Soil $\delta^{15}N$ patterns in old-growth forests of southern Chile as integrator for N-cycling

PASCAL BOECKX*†, LEANDRO PAULINO‡, CARLOS OYARZÚN§, OSWALD VAN CLEEMPUT† and ROBERTO GODOY‡

†Laboratory of Applied Physical Chemistry – ISOFYS, Ghent University, Coupure 653, 9000 Gent, Belgium
‡Instituto de Botánica, Universidad Austral de Chile, Casilla 657, Valdivia, Chile
§Instituto de Geociencias, Universidad Austral de Chile, Casilla 657, Valdivia, Chile

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Old-growth forests of southern Chile represent an important reserve of temperate (rain) forests in the world. Wetter and colder forest ecosystems appear to be more efficient in conserving and recycling N such that mostly non-plant available N species are lost, which could be indicated by more depleted $\delta^{15}N$ values of the soil and plants. Hydrological N loss from the old-growth forests in southern Chile occurs mainly via dissolved organic nitrogen and not via dissolved inorganic N. Forest disturbances (e.g. fire, clear-cutting or enhanced N deposition) cause (abrupt) changes in ecosystem N-cycling processes. In this study, we hypothesized that $\delta^{15}N$ signatures of soil profiles under old-growth forests could be used as an integrator for ecosystem N-cycling, and changes of these $\delta^{15}N$ profiles could be valuable to assess ecosystem resilience towards disturbances. Six old-growth forests were selected in the phytogeographical region of the Valdivian rain forest in southern Chile. One of the sites has been partly burned in February 2002. First, we observed that ecosystems with higher mean annual precipitation and lower mean annual temperature were relatively more depleted in $^{15}N$. Secondly, we found that a forest fire caused a 100-fold increase of the nitrate export and induced an enrichment of the soil $\delta^{15}N$ signal in the upper 20 cm.

Keywords: Chile; Ecosystems; Forests; Natural abundance; Nitrogen-15; Nitrogen cycle

1. Introduction

Old-growth forests of southern Chile represent an important reserve of pristine temperate forests in the world. The precipitation chemistry in southern Chile still reflects a close approximation of preindustrial conditions [1]. It has been shown that hydrological N loss from these unpolluted forests occurs mainly via dissolved organic nitrogen (DON), whereas dissolved inorganic nitrogen (DIN, $\text{NO}_3^- + \text{NH}_4^+$) is the main N loss process of more polluted forests [2]. Perakis and Hedin [2] estimated that the hydrological N loss from unpolluted forests in southern Chile and Argentina ranged between 0.2 and 3.5 kg N ha$^{-1}$ year$^{-1}$, of which less
than 5% is NO$_3^-$-N. The DON/DIN ratio of the hydrological N loss from these old-growth forests seemed to be controlled by the rainfall intensity. The higher the rainfall, the higher is the DON/DIN ratio [2]. MacDonald et al. [3] showed that NO$_3^-$ leached from European N-polluted forests cover a range between 1 and 40 kg N ha$^{-1}$ year$^{-1}$, which is largely controlled by N input and ecosystem N enrichment (C/N ratio).

Moreover, it has been shown that wetter and colder ecosystems appear to be more efficient in conserving and recycling N, such that mostly non-plant available N species are lost, which could be indicated by more depleted $\delta^{15}$N values [4]. The authors showed that $\delta^{15}$N signatures decreased with increasing mean annual precipitation (MAP) and decreasing mean annual temperature (MAT) of the ecosystem. Thus, ecosystems, which have a more open N cycle, tend to be isotopically enriched because of N losses via fractionating pathways [5].

Large-scale disturbances of forest ecosystems change N cycle processes. A clear-cutting experiment on a temperate forest ecosystem in North America demonstrated a significant enrichment in soil and plant $^{15}$N associated with enhanced nitrification and nitrate losses over the catchment. Nevertheless, in the long-term, nitrate losses and $\delta^{15}$N values returned to initial conditions [6]. Our previous work on $\delta^{15}$N ($\%e$) and total N (TN, $\%$N) profiles in forest soils has shown that a shift of the $\delta^{15}$N versus TN relation towards more enriched $\delta^{15}$N values could be used as an indicator to distinguish between forests with and without NO$_3^-$ losses [7]. These forest soils received an N deposition up to 44 kg N ha$^{-1}$ year$^{-1}$ and leached considerable amounts of NO$_3^-$ (up to 18.5 kg N ha$^{-1}$ year$^{-1}$) [8, 9].

General interpretations of $\delta^{15}$N signatures for (forest) ecosystem functioning and N-cycling are not always unambiguous. The behavior of $^{15}$N in soils and plants might be too complex to permit the use of variations of its natural abundance as indicator to explore N-cycling in natural ecosystems [10, 11]. Nevertheless, N isotopes may reflect a time-integrated measure of the controls on the ecosystem N cycle [12]. Högberg [13], Högberg and Johannisson [14], and Vervaet et al. [7] showed that $\delta^{15}$N data in forests could be correlated to N losses. Recently, Gebauer and Meyer [15] successfully used $\delta^{15}$N and $\delta^{13}$C data to calculate N and C gains in orchids.

In this article, we propose a conceptual model for DON and DIN losses from forest ecosystems (figure 1). Nitrate and to a lesser extent NH$_4^+$ that is lost from forest soils, is depleted in $^{15}$N [10, 16, 17]. Thus, when NO$_3^-$ is being lost from the soil profile (either via leaching or denitrification), the whole soil N pool becomes progressively more enriched in $^{15}$N. As a

![Figure 1. Conceptual ecosystem integrator for the identification of the type of hydrological N loss and ecosystem resilience in old-growth forests ecosystems based on the shift of the exponential relation between $\delta^{15}$N of the soil total N pool (TN).](image-url)
result, a gradual upward shift of the $\delta^{15}$N versus TN relation (arrow upwards in figure 1) is expected. Analysis of $\delta^{15}$N in DON is very uncommon. However, some researchers indicated that DON is enriched in $^{15}$N [18–20]. It is suggested that $\delta^{15}$N of DON in groundwater appears to emulate the soil $\delta^{15}$N trend. The $\delta^{15}$N enrichment of DON is also in agreement with the suggestion of Perakis and Hedin [21] that DON loss does not originate directly from active microbial turnover, but most probably from older, relatively enriched soil organic N. Thus, accordingly, loss of $^{15}$N-enriched DON is expected to shift the $\delta^{15}$N–TN relation (downwards arrow in figure 1) downward, indicating a $^{15}$N depletion of the whole soil N pool.

In this study, we tested the hypothesis whether variations in the $\delta^{15}$N–TN relationship in soil profiles under old-growth forests of southern Chile could be used as an ecosystem indicator (1) to differentiate between watersheds draining high or low DON/DIN ratios and (2) to assess ecosystem resilience towards disturbances (i.e. fire).

2. Materials and methods

$\delta^{15}$N and TN profiles have been established for six old-growth forests of southern Chile. All forests are situated in the phytogeographical region of the Valdivian rain forest (37 to 43°S) [20]. From west to east, this region can be divided into the Coastal Mountains near the Pacific Ocean, the Central Depression, and the Andean Mountains. The area is characterized by a strong increasing MAP gradient from west to east [22]. The following forest ecosystems have been selected (figure 2): *Nothofagus nitida* (Phil) Krasser, (NN); *Nothofagus betuloides* (Mirb.) Oerst (NBC) and *Fitzroya cupressoides* (Mol.) Johnst. (FC) in the Natural Monument *Alerce Costero* in the Coastal Mountains; *Nothofagus obliqua* (Mirb.) Oerst. (NO) in *Paillaco* in the Central Depression; *Nothofagus betuloides* (Mirb) Oerst. (NBA) in Puyehue National Park in the Andean Mountains; and an *Araucaria araucana* (Mol.) K. Koch – *Nothofagus* forest ecosystem (AN) in Tolhuaca National Park in the Andean Mountains. Part of the latter ecosystem has been affected by a wildfire in February 2002 and burned and unburned plots have been selected. All *Nothofagus* spp., except the deciduous *N. obliqua*, are evergreen. *F. cupressoides* and *A. araucana* are evergreen conifers.

In January 2001, the NN, NBC, NO, and NBA sites were sampled. In August 2001, the FC site was sampled. In June 2003, the AN sites have been sampled. The sampling sites in the Coastal Mountains (NN, NBC, and FC) are located in the Natural Monument *Alerce Costero* (40°12′ S, 73°26′ W) (figure 2). The area is situated on an undulating plateau at 800–1000 m a.s.l. The soils consist of metamorphic schists and are classified as Luvisols. The Coastal Mountains have a maritime, humid temperate climate [20]. The soil profiles at these sites are shallow (40 cm for *N. nitida* and 30 cm for *N. betuloides* and *F. cupressoides*). In the Central Depression, the NO site near *Paillaco* (40°07′ S, 72°51′ W, 160 m a.s.l.) was selected (figure 2). The soils are deep, originating from volcanic ash and classified as Andisols. The climate is classified as temperate. The NBA site (40°47′ S, 72°12′ W, 920 m a.s.l.) is located in the valley of *Antillanca* in Puyehue National Park in the Andean Mountains (figure 2). The climate is classified as rainy temperate [1]. The soils are deep, originate from volcanic ash and are classified as Andisols [23]. A burned and unburned section of AN were sampled in Tolhuaca National Park (38°10′ to 38°15′ S, 71°40′ to 71°50′, 1100–1300 m a.s.l.) in the Andean Mountains. The climate is temperate-cold and the soils are deep Andisols derived from volcanic ash.

The general characteristics of the experimental sites of the selected forest ecosystems are given in table 1. An extensive description of climate, physical environment, habitats and forest types of these ecosystems has been given in refs. [24–26].
Figure 2. Location of the different experimental forest ecosystems in southern Chile: *N. nitida* (NN), *N. betuloides* (NBC), *F. cupressoides* (FC) in the Natural Monument Alerce Costero in the Coastal Mountains, *N. obliqua* (NO) near Paillaco in the Central Depression, *N. betuloides* (NBA) in Puyehue National Park in the Andean Mountains, and a burned and unburned section of an *A. araucana*–*Nothofagus* forest (AN) in Tolhuaca National Park in the Andean Mountains.

From a soil profile, samples of the mineral soil were collected in layers of 2 cm until a depth of 30 cm and every 5 cm between 30 and ~50 cm or until the bedrock was reached. For all ecosystems, one soil profile has been sampled, except for the burned and unburned AN ecosystem, which have been sampled at three different plots. The soils were air-dried and sieved (2 mm) to remove litter and root residues. At the same time dead leaves (from the soil surface) and fine roots (~10 cm depth) of the dominant plant species were sampled and air-dried. All samples were ground and homogenized in a planetary ball mill (PM 400, Retsch, Germany) and transferred into tin capsules for analysis of TN and δ15N. Nitrogen-15 and TN were measured using an elemental analyzer (ANCA-SL, PDZ-EUROPA, UK) connected to an Isotope Ratio Mass Spectrometer (20-20, PDZ-EUROPA, UK). Analyses were triplicated. In Vervaet et al. [7] and for the AN ecosystem, in this study, we showed that there was no large difference between δ15N profiles taken at one location (three repetitions per location). Therefore, only one soil profile was sampled per forest type in NN, NBC, FC, NO, and NBA.

The natural abundance of 15N (δ15N) is expressed in per mil (‰) and is a relative measurement against the 15N/14N ratio in an international standard (N2 in air) equation (1).

\[
\delta^{15}N = \frac{(^{15}N/^{14}N)_{\text{sample}} - (^{15}N/^{14}N)_{\text{standard}}}{(^{15}N/^{14}N)_{\text{standard}}} \times 1000
\]  

The working standard used was flour with a δ15N value of 2.69 ± 0.15 ‰ versus N2 in air and TN content of 1.75 ± 0.04 % (both values were certified by Iso-Analytical, UK).
Table 1. General characterization of the different experimental forest ecosystems in southern Chile: *N. nitida* (NN), *N. betuloides* (NBC), *F. cupressoides* (FC) in the Coastal Mountains, *N. obliqua* (NO) in the Central Depression, *N. betuloides* (NBA) in the Andean Mountains, and a burned and unburned section of an *A. araucana–N. obliqua* forest (AN) in the Andean Mountains.

<table>
<thead>
<tr>
<th></th>
<th>NN</th>
<th>NBC</th>
<th>FC</th>
<th>NO</th>
<th>NBA</th>
<th>AN burned</th>
<th>AN burned</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm)</td>
<td>4097</td>
<td>4097</td>
<td>4097</td>
<td>1657</td>
<td>7111</td>
<td>3173</td>
<td>3173</td>
</tr>
<tr>
<td>MAT (°C)</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>12</td>
<td>4.5</td>
<td>8.6</td>
<td>8.6</td>
</tr>
<tr>
<td>Bulk N precipitation (kg N ha(^{-1}) year(^{-1}))</td>
<td>4.6</td>
<td>11.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIN(^a)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.97</td>
<td>2.5</td>
<td>3.6</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>DON(^a)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.74</td>
<td>2.1</td>
<td>8.2</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Through fall N (kg N ha(^{-1}) year(^{-1}))</td>
<td>2.64</td>
<td>2.64</td>
<td>2.64</td>
<td>4.76</td>
<td>3.07</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>DIN(^a)</td>
<td>1.07</td>
<td>1.07</td>
<td>1.07</td>
<td>4.92</td>
<td>5.51</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>DON(^a)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.58</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Soil N percolation (kg N ha(^{-1}) year(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIN(^a)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.56</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Run off N (kg N ha(^{-1}) year(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIN(^a)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>3.06</td>
<td>n.d.</td>
<td>0.89</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>DON(^a)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>1.04</td>
<td>n.d.</td>
<td>5.24</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>pH(\text{H}_2\text{O})(^b)</td>
<td>4.0 (20)</td>
<td>4.1 (20)</td>
<td>3.6 (20)</td>
<td>6.0 (24)</td>
<td>5.0 (10)</td>
<td>5.3 (20)</td>
<td>5.0 (20)</td>
</tr>
<tr>
<td>Total C (%)(^b)</td>
<td>14.8 (20)</td>
<td>8.7</td>
<td>11.6</td>
<td>11.2 (24)</td>
<td>12.3 (10)</td>
<td>11.7 (20)</td>
<td>12.0 (20)</td>
</tr>
<tr>
<td>Total N (%)(^b)</td>
<td>0.92 (20)</td>
<td>0.39</td>
<td>0.46</td>
<td>0.82 (24)</td>
<td>0.56 (10)</td>
<td>0.47 (20)</td>
<td>0.56 (20)</td>
</tr>
<tr>
<td>C/N</td>
<td>16.1</td>
<td>22.3</td>
<td>25.0</td>
<td>13.6</td>
<td>22.0</td>
<td>24.9</td>
<td>21.3</td>
</tr>
<tr>
<td>Soil type</td>
<td>Luvisol</td>
<td>Luvisol</td>
<td>Luvisol</td>
<td>Andisol</td>
<td>Andisol</td>
<td>Andisol</td>
<td>Andisol</td>
</tr>
</tbody>
</table>

Note: MAP, Mean Annual Precipitation; MAT, Mean Annual Temperature; n.d., not determined.
\(^a\)DON and DIN data are adapted from refs. [21, 34]. DIN data of FC include only NO\(^−_3\).
\(^b\)The soil depth in cm for which pH, total C, total N, and C/N were measured is given between brackets. All pH, C, and N data are adapted from refs. [23], except for NBC, NN, FC (measured in this study), and AN (CONAF, personal communication).

3. Results and discussion

In general, the \(\delta^{15}N\) profiles under the *Nothofagus* spp. (NN, NBC, NO, and NBA) increased from \(\delta^{15}N\) values of approximately \(-4\%e\), at the surface (0–2 cm), until approximately 5–10\%e deeper in the soil (figure 3). With the exception of the NBC site, all \(\delta^{15}N\) values of the surface layers were negative. The FC site showed a \(\delta^{15}N\) value of \(-2.53\%e\) in the upper 2 cm and it increased gradually until 2.20\%e at 30 cm depth (figure 3). The \(\delta^{15}N\) values in the surface layers are typical data for temperate ecosystems [15]. The unburned and burned AN site showed a \(\delta^{15}N\) value of \(-1.71\) and \(3.92\%e\), respectively, in the upper 2 cm and it increased gradually until 3.93 and 6.03\%e, respectively, at 50 cm depth (figure 4). The shift of the \(\delta^{15}N\) profile in the burned AN site corresponds to the conceptual model as suggested by Högberg [10], because of the forest fire the \(\delta^{15}N\)-depleted surface layer has been partially consumed and an increase in nitrification after the fire may provide \(15^N\)-enriched NH\(^+\)\(_4\) and cause a loss of \(15^N\)-depleted NO\(^−_3\) (see also further).

In some soil profiles the \(\delta^{15}N\) values showed some scattering in the upper 15 cm. The scattering can be attributed to isotopic mixing of freshly added litter (depleted in \(15^N\)) and SOM (more enriched in \(15^N\)) [9]. From table 2, it can be seen that for all forests the \(\delta^{15}N\) value of leaves and fine roots were depleted in \(15^N\) relative to the soil data. Thus, in general, litter fall provides the soil with a continuous input of \(15^N\)-depleted organic N. The observed increase of the \(\delta^{15}N\) signatures in the soil profiles is the result of isotopic fractionation during decomposition and removal of depleted inorganic N via plants, microbes, or leaching. As
Figure 3. Soil $\delta^{15}N$ (‰) profiles for five forest ecosystems of southern Chile: *N. nitida* (NN), *N. betuloides* (NBC), *F. cupressoides* (FC) in the Coastal Mountains, *N. obliqua* (NO) in the Central Depression, and *N. betuloides* (NBA) in the Andean Mountains.

Recalcitrant N accumulates with increasing soil depth, this process contributes to the increase in $\delta^{15}N$ down the profile.

The TN content of all sites decreased with depth (data not shown). The *Nothofagus* ssp. forests (NN, NBC, NO, NBA) showed a TN content in the surface layer of 2.29–0.42% and decreased gradually from 0.23% to 0.07%. The deciduous *Nothofagus* site (NO) had a higher TN, owing to cyclic litter fall with rapid decomposition (Godoy et al., unpublished data, [27]). The TN content of the FC stand ranged from 0.90% to 0.20%. The unburned and burned AN site had a TN content of 0.74% and 0.60%, respectively, in the upper 2 cm and it decreased gradually to 0.28% and 0.31%, respectively, at 50 cm depth (data not shown).

The increase of $\delta^{15}N$ and decrease of TN with depth has also been observed elsewhere [28–33]. However, in none of these studies the $\delta^{15}N$ profiles was measured in such a detail as in this study. In figure 5, the exponential correlation between $\delta^{15}N$ (‰) and TN (% dry weight) of all the soil profiles, except the AN site, is shown.

Figure 4. Soil $\delta^{15}N$ (‰) profiles for an unburned (filled bullets) and burned (February 2002, empty bullets) *A. araucaria–Nothofagus* forest ecosystem in Tolhuaca National Park in the Andean Mountains.
Table 2. $\delta^{15}N$ values (‰) of leaves and fine roots of the dominant plant species in the experimental forest ecosystems in southern Chile: *N. nitida* (NN), *N. betuloides* (NBC), *F. cupressoides* (FC) in the Coastal Mountains, *N. obliqua* (NO) in the Central Depression, *N. betuloides* (NBA) in the Andean Mountains, and *A. araucana–Nothofagus* (AN) in the Andean Mountains.

<table>
<thead>
<tr>
<th>Location</th>
<th>Plant species/life form</th>
<th>Leaves</th>
<th>Fine roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td><em>N. nitida/tree</em></td>
<td>−4.9</td>
<td>−3.7</td>
</tr>
<tr>
<td></td>
<td><em>Podocarpus nubigena/tree</em></td>
<td>−5.4</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td><em>Chusquea spp./shrub</em></td>
<td>−1.7</td>
<td>n.d.</td>
</tr>
<tr>
<td>NBC</td>
<td><em>N. betuloides/tree</em></td>
<td>−8.2</td>
<td>−7.5</td>
</tr>
<tr>
<td></td>
<td><em>Drimys winteri/tree</em></td>
<td>−7.9</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td><em>Chusquea spp./shrub</em></td>
<td>−6.2</td>
<td>n.d.</td>
</tr>
<tr>
<td>FC</td>
<td><em>Fitzroya cupressoides/tree</em></td>
<td>−5.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>NBA</td>
<td><em>N. betuloides/tree</em></td>
<td>−5.4</td>
<td>−10.7</td>
</tr>
<tr>
<td></td>
<td><em>Chusquea spp./shrub</em></td>
<td>−7.9</td>
<td>−7.3</td>
</tr>
<tr>
<td></td>
<td><em>Saxegothaea conspicua/tree</em></td>
<td>−5.2</td>
<td>n.d.</td>
</tr>
<tr>
<td>AN (unburned)</td>
<td><em>A. araucana/tree</em></td>
<td>−1.6</td>
<td>n.d.</td>
</tr>
<tr>
<td>AN (burned)</td>
<td><em>N. alpina/tree</em></td>
<td>3.1</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

The $\delta^{15}N$ value in a specific compartment of an ecosystem (e.g. a soil layer) can only vary if $^{15}N$ is leaving or entering that compartment. The forest floor is provided with $^{15}N$ depleted organic N via litter fall. In forests, N leaves the soil layer via plant uptake, microbial transformations or via DIN and DON leaching. Because of the low pH, denitrification (unpublished) and NH$_3$ losses are usually negligible in forests. Drained N losses are indicative for an open N cycle. The MAP and MAT of the ecosystem could trigger the openness of the N cycle [4]. These authors indicated that with increasing MAP and decreasing MAT ecosystem N efficiency is increasing resulting in relatively more depleted $\delta^{15}N$ values. This observation could be confirmed for the old-growth forest ecosystems of our study. The MAP and MAT in *Puyehue, Alerce Costero*, and *Paillaco* are 7111, 4097, and 1657 mm year$^{-1}$, respectively, and 4.5, 8, and 12 $^\circ$C, respectively (table 1). The NBA ecosystem showed the lowest $\delta^{15}N$ enrichment (figures 3 and 5), the highest MAP and lowest MAT. The NN, NBC, and FC ecosystems showed intermediate soil $\delta^{15}N$, MAP, and MAT data. The NO ecosystem showed the most enriched soil $\delta^{15}N$ data, the lowest MAP, and highest MAT. This trend was more or less followed by the leaf and root $\delta^{15}N$ data (table 2). Amundson et al. [4] also suggest that with increasing MAP and decreasing MAT mostly non-plant available N species (DON) are subject to (leaching) losses. In addition, Handley et al. [18] reported that rainfall could at least partly influence the whole soil $\delta^{15}N$ signature.

Here, we hypothesize that the type of N losses as induced by MAP (and MAT) would in turn result in a shift of the $\delta^{15}N$ signatures of the soil profile (figure 1). It should be noted, however, that soil $\delta^{15}N$ signatures are also determined by soil age and parent material, topographical position and (anthropogenic) disturbances, which might bias the comparison of soil $\delta^{15}N$ of different ecosystems. However, here, we want to focus on changes of ecosystem $\delta^{15}N$ patterns, which could be used as an integrator of long-term N-cycling or ecosystem resilience towards disturbances [12]. DIN and DON losses were not measured for all old-growth forest described in this study, but can also be predicted using the approach as suggested in Perakis and Hedin [2]. These authors suggest a correlation between the hydrologic loss of DON and DIN across a range of precipitation inputs to unpolluted forests in southern Chile and Argentina. Using this approach, it was predicted that for *Puyehue, Alerce Costero* and *Paillaco*, the DON and
Figure 5. Soil $\delta^{15}$N (‰) versus TN (%N) relation for five forest ecosystems of southern Chile. The trendline equations are from top to bottom for NO, FC, NN together with NBC, and NBA.

DIN loss (kg N ha$^{-1}$ year$^{-1}$) was approximately 4.1 and 0.6, 1.8 and 0.3, and 0.5 and 0.1, respectively. As a result, the DON and DIN losses from the old-growth forest in this study is expected to increase in the following order: NBA $>$ NN, NBC, and FC $>$ NO. At the same time, the DON/DIN ratio decreases from 7 over 6 to 5.

Oyarzún and Godoy [23] reported a DON loss for the NBA site of 5.2 kg N ha$^{-1}$ year$^{-1}$. Oyarzún et al. [34] found a DON loss from FC of 1.0 kg N ha$^{-1}$ year$^{-1}$. For the NO site only percolation losses (measured at 80 cm depth in the soil profile) exist. The DON percolation was 0.6 kg N ha$^{-1}$ year$^{-1}$ [21]. Therefore, these data largely confirm the DON losses as calculated with the approach of Perakis and Hedin [2]. The DIN (kg N ha$^{-1}$ year$^{-1}$) losses for NBA, NO, and FC were 0.9 (NO$_X$N = 0.6), 0.6 (NO$_X$N = 0.3), and 3.1 (NO$_X$N = 3.1, NH$^+_4$ was not determined), respectively. Here, the agreement with the approach of Perakis and Hedin [2] is less clear for the FC site.

A closer look at figure 5 shows that the three MAP–MAT sub-regions can be distinguished. Relatively seen, the $\delta^{15}$N versus TN curve of the NBA site is shifted downwards, whereas one of the NO site is shifted upwards. The data of the NBC, NN, and FC sites showed intermediate values. Therefore, DON/DIN loss ratios from old-growth forests of southern Chile could be reflected by the $\delta^{15}$N patterns, which responded to the MAP and MAT of the ecosystem.

In figures 4 and 6, the soil $\delta^{15}$N profiles of the burned and unburned AN ecosystem are shown. It is clear that the curve of the burned site has shifted upwards or towards more enriched $\delta^{15}$N values. Also the enrichment factor ($\varepsilon$), calculated according to Mariotti et al. [35], increased from $-6.73$ ‰ to $-4.33$ ‰ in the unburned and burned site, respectively. It is suggested that this shift towards more enriched $\delta^{15}$N values is induced by a loss of $^{15}$N depleted N species (e.g. NO$_X$, NO$_X^-$, and peroxyacetyl-nitrate) after the forest fire in February 2002. We hypothesize that this upward shift will continue until excess NO$_X^-$-export from the burned plot comes to an end. Currently (June 2003), the burned site is exporting about 100-fold more nitrate than the undisturbed site (data not shown). Excess nitrate export and enrichment of the soil and plant $\delta^{15}$N values have also been observed by Pardo et al. [6] after a clear-cut. The regenerating $N.~alpina$ and $N.~procera$ in the burned AN site showed a $\delta^{15}$N value of 3.10 ± 0.58 ‰. This is the only enriched $\delta^{15}$N value we observed so far for Nothofagus trees and could further support the hypothesis of $^{15}$N enrichment of the forest ecosystem after burning [10]. Subsequently,
and subject to follow-up research, we expect that the $\delta^{15}$N–TN relation will gradually move back towards the unburned situation because of the production of more $^{15}$N-depleted organic matter from the regenerating vegetation. This hypothetical development of $\delta^{15}$N of foliage in case of burning has also been suggested by Högberg [10]. This evolution has, however, never been proved in the field. When this would be the case, soil $\delta^{15}$N profiles could be a useful tool to assess the resilience of forest ecosystems towards (heavy) disturbance, such as fire and clearfelling. Finally, it is also clear that also the $\delta^{15}$N–TN curve for the unburned AN site (figures 4 and 6), having an MAP of 3173 mm and a MAT of 8.6 °C (table 1) falls within the same range as observed for the forest ecosystems in the Coastal Mountains with a comparable MAP and MAT (figure 3, table 1).

4. Conclusion

The $\delta^{15}$N signature in soil profiles under pristine old-growth forests of southern Chile increased with depth. In all cases, an exponential correlation was found between $\delta^{15}$N and TN. Evidence is emerging that the relative position of the $\delta^{15}$N–TN curve could be a potential indicator for ecosystem N-cycling in old-growth forests in southern Chile. This approach could be promising to assess ecosystem resilience after heavy disturbances such as wildfires or long-term disturbances such as enhanced N deposition, which gradually alters the N-cycle of the ecosystem. More $\delta^{15}$N data of soil profiles are needed to fully confirm and test this hypothesis.

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