Biogeochemistry (2011) 102:195–208
DOI 10.1007/s10533-010-9434-5

Stable N isotope composition of nitrate reflects N transformations during the passage of water through a montane rain forest in Ecuador

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Received: 22 September 2009 / Accepted: 19 March 2010 / Published online: 11 April 2010

Abstract Knowledge of the fate of deposited N in the possibly N-limited, highly biodiverse north Andean forests is important because of the possible effects of N inputs on plant performance and species composition. We analyzed concentrations and fluxes of NO_3^-–N, NH_4^+–N and dissolved organic N (DON) in rainfall, throughfall, litter leachate, mineral soil solutions (0.15–0.30 m depths) and stream water in a montane forest in Ecuador during four consecutive quarters and used the natural ^{15}N abundance in NO_3^- during the passage of rain water through the ecosystem and bulk $\delta^{15}N$ values in soil to detect N transformations. Depletion of ^{15}N in NO_3^- and increased NO_3^-–N fluxes during the passage through the canopy and the organic layer indicated nitrification in these compartments. During leaching from the organic layer to mineral soil and stream, NO_3^- concentrations progressively decreased and were enriched in ^{15}N but did not reach the $\delta^{15}N$ values of solid phase organic matter ($\delta^{15}N = 5.6$–6.7‰). This suggested a combination of nitrification and denitrification in mineral soil. In the wettest quarter, the $\delta^{15}N$ value of NO_3^- in litter leachate was smaller ($\delta^{15}N = -1.58$‰) than in the other quarters ($\delta^{15}N = -9.38 \pm$ SE 0.46‰) probably because of reduced mineralization and associated fractionation against ^{15}N. Nitrogen isotope fractionation of NO_3^- between litter leachate and stream water was smaller in the wettest period than in the other periods probably because of a higher rate of denitrification and continuous dilution by isotopically lighter NO_3^-–N from throughfall and nitrification in the organic layer during the wettest period. The stable N isotope composition of NO_3^- gave valuable indications of N transformations during the passage of water through the forest ecosystem from rainfall to the stream.

Keywords Denitrification · ^{15}N natural abundance · Nitrate · Nitrification · Terrestrial N cycling · Tropical montane forest

Abbreviations

DON	Dissolved organic nitrogen
DRY	Period between October 2006 and December 2006
INT1	Period between July 2006 and September 2006
INT2	Period between January 2007 and March 2007
LL	Litter leachate
RF	Incident rainfall

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SE	Standard error
SS15	Soil solution at the 15 cm depth of the mineral soil
SS30	Soil solution at the 30 cm depth of the mineral soil
SW	Stream water
TF	Throughfall
WET	Period between April 2007 and July 2007
$\varepsilon_{amm+nit}$	Isotope enrichment factor during ammonification and nitrification
ε_{denit}	Isotope enrichment factor during denitrification

Introduction

Tropical montane forests are believed to be frequently deficient in N and therefore should be characterized by closed cycling of N with only small N losses at the catchment level (Marrs et al. 1988; Tanner et al. 1998). However, there are also indications that other elements including P, base metals and even trace elements limit or co-limit plant growth in the tropical montane forest (Tanner et al. 1998; Wilcke et al. 2002, 2008; Boy et al. 2008a). Nitrogen deposition is expected to increase in the tropics in the near future (Galloway et al. 2004). This might have a fertilizing effect on the tropical montane forest with unknown consequences for biodiversity (Vitousek et al. 1997; Matson et al. 1999; Wassen et al. 2005; Boy et al. 2008a). However, if N was not limiting and therefore not fully retained, N exports might increase, resulting in either increased nitrate concentrations in freshwater or increased emissions of the greenhouse gas N_2O.

Knowledge of important N transformations in the various compartments of the tropical montane forest ecosystem is therefore essential to assess ecosystem responses to elevated N deposition (Matson et al. 1999). The cycling of N in terrestrial environments includes atmospheric inputs via N_2 fixation or wet and dry deposition of various N species, which are assimilated by plants and microbes and transformed to other N species in biomass. Organic N in dead biomass is mineralized to NH_4^+ by soil bacteria via ammonification, which is then either taken up by plants or microbes or nitrified to NO_3^- by chemoautotrophic bacteria or heterotrophic fungi. While NH_4^+ is retained in most soils, NO_3^- tends to be leached easily and hence possibly pollutes freshwaters. Nitrate can also be reduced microbially by denitrification. Denitrification is the multi-step reduction of NO_3^- typical for anaerobic conditions in soils resulting in gaseous export of reaction products (i.e. NO, N_2O and N_2) (Kendall 1998).

The complete assessment of the terrestrial N cycle is difficult since fluxes of N occur in dissolved, gaseous and particulate phase and various N transformations proceed simultaneously. The measurement of gaseous N emissions from soils requires a high spatial and temporal resolution to allow for extrapolation to ecosystem scales. Furthermore, measured gaseous soil N emissions do not include N_2, which are estimated to amount to $\sim$28 Tg y^{-1} N (Houlton and Bai 2009). Generally, the cycling of N is mediated biologically and transformations of N can be described as kinetic reactions accompanied by isotope fractionations as long as reactions are incomplete (Nadelhoffer and Fry 1988; Kendall 1998). The size of isotope fractionation depends on substrate availability, reaction pathway, reaction kinetics, reaction progress, redox conditions and composition of the microbial community. While N_2 fixation, ammonification and plant uptake are accompanied by small fractionations, multi-step reactions like nitrification and denitrification discriminate intensely against ^{15}N (Blackmer and Bremner 1977; Mariotti et al. 1981). While isotope fractionations of N transformations have been studied more frequently in laboratory experiments (Mariotti et al. 1981; Högberg 1997; Kendall 1998; Granger et al. 2008), field studies in terrestrial environments are still scarce, especially in the tropics (Houlton et al. 2006). As field studies usually do not allow to control for all processes contributing to isotope fractionations, the interpretations of isotope data collected in field studies inherently are more uncertain than those of controlled laboratory studies.

Studies on N transformations by means of stable isotopes were either limited to temperate regions or were mostly conducted after tracer applications (Koba et al. 1997; Perakis and Hedin 2001; Oelmann et al. 2007; Spoelstra et al. 2007) with the latter being accompanied by ecosystem disturbance. In the tropics, stable isotope studies on N transformations have been either limited to lowland rain forests or focused only on single processes of the N cycle or selected

ecosystem compartments (Marrs et al. 1988; Vitousek and Matson 1988; Hall and Matson 2003; Soethe et al. 2006). The same holds true for the few studies conducted in tropical montane forests (Hietz et al. 2002; Houlton et al. 2006; Kiese et al. 2008).

The objectives of our study were (i) to infer the importance of different N transformation processes from shifts of $\delta^{15}N$ values in NO_3^- during its dissolved transport through the ecosystem and (ii) to determine the seasonal variability of N transformations in a tropical montane rain forest in Ecuador.

Study site

The study site is a 20 m-long transect on a steep slope (30–60°) at 1950–1960 m a.s.l. adjacent to the draining stream between the cities of Loja and Zamora in the Andes of south Ecuador (4°00′S, 79°05′W) which is entirely covered by old-growth forest.

Annual rainfall volume between July 2006 and June 2007 was 2315 mm at 1910 m a.s.l. with a single maximum in the southern hemispheric winter and no distinct dry season. At this elevation, contribution of horizontal rain is negligible and the mean annual temperature is 15°C (Bendix et al. 2004) with only small variations throughout the year.

The bedrock consists of weakly metamorphosed Palaeozoic schists and sandstones and some quartz veins. Soils have developed from periglacial cover beds or postglacial landslides composed of varying bedrock materials. The soils are folic Cambisols (dystric) and haplic Cambisols (dystric) (IUSS Working Group WRB 2006). All mineral soils had a loamy skeletal texture and pH values between four and five. Typical for soils in the research area is a thick organic layer consisting of Oi, Oe and Oa horizons, where most of the plant-available nutrients are concentrated (Wilcke et al. 2002). The thickness of the organic layer varied between 0.11 and 0.24 m.

According to Bruijnzeel and Hamilton (2000) the forest can be classified as a tropical Lower Montane Forest, characterized by a high abundance of epiphytes and bryophytes. The most abundant tree families are Lauraceae, Rubiaceae, Melastomataceae, Podocarpaceae and Euphorbiaceae. A map and more detailed information on the study site can be found in Wilcke et al. (2001) and Homeier (2004).

Materials and methods

Sampling

Soil was sampled from 0.5 m wide and at least 1 m deep pits dug with a hand spade. Each horizon was sampled from the front wall of the pit in a representative manner. To sample organic soil horizons, a rectangular part of the organic layer was cut out and Oi, Oe and densely rooted Oa1 and sparsely rooted Oa2 horizons were separated from top to bottom with a machete. Soil samples were dried to constant mass at 70°C and mineral soil horizons were further sieved to <2 mm. For elemental and isotope analysis, soil samples were ground with a ball mill (PM 200, Resch, Germany).

Water samples of incident rainfall (RF), throughfall (TF), litter leachate (LL), mineral soil solutions at the 0.15 m (SS15) and 0.30 m (SS30) depths, respectively and stream water (SW) were collected weekly between July 2006 and June 2007. The gauging station for RF was located on a forest clearing close to the transect at similar altitude and consisted of five collectors. Our RF measurement included wet deposition and the soluble part of coarse particulate dry deposition because the collectors were continuously open. However, the aerosol trapping capacity of collectors is small relative to the forest canopy. Throughfall was sampled with 20 collectors placed randomly along the transect. Collectors for RF and TF were fixed 2 l PE sampling bottles with circular funnels of 0.115 m diameter and the funnel opening was at 1 and 0.3 m height above the soil, respectively. To prevent contamination with coarse organic matter, bottle openings were covered by a PE net (0.5 mm mesh width). To prevent evaporation, funnels were equipped with table-tennis balls and bottles were wrapped with aluminum foil. Litter leachate was collected by one zero-tension lysimeter at ridge, middle-slope and lower-slope position, respectively. Collectors for LL consisted of 0.20 × 0.14 m plastic boxes covered with a PE net (0.5 mm mesh width). The boxes were connected to 2 l PE sampling bottles with plastic hoses. Zero-tension lysimeters were installed by slipping them in below the organic layer parallel to the surface from a soil pit. The organic layer was not disturbed by the installation and most roots in the organic layer remained intact (Wilcke et al. 2001). Mineral soil solutions were sampled

continuously by suction cups (mullet suction cups, 1 ± 0.1 µm pore size, UMS, Munich, Germany) with a vacuum pump connected to a manometer. Vacuum was held permanently between 200 and 400 mbar. Suction cups were connected to glass bottles stored in a plastic box with a plastic tube. Stream water was sampled manually in the middle of the stream at the foot of the transect on each sampling day.

For each sampling date, volume-weighted samples of RF, TF, and LL were created in the field from all respective collectors, mixed in 2.5 l PE bottles and transported to the field laboratory. In the field laboratory, samples were filtered through 4–7 µm ashless white ribbon filters (595 ½, Schleicher, Schuell, Germany) and mixed to volume–weighted aliquots of each sample type for each month. Volume–weighting for SS15 and SS30 was based on the available weekly sampling volumes. Weighting of SW samples was based on the weekly measured water levels at a weir 200 m downstream of the transect studied. Between sampling dates, samples were stored frozen in 5 l plastic canisters in the dark.

As we faced difficulties in obtaining a mass of N above the detection limit for $\delta^{15}N$ of NO_3^-, we were forced to combine samples of three consecutive months after processing of NO_3^- to pure $AgNO_3$ (see below). The combination was not weighted because the individual $AgNO_3$ yields of the monthly samples were small. The combined months correspond to seasonal rainfall conditions in the research area. The period July to September 2006 is termed season INT1, the period October to December 2006 season DRY, the period January to March 2007 season INT2, and the period April to June 2007 season WET. While INT1 and INT2 were characterized by average monthly rainfall volumes (150–200 mm month^{-1}), DRY was characterized by lower-than-average rainfall volumes (<150 mm month^{-1}) and WET was characterized by higher-than-average rainfall volumes (>200 mm month^{-1}). Some samples were excluded from the combination because processing failed and samples for March 2007 were not collected (Table 1).

Table 1 Temporal correspondences of bulked seasonal samples for ^{15}N analysis in NO_3^-

Season	Period	Sample type	Combined monthly samples		
INT1	July 06–Sep 06	RF	x	Aug 06	Sep 06
		TF	July 06	Aug 06	Sep 06
		LL	July 06	Aug 06	Sep 06
		SS15	July 06	Aug 06	Sep 06
		SS30	July 06	x	Sep 06
		SW	July 06	Aug 06	Sep 06
DRY	Oct 06–Dec 06	RF	Oct 06	Nov 06	Dec 06
		TF	Oct 06	Nov 06	Dec 06
		LL	Oct 06	Nov 06	Dec 06
		SS15	x	Nov 06	n.a.
		SS30	x	Nov 06	Dec 06
		SW	Oct 06	Nov 06	Dec 06
INT2	Jan 07–Mar 07	RF	Jan 07	Feb 07	n.a.
		TF	Jan 07	Feb 07	n.a.
		LL	Jan 07	Feb 07	n.a.
		SS15	Jan 07	Feb 07	n.a.
		SS30	x	Feb 07	n.a.
		SW	Jan 07	Feb 07	n.a.
WET	Apr 07–June 07	RF	Apr 07	May 07	June 07
		TF	Apr 07	May 07	June 07
		LL	Apr 07	May 07	June 07
		SS15	Apr 07	May 07	June 07
		SS30	Apr 07	May 07	June 07
		SW	n.a.	May 07	June 07

x excluded, *n.a.* not available

Chemical analyses

Soil samples were packed into tin boats (Elementar, Hanau, Germany) and analyzed with Elemental Analyzer (EA; VarioEL III, Elementar) coupled online to an Isotope Ratio Mass Spectrometer (IRMS; IsoPrime, GV Instruments, Manchester, England) for C and N concentrations and $\delta^{15}N$. We used the isotope reference materials IAEA N-1 ($\delta^{15}N = +0.4‰$) and IAEA N-2 ($\delta^{15}N = +20.3‰$) (IAEA, Vienna, Austria) to calibrate reference gases of the IRMS for $\delta^{15}N$ and repeated calibration after a set of 10 samples. We used five subsequent samples of sulfanilic acid (Merck, Darmstadt, Germany) to calibrate the EA. The five measured samples of sulfanilic acid yielded an analytical precision of <0.3‰ for $\delta^{15}N$.

Monthly samples of RF, TF, LL, SS15, SS30, and SW were analyzed for concentrations of NH_4^+–N, NO_3^-–N and total dissolved N (TDN) with a Continuous Flow Analyzer (CFA; AutoAnalyzer 3, Bran + Luebbe, Norderstedt, Germany). Nitrate concentration was analyzed photometrically after

reduction to nitrite and therefore contains a small unknown contribution of nitrite. Concentrations of NH_4^+–N were analyzed photometrically as 5-aminosalicylate after a modified Berthelot reaction. Concentrations of TDN were determined after oxidation with $K_2S_2O_8$, followed by a reduction as described for NO_3^-–N. Detection limits were 0.039 mg l^{-1} for NH_4^+–N and 0.045 mg l^{-1} for NO_3^-–N and TDN, respectively. For NO_3^-–N, NH_4^+–N and TDN concentrations, 3, 5 and 0 out of 71 samples were below the detection limit and were set to the detection limit. Concentrations of dissolved organic N (DON) were calculated as the difference between TDN and the sum of inorganic N (NO_3^-–N + NH_4^+–N), with three out of 71 samples having negative concentrations that were corrected to zero.

To determine $\delta^{15}N$ of NO_3^-, samples were processed according to the method of Silva et al. (2000) with slight modifications. Before pouring the solutions through an anion exchange resin (AG1x8, Cl^- form, BioRad, Ismaning, Germany), solutions were acidified to a pH value of two by adding 3 mol l^{-1} HCl to protonate dissolved organic compounds and prevent their accumulation on anion exchange sites. Anions were stripped from anion exchange resins by adding 15 ml of 3 mol l^{-1} HCl in five increments. The resulting solution was neutralized with Ag_2O (Silberoxid 99+, Merck). Precipitating AgCl was removed by vacuum filtration with 0.45 µm cellulose–acetate membranous filter (Sartorius, Göttingen, Germany). Since we initially aimed at also analyzing $\delta^{18}O$ of NO_3^-, sample processing was further conducted as described by Silva et al. (2000). Resulting solutions were sublimated with a freeze-drier (Alpha 1-2D, Christ, Osterode am Harz, Germany). However, resulting salts were contaminated with O from an unknown source (data not shown).

Resulting $AgNO_3$ was analyzed for N concentrations and $\delta^{15}N$ with EA-IRMS by combustion as described above, but silver boats (Elementar) instead of tin boats were used. Furthermore, we had problems in analyzing small amounts of nitrate salts (data not shown) and therefore only included results from measurements without replication in our data set if we were able to use a minimum amount of 150 µg N, for which a linear response of the IRMS could be proven. Samples with lower masses for determination were only included if repeated measurements with a minimum of 60 µg N per measurement were available.

The average recovery of NO_3^-–N was 85 ± 9.1% (mean ± standard error, SE) and 82 ± SE 9.2% when excluding samples below the detection limit. The quality of the calculation of recoveries was biased by the unweighted combination of monthly samples to seasonal samples.

Calculations and statistical tests

To estimate fluxes of NO_3^-–N and NH_4^+–N we multiplied the respective water flux with the concentrations of the sample type. The water flux of RF and TF was recorded during sampling. For LL, SS15 and SS30, we estimated the water flux by regression on throughfall volumes using Eqs. 1–3 (n = 114, based on own unpublished data). We did not calculate fluxes of SW.

$$LL = 0.7293 * TF + 1.5981 \left(r^2 = 0.9397\right) \quad (1)$$

$$SS15 = 0.5929 * TF + 4.3731 \left(r^2 = 0.7864\right) \quad (2)$$

$$SS30 = 0.4620 * TF + 7.2960 \left(r^2 = 0.5064\right) \quad (3)$$

The abundances of ^{15}N are given in the δ-notation and refer to the international standard Vienna-Air. Since we did not know the isotope signature of NH_4^+–N in LL, we were not able to determine isotope enrichment factors solely for nitrification, but estimated in situ isotope enrichment during the overall process of ammonification and nitrification in the organic layer ($\varepsilon_{amm+nit}$) with Eq. 4:

$$\varepsilon_{amm+nit} = \delta^{15}N_{LL} - \delta^{15}N_{organic\ layer} \quad (4)$$

where $\delta^{15}N_{LL}$ is $\delta^{15}N$ value of NO_3^- in LL and $\delta^{15}N_{organic\ layer}$ is the mean bulk $\delta^{15}N$ value of the organic layer. This calculation is based on the following assumptions: (1) NH_4^+ and NO_3^- originate solely from mineralization of the organic layer (i.e., deposition with TF is negligible), (2) no isotope fractionation occurs during plant uptake and microbial immobilization of inorganic N, (3) the change in size of the N pool of the organic layer due to mineralization is negligible and changes in bulk $\delta^{15}N$ values during those reactions only occur on long time scales, and (4) NH_4^+ is continuously produced so that the NH_4^+ pool is infinite and we therefore assume that $\delta^{15}N$ values of NO_3^- in LL reflects the instantaneous product of nitrification. According to Mariotti et al. (1981) this calculation holds true for small ε values.

We also estimated the in situ isotope enrichment factors for denitrification (ε_{denit}) of NO_3^- leached in soil with Eq. 5.

$$\varepsilon_{denit} = \left(\delta^{15}N_y - \delta^{15}N_x\right)/\ln\left(c_y\ c_x^{-1}\right) \quad (5)$$

where $\delta^{15}N$ refers to $\delta^{15}N$ of NO_3^-, c refers to the NO_3^-–N concentrations and the subscripts denote the respective sample types with x preceding y hydrologically. Note that ε_{denit} refers to the ^{15}N enrichment in the substrate (i.e., the residual NO_3^- fraction) and is therefore negative. This is only a rough estimate because we assume that (1) no other NO_3^- sinks than denitrification exist during leaching and (2) no nitrification occurs in the leaching zones. We also calculated ε_{denit} for mineral soils, although we do not know the amounts of NO_3^-–N being leached vertically and laterally, respectively.

Because sample size was small, we used the non-parametric Mann–Whitney *U*-test to detect significant differences between sample groups and the Wilcoxon matched-pairs test for significant differences between variables. Coefficients of correlation (*r*) refer to Pearson. All statistical analyses were performed with STATISTICA 7.0 (StatSoft Inc., 2004; Tulsa, OK, USA).

Results

Nitrogen concentrations, C:N ratios and $\delta^{15}N$ values of soils

In the mineral soil, N concentrations decreased with depth, but increased with depth in the organic layer (Table 2). In the organic layer, the C:N ratios of soil organic matter (SOM) of Oi and Oe horizons differed from Oa2 horizons ($p < 0.01$). While C:N ratios in Oa and Ah horizons were similar, C:N ratios decreased with depth to the Bw horizon, but did not change further in deeper soil. Nitrogen concentrations and C:N ratios were significantly lower in Oi and Oe horizons than in Oa2 horizons (Table 2). While $\delta^{15}N$ values correlated with C:N ratios in the organic layer ($r = -0.75$; $p < 0.01$), this was not true in mineral soil. There was a significant increase in the $\delta^{15}N$ value at the transition of the organic layer to the mineral soil of about 2.5‰.

Table 2 Mean and standard error (in parantheses) of C and N concentrations and bulk $\delta^{15}N$ values of soil horizons

Horizon	C [g kg^{-1}]	N [g kg^{-1}]	C:N	$\delta^{15}N$ [‰]	n
Oi	440 (16)	18 (0.95)	24 (2.1)	+1.27 (0.33)	3
Oe	446 (21)	22 (1.4)	21 (2.1)	+1.38 (0.44)	3
Oa1	394 (32)	25 (2.1)	16 (0.63)	+2.26 (0.51)	3
Oa2	367 (21)	24 (0.91)	15 (0.96)	+3.02 (0.50)	3
Ah	30 (2.0)	2.1 (0.10)	14 (0.46)	+5.62 (0.30)	3
BwAh	15 (0.66)	1.1 (0.02)	13 (0.37)	+6.52 (0.04)	2
Bw	5.5 (0.75)	0.63 (0.02)	8.6 (1.2)	+6.69 (0.43)	3
C	2.2 (0.73)	0.25 (0.03)	9.3 (3.5)	+5.97 (0.45)	3

Dissolved N concentrations and fluxes

Concentrations and fluxes of NH_4^+–N, NO_3^-–N and DON and differences among sample types in the quarters studied are shown in Figs. 1, 2, respectively. Compared with NH_4^+–N and DON, concentrations and fluxes of NO_3^-–N in RF were more variable. Concentrations and fluxes of NO_3^-–N were lower in WET than in all other seasons and in INT1 than in INT2. This resulted in lower bulk deposition in DRY and WET than in INT1 and INT2 ($p < 0.05$).

Fluxes of NH_4^+–N with TF were lower in INT1 and DRY than in WET ($p < 0.05$). The quarter INT1 was characterized by significantly lower NO_3^-–N concentrations and fluxes in TF than in other quarters. Furthermore, INT1 was the only quarter having lower concentrations of NO_3^-–N than NH_4^+–N in TF. Throughfall volume correlated with TF fluxes of NH_4^+–N ($r = 0.65$; $p < 0.05$) and DON ($r = 0.71$; $p < 0.05$).

In LL, NH_4^+–N concentrations and fluxes varied little from INT1 to INT2 with a peak in January 2007 but declined sharply in WET to levels far below those of the other quarters ($p < 0.05$). A similar pattern was observed for NO_3^-–N concentrations in LL, which also declined in WET compared to other seasons ($p < 0.05$), but not for NO_3^-–N fluxes. In all seasons, NO_3^-–N concentrations in LL were higher than NH_4^+–N concentrations ($p < 0.05$) and correlated significantly with each other ($r = 0.70$; $p < 0.05$). Concentrations of DON were lowest in INT1. Leaching of DON from the organic layer increased throughout the observation period and was higher in INT2 and WET than in INT1 and DRY ($p < 0.05$). In LL, the DON flux correlated with seepage ($r = 0.80$; $p < 0.01$).

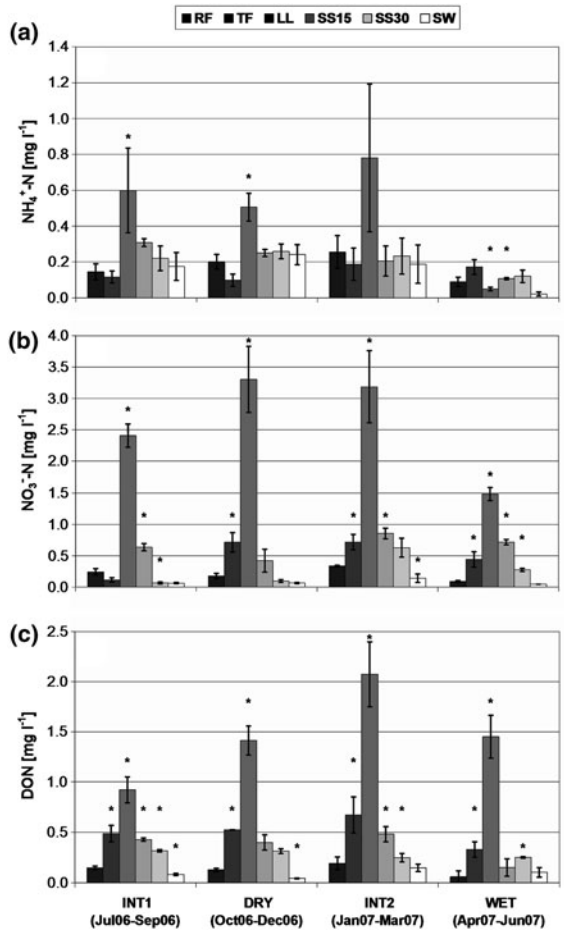

Fig. 1 Mean concentration ± SE of **a** NH_4^+–N, **b** NO_3^-–N and **c** DON of ecosystem solutions in the four studied quarters (n = 3, except for SS15 in DRY and SW in WET n = 2), *asterisks* mark differences to the spatially preceding ecosystem flux ($p < 0.05$)

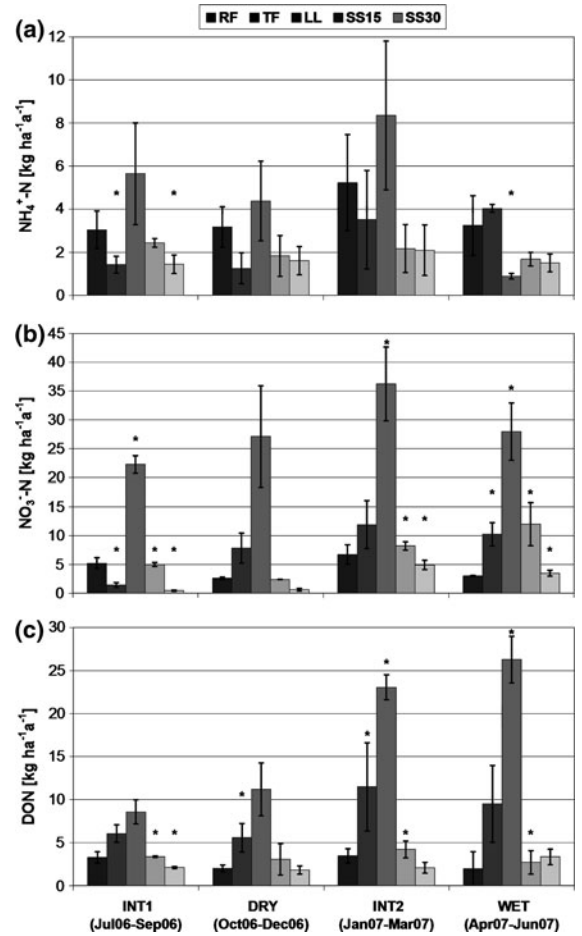

Fig. 2 Mean fluxes ± SE of **a** NH_4^+–N, **b** NO_3^-–N and **c** DON of ecosystem solutions in the four studied seasons (n = 3, except for SS15 in DRY and SW in WET n = 2), *asterisks* mark differences to the spatially preceding ecosystem flux ($p < 0.05$)

Concentrations of NH_4^+–N in SS15 varied little, but were lower in WET than in INT1 ($p < 0.05$). While concentrations of NO_3^-–N in SS15 did not vary significantly among quarters. NO_3^-–N fluxes showed a seasonal variability with smaller fluxes in INT1 than in INT2 and WET ($p < 0.05$). In mineral topsoil, translocation of NO_3^- was highly correlated with leachate volume ($r = 0.91$; $p < 0.001$). Concentrations of DON in SS15 correlated with NH_4^+–N ($r = 0.71$; $p < 0.05$) and were consequently also lowest in WET.

Concentrations of NH_4^+–N in SS30 were lower in WET than in DRY ($p < 0.05$). Concentrations of NO_3^-–N were highly variable peaking in INT2. The differences in NO_3^-–N concentrations were significant between INT2 and all other seasons and also between WET and INT1 and DRY. Except for a lacking difference between INT2 and WET, this also holds true for NO_3^-–N fluxes. Although we did not observe a seasonal variation in DON concentrations and fluxes, DON fluxes correlated with leachate volume ($r = 0.94$; $p < 0.001$).

Concentrations of NH_4^+–N in SW were consistently low in all seasons. This also applies to NO_3^-–N concentrations, but a small increase occurred in INT2. Concentrations of DON varied little in SW but increased slightly in INT2.

$\delta^{15}N$ values of dissolved NO_3^- in ecosystem fluxes

Along the hydrological pathway, $\delta^{15}N$ values of NO_3^- decreased from RF (+0.01 ± SE 1.6‰; n = 3) via TF (−3.1 ± 2.2‰; n = 4) to LL (−7.4 ± 2.0‰; n = 4) and then increased via SS15 (+0.04 ± 1.2‰; n = 2), SS30 (+1.1 ± 1.7‰; n = 2) to SW (+4.4 ± 0.53‰; n = 3). This typical distribution of $\delta^{15}N$ values was observed for DRY and INT2 but was modified in WET, where $\delta^{15}N$ values of NO_3^- did not vary as much as in other quarters among different ecosystem fluxes (Fig. 3).

The $\delta^{15}N$ values of NO_3^- in RF were lowest in INT1 and highest in INT2. In TF, $\delta^{15}N$ values of NO_3^- were consistently depleted in ^{15}N relative to RF except in INT1. The difference in $\delta^{15}N$ values of NO_3^- between RF and TF was most pronounced in INT2. There was a marginally significant correlation between NH_4^+–N concentrations in RF and $\delta^{15}N$ values of NO_3^- in TF ($r = -0.91$; $p = 0.09$). In all seasons, NO_3^- in LL was depleted in ^{15}N relative to all other ecosystem fluxes. While $\delta^{15}N$ values of NO_3^- in LL were generally strongly negative and varied little among INT1, DRY and INT2, an increase by 6–8‰ occurred in WET. The $\delta^{15}N$ values of NO_3^- in LL correlated with monthly throughfall volumes ($r = 0.99$, $p < 0.05$). Based on Eq. 4, the fractionation factors of ammonification + nitrification ($\varepsilon_{amm+nit}$) in the organic layer were −11.5, −12.1, −10.5 and −3.6‰ in INT1, DRY, INT2 and WET, respectively. The $\varepsilon_{amm+nit}$ values correlated with the natural logarithm of NH_4^+–N concentrations in LL ($r = -0.95$; $p < 0.05$). The $\delta^{15}N$ values of NO_3^- in SS15 were only available for INT1 and WET. Nitrate in SS15 was enriched in ^{15}N relative to LL in both seasons and the $\delta^{15}N$ value of NO_3^- in SS15 was higher in INT1 than in WET. According to Eq. 5, the ^{15}N enrichment of NO_3^- between LL and SS15 corresponds to fractionation factors (ε_{denit}) of −8.1‰ and −0.6‰ for INT1 and WET, respectively. For SS30, $\delta^{15}N$ values of NO_3^- were only available for INT2 and WET. In INT1, the $\delta^{15}N$ value of NO_3^- in SS30 was far higher than in LL. In WET, the $\delta^{15}N$ value of NO_3^- in SS30 was higher than in LL and in SS15. The ^{15}N fractionation (ε_{denit}) according to Eq. 5 was −4.9 and −2.6‰ between LL and SS30 in INT2 and WET, respectively, and −4.1‰ between SS15 and SS30 in WET. In SW, $\delta^{15}N$ values of NO_3^- were highest of all ecosystem fluxes in all quarters (no data for INT2). The highest ^{15}N enrichment of NO_3^- in SW occurred in DRY and the lowest in WET. Again applying Eq. 5, the isotope fractionation factor (ε_{denit}) between LL and SW was −3.8, −3.9 and −1.5‰ in INT1, DRY and WET, respectively. The $\delta^{15}N$ values of NO_3^- of all sample types in all seasons correlated with ln c(NO_3^-–N) while the relationship of the inverse of NO_3^-–N concentrations with $\delta^{15}N$ values of NO_3^- precluded regression analysis because of dislocated data clouds (Fig. 4).

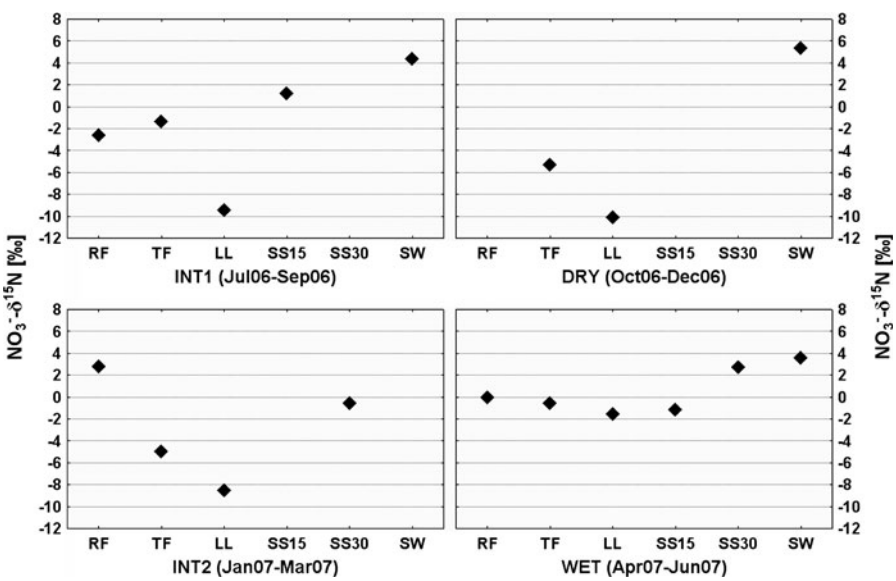

Fig. 3 $\delta^{15}N$ values of NO_3^- values in rainfall (*RF*), throughfall (*TF*), litter-leachate (*LL*), soil-solution at 15 cm (*SS15*) and 30 cm (*SS30*) soil depth, respectively, and stream water (*SW*) in the four studied quarters. Analytical precision was smaller 0.3‰ for repeated measurements of sulfanilic acid (n = 5)

Discussion

Shifts of $\delta^{15}N$ values as indicator of N transformations

Nitrate in RF had typical $\delta^{15}N$ values for areas with low N deposition from anthropogenic sources (Heaton 1987; Freyer 1991; Garten 1992; Fig. 3). Nitrification might have resulted in the release of NO_3^- to the throughfall and the discrimination against ^{15}N in NO_3^- in the canopy in DRY, INT2 and WET (Figs. 2, 3). The occurrence of nitrification is also in line with the observed retention of atmospheric NH_4^+ in the canopy in INT1, DRY and INT2 (Figs. 1, 2). Deposition of NH_4^+ likely stimulated nitrification and related isotope fractionation in the canopy. In tropical and temperate forests, ammonification of organic matter accumulated in the canopy and nitrification at leaf surfaces, respectively, have already been shown (Vance and Nadkarni 1990; Wilson 1992). The high abundance of epiphytes in the canopy and the high litterfall in our research area (Wilcke et al. 2002) result in the accumulation of organic matter in the canopy that can be mineralized. The observation of Sah and Brumme (2003) that nitrification explained ^{15}N fractionation in NO_3^- during canopy passage in a N-saturated beech forest corroborates our interpretation.

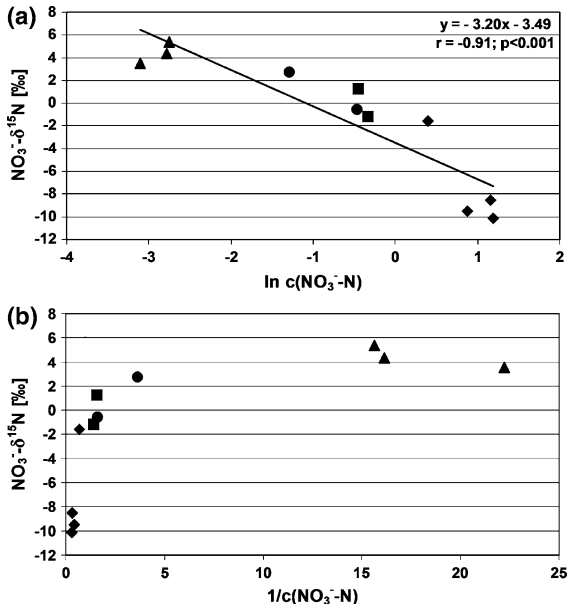

Fig. 4 Relationship of **a** logarithm of NO_3^-–N concentration and **b** reciprocal NO_3^-–N concentration with $\delta^{15}N$ value of NO_3^- as an indication of denitrification and mixing, respectively, in LL (*diamonds*), SS15 (*rectangles*), SS30 (*circles*) and SW (*triangles*)

Depletion in ^{15}N in NO_3^- and increase in NO_3^- concentrations in LL relative to TF revealed that in the organic layer considerable nitrification took place in spite of wide C:N ratios. This can be attributed to the large N stocks which result in the release of large amounts of NO_3^- by ammonification and nitrification although mineralization rates are limited by climatic and edaphic conditions (Wilcke et al. 2002, Figs. 1, 3). Furthermore, concentrations and fluxes of NH_4^+ remained low very likely because of NH_4^+ consumption by nitrifying bacteria. Nitrogen isotope fractionation during ammonification and nitrification as indicated by the calculated $\varepsilon_{amm+nit}$ values (Eq. 4) in INT1, DRY and INT2 was smaller or in the lower range of isotope fractionation of nitrifying bacteria ($\varepsilon = -12$ to -27‰) determined in laboratory incubations (Shearer and Kohl 1986) but similar to other field studies. In a field study, Mayer et al. (2001) found a ^{15}N depletion by -6 and -9‰ in organic layers below beech and spruce, respectively, in northwestern Germany. Mayer et al. (2001) reported an even stronger fractionation of N isotopes and NH_4^+ limitation of the fractionation when incubating the soil in the laboratory. In situ isotope fractionation in our study might have been more pronounced than in northwestern Germany because of better mineralization conditions (e.g., higher temperatures, more favorable C:N ratios) and the large N stock in the organic layer. Spoelstra et al. (2007) determined ε for nitrification as -6.6 to -12.9‰ in Ontario with no NH_4^+ limitation under artificial rain which was free of inorganic N. Since we did not exclude deposition from the atmosphere in our study, calculated $\varepsilon_{amm+nit}$ are underestimated, because mixing of NO_3^- from RF and TF with freshly nitrified N from the organic layer would likely have increased $\delta^{15}N$ of NO_3^- in LL. An in situ determination of isotope fractionation during nitrification is complicated by simultaneous consumption and ^{15}N enrichment of NH_4^+ as a consequence of the nitrification, NH_4^+ assimilation and temperature effects on fractionation factors.

Nitrate-$\delta^{15}N$ values in mineral soil solution were depleted relative to $\delta^{15}N$ values of mineral soil and N isotope fractionation associated with nitrification could be responsible for this pattern. Hypothetically applying Eq. 4 to the corresponding bulk $\delta^{15}N$ values

of the mineral soil horizons at 0.10–0.30 m depth would result in $\varepsilon_{amm+nit}$ of −4.8 to −7.3‰ in SS15 for INT1 and WET, respectively, and −7.4 and −4.1‰ in SS30 for INT2 and WET, respectively. However, our estimates of $\varepsilon_{amm+nit}$ are biased by the fact that NO_3^- leaching from the organic layer into the mineral soil was not neglible, as suggested by the correlation of NO_3^-–N concentrations in SS15 and throughfall volumes. Leached NO_3^- from the organic layer would have lowered $\delta^{15}N$ of NO_3^- in soil solutions, thus the real $\varepsilon_{amm+nit}$ are likely less negative than our estimates.

Decreasing NO_3^-–N concentrations and fluxes in the order LL > SS15 > SS30 (Figs. 1, 2) suggest that there was a NO_3^- sink in the mineral soil. Potential sinks of NO_3^- include adsorption, assimilation by soil microorganisms and the sparse roots in the mineral soil, lateral leaching and denitrification. To further explore the potential role of denitrification, we calculated ε_{denit} values (Eq. 5). Our estimated ε_{denit} between LL and SS15 of −8.1 in INT1 was less negative but in the same order as in a study of Houlton et al. (2006) who found an ε value for denitrification of −13.2‰ in soil cores which were placed in a tropical montane forest in Hawaii. In the experiment of Houlton et al. (2006), however, root uptake and nitrification was experimentally excluded. In another field study, Mariotti et al. (1988) determined ε_{denit} values of −4.7 to −5.0‰ in an aquifer in France. Using a method which was comparable to our approach, Koba et al. (1997) found ε_{denit} values of −5.6 to −6.0‰ for intermittent denitrification in a wet soil with no strong anaerobic conditions between 0.3 and 0.7 m depth in a field study in Japan, which is comparable to our estimated ε_{denit} values between LL and SS30 of −4.9 to −2.6‰ in INT2 and WET, respectively, and of −4.1‰ between SS15 and SS30 in WET. Both authors attributed the low fractionation factors to diffusion-limited denitrification. Recent research has highlighted the importance of hot spots and hot moments for denitrification (McClain et al. 2003). In both cases, the rate constant of denitrification is high and thus associated with small fractionation (Mariotti et al. 1988). However, the experimental design does not allow us to distinguish between diffusion-limitation or mixing with mineral soil-borne nitrate, but most probably our results include both processes.

In SW, $\delta^{15}N$ values of NO_3^- was consistently high and similar to the $\delta^{15}N$ values of inorganic N of NO_3^--dominated streams in the Barro Branco watershed in Amazonia (4.52 ± SD 0.8‰, Brandes et al. 1996, Fig. 3). According to Mariotti et al. (1981, 1988), denitrification can be described as a Rayleigh fractionation (i.e., a significant correlation of the logarithm of NO_3^-–N concentrations and the $\delta^{15}N$ of NO_3^-), while mixing of two NO_3^- sources leads to a significant correlation of reciprocal NO_3^-–N concentrations and $\delta^{15}N$ of NO_3^-. Therefore, denitrification between LL, SS15, SS30 and SW likely caused decreasing concentrations and increasing $\delta^{15}N$ values of NO_3^- (Fig. 4a). A mixing model described this pattern poorly (Fig. 4b). This model of denitrification assumes vertical transport of NO_3^- from LL, via SS15 and SS30 to SW. A theoretical application of both models, i.e. denitrification versus mixing, to lateral leaching from LL to SW (i.e., exclusion of SS15 and SS30) still favored denitrification ($r = -0.95$; $p < 0.001$) over mixing as the dominant process driving ^{15}N enrichment of NO_3^-. However, the model does not account for other (non-fractionating) sinks for NO_3^- during leaching.

Seasonal variability of N transformations

The small seasonal variations in N deposition and $\delta^{15}N$ values of NO_3^- in RF (Fig. 3) might have resulted from a combination of differing geographical origins of air parcels and associated varying N loads, varying atmospheric trajectories and varying reaction kinetics during NO_3^- production and washout in thunderstorms (Kendall 1998). A significant increase in N deposition in the research area is derived from biomass burning in the Amazonian basin except for a short period between April and July (Boy et al. 2008a), which was also reflected in our study by lower NO_3^- deposition in WET. As shown by Hastings et al. (2003) for rainfall on the Bermuda islands, elevated NO_x concentrations resulting from fossil fuel consumption in the USA, lead to a decrease in $\delta^{15}N$ values of NO_3^- compared to NO_3^- generated by lightning. We only observed small increases in NO_3^- deposition in INT1 and INT2, but the $\delta^{15}N$ of NO_3^- in INT2 was higher than in other quarters. The higher deposition and the lower $\delta^{15}N$ values of NO_3^- in INT1 might have resulted from N emissions by biomass burning, because gaseous HNO_3 in the atmosphere is depleted in ^{15}N relative to rain water by equilibrium isotope fractionation (Moore 1977). However, the difference in

NO_3^-–N fluxes and $\delta^{15}N$ values of NO_3^- in RF and TF in INT2 is probably too large to be explained by gaseous dry deposition. The few available studies on aerosols containing NO_3^- showed a ^{15}N enrichment relative to NO_3^- in rain water (Moore 1977; Heaton et al. 1997). Garten (1992) therefore attributed higher $\delta^{15}N$ values of NO_3^- in throughfall than in rainfall to particulate dry deposition. Consequently, particulate dry deposition cannot explain ^{15}N depletion of NO_3^- during canopy passage in DRY, INT2 and WET, but might have contributed to higher $\delta^{15}N$ values of NO_3^- in TF in INT1. In the latter case, particulate dry deposition was not followed by leaching but by retention in the canopy. In a previous study Boy et al. (2008a) also observed N retention in the canopy during elevated N deposition and explained this unexpected observation by the simultaneous deposition of one or several co-limiting nutrients (e.g., Mn) also emitted from biomass-burning which stimulated N uptake in the canopy. In our study, it remains, however, unclear whether NO_3^- uptake in the canopy was stimulated by dry deposition of co-limiting nutrients or whether nitrification was interrupted in INT1. The latter might have resulted from a pool depletion because of intense leaching of mineralizable substrates and NH_4^+ in the preceding wet phase as indicated by correlation of NH_4^+–N and DON fluxes with throughfall volume. High precipitation rates and associated leaching in WET might also have contributed to lower fractionation during nitrification or caused a leaching of conserved NO_3^--bearing particles (with higher $\delta^{15}N$) from the canopy in WET. Furthermore, short-term variations of canopy N transformations could not be detected with our experimental design relying on bulked three month samples, but might have been of high relevance.

The correlation of $\delta^{15}N$ values of NO_3^- in LL with throughfall volume pointed at a climatically driven variability of N transformations. Isotope fractionation during ammonification and nitrification was high in INT1, DRY and INT2 but low in WET. The higher $\delta^{15}N$ value of NO_3^- in LL in WET did not coincide with significantly lower NO_3^- leaching but with reduced NH_4^+ leaching. Therefore, it can be assumed that ammonification rather than nitrification was inhibited during high rainfall conditions, possibly because of intensified leaching of DON which is one of the substrates for ammonification, or lateral export of NH_4^+. In contrast, Kiese et al. (2008) found increased gross nitrification rates in wet seasons in a tropical montane forest in Australia on a better drained soil than at our study site. The (marginally significant) correlation of the logarithm of the NH_4^+ concentrations and $\delta^{15}N$ of NO_3^- in LL supported the assumed limitation of isotope fractionation during nitrification by reduced NH_4^+ availability in WET. The concentration of NH_4^+ is usually increased by frequent drying and rewetting cycles, because of the release of microbial organic N during drying and subsequent ammonification (Fierer and Schimel 2002). Therefore, more NH_4^+ was available in INT1, INT2, and DRY than in WET. Furthermore, lateral flow in the organic layer following water saturation of the soil is important at our study site (Goller et al. 2005; Boy et al. 2008b), which is likely to have occurred in WET. Lateral flow might have flushed NH_4^+ quickly to the stream and consequently reduced its availability. Increased leaching of NO_3^-, because of high rainfall rates and its high mobility in soil, cannot be excluded and could have compensated reduced nitrification. We can rule out sorption/desorption processes as explanation for the reduced ^{15}N depletion in WET because these processes are not associated with N isotope fractionation in soil NO_3^- (Clay et al. 2004).

In WET, the difference in $\delta^{15}N$ values of NO_3^- between LL and SS15 was small and NO_3^-–N fluxes in SS15 were highest of all quarters although still lower than in LL (Figs. 2, 3). This suggested that NO_3^- leaching through the mineral topsoil dominated the $\delta^{15}N$ values of NO_3^- in the rainy season. The fact that NO_3^- fluxes decreased significantly between LL and SS15, however, suggests that there must have been NO_3^- losses between LL and SS15 which did not result in changes of the $\delta^{15}N$ values of NO_3^- (Figs. 2, 3). As weather conditions in WET were likely to lead to episodic water saturation favoring lateral flow in topsoil (Boy et al. 2008b), unaccounted lateral NO_3^- losses can contribute to explain the decreasing NO_3^- fluxes between LL and SS15. In the subsoil (SS30), the increase of $\delta^{15}N$ values of NO_3^- and the simultaneous further decrease in NO_3^- fluxes, in contrast suggests enhanced denitrification during WET. The elevated NO_3^-–N concentrations and fluxes in INT1 and INT2 than in DRY could at least partly be attributable to stimulated nitrification because of the rewetting of mineral soil. If the ^{15}N enrichment in NO_3^- between SS15

and SS30 in WET, which was comparable to the ^{15}N enrichment in NO_3^- between LL and SS30 in INT2, was interpreted as the consequence of denitrification it could be concluded that denitrification occurred to a larger extent in the subsoil during wet conditions, and in the topsoil during intermediate soil moisture conditions. Seasonal variability of isotope fractionation during denitrification to N_2O in soils was reported previously (−11 to −34‰, ^{15}N depletion in N_2O relative to NO_3^-, Mandernack et al. 2000). The ε_{denit} values of Mandernack et al. (2000) are not directly comparable to ours because the authors reported the fractionation of several chemical reaction steps while we report fractionation during the first reaction step only. However, both studies reflect the importance of climatic conditions for reaction kinetics.

Conclusions

The analysis of $\delta^{15}N$ values of NO_3^- in aqueous ecosystem fluxes in combination with concentrations and fluxes of dissolved N species and $\delta^{15}N$ values of SOM allowed us to characterize N transformations an their seasonal variation in a tropical montane forest. While the canopy and organic layers were dominated by nitrification associated with increasingly lighter NO_3^--$\delta^{15}N$ values between RF and LL, decreasing concentrations and increasing $\delta^{15}N$ values of NO_3^- during leaching to the draining stream could be described as a Rayleigh fractionation and hence indicated denitrification in addition to further nitrification in the mineral soil. The ^{15}N enrichment of NO_3^- was highest in mineral topsoil with additional fractionation in mineral subsoil and presumably riparian zones during low and average rainfall conditions, but also occurred in the mineral subsoil in the wettest season, probably because of high leaching rates. The isotope effects which we attributed to denitrification were small, probably because of co-occurring nitrification in the mineral soil and mixing of mineral-soil derived with organic layer-derived NO_3^-.

While NO_3^- leaching from the organic layer was similar throughout the year, compared to other climatic quarters ammonification seemed to be reduced in the wettest quarter resulting in a lower ^{15}N fractionation during nitrification. Nitrate was released from the canopy by nitrification in three out of four periods. Retention of N in the canopy coincided with isotope discrimination against ^{15}N during the canopy passage of NO_3^- and with the typical period of elevated dry deposition resulting from biomass-burning in Amazonia suggesting that the deposition of co-limiting nutrients stimulated N uptake in the canopy and reduced NO_3^- release. Denitrification in soil was not limited to high rainfall conditions but rainfall conditions likely influenced the depth of the most active denitrifying zone in soil.

Acknowledgments We gratefully thank Nature and Culture International (NCI) in Loja, Ecuador, for providing the study area and the research station and the Instituto Forestal Areas Naturales y Vida Silvestre (INEFAN) for the study permission. We furthermore thank the Earth System Science Research Center of the Johannes Gutenberg University Mainz for improving our laboratory equipment. This study was funded by the Deutsche Forschungsgemeinschaft (DFG-FOR 816, Wi1601/8-1).

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