

Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment

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Phosphorus (P) is generally considered the most common limiting nutrient for productivity of mature tropical lowland forests growing on highly weathered soils^{1–5}. It is often assumed that P limitation also applies to young tropical forests, but nitrogen (N) losses during land-use change may alter the stoichiometric balance of nutrient cycling processes. In the Amazon basin, about 16% of the original forest area has been cleared⁶, and about 30–50% of cleared land is estimated now to be in some stage of secondary forest succession following agricultural abandonment⁷. Here we use forest age chronosequences to demonstrate that young successional forests growing after agricultural abandonment on highly weathered lowland tropical soils exhibit conservative N-cycling properties much like those of N-limited forests on younger soils in temperate latitudes. As secondary succession progresses, N-cycling properties recover and the dominance of a conservative P cycle typical of mature lowland tropical forests re-emerges. These successional shifts in N:P cycling ratios with forest age provide a mechanistic explanation for initially lower and then gradually increasing soil emissions of the greenhouse gas nitrous oxide (N₂O). The patterns of N and P cycling during secondary forest succession, demonstrated here over decadal timescales, are similar to N- and P-cycling patterns during primary succession as soils age over thousands and millions of years, thus revealing that N availability in terrestrial ecosystems is ephemeral and can be disrupted by either natural or anthropogenic disturbances at several timescales.

Ecologists have long noted that tropical forests growing on highly weathered soils exhibit conservative P-cycling processes, whereas conservative N-cycling properties are more common on younger soils, including most temperate forests and montane forests^{1,2}. This pattern was demonstrated along a soil age chronosequence in the Hawaiian Islands³, where N, which is derived primarily from the atmosphere, is in short supply in the youngest volcanic soils and gradually accumulates as soils age. In contrast, rock-derived P is more abundant in young soils but becomes bound in unavailable forms to soil minerals as soil weathering proceeds over thousands and millions of years. In global-scale analyses, the N:P ratios of green foliage⁴ and litterfall⁵ of mature forests have been shown to increase with decreasing latitude, indicating generally increasing P conservation and decreasing N conservation with soil age.

Although these stoichiometric generalizations seem robust for mature forests, accelerating land-use change is altering tropical landscapes worldwide, and the consequences for nutrient cycling in secondary forests are unclear⁸. Secondary tropical forests are playing an increasingly important part in maintaining genetic diversity⁹ and hydrological functioning of altered landscapes¹⁰, but biogeochemical processes remain poorly studied in tropical secondary forest succession.

Forest clearing causes an initial loss of nutrients from Amazonian terrestrial ecosystems through fire, erosion, soil emissions of gases, harvesting of timber and hydrologic leaching of nutrients^{8,11}. Additional losses occur as cattle or crops are harvested and as fire is used as a management tool to prepare fields for planting and to control pasture weeds¹². Both N and P can be lost as particulates during biomass burning¹³, but, in contrast to P, N is also volatilized as a gas. Nitrate also generally leaches from soils more readily than does phosphate. As a consequence of these N losses, net N mineralization, net nitrification, nitrate leaching and soil efflux of N₂O often decline as tropical cattle pastures age^{14–17}. Fertilization can maintain agricultural productivity, but where fertilization is not economically viable, the cleared land is often abandoned and a secondary forest begins to grow. Aggrading forests create a strong demand for essential plant nutrients. The objective of this study was to use space-for-time substitutions in secondary forest age chronosequences to describe patterns in the indicators of N and P nutrient cycling during tropical secondary forest succession.

Three forest-age chronosequences, including stands ranging in age from 3 to 70 yr and remnant mature forests, were established in the Brazilian state of Pará, in eastern Amazonia. A complete set of seven indicators of N-cycling rates was obtained for the chronosequence in our main study site in the municipality of São Francisco do Pará. To provide true replication, a second chronosequence was established in the municipality of Capitão Poço, and a third chronosequence was constructed from previously published studies on a ranch in the municipality of Paragominas. The São Francisco do Pará and Paragominas municipalities are about 200 km apart, with Capitão Poço roughly in the middle. Not all of the indicators could be measured in the second and third chronosequence, but each indicator is represented by at least two replicate chronosequences. Soil texture varied among the chronosequences, but was relatively uniform within each chronosequence (Supplementary Table 1). The dominant vegetation of the region was once moist lowland tropical forest, but is now a mosaic of secondary forests, agricultural fields, cattle pastures and tree crops¹⁸ (see Supplementary Information for more site information).

All of the indicators derived from analysis of green foliage, litter-fall, soil and trace gas emissions are consistent with a conservative N cycle in the young successional forests, recovery of N-cycling processes as succession proceeds, and a leaky N cycle in advanced stages of secondary succession and in mature forests (Fig. 1). The log-linear relationships indicate that the largest changes in N-cycling indicators occur early during succession and that the rate of change declines as the secondary forests mature.

The first indicator, foliar ¹⁵N, increases with increasing forest age in both São Francisco do Pará and Capitão Poço chronosequences

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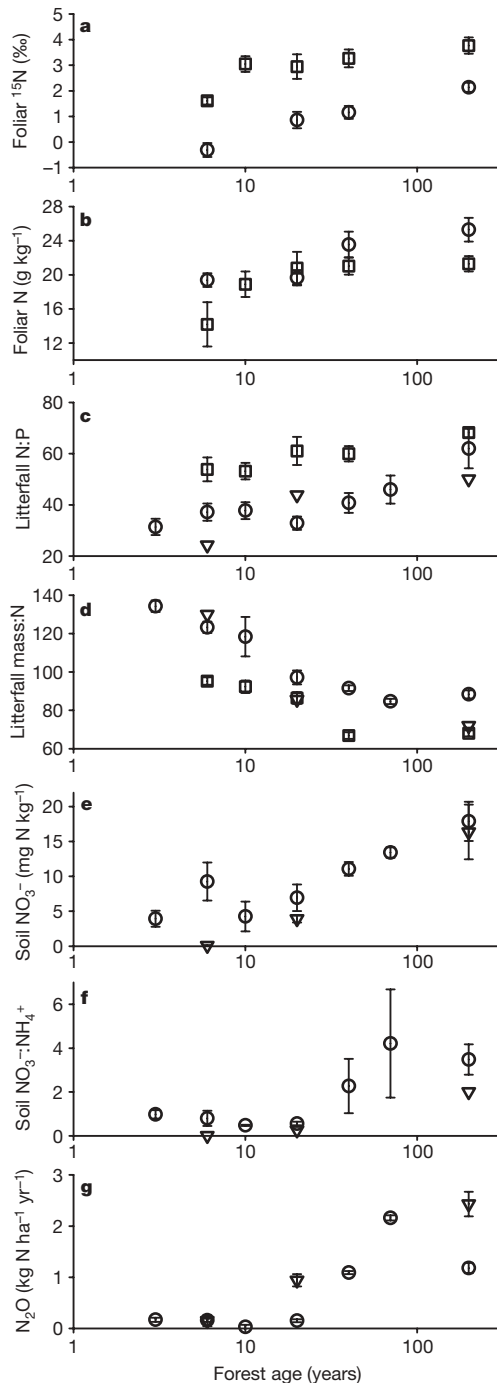


Figure 1 | Indicators of N and P cycling along secondary successional forest chronosequences. a–g, Foliar delta ^{15}N (a); foliar N concentration (b); litterfall N:P ratios (c), litterfall mass-to-N ratios (d); soil nitrate (e); soil nitrate:ammonium ratios (f); and annual soil emissions of nitrous oxide (g). Error bars indicate s.e.m. within each forest age at each chronosequence location. The chronosequences are represented as circles for São Francisco do Pará, squares for Capitão Poço and triangles for Paragominas. The effect of forest age, either as a ranking factor or as the logarithm of age (assuming an age of 200 yr for the mature forest; see Methods) is significant in analysis of covariance (ANCOVA) for all seven indices ($P < 0.05$; see Supplementary Table 3 for all P values). The site-effect is significant for foliar ^{15}N , litterfall N:P, litterfall mass:N and N_2O . Results are nearly identical when the mature forests (of unknown age) are omitted from the analysis, except that the age-effect is not significant for N_2O and is only marginally significant for the nitrate:ammonium ratio ($P = 0.119$ and $P = 0.057$, respectively).

(Fig. 1a). The foliar ^{15}N values of the mature forests of these sites are within the range commonly measured in mature tropical forest foliage ($3.7\text{‰} \pm 3.5$; error of one standard deviation¹⁹), but the values in the youngest forest at São Francisco do Pará (-0.5‰) are as low as those usually measured in temperate forest foliage ($-2.8\text{‰} \pm 2.0$; error of one standard deviation¹⁹). Enriched foliar ^{15}N in the older forests is indicative of a leaky N cycle, in which isotopically light N is lost from the ecosystem owing to fractionation during nitrification and denitrification, leaving isotopically enriched N behind²⁰. In contrast, little N is lost in the conservative N cycle of young secondary forests, resulting in little N fractionation or ^{15}N enrichment. Increasing concentrations of fresh foliar N concentrations with increasing forest age (Fig. 1b), provide a second indicator of increasing N availability as secondary forest succession progresses.

Third, the N:P ratios of fine litterfall increased with forest age (Fig. 1c) for all three chronosequences. The mean litterfall N:P ratios in the mature forests (50–68) bound the global mean of 62 for tropical forests⁵. In contrast, the mean litterfall N:P values of 31 and 24 in the youngest forests of the São Francisco do Pará and Paragominas chronosequences are closer to the global mean of 29 for broadleaf temperate forests⁵. The mean N:P ratio in fresh foliage ranged from 27 to 35 and did not vary systematically across forest ages. Hence, only in the advanced successional and mature forests did the trees reabsorb more P relative to N before leaf abscission. Fourth, the litterfall mass:N ratio also declined with forest age (Fig. 1d). The values in the young forests (100–140) are similar to N-limited temperate forests, whereas the advanced and mature forests values (68–88) are common for mature lowland tropical forests¹. Although both foliar N and P may be reabsorbed before leaf fall, the balance shifts from N economy to P economy in litterfall during secondary forest succession.

Fifth, extractable soil nitrate increased with forest age (Fig. 1e), indicating increasing availability of soil N as the forests mature. Ammonium (which tended to decrease with forest age, but not significantly so) is often the dominant form of inorganic soil N in N-limited systems, whereas nitrate accumulates where available N is more abundant^{14,21}. Hence, in a related sixth index, the ratio of extractable nitrate to extractable ammonium increased from values ≤ 1 for forests ≤ 20 yr to values > 1 for forests ≥ 40 yr (Fig. 1f).

Seventh, although the more clay-rich Paragominas site exhibited higher soil emissions of N_2O than the sandy São Francisco do Pará site, N_2O emissions increased with forest age at both sites (Fig. 1g). Higher rates of nitrate leaching in mature forests compared to young forests have also been measured at the Paragominas site²². These differences in N losses across forest ages reflect current biogeochemical fluxes and are consistent with the time-integrated indicator of ecosystem N loss and fractionation provided by foliar ^{15}N analyses²⁰, which reflects decades of previous slash-and-burn management in the young forests and decades of regrowth in the older forests.

These results have important implications for rates of regrowth of secondary forest and the sustainability of slash-and-burn agriculture, which depends on the accumulation of nutrients in fallow vegetation as the source of nutrients for the next cycle of slash-and-burn agriculture. Rates of secondary forest growth in Amazonia have been inversely correlated with the number of fires during the agricultural phases²³. A large fraction of biomass N is often lost during fires¹³, depleting the pool of actively cycling ecosystem N and provoking a N limitation and a conservative N cycle after repeated fire⁸. The time needed between cycles to re-accumulate needed nutrients for the next agricultural phase increases when the pool of available nutrients, such as available N, declines with each fire cycle. Further evidence for nutrient limitation comes from nutrient amendment experiments in which biomass accumulation in young Amazonian secondary forests responded to N and not P additions in one study²⁴ and showed species-specific responses in another study²⁵.

Although lower emissions of N_2O in secondary compared to mature tropical forests have been previously observed^{14,17,26,27}, here

we show that increasing emissions of N_2O as the successional forests age can be understood in terms of gradual recuperation of several N-cycling processes. A legacy of large N losses during an agricultural phase would probably result in slow rates of recovery of N cycling⁸ and slow rates of increases of N_2O emissions during secondary succession. Conversely, acceleration of N-cycle recuperation and rapid increases in N_2O emissions could result from the emergence of a dominant N-fixing species during secondary forest succession²⁷.

Recent isotopic evidence suggests that biological N fixation may contribute significantly to recuperation of the N cycle during the first 25 yr of secondary succession of Amazonian forests²⁸, although quantitative estimates are poorly constrained. In our study, however, no difference in foliar ^{15}N enrichments between legume and non-legume species was observed (Supplementary Table 2), although leguminous species had higher foliar N concentrations and lower C:N ratios. Leguminous tree species in closed-canopy tropical forests often exhibit N-rich tissues without necessarily fixing significant amounts of nitrogen²⁹. We can neither rule out nor support the importance of N fixation in this study. Atmospheric deposition inputs of nitrogen in this region ($2\text{--}6\text{ kg ha}^{-1}\text{ yr}^{-1}$; ref. 22) are in the same range of $3\text{--}8\text{ kg ha}^{-1}\text{ yr}^{-1}$ that accumulates in woody biomass of these successional forests, but are not enough to account for about $11\text{ kg ha}^{-1}\text{ yr}^{-1}$ that accumulates in foliar biomass during canopy development^{22,24}. The top 10 cm of mineral soil contains ample organic-N stocks ($\geq 1000\text{ kg ha}^{-1}$ of N atoms) that could supply the regrowing forest if a small fraction is gradually mineralized to a bioavailable form, and considerably more is present at lower soil depths²². The rate of recuperation of N-cycling processes during secondary succession may reflect, in part, the kinetics of mobilization of recalcitrant forms of soil N to an actively cycling N pool, as well as the legacy of the degree of degradation during agricultural phases.

The patterns of N and P cycling shown here for secondary succession parallel those previously demonstrated for primary succession. Actively cycling N in terrestrial ecosystems can be lost either by land-use change, such as forest clearing, burning and agricultural practices, or by natural processes such as fires, landslides, glaciers and volcanic activity. Just as accumulation of total ecosystem N alleviates an N limitation as soils age over thousands and millions of years, actively cycling N accumulates over decades and centuries during secondary forest succession, resulting in a similar successional trajectory from a conservative N cycle following agricultural abandonment to the leaky-N and conservative-P cycles expected in mature lowland tropical forests on old soils.

METHODS SUMMARY

In the São Francisco do Pará municipality, two farms were selected to represent each forest age of 3, 6, 10, 20, 40 and 70 yr. In the Capitão Poço municipality, one farm was identified for each forest age of 6, 10, 20 and 40 yr. Two $20\text{ m} \times 20\text{ m}$ plots were established on each farm. These forests were regrowing after abandonment of crop fields. Two plots were also established in each remnant mature forest at São Francisco do Pará and Capitão Poço. Measurements were made from October 2000 through to June 2002 in São Francisco do Pará, and from February 2004 to January 2005 at Capitão Poço. For the Paragominas chronosequence, data from previous studies^{17,22,24} were assembled to construct a chronosequence that included 6- and 20-yr-old forests on abandoned cattle pastures and a mature forest, all on the same soil type and ranch. An ANCOVA was used to test the effects of site (São Francisco do Pará, Capitão Poço and Paragominas) and forest age on mean values of each N and P indicator for each site and age. Because of uncertainty of the ages of the mature forests, the effects of forest age were analysed three ways. First, age was converted to a rank score from 1 to 7 (3-, 6-, 10-, 20-, 40- and 70-yr-old secondary forests and the mature forest, respectively). Second, the ages of the mature forests were conservatively estimated at 200 yr, and the logarithm of forest age was used as a continuous variable. Third, this analysis was repeated with the data from mature forests excluded to test the effects of log-age only in the successional forests of known age. The minor differences in results of these statistical tests are presented in the Fig. 1 legend and in Supplementary Table 3.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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conducted laboratory analyses of soils and litter. A.M.F. collected foliar samples at São Francisco and conducted isotopic analyses, under the supervision of J.P.H.B.O. and G.B.N. F.Y.I. led and R.T.S. assisted with field measurements of trace gas fluxes. S.N.H. conducted litterfall studies and E.C.L. collected soils and fresh foliage at Capitão Poço, both under the supervision of I.C.G.V. A.M.F., F.Y.I., J.P.H.B.O., G.B.N., and L.A.M. contributed to an early draft of the manuscript in Portuguese, and L.A.M. supervised the work of A.M.F. I.C.G.V. established the chronosequence study.

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METHODS

Site descriptions. The remnant mature forests in the municipalities of São Francisco do Pará and Capitão Poço are located at 1° 15' S, 47° 47' W and 02° 12' S, 47° 22' W, respectively, and the secondary forests are located in those vicinities. The Fazenda Vitoria ranch in the municipality of Paragominas is located at 2° 59' S, 47° 31' W. Mean annual precipitation is 2,200 mm for São Francisco do Pará and Capitão Poço and 1,800 mm for Paragominas. The dominant soils are Typic Hapludults in São Francisco do Pará and Capitão Poço, and Typic Hapustox at the Paragominas ranch (see Supplementary Table 1 for surface soil characteristics). Human settlement in this region expanded during the era of rubber extraction in the late nineteenth century. Government programmes for distributing rural lands for agricultural development, mostly by small landholders, increased forest clearing in the mid-twentieth century. Many farms have now undergone nine or more cycles of slash-and-burn agriculture¹⁸. Cattle ranching and logging were the main forces of deforestation in the 1960–80s in Paragominas, including the ranch used in this study, which was established in 1969 (ref. 22).

Method details. Soil emissions of N₂O were measured at São Francisco do Pará and Paragominas using syringe sampling of static chambers and gas chromatography with an electron capture detector¹⁷. Three chamber fluxes were measured per date in each of the twenty-six plots at São Francisco do Pará, with five dates in each of the dry and wet seasons at São Francisco do Pará. Three collections (0.25 m² per collection) of fine litterfall were made monthly for a year in each plot at both São Francisco do Pará and Capitão Poço. Soil inorganic N was extracted in 1 M KCl from triplicate soil samples collected from the top 10 cm in each plot during the rainy season. Fresh foliar samples were collected in the 6-, 20- and 40-yr-old successional forests and the mature forest of São Francisco do Pará and Capitão Poço. At São Francisco do Pará, fully expanded leaves were collected for the dominant species at each site, according to the species importance values indices¹⁸. At Capitão Poço, all leaves within 1 m × 2 m miniplots were harvested and composited. Finely ground foliar samples were analysed for C and N concentrations using a Carlo-Erba CHN analyser, for C and N stable isotope ratios using a Delta Plus ThermoQuest-Finnigan mass spectrometer, and for P by acid digestion followed by colorimetric spectrophotometry.