Stable isotope signatures of soil nitrogen on an environmental-geomorphic gradient within the Congo Basin

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Abstract.

Nitrogen (N) availability can be highly variable in tropical forests on a regional and on a local scale. While environmental gradients influence N cycling on a regional scale, topography is known to affect N availability on a local scale. We compared stable isotope signatures ($\delta^{15}N$) of soil profiles in tropical lowland forest, tropical montane forest, and subtropical Miombo woodland within the Congo Basin as a proxy to assess ecosystem-level differences in N cycling. Furthermore, we examined the effect of surface slope angles on $\delta^{15}N$ in the same forests to quantify local differences induced by topography. Soil $\delta^{15}N$ profiles indicated that the N cycling in in the montane forest is more closed and dominated by organic N turnover, whereas the lowland forest and Miombo woodland experienced a more open N cycle dominated by inorganic N. Furthermore, our results show that slope angles only affects the soil $\delta^{15}N$ signature in the Miombo forest, which is prone to erosion due to the lower vegetation cover and intense rainfalls at the onset of the wet season. Lowland forest, on the other hand, with a flat topography and protective vegetation cover, showed no influence of topography on soil N cycling. Values from the montane forest showed high variability in stable isotope signatures, but they were not constrained by topography. A pan-tropical analysis of soil $\delta^{15}N$ values (i.e. from our study and the literature) reveals that soil $\delta^{15}N$ is best explained by factors controlling erosion, namely mean annual precipitation, leaf area index, and slope angles. The erosive forces vary immensely between different tropical forest ecosystems and our results highlight the need of more spatial coverage of N-cycling studies in tropical forests, to further elucidate the local impact of topography on N cycling in this biome.

1 Introduction

The nutrient status of forests is an important determinant for the allocation of sequestered carbon (C) to biomass (Vicca et al., 2012), with nitrogen (N) and/or phosphorus (P) limitation potentially constraining net primary productivity (NPP) (Alvarez-

Clare et al., 2013; Fernández-Martínez et al., 2014; Townsend et al., 2011; Vitousek et al., 2010). In addition, models show that the increase of primary production due to CO_2 fertilization will be constrained by nutrient limitations (Wieder et al., 2015). Therefore, understanding nutrient cycling in forests globally is key to assess present and future forest productivity (Townsend

35   et al., 2011; Wieder et al., 2011). However, given their major role in the global C cycle (Lewis, 2006; Malhi and Grace, 2000), quantifying nutrient supply and availability seems especially important for tropical forests.

Up-to-date, research has revealed high diversity in nutrient cycling with substantial variation from regional to local scales throughout the tropics. Old and highly weathered soils in tropical lowland forests are typically considered to be rich in N and

40   poor in rock-derived P (e.g. Vitousek 1984). In contrast, tropical montane forests that lie on steep terrain are subjected to geomorphic processes that rejuvenate soils with bedrock nutrients through uplift and erosion. In regions with a moderate uplift, P depletion is unlikely to occur due to this rejuvenation (Porder et al., 2007). As a result of reduced N supply in higher altitudes, due to slower N mineralization processes at lower temperatures, it is more likely that plant growth is limited by N availability in tropical montane forest (Bauters et al., 2017; Soethe et al., 2008; Tanner et al., 1998). It is important that nutrient availability

45   in tropical forests are not being generalized and differentiations between different ecosystems within the tropics are being made. Therefore, analysis of N cycling within ecological gradients are necessary. The "openness of the N cycle" is a good metric to describe cycling differences across ecosystems with contrasting N availabilities. While in N limited ecosystems the internal cycling and recycling of N is relatively more important than the absolute in- and outputs (closed N cycle), the opposite is true for ecosystems that are rich in N (open N cycle) (Boeckx et al., 2005).

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Furthermore, at the local scale, topography has been identified as a main determinant for both biogeochemical and ecological variability in tropical forests (Asner et al., 2015; Hofhansl et al., 2020) in part through its effect on geomorphic process activity. In intact forest, slope gradient is the main control on physical erosion intensity at the hillslope scale (Roering et al., 2001). Accumulated topsoil N has shown to be affected by physical erosion (Amundson et al., 2003; Hilton et al., 2013; Perakis et

55   al., 2015; Weintraub et al., 2015), resulting in a close relation between nutrient availability and geomorphic process rates. Therefore, while at regional scales mainly climatic and environmental gradients are responsible for differences in nutrient availabilities (Prescott, 2002; Read, 1991), hydro-geomorphic gradients seem to be a main determinant of N cycling at the local scale (Amundson et al., 2003). Recently, studies from Taiwan and Costa Rica have suggested that erosion has a significant control on N cycling (Hilton et al., 2013; Weintraub et al., 2015). They found indications of lower N availability and more

60   closed N cycle in steeper sloping positions. This effect has only been reported from more geomorphic active sites of the tropics (Costa Rica and Taiwan) and the magnitude of this effect in more stable landscapes is unknown calling for a consistent study across geomorphic gradients in the tropics.

The stable isotope composition of N ($\delta^{15}N$) is a useful proxy to assess various N cycling processes (Amundson et al., 2003;

65   Martinelli et al., 1999). Craine et al. (2015) synthesized global soil $\delta^{15}N$ data to understand patterns of N cycling and show

how soil δ15N values give insights in input and output pathways of N in the system (Högberg, 1990; Högberg and Johannisson, 1993). As such, climatic conditions such as rainfall (Austin & Vitousek, 1998; Boeckx et al., 2005), mean annual temperature (Boeckx et al., 2005) and soil moisture (Handley et al., 1999) all influence soil N isotopic signature. Generally, soil δ15N also signals the openness of the N cycle (Boeckx et al., 2005). On a local scale, soil erosion alters the δ15N signature and leads to

70 more depleted values (Hilton et al., 2013; Weintraub et al., 2015). Because soil erosion is a non-fractionating process, the combined fractionation of a system decreases when soil erosion becomes more important relative to fractionating N losses, such as denitrification. In this case, the isotopic signature of the remaining N in the system more closely resembles the signature of the N inputs compared to systems with less erosion.

75 The objectives of this study were 1) to assess the differences in N availability and N openness in three different forest types along an environmental-geomorphic gradient in the Congo Basin and 2) evaluate the extent and 3) drivers of physical erosion and its effects on N availability in these forests. To answer these objectives, soil N content and the stable isotope compositions were measured in soil profiles taken from different topographical positions in three different forest ecotypes. We used δ15N signatures as a proxy that integrates N transformations for differences in N availability and the openness of a system. We

80 hypothesized N availability and openness of N cycle would be highest in lowland tropical forest, that δ15N signatures would be lower on steeper slopes, and that this effect would be more pronounced in the montane and Miombo forests, where the erosional forces are higher compared to the lowland forest.

2 Methods

2.1 Site description

85 We selected representative sites for the Congo Basin's major forest ecotypes (tropical montane forest, tropical lowland forest, and the semi-dry Miombo woodland). The montane forest site is situated in the Kahuzi-Biéga National Park (2.344° S, 28.746° E), northwest of the city of Bukavu in the eastern part of Democratic Republic of the Congo (DRC). Two forest subtypes are present in the Park: montane mixed forest and monodominant bamboo forest. Soil samples were taken in the mixed forests. Soils are classified as Ferralsols/Acrisols (Bauters et al., 2019) (Table 1). The topography of the sampled forest is characterized

90 by steep slopes (Figure 1). Mean annual temperature is ~15°C and mean annual rainfall is ~1970 mm (Fick and Hijmans, 2017). Rainfall is seasonal with two peaks in April and October and a dry season that lasts from June to September.
Samples from the tropical lowland forest were taken in the Yoko Forest Reserve (0.291° N, 25.295° E), south of the city of Kisangani. The Yoko Forest Reserve consists of two forest subtypes: a mixed lowland tropical forest and a monodominant forest, where the *Gilbertiodendron dewevrei* species comprises more than 60 % of the basal area. Soils in this area are classified

95 as deeply weathered Ferralsols (Van Ranst et al., 2010). The sampling site exhibited low variation in elevation and contained mainly gentle slopes (Figure 1). Samples were taken between altitudes of 440 – 460 m a.s.l. Mean annual temperature is ~24°C

and mean annual rainfall is ~1760 mm (Fick and Hijmans, 2017) (Table 1). Yoko experiences a short wet season from March to May and a longer one from August to November.

For the Miombo woodland, a site 50 km north of the city of Lubumbashi was selected (11.212° S, 27.233° E). A pristine
100 Miombo is characterized by deciduous woodland dominated by trees of the *Brachystegia, Julbernadia* and *Isoberlinia* genera (Campbell, 1996). As charcoal production and seasonal fires are a common occurrence, soil sampling in areas with recent charcoal production activities was avoided. We classified the soils as Acrisols and Cambisols. The topography is mostly flat and the elevation ranges between 1260 and 1310 m a.s.l (Figure 1). This region experiences a wet season from October to April, with a peak rainfall in December. Mean annual rainfall is ~1250 mm and mean temperature is ~21 °C (Fick and Hijmans,
105 2017)(Table 1).
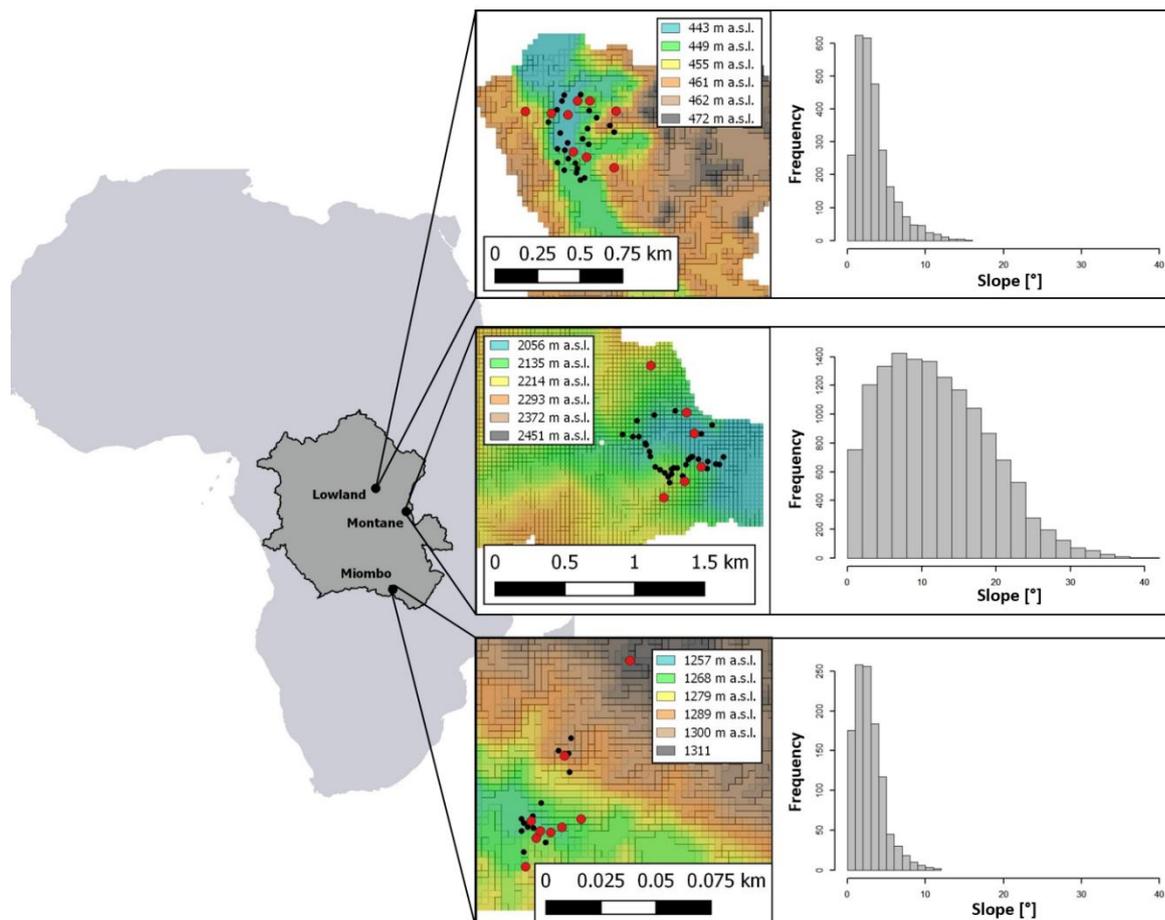
Figure 1: Location of the sampling sites; from left to right: dark grey area indicates the Congo Basin within Africa with forest site locations, red dots indicate locations of 1m soil profiles within each forest site, black dots indicate locations of topsoil sampling positions (0-20 cm); histograms show slope distribution of the respective sampling site.

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Table 1: Site characteristics of the three forest types. The range of altitudes of the sampled profiles and top soil samples is shown. The slope indicates the average slope ± standard deviation from all the samples taken at the respective forest site. MAP is the mean annual precipitation and MAT is the mean annual temperature extracted from the WorldClim database (average MAP for the years 2010-2018). EC represents the mean erosion coefficient values calculated from equation 1. LSI is the landslide hazard risk for each
115 **site extracted from the Global Landslide Hazard Map (Worldbank). LAI is the mean annual leaf area index, extracted from the Copernicus LAI-300m database (European Environment Agency).**

Forest type	Latitude [°]	Longitude [°]	Altitude [m a.s.l.]	Slope [°]	MAP [mm]	MAT [°C]	EC	LSI	Soil type	LAI
Montane	-2.344	28.746	2020 - 2180	4.3 ± 2.3	1970	15	2.79	3.96	Ferralsols/Acrisols	5.42
Lowland	0.291	25.295	440 - 460	22.8 ± 8.5	1760	24	1.22	1	Ferrasols	4.15
Miombo	-11.212	27.233	1260 - 1310	3.9 ± 2.2	1250	21	20.11	1	Arcrisols/Cambisols	0.83

2.2 Sampling

120 To analyze differences in soil N cycling between forest sites, soil samples were taken from soil profiles at different slope positions in the catchments (slope, shoulder and ridge) in order to better represent the catchment as a whole. Nine soil profiles were dug and sampled in both the lowland and Miombo forests. Due to difficult terrain within the montane forest, only six soil profiles were sampled (Figure 1). For each 1 m-profile, soil samples from nine different depths were taken (0-2 cm, 2-4 cm, 4-6 cm, 6-10 cm, 10-15 cm, 15-20 cm, 20-30 cm, 30-50 cm and 50-100cm). To calculate N stocks, bulk density of the soil was
125 determined at five depths of each soil profile using a 100 cm³ bulk density ring. The bulk density samples were dried at 105°C for 48h and then weighed. To analyze the effect of surface slope angles on stable isotopic signature of soil δ^{15}N, additional topsoil samples (bulk sample from the top 0-20 cm) were taken from throughout the catchments at locations with varying slopes (montane: n=27; lowland: n=24; Miombo: n=21) (Figure 1). Profile- and topsoil sample positions were recorded with a GPS (GPSMAP 60CSx; Garmin; accuracy <5m) and slope angles were estimated using 30-m SRTM derived digital elevation
130 models (DEM) (NASA JPL) which were smoothed with a low-pass filter.

2.3 Lab analysis

Soil samples were air dried and subsequently sieved through a 2 mm sieve to remove roots and organic residues. To homogenize, soil samples were milled and analyzed for C and N content and ^{15}N isotopes using an elemental analyzer (Automated Nitrogen Carbon Analyzer; SerCon; Crewe, UK) interfaced with an Isotope Ratios Mass Spectrometer (IRMS;
135 20-20, SerCon). Stable isotope ratios are expressed in delta notation against AIR-N$_2$ standard. N stocks were calculated using mass-based N content, multiplied by the bulk density of the respective depth and integrated over the whole profile depth.

2.4 Inter-site comparison

For a better inter-site comparison of the erosional effect we estimated a soil loss coefficient. In addition to slope, soil erosion is controlled by vegetation cover and rainfall characteristics. To incorporate these additional factors, we calculated an erosion
140 coefficient (EC) for each surface soil sample. To calculate the EC, we adapted the numerical model for sediment detachment (kg m^{-2} yr^{-1}) from Pelletier (2012):

$$EC = S^{\frac{5}{4}} R_a e^{-L_a} \qquad\qquad (Eq.\ 1)$$

Where S is the tangent of the mean hillslope angle of all samples, R_a the mean annual precipitation (MAP), and L_a the mean annual leaf area index (LAI). Numerical values for LAI were extracted from the Copernicus LAI-300m satellite images produced by the Earth Observation program of the European Commission (European Environment Agency) for the year 2019.
145 MAP data were extracted from the WorldClim database and averaged for the years 2010-2018 (Fick and Hijmans, 2017).

For a pan-tropical comparison, data points from Costa Rica (Weintraub et al., 2015) and Taiwan (Hilton et al., 2013) were extracted from the supporting material of the representative studies.

2.5 Statistical analysis

150 To assess the effect of forest type and soil depth on δ^{15}N and mass-based C:N ratio, linear mixed effects models were fitted, controlling for topographic position via a random intercept. A third model was fitted for N-stocks. Since stocks are integrated over the whole soil profile, soil depth was omitted as an explanatory variable in the third model, including only the effect of forest type as a fixed effect, while controlling for topographic position via a random intercept. All models were fitted using maximum likelihood methods via the lmerTest package (Kuznetsova et al., 2017). To assess the relation between slope angle
155 and δ^{15}N of the spatial surface samples, linear regressions were applied. Statistical analyses were conducted using the R-software (R Development Core Team, 2019).

Furthermore, a structural equation model (SEM) was applied to examine the relative direct and indirect importance of different variables on the soil δ^{15}N values of the five tropical forests. The SEM model was generated using the *lavaan* package version 0.6 (Rosseel, 2012) in the R-software.

3 Results

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The montane forest showed the highest N stock with mean value (mean ± standard deviation (SD)) of 1.47 ± 0.61 t N ha^{-1}. N stocks were lower in the lowland forest and Miombo woodland, with 0.87 ± 0.11 and 0.73 ± 0.30 t N ha^{-1}, respectively (Table 2). The montane forest and Miombo woodland soils exhibited similar C:N ratios with 11.4 ± 1.1 in the montane and 11.2 ± 1.8 in the Miombo, while the C:N ratio in the lowland forest was lower with a mean value of 7.4 ± 0.5 (Table 2). Average bulk N
165 and C:N values over the whole profile are reported in Figure S2. In general, soil profiles of the Miombo woodlands showed

the lowest δ^{15}N values, while the lowland forest displayed higher δ^{15}N values, especially in top soils (Figure 2). Lowland values increased steadily until a depth of 10 cm and decreased from there again until 100 cm depth. δ^{15}N in the Miombo woodlands showed the highest enrichment at a depth of 30 cm. Isotopic values of the montane forest varied considerably (large confidence intervals, Figure 2), but showed a general steady enrichment with increasing depth (Figure 2). Spatial topsoil

170   samples from each catchment (0 – 20 cm) showed highest δ^{15}N values in the lowland forest (mean ± SD; 7.55 ± 0.69 ‰), while the montane forest displayed slightly lower values (6.48 ± 1.42 ‰). The Miombo woodland was most depleted in δ^{15}N (2.93 ± 0.46 ‰) (Figure 2b). The topsoil samples from the montane forest showed highest variability in δ^{15}N values, ranging from 3.71 – 10.72 ‰ with a CV of 22%, whereas the Miombo showed a CV of 16% and the lowland forest a CV of 9%. Slope angle did not have a significant effect on topsoil δ^{15}N in the lowland and montane forests (p=0.8 for lowland and p=0.57 for

175   montane forest, respectively; Figure 3). In contrast, topsoil δ^{15}N significantly decreased with increasing sloping angles in the Miombo (r^2=0.30, Figure 3). The Miombo woodland displayed the highest average EC (20.11), whereas the montane (2.77) and lowland (1.22) forests showed clearly lower values (Table 1).
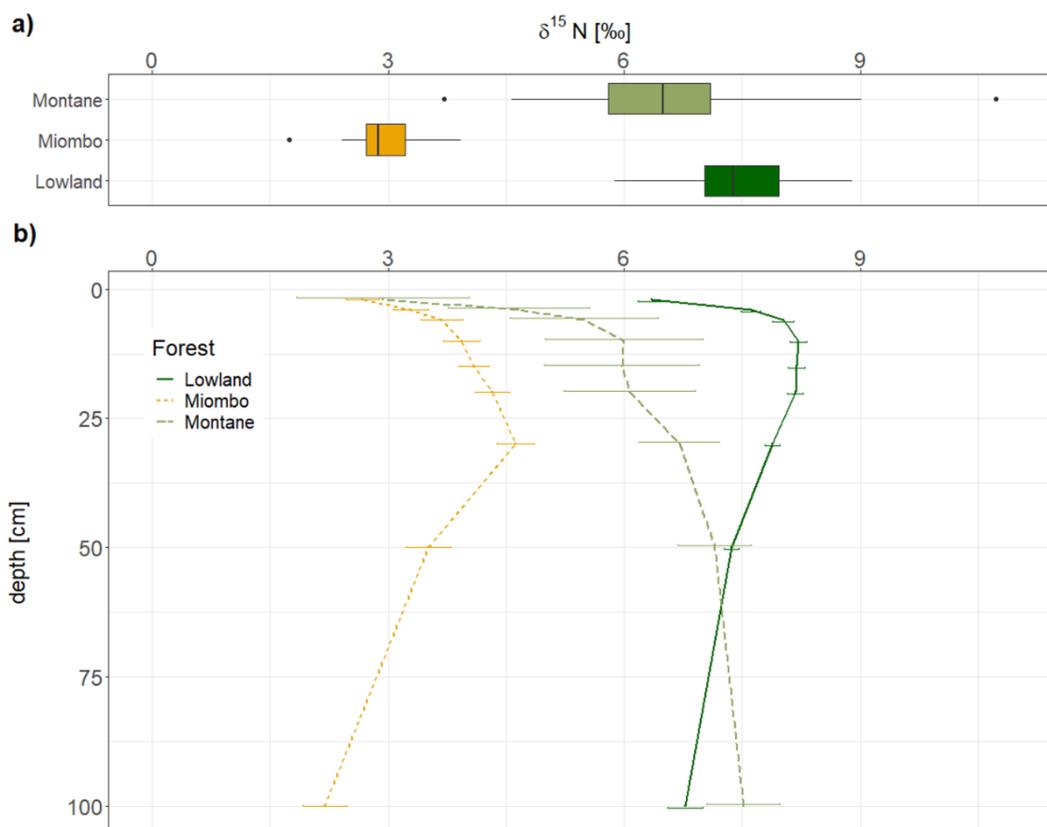
Figure 2: a) Boxplots of the δ^{15}N of the top soil (0-20 cm) samples from the montane, lowland and Miombo forest sites. b) δ^{15}N
180   **profiles from the different forest types. Lines showing the means of the replicates from one sampling site. Error bars indicating standard errors.**
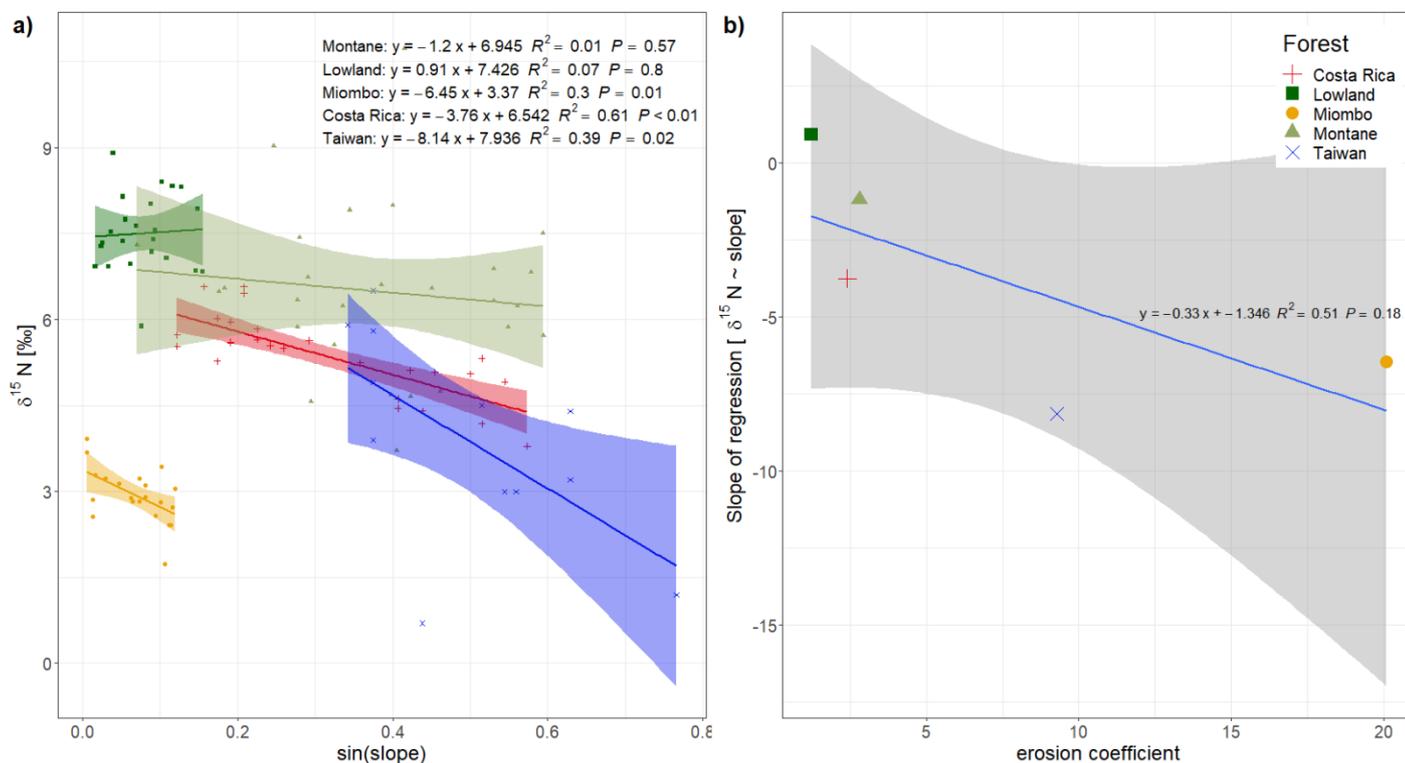
Figure 3: a) Soil δ^{15}N values vs sinus of the slope from the spatial sampling of topsoil (0-20 cm) in DRC, including data from Costa Rica (Weintraub et al., 2015) and Taiwan (Hilton et al., 2013); **b)** slope of the regressions from panel (a) plotted against the mean erosion coefficient of each study site.

4 Discussion

4.1 Differences of N cycling between forest types

At present, relatively little information on δ^{15}N data for soils in tropical forests is available. This holds especially for Central Africa, where almost no data is reported. Rütting et al. (2015) is one of the few studies that reported on δ^{15}N from montane tropical forests in Rwanda. Their values for topsoils (0-5 cm) range between 3.6 and 4.6 ‰ and are similar to the values reported in this study for the montane forest (mean of 4.4 ‰ for 0-6 cm depth in this study). δ^{15}N values from tropical mountain forests of Taiwan are also in the same range, with a mean value of 4 ‰ for the upper 10 cm of the soil profile (Hilton et al., 2013). Jamaican soils from tropical montane forests are much more depleted in ^{15}N, with values from -1.6 to 1.5 ‰ for depths down to 20 cm (Brearley, 2013). Top soils from an altitudinal gradient in Borneo were also more depleted, with a mean value of 1.6 ‰ (Kitayama and Iwamoto, 2001). Values reported for tropical lowland forests are available for soils in Costa Rica where top soils (0-10 cm) showed values of 4.7 ‰ (Osborne et al., 2017) and a mean value of 5.9 ‰ for soils from 0-20 cm depth (Weintraub et al., 2015). Higher erosion, due to high rainfall and uplift rates (1.7-8.5 mm yr^{-1}), may explain why these values of lowland forest in Costa Rica are more depleted in the heavier N isotope compared to the lowland forest in Yoko from

this study (7.55 ± 0.69 ‰ for 0 – 20 cm soil samples) because the residence time of soil N decreases with erosion and hence

200   soil rejuvenation (Amundson et al., 2003). The $\delta^{15}N$ reported here for the Miombo woodland (2.68 – 4.32 ‰ between 0 – 20 cm, Figure 2) falls within the same range as values reported by Wang et al. (2013) from a moist woodland savanna in Zambia (2.6 – 4.8 ‰), but are lower compared to values reported from a dry forest in the Cerrado Savanna of Brazil (6.3 – 10.8 ‰, Bustamante et al., 2004).

Soil $\delta^{15}N$ is related to the residence time of ecosystem N. Higher rates of mineralization, nitrification and dentification leaves

205   enriched N in the remaining soil organic matter (SOM), while the more depleted mineral N is removed via plant uptake or denitrification and transported down into deeper soil layers by leaching (Handley et al., 1999; Hobbie and Ouimette, 2009). Over the whole profile, the Miombo woodland showed highest depletion of ^{15}N (Figure 2), which indicates a slower N turnover and a more closed N cycle compared to the lowland and montane forest. However, topsoil $\delta^{15}N$ is influenced by the isotopic signature of incoming litter fall, inputs through roots, and N fixation (Craine et al., 2015). Downward transport processes and

210   transformation of SOM influences the $\delta^{15}N$ in deeper soil layers (Baisden et al., 2002). Decomposition processes enrich the remaining SOM in soils with the heavier ^{15}N isotope (Billings & Richter, 2006; Compton et al., 2007). In this study, the Miombo woodland showed slightly lower $\delta^{15}N$ values for the topsoil (0-2 cm, 2.66 ‰) than the montane forest (2.94 ‰), while the lowland forest showed a more enriched signature (6.35 ‰). As topsoil ^{15}N signature is mostly governed by N inputs, the Miombo and montane forest are likely to receive depleted N inputs. Furthermore, the presence of N_2-fixation depletes $\delta^{15}N$ in

215   top soils (Robinson, 2001), as it imports atmospheric N with an isotopic signature of 0‰, and might explain the low values in the Miombo woodland. Indeed, previous studies have shown that N_2-fixation is a more important process in tropical woodlands than in tropical forests (Hogberg & Alexander 1995), where less trees of the Fabacaea family are present (Malmer and Nyberg, 2008). Furthermore, it is reported that symbiotic N_2-fixation is downregulated in old-growth tropical lowland forest (Bauters et al., 2016).

220   Soil $\delta^{15}N$ profiles of the lowland forest and the Miombo woodland showed a $\delta^{15}N$ peak at 10 cm and 30 cm depths, respectively, indicating the highest microbial activity and/or leaching of soil N in this layer. Montane forest showed a similar peak at the same depth, indicating high turnover or leaching in this soil layer as well; however, from 20 cm onwards $\delta^{15}N$ increased even further to the highest values at 100 cm depth. Hobbie & Ouimette (2009) presented a conceptual model for processes that influence $\delta^{15}N$ values in a profile. According to this model, a soil profile with increasing ^{15}N values with depth (i.e. the case

225   of the montane forest) is indicative of an N-limited ecosystem or dominated by organic N cycling. Although, we found highest N stocks in the montane forest, higher foliar C:N ratios of tropical forests with increasing altitude (Bauters et al., 2017) are indicating, however, that this ecosystem experiences lower N availability and is dominated by organic N cycling. In Rwandan and Ecuadorian forests, Bauters et al. (2017) concluded from leaf $\delta^{15}N$ altitudinal gradients that N-cycling becomes more closed with increasing elevation and hypothesized that organic N sources for plants become more important in ecosystems

230   with lower temperatures. Furthermore, very high mineralization rates have been measured in the montane forests of Kahuzi-Biéga, which indicates a high organic matter turnover in these forests (Bauters et al., 2019). On the other hand, a profile with higher $\delta^{15}N$ values in the root layers and decreasing $\delta^{15}N$ in subsequent depths (similar to the lowland and Miombo profiles)

is more likely to occur in systems with less N limitation and more inorganic N cycling (Hobbie and Ouimette, 2009). Indeed, Bauters et al. (2019) found indication of mineral N excess in the Yoko lowland forest. In such systems, $\delta^{15}N$ depleted N-

235 species are transported downwards through the soil column, resulting in an accumulation of depleted ^{15}N in lower layers. Understanding N cycles in tropical forests is important for a better assessment of the productivity of these ecosystems. Although N stocks were highest in the montane forest, the $\delta^{15}N$ profile in this forest indicate a more closed N cycling system with mainly organic N cycling, which is less bioavailable. On the other hand, the lowland forest and the Miombo woodland seem to have a more open N cycle due to excess of available N. Furthermore, lowland forest in Central Africa are reported to

240 experience high atmospheric N depositions (Bauters et al., 2018) and the Miombo woodlands have more likely additional inputs from N_2-fixation.

Table 2: Fixed effects estimates for $\delta^{15}N$, N-stocks and C:N ratios. Fixed effects include forest type (Lowland, Montane and Miombo) and soil depth in cm (for $\delta^{15}N$ and C:N). N-stocks are for top 100 cm of the soils. For each effect, estimated standard error and
245 **estimated P-values is given, along with the estimated marginal (m) and conditional (c) R^2adj (Nakagawa and Schielzeth, 2013).**

Response	Effect	Estimate	SE	P-value	$R^2_{adj,m}$	$R^2_{adj,c}$
$\delta^{15}N$ [‰]	Intercept: Lowland	7.716	0.10	< 0.001	0.65	0.65
	Montane	-1.818	0.18	< 0.001		
	Miombo	-4.078	0.16	< 0.001		
	depth	-0.002	0.00	0.26		
C:N	Intercept: Lowland	7.961	0.16	< 0.001	0.57	0.58
	Montane	3.935	0.26	< 0.001		
	Miombo	4.198	0.22	< 0.001		
	depth	-0.015	0.00	< 0.001		
N [kg m^{-2}]	Intercept: Lowland	1.472	0.17	< 0.001	0.36	0.36
	Montane	0.653	0.27	0.160		
	Miombo	-0.349	0.24	0.03		

4.2 Influence of topography on soil N

In contrast to the Costa Rican tropical lowland forests (Weintraub et al., 2015), slope angle was found to have no effect on
250 $\delta^{15}N$ in the lowland forest of this study (Figure 3a). Topography is one of the main factors controlling physical erosion processes (Hilton et al., 2013; Weintraub et al., 2015). There are no steep slopes in the lowland forest (max. slope sampled 9°) and thus, there is a low potential for physical erosion. This is also indicated by a lower EC (1.22) in the lowlands compared to the coefficient in Costa Rica (2.39). Although the Miombo woodland is located in an area, with similar topography to the

lowland forest (Figure 1), the EC from this area is much higher (20.11). This can be explained by the fact that the forest cover
in the Miombo is less dense than the tropical forests and this increases the exposure of the soil surface to the energetic input
of rainfall. Furthermore, the loss of understory vegetation from fire, which occurs frequently during the dry season (Campbell,
1996), leaves the soil unprotected from erosion at the onset of the rainy season. The spatial topsoil samples of the Miombo
woodland clearly indicate a significant effect of slope angles on $\delta^{15}N$ (Figure 3a). The $\delta^{15}N$ values in the montane forest,
situated on steep terrain, tend to decrease with increasing slope angle, however, the visible trend is not statistically significant
(p value of 0.57, Figure 3a). Furthermore, we found a higher variability in $\delta^{15}N$ values of the soil samples in the montane forest
(CV of 22%) compared to the Miombo (CV of 15%) and lowland forest (CV of 9%). While slope angle is a commonly used
predictor of soil erosion potential, the curvature of the land surface (i.e. convex or concave) has also been shown to affect soil
erosion at a local scale (Stefano et al., 2000). The specific hydrology seems to control the erosive mechanism, as slope gradients
have been found to influence the rate of soil erosion through overland flow, while curvatures affect diffusive processes, such
as splash erosion (Stefano et al., 2000). However, while a trend of lower $\delta^{15}N$ on more convex landscapes in the montane forest
is visible (Figure S1), no statistical significance was found either, as samples were not equally distributed over the range of
curvatures. In addition, because our sampling strategy was focused on the slopes, sample sites were biased towards concave
curvatures.

Comparing the effect of slope angles on topsoil $\delta^{15}N$ of the study sites from the Congo Basin with the effect observed in
tropical forests of Costa Rica (Weintraub et al., 2015) and Taiwan (Hilton et al., 2013) shows that the magnitude of the effect
is related to the mean erosion coefficient in these ecosystems (Figure 3b). Sites with a high erosion coefficient show a higher
slope of the regression *$\delta^{15}N$ ~slope angles* compared to sites with a low erosion coefficient, implying a bigger effect on soil
$\delta^{15}N$ in more geomorphic active sites. As the dense tropical lowland forests of the Congo Basin do not represent high EC
values, the local effect of erosion on soil $\delta^{15}N$ signatures seems negligible. Although the montane forest shows a moderate EC,
due to the steep topography, the high variability in soil N masks the potential effect of topography on $\delta^{15}N$ in our dataset. The
estimation of EC based on equation 1 assumes that splash and overland flow are the dominant erosion processes but this may
underestimate erosional forces at higher hillslope angles (Pelletier, 2012). At higher hillslope angles, landslides will be more
important erosional processes than overland flow. This might indicate that at sites with high risks of landslides (as shown in
the landslide hazard index (LSI) in Table 1) are even more prone to physical soil loss than the EC would indicate. In this case
especially the forest in Taiwan (LHI of 4), the montane forest (LHI of 3.96) and the forest in Costa Rica (LHI of 3.65)
experience a high risk of landslides and total soil loss in this forest might even be higher than predicted by the EC.

We applied a SEM to assess the driving factors of the top soil $\delta^{15}N$ variability between different tropical forest sites (Figure
4). On a global scale, soil $\delta^{15}N$ is reported to be mainly influenced by climatic factors (MAT and MAP) and soil properties,
such as soil C and clay content (Craine et al., 2015a). Our results showed that soil C had only a marginal influence (lower
coefficient compared to slope, MAP and LAI) on tropical soil $\delta^{15}N$ and is only significantly influenced by MAT and not MAP.
Soil $\delta^{15}N$ in these tropical forests seems to be primarily influenced by factors controlling erosion (MAP, LAI and slope). Beside
the direct effect of MAP on soil $\delta^{15}N$, there is also a significant indirect effect visible, where sites with higher MAP experience

also higher LAI values (Figure 4). It was not possible to analyze the effect of clay content on $\delta^{15}N$ because the data were not available for all the samples. Although our data showed only for the Miombo a significant effect of topography on the soil
290    $\delta^{15}N$ values, we can highlight that especially in geomorphic active sites, erosion needs to be taken into account to explain local differences in soil $\delta^{15}N$ values.
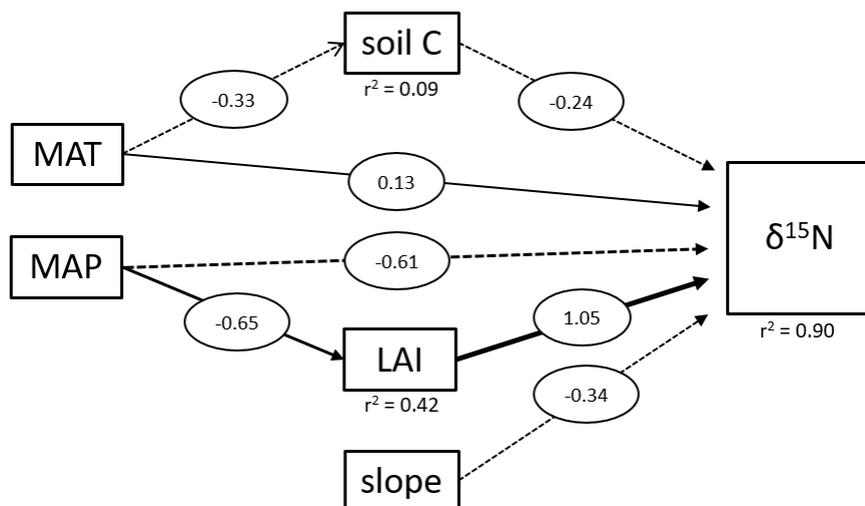
Figure 4: Structural equation model of direct and indirect effects on tropical soil $\delta^{15}N$ values. The diagram shows all significant relationships. MAT represents the mean annual temperature in °C, MAP the mean annual precipitation in mm and LAI the leaf
295   **area index. Soil C is the carbon content of the soil samples and slope the slope angles of the sampling site.**

5 Conclusion

The three tropical forest ecosystems in the Congo Basin each showed a distinct $\delta^{15}N$ soil profile. The $\delta^{15}N$ soil profiles in the montane forest indicate a closed N cycle, which supports the common theory of decreasing N availability at higher altitudes. In contrast, the $\delta^{15}N$ soil profiles indicated that the lowland forest and Miombo woodland tended to have more open N cycles,
300   which were mostly dominated by inorganic N cycling. The Miombo woodland showed the lowest $\delta^{15}N$ values, which is most plausibly explained by enhanced N fixation due to the plant species assemblage. Furthermore, topsoil $\delta^{15}N$ signatures across our and literature sites of the tropics suggests that a significant amount of the observed variability can be explained by erosional processes. Rainfall, vegetation cover, and topography are the main factors to explain $\delta^{15}N$ variability between five different tropical forest sites. Within the Congo Basin, only the Miombo woodland showed an influence of slope on soil $\delta^{15}N$ signature,
305   whereas in the lowland forest, the comparably low MAP, dense vegetation, and flat topography likely limit erosion and resulted in the lack of a correlation between the $\delta^{15}N$ signature of soil N and slope angle. In general, this study highlights the possible importance of soil erosion on N cycling in tropical forests; however, not all sites are similarly impacted. Therefore, soil erosion has to be taken into account while interpreting of soil $\delta^{15}N$ values as a proxy for N cycling. It underlines the importance of further spatial field studies in the tropics given the observed high variabilities between different tropical forest types.

Data availability

All data used in this study were published at Zenodo and are available under http://doi.org/10.5281/zenodo.4113895.

Authors contribution:

SB, MBauters, PB and KvO designed the research project. SB and MBauters carried out Field- and laboratory work. Data analysis and interpretation was performed by SB, MBauters, KvO, TWD, MBarthel, JS and PB. SB wrote the manuscript with contributions from all co-authors.

Competing interests:

The authors declare no conflict of interest.

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References

Alvarez-Clare, S., Mack, M. C. and Brooks, M.: A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest, Ecology, 94(7), 1540–1551, doi:10.1890/12-2128.1, 2013.

Amundson, R., Austin, A. T., Schuur, E. A. G., Yoo, K., Matzek, V., Kendall, C., Uebersax, A., Brenner, D. and Baisden, W. T.: Global patterns of the isotopic composition of soil and plant nitrogen, Global Biogeochem. Cycles, 17(1), doi:10.1029/2002GB001903, 2003.

Asner, G. P., Anderson, C. B., Martin, R. E., Tupayachi, R., Knapp, D. E. and Sinca, F.: Landscape biogeochemistry reflected in shifting distributions of chemical traits in the Amazon forest canopy, Nat. Geosci., 8(7), 567–575, doi:10.1038/ngeo2443, 2015.

Austin, A. T. and Vitousek, P. M.: Nutrient dynamics on a precipitation gradient in Hawai'i., Oecologia, 113, 519–529, doi:10.1007/978-3-642-78568-9_6, 1998.

Baisden, W. T., Amundson, R., Brenner, D. L., Cook, A. C., Kendall, C. and Harden, J. W.: A multiisotope C and N modeling

analysis of soil organic matter turnover and transport as a function of soil depth in a California annual grassland soil chronosequence, Global Biogeochem. Cycles, 16(4), 82-1-82–26, doi:10.1029/2001gb001823, 2002.

Bauters, M., Mapenzi, N., Kearsley, E., Vanlauwe, B. and Boeckx, P.: Facultative nitrogen fixation by legumes in the central Congo basin is downregulated during late successional stages, Biotropica, 48(3), 281–284, doi:10.1111/btp.12312, 2016.

340 Bauters, M., Taveirne, C., Van Der Heyden, D., Boeckx, P., Verbeeck, H., Demol, M., Bruneel, S. and Cizungu, L.: Parallel functional and stoichiometric trait shifts in South American and African forest communities with elevation, Biogeosciences, 14(23), 5313–5321, doi:10.5194/bg-14-5313-2017, 2017.

Bauters, M., Drake, T. W., Verbeeck, H., Bodé, S., Hervé-Fernandez, P., Zito, P., Podgorski, D. C., Boyemba, F., Makelele, I., Ntaboba, L. C., Spencer, R. G. M. and Boeckx, P.: High fire-derived nitrogen deposition on central African forests,
345 Proc. Natl. Acad. Sci., 1–6, doi:10.1073/pnas.1714597115, 2018.

Bauters, M., Verbeeck, H., Rütting, T., Barthel, M., Bazirake Mujinya, B., Bamba, F., Bodé, S., Boyemba, F., Bulonza, E., Carlsson, E., Eriksson, L., Makelele, I., Six, J., Cizungu Ntaboba, L. and Boeckx, P.: Contrasting nitrogen fluxes in African tropical forests of the Congo Basin, Ecol. Monogr., 0(0), 1–15, doi:10.1002/ecm.1342, 2019.

Billings, S. A. and Richter, D. D.: Changes in stable isotopic signatures of soil nitrogen and carbon during 40 years of forest
350 development, Oecologia, 148(2), 325–333, doi:10.1007/s00442-006-0366-7, 2006.

Boeckx, P., Paulino, L., Oyarzún, C., Cleemput, O. V. A. N. and Godoy, R.: Soil δ 15 N patterns in old-growth forests of southern Chile as integrator for N-cycling, Isotopes Environ. Health Stud., 41(3), 249–259, 2005.

Brearley, F. Q.: Nitrogen stable isotopes indicate differences in nitrogen cycling between two contrasting Jamaican montane forests, Plant Soil, 367(1–2), 465–476, doi:10.1007/s11104-012-1469-z, 2013.

355 Bustamante, M. M. C., Martinelli, L. A., Silva, D. A., Camargo, P. B., Klink, C. A., Domingues, T. F. and Santos, R. V.: 15N natural abundance in woody plants and soils of Central brazilian savannas (cerrado), Ecol. Appl., 14(4), 200–213, doi:10.1890/01-6013, 2004.

Campbell, B.: The Miombo in Transition: Woodlands and Welfare in Africa, Bogor, Indonesia, 1996.

Compton, J. E., Hooker, T. D. and Perakis, S. S.: Ecosystem N distribution and δ15N during a century of forest regrowth after
360 agricultural abandonment, Ecosystems, 10(7), 1197–1208, doi:10.1007/s10021-007-9087-y, 2007.

Craine, J. M., Elmore, A. J., Wang, L., Augusto, L., Baisden, W. T., Brookshire, E. N. J., Cramer, M. D., Hasselquist, N. J., Hobbie, E. A., Kahmen, A., Koba, K., Kranabetter, J. M., Mack, M. C., Marin-Spiotta, E., Mayor, J. R., McLauchlan, K. K., Michelsen, A., Nardoto, G. B., Oliveira, R. S., Perakis, S. S., Peri, P. L., Quesada, C. A., Richter, A., Schipper, L. A., Stevenson, B. A., Turner, B. L., Viani, R. A. G., Wanek, W. and Zeller, B.: Convergence of soil nitrogen isotopes across
365 global climate gradients, Sci. Rep., 5, 1–8, doi:10.1038/srep08280, 2015a.

Craine, J. M., Brookshire, E. N. J., Cramer, M. D., Hasselquist, N. J., Koba, K., Marin-Spiotta, E. and Wang, L.: Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils, Plant Soil, 396(1–2), 1–26, doi:10.1007/s11104-015-2542-1, 2015b.

Fernández-Martínez, M., Vicca, S., Janssens, I. A., Sardans, J., Luyssaert, S., Campioli, M., Chapin, F. S., Ciais, P., Malhi,

370   Y., Obersteiner, M., Papale, D., Piao, S. L., Reichstein, M., Rodà, F. and Peñuelas, J.: Nutrient availability as the key regulator of global forest carbon balance, Nat. Clim. Chang., 4(6), 471–476, doi:10.1038/nclimate2177, 2014.

Fick, S. E. and Hijmans, R. J.: WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas, Int. J. Climatol., 37(12), 4302–4315, doi:10.1002/joc.5086, 2017.

Handley, L. L., Austin, A. T., Robinson, D., Scrimgeour, C. M., Raven, J. A., Heaton, T. H. E., Schmidt, S. and Stewart, G.

375   R.: The 15N natural abundance (δ 15N) of ecosystem samples reflects measures of water availability, Aust. J. Plant Physiol., 26(2), 185–199, doi:10.1071/PP98146, 1999.

Hilton, R. G., Galy, A., West, A. J., Hovius, N. and Roberts, G. G.: Geomorphic control on the δ15N of mountain forests, Biogeosciences, 10(3), 1693–1705, doi:10.5194/bg-10-1693-2013, 2013.

Hobbie, E. A. and Ouimette, A. P.: Controls of nitrogen isotope patterns in soil profiles, Biogeochemistry, 95(2), 355–371,

380   doi:10.1007/s10533-009-9328-6, 2009.

Hofhansl, F., Chacón-Madrigal, E., Fuchslueger, L., Jenking, D., Morera-Beita, A., Plutzar, C., Silla, F., Andersen, K. M., Buchs, D. M., Dullinger, S., Fiedler, K., Franklin, O., Hietz, P., Huber, W., Quesada, C. A., Rammig, A., Schrodt, F., Vincent, A. G., Weissenhofer, A. and Wanek, W.: Climatic and edaphic controls over tropical forest diversity and vegetation carbon storage, Sci. Rep., 10(1), 1–11, doi:10.1038/s41598-020-61868-5, 2020.

385   Hogberg, P. and Alexander, I. J.: Roles of Root Symbioses in African Woodland and Forest : Evidence from 15N abundance and foliar analysis, J. Ecol., 83(2), 217–224, 1995.

Högberg, P.: Forests losing large quantities of nitrogen have elevated 15N:14N ratios, Oecologia, 84(2), 229–231, doi:10.1007/BF00318276, 1990.

Högberg, P. and Johannisson, C.: ^{15}N Abundance of forests is correlated with losses of nitrogen, Plant Soil, 157(1), 147–150,

390   1993.

Kitayama, K. and Iwamoto, K.: Patterns of natural 15N abundance in the leaf-to-soil continuum of tropical rain forests differing in N availability on Mount Kinabalu, Borneo, Plant Soil, 229(2), 203–212, doi:10.1023/A:1004853915544, 2001.

Lewis, S. L.: Tropical forests and the changing earth system, Philos. Trans. R. Soc. B Biol. Sci., 361(1465), 195–210, doi:10.1098/rstb.2005.1711, 2006.

395   Malhi, Y. and Grace, J.: Tropical forests and atmospheric carbon dioxide, Trends Ecol. Evol., 15(8), 332–337, doi:10.1016/S0169-5347(00)01906-6, 2000.

Malmer, A. and Nyberg, G.: Forest and water relations in miombo woodlands : need for understanding of complex stand management, Finnish For. Res. Inst., 98, 70–86, 2008.

Martinelli, L. A., Piccolo, M. C., Townsend, A. R., Vitousek, P. M., Cuevas, E., McDowell, W., Robertson, G. P., Santos, O.

400   C. and Tre: Nitrogen stable isotopic composition of leaves and soil: Tropical versus temperate forests, Biogeochemistry, 46, 45–65, 1999.

Nakagawa, S. and Schielzeth, H.: A general and simple method for obtaining R2 from generalized linear mixed-effects models, Methods Ecol. Evol., 4(2), 133–142, doi:10.1111/j.2041-210x.2012.00261.x, 2013.

Osborne, B. B., Nasto, M. K., Asner, G. P., Balzotti, C. S., Cleveland, C. C., Sullivan, B. W., Taylor, P. G., Townsend, A. R. and Porder, S.: Climate, Topography, and Canopy Chemistry Exert Hierarchical Control Over Soil N Cycling in a Neotropical Lowland Forest, Ecosystems, 20(6), 1089–1103, doi:10.1007/s10021-016-0095-7, 2017.

Pelletier, J. D.: A spatially distributed model for the long-term suspended sediment discharge and delivery ratio of drainage basins, J. Geophys. Res. Earth Surf., 117(2), 1–15, doi:10.1029/2011JF002129, 2012.

Perakis, S. S., Tepley, A. J. and Compton, J. E.: Disturbance and Topography Shape Nitrogen Availability and δ15N over Long-Term Forest Succession, Ecosystems, 18(4), 573–588, doi:10.1007/s10021-015-9847-z, 2015.

Porder, S., Vitousek, P. M., Chadwick, O. A., Chamberlain, C. P. and Hilley, G. E.: Uplift, erosion, and phosphorus limitation in terrestrial ecosystems, Ecosystems, 10(1), 158–170, doi:10.1007/s10021-006-9011-x, 2007.

Prescott, C. E.: The influence of the forest canopy on nutrient cycling, Tree Physiol., 22(15–16), 1193–1200, doi:10.1093/treephys/22.15-16.1193, 2002.

Van Ranst, E., Baert, G., Ngongo, M. and Mfuka, P.: Carte pédologique de Yangambi, planchette 2: Yangambi, échelle 1:50.000, UGent; Hogent; UNILU; UNIKIN, Gent., 2010.

Read, D. J.: Mycorrhizas in ecosystems, Experientia, 47, 376–391, doi:10.1016/0006-2952(93)90100-B, 1991.

Robinson, D.: δ15N as an integrator of the nitrogen cycle, Trends Ecol. Evol., 16(3), 153–162, doi:10.1016/S0169-5347(00)02098-X, 2001.

Roering, J. J., Kirchner, J. W. and Dietrich, W. E.: Hillslope evolution by nonlinear, slope-dependent transport: Steady state morphology and equilibrium adjustment timescales, J. Geophys. Res. Solid Earth, 106(B8), 16499–16513, doi:10.1029/2001jb000323, 2001.

Rosseel, Y.: lavaan: An R Package for Structural Equation Modeling, J. Stat. Softw., 48(2), 1–36, 2012.

Rütting, T., Ntaboba, L. C., Roobroeck, D., Bauters, M., Huygens, D. and Boeckx, P.: Leaky nitrogen cycle in pristine African montane rainforest soil, Global Biogeochem. Cycles, 29, 1754–1762, doi:10.1111/1462-2920.13280, 2015.

Soethe, N., Lehmann, J. and Engels, C.: Nutrient availability at different altitudes in a tropical montane forest in Ecuador, Journal of Tropical Ecology, 24, 397–406, doi:10.1017/S026646740800504X, 2008.

Stefano, C. Di, Ferro, V., Porto, P. and Tusa, G.: Slope curvature influence on soil erosion and deposition processes, Water Resour. Res., 36(2), 607–617, 2000.

Tanner, E. V. J., Vitousek, P. M. and Cuevas, E.: Experimental investigation of nutrient limitation of forest growth on wet tropical mountains, Ecology, 79(1), 10–22, 1998.

Townsend, A. R., Cleveland, C. C., Houlton, B. Z., Alden, C. B. and White, J. W. C.: Multi-element regulation of the tropical forest carbon cycle, Front. Ecol. Environ., 9(1), 9–17, doi:10.1890/100047, 2011.

Vicca, S., Luyssaert, S., Peñuelas, J., Campioli, M., Ill Chapin, F. S., Ciais, P., Heinemeyer, A., Högberg, P., Kutsch, W. L., Law, B. E., Malhi, Y., Papale, D., Piao, S. L., Reichstein, M., Schulze, E. D. and Janssens, I. A.: Fertile forests produce biomass more efficiently, Ecol. Lett., 15, 520–526, doi:10.1111/j.1461-0248.2012.01775.x, 2012.

Vitousek, P. M., Porder, S., Houlton, B. Z. and Chadwick, O. a: Terrestrial phosphorus limitation : mechanisms , implications,

and nitrogen – phosphorus interactions, Ecol. Appl., 20(1), 5–15, doi:10.1890/08-0127.1, 2010.

Wang, L., Okin, G. S., D'Odorico, P., Caylor, K. K. and Macko, S. A.: Ecosystem-scale spatial heterogeneity of stable isotopes

440   of soil nitrogen in African savannas, Landsc. Ecol., 28(4), 685–698, doi:10.1007/s10980-012-9776-6, 2013.

Weintraub, S. R., Taylor, P. G., Porder, S., Cleveland, C. C., Asner, G. P. and Townsend, A. R.: Topographic controls on soil

nitrogen availability in a lowland tropical forest, Ecology, 96(6), 1561–1574 [online] Available from: c:%5CPDfs%5C14-

0834.1.pdf, 2015.

Wieder, W. R., Cleveland, C. C. and Townsend, A. R.: Throughfall exclusion and leaf litter addition drive higher rates of soil

445   nitrous oxide emissions from a lowland wet tropical forest, Glob. Chang. Biol., 17(10), 3195–3207, doi:10.1111/j.1365-

2486.2011.02426.x, 2011.

Wieder, W. R., Cleveland, C. C., Smith, W. K. and Todd-Brown, K.: Future productivity and carbon storage limited by

terrestrial nutrient availability, Nat. Geosci., 8(6), 441–444, doi:10.1038/NGEO2413, 2015.

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