Thermal disruption of soil bacterial assemblages decreases diversity and assemblage similarity

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Abstract. The metabolic theory of ecology assumes that rates of selection and adaptation for organisms are functions of temperature. Niche theory predicts that strong selection pressure should simplify assemblages as species are extirpated and taxa pre-adapted for the new environment thrive. Here, we use closed mesocosms to test the prediction that higher temperatures decrease species richness and increase assemblage similarity more and faster than lower temperatures. We incubated two temperate forest soil types at constant temperatures from 10° to 35°, destructively sampling mesocosms at 30, 180, and 440 d. We quantified taxonomic richness and assemblage similarity of soil bacteria using 16S rRNA gene amplicons. As predicted, mesocosms at higher temperatures lost more taxa than those at lower temperature. Contrary to predictions, the simplified assemblages at higher temperatures became less similar to each other over time. After 440 d of incubation, the number of taxa lost was a linear function of the difference between treatment temperature and site mean annual temperature, while assemblage similarity decreased as an accelerating function of this temperature difference.

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INTRODUCTION

A dominant theme in global change biology is the role of temperature as a selective filter where the temporally stable species in an assemblage have non-negative fitness and thus maintain population sizes at the temperatures they experience at that place and time. A corollary of a species' thermal niche matching their environments is that as environments change, assemblages should change predictably, with extirpations of those species with thermal niches further from

the new temperature regime, and increases in and/or colonization by species adapted to this new environment.

Soil bacteria are vital to ecosystems (Bell et al. 2005), yet the temporal stability of diversity and composition of soil microbial assemblages has not been well characterized. Most such studies focus on the dynamics of whole assemblage traits (e.g., respiration or microbial biomass, e.g., Bradford et al. 2008, de Vries and Shade 2013, Pold et al. 2016, Romero-Olivares et al. 2017). Given their immense diversity and short generation times, microbial assemblages should be able to react to changes in climate, especially temperature, faster than macrobes (Wallenstein and Hall 2011).

Predicting the dynamics of hyper-diverse microbial assemblages in a warming world is a major challenge to microbial ecology, one compounded by taxon-specific responses to temperature (Davis 1981, Bradford et al. 2008, Pold et al. 2016), the increased number of potential species interactions (Widder et al. 2016), and functional redundancy of microbial taxa (Wohl et al. 2004). Adding to these difficulties, temperature can have two major effects on microbial assemblage dynamics. First, temperature is a template for selection and adaptation (Garcia-Pichel et al. 2013). Second, temperature delimits generation time of microbes (Ratkowsky et al. 1982) and thus the rates at which species filtering and adaptation can occur.

Generation time in microbes scales with temperature (Ratkowsky et al. 1982). For example, generation time in *Escherichia coli* varies 2 orders of magnitude (from ~1.75 d to 21 min per generation) from 10°C to 40°C (Ingraham 1958). While cold temperatures can be fatal to bacteria (e.g., Rivkina et al. 2000), many have evolved the ability to survive cold temperatures (Walker et al. 2006, Schimel et al. 2007). Thus at lower (but not fatally low) temperatures, bacterial assemblages should change little, as they are dormant, less metabolically active, and not changing in abundance, which we will call "the cold-storage effect." At higher temperatures, bacterial assemblages have the capacity to change quickly as higher metabolic activity can alter resource concentrations and faster generation times can quickly result in large shifts in assemblage structure and diversity.

If the match between an environment's temperature and the thermal ecology of potential taxa ultimately determines taxon composition (e.g., through species filtering), then assemblages in a closed system (i.e., with no immigration) should change more and faster at higher temperatures. Higher temperatures should lead to more extirpations of species with relatively low critical thermal maxima, increasing the relative abundance of species (and therefore the likelihood of being sampled) with relatively high critical thermal maxima. Thus at higher temperatures, mesophilic and thermophilic bacteria (Rothschild and Mancinelli 2001) should do well relative to psychrophiles (Siddiqui et al. 2013). Higher temperatures also allow faster replacement, via decreased generation time for the species that have not yet hit their critical thermal maxima. Thermal niches matching home environments also mean that the change should be predictable, as environmental filtering would leave species from the subset of taxa that can survive the higher temperatures. Thus, without immigration and with a greater increase in temperature, microbial assemblages should lose more species and become more similar in composition.

If, on the other hand, taxa do not vary in their thermal ecology and share resource needs (i.e., neutral theory; Hubbell 2001), then the temporal dynamics (of a closed system) would be driven by changing resource concentrations. With higher metabolic rates driving faster resource consumption, assemblage change would be faster but follow a random walk where assemblages can become more different from each other over longer time periods and at higher temperatures.

Here, we use mesocosms stocked with soil (and their constituent bacterial flora) from two coniferous forests to examine how the diversity and compositional similarity of bacterial flora change over time across temperatures from 10°C to 35°C. Our expectation was that, through species filtering and/or adaptation, (1) individual site by temperature treatments (e.g., Niwot 35°C) would become more similar to each other over time; (2) that this homogenization would occur fastest at higher temperatures; and that (3) this homogenization will be more complete and happen more rapidly for soils with a greater temperature difference between the mean annual temperature of their home environments and our temperature treatments.

MATERIALS AND METHODS

Soil collection and incubation

We collected ~30 L of soil from the top 10 cm of mineral soils from two sites in July 2013. The first was from old-growth forest at H.J. Andrews Experimental Forest LTER in Oregon, USA (HJA), at 860 m elevation. The second site was from a spruce forest at Niwot Ridge LTER in Colorado, USA (NWT), at 3186 m elevation. HJA has a mean annual temperature (MAT) of 8.5°C, and NWT has a MAT of –3.2°. See https://lterne t.edu/site-characteristics for additional characteristics of the two sites. Soils were taken to Norman, OK, and incubated within 72 h of collection. Total carbon (C) and total nitrogen (N) in the soils were measured by a LECO TruSpec Carbon and Nitrogen Analyzer (LECO Corporation, St. Joseph, Michigan, USA) at the Soil, Water and Forage Analytical Laboratory at Oklahoma State University (Stillwater, Oklahoma, USA).

Each mesocosm was comprised of 500 mL of homogenized, sieved soil placed in a 900-mL plastic cup with a tight-fitting lid. A piece of sterile cotton was used as a stopper in a 1-inch hole drilled through the lid to allow ventilation. Each mesocosm was weighed at the start of the experiment, and that weight was maintained throughout the experiment by adding distilled water.

For both soil types, we incubated nine mesocosms at temperatures of 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C. We sampled the homogenized, sieved soils at time zero (i.e., before incubation) and then sampled three of each temperature treatment at 30, 180, and 440 d. Sampled soils were stored at −80°C until all were completed and analyzed.

Molecular methods

DNA extraction.—Ten grams of soil was collected from different parts of each well-mixed soil sample and ground in liquid nitrogen. One gram of this sample was used for microbial assemblage DNA extraction by DNeasy Power-Soil DNA Isolation Kit (QIAGEN, Hilden, Germany).

Library preparation and sequencing.—A two-step PCR amplification method was used for PCR product library preparation as described previously

(Wu et al. 2015). In the first-step PCR, the standard primers were used to amplify the V4 region of prokaryotic 16S rRNA genes (515F [5′-GTG CCAGCMGCCGCGGTAA-3′] and 806R [5′-GGA CTACHVGGGTWT CTAAT-3′]). In the second-step PCR, phasing primers were designed and used to increase the base diversity in sequences of sample libraries. PCR amplification and purification were the same as reported previously (Wu et al. 2015), except amplification cycles (10 cycles in the first step and 20 cycles in the second step for the 16S rRNA gene). Sample libraries were then sequenced by a MiSeq platform (Illumina, San Diego, California, USA) as described previously (Caporaso et al. 2012).

Data processing.—Sequencing data generated from MiSeq were processed to combine paired-end reads and to filter out poorly overlapped and unqualified sequences by using a Galaxy pipeline at http://zhoulab5.rccc.ou.edu:8080. After demultiplexing of raw fastq data (barcode error is set as zero) and primer trim, the reads with average quality score <20 were removed by Btrim (Kong 2011, Caporaso et al. 2012) and the paired-end reads were combined by Flash (Magoč and Salzberg 2011). Then, sequences containing N (unidentified base) or with length out the range 240–260 bp (without primers) were removed. Chimeras were detected by UCHIME (Edgar et al. 2011) and OTUs were generated by UCLUST (Edgar 2010) with 97% sequence similarity threshold. The reference databases of 16S rRNA genes were from Silva SSU 128 release (Edgar 2010, Quast et al. 2013). OTUs detected in negative control samples were removed as artifacts and/or contaminants. OTUs were identified taxonomically using the RDP classifier based on 16S rRNA training set 16 (Wang et al. 2007). The representative sequences were used to build phylogenetic tree by FastTree (Price et al. 2009, 2010) after aligned by PyNAST (Caporaso et al. 2010). Global singletons were removed as they may be sequencing errors and are unlikely to affect ecological metrics (Brown et al. 2015) and samples were rarefied to the same sequencing depth (20,000 reads) before further analysis.

Statistics

All analyses were performed in R version 2.15.1 (R Core Team 2012). To examine the overall effects of site, days, and temperature on

diversity and assemblage similarity, we used generalized linear models. For OTU richness, we used the Poisson distribution with a log link. As Chao diversity estimates were highly and positively correlated with OTU counts ($r^2 = 0.97$; see Appendix S1: Fig. S2), we used S (i.e., OTU richness) for analyses. For Bray-Curtis similarity (which can range from 0 to 1), we used a quasi-binomial distribution with a probit link. We entered site (Niwot or HJA), temperature (10°C, 15°C, 20°C, 25°C, 30°C, and 35°C), and number of days (0, 30, 180, and 440). To allow temperature to be a continuous variable, we assigned time zero samples 22.5°C, the mean of all our temperature treatments. While choosing this temperature for time zero is arbitrary, the results of interest are how diversity and similarity change across temperature and time.

As a large number of OTUs were rare, to make certain that differences in assemblage composition were not the result of rare species (Shade et al. 2014) and thus large numbers of zeros in the presence–absence matrix, we repeated the Bray-Curtis similarity analysis using the taxa that were among the 200 most numerically abundant taxa from each site by temperature treatment.

To understand the importance of relative change in temperature on assemblage dissimilarity, we performed linear and quadratic regression on the difference in temperature between the treatment and MAT of the site. All treatments were higher than the MAT of both sites, but as the MAT of Niwot was colder than HJA, the relative changes were larger. To choose between the linear and quadratic models, we used ΔAIC where a decrease in ΔAIC ≥ 2 with the addition of the quadratic term indicated a better model (Hilborn and Mangel 2013). We expected that higher temperatures and higher temperatures relative to MAT would have the greatest decrease in diversity and highest increase in assemblage similarity due to the stronger selection for heat-tolerant taxa.

RESULTS

Our methods detected a total of 105,146 OTUs at 97% sequence identity with 63,643 OTUs from Niwot soils and 69,372 OTUs from HJA soils. Generally, OTU richness declined more and assemblage similarity decreased more for (1) higher incubation temperatures, (2) longer incubation times, and (3) soils from the colder forest, Niwot (Table 1, Fig. 1).

Soils lost OTUs over time especially at higher temperatures

Both sites lost OTUs at higher incubation temperatures, but soils from Niwot lost more OTUs than those from HJA. At HJA, the average OTU richness at time zero was (mean ± SD) 5650 ± 305 OTUs (Fig. 1a). After 180 d of incubation, OTU richness of HJA soils declined significantly for the two highest temperatures. At 30°C, OTU richness had declined by 9% to 5196 ± 23 OTUs, while at 35°C, it had declined by 17% to 4673 ± 370 OTUs. These declines continued at these temperatures after 440 d of incubation with 30°C losing 15% (4813 ± 504 OTUs) and 35°C losing 44% (3175 ± 880 OTUs) of the initial OTU richness. The 25°C incubation did not change significantly in OTU richness. After 440 d of incubation, OTU richness for HJA soils increased significantly for the three coldest temperatures (10°C = 10%, 15°C = 16%, and 20°C = 18%). As there were no significant increases for the three coldest treatments at Niwot, these increases in OTU richness were likely due to increases of population sizes of rare species not detected at time zero, not immigration or contamination.

Table 1. Results of generalized linear model of the effects of temperature, number of days, and site on OTU richness (using a Poisson distribution) and Bray-Curtis dissimilarity (using a quasi-binomial distribution).

	OTU richness				Bray-Curtis dissimilarity			
	Coef.	SE	z value	Pr(>|z|)	Coef.	SE	t value	Pr(>|t|)
Intercept	9.16	0.004	2288	<0.001	−0.30	0.025	−12.32	<0.001
Temperature	−0.016	0.0002	−105.6	<0.001	0.018	0.0009	19.93	<0.001
Days	−0.0007	7.5×10^{-6}	−92.32	<0.001	0.0014	0.00005	25.29	<0.001
Site(Niwot)	−0.17	0.003	−66.11	<0.001	0.27	0.015	13.70	<0.001

Note: Coef., coefficient estimated by the model; SE, the standard error of the coefficient.
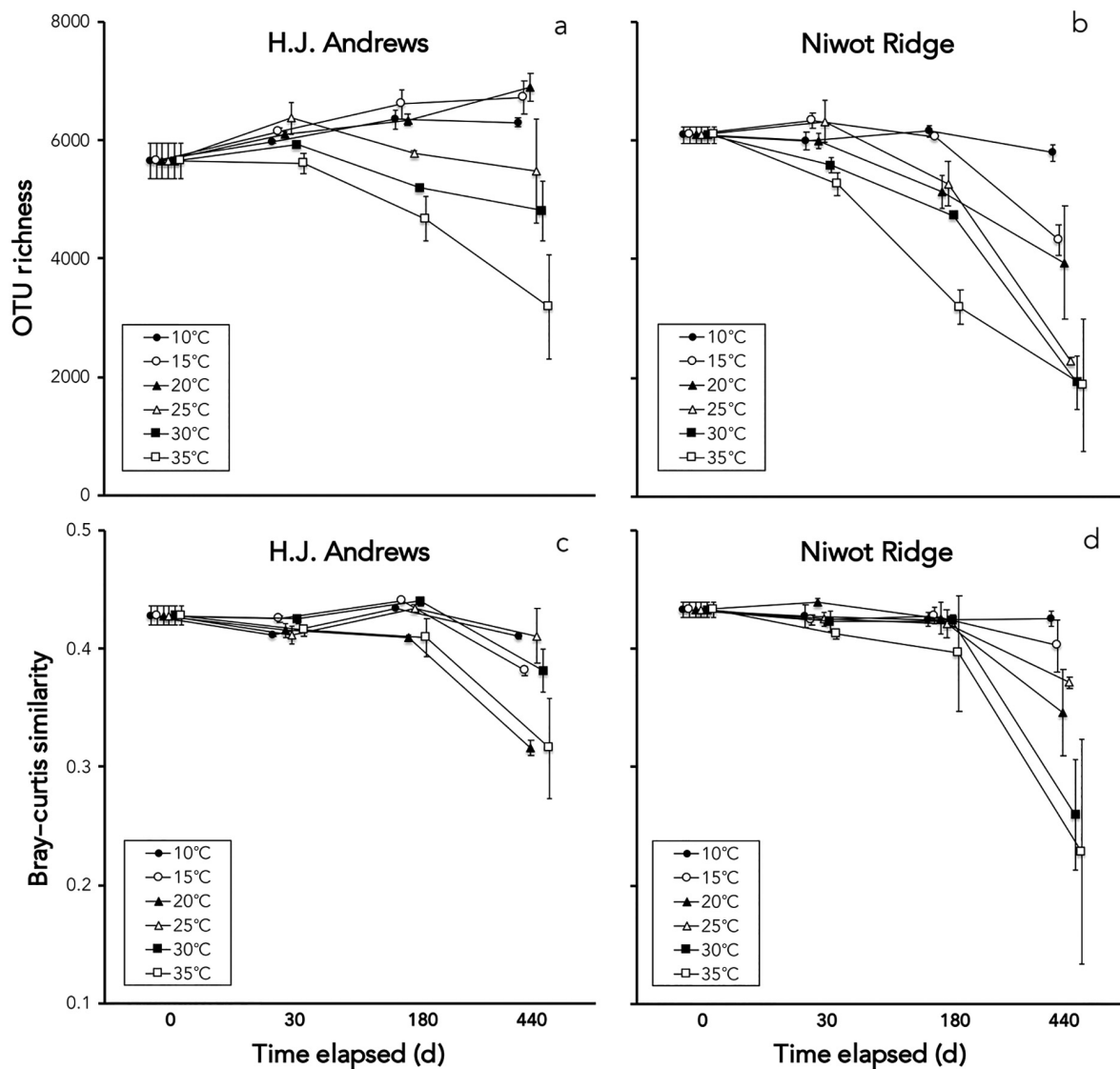
Fig. 1. Soil bacterial assemblages in closed mesocosms lost more OTUs at higher temperature and longer incubation periods at HJA (a) and more so at Niwot (b), having one-half (HJA) and one-third (Niwot) of the starting OTU richness at 35°C after 440 d of incubation. These assemblages also became more dissimilar at higher temperatures and longer incubation periods at HJA (c) but more so at Niwot (d). The *x*-axis is not proportional.

Over the span of the experiment (i.e., 440 d), Niwot OTU richness decreased significantly for all but the coldest temperature (10°C). By day 180, diversity had decreased significantly (13.5–47.7%) at 25°C, 30°C, and 35°C; by day 440, soils in the 15°C and 20°C growth chambers had also decreased significantly (29.2–34.7%), while soils at warmer temperatures continued to lose OTUs.

Between-mesocosm similarity of communities decreased at warmer temperatures

Mean Bray-Curtis similarities started near 0.43 for both sites (0.428 for HJA and 0.432 for Niwot; Fig. 1c and d) and had not changed after 180 d. After 440 d, all soil assemblages were significantly less similar than day 0 except the coldest (10°C) treatments (Fig. 1c, d, Table 1) and HJA soils at 25°C. In Niwot soils, similarity generally

decreased as a function of temperature. HJA showed a more complex pattern where 20°C and 35°C decreased in similarity more than the 25°C and 30°C mesocosms.

When we focused on only the most common OTUs, the outcome—decreased similarity over time at the higher temperatures (Appendix S1: Fig. S1)—was also observed. For soils from the colder Niwot site, the coldest temperature (10°C) did not decrease significantly in similarity after 440 d. At HJA, the three coldest temperatures (10°C, 15°C, and 20°C) also did not become less similar after 440 d. Early in the experiment, some of the colder treatments became *more* similar to each other (Niwot 15°C and 25°C and HJA 10°C, 15°C, and 25°C at 30 d; Niwot 10°C and HJA 20°C at 180 d), but none were significantly more similar at 440 d.

Differences between MAT and treatment

We next tested the proposition that community responses would be more proportional to the deviation of temperature of the growth chamber from the average temperature experienced in the home environment. Using relative temperature change, the difference between the site MAT and the treatment, OTU richness declined over 440 d of incubation as a linear function of temperature increase (OTUs = 1999−171.9 × Δ°C, R^2 = 0.87, P < 0.0001, AIC = 196.7; Fig. 2a). Adding the quadratic term did not significantly lower the AIC. Bray-Curtis similarity showed an accelerating decrease with temperature (ΔBC = 0.02−(2.2 × 10^{-3} × Δ°C) + (2.0 × 10^{-4} × Δ°C)^2, R^2 = 0.90, P < 0.0001, AIC = −52.8; Fig. 2b) with the quadratic term significantly better than the linear model (linear AIC = −44.8).

In summary, we detected fewer OTUs in our closed mesocosms at higher temperatures and with longer incubation times. With these increased losses, mesocosms became less similar as a function of both absolute and relative temperature increases.

DISCUSSION

Here, we tested two predictions for the dynamics of soil microbial assemblages in a warming world. Consistent with the tenets of metabolic and thermal ecology, assemblage dynamics—
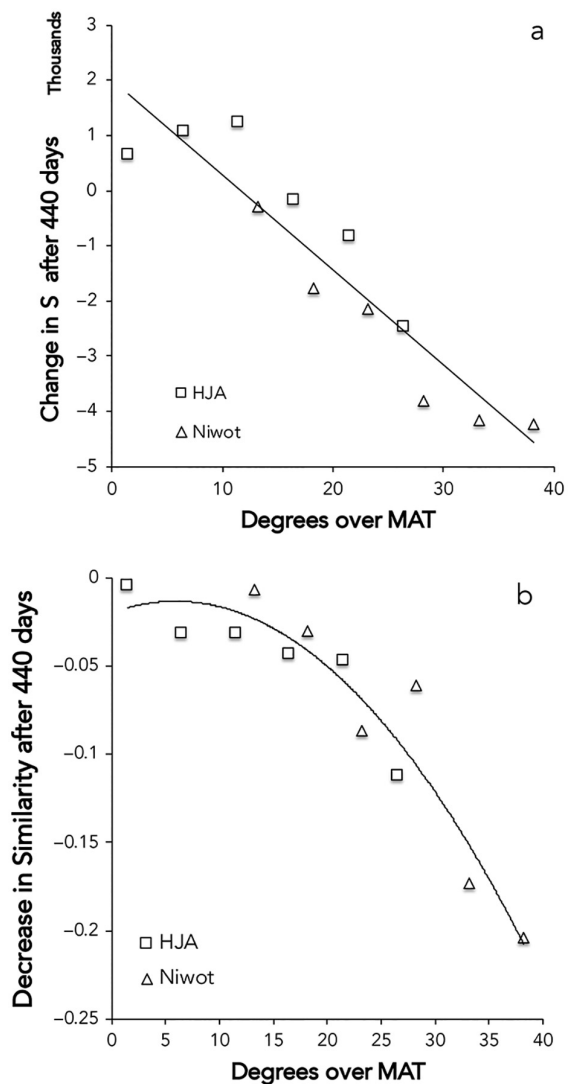

Fig. 2. (a) Relative temperature change, the difference between experimental treatment temperatures and the MAT of the sites in degrees C (δ°C), is predictive of both the number of species lost and increase in Bray-Curtis dissimilarity over 440 d. The decrease in OTUs is a linear function of temperature difference (δOTUs = 1999 − 171.9 × δ°C, R^2 = 0.87, P < 0.0001, AIC = 196.7). Adding a polynomial term did not significantly improve this fit (AIC of quadratic regression = 197.7). (b) The decrease in Bray-Curtis similarity is accelerating with temperature difference as the quadratic regression (δBC = −0.02 + (2.2 × 10^{-3} × δ°C) − (2.0 × 10^{-4} × δ°C)^2, R^2 = 0.90, P < 0.0001, AIC = −52.8) is a significant improvement over the linear model (AIC = −44.8).

driven by extinctions in these closed systems—accelerated with temperature. The nature of these assemblage dynamics, where extreme temperatures over more than a year of incubation did not favor a consistent subset of—presumably thermophilic—taxa. Instead, the assemblages became significantly less similar. Combined, our results suggest that changes in microbial communities due to warming may be difficult to predict.

In general, assemblages became less similar over time, and this happened faster and to a greater degree at higher temperatures. These changes were not driven solely by the loss of rare species. When considering only the most abundant taxa, similarity decreased more at the highest temperatures and more for the soils from Niwot (Appendix S1: Fig. S1). The coldest assemblages changed very little, as would be expected when there is relatively little metabolic activity. This cold-storage effect thus maintained assemblage diversity and similarity.

There may be a threshold temperature for the cold-storage effect; thus, with climate change, colder places may see the largest change if local temperatures rise above that threshold. Assemblages became more dissimilar as an accelerating function of the difference between their MAT and our temperature treatments, and this relationship appears to inflect around 20°C over MAT (Fig. 2b).

While we only compare two sites here, it is notable that the results are consistent with the importance of relative temperature differences, not solely the absolute temperature of treatments. The MAT of HJA is 11.7°C warmer than Niwot, and the assemblages from HJA were more robust to higher temperatures. The implication of this is that these assemblages may be locally adapted to the temperatures they had experienced, and thus, greater changes in temperature should lead to greater simplification.

Temperature as a template for and a driver of rates of selection

As absolute and relative temperature increases, mesocosms lost more OTUs and became less similar and did so more quickly. These results suggest that at higher absolute temperature and higher relative temperature change, temperature acts both a selective template filtering species and also the driver of the rate at which that

selection occurs. Increasing the metabolic rates of a microbial assemblage decreases generation time and increases rates of resource use (e.g., Ratkowsky et al. 1982). Thus, both niche and neutral theories predict that individual turnover (birth–death) will increase at higher temperatures.

Our initial assumption was that the stronger selection from higher temperatures would simplify the microbial assemblages, selecting against psychrophilic OTUs and selecting for the subset of thermophilic taxa. The decrease in OTU richness, as well as the faster rates of decline at highest temperatures, indicates that higher temperatures are a strong selective agent leading to lower OTU richness at faster rates. That said, we assumed this selective simplification would be non-random, selecting for more similar assemblages of warm-adapted taxa. We did not observe this. At the highest temperatures, both whole assemblages and the most abundant taxa from those assemblages became more different from each other over time.

Caveats

By design, our temperature treatments were not realistic (e.g., no daily or seasonal variation, temperatures much higher than predicted by global warming models, no intentional re-immigration). Our goal was to focus solely on the patterns and rates of one ecological process, extinction. As there is no intentional immigration in this system, the decrease in similarity could be due to the lack of re-immigration. In variable but open environments (e.g., the ocean), bacterial assemblage diversity and composition were predictable at interannual scales (Gilbert et al. 2012). That said, these results may be informative as it is rare that you can isolate and replicate systems and quantify the role of extinction alone, and, as we have shown for the first time, the temperature dependence of this extinction rate.

These two sites differed in more than just temperatures. Niwot soils had significantly lower total carbon than HJA (see Appendix S1: Table S1), and we did not supplement carbon resources over this experiment. Thus, it is possible that that the differences seen between Niwot and HJA, where Niwot lost more OTUs, have more to do with the Niwot mesocosms running out of carbon faster than HJA. That said, if this process were driven by selection due to limiting

carbon, we would again expect the assemblages to become more similar over time, as low-carbon specialists would comprise a larger portion of the assemblage.

When framed by the suggestion that "everything is everywhere, but the environment selects" (Baas Becking 1934, quoted in De Wit and Bovier 2006), these results indicate that one or both of these suggestions are false. These mesocosms closed to the immigration of novel and re-immigration of extirpated species appear to either have vastly different starting biotas (i.e., everything is not everywhere) or that the environmental selection is merely temperature-driven extinction rates that are not driven by species traits (i.e., are neutral dynamics; sensu Hubbell 2001).

CONCLUSIONS

Our results show that higher temperatures can lead to rapid simplification of soil microbial assemblages through extinction. The magnitude of species lost appears to be a linear function of the difference between our temperature treatment and the temperatures the soil microbes experience in situ. The extinctions show no obvious directional selection for these assemblages. In these closed systems, this assemblage-level thermal churn, where higher temperatures increase the rates of assemblage dynamics and send microbial assemblages on seemingly random walks to multiple final states, appears to be an accelerating function of the temperature.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2598/full