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Biological Nitrogen Fixation

Sarah A. Batterman[1]* *and Nina Wurzburger*[2]

ABSTRACT. Biological nitrogen fixation supplies the largest quantity of new, natural nitrogen to terrestrial ecosystems, but little is known about its function and controls in tropical forests. Recent work at Barro Colorado Island and the facilities of the Smithsonian Tropical Research Institute has made substantial advances in our understanding of this process. Nitrogen fixation is performed by bacteria associated with leguminous trees and by free-living bacteria in soils, litter, and tree canopies. Research indicates that high nitrogen availability can suppress fixation, while other elements, such as phosphorus and molybdenum, and environmental factors, such as energy and carbon dioxide concentration, control rates of fixation. Tropical trees with symbiotic associations with nitrogen-fixing bacteria use facultative fixation, adjusting fixation to their nitrogen demand versus soil supply. Furthermore, tree species differ in their rates and timing of symbiotic nitrogen fixation. These findings have inspired research on nitrogen fixation in other tropical forests and have contributed substantial new understanding to tropical forest biogeochemical cycling and function.

Keywords: biodiversity; biological nitrogen fixation; biogeochemical cycling; nutrient limitation; nitrogen; phosphorus; tropical forest

INTRODUCTION

Biological nitrogen fixation—the biological conversion of atmospheric dinitrogen into a bioavailable form—has long been invoked as a critical process for regulating ecosystem function. It is the primary natural pathway by which new nitrogen enters ecosystems, thereby promoting ecosystem development in primary succession and allowing ecosystems to recover from disturbance during secondary succession. Within tropical forests, several distinct organisms contribute to biological nitrogen fixation, including free-living microbes that live in the soil and leaf litter, cyanobacteria that live in the canopy, and bacteria that form a symbiotic relationship with roots of certain plant species. Until recently, however, we have had little empirical evidence about how biological nitrogen fixation in tropical forests is governed over space and time. Over the past two decades, research at Barro Colorado Island (BCI) and nearby facilities of the Smithsonian Tropical Research Institute has advanced our understanding of this key ecosystem process and has made major contributions to the field of tropical biogeochemistry.

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NITROGEN FIXATION NICHES AND THE NITROGEN PARADOX OF TROPICAL FORESTS

Scientists have long assumed that tropical forests are nitrogen rich because of the abundance of leguminous trees (Jenny, 1950; Vitousek, 1984; Vitousek and Howarth, 1991; Martinelli et al., 1999; Hedin et al., 2003; Davidson et al., 2007; Brookshire et al., 2012), which can form a symbiotic association with nitrogen-fixing bacteria. However, this presents a paradox: if available soil nitrogen is sufficient, nitrogen-fixing trees should be outcompeted by non-fixing trees because of the high energetic cost of fixation (Hedin et al., 2009). Over the past two decades, research in Panamanian forests has revealed that although nitrogen-fixing trees are abundant across forest ages, trees only fix nitrogen at appreciable rates in young secondary forests or in treefall gaps in mature forests (Barron et al., 2011; Batterman et al., 2013a; Wurzburger and Hedin, 2016). This suggests that nitrogen-fixing trees can up- or downregulate fixation depending on their nitrogen demand and available soil nitrogen—a facultative fixation strategy (Barron et al., 2011). Tropical symbiotic fixers that use facultative fixation therefore can persist at high abundances later in succession despite nitrogen richness in the ecosystem (Hedin et al., 2009). This facultative strategy contrasts with the obligate strategy typical of many temperate and boreal nitrogen-fixers that fix nitrogen at a constant rate, regardless of plant nitrogen demand and soil nitrogen supply (Menge et al., 2009; Sheffer et al., 2015). Findings from Panama spurred a substantial body of theoretical work on facultative versus obligate versus the possibility of other strategies (Menge et al., 2009, 2015; Sheffer et al., 2015) as well as research on the role of symbiotic nitrogen fixers in forest recovery in Costa Rica, Trinidad, and Brazil (Sullivan et al., 2014; Winbourne et al., 2018; Brookshire et al., 2019; Taylor et al., 2019; Wong et al., 2020).

If mature tropical forests are nitrogen rich and most leguminous trees are not actively fixing, where could the nitrogen richness come from? Two free-living sources of biological nitrogen fixation could provide substantial inputs of new nitrogen independent of soil nitrogen status: (1) asymbiotic fixers that rely on organic carbon for their energy are favored in the leaf litter layer, which has a high C: N ratio, and lies just above the mineral soil (Wurzburger et al., 2012); and (2) cyanobacteria that fix nitrogen proliferate in the canopy of tropical trees where high light availability drives high nitrogen demand (Stanton et al., 2019). Other sources of fixed nitrogen that have not been studied in Panama but that may also be important in tropical forests include free-living fixers in woody debris, on leaf surfaces (epiphylls), and in the guts of ants, and symbiotic fixers associated with cycads and leguminous lianas. The tropical nitrogen paradox therefore could be explained by the dominance of new nitrogen inputs from symbiotic fixers in early succession and in mature forest treefall gaps, and the later importance of asymbiotic sources from the leaf litter layer, canopy, and other sources as forests age and with time following disturbance.

Current best estimates indicate that symbiotic nitrogen fixation can provide up to 30 kg N ha^{-1} yr^{-1} in younger forests and ~2 kg N ha^{-1} yr^{-1} in mature forests (Batterman et al., 2013a), leaf litter fixation provides <0.5 kg N ha^{-1} yr^{-1} (Barron et al., 2009), and canopy fixation could provide up to 5 kg N ha^{-1} yr^{-1} (Stanton et al., 2019). Although local nitrogen availability and nitrogen fixer identity (i.e., symbiotic versus asymbiotic) drive these broad patterns in fixation over space and time, other elements contribute to the regulation of fixation.

CONTROLS ON BIOLOGICAL NITROGEN FIXATION

Ecological theory suggests that biological nitrogen fixation is regulated by phosphorus in addition to nitrogen due to the large amount of adenosine triphosphate (ATP) required to break the triple bond of dinitrogen (Vitousek and Howarth, 1991). This theory is particularly relevant to highly weathered, phosphorus-poor soils that often characterize tropical forests. A more recent alternative theory suggests that nitrogen fixation evolved as a mechanism to acquire phosphorus because enzymes that liberate phosphorus require nitrogen for their synthesis (Houlton et al., 2008). Few studies, however, had tested how biological nitrogen fixation and phosphorus interact. Work from BCI and Panama filled a key knowledge gap by testing these theories as well as by expanding the consideration of how other elements, like molybdenum and carbon, might further govern fixation.

Field and laboratory experiments demonstrated that nitrogen and phosphorus interact to regulate nitrogen fixation such that fixation is suppressed by high available soil nitrogen but is limited by available phosphorus (Barron et al., 2009, 2011; Wurzburger et al., 2012; Batterman et al., 2013b; but see Batterman et al., 2018). At face value, the finding that fixation is limited by available phosphorus does not conform to the theory that fixation evolved to promote phosphorus acquisition in tropical forests. To probe this question directly, several investigators quantified phosphatase activity in the rhizosphere of nitrogen-fixing trees and found that activities did not increase with nitrogen fixation activity and that higher phosphatase activity in the rhizosphere of fixers versus non-fixers was observed only in certain species (Batterman et al., 2013b; Batterman et al., 2018; Nasto et al., 2019; see also Soper et al., 2019 from Costa Rica). However, recent findings suggest that fixers may uniquely access otherwise-unavailable soil phosphorus pools by changing the soil C: N ratios, pH, and microbial community composition, which enhances soil weathering beneath their canopies (Epihov et al., 2021). These findings suggest that fixers may indeed have evolved strategies for overcoming low-phosphorus tropical soils.

In addition to phosphorus constraining nitrogen fixation, research in Panama has examined the potential importance of molybdenum and carbon dioxide (CO_2) as limiting elements. Molybdenum is thought to be particularly important because it is a co-factor in the nitrogenase enzyme that catalyzes the fixation reaction. Elevated CO_2 could stimulate fixation by increasing carbon efficiency and providing more energy for plants to allocate to their bacterial symbionts (Cernusak et al., 2011). Indeed, symbiotic fixation rates in seedlings were stimulated by molybdenum (Wurzburger and Hedin, 2016; Trierweiler et al., 2018) and by rising concentrations of atmospheric CO_2 (Trierweiler et al., 2018; Nasto et al., 2019). Fixation by free-living fixers in leaf litter and the canopy was similarly stimulated by molybdenum (Barron et al., 2009, 2011; Wurzburger et al., 2012; Stanton et al., 2019).

These findings from Panama stimulated a host of research on the constraints on biological nitrogen fixation. Free-living fixation rates have been found to vary by tree species, to be constrained by phosphorus (Reed et al., 2008, 2011), and to sometimes, but not always, correlate with molybdenum availability (Wurzburger et al., 2012; Reed et al., 2013; Wong et al., 2021). Differences in the type and degree of nutrient limitation may be explained by the mineralogy of soil parent materials. For example, in Belize, iron limited free-living fixation rates in soils derived from high-pH limestone bedrock (Winbourne et al., 2018). For symbiotic fixation, recent work from Costa Rica and Brazil identifies roles for light and water as regulating factors (Taylor and Menge, 2018; Winbourne et al., 2018; McCullogh et al., 2021). In Panama, however, there was no seasonality in fixation rates between the wet and dry seasons, which differ substantially in both light availability and rainfall (Barron et al., 2011). And, finally, intriguing new evidence from BCI and Panama suggest a key role of herbivory in governing fixation rates (Batterman et al., in review; Barker 2020; Barker et al., 2022). Thus, nitrogen appears to regulate where, when, and which fixer is fixing in tropical forests, but other elements and environmental conditions control how much nitrogen is fixed, further creating nitrogen hot spots in the tropical landscape.

SPECIES DIFFERENCES IN SYMBIOTIC NITROGEN FIXATION

Finally, diversity of leguminous fixers is high in tropical forests, yet biogeochemical theory generally assumes that all fixer species function similarly. Research from Panama challenges this assumption. Although legumes are highly diverse and account for around 9% of tree basal area on the BCI 50-hectare plot (Losos and Leigh, 2004), only a few species dominate fixation activity, suggesting they serve as “superfixers” across the landscape (Wurzburger and Hedin, 2016). Such a pattern of high fixation activity isolated to certain locations of the landscape could be caused by differences in per-area or per-tree fixation rates (Wurzburger and Hedin, 2016; Batterman et al., 2018; Nasto et al., 2019) as well as differences in the abundance of nitrogen-fixing tree species. These fixation hot spots may change over time, such that different species dominate in abundance and fixation activity early versus late in succession (Batterman et al., 2013a) or in treefall gaps versus intact mature forests. Research in Panama has also found that symbiotic nitrogen fixer species differ in their strategies for acquiring phosphorus, such as phosphatase activity and mycorrhizal colonization (Wurzburger and Hedin 2016; Batterman et al., 2018; Nasto et al., 2019), which suggests that nitrogen fixers employ a suite of strategies to acquire limiting nutrients.

These findings from Panamanian forests have led to new research identifying species differences in fixation, including at sites in Costa Rica (McCulloch et al., 2021), Brazil (Winbourne et al., 2018; Wong et al., 2020), and Trinidad (Brookshire et al., 2019; Currey et al., 2021). Although our knowledge of functional diversity in the symbiotic nitrogen-fixing trees has burgeoned, we know little about diversity in the function of lianas (Collins et al., 2016) or microbial fixers, both as symbiotic partners and as free-living organisms. Intriguing evidence points to the symbiotic bacteria being highly diverse on BCI (Parker, 2008); however, we do not know how these symbiotic relationships emerge or how the bacterial species identity affects fixation rates.

CONCLUSION

We have made many critical advances in our understanding of biological nitrogen fixation from work on BCI and the surrounding forests of Panama, including a backdrop of substantial research on the evolution of the dominant symbiotic nitrogen-fixer plant genus, *Inga* (e.g., Coley and Kursar, 2014; Coley 2024). We have learned that fixation is heterogeneous across many niches, space, and time and that tropical symbiotic nitrogen fixers are dynamic in their fixation rates even over their own lifetimes through the use of facultative nitrogen fixation. We have also developed a more nuanced understanding for how nutrients govern biological nitrogen fixation and how fixation will change with rising CO_2. However, many questions remain: What are the intricacies of the legume-rhizobia relationship? What is the role of herbivory and trophic interactions in shaping fixer abundances and fixation rates? How do the diverse strategies of symbiotic fixers affect ecosystem scale inputs of nitrogen? Undoubtedly, exciting new findings about the many unresolved questions about biological nitrogen fixation will emerge in the coming years from BCI and surrounding Panama forests.

REFERENCES

Barker, W., L. S. Comita, O. L. Phillips, and S. A. Batterman. 2020. Chapter 4: Know Thy Neighbour: Herbivory Contributes to Greater Negative Density-Dependent Effects for Fixer Seedlings Compared to Non-fixers in a Tropical Forest. In The role of herbivory in governing tropical nitrogen fixation. PhD Thesis, University of Leeds.

Barker, W., L. S. Comita, S. J. Wright, O. L. Phillips, B. E. Sedio, T. R. Baker, and S. A. Batterman. 2022. Widespread Herbivory Cost in Tropical Nitrogen-Fixing Tree Species. *Nature*, 612: 483–487. :

Barker, W., K. Winter, S. Palmer, O. Phillips, D. Ashley, A. Virgo, J. Aranda, and S. Batterman. 2020. Chapter 3: A Quicker Fix: Herbivory May Stimulate Nitrogen Fixation Rates in Tropical Forests. In The role of herbivory in governing tropical nitrogen fixation. PhD Thesis, University of Leeds.

Barron, A. R., D. W. Purves, and L. O. Hedin. 2011. Facultative Nitrogen Fixation by Canopy Legumes in a Lowland Tropical Forest. *Oecologia*, 165: 511–520.

Barron, A. R., N. Wurzburger, J. P. Bellenger, S. J. Wright, A. M. L. Kraepiel, and L. O. Hedin. 2009. Molybdenum Limitation of Asymbiotic Nitrogen Fixation in Tropical Forest Soils. *Nature Geoscience*, 2: 42–45.

Batterman, S. A., S. Costa, F. West, J. S. Hall, M. van Breugel, D. Medvigy, and L. O. Hedin. In review. Herbivory Imposes Major Cost to Tropical Nitrogen-Fixing Tree Species.

Batterman, S. A., J. S. Hall, B. L. Turner, L. O. Hedin, J. K. L. Walter, P. Sheldon, and M. van Breugel. 2018. Phosphatase Activity and Nitrogen Fixation Reflect Species Differences, Not Nutrient Trading or Nutrient Balance, Across Tropical Rainforest Trees. *Ecology Letters*, 21: 1486–1495.

Batterman, S. A., L. O. Hedin, M. van Breugel, J. Ransijn, D. J. Craven, and J. S. Hall. 2013a. Key Role of Symbiotic Dinitrogen Fixation in Tropical Forest Secondary Succession. *Nature*, 502: 224–227.

Batterman, S. A., N. Wurzburger, and L. O. Hedin. 2013b. Nitrogen and Phosphorus Interact to Control Tropical Symbiotic N_2 Fixation: A Test in *Inga punctata*. *Journal of Ecology*, 101: 1400–1408.

Brookshire, E. N., L. O. Hedin, J. D. Newbold, D. M. Sigman, and J. K. Jackson. 2012. Sustained Losses of Bioavailable Nitrogen from Montane Tropical Forests. *Nature Geoscience*, 5: 123–126.

Brookshire, E. N. J., N. Wurzburger, B. Currey, D. N. L. Menge, M. P. Oatham, and C. Roberts. 2019. Symbiotic N Fixation Is Sufficient to Support Net Aboveground Biomass Accumulation in a Humid Tropical Forest. *Scientific Reports*, 9: 7571.

Cernusak, L. A., K. Winter, C. Martinez, E. Correa, J. Aranda, M. Garcia, C. Jaramillo, and B. L. Turner. 2011. Responses of Legume Versus Non-Legume Tropical Tree Seedlings to Elevated CO_2 Concentration. *Plant Physiology*, 157: 372–385.

Coley, P. D. 2024. Herbivores, Plant Defenses, and Tree Diversity. In *The First 100 Years of Research on Barro Colorado: Plant and Ecosystem Science*, ed. H. C. Muller-Landau and S. J. Wright, pp. 389–392. Washington, D.C.: Smithsonian Institution Scholarly Press.

Coley, P. D., and T. A. Kursar. 2014. On Tropical Forests and Their Pests. *Science*, 343: 35–36.

Collins, C. G., S. J. Wright, and N. Wurzburger. 2016. Root and Leaf Traits Reflect Distinct Resource Acquisition Strategies in Tropical Lianas and Trees. *Oecologia*, 180: 1037–1047.

Currey, B., M. P. Oatham, and E. N. J. Brookshire. 2021. Negative Trait-Based Association Between Abundance of Nitrogen-Fixing Trees and Long-Term Tropical Forest Biomass Accumulation. *Journal of Ecology*, 109: 966–974.

Davidson, E. A., C. J. Reis de Carvalho, A. M. Figueira, F. Y. Ishida, J. P. H. B. Ometto, G. B. Nardoto, R. T. Sabá, S. N. Hayashi, E. C. Leal, I. C. G. Vieira, and L. A. Martinelli. 2007. Recuperation of Nitrogen Cycling in Amazonian Forests Following Agricultural Abandonment. *Nature*, 447: 995–998.

Epihov, D. Z., K. Saltonstall, S. A. Batterman, L. O. Hedin, J. S. Hall, M. van Breugel, J. R. Leake, and D. J. Beerling. 2021. Legume-Microbiome Interactions Unlock Mineral Nutrients in Regrowing Tropical Forests. *Proceedings of the National Academy of Sciences of the United States of America*, 118: e2022241118.

Hedin, L. O., E. N. J. Brookshire, D. N. L. Menge, and A. R. Barron. 2009. The Nitrogen Paradox in Tropical Forest Ecosystems. *Annual Review of Ecology Evolution and Systematics*, 40: 613–635.

Hedin, L. O., P. M. Vitousek, and P. A. Matson. 2003. Nutrient Losses Over Four Million Years of Tropical Forest Development. *Ecology*, 84: 2231–2255.

Houlton, B. Z., Y. P. Wang, P. M. Vitousek, and C. B. Field. 2008. A Unifying Framework for Dinitrogen Fixation in the Terrestrial Biosphere. *Nature*, 454: 327–330.

Jenny, H. 1950. Causes of the High Nitrogen and Organic Matter Content of Certain Tropical Forest Soils. *Soil Science*, 69: 63–69.

Losos, E. C., and E. G. Leigh. 2004. *Tropical Forest Diversity and Dynamism: Findings From a Large-Scale Plot Network*. Chicago: University of Chicago Press.

Martinelli, L. A., M. C. Piccolo, A. R. Townsend, P. M. Vitousek, E. Cuevas, W. McDowell, G. P. Robertson, O. C. Santos, and K. Treseder. 1999. Nitrogen Stable Isotopic Composition of Leaves and Soil: Tropical Versus Temperate Forests. *Biogeochemistry*, 46: 45–65.

McCulloch, L. A., D. Piotto, and S. Porder. 2021. Drought and Soil Nutrients Effects on Symbiotic Nitrogen Fixation in Seedlings from Eight Neotropical Legume Species. *Biotropica*, 53: 703–713.

Menge, D. N. L., S. A. Levin, and L. O. Hedin. 2009. Facultative Versus Obligate Nitrogen Fixation Strategies and Their Ecosystem Consequences. *American Naturalist*, 174: 465–477.

Menge, D. N. L., A. A. Wolf, and J. L. Funk. 2015. Diversity of Nitrogen Fixation Strategies in Mediterranean Legumes. *Nature Plants*, 1: 1–5.

Nasto, M. K., K. Winter, B. L. Turner, and C. C. Cleveland. 2019. Nutrient Acquisition Strategies Augment Growth in Tropical N_2-Fixing Trees in Nutrient-Poor Soil and Under Elevated CO_2. *Ecology*, 100: e02646.

Parker, M. A. 2008. Symbiotic Relationships of Legumes and Nodule Bacteria on Barro Colorado Island, Panama: A Review. *Microbial Ecology*, 55: 662–672.

Reed, S. C., C. C. Cleveland, and A. R. Townsend. 2008. Tree Species Control Rates of Free-Living Nitrogen Fixation in a Tropical Rain Forest. *Ecology*, 89: 2924–2934.

Reed, S. C., C. C. Cleveland, and A. R. Townsend. 2013. Relationships Among Phosphorus, Molybdenum and Free-Living Nitrogen Fixation in Tropical Rain Forests: Results from Observational and Experimental Analyses. *Biogeochemistry*, 114: 135–147.

Reed, S. C., C. C. Cleveland, A. R. Townsend, D. J. Futuyma, H. B. Shaffer, and D. Simberloff. 2011. Functional Ecology of Free-Living Nitrogen Fixation: A Contemporary Perspective. *Annual Review of Ecology, Evolution, and Systematics*, 42: 489–512.

Sheffer, E., S. Batterman, S. Levin, and L. Hedin. 2015. Biome-Scale Nitrogen Fixation Strategies Selected by Climatic Constraints on Nitrogen Cycle. *Nature Plants*, 1: 15182.

Soper, F. M., M. K. Nasto, B. B. Osborne, and C. C. Cleveland. 2019. Nitrogen Fixation and Foliar Nitrogen Do Not Predict Phosphorus Acquisition Strategies in Tropical Trees. *Journal of Ecology*, 107: 118–126.

Stanton, D. E., S. A. Batterman, J. C. Von Fischer, and L. O. Hedin. 2019. Rapid Nitrogen Fixation by Canopy Microbiome in Tropical Forest Determined by Both Phosphorus and Molybdenum. *Ecology*, 100: e02795.

Sullivan, B. W., W. K. Smith, A. R. Townsend, M. K. Nasto, S. C. Reed, R. L. Chazdon, and C. C. Cleveland. 2014. Spatially Robust Estimates of Biological Nitrogen (N) Fixation Imply Substantial Human Alteration of the Tropical N Cycle. *Proceedings of the National Academy of Sciences of the United States of America*, 111: 8101–8106.

Taylor, B. N., R. L. Chazdon, and D. N. L. Menge. 2019. Successional Dynamics of Nitrogen Fixation and Forest Growth in Regenerating Costa Rican Rainforests. *Ecology*, 100: e02637.

Taylor, B. N., and D. N. L. Menge. 2018. Light Regulates Tropical Symbiotic Nitrogen Fixation More Strongly Than Soil Nitrogen. *Nature Plants*, 4: 655–661.

Trierweiler, A. M., K. Winter, and L. O. Hedin. 2018. Rising CO_2 Accelerates Phosphorus and Molybdenum Limitation of N_2 Fixation in Young Tropical Trees. *Plant and Soil*, 429: 363–373.

Vitousek, P. M. 1984. Litterfall, Nutrient Cycling, and Nutrient Limitation in Tropical Forests. *Ecology*, 65: 285–298.

Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen Limitation on Land and in the Sea: How Can It Occur? *Biogeochemistry*, 13: 87–115.

Winbourne, J. B., A. Feng, L. Reynolds, D. Piotto, M. G. Hastings, and S. Porder. 2018. Nitrogen Cycling During Secondary Succession in Atlantic Forest of Bahia, Brazil. *Scientific Reports*, 8: 1377.

Wong, M. Y., C. Neill, R. Marino, D. V. Silverio, P. M. Brando, and R. W. Howarth. 2020. Biological Nitrogen Fixation Does Not Replace Nitrogen Losses After Forest Fires in the Southeastern Amazon. *Ecosystems*, 23: 1037–1055.

Wong, M. Y., C. Neill, R. Marino, D. Silverio, and R. W. Howarth. 2021. Molybdenum, Phosphorus, and pH Do Not Constrain Nitrogen Fixation in a Tropical Forest in the Southeastern Amazon. *Ecology*, 102: e03211.

Wurzburger, N., J. P. Bellenger, A. M. L. Kraepiel, and L. O. Hedin. 2012. Molybdenum and Phosphorus Interact to Constrain Asymbiotic Nitrogen Fixation in Tropical Forests. *PLoS ONE*, 7: e33710.

Wurzburger, N., and L. O. Hedin. 2016. Taxonomic Identity Determines N_2 Fixation by Canopy Trees Across Lowland Tropical Forests. *Ecology Letters*, 19: 62–70.