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Contrasting nitrogen fluxes in African tropical forests of the Congo Basin

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Abstract. The observation of high losses of bioavailable nitrogen (N) and N richness in tropical forests is paradoxical with an apparent lack of N input. Hence, the current concept asserts that biological nitrogen fixation (BNF) must be a major N input for tropical forests. However, well-characterized N cycles are rare and geographically biased; organic N compounds are often neglected and soil gross N cycling is not well quantified. We conducted comprehensive N input and output measurements in four tropical forest types of the Congo Basin with contrasting biotic (mycorrhizal association) and abiotic (lowland–highland) environments. In 12 standardized setups, we monitored N deposition, throughfall, litterfall, leaching, and export during one hydrological year and completed this empirical N budget with nitrous oxide (N_2O) flux measurement campaigns in both wet and dry season and in situ gross soil N transformations using ^{15}N-tracing and numerical modeling. We found that all forests showed a very tight soil N cycle, with gross mineralization to immobilization ratios (*M*/*I*) close to 1 and relatively low gross nitrification to mineralization ratios (*N*/*M*). This was in line with the observation of dissolved organic nitrogen (DON) dominating N losses for the most abundant, arbuscular mycorrhizal associated, lowland forest type, but in contrast with high losses of dissolved inorganic nitrogen (DIN) in all other forest types. Altogether, our observations show that different forest types in central Africa exhibit N fluxes of contrasting magnitudes and N-species composition. In contrast to many Neotropical forests, our estimated N budgets of central African forests are imbalanced by a higher N input than output, with organic N contributing significantly to the input-output balance. This suggests that important other losses that are unaccounted for (e.g., NO_x and N_2 as well as particulate N) might play a major role in the N cycle of mature African tropical forests.

Key words: *^{15}N tracing; central African tropical forest; Congo Basin; gross N rates; N balance; N deposition; N losses; nitrogen cycle; organic nitrogen; tropical forests.*

Introduction

Tropical forests dominate the terrestrial carbon (C) cycle, accounting for about one-third of the global terrestrial gross primary productivity (Beer et al. 2010). Furthermore, intact tropical forests have been reported to sequester the equivalent of about one-half the total terrestrial C sink (Pan et al. 2011). However, recent work has suggested an increasing role of nutrients limiting the productivity of ecosystems; both data and modeling efforts have shown that carbon dioxide (CO_2) uptake by terrestrial ecosystems strongly depends on nutrient availability (Bonan 2008, Peñuelas et al. 2013, Fernandez-Martinez et al. 2014, Wieder et al. 2015). This has instigated the implementation of the effects of nutrient stocks and cycling in land-surface models (Wang et al. 2010, Zaehle and Friend 2010, Goll et al. 2012, 2017) and stressed the need for a profound and mechanistic

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understanding of nutrient cycling effects on the C cycle. Nitrogen (N) is of particular interest, being one of the important macronutrients to sustain plant life. However, the cycling of N is poorly quantified for tropical forest ecosystems, particularly in the Congo Basin.

The nitrogen paradox of tropical forests

Tropical forests are regarded as N-rich ecosystems, where N is cycled in excess. Much like discussed by Taylor et al. (2015), this is supported by multiple lines of evidence such as the high N:P ratio in canopy leaves (McGroddy et al. 2004, Fyllas et al. 2009), high dissolved inorganic N (DIN) losses from streams (Brookshire et al. 2012*a*), high ^{15}N natural abundance in tropical forest soils (Bai and Houlton 2009), and extensive reviews on N cycling in tropical forests (Vitousek 1984, Vitousek and Sanford 1986, Bruijnzeel 1991). The generally high losses of bioavailable N from forest catchments imply the existence of an N input sustaining these losses (Hedin et al. 2009). Although symbiotic biological nitrogen fixation (BNF) is traditionally put forward as a major source of N for natural ecosystems, several studies have now concluded that this process might be down-regulated in N-rich environments, such as old-growth wet tropical forests (Barron et al. 2011, Bauters et al. 2016). This paradoxical sustained BNF has subsequently been explained by spatial heterogeneity where N poor niches are decoupled from the N richness, and where symbiotic or asymbiotic BNF can be maintained (Reed et al. 2011, Menge and Levin 2017). Hence, tropical forests are regarded as a "leaky nitrostat" (Hedin et al. 2009), with an open N cycle, where N input via BNF is flexibly up-regulated when and where local soil N deficiencies occur.

However, the data at the very basis of the nitrogen paradox, i.e., observations of sustained N loss in tropical rainforests, are actually few and of varying quality. First, some of the export budgets have reported DIN losses only (Appendix S1: Table S1), leaving both dissolved organic and particulate organic N (DON and PON) unquantified, although these have proven to be of extreme importance in the overall N balance of some tropical (Taylor et al. 2015, Gücker et al. 2016, Brookshire et al. 2017) and temperate (Perakis and Hedin 2002) forest ecosystems. Second, research results from both montane and lowland tropical forests have not been differentiated but rather just generalized. In parallel to the vision of N-rich and P-poor lowland rainforests, ecologists have a longstanding assumption of tropical high-altitude forests being rather limited by N than P (Tanner et al. 1998, Santiago 2015). Hence, lumping all "tropical forest" catchments should be cautioned against. A final important assumption of the N paradox is that there is low N deposition in most remote sites. Again here, recent studies have shown that DON deposition can contribute largely to total N deposition although it has been ignored in most studies and that the atmospheric N deposition in central Africa is much higher than expected from simulations (Mace et al. 2003, Cape et al. 2011, Cornell 2011, Bauters et al. 2018). The few studies that include DON deposition in N balances usually come up with N inputs that could outbalance reported N export ranges (Appendix S1: Table S2). Meanwhile in modeling efforts, most often only "reactive," inorganic N deposition is analyzed or simulated. Finally, the combination of empirical field measurements of N input and output, to at least attempt to achieve a local N budget balance, is extremely rare for tropical forests (Bruijnzeel 1991). This biases our perception of forest N cycling, since it depends on geographic location (deposition; Dentener et al. 2006), forest type (Staelens et al. 2011), climate (Weintraub et al. 2016), and topography (Weintraub et al. 2014). The availability of studies that extensively characterize the N cycle and its fluxes in tropical forests is also geographically biased, being studied in Costa Rica (Taylor et al. 2015), Hawaii (Vitousek 1984, Hedin et al. 2003), and some other sites in South America and Southeast Asia (Bruijnzeel 1991), with very few data on African tropical forest (Galy-Lacaux et al. 2014).

Tropical forest nitrogen cycling in contrasting abiotic environments

Soil types and/or climate affect N cycling of natural ecosystems (Vitousek 1984, Vitousek and Matson 1988, Vitousek et al. 1995). Simple proxies integrating the local N cycle have been widely assessed in tropical forest environments: litterfall (Vitousek 1984), topsoil $\delta^{15}N$ (Craine et al. 2009, Mayor et al. 2014), canopy stoichiometry (Vitousek et al. 1995, Asner et al. 2015), N losses (Hedin et al. 2003, Brookshire et al. 2012*b*, Gücker et al. 2016), and net soil N transformations (Vitousek and Matson 1988). Although, most of these proxies are considered integrative, they only offer limited insight in the mechanisms behind contrasting N cycle patterns between ecosystems. For example, net rates of N mineralization and nitrification (sensu Davidson et al. 1992) are not necessarily correlated with the gross N mineralization and nitrification rates, and hence only offer a limited view on the soil N cycle (Davidson et al. 1992). Additionally, gross N transformation rates need to be assessed in situ, since lab-based or disturbed soil assessments render non-representative rates (Booth et al. 2006, Arnold et al. 2008, Gütlein et al. 2016). However, quantifying the bulk of these N fluxes—combining patterns in N inputs, N losses, and gross soil N rates—are very labor intensive and logistically challenging in remote places, and therefore very rare in tropical forests (see, e.g., Gerschlauer et al. [2016] for a review of in situ gross N cycle studies of tropical forests). Nevertheless, the contradictions in existing observations of N loss patterns across tropical forest catchments prove that there is a lot to be learned from "holistic" and integrative studies in contrasting environments (Brookshire et al. 2012*b*, Taylor et al. 2015, Gücker et al. 2016).

Tropical forest nitrogen cycling in contrasting biotic environments

In addition to abiotic controls on N cycling, a vital component in the N cycle are the organisms involved in it. Due to the inherent metabolic need of all organisms, ranging from microbes over soil fauna to plants to build in both C and N, organismal growth is inherently linked to N cycle rates. Different studies have pointed to the impacts and effects of plant species on local (Reed et al. 2008, Bauters et al. 2017*b*, Menge and Levin 2017) or landscape-scale biogeochemistry (Knops et al. 2002). However, few studies have directly compared N inputs, outputs, and soil N cycling across forest types, where geography, and thereby climate, soil, and geology, were kept constant. The co-occurrence of both monodominant forests consisting of *Gilbertiodendron dewevrei* (De Wild.) J. Léonard with the lowland mixed forest, and monodominant bamboo forests with the montane mixed forest, on the African continent offer great conditions to asses N cycle differences because of different biotic composition. On both locations, both forest types are climax ecosystems but represent a distinct set of functional traits (Peh et al. 2011*a*). These traits are only proxies for the underlying life-history of plants (Díaz et al. 2015), hence the real mechanisms of monodominance are to be found in underlying processes that are expressions of these traits. For *Gilbertiodendron* forest, for example, authors have suggested that the association with ectomycorrhizal fungi (EcM) is underlying the phenomenon of monodominance (Peh et al. 2011*a, b*, Corrales et al. 2016, Kearsley et al. 2017). One study has reported differences in the local N cycle from a monodominant vs. mixed forest in the Neotropics and concluded that monodominant forest systems are promising model systems to explore the organization of the tropical N cycle, and the consequences of ecological trait assembly on ecosystem functioning in general (Brookshire and Thomas 2013). Consequently, a more elaborate study aiming at quantifying N fluxes in both mixed and monodominant forests would further unravel (1) the potential variability of N cycling due to biotic drivers and (2) how monodominance can establish in a highly diverse tropical forest biome.

Aim and hypotheses

With this study, we aimed to gain insight in the N cycle of tropical forests by making empirical N budgets for different forest types in a poorly documented region. We specifically aimed to answer three main questions: (1) How does N cycling differ in contrasting abiotic environments of lowland vs. montane tropical forests? (2) How does forest type within each geographical location affect the N cycle, i.e., what is the variability that can be expected from a change in biotic forest composition? (3) How does the N cycle of tropical forests in the Congo basin compare to the better-documented South American and Southeast Asian tropical forests?

To answer these questions, we quantified components of the N cycle in four contrasting forest types in the tropical forest of the Congo basin (Democratic Republic of the Congo). We monitored forest N fluxes fortnightly in triplicate in lowland mixed forest, lowland monodominant forest, montane mixed forest, and montane monodominant forest during one hydrological year and complemented these measurements with ^{15}N tracing to quantify in situ gross N dynamics in the soil and N_2O emission via intensive field campaigns in dry and wet seasons. Altogether, this approach aims for an approximate empirical N budget with insights into soil dynamics in four contrasting forest types at two contrasting locations.

Material and Methods

Study sites

The study was carried out in intact old-growth forests at two geographical locations (lowland and montane) in the Congo Basin (DR Congo). At both locations, we assessed two forest types with three repetitions of the experimental setup per forest type (totaling $n = 12$). The lowland sites are situated in the tropical forest near Yoko village, roughly 30 km south of Kisangani, Tshopo province, DR Congo (Table 1; Appendix S1: Table S3, Fig. S1) with mean annual rainfall of 1,800 mm and average temperature of 24.2°C. Vegetation at the lowland location is classified as semi-deciduous rainforest, and the climate falls within the Af-type (tropical rainforest climate), following the Köppen-Geiger classification. Soils in the region are typical deeply weathered and nutrient-poor Ferralsols (Van Ranst et al. 2010), with very limited elevational differences and gentle slopes. The site has two dominant forest types, lowland mixed forest (LMF) and lowland monodominant forest (LMoF), where >60% of the basal area consists of one species *Gilbertiodendron dewevrei* (De Wild.) J. Léonard. The montane forest is situated in the Kahuzi-Biéga National Park, roughly 30 km northwest of Bukavu, South-Kivu province, DR Congo (Table 1; Appendix S1: Table S3, Fig. S1). The national park is part of the Albertine Rift region, with an altitude ranging from 650 to 3,320 m above sea level (asl). Annual rainfall is between 1,500 and 2,000 mm, with a mean annual temperature of 20°C. Most of the accessible part of the park is located around 2,200 m asl, where two main forest types can be found: montane mixed forest (MMF) and monodominant bamboo forest (MMoF). The latter is characterized by the dominance of the bamboo *Yushania alpine* (K.Schum.) W.C.Lin. Soils in the montane region are Ferralsols/Acrisols, with comparable high sand and silt content compared to the lowland sites. We established three plots per forest type of 40 by 40 m, where throughfall, litterfall, and soil solution were sampled. This resulted in four sets of three study plots in LMF, LMoF, MMF, and MMoF, at two locations

TABLE 1. Site characteristics of the different study plots.

	Lowland		Montane	
Type	LMF	LMoF	MMF	MMoF
Elevation (m above sea level)	448.6 ± 4.5	452.7 ± 8.99	2250.7 ± 38.73	2289.0 ± 8.8
Soil class	Ferralsol	Ferralsol	Ferralsol/Acrisol	Ferralsol/Acrisol
Texture				
Sand (%)	73.5 ± 2.7	69.5 ± 2.2	48.4 ± 12.8	62.5 ± 14.4
Silt (%)	22.2 ± 2.7	26.2 ± 2.2	44.4 ± 10.5	35.6 ± 13.7
Clay (%)	4.3a,b ± 0.6	4.3a,b ± 0.4	7.2a ± 2.4	1.9b ± 0.7
C (%)	1.74a ± 0.36	2.77a ± 0.71	14.35b ± 1.7	13.85b ± 3.12
N (%)	0.12a ± 0.02	0.18a ± 0.04	1.31b ± 0.17	1.13b ± 0.18
C:N	14.4a,c ± 1.1	15.0a ± 0.9	11.0b ± 0.1	12.1b,c ± 0.8
pH-H_2O	4.3a,b ± 0.5	3.5a ± 0.2	5.2b ± 0.6	4.1a,b ± 0.1
$\delta^{15}N$ (‰)	10.1a ± 0.9	7.6b ± 0.6	5.6b,c ± 0.6	3.9c ± 0.6

Notes: Texture, carbon (C), nitrogen (N), and all soil variables were measured on composite samples of the top 5 cm of soil in all plots in the lowland mixed forest (LMF), lowland monodominant forest (LMoF), montane mixed forest (MMF), and montane monodominant forest (MMoF). Values are means ± SD. Different lowercase letters indicate statistically significant differences.

(lowland and highland). At both locations, the mixed forest type represents the dominant vegetation type, while the monodominant forests are less abundant.

Water and litter sampling and analysis

Throughfall and bulk precipitation were collected fortnightly using polyethylene (PE) funnels supported by a wooden pole of 1.5 m height to which a PE tube was attached and draining into 5-L PE container. A nylon mesh was placed in the neck of the funnel to avoid contamination by large particles. The container was buried in the soil and covered by leaves to avoid the growth of algae and to keep the samples cool. We installed eight throughfall collectors in each plot as two rows of four collectors, with approximately 8 m distance between all collectors. The soil solution was sampled per study plot by four lysimeters at 20 cm depth, four lysimeters at 40 cm depth and three lysimeters at 80 cm depth. Suction cup lysimeters consisted of a PVC tube fitted with a porous ceramic cup (Eijkelkamp Soil and Water, Giesbeek, the Netherlands) and connected to a buried opaque 2-L glass bottle by a PE tube. A pressure of −500 hPa was applied on each sampling occasion, using a portable vacuum pump (Prenart Equipment, Copenhagen, Denmark). On every sampling occasion, the water volume in each collector was measured in the field, and recipients, funnels, and mesh were replaced and rinsed with distilled water. A volume-weighted composite sample of the devices per plot was made. All samples were stored in a freezer immediately and sent in batch to Belgium for chemical analysis. The volume-weighted composite samples were first filtered using a nylon membrane filter of 0.45 μm before freezing. NH_4^+ was determined colorimetrically by the salycilate-nitroprusside method (Mulvaney 1996) on an autoanalyzer (AA3; Bran and Luebbe, Norderstedt, Germany). NO_3^- was determined colorimetrically using the same autoanalyzer in form of NO_2^- after reduction of NO_3^- in a Cd–Cu column followed by the reaction of the NO_2^- with N-1-napthylethylenediamine to produce a chromophore. Additionally, the total dissolved nitrogen (TDN) was determined by adding 1:1 oxidizing solution of NaOH, H_3BO_3, and $K_2S_2O_8$, and putting it in an autoclave for 1 h at 121°C in order to convert NH_4^+ and dissolved organic N (DON) into NO_3^- (Lachouani et al. 2010). Exchangeable cations (K, Ca, Mg, and Na) were determined by atomic absorption spectrophotometry (Eppendorf, Netheler & Hinz GmbH, Hamburg, Germany). Chloride (Cl^-) was measured in the samples by Ion Chromatography (Thermo-Scientific, Pittsburgh, Pennsylvania, USA).

Additionally, stream water was sampled in a nearby stream at both locations (lowland and mountains), where a V-notch weir (90°) was installed to survey the river water flux and stream water composition. The flow rate was estimated using a bucket and a stopwatch at every sampling occasion. Additionally, a water level height data logger (WT-HR 1500; Trutrack, Christchurch, New Zealand) was installed approximately 2 m upstream of the V-notch, logging the water level every two hours. Overall, the data presented here comprises sampling from October 2015 to October 2016 in the lowland site and from December 2015 to December 2016 in the montane site. The catchment area corresponding to the drainage at the outlet point was determined using ASTER data (ASTGTM v2, 30-m resolution, https://lpdaac.usgs.gov/dataset_discovery/aster), and a D8 flow accumulation algorithm using Whitebox GAT and QGis version 2.18 (*available online*).[9] Subsequently, relations were fitted for the logger height and the stream flow at the sampling dates, and the accumulated drainage flow was calculated using the logged water heights.

Litterfall traps were set up parallel to the throughfall collectors, in the same setup scheme; i.e., two rows of four litterfall traps at approximately 8 m distance between each other. The traps were sampled every two weeks and the collected samples were dried immediately

[9] http://www.qgis.org

after sampling. Branches with diameter >2 cm were discarded, since we were interested in fine litterfall. After drying for 24 h at 70°C, the samples were transported to Belgium for analysis. For the lowland location, all samples were ground for homogenization and subsequently analyzed on an elemental analyzer (ANCA-SL; SerCon, Crewe, UK) coupled to an IRMS (20-20; SerCon). Total N and C litterfall flux were determined by multiplying the N and C content of every sample with the subsequent dry mass of the total sample. For the montane forest types, the samples were pooled per setup and per month, a subset of three sampling dates per study site ($n = 6$) was analyzed, and the average N content was multiplied with the study site's respective litterfall weight.

^{15}N tracing experiment for gross soil N dynamics

In parallel to the monitoring of the 12 plots, a specific in situ ^{15}N labeling experiment using the virtual soil core approach (Rütting et al. 2011) was conducted in August 2016 in the three LMF plots and one LMoF plot (onset of the wet season) and in April 2017 in the three MMF and three MMoF plots (onset of the wet season). The labeling experiment allows an assessment of in situ gross N dynamics in an undisturbed system. Within each plot, we replicated the experiment three times. As such, per replication, we selected two rows of five labeling spots parallel to each other. Subsequently, we simultaneously labeled one row with a $^{14}NH_4{}^{15}NO_3$ and the other with $^{15}NH_4{}^{14}NO_3$ solution, both with 98% ^{15}N atom%. Both solutions contained the same concentrations of NH_4^+ and NO_3^- and were added in the same amount. This was done by using specifically designed, handmade injection devices of 19 1-mL injections and a spatially homogenous pattern, in the top 7 cm of the soil. The labeling spots were then subsequently sampled at different time steps after labeling (8 h, 24 h, 48 h, and 72 h for the lowland sites; 2 h, 8 h, 24 h, 48 h, and 72 h for the montane sites). Sampling was done by taking the "inner soil core" in the center of the labeling area, to avoid border effects of the labeling. Immediately after sampling, the soil samples were transferred to the field laboratory and extracted by shaking 60 g of the sample with 120 mL of 1 mol/L KCl. After exactly 1 h, the extract was filtered through filter paper (MN615; Macherey-Nagel, Darmstadt, Germany), and the extracts were exported to Belgium and Sweden for analysis. As described above, NO_3^- and NH_4^+ concentrations were determined colorimetrically using an auto analyzer. For the Yoko samples, the ^{15}N contents of both nitrogen species were analyzed after conversion to N_2O (Hauck 1982, Stevens and Laughlin 1994), using a coupled trace gas preparation unit (ANCA-TGII; PDZ Europa, Chesire, UK) and an Isotope Ratio Mass Spectrometer (IRMS) (20-20; Sercon). For samples from Kahuzi-Biega, an automatized sample preparation unit coupled to a quadrupole mass spectrometer (GAM 400; InProcess, Bremen, Germany) was used (Stange et al. 2007). The NH_4^+ was oxidized to N_2 with a NaOBr solution in an alkaline medium and NO_3^- was reduced to NO with a $V(III)Cl_3$ solution in an acidic medium (HCl). The results of the analyses were used in the numerical ^{15}N tracing model Ntrace, which is coded in MatLab (Version 7.13; The MathWorks, Natick, Massachusetts, USA) and has several advantages over the classic pool dilution techniques (Rütting et al. 2011). This model uses Mon*t*e Carlo sampling techniques for parameter estimation, and as such estimates the fluxes of a prior defined set of N pools and transformations. For the latter, we assumed the organic NH_4^+ and NO_3^- pool and the major known fluxes between those, i.e., mineralization, NH_4^+ immobilization, nitrification, dissimilatory reduction of nitrate to ammonium (DNRA) and NO_3^- immobilization. Model fits were evaluated using the Akaike Information Criterion, and the resulting shape of parameter distribution functions (Rütting et al. 2011).

We characterized the topsoils of each study site by taking composite samples of five points within each plot of the 0–5 cm depth layer. Samples were dried for 48 h at 60°C. Roots were picked out of the soil samples before grinding the soil for analyses. Carbon and N concentrations, along with the $\delta^{15}N$ of the soils, were analyzed using an elemental analyzer (Automated Nitrogen Carbon Analyzer; SerCon, Crewe, UK), interfaced with an Isotope Ratios Mass Spectrometer (IRMS; 20-20, SerCon). The soil pH (pH_{H20}) of each sample was determined by using a glass electrode (Model 920A; Orion, Cambridge, UK) after suspension of 14 mL soil in 70 mL distilled water (Table 1). Soil texture was determined on a laser defraction particle size analyzer (LS 13 320; Beckman Coulter, Brea, California, USA) after dissolving 1 mg (lowland) and 0.25 mg (highland) of soil in 4 mL of 10% Na-hexametaphosphate, shaking on an orbital shaker for 3 h, and subsequent sonication for 1 min. Particle size determination was done according to the classification system of the United States Department of Agriculture (USDA).

N_2O fluxes

N_2O fluxes were measured daily during one week at the end of the dry season (September–October 2016) and during one week within the wet season (April–May 2017) at one of the mixed and monodominant lowland sites and at one site in the montane mixed forest. For logistical reasons, it was not possible to organize N_2O sampling in the montane monodominant forest. Three non-steady-state (static) chambers (Hutchinson and Mosier 1981, De Klein and Harvey 2015) made of PVC were installed at each site at least 24 h before the first gas sample was taken (diameter = 330 mm, h = 280 mm). For individual flux measurements, chambers were closed for the duration of 1 h and gas samples taken at four evenly spread time points in steps of 20 min from the chamber headspace (0, 20, 40,

60 min) using a syringe. At each time point, 20 mL of sample was stored in pre-evacuated 12 mL vials (Exetainer, Labco, Lampeter, UK) and later transported to ETH Zurich for analysis. The samples were analyzed for N_2O using gas chromatography (456-GC; Scion Instruments, Livingston, UK) and fluxes calculated according to

$$F = \frac{\mathrm{VP}}{\mathrm{RST}} \frac{\Delta C}{\Delta t}$$

where $\frac{\Delta C}{\Delta t}$ denotes the rate of change in concentration (slope of linear regression model), V is the volume of static chamber, R is the gas constant (0.08206 [L atm/K mol]), S is the area, P is the pressure, and T is the temperature. Each chamber was equipped with a thermocouple and temperature measured at each sampling using a handheld reader (HH-25TC, Type T, Omega Engineering, Stamford, USA). Yearly, average flux was calculated by upscaling from these two weeks of data per site, assuming relatively stable soil temperature and soil moisture regimes in these latitudes.

Data processing and analysis

We used plot-averaged values for the volume of the bulk rainfall, throughfall, and lysimeter collectors. The water flux for bulk rainfall and throughfall was calculated by dividing the average water volume by the surface area of the collector. Element deposition was consecutively calculated by multiplying the water volume with the element concentration in that volume. The leaching flux at the level of the suction cup lysimeters was calculated using the Chloride Mass Balance (CMB) method for the lowland cluster (De Schrijver et al. 2004). This method is based on the assumption of conservation of mass between the input of atmospheric chloride and the chloride flux in the subsurface (Eriksson and Khunakasem 1969). The nutrient concentration in the collected water was used to calculate the total nutrient output per surface area of the catchment. For the montane sites, we used sodium as an inert tracer instead of the CMB method because we found very high chloride depositions, supposedly from the Nyiragongo volcano outgassing (Virunga National Park, North Kivu), roughly 100 km north of the montane site. This suggests that substantial retention mechanisms in the soil are impeding the use of CMB for these sites. Due to the lack of relation between N concentration and flow rate, we calculated catchment-scale export by multiplying the accumulated discharge with the concentrations (arithmetic mean, minimum, and maximum) of N measured across all stream samples to estimate the catchment scale losses. The catchment scale losses are assumed to be integrative for the losses under the most dominant forest types, being respectively LMF and MMF in the lowland and the highland site. The overall water balance was evaluated at both locations by comparing the resulting evapotranspiration per area with the simulated evapotranspiration from Global Land Evaporation Amsterdam Model (GLEAM; Miralles et al. 2011, Martens et al. 2017).

For the plot-level data, we conducted one-way Analysis of Variance (ANOVA), with additional Tukey's Honestly Significant Difference (HSD) post hoc testing. When the underlying assumptions were not met, based on Bartlett's test for homogeneity of variance and Shapiro test for normality, the data were log-transformed. Kruskal-Wallis and a subsequent Dunn's test were used to analyze the data that did not meet the ANOVA assumptions after transformation. Significance was determined as $P < 0.05$. All analyses were done using the R software (The R Core Team 2018). We want to stress that, due to the small sample sizes, the statistical test results must be interpreted with caution. Measurements done on only one of the plots per forest type (N_2O, and gross N cycling rates in LMoF) where excluded from statistical analysis.

Results

Hydrology

Over the course of a year, the lowland sites showed throughfall volumes of 1,765 ± 28 and 1,993 ± 87 mm water (mean ± SD) in the LMF and LMoF, respectively, and 2,131 mm of rainfall in the open field. This concurs with an average of 17% canopy interception evaporation in the mixed forest plots, while only 6% in the monodominant plots. The water export from the catchment through river discharge was calculated for the period from 1 April 2015 to 23 January 2016, because of data logger failure after 23 January 2016. For this period, 545 mm water left the 30.1 ha catchment at the outlet point, being 41% of the incident rainfall (open field) during that period. Extrapolating this value for the rest of the hydrological year resulted in a total evapotranspiration of 1,257 mm. For the montane sites, throughfall amounted to 1,627 ± 127 and 1,767 ± 55 mm for the MMF and MMoF, respectively, with open field rainfall of 1,802 mm. MMF and MMoF showed canopy evaporation of respectively 18% and 9%. Catchment scale export through river discharge was monitored from 5 September 2015 to 9 September 2016, and 497 mm left the 11.5 ha catchment, which was roughly 27% of the incident rainfall (Appendix S1: Table S4), resulting in annual total evapotranspiration of 1,315 mm. We evaluated our water balance by comparing calculated with simulated evapotranspiration using GLEAM (Appendix S1: Table S4; Miralles et al. 2011, Martens et al. 2017), revealing that the resulting evapotranspiration values from our empirical data are within the order of magnitude of the GLEAM estimates (Appendix S1: Table S4). Stem flow was not assessed in this study due to logistic constraints, but it has been shown to be only of minor importance to the water balance of tropical forests (Schellekens et al. 2000).

N input, output, and litterfall

Bulk deposition of TDN was similar at both geographic locations, being respectively 18.2 and 21.2 kg·ha^{-1}·yr^{-1}. Canopy passage added a variable amount of N, hence total throughfall N input varied among the different systems: 53.1 ± 3.2, 37.5 ± 10.0, 37.7 ± 0.7 and 27.2 ± 6.7 kg·ha^{-1}·yr^{-1} in, respectively, LMF, LMoF, MMF, and MMoF, with significantly lower NH_4^+ throughfall loads in LMoF, lower ($P < 0.05$) NO_3^- throughfall loads in MMoF, and significantly lower DON throughfall loads in MMF and in MMoF. This resulted in the significantly highest TDN deposition loads in LMF and MMF. The calculated losses of TDN in the dominant forest type at each location (LMF and MMF) via leaching at 80 cm soil depth were in the same order of magnitude, i.e., 11.5 ± 2.8 and 15.5 ± 5.5 kg·ha^{-1}·yr^{-1} for LMF and MMF, respectively. TDN river export at both locations was 7.3 and 7.2 kg·ha^{-1}·yr^{-1} for LMF and MMF (Table 2), respectively. The dissolved N species composition changed through the ecosystems, from throughfall to catchment export, and varied across the different forest types (Tables 2 and 3, Fig. 1). Lowland forests, in general, show elevated export of N in the form of DON. This was confirmed by the leaching data from the most abundant forest type, LMF, showing a DON leasing loss at 80 cm depth that was significantly higher than in all other forest types (Table 2), resulting also in a significant higher DON and lower NO_3^- fraction in those losses (Table 3). However, LMoF showed a distinct leaching pattern, with NO_3^- being by far the most abundant form of N output. Likewise, the montane forests showed a very high stream NO_3^- runoff (Table 3, Fig. 1). In the montane forests, we found that MMoF showed different leaching patterns with less pronounced

Table 2. Calculated yearly nitrogen budgets for lowland mixed forest (LMF), lowland monodominant forest (LMoF), montane mixed forest (MMF), and montane bamboo forest (MMoF).

	Lowland		Montane	
Source	LMF	LMoF	MMF	MMoF
Wet deposition				
NH_4^+	2.4		9.6	
NO_3^-	2.8		5.8	
DON	13		5.8	
TDN	18.2		21.2	
Throughfall				
NH_4^+	12.2a ± 2.3	4.8b ± 0.1	9.4a ± 1.8	12.5a ± 4.6
NO_3^-	14.3a ± 2.0	12.0a ± 0.5	13.9a ± 2.2	5.9b ± 1.6
DON	26.6a ± 1.6	20.7a,b ± 2.3	14.4b ± 0.3	8.8c ± 2.2
TDN	53.1a ± 3.2	37.5b ± 4.2	42.1a,b ± 0.8	30.4b ± 7.4
N in Litterfall	203a ± 11	193a,b ± 28	250a ± 20	132b ± 28
Leaching at 20 cm				
NH_4^+	2.9 ± 1.9	4.8 ± 0.1	2.0 ± 1.1	1.1 ± 0.2
NO_3^-	6.6a ± 3.1	12.0a ± 0.5	19.2a ± 12.6	1.5b ± 1.4
DON	16.0 ± 9.7	20.7 ± 2.3	6.5 ± 4.2	2.7 ± 1.0
TDN	25.5 ± 15.4	37.5 ± 10.0	27.7 ± 17.7	5.2 ± 2.0
Leaching at 40 cm				
NH_4^+	8 .0 ± 7.0	3.8 ± 4.4	1.8 ± 1.0	0.7 ± 0.2
NO_3^-	6.3a,b ± 3.9	18.9b ± 16.7	12.8a,b ± 14.7	1.3a ± 0.8
DON	9.4 ± 2.8	9.6 ± 8.7	2.7 ± 2.54	1.2 ± 0.6
TDN	23.7a ± 7.1	32.3a ± 29.4	17.3a ± 16.3	3.2b ± 1.6
Leaching at 80 cm				
NH_4^+	2.4 ± 1.0	2.0 ± 1.1	0.9 ± 0.5	1.9 ± 2.1
NO_3^-	2.2 ± 0.7	9.7 ± 6	12.7 ± 8.2	2.4 ± 2.7
DON	6.9a ± 1.7	3.3b ± 1.2	1.9b ± 1.19	0.8b ± 0.5
TDN	11.5 ± 2.8	15.0 ± 5.5	15.5 ± 9.7	5.1 ± 3.4
Stream losses†				
NH_4^+	0.7 (0.0–1.7)		1.4 (0.4–8.3)	
NO_3^-	0.4 (0.0–0.9)		3.8 (0.2–7.9)	
DON	6.2 (0.5–18.5)		2 (0.4–4.9)	
TDN	7.3 (0.5–21.1)		7.2 (1–21.1)	

Notes: All numbers are in kg N·ha^{-1}·yr^{-1}. All numbers show the arithmetic mean with standard deviation, except for the stream losses, which shows the arithmetic mean with minimum and maximum in parentheses. Significant differences across forest types are indicated by different letters per type ($P < 0.05$).

†Values for stream losses are for lowland and montane forests.

TABLE 3. The fraction of the different dissolved nitrogen species at both geographic locations and all four forest types, with throughfall and leaching composition in both sampled forest types per location.

	Lowland		Montane	
Source	LMF	LMoF	MMF	MMoF
Throughfall				
NH_4^+	0.23a,b ± 0.04	0.13a ± 0.01	0.25b ± 0.05	0.45c ± 0.06
NO_3^-	0.27a,b ± 0.05	0.32a ± 0.03	0.37a ± 0.05	0.22b ± 0.01
DON	0.50a ± 0.02	0.55a ± 0.04	0.38b ± 0.02	0.33b ± 0.06
Leaching at 20 cm				
NH_4^+	0.12a,b ± 0.05	0.08a ± 0.01	0.08a ± 0.01	0.23b ± 0.10
NO_3^-	0.28a ± 0.07	0.6b ± 0.09	0.69b ± 0.01	0.24a ± 0.15
DON	0.60a ± 0.11	0.32b,c ± 0.10	0.24b ± 0.00	0.53a,c ± 0.10
Leaching at 40 cm				
NH_4^+	0.31 ± 0.21	0.1 ± 0.03	0.13 ± 0.05	0.24 ± 0.07
NO_3^-	0.26a ± 0.10	0.6b ± 0.03	0.63b ± 0.17	0.37a,b ± 0.12
DON	0.44 ± 0.31	0.3 ± 0.02	0.23 ± 0.12	0.39 ± 0.04
Leaching at 80 cm				
NH_4^+	0.20a,b ± 0.04	0.14a,b ± 0.06	0.06a ± 0.01	0.38b ± 0.30
NO_3^-	0.19a ± 0.03	0.62b ± 0.17	0.81b ± 0.02	0.41a,b ± 0.25
DON	0.61a ± 0.07	0.25b ± 0.14	0.13b ± 0.01	0.20b ± 0.11
Stream losses†				
NH_4^+	0.09		0.19	
NO_3^-	0.05		0.53	
DON	0.85		0.28	

Notes: All numbers represent the fraction of the total budget numbers per forest type. All numbers show the arithmetic mean with standard deviation. Significant differences across forest types are indicated by different letters per type ($P < 0.05$).

†Values for stream losses are for lowland and montane forests.

dominance of NO_3^- loss. Moreover, NH_4^+ was more prevalent in throughfall and leaching compared to MMF. Additionally, both throughfall inputs and hydrological losses of N were lower in the bamboo soil. Litterfall was significantly lower in MMoF compared to LMF and MMF, while similar across LMF, LMoF, and MMF (Table 2).

Gross N soil transformations

The ^{15}N labeling experiment showed a high consistency of gross rates within each experimental plot, but a low consistency across plots within the same forest type (Appendix S1: Table S5), although the gross mineralization to immobilization (*M*/*I*) ratios were more consistent within LMF, MMF, and MMoF (Table 4). The montane sites showed significantly lower *N*/*M* ratios compared to the lowland sites, but across all sites NO_3^- production was more or less balanced by NO_3^- consumption, with dissimilatory nitrate reduction to ammonium (DNRA) consuming relatively more NO_3^- in the montane sites, while being negligible in the lowland sites (Table 4). Overall, the highest gross mineralization rates were found in both montane sites (94.9 and 43.0 μg $N \cdot g^{-1} \cdot d^{-1}$, respectively), and at all sites, NH_4^+ immobilization was by far the most important NH_4^+ consumption process. For both lowland and montane locations, the monodominant plots showed similar *M*/*I* and *N*/*M* ratios as the mixed. However, both lowland sites (LMF, LMoF) showed a higher *N*/*M* ratio compared to the montane sites. Overall, all sites displayed a very tight soil N cycling, with *M*/*I* ratios that were close to 1 across all sites.

Soil N_2O fluxes

We found similar flux rates during the dry and the wet season at LMF and LMoF with a total median emission of 1.99 and 1.75 kg $N \cdot ha^{-1} \cdot yr^{-1}$, respectively (0.23 and 0.20 $nmol \cdot m^{-1} \cdot s^{-1}$). The MMF site, however, showed much higher fluxes during the wet season with an average total median emission of 3.45 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (0.39 $nmol \cdot m^{-1} \cdot s^{-1}$) across both seasons.

DISCUSSION

Comparing lowland and montane mixed forest (LMF vs. MMF)

In general, lowland tropical forests are considered to be N rich and P limited (Hedin et al. 2009). The combination of high N deposition rates (Bauters et al. 2018) and the downregulation of symbiotic BNF (Bauters et al. 2016) suggests that lowland tropical forests in the Congo Basin are indeed N rich. Moreover, recent work has shown that N availability is lower in high-altitude than in lowland African and Neotropical forest (Tanner et al. 1998, Bauters et al. 2017*a*), but does not per se indicate N limitation in montane forest. Bulk wet

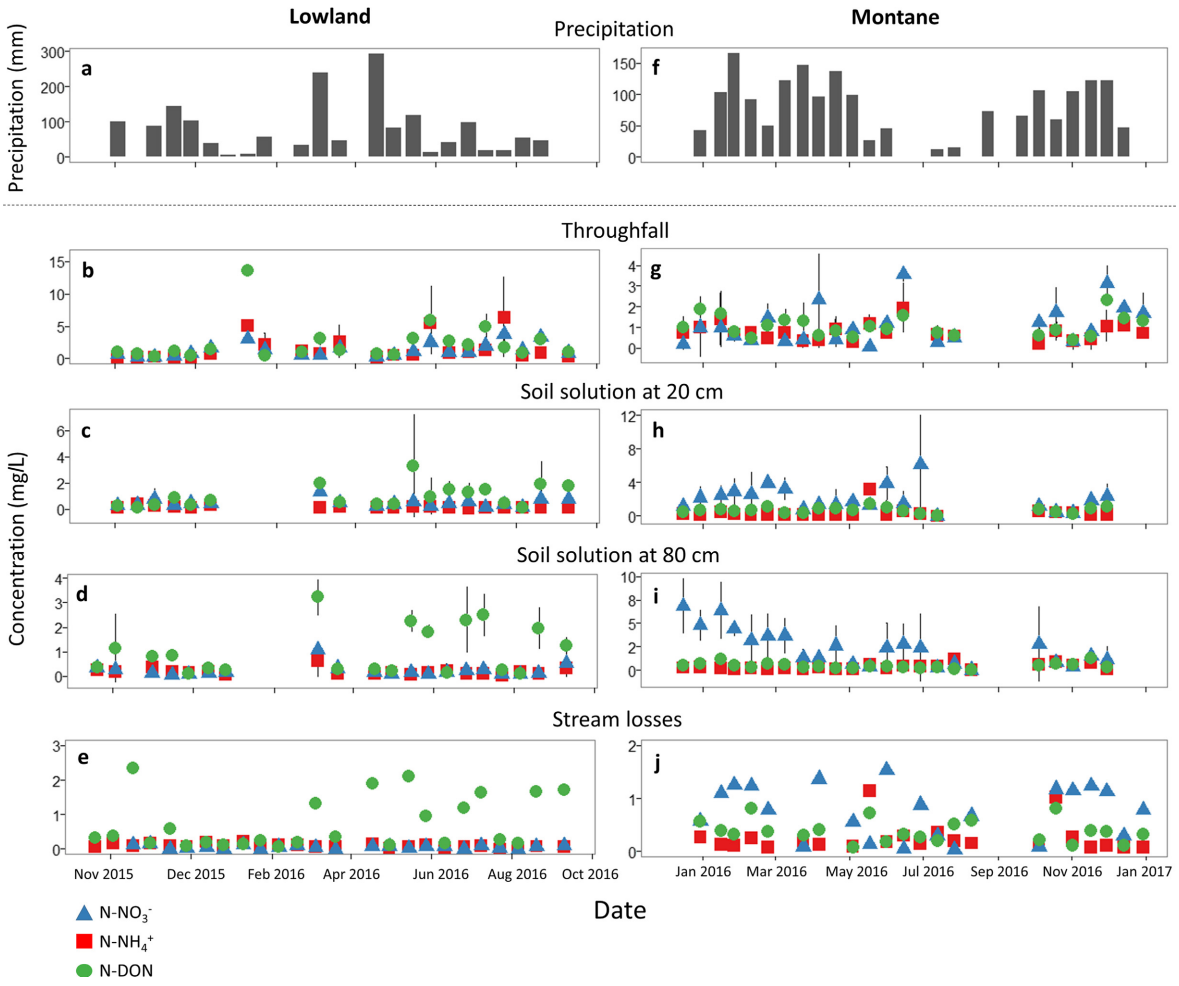

FIG. 1. (a, f) Precipitation and (b, g) composition of nitrogen species over one hydrological year of throughfall, (c, h) soil solution at 20 and (d, i) 80 cm depth, and (e, j) stream losses, respectively, for the lowland mixed (a–e) and the montane mixed forest (f–j). Blue triangles are nitrate, red squares are ammonium and green circles dissolved organic N (DON). Error bars show the standard deviation on the arithmetic mean of the three monitoring plots per forest type. Note that the *y*-axes of lowland and montane plots have different ranges.

TABLE 4. Gross soil N transformations (μg $N \cdot g^{-1} \cdot d^{-1}$), including dissimilatory nitrate reduction to ammonium (DNRA), for lowland mixed forest (LMF), lowland monodominant forest (LMoF), the montane mixed forest (MMF) and the montane bamboo forest (MMoF), gross mineralization to total immobilization ratios (M/I), and gross nitrification to gross mineralization ratios (N/M).

N fluxes	LMF	LMoF	MMF	MMoF
Gross NH_4^+ production				
Mineralization	5.62a (3.70)	8.00	94.87b (24.61)	43.00b (16.63)
DNRA	0.05a (0.03)	0.03	0.94b (0.85)	0.98 (0b.41)
Gross NH_4^+ consumption				
Nitrification	1.47 (0.35)	1.51	1.23 (1.06)	2.38 (1.42)
NH_4^+ immobilization	4.27a (3.99)	6.65	97.00b (23.78)	42.00a,b (12.83)
Gross NO_3^- production				
Nitrification	1.47 (0.35)	1.51	1.23 (1.06)	2.38 (1.42)
Gross NO_3^- consumption				
DNRA	0.05a (0.03)	0.03	0.94b (0.85)	0.98b (0.41)
NO_3^- immobilization	1.36 (0.36)	1.57	0.83 (1.43)	0.79 (1.37)
M/I	0.99	0.97	0.97	0.99
N/M	0.26a	0.19	0.01b	0.06a,b

Notes: The rates show the mean and standard deviations over the three sites for LMF, MMF, and MMoF, while only one site was included in the tracer study in the LMoF. Significant differences across forest types are indicated by different letters per type ($P < 0.05$).

deposition and catchment-scale export found in this study are very similar across both locations. However, the most abundant forest types at both the lowland and the highland site (i.e., respectively, lowland mixed and montane forest) showed a striking difference in composition of hydrological N losses, as well as gross N transformation rates.

In the lowland forests, soil pore water became gradually dominated by DON with leaching depth, which was confirmed by the catchment-scale export. Although, traditionally, DIN has been regarded as the main hydrological N output from tropical forests (Bruijnzeel 1991, Schwendenmann and Veldkamp 2005, Brookshire et al. 2012*a*), findings of DON dominated export from river catchments in lowland tropical forest have been reported as well (Neill et al. 2001, Taylor et al. 2015, Gücker et al. 2016). A closer look at measured values in the literature indicates that DON losses are almost always at least as high as DIN losses in lowland tropical forest (Appendix S1: Table S1). This is in contrast to the general concept that nitrification and subsequent NO_3^- leaching is the primary mechanism for N export in lowland tropical forest (Bruijnzeel 1991, Neill et al. 2001, Hedin et al. 2003, Corre et al. 2010). This observation is also supported by the gross N dynamics we found in the lowland forest. While the absolute gross rates across the three LMF sites were highly variable, the *M*/*I* ratios were very consistent around 1, pointing at an almost complete re-immobilization of mineralized N. Additionally, absolute mineralization rates for LMF sites were on the low side of reported results from pool dilution experiments (see Appendix S1: Table S6 for an overview) but in the same order of magnitude of some recent work in tropical lowland forest (Silver et al. 2005, Sotta et al. 2008, Wieder et al. 2013, Allen et al. 2015). The rates and ratios are closely resembling reported rates from lowland tropical forest in Costa Rica (Wieder et al. 2013), where a very similar dominance of organic N losses was found (Taylor et al. 2015). Furthermore, only a small fraction of the mineralized N was nitrified in the lowland forest, resulting in an *N*/*M* ratio of 0.26 and thus low soil pore water NO_3^-. This soil water NO_3^- is directly available for plant uptake, supposedly resulting in low NO_3^- leaching in LMF. Generally, these transformation ratios suggest a very tight turnover of soil N that is controlled by the microbial activity through high immobilization (of $NH4^+$) and a relative low nitrification.

The observations from lowland forest are in stark contrast to the high soil pore water and stream water NO_3^- concentrations observed in the montane forest with comparatively low DON losses. Previous reports have indeed also shown high NO_3^- losses from some Neotropical montane forests, which has led to the belief that many tropical montane forests are not N-limited (Mcdowell and Asbury 1994, Brookshire et al. 2012*b*, Rütting et al. 2014). This is further supported by the lower soil C:N ratio in the montane compared to lowland forest, which is an indicator of higher soil available N (Appendix S1: Table S6). While we found similar *M*/*I* ratios in MMF, the very low *N*/*M* ratio of 0.01 suggests that almost none of the mineralized N was nitrified. We must, however, acknowledge that this low *N*/*M* ratio is mainly driven by the very high mineralization rates, while the absolute gross nitrification rates were comparable to the lowland. Indeed, these high gross mineralization rates in montane forests suggest a much higher absolute organic matter turnover in montane forest compared to lowland forest, which is in contrast with earlier research on net mineralization rates (Marrs et al. 1988, Vitousek and Matson 1988). However, it does corroborate the recent finding that gross soil N mineralization is mainly controlled by organic matter content of the soil (Figueiredo et al. 2016). We find the same positive relation between gross mineralization and soil C and N content, and a negative relation between gross mineralization and soil C:N (Tables 1 and 4), which is in accordance with a meta-analysis on gross soil N transformations (Booth et al. 2005). This further reiterates that gross transformations are mainly determined by the quantity and quality of the organic matter, an effect that dominates any climatic effects on gross soil N transformations.

Overall, we cannot reliably compare ecosystem N uptake of LMF and MMF, since only N cycling via litterfall was assessed, ignoring amongst others woody and fine root productivity. Recent work has shown that net primary productivity (NPP) decreases in high-altitude forests compared to lowland forest (Malhi et al. 2016). If a similar lower NPP would be also be true in our MMF sites, than this could further explain DIN-dominated losses in our montane sites, via a decreased uptake of the available NO_3^-. However, the observation of DIN-dominated losses in montane forest vs. DON-dominated losses in lowland forest can currently not be directly explained by our gross soil N transformation rates. We must stress, however, that the gross N transformations were only assessed in the topsoil, which does not necessarily reflect processes in the deeper soil layers. Furthermore, the microbial community at both locations seems to very efficiently retain N in the upper soil layers, and this tight N cycling takes place in an N rich environment, given the high atmospheric N deposition and N mineralization rates. In fact, the contrast in N species losses in LMF and MMF seems to suggest that the much higher gross N rates in MMF lead to an increased susceptibility for NO_3^- leaching, while the DON losses from LMF might point to an actual excess of mineral N. Additionally, there are abiotic soil processes that might play an important role in these sites. Nitrogen addition experiments in old-growth N-rich forests have shown that abiotic sorption of NO_3^- plays an important role in dampening the expected NO_3^- hydrological losses (Lohse and Matson 2005). Additionally, there is an increasing awareness about abiotic conversion of nitrification intermediates to gaseous N in conditions of low pH and high Mn or Fe content, which might be an important unaccounted abiotic loss pathway (Heil et al. 2016, Liu et al. 2017).

Hence, physicochemical abiotic parameters might play an important role in retaining or processing NO_3^- in the upper soil layer, and the in situ ^{15}N tracing method is not able to differentiate between biotic or abiotic NO_3^- immobilization or processing. Therefore abiotic NO_3^- immobilization via the ferrous wheel hypothesis, Fe-ammox or NO_3^- adsorption to variable-charge soils might also contribute to the reported NO_3^- immobilization rates (Davidson et al. 2003, Lohse and Matson 2005, Jiang et al. 2015).

Local differences in N cycling linked to biotic differences (LMF vs. LMoF; MMoF vs. MMF)

The intriguing monodominance of *Gilbertiodendron dewevrei* in the monodominant lowland forest type has been discussed broadly during the last decade (Torti et al. 2001, Peh et al. 2011*b*, Cassart et al. 2016, Kearsley et al. 2017). One of the hypotheses trying to explain the underlying mechanism for monodominance is that a specific combination of *Gilbertiodendron dewevrei* traits and strategy ensures a low soil N availability and hence acts as an environmental filter for other species to establish under mature stands, as also observed in monodominant forests in South America (Torti et al. 2001, Brookshire and Thomas 2013). One study from LMoF vs. LMF forests in central Africa has suggested that a slower litter decomposition in LMoF drives SOC build-up (Cassart et al. 2016) given a similar litterfall (Table 2), and hence a more closed N cycle. This corroborates with the significantly lower $\delta^{15}N$ we found in the topsoil of LMoF. Furthermore, *Gilbertiodendron* associates with ectomycorrhiza (EcM), which in turn are known facilitators of plant growth via organic N uptake. This is in contrast with LMF, which is predominantly associated with arbuscular mycorrhizal (AM) fungi. As such, low soil DIN levels have been reported from EcM monodominant forest stands, presumably driven by the EcM-mediated drawdown of the readily mineralizable DON pool (Corrales et al. 2016). Direct evidence for this is lacking, in part, because the typical plant $\delta^{15}N$-depletion that normally results from the EcM symbiosis as observed in the temperate forest is apparently absent in lowland tropical forest (Mayor et al. 2015). Nevertheless, insights via structural equation modeling, the high degree of root colonization, and the observations of $\delta^{15}N$-enrichment of EcM sporocarps in similar sites suggest an EcM-mediated N-acquisition in EcM forests in lowland tropics (Tedersoo et al. 2012, Mayor et al. 2015). Building on this, the differing pattern of N losses between lowland AM and EcM forest sites indeed suggests that their N-acquisition strategy is different. Hence, the switch from DON to NO_3^- dominated losses in LMoF has two possible explanations: (1) the ability of monodominant forest for EcM-mediated scavenging of DON, leaving the DIN pool to leach, or (2) higher net mineralization and hence less efficient immobilization under EcM-dominated forests. The results from our ^{15}N tracing experiment revealed that there are no significant differences in gross N cycling in monodominant forest vs. mixed forest. Accordingly, both *M*/*I* and *N*/*M* ratios indicated tight N cycling in monodominant and mixed forest, with relatively low nitrification. In conclusion, our data suggest that the dominance of NO_3^- leaching under the monodominant forest in central African forests is caused by a reduced uptake of NO_3^- by plants or EcM, and we conclude that altered N cycling might not be the mechanism driving the competitive advantage of these EcM communities in Afrotropical forests. Indeed, although the N fluxes are altered under EcM communities, other nutrients than N (e.g., P) might be promoting monodominance in lowland forests (Newbery et al. 1997, Chuyong et al. 2000, Kuyper 2012).

The montane sites, MMF and MMoF, showed a striking difference in throughfall deposition loads, supposedly caused by either decreased dry deposition or decreased canopy leaching of N under the thin bamboo canopy. These lower deposition loads might explain the lower soil NO_3^- quantities leaching out under the monodominant montane forest, by which the relative contribution of NH_4^+ in the leachate composition increased. As such, the hydrological losses of the monodominant montane forest were not NO_3^- dominated, in contrast with the montane mixed forest.

Overall, the results from different forest types at both locations (lowland and upland) show that biotic differences in forest composition can strongly affect N cycling within the ecosystem. Via the differences exhibited above, we show that important contrasts can arise along the entire critical zone of the ecosystem, i.e., in throughfall, soil N transformations, and, subsequently, leaching patterns. This finding complicates the upscaling of N cycling for both empirical and modeling efforts and shows that biotic forest composition needs to be explicitly considered.

Nitrogen budget for African tropical forests

Dry deposition is challenging to assess, and the methods available lead to highly variable results (Hofhansl et al. 2011), hence we did not assess it directly here. However, there is strong evidence from the lowland sites that important proportions of dry deposition are exogenous (fire-derived) N inputs, resulting in a total deposition on the Congo basin's tropical forest that is substantially higher than expected, with an important organic component. Additionally, N_2O emissions from both the dry and the wet season in one of the LMF, LMoF, and MMF sites resulted in rough estimates of 1.99, 1.75, and 3.45 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$, respectively. Altogether for LMF, this amounted to 18.2–53.1 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ throughfall with 10.1 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ losses, of which nearly 80% were hydrological, resulting in an imbalance of roughly 8–40 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$. For the other sites, not taking into account dry deposition, a

similar minimum imbalance of roughly 10 kg $N \cdot ha^{-1} \cdot yr^{-1}$ was estimated. Although this specific study did not directly assess BNF, we can reliably adopt results from a recent study in an old-growth forest resembling closely our LMF sites. At roughly 200 km from this study's lowland cluster, Bauters et al. (2016) concluded from an absolute absence of nodules on roots of N fixers, that symbiotic BNF is probably downregulated in lowland mature forest. This leaves the asymbiotic, free-living, nitrogen fixation as a big unknown in our study sites (Hedin et al. 2009). The question that arises here, however, is whether free-living nitrogen fixation still takes place in the studied forest types, which are already showing a higher input than output. In other words, are there N-poor niches for asymbiotic BNF to take place in these sites, given the relatively high N deposition they are subjected to? Thus, for these central African forests sites, we found an apparent lack of N output and question the fate of these high N deposition loads (Fig. 2). This is in sharp contrast with the general concept of "N-leaky" tropical forest, based on observations from mainly Neotropical forests (Hedin et al. 2009), where an

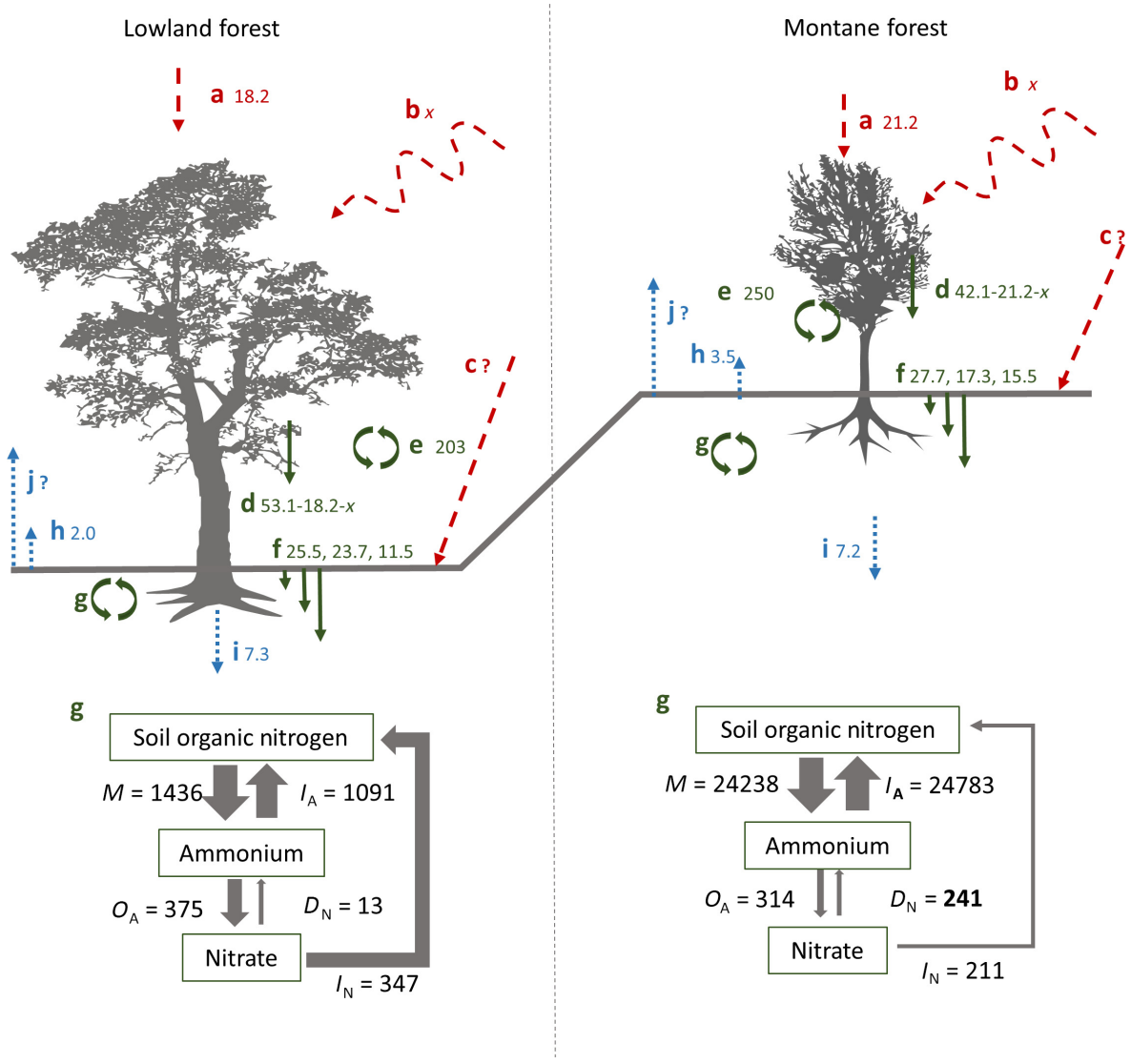

FIG. 2. The nitrogen cycle in lowland mixed forest (left) and montane mixed forest (right) in the Congo basin with (a) bulk deposition, (b) dry deposition on top of the canopy, (c) N fixation, (d) canopy leaching, (e) litterfall, (f) lysimeter leaching at 20, 40, and 80 cm depth, (g) the soil gross N dynamics in detail shown in the lower part of the figure; gross mineralization (M), autotrophic nitrification (O_A), NH_4^+ immobilization (I_A), NO_3^- immobilization (I_N), and dissimilatory NO_3^- reduction to NH_4^+ (D_N), (h) N_2O emissions, (i) catchment scale hydrological export, and (j) N_2 emissions. All numbers, except those in g, show total dissolved nitrogen (TDN), and all numbers expressed kg $N \cdot ha^{-1} \cdot yr^{-1}$. Red (dashed) arrows are inputs, blue (dotted) outputs and gray (solid) arrows are internal fluxes; x represents the unknown dry deposition.

apparent lack of input was noted. Hence, we conclude that including organic N in the forests' inputs and outputs results in an N balance that is in contrast with the current concept of low-input–high-hydrological losses in tropical forests.

There are two outputs that have been shown to be important in other tropical forest catchments and that were not assessed in this study, namely (1) the particulate organic nitrogen (PON) export and (2) other gaseous N species (NO_x and N_2). The importance of PON for budgets has been shown for geomorphically active lowland forests recently (Taylor et al. 2015). Additionally, gaseous N losses other than N_2O, on the other hand, are also likely to play an important role in the overall N budget. (Chemo-)denitrification (i.e., the reduction of NO_3^- to NO, N_2O, or N_2 under anoxic conditions) has been hypothesized to be a major loss pathway for some tropical forests (Houlton et al. 2006). However, N_2 is only rarely assessed in the field, because it is notoriously difficult to measure due to the high background concentrations of N_2. Likewise, Templer et al. (2008) tracked only 55% of the produced NO_3^- in plant uptake, leaching, DNRA, N_2O fluxes, and microbial N uptake during their ^{15}N tracing experiment in Puerto Rican tropical forest, and concluded that other sinks, such as denitrification to N_2, might have largely removed the remaining 45%. In soils with high organic matter content and under wet conditions, denitrification is likely to convert most of the NO_3^- to N_2 (Potter et al. 1996), hence N_2 could be a drastically underestimated loss (Davidson et al. 2000, Holtgrieve et al. 2006). Empirical estimations of N_2 production from old tropical forests were roughly four times higher than N_2O production (Hedin et al. 2003), and up to five times in process-based models (Bai and Houlton 2009). Moreover, recent isotopic studies and ecosystem models have pointed out that denitrification losses in humid tropical forests make up a large fraction of total ecosystem N losses (Houlton et al. 2006, Brookshire et al. 2017). Others have shown that terrestrial denitrification fluxes are underestimated by up to 98%, and that N gaseous export exceeds NO_3^- by a factor six (Fang et al. 2015). Several processes might play a role for N_2 losses, including ammonium oxidation coupled to nitrite reduction (anammox; Xi et al. 2016). In specific for these sites, however, it seems more likely that ammonium oxidation coupled to iron reduction (i.e., Feammox) or other forms of chemo-denitrification play an important role in soils with high Fe content (Yang et al. 2012, 2015, Xi et al. 2016) and low pH (Heil et al. 2016, Liu et al. 2017), in which case denitrification would be partly decoupled from biological activity; but further observations are needed to assess this. Altogether, developing novel methods to measure N_2 in situ is vital to understand both the N balance of tropical forests and to gain insight in the driving factors behind N_2O:N_2 partitioning during nitrification and (chemo) denitrification.

Conclusions

How does the abiotic environment affect N cycling in tropical forests by comparing montane and lowland forest?

Our study showed low NO_3^- losses, but high DON losses and a gross mineralization to immobilization ratio of nearly 1 N-rich lowland tropical forest. This implies that this tropical forest soil efficiently retain N, with high internal gross N cycling rates. Instead, we found a more open soil N cycle showing higher N_2O emissions combined with higher NO_3^- losses under montane forest, which is traditionally considered N limited. The gross soil N rates seemed fully determined by the soil organic matter content and quality, rendering the gross rates to be an order of magnitude higher in the montane vs. the lowland forest, which was a surprising finding. Overall, these highly different soil N transformation rates and the distinct leaching patterns were the most apparent differences, but the N budgets for both forest types overall rendered a very similar imbalance, with an apparent missing N output.

How does forest type within each geographical location affect the N cycle, i.e., what is the variability that can be expected from a change in biotic forest composition?

Biologically distinct forest types within the same environment also showed very contrasting leaching compositions. N leaching under a lowland EcM-dominated forest was NO_3^- dominated, in fact, much like the montane mixed forest, but in contrast with lowland AM-dominated forest. Additionally, both of our montane sites showed differences in throughfall composition that might cause a compositional shift in the leachate. Overall, we argue that N flux differences (input – cycling – output) inferred from biotic contrasts might be as important as those that arise from environmental contrasts.

How does the N cycle of tropical forests in the Congo basin compare to the better-documented South American and Southeast Asian tropical forests?

Independent from all abiotic/biotic differences, and contrary to studies from the Neotropics, the hydrological and N_2O losses in all of our studied forest types were substantially lower than the minimum input levels. Indeed, this reiterates the importance of quantifying all N fluxes and including organic nitrogen in balances and leaves us with questions on the ultimate fate of the high N deposition in central African forests (Fig. 2). Furthermore, we conclude that either particulate N loss or (chemo-)denitrification might be one of the keystone parameters to close the N budget of African tropical forest, bridging the discrepancy between high N input and output through unaccounted losses of N_2 and/or NO and particulate N.

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Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecm.1342/full