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Barro Colorado Nature Monument

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60

A Factorial Nitrogen, Phosphorus, and Potassium Addition Experiment Conducted in a Mature Lowland Tropical Forest in the Barro Colorado Nature Monument

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ABSTRACT. We initiated a factorial nitrogen-phosphorus-potassium addition experiment in old-growth forest growing on an infertile Oxisol in 1998. The experiment provides clear evidence that each added nutrient limits multiple plant and ecosystem functions. All three added nutrients limit tissue nutrient concentrations, allocation to roots, and seedling growth. Phosphorus also limits soil microbial biomass, stand-level fine litter productivity, reproductive effort by trees, and soil and litter invertebrate abundance. Potassium also limits stomatal function and soil and litter invertebrate abundance. The experiment provides no evidence for diversity declines associated with eutrophication. Rather, added nutrients increased soil microbial diversity and soil and litter invertebrate diversity. Going forward, we anticipate additional lagged responses by long-lived tree species adapted to infertile soil and a shift in tree species composition to species adapted to more fertile soils. We plan further studies to explore how tropical forests maintain high productivity despite impoverished soils.

Keywords: insect abundance; insect diversity; limitation; microbial biomass; microbial diversity; nitrogen; phosphorus; potassium; productivity; seedling growth

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INTRODUCTION

Many tropical forests maintain high levels of productivity despite growing on impoverished soils (Muller-Landau et al., 2021). A longstanding paradigm holds that phosphorus (P) and/or a major cation—such as potassium (K), calcium, or magnesium—but not nitrogen (N) limit productivity on highly weathered tropical soils (Vitousek and Sanford, 1986). This paradigm is motivated by extensive leaching of all nutrients from highly weathered soils, while N is replenished through biological fixation of atmospheric dinitrogen by cyanobacteria and bacteria that prosper in the warm, wet climates that characterize tropical forests (Walker and Syers, 1976; Hedin et al., 2009). Previous N and P addition experiments conducted in tropical forests provide limited support for the paradigm, with similar strong positive responses to both N and P addition; however, many of these experiments failed to characterize local soils or took place in forests

growing on relatively fertile soils or in rapidly growing secondary forests where nutrient demand is large (reviewed by Ostertag and DiManno, 2016; Wright, 2019). We initiated a replicated, factorial N-P-K addition experiment in old-growth forest growing on a highly weathered Oxisol to determine which, if any, nutrient limits productivity and other ecosystem functions and to study how trees maintain high levels of productivity despite impoverished soils. Here, we synthesize responses through the 24th year of the experiment, providing clear evidence that each added nutrient limits multiple components of the ecosystem.

STUDY SITE AND EXPERIMENTAL DESIGN

The study site supports 30- to 40-m tall, species-rich, old-growth forest on the Gigante Peninsula in the Barro Colorado Nature Monument (9°06'31" N, 79°50'37" W). The most recent fires occurred 600 to 900 years ago, and paleobotanists have not detected any evidence of agriculture (McMichael et al., 2024). Soil inorganic N availability is high, while P and K availability are exceptionally low for the region (Yavitt et al., 2009). The soil corresponds to the most highly weathered red light clay soils of Barro Colorado Island (BCI; Yavitt, 2024), but the basalt bedrock, which is not present on BCI, has unusually low concentrations of P and K (Wright et al., 2011). Phosphorus availability influences local distributions of nearly half of the tree species in central Panama (Condit et al., 2013), and the study site is dominated by tree species associated with P-impooverished soils (Wright et al., 2018).

The experimental design includes four replicates of eight factorial combinations of N, P, and K addition (control, +N, +P, +K, +NP, +NK, +PK, +NPK). We add fertilizer by hand in four equal doses each wet season with 6–8 weeks between applications. Nitrogen is added as urea, P as triple superphosphate, and K as potassium chloride. Annual doses are 125 kg N ha⁻¹ yr⁻¹, 50 kg P ha⁻¹ yr⁻¹, and 50 kg K ha⁻¹ yr⁻¹, which equals 69%, 470%, and 88% of annual inputs from fine litter at a nearby site (3 km) on BCI, respectively. We add P at elevated levels because the highly weathered soil adsorbs large quantities of P in forms unavailable to plants and microbes (Mirabello et al., 2013). Companion experiments at the same site add the remaining macronutrients (calcium, magnesium, and sulfur) and micronutrients (Kaspari et al., 2008; Barron et al., 2009) and manipulate fine litter inputs of nutrients and organic matter (Tanner et al., 2024).

SOIL RESPONSES

The addition of a nutrient increased its extractable (henceforth available) concentrations in surface soils (see Table 1 for references). Potassium addition quickly doubled K⁺ availability. Nitrogen addition quickly doubled nitrate (NO₃⁻) availability but did not affect ammonium (NH₄⁺) availability probably because microbes rapidly nitrify NH₄⁺ producing NO₃⁻. In contrast,

P addition increased phosphate (PO₄⁻³) availability gradually, with minimal increases in the first 3 years, a doubling in the 4th year, and a 29-fold increase by the 10th year. This delay reflects gradual saturation of sites that form insoluble bonds with PO₄⁻³ in strongly weathered soils (Mirabello et al., 2013). An interaction between N and P addition developed after P availability increased, with reduced NH₄⁺ and NO₃⁻ availability where P and N were added together. This suggests plants and microbes increased N uptake and retention after P limitation was relieved (Mayor et al., 2014a; Turner and Wright, 2014) and is consistent with increased microbial gene activity associated with N acquisition after 17 years of P addition (Yao et al., 2018).

Nitrogen addition acidified the soil, which affected availabilities of several other nutrients (see Table 1 for references). Soil pH declined by 0.6 pH units over the first four years of N addition and then held steady through 20 years (Fig. 1). Two possible causes of acidification are production of H⁺ during nitrification of added ammonium and loss of buffering capacity caused by leaching losses of base cations (calcium, magnesium, K, sodium) with mobile nitrate. After nine years of N addition, concentrations of nitrate in soil solution at 1.5-m depth averaged 93-fold greater in +N plots than in control plots, thus confirming substantial leaching losses (Corre et al., 2010). Potassium leaches away with nitrate reducing K⁺ availability and, where K and N are added together, K⁺ displaces other cations in leachate, further exacerbating K⁺ losses causing an N*K interaction (Table 1). Acidification was also associated with reduced calcium availability, increased aluminum toxicity,

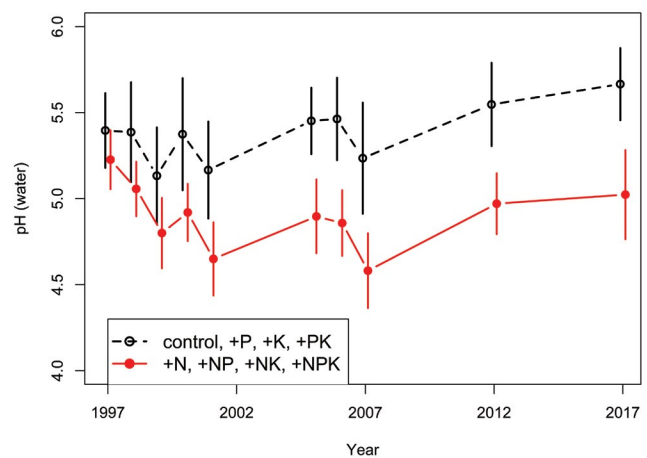


FIGURE 1. Nitrogen (N) addition acidified the soil. Mean soil pH in water declined over the first four years of N addition (1998 through 2001) and fluctuated in parallel between N treatments after 2001. The N treatment difference averaged 0.6 pH units after 2001. Results for 1997 predate N addition. Means (± 2 SE) are presented for 16 -N plots and 16 +N plots across treatments, as indicated in the legend.

TABLE 1. The responses of pH and extractable concentrations of selected elements in surface soils (0 to 10 cm depth) to factorial addition of nitrogen (N), phosphorus (P), and potassium (K). None indicates no statistically significant effect(s).

Response (methods)	Main effects	Interactions	References ^a
pH (in water)	+N: reduced from 5.4 to 4.8 +P: none +K: none	none	1, 2, 3
Extractable NH ₄ ⁺ (K ₂ SO ₄ extract)	+N: none +P: none +K: none	N*P: +NP reduced NH ₄ ⁺ availability by year 10	2, 3
Extractable NO ₃ ⁻ (K ₂ SO ₄ extract)	+N: approximately doubled +P: none +K: none	N*P: +P reduced the +N increase by year 10	2, 3
Extractable PO ₄ ⁻³ (Mehlich-III)	+N: none +P: increased 29-fold by 10th year +K: none	none	2, 3
Extractable K ⁺ (Mehlich-III and 1 M NH ₄ Cl)	+N: reduced +P: none +K: approximately doubled	N*K: +K amplified the +N reduction	2, 3
Extractable Ca ⁺² (Mehlich-III)	+N: reduced by 25% +P: none +K: none	none	3
Extractable Al ⁺³ (Mehlich-III)	+N: increased by 18% +P: none +K: none	none	3

^a References: 1. Corre et al. (2010); 2. Yavitt et al. (2011); 3. Turner et al. (2013).

and altered availabilities of selected micronutrients (Table 1; Yavitt et al., 2011; Turner et al., 2013). The liberation of Al⁺³ further acidifies soil through formation of aluminum hydroxides. Acidification is an inevitable consequence of N addition in highly weathered tropical soils (Tian and Liu, 2015) and complicates the interpretation of microbial and plant responses to N addition (Wright, 2019).

The N cycle became leakier over the first four years of N addition. Relative to controls, N-oxide emissions (NO and N₂O) increased by 58%, 105%, and 254% after 1, 2, and 9–10 years of N addition, respectively, and accounted for 10% of added N in years 9 and 10 (Koehler et al., 2009a, 2012; Corre et al., 2014). Nitrogen addition also increased gross N mineralization (organic N to NH₄⁺), nitrification (NH₄⁺ to NO₃⁻), and NO₃⁻ leaching losses (Corre et al., 2010). Nitrogen isotope ratios (δ¹⁵N) provide additional insight. Biological reactions

fractionate against the heavier ¹⁵N isotope and increasingly concentrate ¹⁵N in soluble soil N as N supplies increase. This mechanism raised the δ¹⁵N of extractable soil nitrate from -0.50‰ in control plots to 3.60‰ in +N plots even though the δ¹⁵N of our urea fertilizer is -2.22‰ (Mayor et al., 2014a). The δ¹⁵N of leaf litter increased from 0.5 to 1.5‰ in +N plots over the first 4 years as plants increasingly fractionated against ¹⁵N then held steady through 10 years of N addition (Corre et al., 2010). Gradual N cycle responses to N addition parallel gradual phosphate availability responses to P addition suggesting colimitation by both nutrients.

Tropical forest soils hold globally significant amounts of carbon (C) in organic matter and take up 6.2 Tg of methane, a potent greenhouse gas, each year (Veldkamp et al., 2013). Nitrogen addition stimulated methane consumption by methanotrophs, but it did not affect methane uptake because clay-rich soils

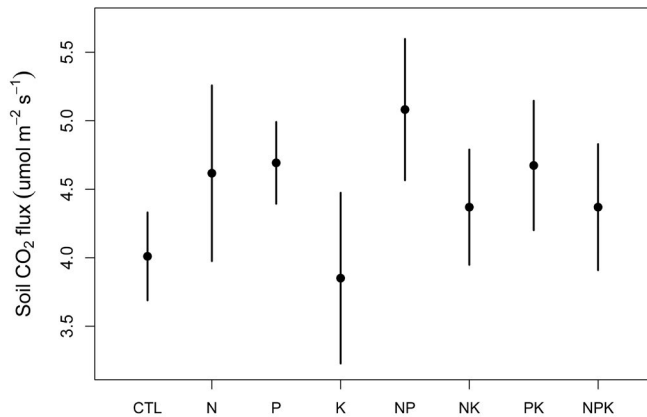


FIGURE 2. Soil respiration (means ± 1 SE) was unaffected in the fifth year of nutrient addition. The horizontal axis presents the eight treatments of our factorial nitrogen (N), phosphorus (P), and potassium (K) addition experiment. We measured soil respiration three times at four random locations in the inner 30×30 m of every experimental plot on five dates between March and October 2002, using a soil cuvette and LI-6400 infrared gas analyzer (Licor, Nebraska). We installed 10-cm diameter polyvinyl chloride collars to 10-cm depth one year before measurements began.

limited diffusion of atmospheric methane into the soil (Koehler et al., 2012; Veldkamp et al., 2013). Each year, tropical forests cycle more than 10% of atmospheric carbon dioxide (CO_2) through photosynthesis and respiration, with soil respiration being the second-largest CO_2 flux (Malhi, 2005). Soil respiration did not respond to any nutrient after 5 years (Fig. 2) nor to N addition after 10 years (Koehler et al., 2009b). Finally, soil organic C concentrations and total amounts did not respond after 10 years of nutrient addition (Turner et al., 2015). Neither N, P, nor K addition affected soil C dynamics on a decadal time scale.

MICROBIAL AND INVERTEBRATE RESPONSES

Phosphorus limits soil microbial communities. Phosphorus addition increased bacterial taxonomic richness and microbial biomass concentrations of C, N, and especially P (Table 2, rows A–D) and reduced microbial turnover rates (Gross et al., 2015). Single-celled myxomycetes (or plasmodial slime molds) are predators of bacteria. Phosphorus addition increased myxomycete abundance (Table 2, row H) suggesting bottom-up control of a microbial trophic cascade.

Enzyme activity and gene abundances associated with P acquisition confirm P limitation. Soil phosphatase activity declined by 65% following P addition (Turner and Wright, 2014). Phytase catalyzes phosphate release from phytate, which is the most

recalcitrant P-containing compound in soil organic matter. A comparison of control and P-addition treatments detected more than 100 new phytase genes, which is a substantial addition to known microbial phytase genes (Yao et al., 2018). Recalcitrant P substrates are important sources of P for soil microbes in these P-deficient soils. Phosphorus addition also altered gene abundances of enzymes involved in C, N, and sulfur cycles, suggesting changes in allocation to maintain stoichiometric ratios after P limitation is alleviated (Yao et al., 2018).

Nitrogen and K addition had fewer effects on soil microbial communities (Table 2, rows A–E). Nitrogen addition stimulated methane uptake by methanotrophs (Veldkamp et al., 2013). Nitrogen addition also reduced bacterial taxonomic richness, and P addition ameliorated this reduction (Table 2, row D). This finding parallels a marginally significant N*P interaction for soil pH (Turner et al., 2013), suggesting that the reduction in soil pH associated with N addition (Fig. 1) might directly limit bacterial taxonomic richness. In forest floor litter, N addition did not affect pH or bacterial taxonomic richness (Table 2, row F; Walker et al., 2019). The contrasting responses of pH and bacterial taxonomic richness in soil versus litter is consistent with the hypothesis that acid soils reduce bacterial taxonomic richness.

Nutrient addition had additional effects on microbial communities in the forest floor litter layer. In the parallel micronutrient addition experiment, molybdenum, which is a cofactor in the N-fixing enzyme nitrogenase, stimulated N fixation by heterotrophic bacteria (Barron et al., 2009). Fungal taxonomic richness in the litter layer increased in response to N, K, and P addition alone but not when combined with a second nutrient (Table 2, row G). Bottom-up control of microbial communities is widespread.

Nutrient addition did not affect the taxonomic richness of bacteria living inside leaves (Table 2, row J). Each pairwise combination of nutrients had statistically significant effects on taxonomic composition of foliar endophytic bacteria; however, minimal variation was explained (R^2 values < 0.01 ; Griffin et al., 2019).

Phosphorus and K both limited invertebrates in superficial soil and the litter layer. Phosphorus addition and K addition both increased abundance and species richness, while N addition reduced abundance significantly (Table 2, rows K–L). Phosphorus addition ameliorated this effect of added N where P and N were added together (Table 2, rows K–L). Phosphorus addition also increased ant recruitment to baits, while N addition reduced nest density of a dominant ant species (Bujan et al., 2016, 2019). The species richness of litter ants was unaffected (Table 2, row M).

Two broad patterns emerge across microbial and invertebrate responses. First, P addition increased the abundance of every taxonomic group examined, including microbes, slime molds, and invertebrates in the soil and leaf litter (Table 2, rows A–C, H, K). Second, there was virtually no evidence for declines in species richness following nutrient addition (Table 2, rows D–G, I–J, L–M). Instead, species richness increased with P addition for soil bacteria and with P and K addition for soil and litter invertebrates (Table 2, rows D, K, L). This result contrasts with reductions in diversity associated with eutrophication in freshwater

TABLE 2. Responses of microbes and invertebrates to factorial nitrogen (N), phosphorus (P), and potassium (K) addition. None indicates no statistically significant effect(s).

Row – response (units)	Main effects	Interactions	References ^a
A – Soil microbial C (mg C kg ⁻¹ soil)	+N: none +P: increased by 13% +K: none	none	1
B – Soil microbial N (mg N kg ⁻¹ soil)	+N: none +P: increased by 21% +K: none	none	1
C – Soil microbial P (mg P kg ⁻¹ soil)	+N: none +P: increased by 49% +K: none	none	1
D – Soil bacterial species richness ^a	+N: reduced +P: increased +K: none	N*P: +P ameliorated the +N reduction	2
E – Soil fungal species richness ^a	+N: none +P: none +K: none	none	2
F – Litter bacterial species richness ^a	+N: none +P: none +K: none	none	3
G – Litter fungal species richness ^a	+N: none +P: none +K: none	N*K and N*P: increased by one but not by both nutrients	4
H – Litter myxomycete abundance	+N: none +P: increased by 23% +K: none	none	5
I – Litter myxomycete species richness	+N: none +P: none +K: none	none	5
J – Species richness of foliar bacterial endophytes	+N: none +P: none +K: none	none	6
K – Soil/litter invertebrate abundance	+N: reduced +P: increased +K: increased	N*P: +P ameliorated the +N reduction	2
L – Soil/litter invertebrate species richness	+N: none +P: increased +K: increased	N*P: +P ameliorated a non- significant +N reduction	2
M – Litter ant species richness	+N: none +P: none +K: none	none	3

^a References:

1. Turner and Wright (2014)
2. Kaspari et al. (2017)
3. Kaspari et al. (2010)
4. Kerekes et al. (2013)
5. Walker et al. (2019)
6. Griffin et al. (2019)

communities and many plant communities. We evaluated microbial and invertebrate responses after 9–17 years of nutrient addition, giving ample time for these short-lived organisms to respond numerically. We conclude that P limits many microbial and invertebrate taxa in the soil and leaf litter. We speculate that the absence of eutrophication-driven declines in species richness reflects some combination of rapid evolution in microbes, species replacement enabled by the hyperdiversity of tropical microbes and invertebrates, and niches enabled by spatial heterogeneity at the spatial scales of these smallest of all organisms.

MYCORRHIZAL RESPONSES

Arbuscular mycorrhizal fungi (AMF) colonize roots and provide their hosts with nutrients in exchange for carbon. Nutrient addition is expected to reduce benefits for hosts, allocation by hosts to AMF, and percentage of root length colonized by AMF (Treseder, 2004). Nitrogen addition reduced a lipid biomarker of AMF activity by 30%, colonization of roots of selected tree species by 18%, and colonization of roots in bulk soil samples by 6% (Sheldrake et al., 2018; Wurzbürger and Wright, 2015). Phosphorus addition reduced the AMF biomarker by 25%, colonization of tree roots by 18%, and colonization of *Philodendron fragrantissimum* (Araceae) roots, but it increased colonization of roots in bulk soil samples by 8% (Wurzbürger and Wright, 2015; Sheldrake et al., 2018; Woods et al., 2018). Potassium addition had no significant effects on AMF. We conclude AMF contribute to plant N and P nutrition but not K nutrition. The minimum mean percentage of root length colonized for any treatment was 42% across the three root colonization studies. This result suggests (1) our treatments do not completely remove benefits associated with N or P nutrition; (2) AMF provide benefits other than N and P nutrition (Herre et al., 2007); and/or (3) plants have limited control over allocation to AMF. Nitrogen and especially P addition altered AMF species composition (Sheldrake et al., 2017, 2018). Altered AMF species composition with N and P addition raises the possibility that relatively parasitic AMF species replace relatively symbiotic AMF species, with plants having limited control over allocation to AMF.

PLANT RESPONSES

Nutrient addition increased tissue concentrations of the added nutrient. For two studies of sun-exposed leaves of canopy tree species, the addition of the same nutrient increased mean tissue concentrations by 3.1% and 5.0% for N, 18% for K, and 68% and 38% for P (Mayor et al., 2014b; Wright et al., 2011). For fine roots from bulk soil samples (mostly canopy trees and lianas), the addition of the same nutrient increased tissue concentrations by 2.9% for N, 13% for K, and 140% for P (Wurzbürger and Wright, 2015). For deeply shaded, understory seedlings of five tree species, the addition of the same nutrient increased mean

tissue concentrations for new leaves, mature leaves, roots, and stems by 7.4%, 12%, 10%, and 13% for N; 8.7%, 37%, 15% and 33% for P; and 4.4%, 3.5%, 8.7%, and –5.0% (the only decrease) for K, respectively (Santiago et al., 2012; Schreeg et al., 2014). Tissue concentration responses were consistently strong for P and modest for N and K.

These nutrient-specific responses integrate nutrient availability at the experimental site, which is high for N and low for P and K, and relative rates of leaching from living tissues. Rainwater K concentrations increase by an average of 1100% as rain passes through vegetation canopies (reviewed by Parker, 1983). In contrast, canopy foliage often absorbs N and P from rainwater (Parker, 1983). We speculate that tissue N concentrations approach optimal levels in our control treatment (Mayor et al., 2014b), dramatic increases in tissue P concentrations reflect P limitation, and rapid leaching prevents dramatic increases in tissue K concentrations. It remains to be determined whether the observed increases in tissue nutrient concentrations represent luxury consumption or relieve nutrient limitation of plant function.

Nitrogen, K, and especially P addition caused statistically significant physiological responses. Whole metabolome responses include increased allocation to secondary metabolites and defense with P addition in two canopy tree species and increased allocation to primary metabolites with N addition in an understory species (Gargallo-Garriga et al., 2017). Photosynthetic rates increased with N addition, stomatal conductance increased with P and K addition, and leaf internal CO₂ concentrations increased with K addition for deeply shaded seedlings of the canopy tree *Alseis blackiana* (Pasquini and Santiago, 2012). Phosphorus is known to increase biochemical efficiency of the photosynthetic light reactions, and P addition increased maximum electron transport rates for deeply shaded seedlings of 13 tree and liana species (Pasquini et al., 2015). Potassium addition reduced this last effect of P addition, which is likely linked to altered stomatal control with K addition (Pasquini et al., 2015). These widespread physiological responses raise the likelihood that productivity also responds.

Allocation complicates productivity responses. Leaves, roots, and stems coordinate acquisition of all essential resources, and the addition of limiting nutrients could alter allocation as well as production. Allocation to leaves should increase if added nutrients relieve carbon limitation but remain unchanged if carbon is not limiting or even decrease if added nutrients raise per leaf productivity. Allocation to roots might increase if a second soil resource is limiting but should decrease otherwise. Allocation to wood must support any increase in leaf biomass or tree heights. Elevated allocation to storage and defense and concomitant slow growth and low-mortality rates characterize long-lived trees adapted to infertile soils. This raises the possibility that added nutrients might be acquired and stored for many years before productivity responds.

Observed productivity responses are consistent with these allocation and life-history considerations. Belowground, the first four years of K addition reduced allocation to roots in tree

seedlings and stand-level fine root biomass and increased stand-level fine root turnover rates (Wright et al., 2011; Yavitt et al., 2011; Santiago et al., 2012). Fine root biomass also declines with K availability across a natural soil fertility gradient in central Panama (Cusack and Turner, 2021). We speculate leaching losses of mobile K⁺ ions drive allocation to fine roots in K-poor soils in humid tropical forests (Wright and Kitajima, 2022). Roots responded more slowly to N and P addition. After 13 years, the smallest stand-level fine root biomass and largest specific root lengths (cm g⁻¹) occurred where N, P, and K were added together (Wurzburger and Wright, 2015). The addition of all three macronutrients eventually reduced fine root biomass, with the strongest and most rapid reductions for K addition.

Allocation shifts away from roots will contribute to increased aboveground productivity. Seedling height growth increased significantly with the addition of K alone and N and P together (Santiago et al., 2012). With this exception, statistically significant aboveground productivity responses have been restricted to P addition. Early reports of significant increases in the reproductive fraction of fine litter with N addition and tree diameter growth with N and K together (Kaspari et al., 2008; Wright et al., 2011) were not sustained as additional data became available (Wright et al., 2018; Fortier and Wright, 2021). In contrast, P addition has caused sustained increases of 20% for community-wide fine litter production and 43% for reproductive effort across 38 tree species (Wright et al., 2011; Fortier and Wright, 2021). The experiment already provides clear evidence for P limitation of stand-level productivity.

The strong seedling growth responses to N, P, and K suggest that, with time, stand-level productivity responses might also emerge for all three nutrients. We speculate that deeply shaded seedlings are particularly prone to nutrient limitation because limited carbon budgets limit root function (Yavitt and Wright, 2008). In contrast, the canopy trees and lianas that dominate stand-level productivity access full sunlight to sustain root function and nutrient acquisition. In addition, elevated allocation to storage and defense characterize plants adapted to infertile soils and added nutrients might be stored for many years before productivity responses emerge. For these reasons, we anticipate stronger stand-level responses to all three added nutrients as the experiment continues.

CONCLUSIONS

Phosphorus, K, and N each limit multiple components of an old-growth tropical forest growing on a highly weathered Oxisol. All three nutrients limit plant tissue nutrient concentrations, allocation to fine roots, and seedling growth rates. In addition, K limits stomatal behavior and soil and litter invertebrates, and P limits a wide range of plant functions, soil and litter invertebrates, and soil microbes. Added nutrients invariably increased the diversity as well as abundance of soil microbes and soil and litter invertebrates.

The experiment presents new opportunities as it enters its 25th year. New approaches will be needed to separate the effects of N availability and altered pH for soil microbes and possibly fine roots. New studies will compare function with and without added nutrients to determine how tropical forests maintain high levels of productivity on highly weathered soils. As one example, comparison of control and P-addition treatments identified more than 100 new microbial genes involved in the breakdown of phytate. We now know soil microbes exploit the most recalcitrant P-containing organic compound on P-impooverished tropical forest soils (Yao et al., 2018). Finally, we anticipate lagged responses by long-lived trees adapted to impoverished soil, possibly including increases in wood production and aboveground productivity responses to N, P, and K addition.

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REFERENCES

- Barron, A. R., N. Wurzburger, J. P. Bellenger, S. J. Wright, A. M. Kraepiel, and L. O. Hedin. 2009. Molybdenum Limitation of Asymbiotic Nitrogen Fixation in Tropical Forest Soils. *Nature Geoscience*, 2: 42–45
- Bujan, J., S. J. Wright, and M. Kaspari. 2016. Biogeochemical Drivers of Neotropical Ant Activity and Diversity. *Ecosphere*, 7: e01597. <https://doi.org/10.1002/ecs2.1597>
- Bujan, J., S. J. Wright, and M. Kaspari. 2019. Biogeochemistry and Forest Composition Shape Nesting Patterns of a Dominant Canopy Ant. *Oecologia*, 189: 221–230. <https://doi.org/10.1007/s00442-018-4314-0>
- Condit, R., B. M. J. Engelbrecht, D. Pino, R. Pérez, and B. L. Turner. 2013. Species Distributions in Response to Individual Soil Nutrients and Seasonal Drought Across a Community of Tropical Trees. *Proceedings of the National Academy of Sciences of the United States of America*, 110: 5064–5068. <https://doi.org/10.1073/pnas.1218042110>
- Corre, M. D., J. P. Sueta, and E. Veldkamp. 2014. Nitrogen-Oxide Emissions from Tropical Forest Soils Exposed to Elevated Nitrogen Input Strongly Interact with Rainfall Quantity and Seasonality. *Biogeochemistry*, 118: 103–120.
- Corre, M. D., E. Veldkamp, J. Arnold, and S. J. Wright. 2010. Impact of Elevated N Input on Soil N Cycling and Losses in Old-Growth Lowland and Montane Forests in Panama. *Ecology*, 91: 1715–1729.
- Cusack, D. F., and B. L. Turner. 2021. Fine Root and Soil Organic Carbon Depth Distributions Are Inversely Related Across Fertility and Rainfall Gradients in Lowland Tropical Forests. *Ecosystems*, 24: 1075–1092. <https://doi.org/10.1007/s10021-020-00569-6>
- Fortier, R., and S. J. Wright. 2021. Nutrient Limitation of Plant Reproduction in a Tropical Moist Forest. *Ecology*, 102: e03469. <https://doi.org/10.1002/ecy.3469>
- Gargallo-Garriga, A., S. J. Wright, J. Sardans, M. Pérez-Trujillo, M. Oravec, K. Vecerova, O. Urban, M. Fernandez-Martinez, T. Parella, and J. Penuelas. 2017. Long-Term Fertilization Determines Different Metabolomic Profiles and Responses in Saplings of Three Rainforest Tree Species with Different Adult Canopy Position. *PLoS ONE*, 12: e0177030. <https://doi.org/10.1371/journal.pone.0177030>

- Griffin, E. A., J. G. Harrison, S. W. Kembel, A. A. Carrell, S. J. Wright, and W. P. Carson. 2019. Plant Host Identity and Soil Macronutrients Explain Little Variation in Sapling Endophyte Community Composition: Is Disturbance an Alternative Explanation? *Journal of Ecology*, 107: 1876–1889. <https://doi.org/10.1111/1365-2745.13145>
- Gross, A., B. L. Turner, S. J. Wright, E. V. J. Tanner, M. Reichstein, T. Weiner, and A. Angert. 2015. Oxygen Isotope Ratios of Plant Available Phosphate in Tropical Forest Soils. *Soil Biology & Biochemistry*, 88: 354–361. <https://doi.org/10.1016/j.soilbio.2015.06.015>
- 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. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110246>
- Herre, E. A., L. C. Mejia, D. A. Kylo, E. Rojas, Z. Maynard, A. Butler, and S. A. V. Bael. 2007. Ecological Implications of Anti-Pathogen Effects of Tropical Fungal Endophytes and Mycorrhizae. *Ecology*, 88: 550–558.
- Kaspari, M., J. Bujan, M. D. Weiser, D. Ning, S. T. Michaletz, Z. L. He, B. J. Enquist, R. B. Waide, J. Z. Zhou, B. L. Turner, and S. J. Wright. 2017. Biogeochemistry Drives Diversity in the Prokaryotes, Fungi, and Invertebrates of a Panama Forest. *Ecology*, 98: 2019–2028. <https://doi.org/10.1002/ecy.1895>
- Kaspari, M., M. N. Garcia, K. E. Harms, M. Santana, S. J. Wright, and J. B. Yavitt. 2008. Multiple Nutrients Limit Litterfall and Decomposition in a Tropical Forest. *Ecology Letters*, 11: 35–43. <https://doi.org/10.1111/j.1461-0248.2007.01124.x>
- Kaspari, M., B. S. Stevenson, J. Shik, and J. F. Kerekes. 2010. Scaling Community Structure: How Bacteria, Fungi, and Ant Taxocenes Differentiate Along a Tropical Forest Floor. *Ecology*, 91: 2221–2226.
- Kerekes, J., M. Kaspari, B. Stevenson, R. H. Nilsson, M. Hartmann, A. Amend, and T. D. Bruns. 2013. Nutrient Enrichment Increased Species Richness of Leaf Litter Fungal Assemblages in a Tropical Forest. *Molecular Ecology*, 22: 2827–2838.
- Koehler, B., M. D. Corre, K. Steger, R. Well, E. Zehe, J. P. Sueta, and E. Veldkamp. 2012. An In-depth Look Into a Tropical Lowland Forest Soil: Nitrogen-Addition Effects on the Contents of N₂O, CO₂ and CH₄ and N₂O Isotopic Signatures Down to 2-m Depth. *Biogeochemistry*, 111: 695–713.
- Koehler, B., M. D. Corre, E. Veldkamp, and J. P. Sueta. 2009a. Chronic Nitrogen Addition Causes a Reduction in Soil Carbon Dioxide Efflux During the High Stem-Growth Period in a Tropical Montane Forest but No Response from a Tropical Lowland Forest on a Decadal Time Scale. *Biogeosciences*, 12: 2973–2983.
- Koehler, B., M. D. Corre, E. Veldkamp, H. Wullaert, and S. J. Wright. 2009b. Immediate and Long-Term Nitrogen Oxide Emissions from Tropical Forest Soils Exposed to Elevated Nitrogen Input. *Global Change Biology*, 15: 2049–2066. <https://doi.org/10.1111/j.1365-2486.2008.01826.x>
- Malhi, Y. 2005. The Carbon Balance of the Tropical Forest Biome. In *The Carbon Balance of Forest Biomes*, ed. H. Griffiths and P. G. Jarvis. pp. 217–234. Oxford: Taylor & Francis.
- Mayor, J. R., S. J. Wright, A. G. Schuur, M. E. Brooks, and B. L. Turner. 2014a. Stable Nitrogen Isotope Patterns of Trees and Soils Altered by Long-Term Nitrogen and Phosphorus Addition to a Lowland Tropical Rainforest. *Biogeochemistry*, 119: 293–306. <https://doi.org/10.1007/s10533-014-9966-1>
- Mayor, J. R., S. J. Wright, and B. L. Turner. 2014b. Species-Specific Responses of Foliar Nutrients to Long-term Nitrogen and Phosphorus Additions in a Lowland Tropical Forest. *Journal of Ecology*, 102: 36–44. <https://doi.org/10.1111/1365-2745.12190>
- McMichael, C. N. H., and M. B. Bush. 2024. The Fire History of Old-Growth Forest in the Barro Colorado Nature Monument, Panama. In *The First 100 Years of Research on Barro Colorado: Plant and Ecosystem Science*, ed. H. C. Muller-Landau and S. J. Wright, pp. 41–46. Washington, D.C.: Smithsonian Institution Scholarly Press.
- Mirabello, M. J., J. B. Yavitt, M. Garcia, K. E. Harms, B. L. Turner, and S. J. Wright. 2013. Soil Phosphorus Responses to Chronic Nutrient Fertilisation and Seasonal Drought in a Humid Lowland Forest, Panama. *Soil Research*, 51: 215–221. <https://doi.org/10.1071/sr12188>
- Muller-Landau, H. C., K. C. Cushman, E. E. Arroyo, I. Martinez Cano, K. J. Anderson-Teixeira, and B. Backiel. 2021. Patterns and Mechanisms of Spatial Variation in Tropical Forest Productivity, Woody Residence Time, and Biomass. *New Phytologist*, 229: 3065–3087.
- Ostertag, R., and N. M. DiManno. 2016. Detecting Terrestrial Nutrient Limitation: A Global Meta-Analysis of Foliar Nutrient Concentrations after Fertilization. *Frontiers in Earth Science*, 4. <https://doi.org/10.3389/feart.2016.00023>
- Parker, G. G. 1983. Throughfall and Stemflow in the Forest Nutrient Cycle. *Advances in Ecological Research*, 13: 57–133.
- Pasquini, S., and L. Santiago. 2012. Nutrients Limit Photosynthesis in Seedlings of a Lowland Tropical Forest Tree Species. *Oecologia*, 168: 311–319.
- Pasquini, S. C., S. J. Wright, and L. S. Santiago. 2015. Lianas Always Outperform Tree Seedlings Regardless of Soil Nutrients: Results from a Long-Term Fertilization Experiment. *Ecology*, 96: 1866–1876. <https://doi.org/10.1890/14-1660.1>
- Santiago, L. S., S. J. Wright, K. E. Harms, J. B. Yavitt, C. Korine, M. N. Garcia, and B. L. Turner. 2012. Tropical Tree Seedling Growth Responses to Nitrogen, Phosphorus and Potassium Addition. *Journal of Ecology*, 100: 309–316.
- Schreeg, L. A., L. S. Santiago, S. J. Wright, and B. L. Turner. 2014. Stem, Root, and Older Leaf N:P Ratios Are More Responsive Indicators of Soil Nutrient Availability Than New Foliage. *Ecology*, 95: 2062–2068.
- Sheldrake, M., N. P. Rosenstock, S. Mangan, D. Revillini, P. A. Olsson, E. Verbruggen, E. V. J. Tanner, B. L. Turner, and S. J. Wright. 2018. Responses of Arbuscular Mycorrhizal Fungi to Long-Term Inorganic and Organic Nutrient Addition in a Lowland Tropical Forest. *ISME Journal*, 12: 2433–2445. <https://doi.org/10.1038/s41396-018-0189-7>
- Sheldrake, M., N. P. Rosenstock, D. Revillini, P. A. Olsson, S. J. Wright, and B. L. Turner. 2017. A Phosphorus Threshold for Mycoheterotrophic Plants in Tropical Forests. *Proceedings of the Royal Society B: Biological Sciences*, 284. <https://doi.org/10.1098/rspb.2016.2093>
- Tanner, E. V. J., C. Rodassana, L. M. Bréchet, A. G. Vincent, S. Leitman, S. J. Wright, and E. J. Sayer. 2024. The Gigante Litter Manipulation Experiment. In *The First 100 Years of Research on Barro Colorado: Plant and Ecosystem Science*, ed. H. C. Muller-Landau and S. J. Wright, pp. 459–554. Washington, D.C.: Smithsonian Institution Scholarly Press.
- Tian, D., and S. Liu. 2015. A Global Analysis of Soil Acidification Caused by Nitrogen Addition. *Environmental Research Letters*, 10: 024019.
- Treseder, K. K. 2004. A Meta-Analysis of Mycorrhizal Responses to Nitrogen, Phosphorus, and Atmospheric CO₂ in Field Studies. *New Phytologist*, 164: 347–355.
- Turner, B. L., and S. J. Wright. 2014. The Response of Microbial Biomass and Hydrolytic Enzymes to a Decade of Nitrogen, Phosphorus, and Potassium Addition in a Lowland Tropical Rain Forest. *Biogeochemistry*, 117: 115–130. <https://doi.org/10.1007/s10533-013-9848-y>
- Turner, B. L., J. B. Yavitt, K. E. Harms, M. N. Garcia, T. E. Romero, and S. J. Wright. 2013. Seasonal Changes and Treatment Effects on Soil Inorganic Nutrients Following a Decade of Fertilizer Addition in a Lowland Tropical Forest. *Soil Science Society of America Journal*, 77: 1357–1369. <https://doi.org/10.2136/sssaj2012.0128>
- Turner, B. L., J. B. Yavitt, K. E. Harms, M. N. Garcia, and S. J. Wright. 2015. Seasonal Changes in Soil Organic Matter After a Decade of Nutrient Addition in a Lowland Tropical Forest. *Biogeochemistry*, 123: 221–235. <https://doi.org/10.1007/s10533-014-0064-1>
- Veldkamp, E., B. Koehler, and M. Corre. 2013. Indications of Nitrogen-Limited Methane Uptake in Tropical Forest Soils. *Biogeosciences*, 10: 5367–5379.
- Vitousek, P. M., and R. L. Sanford. 1986. Nutrient Cycling in Moist Tropical Forest. *Annual Review of Ecology and Systematics*, 17: 137–167.
- Walker, L. M., M. Cedeno-Sanchez, F. Carbonero, E. A. Herre, B. L. Turner, S. J. Wright, and S. L. Stephenson. 2019. The Response of Litter-Associated Myxomycetes to Long-Term Nutrient Addition in a Lowland Tropical Forest. *Journal of Eukaryotic Microbiology*, 66: 757–770. <https://doi.org/10.1111/jeu.12724>
- Walker, T. W., and J. K. Syers. 1976. Fate of Phosphorus During Pedogenesis. *Geoderma*, 15: 1–19. [https://doi.org/10.1016/0016-7061\(76\)90066-5](https://doi.org/10.1016/0016-7061(76)90066-5)
- Woods, C. L., S. J. DeWalt, C. L. Cardelus, K. E. Harms, J. B. Yavitt, and S. J. Wright. 2018. Fertilization Influences the Nutrient Acquisition Strategy of a Nomadic Vine in a Lowland Tropical Forest Understory. *Plant and Soil*, 431: 389–399. <https://doi.org/10.1007/s11104-018-3772-9>
- Wright, S. J. 2019. Plant Responses to Nutrient Addition Experiments Conducted in Tropical Forests. *Ecological Monographs*, 89: e01382. <https://doi.org/10.1002/ecm.1382>
- Wright, S. J., and K. Kitajima. 2022. Leaf Ontogeny and Phenology Influence Nutrient, Moisture and Light Limitation of Ecosystem Productivity in Tropical Forests. *Tree Physiology*, 42: 919–921. <https://doi.org/10.1093/treephys/tpac020>
- Wright, S. J., B. L. Turner, J. B. Yavitt, K. E. Harms, M. Kaspari, E. V. J. Tanner, J. Bujan, E. A. Griffin, J. R. Mayor, S. C. Pasquini, M. Sheldrake, and M. N. Garcia. 2018. Plant Responses to Fertilization Experiments in Lowland, Species-Rich, Tropical Forests. *Ecology*, 99: 1129–1138.

- Wright, S. J., J. B. Yavitt, N. Wurzburger, B. L. Turner, E. V. J. Tanner, E. J. Sayer, L. S. Santiago, M. Kaspari, L. O. Hedin, K. E. Harms, M. N. Garcia, and M. D. Corre. 2011. Potassium, Phosphorus, or Nitrogen Limit Root Allocation, Tree Growth, or Litter Production in a Lowland Tropical Forest. *Ecology*, 92: 1616–1625.
- Wurzburger, N., and S. J. Wright. 2015. Fine-Root Responses to Fertilization Reveal Multiple Nutrient Limitation in a Lowland Tropical Forest. *Ecology*, 96: 2137–2146. <https://doi.org/10.1890/14-1362.1.sm>
- Yao, Q. M., Z. Li, Y. Song, S. J. Wright, X. Guo, S. G. Tringe, M. M. Tfaily, L. Pasa-Tolic, T. C. Hazen, B. L. Turner, M. A. Mayes, and C. L. Pan. 2018. Community Proteogenomics Reveals the Systemic Impact of Phosphorus Availability on Microbial Functions in Tropical Soil. *Nature Ecology and Evolution*, 2: 499–509. <https://doi.org/10.1038/s41559-017-0463-5>
- Yavitt, J. B. 2024. Soils of Barro Colorado Island. In *The First 100 Years of Research on Barro Colorado: Plant and Ecosystem Science*, ed. H. C. Muller-Landau and S. J. Wright, pp. 29–39. Washington, D.C.: Smithsonian Institution Scholarly Press.
- Yavitt, J. B., K. E. Harms, M. N. Garcia, M. J. Mirabello, and S. J. Wright. 2011. Soil Fertility and Fine Root Dynamics in Response to 4 Years of Nutrient (N, P, K) Fertilization in a Lowland Tropical Moist Forest, Panama. *Austral Ecology*, 36: 433–445. <https://doi.org/10.1111/j.1442-9993.2010.02157.x>
- Yavitt, J. B., K. E. Harms, M. N. Garcia, S. J. Wright, F. He, and M. J. Mirabello. 2009. Spatial Heterogeneity of Soil Chemical Properties in a Lowland Tropical Moist Forest, Panama. *Australian Journal of Soil Research*, 47: 674–687. <https://doi.org/10.1071/sr08258>
- Yavitt, J. B., and S. J. Wright. 2008. Seedling Growth Responses to Water and Nutrient Augmentation in the Understorey of a Lowland Moist Forest, Panama. *Journal of Tropical Ecology*, 24: 19–26. <https://doi.org/10.1017/s0266467407004713>