

# Microbial diversity decline and community response are decoupled from increased respiration in warmed tropical forest soil

## Electronic Supplementary Material

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This document contains Supplementary methods, results, tables, and figures for the manuscript. Large tables are provided as additional **Supplementary Dataset** files. See [Appendix 1](#) for more details. The source code for this PDF—including all figures, tables, and data sets—can be found here: <https://github.com/sweltr/high-temp/tree/main/paper/ESM>. An HTML version of this file, including all Supplementary Datasets, can be found on the project website at <https://sweltr.github.io/high-temp/som.html>.

## Data & Code Availability

We provide additional data products and code through online repositories (**Supplementary Table 1**). For further details, please see the Data Availability page on the project website at <https://sweltr.github.io/high-temp/data-availability.html>.

**Supplementary Table 1** | Publicly available data and data products.

url	archive	content
<a href="https://doi.org/10.25573/data.c.5667571">https://doi.org/10.25573/data.c.5667571</a>	Figshare	collection of data and data products.
<a href="https://zenodo.org/badge/latestdoi/368915237">https://zenodo.org/badge/latestdoi/368915237</a>	Zenodo	reproducible workflows in R Markdown format.
<a href="https://doi.org/10.25573/data.14686665">https://doi.org/10.25573/data.14686665</a>	Figshare	Raw 16S rRNA data for each sample (before removing primers).
<a href="https://doi.org/10.25573/data.14686755">https://doi.org/10.25573/data.14686755</a>	Figshare	Raw ITS data for each sample (before removing primers).
<a href="https://www.ebi.ac.uk/ena/browser/view/PRJEB45074">https://www.ebi.ac.uk/ena/browser/view/PRJEB45074</a>	European Nucleotide Archive	study accession number PRJEB45074 (ERP129199) for all sequencing data (primers removed).
<a href="https://www.ebi.ac.uk/ena/browser/view/ERS6485270-ERS6485284">https://www.ebi.ac.uk/ena/browser/view/ERS6485270-ERS6485284</a>	European Nucleotide Archive	16S rRNA sample accession numbers (ERS6485270-ERS6485284, primers removed).
<a href="https://www.ebi.ac.uk/ena/browser/view/ERS6485285-ERS6485299">https://www.ebi.ac.uk/ena/browser/view/ERS6485285-ERS6485299</a>	European Nucleotide Archive	ITS sample accession numbers (ERS6485285-ERS6485299, primers removed).

## Supplementary Methods

### DNA extraction & sequencing

Samples were named by combining the plot number (P01–P10) with the treatment (C = control, W = warming), the temperature (0 = no warming, 3 = +3°C warming, and 8 = +8°C warming), and the plot pairing designation (A–E). For example, **P07\_W3D** is the sample from plot #7 that was warmed by +3°C. This sample is part of group **D** which contains P07\_W8D (warmed by +8°C) and P08\_C0D (the control sample for this group) (**Supplementary Table 2**).

**Supplementary Table 2 | Sample Details.**

Sample ID	Plot	Depth (cm)	Treatment	Pair
P01_W3A	P01	00_010	+3°C	A
P01_W8A	P01	00_010	+8°C	A
P02_C0A	P02	00_010	Control	A
P03_W3B	P03	00_010	+3°C	B
P03_W8B	P03	00_010	+8°C	B
P04_C0B	P04	00_010	Control	B
P05_W3C	P05	00_010	+3°C	C
P05_W8C	P05	00_010	+8°C	C
P06_C0C	P06	00_010	Control	C
P07_W3D	P07	00_010	+3°C	D
P07_W8D	P07	00_010	+8°C	D
P08_C0D	P08	00_010	Control	D
P09_W3E	P09	00_010	+3°C	E
P09_W8E	P09	00_010	+8°C	E
P10_C0E	P10	00_010	Control	E

DNA was extracted using the DNeasy Powersoil kit (Qiagen). Bacterial and fungal communities were amplified using a two-stage PCR protocol. Locus specific primers used for PCR1 included the Illumina sequencing primer sequence on their 5' ends. For bacteria, we amplified the V4 hypervariable region of the 16S rRNA gene with the 515F–806R<sup>1</sup> primer pair (**Supplementary Table 3**). For fungi, we amplified the first internal transcribed spacer (ITS1) region of the rRNA operon with the primers ITS1F<sup>2</sup> and ITS2<sup>3</sup> (**Supplementary Table 3**).

**Supplementary Table 3 |** Primer sequences for 16S rRNA & ITS gene amplification.

Data set	Primer name	Primer sequence
<b>16S rRNA</b>		
	515F	GTGCCAGCMGCCGCGTAA
	806R	GGACTACHVGGGTWTCTAAT
<b>ITS</b>		
	ITS1f	CTTGGTCATTAGAGGAAGTAA
	ITS2	GCTGCGTTCTCATCGATGC

We used Platinum 2X Mastermix (Thermo) in PCR reactions with a final volume of 12.5µl with 25 cycles using a 50°C annealing temperature for both loci. PCR2 used 2µl of PCR1 as template and added on remaining Illumina adaptors and index sequences. PCR2 products were cleaned and normalized using PCR Normalization plates (CharmBiotech, USA) and pooled libraries concentrated using AMPure beads (Beckman Coulter, USA). Libraries were sequenced on an Illumina MiSeq with 250bp paired end reads.

## Processing microbial community data

Reads in both data sets were trimmed of forward and reverse primers using cutadapt<sup>4</sup> (v1.18) following an initial filtering step that removed reads with ambiguous bases. Primer sequences with more than 12% error rate ( $-error-rate = 0.12$ ) were discarded. Reads were then processed using DADA2<sup>5</sup> (v1.16.0) within R<sup>6</sup> (v4.1.0). Reads were dropped from the data set if they had three or more expected errors ( $maxEE = 2$ ), at least one base with very low quality ( $truncQ = 2$ ), or at least one position with an unspecified nucleotide ( $maxN = 0$ ). Based on visual inspection of quality plots, only the forward reads from the 16S rRNA data were retained, while both forward and reverse reads were retained in the ITS data set. Remaining reads were dereplicated before inferring amplicon sequence variants (ASVs). We used ASVs over traditional OTUs because ASVs provide single nucleotide resolution, thus providing more detailed resolution when examining treatment effects. Paired-end reads (ITS only) were merged and read pairs that did not match exactly across at least 12 base pairs ( $minOverlap = 12$ ) were discarded. For the 16S rRNA data we retained amplicons between 230 and 235 base pairs and for the ITS data we retained amplicons between 100 and 450 base pairs. Reads were then screened for chimeras (method = consensus). Taxonomy for the 16S rRNA data set was assigned to each ASV using the naive Bayesian classifier<sup>7</sup> against the Silva reference database<sup>8</sup> (Silva\_nr\_v138\_train\_set version 138). For taxonomic classification of the ITS data set, we used the naive Bayesian classifier<sup>7</sup> against the UNITE<sup>9</sup> database, specifically the UNITE general FASTA release for Fungi (v. 04.02.2020)<sup>10</sup>. **Supplementary Dataset1** and **Supplementary Dataset2** contain the ASV table, taxonomic assignments, and unique sequences for the 16S rRNA and ITS data sets, respectively. The complete DADA2 workflow is available here: <https://sweltr.github.io/high-temp/dada2.html>. Prior to community analysis of the 16S rRNA data set, ASVs classified as chloroplasts, mitochondria, or Eukaryota, or ASVs that remained unclassified (i.e., NA) at the kingdom level, were removed from the data set using the phyloseq package<sup>11</sup> (v1.36.0) in R<sup>6</sup>. No curation was performed on the ITS data set since all ASVs could be classified to kingdom level (Fungi). The complete data set preparation workflow is available here: <https://sweltr.github.io/high-temp/data-prep.html>.

## Filtering

We applied three complementary methods of prevalence filtering to the 16S rRNA and ITS data sets. The complete filtering workflow is available here: <https://sweltr.github.io/high-temp/filtering.html>.

- i) Sample-wise filtering with arbitrary functions. We used the `genefilter_sample` function (phyloseq package<sup>11</sup>, v1.36.0) to remove ASVs represented by fewer than 5 reads and/or present in less than 20% of samples.
- ii) PERFect (PERmutation Filtering test for microbiome data)<sup>12</sup> (v0.2.4) filtering. Here we used the function `PERFect_sim` with the alpha parameter set to 0.05 for the 16S rRNA data and 0.1 for the ITS data.
- iii) PIME (Prevalence Interval for Microbiome Evaluation)<sup>13</sup> (v0.1.0) filtering. We rarefied all samples to even depths (per the developer's recommendation) then split the data sets by predictor variable (temperature treatment) using `pime.split.by.variable`. We then calculated the prevalence intervals with `pime.prevalence` and used `pime.best.prevalence` to calculate the best prevalence. The best prevalence interval was selected when the out-of-bag (OOB) error rate first reached zero or close to zero. The most prevalent ASVs (at the best interval) were retained from each split. Splits were merged to obtain the final PIME data set.

## Alpha diversity estimates

To account for presence of rare sequence variants caused by sequencing errors (or other technical artifacts), we used Hill numbers<sup>14</sup> for alpha diversity estimates. Hill numbers allow the weight put on rare versus abundant sequence variants to be scaled while providing intuitive comparisons of diversity levels using *effective number of ASVs* as a measuring unit. This approach allows for balancing the over representation of rare ASVs that might be inflated due to sequencing errors. We calculated three Hill numbers (using the R package `hilldiv`<sup>15</sup>) that weigh common ASVs differently: (i) Observed richness, where  $q\text{-value} = 0$ ; (ii) Shannon exponential ( $q\text{-value} = 1$ ), which weighs ASVs by their frequency; and (iii) Simpson multiplicative inverse ( $q\text{-value} = 2$ ), which over

weighs abundant ASVs. We report all three metrics of alpha diversity, while acknowledging that each metric is based on (measured) relative abundance and can be subject to bias due to variation in extraction efficiency and sequencing depth, especially when detecting rare taxa (or ASVs)<sup>16</sup>. However, we also recognize that each metric provides complementary information, by varying in their relative sensitivity towards rare and common species<sup>17</sup>. We therefore interpret alpha diversity metrics in terms of changes in diversity due to changes in rarer ASVs (observed richness) and due to changes in more proportionally abundant ASVs (Shannon and inverse Simpson). Next, we assessed whether the alpha diversity estimates were normally distributed using both the Shapiro-Wilk Normality test<sup>18</sup> and the Bartlett Test of Homogeneity of Variances<sup>19</sup>. If the p-values from both tests were not significant ( $p > 0.05$ ), we accepted the null hypothesis that the results were normally distributed. If one or both of the p-values were significant ( $p < 0.05$ ), we rejected the null hypothesis. For parametric data we tested for significance across treatments using ANOVA followed by Tukey post-hoc test. For non-parametric data we tested for significance across treatments using Kruskal-Wallis followed by Dunn test with Benjamini-Hochberg correction. All tests were performed using the vegan package<sup>20</sup> in R<sup>6</sup>. The complete alpha diversity workflow is available here: <https://sweltr.github.io/high-temp/alpha.html>.

## Beta diversity estimates

To test for significance between temperature treatments, we performed the following steps for the 16S rRNA and ITS data sets. First, we transformed the sample counts to relative abundance. We then generated distance matrices using the phyloseq<sup>11</sup> function `phyloseq::distance`. For the 16S rRNA data, we used unweighted and weighted UniFrac<sup>21</sup>. For the ITS data, we used Jensen-Shannon Divergence<sup>22</sup> and Bray-Curtis dissimilarity<sup>23</sup>. Next, we calculated beta dispersion using the `betadisper` function from the vegan package<sup>20</sup>. Then we used the function `permuteTest` to run a Permutation test for homogeneity of multivariate dispersions<sup>24</sup>. If beta dispersion tests were not significant, we ran a PERMANOVA<sup>25</sup> using `adonis` (PERMANOVA assumes equal dispersion), otherwise we used Analysis of Similarity<sup>26</sup> (`anosim`), both available in the vegan package<sup>20</sup>. Ordination plots were generated for each distance matrix using Principal Coordinate Analysis<sup>27</sup> (PCoA). The complete beta diversity workflow is available here: <https://sweltr.github.io/high-temp/beta.html>.

## Differentially abundant ASVs

Differentially abundant ASVs across temperature treatments (PIME filtered data sets) were identified using (i) the `1abds` package<sup>28</sup> (v.2.0-1)—to run Dufrene-Legendre Indicator Species Analysis (ISA)—and (ii) the microbiomeMarker package<sup>29</sup> (v.0.0.1.9000) to run linear discriminant analysis (LDA) effect size (LEfSe)<sup>30</sup>. ISA calculates the indicator value (fidelity and relative abundance) of ASVs in treatment groups. For the 16S rRNA and ITS data, we set the p-value cutoff to 0.5. For all other parameters the default values were used. For the LEfSe analysis we used pre-sample normalization of the sum of the values to  $1e^{+06}$  (norm = “CPM”), set the LDA score cutoff to 2 (`lda_cutoff` = 2), set the p-value cutoff of Wilcoxon test to 0.05 (`wilcoxon_cutoff` = 0.05), and the p-value cutoff of Kruskal-Wallis test to 0.05 (`kw_cutoff` = 0.05). The complete differentially abundant workflow is available here: <https://sweltr.github.io/high-temp/da.html>.

## Multivariate analysis

Here we compare the environmental metadata (**Supplementary Dataset3**) with both the PIME filtered 16S rRNA and the ITS community data. The complete multivariate workflow is available here: <https://sweltr.github.io/high-temp/metadata.html>. Each workflow contained the same major steps:

- 1) **Metadata normality tests:** We used Shapiro-Wilk Normality Test<sup>18</sup> to test whether each metadata parameter was normally distributed.

- 2) **Normalize parameters:** Use the R package `bestNormalize`<sup>31,32</sup> and default parameters to find and execute the best normalizing transformation for non-parametric metadata parameters identified in step #1. The function tested the following normalizing transformations: arcsinh, Box-Cox, Yeo-Johnson, Ordered Quantile (ORQ) normalization, log transformation, square-root, and exponential. Once the non-parametric parameters were transformed, we reran the normality tests.
- 3) **Partition metadata:** Next we split the metadata parameters into three groups: **a)** environmental and edaphic properties; **b)** microbial functional responses; and **c)** temperature adaptation properties.
  - a) **Environmental and edaphic properties:** AST, H<sub>2</sub>O, N, P, Al, Ca, Fe, K, Mg, Mn, Na, TEB, ECEC, pH, NH<sub>4</sub>, NO<sub>3</sub>, PO<sub>4</sub>, DOC, DON, DOCN.
  - b) **Microbial functional responses:** micC, micN, micP, micCN, micCP, micNP, AG<sub>ase</sub>, BG<sub>ase</sub>, BP<sub>ase</sub>, CE<sub>ase</sub>, P<sub>ase</sub>, N<sub>ase</sub>, S<sub>ase</sub>, XY<sub>ase</sub>, LP<sub>ase</sub>, PX<sub>ase</sub>, CO<sub>2</sub>, enzCN, enzCP, enzNP.
  - c) **Temperature adaptation:** AG<sub>Q10</sub>, BG<sub>Q10</sub>, BP<sub>Q10</sub>, CE<sub>Q10</sub>, P<sub>Q10</sub>, N<sub>Q10</sub>, S<sub>Q10</sub>, XY<sub>Q10</sub>, LP<sub>Q10</sub>, PX<sub>Q10</sub>, CUE<sub>cn</sub>, CUE<sub>cp</sub>, NUE, PUE, T<sub>min</sub>, SI.

The following parameters were collected but not used in the analysis: minPO<sub>4</sub>, minNH<sub>4</sub>, minNO<sub>3</sub>, minTIN.

- 4) **Autocorrelation tests:** Then we tested all possible pair-wise comparisons of the normalized metadata (from step #2) for each group (step #3) to identify potential autocorrelated parameters.
- 5) **Remove autocorrelated parameters.** Based on the results of step #4.
- 6) **Dissimilarity correlation tests:** We used Mantel Tests to determine if any metadata groups were significantly correlated with community data. We generated Bray-Curtis<sup>23</sup> distance matrices for the community data and Euclidean distance matrices for each metadata group. We performed Mantel tests<sup>33,34</sup> for all comparisons using the function `mantel` from the `vegan`<sup>20</sup> package.
- 7) **Best subset of variables:** We determined which metadata parameters (from each group) were the most strongly correlated with the community data using the `bioenv` function from the `vegan`<sup>20</sup> package where method = “spearman”, index = “bray”, and metric = “euclidean”.
- 8) **Distance-based Redundancy Analysis (dbRDA):** We performed ordination analysis of samples and metadata vectors for each of the three metadata subsets using the following recipe:
  - i) Run `rankindex`<sup>35</sup> in the `vegan` package to compare metadata and community dissimilarity indices for gradient detection. This aids in the selection of the best dissimilarity metric to use. Here we tested the following metrics: Euclidean, Manhattan, Gower, Bray–Curtis, and Kulczynski.
  - ii) Run `capscale` in the `vegan`<sup>20</sup> package for distance-based redundancy analysis.
  - iii) Run `envfit` to fit environmental parameters onto the ordination. This function basically calculates correlation scores between the metadata parameters and the ordination axes.
  - iv) Select metadata parameters significant for `bioenv` (see above) and/or `envfit` analyses.
  - v) Plot the ordination and vector overlays.

## Supplementary Results

### Tracking reads through DADA2 workflow

#### 16S rRNA data

The processed and curated 16S rRNA data set contained 937,761 high-quality reads, with a range of 25,151–86,600 reads per sample (mean 62,443). Modelling and error correcting amplicon errors inferred 20,332 ASVs, 19% of which were doubletons. After removing reads classified as mitochondria, chloroplast, or Eukaryota, the data set contained 20,173 ASVs and 936,640 reads—with a range of 25,088–86,600 reads (mean 62,443) and 813–3065 (mean 2063) ASVs per sample (**Supplementary Table 4**).

**Supplementary Table 4** | Tracking read changes through DADA2 workflow (16S rRNA).

Sample	raw <sup>1</sup>	pre filt <sup>2</sup>	cut <sup>3</sup>	filter <sup>4</sup>	denoiseF <sup>5</sup>	nonchim <sup>6</sup>	final <sup>7</sup>	asvs <sup>8</sup>
P01_W3A	85489	85482	85402	79804	70434	68037	67902	2371
P01_W8A	77645	77642	77470	72760	65950	60535	60509	1610
P02_C0A	87091	87085	86878	81240	71961	70436	70412	2272
P03_W3B	93065	93053	92851	86732	78067	75183	75123	2356
P03_W8B	66401	66367	65498	60860	54822	53437	53347	1575
P04_C0B	67810	67800	67617	63037	54678	53813	53770	2040
P05_W3C	58266	58259	58006	54127	47868	47070	47023	1663
P05_W8C	120892	120842	117015	109421	100026	86841	86600	2124
P06_C0C	101599	101592	101363	94740	83190	81219	81150	3065
P07_W3D	76781	76776	76583	71678	64031	61742	61632	2010
P07_W8D	79032	79022	78724	73639	65125	62202	62117	2157
P08_C0D	77599	77591	77133	72263	63248	62171	62136	2441
P09_W3E	95566	95564	95310	89315	79799	76299	76231	2386
P09_W8E	95182	94457	44250	33000	31620	25151	25088	813
P10_C0E	67583	67579	67432	62598	54075	53625	53600	2066

**Column descriptions.**

<sup>1</sup> raw: number of initial reads; <sup>2</sup> pre filt: after removing reads with ambiguous bases;

<sup>3</sup> cut: after removing primers; <sup>4</sup> filter: after filtering;

<sup>5</sup> denoiseF: forward reads after denoising; <sup>6</sup> nonchim: after removing chimeras;

<sup>7</sup> final: final read totals; <sup>8</sup> asvs: total ASVs.

## ITS data

The processed and curated ITS data set contained 491,143 high-quality reads, with a range of 80–64,636 reads per sample (mean 32,743). Modelling and error correcting amplicon errors inferred 3357 ASVs, 2.1% of which were doubleton ASVs. After removing 2 samples with low read counts (< 500 reads), the data set contained 3357 ASVs and 490,767 reads—with a range of 9172–64,636 reads (mean 37,751) and 335–1017 (mean 757) ASVs per sample (Supplementary Table 5).

**Supplementary Table 5** | Tracking read changes through DADA2 workflow (ITS).

Sample	raw <sup>1</sup>	pre filt <sup>2</sup>	cut <sup>3</sup>	filter <sup>4</sup>	denoiseF <sup>5</sup>	denoiseR <sup>6</sup>	merge <sup>7</sup>	nonchim <sup>8</sup>	final <sup>9</sup>	asvs <sup>10</sup>
P01_W3A	50894	50882	50419	35999	32635	33389	29795	29794	29794	945
P01_W8A	70734	70649	68075	48230	47150	47059	41880	41880	41880	719
P02_C0A	74581	74557	73994	58956	58018	58130	51144	51144	51144	1011
P03_W3B	60006	59953	58146	42563	42253	42151	38442	38374	38374	765
P03_W8B	31174	31029	25782	17548	17132	17210	14188	14188	14188	349
P04_C0B	88732	88694	87532	68259	64797	67613	56594	56593	56593	1017
P05_W3C*	809	797	412	308	302	302	296	296	296	
P05_W8C	81908	81671	74669	48518	41357	46321	35713	35600	35600	616
P06_C0C	67419	67399	66656	51055	50634	50362	45851	45850	45850	954
P07_W3D	70123	70073	68474	43471	42194	42074	36121	36063	36063	867
P07_W8D	15435	15417	14955	9488	9426	9427	9172	9172	9172	335
P08_C0D	60799	60604	54534	41563	41243	41077	38392	38371	38371	702
P09_W3E	55927	55764	51382	35722	35410	35168	29155	29102	29102	745
P09_W8E*	1418	1383	141	85	84	82	80	80	80	
P10_C0E	96531	96492	95256	80877	68681	70013	64805	64636	64636	812

**Column descriptions.**

<sup>1</sup> raw: number of initial reads; <sup>2</sup> pre filt: after removing reads with ambiguous bases;

<sup>3</sup> cut: after removing primers; <sup>4</sup> filter: after filtering; <sup>5</sup> denoiseF: forward reads after denoising; <sup>6</sup> denoiseR: reverse reads after denoising; <sup>7</sup> merge: after merging paired-end reads; <sup>8</sup> nonchim: after removing chimeras; <sup>9</sup> final: final read totals;

<sup>10</sup> asvs: total ASVs. \* These samples were removed from final analysis.

## Filtering

### 16S rRNA data

The curated 16S rRNA data set contained 936,640 reads and 20,173 ASVs. Applying arbitrary filtering—removing ASVs represented by fewer than 5 reads and/or present in less than 20% of samples—reduced the number of reads by 22% and the number of ASVs by 90%. Similarly, PERFect filtering reduced the number of reads by 22% and the number of ASVs by 92% (**Supplementary Table 6**). Rarefying the FULL data set to even read depths (25,088 reads/sample) prior to PIME filtering removed 2741 ASVs, reducing the number of reads by 60% and the number of ASVs by 14%. Baseline noise detection indicated the out-of-bag (OOB) error rate was 0.67. Splitting the rarefied data by predictor variable (temperature treatment) resulted in a total of 7883 ASVs for the Control group, 7173 ASVs for the +3°C group, and 6027 ASVs for the +8°C group. The Best Prevalence interval, calculated using the function `pime.best.prevalence`, was 50%, the first interval where the OOB error rate dropped to 0%. At this interval (compared to the FULL data set), the number of reads was reduced by 75% and the number of ASVs by 95% (**Supplementary Table 6**).

**Supplementary Table 6** | Results of different filtering approaches (16S rRNA).

Description	subset by treatment	no. samples	total reads	total asvs
FULL data set		15	936640	20173
	Control	5	321068	9026
	+3°C	5	327911	8154
	+8°C	5	287661	6735
Arbitrary filter		15	729973	1822
	Control	5	247405	1592
	+3°C	5	266549	1682
	+8°C	5	216019	1399
PERFect filter		15	731508	1659
	Control	5	243480	1254
	+3°C	5	262133	1339
	+8°C	5	225895	1336
PIME filter		15	232532	1058
	Control	5	80595	752
	+3°C	5	84976	692
	+8°C	5	66961	355

### ITS data

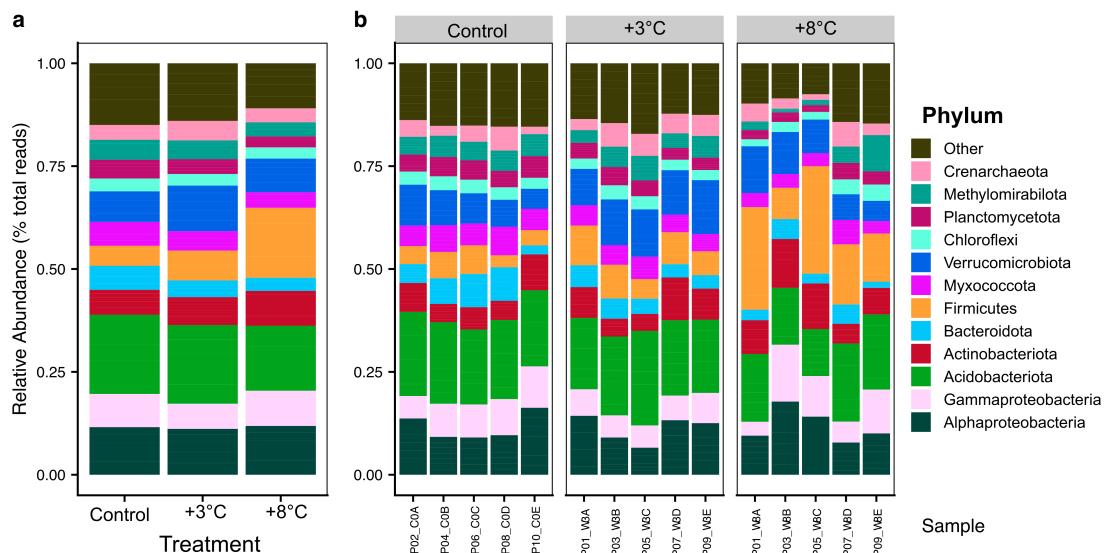
The curated ITS data set contained 490,767 reads and 3355 ASVs. Applying arbitrary filtering—removing ASVs represented by fewer than 5 reads and/or present in less than 20% of samples—reduced the number of reads by 21% and the number of ASVs by 76%. PERFect filtering reduced the number of reads by 26% and the number of ASVs by 91% (**Supplementary Table 7**). Rarefying the FULL data set to even read depths (9172 reads/sample) prior to PIME filtering removed 298 ASVs, reducing the number of reads by 76% and the number of ASVs by 9%. Baseline noise detection indicated the out-of-bag (OOB) error rate was 0.39. Splitting the rarefied data by predictor variable (i.e., temperature treatment) yielded a total of 1932 ASVs for the Control group, 1682 ASVs for the +3°C group, and 1306 ASVs for the +8°C group. The Best Prevalence interval, calculated using the function `pime.best.prevalence`, was 55%, the first interval where the OOB error rate dropped to 0%. At this interval (compared to the FULL data set), the number of reads was reduced by 86% and the number of ASVs by 87% (**Supplementary Table 7**).

**Supplementary Table 7 |** Results of different filtering approaches (ITS).

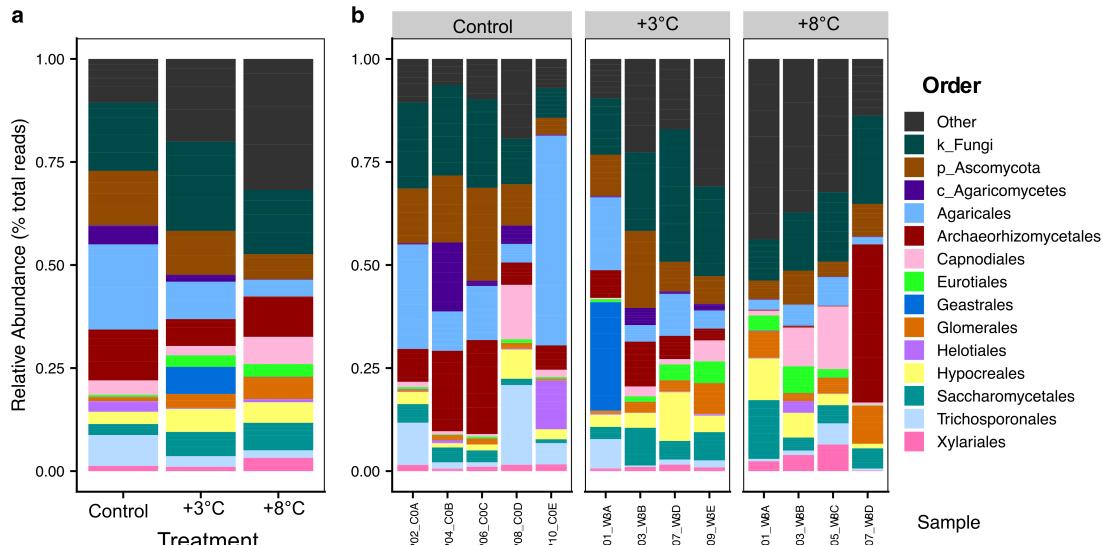
Description	subset by treatment	no. samples	total reads	total asvs
FULL data set		13	490767	3355
	Control	5	256594	2397
	+3°C	4	133333	1998
	+8°C	4	100840	1376
Arbitrary filter		13	385643	816
	Control	5	208940	769
	+3°C	4	108219	780
	+8°C	4	68484	556
PERFect filter		13	361399	306
	Control	5	208458	264
	+3°C	4	92836	257
	+8°C	4	60105	167
PIME filter		13	67665	474
	Control	5	30009	315
	+3°C	4	20827	234
	+8°C	4	16829	138

## Taxonomic diversity of microbial communities

Bacterial diversity was largely comprised of Proteobacteria (Alpha, Gamma), Acidobacteriota, Actinobacteriota, Bacteroidota, Firmicutes, Myxococcota, Verrucomicrobiota, Chloroflexi, and Planctomycetota. Methylophilobactera and Crenarchaeota were the dominant phyla of Archaeal diversity (**Supplementary Figure 1**). For a breakdown of dominant bacterial phyla by family, see [Appendix 2](#). Fungal diversity was largely comprised of Ascomycota, Basidiomycota, and Glomeromycota (**Supplementary Figure 2**). The complete taxonomic workflow is available here: <https://sweltr.github.io/high-temp/taxa.html>.



**Supplementary Figure 1 |** Top bacterial/archaeal phyla (unfiltered data). (a) Collapsed by temperature treatment. (b) Samples faceted by temperature treatment.



**Supplementary Figure 2** | Top fungal orders (unfiltered data). (a) Collapsed by temperature treatment. (b) Samples faceted by temperature treatment.

### Alpha diversity of microbial communities

Shapiro-Wilk Normality and Bartlett tests indicated all data was normally distributed except for Shannon exponential estimates of the 16S rRNA PIME filtered data. Differences in alpha diversity assessed using analysis of variance (ANOVA) followed by Tukey HSD post hoc tests (normally distributed data) or Kruskal-Wallis followed by Dunn test with Benjamini-Hochberg correction (non-normally distributed data).

See subsequent pages for results from the 16S rRNA data set (**Supplementary Table 8**, **Supplementary Table 9**, and **Supplementary Figure 3**) and the ITS data set (**Supplementary Table 10**, **Supplementary Table 11**, and **Supplementary Figure 4**)

## 16S rRNA data

**Supplementary Table 8** contains the results of alpha diversity estimates for different filtering methods. In **Supplementary Table 9** we report the results of the Shapiro-Wilk Normality and Bartlett tests (and results of post-hoc analysis) for each Hill number. **Supplementary Figure 3** contains alpha diversity plots comparing the different filtering methods for Hill numbers.

**Supplementary Table 8 |** Hill numbers for 16S rRNA samples.

Sample	Observed richness				Shannon exponential				Simpson multiplicative inverse			
	FULL	FILT	PERFect	PIME	FULL	FILT	PERFect	PIME	FULL	FILT	PERFect	PIME
P01_W3A	2371	831	707	502	760.7	385.1	353.4	226.3	200.3	128.0	123.6	84.2
P01_W8A	1610	620	563	322	236.1	127.8	126.5	59.2	25.5	18.9	19.1	12.0
P02_C0A	2272	884	737	590	781.1	437.1	397.4	291.2	261.5	178.5	171.6	129.3
P03_W3B	2356	922	764	596	686.8	373.7	337.3	235.0	159.4	108.7	103.8	75.8
P03_W8B	1575	422	411	224	501.0	181.0	211.9	90.1	153.3	69.2	82.6	38.6
P04_C0B	2040	802	663	570	797.8	439.4	388.4	309.2	272.0	178.8	166.8	130.7
P05_W3C	1663	680	574	499	578.8	329.4	299.2	246.1	201.2	139.5	132.6	111.0
P05_W8C	2124	572	661	289	307.3	102.4	146.9	47.9	27.3	14.9	18.5	9.6
P06_C0C	3065	1011	857	664	1098.4	518.7	481.3	324.7	309.4	182.0	179.7	120.6
P07_W3D	2010	843	725	516	598.8	353.4	327.5	208.0	146.8	103.7	99.7	67.5
P07_W8D	2157	771	671	276	560.8	284.2	270.3	88.1	91.5	60.2	60.2	24.4
P08_C0D	2441	813	681	594	973.3	464.8	419.8	331.3	394.2	230.0	220.5	168.7
P09_W3E	2386	874	751	563	680.1	352.7	334.5	224.4	168.5	110.3	109.4	76.5
P09_W8E	813	376	332	170	375.3	165.6	149.9	67.9	120.2	55.7	56.8	26.4
P10_C0E	2066	670	571	491	886.8	416.5	379.4	298.3	427.6	242.8	231.4	174.7

FULL = unfiltered data set; FILT = arbitrary filtering where nreads > 5 and prevalence > 20%; PERFect = PERFect filtering; PIME = PIME filtering

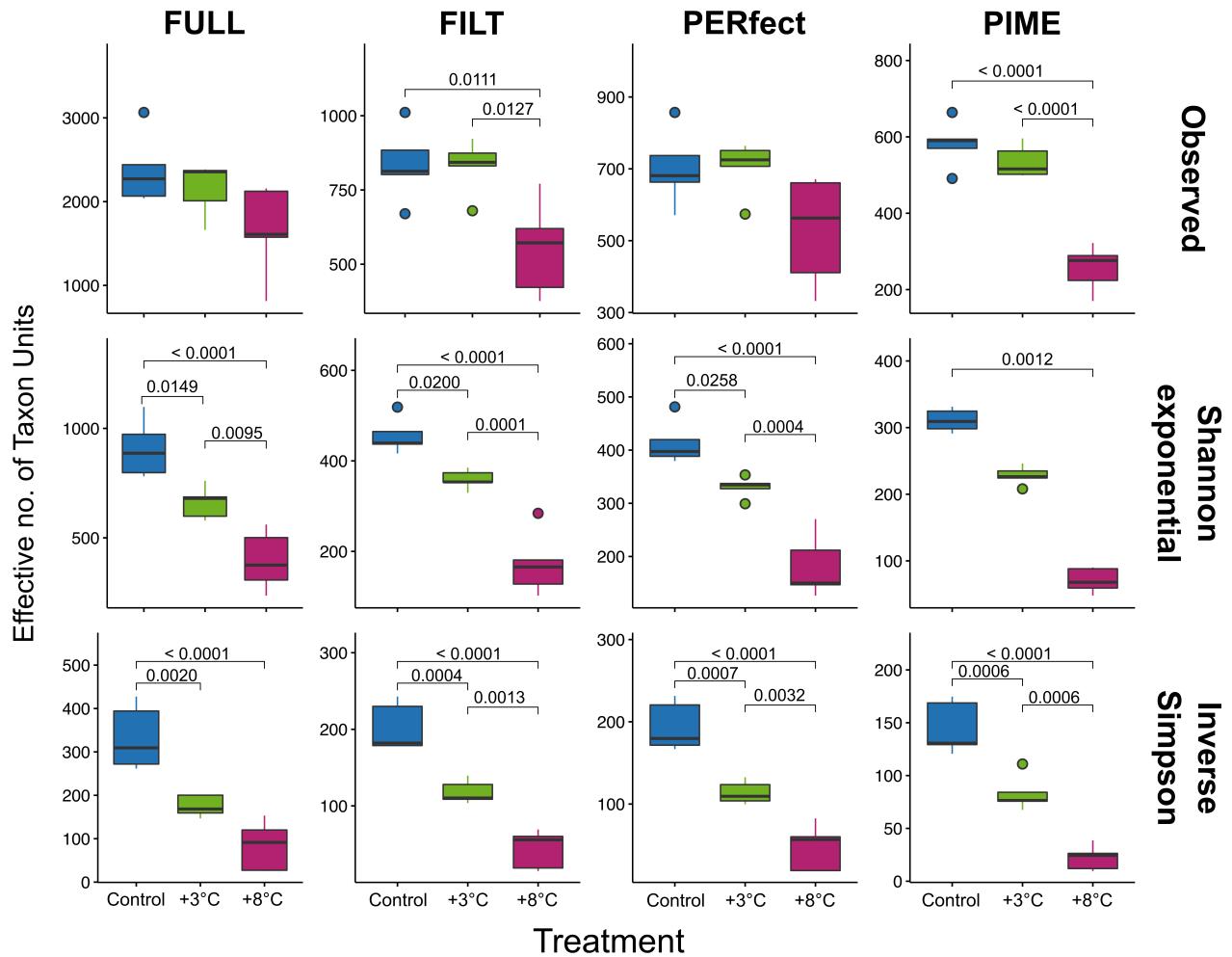
**Supplementary Table 9 |** Summary of 16S rRNA significant tests. Posthoc p-values adjusted for multiple comparisons.

metric <sup>1</sup>	data set <sup>2</sup>	pval_shap <sup>3</sup>	pval_bart <sup>4</sup>	method <sup>5</sup>	posthoc method <sup>6</sup>	posthoc pval <sup>7</sup>
Observed	FULL	0.268	0.599	ANOVA	Tukey post-hoc test	6.05e-02
Observed	FILT	0.367	0.585	ANOVA	Tukey post-hoc test	6.08e-03
Observed	PERFect	0.191	0.437	ANOVA	Tukey post-hoc test	5.05e-02
Observed	PIME	0.055	0.755	ANOVA	Tukey post-hoc test	1.40e-06
Shannon exponential	FULL	0.994	0.490	ANOVA	Tukey post-hoc test	6.27e-05
Shannon exponential	FILT	0.230	0.107	ANOVA	Tukey post-hoc test	2.60e-06
Shannon exponential	PERFect	0.331	0.159	ANOVA	Tukey post-hoc test	7.10e-06
Shannon exponential	PIME	0.037	0.880	Kruskal-Wallis	Dunn test	1.93e-03
Inverse Simpson	FULL	0.584	0.155	ANOVA	Tukey post-hoc test	4.67e-05
Inverse Simpson	FILT	0.673	0.413	ANOVA	Tukey post-hoc test	1.30e-06
Inverse Simpson	PERFect	0.747	0.348	ANOVA	Tukey post-hoc test	3.20e-06
Inverse Simpson	PIME	0.370	0.371	ANOVA	Tukey post-hoc test	1.00e-06

**Column descriptions.** <sup>1</sup> metric: Hill number; <sup>2</sup> data set: FULL = unfiltered data set; FILT = arbitrary filtering where nreads > 5 and prevalence > 20%; PERFect = PERFect filtering; PIME = PIME filtering

<sup>3</sup> pval\_shap: p-value of Shapiro-Wilk Normality test; <sup>4</sup> pval\_bart: p-value of Bartlett Test of Homogeneity of Variances;

<sup>5</sup> method: Selected significance test; <sup>6</sup> posthoc method: Selected posthoc test; <sup>7</sup> posthoc pval: Posthoc p-value;



**Supplementary Figure 3** | Alpha diversity estimates of 16S rRNA communities. The centre line of each box plot represents the median, the lower and upper hinges represent the first and third quartiles and whiskers represent  $\pm 1.5$  the interquartile range. Shapiro-Wilk Normality Test and Bartlett Test of Homogeneity of Variances normality tests indicated all data was normally distributed except for Shannon exponential estimates of the PIME filtered data. Differences in alpha diversity assessed using analysis of variance (ANOVA) followed by Tukey HSD post hoc tests (normally distributed data) or Kruskal-Wallis followed by Dunn test with Benjamini-Hochberg correction (non-normally distributed data). Only significant differences (p-values adjusted for multiple comparisons) between treatments and controls are shown in plots, where  $n = 5$  for each treatment.

## ITS data

**Supplementary Table 10** contains the results of alpha diversity estimates for different filtering methods. In **Supplementary Table 11** we report the results of the Shapiro-Wilk Normality and Bartlett tests (and results of post-hoc analysis) for each Hill number. **Supplementary Figure 4** contains alpha diversity plots comparing the different filtering methods for Hill numbers.

**Supplementary Table 10 |** Hill numbers for ITS samples.

Sample	Observed richness				Shannon exponential				Simpson multiplicative inverse			
	FULL	FILT	PERFect	PIME	FULL	FILT	PERFect	PIME	FULL	FILT	PERFect	PIME
P01_W3A	945	499	148	181	55.9	33.8	18.5	14.8	10.7	8.4	6.8	5.6
P01_W8A	719	344	98	123	141.5	79.0	31.8	31.6	42.4	28.1	17.3	12.5
P02_C0A	1011	494	147	238	112.5	64.0	38.1	43.3	36.1	26.2	21.9	19.9
P03_W3B	765	423	136	196	132.9	83.0	45.7	56.5	44.1	29.1	24.4	26.9
P03_W8B	349	204	64	109	112.1	72.2	26.2	42.8	49.0	36.4	17.8	23.7
P04_C0B	1017	471	161	229	60.9	43.3	25.2	39.5	17.6	15.5	12.5	20.6
P05_W8C	616	296	90	131	157.5	76.6	34.2	35.1	41.8	20.7	15.3	11.5
P06_C0C	954	486	157	246	99.5	58.4	34.2	35.2	23.9	16.5	14.8	13.1
P07_W3D	867	492	147	205	160.4	102.9	46.3	48.0	56.2	42.8	27.2	22.5
P07_W8D	335	223	76	97	35.0	28.0	16.9	18.1	13.7	12.3	9.1	9.9
P08_C0D	702	345	105	198	87.2	46.7	25.7	30.7	25.5	17.9	14.2	14.2
P09_W3E	745	390	118	196	232.7	133.6	51.9	66.6	96.0	61.7	33.9	34.9
P10_C0E	812	404	143	203	24.6	14.7	11.1	8.1	4.9	4.0	3.8	2.5

FULL = unfiltered data set; FILT = arbitrary filtering where nreads > 5 and prevalence > 20%; PERFect = PERFect filtering; PIME = PIME filtering

**Supplementary Table 11 |** Summary of ITS significant tests. Posthoc p-values adjusted for multiple comparisons.

metric <sup>1</sup>	data set <sup>2</sup>	pval shap <sup>3</sup>	pval bart <sup>4</sup>	method <sup>5</sup>	posthoc method <sup>6</sup>	posthoc pval <sup>7</sup>
Observed	FULL	0.128	0.516	ANOVA	Tukey post-hoc test	6.14e-03
Observed	FILT	0.115	0.938	ANOVA	Tukey post-hoc test	2.36e-03
Observed	PERFect	0.162	0.667	ANOVA	Tukey post-hoc test	1.09e-03
Observed	PIME	0.134	0.454	ANOVA	Tukey post-hoc test	7.40e-06
Shannon exponential	FULL	0.846	0.445	ANOVA	Tukey post-hoc test	2.21e-01
Shannon exponential	FILT	0.934	0.363	ANOVA	Tukey post-hoc test	1.39e-01
Shannon exponential	PERFect	0.919	0.555	ANOVA	Tukey post-hoc test	1.87e-01
Shannon exponential	PIME	0.972	0.436	ANOVA	Tukey post-hoc test	3.46e-01
Inverse Simpson	FULL	0.184	0.126	ANOVA	Tukey post-hoc test	1.82e-01
Inverse Simpson	FILT	0.349	0.159	ANOVA	Tukey post-hoc test	1.83e-01
Inverse Simpson	PERFect	0.961	0.236	ANOVA	Tukey post-hoc test	2.05e-01
Inverse Simpson	PIME	0.955	0.477	ANOVA	Tukey post-hoc test	3.42e-01

**Column descriptions.**

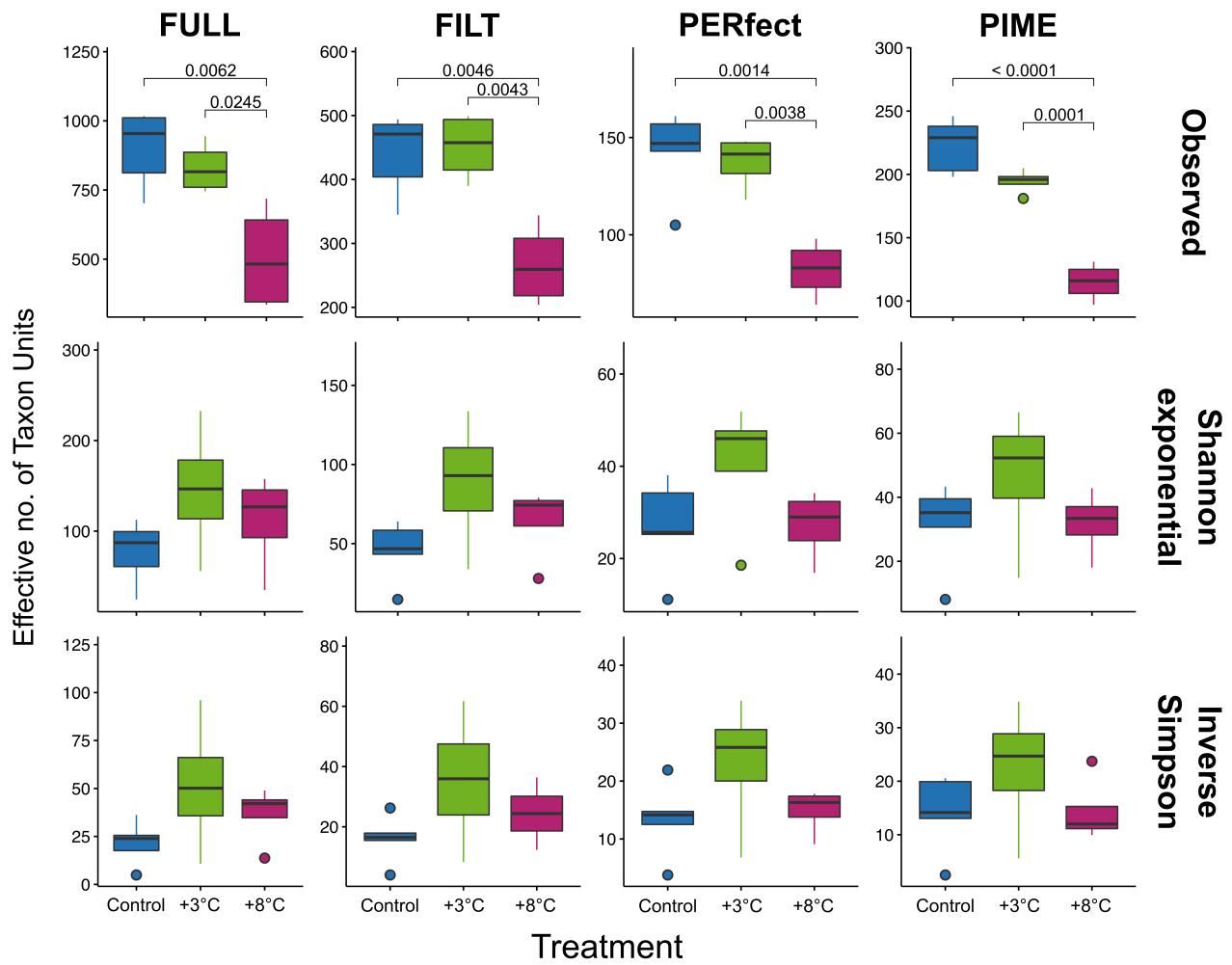
<sup>1</sup> metric: Hill number;

<sup>2</sup> data set: FULL = unfiltered data set; FILT = arbitrary filtering where nreads > 5 and prevalence > 20%; PERFect = PERFect filtering; PIME = PIME filtering

<sup>3</sup> pval\_shap: p-value of Shapiro-Wilk Normality test;

<sup>4</sup> pval\_bart: p-value of Bartlett Test of Homogeneity of Variances; <sup>5</sup> method: Selected significance test;

<sup>6</sup> posthoc method: Selected posthoc test; <sup>7</sup> posthoc pval: Posthoc p-value;



## Beta diversity of microbial communities

To test for significance between treatment groups, we calculated the beta dispersion (using the `betadisper` function, `vegan` package) for each 16S rRNA distance matrix (unweighted and weighted UniFrac) and each ITS distance matrix (Jensen-Shannon and Bray-Curtis). We then used the function `permuteTest` to run a Permutation Test for Homogeneity of multivariate dispersions against the results of each beta dispersion test (**Supplementary Table 12**, **Supplementary Table 13**). If the results were not significant (i.e., p-value > 0.05) we ran a PERMANOVA using `adonis` (PERMANOVA assumes equal dispersion), otherwise we used Analysis of Similarity (ANOSIM). **Supplementary Table 14** contains the results of the significance tests.

**Supplementary Table 12** | Results of beta dispersion & permutation test for homogeneity of multivariate dispersions (16S rRNA).

Description	distance metric	p-value	selected test
FULL data set	unweighted UniFrac	0.013	ANOSIM
	weighted UniFrac	0.004	ANOSIM
Arbitrary filter	unweighted UniFrac	0.002	ANOSIM
	weighted UniFrac	0.004	ANOSIM
PERFect filter	unweighted UniFrac	0.001	ANOSIM
	weighted UniFrac	0.003	ANOSIM
PIME filter	unweighted UniFrac	0.474	ADONIS
	weighted UniFrac	0.012	ANOSIM

**Supplementary Table 13** | Results of beta dispersion & permutation test for homogeneity of multivariate dispersions (ITS).

Description	distance metric	p-value	selected test
FULL data set	Jensen-Shannon	0.745	ADONIS
	Bray-Curtis	0.696	ADONIS
Arbitrary filter	Jensen-Shannon	0.920	ADONIS
	Bray-Curtis	0.864	ADONIS
PERFect filter	Jensen-Shannon	0.865	ADONIS
	Bray-Curtis	0.819	ADONIS
PIME filter	Jensen-Shannon	0.704	ADONIS
	Bray-Curtis	0.756	ADONIS

**Supplementary Table 14** | Summary of beta diversity significant tests. Where beta dispersion tests were not significant, we used Permutational multivariate analysis of variance (PERMANOVA) to calculate dissimilarity among treatment groups. Where beta dispersion tests were significant, we used Analysis of Similarity (ANOSIM).

Data set	Distance metric	(FULL)	(FILT)	(PERFect)	(PIME)
<b>16S rRNA</b>					
	unweighted UniFrac	0.003	0.003	0.003	0.001
	weighted UniFrac	0.001	0.001	0.001	0.001
<b>ITS</b>					
	Jensen-Shannon divergence	0.036	0.030	0.063	0.002
	Bray-Curtis dissimilarity	0.047	0.028	0.079	0.003

## Differentially abundant ASVs

Indicator Species Analysis (ISA) of the 16S rRNA data set identified 251 differentially abundant (DA) ASVs. Of those, 154 ASVs were enriched in the Control samples, 82 in the +3°C treatment, and 15 in the +8°C treatment (**Supplementary Dataset4**). Linear discriminant analysis (LDA) effect size (LEfSe) identified 676 DA ASVs with an LDA score > 2.0 and a p-value < 0.05. Of those, 355 ASVs were enriched in the Control samples, 227 in the +3°C treatment, and 94 in the +8°C treatment (**Supplementary Dataset5**).

ISA of the ITS data set identified 203 DA ASVs. Of those, 54 ASVs were enriched in the Control samples, 95 in the +3°C treatment, and 54 in the +8°C treatment (**Supplementary Dataset6**). LEfSe identified 228 DA ASVs with an LDA score > 2.0 and a p-value < 0.05. Of those, 52 ASVs were enriched in the Control samples, 107 in the +3°C treatment, and 69 in the +8°C treatment (**Supplementary Dataset7**).

## Multivariate analysis

### Normality tests & parameter normalization

We used Shapiro-Wilk Normality Test<sup>18</sup> to determine which of the 61 metadata parameters were or were not normally distributed. For the 16S rRNA data we needed to transform 25 metadata parameters (p-value < 0.05) and for the ITS data, 21 metadata parameters needed transformation (p-value < 0.05). Please see the project website for the specific parameters that were transformed and the method of transformation used in each case (<https://sweltr.github.io/high-temp/metadata.html>). For both community data sets, `bestNormalize` was unable to find a suitable transformation for `A1` and `Fe`. This is likely because there was very little variation in these parameters and/or there were too few significant digits.

### Removing autocorrelated parameters

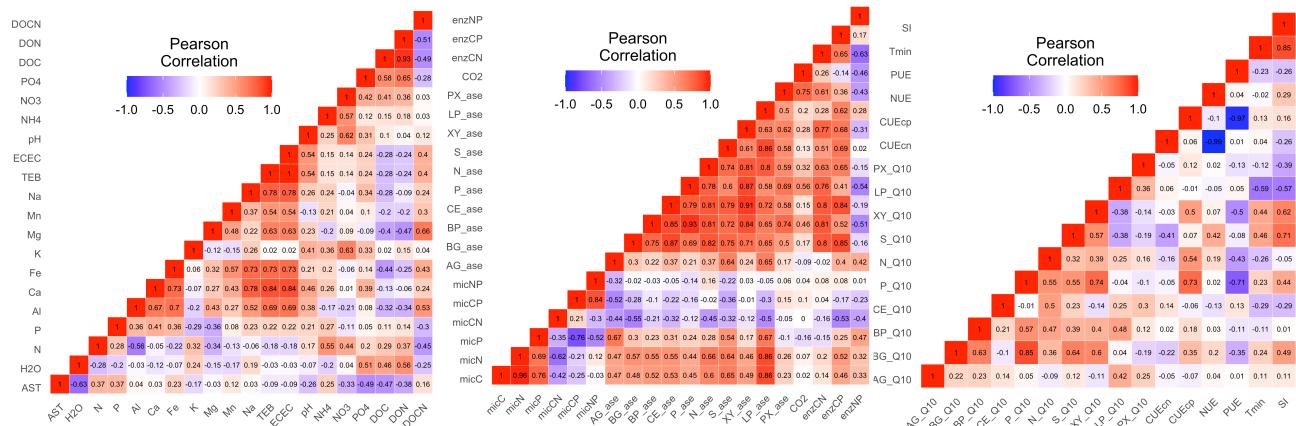
Based on autocorrelation tests between the metadata and community data (**Supplementary Figure 5, Supplementary Figure 6**), we removed the following parameters:

Environmental and edaphic properties: TEB, DON, Na, Al, Ca.

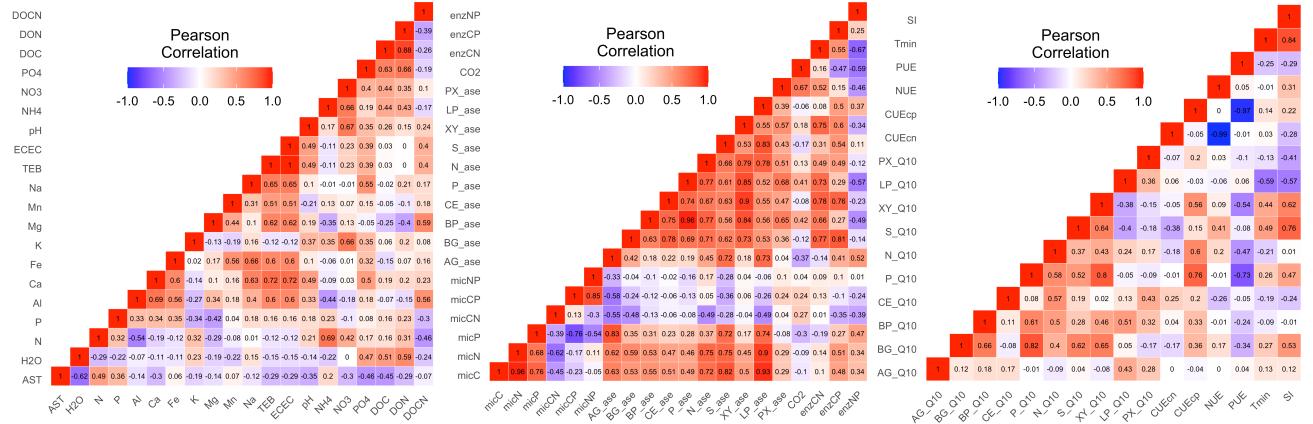
Microbial functional responses: micN, micNP, enzCN, enzCP, BP<sub>ase</sub>, CE<sub>ase</sub>, LP<sub>ase</sub>, N<sub>ase</sub>, P<sub>ase</sub>.

Temperature adaptation: NUE, PUE, SI.

We removed P<sub>Q10</sub> (temperature adaptation) from the ITS analysis based on the autocorrelation tests.



**Supplementary Figure 5 | Autocorrelation plots (16S rRNA) against: (left) environmental & edaphic properties; (middle) microbial functional responses; & (right) temperature adaptation.**



**Supplementary Figure 6 |** Autocorrelation plots (ITS) against: (left) environmental & edaphic properties; (middle) microbial functional responses; & (right) temperature adaptation.

### Dissimilarity correlation tests

We used Mantel Tests to determine if any metadata groups were significantly correlated with 16S rRNA or ITS community data (**Supplementary Table 15**).

**Supplementary Table 15 |** Mantel tests for 16S rRNA & ITS data compared to each of the three metadata groups. Significant differences denoted by p-values  $< 0.05$ .

Data set	edaphic properties	soil functional response	temperature adaptation
16S rRNA	0.003	0.180	0.001
ITS	0.002	0.288	0.001

### Best subset of variables

The `bioenv` function found the following metadata parameters (normalized with autocorrelated data removed) significantly correlated with community data (results of Mantel tests shown in parentheses).

#### Environmental and edaphic properties

**16S rRNA:** AST ( $r = 1.0, p = 0.001$ ).

**ITS:** AST ( $r = 1.0, p = 0.001$ ).

#### Microbial functional responses

**16S rRNA:** AG<sub>ase</sub> ( $r = 0.559, p = 0.001$ ), enzNP ( $r = 0.462, p = 0.006$ ), S<sub>ase</sub> ( $r = 0.614, p = 0.001$ ), PX<sub>ase</sub> ( $r = 0.612, p = 0.001$ ), XY<sub>ase</sub> ( $r = 0.456, p = 0.002$ ).

**ITS:** enzNP ( $r = 0.553, p = 0.001$ ), PX<sub>ase</sub> ( $r = 0.685, p = 0.001$ ), XY<sub>ase</sub> ( $r = 0.505, p = 0.002$ ).

#### Temperature adaptation

**16S rRNA:** CUE<sub>cp</sub> ( $r = 0.325, p = 0.013$ ), LP<sub>Q10</sub> ( $r = 0.377, p = 0.005$ ), P<sub>Q10</sub> ( $r = 0.518, p = 0.001$ ), S<sub>Q10</sub> ( $r = 0.440, p = 0.001$ ), and T<sub>min</sub> ( $r = 0.404, p = 0.005$ ).

**ITS:** XY<sub>Q10</sub> ( $r = 0.726, p = 0.001$ ), T<sub>min</sub> ( $r = 0.616, p = 0.001$ ).

## **Distance-based Redundancy Analysis (dbRDA)**

In all cases (i.e., both community data sets against each of the three metadata subsets), `rankindex`<sup>35</sup> indicated that Bray-Curtis was best dissimilarity metric to use. Based on these results, we set `dist = "bray"` for each dbRDA analysis using `capscale`. Due to issue pertaining to degrees of freedom, we needed to remove some metadata parameters from specific groups. From the 16S rRNA analysis, we removed Mg and Mn (environmental and edaphic properties). From the ITS analysis, we removed Mg, Mn, Na, Al, Fe, and K (environmental and edaphic properties) and  $S_{Q10}$  (temperature adaptation). Next, we used the vegan function `envfit` to fit environmental parameters onto the ordination. This function calculates correlation scores between metadata parameters and ordination axes. `envfit` found the following parameters were significantly correlated with community data (Goodness of fit statistic/squared correlation coefficient and empirical p-values for each variable shown in parentheses).

### Environmental and edaphic properties

**16S rRNA:** AST ( $r^2 = 0.829$ ,  $p = 0.001$ ), H<sub>2</sub>O ( $r^2 = 0.519$ ,  $p = 0.010$ ), DOC ( $r^2 = 0.446$ ,  $p = 0.024$ ).  
**ITS:** AST ( $r^2 = 0.485$ ,  $p = 0.037$ ), DOC ( $r^2 = 0.535$ ,  $p = 0.028$ ).

### Microbial functional responses

**16S rRNA:** AG<sub>ase</sub> ( $r^2 = 0.444$ ,  $p = 0.026$ ), BG<sub>ase</sub> ( $r^2 = 0.560$ ,  $p = 0.007$ ), S<sub>ase</sub> ( $r^2 = 0.737$ ,  $p = 0.002$ ), XY<sub>ase</sub> ( $r^2 = 0.519$ ,  $p = 0.009$ ), PX<sub>ase</sub> ( $r^2 = 0.764$ ,  $p = 0.001$ ), CO<sub>2</sub> ( $r^2 = 0.504$ ,  $p = 0.013$ ), enzNP ( $r^2 = 0.624$ ,  $p = 0.004$ ).  
**ITS:** micP ( $r^2 = 0.693$ ,  $p = 0.002$ ), micCP ( $r^2 = 0.583$ ,  $p = 0.016$ ), AG<sub>ase</sub> ( $r^2 = 0.506$ ,  $p = 0.037$ ), PX<sub>ase</sub> ( $r^2 = 0.500$ ,  $p = 0.035$ ), enzNP ( $r^2 = 0.547$ ,  $p = 0.014$ ).

### Temperature adaptation

**16S rRNA:** S<sub>Q10</sub> ( $r^2 = 0.496$ ,  $p = 0.015$ ), XY<sub>Q10</sub> ( $r^2 = 0.373$ ,  $p = 0.049$ ), LP<sub>Q10</sub> ( $r^2 = 0.413$ ,  $p = 0.041$ ), T<sub>min</sub> ( $r^2 = 0.446$ ,  $p = 0.030$ ).  
**ITS:** XY<sub>Q10</sub> ( $r^2 = 0.617$ ,  $p = 0.010$ ), CUE<sub>cp</sub> ( $r^2 = 0.479$ ,  $p = 0.035$ ), T<sub>min</sub> ( $r^2 = 0.475$ ,  $p = 0.028$ ).

# Appendices

## Appendix 1: Description of Supplementary Datasets

For this study, **Supplementary Datasets** are text files that were too large to include in the Supplementary Material. The individual files can be downloaded from the journal's website. Below are descriptions for each Supplementary Data item.

### Supplementary Dataset1

**Description:** Output from the **16S rRNA** DADA2 workflow before manual curation. Table is a tab delimited text file containing information for 20,332 ASVs. The first column is the unique ASV ID, followed by the read counts for each sample, ASV taxonomic lineage (Kingdom to Genus), and finally the unique ASV sequence.

**Filename** Supplementary\_Dataset1.txt

### Supplementary Dataset2

**Description:** Output from the **ITS** DADA2 workflow before manual curation. Table is a tab delimited text file containing information for 3357 ASVs. The first column is the unique ASV ID, followed by the read counts for each sample, ASV taxonomic lineage (Kingdom to Genus), and finally the unique ASV sequence.

**Filename** Supplementary\_Dataset2.txt

### Supplementary Dataset3

**Description:** Complete **metadata** information collected in this study. Tab delimited text file containing data for 61 metadata parameters (before normalization) associated with each sample. The first column is the sample ID, followed plot number (1–10), treatment (control or warm), temperature (0°C, +3°C, +8°C), plot pair ID (A–E), and collection season (W = rainy season). Subsequent columns contain values for all metadata parameters.

**Filename** Supplementary\_Dataset3.txt

### Supplementary Dataset4

**Description:** Differentially abundant (DA) ASVs from the **16S rRNA** data identified using Indicator Species Analysis (ISA) against the PIME filtered data set. Tab delimited text file of all 251 DA ASVs between temperature treatments.

**Filename** Supplementary\_Dataset4.txt

Description of table headers:

- **ASV\_ID** ASV name.
- **group** Sample group ASV is enriched in.
- **indval** Indicator value from Dufrene-Legendre Indicator Species Analysis.

- **pval** p-value from Dufrene-Legendre Indicator Species Analysis.
- **freq** Total number of samples where ASV was detected.
- **freq\_C0** Total number of Control samples where ASV was detected.
- **freq\_W3** Total number of +3°C samples where ASV was detected.
- **freq\_W8** Total number of +8°C samples where ASV was detected.
- **reads\_total** Total reads in data set.
- **reads\_C0** Total reads in Control samples.
- **reads\_W3** Total reads in +3°C samples.
- **reads\_W8** Total reads in +8°C samples.

The remaining columns contain lineage information for each ASV followed by its' unique sequence.

### **Supplementary Dataset5**

**Description:** Differentially abundant (DA) ASVs from the **16S rRNA** data identified using linear discriminant analysis (LDA) effect size (LEfSe) against the PIME filtered data set. Tab delimited text file of all 676 DA ASVs between temperature treatments.

<b>Filename</b> Supplementary_Dataset5.txt
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Description of table headers:

- **ASV\_ID** ASV name.
- **group** Sample group ASV is enriched in.
- **lدا** Linear discriminant analysis (LDA) scores.
- **pval** p-value from LEfSe analysis.
- **freq** Total number of samples where ASV was detected.
- **freq\_C0** Total number of Control samples where ASV was detected.
- **freq\_W3** Total number of +3°C samples where ASV was detected.
- **freq\_W8** Total number of +8°C samples where ASV was detected.
- **reads\_total** Total reads in data set.
- **reads\_C0** Total reads in Control samples.
- **reads\_W3** Total reads in +3°C samples.
- **reads\_W8** Total reads in +8°C samples.

The remaining columns contain lineage information for each ASV followed by its' unique sequence.

### **Supplementary Dataset6**

**Description:** Differentially abundant (DA) ASVs from the **ITS** data identified using Indicator Species Analysis (ISA) against the PIME filtered data set. Tab delimited text file of all 203 DA ASVs between temperature treatments.

<b>Filename</b> Supplementary_Dataset6.txt
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Description of table headers:

- **ASV\_ID** ASV name.
- **group** Sample group ASV is enriched in.
- **indval** Indicator value from Dufrene-Legendre Indicator Species Analysis.
- **pval** p-value from Dufrene-Legendre Indicator Species Analysis.
- **freq** Total number of samples where ASV was detected.

- **freq\_C0** Total number of Control samples where ASV was detected.
- **freq\_W3** Total number of +3°C samples where ASV was detected.
- **freq\_W8** Total number of +8°C samples where ASV was detected.
- **reads\_total** Total reads in data set.
- **reads\_C0** Total reads in Control samples.
- **reads\_W3** Total reads in +3°C samples.
- **reads\_W8** Total reads in +8°C samples.

The remaining columns contain lineage information for each ASV followed by its' unique sequence.

### **Supplementary Dataset7**

**Description:** Differentially abundant (DA) ASVs from the **ITS** data identified using linear discriminant analysis (LDA) effect size (LEfSe) against the PIME filtered data set. Tab delimited text file of all 228 DA ASVs between temperature treatments.

**Filename** Supplementary\_Dataset7.txt

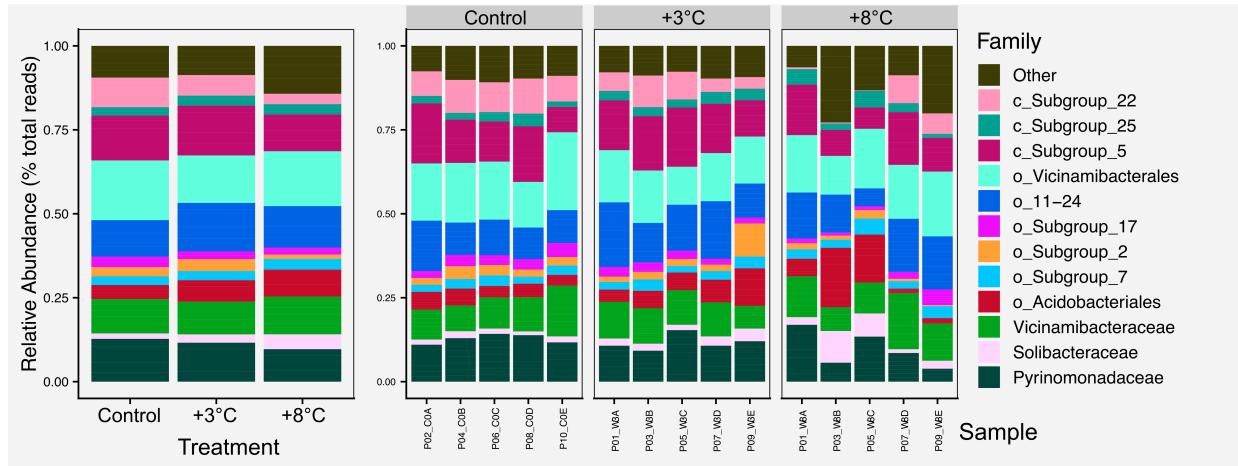
Description of table headers:

- **ASV\_ID** ASV name.
- **group** Sample group ASV is enriched in.
- **lda** Linear discriminant analysis (LDA) scores.
- **pval** p-value from LEfSe analysis.
- **freq** Total number of samples where ASV was detected.
- **freq\_C0** Total number of Control samples where ASV was detected.
- **freq\_W3** Total number of +3°C samples where ASV was detected.
- **freq\_W8** Total number of +8°C samples where ASV was detected.
- **reads\_total** Total reads in data set.
- **reads\_C0** Total reads in Control samples.
- **reads\_W3** Total reads in +3°C samples.
- **reads\_W8** Total reads in +8°C samples.

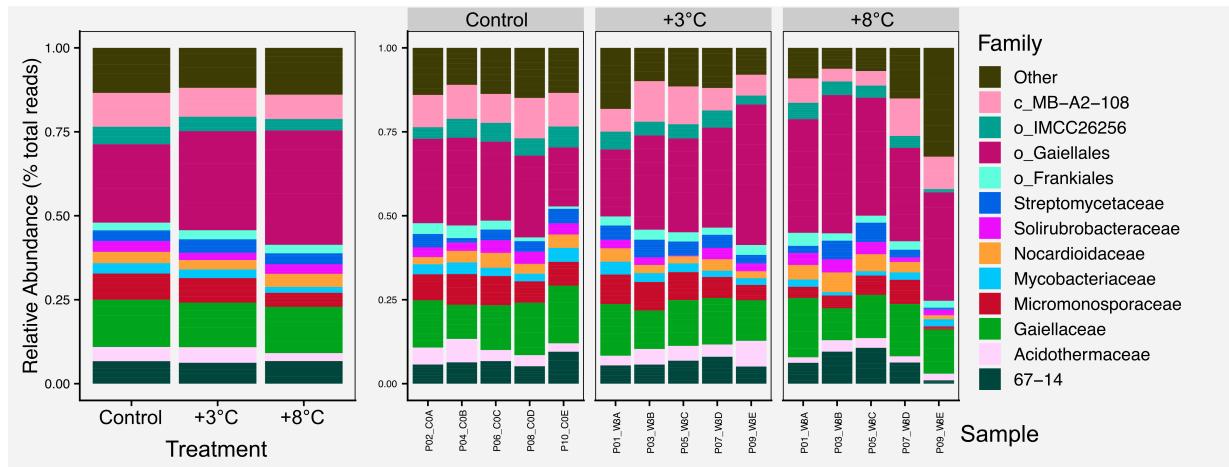
The remaining columns contain lineage information for each ASV followed by its' unique sequence.

## Appendix 2: Family-level bacterial charts

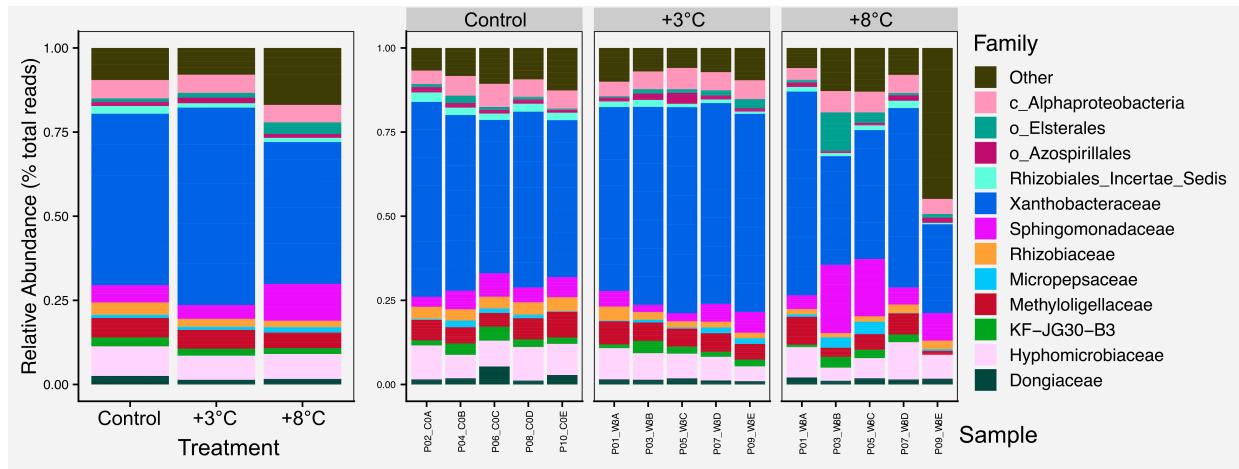
Top twelve (12) families of abundant bacterial phyla. Remaining taxa are grouped in *Other*. In cases where ASVs could not be classified to family level, abundance data was calculated for the next highest taxonomic rank, denoted by the prefix **rank abbreviation** plus **underscore** (e.g., *c*\_ is Class). As above, relative abundance of taxa based on the full, unfiltered data set. Left plots show taxa collapsed by temperature treatment while right plots show individual samples faceted by temperature treatment. Taxa are ordered first by rank and then alphabetically. The same color palette displayed in the same order was used for each plot.



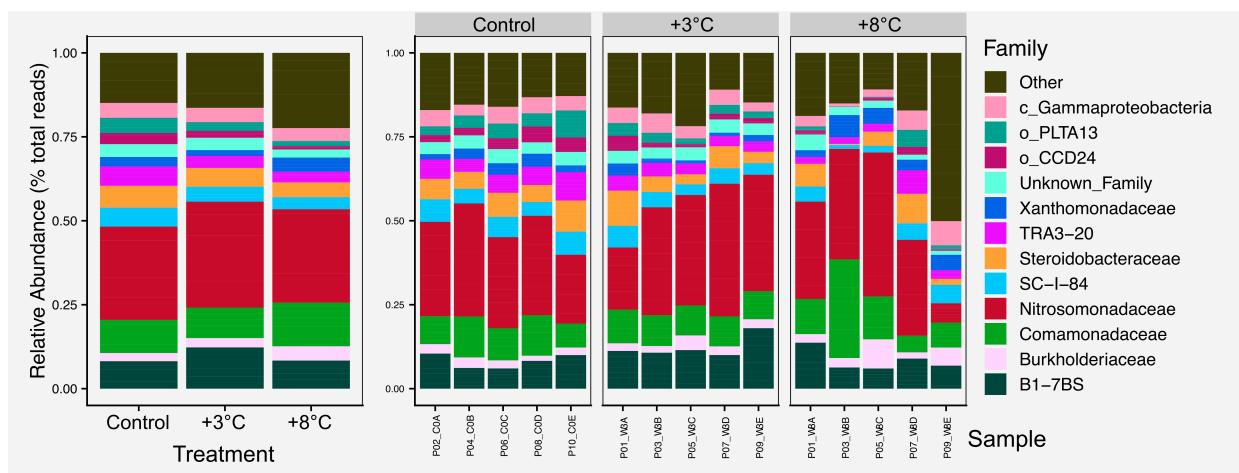
Supplementary Figure 7 | Acidobacteriota family plots.



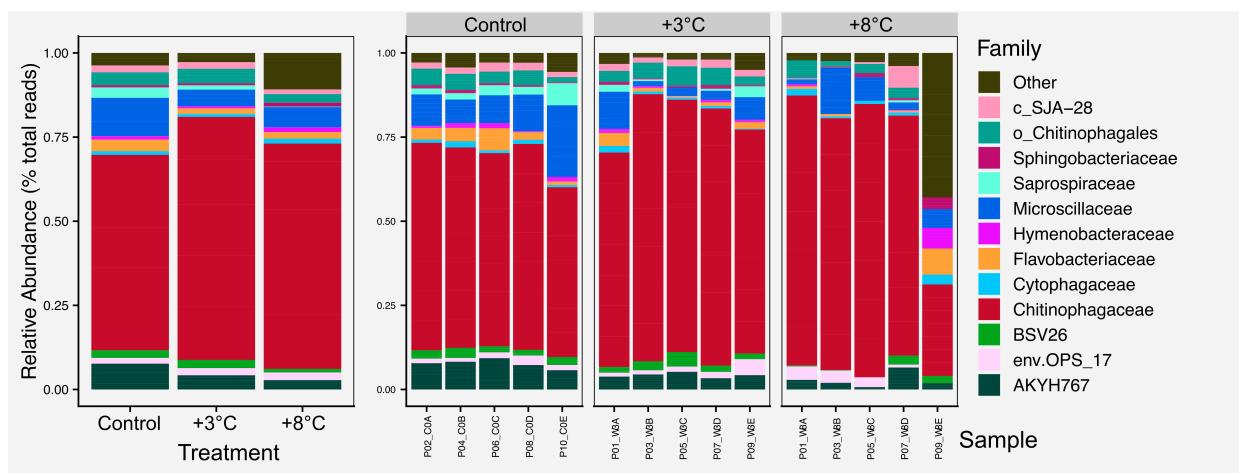
Supplementary Figure 8 | Actinobacteriota family plots.



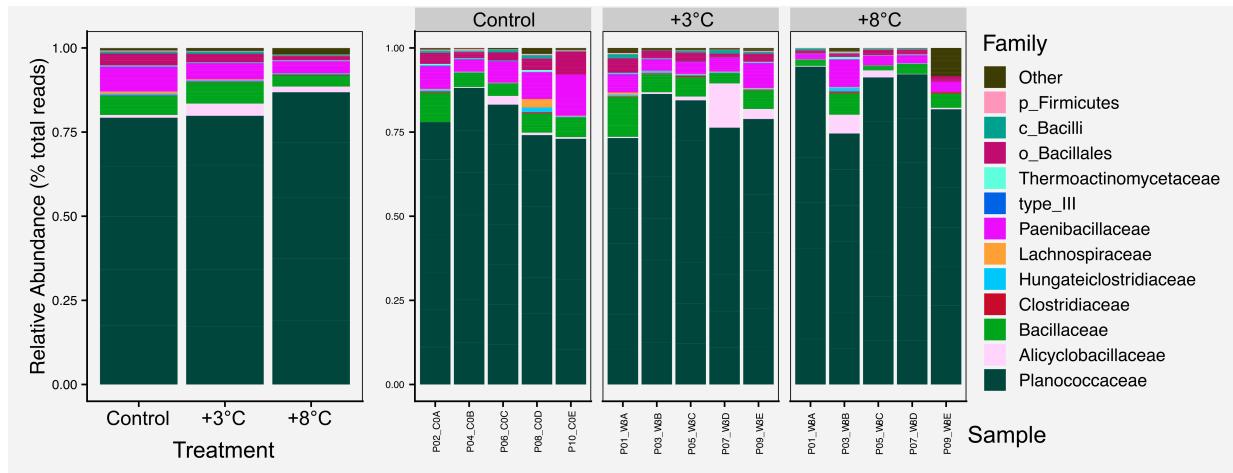
**Supplementary Figure 9 |** Alphaproteobacteria family plots.



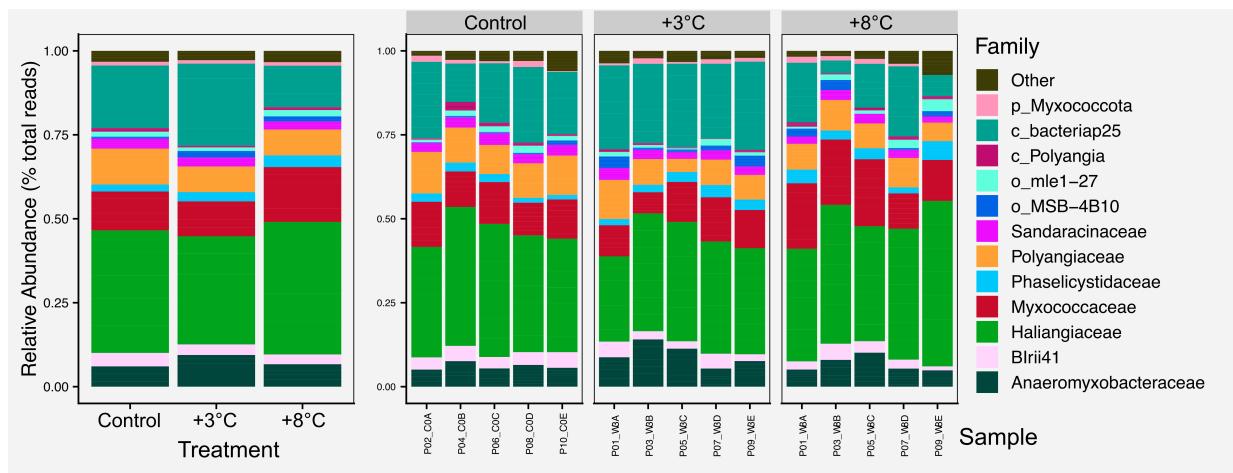
**Supplementary Figure 10 |** Gammaproteobacteria family plots.



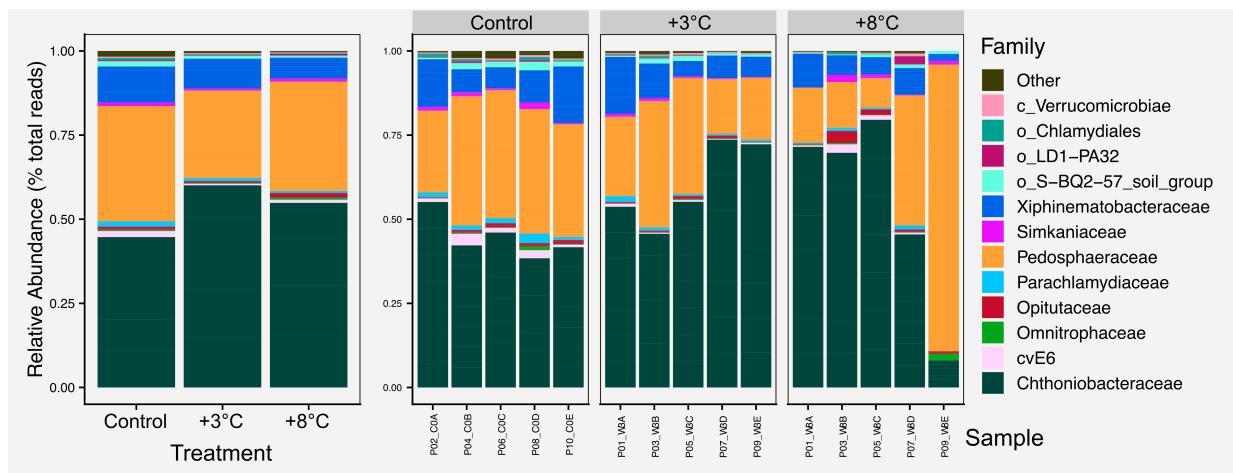
**Supplementary Figure 11 |** Bacteroidota family plots.



**Supplementary Figure 12 | Firmicutes family plots.**



**Supplementary Figure 13 | Myxococcota family plots.**



**Supplementary Figure 14 | Verrucomicrobiota family plots**

## References

1. Caporaso, J. G. *et al.* Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences* **108**, 4516–4522 (2011).
2. Gardes, M. & Bruns, T. D. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**, 113–118 (1993).
3. White, T. J., Bruns, T., Lee, S., Taylor, J., *et al.* Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* **18**, 315–322 (1990).
4. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal* **17**, 10–12 (2011).
5. Callahan, B. J. *et al.* DADA2: High-resolution sample inference from illumina amplicon data. *Nature Methods* **13**, 581 (2016).
6. Team, R. C. R: A language and environment for statistical computing.
7. Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**, 5261–5267 (2007).
8. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* **41**, D590–D596 (2012).
9. Nilsson, R. H. *et al.* The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research* **47**, D259–D264 (2019).
10. Abarenkov, K. *et al.* UNITE general FASTA release for fungi. (2020).
11. McMurdie, P. J. & Holmes, S. Phyloseq: An r package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**, e61217 (2013).
12. Smirnova, E., Huzurbazar, S. & Jafari, F. PERFect: PERmutation filtering test for microbiome data. *Biostatistics* **20**, 615–631 (2019).
13. Roesch, L. F. W. *et al.* PIME: A package for discovery of novel differences among microbial communities. *Molecular Ecology Resources* **20**, 415–428 (2020).
14. Alberdi, A. & Gilbert, M. T. P. A guide to the application of hill numbers to DNA-based diversity analyses. *Molecular Ecology Resources* **19**, 804–817 (2019).
15. Alberdi, A. & Gilbert, M. T. P. Hilldiv: An r package for the integral analysis of diversity based on hill numbers. *bioRxiv* 545665 (2019).
16. Bálint, M. *et al.* Millions of reads, thousands of taxa: Microbial community structure and associations analyzed via marker genes. *FEMS Microbiology Reviews* **40**, 686–700 (2016).
17. Roswell, M., Dushoff, J. & Winfree, R. A conceptual guide to measuring species diversity. *Oikos* **130**, 321–338 (2021).
18. Shapiro, S. S. & Wilk, M. B. An analysis of variance test for normality (complete samples). *Biometrika* **52**, 591–611 (1965).
19. Bartlett, M. S. Properties of sufficiency and statistical tests. *Proceedings of the Royal Society of London. Series A-Mathematical and Physical Sciences* **160**, 268–282 (1937).
20. Oksanen, J. *et al.* Vegan: Community ecology package. *R package version* **2**, (2012).
21. Chen, J. *et al.* Associating microbiome composition with environmental covariates using generalized UniFrac distances. *Bioinformatics* **28**, 2106–2113 (2012).
22. Fuglede, B. & Topsøe, F. Jensen-shannon divergence and hilbert space embedding. in *International symposium on information theory, 2004. ISIT 2004. proceedings*. 31 (IEEE, 2004).

23. Bray, J. R. & Curtis, J. T. [An ordination of the upland forest communities of southern Wisconsin](#). *Ecological Monographs* **27**, 326–349 (1957).
24. Legendre, P., Oksanen, J. & ter-Braak, C. J. [Testing the significance of canonical axes in redundancy analysis](#). *Methods in Ecology and Evolution* **2**, 269–277 (2011).
25. Legendre, P. & Anderson, M. J. [Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments](#). *Ecological Monographs* **69**, 1–24 (1999).
26. Clarke, K. R. [Non-parametric multivariate analyses of changes in community structure](#). *Australian Journal of Ecology* **18**, 117–143 (1993).
27. Gower, J. C. [Some distance properties of latent root and vector methods used in multivariate analysis](#). *Biometrika* **53**, 325–338 (1966).
28. Roberts, D. W. & Roberts, M. D. W. [Package ‘labdsv’](#). *Ordination and Multivariate* **775**, (2016).
29. Cao, Y. [microbiomeMarker: Microbiome biomarker analysis](#). *R package version 0.0.1.9000*, (2020).
30. Segata, N. *et al.* [Metagenomic biomarker discovery and explanation](#). *Genome Biology* **12**, 1–18 (2011).
31. Peterson, R. A. & Cavanaugh, J. E. [Ordered quantile normalization: A semiparametric transformation built for the cross-validation era](#). *Journal of Applied Statistics* (2019).
32. Peterson, R. A. [Finding optimal normalizing transformations via bestNormalize](#). *The R Journal* (2021).
33. Mantel, N. [The detection of disease clustering and a generalized regression approach](#). *Cancer Research* **27**, 209–220 (1967).
34. Legendre, P. & Legendre, L. [Numerical ecology](#). (Elsevier, 2012).
35. Faith, D. P., Minchin, P. R. & Belbin, L. [Compositional dissimilarity as a robust measure of ecological distance](#). *Vegetatio* **69**, 57–68 (1987).