Microbial diversity of extreme habitats in human homes

Amy M. Savage, Justin L Hills, Katherine Driscoll, Daniel J Fergus, Amy M Grunden, Robert R Dunn

Background: High throughput sequencing techniques have opened up the world of microbial diversity to scientists, and a flurry of studies in the most remote and extreme habitats on earth have begun to elucidate the key roles of microbes in ecosystems with extreme conditions. These same environmental extremes can also be found closer to humans; in fact, they can be found in our homes. Here, we used high throughput sequencing techniques to assess microbial diversity in the extreme environments inside human homes (e.g. dishwashers, hot water heaters, washing machine bleach reservoirs, etc.). We focused on habitats in the home with extreme temperature, pH and chemical environmental conditions.

Results: We found that although these habitats supported a lower diversity of microbes than less extreme habitats in the home, there were still diverse microbial assemblages in extreme home environments. Habitats with extreme temperatures alone appeared to be able to support a greater diversity of microbes than habitats with extreme pH or extreme chemical environments alone. Microbial diversity was lowest when habitats had both extreme temperature and one of these other extremes. This interactive effect was strongest when habitats had both extreme temperatures and extreme pH. Under these conditions, taxa with known associations with extreme conditions dominated.

Conclusions: Our findings highlight the importance of examining interactive effects of multiple environmental extremes on microbial communities. Inasmuch as taxa from extreme environments can be both pathogens and industrially useful, our findings also suggest future work to understand both the threats and opportunities posed by the life in these habitats.

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Abstract:

Background: High throughput sequencing techniques have opened up the world of microbial diversity to scientists, and a flurry of studies in the most remote and extreme habitats on earth have begun to elucidate the key roles of microbes in ecosystems with extreme conditions. These same environmental extremes can also be found closer to humans; in fact, they can be found in our homes. Here, we used high throughput sequencing techniques to assess microbial diversity in the extreme environments inside human homes (e.g. dishwashers, hot water heaters, washing machine bleach reservoirs, etc.). We focused on habitats in the home with extreme temperature, pH and chemical environmental conditions.

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Conclusions: Our findings highlight the importance of examining interactive effects of multiple environmental extremes on microbial communities. In as much as taxa from extreme

environments can be both pathogens and industrially useful, our findings also suggest future work to understand both the threats and opportunities posed by the life in these habitats.

Keywords: Community Structure, Extreme environments, Human Homes, Interactive effects, Microbial Diversity

Background:

The innovation of culture-independent, high-throughput sequencing techniques has facilitated the discovery of high microbial diversity in many habitats once considered inhospitable to life (Rothschild and Mancinelli 2001). The species in these environments are frequent targets for the discovery of useful enzymes (Niehaus et al. 1999, van den Burg 2003, Elleuche et al. 2014), as well as key insights into the evolution of microbial metabolism (Valentine 2007, Hoehler and Jorgensen 2013). Often overlooked, however, is that the attributes that define many of the most extreme habitats on Earth, such as extremes of temperature, pH, water activity, or low nutrient levels, can also be found more immediate to everyday experience. Human homes, for example, contain microhabitats as hot, acidic, basic or as salty as any encountered elsewhere on Earth (Martin et al. 2015).

We know of only two extreme habitats within homes where microbial diversity has been studied to date, and in both cases culture-dependent techniques were used. In 1973, Brock and Boylen discovered a species of the genus *Thermus* (*T. aquaticus*) living in hot water heaters. Species of this genus had previously been known only from hot springs (Brock and Boylen 1973). The other studies that have considered extreme environments in the home are studies of tap water. Tap

water is hospitable in terms of its abiotic conditions (e.g. temperature, pH, toxicity) but is very low in nutrients and so was long assumed to be relatively devoid of life; until, that is, it was studied. Tap water has now been shown to contain many species of bacteria capable of surviving in low nutrient environments (Kalmbach et al. 1997, Szewzyk et al. 2000, Boe-Hansen et al. 2002). If life exists in hot water heaters and tap water, it seems possible and even likely that many extreme habitats in homes sustain life. In fact, homes have the potential to replicate a very broad range of many conditions seen in the world more generally. That the environmental extremes imposed by these conditions in homes (cold, hot, acidic, alkaline, wet or dry) delineate which species are present seems inevitable. That they are lifeless is unlikely.

Here, we used culture-independent, high-throughput sequencing to address the following questions: (1) What is the relative diversity of microbes under extremes of temperature, pH and chemical environments of southeast US homes and how does it compare to habitats without each extreme conditions? Additionally, Harrison et al. (2013) recently argued that because many extreme environments include simultaneous extremes in multiple environmental factors, interactive effects of these multiple sources of extreme conditions are likely to be important determinants of microbial diversity in extreme environments. Therefore, we asked (2) how do multiple, simultaneous extreme conditions influence microbial diversity in human homes? Finally, we asked (3) which microbial genera from the broader home (Dunn et al. 2013) fail to persist in extreme home habitats, and which microbial genera persist only in these extreme habitats?

Results and Discussion:

What is the relative diversity of microbes in extreme temperature, pH and chemical environments of southeast US homes and how does it compare to habitats without each extreme condition?

The rarefied OTU richness in habitats with extreme temperatures was more than twice as high as in habitats with extreme pH (73 *vs.* 33) and almost three times as high as habitats with extreme chemical environments (27.6; Fig. 1). Habitats with extreme temperatures also had higher OTU richness than habitats with intermediate temperatures (Fig. 2a). Conversely, previous research indicates that the diversity in habitats with either extremely high or extremely low temperatures is generally low, and dominated by a small number of abundant bacterial species (Lewin et al. 2013). For example, Sharp et al. (2014) recently found that OTU richness in hydrothermal vents peaked at intermediate temperatures (24˚C), with reduced OTU richness in extremely hot or cold environments (Sharp et al. 2014). We did not detect significant differences in the rarefied species richness of microbes in extreme *vs.* neutral pH conditions; however, the marginally non-significant trend suggests that extreme pH environments also had higher microbial diversity than neutral habitats (Fig. 2b). Recent studies have demonstrated that pH is a key predictor of microbial diversity in both extreme environments, such as acid mine drainage sites (Kuang et al. 2013), and less extreme environments, such as tropical soils (Tripathi et al. 2012). In both cases, habitats with neutral pH had higher microbial diversity than those with a pH higher or lower than neutral. Thus, we again found different patterns in extreme home environments compared to other studies comparing extreme and non-extreme habitats. One possible explanation for the difference between the two studies is that human-associated microbes are present in home environments with intermediate temperatures. Perhaps these species are able to dominate habitats with intermediate, but not extreme, conditions. Alternatively, the lower diversity in habitats with intermediate temperatures and neutral pH in our study could be due to the occurrence of extreme conditions along different axes (e.g. intermediate temperature, but extreme pH or chemical habitats).

In contrast, habitats with extreme chemicals had significantly lower accumulated OTU richness than habitats without these extreme conditions (Fig. 2c). Extreme chemical environments are poorly studied and understood (Rothschild and Mancinelli 2001). However, our data suggest that they could act as strong filters in extreme environments.

How do multiple, simultaneous extreme conditions influence microbial diversity in extreme home environments?

Many of the habitats in this study were characterized by more than one extreme environmental condition. Therefore, we also examined the potential for interactive effects of multiple, simultaneous extreme conditions on microbial diversity. Due to limited replication across all environmental extremes, we were only able to examine extreme pH and chemical habitats with and without extreme temperatures. We used an ordination framework to examine these interactive effects (see methods).

We found significant interactions between extreme temperature and both extreme pH (PerMANOVA: P=0.0001; Figure 3a) and extreme chemical (PerMANOVA: P=0.0001; Figure 3b) environments for OTU composition. Specifically, when temperatures were intermediate, there were no significant differences in microbial composition in extreme *vs.* neutral pH habitats (pairwise PerMANOVA: P>0.05). However, when temperatures were extreme, there was a very large difference between the composition of microbes in extreme pH habitats, compared to neutral habitats (pairwise PerMANOVA: P=0.0001; Fig. 3a). The five genera that contributed the most to differences between these two habitat types were *Parascardovia, Micrococcus,* an unknown genus from Sphingomonadaceae, *Rothia,* and *Brachybacterium.* Most of these genera are associated with humans (Oshima et al. 2015, Gueimonde et al. 2012, Kloos et al. 1975, Kocur et al. 2006, Vaccher et al. 2007, Uchibori et al. 2012). Sphingomonadaceae are

widespread in aquatic habitats, including drinking water (Vaz-Moreira et al. 2011), but also other aquatic environments (e.g. tree holes-Xu et al. 2008). *Brachybacterium* is usually associated with marine environments (Ward and Boru 2006), including Antarctic sea ice (Junge et al. 1998). However, it was recently detected in an urban shopping center (Tringe et al. 2008). All of these genera were more common in habitats with extreme temperatures and neutral pH than they were in habitats with both extremes.

The interaction between temperature and chemical extremes was slightly different. Microbial composition was indistinguishable between the habitats that only had one extreme condition-regardless of whether it was temperature or chemicals that were extreme. However, habitats with both extreme temperatures and extreme chemicals had significantly different microbial composition compared to all other groups (pairwise perMANOVA; P=0.001). Habitats with intermediate temperatures and no chemicals were also significantly different from all other groups in terms of microbial composition, with the biggest differences occurring between habitats with both extremes and those with neither extreme (Fig. 3b). The five genera that contributed the most to compositional difference between these two habitats were *Methylobacterium,* an unknown genus of Moraxellaceae, *Sejonia,* an unknown genus of Sphingomonadaceae, and *Flavobacterium.* With the exception of the unknown genus of Moraxellaceae, which was more common in extreme chemical and temperature environments, all of these genera were more common in the habitats without temperature and chemical extremes. Moraxallaceae have been found in other extreme environments, including deep sea sediments (Maruyama et al. 1997). Although it was more common in our less extreme environments, *Sejonia* is better known from Antarctic ice (Yi et al. 2005). Sphingomonadaceae as described above are common to aquatic habitats. *Methylobacterium* is a widespread habitat generalist that

160 is facultatively methyltrophic (Green 2006). Finally, *Flavobacterium* is common in freshwater
161 and marine ecosystems but tends to flourish in cold environments with high salinity (Bernardet
162 and Bowman 2006).

163

164 *Which microbial genera differentiate extreme home habitats from the rest of the home?*

165 After removing all human-associated microbes (above), there were a total of 241 unique genera
166 in the broader homes dataset (Dunn et al. 2013). Our extreme samples contained 135 of the
167 remaining broader homes genera, but ~44% of the genera found in the broader homes were
168 absent from our extreme home samples (Supp. Table 3), the absence of which might simply be
169 due to the larger number of samples in Dunn et al. (2013). More interestingly, we found 20
170 genera present among our samples that were absent from the broader homes dataset. Nine of
171 these genera were found in all three categories of extreme environments (Table 1); one genus
172 (*Solibacter*) was absent from habitats with extreme pH, but occurred in both extreme chemical
173 and temperature environments. *Solibacter* is a common and abundant soil microbe, especially in
174 tropical regions (Guan et al. 2013, Wang et al. 2015). There was also one genus
175 (*Brevundimonas*) that was absent from extreme chemical environments, but present in both
176 extreme temperature and extreme pH environments; *Brevundimonas* is one of the only genera
177 thought to be able to survive the low temperatures and ionizing radiation on Mars (Dartnell et al.
178 2010). There were three genera (*Azobacteroides, Elizabethkingia,* and *Xiphinematobacter*) that
179 occurred in both extreme pH and chemical environments that were absent in extreme
180 temperature environments. Both *Azobacteroides* and *Xiphinematobacter* are gut symbionts of
181 invertebrates; *Azobacteroides* is commonly found inside the protozoan symbionts of termites
182 (Noda et al. 2007), and *Xiphinematobacer* is an endosymbiont of nematodes (Vandekerckhove et

al. 2000). In invertebrate guts these microbes likely experience extreme chemical and pH environments frequently, while being relatively protected from temperature stress. *Elizabethkingia* is a cosmopolitan genus, with species that are endosymbionts of mosquitos (Kämpfer et al. 2011), and others that are pathogens of both humans (Ceyhan and Celik 2011) and frogs (Xie et al. 2009). There was one genus that was only found in extreme chemical environments (*Helcococcus*). Interestingly, members of the genus *Helcococcus* possess the ability to degrade detergents. In fact, the detergent Tween-80 can be added to media to enrich *Helcococcus* (Collins et al. 1993, Chagla et al. 1998). Finally, we found 5 genera (*Brochothrix, Buchnera, Polynucleobacter, Ralstonia,* and *Thermicanus*) unique to extreme temperature environments. *Brochothrix* is a common spoilage bacterium in meat (Rattanasomboom et al. 1999). *Buchnera* is a widespread aphid endosymbiont (Shigenobu et al. 2000). The genus *Polynucleobacter* includes both free-living species and species that are endosymbionts of nematodes (Vannini et al. 2007). *Ralstonia metallidurans* is a bacterium specifically adapted to toxic metal environments (Mergeay et al. 2003). Other species of *Ralstonia* have been shown to be effectively controlled using high temperature treatments in commercial crops (Kongkiattikajorn and Thepa 2007). In our study, *Ralstonia* were collected in both high and low temperature environments. Finally, *Thermicanus* is, as its name suggest, a thermophilic bacterial genus (Wrighton et al. 2008).

Conclusions:

This study has provided a glimpse into the microbial diversity that lives in habitats of human homes similar in their extreme temperature, pH and chemical conditions to some of the most extreme habitats on Earth. We discovered that these conditions have lower diversity than the surrounding home environment; yet tens of bacterial lineages can be found in these extreme

habitats of the human home, including many taxa with known associations with extreme conditions. Habitats with extreme temperatures alone appear to be able to support a greater diversity of microbes than habitats with extreme pH or extreme chemical environments alone. Microbial diversity is significantly lowest when habitats have both extreme temperature and one of these other extremes. A key next step is understanding which of the relatively few species that are found in these poly-extreme environments in the home are metabolically active there and both whether these polyextreme taxa pose health threats (as was recently suggested by Gümral et al. 2015) and/or might be useful industrially.

Methods:

Sampling extreme home environments

We sampled extreme environments in six houses in the Raleigh-Durham metropolitan area (Supp. Fig 1). In each house, we used dual-tipped sterile BBL™ CultureSwabs™ or 50mL conical tubes to swab or collect water from each of 10 standardized extreme locations in homes. The sites sampled in all six houses included environments that were extreme in terms of their temperature, pH and chemical environments (Supp. Table 1). Samples were preserved at -20º C immediately after collection.

Isolating and identifying microbes in extreme home environments

Genomic DNA was extracted from all samples using the MoBio Power Soil DNA extraction kit (MoBio, Carlsbad, CA) as described previously (Fierer et al., 2008; Lauberet al., 2009). For swabs, the tips were placed in PowerBead tubes containing solution C1 and swirled vigorously for approximately 10 seconds to release contents and removed. Water samples were thawed and

filtered using Corning 50mL 0.22um cellulose acetate filters after which the filters were added to the PowerBead tubes. The extractions were subsequently performed as directed by the manufacturer, except that the final elution was performed in 50µl of 70º C C6 elution buffer. Because the water samples were frozen prior to filtering and extraction, the results reported for the water samples likely under-represents the true diversity of taxa in those environments.

We used methods described in Bates et al (2011) to amplify bacterial and archaeal DNA from the samples collected from homes and six negative controls. Briefly, amplicons were produced by PCR with universal bacterial/archaeal 515F and 806R primers to which Roche 454 B pyrosequencing adapters had been added, as described in Hulcr et al. (2012). The 515F primer contained an additional 12-bp barcode sequence for individual sample identification. All the samples were amplified by triplicate PCR reactions, cleaned using the UltraClean-htp 96-well PCR Clean-up kit (MoBio), and quantified with a Quant-iT PicoGreen dsDNA Assay kit (Invitrogen). Equimolar amounts of each sample were pooled into a single sample to sequence. DNA pyrosequencing was performed at Selah Clinical Genomics Center at Innovista (University of South Carolina, USA) using a Roche Genome Sequencer 454 FLX system to facilitate comparison to previous related work that utilized this platform (Dunn et al, 2013). Though these methods here do not distinguish living from recently dead cells with the comparative approach used here we presume that taxa frequently identified in one habitat but rare or absent in most others are likely surviving in the more frequent habitat. The sequences were submitted to NCBI (SRA accession number SRP071677).

The QIIME analysis package (Caporaso et al, 2010a) was used to process and analyze the barcoded microbial amplicon sequences. Sequences were quality filtered to a minimum quality score of 25 with no unambiguous bases and sorted to each sample by the 12 bp barcodes. The

454 pyrosequencing produced 197,305 reads that passed the quality screening. Sequences were grouped into Operational Taxonomic Units (OTUs) that shared at least 97% sequence similarity. A representative sequence was taken for each OTU group and PyNAST (Caporaso et al, 2010b) was employed to align these representative sequences to the Greengenes database (DeSantis et al, 2006) and the taxonomic identity of each OTU was determined using the RDP Classifier (Wang et al, 2007). Phylotypes were considered to be contaminants if they were seen in at least two of the six negative control samples. After removing contaminant sequences and singletons, the number of quality-filtered reads per sample was between 6 and 5861 (median=2306).

Analysis of the relative diversity of microbes in extreme temperature, pH and chemical environments of homes and how it compares to habitats without each extreme condition

We compared microbial species accumulation among three extreme variables in homes: temperature, pH, and chemical extremes. Temperature was classified on a scale of 1-5, with 1 representing the coldest environments and 5 representing the hottest environments. We then binned 1 and 5 into an extreme temperature category and 2-4 into an intermediate temperature category. Similarly, environments were classified as acidic, basic or neutral and then binned into extreme pH (acidic or basic environments) *versus* neutral environments. Finally, chemical extremes were those environments characterized by the presence of detergent, bleach, metals, ammonia, or natural gas (Supp. Table 2).

We used EstimateS v. 9.1.0 (Colwell 2013) to construct individual-based species accumulations for all three extreme environments and their non-extreme counterparts. For these curves, reads were used as individuals and the curves were constructed using 1000 iterations. To formally assess differences in accumulated species by read, we used $\pm$ 95% confidence intervals for each curve.

Assessing how multiple, simultaneous extreme conditions influence microbial diversity in human homes

We were interested in testing the hypothesis that interactive effects of multiple, simultaneously extreme environmental conditions are important determinants of microbial diversity in extreme home environments (Harrison et al. 2013). Our study included multiple samples with more than one environmental extreme (Supp. Table 1); however, we only had sufficient replication to assess this hypothesis for 2-way interactions between extremely high temperatures and extreme pH as well as high temperature and chemical environments. Because number of reads varied significantly among different environmental extremes, we could not use a standard 2-way ANOVA. Instead, we assessed these effects using an ordination framework.

We visualized the composition of bacteria from extreme habitats in homes using non-metric multidimensional scaling ordination (NMDS) in Primer-E v.7.0.9 with PerMANOVA +1 (Clarke & Gorley, 2015). To do this, we first constructed NMDS plots with 100 restarts and a Type I Kruskal fit scheme based on a Dissimilarity matrix of Bray-Curtis distances. To assess the relationship between temperature (extreme *vs.* intermediate) and the other extremes (pH: extreme *vs.* neutral; chemicals: extreme *vs.* none), we conducted a permuted multivariate analysis of variance (PerMANOVA) test with temperature class and either pH or chemical class and their interaction as factors, 9,999 iterations and Type III sums of squares. Thus, we conducted two separate analyses; to account for the additional error associated with multiple tests, we used a revised $\alpha=0.05/2=0.025$ as our cut-off for statistical significance. When interactions were significant, we conducted pairwise PerMANOVA to determine which treatment combinations significantly differed from one another. Finally, we conducted SIMPER analyses for each

296 significant treatment combination to determine the OTUs that contributed the most to pairwise
297 between-group differences in ordination space.

298

299 *Determining which microbial genera differentiate extreme home habitats from the rest of the*
300 *home*

301 We were particularly interested in microbes that are not associated with humans, so we removed
302 human-associated OTU's from our dataset. We identified these human-associated OTU's using
303 databases that identified human gut (Flores et al. 2014) and skin (Urban et al. 2016)
304 microbiomes. OTU's that occurred in at least 80% of the samples in those databases were
305 considered human-associates and excluded from our analyses of the microbial diversity of
306 extreme habitats in human homes. We then removed any OTU's that occurred less than 20 times
307 in our samples to reduce the possibility of spurious results from the sequencing process. Thus,
308 our assessments of microbial diversity are conservative.

309 We compared the occurrences of microbes in our samples to those reported in less extreme home
310 environments (Dunn et al. 2013).. We first determined the identity of microbes that were absent
311 from the broader homes dataset, but present in extreme environments and then tabulated the
312 extreme habitat(s) in which they were present. Likewise, we identified the non-human associated
313 microbes that were present in the broader home environment, but absent from all extreme
314 environments in our samples.

315 **Author contributions:** AMS conducted analyses of microbial community data and drafted the
316 manuscript, JLH and KD conceived of the study, collected microbial samples, assigned extreme
317 classifications to each home environment, and assisted with sample isolations; DJF isolated

samples and identified microbes, conducted sequence analyses and processed QIIME data; AMG assisted with design and collection of samples and participated in sequence analyses; and RRD provided support for all stages of the project development in addition to financial support (A.P. Sloan Microbiology of the Built Environment Program).

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Figure Legends:

Figure 1: Rarefaction curves for each extreme environment, expressed as number of OTU by number of reads from sequencing. Each curve was constructed using 1000 iterations, and the dotted lines represent 95% confidence intervals.

Figure 2: Comparison of rarefaction curves between extreme and non-extreme habitats. (a) extreme *vs.* intermediate temperatures, (b) extreme *vs.* neutral pH environments, and (c) extreme chemicals present *vs.* absent. Rarefaction curves are expressed as number of OTU by number of reads from sequencing. Each curve was constructed using 1000 iterations, and the dotted lines represent 95% confidence intervals.

Figure 3: NMDS ordinations OTU occurrence by (a) Temperature & pH and (b) Temperature & chemical environments in the home. Symbols represent centroids ± 1 SE. 2-D stress was 0.18.

503

504

505 **Tables:**

506 Table 1**:** Summary of occurrences of microbes that were present in samples from extreme home
507 environments, but absent from the broader home samples. Each X indicates that the genus was
508 found in a given extreme environment.

509

510 **Supplementary Tables and Figures:**

511 Raw Data: Output file from QIIME at the genus level (L6).

512 Supp. Table 1: Description of sample locations. Standardized locations were sampled in all 6
513 houses, while special locations were only sampled in a subset of the houses (due to availability
514 of samples across houses)

515

516 Supp. Table 2: Classifications of sampled extreme home environments based upon temperature,
517 pH and chemical conditions.

518

519 Supp. Table 3: List of non-human associated microbes in extreme and non-extreme (Dunn et al.
520 2013) home habitats

521

522 Supp. Figure 1: Map of houses that were sampled for the study

523

524

525

Table 1(on next page)

Table 1

Summary of occurrences of microbes that were present in samples from extreme home environments, but absent from the broader home samples. Each X indicates that the genus was found in a given extreme environment. t&#65533;h6\0&#65533;>

Table 1: Summary of occurrences of microbes that were present in samples from extreme home environments, but absent from the broader home samples. Each X indicates that the genus was found in a given extreme environment.

Genus	Extreme Temperatures	Extreme pH	Extreme Chemical
Brochothrix	X		
Buchnera	X		
Polynucleobacter	X		
Ralstonia	X		
Thermicanus	X		
Helcococcus			X
Solibacter	X		X
Brevundimonas	X	X	
Azobacteroides		X	X
Elizabethkingia		X	X
Xiphinematobacter		X	X
Azospira	X	X	X
Brachybacterium	X	X	X
Enhydrobacter	X	X	X
Gluconobacter	X	X	X
Oligella	X	X	X
Parascardovia	X	X	X
Photobacterium	X	X	X
Propionibacterium	X	X	X
Salinibacterium	X	X	X

1

Figure 1: Comparison among all extreme environments

Rarefaction curves for each extreme environment, expressed as number of OTU by number of reads from sequencing. Each curve was constructed using 1000 iterations, and the dotted lines represent 95% confidence intervals.],"exsi6

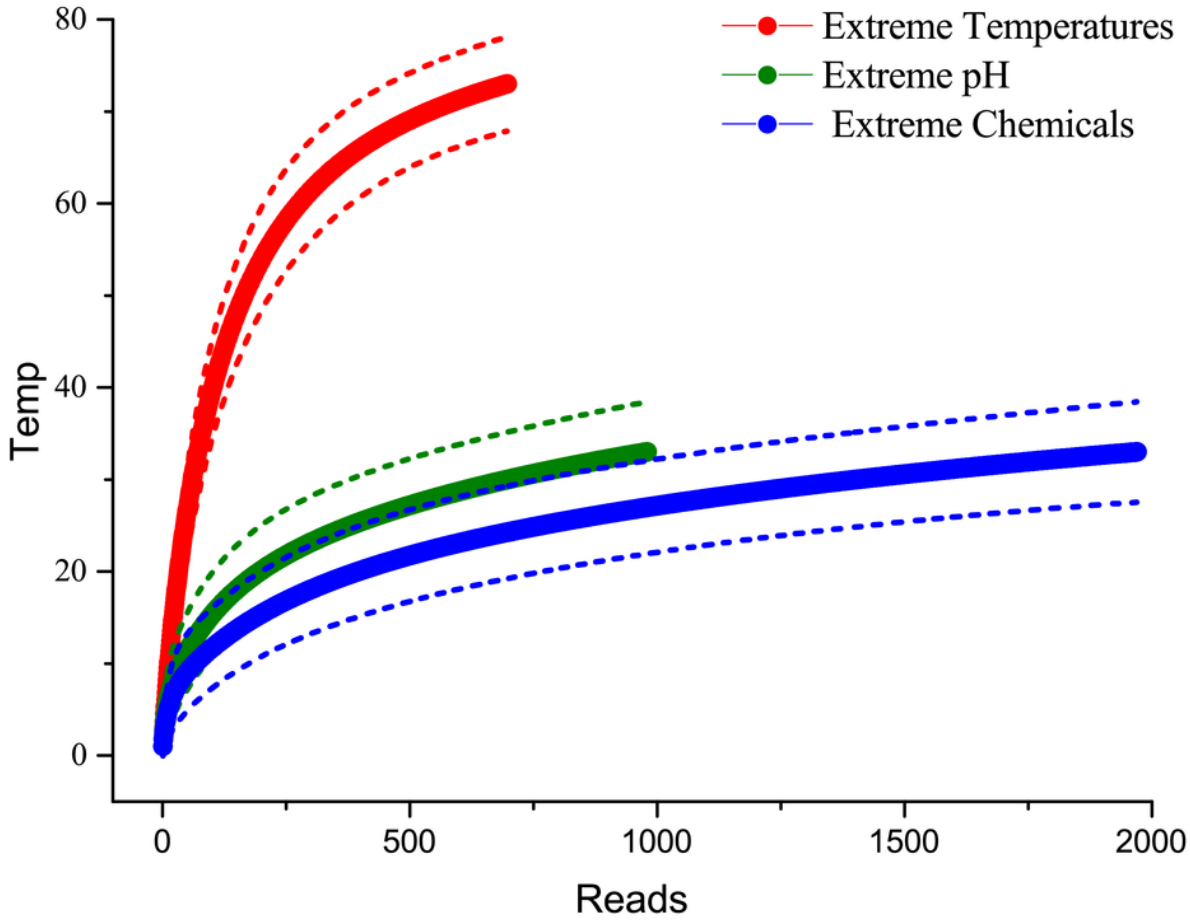

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Figure 2a: Extreme vs. intermediate temperatures

Comparison of rarefaction curves between extreme and non-extreme habitats. (a) extreme *vs.* intermediate temperatures, (b) extreme *vs.* neutral pH environments, and (c) extreme chemicals present *vs.* absent. Rarefaction curves are expressed as number of OTU by number of reads from sequencing. Each curve was constructed using 1000 iterations, and the dotted lines represent 95% confidence intervals. s7.add[i6? >

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Figure 2b: Extreme vs. neutral pH environments

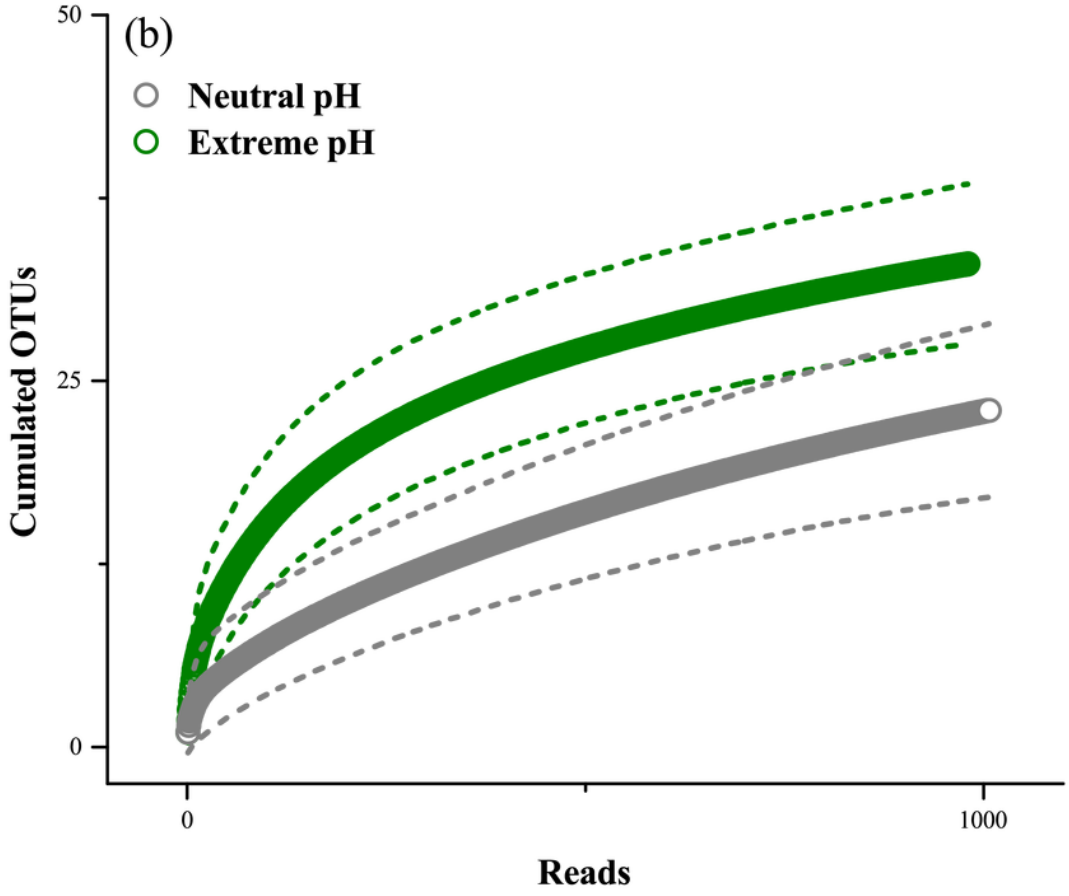

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Figure 2c: Extrme chemicals present vs. absent

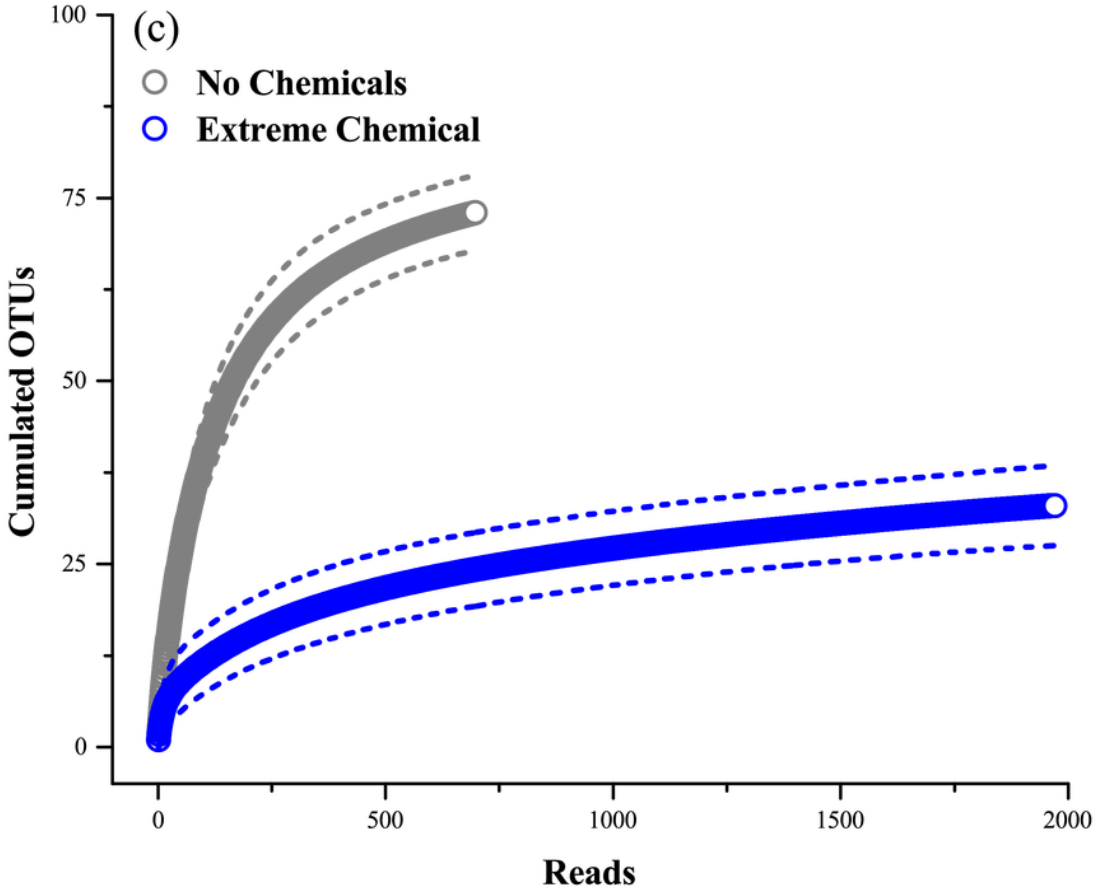

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Figure 3a: Temperature & pH

NMDS ordinations OTU occurrence by (a) Temperature & pH and (b) Temperature & chemical environments in the home. Symbols represent centroids ± 1 SE. 2-D stress was 0.18. and wi6&#65533;6&#65533;>

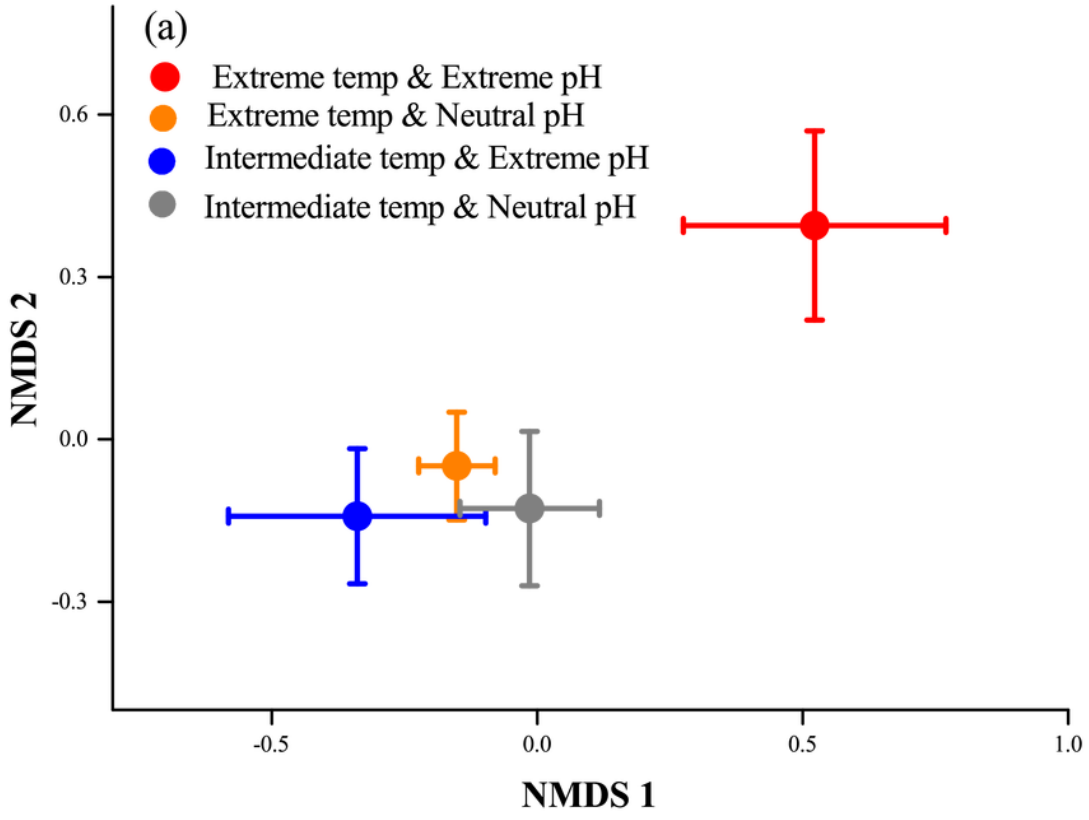

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Figure 3b: Temperature & chemical environments