The Pennsylvania State University

The Graduate School

"THE EFFECT OF ENVIRONMENTAL HETEROGENEITY ON BACTERIA WITHIN AN AGRICULTURAL SOIL COMMUNITY"

A Thesis in

Ecology

by

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Submitted in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science

May 2022

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Abstract

Ecosystem disruptions such as climate and land use change have the potential to disproportionately challenge certain organisms, resulting in lowered biodiversity, as well as other changes to community composition. The disproportionate impact on some organisms can be explained by inter-organism differences in niche breadth, with specialist organisms being more impacted than generalists, as their more restricted potential niche space limits their ability to persist under heterogeneous conditions. These patterns have been observed across multiple types of organisms; however, the effect of increasing environmental heterogeneity on microorganisms has not been well documented, despite the importance of microorganisms in providing many essential ecosystem services.

In this thesis, I demonstrate the impact of using active selection gradients to collect microbial generalists of different types and see the impact of environmental heterogeneity on the bacterial community. In the first chapter, I apply temperature heterogeneity to soil microcosms containing microbiomes to detect the impacts of oscillating temperatures on the composition of both resident microorganisms and those that can actively colonize new environments. The goals of this study were to assess changes to bacterial composition in an agricultural soil under different temperature regimes and to identify potential thermal generalist taxa. Soil microcosms were either maintained at a constant temperature of 10°C or 30°C or received alternating temperature treatments, transitioning between 10°C and 30°C. After 4 rounds of passaging, each with 2 weeks of incubation for a total experiment time of 8 weeks, we assessed the composition of bacteria through 16S rRNA gene sequencing. The alpha diversity of microbiomes receiving alternating temperatures did not differ significantly differ from those kept at constant temperature, but these treatments did differ significantly in composition. Many taxa persisted under the various temperature regimes, likely through mechanisms like dormancy, showing resilience to changing temperature conditions. Taxonomic patterns of thermal generalism were found at the genus level, indicating that traits related to temperature are conserved at lower taxonomic levels. Additionally, strong thermal-generalist colonists were identified across

temperature regimes and showed patterns at the class level, which may be related to traits involved in successful colonization.

In the second chapter of this thesis, I describe an experiment in which we investigate the impact of pH heterogeneity on bacterial composition. The aims of this research were to determine the impact of pH shifts on soil microbial diversity and composition and to identify potential pH generalist taxa. A mid-pH soil (5.69) was placed within sterilized low-, mid-, and high-pH soils to select for pH generalists, maintained at 20°C, and destructively sampled at 2, 4, 8, and 16 weeks. Additionally, microcosms harvested at 8 weeks were used to seed plates filled with sterile soil from the opposite end of the pH spectrum to maximize pH heterogeneity and were maintained for an additional 8 weeks before sampling. 16S rRNA gene sequencing was performed, revealing no significant impact of pH regime change on alpha diversity in plates either maintained in the same pH condition for 16 weeks or those transplanted at 8 weeks, although comparison to the source soil revealed that the act of transplanting significantly reduced biodiversity. Microcosms differed significantly in composition due to pH regime and distinct patterns of pH tolerance were found at higher taxonomic levels.

Overall, the results of this research build upon previous studies investigating the impact of changing conditions on soil microbiomes and add perspective on how changing temperature and pH conditions may impact bacterial communities within agricultural soils in a temperate region. The identification of putative generalists may be used to better predict taxa resilient to environmental change, as well as identify potentially desirable taxa for development as microbial products. This work has also shown patterns of trait conservation at different taxonomic levels, building upon our understanding of how genetic complexity is related to taxonomic levels of gene conservation. Understanding how bacterial communities change and which taxa are likely to be more impacted than others may better inform future experiments investigating functional differences between selected and original communities. Potential avenues of research could involve evaluating rates of nutrient cycling in resulting communities to better understand the relationship between community

composition and ecological function. This work provides an example of how microcosm experiments can be used to study core ecological concepts, such as the relationship between niche breadth and environmental heterogeneity, and tradeoffs between generalists and specialists, among others, although traits common in microbial life may change some patterns compared to larger organisms.

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Acknowledgements

I would like to thank the members of the Microbiome Manipulation Lab, especially my advisor Terrence Bell, for their help and support during my time at Penn State. I would also like to thank my committee members, Emily Davenport, Michela Centinari, and Paul Esker for their time and advice. I'm also grateful for the patience and love of my partner, friends, and family. Above all, I'd like to thank my parents for doing everything they could to make my education a priority.

This work was supported by the United States Department of Agriculture National Institute of Food and Agriculture (grant no. 2020-67013-30865). Findings and conclusions do not necessarily reflect the view of the funding agency.

Chapter 1: The impact of thermal heterogeneity on bacteria within an agricultural soil community

Introduction

As ecosystem disruptions due to climate and land-use change continue and in some cases, increase in magnitude, frequency, and unpredictability, certain organisms are expected to perform better than others (Trenberth, 2012; United Nations, 2018). Generalist organisms, defined by a wide niche breadth, are expected to be favored under heterogeneous conditions, while organisms with more narrow niche breadths, i.e. specialists, are expected to be more severely impacted by heterogeneity due to an inability to persist under changing conditions (Hutchinson, 1953; Roughgarden, 1974; Kassen, 2002; Ma and Levin, 2006; Devictor, Julliard and Jiguet, 2008; Pandit *et al.*, 2015). Environmental heterogeneity has been linked to species loss, decreases in biodiversity, and shifting community composition in multiple species including butterflies, birds, bats, and fishes (Le Viol *et al.*, 2012; Börschig *et al.*, 2013; Büchi and Vuilleumier, 2014; Stuart-smith *et al.*, 2021).

These concepts have not been widely explored in microbiomes (i.e. assemblages of microorganisms inhabiting defined environments), despite the important role of microbes in mediating key ecosystem services, such as nutrient cycling (Jansson and Hofmockel, 2020). Changes in microbiomes may also have cascading effects on the larger community. Soil microbiomes may respond to changing conditions in similar patterns to those of larger organisms, with a loss of specialists, reduced biodiversity, and shifting community composition and dynamics. However, certain traits widespread in microbial life, such as dormancy and horizontal gene transfer, may alter these patterns by allowing persistence in non-ideal environments or the uptake of ecologically relevant genes from other taxa (Allison and Martiny, 2009; Lennon and Jones, 2011; Harrison and Brockhurst, 2012).

Another way in which larger organisms and bacteria may differ in their response to external conditions has to do with genome size and evolutionary time scales. In a

study of ubiquitous bacteria, Barberán *et al.*, 2014 found that habitat breadth was positively related to genome size. This potentially indicates that a mechanism by which bacteria cope with changing conditions is through an increased repertoire of metabolic capabilities stored in a larger genome, although larger genomes have been linked with increased energetic costs of maintenance (Ranea *et al.*, 2005). Shorter generational times in bacteria may also enable for more a fine-tuned response to conditions at scales in which larger organisms can't respond, providing an opportunity for evolutionary response.

Bacteria have been described as generalists and specialists of many variables, often metabolic capability (Bell and Bell, 2020). It's important to note that applying these terms can be complicated, and requires nuance and specificity (Loxdale and Harvey, 2016; Bell and Bell, 2020). Niche breadth is often a continuum across species, meaning that one bacterium may be described as a generalist compared to another, but as a specialist in comparison to some other bacterium. Organisms also exist along many niche axes, meaning that the same organism can be described as a generalist of one variable and a specialist of another (Figure 1.1).

One environmental variable that is expected to change under future climate scenarios, and that will likely impact soil microbiomes (at least in the top layers of soil where atmospheric conditions are influential) is temperature. Bacterial life is directly influenced by environmental temperatures in several ways. Temperature is known to impact the structural integrity of membranes, the behavior of many proteins and lipids, and overall metabolic rates (Reizer, Grossowicz and Barenholz, 1985; Lepock, Frey and Inniss, 1990; Shivaji and Prakash, 2010). Changes in temperature can also cause cells to shift competitive dynamics and life strategies, as shown by Leroi, Bennett and Lenski, 1994. These authors showed that prior acclimation of *Escherichia coli* to a relatively high temperature (41.5°C) induced a phenotypic change that resulted in increased fitness relative to the ancestral strain at the higher temperature; however, at the baseline temperature (32°C), this protective response often, but not always, caused physiological stress to the cell, showing that adaptation to a new environment may or may not reduce fitness in an environment that the organism was previously well-

adapted to. Robador et al. (2009) showed that experimental evolution of Arctic sulfate-reducing bacteria to different temperatures led to differences in metabolic rates across temperatures, changing the activation energy required by many sulfate-reducing proteins and shifting competitive dynamics. Many of the structures and functions that are directly impacted by temperature are universal across bacterial life (e.g., maintenance of membranes), so we would expect virtually every member of a microbiome to be impacted by differences in temperature regimes.

Figure 1.1

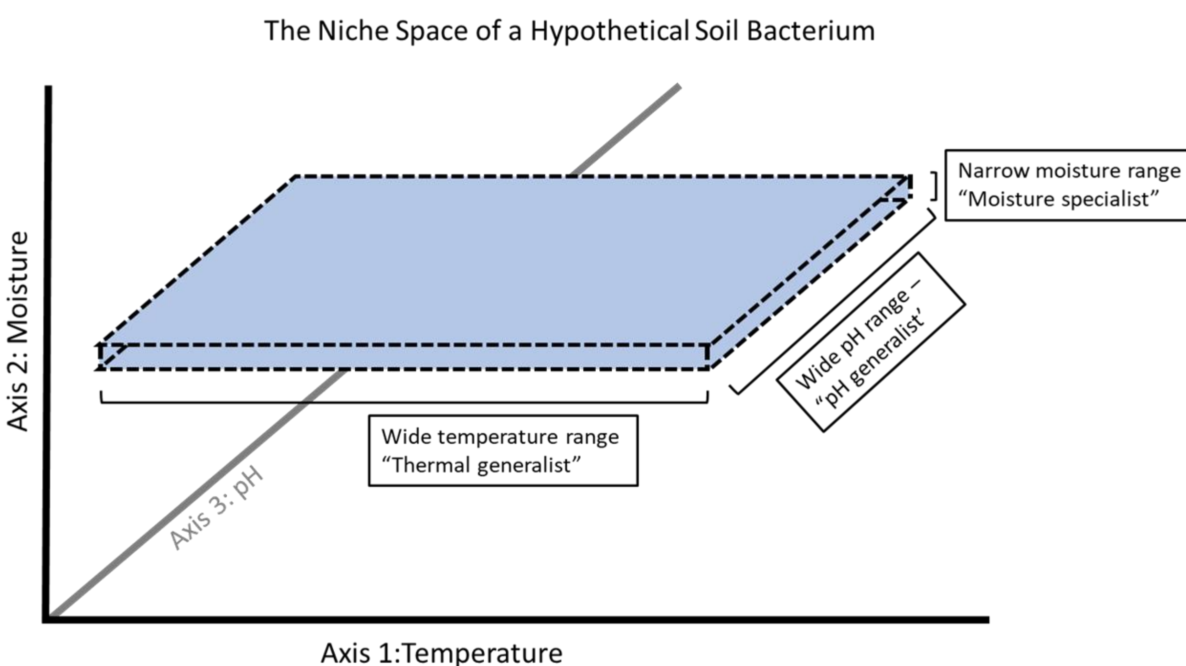

Figure 1.1: Niche breadth of a hypothetical soil bacterium is shown in three dimensions. The hypothetical soil bacterium shows a relatively wide niche breadth along the x and z axes (temperature and pH), but a limited range in the y axis (moisture), making it a generalist of temperature and pH and a specialist of moisture conditions.

In addition to studies on isolates and small assemblages, the composition of complex microbiomes has often been shown to change in response to temperature, although this is not always the case. Several studies show significant changes in alpha diversity in response to warming (Wu *et al.*, 2015; Luláková *et al.*, 2019), while others show no significant response (Xiong *et al.*, 2014). These patterns are seemingly dependent on chosen temperature regime, as was the case in Luláková *et al.* (2019).

Other influential variables limiting comparison include the timespan of the experiment and soil type, which was shown in Luláková *et al.* (2019) and a survey-based experiment by Zhou *et al.* (2020). Beta diversity is typically impacted by warming experiments, with changes found between temperature regimes with only 2°C difference, such as in the case of the 15-month warming experiment by Xiong et al. (2014) and in a 14-day temperature shift of 4°C to 40°C (Luláková *et al.*, 2019).

Communities under distinct temperature conditions have also been seen to change, showing taxonomic patterns that may be related to the level of conservation of relevant genes; in this case, genes pertaining to temperature. In a study on the microbiota of Pacific oysters, the microbiome was sampled at both 8°C and 22°C (Lokmer and Mathias Wegner, 2015). Shifts at lower taxonomic levels were seen under different temperature treatments, while composition of higher taxonomic levels remained relatively constant in what the authors term “dynamic stability”. Similar studies have been performed in anaerobic reactor systems, marine sponge microbiomes, and soils, with each showing taxonomic shifts in response to temperature (Carballa *et al.*, 2011; Xiong *et al.*, 2014; De Vrieze *et al.*, 2015; Wu *et al.*, 2015; Luláková *et al.*, 2019). These patterns may indicate that genes related to temperature are conserved at certain taxonomic levels, as described by Martiny *et al.* (2015). In this paper, a hierarchy of conservation of traits is proposed to be related to the relative complexity of the gene(s) required. In the case of thermal tolerance, genes involved have been observed to be relatively simple in comparison to the genetic framework associated with pH, for example (Martiny *et al.*, 2015). This may indicate that thermal tolerance traits are conserved at lower taxonomic levels, perhaps at the genus level, whereas more complex traits are conserved at higher taxonomic levels (Martiny *et al.*, 2015). If traits related to temperature are conserved at lower taxonomic levels, patterns of thermal generalism and specialism may be seen at lower taxonomic levels.

The identification of generalist bacterial groups may be desirable for several reasons. Study of these generalists may also allow for investigation into potential tradeoffs in maintaining generalist or specialist abilities, as well as better prediction of generalist invasive species and pathogens. We may also increase our understanding of

traits related to generalism in bacteria, such a horizontal gene transfer, and traits related to generalism in all organisms, such as enhanced genetic plasticity. Generalists of important ecological variables may also be more desirable targets for development as microbial products in medicine, agriculture, and other industries.

To investigate the impact of thermal heterogeneity on soil bacterial composition and to identify putative thermal generalists, we have developed a novel system for applying environmental filtering to soil microcosms. Because temperature is known to impact virtually all bacterial life, including at the community level, we can leverage environmental filtering by temperature to collect communities with a wider observed range of thermal tolerance. With this approach, we collected soil microbiomes that persist (Survivors) or colonize (Colonizers) under homogeneous or heterogeneous temperature conditions. Following conditioning under multiple temperature regimes, soils were sampled and 16S rRNA gene sequencing performed to characterize bacterial composition. Because standard sequencing approaches do not distinguish between active, dormant, and dead cells, multiple microcosm types and sampling dates were used to distinguish between bacteria that had likely persisted under the applied conditions and those that could actively colonize sterile soil. Bacteria identified from fluctuating temperature treatments are putative thermal generalists, since they were shown to persist or grow across a relatively wide range of soil temperatures (10°C-30°C). Because soil microbiomes are complex, the resulting bacteria may achieve thermal generalism through traits within their genomes or through interactions within the community. Our expectations were that 1) thermal heterogeneity would disadvantage specialist organisms, resulting in lowered biodiversity under fluctuating temperature conditions and 2) community composition would shift in response to temperature regime, allowing us to identify potential thermal generalists and taxonomic patterns associated with thermal generalism. We also expected Colonizers to be less diverse than Survivors because of the added difficulty of being active in a range of temperature conditions.

Hypotheses:

1. Community composition will be impacted by different temperature regimes.

   a. Alpha diversity of communities under heterogeneous temperature regimes will decrease.
   b. Patterns by which community composition changes under temperature regimes will be found at lower taxonomic levels.
2. Colonizer communities will be less diverse than Survivor communities.

Methods

Environmental filtering

Soil from an organic agricultural field at the Russell E. Larson Agricultural Research Center at Rock Springs, Pennsylvania Furnace, Pennsylvania, USA (40°43'12.4"N 77°55'40.1"W) was collected on November 9th, 2019. This soil was sieved to 0.2”. Because the soil was collected from relatively cold conditions (11°C at the time of collection), one portion of the soil was stored at 20°C for a week to allow for adjustment to temperature between thermal bounds of 10°C and 30°C to reduce the influence of pre-adaptation of the bacterial community to colder conditions. Another portion of the soil was autoclaved on three subsequent days at 121°C and 20lbs/in^2 and for 90 minutes each time, starting three days before microcosm construction to minimize potential contamination.

Several temperature ranges were tested to ensure sufficient growth, determined by DNA concentration of soil extractions in two-week time periods. We chose 10°C and 30°C to be the low and high thermal bounds, respectively, with four total temperature regimes applied (Figure 1.1). Two temperature regimes were heterogeneous and were maintained at either 10°C or 30°C and oscillated to the other temperature every two weeks for a total of two complete cycles and a timespan of 8 weeks. Both heterogeneous temperature regimes (i.e., cold → hot and hot → cold) were present to control for priority effects of the first temperature condition. Control temperature regimes were maintained at either 10°C or 30°C for the 8-week experiment. Samples were collected at the end of every two-week period.

Figure 1.2

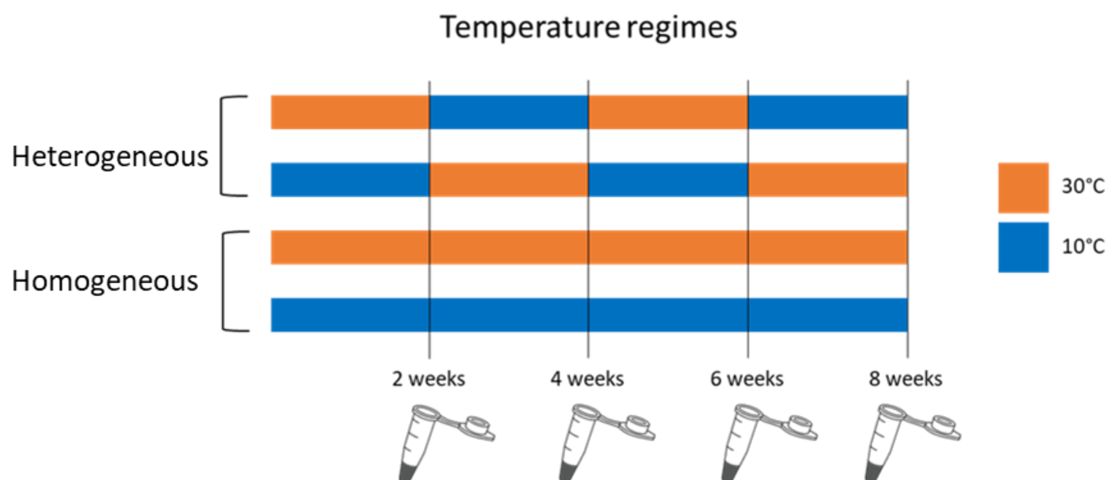

Figure 1.2: Visual representation of temperature regimes with color indicating temperature, Eppendorf tubes indicating sampling points, and time represented along the x axis.

Microcosms were created within 60mm x 15mm Petri dishes. Two microcosm types were created 1) to filter for bacteria capable of persisting under different temperature regimes (Survivors) and 2) to filter for active colonizers under different temperature regimes (Colonizers). The first plate type, Survivor, was composed of 100% sieved bulk soil and saturated with sterile water. These plates were sampled every two weeks for the entire 8-week period of the experiment. The second plate type, Colonizer, was composed of roughly 5% sieved bulk soil within an ethanol-sterilized nylon inoculum bag with 95% of sieved and triple-autoclaved soil. The nylon inoculum bag was placed on one side of each plate, and soil was taken from the opposite side of the plate to collect only bacteria that had colonized the sterile soil (Figure 1.2). The collected soil was split into two portions; one portion was collected for sequencing and the other was used to construct another nylon inoculum bag. This new nylon inoculum bag was then placed in a new plate of sterile soil and placed in the relevant temperature condition for the next 2-week period. Both Survivor and Colonizer plates were sealed with a layer of Parafilm to prevent contamination while still allowing air exchange. Plates were weighed at 2-week intervals and, if necessary, rehydrated with sterile water to achieve saturation.

Figure 1.3

Figure 1.3: Colonization microcosm. Darker brown area is the nylon inoculum bag. Red oval shows where sampling and creation of the next inoculum bag will occur.

Three control plate treatments were added. The first, termed ‘Nutrient-Depletion’, was constructed from 50% non-sterilized soil and 50% sterilized soil, separated by an ethanol-sterilized nylon barrier. By comparing the results of the Nutrient-Depletion and Survivor plates, we were able to determine whether nutrient depletion of the soil shaped the community throughout the experiment and potentially better design future experiments. The second control, Negative-Survivor, corresponds with the Survivor treatment and is 100% sterilized soil. The third control, the Negative-Colonizer treatment, is 95% sterilized soil with 5% sterilized soil within an ethanol-sterilized nylon inoculum bag. Like the Colonizer treatment, soil from outside the inoculum bag was used to inoculate another 95% sterile soil plate at the end of every two-week period. The purpose of this control was to determine the identity of inadvertent invaders of the transferring process. Triple autoclaving is one of the best available methods for sterilizing soil, but it’s important to note that we and others have observed microbial grow-back at longer timescales (8+ weeks), although this is typically restricted to around 1-5 distinct taxa (unpublished results).

Sequencing

DNA was extracted from 0.25-0.35g of soil using the NucleoSpin 96 Soil DNA extraction kit from Macherey-Nagel (Bethlehem, PA, USA) according to the manufacturer's instructions. Extractions were prepared and sequenced by GenomeQuebec with Illumina NovaSeq 6000 sequencing platform (2 x 250). The v4 region of the 16S rRNA gene was amplified with 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'- GGACTACNVGGGTWTCTAAT-3') Illumina primer pairs (Apprill *et al.*, 2015; Parada, Needham and Fuhrman, 2016). All data analyses were performed in R. Pre-processing was performed using the DADA2 pipeline, modified to run samples in batches after estimating error rates, using the same parameters as in Callahan *et al.* (2016). The only modifications to parameters used in Callahan *et al.* (2016) were when truncating reads and removing chimeras because of high-quality reads and NovaSeq's tendency to over-report chimeras (Costello *et al.*, 2017, unpublished results). The full pipeline can be found at https://github.com/ryanc16/dada2-batched-pipeline.

Rarefaction was applied before calculating Chao and Shannon diversity indices or Bray-Curtis dissimilarity distances to control for uneven sampling depth since sequencing depth has a large impact on the discovery rate of rare microorganisms. Chao and Shannon diversity indices were calculated in R version 3.5.2 (R Core Team 2018) with package Phyloseq (McMurdie and Holmes, 2013). Base R was used to calculate Fisher's ANOVA to detect significant differences between samples for both Chao and Shannon diversity measures. Ordinations and PERMANOVA (nonparametric permutational multivariate analysis of variance) were performed with R packages Phyloseq and vegan 2.5-6 (Jari Oksanen *et al.*, 2020). Bray-Curtis dissimilarity measurements were plotted on an NMDS (non-multidimensional scale) plot. PERMANOVA was conducted to evaluate the impact of variables temperature regime, plate type, and time-point on bacterial community structure. The full PERMANOVA model was Bray-Curtis dissimilarity ~ TempRegime*PlateType*TimePoint. The non-rarefied Phyloseq object was transformed to represent 100% relative abundance and converted to a data frame. Base R and ggplot2 were used to create bar plots representing community composition (Wickham, 2016). Taxonomic patterns of trait conservation were examined following rarefaction by aggregating by different taxonomic

levels. PERMANOVA was used to find the taxonomic level by which resulting communities were most distinct by temperature regime. P values were adjusted for multiple comparisons using the False Discovery Rate method (Benjamini and Hochberg, 1995) and R command p.adjust.

First replicates and Source Soil were lost due to sequencing errors. Extracted DNA from Source Soil was amplified with 515F and 806R primers and sequenced by Illumina MiSeq (2 x 250). Source soil was not used for any calculations because of the inherent differences between sequencing preparation methods and differences between NovaSeq and MiSeq sequencing technologies. Figures with Source Soil for reference are included in Appendix A.

Identifying putative generalists

Putative thermal generalists were identified after removing genera below 1% relative abundance and looking across temperature regimes for presence/absence. A 1% relative abundance threshold was used to prevent the misidentification of rare or contaminant taxa as putative generalists. Several genera were identified as being able to persist or colonize above this threshold in all temperature regimes and were identified as putative thermal generalists.

Contaminants

Contamination found in Negative-Survivor and Negative-Colonizer plates was examined by DNA yield per gram of soil as a proxy for biomass. Optical density, measured by Qubit Fluorometer, was measured for two randomly selected replicants from each plate type, temperature regime, and time-point. Both Chao and Shannon alpha diversity were calculated for negative control communities and compared to scores from treatments communities. Fisher's ANOVA was applied to both DNA yield and alpha diversity measures to detect significant differences between treatments and corresponding negative controls. Prevalent genera found negative controls (genera above 1% relative abundance) were identified and noted if the genus was also identified as a putative generalist within treatment communities.

Results

Following initial processing, we observed 267,157 ASVs (actual sequence variants) from 6,667,612 total reads. 87,961 singletons were present, comprising roughly 32.57% of all ASVs. 77% of reads were resolved at the genus level. The magnitude of these values is characteristic of NovaSeq data (Karadayı, 2021; Mei *et al.*, 2022).

Contaminants

Negative controls Negative-Survivor and Negative-Colonizer showed significant contamination either through imperfect sterilization of soil or through introduction at other points in microcosm construction or management. Fisher's ANOVA showed that Survivor and Negative-Survivor communities were typically distinct by estimated biomass and both alpha diversity measures (Figure 1.4A, B). In contrast, estimated biomass and both alpha diversity measures were rarely found to be distinct between Colonizers and either Negative-Survivors or Negative-Colonizers (Figure 1.4A, B).

Bray-Curtis dissimilarity distances were calculated between all plate type communities and visualized on an NMDS chart (Figure 1.4C). Full model PERMANOVA indicated that community composition was influenced by plate type among all samples ($R^2_{PlateType}$=0.07077, p<0.001), along with other variables (Appendix A, Table 2). Survivor and Nutrient-Depletion communities clustered most closely together with PERMANOVA indicating that plate type was influential in determining community composition ($R^2_{PlateType}$=0.01484, p<0.001). Colonizer communities overlapped with Negative-Survivor and Negative-Colonizer communities. Full PERMANOVA showed that Colonizer communities were distinct in community composition from negative controls ($R^2_{PlateType}$=0.04379, p<0.001).

Contaminants from Negative-Survivor communities primarily came from phylum *Proteobacteria*, making up an average of 77.7% of contaminants. *Proteobacteria* was followed in abundance by *Firmicutes* (22.9%), *Bacteroidetes* (12.8%), and *Planctomycetes* (8.75%). The most abundant genera across all Negative-Survivor communities were *Massilia* (20.0%), *Escherichia/Shigella* (18.2%), *Janthinobacterium*

(10.3%), and *Lysobacter* (6.1%) from *Proteobacteria*. *Proteobacteria* was also the dominant phylum in Negative-Colonizer communities (63.0%), followed by *Firmicutes* (23.4%), *Actinobacteria* (13.8%), *Bacteroidetes* (13.0%), and *Gemmatimonadetes* (12.6%). The most abundant genera in Negative-Colonizer communities included *Massilia* (24.5%) and *Escherichia*/*Shigella* (9.2%), as well as *Oxalicibacterium* (4.9%) from phylum *Proteobacteria*, and *Pseudarthrobacter* (5.7%) and *Paenibacillus* (5.8%) from phyla *Actinobacteria* and *Firmicutes*, respectively.

Figure 1.4

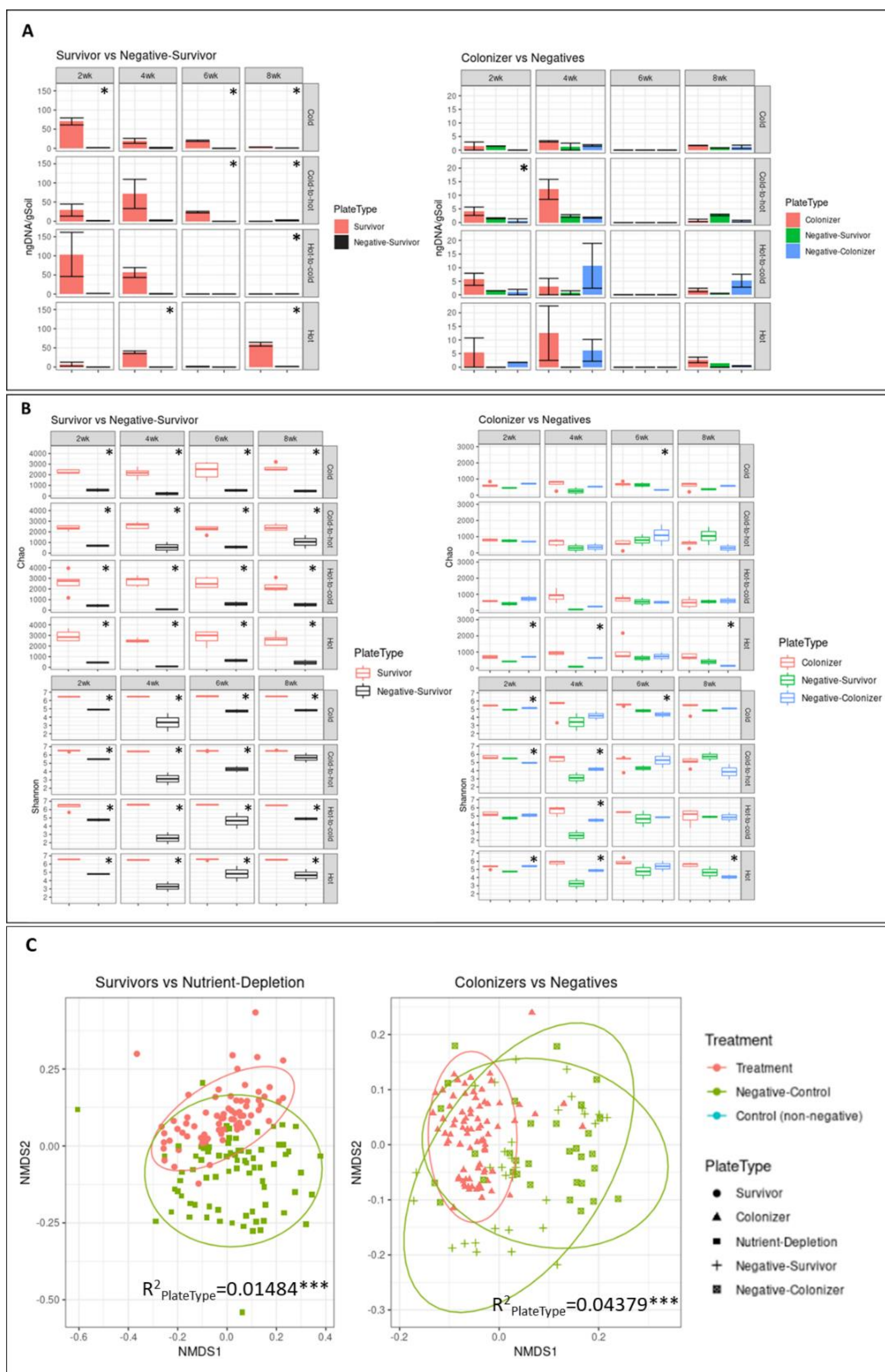

Figure 1.4: Comparing treatments and controls. (A) ng DNA/g soil yield from extraction estimated by optical density, (B) Chao and Shannon alpha diversity measures, (C) Bray-Curtis dissimilarity distances visualized on NMDS charts with $R^2_{PlateType}$ from PERMANOVA. * indicates a significant difference between treatment and corresponding controls, *** indicates $p<0.001$

Alpha diversity

Alpha diversity was calculated by both Chao and Shannon indices following rarefaction. Temperature regime did not typically significantly impact estimates of biodiversity by either index for any plate type or at any time-point, in contrast to our expectations from Hypothesis 1a (Figure 1.5). The one exception occurred when calculating Chao richness in Nutrient Depletion, a control, in the final time-point (Fisher's ANOVA, F=3.609, p=0.036). Mean diversity in Survivor plates was higher than in Colonizer plates (Fisher's ANOVA, F_{Chao}=148, p<0.001, $F_{Shannon}$=106.1, p<0.001).

Figure 1.5

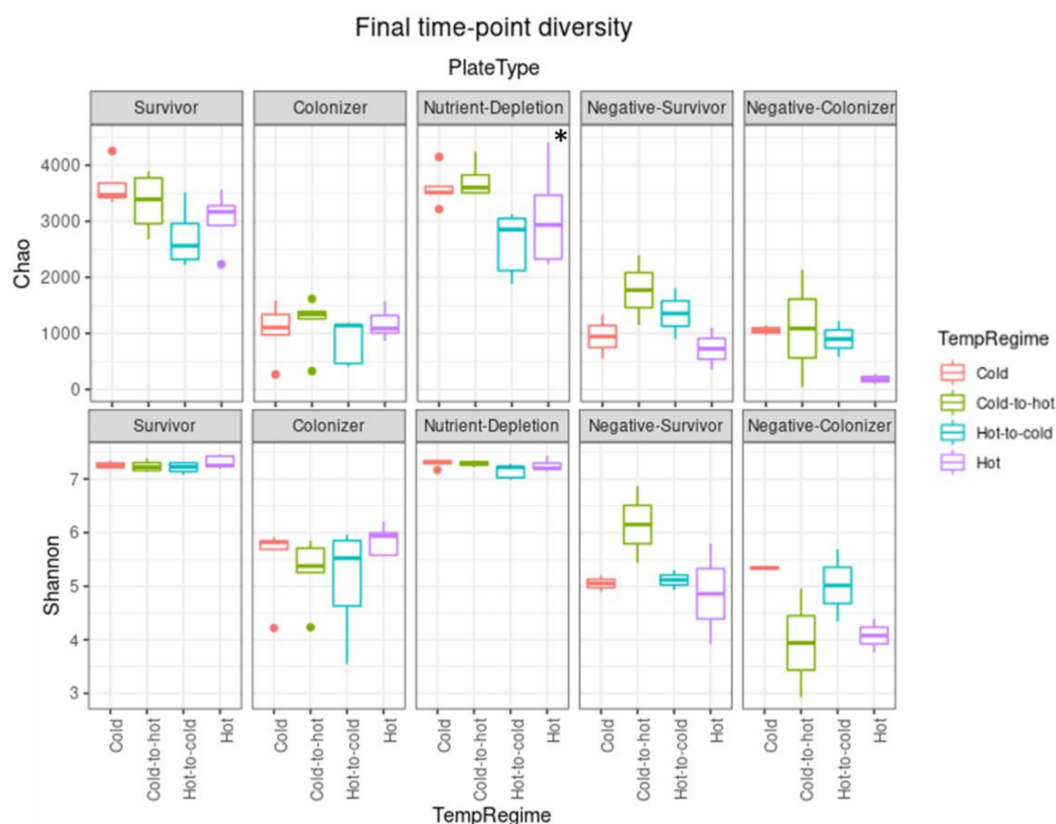

Figure 1.5: Chao and Shannon diversity indices for final time-point of each plate temperature regime and plate type.

Beta diversity

Bray-Curtis dissimilarity distances between samples were calculated following rarefaction and visualized on non-metric multi-dimensional scaling (NMDS) plots. A full model for the dataset containing all variables was calculated with Bray-Curtis distances

by PERMANOVA, showing that temperature regime, plate type, and time-point all significantly influenced bacterial composition ($R^2_{TempRegime}$=0.02233*, $R^2_{PlateType}$=0.07077*, $R^2_{TimePoint}$=0.0119*, *p<0.001, Appendix A, Table 2). Across the final time-point, temperature regime and plate type were both significant ($R^2_{TempRegime}$=0.04513, p<0.001; $R^2_{PlateType}$=0.14113, p<0.001).

When plate type was controlled for within the final time-point, the effect of temperature regime was even more pronounced for all plate types. Temperature regime explained 22.782% of the variation between Survivor communities in the final time-point (p<0.001) and 23.234% of the variation between Colonizer communities in the final time-point (p<0.001, Figure 1.6). The impact of temperature regime on both Survivor and Colonizer communities across all time-points varied. In the case of Survivor communities, all R^2 values occurred around 23% ($R^2_{TimePoint1}$=0.23302*, $R^2_{TimePoint2}$=0.20467*, $R^2_{TimePoint3}$=0.19981*, $R^2_{TimePoint4}$=0.22782*, *p<0.001), showing that the effect of temperature regime was consistent throughout all time-points. However, the impact of temperature regime generally decreased in Colonizer communities across time-points ($R^2_{TimePoint1}$=0.39672*, $R^2_{TimePoint2}$=.28533*, $R^2_{TimePoint3}$=.32701*, $R^2_{TimePoint4}$=0.23234*, *p<0.001), indicating that temperature regime was more important in earlier time-points for Colonizer communities.

Figure 1.6

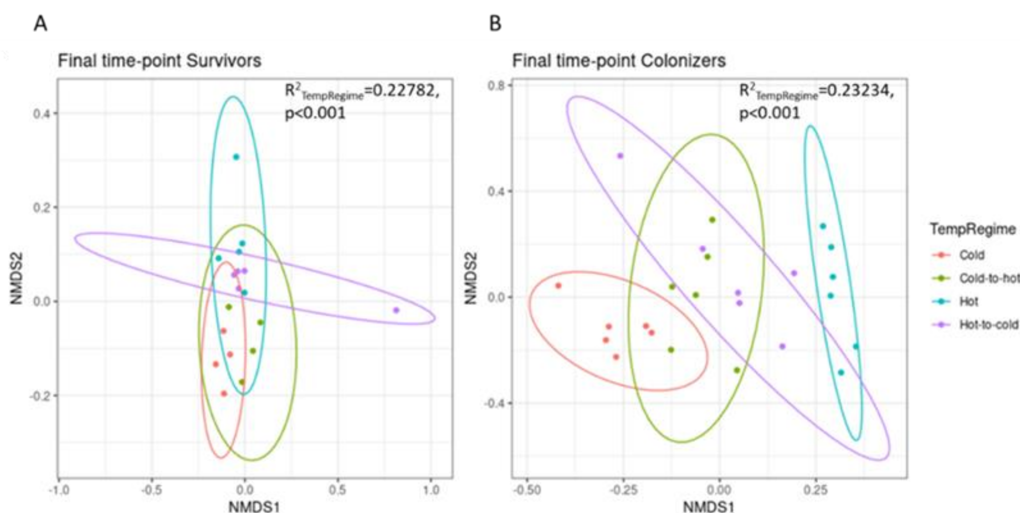

Figure 1.6: Bray-Curtis ordination of final time-point (A) Survivor plates and (B) Colonizer plates by temperature regime.

PERMANOVAs were also run after resolving to various taxonomic ranks to see if communities were more distinct by temperature regime at certain taxonomic levels (Table 1.1). The false discovery rate method was used to correct for multiple comparisons. In Survivor communities, the strongest clustering was found at the genus level (R^2=0.6121, p=0.0015). R^2 values for Survivor communities decreased at higher taxonomic levels, indicating that there was more variation between temperature regimes at lower taxonomic levels. In contrast, the strongest clustering for Colonizer communities was found at the class level (R^2=0.70798, p<0.001), indicating that the strongest dissimilarities between Colonizer communities were found at this taxonomic level.

Table 1.1

	Phylum	Class	Order	Family	Genus	ASV
Survivors	R^2=0.49961, p=0.005	R^2=0.55146, p=0.0024	R^2=0.54665, p=0.0015	R^2=0.58424, p=0.0015	R^2=0.6121, p=0.0015	R^2=0.22782, p=0.0015
Colonizers	R^2=0.47234, p=0.001	R^2=0.70798, p=0.001	R^2=0.41309, p=0.001	R^2=0.40644, p=0.001	R^2=0.40526, p=0.001	R^2=0.23234, p=0.001

Table 1.1: PERMANOVA results from final time-point Survivors and Colonizers at different taxonomic levels, with p values adjusted for multiple comparisons with the False Discovery Rate method.

Composition

All plate type communities were consistently dominated by *Proteobacteria* at all time-points and temperature regimes, along with many of the negative controls (Figure 1.3). In Survivor communities, *Proteobacteria* made up 39.34%-59.95% of reads, typically followed in prevalence by *Acidobacteria* (12.29%-19.90%), *Actinobacteria* (9.39%-13.09%), and *Chloroflexi* (9.25%-1.829%). Some phyla were only detectable in certain temperature regimes, such as *Elusimicrobia* at 0.00319% relative abundance in hot-constant conditions, and *Patescibacteria* and *Halanaerobiaeota* at 0.00191% and 0.000636% in cold-constant conditions. No unique phyla were found in either fluctuating temperature regime at >1% prevalence. In fact, community composition for fluctuating temperature regimes was typically intermediate to hot and cold-constant temperature regimes, showing a range of taxa responses to temperature.

Colonizer communities were further dominated by *Proteobacteria* (76.283%-99.581%), followed by *Bacteroidetes* (1.777%-9.707%), and *Firmicutes* (1.322%-8.224%), indicating that these phyla contain members capable of colonizing under these conditions. While fluctuating temperature regimes in Survivor communities were typically intermediate in composition to both constant temperature regimes, in Colonizer communities this was less true, with *Proteobacteria* being most prevalent in the two fluctuating treatment communities. *Massilia*, a genus found in *Proteobacteria*, was especially prevalent in Colonizer communities at 25.43%-74.41%, compared to the maximum abundance of 3.45% obtained in Survivor communities regardless of temperature regime, indicating successful colonization under a range of 10°C to 30°C.

Putative generalists

Several genera were identified as persisting or colonizing under all temperature regimes at above 1% relative abundance in both Survivor and Colonizer communities. Within Survivor communities, genera *Arenimonas*, *Luteimonas*, and *Lysobacter* from *Gammaproteobacteria*, *Sphingomonas* and *Skermanella* from *Alphaproteobacteria*, *Pseudarthrobacter* from *Actinobacteria*, and *RB41* from *Acidobacteria* were identified as putative thermal generalists, persisting at above 1% relative abundance in all temperature regimes. These putative generalist Survivors together made up 2.5% of genera detected and accounted for 34.5% of all reads in Survivor communities. All genera except *Lysobacter* had significantly more reads than either negative control when read counts were compared by Fisher's ANOVA (Appendix A, Figure 6). Of these putative thermal generalist Survivor genera, three were not prevalent as contaminants of negative control communities; *Arenimonas*, *Luteimonas*, and *Skermanella*.

Genera found to colonize at above 1% relative abundance in all temperature regimes included *Massilia*, *Pseudomonas*, and *Escherichia*/*Shigella*, all from *Gammaproteobacteria*. Notably, all three of these genera were found to be significant (above 1% relative abundance) contaminants in Negative-Survivor and Negative-Colonizer communities. Together, these genera made up 1.8% of detected genera and 36.5% of reads found in all Colonizer communities. Fisher's ANOVA of read counts by plate type for each of these genera did not indicate any significant differences between

Colonizer and negative communities, although all three genera were more prevalent in Colonizer communities (Appendix A, Figure 7).

A threshold of 1% relative abundance was chosen to prevent misidentification of dead, rare, and contaminant taxa as putative thermal generalists. Relaxing this threshold to 0.1% increased the number of putative thermal generalists, the proportion of putative thermal generalists in the community, and the proportion of reads from putative generalists (Appendix A, Table 5).

Figure 1.7

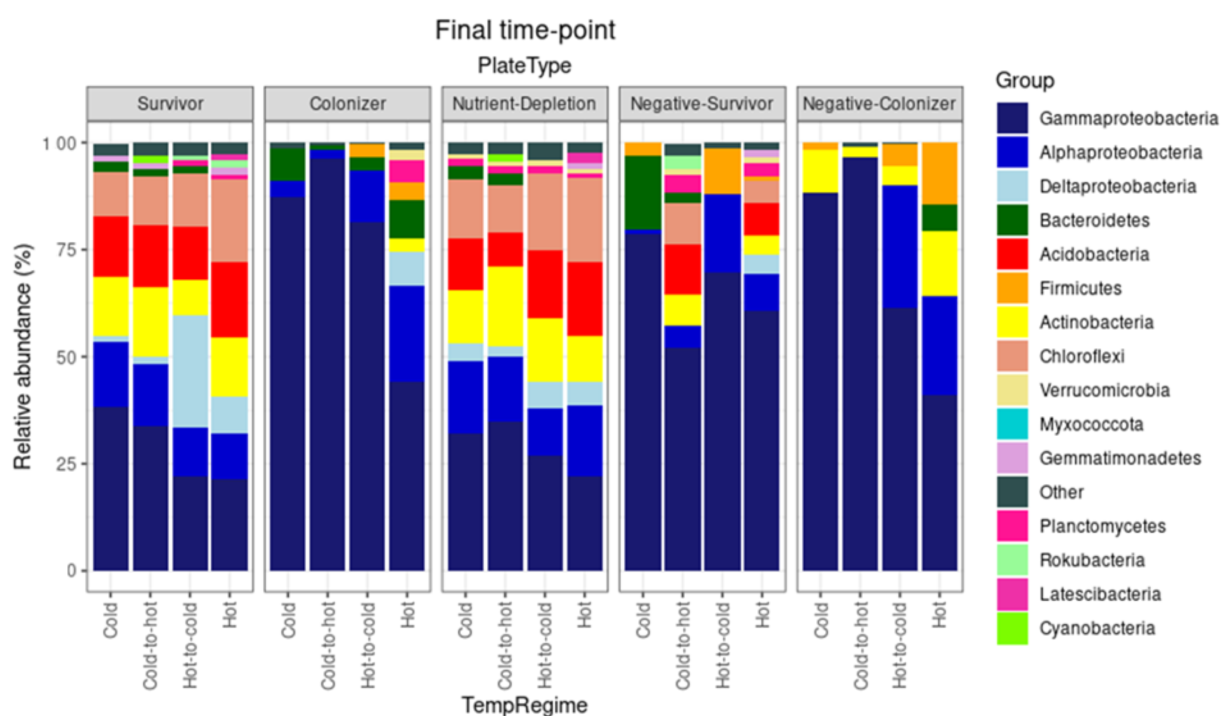

Figure 1.7: 100% relative abundance plots for all phyla above 1% prevalence in all temperature regimes (vertical), plate types (horizontal), and time-points (x-axis).

Discussion

Overall, our results show that temperature regime had a significant impact on soil bacterial community composition in both Survivor and Colonizer communities. No significant impact on community alpha diversity was found in either plate type, showing that biodiversity was not significantly impacted by temperature regime. Patterns of thermal tolerance were found at the genus level in Survivors, while Colonizer

communities clustered most distinctly at a higher taxonomic level, indicating the importance of other traits in colonization in any temperature condition.

Hypothesis 1: Community composition will be impacted by different temperature regimes.

Bacterial composition in both Survivor and Colonizer communities was distinctly impacted by temperature regime, explaining 22.782 and 23.234% of the variance between communities at the last time-period respectively. This significant impact of temperature regime on resulting communities agrees with what has been found in previous studies (Xiong *et al.*, 2014; Wu *et al.*, 2015; Luláková *et al.*, 2019), although direct comparison is limited for several reasons. These microcosm studies were focused mainly on the effect of warming rather than heterogeneity (Xiong *et al.*, 2014; Wu *et al.*, 2015; Luláková *et al.*, 2019), while other studies looking at communities under various temperature conditions have generally been observational (Z. Zhou *et al.*, 2016; J. Zhou, Wang and Luo, 2020). Additionally, many investigations of fluctuating temperatures have been performed on soils predicted to experience the most drastic change in temperature under global warming, such as those in arctic and alpine regions (Wu *et al.*, 2015; Luláková *et al.*, 2019), rather than agricultural and temperate soils. Initial bacterial composition, soil type, temperature regimes, and time periods used in other studies also limit direct comparison to this study.

However, several studies that use multiple soils and temperature conditions show that patterns between temperature change and bacterial composition are often shared across microbiomes and soil types. An example of this is in Luláková et al. (2019), in which two alpine surface soils were warmed to 4°C, 15°C, 25°C, and 40°C over 14 days. Both surface soils showed similar impacts of temperature increase, although with distinct differences between them, including initial bacterial diversity and other physiochemical differences, indicating that different microbiomes and soil types can show similar patterns at different temperatures. In another study, bacteria experiencing a 1°C or 2°C increase over 15 years were also significantly different in composition, indicating that both the time and magnitude of temperature change can be influential (Xiong *et al.*, 2014). Those results, along with the results of this study,

indicate that the impact of temperature change on soil bacterial communities varies based on several variables, but that temperature change in general is influential for bacterial composition. This experiment builds on the existing body of literature by providing an experimental example of short-term temperature change on a single agricultural soil community from a temperate region.

Survivor communities of all temperature regimes shared most of the same phyla, indicating widespread persistence across temperature conditions, although dead cells may have been detected as well. Persistence may have occurred because of the ability to form dormancy structures, a prevalent trait across bacteria (Lennon and Jones, 2011). Dominant phyla in Survivor communities were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Chloroflexi*. Several genera were found to exist above 1% relative abundance in all temperature regimes, often belonging to these dominant phyla. Some of the putative generalist genera are known to have desirable metabolic abilities, such as the recorded ability of *Sphingomonas* strains to break down certain environmental contaminants (Ni'matuzahroh, M. Gilewicz, M. Guilano, 1999), potentially making these taxa desirable as microbial products.

Proteobacteria, *Bacteroidetes*, and *Firmicutes* became more prevalent in Colonizer communities, showing the presence of successful colonists within these phyla. Compared to Survivor communities, Colonizer communities were less diverse, showing the added difficulties of colonizing under variable temperature regimes. Several genera were found above 1% relative abundance in all Colonizer temperature regimes, all from class *Gammaproteobacteria*. Successful putative thermal generalist colonists *Escherichia*, *Pseudomonas*, and *Massilia* were often significantly more abundant in Colonizer communities than Survivors, hinting at possible tradeoffs between generalism and competitive ability in these genera.

Hypothesis 1a: Alpha diversity of bacteria under heterogeneous temperature regimes will decrease.

In contrast to the expectation of reduced biodiversity under heterogeneous temperature regimes, the resulting communities in both Survivor and Colonizer plate

types showed no significant changes in biodiversity by temperature regime. In the Survivor communities, a possible explanation is that bacteria largely persisted through certain characteristics such as dormancy, which is widespread in bacteria (Lennon and Jones, 2011). This contrasts with some studies that indicate a significant change in biodiversity under different temperature regimes, while corresponding to results found in others that suggest no significant change, although interpretations are again limited by a variety of other variables, such as the magnitude of the temperature change and the speed at which it was achieved. In the case of the Luláková *et al.* (2019) study on two alpine surface soils, alpha diversity was not significantly changed between communities at 4°C, 15°C, and 25°C, but was significantly lower at 40°C (2019). This indicates that certain ranges of temperatures, likely those further outside of a community's typical conditions, are more likely to impact alpha diversity than temperatures that are more typical for the community. Taken in this context, our results may indicate that fluctuation between temperatures in the range of 10°C to 30°C do not negatively impact the bacterial diversity of this temperate, agricultural soil, although those trends may change based on the timespan of the fluctuating conditions.

Hypothesis 1b: Patterns by which bacterial composition changes under temperature regimes will be found at lower taxonomic levels.

Aggregating by different taxonomic levels revealed different strengths of clustering for both Survivor and Colonizer communities. Because traits related to temperature are expected to be conserved at a relatively shallow degree because of the relative simplicity of pertinent genes (Martiny *et al.*, 2015) we expected to see the strongest trends at the genus level. In Survivor communities, the strongest clustering was found at the genus level in agreement with Martiny *et al.* (2015) indicating that traits related to temperature are conserved at lower taxonomic levels compared to other, more complex traits, like those related to pH. In contrast to traits conferred by more complex genetic architecture, traits enabling thermal generalism may be more likely to arise independently in lower taxonomic levels.

Colonizer communities clustered most strongly at the class level, indicating the importance of more complex traits involved in successful colonization. Previous

literature has shown the importance of traits such as phenotypic plasticity or motility in mediating successful bacterial colonization (Thompson *et al.*, 2005; Mallon, Van Elsas and Salles, 2015; Kaminsky *et al.*, 2018). In larger organisms, traits related to growth and reproductive strategy have been linked to successful colonization (Sakai *et al.*, 2001), which may also be true for bacteria. This may mean that Colonizer communities, in addition to being sorted by temperature tolerance at lower taxonomic levels, were also sorted by traits conserved at higher taxonomic levels. These traits could be phenotypic plasticity, motility, or traits related to metabolism or reproductive strategy, resulting in strong clustering at the class level.

Hypothesis 2: Colonizer communities will be less diverse than Survivor communities as certain taxa incapable of colonization under certain thermal conditions become less abundant.

Bacteria were significantly less diverse in Colonizer communities than in Survivor communities based on both Chao and Shannon diversity measures. This agrees with previous research indicating the relatively rare success of colonization (Thompson *et al.*, 2005; Mallon, Van Elsas and Salles, 2015; Kaminsky *et al.*, 2018), as well as previously-mentioned studies that show relatively high levels of persistence across temperature conditions (Xiong *et al.*, 2014; Wu *et al.*, 2015; Luláková *et al.*, 2019). A limitation of this experimental design is that successful colonization must have occurred within a 2-week span of time to be collected for sequencing, potentially excluding slower-growing colonists. However, studies on larger organisms have typically shown that successful colonists are often faster-growing (Sakai *et al.*, 2001).

<u>Identifying putative generalists</u>

Several genera were identified as putative generalist survivors or colonizers, occurring above 1% relative abundance in all temperature regimes. Several of these genera have been proposed for use in bioremediation, such as *Sphingomonas* (Ni'matuzahroh, M. Gilewicz, M. Guilano, 1999). Others belong to groups of interest such as *Massilia* in family *Oxalobacteraceae*, which contains several known diazotrophs. Several genera identified as putative thermal generalists were also prevalent (>1% relative abundance) as contaminant taxa in Negative-Survivor and

Negative-Colonizer communities. Importantly, all putative generalist taxa were more abundant in treatment communities than negatives, except *Lysobacter*, often significantly so, increasing our confidence that these taxa are the result of treatments and not just environmental contaminants.

Conclusion

In this study, where soil microcosms were maintained under either heterogeneous or homogeneous temperature regimes, we found that bacterial composition was significantly influenced by temperature regime. This agrees with previous literature, although direct comparison is limited by variables including chosen temperature regime, timespan, soil type, and starting bacterial composition. We found that alpha diversity was not significantly impacted by temperature regime for either community type, indicating that biodiversity does not decrease under these conditions for either Survivors or Colonizers, either by traits such as dormancy, or an increased thermal tolerance among successful colonizers. Taxonomic patterns were found at the genus level in Survivor communities, indicating that traits related to thermal tolerance are relatively shallowly conserved. Taxonomic patterns were found at the class level in Colonizer communities, indicating a more deeply conserved trait relevant to successful colonization under these conditions. We also found that bacteria are more capable of persisting rather than colonizing under various temperature conditions, as indicated by a significant difference in Survivor and Colonizer alpha diversity, regardless of temperature regime.

Fluctuating temperature regimes did not significantly impact bacterial diversity in this context. However, these communities were different based on temperature regime, showing variable thermal niche breadth among constituent members. Overall, bacterial composition shifted from temperature extremes, with fluctuating temperatures showing intermediate composition. Many phyla showed thermal generalism, likely through dormancy. Certain phyla such as *Proteobacteria* showed a combination of thermal generalism and successful colonization, with a specific example of this being *Massilia*. Patterns of thermal generalism were found at the genus level in Survivor communities

and at the order level in Colonizer communities, indicating differential conservation of certain traits.

Future work

Results from this study could be expanded upon by environmentally filtering by multiple variables, especially those likely to significantly change in the future, as communities may react differently to different variables. The use of multiple environmental filtering regimes may also reveal characteristics shared between generalists of different variables, such as an increased proclivity for horizontal gene transfer or the maintenance of a larger genome, perhaps showing that generalists of one variable are likely to be generalists of another, or 'multi-generalists'. Because environments rarely change by one measure alone, this might be a better representation of how environmental heterogeneity impacts bacteria.

Another potential avenue of exploration has to do with ecosystem functioning. In microbial ecology, the relationship between biodiversity and ecosystem function is unclear. Bacteria are incredibly important for biogeochemical cycling and other ecosystem services globally, making the understanding of these relationships relevant. Future studies could explore how communities filtered by fluctuating temperature conditions differ in important ecological functions, such as carbon cycling.

Limitations

There are certain limitations to this experiment. Soil is imperfectly sterilized by triple-autoclaving regimes, resulting in microbial grow-back over the time in which this experiment occurred and limiting our ability to discern between active colonists and dormant surviving bacteria. Current sequencing technologies also do not distinguish between dead, dormant, or active cells, meaning that it is possible that a degree of dead cells may have been collected. In this design, strains included in Colonizer samples had to have colonized within a two-week period, potentially excluding slower-growing colonizers. Another potential limitation is that temperature cannot be wholly separated from other environmental variables like moisture and salinity.

Funding

This work was supported by the United States Department of Agriculture National Institute of Food and Agriculture (grant no. 2020-67013-30865). Findings and conclusions do not necessarily reflect the view of the funding agency.

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Chapter 2: The impact of pH heterogeneity on bacteria within an agricultural soil community

Introduction

Specialist organisms, defined by having a relatively narrow niche breadth are expected to be more impacted by increased environmental heterogeneity (Hutchinson, 1953; Roughgarden, 1974; Kassen, 2002; Ma and Levin, 2006; Devictor, Julliard and Jiguet, 2008; Pandit *et al.*, 2015). Patterns of increased vulnerability of specialist organisms to changes in environment have been found in bird, fish, and butterfly communities, among others (Le Viol *et al.*, 2012; Börschig *et al.*, 2013; Büchi and Vuilleumier, 2014; Stuart-smith *et al.*, 2021). Bacterial communities may also be impacted by environmental heterogeneity, with specialists being disadvantaged, although traits such as widespread dormancy and horizontal gene transfer in microbial life may change these patterns. Bacterial communities, especially those in the soil, provide crucial ecosystem services such as nutrient cycling and decomposition, making prediction of impacts of environmental heterogeneity on bacterial communities of significant interest.

As with temperature (Chapter 1), pH is an environmental variable that is likely to diverge from historical norms and has a significant impact on microbial life, altering cellular processes and community composition (Hughes, Cullum and Bennett, 2007; Lauber *et al.*, 2009; Rousk *et al.*, 2010; Krulwich, Sachs and Padan, 2011). Soil pH will likely be influenced by future changes in weather and land management, potentially increasing in variability with space and time (Rengel, 2011). Soil pH can vary dramatically, even on the scale of a few feet, and often changes temporally, depending on weather events like rain, as well as land management practices (Serrano *et al.*, 2010; Kumhálová *et al.*, 2011). This can create a patchwork of distinct communities shaped in part by pH across time and space.

Different pH conditions can challenge bacteria at a cellular level. Environmental pH impacts the structural integrity of membranes, affecting the ability to transport vital

substances and disrupt protein action from both inside and outside the cell (Krulwich, Sachs and Padan, 2011). It has been found that pH impacts protein formation and action (Somero, 1986). Within single lineages, adaptations to extreme pH can include changes to the cell membrane and carefully controlled intracellular conditions to maintain pH homeostasis, especially with respect to the cytosol, and differential enzyme formation and expression (Hughes, Cullum and Bennett, 2007; Krulwich, Sachs and Padan, 2011). Environmental pH levels may also influence the ability to maintain dormancy structures by altering cytosolic conditions, impacting cellular activities related to dormancy (Pu *et al.*, 2019).

Environmental pH has also been documented to change bacteria at the community level. In soils, differences in pH have been seen to have strong impacts on bacterial composition (Rousk, Bååth, et al.; Lauber et al.; Högberg et al. 2006). For example, Rousk *et al.*, 2010 surveyed a field that had been subject to a long-term liming experiment and had a pH range of 4.0 to 8.3, finding that pH explained 29.1% of variation between different bacterial assemblages (Rousk *et al.*, 2010). In a survey of North and South American soils, Lauber and colleagues found that pH was significantly correlated with differences in bacterial composition, explaining 79% of the variation between soil samples (Lauber *et al.*, 2010). However, these studies are typically survey-based, limiting the ability to control for available bacterial pools and other soil characteristics. Survey-based studies cannot confirm the range of pH tolerance available to constituent taxa or how community dynamics may shift due to differences in pH tolerance.

Tolerance to a particular pH range may be more highly conserved than tolerance to a particular temperature range. Due to the complexity of genes related to pH, it has been suggested that pH tolerance is conserved at higher taxonomic levels than temperature (Martiny 2015). This may impact the likelihood of horizontal gene transfer of these traits, as more complex traits are less likely to be transferred in full with horizontal gene transfer, reducing the likelihood that these traits will be functional (Jain, Rivera and Lake, 1999; Cohen, Gophna and Pupko, 2011). In previous studies, certain taxa have been observed to be differentially impacted by pH conditions. In a survey-

based study performed by Rousk *et al.* (2010), certain phyla and classes were seen to display contrasting relationships with changing pH conditions. For example, they found that *Acidobacteria* subgroup 16 and *Alphaproteobacteria* were less sensitive to pH range, occurring at similar levels at a pH of 4 to 8.3. In contrast, *Acidobacteria subgroup 3* significantly decreased in higher pH conditions while *Gammaproteobacteria* significantly increased. The distinct and variable higher-order taxonomic patterns found in this study provide additional evidence to a higher level of conservation for genes related to pH.

We aimed to investigate the impact of pH heterogeneity on bacterial composition in an agricultural soil by transplanting non-sterile soil into sterile soils with a range of pH levels (5.0, 5.77, and 6.72). The use of both Survivor and Colonizer plate types allows for differentiation between cells that either persist or colonize under different pH conditions, respectively. Bacteria found under all pH conditions may also be tentatively identified as potential pH generalists. Our expectations are that bacterial composition will be impacted by pH, with certain taxa being differentially sensitive due to differences in their niche breadth along the pH axis. We expect to see community composition change as bacteria respond to pH, with shifts largely among higher taxonomic levels due to the proposed complexity of genes related to pH. Alpha diversity is also likely to decrease as pH-specialist taxa are disadvantaged due to unfavorable pH conditions. Additionally, we expect that Colonizer communities should be less diverse than Survivor communities, due to the additional requirements of colonization, but that Colonizer communities should increase in biodiversity with time as slower-growing colonists move into the sterilized soil.

Hypotheses:

1. Differences in soil pH in the recipient microcosms will impact the bacterial composition of resulting communities.
    a. Alpha diversity under heterogeneous pH conditions will decrease.
    b. Taxonomic patterns of pH tolerance will be most apparent at higher taxonomic levels.
2. Colonizer communities will contain less diversity than Survivor communities.

Methods

Environmental Filtering

Soils were collected from the Russell E. Larson Agricultural Research Farm, located in Pennsylvania Furnace, Pennsylvania, USA (Appendix B, Figure 1). These soils had pH levels of 4.8, 5.69, and 6.55 prior to autoclaving (Appendix B, Table 1). Soils were collected within a 300m radius of each other to control for the effects of other dissimilarities in the soils (e.g., nutrient levels). Soils were subsequently sieved to 0.2" for texture uniformity and stored at 4°C. A portion of these soils were autoclaved for 90 min at 121°C and 20lbs/in^2 three times, with an autoclave session each consecutive day. After autoclaving, soil pH levels were 5.0, 5.77, and 6.72 respectively.

Microcosms were assembled with either low, mid, or high-pH triple-autoclaved soil in 100mm x 25mm Petri dishes. We chose to use the non-autoclaved mid-pH soil (pH 5.69) as the source soil because it was intermediate to the post-autoclaving pH levels of 5.0 (low-pH) and 6.72 (high-pH). The unsterilized source soil was placed into ethanol-sterilized tubular nylon inoculum bags to maximize surface area interaction with the surrounding environment. This inoculum bag was then placed in soil microcosms, constituting 20% of the mass within the plate (Figure 1.1) and saturated with sterile water. These plates, termed "Single-transplants" were sampled destructively at 2, 4, 8, and 16 weeks. Additionally, portions of the plates sampled at 8 weeks were used to inoculate the reciprocal pH extreme (termed "Double-transplants"), allowing for increased heterogeneity, and were sampled at 16 weeks.

Figure 2.1

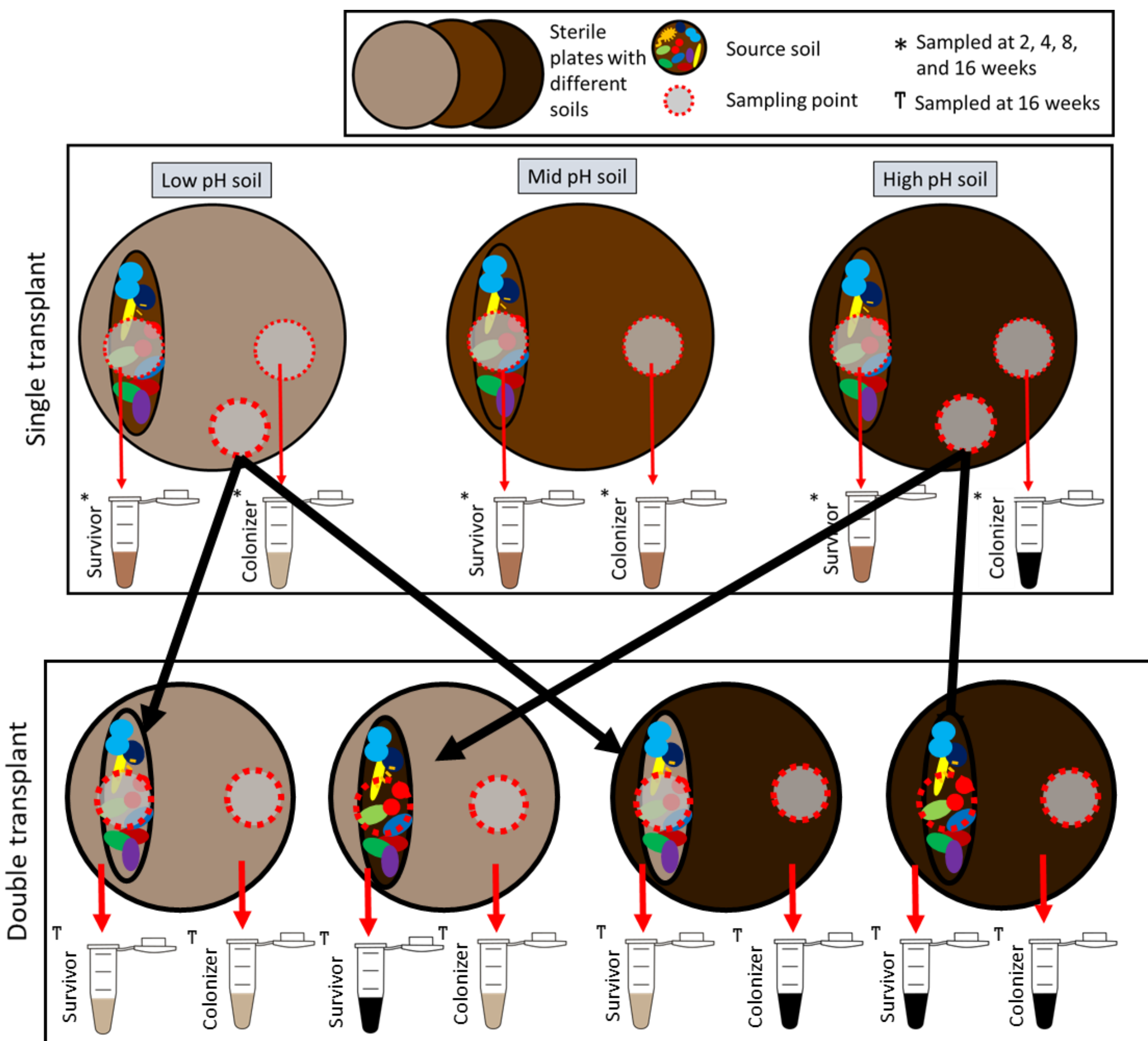

Figure 2.1: A visual representation of the environmental filtering by pH design, with light brown representing the low pH soil, medium brown representing the mid pH soil, and dark brown representing the high pH soil. Initial plates will be sampled destructively at 2, 4, 8, and 16 weeks. Double transplant plates will be transferred to a new bed of sterile soil at 8 weeks and sampled again at 16 weeks.

Sequencing

DNA was extracted from 0.25-0.35g of soil with NucleoSpin 96 Soil DNA extraction kit from Macherey-Nagel (Bethlehem, PA, USA) according to manufacturer's instructions. The v4 region of the 16S rRNA gene was then amplified with 505F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACVSGGGTATCTAAT-3')

Illumina primers (Apprill *et al.*, 2015; Parada, Needham and Fuhrman, 2016). Illumina Nextera XT primers were used for indexing. Samples, as well as any negative controls that amplified (57.8%), were then pooled and sent to the Pennsylvania State University Genomics core, where they were sequenced on the Illumina MiSeq platform (2 x 250).

Data analysis was performed in R 3.5.2 (R Core team 2018), with pre-processing performed using the DADA2 pipeline (Callahan *et al.*, 2016). Reads were truncated at 250 and 220 base pairs according to quality profiles. Alpha and beta diversity were both calculated with Phyloseq after removing samples with less than 5,000 reads and rarefying using Phyloseq's rarefy_even_depth to correct for uneven sampling (McMurdie and Holmes, 2013). Phyloseq's estimate_richness command was used to calculate Chao and Shannon diversity, and ANOVA was found with vegan's aov command to determine significant differences. Beta diversity was calculated using Phyloseq's distance command with Bray-Curtis distances as the distance parameter. PERMANOVA was then performed with vegan's adonis command (Jari Oksanen *et al.,* 2020). The Phyloseq object, including low abundance samples (reads<5000), was transformed to show relevant abundance and converted to a data frame. Base R and ggplot2 were used to create figures. PERMANOVA was applied after aggregating at different taxonomic ranks to detect the taxonomic rank at which communities under different pH regimes were most distinct. The False Discovery Rate method (Benjamini and Hochberg, 1995) was used to adjust p values following multiple comparisons to prevent type I errors.

<u>Identifying putative generalists</u>

Amplicon sequence variants (ASVs) that were less than 1% relative abundance were then removed from the data frame and inspected to identify groups present in all pH regimes. 1% relative abundance was chosen as a somewhat arbitrary threshold when identifying putative pH generalists to avoid misidentifying rare, dead, or contaminant taxa. Genera found to persist or colonize at above 1% relative abundance in all pH regimes were identified as putative pH generalists.

<u>Contaminants</u>

Optical density of all treatments and controls was measured by Qubit Fluorometer to approximate DNA yield of extractions according to the manufacturer's instructions (Invitrogen, Waltham, MA, USA). Alpha diversity was measured by Chao and Shannon alpha diversity indices. Fisher's ANOVA was applied to detect significant differences between treatments and corresponding negatives. Bray-Curtis dissimilarity distances were calculated for the resulting communities and PERMANOVA applied to determine differences between treatments and negatives.

Results

After pre-processing, the resulting dataset contained 13,782 ASV's from 13,194,481 total reads. Of all reads, 70.58% could be resolved to the genus level.

Contaminants

Negative-Survivor and Negative-Colonizer controls were found to have some amount of contaminant taxa through either imperfect sterilization of soil by triple autoclaving or introduction during environmental filtering. Fisher's ANOVA was used to look for significant differences between biomass (using DNA yield as a proxy) and alpha diversity measures of treatment and negative communities. No significant differences in biomass were seen between Survivors and Negative-Survivors or Colonizers and Negative-Colonizers at any time-point or under any pH condition (Figure 2.2A). Both Chao and Shannon alpha diversity measurements were typically distinct between Survivors and Negative-Survivors, and between Colonizers and Negative-Colonizers, especially at later time-points (Figure 2.2B).

Bray-Curtis dissimilarity distances of all resulting communities were visualized with an NMDS plot. Survivor and Colonizer communities clustered distinctly with PERMANOVA showing that community composition of all Survivor and Colonizer communities were significantly influenced by plate type ($R^2_{PlateType}=0.21368$, $p<0.001$, Figure 2.2C). Negative control communities clustered together, with plate type explaining less variation between communities ($R^2_{PlateType}=0.0157$, $p<0.001$). Contaminants in both negative control communities largely came from phyla *Firmicutes* (average of 72.2%), *Proteobacteria* (19.2%), *Bacteroidetes* (3.9%), and *Actinobacteria*

(3.4%). The most prevalent genera of contaminants included *Sphingoaurantiacus* (18.4%), *Massilia* (16.4%), and *Lysobacter* (15.2%) from phylum *Proteobacteria*, and *Pedobacter* (16.2%) from *Bacteroidetes*.

Figure 2.2

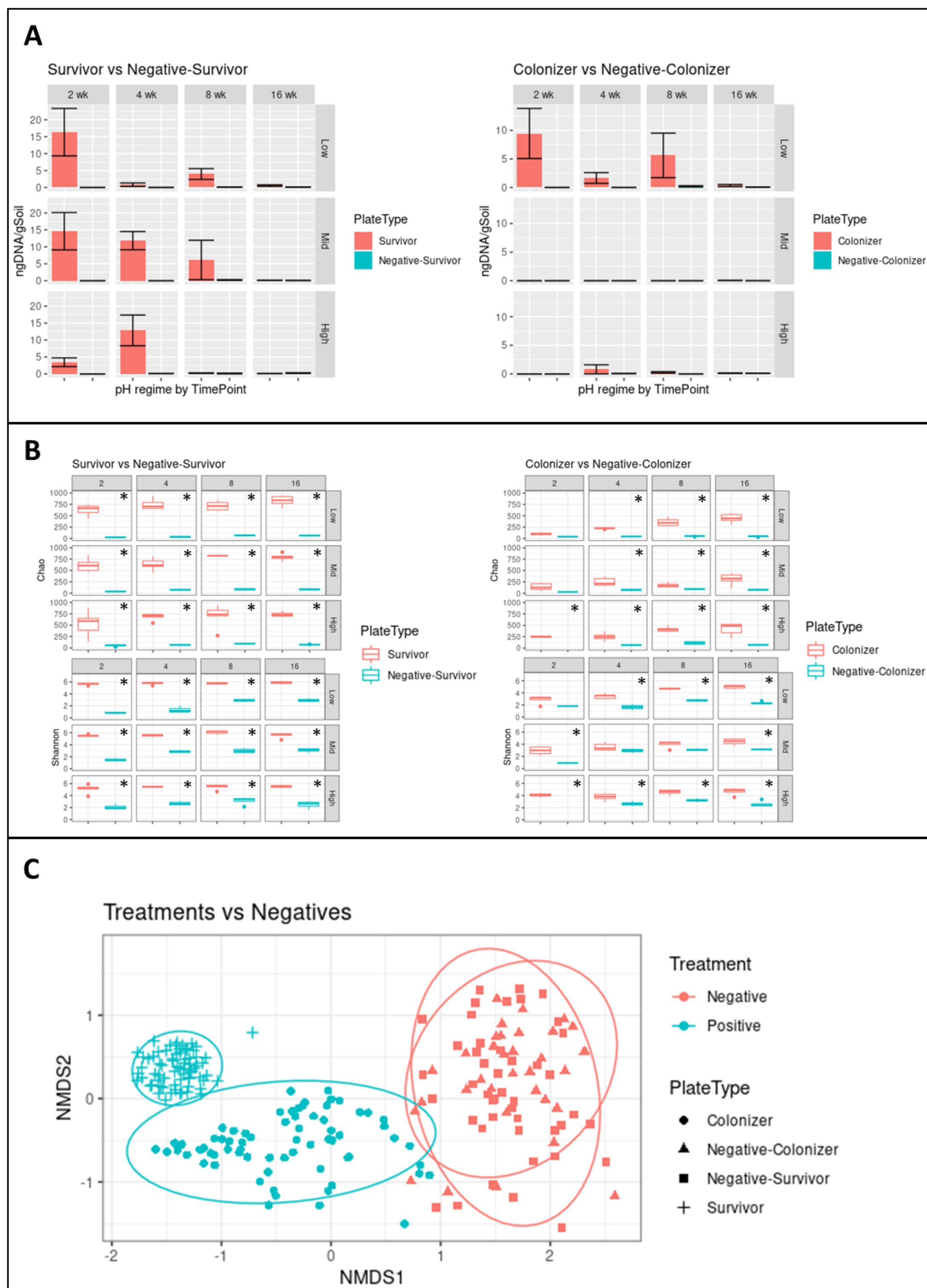

Figure 2.2: Comparing treatment and negative control communities. (A) ng DNA/g soil yield from extraction estimated by optical density, (B) Chao and Shannon alpha diversity measures, (C) Bray-Curtis dissimilarity distances between treatment and control communities. * indicates significant difference between groups.

<u>Alpha diversity</u>

Alpha diversity levels of treatment communities were not typically significantly influenced by pH regime. Instead, biodiversity among final time-point Survivor communities was found to be significantly impacted by transplant number (Fisher's ANOVA, F_{Chao}=213, p<2e-16, $F_{Shannon}$=91.5, p=8.87e-12) (Figure 2.3). Single-transplant Survivor communities were more diverse than Double-transplant Survivor communities, a negative relationship between diversity score and number of transplant events. Diversity scores of Single transplant Survivors were not significantly impacted by pH regime (F_{Chao}=1.642, p=0.229, $F_{Shannon}$=2.547, p=0.114). In Double transplant Survivors, pH regime was only significantly impacted by Shannon diversity index (Fisher's ANOVA, $F_{Shannon}$=3.453, p=0.036). Interestingly, the first pH condition of Double transplant Survivors was found to be more influential than the second by Shannon diversity (Fisher's ANOVA, $F_{Shannon}$=12.77, p=0.0016) but not Chao (Fisher's ANOVA, F_{Chao}=0.226, p=0.639). End pH regime was never significant in calculating diversity for Double transplant Survivors (F_{Chao}=0.226, p=0.639, $F_{Shannon}$=0.005, p=0.946) indicating that priority effects are influential in determining community diversity of Survivor communities.

Colonizer communities were significantly less diverse than Survivor communities (Fisher's ANOVA, F_{Chao}=172.1, p<2e-16, $F_{Shannon}$=184.4, p<2e-16), and again were not typically significantly impacted by pH regime. Within Colonizer communities, Transplant number was again found to be influential (Fisher's ANOVA, F_{Chao}=25.14, p =1.44e-05, $F_{Shannon}$=29.69, p=3.79e-06). Within Single transplant Colonizers, pH was not significantly different (Fisher's ANOVA, F_{Chao}=1.752, p=0.207, $F_{Shannon}$=2.096, p=0.157). Double transplant communities were also found to not be significantly impacted by pH regime (Fisher's ANOVA, F_{Chao}=1.572, p=0.235, $F_{Shannon}$=1.102, p=0.377). In Double transplant communities, the starting pH condition was significant by Chao (Fisher's ANOVA, F_{Chao}=25.14, p=1.44e-05) but not Shannon (Fisher's ANOVA, $F_{Shannon}$=3.738, p=0.0691), again hinting at the impact of priority effects on final time-point diversity. Furthermore, Single transplant Colonizer communities became significantly more

diverse as time went on, showing colonization success on different time scales. Fisher's ANOVA on both Chao and Shannon diversity indices were significant by time-point for Low and High-pH conditions (Low: F_{Chao}=22.53, p=1.27e-06, $F_{Shannon}$=27.09, p=3.02e-07; High: F_{Chao}=7.554, p=0.00144, $F_{Shannon}$=4.978, p=0.00968). Chao diversity was significantly impacted by time-point for Mid-pH communities (Fisher's ANOVA, F_{Chao}=4.666, p=0.0125) but not Shannon (Fisher's ANOVA, $F_{Shannon}$=2.696, p=0.0734).

Figure 2.3

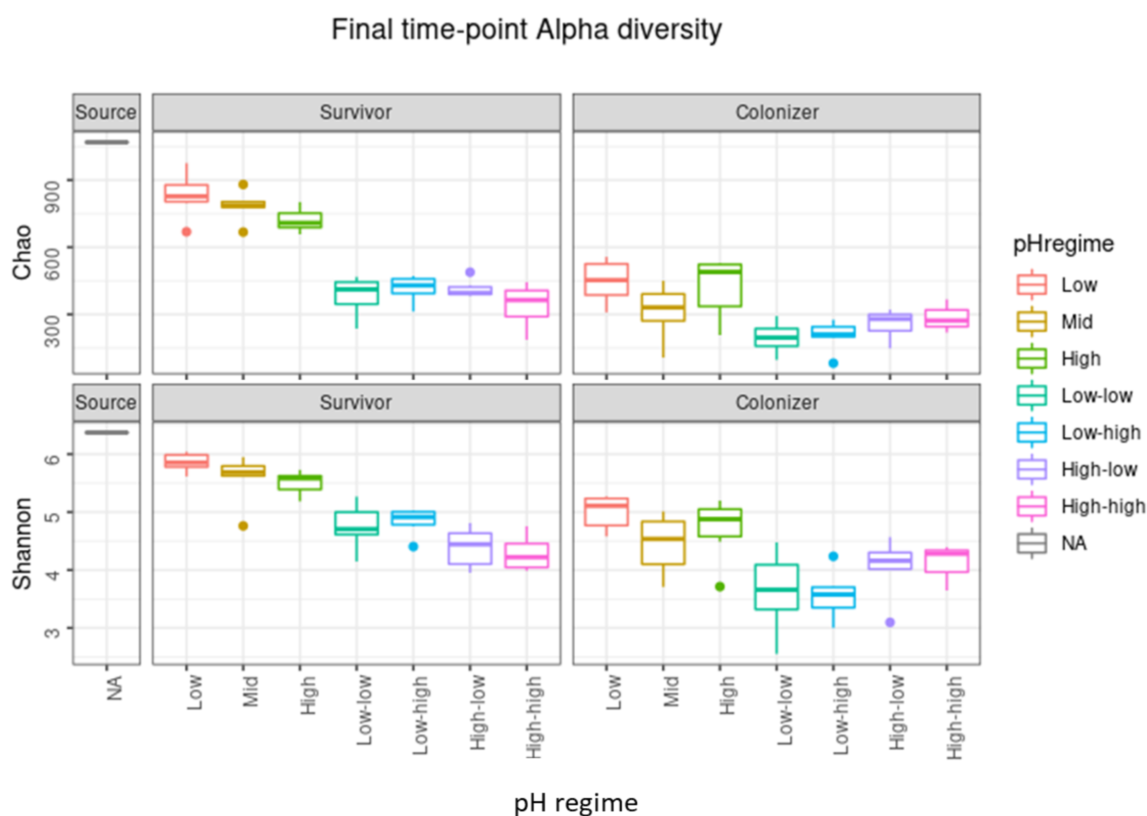

Figure 2.3: Chao and Shannon diversity scores of final time-point samples compared to source soil (grey).

Beta diversity

Bacterial community composition was found to be significantly impacted by pH regime. Bray-Curtis dissimilarity distances were calculated at the ASV level following removal of low-abundance samples and rarefaction and illustrated on NMDS plots. A full model showed significant impacts of pH regime, transplant number, plate type, time-point, and treatment/negative status on community composition ($R^2_{pHregime}$=0.04987*,

$R^2_{TransplantNumber}$=0.06063*, $R^2_{PlateType}$=0.04874*, $R^2_{Time-point}$=0.03081*, $R^2_{Treatment/NegativeStatus}$=0.08463*, *p=0.001), along with significant interactions between variables (Appendix B, Table 2). Within final time-point samples, PERMANOVA indicated that plate type, pH regime, and transplant number were significant ($R^2_{PlateType}$=0.13864, p=0.001, $R^2_{pHregime}$=0.07681, p=0.001, R^2=0.0.05827, p=0.001), as well as interactions between all three variables (Appendix B, Table 3).

The impact of pH regime on bacterial composition could be more clearly seen within treatment types (Figure 2.3). Among just the final time-point Survivor communities (Figure 2.3A), pH regime explained 24.388% of the variation (p=0.001). Transplant number was again found to be influential in determining community composition (R^2=0.25718, p=0.001). Within the Single transplant Survivor communities, pH regime explained 24.930% of the variation (p=0.001), with mid-pH communities being intermediate between low-pH and high-pH communities. In the Double Transplant Survivor communities pH regime was even more influential, explaining 37.459% of the variation (p=0.001). These communities clustered strongly by starting pH conditions (Low-low & Low-high vs High-high & High-low), but not end pH conditions, again showing a significant impact of starting pH condition.

Among final time-point Colonizer communities, pH regime was found to be influential, explaining 25.6% of the variation between communities (p=0.001) (Figure 2.4B). Notably, three replicates from the High-High pH treatment were excluded due to low abundance. Transplant number was again found to be influential (R^2=0.12597, p=0.001). Within Single Transplant Colonizer communities, pH regime explained 28.287% of the variation (p=0.001), while in Double Transplant Colonizer communities, pH regime explained 29.877% (p=0.001). Compared to the Survivors, Colonizer communities of different transplant numbers clustered with more overlap, hinting that taxa good at colonization are also more tolerant of the transplanting process and different pH conditions. Double transplant Colonizers clustered more tightly by starting pH condition (R^2=0.0888, p=0.028), but were also significant for end pH condition (R^2=0.14946, p=0.001). Compared to Double transplant Survivors, the end pH condition was more influential in final community composition than starting pH condition.

Figure 2.4

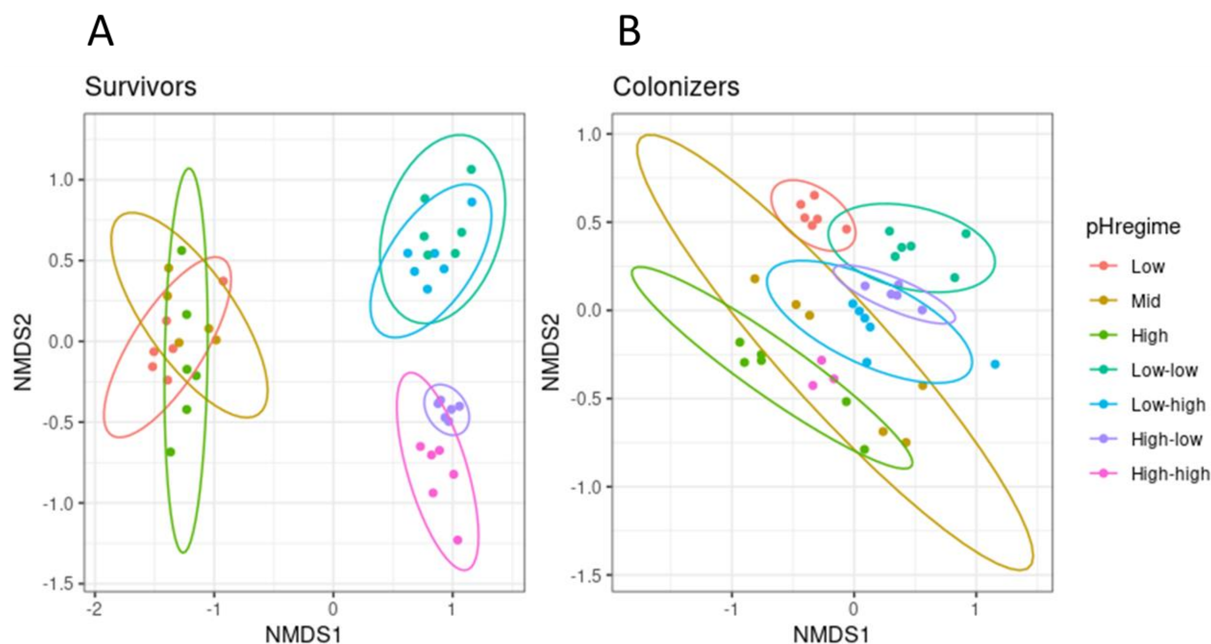

Figure 2.3: Bray-Curtis dissimilarity distances represented on NMDS plot for all final time-point A) Survivor plates and B) Colonizer plates with color indicating pH regime.

Clustering at taxonomic levels

Complex traits, such as those related to pH tolerance, are expected to be conserved at higher taxonomic levels due to the unlikelihood of generating these traits or acquiring them by horizontal gene transfer (Jain, Rivera and Lake, 1999; Cohen, Gophna and Pupko, 2011; Martiny *et al.*, 2015). Bacterial composition was resolved by taxonomic levels and PERMANOVA was applied to determine the taxonomic level at which communities were most distinct.

In Survivor communities, clustering was most distinct at higher taxonomic levels. Within Single transplant Survivor communities, the most distinct clustering was found at the class level (R^2=0.26592, p=0.033), although R^2 values for several other taxonomic levels were similar (Table 2.1). Double transplant Survivor communities clustered most distinctly at the order level (R^2=0.43886, p=0.001) but also had similar R^2 values at different taxonomic levels.

Colonizers were found to cluster most distinctly at lower taxonomic levels. In Single transplant Colonizer communities, the level at which the most distinct clustering was observed was at the ASV level, although values for genus and family were similar. Double transplant Colonizer communities showed the most variation between pH regimes at the family level, although other taxonomic levels gave similar values. This may point to increased importance of traits related to successful colonization rather than solely pH tolerance.

Table 2.1

	Phylum	Class	Order	Family	Genus	ASV
Survivors, total	R^2=0.57848, p=0.001	R^2=0.56434, p=0.001	R^2=0.58431, p=0.001	R^2=0.57973, p=0.001	R^2=0.55925, p=0.001	R^2=0.50106, p=0.001
Survivors, Single Transplant	R^2=0.24732, p=0.08	**R^2=0.26592, p=0.0396**	R^2=0.23638, p=0.039	R^2=0.23097, p=0.039	R^2=0.21813, p=0.036	R^2=0.2493, p=0.006
Survivors, Double Transplant	R^2=0.38127, p=0.001	R^2=0.41022, p=0.001	**R^2=0.43886, p=0.001**	R^2=0.40812, p=0.001	R^2=0.41363, p=0.001	R^2=0.37459, p=0.001
Colonizers, total	R^2=0.33224, p=0.189	R^2=0.35953, p=0.006	R^2=0.37059, p=0.003	R^2=0.40913, p=0.003	R^2=0.41042, p=0.003	R^2=0.38196, p=0.003
Colonizers, Single Transplant	R^2=0.22691, p=0.093	R^2=0.23326, p=0.0588	R^2=0.23108, p=0.0588	R^2=0.26114, p=0.01	R^2=0.28226, p=0.006	**R^2=0.28287, p=0.006**
Colonizers, Double Transplant	R^2=0.22485, p=0.189	R^2=0.31257, p=0.006	R^2=0.30293, p=0.003	**R^2=0.31726, p=0.003**	R^2=0.29631, p=0.003	R^2=0.29877, p=0.003

Table 2.1: Bray-Curtis PERMANOVA values by each final time-point treatment type and taxonomic level. Bold values indicate highest significant ($p<0.05$) R^2 value. P values were adjusted according to the False Discovery Rate method.

Composition

Bacterial composition was examined by looking at 100% relative abundance across treatments (Figure 2.5). Eleven phyla were represented above 1% relative abundance in the source soil, with the most abundant phyla being *Proteobacteria* (29.5%), followed by *Actinobacteria* (24.9%), and *Acidobacteria* (10.4%). 1% relative

abundance was chosen as an arbitrary cutoff to prevent misidentification of dead cells or contaminants as pH generalists in samples.

Single transplant Survivors of each pH regime included the same major taxonomic groups as the source soil. This pattern shows that most higher-level taxa found in the source soil can persist in another pH regime. In Single transplant Survivors, *Proteobacteria* ranged from 33.4-39.6% of the community and was typically followed in abundance by *Firmicutes* (18.5-33.4%) and *Acidobacteria* (5.9-11.7%). Double transplant Survivors averaged 6.25 phyla above 1% relative abundance, showing a loss of roughly half the phyla found in the source soil. Final time-point communities were distinct by pH regime, with Low-low and High-low communities being more similar, while Low-high and High-high communities were more similar. Low-low and High-low communities had five phyla above 1% relative abundance while Low-high and High-high communities had 7-8 phyla present above 1% relative abundance. *Proteobacteria* was also dominant in Double transplant Survivors (35.7-46.6%). *Firmicutes* was the second most abundant phylum (23.7-38.3%) but was followed by *Bacteroidetes* (5.4-30.9%) instead of *Acidobacteria*, which was not present above 1% relative abundance. Overall, this indicates that many phyla can persist under varying pH conditions, but fewer are tolerant to being transplanted to another soil condition.

Both Colonizer treatment communities had less phyla above 1% relative abundance compared to the 11 phyla in the source soil. Colonizers were also distinct by transplant number. An average of six phyla were present above 1% relative abundance in the Single transplant Colonizers, while Double transplant Colonizers averaged 4.5 phyla, showing a loss of roughly half the phyla introduced from the source soil. The most abundant phyla in the Single transplant Survivors were *Proteobacteria* (38.7-49.6%) and *Firmicutes* (15.3-38.7%). In Double transplant Survivors *Proteobacteria* was more abundant (55.1-68.4%), followed by *Firmicutes* (19.5-28.7%).

It’s also worth noting that common patterns are found between both Survivor and Colonizer plate types, showing that patterns of pH tolerance are often shared between bacteria that can persist and those that can colonize. Certain taxa show patterns of pH preference across both plate types, such as *Bacteroidetes*, which is most abundant in

conditions that start at high pH. Many other phyla show intolerance of transplantation across both plate types, including *Acidobacteria* and *Gemmatimonadetes*. In both plate types, phyla *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* together become more prevalent in Double transplant communities showing tolerance to transplantation.

Figure 2.5

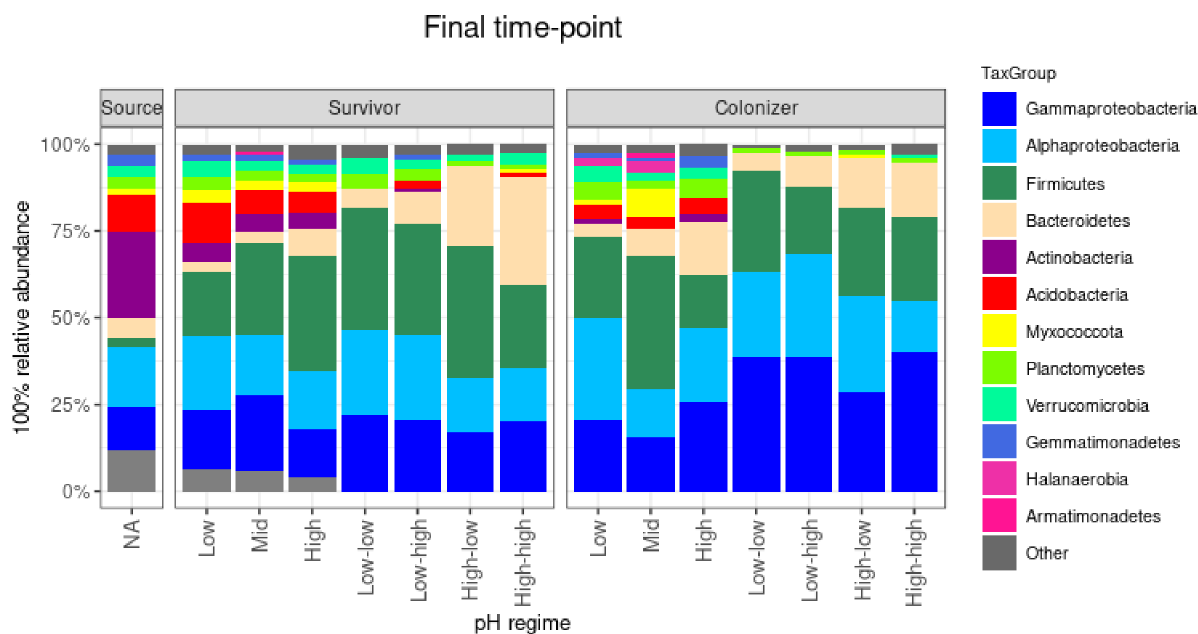

Figure 2.5: Relative abundance composition of final time-point Survivors and Colonizers by Plate Type, pH regime, and with color representing taxonomic groups Phylum or Class, in the case of Proteobacteria.

<u>Putative generalists</u>

The data was parsed for genera above 1% relative abundance in most or all pH regimes to identify putative generalists. 1% was again chosen as an arbitrary cutoff to prevent misidentification of dead cells or contaminants as generalist.

Genera found at above 1% relative abundance in Survivor communities in all pH regimes were *Clostridium sensu stricto 8* and *10* from *Firmicutes*. Genera found in most pH regimes were also from more abundant taxonomic groups. These genera include *Azospirillum* and *Sphingomonas* from *Alphaproteobacteria*, *Noviherbaspirillum* from *Gammaproteobacteria*, *Ruminiclostridium* from *Firmicutes*, and *Flavisolibacter* from *Bacteroidetes*. *Thermomonas* from *Gammaproteobacteria* was found above 1% relative

abundance in all Single transplant pH regimes, while *Chthoniobacter* from *Verrucomicrobia* was found in all Double transplant regimes. Among all of these genera found above 1% relative abundance in all Single transplant pH regimes, *Azospirillum*, *Flavisolibacter*, *Noviherbaspirillum*, and *Thermomonas* were found to have significantly more reads than negative controls by Fisher's ANOVA (Appendix B, Figure 4). Double transplant Survivor plates showed more contamination in negative controls. Fisher's ANOVA on Double transplant Survivor read counts from genera *Clostridium sensu stricto 8* and *Flavisolibacter* were significantly higher in treatments than negatives. All putative generalist genera had more reads in Survivors than negatives. Putative pH generalist genera identified in Single transplant treatments constituted 0.26% of detected genera and 6.60% of reads.

In Colonizer communities, genera found above 1% relative abundance in all pH regimes included *Azospirillum* from *Alphaproteobacteria* and *Noviherbaspirillum* from *Gammaproteobacteria,* which were also found in many of the Survivor communities. All pH regime communities in Single transplant treatments were found to contain at least 1% relative abundance of *Bryobacter* from *Acidobacteria*, *Chthoniobacter* from *Verrucomicrobia*, *Ramlibacter* from *Gammaproteobacteria*, both *Clostridium sensu stricto 8* and *10* from *Firmicutes*, and *Phenylobacteria* from *Alphaproteobacteria*. Double transplant genera included *Achromobacter*, *Klebsiella*, and *Massilia* from *Gammaproteobacteria* and *Caulobacter* from *Alphaproteobacteria*, as well as genera found in all pH regimes. Comparison of putative generalist read counts by Fisher's ANOVA showed that *Azospirillum*, *Bryobacter*, *Klebsiella*, and *Noviherbaspirillum* were significantly more abundant in Single transplant Colonizers than negatives, *Achromobacter* and *Klebsiella* had significantly more read counts in Double transplant Colonizers (Appendix B, Figure 5). Genera identified as putative pH generalist Colonizers made up 0.40% of detected genera and 12.0% of reads.

A threshold of 1% relative abundance was used when identifying putative pH generalists to avoid identifying dead or contaminant taxa as generalists. If a threshold of 0.1% was used instead, the proportion of the community that is generalist increases as well as the proportion of reads attributed to putative generalists (Appendix B, Table 6).

Discussion

There was a significant impact of pH regime on bacterial composition, consistent with previous studies (Högberg, Högberg and Myrold, 2007; Aciego Pietri and Brookes, 2008; Nicol *et al.*, 2008; Lauber *et al.*, 2009; Rousk *et al.*, 2010; Zhalnina *et al.*, 2014). Survivors under different pH regimes clustered most distinctly at the class and order level, indicating that traits related to pH are conserved at higher taxonomic levels. Single transplant Colonizers under different pH regimes clustered best at lower taxonomic levels, while Double transplant Colonizers clustered at higher taxonomic levels, although R^2 values were often similar at different taxonomic levels. This potentially shows impacts of variables inherently related to pH (i.e., nutrient availability) that may be conserved at different taxonomic levels. Several genera were identified as putative pH generalists showing some overlap with thermal generalists found in Chapter 1.

Hypothesis 1: Difference in soil pH in the recipient microcosms will impact the bacterial composition of resulting communities.

Both Survivor and Colonizer communities were significantly impacted by pH regime, with pH explaining 24.388% and 25.6% of the variance between all final time-point communities respectively. Previous studies also indicated the influence of pH regime on community composition, although the extent of the influence varied by soil type, pH range, and time span, among other variables (Rousk, Bååth, et al.; Lauber et al.; Högberg et al. 2006; Nicol et al.; Aciego Pietri and Brookes; Zhalnina et al.). Our study builds on previous work by providing an example of active environmental filtering on a single representative community. In comparison to survey-based approaches, having the same source community in this experiment enables us to determine the impact of a novel pH condition on a single community and allows us to track the pH tolerances of different taxa in the community. This is also an example of a more limited pH range compared to other experiments, showing the impact of less extreme changes in pH conditions.

Most phyla were found to persist after transplanting to a different pH condition, perhaps due to shared mechanisms such as dormancy, although dead cells may be detected as well. Several phyla showed distinct patterns in pH tolerance, such as *Bacteroidetes*, which has also been observed in some studies (Mod *et al.*, 2021), but not all (Zhalnina *et al.*, 2014; Jiao and Lu, 2020). Roughly half of the phyla found in Single transplant Survivor communities were absent in Double transplant Survivors, indicating the added difficulty of a subsequent transplant, even into the same pH range soil. The high level of persistence in Single transplant Survivor communities may indicate potential resilience to future changes in pH as members in the community may be able to become dormant under unfavorable circumstances, but not be removed from the community. The Double transplant Survivor communities may show that this resilience decreases with further disturbance, and that priority effects have a strong impact on community composition.

Fewer phyla were found to successfully colonize under a range of pH conditions, likely reflecting the added difficulties of active growth and colonization. Phyla seen to colonize under a range of pH conditions include *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Planctomycetes*, all of which were also successful Survivors. Fewer phyla were detected in Double transplant Colonizers, again indicating the difficulty of colonization and the impact of a second transplant event. End pH regime was more influential than starting pH regime in determining Double transplant Colonizer composition. End pH condition explained 14.946% of the variation, while starting pH condition explained 8.88%, showing that the final pH regime was significant for community composition. This may be because different traits or strategies are involved in colonizing low versus high pH conditions, perhaps due to differences in membrane potential and requirements for intracellular homeostasis under either condition.

Hypothesis 1a: Alpha diversity under heterogeneous pH conditions will decrease.

Alpha diversity did not significantly change by pH regime in either Survivor or Colonizer communities. Certain taxa were only detected in some pH conditions, potentially indicating a narrow range of acceptable pH conditions. Other taxa seemed to be released from some competitive pressure and became more abundant, stabilizing

overall diversity. A potential explanation for the lack of significant changes in diversity by pH regime may be in the widespread ability of bacteria to form dormancy structures (Lennon and Jones, 2011). In previous studies, pH has often been associated with diversity, with increased diversity at higher or more neutral pH (Högberg et al. 2006; Fierer and Jackson; Lauber et al.; Rousk, Bååth, et al.; Jiao and Lu; Mod et al.). The pH range used in previous studies was wider than the pH ranges used here, showing how diversity is impacted by more dramatic changes in pH. These studies were also survey-based, meaning that the bacterial assemblages present in those soils were sampled, rather than tested from one source soil. This could mean that pH has an even more significant impact on diversity as time goes on whereas shorter time periods of pH change may not significantly impact biodiversity.

Hypothesis 1b: Taxonomic patterns of pH tolerance will be most apparent at higher taxonomic levels.

Traits related to pH have been proposed to be conserved at higher taxonomic levels because of their relative complexity (Jain, Rivera and Lake, 1999; Cohen, Gophna and Pupko, 2011; Martiny *et al.*, 2015). We investigated the level at which traits related to pH were conserved by aggregating at different taxonomic levels to find the level at which communities were most distinct. In the case of the Survivor communities, the strongest clustering was found at higher taxonomic levels, such as class and order, supporting the idea of higher taxonomic conservation of pH. However, Survivor communities clustered at roughly the same strength at a range of taxonomic levels. This may indicate a range in the complexity of traits related to pH and the environmental variables associated with pH, like nutrient availability. For example, adaptations to pH have included changes to proton and non-proton pumps, as well as other transport structures such as antiporters (Krulwich, Sachs and Padan, 2011). Bacteria may also modify their protein expression and synthesize signaling molecules to cope with and detect changes in pH (Krulwich, Sachs and Padan, 2011). Other changes may include response to different nutrients related to pH, resulting in different protein regulation and tolerance to toxins including antibiotics (Stocker *et al.*, 2008; Nguyen *et al.*, 2011). This variety of traits may be conserved at different taxonomic levels.

Colonizer communities typically clustered most strongly at lower taxonomic levels such as at the family and ASV level, although values were similar across taxonomic levels. This may indicate that some traits related to successful colonization in these conditions are relatively simple and are conserved at lower taxonomic levels, resulting in sorting at multiple taxonomic levels. In addition to traits related to pH, Colonizers may need other attributes such as strategies for faster growth, tolerance of soil toxins created by autoclaving, or the ability to metabolize different nutrients, all of which may be conserved at different taxonomic levels.

Hypothesis 2: Colonizer communities will contain less diversity than Survivor communities.

Colonizer communities were significantly less diverse than Survivor communities, reflecting the difference between persistence and active colonization and growth under adverse pH conditions (Krulwich, Sachs and Padan, 2011). Successful Colonizers under any conditions included more abundant phyla such as Proteobacteria, Firmicutes, and *Bacteroidetes*. Genus *Massilia* from *Proteobacteria* was found to be a strong colonizer regardless of pH and was also identified as a strong colonizer under different temperature regimes in Chapter 1, making it a potential multi-generalist. Unsurprisingly, the diversity of Colonizer communities increased with time (Appendix B, Figure 3) showing the collection of both fast and relatively slow-growing successful colonizers over a 16-week timespan. Interestingly, alpha diversity of Colonizer communities was more related to starting pH conditions, indicating priority effects, while Bray-Curtis dissimilarity distances clustered most strongly when separated by the ending pH conditions.

Identifying putative generalists

Several genera were found in all or most pH conditions, showing a potential pH generalist ability. Some of these putative generalists, such as *Azospirillum*, *Sphingomonas*, and *Noviherbaspirillum*, have strains reported to perform desirable functions for agriculture, bioremediation, and other purposes (Ni'matuzahroh, M. Gilewicz, M. Guilano, 1999; Steenhoudt and Vanderleyden, 2000; Ishii *et al.*, 2017). Additionally, some of these genera are related to other beneficial bacteria, such as

putative pH generalist and successful colonizer *Massilia* from family *Oxalobacteraceae*, which contains several diazotrophs. Several putative pH generalists identified are associated with human disease, such as *Achromobacter* and *Klebsiella* (Appelbaum and Campbell, 1980; Duggan *et al.*, 1996; Chang *et al.*, 2022). The two taxa that were found in Survivor communities under all pH regimes, *Clostridium sensu stricto 8* and *10*, are known for being anaerobic fermenters (Yang, Yin and Wang, 2019).

It's important to note that several of these putative pH generalists were prevalent contaminants (>1% relative abundance) in negative control communities. Typically, putative generalists are more abundant in either Survivor or Colonizer communities than negative controls, although not always by a significant margin. Certain putative generalist taxa Clostridium sensu stricto 10 and 8, and Chthoniobacter were sometimes more prevalent in negative controls. It’s possible that these taxa invaded microcosms of all pH conditions, making them both active and generalist, but there is no certainty that this is the case.

Multi-generalists

Sphingomonas and *Massilia* were found to be putative pH and thermal generalist Survivors and Colonizers, respectively. Based on these results, *Sphingomonas* and *Massilia* may be tentatively identified as generalists of multiple variables. Notably, both genera are Gram-negative, as well as other putative thermal and pH generalist taxa, such as *Escherichia*/*Shigella* and *Klebsiella*. It’s possible that successful generalist taxa, or at least taxa of variables like temperature and pH, could benefit from the protective outer membrane of Gram-negative bacteria, allowing them to persist or colonize in challenging conditions. Generalists of multiple variables, or “multi-generalists”, may achieve generalism of different variables by shared mechanisms. One mechanism could be increased genome size, which was found in a study of ubiquitous bacteria (Barberán *et al.*, 2014) and may enable generalist lifestyles through increased retention of ecologically relevant traits. Another potential mechanism related to multi-generalism may be increased genetic plasticity, which has been found in larger organisms including grasses and arthropods (Griffith and Sultan, 2012; Snoeck *et al.*, 2018), and has often been theorized to include maintenance tradeoffs limiting total fitness (Murren *et al.*,

2015). Changing life strategies, such as reproductive timing and effort, have also been linked to generalism in macrovertebrates (Verberk, van der Velde and Esselink, 2010). These mechanisms may be found among bacterial life and can potentially highlight ecological similarities and dissimilarities between microbial life and larger organisms.

Conclusion

This experiment builds upon past work by allowing us to see the impact of short-term pH change on one model soil community and directly test pH tolerance range. We found that, in agreement with previous results, pH was a powerful predictor of community composition and that phyla show different levels of sensitivity to pH conditions. In contrast to some previous work, alpha diversity was not significantly impacted by pH regime, potentially showing the role of dormancy and other traits in preserving biodiversity across shorter time scales and smaller environmental variations.

In this study, we found that Survivor communities under different pH conditions clustered most strongly at higher taxonomic levels such as class and order, supporting the idea that the relatively complex genes related to pH tolerance are conserved at higher taxonomic levels. Colonizer communities were seen to cluster similarly across taxonomic levels, indicating a range of traits conserved at different levels involved with successful colonization. We also saw impact of the first pH condition on alpha diversity within both Survivors and Colonizers. Interestingly, the diversity of Colonizer communities was more strongly related to starting pH regime while relative abundance was more attributable to final pH regime.

We also identified putative pH generalists that capable of persisting or actively colonizing under a range of pH conditions. Some putative generalist taxa were found along a wide range of temperature in Chapter 1, as well as in a wide range of pH conditions, making them putative generalists of multiple variables or ‘multi-generalists’. Understanding the ecology of generalist bacteria, especially interactions between them and likely environmental changes may help to improve the use of beneficial bacteria, prediction of pathogens, and improve our understanding of the ecology of close relatives.

Future work

Future research building upon this work could involve filtering by other influential environmental conditions to understand more about the ecology of generalists and the mechanisms by which generalism is achieved. Genera *Sphingomonas* and *Massilia* may be favorable targets for this research because thermal and pH generalism have now been tentatively shown within both genera. Other research arcs could investigate the impact of pH on the biodiversity-ecosystem function relationship. We now have evidence of a significant shift in the community in response to short term pH changes in this soil type, which could potentially impact function (Olson, 1993; Zhou, Wang and Luo, 2020).

Limitations

In this research we used different soils to create pH regimes. Previous studies have examined the impact of pH through liming soils (Andersson and Nilsson, 2001; Pawlett *et al.*, 2009) or by surveying systems where the soil had been managed through liming for decades (Aciego Pietri and Brookes, 2008; Rousk, Brookes and Bååth, 2009; Zhalnina *et al.*, 2014). The choice to use different soils in this experiment allowed us to avoid the effects of liming and provide perspective in other soil types but did introduce other variables that could impact the microbial communities. However, a lot of the variables known to vary between these soil samples are variables that are influenced by pH (Appendix B, Table 1), meaning that impact on the community could be an indirect impact of different pH conditions.

Another consideration is the relationship between pH and nutrient availability. pH has a considerable impact on nutrient availability (Devau *et al.*, 2009; Zhalnina *et al.*, 2014; Mod *et al.*, 2021), meaning that the effects of pH cannot be wholly separated from the impact of different nutrient availability. Because of this relationship, bacteria may be directly influenced by pH or indirectly influenced by pH through nutrient availability.

Other limitations of this research include technical aspects, such as avoiding contamination and primer and other molecular biases against certain taxonomic groups. Transplantation was a significant driver in decreasing biodiversity, meaning that some

bacteria were lost because of the rapid change in pH conditions. These taxa may have persisted if pH regime change was more gradual, potentially limiting identification of some putative pH generalists.

Funding

This work was supported by the United States Department of Agriculture National Institute of Food and Agriculture (grant no. 2020-67013-30865). Findings and conclusions do not necessarily reflect the view of the funding agency.

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Appendix A: Supplementary information from Chapter 1

Figure 1:

Soil sampling location at the Russell E. Larson Agricultural Research Center in Pennsylvania Furnace, PA.

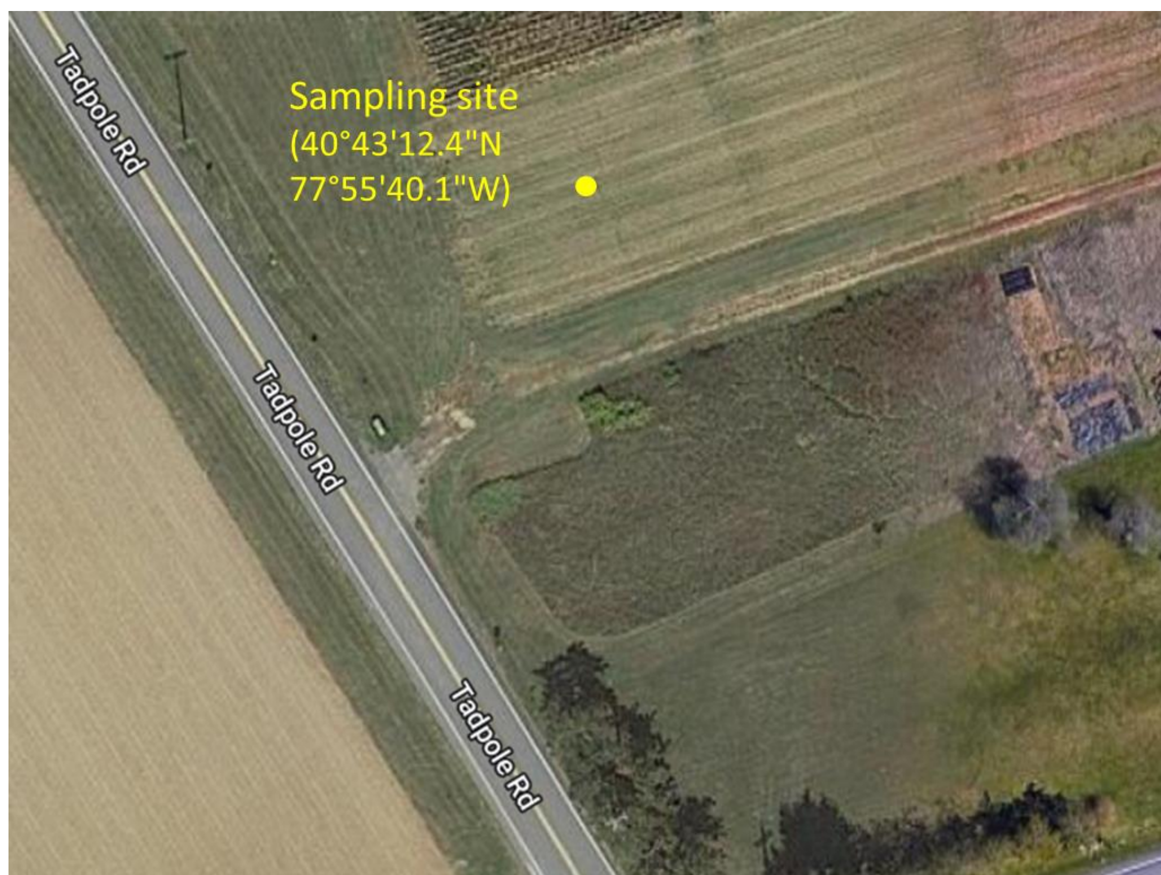

Table 1:

Soil physiochemical characteristics

pH	Phosphorus ppm	Potassium ppm	Magnesium ppm	Calcium ppm
7.07	43	200	135	1470.1
Acidity (meq/100g)	**CEC (meq/100g)**	**K% Saturation of CEC**	**Mg% Saturation of CEC**	**Ca% Saturation of CEC**
0	9	5.7	12.5	81.8
Zinc ppm	**Copper ppm**	**Sulfur ppm**	**Organic Matter %**	**Nitrogen %**
2	3.3	21.9	2.63	0.141

Table 2:

Read-out from PERMANOVA applied to Bray-Curtis dissimilarity distances of all samples by all relevant variables, including controls.

Full model: adonis(formula = ps_bray ~ TempRegime * PlateType * TimePoint, data = sampledf)

Permutation: free
Number of permutations: 999
Terms added sequentially (first to last)

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	
TempRegime	4	3.612	0.90294	2.1744	0.02233	0.001	***
PlateType	4	11.445	2.86117	6.8901	0.07077	0.001	***
Time-point	3	1.925	0.64177	1.5455	0.01191	0.001	***
TempRegime:PlateType	12	8.628	0.71904	1.7315	0.05336	0.001	***
TempRegime:Time-point	9	4.29	0.47663	1.1478	0.02653	0.001	***
PlateType:Time-point	12	6.648	0.55401	1.3341	0.04111	0.001	***
TempRegime:PlateType:Time-point	36	16.37	0.45472	1.095	0.10123	0.001	***
Residuals	262	108.798	0.41526		0.67277		
Total	342	161.716			1		

Signif. codes: 0 ‘***’	0.001	‘	**’	0.01 ‘*’ 0	.05 ‘.’	0.1 ‘ ’	1

Table 3:

Read-out from PERMANOVA applied to Bray-Curtis dissimilarity distances of all final time-point (8wk) samples, including controls.

Model: adonis(formula = ps_bray ~ TempRegime * PlateType, data = sampledf)

Permutation: free
Number of permutations: 999
Terms added sequentially (first to last)

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	

TempRegime	3	1.704	0.56807	1.4493	0.04513	0.001	***
PlateType	4	5.337	1.33433	3.4043	0.14133	0.001	***
TempRegime:PlateType	12	6.031	0.50259	1.2823	0.1597	0.001	***
Residuals	63	24.693	0.39196		0.65385		
Total	82	37.766			1		

Figure 2:

Chao and Shannon alpha diversity measures of all final time-point communities by plate type and temperature regime, including Source Soil, sequenced by a different method because of a previous sequencing error.

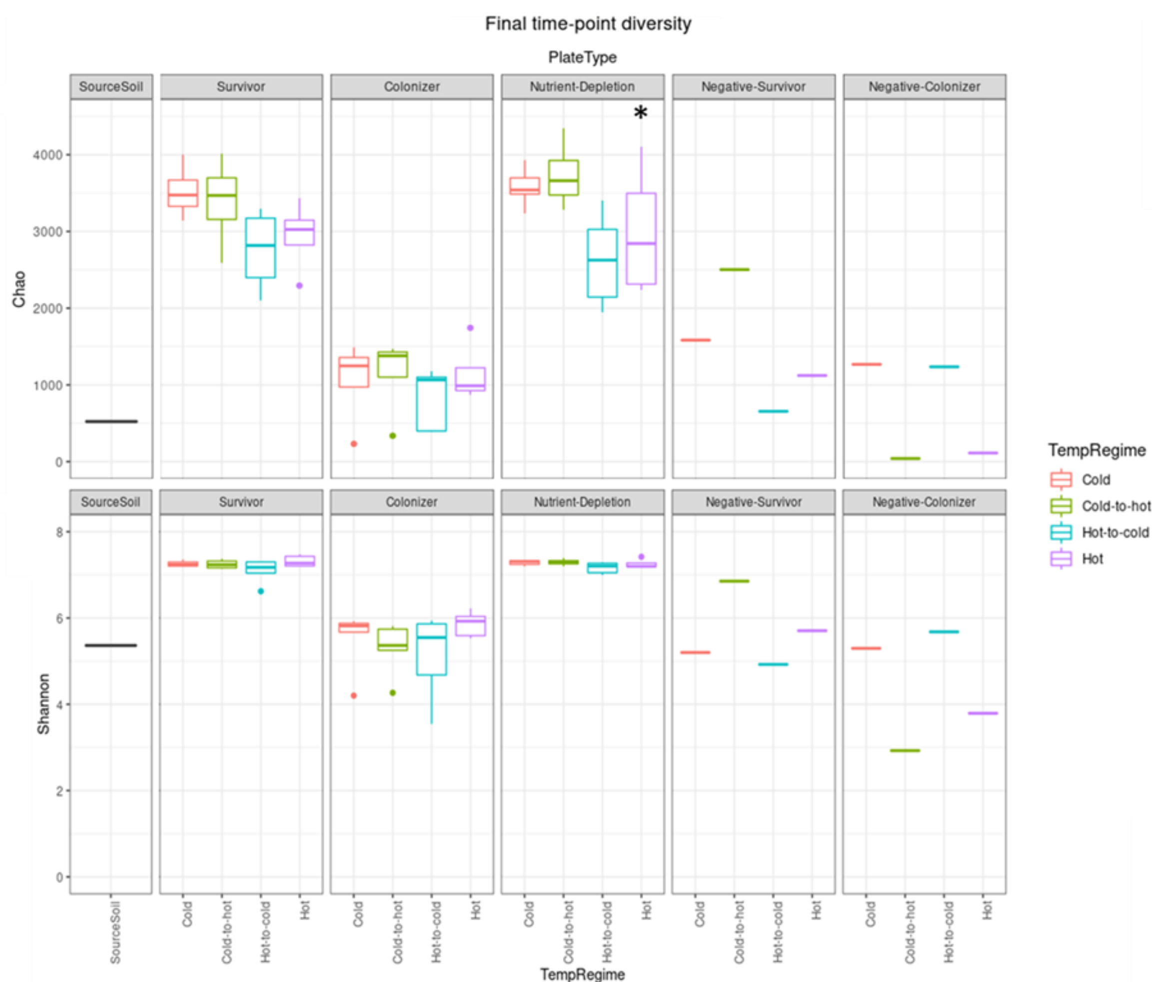

Figure 4:

Bray-Curtis dissimilarity distances of final time-point samples (all plate types), including Source Soil, which was sequenced by a different method due to a previous sequencing error.

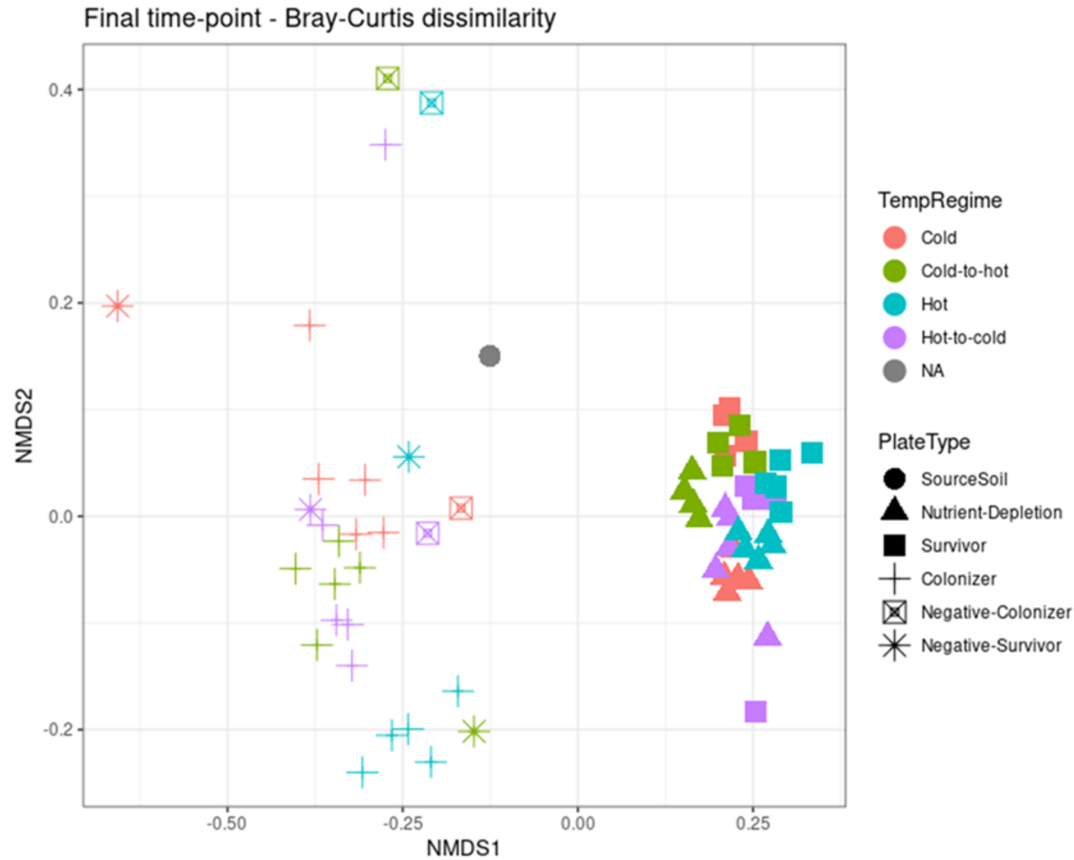

Figure 5:

Final time-point 100% relative abundance of final time-point samples with Source Soil (sequenced by another method because of a previous sequencing error) for perspective.

Table 4:

PERMANOVA of individual phyla within final time-point Survivors and Colonizers with p values adjusted for multiple comparison by the False Discovery Rate method to view more thermally sensitive phyla. Highlighted cells indicate significant difference in phylum community composition at different taxonomic levels due to temperature regime.

Survivors		Taxonomic level					
Taxonomic Group		Phylum	Class	Order	Family	Genus	ASV
Proteobacteria	R^2	0.40213	0.6564	0.66708	0.66253	0.64852	0.22718
	p	0.066	0.0024	0.0015	0.0015	0.0015	0.0015
Actinobacteria	R^2	0.18973	0.30855	0.37047	0.421	0.42308	0.20385
	p	0.072	0.072	0.072	0.072	0.072	0.197

Acidobacteria	R^2	0.46154	0.35885	0.37085	0.37085	0.36356	0.19365
	p	0.46154	0.44502	0.44502	0.44502	0.44502	0.44502
Chloroflexi	R^2	0.22735	0.21654	0.22792	0.24037	0.25511	0.19287
	p	0.2484	0.278	0.2484	0.2484	0.2484	0.2484
Bacteroidetes	R^2						0.21503
	p						0.006
Colonizers		Taxonomic level					
Taxonomic Group		Phylum	Class	Order	Family	Genus	ASV
Proteobacteria	R^2	0.54844	0.74575	0.35316	0.35087	0.41498	0.2357
	p	0.066	0.0024	0.0015	0.0015	0.0015	0.0015

Figure 6:

Read counts of identified putative thermal generalist genera found in Survivors, compared to negative controls by Fisher's ANOVA. * indicates significant difference between Survivors and both negatives.

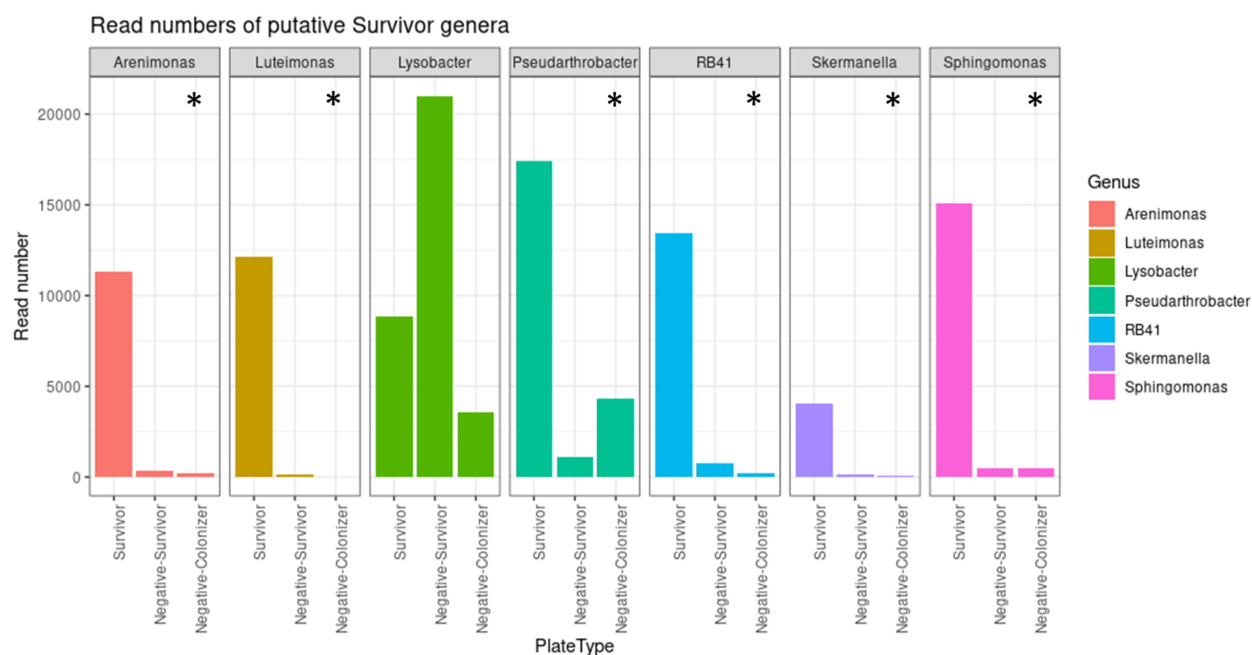

Figure 7:

Read counts of identified putative thermal generalist general found in Colonizer plates compared to negative controls by Fisher's ANOVA. No significant differences were found between read counts.

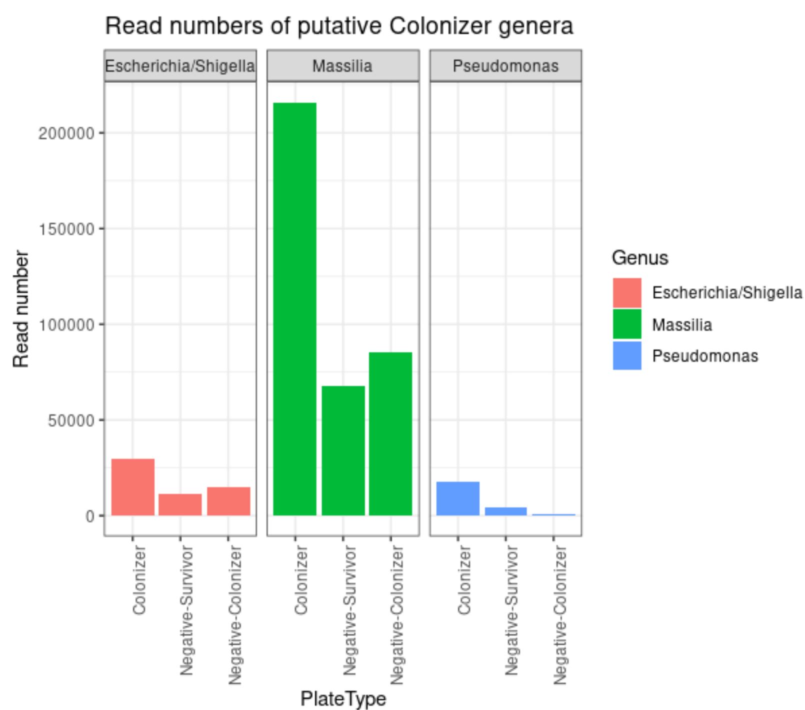

Table 5:

Proportion of putative generalist taxa and their read counts by threshold

	1% threshold	0.1% threshold
Survivors:		
Putative generalist genera/all genera detected (%)	21.2%	20.6%
Putative generalist genera/all genera detected above 1% in any treatment (%)	2.5%	10.1%
Putative generalist genera read counts/all read counts (%)	34.5%	47.8%
Colonizers:		
Putative generalist genera/all genera detected (%)	6.7%	5.4%
Putative generalist genera/all genera detected above 1% in any treatment (%)	1.8%	3.5%
Putative generalist genera read counts/all read counts (%)	36.5%	38.8%

Appendix B: Supplementary information from Chapter 2

Figure 1:

Sampling map at the Russell E. Larson Agricultural Research Center. High and mid pH soils were collected from opposite corners of the same field, while the low pH soil was collected from another.

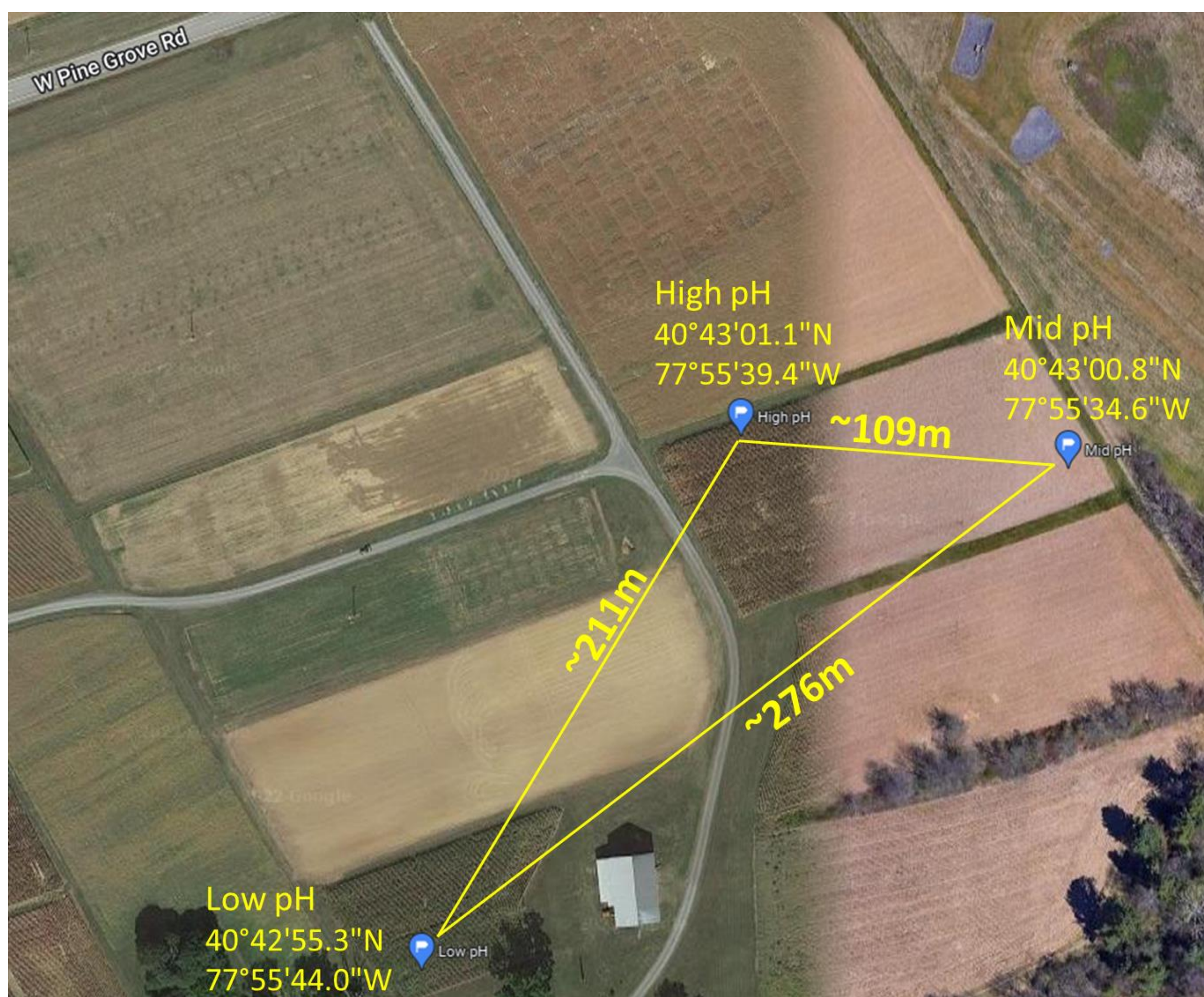

Table 1: Soil physiochemical characteristics. Note that mid and high pH soils (taken from opposite corners of the same field) are more similar than low pH soil.

Soil Id	pH	P ppm	K ppm	Mg ppm	Ca ppm	Acidity index meq/100 g	CEC meq/100 g	K percent saturation
Low	4.8	108	189	84	620.3	6.9	11.2	4.3
Low (sterile)	5	133	161	83	524.2	6.3	10	4.1
Mid	5.69	62	123	155	982.4	3.4	9.9	3.2

Mid (sterile)	5.77	77	97	129	778.7	2.8	8	3.1
High	6.55	36	100	204	1767.9	2	12.8	2
High (sterile)	6.72	55	94	135	1688.7	2	11.8	2
Soil Id	**Mg percent saturation**	**Ca percent saturation**	**Zinc ppm**	**Cu ppm**	**Sulfur ppm**	**% Organic Matter**	**Nitrogen %**	
Low	6.3	27.7	3.5	2.9	15.4	3.16	0.191	
Low (sterile)	6.9	26.1	1.4	0.6	38.7	2.98	0.195	
Mid	13	49.5	1.5	2	40.5	2.95	0.198	
Mid (sterile)	13.4	48.6	0.6	0.4	55.1	2.84	0.188	
High	13.3	69.1	1.3	1.8	31.4	3.06	0.207	
High (sterile)	9.5	71.5	0.9	0.6	45.6	3.1	0.207	

Table 2:

Qubit data of DNA extraction averages by treatment group. Treatment/negative control pairs where the negative control yielded more DNA than the treatment are highlighted.

Group	**Treatment** (ng DNA/g soil)	**Negative control** (ng DNA/g soil)	**Difference** (Positive ng DNA/g soil - Negative ng DNA/g soil)
Single transplant			
Single transplant, Survivor, Low-pH, Week 2	16.335	0.004	16.331
Single transplant, Colonizer, Low-pH, Week 2	9.448	0.000	9.448
Single transplant, Survivor, Mid-pH, Week 2	14.603	0.000	14.603
Single transplant, Colonizer, Mid-pH, Week 2	0.000	0.000	0.000
Single transplant, Survivor, High-pH, Week 2	3.443	0.000	3.443
Single transplant, Colonizer, High-pH, Week 2	0.000	0.000	0.000
Single transplant, Survivor, Low-pH, Week 4	0.819	0.005	0.814
Single transplant, Colonizer, Low-pH, Week 4	1.668	0.000	1.668
Single transplant, Survivor, Mid-pH, Week 4	11.819	0.000	11.819
Single transplant, Colonizer, Mid-pH, Week 4	0.000	0.000	0.000
Single transplant, Survivor, High-pH, Week 4	12.830	0.087	12.743
Single transplant, Colonizer, High-pH, Week 4	0.796	0.054	0.742
Single transplant, Survivor, Low-pH, Week 8	3.983	0.120	3.863
Single transplant, Colonizer, Low-pH, Week 8	5.619	0.193	5.426
Single transplant, Survivor, mid-pH, Week 8	6.121	0.209	5.912
Single transplant, Colonizer, mid-pH, Week 8	0.000	0.000	0.000
Single transplant, Survivor, High-pH, Week 8	0.210	0.120	0.090
Single transplant, Colonizer, High-pH, Week 8	0.256	0.005	0.251
Single transplant, Survivor, Low-pH, Week 16	0.557	0.122	0.436
Single transplant, Colonizer, Low-pH, Week 16	0.338	0.072	0.267
Single transplant, Survivor, Mid-pH, Week 16	0.084	0.050	0.034

Single transplant, Colonizer, Mid-pH, Week 16	0.033	0.005	0.027
Single transplant, Survivor, High-pH, Week 16	0.095	0.229	-0.134
Single transplant, Colonizer, High-pH, Week 16	0.116	0.093	0.024
Double transplant			
Double transplant, Survivor, Low-pH-to-low-pH, Week 16	0.277	0.379	-0.102
Double transplant, Colonizer, Low-pH-to-low-pH, Week 16	0.077	0.058	0.019
Double transplant, Survivor, Low-pH-to-high-pH, Week 16	0.234	0.298	-0.065
Double transplant, Colonizer, Low-pH-to-high-pH, Week 16	0.058	0.113	-0.055
Double transplant, Survivor, High-pH-to-low-pH, Week 16	0.076	0.350	-0.274
Double transplant, Colonizer, High-pH-to-low-pH, Week 16	0.053	0.331	-0.278
Double transplant, Survivor, High-pH-to-high-pH, Week 16	0.178	0.060	0.118
Double transplant, Colonizer, High-pH-to-high-pH, Week 16	0.074	0.064	0.010

Table 3:

Full model PERMANOVA read-out of all samples at all time points and plate types.

Model: adonis(formula = ps_bray ~ Experiment*Treatment*PlateType*SoilType*HeterogeneityLevel*Time-point*Repetition,data = sampledf)

Terms added sequentially (first to last)							
	Df	Sums Of Sqs	MeanSqs	F.Model	R2	Pr(>F)	
Transplant number	4	7.013	1.7533	7.722	0.06063	0.001	***
Treatment	1	9.79	9.7898	43.118	0.08463	0.001	***
PlateType	1	5.638	5.6384	24.833	0.04874	0.001	***
SoilType	5	5.769	1.1537	5.081	0.04987	0.001	***
Time-point	1	3.564	3.5641	15.698	0.03081	0.001	***
Repetition	1	0.288	0.2883	1.27	0.00249	0.115	
Transplant number:Treatment	1	2.294	2.2937	10.102	0.01983	0.001	***
Transplant number:PlateType	1	2.146	2.1458	9.451	0.01855	0.001	***
Treatment:PlateType	1	2.96	2.9604	13.039	0.02559	0.001	***
Treatment:SoilType	5	4.372	0.8744	3.851	0.03779	0.001	***
PlateType:SoilType	5	2.978	0.5957	2.624	0.02575	0.001	***
Treatment:Time-point	1	1.914	1.9143	8.431	0.01655	0.001	**

							*
PlateType:Time-point	1	0.909	0.9085	4.001	0.00785	0.001	***
SoilType:Time-point	2	1.15	0.5748	2.532	0.00994	0.001	***
Transplant number:Repetition	1	0.262	0.2621	1.154	0.00227	0.227	
Treatment:Repetition	1	0.585	0.5848	2.576	0.00506	0.001	***
PlateType:Repetition	1	0.18	0.1801	0.793	0.00156	0.78	
SoilType:Repetition	5	1.467	0.2934	1.292	0.01268	0.017	*
Time-point:Repetition	1	0.287	0.2871	1.264	0.00248	0.129	
Transplant number:Treatment:PlateType	1	1.193	1.1927	5.253	0.01031	0.001	***
Treatment:PlateType:SoilType	5	1.674	0.3349	1.475	0.01447	0.002	**
Treatment:PlateType:Time-point	1	0.984	0.9837	4.332	0.0085	0.001	***
Treatment:SoilType:Time-point	2	1.196	0.5979	2.633	0.01034	0.001	***
PlateType:SoilType:Time-point	2	0.563	0.2815	1.24	0.00487	0.095	.
Transplant number:Treatment:Repetition	1	0.431	0.4314	1.9	0.00373	0.017	*
Transplant number:PlateType:Repetition	1	0.202	0.2017	0.888	0.00174	0.577	
Treatment:PlateType:Repetition	1	0.141	0.1412	0.622	0.00122	0.985	
Treatment:SoilType:Repetition	5	2.323	0.4646	2.046	0.02008	0.001	***
PlateType:SoilType:Repetition	5	0.738	0.1476	0.65	0.00638	1	
Treatment:Time-point:Repetition	1	0.376	0.3765	1.658	0.00325	0.025	*
PlateType:Time-point:Repetition	1	0.164	0.1637	0.721	0.00141	0.899	
SoilType:Time-point:Repetition	2	0.601	0.3003	1.323	0.00519	0.051	.
Treatment:PlateType:SoilType:Time-point	2	0.526	0.2631	1.159	0.00455	0.188	
Transplant number:Treatment:PlateType:Repetition	1	0.166	0.166	0.731	0.00144	0.864	
Treatment:PlateType:SoilType:Repetition	5	0.549	0.1097	0.483	0.00474	1	
Treatment:PlateType:Time-point:Repetition	1	0.167	0.1667	0.734	0.00144	0.88	
Treatment:SoilType:Time-point:Repetition	2	0.831	0.4157	1.831	0.00719	0.002	**
PlateType:SoilType:Time-point:Repetition	2	0.321	0.1606	0.707	0.00278	0.977	
Treatment:PlateType:SoilType:Time-point:Repetitio	n 2	0.38	0.19	0.837	0.00329	0.818	
Residuals	214	48.589	0.227		0.42003		
Total	296	115.68			1		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05	‘.’ 0	.1 ‘ ’ 1					

Table 4:

Final time-point full PERMANOVA

Model: adonis(formula = ps_bray ~
Experiment*Treatment*PlateType*SoilType*Heteroge neityLevel*Time-point*Repetition,data = sampledf)

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Transplant number	1	3.013	3.01264	11.8922	0.05827	0.001	***
PlateType	3	7.169	2.38954	9.4326	0.13864	0.001	***
pHregime	5	3.971	0.79426	3.1353	0.07681	0.001	***
Transplant number:PlateType	3	3.999	1.333	5.2619	0.07734	0.001	***
PlateType:pHregime	15	6.701	0.44676	1.7636	0.12961	0.001	***
Residuals	106	26.853	0.25333		0.51934		
Total	133	51.706			1		

Figure 2:

100% relative abundance of Negative-Survivor and Negative-Control plates at the phylum level show that at the phylum level, negatives were similar to corresponding treatments at this level, but examination at lower taxonomic levels reveals differences in composition.

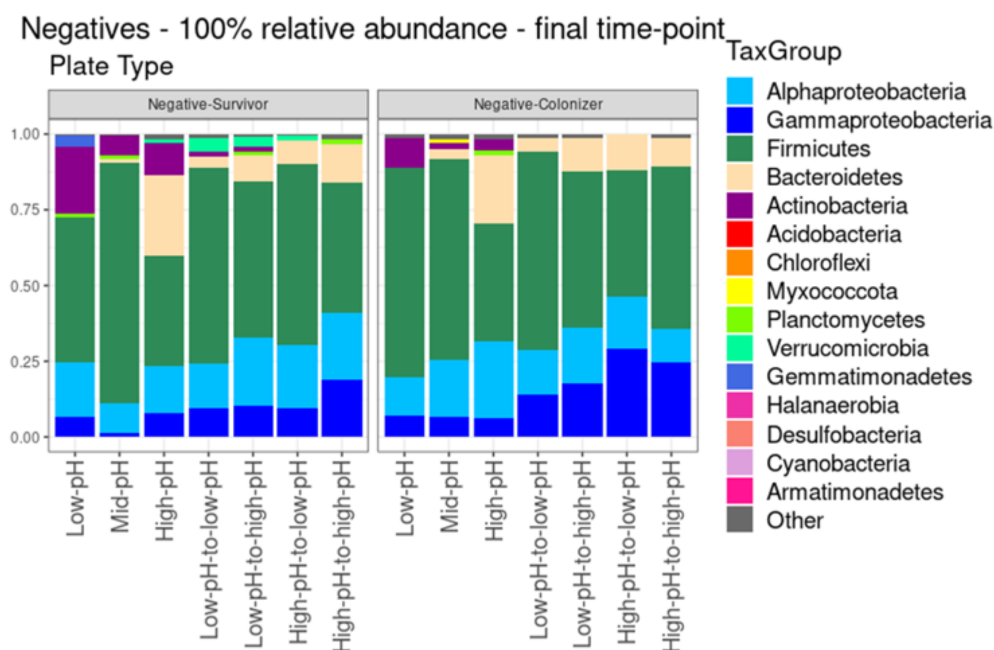

Table 5:

R^2 and p values from each major phylum clustered at each taxonomic level with first significant value going down taxonomic levels highlighted. p values are corrected for multiple comparisons by the False Discovery Rate method.

Survivors - SingleTransplant, final time-point					
Phylum/Class	**Class R2**	**Order R2**	**Family R2**	**Genus R2**	**ASV R2**
Proteobacteria	R2=0.14648, p=0.442	R2=0.11817, p=0.442	R2=0.12764, p=0.442	R2=0.14797, p=0.442	R2=0.15495, p=0.442
Gammaproteobacteria	NA	R2=0.10486, p=0.495	R2=0.11972, p=0.495	R2=0.16961, p=0.28	R2=0.16109, p=0.28
Alphaproteobacteria	NA	R2=0.14882, p=0.305	R2=0.14952, p=0.305	R2=0.13734, p=0.305	R2=0.15118, p=0.305
Firmicutes	R2=0.28993, p=0.047	R2=0.32428, p=0.03	R2=0.32433, p=0.03	R2=0.25682, p=0.0375	R2=0.40267, p=0.005
Bacteroidetes	R2=0.43607, p=0.01	R2=0.28601, p=0.012	R2=0.30852, p=0.005	R2=0.26208, p=0.005	R2=0.31226, p=0.005
Planctomycetes	R2=0.14262, p=0.428	R2=0.14856, p=0.428	R2=0.15122, p=0.428	R2=0.11984, p=0.428	R2=0.15439, p=0.31
Acidobacteria	R2=0.48129, p=0.0025	R2=0.040915, p=0.0025	R2=0.45448, p=0.003	R2=0.48403, p=0.003	R2=0.27555, p=0.003
Actinobacteria	R2=0.03784, p=0.824	R2=0.10909, p=0.68	R2=0.11047, p=0.68	R2=0.12144, p=0.68	R2=0.14393, p=0.68
Colonizers - SingleTransplant, final time-point					
Phylum/Class	**Class R2**	**Order R2**	**Family R2**	**Genus R2**	**ASV R2**
Proteobacteria	R2=0.17001, p=0.201	R2=0.16235, p=0.201	R2=0.22989, p=0.0117	R2=0.2568, p=0.0025	R2=0.23339, p=0.0025
Gammaproteobacteria	NA	R2=0.09726, p=0.569	R2=0.23668, p=0.0147	R2=0.28059, p=0.002	R2=0.23779, p=0.002
Alphaproteobacteria	NA	R2=0.19497, p=0.06	R2=0.21018, p=0.048	R2=0.21198, p=0.018	R2=0.23023, p=0.004
Firmicutes	R2=0.21029, p=0.092	R2=0.30733, p=0.0217	R2=0.30794, p=0.0217	R2=0.268, p=0.0363	R2=0.35376, p=0.005
Bacteroidetes	R2=0.43284, p=0.0083	R2=0.24217, p=0.028	R2=0.24734, p=0.025	R2=0.33097, p=0.0025	R2=0.32341, p=0.0025
Planctomycetes	R2=0.1574, p=0.221	R2=0.24179, p=0.02	R2=0.25589, p=0.02	NA	R2=0.229, p=0.008
Acidobacteria	R2=0.23308, p=0.055	R2=0.29272, p=0.0075	R2=0.25453, p=0.025	R2=0.23672, p=0.0325	R2=0.2811, p=0.005
Actinobacteria	R2=0.10919, p=0.6113	R2=0.09392, p=0.676	R2=0.15811, p=0.19	R2=0.20561, p=0.015	R2=0.19079, p=0.015

Figure 3:

Chao and Shannon alpha diversity metrics for all Single transplant Colonizers by time-point, showing that the diversity of Colonizers generally increased with time.

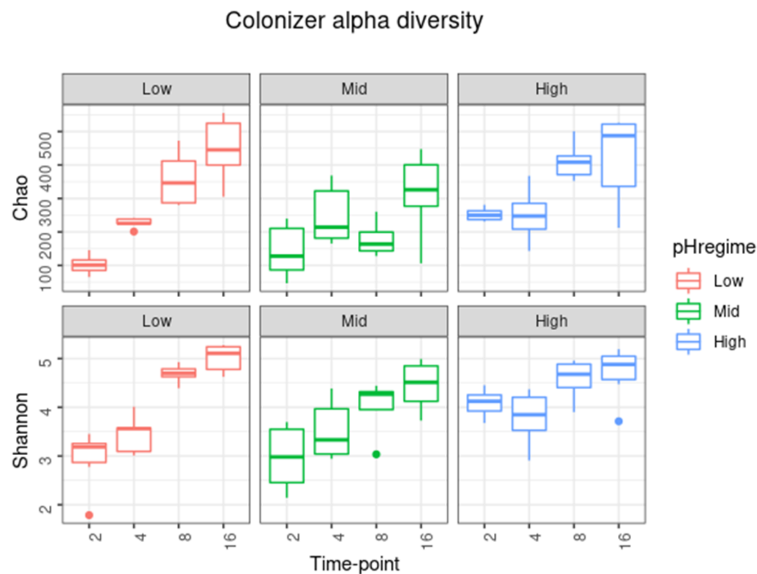

Figure 4:

Read counts of putative pH generalist genera found in Survivor communities compared to negative control communities. Significance when comparing read counts by Fisher's ANOVA is indicated with asterisks.

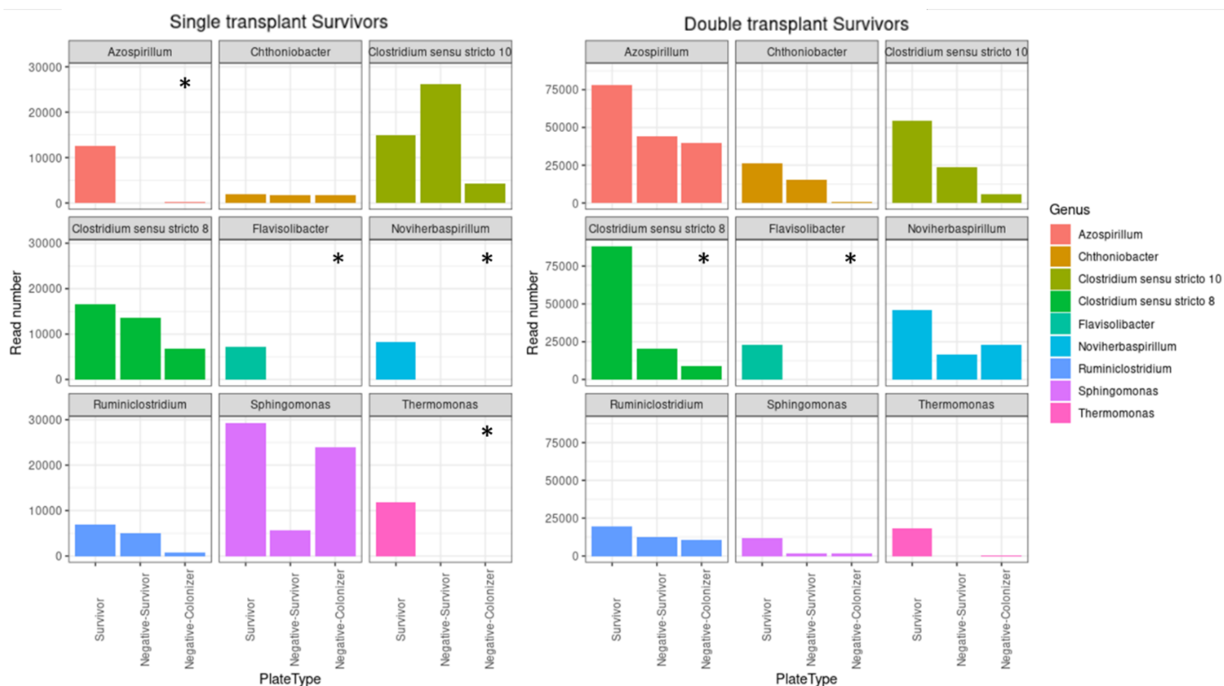

Figure 5:

Read counts of putative pH generalist genera found in Colonizer communities compared to negative control communities. Significance when comparing read counts by Fisher's ANOVA is indicated with asterisks.

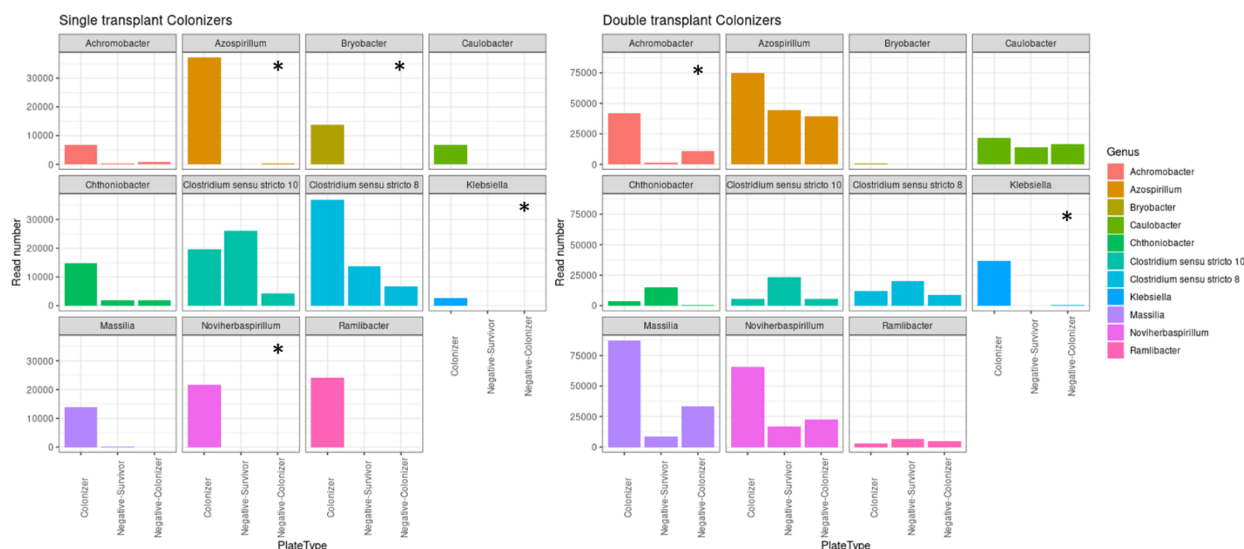

Table 6:

Putative generalists at different thresholds for identification.

	1% threshold	0.1% threshold
Survivors:		
Putative generalist genera/all genera detected (%)	4.2%	4.2%
Putative generalist genera/all genera detected above 1% in any treatment (%)	0.3%	0.4%
Putative generalist genera read counts/all read counts (%)	6.6%	12.0%
Colonizers:		
Putative generalist genera/all genera detected (%)	15.9	4.9%
Putative generalist genera/all genera detected above 1% in any treatment (%)	4.7%	1.4%
Putative generalist genera read counts/all read counts (%)	31.5%	23.5%

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Education

The Pennsylvania State University, 2018 – 2022 **State College, PA**
Master's candidate in Ecology, Huck Institute of the Life Sciences
Advised by Terrence Bell, Ph.D.
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Colorado Mesa University, 2012-2016 **Grand Junction, CO**
BS in Biological Sciences, minor in Chemistry
Advised by Matthew Stansbury, Ph.D.

Research Experience

The Microbiome Manipulation Lab, 2018-ongoing **State College, PA**
Graduate Researcher
Research Interests: Microbial ecology, applied microbial ecology, microbial product development, microbial ecosystem function

Monsanto Research and Development – Pest and Pathogen Control, 2017-2018 **Chesterfield, MO**
Biologist I

- Conducted plant pathological assays for pesticide formulation development
- Maintained fungal and bacterial stocks

Stansbury Lab, 2014-2016 **Grand Junction, CO**
Undergraduate Researcher

- Functional divergence of *luciferase* paralogs in *Photuris pennsylvanica*

Publications

- **DePriest M**, Bell TH ""Environmental filtering by temperature regime doesn't reduce alpha diversity in an agricultural soil but does change composition" *In prep.*
- King WL, Yates C, Trexler RV, **DePriest M**, Fleishman S, Grandinette E, Isbell S, Kaminsky LM, Sutherland J, Bell TH "Bacterial succession from a microscale perspective" *In prep*