Supporting Information:

In situ stimulation of thiocyanate biodegradation through phosphate amendment in gold mine tailings water

Mathew P. Watts[1], Han M. Gan[2,3,4], Lee Y. Peng[3, 4], Kim-Anh Lê Cao[5] and John W. Moreau[1, *]

[1]School of Earth Sciences, The University of Melbourne, Parkville, Vic., Australia

[2]Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Geelong, Vic, Australia

[3]School of Science, Monash University Malaysia, Bandar Sunway, Petaling Jaya, Selangor, Malaysia

[4]Genomics Facility, Tropical Medicine and Biology Platform, Monash University Malaysia, Bandar Sunway, Petaling Jaya, Selangor, Malaysia

[5]Melbourne Integrative Genomics and the School of Mathematics and Statistics, The University of Melbourne, Parkville, Vic., Australia

*Corresponding Author : jmoreau@unimelb.edu.au

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PCR protocol

The bacterial 16S rRNA genes were amplified separately, according to the following PCR protocol; initial denaturing at 98°C for 30 seconds, followed by 25 cycles of denaturing for 10 seconds at 98°C, annealing for 30 seconds at 60°C and extension for 1 minute at 65°C, followed by a final extension for 2 minutes at 65°C. The resulting amplicons were then purified using 0.8x volume of Agencourt AMPure XP beads (Beckman Coulter, Brea, CA), respectively followed by a 80% ethanol wash. The second-half of the Illumina adapter along with unique 8-bp dual index barcode (Nextera XT Index i7 and i5) were added to the amplicons via another round of PCR under the following thermal cycler conditions; denatured at 98°C for 30 seconds, followed by 8 cycles of 10 seconds at 98°C and extension for 1 minute and 15 seconds for 65°C and a final extension for 2 minutes at 65°C. The resulting amplicons were again purified using 0.8X volume of Agencourt AMPure XP beads and washed with 80% ethanol.

The archaeal 16S rRNA gene was amplified using the initial PCR protocol; initial denaturation step for 3 minutes at 95°C, followed by 25 cycles of 30 seconds at 95°C, 30 sec at 57°C, 1 minute at 72°C, and final elongation for 1 minutes at 72°C. The resulting PCR products were then purified using 0.7X volume ratio of Agencourt AMPure XP beads and washed with 80% ethanol.. This was followed by a second round of PCR; initial denaturation step for 3 minutes at 95°C, followed by 25 cycles of 30 seconds at 95°C, 30 seconds at 53°C, 30 seconds at 72°C, and final elongation for 1 minutes at 72°C. The products of this second round of PCR was again purified using 0.7X volume ratio of Agencourt AMPure XP beads, followed by a 80% ethanol wash . The resulting archaeal 16S amplicons were ligated to the Nextera XT i7 and i5 index primers , according to the protocol; initial denaturation for 3 minutes at 95°C, followed by 5 cycles of 30 seconds at 95°C, 30 seconds at 55°C, 30 seconds at 72°C, and final elongation for 1 minute at 72°C. The final PCR product was again subjected to 0.7X volume ratio Agencourt AMPure XP beads purification and a 80% ethanol wash.

The 18S rRNA genes were amplified according to the following PCR protocol; initial denaturing at 98°C for 30 seconds, followed by 25 cycles of denaturing for 10 seconds at 98°C, annealing and

extension for 1 minute at 65°C, followed by a final extension for 2 minutes at 65°C. The 18S rRNA amplicons were then purified using 1.0X volume of Agencourt AMPure XP beads, respectively followed by a 80% ethanol wash. The second-half of the Illumina adapter along with unique 8-bp dual index barcode were added to the amplicons via another round of PCR under the following thermal cycler conditions; denatured at 98°C for 30 seconds, followed by 8 cycles of 10 seconds at 98°C and extension for 1 minute and 15 seconds for 65°C and a final extension for 2 minutes at 65°C. The resulting amplicons were again purified using 0.8X volume of Ampure XP bead and washed with 80% ethanol.

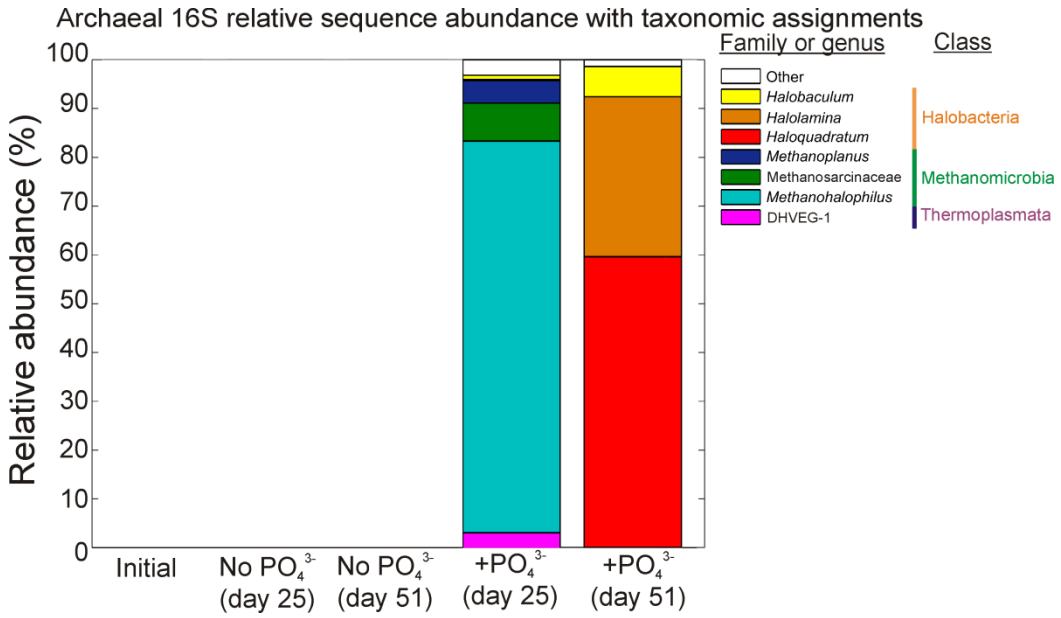

Figure S1. Microbial community analysis of surface water samples based on archaeal 16S rRNA gene sequences. Relative 16S rRNA gene sequence abundance, assigned to taxa which make up ≥2% of sequences in any of the samples, of the samples with positive amplification; $+PO_4^{3-}$ at 25 and 51 days.

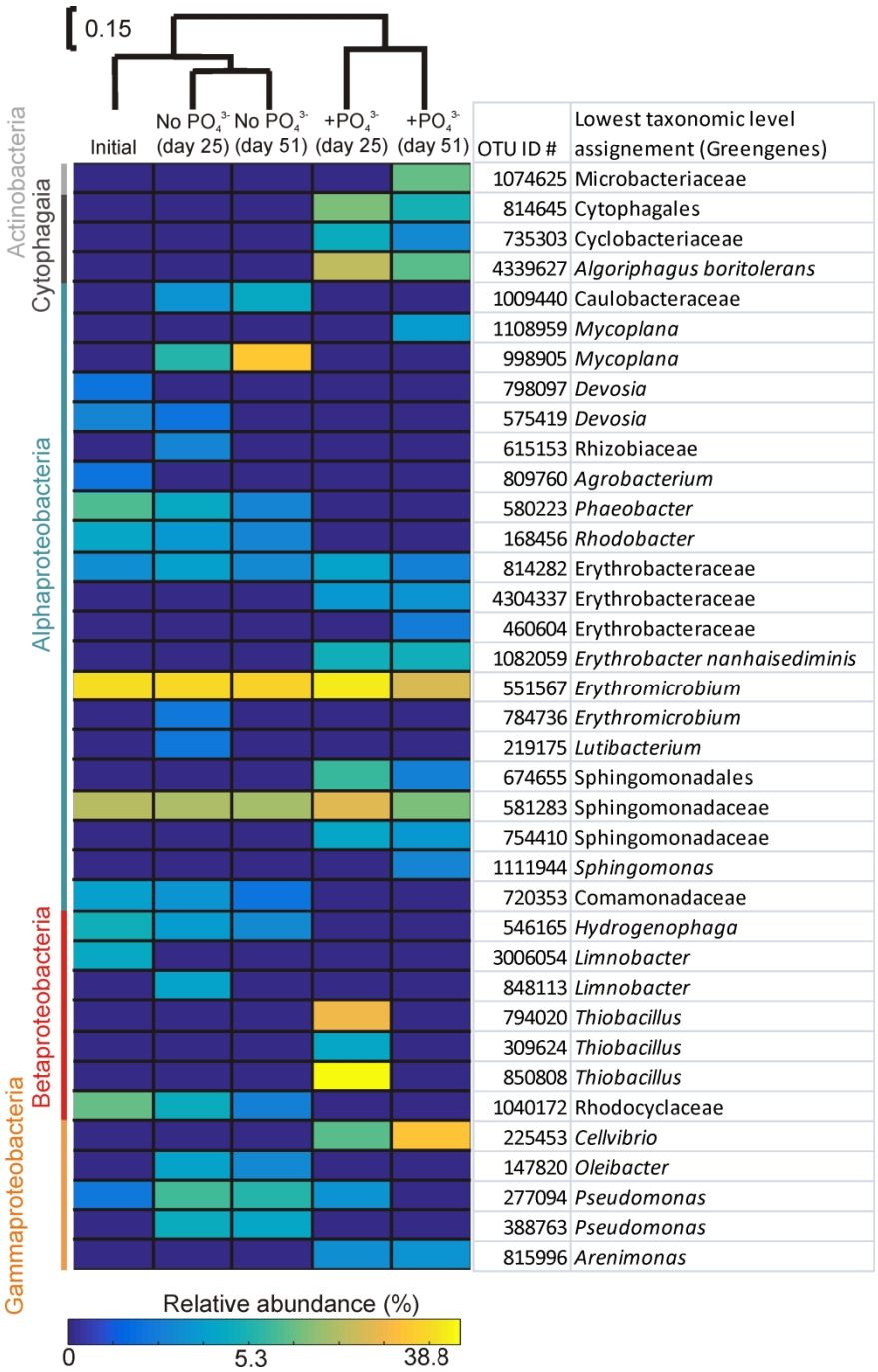

Figure S2. Heatmap of the $\log_{10+1}$ scale relative abundance of bacterial 16S rRNA gene sequences assigned to OTUs, at 97% sequence similarity, which represent greater than 1% in any sample. Class level distinctions are given to the left of the OTU map and the Greengenes assigned OTU ID# and lowest taxonomic assignment is given in a table to the right. The dendrogram represents Bray-Curtis dissimilarity of the microbial communities in the initial surface water and the incubated samples, in the presence and absence of phosphate, with the scale bar denoting similarity as a fraction.

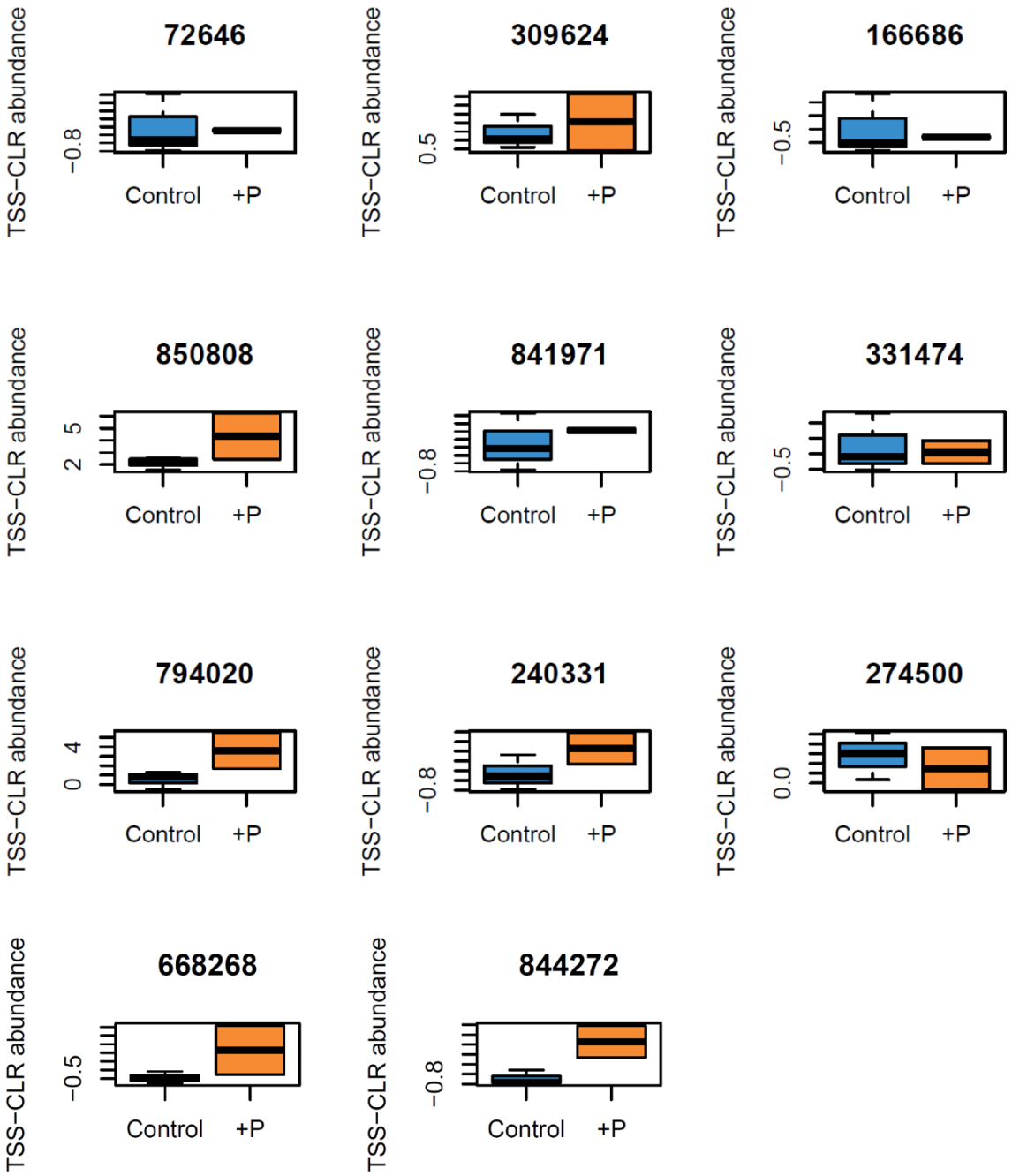

Figure S3. Boxplots with respect to no-PO_4^{3-} control and +P treatment of OTU abundance (total sum scaling normalized centered log ratio (CLR) transformed data). Each plot represents a thiobacilli OTU with an OTU reference ID.

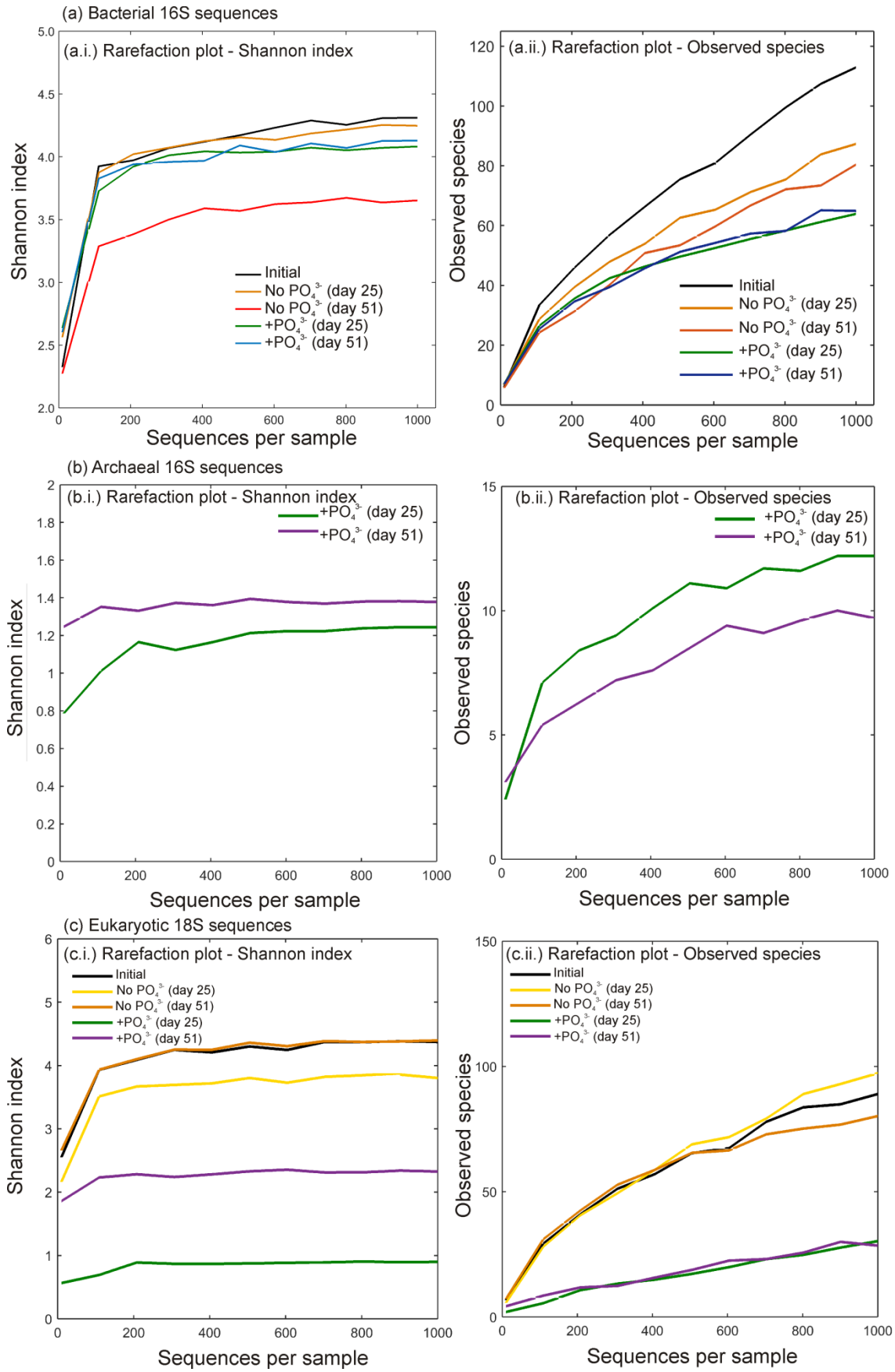

Figure S4. Rarefaction curve of the bacterial 16S rRNA (a), archaeal 16S rRNA and eukaryotic 18S rRNA gene sequence data subject to alpha-diversity analysis by the Shannon index (i) and observed species (ii).

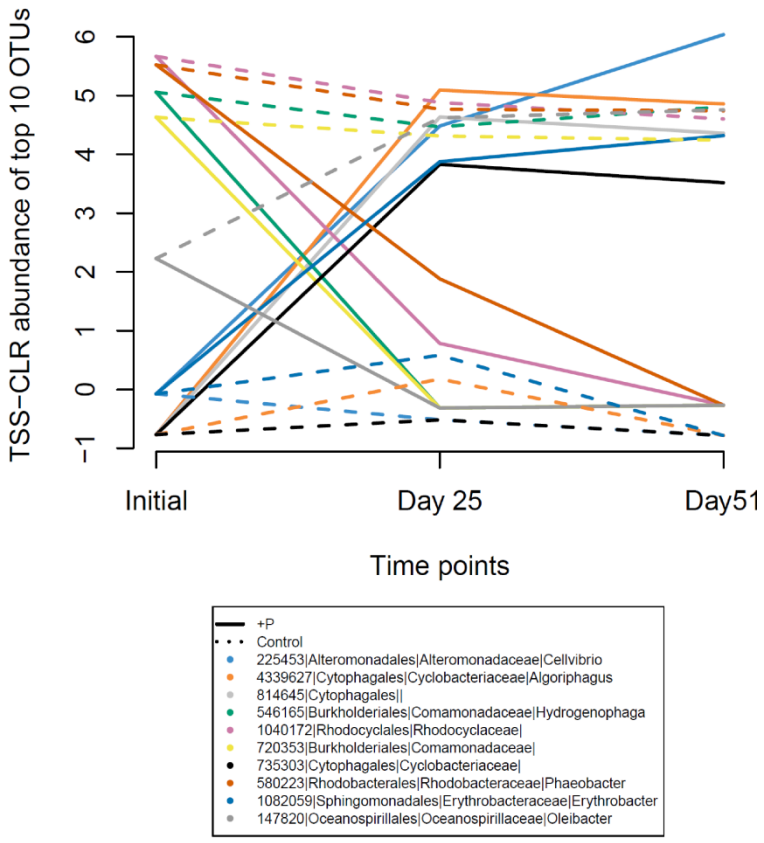

Figure S5. Top 10 OTUs driving the variation between the treatments (no-PO_4^{3-} control and +P) presented in the PCA plot in Fig. 3 with respect to time. Solid lines represent +P and dashed lines no-PO_4^{3-} control, color correspond to a given OTU as refered in the legend.

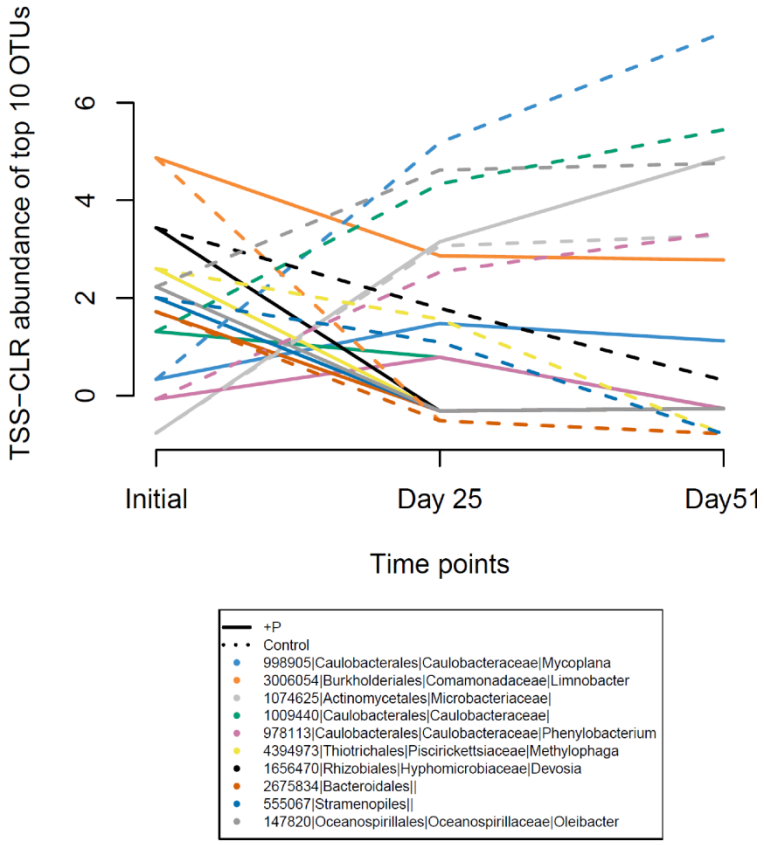

Figure S6. The top 10 OTUs driving the difference between the time points presented in the PCA plot (component 2) in Fig. 3. Note the solid lines represent +P and dashed lines the no-PO_4^{3-} control.

Table S1. Closest GenBank phylogenetic relatives of 16S Bacterial OTUs that comprised ≥2% of total sequence abundance in any samples.

Geengenes assignment (lowest taxanomic classification)	Closest phylogenetic relative (GenBank)	Accession number	Sequence similarity (%)
Microbacteriaceae	*Leifsonia* sp. 8_1Ka	EF540450	99%
Cytophagales	*Bacteroidetes* bacterium 4_C16_35	EF540508	99%
Algoriphagus boritolerans	*Bacteroidetes* bacterium 4_C16_35	EF540508	99%
Caulobacteraceae	*Phenylobacterium falsum* strain AC-49	NR_042277	99%
Mycoplana	*Brevundimonas subvibrioides* strain HMF3828	KT983987	98%
	Brevundimonas variabilis	HF913440	98%
Mycoplana	*Brevundimonas* sp. 3X/A02/233	AY576767	99%
Phaeobacter	*Seohaeicola saemankumensis* strain SD-15	NR_044437	99%
Rhodobacter	*Paracoccus* sp. Spegb8	KC462963	99%
	Pseudorhodobacter wandonensis strain WT-MW11	NR_109461	99%
Erythrobacteraceae	*Porphyrobacter sanguineus* strain NJES-79	KR140256	98%
Erythrobacteraceae	*Porphyrobacter sanguineus* strain NJES-79	KR140256	99%
Erythrobacter nanhaisediminis	*Erythrobacter vulgaris* strain 707B6	KP713661	99%
	Erythrobacter nanhaisediminis strain HME8335	JQ762409	99%
	Erythrobacter aquimaris strain D4017	FJ161254	99%
Erythromicrobium	*Porphyrobacter sanguineus* strain ATCC 25661	AB062106	99%
Sphingomonadales	*Sphingopyxis fribergensis* strain Kp5.2	NR_137271	98%
	Sphingopyxis chilensis strain CHS13	KP895806	98%
	Sphingopyxis panaciterrae strain		98%
	M_Sw_oHS_07/10_4_1(2)	KF777401	
	Sphingopyxis alaskensis strain WWH70	KC921184	98%
Sphingomonadaceae	*Porphyrobacter sanguineus* strain ATCC 25661	AB369190	99%
Sphingomonadaceae	*Porphyrobacter sanguineus* (ATCC 25661)	AB369190	99%
Comamonadaceae	*Acidovorax defluvii* strain DT46	KC920943	99%
Hydrogenophaga	*Hydrogenophaga* sp. R-38517	FR691429	99%
Limnobacter	*Limnobacter thiooxidans* strain Nb15RA-1	KP296193	99%
Limnobacter	*Limnobacter thiooxidans* strain Nb15RA-1	KP296193	99%
Thiobacillus	*Thiobacillus thioparus* strain THI 111	NR_117864	98%
Thiobacillus	*Thiobacillus thioparus* strain THI 111	NR_117864	98%
Rhodocyclaceae	Bacterium SL4.29	DQ517124	99%
Cellvibrio	*Cellvibrio* sp. EP_S_20	KJ642548	99%

Oleibacter	*Thalassolituus marinus* strain IMCC1883	HM569768	94%
Pseudomonas	*Pseudomonas toyotomiensis* strain SW237	KC178914	99%
	Pseudomonas mendocina strain L6	KT834848	99%
	Pseudomonas asplenii strain BW7	KP684084	99%
	Pseudomonas chengduensis strain BW20	KP684064	99%
	Pseudomonas alcaliphila strain BW1	KP684063	99%
	Pseudomonas indoloxydans strain F3-54	KP462876	99%
	Pseudomonas alcaligenes strain NWU3	KM882604	99%
	Pseudomonas oleovorans strain D9	KM488459	99%
	Pseudomonas trautweinii strain T	KF908035	99%
	Pseudomonas nitroreducens strain X14-1-1	JX987715	99%
	Pseudomonas pseudoalcaligenes strain CLR9	KF478199	99%
Pseudomonas	*Pseudomonas knackmussii*	KX013427	99%
	Pseudomonas stutzeri strain LC2-8	FJ859914	99%
	Pseudomonas chloritidismutans strain K4-1	LN854586	99%
	Pseudomonas xanthomarina strain ZJY-488	KP282799	99%
Arenimonas	*Arenimonas* sp. s2-21	KT619125	99%

Table S2. Closest GenBank phylogenetic relatives of 16S Archaeal OTUs that make up ≥1% of total sequence abundance in any samples.

Geengenes assignment (lowest taxanomic classification)	Closest phylogenetic relative (GenBank)	Accession number	Sequence similarity (%)
Methanohalophilus halophilus	*Methanohalophilus halophilus* strain Z-7982	CP017921.1	99%
	Methanohalophilus portucalensis strain Z-7301	KT285316.1	99%
	Methanohalophilus mahii strain DSM 5219	NR_074239.1	99%
Haloquadratum	*Haloquadratum walsbyi* strain JCM 12705	NR_113463.1	99%
Halolamina	*Halolamina* sp. YKT1	KU051660.1	99%
Methanosarcinaceae	*Methanococcoides methylutens* MM1	CP009518.1	95%
Halobaculum	*Halobaculum magnesiiphilum* strain MGY-184	NR_113365.1	98%
Methanoplanus	*Methanoplanus limicola* strain DSM 2279	NR_044727.1	99%
DHVEG-1	*Euryarchaeote* J4.75-12	AF199378.1	95%
Methanomicrobiales	*Methanocalculus chunshungensis*	AY234332.1	99%
Halophilic MK62-1	*Haloparvum sedimenti*	LC163946.1	99%

Table S3. Closest GenBank phylogenetic relatives of 18S Eukaryotic OTUs that make up ≥10% of total sequence abundance in any samples.

Geengenes assignment (lowest taxanomic classification)	Closest phylogenetic relative (GenBank)	Accession number	Sequence similarity (%)
Chlamydomonas	*Chlamydomonas* sp. ex Foraminifera MH-2008	FM877716.1	96%
Chlamydomonas	*Chlamydomonas* sp. ex Foraminifera MH-2008	FM877716.1	96%
Singhamoeba sp. COHH 15	*Spumella* sp. GOT220	EF027354.1	100%
Singhamoeba sp. COHH 15	*Paratetramitus jugosus*	M98050.1	99%
Tetramitus sp. F5-4	*Naegleria* sp. ATCC 30294	AF011457.1	100%
Chrysophyceae	*Cercozoa* sp. Panama49	EU709240.1	91%
Pythium capillosum	*Pythium pectinolyticum* voucher CBS122643	HQ643739.2	99%
	Pythium flevoense strain CBS 234.72	AY598691.2	99%
	Pythium capillosum strain CBS 222.94	AY598635.2	99%
	Pythium aquatile strain CBS 215.80	AY598632.2	99%
Acanthamoeba sp. EI1	*Acanthamoeba triangularis*	AF316547.2	99%
	Acanthamoeba lugdunensis	AF005995.1	99%
	Acanthamoeba polyphaga JAC/S2 ATCC 50372	U07415.1	99%

Table S4. Summary statistics for thiobacilli OTUs, including raw *p*-values for Welsh's t-test, fold change when amended with PO_4^{3-} and standard deviation (SD) in the $+PO_4^{3-}$ and no PO_4^{3-} samples

OTU	*p* value raw	Fold change	SD $+PO_4^{3-}$	SD no-PO_4^{3-}
72646	0.90	-0.06	0.03	0.74
309624	0.76	0.65	2.28	0.98
166686	0.70	-0.29	0.03	1.14
850808	0.45	2.19	2.68	0.52
841971	0.50	0.17	0.03	0.36
331474	0.80	-0.18	0.52	0.96
794020	0.34	3.03	2.68	0.95
240331	0.32	0.51	0.46	0.36
274500	0.65	-0.67	1.50	1.20
668268	0.46	1.61	2.01	0.34
844272	0.24	0.74	0.46	0.15

Table S5. Diversity measurements at selected timepoints.

		Bacteria		**Archaea**		**Eukaryotes**	
Sample	Timepoint (days)	Shannon index (1000 sequences per sample)	Observed species (1000 sequences per sample)	Shannon index (1000 sequences per sample)	Observed species (1000 sequences per sample)	Shannon index (1000 sequences per sample)	Observed species (1000 sequences per sample)
Initial	0	4.3 ± 0.1	113 ± 8	-	-	4.4 ± 0.1	89 ± 3
No PO_4^{3-}	25	4.3 ± 0.0	87 ± 5	-	-	3.8 ± 0.1	97 ± 3
	51	3.7 ± 0.0	80 ± 4	-	-	4.4 ± 0.1	80 ± 5
$+PO_4^{3-}$	25	4.1 ± 0.1	64 ± 5	1.2 ± 0.1	12 ± 1	0.9 ± 0.1	30 ± 4
	51	4.1 ± 0.1	65 ± 4	1.4 ± 0.1	10 ± 1	2.3 ± 0.1	29 ± 3