

Mechanisms for retention of bioavailable nitrogen in volcanic rainforest soils

DRIES HUYGENS^{1,2*}, PASCAL BOECKX², PAMELA TEMPLER³, LEANDRO PAULINO⁴,
OSWALD VAN CLEEMPUT², CARLOS OYARZÚN⁵, CHRISTOPH MÜLLER⁶ AND ROBERTO GODOY⁷

¹Instituto de Ingeniería Agraria y Suelos, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

²Laboratory of Applied Physical Chemistry (ISOFYS), Ghent University, Coupure 653, B-9000 Gent, Belgium

³Department of Biology, Boston University, 5 Cummington Street, Boston, Massachusetts 02215, USA

⁴Departamento de Suelos y Recursos Naturales, Universidad de Concepción, Casilla 537, Chillán, Chile

⁵Instituto de Geociencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

⁶School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland

⁷Instituto de Botánica, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

*e-mail: Dries.Huygens@UGent.be

Published online: 11 July 2008; doi:10.1038/ngeo252

Nitrogen cycling is an important aspect of forest ecosystem functioning. Pristine temperate rainforests have been shown to produce large amounts of bioavailable nitrogen, but despite high nitrogen turnover rates, loss of bioavailable nitrogen is minimal in these ecosystems. This tight nitrogen coupling is achieved through fierce competition for bioavailable nitrogen by abiotic processes, soil microbes and plant roots, all of which transfer bioavailable nitrogen to stable nitrogen sinks, such as soil organic matter and above-ground forest vegetation. Here, we use a combination of *in situ* ¹⁵N isotope dilution and ¹⁵N tracer techniques in volcanic soils of a temperate evergreen rainforest in southern Chile to further unravel retention mechanisms for bioavailable nitrogen. We find three processes that contribute significantly to nitrogen bioavailability in rainforest soils: heterotrophic nitrate production, nitrate turnover into ammonium and into a pool of dissolved organic nitrogen that is not prone to leaching loss, and finally, the decoupling of dissolved inorganic nitrogen turnover and leaching losses of dissolved organic nitrogen. Identification of these biogeochemical processes helps explain the retention of bioavailable nitrogen in pristine temperate rainforests.

In pristine temperate forest ecosystems, a combination of high bioavailable nitrogen (N) production and minimal N losses has been observed^{1–6}. Bioavailable N comprises all organic and mineral N species that are readily available for microbial and/or plant uptake⁷ (Fig. 1). Depolymerization of soil organic matter (SOM) by heterotrophic bacteria and fungi is the main driver of N cycling in N-limited ecosystems⁷. This grazing step is critical, as it controls the conversion of passive N into bioavailable N species. Some heterotrophic fungi release NO₂⁻ and NO₃⁻ directly from the breakdown of recalcitrant organic compounds⁸. The role and relative magnitude of heterotrophic nitrification in N-limited ecosystems remain largely uncertain^{9,10}, but are most likely dependent on the quality and availability of substrates¹¹. Temperate evergreen forest soils have high concentrations of lignin, polyphenols and other secondary plant substances^{12,13}, suggesting that specialist microorganisms may be dominant in the N depolymerization process^{11,14}.

Besides biotic N immobilization^{3,15}, dissimilatory nitrate reduction to ammonium (DNRA) has been hypothesized to play a key role in retention of bioavailable N in forests that are located in geographic areas with large rainfall amounts^{6,16}. DNRA redirects N flow towards NH₄⁺, thereby reducing soil NO₃⁻ concentrations and thus substrate availability for N loss through leaching or denitrification. N leaching in pristine forest ecosystems mainly occurs as dissolved organic N (DON)⁴. Although DON can be formed from the biological or physical association of

NO₃⁻ with dissolved organic matter^{3,17} (DOM), DON losses have been suggested to be controlled by soil dynamics operating independently of dissolved inorganic N (DIN) turnover^{2,3,5,18}. The processes regulating NO₃⁻ versus DON retention in pristine forest soils are still not completely understood^{5,18}.

We used a combination of *in situ* ¹⁵N isotope dilution and ¹⁵N tracer techniques in volcanic soils of a pristine, evergreen *Nothofagus betuloides* rainforest to elucidate process-specific N cycling and retention pathways. This rainforest is located in the temperate climate region of southern Chile, an area still reflecting undisturbed, pre-industrial environmental conditions¹⁹. Our results indicate that these forest soils retained a significant amount of NH₄⁺ and NO₃⁻ one year following ¹⁵N addition (approximately 84 and 69%, respectively). We found that heterotrophic nitrification dominates (96%) total NO₃⁻ production, and contributed 10% to total mineral N production. A significant proportion of NO₃⁻ produced through heterotrophic nitrification was quickly converted to NH₄⁺ through DNRA (29%) or immobilized into different organic N pools (67%). DNRA is linked to heterotrophic nitrification and contributes 5% to NH₄⁺ production. Moreover, we document a mechanism for retention of bioavailable N through NO₃⁻ immobilization into soluble organic N (SON) pools that show a high sorption affinity in volcanic soils. These adsorbed N compounds have a long residence time in the soil profile, allowing decomposition by the microbial community^{20,21}. Lysimeter ¹⁵N measurements in field plots sprayed

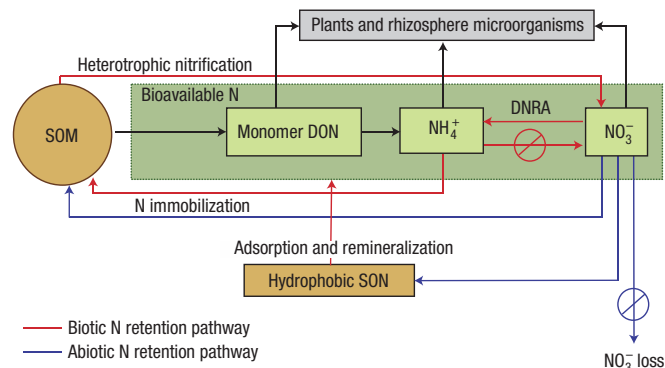


Figure 1 Conceptual model for the nitrogen (N) cycle in volcanic soils of temperate rainforests (modified from Schimel and Bennett⁷). The N transformation pathways that optimize nitrogen bioavailability in these soils are indicated in colour (DON = dissolved organic N, DNRA = dissimilatory nitrate reduction to ammonium, SOM = soil organic matter, SON = soluble organic N, NH_4^+ = ammonium N, NO_3^- = nitrate N; N pools in the green area are defined as bioavailable N forms).

with ^{15}N -DIN tracers showed the absence of ^{15}N -DON losses, providing direct evidence that DON leaching losses do not result from DIN turnover. The documented internal N transformation pathways lead to significant amounts of N retention, which optimizes N bioavailability in volcanic soils of pristine, evergreen rainforests (Fig. 1).

NITROGEN POOLS, TURNOVER AND FATE

SOM of the volcanic *Nothofagus* soils was the largest soil N pool (97.8% of total N), followed by soluble organic matter (SON, 1.6%), NH_4^+ (0.4%), microbial biomass N (MBN; 0.3%) and NO_3^- (0.03%) (Table 1). Soil water N concentrations showed a non-significant decrease with depth ($p > 0.05$) (Table 2). The relative contribution of the different soil water pools remains largely constant with depth (Table 2).

One year following addition of trace amounts of ^{15}N , total plot recovery was $84 \pm 8\%$ and $69 \pm 5\%$ for ^{15}N - NH_4^+ and ^{15}N - NO_3^- , respectively (Tables 3 and 4). A significant ($p < 0.05$) decrease in ^{15}N recovery was noticed after day 98 and day 5 for $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$, respectively. For both ^{15}N labels, SOM seemed to be the dominant sink, in the short (1–5 days) and long term (356 days). Five days after ^{15}N application, only $8.3 \pm 0.5\%$ (for $^{15}\text{NH}_4^+$) and $0.14 \pm 0.07\%$ (for $^{15}\text{NO}_3^-$) of the added ^{15}N was recovered in the labelled NH_4^+ and NO_3^- pool, respectively. Microbial biomass N was a large, immediate sink for $^{15}\text{NH}_4^+$ ($12.3 \pm 1.1\%$ after 1 day), but not for $^{15}\text{NO}_3^-$ ($1.3 \pm 0.3\%$). SON was a short-term sink for $^{15}\text{NO}_3^-$ ($21.2 \pm 1.1\%$ after 1 day), contrary to $^{15}\text{NH}_4^+$ ($-2.5 \pm 2.4\%$). Moreover, $^{15}\text{NO}_3^-$ immobilization occurred preferentially into the operationally defined hydrophobic SON pool ($69 \pm 5.7\%$ and $91 \pm 4.0\%$ of total ^{15}N recovery in SON at day 1 and day 5, respectively). Recovery in the roots seemed to be constant over the experimental period (one year), without showing differences between ^{15}N treatments, and varied between 5.2% and 7.5% of the applied ^{15}N .

Rates of production and consumption associated with the NH_4^+ pool were much greater than those associated with the NO_3^- pool (Table 5). Heterotrophic nitrification dominated total NO_3^- production ($96 \pm 10\%$), and accounted for 10% of total mineral N production. DNRA accounted for $29 \pm 8\%$ of NO_3^- consumption and $5.1 \pm 0.9\%$ of NH_4^+ production. Autotrophic nitrification

Table 1 Physico-chemical properties of the sampled 0–10 cm soil layer. TC = total carbon content, TN = total nitrogen content; NH_4^+ = extractable ammonium, NO_3^- = extractable nitrate, SON = extractable soluble organic nitrogen, MBN = microbial biomass nitrogen, SOM = soil organic matter nitrogen. Analysis carried out on natural abundance samples, $n = 8$, with standard errors in brackets.

pH	5.0	(0.2)
Bulk density (g cm^{-3})	0.22	(0.02)
TC (%)	15.1	(1.4)
TN (%)	0.75	(0.06)
$\text{NH}_4^+\text{-N}$ ($\mu\text{g N g}^{-1}$)	27.2	(2.8)
$\text{NO}_3^-\text{-N}$ ($\mu\text{g N g}^{-1}$)	2.0	(0.1)
SON ($\mu\text{g N g}^{-1}$)	119.0	(7.8)
MBN ($\mu\text{g N g}^{-1}$)	19.7	(2.8)
SOM ($\mu\text{g N g}^{-1}$)	7431	(293)
Root N ($\mu\text{g N g}^{-1}$)	88.7	(4.1)

represented only $0.4 \pm 2.6\%$ of total NH_4^+ consumption. NH_4^+ and NO_3^- immobilization into organic N pools were the dominant DIN consumption fluxes ($89 \pm 23\%$ and $67 \pm 19\%$, respectively).

The recovery of Br^- , a hydrological tracer that is used to check preferential flow paths in the watershed, confirmed that the hydrological flow paths of the ^{15}N tracer solutions passed through the suction tension lysimeter draining area (data not shown). Applied ^{15}N - NH_4^+ was not detected in the soil water over the entire study period. Applied ^{15}N - NO_3^- was only detected in the soil water after the first rain event. The observed ^{15}N losses occurred as NO_3^- . After the first rainfall event, enrichments in ^{15}N - NO_3^- of the soil water equalled $0.30 \pm 0.10\%$, $1.80 \pm 0.26\%$ and $0.017 \pm 0.007\%$ (expressed as at.% excess), at a depth of 10, 20 and 50 cm, respectively. We found no detectable ^{15}N tracer in the DON leached at any time after ^{15}N pulse addition (data not shown). Soil water N concentrations after ^{15}N application did not differ from background values (data not shown).

BUILDING ON PREVIOUSLY OBTAINED RESULTS

The results of our work, in conjunction with previous work, suggest that a combination of microbial and abiotic retention processes lead to the long-term sustained N retention of temperate evergreen rainforest soils (Fig. 1). Previous work on N cycling processes in pristine temperate forest soils of southern Chile demonstrated (1) high rates of NH_4^+ turnover, but slow NO_3^- dynamics^{3,6}, (2) the inhibition of autotrophic nitrification leading to a dominance of heterotrophic nitrification for NO_3^- production processes under background N conditions and increased N inputs rates⁵, (3) the importance of microbial biomass and slowly cycling SOM pools as a sink for mineral N additions in the short term (weeks) and long term (years), respectively^{3,6} and (4) the occurrence of DNRA under controlled laboratory conditions⁶. Building on this earlier work, our results contribute to a better understanding of the process-specific pathways that optimize N bioavailability in volcanic soils of a temperate evergreen rainforest. We indicate (1) the importance of heterotrophic nitrification as a bioavailable N production process, and (2) abiotic NO_3^- transformations into NH_4^+ and DON pools that show a high adsorption affinity to the soil matrix.

HETEROTROPHIC NITRIFICATION

In N-limited ecosystems where carbon (C) is abundantly present, microbial immobilizers are more competitive for NH_4^+ than autotrophic nitrifiers²². This explains the low autotrophic nitrification rates ($0.06 \mu\text{g N g}^{-1} \text{d}^{-1}$) and high immobilization of NH_4^+ observed in this study ($20.5 \mu\text{g N g}^{-1} \text{d}^{-1}$), similar to the

Table 2 Nitrogen concentrations of the different soil groundwater N pools, and their percentile contribution to total dissolved N at different depths. NH_4^+ = ammonium, NO_3^- = nitrate, hydrophilic DON = operationally defined hydrophilic dissolved organic nitrogen, hydrophobic DON = operationally defined hydrophobic dissolved organic nitrogen. Analysis carried out on natural abundance samples, $n = 4$, with standard errors of the spatial variation in brackets.

	N concentration ($\mu\text{g N l}^{-1}$)						% of total dissolved N					
	10 cm depth		20 cm depth		50 cm depth		10 cm depth		20 cm depth		50 cm depth	
NH_4^+	26	(8)	23	(9)	16	(3)	12	(5)	12	(4)	15	(2)
NO_3^-	71	(15)	53	(15)	26	(3)	32	(2)	28	(2)	24	(2)
Hydrophilic DON	73	(22)	50	(10)	43	(7)	33	(5)	29	(3)	39	(5)
Hydrophobic DON	53	(13)	55	(10)	24	(5)	24	(4)	31	(3)	22	(5)
Total dissolved N	224	(56)	180	(45)	108	(9)						

Table 3 Recovery (%) of the applied $^{15}\text{NH}_4^+$ as a function of time for measured N pools. NH_4^+ = ammonium N, NO_3^- = nitrate N, SON = soluble organic N, ho-SON = hydrophobic soluble organic N, hi-SON = hydrophilic soluble organic N, SOM = soil organic matter, roots = root N, whole plot = total recovery in the measured plot. $n = 8$; avg = average; s.e. = standard error, ND = not determined as total SON was not significantly different ($p < 0.05$) from zero; significant differences ($p < 0.05$) are indicated by different letters that is, values having different letters are significantly different from each other; values having the same letter are not significantly different.

	$^{15}\text{NH}_4^+$ recovery (%)																		
	NH_4^+		NO_3^-		SON		ho-SON		hi-SON		MBN		SOM		Roots		Whole plot		
	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	
Time (days)	1	45.5	1.6	0.03	0.01	-2.5	2.4	ND	ND	ND	ND	12.4	1.1	49.8	4.4	5.2	0.3	111.0 ^a	4.8
	5	8.3	0.5	0.02	0.01	1.1	0.8	ND	ND	ND	ND	1.3	0.6	78.4	5.2	7.5	0.3	94.3 ^{a,b}	5.0
	98	1.3	0.1	0.05	0.01	0.9	0.4	ND	ND	ND	ND	0.9	0.3	66.3	6.2	6.7	0.5	77.0 ^b	6.2
	265	0.7	0.2	0.02	0.01	0.5	0.1	ND	ND	ND	ND	0.1	0.2	70.2	5.0	6.0	0.6	79.0 ^b	5.3
	357	0.7	0.1	0.02	0.02	-0.6	0.1	ND	ND	ND	ND	0.2	0.2	77.3	7.6	5.7	0.5	83.6 ^b	8.1

results of Perakis and Hedin³. Fluctuating redox conditions, which can potentially limit autotrophic nitrifiers²³, were not observed in the *in situ* incubated soils (see Supplementary Information, Table S1). Hence, our results indicate that nitrification is almost entirely heterotrophic in this forest ecosystem ($2.27 \mu\text{g N g}^{-1} \text{d}^{-1}$). The dominance of heterotrophic over autotrophic nitrification fluxes has been observed in a range of undisturbed forest ecosystems located on different geologic substrates^{5,9,24,25}. For this, we believe that a prominent contribution of heterotrophic nitrification to total NO_3^- production is widespread in organic layers of pristine forest soils, irrespective of soil mineralogy. We argue that heterotrophic nitrification should be considered in conceptual N cycling models in pristine forest ecosystems (Fig. 1).

Heterotrophic nitrification contributes to about 10% of total mineral N production in these forest soils. In contrast to autotrophic nitrification, the functional role and significance of heterotrophic nitrification remain largely unknown⁹. Heterotrophic nitrification may contribute to bioavailable N production because some heterotrophic nitrifiers show superior degrading capabilities for recalcitrant SOM originating from evergreen leaves, such as lignin and polyphenols^{9,13,14}. Lignin and lignin-derived phenol degradation is an important control on depolymerization and dissolved organic monomer production in forest soils^{26,27}. Hence, heterotrophic nitrification contributes to the production of bioavailable N from a N substrate that is to a large extent inaccessible to other soil biota¹⁴. The fact that NO_3^- is highly susceptible to N losses indicates the necessity to understand the relative magnitude of NO_3^- consumption processes in this pristine rainforest soil.

NITRATE RETENTION PATHWAYS

Although NO_3^- turnover is only about one tenth of the total mineral N turnover in this forest (Table 5), total gross nitrification

is comparable to that of many polluted and unpolluted forest ecosystems¹⁰. This observation indicates the necessity of an in-depth investigation of soil NO_3^- transformation pathways to gain insight into soil–plant–soil water dynamics and water quality of nearby aquatic ecosystems. In this forest soil, a combination of DNRA and abiotic nitrate immobilization into organic N pools (29% and 67% of total NO_3^- consumption, respectively) ensures NO_3^- retention. The observed NO_3^- retention mechanisms in this study are different from those of Perakis and Hedin³, who found microbial assimilation as an effective NO_3^- retention mechanism¹⁵ in non-volcanic south Chilean soils developed from highly weathered schists. Besides potential influences of differences in NH_4^+ pool sizes¹⁵, this difference might be related to soil type and mineralogy, factors that strongly influence the behaviour and retention of inorganic N compounds²⁸. Volcanic soil material exerts a strong influence on anion sorption²⁹, charge development and microaggregation associated with anaerobic microsite formation³⁰, potentially favouring DNRA¹⁶ and/or abiotic NO_3^- immobilization^{17,28} in these volcanic rainforest soils.

Similar to our previous laboratory study⁶, this field study documents the occurrence of DNRA in N-limited, temperate evergreen rainforests. In these soils, DNRA is responsible for 29% of total NO_3^- consumption, with absolute values ($1.0 \mu\text{g N g}^{-1} \text{d}^{-1}$) similar to documented values in the literature^{16,23,31,32}. DNRA accounts for 5% of total NH_4^+ production ($19.6 \mu\text{g N g}^{-1} \text{d}^{-1}$) and for 4% of total mineral N retention. DNRA keeps N in an immediately bioavailable N form, NH_4^+ , which is less susceptible to ecosystem N loss such as leaching or gaseous N production from NO_3^- (Fig. 1). In the framework of bioavailable N retention, DNRA is an effective mechanism to compete with denitrification^{23,31,32}. DNRA is favoured by high levels of available carbon^{16,23}, a condition that restricts autotrophic nitrification in N-limited forest ecosystems²². Hence, DNRA depends directly on heterotrophic

Table 4 Recovery (%) of the applied $^{15}\text{NO}_3^-$ as a function of time for measured N pools. NH_4^+ = ammonium N, NO_3^- = nitrate N, SON = soluble organic N, ho-SON = hydrophobic soluble organic N, hi-SON = hydrophilic soluble organic N, SOM = soil organic matter, roots = root N, whole plot = total recovery in the measured plot. $n = 8$; avg = average; s. e. = standard error, ND = not determined as total SON was not significantly different ($p < 0.05$) from zero; significant differences ($p < 0.05$) are indicated by different letters that is, values having different letters are significantly different from each other; values having the same letters are not significantly different.

	$^{15}\text{NO}_3^-$ recovery (%)																		
	NH_4^+		NO_3^-		SON		ho-SON		hi-SON		MBN		SOM		Roots		Whole plot		
	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	
Time (days)	1	6.4	0.6	0.03	0.04	21.2	1.1	15.0	1.6	6.2	1.0	1.3	0.3	58.6	2.7	5.7	0.5	93.2 ^a	2.4
	5	2.0	0.2	0.14	0.07	12.0	0.8	11.1	1.1	0.9	0.4	0.7	0.2	51.7	3.8	5.3	0.4	71.9 ^b	4.3
	98	0.4	0.1	-0.07	0.01	1.6	0.5	ND	ND	ND	ND	0.8	0.2	58.8	4.2	6.3	0.4	67.7 ^b	4.0
	265	0.3	0.1	0.02	0.01	3.9	2.5	ND	ND	ND	ND	0.5	0.3	49.9	4.8	5.4	0.4	60.0 ^b	3.6
	357	0.5	0.1	0.08	0.03	1.1	0.4	ND	ND	ND	ND	0.1	0.1	61.9	4.3	5.9	0.4	69.5 ^b	5.0

Table 5 Ammonium (NH_4^+) and nitrate (NO_3^-) production and consumption fluxes ($\mu\text{g N g}^{-1} \text{d}^{-1}$) and their % contribution to the total N pool production or consumption (standard errors in brackets, $n = 8$).

N flux	$\mu\text{g N g}^{-1} \text{soil d}^{-1}$		% contribution	
Gross NO_3^- production	2.32 (0.22)			
Autotrophic nitrification	0.06 (0.22)		4.1	(9.8)
Heterotrophic nitrification	2.27 (0.31)		95.9	(9.8)
Gross NO_3^- consumption	4.20 (0.76)			
Dissimilatory nitrate reduction to ammonium	1.00 (0.20)		28.7	(8.2)
NO_3^- immobilization into organic N pools	3.00 (1.27)		67.0	(18.9)
Gross NH_4^+ production	21.00 (3.60)			
Dissimilatory nitrate reduction to ammonium	1.00 (0.20)		5.1	(0.9)
Gross NH_4^+ consumption	19.63 (3.73)			
Autotrophic nitrification	0.06 (0.22)		0.4	(2.6)
NH_4^+ immobilization into organic N pools	20.54 (4.63)		88.9	(23.0)

nitrification for substrate generation, suggesting that both pathways are functionally linked in N-limited forests (Fig. 1).

Similar to previous work^{3,17,33}, a large proportion (67%) of the NO_3^- produced was immobilized by SOM and SON pools ($3.0 \mu\text{g N g}^{-1} \text{d}^{-1}$). Low amounts of microbial uptake of $^{15}\text{NO}_3^-$ (Table 4) suggest the dominance of abiotic NO_3^- immobilization processes in organic N pools in this soil. In this study, the preferential transformation of NO_3^- into (operationally defined) hydrophobic SON is demonstrated (Fig. 1). This finding is consistent with the 'ferrous wheel hypothesis'¹⁷ and/or other mechanisms of rapid NO_3^- reduction in forest soils³⁴. The ferrous wheel mechanism¹⁷ describes the formation of (hydrophobic) phenolic compounds and lignin derivatives during NO_3^- immobilization. A recent study³⁵ refutes this hypothesis by claiming that earlier evidence^{17,33} had analytical artefacts during NO_3^- concentration measurements. However, in our study, NO_3^- concentrations were quantified using an imidazole buffer, which does not affect the analytical precision³⁵. As such, the occurrence of the ferrous wheel hypothesis cannot be ruled out in this forest soil. We speculate that volcanic Andisols, characterized by high Fe^{2+} concentrations, high levels of dissolved organic carbon and the presence of anaerobic microsites, offer ideal conditions for the occurrence of these NO_3^- condensation reactions into SON.

ORIGIN OF N LEACHING LOSSES

We found that the dominant form of N leaching was DON (Table 2), as observed elsewhere in unpolluted forests of Chile⁴. As anthropogenic N loads have the potential to activate DON loss pathways¹⁸ affecting downstream water quality³⁶, the fate of

SON products originating from DIN turnover processes should be assessed in soil water. The relatively low soil water N concentrations in this study (Table 2) were in accordance with those of Oyarzún *et al.*³⁷. High retention of inorganic and organic N compounds has previously been observed in A and B soil horizons, rich in Fe and Al oxyhydroxides²⁸. Perakis *et al.*⁵ showed DIN retention of up to 62% in the 0–25 cm soil layer of a non-volcanic south Chilean forest soil, and this at N addition rates up to $160 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Plant uptake processes, microbial assimilation and N adsorption processes retain a significant amount of N in the soil–plant ecosystem³⁵. In our study site, N transformation pathways were shown to transfer NO_3^- into SON (Table 4). Despite the occurrence of the DIN–SON transformation process, no leaching losses of these DIN turnover products were observed in the soil water. NO_3^- mainly turns over in (operationally defined) hydrophobic SON, strongly adsorbed to the soil matrix³⁸, preventing NO_3^- turnover products leaching from the soil profile. This indicates the occurrence of an extra mechanism for N retention in these volcanic rainforest soils. Jardine *et al.*³⁸ indicated the preferential adsorption of hydrophobic over hydrophilic DOM to soil Al and Fe oxyhydroxides, typical for volcanic ash soils. Moreover, adsorption of hydrophobic organic compounds to Al and Fe oxyhydroxide surfaces is to a main extent irreversible³⁹. Adsorbed organic N compounds are concentrated in microsites where bacteria and fungi might exude exoenzymes to slowly hydrolyse this substrate, resulting in the production of either resynthesized microbial organic compounds or inorganic mineralization products^{20,21}. Hence, this pathway reintroduces adsorbed hydrophobic SON into the bioavailable N pool (Fig. 1).

Early work on organic leaching losses from forest ecosystems already suggested that much of the DOM draining from the forest floor originates from recalcitrant SOM pools⁴⁰. On the basis of analysis of C/N ratios and charge density stoichiometry of dissolved organic carbon, Hedin *et al.*² supported this finding in south Chilean rainforests on non-volcanic soils with a special focus on DON. Studies^{3,5} showing a high ¹⁵N-DIN retention in soil-plant ecosystem compartments of non-volcanic south Chilean forest ecosystems lend further support to this theory. This study integrates results on ¹⁵N tracer recovery in the soil, soil water and plant compartment in a N-limited evergreen volcanic rainforest. Using this integrated approach, we showed that DIN inputs do not end up in the soil water compartment. Hence, we provide direct evidence that DON losses in evergreen volcanic rainforests do not result from the turnover of actively cycling soil DIN pools, but originate rather from bio-unavailable compounds leaching from slow-turnover SOM pools^{2,3,5,40}.

INCREASED INSIGHT INTO ECOSYSTEM N RETENTION

This field study indicates that N transformation pathways in volcanic rainforest soils optimize N bioavailability (Fig. 1). We document that three mechanisms contribute to the retention of bioavailable N in volcanic soils of an evergreen, rainforest soil: (1) heterotrophic nitrification, a mineral N production process carried out by microbial communities that mobilize recalcitrant evergreen organic material, (2) DNRA, a conservation mechanism for NO₃⁻, which is functionally linked to heterotrophic nitrification and (3) bioavailable N retention through NO₃⁻ turnover into SON fractions that show a high sorption affinity in volcanic soils. As indicated by ¹⁵N mass balances in soil, water and vegetation compartments, efficient bioavailable N recycling prevents leaching losses of DIN and its turnover products. This confirms earlier hypotheses that DON leaching losses do not cycle through active soil N pools. We presume that these 'alternative' N transformation pathways may be more widespread than previously considered in pristine temperate forest ecosystems.

METHODS SUMMARY

The study site is located in the Andean mountain range, Antillanca, southern Chile (40°47' S, 72°12' W). The vegetation is characterized as a *Nothofagus betuloides* forest. The soil is classified as Umbric Vitric Andosol⁴¹. Further details concerning the site description can be found in Table 1 and Supplementary Information, Methods SA.

In a ¹⁵N pulse-chase experiment, 20 3 × 2 m plots were established in a representative 50 × 30 m area of the *N. betuloides* forest. Eight subplots were sprayed with a ¹⁵N-NH₄⁺ solution, eight were sprayed with a ¹⁵N-NO₃⁻ solution and four were sprayed with a bromide solution. Soil sampling of the ¹⁵N tracer experiment took place 1 day before ¹⁵N application (background sampling), and 1, 5, 98, 265 and 357 days after ¹⁵N application (six time points over a 1 year period). The top 10 cm of the soils (O horizon) were sampled using disposable polyvinyl chloride tubes (*n* = 8). Bulk densities were determined by pushing steel cylinders (200 cm³) into the undisturbed 0–10 cm soil layer (8 replicates per ¹⁵N treatment). In four ¹⁵N-NH₄⁺ and four ¹⁵N-NO₃⁻ subplots, zero-tension lysimeters were used to sample soil water at a depth of 10 and 20 cm. Ceramic suction tension lysimeters (1900 Soil Water Samplers, Soil Moisture) were installed in four ¹⁵N-NH₄⁺, four ¹⁵N-NO₃⁻ subplots and the four Br⁻ plots at a depth of 50 cm. Lysimeters were sampled 1 day before ¹⁵N application to obtain background N concentration values (*n* = 4). For the first month after ¹⁵N application, soil water samples were taken the day after each rain event. Time intervals between soil water sampling increased towards the end of the 1 year experimental period. Further details concerning the experimental set-up of the ¹⁵N pulse-chase experiment can be found in Supplementary Information, Methods SB.

Gross rates of N cycling were determined in the 0–10 cm layer through homogeneous ¹⁵N injection⁴² in intact soil cores in the field⁴³ (*n* = 8). Further

details concerning the set-up of the ¹⁵N core injection experiment can be found in Supplementary Information, Methods SC.

Soil samples were sieved shortly after sampling over a 2 mm sieve while live roots were removed, washed in a 0.01 M KCl solution and dried at 60 °C. The sieved soil sample was rehomogenized and split into three subsamples: (1) approximately 20 g wet soil was dried at 60 °C (18 h) for soil moisture determination, (2) approximately 30 g wet soil was used for microbial biomass N determinations and (3) approximately 200 g wet soil was used for KCl extraction. MBN determinations were carried out using the 'chloroform labile nitrogen' method for field samples³. Soils were extracted using a 0.1 M KCl solution of respectively 30 ml and 150 ml for (2) and (3). We calculated concentrations and ¹⁵N enrichments of the extractable SON as extractable total dissolved nitrogen (TDN) minus extractable NH₄⁺-N and NO₃⁻-N. When ¹⁵N enrichments of the SON were significantly different from zero, SON was further separated into an operationally defined hydrophobic and hydrophilic SON fraction⁴⁴, and isotopically corrected for DIN species. Nitrogen in the roots was calculated as the %N of fine roots multiplied by the root biomass. SOM N was calculated as the difference between root-free soil N minus extractable TDN minus MBN. Further details concerning soil sample processing and calculations can be found in Supplementary Information, Methods SD.

Lysimeter solution sample (~500 ml) TDN content was split into NH₄⁺-N, NO₃⁻-N and operationally defined hydrophobic and hydrophilic DON, using a combination of dialysis⁴⁵ and DON fractionation⁴⁴ methods. Further details concerning soil water processing and calculations can be found in Supplementary Information, Methods SE.

Ammonium in the soil and water extracts was determined colourimetrically by the salicylate-nitroprusside method⁴⁶. Nitrate was determined colourimetrically through NO₂⁻ reduction and subsequent imidazole buffered reaction with N-1-naphthylethylenediamine. The ¹⁵N contents of NH₄⁺ and NO₃⁻ were analysed after conversion to N₂O (refs 47,48), and measured using a trace-gas preparation unit (ANCA-TGII, PDZ Europa), coupled to an isotope ratio mass spectrometer (20-20, SerCon). Solid soil samples were ground with a planetary ball mill (PM400, Retsch) for total nitrogen, ¹⁵N and total carbon analysis with an elemental analyser (ANCA-SL, PDZ Europa), coupled to an isotope ratio mass spectrometer (20-20, SerCon). TDN concentrations and ¹⁵N enrichments were determined according to Huygens *et al.*⁴⁹. Further details on analytical Methods can be found in Supplementary Information, Methods SF.

Gross mineralization, nitrification and immobilization fluxes were calculated according to Davidson *et al.*⁴³. DNRA, autotrophic nitrification and mineral immobilization rates were calculated using a combination of ¹⁵N isotope dilution and ¹⁵N tracer techniques¹⁶. A detailed description of the DNRA calculation method is given in the Supplementary Information, note 'DNRA calculations and assumptions'. Heterotrophic nitrification was calculated as the difference between gross NO₃⁻ production and autotrophic nitrification⁵⁰. Further details on N flux calculations can be found in Supplementary Information, Methods SG.

Received 31 August 2007; accepted 16 June 2008; published 11 July 2008.

References

- Vitousek, P. M. & Reiners, W. A. Ecosystem succession and nutrient retention: A hypothesis. *Bioscience* **25**, 376–381 (1975).
- Hedin, L. O., Armesto, J. J. & Johnson, A. H. Patterns of nutrient loss from unpolluted, old-growth temperate forests: Evaluation of biogeochemical theory. *Ecology* **76**, 493–509 (1995).
- Perakis, S. S. & Hedin, L. O. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, southern Chile. *Ecology* **82**, 2245–2260 (2001).
- Perakis, S. S. & Hedin, L. O. Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. *Nature* **415**, 416–419 (2002).
- Perakis, S. S., Compton, J. E. & Hedin, L. O. Nitrogen retention across a gradient of ¹⁵N additions to an unpolluted temperate forest soil in Chile. *Ecology* **86**, 95–105 (2005).
- Huygens, D. *et al.* Soil nitrogen conservation mechanisms in a pristine south Chilean *Nothofagus* forest ecosystem. *Soil Biol. Biochem.* **39**, 2448–2458 (2007).
- Schimel, J. P. & Bennett, J. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology* **85**, 591–602 (2004).
- Wood, P. M. Autotrophic and heterotrophic mechanisms for ammonia oxidation. *Soil Use Manag.* **6**, 78–79 (1990).
- De Boer, W. & Kowalchuk, G. A. Nitrification in acid soils: Micro-organisms and mechanisms. *Soil Biol. Biochem.* **33**, 853–866 (2001).
- Booth, M. S., Stark, J. M. & Rastetter, E. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecol. Monogr.* **75**, 139–157 (2005).
- Killham, K. Heterotrophic nitrification. *Spec. Publ. Soc. Gen. Microb.* **20**, 117–126 (1986).
- Gallet, C. & Lebreton, P. Evolution of phenolic patterns in plants and associated litters and humus of a mountain forest ecosystem. *Soil Biol. Biochem.* **27**, 157–165 (1995).
- Aerts, R. The advantages of being evergreen. *Trends Ecol. Evol.* **10**, 402–407 (1995).
- Bending, G. D. & Read, D. J. Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. *Mycol. Res.* **101**, 1348–1354 (1997).
- Stark, J. M. & Hart, S. C. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* **385**, 61–64 (1997).

16. Silver, W. L., Herman, D. J. & Firestone, M. K. Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. *Ecology* **82**, 2410–2416 (2001).
17. Davidson, E. A., Chorover, J. & Dail, D. B. A mechanism of abiotic immobilization of nitrate in forest ecosystems: The ferrous wheel hypothesis. *Glob. Change Biol.* **9**, 228–236 (2003).
18. Brookshire, E. N. J., Valett, H. M., Thomas, S. A. & Webster, J. R. Atmospheric N deposition increases organic N loss from temperate forests. *Ecosystems* **10**, 252–262 (2007).
19. Weathers, K. C. & Likens, G. E. Clouds in southern Chile: An important source of nitrogen to nitrogen-limited ecosystems? *Environ. Sci. Technol.* **31**, 210–213 (1997).
20. Guggenberger, G. & Kaiser, K. Dissolved organic matter in soil: Challenging the paradigm of sorptive preservation. *Geoderma* **113**, 293–310 (2003).
21. Qualls, R. G. & Haines, B. L. Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. *Soil Sci. Soc. Am. J.* **56**, 578–586 (1992).
22. Vitousek, P. M. Nutrient cycling and nutrient use efficiency. *Am. Nature* **199**, 533–572 (1982).
23. Pett-Ridge, J., Silver, W. L. & Firestone, M. K. Redox fluctuations frame microbial community impacts on N-cycling rates in a humid tropical forest soil. *Biogeochemistry* **81**, 95–110 (2006).
24. Burton, J., Chen, C. R., Xu, Z. H. & Ghadiri, H. Gross nitrogen transformations in adjacent native and plantation forests of subtropical Australia. *Soil Biol. Biochem.* **39**, 426–433 (2007).
25. Pedersen, H., Dunkin, K. A. & Firestone, M. K. The relative importance of autotrophic and heterotrophic nitrification in a conifer forest soil as measured by ¹⁵N tracer and pool dilution techniques. *Biogeochemistry* **44**, 135–150 (1999).
26. Kaiser, K., Guggenberger, G., Haumaier, L. & Zech, W. Seasonal variations in the chemical composition of dissolved organic matter in organic forest floor layer leachates of old-growth Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) stands in northeastern Bavaria, Germany. *Biogeochemistry* **55**, 103–143 (2001).
27. Kalbitz, K., Kaiser, K., Bargholz, J. & Dardenne, P. Lignin degradation controls the production of dissolved organic matter in decomposing foliar litter. *Eur. J. Soil Sci.* **57**, 504–516 (2006).
28. Qualls, R. G. Comparison of the behavior of soluble organic and inorganic nutrients in forest soils. *For. Ecol. Manag.* **138**, 29–50 (2000).
29. Strahm, B. D. & Harrison, R. B. Mineral and organic matter controls on the sorption of macronutrient anions in variable-charge soils. *Soil Sci. Soc. Am. J.* **71**, 1926–1933 (2007).
30. Huygens, D., Boeckx, P., Van Cleemput, O., Oyarzun, C. E. & Godoy, R. Aggregate and soil organic carbon dynamics in south Chilean Andisols. *Biogeosciences* **2**, 159–174 (2005).
31. Bengtsson, G. & Bergwall, C. Fate of ¹⁵N labelled nitrate and ammonium in a fertilized forest soil. *Soil Biol. Biochem.* **32**, 545–557 (2000).
32. Silver, W. L., Thompson, A. W., Reich, A., Ewel, J. J. & Firestone, M. K. Nitrogen cycling in tropical plantation forests: Potential controls on nitrogen retention. *Ecol. Appl.* **15**, 1604–1614 (2005).
33. Dail, D. B., Davidson, E. A. & Chorover, J. Rapid abiotic transformation of nitrate in an acid forest soil. *Biogeochemistry* **54**, 131–146 (2001).
34. Aber, J. D. *et al.* Nitrogen saturation in temperate forest ecosystems: Hypothesis revisited. *Bioscience* **48**, 921–934 (1998).
35. Colman, B. P., Fierer, N. & Schimel, J. P. Abiotic nitrate incorporation in soil: Is it real? *Biogeochemistry* **84**, 161–169 (2007).
36. Seitzinger, S. P. & Sanders, R. W. Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. *Mar. Ecol. - Prog. Ser.* **159**, 1–12 (1997).
37. Oyarzún, C. E., Godoy, R., De Schrijver, A., Staels, J. & Lust, N. Water chemistry and nutrient budgets in an undisturbed evergreen rainforest of southern Chile. *Biogeochemistry* **71**, 107–123 (2004).
38. Jardine, P. M., Weber, N. L. & McCarthy, J. F. Mechanisms of dissolved organic-carbon adsorption on soil. *Soil Sci. Soc. Am. J.* **53**, 1378–1385 (1989).
39. Kaiser, K. & Zech, W. Release of natural organic matter sorbed to oxides and a subsoil. *Soil Sci. Soc. Am. J.* **63**, 1157–1166 (1999).
40. Qualls, R. G., Haines, B. L. & Swank, W. T. Fluxes of dissolved organic nutrients and humic substances in a deciduous forest. *Ecology* **72**, 254–266 (1991).
41. IUSS Working Group WRB. World Soil Resources Report No. 103, (2006).
42. Andersen, M. K. & Jensen, L. S. Low soil temperature effects on short-term gross N mineralisation-immobilisation turnover after incorporation of a green manure. *Soil Biol. Biochem.* **33**, 511–521 (2001).
43. Davidson, E. A., Hart, S. C., Shanks, C. A. & Firestone, M. K. Measuring gross nitrogen mineralization, immobilization, and nitrification by ¹⁵N isotopic pool dilution in intact soil cores. *J. Soil Sci.* **42**, 335–349 (1991).
44. Leenheer, J. A. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewater. *Environ. Sci. Technol.* **15**, 578–587 (1981).
45. Vandenbruwane, J., De Neve, S., Qualls, R. G., Salomez, J. & Hofman, G. Optimization of dissolved organic nitrogen (DON) measurements in aqueous samples with high inorganic nitrogen concentrations. *Sci. Total Environ.* **386**, 103–113 (2007).
46. Mulvaney, R. L. in *Methods of Soil Analysis* (ed. Sparks, D. L.) 1123–1184 (ASA and SSSA, Madison, 1996).
47. Hauck, R. D. in *Methods of Soil Analysis* (eds Page, A. L., Miller, R. A. & Keeney, D. R.) 735–779 (ASA and SSSA, Madison, 1982).
48. Stevens, R. J. & Laughlin, R. J. Determining ¹⁵N in nitrite or nitrate by producing nitrous oxide. *Soil Sci. Soc. Am. J.* **58**, 1108–1116 (1994).
49. Huygens, D. *et al.* On-line technique to determine the isotopic composition of total dissolved nitrogen. *Anal. Chem.* **79**, 8644–8649 (2007).
50. Barraclough, D. & Puri, G. The use of ¹⁵N pool dilution and enrichment to separate the heterotrophic and autotrophic pathways of nitrification. *Soil Biol. Biochem.* **27**, 17–22 (1995).

Supplementary Information accompanies this paper on www.nature.com/naturegeoscience.

Acknowledgements

This research was supported by the Fund for Scientific Research - Flanders (Belgium) (FWO, G.0426.04) and a Bilateral Scientific and Technological Cooperation between Flanders and Chile (BOF, UGent). R.G. would like to thank the National Commission for Scientific and Technological Research - Chile (FONDECYT). Support during field campaigns was provided by Y. Rivas, G. Guevara, P. Etcheverría, Y. Ugarte, E. Padilla, L. Almonacid and J. Peters. We are grateful to the CONAF, especially to N. Pacheco, for supporting our research. We acknowledge E. Gillis, K. Van Nieuland and J. Vermeulen for isotope analyses.

Author contributions

All authors worked out the study aims, discussed the results and edited/commented on the manuscript; D.H., P.B., L.P., C.O. and R.G. participated in field sampling campaigns; D.H., P.B. and P.T. prepared experimental set-up and scientific protocols. D.H. wrote the manuscript, analysed water samples and carried out data analysis; D.H., P.B. and R.G. supervised the project; P.T. provided expertise on ¹⁵N field work and techniques during a scientific exchange programme of D.H.

Author information

Reprints and permission information is available online at <http://npg.nature.com/reprintsandpermissions>. Correspondence and requests for materials should be addressed to D.H.