

Key role of symbiotic dinitrogen fixation in tropical forest secondary succession

Sarah A. Batterman¹, Lars O. Hedin¹, Michiel van Breugel², Johannes Ransijn^{3,4}, Dylan J. Craven^{5†} & Jefferson S. Hall²

Forests contribute a significant portion of the land carbon sink, but their ability to sequester CO₂ may be constrained by nitrogen^{1–6}, a major plant-limiting nutrient. Many tropical forests possess tree species capable of fixing atmospheric dinitrogen (N₂)⁷, but it is unclear whether this functional group can supply the nitrogen needed as forests recover from disturbance or previous land use¹, or expand in response to rising CO₂ (refs 6, 8). Here we identify a powerful feedback mechanism in which N₂ fixation can overcome ecosystem-scale deficiencies in nitrogen that emerge during periods of rapid biomass accumulation in tropical forests. Over a 300-year chronosequence in Panama, N₂-fixing tree species accumulated carbon up to nine times faster per individual than their non-fixing neighbours (greatest difference in youngest forests), and showed species-specific differences in the amount and timing of fixation. As a result of fast growth and high fixation, fixers provided a large fraction of the nitrogen needed to support net forest growth (50,000 kg carbon per hectare) in the first 12 years. A key element of ecosystem functional diversity was ensured by the presence of different N₂-fixing tree species across the entire forest age sequence. These findings show that symbiotic N₂ fixation can have a central role in nitrogen cycling during tropical forest stand development, with potentially important implications for the ability of tropical forests to sequester CO₂.

The recognition that large proportions of tropical forests are recovering from cutting, agricultural land use or natural disturbances^{1,9,10} raises fundamental questions about limits on the tropical carbon sink. Nitrogen may constrain the post-disturbance recovery of vegetation and soil carbon pools^{1,3,11,12} and may limit forest response^{6,8} to rising CO₂, but the extent to which biological N₂ fixation can relieve these constraints remains unresolved^{3,13,14}. Tree species capable of symbiotic N₂ fixation are abundant in many tropical forests, but recent observations show that, depending on local conditions, individual trees may or may not produce the symbiotic nodules where fixation occurs¹⁵, that phosphorus may ultimately constrain fixation in tropical soils^{8,16–18} and that some tropical forests contain a low diversity of N₂-fixing species^{7,19}.

It is therefore crucial to resolve whether N₂-fixing trees can relieve nitrogen limitation and ensure fixation as an ecosystem service²⁰ during tropical forest secondary succession. The existence of a feedback between post-disturbance forest regrowth and N₂ fixation could influence not only the limits to forest CO₂ uptake but also how we understand and model potentially nonlinear interactions between land use, nitrogen cycling and the land carbon sink^{2,3,21}.

Here we use a replicated, natural ecosystem experiment of forest recovery from land use across Panamanian forests to ask, first, whether symbiotic N₂ fixation can overcome the nitrogen deficiency imposed by rapid biomass accumulation during secondary succession; and, second, whether ecosystem-scale fixation depends on the identity and diversity of N₂-fixing species.

The Agua Salud Project encompasses approximately 15 km² of tropical moist forests²² on phosphorus-poor oxisols similar to soils in large

tropical regions worldwide²³ (Supplementary Note 1 and Supplementary Table 1). We examined 12 randomly chosen 0.2-ha plots abandoned from pasture 5, 12 or 30 years ago (four replicate plots per age), plots abandoned approximately 80 years ago (two 1.0-ha plots), and forests near mature status (approximately 300 years; two 1.0-ha plots).

We measured diameters of 13,372 trees from 297 identified tree species, and collected 244 trees to develop allometric biomass equations for the 26 dominant species²² (Methods, Supplementary Note 2 and Supplementary Table 2). Fixers constituted 2.6–14.5% of basal area (Table 1), which is at the low end for many tropical forests, and several of our most abundant species are widespread throughout the neotropics⁷. Comparison between 2009 and 2011 measures enabled us to quantify directly individual tree growth rates and changes in community composition. Biomass carbon pools were calculated from tree allometries (sum of leaves, wood and roots; Supplementary Note 2, Supplementary Fig. 1 and Supplementary Table 2); leaf nutrients across 55 species were similar to other forests²⁴.

We determined fixation in 268 trees (approximately 30% of fixers in all plots) across the nine most abundant species (>70% of fixer basal area across plots; Supplementary Notes 3 and 4). For each tree we quantified active N₂-fixing nodules beneath the canopy (nodulation; g m⁻²) and estimated the fixation rate based on ¹⁵N-tracer incubations or acetylene reduction (Supplementary Note 3 and Supplementary Table 4). This enabled us to scale up fixation to the ecosystem, by summing across all individual N₂ fixers in all plots using species- and forest-age-specific data on nodulation and per-nodule biomass fixation (scenario number 4 in Supplementary Note 3).

We observed rapid forest regrowth and biomass carbon accumulation across the chronosequence (Fig. 1a and Supplementary Table 3). The rate of biomass accumulation (that is, net biomass carbon increase per area and forest age) was exceptionally high in young forests, such that our 12-year forests contained >40% (>50,000 kg C ha⁻¹; >4,100 kg C ha⁻¹ yr⁻¹) of the biomass present in the 300-year forests (approximately 120,500 kg C ha⁻¹). Biomass carbon accumulation decelerated in older forests (black line, Fig. 1a), with a theoretical long-term equilibrium biomass that compared well with eleven old-growth Panamanian forests²⁵ (red line, Fig. 1a).

We also observed a dramatic trend in N₂ fixation across the forest chronosequence (Fig. 1b, Supplementary Fig. 2 and Supplementary Table 5). Ecosystem nodulation and total N₂ fixation increased steeply to a maximum in our 12-year forests (29 kg N ha⁻¹ yr⁻¹), and then decreased to low levels in 80-year forests. At 300 years, fixation recovered to 8% of maximum (Table 1, using scenario number 4 in Supplementary Note 3). Because our sampling was limited to specific forest ages, we cannot evaluate whether even higher fixation may have occurred between 5 and 30 years of forest age.

Highest nodulation and fixation coincided with the period of most rapid plant biomass accumulation (Fig. 1a), implying that nodulation and N₂ fixation were induced by high demand for nitrogen imposed by

¹Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544, USA. ²Smithsonian Tropical Research Institute, Balboa, Ancón, Panamá, Panamá. ³Forest Ecology and Forest Management Group, Centre for Ecosystem Studies, Wageningen University, 6700 AA Wageningen, The Netherlands. ⁴Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark. ⁵School of Forestry and Environmental Studies, Yale University, New Haven, Connecticut 06511, USA. †Present address: German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany.

Table 1 | Properties of N₂ fixers and fixation at the ecosystem, community and individual scales during succession

Variable	Forest age				
	5 years (4 plots)	12 years (4 plots)	30 years (4 plots)	80 years (2 plots)	300 years (2 plots)
Ecosystem: total N ₂ fixation (kg N ha ⁻¹ yr ⁻¹)	10 (4)	29 (12)	11 (6)	0.3 (0.1)	2.2 (0.2)
Community: fixer basal area (per cent of total)	5.0 (1.7)	14.5 (1.1)	5.4 (2.3)	2.6 (0.9)	5.8 (1.4)
Community: fixer versus non-fixer recruitment (per cent per 2 years)	61 versus 5.2	0.51 versus -2.9	-1.9 versus -1.15	NA	NA
Individual: ratio of fixer to non-fixer growth rate	9.2 (3.7)	2.3 (0.6)	1.1 (0.9)	NA	NA

Numbers represent mean (s.e.m.). Ecosystem total N₂ fixation is calculated based on species- and forest-age-specific data for both nodulation and per-nodule biomass fixation (scenario number 4 in Supplementary Note 3). Recruitment was calculated as the per cent of trees remaining in 2011 compared to 2009. Positive numbers indicate increasing abundance (that is, positive recruitment), and negative numbers signify that the group is decreasing (that is, self-thinning). NA, numbers not available because the forest inventory was limited to 1 year. Growth rate was calculated as the average difference in per-individual biomass between 2009 and 2011. Values of greater than 1 indicate greater growth of fixers relative to non-fixers, whereas values of less than 1 indicate that fixers are growing slower relative to non-fixers.

the regrowing forest. In turn, fixation decreased between the 12-, 30- and 80-year-old forests, coincident with a progressive slowdown of biomass accumulation; fixation in 300-year-old forests was similar to independent measures¹⁵ from nearby old-growth forests (red point, Fig. 1b).

These ecosystem trends in N₂ fixation were corroborated by independent observations at scales of plant community composition and individual plant growth (Table 1). At the community scale, relative fixer abundance (per cent of total basal area) displayed the same trend as ecosystem nodulation and fixation: a steep increase to a peak in 12-year forests, followed by a decrease to low levels in forests of 80 years and older. At the individual scale, fixers accumulated biomass ninefold faster than non-fixers at 5 years into forest recovery, but the fixer growth advantage declined to twofold in 12-year-old forests, and became equivalent to non-fixers in 30-year-old forests. This fixer growth advantage was also apparent at the community scale in that recruitment of fixers greatly exceeded non-fixers (61% versus 5%) in

young forests, but decreased to similar levels in the progressively older forests. The partial recovery of fixation and percentage basal area of fixers in 300-year-old forests was due to increased abundance and per-individual fixation of *Tachigali versicolor* (discussed below; see also Supplementary Note 4).

We next asked how these emergent patterns in fixation depended upon the identity of N₂-fixing species. When assessed across all plots, individual species ranged approximately 200-fold in total nodulation and fixation (Fig. 2a and Table 2), with two species contributing >65% of all nodulation: *Inga coccleensis* and *T. versicolor*.

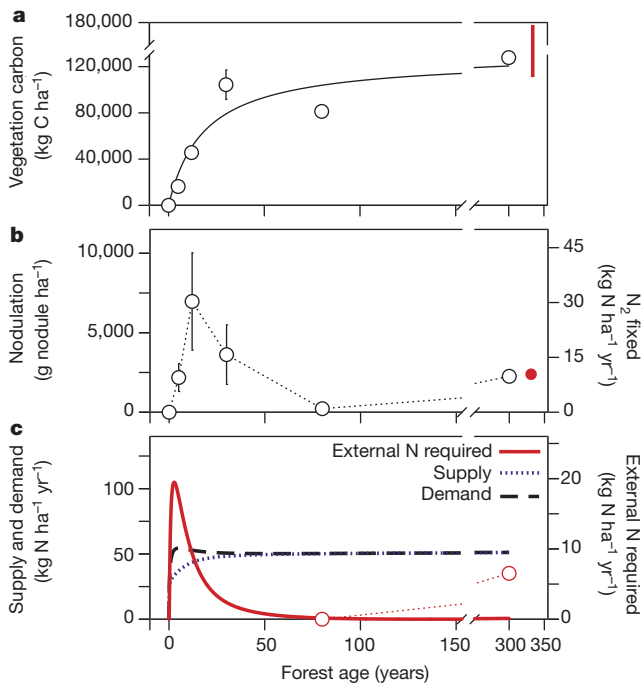


Figure 1 | Biomass carbon accumulation and dinitrogen fixation in forests recovering from land use. **a**, Sum of above- and belowground vegetation carbon (circles). Error bars (means \pm s.e.m.) are invisible when replicates were similar. The fitted Michaelis-Menten curve was used to determine net nitrogen demand for the model, below. Red bar identifies the biomass range observed across 11 (ref. 25) old-growth Panamanian forests. **b**, Nodule biomass and N₂ fixed (mean \pm s.e.m.). Fixation differs slightly from Table 1 because constant per-nodule biomass fixation rate is assumed (scenario number 1, Supplementary Note 3). Red point identifies fixation in nearby mature forests¹⁵. **c**, Modelled plant nitrogen demand (black), soil nitrogen supply (blue) and external nitrogen required from N₂ fixation (red). Dotted red line illustrates that fixation increases with disturbance in mature forests. $n = 4$ 0.2-ha plots for 5–30 years, $n = 2$ 1.0-ha plots for 80–300 years.

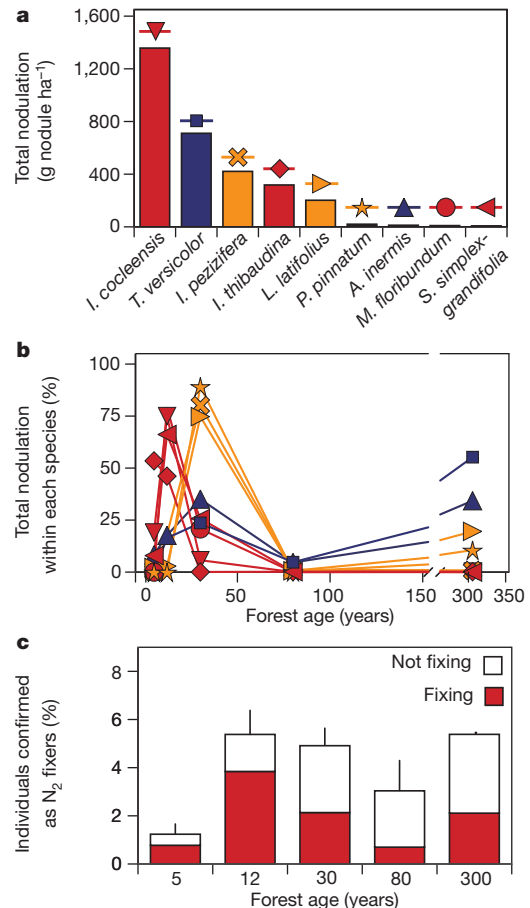


Figure 2 | Individual species contributions to ecosystem fixation. **a**, Calculated total nodulation by species: *Inga coccleensis* ($n = 64$), *Tachigali versicolor* ($n = 36$), *Inga pezzifera* ($n = 29$), *Inga thibaudiana* ($n = 30$), *Lonchocarpus latifolius* ($n = 21$), *Platymiscium pinnatum* ($n = 12$), *Andira inermis* ($n = 30$), *Machaerium floribundum* ($n = 11$) and *Swartzia simplex-grandifolia* ($n = 35$). **b**, Observed percentage of total fixation for each species at each forest age, and the species that specialize on young (red), mid-successional (orange) and mature (blue) forests (colours and symbols are same as in **a**). n values are given in Supplementary Data. **c**, Proportion of trees with known ability to fix that are currently fixing (red) and not fixing (white). $n = 4$ 0.2-ha plots for 5–30 years, $n = 2$ 1-ha plots for 80–300 years. Error bars indicate s.e.m.

Although six of the nine N₂-fixing species were present throughout the chronosequence, individual species differed in when they fixed the most and were most abundant (Fig. 2b and Table 2). One group dominated by *I. coccleensis* fixed primarily in the youngest forests, a second group in 30-year-old forests (dominated by *Inga pezizifera*), and a third group in mature forests (dominated by *T. versicolor*). We infer that these forests depend on a diverse suite of tree species that partition the biogeochemical niche²⁶ for fixation over the course of biomass recovery.

We further asked whether our observed fixation trend was generated by, first, N₂-fixing species that always fix but are progressively excluded from the community as nitrogen is replenished (that is, obligate fixation); or, second, N₂-fixing species that persist over time but that turn fixation on or off depending on their nitrogen balance (that is, facultative fixation). This distinction is significant because the first theory implies that the ecosystem capacity for fixation disappears over time, whereas the second theory implies that the capacity is functionally ensured throughout forest succession.

Our results support the second theory. The proportion of trees with confirmed ability to fix remained relatively stable in forests 12 years and older, yet the proportion that expressed fixation declined from 71% at 12 years to 23% at 80 years (Fig. 2c and Table 2). Furthermore, even when fixing, individuals varied their investment in fixation and nodulation across at least two orders of magnitude independent of species (Table 2), consistent with facultative fixation at the individual scale. These dynamics indicate that fixers can buffer forest nitrogen supply by upregulating fixation when nitrogen is low^{15,27}.

Our findings identify an ecosystem-scale feedback between forest regrowth, vegetation nitrogen demand and the supply of new nitrogen ensured by N₂ fixers. The results imply that nitrogen limitation is the mechanism favouring fixers and fixation in young forests, but the drop

in fixation in older forests could result from either an alleviation of nitrogen-limitation or a decrease in light available to fixers. However, light does not explain the approximately 99% decline between maximum and minimum fixation rates (12 versus 80 years), because the proportion of fixers with canopy access to sunlight remained high over time (51% versus 45%, respectively; Supplementary Note 8 and Supplementary Table 7).

We evaluated the nitrogen feedback using a mass-balance model that captures the mechanisms of plant nitrogen demand (net leaf, wood and fine-root growth), plant–soil nitrogen recycling, nitrogen inputs (deposition and fixation) and nitrogen losses (Methods, Supplementary Notes 5–7, Supplementary Box 1, Supplementary Table 6 and Supplementary Figs 3, 6 and 7). When the model was run with the observed pattern of biomass carbon and nitrogen accumulation (fitted line in Fig. 1a), it revealed, first, that the nitrogen demand imposed by vegetation growth greatly exceeded the capacity of internal recycling to supply plant nitrogen in the first approximately 25 years (dashed versus dotted lines, Fig. 1c); second, that the resulting nitrogen deficit (red line, Fig. 1c) was highest when biomass accumulation was steepest in early succession, and declined as biomass accumulation decelerated over time (red line in Fig. 1c versus black line in Fig. 1a); and, third, that the overall shape of the resulting nitrogen deficit recreated the observed changes in fixation across our plots (red line in Fig. 1c versus black line in Fig. 1b). The field data indicated a lag in fixation in our youngest forests, compared to the model, probably caused by slow establishment and growth of the early tree community (Supplementary Note 6 and Supplementary Figs 4 and 5).

Although fixation would be downregulated as the plant–soil nitrogen cycle becomes increasingly balanced (Fig. 1c), we nevertheless observed low but continued fixation in 300-year-old forests by the gap specialist, *T. versicolor*. This indicates that the feedback between

Table 2 | Properties of individual species at different stages of forest recovery

Forest age (years)	Species (from left to right in decreasing order of importance for N ₂ fixation at the ecosystem level)								
	<i>I. coccleensis</i>	<i>T. versicolor</i>	<i>I. pezizifera</i>	<i>I. thibaudiana</i>	<i>L. latifolius</i>	<i>P. pinnatum</i>	<i>A. inermis</i>	<i>M. floribundum</i>	<i>S. simplex-grandifolia</i>
Abundance, given as the number of trees per species per ha; the total number of trees of any species per ha is given in parentheses beside forest age									
5 (13,806)	230	0	10	135	65	0	95	0	30
12 (6,145)	460	5	80	150	235	0	25	7	140
30 (3,925)	70	95	100	15	5	60	70	8	165
80 (2,240)	8	31	22	7	5	3	4	0	5
300 (1,569)	9	48	12	6	18	6	24	0	0
Trees that were N ₂ -fixing (per cent of trees examined)									
5	62	NA	NA	82	83	NA	17	NA	100
12	91	100	81	87	60	NA	50	22	40
30	56	53	54	100	100	72	17	50	25
80	0	48	33	NA	25	0	17	NA	0
300	8	72	25	NA	45	50	30	NA	0
Mean nodulation rate for N ₂ -fixing trees (min–max) (g nodule m ⁻² under tree canopy)									
5	1.02 (0.06–9.34)	NA	NA	1.07 (0.11–6.66)	1.17 (0.04–3.00)	NA	0.18	NA	0.82
12	1.93 (0.15–7.79)	8.12	1.35 (0.2–12.87)	1.43 (0.30–8.40)	0.34 (0.18–0.49)	NA	0.15	1.54 (0.15–16.01)	0.63 (0.08–3.66)
30	1.81 (0.02–4.78)	6.73 (0.30–31.87)	3.09 (0.25–8.03)	1.18 (0.3–3.78)	9.05	1.08 (0.27–2.26)	0.27 (0.21–0.36)	4.94	0.55 (0.11–3.23)
80	NA	1.82 (0.44–2.95)	0.79	NA	0.19	NA	0.11	NA	NA
300	3.33	3.06 (0.18–16.01)	0.46	NA	0.99 (0.32–5.62)	1.18 (0.14–9.96)	0.18 (0.06–0.39)	NA	NA
Mean fixation rate for N ₂ -fixing trees* (s.e.m.) (g N m ⁻² yr ⁻¹ under tree canopy)									
5	4.6 (2.2)	NA	NA	4.8 (2.1)	0.8 (0.8)	NA	0.5 (NA)	NA	2.2 (NA)
12	8.7 (2.4)	7.4 (NA)	6.5 (2.8)	6.5 (2.8)	0.2 (0.1)	NA	0.4 (NA)	4.1 (NA)	1.7 (0.3)
30	8.2 (4.8)	6.1 (3.6)	5.3 (NA)	5.3 (NA)	6.4 (NA)	2.9 (1.4)	0.7 (NA)	13.2 (NA)	1.5 (1.2)
80	NA	1.7 (0.3)	2.6 (NA)	NA	0.1 (NA)	NA	0.3 (NA)	NA	NA
300	11.1 (NA)	2.8 (1.1)	1.6 (NA)	NA	0.7 (0.2)	3.1 (NA)	0.5 (NA)	NA	NA

* Uses the species- and forest-age-specific per-nodule biomass fixation rate. NA, data not available because the species was either absent or not measured, or because the error could not be calculated due to insufficient replication. The number of trees sampled for Table 2 is given in the Supplementary Data for Fig. 2b.

fixation and soil nitrogen deficiency also can occur in tree-fall gaps¹⁵ that begin to form as stands mature and become less uniform; *T. versicolor* grows rapidly when such light gaps form.

Our findings offer an example in which a vital ecosystem function emerges from the properties of individual species. We estimate that fixers provided >50% of the nitrogen needed to support the sequestration of 50,000 kg of plant carbon per hectare over the first 12 years of forest recovery. The presence of such high fixation rates on the phosphorus-poor Agua Salud soils implies that phosphorus may be less of a constraint on tropical fixation than often assumed in theories or models. Other factors including competition for light or soil molybdenum²⁸ may ultimately limit fixers and/or fixation. Most fundamentally, our results offer a mechanism by which tropical forests can avoid nitrogen constraints caused by disturbance or land-use transition, or that are predicted to occur in response to rising atmospheric CO₂.

METHODS SUMMARY

We sampled 16 forest plots at different stages of recovery from agriculture to intact forest within the 15 km² Agua Salud Project and in the nearby Soberania National Park. All plots had similar climate and were on soils (oxisols) with similar chemical and physical characteristics. In each plot, we measured the diameter of trees and calculated aboveground and belowground carbon and nitrogen biomass using allometric equations developed from 244 trees collected at our sites. We analysed leaf carbon and nitrogen contents from 55 common species across our plots, and used standard conversion factors to estimate carbon and nitrogen biomass across leaf, roots and wood tissues of each individual tree. To determine whole-ecosystem biomass carbon and nitrogen, we summed across all individuals of each species in each plot.

We quantified fixation in individuals and at the ecosystem scale by measuring nodule biomass for 268 trees across the 9 most abundant N₂-fixing species, and per-nodule biomass fixation rates derived from ¹⁵N incorporation and acetylene reduction. We calculated fixation at the scale of the ecosystem across both individual species (sum of all individuals of a given species) and across all species combined (sum of all individuals from all N₂-fixing species).

We developed a mass-balance plant–soil ecosystem model in which plant nitrogen demand and plant litter input to soils was externally forced by the observed carbon accumulation curve across our chronosequence. We considered a plant-available and a plant-unavailable soil pool, and a ‘passive’ soil pool that turned over on a timescale greater than the successional dynamics of this study (and thus was considered external to the plant–soil model). Nitrogen can enter the system through inputs from deposition, the slow mineralization of the externalized passive pool or N₂ fixation, which is determined as the difference between plant nitrogen demand and soil nitrogen supply.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 28 November 2012; accepted 2 August 2013.

Published online 15 September 2013.

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Supplementary Information is available in the online version of the paper.

Acknowledgements We thank S. Adelberg and K. Zelazny for assisting with data collection, M. Baillon and A. Hernandez for botanical identifications, N. Wurzbarger and A. Barron for species-specific N₂ fixation rates, J. Sprent for advice about N₂-fixing trees and P. Reich for comments. This work was supported by grants to L.O.H. from the National Science Foundation (NSF; DEB-0614116), the National Oceanic and Atmospheric Association (NOAA; grant NA17RJ262–344), the Cooperative Institute for Climate Science of Princeton University and the Carbon Mitigation Initiative of Princeton University; and to S.A.B. from the Smithsonian Tropical Research Institute (STRI). It is a contribution to the Agua Salud Project (ASP), a collaboration among STRI, the Panama Canal Authority (ACP) and the National Environmental Authority of Panama (ANAM). ASP funding came from the HSBC climate partnership, STRI, the Frank Levinson Family Foundation, the Motta Family Foundation and an anonymous donor.

Author Contributions S.A.B., L.O.H., J.S.H. and M.v.B. designed the project. S.A.B. conducted N₂-fixation-related field work. J.R., M.v.B. and J.S.H. provided allometry data; D.J.C. provided plant nutrient data. S.A.B. and L.O.H. wrote the paper. All authors commented on the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.A.B. (sbatterman@princeton.edu).

METHODS

Study sites. We sampled 16 forest plots at different stages of recovery from agriculture to intact forest within the 15-km² Agua Salud Project²² and in Soberania National Park approximately 3 km from Agua Salud. Soils across all sites were highly phosphorus-poor and clay-rich oxisols derived from basalt parent material, with no significant differences in carbon, nitrogen, phosphorus or cations across forest age ($P > 0.05$, ANOVA, d.f. = 4 for total carbon, total nitrogen, carbon:nitrogen, total phosphorus; and d.f. = 2 for Mg, K, Ca, Al and extractable phosphorus) (Supplementary Table 1). All sites were classified as tropical moist forest, received similar annual rainfall (2,700 mm; S. Paton, personal communication) with a December-to-April dry season, and shared a diverse community of tree species (>297). Forests contained 24 N₂-fixing tree taxa, many of which are commonly found throughout neotropical forests. We measured all biological and soil properties in the wet season.

Sampling approach. We selected sites to create a replicated sequence of forest recovery ages, with all sites <80 years old recovering from grazing, and sites 80 and 300 years old recovering from either cutting alone or cutting followed by grazing and/or non-intensive agriculture^{29,30}. We used a standard chronosequence approach³¹ in which space-for-time substitution is used to infer changes in ecosystem properties at temporal scales that exceed the limit of direct observation, and which assumes that sites share similar initial conditions and similar dynamics over time. Our forests and soils are very similar in composition and historical origin, but, depending on forest response to CO₂ fertilization and/or temperature change (at present unknown), increased atmospheric CO₂ or temperature may have introduced a historic bias in the rate of recovery in younger forest stands.

We adjusted area and number of plots to account for differences in tree density (13,800 versus 1,500 trees per ha in 5- versus 300-year-old forests, respectively; Table 2), landscape variability in community composition (high in young forests), biomass accumulation (high in young forests; Fig. 1a) and the influence of localized forest gap dynamics (absent in young forests but common in 300-year-old forests). Note that in mature forest it is not possible to conceptually or visually separate 'gaps' (unless they are only a few years old) from 'mature forest'. We sampled four replicate 0.2-ha plots in all forests <80 years, and two replicate 1.0-ha plots in the 80- and 300-year-old forests. In plots <80 years, we measured every tree with a diameter at breast height (DBH) of ≥ 5 cm, and half of all trees with a DBH of 1 to 5 cm; in 80- and 300-year-old plots we measured every tree with a DBH of ≥ 10 cm and two-fifths of trees with a DBH of 1 to 10 cm in 2008. We sampled all plots of <80 years in age in 2009 and 2011; this enabled us to reliably calculate differences in individual tree growth rates between fixers and non-fixers, and to quantify changes in community composition during the period of highest biomass accumulation and strongest competition for light. We did not re-sample our older sites because growth calculated from DBH becomes reliable only in large trees over longer timescales.

Biomass calculations. We used a standard allometric approach to quantify biomass allocation to leaves, wood and fine roots across all plots (Supplementary Fig. 1 and Supplementary Tables 2 and 3). For each of our 26 most common species (contributing approximately 75% of total basal area across all plots) we developed equations (all $R^2 > 0.85$) for aboveground wood and leaf biomass by collecting 244 trees <26 cm DBH: $B_{x,s} = Y_{x,s} \times \text{DBH}^{(Z_{x,s})}$, where B is biomass, Y is a proportionality coefficient, Z is a scaling exponent, x is leaf or wood, and s is species²² (Supplementary Table 2). For all other species, we used a general equation based on all species combined (Supplementary Table 2). Roots and the sum of aboveground and belowground wood were calculated as described below. For trees with a DBH of ≥ 26 cm, we used a general equation for aboveground tropical forest biomass²⁵ (AGB): $\text{AGB} = A \times e^B$, where $B = -3.742 + 3.45 \times \log(\text{DBH}) - 0.148 \times \log(\text{DBH})^2$ and A is a coefficient for wood density. We calculated biomass allocation to leaves versus roots versus wood, as follows: leaf area index equilibrating at approximately 0.2 kg N m⁻² after canopy closure; fine roots equal to leaf biomass³²; coarse roots from a published allometric relation with stem biomass derived from the global Luysaert database³³; and total wood = AGB - leaf biomass + coarse root biomass. Biomass was summed across all trees in each plot and converted to carbon based on the average carbon content (0.47) in our forests³⁴.

Tissue nutrient pools. We determined biomass, and biomass carbon and nitrogen pools (Supplementary Table 3), by summing the biomass of individual trees (estimated with allometric equations as described above) across all individuals and species and applying the nitrogen content of tissues that we determined from our forests and the literature. We measured species-specific leaf nitrogen content of leaves from the 55 most common species of trees across the Agua Salud forest sites³⁵. Leaf nitrogen contents were within the range found at other neotropical sites^{24,36}. To calculate the total leaf nitrogen pool size, we found the community abundance-weighted average leaf nitrogen content and applied that to our total leaf biomass pool size. We used a standard nitrogen content of wood derived from the literature³⁷ in the lower (and conservative, for our calculations) range of

tropical wood nitrogen contents³⁸, and we approximated fine-root nitrogen content as being equal to leaves. Total forest biomass nitrogen was determined as the sum of leaf, wood, and root nitrogen.

Dinitrogen fixation. We directly quantified nodule biomass and N₂ fixation by collecting 2,334 soil cores (5.5 cm in diameter, 10 cm deep) randomly from the rooting zone beneath each individual tree using crown area to approximate root area in the rainy season of 2010 (refs 15, 39). To account for differences in rooting area with tree size, we collected 6 cores for trees with DBH of 1–5 cm, 8 cores for trees with DBH of 5–10 cm, 10 cores for trees with DBH of 10–20 cm and 12 cores for trees with DBH > 20 cm, consistent with our sampling method that we developed over the past 9 years. All nodules were cut open to confirm nitrogenase activity, and total nodule biomass was expressed per soil area of each tree (g nodule m⁻²) and per individual tree (g nodule tree⁻¹). Because natural abundance ¹⁵N/¹⁴N ratios cannot necessarily resolve individual tree N₂ fixation⁴⁰, we quantified nodulation from June to August 2010 and nodule mass-specific fixation using ¹⁵N-tracer incubations of several species of *Inga*¹⁵ and acetylene reduction assay (ARA) measures in 15 species of fixers across different growth conditions in Panamanian forests between 2003 and 2012 (Supplementary Table 4). We expanded these measures to the plot scale as follows: we scaled average species-specific nodule biomass (g nodule m⁻²) to the rooting area for each individual tree and summed across all trees in each plot; for the species for which we did not directly quantify nodulation (<30% of fixer basal area), we used the average nodule biomass per area from all species. When evaluating percent nodulation across species (Fig. 2c) and the fraction of trees that were currently fixing, we considered only trees for which we had directly measured fixation.

We calculated N₂ fixation by multiplying nodule biomass by per-nodule biomass fixation rate from the two groups above, and evaluated the potential effect of species- and forest-age-specific per-nodule biomass fixation rates (discussed in Supplementary Note 3). Total ecosystem fixation rates were relatively insensitive to variations in these assumptions; we therefore report species- and forest-age-specific data on both nodulation and per-nodule biomass fixation in Table 1 and compare the alternative scenarios in Supplementary Note 3.

Model description. Our model evaluates the effect of the observed biomass accumulation trend (line in Fig. 1a; quantified from allometric measures of all individual trees across all species and all plots) on the internal forest N cycle. The model (Supplementary Box 1) tracks nitrogen mass balances (as kg N ha⁻¹) in plant pools of leaves (N_L), wood (N_W) and fine roots (N_R), as well as in plant available (N_A) and plant unavailable (N_U) forms in soil:

$$B_x = B_x^* t / (t^* + t) \quad (1)$$

$$N_L = N_R = \omega_L B_L \quad (2)$$

$$N_W = \omega_W (B_T - B_L - B_R) \quad (3)$$

$$dN_A/dt = I - \text{plant N demand} + mN_U - k(N_A) + r\alpha(\mu_L N_L(t) + \mu_R N_R(t)) \quad (4)$$

$$dN_U/dt = \beta\mu_W N_W(t) - m(N_U) + r(1 - \alpha)(\mu_L N_L(t) + \mu_R N_R(t)) \quad (5)$$

Equation (1) describes the observed net plant biomass accumulation (B) over time (t) fitted as a Michaelis–Menten equation based on the tissue-specific allometric calculations outlined above (Fig. 1a and Supplementary Note 2), and where B_x^* is the equilibrium maximum biomass, t_x^* is the half-saturation constant and x refers to tissue pool (L , leaves; R , fine roots; W , wood; T , total biomass). Following standard convention^{9,32,33}, we assumed equal quantities of nitrogen in leaf (N_L) and fine root (N_R) biomass, and calculated both pools as leaf biomass times the fraction of nitrogen in leaf biomass (ω_L) (equation (2)). We calculated nitrogen in wood as the product of wood biomass (total plant biomass minus leaf and fine-root biomass) and the fraction of nitrogen in wood (ω_W) (equation (3)). Equation (4) gives the time-dependent change in the plant-available soil nitrogen pool, as a function of external nitrogen input (I), the plant nitrogen growth demand, the input mineralization rate (m) from plant-unavailable soil nitrogen, the loss rate (k) from the plant-available soil nitrogen pool, and the input rates of plant leaf (μ_L) and fine-root (μ_R) turnover, where r is the nitrogen fraction not retranslocated and α is the

fraction entering the soil-available pool. Plant nitrogen demand was calculated from equation (1) applied to all tissue pools and corrected for annual turnover (adjusted for retranslocation) of leaf and fine-root nitrogen. Equation (5) gives the time-dependent change in the plant-unavailable soil nitrogen pool, as a function of the input rate from wood mortality (μ_W), where β identifies the fraction of wood mortality entering the plant-unavailable pool; the loss mineralization rate (m) to the plant-available pool; and the input rates of nitrogen from leaf (μ_L) and fine-root (μ_R) turnover.

We simplified our model by externalizing all soil nitrogen with mean residence time (MRT) that exceeded the dynamics of interest in this study (taken as >30 years). We assumed that this soil nitrogen is large in size and originated from wood, with the flux calculated as a loss fraction ($1 - \beta$) of wood mortality. We also considered a slow but constant mineralization input from this external pool, which we added to the external input term (I). We show in Supplementary Information that this simplification offered a good approximation of more complex models in which we explicitly tracked a passive soil nitrogen pool following the standard approach of CENTURY-type models.

The external nitrogen required (that is, N_2 fixation) was calculated as the difference between plant nitrogen demand and soil nitrogen supply. A positive value indicates that the internal nitrogen supply could not match the demands of the recovering forest, thus necessitating additional nitrogen inputs from symbiotic fixation. A negative balance, in turn, indicates that internal supplies were sufficient or exceeded forest demand.

Model parameterization. We parameterized the model using values reasonable for our Panamanian forests as summarized in Supplementary Table 6. We used the tissue nitrogen contents (percentage nitrogen) that we determined from our data and the literature as described above. We allowed leaves to turn over annually, which is conservative relative to our observations across the youngest forests, as faster turnover would increase external nitrogen required.

We doubled the rate of wood turnover (from 0.02 to 0.04 per year) to evaluate the effect of disturbance on fixation in mature forests (shown as dotted red line in

Fig. 1c). Because we are simulating forest carbon recovery during secondary succession, we started the plant-unavailable nitrogen pool at 60% of equilibrium size; we show in Supplementary Information that increasing the pool size to 100% does not alter the dynamics of the system.

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