INTERPLAY BETWEEN MICROBIOME AND TEMPERATURE IN *TENEBRIO MOLITOR*

by

Samuel R. Tye, B.S.

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Committee Members:

Camila Carlos-Shanley, Chair

Dittmar Hahn

Chris Nice

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LIST OF ABBREVIATIONS

Abbreviation	Description
TSA	Trypticase Soy Agar, General Growth Medium
BEA	Bile Esculin Agar, Selective and Differential Medium
PDA	Potato Dextrose Agar, General Growth Medium
CFU	Colony Forming Units, Countable Colonies on a Plate
PBS	Phosphate Buffered Saline, Buffer Solution
TAE	Tris-Chloride, Acetic Acid, and EDTA, Buffer Solution
PCR	Polymerase Chain Reaction, Amplifies Portions of DNA
ASV	Amplicon Sequence Variant, Feature-Level Sequence Variant
OTU	Operational Taxonomic Unit
GLM	General Linear Model, Statistical Method

I. INTRODUCTION

Arthropods and the Environment

Arthropods are a critically important part of ecosystems across the planet. Many insects are agriculturally important because they help pollinate crops. Many people consider members of the family Apidae when they think of pollinators, but bees are not the only arthropods that pollinate plants. Arthropods tend to have a specialized relationship with plants that extends beyond simple pollination. Places with a greater plant richness also tend to harbor a greater variety of arthropods. Arthropods often have relationships with the plants that they live with. Due to the direct correlation between arthropod species richness and plant species richness in their environments, habitat loss across the planet is contributing greatly to the mass decline in arthropod diversity (Villanueva-López *et al.,* 2019).

Arthropods are poikilothermic organisms, meaning they are for the most part not able to control their own body temperature and it is at the mercy of the environment. Insects tend to be slower and less active when they are cold or below their optimal temperature, and they tend to become more active as they warm up or reach their optimal temperature (Fitzgerald *et al.,* 2021). Suboptimal temperatures could also interfere with reproduction and alter the physiology of offspring as has been shown in *Drosophila obscura* (Domenech *et al.,* 2022). There are arthropods which are suited for different temperatures, and there are many adaptations to help them overcome the challenges in their environment. The ghost moth (*Hepialus xiaojinensis*) for example is a cold-adapted stenothermal moth inhabiting the cold meadows in Tibet. Ghost moths have been observed maintaining feeding and growth at 0°C thanks to various biochemical and metabolic mechanisms (Zhu *et al.,* 2016). Similarly, there are small arthropods such as shrimps living near deep sea thermal vents who have adaptations

that let them thrive in that ecosystem (Zhu *et al.,* 2020).

Certain arthropods such as *Hexagenia* species (mayflies) can be bioindicators of a healthy environment because of how sensitive they are to pollution or otherwise adverse conditions (Edsall *et al.,* 2001). The majority of the *Hexagenia* lifecycle takes place aquatically, and are sensitive to changes in their environment. They spend most of their lives just under the surface of soft bottomed habitats underwater depths of less than 20m. Mating swarms of these flies are synonymous with healthy ecosystems because of their sensitivity to changes in their environment (Edsall *et al.,* 2001). Typically, when there are healthy numbers of these species it is a sign that there is not an issue with pollution in the area. In addition to this wetland mayfly bioindicator, certain beetles such as members of the family *Carabidae* can also be bioindicators (Burgio *et al.,* 2015). Functional biodiversity is associated with the mutual relationship between certain flora and fauna in an environment, and the efficacy of the function is often related to biodiversity (Altieri, 1999). For example, conservation of biodiversity is one of the primary goals of sustainable farming, and it has been noted that drastic declines in biodiversity in range and abundance have been reported across farmland in Europe which reduces overall functional biodiversity. Functional biodiversity is a term that refers to the wide range of functions that various organisms perform in their ecosystems. The outcome of functional biodiversity is to enhance the functionality of ecosystems via functions such as pollination, pest control, disease suppression, and nutrient cycling (Burgio *et al.,* 2015).

Although arthropods perform many critical roles in the environment, certain species may be known to cause harm. Certain endemic pest species can cause problems for farmers, however there are many cases where these local pests can be mitigated by properly rotating

crops or using various means of control for the pest species (Gupta *et al.,* 2020). The species that cause real damage to the environment and people are typically invasive species such as the spotted lanternfly (Maino *et al.,* 2021). Many of the invasive species within arthropods belong to the insects. Insects such as lantern flies and longhorn beetles are invasive and kill large swaths of trees, which irreparably change ecosystems (Maino *et al.,* 2021; Yamasako *et al.,* 2022).

Impact of Temperature on Microbes

Many of the organisms that people tend to think about when they consider microbial life are bacteria on or inside of humans or microbes that may make us and livestock sick (Lepesteur, 2021; Middlebrook *et al.,* 2022). Many of the organisms that are pathogenic to humans grow very well at 37°C, which would all be considered mesophiles (Ogawa *et al.,* 2021). Likewise, there are also organisms that must grow at much lower or higher temperatures. Organisms that require very cold temperatures are psychrophilic organisms, while organisms that require temperatures at or above 55°C are thermophilic (Wang *et al.,* 2022; Ljungqvist *et al.,* 2022). Examples of these types of organisms in nature can be found in extreme environments like a frozen tundra, or in boiling hot springs.

Microbial organisms cannot generate heat for themselves the same way that mammals can, so they are typically at the mercy of the environment where they exist. There are however some ways for microbes to mitigate the impact of the environment such as taxa that form biofilms, spores, or capsules. Usually, microbes grow the best at a specific temperature, known as their thermal optimum (Mira *et al.,* 2022). Organisms that are grown far outside their thermal optimum may grow extremely slowly or perhaps not at all. Many microbes are

very sensitive to temperature, as they do not all have the means to overcome this obstacle. Despite this, there are some organisms that can produce defensive structures such as capsules and spores (Wan *et al.,* 2022). One example of a microbe that can tolerate cold temperatures are members of the genus *Psychrobacter* which have metabolic adaptations to allow them to thrive in the cold (Wang *et al.,* 2022). Another example of a microbe overcoming temperature is another bacterium known as *Pseudomonas syringae*. This bacterium *P. syringae* can produce proteins that raise the freezing point of water, so it creates ice crystals above freezing temperatures. *P. syringae* uses this to its advantage by using this ice crystal formation to puncture plant cell walls where it grows and use the nutrients from the lysed plant cells to grow (Santamaría-Hernando *et al.,* 2022).

Another good example of the importance of temperature on microbes can be seen from space when cyanobacterial blooms occur in large bodies of water. Blue or green algae are cyanobacteria growing in the water, and they are temperature sensitive microbes that produce much of Earth's breathable oxygen. These algal blooms in aquatic environments can be triggered by raising temperatures and an influx of nutrients. Oxygen is depleted in bodies of water with large blooms as the cyanobacteria are decomposed after death leading to anoxic zones that kill wildlife and create dead zones in aquatic systems (Gacheva *et al.,* 2013; Huang *et al.,* 2022).

Microbe-host Interactions: the Microbiome

Gut microbiomes are important across all walks of life because they do many different things for their hosts. In some mammalian hosts such as cows or other ruminants, the gut microbiome provides them with the ability to further digest plant matter by catabolizing some

forms of cellulose (Clemmons *et al.,* 2018). Many plants rely on the rhizobiome that inhabits the area around their roots, or rhizosphere, to increase the bioavailability of metal ions or other molecules to the host plant (Garcia-Gonzalo *et al.,* 2017; Ortiz *et al.,* 2020). Some arthropods such as roaches can reuse their nitrogenous waste thanks to a *Blattabacterium,* and in termite hindguts the protist *Pseudotrichonympha* catabolizes lignocellulose (del Campo *et al.,* 2017; Noda *et al.,* 2020). Some insects like mosquitos rely on a particular gut microbiome to grow properly as larvae (Valzania *et al.,* 2018). Other studies have reported that gut microbiome diversity in larval and adult mosquitos is low, implying that some key organisms are involved in their growth (Coon *et al.,* 2016). It is estimated that roughly 15% of insects harbor a mutualistic bacterial endosymbiont (*Blattabacterium cuenoti*) within specialized cells inside the insect's fat bodies (Rosas *et al.,* 2018). Many of the bacterial-insect symbionts that have a mutualistic relationship with insects belong to gamma and beta proteobacteria, while many opportunistic pathogens that have a parasitic relationship with the host belong to alpha proteobacteria and firmicutes (Provorov *et al.,* 2017). There are many different examples of symbiotic relationships between microbes and insects, but the extent and exact nature of many of these relationships remains a subject where further research is required.

Bacteria are not the only organisms that make up a gut microbiome: there are also eukaryotes that play important roles. Although there is typically a greater abundance of prokaryotes in the gut than eukaryotes, the eukaryotes still play important roles similar to the prokaryotes (Hooks *et al.,* 2019). Diet heavily influences microbiome composition (Frago *et al.,* 2012; He *et al.,* 2021). One example of such an influence is made by Li *et al*. who demonstrated a strong link between the microbiome composition of leaves and the caterpillars that ate them (Li *et al.,* 2020). It is known that factors in the host's environment such as diet heavily

influence host gut microbiome and genetic factors contributed by the host influence bacterial gut composition, but little is known about how these factors impact the eukaryotes found in gut microbiomes (Ramayo-Caldas *et al.,* 2020). There is also a gap in our understanding of cross kingdom interactions in the gut of insects. An example of important gut eukaryotes in arthropods has previously been mentioned referring to the role that *Pseudotrichonympha* plays in breaking down lignocellulose in termite hindguts (del Campo *et al.,* 2017). Another example of cross kingdom interaction in a gut microbiome is suggested in a paper by Sapkota et al. where bacterivorous protists may be keystone species in the guts of earthworms (Sapkota *et al.,* 2020). One interaction that may not be so clear is the one between population density and microbiome. A study by Mogouong *et al.* suggested that the relative abundance and composition of certain taxa are influenced by the host population density (Mogouong *et al.,* 2020). More research is required of cross-kingdom gut microbiome interactions, as many keystone eukaryote species may be discovered.

Microbiomes are typically complex and rich in a wide variety of taxa (Adair *et al.,* 2017; Thomson *et al.,* 2017). Within the literature, there is a gap in the knowledge that confounds the exact nature of the relationship between microbiome and host. The interactions within microbiome and between microbiome and host are often complex (King *et al.,* 2016; Weldon *et al.,* 2020). There is evidence however that the interaction between various genotypes in the microbiome can explain more phenotypic variation than species-species interactions (Smee *et al.,* 2021). The study conducted by Smee *et al*. highlights the importance of studying the coevolutionary development of these specific genotypic interactions in addition to the interspecies or cross-kingdom interactions amongst microbiomes.

Microbiomes do not always refer to the gut, as is the case in the Suen *et al.* 2010 study with leaf-cutter ants. This study demonstrated the importance of the leafcutter ant fungal garden microbiome in cellulose degradation (Suen *et al.,* 2010). Cellulose contained within plant cell walls makes up the largest reservoirs of organic carbon on planet Earth (Stricklen 2008). The 2010 Suen *et al.* study also found the leafcutter and fungal garden microbiome to show close similarity with bovine rumen, raising more questions about the evolution of cellulose degrading microbial communities such as how they evolved cellulose degrading abilities in separate environments under different pressures.

Insects and their Gut Microbiome

Arthropods are the most diverse Phylum of animals on the planet, but they are currently facing a massive loss of diversity and biomass worldwide. In addition to this loss, many insects are changing their ecological range by moving into and inhabiting habitats that they normally would not (Bell *et al.,* 2020). Insects fill crucial niches in many different ecosystems, so their decline is becoming increasingly noticeable (Kawahara *et al.,* 2021). Insects are poikilothermic organisms, meaning that they cannot control their own body temperature physiologically. This in turn means that their gut microbiome is at the mercy of the temperature in the environment of the host (Kokou *et al.,* 2018). The gut microbiome is composed of a cross-kingdom microbial conglomerate of organisms that serve multiple known essential roles in many different animal species like metabolism or immunity, and arthropods are no different. Similarly, the gut metagenome is composed of all the genes of the gut microbial community and is indicative of all the microbes present in the sample (Ohue *et al.,* 2019). The gut microbiome serves multiple known essential roles in many different animal

species like metabolism or immunity, and arthropods are no different. It is already known that some arthropods such as roaches can reuse their nitrogenous waste thanks to a *Blattabacterium* and in termite hindguts the protist *Pseudotrichonympha* allows them to digest lignocellulose (Noda et al., 2020; del Campo *et al.,* 2017). There is a clear gap in our understanding of the relationship between insect gut microbiome, host lifecycle, and host tolerance to temperature stress (Gupta *et al.,* 2020). The gut microbiome confers some benefits to the insect host and is directly influenced by environmental temperatures. This is the reason why understanding the relationship between gut microbiome and host is important for studying changes in insect ecology and lifecycle across the globe (Arango *et al.,* 2020; Fischbach *et al.,* 2018).

Some bacteria may be considered inactive if they undergo a physiological change from vegetative cells to spores, and they may become competitive if conditions allow them to germinate. This is because the most competitive bacteria sequester more space and resources than their less competitive counterparts, ultimately influencing the abundance of taxa in a particular environment. Some bacteria can even enter a state of dormancy. Dormancy is a state that many microbes can enter when they experience unfavorable conditions in their environment. This is a reversible state of low metabolic activity. These dormant microbes can create what is called a 'seed bank' which is comprised of organisms that could be revived following a favorable environmental change (Lennon and Jones, 2011). Discerning between active or dormant taxa is currently one of the biggest limitations to culture-independent microbial community analysis. Community composition analysis using DNA-based methods such as 16S rRNA sequencing or shotgun metagenomics have the pitfall of not differentiating between living, dead, or dormant cells (Burkert *et al.,* 2019).

Poikilotherms such as insects are for the most part unable to control their body temperature physiologically and are thus at the mercy of their environment (Makarieva *et al.,* 2005). If the insect microbiome impacts lifecycle and drastically shifts across various temperatures, then a transforming climate is one reason why insect microbiomes and ecology are changing. Understanding the relationship between insects and their microbiome will better provide researchers with the information necessary to study these population declines.

Impacts of Captivity

We humans have undoubtedly touched most of the globe with our influence. Part of human interference includes captivity for scientific purposes or otherwise. The impact of captivity on animal models is therefore a highly relevant field of study, deserving of more attention and research. An example of such research with mammal models is the preliminary study by Harbers *et al*., which found that wild and captive boars (*Sus scrofa*) had significantly different bone densities and body mass (Harbers *et al*., 2020). Aside from studies into the impact of captivity on growth and development, another major area of interest is reproduction. Understanding the impact of captivity on reproduction is essential for the rehabilitation and preservation of endangered species. A study by Peng *et al.,* investigated the impact of reproductive behavior in the endangered giant panda and found that the area they were kept in significantly influences the frequency of reproductive behavior (Peng *et al.,* 2006). In addition to space, diet has also been shown to have an influence on reproductive performance. Using a catadromous fish model (*Anguilla anguilla*), Butts *et al.,* found that dietary amino acids significantly impact sperm motility (Butts *et al.,* 2020). Research with captive and wild griffon vultures (*Gyps fulvus*) showed that blood cell composition is significantly different between

wild and captive vultures after a prolonged period of captivity (Giambelluca *et al.*, 2017). There is clearly plenty of evidence that captivity has a significant impact on animals in diverse vertebrate models, and the same research is necessary for invertebrate models. Organisms in captivity tend to experience different selective pressures and stressors than in the wild. For example, a study by Schmidt *et al.,* found that plecopterans were sensitive to the 2°C difference in temperature between the river and the water in their laboratory (Schmidt *et al.*, 2018). Furthermore, a study by Olzer *et al.,* found that house crickets (*Acheta domesticus*) are roughly 1.5 times more aggressive in captivity than in the wild (Olzer *et al.,* 2019).

There is plenty of evidence to suggest that captivity impacts both vertebrate and invertebrate animals physiologically, but there is a gap in the knowledge in how captivity impacts the microbiome, specifically the microbiome of insects. The microbiome of mammalian models changes according to diet and environment, so it is not unreasonable to hypothesize that the microbiome of invertebrate models would follow the same trend (Schwab *et al.,* 2011). There are undoubtedly a wide variety of factors involved in captivity, meaning there is more work to be done to disseminate the impact that these factors of captivity have on research organisms, organisms in rehabilitation, and even pets.

Broader Impacts

Insects are facing an almost silent mass extinction across the globe. Changing climate may be impacting arthropods in more ways than just one. In a recent paper by Halsch *et al.* suggest that changing climate is contributing not only to a change in insect ecology, but also to the massive population declines that insects have been experiencing (Halsch *et al.*, 2020). This project offers some insight into what is changing in the microbial world that may be

contributing to this mass extinction. The data from this project will be used in future insect microbiome studies and can help bridge the gap in our understanding of how microbes may be playing a role in insect decline.

Using *Tenebrio molitor* as a model arthropod organism, I compared the lifespan, tolerance to heat stress, weight, and successful eclosion in organisms with and without Rifampin disrupted gut microbiomes. I hypothesize that the gut microbiome of *T. molitor* is critical to a normal lifecycle. This data will provide more insight into how important microbes in the gut are to arthropods. With a more robust understanding of how important microbes are to insects, better care could be taken with agriculture and the environment with respect to how certain projects may disrupt the gut of insects. The results of my research will provide further evidence for the importance of gut bacteria to insect lifecycles and will lead to future studies into host- microbiome interactions in arthropods.

The common yellow mealworm, *T. molitor*, is also a potential source of food (Rumbos *et al.,* 2020). Further establishing this organism as a model arthropod would help to normalize raising these insects in more than just lab settings. The yellow mealworm has the potential to replace the high-cost fish and soya bean substrate that is commonly fed to chickens in the poultry industry (Selaledi *et al.,* 2019). In addition to serving as a high-protein feed substrate for the poultry industry, *T. molitor* also has the potential to serve as a protein source for humans. Bread is an important food for people across the planet, and traditionally it is made with a wheat flour. A paper published by Roncolini *et al.* suggests that *T. molitor* dried and crushed into a powder can be used to supplement 5-10% of the wheat flour in a bread recipe without changing the texture, integrity, or flavor or the bread (Roncolini *et al.,* 2019). There is great value in supplementing expensive agricultural substrates for the poultry industry and

reducing the use of wheat flour with the addition of *T. molitor* as a food source.

The utility of *T. molitor* extends beyond just using them to study insect microbiomes, temperature responses, or their potential as a food source. Mealworms also have the potential for biodegradation. A study by Yang *et al.*, demonstrated mealworms' ability to consume and to an extent depolymerize polypropylene plastic with the presence of bacteria from the genus *Kluyvera* in their gut microbiome (Yang *et al.*, 2021). In addition to plastic degradation, *T. molitor* has also been shown to break down the inedible spent mushroom substrate left over after the edible mushrooms have been harvested (Li *et al.*, 2020). An example of a future study with *T. molitor* may take advantage of its potential for biodegradation and as a food source by rearing the mealworms on agricultural waste such as spent mushroom substrate and using these larvae to make mealworm flour.

Expected Outcomes and Pitfalls

The purpose of this project was to bridge the gap in understanding between the interaction of insects and their gut microbiomes as it relates to temperature. I expected to see a difference in lifecycle stages between temperatures among the two cohorts of *T. molitor*. More specifically, I expected to see a reduction in lifespan and a difference in tolerance to temperature stress between cohorts of *T. molitor* with disrupted microbiomes, and undisrupted microbiomes. I anticipated that not all larvae would consume their food equally, but the error of this confounding variable was reduced by increasing the sample size. I also anticipated that the microbiome would be initially disrupted in the antibiotic treatment but would eventually return to normal by the end of the experiment. Despite the microbiome recovering from the disruption, the host may still have been negatively impacted by the treatment.

It is difficult to classify microbial communities to the species level. So, in addition to targeted sequencing in the future, subsequent studies will also employ shotgun metagenomics to characterize the gut microbiomes more thoroughly. One advantage to using shotgun metagenomics over other methods is that both prokaryotic and eukaryotic genomes will be analyzed simultaneously, as opposed to introducing error by splitting the sample in half and performing both 16S rRNA and 18S rRNA sequencing separately. Characterizing the gut metagenome with shotgun metagenomics will provide insight into which organisms are most prevalent in each treatment, and their relationship with host physiology. Shotgun metagenomics is great for elucidating the entire microbial community of a sample but is limited in part by its inability to differentiate between living and dead cells.

Aims

I hypothesize that the use of Rifampin in the food substrate of *T. molitor* larvae would disrupt the gut microbiome without significantly impacting the lifecycle of the host organism. The reason for knowing this is to study the impact that temperature has on *T. molitor* with and without disrupted microbiomes. I hypothesize that a disrupted gut microbiome reduces the ability of *T. molitor* to respond to temperature stress. Significantly different gut microbiomes coupled with reduced survivability would provide evidence to suggest that a normal gut microbiome provides some benefit for *T. molitor* to survive heat shock events. This research could be expanded to other orders of arthropods to elucidate the impact that warming climates have on declining arthropod species and their host-microbiome relationships.

Two aims were made to test these hypotheses in this study.

Aim 1: Observe the impact that a Rifampin-disrupted microbiome has on *T. molitor* life cycle under different temperatures.

Aim 2: Characterize and compare *T. molitor* lifecycle and gut bacteriome when treated with different antibiotics and exposed to a heat shock.

II. INTERPLAY BETWEEN MICROBIOME AND TEMPERATURE IN TENEBRIO MOLITOR

INTRODUCTION

Phylum Arthropoda and Tenebrio molitor

Arthropods are the most diverse phylum of animals on the planet; however, they are suffering mass extinctions across the globe. Insects fill crucial niches in many different ecosystems; thus, their decline is consequential (Kawahara *et al.*, 2021). Insects are known as poikilothermic organisms, which means they cannot physiologically control their own body temperature outside of various behavioral traits. This means their gut microbiome is subject to changes in environmental temperatures (Kokou *et al.*, 2018). As the climate steadily warms, impacts on these animals are ultimately unknown and changing temperatures will impact arthropods in unforeseen ways. There is already evidence that changing climate is contributing to diverging insect ecology as insects are being found outside their normal expected range, and to population declines (Bell *et al.*, 2020). Findings from this research will elucidate, in part, the impact that microbes have on insects and their role in the extinction process. Without further research, the reality of this mass extinction may not be fully realized until it is too late.

Gut microbiomes are composed of a cross-kingdom microbial conglomerate, including bacteria, viruses, protists, and fungi. This organismal conglomerate serves multiple known roles in many different animal species, including essential functions like digestion. Microbiomes play critical roles in metabolism and immunity in many different organisms, including arthropods. Major examples of these benefits include *Blattabacterium* species assisting roaches to reuse their nitrogenous waste, in termite hindguts the protist *Pseudotrichonympha* catabolizes lignocellulose, and mosquitos utilize a specialized gut microbiome to develop properly as larvae (del Campo *et al.*, 2017: Noda *et al.*, 2020: Valzania

et al., 2018). It is estimated that roughly 15% of insects harbor a mutualistic bacterial endosymbiont (*Blattabacterium cuenoti*) within specialized cells inside the insect's fat bodies (Rosas *et al.,* 2018). Many of the bacterial-insect symbionts that have a mutualistic relationship with insects belong to gamma and beta proteobacteria, while many opportunistic pathogens that have a parasitic relationship with the host belong to alphaproteobacteria and firmicutes (Provorov *et al.,* 2017). There are many different examples of symbiotic relationships between microbes and insects, but the extent and exact nature of many of these relationships remains a subject where further research is required. Understanding the relationship between gut microbiome and host is important for studying changes in insect ecology and lifecycle due to a changing climate (Arango *et al.,* 2020; Fischbach *et al.,* 2018).

Normal gut flora or the normal gut microbiome of an organism helps metabolize what is consumed in the diet, and as such will change as diet does (Lou *et al.,* 2021; Przemieniecki *et al.,* 2020). It has been documented that *T. molitor* can consume and survive with plastic in their diet, suggesting that the microbes present in the gut with this diet assist in plastic metabolism (Liu et al., 2022). The normal gut flora of *T. molitor* includes the genus *Spiroplasma,* which appears to normally remain in high abundance (Wang *et al.,* 2022; Khanal *et al.,* 2023). Despite *Spiroplasma* having a history in the literature for being an insect or plant pathogen, a thorough phylogenetic analysis of the *Spiroplasma* in *T. molitor* appears to be distinct from previously identified members of this genus (Jung *et al.,* 2014). The ability of *T. molitor* to survive on plastic and the prevalence of a novel *Spiroplasma* species in the gut microbiome warrant future research into the metabolic activity of *Spiroplasma* and polystyrene depolymerization.

This research will help bridge the gap in knowledge between temperature, microbiomes, and insect life cycle. Using *Tenebrio molitor* as a model arthropod organism, I compared the lifespan, tolerance to heat stress, weight, and the prokaryotic gut composition in organisms with and without disrupted gut microbiomes. I hypothesized that the gut microbiome of *T. molitor* changes significantly with a sudden change in temperature, and the host response to heat shock will differ based on the level of microbiome disruption. These data will provide insight into how microbes in the gut of arthropods respond to temperature stress and interact with the host. The results of this research will provide further evidence for the importance of gut bacteria to insect lifecycles and will inform future studies into insect conservation.

Future studies with *T. molitor* could elucidate their utility as a food source (Rumbos *et al.,* 2020). Mealworms also have potential for biodegradation of certain otherwise recalcitrant materials. Mealworms have demonstrated an ability to consume and at least partially depolymerize polypropylene plastic when bacteria from the genus *Kluyvera* are present in their gut microbiome (Yang *et al.,* 2021). In addition to plastic degradation, *T. molitor* has also been shown to break down the inedible spent mushroom substrate left over after edible mushroom harvest (Li *et al.,* 2020). Examples of future *T. molitor* studies include biodegradation of plastics and agricultural waste, potential as human or agricultural food source, and further insect microbiome studies. Stigma surrounds *T. molitor* as it is considered a pest organism (Dastranj *et al.,* 2013). This misnomer could be corrected through the normalization of *T. molitor* farming for the various applications to which they are suited.

A common darkling beetle, *T. molitor*, is an excellent organism to use as a model to explore the relationships between insect lifecycle, gut microbiome, and temperature. Larvae of *T. molitor* have a wide variety of uses in and outside of the lab. Because of the ease of rearing mealworms and the wide variety of substrates they can consume, many people use them outside of the lab as a cheap nutrient source (Roncolini *et al.,* 2019; Rumbos *et al.,* 2020). Many pet owners who have animals that would eat mealworms may also raise *T. molitor* larvae as feed because rearing mealworms is a cheap and easy alternative to more expensive animal feeds (Selaledi *et al.,* 2019). Replacing some expensive animal feed with less expensive options such as supplementing with *T. molitor* is a reasonable option to save money. There is merit in utilizing *T. molitor* as a model arthropod organism for the purposes of agriculture and industry.

Stigma of Tenebrio molitor as a Pest

Another pitfall in this study is the stigma over *T. molitor* being considered an international pest organism (Dastranj *et al.*, 2013). The stigma stems from the fact that *T. molitor* is commonly found infesting stores of grain (Vigneron *et al.,* 2019; Jehan *et al.,* 2022). This misnomer could however be corrected with the normalization of *T. molitor* farming, as there is a growing desire to utilize mealworms for their high nutrient content (Vigneron *et al.,* 2019). There is already plenty of evidence that the pest-stigma of *T. molitor* is changing by the way people are now utilizing this organism (Roncolini *et al.*, 2019; Rumbos *et al.*, 2020; Selaledi *et al.,* 2019). As more people feel comfortable integrating *T. molitor* into their diets, a cheaper protein source will become available to the world.

MATERIALS AND METHODS

Experiment 1: Experimental Design

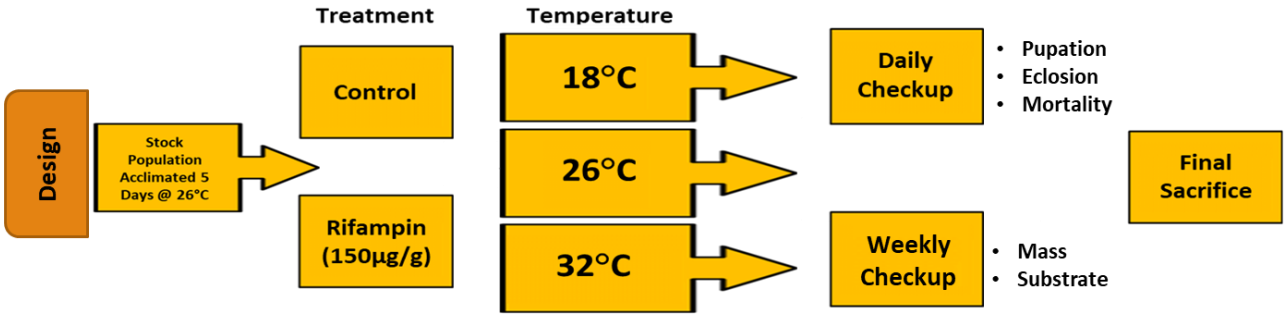

Figure 1.01: Experimental design for the first experiment. Fresh substrate was added to each replicate once per week. Larval guts extracted from sacrificed larvae to be used in downstream applications.

Experiment 1: Tenebrio molitor Stock Population

The initial stock population of *T. molitor* was purchased online from Exotic Nutrition™. Once the larvae arrived at the lab, they were placed in a plastic box and any dead were removed. A thick layer of Vaseline was placed at the top of the box to prevent any larvae from scaling the side of the container and escaping. Small holes were placed in the lids of the containers to promote gas exchange within their enclosure. The stock population was then placed inside an incubator at 27°C and allowed to acclimate for at least 1 week before used for any experimentation. Humidity within the incubator was maintained between 55% and 60% by plastic tubs filled with water. The stock population was fed a diet of wheat bran and celery, with the celery being replaced every 2 days.

Experiment 1: Rearing T. molitor Larvae

The stock population of about 4,000 *T. molitor* was raised in a large plastic tub that has 6 holes drilled in both sides to facilitate air circulation. They live in ground oats that line the bottom of the tub about 5cm deep. If new larvae are brought into the lab, they must all be acclimated at 26°C for at least 5 days, and then the population is split in half to acclimate some with oats supplemented with 150µg/g Rifampin before they can be used in the experiment. Dead individuals and exoskeletons are removed from the container twice per week to keep the enclosure clean and further reduce the risk of fungal infection and mite infestation. Moisture inside of the enclosure is kept at a minimum to discourage the spread of fungus. In the event of a mite infestation, all organisms are removed and placed in secondary containment while their enclosure is thoroughly cleaned. To prevent escaping mites, a thin layer of petroleum jelly was spread along the inside of the wall of the enclosure and replaced, as necessary.

Experiment 1: Treatment, Feeding, and Growth Conditions

Acclimated larvae similar in size were chosen for the experiment. Sterile petri dishes were used to house 5 larvae each with 1g of the assigned substrate. Each plate was provisioned two substrate types which are ground oats or ground oats mixed with (150µg/g) Rifampin. It was determined that the ideal temperature range for *T. molitor* is between 25°C and 28°C (Ribeiro *et al.*, 2018). Individuals are grown in these two substrate types at three different temperatures: below ideal at 18°C, ideal at 26°C, and above ideal at 34°C. Each temperature treatment contained 32 plates of each group, bringing the total plates per temperature to 64, and the total plates per experiment to 192. Therefore, there are 160 larvae

in each group, 320 larvae per temperature treatment, and 960 larvae per experiment. This amount was picked due to the practicality of two people collecting data daily. The larvae were counted daily, and rates of pupation, adult emergence, and death were recorded. Adult beetles were sacrificed at -20°C in Eppendorf tubes stored at -80°C for future analysis upon emergence. The mass of each larva in a plate was taken every 5 days and was divided by the number of larvae in the dish to record the average larval mass per dish.

Experiment 1: Data Collection

Prior to beginning the experiment, 50 total acclimated larvae were dissected following the gut extraction protocol as seen in experiment 2, and each gut was stored at -80°C for future analysis. Data was collected from both the control and antibiotic plates at each temperature daily, and mass data was collected from the larvae every 5 days. The data collected daily was instar count, pupation count, larval deaths, pupal deaths, adult emergence, and adult physical appearance. Adults were immediately sacrificed in Eppendorf tubes at -80°C upon emergence for microbiome analysis. Adults with obvious phenotypic abnormalities were photographed before they were sacrificed and stored. At day 15 (halfway through the experiment), 60 larvae were removed and sacrificed at -20°C preceeding the gut extraction protocol where the individual guts were stored at 80°C for microbiome analysis. The final T2 sacrifice was performed on the last day of the experiment. A total of 20 guts were extracted from larvae in each treatment, and the remaining larvae were stored whole at -80°C for future analysis.

Experiment 1: T. molitor Lifecycle Analysis

All data was collected daily except for the larval mass data which was collected weekly. Numbers of instars, pupations, eclosions, and deaths were recorded daily. Eclosed beetles were immediately weighed, sacrificed in ethanol, and stored at -20°C for future gut analysis. Once weekly, total larval mass was recorded for each plate, and averaged to determine mean larval mass per cohort. General linear models were used to determine statistical significance for the lifecycle data: growth, pupation, eclosions, and mortality.

Experiment 2: Experimental Design

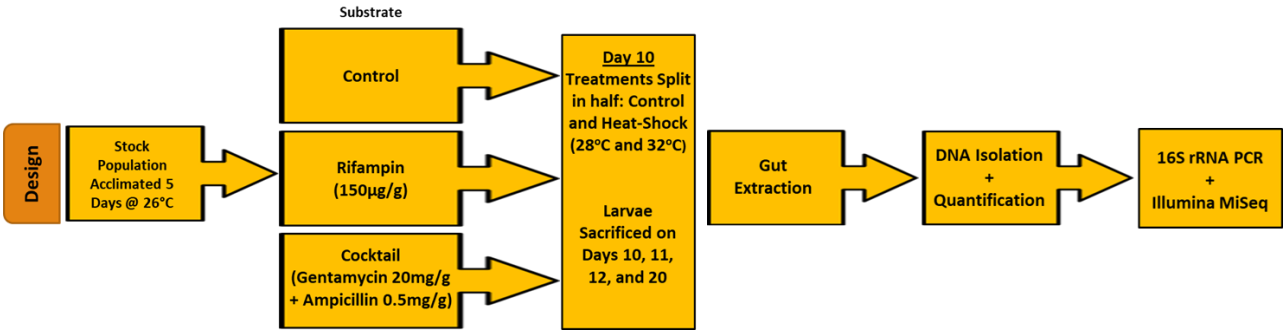

Figure 2.01: Experimental design for experiment 2. Following the Day 10 heat-shock treatment, no new substrate was given to the organisms.

Experiment 2: Experimental Setup

Two days prior to the start of the experiment, the materials and incubators were prepared to minimize any confounding factors on the first day of the experiment. To prepare, two incubators were set to 28°C, and 32°C respectively. Large containers of water were placed inside the incubators and refilled as necessary to maintain humidity between 55% and 60% throughout the entirety of the experiment. Plastic containers with a volume of 225g were used

as independent enclosures for each group of larvae, holes were made in each container lid to facilitate ventilation. The larvae were all obtained from a company called Exotic Nutrition™ at the same developmental stage and were acclimated in the lab at their ideal temperature (27^{o}C). Larvae of approximately the same length that appeared healthy were chosen for the experiment. A total of 750 healthy larvae were picked for the experiment and divided into 6 groups of 125 larvae at random. These 6 groups of 125 larvae were randomly assigned a group (Group 1, Group 2, Group 3, Group 4, and Group 6), and placed in their 225g plastic container with 40g of their respective wheat substrate and ~10g of washed celery as described in the substrate preparation section. On the tenth day of the experiment half of the larvae were transferred to an incubator set to 32°C. The groups moved to the increased temperature were 2, 4, and 6 and kept there until the experiment concluded on the twentieth day. Groups were not given antibiotics following the heat-shock treatment.

E*xperiment 2: Substrate Preparation*

Three separate substrates were created using baked wheat bran as the base. The wheat bran base was baked at 93^{o}C (200^{o}F) for 45 minutes prior to use in the experiment, and 250g of each substrate type was necessary for the entirety of the experiment. The substrate used for the first 2 groups (Group 1 and Group 2) acted as the control and had no antibiotics mixed in with it. The wheat bran substrate used for the second 2 groups (Group 3 and Group 4) was mixed with rifampin to a final concentration of 150µg/g rifampin to wheat bran. Wheat bran substrate for the third set of 2 groups (Group 5 and Group 6) was mixed with gentamycin to a final concentration of 20mg/g, and ampicillin to a final concentration of 0.5mg/g. Substrates were stored for the duration of the experiment at the storage temperature of their antibiotics.

Celery stalks were added to the substrate in each container of the experiment after a thorough 1 minute washing protocol to enrich their diet. The celery was first rinsed with DI water, then vigorously rinsed in a 1% sodium hypochlorite solution. The sodium hypochlorite solution was thoroughly rinsed off with more DI water until the chemical scent was gone in its entirety. Approximately 10g of this surface sterilized celery was used in each group's container and was changed every 2 days of the experiment.

Experiment 2: Data Collection

On the first day of the experiment the individual mass of 20 random larvae per group was recorded, and then again on days 10 and 20. Larval mortality was recorded every 2 days for the first 10 days, then once more on day 20. All dead larvae were preserved in tubes at -80 ^{o}C for potential downstream applications. The total number of pupations were recorded on day 20, but none were allowed to eclose following the experiment. On the first day of the heat shock treatment, day 10, the mass of 20 larvae selected at random from each group was recorded, and a total of 40 larvae were sacrificed from each group on days 10, 11, 12, and all remaining larvae on day 20. From each collection of 40 larvae: 6 were dissected following the gut extraction protocol to be stored for DNA extraction, 30 were flash frozen in liquid nitrogen, and 4 were dissected following the gut extraction protocol and used in downstream applications.

Experiment 2: Rearing T. molitor Larvae

The stock population of *T. molitor* larvae was raised in a large plastic tub full of a wheat bran substrate. The tub with substrate was placed inside an incubator set to 27 ^{o}C and humidity was maintained between 50% and 60% with large tubs of water. Water was

replaced as it evaporated to ensure humidity remained constant. The larvae were also fed a large piece of celery which was washed according to the celery washing protocol. Once per week all leftover exoskeletons and dead were removed to maintain a healthy stock population. Substrate was changed out in its entirety once per month to reduce waste buildup in the stock population.

Experiment 2: Gut Extraction

Guts were extracted from larvae after knocking them out. The larvae were first knocked out by placing them inside the -20^{o}C freezer for 1 minute and 30 seconds. Once the larvae were no longer moving, they were taken to the biosafety enclosure and placed into a jar of 70% ethanol. Individual larvae were removed from the ethanol to a sterile dissection area where the very last body segment of the larvae was aseptically cut off with a scalpel. The larvae are handled with two sets of sterile forceps where one holds the body while the other carefully pulls the hindgut out of the last body segment. Guts are stored in sterile 2mL Eppendorf tubes at -80^{o}C until downstream applications.

Experiment 2: DNA Extraction and Quantification

Guts stored from the gut extraction protocol were used for DNA extraction and downstream applications. The extraction was performed using the QIAamp BiOStic Bacteremia DNA Kit™ following the protocol with an adjustment. The adjustment made was to include the use of a product called Zymo Spike-In Control II™. This spike-in control was used prior to the DNA extraction step in all samples. Each gut sample received 20µL of the spike-in control prior to any cell lysis. Another change to the DNA extraction protocol was to

use a bead beating step in lieu of using a horizontal vortex adapter in step 4 of the original Qiagen protocol. Once all the DNA was extracted from the samples, the concentration was read using a Qubit-4 Fluorometer following the protocol without any adjustments.

Experiment 2: 16S rRNA PCR

The polymerase chain reaction (PCR) was performed with tagged MiSeq primers specific for the V4 region of the prokaryotic 16S rRNA gene ((primers: forward – GTGCCAGCMGCCGCGGTAA; reverse – GGACTACHVGGGTWTCTAAT) (Kozich et al., 2013). Each reaction was done in a 24µl volume with 12µl KAPA HiFi HotStart ReadyMix (2X) (KAPA Biosystems, Boston, Ma, United States), 1.5µl NanoPure water, 1µl forward primer (10µM), 1µl reverse primer (10µM), and 8µl diluted DNA (20ng/µl). Under the following conditions, all samples were run in triplicate: initial denaturation at 95°C for 3 min, 25 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and final elongation at 72°C for 5 min. A control reaction with non-template DNA was included and submitted for sequencing.

Experiment 2: Gel Electrophoresis and PCR Cleanup

Agarose gel electrophoresis was used to confirm the presence of DNA of the proper size. All agarose gels used in this experiment were 1.5% agarose unless otherwise stated. The buffer used to mix the gels was 1X TAE. A 200mL flask was used to combine 60mL of 1X TAE with 0.9g of agarose. The flask was swirled around and placed inside a 1,000-watt microwave where the solution was microwaved in 30 second intervals until the agarose was completely dissolved in the solution. Once the agarose was dissolved, the flask was allowed to cool so it was comfortable to the touch, but not yet solidified. Once the flask was comfortable

to the touch a 1:200 ratio of EZ-Vision® Bluelight DNA Dye™ by VWR to agarose solution was created by adding EZ-Vision® Bluelight DNA Dye™ to the flask and mixing by gently swirling. Once the EZ-Vision® Bluelight DNA Dye™ was mixed into the solution, it was poured into a gel cast with the proper combs and solidified for approximately 20 minutes. The amount of sample to add to the well depends on the size of the comb, but for this experiment 5µL of each sample was combined with 2µL of 6X TriTrack DNA Loading Dye™ and 7µL total was loaded into each well. All gels were run at 90V for 30 minutes unless otherwise specified. The gels were imaged using a UV screen where an orange plastic shield protected the eyes from UV exposure.

The amplicons from the second round of PCR were combined and run through the Exo- SAP IT protocol. It is important to keep the Exo-SAP IT reagent on ice during the entirety of the protocol. The PCR cleanup solution consists of 5µL of the second reaction amplicon and 2µL of the Exo-SAP IT reagent. The Exo-SAP IT protocol on the thermocycler runs at 37°C for 15 minutes and then 80°C for 15 minutes. The Exo-SAP IT products were all stored at -20°C until they were used for downstream applications.

Experiment 2: Size Selection and Sequencing

The size selection process takes advantage of gel electrophoresis by running purified DNA through a gel and pulling out bands at the proper size of the DNA with a pipette. The purified library was loaded onto a pre-cast E-Gel™ Size Select™ II, 2% agarose gel and run until the band of interest bled into the second open well on the gel. The band of interest is determined by measuring it with the ladder based on size. The DNA was stored in a PCR tube for downstream applications. The sequencing takes place on the Illumina MiSeq platform.

Experiment 2: 16S rRNA Data Analysis

The free statistical code software known as R was used to process the raw data output from the Illumina MiSeq platform. Quality control for the MiSeq reads was accomplished using the FastQC package in R to remove low quality reads. Read trimming and merging was done with the DADA2 package in R, and the taxonomic database used was SILVA. The free online software MicrobiomeAnalyst was used to generate figures and perform statistical analysis with the data. Data parameters on MicrobiomeAnalyst were kept default except the low-count filter was reduced to 15%. Data was scaled logarithmically and not rarified. Alpha diversity in the Chao1 index was calculated alongside a Kruskal-Wallis test to determine statistical significance of the diversity within groups. Beta diversity in the Bray-Curtis index was calculated alongside a PCoA. Relative abundance was graphed and statistically analyzed with a PERMANOVA. Individual amplicon sequences are separated into amplicon sequence variants (ASVs) which are each unique sequences. These ASVs are combined if they match at a threshold of 97% or greater into operational taxonomic units (OTUs). The OTUs were evaluated at the genus level, and a single-factor analysis of three selected taxa (*Escherichia-Shigella, Spiroplasma*, and *Staphylococcus*) using DESeq2 as a tool. DESeq2 is a package that employs an empirical Bayes approach to normalization and differential analysis of high-dimensional count data.

RESULTS

Results from the first 30-day pilot experiment are reflected in **Tables 1.2-1.5**, and in **Figures 1.1-1.4**. The ANOVA results in **Table 1.1** correspond to **Figure 1.1** where total larval growth was averaged for each treatment and plotted with standard deviation error bars. The average growth was roughly the same in the control cohorts at 18°C and 26°C, but the

control cohort at 34°C did not show as much growth. Furthermore, the control cohorts grew more than the antibiotic cohorts at every temperature except 34°C. The antibiotic cohorts all grew a similar amount across the 3 temperatures. The cohort that grew the least overall is the control cohort at 34°C. The results of a general linear model to determine if treatment, temperature, or both could predict pupation are found in **Table 1.2**. There are no significant factors in **Table 1.2**, visualized in **Figure 1.2** which illustrates the total number of pupations obtained over the course of the experiment. More pupations are seen at 26°C than either of the other temperatures. The fewest number of pupations were observed at 34°C. Average eclosions are represented in **Figure 1.3** and tested with a general linear model represented in **Table 1.3** to determine if eclosion could be predicted by treatment, temperature, or both. The number of eclosions at 26°C is significantly higher in both cohorts than either 18°C or 34°C, but there was not a significant difference between cohorts. Very few eclosions were observed at 18°C, and they were only counted in the control cohort as the antibiotic cohort had 0. Very few eclosions were seen in the control cohort at 34°C, however all the pupae in the antibiotic cohort eclosed. Average number of deaths are illustrated in **Figure 1.4** and tested by a general linear model in **Table 1.4** to determine if temperature, treatment, or both could be used to predict death. At 18°C significantly more larvae in the control cohort died than the antibiotic cohort. At 26°C however, there is not a significant difference in deaths between the control and antibiotic cohorts. The total deaths in the 34°C for the antibiotic cohort are significantly higher than the control cohort, and both cohorts at all other temperatures. A trend appears to take place between **Figures 1.1-1.4** where the control cohorts at 18°C and 26°C all have higher response variables than their antibiotic counterparts, but the opposite is true at 34°C.

Data displayed in **Table 2.1** reflects the results of a 1-way PERMANOVA on bacterial

genera across all treatments using day as a factor, while **Table 2.2** reflects the same test with temperature as a factor. The cocktail held significant P-values against the control and the rifampin treatments separately, but the control and rifampin treatments did not have significant P-values with one another. The total sum squares are highest on Day 20 and Day 12 implying the greatest variation attributed to the standard error. The highest variation between samples is reflected by the F statistic on Day 11 and Day 12. Similarly, the variation between samples is lowest at the start of the heat shock experiment but is highest on Day 11, 1 day after the heat shock treatment. Alpha-diversity with a Chao1 index of genera across all days is displayed in **Figure 2.1**, and Kruskal-Wallis was used to test the diversity within groups denoted in **Table 2.3**. Beta diversity across all days is reflected in **Figure 2.2** and is a visual representation of the data in **Table 2.4**. The community composition of bacterial taxa at the level of genera is represented in **Figure 2.3** through **Figure 2.6.** The relative abundance for Day 10 is reflected in **Figure 2.3,** Day 11 is reflected in **Figure 2.4,** Day 12 is reflected in **Figure 2.5,** and Day 20 is reflected in **Figure 2.6.** Referring to **Table 2.1** across all days, the taxa in the cocktail treatment differed significantly from the taxa in the control and the Rifampin treatment however the control and Rifampin treatments did not significantly differ from one another. Referring to **Table 2.2**, the cocktail produced significant P-values against the control and Rifampin treatments, but the control and Rifampin treatments were not significantly different from one another. The total sum of squares and F statistic were highest in the 32°C treatment, meaning that the variation due to standard error and the variation between samples was the greatest.

Some significant differences occur in taxa between the different treatments, particularly in *Escherichia-Shigella*, *Spiroplasma*, and *Staphylococcus*. Single-factor ANOVA

reflected in **Table 2.5** was performed to determine how, if at all, the abundance of each taxon changed between treatments after the heat shock. **Figures 2.7-2.9** are log transformed counts of these taxa separated by day. The log-transformed counts of *Escherichia-Shigella* in **Table 2.5** and **Figure 2.7** illustrate that their abundance was significantly different between treatments on Day 10. Log transformed counts of *Spiroplasma* are displayed in **Figure 2.8** and **Table 2.5**, where their abundance is significantly different among groups on all days except Day 10. However, based on **Figure 2.9** and **Table 2.5**, the abundance of *Staphylococcus* was relatively low and insignificant until Day 20 where abundance was highest overall except in the cocktail heat-shock group.

Table 1.1: General linear model with a Poisson distribution to determine if the factors treatment, temperature, or both can be used to predict growth. Displayed are the P values, total Sum- Squares, and F statistics.

Growth	P	Sum-Sq	F
Treatment	0.154	0.0098	2.085
Temperature	0.459	0.00262	0.557
Treatment x Temperature	0.125	0.01143	2.431

Table 1.2: General linear model with a Poisson distribution to determine if the factors treatment, temperature, or both could be used to predict pupation. Displayed are P values, total Sum- Squares, and F statistic for each factor.

Pupation	P	Sum-Sq	F
Treatment	0.631	0.42	0.233
Temperature	0.481	0.9	0.504
Treatment x Temperature	0.348	1.6	0.897

Table 1.3: General linear model with a Poisson distribution to determine if the factors treatment, temperature, or both could be used to predict eclosion. Displayed are P values, total Sum- Squares, and F statistic for each factor.

Eclosion	P	Sum-Sq	F
Treatment	0.602	0.42	0.275
Temperature	0.309	1.6	1.055
Treatment x Temperature	0.129	3.6	2.373

Table 1.4: General linear model with a Poisson distribution to determine if the factors treatment, temperature, or both could be used to predict death. Displayed is P, total Sum-Squares, and F statistic for each factor.

Mortality	P	Sum-Sq	F
Treatment	0.8127	0.017	0.057
Temperature	0.5621	0.1	0.34
Treatment x Temperature	**0.0233**	**1.6**	**5.441**

Table 2.1: One-way PERMANOVA of *T. molitor* gut bacteria with 9999 permutations using day as a factor. Significance in each treatment was determined using the Bray-Curtis index with Bonferroni-corrected P-values of ($P < 0.05$) denoting significance. Gut amplicon sequence variants (ASVs) are compared across each treatment across all days. Total sum of squares reported to show variation attributed to the error, and F statistic reported to show the ratio of variances between samples.

	Cocktail x Control	**Control x Rifampin**	**Rifampin x Cocktail**	**Total Sum squares**	**F**
Day 10	0.3335	0.0632	0.217	6.456	1.429
Day 11	0.0941	**0.0001**	**0.0001**	8.272	8.44
Day 12	0.545	**0.0006**	**0.0003**	7.258	5.443
Day 20	**0.0023**	0.1999	**0.0169**	9.049	2.886
All Days	**0.0001**	0.1061	**0.0001**	34.83	7.93

Table 2.2: One-way PERMANOVA of *T. molitor* gut bacteria with 9999 permutations using temperature as a factor. Significance in each treatment was determined using the Bray-Curtis index with Bonferroni-corrected p-values of ($P < 0.05$) denoting significance. Gut amplicon sequence variants (ASVs) are compared across each treatment across all days. Total sum of squares reported to show variation attributed to the error, and F statistic reported to show the ratio of variances between samples.

	Cocktail x Control	**Control x Rifampin**	**Rifampin x Cocktail**	**Total Sum squares**	**F**
28°C	0.2458	**0.0012**	**0.0007**	15.5	3.486
32°C	**0.0001**	0.2161	**0.0001**	18.66	6.176

Table 2.3: Chao1 index alpha-diversity with Kruskal-Wallis test. Alpha-diversity measures were most significant ($P<0.05$) with high Kruskal-Wallis scores on Day 11 and Day 12.

Time	**P**	**Kruskal-Wallis**
Day 10	0.19662	4.682
Day 11	**0.020464**	**13.331**
Day 12	**0.042593**	**11.484**
Day 20	0.082871	9.7424

Table 2.4: PERMANOVA of *T. molitor* gut bacteria beta diversity between treatments on each day. Significance in each treatment was determined using the Bray-Curtis index with Bonferroni- corrected bolded p-values of ($P < 0.05$) denoting significance. The R-squared value was reported to show the proportion of variance for beta diversity accounted for by treatment on each day, and F statistic reported to show the ratio of variances between samples.

Time	P	R-Squared	F
Day 10	0.316	0.19799	1.152
Day 11	**0.001**	**0.76133**	**12.76**
Day 12	**0.001**	**0.6196**	**5.5379**
Day 20	**0.001**	**0.4937**	**4.6805**

Table 2.5: Single-factor DESeq2 analysis of chosen genera on each day. Results at the taxonomic level of genera with bolded P values of ($P < 0.05$) denoting significance. Box plots of this data can be found in **Figure 2.7** through **Figure 2.9**.

Time	*Escherichia-Shigella*	*Spiroplasma*	*Staphylococcus*
Day 10	**0.010286**	0.73052	0.15135
Day 11	0.93331	**2.42E-23**	0.37677
Day 12	0.72767	**1.61E-10**	0.2469
Day 20	0.41672	**0.010581**	**1.48E-16**

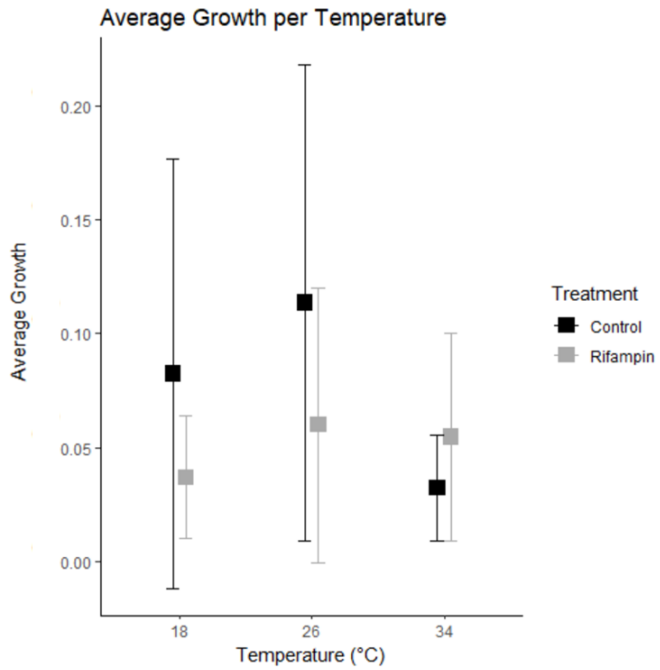

Figure 1.1: Box and whisker plot for average growth for each temperature and treatment with standard deviation bars. Both cohorts saw greater growth at the 26°C treatment than all other treatments. The control cohort saw greater growth in the 18°C and the 26°C than their respective antibiotic cohorts, but the opposite is seen in the 34°C treatment. (P-values; Treatment: 0.154, Temperature: 0.459, Treatment & Temperature: 0.125)

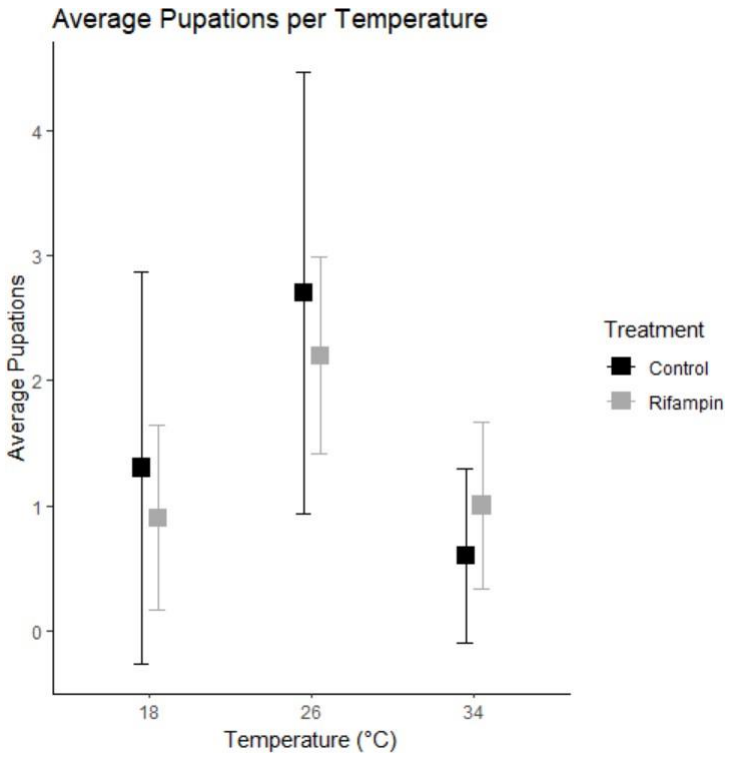

Figure 1.2: Average number of pupations with standard deviation bars. Both treatments saw more pupations at 26°C than all other treatments. The control treatment saw more pupations in the 18°C and the 26°C than the antibiotic treatment, but the opposite is seen at 34°C. (P-values; Treatment: 0.631, Temperature: 0.481, Treatment & Temperature: 0.348)

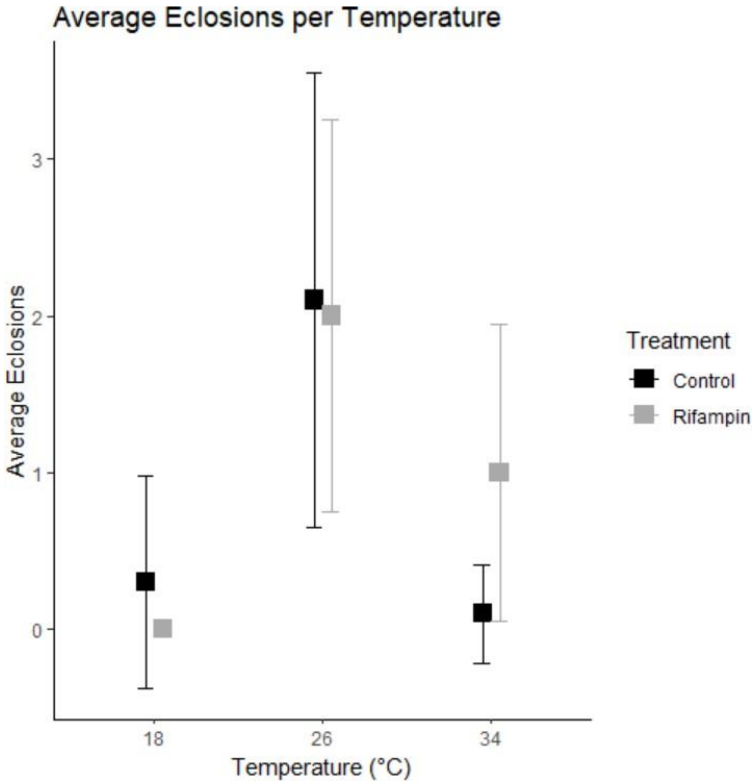

Figure 1.3: Total number of eclosions over 30 days with standard deviation bars. Significantly more eclosions are observed at 26°C than at any other temperature. There were no eclosions seen in the antibiotic cohort at 18°C, and very few seen in the control cohort. The opposite can be said for the 34°C treatment, where fewer eclosions were seen in the control cohort than the antibiotic cohort. (P-values; Treatment: 0.602, Temperature: 0.309, Treatment & Temperature: 0.129)

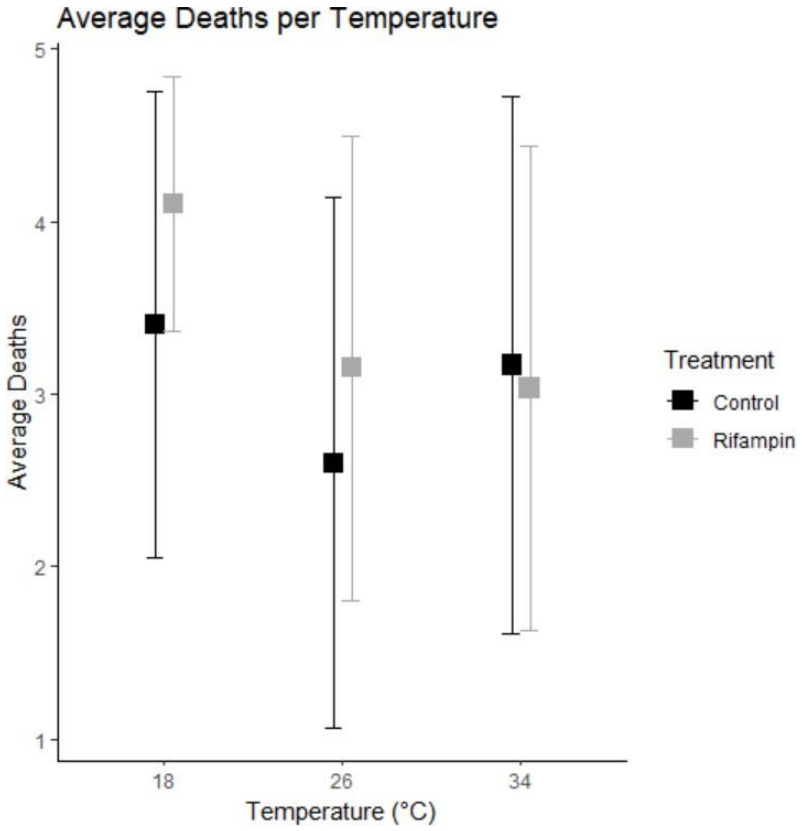

Figure 1.4: The average number of deaths with standard deviation bars. At 18°C and 26°C the average number of deaths is slightly higher in the Rifampin cohorts than the control cohorts, but the opposite is true at 34°C. (P-values; Treatment: 0.8127, Temperature: 0.5621, Treatment & Temperature: 0.0233)

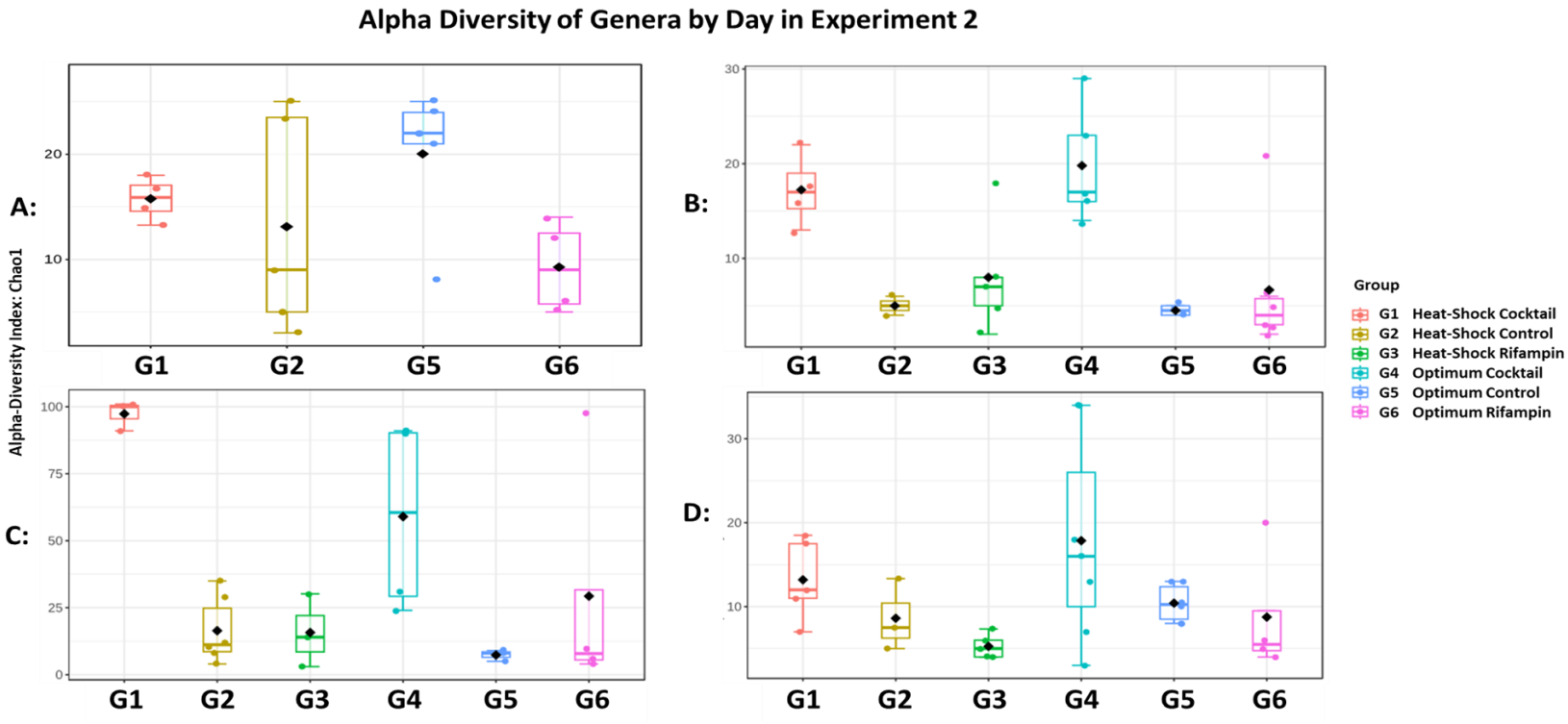

Figure 2.1: Chao1 alpha-diversity measure with Kruskal-Wallis statistics. Graphs are separated by; Day 10 (A), Day 11 (B), Day 12 (C), and Day 20 (D). Groups 3 and 4 were omitted from Day 10 due to insufficient sample size. Kruskal-Wallis results for this test are found in **Table 2.3**.

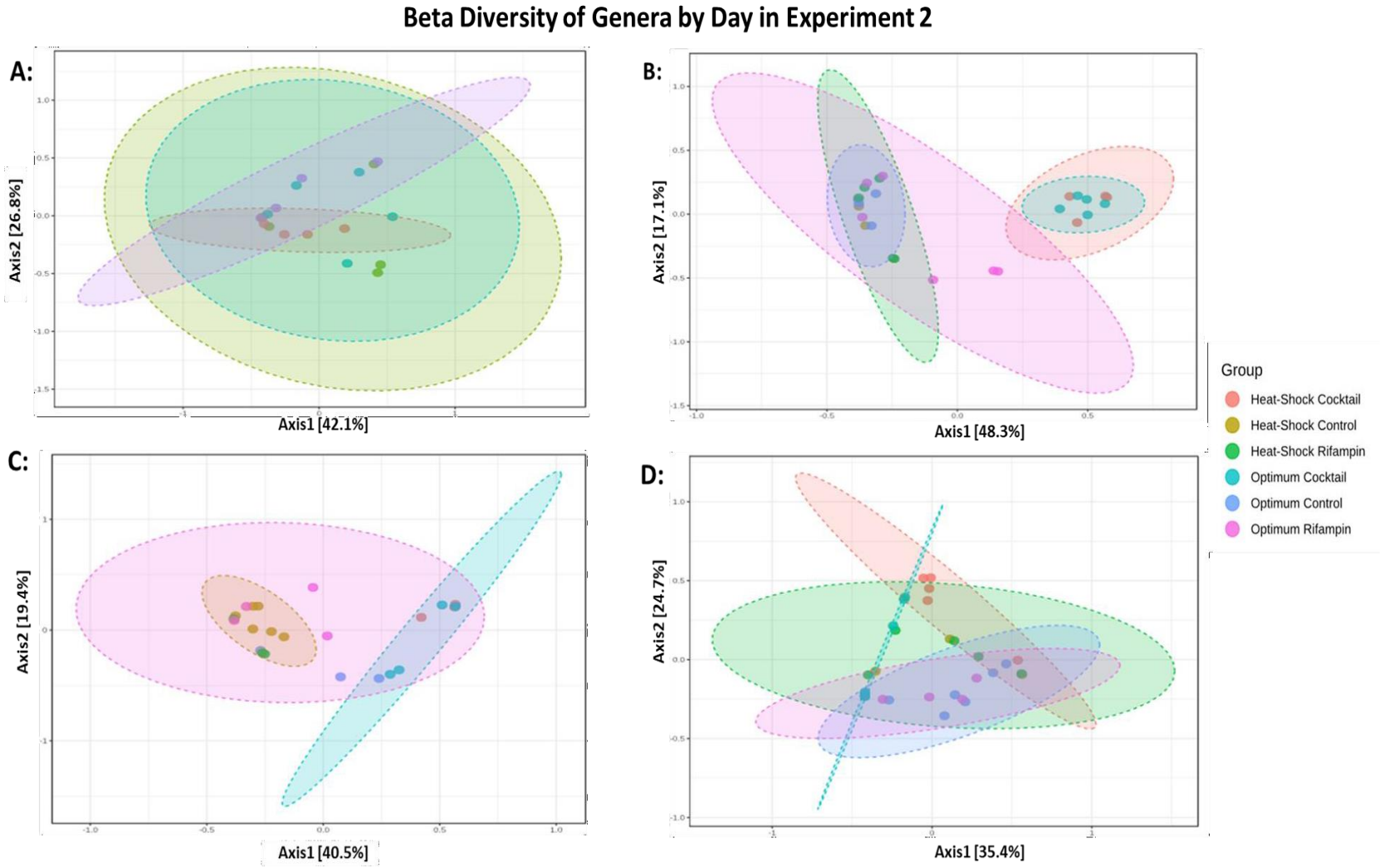

Figure 2.2: Bray-Curtis Index PCoA of genera across Day 10 (A), Day 11 (B), Day 12 (C), and Day 20 (D). Beta diversity of genera appear most similar on Days 10 and 20. PERMANOVA was done to assess similarity between the genera in each treatment reflected in **Table 2.4**. Ellipses show the 95% confidence interval from the centroid.

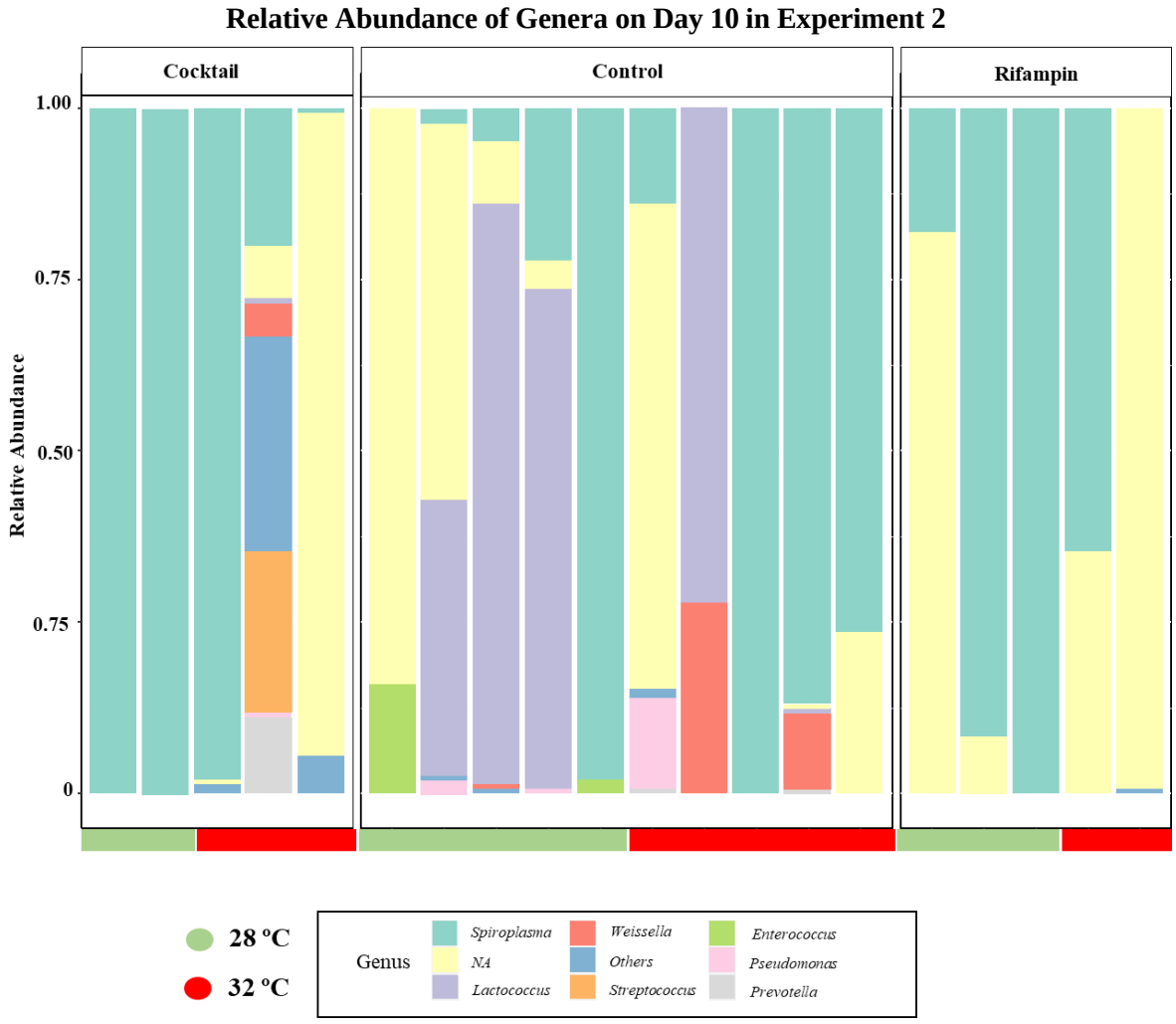

Figure 2.3: Relative abundance of genera separated by substrate and temperature treatment for Day 10. Each column represents the taxa in each sample, and each group of columns represents a substrate treatment. Samples from the 28°C temperature treatment are seen first and in green, while the 32°C temperature treatment comes second.

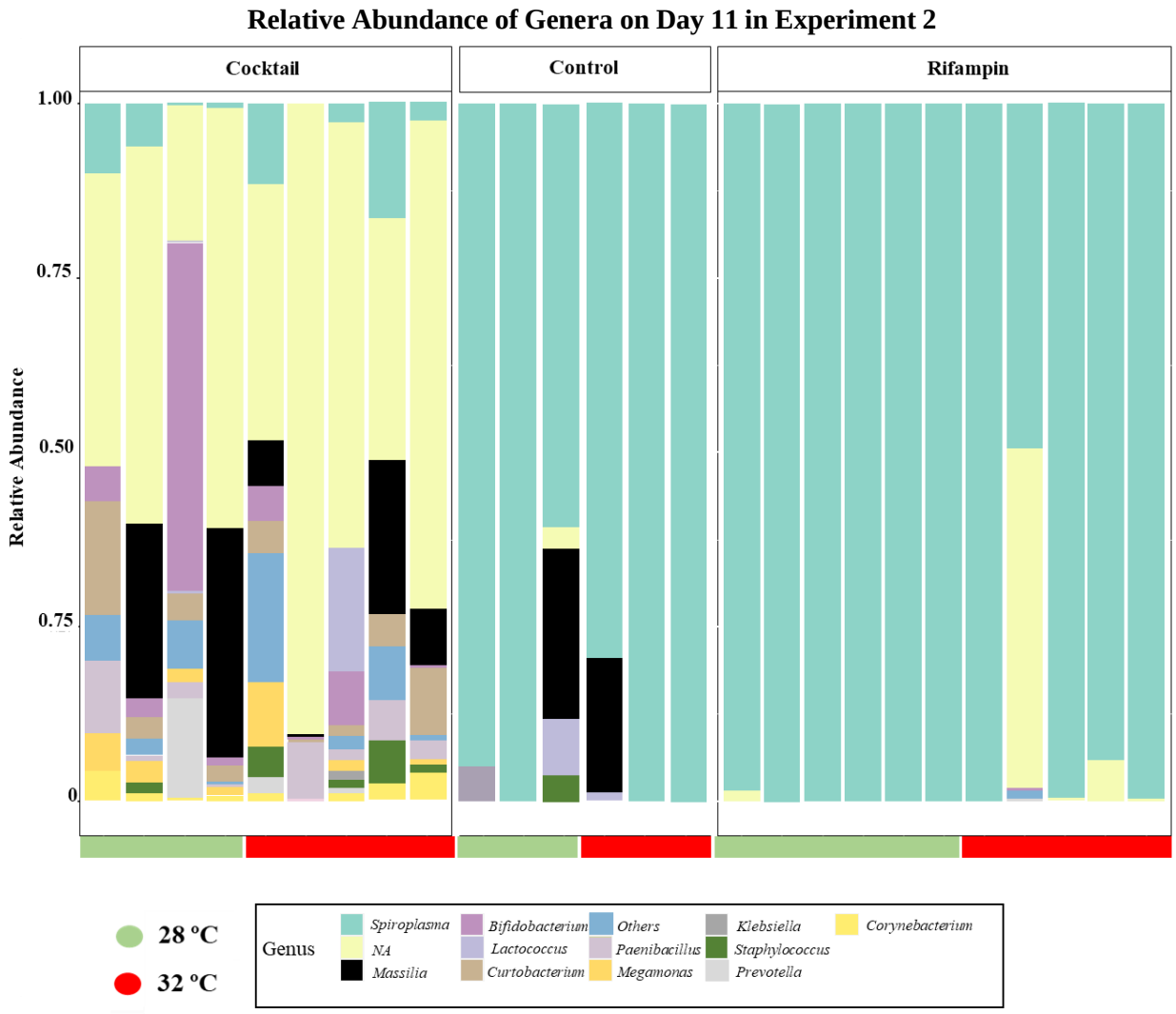

Figure 2.4: Relative abundance of genera separated by substrate and temperature treatment for Day 11. Each column represents the taxa in each sample, and each group of columns represents a substrate treatment. Samples from the 28°C temperature treatment are seen first and in green, while the 32°C temperature treatment comes second.

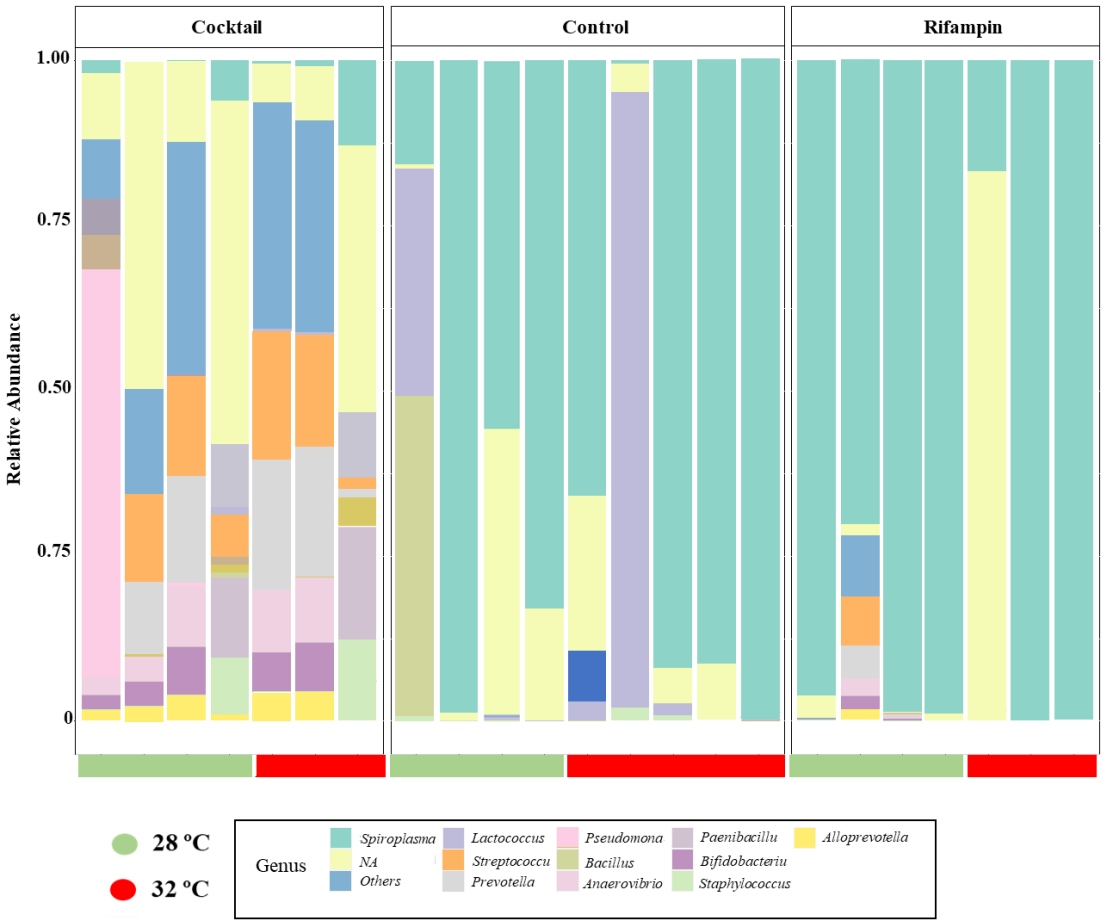

Figure 2.5: Relative abundance of genera separated by substrate and temperature treatment for Day 12. Each column represents the taxa in each sample, and each group of columns represents a substrate treatment. Samples from the 28°C temperature treatment are seen first and in green, while the 32°C temperature treatment comes second.

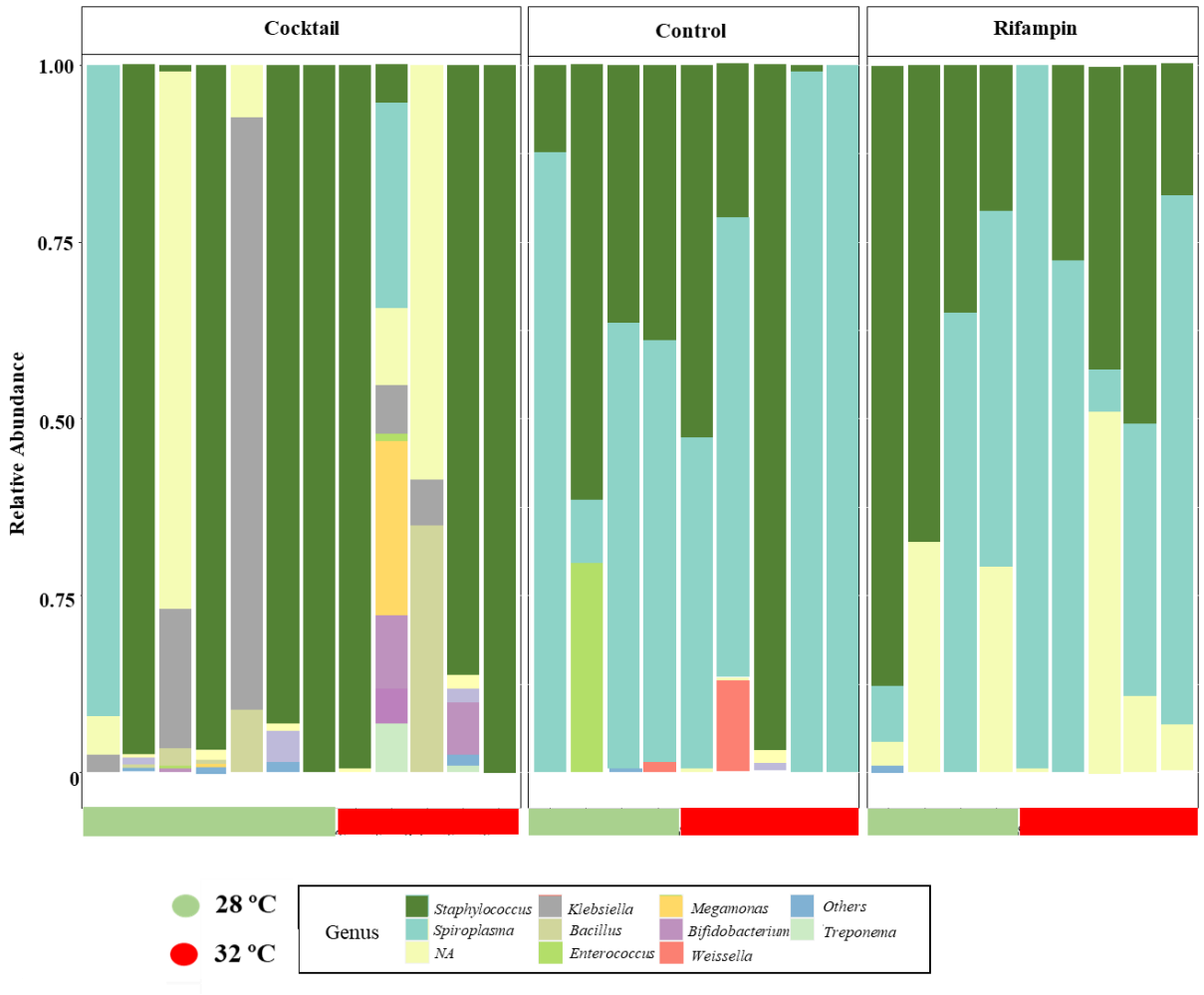

Figure 2.6: Relative abundance of genera separated by substrate and temperature treatment for Day 20. Each column represents the taxa in each sample, and each group of columns represents a substrate treatment. Samples from the 28°C temperature treatment are seen first and in green, while the 32°C temperature treatment comes second.

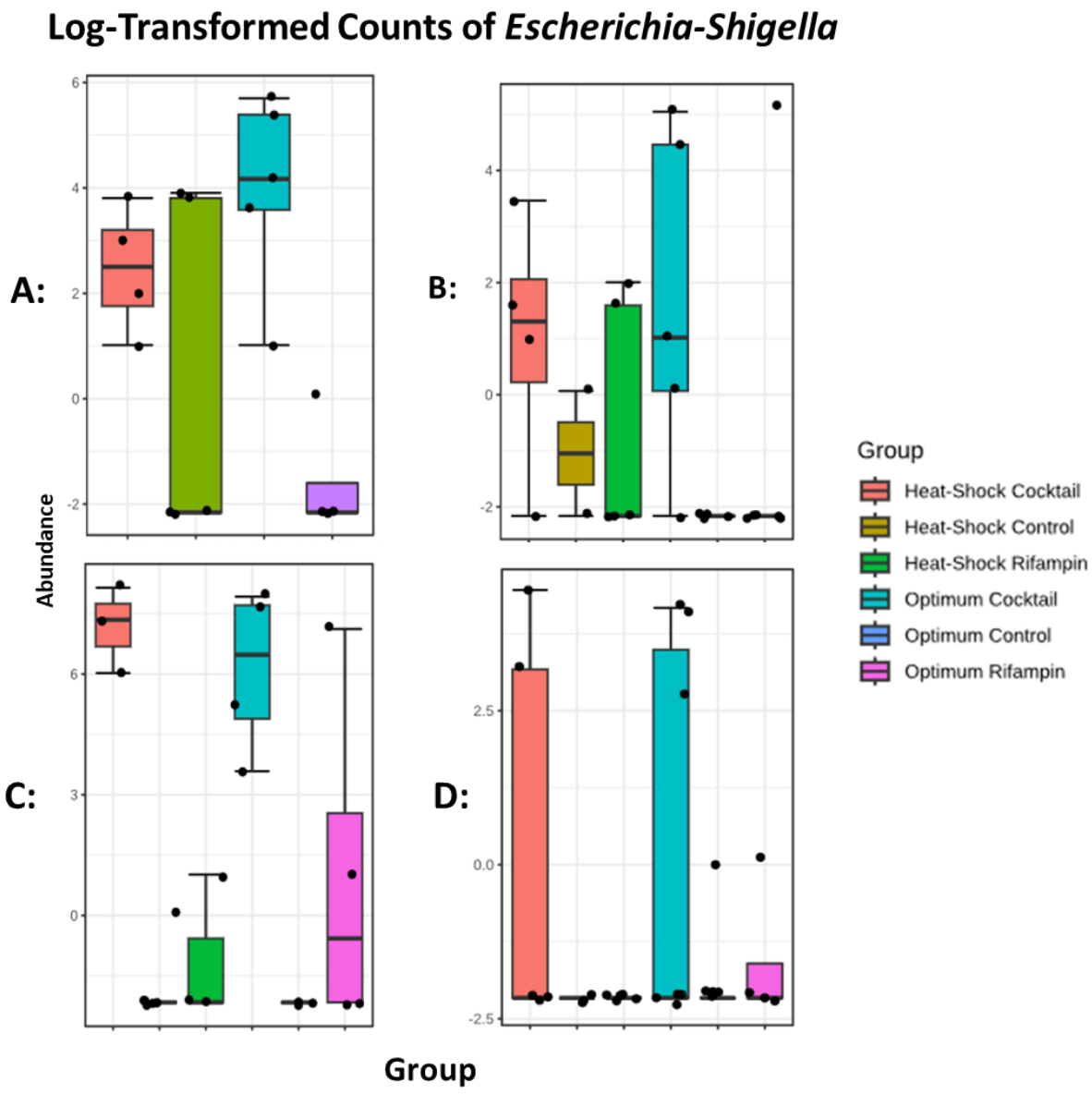

Figure 2.7: Displayed are log transformed counts of *Escherichia-Shigella* on the day of and following the heat shock experiment in 6 groups across 4 days. The days are arranged as Day 10 (A), Day 11 (B), Day 12 (C), and Day 20 (D). Error bars show standard deviation.

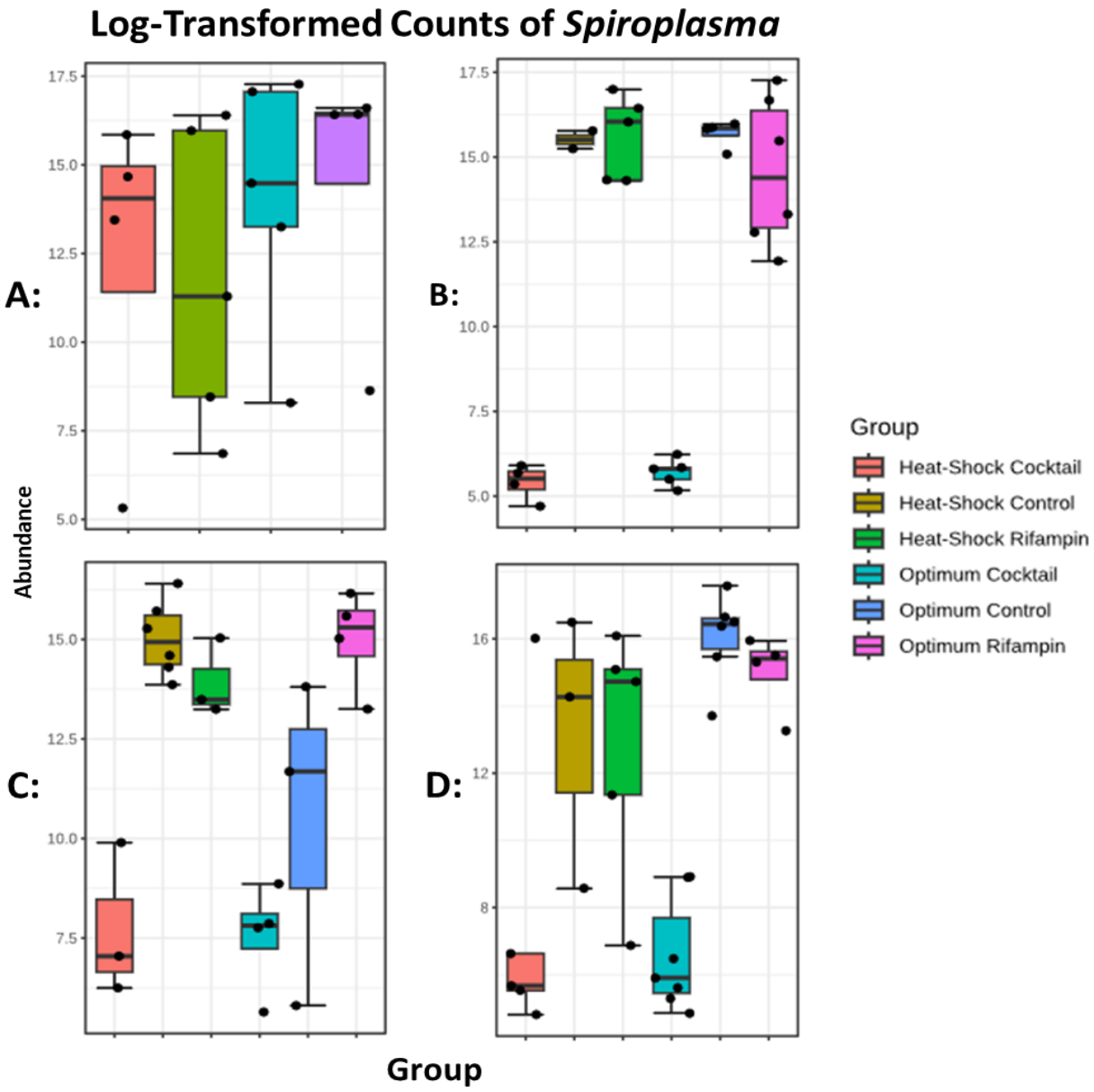

Figure 2.8: Displayed are log transformed counts of *Spiroplasma* on the day of and following the heat shock experiment in 6 groups across 4 days. The days are arranged as Day 10 (A), Day 11 (B), Day 12 (C), and Day 20 (D). Error bars show standard deviation.

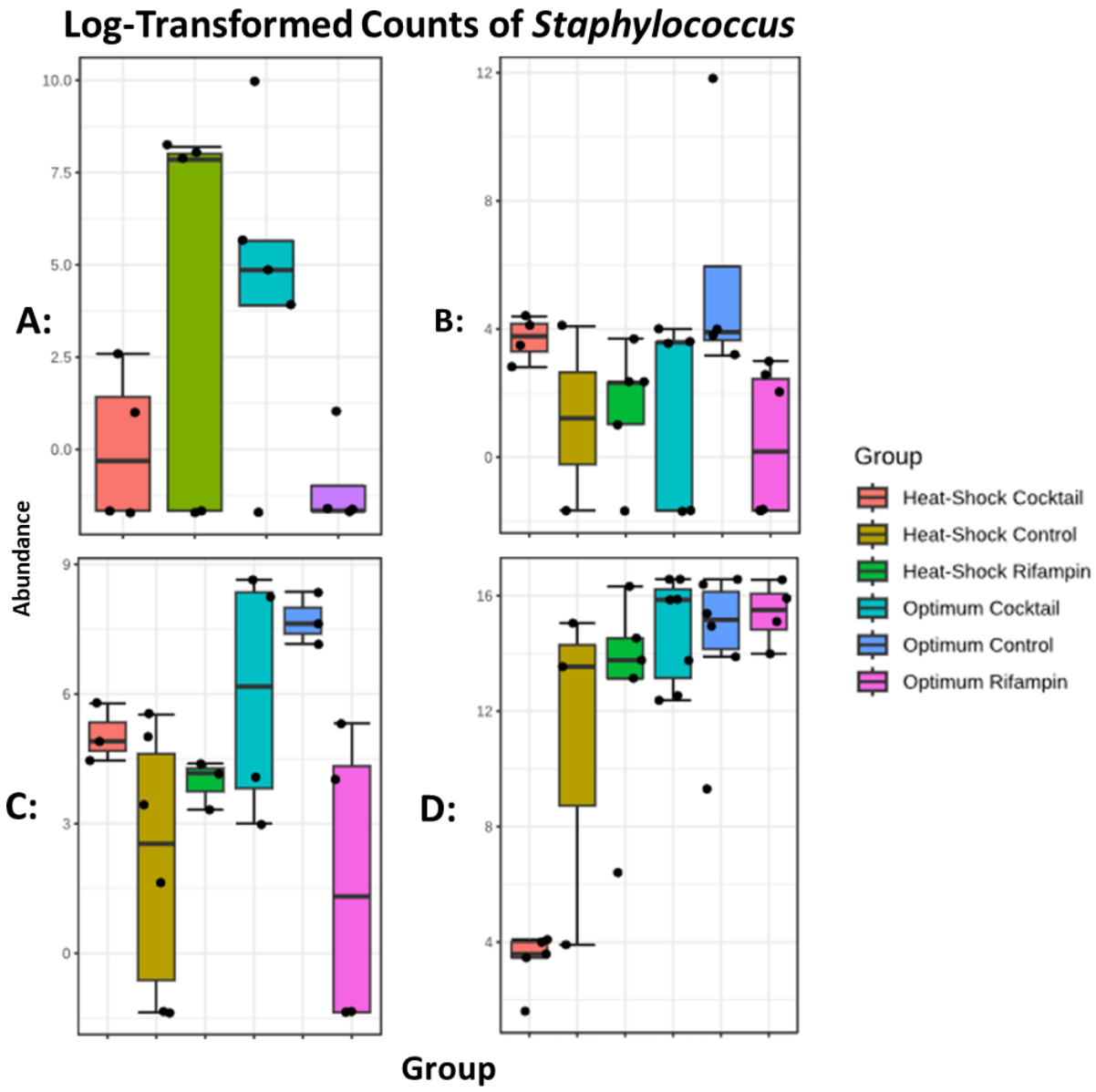

Figure 2.9: Displayed are log transformed counts of *Staphylococcus* on the day of and following the heat shock experiment in 6 groups across 4 days. The days are arranged as Day 10 (A), Day 11 (B), Day 12 (C), and Day 20 (D). Error bars show standard deviation.

DISCUSSION

Based on the results from the first experiment, it appears that temperature or Rifampin alone were not enough to significantly impact the lifecycle of *T. molitor*, however when combined these effects impacted overall mortality. There is evidence to suggest that there are temperature-associated limits to body size in arthropods (Makarieva *et al.,* 2005). It is because of data like this that the idea surrounding growth in the first experiment was that the cold-exposed *T. molitor* larvae would reflect significantly reduced growth compared to the control or hot-exposed larvae. Additionally, the first experiment served to elucidate the impact of prolonged temperature exposure on *T. molitor* larvae with and without Rifampin-disrupted microbiomes. As temperature increases, I expected to see changes in lifespan, tolerance to temperature stress, and significantly different microbiomes between *T. molitor* fed substrate with or without Rifampin. For the first experiment I predicted a significant difference between the temperatures, but I did not expect to see much of a difference between the treatments at the same temperature. The results from the general linear model in **Table 1.1** suggests that neither the temperature or Rifampin treatment or both had a significant effect on growth, reflected again in **Figure 1.1** where it appears the control group grew better at 18°C and 26°C, but the Rifampin group grew slightly more than the control at 32°C. Based on **Table 1.2**, a similar pattern can be seen for pupation where neither temperature, treatment, or both can be used to predict pupation. The same pattern for growth is seen for pupation as reflected in **Figure 1.2** where more organisms overall pupated at 18°C and 26°C from the control treatment than the Rifampin treatment, but not significantly more. The opposite trend still holds true at 34°C with more organisms in the Rifampin treatment pupating than the control. This is expected as 26°C is well within the ideal temperature for *T. molitor*, however finding the fewest number of

pupations at 34°C was surprising because typically organisms are more metabolically active in warmer temperatures. It was expected to see fewer pupations at 18°C where the organisms react to the cold by reducing their overall metabolic and reproductive activity (Jehan *et al.,* 2022). The eclosion model for the first experiment is displayed in **Table 1.3** keeps up the trend that treatment and temperature cannot be used to predict the number of eclosions, visualized in **Figure 1.3** where overall more organisms eclosed at 26°C than other temperatures. This is expected, as 26°C is well within their ideal temperature range. There was not a significant difference at the control temperature of 26°C between the treatments, implying that the disrupted microbiome did not significantly impact the ability of *T. molitor* to eclose. The final metric measured for *T. molitor* lifecycle in the first experiment is mortality and if treatment, temperature, or both could be used to predict it. The general linear model in **Table 1.4** reflects the impact of the factors on mortality and shows that while individually treatment or temperature cannot be used to predict mortality, the combined effect of both factors can. The average deaths recorded in the first experiment are reflected in **Figure 1.4**, where it appears the fewest overall deaths unsurprisingly occur in the control group at the control temperature. It is possible that the disrupted microbiome altered the response of *T. molitor* to temperature stress. Based on the results obtained in the first experiment, it appears that the factors temperature and treatment did not significantly impact the overall health of the larvae except for the combined factors in the case of mortality. Given the results of the first experiment, it appears that overall the organisms do the best at their ideal temperature in the control conditions, however not significantly better. It was for this reason that the second experiment was done to determine how the microbiome was changing in response to antibiotic and temperature stress, and to see how it recovers over time from a stress event. The second

experiment focused on using the ideal temperature of 26°C and 34°C as a temperature point for a heat shock.

After determining that Rifampin treatments in the first experiment did not appear to significantly impact the health of *T. molitor,* a second experiment was designed with an antibiotic cocktail composed of gentamycin and ampicillin to target a wider variety of bacteria in the gut. The idea here was to elucidate how a reduction in gut microbial diversity changes in response to a heat shock event. The data in **Table 2.1** illustrates the results of a 1-way PERMANOVA on the genera across the different days, while the data in **Table 2.2** reflects the same test using temperature as a factor. The cocktail was significantly different from the other two treatments concerning both of the factors day and temperature. The total sum of squares in **Table 2.1** are highest on Days 20 and 12, where the greatest variation attributed to the standard error is found. In other words, the greatest variation occurred 2 days after the heat shock and remained until the conclusion of the experiment on Day 20. The variation between samples was also highest on Day 11, immediately after the heat shock occurred. Alpha diversity with the Chao1 index is displayed in **Figure 2.1** with a Kruskal-Wallis test reflected in **Table 2.3**. The beta diversity of genera across the days is reflected in **Figure 2.2**. Beta diversity refers to the degree of a change in community composition in relation to their environment between separate communities, while alpha diversity refers to the community diversity within samples or groups in this case (Gail *et al.,* 2020). This difference is perhaps due to a difference in community recovery. As the gut microbiome replenished itself, the most competitive taxa were different than they were prior to and immediately after the heat shock. Comparing the genera across the control temperature and the heat shock groups in **Table 2.2** the cocktail was significantly different in composition than both the control and Rifampin

treatments like in **Table 2.1**. Based on the Kruskal-Wallis test reflected in **Table 2.3**, and the alpha diversity plots in **Figure 2.1** it is clear that there is an effect on diversity within samples due to temperature. The alpha diversity is statistically different on Day 11 and Day 12, but not on Day 10 or Day 20. It is unsurprising that the beta diversity between days changes slightly, with the greatest difference being on Day 20. The diversity between the group communities is changing in response to the heat shock and seems to return to a similar level of diversity by the 20^{th} day. The total sum of squares and the F statistic were the highest at 32°C, implying that the variation between samples was the greatest in the groups that were heat shocked. This makes sense because the community changes would not be identical with a heat shock event, while the communities in the control groups would remain relatively the same. The variation between samples was almost twice as high in the 32°C treatment than in the 26°C treatment implying a greater disturbance to the microbiome at elevated temperatures than at the ideal. The alpha diversity measuring the difference in bacterial community within a sample, or group, reflected in **Table 2.3** and **Figure 2.1** indicate that the diversity of genera within each group were only statistically significant based on a Kruskal-Wallis test on Day 11 and Day 12. Based on **Figure 2.1** (B) and (C) denoting Day 11 and Day 12 respectively, the diversity was greatest in Group 1 and Group 4. Group 1 and 4 both belong to the cocktail treatment, implying that the diversity was greatest due to the wide variety of organisms the antibiotic cocktail killed, and not because of temperature. Based on the beta diversity over time in **Table 2.4** and **Figure 2.2**, the antibiotic cocktail treatment was significantly different from the control and Rifampin treatments on Day 11 (B), Day 12 (C), and Day 20 (D) following the heat shock on Day 10 (A). Interestingly however, the control and Rifampin groups were not statistically significant from one another. This is unexpected because the Rifampin would

presumably alter the gut bacteriome enough to be different from the control, but this was not the case in this study. A possible explanation for the total sum of squares in **Table 2.4** being highest on Day 20 is that Day 20 is ten days following the heat shock treatment and the organisms have had time to acclimate to their new conditions, causing a degree of variation due to the error. For the second experiment, I predicted that the microbiome would be initially disrupted in the antibiotic treatment but would eventually return to normal by the end of the experiment and that the microbiomes between heat shock groups will remain significantly different.

A single factor DESeq2 analysis of three selected genera was performed and is reflected in **Table 2.5**, where bolded values denote significance. Three of the taxa that appeared to change the most (*Escherichia-Shigella*, *Spiroplasma*, and *Staphylococcus*) on and following the heat shock were picked. The first of the genera that was analyzed was *Escherichia-Shigella* due to the way its abundance changed over time but also because this would be a significant taxon to consider when rearing *T. molitor* as a food source. The abundance of *Escherichia-Shigella* significantly differed within groups on Day 10 according to **Table 2.5**. Based on Figure 2.7, the abundance of Escherichia-Shigella was highest in both cocktail treatments, implying that temperature was not really a big factor here. This is perhaps due to the ability of this taxa to out compete the others given the antibiotic cocktail widely disrupted the microbiome. The second genera to be analyzed was *Spiroplasma* which significantly differed between treatments on Day 11, Day 12, and Day 20. The log transformed counts of *Spiroplasma* across all days can be found in **Figure 2.8**, where the abundance appears to be lowest in the cocktail treatment across all days. Based on the relative abundance in **Figure 2.3** through **Figure 2.6**, it appears that the counts of *Spiroplasma* and

Staphylococcus are evenly distributed across both temperatures in each treatment. There is, however, merit in looking into other genera as well in further downstream analysis but that falls outside the scope of this work. Based on the information in **Table 2.5** and **Figure 2.9**, counts of *Staphylococcus* do not significantly differ between treatments on any given day except on Day 20. However, the relative abundance of *Staphylococcus* appears to be greater at 32°C on Day 11 directly following the heat shock, specifically in the optimum control group as seen in **Figure 2.9**. A possible reason for this is because the *Staphylococcus* may have been more active and worked to out compete other taxa directly following the heat shock, but not competitive enough to outgrow the other genera in the community. It can be clearly seen in **Figure 2.9** that the abundance of *Staphylococcus* gradually increases by Day 20 (D) except for the heat-shocked cocktail group. The results of the second experiment were expected in the respect that the antibiotic treatment significantly altered the bacterial communities in the larvae, however the microbiomes did not seem to recover by the end of the experiment quite like I expected. The diversity within samples denoted by alpha diversity in **Table 2.3** and **Figure 2.1** suggested that the highest within-group diversity occurred due to the effect antibiotics had rather than the temperature. The beta diversity in **Figure 2.2** from Day 10 (A) to Day 20 (D) appeared to return to a similar state but looking at the difference in actual taxa recorded in **Figure 2.3** through **Figure 2.6** the relative abundance of each genus had changed. This suggests that the abundance of these genera was altered as a direct result of the antibiotic treatment in the substrate rather than by temperature.

Disseminating the relationship between the gut microbiome and host in arthropods is a critical step in a comprehensive understanding of these organisms for the benefit of agriculture, industry, and the environment. There are many arthropods that can be raised for

food, or that can invade farmland. There are many invasive species throughout the kingdoms of life. Examples of these invasive species include aquatic invertebrates such as the zebra mussel, invasive plants like non-native flowering angiosperms, and other various invasive vertebrate species like released pets (Dzialowski, 2013; Nguyen *et al.*, 2021; Tedeschi *et al.*, 2021). Invasive and pest arthropod species pose a significant threat to agricultural industries across the globe, and understanding their relationship with temperature, environment, and microbiome is imperative to safely mitigating their impact on human activities. There is a concern that the increasing diversity of invasive arthropod species may pose a problem large enough to resist attempts at pest eradication (Suckling *et al.*, 2019). Understanding host-microbiome interactions and responses to temperature stress in *T. molitor* may help elucidate the role that microbes and climate play in the spread and diversification of invasive species.

The outcome of this research contributes to understanding the importance and interaction of temperature, microbiome, and insect lifecycle in *T. molitor*. Further establishing *T. molitor* as a model arthropod organism will normalize the mass production of mealworms and could help make mealworm products more available to individuals and corporations for use as a protein supply. Raising *T. molitor* is economical but has the potential to contribute significantly to the available protein. Increasing protein availability could support both individuals and farmers. Individuals could incorporate a cheap protein into their diet which creates a market for rearing *T. molitor*. Farmers and ranchers could incorporate *T. molitor* into their animal feed which could reduce the cost of reared animals by cheaply supplementing their food. Since they can breakdown waste in soil, they may also decrease the dependence of farms on fertilizers which pose a threat to the environment. For example, farmers in the poultry industry increase their profits by spending less on expensive poultry substrate.

Wheat and soya bean farms take up a lot of space, by reducing the use of these resources and supplementing some of them with sustainable mealworm farms, the land could be put to better use and could help reduce the impact of farming on the environment. The use of *T. molitor* also has the potential to reduce the production of greenhouse gasses in meat production by supplementing high carbon feed with less impactful protein sources. A reduction in the demand for high-cost protein sources in agriculture would ultimately reduce their environmental impact by simply producing less and using fewer resources. They could also reduce the need for fertilizer which causes nutrient pollution to the land and waterways. Lastly, this research has the capacity to indirectly benefit people. By identifying the impact of temperature and keystone gut microbiome species, inferences can be made about insect health. In addition to practicing more sustainable agriculture, the implications of *T. molitor* as a direct source of food for people are difficult to ignore.

CONCLUSION

The purpose of this project was to bridge the gap in understanding between temperature, gut microbiome, and food safety of *T. molitor*, and to further establish *T. molitor* as a model insect organism. Many people do not have access to adequate quantities or sustainable sources of food. Additionally, in recent years, there has been a massive worldwide decline in insects across the globe. There is a unique opportunity to study both problems at the same time with this research by using a common yellow mealworm, *T. molitor*. This organism could be used as a model organism to study the individual and compounding effects of temperature and microbiome on insect lifecycles. There is a massive untapped potential for using *T. molitor* as a regular source of protein around the world. In addition, studying the

impact of temperature and microbiome on mealworms could help shed some light on the massive decrease in agriculturally relevant insect biomass on Earth. By studying the effects of temperature and microbiome composition on *T. molitor*, more sustainable rearing methods may be identified. In addition, characterizing the microbiome of *T. molitor* will help to identify bacteria that are crucial for a healthy mealworm lifecycle. Future experiments could be done by providing the mealworms a food substrate inoculated with a probiotic bacterium isolated from guts of healthy larvae to promote healthier growth. Identifying keystone species in the gut microbiome in one insect could also offer insight into which bacteria are most helpful for other insect species. By understanding the impact of temperature and microbiome on insect lifecycles, sustainable methods of growing healthy insects can be developed.

APPENDIX SECTION

Appendix Table 1: Complete single-factor DESeq2 analysis of Day 10 in experiment 2. The order of amplicon sequence variants (ASVs) are organized based on order of greatest abundance to lowest abundance.

ASV #	P-Values	FDR	log2FC	lfcSE	Phylum	Class	Order	Family	Genus
ASV_10	6.62E-12	2.25E-10	-19.057	2.7757	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Weissella*
ASV_6	1.64E-11	2.79E-10	-19.253	2.8587	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Lactococcus*
ASV_19	4.76E-11	5.40E-10	19.488	2.9626	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
ASV_5	1.67E-09	1.42E-08	-17.413	2.889	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Lactococcus*
ASV_3	2.93E-08	1.87E-07	10.517	1.8966	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Erwiniaceae*	*NA*
ASV_8	3.30E-08	1.87E-07	-16.032	2.902	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*NA*
ASV_9	3.53E-06	1.71E-05	-14.307	3.0853	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*
ASV_23	6.34E-05	0.00026946	9.8603	2.4652	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*NA*	*NA*
ASV_65	0.013262	0.0501	-4.9955	2.017	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*Escherichia-Shigella*
ASV_27	0.026251	0.083894	-5.6416	2.5384	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_108	0.027142	0.083894	-4.9414	2.2365	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Kaistiaceae*	*Kaistia*
ASV_47	0.1116	0.31621	-4.4411	2.7913	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_2	0.1395	0.35579	-4.0124	2.7153	*Firmicutes*	*Bacilli*	*Staphylococcales*	*Staphylococcaceae*	*Staphylococcus*
ASV_37	0.1465	0.35579	-4.2816	2.9488	*Actinobacteriota*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*
ASV_24	0.25252	0.55742	-2.2169	1.9374	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_97	0.27172	0.55742	-2.2541	2.0509	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Hafniaceae*	*Hafnia-Obesumbacterium*
ASV_32	0.27871	0.55742	-2.1313	1.9675	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_12	0.31204	0.58941	-2.9016	2.8702	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
ASV_39	0.39089	0.66925	-1.8771	2.1878	*Actinobacteriota*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*
ASV_46	0.39367	0.66925	-2.5129	2.946	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Ruminococcaceae*	*Faecalibacterium*
ASV_117	0.44271	0.69054	-1.6561	2.1575	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*Citrobacter*
ASV_16	0.44682	0.69054	-1.8764	2.4666	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_1	0.57114	0.81594	0.98967	1.7474	*Firmicutes*	*Bacilli*	*Entomoplasmatales*	*Spiroplasmataceae*	*Spiroplasma*
ASV_113	0.57596	0.81594	-1.4307	2.558	*Fusobacteriota*	*Fusobacteriia*	*Fusobacteriales*	*Fusobacteriaceae*	*Cetobacterium*
ASV_83	0.62737	0.85323	-0.94885	1.9547	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*NA*
ASV_56	0.69073	0.90326	-1.3299	3.3426	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Ligilactobacillus*
ASV_36	0.82564	0.91493	0.40132	1.8218	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*NA*
ASV_90	0.84472	0.91493	-0.60859	3.1072	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*
ASV_54	0.86139	0.91493	0.35894	2.0557	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Megamonas*
ASV_119	0.875	0.91493	0.48346	3.0732	*Firmicutes*	*Bacilli*	*Staphylococcales*	*Staphylococcaceae*	*Staphylococcus*
ASV_26	0.88029	0.91493	-0.6086	4.0411	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
ASV_109	0.88178	0.91493	-0.60859	4.0924	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*
ASV_138	0.88802	0.91493	0.44218	3.1404	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Xanthobacteraceae*	*Xanthobacter*
ASV_161	0.96684	0.96684	0.11112	2.6728	*Planctomycetota*	*Planctomycetes*	*Planctomycetales*	*Rubinisphaeraceae*	*SH-PL14*

Appendix Table 2: Complete single-factor DESeq2 analysis of Day 11 in experiment 2. The order of amplicon sequence variants (ASVs) are organized based on order of greatest abundance to lowest abundance.

ASV #	P-Values	FDR	log2FC	lfcSE	Phylum	Class	Order	Family	Genus
ASV_1	1.77E-19	8.86E-18	12.219	1.3537	*Firmicutes*	*Bacilli*	*Entomoplasmatales*	*Spiroplasmataceae*	*Spiroplasma*
ASV_6	2.04E-06	5.10E-05	-16.308	3.4338	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Lactococcus*
ASV_48	0.0033853	0.056422	-7.9172	2.7018	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Massilia*
ASV_82	0.070808	0.88509	-5.7514	3.1834	*Firmicutes*	*Bacilli*	*Paenibacillales*	*Paenibacillaceae*	*Paenibacillus*
ASV_164	0.11516	0.92341	-4.2559	2.7014	*Actinobacteriota*	*Actinobacteria*	*Corynebacteriales*	*Corynebacteriaceae*	*Corynebacterium*
ASV_121	0.12443	0.92341	-4.2993	2.7982	*Actinobacteriota*	*Actinobacteria*	*Corynebacteriales*	*Corynebacteriaceae*	*Corynebacterium*
ASV_54	0.13635	0.92341	-3.828	2.5699	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Megamonas*
ASV_239	0.18537	0.92341	-4.9191	3.7142	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Massilia*
ASV_67	0.22559	0.92341	-2.5712	2.1218	*Actinobacteriota*	*Actinobacteria*	*Micrococcales*	*Microbacteriaceae*	*Curtobacterium*
ASV_11	0.23729	0.92341	-3.9891	3.3755	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*Klebsiella*
ASV_130	0.23746	0.92341	-3.6903	3.1238	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Massilia*
ASV_95	0.24352	0.92341	-4.1872	3.5904	*Actinobacteriota*	*Actinobacteria*	*Micrococcales*	*Microbacteriaceae*	*Curtobacterium*
ASV_8	0.2661	0.92341	-4.5318	4.0751	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*NA*
ASV_3	0.29529	0.92341	-1.9443	1.8577	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Erwiniaceae*	*NA*
ASV_37	0.31742	0.92341	-3.1806	3.1813	*Actinobacteriota*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*
ASV_100	0.39098	0.92341	4.189	4.8831	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Megamonas*
ASV_234	0.43823	0.92341	-3.5387	4.565	*Actinobacteriota*	*Actinobacteria*	*Micrococcales*	*Sanguibacteraceae*	*Sanguibacter-Flavimobilis*
ASV_66	0.45826	0.92341	3.4069	4.5933	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_2	0.46947	0.92341	-1.2626	1.7455	*Firmicutes*	*Bacilli*	*Staphylococcales*	*Staphylococcaceae*	*Staphylococcus*
ASV_189	0.47663	0.92341	-2.4115	3.3882	*Proteobacteria*	*Gammaproteobacteria*	*Gammaproteobacteria Incertae Sedis*	*Unknown Family*	*Acidibacter*
ASV_93	0.50163	0.92341	-3.2452	4.8297	*Actinobacteriota*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*
ASV_74	0.51207	0.92341	3.2108	4.8974	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_36	0.54683	0.92341	1.1678	1.9382	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*NA*
ASV_336	0.58173	0.92341	-2.6453	4.8021	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*NA*
ASV_51	0.58247	0.92341	-2.6147	4.7558	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Veillonellaceae*	*Dialister*
ASV_63	0.61836	0.92341	2.4443	4.9065	*Spirochaetota*	*Spirochaetia*	*Spirochaetales*	*Spirochaetaceae*	*Treponema*
ASV_263	0.61891	0.92341	-2.329	4.6823	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Beijerinckiaceae*	*Methylobacterium-Methylorubrum*
ASV_168	0.62854	0.92341	-1.7916	3.7032	*Actinobacteriota*	*Actinobacteria*	*Micrococcales*	*Micrococcaceae*	*Micrococcus*
ASV_188	0.65345	0.92341	-2.1569	4.8039	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_32	0.66757	0.92341	1.4644	3.4097	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_46	0.68507	0.92341	1.9918	4.9113	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Ruminococcaceae*	*Faecalibacterium*
ASV_87	0.70725	0.92341	-1.8173	4.839	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_210	0.72382	0.92341	-1.5444	4.3708	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Xanthobacteraceae*	*Bradyrhizobium*
ASV_28	0.73856	0.92341	1.64	4.9136	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Anaerovibrio*
ASV_315	0.74663	0.92341	-1.5719	4.8653	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Tepidimonas*
ASV_5	0.75603	0.92341	0.90324	2.9071	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Lactococcus*
ASV_118	0.77351	0.92341	1.4065	4.8872	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*NA*
ASV_9	0.78896	0.92341	-1.1735	4.3841	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*
ASV_71	0.79339	0.92341	-1.2773	4.877	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_24	0.8285	0.92341	0.9283	4.2853	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_65	0.83801	0.92341	-0.56936	2.785	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*Escherichia-Shigella*
ASV_57	0.84953	0.92341	0.93342	4.9202	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_69	0.84953	0.92341	0.93343	4.9202	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_109	0.84953	0.92341	0.93343	4.9202	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*
ASV_153	0.84953	0.92341	0.93345	4.9202	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Catenisphaera*
ASV_16	0.84954	0.92341	0.93341	4.9202	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_52	0.8881	0.92652	-0.69052	4.9077	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Ruminococcaceae*	*Faecalibacterium*
ASV_62	0.91969	0.92652	0.22769	2.2582	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*NA*
ASV_39	0.92528	0.92652	-0.24133	2.5731	*Actinobacteriota*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*
ASV_61	0.92652	0.92652	0.4065	4.4079	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Dorea*

Appendix Table 3: Complete single-factor DESeq2 analysis of Day 12 in experiment 2. The order of amplicon sequence variants (ASVs) are organized based on order of greatest abundance to lowest abundance.

ASV #	P-Values	FDR	log2FC	lfcSE	Phylum	Class	Order	Family	Genus
ASV_1	9.25E-09	2.04E-06	10.197	1.7753	*Firmicutes*	*Bacilli*	*Entomoplasmatales*	*Spiroplasmataceae*	*Spiroplasma*
ASV_8	1.77E-05	0.0019518	-18.079	4.2128	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*NA*
ASV_12	0.00048158	0.035316	-13.001	3.7243	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
ASV_29	0.011337	0.62355	-13.949	5.5089	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*
ASV_14	0.097341	0.99512	-9.1332	5.5089	*NA*	*NA*	*NA*	*NA*	*NA*
ASV_109	0.10981	0.99512	-6.5841	4.1175	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*
ASV_3	0.12792	0.99512	4.0787	2.6792	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Erwiniaceae*	*NA*
ASV_85	0.18432	0.99512	-7.1265	5.3681	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*
ASV_114	0.22141	0.99512	-5.687	4.6508	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Massilia*
ASV_108	0.22941	0.99512	-4.664	3.8806	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Kaistiaceae*	*Kaistia*
ASV_95	0.2738	0.99512	-4.6267	4.2278	*Actinobacteriota*	*Actinobacteria*	*Micrococcales*	*Microbacteriaceae*	*Curtobacterium*
ASV_144	0.28211	0.99512	-4.6527	4.3257	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Agathobacter*
ASV_143	0.29363	0.99512	-4.1813	3.9814	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*
ASV_97	0.34377	0.99512	-3.4748	3.6702	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Hafniaceae*	*Hafnia-Obesumbacterium*
ASV_83	0.34798	0.99512	-3.6095	3.846	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*NA*
ASV_220	0.36263	0.99512	-4.3062	4.7302	*Actinobacteriota*	*Actinobacteria*	*Micrococcales*	*Microbacteriaceae*	*Frondihabitans*
ASV_158	0.3908	0.99512	-4.3633	5.0845	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium sensu stricto 1*
ASV_62	0.43872	0.99512	-2.2233	2.8712	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*NA*
ASV_325	0.44761	0.99512	4.1606	5.4788	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Butyricicoccaceae*	*UCG-009*
ASV_250	0.44891	0.99512	-4.0665	5.3702	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*NA*
ASV_216	0.4502	0.99512	-3.9255	5.1987	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Succinivibrionaceae*	*Succinivibrio*
ASV_61	0.4554	0.99512	-2.5906	3.4706	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Dorea*
ASV_180	0.47779	0.99512	-2.6169	3.6865	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_5	0.47861	0.99512	-1.6954	2.3928	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Lactococcus*
ASV_99	0.4812	0.99512	-2.3566	3.3457	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_175	0.48135	0.99512	-2.8433	4.038	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Solobacterium*
ASV_79	0.48687	0.99512	-2.5285	3.6366	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_280	0.48959	0.99512	-3.711	5.3709	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	*Stenotrophomonas*
ASV_251	0.4926	0.99512	-3.6854	5.3709	*Actinobacteriota*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*
ASV_219	0.49274	0.99512	-2.8647	4.1762	*Firmicutes*	*Clostridia*	*Peptostreptococcales-Tissierellales*	*Anaerovoracaceae*	*Mogibacterium*
ASV_151	0.49361	0.99512	-2.7245	3.9798	*Firmicutes*	*Negativicutes*	*Acidaminococcales*	*Acidaminococcaceae*	*Phascolarctobacterium*
ASV_226	0.49838	0.99512	-3.6364	5.371	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_67	0.51365	0.99512	-2.4309	3.7217	*Actinobacteriota*	*Actinobacteria*	*Micrococcales*	*Microbacteriaceae*	*Curtobacterium*
ASV_137	0.51581	0.99512	-2.3966	3.6882	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_90	0.51795	0.99512	-2.0993	3.247	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*
ASV_72	0.51991	0.99512	-1.9685	3.0592	*Firmicutes*	*Bacilli*	*Paenibacillales*	*Paenibacillaceae*	*Paenibacillus*
ASV_2	0.52071	0.99512	1.5057	2.3444	*Firmicutes*	*Bacilli*	*Staphylococcales*	*Staphylococcaceae*	*Staphylococcus*
ASV_51	0.52167	0.99512	-2.2996	3.5889	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Veillonellaceae*	*Dialister*
ASV_192	0.5232	0.99512	-2.618	4.1008	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Lachnoclostridium*
ASV_215	0.52344	0.99512	-2.7151	4.2553	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*[Eubacterium] hallii group*
ASV_211	0.53276	0.99512	-2.56	4.1038	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_184	0.53526	0.99512	-2.8298	4.5642	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotellaceae NK3B31 group*
ASV_145	0.53665	0.99512	-3.3192	5.3718	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Beijerinckiaceae*	*Bosea*
ASV_86	0.5388	0.99512	-2.3839	3.8785	*Actinobacteriota*	*Coriobacteriia*	*Coriobacteriales*	*Atopobiaceae*	*NA*
ASV_50	0.54534	0.99512	-2.1407	3.5397	*Spirochaetota*	*Spirochaetia*	*Spirochaetales*	*Spirochaetaceae*	*Treponema*
ASV_185	0.54657	0.99512	-2.4388	4.0451	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Holdemanella*
ASV_76	0.55471	0.99512	-2	3.3856	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Megamonas*
ASV_224	0.55801	0.99512	3.1612	5.3963	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*

ASV #	P-Values	FDR	log2FC	lfcSE	Phylum	Class	Order	Family	Genus
ASV_113	0.55902	0.99512	-2.5507	4.3655	*Fusobacteriota*	*Fusobacteriia*	*Fusobacteriales*	*Fusobacteriaceae*	*Cetobacterium*
ASV_127	0.56011	0.99512	-2.1366	3.6669	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Sutterellaceae*	*Sutterella*
ASV_139	0.56144	0.99512	-2.2954	3.9529	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Sarcina*
ASV_100	0.56346	0.99512	-2.1152	3.6614	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Megamonas*
ASV_57	0.56527	0.99512	-2.0065	3.4894	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_70	0.57198	0.99512	-1.9702	3.4863	*Actinobacteriota*	*Coriobacteriia*	*Coriobacteriales*	*Atopobiaceae*	*Libanicoccus*
ASV_273	0.57517	0.99512	-3.0112	5.3728	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Ligilactobacillus*
ASV_102	0.57535	0.99512	-1.8418	3.2879	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Coprococcus*
ASV_161	0.57671	0.99512	-2.9991	5.3728	*Planctomycetota*	*Planctomycetes*	*Planctomycetales*	*Rubinisphaeraceae*	*SH-PL14*
ASV_176	0.57698	0.99512	-2.9915	5.3629	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Tyzzerella*
ASV_252	0.57776	0.99512	-2.9026	5.2144	*Actinobacteriota*	*Actinobacteria*	*Micrococcales*	*Micrococcaceae*	*Rothia*
ASV_183	0.58	0.99512	-2.3105	4.1751	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotellaceae UCG-001*
ASV_63	0.5834	0.99512	-1.9885	3.6259	*Spirochaetota*	*Spirochaetia*	*Spirochaetales*	*Spirochaetaceae*	*Treponema*
ASV_37	0.58935	0.99512	-1.7206	3.1877	*Actinobacteriota*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*
ASV_69	0.59091	0.99512	-1.9822	3.6876	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_73	0.5939	0.99512	-1.8492	3.4682	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_96	0.59491	0.99512	-1.9519	3.6708	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Anaerovibrio*
ASV_88	0.60119	0.99512	-1.8372	3.515	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Lachnoclostridium*
ASV_174	0.60834	0.99512	-1.9105	3.7282	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_24	0.61011	0.99512	-1.5942	3.1264	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_166	0.61129	0.99512	-2.1932	4.3154	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Aeromonadaceae*	*Aeromonas*
ASV_98	0.62109	0.99512	-1.9247	3.8938	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_140	0.62189	0.99512	-1.9586	3.9714	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Oribacterium*
ASV_118	0.62249	0.99512	-1.9898	4.0415	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*NA*
ASV_32	0.623	0.99512	-1.6746	3.4064	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_106	0.62434	0.99512	-1.912	3.9044	*Actinobacteriota*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*
ASV_81	0.6296	0.99512	-1.7531	3.6349	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelatoclostridiaceae*	*Catenibacterium*
ASV_75	0.63209	0.99512	-1.8635	3.8921	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*NA*
ASV_124	0.63331	0.99512	-1.9109	4.0054	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*[Ruminococcus] gnavus group*
ASV_197	0.63473	0.99512	-1.9453	4.0948	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Rikenellaceae*	*Rikenellaceae RC9 gut group*
ASV_149	0.63476	0.99512	-2.112	4.4461	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_39	0.63498	0.99512	-1.6685	3.5147	*Actinobacteriota*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*
ASV_48	0.63568	0.99512	-1.7091	3.6075	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Massilia*
ASV_129	0.63689	0.99512	-1.5585	3.3016	*Firmicutes*	*Clostridia*	*Peptostreptococcales-Tissierellales*	*Peptostreptococcaceae*	*Peptoclostridium*
ASV_138	0.63876	0.99512	-2.3202	4.9425	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Xanthobacteraceae*	*Xanthobacter*
ASV_28	0.63905	0.99512	-1.6801	3.582	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Anaerovibrio*
ASV_152	0.64194	0.99512	-1.8736	4.0293	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Sutterellaceae*	*Sutterella*
ASV_36	0.64382	0.99512	-1.0943	2.3667	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*NA*
ASV_341	0.64661	0.99512	-2.4595	5.3646	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Vagococcaceae*	*Vagococcus*
ASV_182	0.64917	0.99512	-1.7066	3.7514	*Firmicutes*	*Negativicutes*	*Acidaminococcales*	*Acidaminococcaceae*	*Phascolarctobacterium*
ASV_92	0.65054	0.99512	-1.6485	3.639	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_293	0.65258	0.99512	-2.4199	5.3753	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Lachnoclostridium*
ASV_195	0.65292	0.99512	-1.9302	4.2921	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Faecalitalea*
ASV_107	0.65853	0.99512	-1.6427	3.7169	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_190	0.65854	0.99512	-1.9837	4.4889	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_310	0.66191	0.99512	-2.2919	5.2412	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Oribacterium*
ASV_244	0.66429	0.99512	-1.6691	3.8459	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*
ASV_77	0.66467	0.99512	-1.5879	3.6631	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*NA*
ASV_9	0.6657	0.99512	-1.758	4.069	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*
ASV_237	0.6661	0.99512	-1.9791	4.5865	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*[Ruminococcus] gnavus group*
ASV_132	0.66993	0.99512	-1.6812	3.9443	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Pasteurellaceae*	*NA*
ASV_142	0.67192	0.99512	-1.7155	4.0507	*Campylobacterota*	*Campylobacteria*	*Campylobacterales*	*Helicobacteraceae*	*Helicobacter*
ASV_171	0.67454	0.99512	-1.7378	4.1384	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*

ASV #	P-Values	FDR	log2FC	lfcSE	Phylum	Class	Order	Family	Genus
ASV_204	0.67764	0.99512	-1.6036	3.8576	*Actinobacteriota*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*
ASV_65	0.68093	0.99512	-1.0605	2.5791	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*Escherichia-Shigella*
ASV_66	0.68335	0.99512	-1.3545	3.3208	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_173	0.68372	0.99512	-1.5296	3.7547	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium sensu stricto 1*
ASV_47	0.68384	0.99512	-1.464	3.5952	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_71	0.69112	0.99512	-1.5486	3.8974	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_52	0.69269	0.99512	-1.5215	3.8499	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Ruminococcaceae*	*Faecalibacterium*
ASV_94	0.69348	0.99512	-1.4486	3.6754	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_311	0.69445	0.99512	-2.1122	5.3771	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Pasteurellaceae*	*NA*
ASV_257	0.69587	0.99512	-1.9643	5.025	*Actinobacteriota*	*Actinobacteria*	*Corynebacteriales*	*Corynebacteriaceae*	*Corynebacterium*
ASV_101	0.69652	0.99512	-1.2104	3.1033	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Fusicatenibacter*
ASV_343	0.70098	0.99512	2.0631	5.3726	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Roseburia*
ASV_221	0.70298	0.99512	-1.6316	4.2791	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*
ASV_49	0.7047	0.99512	-1.3645	3.6005	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Anaerovibrio*
ASV_120	0.71065	0.99512	-1.3794	3.7184	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_84	0.71874	0.99512	-1.4045	3.8997	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_235	0.72513	0.99512	1.5679	4.459	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*NA*
ASV_153	0.72579	0.99512	-1.5842	4.5168	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Catenisphaera*
ASV_103	0.72941	0.99512	-1.353	3.9116	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Butyricicoccaceae*	*NA*
ASV_179	0.73016	0.99512	-1.5772	4.5728	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Succinivibrionaceae*	*Anaerobiospirillum*
ASV_170	0.7317	0.99512	-1.4166	4.1316	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotellaceae NK3B31 group*
ASV_362	0.73416	0.99512	-1.8268	5.3792	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*[Ruminococcus] torques group*
ASV_134	0.73564	0.99512	-1.3622	4.0347	*Firmicutes*	*Negativicutes*	*Acidaminococcales*	*Acidaminococcaceae*	*Phascolarctobacterium*
ASV_56	0.7367	0.99512	-1.2047	3.5831	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Ligilactobacillus*
ASV_351	0.73795	0.99512	-1.7959	5.3678	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Ruminococcaceae*	*Ruminococcus*
ASV_74	0.7384	0.99512	-1.1496	3.4423	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_117	0.73853	0.99512	-1.3518	4.0497	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*Citrobacter*
ASV_93	0.74748	0.99512	-1.1333	3.5199	*Actinobacteriota*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*
ASV_245	0.74777	0.99512	-1.6653	5.1785	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*
ASV_125	0.75176	0.99512	-1.2443	3.9336	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_68	0.75364	0.99512	-1.0933	3.4835	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Succinivibrionaceae*	*Anaerobiospirillum*
ASV_205	0.75465	0.99512	-1.3343	4.2694	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_122	0.76607	0.99512	-1.1743	3.947	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_104	0.76618	0.99512	-1.1636	3.9131	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_27	0.76648	0.99512	-0.97609	3.2867	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_150	0.76729	0.99512	-1.1838	4.0002	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Holdemanella*
ASV_46	0.76916	0.99512	-0.91361	3.1132	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Ruminococcaceae*	*Faecalibacterium*
ASV_172	0.76977	0.99512	-1.1936	4.0782	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*NA*
ASV_165	0.77133	0.99512	-1.3248	4.5583	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_292	0.77534	0.99512	-1.5093	5.2887	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*[Ruminococcus] gnavus group*
ASV_87	0.77592	0.99512	-1.1087	3.8952	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_116	0.77611	0.99512	-1.1469	4.0327	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_55	0.77689	0.99512	-1.0628	3.7503	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_141	0.77859	0.99512	-0.89458	3.1818	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_196	0.77973	0.99512	-1.3142	4.699	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Holdemanella*
ASV_54	0.78612	0.99512	-0.87044	3.2078	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Megamonas*
ASV_236	0.79023	0.99512	-1.0282	3.8653	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*NA*
ASV_324	0.79435	0.99512	-1.3936	5.3463	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*NA*
ASV_169	0.79717	0.99512	-1.0367	4.0338	*Firmicutes*	*Bacilli*	*RF39*	*NA*	*NA*
ASV_16	0.80473	0.99512	-0.81568	3.2994	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_119	0.805	0.99512	-0.77582	3.1425	*Firmicutes*	*Bacilli*	*Staphylococcales*	*Staphylococcaceae*	*Staphylococcus*
ASV_147	0.80752	0.99512	-0.87444	3.5893	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_168	0.80806	0.99512	-0.9206	3.7896	*Actinobacteriota*	*Actinobacteria*	*Micrococcales*	*Micrococcaceae*	*Micrococcus*

ASV #	P-Values	FDR	log2FC	lfcSE	Phylum	Class	Order	Family	Genus
ASV_112	0.8081	0.99512	-0.95665	3.9389	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_217	0.81057	0.99512	-1.0268	4.2839	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*NA*
ASV_160	0.81428	0.99512	-0.94425	4.0196	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*[Ruminococcus] torques group*
ASV_189	0.81597	0.99512	-1.1689	5.0223	*Proteobacteria*	*Gammaproteobacteria*	*Gammaproteobacteria Incertae Sedis*	*Unknown Family*	*Acidibacter*
ASV_222	0.81716	0.99512	-1.0049	4.3465	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Sutterellaceae*	*Sutterella*
ASV_241	0.81972	0.99512	-1.0227	4.4872	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotellaceae UCG-003*
ASV_164	0.8239	0.99512	-0.85522	3.8432	*Actinobacteriota*	*Actinobacteria*	*Corynebacteriales*	*Corynebacteriaceae*	*Corynebacterium*
ASV_6	0.82508	0.99512	1.1368	5.1436	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Lactococcus*
ASV_207	0.82522	0.99512	-1.1598	5.2521	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Megamonas*
ASV_229	0.82529	0.99512	1.0686	4.841	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*
ASV_157	0.82765	0.99512	-0.9719	4.464	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_146	0.82792	0.99512	-0.81664	3.7568	*Fusobacteriota*	*Fusobacteriia*	*Fusobacteriales*	*Fusobacteriaceae*	*Fusobacterium*
ASV_115	0.83483	0.99512	-0.8252	3.9575	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Ruminococcaceae*	*Subdoligranulum*
ASV_23	0.83945	0.99512	-0.79617	3.9298	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*NA*	*NA*
ASV_155	0.84375	0.99512	-0.97863	4.9653	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Veillonellaceae*	*Megasphaera*
ASV_191	0.84499	0.99512	-0.81086	4.1473	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_246	0.84647	0.99512	-0.96957	5.0075	*Actinobacteriota*	*Coriobacteriia*	*Coriobacteriales*	*Atopobiaceae*	*Olsenella*
ASV_272	0.84865	0.99512	1.0218	5.3546	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_123	0.85208	0.99512	-0.9963	5.343	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
ASV_11	0.85327	0.99512	-0.7461	4.0342	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*Klebsiella*
ASV_198	0.85328	0.99512	-0.71555	3.8693	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*NA*
ASV_301	0.8562	0.99512	-0.9764	5.3883	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*NA*
ASV_262	0.86737	0.99512	-0.89405	5.3534	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Burkholderiaceae*	*Lautropia*
ASV_178	0.86825	0.99512	-0.77072	4.6463	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Dorea*
ASV_285	0.87209	0.99512	-0.82426	5.1194	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*NA*
ASV_329	0.87404	0.99512	-0.84452	5.3272	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_80	0.87427	0.99512	0.85002	5.3719	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
ASV_228	0.87981	0.99512	-0.80301	5.3107	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Anaerovibrio*
ASV_136	0.88017	0.99512	-0.59376	3.9386	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Paraprevotella*
ASV_121	0.88035	0.99512	0.60624	4.0277	*Actinobacteriota*	*Actinobacteria*	*Corynebacteriales*	*Corynebacteriaceae*	*Corynebacterium*
ASV_194	0.88619	0.99512	-0.67124	4.6899	*Campylobacterota*	*Campylobacteria*	*Campylobacterales*	*Helicobacteraceae*	*Helicobacter*
ASV_128	0.88676	0.99512	-0.59322	4.1658	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
ASV_181	0.88936	0.99512	-0.72974	5.2455	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Lachnoclostridium*
ASV_268	0.89121	0.99512	-0.62007	4.5337	*Bacteroidota*	*Bacteroidia*	*Chitinophagales*	*Chitinophagaceae*	*Vibrionimonas*
ASV_274	0.89359	0.99512	0.66765	4.9915	*Actinobacteriota*	*Coriobacteriia*	*Coriobacteriales*	*Atopobiaceae*	*Libanicoccus*
ASV_299	0.89473	0.99512	0.7066	5.3398	*Firmicutes*	*Bacilli*	*Acholeplasmatales*	*Acholeplasmataceae*	*Anaeroplasma*
ASV_177	0.89906	0.99512	0.46231	3.6446	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*[Ruminococcus] gauvreauii group*
ASV_247	0.89955	0.99512	-0.62915	4.9843	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium sensu stricto 1*
ASV_287	0.90533	0.99512	-0.63595	5.3474	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Oribacterium*
ASV_193	0.90924	0.99512	0.39504	3.4655	*Fusobacteriota*	*Fusobacteriia*	*Fusobacteriales*	*Fusobacteriaceae*	*Cetobacterium*
ASV_283	0.90944	0.99512	0.60604	5.3283	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*
ASV_332	0.9095	0.99512	0.60665	5.3372	*Firmicutes*	*Negativicutes*	*Acidaminococcales*	*Acidaminococcaceae*	*Phascolarctobacterium*
ASV_131	0.91206	0.99512	-0.43748	3.9613	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_199	0.91309	0.99512	-0.4567	4.1843	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_148	0.91416	0.99512	0.40146	3.7242	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*NA*
ASV_126	0.91487	0.99512	-0.42386	3.9651	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_313	0.91967	0.99512	0.53841	5.3386	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Howardella*
ASV_367	0.92042	0.99512	-0.53903	5.3955	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*NA*
ASV_286	0.92239	0.99512	-0.51992	5.3368	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Pasteurellaceae*	*Pasteurella*
ASV_167	0.9243	0.99512	0.3558	3.7447	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*
ASV_276	0.9301	0.99512	-0.44303	5.0508	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Butyricicoccaceae*	*UCG-009*
ASV_214	0.93962	0.99512	-0.26249	3.4651	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*
ASV_288	0.94668	0.99512	-0.35802	5.3534	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Ruminococcaceae*	*Faecalibacterium*

ASV #	P-Values	FDR	log2FC	lfcSE	Phylum	Class	Order	Family	Genus
ASV_316	0.94984	0.99512	-0.33969	5.3996	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*NA*
ASV_10	0.95039	0.99512	0.24974	4.0143	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Weissella*
ASV_154	0.96043	0.99512	0.19895	4.0099	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Leuconostoc*
ASV_162	0.96783	0.99512	-0.16743	4.1519	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Neisseriaceae*	*Neisseria*
ASV_255	0.97148	0.99512	-0.19124	5.3489	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Blautia*
ASV_200	0.97206	0.99512	0.12543	3.5816	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotellaceae Ga6A1 group*
ASV_133	0.97469	0.99512	0.10856	3.4212	*Fusobacteriota*	*Fusobacteriia*	*Fusobacteriales*	*Fusobacteriaceae*	*Fusobacterium*
ASV_323	0.97619	0.99512	0.14251	4.7745	*Actinobacteriota*	*Coriobacteriia*	*Coriobacteriales*	*Atopobiaceae*	*NA*
ASV_302	0.97866	0.99512	-0.14335	5.3589	*Firmicutes*	*Bacilli*	*Staphylococcales*	*Gemellaceae*	*Gemella*
ASV_206	0.98233	0.99512	-0.10978	4.9575	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Oscillospiraceae*	*Colidextribacter*
ASV_240	0.99413	0.99512	-0.03205	4.3596	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Veillonellaceae*	*NA*
ASV_271	0.99474	0.99512	0.028468	4.3177	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_210	0.99512	0.99512	-0.032809	5.36	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Xanthobacteraceae*	*Bradyrhizobium*

Appendix Table 4: Complete single-factor DESeq2 analysis of Day 20 in experiment 2. The order of amplicon sequence variants (ASVs) are organized based on order of greatest abundance to lowest abundance.

ASV #	P-Values	FDR	log2FC	lfcSE	Phylum	Class	Order	Family	Genus
ASV_2	6.91E-17	3.66E-15	11.61	1.3907	*Firmicutes*	*Bacilli*	*Staphylococcales*	*Staphylococcaceae*	*Staphylococcus*
ASV_23	4.49E-14	1.19E-12	-28.588	3.7886	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*NA*	*NA*
ASV_64	2.46E-06	4.34E-05	9.0127	1.9128	*Firmicutes*	*Bacilli*	*Staphylococcales*	*Staphylococcaceae*	*Staphylococcus*
ASV_12	4.10E-06	5.44E-05	-24.869	5.3992	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
ASV_11	8.54E-06	8.66E-05	-15.467	3.4748	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*Klebsiella*
ASV_48	9.81E-06	8.66E-05	-23.871	5.3989	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Massilia*
ASV_5	0.00011489	0.00086992	-16.261	4.2161	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Lactococcus*
ASV_10	0.0019167	0.012698	15.233	4.9093	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Weissella*
ASV_1	0.0065031	0.038296	4.703	1.7282	*Firmicutes*	*Bacilli*	*Entomoplasmatales*	*Spiroplasmataceae*	*Spiroplasma*
ASV_15	0.015528	0.082297	-7.4957	3.0976	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*
ASV_53	0.022103	0.1065	12.282	5.3666	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*NA*	*NA*
ASV_3	0.033129	0.14632	-3.6084	1.6937	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Erwiniaceae*	*NA*
ASV_19	0.072238	0.29451	-7.3234	4.0739	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
ASV_163	0.21354	0.75538	6.685	5.3743	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*
ASV_32	0.21379	0.75538	3.2421	2.6079	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_58	0.22941	0.75991	-6.5795	5.4743	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*NA*
ASV_60	0.24418	0.76127	-6.3754	5.4743	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*NA*
ASV_26	0.27163	0.78267	-5.9229	5.3877	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
ASV_43	0.28085	0.78267	-5.9036	5.4743	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*NA*
ASV_44	0.29535	0.78267	-5.7287	5.4743	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*NA*
ASV_65	0.326	0.783	-4.1396	4.2146	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*Escherichia-Shigella*
ASV_91	0.33845	0.783	-5.2402	5.4743	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*NA*
ASV_14	0.33979	0.783	-3.7062	3.8825	*NA*	*NA*	*NA*	*NA*	*NA*
ASV_27	0.39851	0.84064	4.2787	5.0679	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_46	0.40242	0.84064	-4.0447	4.8306	*Firmicutes*	*Clostridia*	*Oscillospirales*	*Ruminococcaceae*	*Faecalibacterium*
ASV_28	0.41239	0.84064	3.7075	4.523	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Anaerovibrio*
ASV_81	0.44768	0.85461	-3.2404	4.2677	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelatoclostridiaceae*	*Catenibacterium*
ASV_50	0.45525	0.85461	-4.0307	5.3981	*Spirochaetota*	*Spirochaetia*	*Spirochaetales*	*Spirochaetaceae*	*Treponema*
ASV_121	0.48347	0.85461	-2.1742	3.1027	*Actinobacteriota*	*Actinobacteria*	*Corynebacteriales*	*Corynebacteriaceae*	*Corynebacterium*
ASV_54	0.48374	0.85461	-2.5511	3.6429	*Firmicutes*	*Negativicutes*	*Veillonellales-Selenomonadales*	*Selenomonadaceae*	*Megamonas*
ASV_37	0.51274	0.87662	-2.1682	3.3123	*Actinobacteriota*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*
ASV_55	0.53176	0.88073	-2.7885	4.4593	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Alloprevotella*
ASV_107	0.58795	0.89189	2.9332	5.4138	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*
ASV_39	0.60267	0.89189	-1.6117	3.096	*Actinobacteriota*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*
ASV_24	0.61347	0.89189	1.5422	3.0531	*Bacteroidota*	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella_9*
ASV_36	0.62171	0.89189	0.99509	2.0167	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*NA*
ASV_16	0.62264	0.89189	1.4404	2.927	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*
ASV_113	0.73489	0.99445	-1.8258	5.3918	*Fusobacteriota*	*Fusobacteriia*	*Fusobacteriales*	*Fusobacteriaceae*	*Cetobacterium*
ASV_213	0.79063	0.99445	-1.4458	5.4456	*Actinobacteriota*	*Acidimicrobiia*	*Microtrichales*	*NA*	*NA*
ASV_225	0.79063	0.99445	-1.4457	5.4456	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Kaistiaceae*	*Kaistia*
ASV_9	0.84487	0.99445	-0.68681	3.5101	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*
ASV_176	0.86475	0.99445	0.9273	5.4442	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Tyzzerella*
ASV_61	0.86782	0.99445	-0.90051	5.4107	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*Dorea*
ASV_138	0.89684	0.99445	-0.6786	5.2339	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Xanthobacteraceae*	*Xanthobacter*
ASV_62	0.95136	0.99445	-0.18695	3.0646	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*NA*
ASV_161	0.95731	0.99445	0.29037	5.425	*Planctomycetota*	*Planctomycetes*	*Planctomycetales*	*Rubinisphaeraceae*	*SH-PL14*
ASV_83	0.96637	0.99445	0.19895	4.7186	*Firmicutes*	*Clostridia*	*Lachnospirales*	*Lachnospiraceae*	*NA*
ASV_117	0.99442	0.99445	0.037758	5.3994	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	*Citrobacter*
ASV_156	0.99443	0.99445	0.037742	5.4064	*Actinobacteriota*	*Actinobacteria*	*Micrococcales*	*Micrococcaceae*	*Kocuria*
ASV_63	0.99445	0.99445	0.037956	5.453	*Spirochaetota*	*Spirochaetia*	*Spirochaetales*	*Spirochaetaceae*	*Treponema*
ASV_158	0.99445	0.99445	0.037916	5.453	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium sensu stricto 1*
ASV_195	0.99445	0.99445	0.037919	5.453	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Faecalitalea*
ASV_208	0.99445	0.99445	0.037914	5.453	*Verrucomicrobiota*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Akkermansiaceae*	*Akkermansia*

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