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Functional role of DNRA and nitrite reduction in a pristine south Chilean *Nothofagus* forest

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Abstract Nitrite (NO_2^-) is an intermediate in a variety of soil N cycling processes. However, NO_2^- dynamics are often not included in studies that explore the N cycle in soil. Within the presented study, nitrite dynamics were investigated in a *Nothofagus betuloides* forest on an Andisol in southern Chile. We carried out a ^{15}N tracing study with six ^{15}N labeling treatments, including combinations of NO_3^-, NH_4^+ and NO_2^-. Gross N transformation rates were quantified with a ^{15}N tracing model in combination with a Markov chain Monte Carlo optimization routine. Our results indicate the occurrence of functional links between (1) NH_4^+ oxidation, the main process for NO_2^- production (nitritation), and NO_2^- reduction, and (2) oxidation of soil organic N, the dominant NO_3^- production process in this soil, and dissimilatory NO_3^- reduction to NH_4^+ (DNRA). The production of NH_4^+ via DNRA was approximately ten times higher than direct mineralization from recalcitrant soil organic matter. Moreover, the rate of DNRA was several magnitudes higher than the rate of other NO_3^- reducing processes, indicating that DNRA is able to outcompete denitrification, which is most likely not an important process in this ecosystem. These functional links are most likely adaptations of the microbial community to the prevailing pedo-climatic conditions of this *Nothofagus* ecosystem.

Keywords *Nothofagus betuloides* · ^{15}N tracing model · Nitrite (NO_2^-) · N retention · Dissimilatory nitrate reduction to ammonium (DNRA) · Markov chain Monte Carlo sampling

Abbreviations

Anammox	Anaerobic ammonium oxidation
DNRA	Dissimilatory nitrate reduction to ammonium
GWC	Gravimetric water content

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MCMC	Markov chain Monte Carlo
MOM	Macro organic matter
PDF	Parameter density function
SOM	Soil organic matter
WFPS	Water filled pore space

Introduction

Nitrogen (N) is a key element for ecosystem productivity. In natural, N-limited ecosystems the amount of N available for plant and microbial uptake is dependent on the balance between inputs and outputs of N. *Nothofagus* forests in southern Chile are characterized by very low N depositions (Holland et al. 1999). Thus, ecosystem productivity is inversely related to the amount of N lost from the system. In moderately acid soils, leaching of NO_3^- and gaseous N emissions are likely to be the most important N loss mechanisms. Both processes are dependent on the availability of nitrate (NO_3^-). Therefore, soil N processes that transfer NO_3^- into more stable N forms ensure long-term ecosystem sustainability. Recently, we showed that DNRA in combination with subsequent ammonium (NH_4^+) immobilization is an important mechanism for mineral N retention in an N-limited undisturbed *Nothofagus* forest ecosystem in southern Chile (Huygens et al. 2007). As DNRA occurs under similar low-oxygen conditions as denitrification (Tiedje et al. 1982), both processes are in competition for the available NO_3^- and can affect each other (Knowles 1982; Silver et al. 2001, 2005). Denitrification is next to nitrification the main pathway of nitrous oxide (N_2O) production in forest ecosystems (Speir et al. 1999). Therefore a high DNRA rate has the potential to reduce gaseous N losses (Huygens et al. 2007). Undisturbed *Nothofagus* forests are characterized by small N_2O production from denitrification and no net N_2O emissions (Price et al. 2004; Speir et al. 1999). In our previous study (Huygens et al. 2007) we compared the rate of DNRA with NO_3^- immobilization and concluded that gaseous N losses via denitrification are presumably low in the studied ecosystem. However, in order to show unambiguously that DNRA can successfully compete with denitrification it is important to compare directly the gross transformation rates of both processes. Our approach relies on the determination of the nitrite (NO_2^-) dynamics in soil. Nitrite is an important intermediate in denitrification and both, heterotrophic and autotrophic nitrification (Müller et al. 2006). The reduction of NO_3^- to NO_2^- is the first step in the denitrification sequence and provides a measure of the potential for denitrification in soil (Betlach and Tiedje 1981).

Inhibition of autotrophic nitrification has been postulated as another key mechanism to prevent N losses from forest ecosystems (Vitousek et al. 1979). Several studies showed that autotrophic nitrification is lower than the heterotrophic pathway in temperate forest soils (Hart et al. 1997; Schimel et al. 1984). In old-growth forests ("climax ecosystems") the inhibition of autotrophic nitrification is caused by the absence of *Nitrobacter*, a bacterial group responsible for the NO_2^- oxidation to NO_3^- (Rice and Pancholy 1972). Furthermore, other processes such as nitrifier-denitrification (Wrage et al. 2001), chemo-denitrification, a non-enzymatic chemical reaction of NO_2^- with organic N (Paul 2007; Sprent 1987), nitrosation, a mechanism of NO_2^- reaction with phenols to form organic N (Azhar et al. 1986; Paul and Clark 1996), self-decomposition to NO_3^- under acidic conditions (Van Cleemput and Baert 1976) and the recently discovered anaerobic NH_4^+ oxidation (anammox) (Mulder et al. 1995; Paul 2007) are also involved in the soil NO_2^- dynamics and in the production of gaseous N components. The arguably most important process for N_2O production in soils under high moisture conditions is NO_3^- reduction via NO_2^- to gaseous N (Russow et al. 2000; Van Cleemput and Samater 1996; Venterea and Rolston 2000). NO_2^- is sometimes considered as an intermediate in biochemical models (Betlach and Tiedje 1981; Cho and Mills 1979) but rarely in ecosystem N cycling models (Burger and Jackson 2004; Schimel and Bennett 2004).

The current "state-of-the-art" technique to quantify simultaneously occurring gross N transformation rates in soils are ^{15}N tracing studies in combination with parameter optimization routines (Mary et al. 1998; Müller et al. 2004, 2007). However, to our knowledge no ^{15}N tracing study so far has considered the functional role of NO_2^- dynamics and related N transformations either in these ecosystems or in any other natural ecosystem. Recent progress in ^{15}N tracing technology allows the simultaneous optimization of large number of parameters which is a

prerequisite to analyze complex models in ^{15}N tracing studies, which include NO_2^- dynamics (Müller et al. 2007; Rütting and Müller 2008). The main advantage of ^{15}N tracing models over the commonly used dilution technique (Stark 2000) is that process-specific N rates such as pool specific mineralization, autotrophic and heterotrophic pathways of nitrification or DNRA can be identified simultaneously (Rütting and Müller 2007).

Here we present results from a ^{15}N tracing study using soil from a pristine, unpolluted *Nothofagus betuloides* forest in southern Chile. The aim of our study was to explore the functional role of DNRA in relation to the NO_2^- dynamics and of NO_2^- reduction processes in this soil. In particular we were interested in "functional links", which we define as metabolic connections in the form of enzymatic chain reactions which are inherent in microbial networks or in single organisms. We hypothesize that DNRA is able to compete successfully with denitrification for the available NO_3^- and that the oxidation of NO_2^- to NO_3^- in the nitrification pathway is inhibited in the old-growth forest.

Materials and methods

Study site

The study site is located at 900 m a.s.l. in the Andean mountains, Antillanca, southern Chile (40°47′ S, 72°12′ W). The local climate is characterized by average annual temperature of 4.5°C and mean annual precipitation around 7,000 mm. The forest vegetation is dominated by evergreen *Nothofagus betuloides* with an average tree age of 325 years (Godoy et al. 2001). Total annual bulk N deposition amounts to 11.8 kg N ha^{-1} in form of dissolved organic nitrogen (8.2 kg N ha^{-1}) and dissolved inorganic nitrogen (3.6 kg N ha^{-1}) (Oyarzún et al. 2004). The initial mineral N concentration measured in the soil samples was 441 μg NH_4^+–N, 5.5 μg NO_3^-–N and 0.05 μg NO_2^-–N g^{-1} soil.

The soil of the forest is classified as Umbric Vitric Andosol (IUSS Working Group WRB 2006), with typical variable charge characteristics (i.e., pH dependent) as a result of their particular clay mineral and organic matter content (Nanzyo et al. 1993; Radcliffe and Gillman 1985). Main soil characteristics are listed in Table 1. More detailed information for the study site can be found in Huygens et al. (2007) for soil characteristics and in Oyarzún et al. (2004) for water and soil chemistry.

Table 1 Main chemical and physical soil properties of the unpolluted south Chilean *Nothofagus betuloides* mineral soil A horizon (0–10 cm)

Bulk density (g cm^{-3})	0.52
pH	5.0
Total carbon (g kg^{-1})	134
Total nitrogen (g kg^{-1})	6.8
Fe-ox (g kg^{-1})[a]	10.8
Textural composition (%)	
Sand (0.02–2 mm)	71
Silt (2–20 μm)	23
Clay (<2 μm)	6
CEC_T (cmol kg^{-1})[b]	5.7

[a] Ammonium oxalate extractable Fe

[b] Cation exchange capacity determined via $Ca^{2+} + Al^{3+}$ adsorption

^{15}N tracing experiment

This study was carried out in conjunction with the study by Huygens et al. (2007). To analyze the dynamics of NO_2^- we considered three additional ^{15}N labeling treatments, where ^{15}N enriched NO_2^- was added (see below). Furthermore the concentrations and ^{15}N excess of NO_2^- were analyzed in all ^{15}N labeling treatments.

Soil samples were taken in autumn 2004 following a stratified random procedure. In a representative forest area (25 × 20 m), 10 grids (5 × 5) were established. From each grid, three subsamples (a, b and c) were taken from the mineral A horizon (0–10 cm). The ten subsamples a, b, and c were compiled to obtain three replicates (subsamples composited per replicate). The soil was sieved (<2 mm) and dried to gravimetric water content (GWC) of about 30%, and stored for two months at 5°C. One week prior to ^{15}N additions, the soils were pre-incubated at a water-filled-pore-space (WFPS) of 45% (GWC = 69.6% on average).

There were in total six different ^{15}N treatments (Table 2), each with three replicates, of which either NH_4^+, NO_2^-, NO_3^- or a combination of the various moieties were labeled with ^{15}N at 98 atom% excess.

Table 2 Labeling treatments in the ^{15}N tracing study with soil from a pristine south Chilean *Nothofagus* forest

Treatment	NH_4^+	NO_2^-	NO_3^-
1	**^{15}N**	**^{15}N**	^{14}N
2	^{14}N	**^{15}N**	^{14}N
3	^{14}N	**^{15}N**	**^{15}N**
4	**^{15}N**	^{14}N	^{14}N
5	^{14}N	^{14}N	**^{15}N**
6	**^{15}N**	^{14}N	**^{15}N**

Nitrogen was applied at a rate of 50 μg NH_4Cl–N g^{-1}, 5 μg $NaNO_2$–N g^{-1} and 5 μg KNO_3–N g^{-1} dry soil in 8 ml solutions. The analysis of ^{15}N enrichment experiments is based on the assumption of a homogenous mixing of the isotopes (^{14}N and ^{15}N) in soil (Kirkham and Bartholomew 1954). Therefore, we thoroughly mixed the soil after N application, which provides a sufficient mixing (Luxhøi et al. 2003). The bulk densities were adjusted to field values (i.e., 0.51 g cm^{-3}, resulting in 50% WFPS). Temperature (15°C) and moisture content were kept constant during the entire experiment. Soil was extracted 0.3, 2, 4, 7 and 12 days after N application with 180 ml 2 M KCl solution, shaken for 120 min.

NH_4^+ in the extract was determined colorimetrically by the salycilate-nitroprusside method (Mulvaney 1996) on an auto-analyzer (AA3, Brann and Luebbe, Germany). Using the same auto-analyzer, NO_2^- was determined colorimetrically after a reaction with *N*-1-napthylethylenediamine to produce a chromophore. NO_3^- was determined, as NO_2^-, after on-line conversion in a Cd–Cu reductor. The NO_3^- results were corrected for NO_2^- present in the soil samples. The ^{15}N contents of NH_4^+, NO_2^- and NO_3^- were analyzed after conversion to N_2O (Hauck 1982; Saghir et al. 1993; Stevens and Laughlin 1994) on a trace gas preparation unit (ANCA-TGII, PDZ Europa, UK), coupled to an Isotope Ratio Mass Spectrometer (IRMS) (20-20, SerCon, UK).

Soil organic matter fractionation

Using a modified method of Meijboom et al. (1995), we isolated five physical soil organic matter (SOM) fractions from the previously extracted soil (at each extraction time). The 2 mm sieved soil was wet-sieved over a set of three sieves (250, 150 and 50 μm). The 50–150 μm size fraction was collected and dried. The size fraction <50 μm was collected in buckets and determined after one day/night of sedimentation. The soil material on the top sieves (>150 μm) was washed into buckets and swirled with a jet of deionized water. The mineral fraction was collected from the bottom of the bucket, whereas macro-organic matter (MOM) was collected in the water level. All fractions were dried for 48 h at 45°C, and ground with a planetary ball mill (PM400, Retsch, Germany) for total N and ^{15}N analysis with an elemental analyzer (ANCA-SL, PDZ Europa, UK), coupled to an IRMS (20-20, SerCon, UK). For the ^{15}N tracing analysis two conceptual SOM pools, a labile SOM (N_{lab}) and a more recalcitrant SOM (N_{rec}) pool, were compiled (Huygens et al. 2007). As indicated by Hassink (1995) the macro-organic matter (MOM) fraction is an active, microbial available N pool, which we used to characterize the N_{lab} pool in the ^{15}N tracing model (Fig. 1). The stable fraction of the SOM was compiled from the mineral fraction 150–2,000 μm and the size fractions 150–50 μm and <50 μm, all of them offering physical SOM protection (Huygens et al. 2005). The N_{rec} pool in the model is considered not to be an inert N pool, but is more resistant to N mineralization relative to the labile fraction. Both SOM pools contain organic N compounds and its associated microbial biomass N. The potential to use physically isolated SOM fractions as functional pools in modeling approaches has been demonstrated previously (Elliott et al. 1996; Skjemstad et al. 2004; Smith et al. 2002; Zimmermann et al. 2006).

^{15}N tracing model

To quantify the simultaneously occurring gross N transformation rates we used the ^{15}N tracing analysis tool by Müller et al. (2007). The ^{15}N tracing model (Fig. 1) is based on the model by Müller et al. (2004). We added the modifications by Huygens et al. (2007), consisting of a second NH_4^+ immobilization and adsorption-release dynamics between free and adsorbed NH_4^+, as well as a sub-model for the NO_2^- dynamics (Müller et al. 2006; Rütting and Müller 2008). Similar to previous studies (e.g., Müller et al. 2004), NO_3^- turnover fluxes are only linked to the more recalcitrant organic N pool (N_{rec}) in the model set-up. This can be explained by the fact that

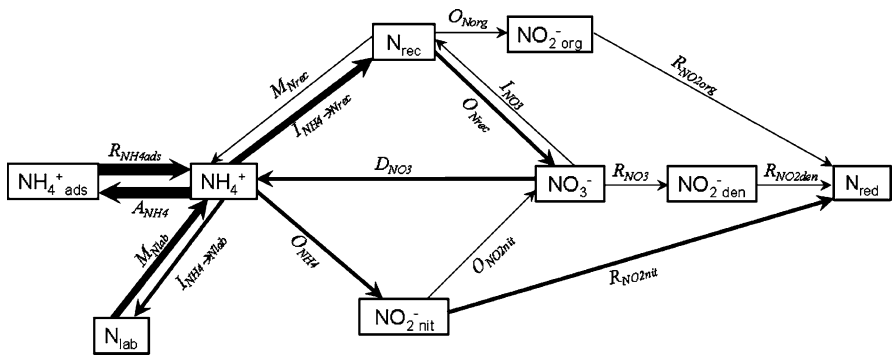

Fig. 1 Conceptual ^{15}N tracing model to analyze gross N transformations (N_{lab} = labile soil organic N, N_{rec} = recalcitrant soil organic N, NH_4^+ = ammonium, NO_3^- = nitrate, $NH_4^+{}_{ads}$ = adsorbed NH_4^+, NO_2^- = nitrite sub-pools, N_{red} = reduced NO_2^-). The thicknesses of the *arrows* represent the relative importance of each transformation (for further explanation of N transformations and parameter values see Table 3)

mineralization-immobilization turnover of NO_3^- is dominated by fungi, which prefer NO_3^- as their N source (Marzluf 1997) and recalcitrant organic compounds as energy source (Paul 2007). A description of the N transformations and the kinetic settings are presented in Table 3. The NO_2^- sub-model considered three separate process-specific NO_2^- pools, i.e., $NO_2^-{}_{nit}$ (intermediate in the oxidation of NH_4^+ to NO_3^-), $NO_2^-{}_{den}$ (intermediate in the reduction of NO_3^- to gaseous N) and $NO_2^-{}_{org}$ (derived from the oxidation of organic N). The first two processes are expected to be the main sources of NO_2^- in soils (Russow et al. 2000; Sprent 1987). However, in a previous study it was shown that NO_2^- produced from organic N is an important NO_2^- pool in grassland soil (Müller et al. 2006; Rütting and Müller 2008). This process was included in the sub-model because a range of micro-organisms are able to oxidize various organic N forms to NO_2^- (Doxtader and Alexander 1966; Sprent 1987). The reduction of all three NO_2^- sub-pools is considered to result in the formation of N_{red}, which is a theoretical pool in the model combining gaseous N species (e.g., N_2O, NO, NO_2, and N_2) as well as other NO_2^- consuming processes such as organic N produced via fixation of NO_2^- (Azhar et al. 1986; Smith and Chalk 1979). The final model used for data analysis contains nine N pools and 16 N transformations (Fig. 1). During this study several modifications of the ^{15}N tracing model were tested. These modifications include DNRA pathway via $NO_2^-{}_{den}$ pool and heterotrophic nitrification via $NO_2^-{}_{org}$ pool. Previously we also tested a version where $NO_2^-{}_{org}$ was simultaneously produced from N_{rec} and N_{lab} (Rütting and Müller 2008). However, all tested modification did not result in a better fit between model and experimental data (data not shown) and were therefore not considered for final data analysis. The ^{15}N tracing analysis tool calculates gross N transformation rates by simultaneously optimizing the model parameters of the various N transformations with a Markov chain Monte Carlo (MCMC) sampling algorithm (Müller et al. 2007) which is useful to analyze data with complex models that contain large number of parameters (Rütting and Müller 2007, 2008). The optimization of the model parameters is guided by the minimization of the difference between modeled and measured data, i.e., minimizing a misfit function f (**m**) in form of a quadratic weighted error (Müller et al. 2007).

Statistical analysis

The data were supplied to the ^{15}N tracing model as averages ± standard deviations of the experimental measurements (three replicates). The optimization procedure results in a probability density function (PDF) for each model parameter, which are significantly different from zero. From the PDFs, average values and standard deviations for the transformation parameters are calculated (Müller et al. 2007). Furthermore, parameters which are actually zero can be identified and consequently excluded from the model (Müller et al. 2007). Therefore the selection of N transformations and parameters is not arbitrary but based on analysis results of several model runs. For N transformations following first-order kinetics,

Table 3 Description of model parameters and optimized values (mean and standard deviation), using a Markov chain Monte Carlo (MCMC) method, and average N transformation rates (mean and standard deviation) for a pristine south Chilean *Nothofagus* forest on an Andisol

Transformation	Description	Kinetics (units)[a]	Parameter value		N rate (μg g^{-1} d^{-1})	
			Mean	SD	Mean	SD
$I_{NH_4 \rightarrow Nlab}$	Immobilization of NH_4^+ to N_{lab}	0 (μmol g^{-1} h^{-1})	0.00157	0.00002	0.528	0.007
$I_{NH_4 \rightarrow Nrec}$	Immobilization of NH_4^+ to N_{rec}	0 (μmol g^{-1} h^{-1})	0.00797	0.00024	2.681	0.082
I_{NO_3}	Immobilization of NO_3^- to N_{rec}	0 (μmol g^{-1} h^{-1})	0.00002	0.00000	0.006	0.001
M_{Nrec}	Mineralization of N_{rec} to NH_4^+	0 (μmol g^{-1} h^{-1})	0.00012	0.00006	0.039	0.021
M_{Nlab}	Mineralization of N_{lab} to NH_4^+	1 (h^{-1})	0.00018	0.00004	3.690	0.828
O_{NH_4}	Oxidation of NH_4^+ to ${NO_2^-}_{nit}$	1 (h^{-1})	0.00005	0.00000	0.665	0.023
O_{Nrec}	Oxidation of N_{rec} to NO_3^-	0 (μmol g^{-1} h^{-1})	0.00127	0.00005	0.428	0.016
D_{NO_3}	Dissimilatory NO_3^- reduction to NH_4^+	0 (μmol g^{-1} h^{-1})	0.00106	0.00005	0.355	0.016
A_{NH_4}	Adsorption of NH_4^+ to ${NH_4^+}_{ads}$	1 (h^{-1})	0.00328	0.00020	40.285	2.453
R_{NH_4ads}	Release of adsorbed NH_4^+ to NH_4^+	1 (h^{-1})	0.00463	0.00032	42.674	2.987
O_{NO_2nit}	Oxidation of ${NO_2^-}_{nit}$ to NO_3^-	1 (h^{-1})	0.00282	0.00042	5.5E−05	8.3E−06
O_{Norg}	Oxidation of N_{rec} to ${NO_2^-}_{org}$	1 (h^{-1})	3.7E−07	7.8E−09	0.044	0.001
R_{NO_3}	Reduction of NO_3^- to ${NO_2^-}_{den}$	1 (h^{-1})	7.7E−08	2.4E−08	1.6E−05	4.8E−06
R_{NO_2nit}	Reduction of ${NO_2^-}_{nit}$ to N_{red}	1 (h^{-1})	34.76489	0.36870	0.677	0.007
R_{NO_2den}	Reduction of ${NO_2^-}_{den}$ to N_{red}	1 (h^{-1})	0.04728	0.01604	1.9E−05	6.5E−06
R_{NO_2org}	Reduction of ${NO_2^-}_{org}$ to N_{red}	1 (h^{-1})	0.06168	0.00079	0.044	0.001

All parameter values are significant different from zero

[a] Kinetics: 0 = zero order, 1 = first order

average rates were calculated by integrating the gross N rates over the experimental period divided by the total time (Rütting and Müller 2007).

In addition a correlation matrix was calculated to find groups of N transformations that tend to be constrained together and thereby identify processes that are closely linked. Transformations with correlation coefficients $r \geq |0.8|$ were considered to be strongly correlated whereas $|0.8| > r \geq |0.5|$ indicate medium correlations (Fahrmeir et al. 2004).

Presentation of results and model set-up

The experimental values are presented as arithmetic means ± one standard deviation. The MCMC algorithm is programmed in the software MatLab (Version 7.3, The MathWorks Inc.) and calls in each iteration the ^{15}N tracing model which is separately set up in Simulink (Version 6.5, The MathWorks Inc.), a companion software to MatLab. All six different ^{15}N treatments (Table 2) were analyzed in one optimization run. The initial pool sizes and the ^{15}N contents of the different model pools were obtained by extrapolating the data for 0.3 and 2 days back to time point zero (Müller et al. 2004).

Results

N pool sizes and ^{15}N enrichments

The measured and modeled values of the soil N concentrations and their ^{15}N enrichments are presented in Fig. 2 for mineral N forms and in Fig. 3 for the two organic N fractions.

During the experimental period the different N pools remained more or less constant in size, indicating low net N transformation rates. At the first extraction (8 h) the measured NO_2^- concentration was very low (0.027 μg N g^{-1} soil on average) in comparison to the added amount. A fast decline in the $^{15}NO_2^-$ enrichments after labeling this pool with ^{15}N, suggests a fast inflow of unlabelled N and a high turnover of NO_2^- (Fig. 2b, e). Furthermore, labeling only NO_2^- did not result in a significant ^{15}N enrichment of the measured NH_4^+ pool in contrast

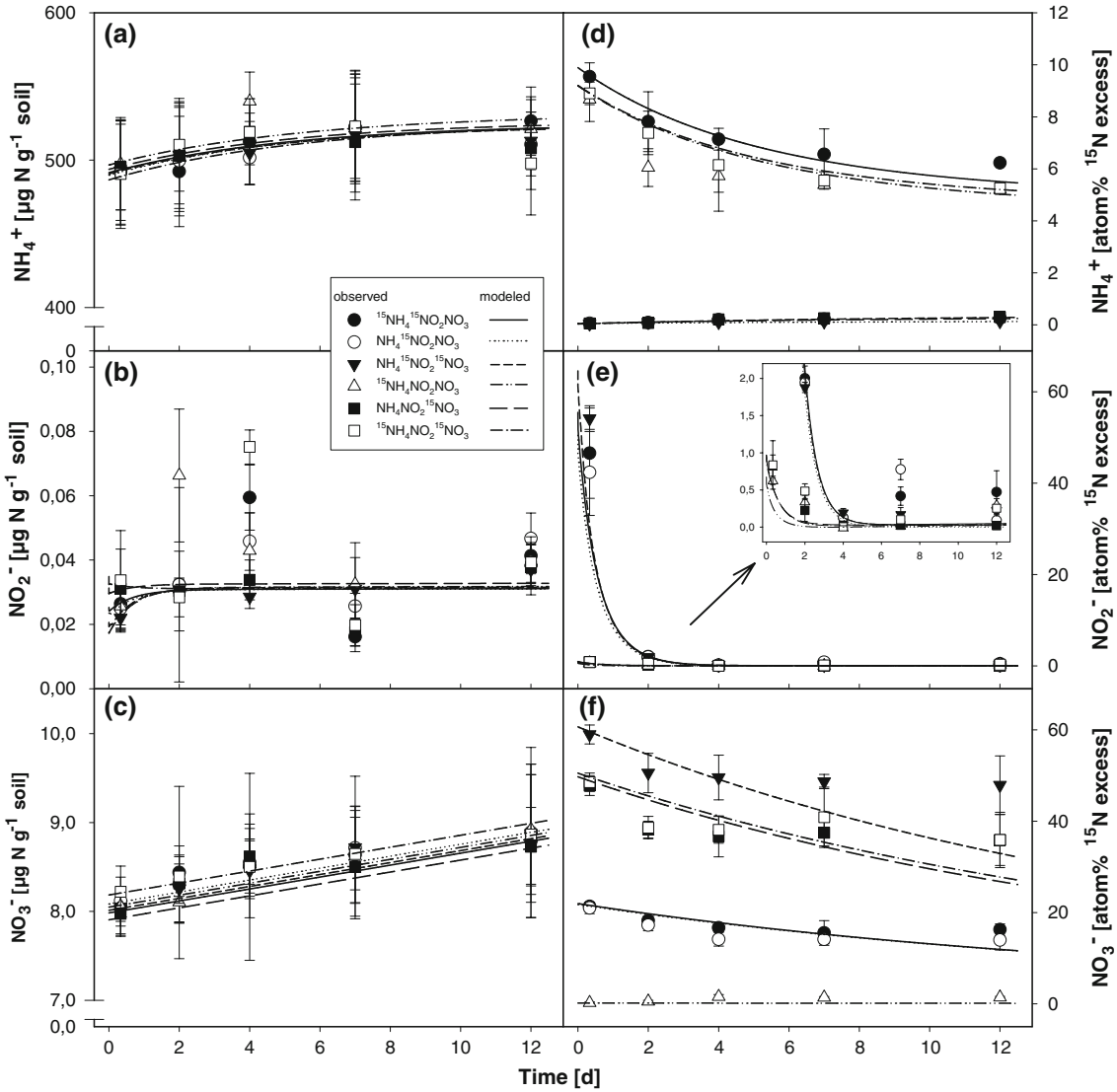

Fig. 2 Measured and modeled concentrations and ^{15}N enrichments of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) of an Andisol after application of 60 µg N g^{-1} soil as $NH_4/NO_2/NO_3$ at 98 atom% ^{15}N excess (*symbols* represent average measured values ± one standard deviation; *lines* represent modeled values)

to NO_3^-, which was labeled rapidly (Fig. 2d, f). This indicates a fast initial transformation of NO_2^- to NO_3^- in this soil. At the same time both measured fractions of SOM were slightly enriched in ^{15}N (Fig. 3c, d) which points toward a fast incorporation of NO_2^- into organic material.

The aim of the optimization algorithm is to minimize a cost function which takes into account the actual standard deviations of the observed values. Depending on the standard deviations the algorithm may not hit exactly the observed average values. In general the ^{15}N tracing model (Fig. 1) was able to reproduce the measured N dynamics in the Andisol from a *Nothofagus* forest, as indicated by a close fit between the model and the experimental data (Figs. 2, 3). Only small deviations were observed for the NO_3^- and NO_2^- concentrations (Fig. 2b, c). However, considering the high number of measured variables (5 pools, each concentration and ^{15}N excess) and the high variability of NO_2^- concentrations in this soil the fit is satisfactory and generally within the uncertainty range of the measured data.

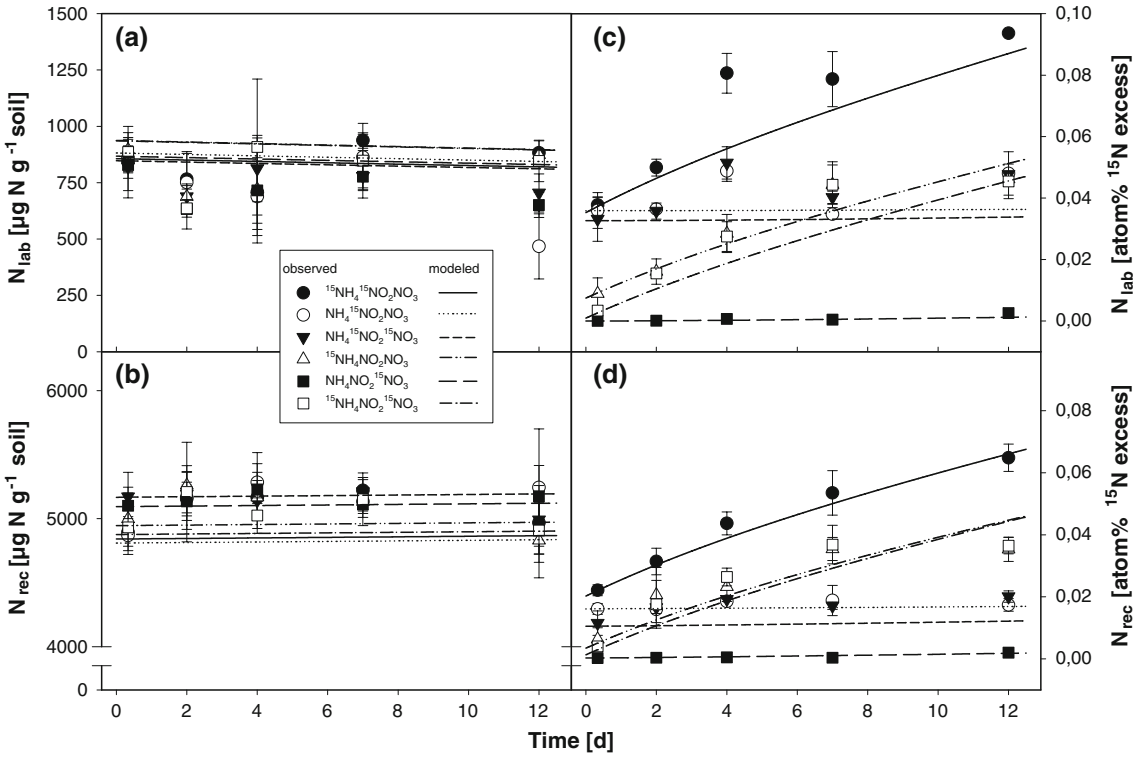

Fig. 3 Measured and modeled concentrations and ^{15}N enrichments of two organic N pools (N_{lab} and N_{rec}) of an Andisol after application of 60 µg N g^{-1} soil as $NH_4/NO_2/NO_3$ at 98 atom% ^{15}N excess (*symbols* represent average measured values ± one standard deviation; *lines* represent modeled values)

Gross N transformation rates

The highest N transformation rates in the Andisol were related to the exchange between free and adsorbed ammonium (NH_4^+ and $NH_4^+{}_{ads}$ pool) (Table 3; Fig. 1). This exchange reaction was responsible for 90% of the NH_4^+ dynamics. The average total NO_2^- production amounted to 0.71 µg N g^{-1} soil day^{-1}, which was dominated by NH_4^+ oxidation (O_{NH_4}) (Table 3). This pathway contributed 93.7% to the overall NO_2^- production, while the contribution of denitrification (R_{NO_3}) was nearly zero in this soil (contribution < 0.1%). In addition, about 6.3% of the total NO_2^- production was related to the oxidation of organic N to NO_2^- (Table 3). The estimated gross production of N_{red} amounted on average to 0.72 µg N g^{-1} soil day^{-1}, of which 93.9% was related to the nitrification pathway (R_{NO_2nit}). After a peak N_{red} production (i.e., reduction of NO_2^-) at $t = 0$, the subsequent production was more or less constant (Fig. 4). Besides NO_2^- reduction, the oxidation of $NO_2^-{}_{nit}$ to NO_3^- was the only other NO_2^- consuming process amounting however to less than 0.1% of total NO_2^- consumption over the experimental period. Nitrate was almost exclusively produced (>99.9%) by oxidation of organic N to NO_3^- (Table 3). The consumption of NO_3^- was dominated by the process of DNRA (D_{NO_3}), which was responsible for 98.4% of the total NO_3^- consumption (Table 3; Fig. 1). The other two NO_3^- consuming processes, immobilization (I_{NO_3}) and NO_3^- reduction to $NO_2^-{}_{den}$ (R_{NO_3}) amounted only to 1.6 and <0.1% of the total NO_3^- consumption, respectively.

Correlations among N transformation rates

In one sixth of all cases a significant correlation between two model parameters was observed (Table 4). The tightest correlation was found between the production and reduction of $NO_2^-{}_{den}$ (R_{NO_3} and R_{NO_2den}; $R = 0.87$). In the nitrification pathway, the production of $NO_2^-{}_{nit}$ (O_{NH_4}) was positively correlated with the reduction (R_{NO_2nit}; $R = 0.66$) but not

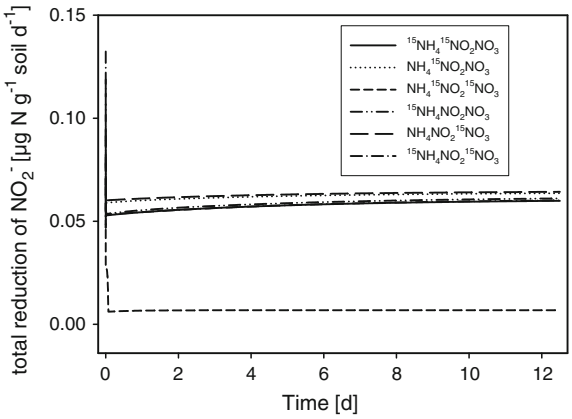

Fig. 4 Total NO_2^- reduction rates ($R_{NO_2} = R_{NO_2org} + R_{NO_2nit} + R_{NO_2den}$) to N_{red} of an Andisol after application of 60 µg N g^{-1} soil as $NH_4/NO_2/NO_3$ at 98 atom% ^{15}N excess

with the oxidation of $NO_2^-{}_{nit}$ to NO_3^- (O_{NO_2nit}). On the other hand, no correlation existed between the production and consumption of $NO_2^-{}_{org}$ (Table 4). Another significant correlation was observed between the processes related to the dynamics between NH_4^+ and NH_4^+ exchange sites (A_{NH_4} and R_{NH_4ads}; $R = 0.80$) and between oxidation of recalcitrant organic N and DNRA (O_{Nrec} and D_{NO_3}; $R = 0.78$). Furthermore, both DNRA and O_{Nrec} were negatively correlated with the reduction of NO_3^- (R_{NO_3}) and NO_2^- (R_{NO_2den}) during denitrification. In contrast, the immobilization of NO_3^- (I_{NO_3}) was positively correlated with these two transformations (Table 4).

Discussion

Turnover of NH_4^+

The measured NH_4^+ concentrations ($\sim$500 µg N g^{-1} soil; Fig. 2a) are at the upper end of NH_4^+ concentrations that can be found in natural soils (Booth et al. 2005) and is possibly caused by the storage of the soil samples. Similar NH_4^+ concentrations are reported for other forest soils (Fitzhugh et al. 2003a, b; Hackl et al. 2004). However, there is no evidence that the high NH_4^+ concentrations affected the gross N transformation rates in this Andisol. In particular the rate of NH_4^+ oxidation (O_{NH_4}), which reacts very sensitively to NH_4^+ concentrations (Shaviv 1988), remained low and characteristic for background rather than elevated NH_4^+ concentrations (Table 3).

The total NH_4^+ production was similar to the value for an old-growth south Chilean forest on Cambisol (Perakis and Hedin 2001). Approximately 90% of the total N flow in the soil was related to the adsorption—release dynamics of NH_4^+ with exchange sites ($NH_4^+{}_{ads}$) (Table 3). The NH_4^+ adsorption removed N from the dissolved pool but kept it in a potentially available form (Tamm 1991), providing a highly effective mechanism for buffering excess NH_4^+ and has the advantage that stored NH_4^+ can become available on demand.

Low N mineralization rates have been hypothesized as a mechanism to prevent N losses from N-limited forest ecosystems (Vitousek et al. 1979). The total gross N mineralization rate ($M_{Nlab} + M_{N\text{-}rec}$) in the current study (3.7 µg N g^{-1} day^{-1}; Table 3) is lower than observed previously for the same soil (Huygens et al. 2007) but is in line with the general finding that gross N mineralization in pristine southern hemisphere forests are below 10 µg N g^{-1} day^{-1} (Booth et al. 2005; Parfitt et al. 2002). Dissimilatory NO_3^- reduction to NH_4^+ (D_{NO_3}) was responsible for almost 9% of total microbial NH_4^+ production (excluding the adsorption-release characteristic of this soil). The supply of NH_4^+ via this pathway was about ten times higher than direct mineralization of recalcitrant soil organic matter (Table 3). This shows that NH_4^+ production may not necessarily follow the classical pathway via direct mineralization of SOM. Nitrogen cycling paradigms such as the ones postulated by Schimel and Bennett (2004) should also consider the NH_4^+ production pathway via organic N oxidation to NO_3^- and subsequent reduction to NH_4^+.

The difference in total gross mineralization between the present study and our previous analysis (Huygens et al. 2007) highlights the importance of NO_2^- dynamics on the estimation of simultaneously occurring soil N transformations, even those not directly related to NO_2^- dynamics. Similar observations have been made in permanent grassland soils (Müller et al. 2007; Rütting and Müller 2008). This shows that the consideration of NO_2^- data in ^{15}N tracing models constrains the search for parameter constellations and thus allows a more precise quantification of gross rates for all transformations of the N cycle.

Table 4 Correlation matrix for all N transformations estimated with a Markov chain Monte Carlo (MCMC) method

	R_{NO_2org}	R_{NO_2den}	R_{NO_2nit}	R_{NO_3}	O_{Norg}	O_{NO_2nit}	R_{NH_4ads}	A_{NH_4}	D_{NO_3}	O_{Nrec}	O_{NH_4}	M_{Nlab}	M_{Nrec}	I_{NO_3}	$I_{NH_4\rightarrow Nrec}$
$I_{NH_4\rightarrow Nlab}$	−0.05	0.28	0.01	0.25	0.01	−0.38	**0.64**	**0.58**	−0.20	−0.17	−0.07	−0.28	−0.05	0.31	−0.04
$I_{NH_4\rightarrow Nrec}$	0.17	−0.14	0.03	−0.12	−0.17	−0.04	−0.08	−0.00	−0.10	−0.15	0.11	0.22	−0.09	−0.22	
I_{NO_3}	−0.28	**0.77**	0.02	**0.60**	0.16	−0.28	**0.64**	0.40	−0.32	−0.31	0.03	**−0.78**	−0.21		
M_{Nrec}	−0.08	−0.44	−0.04	−0.14	0.21	0.10	0.04	−0.18	0.21	0.27	−0.06	−0.31			
M_{Nlab}	0.28	**−0.60**	0.04	**−0.63**	−0.22	0.33	**−0.65**	−0.30	0.30	0.26	0.04				
O_{NH_4}	−0.15	0.11	**0.66**	0.13	0.16	−0.03	−0.08	−0.04	−0.04	−0.06					
O_{Nrec}	0.07	**−0.59**	−0.03	**−0.61**	−0.06	0.27	−0.25	−0.17	**0.78**						
D_{NO_3}	0.09	**−0.59**	−0.01	**−0.64**	−0.07	0.30	−0.25	−0.17							
A_{NH_4}	0.03	0.34	0.02	0.26	−0.01	**−0.51**	**0.80**								
R_{NH_4ads}	−0.14	**0.51**	−0.00	0.49	0.08	*−0.52*									
O_{NO_2nit}	0.08	−0.47	−0.03	−0.36	−0.04										
O_{Norg}	−0.45	0.14	0.08	0.19											
R_{NO_3}	−0.20	**0.87**	0.06												
R_{NO_2nit}	−0.08	0.05													
R_{NO_2den}	−0.24														

Medium and strong correlations ($r > |0.5|$) are bold

Vitousek et al. (1979) postulated inhibition of NH_4^+ oxidation as an effective mechanism to avoid N losses from ecosystems. Our analysis showed that O_{NH_4} was only responsible for a small amount of NH_4^+ consumption (1.5%; Table 3) which supports previous results on forest soils (Hart et al. 1997; Schimel et al. 1984). Moreover, immobilization of NH_4^+ into N_{rec} was six-times higher than into the N_{lab} pool (Table 3), which may provide an effective N sequestration mechanism and may support long-term ecosystem productivity (Pepper et al. 2007).

Production and consumption of NO_3^-

The studied Andisol is characterized by lower NO_3^- than NH_4^+ concentrations (Fig. 2) indicating inherently a closed N cycle (Davidson et al. 2000; Pérez et al. 1998). Moreover, the observed NO_3^- turnover was lower than the NH_4^+ turnover (Table 3; Fig. 1), which contributes to a better N retention (Doff Sotta et al. 2008). In the ^{15}N tracing model three microbial consumption processes for NO_3^-, namely immobilization into N_{rec}, denitrification and DNRA, were considered (Fig. 1).

Similar to our previous study (Huygens et al. 2007), DNRA (D_{NO_3}) was the most important NO_3^- consumption process and the main mechanism for NO_3^- conversion to NH_4^+ showing the high potential for DNRA in the Andisol. The calculated DNRA rates amounts 0.36 ± 0.02 μg g^{-1} day^{-1}, similar to values documented by Silver et al. (2001) (0.6 μg N g^{-1} day^{-1}) in tropical forest soils. DNRA has also been documented in several other forest ecosystems in different climates (Bengtsson and Bergwall 2000; Doff Sotta et al. 2008; Pett-Ridge et al. 2006; Silver et al. 2003). In contrast to DNRA, denitrification (R_{NO_3}) was negligible in this Andisol (Table 3). This indicates that DNRA out-competes denitrification (Pett-Ridge et al. 2006; Silver et al. 2001, 2005) and at the same time is a key process for NO_3^- retention. The most obvious advantage of DNRA over other NO_3^- consuming processes is, that N is transferred into NH_4^+, another plant available N form which is not prone to N losses under acidic conditions and therefore leads to conservation of mineral N in soils (Nijburg and Laanbroek 1997). It should be noted that our laboratory experiment was performed at a WFPS of 50%, which is lower than the favorable conditions for denitrification (Linn and Doran 1984). Therefore, our experiment might underestimate the in situ denitrification rate, where high annual precipitation gives rise to large anoxic micro-sites. DNRA might likewise be underestimated in the present laboratory study. This is supported by recent results from an in-filed study in the same forest soil where almost three times higher DNRA rates were observed

(1.0 ± 0.2 μg N g^{-1} day^{-1}) (Huygens et al. 2008). As proposed by Burger and Jackson (2004), a rapid conversion of NO_3^- to NH_4^+ via NO_3^- immobilization and subsequent remineralization can be an alternative pathway for NO_3^- reduction to ammonium. Quick immobilization—remineralization might occur in a small SOM sub-pools and thus could be masked by larger pools (Piñeiro et al. 2006). However, as none of the five different SOM fractions we analyzed had higher ^{15}N excess than NH_4^+ (data not shown), we expect this pathway to be of limited importance in this soil. Silver et al. (2001, 2005) came to similar conclusions for an upland tropical forest which is characterized by high annual precipitation.

Less than 1% of the NO_3^- production was derived from oxidation of NH_4^+ via the $NO_2^-{}_{nit}$ pool while oxidation of organic N to NO_3^- (i.e., heterotrophic nitrification) was the dominant NO_3^- production pathway (Fig. 1), which is in line with several ^{15}N labeling studies on acidic forest soils (Burton et al. 2007; Grenon et al. 2004; Pedersen et al. 1999; Schimel et al. 1984; Zeller et al. 2007). Heterotrophic nitrification is predominantly carried out by fungi (Landi et al. 1993) which gain energy from compounds that belong to more recalcitrant organic N pools in soil (Paul 2007). Therefore, NO_3^- production via organic N oxidation is often carried out by acid tolerant fungi in forest soils (Eylar and Schmidt 1959; Stroo et al. 1986).

Our analysis shows a significant correlation between the gross rates of O_{Nrec} and D_{NO_3} ($R = 0.78$; Table 4). This further supports a functional link between oxidation of recalcitrant organic N to NO_3^- (i.e., heterotrophic nitrification) and subsequent reduction via DNRA to NH_4^+. This means that established ecosystems are characterized by microbial communities with energetically favorable pathways for NH_4^+ production and pathways that prevent N losses via leaching and/or gaseous N production. To our knowledge no other study so far had reported a similar functional link between heterotrophic NO_3^- production and DNRA.

Dynamics of NO_2^-

In the current study we present a detailed process-based analysis of NO_2^- dynamics in a natural ecosystem. $^{15}NO_2^-$ has previously been used to investigate the fate of NO_2^- in soils (Burns et al. 1995; Fitzhugh et al. 2003a, b; Nelson and Bremner 1969) including production of gaseous N (Russow et al. 2000; Venterea 2007). However, to our knowledge no study so far has performed a detailed tracing experiment to quantify the various gross N transformations related to NO_2^- production and consumption in soil. The ^{15}N tracing model (Fig. 1) developed in the present study separates total NO_2^- into process-specific sub-pools (Müller et al. 2006) which are most likely associated with different soil micro-sites (Van Cleemput and Samater 1996). Despite this additional complexity, the model was able to reproduce the measured concentration and ^{15}N enrichment of NO_2^- appropriately (Fig. 2b, e). We increased the NO_2^- pool by 90 times its background concentration (addition of 5 μg N g^{-1} soil to 0.055 μg NO_2^-–N g^{-1} soil) to demonstrate the potential for NO_2^- production and consumption in this soil. This increase is similar to other experiments (Fitzhugh et al. 2003a). However, the measured NO_2^- concentrations at the first soil sampling (8 h after labeling) were in the range of background concentrations. Similar observations have been made by Islam et al. (2008) in acidic soils, where significant NO_2^- losses occurred directly after additions of NO_2^-. Possible mechanisms, which are responsible for this phenomenon, are chemical fixation of NO_2^- by SOM or self-decomposition to gaseous N forms (Fitzhugh et al. 2003b; Islam et al. 2008). The latter pathway may has been responsible for the large amount of NO_2^- that was reduced to the theoretical N_{red} pool shortly after N application (Fig. 4). Furthermore, the large amounts of extractable Fe (Table 1) in the Andisol may also have promoted NO_2^- self-decomposition to NO_3^- immediately after N addition (Van Cleemput and Baert 1984; Van Cleemput and Samater 1996). Evidence for this process comes from ^{15}N enrichment of NO_3^- 8 h after NO_2^- labeling at the first extraction (Fig. 2f). As we made no extraction prior to 8 h, we cannot clarify the exact mechanisms for this rapid conversion of NO_2^- to NO_3^-. The suggested rapid abiotic transformations of NO_2^- to NO_3^- and SOM are taken care of by the model set-up, as the initial model pools are interpolated from the first two measurements (see above). The effect of NO_2^- additions on potential stimulation of microbial activity is still unclear (Fitzhugh et al. 2003a). However, we did not find any evidence that the NO_2^- application caused an

unusual behavior of the N transformations in the system during the rest of the experimental period where NO_2^- concentrations were at background levels (Fig. 2b).

Production of NO_2^-

The fast dilution of applied $^{15}NO_2^-$ indicated a rapid production of unlabeled NO_2^- entering the overall NO_2^- pool (Fig. 2e). Almost 94% of the NO_2^- produced during the 12 day experimental period was related to the process of NH_4^+ oxidation, which is in line with previous findings that nitrification is the dominant NO_2^- producing process in a variety of soils (Burns et al. 1996; Russow et al. 2000). As only a small amount of this produced NO_2^- is further oxidized to NO_3^- (Table 3) it seems possible that *Nitroso-* but not *Nitro*-Bacteria are active in this Andisol. Support for this hypotheses comes from investigations in old-growth forests, where autotrophic nitrification was inhibited by the absence of *Nitrobacter* (Rice and Pancholy 1972). Furthermore, Archaea could also be partly responsible for NH_4^+ oxidization in this ecosystem (Leininger et al. 2006). Denitrification had, in contrast to other findings (Burns et al. 1996; Russow et al. 2000), only a negligible contribution to the total NO_2^- production in this Andisol (Table 3) which is in line with findings by Smith et al. (1997). Instead, oxidation of organic N to NO_2^- was responsible for more than 6% of the total NO_2^- production. This organic pathway of NO_2^- production has been documented before (Doxtader and Alexander 1966) but usually ignored in studies investigating the soil NO_2^- dynamics. However, recent findings indicate that this organic NO_2^- production process is likely to be more important than previously believed (Rütting and Müller 2008). The Andisol soil is iron-rich (Table 1) and therefore NO_2^- production could also have been catalyzed by Fe-ions as proposed by the "ferrous wheel hypothesis" (Davidson et al. 2003, 2008). Any NO_2^- production via this process is derived from abiotic reduction of NO_3^- and therefore would have been part of the NO_3^- reduction rate (R_{NO_3}), which may therefore be a combined rate of biotic (denitrification) and abiotic ("ferrous wheel hypothesis") reactions. Since R_{NO_3} is only a tiny rate in this Andisol we can exclude both processes from having a major contribution in this study.

Consumption of NO_2^-

The highest consumption of NO_2^- in our study was related to the reduction of nitrification-related NO_2^- ($NO_2^-{}_{nit}$) to N_{red} (Table 3). Moreover, our results indicate that a functional link exists between NH_4^+ oxidation and $NO_2^-{}_{nit}$ reduction ($R = 0.66$; Table 4) rather than oxidation to NO_3^-. The kinetic parameters for $NO_2^-{}_{nit}$ reduction (R_{NO_2nit}) and $NO_2^-{}_{nit}$ oxidation (O_{NO_2nit}) differed by four orders of magnitude (i.e., 34.8 and 0.003 h^{-1}, respectively, Table 3) indicating that R_{NO_2nit} will easily out-compete O_{NO_2nit} for the available NO_2^-. The exact nature of R_{NO_2nit} could not be identified within the scope of this study. However, processes such as nitrifier-denitrification (Wrage et al. 2001), nitrosation with phenolic compounds (Azhar et al. 1986), chemo-denitrification (Chalk and Smith 1983) and the anaerobic ammonium oxidation (anammox) (Mulder et al. 1995) could all be partly responsible. More detailed studies regarding the function of phenols and detailed analyses of process-based gaseous N dynamics in this soil are required to unravel the N transformations related to the production of N_{red}. The rapid NO_3^- enriched with ^{15}N after addition of ^{15}N labeled NO_2^- (Fig. 2f) cannot be explained by the low rate of O_{NO_2nit}. Perhaps, more than one transformation process is responsible for O_{NO_2nit} whereas one may be a fast abiotic (i.e., self-decomposition) and the second a slower biotic (i.e., autotrophic nitrification) process. Similar findings were observed for NO_3^- immobilization in temperate forest soils (Berntson and Aber 2000). Further studies are required to investigate the exact nature of processes, which are responsible for these findings.

Over the experimental period (12 days) we estimated a total NO_2^- reduction of 8.6 μg N g^{-1} soil (Table 3), which amounts to 0.14% of the total N present prior to the experiment (average total N = 6,342 μg N g^{-1} soil). The product of the NO_2^- reduction processes is a theoretical pool (N_{red}), which may contain in addition to gaseous N species (N_2O, NO, NO_2, N_2) also NO_2^- consumed by other biotic and abiotic processes as discussed above. Consequently N_{red} is not equal to gaseous N losses from the soil. Furthermore, based on the mass balance we did not find any evidence for N loss from the ecosystem. The strong competition for NO_3^- in the soil may explain why gaseous N losses from these

Nothofagus forests are negligible (Perakis and Hedin 2001). Further studies are needed to clarify the exact mechanisms of gaseous N production and emission in these *Nothofagus* ecosystems.

Conclusions

With the present study we confirmed our previous findings (Huygens et al. 2007) that pristine *Nothofagus* forests are characterized by negligible N losses due to a tight N cycle with high turnover rates and NO_3^- consumption dominated by DNRA. Here, we show that DNRA has the potential to out-compete denitrification for the available NO_3^-. However, further direct measurements of denitrification are required to confirm our results. With the present study we provide evidence that a functional link exists between (1) heterotrophic nitrification and DNRA as well as (2) between NH_4^+ oxidation (nitritation) and subsequent NO_2^- reduction. To the best of our knowledge no other study has reported similar findings in any ecosystem. Therefore, more studies in various ecosystems are required to unambiguously prove if theses functional links are a general pattern in terrestrial soil. The preference of $NO_2^-{}_{nit}$ reduction over oxidation to NO_3^- is most likely related to the development of functionally linked microbial community structures which have adapted to the temporally prevailing anoxic conditions in this soil. This is in line with our observation that DNRA successfully out-competes immobilization and denitrification for the available NO_3^-, i.e., processes requiring reducing conditions are strongly supported in this Andisol.

Nitrite is an important intermediate in several N transformation processes (Russow et al. 2000; Sprent 1987; Van Cleemput and Samater 1996; Venterea and Rolston 2000) and we showed that ignoring NO_2^- dynamics may lead to erroneous estimates of gross N rates. Including NO_2^- dynamics in ^{15}N tracing studies provides us with more detailed and arguably more realistic N cycle models (Rütting and Müller 2008). Additionally, NO_2^- data constrain the search for parameter constellations and thus allows a more precise quantification of gross N transformation rates even for rates which are not directly involved in the NO_2^- dynamics.

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