Soil Biology and Biochemistry 130 (2019) 43–54

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Phosphate addition increases tropical forest soil respiration primarily by deconstraining microbial population growth

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ARTICLE INFO

Keywords:
Metagenomics
Metatranscriptomics
Soil respiration
Phosphorus limitation
Tropical ecosystem
Enzyme activities
Microbial ecology

ABSTRACT

Tropical ecosystems are an important sink for atmospheric CO_2; however, plant growth is restricted by phosphorus (P) availability. Although soil microbiota facilitate organic P turnover and inorganic P mobilization, their role in carbon-phosphorus coupled processes remains poorly understood. To advance this topic, soils collected from four sites representing highly weathered tropical soils in the El Yunque National Forest, Puerto Rico were incubated with exogenous PO_4^{3-} under controlled laboratory conditions. P amendment increased CO_2 respiration by 14–23% relative to control incubations for soils sampled from all but the site with the greatest total and bioavailable soil P. Metatranscriptomics revealed an increase in the relative transcription of genes involved in cell growth and uptake of other nutrients in response to P amendment. A new methodology to normalize gene expression by population-level relative (DNA) abundance revealed that the pattern of increased transcription of cell growth and division genes with P amendment was community-wide. Soil communities responsive to P amendment possessed a greater relative abundance of α-glucosyl polysaccharide biosynthesis genes, suggestive of enhanced C storage under P-limiting conditions. Phosphorylase genes governing the degradation of α-glucosyl polysaccharides were also more abundant and increased in relative transcription with P amendment, indicating a shift from energy storage towards growth. Conversely, microbial communities in soils nonresponsive to P amendment were found to have metabolisms tuned for the phosphorolysis of labile plant-derived substrates, such as β-glucosyl polysaccharides. Collectively, our results provided quantitative estimates of increased soil respiration upon alleviation of P constraints and elucidated several underlying ecological and molecular mechanisms involved in this response.

1. Introduction

Despite covering < 10% of the global land surface, tropical ecosystems account for one third of terrestrial net primary productivity and harbor one fourth of terrestrial biosphere carbon (C) (Bonan, 2008). Tropical ecosystems also play a major role in offsetting anthropogenically-emitted carbon dioxide (CO_2) through enhanced plant uptake (Bala et al., 2007). However, CO_2 uptake is limited by the availability of other essential nutrients, especially phosphorus (P) (Oren et al., 2001; Beedlow et al., 2004; Cleveland et al., 2011). In many highly weathered tropical soils, P is often regarded as the primary limiting nutrient for biological activities (Vitousek, 1984; Tanner et al., 1998). Belowground microbial activities are vital to the scavenging of inorganic P and for the turnover of P-containing plant and microbial detritus, thereby increasing P availability for both plants and microorganisms. While several previous studies have focused on how P availability constrains aboveground communities and C cycling, microbial activities involved in P cycling and soil organic carbon (SOC) turnover (CO_2 release) remain much less understood (Turner and Wright, 2014).

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https://doi.org/10.1016/j.soilbio.2018.11.026
Received 7 June 2018; Received in revised form 27 November 2018; Accepted 28 November 2018
Available online 29 November 2018
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P content has been found to regulate microbial activities in tropical forests (Cleveland et al., 2002; Cleveland and Townsend, 2006), and prior studies have shown that alleviating P constraints can result in enhanced soil microbial growth and activity. For instance, in a decade-long nutrient-fertilization experiment in Panama, soil microbial biomass carbon (MBC) responded to added P but not to other nutrients (Turner and Wright, 2014). While a 25% increase in fine litterfall from added P could have contributed to the observed increase in MBC (Wright et al., 2011), another study conducted at an adjacent site found no change in MBC when soil was supplemented with doubled plant litter inputs (Sayer et al., 2012). Therefore, the increased MBC in these studies is presumably attributable to direct microbial P usage. However, a mechanistic understanding of how the added P promoted microbial growth and which gene(s) can serve as biomarkers for this response remains elusive. Such biomarkers could be important for improved modeling and managing of P-limited tropical soil ecosystems.

Given that other nutrients are present in excess or are otherwise biologically obtainable, P-limitation could constrain microbial biomass directly by limiting the *de-novo* biosynthesis of P-containing 'cell infrastructure components', such as DNA, RNA, glycerophospholipids, ATP, and NADPH. Cultivated microorganisms adapted to P limitation are known to suppress their cell replication mechanisms while exhibiting a state of dormancy induced by P starvation (Drebot et al., 1990). During states where nutrients such as P are limiting and organic matter substrates are available in excess, microbiota have been shown to biosynthesize and accumulate α-glucosyl polymers such as glycogen and trehalose to serve as readily accessible energy sources for when nutrient conditions improve (Zevenhuizen, 1966; Lilie and Pringle, 1980). However, it remains unclear if the previous findings based mostly on lab-scale experiments with microbial isolates apply to *in-situ* soil conditions involving highly diverse substrates and complex traits of microbial communities, or what other traits low-P-adapted soil microorganisms possess.

The high diversity of soil communities compared to other environments (Rodriguez and Konstantinidis, 2014) and large degree of compositional heterogeneity makes consistent, reproducible patterns difficult to ascertain. To address these challenges, 'multi-omics' approaches have the potential to improve ecological interpretations and provide a more resolved quantification of microbial response compared to DNA-only approaches (Hultman et al., 2015; Coolen and Orsi, 2015; Mackelprang et al., 2016). For instance, by relating transcriptional activity to *in-situ* DNA abundance, immediate transcriptional responses can be evaluated at greater resolution and at both the whole-community and the individual genome/population levels. Metatranscriptomic or multi-omics approaches can also uncover shifts in microbial activities that aren't well reflected by corresponding gene frequency changes in metagenomes (DNA level), particularly in cases of sudden changes or short-lived pulses that are not accompanied by growth/replication (Singer et al., 2017).

In this study, we evaluated how highly weathered tropical soils respond acutely to the addition of a readily available P source. Triplicate soil samplings were collected from four locations in the El Yunque National Forest in Puerto Rico. Short-term laboratory incubation experiments were employed to relate heightened soil respiration from the amendment of exogenous phosphate to changes in microbial community structure and transcriptional activity assessed by metagenomics and metatranscriptomics, respectively, and to enzyme activities involving soil organic carbon (SOC) and soil organic phosphorus (SOP) mineralization. Our goal was to address the following hypotheses: 1) the microbial community in a P-limited soil will possess traits indicative of more abundant, diverse, and active mechanisms of P acquisition or mechanisms for the retention of organic matter substrates, and 2) greater soil respiration resulting from P amendment will be relatable to prior *in-situ* P limitation and to increased expression of genes involved in central anabolic and catabolic activities.

2. Materials and methods

Methods for soil physical and chemical measurements can be found in the supplemental material.

2.1. Study site description, sample collection, and sample transport

The Luquillo Experimental Forest within the El Yunque National Forest in northeastern Puerto Rico is classified as a subtropical wet forest. Oxisol soils weathered from volcaniclastic sediments were collected from 350 m above sea level (masl) at the El Verde Field Station (18°19′16″N, 65°49′12″W) near the town of Río Grande (Silver et al., 1999; Mage and Porder, 2013). Ultisol soils weathered from volcaniclastic sandstones were collected from 400 masl from the Bisley Experimental Watershed near the Sabana Field Research Station (18°19′32″N, 65°43′44″W) and the town of Luquillo (McGroddy and Silver, 2000; Liptzin and Silver, 2009; Wood et al., 2016). Both locations are associated with the US Forest Service Luquillo Experimental Forest. The sites receive around 3500 mm of rainfall per year and have a mean annual temperature of ~23 °C. Common forest tree species include *Prestoea montana* (Graham) G. Nicholson (palm) and *Dacryodes excelsa* Vahl (tabonuco). The topography consists of numerous small-scale ridges and valleys (Scatena, 1989; Mage and Porder, 2013). At El Verde (E) and at Bisley (B) watersheds, samples were collected from valley (V) and ridgetop (R) locations on January 12–13, 2016 (Fig. S1). Triplicate soil samplings of ~1 kg were taken at a depth of 10 cm using a small trowel. The soils were shipped overnight on blue ice to ORNL, where they were stored at 4 °C until the start of the incubation experiments (< 2 weeks after receipt).

2.2. Incubation procedure

Incubation experiments were conducted on two automated MicroOxymax Respirometer Systems (Columbus Instruments, Columbus, OH) capable of monitoring CO_2, including a 20-flask capacity system at Oak Ridge National Laboratory (ORNL) and a 30-flask capacity system located at the University of Tennessee at Knoxville (UT). For each incubation, ~100 g of wet soil was added to a sterilized 250 mL incubation vessel. Three mL of sodium phosphate solution (equivalent to 30 μg P g soil^{-1}) was evenly distributed across P amended (P+) soils; 3 mL of molecular grade water was evenly distributed across control (P−) incubations. This readily available form of P was used in order to study the straightforward and immediate response of soils to an alleviation of prior nutrient constraints. Incubations were held at 27 °C, reflecting the average daily temperature in the nearby city of San Juan, Puerto Rico from May–October (The Southeast Regional Climate Center; http://www.sercc.com). The first incubation experiment conducted at ORNL lasted for ~6 days and included 2 of the 3 triplicate field samplings from each of the four sites to compare respiration under P+ and P− conditions (Fig. S2). Two autoclaved soils and two empty jars were incorporated to ensure that measured gases resulted from biological activity. Headspace gases were measured and logged every ~2.16 h. A second incubation experiment was performed similarly at UT that included all three replicate field samplings from each of the four locations and lasted ~14 days; gas measurements were logged every ~2.96 h for the UT setup. At the conclusion of each experiment, soil subsamples from each jar were immediately subjected to liquid nitrogen prior to storage at −80 °C in order to preserve soil community RNA.

2.3. Soil enzyme assays

Enzyme assays were performed on field, control (P−) incubation, and P-amended (P+) incubation soil samples by measuring the activities on target substrates labeled with 4-methylumbelliferone (MUB). See Fig. S2 for a diagram of incubation soils used for enzyme activity

analysis. The substrates in our study included 4-MUB-α-D-glucopyranoside for α-glucosidase (AG), 4-MUB-β-D-glucopyranoside for β-glucosidase (BG), 4-MUB-β-D-cellobioside for β-D-cellubiosidase (CB), 4-MUB-N-acetyl-β-D-glucosaminide for N-acetyl β-glucosaminidase (NAG), 4-MUB-β-D-xylopyranoside for β-xylosidase (XYL), 4-MUB-phosphate for phosphomonoesterase (PHOS), and bis-4-MUB-phophate for phosphodiesterase (DIPHOS). For each soil sample, soil equivalent to 2.75 g of dry weight was measured. Sodium acetate buffer (50 mM) was adjusted to match the pH of each soil sample. The soil sample was subsequently mixed with 91 mL of the pH-adjusted sodium acetate buffer. The soil slurry was divided into three technical replicates. Each well in the 96-well plate was filled by 800 µl of the soil slurry. For standard plates, each row of the wells was further mixed with 200 µl of MUB solutions of the concentrations of 2.5, 5, 10, 25, 50, 100 µM respectively. For enzyme reaction plate, each row of the wells was mixed with 200 µl of each MUB labeled substrate (200 µM). The incubation at 26 °C was performed on both standard and enzyme reaction plates for 3 h. Subsequently, the plates were taken out of the incubator and centrifuged for 8 min at 1500 rpm. From each well, 250 µl of the supernatant was transferred to a clean reading plate. The reaction was terminated by adding 5 µl of 0.5 M NaOH. Measurements for reading plates were conducted on Tecan Infinite M200 microplate reader for the absorbance at 365 nm. The enzyme activities were quantified as nmol/gram-dry-soil/hour based on the regression lines of those standard curves. Relative phosphomonoesterase activity was comparable between soils. Thus, enzyme activities were evaluated as a ratio between SOC-degrading enzyme activities (namely, those of α-glucosidase, β-glucosidase, β-D-cellubiosidase, N-acetyl β-glucosaminidase, and β-xylosidase) and phosphodiesterase (DIPHOS) activity (e.g., β-glucosidase:DIPHOS), an approach used in previous studies to compare enzyme activities between soils (Sinsabaugh et al., 2009; Ramirez et al., 2012; Turner and Wright, 2014).

2.4. Soil RNA and DNA isolation, library preparation, and sequencing

Soil DNA and total RNA was simultaneously extracted from incubation endpoint samples demarcated in Fig. S2 using the RNA PowerSoil Total RNA Isolation Kit and the RNA PowerSoil DNA Elution Accessory Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to manufacturer's protocol, using ~0.5 g of soil for each extraction. Total RNA samples were treated with DNase using the TURBO DNA-free kit (Ambion, Austin, TX). Elimination of DNA from RNA samples was confirmed by PCR amplification of the 16S rRNA gene using universal primers 8F and 1492R, followed by gel-electrophoresis. RNA integrity was assessed with an Agilent 2100 Bioanalyzer instrument (Agilent Technologies, Santa Clara, CA), using the Agilent RNA 6000 Pico Kit. Generally, total RNA samples with a RIN (RNA integrity number) of < 7 (as determined by Agilent Bioanalyzer software) were discarded. Following DNA digestion and evaluation of RNA integrity, two to four replicate extractions were pooled for each sample in order to obtain enough high quality RNA and to overcome high sample-to-sample heterogeneity, which is characteristic of soil microbial communities. Ribosomal RNA (rRNA) was depleted from total RNA using the Ribo-Zero rRNA Removal Kit (Bacteria) (Illumina, San Diego, CA) and cDNA libraries were constructed using the ScriptSeq v2 RNA-Seq Library Preparation Kit (Illumina, San Diego, CA) according to manufacturer's instructions, but with two modifications: RNA was fragmented for 1 min instead of 5 in step 3.A, and AMPure XP purification (step 3.F.1) was performed twice in order to reduce the amount of primer-dimers generated during amplification of cDNA.

The quality of each library was inspected on an Agilent 2100 Bioanalyzer, using the Agilent High Sensitivity DNA kit. RNA and DNA quantification were achieved using Qubit RNA HS Assay Kit and Qubit dsDNA HS Assay Kit, respectively (Thermo Fisher Scientific). Dual-indexed DNA sequencing libraries were prepared using the Illumina Nextera XT DNA library prep kit according to manufacturer's instruction except the protocol was terminated after isolation of cleaned double-stranded libraries. Library concentrations were determined by fluorescent quantification using a Qubit HS DNA kit and Qubit 2.0 fluorometer (ThermoFisher Scientific) according to the manufacturer's instructions and samples were run on a High Sensitivity DNA chip using the Bioanalyzer 2100 instrument (Agilent) to determine library insert sizes. DNA and cDNA libraries were sequenced at the Georgia Institute of Technology High Throughput DNA Sequencing Core on an Illumina HiSeq 2500 instrument in the rapid run mode for 300 cycles (150 bp paired-end). Additional sequencing of the DNA libraries was performed on an Illumina NextSeq 500 instrument, also in the high output mode for 300 cycles (150 bp paired-end). Adapter trimming and demultiplexing of sequences was carried out by either Illumina instrument. Two metagenomes were sequenced from each of El Verde ridge (ER) P−, ER P+, Bisley ridge (BR) P−, BR P+, and Bisley valley (BV) P− incubations, and two metatranscriptomes were evaluated from the same ER P−, ER P+, BR P−, and BR P+ incubation samples that were used for metagenomic sequencing (see Fig. S2). Metagenomic and metatranscriptomic datasets were deposited in the European Nucleotide Archive under project PRJEB23349.

2.5. Bioinformatics data analysis

Both metagenomic and metatranscriptomic paired-end reads were merged using PEAR (Zhang et al., 2014) (options: -p 0.001). All merged and non-merged reads were then quality-trimmed with the SolexaQA package (Cox et al., 2010) (options: -h 17; > 98% accuracy per nucleotide position). Trimmed sequences used for downstream functional annotation were truncated to 150 bp to avoid read-length biases. SortMeRNA was used with default settings to identify and remove residual rRNA sequences from sample metatranscriptomes (i.e., those remaining after rRNA subtraction) (Kopylova et al., 2012). Protein-prediction for short-reads was performed with FragGeneScan (Rho et al., 2010) (Illumina 1% error model). Resulting amino acid sequences were searched against Swiss-Prot database (UniProt Consortium, 2015; downloaded on 11-27-2016), using blastp (BLAST + version 2.2.28) (options: -word_size 4, cutoff: bit score ≥55) (Camacho et al., 2009). Corresponding gene and enzyme accession IDs for each Swiss-Prot entry was obtained from http://www.uniprot.org/downloads to consolidate the annotations. Taxonomic composition was determined using 16S rRNA gene fragments recovered from metagenomes using SortMeRNA (Kopylova et al., 2012), which were annotated and summarized at the phylum-level as described previously (Johnston et al., 2016). Metagenomic datasets belonging to the same site (e.g., Bisley valley) were combined for co-assembly (combining data from multiple sample metagenomes to increase coverage) with idba_ud (Peng et al., 2012) (options: –mink 55 –maxk 123 –step 4). Resulting contigs ≥1 kbp were retained for further analysis. Protein-encoding genes of the assembled contigs were predicted with Prodigal (Hyatt et al., 2010) and were searched against the Swiss-Prot and TrEMBL database as described above (UniProt Consortium, 2015).

Metatranscriptomes were checked for DNA contamination by mapping short-read transcripts to assembled contigs and determining strand-specificity (consistency in sense/antisense orientation) for the 1000 most transcriptionally-active genes. Samples with < 95% average strand-specificity were not used for analysis. One sample metatranscriptome, El Verde ridge sampling 1 control incubation, failed this criterion. Thus, seven metatranscriptomes (n = 3 for P− and n = 4 for P+ incubations) were used to compare P− and P+ incubations from P-responsive ridge soils.

To evaluate the transcriptional activity of individual bacterial populations/genomes, the following methodology for integrating metagenome and metatranscriptome datasets obtained from the same subsamples was used. First, assembled contigs (from DNA) encoding ≥5 sequential genes of the cell division and cell wall operon (*dcw*) (Mingorance et al., 2004), including genes *ftsAIQWYZ*, *murBCDEFG*,

mraY, and *ddl*, were identified. These (distinct) versions of the *dcw* operon typically differed by 5% or more nucleotide sequence divergence and thus, represented distinct populations. Because more cells (higher DNA abundance) would likely result in more mRNA transcripts originating from these cells without necessarily an increase in the rate of gene transcription, an approach to normalize population-specific transcriptional activity to *in-situ* (DNA) abundance was employed. Specifically, the transcriptional coverage of each individual *dcw* operon was divided by its DNA sequence coverage as a proxy for relative abundance (i.e., how many times a nucleotide base is sampled/covered by sequencing reads, normalized by library sequencing depth). Metagenome and metatranscriptome coverage of assembled contigs was determined using MegaBLAST (Camacho et al., 2009), retaining only reads that matched at ≥98% nucleotide identity, ≥100 bp match, and ≥90% of the short-read length. Relatedness between individual *dcw* operons was determined by aligning full-length assembled and reference *murG* amino acid sequences (obtained from Uniprot.org) using MAFFT (Katoh and Standley, 2013). Phylogenetic distances from the resulting alignment were determined using PhyML 3.0 (Guindon et al., 2010). Visualization of the resulting *murG* sequence-based phylogenetic tree(s), coupled to the DNA-normalized transcriptional activity, were generated using the web-based software iTOL (Letunic and Bork, 2016).

2.6. Statistical analyses

Paired *t*-test was performed to evaluate differences in CO_2 respiration between P− and P+ incubations for all four sampling locations, individually. ANOVA with Tukey's HSD was used to distinguish sampling locations that differed significantly by soil physiochemical indices, enzyme activity measurements, and the relative abundances of microbial phyla. Further, paired *t*-test was used to identify significant differences in enzyme activity between P− and P+ soils from Bisley ridge and El Verde ridge. Tables with raw counts of genes (based on Swiss-Prot annotations; UniProt Consortium, 2015) were processed with the edgeR software package (Robinson et al., 2010) to identify significant, differentially abundant genes between sample groups (P-responsive ridge vs. P-nonresponsive BV metagenomes) or treatments (P− vs. P+ incubation metatranscriptomes). In edgeR, the calcNormFactors() function was used to correct for normalization issues, such as differences in library size. To better assess transcriptional differences resulting from P+, a paired design was adopted so that baseline differences between sampling sites were subtracted out. P-values from metagenome edgeR analysis were transformed to account for false discovery rate using Benjamini–Hochberg correction (Benjamini and Hochberg, 1995). P-value correction was not performed for metatranscriptome results due to the small number of datasets used for comparison, attributable to the difficulty in obtaining high quality RNA from soils. To account for the latter, analysis and reporting of metatranscriptome-derived results focused primarily on consistent transcriptome responses between multiple genes involved in the same pathway, operon, or functional category. Many such broad categories or groups showed a high degree of consistency, as demonstrated in the results section. Functional annotations with an average relative abundance < 1E-6 for metagenomes and < 1E-5 for metatranscriptomes were not considered for comparisons between sample groups.

3. Results

3.1. Relationships between environmental indices and CO_2 respiration during incubation

Available soil phosphorus varied significantly among study locations (ANOVA, adj. p-value < 0.05); it was lowest in Bisley ridge (BR) and El Verde valley (EV), intermediate in El Verde ridge (ER), and greatest in Bisley valley (BV) (Table 1). A positive correlation between available P and microbial biomass phosphorus (MBP) was observed in ridge soils (R^2 = 0.82) (Fig. S3a). Positive correlations were also observed between MBP and microbial biomass carbon (MBC) (R^2 = 0.77) and microbial biomass nitrogen (MBN) in ridge soils (R^2 = 0.60) (Fig. S3ef). P amendment (P+) significantly increased CO_2 respiration for EV, ER, and BR soils (+14%, +14%, and +23% mean increase from five replicate incubations, respectively; p-value < 0.05), thus, these soils are subsequently referred to as “P responsive”. P+ resulted in a non-significant increase for BV soils (+5%), thus the BV soils are subsequently referred to as “P nonresponsive” (Fig. 1). The increase in CO_2 respiration with P addition (percent increase vs. CO_2 emitted from P−/control incubation) was positively correlated with MBC:MBP ratio in ridge soils (R^2 = 0.86) (Fig. S3d), i.e., soils with stronger P limitation based on the MBC:MBP ratio responded the most. The above correlations were not observed with Bisley valley soils, with the exception of those between MBP, MBC, and MBN. Also for ridge soils, those with greater measured MBP respired more total (absolute amount) CO_2 after 14 days (R^2 = 0.80) (Fig. S3b) and those with a greater MBC:MBP ratio respired less CO_2 after 14 days (R^2 = 0.85) (Fig. S3c).

3.2. Organic carbon degrading and phosphoesterase enzyme activities

Enzyme assays, metagenomic sequencing, metatranscriptomic sequencing efforts focused on the same soil subsamples collected at incubations endpoints. Enzyme assays and ‘omics efforts included P-responsive ER and BR and P-nonresponsive BV soils but not EV soils due to difficulty in obtaining high quality RNA from the latter (Fig. S2). Enzyme assays were also performed on non-incubated field soils corresponding to the same soil inocula used for the incubations.

Enzyme activities were assessed based on the ratio between SOC-degrading enzyme activities (namely, those of α-glucosidase, β-glucosidase, β-D-cellubiosidase, N-acetyl β-glucosaminidase, and β-xylosidase) and phosphodiesterase (DIPHOS) activity as suggested previously (Sinsabaugh et al., 2009; Ramirez et al., 2012; Turner and Wright, 2014). DIPHOS activity was ~2.5- to ~54-fold greater than that of SOC-degrading enzymes in P-responsive ridge soils, for both field and incubated soils (Fig. 2). Activity ratios were significantly greater for all five SOC-degrading enzyme classes for BV soils compared to ridge soils (~4.5 times greater, on average; adj. p-value < 0.05 ANOVA). Further, P+ ridge incubation soils demonstrated a significant increase in relative β-D-cellubiosidase activity (+22%; p-value < 0.05, paired *t*-test) and near-significant increases in α-glucosidase (+46%; p-value < 0.1) and β-glucosidase (+5%; p-value < 0.1) activities compared to field and P− incubation samples.

3.3. Compositional characteristics of the soil communities

Ten metagenome libraries representing ER, BR, and BV incubation soils, and eight metranscriptomes representing P-responsive ER and BR control (P−) and P amendment (P+) incubations, were sequenced (see Fig. S2). One ER control incubation with high DNA contamination was discarded and not used for comparisons. On average, 5.2 Gbp of raw sequencing effort was obtained for each metatranscriptome and 16.3 Gbp for each metagenome. Following paired-end read merging, quality trimming, and filtering of rRNA sequences (for metatranscriptomes), 2.0 Gbp of metatranscriptomic and 12.2 Gbp of metagenomic data was used for downstream analyses.

Using Nonpareil 3 (options: -T kmer -X 100000) (Rodriguez-R et al., 2018), a bioinformatics tool to determine how much of the extracted DNA was sequenced based on the level of redundancy among the resulting metagenomic reads, the microbial community in BV soils was more diverse than the ER and BR soils (Fig. S4). The estimated sequencing depth needed to achieve 75% read redundancy for the ER, BR, and BV extracted microbial community DNA was 14.3, 15.0, and 23.4 Gbp, respectively. This redundancy level (~40–60%) is appropriate for comparisons between metagenomes, with un-sampled diversity presumably representing comparatively rarer taxa (Rodriguez-R and

Table 1
Summary of chemical, biological, and physical measurements performed on field soils. Values are given as the mean ± the standard error, derived from measurements on triplicate samplings for each site. Superscript letters are used to distinguish sample groups that were significantly different (adj. p-value < 0.05); values with superscript letters differing from letters assigned to other values designate statistically-significant differences between soils for that measurement, as determined with ANOVA in conjunction with Tukey's HSD test.

Study Site	pH	Soil Moisture %	NH4—N (mg/kg)	NO3—N (mg/kg)	Total P (mg/kg)	Total C (g/kg)	Total N (g/kg)
El Verde Ridge	4.65 ± 0.08 a	0.36 ± 0.01 a	43.8 ± 10.8 ab	6.99 ± 0.55 a	334.0 ± 9.08 b	6.64 ± 0.32 b	0.50 ± 0.02 c
Bisley Ridge	4.82 ± 0.12 a	0.41 ± 0.02 a	65.5 ± 18.2 b	1.23 ± 0.97 a	206.3 ± 14.5 a	5.33 ± 0.42 b	0.38 ± 0.03 b
El Verde Valley	5.41 ± 0.06 b	0.45 ± 0.04 a	10.3 ± 1.1 a	9.21 ± 3.50 a	364.5 ± 27.8 b	3.07 ± 0.35 a	0.25 ± 0.03 a
Bisley Valley	5.40 ± 0.17 b	0.45 ± 0.02 a	37.4 ± 9.0 ab	11.4 ± 8.74 a	447.8 ± 15.0 c	5.24 ± 0.42 b	0.39 ± 0.02 b

Measurements from Mehlich-1 (mg/kg dry soil)

Study Site	Ca	K	Mg	Mn	Zn	P
El Verde Ridge	136.5 ± 17.0 a	45.6 ± 3.7 b	121.0 ± 11.4 a	18.4 ± 4.7 a	4.5 ± 1.3 a	1.9 ± 0.1 b
Bisley Ridge	266.6 ± 78.0 a	16.7 ± 0.5 a	205.3 ± 26.5 b	42.4 ± 25.8 a	2.1 ± 0.8 a	0.9 ± 0.1 a
El Verde Valley	652.3 ± 31.6 b	40.7 ± 5.1 b	356.0 ± 21.9 c	41.2 ± 8.9 a	1.8 ± 0.2 a	0.8 ± 0.3 a
Bisley Valley	842.2 ± 93.7 b	15.6 ± 4.2 a	301.9 ± 6.3 c	35.5 ± 6.9 a	2.4 ± 0.1 a	3.0 ± 0.3 c

Microbial biomass indices — Soil texture analysis

Study Site	MBC (mg/g dry soil)	MBP (ug/g dry soil)	MBN (mg/g dry soil)	Sand (%)	Clay (%)	Silt (%)
El Verde Ridge	1.78 ± 0.19 c	5.59 ± 0.54 b	0.28 ± 0.04 b	16.5 ± 5.9 a	49.1 ± 3.1 b	34.5 ± 3.6 a
Bisley Ridge	1.05 ± 0.16 b	2.26 ± 0.50 a	0.18 ± 0.03 ab	3.5 ± 1.8 a	63.4 ± 1.6 c	33.5 ± 2.8 a
El Verde Valley	0.44 ± 0.02 a	0.23 ± 0.07 a	0.08 ± 0.00 a	7.0 ± 4.4 a	42.1 ± 3.7 ab	50.9 ± 1.1 b
Bisley Valley	0.61 ± 0.09 ab	1.80 ± 0.60 a	0.10 ± 0.02 a	20.1 ± 2.8 a	33.9 ± 1.6 a	46.1 ± 1.6 b

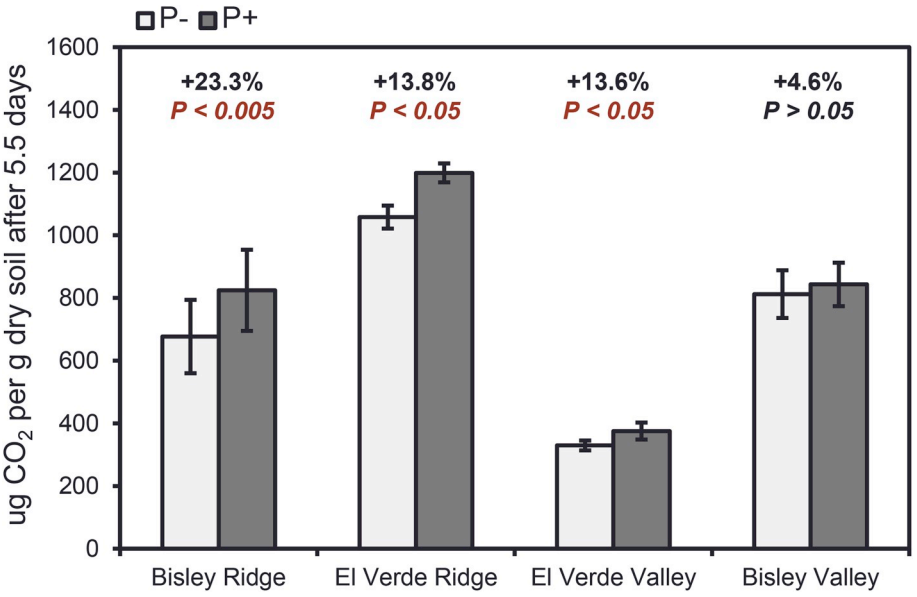

Fig. 1. Cumulative CO_2 respired after ~5.5 days of incubation for soils without (P−, light gray) and with (P+, dark gray) exogenously added phosphorus. Bars denote the mean CO_2 respired (+/− the standard error of the mean) for control incubations vs. incubations amended with exogeneous phosphate as 30 mg PO_4^{3-}-P per kg, for soils sampled from each of the four locations, based on five replicate incubations for each site and experimental condition. See also Fig. S2 for incubation layout.

Konstantinidis, 2014; Zhang et al., 2017).

The composition of bacterial phyla derived from metagenomic 16S rRNA gene fragments was comparable between ridge locations, and more distinct between ridge and BV soils, consistent with the Nonpareil diversity results mentioned above (Fig. S5; Fig. S6b). Considering phyla that represented ≥1% of the community for at least one metagenome, only *Verrucomicrobia* abundance differed significantly between ER and BR (10.4% vs. 5.6% for ER and BR soils, respectively; adj. p-value < 0.05). In contrast, the abundance of several phyla differed significantly between ridge and BV soils, including *Chloroflexi* (2.2% vs. 7.2% for ridge and BV soils, respectively), *Firmicutes* (1.0% vs. 0.5%), *Gemmatimonadetes* (0.2% vs. 1.3%), *Latescibacteria* (0% vs. 1.4%), *Nitrospirae* (0.1% vs. 4.3%), *Planctomycetes* (8.2% vs. 6.8%), and *Proteobacteria* (40.4% vs. 35.9%). Mash, a tool that uses kmer composition for β-diversity calculations based on whole metagenomes (not just 16S) (Ondov et al., 2016), also revealed greater similarity between ER and BR, than between ER/BR and BV (Fig. S6a).

Analyses of community composition revealed a large degree of overlap, in general, between P+ and P− incubation metagenomes (Fig. 3; Fig. S5; Fig. S6). Thus, comparisons between P+ and P− incubation soil communities were limited to metatranscriptomic data analyses.

3.4. Abundance and transcriptional activity of α-glucosyl polymer biosynthesis, anabolic and catabolic process, and cell division genes

Genes involved in the biosynthesis of purines (*purABCDEFHKMT*, *guaAB*), pyrimidines (*carAB*, *pyr1BCDEFHG*, *ndk*, *nrdAB*), and glycerophospholipids (*tpiA*, *gspA*, *plsBCXY*, *pls*, *cdsA*) generally increased in relative transcription with P+ (vs. P− incubation soils) (Fig. 4). The relative transcription of genes encoded by the universal *dcw* operon also generally increased with P+ (Fig. 4; see category 'cell division and peptidoglycan biosynthesis'). A multi-omics approach normalizing transcriptional activity by *in-situ* population DNA abundance and

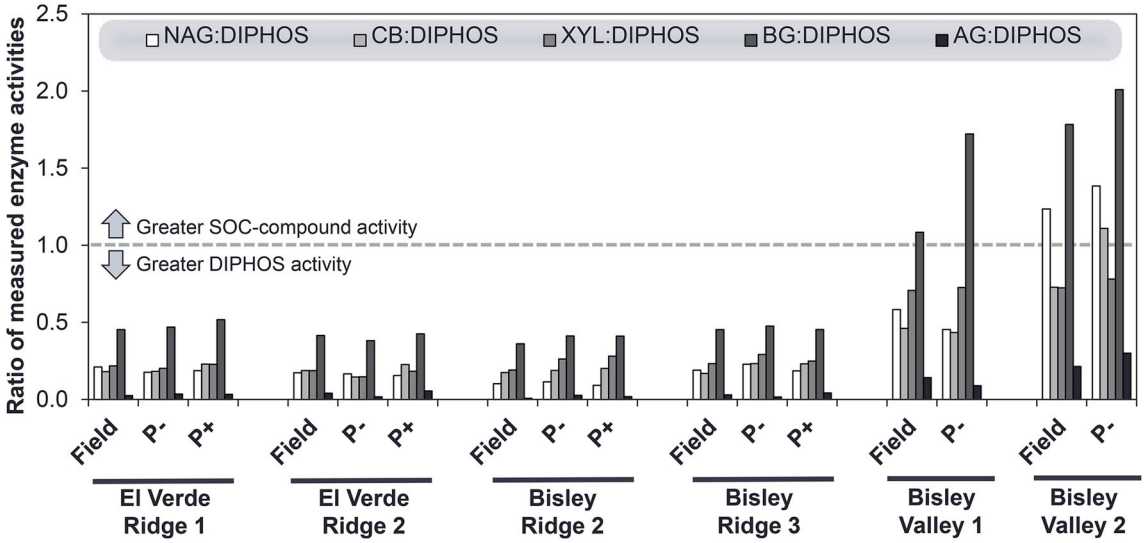

Fig. 2. Relative enzymatic activity for soils from field (Field), control incubations (P−), and incubations amended with phosphorus (P+). Values are expressed as the ratio between various soil organic carbon-specific enzyme activities and the activity of phosphodiesterase (DIPHOS; see key on the top). Labels are AG:α-glucosidase, BG:β-glucosidase, CB:β-D-cellubiosidase NAG:N-acetyl-β-glucosaminidase, XYL:β-xylosidase, DIPHOS:Phosphodiesterase. Incubation endpoints and the corresponding field soils used for enzyme assay analysis can be found in Fig. S2. Numbers (i.e., 1, 2, or 3) proceeding site names correspond to the unique ~1 kg replicate samplings taken at each site.

metatranscriptome dataset size demonstrated that heightened transcription of genes for microbial cell division and growth with P+ was community-wide as opposed to limited to only a few populations (Fig. 5). Specifically, > 85% of *dcw* operons had greater DNA-normalized relative transcription under P+ (vs. P−); the relative total transcription of all recovered *dcw* segments was 57% and 87% greater with P+ (vs. P−) for ER and BR soils, respectively.

The relative DNA abundances of genes for α-glucosyl polymer biosynthesis and phosphorylases acting on α-glucosyl polymers were generally greater in P-responsive ridge metagenomes compared to P-nonresponsive BV (Fig. 3). The opposite trend was found for phosphorylase genes specific to polymers with β-glycosidic bonds. Further, the relative transcription of all phosphorylases acting on carbohydrates increased with P+ in ridge soil metatranscriptomes (Fig. S7). A survey of metagenome-derived contigs revealed that many microbiota in P-responsive soils possessed genomic segments encoding genes for the debranching and phosphorolysis of large, α-glucosyl polymers, such as glycogen debranching enzyme (EC 3.2.1.196) and glycogen/maltodextrin phosphorylase (EC 2.4.1.1). These were often adjacent to genes involved in core catabolic functions and/or the formation of several biosynthetic precursors relevant to cell growth, such as genes involved in the formation of D-ribose 5-P (for nucleotide and ATP biosynthesis), D-erythrose 4-P (for amino acid biosynthesis), and glycerone phosphate (for phospholipid biosynthesis) (See Fig. 6a for conceptual metabolic pathway diagram and Fig. 6b for recovered genomic segments encoding these functions).

To evaluate the transcriptional activity of these common carbon catabolic and anabolic pathways, genomic segments possessing ≥5 sequential genes related to glycogenolysis, (*glgX*, *malQ*, *pgm*, and phosphorylases specific to glycogen/maltodextrin [EC 2.4.4.1]), the initial stages of glycolysis (*pgi*, *pfkA*, and *fba*), and/or the pentose phosphate pathway (*pgl*, *gnd*, *rpe*, *rpiA/ywlf*, *tkt*, and *tal*) were targeted for further analysis. As an additional stipulation, only segments with ≥2 genes belonging to the glycogenolysis category were retained. P+ increased the transcriptional coverage of these recovered segments by +250% and +103%, on average, for ER and BR soil incubations communities, respectively. Further, genes involved in electron transport chain processes (energy production), such as bacterial cytochrome c-type biogenesis (*ccmABCEFH*), cytochrome bd-II ubiquinol oxidase (*cydAB*), cytochrome bo(3) ubiquinol oxidase (*cyoABC*), succinate dehydrogenase (*sdhAB*), and fumarate reductase (*frdAB*), generally increased in relative transcription with P+ (Fig. S8).

3.5. Abundance and transcriptional activity of nutrient acquisition and storage genes

While the relative DNA abundances of genes encoding for phosphate transport ATPase complex (*pstABC*) were not significantly different between sites, that of *pstS* (encoding phosphate-binding protein) was 42.6% greater in ridge incubation metagenomes relative to BV (Fig. 3; adj. p-value < 0.05). The phosphate ABC transporter membrane subunit genes (*pstAC*) increased in relative transcription with P+ (Fig. 4), but *pstB* and *pstS* (encoding ATP-binding protein) decreased (p-value < 0.05). The relative abundance of *ppx* (encoding exopolyphosphatase), a polyphosphatase with strong preference for long-chain polyphosphates, was 89.7% greater in BV incubation metagenomes (adj. p-value < 0.05). Genes involved in the uptake, fixation, and/or assimilation other nutrients generally increased in relative transcription with P+. This included genes involved in N fixation (*nifDHK*), ammonium import (*nrgA*), assimilatory nitrate reduction (*nasABCD*, *nir*), sulfur binding and import (*cysATW*, *sbp*), and potassium import (*kdpABC*).

3.6. Transcriptional activity of cellular stress and maintenance genes

Given the increase in relative expression of genes involved in energy production and cell growth with P addition mentioned above, it was interesting to evaluate which genes decreased in relative transcription. The most consistent pattern across ridge soils was a significant decrease in the relative transcription of nearly every ribosomal protein in P+ incubations (Fig. S8; p-value < 0.05) and in ribosome-related functions, such as ribosomal recycling factor (*frr*). The relative transcription of genes related to cellular stress or maintenance, such as for protecting against reactive oxygen species (*prdx*, *grx*, *sodCFMN*), protein degradation (*lon, clpACPS*), molecular chaperones (*CH60*, *CH10*, *clpB*, *dnaKJ*), and cold or heat-shock proteins (*cpsABCE*, *hspBCDH*, *idpA*), were also lower in P+ soil metatranscriptomes (Fig. S8).

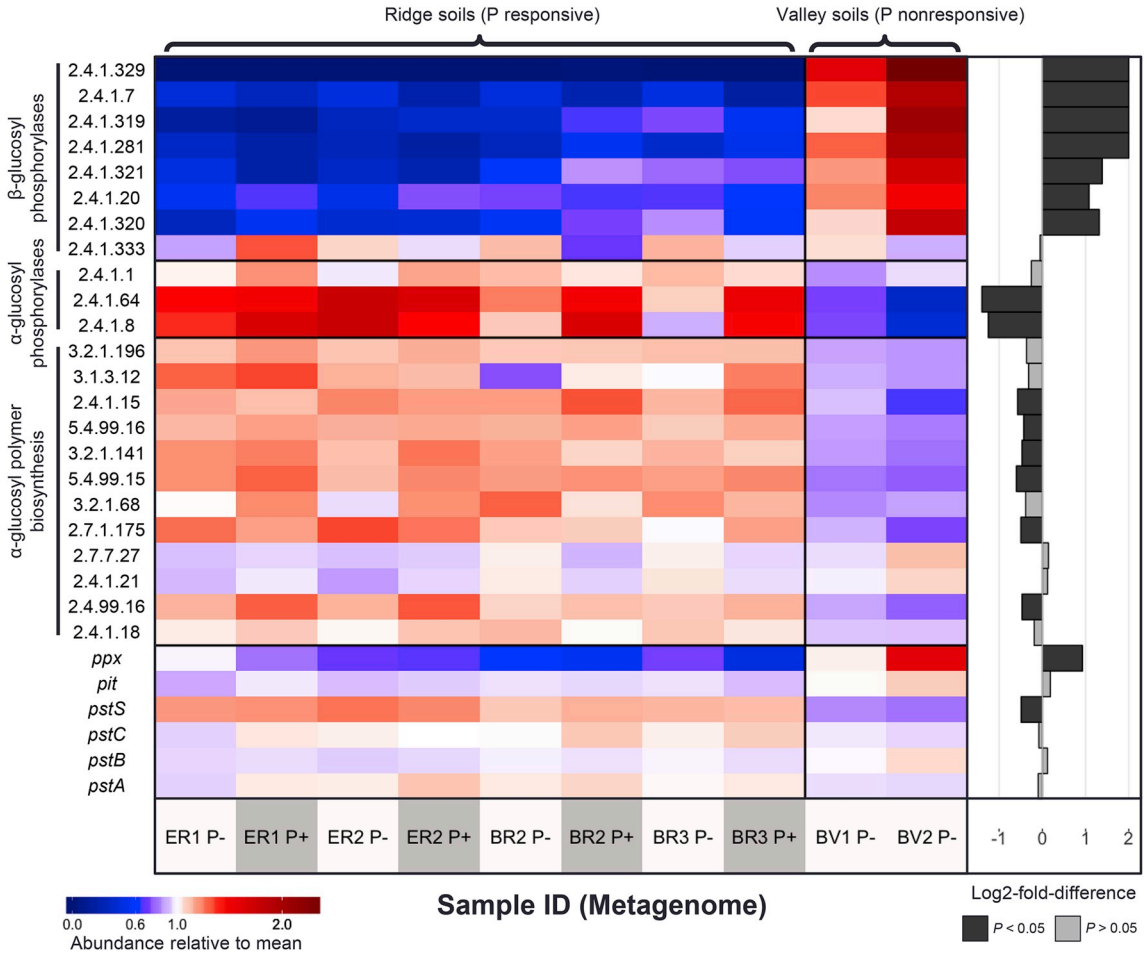

Fig. 3. Heatmap showing the relative DNA abundances of genes involved in P uptake and storage, α-glucosyl polymer synthesis, and phosphorolysis of α- and β-glucosyl substrates. Heatmap depicts the relative abundances of genes or enzyme comission IDs (y-axis; rows) in each metagenome (x-axis, columns), normalized by the mean abundance of the gene across all metagenomic datasets (scale on bottom left; more red denotes greater relative abundance). Sub-plots on the right of each heatmap represent the log2-fold difference between ridge and BV soil metagenomes, and are colored by whether or not differences were statistical significant (see figure key). A log2-fold change of +2 was set as the maximum value for the right panel for presentation purposes (some values from enzyme IDs under 'β-glucosyl specific phosphorylase' were greater than +2). Abbreviations: El Verde ridge (ER), Bisley ridge (BR), and Bisley valley (BV) soils; numbers (i.e., 1, 2, or 3) proceeding site names correspond to the unique ~1 kg replicate samplings taken at each site; P+ and P− correspond to soil incubations with and without the addition of exogenous PO_4^{3-}, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

Phosphorus is often regarded as the primary nutrient limiting biological activities in tropical ecosystems (Vitousek, 1984; Tanner et al., 1998), yet many uncertainties remain concerning how P availability governs tropical soil microbial community composition and activities (Turner and Wright, 2014). In this study, P amendment (P+) of lab-incubated soils increased CO_2 respiration by 14–23% for soils sampled from all but the site with the greatest total and bioavailable P; ER, BR, and EV were responsive to P+ while BV was not (Table 1; Fig. 1). The results obtained here were consistent with previously measured soil carbon flux response following P fertilization (Cleveland and Townsend, 2006) and a 20% and 16% increase in litter and soil respiration, respectively, following 158 days of P fertilization in soils from French Guiana (Fanin et al., 2012). P-responsive ER and BR soils were found to be more similar in overall microbial community taxonomic and sequence-based composition relative to P-nonresponsive BV soils (Fig. S5, S6ab). Although BV soils had the greatest total and bioavailable soil P content and were not responsive to P+, their P concentrations are still often regarded as sufficiently low as to limit plant growth (Powers et al., 2004). Thus, it is likely that BV soil communities are constrained by other nutrients, such as potassium, which was lowest in BV soils. BV soil communities could also be constrained by comparatively greater sand content (Table 1), which often corresponds to lower soil microbial biomass (Kaiser et al., 1992). Consistent with this, BV soil MBC was lower or comparable to ER and BR soil MBC (Table 1).

Metatranscriptomics revealed that P+ of ridge soils increased the relative transcription of numerous genes involved in cell growth and replication, including genes involved in cell division and the biosynthesis of peptidoglycan, glycerophospholipids, and purines and pyrimidines (DNA, RNA, ATP, etc. precursors) (Fig. 4). A population-level investigation of cell division and cell wall (*dcw*) operon gene expression indicated that removal of prior P nutrient constraints universally increased the relative transcription of genes involved in cell growth and proliferation, at least for the abundant taxa whose genome sequences could be adequately recovered (Fig. 5).

In addition to the acute growth response with P+, there were positive correlations between field soil measurements of available P and MBP, MBC, and MBN for ridge soils (Fig. S3), reflecting greater sustained biomass with greater long-term soil P. These results are consistent with previous studies showing an increase in microbial biomass

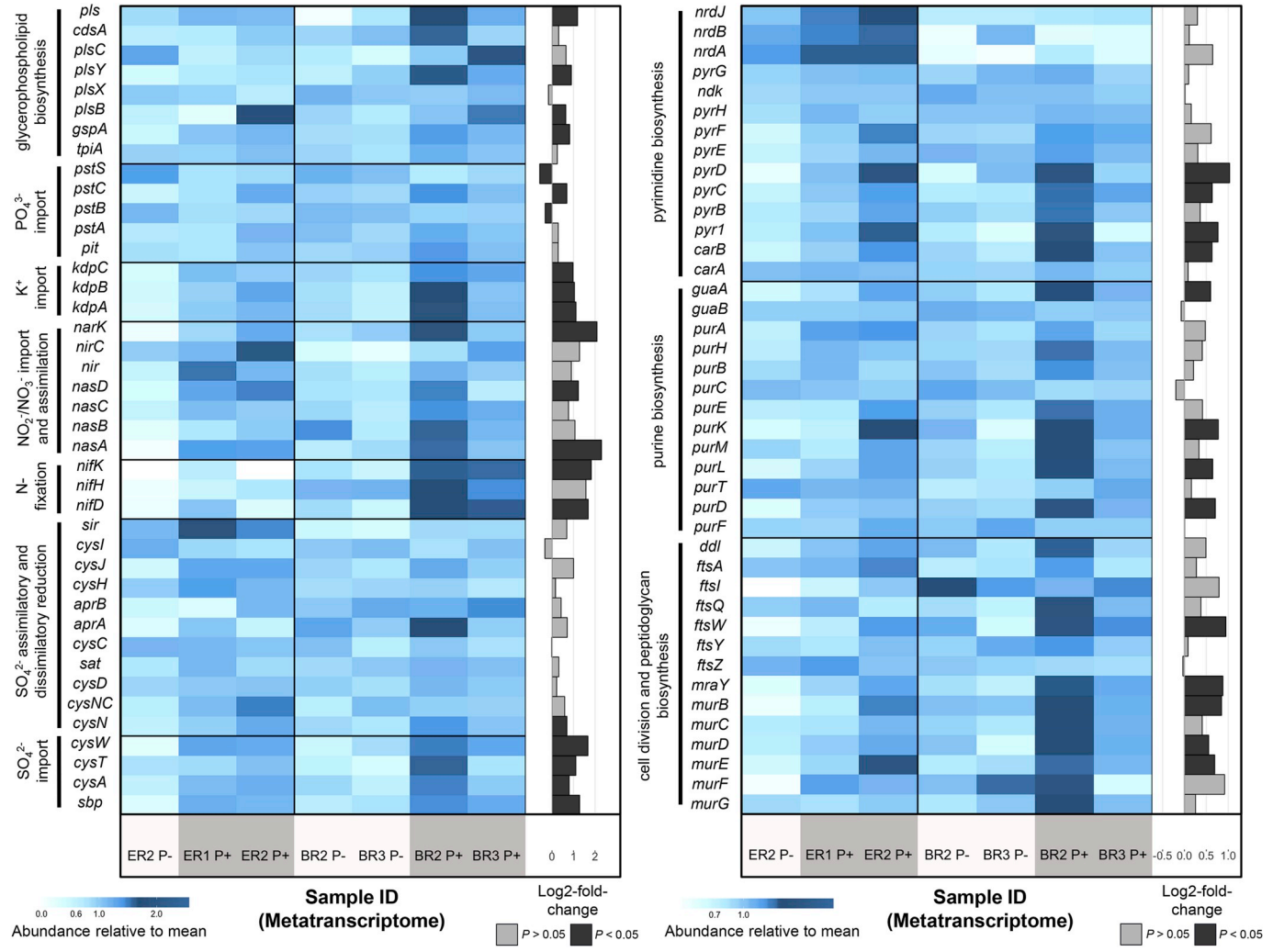

Fig. 4. Heatmap showing the relative transcriptal activity of genes involved in nutrient uptake and assimilation, cell division, and cell component biosynthesis. Heatmap depicts the relative transcription (i.e., abundance) of genes or enzyme comission IDs (y-axis; rows) in each metatranscriptome (x-axis, columns), normalized by the mean abundance of the gene across all metatranscriptomes (scale on bottom left; darker blue denotes greater transcriptional activity). Sub-plots on the right of each heatmap represent the log2 fold-change between ridge P+ and P− soil incubation metatranscriptomes, and are colored by whether or not the differences between P+ and P− soil incubations were statistically significant (see figure key). Abbreviations: El Verde ridge (ER) and Bisley ridge (BR) soils; numbers (i.e., 1, 2, or 3) proceeding site names correspond to the unique ~1 kg replicate samplings taken at each site; P+ and P− correspond to soil incubations with and without the addition of exogenous PO_4^{3-}, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

with long-term P fertilization at tropical field locations (Liu et al., 2012; Turner and Wright, 2014). In the Turner and Wright (2014) study, the authors suggested that increased microbial biomass could be due to increased plant litter inputs resulting from P fertilization, but remarked that at an adjacent site, in which soil was supplemented with doubled plant litter inputs, no increase in microbial biomass was observed (Sayer et al., 2012). Thus, while the amount and quality of organic carbon is traditionally thought to constrain soil microbial biomass and activities (Wardle, 1992; Demoling et al., 2007), our results suggest that P also directly constrains microbial cell growth and proliferation in P-limited tropical soils. This is likely due to the essentiality of P for many core cellular infrastructure components, such as DNA, RNA, ATP, NADPH, and glycerophospholipids. In order to sustain enhanced growth, microbes would also need to assimilate other nutrients and heighten catabolic functions. Accordingly, genes involved in nutrient import and/or assimilation and energy-yielding catabolism generally had greater relative transcription with P+ (Fig. 4; Fig. S8).

Previous research has demonstrated how conditions of excess organic carbon but inadequate P can result in the accumulation of intracellular α-glucosyl polymers in certain microbial isolates (Zevenhuizen, 1966; Dephilippis et al., 1992; Woo et al., 2010). Accordingly, genes involved in the biosynthesis of α-glucosyl polymers had greater relative abundances in P-responsive ridge soils relative to P-nonresponsive BV soil metagenomes (Fig. 3). Phosphorylase genes specific to α-glucosyl polymer degradation were also more abundant in ridge soils. Soil microbiota that accumulate α-glucose polymers under P-limiting, carbon-rich conditions could benefit from metabolizing α-glucosyl substrates with phosphorylase enzymes when P becomes (more) available because phosphorylase activities are impaired at low P concentrations (Derensy-Dron et al., 1999; Jaito et al., 2014). Hence, such mechanisms could serve as a direct control over the metabolism of stored organic substrates and downstream catabolic and anabolic activities. Further, we identified several genomic segments encoding functions to degrade α-glucosyl substrates including glycogen/maltodextrin phosphorylase, accompanying genes involved in glycolysis and/or the pentose phosphate pathway (Fig. 6). These segments could represent a streamlined pathway where stored organic substrates are utilized to generate major biosynthetic precursors and heighten cellular

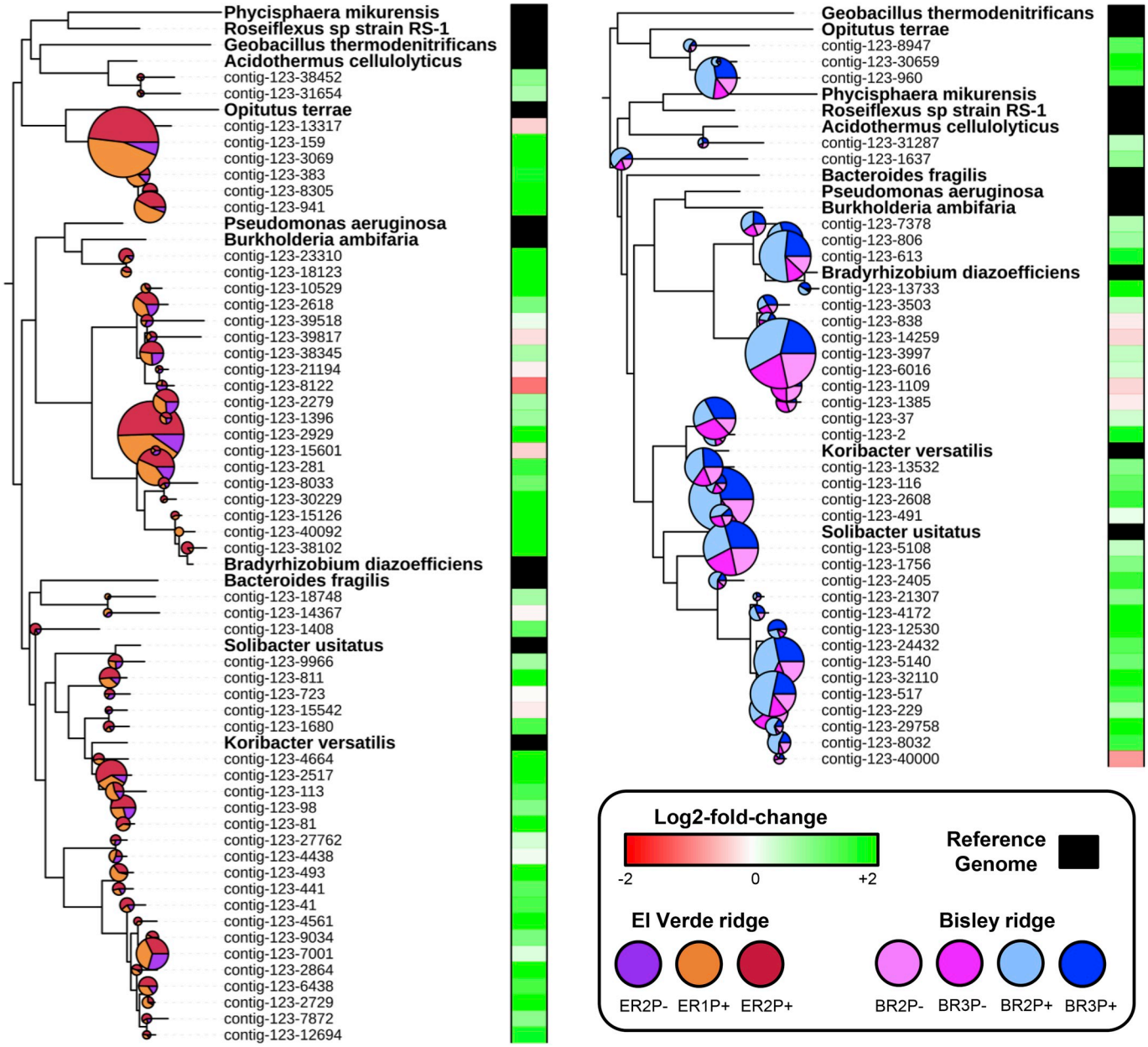

Fig. 5. Population-specific transcriptional activity of the common cell division and cell wall (*dcw*) operon for El Verde ridge (left) and Bisley ridge (right) community co-assemblies. The phylogenetic tree was constructed using aligned *murG* sequences and included reference sequences from Uniprot databases (in black). Pie charts represent the relative DNA-normalized transcriptional activity in each control (P−) and P-amended incubation samples (P+) for each unique *dcw* operon recovered (representing each branch). The size of each pie chart is proportional to the mean relative (DNA) abundance of the corresponding *dcw* segment. The right box (green/red) represents the log2-fold difference in activity (ratio) between P− and P+ samples for each sequence/population (green denotes greater activity in P+ vs. P−). A log2-fold change of +2 was set as the maximum value for presentation purposes (e.g., some positive values were slightly greater than +2). Abbreviations: El Verde ridge (ER) and Bisley ridge (BR) soils; numbers (i.e., 1, 2, or 3) proceeding site names correspond to the unique ~1 kg replicate samplings taken at each site. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

activities through energy-yielding catabolism upon conditions favorable for cell proliferation and growth. Enhanced usage of stored organic substrates, as evidenced by increased relative transcription of these recovered pathways (Fig. 6a) and all surveyed carbohydrate-active phosphorylase genes (Fig. S7), likely contributed to elevated CO_2 respiration with P+. Consistent with these interpretations, P amendment of ridge soils increased measured α-glucosidase (responsible for the cleavage of terminal glucose residues from a variety of α-glucosyl substrates) activity by +46%, making it the most P-responsive SOC-degrading enzyme assessed in this study. Therefore, metatranscriptomics provided, in general, consistent results with enzyme assay analysis, albeit the former offered higher resolution and sensitivity. Ridge soils with larger MBC:MBP also exhibited greater respiration response to P+ (Fig. S3d). These results could further indicate a greater amount of stored α-glucosyl substrates undergoing microbial metabolism following P amendment.

Conversely, phosphorylase genes specific to β-glucosyl polymers such as cellulose and hemicellulose were more abundant in P-non-responsive BV soil metagenomes (Fig. 3). Direct metabolism of plant-derived substrates using phosphorolysis could be metabolically advantageous under conditions of greater available soil P because it conserves ATP that is otherwise needed to phosphorylate glucose with glucokinase following hydrolysis. These results implicate phosphorylase genes for different substrate classes (i.e., β-glucosyl vs. α-glucosyl

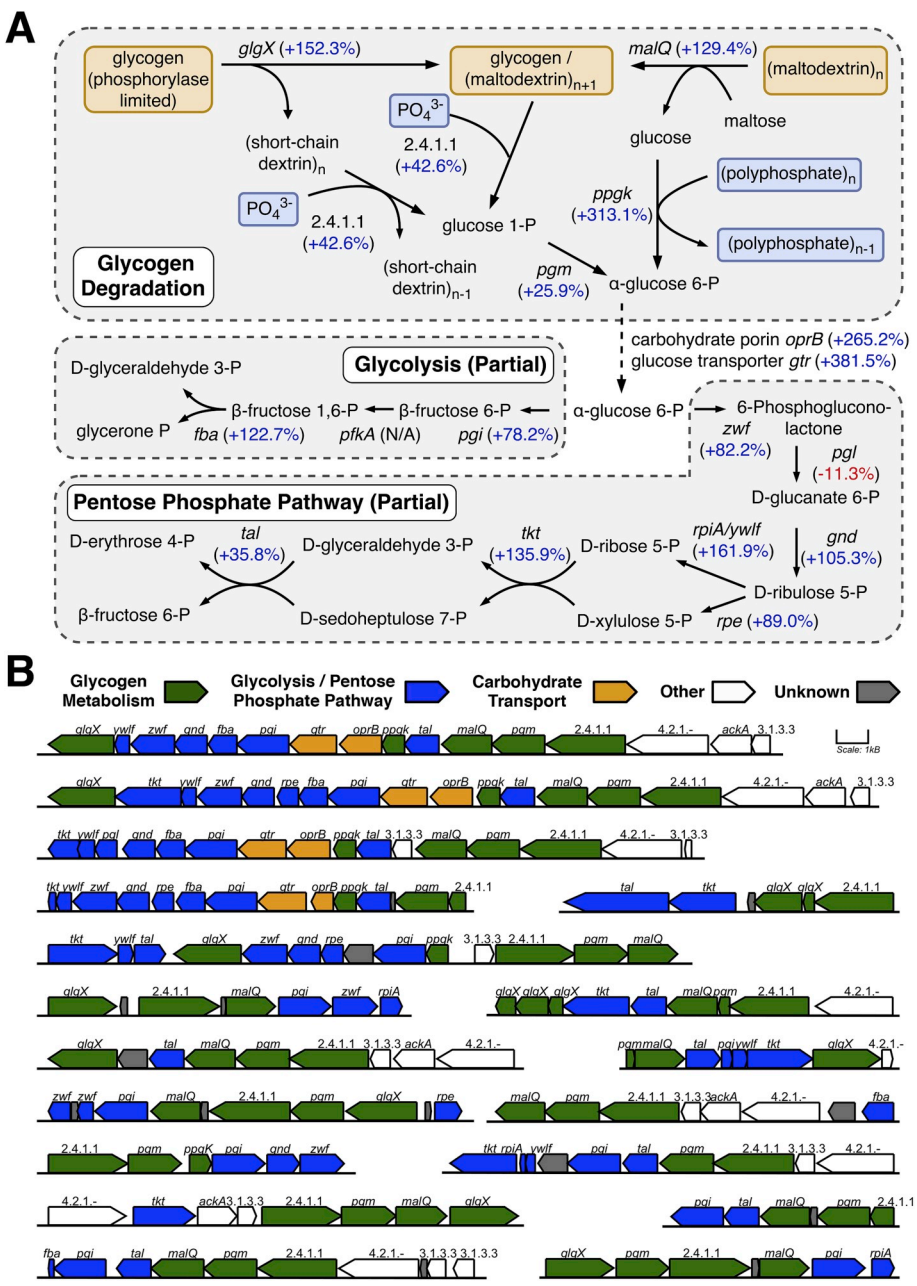

Fig. 6. (A) Pathway diagram and (B) gene organization in recovered genomic segments reflecting glycogen metabolism, glycolysis, and the pentose phosphate pathway. Percentages given in blue or red (in panel A) represent the average increase or decrease in the relative transcription of the corresponding genes, respectively (i.e. the change in transcription with P amendment vs. control incubations). Gene tracks (in panel B) represent unique contigs assembled from Bisley and El Verde ridge metagenomes that encoded genes of interest. Continuous black lines under the gene boxes are used to signify unique, continuous genomic segments. Gene Graphics (Harrison et al., 2017) was used to produce 'gene track' illustrations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

polymers) as a trait reflecting soil P bioavailability, and provide mechanistic insights into how microbial growth and activity can be coupled to, and potentially regulated by, soil P conditions. Consistent with these interpretations, the relative abundances of proteins and functional genes for glycogen phosphorylase were recently found to be much greater in unfertilized relative to experimentally fertilized Panamanian soils (Yao et al., 2018). Thus, despite our evaluation of tropical soil microbial communities being limited to three sampling sites, differences in the relative abundances of phosphorylase genes are likely attributable to soil P availability and are applicable to other tropical forest ecosystems.

Among several phyla that differed in abundance between ridge and BV soils, *Gemmatimonadetes* was 7.5-fold more abundant in BV communities (Fig. S5). While few members from *Gemmatimonadetes* have been cultivated to date, it is thus far represented mostly by organisms capable of polyphosphate accumulation under conditions of higher P but otherwise unfavorable for growth (Zhang et al., 2003; DeBruyn et al., 2013; Pascual et al., 2016). Further, there was a 2-fold greater relative abundance of *ppx* (encoding exopolyphosphatase, a polyphosphatase specific to long-chain polyphosphates) in BV metagenomes, consistent with more bioavailable P for BV soils (Fig. 3). In contrast, the relative abundance of *pstS* (encoding phosphate-binding protein) was greater in ridge soil metagenomes and was also relatively less transcribed with P+. Further, phosphodiesterase activity was greater than the activities of organic carbon degrading enzymes for ridge soils, which was also consistent with previous evaluations of tropical soil phosphoesterase activity (Turner and Wright, 2014; Yao et al., 2018). Taken together, microbial adaptation to P limitation likely includes greater reliance on inorganic phosphate binding and organic phosphorus recycling and lower abundance of taxa and traits involving accumulation of long-chain polyphosphates.

The relative transcription of genes involved in protein maintenance and turnover, environmental stress tolerance, and ribosome biogenesis and recycling were greater in control (P−) incubation soils (Fig. S8). These findings are consistent with previous studies showing high overlap in cellular protective mechanisms operating under nutrient

starvation and other stress types (Spence et al., 1990; Givskov et al., 1994). Studies on microbial isolates have found that nutrient-starved cells were more resistant to oxidative stress and displayed greater thermotolerance than actively growing cells (Rockabrand et al., 1995; Petti et al., 2011), presumably due to the need to survive environmental fluctuations over larger generation times. Heightened ribosome production and recycling under P-limited conditions may reflect a continual need to divert P used for rRNA for other cellular functions, and vice-versa (i.e., from those uses back to translational machinery, such as rRNA). Barnard et al. (2013) demonstrated how soil microbiota adapted to oscillating growth conditions often accumulate ribosomes during stationary growth phases, presumably to respond more rapidly upon more favorable nutrient availability. The aforementioned study also reported a decline in ribosome levels during conditions of elevated growth for responsive taxa. Altogether, greater relative expression of genes involved in cell upkeep, environmental stress tolerance, and core functionality may reflect suboptimal growth conditions.

The novel strategy introduced here of normalizing population-specific transcriptional activity to *in-situ* DNA abundance can be useful for comparing community metatranscriptomes where high compositional heterogeneity is an inherent environmental feature. For instance, the transcription of certain functions could be evaluated against baseline cellular expression, activities, and between functions where contrasting modes of response are expected. This approach could also be combined with qPCR or inclusion of internal standards prior to nucleic acid extraction in order to estimate absolute abundances of target genes or genomes, if desirable. Finally, difficulty in obtaining high quality RNA resulted in a limited number of datasets and fewer sites represented than intended. To account for this, our reporting of results was heavily concentrated on consistent responses observed between functions involved in the same pathway, operon, or functional category. While many consistencies were observed, general methodological improvements in the recovery and sequencing of high quality RNA from soils will enable more robust and resolved evaluations to be performed in future studies.

5. Conclusions

In this study, community composition (determined with metagenomics) and activity assessments (including CO_2 respiration, enzyme assays for organic carbon and phosphorus decomposition, and metatranscriptomics) together revealed how tropical soil microbiota respond acutely to an alleviation of P constraints. Functional signatures revealed community traits reflecting long-term adaptation to growth-restricting P conditions. This included the abundances of genes for α-glucosyl polymer biosynthesis, phosphorylases specific to stored vs. plant-derived substrates, and nutrient acquisition, which could serve as biomarkers of microbial adaptation or response to P nutrient availability. Genomic indicators of environmental change or nutrient conditions serve as candidates for incorporation into genome-informed ecosystem models. Further, the gene and partial genome sequences reported here could provide reference data for PCR assays to monitor the abundance of specific indicator genes for nutrient status in field samples. It would also be important to test these findings against other tropical forest locations such as long-term fertilization studies in order to better distinguish between the long-term and acute effects of greater P availability, and to determine how the strength and consequences of P limitation compare to other major nutrients (N, Ca, K, etc.).

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This research was sponsored by the Oak Ridge National Laboratory Directed Research and Development Program (to MAM); the ORNL 'GO! Student Program' (to ERJ); and the US National Science Foundation (award 1356288 to KTK). ORNL is managed by the University of Tennessee-Battelle, LLC, under contract DE-AC05-00OR22725 with the U.S. Department of Energy. We thank Grizelle Gonzalez of the US Forest Service International Institute of Tropical Forestry and Jess Zimmerman of the University of Puerto Rico for their assistance in obtaining soils from the US Forest Service El Yunque National Forest, and Carlos Torrens of the US Forest Service for his assistance collecting the Bisley Watershed samples. We would also like to thank the Partnership for an Advanced Computing Environment (PACE) at the Georgia Institute of Technology, which enabled the computational data analysis reported in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2018.11.026.

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