

**A comparison of the stable isotopic ecology of
eastern, western, and pre-human forest ecosystems
in the South Island of New Zealand**

*A thesis submitted in partial fulfilment
of the requirements for the degree of*

Master of Science in Environmental Science

at the

The University of Canterbury

By

Olivia Rose Johnston

University of Canterbury 2014

Frontispiece



Okarito Forest, Westland

Contents

Acknowledgements.....	vii
Abstract.....	ix
List of figures and tables.....	xi
Terminology used	xiv
Chapter 1: Introduction.....	1
1.1 Vegetation history of the South Island	1
1.2 Stable isotope concepts and terminology	3
1.3 C and N stable isotopes as tracers of ecosystem processes	4
1.4 Sources of foliar C stable isotopic variation.....	7
1.4.1 Spatial variation	8
1.4.2 Temporal variation.....	9
1.4.3 Species variation	9
1.5 Sources of foliar N isotopic variation	11
1.5.1 Spatial variation	11
1.5.2 Temporal variation.....	13
1.5.3 Species variation	13
1.6 Detection of changes in C and N cycling resulting from human activities.....	15
1.7 Fossil vegetation stable isotopic analysis.....	18
1.8 Using C:N as a probe for N status at different sites.....	20
1.9 Objectives.....	21
Chapter 2: Study forest description	23
2.1 Introduction	23
2.2 West Coast forests	24
2.2.1 Climate	24
2.2.2 Geology and soils	25
2.2.3 Vegetation.....	26
2.2.4 Saltwater Forest – a West Coast historically logged, disturbed forest.....	27
2.2.5 Okarito Forest – a West Coast unlogged forest, with limited human impacts.....	28
2.3 Eastern forests	29
2.3.1 Climate	29
2.3.2 Geology and soils	30
2.3.3 Riccarton Bush – an eastern lowland, unlogged, but highly disturbed and isolated forest.....	32
2.3.4 Pyramid Valley – fossil leaves from an eastern undisturbed, pre-human forest.	34
2.4 Forest selection.....	36
Chapter 3: Methods	39
3.1 Field methods	39
3.2 Mass spectrometer preparation and analysis of leaf stable isotopic composition	43
3.3 Radiocarbon dating.....	44
3.4 Scanning electron microscopy	44
3.5 Data quality control	45
3.6 Statistical analysis	47
Chapter 4: Description of leaf composition	49

4.1 Introduction	49
4.2 Modern forests	49
4.2.1 Eastern and western forest comparison.....	55
4.2.2 Comparison between the logged Saltwater Forest and the unlogged Okarito forest.....	56
4.3 North Canterbury fossil forest	57
4.3.1 Pyramid Valley foliar stable isotopic description.....	58
4.3.2 Comparison of fossil vegetation with modern vegetation	64
4.4 Measurement uncertainty	64
4.5 Summary	68
Chapter 5: Discussion	71
5.1 Introduction	71
5.2 Foliar $\delta^{13}\text{C}$ values.....	72
5.3 Foliar $\delta^{15}\text{N}$	74
5.4 Foliar C:N.....	76
5.5 Eastern and western forest comparison.....	76
5.5.1 Foliar $\delta^{13}\text{C}$ in eastern and western forests.....	77
5.5.2 Foliar $\delta^{15}\text{N}$ and C:N in eastern and western forests	78
5.6 Historically-logged forest and unlogged forest comparison.....	82
5.6.1 $\delta^{13}\text{C}$ in logged and unlogged forests	82
5.6.2 Foliar $\delta^{15}\text{N}$ and C:N in a logged forest versus, unlogged forest	84
5.6.3 Species composition	86
5.6.4 Summary of logging impacts to leaf stable isotopic composition	87
5.7 Pyramid Valley extinct forest	87
5.7.1 Age of fossil material.....	87
5.7.2 Integrity of fossil leaf stable isotopic composition	88
5.7.3 Comparison of fossil Pyramid Valley with Riccarton Bush vegetation	93
5.7.4 Summary of fossil data.....	97
5.8 Effects of human disturbance	98
5.8.1 4500 years ago until now	98
5.8.2 Fragmentation, forest isolation and logging.....	99
5.8.3 The next step in human disturbance	101
5.9 Limitations.....	102
5.11 Summary	104
Chapter 6: Conclusions and future research	105
6.1 C and N stable isotopic differences between eastern and western forests	105
6.2 Stable isotopes as indicators of cycles in logged and unlogged forests	106
6.3 Fossil and modern material had different foliar $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N values	106
6.4 Future research.....	107
6.5 Summary	108
References.....	109
Appendices.....	119
Appendix 1: Raw data from stable isotopic analyses of the modern leaf material from Okarito Forest, Saltwater Forest, and Riccarton Bush.....	119
Appendix 2: Raw data from stable isotopic analyses of the fossil leaf material from Pyramid Valley, North Canterbury.....	131

Acknowledgements

I wish to express my gratitude to my fantastic supervisory team, Richard Holdaway, David Hawke and Travis Horton. Richard inspired me from day one, had it not been for his persistence I would not have taken on a masters. I wish to thank him for all the time, and the many chai lattes, he has invested in me, not to mention the kind words of encouragement when I needed them the most. David was always willing to help me, be it with my field work on the West Coast, emailing a reply to another one of my questions, re-reading chapters, or biking across town to meet me. His expertise was of great assistance to my research. Travis provided me with the stable isotope knowledge to collect, process, and prepare samples for analyses. The many hours he spent in the stable isotope laboratory either fixing the mass spectrometer, or running my samples, was really appreciated.

Many thanks must go to the people that helped out with my research. Lara Hawke was a great field assistant and took fantastic notes for me. Laboratory technicians, Chris Grimshaw, Jan McKensie, and Neil Andrews, each assisted me with various parts of my project by supplying technical advice and equipment. David Norton taught me how to correctly identify the species I had collected, which was an essential task, and I am thankful for all the time he saved me trying to identify them. Elena Moltchanova assisted me my statistical analysis so I would also like to thank her.

I was fortunate enough to receive several study grants throughout my research, the organisations of which, I am delighted to thank. The Ngāi Tahu Research Center, the Sir Robertson Stewart Trust, the Ngāi Tahu Kā Pūtea, and the University of Canterbury, School of Biological Sciences and Department of Geological Sciences, all deserve a huge thank you, as they made my study possible.

Thank you to the Department of Conservation for providing sampling location advice and approving my collection permit for the Saltwater and Okarito forests. John Moore and the Riccarton Bush Trust also deserve my thanks for allowing me to sample leaf material from Riccarton Bush.

Lastly I want to acknowledge my amazing family and friends, who have given me many laughs and unconditional support through my university years, particularly my fiancé, Damian Cranney, who has shown me more love and encouragement than anyone could ask for.

Abstract

New Zealand forests have been reduced and degraded by gross removal, logging, and the effects of mammals introduced by Polynesian and European settlers. These changes increase the value of the remaining forests, so information on the effects of these disturbances will be useful to inform the management of forest protection. Integrated measurements of C and N cycling within forests can be obtained using foliar stable isotope ratios, which may detect differences between forests resulting from natural or anthropogenic disturbances. This thesis characterises the stable isotopic composition distribution and likely drivers of isotopic variation of vegetation in several central South Island forests, and provides a baseline for future ecological New Zealand studies of present and pre-human vegetation. The largest detected stable isotope variation in modern leaf material was that of $\delta^{15}\text{N}$ values between the eastern and western podocarp-broadleaf forests. This variation was probably controlled by the lower soil N availability associated with the high rainfall of western forests causing low $\delta^{15}\text{N}$ values ($-8.5 \pm 3.5 \text{ ‰}$) relative to an eastern forest ($+1.6 \pm 1.3 \text{ ‰}$) and global temperate forests (average $-2.8 \pm 2.0 \text{ ‰}$ (Martinelli *et al.* 1999)). The significant but slightly higher mean $\delta^{15}\text{N}$ (0.6 ‰) of a historically selectively logged forest (Saltwater Forest) in comparison to the mean in an unlogged forest (Okarito Forest), on the West Coast, could be attributed to either alteration to N cycling from logging, site differences in topography, or local soil N differences between the forests. Although $\delta^{13}\text{C}$ showed no significant geographical variation, the well-described ‘canopy effect’ was observed in all modern forests, manifested as a positive covariation between $\delta^{13}\text{C}$ and vegetation height. Similarly, large taxon-specific differences were observed between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in both modern and fossil leaves. Well-preserved fossil leaves, from sediments c. 4500 years B.P in Pyramid Valley, North Canterbury, had higher $\delta^{13}\text{C}$ (4.2 ‰) and $\delta^{15}\text{N}$ (2.5 ‰) values than modern vegetation from Riccarton Bush, Christchurch. The difference between ecosystems spanning several millennia probably reflects ecosystem-scale changes in C and N cycling within New Zealand forests following human arrival, particularly from the degradation caused by invasive animals.

List of figures and tables

Figure 1.1 C ₃ photosynthesis, showing the diffusion of CO ₂ through the stoma which accounts for a CO ₂ fractionation of 4 ‰ and carboxylation which accounts for 29 ‰. This fractionation changes the stable isotopic composition of atmospheric CO ₂ (-8 ‰) to that of leaf CO ₂ (-28 ‰). Figure reproduced from O’Leary (1988), and annotated using Dawson <i>et al.</i> (2002).	5
Figure 1.2 Foliar $\delta^{15}\text{N}$ is an integrated measure of N cycling within an ecosystem. Many different N pools exist, each with different $\delta^{15}\text{N}$ values as a result of fractionation during each chemical process, any of which may contribute to foliar $\delta^{15}\text{N}$. Figure reproduced from Dawson <i>et al.</i> (2002).	6
Figure 2.1: South Island sampled forest locations in New Zealand.	23
Table 2.1 Annual climatic information for each modern forest area, averaged from 2009-2012 (CliFlo 2013).	24
Figure 2.2 Location of West Coast forests and each sample transect within Okarito Forest (2,3) and Saltwater Forest (1,4).	25
Figure 2.3 Regional location of Riccarton Bush and the fossil deposit at Pyramid Valley, in Canterbury.	30
Figure 2.4 Location of Pyramid Valley, showing ridges of outcropping Mt Brown, Amuri and Weka Pass formations (photograph: R. Holdaway, pers. comm. May 2012).	32
Figure 2.5 Forest vegetation zones in Riccarton Bush in 1995. Zones are; dense kahikatea forest (1), broadleaved forest with scattered kahikatea (2), kahikatea/cabbage tree forest (3), dense broadleaf forest (4), and cleared and replanted forest fringe (5). Figure from Norton (1995).	34
Figure 3.1 Riccarton Bush site locations (●) within both transect 5 and 6. Photography shows patches of different forest compositions (as described in section 2.3.3) within a highly disturbed isolated forest fragment. Coordinate system WGS84 (zone 59), Google Earth 2013.	40
Figure 3.2 Okarito site locations (●) within each transect, and several representative forest characteristics. Coordinate system WGS84 (zone 59), Google Earth 2013.	41
Figure 3.3 Saltwater Forest site locations (●) within each transect. Historic periods of sustainable yield logging since 1981 are shown (radial tree pattern shows past selective logging, small cleared patches shows past coupe logging). Coordinate system WGS84 (zone 59), Google Earth 2013.	42
Figure 4.1 Mean foliar $\delta^{13}\text{C}$ for each vegetation height level (0.1, 1.5 and 3.5 m) in the three modern forests: Okarito Forest, open; Saltwater Forest, single-hatched; Riccarton Bush, cross-hatched.	50
Figure 4.2 Mean foliar C:N ratio at three vegetation height levels (0.1, 1.5, and 3.5 m) for leaf samples from: Okarito Forest, clear; Saltwater Forest, single-hatched; Riccarton Bush, cross-hatched.	51

Figure 4.3 Okarito Forest foliar stable isotopic composition (mean \pm SD) for taxa sampled: 1, *Phyllocladus alpinus*; 2, *Weinmannia racemosa*; 3, *Kunzea ericoides*; 4, *Pseudopanax crassifolius*; 5, *Neomyrtus pedunculata*; 6, *Quintinia serrata*; 7, *Dacrydium cupressinum*; 8, *Manoao colensoi*; 9, *Metrosideros umbellata*; 10, *Coprosma foetidissima*; 11, *Podocarpus totara*. 52

Figure 4.4 Okarito Forest foliar C:N measurements (mean \pm SD) for taxa sampled: 1, *Phyllocladus alpinus*; 2, *Weinmannia racemosa*; 3, *Kunzea ericoides*; 4, *Pseudopanax crassifolius*; 5, *Neomyrtus pedunculata*; 6, *Quintinia serrata*; 7, *Dacrydium cupressinum*; 8, *Manoao colensoi*; 9, *Metrosideros umbellata*; 10, *Coprosma foetidissima*; 11, *Podocarpus totara*. 52

Table 4.1 Species presence (x indicates presence) at Okarito Forest, Saltwater Forest, and Riccarton Bush. 53

Figure 4.5 Isotope biplot (individual sample point, mean, SD) of modern foliar material for Riccarton Bush, cross, Okarito Forest, circle, and Saltwater Forest, triangle. 54

Table 4.2 Descriptive statistics of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N, for all 3 modern forests and fossil leaf material. NOTE: Modern $\delta^{13}\text{C}$ values have not been adjusted for the Suess Effect (+1.3 ‰) in this table. 55

Figure 4.6 Foliar $\delta^{15}\text{N}$ correlation with foliar C:N ratios (sample values, forest mean, SD) with linear fits, for Riccarton Bush; green circles (thinnest line), Saltwater Forest; red triangles (second thinnest line), Okarito Forest; blue crosses (second thickest line), and Pyramid Valley; yellow diamonds (thickest line). 56

Figure 4.7 Calibration of radiocarbon dates with OxCal 4.2.3 (Bronk Ramsey 2009), using the SHCal13 atmospheric curve (Hogg *et al.* 2013), shows the likely age of each of the two samples analysed are c. 4400 Cal years B.P. 59

Figure 4.8 Isotope biplot (sample values, means, SD) of foliar material for Riccarton Bush; crosses, Okarito Forest; squares, Saltwater Forest, triangles, (All modern foliar $\delta^{13}\text{C}$ values account for the Suess Effect, +1.3 ‰), and Pyramid Valley fossil material, diamonds. The global temperate forest average foliar $\delta^{15}\text{N}$ (Martinelli *et al.* 1999) is shown by a dashed line. 59

Figure 4.9 Multi-axis graph showing trends between the gel layers and foliar $\delta^{15}\text{N}$, (diamonds, long-dashed line), $\delta^{13}\text{C}$ (circles, solid line), and C:N ratios (triangles, dashed line) of fossil vegetation from Pyramid Valley. 60

Figure 4.10 Isotope biplot of Pyramid Valley fossil vegetation (sample values, mean, SD) for each taxonomic group (dicot assemblage; triangle, matai; diamond, *rubus* sp; square, unknown species group; circle). 61

Figure 4.11 Pyramid Valley fossil leaf C:N values (mean, SD) of taxonomic groups (dicot assemblage; triangle, Matai; diamond, *rubus* sp.; square, unknown species group; circle). 62

Figure 4.12 Scanning electron microscopy image showing exceptional preservation of a matai leaf. 63

Table 4.3 Analytical statistics for each set of 5 sub-samples, taken from a random selection of samples in the present study. 65

Figure 4.13 Box plot of C:N for each set of sample replicates (each sample has 5 sub-samples) for 12 random vegetation samples. 66

Figure 4.14 Mean foliar $\delta^{13}\text{C}$ vs. standard deviation for each set of 5 subsamples, with a linear regression line fitted. 67

Figure 4.15 Mean foliar $\delta^{15}\text{N}$ vs. standard deviation for each set of 5 subsamples, with a linear regression line fitted. 67

Figure 4.16 Mean C:N ratio vs. standard deviation for each set of 5 sub-samples, with a linear regression line fitted. 68

Figure 5.1 Stable isotope biplot of mean modern Riccarton Bush vegetation (light green/open circle; +1.3 ‰ added to $\delta^{13}\text{C}$ to account for the Suess Effect) and fossil Pyramid Valley vegetation (dark green, open triangle), in comparison to the bone gelatin of Pyramid Valley fossil moa (R. Holdaway, pers. comm. 20 Feb 2014) (blue, solid triangle; *Pachyornis elephantopus*, solid circle; *Emeus crassus*, solid square; *Euryapteryx curtus*), and fossil Finsch's duck from various New Zealand locations (Holdaway *et al.* 2013) (red, solid diamond; rainfall > 1500 mm yr⁻¹, open diamond; rainfall < 1500 mm yr⁻¹). 93

Terminology used

Fossil vegetation

The term ‘fossil’ has been used in the sense that the leaves from Pyramid Valley have remained intact well past their normal survival time, but no mineralisation has occurred, so although the leaves are fossils, they are not fossilised in the conventional sense.

Stable isotopic terminology

Conventions were followed as demonstrated by Coplen (2011), except for several circumstances where university laboratory conventions were preferred, specifically for some axis labels of figures.

Taxonomy names

The appropriate scientific species names were used for the identified vegetation, using Dawson & Lucas (2012) as a guide.

Chapter 1: Introduction

1.1 Vegetation history of the South Island

An extensive tectonic history resulting in rapid tectonic uplift c. 5 - 2 million years (Ma) built the Southern Alps and shaped the topography of the South Island of New Zealand (King 2000). This has been a primary control on the development and spatial distribution of the present vegetation and forest cover in the South Island, as it provided elevation and exposure to variable environmental conditions. Abrupt global climatic fluctuations defined the beginning of the Quaternary c. 2.5 Ma, with rapid cooling which initiated glacial development in the South Island, and caused the retreat of the once-dominant sub-tropical forest and in turn the expansion of grass and shrub land cover (McGlone 2004). There followed more than 50 warming and cooling cycles through the past 2.5 Ma, and each fluctuation influenced the type and distribution of the South Island's vegetation (Petit *et al.* 1999; McGlone 2004).

The end of the last glacial phase was the Last Glacial Maximum (LGM) c. 26.5 thousand years (ka) before present (B.P.) to 19 ka B.P. (Clark *et al.* 2009). The climate of this glaciation fluctuated, containing various periods of interstadials, glacial advances and abrupt climatic changes (Williams 1996). During the LGM summer temperatures averaged up to 1.9 °C cooler than modern temperatures which caused different vegetation and forest distributions from the present, such as forest tree lines 600-1000 m below those of the present (Hellstrom *et al.* 1998; McGlone 2004; Marra *et al.* 2006). Wind and frost exposure, fire, and precipitation also contributed to the vegetation distribution. This dry harsh environment of the LGM contributed to a scarcity of forest vegetation and caused subalpine grass/shrub land vegetation cover from sea level to 800 m above sea level (masl) in some places in New Zealand (Pillans *et al.* 1993). Hardy tree species such as the silver beech (*Nothofagus menziesii*) were among the few forest trees to survive through this cold

period (McGlone 2004). At the end of the LGM warming conditions became more favourable for forest growth, which triggered rapid increase in forest productivity at c. 15 ka BP (Hellstrom *et al.* 1998).

At c. 3 ka B.P. unbroken forest covered 85-90% of New Zealand land, with the remainder covered in shrubs and grass on low-lands, river terraces, valley floors, steep cliffs, and infertile soils (McGlone 1989). Most lowland forests were conifer-broadleaf forests, as each forest was characterised by a dominant podocarp species such as matai (*Prumnopitys taxifolia*), totara (*Podocarpus totara*), or kahikatea (*Dacrycarpus dacrydioides*), and upland forest was predominantly beech (*Fagus* family) forest. The climate was mild during this period, with occasional natural disturbances to forest structure, and slowly a transition into a drier, frost and disturbance prone climate, occurred (McGlone 1989).

Forest distribution has been drastically altered since the arrival of Polynesians c. 1000 years B.P. in the South Island (McGlone 1989). Although climatic changes occurred simultaneously it was unarguably human forest burning that caused the loss of over half of lowland and montane forests (McGlone 1989; Ewers *et al.* 2006). Vegetation productivity significantly increased at c. 700 years B.P. and the impact of animal species from invasions have altered vegetation composition from its pre-human state (McGlone 1989; Atkinson & Cameron 1993). The arrival of Europeans in the early 19th century initiated further forest clearing of lowlands, which rapidly increased with population from the 1870's (Ewers *et al.* 2006). Forest was further demised during conversions to farmland (Stevens *et al.* 1988) and plantations (Fleet 1986). The introduction of herbivores, primarily deer (*Cervus* sp.), goats (*Rupicapra rupicapra*), pigs (*Sus* sp.) and possums (*Trichosurus vulpecula*), following European arrival to New Zealand, has been detrimental to the forest cover, as unlike most countries, forests evolved without grazing and browsing animals (Cockayne 2011). Localised changes to forest ecosystems from humans are significant and will be described in the following chapter.

1.2 Stable isotope concepts and terminology

Stable isotope analysis is a powerful ecological tool as it provides an integrated quantitative measure of C and N cycling (Fry 2006), which contrasts with techniques that calculate single aspects of ecosystem health and provide only “snapshots” of ecological processes, such as species richness or canopy structure measures. The basis of the approach is straightforward but the application can be less so, as many variables alter the stable isotopic composition of ecosystem materials (Ometto *et al.* 2006).

Most elements exist in several different forms (“isotopes”) because of variation in the number of neutrons in the nucleus. An isotope is distinguished by its mass number, for example the carbon (C) isotope ^{13}C has 6 protons and 7 neutrons giving it a mass of 13, whereas ^{12}C has 6 protons and 6 neutrons giving it an atomic mass of 12. Stable isotopes do not spontaneously decay into other isotopes over time, unlike unstable radioactive nuclides characterised by extreme neutron:proton ratios.

When measuring stable isotope ratios for elements such as C or nitrogen (N), these are expressed in delta (δ) notation, in parts per thousand (‰) relative to one of a range of internationally accepted standard materials (Fry 2006; Sulzman 2007). $\delta^{13}\text{C}$ is measured relative to Vienna Pee Dee Belemnite (VPDB) (Werner & Brand 2001; Dawson *et al.* 2002; Fry 2006) and $\delta^{15}\text{N}$ is measured using air as the standard reference material, as the atmospheric N pool is so large it is considered to be globally constant: no known process can alter it (Werner & Brand 2001; Fry 2006).

The equation to determine $\delta^{13}\text{C}$ is as follows, and can also be used for determining $\delta^{15}\text{N}$ values by substituting ^{15}N and ^{14}N instead of ^{13}C and ^{12}C (Mook 1973; O’Leary 1981; Farquhar *et al.* 1982; Fry 2006).

$$\delta^{13}\text{C} = \{((^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{standard}}) / (^{13}\text{C}/^{12}\text{C})_{\text{standard}}\} \times 1000$$

Fractionation is the term given for changes in the partitioning of heavy and light isotopes from a source to a product, during biogeochemical processes. Fractionation occurs because isotopes of the same element behave differently to one another due to different masses. During kinetic reactions lighter isotopes react faster than their corresponding heavy isotopes, and in exchange reactions concentration of the heavy isotope occurs where bonds are the strongest. These reactions alter the concentration of one isotope relative to the other one, in the product material (Dawson *et al.* 2002). For example, during photosynthesis ^{12}C is preferentially incorporated into leaf material, consequently $\delta^{13}\text{C}$ of atmospheric CO_2 (c. -8 ‰) is higher than in plant material (c. -28 ‰) (Dawson *et al.* 2002).

Isotopes of a given element each undergo the same reaction but the rate of the reaction varies between stable isotopes depending on their mass, which enables isotopes to trace ecological interactions, processes, and activities within ecosystems. Stable isotopes used in the present study are ^{13}C , ^{12}C , ^{15}N , and, ^{14}N , as these give an integrated measurement of ecosystem C and N cycling (Peterson & Fry 1987; Fry 2006; Sulzman 2007). The isotopic ratios of a range of elements appropriate to the material and to the research question, can be obtained from many materials using a mass spectrometer such as; leaves, feathers, bone (carbonate and collagen), teeth (enamel), eggshell, soil, or water (West *et al.* 2006).

1.3 C and N stable isotopes as tracers of ecosystem processes

The role of stable isotopes as naturally occurring ecological recorders as well as the automation of their analysis has recently driven research in this field, and among the key findings is a greater understanding of C and N cycling at various spatial and temporal scales (West *et al.* 2006; Michener & Lajtha 2007). Stable isotope values act as tracers that integrate C or N cycling through the ecosystem so provide a non-invasive and practical tool for monitoring the dynamics of these nutrient cycles (Houlton *et al.* 2007). Stable isotope measurements may be used to inform the management,

sustainable use, and conservation of ecosystems from human economic exploitation, such as logging and farming, which causes degradation of forest ecosystems (Rounick & Winterbourn 1986; Andersson *et al.* 2000).

Environmental and internal leaf conditions of photosynthesis account for variation in foliar $\delta^{13}\text{C}$, and with care these values may act as a quantitative measure of the environmental effects on C cycling (Ometto *et al.* 2006; West *et al.* 2006). During photosynthesis, as leaf CO_2 is derived from atmospheric CO_2 , fractionation occurs in two stages; diffusion through the leaf stoma which accounts for a c. 4 ‰ change, and carboxylation with the rubisco photosynthetic enzyme which accounts for a c. 29 ‰ change (Fig. 1.1) (O'Leary 1981, 1988; Farquhar *et al.* 1982). The combined fractionation causes a change from -8 ‰ in atmospheric CO_2 , to -28 ‰ in leaf tissue (Fig. 1.1) (O'Leary 1981, 1988; Dawson *et al.* 2002). The closing of leaf stoma is controlled by water stress, light, and N limitation, so the amount of fractionation and the foliar $\delta^{13}\text{C}$ value depend on environmental variables that control stomatal opening (O'Leary 1981; West *et al.* 2006).

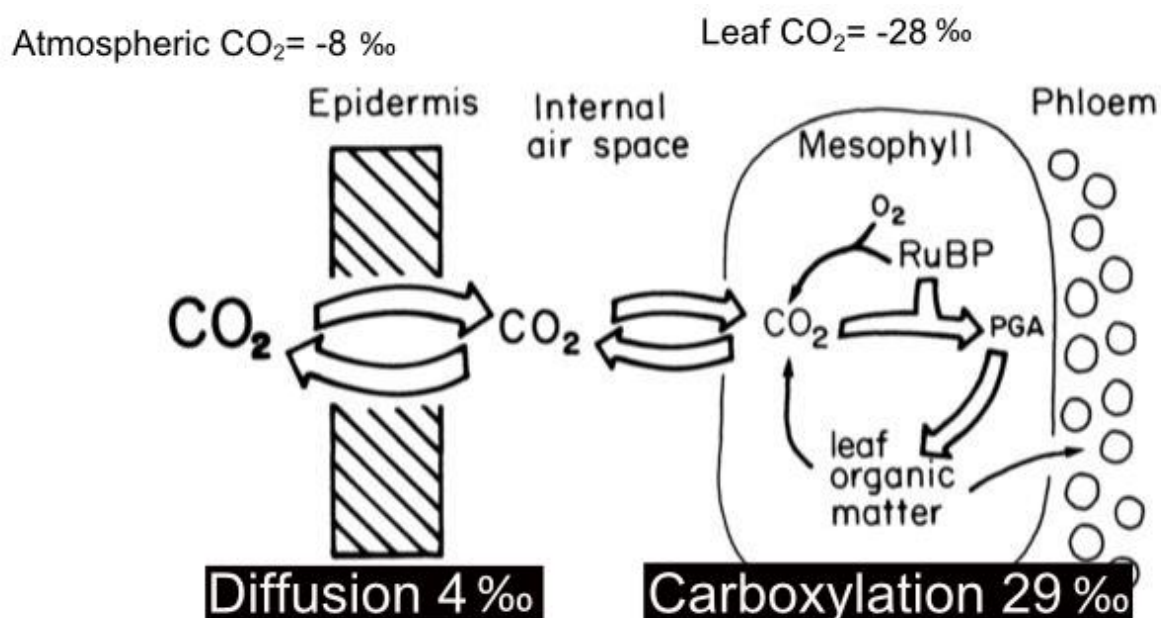


Figure 1.1 C_3 photosynthesis, showing the diffusion of CO_2 through a stoma which accounts for a CO_2 fractionation of 4 ‰ and carboxylation which accounts for 29 ‰. This fractionation changes the stable isotopic composition of atmospheric CO_2 (-8 ‰) to that of leaf CO_2 (-28 ‰). Figure reproduced from O'Leary (1988), and annotated using Dawson *et al.* (2002).

N is isotopically homogenous in the atmosphere, but in other materials $\delta^{15}\text{N}$ values may range between 10 ‰ and -20 ‰ as a result of fractionation during N cycling (Fry 2006). Fractionation occurs during each process of N cycling that transforms N into different forms, such as the many oxidation and reduction steps, N mineralisation, nitrification, denitrification and ammonia volatilization (Mariotti *et al.* 1981; Robinson 2001; Kahmen *et al.* 2008) (Fig. 1.2). Therefore, foliar $\delta^{15}\text{N}$ measurements are integrative and reflect a combination of biogeochemical processes of external N sources and tree physiological mechanisms, so may be used as tracers of N cycling within terrestrial ecosystems (Högberg 1997; Robinson 2001; Evans 2001; West *et al.* 2006; Marshall *et al.* 2007; Kahmen *et al.* 2008), and can also identify impacts to N cycling from former land uses and disturbances (Craine *et al.* 2009; Callesen *et al.* 2013).

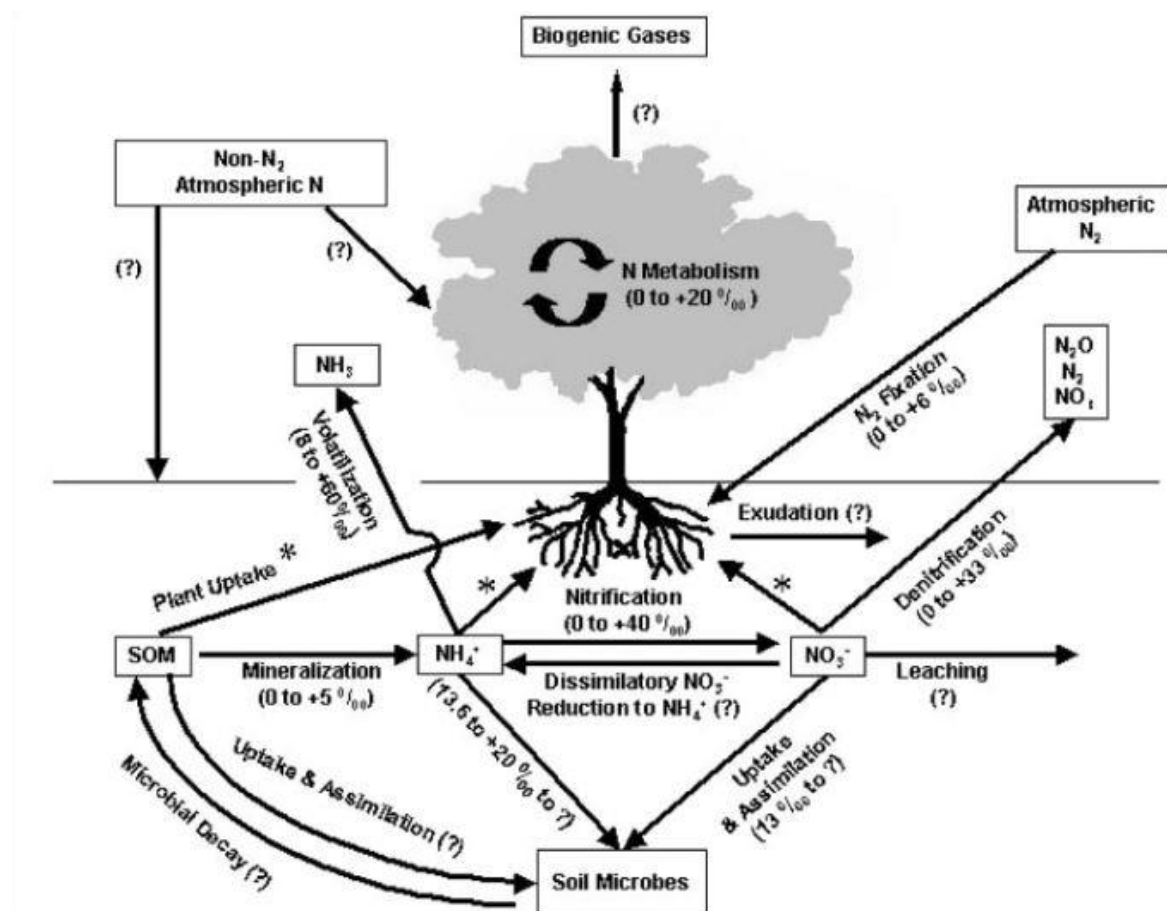


Figure 1.2 Foliar $\delta^{15}\text{N}$ is an integrated measure of N cycling within an ecosystem. Many different N pools exist, each with different $\delta^{15}\text{N}$ values as a result of fractionation during each chemical process, any of which may contribute to foliar $\delta^{15}\text{N}$. Figure reproduced from Dawson *et al.* (2002).

Both C and N cycling dominate forest ecosystem processes as these nutrients are pivotal in plant function, growth, and distribution. Therefore measurements of C and N provide a more complete depiction of ecosystem processes when analysed together (Dawson *et al.* 2002; Fry 2006). The leaf is an appropriate tissue to use for C and N cycling analysis, as leaves are easily sampled and yield valuable information on ecology, environment and vegetation dynamics (Ometto *et al.* 2006).

1.4 Sources of foliar C stable isotopic variation

The effects of environmental and internal leaf variations on photosynthesis which alter vegetation $\delta^{13}\text{C}$ can be expressed in the equation below, which shows the combination of several independent effects that contribute to foliar $\delta^{13}\text{C}$ values (Farquhar *et al.* 1982; Heaton 1999).

$$\delta^{13}\text{C}_p = \delta^{13}\text{C}_a - a - (b - a) \times (p_i \div p_a) + d$$

where:

a = Isotopic fractionation during diffusion of CO_2 through stomata

b = Isotopic fractionation during carboxylation

p_i = Concentration of intracellular CO_2

p_a = Concentration of ambient atmospheric CO_2

d = Includes a range of species specific effects

Specific leaf traits may correlate with one another, for example intracellular CO_2 levels are indicative of $\delta^{13}\text{C}$ and water use efficiency of leaf tissue (Marshall & Zhang 1994; Marshall *et al.* 2007). Any number of environmental variables may influence the $\delta^{13}\text{C}$ of a given leaf sample by altering values in the equation. The possible effects of these variables will be described in the following sections on sources of spatial, temporal, and species variation which must be considered in the present study to determine the likely sources of any $\delta^{13}\text{C}$ differences between and within forests.

1.4.1 Spatial variation

The 'canopy effect' is a term given to the increase in $\delta^{13}\text{C}$ values as vegetation height increases in the canopy (Medina & Minchin 1980; Garten & Taylor 1992; Cerling *et al.* 2004; Ometto *et al.* 2006; Kranabetter *et al.* 2010; Crowley *et al.* 2012). The 'canopy effect' occurs because as height decreases in the canopy, light levels are reduced so photosynthetic rates are lower and a greater amount of fractionation occurs during carbon fixation (Medina & Minchin 1980; Garten & Taylor 1992). There is also a higher proportion of respired, and ^{13}C depleted, CO_2 at lower levels under the canopy, which results in decreased $\delta^{13}\text{C}$ values (Silveira *et al.* 1989). Although this is a common effect it may be complicated with other sources of variation, such as the different inter-taxa responses to light and CO_2 changes (Kranabetter *et al.* 2010).

The amount of available water affects tree metabolic processes and in turn influences the amount of carbon fractionation within leaf tissues (Garten & Taylor 1992; Stewart *et al.* 1995). For example, humidity changes with location and through time which causes an increase in foliar $\delta^{13}\text{C}$ in low humidity conditions because stomata close from the decrease in stomatal conductance (Garten & Taylor 1992).

Similarly, soil composition and structure effect foliar $\delta^{13}\text{C}$. Soil compaction (Gomez *et al.* 2002) and soil warming (Melillo *et al.* 2002) both alter stomatal conductance, because the substrate water dynamics are altered. These soil characteristics may vary at small spatial scales, which would also cause foliar $\delta^{13}\text{C}$ variation at small spatial scales.

Topographic, altitudinal and latitudinal variation all affect foliar $\delta^{13}\text{C}$ (Körner *et al.* 1991; Garten & Taylor 1992; Marshall & Zhang 1994) because spatial differences alter environmental conditions such as temperature (Heaton 1999), rainfall (Schulze *et al.* 1998), salinity (Poss *et al.* 2000), and atmospheric pressure (Körner *et al.* 1991), which in turn change leaf photosynthetic processes.

Even individual tree factors, such as tree morphology, influence foliar $\delta^{13}\text{C}$ because branch length and the aspect of sun exposure may alter water hydraulic dynamics and stomatal conductance within the leaf cells (Waring & Silvester 1994; Heaton 1999).

1.4.2 Temporal variation

The $\delta^{13}\text{C}$ of atmospheric CO_2 has fluctuated through time as a result of changes in biological, chemical, and geological processes within the earth system. Over the past 200 years, the $\delta^{13}\text{C}$ of atmospheric CO_2 has decreased by c. 1.5 ‰ from the input of ^{13}C depleted CO_2 caused by anthropogenic activities such as fossil fuel burning and changes in land management, but also from natural decomposition (Heaton 1999). This change of $\delta^{13}\text{C}$ through time, known as the Suess Effect (Suess 1955), must be considered when comparing stable C isotopic values of different time periods (Peterson & Fry 1987; Heaton 1999; Fry 2006)

Foliar $\delta^{13}\text{C}$ within tree tissues may vary by ± 1 ‰ seasonally and annually (Heaton 1999). Such seasonal and annual climatic variations which lead to $\delta^{13}\text{C}$ variations over short time intervals, testifies to the high level of climatic control on plant physiology. Life history stage may also account for short term $\delta^{13}\text{C}$ variation in some species, as tree and leaf mechanisms may change throughout the life and age of a tree or leaf (Donovan & Ehleringer 1992). Life stage may particularly influence trees with different adult and juvenile forms, such as *Pseudopanax* sp.

1.4.3 Species variation

The extent of a given response to an environmental variable differs between plant taxa, so there is a wide range of $\delta^{13}\text{C}$ values in leaf material (O'Leary 1981; Adams & Grierson 2001). The most significant source of inter-taxa variation is that of foliar $\delta^{13}\text{C}$ between taxa that have different photosynthetic pathways (Garten & Taylor 1992; Ometto *et al.* 2006; Marshall *et al.* 2007). The

photosynthetic mechanism is such a large source of variation, because of different fractionation steps during photosynthesis, that those using the C_3 pathway such as temperate trees (-22 to -34 ‰), C_4 plants such as grasses (-6 to -19 ‰), and succulents using crassulacean acid metabolism (CAM) pathways, can all be distinguished from one another (Smith & Epstein 1971; O'Leary 1988; Schweizer *et al.* 1999). The variation in $\delta^{13}C$ values between vegetation using different photosynthetic mechanisms shows the importance of comparing vegetation using only one pathway in ecological stable isotope studies. The present study deals with only C_3 tree species as the only C_4 plants in New Zealand are grasses (Campbell *et al.* 1999). However, variation is also present within taxa of a given photosynthetic pathway because of species variation in the uptake of CO_2 (Heaton 1999).

Water use efficiency varies among C_3 species, which also affects the $\delta^{13}C$ of their leaves. Water use efficiency is often measured by $\delta^{13}C$ as these values are correlated and both are dependent on intracellular CO_2 levels (Stewart *et al.* 1995; Marshall *et al.* 2007). The significantly lower $\delta^{13}C$ of herbaceous species compared to perennial species, and deciduous species in comparison to evergreen species, are partially because of differences in water use efficiency (Smedley *et al.* 1991). Other species differences contribute to the variation of $\delta^{13}C$ values, these include differences in the tree's ratio of CO_2 assimilation to transpiration and the rooting depths in the different groups (Smedley *et al.* 1991; Garten & Taylor 1992; Marshall & Zhang 1994).

Finally, there is high heritability of foliar $\delta^{13}C$ in trees which causes $\delta^{13}C$ variation in leaves (Xu *et al.* 2003). This genetic variation may determine how certain species, individuals, and families respond to changes in environmental variables that affect foliar $\delta^{13}C$ (Heaton 1999; Xu *et al.* 2003).

1.5 Sources of foliar N isotopic variation

Foliar $\delta^{15}\text{N}$ variation arises from the influence of environmental and taxa variation. Such variation is present over a number of scales, and is particularly exacerbated by the extreme mobility of N within the environment (Högberg 1997; Craine *et al.* 2009) (Fig. 1.2). Various spatial, temporal and taxonomic sources of variation in foliar $\delta^{15}\text{N}$ will be described in the following section, as a basis for the interpretation of variation patterns in the data from the present study.

1.5.1 Spatial variation

Spatial variation of foliar $\delta^{15}\text{N}$ occurs because of variation in forest structure, composition, and environmental processes at many spatial scales, from within-forest variation driven by local differences, to regional and global variation (Craine *et al.* 2009).

Forest structure aspects such as edge effects may alter foliar $\delta^{15}\text{N}$ from the variation of environmental conditions with respect to the forest edge, such as taxonomic representation, nutrient and water availability, or N pool sources (Crowley *et al.* 2012). Vegetation height and $\delta^{15}\text{N}$ are often positively correlated in forests (Ometto *et al.* 2006), however unlike $\delta^{13}\text{C}$, this is not thought to be a partial result of the degree of canopy closure altering light levels (Cerling *et al.* 2004), but an effect of temperature conditions on stomatal conductance within leaves (Johnson & Berry 2013).

Under most conditions leaves acquire the majority of N directly from the soil, so any factors that may influence soil N composition or pools, will also affect foliar $\delta^{15}\text{N}$ (Rennenberg *et al.* 1996; Ometto *et al.* 2006; Marshall *et al.* 2007; Kahmen *et al.* 2008; Craine *et al.* 2009; Callesen *et al.* 2013). Soil characteristics and N pools vary spatially because of site fertility, nitrification, leaf litter N pools, sub-soil nitrate concentration, former land-use, and differences in N leaching (Callesen *et al.* 2013).

N availability differences also cause differences in $\delta^{15}\text{N}$, and because N availability is increasing in ecosystems worldwide from human alteration, these changes may have severe consequences to N cycling and foliar $\delta^{15}\text{N}$ values (Gruber & Galloway 2008). Direct uptake of N from the atmospheric pool often occurs in N limited environments and can result in strongly depleted $\delta^{15}\text{N}$ values if gaseous ammonia is a principal source of N (Rennenberg *et al.* 1996; Hobbie *et al.* 2000; Tozer *et al.* 2005; Johnson & Berry 2013). The different N sources of tree N are difficult to separate, and these often contribute to the unexplained variation in $\delta^{15}\text{N}$ between leaves from different locations (Boyce *et al.* 1996).

Soil age influences the $\delta^{15}\text{N}$ values in leaf material, so has implications for comparing forests with different substrate ages (Vitousek *et al.* 1990; Hobbie *et al.* 2000). In a Hawaiian system foliar $\delta^{15}\text{N}$ of *Metrosideros polymorpha* increased with an increasing substrate age (Vitousek *et al.* 1990), due to the faster loss of forest floor ^{14}N than ^{15}N to the atmospheric pool during decomposition. This fractionation effect increases at depth in soils because of the corresponding age profile, and may alter foliar isotopic content depending on tree rooting depth and the specific store(s) of plant-available N that are accessed (Fry 2006).

Latitudinal and altitudinal variation cause foliar $\delta^{15}\text{N}$ variation because of the associated climatic differences. Temperature gradients may account for such changes in many cases (Craine *et al.* 2009). However, differences in mycorrhizal fungi assemblages at different ambient temperatures may explain this spatial variation with location, and would confound any temperature effect (Craine *et al.* 2009). Mean annual precipitation also varies with latitude and altitude and affects leaf $\delta^{15}\text{N}$ values by altering soil $\delta^{15}\text{N}$ and consequently $\delta^{15}\text{N}$ values generally increase as annual precipitation decreases (Heaton 1987; Schulze *et al.* 1998; Xu *et al.* 2003)

1.5.2 Temporal variation

Short term changes, such as annual precipitation variation, have significant effects on the cycling of N in an ecosystem, therefore affecting vegetation $\delta^{15}\text{N}$ values (Aranibar *et al.* 2004). Conversely, the magnitude of change in seasonal rainfall often is not large enough to alter $\delta^{15}\text{N}$ (Ometto *et al.* 2006).

Over longer time scales such as thousands and millions of years global N cycling has responded sensitively to the earth's climatic fluctuations, particularly from changes in temperature and precipitation (Gruber & Galloway 2008). Recently changes associated with human activities have altered N cycling, for example, N fertilization may have doubled the rate of N input to terrestrial N cycles (Vitousek & Aber 1997). Effects such as this may be large enough to alter the $\delta^{15}\text{N}$ of local, regional, and even global atmospheric N pools (Gruber & Galloway 2008), which would in turn influence foliar $\delta^{15}\text{N}$. Similarly, natural disturbances and the frequency of which, such as drought, flood, or fire, may alter N cycling (Vitousek & Aber 1997). For example, the intensity of forest fires changes soil N levels, and with more intense fires larger volumes of N are lost by volatilisation which decreases foliar $\delta^{15}\text{N}$ (Cook 2001; Certini 2005). This shows that historic burning lowers $\delta^{15}\text{N}$, so high values of $\delta^{15}\text{N}$ are not possible in forests with a history of burning (Cook 2001). The N cycle also changes after logging, and evidence of altered N cycling may still exist in regenerated forests over a century after logging (Goodale & Aber 2001).

1.5.3 Species variation

Taxa-specific responses to environmental variables cause variation of foliar $\delta^{15}\text{N}$ between taxon (Callesen *et al.* 2013). Between-taxon foliar $\delta^{15}\text{N}$ variation has been recorded previously in tree species, including some of those used in the present study (D. Hawke, pers. comm. 2013). Many studies have attempted to define the amount and reasons for inter-taxon $\delta^{15}\text{N}$ variation (eg., Michelsen *et al.* 1996; Templer *et al.* 2007; Kahmen *et al.* 2008; Callesen *et al.* 2013). Among many

possible drivers, different species will access different soil N pools in different proportions which results in differences in the absorbed ratio of NO_3^- to NO_4^+ , and in turn influences foliar $\delta^{15}\text{N}$. The specific relationship between N source composition and foliar $\delta^{15}\text{N}$ values may vary regionally and globally, so is difficult to determine (Kahmen *et al.* 2008). The species composition of surrounding vegetation also affects foliar $\delta^{15}\text{N}$ because each species may utilise soil N differently, which alters the soil N pool and the N available to other individuals in the assemblage (Kahmen *et al.* 2008).

Foliar $\delta^{15}\text{N}$ often differs significantly between N-fixing plants and those that do not fix N (Marshall *et al.* 2007). Generally legumes have a $\delta^{15}\text{N}$ near 0 ‰ because of symbiotic N fixation of atmospheric N which is also 0 ‰, whereas if these taxa are compared to non-fixing species in the same stand, they will have lower $\delta^{15}\text{N}$ values (Hobbie *et al.* 2000; Robinson 2001). However, this is not always the case as some studies have shown no significant difference in foliar $\delta^{15}\text{N}$ between legume and non-legume trees (Ometto *et al.* 2006).

Fungi affects soil N pools which cause trees with fungal associations, such as mycorrhizae, to often have foliar $\delta^{15}\text{N}$ values up to 8 ‰ lower than plants lacking mycorrhizae (Michelsen *et al.* 1998; Hobbie *et al.* 2000; Hobbie & Colpaert 2003; Callesen *et al.* 2013). Conversely, sometimes there is no difference in foliar $\delta^{15}\text{N}$ between taxa with different mycorrhizal fungi associations (Hogberg & Alexander 1995). The effect of mycorrhizae may vary globally, with different suites of plant taxa or inconsistencies, which shows that the reliance of trees on mycorrhizae derived N often depends on the nutrient limitations of the environment (Hobbie *et al.* 2000).

Finally, genetic variation affects the expression of foliar $\delta^{15}\text{N}$, but very few studies have isolated the amount of genetic influence as it is difficult to exclude variation associated with environmental influences on foliar $\delta^{15}\text{N}$ (Xu *et al.* 2003).

1.6 Detection of changes in C and N cycling resulting from human activities

The effects of human alteration on biogeochemical systems within landscapes are poorly understood (Raich & Schlesinger 1992), but it is accepted that nutrient cycling through soil and vegetation within forest ecosystems may be altered by anthropogenic activities (Prescott 2002; Thiffault *et al.* 2011; LeDuc *et al.* 2013; Callesen *et al.* 2013; Mudge *et al.* 2014). Vegetation and soil are closely linked through nutrient cycling, particularly N, so changes observed in the multiple soil N pools are also likely to influence vegetation composition (Callesen *et al.* 2013).

Foliar stable isotopic ratios may be used to detect long-term plant responses from changes to C and N cycling at the ecosystem level or higher (Gebauer & Schulze 1991; Goodale & Aber 2001). For example, changes in atmospheric CO₂ levels (Bassirirad & Constable 2003) or changes from disturbance events (McLauchlan *et al.* 2007) are often reflected in foliar $\delta^{15}\text{N}$. Common human activities that may alter C and N cycles include logging, grazing, farming or burning, but often the specific causes of stable isotope differences are difficult to determine. Grazing potentially enriches ^{15}N in vegetation and in its consumers, but it is difficult to separate the effect from those resulting from differences in aridity (Schulze *et al.* 1998) and it is more likely that these detected $\delta^{15}\text{N}$ differences depend on past fire disturbance (Cook 2001), such as the shift in the soil N pool accessed by vegetation after burning that was found by LeDuc *et al.* (2013). However, this effect is not consistent as Schulze *et al.* (1998) found no change foliar $\delta^{15}\text{N}$ values in eucalypts following annual burning.

Many different impacts of logging influence N and C cycling, which in some cases is not indicated in the stable isotopic ratios of vegetation (Guehl *et al.* 1998), but often, differences are observed in these ratios in foliar and soil material of logged and unlogged forests (Goodale & Aber 2001; Cook 2001; Certini 2005; McLauchlan *et al.* 2007).

Trees contain a large proportion of the forest ecosystem biomass, so with their removal recyclable nutrients and other organic materials are also lost (Guehl *et al.* 1998; McLauchlan *et al.* 2007; Will & Neary 2007). Removal of logs modifies N cycling by altering plant N uptake, substrate N availability, soil temperature, and soil moisture (Parfitt *et al.* 2001). Soil N increases following harvesting because of the decline of tree N uptake, an increased rate of decomposition from gap exposure, and the reduction of C inputs causing a decline in N assimilation by microbial biomass (Parfitt *et al.* 2001; Prescott 2002). However, in temperate environments, there is usually a lag of many years before the N in cutover material actually appears in a form available to trees (Parfitt *et al.* 2001). It was previously thought that the common increase in available soil N following logging was a result of rapid decay of the debris, but the lower soil C relative to N may cause the observed N availability increase (Prescott 2002). Post-harvest leaching is not dominant often because of the increase in weeds and microbial biomass which conserves the soil N (Parfitt *et al.* 2001). However, a long term loss of soil N after tree harvesting has sometimes been reported because the cutover residue may overload the soil N pool and contribute to mineral leaching of N into waterways. In turn, this leaching alters the forest N store and has potential detrimental flow-on effects such as eutrophication of water bodies (Thiffault *et al.* 2011). The effects to nutrient cycling are similar whether individual or multiple stems are cut in each patch logged, so N cycle changes may be similar between different forests logged using different techniques (Prescott 2002). Up to 20 years after logging, forest productivity may remain decreased because of impaired N and phosphorus availability from changes to these cycles (Thiffault *et al.* 2011).

Soil C, as with N, does not change in predictable ways after forest removal: some temperate forests have exhibited increases in soil C and some show decreases, post-harvest (Nave *et al.* 2010; Thiffault *et al.* 2011). The differences are likely to result from variable soil types, and forest species compositions, and make it difficult to predict any directional changes in C storage in soil and litter in harvested areas (Nave *et al.* 2010).

As with natural wind-throw, selective logging creates canopy gaps which changes local nutrient cycles (Coates & Burton 1997). Removal or alteration of the density of the canopy often results in heterogeneity and changes to N cycling, from the direct and indirect effects of logging. Indirect effects of canopy removal include the death and decay of roots, changes in vegetation composition, or the disruption of surface soil (Prescott 2002), and a significant direct effect is often the changes in light exposure to understory species. These changes may alter the availability of soil N, leading to changes in the soil N pools and those accessed by trees, and resulting in altered foliar $\delta^{15}\text{N}$ in logged regenerating forest, even at the scale of individual leaves in managed tree stands (Coates & Burton 1997; McLauchlan *et al.* 2007).

Post-disturbance trajectories must be considered to understand N and C cycle changes following a disturbance (McLauchlan *et al.* 2007). Once an ecosystem has been altered, removing the source of degradation alone will often not be enough to revert the ecosystem back to its original state, this is particularly likely if abiotic factors or processes are effected, which would leave lasting effects and reducing ecosystem resilience to future disturbances (Milchunas & Lauenroth 1995; Hobbs & Norton 1996; Folke *et al.* 2004). Therefore, because recovery trajectories follow disturbances, the results obtained from any study will depend on when sampling is performed in relation to when and if changes in C or N cycling are observed, which may be different periods of times between and within ecosystems (Milchunas & Lauenroth 1995; Callesen *et al.* 2013). For example, no difference in soil N was detected between burnt savannah grassland and grassland unburnt for 3-4 years, a result that was likely because the time following burning was too short to show differences in N cycling within the soil (Cook 2001). Interpretations of results must consider the consequences of the length of time passed since a disturbance, and recognise that sampling at one point in time reflects only the visible effects at that time and can often not inform on the entire progression of ecosystem recovery.

In summary, the effects to N and C cycling from logging provide a basis for understanding how logging – and different intensities of logging- may alter nutrient balances in forests and have flow on

effects on the long term health of the soil and vegetation (McLauchlan *et al.* 2007). Stable isotopic analysis may be used to detect not only anthropogenic changes in C and N cycling in present systems, but also to investigate past environments by analysing fossil material- especially well-preserved material (Xiao *et al.* 2013), which will be described in the following section.

1.7 Fossil vegetation stable isotopic analysis

The dual N and C isotope analysis of foliar material to detect N and C cycle characteristics may be applied to fossil leaf material if the material is well-preserved. To obtain information on past C and N cycling direct observation is impossible, so the only way to research the past ecosystems is by using indirect methods, such as stable isotopic analysis of fossil material. Factors influencing fossil foliar isotopic composition may change over time, but, if fossil composition has not been altered these data may be compared with measurements on leaves from the present environment to determine any differences through time. Isotopic data from fossils can be used as proxies for palaeoclimate reconstruction (Wolfe 1995), to determine changes that have occurred through time, for example the changes to the atmospheric CO₂ reservoir (Cerling *et al.* 1998). As there is such a large difference between $\delta^{13}\text{C}$ values in plants with C₃ and C₄ photosynthetic pathways, this can be used to determine vegetation composition changes through time by identifying the proportion of each, which is better than determining vegetation composition by making inferences based on the isotopic signatures of tree consumers (DeNiro & Hastorf 1985; Cerling *et al.* 1998).

The palaeodiet of animals can be inferred from the C stable isotopic composition of preserved vegetation, and vice versa (DeNiro & Epstein 1981; Burrows 1989; Cerling *et al.* 2003). This is possible because the isotopic composition of fossil bone and teeth depend on the isotopic composition of the animal's diet (Cerling *et al.* 2004). However, this method requires the knowledge of the animal's trophic role in an ecosystem.

Pyramid Valley, the fossil site from which the leaf material used in this study was sourced, contains preserved leaves, bones, mollusc shells and an array of micro fossils including ostracods and cladocerans (Deevey 1955). The vegetation composition formerly surrounding the site has been inferred from pollen found in the lake bed gel (Moar 1970) and from macrofossils and moa gizzard contents, which have provided at least a partial inventory of the vegetation between 5000 and 1000 B.P. (Burrows 1989; Holdaway & Worthy 1997). Stable isotopic analysis of well-preserved fossil vegetation from the Pyramid Valley deposit will characterise the stable isotopic composition, and N and C cycling, in an ecosystem unaltered by human activities, in New Zealand.

There are potential problems in interpreting stable isotopic signatures of fossil vegetation (Heaton 1999) which must be considered when comparing isotopic composition of modern and fossil material (Heaton 1999). The isotopic carbon composition may differ in vegetation by up to 2 ‰ from the effect of glacial and interglacial changes through time and up to 1.5 ‰ from century- scale changes (Heaton 1999). However, if present, decay is a major source of bias in stable isotopic studies of fossil vegetation, because the process alters the ratio of cellulose to lignin to polysaccharides, which in turn alters the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (DeNiro & Hastorf 1985; Arens *et al.* 2000). Beerling (1996) claims that stable isotope measurements on modern leaves cannot be compared to those on fossil leaves because of the degree of diagenetic alteration of the isotope ratios through time. However, the depositional environment in the Pyramid Valley deposit is exemplary, with enough of the highly labile chlorophyll present to give the leaves a natural green colour, and with the leaf structure intact. The lake bed material is an anoxic gel formed in the breakdown of products of terrestrial and aquatic plants and not inorganic sediments such as those used by Beerling (1996) for $\delta^{13}\text{C}$ values or Xiao *et al.* (2013) for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of leaves.

The stable isotopic analysis of fossil leaves from Pyramid Valley presented in this study allows the pre-settlement ecosystem surrounding the Pyramid Valley site to be better understood, and compared with that of modern forest vegetation in Canterbury.

1.8 Using C:N as a probe for N status at different sites

C to N molar ratios (C:N) are determined from measurements of the total C and total N within samples, during stable isotope analysis. The ratio may be used in analysis of N cycling in ecosystems by giving a measure of the N availability (Vitousek 1982). In conjunction with stable isotope measurements, the ratio provides a more detailed investigation of N cycling than the isotope data alone. C:N ratios of soil offer a further source of information on the linkage between soil and vegetation N cycling (Ollinger *et al.* 2002). For example Cloern *et al.* (2002) found that seasonal fluctuations in the C:N ratio accompanied seasonal fluctuations in C and N stable isotopic composition in the foliage of some species, showing that N availability may change throughout the year in some ecosystems.

In general a low foliar C:N ratio indicates high N availability in the ecosystem and conversely a high C:N ratio suggests low N availability, to the extent that it may be a limiting factor for plant growth (Vitousek 1982). In terrestrial systems, when the ratio of C:N is high, N from decomposing leaves in the litter will be retained by decomposers, resulting in lower soil N availability. When the ratio is low, decomposers are not N limited so they release inorganic N into the soil, increasing N availability (Vitousek 1982). In addition to these general properties, the C:N ratio can also discriminate between the N from the oceanic food web from the N from terrestrial N sources (Schubert & Calvert 2001).

The consideration of C:N values may also help to identify differences in N cycles between anthropogenically-altered, and apparently unaltered ecosystems. For example, Goodale & Aber (2001) found lower C:N values in old growth stands compared to those with a history of logging or burning, indicating that N cycling was affected by century-scale disturbances. In the present study, the C:N ratio will be used to identify the possible N cycle differences between logged and unlogged forests on the West Coast of the South Island and between the eastern and western forests.

1.9 Objectives

The overall aim of the present study was to define the spatial and temporal distribution of C and N foliar stable isotopic ratios and from this data determine if differences can be detected in N and C cycling, between specific temperate South Island, New Zealand forest ecosystems.

To achieve this aim, the following objectives were formed:

- 1) Examine the C and N stable isotopic variation of foliar material between eastern and western forests in the South Island of New Zealand and identify any significant variation sources between the coasts.
- 2) Determine if the foliar stable isotopic ratios are different between a regenerating historically-logged forest and an adjacent unlogged forest, to assess if logging has altered C or N cycling.
- 3) Identify the C and N stable isotopic ratios of preserved leaf material from a swamp deposit in Pyramid Valley, North Canterbury, and if possible, compare these results to modern vegetation to assess the potential C and N alterations that have occurred since pre-human periods.

Although the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N values are ultimately mediated at the cellular level within leaf material, they may be altered by many environmental factors which makes interpreting these values difficult. However, the growing literature on the known sources of stable isotopic ratio variation in plants and vegetation, at different scales, provides the basis for interpreting the results from modern day stable isotopic studies (West *et al.* 2006; Craine *et al.* 2009). Foliar stable isotopic ratios may prove to be a good indicator of changes to C and N cycles between different environments. The present study will provide insight on the natural isotopic variation of forest vegetation under the primary climatic systems (wet west versus dry east) in the South Island. It will also indicate any detectable variations associated with different levels of anthropogenic forest alteration, and provide

a baseline for future studies on forest N and C cycle processes here, elsewhere in New Zealand and internationally.

Chapter 2: Study forest description

2.1 Introduction

Four podocarp (*Podocarpaceae*) forests were selected; two were West Coast modern forests (Saltwater Forest and Okarito Forest), an eastern coast modern forest (Riccarton Bush), and an eastern fossil forest site (Pyramid Valley) (Fig. 2.1). All forests have different levels of human driven disturbance, ranging from none in the pre-human fossil forest to extensive disturbance in the historically logged Saltwater Forest. This chapter reports the characteristics of each forest and why they were selected for the present study.

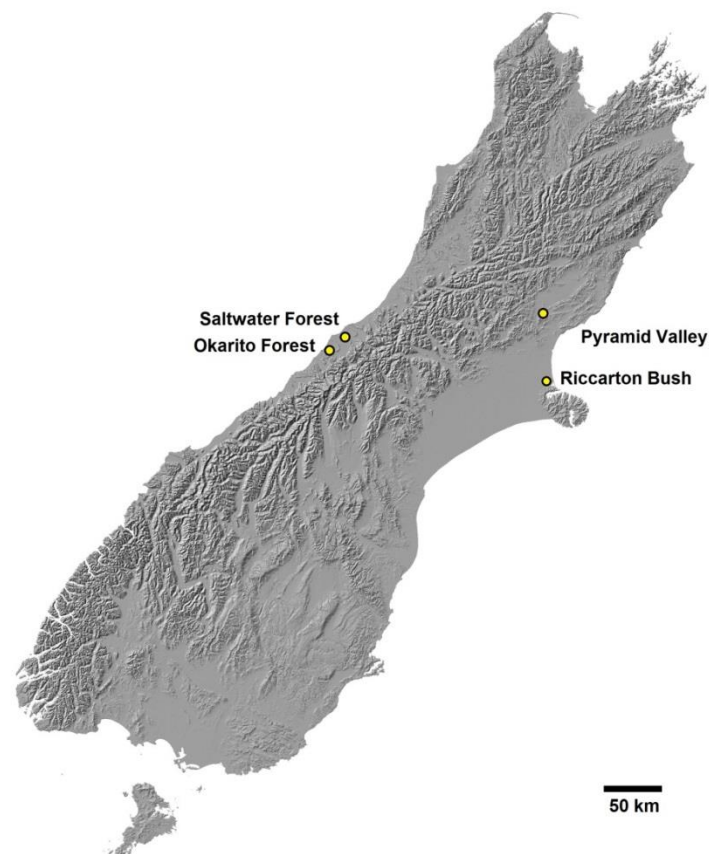


Figure 2.1 South Island sampled forest locations in New Zealand.

2.2 West Coast forests

2.2.1 Climate

New Zealand is located in the zone of Southern Hemisphere mid-latitude westerlies, where low and high pressure systems progress from west to east (Ryan 1995). Air masses rise over the Southern Alps, resulting in the highest precipitation in West Coast forests, of any in New Zealand (Peat 1987; Almond 1996). For example, the average annual precipitation at Okarito, near the two West Coast forests used in this study, for 2009-2012 was > 3000 mm (CliFlo 2013), far more than the < 700 mm that the eastern coast received (Tab. 2.1). Temperatures on the West Coast are mild (Tab. 2.1) with a mean annual air temperature of c. 12 °C and the sunshine hours are relatively high as the West Coast receives c. 1880 hours per annum. Together with the high rainfall but lack of seasonality in the rainfall, and the mild temperatures, these climatic factors characterise the environment of the temperate West Coast podocarp forests (Ryan *et al.* 2012).

Temperature decreases 4.7 °C per km of altitudinal increase, which is a primary control of the vegetation distribution on the West Coast (Ryan *et al.* 2012). Saltwater Forest and Okarito Forest are on flat land < 5 km from the coast and only 20 km apart (Fig 2.2), therefore, they experience very similar climatic conditions.

Table 2.1 Annual climatic information for each modern forest area, averaged from 2009-2012 (CliFlo 2013).

Study Forest	NIWA Climate station location	Station ID	Mean precipitation (mm)	Mean January temperature (°C)	Mean July temperature (°C)
Riccarton Bush	Christchurch, Kyle St	24120	655.2	17.6	6.2
Okarito/Saltwater	Okarito	4053	3104.3	15.4	7.4

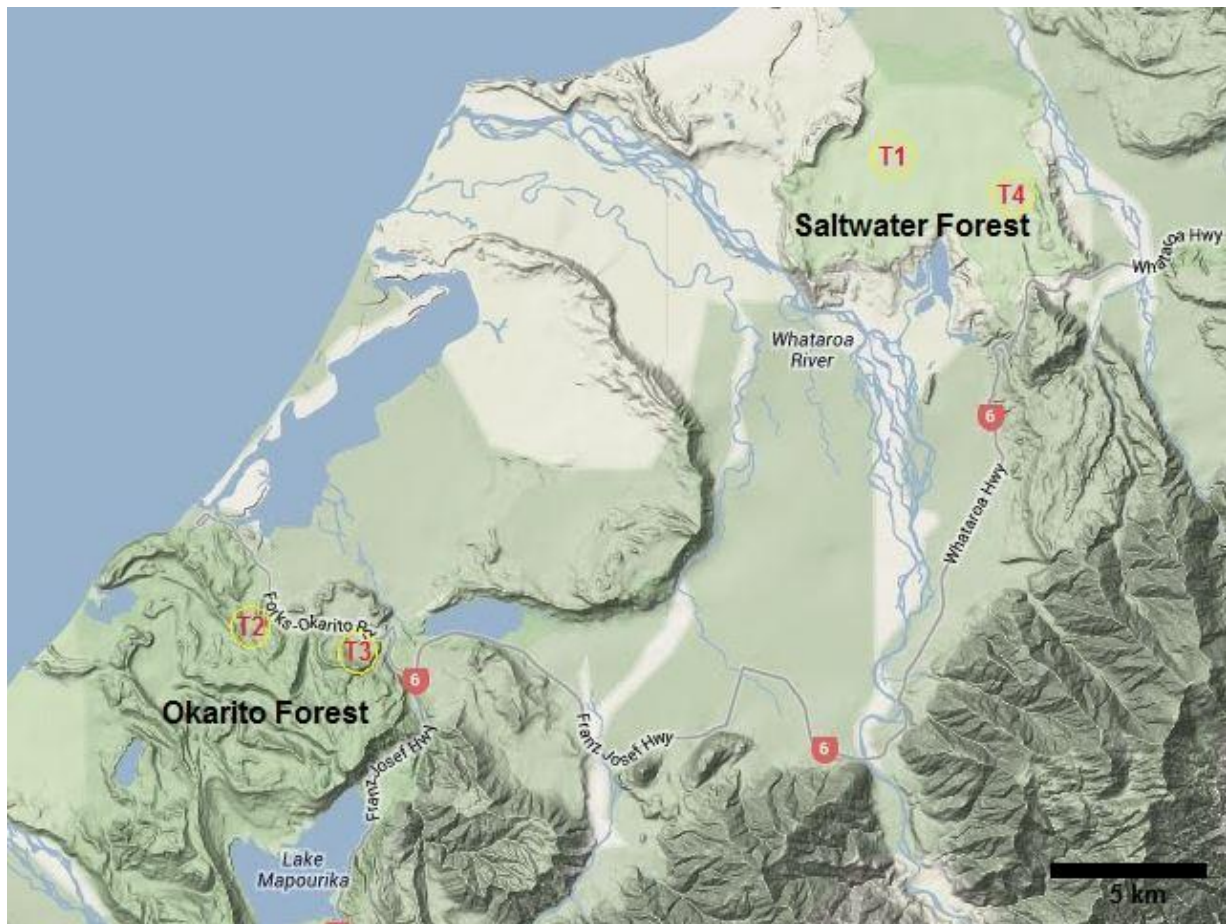


Figure 2.2 Location of West Coast forests and each sample transect within Okarito Forest (2,3) and Saltwater Forest (1,4).

2.2.2 Geology and soils

The underlying geology and soil type are important determinants of the vegetation composition and structure in forest ecosystems (Brown & Weeber 1995). West Coast soils are dominated by fluvial deposits including a number of glacial outwash gravels, diamictons, and moraines, formed of material transported from the rapidly eroding Southern Alps. Saltwater Forest and Okarito Forest are located on weathered outwash gravel plains and moraines from the most recent (Otiran) glaciation, which ended between 14 ka and 16 ka (Collins 1986; Almond 1996). The three oldest moraines in the area underlay these forests, with younger outwash gravels on top, so the soil age

ranges from 22 ka to 14 ka in the parts of the two forests that were sampled (Mew & Palmer 1989; Almond 1996).

West Coast soils are complex: they incorporate old, buried soils and loess sheets as well as podzols and paleosols created as a result of the mild, high rainfall climate (Almond 1996). Humified alluvial gravels at a depth of 40-60 cm are overlain by a grey silt loam. Below the humus, there are iron pans in some locations within the gravels (Rogers 1995). These soils are nutrient-leached and infertile as a result of the high water table maintained by the regular rainfall (Rogers 1995). The low-relief, hummocky, topography of Saltwater Forest includes small alluvial channels with poorly drained soils (Almond 1996). Okarito Forest has better drainage than Saltwater Forest, as it has a more varied topography because of the local variation in the accumulation of glacial deposits.

2.2.3 Vegetation

The natural vegetation of the central West Coast through the Holocene was primarily lowland podocarp forest to 400 m above sea level (masl) (Ryan *et al.* 2012). The present forests are a mosaic patchwork with the vegetation varying in age and structure across short distances within these forests. This is a result of natural disturbances such as snowfall, wind-throw, and earthquakes, natural variations in soil and topography throughout Saltwater Forest, and also different sequences of past logging disturbances in the area (Collins 1986; Norton & Leathwick 1990; Almond 1996; James & Norton 2002). There is a notable absence of *Nothofagus* spp. (southern beech) which are present throughout most of the rest of New Zealand (Ryan *et al.* 2012). Several forests in this Westland area have been used for extractive forestry, and cleared lands have been used for agriculture since European settlement in the 1860s and 1870s (Bennett 1980).

2.2.4 Saltwater Forest – a West Coast historically logged, disturbed forest.

Saltwater Forest (43°07'S, 170°24'E) is c. 8000 ha of predominantly rimu (*Dacrydium cupressinum*) forest between the Poerua and Whataroa rivers in the Hari hari Ecological District (Fig. 2.2).

The extensive logging history of Saltwater Forest began in the 1860s with the arrival of gold prospectors and provided a substantial proportion of rimu timber harvested from the region (James & Norton 2002). Unsustainable initial harvesting progressed to strip-felling of 4 ha sections with both skidders and high lead cables, in an attempt to make the logging more sustainable. In 1981, coupe logging was trialled, where 0.2-2 ha sections were clear-felled then replanted with seedlings (James & Norton 2002). From 1984, stems were selectively logged in a further attempt at sustainable management (James 1987; Spurr *et al.* 1992; Norton 2002; James & Norton 2002). Soil and water tables were extensively damaged by the early intensive ground-based harvesting operations, and these effects may still influence the growth of vegetation on these soils today (James & Norton 2002)

In 2002, all logging ceased to preserve the remainder of Saltwater Forest (Sampson *et al.* 2007), which resulted in regeneration throughout the forest. However, the nutrient cycles in the ecosystem may have been altered significantly by the effects of machinery and vegetation damage associated with tree removal. Therefore, the ecosystem is unlikely to be functioning in the same way as it was before logging.

Presently, Saltwater Forest is composed of the emergent rimu, over a discontinuous canopy (at c. 30 m) of other conifers, mainly miro (*Prumnopitys ferruginea*) and silver pine (*Dacrydium colensoi*), with some *Phyllocladus* and totara (*Podocarpus totara*), as well as various angiosperms. The understorey is mainly composed of conifer seedlings, angiosperm shrubs, lianes, epiphytes, and forest floor ferns and mosses (Peat 1987; Norton 2002).

As noted above, the logging has significantly disturbed the forest and the soils, which provides an ideal setting for comparing the effects of disturbance on forest nutrient cycling, in comparison with the unlogged Okarito Forest nearby.

2.2.5 Okarito Forest – a West Coast unlogged forest, with limited human impacts.

Okarito Forest (sometimes called South Okarito Forest) (43°14'S, 170°10'E) is slightly larger (Okarito Forest c. 9000 ha) than Saltwater Forest and is also located in the Hari Hari Ecological District (Fig. 2.2). The sampled section of Okarito Forest was the unlogged part to the south of Forks-Okarito Road, which is not to be confused with the previously logged North Okarito Forest. Previous research has often focused on the effects of logging on North Okarito Forest and on the Okarito Lagoon (Pearce & Griffiths 1980).

Vegetation in Okarito Forest is very similar to that described for Saltwater Forest, as it is a podocarp forest that is rimu dominated with miro, silver pine and kahikatea (*Dacrycarpus dacrydioides*), as well as various broadleaf tree species which form a multi-tiered canopy rainforest.

Okarito Forest is in the Westland National Park and is administered by the Department of Conservation (DoC). Although the area sampled has never been logged, it may have been affected by small-scale human activities but these areas were avoided when the understorey leaves were sampled. Several small patches of manuka (*Leptospermum scoparium*) scrubland and fern along Forks-Okarito Road showed where vegetation had been burnt. Numbers of introduced mammals are controlled by trapping but the construction of small walking tracks will have affected drainage and provided access points for invasion by pest and weed species (Sun & Walsh 1998).

Okarito Forest is, however, relatively undisturbed in comparison to both the logged Saltwater Forest, and the unlogged but disturbed and isolated forest fragment at Riccarton Bush on the eastern coast. Therefore, it is useful to compare the stable isotope ratios (and hence C and N cycles) between these

forests to reveal potential differences to N and C cycling as a result of severe disturbances such as logging or forest removal to the point of isolation.

2.3 Eastern forests

2.3.1 Climate

Both Riccarton Bush and the fossil site, Pyramid Valley, are located in Canterbury (Fig. 2.3), in the rain shadow of the Southern Alps. Precipitation comes mainly from the southwest and the strongest winds are northwesterlies or southwesterlies, with the latter being more frequent (Ryan 1995). The rain shadow results in eastern coastal plains and downlands receiving a relatively low rainfall, for example, the Christchurch mean annual rainfall of <700 mm of rain, is evenly spread throughout the year, and only 20% of that received by the West Coast (Tab. 2.1). Christchurch has higher summer and cooler winter temperatures than the western side of the island (Tab. 2.1). The eastern lowlands often experience air, and more commonly ground, frosts (Ryan 1995). Annual temperature and rainfall have fluctuated since the end of the Otira glaciation and from the mid-1860s mean annual temperatures in Canterbury have tended to increase.

The present climate at Pyramid Valley in the Hawarden Basin is similar to that at Riccarton Bush, but this area experiences drier summers and southerlies which bring snow in most winters (Holdaway & Worthy 1997). The climate of Pyramid Valley when the fossil leaves were deposited was likely similar to that at present, but with milder extremes (Burrows 1980).

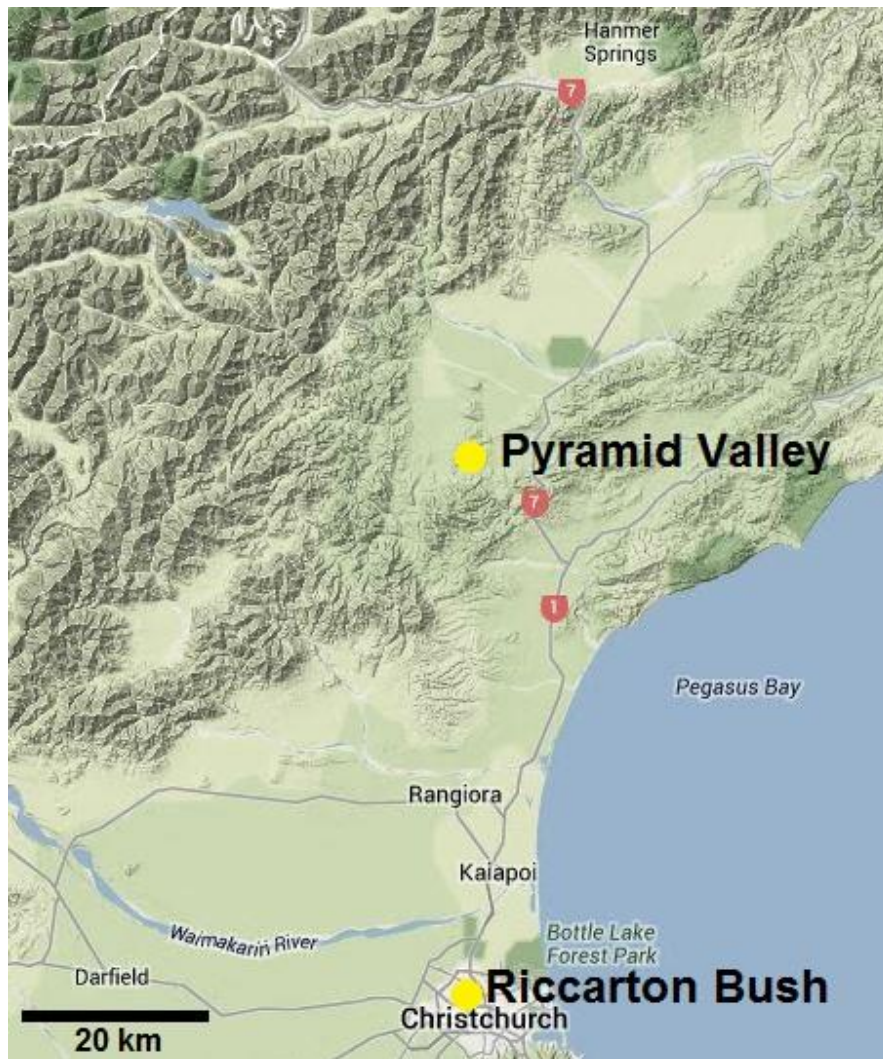


Figure 2.3 Regional location of Riccarton Bush and the fossil deposit at Pyramid Valley, in Canterbury.

2.3.2 Geology and soils

Glacial and fluvial processes eroded the old marine sediments of the Jurassic greywacke basement as the Southern Alps rose over the past 4-6 million years, and transported the material eastwards, depositing it on younger rock which formed the foundations of the Canterbury Plains. Climatic fluctuations over the past 2.5 Ma have caused glacial advances and retreats, contributing sediments to the major rivers. The numerous fluvial channels of the Waimakariri River moved back and forth across the plains to the north of Banks Peninsula, dropping relatively fertile silts on the sands and gravels of the Canterbury Plains (Brown & Weeber 1995).

Riccarton Bush is on the lowest and youngest of the four main river terraces of the Canterbury Plains (Webb 1995). The soils underlying Riccarton Bush consist of layers of sand, silt and clay deposited by successive floods over the past 5000 years (Cox 1963; Vucetich 1969; Webb 1995). In the north-eastern section the overlying sediments are younger than in the rest of Riccarton Bush as that area is closest to the tributary and received debris directly from floods. The top 3 m of sediments are the same throughout Riccarton Bush, but the individual layers vary in thickness. The soil horizon is a clay loam, varying in thickness from 0.4 to 1.2 m in the forest (Webb 1995). The soil is relatively young (less than 3500 years old) so there is little variation in weathering and leaching. Soil characteristics have controlled the type of forest formed on the Canterbury Plains, this is shown by the presence of kahikatea in Riccarton Bush. This species requires damp conditions yet Christchurch is subject to summer drought, the high water table in this area, which was formerly a swamp, has sustained the deep-rooted kahikatea (Brown & Weeber 1995; Webb 1995).

The fossil deposit at Pyramid Valley is in a depression between westward dipping Amuri Limestone and Weka Pass Limestone formations on the eastern side, and Mt Brown limestone formation on the western side (Moar 1970) (Fig. 2.4). The present pond is maintained at c. 1 m deep, but the pond was drained before 1900 following European settlement and the area of *Carex secta* used as pasture until the 1970s. Moar (1970), Gregg (1972) and Johnston (2013) have described the sediments within the swamp. The present surface is formed in a black peat that ranges from 100 – 600 mm deep. Beneath that is a grey organic material, underlain by yellow, orange, and brown laminae reaching to the base of the swamp deposit formed by 100– 200 mm of bluish clay resting on a lower black layer of peat. The ‘sediments’ are all primarily amorphous organic material, with < 10 % inorganics, except for mineral layers above and below the lower peat (Moar 1970).



Figure 2.4 Location of Pyramid Valley, showing ridges of outcropping Mt Brown, Amuri and Weka Pass formations (photograph: R. Holdaway, pers. comm. May 2012).

2.3.3 Riccarton Bush – an eastern lowland, unlogged, but highly disturbed and isolated forest.

The 7 ha Riccarton Bush (*Putaringamotu*) (43°31'40"S, 172°35'45"E) is one of the few remaining fragments of the podocarp-broadleaf forest that once covered most of the Canterbury Plains and the eastern downlands of New Zealand's South Island (Holloway 1969) (Fig. 2.3). Riccarton Bush is an ideal research site because it is easily accessible and Maori and early European disturbances to the forest have been well documented (Molloy & Brown 1995). In 1904, Leonard Cockayne, an eminent early New Zealand botanist, claimed that Riccarton Bush was the last piece of forest of its kind on the face of the earth (Molloy & Brown 1995), and this judgement is probably correct.

The structure and floristic composition of Riccarton Bush have changed over time as a result of natural events and human activities (Norton 1995). The post-glacial vegetation on the Canterbury

Plains was influenced by pre-European fires, floods, and sea level fluctuations (Molloy and Brown 1995; Norton 1995). This 3000-year-old forest stand was one of only two remaining on the Canterbury Plains when the first Europeans sighted it in 1836. Polynesian fires had removed the rest in the 14th century (Molloy 1969). Since the Deans brothers settled at Riccarton Bush in 1843, and the arrival of colonists after 1850, more vegetation was lost from wood collection, farming conversion of the fertile land, and a new dominance of introduced species (Molloy & Brown 1995). When Riccarton Bush was surveyed in 1849 only 22 ha remained (Molloy & Brown 1995), of which 7 ha now remain (Norton 1995). During the same period, both the Papanui Bush and Oxford Bush were destroyed by logging and fire. Recent human activities such as city development have prevented the floods which formerly replenished nutrients, and lowered the water table, contributing to further degradation of the Riccarton Bush forest (Norton 1995). Further changes to the ecosystem are inevitable, but these will progress slowly if degradation is slowed as is being attempted by forest management (Holloway 1969). Currently the forest is managed by small-scale planting, irrigation to maintain the water level, and pest and weed control. A predator-proof fence now excludes introduced mammals, but introduced birds are common in the reserve.

Not just the forest size but also the structure of Riccarton Bush has changed through time (Norton 1995). Increased edge effects from the size reduction, to the point where Riccarton Bush has become completely isolated, have dramatically reduced the number of species, from 106 to 67, between 1870 and 1993 (Norton 2002). An account from 1851 reports that the dominant tree species were kahikatea, rimu, pokaka (*Eleaocarpus hookerianus*), kowhai (*Sophora* spp.), and totara (Molloy & Brown 1995), which are also the dominant species today. In 1995, Riccarton Bush contained several areas of distinctly different forest structure, as mapped by Norton (1995) (Fig. 2.5). Spatial variation would have been present hundreds of years ago on a larger scale, and are likely to have included different patterns to those of today, throughout the region (Norton 1995).

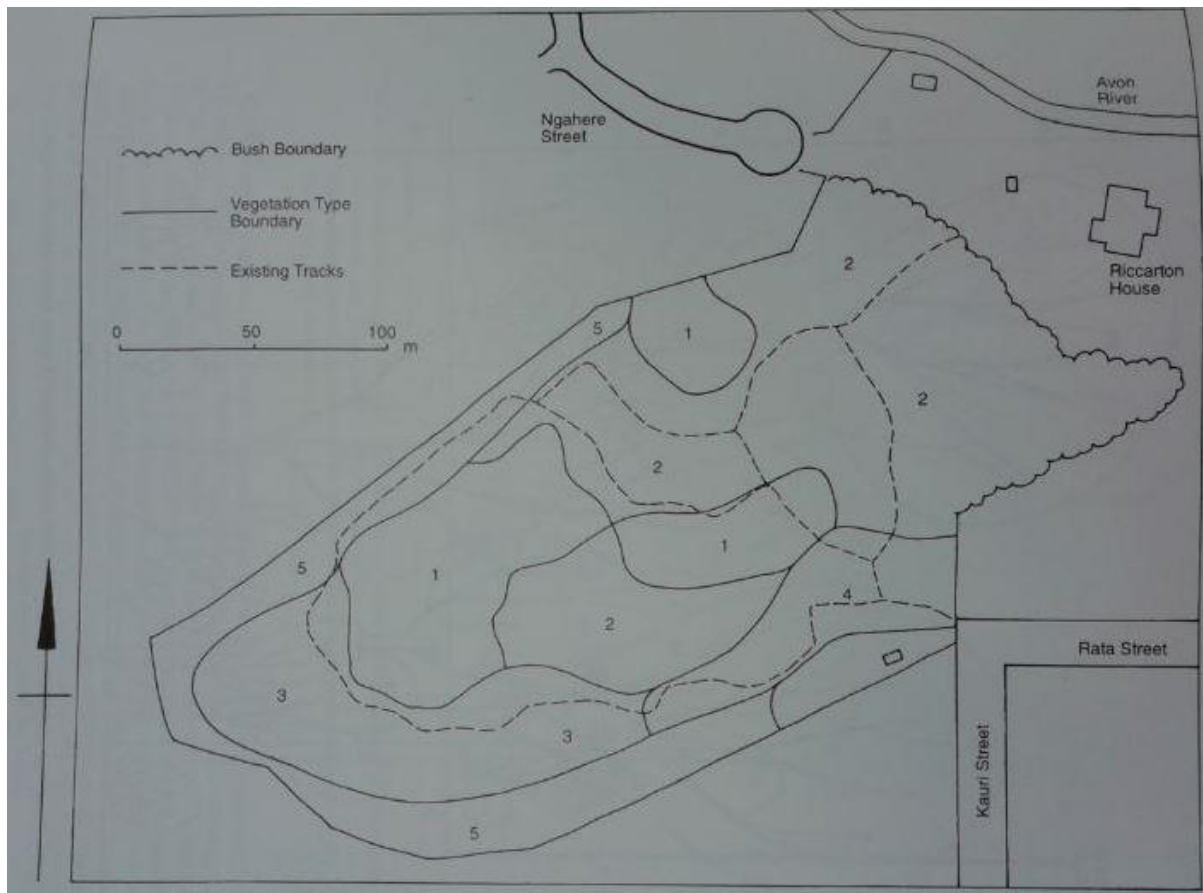


Figure 2.5 Forest vegetation zones in Riccarton Bush in 1995. Zones are; dense kahikatea forest (1), broadleaved forest with scattered kahikatea (2), kahikatea/cabbage tree forest (3), dense broadleaf forest (4), and cleared and replanted forest fringe (5). Figure from Norton (1995).

2.3.4 Pyramid Valley – fossil leaves from an eastern undisturbed, pre-human forest.

Pyramid Valley (42°58'22.7"S, 172°35'49.9"E) is at 330 masl, set amongst low hills, north of the Doctors Hills, and at the southern edge of the Hawarden Basin, North Canterbury, South Island (Fig. 2.3). A late Holocene c. 5000 to 600 B.P swamp and lake deposit (Holdaway & Worthy 1997) occupies a small basin between the two limestone ridges (Fig. 2.4). Parts of the lake bed have been excavated at intervals between 1937 and 1973. The excavations revealed the remains of birds and other vertebrate fauna and well-preserved plant material from layers of amorphous anoxic organic gel-like material (Johnston 2013). Over 180 partial skeletons of the extinct moa (Aves:

Dinornithiformes) have been uncovered (Scarlett 1969; Holdaway & Worthy 1997). These and the other important aspects of the deposit led the Hodgen family, on whose farm it lies, to have it covenanted under the Queen Elizabeth II National Trust and preserved in perpetuity.

The vegetation and perfectly preserved material of many other taxa provide an insight into the fauna, flora and environment of North Canterbury in the late Quaternary before human colonisation (Holdaway & Worthy 1997). During the period represented by the deposit the surrounding vegetation was mixed podocarp-broadleaf forest, with emergent matai, and a canopy and sub-canopy of broadleaf species (Molloy 1969; Burrows 1989). The exposed higher ridges and south-facing slopes may have been covered by shrublands and grassland (Moar 1970; Holdaway & Worthy 1997) and tall forest trees were likely to have been limited to the valleys because of seasonal droughts and cold southerly storms (Holdaway & Worthy 1997). The vegetation in the surrounding area probably varied greatly in time and space because complex environmental conditions resulted in microclines over relatively short distances (Harris 1969). Supporting this interpretation is the presence of a wide range of taxa each suited to different habitats, in the single deposit (Holdaway & Worthy 1997). Molloy (1969) suggested that the composition of the vegetation indicates that the lake lay on an ecotone between lowland podocarp-hardwood forest and sub-montane podocarp-beech forest .

The nearest forest to Pyramid Valley today is the remnant high country beech forest in the foothills to the west. The current vegetation around the lake is primarily composed of introduced pasture and shelter belt species, with the usual weeds of farmland. The only native vegetation that persists is some *Carex secta* tussocks at the southern end and a solitary cabbage tree (*Cordyline australis*).

Native shrubs have been planted within the fenced covenant area but the heavy sward of introduced grasses has choked most of the plantings (Fig. 2.4). The pond over the swamp is maintained in all but the driest years to preserve the anoxic conditions within the sediments.

2.4 Forest selection

Several factors were considered when selecting the forests to be sampled for this study. First, because these are natural forests and not experimentally manipulated plots, they were the only ones with the appropriate histories and locations. The forests were selected on the basis of being at a similar latitude, as latitude is one of the primary controls of foliar stable isotopic ratios (Körner *et al.* 1991).

For the West Coast forests, it was desirable to select forests of the same composition and on the same substrate, to minimise the possible extent of variation in isotopic signatures resulting from factors other than the presence or absence of logging. As the modern forests were going to be compared to the fossil flora at Pyramid Valley, podocarp-broadleaf forest, beech (*Nothofagus*) forests were not considered.

To determine the effect of human activities on N and C cycling a relatively unaltered forest adjacent to a historically selectively logged forest was required. Both forests had to be easily accessible as there were time constraints on sampling. Relatively flat sites that would be reasonably easy to sample were desirable. The staff of the Westland Conservancy of the Department of Conservation assisted in the selection of the two forests. Based on their knowledge of the area, they were able to suggest areas of forest that met these criteria, which were the unlogged Okarito Forest and the logged Saltwater Forest.

The understorey of Riccarton Bush was sampled because it is the only remaining area of lowland forest on the eastern coast at a similar latitude to those on the West Coast, with which to make the comparison between the effects of the different rainfall regimes on the stable isotope values. To the east of the foothills of the Southern Alps, most of the forest was burnt by early Polynesians, and most of the rest by European settlers (Molloy & Brown 1995), so options for sampling podocarp-broadleaf forests were severely restricted. Although the dominant podocarp species in Riccarton Bush is kahikatea there are no rimu forests in the area.

Pyramid Valley was included in the present study to provide not just a temporal aspect to determine if stable isotopes can be used to detect changes in C and N cycling through time, but also to determine if the arrival of humans and the organisms they introduced initiated changes in the remaining forests. Also, this is the only deposit in the area known to contain so many extraordinarily preserved forest species.

Practicality of sampling is a major factor to be considered in planning any project. It would have been preferable to have sampled from more forests throughout New Zealand but the time and resources available precluded extending beyond the four chosen. These were sufficient, however, to achieve the aims of the study.

Chapter 3: Methods

3.1 Field methods

Saltwater Forest and Okarito Forest vegetation was sampled along transects in mid-January 2013, and Riccarton Bush (Christchurch) was sampled late-February 2013. Sampling of the material from all modern forests was conducted over the same season to reduce any effect of seasonality variation. Two transects were set out in each forest, each was c. 300 m, began c. 20 m from an access road, and had four or five sample sites evenly spaced within each transect (Fig. 3.1, 3.2, and 3.3). At each 4 m diameter sample site, leaves of many species, leaf litter, and soil material were collected at three sampling levels within the understorey (0.1 m, 1.5 m and 3.5 m above the ground) using loppers. Five to ten samples were collected at each height within each sample site depending on the availability of different tree species. Surface leaf litter and soil samples (to 15 cm depth) were collected from the ground with a trowel and pooled from three areas within each sample site. A general site description was conducted which often included GPS location, an estimation of major stem spacing distance, average canopy height, topography observations, and any other distinctive features. Panoramic photographs were taken at each site to document these characteristics.

The randomisation of transects and the sample sites within them, was important as the samples collected must be representative of the forest for results to be useful at a forest scale. Transects were randomised within the forests as much as accessibility and topography challenges would allow.

Fossil leaves were naturally preserved in several layers in >90 % amorphous gel composed of the degradation products of freshwater and terrestrial vegetation (Johnston 2013). To sample the fossil vegetation, blocks of preserved lake bed gel were excavated at Pyramid Valley in 2008 (Johnston 2013). The blocks were cut out of the wall of an excavation pit at the northern end of the current

Pyramid Valley lake bed, and the orientation and location were recorded for future reference. The gel blocks were stored frozen at -20 °C for five years, until required for the present study.

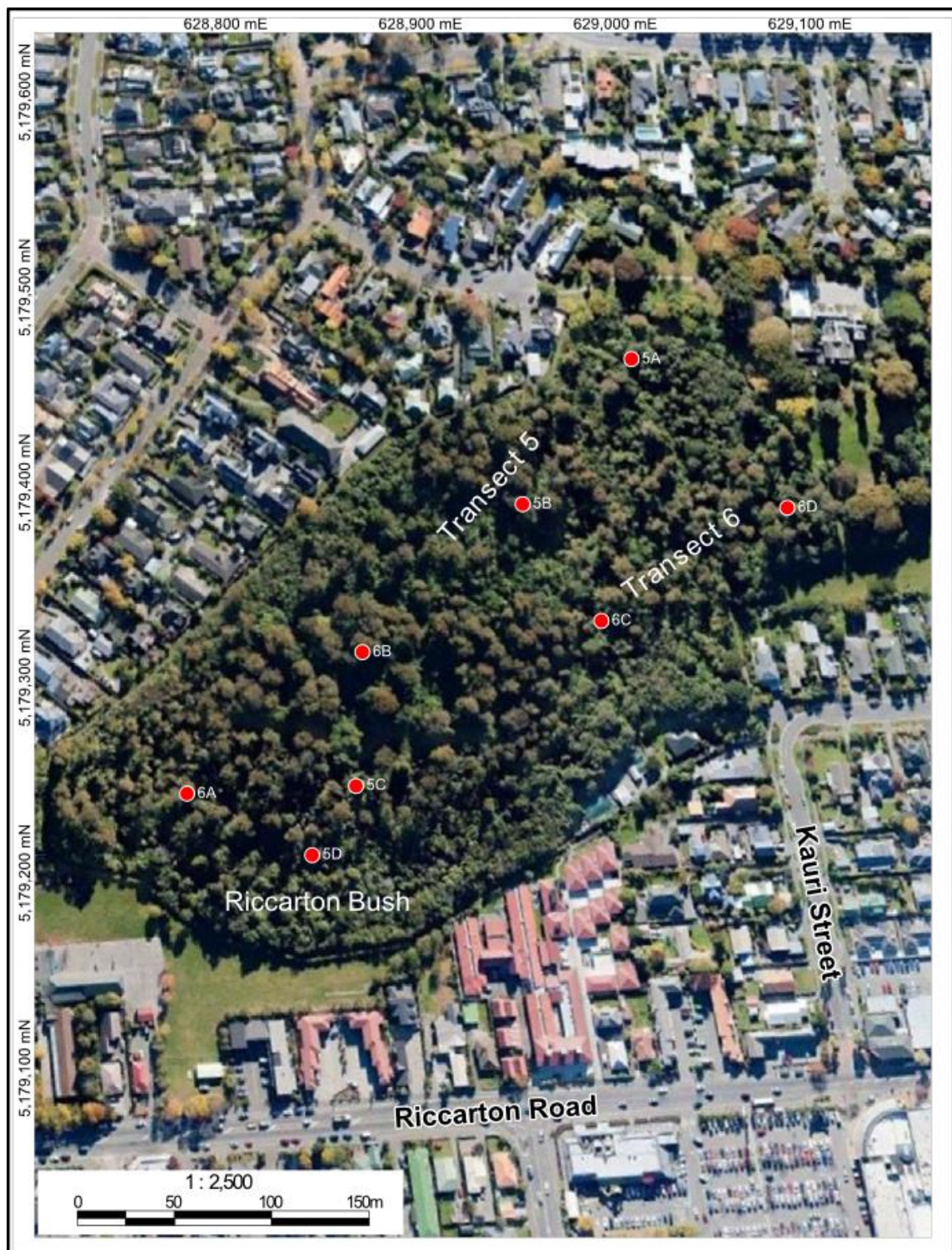


Figure 3.1 Riccarton Bush site locations (●) within both transect 5 and 6. Photography shows patches of different forest compositions (as described in section 2.3.3) within a highly disturbed isolated forest fragment. Coordinate system WGS84 (zone 59), Google Earth 2013.

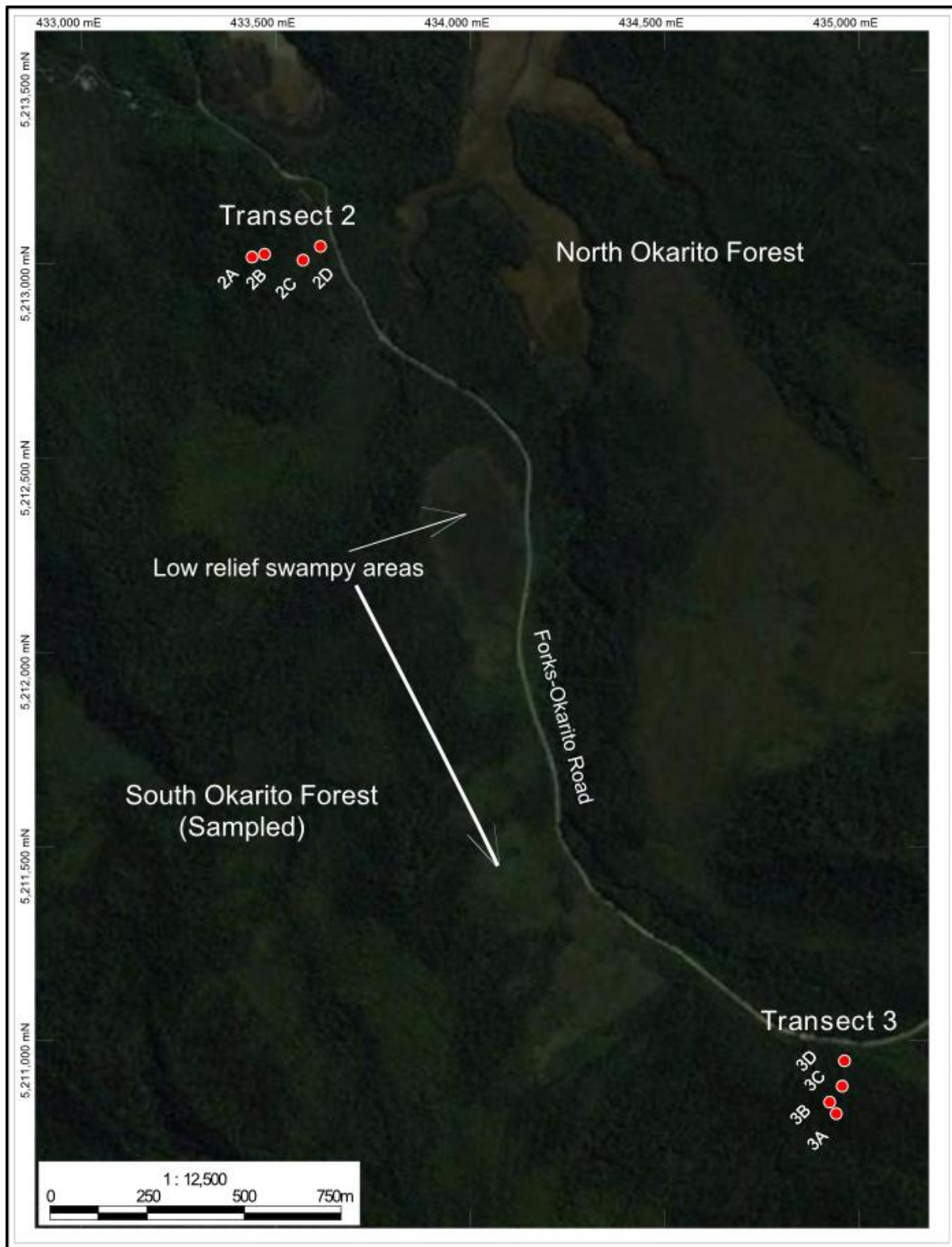


Figure 3.2 Okarito site locations (●) within each transect, and several representative forest characteristics. Coordinate system WGS84 (zone 59), Google Earth 2013.

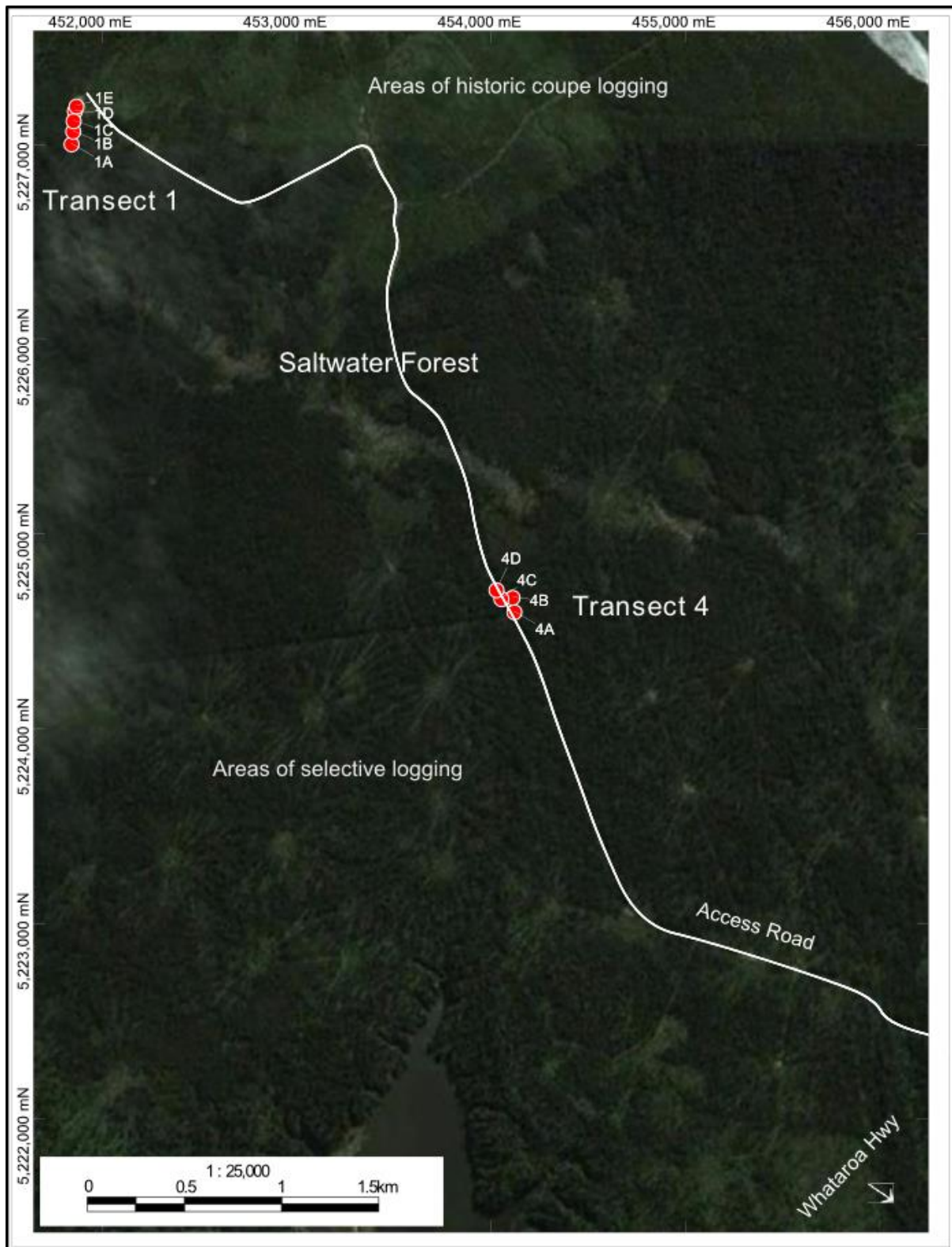


Figure 3.3 Saltwater Forest site locations (●) within each transect. Historic periods of sustainable yield logging since 1981 are shown (radial tree pattern shows past selective logging, small cleared patches shows past coupe logging). Coordinate system WGS84 (zone 59), Google Earth 2013.

3.2 Mass spectrometer preparation and analysis of leaf stable isotopic composition

Modern leaf samples collected in sealed bags were identified (Crowe 2009; Dawson *et al.* 2002; Dawson & Lucas 2012), and their wet mass was measured on a top loading balance (± 0.01 g) 1-3 weeks following field collection. Leaves were dried quickly, to a constant mass at <60 °C in a thermostatically controlled oven (≥ 72 h), to minimise decomposition processes and retain the isotopic integrity of the leaves. Dried samples were stored in sealed plastic bags in a cool dark cupboard until further processing (Poss *et al.* 2000; Hawke & Newman 2007).

Multiple leaves from each sample bag were ground together to a fine powder with liquid N using a pestle and mortar (Teece & Fogel 2004). Powders were transferred with a spatula from the pestle into sample tubes, and the equipment was thoroughly cleaned with ethanol between samples. Powdered samples were re-dried at $40-50^{\circ}\text{C}$ (≥ 24 h) to remove any moisture obtained during powdering, then sealed and stored in a cool dark cupboard for several weeks until required.

A block of preserved Pyramid Valley gel was defrosted and stored under argon gas, so that material was accessible for leaf removal, but so that any decay was prevented. Tweezers were used to extract leaves from the gel layer they were found in, which was recorded to the nearest cm in the gel block used. Fossil leaves were rinsed in MilliQ water and lightly cleaned to remove any adhering sediment (Xiao *et al.* 2013). Photographs of leaves for identification and record keeping were taken. Leaves were pooled by taxa within the layer they were found in, and were then oven dried in a thermostatically controlled oven at 50°C (≥ 42 h). Several large leaves, of unknown taxa, were not pooled as alone they provided enough material for stable isotope analysis. Small leaves of the same layer and species were ground together in a pestle and mortar with liquid nitrogen to form a fine powder, as was done for the modern leaf samples.

Powdered samples of modern and fossil material were sub-sampled by scooping a small amount (800-3000 mg) of powder from a sample tube. The sub-sample was weighed and enclosed in a tin

capsule (4x6 mm) and placed into a labelled tray ready for analysis of each tray in the mass spectrometer. Between each sample equipment was thoroughly cleaned with ethanol to prevent any cross-contamination.

Stable isotopic compositions were determined using a Thermo Finnigan Delta V plus isotope ratio mass spectrometer coupled to a Costech elemental combustion system 4010, under continuous flow conditions, in the stable isotope laboratory, Department of Geological Sciences, University of Canterbury. C and N isotope compositions were normalised to the VPDB and Air scales, based on replicate analysis of IAEA-N-1, IAEA-N-2, IAEA-CH-3, NBS-22, and an internal calibrated acetanilide standard.

3.3 Radiocarbon dating

Two individual fossil leaf samples from the highest and lowest gel layers that were sampled in this study, were submitted to the Rafter Radiocarbon Laboratory, National Isotope Center, GNS Science, for radiocarbon dating to constrain the age span of the Pyramid Valley fossil leaves. One leaf was taken from the 5th and 16th layer of the gel, which equates to a sample at 120 cm and 130 cm below the Pyramid Valley lake bed surface when the gel was excavated. Leaves were removed from the gel and placed in individual sample tubes with argon gas, to prevent decay before analysis with an accelerator mass spectrometer at GNS Science.

3.4 Scanning electron microscopy

Scanning electron microscopy (SEM) was used to photograph fossil leaf specimens to obtain a visual representation of the level of leaf preservation. Selected fossil leaves were mounted onto slides and coated in gold, then photographed with the Leica 440 Scanning Electron Microscope in the School of Biological Sciences, University of Canterbury.

3.5 Data quality control

The potential for isotopic fractionation is present at every stage and in every detail of the process of obtaining stable isotopic values, hence the need for control and international protocols for analyses (Werner & Brand 2001). International reference materials used as standards were important as relative values are more reliable and comparable than an absolute determination, which reduces any error (Werner & Brand 2001). Care was required during all stages of the research process to minimise sources of variation and error in the stable isotopic measurements.

Although the effect of natural variables (e.g. vegetation height) on stable isotopic composition will often far outweigh any mass spectrometry error, this analytical uncertainty is often overlooked by ecologists. This may have confounding effects if the variation in the comparison being measured is small (Jardine & Cunjak 2005; Hawke *et al.* 2013).

The two kinds of uncertainty that contribute to the overall error for each stable isotope measurement are the error associated with the variation within mass spectrometer sample batches, and laboratory error between batches. Within-batch variation is a combined measure of the between leaf variance, within leaf variance, processing method error, and sub-sampling error. Between-batch error is determined by comparing the control sample results across batches to ensure there was no sample concentration-dependant calibration with the mass spectrometer and to account for any differences between sample runs (Hawke *et al.* 2013).

Blind to the lab analyst, five sub-samples of 10 random single samples, were submitted for mass spectrometer measurements. These sub-samples were then compared and the variation within each sample was calculated and averaged between sub-sampled groups to give a combined measurement of the error expected within each run. Acetanilide was included several times in each mass spectrometer sample batch as the in-house reference material, and was used to determine the variability between batches to ensure no processing drift occurred with time through each batch (Werner & Brand 2001; Jardine & Cunjak 2005).

Vigorous cleaning between the grinding and massing of different samples, using ethanol, *kim* wipes and canned air was conducted to prevent cross contamination. Samples were appropriately labelled throughout the processing; including the sample bags in the field and all sample tubes in the laboratory, to further prevent any contamination.

Although ecological samples are never completely homogenous, and there will always be variance between subsamples (Jardine & Cunjak 2005), heterogeneity within each sample was decreased by using all the leaves off each branch collected, and grinding them to a fine powder.

Initial sample masses were recorded and relatively constrained for each sample to test for any effects of mass on the stable isotopic value, and obtain a mass which ensures the peak height of both C and N gasses are within an appropriate range which is similar to the standards (Jardine & Cunjak 2005). Output graphs which showed the gases released from the sample, were examined to ensure there was no drift with time during each run in the mass spectrometer (Werner & Brand 2001). Materials with high C content relative to N, such as leaves, ideally should be analysed separately to achieve greater precision, but analysing them together is more practical and is widely accepted. Since C and N were analysed together care was taken with the mass of the standards to ensure there was complete coverage of the peak height of both gasses (Jardine & Cunjak 2005).

The present stable isotopic analysis was conducted on a single mass spectrometer, as instruments are different and measurements are not consistent between different laboratories, although theoretically they should be (Werner & Brand 2001; Mill *et al.* 2008). Using one machine also minimises the time between sample runs and reduces any error associated with time dependency (Mill *et al.* 2008).

In summary, these methods show the consideration and care taken to ensure the results were reliable and influenced by the smallest amount of uncertainty and unexplained variation, as was possible.

3.6 Statistical analysis

The statistical package R 3.01 (R Core Team 2013) was used for the majority of statistical analyses, with only initial exploratory statistics conducted with Microsoft Excel 2010. Many statistical tests were used to determine if there were any significant differences between certain groups and factors, or trends within the data. Careful consideration of factors that had to be included in any models was required, to control for effects that they may have on the response variables.

Statistical analysis mainly involved comparisons between two appropriate models using mixed effects modelling. One included the factor being tested and one excluded it. If the two models were significantly different when tested with a simple ANOVA test, this indicated the factor tested was significant as there was a significant difference between at least two of the groups included in the factor. P-values quoted in the results section were from these model comparison tests, unless otherwise stated. Significance tests were conducted at the 95 % confidence level ($\alpha = 0.05$), and assumptions of the models were tested, for instance, to ensure a homogeneous variance.

The graphing programme Grapher (Grapher 2012) was used to visually represent the data. The layout of many graph axis are not conventional, as it was more intuitive that $\delta^{13}\text{C}$ was placed on the y-axis as this correlated to canopy height, which is a vertical measure (Cerling *et al.* 2004). Graphs that compare modern and fossil data have been adjusted to consider anthropogenic alteration of atmospheric $\delta^{13}\text{C}$ through the industrial revolution (Suess Effect) by increasing all modern $\delta^{13}\text{C}$ values by 1.3 ‰ (Suess 1955).

To calibrate the two radiocarbon ages to calendar years, Oxcal 4.2.3 computing software (Bronk Ramsey 2009) was used, with the SHCal13: Southern Hemisphere calibration curve (Hogg *et al.* 2013).

Samples within and between runs were analysed to calculate the amount of variation expected from these sources and to obtain an overall uncertainty amount for each measurement, as described above, which are presented in the following results section.

Chapter 4: Description of leaf composition

4.1 Introduction

Similarities and differences in foliar stable isotopic composition and C:N within and between the three modern forests and the fossil vegetation of Pyramid Valley, are described in this section. These results characterise the foliar stable isotopic composition of the selected South Island forests and compare those between the eastern and western coasts, a historically logged forest and an unlogged forest, and a pre-human fossil forest and the modern forests.

4.2 Modern forests

Within all three modern forests, both foliar $\delta^{13}\text{C}$ ($P=0.0000$, $\chi^2=145.66$, $df=6$) and foliar $\delta^{15}\text{N}$ ($P=0.0012$, $\chi^2=21.951$, $df=6$) varied significantly with vegetation height in samples taken between 0 and 3.5 m above the forest floor. However, $\delta^{15}\text{N}$ of leaf material at 0.1 m and 1.5 m was the only group which showed a significant difference ($P=0.0004$, $\chi^2=18.248$, $df=3$), so there was no distinct relationship between $\delta^{15}\text{N}$ and vegetation height in the lower 3.5 m of the forest. In contrast, foliar $\delta^{13}\text{C}$ decreased as height increased, between all three sampled height levels in all three forests, which was shown by the significantly different $\delta^{13}\text{C}$ values between each understorey level (Fig. 4.1) (0.1 m and 1.5 m: $P=0.0000$, $\chi^2=82.856$, $df=3$, 1.5 m and 3.5 m: $P=0.02006$, $\chi^2=9.831$, $df=3$, 0.1 m and 3.5 m: $P=0.0000$, $\chi^2=142.1$, $df=3$).

The C:N ratio of the vegetation within the understorey varied significantly with height in all three forests ($P=0.0023$, $\chi^2=20.494$, $df=6$). The ratio differed significantly between vegetation at 0.1 m and 1.5 m ($P=0.0133$, $\chi^2=10.721$, $df=3$), and between 0.1 m and 3.5 m ($P=0.0051$, $\chi^2=12.791$, $df=3$), but there was no significant difference between the C:N ratios of leaves at 1.5 m and 3.5 m

($P=0.1954$, $\chi^2=4.6971$, $df=3$). Even then, any significance was subtle and the effect of height within the understorey on C:N was small (Fig. 4.2), and is not dealt with further here.

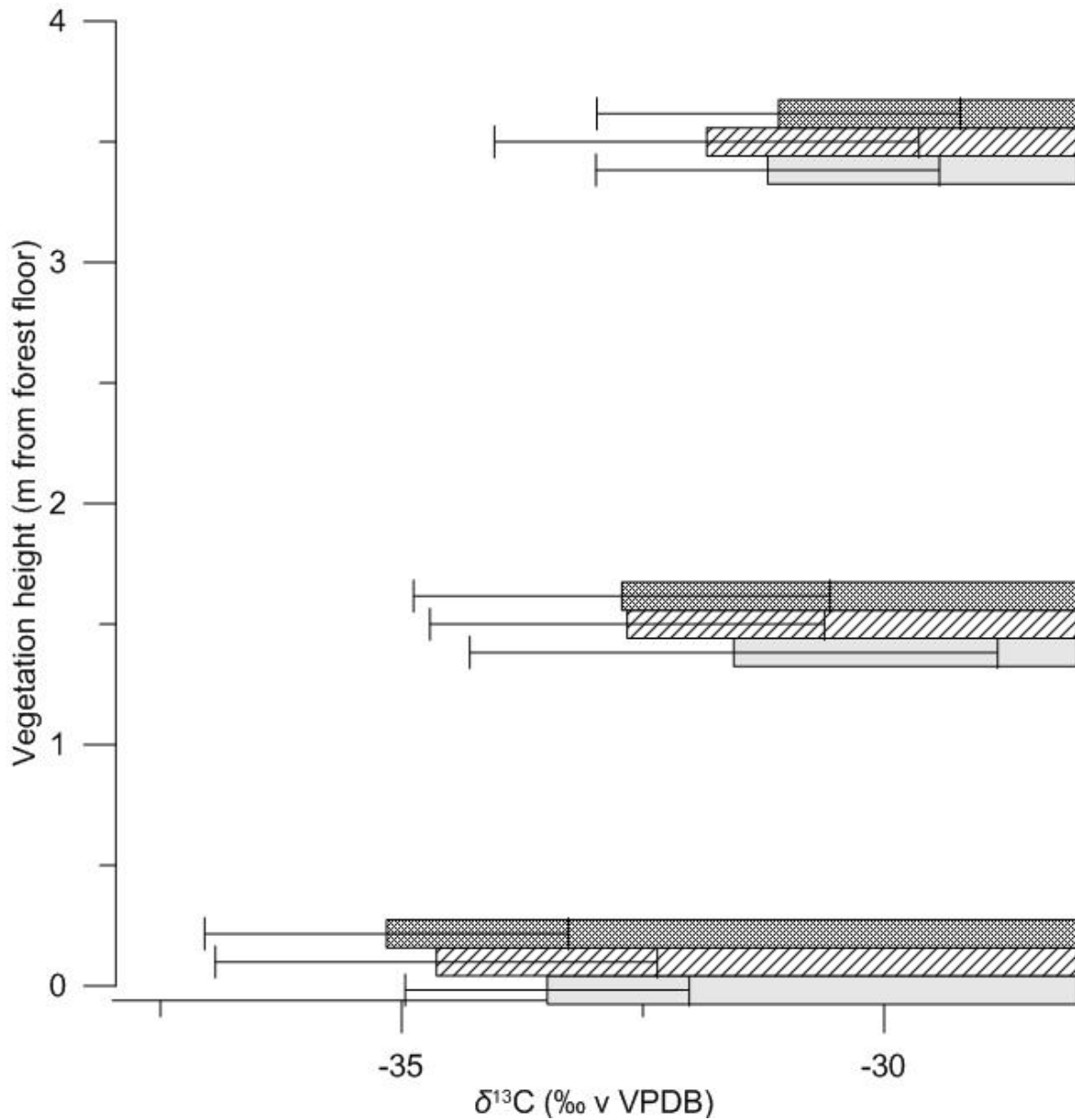


Figure 4.1 Mean foliar $\delta^{13}\text{C}$ for each vegetation height level (0.1, 1.5 and 3.5 m) in the three modern forests: Okarito Forest, open; Saltwater Forest, single-hatched; Riccarton Bush, cross-hatched.

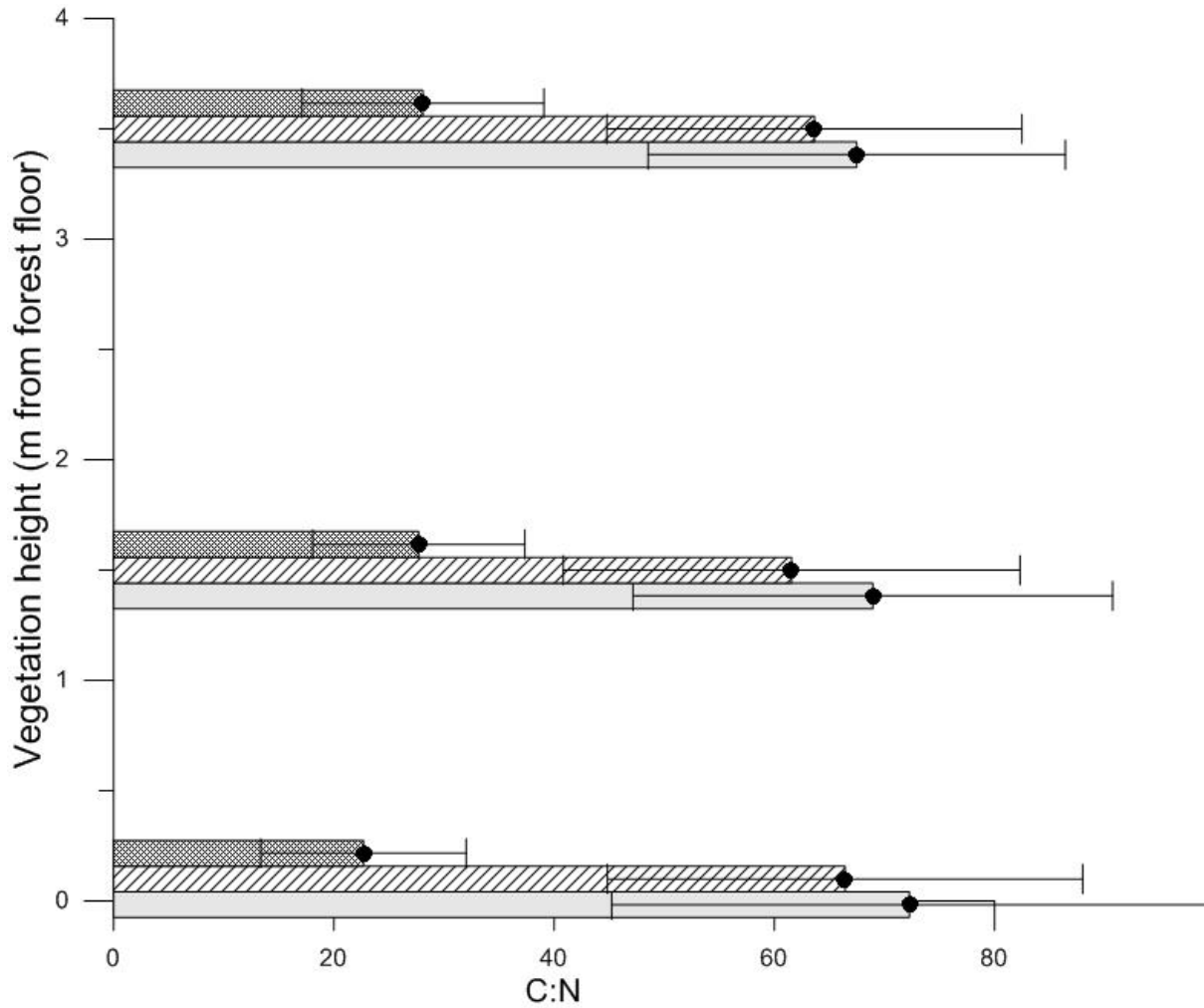


Figure 4.2 Mean foliar C:N ratio at three vegetation height levels (0.1, 1.5, and 3.5 m) for leaf samples from: Okarito Forest, clear; Saltwater Forest, single-hatched; Riccarton Bush, cross-hatched.

There were species differences in both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in leaf material within each forest (e.g., Okarito Forest, Fig. 4.3). More interspecific variation was shown in $\delta^{15}\text{N}$ (species means differed by up to 8.7 ‰), than in leaf $\delta^{13}\text{C}$ within Okarito Forest (species means differed by up to 4 ‰) (Fig. 4.3). Species was also a good predictor of the foliar C:N ratio, in all modern forests (e.g., Okarito Forest, Fig. 4.4). Types of species present differed between forests (Tab. 4.1), and sample sizes of each species were small, so their exact effect could not accurately be determined from statistical tests in this study.

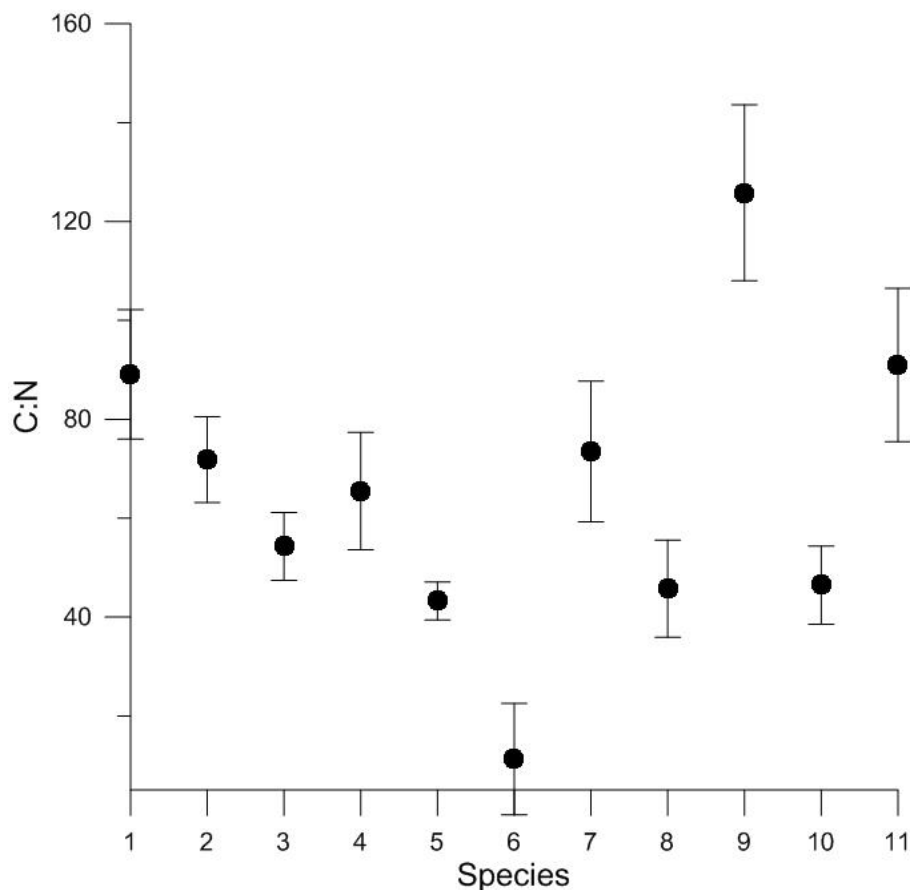
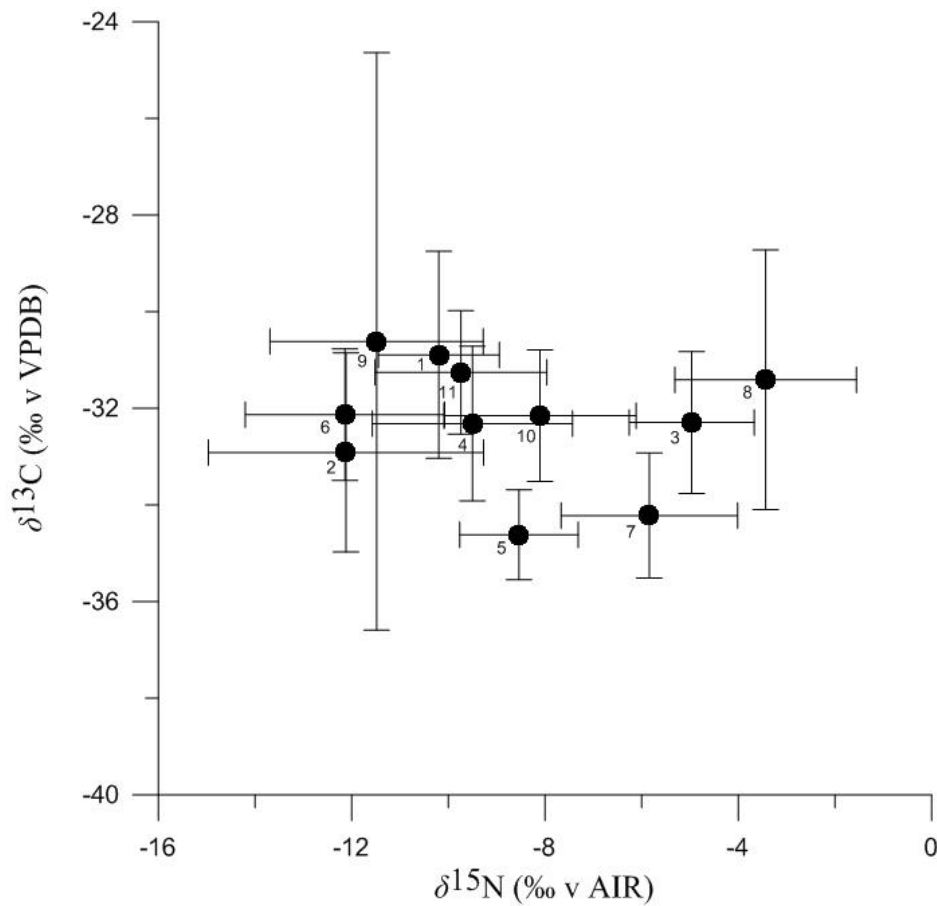


Table 4.1 Species composition differences (x indicates presence) in Okarito Forest, Saltwater Forest, and Riccarton Bush.

Scientific species name	Common name	Okarito	Saltwater	Riccarton
<i>Carpodetus serratus</i>	putaputaweta		x	
<i>Coprosma foetidissima</i>	stinkwood	x	x	
<i>Coprosma pedicellata</i>	swamp coprosma		x	
<i>Coprosma robusta</i>	karamu			x
<i>Coprosma rotundifolia</i>	round leaved coprosma			x
<i>Cordyline banksii</i>	forest cabbage tree	x		
<i>Dacrycarpus dacrydioides</i>	kahikatea			x
<i>Dacrydium cupressinum</i>	rimu	x	x	
<i>Elaeocarpus hookerianus</i>	pokaka		x	x
<i>Griselinia littoralis</i>	papauma		x	
<i>Hoheria angustifolia</i>	narrow leaved houhere		x	
<i>Kunzea ericoides</i>	kanuka	x		
<i>Leptospermum scoparium</i>	manuka	x		
<i>Lophomyrtus obcordata</i>	mapou	x	x	x
<i>Manoao colensoi</i>	silver pine	x	x	
<i>Melicope simplex</i>	poataniwha			x
<i>Melicytus ramiflorus</i>	whitey wood/ mahoe			x
<i>Metrosideros umbellata</i>	southern rata	x		
<i>Myrsine divaricata</i>	weeping matipo		x	
<i>Neomyrtus pedunculata</i>	rohutu	x	x	
<i>Parsonsia heterophylla</i>	kaihia			x
<i>Phyllocladus alpinus</i>	celery pine	x	x	
<i>Pittosporum eugenioides</i>	lemonwood			x
<i>Plagianthus regius</i>	ribbonwood			x
<i>Podocarpus totara</i>	totara	x		
<i>Prumnopitys ferruginea</i>	miro	x	x	
<i>Pseudopanax crassifolius</i>	lancewood	x	x	
<i>Pseudowintera colorata</i>	mountain pepper tree		x	
<i>Quintinia serrata</i>	tawheowheo	x	x	
<i>Raukawa simplex</i>	raukawa		x	
<i>Streblus heterophyllus</i>	milk tree			x
<i>Weinmannia racemosa</i>	kamahi	x	x	

Within-forest foliar stable isotopic variation is presented in Fig. 4.5, for each modern forest. The range of $\delta^{15}\text{N}$ values in Saltwater and Okarito was >15 ‰, but the range in leaves from Riccarton Bush showed a more confined spread of 6 ‰. The range of $\delta^{13}\text{C}$ values was c.15 ‰ in all three modern forests.

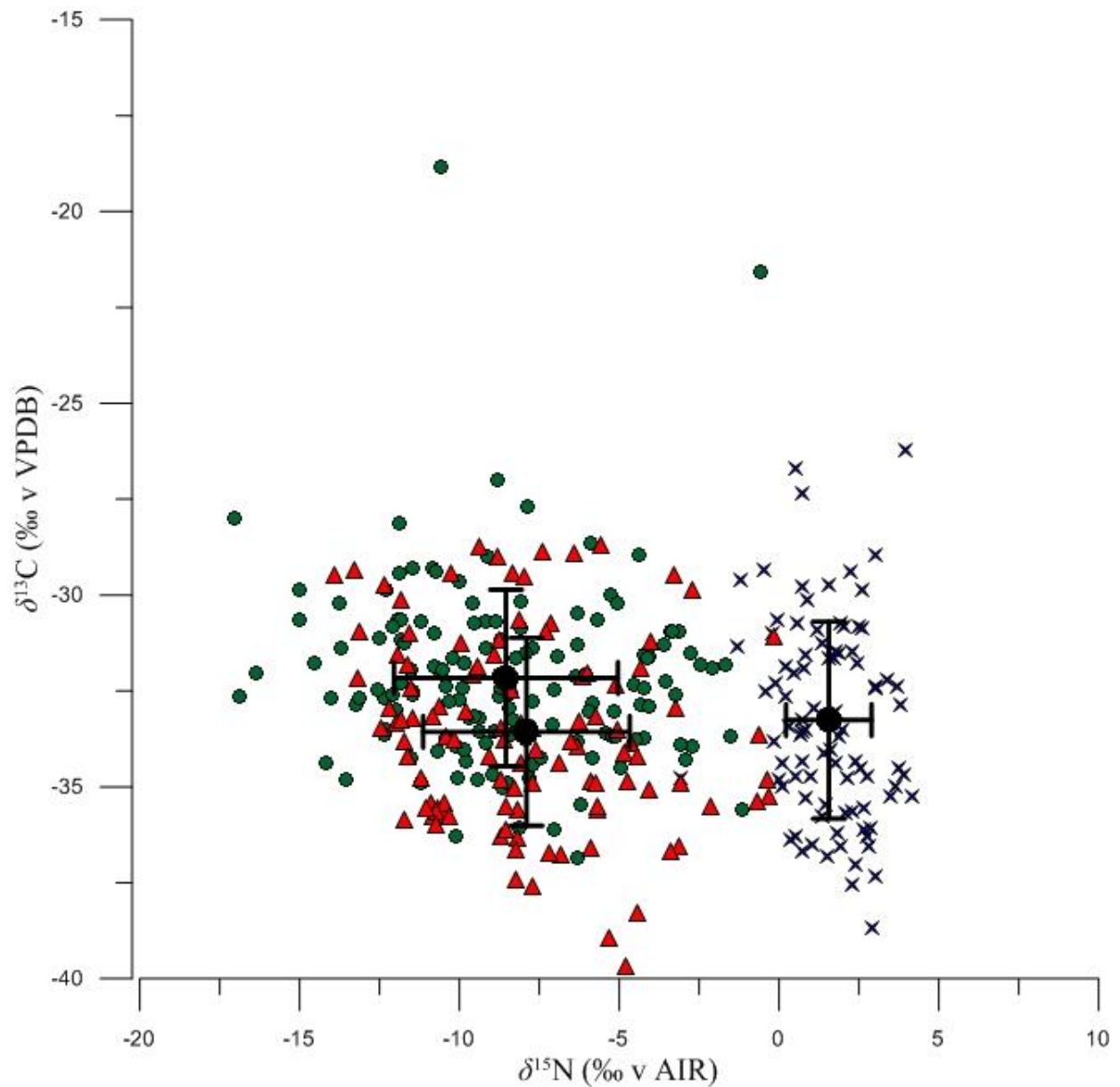


Figure 4.5 Isotope biplot (individual sample point, mean, SD) of modern foliar material for Riccarton Bush; cross, Okarito Forest; circle, and Saltwater Forest; triangle.

The significant effect of vegetation height within the forest and plant species on the response variables indicates that these factors must be controlled for in the remainder of the analysis, to avoid confusing their effects with those of other environmental factors.

4.2.1 Eastern and western forest comparison

The foliar $\delta^{13}\text{C}$ values for the three modern forests did not differ significantly ($P=0.4082$, $\chi^2=6.1357$, $\text{df}=6$) (Tab. 4.2). The positive correlation between vegetation height and $\delta^{13}\text{C}$ was detected in forests on both the wet western coast and the drier eastern coast. The vegetation at Riccarton Bush showed a greater increase in $\delta^{13}\text{C}$ with increasing height, than the vegetation in either of the West Coast forests (Fig. 4.1).

Table 4.2 Descriptive statistics of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N, for all 3 modern forests and fossil leaf material. NOTE: Modern $\delta^{13}\text{C}$ values have not been adjusted for the Suess Effect (+1.3 ‰) in this table.

Forest	<i>n</i>	$\delta^{13}\text{C}$ (mean \pm SD, ‰)	$\delta^{15}\text{N}$ (mean \pm SD, ‰)	C:N (mean \pm SD)
Riccarton Bush	89	-33.3 ± 2.6	1.6 ± 1.3	25.8 ± 10.1
Saltwater Forest	108	-33.6 ± 2.5	-7.9 ± 3.2	64.3 ± 20.9
Okarito Forest	146	-32.2 ± 2.3	-8.5 ± 3.5	69.7 ± 23.1
Pyramid Valley (Fossil)	44	-27.8 ± 1.6	3.8 ± 0.7	35.5 ± 8.7

However, the foliar $\delta^{15}\text{N}$ of vegetation from Riccarton Bush was significantly higher (>9.4 ‰) than that in either of the West Coast forests (Saltwater Forest; $P=0.0002$, $\chi^2=19.904$, $\text{df}=3$, Okarito Forest; $P=0.0004$, $\chi^2=18.157$, $\text{df}=3$) (Tab. 4.2, Fig. 4.5). The variation from species, vegetation height, transect, and site location, were all controlled for by inclusion in the mixed effects model.

Similarly, foliar C:N ratios differed significantly ($P=0.0013$, $\chi^2=13.352$, $\text{df}=2$) between the three modern forests. Leaf C:N was significantly lower in Riccarton Bush, near the East Coast than in either West Coast forest (Okarito Forest; $P=0.0002$, $\chi^2=19.238$, $\text{df}=3$, Saltwater Forest; $P=0.0009$, $\chi^2=16.552$, $\text{df}=3$) (Fig. 4.6). The lower C:N ratio showed that leaves in Riccarton Bush were richer in N than those in either Saltwater or Okarito forests on the West Coast.

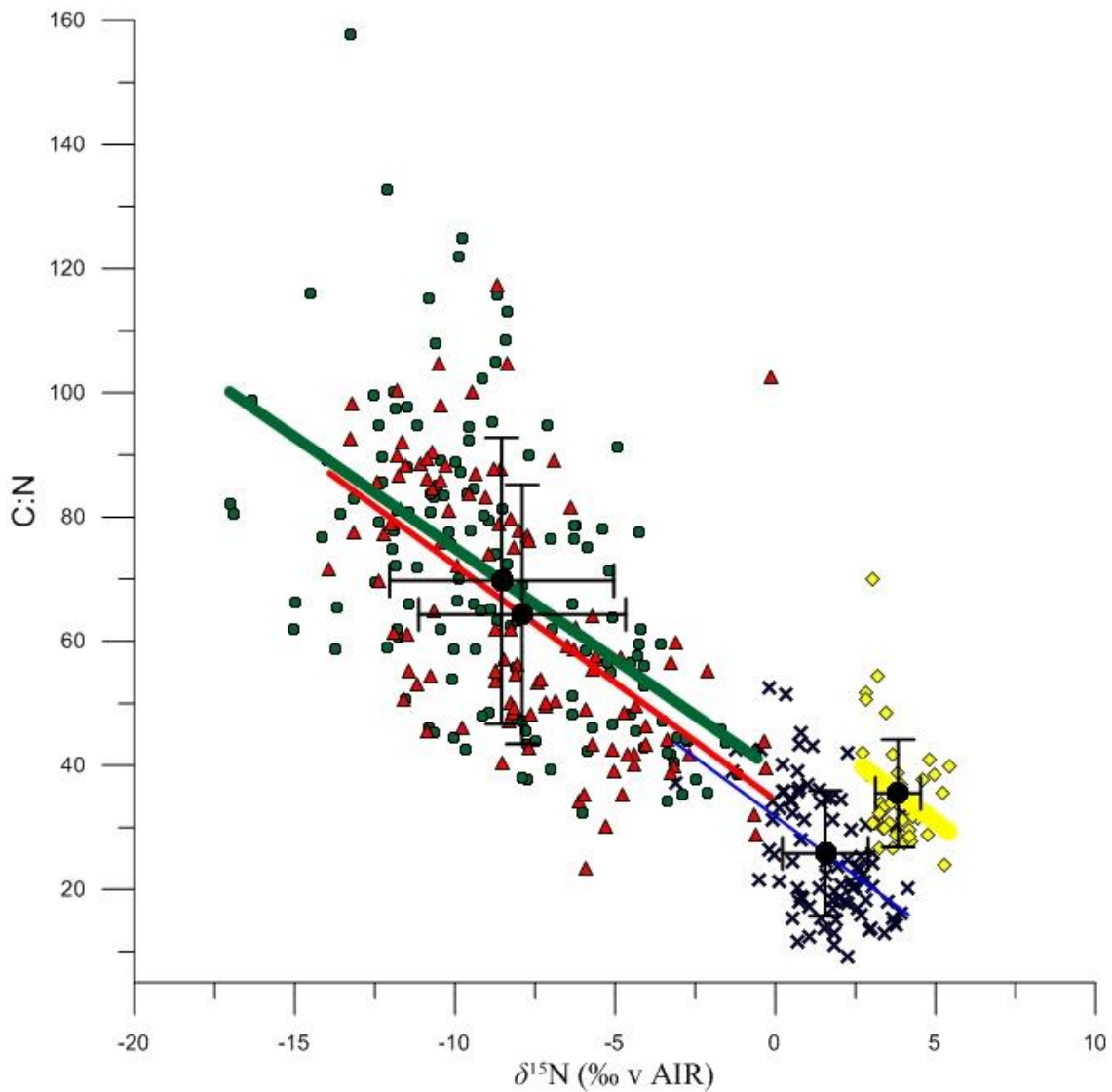


Figure 4.6 Foliar $\delta^{15}\text{N}$ correlation with foliar C:N ratios (sample values, forest mean, SD) with linear fits, for Riccarton Bush; green circles (thinnest line), Saltwater Forest; red triangles (second thinnest line), Okarito Forest; blue crosses (second thickest line), and Pyramid Valley; yellow diamonds (thickest line).

4.2.2 Comparison between the logged Saltwater Forest and the unlogged Okarito forest

Historic logging in Saltwater Forest may have caused the field-observed lower density of the undergrowth compared to the unlogged Okarito Forest. This may reflect an altered forest structure, but this study focused on quantifying possible differences in the isotopic ratios within leaves to determine if past management of the forest altered nutrient flows within the forest. Okarito Forest,

which is apparently relatively unaltered from its pre-European structure, was used as a baseline for a comparison of the logged Saltwater Forest.

There was no significant difference of foliar $\delta^{13}\text{C}$ ($P=0.5558$, $\chi^2=2.081$, $df=3$) or C:N ($P=0.4136$, $\chi^2=2.8607$, $df=3$) in leaf material between Saltwater Forest, and that from Okarito Forest (Fig. 4.6). As previously mentioned, foliar $\delta^{13}\text{C}$ was correlated with vegetation height within both forests, and although the mean $\delta^{13}\text{C}$ at each vegetation level was consistently higher in Saltwater Forest, the foliar $\delta^{13}\text{C}$ did not differ significantly between forests (Fig. 4.1). There was also no difference in foliar $\delta^{13}\text{C}$ at each height level between the logged and unlogged forest indicating any changes in forest structure were not recorded in the bottom 3.5 m of the understorey (Fig. 4.1).

There was a significant difference in $\delta^{15}\text{N}$ between vegetation in the logged Saltwater Forest and that in the unlogged Okarito Forest ($P=0.0181$, $\chi^2=10.054$, $df=3$). The Okarito Forest vegetation had a lower mean $\delta^{15}\text{N}$ than that in Saltwater Forest, therefore the unlogged forest was more depleted in ^{15}N than leaf material in the logged forest (Tab. 4.2). Although the difference was statistically significant, it was very slight (0.6 ‰) in absolute terms between the two forests (Fig. 4.5).

There were species differences between the two forests, but these were small in comparison to those between the western and eastern forests: most of the dominant species were the same in Okarito and Saltwater forests (Tab. 4.1).

4.3 North Canterbury fossil forest

The vegetation preserved in the Pyramid Valley lake bed deposit was derived from a forest that was destroyed about 700 years ago (Molloy 1969). As shown by the radiocarbon ages on leaves at the upper and lower boundaries of the sequence containing most of the vegetation, the material represents the forest surrounding the lake c. 4500 years B.P.

A comparison of the stable isotopic composition of well-preserved fossil leaf material from the Pyramid Valley deposit with the living samples from a surviving fragment of the eastern forests (Riccarton Bush) and from the two western forests, should provide information on any nutrient cycle differences between the present forests and those from a pre-human forest ecosystem. In the following section the age, stable isotopic composition and the C:N ratio of the fossil material from Pyramid Valley is described.

4.3.1 Pyramid Valley foliar stable isotopic description

Two intact leaves of matai samples extracted from laminated amorphous organic matter near the bottom of the sequence at Pyramid Valley (Johnston 2013) and subjected to the AMS ^{14}C dating at the Rafter Laboratory, GNS Science, Lower Hutt, yielded conventional radiocarbon ages of 3990 ± 63 (NZA 55053) and 3989 ± 57 (NZA 55052) years BP for leaves from 120 cm and 130 cm, respectively, below the current lake bed surface. Calibrated using the SHCAL curve (OxCal 4.2.3) (Bronk Ramsey 2009), these ages corresponded to the period 4220-4520 calendar years BP (Fig. 8A). Although the ages yielded apparently near identical calendar ages, the leaves were separated by 100 mm of laminated material. The laminae average 0.4 mm thick, which corresponds to the calculated rate of accumulation for the deposit (Johnston 2013), and so the leaves were laid down c. 250 years apart. A basic Bayesian statistical analysis (data not presented) of the calibrated ages, supports a potential time gap of that magnitude between the samples. Regardless, the ages show that the fossil material analysed represents the forest surrounding the site just over 4000 years ago.

The fossil leaf material from the Pyramid Valley deposit yielded isotopic values that were different from those in any of the three living forests (Tab. 4.2, Fig. 4.8). The $\delta^{13}\text{C}$ of samples (-27.8 ± 1.6 ‰) ranged from -24.9 ‰ to -30.5 ‰ and $\delta^{15}\text{N}$ ($+3.8 \pm 0.7$ ‰) ranged from +2.7 ‰ to +5.4 ‰ (Tab. 4.2, Fig. 4.8).

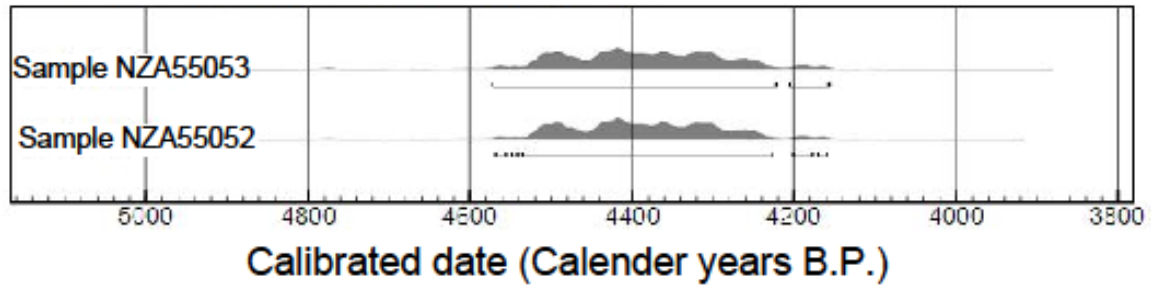


Figure 4.7 Calibration of radiocarbon dates with OxCal 4.2.3 (Bronk Ramsey 2009), using the SHCal13 atmospheric curve (Hogg *et al.* 2013), shows the likely age of each of the two samples analysed are c. 4400 Cal years B.P.

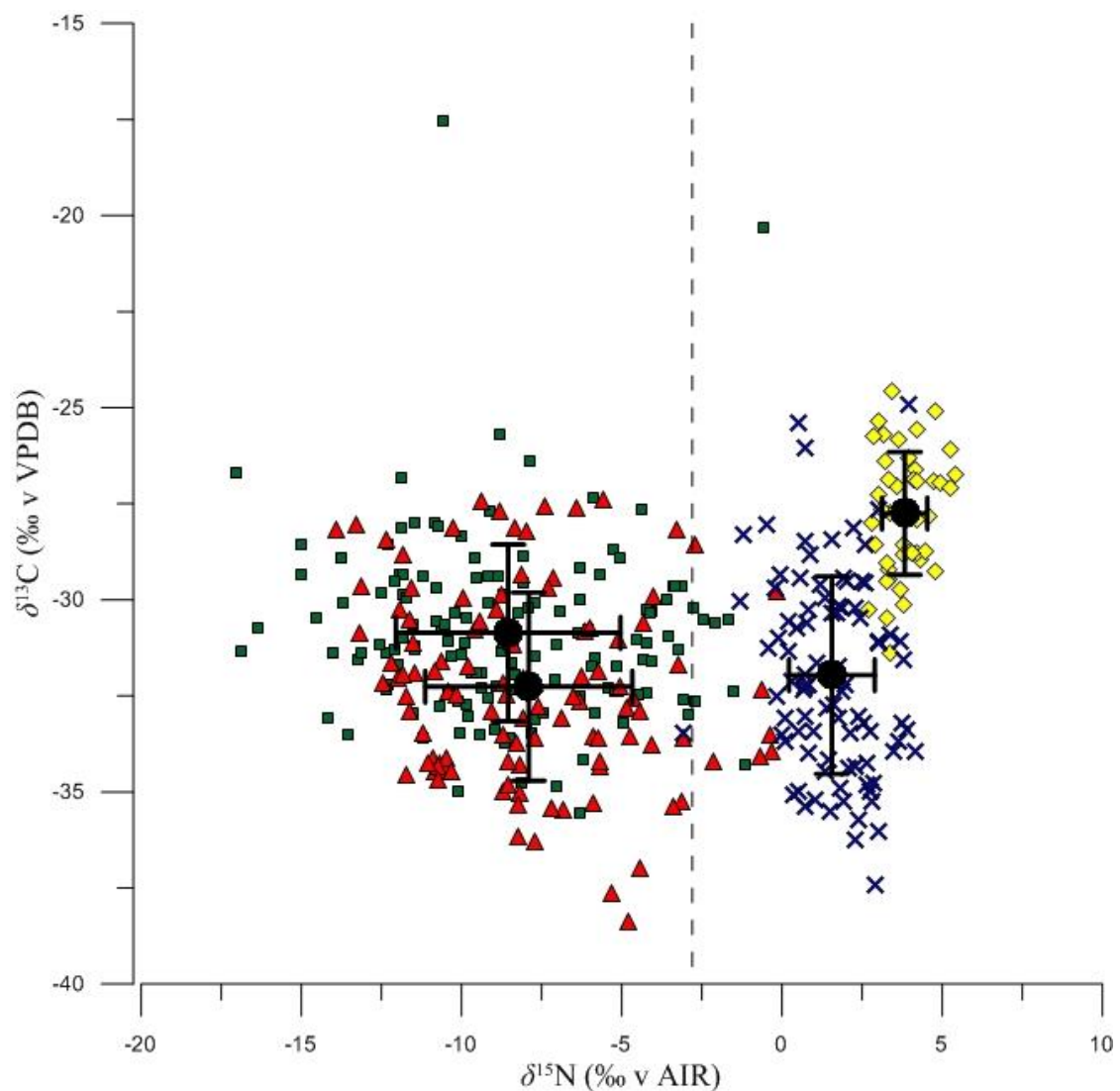


Figure 4.8 Isotope biplot (sample values, means, SD) of foliar material for Riccarton Bush; crosses, Okarito Forest; squares, Saltwater Forest, triangles, (all modern foliar $\delta^{13}\text{C}$ values account for the Suess Effect, +1.3 ‰), and Pyramid Valley fossil material, diamonds. The global temperate forest average foliar $\delta^{15}\text{N}$ (Martinelli *et al.* 1999) is shown by a dashed line.

There was no relationship between layer number (sequence of layers in the stratigraphy that the fossils were removed from) and $\delta^{15}\text{N}$ (two-way ANOVA: $P=0.1757$, $F\text{-value}=1.908$, $df=1$), or $\delta^{13}\text{C}$ (two-way ANOVA: $P=0.218$, $F\text{-value}=1.573$, $df=1$), or C:N (two-way ANOVA: $P=0.4035$, $F\text{-value}=0.714$, $df=1$) (Fig. 4.9). These results suggest that there was no change over the 250 years represented by the material. In turn, the similar values suggested that the distinctive stable isotopic composition of the leaves was not an artefact of decay. No layer-species interactions were significant either ($\delta^{15}\text{N}$; $P=0.4149$, $F\text{-value}=0.976$, $df=3$. $\delta^{13}\text{C}$; $P=0.279$, $F\text{-value}=1.332$, $df=3$. C:N; $P=0.0762$, $F\text{-value}=2.486$, $df=3$), so the entire vegetation sample appears to indicate stable N and C cycles during that period.

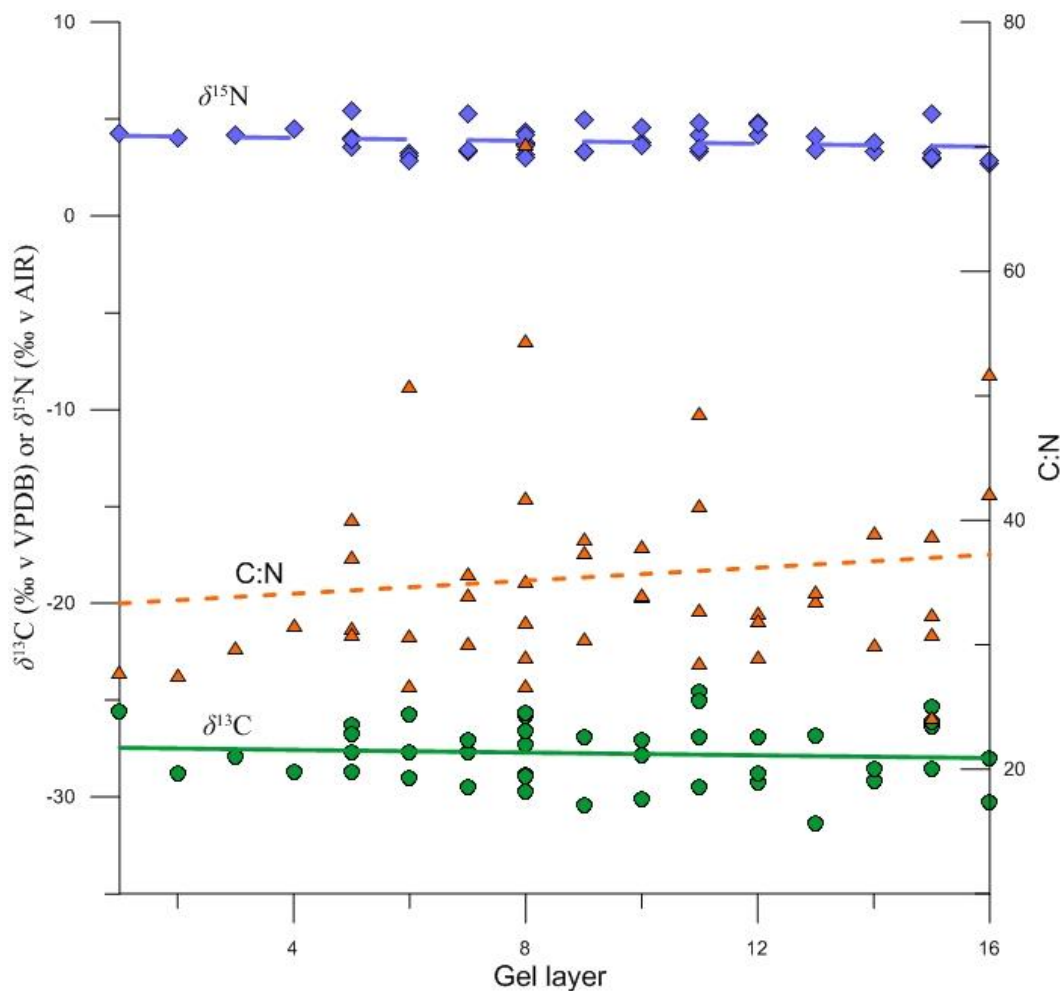


Figure 4.9 Multi-axis graph showing trends between the gel layers and foliar $\delta^{15}\text{N}$, (diamonds, long-dashed line), $\delta^{13}\text{C}$ (circles, solid line), and C:N ratios (triangles, dashed line) of fossil vegetation from Pyramid Valley.

However, there were significant differences in foliar $\delta^{13}\text{C}$ between that of the emergent podocarps (matai), a liane (*Rubus* sp.), and other pooled dicotyledonous trees and shrubs (Two-way ANOVA: $P=0.0000$, $F\text{-value}=26.901$, $df=3$). Of foliar $\delta^{13}\text{C}$ in the known species groups, the unidentified pooled species group only differed significantly from the dicots (Tukey's honest significance test, $P<0.05$ indicates a difference) (Fig. 4.10).

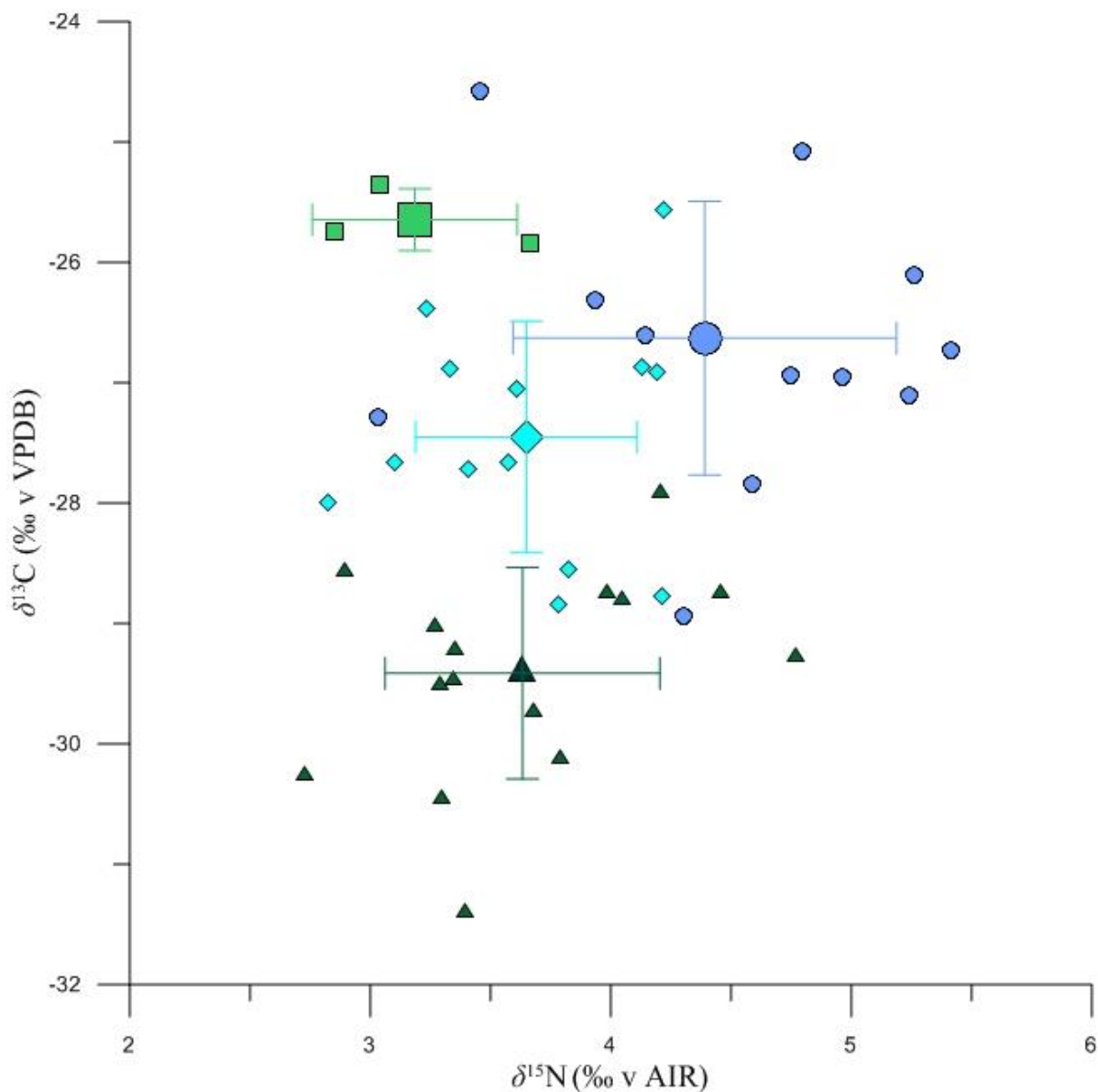


Figure 4.10 Isotope biplot of Pyramid Valley fossil vegetation (sample values, mean, SD) for each taxonomic group (dicot assemblage; triangle, matai; diamond, *Rubus* sp; square, unknown species group; circle).

Although there appeared to be significant differences in $\delta^{15}\text{N}$ between species groups (Two-way ANOVA: $P=0.0032$, $F\text{-value}=5.528$, $df=3$), the unknown species group was the only group that significantly differed from the known taxa groups, so from the known fossil taxa there was no effect of species group on $\delta^{15}\text{N}$ in the fossil leaves (Tukey's honest significance test, $P<0.05$ indicates a difference) (Fig. 4.10).

There were no differences in C:N between the fossil species sampled (Two-way ANOVA, $P=0.0695$, $F\text{-value}=2.569$, $df=3$) (Fig. 4.11), but a larger sample size may have demonstrated some difference because the significance level was very close to 0.05.

Scanning electron microscopy showed the exceptional level of leaf preservation of fossil material, as leaves were intact and showed structures such as stomata (Fig 4.12).

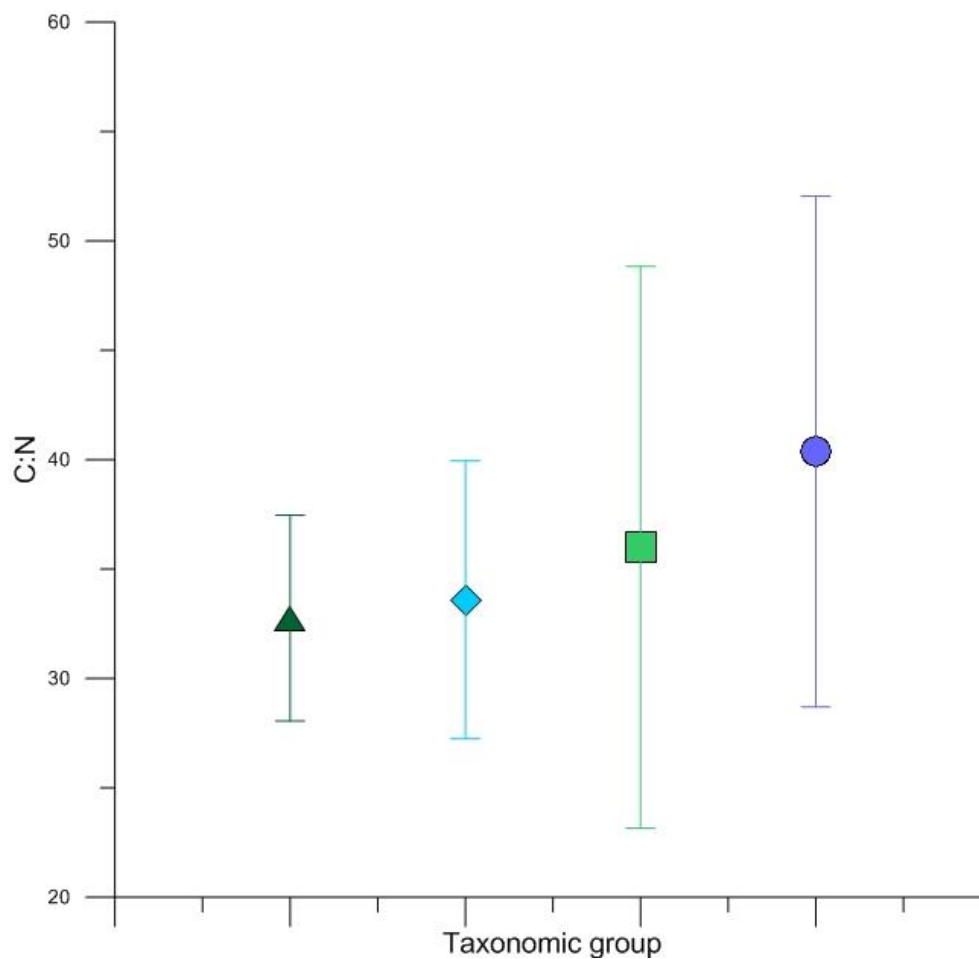


Figure 4.11 Pyramid Valley fossil leaf C:N values (mean, SD) of taxonomic groups (dicot assemblage; triangle, Matai; diamond, *rubus* sp.; square, unknown species group; circle).

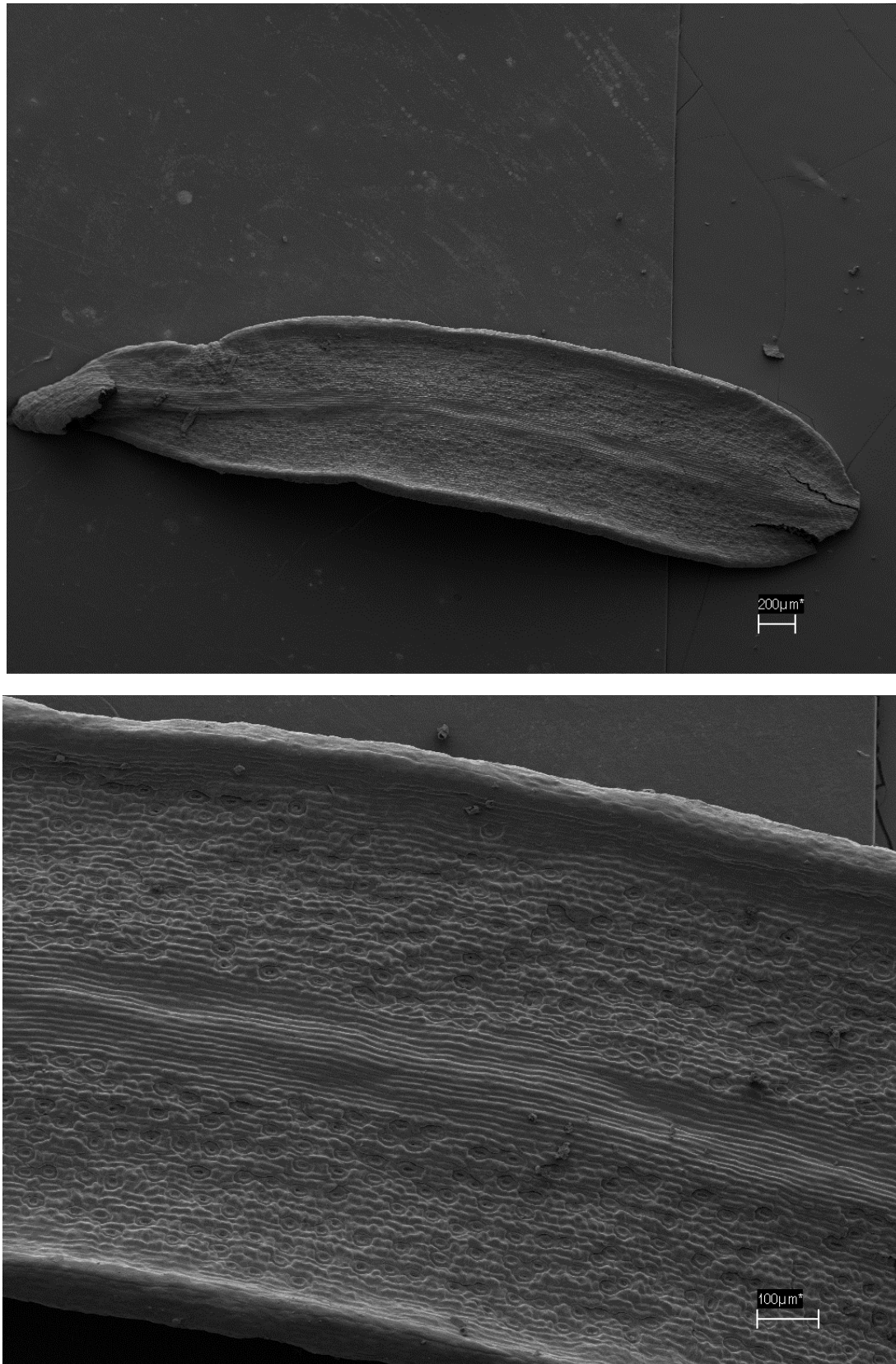


Figure 4.12 Scanning electron microscopy images showing the exceptional preservation of a matai leaf with well-defined stomata and guard cells.

4.3.2 Comparison of fossil vegetation with modern vegetation

The fossil vegetation from 4000 years B.P. at Pyramid Valley had higher values of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than the vegetation in the modern eastern forest remnant, Riccarton Bush (Fig. 4.8). Two outliers in the Riccarton bush data lay within the range of the fossil material, but the means were highly significantly different (Fig. 4.8).

In comparison to the temperate $\delta^{15}\text{N}$ global average collated from many studies (Martinelli *et al.* 1999), the western forests had lower $\delta^{15}\text{N}$ values, and the eastern forests had higher $\delta^{15}\text{N}$ values (Fig. 4.8).

Common features of all three modern forests included the significant negative correlation between foliar $\delta^{15}\text{N}$ and C:N (Fig. 4.6), which showed there is likely to be a relationship between these variables irrespective of environmental differences.

A comparison between the C:N ratio of foliage from the three modern forests and the fossil material showed that the Pyramid Valley leaves were most similar in their C:N to those from Riccarton Bush (Tab. 4.2, Fig. 4.6). The fossil leaves had a higher mean C:N (so lower N availability) (35.5 ± 8.7) in comparison to leaves from Riccarton Bush (25.8 ± 10.1), but the mean was still within the range for Riccarton Bush C:N (Fig. 4.6). Foliage from Saltwater Forest (64.3 ± 20.9) and Okarito Forest (69.7 ± 23.1) had higher mean values of C:N than either Riccarton Bush and Pyramid Valley (Fig. 4.6).

4.4 Measurement uncertainty

The combined within- and between-run errors give the total error expected for each stable isotopic value. The within-run variation includes within leaf heterogeneity, between leaf heterogeneity, and processing heterogeneity and was calculated from the standard deviation of each group of sub-samples, which yielded an average standard deviation of 0.21 ‰ for $\delta^{13}\text{C}$ and 0.19 ‰ for $\delta^{15}\text{N}$ (Tab.

4.3). Between-run variation was calculated from the stable isotopic values of an acetanilide standard, which was measured several times in all runs. The standard deviations of the means from multiple samples in all 8 runs were 0.25 ‰ for $\delta^{13}\text{C}$ and 0.02 ‰ for $\delta^{15}\text{N}$. Together, these results gave an overall analytical measurement uncertainty of 0.28 ‰ for $\delta^{13}\text{C}$ and 0.19 ‰ for $\delta^{15}\text{N}$, which were negligible in comparison to environmental effects measured in leaf stable isotopic ratios.

The standard deviation of the mean C:N values for each set of sub-samples was between 0.83 and 8.23 (Tab. 4.3) which gave a mean standard deviation of 3.81. The variation for each sample is shown in Fig. 4.13.

Table 4.3 Analytical statistics for each set of 5 sub-samples, taken from a random selection of samples in the present study.

Sample number	Species	Mean $\delta^{13}\text{C}$ (‰ v VPDB)	SD of $\delta^{13}\text{C}$ (‰ v VPDB)	Mean $\delta^{15}\text{N}$ (‰ v Air)	SD of $\delta^{15}\text{N}$ (‰ v Air)	Mean C:N	SD of C:N
Modern leaf material							
T1A8	<i>Griselinia littoralis</i>	-30.71	0.34	-7.16	0.23	50.10	5.99
T2A5	<i>Phyllocladus alpinus</i>	-33.24	0.13	-8.35	0.39	112.94	2.68
T2C4	<i>Leptospermum scoparium</i>	-32.49	0.09	-7.03	0.15	76.44	3.63
T2D8	<i>Kunzea ericoides</i>	-33.05	0.19	-5.93	0.25	58.52	3.05
T3C13	<i>Dacrydium cupressinum</i>	-33.68	0.20	-5.21	0.16	71.20	5.65
T3D14	<i>Kunzea ericoides</i>	-33.02	0.78	-5.14	0.32	55.00	3.18
T4A17	<i>Phyllocladus alpinus</i>	-28.73	0.39	-9.38	0.10	87.05	8.23
T4A20	<i>Weinmannia racemosa</i>	-30.95	0.17	-13.15	0.23	77.44	5.67
T4C6	<i>Quintinia serrata</i>	-34.86	0.25	-5.91	0.21	48.90	4.62
T5C3	<i>Pittosporum eugenioides</i>	-27.33	0.27	0.73	0.12	17.98	1.50
T6A14	<i>Coprosma robusta</i>	-30.71	0.34	0.55	0.22	32.23	0.83
Fossil leaf material							
L8unk.B	Unknown sp.	-26.61	0.49	4.14	0.34	34.97	2.17
L13matai	<i>Prumnopitys taxifolia</i>	-26.87	0.12	4.13	0.25	34.15	2.31

To ensure there was no dependence of each response variable on the standard deviation of each set of sub-samples, the relationships were graphed and linear regression tests performed: if the slope of the regression differed significantly from 0, at the significance level of 0.05 (α) there was a relationship. The $\delta^{13}\text{C}$ value did not depend on the within-sample variation ($P=0.7478$, $F=0.1087$, $df=1$) (Fig. 4.14), nor did the $\delta^{15}\text{N}$ ($P=0.6682$, $F=0.1940$, $df=1$) (Fig. 4.15), which shows the variances were not affected by the size of the isotopic signal. Conversely, the within-sample variation and the mean were correlated in the C:N ratio (Fig. 4.16), which suggested that C:N values were less robust than the stable isotope values, as the amount of variation within the sample depends on the value of the sample. None of the linear regressions violated any of the assumptions required for parametric tests.

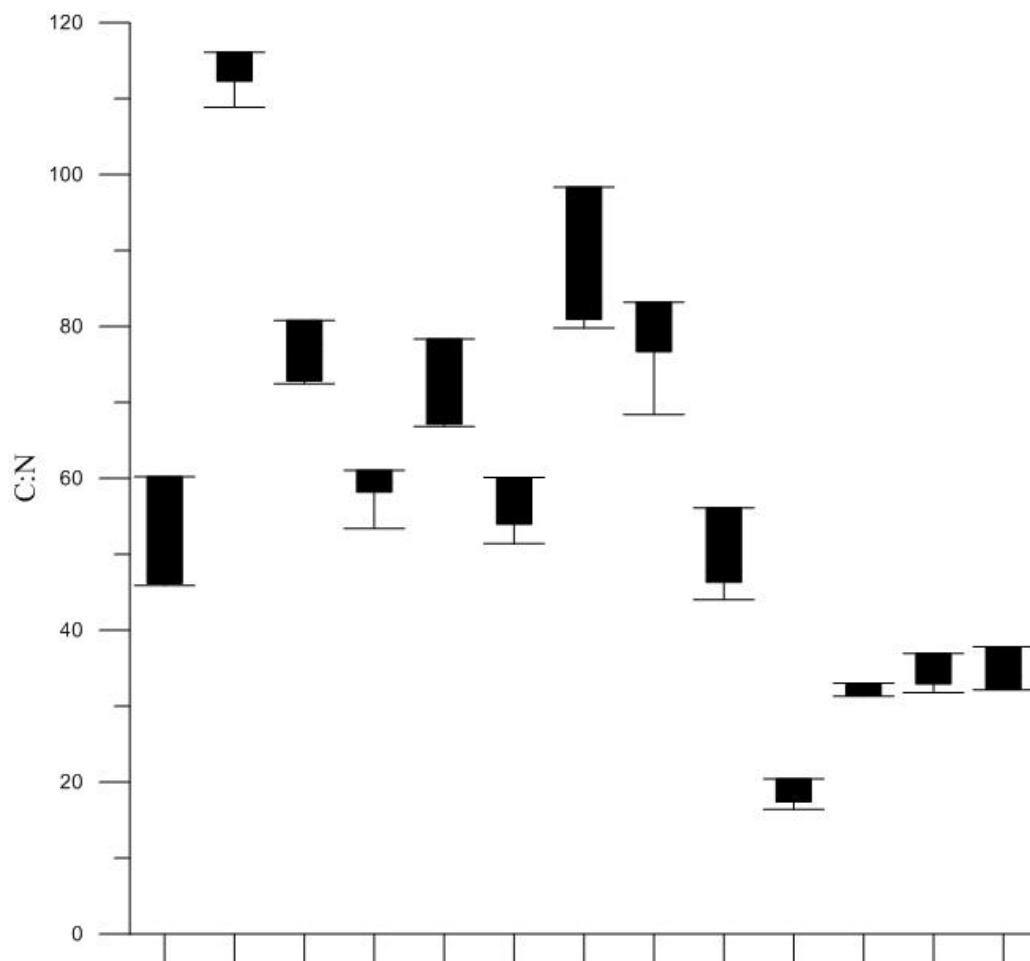


Figure 4.13 Box plot of C:N for each set of sample replicates (each sample has 5 sub-samples) for 12 random vegetation samples.

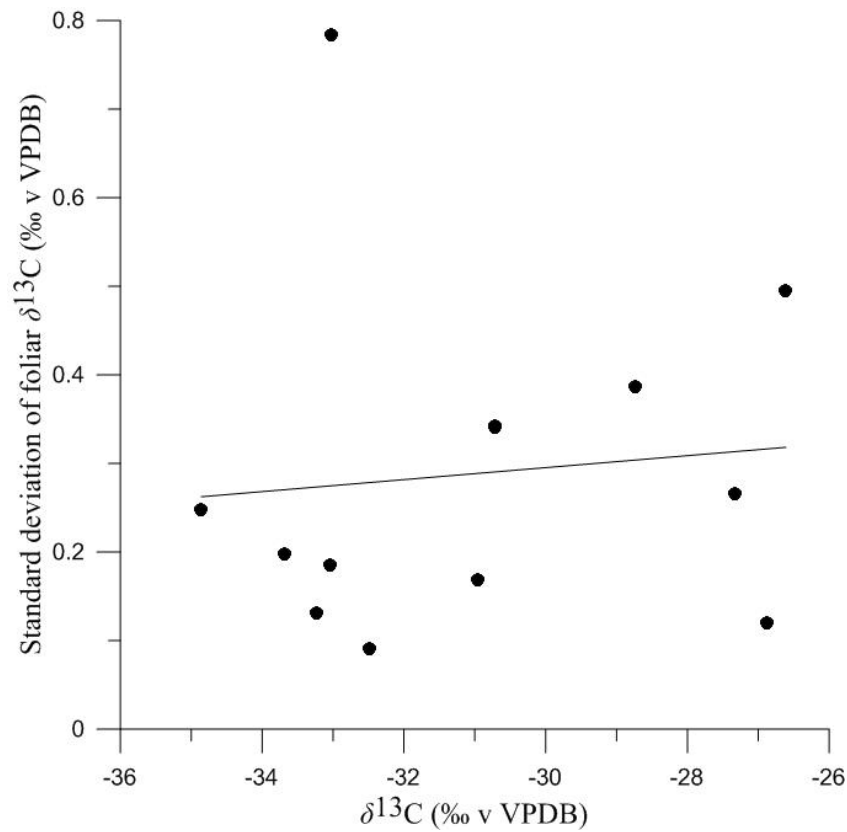


Figure 4.14 Mean foliar $\delta^{13}\text{C}$ vs. standard deviation for each set of 5 subsamples, with a linear regression line fitted.

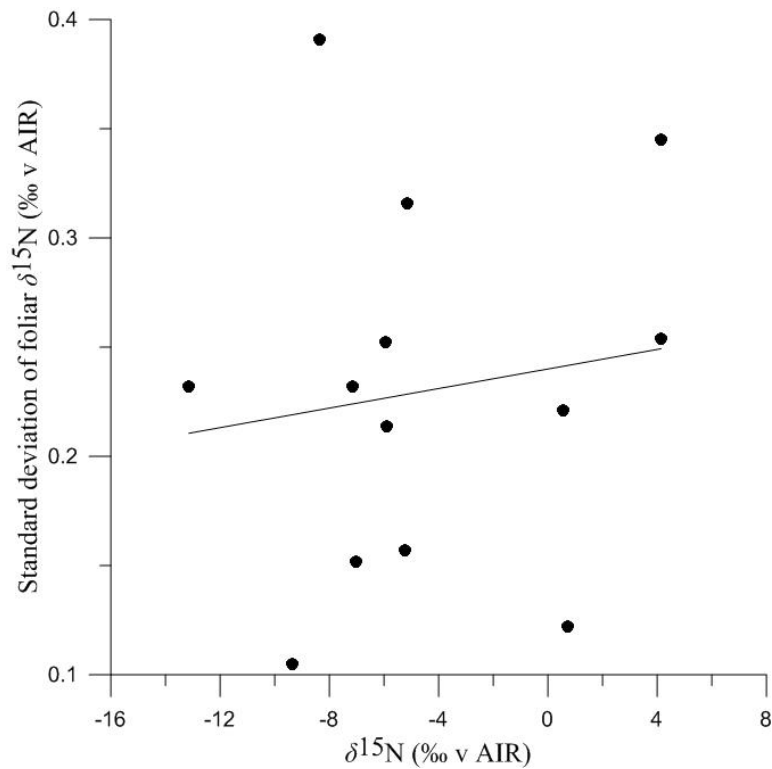


Figure 4.15 Mean foliar $\delta^{15}\text{N}$ vs. standard deviation for each set of 5 subsamples, with a linear regression line fitted.

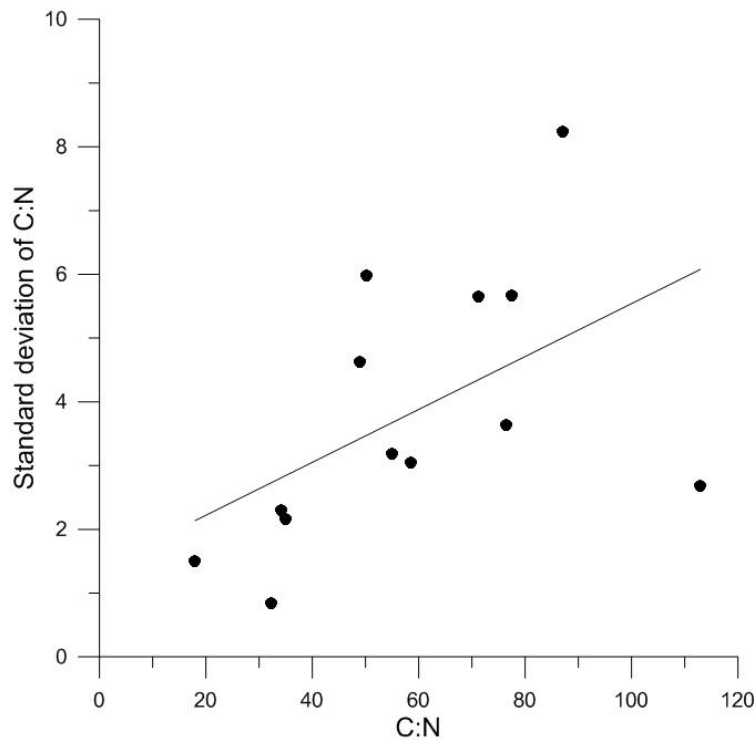


Figure 4.16 Mean C:N ratio vs. standard deviation for each set of 5 sub-samples, with a linear regression line fitted

4.5 Summary

In summary, foliar $\delta^{13}\text{C}$ did not vary between any of the forests, but vegetation height and species explained significant amounts of the $\delta^{13}\text{C}$ variation. There were significant differences in the foliar $\delta^{15}\text{N}$ values of understorey vegetation in the modern forests between eastern and western forests, as well as between the logged and unlogged forests. Foliar $\delta^{15}\text{N}$ values varied slightly with species, but there was no effect of vegetation height above the forest floor.

Vegetation height and species also explained significant amounts of the variation in C:N values of leaf material in all three modern forests. The eastern and western forest vegetation differed significantly in their C:N values, but there was no difference in the ratio between understorey vegetation in the logged and unlogged forests.

The fossil leaves were likely to have been deposited in Pyramid between 4220 and 4520 calendar years B.P. The stratigraphic layer, and hence time spent in situ, had no effect on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, or C:N

values. Species- or groups of species had a significant effect on the $\delta^{13}\text{C}$ values of the fossil leaves. In comparison with modern leaves, the fossil leaves had higher values of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than vegetation in any of the modern forests. Fossil material C:N (and N availability) was higher than Riccarton Bush, but lower than in the vegetation from the two West Coast forests. Leaf $\delta^{15}\text{N}$ and C:N were correlated in all forests in the present study, including the fossil vegetation.

Measurements of standard materials and on the variance within and between mass spectrometer runs, showed only a very small variation in measurements which was attributable to natural sample variation and processing error for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Here, the results of this study were presented, in the following chapter the significance of these results are discussed in relation to the original study objectives.

Chapter 5: Discussion

5.1 Introduction

New Zealand has unique native forests whose area has been reduced and the structure and composition degraded by the activities of humans and their commensal animals since the arrival of Polynesians c. 700 years ago. The protection of the remaining native forests depends on the knowledge of forest population dynamics and nutrient cycles to inform their conservation (McGlone 1989; Ewers *et al.* 2006). Often studies focus on forest composition, but few focus on the invisible underlying ecosystem processes that control the structure and composition, such as C and N cycling (Hobbs & Norton 1996). As foliar $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N can be used as integrators of the C and N cycles (Rounick & Winterbourn 1986; Robinson 2001; West *et al.* 2006), the present study measures these values in forests within different precipitation regimes and different histories of anthropogenic alteration within the South Island.

Field and laboratory studies elsewhere have shown that foliar $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N are controlled by many environmental variables, even though the effect of which is ultimately controlled by internal leaf variables at a cellular level (e.g., Heaton 1999; West *et al.* 2006). Interpretations from previous studies were used here to suggest likely controls on the stable isotopic and C:N variation within and between the understorey vegetation in different forest ecosystems, to further the understanding of N and C cycling. This study contributes information of global interest by characterising the stable isotopic composition at several heights in the understorey, in wet and dry forests and forests at varying levels of human-mediated degradation. A new stable isotopic record is revealed in pre-human vegetation, one that is considerably different to any known present New Zealand forest.

5.2 Foliar $\delta^{13}\text{C}$ values

The most consistent variation in leaf $\delta^{13}\text{C}$ is between those from plants of different photosynthetic pathways, as values for C_3 species typically range between -24 to -34 ‰, and in C_4 species from -6 to -19 ‰ (Smith & Epstein 1971). The New Zealand vegetation is dominated by C_3 vegetation: in the present study, foliar $\delta^{13}\text{C}$ values ranged from -18.8 ‰ to -39.7 ‰, values typical of C_3 species, perhaps with a somewhat larger range than usual. Foliar $\delta^{13}\text{C}$ is a combined measure of internal plant physiological properties, including photosynthetic pathway, as well as external environmental characteristics (O'Leary 1981). The uptake of atmospheric CO_2 is governed by the opening of leaf stomata, which is affected by water stress, N limitation, and light levels, which are controlled by environmental variables: all therefore influence foliar $\delta^{13}\text{C}$ values. The extent of the responses to such environmental variables differs between plant taxa, -which explains the wide $\delta^{13}\text{C}$ variation not just between species of different photosynthetic pathways but also between C_3 species (O'Leary 1981).

Foliar $\delta^{13}\text{C}$ values ranging from -33 ‰ to -26 ‰ have been measured in New Zealand commercial pine forests in Waikato, which have a moderate annual rainfall of 1200 mm (Waring & Silvester 1994). In comparison to this pine forest data, those from the present study include a larger range of values because they cover a wider range of environmental conditions between forests and a larger number of taxa (Körner *et al.* 1991; Heaton 1999). Foliar $\delta^{13}\text{C}$ values from podocarp/broadleaf forest at Mason Bay, Stewart Island, and tupare (*Olearia lyallii*) forest at The Snares Islands, NE of New Zealand (with a large contribution of guano from the breeding seabird population), showed a similar range of values to the pine forest study and the present study (-32.7 ‰ to -24.6 ‰)(Hawke & Newman 2007). Together these studies characterise the general range of expected foliar $\delta^{13}\text{C}$ values in New Zealand forests.

The frequently described 'canopy effect', where foliar $\delta^{13}\text{C}$ increases as vegetation height increases, reaching a maximum in the canopy leaves (Medina & Minchin 1980; van der Merwe & Medina 1991;

Garten & Taylor 1992; Cerling *et al.* 2004; Ometto *et al.* 2006; Kranabetter *et al.* 2010; Crowley *et al.* 2012), was detected in the understorey vegetation in all three modern forests in the present study. The greater amount of respired CO₂ depleted in ¹³C (particularly from soil respiration) and the lower light levels which alter C isotope fractionation during carboxylation, in vegetation at low heights, causes lower vegetation $\delta^{13}\text{C}$ values at lower heights in forests (Silveira *et al.* 1989; Garten & Taylor 1992). The foliar $\delta^{13}\text{C}$ values in the Ituri Forest, Democratic Republic of Congo ranged from an average of -25 ‰ at the top of the canopy to an average of -36 ‰ in the understorey (Cerling *et al.* 2004), and on the Rio Negro tributary of the Amazon they ranged from -27 ‰ to -31 ‰ at the top of the canopy to -27 ‰ to -36 ‰ in the understorey (Medina & Minchin 1980; van der Merwe & Medina 1991). Although most canopy effect studies, including the Ituri Forest and Amazon, cover the entire forest height, the present study shows that the 'canopy effect' was present even within the bottom 3.5 m of the understorey.

Taxon is known to affect the $\delta^{13}\text{C}$ values of vegetation (Smedley *et al.* 1991, Garten & Taylor 1992, Stewart *et al.* 1995; Marshall *et al.* 2007), often accounting for 4 ‰ of variation in $\delta^{13}\text{C}$ within forests (Körner *et al.* 1991). Stable isotopic variation resulting from differences in tree assemblage composition was observed in the present study. It was not possible, however, to determine the contribution of taxon differences to the total $\delta^{13}\text{C}$ variation because the forests differed significantly in species composition, and too many taxa were included with different amounts of each sampled. However, of the taxa data obtained the variation between taxa in Okarito Forest suggests that this variable accounted for a maximum of c. 5 ‰ variation in $\delta^{13}\text{C}$. Defining the inter-taxon variation was explored to control for the effect of species on stable isotopic variation between logged and unlogged and between eastern and western forests.

5.3 Foliar $\delta^{15}\text{N}$

As with $\delta^{13}\text{C}$, different taxa exhibit different $\delta^{15}\text{N}$ isotopic signals as a consequence of different structural and physiological mechanisms of N uptake (Callesen *et al.* 2013). The $\delta^{15}\text{N}$ of soil N pools control foliar $\delta^{15}\text{N}$, because each pool has a different isotopic signature, and trees may access a variety of N pools (Evans 2001). Many spatially variable environmental factors fluctuate over short spatial distances which alter N pools and N availability within soil, and in turn influence foliar $\delta^{15}\text{N}$, making it difficult to identify the drivers of foliar $\delta^{15}\text{N}$ variation (Mariotti *et al.* 1981; Kahmen *et al.* 2008).

Different taxa exhibit different leaf $\delta^{15}\text{N}$ values (Michelsen *et al.* 1996; Templer *et al.* 2007; Kahmen *et al.* 2008; Callesen *et al.* 2013), and the differences typically account for < 5 ‰ of the variation in $\delta^{15}\text{N}$ in tropical and temperate ecosystems. However, in low N ecosystems such as Arctic tundra species differences may reach 10 ‰ (Naelhoffer *et al.* 1996; Wang *et al.* 2014). Taxa variation is greater in N limited forests (Schulze *et al.* 1994; Naelhoffer *et al.* 1996) which may account for the variation of up to 9 ‰ between taxa in the N limited West Coast forests. However, there were insufficient data on individual taxa to determine any taxon effect in the present study, or if the same species differed in $\delta^{15}\text{N}$ between forests.

Martinelli & Piccolo (1999), based a review on many individual studies, which provided a mean value of $\delta^{15}\text{N}$ for temperate forests of -2.8 ± 2 ‰, 4 ‰ less than the mean foliar $\delta^{15}\text{N}$ in the Riccarton Bush understorey ($+1.6 \pm 1.3$ ‰) and > 5 ‰ higher than that in understorey of the Saltwater Forest (-7.9 ± 3.2 ‰) and Okarito Forest (-8.5 ± 3.5 ‰). All three forest sites in the present study are in “temperate” latitudes, so generally would be expected to have $\delta^{15}\text{N}$ values close to the temperate forest average. Foliar $\delta^{15}\text{N}$ values in tropical forests average $+3.7 \pm 3.5$ ‰ (Martinelli *et al.* 1999), almost identical to the values measured in the well-preserved leaves from Pyramid Valley ($+3.8 \pm 0.7$ ‰). However Pyramid Valley is at nearly the same (temperate) latitude as the modern forests in this study and was certainly not a tropical forest, even though the physiognomy of the forest trees so far

as the vegetation is known, was more akin to those of tropical than classical temperate forests (McGlone 1989).

African and Amazonian forests exhibit similar $\delta^{15}\text{N}$ values to those in Riccarton Bush and Pyramid Valley. Foliar material from a variety of arid environments in South Africa, each receiving 50 to 1300 mm of rainfall annually, has $\delta^{15}\text{N}$ values from -1 ‰ to 6 ‰ (Heaton 1987). An Amazonian lowland tropical rainforest (5° north of the equator and receives 2200 mm of rainfall each year) ranges from -0.3 ‰ to + 3.2 ‰ (Guehl *et al.* 1998). However, foliar $\delta^{15}\text{N}$ in the Ituri Forest (a multi-tier forest about 1° north of the equator) averages + 5.4 ‰ (Cerling *et al.* 2004), which is higher than any of the $\delta^{15}\text{N}$ means of that in the present study. Evergreen species within high latitude taiga forests (Alaska, 64° north of the equator) exhibit average $\delta^{15}\text{N}$ values of -3 ‰ to -9 ‰ (Kielland *et al.* 1998), often averaging -7 ‰ because of N limitation in these high latitude forests (Schulze *et al.* 1994). In comparison to the low latitude evergreen West Coast forests these values from the N limited taiga forests were very similar. All of these studies give an indication of the variation of global forest foliar $\delta^{15}\text{N}$, and in particular show the low foliar $\delta^{15}\text{N}$ values in the West Coast are unusual for temperate forests, but very similar to that found in high latitude boreal N limited forests.

Broadleaf vegetation in New Zealand on unfertile sand dunes on Stewart Island had a low $\delta^{15}\text{N}$ mean value of -6.1 ‰ (Hawke & Newman 2007). Although a different life form to trees, lichens and algae in the Rotorua geothermal area had exceptionally low $\delta^{15}\text{N}$ values typically -20 ‰ because this vegetation was strongly reliant on atmospheric N assimilation (Tozer *et al.* 2005). Both of these studies were conducted in low-N available environments, indicating this reduces $\delta^{15}\text{N}$ values and that other depleted environments are present in New Zealand, although likely for different reasons than the West Coast forests, which has N limited leached soils from high annual precipitation.

5.4 Foliar C:N

Leaf C:N values indicate forest N availability, as high ratios such as those in tundra environments reflect low N-availability and low ratios indicate high N-availability (Vitousek 1982; Naelehofer *et al.* 1996; Hobbie *et al.* 2000; Tozer *et al.* 2005; Hawke & Newman 2007). Leaf C:N ratios are indicative of ecosystem C:N. If a favourable nutrient composition is present, this will flow through the ecosystem and benefit the health of its constituents, for example, increased macronutrient intake enhances the reproduction success of a predatory insect (Barry & Wilder 2013).

C:N ratios of modern and fossil material measured ranged from 25.8 to 69.7. Vegetation in Saltwater and Okarito forests had high mean leaf C:N values (Saltwater, 64.3 ± 20.9 ; Okarito, 69.7 ± 23.1) compared with those of vegetation in Riccarton Bush (25.8 ± 10.1) and the fossil vegetation from Pyramid Valley (35.5 ± 8.7), indicating the West Coast environments are likely to have relatively lower levels of available N.

C:N ratios in all the forests, particularly the West Coast forests, exhibited high variances which suggests that the factors controlling N availability varied at local scales within each forest. As high $\delta^{15}\text{N}$ of leaf material is associated with high N availability, these two response variables are correlated (Högberg 1997; Hobbie *et al.* 2000), as observed in all the modern and fossil material in the present study.

5.5 Eastern and western forest comparison

Eastern and western forests at similar latitudes in the South Island were compared to characterise the effect on stable isotopic composition of the different environmental conditions, primarily precipitation, between the wet western coast and the dry eastern coast. The data presented here revealed no difference in foliar $\delta^{13}\text{C}$ between forests growing under these different rainfall regimes, but $\delta^{15}\text{N}$ and C:N, differed significantly between coasts.

5.5.1 Foliar $\delta^{13}\text{C}$ in eastern and western forests

Although the forests were very different in composition and were growing on different soils and under different rainfall regimes, foliar $\delta^{13}\text{C}$ values in Riccarton Bush on the eastern coast (-33.3 ± 2.6 ‰) were the same as those in both Saltwater (-33.6 ± 2.5 ‰) and Okarito forests (-32.2 ± 2.3 ‰) on the West Coast. Similarly, Hawke & Newman (2007) found no difference between foliar $\delta^{13}\text{C}$ in an N limited forest on Stewart Island and that in an N-enriched (sea-bird guano supplemented) forest on The Snares, but that $\delta^{15}\text{N}$ in the vegetation differed significantly. In N-limited environments, such as the South Island's West Coast, $\delta^{13}\text{C}$ often decreases with increasing $\delta^{15}\text{N}$ (Clay *et al.* 2001), but no $\delta^{13}\text{C}$ difference was observed.

Leaf C is derived primarily from atmospheric CO_2 , which has a relatively constant stable isotopic composition at any one time (Fry 2006). Foliar $\delta^{13}\text{C}$ varies when internal photosynthetic processes change due to varying environmental conditions such as water availability, humidity, or light, which alter leaf processes and stomatal activity resulting in differential fractionation during photosynthesis (Garten & Taylor 1992; Stewart *et al.* 1995; Schulze *et al.* 1998). Annual precipitation affects foliar $\delta^{13}\text{C}$ (Garten & Taylor 1992; Stewart *et al.* 1995; Schulze *et al.* 1998), but this was not detected in foliar $\delta^{13}\text{C}$ between the coasts even though rainfall on the West Coast averages > 4 times the annual precipitation near Christchurch (Cliflo 2013).

There was a discrimination in $\delta^{13}\text{C}$ below an annual mean precipitation of 475 mm, across a continental-scale rainfall transect in Northern Australia, which suggests that $\delta^{13}\text{C}$ may be affected by aridity below a threshold precipitation (Schulze *et al.* 1998). However, for the same range of annual precipitation, a linear relationship between average precipitation and foliar $\delta^{13}\text{C}$ was found in Queensland, Australia (Stewart *et al.* 1995). Factors other than precipitation, such as fire frequency gradients, may be responsible for the differences between these Australian studies (Stewart *et al.* 1995). In the data presented here, all forests are in areas of moderate to high annual precipitation which may be one reason that no $\delta^{13}\text{C}$ discrimination was detected between the modern New

Zealand forests. Any differences between the two coasts were not large enough to alter understorey foliar $\delta^{13}\text{C}$ between the forests on either coast of the South Island.

Soils in Riccarton Bush are very young, less than 5 ka (Cox *et al.* 1963), whereas soils in Saltwater Forest and Okarito Forest are considerably older, ranging in age from 22 ka to 14 ka, and highly nutrient leached (Almond 1996), so any soil age relationship with $\delta^{13}\text{C}$ had to be considered. Foliar $\delta^{13}\text{C}$ often increases with soil age (Vitousek *et al.* 1990) but not in the present study as soil age had no effect on the foliar $\delta^{13}\text{C}$ of present day vegetation.

Foliar $\delta^{13}\text{C}$ increased with understorey vegetation height in forests on both coasts, with no obvious differences between the forests which may indicate similar structures. The density of the upper canopy will affect leaf $\delta^{13}\text{C}$ within the understorey by controlling the amount of sun light that reaches the lower canopy and undergrowth vegetation (Crowley *et al.* 2012). If there were any differences in canopy structure between eastern and western forests, it did not affect understorey foliar $\delta^{13}\text{C}$ in the sampled height range, despite the Riccarton Bush sub-canopy vegetation exhibiting visually more pronounced layers than those of the two West Coast forests.

5.5.2 Foliar $\delta^{15}\text{N}$ and C:N in eastern and western forests

The significant difference in foliar $\delta^{15}\text{N}$ between eastern ($+1.6 \pm 1.3 \text{ ‰}$) and western forests (Okarito Forest, $-8.5 \pm 3.5 \text{ ‰}$; Saltwater Forest, $-7.9 \pm 3.2 \text{ ‰}$), and C:N between eastern (25.8 ± 10.1) and western forests (Okarito Forest, 69.7 ± 23.1 ; Saltwater Forest, 64.3 ± 20.9), reflected spatial differences in N-availability between forests and that C:N and $\delta^{15}\text{N}$ were correlated (Vitousek *et al.* 1989; Hobbie *et al.* 2000).

The major environmental difference between the coasts was the lower precipitation (655 mm mean annual rainfall) in Riccarton Bush on the eastern coast, in comparison to the wet Saltwater and Okarito forests on the western coast (3104 mm mean annual rainfall) (CliFlo 2013). The higher mean

$\delta^{15}\text{N}$ in the drier forest (Riccarton Bush) was consistent with the results of studies elsewhere indicating that vegetation $\delta^{15}\text{N}$ is inversely related to mean annual precipitation increases (Heaton 1987; Schulze *et al.* 1998; Amundson *et al.* 2003; Xu *et al.* 2003). A $\delta^{15}\text{N}$ increase of 0.4 ‰ for every 100 mm increase in annual precipitation was shown between the two coasts, which was supported by Heaton (1987) whose results suggested that drier environments in southern Africa have the exact same relationship between precipitation and $\delta^{15}\text{N}$.

Low mean foliar $\delta^{15}\text{N}$ and high C:N values are typical of very N limited environments such as the Alaskan boreal forest (0 ‰ to -11 ‰)(Hobbie *et al.* 2000), tundra (-8 ‰ to -6 ‰) (Naelehofer *et al.* 1996), and Hawaiian rainforests on volcanic soils of various ages (-10 ‰ to + 1 ‰) (Vitousek *et al.* 1989). A New Zealand N limited forest growing on sand dunes on Stewart Island (-6.1 ‰)(Hawke & Newman 2007), showed a similar level of foliar $\delta^{15}\text{N}$ depletion as the West Coast forests as that seen in the present study. The N limitation of West Coast forests results from the high precipitation which leaches plant-available N from the soil (Norton & Leathwick 1990), in contrast to the situation in the more fertile alluvium-rich soils of the East Coast Riccarton Bush.

The strong correlation between $\delta^{15}\text{N}$ and C:N may result from a N reliance on mycorrhizal fungi (Hobbie *et al.* 2000; Xu *et al.* 2003; Callesen *et al.* 2013). If N availability is low, vegetation must access other N sources, which can cause a tree to depend on the uptake of mycorrhizal-derived N resulting in low foliar $\delta^{15}\text{N}$ (Hobbie *et al.* 2000). Therefore, it is likely that the spatial difference in mean annual precipitation between the eastern and western forests, resulted in differences in N-availability from altered mycorrhizal-plant interactions which lead to differences in the uptake of ^{15}N and a lower foliar $\delta^{15}\text{N}$ in West Coast forests (Heaton 1987; Hobbie *et al.* 2000; Hobbie & Colpaert 2003; Xu *et al.* 2003).

Another possible source of leaf N in N limited soils is diffusively assimilated $\text{NH}_{3(\text{g})}$ from the atmosphere (Vitousek & Aber 1997; Tozer *et al.* 2005; Wang *et al.* 2014). When plants rely on this source of N, leaves become depleted in ^{15}N to levels matching the extent of foliar depletion in West

Coast forests (Vitousek & Aber 1997; Tozer *et al.* 2005; Johnson & Berry 2013). Lichens in the Rotorua geothermal area (New Zealand) overcome the low N-availability of their substrate by deriving a large component of their N from diffusive assimilation of atmospheric $\text{NH}_{3(g)}$, and display $\delta^{15}\text{N}$ values as low as -24 ‰ (Tozer *et al.* 2005). Taxonomically “higher” plants, such as those in the present study, may also exhibit this type of N uptake if they are growing on low N soils (Tozer *et al.* 2005), which may contribute to the low western forest $\delta^{15}\text{N}$.

The contribution of ^{15}N -enriched marine spray may increase foliar $\delta^{15}\text{N}$ at coastal sites, which, with the dominant westerly winds (Ryan *et al.* 2012) and proximity to the ocean, may be expected in Okarito and Saltwater Forests (Heaton 1987). However, no marine effect was identified in the present study as the $\delta^{15}\text{N}$ values were lower in the West Coast vegetation in comparison to those in Riccarton Bush, which is located further inland and also subjected to westerly winds. Any salinity effect was masked by factors, such as precipitation, that resulted in lower $\delta^{15}\text{N}$ values in the West Coast forests.

Species composition of a forest and the relative abundance of each species effects soil N pools. As the species compositions differ significantly between the eastern forests and West Coast forests, this effect likely explains some of the differences in foliar $\delta^{15}\text{N}$ between the coasts (Kahmen *et al.* 2008). Further contributions to the differences may result from each species having slightly different physiologies and environmental responses, favouring uptake of ^{15}N in different proportions from different N pools (Hobbie *et al.* 2000; Hobbie & Colpaert 2003; Kahmen *et al.* 2008). However, the difference in $\delta^{15}\text{N}$ of over 9 ‰ between the eastern and western forests is too large for species differences to be the sole cause of forest $\delta^{15}\text{N}$ differentiation between coasts.

Soils of different ages contain mineral stores at different depths (Vitousek *et al.* 1989; Hobbie *et al.* 2000; Amundson *et al.* 2003). As soil age increases from 10 ka, the foliar N-concentration generally increases along with N-availability and $\delta^{15}\text{N}$ (Vitousek *et al.* 1995; Amundson *et al.* 2003; Parfitt *et al.* 2005). Riccarton Bush soils are 9-17 ka younger than the Okarito and Saltwater forest soils, yet its

vegetation exhibits a higher $\delta^{15}\text{N}$, so the predicted relationship with soil age was not present. However, the trees within each forest may be accessing different N pools at different depths, which could contribute to the foliar $\delta^{15}\text{N}$ differentiation between eastern and western forests (McKane *et al.* 1990; Fry 2006) and the higher values on the younger soil. Soil N content also decreases with soil age in temperate high rainfall and poorly-drained forests, such as the West Coast forests, which leads to lower foliar $\delta^{15}\text{N}$ (Vitousek *et al.* 1995; Parfitt *et al.* 2005), which could contribute to the reversed relationship with soil age found in the present study. The difference between the foliar $\delta^{15}\text{N}$ values in the eastern and western forests are therefore likely to result from the difference in annual precipitation, and not from differences in soil age.

NO_3^- is the largest N pollutant in the present atmosphere that may have a considerable deposition effect on vegetation, because fossil fuel combustion contributes to the concentration of NH_3 in the local atmosphere and NH_4^+ in the regional atmosphere (Krupa 2003). As a result of altered N composition, vegetation in heavily polluted areas may be depleted in ^{15}N by up to 2 ‰ relative to vegetation in sites remote from industry and transport (Tozer *et al.* 2005). As Riccarton Bush is close to a main city, pollution may lower foliar $\delta^{15}\text{N}$ in the forest understorey, but the Riccarton bush samples exhibited a high $\delta^{15}\text{N}$ in comparison to values in the remote western forests. Sampling was not adequate as a basis for assessing the effect of NO_3^- on leaf stable isotopic composition, as the east-west environmental differences probably masked any effect leading to lower $\delta^{15}\text{N}$.

Variation in C:N ratios in both West Coast forests was double those in both the modern and the fossil eastern forests. This greater within-forest variability in the West Coast forests probably results from the lower availability of N, as exhibited by similarly N-depleted tundra in Arctic, Alaska (Naelhoffer *et al.* 1996).

From the consideration of all the potential factors that could influence foliar $\delta^{15}\text{N}$ between western and eastern forests, the high annual precipitation on the West Coast is almost certainly the main environmental driver of the low foliar $\delta^{15}\text{N}$ and high C:N values there compared to those on the

eastern coast. High rainfall leads to low N-availability so plants must rely on mycorrhizal fungi-plant interactions or uptake of gaseous NH_3 , or both, to obtain N. Other environmental factors may contribute to costal spatial differences in $\delta^{15}\text{N}$, but these were probably masked by the large effect of altered N uptake from different pools between the eastern and western forests.

5.6 Historically-logged forest and unlogged forest comparison

Logging often has long-term effects to ecosystems, far beyond the death of the trees removed (Thiffault *et al.* 2011). Effects to foliar stable isotopic composition from historic selective logging in Saltwater Forest were determined by comparing it with the unlogged Okarito Forest. Following a disturbance, ecosystems change through time as they recover, and the changes continually alter the cycling of N and C within the forest (Milchunas & Lauenroth 1995; Nave *et al.* 2010). The results of the present study show the state of the ecosystem in its present form, and cannot be used to generalise as to the entire effects of selective logging on N and C cycling. At the time of sampling there was no difference in foliar $\delta^{13}\text{C}$ or C:N between the selectively logged Saltwater Forest and the unlogged Okarito Forest, but a small difference was detected in foliar $\delta^{15}\text{N}$.

5.6.1 $\delta^{13}\text{C}$ in logged and unlogged forests

Environmental changes resulting from logging may affect intra-leaf mechanisms during the uptake of C from the atmosphere which would influence foliar $\delta^{13}\text{C}$ (O'Leary 1981). However, the lack of detectable differences in foliar $\delta^{13}\text{C}$ between the historically selectively logged Saltwater Forest and the unlogged Okarito Forest agreed with the observations of Guehl *et al.* (1998), who found no differences in stable isotopic measurements in cut and un-cut forest in the Amazon.

Gaps created in the canopy alter the forest structure and increase the light exposure to the understorey vegetation, which tends to increase the foliar $\delta^{13}\text{C}$ in low forest vegetation (Uhl & Vieira 1989; Garten & Taylor 1992; Andersson *et al.* 2000; Cerling *et al.* 2004; Kranabetter *et al.* 2010). As there was no difference in the relationship between foliar $\delta^{13}\text{C}$ and vegetation height in the logged and unlogged forests, logging has not altered the foliar $\delta^{13}\text{C}$ isotope structure of the lowest 3.5 m of the understorey. This may indicate that although logging occurred in the past, the forest structure has recovered from any alterations, or that structural differences exist but had not affected foliar $\delta^{13}\text{C}$. Species differences between the two forests may also have masked any effect of foliar $\delta^{13}\text{C}$ differences in the structure of the understorey with the small sample sizes of each species and the differences in species composition of the forests.

During logging, mechanically-induced soil compaction influences forest stands because of changes to the availability of water to the trees, which results in changes in nutrient uptake and may change foliar $\delta^{13}\text{C}$ by up to $\pm 1\text{‰}$ (depending on soil type) (Gomez *et al.* 2002). For example, altered water availability makes seed germination more difficult and the nutrient supply to large trees is decreased (Kozlowski 1999). As there was no significant difference in foliar $\delta^{13}\text{C}$ between the two West Coast forests, there was no evidence that soil compaction affected the processes controlling foliar $\delta^{13}\text{C}$ in Saltwater Forest. However, any effects of soil compaction may have declined during the 12-150 years since the forest was logged.

The absence of any difference in $\delta^{13}\text{C}$ between the logged and unlogged forests, and the identification of the same relationship between $\delta^{13}\text{C}$ and vegetation height in the lower vertical range of the understorey between the logged and unlogged forests, suggests that logging has not significantly altered the processes that drive foliar $\delta^{13}\text{C}$. However changes to these processes since logging may remain undetected by measurements of foliar $\delta^{13}\text{C}$ in the present study as sampling did not span the entire period since logging ceased.

5.6.2 Foliar $\delta^{15}\text{N}$ and C:N in a logged forest versus, unlogged forest

Logging modifies N-cycling and changes soil N, $\delta^{15}\text{N}$ and N-availability from altered soil temperature, soil moisture, and plant N uptake (Guehl *et al.* 1998; Goodale & Aber 2001; Parfitt *et al.* 2001; Prescott 2002). Although there are no universal effects to soil N following logging, a loss of N in the form of nitrate from soils after logging is common (Högberg 1997; Parfitt *et al.* 2001; Peri *et al.* 2012), which increases the $\delta^{15}\text{N}$ in the remaining soil. In the longer term, decay of the cutover residue may alter N cycling, increasing the soil N pool and the loss of N, whilst contributing to mineral leaching of N into waterways (Uhl & Vieira 1989; Vitousek *et al.* 1989; Guehl *et al.* 1998; Thiffault *et al.* 2011). Therefore, in general logging eventually causes loss of soil N, so the logged Saltwater Forest might be expected to have a higher $\delta^{15}\text{N}$ than the unlogged Okarito Forest.

The foliar $\delta^{15}\text{N}$ in the historically-logged Saltwater Forest was slightly but significantly higher ($-7.9 \pm 3.2 \text{ ‰}$), than in the unlogged Okarito Forest ($-8.5 \pm 3.5 \text{ ‰}$). However, whether this resulted from the logging is unclear because generally changes in $\delta^{15}\text{N}$ follow changes in N availability but there was no difference in foliar C:N between the logged Saltwater (64.3 ± 20.9) and unlogged Okarito (69.7 ± 23.1) forests. Several factors may have led to these results, which will be detailed in this section.

Wetter forests have lower vegetation $\delta^{15}\text{N}$ values (Peri *et al.* 2012) so the drier soils on the undulating terrain beneath Okarito Forest would be expected to be associated with higher foliar $\delta^{15}\text{N}$ than that growing on the often water-logged soils of the flatter terrain under Saltwater Forest. Soil moisture affects both the redox status of the soil (Schuur & Matson 2001), and the ability for N-containing gases to diffuse from the soil (Ruser *et al.* 2006). If net drainage is high in comparison to net drainage in drier sites there will be a larger associated loss of N in the wet site (Parfitt *et al.* 2005). This N loss, coupled with reduced tree water use efficiency, on wet sites, causes depletion of $\delta^{15}\text{N}$ in soil and vegetation (Peri *et al.* 2012). However, Saltwater Forest on wetter soils had a higher $\delta^{15}\text{N}$ than Okarito Forest on slightly drier soils, and there was no difference in N availability. Although

soil moisture differences may have contributed to the observed difference in foliar $\delta^{15}\text{N}$ between the forests, the underlying mechanisms are unknown as the results are opposite to expectation.

Impacts to the soil from logging would have affected the foliar $\delta^{15}\text{N}$ in Saltwater Forest (Gomez *et al.* 2002). For example, the vegetation there may be accessing different N-pools to those accessed by the Okarito Forest vegetation as a result of alteration of soil N pools by activities associated with logging. Alternatively, the difference in foliar $\delta^{15}\text{N}$ between the forests was so small that it may result from natural spatial variation in N-pools. Soil compaction and disturbance resulting from logging operations probably affected many parts of Saltwater Forest during logging, which may alter microbial processes (Guehl *et al.* 1998) and levels of plant-available water (Gomez *et al.* 2002). However, if microbial processes had been significantly affected by compaction, leaf $\delta^{15}\text{N}$ values would have been lower and C:N would have been higher in the logged forest than the unlogged forest (De Neve & Hofman 2000), which was not the case.

Inorganic N available to roots is more limited on slopes because of the downward migration of soil through time. Plants compensate by taking up isotopically light ammonium, which results in lower $\delta^{15}\text{N}$ values in vegetation on slopes (Garten 1993; Amundson *et al.* 2003). Although the slopes are slight, the topography of the unlogged Okarito Forest may account for the lower $\delta^{15}\text{N}$ values of vegetation compared to the flat Saltwater Forest (Garten 1993; Amundson *et al.* 2003).

As well as soil differences, canopy effects contribute to the levels of foliar $\delta^{15}\text{N}$ and C:N. The effects include factors such as the alteration in N availability resulting from canopy gaps generated by logging (Goodale & Aber 2001; Prescott 2002). However, this effect was not apparent in the present comparison, as there was no difference between C:N in forests. It is possible that the time elapsed since the disturbance was sufficient for enough gap closure to allow N-availability to return to normal levels. The lack of difference in C:N between the forests may also indicate that changes to the canopy from selective logging were minimal.

It is not clear what the driver is for the different foliar $\delta^{15}\text{N}$ between the historically logged Saltwater Forest and the unlogged Okarito Forest. The difference of 0.6 ‰ is so small it could be attributed to one or multiple forest differences that slightly alter foliar $\delta^{15}\text{N}$. Therefore although this difference was statistically significant, the present study cannot determine if historic selective logging has altered foliar $\delta^{15}\text{N}$.

5.6.3 Species composition

Okarito and Saltwater Forests have different species compositions, although they both grow on the same glacial soils of the same age and are only 20 km apart (Almond 1996). Differences in soil moisture resulting from the subtly different terrains may have driven the differences in composition, as different taxa have different responses to waterlogging and relative drought. The species which are present influences the stable isotopic composition of leaf material and the vegetation as a whole (Körner *et al.* 1991; Marshall *et al.* 2007; Callesen *et al.* 2013). As a result, the taxa which were present in each forest could have contributed to the difference in foliar $\delta^{15}\text{N}$, although no difference were found in the $\delta^{13}\text{C}$ or C:N values. Human activities such as logging may alter species composition long after initial ecosystem degradation (Lóšková *et al.* 2013). However, the species composition differences in Saltwater Forest when compared with Okarito Forest, were likely to be a result of the different local environments. The structure of the two forests differed, perhaps as a result of the different drainage regimes affecting species composition: the understorey vegetation in Okarito Forest canopy was dense, with few gaps, whereas the patchy Saltwater Forest canopy had many gaps. These structural differences would affect the amount of light the sampled understorey vegetation received, in turn affecting the foliar stable isotopic composition (Ometto *et al.* 2006). Even though $\delta^{13}\text{C}$ was correlated with vegetation height in both forests, $\delta^{13}\text{C}$ values in Okarito Forest were consistently higher at each sampled height.

5.6.4 Summary of logging impacts to leaf stable isotopic composition

No major long-term effects on C cycling were identified by significant shifts in foliar $\delta^{13}\text{C}$ or C:N as a result of logging in Saltwater Forest. This is not to say that no other significant environmental effects may remain, indeed, as foliar $\delta^{15}\text{N}$ was higher in the logged forest than in the unlogged Okarito Forest, it is possible that logging has altered the N cycle there. The small but significant difference in foliar $\delta^{15}\text{N}$ between the two forests suggests that the long term effects of logging on the N cycle within Saltwater Forest was probably small, but further work is needed, including on soil nutrient cycling and an extension of the analysis to canopy trees.

5.7 Pyramid Valley extinct forest

The stable isotopic composition of well-preserved leaf material dated at c. 4500 years B.P. provided insights into C and N cycling in the pre-human ecosystem at Pyramid Valley. A comparison of the isotopic composition of the fossil leaves with the leaves from modern forests revealed that the Pyramid Valley forest had a distinct isotopic signature. The present foliar stable isotopic analysis allows us to span the time gap between the pre-human ecosystem and present forest ecosystems.

5.7.1 Age of fossil material

The radiocarbon chronology for the Pyramid Valley deposit shows that the sampled material was laid down between 4200 and 4500 years B.P. which constraints the period of interest for the present study. If the 0.4 mm laminae reflect annual deposition (Johnston 2013) the 100 mm between the radiocarbon samples would span c. 250 years. Therefore the fossil leaves sampled for this study represent the vegetation and state of the C and N cycles at Pyramid Valley over a relatively short time period several thousand years before Polynesian settlement.

As the samples radiocarbon dated were individual leaves, they provide an estimate of the age of the laminae enclosing them but not necessarily of all leaves between the same laminae, if they came from old litter or were reworked from older laminae. However, as vertical migration is unlikely in the amorphous organic gel, the dated leaves provide an adequate time scale for the leaves sampled for stable isotopic analysis from Pyramid Valley.

The sampled leaves spanned a relatively short period so little or no difference was expected in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, or C:N measurements of leaves from between different laminae, assuming that the ecosystem was relatively stable over the sampled c. 250 year period. Any major changes in the environmental conditions that control the response variables, such as availability of N, canopy cover, or precipitation, should be apparent in the isotopic data.

5.7.2 Integrity of fossil leaf stable isotopic composition

The integrity of the measurements from the fossil leaves would be affected by changes in composition of the leaves after death as diagenesis may alter the stable isotopic ratio of leaf material (Nguyen Tu *et al.* 2004). Evidence suggests, however, that the stable isotopic ratios of the fossil vegetation in the present study were not significantly altered following deposition as decomposition was inhibited by the anoxic conditions of preservation. Any differences in stable isotopic composition between modern and fossil material are unlikely to be artefacts of diagenesis or decay.

The mean foliar $\delta^{13}\text{C}$ of understorey vegetation in the closest modern lowland forest to Pyramid Valley, Riccarton Bush, was 4.2 ‰ lower than for the Pyramid Valley samples and the mean foliar $\delta^{15}\text{N}$ value was 2.3 ‰ lower than the fossil vegetation. If there were no environmental differences- particularly differences that affected the C and N cycles- between the modern and fossil environments, decay and taxonomic variation should explain all of this.

Other studies show mixed results, as decay was not significant in bulk leaf tissue in a study of fossil *Quercus* leaves (Nguyen Tu *et al.* 2004), but Xu *et al.* (2003) found decay was present and significantly affected leaf $\delta^{13}\text{C}$ values. Different levels of degradation and degradation rates may also be influenced by environmental factors, such as sun exposure (Xiao *et al.* 2013). Some level of decay might be expected in the fossil leaves before they were deposited within the anoxic lake bed as any decay process since the removal of the photosynthesising leaf from the tree may alter this (Rundgren *et al.* 2003). These may include senescence, abscission, ground deposition, water or wind transport to the lake, sinking, or diagenesis (Rundgren *et al.* 2003).

However fossil leaves may retain their isotopic integrity in many instances (Degens 1969; Balesdent & Mariotti 1996), which appears to be the case in the Pyramid Valley material. Physical inspection of individual fossil leaves showed they could be identified by direct comparison with modern examples, including in aspects of shape and colour such as characteristic darkening of the lamina in *Myrsine divaricate* leaves towards the petiole. Similarly, scanning electron microscopy showed the amount of degradation was very small, as leaves were intact and some had insect eggs that were still attached to leaves, and chewed surfaces and edges retained all the remaining cell walls and other structures, including stomata and their guard cells.

Early degradation decreases $\delta^{13}\text{C}$ from the preferential removal of cellulose, leaving the leaf (Benner *et al.* 1987; Rundgren *et al.* 2003; Hawke *et al.* 2013) or wood (Schleser *et al.* 1999) material lignin-enriched. Decomposition of *Spartina alterniflora* for over a year as leaf litter, showed a small $\delta^{13}\text{C}$ decrease of 0.1 ‰ and buried samples showed a decrease of up to 2 ‰ (Ember *et al.* 1987). Similarly, a $\delta^{13}\text{C}$ decrease of up to 3 ‰ at the water-sediment interface was found during decomposition of leaf material in a 120 day laboratory study (Lehmann *et al.* 2002). However, no change in $\delta^{13}\text{C}$ was observed over a 120 day period in a similar study (Schweizer *et al.* 1999). There are few studies that analyse the behaviour of C and N during leaf decay, and of those present, none measure leaf stable isotopic ratios under the same conditions experienced by leaves during

preservation at Pyramid Valley. However, the evidence presented here suggests decay may decrease foliar $\delta^{13}\text{C}$ by a maximum of 3 ‰, and Pyramid Valley leaves showed a 4.5 ‰ higher $\delta^{13}\text{C}$ than modern leaf material, so unless unknown decay processes are present, it is unlikely decay has compromised the C stable isotopic integrity of the fossil leaves.

Fermentation from microbial respiration is a process that may increase $\delta^{13}\text{C}$ in leaves during preservation in an anoxic environment (Rundgren *et al.* 2003). Although fermentation may have contributed to the difference in modern and fossil leaves in the present study, the effect could not be large enough to account for the $\delta^{13}\text{C}$ increase by 4.5 ‰ in fossil vegetation (Rundgren *et al.* 2003).

Degradation processes may alter fossil $\delta^{15}\text{N}$ (Xiao *et al.* 2013) as Melillo *et al.* (1989) showed that leaf litter may decrease in $\delta^{15}\text{N}$ by 1 ‰ in the first 20 months following tree removal of leaves. Similarly, Lehmann *et al.* (2002) found a 3 ‰ increase of $\delta^{15}\text{N}$ during oxic decay and a decrease of 3 ‰ during anoxic decay. These studies show that different decay processes may be present which drive $\delta^{15}\text{N}$ values in opposite directions. The magnitude of fractionation during leaf decay in other studies is large enough to cover the 2.2 ‰ higher $\delta^{15}\text{N}$ values of fossil material, compared to modern material in the present study, but decay may not necessarily be the cause of the difference, which is supported by other data in the present study.

C:N ratios act as reliable indicators of leaf decomposition, as a rapid decrease in leaf C and an increase in N from microbial activity, occurs during decomposition, leading to lower C:N values if decay was significant (Melillo *et al.* 1989; Rundgren *et al.* 2003). Melillo *et al.* (1989) showed a C:N decrease of leaf litter from 170 to less than 86 over a 77 month period. Although the distribution of foliar C:N ratios overlap, Pyramid Valley fossil material had a mean C:N ratio 10 higher than that of the modern Riccarton Bush material, therefore as there was no extreme decrease in C:N with time there is no evidence from C:N data that decay was present (Melillo *et al.* 1989).

The lack of a relationship between stratigraphic position and C:N in Pyramid Valley lake material, suggests that there was no post-burial decay, otherwise leaves deposited for longer, lower in the stratigraphy, would have lower C:N ratios (Rundgren *et al.* 2003). Rapid burial in anoxic conditions is essential for good leaf preservation (Rundgren *et al.* 2003), as was present during the deposition at Pyramid Valley as leaves remained intact and the high levels of H₂S attest to the anoxic conditions present.

In a separate study, the preservation of chlorophyll in Pyramid Valley matai leaves was examined by comparison with present-day matai using chloroform/methanol extraction, purification using thin layer chromatography, and quantification using UV-visible spectrophotometry (Villanueva & Hastings 2000). The results showed a clearly identifiable band corresponding with chlorophyll A, with concentrations 0.5 % that of living leaves (C. Wells & D. Hawke, pers. comm. 9 Dec 2013). As chlorophyll is lost very rapidly from decomposing leaves, the presence of a measurable UV-spectrum, even at such low levels, was strong evidence that the biochemistry of the leaves were intact and hence that the stable isotopic composition had not been dramatically altered. A similar technique also showed that chlorophyll was not lost down-core from an anoxic suite of varves (Villanueva & Hastings 2000), which shows anoxic conditions like those present in the Pyramid Valley amorphous organic gel prevent, or least greatly limit, vegetation decay over periods of several thousand years.

Three moa species were deposited in the Pyramid Valley swamp from c. 5000 B.P to 600 B.P (Holdaway & Worthy 1997) and these fed on the same vegetation community that the fossil leaves were part of c. 4000 B.P. (shown by fossil moa gizzard contents (Burrows 1989)). The stable isotopic composition of an animal's diet determines that of the animal, which enables isotopic maps to show the distribution of trophic levels (DeNiro & Epstein 1981; Kelly 2000) (Fig. 5.1). Therefore, by comparing the moa bone stable isotopic composition to that of the fossil leaves, their relationship can be determined to externally check whether decay had affected the isotopic composition of the

fossil leaves or if the leaf composition is that of the expected diet. $\delta^{15}\text{N}$ values increase from 1 to 5 ‰ with each trophic level (Kelly 2000). The mean $\delta^{15}\text{N}$ of bone gelatin of Pyramid Valley moa of c. 9 ‰ (Fig. 5.1), hence their diet would be expected to lie between 4 and 8 ‰. Pyramid Valley fossil material lies within this range (4 ‰), but the modern Riccarton Bush material does not (1.5 ‰) (Fig. 5.1). This suggests the moa fed on leaves of the same composition as the fossil leaves and they cannot have fed on vegetation with an isotopic composition the same as Riccarton Bush.

Although $\delta^{13}\text{C}$ does not change specifically with trophic level, and for terrestrial vertebrates is often used to determine if a diet included predominantly C_3 or C_4 taxa, the $\delta^{13}\text{C}$ of animal tissues are usually 2 ‰ and no more than 6 ‰ higher than that of their diet (Kelly 2000). The $\delta^{13}\text{C}$ values of the moa bone gelatin had $\delta^{13}\text{C}$ values clustered around -24 ‰ (Fig. 5.1), so the expected $\delta^{13}\text{C}$ of their diet would lie between -24 and -30 ‰. The $\delta^{13}\text{C}$ of the Pyramid Valley vegetation (c. -28 ‰) was within the expected moa diet range, whereas the modern Riccarton Bush material (c. -32 ‰) was 2 ‰ greater (Fig. 5.1) than the maximum difference between herbivores and their diet (correcting for Suess Effect). Similarly, the isotopic values for the vegetarian Finsch's duck (*Chenonetta finschi*) (Fig. 5.1) were within the range expected for consumption of vegetation with the values exhibited by the material from Pyramid Valley, and not that from Riccarton Bush. Comparison of the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of the gelatin of moa and Finsch's duck, shows that, at an ecosystem level, the fossil vegetation stable isotopic ratios bear the expected relationship with those of the animals that consumed it, and not with those of the modern vegetation. This is strong supporting evidence that the values for the fossil vegetation are not artefacts of decay, but represent the isotopic composition of the Pyramid Valley leaf material that grew c. 4500 years ago in North Canterbury.

All evidence suggests that decay had not compromised the stable isotopic composition of leaf material from Pyramid Valley and consequently, data on the fossil material can be compared reliably to those from modern plants. Any differences between isotopic measurements from the two ecosystems should reflect differences in the C and N cycles at the different time.

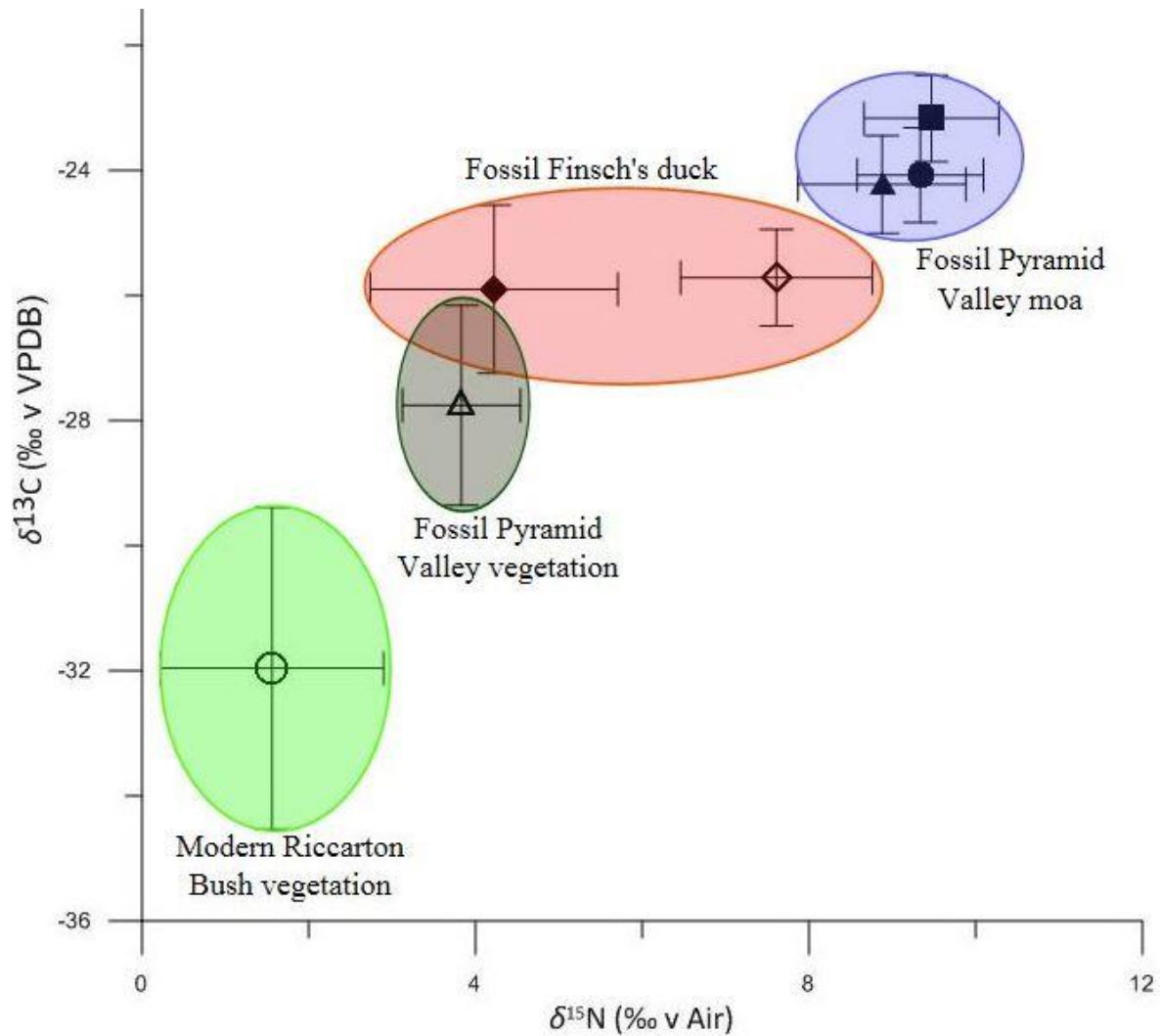


Figure 5.1 Stable isotope biplot of mean modern Riccarton Bush vegetation (light green/open circle, +1.3 ‰ added to $\delta^{13}\text{C}$ to account for the Suess Effect) and fossil Pyramid Valley vegetation (dark green, open triangle), in comparison to the bone gelatin of Pyramid Valley fossil moa (R. Holdaway, pers. comm. 20 Feb 2014) (blue, solid triangle; *Pachyornis elephantopus*, solid circle; *Emeus crassus*, solid square; *Euryapteryx curtus*), and fossil Finsch's duck from various New Zealand locations (Holdaway *et al.* 2013) (red, solid diamond; rainfall > 1500 mm yr⁻¹, open diamond; rainfall < 1500 mm yr⁻¹).

5.7.3 Comparison of fossil Pyramid Valley with Riccarton Bush vegetation

Comparing isotopic data from modern Riccarton Bush material with that from the pre-human layers in Pyramid Valley will provide information on the variations that have occurred in foliar $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N. This will allow changes to the regional C and N cycles, between 4000 B.P. and the present time, to be determined. The impact of human settlement, with its attendant factors including

introduced mammals and the extinction or great diminution of native organisms on N and C cycling is generally unknown. Changes in the past may provide knowledge important to the management of remaining New Zealand forest (Swetnam 1999). Although it is likely the eastern Holocene forests grew under a climate similar to that of today (Burrows 1980), the differences between modern and fossil material could be attributed to either environmental changes over time, or pre-existing environmental differences (such as terrain or precipitation) at each forest location.

5.7.3.1 Difference in foliar $\delta^{13}\text{C}$ between fossil and modern vegetation

The pre-human vegetation had higher values of $\delta^{13}\text{C}$ ($-27.8 \pm 1.6 \text{ ‰}$) than the modern vegetation ($-32.0 \pm 2.6 \text{ ‰}$) which could be the result of changes to the New Zealand terrestrial ecosystem C cycle caused by the arrival effects of humans. Any climatic differences in site conditions between Riccarton Bush and Pyramid Valley before human arrival are unlikely to account for the 4.8 ‰ difference in the $\delta^{13}\text{C}$ of the fossil vegetation and modern understorey vegetation.

Differences in the sampling protocols may have contributed to the difference in measurements between the fossil and modern vegetation. The modern material was collected from the lowest 3.5 m of the understorey vegetation within forests, but the vertical position of the fossil vegetation, the proximity of the leaf to the forest edge, and its means of transport into the lake are unknown. The excellent state of preservation of the fossil vegetation suggests leaves were deposited proximal to their source, and likely came from local vegetation at or near the lake edges. If the material came from the canopy, this could partially explain the higher $\delta^{13}\text{C}$ values as this can account for a 3 to 4 ‰ higher value of $\delta^{13}\text{C}$ relative to that of ground vegetation (Heaton 1999). Conversely, if trees at the forest edge, which receive more light than the understory, contributed to the sampled fossil vegetation, $\delta^{13}\text{C}$ would be higher (Kapos *et al.* 1993; Heaton 1999). As the fossil leaves were found in a 100 mm layered sequence that covers at least 250 years, it is possible the leaves came from a

range of heights over that time period. Although some $\delta^{13}\text{C}$ variation may be accounted for by the multiple height sources of the fossil leaves, it is unlikely to account for the entire difference between the modern and fossil leaves, given the complete separation of the distributions on the isotope bi-plots.

Differences in species composition may account for some of the difference in $\delta^{13}\text{C}$ between forests as the $\delta^{13}\text{C}$ of modern vegetation may account for differences up to a maximum of c. 4 ‰ (Heaton 1999). Most of the fossil material measured was matai, but there were no matai in the modern forest understoreys with which to directly compare the same species of fossil and modern material. However, juvenile kahikatea, which is also in the family Podocarpaceae, from Riccarton bush had an average $\delta^{13}\text{C}$ of -31.9 ± 2.7 ‰, c. 4 ‰ lower than the fossil matai (-27.5 ± 1.0 ‰). This difference between trees of the same family suggests differences in species composition are unlikely to be the cause of the observed differences in $\delta^{13}\text{C}$ between the two forests.

Foliar $\delta^{13}\text{C}$ is often lower at higher altitudes but the difference in altitude between Pyramid Valley (330 masl), and Riccarton Bush (20 masl) is too small to contribute more than a fraction of the observed difference between the modern and fossil vegetation (Vitousek *et al.* 1990; Marshall & Zhang 1994).

As the fossil leaves had higher $\delta^{13}\text{C}$ values than the vegetation from any of the three modern forests, the maximum combined effects of species composition differences and decay could explain the difference in $\delta^{13}\text{C}$ between vegetation from the Pyramid Valley fossil ecosystem and the Riccarton Bush modern ecosystem. However, a lack of evidence for decay in the fossil leaves as well as the intra-family comparison between the modern and fossil podocarps, suggest that taxon and decay contributed little to the difference in $\delta^{13}\text{C}$. Therefore, it is likely the difference in C and N stable isotopic composition between the fossil and modern vegetation reflects changes in the cycles of those elements in the past 4000 years in New Zealand.

5.7.3.2 Difference in $\delta^{15}\text{N}$ and C:N between fossil and modern material

As discussed for $\delta^{13}\text{C}$ values above, foliar $\delta^{15}\text{N}$ values can also be affected by many factors which makes it difficult to determine the source of the difference in $\delta^{15}\text{N}$ between the modern Riccarton Bush ($+1.6 \pm 1.3 \text{ ‰}$) and the fossil Pyramid Valley material ($+3.8 \pm 0.7 \text{ ‰}$). As the Pyramid Valley material grew before humans arrived in New Zealand, and before most anthropogenic environmental change globally, this material may provide a basis for comparison with foliar $\delta^{15}\text{N}$ in the modern vegetation to determine if the local N cycle has changed over the past 4500 years.

N-availability is correlated with $\delta^{15}\text{N}$ and also varies for many reasons, with significant differences in leaf C:N often apparent in adjacent trees. Fossil leaves from Pyramid Valley had a higher average C:N (35.5 ± 8.7) than Riccarton Bush (25.8 ± 10.1), so also a lower N availability. Although the within-forest variation of C:N was high, the measured difference in N availability could be related to different soil N processes in the two forests.

The modern kahikatea from Riccarton Bush had lower $\delta^{15}\text{N}$ ($0.5 \pm 0.2 \text{ ‰}$) than the fossil matai at Pyramid Valley ($3.7 \pm 0.5 \text{ ‰}$), both of which are podocarps. Although differences in species composition may account for some of the differences between the fossil and modern site, this is unlikely to contribute greatly to the difference in $\delta^{15}\text{N}$ because species from the same family would usually exhibit closer $\delta^{15}\text{N}$ values if this was so (Callesen *et al.* 2013). Also, the number of trees that contributed leaves to the fossil sampled material is unknown although with several species present leaves obviously came from several different trees and from different parts of the forest profile.

Riccarton Bush is now surrounded by the built up area Christchurch City, so the contribution of atmospheric NO_3 from recent fossil fuel pollution may have contributed to the lower $\delta^{15}\text{N}$ values of the modern Riccarton Bush vegetation in comparison to that of the pre-human fossil material (Tozer *et al.* 2005). The extent of the contribution from the N pollution to the lower Riccarton bush $\delta^{15}\text{N}$ values could not be determined from the data collected for this study.

As the fossil vegetation likely grew on the forest edges, the deeper rooting and uptake of N from different pools in edge vegetation often decreases $\delta^{15}\text{N}$ (Crowley *et al.* 2012). The modern sampled material was from the forest interior so this within-forest location difference may have contributed to the difference between fossil and modern vegetation $\delta^{15}\text{N}$ values, but if present, this effect was masked as the fossil $\delta^{15}\text{N}$ remained higher than the modern understorey vegetation.

Riccarton Bush and Pyramid Valley have different geological foundations and sediments. Pyramid Valley occupies a basin between out-cropping Weka Pass limestone and a ridge of Mt Brown sandy limestone (Pearce & Griffiths 1980). The local soils are derived from the weathering of these formations and aeolian sediments from adjacent mountains and braided riverbeds (Pearce & Griffiths 1980). Riccarton Bush is underlain by layers of geologically young alluvium (Cox 1963). The effects of these differences in soil (and basement material) on the C:N and $\delta^{15}\text{N}$ of the forests is unknown.

The higher $\delta^{15}\text{N}$ and C:N values from Pyramid Valley compared to those from the understory of Riccarton Bush, although mediated by internal leaf processes, are likely to partially reflect environmental changes since the arrival of humans. This is particularly so because soil N processes are dynamic and are affected by many factors (Högberg 1997) such as the addition of soluble N, soil compaction, removal of vegetation, and water supply, changes to all of which accompanied the spread of human activities, and would have flow on impacts to leaf nutrient composition.

5.7.4 Summary of fossil data

Because of the sites' close proximities and similar climates, the isotopic ratios of the Riccarton Bush foliar material was expected to be similar to those of the fossil vegetation from Pyramid Valley. Environmental changes over the past 4500 years including those resulting from the effects of humans over the past 700 years have most likely contributed to changes leading to lower $\delta^{13}\text{C}$, $\delta^{15}\text{N}$

and C:N of leaf material in the modern ecosystems when compared to those in the former forest at Pyramid Valley. The differences between the living and past forest stable isotopic composition suggest that C and N cycling within modern forests are no longer functioning in the same way as that which surrounded Pyramid Valley before human arrival.

5.8 Effects of human disturbance

Foliar stable isotopic ratios of a series of present forests were measured to determine whether various levels of human alteration have affected their C and N cycles: the fossil forest of Pyramid Valley, pre-dating any human degradation; the unlogged but highly modified modern Riccarton Bush; the relatively unaltered modern Okarito Forest; and the regenerated but historically selectively logged Saltwater Forest. Forests with different levels of human impacts within the present study and other studies can be compared to interpret the effects that human activities in forests may have had to foliar stable isotopic composition and forest ecosystems.

5.8.1 4500 years ago until now

Foliar $\delta^{13}\text{C}$ has fluctuated over the past 4000 years, but mostly over the ranges of 1 ‰ and not exceeding 2 ‰ (Heaton 1999), which suggests that other (probably local) environmental effects contributed to the difference in $\delta^{13}\text{C}$ between the eastern forests over that timescale. Similarly, the $\delta^{15}\text{N}$ of marine sediments has varied by up to 1 ‰ over the past several thousand years (Gruber & Galloway 2008), which again shows the likely extent of $\delta^{15}\text{N}$ variation accounted resulting from global scale changes in N cycling.

The most significant driver of change for New Zealand forest ecosystems in the past several thousand years, after the recovery from the Otrian Glaciation, was the arrival of humans whose

activities have been detrimental to forest environments (McGlone 1989; Johnson 2009; Lee *et al.* 2010). Investigating the changes that occurred in the past provides a reference for assessing modern patterns and processes of stable isotope ecology (Swetnam 1999). Since their arrival, humans have reduced forest cover in New Zealand by at least 71 %, with much if not most of the loss, particularly in the South Island having happened before the end of the 14th century (Ewers *et al.* 2006). The differences between the foliar stable isotopic ratios in the fossil vegetation in comparison to those in modern material suggests that the C and N cycles in the past and present ecosystems were different. It is likely that human influences, especially the introduction of predatory and herbivorous mammals and other organisms, have affected processes within the forests, especially forests composition, demographics, and the recycling of nutrients, which have permanently altered the N and C cycles. Those changes are shown as altered foliar stable isotopic ratios that integrate the various, often opposed, effects. Research of this nature is rarely possible as it is difficult to get reliable foliar stable isotopic ratio values from past ecosystems.

5.8.2 Fragmentation, forest isolation and logging

Human activities have fragmented, and the animals they have introduced have altered the structure of native New Zealand forests over the past 700 years (Ewers *et al.* 2006; Johnson 2009). The effects of Pacific rats (*Rattus exulans*) on forest demographics through direct predation on flowers, seeds, and seedlings (Campbell & Atkinson 2002), and the removal of sea-bird derived N from rat predation of these birds, have probably been under-estimated. Forest burning (McWethy *et al.* 2010), initially for unknown reasons, but probably including a proportion of accidental ignition, removed upwards of 50 % of the original forest in the South Island, including most of the drier eastern forest (Molloy 1969). Further burning to clear land for crops and the more recent logging, has destroyed forests, replacing them with completely different communities (Duncan 1991). These changes will have likely altered N and C cycling in any catchment. Disturbances have caused the complete isolation of

Riccarton Bush from extreme forest fragmentation processes and this is the sole remaining fragment of podocarp forest on the Canterbury Plains. Saltwater Forest has also suffered disturbance, though from a long and varied logging history. There may be evidence of N and C cycle changes in the stable isotopic ratios of vegetation in these remaining forests even well after any disturbance has ceased (Högberg 1997; McLauchlan *et al.* 2007).

Fragmentation increases edge effects and alters C and N cycling in fragmented and isolated forests (Kapos *et al.* 1993; Crowley *et al.* 2012). Although Riccarton Bush is the sole remaining fragment, so is an isolated forest, it will likely suffer from edge effects. The effect of human-mediated fragmentation of forests on the C and N cycles as evidenced by foliar stable isotopic ratios could not be measured in the forests used in the present study, as there are no intact remaining lowland forests on the Canterbury Plains with which to compare measurements from the isolated forest at Riccarton Bush (Ewers *et al.* 2006). As the vegetation in Riccarton Bush had the same $\delta^{13}\text{C}$ as that in the West Coast forests, the greater proportion of edge in the fragment from removal of surrounding forest did not seem to have influenced the local $\delta^{13}\text{C}$, although Kapos *et al.* (1993) has exhibited an increase in foliar $\delta^{13}\text{C}$ at edges. Heterogeneity of N pools at small spatial scales underlies the complexity of N cycle processes (Robertson *et al.* 1988), so edge effects may not be detected in foliar $\delta^{15}\text{N}$. However, Crowley *et al.* (2012) found that foliar $\delta^{15}\text{N}$ was lower at forest edges because of the difference in environmental variables, which may have contributed to the relatively low $\delta^{15}\text{N}$ values in Riccarton Bush. As edge effects decrease with distance from the edge (van der Merwe & Medina 1991; Norton 2002), the sample sites in Riccarton Bush may have been sufficiently distant for them to be representative of a more closed forest.

Among other effects, logging creates canopy gaps which may result in adjacent vegetation becoming enriched in ^{13}C (Cerling *et al.* 2004). Although selective logging severely affected the physical structure of Saltwater forest (James & Norton 2002) the activity was not reflected in the foliar C stable isotopes, as no differences were detected in either the mean $\delta^{13}\text{C}$ or the relationship between

$\delta^{13}\text{C}$ and vegetation height, between the unlogged Okarito Forest and the selectively logged Saltwater Forest. Any differences in foliar $\delta^{13}\text{C}$ between the logged and unlogged forests may have been swamped by differences between plant taxa. Therefore, although there was no correlation between selective logging and foliar $\delta^{13}\text{C}$ values, further work controlling for taxon and drainage effects will be necessary before the effects of logging on C cycling in these forests can be understood. A small difference in foliar $\delta^{15}\text{N}$ and no difference in C:N was found between the logged and unlogged forest, so further study is required, particularly of the N pools in soil, to determine if the difference in foliar $\delta^{15}\text{N}$ reflected the general state of the N cycle in the ecosystem and if this had changed as a result of logging.

5.8.3 The next step in human disturbance

Pine (*Pinus* spp.) plantation forests may be interpreted as the 'next step' in human modification of forest ecosystems as instead of logging native forests and allowing regeneration, pine trees are repeatedly replanted after cutting at commercial maturation. Waring & Silvester (1994) measured foliar $\delta^{13}\text{C}$ values in North Island *P. radiata* plantations which ranged from c. -26 to -30 ‰ and with up to 4 ‰ variation that they attributed to the aspect of the branch relative to the sun. The mean $\delta^{13}\text{C}$ for the Pyramid Valley fossil vegetation lay within the range of $\delta^{13}\text{C}$ values for this pine plantation range, which indeed was the closest of any modern forest to the fossil vegetation measured in the present study. As there was no difference in the understorey $\delta^{13}\text{C}$ between the forests on the eastern and western coasts of the South Island, the 4 ‰ higher values in the North Island pine forest was unexpected. The driver of the $\delta^{13}\text{C}$ difference could result from the repeated logging of exotic species altering the local C cycle or perhaps it could reflect a similarity in structure of the open grid planting of the pine plantation to that of the forest at Pyramid Valley 4000 years ago, i.e. that the extinct fossil forest incorporated more "gap" than is present in the remnant indigenous forests today. It is also possible that the different shapes of introduced *Pinus* trees and

New Zealand forest trees allows greater mixing of sub-canopy air with that outside, under conical conifers, than under the spreading canopies of native trees, which may be responsible for the 4 ‰ difference between South Island and North Island plantation forests.

No studies were found on any foliar $\delta^{15}\text{N}$ data from logged plantations in New Zealand. However such forests often suffer from N deficiencies which limits their productivity, and increases soil C:N ratios because C is lost preferentially to N (Johnson 1995; Davis *et al.* 2012). This low N availability would be passed onto leaf material, likely decreasing foliar $\delta^{15}\text{N}$.

Future changes to stable isotopic composition and C and N cycling are inevitable but their directions and magnitudes cannot be predicted, especially those resulting from changes in climatic conditions from anthropogenic global warming. A 4.6 ‰ decline of atmospheric C is predicted by some (Diefendorf *et al.* 2010), which would greatly affect foliar $\delta^{13}\text{C}$. There are indications that as soil temperatures increase, both N availability and foliar $\delta^{15}\text{N}$ will also increase (Melillo *et al.* 2002). As for C cycling, soil warming will increase vegetation C uptake (Melillo *et al.* 2002), and would probably result in changes to foliar $\delta^{13}\text{C}$. Other effects will accompany global warming, and if climatic conditions change enough it is inevitable that N and C cycling, and in turn foliar stable isotopic composition, will also change.

5.9 Limitations

The study was planned to provide a robust and comprehensive data set, given the limitations of time and resources, but it was obviously not possible to cover all potential sources of variation thus interpretations were made within these bounds.

The total number of foliar samples was limited by the cost of stable isotopic measurements and time constraints. A more complete spatial sampling of each forest would have been desirable, but would have required more and longer transects, more sites per transect, or sampling in the sub-canopy and

the canopy as well. The sampling programme was chosen to provide data on the range of study objectives, within the available resources.

Material from each transect in the modern forests was sampled as close as 20 m to the road edge, however edge effects may extend 50-100 m into a forest (Norton 2002). Transects should probably have been sited at least 100 m from the forest edge to avoid edge effects on the stable isotopic composition of leaf material, but these distances were not practical. In addition, creation of the roads and edges are some of the disturbance features in these forests so the areas around them perhaps should not be excluded.

It would obviously have been preferable to sample a modern forest at Pyramid Valley as a comparison to the fossil material from the same location, to exclude any spatial effects from sampling but there are no podocarp-broadleaf forests left in that area, resultant from Polynesian burning and the clearance of remnants in the 19th century. The vegetation in the area is now almost entirely composed on introduced agricultural and weed species. Riccarton Bush was closest and only appropriate remaining forest available for comparison to the fossil vegetation from Pyramid Valley.

There were potential sources of error in the comparison of both the modern and the fossil vegetation, which were detailed in the fossil and modern material comparison section. These mainly include the unknown origin (height, distance from forest edge, position relative to the sun) and season of growth of each fossil leaf (Heaton 1999).

Although global referencing materials are used for stable isotopic measurements, inter-laboratory variation must be considered when comparing the results of several studies to one another (Mill & Sweeting 2008). Therefore unexplained error is present in comparisons of data sets, particularly in $\delta^{15}\text{N}$ values.

5.11 Summary

As C and N cycles vary under the influence of many different environmental factors and are ultimately mediated by internal leaf processes, it was difficult to determine the exact factors causing stable isotopic variation. However, by comparing other studies to the magnitude of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ variation that were found in the South Island forests, this revealed the likely sources of variation N and C cycle variation within and between forests that differ in location, disturbance history, or through time. The conclusions that can be drawn from these analyses are presented in the following chapter.

Chapter 6: Conclusions and future research

The present study provides a baseline for future stable isotope studies of New Zealand forests, and for similar research on ecosystems and trophic level analyses of animal diets. Differences and similarities of C and N cycling have been identified between forests of different levels of anthropogenic alteration, and between forests c. 4500 years ago and the present. Importantly, the study shows that foliar stable isotope ratios can be used to detect often invisible spatial and temporal differences of C and N cycling, and is more effective at detecting these differences than any resulting from relatively short-term alteration to forests such as that from logging.

6.1 C and N stable isotopic differences between eastern and western forests

The similarity between foliar $\delta^{13}\text{C}$ ratios from the Okarito and Saltwater Forests on the West Coast and Riccarton Bush on the eastern coast suggests that the environmental differences, particularly precipitation, did not affect C cycling. In contrast, foliar $\delta^{15}\text{N}$ values were extremely low in the two wet western forests compared to those in Riccarton Bush on the drier eastern coast. Globally, $\delta^{15}\text{N}$ variation is often the result of a rainfall gradient. It is likely that the high rainfall on the West Coast, leached plant available N from soils and altered the source of N uptake, which in turn altered N cycling. The reduced N availability, shown by the high C:N ratios, also supports this idea. In a global context, the West Coast $\delta^{15}\text{N}$ and C:N values were characteristic of very N-limited environments such as that of tundra.

The effects of different taxa and understorey height on the stable isotope ratios were examined to control for these factors while testing the effect of the variables of interest. The data obtained for

these sources of variation were preliminary as this was not a primary aim of the present study. In all three modern forests the well-documented 'canopy effect' was observed by the positive relationship of foliar $\delta^{13}\text{C}$ with understorey height. From the limited data on the possible effects of vegetation composition, inter-species differences in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N, were found in material from the three modern forests and the fossil material. These findings agree with previous studies, but the sample sizes were small, so a manipulative experiment to explore the differences between taxa would be required to test the effects between taxa used in this study.

6.2 Stable isotopes as indicators of cycles in logged and unlogged forests

The $\delta^{15}\text{N}$ of the understorey vegetation of the historically logged Saltwater Forest was slightly, but significantly, higher than that of the unlogged Okarito Forest, which is possibly from logging-induced environmental alterations to N cycling. Comparatively, no difference was found in foliar $\delta^{13}\text{C}$ or its vertical distribution between the logged and unlogged forest, suggesting that there were no long term effects to the C cycle so far as could be determined from the foliar stable isotope ratios at the time sampled. Similarly, there was no difference of C:N ratios between the logged and unlogged forests, suggesting that the availability of N was similar in the two forests and that logging, therefore, has probably not altered the N and C cycles to the extent of affecting foliar C:N ratios. From the stable isotope data obtained it is apparent that the effect to $\delta^{15}\text{N}$ from logging is small compared to that from climatic controls such as precipitation.

6.3 Fossil and modern material had different foliar $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N values

The c. 4000 B.P. Pyramid Valley fossil leaves had significantly higher values of both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ than any of the modern material, and the C:N ratios were lower than in the West Coast forests but

higher than in Riccarton Bush. These differences between fossil and modern material reflect major changes in the N and C cycles between 4000 years ago and the present. The major change within this time period that may account for the observed differences in nutrient cycling was the arrival of humans to New Zealand. Human activities caused changes to the forest structure, introduced pest species including mammals and rats, eliminated moa megafauna, and removed seabirds and the large guano N source they provided to forests. These combined effects likely caused changes to nutrient cycling in New Zealand, resulting in the different foliar stable isotopic composition between modern and pre-human forests.

6.4 Future research

Although the amount of foliar stable isotopic data obtained was large for an MSc study, the isotopic composition of soil N pools would be a worthwhile focus for future studies. This would better constraint the sources of foliar $\delta^{15}\text{N}$ variation within and between forests by determining the amount of variation explained by differences in soil N pools.

Given that several types of logging were conducted in different parts of Saltwater Forest, a greater clarity of the effects to stable isotopic composition and N cycling from logging would be achieved if a more extensive spatial study was conducted. Such a study could cover not just different types of logging but also areas that were logged over different time frames, to gain a temporal perspective on the changes to N cycling that may occur as an ecosystem recovers from such a disturbance.

Extending the sampling to include the sub-canopy, canopy, and emergent vegetation, beyond the understorey sampled in the present study would greatly improve the understanding of the $\delta^{13}\text{C}$ stable isotopic forest structure. In addition, it would determine if the logging affected the forest structure sufficiently to remove the 'canopy effect' from the higher levels of vegetation, although it was still present in the lower levels.

A study of selected New Zealand native tree species in an experimental context would be useful to isolate the effect of species on stable isotopic composition, as this was not fully achieved in the present study. Higher taxonomic groupings could be used to compare forests as the effect of significant environmental differences (particularly rainfall) between the coasts, causes taxon presence to differ.

6.5 Summary

In summary, this study has contributed to the efforts of the stable isotopic community by providing a baseline for future stable isotope studies on New Zealand terrestrial ecosystems. It provides a new insight into the similarities and differences in the N and C cycles under different conditions and at different times. Overall, an extensive record of the C and N foliar stable isotopic composition of forests was revealed in the South Island for forests on the eastern and western coasts, and forests affected by different levels of human modification, including the first stable isotope results of a pre-human forest in New Zealand.

References

- Adams, M. A.; Grierson, P. F. 2001: Stable isotopes at natural abundance in terrestrial plant ecology and ecophysiology: an update. *Plant Biology* 3: 299–310.
- Almond, P. C. 1996: Loess, soil stratigraphy and Aokautere Ash on Late Pleistocene surfaces in south Westland, New Zealand: Interpretation and correlation with the glacial stratigraphy. *Quaternary International* 34: 163–176.
- Amundson, R.; Austin, A. T.; Schuur, E. A. G.; Yoo, K.; Matzek, V.; Kendall, C.; Uebersax, A.; Brenner, D.; Baisden, W. T. 2003: Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochemical Cycles* 17: 1–10.
- Andersson, F.; Feger, K.; Hüttel, R. 2000: Forest ecosystem research—priorities for Europe. *Forest Ecology and Management* 132: 111–119.
- Aranibar, J.; Otter, L.; Macko, S. 2004: Nitrogen cycling in the soil–plant system along a precipitation gradient in the Kalahari sands. *Global Change Biology* 10: 359–373.
- Arens, N.; Jahren, A.; Amundson, R. 2000: Can C₃ plants faithfully record the carbon isotopic composition of atmospheric carbon dioxide? *Paleobiology* 26: 137–164.
- Atkinson, I. A.; Cameron, E. K. 1993: Human influence on the terrestrial biota and biotic communities of New Zealand. *Trends in Ecology & Evolution* 8: 447–451.
- Balesdent, J.; Mariotti, A. 1996: Measurements of soil organic matter turnover using ¹³C natural abundance. In: Boutton, T. W.; Yamasaki, S. I. ed. *Mass Spectrometry of Soils*. New York, USA. Marcel Dekker. 83–111 p.
- Barry, K. L.; Wilder, S. M. 2013: Macronutrient intake affects reproduction of a predatory insect. *Oikos* 122: 1058–1064.
- Bassirirad, H.; Constable, J. 2003: Widespread foliage $\delta^{15}\text{N}$ depletion under elevated CO₂: inferences for the nitrogen cycle. *Global Change Biology* 9: 1582–1590.
- Beerling, D. 1996: ¹³C discrimination by fossil leaves during the late-glacial climate oscillation 12–10 ka BP: measurements and physiological controls. *Oecologia* 108: 29–37.
- Benner, R.; Fogel, M.; Sprague, E.; Hodson, R. 1987: Depletion of C in lignin and its implications for stable carbon isotope studies. *Nature* 329: 708–710.
- Bennett, P. L. 1980: West Coast communities- a study of their history, settlement pattern and functions. Christchurch, New Zealand. Ministry of Works and Development. 175 p.
- Boyce, R.; Friedland, A.; Chamberlain, C.; Poulson, S. 1996: Direct canopy nitrogen uptake from ¹⁵N-labeled wet deposition by mature red spruce. *Canadian Journal of Forest Research* 26: 1539–1547.
- Bronk Ramsey, C. 2009: Bayesian analysis of radiocarbon dates. *Radiocarbon* 51: 337–360.
- Brown, L.; Weeber, J. 1995: Geology and groundwater. In: Molloy B. ed. *Riccarton Bush: Putaringamotu*. Christchurch, New Zealand. Caxton Press. 37–58 p.
- Burrows, C. 1980: Some empirical information concerning the diet of moas. *New Zealand Journal of Ecology* 3: 125–130.
- Burrows, C. J. 1989: Moa browsing: evidence from the lake sediments and vegetation. *New Zealand Journal of Ecology* 12: 51–56.
- Callesen, I.; Nilsson, L. O.; Schmidt, I. K.; Vesterdal, L.; Ambus, P.; Christiansen, J. R.; Högberg, P.; Gundersen, P. 2013: The natural abundance of ¹⁵N in litter and soil profiles under six temperate tree species: N cycling depends on tree species traits and site fertility. *Plant and Soil* 368: 375–392.
- Campbell, D.; Atkinson, I. A. 2002: Depression of tree recruitment by the Pacific rat (*Rattus exulans* Peale) on New Zealand's northern offshore islands. *Biological Conservation* 107: 19–35.

- Campbell, B. D.; Mitchell, N. D.; Field, T. R. O. 1999: Climate profiles of temperate C₃ and subtropical C₄ species in New Zealand pastures. *New Zealand Journal of Agricultural Research* 42: 223–233.
- Cerling, T. E.; Ehleringer, J. R.; Harris, J. M. 1998: Carbon dioxide starvation, the development of C₄ ecosystems, and mammalian evolution. *Philosophical Transactions of the Royal Society of London* 353: 159–170.
- Cerling, T. E.; Harris, J. M.; Passey, B. H. 2003: Diets of East African bovidae based on stable isotope analysis. *American Society of Mammalogists* 84: 456–470.
- Cerling, T. E.; Hart, J. A.; Hart, T. B. 2004: Stable isotope ecology in the Ituri Forest. *Oecologia* 138: 5–12.
- Certini, G. 2005: Effects of fire on properties of forest soils: a review. *Oecologia* 143: 1–10.
- Clark, P. U.; Dyke, A. S.; Shakun, J. D.; Carlson, A. E.; Clark, J.; Wohlfarth, B.; Mitrovica, J. X.; Hostetler, S. W.; McCabe, A. M. 2009: The Last Glacial Maximum. *Science* 325: 710–714.
- Clay, D. E.; Engel, R. E.; Long, D. S.; Liu, Z. 2001: Nitrogen and water stress interact to influence carbon-13 discrimination in wheat. *Soil Science Society of America* 65: 1823–1828.
- CliFlo 2013. The national climate database website, NIWA.
- Cloern, J.; Canuel, E.; Harris, D. 2002: Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnology and Oceanography* 47: 713–729.
- Coates, K. D.; Burton, P. J. 1997: A gap-based approach for development of silvicultural systems to address ecosystem management objectives. *Forest Ecology and Management* 99: 337–354.
- Cockayne, L. 2011: The vegetation of New Zealand. Cambridge, United Kingdom. Cambridge University Press. 582 p.
- Collins, R. J. 1986. The relationships between landform, soils and vegetation in South Westland terrace rimu forests, New Zealand. MSc thesis. Univeristy of Canterbury.
- Cook, G. D. 2001: Effects of frequent fires and grazing on stable nitrogen isotope ratios of vegetation in northern Australia. *Austral Ecology* 26: 630–636.
- Coplen, T. 2011: Guidelines and recommended terms for expression of stable isotope ratio and gas ratio measurement results. *Rapid Communications in Mass Spectrometry* 25: 2538–2560.
- Cox, J. E.; Mead, C. B. 1963: Soil evidence relating to the post-glacial climate on the Canterbury Plains. *Proceedings of the New Zealand Ecological Society* 10: 23–38.
- Craine, J. M.; Elmore, A. J.; Aida, M. P. M.; Bustamante, M.; Dawson, T. E.; Hobbie, E. A.; Kahmen, A.; Mack, M. C.; McLauchlan, K. K.; Michelsen, A.; Nardoto, G. B.; Pardo, L. H.; Peñuelas, J.; Reich, P. B.; Schuur, E. A. G.; Stock, W. D.; Templer, P. H.; Virginia, R. A.; Welker, J. M.; Wright, I. J. 2009: Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *The New Phytologist* 183: 980–992.
- Crowe, A. 2009: Which native tree? New ecology edition. New Zealand. Penguin NZ. 64 p.
- Crowley, B. E.; McGoogan, K. C.; Lehman, S. M. 2012: Edge effects on foliar stable isotope values in a Madagascan tropical dry forest. *PloS One* 7: e44538.
- Davis, M.; Coker, G.; Watt, M.; Graham, D.; Pearce, S.; Dando, J. 2012: Nitrogen leaching after fertilising young *Pinus radiata* plantations in New Zealand. *Forest Ecology and Management* 280: 20–30.
- Dawson, J.; Lucas, R.; 2012. Field guide to New Zealand's native trees. Nelson, New Zealand. Craig Potton Publishing. 436 p.
- Dawson, T. E.; Mambelli, S.; Plamboeck, A. H.; Templer, P. H.; Tu, K. P. 2002: Stable Isotopes in plant ecology. *Annual Review of Ecology and Systematics* 33: 507–559.
- Deevey, E. S. 1995: Paleolimnology of the upper swamp deposit, Pyramid Valley. *Records of the Canterbury Museum* 6: 291–344.
- Degens, E. T., 1969; Biogeochemistry of stable carbon isotopes. In: Eglinton, G.; Murphy, M. T. ed. Organic Geochemistry: Methods and Research. Berlin, Germany. Springer. 304–329 p.

- De Neve, S.; Hofman, G. 2000: Influence of soil compaction on carbon and nitrogen mineralization of soil organic matter and crop residues. *Biology and Fertility of Soils* 30: 544–549.
- DeNiro, M.; Epstein, S. 1981: Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45: 341–351.
- DeNiro, M. J.; Hastorf, C. A. 1985: Alteration of N and C ratios of plant matter during the initial stages of diagenesis: Studies utilizing archaeological specimens from Peru. *Geochimica et Cosmochimica Acta* 49: 97–115.
- Diefendorf, A. F.; Mueller, K. E.; Wing, S. L.; Koch, P. L.; Freeman, K. H. 2010: Global patterns in leaf ^{13}C discrimination and implications for studies of past and future climate. *Proceedings of the National Academy of Sciences of the United States of America* 107: 5738–5743.
- Donovan, L.; Ehleringer, J. 1992: Contrasting water-use patterns among size and life-history classes of a semi-arid shrub. *Functional Ecology* 6: 482–488.
- Duncan, R. 1991: Disturbance, succession and the coexistence of species in a lowland podocarp forest, South Westland, New Zealand. Unpublished MSc thesis, University of Canterbury.
- Ember, L.; Williams, D.; Morris, J. 1987: Processes that influence carbon isotope variations in salt marsh sediments. *Marine Ecology Progress Series* 36: 33–42.
- Evans, R. D. 2001: Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant Science* 6: 121–126.
- Ewers, R. M.; Kliskey, A. D.; Walker, S.; Rutledge, D.; Harding, J. S.; Didham, R. K. 2006: Past and future trajectories of forest loss in New Zealand. *Biological Conservation* 133: 312–325.
- Farquhar, G.; O’leary, M.; Berry, J. 1982: On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Functional Plant Biology* 9: 121–137.
- Fleet, H. 1986: The concise natural history of New Zealand. Auckland, New Zealand. Heinemann Publishers. 275 p.
- Folke, C.; Carpenter, S.; Walker, B.; Scheffer, M.; Elmqvist, T.; Gunderson, L.; Holling, C. S. 2004: Regime shifts, resilience, and biodiversity in ecosystem management. *Annual Review of Ecology, Evolution, and Systematics* 35: 557–581.
- Fry, B. 2006: Stable Isotope Ecology. USA. Springer Publishers. 308 p.
- Garten, C. 1993: Variation in foliar ^{15}N abundance and the availability of soil nitrogen on Walker Branch watershed. *Ecology* 74: 2098–2113.
- Garten, C.; Taylor, G. 1992: Foliar $\delta^{13}\text{C}$ within a temperate deciduous forest: spatial, temporal, and species sources of variation. *Oecologia* 90: 1–7.
- Gebauer, G.; Schulze, E. 1991: Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. *Oecologia* 87: 198–207.
- Gomez, G. A.; Singer, M. J.; Powers, R. F.; Horwath, W. R. 2002: Soil compaction effects on water status of ponderosa pine assessed through $^{13}\text{C}/^{12}\text{C}$ composition. *Tree physiology* 22: 459–467.
- Goodale, C.; Aber, J. 2001: The long-term effects of land-use history on nitrogen cycling in northern hardwood forests. *Ecological Applications* 11: 253–267.
- Grapher 2012. Graphing System version 9.6.1001. Colorado, USA. Golden Software Inc.
- Gregg, D. R. 1972. Holocene stratigraphy and moas at Pyramid Valley, North Canterbury, New Zealand. *Records of the Canterbury Museum* 9: 151–159.
- Gruber, N.; Galloway, J. N. 2008: An Earth-system perspective of the global nitrogen cycle. *Nature* 451: 293–296.
- Guehl, J. M.; Domenach, A. M.; Bereau, M.; Barigah, T. S.; Casabianca, H.; Ferhi, A.; Garbaye, J. 1998: Functional diversity in an Amazonian rainforest of French Guiana. *Oecologia* 116: 316–330.

- Harris, W. F. 1969: The past history of the vegetation. *In*: Knox, G. A. *ed.* The natural history of Canterbury. Christchurch, New Zealand. Canterbury Branch of the Royal Society of New Zealand. 334-339 p.
- Hawke, D.; Newman, J. 2007: Carbon-13 and nitrogen-15 enrichment in coastal forest foliage from nutrient-poor and seabird-enriched sites in southern New Zealand. *New Zealand Journal of Botany* 45: 309-315
- Hawke, D. J.; Ni, X.; Cheng, C.; Shigemitsu, N. 2013: Closing the circle: how ecologists can prepare their own quality control material to increase confidence in stable isotope data. *Methods in Ecology and Evolution*. Published online doi: 10.1111/2041-210X.12087.
- Heaton, T. 1987: The $^{15}\text{N}/^{14}\text{N}$ ratios of plants in South Africa and Namibia: relationship to climate and coastal/saline environments. *Oecologia* 74: 236-246.
- Heaton, T. 1999: Spatial, species, and temporal variations in the $^{13}\text{C}/^{12}\text{C}$ ratios of C_3 plants: implications for palaeodiet studies. *Journal of Archaeological Science* 26: 637-649.
- Hellstrom, J.; McCulloch, M.; Stone, J. 1998: A detailed 31,000-year record of climate and vegetation change, from the isotope geochemistry of two New Zealand speleothems. *Quaternary Research* 178: 167-178.
- Hobbie, E.; Colpaert, J. 2003: Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. *New Phytologist* 157: 115-126.
- Hobbie, E.; Macko, S.; Williams, M. 2000: Correlations between foliar $\delta^{15}\text{N}$ and nitrogen concentrations may indicate plant-mycorrhizal interactions. *Oecologia* 122: 273-283.
- Hobbs, R.; Norton, D. 1996: Towards a conceptual framework for restoration ecology. *Restoration Ecology* 4: 93-110.
- Holloway, J. T. 1969: The native forests. *In*: Knox, G. A. *ed.* The natural history of Canterbury. Christchurch, New Zealand. Canterbury Branch of the Royal Society of New Zealand. 218-225 p.
- Högberg, P. 1997: Tansley review No. 95 ^{15}N natural abundance in soil-plant systems. *New Phytologist* 137: 179-203.
- Högberg, P.; Alexander, I. 1995: Roles of root symbioses in African woodland and forest: evidence from ^{15}N abundance and foliar analysis. *Journal of Ecology* 83: 217-224.
- Hogg, A.; Hua, Q.; Blackwell, P. 2013: SHCAL13 Southern hemisphere calibration, 0-50,000 years CAL BP. *Radiocarbon* 55: 1889-1903.
- Holdaway, R.; Worthy, T. 1997: A reappraisal of the late Quaternary fossil vertebrates of Pyramid Valley Swamp, North Canterbury, New Zealand. *New Zealand Journal of Zoology* 24: 69-121.
- Holdaway, R. J.; Williams, M.; Hawke, D. J. 2013: A comparison of the pre-human and present isotopic niches of brown teal (*Anas chlorotis*): implications for conservation. *Notornis* 60: 233-244.
- Houlton, B. Z.; Sigman, D. M.; Schuur, E. A. G.; Hedin, L. O. 2007: A climate-driven switch in plant nitrogen acquisition within tropical forest communities. *Proceedings of the National Academy of Sciences of the United States of America* 104: 8902-8906.
- James, I. L. 1987: Silvicultural management of the rimu forests of South Westland. Rotorua, New Zealand. Forest Research Institute. 32 p.
- James, I. L.; Norton, D. A. 2002: Helicopter-based natural forest management for New Zealand's rimu (*Dacrydium cupressinum*, Podocarpaceae) forests. *Forest Ecology and Management* 155: 337-346.
- Jardine, T.; Cunjak, R. 2005: Analytical error in stable isotope ecology. *Oecologia* 144: 528-533.
- Johnson, C. 1995: Soil nitrogen status 8 years after whole-tree clear-cutting. *Canadian Journal of Forest Research* 25: 2346-1355.
- Johnson, C. N. 2009: Ecological consequences of Late Quaternary extinctions of megafauna. *Proceedings of the Royal Society of Biological Sciences* 276: 2509-2519.

- Johnson, J.; Berry, J. 2013: The influence of leaf-atmosphere NH_3 (g) exchange on the isotopic composition of nitrogen in plants and the atmosphere. *Plant, Cell & Environment* 3: 1783–1801.
- Johnston, A. 2013: A high resolution, multi-proxy analyses of the palaeolimnology of Pyramid Valley, North Canterbury. Unpublished MSc thesis. Christchurch, New Zealand. The University of Canterbury.
- Kahmen, A.; Wanek, W.; Buchmann, N. 2008: Foliar $\delta^{15}\text{N}$ values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia* 156: 861–870.
- Kapos, V.; Ganade, G.; Matsui, E.; Victoria, R. L. 1993: ^{13}C as an indicator of edge effects in tropical rainforest reserves. *Journal of Ecology* 81: 425–432.
- Kelly, J. F. 2000: Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* 78: 1–27.
- Kielland, K.; Barnett, B.; Schell, D. 1998: Intraseasonal variation in the $\delta^{15}\text{N}$ signature of taiga trees and shrubs. *Canadian Journal of Forest Research* 28: 485–488.
- King, P. R. 2000: Tectonic reconstructions of New Zealand: 40 Ma to the Present. *New Zealand Journal of Geology and Geophysics* 43: 611–638.
- Körner, C.; Farquhar, G.; Wong, S. 1991: Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. *Oecologia* 88: 30–40.
- Kozłowski, T. 1999: Soil compaction and growth of woody plants. *Scandinavian Journal of Forest Research* 14: 596–619.
- Kranabetter, J. M.; Simard, S. W.; Guy, R. D.; Coates, K. D. 2010: Species patterns in foliar nitrogen concentration, nitrogen content and ^{13}C abundance for understory saplings across light gradients. *Plant and Soil* 327: 389–401.
- Krupa, S. 2003: Effects of atmospheric ammonia (NH_3) on terrestrial vegetation: a review. *Environmental Pollution* 124: 179–221.
- LeDuc, S. D.; Rothstein, D. E.; Yermakov, Z.; Spaulding, S. E. 2013: Jack pine foliar $\delta^{15}\text{N}$ indicates shifts in plant nitrogen acquisition after severe wildfire and through forest stand development. *Plant and Soil*. DOI 10.1007/s11104-013-1856-0
- Lee, W. G.; Wood, J. R.; Rogers, G. M. 2010: Legacy of avian-dominated plant–herbivore systems in New Zealand. *New Zealand Journal of Ecology* 34: 28–47.
- Lehmann, M.; Bernasconi, S.; Barbieri, A.; McKenzie, J. 2002: Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. *Geochimica et Cosmochimica Acta* 66: 3573–3584.
- Lóšková, J.; Ľuptáčík, P.; Miklisová, D.; Kováč, Ľ. 2013: The effect of clear-cutting and wildfire on soil Oribatida (Acari) in wind thrown stands of the High Tatra Mountains (Slovakia). *European Journal of Soil Biology*. In Press.
- Mariotti, A.; Germon, J.; Hubert, P.; Kaiser, P. 1981: Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. *Plant and Soil* 430: 413–430.
- Marra, M. J.; Shulmeister, J.; Smith, E. G. C. 2006: Reconstructing temperature during the Last Glacial Maximum from Lyndon Stream, South Island, New Zealand using beetle fossils and maximum likelihood envelopes. *Quaternary Science Reviews* 25: 1841–1849.
- Marshall, J. D.; Brooks, J. R.; Lajtha, K. 2007: Sources of variation in the stable isotopic composition of plants. In: Michener, R.; Lajtha, K. ed. *Stable isotopes in ecology and environmental science*. USA, Blackwell Publishing Ltd. 22–60 p.
- Marshall, J.; Zhang, J. 1994: Carbon isotope discrimination and water-use efficiency in native plants of the north-central Rockies. *Ecology* 75: 1887–1895.
- Martinelli, L.; Piccolo, M.; Townsend, A. R.; Vitousek, P.; Cuevas, E.; McDowell, W.; Robertson, G. P.; Santos, O.; Treseder, K. 1999: Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests. *Biochemistry* 46: 45–65.

- McGlone, M. 1989: The Polynesian settlement of New Zealand in relation to environmental and biotic changes. *New Zealand Journal of Ecology* 12: 115–129.
- McGlone, M. 2004: Vegetation history of the South Island high country. Landcare Research Contract Report. LC0304/065.
- McKane, R.; Grigal, D.; Russelle, M. 1990: Spatiotemporal differences in ^{15}N uptake and the organization of an old-field plant community. *Ecology* 71: 1126–1132.
- McLauchlan, K. K.; Craine, J. M.; Oswald, W. W.; Leavitt, P. R.; Likens, G. E. 2007: Changes in nitrogen cycling during the past century in a northern hardwood forest. *Proceedings of the National Academy of Sciences of the United States of America* 104: 7466–7470.
- McWethy, D. B.; Whitlock, C.; Wilmshurst, J. M.; McGlone, M. S.; Fromont, M.; Li, X.; Dieffenbacher-Krall, A.; Hobbs, W. O.; Fritz, S. C.; Cook, E. R. 2010: Rapid landscape transformation in South Island, New Zealand, following initial Polynesian settlement. *Proceedings of the National Academy of Sciences of the United States of America* 107: 21343–21348.
- Medina, E.; Minchin, P. 1980: Stratification of $\delta^{13}\text{C}$ values of leaves in Amazonian rain forests. *Oecologia* 45: 377–378.
- Melillo, J. M.; Aber, J. D.; Linkins, A. E.; Ricca, A.; Fry, B.; Nadelhoffer, K. J. 1989: Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic matter. *Plant and Soil* 115: 189–198.
- Melillo, A. J. M.; Steudler, P. A.; Aber, J. D.; Newkirk, K.; Lux, H.; Bowles, F. P.; Magill, A.; Ahrens, T.; Morrisseau, S.; Science, S.; Series, N.; Dec, N. 2002: Soil warming and carbon-cycle feedbacks to the climate system. *Science* 298: 2173–2176.
- Mew, G.; Palmer, R. W. P. 1989. Comparison of soil groups in north and south Westland, and the implications for land use. *Journal of the Royal Society of New Zealand* 19: 405–417.
- McLauchlan, K.K.; Craine, J.M.; Oswald, W.W.; Leavitt, P.R.; Likens, G.E. 2007: Changes in nitrogen cycling during the past century in a northern hardwood forest. *Proceedings of the National Academy of Sciences of the United States of America* 104: 7466–7470.
- Michelsen, A.; Quarmby, C.; Sleep, D.; Jonasson, S. 1998: Vascular plant ^{15}N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. *Oecologia* 115: 406–418.
- Michelsen, A.; Schmidt, I.; Jonasson, S.; Quarmby, C.; Sleep, D. 1996: Leaf ^{15}N abundance of subarctic plants provides field evidence that ericoid, ectomycorrhizal and non-and arbuscular mycorrhizal species access different sources of. *Oecologia* 105: 55–63.
- Michener, R.; Lajtha, K. 2007: Stable isotopes in ecology and environmental science, 2nd edition. Singapore, Blackwell Publishing. 594 p.
- Milchunas, D.; Lauenroth, W. 1995: Inertia in plant community structure: state changes after cessation of nutrient-enrichment stress. *Ecological Applications* 5: 452–458.
- Mill, A.; Sweeting, C. 2008: Mass-spectrometer bias in stable isotope ecology. *Limnology and Oceanography* 6: 34–39.
- Moar, N.T. 1970: A new pollen diagram from Pyramid Valley swamp. *Records of the Canterbury Museum* 8: 455–461.
- Molloy, B. P. J. 1969: Recent history of the vegetation. In: Knox, G. A. ed. The natural history of Canterbury. Christchurch, New Zealand. Canterbury Branch of the Royal Society of New Zealand. 340–360 p.
- Molloy, B.; Brown, L. 1995: Vegetation history. In: Molloy, B. ed. Riccarton Bush: Putaringamotu. Christchurch, New Zealand. Caxton Press. 85–115 p.
- Mook, W. G. 1973: The measuring procedure and corrections for the high-precision mass-spectrometric analysis of isotopic abundance ratios, especially referring to carbon, oxygen and nitrogen. *International Journal of Mass Spectrometry and Ion Physics* 12: 273–298.
- Mudge, P. L.; Schipper, L. A.; Baisden, W. T.; Ghani, A.; Lewis, R. W. 2014: Changes in soil C, N and $\delta^{15}\text{N}$ along three forest-pasture chronosequences in New Zealand. *Soil Research* 52: 27–37.

- Naelhoffer, K.; Shaver, G.; Fry, B.; Giblin, A.; Johnson, L.; Mckane, R. 1996: ^{15}N natural abundances and N use by tundra plants. *Oecologia* 107: 386–394.
- Nave, L. E.; Vance, E. D.; Swanston, C. W.; Curtis, P. S. 2010: Harvest impacts on soil carbon storage in temperate forests. *Forest Ecology and Management* 259: 857–866.
- Nguyen Tu, T. T.; Kürschner, W. M.; Schouten, S.; Bergen, P. F. Van. 2004: Leaf carbon isotope composition of fossil and extant oaks grown under differing atmospheric CO_2 levels. *Palaeogeography, Palaeoclimatology, Palaeoecology* 212: 199–213.
- Norton, D. 1995: Forest structure and processes. In: Molloy, B. ed. Riccarton Bush: Putaringamotu. Christchurch, New Zealand. Caxton Press. 116–127 p.
- Norton, D. 2002: Edge effects in a lowland temperate New Zealand rainforest. *DoC Science Internal Series* 27.
- Norton, D. A.; Leathwick, J. R. 1990: The lowland vegetation pattern, South Westland, New Zealand 1. Saltwater Ecological Area. *New Zealand Journal of Botany* 28: 41–51.
- O’Leary, M. 1981: Carbon isotope fractionation in plants. *Phytochemistry* 20: 553–567.
- O’Leary, M. 1988: Carbon isotopes in photosynthesis. *Bioscience* 38: 328–336.
- Ollinger, S.; Smith, M.; Martin, M. 2002: Regional variation in foliar chemistry and in cycling among forests of diverse history and composition. *Ecology* 83: 339–355.
- Ometto, J. P. H. B.; Ehleringer, J. R.; Domingues, T. F.; Berry, J. A.; Ishida, F. Y.; Mazzi, E.; Higuchi, N.; Flanagan, L. B.; Nardoto, G. B.; Martinelli, L. A. 2006: The stable carbon and nitrogen isotopic composition of vegetation in tropical forests of the Amazon Basin, Brazil. *Biogeochemistry* 79: 251–274.
- Parfitt, R.; Ross, D.; Coomes, D. 2005: N and P in New Zealand soil chronosequences and relationships with foliar N and P. *Biogeochemistry* 75: 305–328.
- Parfitt, R.; Salt, G.; Saggart, S. 2001: Post-harvest residue decomposition and nitrogen dynamics in *Pinus radiata* plantations of different N status. *Forest ecology and management* 154: 55–67.
- Pearce, A.; Griffiths, A. 1980: Effects of selective logging on physical water quality in small streams, Okarito Forest. *Journal of Hydrology* 1: 60–67.
- Peat, N. 1987: Forever the forest: a West Coast story. Auckland, New Zealand. Hodder and Stoughton. 92 p.
- Peri, P. L.; Ladd, B.; Pepper, D. a.; Bonser, S. P.; Laffan, S. W.; Amelung, W. 2012: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope composition in plant and soil in Southern Patagonia’s native forests. *Global Change Biology* 18: 311–321.
- Peterson, B.; Fry, B. 1987: Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18: 293–320.
- Petit, J.; Jouzel, J.; Raynaud, D.; Barkov, N. 1999: Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* 399: 429–436.
- Pillans, B.; McGlone, M.; Palmer, A. 1993: Maximum in central and southern North Island, New Zealand: a paleoenvironmental reconstruction using the Kawakawa Tephra Formation as a chronostratigraphic. *Palaeogeography, Palaeoclimatology, Palaeoecology* 101: 283–304.
- Poss, J. A.; Grattan, S. R.; Suarez, D. L.; Grieve, C. M. 2000: Stable carbon isotope discrimination: an indicator of cumulative salinity and boron stress in *Eucalyptus camaldulensis*. *Tree physiology* 20: 1121–1127.
- Prescott, C. E. 2002: The influence of the forest canopy on nutrient cycling. *Tree physiology* 22: 1193–1200.
- Raich, J. W.; Schlesinger, W. H. 1992: The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* 44B: 81–99.
- R Core Team. 2013: R: A language and environment for statistical computing. Vienna, Austria. R Foundation for Statistical Computing.
- Rennenberg, H.; Schneider, S.; Weber, P. 1996: Analysis of uptake and allocation of nitrogen and sulphur compounds by trees in the field. *Journal of Experimental Botany* 47: 1491–1498.

- Robertson, G. P.; Huston, M. A.; Evans, F. C. 1988: Spatial variability in a successional plant community: patterns of nitrogen availability. *Ecology* 69: 1517–1524.
- Robinson, D. 2001: $\delta^{15}\text{N}$ as an integrator of the nitrogen. *Trends in Ecology & Evolution* 16: 153–162.
- Rogers, H. M. 1995: Along a soil drainage gradient for a lowland temperate rainforest, South Westland, New Zealand. PhD thesis. University of Canterbury.
- Rounick, J.; Winterbourn, M. 1986: Stable carbon isotopes and carbon flow in ecosystems. *Bioscience* 36: 171–177.
- Rundgren, M.; Loader, N. J.; Hammarlund, D. 2003: Stable carbon isotope composition of terrestrial leaves: inter- and intraspecies variability, cellulose and whole-leaf tissue difference, and potential for climate reconstruction. *Journal of Quaternary Science* 18: 583–590.
- Ruser, R.; Flessa, H.; Russow, R.; Schmidt, G.; Buegger, F.; Munch, J. C. 2006: Emission of N_2O , N_2 and CO_2 from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. *Soil Biology and Biochemistry* 38: 263–274.
- Ryan, A. 1995: Climate and weather. In: Molloy, B. ed. Riccarton Bush: Putaringamotu. Christchurch, New Zealand. Caxton Press. 69–84 p.
- Ryan, M. T.; Dunbar, G. B.; Vandergoes, M. J.; Neil, H. L.; Hannah, M. J.; Newnham, R. M.; Bostock, H.; Alloway, B. V. 2012: Vegetation and climate in Southern Hemisphere mid-latitudes since 210 ka: new insights from marine and terrestrial pollen records from New Zealand. *Quaternary Science Reviews* 48: 80–98.
- Sampson, K. A.; Goodrich, C. G.; Taylor, N. C. 2007: A social assessment of community response to forest policy change in South Westland, New Zealand. *Society & Natural Resources* 20: 199–212.
- Scarlett, R. J. 1969: Moas and Other Extinct Birds. In: Knox, G.A. ed. The Natural History of Canterbury. Christchurch, New Zealand. Canterbury Branch of the Royal Society of New Zealand. 565–568 p.
- Schleser, G. H.; Frielingdorf, J.; Blair, A. 1999: Carbon isotope behaviour in wood and cellulose during artificial aging. *Chemical Geology* 158: 121–130.
- Schubert, C.; Calvert, S. 2001: Nitrogen and carbon isotopic composition of marine and terrestrial organic matter in Arctic Ocean sediments: implications for nutrient utilization and organic matter composition. *Deep Sea Research* 48: 789–810.
- Schulze, E.; Chapin, F. S.; Gebauer, G. 1994: Nitrogen nutrition and isotope differences among life forms at the northern treeline of Alaska. *Oecologia* 100: 406–412.
- Schulze, E.; Williams, R.; Farquhar, G. 1998: Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. *Australian Journal of Plant Physiology* 25: 423–425.
- Schuur, E.; Matson, P. 2001: Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. *Oecologia* 128: 431–442.
- Schweizer, M.; Fear, J.; Cadisch, G. 1999: Isotopic ^{13}C fractionation during plant residue decomposition and its implications for soil organic matter studies. *Rapid Communications in Mass Spectrometry* 13: 1284–1290.
- Silveira, L. Da; Sternberg, L.; Mulkey, S.; Wright, S. 1989: Ecological interpretation of leaf carbon isotope ratios: influence of respired carbon dioxide. *Ecology* 70: 1317–1324.
- Smedley, M.; Dawson, T.; Comstock, J. 1991: Seasonal carbon isotope discrimination in a grassland community. *Oecologia* 85: 314–320.
- Smith, B.; Epstein, S. 1971: Two categories of $^{13}\text{C}/^{12}\text{C}$ ratios for higher plants. *Plant physiology* 47: 380–384.
- Spurr, E.; Warburton, B.; Drew, K. W. 1992: Bird abundance in different-aged stands of rimu (*Dacrydium cupressinum*)-implications for coupe-logging. *New Zealand Journal of Ecology* 16: 109–118.

- Stevens, G.; McGlone, M.S.; McCulloch, B. 1988: Prehistoric New Zealand. Auckland, New Zealand. Heinemann Reed. 174 p.
- Stewart, G. R.; Turnbull, M. H.; Schmidt, S.; Erskine, P. D. 1995: ^{13}C Natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability. *Australian Journal of Plant Physiology* 22: 51–55.
- Suess, H. E. 1955: Radiocarbon concentration in modern wood. *Science* 122: 415–417.
- Sulzman, E. W. 2007: Stable isotope chemistry and measurement: a primer. In: Michener, R.; Lajtha, K. ed. Stable isotopes in ecology and environmental science. Hong Kong. Blackwell Publishing Ltd. 1–21 p.
- Sun, D.; Walsh, D. 1998: Review of studies on environmental impacts of recreation and tourism in Australia. *Journal of Environmental Management* 53: 323–338.
- Swetnam, T. 1999: Applied historical ecology: using the past to manage for the future. *Ecological applications* 9: 1189–1206.
- Teece, M. A.; Fogel, M. L. 2004. Preparation of ecological and biochemical samples for isotope analysis. In: de Groot, P. A. ed. Handbook of stable isotope analytical techniques- volume 1. Amsterdam, The Netherlands. Elsevier. 177–202.
- Templer, P. H.; Arthur, M. A.; Lovett, G. M.; Weathers, K. C. 2007: Plant and soil natural abundance $\delta^{15}\text{N}$: indicators of relative rates of nitrogen cycling in temperate forest ecosystems. *Oecologia* 153: 399–406.
- Thiffault, E.; Hannam, K.; Paré, D. 2011: Effects of forest biomass harvesting on soil productivity in boreal and temperate forests—a review. *Environmental Reviews* 309: 278–309.
- Tozer, W. C.; Hackell, D.; Miers, D. B.; Silvester, W. B. 2005: Extreme isotopic depletion of nitrogen in New Zealand lithophytes and epiphytes; the result of diffusive uptake of atmospheric ammonia? *Oecologia* 144: 628–635.
- Uhl, C.; Vieira, I. 1989: Ecological impacts of selective logging in the Brazilian Amazon: a case study from the Paragominas region of the state of Pará. *Biotropica* 21: 98–106.
- van der Merwe, N.; Medina, E. 1991: The canopy effect, carbon isotope ratios and foodwebs in Amazonia. *Journal of Archaeological Science* 18: 249–259.
- Villanueva, J.; Hastings, D. 2000: A century-scale record of the preservation of chlorophyll and its transformation products in anoxic sediments. *Geochimica et Cosmochimica Acta* 64: 2281–2294.
- Vitousek, P. 1982: Nutrient cycling and nutrient use efficiency. *American Naturalist* 119: 553–572.
- Vitousek, P.; Aber, J. 1997: Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Society of America* 7: 737–750.
- Vitousek, P.; Field, C.; Matson, P. 1990: Variation in foliar $\delta^{13}\text{C}$ in Hawaiian *Metrosideros polymorpha*: a case of internal resistance? *Oecologia* 84: 362–370.
- Vitousek, P.; Shearer, G.; Kohl, D. 1989: Foliar ^{15}N natural abundance in Hawaiian rainforest: patterns and possible mechanisms. *Oecologia* 78: 383–388.
- Vitousek, P.; Turner, D.; Kitayama, K. 1995: Foliar nutrients during long-term soil development in Hawaiian montane rain forest. *Ecology* 76: 712–720.
- Vucetich, C. G. 1969: Soils of Canterbury. In: Knox, G. A. ed. The natural history of Canterbury. Christchurch, New Zealand. Canterbury Branch of the Royal Society of New Zealand. 44–67 p.
- Wang, A.; Fang, Y. T.; Chen, D. X.; Koba, K.; Makabe, A.; Li, Y. D.; Luo, T. S.; Yoh, M. 2014: Variations in nitrogen-15 natural abundance of plant and soil systems in four remote tropical rainforests, southern China. *Oecologia* 174: 567–580.
- Waring, R. H.; Silvester, W. B. 1994: Variation in foliar $\delta^{13}\text{C}$ values within the crowns of *Pinus radiata* trees. *Tree Physiology* 14: 1203–1213.
- Webb, T. 1995: Soils and landforms. In: Molloy, B. ed. Riccarton Bush: Putaringamotu. Christchurch, New Zealand. Caxton Press. 59–68 p.
- Werner, R. A.; Brand, W. A. 2001: Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry* 15: 501–519.

- West, J. B.; Bowen, G. J.; Cerling, T. E.; Ehleringer, J. R. 2006: Stable isotopes as one of nature's ecological recorders. *Trends in Ecology & Evolution* 21: 408–414.
- Will, G. M.; Neary, D. G. 1977: Forest management practices in Westland/Nelson in relation to nutrient cycling, nutrient losses in stream flow, and the need for fertilizers. Rotorua, New Zealand Forestry Services. 13 p.
- Williams, P. 1996: A 230 ka record of glacial and interglacial events from Aurora Cave, Fiordland, New Zealand. *New Zealand Journal of Geology and Geophysics* 39: 225–241.
- Wolfe, J. 1995: Paleoclimatic estimates from Tertiary leaf assemblages. *Annual Review of Earth and Planetary Sciences* 23: 119–142.
- Xiao, L.; Yang, H.; Sun, B.; Li, X.; Guo, J. 2013: Stable isotope compositions of recent and fossil sun/shade leaves and implications for palaeoenvironmental reconstruction. *Review of Palaeobotany and Palynology* 190: 75–84.
- Xu, Z.; Prasolova, N.; Lundkvist, K.; Beadle, C.; Leaman, T. 2003: Genetic variation in branchlet carbon and nitrogen isotope composition and nutrient concentration of 11-year-old hoop pine families in relation to tree growth in subtropical Australia. *Forest Ecology and Management* 186: 359–371.

Appendices

Appendix 1: Raw data from stable isotopic analyses of the modern leaf material from Okarito Forest, Saltwater Forest, and Riccarton Bush.

Sample ID (transect, site, sample)	Forest (O: Okarito Forest, S: Saltwater Forest, R: Riccarton Bush)	Vegetation height (m from floor)	Species	Dried and ground sample mass (mg)	$\delta^{13}\text{C}$ (‰ v VPDB)	$\delta^{15}\text{N}$ (‰ v Air)	% N	% C	C:N
T2A2	O	0.1	<i>Prumnopitys ferruginea</i>	1.3	-32.78	-10.34	0.56	46.79	83.40
T2A3	O	0.1	<i>Weinmannia racemosa</i>	1.1	-34.83	-13.56	0.60	48.15	80.36
T2A4	O	0.1	<i>Coprosma foetidissima</i>	1.2	-32.75	-10.03	0.71	41.97	58.72
T2A5	O	0.1	<i>Phyllocladus alpinus</i>	1.3	-33.24	-8.35	0.47	52.80	112.94
T2A6	O	0.1	<i>Podocarpus totara</i>	1.1	-33.58	-9.38	0.62	52.22	84.56
T2B1	O	0.1	<i>Coprosma foetidissima</i>	1.3	-32.76	-7.71	0.99	42.83	43.30
T2B2	O	0.1	<i>Phyllocladus alpinus</i>	1.3	-31.96	-10.53	0.61	52.00	85.02
T2B3	O	0.1	<i>Podocarpus totara</i>	0.9	-32.68	-12.36	0.47	44.43	94.65
T2B4	O	0.1	<i>Neomyrtus pedunculata</i>	1.0	-34.43	-7.73	1.26	47.51	37.61
T2B5	O	0.1	<i>Dacrydium cupressinum</i>	1.2	-33.48	-7.75	0.86	55.24	64.34
T2B6	O	0.1	<i>Kunzea ericoides</i>	0.9	-33.79	-4.49	0.87	48.99	56.55
T2C1	O	0.1	<i>Dacrydium cupressinum</i>	1.2	-34.88	-8.51	0.63	51.58	81.38
T2C2	O	0.1	<i>Phyllocladus alpinus</i>	1.1	-30.98	-10.79	0.46	52.92	115.15
T2C3	O	0.1	<i>Quintinia serrata</i>	1.3	-32.04	-16.34	0.49	48.72	98.65
T2C4	O	0.1	<i>Leptospermum scoparium</i>	1.8	-32.49	-7.03	0.68	51.55	76.44
T2C5	O	0.1	<i>Podocarpus totara</i>	1.0	-31.79	-9.86	0.43	51.80	121.85

T2D1	O	0.1	<i>Weinmannia racemosa</i>	2.9	-34.37	-14.14	0.66	50.85	76.58
T2D2	O	0.1	<i>Kunzea ericoides</i>	1.1	-31.66	-8.25	0.82	51.31	62.49
T2D3	O	0.1	<i>Phyllocladus alpinus</i>	1.2	-31.85	-10.78	0.67	53.97	80.87
T2D4	O	0.1	<i>Coprosma foetidissima</i>	1.3	-33.93	-10.10	0.78	41.77	53.75
T2D5	O	0.1	<i>Lophomyrtus obcordata</i>	0.9	-34.25	-11.54	0.90	45.54	50.51
T2D6	O	0.1	<i>Manoao colensoi</i>	1.1	-31.92	-2.10	1.47	52.08	35.48
T2D7	O	0.1	<i>Neomyrtus pedunculata</i>	1.3	-34.78	-10.04	1.02	45.13	44.29
T3A1	O	0.1	<i>Neomyrtus pedunculata</i>	1.3	-36.08	-8.12	0.93	43.86	47.36
T3A2	O	0.1	<i>Dacrydium cupressinum</i>	1.6	-35.58	-1.17	1.31	50.43	38.39
T3A3	O	0.1	<i>Phyllocladus alpinus</i>	1.1	-34.82	-9.42	0.75	49.89	66.08
T3A4	O	0.1	<i>Quintinia serrata</i>	1.4	-34.91	-11.20	0.67	47.90	71.78
T3A5	O	0.1	<i>Coprosma foetidissima</i>	1.8	-32.80	-5.85	1.03	43.41	42.28
T3A6	O	0.1	<i>Weinmannia racemosa</i>	1.2	-36.30	-10.11	0.63	47.56	75.68
T3A7	O	0.1	<i>Manoao colensoi</i>	1.7	-33.95	-2.72	1.19	52.17	43.75
T3A8	O	0.1	<i>Metrosideros umbellata</i>	1.7	-35.02	-8.66	0.42	48.34	115.62
T3A9	O	0.1	<i>Podocarpus totara</i>	1.1	-32.96	-8.42	0.45	48.62	108.48
T3B1	O	0.1	<i>Weinmannia racemosa</i>	2.6	-34.04	-9.86	0.70	48.91	69.89
T3B2	O	0.1	<i>Dacrydium cupressinum</i>	1.2	-35.47	-6.22	0.66	51.68	78.63
T3B3	O	0.1	<i>Podocarpus totara</i>	2.8	-32.64	-8.75	0.47	49.04	104.99
T3B4	O	0.1	<i>Neomyrtus pedunculata</i>	1.3	-34.68	-8.94	0.93	45.22	48.54
T3B5	O	0.1	<i>Phyllocladus alpinus</i>	1.1	-31.98	-10.63	0.57	47.34	83.17
T3B5a	O	0.1	<i>Phyllocladus alpinus</i>	3.8	-32.40	-10.43	0.60	53.09	89.16
T3B6	O	0.1	<i>Coprosma foetidissima</i>	2.1	-32.14	-6.36	0.92	44.45	48.28
T3B7	O	0.1	<i>Quintinia serrata</i>	1.5	-33.71	-10.44	0.79	48.86	61.84
T3B8	O	0.1	<i>Manoao colensoi</i>	2.3	-32.59	-3.24	1.28	53.17	41.62
T3C1	O	0.1	<i>Phyllocladus alpinus</i>	2.4	-32.45	-12.53	0.53	52.87	99.66
T3C2	O	0.1	<i>Kunzea ericoides</i>	1.0	-33.90	-3.06	1.14	50.53	44.51
T3C3	O	0.1	<i>Podocarpus totara</i>	1.8	-31.97	-11.50	0.47	46.09	97.82
T3C4	O	0.1	<i>Metrosideros umbellata</i>	2.8	-32.88	-13.25	0.35	55.74	157.82
T3C5	O	0.1	<i>Quintinia serrata</i>	1.2	-33.64	-12.36	0.62	48.74	79.18

T3D1	O	0.1	<i>Manoao colensoi</i>	2.5	-34.29	-2.91	1.41	49.69	35.20
T3D2	O	0.1	<i>Phyllocladus alpinus</i>	2.5	-33.18	-9.54	0.54	51.31	94.40
T3D3	O	0.1	<i>Kunzea ericoides</i>	1.7	-33.70	-5.08	1.09	50.73	46.47
T3D4	O	0.1	<i>Coprosma foetidissima</i>	3.3	-29.28	-10.82	0.94	43.16	45.98
T3D5	O	0.1	<i>Quintinia serrata</i>	3.1	-33.51	-12.28	0.53	47.48	89.51
T3D6	O	0.1	<i>Neomyrtus pedunculata</i>	2.5	-36.14	-7.01	1.14	44.67	39.25
T3D8	O	0.1	<i>Dacrydium cupressinum</i>	2.0	-36.85	-6.33	0.79	52.30	65.85
T2A10	O	1.5	<i>Quintinia serrata</i>	1.2	-32.52	-11.44	0.59	47.97	80.71
T2A11	O	1.5	<i>Neomyrtus pedunculata</i>	1.1	-33.75	-7.91	1.16	44.06	37.88
T2A12	O	1.5	<i>Weinmannia racemosa</i>	1.0	-32.59	-12.09	0.87	51.45	59.08
T2A13	O	1.5	<i>Manoao colensoi</i>	1.3	-31.53	-2.78	1.19	52.71	44.11
T2A7	O	1.5	<i>Prumnopitys ferruginea</i>	1.4	-32.44	-9.91	0.71	47.42	66.39
T2A8	O	1.5	<i>Phyllocladus alpinus</i>	0.9	-30.23	-9.60	0.63	52.31	83.68
T2A9	O	1.5	<i>Pseudopanax crassifolius</i>	0.9	-33.65	-8.41	0.69	48.74	70.58
T2B10	O	1.5	<i>Pseudopanax crassifolius</i>	1.1	-31.18	-8.69	0.89	47.61	53.54
T2B11	O	1.5	<i>Quintinia serrata</i>	1.0	-31.13	-12.49	0.69	47.89	69.35
T2B12	O	1.5	<i>Manoao colensoi</i>	1.2	-31.80	-1.69	1.09	49.81	45.90
T2B7	O	1.5	<i>Kunzea ericoides</i>	2.2	-32.89	-4.08	0.96	53.71	55.92
T2B8	O	1.5	<i>Dacrydium cupressinum</i>	1.9	-31.60	-6.95	0.84	52.14	61.79
T2C10	O	1.5	<i>Manoao colensoi</i>	1.1	-32.07	-8.75	0.66	48.58	74.05
T2C11	O	1.5	<i>Leptospermum scoparium</i>	0.9	-30.22	-5.07	0.81	51.60	63.78
T2C6	O	1.5	<i>Leptospermum scoparium</i>	1.3	-31.59	-4.07	1.00	52.57	52.76
T2C7	O	1.5	<i>Manoao colensoi</i>	1.2	-31.32	-3.58	0.86	51.27	59.60
T2C8	O	1.5	<i>Lophomyrtus obcordata</i>	1.4	-31.26	-11.73	0.77	46.65	60.50
T2C9	O	1.5	<i>Quintinia serrata</i>	1.1	-30.65	-14.98	0.70	46.49	66.10
T2D10	O	1.5	<i>Weinmannia racemosa</i>	1.4	-32.64	-16.90	0.64	51.57	80.38
T2D11	O	1.5	<i>Manoao colensoi</i>	1.0	-30.96	-3.13	1.36	54.85	40.30
T2D12	O	1.5	<i>Podocarpus totara</i>	1.1	-30.66	-11.92	0.46	45.62	100.24
T2D8	O	1.5	<i>Kunzea ericoides</i>	1.6	-33.05	-5.93	0.92	53.45	58.52
T2D9	O	1.5	<i>Quintinia serrata</i>	1.3	-29.85	-12.30	0.59	50.53	85.60

T3A10	O	1.5	<i>Metrosideros umbellata</i>	2.5	-34.35	-9.79	0.40	50.34	124.75
T3A11	O	1.5	<i>Coprosma foetidissima</i>	2.7	-32.06	-6.04	1.39	44.81	32.22
T3A12	O	1.5	<i>Weinmannia racemosa</i>	1.8	-32.57	-8.38	0.67	48.48	72.41
T3A13	O	1.5	<i>Neomyrtus pedunculata</i>	2.0	-34.78	-7.80	0.99	44.80	45.48
T3A14	O	1.5	<i>Podocarpus totara</i>	1.6	-31.38	-7.70	0.50	45.17	89.91
T3A15	O	1.5	<i>Quintinia serrata</i>	2.7	-33.19	-9.51	0.63	49.12	77.83
T3A16	O	1.5	<i>Dacrydium cupressinum</i>	1.0	-34.52	-4.93	0.55	49.99	91.38
T3A17	O	1.5	<i>Manoao colensoi</i>	2.4	-33.68	-1.52	1.21	52.84	43.58
T3B10	O	1.5	<i>Neomyrtus pedunculata</i>	1.9	-33.19	-9.68	1.08	45.86	42.60
T3B11	O	1.5	<i>Podocarpus totara</i>	2.9	-30.69	-9.16	0.45	46.00	102.24
T3B12	O	1.5	<i>Quintinia serrata</i>	2.8	-31.76	-9.85	0.56	48.75	87.13
T3B13	O	1.5	<i>Coprosma foetidissima</i>	2.3	-31.50	-7.91	0.92	43.47	47.00
T3B14	O	1.5	<i>Phyllocladus alpinus</i>	2.3	-30.74	-9.55	0.58	53.62	92.25
T3B15	O	1.5	<i>Dacrydium cupressinum</i>	1.3	-33.82	-6.29	0.66	50.02	76.35
T3B9	O	1.5	<i>Manoao colensoi</i>	1.2	-31.83	-2.47	1.37	51.48	37.69
T3C10	O	1.5	<i>Manoao colensoi</i>	2.0	-32.26	-3.53	1.13	53.06	47.11
T3C11	O	1.5	<i>Kunzea ericoides</i>	1.8	-32.85	-4.31	0.92	53.17	57.51
T3C6	O	1.5	<i>Phyllocladus alpinus</i>	1.6	-29.43	-11.90	0.70	54.21	77.80
T3C7	O	1.5	<i>Quintinia serrata</i>	2.3	-32.28	-11.85	0.72	51.91	72.16
T3C8	O	1.5	<i>Podocarpus totara</i>	1.9	-30.70	-11.17	0.48	45.88	94.68
T3C9	O	1.5	<i>Metrosideros umbellata</i>	2.5	-31.79	-14.52	0.43	49.86	116.10
T3D10	O	1.5	<i>Phyllocladus alpinus</i>	2.6	-27.01	-8.81	0.56	53.70	95.25
T3D11	O	1.5	<i>Manoao colensoi</i>	3.4	-21.60	-0.56	1.31	56.07	42.66
T3D12	O	1.5	<i>Pseudopanax crassifolius</i>	1.1	-32.69	-13.13	0.54	44.92	82.80
T3D13	O	1.5	<i>Metrosideros umbellata</i>	3.6	-18.84	-10.58	0.48	51.85	107.99
T3D14	O	1.5	<i>Kunzea ericoides</i>	1.4	-33.02	-5.14	0.93	51.13	55.00
T3D15	O	1.5	<i>Weinmannia racemosa</i>	2.2	-33.19	-9.37	0.87	50.94	58.60
T3D9	O	1.5	<i>Dacrydium cupressinum</i>	1.2	-34.24	-5.85	0.70	52.54	75.08
T2A14	O	3.5	<i>Podocarpus totara</i>	1.3	-30.70	-8.88	0.74	48.17	65.06
T2A15	O	3.5	<i>Quintinia serrata</i>	1.2	-31.18	-11.82	0.77	47.44	61.90

T2A16	O	3.5	<i>Weinmannia racemosa</i>	1.4	-32.97	-11.98	0.67	49.82	74.86
T2A17	O	3.5	<i>Cordyline banksii</i>	1.4	-28.97	-4.37	1.01	45.99	45.61
T2B13	O	3.5	<i>Pseudopanax crassifolius</i>	1.2	-30.17	-8.08	0.86	47.82	55.78
T2B14	O	3.5	<i>Kunzea ericoides</i>	2.0	-32.41	-4.24	0.91	54.33	59.55
T2B15	O	3.5	<i>Lophomyrtus obcordata</i>	1.8	-31.36	-9.14	1.04	49.95	47.91
T2B16	O	3.5	<i>Phyllocladus alpinus</i>	1.3	-27.71	-7.88	0.78	53.77	68.84
T2B17	O	3.5	<i>Manoao colensoi</i>	2.6	-31.31	-6.32	1.09	55.54	51.13
T2C12	O	3.5	<i>Phyllocladus alpinus</i>	1.3	-28.14	-11.88	0.53	51.50	97.53
T2C13	O	3.5	<i>Quintinia serrata</i>	1.2	-29.87	-15.01	0.78	48.32	61.94
T2C14	O	3.5	<i>Manoao colensoi</i>	1.0	-29.99	-5.25	0.98	55.74	56.64
T2C15	O	3.5	<i>Kunzea ericoides</i>	2.9	-30.63	-5.70	1.18	54.07	45.93
T2C16	O	3.5	<i>Weinmannia racemosa</i>	1.2	-28.00	-17.03	0.61	50.17	82.08
T2D13	O	3.5	<i>Dacrydium cupressinum</i>	0.9	-33.37	-7.11	0.56	52.75	94.77
T2D14	O	3.5	<i>Podocarpus totara</i>	1.1	-29.30	-11.47	0.53	46.67	88.06
T2D15	O	3.5	<i>Kunzea ericoides</i>	1.3	-28.67	-5.91	0.92	53.76	58.51
T2D16	O	3.5	<i>Weinmannia racemosa</i>	1.1	-30.20	-13.73	0.77	45.47	58.71
T3A18	O	3.5	<i>Pseudopanax crassifolius</i>	1.7	-33.87	-9.19	0.78	50.34	64.74
T3A19	O	3.5	<i>Dacrydium cupressinum</i>	1.3	-33.73	-4.23	0.62	48.06	77.61
T3A20	O	3.5	<i>Neomyrtus pedunculata</i>	1.2	-34.24	-7.48	0.99	43.64	43.90
T3A21	O	3.5	<i>Quintinia serrata</i>	2.2	-32.41	-8.70	0.78	49.10	63.32
T3A22	O	3.5	<i>Podocarpus totara</i>	1.9	-30.87	-8.07	0.72	45.32	63.14
T3A23	O	3.5	<i>Weinmannia racemosa</i>	1.8	-33.55	-8.91	0.64	51.19	79.51
T3B16	O	3.5	<i>Quintinia serrata</i>	2.8	-31.66	-10.21	0.62	48.32	77.56
T3B17	O	3.5	<i>Weinmannia racemosa</i>	1.2	-32.61	-11.44	0.74	48.84	65.88
T3B18	O	3.5	<i>Neomyrtus pedunculata</i>	1.3	-34.07	-10.68	0.87	39.28	45.33
T3B19	O	3.5	<i>Kunzea ericoides</i>	1.4	-31.66	-4.12	1.23	52.80	42.90
T3B20	O	3.5	<i>Manoao colensoi</i>	2.6	-32.35	-4.51	1.14	55.10	48.13
T3B21	O	3.5	<i>Podocarpus totara</i>	2.9	-30.49	-6.28	0.59	46.32	78.68
T3C12	O	3.5	<i>Manoao colensoi</i>	2.4	-30.97	-3.35	1.52	51.98	34.22
T3C13	O	3.5	<i>Dacrydium cupressinum</i>	1.9	-33.68	-5.21	0.73	51.89	71.20

T3C14	O	3.5	<i>Podocarpus totara</i>	1.2	-30.65	-11.82	0.57	46.39	80.99
T3C15	O	3.5	<i>Metrosideros umbellata</i>	2.3	-30.81	-12.10	0.40	52.43	132.60
T3C16	O	3.5	<i>Quintinia serrata</i>	1.9	-31.37	-13.70	0.77	50.17	65.52
T3C17	O	3.5	<i>Phyllocladus alpinus</i>	1.3	-29.39	-10.74	0.63	52.92	83.75
T3C18	O	3.5	<i>Kunzea ericoides</i>	1.8	-31.57	-4.21	0.88	54.51	61.85
T3D16	O	3.5	<i>Podocarpus totara</i>	2.0	-28.98	-9.09	0.56	44.72	80.18
T3D17	O	3.5	<i>Phyllocladus alpinus</i>	1.5	-29.64	-9.98	0.60	53.61	88.80
T3D18	O	3.5	<i>Manoao colensoi</i>	1.4	-30.93	-3.39	1.23	51.81	42.03
T3D19	O	3.5	<i>Dacrydium cupressinum</i>	1.9	-33.59	-5.39	0.66	51.28	78.14
T3D20	O	3.5	<i>Quintinia serrata</i>	2.0	-32.67	-14.01	0.55	49.32	89.03
T5A1	R	0.1	<i>Parsonsia heterophylla</i>	1.4	-32.23	3.41	3.62	46.69	12.91
T5A2	R	0.1	<i>Lophomyrtus obcordata</i>	2.1	-35.00	0.13	1.40	46.52	33.18
T5A3	R	0.1	<i>Melicytus ramiflorus</i>	2.0	-36.56	1.90	2.81	42.09	14.98
T5A4	R	0.1	<i>Streblus heterophyllus</i>	1.5	-35.47	1.43	2.69	41.22	15.31
T5A5	R	0.1	Creeper A	1.3	-33.56	0.72	3.57	41.61	11.66
T5B1	R	0.1	<i>Streblus heterophyllus</i>	1.0	-36.20	1.82	3.12	40.27	12.90
T5B2	R	0.1	<i>Melicytus ramiflorus</i>	2.9	-36.07	2.86	2.47	45.59	18.43
T5B3	R	0.1	<i>Plagianthus regius</i>	2.0	-38.70	2.92	3.19	42.56	13.36
T5B4	R	0.1	unknown	1.8	-37.05	2.39	2.20	46.79	21.29
T5C1	R	0.1	<i>Melicytus ramiflorus</i>	1.8	-37.54	2.27	2.82	50.11	17.79
T5C2	R	0.1	<i>Streblus heterophyllus</i>	0.9	-36.27	0.52	3.20	49.30	15.39
T5C3	R	0.1	<i>Pittosporum eugeniioides</i>	1.9	-27.33	0.73	2.54	45.55	17.98
T5C4	R	0.1	<i>Parsonsia heterophylla</i>	1.2	-35.56	2.66	3.18	50.28	15.80
T5C5	R	0.1	unknown	0.9	-35.69	2.06	2.39	49.83	20.86
T5D1	R	0.1	<i>Dacrycarpus dacrydioides</i>	1.0	-36.38	0.35	0.93	47.94	51.53
T5D2	R	0.1	<i>Melicytus ramiflorus</i>	1.0	-34.37	0.12	2.31	48.91	21.15
T5D3	R	0.1	<i>Parsonsia heterophylla</i>	1.8	-34.80	-0.01	1.94	49.74	25.60
T5D4	R	0.1	<i>Coprosma rotundifolia</i>	1.4	-36.56	2.83	1.64	49.78	30.44
T5D5	R	0.1	<i>Plagianthus regius</i>	1.7	-36.51	1.06	2.43	42.15	17.34
T6A1	R	0.1	<i>Lophomyrtus obcordata</i>	1.2	-34.52	3.77	1.54	46.78	30.39

T6A2	R	0.1	<i>Dacrycarpus dacrydioides</i>	1.3	-33.44	0.30	1.50	53.92	35.90
T6A3	R	0.1	<i>Melicytus ramiflorus</i>	1.6	-33.70	1.85	2.37	46.45	19.63
T6A4	R	0.1	<i>Parsonsia heterophylla</i>	1.2	-35.23	4.15	2.33	47.35	20.30
T6B1	R	0.1	<i>Melicytus ramiflorus</i>	1.4	-36.67	0.71	2.14	43.17	20.13
T6B2	R	0.1	<i>Coprosma rotundifolia</i>	1.2	-36.30	2.75	2.41	53.64	22.30
T6B3	R	0.1	<i>Elaeocarpus hookerianus</i>	1.0	-34.76	-3.10	1.51	56.19	37.10
T6B4	R	0.1	<i>Streblus heterophyllus</i>	1.2	-35.27	0.84	2.56	47.31	18.46
T6C1	R	0.1	<i>Parsonsia heterophylla</i>	1.9	-34.71	2.78	2.51	53.46	21.31
T6C2	R	0.1	<i>Lophomyrtus obcordata</i>	1.1	-34.74	0.99	1.39	51.21	36.91
T6C3	R	0.1	<i>Plagianthus regius</i>	1.4	-34.77	2.13	2.60	46.92	18.05
T6C4	R	0.1	<i>Melicytus ramiflorus</i>	1.2	-34.02	1.59	2.19	48.88	22.36
T6D1	R	0.1	<i>Melicytus ramiflorus</i>	2.1	-34.68	3.93	3.05	48.96	16.05
T6D2	R	0.1	<i>Lophomyrtus obcordata</i>	1.8	-34.32	0.70	1.20	47.19	39.16
T6D3	R	0.1	<i>Lophomyrtus obcordata</i>	1.2	-34.73	0.51	1.56	53.20	34.07
T6D4	R	0.1	<i>Streblus heterophyllus</i>	1.8	-36.80	1.53	3.07	42.60	13.87
T5A10	R	1.5	<i>Melicytus ramiflorus</i>	2.5	-37.32	3.00	3.04	41.66	13.71
T5A6	R	1.5	Creeper A	0.8	-31.50	1.86	4.04	44.99	11.14
T5A7	R	1.5	<i>Melicytus ramiflorus</i>	2.4	-32.40	3.69	3.13	45.87	14.65
T5A8	R	1.5	<i>Lophomyrtus obcordata</i>	1.2	-33.66	0.70	1.31	46.80	35.79
T5A9	R	1.5	<i>Pittosporum eugenoides</i>	2.1	-26.68	0.53	1.95	47.73	24.52
T5B5	R	1.5	<i>Melicytus ramiflorus</i>	1.9	-32.39	3.04	2.43	49.51	20.38
T5B6	R	1.5	<i>Streblus heterophyllus</i>	2.1	-34.50	2.57	2.82	48.25	17.09
T5B7	R	1.5	<i>Melicope simplex</i>	1.6	-35.25	3.50	2.94	53.06	18.03
T5C6	R	1.5	<i>Coprosma rotundifolia</i>	2.0	-35.78	1.36	2.50	50.68	20.28
T5C7	R	1.5	<i>Melicytus ramiflorus</i>	1.7	-33.49	0.82	2.71	50.84	18.77
T5C8	R	1.5	<i>Pittosporum eugenoides</i>	1.3	-32.28	-0.11	1.49	50.62	34.06
T5D6	R	1.5	<i>Coprosma rotundifolia</i>	1.8	-34.36	2.38	1.78	52.78	29.58
T5D7	R	1.5	<i>Melicytus ramiflorus</i>	1.5	-30.65	-0.07	1.66	52.00	31.25
T5D8	R	1.5	<i>Dacrycarpus dacrydioides</i>	1.0	-33.25	0.74	1.27	55.17	43.46
T5D9	R	1.5	<i>Coprosma robusta</i>	2.1	-31.90	0.79	1.63	45.91	28.12

T6A10	R	1.5	<i>Coprosma robusta</i>	2.3	-31.66	1.73	1.49	51.64	34.76
T6A5	R	1.5	<i>Coprosma rotundifolia</i>	1.2	-36.11	2.66	1.76	44.73	25.34
T6A6	R	1.5	<i>Dacrycarpus dacrydioides</i>	1.4	-29.79	0.73	1.50	54.60	36.33
T6A7	R	1.5	<i>Lophomyrtus obcordata</i>	1.3	-31.57	0.81	1.18	53.04	45.10
T6A8	R	1.5	<i>Pittosporum eugenioides</i>	1.4	-30.14	0.89	1.59	49.83	31.31
T6A9	R	1.5	<i>Lophomyrtus obcordata</i>	1.1	-30.85	2.59	2.34	55.79	23.82
T6B5	R	1.5	<i>Coprosma rotundifolia</i>	1.8	-35.64	2.27	2.00	49.76	24.89
T6B6	R	1.5	<i>Pittosporum eugenioides</i>	2.1	-31.33	-1.31	1.40	54.60	39.02
T6B7	R	1.5	<i>Elaeocarpus hookerianus</i>	1.4	-33.83	-0.16	2.12	56.21	26.48
T6B8	R	1.5	<i>Melicytus ramiflorus</i>	1.6	-33.03	1.79	2.59	47.34	18.31
T6B9	R	1.5	<i>Pittosporum eugenioides</i>	2.9	-32.53	-0.44	1.27	53.90	42.54
T6C4.1	R	1.5	<i>Melicytus ramiflorus</i>	2.4	-33.53	1.98	2.25	42.05	18.72
T6C4.2	R	1.5	<i>Lophomyrtus obcordata</i>	1.4	-30.93	1.19	1.26	54.23	43.06
T6D5	R	1.5	<i>Lophomyrtus obcordata</i>	1.5	-34.14	1.42	1.36	46.04	33.93
T6D6	R	1.5	<i>Pittosporum eugenioides</i>	1.8	-32.03	0.47	1.60	55.26	34.55
T6D7	R	1.5	<i>Melicytus ramiflorus</i>	1.4	-31.77	2.43	2.48	49.57	20.07
T5A11	R	3.5	<i>Melicytus ramiflorus</i>	2.7	-34.98	3.64	2.87	44.20	15.38
T5A12	R	3.5	<i>Pittosporum eugenioides</i>	2.2	-31.85	0.22	1.44	51.71	35.81
T5A13	R	3.5	Creeper A	1.3	-31.49	2.29	4.73	43.95	9.30
T5B8	R	3.5	<i>Melicytus ramiflorus</i>	2.8	-32.41	3.03	2.12	51.71	24.35
T5C10	R	3.5	Creeper B	1.9	-32.87	3.79	3.65	52.45	14.38
T5C9	R	3.5	<i>Melicytus ramiflorus</i>	1.5	-34.39	1.77	3.04	51.27	16.87
T5D10	R	3.5	<i>Melicytus ramiflorus</i>	2.2	-31.64	1.58	2.09	50.90	24.40
T5D11	R	3.5	unknown	1.0	-29.41	2.23	1.32	55.62	42.10
T5D12	R	3.5	<i>Coprosma rotundifolia</i>	2.5	-31.48	1.78	1.68	52.56	31.31
T5D13	R	3.5	Creeper B	2.3	-30.74	1.92	2.29	54.26	23.72
T5D14	R	3.5	<i>Lophomyrtus obcordata</i>	2.5	-29.73	1.57	1.63	56.70	34.80
T6A11	R	3.5	<i>Dacrycarpus dacrydioides</i>	2.5	-30.82	2.04	1.66	57.57	34.60
T6A12	R	3.5	<i>Melicytus ramiflorus</i>	1.6	-29.88	2.61	2.17	50.12	23.10
T6A13	R	3.5	<i>Lophomyrtus obcordata</i>	3.8	-26.22	3.97	1.62	52.13	32.21

T6A14	R	3.5	<i>Coprosma robusta</i>	1.8	-30.71	0.55	1.41	45.49	32.23
T6A15	R	3.5	Creeper B	1.9	-29.35	-0.49	2.48	53.45	21.53
T6B10	R	3.5	<i>Pittosporum eugenoides</i>	1.9	-29.61	-1.22	1.31	55.58	42.56
T6B11	R	3.5	unknown	2.3	-30.99	-0.19	1.07	56.42	52.56
T6C5	R	3.5	<i>Melicytus ramiflorus</i>	2.5	-28.95	3.04	1.99	51.44	25.82
T6C6	R	3.5	Creeper B	2.0	-32.94	1.08	3.65	45.37	12.43
T6C7	R	3.5	<i>Pittosporum eugenoides</i>	2.1	-32.63	0.21	1.11	44.52	40.06
T6D8	R	3.5	<i>Pittosporum eugenoides</i>	2.2	-31.26	1.36	1.58	56.83	35.99
T6D9	R	3.5	<i>Melicytus ramiflorus</i>	1.5	-30.81	2.48	2.45	50.04	20.40
T1A2	S	0.1	<i>Griselinia littoralis</i>	1.9	-31.56	-11.90	0.78	48.12	61.46
T1A3	S	0.1	<i>Dacrydium cupressinum</i>	1.5	-34.76	-11.20	0.92	48.88	53.01
T1A4	S	0.1	<i>Lophomyrtus obcordata</i>	1.6	-33.22	-11.44	1.01	55.41	55.05
T1A5	S	0.1	<i>Weinmannia racemosa</i>	1.9	-34.19	-9.04	0.69	57.14	83.14
T1A6	S	0.1	<i>Coprosma pedicellata</i>	1.3	-35.49	-8.52	1.28	51.63	40.40
T1A7	S	0.1	<i>Pseudowintera colorata</i>	1.5	-36.32	-8.19	1.07	51.78	48.38
T1B2	S	0.1	<i>Weinmannia racemosa</i>	1.7	-36.10	-8.57	0.65	57.36	87.87
T1B3	S	0.1	<i>Griselinia littoralis</i>	2.2	-33.30	-6.24	0.88	54.91	62.17
T1B4	S	0.1	<i>Manoao colensoi</i>	1.4	-36.27	-8.72	0.92	56.94	61.82
T1C1	S	0.1	<i>Quintinia serrata</i>	1.8	-35.61	-8.18	0.74	55.70	75.11
T1C2	S	0.1	<i>Neomyrtus pedunculata</i>	1.0	-36.79	-6.84	0.94	47.24	50.25
T1C3	S	0.1	<i>Weinmannia racemosa</i>	1.1	-35.04	-8.26	0.73	57.73	79.62
T1C4	S	0.1	<i>Carpodetus serratus</i>	1.7	-35.23	-0.30	1.32	52.38	39.57
T1D1	S	0.1	<i>Pseudowintera colorata</i>	1.7	-32.39	-8.46	1.11	63.60	57.14
T1D2	S	0.1	<i>Weinmannia racemosa</i>	1.5	-33.70	-10.42	0.76	57.91	75.78
T1D3	S	0.1	<i>Griselinia littoralis</i>	1.9	-30.12	-11.83	0.53	52.83	100.51
T1D4	S	0.1	<i>Neomyrtus pedunculata</i>	1.0	-33.04	-9.79	1.15	52.68	45.99
T1D5	S	0.1	<i>Coprosma pedicellata</i>	1.5	-35.98	-10.76	0.98	53.21	54.48
T1E1	S	0.1	<i>Neomyrtus pedunculata</i>	2.7	-31.17	-8.74	1.05	56.12	53.52
T1E2	S	0.1	<i>Weinmannia racemosa</i>	2.0	-32.47	-8.37	0.55	57.97	104.76
T1E3	S	0.1	<i>Cordyline banksii</i>	1.8	-28.87	-7.42	0.93	49.42	53.32

T1E4	S	0.1	<i>Hoheria angustifolia</i>	1.8	-29.42	-8.31	1.20	56.71	47.08
T4A1	S	0.1	<i>Quintinia serrata</i>	1.9	-32.18	-13.19	0.58	57.31	98.25
T4A2	S	0.1	<i>Phyllocladus alpinus</i>	1.3	-31.09	-0.14	0.56	57.63	102.62
T4A3	S	0.1	<i>Manoao colensoi</i>	1.0	-33.46	-8.71	0.91	50.25	55.09
T4A4	S	0.1	<i>Manoao colensoi</i>	1.5	-32.32	-5.10	1.19	50.81	42.62
T4A5	S	0.1	<i>Neomyrtus pedunculata</i>	1.6	-32.91	-10.65	0.82	53.59	65.00
T4A7a	S	0.1	<i>Weinmannia racemosa</i>	1.2	-33.24	-11.81	0.60	53.62	89.89
T4A7b	S	0.1	<i>Weinmannia racemosa</i>	1.6	-32.94	-12.21	0.64	49.52	77.38
T4A7c	S	0.1	<i>Weinmannia racemosa</i>	1.5	-33.32	-11.99	0.58	46.02	78.97
T4A7d	S	0.1	<i>Weinmannia racemosa</i>	2.0	-33.47	-12.43	0.55	47.37	85.60
T4A8	S	0.1	<i>Coprosma foetidissima</i>	1.6	-32.43	-11.50	0.82	50.43	61.16
T4B1	S	0.1	<i>Weinmannia racemosa</i>	1.8	-36.74	-7.19	0.99	49.12	49.42
T4B2	S	0.1	<i>Coprosma foetidissima</i>	1.4	-36.58	-5.90	2.17	51.01	23.50
T4B3	S	0.1	<i>Quintinia serrata</i>	2.2	-37.57	-7.71	1.08	46.53	42.92
T4B4	S	0.1	<i>Prumnopitys ferruginea</i>	1.3	-36.62	-8.25	1.06	53.14	50.11
T4B5	S	0.1	<i>Neomyrtus pedunculata</i>	1.4	-39.69	-4.78	1.30	45.60	35.20
T4B6	S	0.1	<i>Neomyrtus pedunculata</i>	0.9	-38.93	-5.30	1.49	44.84	30.06
T4C1	S	0.1	<i>Weinmannia racemosa</i>	1.2	-36.67	-3.37	1.11	48.93	44.19
T4C2	S	0.1	<i>Neomyrtus pedunculata</i>	1.4	-36.56	-3.14	1.32	52.41	39.75
T4C3	S	0.1	<i>Quintinia serrata</i>	1.4	-35.62	-5.70	0.97	53.75	55.44
T4C4	S	0.1	<i>Pseudopanax crassifolius</i>	0.9	-35.50	-5.66	0.83	47.43	56.96
T4D1	S	0.1	<i>Pseudopanax crassifolius</i>	1.2	-35.78	-10.32	0.74	56.18	75.80
T4D2	S	0.1	<i>Quintinia serrata</i>	1.5	-35.55	-11.05	0.62	54.97	88.65
T4D2a	S	0.1	<i>Quintinia serrata</i>	1.3	-35.66	-10.73	0.59	53.83	90.55
T4D2b	S	0.1	<i>Quintinia serrata</i>	1.6	-35.42	-10.46	0.62	53.33	85.86
T4D2c	S	0.1	<i>Quintinia serrata</i>	2.1	-35.74	-10.47	0.53	52.12	97.86
T4D2d	S	0.1	<i>Quintinia serrata</i>	0.9	-35.86	-11.71	0.63	51.23	81.26
T4D2e	S	0.1	<i>Quintinia serrata</i>	1.6	-35.55	-10.69	0.62	52.62	84.61
T4D2f	S	0.1	<i>Quintinia serrata</i>	1.5	-35.77	-10.85	0.61	52.36	86.11
T4D2g	S	0.1	<i>Quintinia serrata</i>	1.6	-35.75	-10.49	0.49	51.53	104.68

T4D2h	S	0.1	<i>Quintinia serrata</i>	1.3	-35.41	-10.87	0.59	52.47	89.36
T4D3	S	0.1	<i>Weinmannia racemosa</i>	1.9	-34.19	-11.63	0.64	58.76	91.98
T4D4	S	0.1	<i>Raukua simplex</i>	1.0	-37.44	-8.25	0.73	45.14	61.89
T4D5	S	0.1	unknown	0.9	-38.30	-4.41	1.26	50.57	40.25
T1A10	S	1.5	<i>Coprosma pedicellata</i>	2.8	-33.53	-5.05	1.46	56.76	38.98
T1A11	S	1.5	<i>Phyllocladus alpinus</i>	1.9	-29.51	-7.99	0.80	62.36	77.82
T1A12	S	1.5	<i>Weinmannia racemosa</i>	2.3	-32.09	-9.58	0.70	58.19	83.67
T1A8	S	1.5	<i>Griselinia littoralis</i>	1.7	-30.71	-7.16	0.95	47.07	50.10
T1A9	S	1.5	<i>Pseudopanax crassifolius</i>	1.8	-30.65	-8.13	1.13	55.56	49.25
T1B5	S	1.5	<i>Phyllocladus alpinus</i>	1.3	-34.82	-8.70	0.53	61.90	117.41
T1B6	S	1.5	<i>Griselinia littoralis</i>	1.5	-33.80	-6.49	0.89	52.83	59.28
T1B7	S	1.5	<i>Phyllocladus alpinus</i>	2.0	-31.85	-9.44	0.60	60.10	100.10
T1B8	S	1.5	<i>Weinmannia racemosa</i>	2.4	-34.37	-6.88	0.67	60.11	89.14
T1C10	S	1.5	<i>Myrsine divaricata</i>	1.8	-34.82	-0.35	1.28	56.20	43.82
T1C5	S	1.5	<i>Weinmannia racemosa</i>	1.3	-34.92	-7.73	0.73	56.14	76.89
T1C6	S	1.5	<i>Pseudopanax crassifolius</i>	1.9	-33.76	-8.61	0.73	57.87	78.99
T1C7	S	1.5	<i>Quintinia serrata</i>	1.4	-33.47	-7.70	0.73	55.37	76.11
T1C8	S	1.5	<i>Carpodetus serratus</i>	1.1	-33.63	-0.60	1.75	50.50	28.82
T1C9	S	1.5	<i>Elaeocarpus hookerianus</i>	1.3	-33.86	-4.63	1.30	54.20	41.75
T1D6	S	1.5	<i>Pseudowintera colorata</i>	1.6	-30.97	-7.30	1.17	63.03	53.78
T1D7	S	1.5	<i>Coprosma pedicellata</i>	1.3	-31.92	-4.33	1.12	55.33	49.47
T1D8	S	1.5	<i>Weinmannia racemosa</i>	2.2	-31.24	-9.95	0.83	59.77	72.17
T1D9	S	1.5	<i>Griselinia littoralis</i>	1.4	-28.99	-8.78	0.61	53.58	87.70
T1E5	S	1.5	unknown	2.3	-28.68	-5.58	0.98	56.49	57.71
T1E6	S	1.5	<i>Weinmannia racemosa</i>	2.9	-31.55	-8.92	0.81	60.16	73.90
T1E7	S	1.5	<i>Coprosma pedicellata</i>	1.5	-29.49	-3.26	1.45	56.25	38.79
T4A10	S	1.5	<i>Quintinia serrata</i>	2.7	-29.47	-13.92	0.71	51.11	71.53
T4A11	S	1.5	<i>Weinmannia racemosa</i>	2.4	-31.00	-11.55	0.67	59.09	88.21
T4A12	S	1.5	<i>Neomyrtus pedunculata</i>	1.2	-33.18	-10.85	1.04	47.18	45.54
T4A13	S	1.5	<i>Manoao colensoi</i>	2.3	-31.20	-4.02	1.27	55.08	43.35

T4A9	S	1.5	<i>Phyllocladus alpinus</i>	1.8	-28.92	-6.39	0.70	56.69	81.47
T4B10	S	1.5	<i>Griselinia littoralis</i>	1.8	-35.09	-4.06	1.06	49.15	46.21
T4B7	S	1.5	<i>Quintinia serrata</i>	1.6	-33.94	-6.30	0.84	49.22	58.64
T4B8	S	1.5	<i>Pseudopanax crassifolius</i>	1.1	-35.38	-0.66	1.54	49.50	32.11
T4B9	S	1.5	<i>Weinmannia racemosa</i>	2.5	-34.88	-5.73	0.92	59.06	64.01
T4C5	S	1.5	<i>Weinmannia racemosa</i>	2.1	-34.92	-3.09	0.96	57.14	59.82
T4C6	S	1.5	<i>Quintinia serrata</i>	1.7	-34.86	-5.91	0.94	45.76	48.90
T4C7	S	1.5	<i>Lophomyrtus obcordata</i>	0.9	-34.21	-4.41	1.14	47.43	41.78
T4D10	S	1.5	<i>Neomyrtus pedunculata</i>	1.7	-34.85	-4.73	1.08	52.14	48.36
T4D11	S	1.5	<i>Quintinia serrata</i>	2.0	-33.80	-11.73	0.66	57.28	86.57
T4D6	S	1.5	<i>Pseudopanax crassifolius</i>	1.1	-34.05	-7.63	0.98	47.31	48.27
T4D8	S	1.5	<i>Coprosma foetidissima</i>	1.4	-32.13	-6.14	1.56	53.63	34.32
T4D9	S	1.5	<i>Griselinia littoralis</i>	1.1	-33.34	-8.07	0.99	55.64	56.30
T4A14	S	3.5	<i>Pseudopanax crassifolius</i>	2.6	-29.33	-13.26	0.56	51.79	92.46
T4A15	S	3.5	<i>Griselinia littoralis</i>	1.1	-29.41	-10.28	0.63	55.77	88.32
T4A16	S	3.5	<i>Manoao colensoi</i>	2.0	-29.85	-2.70	1.28	53.29	41.78
T4A17	S	3.5	<i>Phyllocladus alpinus</i>	2.6	-28.73	-9.38	0.66	57.53	87.05
T4A18	S	3.5	<i>Quintinia serrata</i>	1.6	-29.73	-12.37	0.72	50.58	69.79
T4A20	S	3.5	<i>Weinmannia racemosa</i>	1.5	-30.95	-13.15	0.65	49.81	77.44
T4B11	S	3.5	<i>Griselinia littoralis</i>	1.1	-34.11	-4.85	0.84	48.01	57.43
T4B12	S	3.5	<i>Lophomyrtus obcordata</i>	1.3	-33.17	-5.71	1.04	44.99	43.43
T4B13	S	3.5	<i>Weinmannia racemosa</i>	1.6	-34.37	-8.09	0.91	49.81	54.75
T4C8	S	3.5	<i>Quintinia serrata</i>	1.4	-32.97	-3.25	0.87	49.04	56.51
T4C9	S	3.5	<i>Weinmannia racemosa</i>	1.7	-35.51	-2.13	0.90	49.90	55.28
T4D12	S	3.5	<i>Coprosma foetidissima</i>	1.7	-32.02	-5.97	1.30	45.90	35.24
T4D13	S	3.5	<i>Weinmannia racemosa</i>	1.8	-33.78	-10.18	0.73	58.74	80.89
T4D14	S	3.5	<i>Pseudopanax crassifolius</i>	1.6	-31.81	-11.61	1.03	51.90	50.55

Appendix 2: Raw data from stable isotopic analyses of the fossil leaf material from Pyramid Valley, North Canterbury.

Sample ID (layer, species)	Taxa grouping	Dried and ground sample mass (mg)	$\delta^{13}\text{C}$ (‰ v VPDB)	$\delta^{15}\text{N}$ (‰ v Air)	% N	% C	C:N
L2dicot	Dicotyledon	0.6	-28.79	4.05	1.69	46.32	27.39
L3dicot	Dicotyledon	1.6	-27.90	4.21	1.66	49.13	29.64
L4dicot	Dicotyledon	1.1	-28.75	4.45	1.66	52.27	31.48
L5dicot	Dicotyledon	2.3	-28.75	3.98	1.91	59.50	31.22
L6dicot	Dicotyledon	2.1	-29.02	3.27	1.93	51.17	26.55
L7dicot	Dicotyledon	2.2	-29.50	3.29	1.44	48.84	33.85
L8dicot	Dicotyledon	2.0	-29.73	3.68	1.39	58.01	41.67
L9dicot	Dicotyledon	1.8	-30.46	3.30	1.78	54.16	30.40
L10dicot	Dicotyledon	1.4	-30.12	3.79	1.54	52.03	33.81
L11dicot	Dicotyledon	1.2	-29.46	3.34	1.73	56.39	32.65
L12dicot	Dicotyledon	2.6	-29.28	4.77	1.83	53.06	28.94
L13dicot	Dicotyledon	2.4	-31.39	3.39	1.53	51.16	33.39
L14dicot	Dicotyledon	3.0	-29.22	3.35	1.63	48.66	29.93
L15dicot	Dicotyledon	2.8	-28.56	2.89	1.28	49.52	38.59
L16dicot	Dicotyledon	1.4	-30.25	2.73	1.26	52.76	42.02
L1matai	<i>Prumnopitys ferruginea</i>	1.0	-25.57	4.22	1.80	49.91	27.67
L5matai	<i>Prumnopitys ferruginea</i>	1.3	-27.66	3.57	1.82	55.94	30.71
L6matai	<i>Prumnopitys ferruginea</i>	1.3	-27.67	3.10	1.91	58.38	30.63
L7matai	<i>Prumnopitys ferruginea</i>	1.5	-27.72	3.41	1.90	57.04	30.02

L8matai	<i>Prumnopitys ferruginea</i>	2.4	-28.84	3.78	1.83	53.03	28.94
L9matai	<i>Prumnopitys ferruginea</i>	1.8	-26.89	3.33	1.68	62.65	37.26
L10matai	<i>Prumnopitys ferruginea</i>	1.5	-27.05	3.61	1.70	57.58	33.83
L11matai	<i>Prumnopitys ferruginea</i>	2.3	-26.90	4.19	1.99	56.65	28.44
L12matai	<i>Prumnopitys ferruginea</i>	2.0	-28.77	4.22	1.82	58.92	32.42
L13matai	<i>Prumnopitys ferruginea</i>	1.4	-26.87	4.13	1.91	65.16	34.15
L14matai	<i>Prumnopitys ferruginea</i>	1.6	-28.55	3.82	1.70	66.10	38.89
L15matai	<i>Prumnopitys ferruginea</i>	1.5	-26.39	3.23	1.81	58.28	32.27
L16matai	<i>Prumnopitys ferruginea</i>	0.9	-27.99	2.82	1.23	63.39	51.66
L6rubus	<i>Rubus</i> sp.	1.1	-25.74	2.85	1.06	53.42	50.63
L8rubus	<i>Rubus</i> sp.	1.7	-25.83	3.66	1.89	50.16	26.61
L15rubus	<i>Rubus</i> sp.	2.1	-25.35	3.04	1.67	51.49	30.76
L5unk.B	unknown	0.6	-26.32	3.93	1.55	57.11	36.89
L5unk.A	unknown	1.3	-26.72	5.42	1.38	55.25	39.92
L7unk.A	unknown	2.2	-27.10	5.24	1.69	60.05	35.63
L8unk.A	unknown	2.3	-28.94	4.30	1.50	47.58	31.64
L8unk.D	unknown	2.7	-25.70	3.20	1.13	61.49	54.32
L8unk.C	unknown	1.1	-27.28	3.03	1.07	74.67	70.09
L8unk.B	unknown	1.3	-26.61	4.14	1.51	52.65	34.97
L9unk.A	unknown	2.3	-26.95	4.96	1.56	60.09	38.43
L10unk.A	unknown	2.2	-27.85	4.59	1.51	57.14	37.77
L11unk.A	unknown	1.2	-24.58	3.45	1.25	60.50	48.47
L11unk.B	unknown	2.3	-25.08	4.80	1.36	55.61	41.02
L12unk.A	unknown	2.5	-26.93	4.75	1.81	57.62	31.87
L15unk.A	unknown	3.4	-26.10	5.26	1.76	42.15	23.99