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Long-Term Change in the Nitrogen Cycle of Tropical Forests

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Materials and Methods
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Long-Term Change in the Nitrogen Cycle of Tropical Forests

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Deposition of reactive nitrogen (N) from human activities has large effects on temperate forests where low natural N availability limits productivity but is not known to affect tropical forests where natural N availability is often much greater. Leaf N and the ratio of N isotopes (δ^{15}N) increased substantially in a moist forest in Panama between ~1968 and 2007, as did tree-ring δ^{15}N in a dry forest in Thailand over the past century. A decade of fertilization of a nearby Panamanian forest with N caused similar increases in leaf N and δ^{15}N. Therefore, our results indicate regional increases in N availability due to anthropogenic N deposition. Atmospheric nitrogen dioxide measurements and increased emissions of anthropogenic reactive N over tropical land areas suggest that these changes are widespread in tropical forests.

Anthropogenic N fixation has approximately doubled atmospheric deposition of reactive N in terrestrial ecosystems globally, with regional variation resulting from differences in the intensity of agriculture, the burning of fossil fuels, and biomass burning (*1*). Many temperate and boreal ecosystems are N limited; in these regions, atmospheric N deposition has caused the acidification of soils and waters, loss of soil cations, a switch from N to P limitation, a decline in the diversity of plant communities adapted to low N availability, and increases in carbon uptake and storage (*2*, *3*). Natural N availability is much greater in many tropical forests than in most temperate forests due to high rates of N fixation by heterotrophic soil microbes and rhizobia associated with legumes, which are abundant in many tropical forests (*4*). Nitrogen deposition is increasing in the tropics, and this region may see the most dramatic increases in the coming decades (*1*). It has been hypothesized that this will acidify soils, deplete soil nutrients, reduce tree growth and carbon storage, and negatively affect biodiversity in tropical forests (*5*, *6*). Yet despite extensive speculation, there remains no direct evidence for changes in the N cycle in tropical forests.

The ratio of stable N isotopes (δ^{15}N) reflects the nature of the N cycle in ecosystems, with higher values indicating greater N availability and a more open N cycle (*7*, *8*). In temperate ecosystems where N deposition is low, leaf N concentrations and the δ^{15}N of leaves and wood decreased during the 20th century, indicating progressive N limitation in response to changes in land use (*9*) and increasing atmospheric CO$_2$ concentrations (*10*). In contrast, wood δ^{15}N values have increased in temperate forests with high rates of N deposition or a history of recent disturbance, suggesting more open N cycles under such conditions (*11*, *12*).

We compared leaves from herbarium specimens (158 species) collected ~40 years ago (~1968) from a tropical moist forest on Barro Colorado Island (BCI), Republic of Panama, with sun and shade leaves (340 species) collected in 2007. Over four decades, leaf δ^{15}N increases averaged 1.4 ± 0.16 per mil (‰) (SEM) and 2.6 ± 0.1‰ when comparing 1960s leaves to conspecific 2007 shade and sun leaves, respectively. Based on their leaf mass per area, 1960s leaves included a mixture of both sun and shade leaves (*13*). The increase in leaf δ^{15}N occurred in both legumes (Fabaceae) and nonlegumes (Fig. 1, A and B). Foliar N concentrations in nonlegumes increased by 7.7 ± 1.9% and 15.2 ± 2.5% when comparing 1960s leaves to 2007 sun and shade leaves, respectively (Fig. 1C). Legumes had substantially greater foliar N concentrations than nonlegumes, and there was no overall change in their foliar N concentration between the 1960s and 2007 (Fig. 1D).

To assess whether the changes detected on BCI are representative of tropical forests more broadly, we determined δ^{15}N in tree rings from three nonleguminous tree species in the Huai Kha Khaeng Reserve, a remote monsoon forest near the Thailand-Myanmar border. Significant increases in δ^{15}N during the past century were detected in all three species (Fig. 2). Similar changes were reported previously for tree rings in two Amazonian rainforest tree species (*14*).

A forest N addition experiment conducted 1 km from BCI provides perspective on the changes in foliar N composition (*15*). Foliar δ^{15}N increased by 0.3 to 1.5‰ in four tree species and by ~0.5 to 1.2‰ in fine litter (*15*), and the N concentration in litterfall increased by 7% (*16*) after 8 to 9 years of fertilization with 125 kg N ha^{-1}year^{-1}. The observed increase in leaf δ^{15}N did not reflect the signal of the N fertilizer, which had a lower δ^{15}N (–2.2‰) than leaves of nonfertilized trees in control plots (*15*) and therefore should have resulted in a decline rather than an increase in foliar δ^{15}N. Nitrogen fertilization also increased NO$_3$ leaching (from 0.01 to 0.93 mg N liter^{-1}), NO flux (from 70 to 196 μg N m^{-2} day^{-1}), and N$_2$O flux (from 448 to 1498 μg N m^{-2} day^{-1}) (*15*), confirming that the increase in leaf δ^{15}N after N fertilization was associated with a more

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open N cycle. Similar increased N losses and higher leaf δ^{15}N have been observed in temperate forests after N fertilization (*17*). Thus, the increases in foliar N and δ^{15}N between the 1960s and 2007 indicate a regional shift to a more open N cycle.

The forests in Panama and Thailand are old-growth forests that have been intensively studied as part of a global network of long-term studies in very large forest plots (www.ctfs.si.edu). Using this information, we tested whether trends other than increased N deposition might plausibly explain the changes in N concentrations and δ^{15}N observed. On BCI, nothing in the forest structure or documented changes since 1982 suggest that it is in a stage of natural breakdown or recovery. There were no significant changes in aboveground biomass, fast- or slow-growing trees, high or low wood density species, large or small seeded species, or large or understory trees (*18*). The most important events resulting in above-average forest disturbance were El Niño–related droughts, which occur approximately every 5 to 10 years and increase tree mortality. At the Thai site, major damage by fire is a rare but recurring event with a strong and widespread disturbance in the mid-1800s and, since then, several strong but localized disturbances of variable intensity in the 1910s, 1940s, and 1960s (*19*). Fire will result in a short peak in N availability, but the documented disturbance events cannot have resulted in the change in N availability we observe mainly in the second half of the 20th century. Precipitation can affect plant δ^{15}N (*20*) but did not change during recent decades on BCI or at the closest weather station to the Thai forest (fig. S2). Nitrogen-fixing plants have a somewhat different δ^{15}N than non–N-fixing plants (as seen in the 0.5 to 1‰ lower δ^{15}N in legumes compared with nonlegumes), so a reduction in N fixation by legumes might result in an increase in ecosystem δ^{15}N. In this case, the difference in leaf δ^{15}N between legumes and nonlegumes should have decreased and leaf N concentrations decreased rather than increased between 1968 and 2007. Also, the proportion of legume trees did not change on BCI (7.25 to 7.52% of stems between 1982 and 2005, data available at www.ctfs.si.edu) and is low at Huai Kha Khaeng (3.17 and 3.05% in 1992 and 1999, respectively).

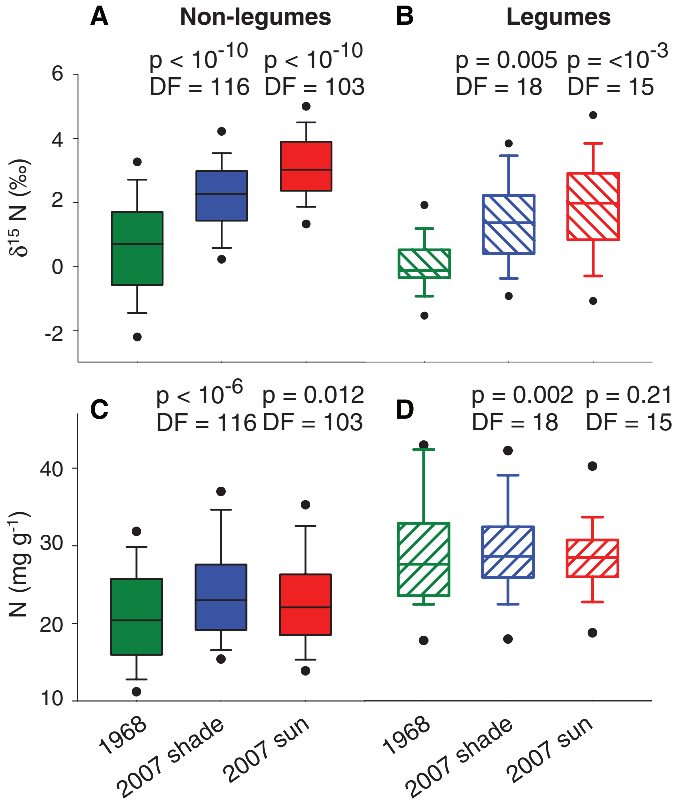

Fig. 1. Leaf δ^{15}N and N concentration in leaves collected ~1968 and 2007 for a tropical moist forest on Barro Colorado Island, Panama. (**A** and **B**) Foliar δ^{15}N and (**C** and **D**) foliar N concentration in leaves of nonlegumes [(A) and (C)] and legumes [(B) and (D)]. Probability (p) values (two-tailed paired Wilcoxon signed rank tests) and degrees of freedom (DF) are for comparisons between leaves collected ~1968 and conspecific sun or shade leaves collected in 2007. Leaf mass per area, which varies with light availability, suggests that herbarium leaves included a mixture of sun and shade leaves.

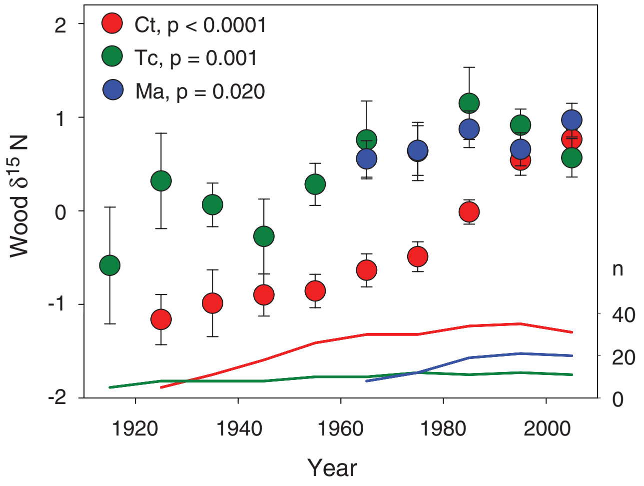

Fig. 2. Increases in wood δ^{15}N in tree rings of three tree species growing in a monsoon forest in the Huai Kha Khaeng Reserve, Thailand. Ct, *Chukrasia tabularis*; Tc, *Toona ciliata*; Ma, *Melia azedarach*. Bars indicate standard errors, and lines represent sample size (n), which decreases for older wood because trees were of different ages. Decades with less than five samples were omitted. Probability (p) values indicate the significance of the decade effect, which was tested using linear mixed-effect models that include tree age (table S1).

The N deposited on BCI is probably from local sources as seen in regionally high tropospheric NO_2 concentrations (fig. S3). Local sources are the increasing shipping traffic through the Panama Canal (fig. S4), which passes BCI, and Panama City, which is 40 km from BCI. The dry forest site in Southeast Asia is located in an area of moderate tropospheric NO_2 concentrations similar to those above Panama, although concentrations are much higher nearby (fig. S5). Our results from Panama and Thailand are likely to apply broadly to tropical forests worldwide, because the largest tropospheric NO_2 concentrations observed over our study sites ($>10^{15}$ molecules cm^{-2}) are recorded over 21, 26, and 12% of tropical Africa, Asia, and America, respectively (fig. S6). Also, atmospheric N deposition recorded <1 km from BCI (9 kg ha^{-1} year^{-1}) (*15*) is similar to model estimates (*21*, *22*) and measurements (*4*) of N deposition in other tropical regions, and N emissions increased in large parts of the tropics (Fig. 3).

Nitrogen deposition over tropical land area increased during the past decades and is projected to increase even further (*1*). This might alter the relative competitive ability of Fabaceae, many of which fix atmospheric N and are naturally N-rich (Fig. 1), leading to shifts in tree species composition. Where N deposition results in increased foliar N, as seen on BCI, the consequence should be increased photosynthetic carbon gain, at least on a leaf-area basis, because foliar N concentration scales with photosynthetic capacity (*23*). This is important for ecosystem models of tropical forests, where the consequences of N deposition are less well understood than for temperate forests (*24*). However, N deposition can also result in soil acidification and altered availability of other nutrients (*5*, *15*), with a potentially negative effect on plant growth. Thus, regional differences in the deposition of N and possibly of other nutrients might contribute to the observed changes in tropical forests (*18*, *25*, *26*) and help to explain regional differences in forest response.

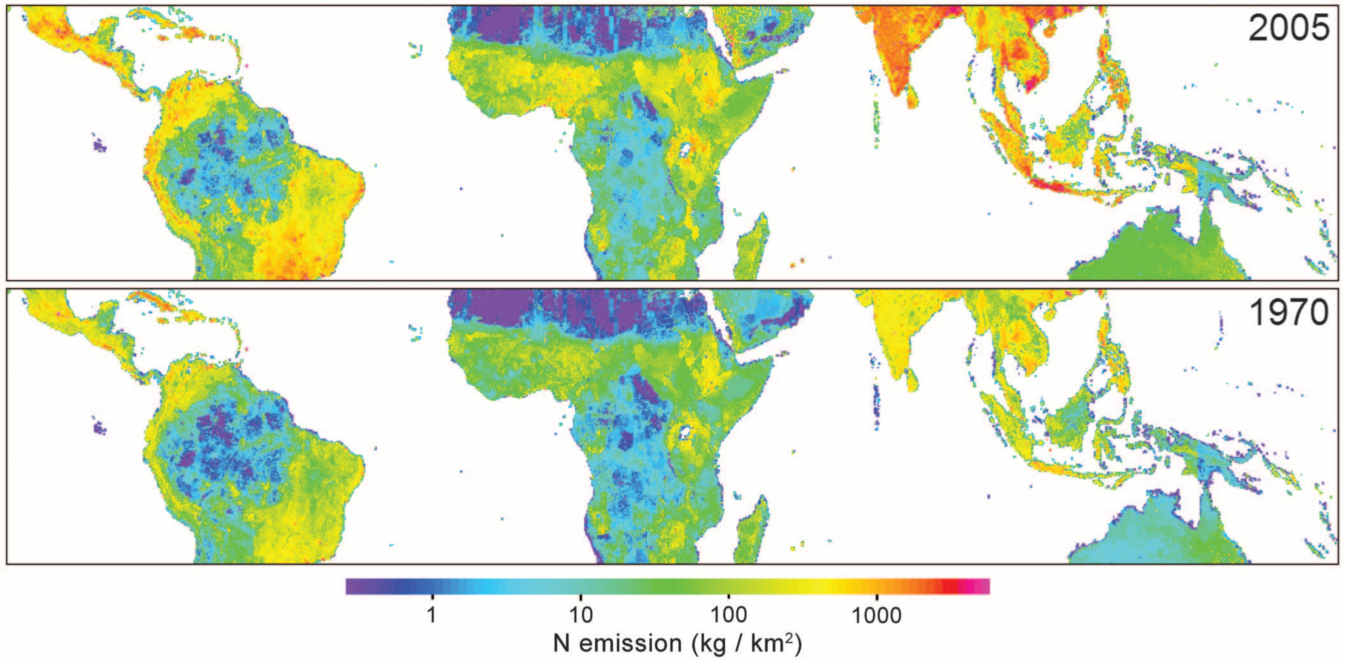

Fig. 3. Anthropogenic N emissions in 2005 and 1970. NO_x and NH_3 emissions per 0.1° grid cell were obtained from European Commission–Joint Research Centre/ Netherlands Environmental Assessment Agency, EDGAR version 4.1, (http://edgar.jrc.ec.europa.eu/) 2010, and were converted to N emissions per surface area.

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Supporting Online Material

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Neural Mechanisms for the Coordination of Duet Singing in Wrens

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Plain-tailed wrens (*Pheugopedius euophrys*) cooperate to produce a duet song in which males and females rapidly alternate singing syllables. We examined how sensory information from each wren is used to coordinate singing between individuals for the production of this cooperative behavior. Previous findings in nonduetting songbird species suggest that premotor circuits should encode each bird's own contribution to the duet. In contrast, we find that both male and female wrens encode the combined cooperative output of the pair of birds. Further, behavior and neurophysiology show that both sexes coordinate the timing of their singing based on feedback from the partner and suggest that females may lead the duet.

Cooperative behaviors are found across taxa and can be critical for survival and reproduction (*1*–*6*). To achieve cooperative performances, brain circuits in each individual must integrate information both from the animal's own self-generated sensory feedback and from sensory cues produced by the partner or partners. We examined how sensory information from these two sources, "autogenous" and "heterogenous" respectively, is integrated in cortical (i.e., pallial) circuits. We used a model system, plain-tailed wrens (*Pheugopedius euophrys*) (*7*), a species of neotropical birds that sing duets in which females and males rapidly alternate syllable production, sounding as if a single bird sang it (see movies S1 and S2) (*8*, *9*).

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