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Research

Declining foliar and litter $\delta^{15}N$ diverge from soil, epiphyte and input $\delta^{15}N$ along a 120 000 yr temperate rainforest chronosequence

Duncan N. L. Menge[1,2], W. Troy Baisden[3], Sarah J. Richardson[4], Duane A. Peltzer[4] and Margaret M. Barbour[4,5]

[1]National Center for Ecological Analysis & Synthesis, 735 State St, Suite 300, Santa Barbara, CA, USA; [2]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA; [3]National Isotope Center, GNS Science, Lower Hutt, New Zealand; [4]Landcare Research, PO Box 40, Lincoln, Canterbury 7640, New Zealand; [5]Faculty of Agriculture, Food and Natural Resources, The University of Sydney, 107 Cobbitty Road, Cobbitty, NSW 2570, Australia

Author for correspondence:
Duncan N. L. Menge
Tel: +1 609 571 7773
Email: dmenge@princeton.edu

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Summary

- Patterns in the natural abundance of nitrogen (N) isotopes (^{15}N and ^{14}N) can help in the understanding of ecosystem processes along environmental gradients, because some processes fractionate against the heavier isotope. We measured $\delta^{15}N$ in many components of the Franz Josef soil chronosequence in New Zealand to see how each component varied along the sequence and within sites, and to see what this variation can tell us about how ecosystem processes such as N losses change with soil age.
- We analyzed $\delta^{15}N$ in foliage from 18 woody species, abscised leaves from seven woody species, three soil horizons, bryophytes, lichens, bulk deposition, and nodules from the N-fixing tree *Coriaria arborea* (Coriariaceae).
- Foliar $\delta^{15}N$ varied significantly across plant species. Foliage and bulk litter became ^{15}N-depleted as soil age increased. Soil N from organic and mineral horizons was significantly more ^{15}N-enriched than bulk litter N at each site. Increasing precipitation also decreased foliar and soil $\delta^{15}N$.
- Comparing input and whole ecosystem $\delta^{15}N$ revealed limited evidence for net fractionation during N losses. These trends are consistent with some combination of increasing fractionation during plant N uptake, mycorrhizal transfer, within-plant processing, and soil decomposition as soils age.

Introduction

Nitrogen (N) availability has strong impacts on community (Tilman, 1987; Zavaleta *et al.*, 2003) and ecosystem (Vitousek & Howarth, 1991; Elser *et al.*, 2007; LeBauer & Treseder, 2008) processes in many systems. Yet despite its importance, ecosystem-scale N cycling is difficult to study because of the number and complexity of ecosystem N pools and fluxes. Increasingly, natural abundance stable N isotope ratios have been used to study N dynamics (Houlton *et al.*, 2006, 2007; Craine *et al.*, 2009). Many ecosystem processes fractionate against heavier isotopes, resulting in ^{15}N-enriched source pools and ^{15}N-depleted sink pools. Therefore, natural abundance N isotopes provide a valuable integrative record of N dynamics (Högberg, 1997; Robinson, 2001).

The degree of fractionation is better known for some N transformations than for others (Dawson *et al.*, 2002; Tcherkez & Hodges, 2008). For example, biological N fixation (Dawson *et al.*, 2002) and nitrate leaching (Houlton *et al.*, 2006) fractionate little, but ammonium volatilization fractionates strongly (Dawson *et al.*, 2002). However, the degree of intrinsic fractionation during some processes is unclear because the observed fractionation depends on the degree of completion of the reaction as well as enzymatic discrimination. If a discriminating enzyme consumes all of its substrate, there is no net fractionation (Dawson *et al.*, 2002). For example, plant uptake of both nitrate and ammonium fractionates when available N concentrations are high or N is not limiting, but not when available N concentrations are low or N is

limiting (Högberg, 1997; McKee *et al.*, 2002; Clarkson *et al.*, 2005).

One use of natural abundance N isotopes is the study of N cycling along environmental gradients such as long-term soil chronosequences. On very young soils, soil N is scarce (Chapin *et al.*, 2002), N fertilization frequently stimulates net primary production (NPP; Vitousek *et al.*, 1993), and symbiotic N fixers are relatively common (e.g. Walker, 1993) and actively fixing dinitrogen gas (e.g. Vitousek & Walker, 1989). By contrast, very old, undisturbed soils are thought to be primarily phosphorus (P)-limited because the major P input – rock weathering – decreases over time, whereas N inputs from atmospheric deposition and some N fixers continue indefinitely (Beadle, 1966; Walker & Syers, 1976; Vitousek & Farrington, 1997; Vitousek, 2004; Wardle *et al.*, 2004). Within the range of P-limited soils, N dynamics can vary. Many old tropical forests are N-replete (Hedin *et al.*, 2009), whereas older temperate forests are often N-poor (Vitousek & Howarth, 1991; Hedin *et al.*, 1995; Perakis & Hedin, 2002), which can have implications for N isotope patterns.

To our knowledge, the patterns of natural abundance N isotopes in soils and plants have been investigated in five chronosequences in three different biomes: boreal forests in Alaska aged 55–225 yr (Hobbie *et al.*, 1999a), boreal forests in Sweden aged hundreds to thousands of years (Hyodo & Wardle, 2009), tropical rainforests in Hawaii aged 28–67 000 yr (Vitousek *et al.*, 1989) and 300–4 100 000 yr (Martinelli *et al.*, 1999), and temperate annual grasslands in California aged 3000–3 000 000 yr (Brenner *et al.*, 2001). Along both Hawaiian chronosequences, the older of which is known to switch from N limitation to co-limitation to P limitation (Vitousek & Farrington, 1997), both foliar and soil $\delta^{15}N$ (see the Materials and Methods section for $\delta^{15}N$ definition) increased with soil age (Vitousek *et al.*, 1989; Martinelli *et al.*, 1999), likely as the result of an increase in losses that fractionate against ^{15}N across the chronosequence (Hedin *et al.*, 2003). Foliar and litter $\delta^{15}N$ increased with forest age in Sweden, likely because of increased N fixation by free-living N-fixers, increased reliance on dissolved organic N, or both (Hyodo & Wardle, 2009). By contrast, foliar $\delta^{15}N$ decreased through early succession in Alaska (Hobbie *et al.*, 1999a), likely because of a shift in the importance of fractionating mycorrhizal N transfer to plants (Hobbie *et al.*, 1999b, 2000), although soil $\delta^{15}N$ showed no consistent pattern (Hobbie *et al.*, 1999a). On the California grassland chronosequence, foliar $\delta^{15}N$ showed no trend with soil age and soil $\delta^{15}N$ increased slightly (Brenner *et al.*, 2001). These studies demonstrate that long-term variation in natural isotope abundance differs among systems and is driven by a range of processes.

The Franz Josef chronosequence on the west coast of New Zealand's South Island is a well-studied chronosequence in temperate forest, a biome for which no chronosequence N isotope data have been reported to our knowledge. Another novel aspect of the Franz Josef chronosequence is that it spans the full range of ecosystem development stages (Peltzer *et al.*, 2010), including young progressive sites (< 10 yr), maximal biomass sites, and relatively old retrogressive sites (120 000 yr) (Richardson *et al.*, 2004). Total soil N : P and nonfixer foliar N : P generally increase with soil age (Stevens, 1968; Richardson *et al.*, 2004), consistent with a switch from N to P limitation. However, soil available N and foliar N decline in the older sites (Richardson *et al.*, 2004, 2005), suggesting that the older sites are N-poor even if they are not N-limited (see also Table 1).

In this study, we examined natural abundance N isotopes across the Franz Josef chronosequence. Specifically, we report $\delta^{15}N$ in foliage and abscised leaves; mineral, organic, and bulk litter soil horizons; lichens and bryophytes; and bulk deposition and actinorhizal root nodules, the last of which is an indication of symbiotic N fixation N inputs. We used these data to resolve two interlinked questions. First, how do each of these pools vary with soil age? And second, how do these pools compare within each site? These data will fill a gap in our knowledge of N isotope patterns along temperate forest chronosequences. Additionally, they will help us to determine the generality of N isotope patterns observed along chronosequences in other biomes.

Materials and Methods

Site description

The Franz Josef chronosequence (43°25′S 170°10′E) is composed of a series of schist outwash surfaces varying in soil age from 0 to > 120 000 yr, formed by repeated glacial advance and retreat over a narrow coastal strip in South Westland, New Zealand (Stevens, 1968; Walker & Syers, 1976; Almond *et al.*, 2001). The climate is wet temperate, with a mean annual temperature at the valley mouth of 10.8°C (Hessell, 1982), and mean annual precipitation (MAP) that ranges from up to 6.5 m near the glacier (surfaces < 12 000 yr) to *c.* 3.5 m on the coastal plains (≥ 12 000 yr) (Table 1). Although rainfall estimates at the sites vary within uncertainty bounds between estimates derived from climate surfaces (Richardson *et al.*, 2004) and meteorological studies (Henderson & Thompson, 1999), all estimates indicate a difference between the younger and older sites of up to 3 m, and precipitation measurements nearby suggest that such extreme gradients are plausible (Griffiths & McSaveney, 1983). Foliar and soil $\delta^{15}N$ correlate negatively with MAP across a number of scales (Handley *et al.*, 1999; Schuur & Matson, 2001; Amundson *et al.*, 2003). Thus, to ensure a conservative test of effects of site age on $\delta^{15}N$, we included the more variable MAP estimates (Richardson *et al.*, 2004) as an independent variable along with site age and sample type. We also note that surfaces younger than the last glacial period (≤ 12 000 yr)

Table 1 Site characteristics and $\delta^{15}N$ measurement species list

	Age (yr) 5 (site 1)	60 (site 2)	130 (site 3)	500 (site 5)	12 000 (site 7)	120 000 (site 9)
Annual precipitation[1] (mm)	6520	6576	6188	6278	3706	3652
Soil pH[1]	6.8	5.6	4.6	3.9	3.9	3.9
Total soil N : P[1] (g g^{-1})	0.07	2.8	6.8	17.5	17.5	33.0
Foliar N : P[1] (g g^{-1})	16.8	15.3	8.2	9.8	13.9	16.1
Nonfixer foliar N : P[1] (g g^{-1})	8.4	14.7	8.2	9.8	13.9	16.1
Maximum tree height[1] (m)	0.9	6.8	7.6	23.8	31.8	11.2
Bulk N deposition[2] (kg ha^{-1} yr^{-1})	1.5	1.5	1.5	1.5	0.9	0.9
N fixation[2] (kg ha^{-1} yr^{-1})	22.1	15.3	5.5	4.7	9.7	2.5
A_{max}[3] (μmol m^{-2} s^{-1})	19.6	16.3	12.5	8.0	5.3	3.9
Dominance weighting[1] (%)						
Coriaria arborea[Nf,e,a]	89*	25*				
Olearia avicenniifolia[e,a]	9*					
Aristotelia serrata[d,a]	2*	21*				
Schefflera digitata[e,a]		46				
Melicytus ramiflorus[e,a]		8*	1*			
Griselinia littoralis[e,a]		< 1	47*	2*		
Coprosma lucida[e,a]			28			
Pseudopanax colensoi[e,a]			11			
Coprosma ciliata[e,a]			2			
Carpodetus serratus[e,a]			1			
Weinmannia racemosa[e,a]			10*	20*	30*	13*
Prumnopitys ferruginea[e,c]				12		
Metrosideros umbellata[e,a]				66*	< 1*	3*
Dacrydium cupressinum[e,c]					44*	14*
Quintinia acutifolia[e,a]					26	14
Manoao colensoi[e,c]						15
Phyllocladus alpinus[e,c]						40
Podocarpus hallii[e,c]						1
% community occupied by sampled species	94	93	81	68	69	80

Site numbers correspond to Richardson *et al.* (2004). Soil N : P is from the upper 10 cm of the mineral horizon. Foliar N : P is dominance-weighted. Bulk N deposition for 60–500 yr sites was assigned the same value as the 5 yr site. N fixation is from *Coriaria arborea*, bryophytes, lichens and bulk litter. A_{max} is the mean maximum photosynthetic rate of species indicated by *s. Dominance weightings were calculated from cover scores (see the Materials and Methods section).

[1]From Richardson *et al.* (2004); [2]from Menge & Hedin (2009); [3]from Whitehead *et al.* (2005).

Species designations (superscripts): a, angiosperm; c, conifer; d, deciduous; e, evergreen; Nf, N fixer.

developed entirely in this climate, whereas older surfaces (> 12 000 yr) experienced cooler temperatures for a large part of their development (Moar & Suggate, 1996) and received atmospheric deposition of loess (Almond, 1996; Almond & Tonkin, 1999).

Clear patterns of forest succession and retrogression are apparent through changes in species composition, tree height and vegetation cover. These forest characteristics are related to strong patterns of plant and soil chemistry that are consistent with a shift from N limitation to P limitation along the chronosequence (Richardson *et al.*, 2004). For instance, N : P in total soil and in live foliage of nonfixing plants – which do not have direct access to atmospheric N_2 – increase with site age (Table 1). We selected six sites that ranged in age from 5 to 120 000 yr. These correspond to sites 1, 2, 3, 5, 7, and 9 from Richardson *et al.* (2004); throughout we use the site numbers from Richardson *et al.* (2004). The sites encompass the full range of ecosystem development stages along the chronosequence, from the progressive phase to the maximal biomass phase to the retrogressive phase (Peltzer *et al.*, 2010). Soil and plant properties of these sites are summarized in Table 1.

Sample collection and processing

New, fully expanded, sunlit canopy leaves were sampled from three trees of the dominant species at each site in March 2002 using orchard cutters for short canopies or a shotgun for taller canopies. We hoped to sample all important species at all sites, but each species only occupies a subset of the sites (Table 1). Therefore, our sampling reflects the best possible coverage of dominant species at each site, and there is an overlap of at least two species between each pair of consecutive sites. Eight species were sampled at more than one site, including some from sites aged 5000 and 60 000 yr, corresponding to sites 6 and 8 in Richardson *et al.* (2004), and an additional 10 species were sampled at a single site each (Table 1). In addition, freshly abscised leaves were collected

in March 2002 from the seven most common species (from underneath the same individuals used for canopy sampling) at sites where they formed a significant part of the canopy. We consider freshly abscised leaves – selected by species from the Oi horizon for comparison with fresh foliage – separately from bulk litter (the entire Oi horizon; see following paragraph). New canopy leaves from eight common species were resampled in January 2003 to test for interannual differences in N isotopic composition. Samples were dried at 70°C to constant mass and ground to a fine powder.

Soil samples from the upper 10 cm were collected in January 2002 from five subplots per site and divided into organic soil (Oa horizon, present only at the three oldest sites) and mineral soil (A horizon or below) (Richardson *et al.*, 2004). Bulk litter (Oi horizon) was sampled in January 2003. Samples were moist-sieved (4 mm), dried at 105°C to constant mass, and ground for analysis.

Samples of *Coriaria* root nodules, bryophytes, and lichens (the latter two from both ground and epiphytic surfaces) were collected along randomly spaced, randomly oriented transects in a subset of sites (Menge & Hedin, 2009). Bryophytes included *Ptychomnium aciculare* (sites 5 and 9) and *Hypnodendron* spp.; lichen species included *Pseudocyphellaria cinnamomea* (sites 1, 3, and 5), *Pseudocyphellaria homoeophylla* (sites 3 and 5), and other *Pseudocyphellaria* and *Sticta* spp. Samples were dried at 60°C to constant mass and ground for analysis. Drying at 60 vs 105°C can alter $\delta^{15}N$ values by up to 0.5‰ relative to each other and 1‰ relative to air-dried controls (Brearley, 2009), so comparisons across sample types (foliage vs soil vs other) should take this into account.

Event-based bulk deposition was sampled from July to December 2004 in open areas near sites 1, 7 and 9 using high-density polyethylene (HDPE) funnels 2 m off the ground connected to dark HDPE bottles on the ground (Menge & Hedin, 2009). Sites 2–5 are within 1.5 km of site one, so three collectors were sufficient. Samples were filtered (0.45 μm) immediately and frozen from ≤ 6 h after collection until they were chemically and isotopically analyzed. Our sampling design does not allow us to capture seasonal or within-canopy variation in $\delta^{15}N$. However, our main trends are much larger than observed seasonal changes in other systems – typically 1–2‰ at most (Ometto *et al.*, 2006; Coletta *et al.*, 2009; Bragazza *et al.*, 2010) – and within-canopy variation reported elsewhere (Bergstrom & Tweedie, 1998) is negligible.

Stable isotope analysis

Leaf and soil samples were analyzed for N content and isotopes at the Waikato Stable Isotope Unit in Waikato, New Zealand, using a Europa Scientific 20/20 (Europa Scientific 20/20, Crewe, UK) isotope ratio mass spectrometer (IRMS). *Coriaria* nodule, bryophyte, and lichen samples were analyzed for N content and isotopes at the Boston University Stable Isotope Laboratory in Boston, MA, USA, using a GV Instruments Isoprime IRMS (GV Instruments, Manchester, UK). The N isotopic composition of bulk deposition TDN was analyzed in the Sigman laboratory at Princeton University (Princeton, NJ, USA), using a modified Finnigan GasBench (Thermo Scientific, Pittsburgh, PA, USA) and DeltaPlus IRMS (Thermo Scientific, Pittsburgh, PA, USA), following the denitrifier method (Sigman, 2001) and persulfate oxidation (Knapp *et al.*, 2005). All machines were calibrated to international standards. All isotope ratios are expressed as $\delta^{15}N$ relative to the atmospheric N_2 standard: $\delta^{15}N = (({}^{15}N/{}^{14}N)_{sample}/({}^{15}N/{}^{14}N)_{standard} - 1) \times 1000$, in ‰ units.

Canopy species dominance weighting

The proportion of the upper sunlit canopy occupied by the species sampled at each site was estimated from visual cover assessments for each species made by Richardson *et al.* (2004). Average cover scores from five subplots of 5 m radius were combined with average maximum tree height for each species to give average cover scores for all species present within the upper third of the maximum tree height for the site. At each site the species sampled formed > 68% of the total cover (Table 1) and > 90% of the upper canopy cover (data not shown). The dominance weighting for each species sampled was then calculated from cover scores as the proportion of total sampled species cover. Dominance weighting values (Table 1) are used to estimate weighted mean foliar $\delta^{15}N$ at each site.

Statistical analyses

All statistical analyses were conducted in R 2.8 (R Development Core Team, 2009), and, unless otherwise stated, were linear regression models performed using the lm function. Two-tailed tests were used throughout, and post-hoc comparisons used Tukey's HSD.

Results

Foliar and abscised leaf $\delta^{15}N$

Foliar $\delta^{15}N$ ($\delta^{15}N_f$) ranged from very ^{15}N-depleted (−14‰) to slightly ^{15}N-enriched (4‰). Overall, site age and species explained 83% of the variation in the foliar $\delta^{15}N$ ($R^2 = 0.83$, $P < 0.0001$). Including MAP in the model increased the R^2 to 0.86, but the MAP main effect was not significant ($P = 0.58$) (see Fig. 1 caption for significance of all effects and interactions). Individual species tended to become more ^{15}N-depleted with soil age, especially in sites of intermediate to old age (Fig. 1). $\delta^{15}N_f$ of *Melicytus ramiflorus* (df = 14; $P = 0.004$), *Dacrydium cupressinum* (df = 29; $P < 0.001$), *Metrosideros umbellata* (df = 20; $P = 0.006$), *Weinmannia racemosa* (df = 46; $P < 0.0001$), and *Quintinia acutifolia*

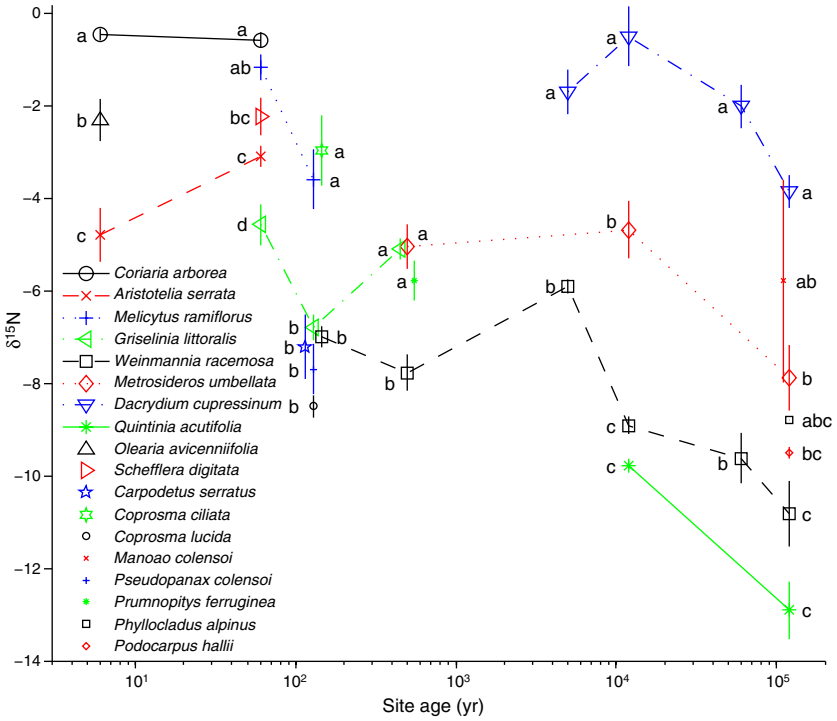

Fig. 1 Foliar $\delta^{15}N$ across the Franz Josef chronosequence. Symbols represent species mean ± SE at each site. Some species at certain sites are displayed with slightly altered ages for readability, but come from the same sites listed in the Materials and Methods section. Note the logarithmic scale of the horizontal axis. Effects and significance of the overall foliar model were A****, S****, P ns, A × S****, A × P***, S × P*** and A × S × P ns, where A is log site age, S is species, P is mean annual precipitation, '×' indicates the interaction term, and the significance codes are: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. The species effect was significant within each site ($P < 0.001$ for ANOVAs at sites aged 5, 60, 130, 500, 5000, 12 000, 60 000, and 120 000 yr, with df within, between species = 21, 2; 25, 4; 27, 6; 23, 3; 7, 1; 31, 3; 6, 1; 36, 6). Letters denote significant ($P < 0.05$) species differences within each site, and thus have no meaning across sites.

(df = 4; $P = 0.008$) all decreased with soil age, whereas *Coriaria arborea* (df = 14; $P = 0.64$) and *Griselinia littoralis* (df = 17; $P = 0.33$) showed no trend, and *Aristotelia serrata* increased (df = 14; $P = 0.02$). *D. cupressinum* ($P = 0.02$) and *M. umbellata* ($P = 0.02$) $\delta^{15}N_f$ were elevated at lower rainfall, whereas *W. racemosa* $\delta^{15}N_f$ was not ($P > 0.05$). However, the MAP × age interaction was significant for *W. racemosa* ($P = 0.007$), with a sharper decrease with age at lower rainfall than at higher rainfall (Fig. 1).

Within each site, $\delta^{15}N_f$ of individual species tended to differ (Fig. 1). Species differences within each site were also consistent across sites (Fig. 1). At each site where they coexisted, the symbiotic N fixer *Coriaria arborea* was more ^{15}N-enriched than *A. serrata*; *M. ramiflorus* was more ^{15}N-enriched than *G. littoralis*; *M. umbellata* was more ^{15}N-enriched than *W. racemosa*; and *D. cupressinum* was more ^{15}N-enriched than *M. umbellata*, *W. racemosa* and *Q. acutifolia*. The sole exception was that *G. littoralis* was more ^{15}N-enriched than *W. racemosa* at the 500 yr site but they did not differ statistically at the 130 yr site.

There was a strong correlation between $\delta^{15}N_f$ and abscised leaf $\delta^{15}N$ within individual trees from 2002, and the fit was very close to the 1 : 1 line (Fig. 2a). Within individual species, there was some variation between 2002 and 2003 foliage (Fig. 2b).

Dominance-weighted foliar $\delta^{15}N$

Dominance-weighted $\delta^{15}N_f$ decreased from −0.70‰ at site 1 to −8.45‰ at site 9 (Fig. 3a). Dominance-weighted $\delta^{15}N_f$ (df = 189) decreased with site age ($P < 0.0001$) and MAP ($P < 0.001$), with a significant interaction between the two ($P = 0.008$). For the model with MAP, $R^2 = 0.38$, whereas $R^2 = 0.31$ for the model without MAP ($P < 0.0001$ for both).

Soil $\delta^{15}N$

In our statistical analysis, soil $\delta^{15}N$ decreased with site age and MAP and varied with soil horizon, with significant age × horizon and age × MAP interactions (Fig. 3a; $R^2 = 0.84$ and 0.73 with and without including MAP; $P < 0.0001$ for both). Soil from the mineral horizon was ^{15}N-enriched (range −0.02–4.10‰) relative to the atmosphere (df = 27; $P = 0.002$). Soil horizons differed from each other within each site. As with plant species, individual soil horizon differences within sites were fairly consistent across sites. Soil from both the mineral (range −0.02‰–4.10‰) and organic (−2.28‰–1.17‰) horizons were more ^{15}N-enriched than bulk litter (−7.11‰ to −0.30‰) at each site, and the mineral horizon was more ^{15}N-enriched than the organic horizon at one site (Fig. 3a). Mineral soil $\delta^{15}N$ (df = 25) had a strong interaction between MAP and site age ($P < 0.001$), decreasing with site age at high precipitation but not changing at low precipitation; main effects showed a strong decrease at elevated MAP ($P < 0.0001$) but no change with site age ($P = 0.40$) (Fig. 3a). Organic soil $\delta^{15}N$ (df = 6; $P = 0.64$) showed no consistent trends with soil age ($P = 0.64$) or MAP ($P = 0.49$). By contrast, bulk litter $\delta^{15}N$ (df = 26) decreased with both soil age ($P < 0.001$) and MAP ($P = 0.04$), similar to dominance-

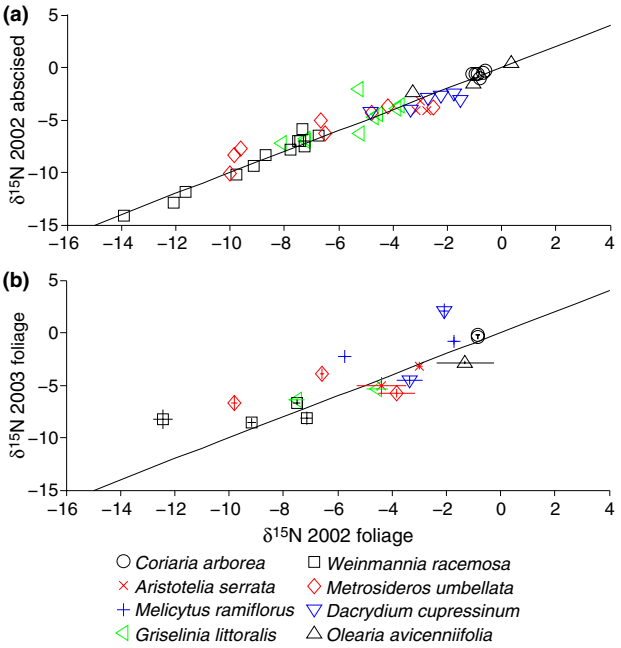

Fig. 2 $\delta^{15}N$ variation during abscission and from year to year. (a) Foliar and abscised $\delta^{15}N$ from the 2002 sampling were highly correlated (df = 45, r^2 = 0.94, P < 0.001). Each point represents a single tree, with species indicated by symbol shape. The line plotted is the 1 : 1 line, which is statistically indistinguishable from the fit (slope ± SE = 0.95 ± 0.04, intercept ± SE = −0.15 ± 0.23). Divergence below this line would indicate fractionation during N resorption, for which there is no strong evidence. (b) Foliar $\delta^{15}N$ in 2002 (horizontal axis) vs 2003 (vertical axis). The species means at each site were well correlated (df = 16, r^2 = 0.65, P < 0.001), but the fit diverged from the 1 : 1 line (slope ± SE = 0.75 ± 0.13, intercept ± SE = −0.47 ± 0.79). Each symbol represents a species mean ± SE. Species are the same as in Fig. 1.

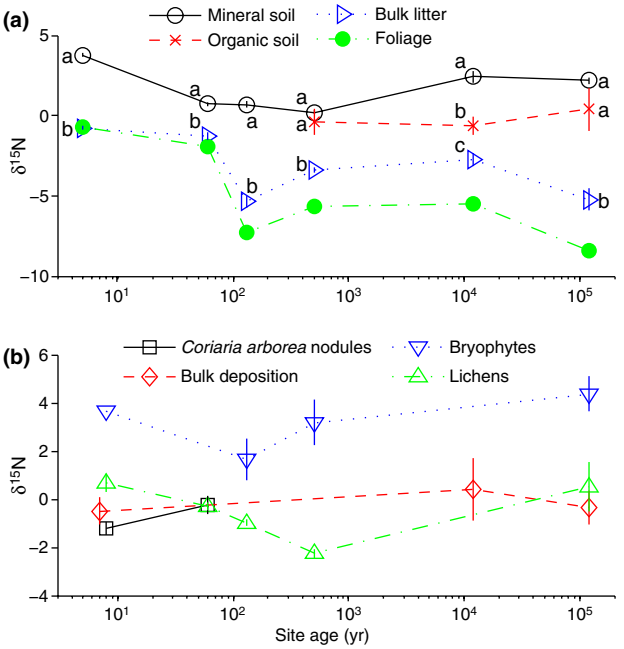

Fig. 3 $\delta^{15}N$ patterns across the Franz Josef chronosequence. (a) Mineral soil, organic soil, and bulk litter mean ± SE are shown by open symbols, and the dominance-weighted foliar mean is shown by closed circles. Effects and significance on soils (excluding foliar means, which are not soil) were A**, H****, P****, A × H**, A × P**, H × P ns, and A × H × P ns, where A is log site age, H is the soil horizon, P is mean annual precipitation, '×' indicates the interaction term, and the significance codes are: ns, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. Letters denote significant (P < 0.05) horizon differences within each site following ANOVAs (P < 0.0001 at each site) with df within, between soil horizons = 8, 1; 8, 1; 8, 1; 9, 2; 11, 2; and 9, 2 for sites aged 5, 60, 130, 500, 12 000, and 120 000 yr. (b) *Coriaria arborea* nodule (A ns), bulk deposition (A ns, P ns), bryophyte (A ns, P ns, A × P ns), and lichen (A ns, P****, A × P ns) means and SE are shown as a function of soil age. Note the logarithmic scale of the horizontal axis of both panels.

weighted plant $\delta^{15}N$ (Fig. 3a) but without a significant interaction (P = 0.54).

N input $\delta^{15}N$

Coriaria arborea nodule $\delta^{15}N$ ranged from −2.30‰ to 1.51‰, and bulk deposition $\delta^{15}N$ ranged from −2.40‰ to 2.90‰ (Fig. 3b). The mean of the two was −0.47‰. There were no significant differences between the two (P = 0.78) or effects of site age (P = 0.34) or MAP (P = 0.75). The full model, with R^2 = 0.13, was not significant (P = 0.56). Excluding the top two and bottom two points gives a range of −2.3‰ to 1.5‰, which we use as our reasonable input range.

Bryophyte and lichen $\delta^{15}N$

Bryophytes were the most ^{15}N-enriched pool ($\delta^{15}N$ range 0.78‰–5.96‰), and did not change with soil age (df = 6; P = 0.17) or MAP (P = 0.67) (Fig. 3b). Lichens ranged around the atmospheric standard (−3.00‰–2.13‰), did not change with soil age (df = 17; P = 0.85), but decreased with MAP (P < 0.0001) (Fig. 3b). For the full lichen $\delta^{15}N$ model, R^2 = 0.72 and P < 0.0001.

Discussion

Precipitation effects

Increasing MAP lowers the $\delta^{15}N$ of dominance-weighted foliage, foliage of two of three species, mineral soil, bulk litter, and lichens across the Franz Josef chronosequence, agreeing with published negative correlations between MAP and foliar and soil $\delta^{15}N$ across a range of scales (Austin & Sala, 1999; Handley *et al.*, 1999; Schuur & Matson, 2001; Amundson *et al.*, 2003; Craine *et al.*, 2009; Liu & Wang, 2010). Because our older sites (≥ 12 000 yr) have lower MAP (3500 mm) than our younger sites (6000–6500 mm; Table 1), the trends

with site age are even stronger than they appear, because the precipitation effect shifts $\delta^{15}N$ up at the older sites.

It is unlikely that water limits NPP at Franz Josef, but periods of soil anoxia are possible. The $\delta^{15}N$ decrease along a rainfall gradient in Maui (Schuur & Matson, 2001) has been attributed to an increasing degree of completion of denitrification resulting from such anoxia (Houlton *et al.*, 2006), such that the fractionation is expressed more in the less wet (2000–3500 mm yr^{-1}) sites than in the wetter (4000–5000 mm yr^{-1}) sites where nearly all nitrate is consumed. At Franz Josef the evidence for net fractionation during N losses is weak (see following section) so this mechanism has limited support, although we cannot rule it out. Another potential explanation for the change with MAP – changes in the $\delta^{15}N$ of N inputs (Heaton, 1987) – seems unlikely given that $\delta^{15}N$ of our bulk deposition inputs (Fig. 3b) does not change with precipitation.

Little evidence for fractionation during N losses

Fractionation during N losses is thought to play a major role in N isotope patterns in some ecosystems (Martinelli *et al.*, 1999; Houlton *et al.*, 2006; Hobbie & Ouimette, 2009), and strongly affects our expectations for our other patterns, so we derived a calculation to estimate net N loss fractionation with our data. We estimated the $\delta^{15}N$ of the entire ecosystem – essentially the weighted average of plant $\delta^{15}N$ and soil $\delta^{15}N$ – using foliar and soil data from Franz Josef, published corrections for soil depth and within-plant $\delta^{15}N$ differences, and a range of ratios of soil N to plant N (see Supporting Information, Methods S1 for details). The $\delta^{15}N$ of the entire ecosystem is equal to the $\delta^{15}N$ of inputs modified by net N loss fractionation during the history of the site, so we compared our ecosystem-level $\delta^{15}N$ estimate with our measured input $\delta^{15}N$ range to estimate net N loss fractionation.

Our reasonable range of net input $\delta^{15}N$ (−2.3‰–1.5‰) is similar to other findings (Handley *et al.*, 1999; Houlton *et al.*, 2006). Total plant $\delta^{15}N$ is quite negative at our older sites, whereas weighted total soil N is somewhat ^{15}N-enriched. Based on our best guesses for soil N to plant N ratios, ecosystem $\delta^{15}N$ is solidly within the input range until site 7, at which point it is slightly outside (0.6‰) the input range (Fig. 4). Therefore, based on our calculation, the evidence for net N loss fractionation is weak, although it is slightly stronger in the older sites. This is consistent with estimates from a global database of soil and plant $\delta^{15}N$ showing little evidence for fractionating N losses in cool, wet biomes (Amundson *et al.*, 2003), suggesting that fractionating losses such as denitrification either do not occur or are not expressed in these biomes. Our observations are also consistent with dissolved organic N leaching being the dominant loss pathway near the Franz Josef sites (McGroddy *et al.*, 2008), although strong P limitation in the oldest sites may make inorganic N losses more likely.

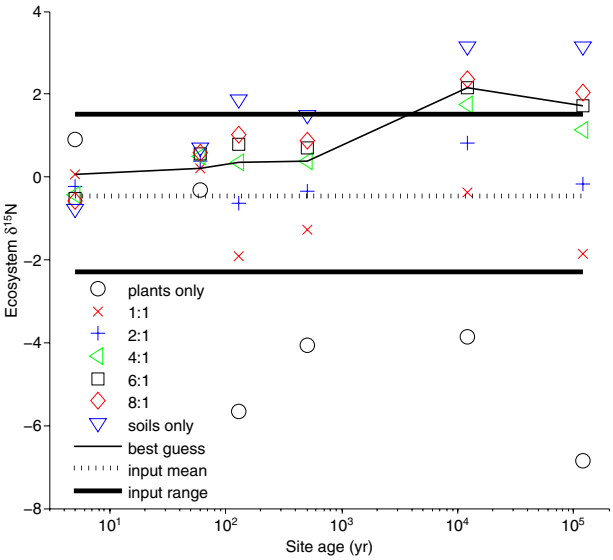

Fig. 4 Calculated ecosystem-level $\delta^{15}N$ relative to input $\delta^{15}N$. Ecosystem $\delta^{15}N$ is calculated as a weighted average of plant N and soil N. Plant $\delta^{15}N$ is weighted foliar N (see Fig. 3) adjusted by +1.6‰ to account for fractionation within the plant. Soil $\delta^{15}N$ is the weighted average of the soil horizons adjusted by +1.4‰ at sites with deep soils to account for ^{15}N-enriched deep soil (see Supporting Information, Methods S1 for details). The seven symbols at each site reflect different ratios of total soil N to total plant N, ranging from 0 : 1 (plants only) to ∞ : 1 (soils only). The thin line is our best guess at ecosystem-level $\delta^{15}N$. The thick horizontal lines are the upper and lower reasonable values of input $\delta^{15}N$ from our measurements of bulk deposition and N fixation. Values above this range indicate net fractionation during N losses over the course of ecosystem development. At sites 1 and 2 both plant $\delta^{15}N$ and soil $\delta^{15}N$ are within the input range of −2.3‰ to 1.5‰, so no ratio of soil N : plant N suggests net N loss fractionation. At sites 3 and 5, soil N : plant N ratios between 1 : 1 and 8 : 1 all yield ecosystem $\delta^{15}N$ values within the input range, but more extreme ratios would yield values outside the input range. At sites 7 and 9 soil N : plant N ratios of 4 : 1 and greater (site 7) and 6 : 1 and greater (site 9) produce ecosystem $\delta^{15}N$ values more ^{15}N-enriched than the input range. Note the logarithmic scale of the horizontal axis.

Litter is depleted relative to organic and mineral soil

The ^{15}N-enrichment from less decomposed (bulk litter) to more decomposed (mineral) soil horizons is consistent within each of our sites, and becomes exaggerated as soil age increases. This pattern is consistent with observations from temperate deciduous forests (Templer *et al.*, 2007), boreal forests (Hyodo & Wardle, 2009), tropical forests (Martinelli *et al.*, 1999), temperate grasslands (Brenner *et al.*, 2001; Baisden *et al.*, 2002), and elsewhere (Hobbie & Ouimette, 2009). All of the $\delta^{15}N$ patterns we observe at Franz Josef depend on complex interactions between multiple processes, discriminations, and degrees of completion, and a full understanding of these drivers would require careful experimentation. However, we can speculate about whether previously identified mechanisms are consistent with our results.

Amundson *et al.* (2003) and Amundson & Baisden (2000) presented simplified ecosystem N cycling using a two-pool model containing plants and soil. In that approach, fractionation may be associated with external losses of N and uptake of N from soil to plants. As noted previously, little or no evidence exists for fractionating external N losses along this chronosequence, so that a two-pool model implies the results we observe are caused by increasing fractionation associated with plant uptake. However, the simplified two-pool approach does not attempt to account for differences among co-occurring litter, organic soil and mineral soil. Some processes within the ecosystem can be expected to cause N isotope discrimination within these layers (Templer *et al.*, 2007). Among these processes, mineralization imparts only limited fractionation against ^{15}N, while nitrification and plant uptake can discriminate more strongly against ^{15}N, leaving behind a pool of ^{15}N-enriched available N (Högberg, 1997; Robinson, 2001; Dawson *et al.*, 2002; Amundson *et al.*, 2003). Successive processes of mineralization, nitrification, and plant uptake of ammonia or nitrate are therefore likely to explain the progressive increase in $\delta^{15}N$ values from litter to organic soil to mineral soil.

Decreasing foliar $\delta^{15}N$ ($\delta^{15}N_f$) with soil age

The $\delta^{15}N_f$ observed at Franz Josef spans a large part of the global range (Amundson *et al.*, 2003; Craine *et al.*, 2009), and is among the largest ranges recorded at a single location (Nadelhoffer *et al.*, 1996; Clarkson *et al.*, 2005). One of the most striking isotopic patterns at Franz Josef is the decrease in $\delta^{15}N_f$ across the chronosequence, which is seen in both the dominance-weighted foliage (Fig. 3a) and many individual species (Fig. 1). Because N retranslocation out of foliage does not fractionate against ^{15}N in these sites (Fig. 2a), $\delta^{15}N_f$ reflects the plant N source pool (e.g. nitrate, ammonium and small organic N molecules in the soil) at each site modified by fractionation against ^{15}N between the soil and foliage. The plant N source $\delta^{15}N$ and fractionation between soil and foliage depend on myriad interacting processes, but it is possible to speculate about the likelihood of potential drivers.

The plant N source pool could become ^{15}N-depleted across the chronosequence if it comes primarily from decomposition of litter. In support of this interpretation, Baisden *et al.* (2002) found that plant N resembled soil pools with rapid turnover in California grasslands. The interpretation that plant N becomes depleted because litter N becomes depleted, however, suffers from a chicken-and-egg problem, as litter N is depleted because plant N is depleted. Therefore, although this feedback can contribute to the foliar pattern, it cannot wholly explain it.

Fractionation during N uptake could also increase with site age. If N is strongly limiting in the early sites, N uptake would be nearly complete, resulting in little uptake fractionation regardless of root or mycorrhizal isotopic discrimination. By contrast, if N is plentiful relative to plant demand in the older, presumably P-limited sites, the expressed fractionation may be greater (Högberg, 1997; Fry *et al.*, 2000; McKee *et al.*, 2002; Clarkson *et al.*, 2005), which could deplete $\delta^{15}N_f$ with increasing soil age. Mycorrhizal N transfer to plants can fractionate strongly (Hobbie *et al.*, 1999a; Craine *et al.*, 2009), and the decreasing overall fertility in the older sites might encourage greater mycorrhizal colonization, which would be consistent with decreasing $\delta^{15}N_f$ across the sites. Finally, although fractionation during within-plant processing can be large (up to 11‰) (Handley & Raven, 1992), it is typically small (< 2‰) (Högberg, 1997; Houlton *et al.*, 2007; Templer *et al.*, 2007), so there is little evidence to suggest change in within-plant fractionation across the sites.

Different isotopic values for different species

The consistent and dramatic isotopic separation of different species' $\delta^{15}N_f$ that we observed at each site is not unique to Franz Josef (Michelsen *et al.*, 1996, 1998; Hobbie *et al.*, 2005; Templer *et al.*, 2007; Kahmen *et al.*, 2008). However, the substantially greater variation in species $\delta^{15}N$ in the older sites, suggesting a possible divergence in plant strategies for acquiring and processing N, differs from other chronosequences (Vitousek *et al.*, 1989; Martinelli *et al.*, 1999; Hobbie *et al.*, 2000; Hyodo & Wardle, 2009). Tree preferences for different forms of nitrogen such as nitrate vs ammonium, (McKane *et al.*, 2002; Templer & Dawson, 2004; Kahmen *et al.*, 2008) – which can differ widely in $\delta^{15}N$ at a given site (Houlton *et al.*, 2007) – could lead to species differences in $\delta^{15}N_f$ (Kahmen *et al.*, 2008). Species differences within sites do not result from different types of mycorrhizal associates – which can influence $\delta^{15}N_f$ (Michelsen *et al.*, 1996, 1998; Craine *et al.*, 2009) – because all species we sampled form arbuscular mycorrhizal associations (McNabb, 1958; Baylis *et al.*, 1963; Hall, 1975; Hurst *et al.*, 2002; Russell *et al.*, 2002). However, different degrees of mycorrhizal infection can also influence $\delta^{15}N_f$ (Hobbie *et al.*, 2005), and might differ between species. If this is the dominant mechanism, we would expect the ^{15}N-enriched species such as *D. cupressinum* and *M. ramiflorus* to have less mycorrhizal infection than the ^{15}N-depleted species such as *Q. acutifolia* and *W. racemosa*. The grouping of plant species by $\delta^{15}N_f$ is intriguing, as it does not correspond to groupings by traits such as leaf mass per unit area or growth rate (S. Richardson, unpublished).

Bryophyte ^{15}N-enrichment

The ^{15}N-enrichment of bryophytes in Franz Josef contrasts with bryophyte $\delta^{15}N$ in Europe (Pearson *et al.*, 2000; Bragazza *et al.*, 2005; Solga *et al.*, 2005; Skinner *et al.*,

2006; Zechmeister *et al.*, 2008) and Central America (Hietz *et al.*, 2002; Wania *et al.*, 2002), which is typically depleted (generally −12‰ to −2‰, but up to 6‰ near NO_x sources). The bryophytes in many of these other studies were not under a canopy, and atmospheric deposition rates were generally high (up to 20 kg N ha^{-1} yr^{-1}); there was a strong correlation between bryophyte $\delta^{15}N$ and atmospheric deposition $\delta^{15}N$. The bryophytes in our study occur as epiphytes, on the forest floor, and on fallen logs, and atmospheric deposition is low (0.9–1.5 kg N ha^{-1} yr^{-1} (Menge & Hedin, 2009)). Thus, N sources also include biological N fixation by cyanobacteria within the mats, leaching from foliage above, and litter deposition directly onto mats. All of these potential sources are ^{15}N-depleted relative to bryophytes (Figs 1–3). Therefore, the ^{15}N-enriched bryophyte signal could result from fractionating N losses from bryophyte mats themselves or fractionating N uptake into aerial plant roots scavenging bryophyte mats. At least one species at Franz Josef – *Metrosideros umbellata* – has aerial roots (Dawson, 1967).

Comparison of soil and foliar results to other chronosequences

Bulk soil shows ^{15}N enrichment with site age in California (Brenner *et al.*, 2001) and Hawaii (Martinelli *et al.*, 1999), but no apparent change with site age in Sweden (Hyodo & Wardle, 2009) and Alaska (Hobbie *et al.*, 1999a). At Franz Josef there is no strong soil $\delta^{15}N$ pattern; there appears to be slight enrichment across the sites in bulk soil $\delta^{15}N$ when soil pools are weighted by N content (Fig. 4), but neither mineral nor organic soil changes with soil age on its own. An increase in net N loss fractionation with site age has been proposed as the explanation for the Hawaiian (Vitousek *et al.*, 1989) and Californian (Brenner *et al.*, 2001) sites. This explanation is consistent with our N loss calculation, which suggests no fractionation during N losses at the younger Franz Josef sites and small amounts of fractionation during N losses at the older sites. All the chronosequences display more ^{15}N enrichment with degree of soil decomposition (Hobbie *et al.*, 1999a; Martinelli *et al.*, 1999; Brenner *et al.*, 2001; Hyodo & Wardle, 2009) (Fig. 3). Discrimination against ^{15}N during some aspect of decomposition, as hypothesized by Templer *et al.* (2007), is the most universal candidate for this broad pattern. Foliage becomes more ^{15}N-depleted with soil age in Franz Josef (Figs 1, 3a), similar to Alaska (Hobbie *et al.*, 1999a) but unlike Hawaii (Vitousek *et al.*, 1989; Martinelli *et al.*, 1999), Sweden (Hyodo & Wardle, 2009) (both more ^{15}N-enriched with site age), and California (Brenner *et al.*, 2001) (no change).

Isotopically, the Franz Josef chronosequence is most similar to the Alaskan chronosequence, and they are similar in other ways as well. Both are primary successional sequences initiated by glacial retreat, both have actinorhizal N fixers that dominate early successional habitats (Chapin *et al.*, 1994; Richardson *et al.*, 2004), and both become nutrient-poor in the older sites. In Alaska mycorrhizal transfer has been implicated as the dominant mechanism explaining decreasing foliar $\delta^{15}N$ with soil age (Hobbie *et al.*, 1999b, 2000, 2005). This could contribute to the pattern at Franz Josef, but we have no hard evidence to evaluate this mechanism, and other factors such as fractionation during N uptake, within-plant processing, or fractionation during soil organic matter decomposition might play roles. The ways in which the Franz Josef chronosequence differs from the Alaskan chronosequence represent an intermediate between the Alaskan and Hawaiian sites, consistent with its intermediate latitudinal location. For example, the old sites at Franz Josef are thought to be P-limited like the old Hawaiian sites but N-poor like the old Alaskan sites, and the bulk soil $\delta^{15}N$ pattern with age lies between the clear rise in Hawaii and the stasis in Alaska.

This study represents the first chronosequence study of N isotopes in temperate forests, helping to fill in the global map of natural abundance N isotope patterns. The clear decrease in foliar and litter $\delta^{15}N$ with soil age, as observed in boreal but not in tropical forests, could result from a number of mechanisms, including fractionation during decomposition or fractionation between the plant N source and foliage, although we currently lack the data to evaluate the likelihood of these mechanisms. The divergence of foliar and litter $\delta^{15}N$ from slower turnover soil N pools appears ubiquitous across forests worldwide, and could result from fractionation during some aspect of decomposition. Soil $\delta^{15}N$ does not rise clearly as it does in tropical forests, corresponding to a lower likelihood of net fractionation during N losses.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Methods S1 Ecosystem-level calculation.

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Supporting Information

Methods S1 Ecosystem-level calculation

Ecosystem-level $\delta^{15}N$, the weighted average of all ecosystem components, equals the $\delta^{15}N$ of inputs modified by net N loss fractionation during the history of the site. We consider the entire ecosystem, and not soil N in isolation (*sensu* Amundson *et al.*, 2003; Houlton *et al.*, 2006), because in contrast these previous studies we cannot assume steady state in our sites and we are concerned with N losses integrated over time rather than instantaneous measurements. Because we measured the $\delta^{15}N$ of the dominant inputs, an estimate of ecosystem-level $\delta^{15}N$ would reveal net N loss fractionation at each site. We assume that plant and soil N are the only major N pools within these forest ecosystems. Excluding bryophytes and lichens from the calculation is justified because Stevens' (1968) estimates of soil N stocks exceed bryophyte and lichen N stocks by well over an order of magnitude (Menge & Hedin, 2009 and D. Menge, unpublished data) and the $\delta^{15}N$ of bryophytes and lichens is similar to soils (within a few per mil). Therefore, if f_P is the fraction of ecosystem N in plants and $(1 - f_P)$ is the fraction in soils,

$$\delta^{15}N_{ecosystem} = f_P\delta^{15}N_{plant} + (1 - f_P)\delta^{15}N_{soil} \qquad \text{Eqn 1}$$

Stevens (1968) reported N stocks in mineral soil, organic soil, and bulk litter at sites two, three, five, and seven, allowing us to calculate a weighted average of soil $\delta^{15}N$ for the upper 10 cm at each of these sites. For site one we used bulk litter $\delta^{15}N$ as soil $\delta^{15}N$ because other soil horizons are negligible. At site nine we assumed the same ratios as site seven. Although there is substantial variation in how deeper soil relates to the top 10 cm (Hobbie & Ouimette, 2009), a global analysis has shown that soil $\delta^{15}N$ to 50 cm depth is well correlated with soil $\delta^{15}N$ to 10 cm depth and about 1.4‰ more ^{15}N-enriched (Amundson *et al.*, 2003). We added this global mean

adjustment of 1.4‰ to our weighted average of 0-10 cm soil $\delta^{15}N$ to calculate overall $\delta^{15}N_{soil}$ at sites three, five, seven, and nine (those with deeper soil).

We calculated weighted foliar $\delta^{15}N$ from our dominance weightings of each species (Table 1). Foliage is typically depleted relative to roots and wood by ~2‰ (Högberg, 1997; Houlton *et al.*, 2007; Templer *et al.*, 2007) (but see Bergersen *et al.*, 1988) and foliage contains an eighth to a quarter of the N in similar trees (Whittaker *et al.*, 1979; Vann *et al.*, 2002), so we added 1.6‰ to foliar $\delta^{15}N$ at each site to estimate $\delta^{15}N_{plant}$. We did not have plant N stock estimates, and thus could not calculate the fraction of ecosystem N in plants, f_P. Ratios of soil N to plant N in similar mature forests in Chile are 4:1 to 8:1 (Vann *et al.*, 2002), and some of our forests are younger with less developed soils, so we calculated ecosystem $\delta^{15}N$ using soil N to plant N ratios of 1:1, 2:1, 4:1, 6:1, and 8:1, as well as the end members of 0:1 (plants only) and ∞:1 (soils only).

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