (Upsdell, 1994) which fits the line taking into account all the data, not just the data from an individual treatment (solid line is 0–75 mm depth and dotted line 75–150 mm depth). Confidence bands are 95 % intervals for the position of the curve. Panel h shows the average rate of change in TKN for different N rates with 95 % confidence intervals (based on all the data in panels a–g).

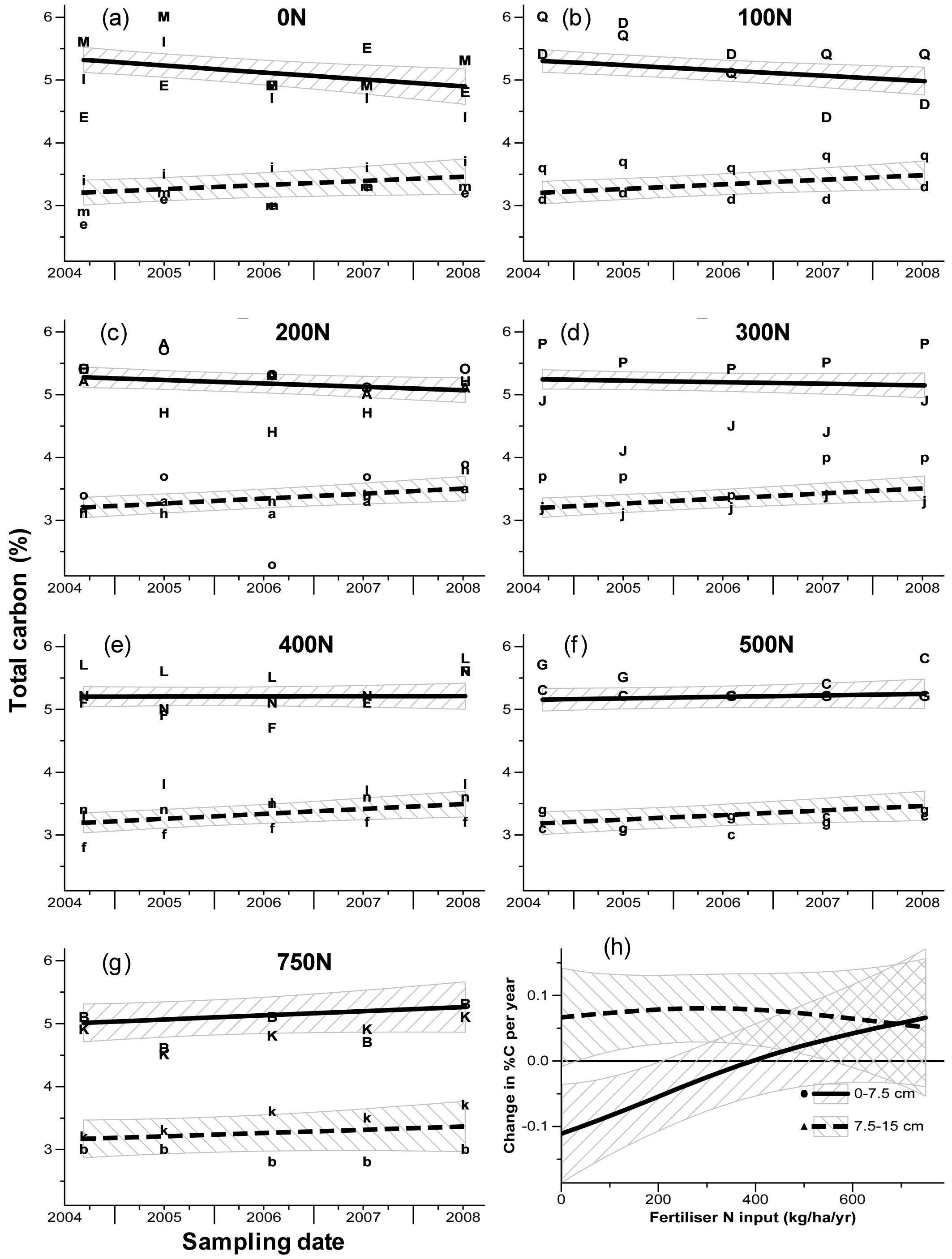

Fig. S6.2. Panels a–g show total carbon (%C) data for soils from individual paddocks in the different treatments. Upper case letters are for the 0–75 mm depth and lower case letters for the 75–150 mm depth. Lines are linear regression lines fitted to the data using a bayesian smoother (Upsdell, 1994) which fits the line taking into account all the data, not just the data from an individual treatment (solid line is 0–75 mm depth and dotted line 75–150 mm depth). Confidence bands are 95 % intervals for the position of the curve. Panel h shows the average rate of change in %C for different N rates with 95 % confidence intervals (based on all the data in panels a–g).

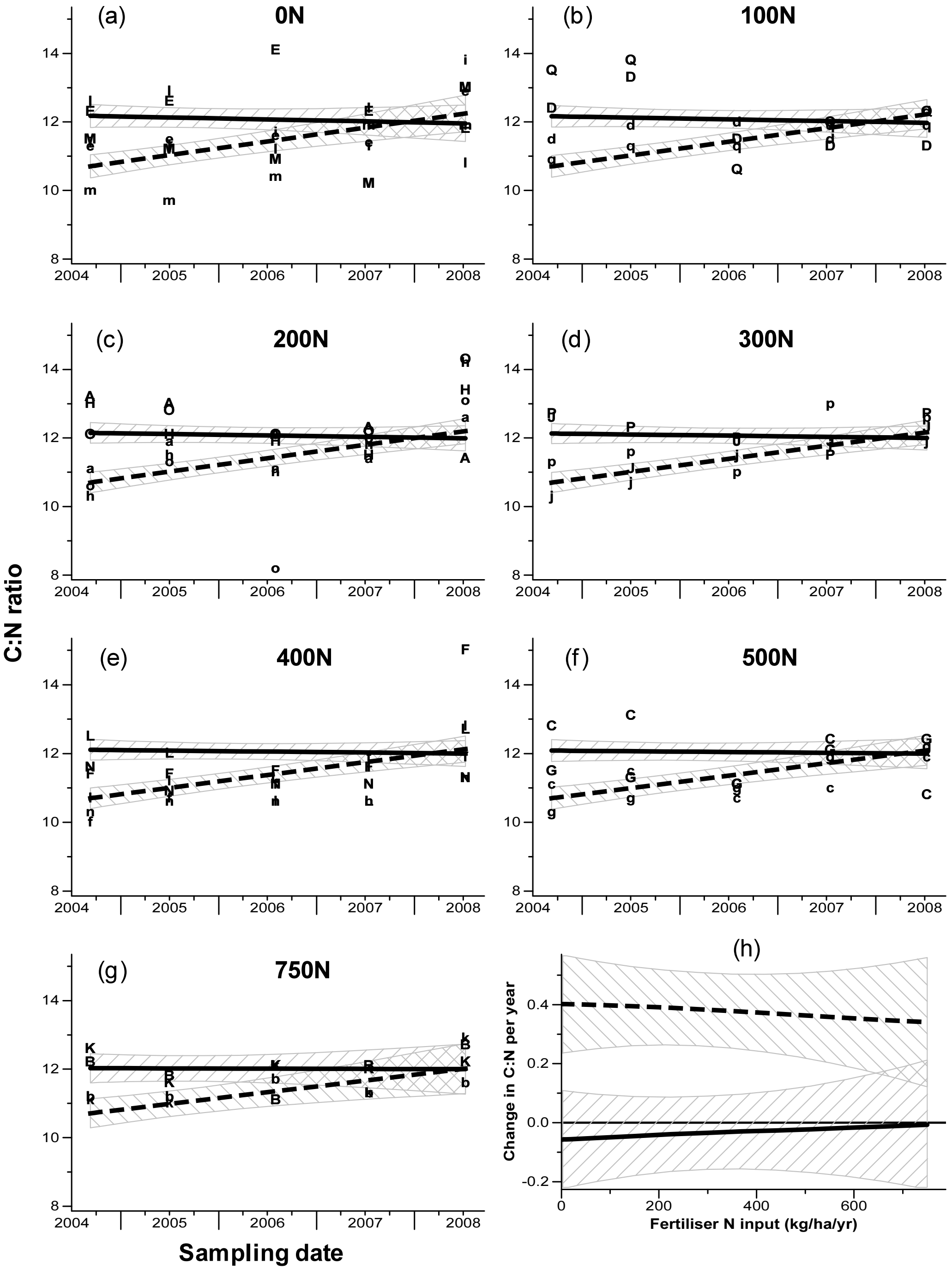

Fig. S6.3. Panels a–g show the C:N ratio for individual paddocks in the different treatments. Upper case letters are for the 0–75 mm depth and lower case letters for the 75–150 mm depth. Lines are linear regression lines fitted to the data using a bayesian smoother (Upsdell, 1994) which fits the line taking into account all the data, not just the data from an individual treatment (solid line is 0–75 mm depth and dotted line 75–150 mm depth). Confidence bands are 95 % intervals for the position of the curve. Panel h shows the average rate of change in the C:N ratio for different N rates with 95 % confidence intervals (based on all the data in panels a–g).

7 Summary and conclusions

7.1 Introduction and review of thesis aim and hypotheses

The ratio of naturally occurring nitrogen isotopes ^{14}N and ^{15}N ($\delta^{15}N$) in soils can provide useful information on N cycle processes (Högberg, 1997; Robinson, 2001). This occurs because the slight difference in mass between the two isotopes means that they behave differently in many physical, chemical and biological processes. In general, ^{14}N reacts faster than ^{15}N, and therefore tends to be preferentially lost during the main N transformation processes in soil (e.g. ammonia volatilisation, nitrification, denitrification or N leaching). Therefore soils with higher N inputs, cycling and losses are often enriched with ^{15}N relative to soils with lower N inputs and losses (Johannisson and Högberg, 1994; Watzka *et al.*, 2006; Stevenson *et al.*, 2010). Recent research in New Zealand by Stevenson *et al.* (2010) revealed a clear separation in $\delta^{15}N$ between land uses; forests (both native and exotic) had the lowest $\delta^{15}N$, while intensive dairy pastures and cropping soils had the highest $\delta^{15}N$, with soils under more extensively managed sheep-beef pastures having intermediate $\delta^{15}N$. The difference in $\delta^{15}N$ between land uses was largely attributed to differences in management intensity and more specifically N inputs, and isotope fractionating N losses, which typically increase from forests through to cropped land. These results suggested that $\delta^{15}N$ had potential to be a useful indicator of past management intensity and long-term N cycling and loss processes that have occurred in soils.

The overarching hypothesis for this thesis was that pastoral soils under intensive management regimes (with high N inputs, cycling and losses) would become progressively enriched with ^{15}N relative to soils under less intensive management. This hypothesis was tested by measuring $\delta^{15}N$ in soils from forest-to-pasture chronosequences, and also archived samples from long-term grazed field trials with different fertiliser or irrigation regimes in New Zealand.

More specific hypotheses tested in this thesis are listed below (see Chapter 1 section 1.3 for additional detail around hypotheses).

7.1.1 Hypotheses

1. Soil δ^{15}N will increase with time following conversion of forest or scrub to pasture, due to increased N inputs and isotope fractionating N losses.

2. Soil δ^{15}N will increase more rapidly in more intensively managed pastures receiving higher rates of superphosphate fertiliser, nitrogen fertiliser or more frequent irrigation.

3. Soil δ^{15}N will be a useful indicator of past management intensity and long-term N inputs, cycling and losses from pastoral systems.

The following section (7.2) will evaluate how these hypotheses were supported (or not supported) by the results in this thesis, and then the final section (7.3) will present recommendations for future research.

7.2 Evaluation of hypotheses

7.2.1 Hypothesis 1

Soil δ^{15}N will increase with time following conversion of forest or scrub to pasture, due to increased N inputs and isotope fractionating N losses.

This hypothesis was tested by analysing soils from four forest-to-pasture chronosequences. Three of the chronosequences were on pumice soils in the Central North Island, where exotic pine forest plantations had been converted to pasture, and the fourth chronosequence was on a podzol soil in Northland, where native scrub had been converted to pasture.

Results from the three forest-to-pasture chronosequences on pumice soils, supported hypothesis 1, with a clear increase in surface soil δ^{15}N between pine forests (2 ‰), and long-term pastures (4.1 ‰). However, results from the Northland chronosequence did not support the hypothesis with no systematic change in δ^{15}N between the scrub and pasture sites. The factors potentially influencing δ^{15}N for the different chronosequences are summarised below.

The increase in δ^{15}N between the pine forests and long-term pastures in the chronosequences on the pumice soil was very likely due to increased N inputs and isotope fractionating N losses (e.g. ammonia volatilisation) from the pasture sites. Total N inputs would have probably been >200 kg ha^{-1} y^{-1} for the pasture sites, but <10 kg ha^{-1} y^{-1} for the forest sites, and research has shown that N losses are generally much higher from pastures than forests (Di and Cameron, 2002; Menneer *et al.*, 2004b). This is further supported by the increase in both total soil N and δ^{15}N under the pastures, because if isotope fractionating N losses had not occurred, immobilisation of N in the soil should have caused δ^{15}N to decrease, since N fixed in association with legumes and fertiliser N generally have δ^{15}N values of ~0 ‰.

Analysis of full soil profiles revealed a significant increase in δ^{15}N between the A horizon and subsoils in the pine forest sites, but no significant difference between the A horizon and subsoil horizons in the long-term pasture sites. This was attributed to ^{15}N enrichment of the A horizons in the pasture sites, and suggested that the difference in δ^{15}N between surface soil and subsoil could also be a useful indicator of past management intensity and N inputs and losses. This profile indicator may be more suitable (than absolute values of surface soil δ^{15}N) for comparing across soil types/locations, where baseline δ^{15}N values differ. This hypothesis needs to be investigated further.

It was unclear why surface soil δ^{15}N did not increase as pasture age increased in the Northland chronosequence, particularly when total N increased, and C:N ratio decreased as would be expected (and as was observed for the chronosequences on the pumice soil). There was considerable variation in δ^{15}N between sampling sites (including the scrub sites), which suggested that site specific factors were important, and that differences in δ^{15}N may have been present prior to pasture establishment. The soil at the Northland site was quite extreme, being old, weathered, and strongly podzolic due to formation under kauri forest with acidic litter. The age of the soil would have meant that even small differences in N cycling (over long time periods) could have led to large pre-existing differences in δ^{15}N between sites, which may have masked management impacts. Another possible explanation is that kauri gum diggers had disturbed the soil profiles, turning subsoil enriched with ^{15}N up into the surface soil in places.

Summary

Results from the three chronosequences on the pumice soil provided strong support for hypothesis 1, and the conclusion of Stevenson *et al.* (2010), which was that the effect of land use, and more specifically N inputs and isotope fractionating losses were the key drivers of large differences in δ^{15}N between forests and pastures (rather than any potential differences caused by the spatial distribution of pasture and forest sites). The results from the chronosequences on pumice soils are expected to be more typical of results for most other soils in New Zealand (than results from the Northland chronosequence), but clearly further research on other soil types would be required to substantiate this suggestion.

7.2.2 Hypothesis 2

Soil δ^{15}N will increase more rapidly in more intensively managed pastures receiving higher rates of superphosphate fertiliser, nitrogen fertiliser or more frequent irrigation.

Hypothesis 2 was tested by analysing archived soils taken from long-term field trials receiving different rates of superphosphate fertiliser, irrigation or nitrogen fertiliser.

<u>*Chapter 4 – Winchmore superphosphate and irrigation trials*</u>

The two longest trials investigated in this thesis were at Winchmore on the Canterbury plains, where different rates of superphosphate and irrigation had been applied for ~50 years. Archived soils from these two trials provided an extremely valuable dataset, with no other studies reporting changes in soil δ^{15}N over such long time periods in grazed pasture systems (and there are few similar studies in other ecosystems). Results from these two trials provided support for hypothesis 2, with δ^{15}N increasing more in treatments receiving higher rates of superphosphate or more frequent irrigation. There were also positive correlations between the average rate of change in soil δ^{15}N, and total pasture production, clover production (thus N fixation) and calculated N losses. These results led to the conclusion that most of the difference in soil δ^{15}N between treatments was due to the influence that fertiliser and irrigation had on pasture production, N fixation by clover, and the flow on effects this had on N cycling and isotope fractionating loss processes (e.g. ammonia volatilisation).

Chapter 5 – North Island hill country superphosphate trials

Soil δ^{15}N was also measured in archived soils from three long-term (15–25 year) superphosphate trials on North Island hill country. Results from these three trials did not support hypothesis 2, with no consistent differences in soil δ^{15}N between treatments. It was not entirely clear why there were no differences in these hill country trials, particularly since treatments were very similar to the treatments in the Winchmore superphosphate trial (where there was a clear treatment effect). The shorter duration of the hill country trials, combined with smaller differences in pasture production between treatments and higher variability due to complex topography, may have contributed to the lack of observed differences between treatments. Differences in erosion between treatments (due to differences in thatch build-up), is another possible explanation. Interestingly, slope and aspect had a significant effect on soil δ^{15}N, with higher values on sheltered east facing slopes at Ballantrae and on easy slopes than steep slopes at Whatawhata and Te Kuiti. These differences in soil δ^{15}N between slopes and aspects were probably related to differences in pasture production, stock grazing behaviour (particularly excreta deposition) and erosion (see Chapter 5, section 5.4.2 for specific details). Because slope and aspect are permanent features of the landscape, differences in soil δ^{15}N could have begun to develop when pastures were first established in the early 1900s, or even prior to that under native vegetation. Therefore differences in soil δ^{15}N between slope and aspect had much longer to develop than any differences caused by different rates of superphosphate. More research is required to better understand the influence of slope and aspect on soil δ^{15}N (see section 7.3.1.2 for suggestions).

Chapter 6 – Nitrogen fertiliser trial

The final trial used to test hypothesis 2 was a N fertiliser trial, where N rates ranging from 0 to 750 kg ha^{-1} y^{-1} were applied to hill country pastures over a 4 year period. As for results from the two Winchmore trials, results from the N fertiliser trial, provided support for hypothesis 2, with $\delta^{15}N$ increasing significantly with time in treatments receiving ≥100 kg N ha^{-1} y^{-1}, and there was a positive correlation ($p<0.001$) between $\delta^{15}N$ and nitrate leaching. This occurred even though the duration of the trial was much shorter than the hill country trials in Chapter 5. The significant effect of treatment was almost certainly a consequence of applying very high rates of N, and showed that $\delta^{15}N$ can respond to treatments in hill country (in contrast to Chapter 5). Rates of change in soil $\delta^{15}N$ were about an order of magnitude higher than in the two Winchmore trials (Chapter 4), which was probably due to the very high N fertiliser inputs. Total soil N and the C:N ratio were not affected by treatment, which suggests that $\delta^{15}N$ may be a more sensitive indicator of past N inputs and losses than soil N or C:N ratio.

Summary

Results from the two ~50 year irrigation and superphosphate trials at Winchmore, and the shorter 4 year N rate trial at Ballantrae provided support for hypothesis 2, with soil $\delta^{15}N$ increasing significantly more in intensively managed treatments receiving higher rates of fertiliser or irrigation. This was attributed to increased N inputs (from fertiliser and clover fixation) and isotope fractionating N losses in the more intensively managed treatments. In contrast, results from the three hill country superphosphate trials did not support hypothesis 2, with no consistent differences in soil $\delta^{15}N$ between treatments. The lack of observed differences in the hill country superphosphate trials may have been due to, the shorter duration of the trials, smaller differences in pasture production between treatments, differences in erosion between treatments (due to differences in thatch build-up), or higher variability due to complex topography. The effect that topography has on soil $\delta^{15}N$ via the influence of factors such as erosion, pasture production, and grazing animal behaviour (particularly excreta deposition), is an interesting avenue for further research.

7.2.3 Hypothesis 3

Soil δ^{15}N will be a useful indicator of past management intensity and long-term N inputs, cycling and losses from pastoral systems.

Soil δ^{15}N could potentially be a useful indicator to add to a suite of soil quality measurements. Surface soil δ^{15}N in particular meets criteria outlined by Sparling and Schipper (2004), of samples being easy to collect, relatively cheap and easy to analyse, and there is an interpretive framework (e.g. high δ^{15}N values = high N losses). Because soil δ^{15}N provides an integrated measure of N cycle processes over long time periods, it could be a very simple and cost effective way of 'ranking' different parcels of land based on past N inputs and losses (particularly when management history is poorly known). If such an indicator could be developed it would be able to assist with targeting mitigation strategies and regulations (aimed at reducing N losses), to areas most at risk of N loss.

Research for this thesis has shown that δ^{15}N in pastoral soils can be influenced by past management intensity and management induced changes in N cycling, with soils at intensively managed sites, with high N inputs and losses, generally having higher δ^{15}N values than less intensively managed soils (Chapters, 3, 4, 6, and also see Stevenson *et al.,* 2010). These positive results supported hypothesis 3, and provided enough data for the development of two preliminary indicators of *"past management intensity and long-term N inputs, cycling and losses from pastoral systems"* (see section 7.2.3.1 for details). However, results from the Northland chronosequence and hill country superphosphate trials (Chapters 3 and 5), did not provide support for hypothesis 3, and along with the literature review, (Chapter 2, section 2.4), demonstrated that natural factors (such as climate, slope and aspect) can have a large influence on soil δ^{15}N, and in some cases natural effects can be greater than management induced changes in soil δ^{15}N. Therefore soil δ^{15}N will probably be most accurate as an indicator of past management intensity and management induced N losses, at sites with the same (or similar) topography, soils and climatic conditions. The difference between surface soil and subsoil δ^{15}N (see section 7.2.3.1), may be a better indicator of past management intensity and management related N losses when comparing across very different soil types or regions etc, but this needs to be investigated further. While the influence of natural factors such as, topography, soil type and climate might complicate the use of soil δ^{15}N as an indicator of past management intensity and management related N losses, soil δ^{15}N has potential to further our understanding of how these 'natural factors' affect N dynamics.

Average rates of change in soil δ^{15}N over the duration of the trials investigated in this thesis, ranged from –0.007 ‰ y^{-1} in the non-fertilised treatment of the Winchmore superphosphate trial, to 0.35 ‰ y^{-1} in the treatment receiving 750 kg N ha^{-1} y^{-1} in the N fertiliser trial at Ballantrae (Table 7.1). In all trials except the N fertiliser trial (Chapter 6), changes in δ^{15}N were <0.1 ‰ y^{-1}(Table 7.1). This suggests that under 'typical' pastoral management regimes, any changes in bulk soil δ^{15}N will probably only be detectable at decadal time scales. Therefore, as stated in hypothesis 3, soil δ^{15}N will be an indicator of long-term management and N dynamics. This will be especially true if trying to detect differences between treatments, when all treatments were increasing (or decreasing) but at slightly different rates. However, if N inputs and losses are high, changes may be detectable over shorter time intervals (e.g. the N fertiliser trial in Chapter 6).

Table 7.1 Average rates of change in soil δ^{15}N from various trials investigated in this thesis.

Trial	Trial duration (years)	Average rate of change in δ^{15}N across all treatments (‰ y^{-1})	Range of rates of change in δ^{15}N (‰ y^{-1})
Forest-to-pasture chronosequences (pumice soil)	~50	0.049	0.049
Winchmore superphosphate trial	51	0.011	-0.007–0.022
Winchmore irrigation trial	43	0.025	0.015–0.034
Ballantrae hill country superphosphate trial	17	0.049	0.011–0.089
Whatawhata hill country superphosphate trial	25	0.020	0.017–0.023
Ballantrae rate of N trial	4	0.181	0.049–0.350

7.2.3.1 Preliminary indicator of past management intensity and N losses

Table 7.2 proposes a preliminary indicator of past management intensity and N inputs and losses for New Zealand soils, based on soil δ^{15}N values. Two separate but related indicators are proposed: (1) absolute values for surface soil δ^{15}N based on results from this thesis and Stevenson *et al.* (2010), and (2) the difference between surface soil and subsoil δ^{15}N values. This second 'profile' indicator is very preliminary and is only based on results from the three forest-to-pasture chronosequences on the pumice soil in Chapter 3, and some other international studies in the literature (see Chapter 3, Table 3.3). The profile indicator is also illustrated in Fig. 7.1 for ease of understanding.

Table 7.2 Suggested preliminary indicators of past management intensity and N inputs and losses for New Zealand soils, based on (1) surface soil δ^{15}N values (~0–100 mm) and (2) the difference between surface and subsoil (~500 mm depth) δ^{15}N values.

(1) Surface soil δ^{15}N (‰)	(2) Surface minus subsoil δ^{15}N (‰)	Past N inputs and losses	Probable past land use and management intensity
<3	<–1.5	Low	Extensive pasture, forests, natural ecosystems
3–5	–1.5–0	Moderate	Moderate intensity pastoral systems
>5	>0	High	Intensive pasture, cropping land

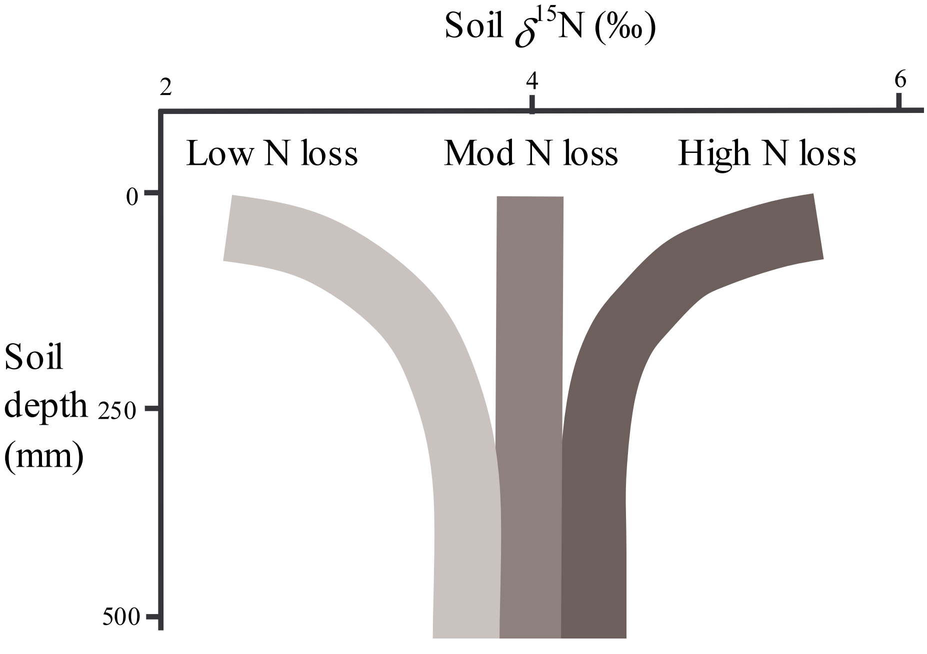

Fig. 7.1 Conceptual diagram showing the expected δ^{15}N profiles in New Zealand soils under pastures with different rates of N loss (based on results from Chapter 3). It is expected that if a forest or low intensity pastoral soil is converted to intensive pasture, the shape of the profile will progress from that under 'low N loss' to that under 'high N loss'. Absolute δ^{15}N values may differ for different soil types, but it is hypothesized that the difference between surface and subsoil δ^{15}N values will be similar. This hypothesis still needs to be tested further.

It is clear that there will be (and are) exceptions to the broad categories proposed in Table 7.2 particularly due to the influence of natural factors such as soil type, topography and climate. More research is required to determine if such general indicators will be useful, or if any indicator would need to be further stratified by 'natural factors' such as soil type or climate.

Soil δ^{15}N could also be thought of as an indicator of the *'duration and intensity of pastoral agriculture'*. This is because soil δ^{15}N values generally increase with time under pasture, and the rate of increase is generally higher in more intensively managed pastures with higher N inputs cycling and losses (Watzka *et al.*, 2006; Stevenson *et al.*, 2010; Chapter 3; Chapter 4; Chapter 6). Soil δ^{15}N may also be a useful indicator of the vulnerability to future N losses, and this suggestion is discussed further in section 7.3.2.2.

It is unlikely that soil δ^{15}N values will be able to provide accurate 'quantitative' estimates of past management intensity and N losses, and rather will probably be most useful as an indicator of 'relative' differences between sites. However, if more measurements are made of the isotopic signature of N inputs and outputs, and these data are combined with data on total N fluxes, there is potential to be able to use modelling to constrain/predict N losses via different pathways.

7.3 Recommendations for further research

Results from this thesis have shown that δ^{15}N changes with time in (some) pastoral soils, can be influenced by management and can be related to N inputs and losses. Therefore soil δ^{15}N may be a useful indicator of past management intensity, and N inputs and losses in pastoral systems (or the 'duration and intensity of pastoral agriculture' at a particular site). The following section outlines some avenues for future research, which could further improve our understanding of soil δ^{15}N (and total N cycle processes) in pastoral systems. Recommendations for future research are divided into two sections: 'basic' research and 'applied' research. The 'basic' research will help answer some important fundamental questions, which will assist with development of the more 'applied' research.

7.3.1 Basic research

7.3.1.1 Improve understanding of effect that soil type has on soil $\delta^{15}N$

Soil δ^{15}N results from the three forest-to-pasture chronosequences on the pumice soil, were quite different to results from the chronosequence on the podzol soil (Chapter 3). The chronosequences on the pumice soil showed the expected increase in δ^{15}N values with pasture age, while there was no clear relationship between pasture age and soil δ^{15}N for the chronosequence on the podzol soil, with a large variation in δ^{15}N values between sites. It would be desirable to carry out similar studies on other soil types, to determine if the ‘expected’ trends observed for the three chronosequences on the pumice soils are more widely applicable, or if high variability on the podzol soil is typical for other soil types in New Zealand. A constraint to such a study is that much of the recent conversion of forest to pasture has been on pumice soils in the Central North Island, although there are definitely some potential sites on the Canterbury Plains. Alternatively, a number of paired site studies of forest and pasture soils have been carried out in New Zealand in the past (mostly focusing on soil C), and these sites could be sampled and soils analysed for δ^{15}N (or any archived soils from these sites could be analysed for δ^{15}N).

There is also a need to improve understanding of the influence soil type has on soil δ^{15}N, to determine if it is valid to compare δ^{15}N values between soil types (when the influence of management on soil δ^{15}N is the research question). The best way to answer this question would be to sample from different soil types, but under the same climatic and management regimes (ideally within the same paddock).

7.3.1.2 Improve understanding of effect that topography has on soil $\delta^{15}N$

Results from the hill country superphosphate trials (Chapter 5) revealed that both slope and aspect can have a greater influence on soil δ^{15}N than different rates of superphosphate fertiliser, and associated changes in pasture production and grazing intensity. This effect of slope and aspect will complicate the use of δ^{15}N as an indicator of past management, and management related N losses, but could provide useful information on N cycling in hill country pasture systems. More detailed studies on the influence of slope and aspect on soil δ^{15}N are warranted, to identify mechanisms driving the observed differences in δ^{15}N (e.g. erosion, pasture production, stock grazing behaviour and excreta deposition). One pertinent question to answer is whether the differences between slopes and aspects were present prior to pasture establishment, or developed subsequently. This question could be answered by sampling from different topographic positions in undisturbed native forests.

7.3.1.3 Measure δ^{15}N of N inputs and losses for pasture systems

Research from this thesis has improved our understanding of how soil δ^{15}N changes with time in pastoral soils (i.e. changes can be detectable on ~decadal time scales), and also identified broad drivers of changes (e.g. management intensity, pasture production and N inputs and losses). There is, however, a need to improve our understanding of the actual mechanisms driving changes in soil δ^{15}N in pastoral systems. This could be achieved by measuring the isotopic composition of the various N inputs, outputs and N pools in pastoral systems as shown in Fig. 2.1, Chapter 2. Determining the isotopic signature of leached N from grazed systems is a particular priority, for two reasons. (1) Leaching is often a major N loss pathway from pastoral systems (Di and Cameron, 2002), but the majority of studies where leachate or stream water δ^{15}N has been determined have been in natural, or ungrazed ecosystems (e.g. Houlton and Bai, 2009). (2) Urine patches are a key mechanism by which N is lost (Monaghan *et al.*, 2007), and urine N is depleted in ^{15}N relative to soil and pasture (Steele and Daniel, 1978). Therefore direct leaching of urine N should theoretically lead to enrichment of soil with ^{15}N. This hypothesis is not in agreement with recent studies in natural ecosystems, where it was concluded that N leaching caused no, or only very minor ^{15}N enrichment of soil (Houlton and Bai, 2009; Koba *et al.*, 2012). Similarly, the δ^{15}N of nitrate observed in some New Zealand streams (Clough *et al.*, 2011; Baisden and Douence, 2012), was similar to the soil δ^{15}N reported in this thesis and Stevenson *et al.* (2010). Measuring the full total N and δ^{15}N budget for a site (or number of sites), would be the best way to improve understanding of the mechanisms driving changes in δ^{15}N in pastoral soils. Conducting such a study at one of the long-term field sites (ideally Winchmore) studied in this thesis would provide additional data for interpretation.

7.3.1.4 *Modelling soil δ^{15}N and total N losses*

If more certainty can be gained around the isotopic composition of N inputs and losses in pastoral systems (as discussed above), there is potential to combine this data with soil δ^{15}N values and total N fluxes, and then use modelling to help constrain the magnitude and/or isotopic composition of any unmeasured N fluxes (e.g. Bai and Houlton, 2009; Houlton and Bai, 2009; Koba *et al.*, 2012). For example, basic modelling could be used to estimate the proportional contribution of leaching and gaseous N losses required to give measured soil δ^{15}N values, if the isotopic composition of N inputs and losses was known. Impacts of erosion processes could also be studied in this way for the hill-country sites.

7.3.1.5 *Do mycorrhizal fungi affect soil δ^{15}N in grazed pastures?*

There has been large amount of research into the effect that mycorrhizal fungi have on soil δ^{15}N, because fungi can cause very strong fractionation when transferring N to plants (N transferred is very depleted in ^{15}N) (Hobbie and Ouimette, 2009; Wallander *et al.*, 2009). Most of this research has been carried out in natural ecosystems. Differences in fertilisation and grazing have been shown to influence fungal abundance and species composition in pastoral systems (Bardgett *et al.*, 2001; Wardle *et al.*, 2004; Parfitt *et al.*, 2010; Parfitt *et al.*, in press-a), but it appears that there has been no research on the effect of mycorrhizal fungi on soil δ^{15}N in grazed pastures. It would interesting to investigate whether the observed effect of fertiliser or irrigation on soil δ^{15}N in this thesis could have been related to fungal abundance and dynamics. Mycorrhizal fungi have been shown to influence the distribution of ^{15}N within the soil profile (Billings and Richter, 2006; Högberg *et al.*, 2011), and therefore in any future studies it would be advisable to sample from greater depths in the profile than most of the studies in this thesis.

7.3.2 *Applied research*

7.3.2.1 *Can soil δ^{15}N be used to identify 'critical source areas' of N loss?*

The focus of this thesis was on how soil δ^{15}N changed with time under different pastoral management regimes, and whether soil δ^{15}N could be a useful indicator of past management intensity and N inputs and losses at farm or paddock scales. Recently there has been an emphasis on improving understanding of nutrient losses from critical source areas (CSA's) within paddocks**,** particularly in hill country (Betteridge *et al.*, 2010). Critical source areas are areas which contribute disproportionately to nutrient losses relative to their size (e.g. stock campsites where a large amount of excreta is deposited). A number of approaches have been used to identify CSA's for N loss, such as, directly measuring nitrification rates (Devantier *et al.*, 2012), mineral N concentrations (Betteridge *et al.*, 2011), actual N leaching losses (Hoogendoorn and Devantier, 2011), while another approach has been to identify where excreta is typically deposited using GPS tracking devices attached to grazing animals (Betteridge *et al.*, 2010; Betteridge *et al.*, 2012). One aim of this research is to be able to predict where these CSA's are so that they could be targeted by mitigation strategies (e.g. nitrification inhibitors) (Betteridge *et al.*, 2012). Directly measuring N losses or where animal excreta is deposited, is time consuming and expensive and can only realistically be carried out over relatively short time periods (i.e. daily to annual time scales). Soil δ^{15}N could also be used to identify CSA's, with results from Chapter 5 showing that soil δ^{15}N was higher on easy slopes where N losses would be expected to be higher, and Hawke (2001) found that soil δ^{15}N could identify sheep campsites. A major advantage of using soil δ^{15}N to identify CSA's is that one soil sample provides an integrated measure of N losses over long-time periods. Soil δ^{15}N could potentially be used in conjunction with direct measurements of N deposition in excreta and N losses to assist in calibrating/validating models to predict the location of CSA's. This is an area that warrants further research.

7.3.2.2 Determine if soil δ^{15}N could predict risk of future N losses

The ability of soil δ^{15}N to be able to provide an indication of past management intensity and N dynamics is useful for improving our understanding of N cycle processes in pastoral systems, and could be useful for modelling etc. However, having an indicator which could predict the vulnerability soils to future N losses would be more useful in a practical sense. For a set management regime, soils with high δ^{15}N may be more vulnerable to N losses than similar soils with lower δ^{15}N. The basis for this hypothesis is twofold. First, soils with high δ^{15}N have likely been subject to high N losses in the past, and therefore it could be simply argued that they will be vulnerable to on-going N losses. Another similar argument (but with some theoretical basis), is that soils with high δ^{15}N are likely to be more ‘saturated’ with nitrogen than soils with low δ^{15}N (Högberg *et al.*, 1996), and will therefore be less able to retain added N (Gundersen *et al.*, 1998; Schipper *et al.*, 2004). This hypothesis could be tested relatively easily by measuring N losses from soils of the same type but with different δ^{15}N values, if all soils were subjected to the same management regime. Such a study might be most suited to a barrel lysimeter approach.

Ultimately, the development of soil δ^{15}N into a useful indicator for determining past N losses, or future vulnerability of pastoral systems to N losses, will depend on our ability to identify situations where the hypothesised relationship of progressive ^{15}N enrichment driven by the level of management intensity occurs, and where it does not. This work has identified a number of situations where the overarching hypothesis of this thesis was generally supported, and some situations where other factors appear to be involved. In the situations where the hypothesis was not supported, it is likely that N loss processes favouring δ^{15}N values equal to or higher than average soil values may have been occurring. This possibility deserves further investigation, and should be evaluated against the possibility that expected increases in δ^{15}N are simply masked by other factors (e.g. variability). Therefore determining the isotopic signature of N inputs and losses (see section 7.3.1.3) is probably one of the key steps to furthering our understanding of soil δ^{15}N in pastoral systems, and the development of a more robust indicator of past, or future vulnerability to N losses from pastoral systems.

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Appendix A: Relationship between soil δ^{15}N, C/N and N losses across land uses in New Zealand

This appendix contains a published journal paper (Stevenson *et al.*, 2010), on which Paul Mudge was a co-author. This paper was produced during the mid-stages of this PhD, and reports δ^{15}N values from soils under a range of land uses in New Zealand. The paper is not directly part of work conducted for this PhD, but was directly aligned and is therefore provided for context. Paul Mudge supplied the lead author (Stevenson) with a literature review on soil δ^{15}N under different land uses, and contributed to interpretation of results and writing of the manuscript.

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Relationship between soil δ^{15}N, C/N and N losses across land uses in New Zealand

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ABSTRACT

Several of the major processes that result in N loss from soil (nitrification, ammonia volatilization, and denitrification) discriminate against ^{15}N and fractionate the stable N isotopes, thus δ^{15}N of ecosystem components has been suggested as an indicator of ecosystem N leakiness. This concept has been applied more successfully to native systems (primarily forest) than to managed systems where N inputs are greater and N cycling processes have potentially been modified. We analysed 210 New Zealand soils (0–100 mm depth) from different land-use systems (increasing in intensity of land use management from indigenous, to plantation forestry, pasture under drystock, pasture under dairy, and cropping) for δ^{15}N and measures of N availability (total N, C/N, and N mineralization) to determine whether increasing intensity of land use management would lead to increased soil δ^{15}N values. Mean soil δ^{15}N differed between land uses with intensively managed cropping having the highest mean soil δ^{15}N (6.2‰) followed by dairy (5.4‰), drystock (3.8‰), forestry (2.8‰) and indigenous forests (2.1‰). Over all land uses there was a negative correlation between δ^{15}N and the soil C/N ratio ($\rho = -0.73$) and regression analysis indicated a relatively strong linear relationship between δ^{15}N and C/N ($r^2 = 0.56$, $P < 0.001$) when cropping sites (where significant loss of soil C had occurred), and sites with C/N ratio >18 were excluded. Typical N balances for each land use showed that total N loss (and in particular fractionating N losses from ammonia volatilization and nitrate leaching) also increased with increasing land-use intensity. Our results indicate that soil δ^{15}N may be a useful tool in assessing potential N losses in different soils.

1. Introduction

Human activity has altered the nitrogen cycle through land-use change, agricultural intensification, and use of fossil fuels (Vitousek et al., 1997; Matson and Vitousek, 2006). The New Zealand landscape is dominated by managed land uses (pastoral land alone accounts for 45% of total land area), and the intensity of management of these lands has increased considerably during the last few decades. For example, N fertilizer addition has increased from approximately 50 Gg N in 1989 to 335 Gg N in 2005 (Parfitt et al., 2006). Increasing N additions may deplete the soils ability to store additional N (Schipper et al., 2004), and loss of reactive N from soil to waters and the atmosphere is becoming increasingly problematic, particularly in areas where intensification of agriculture is occurring (Galloway et al., 2008; Parliamentary Commissioner for the Environment, 2004). Indicators of the extent of N enrichment of soils, and of potential N loss would be beneficial to establish where remedial practices to reduce N loss to surface and ground waters should be focused.

Robinson (2001) suggested that δ^{15}N (the ratio of ^{15}N to ^{14}N in relation to atmospheric N_2) of ecosystem components represents an integrated signal of ecosystem N processes because the isotopic composition of these components is driven by the isotopic composition of inputs and outputs that can be modified by N fractionation within the system. Nitrification, denitrification and ammonia volatilization are processes that fractionate the N isotopes in soil (Delwiche and Steyn, 1970), and loss of N through these processes will generally result in an increase of ^{15}N in the remaining ecosystem components (see Table 1). Thus the δ^{15}N of ecosystem components can represent a measure of N leakiness or the degree of N saturation of a system (Pardo et al., 2006; Högberg, 1990, 1991).

In a study of global patterns in N isotopes of soil and vegetation, Amundson et al. (2003) suggested that $\delta^{15}N_{soil}$ [and $\Delta\delta^{15}N_{(plant-soil)}$] accurately portrays the sensitivity of ecosystems to increased rates of anthropogenically derived N, and implied that ecosystems with high $\delta^{15}N_{soil}$ should be considered sensitive to additional N inputs. Considerable research has been carried out in

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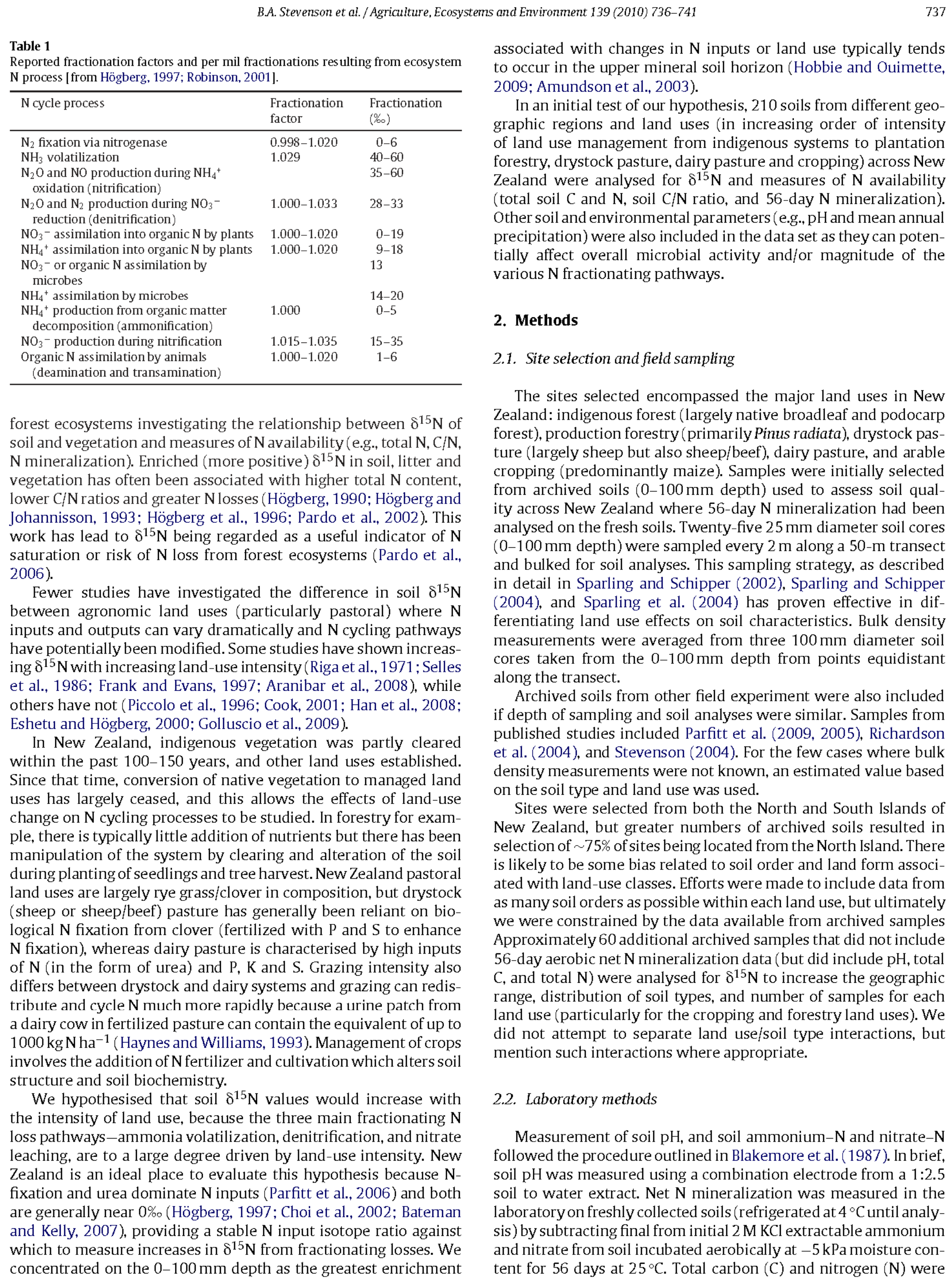

Table 1
Reported fractionation factors and per mil fractionations resulting from ecosystem N process [from Högberg, 1997; Robinson, 2001].

N cycle process	Fractionation factor	Fractionation (‰)
N_2 fixation via nitrogenase	0.998–1.020	0–6
NH_3 volatilization	1.029	40–60
N_2O and NO production during NH_4^+ oxidation (nitrification)		35–60
N_2O and N_2 production during NO_3^- reduction (denitrification)	1.000–1.033	28–33
NO_3^- assimilation into organic N by plants	1.000–1.020	0–19
NH_4^+ assimilation into organic N by plants	1.000–1.020	9–18
NO_3^- or organic N assimilation by microbes		13
NH_4^+ assimilation by microbes		14–20
NH_4^+ production from organic matter decomposition (ammonification)	1.000	0–5
NO_3^- production during nitrification	1.015–1.035	15–35
Organic N assimilation by animals (deamination and transamination)	1.000–1.020	1–6

forest ecosystems investigating the relationship between δ^{15}N of soil and vegetation and measures of N availability (e.g., total N, C/N, N mineralization). Enriched (more positive) δ^{15}N in soil, litter and vegetation has often been associated with higher total N content, lower C/N ratios and greater N losses (Högberg, 1990; Högberg and Johannisson, 1993; Högberg et al., 1996; Pardo et al., 2002). This work has lead to δ^{15}N being regarded as a useful indicator of N saturation or risk of N loss from forest ecosystems (Pardo et al., 2006).

Fewer studies have investigated the difference in soil δ^{15}N between agronomic land uses (particularly pastoral) where N inputs and outputs can vary dramatically and N cycling pathways have potentially been modified. Some studies have shown increasing δ^{15}N with increasing land-use intensity (Riga et al., 1971; Selles et al., 1986; Frank and Evans, 1997; Aranibar et al., 2008), while others have not (Piccolo et al., 1996; Cook, 2001; Han et al., 2008; Eshetu and Högberg, 2000; Golluscio et al., 2009).

In New Zealand, indigenous vegetation was partly cleared within the past 100–150 years, and other land uses established. Since that time, conversion of native vegetation to managed land uses has largely ceased, and this allows the effects of land-use change on N cycling processes to be studied. In forestry for example, there is typically little addition of nutrients but there has been manipulation of the system by clearing and alteration of the soil during planting of seedlings and tree harvest. New Zealand pastoral land uses are largely rye grass/clover in composition, but drystock (sheep or sheep/beef) pasture has generally been reliant on biological N fixation from clover (fertilized with P and S to enhance N fixation), whereas dairy pasture is characterised by high inputs of N (in the form of urea) and P, K and S. Grazing intensity also differs between drystock and dairy systems and grazing can redistribute and cycle N much more rapidly because a urine patch from a dairy cow in fertilized pasture can contain the equivalent of up to 1000 kg N ha^{-1} (Haynes and Williams, 1993). Management of crops involves the addition of N fertilizer and cultivation which alters soil structure and soil biochemistry.

We hypothesised that soil δ^{15}N values would increase with the intensity of land use, because the three main fractionating N loss pathways—ammonia volatilization, denitrification, and nitrate leaching, are to a large degree driven by land-use intensity. New Zealand is an ideal place to evaluate this hypothesis because N-fixation and urea dominate N inputs (Parfitt et al., 2006) and both are generally near 0‰ (Högberg, 1997; Choi et al., 2002; Bateman and Kelly, 2007), providing a stable N input isotope ratio against which to measure increases in δ^{15}N from fractionating losses. We concentrated on the 0–100 mm depth as the greatest enrichment associated with changes in N inputs or land use typically tends to occur in the upper mineral soil horizon (Hobbie and Ouimette, 2009; Amundson et al., 2003).

In an initial test of our hypothesis, 210 soils from different geographic regions and land uses (in increasing order of intensity of land use management from indigenous systems to plantation forestry, drystock pasture, dairy pasture and cropping) across New Zealand were analysed for δ^{15}N and measures of N availability (total soil C and N, soil C/N ratio, and 56-day N mineralization). Other soil and environmental parameters (e.g., pH and mean annual precipitation) were also included in the data set as they can potentially affect overall microbial activity and/or magnitude of the various N fractionating pathways.

2. Methods

2.1. Site selection and field sampling

The sites selected encompassed the major land uses in New Zealand: indigenous forest (largely native broadleaf and podocarp forest), production forestry (primarily *Pinus radiata*), drystock pasture (largely sheep but also sheep/beef), dairy pasture, and arable cropping (predominantly maize). Samples were initially selected from archived soils (0–100 mm depth) used to assess soil quality across New Zealand where 56-day N mineralization had been analysed on the fresh soils. Twenty-five 25 mm diameter soil cores (0–100 mm depth) were sampled every 2 m along a 50-m transect and bulked for soil analyses. This sampling strategy, as described in detail in Sparling and Schipper (2002), Sparling and Schipper (2004), and Sparling et al. (2004) has proven effective in differentiating land use effects on soil characteristics. Bulk density measurements were averaged from three 100 mm diameter soil cores taken from the 0–100 mm depth from points equidistant along the transect.

Archived soils from other field experiment were also included if depth of sampling and soil analyses were similar. Samples from published studies included Parfitt et al. (2009, 2005), Richardson et al. (2004), and Stevenson (2004). For the few cases where bulk density measurements were not known, an estimated value based on the soil type and land use was used.

Sites were selected from both the North and South Islands of New Zealand, but greater numbers of archived soils resulted in selection of ~75% of sites being located from the North Island. There is likely to be some bias related to soil order and land form associated with land-use classes. Efforts were made to include data from as many soil orders as possible within each land use, but ultimately we were constrained by the data available from archived samples Approximately 60 additional archived samples that did not include 56-day aerobic net N mineralization data (but did include pH, total C, and total N) were analysed for δ^{15}N to increase the geographic range, distribution of soil types, and number of samples for each land use (particularly for the cropping and forestry land uses). We did not attempt to separate land use/soil type interactions, but mention such interactions where appropriate.

2.2. Laboratory methods

Measurement of soil pH, and soil ammonium-N and nitrate-N followed the procedure outlined in Blakemore et al. (1987). In brief, soil pH was measured using a combination electrode from a 1:2.5 soil to water extract. Net N mineralization was measured in the laboratory on freshly collected soils (refrigerated at 4 °C until analysis) by subtracting final from initial 2 M KCl extractable ammonium and nitrate from soil incubated aerobically at −5 kPa moisture content for 56 days at 25 °C. Total carbon (C) and nitrogen (N) were determined by dry combustion on air-dried, sieved (<2 mm) and finely ground soils using a Leco 2000 CNS analyser (St Joseph, MI, USA).

Isotopic analysis was on air-dried, sieved (<2 mm) soils that were finely ground using mortar and pestle and any organic or root fragments visible to the naked eye removed. Measurement of soil ^{15}N was performed at the University of Waikato Stable Isotope Unit on a Europa Scientific 20–20 Stable Isotope Analyser with a Europa Scientific ANCA-SL inlet system (precision of ^{15}N analyses was ~0.05‰). Isotopic measurements are presented in the delta (δ) notation:

$$\delta^{15}\mathrm{N} = \left[\frac{R_{\mathrm{sample}}}{R_{\mathrm{std}}} - 1\right] \times 1000 \qquad (1)$$

where R_{sample} is the ^{15}N/^{14}N ratio in the sample and R_{std} is the ^{15}N/^{14}N ratio of the standard (atmospheric N_2) expressed as parts per thousand (‰).

Where applicable, soil measurements were reported on a volumetric basis for comparison across different soil types and land uses where bulk density may differ (Reganold and Palmer, 1995).

2.3. *Statistical analyses*

Statistical analyses were performed on Genstat v 10 (Lawes Agricultural Trust, 2007).

Data did not meet assumptions of equal variance even after normal transformations were used (indigenous and forestry groups had much higher variance than other land use groups). Normal parametric means tests, Fishers protected lsd following ANOVA for comparisons between sequential land-use intensity pairs (e.g., indigenous and forestry or drystock and dairy) and the Bonferroni correction for all other multiple pairwise comparisons, were used as a general guide to assessing differences between land use groups. As an added check, sequential pairwise comparisons were analysed with an unequal variance *t* test (one tailed at $\alpha = 0.05$ for δ^{15}N since we specifically hypothesised that δ^{15}N would increase with increasing intensity of land use, and two tailed for other parameters). Any differences between the two methods are noted in the text. To assess the relationship between all sets of variables, Spearman's Rank Correlation test was performed on all data as this non-parametric approach makes no assumption of linearity between groups. Regression analysis was then used to further explore the relationship between δ^{15}N and specific soil characteristics of interest.

3. Results

Mean soil δ^{15}N differed significantly between nearly all land use combinations (Fig. 1). Indigenous and forestry land uses were significantly different ($P < 0.05$) using the lsd means test but only marginally so ($P = 0.058$) using the unequal variance *t* test. Indigenous systems had the lowest mean soil δ^{15}N value followed by plantation forests, drystock pasture, dairy pasture, and cropping. Other measures of soil chemical and biological characteristics also differed significantly with land use (Table 2). Mean soil C concentration decreased in the order of: dairy, indigenous, drystock, forestry, and cropping.

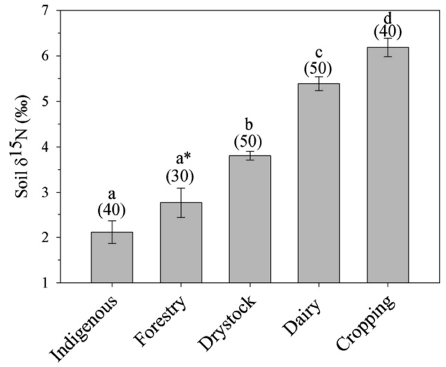

Fig. 1. Mean soil δ^{15}N (0–100 mm) for New Zealand land uses. Error bars represent one standard error of the mean. Differing letter above bars indicate significant differences ($P < 0.05$) between land uses. Numbers in parenthesis indicate the number of samples for each land use. *Indigenous and forestry sites were significantly different by lsd but only marginally so ($P = 0.058$) using an unequal variance *t* test.

Measures of N availability (total N, C/N ratio and N mineralization) all showed dairy soils as having the highest overall N status, but the order in which these measures changed with land use differed somewhat (Table 2). Mean total N concentration decreased in the order of dairy, drystock, indigenous, cropping, and forestry, whereas the mean soil C/N ratio increased in the order of dairy, cropping, drystock, indigenous, and forestry. Mean 56-day N mineralization on dairy and drystock sites was approximately twice that of indigenous, forestry and cropping sites.

Soil C/N ratio ($\rho = -0.73$) and precipitation ($\rho = -0.45$) were negatively correlated with δ^{15}N, whereas total N ($\rho = 0.22$) and pH ($\rho = 0.45$) were positively correlated with δ^{15}N (Table 3). Though statistically significant, Spearman's Rank correlation coefficients (except for C/N) were generally weak (less than 0.50). Other transformed measures of N status (1/total N and natural log total N) commonly plotted against δ^{15}N also showed low correlations with δ^{15}N (data not shown).

Plots of C/N against δ^{15}N are shown in Fig. 2. Using all land uses, linear regression was highly significant ($P < 0.001$) but the r^2 value was relatively low (0.32) and assumption of homoscedasticity technically not met. The relationship between C/N and δ^{15}N was more variable in the cropping land use and sites with higher C/N values (approximately >18). After excluding the cropping land use and sites with soil C/N > 18, the r^2 value for linear regression of C/N against δ^{15}N (N = 140) increased to 0.56 ($P < 0.001$).

4. Discussion

The increases in mean δ^{15}N values across land uses (native vegetation ≤ forestry < drystock < dairy < cropped soils) support our

Table 2
Mean soil (0–100 mm) characteristics by land use (one standard error of the mean in parentheses).

Land use	No. of sites	Total C (mg cm^{-3})	Total N (mg cm^{-3})	C/N ratio	pH	56-day N Min (μg cm^{-3})	δ^{15}N (‰)
Crop	40	40.3 (2.6)a	3.43 (0.20)a	11.8 (0.3)ab	6.30 (0.08)a	52 (7)a	6.2 (0.2)a
Dairy	50	65.7 (2.6)b	6.00 (0.25)b	11.0 (0.2)a	5.87 (0.05)b	131 (8)b	5.4 (0.2)b
Drystock	50	54.8 (2.1)c	4.43 (0.19)c	12.5 (0.2)b	5.70 (0.05)b	127 (10)b	3.8 (0.1)c
Forestry	30	46.8 (3.3)ac	2.80 (0.28)d	18.2 (0.7)c	5.57 (0.10)c	65 (8)a	2.8 (0.3)d
Indigenous	40	64.1 (6.1)bc	3.85 (0.34)ac	16.8 (0.5)c	5.04 (0.12)b	69 (9)a	2.1 (0.3)d*

* Comparison of mean δ^{15}N between forestry and indigenous land uses were significant using lsd but only marginally significant ($P = 0.058$) using unequal variance *t* test.

Table 3
Spearmen's Rank Correlation coefficient (ρ) between soil variables.

δ^{15}N	1						
C/N	-0.73^{***}	1					
Tot C	−0.10	0.00	1				
Tot N	0.22^{***}	-0.42^{***}	0.88^{***}	1			
N Min	0.15	-0.28^{**}	0.36^{***}	0.46^{***}	1		
pH	0.45^{***}	-0.48^{***}	-0.19^{*}	0.04	0.09	1	
Precipitation	-0.45^{***}	0.53^{***}	0.20^{*}	−0.07	-0.34^{***}	-0.28^{**}	1
	δ^{15}N	C/N	Tot C	Tot N	N Min	pH	Precip

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

hypothesis that intensifying land use management results in increased soil δ^{15}N values. The strong correlation between C/N and δ^{15}N suggests that N availability is a primary factor in explaining the increases in δ^{15}N across land uses. Pardo et al. (2006) also concluded from a large-scale study that forest floor δ^{15}N appears to be indicative of N status in forest systems. Reported isotopic values for N fixation (Högberg, 1997) and urea (Choi et al., 2002; Bateman and Kelly, 2007), the major N inputs into New Zealand systems, are near 0‰ (several urea samples we analysed in New Zealand averaged −1.1‰) and this also suggests that enrichment in soil δ^{15}N was generally a result of fractionating N losses within these systems.

Data on N loss for individual sites were not available, so the relationship between increasing δ^{15}N values and the magnitude of fractionating N losses was made by using average values for the different land uses. N cycles in most New Zealand land uses have been reasonably well studied, particularly for pastoral land uses (see for instance Monaghan et al., 2005; Bolan et al., 2004; Houlbooke et al., 2003; Power et al., 2002; Ledgard et al., 1999; Ruz Jerez, 1991; Carran et al., 1982; Neary et al., 1978). Estimates for magnitude of total N loss (and the major N loss pathways) are shown in Table 4. While these are generalised estimates (based both on field measurements and the OVERSEER™ nutrient budgets model), we believe they are sufficient to qualitatively compare δ^{15}N to N loss across the various land uses.

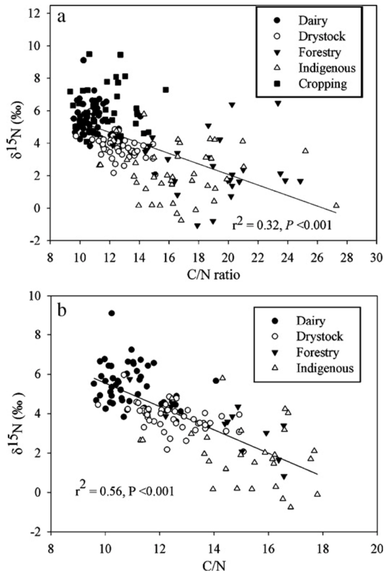

Fig. 2. Relationship between C/N and δ^{15}N for (a) all land uses and (b) excluding the cropping land use and sites where C/N > 18.

Indigenous systems have undergone the least alteration (particularly since atmospheric N deposition in New Zealand is low) and are used as a baseline to compare the effects of land-use change on soil δ^{15}N values. The generally low δ^{15}N values for the indigenous land use is consistent with most other studies of forest systems (see for instance Pardo et al., 2006; Högberg, 1997) and generally reflect low N losses (Table 4) that are typically associated with tight N cycling in N limited systems.

Plantation forestry (predominantly *P. radiata*) had a marginally greater mean δ^{15}N value than indigenous forests possibly because plantation forests in New Zealand have often been planted onto pasture and the slightly higher δ^{15}N values may be a relict feature (see, for instance, Parfitt et al., 2003). Alternatively, many of the plantation forests sampled were second or third rotation forests and some ^{15}N enrichment could be due to increased mineralization rates and nitrate leaching (Pardo et al., 2002) and/or soil disturbance and mixing during harvest and replanting.

There was greater variation in δ^{15}N for the indigenous and exotic forests land uses (Table 1). Chronosequence studies have shown that as forest systems age and become more nutrient limited, foliar nutrients are more closely linked to cycling of organic nutrients (e.g., forest floor material) rather than mineral soil (Vitousek, 1998; Chadwick et al., 1999; Parfitt et al., 2005). We surmise that differences in development of forest systems was responsible for the increased variance, and was the reason there was a better relationship between C/N and δ^{15}N in sites where C/N was less than 18 (Fig. 2). Additionally, N mineralization did not correlate as highly in our study across multiple land uses as has generally been reported for forest systems (Pardo et al., 2006).

Mean drystock pasture δ^{15}N was 1.7‰ enriched and dairy pasture 3.3‰ enriched above indigenous systems. N inputs and outputs were also progressively larger in drystock and dairy land uses than in indigenous systems (Table 4). Urine deposition in particular is likely to have contributed to elevated δ^{15}N in the pasture systems compared to the forests, because urine patches have been shown to cause large losses of N, both from gaseous N losses (primarily ammonia volatilization) and increased leaching (Decau et al., 2003; Clough et al., 1998; Robertson, 1993). In a natural system grazed by ungulates, Frank and Evans (1997) found that soil δ^{15}N from under dung and urine patches was significantly higher than adjacent areas without dung or urine. In the same system, Frank et al. (2004) demonstrated that ammonia volatilization from artificial urine patches caused isotopic fractionation, which led to a measurable increase in soil δ^{15}N after 10 days.

Several comparisons of grazed vs non-grazed systems (some long-term) in non-fertilized grasslands have shown enrichment in grazed systems in the range of 0.7–1.5‰ (Cheng et al., 2009; Neilson et al., 1998; Frank and Evans, 1997), similar to the enrichment in our drystock land use compared with indigenous forest. Total N was greater and C/N ratio lower in the dairy land use in comparison to

Table 4
Typical N inputs and outputs (kg N ha^{-1} $year^{-1}$) for New Zealand land uses.

	Indigenous[a]	Forestry[b]	Drystock[a]	Dairy[a]	Maize[a]
Animal stock units per ha			12	24	
N inputs					
Deposition	2	2	3	5	5
N fixation	2	7	60	100	0
Fertilizer			0	140	230
N outputs					
Products[c]	0	4	11	70	230
Leaching	2	3	12	40	30
All gaseous losses	2	2	14	75	40
Transfer to soil, tracks, etc.			26	60	−65

[a] Adapted from Parfitt et al. (2006) and OVERSEER® model.
[b] Adapted from Kirschbaum et al. (2008).
[c] Includes vegetation or animal products (e.g., wood, crop, meat or dairy products) exported from the system.

the drystock land use. The higher δ^{15}N in dairy pastures compared to drystock pastures of the current study can be attributed to the larger N losses resulting from greater N inputs (Table 4), which in conjunction with increased grazing intensity would provide more opportunity for isotopic fractionation. Mineralization rates, however, were about the same for these two land uses (but significantly greater than all other land uses), and the poor correlation of N mineralization to δ^{15}N values suggests this measure of the labile N pool alone was not an indicator of system leakiness.

The cropping land use had the highest δ^{15}N values, which was likely a result of losses of both C and N from cropped soils. Carbon content of the cropping soils was approximately a third less than indigenous sites, which is consistent with lower C content of cultivated land in comparison to indigenous forest within individual soil types (Schipper and Sparling, 2000). Carbon loss in the surface soil horizon of intensively cultivated soils is a widely noted phenomenon and recent research has suggested that synthetic N fertilization (in excess of crop production) in cultivated lands can decrease SOM-N (Mulvaney et al., 2009). There was no record of organic fertilizer use at sites monitored in our study and the decrease in soil C values suggests losses of soil organic matter (and associated N) were as much responsible for the increase in δ^{15}N values as N availability itself (particularly as there appeared to be greater variation around the C/N vs δ^{15}N regression line for cropping than for most other land uses).

Cropping soils were generally on lower landscape positions, and the cropping land use was the one land use that was dominated by clayey soils. Differences in texture and the lower landscape position (which may result in higher soil water content and larger gaseous N losses) may have also contributed to the large enrichment in δ^{15}N in this land use. Land use/soil type interactions were not specifically explored in our study, and this is an area where further study would be beneficial. We are confident that observed trends in δ^{15}N were largely due to land use affects rather than soil type. For the most common New Zealand soils group, the Brown Soils, δ^{15}N increased from 1.3‰ in indigenous soils to 3.7‰ in drystock soils and 6.1‰ in dairy soils. However, specific soil types are likely to react differently to land-use intensification and it is important to understand these differences for determining how landscapes will react to intensifying land-use pressures.

5. Conclusion

In New Zealand systems, we show that intensifying land use increased soil δ^{15}N values and that the increasing soil δ^{15}N values were negatively correlated to the soil C/N ratio. We posited that land-use intensity largely drives the three main fractionating N loss pathways (nitrate leaching, denitrification and ammonia volatilization) and typical N balances for each land use indicated that total N loss (and in particular losses from ammonia volatilization and nitrate leaching) also increased with increasing land-use intensity. Cropping systems were a minor exception in that soil C loss appeared to be partially responsible for the high δ^{15}N values in that system and highlights the need to consider changes in internal processes as well as inputs and outputs in managed systems.

The soil δ^{15}N of managed systems appears to be a useful indicator of systems that are losing N, particularly in combination with more typical measures of N status such as the C/N ratio. Further work is required to quantify fractionating losses from intensive land-use systems to determine which processes contribute most to increases in soil δ^{15}N. The ability of different soil types to store added N, and the response of soil types in relation to fractionating N losses under intensifying land use are particular questions to address.

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