Michael Kaspari, Jelena Bujan, Michael D. Weiser, Daliang Ning, Sean T. Michaletz, Zhili He, Brian J. Enquist, Robert B. Waide, Jizhong Zhou, Benjamin L. Turner, and S Joseph Wright. Biogeochemistry drives diversity in the prokaryotes, fungi, and invertebrates of a Panama forest. *Ecology*. 2017.

Appendix S1

Materials and Methods

The study site forest is located on the Gigante Peninsula in the Barro Colorado Nature Monument in the Republic of Panama. The 38.4-ha plot (98□60'100" N, 79□50'37" W) supports a diverse (ca. 300 tree species) forest ca. 200 years old. The temperature averages 26□C and annual precipitation averages 2600 mm (Leigh 1999) with a distinct dry season between January and April. The soils, characterized as Endogleyic Cambisols and Acric Nitisols (Koehler et al. 2009) are derived from a basaltic parent material. The soil is low in extractable P (< 1 mg P kg-1, Turner et al. 2013).

We set up a factorial NPK experiment (i.e., 4 plots each of Control, N, P, K, NP, NK, PK, NPK), with annual applications of 125 kg N ha^{-1} as coated urea [$(NH_2)_2CO$]), 50 kg P ha^{-1} as triple superphosphate ([$Ca(H_2PO_4)_2 \cdot H_2O$]), and 50 kg K ha^{-1} as as KCL. In addition four plots received Micronutrients (Scott's S.T.E.M) consisting of HBO_2, $CuSO_4$, $FeSO_4$, $MnSO_4$, $ZnSO_4$ and $(NH_4)_6Mo_7O_{24}$ at 25 kg ha^{-1} $year^{-1}$ plus dolomitic limestone $CaMg(CO_3)_2$ at 230 kg ha^{-1} $year^{-1}$. All plots were fertilized four times a year in the wet season.

We placed the four replicates perpendicular to a 36-m topographic gradient because soil properties (Yavitt et al. 2009) and tree distributions (S. J. Wright, unpublished data) parallel the gradient. The 36 experimental plots each measured 40 x 40 m with all but one pair separated by 40 m or more. Within each replicate, we blocked the N, P, K, and NPK treatments vs. the NP, NK, PK, and control treatments (see Wright et al. (2011) Appendix A). This balanced, incomplete-block design minimizes uncontrolled error associated with spatial variation, enables evaluation of main effects and twoway interactions, but limits power to evaluate the N*P*K interaction (Winer 1971).

Measuring microbial diversity

Microbial diversity was analyzed at the Institute for Environmental Genomics at the University of Oklahoma. (i) DNA extraction. Soil samples were stored at -80°C before use. Microbial genomic DNA was extracted by a freeze-grinding method as described previously (Zhou et al.

1996)and purified by using PowerSoil® DNA Isolation Kit (bead tubes were not used. MO BIO Laboratories, Inc., Carlsbad, CA, USA). Detailed protocol is available at http://ou.edu/content/ieg/tools/protocols.html

(ii) Library preparation and sequencing. A two-step PCR amplification method was used for PCR product library preparation as described previously (Wu et al. 2015). In the first step PCR, the standard primers were used to amplify the V4 region of prokaryotic 16S rRNA genes (515F [5'-GTGCCAGCMGCCGCGGTAA-3'] and 806R [5'-GGACTACHVGGGTWT CTAAT-3']) and the ITS region of fungal rRNA genes (ITS7F [5'-GTGARTCATCGARTCTTTG-3'] and ITS4R [5'-TCCTCCGCTTATTGATATGC-3']) (White et al. 1990), respectively. In the second step PCR, phasing primers were designed and used to increase the base diversity in sequences of sample libraries. PCR amplification and purification were the same as reported previously (Wu et al. 2015), except amplification cycles (10 cycles in the first step and 20 cycles in the second step for 16S; 12 cycles in the first step and 22 cycles in the second step for ITS). Sample libraries were then sequenced by Miseq platform (Illumina, San Diego, CA, USA) as described previously (Caporaso et al. 2012).

(iii) Data processing. Sequencing data generated from MiSeq were processed to combine paired-end reads and to filter out poorly overlapped and unqualified sequences by using a Galaxy pipeline at http://zhoulab5.rccc.ou.edu:8080. After demultiplexing of raw fastq data (barcode error is set as zero) and primer trim, the reads with average quality score less than 20 were removed by Btrim (Kong 2011) and the paired-end reads were combined by Flash (Magoč and Salzberg 2011). Then, sequences containing N (unidentified base) or out the range of length (240-260 for 16S, 100-450 for ITS, without primers) were removed. Chimeras were detected by UCHIME (Edgar et al. 2011) and OTUs were generated by UCLUST (Edgar 2010)with 97% similarity threshold. The reference databases of 16S and ITS were Greengenes (http://greengenes.lbl.gov) (DeSantis et al. 2006)and UNITE+INSDC dataset (https://unite.ut.ee/repository.php#uchime) (Kõljalg et al. 2013), respectively. OTUs were identified taxonomically using RDP classifier (Wang et al. 2007). The sequences of 16S and ITS were resampled to 21,042 per (Caporaso et al. 2010) sample and 10,108 per sample before further analysis, respectively.

We quantify total diversity of bacteria (lumping Archaea and Eubacteria) and fungi. We also quantify diversity of the 19 most common bacterial phyla; and 5 most common fungal phyla.

Measuring invertebrate diversity

After placing soil samples in the cooler, we sifted litter and ca. 0.5cm of mineral soil in each plot through 1cm mesh screens, bagged the siftate, and extracted it for two days in Tulgren funnels with 25 watt bulbs (Bestelmeyer et al. 2000). Tulgren funnels use a heat gradient to drive soil invertebrates downward, where they are collected in 95% EtOH. Invertebrates were examined

under dissecting scopes and identified to species (ants and oribatid mites) and morphospecies (everything else) and grouped into taxa.

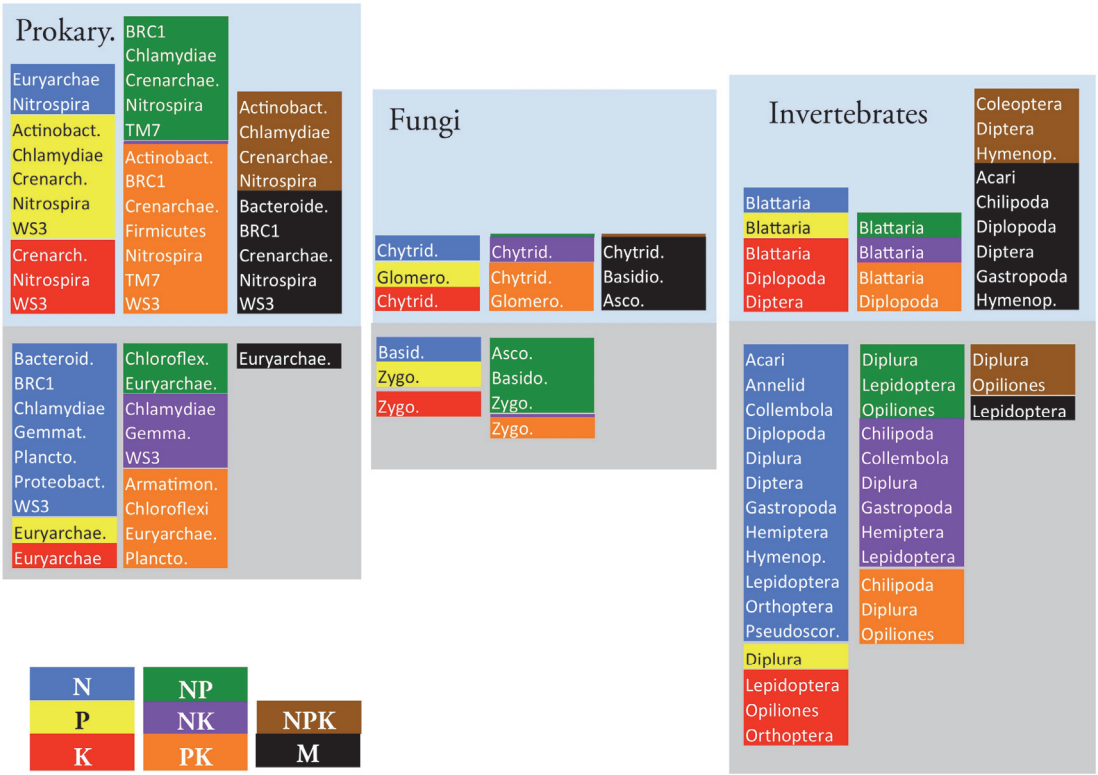

Figure S1. Subtaxa of three clades with effect sizes >1 SD from control (light backgrounds) or <-1 SD from control (dark backgrounds). Treatments are color coded as per bottom left.

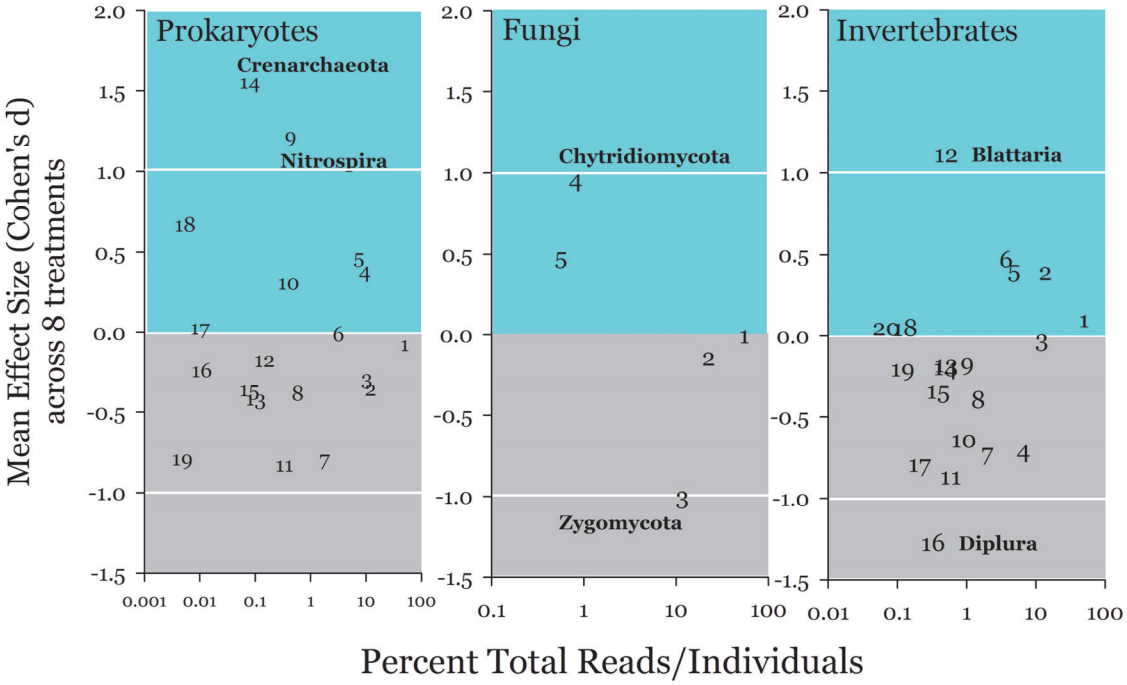

Figure S2. Average responses of bacteria, fungal and invertebrate subtaxa to fertilization. Average Cohen's effect size d (in units of SD), of diversity response to 8 fertilization treatments for the 19 phyla of bacteria, 5 phyla of fungi, and 20 orders of invertebrates. Labeled taxa are those with a $d \geq |1.0|$. **Bacteria**: 1: Proteobacteria 2: Acidobacteria 3: Verrucomicrobia 4: Firmicutes 5: Actinobacteria 6: Bacteroidetes 7: Planctomycetes 8: Gemmatimonadetes 9: Nitrospira 10: Chlamydiae 11: Chloroflexi 12: Cyanobacteria/Chloroplast 13: WS3 14: Crenarchaeota 15: Armatimonadetes 16: OD1 17: BRC1 18: TM7 19: Euryarchaeota. **Fungi:** 1: Ascomycota 2: Basidiomycota 3: Zygomycota 4: Chytridiomycota 5: Glomeromycota. **Invertebrates:** 1:Acari 2: Hymenoptera 3: Coleoptera 4: Collembola 5: Diplopoda 6: Diptera 7: Pseudoscorpions 8: Thysanoptera 9: Araneae 10: Hemiptera 11: Lepidoptera 12: Blattaria 13: Orthoptera 14: Isopoda 15: Annelida 16: Diplura 17: Opiliones 18: Gastropoda 19: Chilipoda 20: Ricineae.

Table S1. Summary of predictions for three hypotheses linking fertilization to community structure.

	Hypothesis		
Response to Nutrient	**Abundance**	**Stress**	**Competition**
Abundance	**Increase**	**Decrease**	**Increase**
Species Richness	**Increase**	**Decrease**	**Decrease**
Species similarity on that plot	**No change**	**No change**	**Increase**

Table S2. Summary of published effects from Gigante Fertilization Experiment. Results with asterisk* reported as "marginally significant at $p<0.10$). "All +N, +P and/or +K plots" represent main effects the result of factorial analyses; N*P, N*K and P*K represent two way interactions; +M represent comparison of 4 micronutrient fertilizations to control.

Treatment	Description	Citation
	Soil biogeochemistry	
All +N plots	Decreased pH by ca. 0.8 units and extractable Ca by 33%. Increased soil moisture and NO_3 extractable Al by 18%, Fe by 10%	(Turner et al. 2013)
All +P plots	Increased microbial biomass (C, N and P), reduced enzymes hydrolyzing phosphate bonds, increased soil organic P, increased pH by ca. 0.1 unit, increased NO_3, extractable P 29-fold,	(Turner and Wright 2014, Turner et al. 2015)
All +K plots	Increased extractable K, Cu	
N*P	P ameliorated ca +N's decline in pH and +N's increase in Nitrate	(Turner et al. 2013)
N*K	N reduced increase in K by 45%	(Turner et al. 2013)
+M	Increased Cu by 90%, decreased Fe by 20%, increased Zn by 105%	(Turner et al. 2013)
Other	Total soil carbon unaffected by N, P, or K	(Turner et al. 2015)
	Decomposition	
All +N plots	No effects	(Kaspari et al. 2008)
All +P plots	Higher cellulose and leaf litter decomposition	(Kaspari et al. 2008)
All +K plots	Higher cellulose decomposition	(Kaspari et al. 2008)
N*P	Adding N to P eliminates +P increase in decomposition	(Kaspari et al. 2008)

+M	Increased leaf litter decomposition	
	Plants	
All +N plots	Reduced fine root biomass when added with P, higher leaf litter N and K	(Kaspari et al. 2008, Wurzburger and Wright 2015)
All +P plots	Higher herbivory on seedlings, increase in fine litter production*, higher leaf litter P and S	(Kaspari et al. 2008, Wright et al. 2011, Santiago et al. 2012)
All +K plots	Higher herbivory on seedlings, higher seedling growth rate, reduced total fine root biomass, higher leaf litter K	(Kaspari et al. 2008, Santiago et al. 2012, Wurzburger and Wright 2015)
+NP	Higher seedling growth rate, reduced fine root biomass	(Santiago et al. 2012, Wurzburger and Wright 2015)
+NK	Increased growth in 1-10cm trees,	(Wright et al. 2011)
P*K	Additive herbivory on +PK plots	(Santiago et al. 2012)
+M	Increased leaf litter N	(Kaspari et al. 2008)
	Consumers	
All +N plots	Bacterial community composition differed from control	(Kaspari et al. 2010)
All +P plots	Bacterial community composition differed from control	(Kaspari et al. 2010)
All +K plots		
N*P	+N and +P tended to increase litter fungal diversity, but +NP decreased it to control levels*	(Kerekes et al. 2013)

N*K	+N and +K tended to increase litter fungal diversity, but +NK decreased it to control levels	(Kerekes et al. 2013)
P*K		
+NPK		
+M		

Table S3. Response of invertebrate abundance to experimental fertilization in a Panama rainforest. General Linearized Mixed Models are used to test a factorial: that N, P, K and their interactions account for variation in species richness, and that the variety of nutrients (1 for N, P, K; 2 for NP, PK, NK, and 3 or more for NPK, and Micronutrients) account for variation in estimates of abundance. Four topographic strata were tested for a block effect and included if significant. F-values provided for fertilizer effects, and appended with asterisks to denote probability > F (*=p<0.05, **p<0.01, ***p<0.001).

					F values					
Group	Analysis	Num df	Den df	Block Sig?	N	P	K	N*P	N*K	P*K
Invertebrate abundance	Factorial	1	22	Yes	6.4*	5.8*	8.6**	6.3*	0.0	0.9
					Number of Kinds					
Invertebrate abundance	Variety	3	29	Yes	0.4					

Table S4. Response of prokaryotes, fungi and invertebrate richness to experimental fertilization in a Panama rainforest. General Linearized Mixed Models are used to test a factorial: that N, P, K and their interactions account for variation in species richness, and that the variety of nutrients (1 for N, P, K; 2 for NP, PK, NK, and 3 or more for NPK, and Micronutrients) account for variation in diversity. Four topographic strata were tested for a block effect and included if significant. F-values provided for fertilizer effects, and appended with asterisks to denote probability > F (*=p<0.05, **p<0.01, ***p<0.001).

Group	Analysis	Num df	Den df	Block Sig?	N	P	K	N*P	N*K	P*K
Prokary	Factorial	1	22	Yes	8.8**	10.1**	0.7	4.4*	0.0	0.1
Fungi	Factorial	1	25	No	2.5	0.1	1.9	0.7	0.4	0.5
Inverts	Factorial	1	22	Yes	3.4	12.3**	5.4*	9.1**	0.9	0.2
					Number of Kinds					
Prokary	Variety	3	29	Yes	0.7					
Fungi	Variety	3	32	No	1.3					
Inverts	Variety	3	29	Yes	3.0*					

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