Human activities, such as the production of ammonium-based fertilizers and the burning of fossil fuels, have led to dramatic changes in the global N cycle over the past century (1). Increased levels of reactive N in the atmosphere have clear ramifications for ecosystems worldwide. Nitrogen is tightly coupled to carbon (C) in global biogeochemical cycles due to the metabolic need of plants and (micro)organisms. Furthermore, recent data analyses and modeling activities have shown that carbon dioxide (CO$_2$) uptake by terrestrial ecosystems strongly depends on nutrient availability (2–4). This finding is important for tropical forests, which dominate the terrestrial C cycle and account for approximately one-third of global terrestrial gross primary productivity (5). This nutrient control on forests highlights the importance of quantifying atmospheric N deposition to understand and predict the global C cycle. This has prompted the Earth system modeling community to improve the implementation of nutrient cycles in their models, with varying consequences for the predicted effects of N deposition on C cycling in tropical forests (6–9). Indeed, model parameterization largely depends on the quantity and quality of empirical data used to constrain the simulations. Recently, global empirical N deposition data mapped from different monitoring networks showed a distinct lack of field-based estimates for the tropics (10). More importantly, the studies that do exist report typically solely the inorganic N inputs, overlooking the organic component in deposition. Recent reviews call attention to the importance of including this atmospheric organic N component, while pointing to a complete lack of data from Africa (11, 12). They conclude that the effect of N deposition can only be understood if the organic component is consistently included and considered.

In short, there is no empirical organic N deposition data for African forests and, consequently, no insight into its potential sources. Some studies have suggested that a large proportion of organic N is sourced from the atmosphere and derived from biomass burning (13, 14). Satellite data coupled to biogeochemical models have identified the African continent as a key region for biomass burning, comprising 65% of the total global burnt area (i.e., 222 Mha yr$^{-1}$) (15). Most of this biomass burning occurs as large seasonal wildfires in the savannas, with an estimated 78% of the tropical reactive N from savanna fire emissions sourced within the African savanna (4.3 of 5.5 Tg N yr$^{-1}$) (16). Additionally, it was found that the net transfer of N from savannas to forests around the equator was larger in Africa than in South America and Asia due to the prevailing equatorward winds that carry emissions into the intertropical convergence zone. These models hint at the potential magnitude and composition of total N deposition on these remote forests, but have not been corroborated by field-based observational data to date. In this study, we directly measured N deposition on the ground in a remote forest in the central Congo Basin. Specifically, we collected biweekly water samples in the open field and under the forest canopy of two}

**Significance**

**Atmospheric N deposition affects productivity and biodiversity of forests worldwide. However, field-based estimates of atmospheric N deposition for tropical forests are extremely sparse. Our results from a monitoring network in the central Congo Basin exceed current regional N deposition simulations. Ultrahigh-resolution mass spectrometry and modeling techniques reveal that savannah biomass burning is the main source for this elevated atmospheric N deposition. Furthermore, a large fraction is deposited as organic N, which is typically not simulated or measured in monitoring networks. These high levels of N deposition have clear ramifications for the ecology and biogeochemistry of the Congo Basin.**


The authors declare no conflict of interest.

This article is a PNAS Direct Submission. Published under the PNAS license.  
1To whom correspondence should be addressed. Email: Marijn.Bauters@UGent.be.  
2Present address: Pontchartrain Institute for Environmental Sciences, Department of Chemistry, University of New Orleans, New Orleans, LA 70148.  
This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1714597115/-/DCSupplemental.
forest types. To unravel the origin of the N, we used both ultrahigh-resolution mass spectrometry and modeling techniques.

Results and Discussion

Monitoring N deposition throughout a hydrological year in the remote forest of the central Congo Basin, we found a high total dissolved nitrogen (TDN) open-field (bulk) deposition of 18.2 kg N ha$^{-1}$ yr$^{-1}$, of which 70% was organic N. Throughfall, the precipitation collected under the forest’s canopy, amounted to 53.1 ± 3.2 kg N ha$^{-1}$ yr$^{-1}$ in mixed forest, and 37.5 ± 4.2 kg N ha$^{-1}$ yr$^{-1}$ in monodominant forest, with roughy 50% organic N in both cases (mean ± SD) (Table S1). The time series of deposition showed a consistent series of peaks at the onset of the rainy seasons in March and September, suggesting a strong contribution from previous dry deposition (Fig. 1). This elevated deposition was not expected at this remote site, because there is no industrial activity or intensive agriculture in the vicinity. Moreover, these measured N deposition rates already surpass the simulated deposition rates for 2030 (17).

To gain further insight into the source and composition of the organic fraction (50–70% of total N deposition) (Table S1), we characterized the compound classes of the organic components using ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). The composition of dissolved organic carbon (DOC) in the three sample types (i.e., open-field rainfall, mixed forest, and monodominant throughfall) showed a striking similarity (Fig. S1). For each sample type, the absolute and subgroup relative abundances of N-containing compounds were consistent (Fig. 2 A–C). The annual average molecular composition plotted in van Krevelen space also yielded, overall, very similar distributions (Fig. 2 D–F and Fig. S1). We found a high number of assigned molecular formulae in all sample types, with an average of 17,096, 16,955, and 15,627 for the open-field rainfall, mixed forest, and monodominant forest throughfall, respectively. Of these formulae, 52% contained at least one N atom on average, resulting in a large number of N-containing compounds with similar composition in all sample types (Fig. S1 and Table S1). One question that arises from these data is to what extent the increased organic N load in throughfall is caused by dry deposition versus canopy leaching. The relative abundances of compound classes in the three sample types share a high percentage of phenolics, closely resembles a previous study of glacier DOM on the Tibetan plateau, where atmospheric deposition was identified as the main source (18). The overall molecular similarity of the two forest types suggests that the throughfall enrichment of N is due to canopy interception of dry deposition rather than canopy leaching, as canopy leaching would be expected to result in a divergent compositional signature between the two forest types. In fact, we found only 2,811 and 2,459 unique formulae in the mixed and monodominant forest throughfall samples, respectively, that were not found in the open-field rainfall samples. Of these formulae, only 1,380 and 1,039 contained at least one N atom. These unique formulae only contributed 0.94% and 0.86% relative abundance to the total DOM signature for the mixed and monodominant forest samples, respectively (Fig. 2 D–F). Collectively, this qualitative compositional evidence suggests that the vast majority of N found in our forest throughfall samples was from atmospheric deposition.

Quantitatively measuring the contribution of canopy exchange and dry deposition in throughfall samples has proven to be a challenging task, especially in the tropics where appropriate methods still need to be developed (19). Rather than using Na$^+$ or Cl$^-$ as an inert tracer (Materials and Methods), we calculated an average condensed aromatic content using FT-ICR-MS data as proxy for fire-derived N deposition component of the organic fraction. Condensed aromatics are formed only through combustion and are therefore not a canopy leaching product (20).

The dissolved organic nitrogen (DON) deposition loads increased linearly with increasing average condensed aromatic content, which suggests that fire-derived dry deposition controls organic N loads in the throughfall (Fig. S2). The difference in N deposition and condensed aromatics content between mixed lowland and monodominant forests might be due to the increased heterogeneity of the mixed forest, which would lead to differing levels of aerosol interception of the canopies. *Gilbertiodendron dewevrei*, the monodominant species constituting a minimum of 60% of the basal area, is characterized by large leaves. Hypothetically, the combination of a homogenous canopy and large leaves might render them less efficient for dry deposition removal from the air, much like the described differences in fine dust removal efficiency between tree species (21). Altogether, these data suggest that organic N deposition found at all sites is predominantly sourced from biomass burning and not the forest canopy itself. We expect that at least part of the inorganic deposition comes from dry deposition as well, but there are no satisfactory techniques to further confirm this using the dataset.

Although the abundance of fires on the savanna borders has been shown before (16), our data suggest that the impact on central African forests in terms of N addition is much higher than expected. To investigate this link, we crossed existing datasets of daily fire pixels (recorded by MODIS satellites) and processed through NASA’s FIRMS system with daily backward wind-trajectories as simulated for the study period by the Hybrid Single Particle Lagrangian Integrated Trajectories (HYSPLIT) model of National Oceanic and Atmospheric Administration (NOAA) (Movie S1). This gave rise to an empirical fire load index (FLI) (Fig. L4), which represents the number of fires that the winds directly above our sites passed over during the week before the sampling. A striking seasonality in this FLI showed that the local dry season is the peak season for fire arrivals for the central African rainforests. This was confirmed by extracting the black carbon column mass density (BCCMD) from NASA’s Modern-Era Retrospective Analysis for Research and Applications version 2 (MERRA 2) model (Fig. L4). Black carbon is a known combustion product, originating from either fossil fuel combustion or biomass burning (20), and hence can also serve as a proxy for the fire load of the atmosphere. The fire load peak zone of the ITCZ shifts from the southern savanna border in January to the northern savanna border in July, inducing shifts in the rainy seasons for the savanna borders. As a result, the dry seasons oscillate inversely from the north to the south, in January to July, and back. During these shifts, the ITCZ passes over the central Congo Basin twice, inducing a bimodal rainfall pattern. It has been shown that the location of the ITCZ and the intertropical confluence on the African continent causes biomass combustion products from the savanna borders to be transported in the direction of the equator during the dry season, and shifts of the ITCZ introduce seasonality in fire regime with latitude (Fig. 1) (22). These field-based observations, combined with molecular signatures of fire-derived organic compounds that correspond well with model-based fire inputs from the atmosphere, raise substantial questions as to the overall impact of high N deposition in central African forests. The fire abundance on the African continent has been chronically high during the last millennia (23, 24), suggesting that N deposition to the Congo Basin has also been high over this period. Additionally, given the agreement between the FLI and the BCCMD (Fig. 1), annual and monthly deposition maps and aerosol transport models suggest that the entire Congo Basin is subject to similar, if not higher, deposition loads (Fig. S3 and Movie S2). Currently available global simulation maps, which are used by modelers for reactive nitrogen loads, predict values for central Africa between 4 and 10 kg dissolved inorganic nitrogen (DIN) ha$^{-1}$ yr$^{-1}$ (25–27). While these estimates correspond well with the DIN loads we

Bauters et al.
found in the open field, they dramatically underestimate total N deposition because organic N is not accounted for, which was also shown by recent simulations (28). Although the bioavailability of this organic N is still being studied (12, 29), the discrepancy between simulations and our data raises questions about the consequences of this high N deposition on forest functioning.
biodiversity, and the regional C budget of central African forests. The latter is of particular importance given that African forests currently represent one of the largest sources of uncertainty for the global CO$_2$ budget (30). It is generally assumed that productivity in lowland tropical forests is not N-limited (31, 32). Moreover, symbiotic N fixation by canopy trees is usually down-regulated in mature central African forests, confirming that N is cycled in excess (33). However, it remains unclear to what extent reactive N loads affect N and C cycling and biodiversity in tropical forests (34, 35). It is possible that the delivery of reactive N impacts forest structure, functioning, and biodiversity via effects on taxa, functional groups, and size classes (36, 37). We therefore suggest an urgent expansion of the monitoring efforts in the Congo Basin, to adjust and improve global and regional estimates of reactive nitrogen loads.

Material and Methods

Study Site. The study was carried out in an old-growth tropical forest of Yoko, roughly 30-km south of Kisangani (00°17′; 25°18′), in the Democratic Republic of the Congo. Vegetation at the study site 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, with very limited elevation differences and gentle slopes. The site has two dominant forest types: mixed lowland tropical forest and Gilbertiodendron-dominated forest, where >60% of the basal area consists of one species, G. dewevrei. Throughfall was sampled at three study plots of 40 x 40 m per forest type.

Additionally, we collected bulk precipitation in one location in the open field at a nearby deforested site.

Data Collection. Samples were collected from September 19, 2015 to September 10, 2016. Throughfall and bulk precipitation was collected using polyethylene funnels supported by a wooden pole of 1.5-m height, on which a polyethylene tube was attached to drain into a 5-L polyethylene 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 with black plastic to prevent the growth of algae and to keep the samples cool to minimize microbial activity. In each plot, we installed eight throughfall collectors in two rows of four, with ∼8-m distance between all collectors. All plots were sampled every fortnight. On each sampling occasion, the water volume in each collector was measured in the field, and used recipients, funnels, and mesh were replaced by new ones, rinsed with distilled water. A volume-weighted composite sample was made per plot. The volume-weighted biweekly composite samples were filtered using a nylon membrane filter of 0.45 μm before freezing, and then immediately stored in a freezer. Finally, the samples were sent in several batches to Ghent University (Belgium) for chemical analysis.

Chemical Analysis and Empirical Data Analysis. After transport to Belgium, NH$_4$$^+$ was determined colorimetrically by the salicylate-nitroprusside method (38) on an autoanalyzer (AA3; Bran and Luebbe). NO$_3$ was determined colorimetrically using the same autoanalyzer as NO$_2$ after reduction of NO$_3$ in a Cd-Cu column, followed by the reaction of the NO$_2$ with N-1-naphthylethylenediamine to produce a chromophore. DON can only be determined indirectly via measurement of TDN and subtraction of DIN, leading to additional analytical uncertainty. As discussed elaborately by Cornell et al. (40), there are three methods that are often used to determine
TN D in water samples: Kjeldahl digestion, persulfate oxidation, and high-temperature catalytic oxidation. At least for marine samples, no systematic biases have been found in method intercomparisons (39, 40). However, there is a risk of underestimating DON, because the main source of error of TDN determination is the incomplete conversion of DON to DIN (40). We used persulfate oxidation for the determination of TDN in the water sample. For this, a 1:1 oxidizing solution of NaOH, H2O2, and K2SO3 was added to the sample, which is subsequently placed in an autoclave at 121 °C for 1 h to convert NH4+ and dissolved DON into NO3− (41). In addition, a subset of samples was taken for bulk precipitation (n = 12), for one of the monodominant plots (n = 19) for further analysis by electrospray ionization (ESI) coupled with FT-ICR MS. Before extraction for FT-ICR MS, dissolved organic carbon (DOC) was analyzed. Therefore, samples were thawed under refrigeration (4 °C), acidified with 12N HCl to a pH of −2, and measured via high-temperature catalytic oxidation in a high-temperature catalytic oxidation. DOC concentrations were determined as the mean of at least three replicate injections, for which the coefficient of variation was <2% (42). Samples were prepared for ESI-FT-ICR MS analysis by the solid-phase extraction (SPE) of DOM method (43). Briefly, filtered samples were acidified to pH 2.3 with HPLC-grade HCl before solid phase extraction on 100-mg Bond Elut PPL (Agilent Technologies) cartridges. Sample volume was adjusted depending on original DOM concentration to obtain constant volume. Negative ions produced by ESI from direct infusion of the methanol extracts at a concentration of 50 μg C mL−1 were analyzed with a 21 T FT-ICR mass spectrometer (Florida State University, Tallahassee) (44). Each signal >6 n RMS baseline noise was assigned a molecular formula with EnviroOrg (45), and classified as condensed aromatic (modified aromaticity index (Alcalculated) > 0.67), polyphenol (0.69 < Alcalculated < 0.76), low oxygen (Al > 0.77, H/C < 0.5), unsaturated, high oxygen (Al < 0.5, H/C > 1.5, O/C > 0.5), unsaturated, low oxygen (Al < 0.5, H/C < 1.5, O/C < 0.5), aliphatic (H/C > 1.5, n = 0), and peptide (H/C > 1.5, n > 0) (18, 46), as defined in the Python script used in the analysis (47).

We used plot-averaged values for the bulk rainfall and throughfall volume data. The water flux for bulk rainfall and throughfall was calculated by dividing the average water volume by the surface area of the collector. Nitrogen deposition (TDN, DIN, and DON) was calculated by multiplying the water volume with the element concentration in that volume. One of the most widely used methods to disentangle dry deposition from canopy leaching is the “canopy budget model,” which assumes that a natural tracer ion (most commonly Na+) is inert when deposited on the canopy. Additionally, the method is based on the calculation of an ion charge balance based cation deposition and computes canopy exchange interactions of NH4+, NO3− (48). However, several assumptions underlying this model seem to be challenged in tropical forest canopies, not least the apparent uptake of Na+ in some cases (49). Additionally, the model offers no theoretical basis for the calculation of organic N compounds from the canopy, other than the assumption that they are equally deposited on the canopy as the tracer ion. Given these factors, we chose not to apply this model here (19).

**Satellite and Wind Trajectory Analysis**

We calculated a FLI, representing the theoretical number of fires that were passed by the wind parcel that arrive above the experimental site. For this, we crossed a daily fire pixel dataset with backward wind trajectories ending at the site. The fire pixel dataset covering the entire African continent was obtained from NASA’s MODIS Collection 6 NRT (50). The backward wind trajectories were generated using the HYSPLIT model provided by the NOAA Air Resources Laboratory (51-54) with the GDAS half-degree archive meteorological dataset. For the entire study period, we generated one trajectory every 6 h, ending above the study site pixel for five different target altitudes (respectively: 40, 500, 1000, 2500, and 5000 m above ground level), going back 1 wk. To cross the datasets, we counted fires within a 25-km zone around the modeled air parcel at each time point going back 24 h. The 24-h period was considered to take into account smoke from smoldering fires that might not have been detected by the VIIRS system at the time point in question. These fire counts were cumulated along the trajectory, resulting in a fire count per trajectory, and hence four fire counts per day. We then calculated the daily fire counts for every fortnight, resulting in the FLI for the concurrent sampling dates. Additionally, we extracted the hourly simulated BCCMD from above our site from the MERRA-2 from the Goddard Earth Observing System Model, v5 (55). We subsequently calculated the average over this time series for all sample dates.

**ACKNOWLEDGMENTS**

We thank S. Vandevoorde for the help with the sample analysis. Some analyses and visualizations used in this paper were produced with the Giovanni online data system, developed and maintained by the NASA/Goddard Earth Sciences Data and Information Services Center. We also acknowledge the use of data and imagery from LANCE FIRMS operated by the NASA/Goddard Space Flight Center/Earth Science Data and Information System with funding provided by NASA/HQ. This research has been supported by the Belgian Research Development Cooperation through VIB- UOS, both through a personal scholarship (to M.B.) and project funding. VIB-UOS supports partnerships between universities and university colleges in Flanders (Belgium) and the South looking for innovative responses to climate change and global and local challenges. Visit www.vlir-uos.be for more information. Acquisition of mass spectra was supported by the National Science Foundation Division of Materials Research (DMR-11-57490). T.W.D., D.C.P., and R.G.M.S. were supported by the Winchester Foundation and partially by National Science Foundation Grant OCE-1464396. P.H.-F. is funded by Programa de Formación de Personal Avanzado Consejo Nacional de Investigaciones Científicas y Tecnológicas, BECAS Chile.