# Biogeochemistry drives diversity in the prokaryotes, fungi, and invertebrates of a Panama forest

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*Abstract.* Humans are both fertilizing the world and depleting its soils, decreasing the diversity of aquatic ecosystems and terrestrial plants in the process. We know less about how nutrients shape the abundance and diversity of the prokaryotes, fungi, and invertebrates of Earth's soils. Here we explore this question in the soils of a Panama forest subject to a 13-yr fertilization with factorial combinations of nitrogen (N), phosphorus (P), and potassium (K) and a separate micronutrient cocktail. We contrast three hypotheses linking biogeochemistry to abundance and diversity. Consistent with the Stress Hypothesis, adding N suppressed the abundance of invertebrates and the richness of all three groups of organisms by ca. 1 SD or more below controls. Nitrogen addition plots were 0.8 pH units more acidic with 18% more exchangeable aluminum, which is toxic to both prokaryotes and eukaryotes. These stress effects were frequently reversed, however, when N was added with P (for prokaryotes and invertebrates) and with added K (for fungi). Consistent with the Abundance Hypothesis, adding P generally increased prokaryote and invertebrate diversity, and adding K enhanced invertebrate diversity. Also consistent with the Abundance Hypothesis, increases in invertebrate abundance generated increases in richness. We found little evidence for the Competition Hypothesis: that single nutrients suppressed diversity by favoring a subset of high nutrient specialists, and that nutrient combinations suppressed diversity even more. Instead, combinations of nutrients, and especially the cation/micronutrient treatment, yielded the largest increases in richness in the two eukaryote groups. In sum, changes in soil biogeochemistry revealed a diversity of responses among the three dominant soil groups, positive synergies among nutrients, and–in contrast with terrestrial plants–the frequent enhancement of soil biodiversity.

*Key words: biogeochemistry; brown food web; richness; soil; tropics.*

## INTRODUCTION

Most of the ca. 25 elements required to grow an organism serve multiple functions and have divergent geographies at multiple spatial scales (Chapin 1980, Elser et al. 1996, Frausto da Silva and Williams 2001, Kaspari and Yanoviak 2009, Kaspari and Powers 2016). Combined, these observations support the hypothesis that shortfalls of multiple elements can constrain ecological abundance and diversity (Kaspari and Powers 2016). This view is gaining increasing credence from field experiments in plant communities (Harpole et al. 2011, Fay et al. 2015).

Such studies of multi-element limitation in soil food webs are rare. Soil food webs are model systems for exploring biogeochemical limitation given the relatively short generation times of bacteria, fungi, and invertebrates (Peters 1983). Moreover, soil degradation (Wall and Six 2015), nitrogen (N) and phosphorus (P) fertilizers (Smith et al. 1999), road salt (Kaspari et al. 2010), heavy metal pollution (Rauch and Pacyna 2009), increased dust mobility (Field et al. 2010) and atmospheric deposition (Matson et al. 1999) are rearranging Earth's biogeochemical gradients. Fertilization experiments, beyond their theoretical interest, have much to say about the effects of humans on the biosphere. Here we report on such an experiment, beginning with three

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hypotheses that relate changes in biogeochemistry to diversity.

### Three bottom-up hypotheses linking biogeochemistry to diversity

Three bottom-up hypotheses assess how changing the supply rates of compounds, $[R_1] \ldots [R_n]$, generates gradients of community diversity. The first two hypotheses share the assumption that increasing the number of individuals in a study plot increases plot diversity since (1) more species are sampled from the local pool, and (2) increasing average population size decreases the rate species are lost from a community (Srivastava and Lawton 1998, Kaspari et al. 2003). Reducing the number of individuals has the opposite effect. The hypotheses differ in what the focal compounds do to the organisms. The Abundance Hypothesis (Srivastava and Lawton 1998, Kaspari et al. 2003) assumes that the focal compounds are necessary to build individuals of a given taxon and each has a low enough supply rate $[R_x]$ such that increasing $[R_x]$ increases the taxon's abundance. Increasing abundance then enhances diversity for the reasons stated previously.

The Stress Hypothesis, in contrast, posits that increasing $[R_x]$ hinders the performance of individuals, reduces population size, and hence decreases diversity. One can imagine two scenarios for compounds that fit the Stress Hypothesis. First, some broad-spectrum toxins like aluminum rarely appear to benefit organisms and hence decrease abundance uniformly when added (Wood 1995, Piña and Cervantes 1996, Alfrey 2012). Second, a compound may promote performance when $[R_x]$ is sufficiently low, but inhibit performance when $[R_x]$ is sufficiently high. Selenium, for example, is required (and can limit plant production) at low concentrations, but becomes inhibitory at high concentrations (Allaway 1986).

The simplest versions of Abundance and Stress Hypotheses are neutral models: all organisms in the species pool share the same biogeochemical niche and show similar performance curves across gradients of $[R_x]$. The third hypothesis, which we, for simplicity, call the Competition Hypothesis (Tilman 1982, Interlandi and Kilham 2001, Cardinale et al. 2009), shares a key premise of the Abundance Hypothesis—that increasing $[R_x]$ increases the number of individuals—but not the assumption that all species benefit uniformly. Consider the simplest case: that of two species, $S_1$ and $S_2$ in a habitat that is a patchwork of two supply rates $[R_{x\ low}]$ and $[R_{x\ high}]$. If $S_1$ possesses traits that allow it to outcompete $S_2$ in $[R_{x\ low}]$ patches; and if $S_2$ possesses traits that allow it to outcompete $S_1$ in $[R_{x\ high}]$ patches; a mosaic of $[R_{x\ low}]$ and $[R_{x\ high}]$ should support both species. This complementary competitive ability, a fundamental tenet of niche theory, leads to a specific prediction: fertilizing a patchy habitat decreases community richness by eliminating the $[R_{x\ low}]$ patches where $S_1$ can always win. For example, fertilizing with N can favor fast growing $[R_{N\ high}]$ weedy species that quickly convert N into biomass and shade out species that would otherwise outcompete these weeds on $[R_{N\ low}]$ plots (Suding et al. 2005). The Competition Hypothesis' logic extends to multiple nutrient limitation: the more $[R_x]$ supply rates are increased, the greater the variety of $[R_{low}]$ patches are lost. This, in turn would result in the loss of a larger fraction of the species pool–that of the $[R_{low}]$ specialists (Interlandi and Kilham 2001, Harpole and Tilman 2007). Like the Stress Hypothesis, the Competition Hypothesis is mechanism for eutrophication, or "The Paradox of Enrichment" (Rosenzweig 1971)—the loss of biodiversity when resources are added to an ecosystem. But it does not act by reducing overall community abundance like the Stress Hypothesis: instead, abundance and biomass are typically highest on fertilized plots.

Combined, the three hypotheses predict differing patterns of abundance, richness, and similarity in response to fertilization (Appendix S1: Table S1). Here we contrast their ability to predict the responses of three, largely consumer, soil taxa—the prokaryotes (archaea and eubacteria), the fungi, and invertebrate animals—to a long-term fertilization experiment in a Panama rainforest. Soil food webs diverge in several relevant ways from the plant studies that have guided terrestrial biogeochemical ecology (Tilman 1982, Harpole et al. 2011). First, soil consumer taxa are not increasingly light-limited when added $R$ increases abundance and biomass. Second, most soil organisms are orders of magnitude smaller than plants. Competitive exclusion, easy to achieve in a $m^2$ plot crowded with herbs and grasses (Chase and Leibold 2002) may be less so in a similar 3-D volume of soil occupied by millions of individuals of bacteria, fungi, and invertebrates (Grundmann 2004, but see Treseder 2008, Ramirez et al. 2012). We explore the abundance and diversity responses of these taxa to fertilizers–N, P, and potassium (K), and a micronutrient cocktail–that frequently depress the diversity of terrestrial plants.

### METHODS

Complete documentation of the experimental design, sample collection, and analytical methods is provided in Appendix S1: Materials and Methods.

Prokaryote, fungal, and invertebrate animal diversity were sampled June and July 2012 from the Gigante Fertilization Experiment (Kaspari et al. 2008, Wright et al. 2011, Turner et al. 2015) in a tropical rainforest in the Republic of Panama. Beginning in 1998, four times a year, a factorial N, P, and K addition was applied to $40 \times 40$ m plots (i.e., four plots each of Control, N, P, K, NP, NK, PK, NPK) as well as four plots with a micronutrient cocktail of B, Cu, Fe, Mn, Zn, and Mo, mainly as sulfates, plus 230 kg/ha of dolomitic limestone, providing calcium (Ca) and magnesium (Mg). A summary of published effects on soil biogeochemistry,

decomposition rates, plant production, and consumer diversity may be found in Appendix S1: Table S2.

Samples in this study came from each of the 36 40 × 40 m plots. Samples came from soil within a 0.25 m² PVC frame adjacent to three litterfall traps in each plot.

### Evaluating diversity of bacteria and fungi

To estimate microbial diversity, we sampled 9, 2 cm cores from each plot that went through the surface litter and down ca. 10 cm in the soil (Oakfield Apparatus Company model HA). Soils were kept on ice in the field, then at −80°C until shipped overnight on dry ice for analysis (Appendix S1: Materials and Methods). We quantified total diversity of prokaryotes (lumping archaea and eubacteria) and fungi. We used Illumina sequencing to identify microbial Operational Taxonomic Units (OTUs) on amplified V4 regions of prokaryotic 162 rRNA genes and ITS regions of fungal rRNA at 97% sequence similarity. We used rarefaction to quantify comparative prokaryote and fungi richness from the pooled soil samples (see Appendix S1). We also quantified the number of OTUs of the 19 most common bacterial phyla and five most common fungal phyla.

### Evaluating abundance and diversity of invertebrates

In each plot, following the removal of the nine soil cores, the remaining litter and loose superficial soil was sifted through 2 cm mesh and placed in a cloth bag. Back at the lab, invertebrates were extracted with a Tullgren funnel (Bestelmeyer et al. 2000). They were sorted taxonomically and counted using a combination of keys to generate species/morphospecies for each plot. We report total diversity (number of species found per plot), and that of 17 orders and three classes of invertebrates (henceforth called Orders) found in at least 10 of the 36 plots.

### Statistics

We used generalized linear mixed models (Proc GLIM-MIX in SAS V 9.2, SAS Institute Inc, Cary, North Carolina, USA) to perform two statistical analyses tailored to the design of the experiment and the nature of the hypotheses. For the invertebrates, we tested if N, P and K generally increased estimates of abundance (Abundance and Competition Hypothesis) or decreased them (Stress Hypothesis) with a factorial analysis for the 32 plots fertilized with N, P, and/or K. The factorial analysis tested for an overall effect of N, P, and K, and pairwise interactions (i.e. e., NP, NK, PK) while taking into account a random block effect based on four topographic strata. We also used a generalized linearized mixed effects model, with topographic blocks, to test if the number of kinds of nutrients added–0 (for control), 1 (for N, P, K), 2 (NP, PK, NK), and 3 or more (for NPK, and Micronutrients)—enhanced (Abundance, Competition Hypothesis) or decreased (Stress Hypothesis) richness. In two cases where the topographic

block failed to account for variation, we dropped it and reran the simplified Generalized Linear Model.

For each group, we used the same methodology to test the hypothesis that N, P, and K enhance diversity (Abundance Hypothesis) or decrease it (Stress, Competition Hypothesis), and if the magnitude of the effect increased with the number of nutrients added.

We used the effect size Cohen's d (Cohen 1988b) to quantify the direction and magnitude of each of the eight fertilizers relative to the control. Cohen's d divides the mean difference of each treatment vs. control by the pooled standard deviation (units are in standard deviations of difference). Effect sizes allow us to standardize and compare the magnitude of fertilizer effects for co-occurring taxa that vary 1,000-fold in species richness (Cohen 1988a). *Sensu* Cohen we refer to $d \geq 1.0$ as a large effect where the mean abundance or diversity of the fertilizer treatment exceeds the 84th percentile of the control. Combined, *P*-values inform our confidence that the patterns are real, effect sizes suggest which patterns are large enough to be interesting (Nuzzo 2014).

We used Ordinary Least Squares Linear Regression (OLS) to test if effect sizes for invertebrate richness increased with abundance as predicted by the Abundance Hypothesis. We also used OLS to test the Competition Hypothesis prediction that as fertilizers increasingly suppress richness, they do so by favoring a smaller and smaller subset of species. We used Jaccard's index (Pielou 1975) to calculate the magnitude of increasing similarity among the four plots for each fertilizer relative to the four control plots.

### Results

Treatment effects on abundance and diversity varied in different ways and magnitudes across the three groups. We first review overall patterns of nutrient response among the three groups, then contrast the specific predictions of the three hypotheses.

### Prokaryote richness increased on plots with added P and decreased with added N

Prokaryote diversity showed a narrow and modest range of responses to fertilization, from a large −1.1 SD decrease on +NK plots to a 0.5 SD increase on +P plots (Fig. 1). When we used a factorial GLMM to search for general responses to N, P, and K, we found P addition tended to increase richness (Appendix S1: Table S4), while N addition reduced richness. K addition had no general effect on prokaryote richness.

### Fungal richness showed no generalized changes with N, P or K additions

Fungal richness showed two strong responses to fertilization, with richness depressed by 1.1 SD on +NP plots and enhanced 1.7 SD on +Micronutrient plots. However,
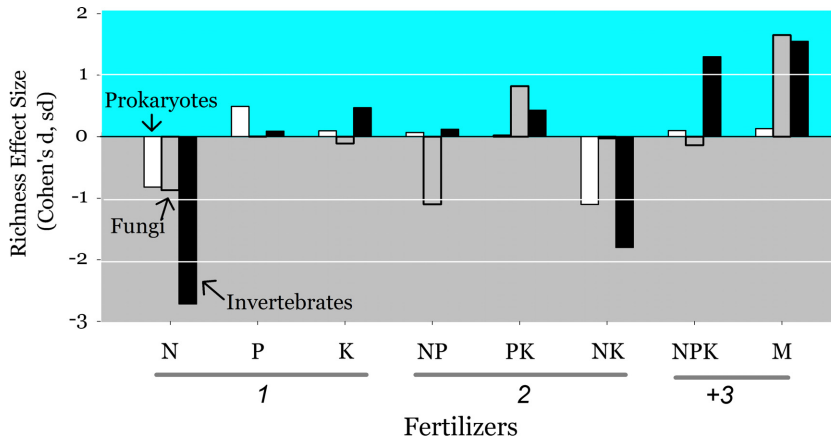
FIG. 1.   Effect size (ES), expressed as Cohen's *d*, of richness for prokaryotes, fungi, and invertebrates in response to 13 yr of fertilization (N = nitrogen, P = Phosphorus, K = Potassium, M = micronutrients). Nutrients are arranged from 1 nutrient to +3 or more nutrients combinations. Values are in units of Standard Deviation above (blue zone) or below (grey zone) values recorded on control plots.

in contrast to the prokaryotes, fungal richness showed no generalized response to N, P, or K addition (Factorial GLMM NS, Appendix S1: Table S4).

### Invertebrate richness increased when P and K were added and decreased with added N

Invertebrate abundance strongly (>1SD) increased on +NPK plots and strongly decreased on +N plots (Fig. 2). The factorial GLMM revealed that addition of N generally suppressed abundance, while the addition of P and K generally enhanced it (Appendix S1: Table S3). An NP interaction revealed how P addition eliminated the deleterious effects of N (Appendix S1: Table S3; Fig. 2). Invertebrate richness responses mirrored those

of invertebrate abundance save for the lack of a generalized suppression of diversity across all N plots (Appendix S1: Table S4).

### Only prokaryote and invertebrate responses were correlated

Prokaryote and invertebrate responses covaried strongly (Fig. 3, *r* = 0.82) driven by large and shared decreases in richness on +N and +NK plots. Responses to fertilization covaried least among the fungi and prokaryotes (Fig. 3, *r* = 0.27) and the fungi and invertebrates (*r* = 0.56). In no case were responses to fertilization reciprocal: fertilizers that increased richness in one group never yielded a strong decrease in another.
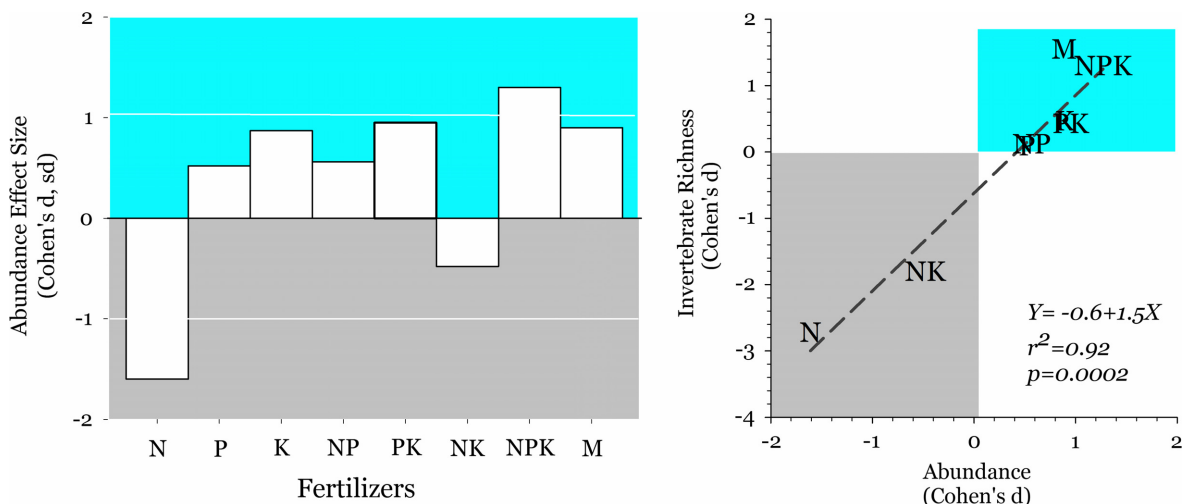


FIG. 2.   Left: Response of arthropod abundance to fertilization, as per Figure 1. Right: Test of the Abundance and Stress Hypothesis' prediction that the species richness of taxon tracks abundance, measured as invertebrates 0.25 m$^{-2}$. Values are presented as effect sizes for each of the 8 fertilization treatments (in units of SD), and the relationship evaluated with LS regression.
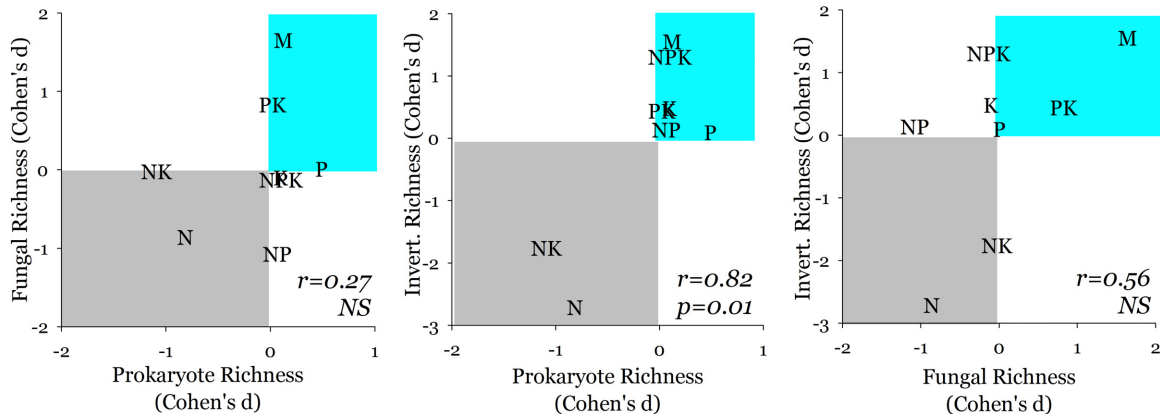
FIG. 3. Comparing responses prokaryote, fungi, and invertebrate richness to 13 years of fertilization using effect sizes (in units of SD). Symbols represent treatments, and those in grey zones are mutually decreasing; those in blue zones are mutually increasing. Pearson correlation coefficient provided.

### Abundance and stress hypotheses predict that abundance drives diversity

The Abundance and Stress hypotheses predict that treatments enhancing abundance enhance richness; and that treatments that reduce abundance reduce richness. OLS regression of richness on abundance revealed a strong positive relationship between estimates of invertebrate abundance and richness ($r^2 = 0.92$, $P = 0.0002$, Fig. 2). One strong (>1 SD) outlier was revealed by the OLS regression: +Micronutrient plots enhanced invertebrate richness above that predicted by abundance.

### Competition hypothesis predicts multiple nutrients are more likely to suppress diversity

We tested the effects of increasing the number of nutrients on diversity in two ways. First, we used a simple Kruskal Wallis test to compare diversity on control versus +Micronutrient plots. Micronutrient plots strongly increased (>1 SD), not decreased, richness for fungi ($P = 0.04$) and invertebrates ($P = 0.052$) and did not differ from controls for bacteria ($P = 0.77$, Fig. 1). We also grouped fertilizer treatments by the number of compounds added ($n = 1$ for +N, +P, +K, $n = 2$ for +NK, +NP, +NK, $n \geq 3$ for +NPK, +Micronutrients). A GLMM analysis showed no effect of nutrient number in a fertilizer on prokaryote or fungi richness, but higher invertebrate richness on $n \geq 3$ plots (Appendix S1: Table S4; Fig. 1).

### Competition hypothesis predicts richness reductions result in a subset of nutrient specialists

Most prokaryote phyla (79% of 19), fungal phyla (100% of 5) and invertebrate orders (80% of 20) showed strong ($d \geq |1\ SD|$) responses to at least one fertilization treatment (Appendix S1: Fig. S1). The fraction varied by group: the majority of prokaryote and fungi phyla increased on at least one fertilization treatment (62% and 56% respectively); the opposite was true for invertebrate orders (61% showed at least one strong decrease on a treatment, $\chi^2 = 8.6$, $P = 0.014$). Moreover, each group had at least one taxon that averaged $d > 1.0$ SD across all eight treatments (Prokaryotes: Crenarchaeota and Nitrospira; Fungi: Chytridiomycota; Invertebrates: Blattaria). Two subgroups averaged $d < -1.0$ SD: the fungus phylum Zygomycota and the invertebrate order Diplura. However, all of these taxa were rare, comprising only 0.1–1% of samples (Appendix S1: Fig. S2).

Despite the widespread evidence for specialization, there was no evidence that nutrient addition reduced richness by favoring a common subset of phyla/orders (OLS regressions p's >0.16, Fig. 4). Instead fertilization uniformly increased plot similarity in the prokaryotes: the contribution of 19 phyla across the four plots for any nutrient treatment was 0.87 SD more similar on average than on controls (Fig. 4). Fungi showed the opposite pattern ($d = -0.57$ SD): a fertilizer treatment disrupted membership on a nutrient treatment's four plots relative to the more predictable composition of control plots (Fig. 4). Invertebrates were intermediate, with an average d of 0.16 SD. In total, the average effect of fertilization on Jaccard dissimilarity differed strongly among the three groups (Kruskal Wallis $P = 0.0013$, Fig. 4) but was not higher in low richness treatments like +N.

## DISCUSSION

Rosenzweig (1971) coined "the paradox of enrichment" to capture a counter-intuitive phenomenon: fertilizing an ecosystem often increased abundance and biomass while reducing diversity. Terrestrial plant communities and aquatic ecosystems, then and now, are rife with examples of how fertilizer runoff or atmospheric deposition can suppress species richness (Smith et al.
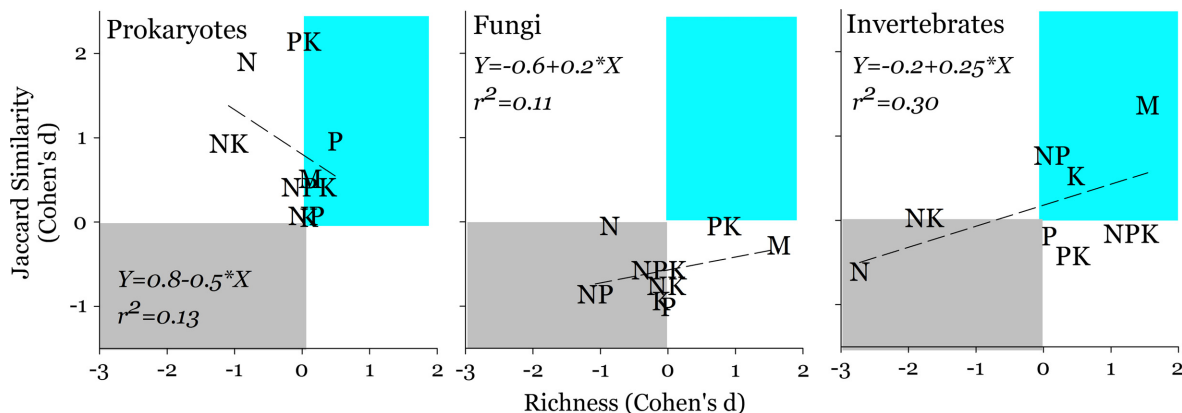
Fig. 4.   Test of the Competition Hypothesis' prediction that experimental decreases in diversity are accompanied by increases in similarity in the abundance of phyla (prokaryotes and fungi) or orders (invertebrates). Responses expressed as effect sizes in units of SD, and curve described by OLS regression.

1999) and how the addition of multiple nutrients decreases richness even more (Harpole and Tilman 2007, Harpole et al. 2016). The Competition Hypothesis arose, in part, to explain this paradox, positing that fertilization eliminates low nutrient niches and favors a subset of species adapted to exploit the remaining high nutrient niches. We found little evidence for the Paradox of Enrichment and Competition Hypothesis among the soil bacteria, fungi, and invertebrates of a well-studied tropical forest (Appendix S1: Table S2). Combinations of nutrients tended to enhance diversity, or at least ameliorate the effects of the one universal suppressor of diversity, nitrogen. Moreover, decreased invertebrate richness followed decreased abundance, consistent with the Stress Hypothesis. Similarly, increased richness tracked enhanced abundance, consistent with the Abundance Hypothesis.

### *Fertilization as a stress: does N release Al as a broad-spectrum toxin?*

This is the first study to our knowledge that documents N-induced reduction in diversity for the prokaryotes, fungi, and invertebrates in the same ecosystem. +N plots decreased soil pH on these plots by ca. 0.8 units (Turner et al. 2013) and pH stress remains a possible mechanism for reduced abundance, although there are many exceptions to such a connection in studies of invertebrates, fungi, and prokaryotes (Mulder et al. 2005, Högberg et al. 2007, Ramirez et al. 2010, Rousk et al. 2010). We suggest that an indirect effect of declining pH–the 18% increase in soil extractable aluminum on +N plots (Appendix S1: Table S2; Turner et al. 2013)– accounts for the uniform decrease among all three groups on +N plots. Al is a broad-spectrum toxin of fungi (Kelly et al. 2005), prokaryotes (Wood 1995, Piña and Cervantes 1996) and animals (Alfrey 2012). Moreover, Al toxicity reduces P absorption across membranes (Sollins 1998, Alfrey 2012) which may help account for

the rebounds in prokaryote and invertebrate richness when P is added with N on +NP plots.

This working hypothesis leads to a number of predictions. First, experimental additions of Al should reduce abundance and diversity of species otherwise likely to decline on +N plots (e.g., the Proteobacteria, Basidiomycota, and Acari, Appendix S1: Fig. S1). Second, the wide variation in Al content across tropical soils (Sollins 1998) should also generate similar geographic variation in soil abundance and diversity. Third, if Al toxicity arises from decreased uptake of P across membranes, then fertilization with N should yield differing results depending on existing quantities of soil P. For example, P availability tends to decrease, while N supplies increase, as one moves toward the older soils near the equator (Walker and Syers 1976, Reich and Oleksyn 2004). N frequently enhances plant productivity in high-P temperate systems (Fay et al. 2015). Our working hypothesis of Al toxicity suggests that N fertilization is more likely to be ineffective, or even inhibitory, to soil richness in the P-poor tropics.

### *Multiple nutrient plots enhanced richness in fungi and inverts, but not prokaryotes*

In clear contrast with studies of plant communities (Harpole and Tilman 2007, Harpole et al. 2016), the largest increase in fungal and invertebrate diversity occurred when multiple nutrients were added to the soil (Fig. 1). Plots receiving multiple nutrients (i.e., +NPK and +Micronutrients) were far more likely to strongly enhance richness of subgroups (21 cases) than decrease it (four cases, Appendix S1: Fig. S1). Consistent with the Abundance Hypothesis, +NPK and +Micronutrient plots recorded strong increases in abundance as well as richness for invertebrates. However, both invertebrates and fungi were 1 SD more diverse on +Micronutrient plots than would be predicted by an OLS curve between abundance and richness effect sizes, while prokaryotes failed to respond to micronutrients at all.

At least two factors may contribute to this pattern. First, fungi and invertebrates share a larger eukaryotic genome (Bentley and Parkhill 2004, Mohanta and Bae 2015). If this results in a larger metabolic network, it could also create a greater simultaneous demand for the metals required to build metalloenzymes (Kaspari and Powers 2016). However, in the invertebrates the +Micronutrient plots enhanced diversity above that predicted by abundance. The stoichiometrically diverse soil biota combine functional traits assembled from different combinations of nutrients (Yoshida 2006, Kaspari and Yanoviak 2009, Mueller et al. 2016). A second working hypothesis is that the biochemical diversity of the soil promotes the number of different kinds of invertebrates that can coexist.

### When the competition hypothesis works

Our results suggest that the well worked out mechanisms linking nutrients and diversity in terrestrial plants may differ from those working in the microbes and invertebrates of the brown food web. For example, in terrestrial and aquatic plant communities N fertilization reduces the frequency of low N patches, favoring a subset of competitors, and shifting the system to light limitation (Bobbink et al. 2010, Simkin et al. 2016). However, in our tropical soil we see little evidence supporting the prediction that N specialists numerically dominate +N plots. Instead, the few subgroups that consistently increased on +N plots tended to increase with *any* fertilizer (Appendix S1: Fig. S1).

We suggest two reasons for the Competition Hypothesis's lack of support in this soil food web. First, aquatic systems are well mixed, matching the hypothesis' assumption that all individuals compete with each other for shared resources (Tilman 1982, Interlandi and Kilham 2001, Cardinale et al. 2009). In a few cubic cm of soil, particles are enclosed in microfilms of moisture and separated by chasms of dead air. The resulting bacterial meta-communities are islands whose inhabitants independently capitalize and exploit any nutrient bonanza. Soils are thus more likely structured as meta-communities that slow competitive exclusion (Chase and Leibold 2002). Consistent with this interpretation, the same NPK plots that promote ant richness in the 3-dimensional volume of the litter, suppress richness at baits on the 2-dimensional surfaces of tree trunks (Bujan et al. 2016). Second, the Competition Hypothesis works by filtering out all but the subset of high-nutrient specialists able to capitalize on a resource pulse. Over thousands of generations–and 13 years encompasses many microbial and invertebrate generations–natural selection is more likely to generate new populations that can fully exploit a novel environment (Lenski et al. 1991, DeLong 2012, Weiser et al. 2017). If so, if the species filtering of the Competition Hypothesis did play out in this experiment, it likely did so in its initial hours, weeks, and years. This is testable.

### The biogeochemical niches of prokaryotes, fungi, and invertebrates

Here we use an experiment to functionally infer the biogeochemical niches of the major soil consumers of a tropical forest. Each group had its own signature response to the eight treatments (Fig. 3). Fungi and prokaryote responses were largely uncorrelated ($r = 0.27$) while those of invertebrates more closely matched the responses of prokaryotes ($r = 0.82$). One working hypothesis for the congruency of prokaryote and invertebrate responses lies in the growing evidence for robust and active bacterial microbiomes in litter invertebrates (Byzov 2006). Fertilizers like +N and +NK that reduce and shape the richness of free-living soil prokaryotes may also make it difficult for litter invertebrates to maintain their optimum microbial consortium.

The incongruence of the prokaryote and fungal responses, in turn, was unexpected, given that many share a common substrate of dead plant tissue (de Boer et al. 2005). We suggest that P and K may differentially favor each in competition for substrate. Investments in P rich ribosomes is a trait of fast growing organisms, and the relatively high growth rates of prokaryotes may allow them to better compete on plots supplemented with P (Gillooly et al. 2005), including the amelioration of +N induced stress seen on +NP plots. In contrast, the fungi's modest increase on +PK plots and rebound from +N lows on +NK plots suggests K enhances relative fungal performance perhaps driven by fungal K efflux ATPases (Benito et al. 2002). Moreover, fine root biomass is reduced on +K plots (Appendix S1: Table S2; Wurzburger and Wright 2015). If this is accompanied by a decrease in the labile carbon of root exudates, +K plots may place bacteria at a disadvantage to many fungi that specialize on more recalcitrant carbon (de Boer et al. 2005). If so, additions of labile carbon like glucose on NK plots should reduce fungal richness.

A second big difference among the three groups was how community similarity responded to fertilization. The Competition Hypothesis predicted that reduction in diversity would increase similarity, as it selected for a subset of similar taxa. We found instead that fertilization tended to make communities more similar than controls in prokaryotes, more dissimilar than controls in fungi, and have no net effect for invertebrates. We do not have a compelling hypothesis as to why this would be.

### Effect sizes are improvements over relative abundance

Our new working hypotheses arise from equal attention to inferential statistics (i.e., *P*-values) and effect sizes. Effect sizes are especially useful with microbial datasets that, when rarified down to a fixed number of reads, lack abundance data, and instead focus on changes in relative abundance. Such a focus can allow small changes in common groups (i.e., the Proteobacteria and Ascomycota that making up 50% of our reads,

Appendix S1: Fig. S2) to generate large relative changes in rare groups. Effect sizes help remedy this artifact by standardizing all experimental responses relative to controls. As a result, effect sizes help identify strong patterns (like the suppression of fungal richness on +NP plots, and bacteria and invertebrate richness on +NK plots, Fig. 1) that are not highlighted by significant *P*-values. At the same time, they reveal that statistically significant increases in bacterial and invertebrate richness on +P plots are modest compared to increases of fungi richness on Micronutrient plots, despite similar *P*-values.

### Caveats and next steps

In the history of this experiment (Appendix S1: Table S2), combinations of nutrients often acted differently than did single nutrient additions. For example, we show that P alleviated the depressive effect of N on bacteria and invertebrate richness; K did the same for fungi. In other studies, adding N to P eliminated the way that P enhanced decomposition (Kaspari et al. 2008); and N, P, and K individually, tended to increase the richness of fungi in the leaf litter, but combinations of N and P, and N and K returned richness to control levels (Kerekes et al. 2013). In other cases, two nutrients had to be added for an effect to show: N and P co-limit seedling growth (Santiago et al. 2012); and N and K co-limit the growth of trees 1–10 cm in diameter (Wright et al. 2011). If the operational question is "Does adding N have a positive effect on abundance and richness?", then the repeated answer from this long-term experiment is "it depends on the existing levels, not only of N, but of P and K as well".

That said, one of our most intriguing results–that adding micronutrients enhances diversity of the eukaryotes more than any other fertilizer–is only consistent with the hypothesis that adding multiple nutrients enhances diversity more than single nutrients. It is possible that only one of the ingredients in our micronutrient cocktail–B, Cu, Fe, Mn, Zn, Mo, Ca, Mg, and S–enhances diversity. The combinatorial explosion of sample size is one of the great challenges when using factorial designs to test for multiple nutrient limitation (Kaspari and Powers 2016). The next step, in this system however, is relatively straightforward: fertilization with single compounds from the micronutrient treatment.

Our focus on the role of biogeochemistry as a template, or ecological filter, is a simplified first step toward understanding these complex food webs. At the same time, we suggest that the rules linking nutrient supply to richness in the soil likely play out in ways that differ from those in aquatic and terrestrial plant communities. Moreover, we are particularly intrigued that a cocktail of "trace elements" generated the strongest increases in richness–among the eukaryotic fungi and invertebrates but not the prokaryotes. In an era of widespread nutrient pollution (Smith et al. 1999, Bobbink et al. 2010, Simkin et al. 2016) the biogeochemical niches of earth's soil

organisms take on a new importance. For example, it has not escaped our notice that the one fungal phylum that uniformly increases with any nutrient addition are the Chytrids, a phylum that includes *Batrachochytrium dendrobatidis*, a pathogen responsible for widespread mortality in amphibian populations (Longcore et al. 1999).

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