

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope composition in plant and soil in Southern Patagonia's native forests

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Abstract

Stable isotope natural abundance measurements integrate across several biogeochemical processes in ecosystem N and C dynamics. Here, we report trends in natural isotope abundance ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in plant and soil) along a climo-sequence of 33 *Nothofagus* forest stands located within Patagonia, Southern Argentina. We measured 28 different abiotic variables (both climatic variables and soil properties) to characterize environmental conditions at each of the 33 sites. Foliar $\delta^{13}\text{C}$ values ranged from -35.4‰ to -27.7‰ , and correlated positively with foliar $\delta^{15}\text{N}$ values, ranging from -3.7‰ to 5.2‰ . Soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values reflected the isotopic trends of the foliar tissues and ranged from -29.8‰ to -25.3‰ , and -4.8‰ to 6.4‰ , respectively, with no significant differences between *Nothofagus* species (*Nothofagus pumilio*, *Nothofagus antarctica*, *Nothofagus betuloides*). Principal component analysis and multiple regressions suggested that mainly water availability variables (mean annual precipitation), but not soil properties, explained between 42% and 79% of the variations in foliar and soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ natural abundance, which declined with increased moisture supply. We conclude that a decline in water use efficiency at wetter sites promotes both the depletion of heavy C and N isotopes in soil and plant biomass. Soil $\delta^{13}\text{C}$ values were higher than those of the plant tissues and this difference increased as annual precipitation increased. No such differences were apparent when $\delta^{15}\text{N}$ values in soil and plant were compared, which indicates that climatic differences contributed more to the overall C balance than to the overall N balance in these forest ecosystems.

Keywords: foliar isotope, *Nothofagus*, rainfall, soil $\delta^{13}\text{C}$, soil $\delta^{15}\text{N}$

Received 16 December 2010 and accepted 13 June 2011

Introduction

Terrestrial nitrogen (N) fluxes have doubled in the last few decades, and anthropogenic N emissions have reached a magnitude similar to the whole amount of N released from the Earth's biosphere (<http://www.millenniumassessment.org>). The feedback mechanisms between the increased N input and the biogeochemical element cycles are complex, including faster growth of some plant species and adverse effects on ecological processes impacted by water and nutrient availability (Bobbink *et al.*, 2010), and a modulation of global biogeochemical cycles such as that of soil carbon (C) (e.g., Agren & Bosatta, 1996; Neff *et al.*, 2002). Hence, there is an increasing need for understanding the response of ecosystems to changes in N and water fluxes. The measurement of the stable isotope composition of carbon and nitrogen can provide insights into nutrient and

moisture dynamics in forest ecosystems (e.g., Garten & van Miegroet, 1994; Nadelhöfer & Fry, 1994; Staddon, 2004), because these isotopes integrate several fundamental biogeochemical processes (Handley *et al.*, 1999).

The discovery that plant tissues contain less ^{13}C than the ambient air has been utilized in global C-cycling studies. In fact, the negative correlation between the stable $\delta^{13}\text{C}$ isotope ratio and intrinsic, photosynthetic water use efficiency (WUE) (Farquhar & Richards, 1984) has led to a wide-spread use of isotopic analyses in plant physiological ecology. In general, when a site is moist and WUE is low, stomatal conductance tends to operate at or near its maximum. Under wet conditions, the intercellular CO_2 concentration is high and plants increasingly discriminate against $^{13}\text{CO}_2$ during photosynthesis, resulting in low tissue $\delta^{13}\text{C}$ values. In contrast, for a given plant species, tissue $\delta^{13}\text{C}$ values are higher and closer to ambient air when WUE is higher under conditions of water stress (Farquhar & Richards, 1984; Farquhar *et al.*, 1989), even if this relationship may not always be linear (Seibt *et al.*, 2008). Formation

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of soil organic matter (SOM) largely preserves the isotopic signal of the plants, with some minor microbial isotope discrimination (Boutton, 1996; Hobbie, 2005). Hence, the stable carbon isotopic signature of soil aggregates could be related to environmental adversity (abiotic stress) in temperate forest and woodland ecosystems (Ladd *et al.*, 2009).

In contrast to the situation with carbon, our understanding of the effects of environmental variables on N isotope discrimination is far from complete. From a soil science point of view any mechanism that leads to loss of N (volatilization, mineralization and leaching, plant uptake) will leave the heavier N isotope behind (Högberg, 1997). In this context, there is evidence that microbial denitrification is a main vector of N loss from tropical rainforests; with a large impact on overall forest N balances (Houlton *et al.*, 2006; Bai & Houlton, 2009). Thus, tropical forest ecosystems with high denitrification rates show elevated $\delta^{15}\text{N}$ values in both plant and soil. Whether similar mechanisms also apply to temperate forests is less clear. Nevertheless, there is increasing evidence that there are global patterns in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ natural abundance of plant tissues and in soils along gradients of precipitation, and in response to other environmental variables such as temperature, soil water content, irradiance, and soil nitrogen availability (Austin & Vitousek, 1998; Handley *et al.*, 1999; Ehleringer *et al.*, 2000; Miller *et al.*, 2001; Sah & Brumme, 2003; Song *et al.*, 2008). In general, plant $\delta^{15}\text{N}$ values decline with increasing annual precipitation, likely due to the mobilization of 'lighter' N for plant nutrition (Craine *et al.*, 2009). However, increases in soil $\delta^{15}\text{N}$ values with increasing moisture supply due to increasing denitrification losses have, to our knowledge, not yet been observed at macro-scale gradients. Few studies have considered both carbon and nitrogen isotope signatures simultaneously, and many authors have reported that there may be large species-dependent variations in isotopic tissue signals (Garten & van Miegroet, 1994; Marshall & Zhang, 1994; Nadelhöfer & Fry, 1994). Controlling plant species composition along environmental gradients would thus be of advantage when using stable isotope signatures as an integrative measure of biogeochemical changes along such gradients.

Southern Patagonia (Santa Cruz and Tierra del Fuego provinces, Argentina) has a wide variety of climate and vegetation types. The most abundant forest type is cool temperate forest dominated by deciduous *Nothofagus pumilio* and/or *Nothofagus antarctica* (frequently used in silvopastoral systems). In Southern Argentina, *Nothofagus* forest cover an area of ca. 335 450 ha, between latitudes 46–56°S, and from sea level to more than 2000 m asl in elevation. Over this geographic range *Nothofagus*

can be found at sites with a wide range of environmental conditions. *Nothofagus pumilio* forest is the main commercial woodland that grows mainly in pure stands, the trees potentially reach a height of 30 m and a diameter of 1.7 m. However, *N. pumilio* does not grow in poorly drained soils and its growth is limited by low fertility. *Nothofagus antarctica* displays the greatest variation and may occur as either tall trees up to 15 m in height at wetter sites with the best growing conditions, or may occur as shrubby trees of ca. 2 m height on rocky, xeric, exposed sites, and/or on poorly drained sites (peat bogs). *Nothofagus betuloides* is another significant dominant forest species in Patagonia and is found in a narrow belt close to the margin of lakes, possibly because the lake margin habitat experiences milder winters. Seedlings of all three *Nothofagus* species typically respond to the creation of canopy gaps by tree falls, and low seedling establishment is often associated with insufficient soil moisture (Veblen *et al.*, 1996). Here, we worked in the primary forest, which is dominated by these three *Nothofagus* species. It is thus possible to find the same dominating *Nothofagus* tree genus along a latitudinal gradient, which is more than 1000 km long and characterized by mean annual precipitation (MAP) ranging from <280 to >880 mm. Studying the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ natural abundance in plants and soil thus promised to give insight into the resource use efficiency of these *Nothofagus* trees along a precipitation gradient. Yet there is a paucity of studies of natural isotope abundance along environmental gradients in *Nothofagus* forests in Patagonia (but see Schulze *et al.*, 1996).

In this study, we measured a large number of abiotic variables to identify first-order controls on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ variation in *Nothofagus* leaves and soil of Patagonia's temperate forest ecosystems. We hypothesized that (i) similar to other biomes plant $\delta^{15}\text{N}$ values in the *Nothofagus* forests would decline with increased moisture supply, because of increased usage of lighter N sources (see above and Craine *et al.*, 2009). As a result (ii) soil $\delta^{15}\text{N}$ values should be higher than those of the plant tissues, and this difference should increase with increasing moisture supply. Furthermore, if moisture is a main driver of these biogeochemical linkages, we hypothesize that (iii) the $\delta^{13}\text{C}$ values of both soil and plants should be closely correlated with the $\delta^{15}\text{N}$ signals, with lower $\delta^{13}\text{C}$ values in the plant at conditions of low WUE, that is, at sites of high MAP.

Methods

Study sites

We measured 28 different abiotic variables at 33 locations (Fig. 1) in Patagonia, Southern Argentina. At each sampling

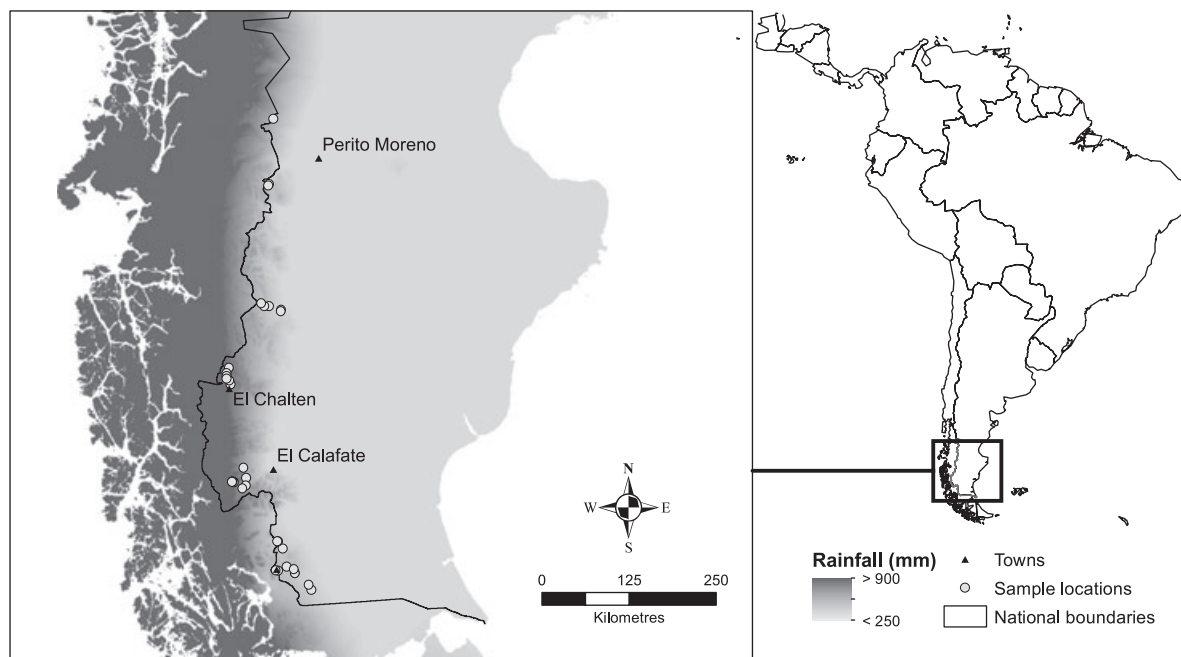


Fig. 1 Site locations of studied *Nothofagus* forests in Patagonia, Southern Argentina.

location, plant, soil, and ecosystem properties were measured in a 20 m × 40 m quadrat. *Nothofagus* forests were sampled in February 2007 and 2008 (at the peak of the Austral summer) across latitudinal (temperature gradient) and longitudinal (rainfall gradient) transects (Table 1). Each stand was dominated by one *Nothofagus* species. We sampled forest communities that occupied a diverse range of topographic positions within the landscape. The height of mature trees ranged from 2 to 26.8 m across transects (Table 1). All sampling sites were either forest or woodland communities dominated by species with the C₃ photosynthetic pathway and all sites were undisturbed for at least 50 years. Data used in the analyses (Appendix S1) were restricted to natural forest ecosystems where soil was the major source of plant N. A digital clinometer (Haglöf, Långsele, Sweden) was used to measure the height of the 10 largest trees within each quadrat.

Climatic variables

Climatic parameters for each site were derived from the WorldClim data set (<http://www.worldclim.org/>) (Hijmans *et al.*, 2005). WorldClim contains geographic surfaces for 19 different climatic parameters that describe rainfall, temperature, and variation in those parameters at a resolution of 30 arc seconds (0.008333°, ca. 1 km). This data set has been used successfully in the Patagonian region in related work (Ladd *et al.*, 2009). The climatic parameters in WorldClim were: mean annual temperature (MAT), mean diurnal temperature range (TM_{DR}), isothermality (T_{ISO}), temperature seasonality (T_{SEAS}), maximum temperature of warmest month (TM_{max_warm_month}), minimum temperature of coldest month (TM_{min_cold_month}), temperature annual range (T_{AR}), mean temperature of wettest quarter (TM_{wetQ}), mean temperature of

driest quarter (TM_{dryQ}), mean temperature of warmest quarter (TM_{warmQ}), mean temperature of coldest quarter (TM_{coldQ}), MAP, precipitation of wettest month (P_{wet_month}), precipitation of driest month (P_{dry_month}), precipitation seasonality (P_{seas}), precipitation of wettest quarter (P_{wetQ}), precipitation of driest quarter (P_{dryQ}), precipitation of warmest quarter (P_{warmQ}), and precipitation of coldest quarter (P_{coldQ}). Incoming solar radiation (Wh m⁻²) was calculated using the Solar Radiation tool in ArcGIS version 9.3 (ESRI, Redlands, CA, USA), using topographic data downloaded from the NASA Shuttle Radar Topography Mission (SRTM) Digital Elevation Model (DEM) of the globe (Jarvis *et al.*, 2008). This DEM uses a 3 arc second resolution (ca. 90 m) so provides a site specific climate variable in the analyses. We also calculated one composite climatic variable for use in statistical analyses because it integrates climatic conditions highly relevant to plant growth. W* represents mean annual water availability (Wynn *et al.*, 2006) (Eqn 1).

$$W^* = (\text{MAP} - Q/(rL)) + 4000, \quad (1)$$

where MAP is mean annual precipitation (mm yr⁻¹), Q is mean annual global solar radiation (J m⁻² yr⁻¹), r is the density of liquid water at 25 °C (1000 kg m⁻³), and L is the latent heat of evaporation of water at 25 °C (2.5 × 10⁶ J kg⁻¹ H₂O). Isothermality was calculated as the 'mean diurnal range' [mean of monthly (T_{max} - T_{min})] divided by the 'annual temperature range' (T_{max} of warmest month - T_{min} of the coldest month); temperature seasonality as 100 × standard deviation of the annual temperature variations (Table 2).

Soil samples

In each 20 m × 40 m quadrat, nine replicate soil samples from the epipedon (0.10 m depth) were collected randomly using a

Table 1 The dominant canopy species at each sample location

Dominant tree species	Long	Lat	MAP (mm)	MAT (°C)	Height (m)	Soil N (mg kg ⁻¹)
<i>Nothofagus antarctica</i>	71°44'58"	51°46'14"	311	6.2	4.9	0.29
<i>N. antarctica</i>	71°42'35"	51°49'58"	314	6.4	4.0	0.35
<i>N. antarctica</i>	72°15'50"	51°13'21"	331	5.9	7.5	0.60
<i>N. antarctica</i>	72°10'58"	51°18'53"	340	5.1	5.1	0.54
<i>N. antarctica</i>	72°03'12"	48°22'18"	346	6.8	1.7	0.21
<i>N. antarctica</i>	71°59'29"	51°37'03"	351	5.1	2.1	0.23
<i>N. antarctica</i>	71°52'55"	46°52'58"	366	5.0	4.6	0.15
<i>N. antarctica</i>	72°08'22"	48°22'08"	377	7.1	5.7	0.36
<i>N. antarctica</i>	71°41'11"	46°03'24"	492	5.4	8.8	0.22
<i>N. antarctica</i>	72°46'54"	50°31'28"	577	7.3	12.3	0.87
<i>N. antarctica</i>	72°47'22"	50°18'23"	592	7.5	5.6	0.33
<i>N. antarctica</i>	72°50'31"	50°33'13"	640	7.2	16.1	0.49
<i>N. antarctica</i>	72°53'19"	49°17'04"	806	7.5	9.6	0.87
<i>N. antarctica</i>	72°54'10"	49°14'17"	822	7.3	4.1	0.17
<i>N. antarctica</i>	72°56'54"	49°10'40"	870	7.3	2.1	0.44
<i>Nothofagus pumilio</i>	71°49'42"	48°25'38"	279	5.9	13.4	0.26
<i>N. pumilio</i>	71°50'18"	48°26'49"	280	5.8	16.0	0.22
<i>N. pumilio</i>	72°16'44"	51°13'21"	350	5.4	14.2	0.16
<i>N. pumilio</i>	71°59'34"	51°37'09"	352	5.2	16.0	0.26
<i>N. pumilio</i>	71°52'00"	46°52'00"	356	5.5	10.4	0.22
<i>N. pumilio</i>	72°00'19"	51°34'26"	383	3.8	20.8	0.45
<i>N. pumilio</i>	51°32'01"	72°08'27"	388	4.5	23.2	0.42
<i>N. pumilio</i>	72°11'17"	48°19'45"	396	6.8	14.6	0.59
<i>N. pumilio</i>	72°21'20"	51°33'55"	481	4.1	17.2	0.45
<i>N. pumilio</i>	72°18'35"	51°34'21"	508	3.4	3.5	0.39
<i>N. pumilio</i>	72°45'24"	50°25'52"	555	7.5	18.5	0.82
<i>N. pumilio</i>	72°53'20"	49°04'52"	814	6.9	16.6	0.24
<i>N. pumilio</i>	73°01'25"	50°27'43"	835	6.7	25.9	0.23
<i>N. pumilio</i>	72°56'38"	49°08'47"	868	7.1	27.7	0.24
<i>N. pumilio</i>	72°57'24"	49°12'40"	873	7.2	18.2	0.47
<i>Nothofagus betuloides</i>	73°01'43"	50°27'56"	844	7.4	14.6	0.18
<i>N. betuloides</i> / <i>N. pumilio</i>	73°01'25"	50°27'43"	836	6.7	26.8	0.24
<i>N. betuloides</i>	72°59'37"	50°28'22"	804	6.8	22.8	0.25

Long, longitude; Lat, south latitude; MAP, mean annual precipitation; MAT, mean annual temperature; height, mean height of 10 mature trees at each sampling location; N, soil nitrogen.

hand auger. Soil samples were pooled into three composite samples that contained an equal proportion of soil from three auger holes ($n = 3$ for each site). Roots and plant debris were removed from soil samples with forceps, after the soil was sieved using 2 mm mesh. Soil samples were oven dried at 40 °C to a constant weight and then stored in the laboratory until processed. Finally, the remaining aggregated soil was finely ground to below 2 µm using a tungsten-carbide mill ($n = 3$ for each site). Several variables were determined for this homogenized soil material. The percentage carbon and nitrogen were measured using a LECO auto-analyzer (St. Joseph, MI, USA). Then we analyzed the carbon and nitrogen isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) (details below). Available phosphorus was quantified using the Colwell method, in which soils are tumbled with a 0.5 M sodium bicarbonate solution (adjusted to pH 8.5) for 16 h at 25 °C employing a soil to solution ratio of 1 : 100 (w/v). The acidified extract was then treated with an ammonium molybdate/antimony trichloride reagent and the

concentration of phosphorus was detected colorimetrically at 880 nm. The pH was determined with an electronic meter immersed in a 1 : 5 mixture of the homogenized soil material and deionized water (w/v). Measurements of the electrical conductivity or resistivity have been applied for soil salinity. The percentages of clay, silt, and sand in each sample were determined using a Malvern Mastersizer 2600 laser particle size analyzer (Malvern Instruments Ltd, Worcestershire, UK).

Plant samples

At each site, three ($n = 3$) replicates of nine expanded sun-exposed leaves were collected from 10 different regeneration trees. Foliage samples were dried in a forced-draft oven at 65 °C for 48 h and ground in a mill containing a 1 mm stainless steel screen. Samples were then used to measure the natural abundance of carbon and nitrogen isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) (details below).

Table 2 Summary statistics from the multiple regression on principal components (PC) that had eigenvalues >1 for soil and foliar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively

Predicted parameter	Statistical parameter	Principal component						
		PC1	PC2	PC3	PC4	PC5	PC6	PC7
Soil $\delta^{13}\text{C}$	Variable with highest factor loading (>0.5)	MAT, MAP; all other input data of climatic extremes	Isothermality; temperature seasonality	%C, %N, pH; contents of Ca, Mg, P	Precipitation seasonality; clay content	Loam content	C/N ratio and K content	Resistance
	SS	28.6	0.30	1.24	0.56	0.05	0.34	0.38
	F-value	47.1	0.50	2.03	0.91	0.09	0.57	0.63
	Pr(F)	0.000	0.486	0.166	0.348	0.771	0.458	0.435
	η^2	61.2	0.65	2.64	1.19	0.11	0.73	0.81
Soil $\delta^{15}\text{N}$	SS	98.2	12.1	17.7	11.7	2.60	1.75	17.1
	F-value	17.6	2.18	3.19	2.10	0.47	0.31	3.06
	Pr(F)	0.0003	0.15	0.86	0.15	0.49	0.57	0.09
	η^2	32.7	4.04	5.91	3.90	0.87	0.58	5.68
	SS	83.9	0.90	3.44	1.75	0.17	1.01	1.19
Foliar $\delta^{13}\text{C}$	F-value	46.5	0.50	1.91	0.97	0.09	0.56	0.66
	Pr(F)	0.000	0.485	0.179	0.334	0.762	0.461	0.423
	η^2	61.0	0.65	2.50	1.27	0.12	0.73	0.86
	SS	71.4	9.51	10.7	10.1	0.87	0.74	11.8
	F-value	19.0	2.53	2.85	2.69	0.23	0.20	3.15
Foliar $\delta^{15}\text{N}$	Pr(F)	0.0002	0.124	0.104	0.114	0.634	0.661	0.088
	η^2	34.2	4.50	5.11	4.82	0.41	0.35	5.66

MAP, mean annual precipitation; MAT, mean annual temperature; SS, sum of squares; Pr, probability; and η values indicate the relative importance (in percentage terms) of each independent variable in explaining variation in the response variable $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$.
 Model soil $\delta\text{C} = -27.0171 - 0.2565 \text{PC}_1 + 0.0422 \text{PC}_2 + 0.0930 \text{PC}_3 + 0.0824 \text{PC}_4 - 0.0328 \text{PC}_5 + 0.0897 \text{PC}_6 - 0.1027 \text{PC}_7$. The multiple r^2 is 0.675, the F-statistic is 7.404 on 7 and 25 df and the P-value is 0.0007565.
 Model soil $\delta\text{N} = 1.7945 - 0.4751 \text{PC}_1 + 0.2667 \text{PC}_2 + 0.3525 \text{PC}_3 + 0.3785 \text{PC}_4 - 0.2318 \text{PC}_5 + 0.2026 \text{PC}_6 + 0.6863 \text{PC}_7$. The multiple r^2 is 0.537, the F-statistic is 4.142 on 7 and 25 df and the P-value is 0.003772.
 Model foliar $\delta\text{C} = -30.6515 - 0.4390 \text{PC}_1 + 0.0727 \text{PC}_2 + 0.1550 \text{PC}_3 + 0.1461 \text{PC}_4 - 0.0586 \text{PC}_5 + 0.1534 \text{PC}_6 - 0.1814 \text{PC}_7$. The multiple r^2 is 0.672, the F-statistic is 7.319 on 7 and 25 df and the P-value is 0.0008266.
 Model foliar $\delta\text{N} = 1.4703 - 0.4051 \text{PC}_1 + 0.2359 \text{PC}_2 + 0.2733 \text{PC}_3 + 0.3508 \text{PC}_4 - 0.1331 \text{PC}_5 + 0.1313 \text{PC}_6 + 0.5711 \text{PC}_7$. The multiple r^2 is 0.551, the F-statistic is 4.387 on 7 and 25 df and the P-value is 0.0027.

Analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

Carbon and nitrogen isotopes were measured using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The gases were separated on a Carbosieve G column (Supelco, Bellefonte, PA, USA) before being introduced to the isotope-ratio mass spectrometry (IRMS). Sample isotope ratios were compared to those of pure cylinder gases injected directly into the IRMS before and after the sample peaks, and provisional delta $\delta^{15}\text{N}_{\text{air}}$ and $\delta^{13}\text{C}$ [Vienna Pee Dee Belemnite (V-PDB)] values calculated. Provisional isotope values were adjusted to bring the mean values of working standard samples, distributed at intervals in each analytical run, to the correct values of the working standards.

The ratio of heavy to light isotopes in the sample material (R_{sample}) was measured using mass spectrometry as the deviation from the isotopic ratio of a standard (R_{std}); where R denotes the ratio of stable carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) isotopes, expressed in δ notation, for example for carbon:

$$\delta^{13}\text{C}(\text{‰}) = (R_{\text{sample}}/R_{\text{std}} - 1) \times 1000. \quad (2)$$

High values of this parameter indicate the enrichment of carbon or nitrogen with its heavy isotope and low values mark the depletion of heavy isotopes relative to the standard. The V-PDB ($\delta^{13}\text{C} = 0$) and atmospheric nitrogen ($\delta^{15}\text{N}_{\text{air}} = 0$) serve as international standards for stable carbon and nitrogen, respectively.

Data analysis

We tested for significant differences in soil and foliar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the main species [*N. pumilio* ($n = 15$), *N. betuloides* ($n = 3$), and *N. antarctica* ($n = 15$) stands] with analysis of variance (ANOVA) using three replicate measurements per site.

The interactive effects of the 12 soil properties and 20 climate variables on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in plant and soil were assessed using principal components analysis (PCA). We used PCA because this method is robust to problems associated with multicollinearity (Toledo *et al.*, 2011). Following Quinn & Keough (2002), those principal components (PCs) that had eigenvalues >1 were regressed against the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in plant and soil using multiple linear regressions. The relative importance of each PC in each regression model (η^2) was then determined by partitioning the sum of squares (after Plaistow *et al.*, 2006). For interpreting the PCA results, Quinn & Keough (2002) suggest that independent variables with factor loading scores >0.7 are strongly correlated to the relevant PC, whereas factor loading scores between 0.4 and 0.6 indicate a moderate correlation, and scores <0.4 a weak or absent correlation.

Results

Soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

For the *Nothofagus* forests evaluated, soil $\delta^{13}\text{C}$ ranged from -29.8‰ to -25.3‰ and soil $\delta^{15}\text{N}$ from -4.8‰ to 6.4‰ . There were no significant ($P = 0.99$) differences

between species in soil $\delta^{13}\text{C}$ values with a mean value of -26.9‰ for *N. pumilio*, -26.8‰ for *N. antarctica*, and -28.2‰ for *N. betuloides* stands. There were also no differences ($P = 0.75$) in soil $\delta^{15}\text{N}$ values between species with mean values of 1.8‰ , 2.2‰ and -1.2‰ for *N. pumilio*, *N. antarctica*, and *N. betuloides*, respectively.

We first used simple correlation analyses to understand variations in soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The results showed that mean annual rainfall already explained up to 79% of the variation in soil $\delta^{13}\text{C}$ values below *N. antarctica* and *N. betuloides* trees, and up to 62% if the values from *N. pumilio* sites were also included (Fig. 2). Overall the soil $\delta^{13}\text{C}$ values decreased linearly by 0.43‰ per 100 mm increase in rainfall. The precipitation effect on the abundance of N isotopes in soil was more variable (R^2 was 0.42–0.49) but larger than for ^{13}C as soil $\delta^{15}\text{N}$ values decreased by 0.9‰ when MAP increased by 100 mm (Fig. 2). The predictions did not improve when partializing MAT in the single regression functions, or when the combined impacts of MAT and MAP were assessed using multiple regression (data not shown). Also, a single regression analyses using mean temperature explained only 27% of the variation in soil $\delta^{13}\text{C}$ and 9% of the variation in soil $\delta^{15}\text{N}$. Likewise, the changes in soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ natural abundances were more related to moisture than to temperature at these sites.

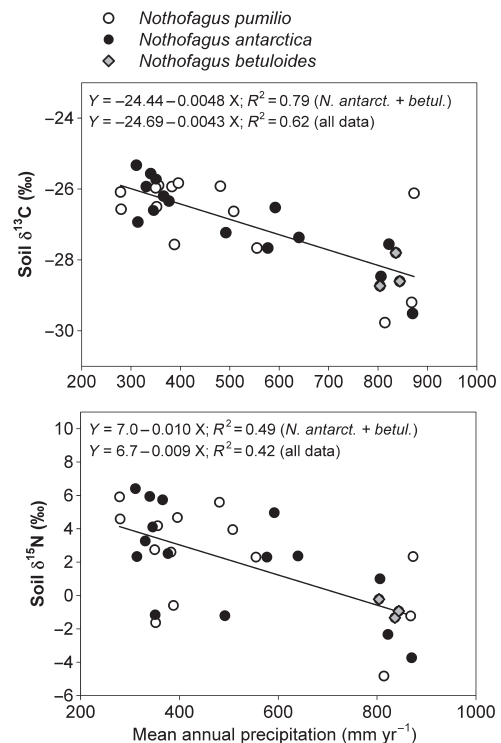


Fig. 2 Changes of soil $\delta^{13}\text{C}$ and soil $\delta^{15}\text{N}$ values along a precipitation gradient in *Nothofagus* forests, Patagonia.

Seven PCs had eigenvalues >1 (see Appendix S2). Together, they explained 67% of variability in the $\delta^{13}\text{C}$ data and 54% of the variability in the $\delta^{15}\text{N}$ values (Table 2). Each PC was characterized by a set of variables with highest factor loadings: climatic parameters largely explained the first PC (MAP, MAT, and others), the second PC was related to temperature variability and lowest minimum temperature, soil chemical properties related to the third and sixth PC. Physical soil properties like texture were main characters of the remaining PCs. Hence, soil properties did not correlate with climatic data along our sampled transects. This facilitated data evaluation and usage of the PC as predictors in multiple regression models.

In general, climatic variables were much better predictors of soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than soil properties (Table 2). Multiple regression using the first seven PCs explained 67% of the variance in soil $\delta^{13}\text{C}$ values across sites (Table 2). The η^2 values indicate that PC1 was the only PC to contribute a significant amount of predictive power to the regression model. The factor loading scores (see Appendix S2) indicate that the environmental factors most closely associated with PC1 were: BIO2 = mean diurnal range [mean of monthly ($T_{\max} - T_{\min}$)], BIO6 = min temperature of coldest month, and BIO12 = annual precipitation and, as outlined above, the latter parameter was able to predict the soil $\delta^{13}\text{C}$ values with reasonable accuracy (Fig. 2).

Multiple regression using the first seven PCs explained 54% of the variance in soil $\delta^{15}\text{N}$ across sites (Table 2). The η^2 values indicate that again PC1 was the most important PC, accounting for 33% of the variation in the data. Yet, the smaller η^2 value for PC1 compared with that for the soil $\delta^{13}\text{C}$ analyses indicated that the other PCs (and environmental variables related to them) contributed to the variability of soil $\delta^{15}\text{N}$, though to a lesser degree than environmental variables associated with PC1 (Table 2).

Foliar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

While foliar $\delta^{13}\text{C}$ ranged from -35.4‰ to -27.7‰ , foliar $\delta^{15}\text{N}$ varied from -3.7‰ to 5.2‰ for the *Nothofagus* stands studied. The foliar $\delta^{13}\text{C}$ was similar across species ($P = 0.89$) with a mean value of -30.5‰ for *N. pumilio*, -30.4‰ for *N. antarctica*, and -32.7‰ for *N. betuloides*. Also, there was no significant difference ($P = 0.66$) between species in foliar $\delta^{15}\text{N}$ with a mean value of -0.9‰ for *N. betuloides*, 1.6‰ for *N. pumilio*, and 1.8‰ for *N. antarctica*.

Multiple regression using the first seven PCs explained 67% of the variance in foliar $\delta^{13}\text{C}$ values and 55% of the variance in foliar $\delta^{15}\text{N}$ across sites (Appendix S2). The η^2 values and the factor loading scores

reveal the same correlation patterns observed for soil $\delta^{13}\text{C}$ (Table 2). Also for foliar $\delta^{15}\text{N}$, the η^2 values indicate that PC1 was the most important PC. However, for nitrogen isotopes in foliage PCs 2, 3, 4, and 7 again also added predictive power to the regression model (5% of variation in the data explained by each of these PCs; see Table 2).

In the *Nothofagus* forests sampled there were strong correlations between foliar $\delta^{13}\text{C}$ and soil $\delta^{13}\text{C}$ (Fig. 3, top) and also between foliar $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ (Fig. 3, lower panel). The relationship between plant and soil isotope composition closely tracked the 1 : 1 relationship for nitrogen isotopes (Fig. 3), with some soils being enriched in ^{15}N while others being depleted in ^{15}N relative to the $\delta^{15}\text{N}$ value of ambient air ($\delta^{15}\text{N} = 0$). However, for carbon isotope composition there was a clear pattern of heavy isotope enrichment (soil relative to plant), which was more pronounced at sites of lower foliar $\delta^{13}\text{C}$ values (Fig. 3, top). This shift in isotopic enrichment from foliar $\delta^{13}\text{C}$ to soil $\delta^{13}\text{C}$ (denoted, $\Delta\delta^{13}\text{C}$) was not a constant parameter but it decreased with increasing foliar $\delta^{13}\text{C}$ values (Figs 3 and 4, top panels). As the latter were influenced by rainfall (see above), the $\Delta\delta^{13}\text{C}$ also became increasingly more negative when MAP increased (Fig. 4, lower panel), that is, the isotopic discrimination during the formation of

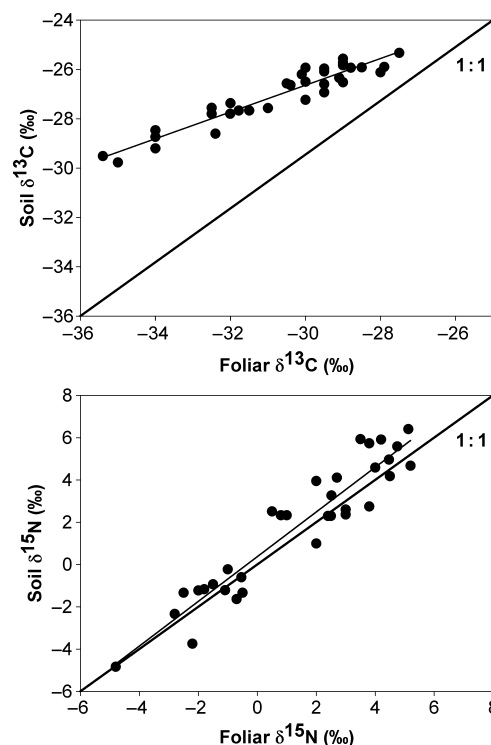


Fig. 3 Correlations between foliar and soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in Patagonian *Nothofagus* forests.

surface SOM was related to climatic variables for C but not for N.

The heavier the isotope natural abundance of C in either soil and plant tissue, however, the more enriched was the sample in the ¹⁵N isotope (Fig. 5). It is, therefore, not only the δ¹³C value that may indicate changes in WUE and ecosystem adversity (Farquhar *et al.*, 1989; Seibt *et al.*, 2008; Ladd *et al.*, 2009), also the δ¹⁵N values must be linked to or at least coincide with these processes via the correlation shown in Fig. 5.

Discussion

Delta ¹³C

For the *Nothofagus* forest evaluated in this study, the key variables explaining the variation in foliar δ¹³C were climatic elements, particularly precipitation. In C₃ plants, foliar δ¹³C is primarily a function of the isotopic composition of source CO₂ and the ratio of intercellular to ambient CO₂ (c_i/c_a) (Farquhar *et al.*, 1989). Changes in c_i/c_a and foliar δ¹³C are associated with changes in A/g, the photosynthetic rate of CO₂ assimilation (A) relative to CO₂ conductance through stomatal aperture and leaf surface boundary layer (g). Any environmental

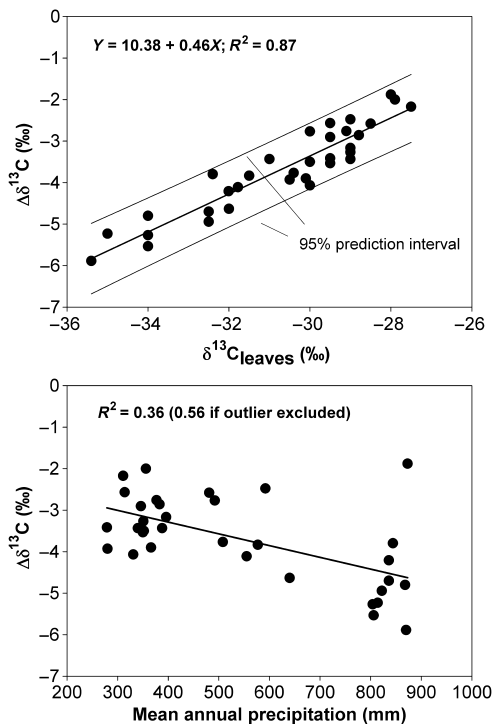


Fig. 4 Degree of isotopic difference between the δ¹³C values in leaves and soil (Δδ¹³C) in relation to (a) the original δ¹³C natural abundance of the leaves (upper figure) and (b) mean annual precipitation (lower figure).

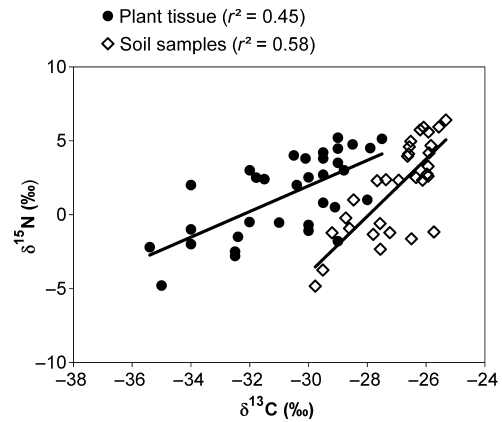


Fig. 5 Relationship between the δ¹⁵N and δ¹³C values in foliar tissue and mineral soils of *Nothofagus* forests, Patagonia.

factor affecting A/g may shift the δ¹³C signal recorded in plant tissues. Variation in foliar δ¹³C values with environment as demonstrated in the present study has been reported previously. For example, Cordell *et al.* (1998) reported a significant increase in foliar δ¹³C values from -29.5‰ at low elevation to -24.8‰ at high elevation due to changes in leaf mass per unit area. Austin & Vitousek (1998) reported δ¹³C values in native-dominated forests of *Metrosideros polymorpha* in Hawaii decreased with increasing annual precipitation from 500 to 5500 mm suggesting differential WUE across the precipitation gradient. Hence, the general picture remains valid also for the *Nothofagus* forests that at low stomatal limitation (i.e., high concentrations of intracellular CO₂ and strong photosynthetic discrimination against CO₂) the foliar δ¹³C values decrease with decreasing WUE.

Plant communities contribute to soil carbon through the deposition of leaf litter, dead root material, and rhizodeposition. Therefore, in the most general terms, the isotopic signature of soil organic carbon (SOC) should reflect the isotopic composition of the source vegetation (litter inputs) (Balesdent *et al.*, 1993; Ehleringer *et al.*, 2000). Our results largely confirm this prediction for Southern Patagonia, that is, similar to foliar δ¹³C also soil δ¹³C values declined with increasing MAP (Fig. 2, top). However, our results also provide some indication that soil processes modify carbon isotope signals in the soil (Bol *et al.*, 1999). From the upper graphs in Figs 3 and 4, it is clear that SOC is enriched in ¹³C relative to plant foliage. We even found systematic differences in the degree of isotopic enrichment (Fig. 4), with productive ecosystems (those with more negative δ¹³C; see Luo *et al.*, 2009) exhibiting a higher relative degree of isotopic enrichment in SOC than in the less productive ecosystems, which also is consistent with Garten *et al.* (2000). Typically any biochemical process, such as

decomposition of SOC, favors the use of the lighter isotope (i.e., ^{12}C), leaving the heavier ^{13}C isotope behind (Hobbie, 2005). Ehleringer *et al.* (2000) reported that $\delta^{13}\text{C}$ differences between vegetation and soil ($\Delta\delta^{13}\text{C}$) are thus expected and suggested that the progressive $\delta^{13}\text{C}$ enrichment of SOM may be related to a gradual shift in the relative contributions of microbial vs. plant components in the residual SOM as well as to the Suess effect (a shift to lighter C in the atmosphere due to burning fossil fuels). Higher microbial portions in SOM mean that the isotopic shift to higher $\delta^{13}\text{C}$ values in soil relative to the plant is more pronounced (more negative $\Delta\delta^{13}\text{C}$), which according to our data increases with increasing MAP, moisture supply (Fig. 4) and thus ecosystem productivity. Here, we analyzed mineral soil only, that is, the SOM is free of recent plant debris. Mineral soil is usually enriched with microbial residues, such as carbohydrate C (Zhang *et al.*, 1998; Amelung *et al.*, 1999), which exhibit higher $\delta^{13}\text{C}$ values than bulk SOM (Derrien *et al.*, 2006). In US grasslands, for example, the contribution of these carbohydrates to mineral-bound SOM was strongly related to MAP (Amelung *et al.*, 1999). Our findings are therefore consistent with reports on the impact of climate on SOM composition and they give support to the assumption that more negative $\Delta\delta^{13}\text{C}$ values at sites of high MAP indicates an increased sequestration of microbially derived SOM in the mineral matrix. We do not see that the Suess effect could have significantly contributed to the results outlined in Fig. 4. An increased incorporation of 'lighter' plant inputs into SOM at the more productive sites should have resulted in less negative $\Delta\delta^{13}\text{C}$ values and not in stronger isotope enrichment relative to current foliar $\delta^{13}\text{C}$ values.

Delta ^{15}N

The causes of variation in N isotope natural abundance across ecosystems are still being investigated (Schulze *et al.*, 1998; Craine *et al.*, 2009; Ladd *et al.*, 2010). Our results (from PCA) are consistent with previous studies in that they demonstrate that many different variables may impact $\delta^{15}\text{N}$ values. Nevertheless, our results support the hypothesis that water availability is an important variable that contributes to variation in N isotope composition across natural temperate forest ecosystems in Patagonia. Houlton *et al.* (2007) similarly showed the importance of water in that study bulk soil $\delta^{15}\text{N}$ in tropical forest communities decreased as precipitation increased from 2200 to 5050 mm per annum. Similarly, Aranibar *et al.* (2004) reported a strong negative relationship between soil $\delta^{15}\text{N}$ and precipitation (from 230 to 978 mm per annum) in the Kalahari region of southern Africa. The regional patterns obtained from our

study are also consistent with the global pattern reported by Handley *et al.* (1999) who showed that the $\delta^{15}\text{N}$ of whole surface mineral soil was best regressed using latitude (a correlate of temperature) and rainfall as independent variables, accounting for 49% of the variation in whole soil $\delta^{15}\text{N}$. Amundson *et al.* (2003) found relatively low explanatory power of climatic variables (MAP and MAT) on soil $\delta^{15}\text{N}$ values at 0.10 m depth ($r^2 = 0.11$), whereas Cheng *et al.* (2009) again stressed climatic controls on ecosystem ^{15}N abundance.

The effects of annual precipitation on soil $\delta^{15}\text{N}$ in this study may be attributable to effects of moisture on N transformations in the soil, for example: (i) adequate moisture may directly stimulate the growth of decomposer communities, (ii) moisture may increase the availability of dissolved soil N (nitrate), and (iii) soil moisture may cause the plant community to shift from predominantly organic to predominantly inorganic N nutrition (Aranibar *et al.*, 2004; Houlton *et al.*, 2007; Kahmen *et al.*, 2008). Kahmen *et al.* (2008) further reported that foliar $\delta^{15}\text{N}$ values were closely related with soil net nitrogen mineralization rates and the uptake ratio of NO_3^- to NH_4^+ in a diverse range of grasses and forbs. The mineral N release that may occur when water is freely available and produces ^{15}N depleted NO_3^- , which then contributes to lower $\delta^{15}\text{N}$ values in the plant tissue. The soil $\delta^{15}\text{N}$ values are correlated with the plant tissue $\delta^{15}\text{N}$ values (Fig. 3, bottom), that is, the soil $\delta^{15}\text{N}$ values follow this trend (Fig. 2, bottom). The data therefore support our hypothesis I: plant $\delta^{15}\text{N}$ values decline with increasing moisture supply. This could have increased plant usage of lighter N sources. Although, all aforementioned processes may well have contributed to lower foliar $\delta^{15}\text{N}$ values at moister sites, they cannot be the major reason for the trends observed because whenever there is a plant uptake of 'light' N forms, the plant should leave the 'heavier' ^{15}N behind. This was clearly not the case, at least, not in the sampled surface soil. Intriguingly, the soil $\delta^{15}\text{N}$ natural abundance did not show any evidence of a systematic enrichment of the heavier isotope relative to plants, as observed for $\delta^{13}\text{C}$, for example (Fig. 3). Hence, we clearly have to reject our first part of hypothesis II that soil $\delta^{15}\text{N}$ values should be higher than those of the plant tissues. For a better mechanistic understanding of the involved processes we may, however, take advantage of the soil isotope analyses.

N isotope fractionation processes in soil can reach a magnitude of up to 35‰ for ammonia volatilization, nitrification, and denitrification (Handley & Raven, 1992; Högberg, 1997). According to the Rayleigh model (Robinson, 2001), all N products emitted to the

environment (NH_3 , N_2O , NO , N_2 , NO_3^-) are depleted in ^{15}N (Högberg, 1997). According to Houlton *et al.* (2006), gaseous N loss by microbial denitrification was the major determinant of N isotopic discrimination across a climate gradient in tropical rain forest. As higher MAP may favor N losses, particularly by denitrification, the moister sites could theoretically have higher soil $\delta^{15}\text{N}$ values. However, in this study they exhibited lower values (Fig. 2), even lacking an isotopic N fractionation during SOM formation (Fig. 3, bottom). Hence, we must also reject the second part of our hypothesis II that increased moisture supply promotes the isotopic enrichment of ^{15}N in SOM. We thus also do not have any indication that N use efficiency might have been worse under conditions of higher MAP than at drier sites in these *Nothofagus* forests. Instead, the close relation to foliar $\delta^{15}\text{N}$ rather suggests that the N was efficiently recycled through SOM so that soil $\delta^{15}\text{N}$ values paralleled those of foliar $\delta^{15}\text{N}$.

An efficient cycling of N through SOM implies that the soil C and N cycles are closely linked. In line with our hypothesis III, soil and foliar $\delta^{13}\text{C}$ values were strongly correlated with their $\delta^{15}\text{N}$ signature (Fig. 5), giving support to the prediction that soil moisture and WUE could be a main driver of both isotopic signals. We propose two mechanisms contributing to their maintenance. First, at high WUE reduced transpiration may limit plant N acquisition, likely due to mass flow constraints of mobile N forms in a drying mineral surface soil (Conroy & Hocking, 1993; McDonald *et al.*, 2002). In contrast, lower restriction in stomatal conductance at sites with higher MAP increases transpiration and thus re-uptake of soil N (mostly NO_3^-) which already has a lower $\delta^{15}\text{N}$ value. At drier sites, however, woody plants may also be more forced to re-utilize water from lower soil depths (Caldwell & Richards, 1989; Caldwell *et al.*, 1998; Jackson *et al.*, 2000; Nie *et al.*, 2011). Usually, $\delta^{15}\text{N}$ values of soils increase with soil depth, because of various stable isotope fractionation processes that accompany N uptake and loss (Delwiche *et al.*, 1979; Yoneyama, 1996; Gioacchini *et al.*, 2006). An increased utilization of subsoil N will thus also result in an increased uptake of heavier N into plant foliar biomass, resulting in higher $\delta^{15}\text{N}$ ratios in the leaves and, when returned to soil, also in the surface soil. It could be some kind of a feedback loop through the subsoil: at higher WUE (and thus higher foliar $\delta^{13}\text{C}$), plants could utilize higher portions of subsoil N (rich in ^{15}N) and, with litter fall, sustain the higher $\delta^{15}\text{N}$ values also in the surface soil. To support or reject this final hypothesis, an analysis of water and nitrogen isotope composition from soil solutions of different depths and related sap flow under different climatic regions might thus warrant future attention.

Conclusion

The results of this study supported our hypotheses that foliar and soil $\delta^{13}\text{C}$ would be less negative (^{13}C enriched) where water availability is limited and that foliar $\delta^{15}\text{N}$ would be less positive (^{15}N depleted) at sites with higher MAP. The observed differences in isotopic N and C contents of leaves and soils across the sites studied was related mainly to water availability variables (precipitation, climatic water balance) and marginally to temperature (MAT and temperature annual range). Both foliar and soil $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signals were closely linked. However, while increased moisture supply also resulted in increased microbial enrichment of soil $\delta^{13}\text{C}$ when MAP increased, soil $\delta^{15}\text{N}$ values closely matched those of the foliar tissue.

We hypothesize that these ecosystems are not losing N in significant amounts, but that foliar N is being efficiently recycled during SOM formation and re-acquisition of plant N, and that these processes are facilitated rather than hindered under moist compared to dryer conditions.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Raw data used in the analyses and data derived from geographic information systems.

Appendix S2. Complete statistical output from Principal Components Analysis.

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