# Long-Term Trends in Nitrogen Isotope Composition and Nitrogen Concentration in Brazilian Rainforest Trees Suggest Changes in Nitrogen Cycle

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Direct or indirect anthropogenic effects on ecosystem nitrogen cycles are important components of global change. Recent research has shown that N isotopes in tree rings reflect changes in ecosystem nitrogen sources or cycles and can be used to study past changes. We analyzed trends in two tree species from a remote and pristine tropical rainforest in Brazil, using trees of different ages to distinguish between the effect of tree age and long-term trends. Because sapwood differed from heartwood in $\delta^{15}N$ and N concentration and N can be translocated between living sapwood cells, long-term trends are best seen in dead heartwood. Heartwood $\delta^{15}N$ in Spanish cedar (*Cedrela odorata*) and big-leaf mahogany (*Swietenia macrophylla*) increased with tree age, and N concentrations increased with age in *Cedrela*. Controlling for tree age, $\delta^{15}N$ increased significantly during the past century even when analyzing only heartwood and after removing labile N compounds. In contrast to northern temperate and boreal forests where wood $\delta^{15}N$ often decreased, the $\delta^{15}N$ increase in a remote rainforest is unlikely to be a direct signal of changed N deposition. More plausibly, the change in N isotopic composition indicates a more open N cycle, i.e., higher N losses relative to internal N cycling in the forest, which could be the result of changed forest dynamics.

## Introduction

Global change is mostly associated with increasing concentrations of atmospheric $CO_2$ and other greenhouse gases triggering climate change, but other anthropogenic processes also affect the local and global environment. Among these, the anthropogenic production of reactive nitrogen through nitrogen fertilization, agricultural $N_2$ fixation, and fossil fuel combustion has far-reaching consequences on ecosystem functions, productivity, biodiversity, and the climate itself (*1*). The global production of reactive nitrogen is estimated

to have doubled relative to preindustrial conditions (*2*), and since reactive nitrogen forms do not remain in the atmosphere for long, local deposition differs strongly depending on regional agricultural and industrial activity. With few measurements in remote areas, deposition and changes in deposition can only be estimated from large-scale models (*2*). As a consequence, there is little information on how much deposition has changed in remote areas and we do not know if this has affected the nitrogen cycle or other ecosystem processes.

Wood or cellulose carbon isotopes reflect the tree responses to atmospheric $CO_2$ increases (*3*), and oxygen isotopes, which are correlated with temperature and rainfall, have been used to detect climate changes during the past centuries (*4*). Wood also contains nitrogen, mainly in the form of cell-wall proteins but also in various extractives. A $^{15}N$ labeling experiment in *Pinus ponderosa* found that most labeled nitrogen was deposited in wood produced the year after the label application, but some nitrogen was translocated to annual rings produced a few years before and after that (*5*). Similar results were obtained for *Fagus sylvatica*, where the peak of labeled $^{15}N$ only extended for three years after labeling (*6*). Thus, tree ring N would be a problematic indicator for short-term (annual) changes in nitrogen inputs or nitrogen cycles, but can be used to detect longer-term trends.

The natural nitrogen isotope composition ($\delta^{15}N$) of ecosystem compartments reflects $^{15}N$ inputs and soil N processes, the latter being affected by losses of $^{15}N$ depleted compounds leading to progressive $^{15}N$ enrichment of soils and plants (*7*). N uptake and assimilation by plants rarely exert an isotope effect (*8*), and plants are therefore expected to reflect the isotopic composition of the plant-available soil N pool. $^{15}N$ enrichment between bulk soil and plants is correlated with the intensity of N cycling (*9*), and long-term changes in plant and soil $\delta^{15}N$ indicate changes in the nitrogen cycle, which is recorded in wood as well as in leaves (*10*).

A decrease in wood $\delta^{15}N$ values during the last decades has been found in several temperate and boreal trees, and was interpreted as evidence for increased deposition of reactive N (*10, 11*), as a sign for decreasing soil N availability due to forest regrowth (*12*), or as a consequence of $CO_2$-induced stimulation of tree growth (*13*). In contrast, increasing wood $\delta^{15}N$ signals have been found after disturbance by logging (*10*), manipulation of the forest water table (*14*), fertilizer N application (*15*), and close to $NO_x$ sources along a motorway (*16*).

Wood C isotopes can be affected by tree age, and wood of young trees can differ from adult wood for several decades after germination (*17*). A bias resulting from this effect can be avoided either by analyzing only wood older than this juvenile phase, provided the length of a juvenile effect is known and there is no additional effect as the tree senesces, or by analyzing wood from trees of the same age produced at different times. As far as we are aware, it is unknown if tree age affects wood $\delta^{15}N$ signals, thus wood $\delta^{15}N$ trends observed in individual trees could either be an effect of tree age or of environmental trends. Without distinguishing between these, interpretations relating to nitrogen deposition or changes in nitrogen cycling should be treated with some caution.

We analyzed N concentration and $\delta^{15}N$ values in wood from two tree species from a Brazilian rainforest with the purpose of assessing N isotope trends in remote tropical rainforests as compared to northern temperate areas with generally higher N deposition. We also tested if N concentrations and isotopic composition are affected by tree age to

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ensure that an observed trend can indeed be interpreted as a change in the N cycle.

## Materials and Methods

**Study Site and Species.** Wood from two important tropical timber species, *Cedrela odorata* L. (Spanish cedar) and *Swietenia macrophylla* King (big-leaf mahogany), was obtained from the indigenous forest reserve "Rio Branco" approximately 50 km northwest of the city of Aripuanã, Mato Grosso, Brazil (10°09′ S, 59°26′ W). The soil is a xanthic ferralsol, the mean air temperature is 22.6 °C, and the annual precipitation is approximately 3000 mm (*18*). The area is characterized by a relatively dry season between May and October with rainfall <100 mm/month from July to September. The primary forest of the study site has been slightly disturbed by logging activities since 1995 with some logged areas subsequently converted to pastures. Within a distance of ca. 25 km from the sampling location, there is almost no agricultural activity apart from small indigenous plots even today, and deforestation around Aripuanã started only in the 1990s.

**Sampling.** Stem discs at breast height were collected after felling in an area of 2.7 km$^2$ in 2001. Leaves were collected from seven different trees of each species in February 2004. The dry season results in distinct growth rings in the two species and the annual character of tree rings and the age of the same sampled trees had previously been analyzed (*19*). To be able to distinguish the effect of tree age from that of long-term environmental changes, we selected 20 *Cedrela* trees between 23 and 154 years old and 11 *Swietenia* trees between 48 and 126 years old. To minimize noise from short-term variations, rings from ten-year segments were pooled, but wood from different individuals was not pooled in order to retain between-tree variation. Also, the $\delta^{15}N$ signal can be diluted by radial transport (*6*), which would make an annual resolution of any environmental signal very difficult. In *Cedrela*, wood from every second ten-year increment (decade), corresponding to wood produced during the 1990s, 70s, 50s, etc., was analyzed; in *Swietenia*, where fewer trees were available, wood from each decade was used.

**Sample Preparation.** Wood samples were ground in a sample mill to pass a 40-mesh screen. For the extraction, 50 mg of ground samples were placed in 2-mL screw-cap plastic vials (Eppendorf, Hamburg, Germany) each with a glass bead. Samples were extracted at 50 °C first with 1 mL of toluene/ethanol (1:1) for 4 h, next with 1 mL of ethanol for 4 h, and finally with deionized water for 1 h following ref *16*. After each extraction, vials were centrifuged at 12000$g$ for 5 min and the supernatant was discarded. Finally the pellets were washed three times with pure methanol and three times with hot water. This removal of soluble compounds, which are more mobile radially than nonsoluble N, can improve the isotopic signal (*6*) and is mostly used for studies on environmental effects on wood N isotopes. To assess the effect of the extraction, nitrogen isotopes were analyzed after extraction treatment and for more than half of the samples also in oven-dried bulk wood.

**Isotope Analysis.** Nitrogen isotope ratios were analyzed at the Department of Chemical Ecology and Ecosystem Research, University of Vienna, with an elemental analyzer (EA 1110, CE Instruments, Milan, Italy) operating in continuous-flow mode and coupled through a ConFlo III interface (Finnigan MAT, Bremen, Germany) to a gas isotope ratio mass spectrometer (Delta$^{PLUS}$, Finnigan MAT). The standard deviation of repeated measurements of a working standard was 0.15‰ and of a low-N wood sample (6−12 $\mu$g N) was 0.37‰. Because the nitrogen concentration in wood is low, we used 10 ± 1 mg of wood samples for $^{15}N$ analysis. Technical details of the isotope analysis are given in the Supporting Information.

**Statistics.** Apart from environmental factors, also the age of the tree can affect cellulose isotope composition (*17*), which could also be the case for N isotopes in wood. We therefore tested both the effect of the age of the tree when the wood was produced (cambial age) and long-term trends (the decade the wood was produced). Linear mixed models (*20*) fit by maximum likelihood were used with individual trees as random factor, cambial age and decade as fixed effects, and N concentration or $\delta^{15}N$ signal as response variable. The models had the form

$$N_{ijk} = \{\beta_0 + \beta1^*D_{ik} + \beta_2{}^*A_{jk} + \beta_3{}^*D_{ik}{}^*A_{jk}\} + \{\mu_{ij} + \varepsilon_{ijk}\}$$

where $N_{ijk}$ is N concentration or $\delta^{15}N$ signal of the *i*th decade in the *j*th cambial age of the *k*th tree, A is cambial age, D is decade, $\beta_0$, $\beta_1$, $\beta_2$, and $\beta_3$ are fixed effects, $\mu$ is the variation at tree level, and $\epsilon$ is the error term.

N and $\delta^{15}N$ data were log-transformed to improve normality and homogeneity of variances and decade was mean-centered. Several samples of *Swietenia*, from either around 1900 or the 1940s, had exceptionally low $\delta^{15}N$ and high N values, which are difficult to explain and result in non-normal and heteroscedastic data. In *Swietenia*, all statistics were calculated with and without these outliers (with $\delta^{15}N$ signals below −1‰). Statistics with all data and with subsets of data are given in the Supporting Information. Possible colinearity between cambial age and decade was checked with the variance inflation factor (*21*), which was always <4, indicating that colinearity is not a problem.

To present the effect of individual factors graphically, we calculated models with only one predictor variable and tested if the residual of this reduced model was significantly correlated with the other predictor. This partial regression is generally somewhat less powerful than the full model.

Differences in N concentration and $\delta^{15}N$ between bulk wood and wood treated with extractives were tested by paired *t* tests, and differences between sapwood and heartwood were tested by two-sided *t* tests. *t* tests were calculated with SPSS 10 (SPSS Inc. Chicago, IL), linear mixed models were calculated with R 2.9.0 (R Development Core Team, http://www.R-project.org).

## Results

Sapwood, which is characterized by lighter color in the two species, extends for ca. 10 years, so that the wood of the past decade was mostly sapwood, the wood of the second past decade was mostly heartwood but with some sapwood, and all other decades were heartwood only. Nitrogen concentration in bulk heartwood was 1.09 ± 0.23 mg g$^{-1}$ dw in *Cedrela* and 0.91 ± 0.22 mg g$^{-1}$ dw in *Swietenia* (Table 1). Sapwood had significantly higher N concentrations in both species in bulk wood as well as in treated wood. Nitrogen concentrations in bulk and treated wood were highly correlated ($r^2 = 0.87$ and 0.89 for *Cedrela* and *Swietenia*, respectively) and treated wood had somewhat but not significantly lower N concentrations (Figure 1). Regression slopes between N concentrations in bulk and treated wood were almost identical for heart- and sapwood (data not shown) and offsets were close to 0, thus the proportion of N extractable in sapwood was not higher than in heartwood.

$\delta^{15}N$ values were higher in sapwood than in heartwood in both species, in bulk wood as well as in treated wood (Table 1). Differences in N and $\delta^{15}N$ between sapwood and heartwood were reduced somewhat by treatment, but remained significant (Table 1). Correlation between $\delta^{15}N$ in bulk and treated wood was poorer ($r^2 = 0.60$ and 0.58 for *Cedrela* and *Swietenia*, respectively) and treated wood had mostly higher $\delta^{15}N$ values (Figure 1). Leaf nitrogen concentration was 20.5 ± 2.4 and 14.9 ± 2.2 mg g$^{-1}$ dw, and leaf $\delta^{15}N$

**TABLE 1. Mean ± SD (n) of Wood Nitrogen Concentration and $\delta^{15}N$ of Two Tropical Trees from a Brazilian Rainforest[a]**

| | *Cedrela odorata* | | | *Swietenia macrophylla* | | |
|---|---|---|---|---|---|---|
| | bulk wood | treated | *p* | bulk wood | treated | *p* |
| | N concentration (mg g$^{-1}$ dw) | | | | | |
| sapwood | 1.71 ± 0.70 (16) | 1.52 ± 0.55 (21) | 0.2315 | 1.31 ± 0.24 (10) | 1.15 ± 0.22 (12) | 0.177 |
| heartwood | 1.09 ± 0.23 (59) | 1.02 ± 0.30 (63) | $2 \times 10^{-9}$ | 0.91 ± 0.22 (42) | 0.94 ± 0.46 (87) | <0.0001 |
| *p* | 0.006 | 0.0007 | | 0.002 | 0.017 | |
| | $\delta^{15}N$ (‰) | | | | | |
| sapwood | 2.92 ± 1.49 (12) | 3.25 ± 1.24 (18) | 0.0015 | 1.92 ± 1.00 (10) | 2.73 ± 1.72 (12) | 0.0017 |
| heartwood | 1.25 ± 1.17 (57) | 2.57 ± 1.09 (59) | 0.0002 | 0.62 ± 1.42 (44) | 1.51 ± 1.28 (87) | 0.0003 |
| *p* | 0.024 | 0.021 | | 0.003 | <0.0001 | |

[a] Sapwood was the outermost decade (1990s), heartwood wood was produced before 1980; extraction treatment ("treated" as opposed to "bulk" wood) was designed to remove soluble forms of N; p indicates significance of Pearson *t*-tests.
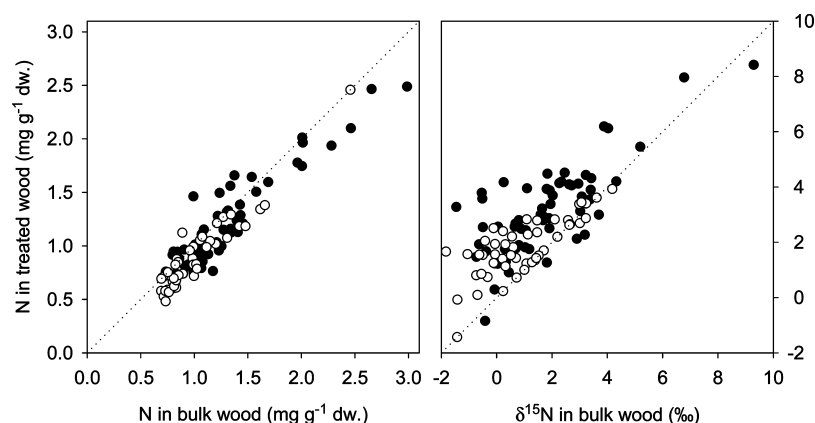


**FIGURE 1. Correlation between bulk wood and wood treated with toluene/ethanol for wood N concentration (left graph) and wood $\delta^{15}N$ signals (right graph) of *Cedrela* (full symbols) and *Swietenia* (empty symbols).**

values were 5.3 ± 2.4‰ and 6.9 ± 1.7‰, in *Swietenia* and *Cedrela*, respectively.

The following analysis is based on treated wood, for which more samples of *Swietenia* have been analyzed. Results for bulk wood are not shown, but generally showed the same trend.

In *Cedrela*, both cambial age and decade had a highly significant effect on wood $\delta^{15}N$ signals, no matter if sapwood or the oldest decades were included or not. Table 2 shows results from heartwood only, model output for samples including sapwood is given in the Supporting Information. The significant decrease of $\delta^{15}N$ with cambial age and independent increase with time is also evident from the partial regression plots (Figures 2 and 3). Excluding sapwood, cambial age and decade both had a significant effect ($p <$ 0.005), but excluding one sample with very high N concentration, the decade effect was no longer significant (Table 3).

In *Swietenia* several samples, mostly from the 1940s with a few in the 1890s and 1950s, had exceptionally low $\delta^{15}N$ values (<−1‰) and high N concentration (mostly >1.5 mg g$^{-1}$ dw, Figure 2). These are difficult to explain, but could be related to any disturbance affecting part of the tree population, and result in inhomogeneous and non-normal variances. With or without sapwood, cambial age had a significant effect on wood $\delta^{15}N$ ($p <$ 0.005) but decade did not. Excluding sapwood and outliers with extremely low $\delta^{15}N$ values resulted in significant effects of cambial age ($p =$ 0.037) and decade ($p =$ 0.013; Table 2). Neither cambial age nor decade had a significant effect on *Swietenia* wood N concentration when sapwood was excluded (Table 3), independent of removing outliers or not. In *Swietenia*, partial regressions, including sapwood, yielded marginally significant correlations between $\delta^{15}N$ residuals and cambial age ($p =$ 0.09; Figure 2) and between $\delta^{15}N$ residuals and decade ($p =$ 0.07, Figure 3).

## Discussion

In *Cedrela* and *Swietenia*, extracting water and lipid soluble substances had little effect on N concentrations though $\delta^{15}N$ signals were about 1‰ higher in treated wood. This contrasts with results from Sheppard and Thompson (*22*), who, using a similar extraction, found less variable and often substantially lower N concentrations in treated wood compared to bulk wood in sapwood but also heartwood of two conifers. Another study found extraction significantly reduced N concentration in *Fagus sylvatica* and somewhat improved the peak of labeled $^{15}N$ (*6*) but this wood was entirely sapwood where living cells contain relatively much soluble N. In retrospect, extraction may not have been necessary for our samples, but, unless the marginal effect of extraction has been shown for a particular tree species, we would recommend removing soluble N, which is not very labor intensive, from tree rings for research on ecosystem nitrogen processes.

Apart from the long-term effects on wood nitrogen discussed below, younger wood in *Cedrela* tended to have higher wood $\delta^{15}N$ signals than older wood produced in the same decade and also had higher N concentrations (Tables 2, 3). In *Swietenia* there was a weaker effect of age on $\delta^{15}N$ and none on N concentrations. Both species regenerate in gaps, small forest clearings generally caused by the death of larger trees, but continue to grow as the gap is replaced by closed rainforest. Thus the difference between juvenile and mature wood in these two species could represent the development from gap phase to mature forest, but other effects such as root access to different N pools could also play a role. If the age-effect is the result of the regeneration niche, we should not expect to see such trend in trees regenerating in mature forests. Our data with 10-year growth segments do not permit us to analyze how long this effect

**TABLE 2. Effect of Cambial Age and the Decade the Wood Was Produced on the $\delta^{15}N$ of Wood from *Cedrela odorata* and *Swietenia macrophylla* Treated to Remove Labile N[a]**

| *Cedrela odorata* | | | |
|---|---|---|---|
| AIC | BIC | logLik | DF |
| −96.4 | −85.5 | 54.2 | 22 |

| fixed effects: | value | SE | p |
|---|---|---|---|
| (intercept) | 2.21 | 0.0358 | 0.0000 |
| camb. age | −0.0022 | 0.00056 | 0.0007 |
| decade | 0.045 | 0.0154 | 0.0075 |
| camb.age * decade | −0.000018 | 0.000216 | 0.93 |

| *Swietenia macrophylla* | | | |
|---|---|---|---|
| AIC | BIC | logLik | DF |
| −132 | −117 | 72.3 | 75 |

| fixed effects: | value | SE | p |
|---|---|---|---|
| (intercept) | 2.00 | 0.032 | 0.0000 |
| camb. age | −0.0012 | 0.00057 | 0.037 |
| decade | −0.016 | 0.0063 | 0.013 |
| camb.age * decade | 0.00012 | 0.00013 | 0.340 |

[a] Only heartwood was selected to avoid a possible effect of sapwood, for *Swietenia* outliers with $\delta^{15}N$ values < −1‰ (see Figure S1) were removed from the analysis, for *Cedrela* only wood produced after 1920 was selected to avoid an unbalanced design as only young cambial wood would be available for the oldest decades (see text and Supporting Information for less stringent selection of data). The analysis is based on a linear mixed-effects model fitted by maximum likelihood with individual trees as random factor.
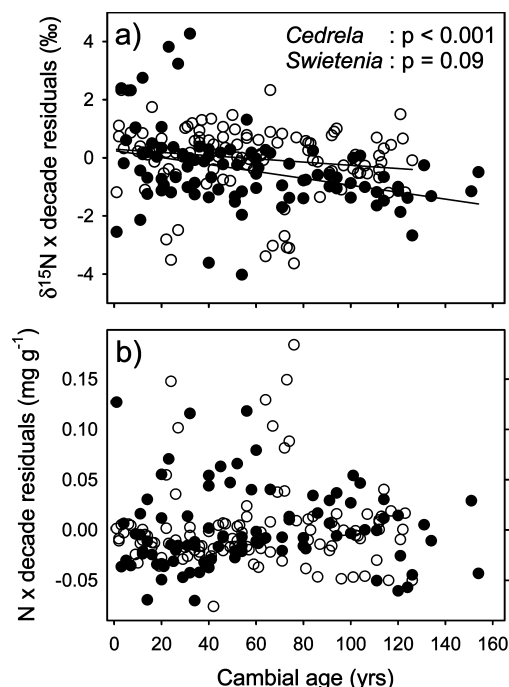


FIGURE 2. Effect of cambial age on wood $\delta^{15}N$ (a) and N concentration (b) in wood after extraction with organic solvents of *Cedrela* (full symbols) and *Swietenia* (empty symbols). The y-axis is the residual of the $\delta^{15}N \times$ decade (a) or N × decade (b) regression, thus a possible decade effect is accounted for in the figures. Original data are shown in the Supporting Information. In *Swietenia*, samples with $\delta^{15}N$ values < −1, which also had very high N concentrations, were rejected from some analyses as explained in the text. "p" indicates Pearson correlation significances for the correlation between cambial age and the residuals.

of a regeneration niche lasts or indeed if it is an effect of different growth condition in the juvenile phase or happening as trees get old. Whatever the cause of an age-effect on wood N isotopes, unless such an effect can be ruled out or accounted for, long-term $\delta^{15}N$ trends in wood of individual trees need to be interpreted with some caution. To avoid confusing a long-term environmental effect with the effect of tree age, trees of different age should be sampled.

In addition to an age effect in heartwood, we found differences in $\delta^{15}N$ and N concentrations between sapwood and heartwood (Table 1), also in treated wood from which labile N had been removed. These differences could result from trends in the environment, but also from an isotope effect during sapwood to heartwood transition, particularly if treatment removes different N fractions than does heartwood formation. Therefore, rejecting sapwood samples may be preferable when looking for long-term environmental effects. As heartwood contains no living cells, N can no longer be translocated between annual rings as in sapwood.

In our study, the increase in $\delta^{15}N$ during the past century was significant, also excluding sapwood and accounting for possible tree age effects. In contrast to our results, most other studies analyzing tree rings from unmanipulated temperate and boreal forests found wood $\delta^{15}N$ to decrease (12, 13, 23), which was explained with increased anthropogenic N deposition (11, 23, 24). In contrast, increases in wood $\delta^{15}N$ values have been related to disturbance by logging (10), fertilization with N (15), traffic along a motorway (16), and change in drainage (14), most of which increase soil N dynamics and thereby increase soil, leaf, and wood $\delta^{15}N$.

Increased deposition of $NO_x$ resulted in wood becoming $^{15}N$-depleted (11, 23−25) or $^{15}N$-enriched (16), depending

on the initial $\delta^{15}N$ of trees and of $NO_x$. Intensified agricultural activities resulted in trees in Switzerland to become more $^{15}N$-depleted (16) as agricultural emissions (mostly $NH_3$ from cattle) have low $\delta^{15}N$ signals. Our study site is remote from anthropogenic emissions. The closest source, large-scale agriculture in Rondônia, is some 200 km from the site, agriculture within 50 km got only off within the past decade of tree growth, and agricultural emissions should have resulted in a decrease rather than the increase in $\delta^{15}N$ we observed. Atmospheric N deposition in the study area is low, with modeled annual rates of <1 kg ha$^{-1}$ in 1860 and ca. 2.5 kg ha$^{-1}$ in the 1990s (2).

At the ecosystem level, $^{15}N$ enrichment has been shown to be caused primarily by enhanced nitrification and N losses (7), because nitrification produces $^{15}N$-depleted $NO_3^-$, which is easily lost through leaching, and $^{15}N$-enriched $NH_4^+$, which tends to be easily lost and thus stays in the soil. Higher rates of nitrification thus result in higher losses, and consequently a greater "openness" of the N cycle, which is the relative importance of N inputs/outputs versus within-system N cycling (26).

Tropical forests have higher leaf and soil $\delta^{15}N$ values than temperate forests (27)—and leaf $\delta^{15}N$ in our two species were in the upper range reported for tropical trees and typical for Amazonian terra firma forests (28)—suggesting generally higher N availability and more open N cycles (7). For the rainforest trees studied the increase in $\delta^{15}N$ values may therefore indicate increased N bioavailability and a more open N cycle, but what could have caused this?

Increasing $CO_2$ concentrations are thought to have affected tropical forest growth (29). Free air $CO_2$ enrichment experiments (FACE) have shown that elevated $CO_2$ can lead to progressive N limitation (30), which should result in more
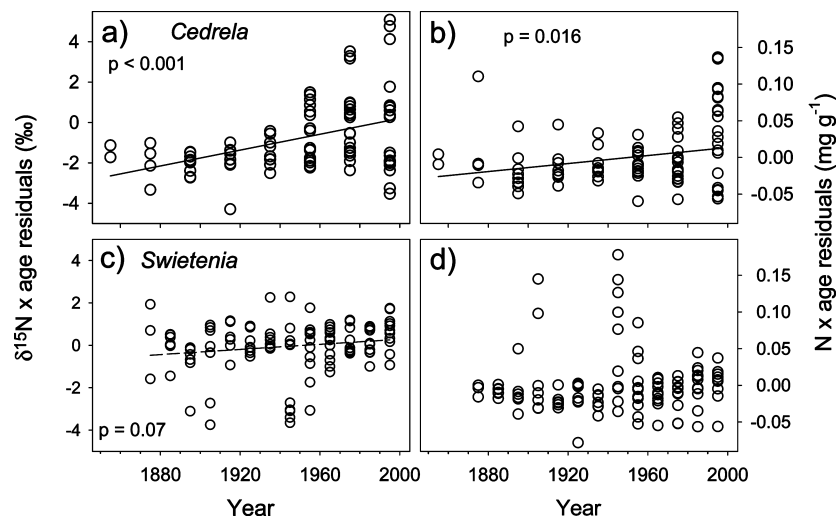
**FIGURE 3.** Trends in $\delta^{15}N$ (a, c) and nitrogen concentration (b, d) in wood after extraction with organic solvents of *Cedrela* (a, b) and *Swietenia* (c, d). The *y*-axis is the residual of the $\delta^{15}N \times$ cambial age (a) or N $\times$ cambial age (b) regression, thus a possible age effect is accounted for in the figures. Original data are shown in the Supporting Information. Solid regression lines show significant (Pearson $p < 0.05$), and broken lines shpw marginally significant ($p < 0.1$) regressions between year and residuals.

**TABLE 3. Effect of Cambial Age and the Decade the Wood Was Produced on the Wood N Concentration from *Cedrela odorata* and *Swietenia macrophylla* Treated to Remove Labile N[a]**

| | *Cedrela odorata* | | |
|---|---|---|---|
| AIC | BIC | logLik | DF |
| −14 | −1.78 | 13.4 | 42 |

| fixed effects: | value | SE | *p* |
|---|---|---|---|
| (intercept) | −2.56 | 0.052 | 0.0000 |
| camb. age | 0.0044 | 0.0008 | 0.0000 |
| decade | −0.017 | 0.012 | 0.170 |
| camb.age * decade | 0.00050 | 0.00029 | 0.086 |

| | *Swietenia macrophylla* | | |
|---|---|---|---|
| AIC | BIC | logLik | DF |
| −311 | −296 | 161 | 73 |

| fixed effects: | value | SE | *p* |
|---|---|---|---|
| (intercept) | 0.0749 | 0.014 | 0.0000 |
| camb. age | 0.00034 | 0.00024 | 0.154 |
| decade | −0.0015 | 0.0027 | 0.570 |
| camb.age * decade | 0.000063 | 0.000054 | 0.246 |

[a] Only heartwood was selected to avoid a possible effect of sapwood (see text and Supporting Information for alternative selection of data). The analysis is based on a linear mixed-effects model fitted by maximum likelihood with individual trees as random factor.

closed N cycles and lower ecosystem $\delta^{15}N$. Leaf $\delta^{15}N$ signatures at higher $CO_2$ concentrations were indeed lower, suggesting lower N losses due to increased plant N demand (*31*). This is contrary to our observations of increasing $\delta^{15}N$ signatures. However, long-term studies of permanent forest plots reported dramatic increases in tree turnover during the past few decades, which means a higher rate of gap formation (*32*). Gap formation as a result of a tree's death results in a pulse of available N and, locally, to increased N losses (*33*), and tree-clearing along *Quercus* stands in Canada resulted in a marked increase in wood $\delta^{15}N$ by 1.5−2.5‰ (*10*). Changes

in forest dynamics are therefore consistent with the increases in wood $\delta^{15}N$ that we found. A more extensive and finer-scaled analysis of trends in wood N and N isotopic composition combined with other evidence of forest disturbance could help resolve the question of altered tree dynamics in tropical forests and generally to understand long-term trends in forest ecosystems.

## Supporting Information Available

Details of the isotope analysis and statistical analysis of alternative models discussed. This information is available free of charge via the Internet at http://pubs.acs.org.

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