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Response of the rare biosphere to environmental disturbance in a highly diverse ecosystem (Zodletone spring, OK, USA)

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Within highly diverse ecosystems, the majority of bacterial taxa are present in low abundance as members of the rare biosphere. The rationale for the occurrence and maintenance of the rare biosphere, and the putative ecological role(s) and dynamics of its members within a specific ecosystem is currently debated. We hypothesized that in highly diverse ecosystems, a fraction of the rare biosphere acts as a backup system that readily responds to environmental disturbances. We tested this hypothesis by subjecting sediments from Zodletone spring, a sulfide- and sulfur-rich spring in southwestern OK, to incremental levels of salinity (1, 2, 3, 4, and 10% NaCl), or temperature (28^0, 30^0, 32^0, and 70^0C), and traced the trajectories of rare members of the community in response to these manipulations using 16S rRNA gene analysis. Our results indicate that multiple rare bacterial taxa are promoted from rare to abundant members of the community following such manipulations and that, in general, the magnitude of such recruitment is directly proportional to the severity of the applied manipulation. Rare members that are phylogenetically distinct from abundant taxa in the original sample (unique rare biosphere) played a more important role in the microbial community response to environmental disturbances, compared to rare members that are phylogenetically similar to abundant taxa in the original sample (non-unique rare biosphere). The results emphasize the dynamic nature of the rare biosphere, and highlight its complexity and non-monolithic nature.

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Abstract

Within highly diverse ecosystems, the majority of bacterial taxa are present in low abundance as members of the rare biosphere. The rationale for the occurrence and maintenance of the rare biosphere, and the putative ecological role(s) and dynamics of its members within a specific ecosystem is currently debated. We hypothesized that in highly diverse ecosystems, a fraction of the rare biosphere acts as a backup system that readily responds to environmental disturbances. We tested this hypothesis by subjecting sediments from Zodletone spring, a sulfide- and sulfur-rich spring in southwestern OK, to incremental levels of salinity (1, 2, 3, 4, and 10% NaCl), or temperature (28^0, 30^0, 32^0, and 70^0C), and traced the trajectories of rare members of the community in response to these manipulations using 16S rRNA gene analysis. Our results indicate that multiple rare bacterial taxa are promoted from rare to abundant members of the community following such manipulations and that, in general, the magnitude of such recruitment is directly proportional to the severity of the applied manipulation. Rare members that are phylogenetically distinct from abundant taxa in the original sample (unique rare biosphere) played a more important role in the microbial community response to environmental disturbances, compared to rare members that are phylogenetically similar to abundant taxa in the original sample (non-unique rare biosphere). The results emphasize the dynamic nature of the rare biosphere, and highlight its complexity and non-monolithic nature.

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Introduction

Microbial communities in nature display a bewildering array of phylogenetic diversity. Our appreciation of the scope of bacterial diversity has been bolstered by the wide utilization of high throughput sequencing technologies in diversity surveys (Carbonetto et al. 2014; de Oliveira & Margis 2015; Giamarellos-Bourboulis et al. 2015; Hassenruck et al. 2015; Jagathrakshakan et al. 2015; Luhrig et al. 2015; Navarrete et al. 2015; Orr et al. 2015; Peng et al. 2015). Pyrosequencing- and Illumina-based studies could yield millions of amplicon sequence reads (usually 16S rRNA) from a single study (Delhaes et al. 2012; Filkins et al. 2012; Goddard et al. 2012; Zhao et al. 2012). Collectively, these studies have revealed that microbial communities in highly diverse samples exhibit a distribution pattern where the majority of bacterial species are present in extremely low numbers (Ashby et al. 2007; Bowen et al. 2012; Logares et al. 2014; Pedros-Alio 2006; Sogin et al. 2006). This fraction of the microbial community has been referred to as the rare biosphere (Sogin et al. 2006).

Multiple studies have examined various characteristics of the rare biosphere, e.g. its proportional size within a specific sample, phylogenetic affiliations of its members, biogeography and ecology of its members, as well as its spatial and temporal dynamics in a specific habitat (Alonso-Saez et al. 2015; Alonso-Saez et al. 2014; Anderson et al. 2015; Gobet et al. 2012; Hugoni et al. 2013; Liu et al. 2015; Reveillaud et al. 2014; Shade & Gilbert 2015; Shade et al. 2014; Youssef et al. 2010). All studies invariably suggest that the rare biosphere does not represent a phylogenetically or functionally monolithic group. For example, studies have shown that members of the rare biosphere exhibit a wide range of phylogenetic novelty (Elshahed et al. 2008; Lynch et al. 2012; Pester et al. 2012; Youssef et al. 2012): While a fraction of the rare biosphere is typically novel (at the phylum, class, order, or family levels), many others are closely related to previously described lineages in other ecosystems. Similarly,

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members of the rare biosphere exhibit a wide range of uniqueness (Elshahed et al. 2008; Galand et al. 2009), with a fraction being unique (i.e. bears no resemblance to other members in the community), while others are very closely related to more abundant members of the community. On a functional level, several studies have documented a large variation in the level of metabolic activity of members of the rare biosphere (by studying rRNA/rDNA transcript to gene ratios) within a single sample (Campbell et al. 2011), ranging from apparent dormancy to disproportionately high metabolic activity (Besemer et al. 2012; Jones & Lennon 2010; Logares et al. 2014; Pester et al. 2010; Pester et al. 2012; Wilhelm et al. 2014).

Maintenance of members of the rare biosphere within a specific ecosystem suggests that its members perform essential ecological functions. As described above, the involvement of members of the rare biosphere in mediating keystone physiological functions has been documented. Another plausible contribution of the rare biosphere to ecosystem functions is by acting as a backup or seed system that responds to various levels of environmental fluctuations, ranging from seasonal changes in temperature, pH, light exposure, and nutrient levels, to more drastic environmental disturbances (e.g. desertification, change in redox potential, hydrocarbon spill) (Crump et al. 2012; Elshahed et al. 2008; Lennon & Jones 2011; Marchant et al. 2002; Taylor et al. 2013; Walke et al. 2014). Under this scenario, environmental disturbances could induce the growth and promotion of specific members of the rare biosphere that are metabolically and physiologically more adapted to the new prevailing condition, and this promotion is coupled to the demotion of formerly abundant members in the community inadequately adapted to the new conditions (Elshahed et al. 2008). Such dynamic process contributes to the functional resilience of a specific ecosystem. It could also explain the observed higher diversity, and functional redundancy in diverse environments, and the retention of the rare

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biosphere in such systems (Shade et al. 2012; Yachi & Loreau 1999).

We reason that such hypothesis could be experimentally evaluated by stepwise subjection of a microbial community to a gradient of environmental disturbances of varying magnitudes, and observing the associated patterns of promotion and demotion within rare and abundant members of the community. Here, sediments obtained from Zodletone spring, a highly diverse anoxic spring in southwestern Oklahoma, USA, were subjected to various incremental degrees of salinity or temperature shifts. The microbial community was examined pre and post enrichment to characterize the dynamics of microbial community shifts associated with such processes. Our results support the proposed functionality and role of the rare biosphere in responding to environmental disturbances, and highlight the role of ecosystem diversity in imparting functional resilience to a specific ecosystem.

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Materials and Methods

Sampling. Sediment samples were collected from Zodletone spring, a sulfide and sulfur-rich spring in southwestern Oklahoma. The spring geochemistry has been previously described in detail (Bühring et al. 2011; Senko et al. 2004). The source of the spring is a contained area (1 m^2) with anaerobic, biomass-laden, and sulfide-rich black viscous sediments, and anoxic, sulfide-rich (8.4 mM) 40-cm water column. Spring source water was also sampled for use in enrichments as detailed below. Samples were stored on ice until returning to the lab, where they were used for the experimental procedures described below within 24h of sampling.

Enrichments preparation. The overall experimental setup is shown in Figure 1. Enrichments were prepared in Balch tubes under anaerobic conditions in an anaerobic chamber (Coy Laboratories, Grass Lake, MI, USA) by adding 1g sediment to 4 ml autoclaved anoxic spring water. For this purpose, spring source water was autoclaved, cooled under a stream of N_2 to maintain anaerobic conditions, and used in all enrichments. To mimic environmental disturbances, either salinity or temperature was varied in the enrichments. Salinity was adjusted by adding NaCl to increase the concentration to 1%, 2%, 3%, 4%, or 10% above ambient concentration (measured at 0.9%) and these enrichments were incubated at room temperature. Temperature was varied by incubating enrichments at 28ºC, 30ºC, 32ºC, or 70ºC. For each condition, triplicate enrichments were incubated for 60 days in the dark. To account for any changes that could occur due to the incubation process itself, triplicate control enrichments were prepared by incubating 1g sediment in 4 ml autoclaved anoxic spring water with no further changes in salinity or temperature.

DNA extraction, PCR amplification and sequencing. Triplicates were pooled, centrifuged at 10,000 xg for 20 minutes and DNA was extracted from the obtained pellet using MoBio

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113 FastDNA Spin kit for Soil (MoBio, Carlsbad, CA) following the manufacturer's instructions, and
114 subsequently quantified using Qubit® fluorometer (Life Technologies, Grand Island, NY).
115 Variable regions V1 and V2 of the 16S rRNA gene were then amplified using barcoded primers
116 for multiplex sequencing using the FLX technology. The forward primer was constructed by
117 adding 454 Roche FLX adaptor A (GCCTCCCTCGCGCCATCAG) to the 27F primer sequence
118 (AGAGTTTGATCCTGGCTCAG) as previously described (Youssef et al. 2010). The forward
119 primer also contained a unique barcode (octamer) sequence for multiplexing. The reverse primer
120 was constructed by adding 454 Roche FLX adaptor B (GCCTTGCCAGCCCGCTCAGT) to the
121 338R primer sequence (GCTGCCTCCCGTAGGAGT). PCRs were conducted in 100 µl volume.
122 The reaction contained 4 µl of the extracted DNA, 1× PCR buffer (Promega, Madison, WI), 2.5
123 mM $MgSO_4$, 0.2 mM dNTPs mixture, 0.5U of the GoTaq flexi DNA polymerase (Promega,
124 Madison, WI), and 10 µM each of the forward and the reverse primers. PCR was carried out
125 according to the following protocol; initial denaturation at 95°C for 5 minutes, followed by 35
126 cycles of denaturation at 95°C for 45 sec, annealing at 52°C for 45 sec, and elongation at 72°C
127 for 30 sec. A final elongation step at 72°C for 5 minutes was included. All PCR reactions were
128 run in at least triplicates, and the resulting products of the expected size were combined and
129 purified using QIAquick PCR cleanup kit (Qiagen Corp., Valencia, CA). Purified PCR products
130 (11-15 µg) were sequenced using FLX technology at the Environmental Genomics Core facility
131 at the University of South Carolina.
132 **Sequence quality filtering, OTU identification, and phylogenetic assignments.** Sequence
133 quality control was handled in mothur (Schloss et al. 2009) as described previously (Youssef et
134 al. 2010). Briefly, sequences with an average quality score below 25, sequences that did not have
135 the exact primer sequence, sequences that contained an ambiguous base (N), sequences having a

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homopolymer stretch longer than 8 bases, and sequences shorter than 80 bp were considered of poor quality and removed from the data set. High-quality reads from each treatment condition were aligned against the Greengenes alignment database using a Needleman-Wunsch pairwise alignment algorithm. Filtered alignments were used to generate an uncorrected pairwise distance matrix, followed by binning the sequences into operational taxonomic units (OTUs) at 3, 6, 8, 10, and 15% cutoffs. For phylogenetic placement, representative OTUs defined at the 3% cutoff ($OTU_{0.03}$) were classified with Greengenes taxonomy scheme using the PyNAST pipeline (Caporaso et al. 2010). Phylum level affiliation of sequences were determined according to the classifier output, and sequences with less than 85% similarity to their closest relative in Greengenes database were considered unclassified.

Defining the rare biosphere. The cutoff for defining rare biosphere is arbitrary and the methods used include relative abundance cutoffs, as well as frequencies of occurrence in a dataset (Youssef et al. 2010). For the sake of this study, we used a more relaxed definition for the rare biosphere, where rare members of the microbial community were identified using an empirical cutoff of $n \leq 10$.

Examining microbial community response to environmental disturbances.

Our analysis had two main goals: To identify differences in the overall microbial community structure and diversity pre and post-enrichment, and to examine and quantify the contribution of various members of the rare biosphere in response to environmental disturbances.

For the first goal (i.e. identification of differences in the overall microbial community structure and diversity due to environmental disturbances), microbial diversity was quantified using various diversity indices e.g. Shannon-Weiner, and Simpson diversity indices, Chao, and ACE estimators of species richness across enrichments. As well, Beta diversity across samples

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was examined using rarefaction curve ranking since this method is not sensitive to sample size as described before (Youssef & Elshahed 2008). Finally, variations in community structure due to disturbances were calculated using Morisita-Horn index. This index was chosen due to its insensitivity to sample size variations (Anderson & Millar 2004). Values obtained were subsequently employed to construct non-metric multidimensional scaling (NMDS) plots for community comparisons using the command nmds in mothur. The proportion of variance (r^2) among communities was estimated from the NMDS plots by first calculating the Euclidean distance between all pairs of data points using the equation; $d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$, where d is the Euclidean distance between 2 points of coordinates (x_1, y_1) and (x_2, y_2) in ordination space. The Euclidean distance was then regressed on the beta-diversity index to estimate r^2.

Our strategy to achieve the second goal (i.e. understanding the contribution of the rare biosphere to environmental disturbances) is based on the identification of the *abundant* members of the community post disturbance in different enrichments, and tracing their origins to the various fractions of the community in the control incubation. This allows for quantifying the contribution of the rare biosphere in the no-treatment control incubation to the microbial community that developed post disturbance. The promotion of specific members of the rare biosphere in the no-treatment control incubation to abundant members of the community post enrichments suggests a role for such members in responding to environmental disturbances. Also, the relative abundances of such members in the post-enrichment community could be regarded as an indicator of the magnitude of importance of this promotion process.

Details of the analysis conducted is shown in Figure 1. First, sequences obtained from the no-treatment control incubation were classified into either “abundant”, “unique rare”, or “non-unique rare” classes using the classification criteria detailed before (Youssef et al. 2010). Briefly,

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182 “abundant” refers to all sequences binned into OTUs with >10 representatives, “unique rare”
183 refers to all sequences binned into OTUs with ≤ 10 representatives and that are phylogenetically
184 distinct from more abundant members of the community in the original enrichments (>85%
185 sequence similarity), while “non-unique rare” refers to all sequences binned into OTUs with ≤ 10
186 representatives and that are phylogenetically similar to more abundant members of the
187 community in the original enrichments (Youssef et al. 2010). We then queried all abundant
188 (n>10) $OTU_{0.03}$ representatives from individual post-disturbance treatments against all sequences
189 recovered from the no-treatment control experiment (14,071 sequences) using local Blastn.
190 Identification of the best “hit” for each abundant $OTU_{0.03}$ representative post-disturbance
191 allowed us to trace its origin in the control sample into either “originating from abundant”,
192 “originating from unique rare”, or “originating from non-unique rare” sequence. Abundant post-
193 disturbance OTUs originating from an “abundant” control sequence represent the “no change”
194 effect, where an abundant member remained abundant post-disturbance. Abundant post-
195 disturbance OTUs originating from a “rare” control sequence represent the “promotion” effect,
196 where a rare member of the original enrichment was promoted to a more abundant OTU post-
197 disturbance. This latter group could be further subdivided into two distinct categories: a.
198 Abundant post-disturbance OTUs “originating from non-unique rare” control sequence. And b.
199 Abundant post-disturbance OTUs “originating from unique rare” control sequence. Based on
200 these results, we calculated the percentage of post-disturbance abundant OTUs that were
201 “originating from abundant”, “originating from unique rare”, or “originating from non-unique
202 rare” sequences, and correlated these values to the severity of enrichment (salinity or
203 temperature) using Pearson correlations.
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Results

Effect of environmental disturbances on overall microbial community. A total of 62,306 high quality sequences were obtained in this study. Estimates of diversity, species richness, evenness, and beta diversity at the species level ($OTU_{0.03}$) in various enrichments are shown in Table 1. In general, the increase in temperature negatively affected the community diversity (Pearson correlation coefficient = -0.82), with the magnitude of diversity loss directly correlated to the prevailing temperature. On the other hand, all salinity treatments resulted in an increase in community diversity compared to the no-treatment control, as evident by diversity ranking estimates obtained (Table 1). However, that increase was not directly correlated to increments in salinity (Pearson correlation coefficient = 0.06).

At the phylum level, 40 distinct bacterial phyla and candidate phyla were identified in this study. The highest phylum level diversity was observed in the no-treatment control enrichment (35 phyla, Table S1, Figure 2). Similar to diversity statistics at the putative species

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level, the number of phyla identified was negatively correlated to the enrichment temperature (Pearson correlation coefficient= -0.66), while salinity had no clear effect on phylum level diversity (Pearson correlation coefficient = -0.09) (Table 1).

To study the effect of disturbances on the overall phylum-level community composition, we utilized likelihood-ratio-Chi-squared test to examine the significant difference between the relative abundances of phyla in the no-treatment control versus the various enrichments. This method failed to identify any significant difference (likelihood ratio $\chi 2 = 99.4$, $p = 1$). Nevertheless, Pearson correlations between specific phyla relative abundance in the various enrichments, and the enrichment condition (salinity or temperature) identified the following patterns. The increase in the enrichment temperature was positively correlated to the percentage abundance of Firmicutes, Aminicenantes (candidate division OP8), Parcubacteria (candidate division OD1), and Thermotogae (Pearson correlation coefficients = 0.98, 0.9, 0.78, and 0.77, respectively), and negatively correlated to the percentage abundances of Bacteroidetes, Marinimicrobia (candidate division SAR406), Verrucomicrobia and Latescibacteria (candidate division WS3) (Pearson correlation coefficients = -0.9, -0.73, -0.77, and -0.79, respectively) (Figure 2 and Table S1). The increase in enrichment salt concentration was positively correlated to the percentage abundance of Firmicutes, and Thermotogae (Pearson correlation coefficients = 0.53, and 0.52, respectively), and negatively correlated to the percentage abundances of Verrucomicrobia and Latescibacteria (candidate division WS3) (Pearson correlation coefficients = -0.6, and -0.73, respectively) (Figure 2 and Table S1).

Shifts in dominant microbial populations post disturbance. To zoom in on the impact of disturbances on the microbial community, we examined the occurrence and magnitude of shifts in the structure of abundant community members post enrichments, compared to the no-

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treatment control. Multiple evidences suggest a shift in the abundant community post disturbance: 1. The proportion of sequences belonging to abundant OTUs (n>10) decreased post enrichment (Table 1), 2. High levels of beta diversity was observed between the no-treatment incubation and all salinity and temperature enrichments. The abundant no-treatment community showed Morisita Horn indices of 0.63±0.07, and 0.57±0.3 for all possible pair wise comparisons with the salinity, and temperature, post-disturbance abundant communities, respectively. Indeed, non-metric multidimensional scaling plot using Morisita-Horn indices clearly shows a shift in the abundant community structure following disturbances (Figure 3), 3. Finally, phylogenetic affiliations of abundant members of the community following disturbances showed marked differences from those in the no treatment control (Figure 4).

Origins of the abundant community members following enrichment support a role for the rare biosphere in ecosystem resilience. We hypothesized that the differences observed in dominant members of the community following disturbances are due to recruitment of organisms from the rare biosphere to become part of the abundant members of the communities. To this end, we sought to identify the origin of all members of the abundant community post disturbance (Figure 1) and determine their origin (abundant, rare unique, and rare non unique) in the no-treatment control community. Our analysis identified three distinct patterns (Table 2): 1. Post-enrichment abundant OTUs that were similar to "abundant" sequences in the no-treatment control. Of the total number of abundant OTUs, those constituted 36.7-91.1% in various conditions. As expected from the community structure analysis, the highest percentages were encountered with the 28ºC and 30ºC incubations. These disturbances resulted in an abundant community structure very similar to the no-treatment control (Figure 3). The percentage of those abundant OTUs decreased as the enrichment salinity, and temperature increased (Pearson

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correlation coefficient = -0.6, and -0.86 for salinity, and temperature, respectively). 2. The rest of the abundant OTUs in the enrichments (8.9-63.3%) were recruited from the rare biosphere, providing direct evidence that the rare biosphere could act as a backup system to respond to environmental disturbances. Within this group, we differentiate between two distinct factions: A. Post-enrichment abundant OTUs that were promoted from "rare non-unique" sequences in the no-treatment control, i.e. rare members of the original community phylogenetically similar to more abundant members of the community. Of the total number of abundant OTUs, those constituted 2.6-10.2% in various conditions. This percentage decreased as the enrichment salinity, and temperature increased (Pearson correlation coefficient = -0.86 for both salinity, and temperature). And B. Post-enrichment abundant OTUs that were promoted from "rare unique" sequences in the no-treatment control, i.e. rare members of the original community phylogenetically distinct from more abundant members of the community. Of the total number of abundant OTUs, those constituted 1.8-60.7% of abundant OTUs in various conditions. This percentage increased as the enrichment salinity, and temperature increased (Pearson correlation coefficient = 0.85, and 0.86 for salinity, and temperature, respectively). Therefore, we argue that, while both factions of the rare biosphere are important in responding to changes in environmental conditions and both act to recruit members to the abundant community, the magnitude of contribution of the "non-unique" rare biosphere to the promotion process was less significant. On the other hand, the "unique" rare biosphere seemed to contribute to the promotion process both when the environmental disturbance was slight, e.g. similar to what would happen during diurnal variation in salinity and temperature, as well as severe, e.g. similar to what would be encountered during seasonal variation in temperature or salinity, or following a drastic change in environmental condition, e.g. drought or fire. This latter effect is so evident in the 10% salt,

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297 and the 70ºC incubation, where 55%, and 61%, respectively of the abundant community was

298 recruited from the "unique" rare biosphere (Table 2).

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Discussion

In this study, we experimentally evaluated the response of microbial community from Zodletone spring source sediments to environmental disturbances (various levels of elevated salinities or temperatures), with emphasis on understanding the role of the rare biosphere in the process. We demonstrate that: 1. Rare bacterial taxa could be promoted to abundant members of the community following environmental manipulations, 2. The magnitude of this promotion process is directly proportional to the severity of the disturbances, and 3. Rare members that are phylogenetically distinct from abundant taxa in the original sample (unique rare biosphere) play a more important role in microbial community response to environmental disturbances.

The results provide experimental proof for several proposed ideas regarding the function and maintenance of the rare biosphere. Prior studies have speculated that the rare biosphere acts as a backup system or a microbial seed bank that preserves microbial community-level function in face of disturbances (Elshahed et al. 2008; Lynch & Neufeld 2015; Reid & Buckley 2011). This fraction of the rare biosphere has been referred to as "conditionally rare taxa". Evidences of the presence of conditionally rare taxa in diverse environments are accumulating (Aanderud et al. 2015; Shade & Gilbert 2015; Shade et al. 2014; Sjostedt et al. 2012; Taylor et al. 2013; Walke et al. 2014). These taxa show cyclical low abundance until conditions become favorable, where they respond to the change and increase in abundance. Previous studies showed that such taxa could make up to 28% of the community (Shade et al. 2014). Conditionally rare taxa contribute greatly to the α-diversity of an ecosystem. The presence of a diverse low-abundance fraction ensures the resilience of the ecosystem, and increases the ecosystem's ability to maintain its functions during environmental changes (Shade et al. 2012; Yachi & Loreau 1999). In the current study, conditionally rare taxa (those that are members of the rare biosphere in the no-

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treatment control incubation, but were promoted to become abundant members in different incubations) constituted 8.9-63.3% of the microbial community under various conditions of elevated temperatures or salinity. This provides direct evidence that this fraction in the rare biosphere acts as a backup or seed system.

Within these conditionally rare taxa that increased in abundance in response to environmental disturbances, we differentiate between two different fractions, those that were promoted from "rare unique" sequences in the no-treatment control, i.e. rare members of the original community phylogenetically distinct from more abundant members of the community, and those that were promoted from "rare non-unique" sequences in the no-treatment control i.e. rare members of the original community phylogenetically similar to more abundant members within the original community. Our results suggest that the rare unique members of the microbial community are more important for ecosystem resilience and response to disturbances, since the majority of conditionally rare taxa identified in various enrichments were unique in the no-treatment control incubation, e.g. 37% to ~61% for temperature incubations ≥ 32ºC, and 37% to ~55% for salt incubations (Table 2). The magnitude of contribution of the rare unique fraction to the abundant community increased with the severity of disturbance (i.e. with the increase in salinity as well as temperature of enrichment). Interestingly, the abundant community in the most severe "unnatural" condition (the enrichment at 70ºC) was mostly (60.7%) made-up from representatives of the "unique" rare biosphere (Table 2), confirming what was shown before in arctic marine sediments (Hubert et al. 2009), and freshwater stratified lakes (Shade et al. 2012) that a fraction of the conditionally rare taxa exhibit greatly reduced metabolic activity under the natural environmental conditions but is able to exploit the "forced" manipulation of conditions (e.g. high temperature incubations of arctic sediments, or complete anoxic conditions in the

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hypolimnion layers of the lake) and become abundant. The "non-unique" rare biosphere, on the other hand, did not significantly contribute to the abundant community post-disturbance, with the highest representation of its members to the post-disturbance abundant community being 10% in the 1% salt enrichment (Table 2), and the magnitude of its contribution to the abundant community decreasing with the severity of disturbance.

In addition to conditionally rare taxa, the post-disturbance abundant community was also partly made-up (36.7-91.1%) of previously abundant members in the no-treatment control, where microorganisms capable of coping with the new condition (increased salt or temperature) without any appreciable loss in fitness remained abundant. It is telling that the highest magnitude of contribution of the pre-disturbance abundant members to the post-disturbance abundant community was observed in the 28ºC (91.1%) and 30ºC (85.6%) enrichments, since these two conditions are the closest to the no-treatment control. While those numbers could possibly be inflated by lingering DNA from cells that were originally abundant in the no-treatment control but that lysed or became inactive during enrichment (what has previously been referred to as taphonomic gradient), the observation that the magnitude of contribution of the pre-disturbance abundant members to the post-disturbance abundant community decreased with the increase in the severity of disturbance is highly logical. The abundant members of the community under certain conditions are those microorganisms that are most adapted to their current environment, and as the conditions change, those members are also expected to change in abundance and/or metabolic activity. That also explains the difference observed in the abundant community structure pre and post disturbance (Figure 4).

Conclusions

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In conclusion, the current study provided direct evidence for the contribution of members of the rare biosphere to the abundant community post environmental disturbances, and hence confirming the notion that a fraction of the rare biosphere acts as a backup system for maintaining ecosystem resilience in face of perturbation. While we show here, similar to previous studies ((Lynch & Neufeld 2015), and references within), that the rare biosphere seed system responds to both periodical (e.g. temperature and seasonal changes), as well as drastic (e.g. what would be encountered in drought or fire) changes in the ecosystem conditions, the disproportionate contribution of rare members whose phylogenetic affiliations are distinct when compared to more abundant members of the community has not been shown before and reinforces the non-monolithic nature of the rare biosphere.

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Figure legends

Figure 1. A flowchart depicting the experimental design. Abundant post-disturbance OTUs originating from an "abundant" control sequence represent the "no change" effect, where an abundant member remained abundant post-disturbance. Abundant post-disturbance OTUs originating from a "rare" control sequence represent the "promotion" effect, where a rare member of the original enrichment was promoted to a more abundant OTU post-disturbance. This latter group could be further subdivided into two distinct categories: a. Abundant post-disturbance OTUs "originating from non-unique rare" control sequence. And b. Abundant post-disturbance OTUs "originating from unique rare" control sequence. * salinity percentage indicates salt concentration above ambient values (calculated at 0.9%).

Figure 2. Heatmap of the percentage abundance of phyla encountered in the different enrichments versus the no-treatment control. Gracilibacteria denotes candidate division GN02, Marinimicrobia denotes candidate division SAR406, Aminicenantes denotes candidate division OP8, Atribacteria denotes candidate division OP9, Parcubacteria denotes candidate division OD1, Saccharibacteria denotes candidate division TM7, and Latescibacteria denotes candidate division WS3. Other CD denotes other candidate divisions including AD3, CV51, KSB3, NC10, WS6, WPS-2, OP1, and OP11.

Figure 3. Non-metric multidimensional scaling based on pairwise Morisita-Horn dissimilarity indices between abundant members. Each symbol represents one enrichment condition. The temperature enrichments abundant communities are shown by (ᘓ) and each is labeled with the temperature. The salinity enrichments abundant communities are shown by (❋). The no-treatment control abundant community is shown by (◷). All salinity post-disturbance abundant communities clustered together away from the no-treatment control incubation, while the effect

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of temperature incubations on the abundant community structure was more complex. Abundant communities following incubations at 28ºC and 30ºC had a structure more similar to the no-treatment control (which was incubated at room temperature) than the abundant community following incubation at 32ºC. The most drastic effect on the abundant community structure was the 70ºC incubation. The 70ºC abundant community clustered alone to the far left of the NMDS plot reflecting a major shift in community structure, and showed an average Morisita Horn index of 0.94±0.04.

Figure 4. Effect of salinity (A), and temperature (B) on the phylogeny of abundant $OTUs_{0.03}$ following enrichment. The Y-axis (logarithmic scale) shows the percentage of abundant OTUs affiliated with each phylum on the X-axis as a fraction of all abundant OTUs encountered in the post-disturbance enrichment. At the species level ($OTU_{0.03}$), abundant OTUs (n>10) in the no-treatment control belonged to the phyla Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Marinimicrobia (candidate division SAR406), Nitrospirae, Aminicenantes (candidate division OP8), Atribacteria (candidate division OP9), Proteobacteria, Spirochaetes, and Thermotogae. This phylogenetic profile of abundant OTUs was maintained in all enrichment conditions with the following exceptions. The increase in the salt concentration of the enrichment (A): 1. Recruited sequences belonging to Chlorobi (1% salt enrichment), and Gemmatimonadetes (2% salt enrichment) to the abundant biosphere. 2. Demoted all sequences belonging to Marinimicrobia (candidate division SAR406), Nitrospirae, and Atribacteria (candidate division OP9) to the rare biosphere. 3. Resulted in the decrease in the number of abundant OTUs belonging to Bacteroidetes, and Spirochaetes, and the increase in the number of abundant OTUs belonging to Firmicutes, Chloroflexi, Actinobacteria, and Thermotogae. On the other hand, the increase in the enrichment incubation temperature (B): 1. Recruited sequences

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592 belonging to Chlorobi (32ºC enrichment) to the abundant biosphere. 2. Demoted all sequences
593 belonging to Chloroflexi, and Nitrospirae to the rare biosphere. 3. Resulted in the decrease in the
594 number of abundant OTUs belonging to Bacteroidetes, and the increase in the number of
595 abundant OTUs belonging to Firmicutes, and Actinobacteria. Also, a significant increase in the
596 percentage of abundant OTUs belonging to Aminicenantes (candidate division OP8) was
597 observed in the 70ºC enrichment, and a significant increase in the percentage of abundant OTUs
598 belonging to Atribacteria (candidate division OP9) was observed in the 28ºC enrichment.
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Figure 1(on next page)

Figure 1

A flowchart depicting the experimental design. Abundant post-disturbance OTUs originating from an “abundant” control sequence represent the “no change” effect, where an abundant member remained abundant post-disturbance. Abundant post-disturbance OTUs originating from a “rare” control sequence represent the “promotion” effect, where a rare member of the original enrichment was promoted to a more abundant OTU post-disturbance. This latter group could be further subdivided into two distinct categories: a. Abundant post-disturbance OTUs “originating from non-unique rare” control sequence. And b. Abundant post-disturbance OTUs “originating from unique rare” control sequence. * salinity percentage indicates salt concentration above ambient values (calculated at 0.9%).

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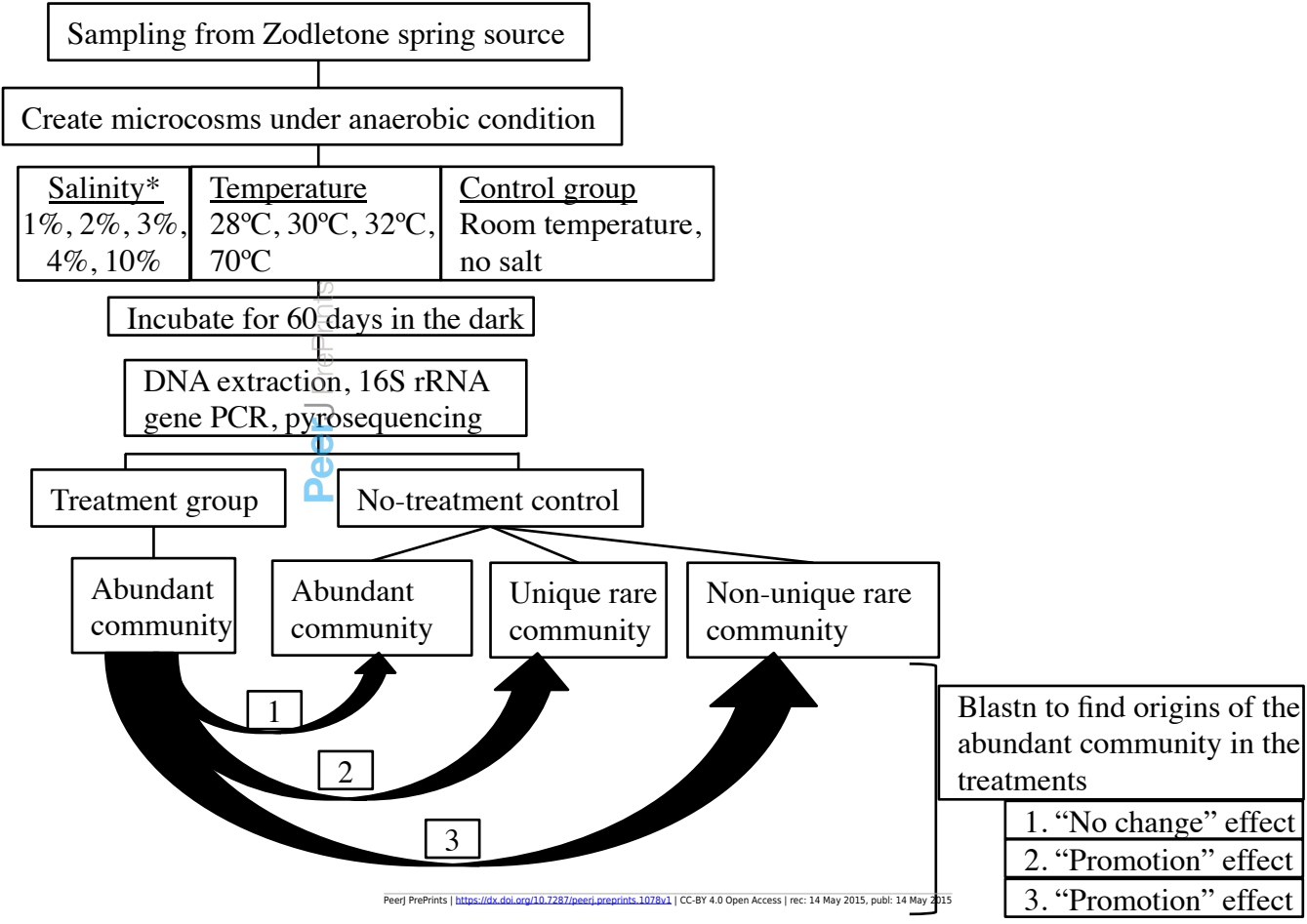

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Figure 2(on next page)

Figure 2.

Heatmap of the percentage abundance of phyla encountered in the different enrichments versus the no-treatment control. Gracilibacteria denotes candidate division GN02, Marinimicrobia denotes candidate division SAR406, Aminicenantes denotes candidate division OP8, Atribacteria denotes candidate division OP9, Parcubacteria denotes candidate division OD1, Saccharibacteria denotes candidate division TM7, and Latescibacteria denotes candidate division WS3. Other CD denotes other candidate divisions including AD3, CV51, KSB3, NC10, WS6, WPS-2, OP1, and OP11.

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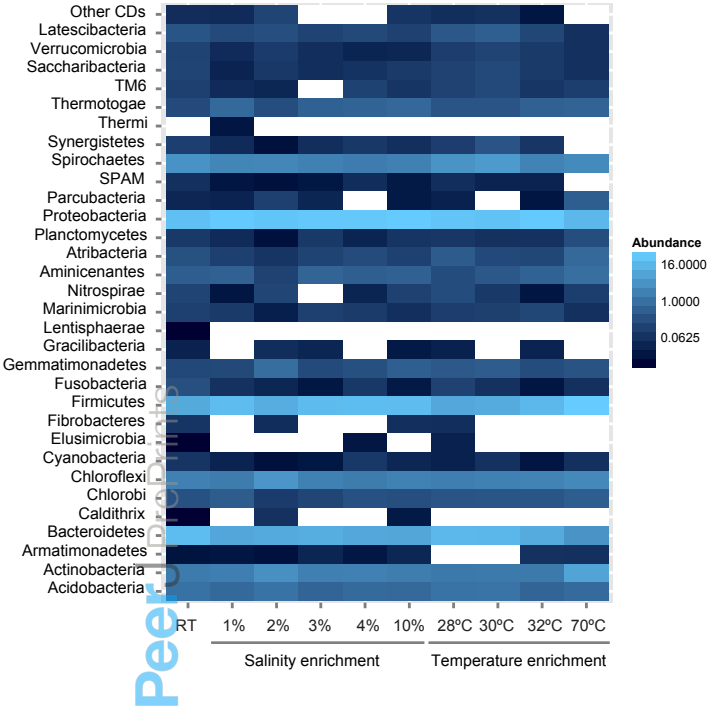

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Figure 3(on next page)

Figure 3

Figure 3. Non-metric multidimensional scaling based on pairwise Morisita-Horn dissimilarity indices between abundant members. Each symbol represents one enrichment condition. The temperature enrichments abundant communities are shown by ( ) and each is labeled with the temperature. The salinity enrichments abundant communities are shown by (¯). The no-treatment control abundant community is shown by (¿). All salinity post-disturbance abundant communities clustered together away from the no-treatment control incubation, while the effect of temperature incubations on the abundant community structure was more complex. Abundant communities following incubations at 28ºC and 30ºC had a structure more similar to the no-treatment control (which was incubated at room temperature) than the abundant community following incubation at 32ºC. The most drastic effect on the abundant community structure was the 70ºC incubation. The 70ºC abundant community clustered alone to the far left of the NMDS plot reflecting a major shift in community structure, and showed an average Morisita Horn index of 0.94±0.04.

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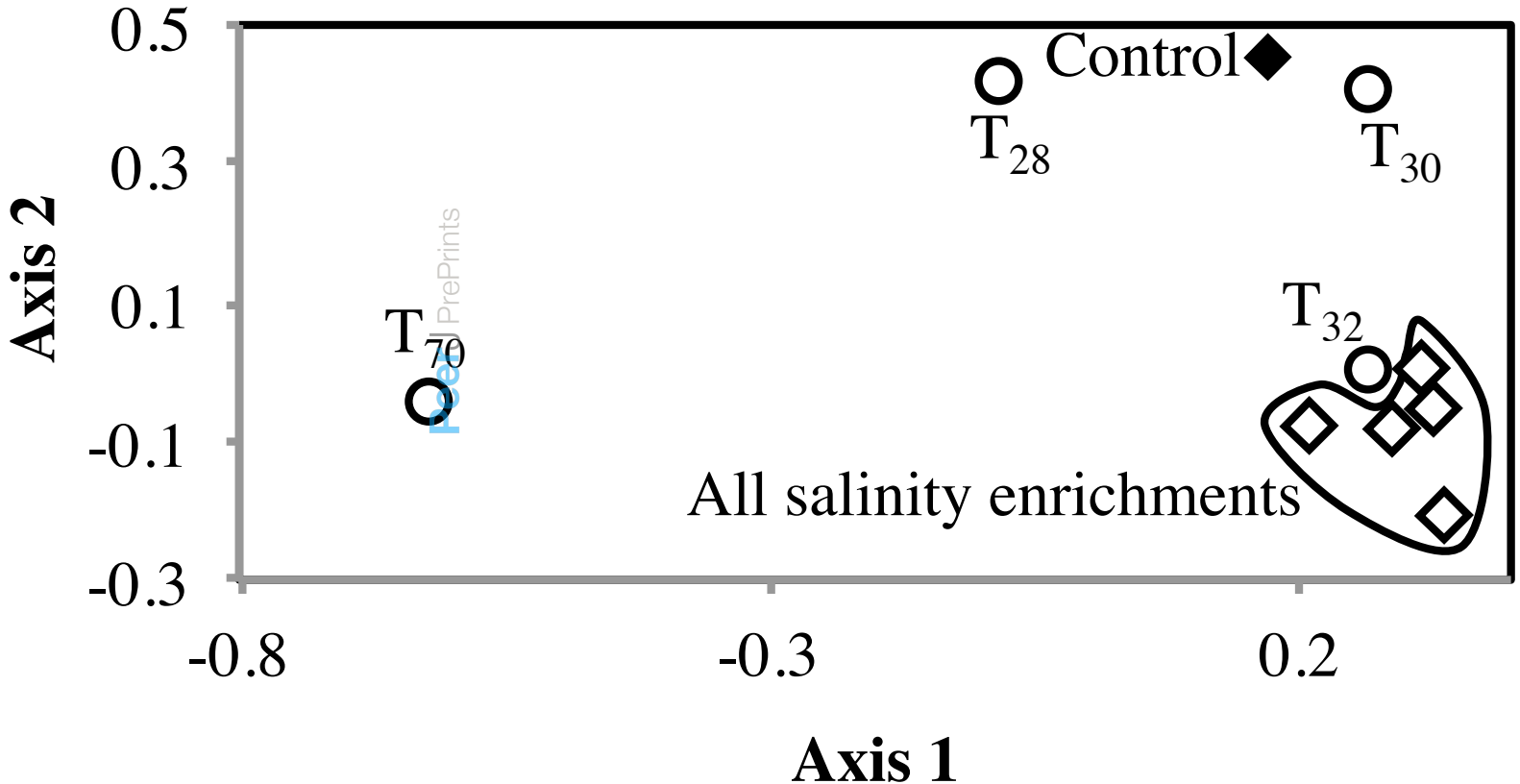

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Figure 4

Figure 4. Effect of salinity (A), and temperature (B) on the phylogeny of abundant $OTUs_{0.03}$ following enrichment. The Y-axis (logarithmic scale) shows the percentage of abundant OTUs affiliated with each phylum on the X-axis as a fraction of all abundant OTUs encountered in the post-disturbance enrichment. At the species level ($OTU_{0.03}$), abundant OTUs (n>10) in the no-treatment control belonged to the phyla Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Marinimicrobia (candidate division SAR406), Nitrospirae, Aminicenantes (candidate division OP8), Atribacteria (candidate division OP9), Proteobacteria, Spirochaetes, and Thermotogae. This phylogenetic profile of abundant OTUs was maintained in all enrichment conditions with the following exceptions. The increase in the salt concentration of the enrichment (A): 1. Recruited sequences belonging to Chlorobi (1% salt enrichment), and Gemmatimonadetes (2% salt enrichment) to the abundant biosphere. 2. Demoted all sequences belonging to Marinimicrobia (candidate division SAR406), Nitrospirae, and Atribacteria (candidate division OP9) to the rare biosphere. 3. Resulted in the decrease in the number of abundant OTUs belonging to Bacteroidetes, and Spirochaetes, and the increase in the number of abundant OTUs belonging to Firmicutes, Chloroflexi, Actinobacteria, and Thermotogae. On the other hand, the increase in the enrichment incubation temperature (B): 1. Recruited sequences belonging to Chlorobi (32ºC enrichment) to the abundant biosphere. 2. Demoted all sequences belonging to Chloroflexi, and Nitrospirae to the rare biosphere. 3. Resulted in the decrease in the number of abundant OTUs belonging to Bacteroidetes, and the increase in the number of abundant OTUs belonging to Firmicutes, and Actinobacteria. Also, a significant increase in the percentage of abundant OTUs belonging to Aminicenantes (candidate division OP8) was observed in the 70ºC enrichment, and a significant increase in the percentage of abundant OTUs belonging to Atribacteria (candidate division OP9) was observed in the 28ºC enrichment.

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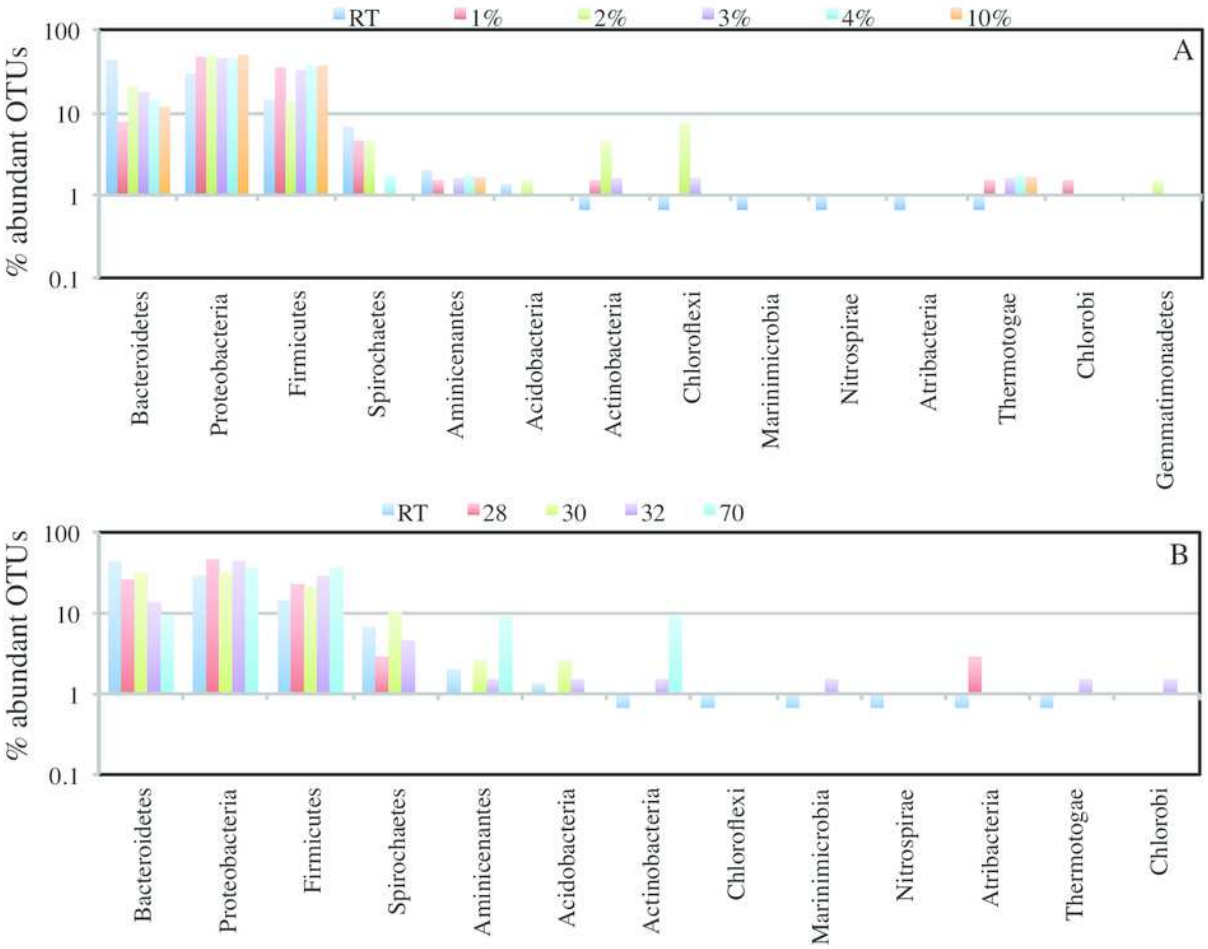

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Table 1(on next page)

Tables 1 and 2

Table 1. Overall diversity estimates in various enrichments versus the no-treatment control incubation. Table 2. Tracing the origins of abundant members in elevated salinities and temperature incubations into various fractions within no treatment control incubation.

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Table 1. Overall diversity estimates in various enrichments versus the no-treatment control incubation.

Treatment	Number of seqs	Alpha Diversity						Beta diversity
		$OTUs_{0.03}$	Number of phyla	% abundant	Simpson	Chao	Ace	Rarefaction curve rank
No-treatment 25ºC, 0.9% salt	14071	4011	35	50.05	0.07	11470	21911	4, 1[a]
Temperature enrichments								
28ºC	3783	1472	29	34.68	0.14	4095	7278	2
30ºC	3398	1393	26	29.78	0.22	3924	6838	3
32ºC	6603	2711	28	37.48	0.10	9532	18095	5
70ºC	1788	674	24	38.26	0.03	1498	2430	1
Pearson[b]			-0.66	-0.38				-0.82
Salt enrichments[c]								
1%	6669	2732	29	38.04	0.08	9643	20765	4
2%	8317	3269	34	40.21	0.07	11261	25532	6
3%	5856	2331	25	39.67	0.09	8811	15943	2
4%	6108	2563	27	36.87	0.09	8727	16535	5
10%	5511	2241	32	37.69	0.11	7146	14708	3
Pearson[b]			-0.09	-0.54				0.06

a: Numbers are rarefaction curve ranks of the no-treatment control incubation compared to the temperature, and the salinity enrichments, respectively.
b: Pearson correlation coefficient between the temperature (ºC)/ salinity (%) in the first column and various indices in the table header.
c: salinity percentage indicates salt concentration above ambient values (calculated at 0.9%).

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Table 2. Tracing the origins of abundant members in elevated salinities and temperature incubations into various fractions within no treatment control incubation.

	% of abundant OTUs in temperature and salinity enrichments recruited from[a]		
	Abundant	Unique rare	Non-unique rare
Temperature enrichment			
28ºC	91.14	1.77	7.08
30ºC	86.96	6.01	7.03
32ºC	58.62	36.93	4.44
70ºC	36.7	60.67	2.63
Pearson [b]	-0.87	0.86	-0.86
Salt enrichment [c]			
1%	45.44	44.34	10.21
2%	56.17	37.4	6.43
3%	47.31	46.1	6.58
4%	47.78	44.76	7.46
10%	41.98	54.55	3.47
Pearson [b]	-0.6	0.85	-0.86

[a]: The percentage of abundant OTUs in the temperature and salt enrichments that had Blastn best "hits" in the no-treatment control belonging to abundant, unique, and non-unique rare fractions. The percentage of abundant OTUs that were recruited from the unique rare fraction of the no-treatment control also includes those abundant OTUs that had no hits in the no-treatment control using the criteria described in Materials and Methods.

[b]: Pearson correlation coefficient between the temperature (ºC)/ salinity (%) in the first column and percentages recruited from the different fractions in the table header.

[c]: salinity percentage indicates salt concentration above ambient values (calculated at 0.9%).

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