Nitrogen Isotope Variation in the Environment: Implications for Interpretation

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Abstract

Natural abundance of ^{15}N varies greatly and unpredictably within and between environments. The unpredictable nature of ^{15}N limits the use of N isotope natural abundance ($\delta^{15}N$) in tracing the flow and fate of N in environments. Recent investigations have, however, revealed consistent and repeatable patterns of ^{15}N in some ecosystem components. These patterns suggest that $\delta^{15}N$ may yet provide a tool to investigate and illuminate ecosystem N cycling processes. Identifying and quantifying the sources of isotopic variation must precede any significant advance in the application of this technique, and to this end an assessment of isotopic variation associated with major ecosystem components has been carried out in this thesis. $\delta^{15}N$ patterns have been established, hypotheses proposed and tested, and conclusions about the application of the technique are presented.

^{15}N patterns in surface and groundwater were measured in a variety of different land-use catchments in an attempt to identify distinct isotopic 'fingerprints'. High levels of ^{15}N variation were measured in both stream and groundwaters, resulting in strongly overlapping land-use 'fingerprints'. Environmental ^{15}N variation in streams and groundwaters was found to be too great to differentiate between land-uses based on $\delta^{15}N$ alone.

In contrast, the artificially ^{15}N enriched signature of effluent N was used to trace its flow and fate, following irrigation, in a forested catchment. The effluent $\delta^{15}N$ signature allowed it to be traced into the major ecosystem components, permitting a first order N budget to be determined for effluent N storage and loss. N sources with significantly different ^{15}N signatures to that of 'background ecosystem N' can therefore be used to trace the flow and fate of N in ecosystems.

During the course of this work a number of higher and lower order plants were observed to have highly depleted (< -8‰) $\delta^{15}N$ signatures. Epiphytes and lithophytes, strongly reliant on atmospheric N sources, were consistently depleted in ^{15}N, with signatures as low as -24‰, measured in a range of environments. A

similar level of depletion was measured in a wide range of plants growing in early primary succession sites (as low as -22.3‰), which could not be accounted for by any abiotic or biotic factor or significantly depleted N source. The absence of any measurable driver of depletion suggested a universal fractionating mechanism which acts in a wide range of environments and vegetation types. Diffusive uptake of atmospheric $NH_{3(g)}$ and the proportional uptake of a supplied N source were two proposed mechanisms that could theoretically account for the level and universal nature of depletion.

Diffusive uptake of atmospheric $NH_{3(g)}$ was tested as a primary fractionating mechanism in plants. Strongly N deficient plants were capable of utilising $NH_{3(g)}$ as a nutritional source, but the level of ^{15}N depletion measured in these plants closely approximated that of the inherent $NH_{3(g)}$ $\delta^{15}N$ signature. No significant additional fractionation is associated with $NH_{3(g)}$ diffusive uptake. Diffusive uptake of atmospheric $NH_{3(g)}$ by plants cannot alone account for the level of depletion measured in early primary succession plant communities.

Proportional uptake of a N source as a primary fractionating mechanism was tested by growing plants in various concentrations and rates of applied N. Fractionation attributed to the proportional uptake of a supplied N source, as a consequence of P limitation or rapid flow over roots, resulted in a significant level of ^{15}N depletion in plants. The level of depletion attributed to this mechanism was, however, not sufficient to account for the level measured in early primary succession plant communities.

Individual ^{15}N fractionating mechanisms cannot alone explain the level of depletion observed in early primary succession plants, however a combination of fractionating mechanisms can. Fractionation attributed to the proportional uptake of an already depleted N source, i.e., wet deposited N, largely accounts for the level of depletion measured in early succession plant communities. This two-step fractionation model can act on both higher and lower plants, independent of ecosystem biotic and abiotic factors. Additional, and less dramatic fractionations attributed to atmospheric $NH_{3(g)}$ uptake, mycorrhizal associations, internal remobilisation, and taxon-specific N acquisition strategies, will contribute to the level of $\delta^{15}N$ depletion.

This thesis presents the first extensive survey of highly depleted $\delta^{15}N$ signatures in terrestrial vegetation. Furthermore, thorough testing of theoretically plausible mechanisms has resulted in a full account of the highly depleted $\delta^{15}N$ signatures measured in a wide range of vegetation types and environments.

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Chapter 1

Introduction

1.1. Introduction

Nitrogen (N) is an essential macronutrient, second only to water availability as the most growth limiting factor in plant production (Mengel and Kirkby 1982). It is well documented that in many areas of the world organic and inorganic N applications are increasing (Mosier et al. 2001). The subsequent increase in production rates following N application may come at the expense of intrinsic environmental quality. Eutrophication of surface waters, and increasing NO_3-N in groundwater, are characteristic ecosystem responses to increased N application (Pierzynski et al. 1994). Rising concerns of excess N on ecosystem function has initiated increased study of the N cycle and its fundamental processes.

The importance of N as an integral component to ecosystem function is illustrated by its presence in so many highly conserved compounds essential to organism growth and operation. N exists in ecosystems in a variety of chemical states from highly oxidised to reduced. The N cycle describes the continuous conversion of these N forms by physical and biological processes.

1.2. The N cycle

The dominant N pool is the atmosphere, of which N_2 comprises 78% by volume. N_2 is generally unavailable to organisms unless it is initially reduced to ammonium ions (NH_4^+) by the process of 'fixation'.

Biological fixation is carried out exclusively by prokaryotic microorganisms, some of which form associations with certain plants such as legumes. The biological conversion of N_2 to NH_3, an energy expensive process, provides N in a form readily utilised by the N_2-fixing organisms or by host plant in exchange for photosynthate. The high energy demanding conversion of N_2 to plant available N

forms is also provided in synthetic fertiliser manufacture (urea) and during lightning storms (NO_3-N).

Assimilation of N into plants occurs principally *via* glutamine synthetase (GS) in the chloroplast to form amide N. NH_4-N and NH_3-N are directly assimilated, as with NO_3-N following conversion to NH_3-N by nitrate and nitrite reductase. Amide N is further converted to glutamate by the reductive glutamine:2-oxoglutarate aminotransferase-(GOGAT)-reaction, the precursor, by transamination, to any other amino acid. Internal N recycling, a natural feature of plant N dynamic processes, provide additional sources of NH_3-N for GS. Under normal conditions, 40% of total N in old leaves is hydrolysed and remobilised to new leaves prior to abscission. GS-GOGAT recycling of this N is crucial in reducing demand for external N supply and the prevention of damage by the highly reactive NH_3-N following remobilisation.

Organic material from a range of sources including plants, animals and their waste products constitute significant inputs to soils. Organic N may comprise up to 95% of soil N and although some organisms have the ability to utilise smaller organic compounds directly, mineralisation provides the mechanism by which inorganic N species are made re-available from organic N for the majority of organisms. Soil organic N compounds range in bioavailability from easily mineralised to unavailable, however all eventually succumb to ammonification (organic N to NH_4-N). Nitrification (NH_4-N to NO_3-N) may follow, producing a further plant available N source, however because of its negative charge it is weakly bound to soil colloids, readily leached to groundwater and eventually to streams, rivers, lakes and the ocean. NO_3-N may also be converted to chemically more reduced gaseous species (N_2O and N_2) by denitrification in anoxic environments, typically waterlogged or compacted soils. Denitrification products are emitted to the atmosphere along with other gaseous N species (e.g. NO, NO_2 and $NH_{3(g)}$) as a consequence of volcanic and biological activity.

The atmospheric N gaseous species include, vapours (hydrated gas molecules) and particles (both solid and liquid). Theses are oxides of N (NO_x, composed of nitric oxide, NO and nitrogen dioxide, NO_2), ammonia ($NH_{3(g)}$) and nitrous oxide (N_2O), peroxy acyl nitrates, nitric acid (HNO_3), ammonium (NH_4^+) and other

inorganic and organic nitrates (NO_3^-). All atmospheric N species are potentially plant available N sources, with $NH_{3(g)}$ the most common and readily available species to plant growth (Krupa 2003). Atmospheric N species are unlikely to contribute significant nutrition to adequately fertilised plants (Raven 1988; Raven et al. 1992), but may when plants are strongly N limited in the absence of conventional root available N sources.

N limited environments range widely, but are characteristically exemplified by primary succession systems. Volcanic materials are classic early primary succession low N substrates, as are geothermally altered soils and exposed rock as a consequence of tectonic movement. Plants growing in low N substrates increase dependence on alternative N sources, including those derived from the atmosphere (Vitousek et al. 1987). Limited N environments provide unique systems to study N cycle and plant N dynamics as limiting N sources reduce the complex N cycle interactions observed in moderate environments.

Stable N isotopes have become increasingly popular with researchers studying N cycle and plant N dynamic processes as they are widely acknowledged as important in explaining both physiological and ecosystem processes over wide spatial and temporal scales (Delwiche and Steyn 1970; Shearer and Kohl 1986; Peterson and Fry 1987; Evans 2001; Robinson 2001; Bedard-Haughn et al. 2003).

1.3. Stable N isotopes

The following review of stable N isotope methodology is based on Högberg (1997) and Bedard-Haughn et al. (2003), within which a more complete analysis of the subject matter is presented.

The element nitrogen exists as two naturally abundant stable isotopes; ^{14}N (mass 14) and the rare, heavier ^{15}N (mass 15), normally at 0.3663% of the total N in any system (Junk and Svec 1958). An extra neutron in ^{15}N accounts for its greater mass, thereby affecting mass dependent properties without altering chemical behaviour. The ratio of ^{15}N:^{14}N, existing in all nitrogenous materials, is expressed in atom %.

$$\text{Atom \% } {}^{15}N = ({}^{15}N/({}^{14}N + {}^{15}N)) * 100$$

Equation 1.1

Atmospheric N_2 atom %, for example, has been repeatedly assessed and found to be remarkably stable at 0.3663% (Mariotti 1983). The small differences in atom % expressed in nitrogenous materials can be easily measured by mass spectrometers to at least 0.00015 atom %. However atom % differences are often numerically cumbersome, so delta (δ) notation is typically used:

$$\delta\ (‰) = (({}^{15}N/{}^{14}N\ (\text{sample}) / {}^{15}N/{}^{14}N\ (\text{standard})) -1) * 1000$$

Equation 1.2

This equation can be approximated by using only ^{15}N values:

$$\delta\ (‰) = ({}^{15}N\ (\text{sample}) / {}^{15}N\ (\text{standard})) -1) * 1000$$

Equation 1.3

Because of its stability and repeatability, the isotopic ratio of atmospheric N_2 (as previously described as 0.3663 atom %) is the standard by which deviations in natural abundance are measured. Atmospheric N_2 is assigned a δ notation of 0, with deviations (measured in parts per thousand, ‰) above or below this value, represented as an enrichment or depletion in ^{15}N, respectively. Typical $\delta^{15}N$ natural abundance falls within the range +10 to -10‰.

1.4. Fractionation

Differences in the ^{15}N:^{14}N ratios measured in nitrogenous materials are a result of 'fractionation', a term used to describe the discrimination of the heavier isotope during equilibrium and bio or physio chemical processes. In all cases, an enrichment of the substrate and a corresponding depletion in the product is observed (assuming incomplete conversion of reactants).

Discrimination of ^{15}N, as a consequence of a larger activation energy required to dissociate an isotopically heavy chemical species when reactions are at equilibrium, is termed the **equilibrium isotopic effect** (Bigeleisen 1965). Most equilibrium isotope effects are typically insignificant, and have little consequence

on naturally occurring $\delta^{15}N$ signatures. The few equilibrium effects that are significant, however, result in well documented strong isotopic fractionation, namely associated with the process of ammonia volatilisation and ion exchange, both of which will be dealt with later and in more detail (see Table 1.1).

Most fractionation effects result from discrimination of ^{15}N as a consequence of its higher mass. Molecules containing the heavier isotope will react or diffuse more slowly and form stronger bonds compared with molecules containing the lighter isotope. This is termed the **kinetic isotope effect** and may also be described by the ratio of the rate constants k_{14}/k_{15}, with k_{14} being larger than k_{15}, resulting in the intrinsic kinetic isotope effect, typically greater than 1 (Fry 1970).

Any single bio or physio chemical N cycle process will have an associated intrinsic equilibrium and/or kinetic isotope effect, with the observed magnitude of the effect represented by α values. α values express the measured isotopic ratio of the reactants (δ_A) divided by the isotope ratios of the products (δ_B) (Högberg 1997).

$$\alpha = \delta_A / \delta_B$$

Equation 1.4

α values typically fall between 1.0000 and 1.0300; α = 1 represents no fractionation, increasing proportionally with deviation from 1. Although rarely encountered, α values <1 reflect a discrimination against ^{14}N. All biotic and abiotic processes within the N cycle express an isotopic effect, with the range of α values for the main processes shown in Table 1.1.

Table 1.1 illustrates the highly variable α values associated with a range of N cycle processes. Potential fractionation attributed to N_2-fixation is minimal, while that of $NH_{3(g)}$ volatilisation is high. The range of α values for each process however, are also highly variable and reflect the inherent difficulty associated with achieving accurate measures of α. α will be constant in a simple unidirectional situation with unlimited substrate and no accumulation of product. When substrate is in limited supply, however, or when a process rate is limited,

the isotopic composition of the instantaneous product, as well as the cumulative product varies during the reaction (Figure 1.1).

Table 1.1 Fraction factors (α values) reported in literature for a range of biotic and abiotic N cycle processes. Compiled from Bedard-Haughn et al. (2003) and Högberg (1997). For more detail refer to these articles.

Process	Fractionation factor (α)
N_2-fixation	0.998 – 1.002
Denitrification	1.000 – 1.033
Mineralisation	≈ 1.000
Nitrification	1.015 – 1.035
NH_4^+ assimilation	1.009 – 1.020
NO_2^- assimilation	1.000 – 1.036
NO_3^- assimilation	1.003 – 1.030
Internal plant metabolism	0.980 – 1.020
$NH_{3(g)}$ volatilisation	1.000 - 1.029

The difficulty in isolating the instantaneous product and analysing its isotopic composition has consequently led researchers to focus on Δδ; the difference between the δ of the substrate (δ_s) and the δ of the product (δ_p) as a consequence of a particular process. This may be also represented as 'ε'; isotopic discrimination. ε describes the isotopic enrichment measured in the product relative to the substrate (in ‰).

$$\varepsilon_{(s/p)} = ((\delta_s - \delta_p) / (1 + \delta_p / 1000))$$

Equation 1.5

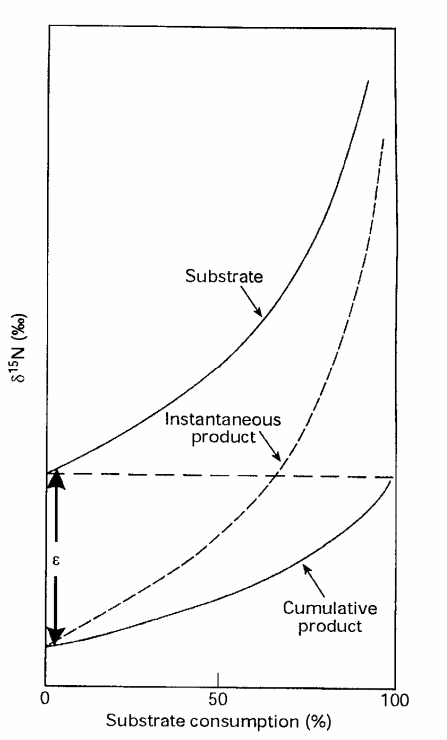
Isotopic discrimination may also be approximated from α values:

$$\varepsilon = 1000(\alpha - 1)$$

Equation 1.6

As with α, ε is not constant, but maximally expressed in the product at the beginning of the reaction, and in the substrates at the end. If a reaction goes to completion in a closed, unidirectional system, there is no net fractionation in the

cumulative product as described by the Rayleigh model of closed systems (Figure 1.1).



1.1).

Figure 1.1. The Rayleigh model describing the relative changes in $\delta^{15}N$ of components during a complete reaction in an isotopically 'closed' system. Figure taken directly from Högberg (1997).

Fractionation following the theoretical Rayleigh model is the central and universal definer of the degree of isotopic fractionation expressed in any ecosystem N cycle reaction. It describes the proportional conversion of a reactant pool into products and governs the strength of fractionation measured in both components as a consequence of this conversion. In theory, this model can fully account for, and explain, isotopic variation in any environmental N cycling process if it approximates an isotopically closed system. Applying this model to N cycle reactions in natural systems however is problematic because a full understanding of process rates and intermediate states are often unknown. In addition, multiple processes acting in series and parallel may mask process fractionations over time. As such, differentiating between fractionation attributed to single processes is difficult. To overcome this, and isolate individual mechanisms, researchers often study single processes *in vitro*. *In vitro* conditions rarely mirror those in natural situations; however they do provide a range of potential α values for individual N cycle and plant N dynamic processes.

1.5. Abiotic and biotic processes resulting in fractionation

The following section reviews α values and fractionation potentials of reactants and products of single abiotic processes. Biological processes and associated fractionation factors are also reviewed. Most biological processes do not consist of a single chemical event but many sub processes, each of which carry potentially fractionating effects. Because of this, it is rare in biological and ecological studies for a $\delta^{15}N$ value to refer to a single process. The level of fractionation measured in a N pool attributed to any N cycling process (regardless of scale) is a function of: 1) the isotopic signatures of inputs and outputs, 2) the input and output flux, 3) specific isotope effects for each N transformation process, and 4) how the system N under investigation is compartmentalised (Högberg 1997).

The following α values must be interpreted with caution and cannot be directly applied to natural situations as these values have been typically derived *in vitro*. Net fractionations ($\delta^{15}N$ values) of the major N cycle and plant N dynamic processes have also been included where possible. Although the described $\delta^{15}N$ values have been determined from field collected material and are more likely to be a 'true' representation of the combined isotopic effects, caution must be applied in assuming similar results in other systems.

1.5.1. Abiotic processes

1.5.1.1. NH_3/ NH_4^+ equilibrium in solution

$NH_4^+{}_{(aq)}$ and $NH_{3(g)}$ are always in equilibrium in a liquid. Urey (1947) showed that the isotope effect attributable to the NH_4^+/NH_3 equilibrium in solution varies greatly, and Högberg (1997) assigns an α value range between 1.020 and 1.027. Variation is attributed to differing solution pH, with lower pH values giving higher α values (refer to section 1.5.1.5 – $NH_{3(g)}$ volatilisation).

1.5.1.2. Diffusion

Diffusion of ions (NH_4^+ and NO_3^-) through a liquid medium is considered to have little effect on isotope fractionation, with Shearer and Kohl (1986) and Högberg (1997) assigning an α value of approximately 1.000. In contrast, diffusion of a

gas through a gas medium has been measured to result in significant isotope fractionation primarily as a consequence of the kinetic effect. Greater diffusion velocity attributed to molecules carrying the lighter isotope compared to the heavy isotope is explained by the kinetic energy (K.E.) equation, where the kinetic energy is equal to half of the molecules mass (m) multiplied by its velocity (v) squared:

$$K.E. = ½mv^2$$

Equation 1.7

Larger molecules, greater difference between the two isotopic masses of a molecule, and longer diffusion pathways result in higher levels of fractionation.

1.5.1.3. Ion exchange

Delwiche and Steyn (1970) found negligible fractionation by ion exchange. This has been confirmed by Högberg (1997) who also reports small, if any fractionation, during this process ($\alpha < 1.005$), but only if full recovery of the exchanged N is obtained. A partial recovery will result in fractionation determined by Rayleigh isotopic principles (see Figure 1.1).

1.5.1.4. Mass flow

There is no isotopic fractionation during mass flow (Högberg 1997).

1.5.1.5. $NH_{3(g)}$ volatilisation

$NH_{3(g)}$ volatilisation is the collective term for any physical or chemical reaction resulting in the loss of $NH_{3(g)}$ from a liquid. The degree of N loss by volatilisation of $NH_{3(g)}$ depends on a number of factors including pH, depth of boundary layer and temperature. The processes of volatilisation involve several steps in which isotopic fractionation can occur:

- Equilibrium between NH_4 and $NH_{3(g)}$ in solution,
- Diffusion of $NH_{3(g)}$ through the liquid to the site of volatilisation,
- The subsequent volatilisation of $NH_{3(g)}$

The $NH_{3(g)} \leftrightarrow NH_4^+{}_{(aq)}$ equilibrium is highly pH dependent. At pH 6 or less, the equilibrium shifts to the right, favouring NH_4^+. Above pH 6, a sudden shift to the

left of the equilibrium is observed, $NH_{3(g)}$ is produced, and N is lost resulting in fractionation. The level of loss determines the degree of fractionation. At pH 8, approximately 10% of the N in the system is in the $NH_{3(g)}$ form, and above pH 10 all the NH_4^+ in the system is converted to $NH_{3(g)}$ (Miers 1992).

The degree of dissociation and solubility of compounds in solution is often dependent on temperature. $NH_{3(g)}$ for example is highly soluble in chilled water, forming the $NH_{3(g)} \leftrightarrow NH_4^+{}_{(aq)}$ equilibrium (Handley and Raven 1992). $NH_{3(g)}$ solubility is inversely proportional to temperature.

The diffusion of $NH_{3(g)}$ away from the liquid to the atmosphere, assuming a short diffusive pathway and the predominance of mass flow, will not result in significant fractionation (Handley and Scrimgeour 1997; Högberg 1997). The depth of the boundary layer affects volatilisation in two ways. Firstly, as the boundary layer above a liquid is reduced, mass flow removes $NH_{3(g)}$, increasing the concentration gradient between the $NH_{3(g)}$ in the solution and $NH_{3(g)}$ in the atmosphere and increasing volatilisation rates. Secondly, as the boundary layer above a liquid is increased, the $NH_{3(g)}$ diffusive pathway and fractionation attributed to gaseous diffusion also increases.

Volatilisation may result in a major or minimal loss of N, and, therefore, fractionation as a consequence of the above sub processes. The compounding effect of these factors on the net fractionation is often large (up to $\alpha = 1.029$), but dependent of the rate limiting factor (Högberg 1997).

1.5.2. Biotic processes

1.5.2.1. N_2-fixation

Högberg (1997) reviewed a number of studies and found that ^{15}N fractionation of N_2-fixers is generally small, and influenced predominantly by bacterial strain, nutrient supply and soil moisture. α values of between 0.998 to 1.002 encompass the range attributed to all known N_2-fixers, indicating minimal fractionation. Field studies confirm minimal fractionation in N_2-fixing plants, usually measuring $\pm$ 2‰ from normal abundance (0‰) (Shearer and Kohl 1986).

1.5.2.2. Denitrification

Measured α values associated with denitrification are variable, but potentially very high, between 1.000 and 1.033 (as depleted as -33‰ in the gaseous products). Variability has been explained as a consequence of the proportion of substrate utilisation, most likely limited by concentrations of available electron donors and acceptors, variations in temperature and the rapid dispersion of the denitrification products (Högberg 1997).

Denitrification is most important in aquatic and wet terrestrial systems, and considered less significant in most well drained soils (Högberg 1997). Denitrification may, however, occur in highly localised environments on a landscape scale, and micro environments in the soil. So, although denitrification is not expected to markedly influence the levels of fractionation in most soil N pools, it may be more widespread than previously considered.

1.5.2.3. Mineralisation

The process of mineralisation results in little if any fractionation if the process goes to completion. As such, Högberg (1997) has assigned it an α value of approximately 1.000. A partial reaction, however, will result in fractionation in both products and reactants, as described by Rayleigh distillation. The variable bio availability of some organic N pools may provide partial mineralisation patterns in products over time. It is the partial process of mineralisation, for example, which has been suggested to account for the commonly observed trend of a 5 to 10‰ decrease in total soil N between surface and sub soils (Blackmer and Bremner 1977; Handley and Scrimgeour 1997).

1.5.2.4. Nitrification

Large α values are associated with the first nitrification step (NH_4^+ to NO_2^-) in pure cultures of *Nitrosomonas europaea*, ranging from 1.015 to 1.036 ($\delta^{15}N$ signatures of 15 to 36‰ in the product NO_2^-). The second nitrification step, NO_2^- to NO_3^- by *Nitrobacter,* is not rate limiting and should not result in further fractionation (Högberg 1997; Shearer and Kohl 1986).

The level of fractionation attributed to these reactions in soils is likely to differ significantly from those in pure culture. A range of factors, including the

concentrations of $NH_{3(g)}$ and NH_4^+, appear to influence the isotopic effect (Mariotti et al. 1981), with increasing NH_4^+ concentration corresponding to increased nitrification α values (although concentrations provided were far in excess of those encountered in normal soils). Fractionation attributed to the first, rate limiting step, is therefore most likely the result of the partial utilisation of a supplied NH_4-N source, as opposed to the inherent fractionation attributed to enzymatic conversion. In field situations, high NH_4-N concentrations are unlikely to exist outside of agricultural situations and the fractionation attributed to nitrification is unlikely to be as excessive as potentially illustrated.

1.5.2.5. Plant N assimilation

Widely varying levels of fractionation have been reported accompanying inorganic N assimilation by plants. Högberg (1997) presents α values as a consequence of this process to range between 1.000 and 1.020.

N is assimilated by plants (almost exclusively) *via* the GS-GOGAT reaction. Most enzymatic steps do not result in significant fractionation, particularly those, like GS, which have a very low k_m (Werner and Schmidt 2002). High fractionation factors are, however, measured in plants following assimilation and found to correlate with illumination, NH_4^+ and NO_3^- concentration (Yoneyama et al. 1991) and plant age (Mariotti et al. 1982). These factors, and any other which drive the partial uptake of a supplied N source in an isotopically 'closed' system, will result in a ^{15}N depletion in the studied plant. The resulting level of fractionation in the studied plant is determined by the amount of source N utilised as illustrated by Rayleigh fractionation principles (Figure 1.1). Yoneyama et al. (2001) measured a maximal fractionation attributed to this mechanism as great as -28.9‰ in rice plants.

No discrimination is measured in plants when all available substrate is utilised. Evans et al. (1996), for example, measured no fractionation between N source and plant $\delta^{15}N$ signature when plants were grown in low N concentration growth medium. This is likely to be the case in most natural systems where the concentrations of inorganic N rarely exceed 1mM.

1.5.2.6. Mycorrhizal association

The role of mycorrhiza in plant N uptake is well documented (Smith and Read 1997). The fractionation in host material as a consequence of this association is much less understood. Mycorrhizal association has been proposed to account for highly depleted foliar $\delta^{15}N$ signatures reported in ecosystems (e.g. Michelsen et al. 1996; 1998; Högberg et al. 1999; Hobbie et al. 1998; 1999; 2000; 2005). Numerous authors propose that mycorrhizal fungi have the ability to access a range of N sources (namely soil organic N), with unique $\delta^{15}N$ signatures, unavailable to non- and endo-mycorrhizal species (e.g. Michelsen et al. 1996; 1998). Alternatively, selective retention of specific N compounds by mycorrhizal fungi during fungal host N transfer has also been suggested to account for a measured depletion in host plants (Högberg et al. 1999; Hobbie et al. 1998; 1999; 2000; 2005). Numerous field observations in which mycorrhizal tissue is consistently enriched in ^{15}N, compared to that of its host plant tissue and soil N pools (Kohzu et al. 2000), add supporting evidence to this hypothesis. The theoretical driver behind this mechanism is the translocation of isotopically depleted fungal metabolites to the host plant. Amino acid biosynthesis may result in amino acids having a lower $\delta^{15}N$ than their precursors (e.g. trasamination of glutamic to aspartic acid results in a product with a $\delta^{15}N$ value 9‰ lower than the source (Evans 2001)), and may be the underlying mechanism driving this trend.

Much of the research supporting the role of mycorrhizal association and the associated depletion of host plants has been carried out in isotopically 'closed' laboratory systems. The reported depleted host plant or mycorrhizal fungi $\delta^{15}N$ signatures resulting from these studies are likely to reflect the fractionation attributed to partial uptake of a supplied source and not directly to mycorrhizal association (e.g. Emmerton et al. 2001a and b; Hobbie et al. (2004)). To overcome this masking mechanism, Hobbie and Colpaert (2003) designed an isotopically 'open' system and compared the fractionation expressed in host plants as a consequence of ecto-mycorrhizal association over and above non-associated plants. The maximum fractionation measured in host plants as a consequence of mycorrhizal fractionation was no more than -2.5‰.

1.5.2.7. N metabolism within plants

Variation in $\delta^{15}N$ between the tissues of individual plants has been frequently reported. Nodular tissue of actinorhizal plants is often found to be slightly depleted relative to the rest of the plant, while legume nodular tissues are more enriched compared with the rest of the plant (Tjepkema et al. 2000). Plant foliage is usually 2-3‰ more depleted than roots in temperate and tropical systems with the level of difference dependent on season and field situation (Högberg et al. 1986; Handley and Scrimgeour 1997). Foliage may be depleted by as much as 7‰ however, in warm and cold desert ecosystems (Evans 2001).

Internal N metabolism has the potential to create differences in $\delta^{15}N$ between plant tissues, and ultimately between plants which vary in their ability to recycle N. Incomplete recovery of N from old leaves prior to abscission (or herbivory) and the incomplete processing of remobilised N from storage are potentially fractionating processes (Yoneyama 1995), resulting in a depletion of ^{15}N in sinks and enrichment in sources. These processes have been suggested to at least partially account for variation in $\delta^{15}N$ between plant tissues and co habiting plants with markedly different $\delta^{15}N$ signatures (Evans 2001).

Klob and Evans (2002) directly tested the fractionation associated with N *reabsorbtion* (conversion and absorption *in situ*) or *reallocation* (physical movement of N from one organ/tissue to another) within different plant tissues in drought deciduous shrubs and deciduous trees. They found that there was substantial reabsorbtion of leaf N before abscission in all species, yet there was no difference between the $\delta^{15}N$ of living *versus* abscised leaves, suggesting no isotopic discrimination with reabsorbtion. Contribution of stored N for leaf production resulted in a significant difference in foliar $\delta^{15}N$ (in some species), suggesting N isotope discrimination may occur with reallocation. However, where differences in foliar $\delta^{15}N$ existed as a consequence of reallocation, it accounted for no more than -3‰.

1.6. $\delta^{15}N$ in the environment

A range of isotopic signatures are measured in ecosystem nitrogenous materials usually falling within the range -10 to +10‰. This range represents the fractionations associated with the myriad of ecosystem abiotic and biotic

processes described in the previous section. Individual ecosystem nitrogenous material $\delta^{15}N$ signatures often fall within more constrained ranges as a consequence of consistent processes and source N $\delta^{15}N$ signatures during their formation. These ranges provide isotopic 'fingerprints' of nitrogenous materials, as illustrated in Figure 1.2.

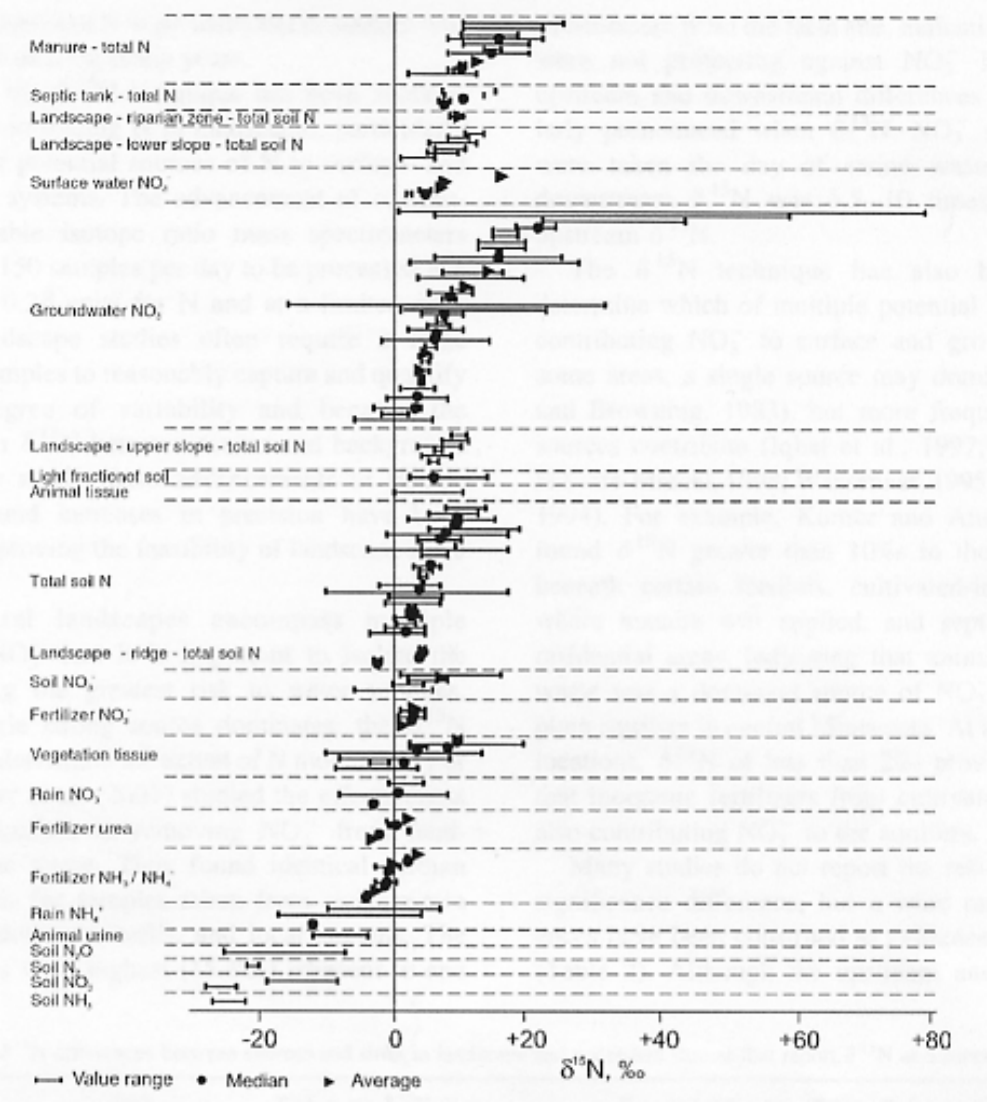


Figure 1.2. Range of $\delta^{15}N$ signatures ('fingerprints') reported in ecosystem nitrogenous materials (N.B. some of the variation presented may also be attributed to fractionation associated with sample preparation and collection prior to analysis.). Figure taken directly from Bedard-Haughn et al. (2003). For referenced studies, refer to this article.

Animal waste $\delta^{15}N$ generally falls between the range +10 to +20‰, significantly higher than the isotopic signature of the vegetation they consume (-8 to +10‰), as a consequence of animal metabolic processes (Steele and Daniel 1978) and $NH_{3(g)}$

volatilisation following its deposit (Frank et al. 2004). It is on the basis of these differences in N source isotopic 'fingerprints' that they have been proposed as natural abundance 'tracers' to follow reaction products in N cycle processes or quantify organism N pool use; the stable N isotope technique. Often, a simple mixing model is employed to determine the proportion of N derived from the N source under study:

$$\%N\ (source) = (\delta^{15}N\ (sample)/\ \delta^{15}N\ (source)) * 100$$

Equation. 1.8

This calculation is comparatively easy to determine when the N source $\delta^{15}N$ is significantly different from the 'background N' (e.g. animal derived N) and enough source N has been added to the system to detect it. Often, however, N sources and background N $\delta^{15}N$ signatures are not so different. Figure 1.2 illustrates this by the overlapping 'fingerprints' of numerous nitrogenous materials. For example, plant $\delta^{15}N$ 'fingerprints' encompass both total soil N and fertiliser N 'fingerprints'. In addition, almost all potential N sources to groundwater fall within its reported range.

A significant difference between N source and background N $\delta^{15}N$ signatures is critical to the application of the stable isotope technique. When the two (or more) components are not significantly different, it is impossible to isotopically differentiate between them. This issue is the main limitation in the application of the stable N isotope methodology to further understand N cycle and plant N dynamic processes.

One method of overcoming $\delta^{15}N$ environmental variability is the employment of isotopic tracers. Isotopic tracers are nitrogenous materials, usually ^{15}N enriched, artificially (typically fertilisers) or naturally (e.g. effluent N as a consequence of N loss during treatment) to provide a unique isotopic signature. Following application of this material to the study system, it becomes incorporated into the overall N cycle and its flow and fate can be traced. Provided enough enriched material is added, the level of enrichment is well above the 'background' $\delta^{15}N$, N pool turnover rates are not too high and the investigated N pools are measurable, the added N can be traced through the system. ^{15}N enrichment not only allows the

flow and fate of the N to be followed, but also changes in ^{15}N enrichment over time can be used to calculate the turnover rate of N in the N pools under investigation. An unfortunate disadvantage to the ^{15}N enrichment method is the high cost of enrichment and often disruptive effects of adding N to a system, limiting its widespread application in N cycle research (Bedard-Haughn et al. 2003).

Natural abundance studies in contrast, are simplistic, inexpensive, undisruptive to the study system and provide an endless array of natural experiments at a range of spatial and temporal scales. These advantages are weighed against the difficulties in overcoming environmental variation considered to be the greatest limitation in successful application of this method in ecosystems (Evans 2001). Reports of repeatable trends in $\delta^{15}N$ within and between ecosystem nitrogenous materials, over and above environmental variation (Robinson 2001), suggest $\delta^{15}N$ may yet reflect and illuminate ecosystem–N cycling and plant N dynamic processes. Quantification and minimisation of environmental variation is the key to successful application of the stable N isotope method in further understanding N cycle processes.

1.6.1. Plant $\delta^{15}N$ trends

Plants are often central components in ecosystem N cycle dynamics. Because of this role their $\delta^{15}N$ signatures have long been suggested as tools providing the ecophysiologist with insights into ecosystem N cycling and plant N dynamic processes. Plant $\delta^{15}N$ signatures generally fall within the range -8 to +10‰ (Figure 1.1), and have been considered by some researchers to reflect their N source $\delta^{15}N$ signatures directly (Shearer and Kohl 1986). This is almost never the case, as plants not only integrate the $\delta^{15}N$ signatures of available N sources, but also the fractionations attributed to partial N source utilisation, uptake mechanisms, stress, genotype and mycorrhizal association (Robinson 2001). In some situations where plants are growing in simple systems with minimal N inputs, e.g. N_2-fixation into primary succession systems (e.g. Kohls et al. 1994) foliar $\delta^{15}N$ signatures approach that of their N sources. The generally held view however, is that in most moderate systems, foliar $\delta^{15}N$ signatures are not reliable indicators of plant N sources (Handley and Scrimgeour 1997; Robinson 2001; Evans 2001).

Despite high levels of environmental variation, foliage $\delta^{15}N$ signatures have been routinely observed to correlate with a range of N sources and ecosystem biotic and abiotic factors over and above the background ecosystem variation. Burning frequency (Schmidt and Stewart 2003; Cook 2001), grazing (Schulze et al. 1999), N availability (Martinelli et al. 1999) and climatic factors, particularly precipitation patterns (Handley et al. 1999 and references within) among others, have been correlated with foliar trends in environments. These observations have led researchers to hypothesise driving mechanisms behind the observed trends.

Handley et al. (1999) reviewed the literature and proposed a unifying and testable theory of ecosystem plant foliar trend determination. Based on the premise that N loss from systems (usually leaching and gaseous losses) result in comparative ecosystem N enrichment, they proposed that ecosystem $\delta^{15}N$ reflects the level of ecosystem N cycle 'openness' where: any factor (including pH, fire, grazing or climatic variables) which decreases the proportional flux of ecosystem N into organic matter storage pool will increase ecosystem N 'openness' and corresponding foliar ^{15}N enrichment relative to atmospheric N_2. This theory satisfactorily accounts for the majority of foliar ^{15}N trends in reported systems. Greatest attention has recently been focused on water availability as the driver of ecosystem 'openness'. Decreasing water availability (typically correlating with decreased precipitation) drives increased ecosystem N 'openness' and a corresponding enrichment in foliar ^{15}N (Handley et al. 1999; Amundson et al. 2003; Swap et al. 2004).

1.6.2. Land-use catchment exported N $\delta^{15}N$

It is well understood that particular land-use practices export more N than others (Cooper et al. 1987). The $\delta^{15}N$ signature of this exported N integrates the isotopic fractionations associated with processes leading to its creation and loss. Predominant, strongly fractionating N cycling processes and the addition of different isotopically labelled N sources occur as a consequence of various land-use management practices. Based on these differences, there is good reason to hypothesise that the $\delta^{15}N$ 'fingerprint' of exported N will be distinctly different between single land-use catchment collecting water bodies.

There are two main N cycling processes that lead to major N losses – and therefore potentially large isotopic fractions - on landscape scales: denitrification and volatilisation of $NH_{3(g)}$. Both processes are associated with high α values (Table 1.1), and therefore potentially depleted $\delta^{15}N$ signatures in their reaction products. Denitrification requires NO_3-N, soluble carbon and an absence of oxygen, often co-occurring in lower landscape positions. Loss of denitrification products results in an enrichment in residual soil N, the level of enrichment dependent on the rate and level of substrate utilisation (Figure 1.1). Follow on effects include enrichment of soil organic matter, plants and groundwater (Sutherland et al. 1993). $NH_{3(g)}$ volatilisation occurs mainly following application of excess N, typically fertilisers or manure, but occurs to a lesser extent naturally from plants and soils (Krupa 2003). The rate and level of substrate utilisation dictates the level of enrichment in the residual N, and is largely dependent on the substrate pH.

N entering the system may also change the net landscape N pools $\delta^{15}N$ signatures. Biological N_2-fixation is a significant N input in natural systems (Vitousek et al. 1987), but is difficult to identify, as it lacks a clear $\delta^{15}N$ signature over and above that of background $\delta^{15}N$ signatures due to its minimal isotopic effect (Table 1.1). N deposition (both wet and dry) may also be significant N inputs in natural systems, particularly in early succession plant communities in the absence of N_2-fixers. Wet and dry deposition $\delta^{15}N$ signatures (NH_4-N) have been reported within the ranges -15 to +10‰ and -20 to +15‰, respectively (Russell et al. 1998; Yeatman et al. 2001). Effects on landscape N pools as a consequence of N deposition however, are likely to be insignificant in all but highly unique situations (Bedard-Haughn et al. 2003). Fertiliser and manure inputs are the most significant source of N to landscapes, particularly influenced by agriculture, with typical $\delta^{15}N$ signatures illustrated in Figure 1.2. Once N is fixed into a landscape, the multiple N cycle transformations, particularly denitrification and volatilisation, change the $\delta^{15}N$ signatures of landscape N pools.

Typically NO_3-N forms the predominant component of exported N as it is the most labile of N forms. When NO_3-N moves from terrestrial to aquatic systems, its $\delta^{15}N$ signature 'should' typically increase as it is transformed via nitrification, denitrification, and preferential leaching of depleted NO_3-N (Handley and Raven

1992). Both denitrification and volatilisation have particularly strong fractionation factors, and depending on the proportion of substrate utilised by each, or both processes, the level of isotopic enrichment measured in the residual N may be high. As such, these processes may lead to large differences in the isotopic signature of the NO_3-N in land-use water bodies. Once the NO_3-N reaches the aqueous environment, it may undergo further transformation processes including denitrification. Clearly, multiple processes with multiple fractionation factors may result in $\delta^{15}N$ signature differences disappearing between source N pools. Multiple fractionating processes and the associated environmental variation is the greatest limiting factor in the usefulness of differentiating between land-use N export $\delta^{15}N$ 'fingerprints' (Bedard-Haughn et al. 2003).

1.7. Aim

As can be observed from the above review, ecosystem abiotic and biotic processes result in a wide range of potential $\delta^{15}N$ values in nitrogenous materials. Although a great level of variation is measured in environmental nitrogenous materials, repeatable patterns of $\delta^{15}N$ are observed suggesting $\delta^{15}N$ signatures may yet reflect and illuminate ecosystem–N cycling processes. Application of the stable N isotope methodology relies exclusively on a significant difference between source N and 'background N' $\delta^{15}N$ signatures. The key driver to further improve the application of this technique is to isolate and/or minimise natural $\delta^{15}N$ variation within measured systems. Furthermore, mechanistic studies testing the drivers of these observed patterns of $\delta^{15}N$ signatures will eventually lead to generally accepted theories explaining the observed trends.

The aim of this research is to explore the level of environmental $\delta^{15}N$ variation in a range nitrogenous material. The resulting chapters examine current literature and critically assess applications of the stable isotopic method at a range of scales, while measuring $\delta^{15}N$ variation within New Zealand systems. Mechanisms are proposed and tested in an attempt to explain the levels of variation measured in the investigated environments.

1.8. Thesis structure

In the following chapter (**Chapter 2: Methods**), the general materials and methods relevant to the following chapters are described for the collection and measurement of N containing environmental materials. Specific experimental design, development of novel methodologies or apparatus description is discussed within following chapters. **Chapter 3** attempts to apply ^{15}N natural abundance to differentiate between collecting water body inorganic N isotopic 'fingerprints' from a range of representative New Zealand land-use management practices. As a consequence, the level of $\delta^{15}N$ variation in land-use N 'fingerprints' is explored. **Chapter 4** takes a similar approach to examine the flow and fate of treated effluent N following its application to a conifer production forest near Rotorua. The isotopically enriched signature of treated effluent is used to determine a first order approximation of effluent N contribution to all significant components of the system including soil, water and vegetation. **Chapter 5** investigates the $\delta^{15}N$ signatures of epiphytes and lithophytes in a range of environments within New Zealand. The unusually depleted signatures measured in these organisms and their dependence on atmospheric N sources is noted. The relatedness of these two variables suggests a mechanism associated with atmospheric N source acquisition. A hypothesis is proposed where a two step fractionation process during the uptake of atmospheric $NH_{3(g)}$ *via* the foliage of highly N stressed plants may account for the unusually depleted $\delta^{15}N$ signatures measured in these organisms. Preliminary testing confirms a high level of depletion attributed to this mechanism of N uptake. **Chapter 6** investigates higher plant $\delta^{15}N$ signatures within early primary succession communities in New Zealand. These organisms are consistently depleted, independent of a range of measured ecosystem biotic and abiotic factors. The universal level of depletion in early succession higher plants, similar levels in higher plants of comparable systems reported in literature, and in lower plants reported in chapter 5, suggests an, as yet, unmeasured mechanism to account for the level of depletion in all systems where depleted vegetation $\delta^{15}N$ signatures are measured. A number of mechanisms are proposed to account for the level of depletion measured. **Chapter 7** tests one of the two hypotheses proposed in the previous chapters: fractionation attributed to the diffusive uptake of atmospheric $NH_{3(g)}$. Two experiments were designed to definitively explore the variation in higher plant $\delta^{15}N$ signatures as a consequence of this mechanism. **Chapter 8** explores a further potential mechanism proposed in chapter 6 to account for the

highly depleted $\delta^{15}N$ signatures in higher plants: the fractionation associated with the partial uptake of a supplied N source. Finally, and in conclusion, **Chapter 9** provides a concluding discussion of the findings from this thesis.

Chapter 2

Methods for collecting and processing of materials for analysis

2.1. Introduction

This chapter describes general sampling protocols and preparation methods used prior to isotope analysis throughout this thesis. Specific methods and experimental procedures are described within the following chapters.

Accurate $\delta^{15}N$ determination is highly dependent on the collection, storage and preparation of samples prior to analysis. Collected samples must be representative of the N-pool under investigation and, therefore, accurately represent its $\delta^{15}N$ signature. Representative sample collection is more difficult for some materials than others. Soils are heterogeneous and are highly dynamic in space and time, particularly on a landscape scale. Liquid samples are, by comparison, homogenous and easier to sample representatively. In addition, incomplete collection of a N-pool, for example incomplete collection of an atmospheric $NH_{3(g)}$ sample, may also result in an erroneous $\delta^{15}N$ signature, reflecting a fractionation attributed to incomplete recovery rather than to its intrinsic $\delta^{15}N$.

Loss of N during sample processing in preparation for analysis is more problematic for some samples than others, too. Some materials, namely liquid samples, require multiple preparation steps prior to analysis, many of which have a high potential for N loss. In contrast, leaf material is comparatively simple to prepare for analysis.

Sample N content must fall within the range 40 – 700 μg-N, for accurate isotopic analysis, but total sample size must not exceed 11 mg to ensure complete

combustion during mass spectrometry. Most sample-N contents fall outside this range and require N-concentrating processes prior to this analysis.

It is important that a thorough understanding of the potential fractionation processes occurring during collection and preparation for each type of sample material is known and understood. The physical nature of the sample dictates the collection method and subsequent handling, ensuring a representative sample, free of fractionation artifacts. The following sections describe the collection, handling, storage, concentration, extraction, final preparation and analysis of samples used in the studies presented in this thesis.

2. 2. Representative Sample collection

2.2.1. Vegetation

Whole-plant analysis provides the most accurate and wholly representative integration of plant $\delta^{15}N$ signature, however this is often logistically difficult and ecologically damaging. Plant $\delta^{15}N$ signatures are typically determined from foliage analysis, and are generally considered to closely approximate whole plant $\delta^{15}N$. Foliage typically provides the easiest sampling point, reflects a recently deposited $\delta^{15}N$ signal, is the largest N pool, and contains the highest concentration of N within the plant (Handley and Scrimgeour 1997).

Where reasonable or required, whole individuals of lichen, moss, algae and higher plants were collected. In this case, mention is made in the text that they are whole plant samples. In most instances, collection of whole individuals was not possible or unnecessary. Representative portions of individual lichen or algal thallus and green tips of the growing gametophytes of individual moss samples were collected. Higher plant foliar collection typically involved removal of the 1st fully expanded new leaf. In most cases leaves were collected from a number of growing points on individual plants, and combined to provide a representative foliar sample.

Following vegetation collection, samples were immediately returned to the laboratory, washed with distilled water to remove contamination, where required,

and dried at 60ºC in a forced air-drying oven for 24 h. Following drying, samples were ground to a fine powder and stored in a desiccator. Ground vegetation samples were accurately weighed into tin capsules prior to isotopic analysis.

The N content of vegetation samples determines the weight required for analysis. The amount of sample N required for accurate analysis by the Waikato Stable Isotope Unit ANCA-SL mass spectrometer is between 40 – 700 µg, but must be contained within no more than 11 mg of total sample weight to avoid incomplete combustion prior to analysis. If the %N of the sample is known (usually by prior %N determination following Kjeldahl digestion), then the following formula can be used to determine the weight of sample required:

$$\text{Sample weight (mg)} = \frac{[\text{N}]}{\%\text{N}} \times 100$$

Equation 2.1

Where [N] is the concentration of N required by the mass spectrometer for accurate analysis, usually 200 µgN.

It is important that equation 2.1 is at least approximated when weighing out samples. For vegetation samples with very low %N contents, the amount of sample required to achieve this N concnetration can often exceed 11 mg. In this situation, the sample must be Kjeldahl-digested and steam distilled to increase the concentration of N in the sample.

2.2.2. Liquid samples

Liquid samples were assumed to be uniform as a consequence of mixing and diffusion, enabling relatively easy representative sample collection. Following collection, liquid samples were pH determined (if required) and immediately transported back to the laboratory. Depending on the N species under investigation, the following protocols were implemented for water samples:

2.2.2.1. Organic-N

Representative samples were Kjeldahl-digested prior to steam distillation and back titration to determine N content. Finally, they were prepared as liquid samples for $\delta^{15}N$ analysis.

2.2.2.2. Inorganic-N

Liquid samples were steam distilled, back titrated and prepared as liquid samples for $\delta^{15}N$ analysis. Where sample N concentrations were too low to distil directly, a concentration step was required. The primary method of concentrating inorganic-N from a dilute liquid sample involved the use of an exchange resin.

2.2.2.3. Exchange resin

On return to the laboratory, samples were prepared for ion exchange by firstly filtering if required (Whatman GF/B filter paper). Extraction of the inorganic-N forms by ion-exchange resin was based on the modified method of Silva et al (2000). A known volume of the filtered sample was passed, under vacuum, through 1 cm^3 resin beds of Dowex 50WX4 100-200 mesh H^+ form ion-exchange resin (NH_4^+) and Dowex 1X8 100-200 mesh, Cl^- form ion-exchange resin (NO_3^-). Prior to sample extraction, the resin bed exchange sites were cleaned and primed with 3 x 5 ml 3M HCl, with excess HCl flushed with 5 ml distilled water. Samples were passed through the resin beds at a rate no greater than 5 ml min^{-1}, and, upon completion, the extracted-N eluted from the resin beds with a further 3 x 5 ml 3M HCl flush. The collected inorganic-N species in HCl were steam distilled and back titrated to determine N concentration and prepared as liquid samples for $\delta^{15}N$ analysis.

2.2.3. Soil samples

Soil samples were collected using profile depth corers and cleaned between individual samples. Upon collection, soil cores were divided into depth profiles: Litter Fermenting Humifying (LFH) layer (= A_o or organic soil), and soil 0-100, 100-200, 200-300 mm, with each section sealed in plastic bags. In the laboratory, the individual soil samples were separated into two subsamples: one KCl extracted sample, to isolate inorganic N species form the soil, the other, air dried and weighed sample, to determine N content for each one. KCl extraction involved a 1:1 (w:w) slurry of soil and 0.1M KCl solution for each sample. The

slurry was shaken for 20 minutes, and the liquid filtered off using Whatman GF/B filter paper, acidified with H_2SO_4 and treated as a liquid sample in preparation for N content determination and $\delta^{15}N$ analysis. The remaining subsample was air dried, and its bulk density determined to obtain total soil mass. It was dried at 60ºC, homogenised and ball milled in preparation for total soil %N and total soil $\delta^{15}N$ analysis. Isotopic analysis was carried out either directly, as with vegetation samples (see section 2.2.1.) or, if N-content was too low, Kjeldahl digested and treated as a liquid sample prior to $\delta^{15}N$ analysis.

2.2.4. Atmospheric $NH_{3(g)}$ samples

Representative sample collection of atmospheric gas is, generally, relatively easy. Other than the logistic difficulties associated with dealing with large volumes of air, gas mixtures are usually well mixed by diffusion, resulting in the target gas being homogenous, at least on a local scale.

Sample collection of low concentration atmospheric gases without introducing fractionation effects during the collection is, in contrast, difficult. Collection for $\delta^{15}N$ analysis requires that all of the target gas is collected in the volume of air sampled, as loss or ineffective collection will result in fractionation. Efficient capture of $NH_{3(g)}$ is particularly difficult as it is a highly active and volatile gas found in often very low atmospheric concentrations (Goldberg 1982). This requires large volumes of air to be passed, in order to collect the target gas in a concentration high enough for analysis.

A novel device was developed to representatively sample atmospheric $NH_{3(g)}$ based on a modified method described by Ferm (1979). This technique, the 'oxalic acid-coated beaded column' technique, involves drawing atmospheric gas via a pump (flow rate of 40 L min^{-1} or 2.4 m^3h^{-1}) through a 500 mm length of 20 mm (internal diameter) PVC tube filled with 4 mm glass beads treated with 3% oxalic acid. Atmospheric NH_x diffuses into the infinite sink generated by the acid, effectively 'scrubbing' the atmosphere sample before being vented. After collection, the beads are placed directly into sample flask, treated as a liquid sample, and analysed for NH_x concentration and $\delta^{15}N$. Knowing the duration of sampling, the flow rate through the apparatus, and the concentration of NH_x in the

collected sample, the concentration of the NHx in the atmosphere can be derived as follows:

$$\text{Volume of gas sampled } (m^3) = \text{Flow rate } (m^3 h^{-1}) \times \text{duration of sampling (h)}$$

Equation 2.2

$$\text{Concentration of } NH_x \text{ in sample } (\mu gN\ m^{-3}) = \frac{NH_x \text{ in sample } (\mu gN)}{\text{Volume of gas sampled } (m^3)}$$

Equation 2.3

The residence time of an atmospheric gas sample was determined by first principles, to ensure enough time for complete recovery of $NH_{3(g)}$. Fick's law was implemented:

$$T_{½} = \frac{d^2}{4 \times D} =$$

$$T_{½} = \frac{(0.0005)^2}{4 \times 1.95 \times 10^{-5}} =$$

$$T_{½} = 3.2 \times 10^{-3} \text{ seconds.}$$

Equation 2.4

Where:

$T_{½}$ is the time required for half of the initial NH_3 concentration to diffuse to the surface of the acid coated beads;

d equals the maximum distance that the NH_3 must diffuse, measured to be 0.0005 m (0.5mm);

D is the diffusion co-efficient for NH_3 (which in this case is approximated by using the diffusion co-efficient for O_2 instead, 1.95×10^{-5} $m^2 s^{-1}$, giving a more conservative residence time).

The residence time of sampled air in the apparatus made to the specifications above was 0.448 s^{-1}, far exceeding the minimal residence time of 3.2×10^{-3} s^{-1} prescribed by Fick's Law to ensure complete recovery of contained $NH_{3(g)}$.

To test the column effectiveness, a standard $NH_{3(g)}$ air/gas mixture (1 mg $NH_{3(g)}$ m^{-3}) was passed through the column, and the recovered $NH_{3(g)}$ $\delta^{15}N$ compared to the standard gas mixture. No significant difference between the $\delta^{15}N$ of recovered $NH_{3(g)}$ and the standard gas mixture (Table 2.1) indicated that this method effectively collects a representative sample of $NH_{3(g)}$ without fractionation.

Table 2.1 $\delta^{15}N$ values of standard $NH_{3(g)}$ and the $\delta^{15}N$ values of recovered standard $NH_{3(g)}$ from the oxalic acid-coated beaded column.

Sample	$\delta^{15}N$	Mean $\delta^{15}N$
5 ml NH_3 gas	+0.26	+0.06
5 ml NH_3 gas	-0.73	
5 ml NH_3 gas	+0.65	
Column washings	+0.72	+0.93
Column washings	+0.87	
Column washings	+1.21	

2.2.5. Fractionation attributed to atmospheric $NH_{3(g)}$ diffusive uptake

Isotopic fractionation attributed to the diffusive uptake of atmospheric $NH_{3(g)}$ was modelled using the acidified capillary matting technique.

Inert fibre glass matting (0.08 m^2) was washed repeatedly with distilled water and soaked in 3% oxalic acid solution. The acidified capillary matting was attached to the bottom inside of an inverted plastic tray ($350 \times 230 \times 50$ mm) and positioned above the ground to avoid wet deposition and soil particulate contamination. The acidified capillary matting was allowed to remain in contact with the atmosphere for between six to 20 days. Recovered $NH_{3(g)}$ concentrations (mg N m^{-2}) from individual cap mat locations provided an alternative atmospheric $NH_{3(g)}$ concentration comparison. Acidified capillary matting recovered $NH_{3(g)}$ $\delta^{15}N$

signatures reflected the inherent $\delta^{15}N$ signature of atmospheric $NH_{3(g)}$ and the fractionation attributed to its diffusive uptake into an infinite sink.

2.3. $\delta^{15}N$ fractionation following collection and during preparation

2.3.1. $\delta^{15}N$ fractionation in samples as a consequence of drying

Forced air-drying of plant material in preparation for %N and $\delta^{15}N$ determination is common practice. A drying temperature of 60°C was maintained to prevent continued respiration associated with low drying temperatures or N transformation that may occur at high temperatures.

2.3.2. $\delta^{15}N$ fractionation in samples as a consequence of N loss during grinding and transfer of liquid samples

N loss by grinding vegetation samples can occur as sample material is left on the internal parts of the mill and dust is lost to the atmosphere. This loss may be considerable, but, as the sample is homogeneous, the loss will not result in fractionation, only in reduction of sample size. Similarly, N loss from liquid samples during transfer between vessels into tin capsules may also result in N loss, but not isotopic fractionation.

2.3.3. $\delta^{15}N$ fractionation in samples as a consequence of Kjeldahl digestion

Kjeldahl digestion is a commonly used method for %N determination, but has limitations in converting all N compounds to NH_4^+. Kjeldahl digestion does not convert NO_3^- to NH_4^+, which, unreacted, remains in solution (Bremner 1965). If total N $\delta^{15}N$ is required of the digested material, lack of NO_3 conversion must be taken into consideration. Devarda's Alloy was used during steam distillation of the digested sample to extract sample NO_3^-.

2.3.4. $\delta^{15}N$ fractionation in samples as a consequence of steam distillation

NH_3 conversion and extraction during steam distillation is a potentially highly fractionating process (Högberg 1997) although insignificant if all sample N is recovered. Previous standardisation with NH_4Cl solution determined least 98% of

sample N was contained within the first 50 ml of collected condensate. At least 50 ml of condensate was collected in subsequent sample analysis to ensure complete recovery of sample N. NO_3-N distillation in the presence of Devarda's Alloy resulted in slightly lower recovery of sample N (no less than 95%) but no significant isotopic fractionation was measured as a consequence.

2.3.5. $\delta^{15}N$ fractionation in samples as a consequence of liquid sample preparation

Preparation of liquid samples for $\delta^{15}N$ analysis involves a number of steps, each with the potential to result in significant isotopic fractionation. The effect of Kjeldahl digestion, steam distillation, and evaporation back to NH_4^+ crystals was determined using NH_4Cl crystals as standard samples (Table 2.3).

A slight enrichment of +0.43‰ was experienced during digestion, distillation, boiling and evaporation, but the difference between the two means was not significant ($p = 0.056$). Given this and the fact that the mass spectrometer error is $\pm 1\delta$, the fractionation effect due to the process of digestion, distillation, boiling and evaporation is considered insignificant.

Table 2.2 Fractionation resulting from the effect of NH_4Cl crystal Kjeldahl digestion, steam distillation, and evaporation back to NH_4^+.

NH_4Cl crystals $\delta^{15}N$ (‰)	**NH_4Cl crystals after digestion, distillation, boiling and evaporation $\delta^{15}N$ (‰)**
0.75	0.64
0.67	1.59
0.26	0.79
0.14	-0.29
0.76	2.46
0.86	-
0.84	-
Mean = 0.61	**Mean = 1.04**

Assessment of freeze drying as a method of liquid sample reduction strongly altered sample N $\delta^{15}N$ signatures (data not shown). Freeze drying was therefore not used as a method of liquid sample reduction prior to analysis.

2.3.6. $\delta^{15}N$ fractionation in samples as a consequence of prolonged drying following evaporation back to NH_4^+

During the liquid sample preparation for $\delta^{15}N$ analysis, distillate from steam distillation is reduced in volume in a forced air drying oven (60°C) and to NH_4^+ crystals in tin capsules at 30°C. This process takes approximately five hours at 60°C. The effect of prolonged drying (3 days at 60°C) on the fractionation of N in samples is unknown. Using NH_4Cl standard solution, the effect of prolonged drying on fractionation was determined (Table 2.3).

Table 2.3 Fractionation resulting from prolonged drying of NH_4^+ crystals at 60°C.

NH_4Cl solution dried to NH_4 crystals in tin capsules at 60°C (5 hours drying time) $\delta^{15}N$ (‰)	**NH_4Cl solution dried to NH_4 crystals in tin capsules and left at 60°C for three days** $\delta^{15}N$ (‰)
-1.54	1.51
-2.59	1.60
-3.17	1.37
Mean = -1.05	**Mean = 1.49**

A significant ($p<0.001$) enrichment of 2.54‰ was experienced in samples after prolonged drying (3 days at 60°C) of samples. This fractionation was avoided by removing the samples from the oven following drying and immediately analysing for $\delta^{15}N$.

2.3.7. $\delta^{15}N$ fractionation in samples as a consequence of KCl extraction

KCl extraction of NO_3^- from soils is not a standardised procedure guaranteed to result in complete NO_3^- recovery. Lindau and Spalding (1984) found that the volume of KCl used affects the efficiency of NO_3^- extraction, and therefore the $\delta^{15}N$ of the extracted NO_3^-. They observed that as the extractant:soil ratios increased from 1:1 to 10:1, the concentration of extracted NO_3^- did too (increasing 1.7 times in the 10:1 compared to that of the 1:1 ratio). $\delta^{15}N$ was also found to increase as the extractant:soil ratio increased (increasing an average of 6‰ in the 10:1 ratio compared to the 1:1 ratio). In this thesis, a 1:1 ratio of KCl

extractant to soil was used in all cases throughout experiments analysing soil extracted inorganic N species.

2.4. Analysis of samples for %N and $\delta^{15}N$ signature at the University of Waikato Stable Isotope Unit

Percentage N and $\delta^{15}N$ analysis of samples were carried out at the University of Waikato Stable Isotope Unit using their Dumas Elemental Analyser (ANCA-SL, Europa Scientific) interfaced to an isotope mass spectrometer (Tracermass, Europa Scientific). Instrument precision and accuracy was periodically tested against two known N isotope standards, IAEA-N1 +0.40‰ and USGS-25 -30.4‰. Instrument mean (n = 5) for N1 is +0.369‰ and for USGS-25; -30‰, confirming both high accuracy and precision in the range of the majority of samples. Duplicate plant samples differ by less than 0.5‰, which is a measure of both instrument and sample homogenisation. Samples were run against reference samples of similar size and drift corrected every ten samples.

Replicate sample analysis was kept to a minimum to reduce unnecessary analysis costs. In addition, most trials were designed to assess $\delta^{15}N$ variation trends over environmental/experimental nutrient gradients, with no need for absolute differences, eliminating the need for statistical replication. Where replication was necessary, plant material replicates differ by no more than 0.5‰, affording additional confidence in the accuracy and precision of single sample analyses.

2.5. Statistical analysis

Determination of absolute $\delta^{15}N$ differences between materials was not usually the aim in any analysis carried out in this investigation. Instead, $\delta^{15}N$ trends over variable environmental and experimental gradients were the focus of interest in a range of nitrogenous materials. Because of this and the high cost of analysis, high levels of replication and statistical differences were generally not required throughout this investigation.

Where statistical analysis or comparisons were appropriate, raw data was analysed and graphed in either Microsoft Excel 2002 edition or Statistica version 6. Statistical significance (p values) of all correlation coefficients (R^2 values) are less than 0.05 (i.e. $p < 0.05$). All statistical comparisons of data groups were compared using independent (by variables), *t*-tests. Significance (p values) of these tests is provided in the text.

Chapter 3

$\delta^{15}N$ 'fingerprints' within land-use catchments

3.1. Introduction

The often adverse environmental effects of anthropogenic land-use have become a public and political issue on a global scale. Human activity has in general greatly altered the N cycle in terrestrial and aquatic systems, resulting in increased N loads to collecting water bodies, such as groundwater, rivers and lakes. Land-use exported N contributes to the eutrophication of these aquatic systems reducing biodiversity and overall water quality for recreation and domestic use (Peters and Meybeck 2000). Land-use practices with high N export rates are under increasing pressure to justify their activities. Agricultural practices, particularly those with high animal N inputs, are generally considered to be the greatest N exporters. Their presence is strongly correlated with increased levels of eutrophication in associated water bodies globally and within New Zealand (McColl 1972; Mitchell 1998).

In New Zealand, local authorities are charged with reducing adverse environmental effects of land-use N to collecting water bodies. Identifying the greatest offending land-use and quantifying N export from these catchments are critical to this end. To date, only gross methods have been applied, usually involving the measurement of N concentrations and flow rates from catchment exiting streams. Differentiating between base line N export above that attributed to the land-use in question is, however, difficult. This issue is further compounded in mixed land-use catchments as N may arise from multiple origins and quantifying individual contributions is not possible. In addition, determining N cycle processes within catchments which are responsible for the loss cannot be identified using this method.

Stable N isotopes have been proposed as a tool to potentially differentiate, and quantify N export between different land-uses (Kohl et al. 1971). The $\delta^{15}N$

signature of land-use exported N represents the integration of specific N input $\delta^{15}N$ signatures and the fractionation effects of intra catchment N cycle processes. Land-use specific N inputs (e.g. animal waste) and intra catchment processes (e.g. nitrification) are therefore expected to provide distinctive isotopic 'fingerprints' in exported N. Where land-use exported N 'fingerprints' are significantly different, a simple mixing model (Equation 1.8) may be used to determine N contributions to a collecting water body in a mixed land-use catchment. This is the stable N isotope method. Following the early study of Kohl et al. (1971) who applied this method to determine fertiliser N contribution to the Mississippi Valley, much criticism has been generated surrounding its reliability. Isotopic 'fingerprints' of N sources (see Figure 1.2) are considered too variable to apply the N isotope methodology to trace their flow and fate in ecosystems (Bedard-Haughn et al. 2003).

Application of the stable N isotope technique in identifying N contributions from land-uses to collecting water bodies is limited by environmental isotopic variation. Numerous studies attempting to apply the stable isotope technique to trace N sources in and out of catchments have succeeded only in highlighting the inherent variation of $\delta^{15}N$ signatures within landscapes. Soil N $\delta^{15}N$, for example, has been reported varying by as much as 20‰ (Black and Waring 1977) which overlaps many isotopic fingerprints of potential N sources to landscapes, including fertiliser N (Figure 1.2). Some reported studies have however managed to trace N through catchments where sources have unquestionably different $\delta^{15}N$ signatures to each other and/or 'background N'. Studies tracing the flow and fate of animal and effluent wastes tend to be the most successful. In these cases, the difference between source and background $\delta^{15}N$ signatures are greater than 5‰, a difference exceeding the level of 'background N' variation in the study systems (Bedard-Haughn et al. 2003). Application of this technique to systems in which N source ^{15}N signatures overlap (Lindau et al. 1997), or are not significantly different to 'background N' (Edwards 1973), are more contentious.

Regardless of the reported variation and disagreements in literature, this technique remains a potentially useful tool in tracing the fate and flow of land-use exported N. There are a number of reasons why N isotopic 'fingerprints' might be significantly different between land-uses. The predominance of animal derived N

inputs in agriculture systems, and the often enriched isotopic signature associated with these N sources (Figure 1.2), are likely to influence the concentration and $\delta^{15}N$ of exported N. Intra-catchment processes and their associated fractionation effects may impart additional differences to isotopic 'fingerprints' of land-use exported N. Processes resulting in an increased flux into inorganic N forms, particularly NO_3-N, the most susceptible N species to leaching loss (Handley and Raven 1992) will most strongly alter exported N levels and $\delta^{15}N$. To date, no assessment of inorganic N $\delta^{15}N$ variation has been carried out for a range of representative New Zealand land-use practices.

Large scale disturbance within catchments is predicted to strongly affect the level of catchment exported N and its $\delta^{15}N$ signature. Clear felling of trees within production pine forested catchments is likely to be one of these processes. Clear felling results in soil disturbance, and large volumes of residual biomass as a consequence of stem wood removal. Following this activity, rates of mineralisation and nitrification are expected to increase the flux of organic N into NO_3-N, which is susceptible to loss via leaching (Handley and Raven 1992; Handley et al. 1999). The death of tree roots following harvest is further predicted to enhance N export by minimising NO_3-N interception prior to loss. To date, no studies have been carried out examining $\delta^{15}N$ trends following this whole catchment disturbance regime in New Zealand.

This investigation was carried out to: 1) identify the level of $\delta^{15}N$ variation in single land-use catchments collecting water bodies and 2) measure trends in exported N concentrations and $\delta^{15}N$ from production forested catchments following whole catchment clear felling. Different land-use practices were expected to provide identifiable isotopic 'fingerprints'. These 'fingerprints' were further expected to be different between catchments as a consequence of specific N inputs and/or intra catchment N cycling process fractionation factors. In-stream activities are likely to contribute to $\delta^{15}N$ variation, however groundwater should provide more reliable measurements of $\delta^{15}N$ land-use 'fingerprints'. Whole catchment clear felling is predicted to strongly alter the level and $\delta^{15}N$ signature of production forested catchment exported N. Increased levels of exported N, particularly that of isotopically depleted NO_3-N, is predicted following whole catchment clear felling.

3.2. Materials and methods

3.2.1. Land-use inorganic N concentration and $\delta^{15}N$ 'fingerprints'

A range of New Zealand representative, well established land-use activities, each within clearly defined single land-use catchments, were chosen for study. Stream and ground water inorganic N species were analysed to determine N concentration and $\delta^{15}N$ variation and define a $\delta^{15}N$ 'fingerprint' associated with each land-use practice:

- Production pine forestry -'young pine'
  - 'mature pine'
- Dry stock farming ('dry stock')
- Native forested ('native')
- Dairy farming ('dairy')

3.2.1.1 Purukohukohu nested sub catchments

The majority of land-use catchments are represented within the Purukohukohu experimental basin (38º 26´S, 176º 13´E), approximately 30 km south of Rotorua. Neighbouring, but hydraulically separated sub catchments are of equal soil type, history and climate, but experience a single, typically New Zealand land-use.

- 'native' (37.2 ha): indigenous mesophyll forest, classified as podocarp/tawa type. A species rich forest, its structure is three tiered: The canopy consists of Rimu (*Dacrydium cupressinum*), 25 – 30 m tall, emerging from a 12 – 25 m canopy dominated by kamahi (*Weinmannia racemosa*), miro (*Prumnopitys ferruginea*), hinau (*Elaeocarpus dentatus*), rewarewa (*Knightia excelsa*) and tawa (*Beilshmiedia tawa*) on the ridgelines and upper catchment. The often sparse sub canopy is dominated by hardwood trees, shrubs and ferns and dominates on lower catchment portions and valleys. The sub canopy, between 2 and 10 m in height, consists of wineberry (*Aristotelia serrata*), tree fuchsia (*Fuchsia excorticata*), mahoe (*Melicytus ramiflorus*), five finger (*Pseudopanax arboreus*), rangiora (*Brachyglottis repanda*), tree nettle (*Urtica ferox*), and

tree fern (*Dicksonia squarrosa*). Valley bottoms are continuously covered in ferns, mosses, liverworts, sedges, and herbs (Beets and Brownlie 1987).

- 'dry stock' (22.5 ha): developed in 1957 into well established pasture grazing sheep and cattle, after original clearing of native forest in the 1920s reverted back to seral scrub. Pasture establishment involved burning and sowing perennial ryegrass (*Lolium perenne*), white clover (*Trifolium repens*) and the application of superphosphate, periodically applied (usually March), equivalent to 30 kgP/ha (Cooper et al. 1987). Invading weed species e.g. ragwort (*Senico jacobaea*), bracken (*Pteridium esculentum*), scotch thistle (*Cirsium vulgare*), and catsear (*Hypochaeris radicata*) periodically co-dominate with Yorkshire fog (*Holcus lanatus*) and cocksfoot (*Dactylis glomerata*). Pasture growth is driven by high levels of clover N_2-fixation and nitrogenous fertilisation (Beets and Brownlie 1987).

- 'young pine' (34.4 ha): as for 'dry stock' but with a land-use change from pasture to pines in 1973. *Pinus radiata*, seven years of age, and almost enclosed canopy at time of investigation, was the second rotation of the originally planted and enclosed catchment (Beets and Brownlie 1987).

- 'mature pine': As with 'young pine', but representing mature *P. radiata* (at least 20 years old).

The Purukohukohu experimental basin soils are typical of the surround area with yellow brown pumice soils overlying a variable topography. The yellow brown soils belong to the Oruanui series, composed of sandy loam, sand and gravel: the parent material originating from Taupo (1850 ±100BP) and older ash showers from Taupo and Okataina volcanic centres (Beets and Brownlie 1987). The soils within and around Purukohukohu experimental basin have typically deep, friable, although structurally weakly developed topsoils. Subsoils are loose and course textured overlying a relatively impermeable base. The soils are both porous and permeable with average infiltration rates of 52 mm/h (pasture soils), 225 mm/h (pine soils) and 600 mm/h (native forest soils) (Beets and Brownlie 1987).

Climate data recorded in the Purukohukohu experimental basin since 1976 describe an average annual rainfall of 1500 mm evenly distributed throughout the year, and average annual temperature of 11ºC (Beets and Brownlie 1987).

Each sub catchment is drained by a clearly defined stream, fed by springs at the head and length of the catchment. Both the 'pasture' and 'young pine' streams can cease flowing for periods during late summer but the 'native' stream is perennial. Stream channels are relatively clear in the 'pine' and 'native' catchments, however a dense mat of floating vegetation (predominantly floating sweet grass: *Glyceria fluitans*) often covers the 'pasture' stream flow channel (Beets and Brownlie 1987).

The almost complete set of land use management practices is complimented by the intensely managed 'dairy' catchment of Toenepi.

3.2.1.2. Toenepi catchment

The Toenepi catchment, located in the Waikato region, contains 29 sub catchments within its 15 km^2 area. Dairying makes up at least 93% of all the sub catchments apart from one, which is dominated by dry stock farming. The main waterway within, and the only one exiting the catchment, is the Toenepi stream arising from springs at the head of the catchment and fed by further springs and surface waterways along its length. The catchment has a predominantly flat topography, overlying three major soil groups. The Topehaehae soils (Gley Soils) are narrowly distributed (13%) and occur generally in the lowest areas adjacent to the main stream and in valleys between hills. The similar Kereone and Kiwithahi soils (Allophanic Soils) account for 47% of the soil types in the catchment, and occur on easy rolling to rolling slopes and on freely draining levees of the plains. The remaining 40% of the soil type distribution within the catchment is accounted for by the Morrinsville soils (Granular Soils) occurring on low rolling down-land topography. These soils have typically high sub soil clay contents, resulting in medium bulk density, porosity, and moderate permeability. The ground water table is shallow, typically between 0.5 and 2.5 m below the surface (Stenger et al. 2003).

3.2.1.3. Sample collection

Three groundwater wells were installed beside stream channels in the four Purukohukohu experimental basin sub catchments. The wells (17.7 mm internal diameter PVC pipe slotted over their entire length) were established to a depth of between 1 to 2.5 m below the surface. Following a settling period of two months, wells were 'developed' by pumping until water became clear. Subsequent analysis of inorganic N concentration and $\delta^{15}N$ signature in ground and stream waters occurred three times over the six month period between January and June 2004. All wells other than numbers 1 and 2 within the 'dry stock' catchment provided consistent water samples for inorganic N concentration and $\delta^{15}N$ signature determination.

The Toenepi catchment contained pre-existing ground water wells as part of an independent ongoing investigation into water quality associated with land-use management practices. Wells (similar dimensions to those established in the Purukohukohu sub catchments) were installed as transects through streams and wetlands one year prior to this investigation (Stenger et al. 2003). A single sampling event in August 2004 included stream water and groundwater from 17 of the pre-installed wells selected within a range of sub catchments.

3.2.2. Clear felling in pine production forested catchments and its effect on inorganic N concentration and $\delta^{15}N$ signature.

Five production pine forestry catchments with single exiting, spring fed, perennial streams were selected prior to clear felling;

- 'Hereperu stream 1', '2', and '3'
- 'Tarawera'
- 'Pinnacles'

The 'Hereperu' streams represent three neighbouring, but hydraulically separated catchments. 'Tarawera' and 'Pinnacles' streams are geographically separated from each other, and the 'Hereperu stream' catchments. All catchments represent a typically free draining pumice soil and similar climatic conditions, separated by a distance of no more than 100 km. All catchments contained *P. radiata* trees of similar stocking and age (c. 25 years old).

To determine the effect of whole catchment clear felling on trends of exported inorganic N concentrations and $\delta^{15}N$ signatures, stream water samples were collected periodically over the nine month period: September 5 2003 to June 10 2004. Within this time frame, four of the five catchments experienced clear felling, the process lasting no more than three days, in which trees were felled and logs prepared *in situ* before hauling off site. Inorganic N concentrations and $\delta^{15}N$ signatures were periodically assessed in all catchment exiting streams over the nine month experimental period. Inorganic N concentration and $\delta^{15}N$ trends were compared between the clear fell catchments and the control ('Pinnacles') catchment, which did not experience clear felling.

3.2.3. Water sample preparation and analysis

All water samples, on return to the laboratory, were immediately filtered (Whatman GF/B) and resin extracted based on the modified method of Silvia et al. (2000). Water samples were passed through 10 mm^3 volume of ion exchange resins (Dowex 1X8 200 mesh and 50WX4-200 mesh) and eluted with 3M HCl using at least five bed volumes. All eluted samples were alkali distilled to recover NH_4-N and NO_3-N (in the presence of Devarda's alloy). Condensate was back titrated against standardised HCl to determine inorganic N concentration then dried in excess acid prior to isotopic analysis. The technique described resulted in no significant isotopic fractionation.

The limited number of replications and sampling events prevent robust statistical analysis. Focus is on mean and standard deviation (sd) of inorganic N concentrations and $\delta^{15}N$ signatures in land-use catchment water bodies. Isotopic 'fingerprints' are defined as the mean and range of inorganic N $\delta^{15}N$. Significant differences between 'fingerprints' is determined by *t*-test comparisons of means where $p < 0.05$. Replication is equally minimal in the clear felling trial. Focus is on the trends in inorganic N concentration and $\delta^{15}N$ signature in catchment exiting streams over the experimental time period as a consequence of clear felling. Variation is compared both within and between individual catchments and against control stream variation.

Most $\delta^{15}N$ values presented are an average of two duplicates with duplicate variation no more than 1‰, a measure of machine and sample preparation precision. Trends exceeding this error are attributed to environmental variation.

3.3. Results

3.3.1. Land-use inorganic N concentration and $\delta^{15}N$ 'fingerprints'

Stream and ground water samples from the experimental catchments exhibited a range of inorganic N concentrations and $\delta^{15}N$ signatures, as follows;

3.3.1.1. NO_3-N concentration

Both stream and groundwater NO_3-N concentrations were highly variable within and between land-use catchments (Table 3.1).

'Dairy' stream water NO_3-N had the highest concentrations (1.37 mg N L^{-1}), 'dry stock' and 'young pine' the lowest averaging 0.03 and 0.08 mgN L^{-1}, respectively. NO_3-N concentration variability was high in all land-use stream waters minimising significant differences between them. NO_3-N concentrations in 'dry stock' were significantly ($p < 0.05$) lower than 'native' and 'mature pine'. 'Young pine' was significantly lower than 'mature pine' and 'native'. 'Dairy', although measuring the highest average NO_3-N stream concentrations, it was not significantly different to all other land-uses.

Land-use groundwater NO_3-N concentrations were always more variable than corresponding stream water NO_3-N concentrations (Table 3.1). Variability resulted in no significant difference between all land-uses except 'young pine' which was significantly lower ($p < 0.05$) than 'native' and 'dry stock'.

Table 3.1. Average NO_3-N concentrations and $\delta^{15}N$ signatures in stream and groundwaters of single land-use catchments. (s.d. = standard deviation)

Land-use	Average NO_3-N concentration in stream water ($mgNL^{-1}$) (s.d.)	Average NO_3-N concentration in ground water ($mgNL^{-1}$) (s.d.)	Average NO_3-N $\delta^{15}N$ in stream water (‰) (s.d.)	Average NO_3-N $\delta^{15}N$ in ground water (‰) (s.d.)
Dry stock	0.03 (±0.05)	1.53 (±0.71)	-1.87 (±5.32)	+4.30 (±0.58)
Native	0.85 (±0.17)	1.40 (±1.07)	-0.01 (±0.47)	+0.96 (±3.32)
Young pine	0.08 (±0.03)	0.07 (±0.09)	+3.13 (±0.42)	-1.99 (±3.54)
Mature pine	0.79 (±0.19)	0.44 (±0.75)	+1.85 (±0.74)	+0.96 (±3.08)
Dairy	1.37 (±2.18)	1.98 (±2.81)	+3.13 (±11.35)	+3.67 (±4.32)

3.3.1.2. NO_3-N $\delta^{15}N$ 'fingerprints'

Average stream water NO_3-N $\delta^{15}N$ signatures within single land-use catchments varied between -1.87 to +3.13‰, a range of approximately 5‰ (Table 3.1). Variation in stream NO_3-N $\delta^{15}N$ signature was greatest in 'dairy' land-use, providing the most extreme $\delta^{15}N$ values in this study during one collection event within a three km stretch of stream. Average stream water NO_3-N $\delta^{15}N$ signatures follow the trend (from most depleted to enriched): 'dry stock' < 'native' < 'mature pine' < 'young pine' = 'dairy'. High levels of variation however, resulted in minimal significant differences between stream NO_3-N $\delta^{15}N$ 'fingerprints' of single land-use catchments (Figure 3.1). Only 'native' stream NO_3-N $\delta^{15}N$ 'fingerprints' are significantly more depleted than 'young' or 'mature pine'.

Average groundwater NO_3-N $\delta^{15}N$ signatures within single land-use catchments varied between -1.99 to +4.30‰, a similar although slightly wider range (6.3‰) to that of stream water NO_3-N $\delta^{15}N$. Variation in groundwater NO_3-N $\delta^{15}N$ signatures was greatest in 'dairy' and lowest in 'dry stock'.

Average groundwater NO_3-N $\delta^{15}N$ signatures follow the trend (from most depleted to enriched): 'young pine' < 'native' = 'mature pine' < 'dairy' < 'dry stock'. Highly variable ground water NO_3-N $\delta^{15}N$ signatures resulted in no significant differences between groundwater NO_3-N $\delta^{15}N$ 'fingerprints' of single

land-use catchments except 'young pine' which was significantly more depleted than 'dry stock' and 'dairy' (Figure 3.1).

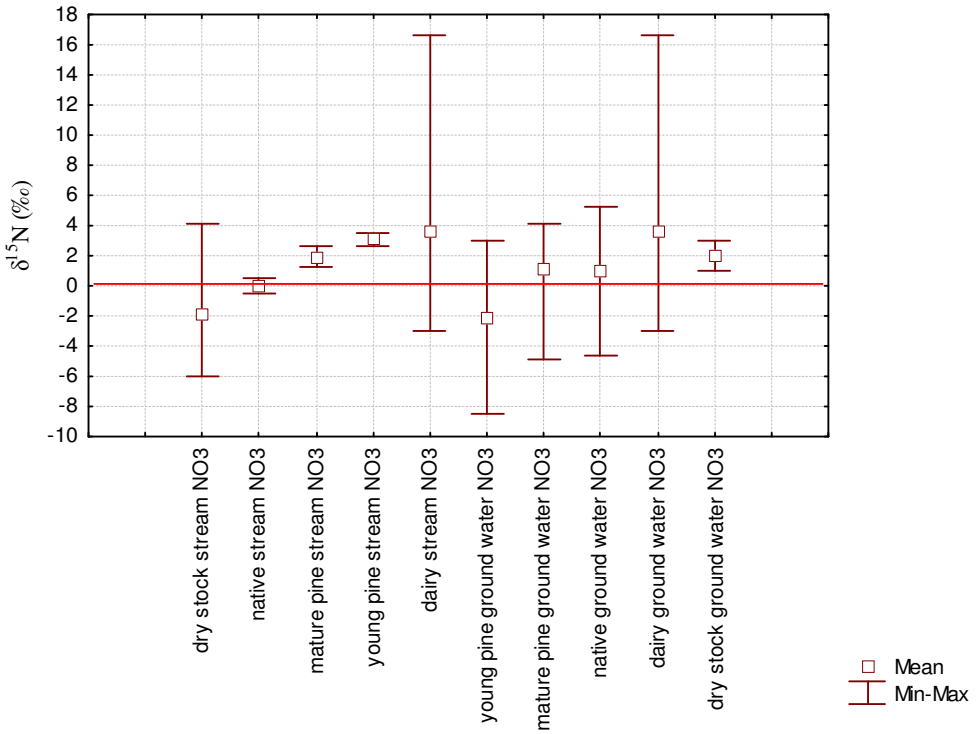


Figure 3.1. NO_3-N $\delta^{15}N$ 'fingerprints' (mean and range) in single land-use catchment stream and groundwaters.

3.3.1.3. NH_4-N concentration

Stream and groundwater NH_4-N concentrations are generally, and as expected, low (Table 3.2). Variation resulted in no significant difference ($p = <0.05$) between all land-use stream NH_4-N concentrations. Similar levels of NH_4-N concentration variation in groundwaters resulted in only 'mature pine' groundwater NH_4-N concentrations significantly ($p < 0.05$) higher than 'young pine' and 'native'.

3.3.1.4. NH_4-N $\delta^{15}N$ 'fingerprints'

Average stream water NH_4-N $\delta^{15}N$ signatures for single land-use catchments ranged between -4.70‰ and -2.08‰ (Table 3.2) following the same trend as stream water NO_3-N (from most depleted to enriched): 'dry stock' < 'native' < 'mature pine' < 'young pine'. The narrow range and high levels of variation resulted in no significant difference between any land-use catchment stream water NH_4-N $\delta^{15}N$ 'fingerprint' (Figure 3.2).

Average groundwater NH_4-N $\delta^{15}N$ signatures fell within the range; -2.02‰ to +3.81‰ (a difference of c. 5.8‰) much wider than that measured in stream NH_4-N $\delta^{15}N$ signatures. However, because of the level of variation, no significant difference was measured between individual land-use groundwater NH_4-N $\delta^{15}N$ 'fingerprints' (Figure 3.2).

Table 3.2. Average NH_4-N concentrations and ^{15}N signatures in stream and groundwaters of single land-use catchments. (s.d. = standard deviation; nd = not determined)

Land-use	**Average NH_4-N concentration in stream water ($mgNL^{-1}$) (s.d.)**	**Average NH_4-N concentration in ground water ($mgNL^{-1}$) (s.d.)**	**Average NH_4-N $\delta^{15}N$ in stream water (‰) (s.d.)**	**Average NH_4-N $\delta^{15}N$ in ground water (‰) (s.d.)**
Dry stock	0.01 (±0.00)	0.08 (±0.09)	-4.70 (±7.64)	-0.44 (±6.96)
Native	0.01 (±0.00)	0.02 (±0.01)	-4.45 (±2.33)	-1.14 (±4.67)
Young pine	0.01 (±0.01)	0.02 (±0.01)	-2.08 (±5.52)	-2.02 (±1.87)
Mature pine	0.01 (±0.01)	0.04 (±0.02)	-2.89 (±1.61)	+3.81 (±5.41)
Dairy	nd	nd	nd	nd

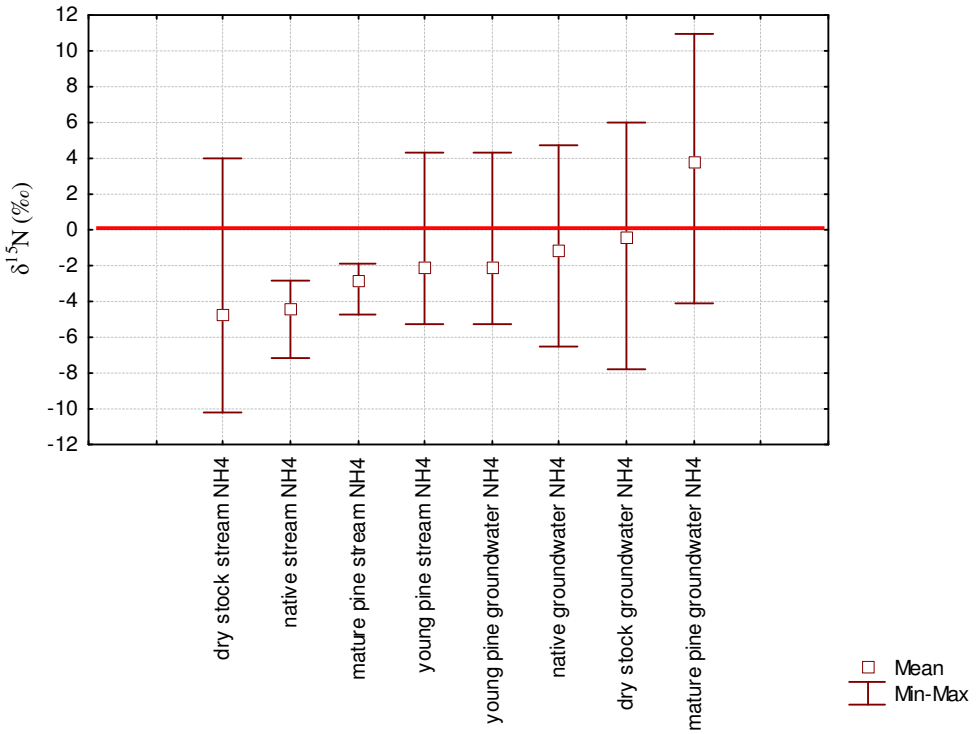


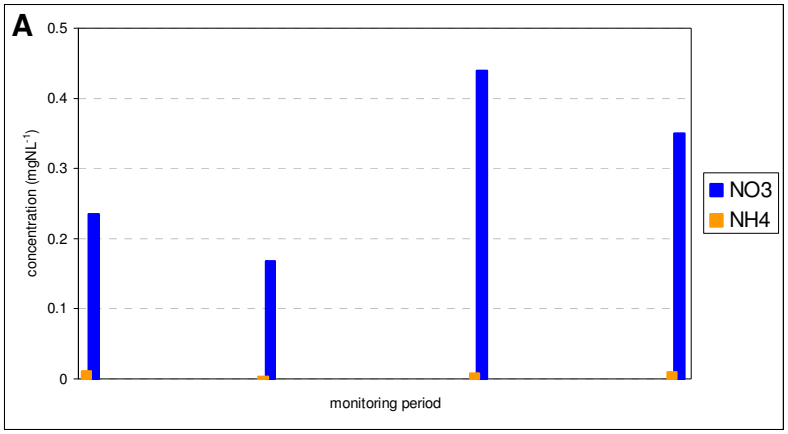
Figure 3.2. NH_4-N $\delta^{15}N$ 'fingerprints' (mean and range) in single land-use catchment stream and groundwaters.

3.3.2. Clear felling in pine production forested catchments and its effect on inorganic N concentration and $\delta^{15}N$ signature.

3.3.2.1. Stream exported inorganic N concentrations following clear felling

NO_3-N concentrations far exceeded NH_4-N concentrations in all catchment streams throughout the monitoring period (Figure 3.3). In all, except one occasion measured in 'Hereperu stream 2' (c. 3.5 months following clear felling), NH_4-N concentration never rose above 0.01 mg N L^{-1}. NO_3-N concentrations ranged between 0.004 to 2 mg N L^{-1} in all catchment streams. 'Hereperu 1' stream consistently had the highest NO_3-N concentrations, and 'Tarawera' stream the lowest. Control stream ('Pinnacles') NO_3-N concentration varied by only c. 0.02 mg N L^{-1} throughout the treatment period.

Following clear felling, two of the four catchments experienced an immediate and dramatic reduction in NO_3-N concentrations; 'Hereperu stream 2' (Figure 3.3D.), and 'Hereperu stream 3' (Figure 3.3E.). The level of NO_3-N concentration reduction in these streams exceeded the level of variation in the 'Pinnacles stream' (Figure 3.3A.) over the monitoring period. Recovery of NO_3-N concentrations to those near pre-clear felling occurred some time within 4 to 6.5 months in 'Hereperu stream 2' and within 4 months in 'Hereperu stream 3'. No clear trend in NO_3-N concentration variation was measured in 'Hereperu stream 1' or 'Tarawera' following catchment clear felling (Figures 3.3B and 3.3E).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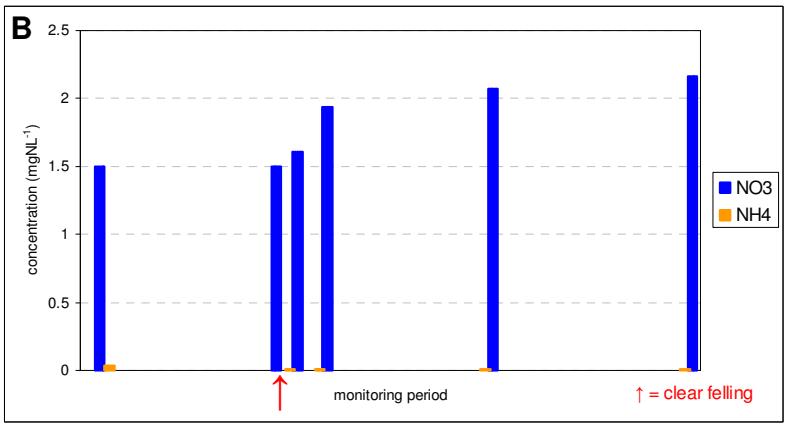
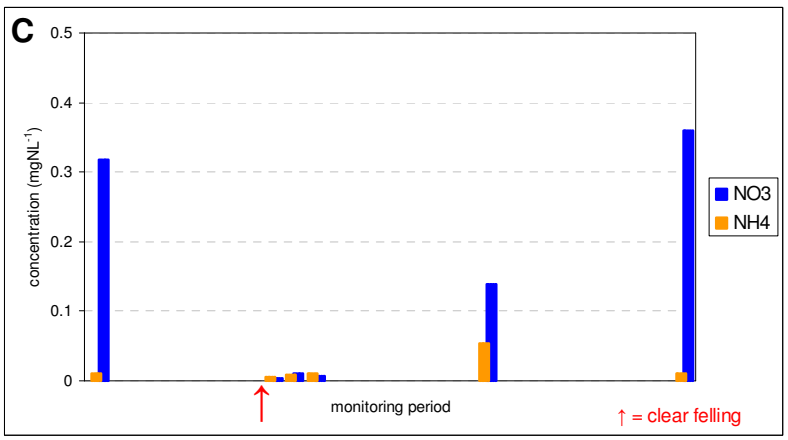
0.4
0.3
0.2
0.1
0
concentration (mgNL-1)
monitoring period
NO3
NH4


B
2.5
2
1.5
1
0.5
0
concentration (mgNL-1)
monitoring period
↑ = clear felling
NO3
NH4


C
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0.3
0.2
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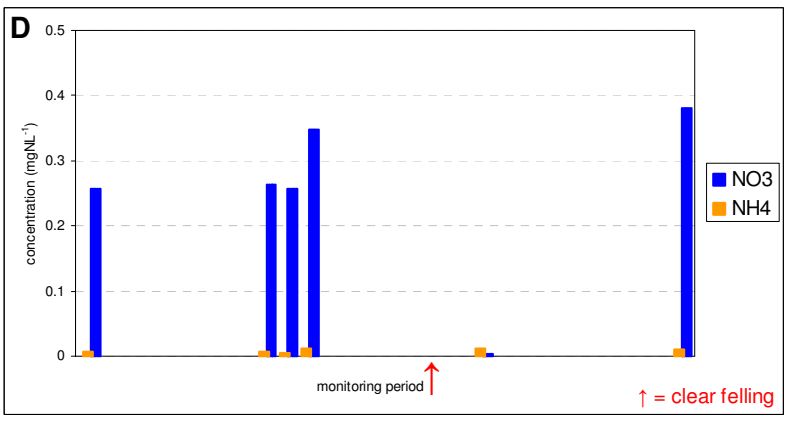
0
concentration (mgNL-1)
monitoring period
↑ = clear felling
NO3
NH4


D
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concentration (mgNL-1)
monitoring period
↑ = clear felling
NO3
NH4

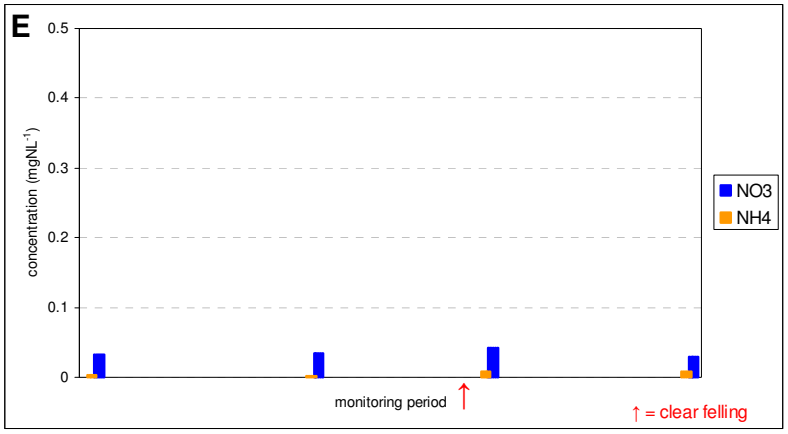


Figure 3.3. Inorganic N concentrations in streams of production forested catchments before and following whole catchment clear felling. A) 'Pinnacles' (control catchment), B) 'Hereperu 1', C) 'Hereperu 2', D) 'Hereperu 3', E) 'Tarawera'. Monitoring period = nine months.

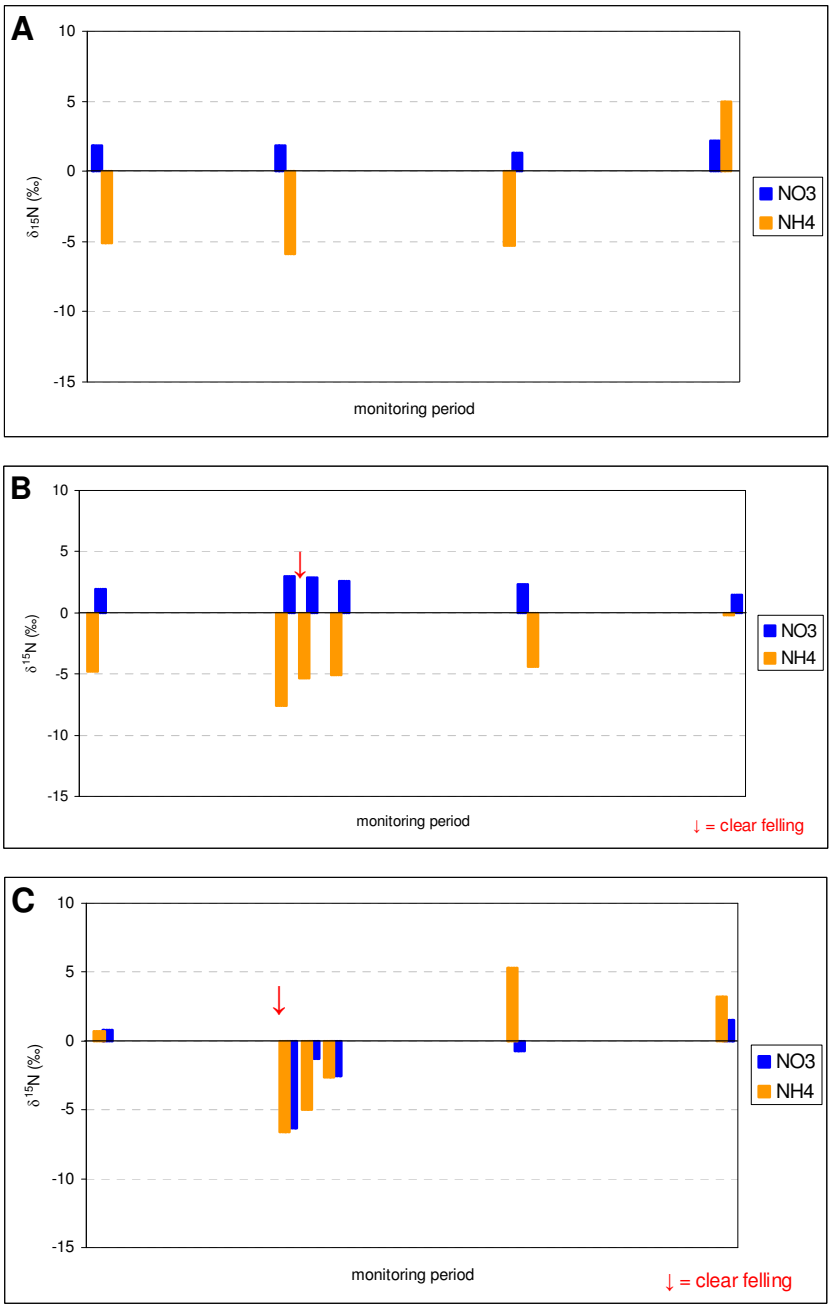
3.3.2.2. Stream exported inorganic N $\delta^{15}N$ signature following clear felling

In all pre-clear felling measurements, NO_3-N $\delta^{15}N$ signatures are more enriched than that of NH_4-N. In most cases, NO_3-N is at least 2‰ more enriched, but in 'Hereperu stream 1', by as much as 10‰ (Figure 3.4B). Inorganic N $\delta^{15}N$ signatures in 'Pinnacles' (control) stream confirms this inorganic N $\delta^{15}N$ trend, where a consistent difference of c. 7‰ between NO_3-N and NH_4-N $\delta^{15}N$, is maintained throughout the trial period until the final measurement where NH_4-N $\delta^{15}N$ increased from -5‰ to +5‰ (Figure 3.4A). Prior to clear felling, NO_3-N and NH_4-N $\delta^{15}N$ signatures averaged +1.9‰ (±0.9‰ sd) and -4.1‰ (±2.3‰ sd), respectively and appears to be the 'normal' exported inorganic N $\delta^{15}N$ signatures.

No obvious changes in NO_3-N or NH_4-N $\delta^{15}N$ signatures were measured in 'Hereperu 1' or 'Tarawera' streams following whole catchment clear felling (Figures 3.4B and 3.4E). In 'Hereperu stream 2' and '3' however, $\delta^{15}N$ signatures of exported inorganic N expressed an immediate isotopic depletion (by as much as 6‰ in 'Hereperu stream 2' and more than 10‰ in 'Hereperu stream 3') immediately following clear felling (Figures 3.4C and 3.4D). In both cases, the depletion in stream N $\delta^{15}N$ signatures coincided with the inorganic N concentration reduction (3.3.2.1.).

Recovery of NO_3-N $\delta^{15}N$ signatures to pre-clear felling levels in 'Hereperu Stream 2' appear to have occurred by the end of the monitoring period (seven months after clear felling), and within four months in 'Hereperu stream 3'. Recovery of NH_4-N $\delta^{15}N$ signatures to pre-clear felling levels appeared to take

much longer. In 'Hereperu stream 2' NH_4-N $\delta^{15}N$ signatures became strongly enriched four months after clear felling, then depleted again, almost recovering to pre-clear felling levels by seven months. NH_4-N $\delta^{15}N$ signatures in 'Hereperu stream 3' also became strongly enriched approximately four months following clear felling, but measurements were discontinued before pre-clear felling $\delta^{15}N$ signatures were achieved (Figure 3.4D).



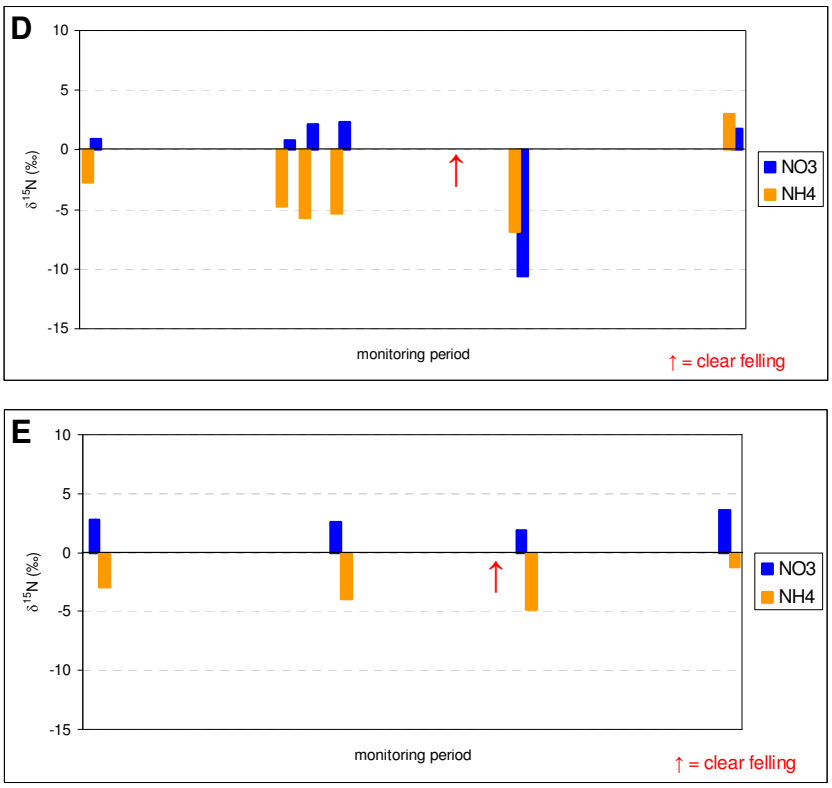


Figure 3.4. Inorganic N $\delta^{15}N$ signatures in streams of production forested catchments before and following whole catchment clear felling. A) 'Pinnacles' (control catchment), B) 'Hereperu 1', C) 'Hereperu 2', D) 'Hereperu 3', E) 'Tarawera'. Monitoring period = nine months.

3.4. Discussion

Stream and groundwaters from a range of New Zealand representative land-uses were collected to determine the natural variation of exported inorganic N concentrations and $\delta^{15}N$ signature variation. A longer monitoring period than that employed here is required to more strongly assess trends and drivers of exported N concentrations and $\delta^{15}N$ variation. However the data gathered in this study allow a general observation of patterns and assessment of variation. Concentrations of NO_3-N for example, although highly variable, exceeded those of NH_4-N in all land-use stream and groundwaters, confirming the dominance of NO_3-N in N export.

Inorganic N concentrations provide a comparative measure of land-use N export. Stream water NO_3-N concentration results suggest a trend (from greatest NO_3-N export to least): 'dairy' > 'native' > 'mature pine' > 'young pine' > 'dry stock'. This trend closely agrees with the findings of Cooper et al. (1987) for stream N export trends from all land-use catchments used in this study, except dairy. NO_3-

N concentration variation is so great however that difference between land-uses are, in the most part, not significant. In-stream processes (e.g. variable rates of utilisation by stream vegetation and denitrification) are likely to account for some level of the measured variation. The unexpectedly low NO_3-N concentration in the 'dry stock' stream, for example, is a likely consequence of NO_3-N stripping by the dense floating in-stream vegetation observed in this land-use catchment stream channel.

Groundwater NO_3-N concentrations follow a generally similar N concentration trend: 'dairy' > 'dry stock' > 'native' > 'mature pine' > 'young pine'. The comparatively high NO_3-N concentrations in 'dry stock' and 'dairy' land-use groundwater's are not surprising given the high level of N input from fertiliser and animal derived N. Ground water NO_3-N concentrations were expected to be less variable in the absence of in-stream processes, however variation equalled, and in many cases exceeded the levels measured in streams. Variation resulted in almost no significant differences between land-use groundwater inorganic N concentrations.

NO_3-N concentrations in 'native' land-use collecting water bodies are surprisingly high. This high concentration is unexpected as undisturbed, stable, and 'natural' systems like 'native' forested catchments are considered to be the most conservative N exporting land-use (Parfitt et al. 2003). To date, no one has accounted for this supposed anomaly, but it may be a consequence of intra catchment processes predominating in mixed, but particularly broad leaf native forests. Broad leaf forests provide a consistent supply of high N:C ratio leaf fall material throughout the year. Favourable mineralisation and nitrification conditions in these forest soils may provide consistent and comparatively high soil NO_3-N concentrations, which is prone to catchment loss (Handley et al. 1999). In contrast, the recalcitrant leaf material of pine vegetation may 'lock up' a large proportion of ecosystem N in litter and soil organic-N forms. In addition, the high nutrient demand from rapidly growing pine trees, especially when young, may further account for the comparatively low inorganic N concentrations in 'pine' land-use catchment water bodies.

High levels of environmental $\delta^{15}N$ variation limited differentiation between land-use practices based on N isotope 'fingerprints' in both stream and groundwaters. In-stream activities may account for variation in stream waters, however similar levels of variation were measured in groundwaters where these processes are assumed to be minimised. The level of variation measured resulted in strongly overlapping isotopic 'fingerprints', inhibiting the application of the stable isotope technique to differentiate between individual land-use catchment N export, even in nested catchments sharing similar abiotic factors.

Variation in groundwater $\delta^{15}N$ is not unique to this study. Temporal variation in groundwater $\delta^{15}N$ has been reported to vary over 4.5‰ as a consequence of seasonal variation in denitrification levels alone (Panno et al. 2001). Spatial variation, as a consequence of complex soil N cycle processes, is highly variable at the landscape scale (Bedard-Haughn et al. 2003) and will also contribute to variation in exported N $\delta^{15}N$. In addition, groundwater $\delta^{15}N$ is strongly altered by its age and depth. Cey et al. (1999) measured $\delta^{15}N$ in groundwater NO_3-N to vary from +4.8‰ at the surface, to +24.8‰ at depth, a reflection of groundwater age and historic land-use. Mixing of groundwaters of different ages prior to catchment export is likely to contribute to isotopic signature variation (Morgenstern et al. 2005). The limited sampling points and replication of groundwater, in addition to the unknown proportion of young and old groundwater mixing, likely accounts for a large portion of measured variation in land-use groundwater $\delta^{15}N$ in this investigation.

Variation in water body inorganic N $\delta^{15}N$ may be minimised by a greater sampling effort and over a longer period of time. Identification of the approximate groundwater age will also establish whether wells are tapping appropriately young, unmixed, groundwaters. Quantifying seasonal trends in $\delta^{15}N$ signatures would also minimise $\delta^{15}N$ variation. These results support those of Robertson (2001) suggesting that further technological advances and interpretative approaches need to be implemented to successfully apply the stable isotopic methodology in determining N export from land-use management practices.

Dramatic whole catchment effects, such as clear felling, are expected to significantly alter concentrations and $\delta^{15}N$ of land-use exported N. Although

significant variation in the trends of exported N concentration and $\delta^{15}N$ were measured in only two of the four monitored catchments, where a response was measured it was clear and consistent; concentrations and $\delta^{15}N$ signatures of exported N immediately decrease as a consequence of whole catchment clear felling. The measured $\delta^{15}N$ depletion in stream NO_3-N was expected. The predicted increase in stream NO_3-N concentration - as a consequence of increased rates of mineralisation and reduced plant NO_3-N interception following clear felling - was however not measured. In contrast NO_3-N concentrations in catchment exiting streams decreased as a consequence of whole catchment clear felling.

The most fitting interpretation of these data suggests that although mineralisation is initiated, it is apparently N limited by the high C:N ratio of clear felling residue (branches and waste stems). Consequently, a high affinity for existing inorganic N sources by mineralising microbes will 'lock up' freely available NO_3-N, explaining the immediate reduction in NO_3-N concentrations following clear felling. Any small loss of inorganic N to the streams during this period will preferentially favour the isotopically lighter $^{14}NO_3$-N and $^{14}NH_4$-N, reflecting the initial product of a large, newly available organic N pool (Handley and Raven 1992).

This modified interpretation conforms to Rayleigh isotopic principles, and accounts for both the reduced concentrations and corresponding depletion measured in exported inorganic N $\delta^{15}N$ signatures from these catchments. Progressive mineralisation of the clear felling residue will provide increasingly higher concentrations of NO_3-N over time and a corresponding increase in the potential loss from the catchment. Higher concentrations of NO_3-N measured in streams some months following clear felling confirm this apparent 'recovery' of the exported inorganic N level over time. This interpretation is however questionable given significant trends were observed in only two of the four monitored catchments.

Quantification of environmental variation is the critical limiting factor in applying the stable N isotope technique to tracing the fate and flow of N within landscapes. This small study further highlights the need to identify sources of environmental

variation and account for them when interpreting landscape scale $\delta^{15}N$ studies. The advantage of this technique however, is that interpretation of empirical data provides insights into intra catchment processes giving rise to land-use N loss. Further quantification of $\delta^{15}N$ variation within landscapes will eventually allow this method to become a critical tool in the management of land-use N export to the environment.

Chapter 4

Using the Stable Isotope ^{15}N to Derive a Budget for Effluent-Derived Nitrogen Applied to Forest*

4.1. Introduction

Increasing nutrient status of surface and ground waters has become a global trend in recent decades. This trend is predominantly attributed to urbanisation and increased intensity of agricultural practices. New Zealand, like many industrialised countries, is no exception. Lake Rotorua for example, an inland eutrophic lake of 80 km^2, formed as a consequence of volcanic activity, has experienced many years of algal blooms and reduced water clarity. Surrounding agricultural land-use and increased urbanisation are the main reasons for deteriorating water quality (Rutherford et al. 1989; Donald et al. 1991). The dominant urban area is the City of Rotorua, situated on the southern shore, which prior to 1991, discharged its municipal effluent directly into the lake.

To improve the water quality of the lake an upgraded treatment plant and a land based effluent irrigation scheme were commissioned in 1991. Rotorua's municipal waste treatment system is based on a modified activated sludge process, designed to remove 80% of N and P. The treated effluent, of which the majority of N occurs as nitrate (NO_3), is pumped 3 km from the city and irrigated onto the Whakarewarewa conifer production forest (for conifer species, see; 'materials and methods'). The 192 ha irrigation area, called the Rotorua Land Treatment System (RLTS) is contained within the Waipa catchment, which flows via the Waipa stream into the Puarenga stream and finally into Lake Rotorua.

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The original design specifications of the RLTS allowed for 312 kg N ha^{-1} yr^{-1} to be applied to the forest, of which 35 kg N ha^{-1} yr^{-1} was expected to be taken up by trees, 65 kg N ha^{-1} yr^{-1} to be denitrified *in situ* and a further 85 kg N ha^{-1} yr^{-1} denitrified in adjacent wetlands (McLay et al. 2000). Complete removal of the applied N was not anticipated and export of N from the RLTS via the Waipa stream was expected to increase, but not above the water right requirement of 169 kg N ha^{-1} yr^{-1} (Cooper and Cooke 1990). Despite a considerable delay, a continuing rise in NO_3 concentrations has been measured in the Waipa Stream since irrigation commenced. This trend has been particularly noticeable since 1994 where a marked seasonal fluctuation in N export from the Waipa stream has been observed with NO_3 concentrations often exceeding the water right requirement during winter. Although suspected, it has been impossible to determine how much of the NO_3 in the stream is directly or indirectly attributed to effluent irrigation.

The increase in N export is partly due to a small increase in loading rates (up to a maximum of 399 kg N ha^{-1} yr^{-1}) (McLay et al. 2000), but is mainly due to the large overestimation of the denitrification capacity of both the upland soils and the lowland wetlands. The upland soils are now shown to denitrify no more than 2 kg N ha^{-1} yr^{-1} (Barton et al. 1999) while the wetlands may only denitrify 37 kg N ha^{-1} yr^{-1} (Tomer et al. 2000).

While some of the applied N is likely to be retained in tree biomass, both above and below ground, the estimated N uptake by closed canopy pine forest is of the order of 35 kg N ha^{-1} yr^{-1} and accounts for but a small proportion of the applied N. The balance between plant storage and export needs to be accounted for. A number of components within the RLTS have the capacity to store N. Conifer ecosystems are capable of storing very large quantities of nitrogen, from 1 t N ha^{-1} for *Pinus radiata* forest growing in sand dunes (Will 1978) to as much as 15 t N ha^{-1} in the case of kauri (*Agathis australis*) forest (Silvester 2000).

The Rotorua soils are free draining volcanic ash soils in which NO_3 is likely to be readily leached beyond the topsoil. NO_3-N levels in a 'high' (88 mm $week^{-1}$) irrigation sub plot within the Forest Research WFIT (Whakarewarewa Forest Irrigation Trial) reached almost 10 g m^{-3} in piezometers (Magesan 1998), close to

the WHO maximum standard for drinking water (11.3 g m^{-3}). Despite the large amounts of N added to these soils, intensive sampling has failed to show significant or consistent N build up in the soil down to 700 mm (McLay 2000). In contrast these soils showed a very significant accumulation of P, especially in the 0-200 mm zone.

A number of factors appear to mitigate against the efficient removal of N in the RLTS:

1. The soils are very permeable, with coarse texture and free draining characteristics thus:
   - the residence time of the effluent in the soil is small
   - upland soils are well aerated and hence do not support significant denitrification
   - the bulk of N (supplied as NO_3), is likely to be rapidly leached beyond the zone of active root uptake.
2. The tree crop species have high nutrient use efficiency (efficient internal N recycling) and thus have a small net demand for N.
3. The plants are not limited by N therefore any litter produced has a low C/N ratio allowing rapid mineralisation, resulting in very low N immobilisation.
4. The lowland wetlands have been shown to be ineffective in denitrification due to 'short circuiting' of groundwater to above-ground seepages in the riparian zone. This is due to the increased hydraulic load from the irrigation activity (Rutherford et al. 1999).

The increase in NO_3-N leaving the RLTS via the Waipa stream is in excess of that predicted and the source of this N is difficult to pinpoint. Despite the evidence showing the N removal processes are less than originally calculated it is notable that the RLTS has managed, on average, to meet the water right requirement of 169 kg N ha^{-1} yr^{-1} leaving in the Waipa Stream. This naturally has raised the issue as to the fate of irrigated N within the RLTS for this situation to occur.

Regional and local governing bodies manage the quality of water bodies like Lake Rotorua. Assessing nutrient contributions from surrounding land-use practices are critical in the monitoring the nutrient load to water bodies. Tracing the source of

nutrients from various land-use practices to surface and ground waters is, however fraught with difficulties. Clearly differentiating between multiple sources in mix land-use catchments, logistics and costs of sampling are major difficulties facing governing bodies in this task.

The stable isotope technique has provided hope as a successful method to trace N through landscapes since it was applied in the 1970s (Kohl et al. 1971). The stable isotope technique ($\delta^{15}N$) relies on observing the small but measurable differences in the ^{14}N:^{15}N ratio (measured in ‰) of N-containing environmental components. Changes in the ratio of these two isotopes are brought about during physical and chemical processes which fractionate against ^{15}N. Processes like denitrification during treatment of effluent result in the N_2 gas product being depleted in ^{15}N and the residual NO_3 enriched in ^{15}N. Processes like these, result in either ^{15}N depleted or enriched materials which can be traced through the environment to collecting water bodies. Success of the technique relies predominantly on the degree of difference between the $\delta^{15}N$ signatures of source-N to that of the background ecosystem N (Bedard-Haughn et al. 2003). Successful applications of the method in literature often involve tracing N sources that have a significantly different signature to that of background N, usually human or animal derived waste, typically enriched in ^{15}N (>10‰) (Komor and Anderson 1993; Aravena et al. 1993; Wassenaar 1995; Fogg 1998; Karr et al. 2001).

Currently the treated effluent has an inorganic N content averaging 6.3 g m^{-3} of which 97% is NO_3-N. Our recent preliminary work shows this NO_3-N has an isotopic signature of +14.12 (±1.40)‰ (n=9, collected during four sampling events over a one year period. All isotopic signatures referenced to N_2 in air. See 'materials and methods') as a result of the treatment process, that can be followed into trees, soil and groundwater (Wilkins 2003). As the majority of control soils and plants have isotope values close to zero it is possible, knowing both the incoming isotopic signal and the background signal, to apportion the effluent N to various compartments, including soil water in the irrigated zone. This work extends that methodology to the RLTS and provides for the first time an overall budget for the fate of N applied to a system over the period of irrigation.

Ammonium (NH_4) in the effluent is also enriched (c. +10‰), but represents only 3% of the inorganic N in the effluent and its contribution is ignored for the purpose of this exercise. The organic N component of the treated effluent has remained consistently low throughout the irrigation period (1.6 g m^{-3}), and has not been separately accounted for.

4.2. Materials and Methods

4.2.1. Location Description

The RLTS is situated within the Whakarewarewa conifer production forest (see below) and the Waipa Stream catchment (38° 10'S, 176° 10'E). The soils are classified as Typic Orthic Allophanic (Hewitt 1998) and are predominately Whakarewarewa and Ngakura Sandy loams (Rijkse 1979). The topography is an essentially north facing valley dissected by several small streams which are moderately incised leaving slopes of no more than 23°, with most less than 12° (Tomer et al. 1997). Valley bottoms have permanent streams and 47 ha of wetlands all flowing to the Waipa Stream which rises 2 km to the east.

4.2.2. Vegetation

The irrigated area is mixed age conifer forest, planted before and during the commencement of irrigation in October 1991. The total irrigated area is 192 ha and consists of 36 ha of 'old' (31 years old) *Pseudotsuga menziesii*, 102 ha of 'young' (12-13 years old) *Pinus radiata* and 54 ha of 'old' (30-33 years old) *Pinus radiata* (Tomer et al. 1997). A variety of understorey weeds are present including *Rubus fruticosus*, *Buddleja salvifolia*, *Leycesteria formosa*, *Pteridium esculentum* and several epiphytes on tree trunks, especially in the irrigated area where overhead sprinkling intercepts the tree trunks.

4.2.3. Effluent Irrigation

The irrigated area is divided into 14 blocks each of about 14 ha where irrigation occurs via high pressure overhead sprinklers throughout the year. The low density, pumice soils with high capacity and infiltration ensure negligible runoff (Tomer et al. 1997). The irrigation regime consists of 2 blocks sprayed for a

period of 2 hours (10 mm). Each block pair has a return period of 12 hours spraying time which means on average each block is sprayed daily.

4.2.4. Sample Collection and Analysis

Sampling was carried out in March 2003. The irrigation area was stratified into three groups, *P. menziesii* and 'young' and 'old' *P. radiata*. Similar control sites (neighbouring forest outside the irrigation zone) were also stratified into the same three groups and sampled to obtain background stable isotope information. Within each stratification samples of foliage, wood and bark, LFH (Litter Fermenting Humifying layer = A_o or organic soil) and underlying soil were sampled for N mass balance and isotopic analysis.

4.2.4.1. Foliage

Crop tree foliage biomass was estimated using published figures and a mean value of 10 t ha^{-1} (Thorn et al. 2000) was used in this study. Single samples of *P. menziesii* and *P. radiata* canopy within each tree crop and control group were collected, dried at 60ºC, ball milled and determined for %N and for ^{15}N isotopic analysis.

Understory species composition and abundance was assessed using multiple transects of $1m^2$ within each tree crop and control group, each transect at least 20m in length. Single representative samples of sub canopy species were destructively sampled dried at 60ºC, weighed, ball milled and determined for %N and for ^{15}N isotopic analysis.

4.2.4.2. Wood and Bark

Total standing wood biomass was estimated from diameter and height measurements made on four stands of trees. The Shinozaki model (Shinozaki et al. 1964) was used to estimate the contribution of all wood in trunk, branches and branchlets. The model tends to slightly overestimate total volume in recently pruned trees. Mass analysis for wood was estimated using a conservative wood density of 0.45 t m^{-3}, and planting density provided by forest managers allowing the determination of standing biomass.

Wood and bark samples were collected (three replicates for each tree crop and control group) using an increment corer and dried at 60ºC. The wood of each tree core was apportioned as outer (sapwood), middle, and inner wood (heartwood). Bark and the apportioned wood cores were ball milled and determined for %N and for ^{15}N isotopic analysis.

4.2.4.3. Soil

LFH and soil 0-100, 100-200, 200-300 mm were sampled and bulk density measurements made to obtain total soil mass for each tree crop and control group. Samples were dried at 60ºC, homogenised, ball milled and determined for %N and for ^{15}N isotopic analysis (three replicates for each tree crop and control group).

4.2.4.4. Ground water and Waipa Stream

Samples of groundwater from piezometers, were obtained from sites adjacent to both irrigated (n=3) and control areas (n=4) on two occasions. Samples of Waipa Stream water were collected at the same time at control sites above effluent irrigation (n=3), and at the gauging station, the point of catchment exit (n=3). Collected water samples were immediately chilled, filtered (Whatman GF/B) and processed. NH_4-N and NO_3-N concentrations were determined using a QuickChem 8000 Automated Ion Analyzer coupled with Omnion FIA (Flow Injection Analysis) software employing standard colorimetric techniques. Where required, liquid samples were concentrated for either NO_3-N and, where measurable NH_4-N, on appropriate ion exchange resins (Dowex 1X8 200 and 50WX4-200) and eluted with 3M HCL using at least five bed volumes. All samples were alkali distilled, and in the case of NO_3 samples were distilled with Devarda's alloy to reduce NO_3 to NH_4. Distilled samples were dried in excess acid prior to mass spectrometry. All samples were routinely run with controls of known size and known ^{15}N content to ensure quality control and rejected if less than 97% recovery achieved. The technique described resulted in no significant isotopic fractionation.

^{15}N isotopic ratios in all samples were determined using an IRMS isotope ratio mass spectrometer (20/20 Europa) coupled to an automated elemental analyser. Isotope ratios can be obtained on samples down to c. 10µg N. $\delta^{15}N$ values were

measured to a precision of better than $\pm$0.5‰ against a secondary standard urea sample which is standardised against air N_2 0‰. Repeated standardisation against international standards indicates that the techniques used at the Waikato Stable Isotope Unit have a precision of $\pm$0.2‰ and an absolute accuracy of $\pm$0.2‰ for optimum sized samples.

4.2.5. Calculations

Following calculation of N mass in each of the plant, soil or water samples, the isotope content can be used to calculate the contribution of effluent N to that fraction using the following equation.

$$\%N \text{ derived from effluent} = (A - B) / (C - B)$$

Equation 4.1

(A) the $\delta^{15}N$ of a measured component within the irrigated area, **(B)** the $\delta^{15}N$ of the same measured component within the control (non-irrigated) area and **(C)** the weighted $\delta^{15}N$ mean of the applied effluent NO_3-N fraction. The ^{15}N enrichment of the effluent NO_3-N has been measured on four different occasions and the mean of these measurements is +14.12 (±1.40)‰ . This $\delta^{15}N$ signature is considerably more enriched than control ecosystem N $\delta^{15}N$ signatures a difference well in excess of most reportedly successful applications of this technique (Bedard-Haughn et al. 2003) providing an excellent tracer of effluent-N into the ecosystem, to measure storage and loss.

4.3. Results

4.3.1. Nitrogen Inputs and Losses

Nitrogen levels in the effluent and in the Waipa Stream have been measured since the start of the irrigation, shown in Figure 4.1. A summation of these plus an extrapolation from the closing date of Figure 4.1, April 2002, until March 2003 when this study commenced, shows that 902 t effluent N has been applied to the forest. Similarly, the total flow of N out of the catchment has been 263 t.

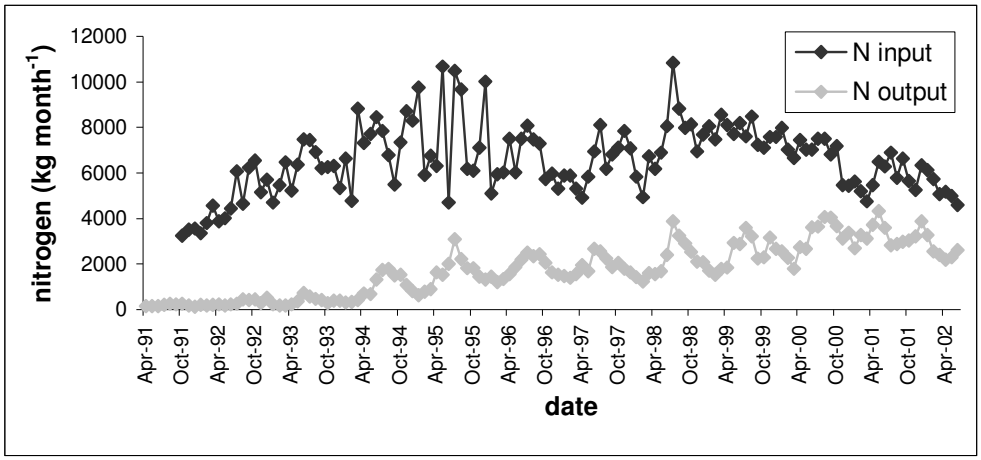


Figure 4.1. Nitrogen inputs and outputs from the Waipa Stream over the effluent irrigation period.

4.3.2. Green Foliage Storage of N

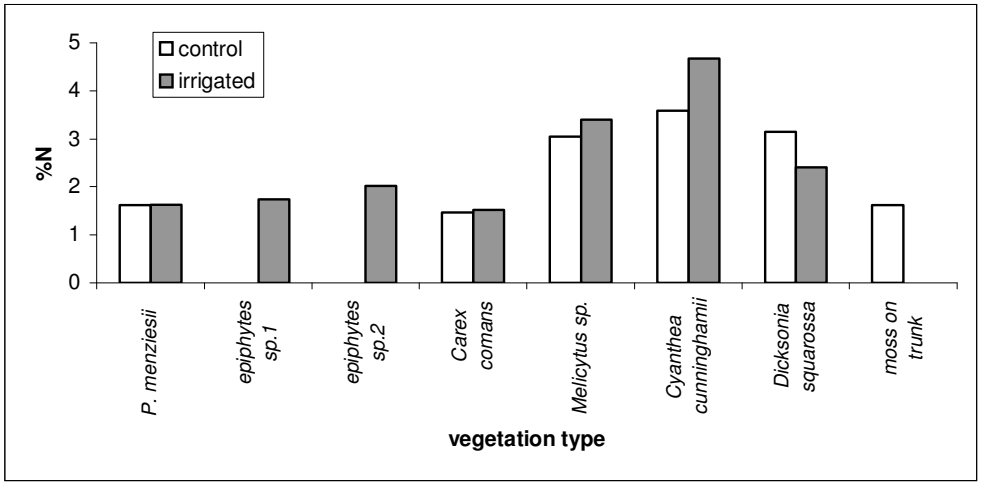
4.3.2.1. Conifer Foliage

The biomass of green conifer foliage is taken as 10 t ha^{-1} across all blocks (see methods) and for irrigated areas the mean N content of *P. menziesii* and *P. radiata* foliage is 1.57% (Figures 4.2A, 4.2B, 4.2C). The ^{15}N isotopic ratio of conifer foliage in irrigated areas is all highly enriched in ^{15}N relative to control conifer foliage (Figures 4.3A, 4.3B, 4.3C). The contribution of effluent N to conifer foliage, derived from Equation 1, varies between 90-100% which equates to 28.5 t or 148 kg N ha^{-1} (Table 4.1).

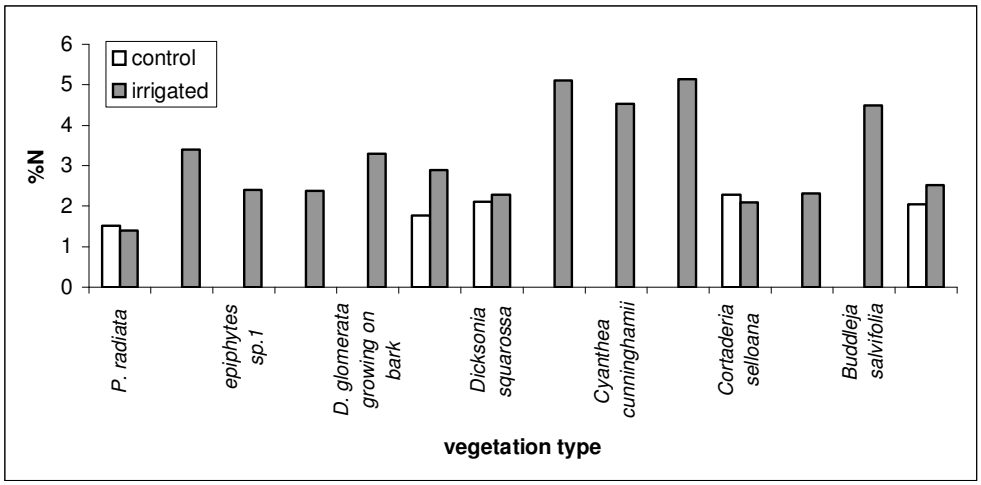
4.3.2.2. Understorey Foliage

There is a diverse range of understorey species in the irrigated area which have, in some cases, very high foliage N levels ranging from 2-5% (Figures 4.2A, 4.2B, 4.2C). Understorey diversity and abundance were significantly lower in control areas. Sampling of understorey within the irrigated area indicated a biomass of approximately 2.5 t ha^{-1} and using an N concentration of 3% gives a total understorey N content of 75 kg N ha^{-1} or 14.4 t N stored in the total understorey. The $\delta^{15}N$ signatures for the understorey (Figures 4.3A, 4.3B, 4.3C) are close to, and often exceed +14.12‰ (average = +13.82 (±1.7) ‰), thus from Equation 1, 98% of the N in these plants is derived from effluent.

A)



B)



C)

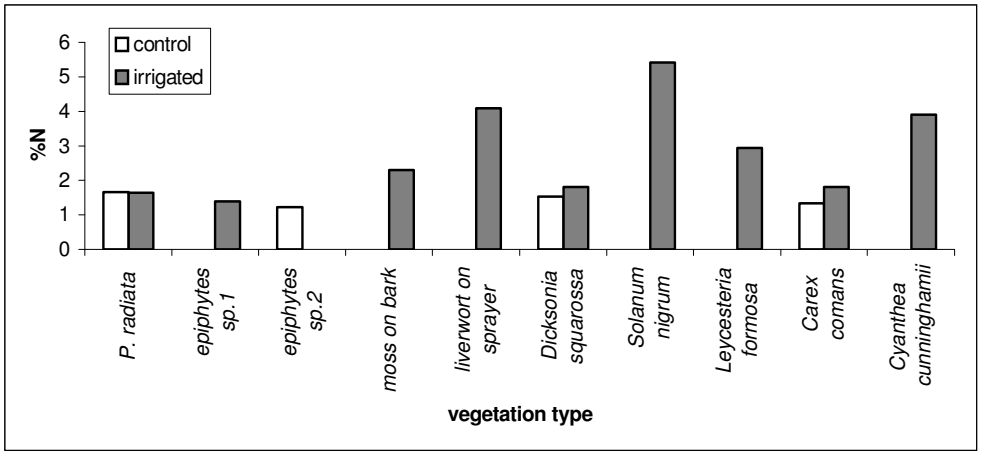
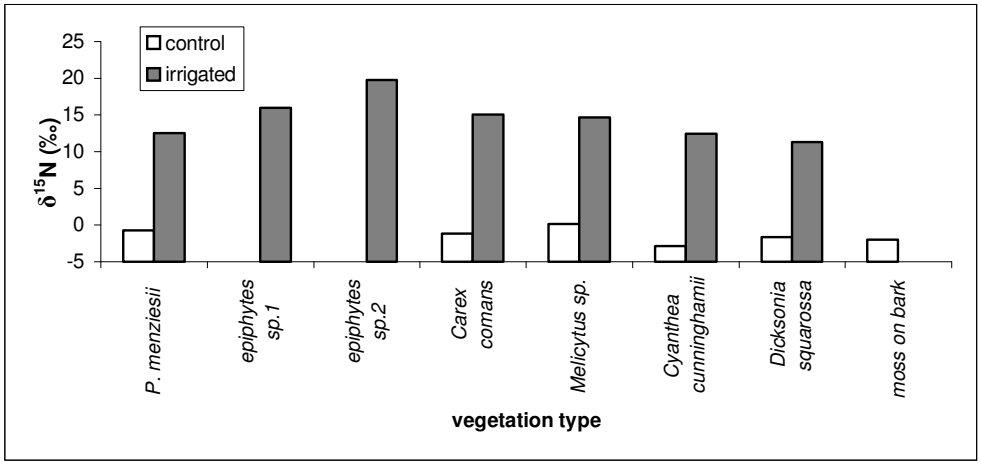
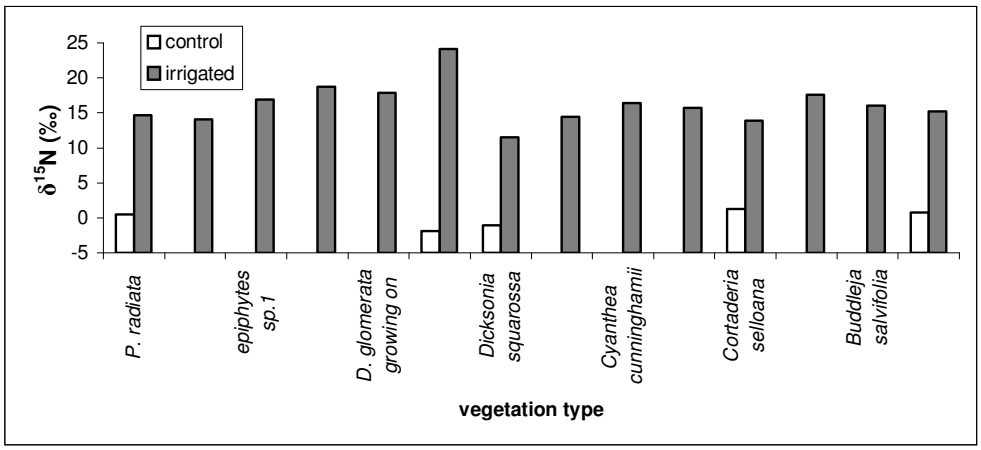


Figure 4.2. %N in the vegetation of A) *P. menziesii* forest (31 years old), B) young (12 years) *P. radiata* forest, and C) old (33 years) *P. radiata* forest. Bars represent single analyses.

A)



B)



C)

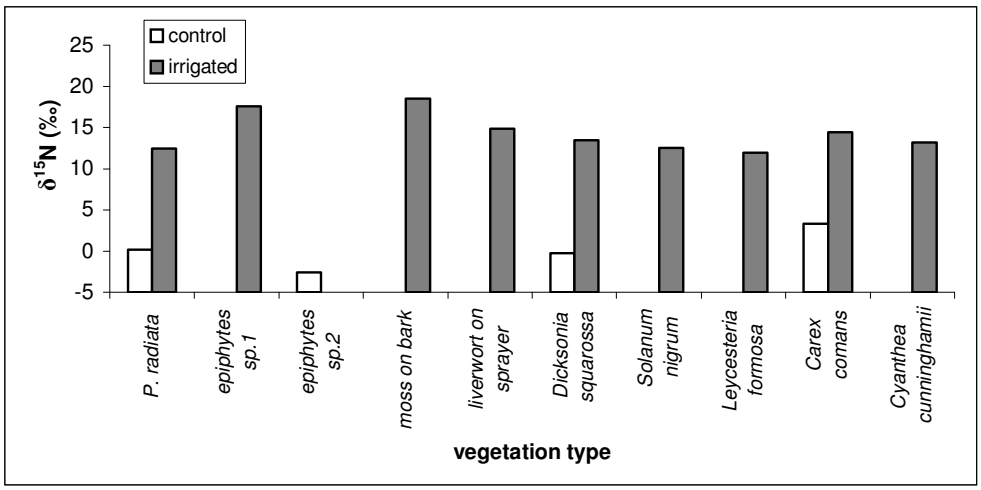


Figure 4.3. $\delta^{15}N$ in the vegetation of A) *P. menziesii* forest (31 years old), B) young (12 years) *P. radiata* forest, and C) old (33 years) *P. radiata* forest. Bars represent single analyses.

Table 4.1. Conifer foliage biomass and contribution of N derived from effluent. $\delta^{15}N$ signature derived from one representative vegetation sample.

Forest type	Total foliage N (kg ha^{-1})	Total foliage N within irrigated area (t)	$\delta^{15}N$ (‰)	N contribution from effluent (%)	Effluent N stored in conifer foliage (t)
P. menziesii	157	5.65	+12.56	89	5.03
P. radiata young	157	16.0	+14.65	100	16.0
P. radiata old	157	8.48	+12.45	88	7.46
				Total N (t)	**28.49**

4.3.3. Standing Biomass Wood Storage of N

Estimated volumes and biomass of wood and bark for the three tree crop groups are presented in Table 4.2.

Table 4.2. Estimated volumes and biomass of wood and bark (from four stands) for the main forest types in the RLTS

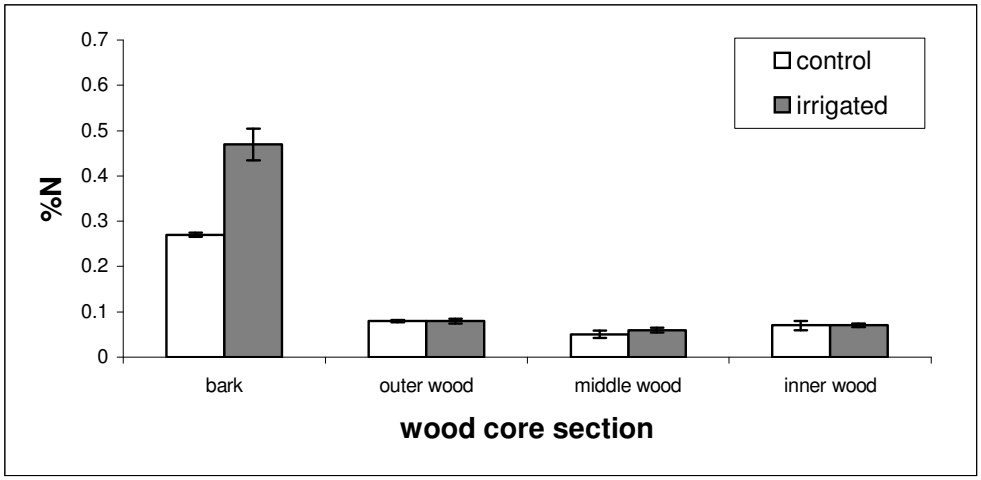
Forest type	Volume (m^3 ha^{-1})		Biomass (t ha^{-1})	
	Wood	Bark	Wood	Bark
P. menziesii	969	53	445	24
P. radiata young	476	26	219	12
P. radiata old	3300	86	1518	40

Wood volume in roots and fallen pruned branches was not measured directly.

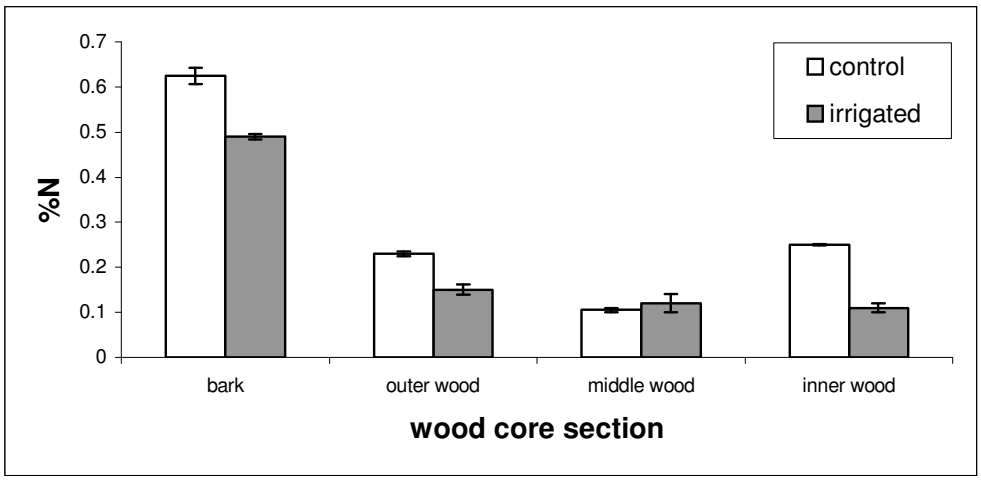
N concentration and $\delta^{15}N$ were measured at four points along increment cores collected from the main forest types within and outside irrigation areas. N concentrations within the increment core followed a typical pattern with values around 0.4-0.5% for bark and 0.1-0.05% in the wood. Sapwood usually contains

more N than heartwood (Figures 4.4A, 4.4B, 4.4C), however, there was little effect of effluent irrigation on wood N content relative to controls except in the bark. N contents and N mass are shown in Table 4.3.

A)



B)



C)

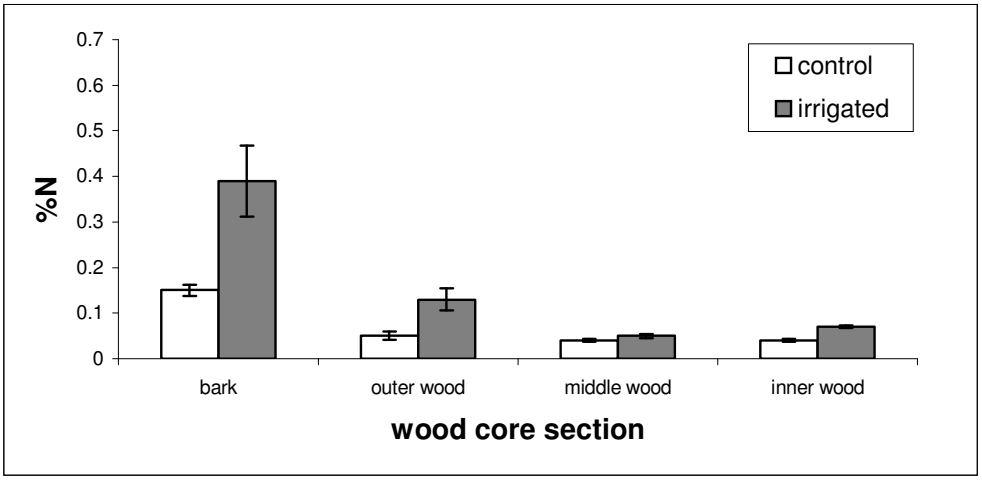


Figure 4.4. %N in the bark and wood of the trees in the A) *P. menziesii* forest (31 years old), B) young (12 years) *P. radiata* forest, and C) old (33 years) *P. radiata* forest. Bars represent means (n=3). Error bars represent s.d.

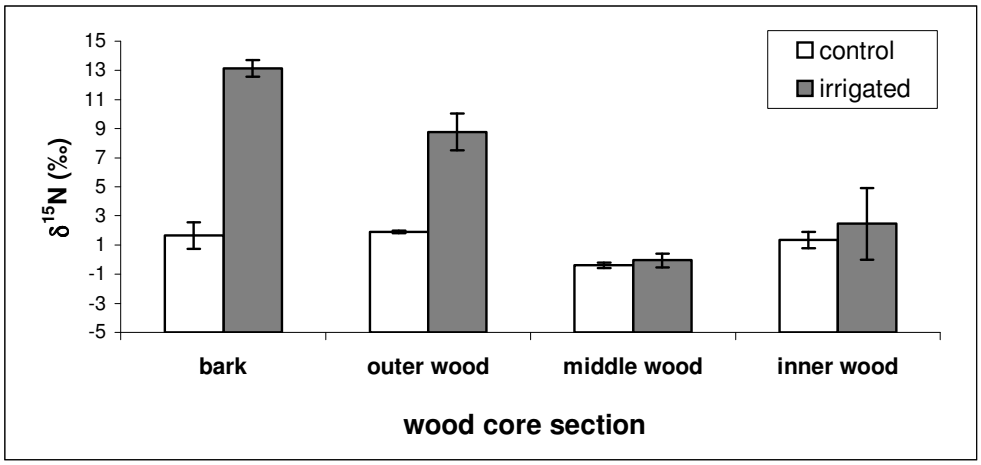
Table 4.3. N concentration and N mass in the wood and bark of irrigated trees. N concentration means derived from three replicates (s.d. in brackets).

Forest type	*Bark*		*Outer wood*		*Middle wood*		*Inner wood*	
	%N	Total N (kg ha^{-1})	%N	Total N (kg ha^{-1})	%N	Total N (kg ha^{-1})	%N	Total N (kg ha^{-1})
P. menziesii	0.47 (0.04)	113	0.08 (0.01)	118	0.06 (0.01)	89	0.07 (0.01)	103
P. radiata young	0.49 (0.01)	59	0.15 (0.02)	109	0.12 (0.04)	88	0.11 (0.02)	80
P. radiata old	0.39 (0.08)	156	0.13 (0.02)	658	0.05 (0.01)	253	0.07 (0.01)	354

The majority of bark and wood samples taken from irrigated trees showed significant ^{15}N isotopic enrichment (Figures 4.5A, 4.5B, 4.5C). As expected the inner wood of *P. menziesii* trees, laid down in these 31 year old trees prior to irrigation was not enriched in ^{15}N to any significant degree (Figure 4.5A). In contrast, the outer wood and bark, laid down after the commencement of irrigation is strongly enriched in ^{15}N indicating a contribution of effluent N. Contrary to *P. menziesii*, the inner wood of older *P. radiata* trees was strongly enriched in ^{15}N indicating a significant movement of N from the active outer wood and bark into the middle and inner wood (Figure 4.5C). Young *P. radiata* trees planted at the time of irrigation commencement show high $\delta^{15}N$ throughout the bark and wood indicating a contribution of effluent N. Calculation of the proportion of N in bark

and wood derived from effluent is given in Table 4.4 and the total effluent N stored in bark and wood is given in Table 4.5.

A)



B)

C)

Figure 4.5. $\delta^{15}N$ in the bark and wood of the trees in the A) *P. menziesii* forest (31 years old), B) young (12 years old) *P. radiata* forest, and C) old (33 years old) *P. radiata* forest. Bars represent means (n=3). Error bars represent s.d.

Table 4.4. Contribution of N derived from effluent in bark and wood. $\delta^{15}N$ means derived from three replicates (s.d. in brackets).

Forest type	*Bark*		*Outer wood*		*Middle wood*		*Inner wood*	
	$\delta^{15}N$ (‰)	**Effluent contribution (%)**	**$\delta^{15}N$ (‰)**	**Effluent contribution (%)**	**$\delta^{15}N$ (‰)**	**Effluent contribution (%)**	**$\delta^{15}N$ (‰)**	**Effluent contribution (%)**
P. menziesii	+13.16 (1.16)	92	+8.77 (2.52)	56	-0.05 (0.05)	0	+2.47 (2.2)	9
P. radiata young	+16.81 (0.63)	100	+16.02 (0.61)	100	+14.56 (0.4)	100	+14.23 (0.78)	100
P. radiata old	+11.8 (2.5)	78	+11.28 (0.77)	81	+6.09 (1.52)	44	+6.38 (1.04)	45

Table 4.5. Total N contribution from effluent in bark and wood

Forest type	Biomass (kg N ha^{-1})	Total N in biomass (kg N)	Effluent contribution to biomass (%)	Stored N from effluent (t)
P. menziesii				
Bark	113	4068	92	3.74
Outer Wood	118	4248	56	2.38
Middle Wood	89	3204	0	0
Inner Wood	103	3708	9	0.33
***P. radiata* young**				
Bark	59	6018	100	6.02
Outer Wood	109	1118	100	11.12
Middle Wood	88	8976	100	8.98
Inner Wood	80	8160	100	8.16
***P. radiata* old**				
Bark	156	8424	78	6.57
Outer Wood	658	35532	81	28.78
Middle Wood	253	13662	44	6.01
Inner Wood	354	19116	45	8.60
			Total (t)	**90.7**

4.3.4. Soil Storage of N

The native pool of N storage in the soils is of the order of 7 t ha^{-1} (Tomer et al. 1997). The work of McLay et al. (2000) indicated that there may be an increase of 750 kg N ha^{-1} but the variation was such that the increase was not statistically significant. Our results showed that N concentrations in RLTS soils followed typical trends; decreasing with depth through the profile. They also showed however that irrigated soils did not have significantly more N than control soils (data not shown).

$\delta^{15}N$ of control soils show a typical increase in enrichment with depth in the profile (Table 4.6). Irrigated soils were significantly more enriched in ^{15}N relative to controls, particularly in the upper profiles, and in some cases even in the lower profiles (Table 4.6). Total soil N in the top 300 mm ranges from 4642 to 7164 kg

ha^{-1}, and using equation 1 the contributions to soil N from effluent N can be determined. In the mineral soils of the irrigated areas 43% of soil N is derived from effluent and in the LFH 87%. A total of 263 t of effluent-derived N is stored in soils, representing 1.37 t ha^{-1} which is twice the amount indicated by McLay et al. (2000) in their mass balance analysis.

Table 4.6. Contribution of effluent N to soil storage. N concentration and $\delta^{15}N$ means derived from three replicates (s.d. in brackets).

Forest type	Soil horizon	Bulk density (kg m^{-3})	% N	Total N (kg ha^{-1})	$\delta^{15}N$ (‰) of treated soils	$\delta^{15}N$ (‰) control soils	N contribution from effluent (%)	N from effluent (kg ha^{-1})	Stored N from effluent (t)
Pinus menziesii	LFH	420	0.57 (0.15)	1676	+6.17 (0.71)	+2.60 (0.99)	31	520	18.72
	0-100 mm	600	0.29 (0.06)	1740	+6.00 (0.39)	+5.21 (0.8)	9	157	5.65
	100-200 mm	550	0.21 (0.06)	1155	+6.58 (0.86)	+6.17 (0.54)	5	58	2.08
	200-300 mm	750	0.19 (0.04)	1425	+6.81 (1.00)	+6.78 (0.34)	0.4	6	0.22
			Total	**5996**					
***Pinus radiata* young**	LFH	120	1.22 (0.68)	732	+12.14 (2.74)	+0.73 (0.28)	87	637	64.97
	0-100	600	0.33 (0.11)	1980	+7.83 (2.22)	+3.08 (0.09)	43	851	86.80
	100-200	920	0.27 (0.07)	2484	+5.27 (0.91)	+5.30 (0.21)	0	0	0
	200-300	820	0.24 (0.06)	1968	+5.38 (0.78)	+5.71 (1.33)	0	0	0
			Total	**7164**					
***Pinus radiata* old**	LFH	200	0.75 (0.44)	750	+9.95 (2.2)	+2.56 (0.4)	64	480	25.92
	0-100	820	0.23 (0.12)	1886	+7.33 (0.77)	+4.99 (0.3)	26	490	26.46
	100-200	920	0.12 (0.02)	1104	+8.04 (0.65)	+5.35 (0.12)	31	342	18.47
	200-300	820	0.11 (0.05)	902	+8.18 (0.34)	+5.73 (1.53)	29	262	14.18
			Total	**4642**				**Total (t)**	**263.47**

4.3.5. Ground water storage of N

Knowles (1995) measured ground water NO_3-N concentrations within the RLTS and found concentrations to be as much as 7 g m^{-3} in irrigated areas and less than 1 g m^{-3} in control areas. Ground water from piezometers within irrigation (n = 3) and control (n = 4) areas were measured on two occasions. The average NO_3-N concentration for ground water within the irrigation areas was 5.0 g m^{-3} and 0.01 g m^{-3} in the control areas, confirming the results of Knowles (1995).

The average $\delta^{15}N$ signature of all samples taken from inside the spray zone is +8.11 (±2.76)‰, while the average outside value is -1.73 (±1.28)‰. Using Equation 1, the contribution of effluent N to the ground water inside the irrigation area is 62%.

The amount of ground water stored in the soil profile has been assessed (Tomer et al. 1997) by gravimetric analysis and TDR (Time Domain Reflectometry). Measurements are of the order of 35-45% gravimetric, with a maximum of 54% during irrigation. As plots are now irrigated daily and the pore space of these soils averages over 50%, it is fair to assume that values of 40% will be maintained throughout the profile. The site runs from 336 m above sea level at stream level to 352 m at ridge tops (Tomer et al. 1997), a 16 m elevation. If an average of 2 m of soil is achieved over the whole 192 ha, then there is a total groundwater volume of 0.4 x 2 m x 192 ha x 10,000 m^2 ha^{-1} = 1.54 x 10^6 m^3 at an average concentration of 5 g m^{-3}, this is a total N of 7.7 t, of which 62% or 4.8 t is from effluent.

4.3.6. Wetland storage of N

The 47 ha of wetland was not intensively sampled but high $\delta^{15}N$ signatures were measured in both wetland waters and plants growing within it, indicating a high contribution of effluent N to these components.

NO_3-N concentrations in water above and below the wetland was 4.5 g NO_3-N m^{-3} with $\delta^{15}N$ signatures of +14.3 and +15.7‰ respectively (single analysis). *Cortaderia fulvida* and *Holcus lanatus* species sampled both above and below the wetland had $\delta^{15}N$ values ranging from +13.1 to +19.9‰ and averaging +15.5 (± 3.1) ‰ (n=4).

Using Equation 1, *over* 100% of the N with both the plants and water can be attributed to effluent N. This unusual situation is likely to be explained by the process of denitrification, usually associated with wetland systems. It is apparent that some level of denitrification is being carried out in the wetland and the remaining N, further enriched in ^{15}N, has been utilised by the wetland plants.

Apportioning a similar green biomass (ie 10 t ha^{-1}) and foliage N concentration (3%, of which 100% is from effluent N) to the wetland as shown for the upland soils, and assuming the soil/root component of the wetland comprises 7 t N ha^{-1} (of which effluent N comprises 30%), and using a 2 m depth of soil, the total contribution of effluent N to vegetation is 13.4 t and to soils is 99 t. Assuming that 60% of the soil pores spaces are filled with water within the wetland, then there is a total groundwater volume of 0.6 x 2 m x 47 ha x 10,000 m^2 ha^{-1} = 5.6 x 10^5 m^3. At an average concentration of 4.5 g NO_3-N m^{-3}, this provides a total NO_3-N load of 2.5 t, of which 100% or 2.5 t is from effluent. All combined the total N storage within the wetland is 115 t.

4.3.7. Denitrification

Denitrification is reported to account for 2 kg N ha^{-1} yr^{-1} in the upland soils (Barton et al. 1999) and 37 kg N ha^{-1} yr^{-1} in the wetland (Tomer et al. 2000) making a total of 23 t of N loss over the 11 year period. High $\delta^{15}N$ measured in both wetland waters and plants confirm the results of Tomer et al. (2000) indicating at least some level of denitrification occurs within the wetland.

4.3.8. Output of N into the Waipa Stream

NO_3-N in water at the gauging station on the Waipa Stream taken on two different occasions gave $\delta^{15}N$ of +12.88 (±0.19)‰ and +12.86 (±0.31)‰. In contrast, $\delta^{15}N$ ratios of NO_3-N in the stream above the irrigated area, collected at the same time, are significantly less enriched at +3.7 (±0.27)‰. Using Equation 1 the calculated contribution of effluent N to current levels of stream NO_3-N is 88%.

4.4. Discussion

Use of the naturally occurring ^{15}N isotope has allowed a reasonable approximation of storage and losses of effluent derived N in the RLTS. The site is naturally highly variable with different species, ages and topography. It has not been possible to sample all components of the RLTS extensively to get a true measure of the variability, however, the picture obtained from such a study is a first order measurement of the contribution of effluent-N to the RLTS. The integrative properties of the trees and soil mean that the long-term accumulation of N in those fractions is preserved and represents a realistic measure of N storage.

The limitations of the expensive isotopic analysis and the time needed to collect and prepare samples mean that these analyses have considerable error terms, some of which have not been explored. Soil heterogeneity is likely to result in the highest error. More intensive sampling of soil, both horizontally and vertically would reduce error in this measured component. On the other hand it must be pointed out that isotopic measurements are, in this type of environment, unequivocal measures of the presence of effluent. Further refinement of sampling by more intensive measurement will certainly increase the precision of measurement, ie. will reduce variability. In our experience however, more extensive sampling is unlikely to greatly change mean values and will not be additionally advantageous to regional and local governing bodies.

Table 4.7 provides an overall N budget for the different components within the RLTS over the 11 years of operation. These values are potentially compromised by changes in isotope ratio in the input over time, for example variable isotopic ratios of the effluent N over the irrigation period. Changes in effluent N $\delta^{15}N$ are likely to have occurred over the irrigation period as effluent treatment (prior to irrigation) has become more efficient over time. Increased efficiency of effluent processing has come in the form of increased removal of N via increased levels of denitrification. Denitrification, as discussed above, results in more enriched $\delta^{15}N$ of the remaining NO_3-N. Therefore current effluent NO_3-N is likely to have a higher $\delta^{15}N$ compared to effluent NO_3-N at the commencement of irrigation. If this is the case, it implies that Equation 1 underestimates effluent N contributions to components of the RLTS, based on effluent-N $\delta^{15}N$ to be +14.12‰. This

underestimate cannot be quantified as historically applied effluent $\delta^{15}N$ isotopic ratios cannot be measured. However, this potential error results in a more conservative estimate of the N stored in the different components of the system, and enhances our confidence in the calculated storage figures.

One variable not addressed is the possibility of further N fractionation in uptake and metabolism within the plant. The small fractionation often observed during uptake of N and metabolism by plants under normal field conditions usually falls within the range of ±2‰, and is usually less than ±1‰. This shift, relative to the enrichment attributed to the contribution of effluent N is insignificant and in fact is likely to further underestimate our calculated contribution of effluent N to the vegetation.

NO_3-N is (and has always been) the largest N component in the applied effluent. It is a readily assimilated fraction of the effluent and can be used as the primary source of N for plant uptake and subsequent storage. Although not included in the analysis, isotopic ^{15}N enrichment figures have been measured for NH_4-N and organic N in the effluent as +9.6‰ and +6.59‰ respectively. Even if these fractions were preferentially utilised by plants over NO_3-N, the signal of these effluent N sources would only act to dilute the ^{15}N ratios in the measured components. This would, in effect, result in an additional underestimate of the calculated N contribution by effluent to the measured components.

Calculated estimates of effluent derived NO_3-N leaving the system via the Waipa Stream is a snapshot taken during six months in one year. The results used here have extrapolated that value back over the full 11 years. We can reliably say that 88% of the NO_3 currently leaving the system is effluent-derived, it is likely however that 88% has not been sustained over the whole period. A gradual increase in NO_3 export via the stream to the current calculated levels has most probably occurred over the irrigation period. The current level of NO_3 export, its gradual increase to this point and proof that it is due directly to effluent is confirmed by the near 10 fold increase in NO_3 concentration measured in the Waipa Stream since irrigation commenced (Figure 4.1).

The overall balance (Table 4.7) shows that 451 t N or 50% of the total incoming N is stored in the forest, the majority in the soil. The wetland accounts for 115 t and

the total storage of 566 t represents 63% of the total N applied. Adding the denitrification losses (estimated from literature) to the storage component, results in over 65% of the total incoming N being accounted for. An estimated 263 t of effluent N has left the catchment via the Waipa stream which is 29% of the incoming N over the 11 year period, leaving a small proportion, 50 t, unaccounted for.

These findings indicate that the RLTS is reaching its capacity to store effluent N at the current rate of application. Although the forest will continue to assimilate effluent N, many of the measured components have reached their capacity for N storage, and it is likely that the proportion of effluent N exiting the stream will increase. The continued application of effluent to the current irrigation area is not sustainable in the long term.

Table 4.7. Nitrogen budget for N inputs and losses (t) over the 11 year irrigation period (Numbers in brackets refer to estimated values not actually measured). Storage values are derived from isotope analyses and represent storage of effluent N only. Losses refer to total N losses from the system.
*(of the 263 t N lost in stream flow 88% or 231 t is directly attributed to effluent)

N Input		**902**	**N Losses**		
Storage:			**Denitrification**	Upland Soils	4
				Wetland	19
				Total	**23**
Forest	Foliage	28	**Stream Flow**		**263***
	Wood/Bark	91			
	Soil	263			
	Roots	(50)			
	Understorey	14			
	Groundwater	4.8			
	Total	**451**			
Wetland	Biomass	(13.4)			
	Soils	(99)			
	Water	2.5			
	Total	**115**			
Total Storage		**566**	**Total Losses**		**286**
			Grand Total		**852**
			Unaccounted		**50**

Chapter 5

Extreme isotopic depletion of nitrogen in New Zealand lithophytes and epiphytes; the result of diffusive uptake of atmospheric ammonia?*

5.1. Introduction

Approximately 50% of total nitrogen (N) deposition from the atmosphere is comprised of the gaseous species NO, NO_2 and NH_3 (Stulen et al. 1998). Of these species NH_3 is the most readily available plant N source, and amongst the most common, especially in natural and unpolluted areas (Krupa 2003). Atmospheric gaseous NH_3 ($NH_{3(g)}$) concentrations in most environments range from 0.02 to 12 µg m^{-3} but may rise considerably near intensive agriculture or for example during forest fires where concentrations up to 250 µg m^{-3} have been recorded (Krupa 2003). Atmospheric $NH_{3(g)}$ is highly labile with short residence times ranging from 2.8 hours to 1-4 days and transport distances of several km (Krupa 2003). Free $NH_{3(g)}$ in the atmosphere readily reacts with acids to give particulate ammonium salts such as $(NH_4)_2SO_4$, the concentrations of which typically mirror $NH_{3(g)}$ concentrations. Ammonium salts can be either immediately deposited in wet or dry deposition or remain suspended in the atmosphere and potentially transported long distances before deposition (Krupa 2003).

There are a variety of natural and anthropogenic sources of $NH_{3(g)}$ in the atmosphere with agriculture being, by far the largest contributor, predominantly via volatilisation from animal wastes, soil organic matter and intensive feedlots (Krupa 2003). Significant $NH_{3\ (g)}$ loss may also occur from plants when internal levels are above compensation point, with well fertilized or senescing plants, and

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plant parts, likely to be net producers of $NH_{3(g)}$ (Farquhar et al. 1980; Raven 1988; Raven et al. 1992; Stulen et al. 1998).

In New Zealand, in areas influenced by oceanic air mass, concentrations of $NH_{3(g)}$ are at the lower end of reported concentration range (Allen et al. 1997). Similarly, inland areas distant from significant agricultural and industrial sources, concentrations of $NH_{3(g)}$ are also likely to be at the lower end of reported concentration range, but ammonium ions (NH_4^+) in precipitation are significant and greatly in excess of NO_x levels (Wilson 1959).

Numerous studies have confirmed the ability of plants to directly assimilate and release $NH_{3(g)}$ to and from the atmosphere. For plants with adequate nutrition it is unlikely that $NH_{3(g)}$ uptake by shoots plays a major role in their N nutrition (Raven 1988; Raven et al. 1992), however when atmospheric $NH_{3(g)}$ concentrations are high, this form of N may be utilised by plants (Krupa 2003). It is also apparent that plants under N-stress may increase there dependence on this N source (Vitousek et al. 1989; Perez-Soba and Van der Eerden 1993; Sutton et al. 1995; Stulen et al. 1998).

The mechanism of $NH_{3(g)}$ uptake into any vegetation involves diffusion from the ambient atmosphere into the apoplast of metabolically active cells followed by assimilation via the primary uptake enzyme glutamine synthetase (GS). Diffusion of ambient atmospheric $NH_{3(g)}$ through the boundary layer of the vegetation is driven by the concentration gradient between the two, with a flux that increases linearly with $NH_{3(g)}$ concentration (Van Hove 1987). Following diffusion, atmospheric $NH_{3(g)}$ readily equilibrates with NH_4^+ in the apoplast, which is close to the *km* of GS operating within the symplast (Farquhar et al. 1980). Unlike higher plants, where the cuticle and stomata play a major role in limiting diffusion processes (Van Hove et al. 1987), lichens, and most probably terrestrial algae, are metabolically active and open to receive atmospheric gases over a wide range of moisture contents (Honegger 1993; Tuba et al. 1996), as well as during the night.

The volatilisation of any $NH_{3(g)}$ from a liquid source to the atmosphere is accompanied by a large isotopic fractionation, as high as 40-60‰ (Högberg 1997;

Robinson 2001) and numerous reports show that atmospheric $NH_{3(g)}$ and NH_4^+ can be significantly depleted in ^{15}N. Isotopic measurements of atmospheric NH_4^+ in precipitation (includes NH_4^+ in solution and particulate NH_4^+ as a consequence of scavenging and interception of particles by the precipitation) is often made by collecting precipitation and measuring the species in solution. Measured NH_4^+ $\delta^{15}N$ in rainwater vary widely between +22‰ and -20‰ (Yeatman et al. 2001). The most depleted signatures are often associated with marine influence, where the emission of $NH_{3(g)}$ from the oceans is proposed as the likely source of these depleted signatures (Yeatman et al. 2001; Jickells et al. 2003).

Isotopic measurements of $NH_{3(g)}$ in the atmosphere are rare, but are summarized in Russell et al. (1998). As with atmospheric NH_4^+, $NH_{3(g)}$ isotopic values range widely between -15 to +25‰. As it is assumed both species generally reflect each others concentration (Krupa 2003), we propose it is also likely they reflect each others isotopic signature. Isotopic measurement of atmospheric $NH_{3(g)}$ is fraught with difficulty in that methods of collection must not result in significant further fractionation attributed to incomplete $NH_{3(g)}$ recovery. This difficulty may account for the limited studies of $NH_{3(g)}$ isotopic determination.

A significantly depleted isotopic signature has been identified in epiphytes in a number of recent studies, ranging typically between -1 to -10‰, with the depleted isotopic signature and the degree of depletion ascribed to their whole or partial dependence on wet and dry atmospheric deposition (Stewart et al. 1995, 2002; Hietz et al. 1999, 2002; Wania et al. 2002; Russow et al. 2004; Solga, et al. 2005). Epiphytic bromeliads, especially the air bromeliads are more negative (*Tillandsia* -11.2‰) than those that have developed an aerial soil. Similarly epiphytes further up the canopy are more negative than those lower down (Hietz et al. 2002; Wania et al. 2002). These results confirm an increasing dependence by these plants on atmospheric N sources with exposure and separation from soil and throughfall. In all the above cases the $\delta^{15}N$ value of the atmospheric N source, either measured or assumed, is proposed to account for the depleted isotopic signals measured in the epiphytes. In almost all cases the degree of depletion measured in the epiphytes exceeds the measured or predicted $\delta^{15}N$ value of the atmospheric N source, the further depletion in the epiphytes relative to the source is attributed to fractionation during N uptake from soil or rain.

Recent work on volatilisation of $NH_{3(g)}$ from urine and guano are proposed to account for significant isotopic depletions in surrounding plants (Erskine et al. 1998; Frank et al. 2004) but the role of this process in low N environments has not been explored. Further, the $NH_{3(g)}$ collection devices used by Erskine are passive, thereby collecting an unknown proportion of the $NH_{3(g)}$ source. Such a collection, as mentioned earlier, will result in isotopic fractionation in the collected product, with the degree of depletion dependent on the proportion of the total available $NH_{3(g)}$ collected.

Our work identifies a range of lithophytic species (vegetation that that grows on rock or stony soil, and derives its nutritional requirements chiefly from the atmosphere) which have significantly more depleted $\delta^{15}N$ values than ever reported, and proposes a model to account for the values. The work has centred on the terrestrial red/brown coloured green alga *Trentepohlia* (Chaetophorales) which, similar to lichens, is commonly found growing on a variety of hard substrates including stone, concrete, glass, asbestos and wood (Rindi and Guiry, 2002). It usually grows in the open and shows a significant predilection for vertical surfaces such as wooden power poles, fence posts and battens, tree bark, walls, rock outcrops and painted masonry where it often indicates the moister polar aspect (Rindi and Guiry, 2003). The alga is able to survive on moist to dry surfaces and has a remarkable capacity to recover from extended desiccation (Gupta and Agrawal 2004). The alga, like other members of the Chaetophorales, forms a differentiated thallus with a prostrate and an erect component, which spreads across the substrate to form an extended mat.

The nutrition of *Trentepohlia* is problematic. It grows on a variety of inert materials such as wood, steel and rock, and while it has a primitive prostrate system and likely assimilates a range of N compounds it, like lichens, is most likely to receive its nutrition from the atmosphere in wet and dry deposition, with preference for NH_x (Abe et al. 2003; Dahlman et al. 2004). The present work arose out of several extreme ^{15}N depleted values we found in *Trentepohlia* and exposed lichens in a variety of geothermal areas in New Zealand. Beyond the epiphytes mentioned above, few papers present measurements of ^{15}N in terrestrial algae or exposed lichens. We report here on a range of ^{15}N values for these plants and atmospheric $NH_{3(g)}$, and develop a model to explain the extreme depletions in

terms of a double fractionation firstly from volatilisation into the atmosphere followed by its diffusive uptake.

5.2. Materials and methods

5.2.1. Sites

Collections were made at sites adjacent to Rotorua (a geothermal area) and the non-geothermal urban centre of Hamilton (100 km north of Rotorua) in North Island, New Zealand. The predominant collection sites near Rotorua were the primary successional domes on Mt Tarawera and an active geothermal site, Te Kopia.

Mount Tarawera, 30 km East of Rotorua, is a recent (1887) eruptive volcano that deposited many km^3 of basaltic lapilli over its summit at 1111 m asl and is the site of active vegetation regeneration by nitrogen fixing plants and low woody vegetation. The basaltic lapilli provide an acutely N deficient substrate supporting a unique pioneer flora.

The geothermal site of Te Kopia is an active gas fumarole area 25 km south of Rotorua in which hydrothermally altered soils support a variety of resistant vegetation. Steam clouds from active vents and fumaroles pervade the site maintaining a very high humidity. Further collections were made at adjacent sites and on a 100 km transect between the typically geothermal urban centre of Rotorua to the non-geothermal urban centre of Hamilton.

The Haultain dairy farm, 15 km north of Hamilton is a typical 12 month grazed farm supporting c. three cows per hectare on mixed clover ryegrass pasture. The pasture is intensively managed, using supplementary urea fertilization on a 21 day rotation.

5.2.2. Atmospheric $NH_{3(g)}$ collection

All methods used to collect N species were rigorously tested to ensure they did not introduce artifactual fractionations. Atmospheric $NH_{3(g)}$ was collected by extracting air and by sampling rainfall representing different components of the atmospheric pool. Free $NH_{3(g)}$ was sampled by pumping air at 40 L min^{-1} through an acid column, modified from the method of Ferm (1979). The column consists of a 500 mm x 20 mm (internal diameter) PVC tube filled with 4 mm glass beads and treated with 3% oxalic acid. Modeling studies and empirical tests showed that oxalic acid effectively traps $NH_{3(g)}$ when both wet and dry and that such a configuration run at 40L min^{-1} (2.4 m^3 h^{-1}) collects all $NH_{3(g)}$ and therefore ensures no fractionation in collection. This method was used to collect $NH_{3(g)}$ from several sites and the $\delta^{15}N$ of the $NH_{3(g)}$ collected is deemed to express the fractionation due to volatilisation from source to atmosphere. Rainfall was collected, and this represents a sampling of both free $NH_{3(g)}$ which has equilibrated with rain and also the NH_4^+ fraction from salts of $NH_{3(g)}$.

5.2.3. Simulation of $NH_{3(g)}$ assimilation

$NH_{3(g)}$ uptake into plants occurs when atmospheric levels of $NH_{3(g)}$ are above the compensation point. Uptake of $NH_{3(g)}$ in *Trentepohlia* is uncomplicated by the presence of stomata, of cuticle and of roots. We postulated that if any plant was going to display a significant N uptake from atmosphere it would be such a plant that grows on N free substrates and is tightly coupled to the atmosphere. In order to model $NH_{3(g)}$ uptake we used a mat of inert fibre glass to simulate the flat thallus of the alga. The mat, commonly used as capillary matting for glasshouse watering, was thoroughly washed and soaked in 3% oxalic acid, as for the glass beads above. The mats, 350 x 230 mm, were fitted underneath a plastic tray to provide shelter from rain and exposed to the atmosphere for six to 20 days. This technique was designed to provide an infinite sink for $NH_{3(g)}$ and thus to simulate diffusive uptake, such as might happen into the algal thallus of *Trentepohlia*.

5.2.4. ^{15}N analysis

Vegetation was removed from substrate, dried and ball milled prior to analysis. In some cases substrate, on which vegetation was growing, was also collected in order to assess its possible role in determining the vegetation isotopic signature. N isotope analysis on substrates, where N content was very low, were conducted

using standard Kjeldahl digestion with Cu and Se catalyst, prior to distillation in excess alkali to recover $NH_{3(g)}$.

NH_x samples were removed from glass beads or from acidified mat by distillation in excess alkali to recover $NH_{3(g)}$. The $NH_{3(g)}$ samples were acidified and evaporated to small volume at 80 ºC and then to complete dryness at 30 ºC. The materials were analyzed for ^{15}N in a Europa 20/20 continuous flow IRMS fitted with an elemental analyser. All procedures were tested against known ^{15}N standards to verify full recovery and absence of fractionation in extraction and recovery.

All isotopic values are expressed as $\delta^{15}N$ values in per mil (‰) with respect to air nitrogen. Instrument precision and accuracy is periodically tested against two known N isotope standards, IAEA-N1 +0.40‰ and USGS-25 -30.4‰. Instrument mean (n=5) for N1 is + 0.369‰ and for USGS-25 is -30.39‰ confirming both high accuracy and precision in the range of the majority of our samples. Duplicate plant samples differ by less than 0.5‰ which is a measure of both instrument and sample precision. Samples were run against reference samples of similar size and drift corrected every 10 samples.

5.3. Results

5.3.1. Preliminary $\delta^{15}N$ results for *Trentepohlia* and lichens

As part of a survey of vegetation on the dome of Mt Tarawera, *Trentepohlia* and two species of the epilithic lichens *Cladia* sp. and *Cladina* sp. were sampled for ^{15}N. In all cases the lithophytes were growing on rock surfaces amongst sparse regenerating woody shrubs in exposed environments. The results (Table 5.1) show the lithophytes are all significantly depleted in ^{15}N.

Table 5.1. $\delta^{15}N$ of lichens and *Trentepohlia* growing on the dome of Mt Tarawera.

Species	N content (%)	$\delta^{15}N$ (‰)
Cladia retipora	0.40	-9.2
Cladina leptoclada	0.35	-6.5
Trentepohlia	0.60	-12.20
Trentepohlia	0.53	-13.32
Trentepohlia	0.97	-16.09
Trentepohlia	0.95	-15.09
Mean		**-12.07**

Mount Tarawera is situated within the Rotorua geothermal area and although there is some adjacent geothermal activity, it is not classed as an active centre. To test whether the isotopic discrimination is associated with geothermal activity, a very active geothermal site at Te Kopia was sampled.

Epiphytic lichens and *Trentepohlia* growing on the stems of the shrub *Kunzea ericoides* var. *microflora* were sampled throughout the site (Table 5.2). The ^{15}N depletions in the sampled vegetation are extreme, and well outside the normal range of natural abundances for plants. To determine whether the close proximity to a geothermal source is responsible for the measured ^{15}N depletions, *Trentepohlia* was collected along a transect from an active fumarole out to a non-active area and assessed for thallus N content and $\delta^{15}N$. All sites show highly depleted N values (Table 5.3) with no relationship to the geothermal source or N content of the thallus.

Table 5.2. $\delta^{15}N$ of *Trentepohlia* and lichens growing at an active geothermal site, Te Kopia.

Species	N content (%)	$\delta^{15}N$ (‰)
Lichen: *Ramalina* sp.	1.1	-18.58
Lichen: *Usnea* sp.	1.0	-20.46
Lichen: *Usnea* sp.	0.9	-20.32
Trentepohlia	2.32	-24.9
Trentepohlia	1.32	-21.4
Trentepohlia	1.93	-19.4
Trentepohlia	1.5	-24.24
Trentepohlia	2.6	-23.57
Trentepohlia	2.3	-24.39
Mean		**-21.92**

Table 5.3. $\delta^{15}N$ of *Trentepohlia* growing on the stems of several shrubs at varying distances from a fumarole.

Distance (m) from active geothermal site	Substrate species	N content (%)	$\delta^{15}N$ (‰)
0	*Kunzea ericoides*	1.5	-24.2
1	"	2.55	-23.5
2	*Cyathodes fasciculatus*	1.52	-21.0
4	"	1.75	-21.5
8	"	2.71	-21.3
13	*Dracophyllum subulatum*	2.28	-24.4
20	"	1.93	-19.4
40	"	2.18	-23.6
Mean			**-22.4**

The epiphytic lichens *Usnea* sp. and *Ramalina* sp. also grow on the host shrubs and these were sampled for N content and $\delta^{15}N$ along a similar distance gradient from an active fumarole (Table 5.4). The lichens show similar extreme depleted ^{15}N values to that of the alga *Trentepohlia.* In both cases there is no relationship to distance from active fumarole, nor is there any relationship with thallus N

content. The mean $\delta^{15}N$ of all the Te Kopia *Trentepohlia* ^{15}N results is -22.4‰ and of lichens is -18.8‰ although these results are significantly different, the extreme isotopic depletion of both and their similar habitat presumes a similar process giving rise to the discrimination.

Table 5.4. $\delta^{15}N$ in the lichens *Usnea* and *Ramalina* at various distances from an active fumarole.

Distance (m) from active geothermal site	Lichen	Lichen N content (%)	$\delta^{15}N$ (‰)
	Usnea	0.87	-19.1
1	"	1.11	-18.6
3	"	1.05	-20.5
5	*Ramalina*	0.89	-20.3
6	*Usnea*	1.12	-18.0
9	*Ramalina*	1.35	-15.1
10	"	0.75	-20.5
15	*Usnea*	0.61	-18.3
20			
Mean			**-18.8**

5.3.2. $\delta^{15}N$ relationship to substrate and geothermal activity

To further test the possible geothermal and substrate origin of the isotopic discrimination, %N and $\delta^{15}N$ of *Trentepohlia* and its substrate were collected along a 100 km transect from the geothermal centre at Rotorua to Hamilton city 10 m asl (remote from geothermal influence). At all nine sites, *Trentepohlia* is highly depleted in ^{15}N with an average of -14.8‰ (Table 5.5). It is apparent there is no relationship between the nitrogen content and $\delta^{15}N$ of the substrate to that of nitrogen content and $\delta^{15}N$ of associated *Trentepohlia*. Similarly there is no relationship with proximity to a geothermal source with *Trentepohlia* samples in Hamilton, having similar ^{15}N depletion to those in Rotorua.

Table 5.5. $\delta^{15}N$ of *Trentepohlia* samples taken along a transect from the geothermal centre of Rotorua to Hamilton city.

Distance from Rotorua (km)	Altitude (m)	Substrate type	Substrate N (%)	Substrate $\delta^{15}N$ (‰)	*Trentepohlia* N content (%)	*Trentepohlia* $\delta^{15}N$ (‰)
2	349	jarra power pole	0.23	+0.20	2.05	-15.8
4	305	wood fence batten	0.22	+13.14	1.96	-15.2
20	365	wood fence batten	0.24	-1.38	2.21	-10.1
26	604	wood fence batten	0.18	+0.90	1.94	-19.6
33	356	rock	0.20	-1.10	1.01	-12.3
45	293	wood fence batten	0.13	+2.73	0.89	-10.3
c. 100	10	fiber glass	ND**	ND**	1.02	-18.6
c. 100	10	painted jarra power	0.37	+59	2.09	-17.8
c. 100	10	pole	0.37	-2.24	0.74	-13.4
		jarra power pole				
					Mean	**-14.8**

*value of +59 was measured in paint, but this has not been verified
** no detectable N in fiber glass

The isotopic values obtained are more depleted than any previously published, and from the above evidence we hypothesize a similar mechanism of atmospheric N assimilation for all the lithophytes and epiphytes at all sites. We propose the diffusive uptake of atmospheric $NH_{3(g)}$, and the associated isotopic fractionation to account for the highly depleted $\delta^{15}N$ values measured.

5.3.3. Rainfall and diffused $NH_{3(g)}$

To test this theory, samples of rainfall NH_x, the simulated diffusive uptake of $NH_{3(g)}$ into acidified mat and *Trentepohlia* were analyzed and compared from two sites: The geothermal area of Te Kopia and the dome of Mt Tarawera. The $\delta^{15}N$ values for each component at Te Kopia and Tarawera are summarized in Figure 5.1.

Although the mean $\delta^{15}N$ values for NH_x in rainfall at both Te Kopia and Tarawera are different, -0.93‰ and -3.24‰ respectively, the difference between their means is small relative to the $\delta^{15}N$ values of acidified mat NH_x and *Trentepohlia* collected at both sites. Assuming rainfall NH_x originates from $NH_{3(g)}$, the depleted isotopic signature of rainfall NH_x is likely to be a result of the fractionation attributed to $NH_{3(g)}$ volatilisation from a source to the atmosphere.

Again, assuming rainfall NH_x $\delta^{15}N$ values represent $NH_{3(g)}$ $\delta^{15}N$, the difference between rain and acidified mat $\delta^{15}N$ values (c. -8‰ for Tarawera and -12‰ at Te Kopia) expresses the very strong fractionation associated with $NH_{3(g)}$ diffusive uptake. The fractionation attributed to the simulated diffusive uptake of $NH_{3(g)}$ into the acidified mat is mirrored in *Trentepohlia* collected at each site (ellipse in Figure5.1), although in both cases the alga is even more depleted.

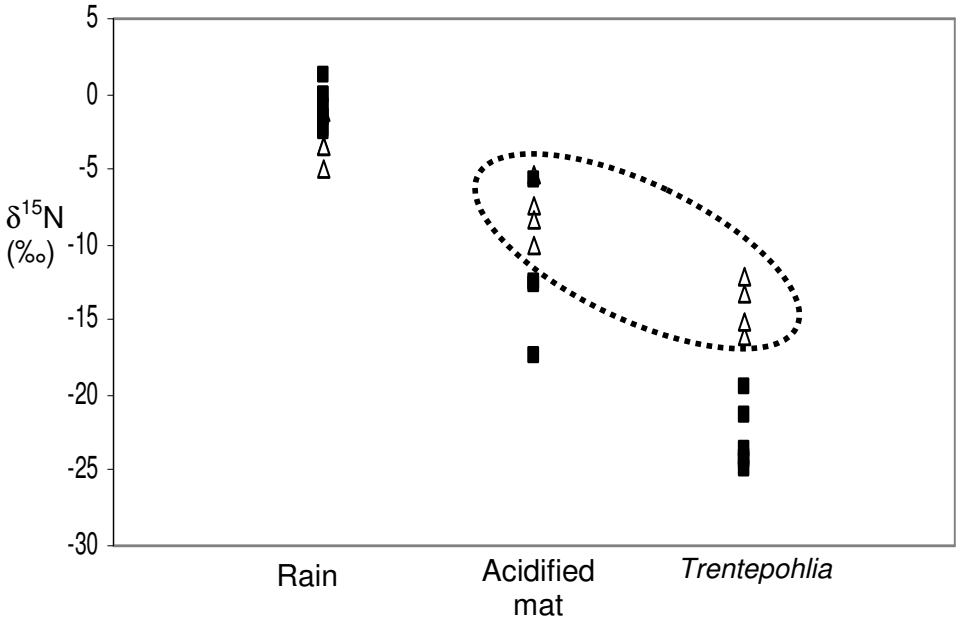


Figure 5.1. $\delta^{15}N$ values (‰) of NH_x in rain, atmospheric $NH_{3\ (g)}$ following diffusive uptake into acidified mat, and *Trentepohlia* at Tarawera (Δ) and Te Kopia (■).

5.3.4. $NH_{3(g)}$ above dairy pasture

To further illustrate this effect we sampled rainfall NH_x, atmospheric $NH_{3(g)}$ and the simulated diffusive uptake of $NH_{3(g)}$ into acidified mat on an intensively managed dairy farm. Rainfall was collected at four sites on the farm, and atmospheric $NH_{3(g)}$ and the simulated diffusive uptake of $NH_{3(g)}$ into acidified mat were sampled over recently grazed and ungrazed pasture (Figure 5.2).

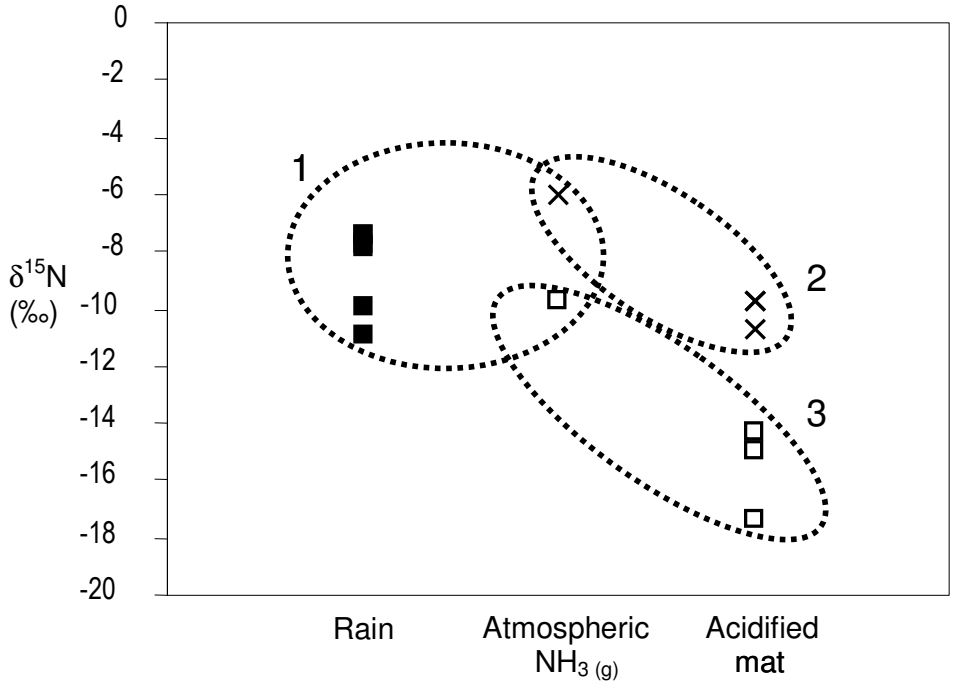


Figure 5.2. $\delta^{15}N$ values (‰) of NH_x in rain, free atmospheric $NH_{3(g)}$, and $NH_{3(g)}$ following diffusive uptake into acidified mat collected on a dairy farm. Rain was collected across the whole farm. Collection of free $NH_{3(g)}$, and $NH_{3(g)}$ following diffusive uptake were carried out in ungrazed (x) and grazed (□) paddocks.

$\delta^{15}N$ values of gaseous $NH_{3(g)}$ over pasture closely reflect the $\delta^{15}N$ values of NH_x in rain (ellipse 1 in Figure 5.2), justifying our use of rain $\delta^{15}N$ values as representative of atmospheric $NH_{3(g)}$ isotopic values. Concentrations of atmospheric $NH_{3(g)}$ were high under both treatments, but significantly higher in grazed (80 µgN m^{-3}) compared to ungrazed (25µgN m^{-3}) (data not shown). High concentrations of volatilised $NH_{3(g)}$ have been measured from cow urine (Petersen et al. 1998), and following fertiliser application (Ping et al. 2000), both likely to be the major source of the atmospheric $NH_{3(g)}$ measured in this case. The depleted $\delta^{15}N$ value of both rainfall NH_x and atmospheric $NH_{3(g)}$ is likely attributed to the fractionation associated with the volatilisation of $NH_{3(g)}$ to the atmosphere from these sources. The further $\delta^{15}N$ depletion measured in the acidified mat mirrors that of the same experiments at Te Kopia and Tarawera (Fig 5.1). Under both ungrazed and grazed treatments, NH_x extracted from the acidified mat is further depleted by c. -4‰ and -5.5‰ respectively, shown in ellipses 2 and 3 in Figure

5.2. This further $\delta^{15}N$ depletion represents the fractionation associated with the diffusive uptake of atmospheric $NH_{3(g)}$ at this site.

5.4. Discussion

The extreme isotopic depletion recorded for these lithophytes has never been reported previously and far exceeds the normal depleted $\delta^{15}N$ recorded for plants of c.-11‰. The values reported here, similar for all sites, regardless of topography, exposure or proximity to a geothermal source, foreshadow a common mechanism of discrimination for these plants which is independent of any obvious environmental factor. We hypothesise that diffusive uptake of $NH_{3(g)}$ into such plants can explain this phenomenon and *Trentepohlia* and lichens provide an ideal model to examine the fractionation as they can rely solely on atmospheric N species for growth, have a high affinity for N, and are uncomplicated by the presence of stomata, cuticle and roots. While there is no doubt that these species may be able to obtain nutrients from a substrate (Abe et al. 2003) it is clear from this study that substrates are often inert. The main source of nutrients for these plants is wet and dry deposition and an explanation for the isotopic abundance must be found there. The limited $\delta^{15}N$ values recorded in this paper and in the recent literature, for $NH_{3(g)}$, indicate it ranges widely and is often depleted. The concentration of atmospheric $NH_{3(g)}$ and its degree of isotopic depletion depends on the fraction of the N source converted to atmospheric $NH_{3(g)}$, climatic conditions, and geographical distance from the source (Krupa 2003).

Epiphytes and lithophytes dependent more or less on wet and dry deposition for their N-nutrition and have been shown to have often highly depleted $\delta^{15}N$ values (Stewart et al. 1995, 2002; Hietz et al. 1999, 2002; Wania et al. 2002; Russow et al. 2004; Solga, et al. 2005). These $\delta^{15}N$ signatures have been attributed to either measured or assumed $\delta^{15}N$ values for atmospheric N-species, many of which are significantly depleted. However in this, and in some of the above studies, the epiphyte isotopic signatures are more depleted than the measured (or assumed) isotopic values of the atmospheric N source. The utilization of an isotopically depleted atmospheric N source, without further fractionation, cannot account for the highly depleted $\delta^{15}N$ values seen in this paper, or in some other similar studies

and another fractionation step must be postulated. In order to explain the large isotopic depletions in these plants we propose a two step fractionation model as shown in Figure 5.3.

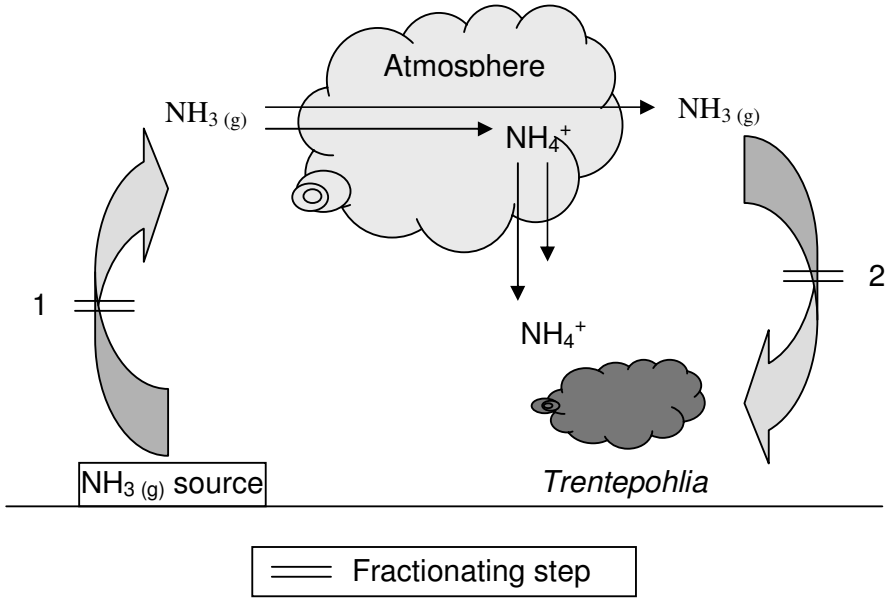


Figure 5.3. Proposed model to account for the depleted isotopic values measured in lithophytic vegetation, in this case *Trentepohlia*. 1) The fractionation factor associated with $NH_{3(g)}$ volatilisation, and 2) that of diffusive $NH_{3(g)}$ uptake.

Ammonia in an aqueous medium volatilises into the atmospheric pool with a potential discrimination of 40-60‰ (Högberg 1997; Robinson 2001) although empirically shown to be c. 15‰ (Heaton et al. 1997). This enters a rather labile atmospheric $NH_{3(g)}$ pool and is capable of being assimilated diffusively by plant cells. Diffusion of $NH_{3(g)}$ into a sink created by the assimilation of $NH_{3(g)}$ by glutamine synthetase is accompanied by a further considerable depletion which, considering the two masses 17 and 18, could be 29‰. The expression of these two large fractionation steps is dependent on the size of the two $NH_{3(g)}$ pools and the extent of the reaction thus the actual fractionation will vary extensively.

Diffusion into the acidified mat is proposed to simulate the potential fractionation experienced by these lithophytes during diffusive uptake of atmospheric $NH_{3(g)}$.

From this study we have shown that atmospheric $NH_{3(g)}$ and NH_x $\delta^{15}N$ signatures are tightly coupled and are typically depleted, most likely as result of volatilisation to the atmosphere from a terrestrial source. A further discrimination follows the diffusive uptake into vegetation of atmospheric $NH_{3(g)}$, clearly shown in Figures 5.1 and 5.2. The $\delta^{15}N$ value of $NH_{3(g)}$ from the acidified mats goes a long way to explaining the highly depleted isotopic signal expressed in the measured vegetation. The fact that the isotopic depletion in the artificial mats never reaches that of the various plants could be due to a further discrimination in the uptake of $NH_{3(g)}$ by GS which is theoretically large but very dependent on the size of the $NH_{3(g)}$ pool (Yoneyama et al. 2003; Werner and Schmidt 2002). What we have attempted to illustrate here is that the negative $\delta^{15}N$ values measured in lithophytes and epiphytes are not solely attributable to a potentially depleted atmospheric N source, but to the additive discriminations inherent in diffusion from a source and diffusion into a sink.

The contribution of other atmospheric N species to the nutrition of *Trentepohlia*, namely oxidized species such as $NO_{3(g)}$, may complicate this model. We do not assume that the species under investigation only assimilate atmospheric $NH_{3(g)}$, in fact they have been shown the ability to utilize both organic and inorganic forms of N (Abe et al. 2003; Dahlman et al. 2004), all of which occur in the atmosphere. However, because 1) NH_x is preferentially utilized and so readily assimilated over and above other forms of N (Raven 1988; Raven et al. 1992; Ritsuko et al. 2002; Abe et al. 2003; Dahlman et al. 2004), 2) $NH_{3(g)}$ is typically the most abundant N species in the atmosphere [particularly in the presence of urine (Peterson et al. 1998; Frank et al. 2004), or in 'unpolluted' sites (Krupa 2003)], and 3) $NH_{3(g)}$ (mass 17/18) produces a large discrimination during diffusion fractionates (Heaton et al. 1997; Högberg 1997; Robinson 2001), we believe atmospheric $NH_{3(g)}$ is the most likely N form assimilated by the lithophytes to explain and model fractionation of ^{15}N during uptake.

Even if atmospheric $NH_{3(g)}$ accounts for only a portion of the N nutrition of the investigated lithophytes [and in most situations is does (Raven 1998; Raven et al. 1992; Stulen 1998)], the degree of fractionation attributed to diffusive uptake of atmospheric $NH_{3(g)}$ is so strong, that uptake of this N source will still be evident in their isotopic ^{15}N signature. Uptake of alternative atmospheric N forms will only

act to dilute the depleted signature attributed to diffusive uptake of atmospheric $NH_{3(g)}$. In fact diffusive uptake of any gaseous atmospheric N species will likely result in a diffusion related isotopic fractionation. For example, concentrations of nitrate collected in rain above dairy farming activity was found to be higher than expected, averaging 0.019 mg NO_3-N L^{-1}. $\delta^{15}N$ values of this nitrate, however were found to be also isotopically depleted (averaging -4.99‰), so utilisation of this N species in rain or in gaseous form is therefore unlikely to significantly alter the model predictions.

It may be argued that extended periods of drying, may in some way, complicate the model proposed here. Although many lithophytes are capable of surviving prolonged periods of desiccation, more than one year in the case of *Trentepohlia* (Gupta and Agrawal 2004), they are generally considered to only efficiently assimilate gaseous N compounds when wet. Because of the high solubility of $NH_{3(g)}$, its concentrations are expected to be low during rain events effectively removing $NH_{3(g)}$ and therefore the fractionation attributed to its diffusive uptake. *Trentepohlia* and lichens however, may remain wetted and metabolically active for extended periods of time following rain events, and rewetting may occur following dew, or even during high humidity periods (Honegger 1993; Tuba et al. 1996). In addition, as these species do not posses stomata, they have access to $NH_{3(g)}$ during periods where higher plants have their stomata closed (eg at night). Furthermore, given the dynamic nature of atmospheric $NH_{3(g)}$ and its rapid concentration recovery even after rain events, we believe these species will still remain wetted enough for gaseous $NH_{3(g)}$ to contribute to nutrition following re-wetting events.

Higher plants have been shown to assimilate atmospheric $NH_{3(g)}$ through their shoots when concentrations exceed their compensation point (Krupa 2003). Plants growing under severe N limiting conditions are also likely to receive a significant proportion of their N nutrition from atmospheric N sources (Vitousek et al. 1989; Perez-Soba and Van der Eerden 1993; Sutton et al. 1995; Stulen et al. 1998), with $NH_{3(g)}$ being the most prominent source (Raven 1988; Raven et al. 1992; Ritsuko et al. 2002; Abe et al. 2003; Dahlman et al. 2004). Although complicated by roots, mycorrhiza, stomata, cuticle and internal recycling, higher plants growing in N-limited conditions are also likely to exhibit the fractionation

attributed to diffusive uptake of atmospheric $NH_{3(g)}$. We hypothesize that the isotopic fractionation associated with diffusive $NH_{3(g)}$ uptake is a significant source of isotopic depletion in all vegetation growing under N limiting conditions.

Chapter 6

$\delta^{15}N$ variation of plants in primary succession

6.1. Introduction

Foliage $\delta^{15}N$ measurements are simple to make and can be thought of as an integration of the many soil and plant uptake processes that occur in ecosystems. As such they contain a wealth of information that as yet defies a full and logical explanation.

Plant $\delta^{15}N$ signatures typically range between -8 and +10‰ (Bedard-Haughn et al. 2003). This range may be measured in co-occurring taxa within a single environment (Nadelhoffer et al. 1996) highlighting the difficulty in differentiating between drivers of isotopic variation. A number of studies have reported that plant foliage ^{15}N values appear to correlate with a range of ecosystem biotic and abiotic factors including burning frequency (Schmidt and Stewart 2003; Cook 2001), grazing (Schulze et al. 1999), N availability (Martinelli et al. 1999) and climatic factors, particularly precipitation (Handley et al. 1999; Amundson et al. 2003; Swap et al. 2004) appearing as repeatable patterns in similar environments. These correlations however are not universal, nor do they have any predictive power in understanding the drivers behind the reported trends.

The search for a theory to explain the repeatable patterns of foliar $\delta^{15}N$ in ecosystems is receiving much attention, and the occurrence of highly depleted $\delta^{15}N$ signatures (< -8‰) in some ecosystems, falling outside the typical range of plant $\delta^{15}N$ signatures, further complicate the interpretation of ecosystem foliar $\delta^{15}N$ patterns (Handley et al. 1999). They are a particularly puzzling phenomenon which is difficult to account for by typical N cycle and plant N dynamic isotopic fractionating mechanisms.

Ecosystems in which highly depleted foliar $\delta^{15}N$ signatures have been measured are comparatively uncommon. They are represented by early pioneer, alpine, and nutrient limited habitats where, in all cases, plant growth is strongly limited. Nadelhoffer et al. (1996) reported a range of c. -8 to +3‰ in plants growing in cold, wet, and strongly N deficient Alaska tundra sites. Michelsen et al. (1996) determined a $\delta^{15}N$ range of -8.8‰ to +2.2‰ in sub-Arctic plants growing in wet, cold and severely nutrient limited heath and fell field sites. In a further study, Michelsen et al. (1998) examined three wet, cold nutrient limited heath sites in Greenland, Siberia and Sweden, and found foliar $\delta^{15}N$ ranged between -9 to +3‰ for all species across all sites. Similar levels of depletion have also been reported in more temperate latitudes including nutrient limited bog vegetation growing in New Zealand, measured between -15.55 and -0.39‰. In this case the level of depletion is inversely related to distance into the bog (Clarkson et al. 2005).

Depleted foliar $\delta^{15}N$ signatures are not however restricted to wet organogenic soil systems. Glacial retreat in Alaska has produced primary successional forest on substrates ranging from 20 to 230 years. Plant species examined on this chronosequence have $\delta^{15}N$ signatures ranging between c. -11 to +2‰, with the most depleted measured in the earliest succession plant communities (Kohls et al. 2003; Hobbie et al 2005). Similar primary successional patterns have been studied on volcanic substrates in Hawaii with foliar signatures ranging between -10.1 to +0.7‰, again the most depleted signatures measured in the earliest succession communities (Vitousek et al. 1989). In addition, slow growing vegetation on the sub-Antarctic Macquarie Island have produced foliar signatures depleted by as much as c. -10‰ (Erskine et al. 1998).

Numerous mechanisms and reasons have been suggested to account for the level of depletion in these systems. Strongly depleted atmospheric N sources (Vitousek et al. 1989; Erskine et al. 1998) or unique organogenic soil N sources (Michelsen et al. 1996; 1998), in addition to differing N acquisition strategies of plant taxonomic groups (Nadelhoffer et al. 1996) have been proposed. Characteristics of the mycorrhizal symbiosis have also been purported to account for the level of depletion measured in some plants. The ability of fungal hyphae to access otherwise unobtainable (and highly depleted) soil N sources (Michelsen et al. 1996; Michelsen et al. 1998; Nadelhoffer et al. 1996) or the preferential retention

of ^{15}N during fungal metabolism and host transfer are the suggested mechanisms (Högberg et al. 1999; Hobbie et al. 1998; 1999; 2000; 2005). In addition, preferential loss of ^{15}N enriched dissolved organic nitrogen (DON), and the resulting $\delta^{15}N$ depletion in residual ecosystem N may account for the highly depleted foliar $\delta^{15}N$ reported in ecosystems (Handley et al. 1999). This widely ranging series of mechanisms have been supposed by various authors to account for the measured level of depletion but a common or unifying hypothesis remains to be identified.

Primary succession is strongly represented in the reported dataset of highly depleted terrestrial systems (Vitousek et al. 1989; Kohl et al. 2003; Hobbie et al 2005). The most depleted signatures are measured in the very early vegetation communities which are reliant solely on the atmosphere for their N nutrition (Vitousek et al. 1989; Handley et al. 1999). As succession proceeds and N_2-fixing species establish (Kohls et al. 2003) and/or soil developmental processes accelerate (Vitousek et al. 1989), the highly depleted foliar $\delta^{15}N$ signatures are diluted and approach the typical plant $\delta^{15}N$ signature range.

Similar levels of isotopic depletion have been measured in epiphytes and lithophytes. The level of depletion reported in epiphytes and lithophytes is apparently inversely proportional to dependence on atmospheric N sources (Chapter 5 and references within). Epiphytes and lithophytes often dominate early succession sites prior to, and throughout the early establishment of higher plants communities. It is within these situations where epiphytes and lithophytes often express the most depleted signatures (Chapter 5). The common level of isotopic depletion and reliance on atmospheric N sources by these organisms suggest a possible universal mechanism accounting for the level of depletion independent of organism type and ecosystem biotic and abiotic factors.

The aim of this study follows from Chapter 5 and examines the range of foliar $\delta^{15}N$ signatures in higher plants growing in primary succession communities in New Zealand. The primary successions investigated encompass a wide range of substrate types and ages, plant taxonomic groups, mycorrhizal associations, geographical and climatic conditions. The wide range of biotic and abiotic factors

represented within these sites allows a closer examination of the drivers of foliar $\delta^{15}N$ signature levels and trends.

6.2. Methods

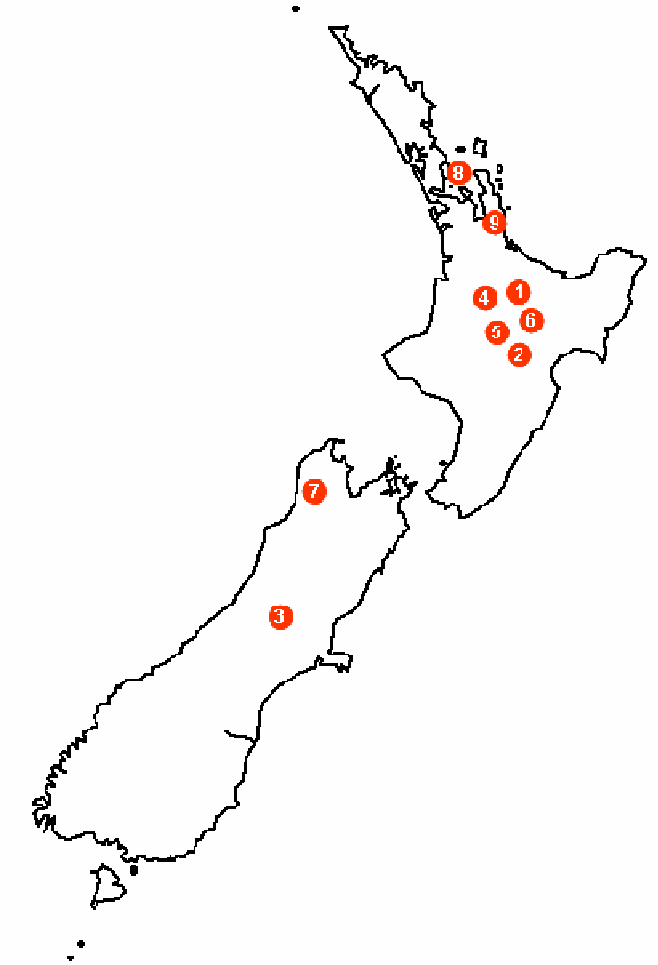
Plant collection sites (Figure 6.1) were selected on the basis of primary successional sequences initiated on young substrates. A wide variety of sites were chosen representing a range of biotic and abiotic factors.

Foliage N concentration and $\delta^{15}N$ signature was assessed on first fully expanded leaves, immediately dried and ground to a fine powder prior to analysis. Often multiple collections occurred at sites over a period of five years. Collected vegetation represented the dominant plant species within the primary succession communities.

Wet deposition, the dominant nutritional source to these communities, was collected in clean water collectors at the inland sites of Mt Tarawera, Mangatepopo Valley and Te Kopia. Wet deposited inorganic N species concentrations and $\delta^{15}N$ signatures were determined by distillation in excess alkali (in the presence of Devarda's alloy in the case of NO_3-N), acidified to small volume at 80ºC and then to complete dryness at 30ºC. This process was tested against known ^{15}N standards to verify full recovery and absence of fractionation. All materials were analysed for ^{15}N in a Europa 20/20 continuous flow IRMS fitted with an elemental analyser.

Because foliar $\delta^{15}N$ trends were empirically observed over environmental gradients and that a large number of sites were sampled, statistical replication was not a primary focus.

6.2.1. Plant collection sites



Collection site	Site name	Substrate type	Substrate age (years)	Elevation (m asl)	Mean annual temp (°C)	Mean annual rainfall (mm)
1	Mt Tarawera	Volcanic lava	119	1111	9.1	1800
2	Mangatepopo Valley	Volcanic lava and glacial deposits	30, 51, 56, 135, and between 2.5 and 1.85 ka	910 – 1470	7.2	2913
3	a) Arthur's Pass b) Porters Pass	Highly mobile alpine colluvial scree	10 - 50	750	8.2	a) 3500 b) 1300
4	Te Kopia	Hydrothermally altered sinter/clay	5 - 20	610	12.8	1410
5	Craters of the moon	Hydrothermally altered sinter/clay	5 - 20	468	11.9	1102
6	Waimangu	Hydrothermally altered sinter/clay	5 - 20	450	12.8	1410
7	Mt Dunn	Ultramafic rock	5 - 50	800	11.2	1600
8	Rangitoto Island	Volcanic lava	250 - 350	30	15.1	1310
9	Kauaeranga River	Alluvial and colluvial boulders	5 - 20	150	15.1	1810

Figure 6.1. Collection site geographical location and abiotic factors

6.2.1.1. Mt Tarawera

Mt Tarawera is a recent (1886) eruptive volcano that deposited 10^8 m^3 of basaltic ash and lapilli over its summit at 1,111 m asl, and is the site of active vegetation regeneration by low, woody N_2- and non-N_2-fixing plants. N has been identified as the major limiting factor in plant colonisation, and has controlled the rates and patterns of vegetation change on Mt Tarawera since its eruption (Clarkson and Clarkson 1995). The Basaltic lapilli provide a uniform, acutely nutrient deficient substrate to plant growth.

Bare substrate is initially colonised by a lichen and herb stage, followed by invasion of ericaceous shrubs and further by the N_2-fixing shrub *Coriaria arborea*. *C. arborea* facilitates succession to broadleaf forest dominated by *Griselinia littoralis* and *Weinmannia racemosa* (Clarkson 1990). Succession on the domes and summit of Mt Tarawera currently consist of sparsely colonised higher plants, predominantly the ericaceous *Dracophyllum subulatum* and endo-mycorrhizal *Leptospermum scoparium* within moss and lichen patches. These plants are either tolerant of low N conditions or show typical N stress symptoms, with yellowing leaves, stunted form and poor vigour.

C. arborea features strongly in vegetation assemblages on Mt Tarawera. Its presence is particularly noticeable at the 'succession front' on the mountain flank, where non-N_2-fixing vegetation succession is visually enhanced in its presence. Yearly invasion and establishment of *C. arborea* ahead of the succession front into the sparsely vegetated dome communities acts to stimulate succession up the mountain, forming 'islands' of closely associated non-N_2-fixing primary successional vegetation (Clarkson 1990). Plants associated with *C. arborea* are greater in size and health, progressively declining with distance from individual *C. arborea*. At distances greater than five meters from *C. arborea*, non-N_2-fixing plants are irregularly and infrequently scattered and exhibit typical N stress symptoms of yellowing leaves, stunted growth and form of the dome vegetation.

6.2.1.2. Mangatepopo Valley

The Mangatepopo Valley, within the Tongariro Cone Complex, is a broad glacial valley flanked by paired moraine ridges. The Tongariro Cone Complex lies at the south western end of the Lake Taupo Volcanic Zone and is an approximately 80 km^3 composite structure built from overlapping material of at least six early cones (erupted between 275 and 65 ka) and 11 cone/vents younger than 25 ka. There is no single summit to the volcanic complex; however the symmetric young cone of Mt Ngauruhoe (2287 m asl) forms the highest point, followed by that of Mt Tongariro (1967 m asl). The saddle between these two cones forms the head of the Mangatepopo Valley.

Mt Tongariro is a strongly weathered and glacially eroded volcano, largely a remnant of the earlier cone development. Growth of the post glacial cone of Ngauruhoe has been dominated by lavas and pryoclastics, contributing to the burying of older cone remnants. Ngauruhoe cone growth has occurred over five phases of activity, over five identifiable time intervals. The oldest phase lavas (phase 1) occurred between 2.5 and 1.85 ka resulting in at least 16 prehistoric lava flows and one block and ash flow present on the lowermost flanks of the cone and valley floor. Subsequent phases have deposited clearly defined lava flows over this first phase material, culminating in the youngest phase (phase 5) eruption in 1975. This activity has produced a wide range of substrate ages; 30, 51, 56, and 135 years old (as in 2005) overlying the prehistoric lava between 2.5 and 1.85 ka old within the valley.

Vegetation succession in Mangatepopo Valley can be visually divided into 'upper' and 'lower valley' assemblages according to the progression of succession. Although vegetation has been initiated throughout the valley, plants above c. 1120 m ('upper valley') are typically small and less frequent than those in the 'lower valley'. The apparent enhanced succession in the 'lower valley' is associated with a sometimes deep organic layer overlying the sandy outwash from the upper valley. Plant growth limit occurs at approximately 1500 m asl.

A wide range of plant taxonomic groups are represented within the Mangatepopo Valley, although mycorrhizal association is more limited (Table 6.1). N_2-fixing species are absent in Mangatepopo Valley.

Mangatepopo Valley provides a plant collection site where a range of biotic and abiotic variables may be driving foliar $\delta^{15}N$ variability. Although substrate material is essentially the same as Mt Tarawera, Mangatepopo Valley does however provide a greater range of substrate ages, and topography (physical properties as a consequence of weathering, glacier activity and alluvial movement). Substrate surfaces range from rough boulders in the youngest substrate materials to gravel pavement in washout areas on the valley floor. The 'moraine' site situated c. 1240 m asl, representing a terminal moraine, contains a wide range of substrate particle sizes and supports a disproportionate number, size and diversity of plant species in the 'upper valley' plant communities.

6.2.1.3. Arthur's/Porters Pass

Arthur's Pass and Porters Pass (c. 750 m asl) in the alpine zone of the Southern Alps, South Island, New Zealand represent formidable environments for primary succession. Plant succession at both sites experience low mean annual temperatures on mobile alpine colluvium exposed as a result of tectonic uplift.

Arthur's Pass and Porters Pass sites largely mirror each other, except in mean annual rainfall, with Porters pass (40 km east of Arthur's pass, in the rain shadow of the Southern Alps), experiencing less than half the annual rainfall of Arthur's Pass. A wide range of plant functional types and the two dominant mycorrhizal associations are present at both collection sites.

6.2.1.4. Te Kopia

The geothermal site of Te Kopia is an active gas fumarole area within the Taupo Volcanic Zone. The predominant geothermal feature, the barren, hydrothermally altered soils, supports small acid sulphate springs, minor fumaroles, mud holes, and dry collapse holes. Te Kopia soils are described as grey to white clays with red brown iron oxide staining, low pH, and often high temperatures (Bignall and Browne 1994). Te Kopia soils have a very low N content but support a variety of resistant vegetation. Vegetation is dominated by the unique *Kunzea ericoides* var. *microflora* with scattered *Styphelia fasciculatus* (Table 6.1). Alternative plant species occur within often deep moss beds on cooler soils.

The geothermal sites of **Waimangu** and **Craters of the moon** are spatially distinct, but representative of geothermally active areas in the Taupo Volcanic Zone supporting similar vegetation communities.

6.2.1.5. Mt. Dun

The Dun mountain massif (1129 m asl), situated c. 20 km inland from the east coast of New Zealand's South Island, provides ultramafic ('serpentine') rock substrate, exposed as a consequence of tectonic movement. Ultramafic substrates are characteristically rich in metallic minerals, particularly magnesium and iron, but also manganese, chromium, nickel and cobalt (Brooks 1987). Primary successional vegetation on these substrates are characteristically stunted, and limited by toxic metal effects (namely nickel and/or magnesium: Robinson et al. 1996), and the unfavourable physical nature of the substrates (sandy, shallow and susceptible to erosion). The ultramafic rock intrudes the older, surrounding volcanic rocks. A sharp vegetation boundary indicates the ultramafic:volcanic substrate interface. Vegetation communities are dominated by a narrow group of plant taxa and mycorrhizal associates (Table 6.1).

6.2.1.6. Rangitoto Island

Rangitoto Island, situated approximately 5 km from the mainland, North Island, is the remnant of a volcanic cone which erupted from the sea in a series of explosions c. 550 years ago. Following its last major eruptions no more than 500 years ago (Lowe et al. 2000), primary succession has been progressively reclaiming the lightweight, clinker like, black lava flows. Succession is dominated by the angiosperm tree *Metrosiderous excelsa*, which is both the first coloniser, and dominant canopy species. With the small survey of vegetation carried out at this collection site a range of plant taxonomic groups, but only one mycorrhizal association, is represented (Table 6.1).

6.2.1.7. Kauaeranga River

The Kauaeranga River drains the high rainfall Pinnacles catchment area, Coromandel Peninsula, North Island, experiencing often massive and devastating flooding events. Each flood event brings down alluvial and colluvial deposits forming a wide flood plain. Within this substrate, few stunted plants exist representing a narrow range of plant taxa and mycorrhizal associates.

Surrounding agricultural land-use contributes to invasion of pasture weeds (Table 6.1).

6.3. Results

$\delta^{15}N$ signatures of higher plants collected within all primary succession ecosystems range between -22.32 to +4.62‰, averaging -10.75‰ (±4.54‰), illustrated in Table 6.1 and Figure 6.2. The level of depletion obtained within most of these sites falls well outside the typical range of vegetation $\delta^{15}N$ signatures and describes a level of natural abundance that has never before been reported. Although these numbers appear widely ranging, duplicate samples are tight, differing no more than 0.5‰ in homogeneous samples, a measure of both the instrument and sample precision (non-homogeneous sample duplicate precision may exceed this range however). The range is instead attributed to environmental variation.

Table 6.1. Collection site, dominant plant species collected, mycorrhizal association, foliar $\delta^{15}N$ signature (‰) and N concentration (%). (NA = not applicable; ND = not determined)

Site	Plant species	Mycorrhizal association	Plant taxonomic group	n	Average foliar $\delta^{15}N$ (‰)	Std. dev. (‰)	Average foliar N concentration (%)	Std. dev. (%)
Tarawera	*Leptospermum scoparium*	Endo-*	Angiosperm tree	13	-12.92	3.69	1.08	0.13
	Gaultheria colensoi	Eric	Angiosperm shrub	34	-11.84	4.11	0.87	0.16
	Dracophyllum subulatum	Eric	Angiosperm shrub	27	-12.85	3.76	1.06	0.13
	Griselinea littoralis	Endo-	Angiosperm tree	7	-5.50	1.69	1.25	0.17
	Pinus contorta	Ecto-	Gymnosperm tree	2	-6.57	1.96	1.57	0.53
		Average			**-11.68**		**1.01**	
		Standard deviation			4.26		0.21	
Mangatepopo	*Leptospermum scoparium*	Endo-	Angiosperm tree	11	-9.62	2.62	1.2	0.20
	Gaultheria colensoi	Eric	Angiosperm shrub	17	-10.27	2.82	0.89	0.13
	Dracophyllum sp.	Eric	Angiosperm shrub	30	-8.38	5.54	0.93	0.12
	Podocarpus nivalis	Endo-	Gymnosperm shrub	3	-12.61	2.63	0.83	0.04

	Calluna vulgaris	Eric	Angiosperm shrub	12	-11.26	2.51	1.1	0.13
	Phyllocladus alpinus	Endo-	Gymnosperm tree	6	-11.72	2.98	0.89	0.06
	Celmisia spectabilis	Endo-	Herb	4	-11.89	4.33	0.79	0.06
	Pentachondra pumila	Endo-	Angiosperm shrub	4	-13.10	1.27	0.90	0.07
	Parahebe sp.	Endo-	Herb	2	-10.88	3.18	0.83	0.06
	Poa sp.	Endo-	Grass	13	-10.19	1.87	0.73	0.27
	Luzula colensoi	Endo-	Sedge	1	-7.18		0.31	
	Sedge sp.	Endo-	Sedge	2	-8.87	3.63	0.87	0.22
		Average			**-10.04**		**0.93**	
		Standard deviation			3.85		0.21	
Arthur's Pass	*Leptospermum scoparium*	Endo-	Angiosperm tree	1	-11.40	NA	0.65	NA
	Dracophyllum sp.	Eric	Angiosperm shrub	1	-5.44	NA	0.90	NA
	Olearia sp.	Endo-	Angiosperm tree	1	-8.65	NA	1.07	NA
	Coprosma sp.	Endo-	Angiosperm shrub	2	-6.35	0.29	0.95	0.04
	Pseudopanax sp.	Endo-	Angiosperm tree	1	-6.94	NA	1.04	NA
	Gaultheria colensoi	Eric	Angiosperm shrub	1	-8.58	NA	0.67	NA
	Celmisia spectabilis	Endo-	Herb	1	-8.27	NA	0.64	NA
	Chionochloa defracta	Endo-	Grass	1	-6.24	NA	0.74	NA
	Hebe sp.	Endo-	angiosperm shrub	1	-10.99	NA	0.79	NA
		Average			**-7.92**		**0.84**	
		Standard deviation			2.04		0.16	
Porters Pass	*Dracophyllum* sp.	Eric	Angiosperm shrub	1	-7.51	NA	0.69	NA
	Gaultheria colensoi	Eric	Angiosperm shrub	1	-7.47	NA	0.87	NA
	Styphelia sp.	Endo-	Angiosperm shrub	1	-6.42	NA	0.71	NA
	Poa sp.	Endo-	Grass	1	-5.59	NA	0.67	NA
	Celmisia spectabilis	Endo-	Herb	1	-7.66	NA	0.55	NA
	Aciphylla	Endo-	Herb	1	-5.29	NA	0.45	NA
		Average			**-6.66**		**0.66**	
		Standard deviation			1.05		0.14	

Arthur's Pass/Porters Pass **		**Average**			**-7.45**		**0.77**	
		Standard deviation			1.81		0.18	
Rangitoto Island	*Griselinea littoralis*	Endo-	Angiosperm tree	1	-13.08	NA	0.54	NA
	Metrosideros excelsa	Endo-	Angiosperm tree	8	-5.33	3.48	0.64	0.1
	Styphelia fasciculatus	Endo-	Angiosperm shrub	1	-6.53	NA	0.64	NA
	Coprosma robusta	Endo-	Angiosperm tree	1	-8.57	NA	1.13	NA
	Leptospermum scoparium	Endo-	Angiosperm tree	2	-5.9	1.26	0.65	0.06
		Average			**-6.36**		**0.67**	
		Standard deviation			3.48		0.16	
Mt. Dun	*Leptospermum scoparium*	Endo-	Angiosperm tree	3	-6.86	3.64	0.85	0.1
	Dracophyllum Subulatum	Eric	Angiosperm shrub	2	-10.78	2.44	0.75	0.08
	Phyllocladus alpinus	Endo-	Gymnosperm tree	2	-10.83	1.07	0.63	0.05
	Chionochloa defracta	Endo-	Grass	3	-7.64	2.12	0.66	0.1
	Exocarpus	Unknown	Root parasite	1	-9.01	NA	0.4	NA
		Average			**-8.7**		**0.7**	
		Standard deviation			2.72		0.15	
Kauaeranga River	*Cortaderia selloana*	Endo-	Grass	2	-1.82	0.47	1.73	0.31
	Senecio jacobaea	Endo-	Herb	2	-0.43	0.74	2.29	0.23
	Leptospermum scoparium	Endo-	Angiosperm tree	1	-0.52	NA	1.09	NA
		Average			**-1.0**		**1.82**	
		Standard deviation			0.86		0.53	
Te Kopia	*Kunzea ericoides*	Ecto-	Angiosperm tree	27	-14.71	3.23	1.31	0.19
	Styphelia fasciculatus	Endo-	Angiosperm shrub	8	-17.29	3.01	1.13	0.11
	Dracophyllum sp.	Eric	Angiosperm shrub	1	-17	NA	0.83	NA
		Average			**-15.35**		**1.26**	
		Standard deviation			3.29		0.2	

Waimangu	*Dracophyllum* sp.	Eric	Angiosperm shrub	1	-16.27	NA	ND	ND
	Kunzea ericoides	Ecto-	Angiosperm tree	1	-16.13	NA	ND	ND
	Styphelia fasciculatus	Endo-	Angiosperm shrub	1	-10.5	NA	ND	ND
	Calluna vulgaris	Eric	Angiosperm shrub	1	-3.84	NA	ND	ND
		Average			**-11.69**		ND	
		Standard deviation			5.88		ND	
Craters of the moon	*Kunzea ericoides*	Ecto-	Angiosperm tree	1	-12.89	NA	ND	ND
	Styphelia fasciculatus	Endo-	Angiosperm shrub	1	-11.58	NA	ND	ND
		Average			**-12.24**		ND	
		Standard deviation			0.93		ND	

* = *L. Scoparium* may form dual ecto- and endo- mycorrhizal associations, but only in the presence of dominating ecto species where continuous inoculation is possible (Moyersoen and Fitter 1999). In the present context, these species are considered endo-mycorrhizal associates.

** = Arthur's Pass and Porters Pass are similar sites separated by 40 km, but with Porters Pass, in the eastern rain shadow of the southern Alps, receiving less than half Arthur's Pass rainfall. Both sub-sites have been combined on this table as the mean foliar $\delta^{15}N$ signatures are not significantly different between the two collection sites: Arthur's Pass -7.92 (±2.04‰; sd) and Porters Pass -6.66 (± 1.05‰; sd).

Plant $\delta^{15}N$ signatures are highly variable across all collection sites (Figure 6.2). Although the mean isotope values may be significantly different between sites, there is enormous variation resulting in a strong overlap in the range of values.

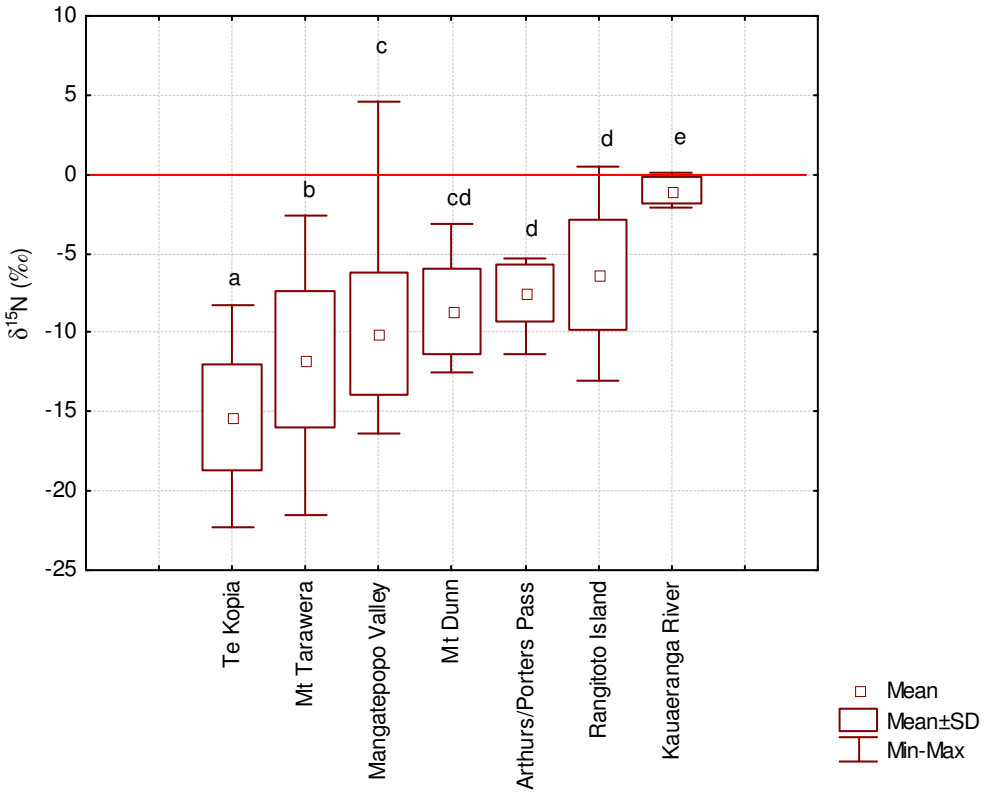


Figure 6.2. Mean and range of foliar $\delta^{15}N$ signatures of all plants at each collection site (different letters denote significant difference ($p < 0.05$) between groups).

A wide range of plant growth forms and taxonomic groups are represented across all collection sites (Table 6.1). Isotopic variation is high across these groupings, all having a similar level of depletion. 'Grasses' proved the exception being significantly ($p < 0.05$) more enriched than 'gymnosperm shrubs', 'angiosperm shrubs' and 'angiosperm trees'. 'Herbs' too were significantly ($p < 0.05$) more enriched than 'angiosperm shrubs' (Figure 6.3).

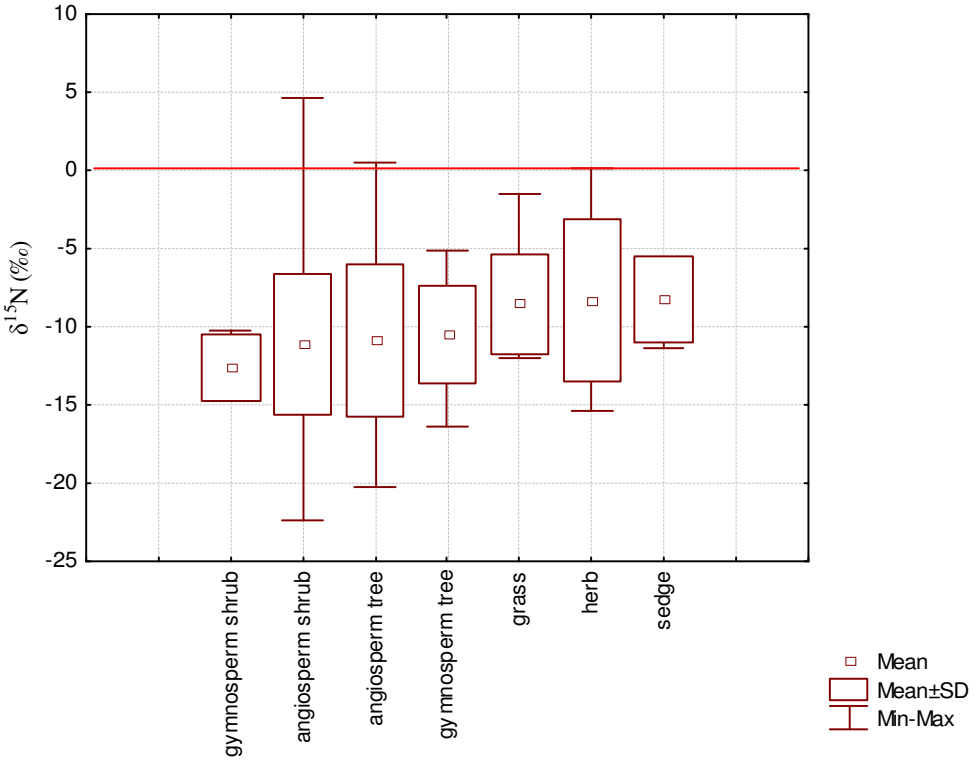


Figure 6.3. Mean and range of foliar $\delta^{15}N$ signatures of all plant growth forms and taxonomic groups across all collection sites.

The major mycorrhizal associations are represented in the combined data set for all collection sites. The mean difference between these major mycorrhizal groups is significant ($p < 0.05$), even though their ranges are very wide (Figure 6.4).

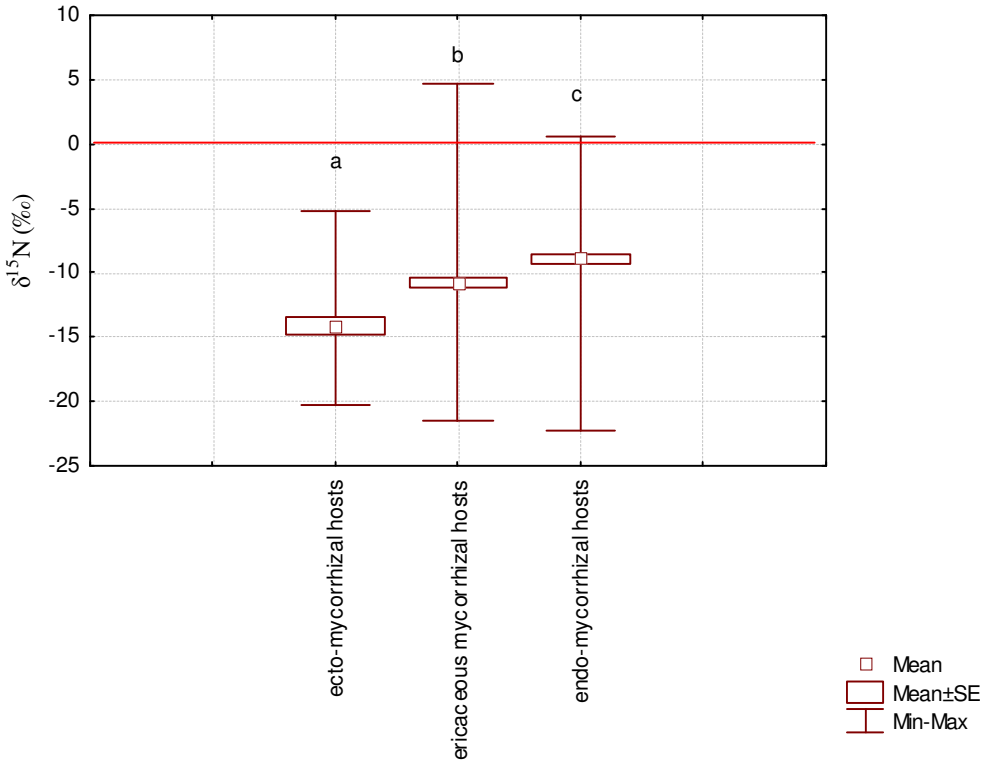


Figure 6.4. Mean and range of foliar $\delta^{15}N$ signatures of mycorrhizal associated host plants across all plant collection sites (different letters denote significant difference ($p < 0.05$) between groups).

Climatic conditions of mean annual rainfall and temperature vary between collection sites. Mean collection site foliar $\delta^{15}N$ is not correlated with either mean annual temperature ($R^2 = 0.1$) or mean annual rainfall ($R^2 = 0.0$). This is most clearly illustrated between Arthur's Pass and Porters pass collection sites, where average foliar $\delta^{15}N$ of the two sites (-7.90 and -6.66‰ respectively), are not significantly different, even though Porters Pass experiences less than half the mean annual rainfall of Arthur's Pass.

Nutrient limitation, particularly N, is clearly similar across all sites, as indicated by low foliar N concentrations, illustrated in Table 6.1 and Figure 6.5. Foliar N concentration is not correlated with $\delta^{15}N$ for the combined data set ($R^2 = 0.006$) suggesting that N limitation alone does not drive the level of isotopic depletion in all plants across all collection sites. Data sets from individual sites do however show apparent correlations between foliar N concentration and $\delta^{15}N$ (Figure 6.5). These correlations will be explored in the individual collection sites examinations below.

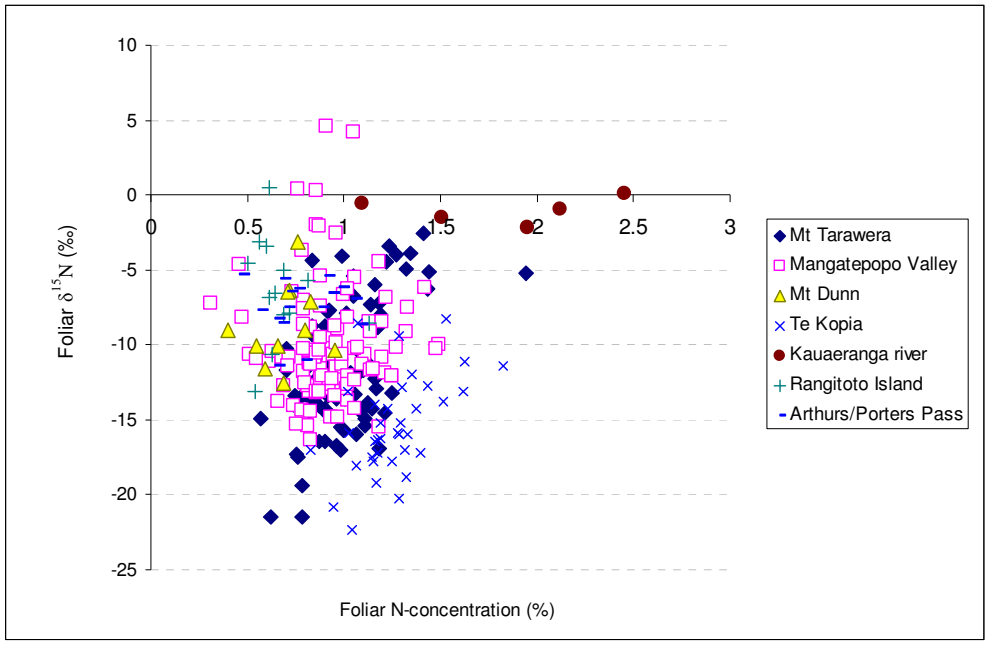


Figure 6.5. Foliar $\delta^{15}N$ signature and N concentration. All plants at all collection sites.

6.3.1. Individual plant collection sites

6.3.1.1. Mt Tarawera

Plants growing on, or near the summit, of Mt Tarawera are visually N deficient, have low foliar N concentration (1.01% ±0.21%) and are highly depleted in ^{15}N (-21.52 to -2.55‰). Foliar N concentration and $\delta^{15}N$ are correlated (Figure 6.6).

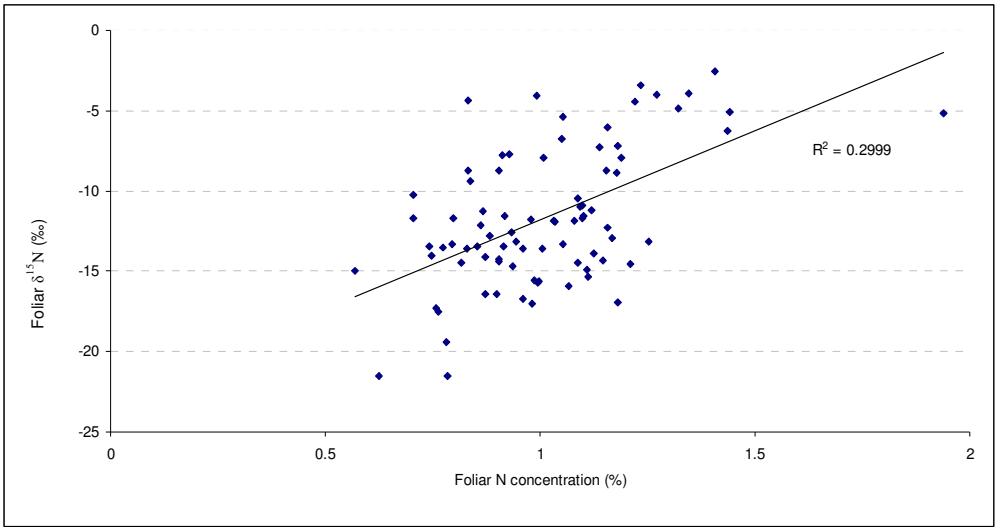


Figure 6.6. Correlation of foliar N concentration (%) and $\delta^{15}N$ signature (‰) of dominant plant species collected across Mt Tarawera

The correlation is driven by proximity to the N_2-fixing plant *C. arborea* with both foliar N concentration and $\delta^{15}N$ positively correlated with distance from the nearest *C. arborea* plant (Table 6.2). Only *Griselinea littoralis* foliar N concentration and $\delta^{15}N$ signature is unaffected by the proximity to *C. arborea*, based however, on a comparatively small sample size (Table 6.1). The correlation shown in Figure 6.6 of plant N concentration therefore does not represent the driver of the isotopic depletion, but simply the result of a two source model of isotopic composition (see 6.4. Discussion).

Table 6.2. Foliar N content, $\delta^{15}N$ signature, and variation (±sd) of predominant non-N_2-fixing plant species growing on Mt Tarawera 'closely associated with *C. arborea*' and the same species 'independent of *C. arborea*'. All foliar $\delta^{15}N$ of plants growing 'independent of *C. arborea*' (except *G. littoralis*) are significantly more depleted ($p < 0.01$) than those of the same plants growing 'closely associated with *C. arborea*'. (ND = not determined)

	Plants growing 'closely associated with *C. arborea*'		**Plants growing 'independent of *C. arborea*'**	
	Foliar N concentration (%) (±sd)	**Foliar $\delta^{15}N$ (‰) (±sd)**	**Foliar N concentration (%) (±sd)**	**Foliar $\delta^{15}N$ (‰) (±sd)**
L. scoparium	1.12 (0.16)	-5.67 (3.15)	1.07 (0.13)	-14.24 (1.71)
D. subulatum	1.24 (0.07)	-6.23 (2.21)	1.03 (0.10)	-14.01 (2.58)
G. colensoi	1.00 (0.19)	-6.87 (4.11)	0.82 (0.11)	-13.64 (2.90)
G. littoralis	1.16 (0.14)	-5.68 (2.25)	1.37 (0.94)	-5.26 (0.94)
P. contorta	ND	ND	1.57 (0.53)	-6.57 (1.95)
Average	**1.10 (0.18)**	**-6.38 (2.32)**	**0.99 (0.21)**	**-13.26 (3.32)**

The three major mycorrhizal associations and plant growth forms are represented in the highly depleted plants growing 'independent of *C. arborea*'. Ecto-mycorrhizal associated gymnosperm trees (although based on a comparatively small data set, n = 2) are significantly more enriched than both ericaceous angiosperm shrubs and endo-mycorrhizal angiosperm trees (Figure 6.7.). The ranges of foliar $\delta^{15}N$ variation measured in all groupings strongly overlap.

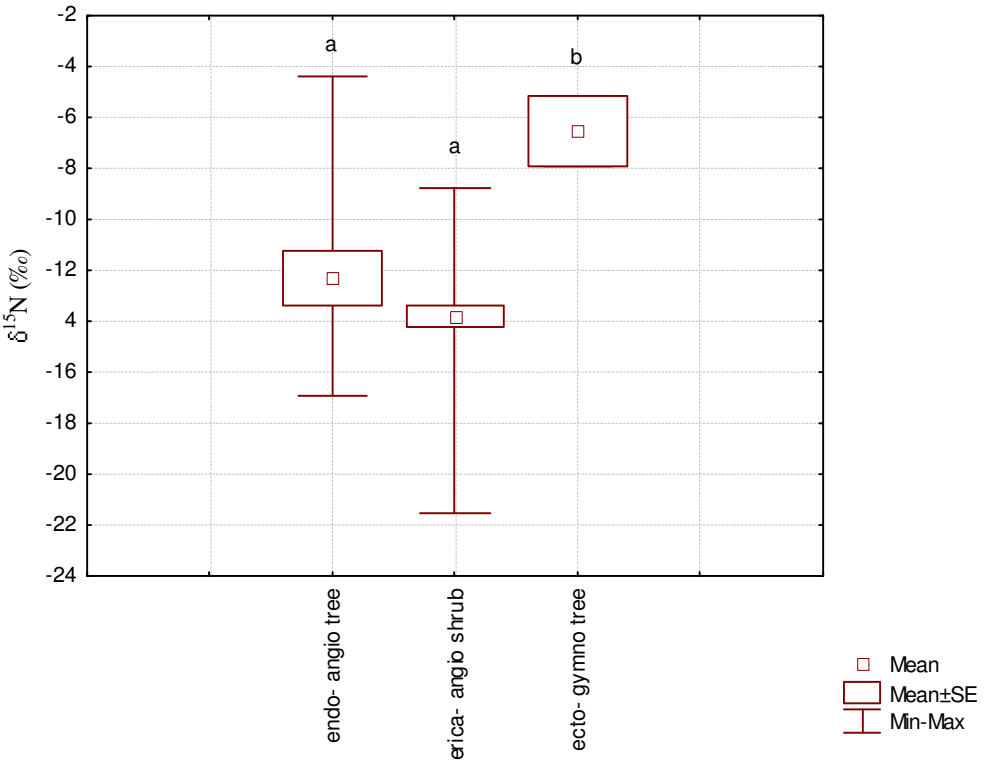


Figure 6.7. Mean and range of foliar $\delta^{15}N$ signatures of plant growth forms and taxonomic groups and mycorrhizal associations of dominant plant species on the domes of Mt Tarawera growing 'independently of *C. arborea*' (different letters denote significant difference ($p < 0.05$) between groups).

6.3.1.2. Mangatepopo Valley

Plants growing in Mangatepopo Valley are similarly N poor (mean foliage N concentration = 0.93% ±0.21%) and highly depleted in ^{15}N (-16.34 to +4.62‰). Foliage N concentration and $\delta^{15}N$ are not correlated, but two groups of plants can be separated on the basis of position within the Valley (Table 6.3). Plants growing in the 'lower valley', below 1120 m asl where vegetation succession is visually enhanced (larger, more frequent and richer species assemblages) have

significantly higher foliar N concentrations and $\delta^{15}N$ compared to those in the 'upper valley'.

Table 6.3. Average foliar N concentrations and $\delta^{15}N$ signatures of dominant plants, growing above and below 1120 m asl in the Mangatepopo Valley.

	Foliar N concentration (%) 'lower valley' (±sd)	Foliar $\delta^{15}N$ (‰) 'lower valley' (±sd)	Foliar N concentration (%) 'upper valley' (±sd)	Foliar $\delta^{15}N$ (‰) 'upper valley' (±sd)
Weighted average	**1.04 (0.20)**	**-7.83 (2.16)**	**0.91 (0.20)**	**-10.47 (3.96)**

A wide range of taxonomic groups, and both ericaceous and endo mycorrhizal associations are represented in the 'upper valley' plant communities. Plant taxonomic group and mycorrhizal association $\delta^{15}N$ are not significantly different within their groupings or between each other (Figure 6.8).

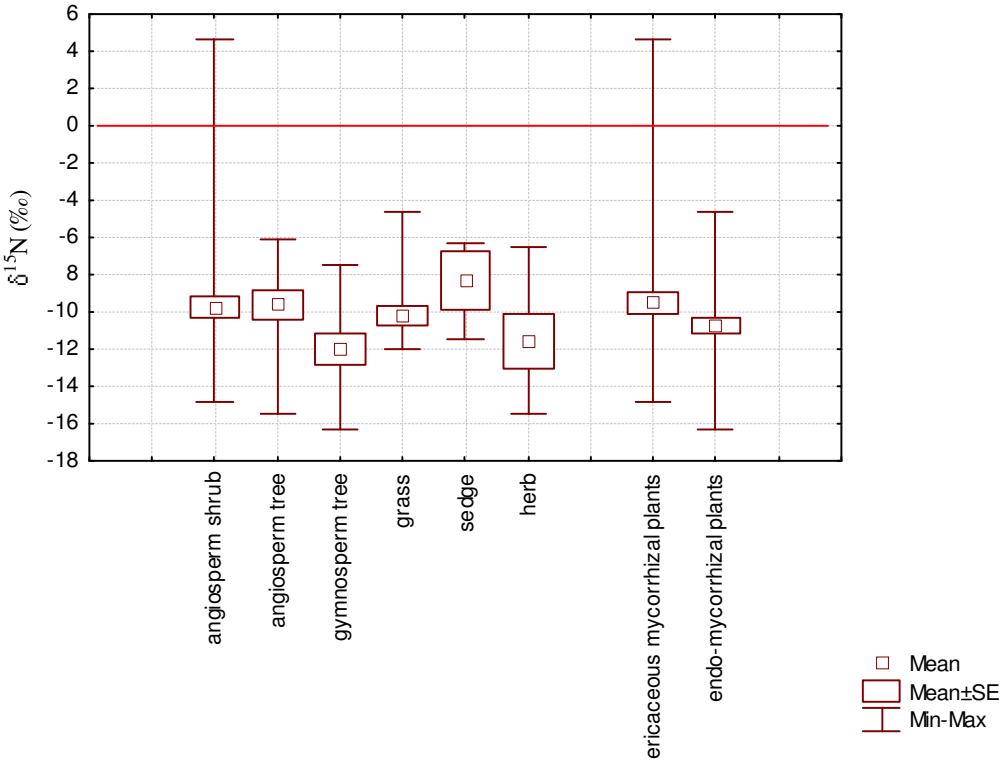


Figure 6.8. Mean and range of foliar $\delta^{15}N$ signatures of plant growth forms and mycorrhizal associated hosts growing above 1120 m asl in Mangatepopo Valley.

A subset of plant species (*L. scoparium*, *D. recurvum*, *G. colensoi*, *Poa* sp. and *C. vulgaris*) growing in Mangatepopo Valley was assessed for foliar P concentration as a potential driver of isotopically depleted foliar $\delta^{15}N$. Foliar P concentrations were low, never more than 0.1% and averaging 0.06% in all species. Foliar P concentrations correlated well with foliar N concentrations, confirming nutrient stress across the Valley, however foliar P concentration, as with N concentration, was not correlated with foliar $\delta^{15}N$ (Figure 6.9).

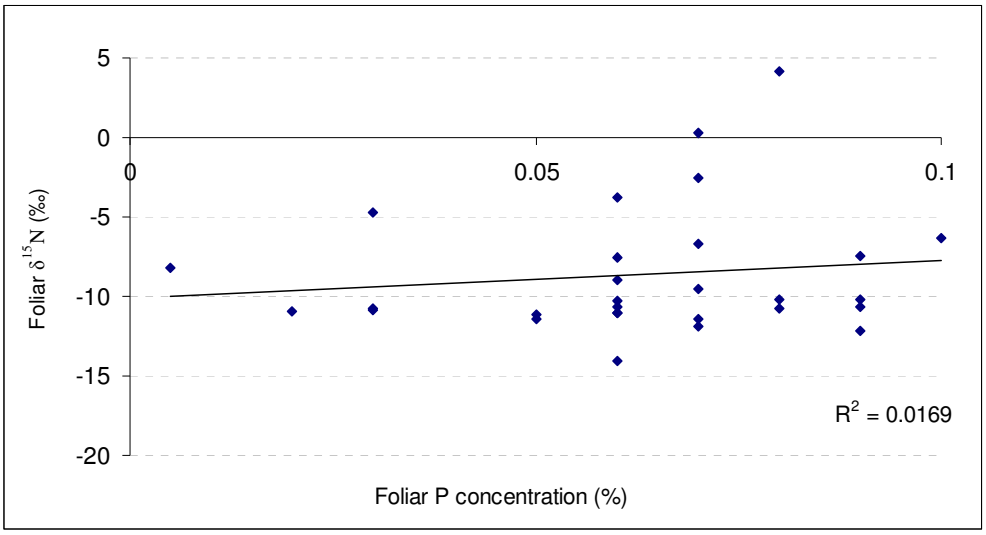


Figure 6.9. Foliar $\delta^{15}N$ and P concentration for a subset of plant species (*L. scoparium*, *D. recurvum*, *G. colensoi*, *Poa* sp. and *C. vulgaris*) growing in Mangatepopo Valley.

Foliar $\delta^{15}N$ signatures of plants growing on different substrate ages, or topographical location in plant communities in the 'upper valley' were not significantly different ($p > 0.05$). The 'moraine' site, positioned c.1270 m asl representing a terminal moraine with a higher proportion of smaller particle substrate size classes, provides habitat for a wider range of plant species with comparatively enhanced health and vigour compared to the surrounding sparse vegetation. The 'moraine' site however provided the most enriched and most depleted $\delta^{15}N$ (+4.62‰ in *D. recurvum* and -16.34‰ in *P. alpinus*), of all plant species within the Mangatepopo Valley.

6.3.1.3. Arthur's/Porters Pass

Primary succession plants growing on the mobile alpine colluvial scree slopes in Arthur's and Porters pass represent a range of plant taxonomic groups and the two dominant mycorrhizal associations (Table 6.1). Plants are N poor (mean foliar N

concentration = 0.77%), and isotopically depleted (mean = -7.45‰) within this collection site (Figure 6.2). Foliar N concentration and $\delta^{15}N$ signature are not correlated ($R^2 = 0.00$).

The dataset is too small to analyse plant taxonomic group differences. There is no statistical difference in average host $\delta^{15}N$ signatures for the two dominant mycorrhizal associations ($p > 0.05$).

6.3.1.4. Rangitoto Island

Rangitoto Island provides a similar volcanic substrate for plant growth as Mangatepopo and Tarawera. Angiosperm trees and endo-mycorrhizal associations dominate this dataset, but are too narrow to make statistical comparisons between them. Average foliar N concentrations of this dataset are however the lowest of any collection site (0.67% ±0.16% sd). Foliar $\delta^{15}N$ signatures, although isotopically depleted (averaging -6.36‰), are significantly more enriched than plants growing on Mt Tarawera or Mangatepopo Valley. Foliar N concentration and $\delta^{15}N$ signature are not correlated in this dataset ($R^2 = -0.03$).

6.3.1.5. Mt. Dun

Mt. Dun provides a unique environment where ultramafic substrate provides physical instability and heavy metal limitation to plant growth. Plant taxa and mycorrhizal associations, although represented, are too narrow to make statistical comparisons. Mt Dun plants have however, low foliar N concentrations and depleted foliar N $\delta^{15}N$ signatures (ranging between -12.5 to -3.11‰). Foliar N concentration and $\delta^{15}N$ are not correlated ($R^2 = 0.02$).

6.3.1.6. Kauaeranga River

The Kauaeranga River provides an apparently alternative N limiting situation with sparse and obviously nutrient stressed vegetation growing within the alluvial flood plain. Plant functional types and mycorrhizal association are not strongly represented within this dataset, limiting statistical analysis. Average foliar N concentrations are the highest recorded across all sites (1.82% ±0.53), although, *Senecio jacobaea*, a typically high N containing species, accounts for the elevated average foliar N concentrations (Table 6.1). Average foliar $\delta^{15}N$ signatures of

vegetation collected from this site are the most significantly enriched of all collection sites (Figure 6.2) averaging -1‰. Foliar $\delta^{15}N$ signature and N concentration are not correlated.

6.3.1.7. Te Kopia

Foliar N contents of plants growing within Te Kopia are amongst the highest measured across all plant collection sites (Table 6.1). In contrast average foliar $\delta^{15}N$ signatures of Te Kopia plants are the most significantly depleted, ranging between -22.32‰ and -8.25‰ (Figure 6.2). The most isotopically depleted foliar $\delta^{15}N$ signature measured across all geographical collection sites is represented at Te Kopia, in *Styphelia fasciculatus*; -22.32‰, one of only two species dominating these geothermal areas. Isotopic depletion of geothermal vegetation within similar geothermal systems is confirmed by single samples of species collected in the geothermal areas of Waimangu and Craters of the moon (Table 6.1) all vegetation $\delta^{15}N$ measured well below the typical range of plant $\delta^{15}N$ signatures.

Foliar N concentrations and $\delta^{15}N$ signatures of vegetation growing in Te Kopia weakly correlate ($R^2 = 0.24$). The correlation is driven by proximity to localised geothermal activity with both N concentration and $\delta^{15}N$ positively correlated with distance to activity (as illustrated in Figure 6.10 for $\delta^{15}N$).

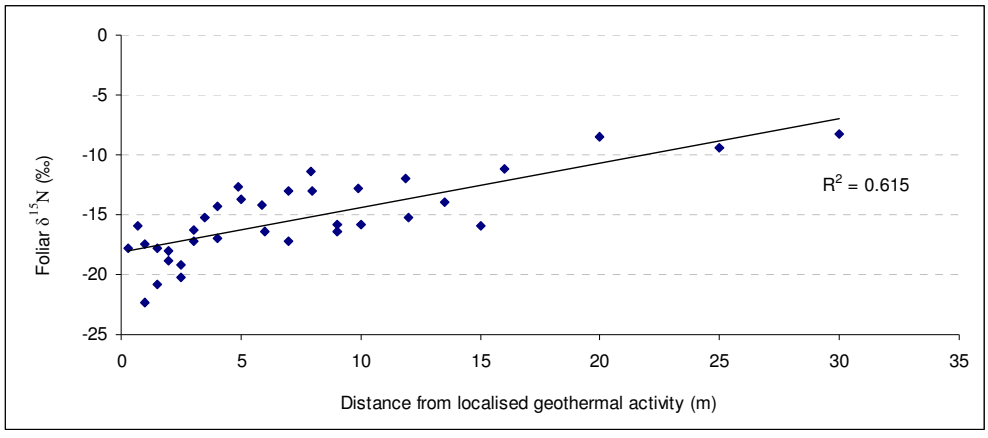


Figure 6.10. Foliar $\delta^{15}N$ signatures of the dominant plant species growing at Te Kopia; *Kunzea ericoides* var. *microflora* and *Styphelia fasciculatus*, with distance from localised geothermal activity (0m) towards the edge of the geothermal area (c. 35m).

The major mycorrhizal associations and a narrow range of plant taxonomic growth forms are represented in the Te Kopia dataset. 'Angiosperm shrubs' are significantly more depleted than 'angiosperm trees' ($p < 0.05$), but with strongly

overlapping $\delta^{15}N$ ranges as illustrated in Figure 6.11. No significant difference between the two mycorrhizal associated host species was measured across this collection site.

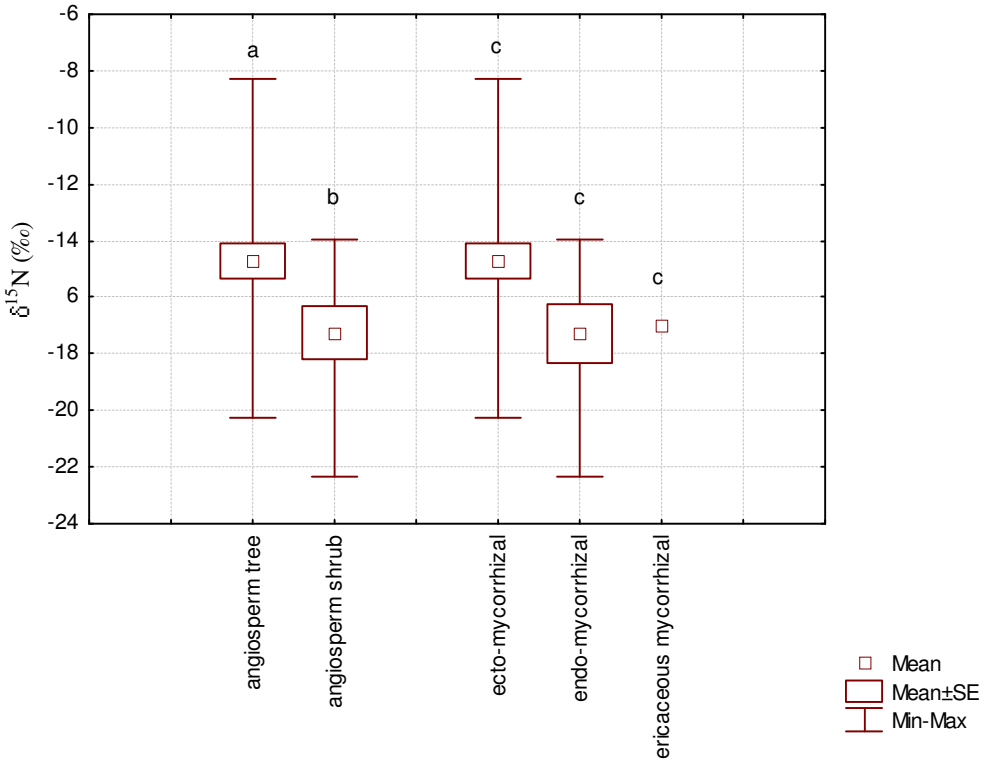


Figure 6.11. Mean and range of foliar $\delta^{15}N$ signatures of plant growth forms and mycorrhizal associated host species collected across Te Kopia (different letters denote significant difference ($p < 0.05$) between groups).

6.3.2. N sources to plants and partitioning of $\delta^{15}N$ within plant and substrate components

Wet deposited inorganic-N species were collected at three of the inland plant collection sites; Mt Tarawera, Mangatepopo Valley, and Te Kopia. Wet deposited NH_4-N (and where collected; NO_3-N) concentrations were not significantly different between collection sites, apparently independent of geographical location. Wet deposited NH_4-N dominated across all sites with average concentrations (0.047 mg L^{-1}), two times that of NO_3-N (0.024 mg L^{-1}).

Wet deposited NO_3-N $\delta^{15}N$ was not significantly different between the two sites where it was collected. In contrast, wet deposited NH_4-N $\delta^{15}N$ signatures were highly variable both within and between sites, ranging between -16.67 and

+0.01‰ (Table 6.4). Site averaged NH_4-N $\delta^{15}N$ signatures were significantly different ($p < 0.05$) between collection sites following the pattern (depleted to enriched): Mangatepopo Valley < Mt Tarawera < Te Kopia. When averaged over all collection sites, however, wet deposited NH_4-N $\delta^{15}N$ (-5.06‰) is not significantly different ($p < 0.05$) to NO_3-N $\delta^{15}N$ (-3.08‰).

Table 6.4. Average concentration and $\delta^{15}N$ signature of wet deposited NH_4-N and NO_3-N species within the Mt Tarawera, Mangatepopo Valley and Te Kopia plant collection areas (ND = no data)

			Wet deposited inorganic-N concentration (mgL^{-1})		**Wet deposited inorganic-N $\delta^{15}N$ (‰)**	
Collection site	**Collection events**	**Number of replicates**	**NH_4-N (±sd)**	**NO_3-N (±sd)**	**NH_4-N (±sd)**	**NO_3-N (±sd)**
Mt Tarawera	Collection 1	4	0.056 (0.01)	ND	-3.24 (1.55)	ND
Mt Tarawera	Collection 2	2	0.020 (0.00)	0.004 (0.00)	-7.63 (0.33)	-3.22 (0.76)
Mangatepopo Valley (and surrounds)	Collection 1	4	0.07 (0.02)	0.03 (0.02)	-14.15 (2.80)	-3.01 (3.61)
Te Kopia	Collection 1	3	0.04 (0.03)	ND	-0.96 (0.86)	ND
Te Kopia	Collection 2	3	0.02 (0.01)	ND	-1.13 (1.28)	ND
Te Kopia	Collection 3	3	0.06 (0.00)	ND	-1.67 (0.71)	ND
Average			**0.047 (0.022)**	**0.024 (0.021)**	**-5.06 (5.40)**	**-3.08 (2.82)**

$\delta^{15}N$ partitioning within plant and substrate components were assessed in an attempt to account for the level of isotopic depletion measured in foliage and determine approximate substrate N $\delta^{15}N$ signatures. A range of sites within Mangatepopo Valley were collected following an altitude transect, representing a range of topographical and substrate age locations.

Foliar, root and substrate material were collected at each site. Complete separation of substrate from root and in particular mycorrhizal hyphal clusters was not possible. Below-ground components were separated into 'root and mycorrhizal hyphae clusters' (containing mostly plant and fungal material) and 'substrate with some root' (consisting predominantly of substrate material).

Replicate plants, and sub-sampled substrates, are tight allowing clear generalised trends of $\delta^{15}N$ compartmentalisation within and across all individual collection sites (Table 6.5). All component $\delta^{15}N$ signatures are variable, but follow the trend (depleted to enriched): 'above ground foliage' < 'root and mycorrhizal hyphae clusters' < 'substrate with some root/fungal material'. The level of isotopic depletion measured in these samples is inversely proportional to its substrate content. This trend suggests root/mycorrhizal N is almost, if not equally, $\delta^{15}N$ depleted as foliage, but diluted by substrate $\delta^{15}N$, with a $\delta^{15}N$ close to 0‰. A near 0‰ signature of substrate N is confirmed by the single sample collected above the vegetation limit (bottom of Table 6.5), in the absence of root/mycorrhizal influence.

Table 6.5. Foliar, root and substrate component $\delta^{15}N$ at various positions within the Mangatepopo Valley as denoted by altitude. (NA = not applicable)

Site elevation up Mangatepopo Valley (m asl)	Site component	$\delta^{15}N$ (‰)	Component average $\delta^{15}N$ (‰)
910	Above ground foliage	-10.59 -6.86 -10.6 -8.12	-9.04
	Root and mycorrhizal hyphae clusters	-3.58 -3.98	-3.78
	Substrate and some root/fungal material	-1.20	-1.20
1016	Above ground foliage	-7.42 -6.13 -4.50 -5.55	-5.90
	Substrate and some root/fungal material	+0.72	+0.72
1112	Above ground foliage	-9.07 -9.15 -9.84	-9.35
	Substrate and some root/fungal material	-0.52 -0.76	-0.64
1269	Above ground foliage	-12.10 -12.05 -8.72 -12.11	-11.25
	Root and mycorrhizal hyphae clusters	-8.61 -10.35 -9.76	-9.57
	Substrate and some root/fungal material	-7.38 -4.7	-6.04
1287	Above ground foliage	-12.36 -11.64 -12.70	-12.23
	Root and mycorrhizal hyphae clusters	-6.03 -7.24	-6.64
	Substrate and some root/fungal material	-4.08 -4.30	-4.19
1304	Above ground foliage	-15.43 -10.23 -11.29 -11.63	-12.15
	Root and mycorrhizal hyphae clusters	-9.60 -8.00	-8.80
	Substrate and some root/fungal material	-6.77 -7.64	-7.21
1350	Above ground foliage	-12.64 -10.35 -11.19	-11.39
	Root and mycorrhizal hyphae clusters	-9.54 -9.66	-9.60
	Substrate and some root/fungal material	-8.54 -9.11 -8.62	-8.76
1390	Above ground foliage	-12.64 -11.28 -12.73	-12.22
	Substrate and some root/fungal material	-5.80 -4.95 -6.52	-5.76
1391	Above ground foliage	-12.53 -13.13 -12.04	-12.57
	Substrate and some root/fungal material	-6.4 -4.6	-5.50
1486	Above ground foliage	-8.63 -10.26	-9.45
	Substrate and some root/fungal material	-7.6 -6.35	-6.98
Above vegetation limit	Substrate with no root or hyphae content	+0.35	NA

6.4. Discussion

Highly depleted (< -8‰) foliar $\delta^{15}N$ signatures are uncommon, but have been reported in a wide range of plant stressed environments (e.g. Nadelhoffer et al. 1996; Hobbie et al. 2005; Erskine et al. 1998) and in epiphytic organisms (Chapter 5). Primary succession plants are strongly represented in the reported dataset of highly depleted terrestrial systems. The level of $\delta^{15}N$ depletion measured in the primary succession plants in this investigation are no exception. In some systems the level of isotopic depletion far exceeds that previously reported in literature for terrestrial plants. The lowest value of -22.32‰ for *Styphelia fasciculatus* in the geothermal areas of Te Kopia is two times more depleted than any previously reported for an angiosperm.

Vegetation growing at one site of apparently recent substrate material, Kauaeranga River, failed to exhibit the level of isotopic depletion measured in all other sites (Figure 6.2). Kauaeranga River collection site represents a river flood plain, and while the floods are massive and bring down enormous volumes of alluvial and colluvial deposits, organic matter is likely to be carried with it. Dead and decaying material, carrying an isotopic signal close to normal abundance, is therefore likely interspersed within this substrate. Kauaeranga River collection site cannot be classed as a true primary successional system.

Highly depleted foliar ^{15}N was measured at all investigated sites where non-N_2-fixing higher plants had recently invaded truly N free substrates. Dilution of highly depleted isotopic signatures of early succession communities has been reported as a consequence of normal successional processes; eg N_2-fixation (Kohls et al. 2003) or soil N development (Vitousek et al. 1989), illustrating a two source model of isotope composition. Similar trends were measured in this study as increasing N inputs from N_2-fixation (Mt Tarawera and proximity to *C. arborea*), soil N development (Mangatepopo Valley and successional progression with increasing altitude), and continuous hydrothermal disturbance (Te Kopia and the hydrothermal gradients from localised geothermal activity) serve to dilute both the N deficiency and the isotope ratio of higher plants in these systems. This is most strongly illustrated in the two plant groupings on Mt Tarawera, where *C. arborea* fixed N dilutes the N stress and depleted $\delta^{15}N$ signature of plants growing

in the early stages of primary succession. The source of this highly depleted $\delta^{15}N$ signature in early succession plants is of great interest.

A range of mechanisms have been suggested to account for the levels of depletion reported in plant stressed environments. Wet deposited N species are likely to be the greatest nutritional source to early successional plants in the absence of substrate N sources. Utilisation of this N source has been attributed to account for the level of $\delta^{15}N$ depletion measured in these systems (Vitousek et al. 1989). Concentrations of NH_4-N, the most dominant wet deposited inorganic N species in this study, agree with those previously measured in inland New Zealand sites (Wilson 1959). $\delta^{15}N$ signatures of wet deposited inorganic N species fall at the lower end of the range reported for wet deposited N species (c. -15 to +10‰ as in Russell et al. 1998), but the average across all sites (c. -4‰) cannot alone account for the level of depletion measured in the higher plants of these systems.

Taxon specific N acquisition strategies, in particular the role mycorrhizal association, have been proposed to account for the high level of isotopic depletion reported in some plant stressed systems (e.g. Högberg et al. 1999; Hobbie et al. 2005). A wide range of plant growth forms, taxonomic groups, and the major mycorrhizal associations are represented within and between the collection sites in this investigation. Endo- and ericaceous mycorrhizal associations dominated across all sites (Table 6.1.), unsurprising as these fungi/host associations predominate in extreme conditions (Körner 1999). The level of isotopic depletion measured within these collection sites is however independent of plant taxonomic group, plant growth form or mycorrhizal association.

A wide range of abiotic factors are represented across the collection sites. Substrate types include volcanic material, ultramafic, sedimentary and geothermally altered materials. The high level of depletion measured in higher plants occurs independently of these substrate types as well as mean annual temperature, altitude and topography. High mean annual rainfall is however a commonality across all sites, with no collection site receiving less than 1000 mm annually.

Increasing mean annual precipitation has been associated with isotopic depletion of plant $\delta^{15}N$ signatures on landscape and global scales (Handley et al. 1999; Amundson et al. 2003; Swap et al. 2004). Plant collection sites in this investigation are generally 'high' rainfall and exhibit depleted foliar $\delta^{15}N$ signatures, conforming to the general trend. Variation in rainfall across all sites does not however correlate with the variation in mean site foliar $\delta^{15}N$. This is clearly illustrated within the Arthur's/Porters Pass collection sites where all abiotic factors are similar but with Porters Pass, in the rain shadow of the Southern Alps, receiving less than half Arthur's Pass rainfall. Mean foliar $\delta^{15}N$ signatures collected within the small, but representative datasets are not significantly different.

Handley et al. (1999) suggested highly depleted plant signatures may be strongly expressed in plant systems where an isotopic branching point, producing a highly depleted product, is operating. If residual N is lost or unavailable, plants utilising this depleted product will reflect its isotopic ratio above all other traditional drivers of plant $\delta^{15}N$. One mechanism proposed as an isotopic branching point by Handley et al. (1999) is the leaching loss of enriched dissolved organic N (DON), leaving residual, plant available N sources isotopically depleted. DON loss may occur in high latitude organogenic soil systems, however given the absence of any soil development, DON loss is unlikely in the early succession systems examined in this study.

No currently proposed mechanism in literature can reasonably account for the level and consistency of depletion measured across the wide range of biotic and abiotic factors measured in this study. The surprisingly similar levels of depletion measured here, and in literature reported studies and the common growth stress experienced by all plants, suggest the level of depletion may be driven by a common, but as yet unknown, universal mechanism. Reports of similar levels of depletion in epiphytes and lithophytes (Chapter 5) further support a universal mechanism, acting independently of organism type.

Utilisation of atmospheric $NH_{3(g)}$ as a source of nutrient and a mechanism to account for the level of depletion in epiphytes and lithophytes was proposed in Chapter 5. Levels of isotopic depletion in epiphytes and lithophytes, as low as

-24‰, are theoretically and empirically accounted for by the double isotopic fractionation of $NH_{3(g)}$ volatilisation to the atmosphere followed by its diffusive uptake. Atmospheric $NH_{3(g)}$ may act as a universal N source to all vegetation if concentrations exceed foliar compensation points. Although higher plant $NH_{3(g)}$ uptake is complicated by roots, stomata, cuticle, and internal recycling, early succession higher plants may be capable of utilising this N source, potentially explaining the level of depletion measured in these systems. This theoretically plausible mechanism may satisfy the universal nature of isotopic depletion in a wide range of primary successional - and alternatively nutrient deficient systems - independent of measured biotic and abiotic factors.

Partial utilisation of a supplied N source is a strongly fractionating process in plants (Yoneyama et al. 2001). The primary successional systems investigated here experience conditions at the extreme end of plant growth tolerance. Multiple growth limiting factors are present in these systems, including temperature, pH, heavy metal concentrations, substrate instability and nutrient limitation. If growth is not primarily limited by N, a partial uptake of a comparatively abundant N source will result in an isotopic depletion in plants. The highly permeable nature of substrate types and therefore the short residence time of wet deposited N sources will contribute to the short time frame N sources are available for plant uptake.

These novel, testable and theoretically plausible mechanisms are capable of explaining the measured level of depletion reported in this investigation. They are presented in light of the inability of currently proposed theories to account for the level and commonality of isotopic depletion measured in these systems, and may operate as isotopic branching points, suggested by Handley et al. (1999). The universal nature and level of depletion in these systems, and those reported in literature suggest further investigation will provide rewarding research and further understanding of ecosystem N cycling and plant N dynamic processes.

Chapter 7

Plant $\delta^{15}N$ response to $NH_{3(g)}$ uptake

7.1. Introduction

Recent focus in ecophysiological studies has been towards a greater understanding of mechanisms controlling plant $\delta^{15}N$ signatures. Most N cycling and plant N dynamic mechanisms result in a plant $\delta^{15}N$ range of -8 to +10‰ (Bedard-Haughn et al. 2003). Reports of highly isotopically depleted $\delta^{15}N$ signatures in a variety of plants outside this range are of particular interest, suggesting that a unique and universal mechanism may be acting on plants in these systems (Chapter 6).

Unusually depleted foliar $\delta^{15}N$ signatures have been measured across a wide range of ecosystem and plant taxonomic groups. Significant isotopic depletions have been measured in wet/cold ecosystems including fell fields, heathlands, tundra and sub-Antarctic islands, with $\delta^{15}N$ signatures as low as -10‰ measured in plants (Nadelhoffer et al. 1996; Michelsen et al. 1996; Erskine et al. 1998). Early primary successional vegetation in Alaska and Hawaii also exhibit highly depleted foliar $\delta^{15}N$, as low as -22.3‰ (Chapter 6 and references within). Higher plants are not alone in their expression of depleted foliar $\delta^{15}N$ signatures; epiphytes and lithophytes have also been measured with very strongly depleted $\delta^{15}N$ signatures (Chapter 5 and references within), particularly when heavily dependent on the atmosphere as a nutritional source. Isotopic depletions in epiphytes and lithophytes, measured as low as -24‰ (Chapter 5) are amongst the lowest measured in natural terrestrial systems.

Numerous mechanisms have been suggested by authors to account for the levels of depletion reported. Chapter 5 proposed atmospheric $NH_{3(g)}$ uptake as a plausible mechanism to account for the level of depletion in epiphytes and lithophytes, and in fact any vegetation reliant on the atmosphere for nutrition.

$NH_{3(g)}$ in particular is one of many atmospheric N species available to plants for growth and represents the most common and readily plant available (Krupa 2003). The double fractionation process of $NH_{3(g)}$ volatilisation to the atmosphere and its subsequent diffusive uptake into foliage theoretically accounts for the level of depletion measured.

Plant uptake of atmospheric $NH_{3(g)}$ is driven by the foliar $NH_{3(g)}$ compensation point. The compensation point describes the atmospheric $NH_{3(g)}$ concentration where no net flux is measured between the leaf and the atmosphere (Farquhar et al. 1980). When atmospheric $NH_{3(g)}$ concentrations exceed the compensation point of foliage, $NH_{3(g)}$ diffuses into the apoplast of living cells and is subsequently assimilated into organic N by glutamine synthetase (GS). Diffusive flux is determined by the concentration gradient between the two, with a flux that increases linearly with $NH_{3(g)}$ concentration (Van Hove et al. 1987). Plant mesophyll NH_3-N concentrations, and therefore $NH_{3(g)}$ compensation points, are dependent on a number of physiological factors: the developmental stage of the plants (or plant part), the activity of GS, and the level of N nutrition (Mattsson and Schjoerring 2002). The cuticle is an effectively impermeable barrier to atmospheric $NH_{3(g)}$ (Van Hove et al. 1987) and therefore, as in CO_2 uptake, stomatal conductance plays a significant role in the diffusive flux of $NH_{3(g)}$ into the apoplast. Following diffusion into the sub-stomatal cavity, atmospheric $NH_{3(g)}$ readily equilibrates with NH_4^+ in the mesophyll apoplast, maintained at a very low concentration by the low K_m of GS operating in the chloroplast (Farquhar et al. 1980).

Foliar $NH_{3(g)}$ compensation points of adequately fertilised higher plants are unlikely to exceed the low atmospheric $NH_{3(g)}$ concentrations measured in unpolluted and non urban areas (Krupa 2003). As a consequence, atmospheric $NH_{3(g)}$ contribution to higher plant N nutrition is unlikely to be significant (Raven et al. 1992). Atmospheric $NH_{3(g)}$ uptake has however been reported to contribute to plant nutrition when its concentration exceeds compensation points, usually as a consequence of polluting levels of $NH_{3(g)}$ (Pérez-soba and Van der Eerden 1993). Alternatively, foliar compensation points are likely linked to the N demand of the plant. Plants experiencing low levels of N supply have been shown to have lower compensation points relative to the same plants growing on higher

N supply (Herrmann et al. 2002; Mattsson and Schjoerring 2002). In addition, Herrmann et al. (2002) reported higher atmospheric $NH_{3(g)}$ uptake rates in plants with a lower shoot total N concentration compared to high N supplied plants. These results suggest that higher plants growing in low nutrient conditions, such as early primary succession communities examined in Chapter 6, may utilise atmospheric $NH_{3(g)}$ as a N nutritional source, even in the presence of low $NH_{3(g)}$ concentrations.

Volatilisation of $NH_{3(g)}$ from terrestrial sources to the atmosphere is a strongly fractionating process (Högberg 1997; Frank et al. 2004). The potential maximal fractionation of -29‰ as a consequence of the two masses ($^{14}NH_{3(g)}$ of 17 c/w $^{15}NH_{3(g)}$ of 18) may be reflected in the initial $NH_{3(g)}$ product. The degree of depletion expressed in a product is dependent on its source $\delta^{15}N$ signature and extent of reaction 'completion' (Högberg 1997). As such, the level of isotopic depletion will vary extensively, but is expected to be more depleted in ^{15}N relative to the source (Högberg 1997, Russell et al. 1998). The initial volatilised $NH_{3(g)}$ product from simulated urine patches of -28‰ reported in Frank et al. (2004) empirically validates this hypothesis. The few measurements of atmospheric $NH_{3(g)}$ $\delta^{15}N$ signatures reported in literature fall within the range -20‰ to +22‰ (Yeatman et al. 2001). The process of diffusion is also equally fractionating (Högberg 1997) and the subsequent diffusion from the atmosphere into leaves is also predicted to result in a potentially highly depleted product. The fractionation attributed to these two processes acting in series easily accounts for the level of depletion measured in early succession plant communities (Chapter 6) and in epiphytes and lithophytes (Chapter 5).

Differentiating between the isotopic fractionation attributed to $NH_{3(g)}$ diffusive uptake over and above the inherent $\delta^{15}N$ signature of $NH_{3(g)}$ is very difficult. If fractionation attributed to one process is known and kept constant, the other may be determined by difference. Continuous measurement of $NH_{3(g)}$ $\delta^{15}N$ in unpolluted, non urban areas of low atmospheric $NH_{3(g)}$ concentration, is logistically very difficult (Chapter 5). A controlled situation to test this method of N acquisition and its consequence to plant $\delta^{15}N$ signatures is required.

The aim of this study was to determine the contribution of atmospheric $NH_{3(g)}$ to nutrition and $\delta^{15}N$ signature of higher plants. Two experiments were conducted. Firstly, **the farm trial**, which explored the $\delta^{15}N$ contribution of probable N sources (namely wet deposited N and atmospheric $NH_{3(g)}$) to N stressed plants. Intense dairy farming systems are high contributors of atmospheric $NH_{3(g)}$ as a consequence of volatilisation from dung and urine (Krupa 2003). This trial was designed to replicate natural ecosystem conditions found in early succession communities, as measured in Chapter 6, however in the presence of varying but typically high atmospheric $NH_{3(g)}$ concentrations. Secondly, **the $NH_{3(g)}$ fumigation trial**, examines the mechanism of foliar uptake of atmospheric $NH_{3(g)}$ to plant N nutrition and $\delta^{15}N$ signature when grown under various nutrient regimes in controlled laboratory conditions.

7.2. Materials and methods

7.2.1. Farm trial

The site chosen to carry out this trial was the Halutain Dairy farm, located 15 km north of Hamilton city, New Zealand. It represents a typical 12 month grazing dairy farm, supporting approximately 3 cows per hectare. The mixed clover ryegrass pasture is intensively managed and urea fertilised, sustaining a stock rotation between 21 days (spring) to 95 days (winter) with supplementary feed. Dairy farming activity is commonly associated with high concentrations of atmospheric $NH_{3(g)}$ (Chapter 5).

In order to expose plants to varying levels of naturally occurring atmospheric $NH_{3(g)}$, a movable trolley device was designed to house growing plants in the field. Two devices were employed: one positioned in paddocks where cows were absent for at least 20 days, with access to elevated (c. 25 $\mu g\ m^{-3}$) atmospheric $NH_{3(g)}$ concentrations ('ungrazed' treatment), the other positioned in paddocks immediately following cow grazing, with access to very high (c. 80 $\mu g\ m^{-3}$) atmospheric $NH_{3(g)}$ concentrations ('grazed' treatment). The trolley device allowed plants to be grown in the presence of atmospheric dry deposition only ('inside' plants) or both wet and dry deposition ('outside' plants). 'Inside plants' were sheltered from airborne particulate N sources. The movable nature of the trolley devices allowed easy movement to treatment paddocks.

Leptospermum scoparium has a wide environmental and nutritional tolerance and is typically found growing in low N substrates of early succession. Previous analysis has shown it to have often highly depleted $\delta^{15}N$ signatures when growing in low N systems (see Chapter 6), and is capable of growing at the extreme low end of higher plant nutrient stress. In addition, its small seed contributes little to plant N, it is long lived and comparatively easy to grow under controlled conditions. It provides an ideal plant to study the effects of $NH_{3(g)}$ uptake on nutrition and $\delta^{15}N$. *L. scoparium* seeds ($\delta^{15}N$ = +1.85‰, N content of 0.76% providing c. 57 μg N $seed^{-1}$) were germinated in medium grained, acid washed sand in 280 x 330 x 40 mm (deep) trays in the laboratory, and provided with de-ionised water as required. One week after germination, the trays of plant material were provided mycorrhizal inoculum (root slurry of field grown, healthy, host plant), taken to the farm and subjected to the 'grazed inside', 'grazed outside', 'ungrazed inside', and 'ungrazed outside' treatments.

Plants were exposed to the treatments for the 10 month period between January and December 2005. In addition to de-ionised water, plants were provided with two litres of nitrogen-deficient (complete-N) nutrient solution per treatment tray each week to ensure N was the limiting nutrient for growth (see nutrient solution section; 7.2.3). Plants of all treatments were harvested periodically over time to determine the effect of treatment on whole plant N concentration and $\delta^{15}N$ signature.

Wet deposition was collected and analysed for inorganic N content and $\delta^{15}N$ signature (Chapter 2) over the 10 month period. In addition, 0.08 m^2 oxalic acid capillary matting (Chapter 5) was exposed, at both 'grazed' and 'ungrazed' sites, to atmospheric $NH_{3(g)}$ for two weekly periods throughout the duration of the experiment. Capillary matting recovered $NH_{3(g)}$ reflects comparative atmospheric $NH_{3(g)}$ concentrations and the proposed fractionation attributed to $NH_{3(g)}$ volatilisation to the atmosphere and its diffusive uptake into an infinite sink.

7.2.2. $NH_{3(g)}$ fumigation trial

L. scoparium plants were prepared for treatment by germination in medium grained, acid washed sand. Following germination, plants were provided complete nutrient solution, mycorrhizal inoculum (root slurry of host tree), and

grown under Na-vapour lamps providing irradiance between 600 and 800 μmol m^{-2} s^{-1}. When plants were five months old, complete nutrient solution was replaced with a complete–N nutrient solution (section 7.2.3). Two months later, plants were carefully removed from the sand growing medium and sealed into 5 L hydroponic growth vessels with Dow Corning 310RTV silicone rubber encapsulate to ensure roots and hydroponic solutions were physically separated from plant aerial parts. Six hydroponic growth vessels were prepared (three treatment and three control), each containing 10 *L. scoparium* individuals (averaging 3.8 mg ± 2.2 mg dry wt, n=27, $\delta^{15}N$ = +1.13‰ ±0.77‰, n = 6). *L. scoparium* plants were supplied with either complete, phosphorous deficient (complete-P), or nitrogen deficient (complete-N) nutrient solutions, physically separated from the atmosphere. Lids of the hydroponic growth chambers could be unsealed and removed to allow weekly nutrient replacement.

The potential of *L. scoparium* to utilise atmospheric $NH_{3(g)}$ as a nutrition source and its effect on whole plant $\delta^{15}N$ signatures was assessed under consistent atmospheric $NH_{3(g)}$ concentration and $\delta^{15}N$ signature. Over 100 days, *L. scoparium* plants, with their corresponding nutrient treatments, were grown in either a 100 L 'control chamber' or similar '$NH_{3(g)}$ fumigation chamber'. $NH_{3(g)}$ scrubbed (see methods 2.2.4.) ambient air at a flow rate of 10 L min^{-1} was maintained through the 'control chamber' over the experimental period. Ambient air with the addition of $NH_{3(g)}$ was passed into the '$NH_{3(g)}$ fumigation chamber' at a rate of 10 L min^{-1}, maintaining a chamber atmospheric $NH_{3(g)}$ concentration of 100 μg $NH_{3(g)}$-N m^{-3}. $NH_{3(g)}$ was supplied to the '$NH_{3(g)}$ fumigation chamber' from a flow regulated tank of 100% pure, compressed $NH_{3(g)}$. Both fumigation chambers were fitted with mixing fans to aid air movement and resealable glass lids. These lids allowed efficient light penetration and periodic access into the chambers but preventing $NH_{3(g)}$ leakage while sealed. Positive pressure drove air flow out of the chamber *via* waste tubing leading away from the experimental area.

Atmospheric $NH_{3(g)}$ concentrations of 100 μg $NH_{3(g)}$-N m^{-3} are considered well above typical $NH_{3(g)}$ foliar compensation points, but below concentrations toxic to plant growth. This concentration remained consistent throughout the duration of the experiment, varying no more than 50 μg above or below 100 μg $NH_{3(g)}$-N m^{-3}.

Atmospheric $NH_{3(g)}$ $\delta^{15}N$ signatures also remained consistent throughout the duration of the experimental period: -3.09‰ (± 0.74‰, n = 12).

Hydroponic nutrient solutions were replaced weekly and aerated by passing ambient, $NH_{3(g)}$ scrubbed air *via* airtight lines, through the fumigation chamber walls and into the hydroponics growth vessels. Waste air was driven by positive pressure through similar lines to waste outside the fumigation chamber.

The experiment was carried out within a glasshouse over the 100 day period; 17th June to the 25th September 2005. Sunlight and day length were augmented with Na-vapour lamps providing no less than 600 μmol m^{-2} s^{-1} over an 18/6 hour day/night period. Temperature remained within the range 15 to 25ºC throughout the trial period.

Contribution of nutrient solution N (μg total N $plant^{-1}$) to experimental plants (Z) can be determined as follows:

$$X - Y = Z$$

Equation 7.1

Where (X) is the average N contribution to plants following treatment in '$NH_{3(g)}$ fumigation chamber' (μg total N $plant^{-1}$) and (Y) is the average N contribution to plants following treatment in 'control chamber' (μg total N $plant^{-1}$).

Contribution (%) of $NH_{3(g)}$-N to total plant N in '$NH_{3(g)}$ fumigation chamber' plants, above that provided by nutrient solution, can therefore be determined by:

Avg. N contribution from $NH_{3(g)}$-N following treatment (%) = 100 – ((Z/X) * 100)

Equation 7.2

Contribution of $NH_{3(g)}$-N $\delta^{15}N$ signature to whole plant N $\delta^{15}N$ signature above that provided by nutrient solution N (as determined by plants growing in the control chamber) may be determined using a mixing model:

$$(A - B/ C) + B$$

Equation 7.3

where (A) is the $\delta^{15}N$ signature of the '$NH_{3(g)}$ fumigation chamber' plant, (B) is the $\delta^{15}N$ signature of the equivalent nutrient treated plant in the 'control chamber', and (C) is the proportional contribution of atmospheric $NH_{3(g)}$ to the treated plants.

7.2.3. Nutrient solutions

Complete

Complete nutrient solution contained (mM): KNO_3 (1), $Ca(NO_3)_2 \cdot 4H_2O$ (2), $NH_4H_2PO_4$ (1), $MgSO_4 \cdot 7H_2O$ (1), $NaEDTA \cdot 2H_2O$ (0.025), $FeCl_3$ (0.025), H_3BO_3 (0.005), $CuSO_4 \cdot 5H_2O$ (0.0025), $MnSO_4 \cdot 1H_2O$ (0.0025), $ZnSO_4 \cdot 7H_2O$ (0.00025), and $Na_2MoO_4 \cdot 2H_2O$ (0.000025).

Phosphorous deficient nutrient solution (complete-P)

Complete-P consisted of complete nutrient solution, as described above, but with 1 mM $NH_4H_2PO_4$ replaced by 1 mM NH_4Cl. Inorganic P contamination (PO_4-P) was calculated based on known P contamination concentrations in chemical stock, at 2.8 μg PO_4-P L^{-1}.

Nitrogen deficient nutrient solution (complete-N)

Complete-N nutrient solution contained (mM): K_2HPO_4 (2), $CaSO_4 \cdot 0.5H_2O$ (1), K_2SO_4 (1), $MgSO_4 \cdot 7H_2O$ (1), $NaEDTA \cdot 2H_2O$ (0.025), $FeCl_3$ (0.025), H_3BO_3 (0.005), $CuSO_4 \cdot 5H_2O$ (0.0025), $MnSO_4 \cdot 1H_2O$ (0.0025), $ZnSO_4 \cdot 7H_2O$ (0.00025), and $Na_2MoO_4 \cdot 2H_2O$ (0.000025).

Inorganic N contamination in the complete-N nutrient solution was measured to average 2.31 μg inorganic N L^{-1} (n = 6) with c. 80% of this in NH_4-N form. $\delta^{15}N$ signatures of NO_3-N and NH_4-N contamination in complete N nutrient solution were determined as -4.46‰ (±1.46%;sd, n = 6) and -3.04‰ (±3.98‰;sd, n=6), respectively.

All nutrient solutions were maintained at pH 5.5.

7.3. Results

7.3.1. Farm trial

To assess the contribution of atmospheric $NH_{3(g)}$ to the nutrition and $\delta^{15}N$ signature of N stressed vegetation, *L. scoparium* plants were grown at the farm site in the presence of varying but high $NH_{3(g)}$ levels in 'grazed' (~80 µg $NH_{3(g)}$-N m^{-3}) and 'ungrazed' (~25 µg $NH_{3(g)}$-N m^{-3}) paddocks. In addition to the varying $NH_{3(g)}$ concentrations at the two treatment locations, plants were exposed to only dry deposition ('inside') and/or wet deposited N species ('outside') over the course of a 10 month experimental period.

7.3.1.1. Plant growth

All plants grown at the farm site increased in dry weight over the 10 month experimental period. Plants growing in the 'grazed outside' treatment typically looked healthier and grew more than plants subjected to other treatments following the 10 month experimental period (Figure 7.1).

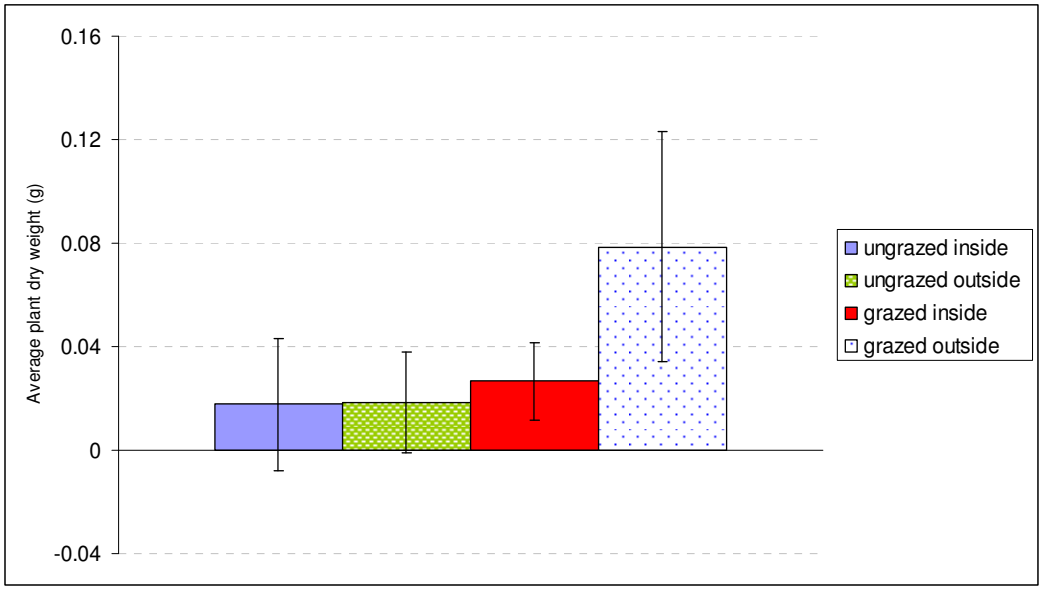


Figure 7.1. Average dry weight of whole plants following the 10 month treatment period. Error bars = sd. n = 10 plants per treatment.

Whole plant N concentration increased (Figure 7.2), with a corresponding decrease in seed N contribution (Figure 7.3) over the 10 month experimental period. The greatest increase in plant N concentration and lowest seed N contribution was most strongly illustrated in plants growing in the 'grazed'

paddocks, particularly those 'outside', exposed to additional wet deposited N sources. In contrast, plants growing in the low $NH_{3(g)}$ concentrations in the 'ungrazed' treatments produced the lowest plant N concentration and greatest seed N contribution to plants. This trend was particularly noticeable in plants growing 'inside', in the absence of wet deposited N species.

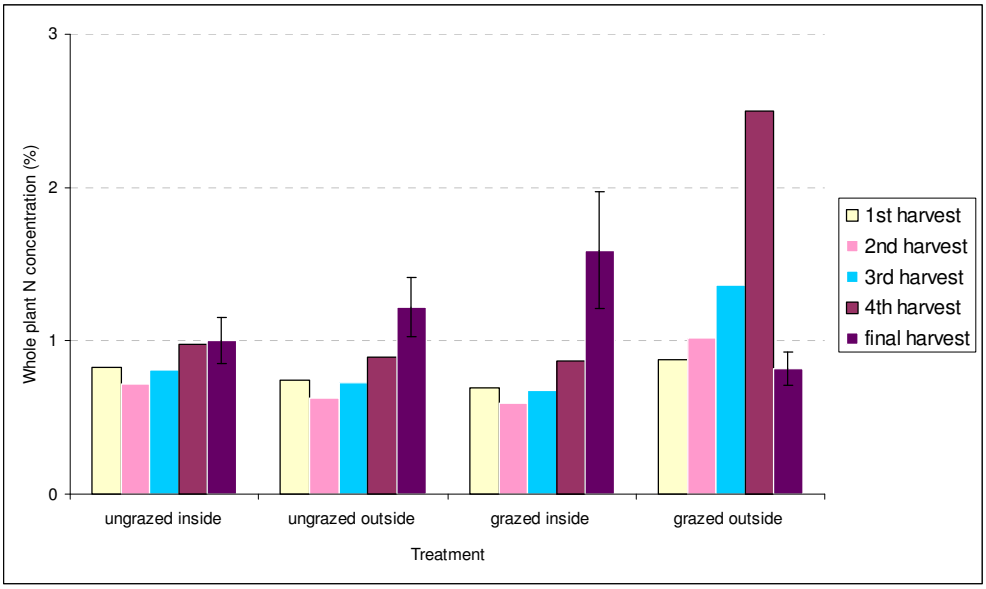


Figure 7.2. Whole plant N concentration (%) of treated plants over the 10 month experimental period. Samples were pooled for each treatment at each harvest (except 'final') because of small sample size. Single samples of each treatment represent a standard deviation for all pooled individuals. Error bars on 'final harvest' = sd. (n = ≥10)

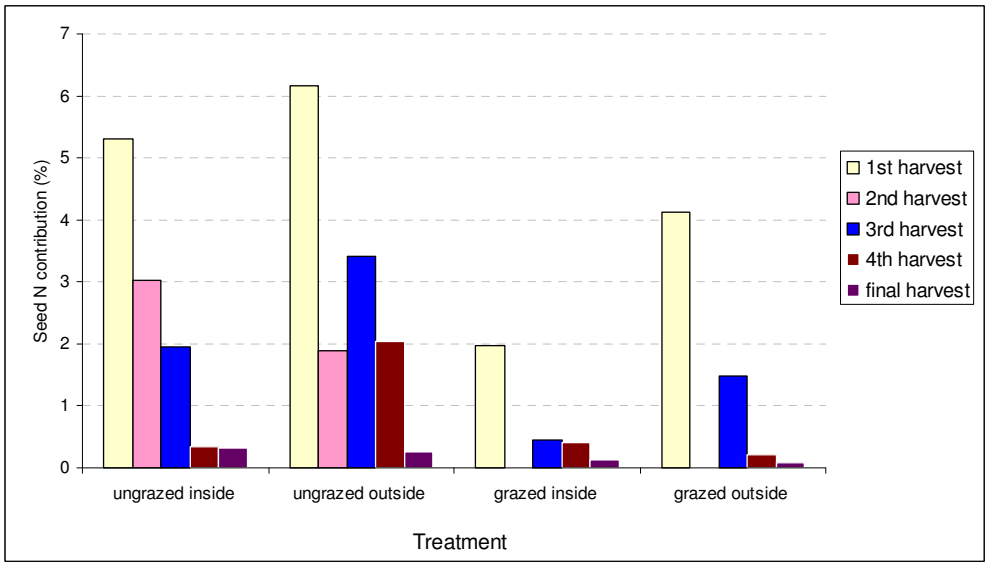


Figure 7.3. Seed N contribution to whole plant N concentration (%) of treated plants over the 10 month experimental period. Individual samples based on average plant and seed N concentration at each harvest. Single samples represent a combined sd for all individuals in each treatment at each harvest.

7.3.1.2. Plant $\delta^{15}N$

Whole plant $\delta^{15}N$ signatures progressively decreased over time resulting in all plant $\delta^{15}N$ signatures significantly more depleted than original seed N at the conclusion of the 10 month experimental period (Figure 7.4). The greatest difference in whole plant $\delta^{15}N$ compared to that of seed N was in 'grazed outside' plants, being as much as 10‰ more depleted than original seed N at final harvest. In contrast, the least difference in whole plant $\delta^{15}N$ compared to that of seed N was in 'ungrazed inside' plants, being only c. 4‰ more depleted than original seed N.

L. scoparium plants growing in the higher atmospheric $NH_{3(g)}$ concentration, 'grazed' treatment, were significantly more isotopically depleted compared to the equivalent plants growing in the comparatively lower atmospheric $NH_{3(g)}$ concentration, 'ungrazed' treatment.

Seed N $\delta^{15}N$ signature (+1.85‰) accounted for less than 0.5% of total plant N following the treatment period, therefore, contributing insignificantly to whole plant $\delta^{15}N$.

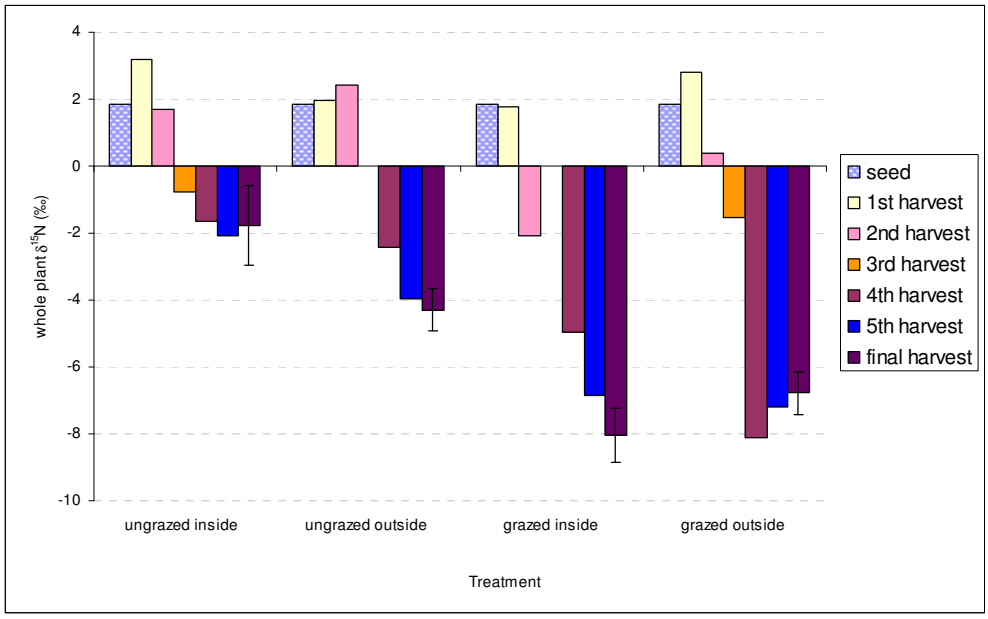


Figure 7.4. Whole plant $\delta^{15}N$ signature (‰) measured in treated plants over the 10 month experimental period. Samples were pooled for seed and each treatment at each harvest (except 'final') because of small sample size. Single samples of each treatment represent a standard deviation for all pooled individuals. Error bars on 'final harvest' = sd. (n = ≥10)

7.3.1.3. Wet deposited inorganic N concentration

Wet deposited NH_4-N concentrations were significantly higher (10 times) than those of NO_3-N across the experimental time period at the farm, averaging 0.40 and 0.04 mg N L^{-1}, respectively (Table 7.1 and Figure 7.5). Concentrations of each wet deposited N species were not significantly different between the two collection sites at the farm (illustrated in Figure 7.5).

Contribution of wet deposited inorganic N to plants growing 'outside' at each treatment location approximated c. 0.04 mg inorganic N $plant^{-1}$ over the 10 month experimental period (based on rainfall (data not shown), inorganic N species concentration tray area, and number of plants per tray). The proportion of this N utilised relative to the amount flushed through the substrate before plants were able to utilise it is, however, unknown.

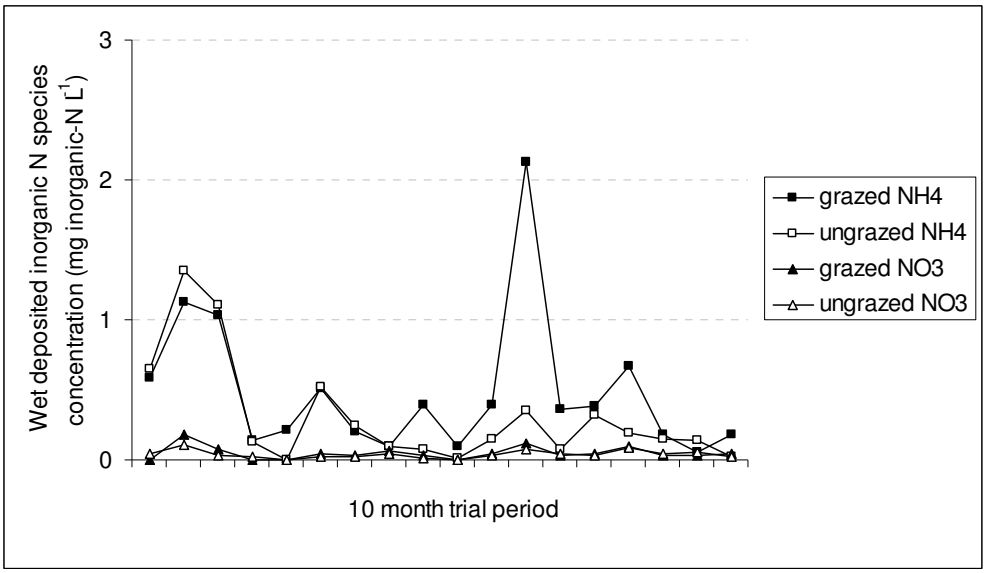


Figure 7.5. Concentrations of wet deposited NH_4-N and NO_3-N collected at 'grazed' and 'ungrazed' locations on the farm over the 10 month experimental period. Each point represents analysis of wet deposition collected over a two weekly interval in 'grazed' and 'ungrazed' paddocks.

Table 7.1. Average wet deposited inorganic N species concentrations over the 10 month experimental period. (n = 18).

	NH_4-N concentration (mg N L^{-1})		**NO_3-N concentration (mg N L^{-1})**	
	Grazed	**Ungrazed**	**Grazed**	**Ungrazed**
Average	**0.49**	**0.31**	**0.05**	**0.04**
sd	0.50	0.37	0.04	0.03

7.3.1.4. Wet deposited inorganic N $\delta^{15}N$ signature

Wet deposited inorganic N $\delta^{15}N$ signatures ranged widely between -14‰ and +11‰ over the treatment period, with the two extremes of this range measured in NH_4-N (Table 7.2 and Figure 7.6). Temporal variation in wet deposited inorganic N species was mirrored between the two collection points but variation was so high that there was no significant difference between wet deposited NH_4-N and NO_3-N $\delta^{15}N$ across the farm. It is noteworthy however, that both wet deposited N species were, on average, isotopically depleted (Table 7.2).

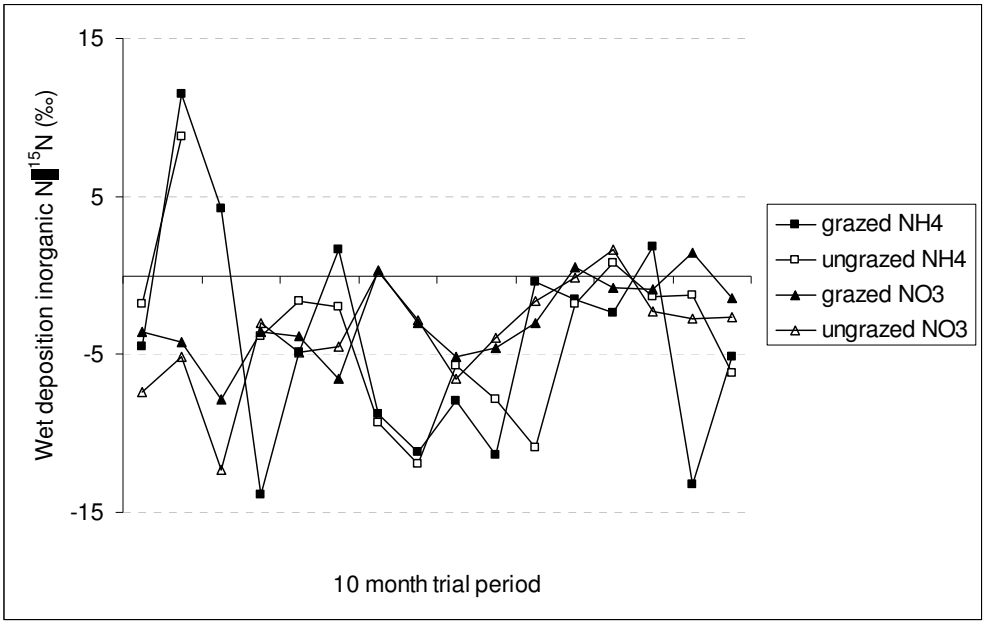


Figure 7.6. $\delta^{15}N$ signatures of wet deposited NH_4-N and NO_3-N collected at 'grazed' and 'ungrazed' locations on the farm over the 10 month experimental period. Each point represents analysis of wet deposition collected over a two weekly interval in 'grazed' and 'ungrazed' paddocks.

Table 7.2. Average wet deposited inorganic N species $\delta^{15}N$ over the 10 month experimental period (n = 16).

	NH_4-N $\delta^{15}N$ (‰)		NO_3-N $\delta^{15}N$ (‰)	
	Grazed	**Ungrazed**	**Grazed**	**Ungrazed**
Average	**-4.14**	**-3.72**	**-2.90**	**-3.64**
sd	6.95	5.21	2.59	3.32

7.3.1.5 Capillary matting recovered $NH_{3(g)}$

Oxalic acid capillary matting provided relative concentrations of atmospheric $NH_{3(g)}$ at the 'grazed' and 'ungrazed' treatment locations over the experimental period. Capillary matting recovered $NH_{3(g)}$ was significantly higher in the 'grazed' compared to the 'ungrazed' paddocks, over the experimental period, reaching a maximum of 100 mg $NH_{3(g)}$-N m^{-2} per mat in 'grazed', compared to 56 mg $NH_{3(g)}$-N m^{-2} per mat in 'ungrazed' treated paddocks (Figure 7.7).

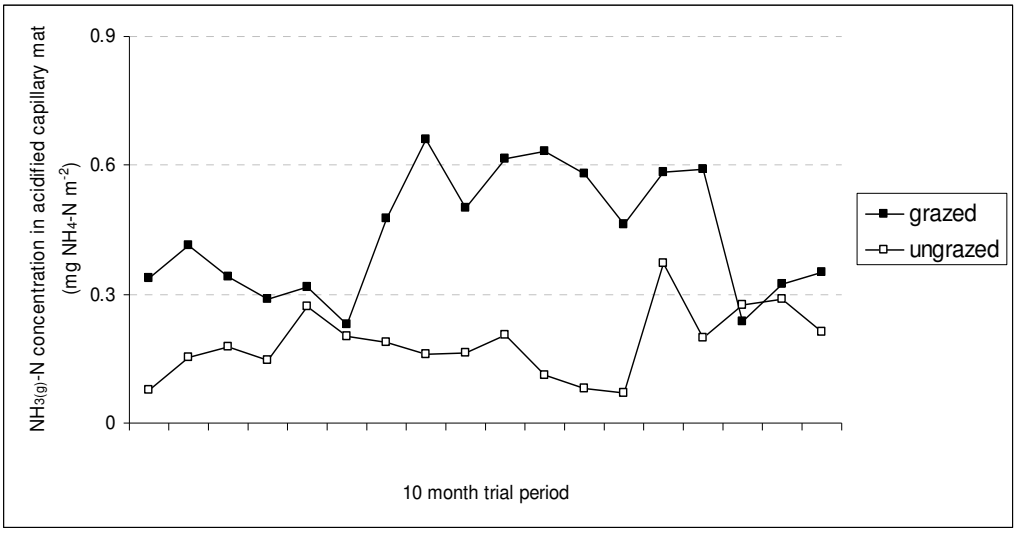


Figure 7.7. Concentration of $NH_{3(g)}$-N recovered from atmospherically exposed oxalic acid capillary matting over the experimental period. Each point represents exposure over a two weekly interval in 'grazed' and 'ungrazed' paddocks.

The $\delta^{15}N$ signature of capillary matting recovered $NH_{3(g)}$ was always highly isotopically depleted, with the most enriched $\delta^{15}N$ signature measured during summer (-9.64‰) and the most depleted during winter (-27.12‰). The level of isotopic depletion measured in capillary matting recovered $NH_{3(g)}$ was not significantly different between the 'grazed' and 'ungrazed' locations, even with the significantly different atmospheric $NH_{3(g)}$ concentrations measured between these two farm positions (Figure 7.8). Capillary matting recovered $NH_{3(g)}$ averaged -21‰ (±3.84‰; sd) over the experimental period.

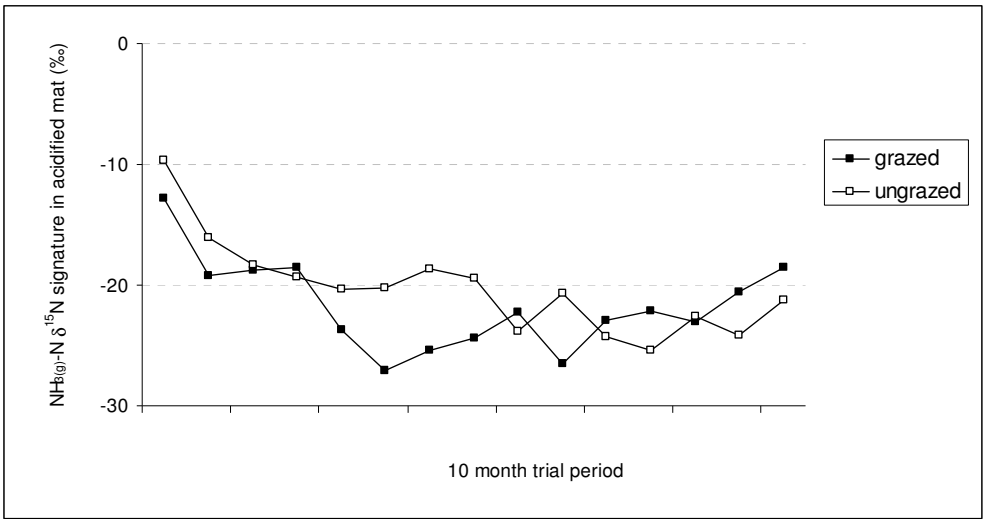


Figure 7.8. $\delta^{15}N$ signature of $NH_{3(g)}$-N recovered from atmospherically exposed oxalic acid capillary matting over the experimental period. Each point represents exposure over a two weekly interval in 'grazed' and 'ungrazed' paddocks.

7.3.2. $NH_{3(g)}$ fumigation trial

To directly assess the mechanism of atmospheric $NH_{3(g)}$ uptake and its contribution to the nutrition and $\delta^{15}N$ signature of plants, *L. scoparium* plants were grown in a range of nutrient solutions, and subjected to atmospheric $NH_{3(g)}$ concentrations above typical foliage $NH_{3(g)}$ compensation points.

7.3.2.1. *L. scoparium* growth response to root nutrient supply

All 'control chamber' *L. scoparium* plants responded visually to nutrient treatment over the 100 day experimental period. Complete-N nutrient plants were stunted, yellow, and expressed generally poor vigour. Complete-P nutrient plants, although larger than those in complete–N nutrient, had red/brown leaves, displayed a high root: shoot ratio and were not as healthy or vigorous as complete nutrient plants. Complete nutrient plants were, as expected, the largest and healthiest.

All *L. scoparium* plants increased in dry weight following exposure to all nutrient treatments over the 100 day experimental period. Plant dry weight increase mirrored that of visual health, following the nutrient treatment pattern: complete > complete-P > complete-N > initial plants (Table 7.3; 'control chamber' plants).

7.3.2.2. *L. scoparium* growth response between '$NH_{3(g)}$ fumigation' and 'control' chambers

L. scoparium plants growing in the '$NH_{3(g)}$ fumigation chamber' subjected to complete-N and complete-P nutrient treatments produced significantly greater dry weights than equivalent nutrient treated *L. scoparium* plants growing in the 'control chamber' (Table 7.3). Complete nutrient treated plants growing in the '$NH_{3(g)}$ fumigation chamber' were not significantly different to the equivalent nutrient treated plants growing in the 'control chamber' (Table 7.3).

Table 7.3. Average dry weight (g) of all *L. scoparium* whole plants growing in all nutrient treatments in the 'control' and '$NH_{3(g)}$ fumigation' chambers following 100 days of treatment. (sd = standard deviation)

		Complete-N		Complete-P		Complete	
	Initial plants	**'control chamber'**	**'NH_3 treatment chamber'**	**'control chamber'**	**'NH_3 treatment chamber'**	**'control chamber'**	**'NH_3 treatment chamber'**
Avg	**0.004**	**0.025**	**0.078**	**0.167**	**0.55**	**10.5**	**3.6**
sd	0.002	0.015	0.059	0.131	0.329	12.304	0.355
n	27	29	25	10	7	2	7

7.3.2.3. *L. scoparium* N concentration response to treatments

Whole plant N concentrations typically increased as nutrient supply increased following the same nutrient treatment pattern as increasing biomass: complete > complete-P > complete-N > initial plants (Figure 7.9). All nutrient treated plant N concentrations were significantly greater than that of initial plants except those exposed to complete-N nutrient in the 'control chamber'.

L. scoparium plants growing in the '$NH_{3(g)}$ fumigation chamber' had significantly higher whole plant N concentrations over the equivalent nutrient treated plants growing in the 'control chamber' at the completion of the 100 day trial period. Complete nutrient treated plants were the exception, which were not significantly different between the two chambers (Figure. 7.9).

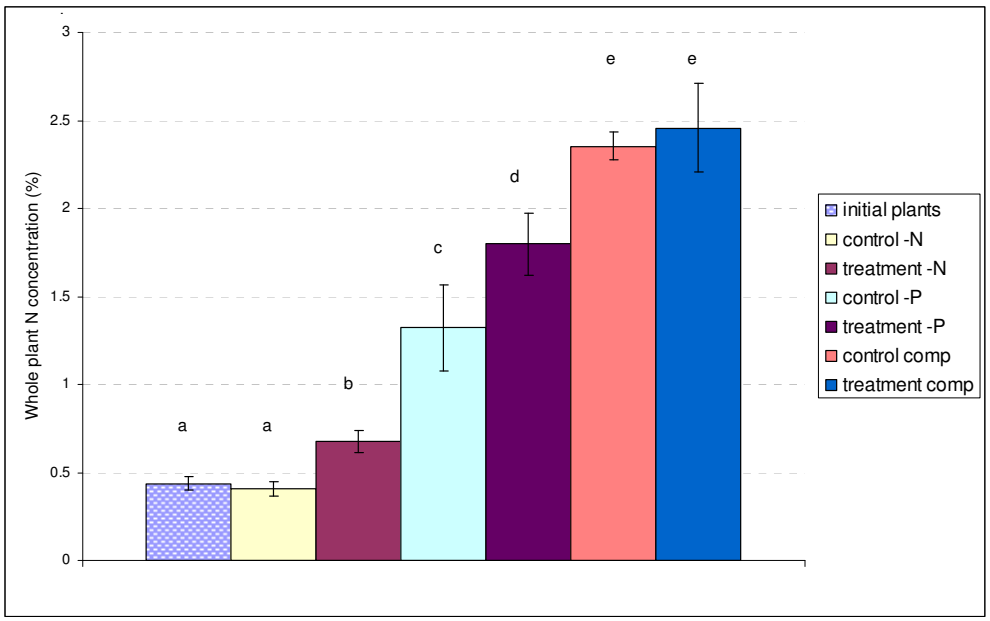


Figure 7.9. Whole plant N concentration (%) of all plants subjected to all nutrient treatments in both 'control' and '$NH_{3(g)}$ treatment chambers' compared to 'initial plants'. Error bars = sd. Different letters denote significant ($p < 0.05$) difference between treated plants (n = 10 plants for all treatments except 'control complete' where n = 2).

Biomass and N concentration increase in 'control chamber' plants following the 100 day trial period, was attributed to nutrient solution supplied N. Using equations 7.1 and 7.2, the average contribution of $NH_{3(g)}$-N to total plant N of '$NH_{3(g)}$ fumigation chamber' plants can be determined. $NH_{3(g)}$-N contributed an additional 17% and 22% to total plant N in complete-N and complete-P nutrient treated '$NH_{3(g)}$ fumigation chamber' plants, respectively.

7.3.2.4. *L. scoparium* $\delta^{15}N$ response to treatments

Whole plant $\delta^{15}N$ signatures of all *L. scoparium* plants ranged between -6.63‰ and +2.73‰, following the 100 day experimental period (Figure 7.10). 'Complete–N' nutrient treated plant $\delta^{15}N$ signatures, growing in both 'control' and '$NH_{3(g)}$ fumigation' chambers, are not significantly different from each other or to the $\delta^{15}N$ signature of the 'initial' plants. 'Complete–P' and 'complete' nutrient treated plants, growing in both 'control' and '$NH_{3(g)}$ fumigation' chambers, were significantly more isotopically depleted than the 'initial' plants by as much as 7.76‰ in the 'control chamber; complete-P' nutrient plants. 'Control chamber; complete' and 'control chamber; complete-P' nutrient treated plants were significantly more isotopically depleted than their equivalent nutrient treated plants in the '$NH_{3(g)}$ fumigation chamber'.

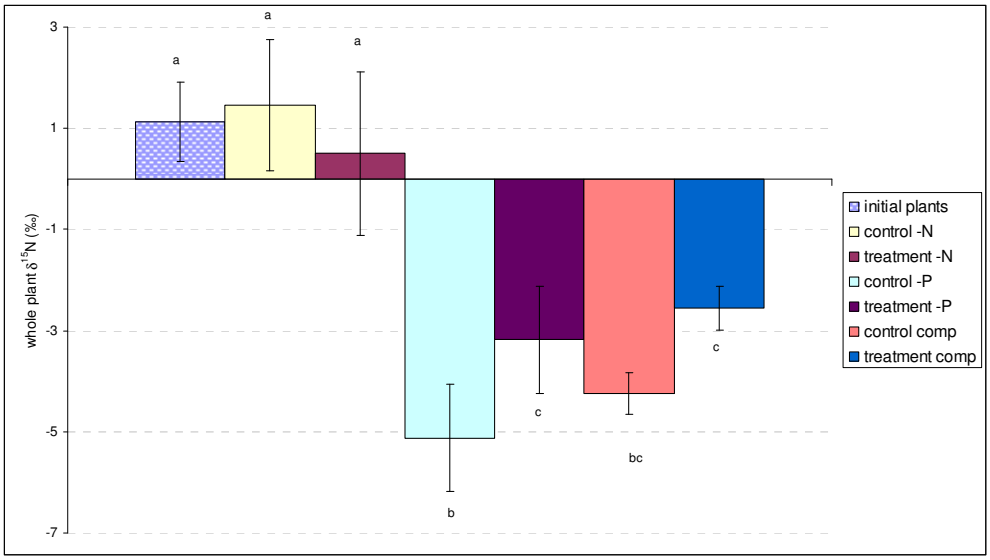


Figure 7.10. Whole plant $\delta^{15}N$ signature of all plants subjected to all nutrient treatments in both 'control' and '$NH_{3(g)}$ fumigation chambers' compared to 'initial' plants. Error bars = sd. Different letters denote significant ($p < 0.05$) difference between treated plants ($n = 10$ plants for all treatments except 'control complete' where $n = 2$).

Contribution of atmospheric $NH_{3(g)}$-N $\delta^{15}N$ signature to whole plant $\delta^{15}N$ (over initial plant N and nutrient N contribution) can be determined using equation 7.3 and the proportional contribution of atmospheric $NH_{3(g)}$ to the nutrition of '$NH_{3(g)}$ treated chamber' plants (section 7.3.2.3.). The fractionation attributed to $NH_{3(g)}$ uptake was calculated for '$NH_{3(g)}$ treated chamber' complete-N and complete-P nutrient treated plants as -5.14‰ and +5.68‰ respectively. Depletion in complete-N plants closely approximates the measured $\delta^{15}N$ signature of the $NH_{3(g)}$ added to the treatment chamber: -3.09‰ (±0.74‰). The enriched signature in the complete-P plants is difficult to account for, but is certainly not close to the level of depletion expected if fractionation accompanied $NH_{3(g)}$ uptake.

7.4. Discussion

Highly depleted foliar $\delta^{15}N$ signatures (< -8‰) have been measured in early succession higher plants (Chapter 6 and references within). The cause of this level of depletion is of interest to plant ecophysiologists, because it falls well outside the range of typical plant $\delta^{15}N$ signatures. Reliance on atmospheric N sources, particularly atmospheric $NH_{3(g)}$, has been proposed to account for the

level of depletion, and this is the first of such a study to directly examine the effect of this mechanism on plant $\delta^{15}N$ signatures.

In this study, both the farm and the $NH_{3(g)}$ fumigation trials confirm the ability of higher plants to utilise gaseous atmospheric N sources (most likely $NH_{3(g)}$) as a nutritional source. The contribution of approximately 20% to the total N of nutrient limited plants in the $NH_{3(g)}$ fumigation trial closely approximated the findings of increased plant N concentration (Pérez-soba and Van der Eerden 1993) and total plant N (Whitehead and Lockyer 1987; Janzen and Bruinsma 1989) in previously reported studies of plant N response following $NH_{3(g)}$ fumigation. The effect of atmospheric $NH_{3(g)}$ uptake on plant $\delta^{15}N$ is more difficult to determine.

The farm trial was designed to replicate plant growth conditions in early succession systems. Wet deposited N species are likely the most significant N source to these plants. Wet deposited N, although depleted, could not directly account for the levels of depletion measured in this trial, particularly to plants growing 'inside' without access to this N source. In the absence of conventional root available N, the only source remaining for plant utilisation in the farm trial was the variable, but high concentrations of atmospheric $NH_{3(g)}$. High concentrations were confirmed, particularly in the 'grazed' paddocks, from the levels of capillary matting recovered $NH_{3(g)}$. The increased growth of 'inside' plants, particularly in the 'grazed' treatment, correlates with the increased atmospheric $NH_{3(g)}$ concentrations. The highest level of depletion measured was in plants with the greatest growth, suggesting that isotope depletion accompanied utilisation of atmospheric $NH_{3(g)}$ as a nutritional source. The level of depletion was inversely proportional to the contribution of atmospheric $NH_{3(g)}$ to N nutrition.

An additional N source to all *L. scoparium* plants was the low inorganic N contaminant in the complete–N nutrient solution. All plants received 2 L of this solution each week, containing an inorganic N contamination of 2.31 $\mu g\ L^{-1}$, with a $\delta^{15}N$ signature of c. -3.5‰. This insignificant N source was unlikely to contribute significantly to plant nutrition, particularly as the majority of delivered nutrient solution passed directly through the highly permeable sand substrate and was lost from the system. In addition, the level of isotopic depletion measured in

the contaminant cannot account for the level of depletion measured in 'grazed inside' or 'outside' plants.

Atmospheric $NH_{3(g)}$ $\delta^{15}N$ signatures were never directly measured at the farm. Logistics of measuring $NH_{3(g)}$ $\delta^{15}N$ signatures is difficult (Chapter 5), and measured values are likely to be highly variable in space and time. Frank et al. (2004) illustrated the range of $\delta^{15}N$ signatures measured in $NH_{3(g)}$ following volatilisation from urine patches over time from -28‰ in day one (strikingly similar to that of capillary matting recovered $NH_{3(g)}$ in this study) to -0.3‰ in day 10 with concentrations of volatilised $NH_{3(g)}$ decreased over the same time period. It is likely that $NH_{3(g)}$ concentrations and $\delta^{15}N$ signatures of volatilised $NH_{3(g)}$ at the farm will mirror these trends.

Acidified capillary matting is a unique method of modelling an organism solely reliant on atmospheric $NH_{3(g)}$ as a nutritional source, with an infinitely low compensation point. The level of isotopic depletion measured in capillary matting recovered $NH_{3(g)}$ (c. -21‰) is the integration of the proposed fractionation effects associated with the volatilisation of $NH_{3(g)}$ to the atmosphere from terrestrial sources and its subsequent diffusive uptake into an infinite sink. However, the level of depletion attributed to each process cannot be differentiated by this method just as it cannot in plants. The significantly more depleted $\delta^{15}N$ and higher levels of $NH_{3(g)}$ assimilated in acidified capillary matting suggests it does not replicate higher plant $NH_{3(g)}$ uptake.

The question remains: are farm trial plants integrating the variable, but potentially highly depleted inherent $\delta^{15}N$ signature of atmospheric $NH_{3(g)}$ over time, or the $\delta^{15}N$ signature attributed to the double fractionating steps of $NH_{3(g)}$ volatilisation and subsequent diffusive uptake? This question was directly tested in the $NH_{3(g)}$ fumigation trial.

The $NH_{3(g)}$ fumigation trial is deficient in its absence of real replication. The $NH_{3(g)}$ fumigation treatment was applied only once. Even so, the results clearly show that atmospheric $NH_{3(g)}$ was utilised by N limited plants, contributing c. 20% to their N nutrition. It is in these plants that a fractionation attributed to the diffusive uptake of $NH_{3(g)}$ would be expected, however no significant isotopic

fractionation was measured in '$NH_{3(g)}$ fumigated' plants above that of 'control' plants. Contribution of atmospheric $NH_{3(g)}$-N $\delta^{15}N$ signature to whole plant $\delta^{15}N$ signature (above initial plant N and nutrient N contribution) was determined at -5.14‰ and +5.68‰ for N and P stressed plants respectively. In complete-N plants, where $NH_{3(g)}$ uptake, and its associated fractionation is likely to be most strongly observed (and not complicated by root supplied N sources) measured fractionation closely approximates the $\delta^{15}N$ of treatment chamber supplied $NH_{3(g)}$: -3.09‰, certainly not close to a likely fractionation attributed to diffusive uptake. This strongly suggests that diffusive uptake of atmospheric $NH_{3(g)}$ does not impart a significant fractionation in plants above the inherent $\delta^{15}N$ of atmospheric $NH_{3(g)}$. The $\delta^{15}N$ signature of farm plants growing 'inside' are therefore likely an integration of the highly variable $\delta^{15}N$ signature of volatilised $NH_{3(g)}$ alone.

A significant level of depletion was measured in all plants except those treated with complete-N. 'Complete-P and 'complete' plants growing in both 'control' and '$NH_{3(g)}$ fumigation' chambers had significant levels of depletion. The level of depletion is not therefore directly related to $NH_{3(g)}$ fumigation, but instead to the partial utilisation of N supplied in the hydroponic solution. Weekly replacement of the nutrient solution resulted in the development of an isotopically 'closed' system, where the partial utilisation of available N was reflected in the $\delta^{15}N$ signature of the receiving plant, according to Rayleigh isotopic principles (Yoneyama et al. 1991).

The results presented here agree with those of Herrmann et al. (2002) and Mattsson and Schjoerring (2002), illustrating that the N demand of plants and foliar compensation points are linked, suggesting plants growing in early succession communities may utilise this N source even when present in low concentrations. Use of this N source will impart a $\delta^{15}N$ signature to plants; that of the inherent signature of atmospheric $NH_{3(g)}$, but not a further fractionation attributed to its diffusive uptake.

Atmospheric $NH_{3(g)}$ $\delta^{15}N$ has been measured as depleted as -28‰ but only in close proximity to abundant $NH_{3(g)}$ sources, e.g. urine patches and penguin guano (Erskine et al. 1998; Frank et al. 2004). Preliminary measurements of

atmospheric $NH_{3(g)}$ $\delta^{15}N$ signatures in early primary succession sites confirms atmospheric $NH_{3(g)}$ $\delta^{15}N$ signatures to be depleted, but not to the level required to account for the depletion in plants (-23‰; Chapter 6). An alternative mechanism is therefore likely to be acting within these systems to account for the level of depletion measured. The depleted signature imparted to plants as a consequence of the partial uptake of supplied N in the $NH_{3(g)}$ fumigation trial may provide a clue.

Partial uptake of a N source in a closed system, according to Rayleigh fractionation principles, has been well established as a strongly fractionating process in plants (Yoneyama et al. 1991). Although 'closed' systems like those of the fumigation trial are unlikely to exist in natural settings, N source uptake limitation by alternative growth limiting factors may approximate an isotopically 'closed' system, resulting in fractionation as a consequence of partial uptake. Partial uptake provides an alternative and testable mechanism, theoretically capable of producing highly depleted foliar $\delta^{15}N$ signatures in terrestrial plants.

Chapter 8

Plant $\delta^{15}N$ response to nutrient stress and the partial uptake of a supplied N source

8.1. Introduction

Plant $\delta^{15}N$ signatures have long been of interest to researchers in the study of plant nitrogen (N) source and acquisition dynamics. Shifts in ^{15}N ratios of plant foliage relative to N sources have been attributed to isotopic fractionations associated with N uptake and subsequent intra plant processes. Each process has its own ^{15}N ratio fractionation potential, dependent on Rayleigh distillation principles, and is often highly variable over time. Under typical field conditions plant $\delta^{15}N$ signatures range between -8 to +10‰ (Bedard-Haughn et al. 2003), with the generally held view that this variation is a consequence of N source $\delta^{15}N$ signatures and fractionation associated with uptake and internal metabolism mechanisms (Evans 2001). Plant $\delta^{15}N$ signatures falling outside this range arouse particular interest to eco-physiologists.

Foliar $\delta^{15}N$ signatures more depleted than -8‰ are uncommon but have been measured in a range of systems including high latitude, equatorial, alpine, geothermal and bog systems (Chapter 6 and references within). Depleted $\delta^{15}N$ signatures have been measured in a wide range of plant growth forms and taxonomic groups represented within these systems and in epiphytes and lithophytes (Chapter 5 and references within). When isotopic depletions of this magnitude fall outside the typical range of plant and soil signatures and naturally occurring plant isotopic fractionating mechanisms, one may wonder what are the mechanisms responsible for this level of isotopic depletion?

Multiple mechanisms have been attributed by various authors to account for the degree of isotopic depletion measured in these studies. The direct utilisation of

highly isotopically depleted N sources has been proposed to account for the level of $\delta^{15}N$ measured. However, in many cases, these depleted N sources have only been supposed, and often where measured, do not account for the level of depletion exhibited in the measured plants (Chapters 6 and 7). Alternatively N uptake and metabolism processes, often resulting in widely variable $\delta^{15}N$ fractionations, are suggested to account for the level of isotopic depletion.

Partial uptake of a N source is among the greatest potential drivers of isotopically depleted $\delta^{15}N$ signatures in plants. Plants, when grown in isotopically closed systems, express isotopic signatures as a consequence of partial uptake based on Rayleigh fractionating principles (Yoneyama et al. 2001). Fractionation attributed to this phenomenon is most strongly measured in the product (plant) when only a small proportion of the reactants (nutrient source) is utilised. Numerous studies have illustrated the fractionation attributed to this mechanism in various organisms, although most do not recognise the mechanism acting (e.g. Emmerton et al. 2001a; 2001b). Isotopic depletions, as low as -28.9‰ in the foliage (Yoneyama et al. 2001), may be attributed to this fractionating mechanism. As plants take up higher proportions of the source N, irrespective of N concentration, N form, and uptake mechanism, whole plant $\delta^{15}N$ begins to reflect the $\delta^{15}N$ of the N source (Yoneyama et al. 1991; 2001; Evans et al. 1996).

Phosphorus (P) stress has been associated with depletions in foliar $\delta^{15}N$ signatures. Isotopic depletions of -8‰ in Mangrove species (McKee et al. 2002) and -15.55‰ in bog species (Clarkson et al. 2005) were found to be correlated with P limitation to plant growth. It is generally accepted that P limitation does not directly result in isotopic depletion. However, the depletion may be a consequence of partial uptake of the comparatively high N source concentration driven by low P concentrations. The strength of the fractionation associated with partial uptake is dependent on the proportion of the available N utilised, with the greatest depletion likely associated with minimal uptake, as seen above.

Plant taxon specific N dynamic processes have been suggested to influence foliar $\delta^{15}N$ signatures in natural ecosystems (Handley and Scrimgeour 1997). Differing N acquisition strategies, root morphology, mycorrhizal association, niche partitioning, N use efficiencies, longevity and overall life history will contribute to

isotopic variation in plants and may result in foliar $\delta^{15}N$ signature differences between co-habiting taxa. Generally, these factors are overlooked by most researchers in preference for the most studied plant taxonomic characteristic: mycorrhizal association.

The role of mycorrhiza in nutrient uptake by plants is well documented (Smith and Read 1997). The fractionation attributed to mycorrhizal association and its effect on plant $\delta^{15}N$ signature, however, has been long debated. A number of authors have suggested mycorrhizal association to account, at least in part, for the highly depleted $\delta^{15}N$ of early primary succession plants (e.g. Hobbie et al. 2005). Significant and consistent isotopic depletion measured in ericaceous and ecto-mycorrhizal associated species, relative to more isotopically enriched endo- and non-mycorrhizal species in single systems, has been proposed as a consequence of their fungal partners (Nadelhoffer et al 1996; Michelsen et al. 1996; 1998). Repeatedly measured ^{15}N enriched mycorrhizal fungal components compared to available soil N pools and associated host plant material, have added weight to this theory. Fractionation and subsequent transfer of the lighter isotope to host plants during fungal metabolism is the proposed mechanism to account for depleted host plant $\delta^{15}N$ signatures (Högberg et al 1999; Hobbie et al. 1998; 1999; 2000; 2005).

Internal metabolism may contribute to isotopic depletion in plants (Yoneyama 1995). Internal N reallocation is a theoretically plausible mechanism to account for intra plant $\delta^{15}N$ variation and an overall, whole plant, isotopic depletion. Fractionation as a consequence of NO_3 reduction, glutamine synthetase (GS)-glutamate synthase (GOGAT) activity, transaminations, and other enzymatic reactions carried out during the process of N remobilisation and recycling are potentially isotopic fractionating, generating products with lower $\delta^{15}N$ values than the reactant pool (Yoneyama 1995; Kolb and Evans 2002). Fractionation as a consequence of these processes has been attributed to account for $\delta^{15}N$ variation between different plant parts and foliage of co-occurring species in laboratory and field studies. In most situations, the difference in $\delta^{15}N$ between roots and leaves is less than 3‰ in deciduous forest and tall grass prairie systems, but can be as great as 7‰ in warm and cold desert ecosystems (Evans 2001). Organ loss, namely older leaves containing ^{15}N enriched N, may at least partially account for an

overall whole plant isotopic depletion measured in the plant material over time (Evans 2001).

Nutrient deficiency (particularly N) is a commonality in all ecosystems where highly depleted foliar $\delta^{15}N$ signatures have been measured (Chapter 6 and references within). Plant N deficiency, although not likely to be the primary driving factor of plant $\delta^{15}N$ depletion itself, may be a prerequisite under which a number of potentially fractionating mechanisms mentioned above may act more strongly. Increased levels of depletion associated with earlier chronosequences (and lower nutrient availability) have been suggested to be driven by a stronger reliance on mycorrhizal association, and the proposed fractionation attributed to its dependence (Hobbie et al. 1998; 1999; 2000; 2005). Internal N reallocation, and its associated fractionation, is likely to be maximised under increasing N stress (Kolb and Evans 2002), as with any fractionation attributed to taxon specific N acquisition strategies (Handley and Scrimgeour 1997). N deficiency may not, however, be the strongest limiting growth factor for plants in early succession communities. Temperature, moisture, pH, and phosphorous availability are also limiting growth in these environments, in addition to the short root exposure time of wet deposited N species as a consequence of high porous substrates. Combined, these factors may result in only a small proportion of wet deposited N utilised by early succession plants, a mechanism which may account for the level of depletion measured in these plants.

The aim of this study was to further investigate the fundamental mechanisms behind N fractionation in plants, in particular, identify mechanisms responsible for a highly depleted $\delta^{15}N$ signature measured in early primary succession plants (Chapter 6). The role of nutrient limitation as a prerequisite for the expression of highly depleted signatures was examined. Partial uptake of a replicated wet deposited N source was also examined as a mechanism to account for the level of depletion measured in a range of nutrient stressed plants. Partial uptake of a supplied N source and the fractionation associated with this mechanism was assessed in conjunction with the additional effects of: 1) nutrient availability, 2) plant species N acquisition strategies, 3) mycorrhizal association, and 4) N reallocation within plants.

8.2. Methods

8.2.1. Long term glasshouse trial

A long term glasshouse experiment was designed to replicate nutrient availability similar to that provided along a primary succession chronosequence. The effects of chronic N and P limitation on foliar N concentration and $\delta^{15}N$ signature were assessed in a range of plant growth form and mycorrhizal associated species. In addition, the effect of N remobilisation and recycling on foliar $\delta^{15}N$ trends was assessed.

A range of soil and sand mixtures were used to simulate a continuum of increasing nutrient availability. Medium grade sand was mixed (v:v) with unsterilised Hamilton sandy loam soil to achieve the following soil percentages: 100, 50, 25, 12, 6, 3, 1.5, 0.75 and 0%. The soil:sand mixtures were placed in free draining plastic pots (165 x 165 x 190mm) and positioned on drip racks to provide a root/air gap and prevent uptake of leachate from neighbouring pots.

Seven species of plant, representing a range of taxonomic groups were grown in the soil:sand substrates over the two year period May 2002 till May 2004 (Table 8.1). Experimental plants were selected primarily on the basis of mycorrhizal association and secondly on accessibility and ease of growth. Many of the plants chosen have been observed with highly isotopically depleted $\delta^{15}N$ signatures in natural ecosystems (Chapter 6; Clarkson et al. 2005). *Leptospermum scoparium*, *Knightia excelsa*, *Coprosma robusta*, and *Pinus radiata* plants were grown from seed, and germinated directly in their respective sand:soil mixture pot. Seeds were sown at a rate to provide at least 10 plants per pot, from which plants were thinned to five and finally two, at two and six months old, respectively. Seedlings (less than 0.5 g dry weight) of *Sporadanthus ferrugineus* were collected from Torehape peat bog, Hauraki plains, New Zealand. Seedlings of *Empodisma minus* and *Calluna vulgaris* were collected from Erua peat bog, neighbouring Tongariro National Park. All seedlings were thoroughly washed of rooting substrate, and transplanted into each sand:soil mixture pot directly at a density of five plants per pot. Plants were later thinned to two per pot at six months old. Representative seeds and/or seedlings were dried, weighed and determined for N content and $\delta^{15}N$ signature.

Table 8.1. Plant species, their taxonomic groups, mycorrhizal associations and seed/seedling characteristics as used in the 'long term glasshouse trial'. Each figure represents at least 5 individuals pooled for one analysis. Each sample represents the mean and sd for the individual plant species.

Plant species	Plant taxonomic group	Mycorrhizal association	Average weight of seed/seedling (g)	Average seed/seedling N concentration (%)	Average seed/seedling $\delta^{15}N$ (‰)
Leptospermum scoparium	Angiosperm tree	Endo*	9.1^{-5}(200)	0.24	0.75
Knightia excelsa	Angiosperm tree	Non	0.02(72)	3.92	2.28
Coprosma robusta	Angiosperm shrub	Endo	4.8^{-3}(100)	0.87	2.28
Empodisma minus	Monocot restiad	Non	0.38(26)	0.67	0.66
Sporadanthus ferrugineus	Monocot restiad	Non	0.15(19)	1.5	-1.66
Pinus radiata	Gymnosperm tree	Ecto	0.03(50)	4.55	2.19
Calluna vulgaris	Angiosperm shrub	Ericoid	0.04(9)	1.62	-3.81

* = *L. Scoparium* may form dual ecto- and endo- mycorrhizal associations, but only in the presence of dominating ecto species where continuous inoculation is possible (Moyersoen and Fitter 1999). In the present context, these species are considered endo-mycorrhizal associates.

Mycorrhizal inoculum was provided to the plants following seed germination or seedling establishment in the form of a root slurry from healthy, field growing host plants. Experimental plants were grown in a glasshouse within their respective sand:soil mixture substrates under natural sunlight conditions. Plants were provided only deionised water (dH_20) as required over the two year period. Periodic pesticide applications were carried out to prevent insect pest outbreaks. Pots were weeded, and dead leaves were removed as required.

Plant nutrient stress was controlled by the provision of nutrient solution to all experimental plants. All plants received 50 ml pot^{-1} $week^{-1}$ of N deficient nutrient solution (section 8.2.4) to ensure limiting N. In addition, a subset of species: *L. scoparium*, *K. excelsa* and *P. radiata*, were prepared and treated as above, but provided with 50 ml pot^{-1} $week^{-1}$ of N & P deficient nutrient solution (section 8.2.4) to test the additional variable of P limitation.

Harvest of the first formed ('young') and pre-abscission ('old') leaves was carried out on the healthiest of the plants representing each species within each treatment

pot at the conclusion of the two year experimental period. Plant material was oven dried at 60ºC, ball milled and analysed for N content (%) and $\delta^{15}N$ signature (‰).

8.2.2. Partial N uptake trial

A laboratory trial was set up to simulate higher plant growth conditions in early primary succession communities. This trial was designed to examine the effect of partial uptake of a supplied N source on whole plant $\delta^{15}N$ signatures, under a range of nutrient limited regimes in early succession substrates. The highly porous nature of early primary succession substrates was replicated by an acid washed sand/pearlite mix (1:1v/v). A sub-set of the plant species used in the glasshouse trial: *L. scoparium*, *K. excelsa* and *P. radiata* were germinated from seed directly in pots (80 mm diameter x 80 mm deep) containing the sand:pearlite substrate and provided only dH_20.

Following germination and establishment, seedlings were thinned to no more than five (*L. scoparium*) or two individuals (*K. excelsa* and *P. radiata*) per pot, provided mycorrhizal innoculum (as above), and dH_20 as required. When plants were 1 month old, they were subjected to a nutrient regime, as described in Table 8.2, over the 4 month period of August 2005 to December 2005.

Plants were grown under Na-vapour lamps providing no less than 500 μmole m^{-2} s^{-1} within a 16/8 hour light/dark period. Pots were positioned on drip racks to provide a root/air gap preventing uptake of leachate from neighbouring pots. All treatment solutions except 'Complete' were applied at a rate of no more than 0.21 ml min^{-1} pot^{-1}, a rate exceeding evapo-transpiration. 'Complete' nutrient treatment solution was applied at a flow rate of no less than 0.5 ml min^{-1} pot^{-1} to ensure excess nutrient solution supply to the roots, a rate greatly exceeding evapo-transpiration. Each plant and treatment were replicated three times.

Following the four month treatment period, plants were harvested, carefully washed to remove all traces of substrate from the root material, assessed for mycorrhizal association, dried, weighed and whole plants assessed for %N and $\delta^{15}N$ signatures.

Table 8.2. Nutrient regime experienced by laboratory nutrient trial plants over the four month experimental period (inorganic N applied during light hours). * = minimum inorganic N applied over the experimental period.

Treatment solution	Description in text	Description	Rate of application (ml min^{-1})	Maximum inorganic N applied over experimental period (mg inorganic N pot^{-1})
De-ionised water	dH_20	Deionised water	≤ 0.21	0
Complete-P (full strength)	-P (full)	Complete nutrient solution containing P at 2.8 µg PO_4-P L^{-1}	≤ 0.21	1377.33
Complete-P (diluted to 25% of full strength)	-P (25%)	Complete nutrient solution containing P at 0.67 µg PO_4-P L^{-1}	≤ 0.21	344.33
Complete-N (full strength)	-N (full)	Complete nutrient solution containing N at 4.62 µg inorganic N L^{-1}	≤ 0.21	0.11
Complete-N (diluted to 50% of full strength)	-N (50%)	Complete nutrient solution containing N at 2.31 µg inorganic N L^{-1}	≤ 0.21	0.06
Complete nutrient (full strength)	Complete	Complete nutrient solution containing N and P at 121 mg inorganic N L^{-1} and 62 mg PO_4-P L^{-1} respectively	≥ 0.50	6558.72*

8.2.3. Mycorrhizal assessment

Mycorrhizal association was carried out on roots of non harvested plants from each treated pot based on the acid fuchsin staining method described in Kormanik and McGraw (1982). Following staining, five 10 mm root sections per plant (including non-mycorrhizal species) were mounted on microscope slides. Five fields of view (20 x magnification) along each of the five root sections were assessed for ecto-mycorrhizal association, scored 0 – 4 on the basis of:

0 = no visible hyphae or physical mycorrhizal root morphology (no infection)

1 = visible physical mycorrhizal root morphology

2 = visible physical mycorrhizal root morphology + external hyphae

3 = visible physical mycorrhizal root morphology + hyphae penetrating root apoplast

4 = clearly visible Hartig net

Roots were also assessed for endo-mycorrhizal association, scored 0 – 4 on the basis of:

0 = no hyphae (no infection)

1 = 1 to 2 points of hyphae penetrating root apoplast

2 = 3 – 5 points of hyphae penetrating root apoplast

3 = hyphae penetrating root apoplast + 1 vesicle or arbuscule

4 = hyphae penetrating root apoplast + > 1 vesicle and/or arbuscule

8.2.4. Nutrient solutions

Complete

'Complete' nutrient solution contained (mM): KNO_3 (2), $Ca(NO_3)_2 \cdot 4H_2O$ (4), $NH_4H_2PO_4$ (2), $MgSO_4 \cdot 7H_2O$ (2), $NaEDTA \cdot 2H_2O$ (0.05), $FeCl_3$ (0.05), H_3BO_3 (0.01), $CuSO_4 \cdot 5H_2O$ (0.005), $MnSO_4 \cdot 1H_2O$ (0.005), $ZnSO_4 \cdot 7H_2O$ (0.0005), and $Na_2MoO_4 \cdot 2H_2O$ (0.00005).

Phosphorus deficient nutrient solution at full strength: '- P (full)'

'-P (full)' nutrient solution contained (mM): KNO_3 (1), $Ca(NO_3)_2 \cdot 4H_2O$ (2), NH_4Cl (1), $MgSO_4 \cdot 7H_2O$ (1), $NaEDTA \cdot 2H_2O$ (0.025), $FeCl_3$ (0.025), H_3BO_3 (0.005), $CuSO_4 \cdot 5H_2O$ (0.0025), $MnSO_4 \cdot 1H_2O$ (0.0025), $ZnSO_4 \cdot 7H_2O$ (0.00025), and $Na_2MoO_4 \cdot 2H_2O$ (0.000025).

Phosphorus deficient nutrient solution at half strength: '- P (25%)'

'-P (25%)' solution was obtained by a two fold dilution of '-P (full)' with dH_2O.

Inorganic P contamination (PO_4-P), although not determined, was calculated based on known P contamination concentrations in chemical stock, calculated at 2.8 and 0.67 µg PO_4-P L^{-1} for '-P (full)' and '-P (25%)', respectively.

Average $\delta^{15}N$ signatures of NO_3-N and NH_4-N in 'complete', '-P (full)' and '-P (25%)' nutrient solutions were determined as +0.70‰ (±0.07‰sd n = 3) and +0.50‰ (±0.01‰sd n=3), respectively.

Nitrogen deficient nutrient solution at full strength: '- N (full)'

'-N (full)' nutrient solution contained (mM): K_2HPO_4 (4), $CaSO_4 \cdot 0.5H_2O$ (2), K_2SO_4 (2), $MgSO_4 \cdot 7H_2O$ (2), $NaEDTA \cdot 2H_2O$ (0.05), $FeCl_3$ (0.05), H_3BO_3 (0.01), $CuSO_4 \cdot 5H_2O$ (0.005), $MnSO_4 \cdot 1H_2O$ (0.005), $ZnSO_4 \cdot 7H_2O$ (0.0005), and $Na_2MoO_4 \cdot 2H_2O$ (0.00005).

Nitrogen deficient nutrient solution at half strength: '- N (50%)'

'-N (50%)' solution was obtained by 50% dilution of the '–N (full)' solution with dH_2O.

Inorganic N contamination in the '-N (full)' and '-N (50%)' nutrient solution averaged 4.62 and 2.31 µg inorganic N L^{-1} respectively (n = 6) with c. 80% of this in NH_4-N form. $\delta^{15}N$ signatures of NO_3-N and NH_4-N contamination in complete N nutrient solution were determined as -4.46‰ (±1.46‰sd n = 6) and -3.04‰ (±3.98‰sd n=6), respectively.

All nutrient solutions were maintained at pH 5.5.

dH_2O

dH_2O was obtained by passing glass distilled tap water through Dowex 50WX4 100-200 mesh (H^+ form) and 1X8 100-200 mesh (C^- form) ion-exchange resins.

Inorganic N contamination in the resin extracted dH_2O was beyond detection limits.

8.2.5. Statistical analysis

Examining absolute differences in $\delta^{15}N$ signatures was not the aim of this investigation, but rather to determine trends in $\delta^{15}N$ in plants over experimental gradients. Because of this and high cost of analysis, replication and statistical analysis was not a strong focus of this investigation.

8.3. Results

8.3.1. Long term glasshouse nutrient trial

A range of plant species were subjected to a N and/or N & P nutrient stress continuum for two years to assess foliar $\delta^{15}N$ signature response in plants under chronic nutrient stress.

8.3.1.1. Growth response in N treated plants

All experimental plants grew over the two year trial period, but exhibited reduced visual growth response (reduction in height, yellowing leaves, stunted form and poor vigour) with increased simulated nutrient stress, illustrated in Figure 8.1.

A dramatic reduction in size and general health were observed in a number of the experimental plant species at the 100% soil substrate treatment (not shown in Figure 8.1). Soil compaction, nutrient exhaustion, poor water retention, and resource competition negatively affected growth of plants in these treatments. As a result, they were highly susceptible to pathogen attack compared to all other treatments and their visual appearance more closely reflected that of the lower soil percentage treatments. Because these plants were subjected to stress outside the scope of the subjected treatments, these plants were dropped from the final experimental analysis.

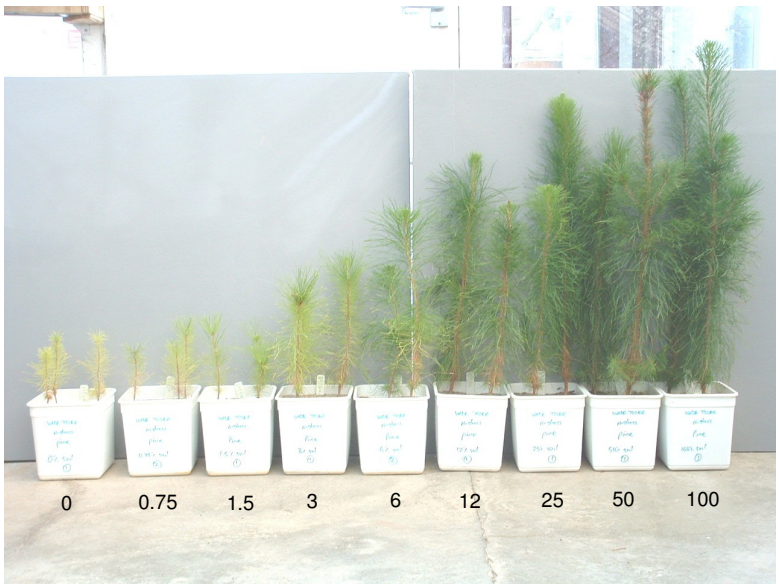


Figure 8.1 Illustration of the visual growth response of *P. radiata* following two years of a N stress continuum. Numbers at bottom refer to the substrate soil percentages.

8.3.1.2. Foliar N concentration in N treated plants

P. radiata had the widest range of foliar N concentrations, between 0.33% at 0% substrate soil to 1.35% at 50% substrate soil. All other plant species foliar N concentrations fell within this range.

Foliar N concentration of all experimental plants increased proportionally with increasing substrate soil concentration following the two year experimental period. Average foliar N concentrations for all plants growing in the 50% soil were almost 50% higher than the average foliage N concentrations for all plants growing in 0% soil (Figure 8.2).

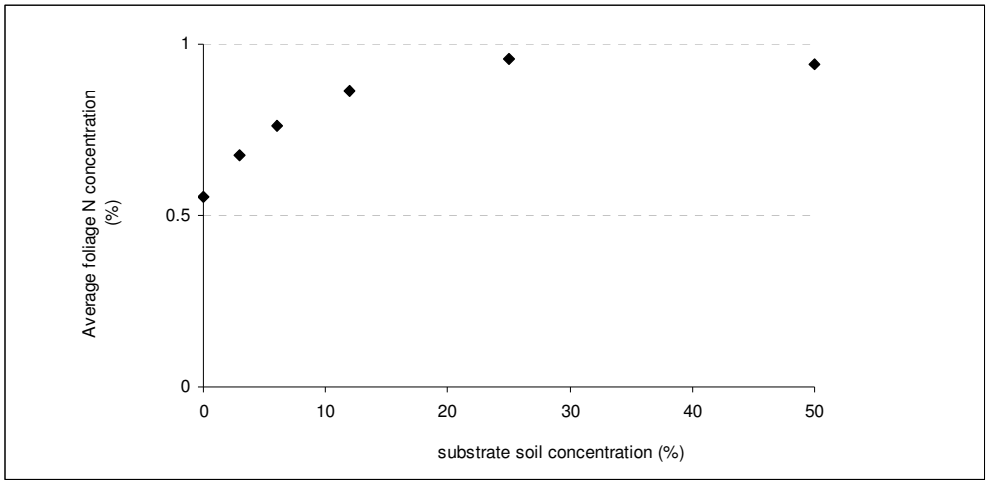


Figure 8.2. Average foliar N concentration of all plants at each substrate soil concentration following two years of growth. Each point represents the average of all plant species (n = 7) at each treatment. Although the sd is large (not shown) the increasing trend with substrate soil concentration is still apparent.

Foliar N concentration response by individual species to increased substrate soil concentration varied (Figure 8.3). The gymnosperm tree *P. radiata* foliar N concentration, for example, increased from 0.33% N at 0% soil to 1.35% N at 50% soil, producing a range of over 1% foliar N concentration. In contrast, the angiosperm shrub *C. vulgaris* foliage N remained essentially unchanged at approximately 0.9%, independent of substrate soil concentration.

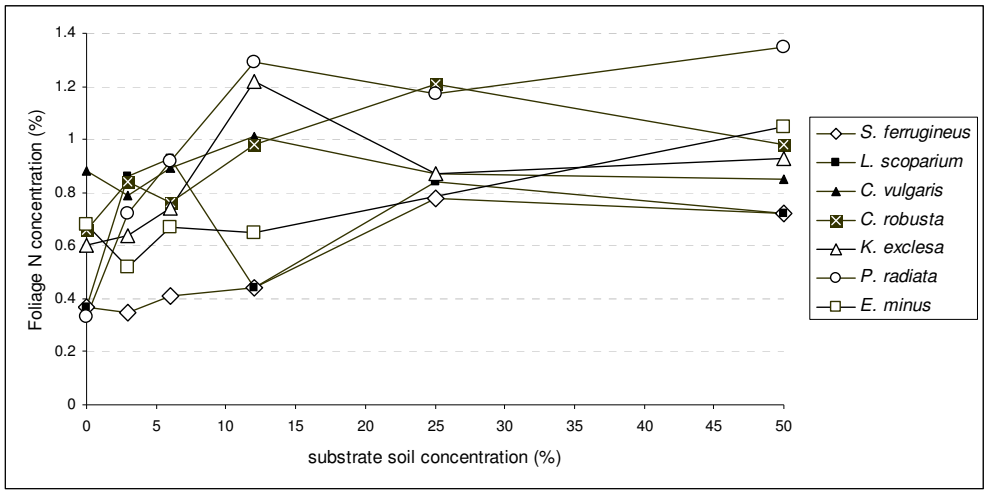


Figure 8.3. Foliar N concentration of individual species in response to increasing substrate soil concentration following two years of growth (each point = sample)

8.3.1.3. Foliar $\delta^{15}N$ signature in N treated plants

Average foliar $\delta^{15}N$ signatures of all species increased when grown in increasing substrate soil concentrations over two years, range between -3.94‰ in *L. scoparium* to +5.47‰ in *E. minus*, a range of almost 9.5‰. Average foliage $\delta^{15}N$ signature of all experimental species was strongly related to substrate soil concentration, becoming progressively enriched with increasing soil concentration (Figure 8.4). A 3.5‰ difference in the average of all plant foliar $\delta^{15}N$ signatures was measured between the 50% and 0% soil substrate treatments.

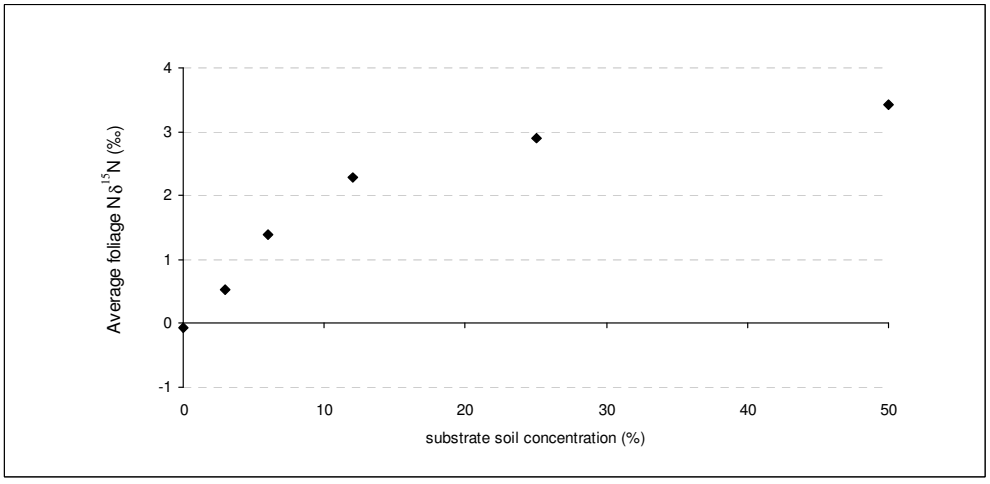


Figure 8.4. Average foliar N $\delta^{15}N$ signature of all plants at each substrate soil concentration following two years of growth. Each point represents the average of all plant species (n = 7) at each treatment. Although the sd is large (not shown) the increasing trend with substrate soil concentration is still apparent.

Foliar $\delta^{15}N$ response to increased substrate soil concentration varied between individual species (Figure 8.5). *P. radiata* foliar $\delta^{15}N$, for example, increased proportionally with soil concentration (until 25% soil), a range of almost 7.5‰, the greatest range in foliar $\delta^{15}N$ signatures measured in an experimental plant in this trial. In comparison, *E. minus* foliar $\delta^{15}N$ increased only 2.3‰ over the range of substrate soil concentrations. It is interesting to note that *E. minus* produced the highest foliar $\delta^{15}N$ signature of all species at both 0% (+3.34‰) and 50% (+5.47‰) soil concentrations, respectively.

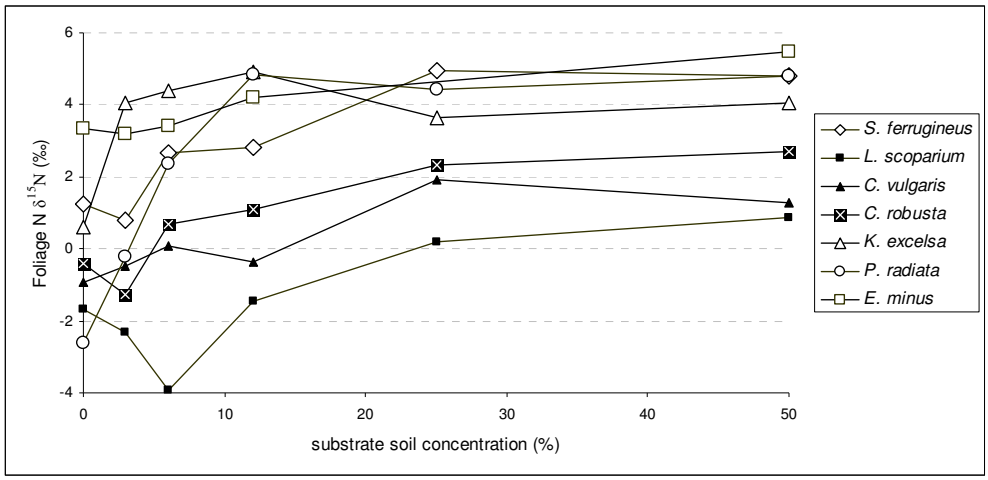


Figure 8.5. Foliar N $\delta^{15}N$ signature of individual plant species in response to increasing substrate soil concentration following two years of growth (each point = sample).

8.3.1.4. Long term glasshouse nutrient trial: limiting N & P treatment

A sub-set of the experimental plants (*L. scoparium*, *K. excelsa* and *P. radiata)* were grown under the same conditions as those under N limitation, but provided complete-N & -P nutrient solution at 50 ml pot^{-1} $week^{-1}$. Following two years of growth under N and P stress, foliar N content (Figure 8.6) and $\delta^{15}N$ signature (Figure 8.7) generally followed the same trend as that of -N treatment; both generally increasing with substrate soil concentration, the strength of which was dependent on plant species.

A wider range of foliar %N values (0.5 to 1.93%) and $\delta^{15}N$ signatures (-6.92 to +4.95 ‰) were measured in the -N & -P treatments over those of the equivalent -N nutrient treated plants, even with the smaller number of plant species in the data set. *L. scoparium* had the widest range in foliar N concentration (0.6 to 1.93%),

but, unlike *K. excelsa* and *P. radiata*, *L. scoparium* foliar N concentration was not strongly related to soil substrate concentration (Figure 8.6). *P. radiata* had the widest range of foliar $\delta^{15}N$ signatures (-6.92 to +4.03‰), with all plant species foliar $\delta^{15}N$ signatures positively correlated with substrate soil concentration (Figure 8.7). The most nutrient stressed plants produce the most depleted $\delta^{15}N$ signatures.

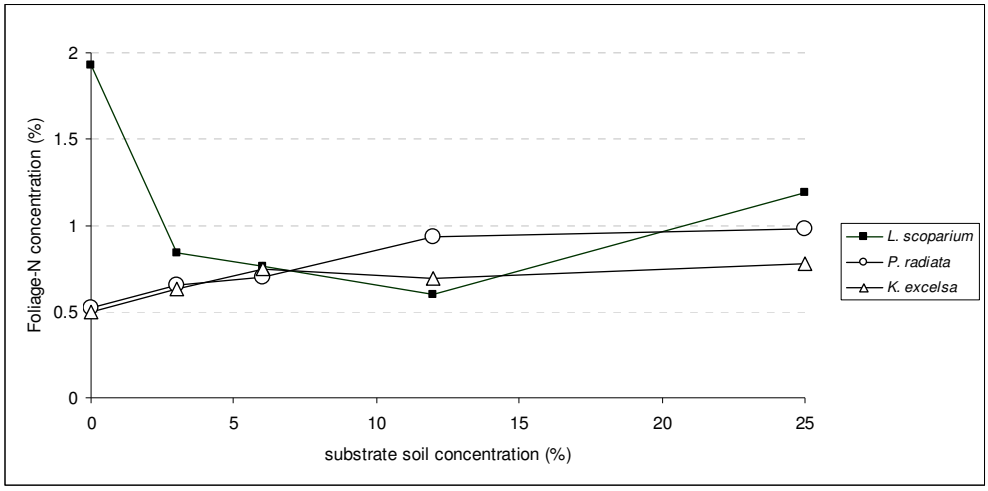


Figure 8.6. Foliar N concentration of individual plant species in response to increasing N and P supply following two years of growth (each point = one sample).

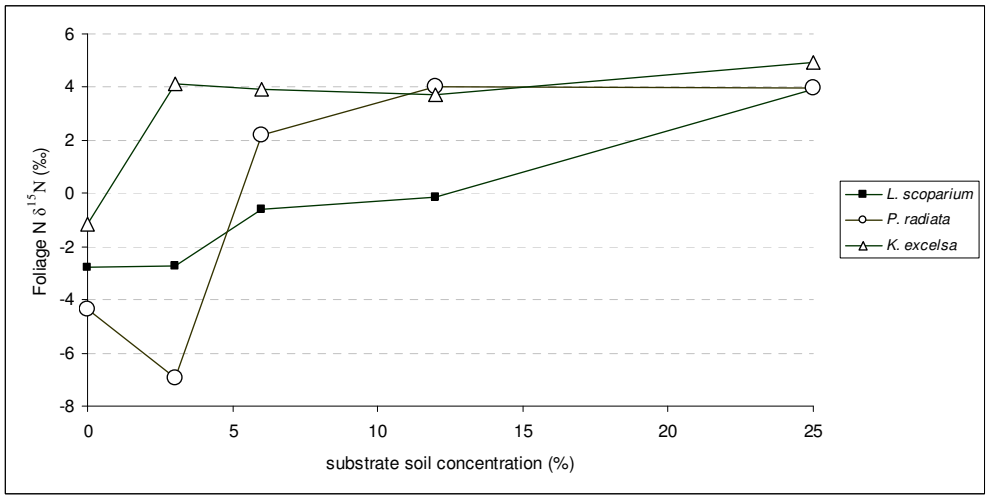


Figure 8.7. Foliar N $\delta^{15}N$ signature of individual plant species in response to increasing N and P supply following two years of growth (each point = one sample).

8.3.1.5. Long term glasshouse nutrient trial: Foliar $\delta^{15}N$ signature and %N correlation

Average foliar N concentration and $\delta^{15}N$ signature of all experimental plants increased with increasing soil concentration, indicating a correlation between nutrient stress and $\delta^{15}N$. The strength of the correlation between nutrient ('N' and 'N and P') stress and foliar $\delta^{15}N$ signature was dependent on plant species. These are ranked in Table 8.3 according to their correlation coefficient.

Table 8.3. Correlation coefficients of all individual experimental plant species between foliar N concentration (for plants experiencing N and N & P stress) and foliar $\delta^{15}N$ signature. ND = not

Plant species	Plant taxa	Mycorrhizal association	R^2 foliar $\delta^{15}N$ vs %N as a consequence of nutrient (N) stress	R^2 foliar $\delta^{15}N$ vs %N as a consequence of nutrient (N and P) stress
P. radiata	Gymnosperm tree	Ecto-	0.98	0.67
S. ferrugineus	Monocot restiad	Non-	0.90	ND
E. minus	Monocot restiad	Non-	0.82	ND
C. robusta	Angiosperm tree	Endo-	0.52	ND
K. excelsa	Angiosperm tree	Non-	0.38	0.79
C. vulgaris	Angiosperm shrub	Ericaceous	-0.02	ND
L. scoparium	Angiosperm tree	Endo-	-0.03	-0.03

The (ecto-mycorrhizal) gymnosperm tree *P. radiata* strongly correlated foliar $\delta^{15}N$ signature with N concentration. This indicated that there was a relationship between the two variables under N stress alone. However this relationship was weakened when the same plants experienced both N and P stress. The monocots and non-mycorrhizal *S. ferrugineus* and *E. minus* also strongly correlated $\delta^{15}N$ and N concentration under N stress alone, but not the non-mycorrhizal (angiosperm tree) *K. excelsa.* However when *K. excelsa* experienced both N and P stress, foliar $\delta^{15}N$ and N concentration relatedness increased. Endo-mycorrhizal association tended to correlate $\delta^{15}N$ with N concentration moderately in the angiosperm tree *C. robusta,* but not at all in the angiosperm tress *L. scoparium* under N stress. The ericaceous *C. vulgaris* like the endo-mycorrhizal *L.*

scoparium, correlated $\delta^{15}N$ very poorly with N concentration. Stress of P in addition to N did not improve the relatedness of $\delta^{15}N$ with N concentration in the endo-mycorrhizal *L. scoparium*.

8.3.1.6. Long term glasshouse nutrient trial: fractionation and internal metabolism

To test the fractionation attributed to internal N reallocation prior to leaf loss, foliar N concentration and $\delta^{15}N$ signature of 'old' leaves, collected from all plant species were compared with 'young' leaves after two years of growth.

8.3.1.6.1. Foliar N concentration

'Old' leaf N concentration of all averaged experimental plant species increased with increasing soil concentration in the substrate, mirroring the trend observed in 'young' leaves (Figure 8.2). 'Old' leaf N concentrations were not significantly different ($p < 0.05$) to those of 'young' leaves at 0% soil substrate concentration, but became significant, and increasingly so, as substrate soil concentrations increased. N & P stressed plants expressed the same trend and level of significance with increasing soil concentration, as with N stressed plants. Both datasets were combined and the trend illustrated in Figure 8.8.

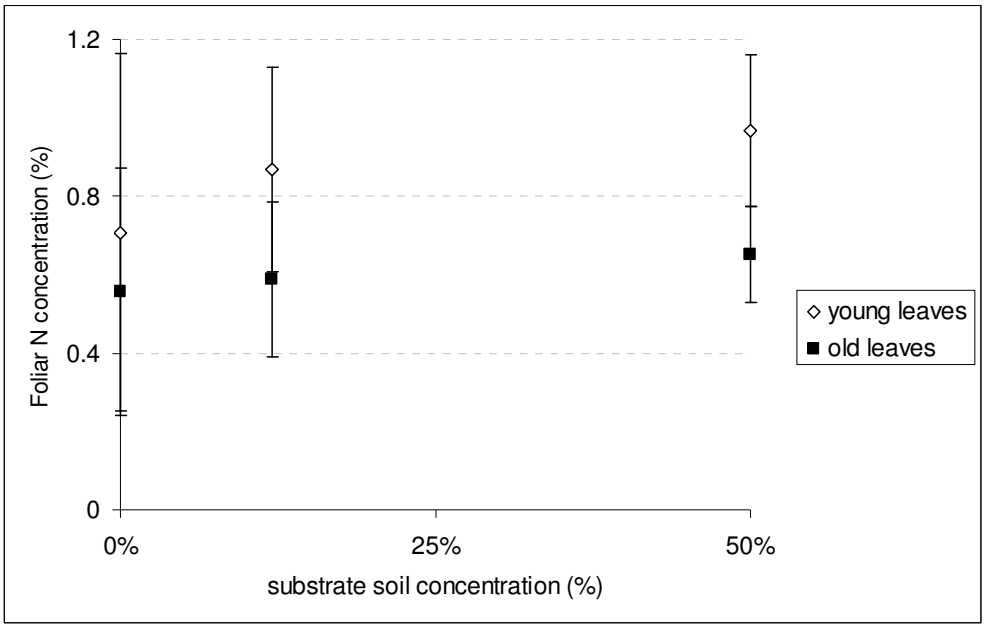


Figure 8.8. Foliar N concentration of 'young' and 'old' leaves of all plant species following two years of growth over a nutrient stress continuum. Error bars = sd (n = 7)

8.3.1.6.2. Foliar N $\delta^{15}N$ signature

'Old' leaf $\delta^{15}N$ signature of all averaged experimental plant species became progressively enriched with increased soil substrate concentration, following the same trend observed in 'young' leaves (Figure 8.4). 'Old' leaf $\delta^{15}N$ signatures, however, were not significantly different ($p > 0.05$) to the $\delta^{15}N$ signature of 'young' leaves in all experimental plants of all substrate soil concentrations (Figure 8.9).

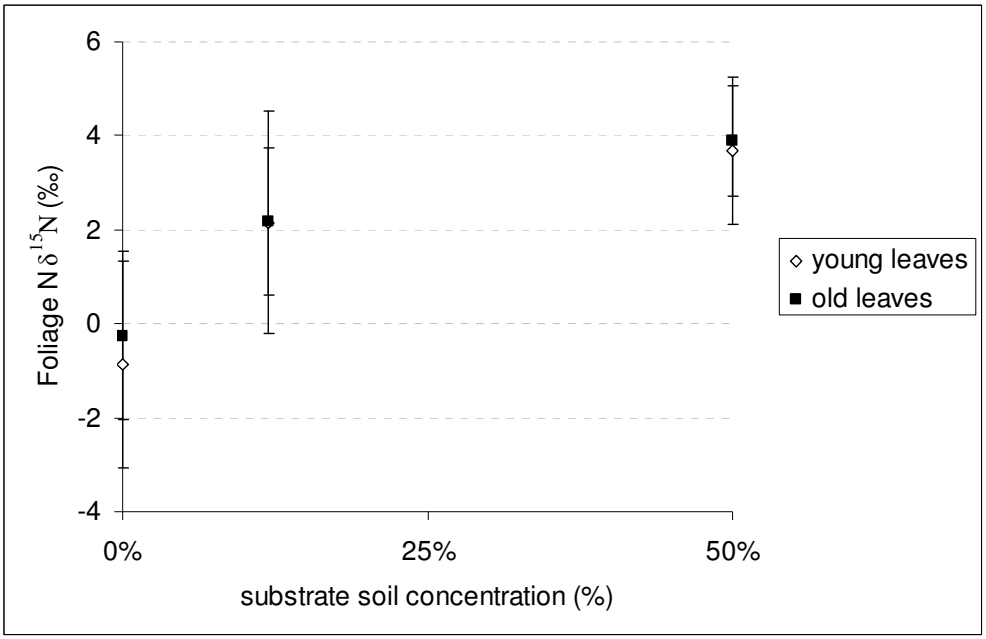


Figure 8.9. Foliar N $\delta^{15}N$ signatures of 'young' and 'old' leaves of all plant species following two years of growth over a nutrient stress continuum. Error bars = sd (n = 7).

8.3.2. Partial uptake trial

A subset of plant species used in the long term glasshouse experiment were subjected to a range of nutrient regimes over a shorter experimental period. In this experiment, the nutrient source concentrations were manipulated so that plants only utilised a proportion of the N supplied. The $\delta^{15}N$ signature of plants was determined as a consequence of partial uptake.

8.3.2.1. Plant growth

Following the four month treatment period, plants experiencing 'complete' nutrient solution were the most vigorous, largest and healthiest. Plants reliant solely on 'dH_2O' were the least healthy and smallest plants. N limited plants

exhibited typical N stress symptoms of yellowing leaves and reduced size almost immediately. P limited plants produced typical P stress symptoms of dark leaf colour particularly in the older leaves.

8.3.2.1.1. Dry weight

All plant species increased in dry weight following nutrient treatment. Dry weight increased proportionally with increased nutrient supply (Figure 8.10), regardless of N or P limitation.

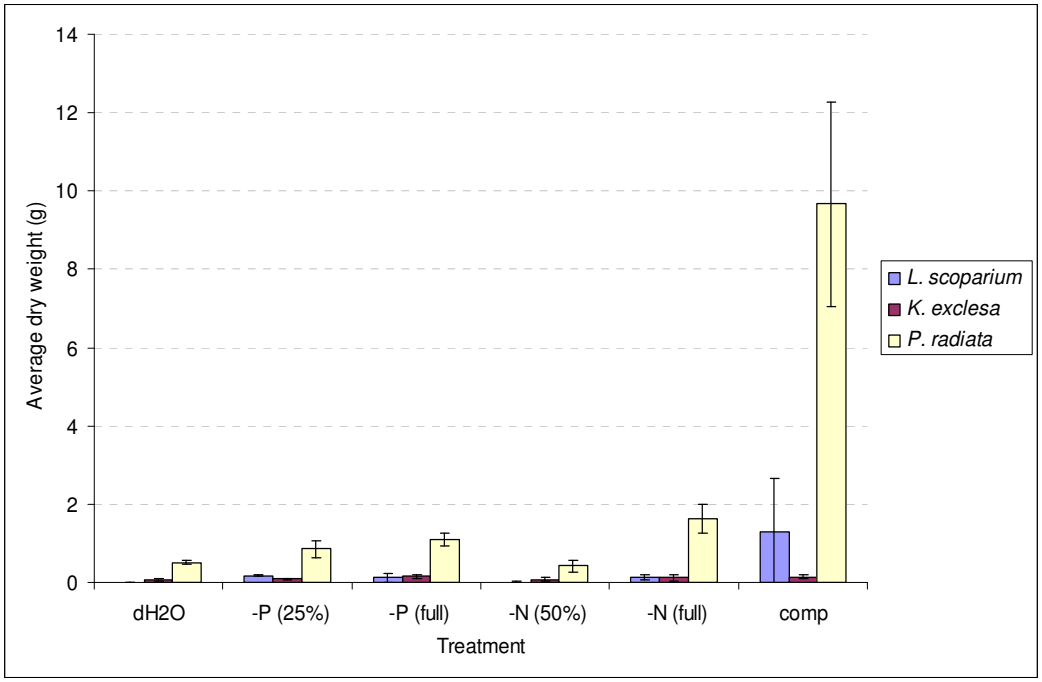


Figure 8.10. Dry weight of harvested plants following nutrient treatments. Error bars = sd (n = 3 for each plant in each treatment except for *K. excelsa* -N (full) where n = 2).

P. radiata produced the greatest increase in dry weight, followed generally by *K. excelsa* and *L. scoparium*. Although *K. excelsa* had a large seed, and therefore potential higher seed N contribution, its growth was generally poor across all treatment conditions, as clearly seen in the 'complete' nutrient treatment, where *K. excelsa* produced the lowest dry weight addition after the four month treatment period.

8.3.2.1.2. Whole plant N concentration

All plant species responded to increased N supply by increasing whole plant N concentration. The lowest whole plant N concentration was measured in plants subjected to 'dH_2O', and highest in 'complete' nutrient treated plants. *L. scoparium* typically had the lowest whole plant N concentration in each treatment compared to *P. radiata* and *K. excelsa*. *K. excelsa* typically had the highest. Whole plant N concentration ranged between 0.34% in *L. scoparium* 'dH_2O', to 3.51% in *K. excelsa* 'complete', with all other whole plant N concentrations falling within this range (Figure 8.11). Similarly, all experimental plant species responded to increased P supply by increasing whole plant N concentration. P stressed plants had typically higher N concentrations than N stressed plants (Figure 8.11).

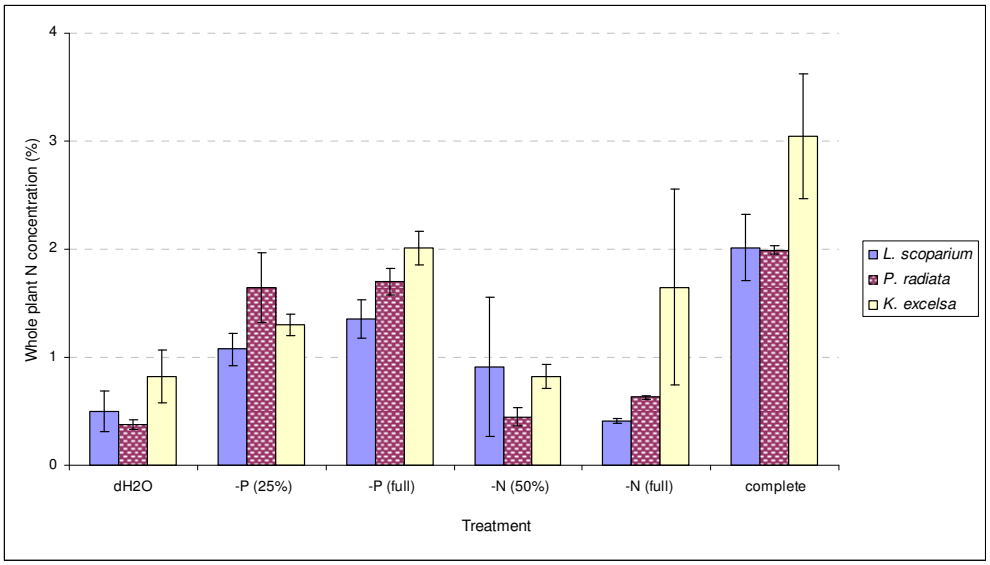


Figure 8.11. Whole plant N concentration following nutrient treatments. Error bars = sd (n = 3 for each plant in each treatment except for *K. excelsa* -N (full) where n = 2).

8.3.2.1.3. Seed N contribution

Seed N contribution to all experimental plant species decreased with increasing nutrient supply (Figure 8.12). *P. radiata* and *K. excelsa* plants relied heavily on seed N as a nutritional source across all nutrient supply treatments, progressively less with increasing nutrient supply. Seed N contribution to *L. scoparium* plants was insignificant except in the 'dH_2O' treatment, attributed to the small seed size and its comparatively low N content.

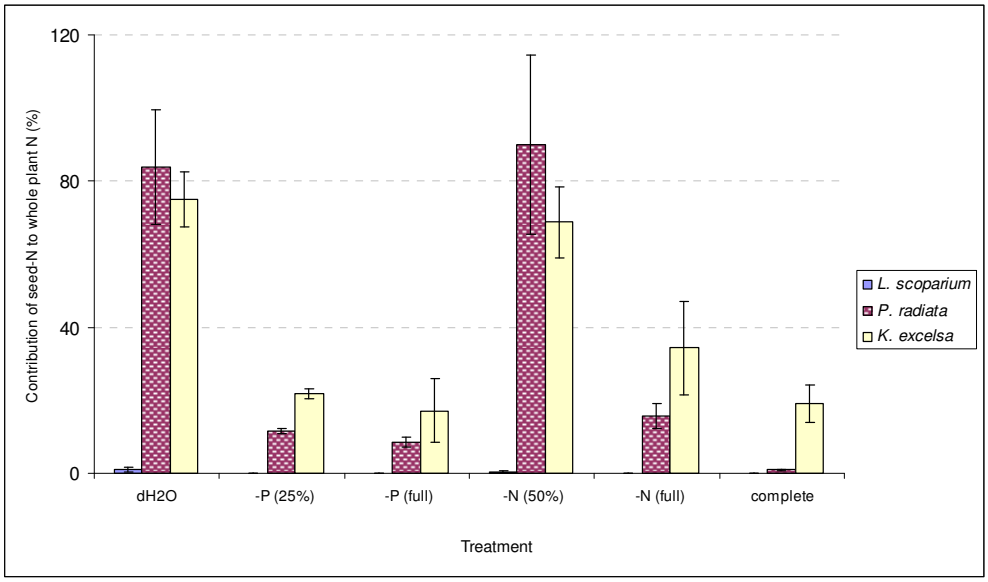


Figure 8.12. Seed N contribution to whole plant N following nutrient treatments. Error bars = sd (n = 3 for each plant in each treatment except for *K. excelsa* -N (full) where n = 2).

8.3.2. Whole plant $\delta^{15}N$ signature

Whole plant $\delta^{15}N$ signatures across all nutrient treatments ranged between -7.3‰ and +3.6‰, with both extremes attributed to *L. scoparium* plants (a range of almost 11‰). Limiting nutrient supply (both N and P) resulted in whole plant $\delta^{15}N$ signatures more closely reflecting seed N $\delta^{15}N$ signatures (c. 2‰ for *P. radiata* and *K. excelsa* and c. 1‰ for *L. scoparium*, Table 8.1). This is most clearly shown in Figure 8.13 for all plant species subjected to 'dH_2O' and '-N (50%)' nutrient treatments. Progressively depleted isotopic signatures were measured in plants as seed N contribution decreased. The greatest deviation from seed N $\delta^{15}N$ was measured in *L. scoparium* experiencing '-P (full)' nutrient treatment, with a measured depletion of c. -7‰. *L. scoparium* plants produced the greatest depletion in plant $\delta^{15}N$ signatures in all treatments, followed by *P. radiata,* with *K. excelsa* plants typically the least depleted, regardless of nutrient limitation or supply.

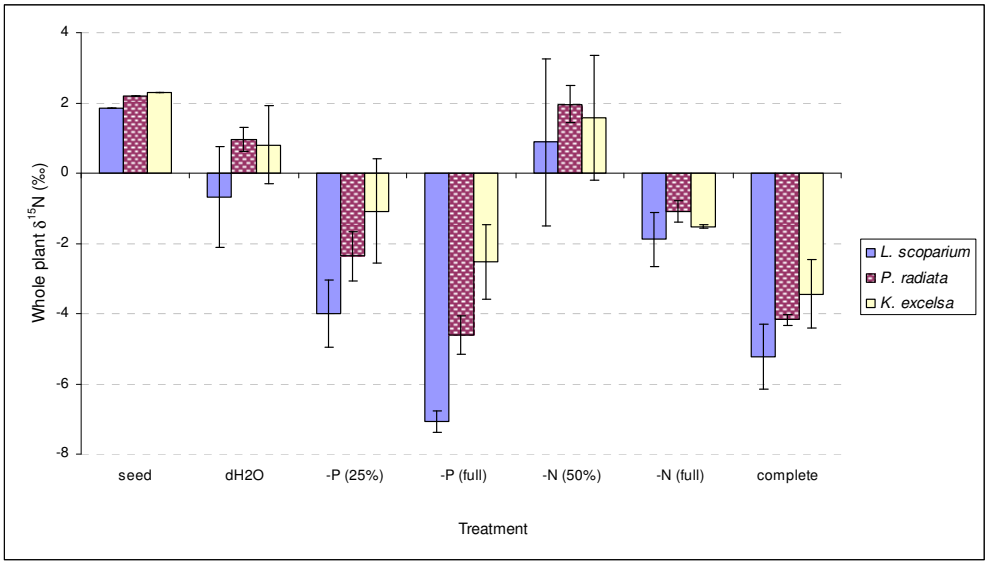


Figure 8.13. Seed and whole plant $\delta^{15}N$ signatures following nutrient treatments. Error bars = sd (n = 3 for each plant in each treatment except for *K. excelsa* -N (full) where n = 2).

8.3.2.3. Correlation of proportion of nutrient utilised and whole plant $\delta^{15}N$ signature

The level of isotopic depletion measured in plants is correlated with the proportion of supplied nutrient utilised for growth (Figure 8.14). Limited N uptake was driven by either N supplied in concentrations excess to plant requirements ('complete' nutrient), or by P limitation ('-P (full)' and '-P (25%)' nutrient treatments). Regardless of limiting process, plants express depletion according to the proportion of supplied N utilised.

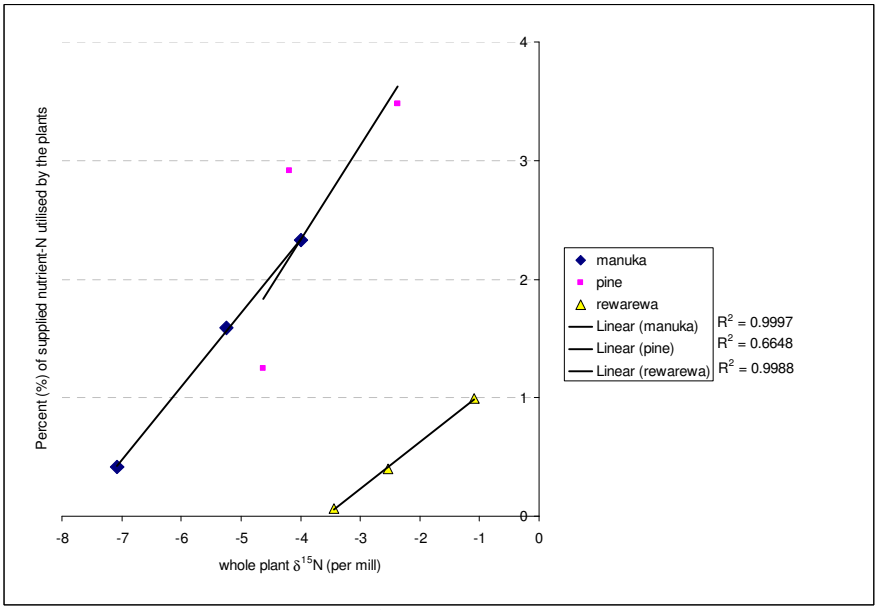


Figure 8.14. Correlation of whole plant $\delta^{15}N$ and the proportion (%) of supplied N utilised by plants following treatment.

8.3.2.4. Mycorrhizal association assessment

Mycorrhizal assessment resulted in no host/mycorrhizal association in any treatment achieving a score of greater than 1. This indicated that no mycorrhizal association of any significance was present in any of the plant species.

8.4. Discussion

Higher plant $\delta^{15}N$ signatures more depleted than -8‰ have been measured in a range of terrestrial ecosystems. Levels of depletion this low fall outside the typical range of plant $\delta^{15}N$ signatures (-8 to +10‰; Bedard-Haughn et al. 2003), and imply a unique and universal mechanism acting over and above conventional N cycle and plant N dynamic processes within these systems. Foliar $\delta^{15}N$ signatures measured in this study fall within the range of variation usually reported as a consequence of fractionation in typical plant N dynamic processes. However, the measured trends allow a number of proposed mechanisms to be examined as drivers of depletion in treated plants.

Nutrient stress is commonly associated with highly depleted foliar $\delta^{15}N$ signatures (Chapter 6; Martinelli et al. 1999), suggesting that nutrient stress maybe linked with depleted foliar $\delta^{15}N$. The glasshouse trial was designed to directly test chronic nutrient stress on foliar $\delta^{15}N$ signatures. Plant response to nutrient stress varied, but the most depleted signatures were always measured in the most nutrient stressed plants. The level of depletion was however small (c. 3.5‰) and nutrient stress, although correlating with more depleted foliar $\delta^{15}N$ signatures, does not alone account for the level of depletion measured in early succession plants (Chapter 6). The driver of this trend is more difficult to isolate.

Fractionation attributed to mycorrhizal association is one of the most strongly purported mechanisms accounting for highly depleted $\delta^{15}N$ in plants (e.g. Michelsen et al. 1998; Hobbie et al. 2005) A range of mycorrhizal associations were represented in the glasshouse trial, but no specific mycorrhizal association provide any clear trends of depletion with increasing nutrient stress. If mycorrhizal association was solely responsible for the generally depleted $\delta^{15}N$ trend with increasing N stress, the level of depletion (-3.5‰) supports the results

of Hobbie and Colpaert (2003) who measured a fractionation attributed to ecto-mycorrhizal association of no more than (-2.5‰) even in highly N limited host plants. Mycorrhizal association is unlikely to drive the level of depletion measured in early succession plants.

Plant species N concentration and $\delta^{15}N$ trends associated with increasing nutrient growth limitation were variable. These trends were apparently independent of mycorrhizal associations; seen between non-mycorrhizal plants (long term glasshouse trial) and in the constant $\delta^{15}N$ trends between plant species in the absence of mycorrhizal association (partial N uptake trial). These differences therefore may be a function of other species specific N acquisition strategies such as differing root architecture and efficiency, the ability to exploit spatially heterogeneous sources, and the ability to store and remobilise previously assimilated N (Handley and Scrimgeour 1997). Numerous reports suggesting mycorrhizal fractionation to account for the level of depletion in plants fail to consider alternative plant specific N acquisition strategies as drivers of plant $\delta^{15}N$. Data from this study suggest that individual plant N acquisition strategies may be more potent drivers of plant $\delta^{15}N$ signature than mycorrhizal association alone and support the cautions highlighted by Handley and Scrimgeour (1997) of interpreting plant $\delta^{15}N$ signatures across nutrient gradients based on only one plant characteristic.

N remobilisation and recycling within plants has also been suggested to account for variation in plant $\delta^{15}N$ signature, particularly increased depletion under nutrient stress (Evans 2001; Kolb and Evans 2002). In this study, limited datasets did not allow statistically meaningful investigation of individual species. However, as a combined data set, all older leaves of all experimental plant species had significantly lower N concentrations compared to younger leaves, implying that N remobilisation and recycling from old to new leaves was occurring. The difference in N content between young and old leaves became progressively less significant as nutrient stress increased, driven by increased N content in young leaves with increasing nutrient supply. The relatively stable N content of older leaves, independent of nutrient stress, reflected an apparent efficient N reallocation from old to young leaves prior to abscission by all experimental plant species, regardless of the level of nutrient stress.

Foliar $\delta^{15}N$ signatures of older leaves became progressively depleted with increasing nutrient stress, following similar trends measured in younger leaves. However, $\delta^{15}N$ signatures of older leaves were not significantly different to that of younger leaves at any level of nutrient stress. This suggests that, although significant N remobilisation occurred in all nutrient treatments, no isotopic fractionation resulted as a consequence. This confirms the results of Kolb and Evans (2002) who found a maximum fractionation in plants attributed to reallocation of -3‰. N remobilisation and recycling within plants cannot account for the level of $\delta^{15}N$ depletion in primary succession plants.

Yoneyama et al. (2001) demonstrated that partial uptake of a supplied N source is a strong driver of whole plant $\delta^{15}N$ signature. The partial uptake trial confirmed this mechanism of plant $\delta^{15}N$ fractionation where plants, limited in their ability to utilise the available N, produced significantly depleted $\delta^{15}N$ signatures. This trend was measured in all plants, regardless of species. It was also measured in all nutrient treatments where N was supplied in excess of requirements; either as a consequence of high concentration or growth limitation by P.

Isotopic fractionation resulting from excessive N supply, as demonstrated in this study, is unlikely to exist in the natural environment, except perhaps in some agricultural systems (Yoneyama et al 2001). However, P limitation, in the presence of adequate N supply as tested here, may occur in natural environments and account, at least in part, for the level of depletion reported in some environments where P, not N, was limiting growth (McKee et al. 2002; Clarkson et al. 2005). N uptake limitation as a consequence of alternative growth limiting factor (e.g. temperature, moisture, pH and P limitation), and/or as a consequence of rapid flow over roots (or thallus, in the case of lower plants) will result in the partial uptake of a N source, with strongly depleted $\delta^{15}N$ signatures reflected in the vegetation.

The level of isotopic depletion measured in plants as a consequence of partial uptake of a supplied N source in this experiment (c. -7‰), begins to approach that of reported studies of depleted plant $\delta^{15}N$ (Chapter 6), but does not fully account for the level of depletion. It is however the only mechanism that comes close to

explaining the level of depletion measured in early primary succession plants, but requires a very low proportion (0.5%) of supplied N (at near 0‰) to obtain this level of depletion. However, coupled with an additional fractionating mechanism, for example partial uptake of an already depleted N source, the combined level of depletion may account for that measured in a range of plants growing in early primary succession

Chapter 9

Concluding discussion

Isotopic fractionation in natural environments is dependent on Rayleigh distillation principles. Because ^{15}N molecules or ions react more slowly than isotopically lighter ^{14}N equivalents, discrimination of ^{15}N may occur during any reaction. The wide range of $\delta^{15}N$ values measured in ecosystems is a function of discrimination during ecosystem N cycling processes. Plant $\delta^{15}N$ signatures represent an integration of source N $\delta^{15}N$ and the fractionations attributed to uptake and internal metabolic processes. Plant $\delta^{15}N$ signatures are traditionally held to range between -8 to +10‰.

Highly depleted (< -8‰) foliar $\delta^{15}N$ signatures are rare, but reported for a wide range of environments including bogs, primary succession plant communities and lower plants. A range of abiotic and biotic factors which are known to drive variations in plant $\delta^{15}N$ are present within all these systems, but no measured factor can directly account for the level of depletion reported here. Exploring mechanisms to explain the level of depletion measured in a wide range of vegetation types and environmental situations became the focus of this thesis.

Handley et al. (1999) has provided the most comprehensive explanation of plant $\delta^{15}N$ drivers in natural systems. Based on the premise that N loss from systems - mostly via leaching and gaseous loss - results in enrichment of residual ecosystem N, and that all N inputs to systems approximate 0‰, they propose ecosystem N 'openness' as a driver of plant $\delta^{15}N$. Any factor which decreases the proportional flux of ecosystem N into organic matter storage components, results in a large pool of inorganic N which is susceptible to leaching and gaseous loss. Accordingly, increased inorganic N loss (increasing ecosystem N 'openness'), drives ^{15}N enrichment in plants. This model satisfactorily accounts for the majority of plant $\delta^{15}N$ trends in reported systems.

Ecosystems in which highly depleted foliar $\delta^{15}N$ signatures have been measured, cannot however be accounted for by this model. To overcome these anomalies, Handley et al. (1999) propose a *pro tempore* hypothesis: in any given system where an 'isotopic branch point' results in an initial product, and that this product is available to plants, they will reflect its highly depleted $\delta^{15}N$. They propose that the fractionating effects of these branching points (where they are present) will dominate over any other driver of plant $\delta^{15}N$. To illustrate their point, and propose a testable mechanism, Handley et al. (1999) suggest the loss of enriched dissolved organic N (DON) will result in highly depleted plant $\delta^{15}N$ signatures in natural systems. This mechanism however, is based on limited evidence and is not mechanistically tested.

Any mechanism proposed to account for the level of depletion measured in vegetation needs to comply with Rayleigh distillation principles. According to the Rayleigh model, the instantaneous product of a reaction will always be comparatively isotopically depleted. The level of depletion is inversely proportional to substrate consumption and the relative mass of the reactants. Therefore, the only theoretically plausible explanation accounting for the level of depletion in these organisms is:

- Their use of a N source which is the immediate product of an isotopic branch point, or
- That the plants themselves act as an isotopic branch point. More specifically, a mechanism acting during internal plant N metabolism or during plant N uptake, that produces an immediate product which is utilised or retained by plants, while the residual substrate N is lost or unavailable.

Numerous authors have suggested highly depleted N sources, the immediate product of an isotopic branch point, account for the level of depletion measured in some plant systems. However, the $\delta^{15}N$ signatures of plants in early succession, and of some lower plants, exceed those of any measured available N source (Chapter 6), reducing support for this explanation. Isotopic fractionation accompanying internal N metabolism and mycorrhizal association also cannot account for the levels of depletion measured in these plants (Chapter 8). The only remaining explanation for this phenomenon is the presence of an isotopic branch

point associated with a N uptake mechanism, where the highly depleted product resulting from this proposed mechanism, is utilised by the vegetation.

Linking the empirical observations to a theoretically sound mechanism to account for the observed level of depletion is a major challenge. A reasonable starting point is examining the abiotic and biotic similarities shared between all highly ^{15}N depleted vegetation. One commonality is their strong growth stress and dependence on atmospheric N sources for nutrition. All plants exhibiting extreme isotopic depletions occur in environments that are at the limits of plant growth. The more stressed the environment and the greater apparent dependence on the atmosphere for nutrition, the greater the isotopic depletion. Addition of alternative N sources, e.g. via N_2-fixation, enhanced soil N processes or exposure to canopy through fall (in the case of lower plants) acts to dilute plant N deficiency and $\delta^{15}N$ depletion. This suggests that a mechanism associated with the uptake of an atmospheric N source by highly N deficient plants, may act as an isotopic branching point. Diffusion of atmospheric $NH_{3(g)}$ – the most dominant and readily available atmospheric gaseous-N species to vegetation – is a strongly fractionating process (Högberg 1997; Frank et al. 2004), and may theoretically account for the level of depletion in plants strongly reliant on this N source for nutrition.

Low N availability however, is not necessarily the primary stress in systems where highly ^{15}N depleted plants have been measured. Early succession plant communities, for example, although N deficient, maybe primarily growth limited by a number of other abiotic factors such as temperature, moisture, pH, and phosphorus availability. In addition, the high porosity and low cation exchange capacity (CEC) of primary successional substrates ensure only a short root exposure time before wet deposited N (the dominant N source in these communities) is lost beyond the root zone. Rapid movement of wet deposition over thalli replicates this mechanism in atmospherically dependent lower plants. Combined, these factors are likely to result in only a small proportion of wet deposited N utilised by these organisms, a mechanism which has the potential to result in highly deleted $\delta^{15}N$ signatures in plants (Yoneyama et al. 2001).

In this thesis, two mechanisms were proposed to account for the levels of depletion measured in vegetation. In light of the above empirical observations, both are theoretically plausible based on Rayleigh distillation principles:

- The double fractionating steps of $NH_{3(g)}$ volatilisation to the atmosphere and its subsequent diffusive uptake into vegetation, and
- The partial uptake of a N source as a consequence of limiting growth factors other than N

Direct testing of both mechanisms revealed that they could produce depleted signatures in plants. Isotopic depletion was expressed by these mechanisms, independent of a range of biotic and abiotic factors, but the maximum fractionation theoretically obtainable was not measured. As such, these mechanisms cannot alone account for the levels of depletion measured in early succession plants. The theoretical level of depletion predicted as a consequence of these mechanisms, but not observed, can be explained. The maximum fractionation attributed to any reaction is observed when only a small amount of the reactants are processed. This highly depleted product will constitute an insignificant proportion of N within a system, and will contribute little to plant nutrition. The products of more complete reactions, although less depleted, will be significantly greater contributors to plant nutrition. The $\delta^{15}N$ signatures of these N sources will dominate plant $\delta^{15}N$, masking more depleted and less ecologically significant N sources, resulting in plant $\delta^{15}N$ values not reaching the potential level of depletion predicted.

Although no single mechanism or N source tested in this investigation was found to fully account for the level of depletion in plants growing in early primary succession sites, one mechanism can account for the greatest part of this depletion. Fractionation attributed to the partial uptake of a N source (near normal abundance) cannot alone account for the level of depletion measured (c. -7‰; Chapter 8), but this fractionation added to an already depleted N source – wet deposited N (-5‰; Chapter 6) – produces a cumulative level of depletion (-12‰), approximating that measured in early succession plants. This two step fractionation can act on both higher and lower plants, independent of ecosystem biotic and abiotic factors and accounts for the greatest portion of depletion expressed in all measured, highly depleted vegetation. Additional and variable

fractionations attributed to; atmospheric $NH_{3(g)}$ uptake (~ -6‰; Chapter 7), mycorrhizal association (up to -2.5‰; Chapter 8; Hobbie and Colpaert 2003), internal N reallocation (up to -3‰; Chapter 8; Kolb and Evans 2002), and differing N acquisition strategies of taxonomic groups (Handley and Scrimgeour 1997) will contribute to the level of $\delta^{15}N$ depletion, and variation, measured in these plants.

Mycorrhizal association has been widely proposed as a mechanism to account for depleted isotopic signatures in plants. Partial transfer of N to the plant and potential fractionation effects during fungal N metabolism are the most likely and therefore suggested processes (eg Hobbie and Colpaert 2003; Hobbie and Hobbie 2006). Although the effect of mycorrhizal association on plant $\delta^{15}N$ was not directly tested here, the results of this investigation do not add evidence to support this theory, suggesting instead that mycorrhizal association is not a strong driver of plant $\delta^{15}N$ depletions. Certainly the level of depletion measured in lithophytes cannot be attributed to mycorrhizal association, and suggests an alternative and universal mechanism acting – independent of mycorrhizal association - to deplete plant $\delta^{15}N$ signatures.

This thesis provides the first extensive survey of highly depleted $\delta^{15}N$ signatures in terrestrial vegetation and describes a level of depletion never previously reported. To explain the level of depletion measured, this study advances the work of Handley et al. (1999) by identifying and thoroughly testing two theoretically plausible mechanisms acting as 'isotopic branch points'. It further quantifies the level of ^{15}N depletion in plants as a consequence of these mechanisms and confirms their potential to drive strongly depleted foliar $\delta^{15}N$ signatures in natural systems. As such, a reasonable account of the level of ^{15}N depletion and variation measured in a range of vegetation types growing in an equally wide range of ecosystems, has been determined. By quantifying and accounting for plant ^{15}N variation in natural systems this thesis allows future research to further illuminate ecosystem N cycling processes that most strongly drive ^{15}N signatures in plants. One research topic deserving immediate attention is an examination of the role of DON loss from systems, proposed by Handley et al. (1999), as a potential mechanism to account for highly depleted plant ^{15}N.

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