UNIVERSITY OF CALIFORNIA,
IRVINE

Biological, Environmental, and Psychological Stress and the Human Gut Microbiome

DISSERTATION

To be submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

In Psychological Science

by

Desiree R. Delgadillo (Chase)

Dissertation Committee:
Associate Professor Sarah D. Pressman Irvine, Chair
Associate Professor Jessica L. Borelli
Associate Professor Katrine L. Whiteson
Associate Professor Michael T. Bailey

2023

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ACKNOWLEDGEMENTS

I am very fortunate to have so many brilliant and beautiful people to thank for helping me on the path to my PhD. Sarah, your adventurous and inquisitive approach to research is more than inspiring, it is mobilizing and contagious. Fortunately, you were brave and curious enough to dive into the exploration of the microbiome with me and I really cannot thank you enough for your belief in me, support, and encouragement. You have challenged me to not only endure the many difficulties and obstacles I have faced along the way, but to power through them and use them as building blocks. With your influence, I have done this and I honestly hope that this is just the beginning of what we might accomplish together. Jessie, I remember the first time I met you at a conference. You had just given a talk and you were surrounded by clamoring fans, and rightly so. Your warmth and kindness were evident then and they are qualities in you that I appreciate deeply and hope to emulate when I become a professor. Your generosity of spirit has helped to sustain and energize me over the years in ways that are unmatched. Combined with all of that, you are also a rigorous and sophisticated scientist. I have so much respect for you and have learned so much from you about research, academia, and life. I very much look forward to exploring more of life and science with you.

I would also like to acknowledge the many contributions of both Mike and Katrine over the years. Katrine, from the moment I showed up on your doorstep, you have connected me to resources and people that have shaped and will influence my work for the rest of my career. You have taught me that the microbiome is a complex and dynamic force with many facets left to explore. I will never forget our car ride to LA and your idea to introduce me to Emeran Mayer. That introduction and your support over the years has set me on a promising course. I appreciate that tremendously. Mike, we have still never met in person but I consider you a mentor and a *hero* in my journey as a psychologist researching the microbiome. We worked together at the most formative stage in my career and you were the catalyst that inspired me to learn complicated statistics so we could explore research questions together. I was basically a stranger to you but you took the time to guide and train me in ways that benefit me every day in my work. I really cannot over-emphasize how impressed and thankful I am by your generosity. Emeran, I read your book while I was preparing to apply for graduate school and it filled me with excitement, purpose, and passion for my current path of research. You made amazing, cutting-edge and complex scientific concepts accessible and fascinating. Thanks to your influence and collaboration, I am determined to produce research that truly helps to ease suffering and improve the quality of life in diverse groups of people. I do not think I could overstate how thankful and excited I am to begin working with you as a mentor at UCLA. I could not have dreamed of a better scenario or outcome.

Importantly, I would like to thank my family and friends. Nicole, together we could take over the world but roller skating is more fun, so let's do that!! Truly, you are inspiring and encouraging and your friendship has made me a better person and scientist. Let's keep making each other healthier and happier for years to come. Marie, I do not think I could count the times

and ways you have supported and strengthened me on this path. If I could give you an award, I would give you 10! Everyone around you benefits from the positivity and brilliance you exude. That is no exaggeration. People like you really do make this world a better place. Amanda, you were the first person to welcome me to UCI! You hired me even though I wasn't a student and then you were patient and kind in my training as an extreme newbie. Without you, I would not be where I am today. I have so much respect for you as a scientist and so much love for you as a friend. Johnny, we created our first published paper together! We were a great team and we wrote some beautiful prose together, much of which was ultimately edited out due to "flowery-ness". I learned a lot from you in that process and you have been a bright light on this path ever since. Your positivity has been a source of strength and I will always appreciate that. Eileen, I could write another, entire dissertation about how amazing you are. Your presence and belief in me have been life giving and have helped me to persevere through big challenges. As far as I am concerned, you have earned a PhD in human awesomeness! I really am in awe of you for too many reasons to number. To my kids, Josiah, Rose, Norah, and Violet. Thank you for sitting through my talks and cheering me on. You are my motivation to succeed and I hope that all of my work paves a path for you that helps you accomplish anything and everything your hearts desire.

VITA

Desiree Delgadillo (Chase)

Department of Psychological Science
University of California, Irvine

EDUCATION

Ph.D. **University of California, Irvine,** Health Psychology, expected 2023
Minor: Affective Science
Biological, Environmental, and Psychological Stress and the Human Microbiome

M.A. **University of California, Irvine,** Social Ecology, 2019
Maternal Expressions of Positive Emotion for Children Predicts Children's Respiratory Sinus Arrhythmia Surrounding Stress

B.A. **Saint Martin's University,** Psychology, 2009
Departmental Honors, *summa cum laude*
Society of Fellows

FELLOWSHIPS, AWARDS AND RESEARCH GRANTS

Latino Excellence Award: UCI's School of Social Ecology Grad Awardee **($1000)** 2023

President's Postdoctoral Fellowship **($60,000 plus $425,000 hiring incentive)** 2023

Social Ecology Outstanding Mentoring Award **($200)** 2019, 2020, 2021, 2022

Graduate/Undergraduate Research Opportunities Program Honorable Mention 2022

TLC Conference Travel Award for Underrepresented Scholars **($1,400)** 2022

Microbiome Initiative Pilot Project Award, *UCI* **($6,300)** 2018

National Science Foundation Graduate Research Fellowship **($138,000)** 2018

Eugene Cota-Robles Fellowship **($74,000)** 2017

Competitive Edge Research Award **($5,000)** 2017

Provost Ph.D. Fellowship, *UCI* **($5,000)** 2017

Dean's Recruitment Fellowship, *UCI* **($5,000)** 2017

PUBLICATIONS

Delgadillo, D. R., Pressman, S. D., Christian, L. M., Galley, J. D., & Bailey, M. T. (2022). Associations between gut microbes and social behavior in healthy 2-year-old children. *Psychosomatic Medicine.*

Delgadillo, D. R., Boparai, S., Pressman, S. D., Goldstein, A., Bureau, J. F., Schmiedel, S., ... & Borelli, J. L. (2021). Maternal expressions of positive emotion for children predicts children's respiratory sinus arrhythmia surrounding stress. *Developmental Psychobiology*.

Diener, E., Pressman, S. D., Hunter, J., & **Delgadillo-Chase, D.** (2017). If, why, and when subjective well-being influences health, and future needed research. *Applied Psychology: Health and Well-Being*, *9*(2), 133-167.

Hunter, J. F., Jones, N. M., **Delgadillo, D. R.,** & Kaveladze, B. (2022). The influence of technology on the assessment and conceptualization of social support. *Quantifying Quality of Life: Incorporating Daily Life into Medicine.* Springer Nature.

Ramirez, V., Martin, L., **Delgadillo, D. R.,** & Pressman, S. D. (2020). Can Positive Affect Alter Physiology?.F. Sirois (1st Edition). *Positive Psychology and Health.* Palgrave Macmillan. Chapter in press.

MANUSCRIPTS IN PREPARATION

Delgadillo, D. R., Borelli, J. L., & Pressman, S. D. Biological, environmental, and psychological stress and the human gut microbiome.

Delgadillo, D. R., Pressman, S. D., & Borelli, J. L. Happy mind, happy gut: Associations between gut microbes and positive emotion.

PRESENTATIONS

**denotes an undergraduate I directly supervised in research*

Delgadillo, D. R., (2022, September)**.** *Associations Between Gut Microbes and Social Behavior in Healthy 2-Year-Old Children.* Paper presented at the The Love Consortium Annual Meeting, Durham, NC.

*Aw, J., **Delgadillo, D. R.**, Pressman, S. D., Patel, J., Carey, A., Black, L., & Gillath, O., (2022, May). *Oxytocin Receptor Polymorphism Variants Affect Perceptions of Social Support.* Poster presented at 29th Annual UCI Undergraduate Research Symposium, University of California Irvine, CA.

Delgadillo, D. R. (2022, March). *Associations Between Gut Microbes and Social Behavior in Healthy 2-Year-Old Children.* Paper presented at the American Psychosomatic Society 79th Annual Meeting, Long Beach, CA.

Delgadillo, D. R. (2020, March). *The Culture of Cuddling: Are Microbes Linked to Social Behavior?* Poster presentation at the American Psychosomatic Society 78th Annual Meeting, Long Beach, CA. (Canceled due to COVID-19)

*Burton, M., Yu, E., Yunusova, A., Vera, J., **Delgadillo, D. R.,** & Borelli, J. L. (2019, May). *Links Between Trait Affect, Relationship Quality, and Diet in the Parent-Child Dyad*. Poster presented at 25th Annual UCI Undergraduate Research Symposium, University of California Irvine, CA.

*Yu, E., Burton, M., Yunusova, A., Vera, J., **Delgadillo, D. R.,** & Borelli, J. L. (2019, May). *Associations Between Trait Affect, Self-Perceived Stress and Diet Among Children*. Poster presented at 25th Annual UCI Undergraduate Research Symposium, University of California Irvine, CA.

Delgadillo, D. R. (2019, May). *Parent-Child Relationships, Positive Emotion Coregulation, and Physiological Reactivity in Children*. Research presentation given at the UCI Psychological Science Departmental Colloquium, Irvine, CA.

*Yu, E., Burton, M., Yunusova, A., Vera, J., **Delgadillo, D. R.,** & Borelli, J. L. (2019, March). *Trait Affect, Self-Perceived Stress and Diet Among Children*. Poster presented at American Psychosomatic Society 77th Annual Scientific Meeting of the American Psychosomatic Society, Vancouver, Canada.

Giesbrecht, G., **Delgadillo, D. R.,** Mayer, E., Christian, L. M., & Tillisch, K. (2019, March). *So...you want to get started in microbiome research. Now what?*" Round Table Discussion Coordinator and Discussant at American Psychosomatic Society 77th Annual Scientific Meeting of the American Psychosomatic Society, Vancouver, Canada.

Delgadillo, D. R., Cross, M. P., & Pressman, S. D. (2016, April). *Do personality traits predict health care seeking behaviors in college students?* Poster presentation at the 96th Annual Convention of the Western Psychological Association, Long Beach, CA.

Acevedo, A., Shader, J., **Delgadillo, D. R**., & Pressman, S. D. (2016, March). *The bigger the better: Greater smile intensity during pain is associated with higher parasympathetic function during stress recovery.* Poster presented at American Psychosomatic Society Research Conference at Westin Hotel, Denver, Colorado.

Delgadillo, D. R., Leger, K., Shader, J., & Pressman, S. D. (2015, May) *Examining the relationship between health locus of control and chronic conditions.* Poster presented at the Psychology Undergraduate Research Conference at UCLA, Los Angeles, CA.

Delgadillo, D. R., Cross, M. P., & Pressman, S. D. (2016, April). *Do personality traits predict health care seeking behaviors in college students?* Poster presented at the Western Psychological Association Research Conference at Westin Hotel, Long Beach, CA.

RESEARCH POSITIONS & EXPERIENCE

Graduate Student Researcher

Stress, Emotion, and Physical Health (STEP) Lab 2017–present
Dept. of Psychological Science, UCI
Advisor: Sarah D. Pressman, Ph.D.

- Designed a correlational study assessing connections between social relationships, positive psychology, and the gut microbiome in adults. Investigated environmental, psychological, and physiological stress in relation to microbial composition. Trained multiple teams of research assistants (the majority of whom were under-represented minorities) to collect, process, and manage microbial, cardiovascular, and psychological datasets. Trained research teams to design recruitment materials and recruit and manage study protocols.
- Utilized biostatistical techniques to explore variations in microbial composition.
- Co-researcher on study examining links between positive psychology, stress, social relationships, and the gut microbiome and cardiovascular function in a community sample of adults.
- Co-designer on a study investigating connections between the practice of Aikido (martial arts) in first-generation college students and leadership ability, empathy, positive emotion, and physical touch. Training a team of research assistants to write documents for the study's Internal Review Board submission, design and implement study protocols, collect and manage data, and recruit and interact with participants.

The Health, Relationships, and Intervention Lab 2017–present
Dept. of Psychological Science, UCI
Principal Investigator: Jessica L. Borelli, Ph.D.
Co-Principal Investigator: Desiree R. Delgadillo, M.A.

- Forged connections with microbiome experts across departments, universities, and the United States to become trained to design and implement microbiome focused study design and protocols. Designed a study to explore links between positive social relationships, positive psychology, stress, and the gut microbiome in 75 mother-child dyads.
- Responsible for designing and managing all microbiome portions of the study, including training multiple teams of research assistants to collect, process, and manage microbial, cardiovascular, and psychological datasets. Trained research teams to design recruitment materials and recruit and manage study protocols. Responsible for dissemination of results.
- Utilized bioinformatic and ecological statistical techniques to explore variations in microbial composition.
- Co-researcher on study examining links between attachment, positive psychology, stress, social relationships, and psychophysiological function in a community sample of children and adults.

Graduate Student Collaborator

Whiteson Lab 2019-February 2023
Dept. of Biological Sciences, UCI
Director: Katrine Whiteson, PhD

- Trained to process and prepare fecal microbial samples in the wet lab
- Processed my own study samples in the wet lab
- Stored, processed, and managed microbial data according to lab protocols

Undergraduate Student Researcher

Stress, Emotion, and Physical Health (STEP) Lab 2014-2017
Dept. of Psychological Science, UCI
Advisor: Sarah D. Pressman, Ph.D.

- Investigated the mitigating effect of positive affect on pain and stress.
- Investigated the effect of smiling on physiological responses to social rejection.
- Led participants through study protocol.
- Measured blood pressure.
- Operated Mindware software.
- Collected physiological responses to the study with electrocardiograph (ECG) equipment.
- Trained new research assistants to use ECG equipment.
- Created stimuli.
- Led subjects through study protocol.
- Collected and entered data.
- Created participant recruitment materials.
- Recruited participants.
- Reviewed relevant literature on positive emotions and health outcomes.

Research Associate and Community Relations Associate 2014- 2017
AVIDA: ADHD Clinical Research Laboratory
Director: Sharon Wigal, Ph.D.

- Participant recruitment. Reviewed relevant literature on ADHD treatments for children.
- Investigated the effect of ADHD treatments on children.
- Collected physiological responses to the study with electrocardiograph (ECG) equipment.
- Research assistant recruitment.
- Created participant recruitment materials.
- Media outreach and website content copywriter.
- Public relations specialist.

STATISTICAL AND SPECIALIZED MICROBIAL STATISTICAL TRAINING

Permutational Analysis of Variance (R)
Analysis of Similarity (R)
Random Forest (R)
Non-metric multidimensional Scaling Ordination (R)
Microbial Bioinformatics Basics (QIIME 2)
Salivary Analyte and Immunoassay Training (sample processing and salivary immunoassay)
Advanced Data Analysis (R and SPSS)

TEACHING EXPERIENCE

Teaching Assistant

Psychology Fundamentals	March 2022 – June 2022
Child Health Psychology	Jan. 2022 – March 2022
Child Clinical Psychology	Sept. 2021 – Dec. 2021
Health Psychology	Aug. 2018 – Sept. 2018

Invited Guest Lectures

"Psychological Processes and the Gut Microbiome" January 2023
Health Psychology
Chapman University

"Emotion and Attachment: Links to the Gut Microbiome and Cardiovascular Responses Surrounding Stress" May 2022
Psychology Fundamentals course
UC Irvine

"Epidemiology and Research" October 2021
Clinical Child Psychology
UC Irvine

"Features of Autism in Children" November 2021
Clinical Child Psychology
UC Irvine

"Mood, Emotion, and the Human Gut Microbiome" August 2018
Health Psychology course
UC Irvine

MENTORSHIP

I have trained numerous undergraduate students, many of whom have been underrepresented minorities. The following describes a select group in which I have worked closely and the skills, experiences, and awards I mentored them through.

Anait Arushanyan 2022-present
- Undergraduate Research Opportunities Award Proposal Preparation
- Internal Review Board document preparation training
- Study design mentorship
- Literature review training

Erin Kim 2022-present
- Undergraduate Research Opportunities Award Proposal Preparation
- Internal Review Board document preparation training
- Study design mentorship
- Literature review training

Morgan McLaughlin 2021-present
- Leadership training as a Study Coordinator for two microbiome studies
- Trained to manage complex microbial datasets
- Taught mind-microbiome study design
- Educated in mind-microbiome literature

Jennifer Aw 2021
- Undergraduate Research Opportunities Award Winner and Presenter
- SPSS statistical software training
- Statistical training: ANOVA, Correlations, and Linear Regression
- Thesis mentor

Guanqiao Yu 2018-2019
- Undergraduate Research Opportunities Award Winner and Presenter with Honors
- Training in academic writing, data collection and management
- SPSS statistical software training
- Statistical training: Correlations and Linear Regression
- Participant Recruitment
- Trained to implement study protocols with mothers and children in their homes
- Trained to instruct participants in microbiome collection
- Taught mind-microbiome study design
- Educated in mind-microbiome literature

Meve Burton 2018-2021
- Undergraduate Research Opportunities Award Winner and Presenter with Honors
- Leadership training as a Study Coordinator
- Taught to interview, train, and manage teams of research assistants
- Training in academic writing, data collection and management

SPSS statistical software training
Taught how to score and create questionnaire scoring syntax
Statistical training: Correlations and Linear Regression
Participant Recruitment and communication
Trained to implement study protocols with mothers and children in their homes
Trained to instruct participants in microbiome collection
Taught mind-microbiome study design
Educated in mind-microbiome literature

Asal Yunusova 2018-2021
Leadership training as a Study Coordinator
Taught to interview, train, and manage teams of research assistants
Poster preparation and presentation
Training in academic writing, data collection and management
Internal Review Board document preparation training
SPSS statistical software training
Statistical training: Correlations and Linear Regression
Participant Recruitment
Trained to implement study protocols with mothers and children in their homes
Trained to instruct participants in microbiome collection
Taught mind-microbiome study design
Educated in mind-microbiome literatures

Diana Latifova 2021
Undergraduate Research Opportunities Award
Taught mind-microbiome study design
Educated in mind-microbiome literatures

Collin Malins 2021
Undergraduate Research Opportunities Award
Taught mind-microbiome study design
Educated in mind-microbiome literatures

Eric Falasiri 2018-2019
Leadership training as a Study Coordinator
Taught to interview, train, and manage teams of research assistants
Participant Recruitment and communication
Trained to implement study protocols with mothers and children in their homes
Trained to instruct participants in microbiome collection
Taught mind-microbiome study design
Educated in mind-microbiome literatures

PROFESSIONAL AND DEPARTMENTAL SERVICE

NSF GRFP Internal Review Committee 2019
School of Social Ecology

Graduate Student Committee 2018
Department of Psychological Science

HUMANITARIAN SERVICE AND VOLUNTEER WORK

Project Hope Alliance 2020
- Volunteered to assist children experiencing homelessness
- Donated time to make meals for low-income families
- Bought and delivered food to those experiencing homelessness

Overseas Outreach Leader for Reef to Outback Volunteer Organization July 2001-Dec. 2003
Townsville, Australia
Director: Kenneth Mulligan
- Lead teams of volunteers providing humanitarian aid to homeless and underprivileged populations in Australia, Samoa and New Zealand.
- Managed group finances, accommodations, travel logistics and humanitarian activities.
- Created a culture within the team environment that maintained efficiency, cohesiveness and high morale.
- Collaborated with local community leaders to maximize time and resources.
- Gave numerous informational and inspirational speeches at humanitarian meetings

Co-director of The Bridge: Community Outreach August 2005- July 2009
Olympia, WA.
- Designed outreach plans
- Recruited and trained volunteers
- Lead teams of volunteers to provide basic necessities for those experiencing homelessness
- Lead teams in random acts of kindness for members of the community
- Built homes with Habitat for Humanity
- Wrote public relations materials

Spoke regularly at humanitarian meetings

ABSTRACT OF THE DISSERTATION

Biological, Environmental, and Psychological Stress and the Human Gut Microbiome

by

Desiree Delgadillo

Doctor of Philosophy in Psychological Science

University of California, Irvine, 2023

Associate Professor Sarah D. Pressman Irvine, Chair

Microbes were the first organisms to evolve on Earth, and inhabit every known environment, including the human gut. Diverse microbial ecosystems are involved in a number of crucial bodily processes including educating the immune system. Interestingly, human microbiome composition and function is also linked to central nervous system activity, specifically, the stress response. Extant literature exploring the stress-microbiome connection in healthy, human adult samples is sparse and primarily tests these links in non-human animals or in clinical human samples, particularly those with depression and anxiety. This dissertation is among the first to explore stress-microbiome links across three stress domains in two samples of healthy adults, specifically, perceived stress, stressful life events, and cardiovascular function surrounding stress as indexed by Respiratory Sinus Arrhythmia (RSA). This vital next step will help researchers better understand the nuanced, dynamic connections between stress types and microbial composition.

We carried out two main studies: Study 1 includes 62 healthy adults, 68% female with a mean age of 37.3 years and Study 2 includes 74 healthy women with a mean age of 41.6 years.

Participants completed surveys assessing stressful life events, perceived stress and completed a laboratory stressor. RSA was collected prior to, during, and following a laboratory stressor. Following the laboratory visit, participants were given fecal collection kits to collect microbiome samples that were assayed for microbial composition.

In Study 1, the low perceived stress group was higher in alpha diversity than the high perceived stress group. Both Study 1 and Study 2 revealed differences in beta diversity between stressful life events groups and Study 1 revealed differences in beta diversity between RSA stress reactivity groups. Differentially abundant microbes between groups are discussed. Further, levels of *Clostridium* were negatively associated with RSA stress reactivity in Study 1 and levels *Escherichia/Shigella* were positively associated with perceived stress in Study 2.

Together, these studies show that stress group membership was differentially linked to microbial composition and that objective assessments of potentially severe stressors such as stressful life events may be more reliably linked to microbial composition (beta diversity) than subjective evaluations of perceived stress. This work provides a foundation for future research that should focus on experimental studies and longitudinal interventions designed to determine bidirectional links between stress types, bacterial species, and metabolic output.

CHAPTER 1: Introduction

Introduction

Microbes permeate virtually every surface of the planet, including the human body. These non-human organisms have shaped human evolution from its nascency and research indicates that microbes impact both physiological and psychological processes (Cryan & Dinan, 2012). Emerging research has found that gut microbes are associated with psychological wellbeing and various psychiatric and neurological conditions including Autism Spectrum Disorder, Parkinson's, and mood disorders such as depression (Mayer et al., 2015). Intriguingly, extant research has also repeatedly revealed associations between levels of stress and microbial composition; however, the majority of studies have been performed in laboratory rats and mice (Bailey et al., 2004; see review by Cryan et al., 2012) and it has been challenging to replicate preclinical findings in human subjects. Particularly, little is known regarding stress-microbiome connections in healthy humans, specifically, whether these connections are universal across various stressor types and whether certain bacteria might be tied to distinct stressors differently.

According to the American Psychological Association (2015), 77% of Americans report experiencing physical symptoms caused by stress, 33% feel they are living with extreme stress and about half of all respondents feel their stress has increased over the past five years. This is even prior to the extreme stress caused by the pandemic (Park et al., 2020). Given these numbers, it is not surprising that upwards of $300 billion dollars is lost annually due to stress related health care costs and missed work (American Psychological Association, 2015), calling for a continuing need for research to better understand stress, its causes and its correlates. As described above, microbiome science has identified intriguing new factors in stress biology. That is, what if some individuals harbor a gut microbial ecosystem that increases stress coping capacity while others host an environment that fosters psychological vulnerability to life's challenges? Could the

microbes in one's gut actually play a role in intensifying or ameliorating the response to stressful experiences or vice versa? Does stress shape which microbes inhabit the gut? Discovering bidirectional links between stress and the gut microbiome could lead to the development of simple, inexpensive interventions and treatments that ease suffering, reduce financial loss and open the possibility of expanding an individual's biological capacity to calibrate stress responses and cope with unavoidable hardships. To inform the design of experimental research, establish directionality, and develop long-lasting, effective interventions, a key first step is to identify which naturally occurring (endogenous) microbial profiles are associated with stress in healthy human adults. Further, if stress and the microbiome are related, does the type of stress matter? In the current study, we will explore whether microbial composition is associated with three different types of stress in two healthy adult samples: psychological stress (i.e., self-reported perceptions of experienced stress and overwhelm), environmental stress (e.g., major life changes such as the death of a loved one or divorce), and physiological changes in response to two different laboratory-based stressors.

Mind-gut Connections

Humans frequently experience gut sensations in response to emotions. For example, when people are nervous, they might say, "I have butterflies in my stomach," or when grief is too intense you might hear that the feeling is "gut wrenching." Early medical doctors and researchers have long known that individuals experience psychological processes in the gut because feelings do in fact, impact gut motility and secretions. One of the first documented instances of this occurred in 1822 when an army surgeon by the name of William Beaumont performed an emergency surgery on a young fur trader, Alexis St. Martin. Martin had accidentally been shot with a musket from less than 3 feet away. The shot created a large

opening on the left side of his abdomen that extended into his stomach leaving a hole the size of an index finger. Beaumont performed life-saving surgery but was unable to completely close the wound, resulting in a fistula -a wound that healed but left a permanent opening- that exposed the inside of Martin's stomach and gave Beaumont the ability to observe internal digestive processes. The surgeon noticed that when Martin became emotionally aroused, his digestive processes slowed (Beaumont, 1883). In a similar example, an army physician in 1946 treated a young man whose intestines were exposed due to a gunshot blast and reported that movement became more active in the small and large intestines in response to psychological stress (Weeks, 1946 as cited in Mayer, 2018). Hundreds of years later, we are now beginning to understand how 'gut wrenching' stress and emotion may lead to changes in motility and secretions in the digestive tract that ultimately shape microbial composition and vice versa. Indeed, stress is closely tied to gastrointestinal disorders such as irritable bowel syndrome (Mayer et al., 2023), gut dysbiosis (De Palma et al., 2014; Karl et al., 2018), and increased intestinal permeability (i.e., leaky gut; see review by Kelly et al., 2015) all of which are bidirectionally linked to microbial composition.

The Discovery of Microbes

Antoni van Leewenhoek was the first person to see bacteria through the use of a self-crafted microscope. Leewenhoek described his first glimpse of microscopic life as 'animalcules' or 'little eels'. It was this discovery that built the foundation of modern medicine's understanding of microbes as the cause of infectious disease (Gest, 2004). Since then, microbes have largely been considered harmful, disease-causing germs that should be killed or managed. Consequently, microbial diversity is declining in industrialized nations and this may be due in part to the use of antibiotics, antiseptics, and chlorinated water (Bello, 2018). However, research shows that many

microbes help to strengthen the immune system (Carding et al., 2015) and perform health relevant tasks such as the digestion and absorption of otherwise indigestible nutrients (Krajmalnik-Brown et al., 2012).

The Importance of the Gut Microbiome for Human Health and Well-being

The human gut microbiome is composed of bacteria, eukaryotes, archaea, viruses and fungi and each individual's microbial profile is as unique as a fingerprint. Although there are hundreds of different species of microbes that could inhabit the gut, it is estimated that approximately 99% originate from 30-40 species (Beaugerie & Petit, 2004; Savage, 1997). Currently, most researchers identify individual microorganisms via DNA gene sequencing. Bacteria are then enumerated in relative abundances, that is, the percentage of each microbe in relation to the total microbial community in one sample or group. In addition to calculations of relative abundances, microbes are also enumerated in raw or absolute counts. Together, these multitudes of diverse microorganisms form an ecosystem within the host that contain bacteria that can have symbiotic or pathogenic relationships with the host.

In some microbiome literature, the term symbiotic is used to describe a relationship between the human host and the organism in which the human obtains health benefits or remains unaffected by the microbe (Eloe-Fadrosh & Rasko, 2013; Haque & Haque, 2017). In contrast, pathogenic bacteria are thought to pose a threat to health in certain host environments. However, terms such as symbiotic and pathogenic are increasingly thought to be too simplistic. For example, only 10% of those infected with *Mycobacterium tuberculosis* (thought to be a pathogenic bacteria) develop pulmonary tuberculosis (Casadevall et al., 2104). Further, so-called symbiotes are not *always* beneficial. An overabundance of a symbiotic microbe in relation to the rest of the bacterial community is sometimes related to ill health in that it reduces overall

diversity within the sample. Further, bacteria thought to be pathogenic may be tolerated in a stable microbial system (Gibson et al., 2014). Indeed, the role that each microbe plays in human health outcomes varies greatly and is dependent upon host genetics, age, and also depends on other microbes present in the ecosystem. Thus, host-microbiome interactions make it difficult to link an entire genus to symbiotic or pathogenic functions. For example, the taxonomic category genus can contain hundreds of species and an even higher number of strains. Some of these may be considered symbionts, while others are considered pathogens. Some genus contains strains that have been shown to carry out symbiotic functions in the literature. *Lactobacillus* and *Bifidobacterium* are examples of genus that contain strains that are thought to optimize immune function and digest nutrients (Carding et al., 2015; Kinross et al., 2011) and are also linked to psychological processes such as adaptive stress responses (Benton et al., 2007). In contrast, some bacteria have been shown to have more pathogenic properties in certain host environments and include strains of *Escherichia coli*, *Streptococcus* spp., *Campylobacter jejuni*, *Gammaproteobacteria, Escherichia/Shigella.* These are bacterial strains are linked to gut dysbiosis, irritable bowel syndrome (Ohman & Simren, 2013), urinary tract infections (Vincent et al., 2010) and diarrhea (Hunter, 2003; Magruder et al., 2019; Nagao-Kitamoto et al., 2016) and to psychological factors such as stress-responses (Biondi, 1997; Dinan & Cryan, 2016; Galland, 2014; Lyte et al., 1992; Sun et al., 2019). As previously mentioned, the expression of symbiotic or pathogenic properties of many bacteria in the human gut depend on the host environment. For example, *Akkermansia* uses the complex carbohydrate mucin in mucus as a food source and thrives when fiber consumption is low (Sonnenberg et al., 2019). However, considerable evidence suggests that *Akkermansia* plays an important role in gut health. While *Akkermansia* is a mucin-degrading bacterium, it is thought that this mechanism stimulates mucus production and

supports gut barrier integrity (Zhou et al., 2017). For that reason, *Akkermansia* will be discussed as symbiotic in this dissertation. However, when I refer to microbes as pathogenic or symbiotic, I do not use these terms definitively but only within the context of prior literature as it pertains to wellbeing in some hosts. I use findings in the extant literature to inform hypotheses; however, the nuances and complexities of host-microbiome interactions should be held as a constant caveat when reading this dissertation (Casadevall et al., 2014). Beyond suggestions of beneficial versus detrimental functions of individual microbes, it is *especially* important to consider the ways in which the composition of the microbial ecosystem *as a whole* might work together to influence wellness.

In many ecosystems, a diversity of species is a hallmark of a resilient, thriving environment, where many members of the system interact to promote stability (Mosca et al., 2016) and this includes the human gut (Bello, 2018). However, in some human body sites such as the vagina (Lehtoranta et al., 2022) and in some cases saliva (Takeshita et al., 2016), lower diversity is associated with poorer health outcomes. In microbiome science, the diversity found within an ecosystem is called alpha diversity while the diversity between ecosystems is called beta diversity. In the human gut, higher alpha diversity is associated with health. In contrast, low gut microbial alpha diversity is not only associated with several poor health outcomes such as diabetes, food allergies, asthma, and obesity but it is also linked to deleterious psychological outcomes such as cognitive and mood disorders (Bello, 2018). The current work explores the ways in which diversity within and between gut microbial ecosystems, might be linked to psychological processes, in particular stress responses. Specifically, I will also assess whether microbial alpha and beta diversity might be linked to the three types of stress.

Defining and Measuring Stress as it Relates to Human Health Indices

Stress is a broad term that is widely used across both popular culture and academic disciplines. But what is stress and how do we accurately measure it? Cohen and colleagues (1998) define stress as "a process in which environmental demands tax or exceed the adaptive capacity of an organism, resulting in psychological and biological changes that may place persons at risk for disease." Building upon this definition, stress is composed of three interrelated domains, 1) objective environmental demand, 2) psychological perceptions and appraisals of environmental demands and 3), biological responses to those demands that result in physiological processes that can become nocuous to health (Cohen et al., 1998). In the discipline of health psychology, each of these stress domains have been studied and measured in various ways.

Measuring stressful life events is one primary way of assessing objective environmental demand. For decades, medical doctors have documented that the onset and progression of disease was linked to the occurrence of extreme demands, grief, and loss (Hinkle & Wolff, 1957). For example, Rahe & Lind (1971) observed that stressful life events were associated with sudden cardiac death. Years later, current research continues to show that stressful life events are linked to numerous health related indices including coronary artery disease (Stantiute, et al., 2013), immune dysregulation, and AIDS progression in HIV patients (Glaser, 2005). Importantly, the physiological impact of stressful life events may also depend on individual perceptions and reactivity (Cohen et al., 1998). Thus, it is not only a major event that calibrates the experience of stress, but also, the appraisal of the event. Appraisals of stress vary by situation and individual. For instance, a great financial loss might feel less stressful for an individual connected to a generous, wealthy family when compared to an individual that lacks a social or

monetary safety net. That is, when the experience or occurrence of a stressor eclipses one's perceived ability to cope, stress appraisals increase (Cohen et al.,1998). One common method used to measure this phenomenon is the Perceived Stress Scale (PSS; Cohen et al., 1983). This instrument assesses how uncontrollable, overwhelming, and unpredictable individuals experience their daily lives (typically over the last month). Measuring this psychological construct is important because it is linked to numerous markers of health, for instance, perceived stress is positively associated with poorer general health (Flores et al., 2008), heart rate, and diastolic blood pressure (Sharma et al., 2013). Additionally, causal links were found in a prospective study of 7066 participants. Results showed that those with high levels of perceived stress are more likely to become overweight, use antihypertensive medication, and are twice as likely to develop diabetes when compared to individuals with low stress perceptions (Rod et al., 2009).

Both objective stressors as well as appraisals of threat trigger an array of biological processes in the body. This provides another method by which stress may be assessed. Examples of biological systems responsive to stressors include the endocrine, immune, and cardiovascular systems. The current study focuses on the cardiovascular system, specifically, functioning of the autonomic nervous system (ANS) in the context of stress. It is well established that autonomic function is impacted by psychological processes; specifically, research suggests that maladaptive stress responses may have long term consequences on cardiovascular health (Steptoe & Kivimaki, 2012). The ANS consists of two branches. The Sympathetic Nervous System (SNS) and the Parasympathetic Nervous System (PNS). The SNS is the branch that mobilizes the organism to "fight or flee" when confronted with challenges or stress in the environment while the Parasympathetic Nervous System (PNS) fosters "rest and digest" functions necessary to replenish physical resources and return an organism to homeostasis following stress (Glick et al.,

1965; Ondicova & Mravec, 2010). If SNS activity is prolonged or inordinate, a situation referred to as allostatic load, it may promote an internal environment that puts the individual at risk for infection, disease or illness (Fisher et al., 2009). Consequently, the PNS may play a particularly important role in future health (Kristal-Boneh et al., 1995).

PNS activity is transmitted via the vagus nerve which stems from the brain, innervates the heart, controls the sinoatrial node, and, important to the current dissertation, regulates gut function (see review by Duan et al., 2018). Respiratory Sinus Arrhythmia (RSA) is one way to gauge cardiovascular vagus nerve activity and serves as an index of heart rate variability. High levels of RSA indicate activation of the PNS and low levels of RSA indicate a withdrawal of PNS activation (Beauchaine, 2015; Reyes del Paso et al., 1993; Katona & Jih, 1975) which allows an organism to mount a response to a potential threat or challenge via the SNS. Specifically, higher RSA reflects greater synchrony between the heart rate and respiration cycles. High resting RSA is positively associated with social and emotional function (Geisler et al., 2013) and ability to respond to environmental demands (Butler et al., 2006; Calkins, 1997) while low resting RSA is associated with anxiety and stress-related disorders (Campbell & Wisco, 2021; Hauschildt et al., 2011). Within a stress framework, RSA is typically highest at rest, lowest during stress, and then eventually returns to baseline levels once the stressor is resolved (Porges et al., 1994).

In sum, the three approaches to measuring stress presented; stressful life events, perceived stress, and physiological responses to stress represent an integrated picture of how objective environmental threats influence health indices (Cohen, et al., 1998). It is valuable to assess all of these because together they comprise the unique but also overlapping features of the human stress experience. Perceived stress is a subjective feeling based on inward appraisals of

threat or challenge. In human assessments, it is focused on emotional experience irrespective of the objective presence or the severity of a real threat. In contrast, stressful life events are an objective measure of the conditions in the environment independent of emotional appraisal. Stressful life events, whether they are perceived as difficult or not, often force an individual to adjust to objective changes in the environment and the collection of these adjustments can require the use of more psychological and physiological resources which may impact health outcomes and indices. As stated previously, the effects of perceived stress, stressful life events, and acute stress can be measured in the body. For instance, RSA measures provide objective, quantifiable assessments gauging physiological preparations for mounting, responding to and recovering from stress, for example, in response to acute laboratory stressors (as relevant to the current study). Indeed, each type of stress presented reflects interrelated but discrete stress domains with differing outcomes related to wellbeing, however, to my knowledge, there is no published work examining environmental, psychological and biological stressors comparing two-samples of healthy humans. Assessing relations between the microbiome and each stressor type will reveal whether these distinct domains are differentially associated with the gut microbiome and could provide targeted insight when designing stress specific intervention studies. Further, extant research across species and various stressor types suggest that stress is a key player in relation to gut health and the architecture of the microbial ecosystem. Indeed, both animal and human research indicates that certain features of the gut microbiome such as diversity, and the presence and abundance of symbiotic and pathogenic bacteria are bidirectionally linked to stress and stress-related disorders (Aroniadis et al., 2017; Michels et al., 2019).

Stress, Stress-related Psychological Processes and the Gut Microbiome

Models of Stress in Animals and How These Relate to Human Stress Concepts

Animal and human research examining mind-microbiome interactions varies in methodological approach and often tests the effects of and associations between probiotics (health promoting microorganisms), antibiotics, the naturally occurring microbiome and psychological variables. Due to the stringent protocols used in laboratory experiments with animals, these studies can also examine the effects of microbial conditions impossible to create in human studies. This includes a total absence of microbes (germ-free animals that contain no microorganisms in or on them) or exposure to specific microbes selected by the researchers on rodent, non-human primate and insect behavioral responses to stressors. Additionally, animal models use methods to experimentally manipulate stress that would be unethical to experimentally assign to humans. For instance, animals are exposed to electric shock (Messaoudi et al., 2011), maternal separation, physical restraint (Sudo et al., 2004), and are placed in cages with an aggressor (Bailey et al., 2011). Many of these conditions are meant to mirror the human stress experience, for example, maternal separation (Bailey & Coe, 1999) has been used to show possible links between early life stress, anxiety, and addiction ((Rana et al., 2014). Further, physical restraint is considered to induce *perceived* stress since there is no real threat to life or limb (Gaudin et al., 1990). While useful as a point of comparison, obviously the human and rat/mouse experience of perceived stress is highly distinct and impossible to generalize clearly across species. That said, animal research fosters greater standardization of other important factors difficult to control in humans that impact microbial composition including genetic variability, diet, exercise, and greater control of environmental conditions making it the ideal setting for studying the microbiome, but not easily replicable in humans. Further, human

neurological structures and processes are much more complex than that of animals, we are unable to measure the animal's actual perception of threat, laboratory results are often not generalizable across species, and while we share some gut microbes in common with other animals, the human gut microbiome is distinct from other species (Amato et al., 2015; Nguyen et al., 2015). In sum, while animal models offer causal insights into mind-microbiome relations, much more work in humans is needed to understand which microbiological underpinnings can be observed in human samples in response to stress and form a basis for the next advancements in intervention work.

The Mind-Microbiome Connection in Animal Research. For over two decades, animal research has shown that microbial composition is linked to stress responses and numerous preclinical studies demonstrate these bidirectional associations (Bailey & Coe, 1999; Bailey et al., 2011; Messaoudi, et al., 2011; Sudo et al., 2004). The current section will highlight key evidence, particularly, those on stress including studies that examine both top-down and bottom-up pathways. For a thorough review of this literature, see reviews by Cryan and colleagues (2019) and Hantsoo & Zemel (2021).

Studies that examine top-down pathways indicate that stressors can shape microbial composition. For instance, in one of the first studies assessing psychological stress and the gut microbiome, the microbiota of infant Rhesus monkeys was significantly altered following the social stress of maternal separation (Bailey & Coe, 1999). Specifically, these monkeys evidenced decreases in the symbiotic bacteria, *Lactobacillus*. Similarly, another early study revealed links between the gut microbiome and social disruption; placement with an aggressive cage mate. In this study, exposure to the stressor resulted in decreased fecal microbiome levels of the bacteria *Bacteroides* and increased levels of the bacterial genus *Clostridium* (Bailey et al., 2011), which

includes both pathogens and normal health associated members. Further, mice exposed to social stressors showed increases in Firmicutes/Bacteroidetes ratios (Gautum, 2018) and decreases in *Bacteroides* (Bailey, Dowd, Galley, Hufnagle, Allen & Lyte, 2011). Notably, Firmicutes and Bacteroidetes are the two most dominant bacterial phyla with some research showing that increased Firmicutes/Bacteroides ratios are associated with poorer health status', specifically, obesity. However, other research reveals contradicting results. Reasons for contradictory findings may be due to neglecting to account for lifestyle factors known to alter microbiota composition or due to differences in methodological approaches in sample processing and sequencing analysis (Magne et al., 2020). Additionally, while the genus *Bacteroides* is generally considered beneficial in the gut, some species can cause pathology when translocated to other body sites. For example, Elliot and colleagues (1999) found that *Bacteroides* species were the most common bacteria identified in necrotizing soft tissue infections (Elliot et al., 1999). In sum, literature examining Firmicutes/Bacteroides ratios reveal mixed findings and the genus *Bacteroides* can express both pathogenic and beneficial functions depending on the host environment (Wexler, 2007). Thus, it is difficult to make inferences based on current research as to how these bacteria might relate to health outcomes and particularly how they might relate to stress.

Research examining bottom-up links between the gut microbiome and the brain also provides promising evidence that microbes can shape the stress response in animals. For example, studies show that certain microorganisms referred to as psychobiotics (probiotics thought to improve psychological outcomes) can mitigate stress responses in animals. Specifically, animals that received a probiotic formula of *Lactobacillus helveticus* and *Bifidobacterium longum* delivered prior to laboratory stressors show reduced anxiety-like

behaviors (e.g., lower levels of burying the electric probe, head stretching and avoiding/approaching the probe) following electric shock when compared to controls (Messaoudi, et al., 2011). Similarly, *Bifidobacterium infantis* was shown to alleviate symptoms of stress in maternally separated rat offspring (Desbonnet et al., 2010; see review by Dinan et al., 2011). Conversely, germ-free mice showed exaggerated stress responses (cortisol levels) to an acute restraint stressor compared to controls (Sudo et al., 2004).

Relatedly, mind-microbiome links are also observed in stress-related disorders such as anxiety and depression. This was shown in experiments in which the gut microbiota of depressed humans was transplanted into rodents. Following the microbial transfer, the rodents displayed significantly increased depressive behaviors (Kelly et al., 2016; Zheng et al., 2016). Interestingly, the influence of microbes has also been shown to promote exploratory behavior, for instance, when the microbiome of bold, exploratory mice was transplanted into anxious mice, the anxious mice displayed the same bold, exploratory behavior as the donor (Cryan & Dinan, 2012; Sharon et al., 2016). Taken together, this body of research indicates that the gut microbiome may be bidirectionally linked with various stress-related psychological processes and these associations are also observed in humans.

The Mind-Microbiome Connection in Human Research. Human research assessing stress-microbiome relations is relatively sparse but findings often mirror those found in animal research and most commonly include the study of probiotics and antibiotics, but also examine the endogenous gut microbiome in relation to stress constructs as will be done in this dissertation. This section will focus on human studies assessing relations between the naturally occurring gut microbiome (and a few key probiotic studies), stress, and stress-related disorders

as they are most relevant to the current work. For a more comprehensive view of this research see reviews by Bailey (2012), Madison & Kiekolt-Glaser (2019), and Hantsoo & Zemel (2021).

A recent review of literature examining relations between various types of stress and the gut microbiome reports that only one study assessed associations between the endogenous microbiome and responses to an acute laboratory stressor (Hantsoo et al., 2019; Hantsoo & Zemel, 2021). This small study of 19 pregnant women found that microbial composition was linked to levels of cortisol in response to the laboratory stressor (Hantsoo et al., 2019). Specifically, cortisol was positively associated with abundances of *Rikenellaceae* and *Dialister* and negatively associated with *Bacteroides*. Similarly, relatively few studies assess relations between stressful life events and microbial composition (Hermes, et al., 2020; Knowles et al., 2008; Nishida et al., 2019). In one such study, it was found that college students' gut bacteria count decreased during exam week when compared to the beginning of the semester (Knowles et al., 2008). Four more studies use self-reports to assess associations between perceived stress and naturally occurring microbial composition in healthy women (Carson et al., 2018; Kleiman et al., 2017) and patient populations (Humbel et al., 2020; Mackner et.al, 2020). However, associations between microbial composition and perceived stress were mixed. For instance, perceived stress was positively associated with *Clostridium* and *Ruminococcus* and negatively associated with *Bifidobacterium* in a sample of 80 healthy women (Carson et al., 2018) while there were no significant associations between these constructs in another sample of healthy women (Kleiman et al., 2017). Additionally, one study assessed various types of stress within the same sample (i.e., stressful life events, cardiovascular function, and cortisol levels) in relation to microbial composition. This cross-sectional study of 93 Belgian children ages 8-16 years found a negative association between stressful life events and microbial alpha (within subject) diversity. Taken

together, these studies broadly suggest that the microbiome is reciprocally linked to stress and that these links vary by stressor and sample type, however research assessing microbiome relations in healthy adults across stress modalities is lacking.

Other stress-related constructs are also linked to naturally occurring microbial composition such as depression, emotional arousal and chronic mood disorders (Jiang et al., 2018; Tillisch, et al., 2017; Zheng et al., 2016). Compared to healthy controls, studies have found that those with generalized anxiety disorder and patients with depression display considerably less microbial diversity (Jiang et al., 2018; Kelly et al., 2017). Further work extends beyond naturally occurring microbiomes and assesses whether probiotic interventions may promote healthier responses to stress and generally improved well-being. A meta-analysis of seven human studies showed improvement in depression, anxiety, and perceived stress in participants that ingested a probiotic compared to those given a placebo (McKean et al., 2017). Similar results were found in another healthy human sample not included in the meta-analysis, in which the probiotic *Bifidobacterium longum* was found to ameliorate daily reported stress and cortisol output in response to a laboratory stressor (Allen et al., 2016). While probiotic research is promising (see review by Dinan, 2013) probiotics are not well defined or regulated (see review by Suez et al., 2019), and their use has not been shown to permanently re-colonize the gut with a non-native microbe (see review by Lerner et al., 2019). That is, since the gut microbiome is relatively stable, and many probiotics are not adapted to the human gut, it usually returns to its baseline composition once probiotic use has ceased. Furthermore, probiotics have been shown to reduce cancer treatment efficacy (Spencer et al., 2021) and to slow the return of healthy gut anaerobes after antibiotic treatment (Suez et al., 2018). This highlights the importance of discovering whether naturally occurring microbial profiles are linked to adaptive stress

responses. Since the current study uses two samples of healthy adults to explore microbiome associations across stressor types, there is strong potential to identify repeatable, stress specific links to the endogenous microbiome. The unveiling of nuanced stress-microbiome connections could pave the way for future researchers to develop interventions specially targeted at vulnerable stress domains and better optimize stress resilient endogenous microbes. This could be accomplished, for example, with prebiotics (groups of nutrients digested by microbes) or high fiber diets, both of which promote the growth of symbiotes (Davani-Davari et al., 2019). This may be because many symbiotes release health promoting byproducts called metabolites when they consume the 'right food' (e.g., prebiotics and fiber) and several of these metabolites are known to promote human gut health and psychological wellbeing (Sun et al., 2016; Yang & Yu, 2018). In sum, mind-microbiome research provides a promising basis for continued exploration of stress ameliorating connections, however, it is important to acknowledge that this science is new and often not in total agreement.

Past research sometimes finds non-significant or conflicting associations between the mind and the microbiome. For instance, one study showed no links between the gut microbiota and perceived stress (Kleiman et al., 2017). Further, Jiang and colleagues (2015) found *increased* diversity in those with depression while other studies showed no differences in microbial diversity between depressed individuals and healthy controls (Naseribafrouei et al., 2014; Zheng, 2016). Additionally, findings from animal research do not always replicate in human samples. For example, the probiotic *Lactobacillus rhamnosus* reduced stress induced corticosterone and depressive and anxious behaviors in mice (Bravo et al., 2011) but failed to mitigate the stress response in a sample of healthy men (Kelly et al., 2017). Replication failures and discrepant findings highlight the need for a judicious awareness of translatability across species (possibly

due to differing neurological structures), differences in statistical approaches across studies and whether important confounds are taken into account (Malan-Muller et al., 2018) such as diet, body mass index (BMI), and sex. Contributing to inconsistencies in the literature, is that extant research is still somewhat diffuse with evidence coming from a wide-array of conditions, behaviors, and self-reports that sometimes examine stress itself but often assess stress-related disorders such as anxiety and depression. The current work is among the first to assess the replicability of microbiome links across three different types of stress (i.e., psychological, environmental, and biological) while adjusting for important confounds (e.g., diet, BMI, and sex) in two samples of healthy adults.

Psychological, environmental, and biological stress (while overlapping) are composed of unique features; the current work will assess whether these various types of stress differentially relate to the microbiome, answering the call of recent work to distinguish between both stressors and stress responses in health psychology research (Crosswell, et al., 2020). Importantly, there are numerous possibilities as to why stress-microbiome connections might be sensitive to the multi-faceted components that distinguish stress domains. For instance, it is possible that links between biological stress and the gut microbiome will be more robustly connected than other stressor types since microbes have co-evolved with human physiology for millennia (Ley et al., 2008) and have also co-developed alongside the individual host's cardiovascular system throughout the lifespan. Connections here may reflect both evolutionary and individual stress-microbiome associations. In contrast, this raises questions as to whether it is the event or the psychological response to these occurrences. Perceived stress can be more transient and this may result in alternate mind-gut associations such as weaker connections or links to different microbes. Alternatively, momentous stress exposures (major stressful life events), regardless of

psychological response, might lead to altered microbial composition since it requires a great deal of objective coping. This type of mind-microbiome correlation is also less likely to raise reverse causality issues since the microbiome is unlikely to *cause* a major stressful life event (e.g., the death of a family member or the loss of a job) to occur. While connections between perceptions of stress and the gut microbiome would not hint at directionality, it is important to note that self-reports are one of the few ways to measure an individual's stress appraisal. While it could be said that perceptions are the most difficult to measure, they are also one of the best ways to gauge the intensity or duration of psychological suffering. In sum, each stress domain provides a distinct assessment of the objective and subjective human stress experience. Assessing each of them allows us the potential to see what types of stress are most tied to the microbiome, or alternatively, whether they all reflect the same pathway and connect in an overlapping (replicating) fashion.

As reviewed, there is preliminary evidence linking various types of stress to microbial composition in both humans and animals. Mind-microbiome research in humans is promising with emerging evidence stemming from a wide array of methodologies and across psychological constructs. However, as mentioned previously, research assessing psychological, environmental, and biological stress in relation to the endogenous gut microbiome within samples of healthy adults is lacking. This gap in the literature is important to address because little is known as to whether certain types of stress connect to the gut microbiome differently. There are few published studies that assess perceptions of stress, stressful life events, and cardiovascular function (Hantsoo & Zemel, 2021; Hauschildt et al., 2011) in relation to naturally occurring microbial composition, however even among these few, most were conducted on patient populations or women exclusively with one study assessing mind-microbiome links across

stressor types; a study administered among a group of children (Michels et al., 2018). The current study will examine more healthy and diverse samples than most previous work and also explore important unanswered questions. Could certain microbes connect differently to perceptions of stress than they do to cardiovascular stress responses or in relation to stressful life events? If so, do the strengths of these possible associations change across stressor type? Further, if the findings of the current work link the microbiome to stress, what are the possible theoretical underpinnings for this discovery?

Theoretical Promise

The human body is thought to collect stress and it is possible that our microbes take similar action. Psychological stress frequently leads to activation and deactivation of multiple, interdependent allostatic systems. Allostasis is defined as "the process of maintaining homeostasis through the adaptive change of the organism's internal environment to meet perceived and anticipated demands" (McEwen & Wingfield, 2003). An example of this is when the sympathetic nervous system is activated in response to an external stressor. The HPA axis triggers the secretion of hormones such as epinephrine, norepinephrine and cortisol. This is adaptive and necessary when confronted by a 'life and limb' or acute type of stress but can become maladaptive when this response is regularly activated by chronic social stressors such as job-related stress or difficult relationships resulting in an accumulation of harmful wear and tear on multiple body systems. This is known as allostatic load (McEwen, 1998). It is possible that the ecological environment and products of the gut microbiome may also act as a third-party allostatic system living within us. Since we are the host to these microbes, the activation of other allostatic systems may influence, communicate and educate microbial communities living within the gut. As we have observed in animal models, chronic stress impacts both the structure and

function of the gut microbiome and this microscopic world within us could also have its own, unique allostatic response and accumulate a stress sensitive allostatic load. Thus, stress is a viable player within these nonlinear systems promoting a biological environment that may facilitate the presence and abundance of certain microbial communities. Could the microbiome be a third-party allostatic system inhabiting humankind? If so, what are the potential pathways linking stress and the gut microbiome?

Mechanisms

There are varying mechanisms that may explain stress-microbiome associations. These include pathways such as the endocrine and immune systems, and microbial metabolic products/signals (Foster et al., 2017; Dinan & Cryan, 2017). Possibly the most compelling is evidence demonstrating that the vagus nerve is a key bidirectional mechanism linking microbial composition to the central nervous system (Bravo et al., 2011; Svensson et al., 2015). This pathway is well demonstrated in vagotomized (an operation that severs one or more branches of the vagus nerve) animals. Specifically, when mice ingested *Lactobacillus* they displayed lower levels of corticosterone, anxious, and depressive behaviors in response to a stressor, however, these effects were not observed in vagotomized mice (Bravo et al., 2011). Interestingly, the vagus nerve can differentiate between pathogenic and non-pathogenic bacteria and can transmit signals that both exacerbate or mitigate anxiety depending on the bacterial stimulus (Forsythe et al., 2014). Human research mirrors these findings. For example, ulcer patients were at higher risk for developing neurological conditions such as Parkinson's disease following truncal vagotomy (removal of a portion of the main trunk of the vagus nerve) when compared to the general population cohort (Svensson et al., 2015) elucidating complex bottom-up pathways. Although vagus nerve fibers are approximately 80-90% afferent and only 10-20% efferent (Aziz &

Thompson, 1998; Vonck & Larsen, 2018), this mechanism also includes top-down directionality. For instance, stressful experiences impact gut motility and secretion via the vagus nerve (Travagli & Anselmi, 2016) highlighting the two-way communication between the mind and the microbiome. But the vagus nerve is only one microbiota-gut-brain (MGB) pathway. Evidence also indicates non-vagal mechanisms are at play in the MGB axis.

The immune system, the endocrine systems, and bacterial metabolic output (including neurotransmitters) are other non-vagal pathways linking the mind and the gut. Cytokines help to regulate the immune system and inflammation (Arango & Descoteaux, 2014) and are able to travel from the gut to the brain if the blood brain barrier is deficient (Dinan & Cryan, 2017). Specifically, levels of Interleukin 1 and Interleukin 6 (known to trigger the release of cortisol via the HPA axis) increase when exposed to bacterial toxins (Yoshioka et al., 1998). Thus, bacterial presence in the gut may be one reason pathogens are linked to stress responses. Beyond stress hormones and immune function, bacteria both produce and absorb neurotransmitters including dopamine and acetylcholine (acetylcholine is a primary neurotransmitter of the PNS; Strandwitz, 2018). Further, the majority of serotonin is produced in the gut and *Bifidobacterium infantis* increases levels of the serotonin precursor tryptophan (Desbonnet et al., 2010). Conversely, microbial growth and colonization is altered by mammalian stress hormones such as epinephrine and norepinephrine (Boyanova, 2019) and microbial diversity is inversely linked to levels of the stress hormone; cortisol in birds (Levin et al., 2016). Indeed, evidence indicates that stress hormones may be another pathway by which microbial composition is shaped. Taken together, evidence linking the mind to the gut supports a cyclical exchange in which the host provides the ecological habitat for microbes and certain microbes may confer either benefits or harm to the host.

Hosting Microbes: Environment Matters

Interestingly, there may be certain microbes that are selected for within a stressed host environment while other microbes thrive in a non-stressed host (Aktipis & Guevara Beltran, 2021). In contrast to pathogenic microbes that appear to benefit from host stress (possible candidates include *E. coli* and *C. jejuni*; Aktipis & Guevara Beltran, 2021), symbiotic microbes are thought to proliferate best when the host is healthy and happy. Indeed, certain symbiotic bacteria are thought to increase social behavior (social proximity is how they are transmitted), promote positive emotion (thought to increase social interactions) and thus facilitate resilience to stress (Aktipis & Guevara Beltran, 2021). *Lactobacillus, Bifidobacterium* (Kuo et al., 2019; Messaoudi et al., 2011; O'callaghan & Van Sinderen, 2016) and *Akkermansia* (Delgadillo et al., 2022) are each linked to adaptive psychosocial responses. For instance, higher levels of naturally occurring *Akkermansia* in human toddlers is linked to better emotion regulation when compared to children with lower levels of *Akkermansia* (Delgadillo et al., 2022). Additionally, a more diverse microbiome is associated with larger social circles, reduced anxiety and less stress (Johnson, 2020). Conversely, *E. coli, Campylobacter jejuni*, and *Gammaproteobacteria* are microbes shown to evolutionarily benefit by exacerbating the stress response in animals (Aktipis & Guevara Beltran, 2021). Aktipis & Guevara Beltran (2021) report that host stress suppresses immune function, is associated with increased intestinal permeability, and increases systemic glucose; all of which promote pathogen growth. Thus, psychological stress loads that persist over time may shape microbial profiles, however, we still do not know how various stressors are differentially linked to diversity or microbial composition.

CHAPTER 2: Aims of the Dissertation

Exploring Links between Stress and the Gut Microbiome: Does Stressor Type Matter?

Past work on the human microbiome and stress is promising but has primarily focused on probiotic interventions and patient populations such as those diagnosed with gut dysbiosis (e.g., irritable bowel syndrome) or stress-related disorders including anxiety and depression (see reviews by Foster & Neufeld et al., 2013; Kelly et al., 2015) with less work assessing stress and the naturally occurring microbiome in healthy samples. Adding to this literature are animal studies that demonstrate causal links in the stress-microbiome connection. For example, in examinations revealing altered stress responses in germ-free mice, vagotomized animals, and in animals that receive fecal transplants (Bravo et al., 2011; Gareau et al., 2010; Schmidt et al., 2020). However, findings from animal research are often not generalizable to human stress experiences described in the current work. Indeed, to my knowledge, there is no published research examining the associations between the gut microbiome and psychological, environmental, and biological stress in a single study assessing two-samples of healthy adults. The current work explores stress-microbiome connections in these two samples to reveal possible patterns across groups and observe whether results replicate across these two studies. This is a vital step in the development of this field because this understanding could inform the next leaps in intervention research. As mentioned earlier, most intervention work on humans is conducted using probiotics and while preliminary findings are promising, it is unlikely that a non-native symbiote can permanently colonize the gut to alter microbial composition (see review by Lerner et al., 2019). Further, probiotics can be harmful in some instances, for example, in post-antibiotic recovery, probiotic use was shown to impede microbial reconstitution (Suez et al., 2018). This leaves open alternate avenues for interventions less explored, such as optimization of the endogenous microbiome with prebiotics or dietary interventions that aim to increase stress

tolerance. However, to do this, we must first have some foundational evidence assessing whether the microbiomes of some individuals are linked to adaptive stress responses and whether these relations change across stressor types. If microbial composition is similar across stressor types, this suggests that the consequences of objective and subjective stress on the microbiome are the same, meaning, the development of an intervention could focus on multiple types of stress at the same time. In contrast, if there are differences between stressor types, this could lead to the development of a targeted approach to intervention that aligns a microbial treatment (e.g., promoting or inhibiting the growth of linked bacteria) with a distinct stressor type. This dissertation will discover whether endogenous gut microbiomes are linked differentially to each stress domain and will lay the foundation necessary for future researchers to discover whether the human microbiome can be recalibrated to mitigate stress.

The current work will explore associations between perceived stress (psychological stress), stressful life events (environmental stress), and cardiovascular stress (biological stress) in relation to levels of microbial diversity and microbial composition in the human gut. I will assess links between each type of stress and relative abundances of microbes within the microbial community in two distinct studies to discover whether findings are replicable and generalizable across each sample. Note that a common theme throughout the study hypotheses presented below is the prediction that microbial composition will be associated with each type of stressor. As we have reviewed, each stressor discussed is linked to numerous health indices (Cohen, 1998). Broadly, biological stress responses are triggered when confronted with a threat, whether perceived or in relation to a stressful life event (Friedman, 1998; Henry, 1993). Therefore, I expect that each stressor type will be linked to microbial composition and that we will see replication of effects across stress domains and in each sample. However, these stress measures

also reflect various durations of time and intensity, for example, in the current study (Study 1 and Study 2) perceived stress offers a measure of the stress participants experienced *"over the last month"*, while RSA (within the context of this study) reflects parasympathetic function surrounding an acute laboratory stressor which lasted for 5-7 minutes. Relatedly, a stressful life event, such as the death of a loved one, potentially combines acute (episodic) and chronic stress, and is typically thought to be very long lasting in its effects (e.g., years). In sum, extant research reveals links between psychological, environmental, biological stress and the gut microbiome. Further, it is established that human physiology is sensitive and responsive to psychological stress (Kemeny, 2003). Thus, I predict that the relation between gut microbes and each stressor type will reflect this sensitivity with varying strengths of associations, therefore, hypotheses will address each stressor individually. Yet, I do not have defined hypotheses beyond what I have outlined thus far and intend to assess these relations in an exploratory manner. For the sake of concision, hypotheses mirrored in both Study 1 and Study 2 are combined below unless otherwise indicated, however, specific aims and analytic approaches for Study 1 and Study 2 will be detailed in the Data Analytic Plan and Results sections. Further, to assess overall diversity and composition in the bacterial community, I will explore stress-microbiome associations in all genera that are present in at least 10% of the samples, however, both hypotheses and aims will provide examples of select bacteria that I predict will be linked to stress (see Table 1).

Bacteria predicted to play a symbiotic role in relation to stress were chosen based on emerging literature linking mitigated stress responses to *Lactobacillus* (Benton et al., 2007; Bravo et al, 2011; Messaoudi et al., 2011) and *Bifidobacterium* (Allen et al., 2016; Messaoudi et al., 2011) and linking higher levels of *Akkermansia* to adaptive psychological processes in children (Delgadillo et al., 2022; Wang et al., 2011). Further, bacteria predicted to play a

pathogenic role within a stress framework were selected based on research connecting *Streptococcus* (Lin et al., 2010; Macerollo et al., 2013), *Clostridium* (Bailey et al., 2011; Mullie et al., 2002), and *Escherichia/Shigella* (see review by Biondi et al., 1997) to exacerbated stress responses. Once again, it is important to emphasize that some of the predicted symbiotic genus contain certain strains that also have pathogenic expressions in particular contexts while some of the predicted pathogenic bacteria contain strains that also have symbiotic expressions that may vary by host. For example, certain strains of *Clostridium* are considered commensal, produce butyrate and are thought to have probiotic properties (Guo et al., 2020). Additionally, *Akkermansia* is a mucin-degrading microbe found primarily in industrialized guts (Smits et al., 2017). Interestingly, levels of *Akkermansia* are high in mouse models lacking dietary fiber (Martens et al., 2008; Sonneneburg et al., 2005). This is important to take into consideration given that fiber consumption is linked to beneficial health outcomes, thus, the role of *Akkermansia* in the human gut as it relates to wellbeing remains broadly undetermined. With all of these complexities in consideration, I used prior research linking stress to select genus to inform the selection of the predicted symbiotic and pathogenic bacteria in Table 1 and to inform the predictions in relevant hypotheses.

Table 1

Bacteria Predicted to Express a Symbiotic or Pathogenic Role in Relation to Stress

Symbiotic (predicted to mitigate stress)	Pathogenic (predicted to intensify stress)
Lactobacillus	*Streptococcus*
Akkermansia	*E. Shigella*
Bifidobacterium	*Clostridium*

Aims & Hypotheses

Psychological Stress

Aim 1 [Study 1 and Study 2]: To determine whether there are differences in alpha diversity or beta diversity between low, mid, and high perceived stress groups

Hypothesis 1: Alpha diversity will be significantly higher in the low perceived stress group when compared to the high perceived stress group.

Hypothesis 2: There will be differences in beta diversity between high and low perceived stress groups.

Aim 2 [Study 1 and Study 2]: To determine whether certain microbes are associated with perceived stress groups and with perceived stress as a continuous measure

Hypothesis 3: There will be differential abundances of certain microbes in perceived low, mid, and high stress groups.

Hypothesis 4: Relative abundance of symbiotic microbes (e.g., *Lactobacillus, Akkermansia*, and *Bifidobacterium*) will have a negative relation with the continuous measure of perceived stress.

Hypothesis 5: Relative abundances of pathogenic bacteria (e.g., *Escherichia/Shigella, Clostridium,* and *Streptococcus*) will have a positive relation with the continuous measure of perceived stress.

Environmental Stress

Aim 3 [Study 1 and Study 2]: To determine whether stressful life events are associated with microbial alpha diversity or beta diversity between low, mid, and high, stressful life events groups

Hypothesis 6: The high stressful life events group will have reduced microbial alpha diversity compared to those in the low stressful life events group.

Hypothesis 7: There will be differences in beta diversity between low, mid, and high stressful life events groups.

Aim 4 [Study 1 and Study 2]: To discover whether certain microbes differentiate low, mid, and high stressful life events groups

Hypothesis 8: Those in the high stress group will significantly differ from the low stress group in certain microbes.

Aim 5 [Study 1 and Study 2]: To determine whether stressful life events are associated with predicted genus

Hypothesis 9: High levels of stressful life events will be associated with reduced relative abundances of symbiotic bacteria.

Hypothesis 9a: There will be positive associations between stressful life events and relative abundances of pathogenic bacteria.

Biological Stress

Aim 6 [Study 1 only]: To determine whether PNS function surrounding acute laboratory stress (assessed via RSA) is linked to microbial alpha diversity (within subject diversity) at rest, in response to or in recovery from a social stressor

Hypothesis 10: There will be differences between low, mid, and high RSA groups and microbial diversity at each RSA measure.

Aim 7 [Study 2 only]: To determine whether PNS function surrounding acute laboratory stress (assessed via RSA) is linked to microbial alpha diversity at rest, or in response to a social stressor

Hypothesis 11: There will be differences between low, mid, and high RSA groups and microbial diversity at each RSA measure.

Hypothesis 11A: I expect that these associations will be weaker compared to Study 1 since the stressor in Study 2 is not directed at the participant.

Aim 8 (Study 1 & Study 2): To discover whether there are differences in beta diversity between low, mid, and RSA groups at each RSA measure

Hypothesis 12: There will be differences in beta diversity between low, mid, and high RSA groups at rest, in reactivity to, and in recovery from a laboratory stressor.

Aim 9 [Study 1 and Study 2]: To discover whether certain microbes differentiate low, mid, and RSA groups at each RSA measure

Hypothesis 13: There will be differences between low, mid, and high RSA groups and certain microbes at each RSA measure.

Aim 10 [Study 1 only]: To determine if RSA at rest, in reactivity to or in recovery from a laboratory stressor will be associated with symbiotic or pathogenic bacteria.

Hypothesis 14: There will be a positive association between RSA at rest, during reactivity and in recovery from an acute laboratory stressor with relative abundances of symbiotic bacteria at each RSA measure.

Hypothesis 15: There will be negative associations between RSA and relative abundances of pathogenic bacteria at each RSA measure.

Aim 11 [Study 2 only]: To determine whether RSA at rest, or in reactivity to a laboratory stressor is associated with symbiotic or pathogenic bacteria

Hypothesis 16: There will be a positive association between RSA and relative abundances of symbiotic bacteria at rest and in reactivity to the stressor.

Hypothesis 17: There will be negative associations between RSA and relative abundances of pathogenic bacteria at rest and in reactivity to the stressor, however, I expect that these associations will be weaker in comparison to Study 1 hypotheses 10 and 11 findings because (as described in the Methods sections below) the acute stressor in Study 1 is aimed directly at the participant while the acute stressor in Study 2 (participant observes their child struggle to solve an impossible puzzle) is directed at the participant's child.

Importance

Some say that we know more about outer space than we know about the microcosm of non-human organisms that reside within every human body. This exciting new frontier is ripe with opportunity for exploration, especially given that very few psychologists have yet to set foot on this uncharted territory. As reviewed, studies on the human microbiome and stress are growing but remain relatively sparse, and diffuse (e.g., commonly assess stress-related disorders, often not stress directly) with the majority of stress specific research primarily conducted on animals. Nevertheless, research to date on both humans and animals is compelling, shows

repeated links between the two constructs and provides a promising foundation for the current dissertation. This work will be among the first to assess whether microbial profiles are linked to perceptions of stress, RSA, and stressful life events in a single study assessing two samples of healthy adults. Findings will inform important next steps in the field, specifically, new interventions. Early intervention work has shown that certain probiotics are linked to adaptive stress responses but these benefits appear to fade once treatment ceases. However, optimizing the naturally occurring microbiome through other intervening measures such as the administration of prebiotics or dietary interventions may be a longer-lasting solution since it would target the growth of potentially symbiotic native inhabitants of the gut. Additionally, examining questions regarding stress-microbiome connections in two diverse human samples will indicate whether results are generalizable, provide a conceptual replication, and will reveal whether the presence or absence of specific microbes might be linked to various types of stress across distinct study designs. Indeed, the concepts, questions and theories addressed in this study, while straight-forward, are innovative and could create avenues by which seminal interventions are developed.

CHAPTER 3: Study 1 - Links Between the Gut Microbiome and Stress in Healthy Adults

Method

Participants

The current study assessed a subset of participants from a larger study on stress, emotion, and health indices. Participants in the larger study were recruited through Craigslist, Facebook, retirement homes, flyers, email, and class announcements (for undergraduate and graduate students at the University of California, Irvine). Individuals in the current study were contacted after participation in the laboratory portion of a larger study via email. The recruitment email contained a $5 gift code as a thank you gift for previous participation and 161 individuals were invited to take part in the at-home microbiome portion of the study. The subset that completed the microbiome portion of the study consisted of 62 adults between the ages of 25-65. This sample size was based on a power analysis with power set at 0.95 and effect size set at 0.22. The sample was 68% female with a mean age of 37.3 years. Participants were compensated $150 for the original larger study and an additional $25 for the microbiome portion follow-up. Individuals were excluded if they were pregnant, had a chronic pain condition, had pulmonary disease, neurological, or psychiatric disorder, had a clinical disorder such as depression or anxiety, had a history of cardiovascular disorder (including hypertension), smoked at the time of recruitment, or regularly took mood altering prescription medication, pain altering medication (e.g., Tylenol, aspirin), cardiovascular function altering medication (e.g., antidepressants, beta blockers, blood pressure altering medication, amphetamines), or four or more medications. Non-English speakers, those that could not perform study tasks, and those that did not own a smartphone were also excluded. The study was approved by the Institutional Review Board at the University of California, Irvine (HS# 2017-3516).

Procedures

There were three distinct parts of the study, 1) baseline assessment, 2) stress reactivity session, and 3) home microbiome assessment. Participants were screened online for study eligibility prior to being scheduled for participation and consented during their baseline assessment. For this dissertation, I draw from the baseline assessment questionnaires completed online and/or at the first in-person study session as well as nurse assessed vitals (height, weight, resting blood pressure).

Approximately one week following the baseline assessment, participants completed the in-lab stress reactivity session. Following a series of stress day questionnaires, participants were connected to an electrocardiogram. Mindware (version 3.1.7) was used to collect and analyze cardiovascular data. Resting baseline RSA was collected for 6 minutes while the participant sat quietly. During this time, participants were instructed to sit still, sit up straight, with legs and arms uncrossed and breathe normally.

Participants then completed the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) in front of a video camera and one judge (research assistant). For this task, participants were given two-minutes to prepare a speech in which they would create an argument explaining why they would be the best candidate for a leadership position at their place of work, club or organization that they were a part of, and then delivered the speech over three minutes in front of the judge and while on camera. Participants were told that their performance would be analyzed by the judge who was an expert trained in public speaking. If the speech was under three minutes, the judge informed the participant that the three minutes were not completed and reminded them that they must fill up the entire three minutes. Participants were permitted to pause, but if they did not continue after 20 seconds, they were told by the judge that they must

continue speaking. During the speech, the judge would say planned critical phrases like, *"You are being too superficial. Please provide additional examples"* and *"You are spending too much time on this aspect; please move on to another strength".* RSA measures were taken throughout the tasks.

Immediately after the speech, the two-minute math task began. The participant was instructed to subtract the number 13 from 1,022 and report their answer verbally. They were told to start over if any mistakes were made. Their time began immediately after the instructions were given and if the participant reported an incorrect answer or was speaking too slowly, the researcher was instructed to say, *"That is incorrect, please start over from 1,022"* and "*Please go as fast as possible."* RSA measures were collected throughout the two-minute math task and during the six-minute undisturbed recovery period that followed.

Participants who completed the baseline assessment and the stress reactivity session were invited by email weeks to months later to participate in the home microbiome portion of the study. The recruitment email contained a $5 Amazon credit code and a thank you for prior involvement. There was no obligation to join the home microbiome session to receive the initial $5, however, participants were informed that they would be compensated with another $20 for their continued involvement. Those that agreed were sent an online study information sheet and questionnaires measuring self-reported stress, diet, and health. They were also mailed a fecal collection kit with detailed instructions designed for public use. All participants were asked not to make any major dietary changes prior to the collection of the microbial sample and to collect and mail the sample within 1-2 weeks of receiving the collection kit. Participants were provided with a pre-paid, pre-addressed box to return the biological specimen according to official United States Postal Service standards. Following at-home collection, the fecal sample was mailed to

UC Irvine for storage and was later sent to another institution and assayed for microbial composition.

Measures

Perceived Stress Scale (PSS)

The current study measured psychological stress using the PSS (Cohen & Williamson,1988). This 10-item measure assessed the frequency in which individuals have perceived stress in the last month on a scale from 0-4. The scale includes items such as, "In the last month, how often have you been upset because of something that happened unexpectedly?", and, "In the last month, how often have you been able to control irritations in your life?", for which participants select *never, almost never, sometimes, fairly often,* or *very often.*

The Holmes and Rahe Life Stress Inventory

The Holmes and Rahe Stress Scale (Holmes & Rahe, 1967) was used to measure exposure to environmental stress. This survey rated 43 potentially stressful life events that individuals may have experienced in the past year. Participants were asked to read through a checklist and answer "*yes*" or "*no*" in regards to whether or not they had experienced occurrences such as "the death of a spouse", "retirement", or a "change in financial state".

Demographics and Body Composition Questionnaires

Participants self-reported demographics (i.e., race/ethnicity, education and income). Weight and height data were collected by a trained nurse. Participant's height and weight was used to calculate body mass index (BMI).

Short Form Health Survey (SF-36)

The Short Form Health Survey (Ware & Sherbourne, 1992) is a commonly administered 36-item quality-of-life measure. Participants were asked to report on their health status by rating

their health on scale from 1-5, as “Excellent” to “Poor”. Specific questions pertaining to general health ratings, bodily pain and physical and mental health issues limiting one’s occupational and social activities were asked. Items were rated on a Likert scale. For example, the scale asks the participants; “Compared to one year ago, how would you rate your health in general now?” with answers ranging from 1-3, “Much better now than one year ago” to “Much worse now than one year ago."

Rapid Eating Assessment for Participants (REAP-S)

The Rapid Eating Assessment for Participants scale (Segal-Isaacson, Rosett, & Gans, 2004) is a survey that assesses nutrition and physical activity patterns. Questions include, “In an average week, how often do you eat less than 2 servings of whole grain products or high fiber starches a day?”, and, “In an average week, how often do you eat less than 2 servings of vegetables a day?”. Answer choices were provided on a Likert scale from 1-3 as follows: “*Usually/often”, “Sometimes”, “Never”*, and *“Does not apply to me”*.

Cardiovascular

Biological stress was assessed using measures of RSA at rest, in reactivity to and in recovery from the laboratory stressor. RSA was collected using electrocardiogram (ECG) and impedance cardiogram (ICG) equipment. Five disposable ECG and ICG electrodes (1.5-inch disposable silver electrodes; Mindware Technologies, Ltd.) were attached to the participant’s torso and placed under the right collar bone, at the anterior point of the sternum, just under the lower right and left ribs, and on the chest near the apex of the heart. Two more disposable leads were placed on the back of the participant on the back of the neck and the lower back. Following placement of the electrodes, signal quality was assessed and, if necessary, adjustments were made to ensure a clear signal. RSA was measured in milliseconds and calculated for each 60-

second segment of data collected. Data collection was initiated and processed using Mindware Version 3.1.7. and for Study 2 BioLab 3.3.1 was used.

Collection and Storage of Stool Samples

Participants were provided with a flushable paper toilet accessory designed to drape from the toilet seat and catch stool prior to exposure to urine or water. Fecal samples were then collected from the paper toilet accessory using sterile plastic applicators and stored in plastic cylindrical collection tubes that contained 2mL of stabilizing fluid. Stabilizing fluid preserved the sample without freezing or refrigeration for up to 60 days. Samples were mailed to UC Irvine and stored in a freezer at approximately -80°C by research personnel and remained there until pyrosequencing was conducted.

Amplicon Sequencing: Study 1 Only

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The samples were processed and analyzed with the ZymoBIOMICS® Service: Targeted Metagenomic Sequencing (Zymo Research, Irvine, CA). DNA extraction was performed using ZymoBIOMICS®-96 MagBead DNA extraction kit. The DNA samples were prepared for targeted sequencing with the *Quick*-16S™ NGS Library Prep Kit (Zymo Research, Irvine, CA). These primers were custom designed by Zymo Research to provide the best coverage of the 16S gene while maintaining high sensitivity. The primer sets used in this project are marked below. *Quick*-16S™ Primer Set V3-V4 (Zymo Research, Irvine, CA)**.**

The sequencing library was prepared using an innovative library preparation process in which PCR reactions were performed in real-time PCR machines to control cycles and therefore limit PCR chimera formation. The final PCR products were quantified with qPCR fluorescence

readings and pooled together based on equal molarity. The final pooled library was cleaned up with the Select-a-Size DNA Clean & Concentrator™ (Zymo Research, Irvine, CA), then quantified with TapeStation® (Agilent Technologies, Santa Clara, CA) and Qubit® (Thermo Fisher Scientific, Waltham, WA).

Control Samples: The ZymoBIOMICS® Microbial Community Standard (Zymo Research, Irvine, CA) was used as a positive control for each DNA extraction, if performed. The ZymoBIOMICS® Microbial Community DNA Standard (Zymo Research, Irvine, CA) was used as a positive control for each targeted library preparation. Negative controls (i.e., blank extraction control, blank library preparation control) were included to assess the level of bioburden carried by the wet-lab process. Sequencing: The final library was sequenced on Illumina® MiSeq™ with a v3 reagent kit (600 cycles) and resulted in an average of 44,832 ± 12,184 reads from the 62 samples. The sequencing was performed with 10% PhiX spike-in. Bioinformatics Analysis: Unique amplicon sequences were inferred from raw reads using the Dada2 pipeline (Callahan et al., 2016). Chimeric sequences were also removed with the Dada2 pipeline. Taxonomy assignment was performed using Uclust from Qiime v.1.9.1. Taxonomy was assigned with the Zymo Research Database, a 16S database that is internally designed and curated, as reference. Independent taxonomic assignment using the Silva database (Quast et al., 2013) was conducted and confirmed identification of common taxa.

Absolute Abundance Quantification*: A quantitative real-time PCR was set up with a standard curve. The standard curve was made with plasmid DNA containing one copy of the 16S gene and one copy of the fungal ITS2 region prepared in 10-fold serial dilutions. The primers used were the same as those used in Targeted Library Preparation. The equation generated by the plasmid DNA standard curve was used to calculate the number of gene copies in the reaction for

each sample. The PCR input volume (2 µl) was used to calculate the number of gene copies per microliter in each DNA sample. The number of genome copies per microliter DNA sample was calculated by dividing the gene copy number by an assumed number of gene copies per genome. The value used for 16S copies per genome is 4. The value used for ITS copies per genome is 200. The amount of DNA per microliter DNA sample was calculated using an assumed genome size of 4.64 x 10^6 bp, the genome size of *Escherichia coli*, for 16S samples, or an assumed genome size of 1.20 x 10^7 bp, the genome size of *Saccharomyces cerevisiae*, for ITS samples. This calculation is shown below:

Calculated Total DNA = Calculated Total Genome Copies × Assumed Genome Size (4.64 × 10^6 bp) × Average Molecular Weight of a DNA bp (660 g/mole/bp) ÷ Avogadro's Number (6.022 x 10^{23}/mole)

Data Availability. Sequence data for 16S metagenomes will be deposited on the National Center for Biotechnology Information (NCBI) sequence read archive (SRA). BioProject accession number will be made public once assigned and R scripts for statistical analysis will be published on GitHub. Data can be requested prior to accession number assignment by sending an email to desic@uci.edu.

CHAPTER 4: Study 2 - Links between the Gut Microbiome and Stress in Healthy Mothers

Method

Participants

The current study recruited mothers of children ages 8 to 12 years old in the Orange and Los Angeles County areas. Research assistants posted advertisements on websites (e.g., Craigslist, public Facebook groups) that parents and children might frequent in the Orange and Los Angeles County areas. Once contacted, research staff briefly explained the requirements, purpose of the study (they were told it was a study on child development), screened participants for eligibility and set up an appointment for the parent-child dyad to participate if qualified. One hundred participants completed the laboratory visit described below and of those, 74 individuals completed the at-home microbiome portion of the study. Specifically, the sample consisted of 74 females with a mean age of 41.6 years; 55.6% White, 23.6% Asian, 11.1% chose more than one race, 7% other, and 2.8% Native Hawaiian. This sample size was based on a power analysis with power set at 0.9 and effect size set at 0.16. Child study data were not included in the current study. Participants were excluded if they were non-English speakers, diagnosed with autism spectrum disorder (ASD), mental retardation (MR), or any other mental or physical diagnoses that would inhibit the ability to understand directions or physically perform study tasks. Mothers with a night shift work schedule were also excluded, due to complications in physiological data collection. Mothers and children received a total of $70 in cash and gift cards for completing both parts of the study. Only mothers' data are included in the current study.

Procedures

The study consisted of two parts, 1) the laboratory visit and 2) the at-home microbiome session. During the laboratory visit, baseline questionnaires were completed and RSA measures were collected at rest and in reactivity to a stressor directed at the participant's child while the

mother observed the child undergo the stressful task. The at-home microbiome session included online self-reported assessments of stress, health and diet. The fecal sample was also collected at-home and mailed to UC Irvine for storage and later sent to another institution to be assayed for microbial composition.

The Laboratory Visit

Upon arrival, parents provided informed consent for participation in the in-lab and at-home portions of the study and permission for their children's participation. Children provided informed assent for participation in both parts of the study but their data were not assessed in the current study. Next, mothers completed surveys measuring stress, health, diet, and psychological variables of interest. The dyad was then brought into the same study room and asked to sit down. Mothers were connected to electrocardiograms and monitored continuously throughout the study. BioLab 3.3.1 was used for data processing and analysis.

Baseline (resting) RSA was collected for 2-minutes in a quiet room with the child present. Following baseline, mothers completed the PCT (Performance Challenge Task) in which the mother watched her child work independently on a series of geometric puzzles modeled after the Block Design task in the WISC III-R (Weschler, 1991). The task included a demonstration, one practice puzzle, and six unsolvable puzzles (participants were unaware of insolvability). Mothers watched children struggle to solve the impossible puzzles for 7-minutes. To underscore failure on the task, a frown face appeared on partially completed puzzles. Mothers and children were also shown a progress bar at the top of the computer screen showing the percentage of puzzles completed correctly. Maternal RSA was measured for the duration of the 7-minute stress task. After completing the study, both members of the dyad were debriefed and informed that the puzzles were impossible to solve.

The At-home Microbiome Session

Upon completion of the in-lab portion of the study, participants were invited to participate in the microbiome portion of the study. Those that consented to participate completed additional stress assessments on-line and received a fecal collection kit in sterile packaging, each containing a collection tube, spatula/ swab utensil, and detailed written instructions. Participants also received pre-paid shipping materials to send their samples back, were instructed not to make any major dietary changes prior to sample collection, and to collect their samples and mail them to UC Irvine within 3-14 days of participation in the study.

Measures

Study 2 used several of the same measures described in Study 1/ the previous section. Please refer to Study 1 measure descriptions for the following assessments also used in Study 2; The Perceived Stress Scale (Cohen & Williamson,1988), the Holmes and Rahe Stress Scale (Holmes & Rahe, 1967), the Short Form Health Survey (Ware & Sherbourne, 1992), and the Rapid Eating Assessment for Participants scale (Segal-Isaacson, Rosett, & Gans, 2004).

Demographics and Body Composition Questionnaires

Participants self-reported demographics (i.e., race/ethnicity, education and income). Following the at-home microbiome sample collection, participants were asked by email to self-report height and weight. Participants height and weight was used to calculate body mass index (BMI).

Cardiovascular

RSA measures were collected using electrocardiogram (ECG) and impedance cardiogram (ICG) equipment. Five disposable ECG and ICG electrodes (1.5-inch disposable silver

electrodes; Mindware Technologies, Ltd.) were attached to the participant's torso and placed under the right collar bone, at the anterior point of the sternum, just under the lower right and left ribs, and on the chest near the apex of the heart. Two more disposable leads were placed on the back of the participant on the back of the neck and the lower back. Following placement of the electrodes, signal quality was assessed and, if necessary, adjustments were made to ensure a clear signal. RSA was measured in milliseconds and calculated for each 60-second segment of data collected. Data collection was initiated and processed using BioLab 3.3.1.

Collection and Storage of Stool Samples

Participants were provided with a flushable paper toilet accessory designed to drape from the toilet seat and catch stool prior to exposure to urine or water. Fecal samples were then collected from the paper toilet accessory using sterile plastic applicators and stored in plastic cylindrical collection tubes that contained 2mL of stabilizing fluid. Stabilizing fluid preserved the sample without freezing or refrigeration for up to 60 days. Samples were mailed to UC Irvine and stored in a freezer at approximately -80°C by research personnel until pyrosequencing was conducted.

Amplicon Sequencing: Study 2 Only

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The samples were processed and analyzed with the ZymoBIOMICS® Service: Targeted Metagenomic Sequencing. ZymoBIOMICS®-96 MagBead DNA Kit was used to extract DNA. The DNA samples were prepared for targeted sequencing with the Quick-16S™ NGS Library Prep Kit. These primers were custom-designed by Zymo Research to provide the best coverage

of the 16S gene while maintaining high sensitivity. The primer sets used in this project were the Quick-16S™ Primer Set V3-V4.

Sequencing Analysis: The sequencing library was prepared using an innovative library preparation process in which PCR reactions were performed in real-time PCR machines to control cycles and therefore limit PCR chimera formation. The final PCR products were quantified with qPCR fluorescence readings and pooled together based on equal molarity. The final pooled library was cleaned up with the Select-a-Size DNA Clean & Concentrator™ (Zymo Research, Irvine, CA), then quantified with TapeStation® (Agilent Technologies, Santa Clara, CA) and Qubit® (Thermo Fisher Scientific, Waltham, WA).

Control Samples: The ZymoBIOMICS® Microbial Community Standard was used as a positive control for each DNA extraction. The ZymoBIOMICS® Microbial Community DNA Standard was used as a positive control for each targeted library preparation. Negative controls (i.e., blank extraction control, blank library preparation control) were included to assess the level of bioburden carried by the wet-lab process. Sequencing: The final library was sequenced on Illumina® MiSeq™ with a v3 reagent kit (600 cycles) and resulted in an average of 37,112 ± 9,708 reads from 74 samples. The sequencing was performed with 10% PhiX spike-in. Bioinformatics Analysis: Unique amplicon sequences were inferred from raw reads using the Dada2 pipeline (Callahan et al., 2016). Chimeric sequences were also removed with the Dada2 pipeline. Taxonomy assignment was performed using Uclust from Qiime v.1.9.1. Taxonomy was assigned with the Zymo Research Database, a 16S database that is internally designed and curated, as reference. Independent taxonomic assignment using the Silva database (Quast et al., 2013) was conducted and confirmed identification of major taxa.

Absolute Abundance Quantification*: A quantitative real-time PCR was set up with a standard curve. The standard curve was made with plasmid DNA containing one copy of the 16S gene and one copy of the fungal ITS2 region prepared in 10-fold serial dilutions. The primers used were the same as those used in Targeted Library Preparation. The equation generated by the plasmid DNA standard curve was used to calculate the number of gene copies in the reaction for each sample. The PCR input volume (2 µl) was used to calculate the number of gene copies per microliter in each DNA sample. The number of genome copies per microliter DNA sample was calculated by dividing the gene copy number by an assumed number of gene copies per genome. The value used for 16S copies per genome is 4. The value used for ITS copies per genome is 200. The amount of DNA per microliter DNA sample was calculated using an assumed genome size of 4.64 x 106 bp, the genome size of *Escherichia coli*, for 16S samples, or an assumed genome size of 1.20 x 107 bp, the genome size of Saccharomyces cerevisiae, for ITS samples. This calculation is shown below:

Calculated Total DNA = Calculated Total Genome Copies × Assumed Genome Size (4.64 × 106 bp) × Average Molecular Weight of a DNA bp (660 g/mole/bp) ÷ Avogadro's Number (6.022 x 1023/mole)

Data Availability. Sequence data for 16S metagenomes will be deposited on the National Center for Biotechnology Information (NCBI) sequence read archive (SRA). BioProject accession number will be made public once assigned and R scripts for statistical analysis will be published on GitHub. Data can be requested prior to accession number assignment by sending an email to desic@uci.edu.

CHAPTER 5: Data Analytic Plan for Study 1 and Study 2

Statistical Approach

Bacterial relative abundances were derived from QIIME (bioinformatics tool used to perform microbiome analysis from raw DNA sequences) and assessed using SPSS and/or R. When data were missing, *t*-tests were conducted on key variables and showed that data were missing at random and met assumptions needed for multiple imputation (Li et al., 2015). In total, data for key variables were imputed for 12 participants in Study 1 and 15 participants in Study 2 resulting in a sample of $N = 62$ and $N = 74$, respectively. Importantly, the pattern of findings did not change when using the imputed versus non-imputed datasets. To preserve statistical power, only bacterial genera that were present in at least 10% of the samples were included in analyses resulting in the exclusion of 90 genera and inclusion of 111 genera in Study 1. Similarly, for Study 2, only bacterial genera that were present in at least 10% of the samples were included in analyses resulting in the exclusion of 98 genera and inclusion of 85 genera for primary analyses. All stress data in Study 1 and Study 2 are continuous but tertiles were calculated to create a 2/3rd group for all high stress groups and a 1/3rd group for all low stress groups. Permutational analysis of variance analyses (PERMANOVA) of Bray-Curtis dissimilarities was used to quantify variation in genus between samples. Specifically, beta diversity was calculated using the adonis2 function in Vegan package 2.6-4 to determine Bray-Curtis distance matrices and conduct PERMANOVA (Oksan et al., 2013) significance testing for compositional data. Nonmetric multidimensional scaling (NMDS) ordination obtained from the 'metaMDS' function in Vegan to plot beta diversity.

Perceived stress, stressful life events, and RSA/physiological stress tertile groups were used for all hypotheses assessing alpha and beta diversity and (when PERMANOVA's were statistically significant) follow-up analyses were conducted to determine which microbes

differentiated stress groups from each other. Specifically, permutational multivariate analysis of variance (PERMANOVA) was conducted to test differences between stress groups. Power analysis for PERMANOVA is not well-established/widely reported in published microbiome literature, however, the stress group sizes in the current studies were comparable and often larger than groups found in extant human stress-microbiome literature (Hantsoo et al., 2019; Mackner et al., 2020; Michels et al., 2019).

Table 2

Summary of Stress Group Membership by Sex in Study 1

Stress Group	Males	Females	Total
Low Perceived Stress	8	14	22
Mid Perceived Stress	6	15	21
High Perceived Stress	3	16	19
Low Stressful Life Events	6	14	20
Mid Stressful Life Events	5	17	22
High Stressful Life Events	6	14	20
Low Reactivity RSA	4	16	20
Mid Reactivity RSA	4	17	21
High Reactivity RSA	9	12	21

Table 3

Summary of Stress Group Membership in Study 2

Stress Group	Total = Females Only
Low Perceived Stress	26
Mid Perceived Stress	23
High Perceived Stress	25
Low Stressful Life Events	24
Mid Stressful Life Events	25
High Stressful Life Events	25
Low Reactivity RSA	24
Mid Reactivity RSA	25
High Reactivity RSA	25

If the groups were significantly different, I attempted to identify differentially abundant microbes using the randomForest 4.7-1.1 package in R (a commonly used machine learning approach to assess which features differentiate groups), however, the results of the Random Forest (RF) for relevant models in both studies had low accuracy rates. Specifically, after carrying out tuning procedures (adjusting relevant parameters) error rates remained between 28.12% to 34.69% in each model, thus, these outcomes were not informative when attempting to identify microbes that differentiated stress groups. Therefore, PERMANOVAs that revealed significant differences between stress groups were probed to discover which microbes differentiated groups using analysis of composition of microbiomes with bias correction (ANCOM-BC); a microbiome specific statistical methodology that accounts for underlying compositional structure in the microbiome. ANCOM-BC corrects for multiple comparisons,

normalizes zero-inflated abundances, and identifies structural zeros when groups are completely missing or nearly completely missing a particular taxon (Lin et al., 2021). The identification of structural zeros is one qualitative assessment used in microbiome work to identify the presence and absence of certain taxa (Bruno et al., 2022; Hawkins et al., 2022). Additionally, ANCOM-BC calculations did not target microbes hypothesized a priori, and as with all previously mentioned analyses, included all genus present in at least 10% of the samples. These analyses were conducted in an exploratory manner; however, the ANCOM-BC approach corrects for multiple comparisons and only adjusted *p*-values are reported.

Correlation and Regression Analyses for Select Genus

Zero-order correlations among perceived stress, stressful life events, RSA baseline, RSA reactivity, RSA recovery and bacterial genera were conducted to test whether these variables were significantly intercorrelated prior to tertile group assignment. These analyses were performed to investigate associations between levels of stress within each stressor type in all 62 participants in Study 1 and all 74 participants in Study 2 and select genus. Further, genus selection was based on extant literature linking certain taxa to stress to reduce multiple comparisons. Measures of select genus were transformed using centered log-ratio (Gloor et al., 2016) to normalize zero-inflated relative abundances, however, raw relative abundance values in significant models (prior to transformation) are reported in Supplemental Tables 1 and 6.

Multiple comparison procedures were used to control for the false discovery rate. False discovery rates (q-values) in genome and metagenome (microbiome) research vary and include q-values such as .15 (Cole et al., 2015). In the current study we considered a p-value < .05 and a q-value < .15 significant as has been done in past similar work (Cole et al., 2015). To probe significant findings further, linear regression models were conducted as used in similar work

(Heym et al., 2019; Pallister et al, 2017). All Linear Models (LM) adjust for sex (in Study 1 only), diet, overall health, BMI, and age. Covariates were chosen based on prior research suggesting that sex (Jašarević et al., 2016), diet (David et al., 2014), overall health, BMI (Crovesy et al., 2020; Ottosson et al., 2018), and age (Cresci et al., 2015) influence gut microbial profiles. This group of covariates will now be referred to as physical covariates. RSA baseline was adjusted for in RSA reactivity analyses for Study 1 and Study 2 and for reactivity and recovery analyses in Study 1. I include two steps per model. Step 1 includes variables for which I adjust for covariates and step 2 includes the predictor of interest. The adjusted R^2 and *p*-values of step 1 of each model and the R^2 change, unstandardized beta coefficient, standard error, and *p*-values of step 2 are reported for each significant model in the Results section below. Descriptive statistics and correlation matrices for variables in statistically significant models in Study 1 and Study 2 are reported in Supplementary Tables 1, 2, 6, and 7.

Overview of Study 1 and Study 2 Microbiota Composition

The relative abundance of microbial families across all samples in Study 1 and Study 2 are depicted in Supplemental Figures 1 and 2. Note that the top 10 most abundant taxa depicted in Supplemental Figures 1 and 2 are similar between Study 1 and Study 2. Specifically, the only differences between the most common bacteria displayed in each study was the inclusion of *Verrucomicrobiaceae* in Study 1 and the inclusion of *Rikenellaceae* in Study 2. Further, the top 10 most abundant taxa in both studies complement research attempting to define a universal core of taxa shared by human individuals. When compared to this data from around the world, namely, El Salvador, Madagascar, Peru, China, Japan, and the United States, the most abundant bacteria in both studies corresponded to the universal core of taxa proposed by Piquer-Estaban and colleagues (2021) with the exception of *Bifidobacteriaceae, Erysipelotrichaceae,*

Peptostreptococcaceae, and *Verrucomicrobiaceae*; bacterial families that include genus and species that tend to be more prevalent in the gut microbiome of those in industrialized countries (Feng et al., 2019; Sonnenberg et al., 2019). Additionally, means and standard deviations of relative abundance values for all select bacteria in Study 1 and Study 2, namely, *Lactobacillus, Akkermansia, Bifidobacterium, Streptococcus, Escherichia/Shigella,* and *Clostridium* prior to log centered ratio transformations are reported in Supplemental Table 8. There were some observable differences between relative abundance and prevalence of select taxa in Study 1 and Study 2. Possible explanations for these differences are explained in detail in the Limitations section. Further, the prevalence of some of the select genus is not well-established in healthy samples, and varies by individual, however, the values displayed in Supplemental Table 8 coincide with research showing that the prevalence of both *Bifidobacterium* (Matsuki et al., 1999) and *Clostridium* (Piquer-Estaban et al., 2022) in the human gut typically exceeds 90% in healthy adults.

Results Organization and Structure

Results will be presented for both studies in parallel, organized by stressor type and related study aim and hypothesis. I will first report the findings for psychological stress indexed using measures of perceived stress. Second, I will report environmental stress as indexed by the occurrence of stressful life events. Finally, I will report findings relating to physiological stress as measured by respiratory sinus arrhythmia (RSA) at rest, in reactivity to and in recovery from a laboratory stressor. At the end of the results section, I will show a summary table reviewing the general pattern of results for hypotheses in both studies, and then will integrate these into the broad discussion.

CHAPTER 6: Results for Study 1 and Study 2

Psychological Stress

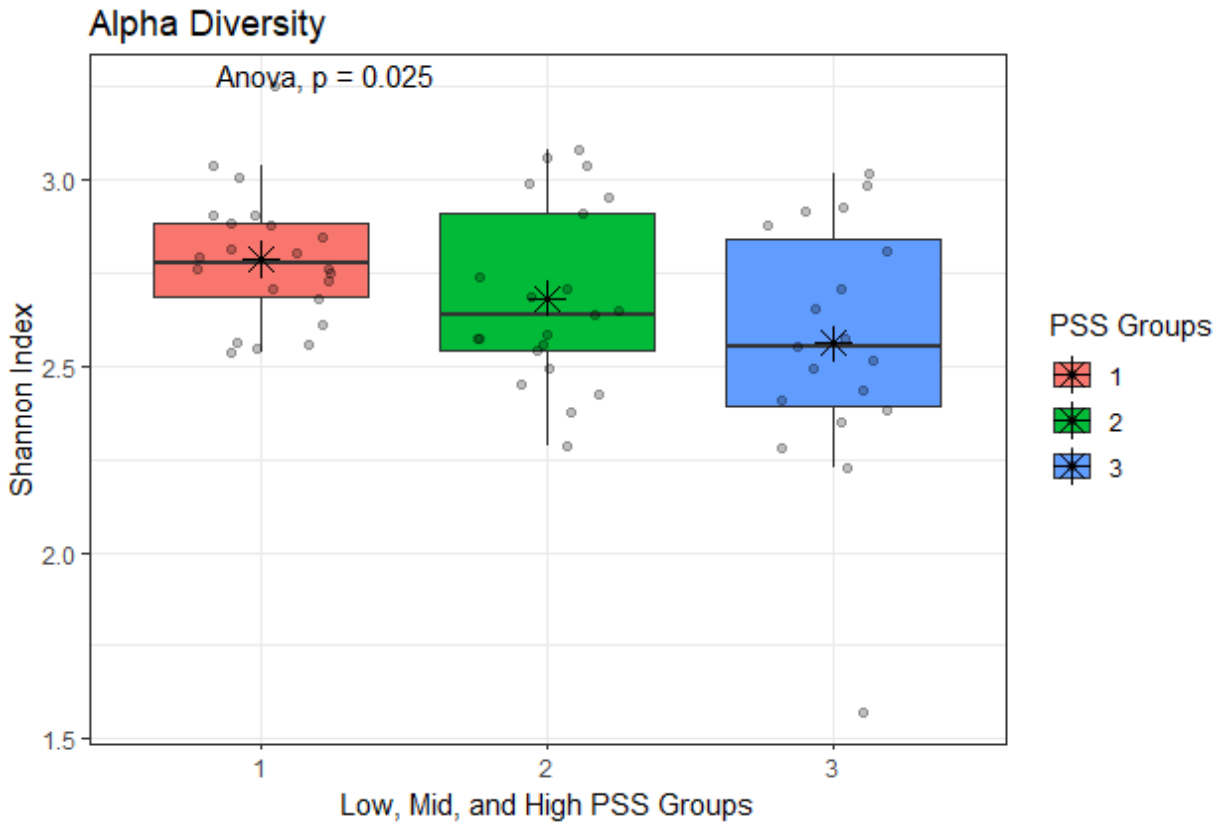
Aim 1 [Study 1 and Study 2]

To Determine Whether there are Differences in Alpha or Beta diversity between Low, Mid, and High Perceived Stress Groups

Significant Differences in Alpha Diversity between Perceived Stress Groups for Study 1. As hypothesized, there were statistically significant differences in alpha diversity between perceived stress groups, but this was only found in Study 1. Specifically, a one-way ANOVA was conducted to test group differences in alpha diversity between perceived stress groups. There were significant differences in alpha diversity between groups at the $p < .05$ level when comparing low, mid, and high perceived stress groups, $F(2,59) = 3.923$, $p = .0251$, in Study 1 as shown in Figure 1. Tukey's HSD Test for multiple comparisons found no statistically significant difference between low and mid stress groups (adj. $p = .5004$) or mid and high stress groups (adj. $p = .3086$), however, the mean value of alpha diversity was significantly different between low and high PSS groups (adj. $p = .0316$, 95% C.I. = -0.4222, -0.0160).

Figure 1

Shannon Diversity of those in the Low Perceived Stress Group is Significantly Higher than those in the High Perceived Stress Group



Note. Box plots showing Shannon diversity across low (pink; group 1), mid (green; group 2), and high (blue; group 3) perceived stress groups in Study 1. All samples were sequenced using 16S sequencing. The center line within each box defines the mean, boxes define the upper and lower quartiles, and whiskers define the interquartile range.

Next, a one-way ANCOVA was conducted to determine whether the statistically significant difference between low, mid, and high perceived stress groups on Shannon diversity remained after controlling for age, sex, BMI, diet, and general health. There was still a significant effect of perceived stress groups on alpha diversity, $F(2, 52) = 5.047$, $p = .0099$. Further, I tested whether group differences would persist when rare taxa (present in less than 10% of the samples) were included in analyses. A one-way ANOVA revealed that the significant pattern remained. There were significant differences in alpha diversity between groups at the $p < .05$ level for the three perceived stress groups, $F(2, 59) = 3.371$, $p = .0414$. In contrast to Study 1, ANOVA revealed no statistically significant differences in alpha diversity by perceived stress group for Study 2.

No Statistically Significant Differences in Microbial Composition between Perceived Stress Groups. Inconsistent with my hypotheses, PERMANOVA revealed that there were no statistically significant differences in beta diversity between perceived stress groups for either study. As a result, I did not follow up with ANCOM-BC calculations.

Aim 2 [Study 1 and Study 2]

To Determine Whether Select Microbes are Associated with Perceived Stress as a Continuous Measure

No Associations between *Lactobacillus, Akkermansia, Bifidobacterium, Streptococcus* or *Clostridium* and Perceived Stress as a Continuous Measure. I hypothesized that there would be associations between the relative abundance of select microbes at different levels of perceived stress as a continuous measure, but Pearson correlations revealed that there were no significant associations between *Lactobacillus, Akkermansia*, *Bifidobacterium, Streptococcus* or

Clostridium and perceived stress in either study. The means and standard deviations for relative abundance and also the prevalence of select microbes are reported in Supplemental Table 8.

***Escherichia/Shigella* Predicts Levels of Perceived Stress.** Finally, consistent with hypothesis 5, I found that levels of *Escherichia/Shigella* significantly, positively predicted perceived stress ($r(72) = .264$, $p = .023$) in Study 2. After adjusting for covariates (adjusted $R^2 = .100$, $p = .032$), *Escherichia/Shigella* remained significantly, positively associated with perceived stress, $\Delta R^2 = 0.047$, $b = .158$, $SE = .883$, $p = 0.049$, 95% CI = .006 - 3.53**.** Inconsistent with Study 2, there were no associations between pathogenic bacteria and perceived stress in Study 1. Supplementary Tables 1 and 2 report descriptive statistics and correlations for key variables in Study 2. The mean relative abundance of *Escherichia/Shigella* (prior to CLR transformation) was .147% with a standard deviation of .371%. These values are also reported in the legend of Supplemental Table 1 and in Supplemental Table 8.

Environmental Stress

Aim 3 [Studies 1 and 2]

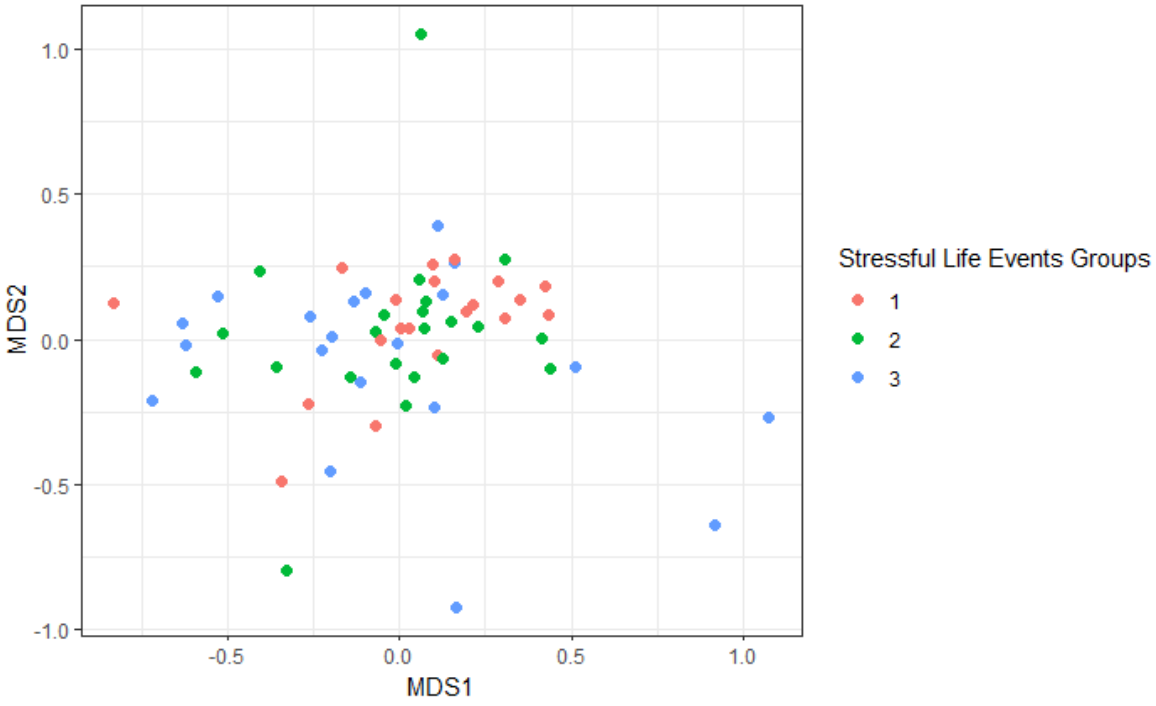
To Determine Whether Stressful Life Events are Associated with Microbial Alpha Diversity or Beta Diversity between Low, Mid, and High, Stressful Life Events Groups

Significant Differences between Stressful Life Events Groups for Beta but not Alpha Diversity in Both Studies. Contrary to hypotheses, there were no significant differences in alpha diversity (Shannon) between environmental stress groups in Study 1 or Study 2. However, Hypothesis 7 was confirmed revealing group differences in stressful life events groups in both studies. First, PERMANOVA revealed that there were significant differences in community structure between high, middle, and low stressful life events groups ($R^2 = 0.0546$, $p = .028$;

Figure 2) in Study 1. After controlling for sex (R^2 = 0.0303, p = .043), the results of the PERMANOVA remained significant (R^2 = 0.0548, p = .024).

Figure 2

Differences in Beta Diversity between Low, Mid, and High Stressful Life Events Groups in Study 1

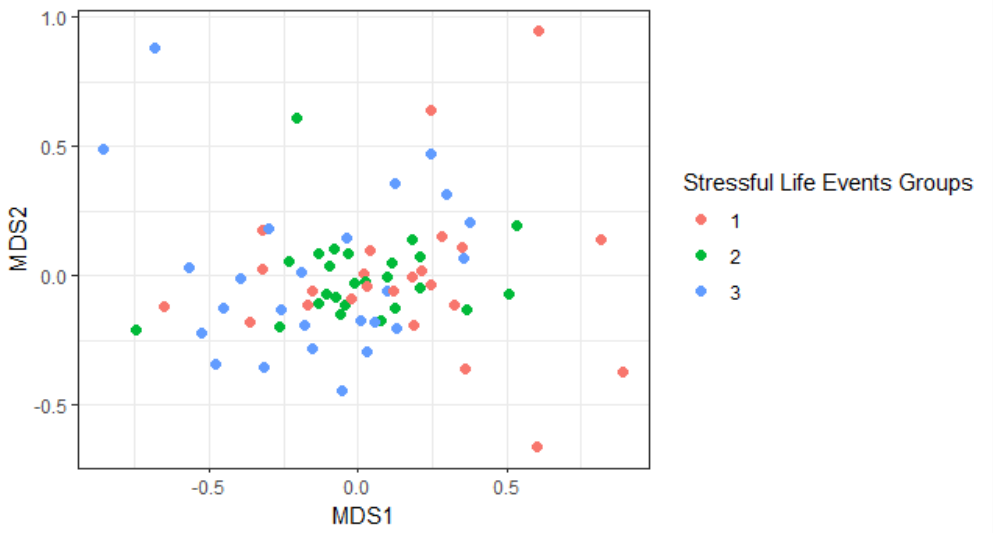


Note. Nonmetric multidimensional scaling (NMDS) ordination using Bray-Curtis dissimilarity to calculate distances on the genus level between stressful life events groups: low (pink: 1), mid (green; 2), and high (blue; 3) in Study 1. Each point represents one sample at one time point. The x and y-axis only provide a reference in which to gauge Bray-Curtis distances in relation to the other samples but are not a meaningful value independently.

Second, Study 2 also showed differences in microbial composition in stressful life events groups. PERMANOVA revealed that there were significant differences in community structure between high, middle, and low stressful life events groups (R^2 = 0.05023, p = .013; Figure 3).

Figure 3

Differences in Beta Diversity between Low, Mid, and High Stressful Life Events Groups in Study 2



Note. Nonmetric multidimensional scaling (NMDS) ordination using Bray-Curtis dissimilarity to calculate distances on the genus level between stressful life events groups: low (pink: 1), mid (green; 2), and high (blue; 3). Each point represents one sample at one time point in Study 2. The x and y-axis only provide a reference in which to gauge Bray-Curtis distances in relation to the other samples but are not a meaningful value independently.

Aim 4 [Studies 1 and 2]

To Discover Whether Certain Microbes Differentiate Low, Mid, and High Stressful Life Events Groups

Differential Abundances of Certain Microbes Distinguished Environmental Stress Groups in Both Studies. Low, mid, and high stressful life events groups showed differences in the presence and abundance of certain microbes in both studies and also significantly differed in levels of absolute abundances of certain genus between stressful life events groups in Study 2. In Study 2, *Gemella* was higher in the low stress group than the high stress group (lfc = -3.76030; adj. p = .0394). Additionally, *Catabacter* was lower in the low stress group than the high stress group (lfc = 5.12483; adj. p = .0394) in Study 2. In Study 1, *Intestinimonas* and *Faecalibacterium/Subdoligranulum* were only absent in the high stressful life events group. In Study 2, *Odoribacter* and *Granulicatella* were only absent in the high stressful life events group. Additionally, only *Eubacterium/Roseburia* was absent in both studies in the high stressful life events groups. A complete report of present/absent outcomes for stressful life events are found in Supplemental Tables 3 and 4.

Aim 5 [Studies 1 and 2]

To Determine Whether Stressful Life Events are Associated with Predicted Genus

No Associations between Select Microbes and Stressful Life Events. Regression analyses were conducted to test whether stressful life events were tied to *Lactobacillus*, *Akkermansia* and *Bifidobacterium*. None of the predicted symbiotic bacteria were associated with levels of stressful life events in Study 1 or Study 2. Similarly, regression analyses found no associations between *Streptococcus, Escherichia/Shigella*, or *Clostridium* and stressful life events.

Biological Stress

Aim 6 [Study 1 only]

To Determine Whether PNS Function Surrounding Acute Laboratory Stress (assessed via RSA) is Linked to Microbial Alpha Diversity at Rest, in Response to or in Recovery from a Social Stressor

No Significant Differences in Alpha Diversity between RSA groups in Study 1. There were no significant differences in alpha diversity (Shannon) between RSA groups at rest, in reactivity to or in recovery from the laboratory stressor in Study 1.

Aim 7 [Study 2 only]

To Determine Whether PNS Function Surrounding Acute Laboratory Stress (assessed via RSA) is Linked to Microbial Alpha Diversity at Rest, or in Response to a Social Stressor

No Significant Differences in Alpha Diversity between RSA Groups in Study 2. There were no significant differences in alpha diversity (Shannon) between RSA physiological stress groups in Study 2 at rest or in response to the laboratory social stressor. Unlike Study 1, this study did not measure RSA recovery from the stressor.

Aim 8 [Studies 1 and 2]

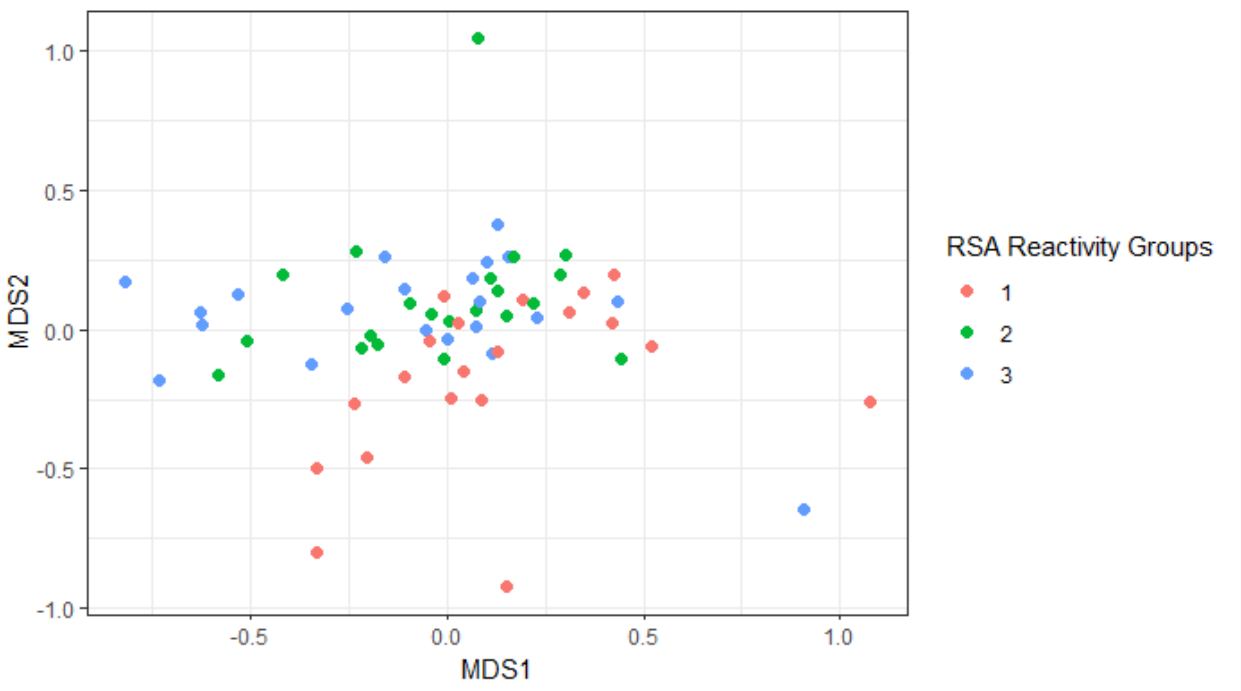
To Discover Whether There are Differences in Beta Diversity between Low, Mid, and High RSA Groups at each RSA Measure (i.e., rest, reactivity and recovery from stress)

Significant Differences between RSA Stress Reactivity Groups for Beta Diversity in Study 1 but not Study 2. RSA was measured at rest, in reactivity to and in recovery from a laboratory stressor. Significant associations were only found during reactivity to the laboratory stressor. Specifically, permutational analysis of variance revealed differences in microbial composition between RSA stress reactivity groups in Study 1 in response to the laboratory stressor. PERMANOVA revealed that there were significant differences in community structure between high, middle, and low RSA reactivity groups (R^2 = 0.0674, p = .005; Figure 4). After

controlling for sex (R^2 = 1.494, p = .123), and baseline RSA (R^2 = .6105, p = .837) the results of the PERMANOVA remained significant (R^2 = 2.138, p = .004). No other RSA stress time points (at rest or in recovery from stress) were found to correlate with beta diversity.

Figure 4

Differences in Beta Diversity between Low, Mid, and High RSA Stress Reactivity Groups in Study 1



Note. Nonmetric multidimensional scaling (NMDS) ordination using Bray-Curtis dissimilarity to calculate distances on the genus level between RSA groups during the laboratory stressor: low (pink: 1), mid (green; 2), and high (blue; 3). Each point represents one sample at one time point for Study 1. The x and y-axis only provide a reference in which to gauge Bray-Curtis distances in relation to the other samples but are not a meaningful value independently.

Aim 9 [Studies 1 and 2]

To Discover Whether Certain Microbes Differentiate Low, Mid, and RSA groups at each RSA Measure

Differential Abundances of Certain Microbes Distinguished RSA Stress Reactivity Groups in Study 1. Differential abundances were due to presence/absence of bacteria according to group membership (see Supplemental Table 5) and differing levels of certain taxa. Specifically, the low-RSA stress reactivity group had higher levels of *Eggerthella* than the mid-RSA reactivity group (lfc = -5.2282; adj. *p*-values = 0.0422) and the high-RSA reactivity group was also significantly lower in *Eggerthella* than the low-RSA reactivity group (lfc= -5.2446; adj. *p*-value = 0.0196). Further, the low-RSA reactivity group was significantly lower in *Lachnoclostridium/Roseburia* (lfc = 5.909; adj. *p*-value = 0.0196) than the high-RSA reactivity group.

In Study 1 *Slackia*, *Blautia/Marvinbryantia*, and *Faecalibacterium/Subdoligranulum* were only absent in the low RSA stress reactivity group while *Denitrobacterium* and *Oxalobacter* were only absent in the high RSA stress reactivity group. A complete report of present/absent outcomes for RSA stress reactivity groups during the laboratory stressor are found in Supplemental Table 5.

Aim 10 [Study 1 only]

To Determine if RSA at Rest, in Reactivity to or in Recovery from a Laboratory Stressor will be Associated with Symbiotic or Pathogenic Bacteria

***Clostridium* Predicts Levels of RSA Stress Reactivity.** Pearson correlations were performed to reveal associations between *Lactobacillus, Akkermansia, Bifidobacterium (i.e., types of predicted symbiotic bacteria),* and levels of RSA. Contrary to my hypothesis, no

predicted symbiotic bacteria were associated with RSA at rest, in reactivity to, or in recovery from the stressor in Study 1. However, Pearson correlations were also performed to reveal associations between *Streptococcus, Escherichia/Shigella, Clostridium (i.e., types of pathogenic bacteria),* and RSA measures surrounding stress. Consistent with my hypothesis, *Clostridium* was significantly negatively associated with stress reactivity RSA ($r(62) = -.369$, $p = .003$). After adjusting for sex, diet, overall health, BMI, age and baseline RSA (adjusted $R^2 = .092$, $p = 0.104$), *Clostridium* remained negatively associated with RSA stress reactivity, $\Delta R^2 = 0.076$, $b = -.484$, $SE = .206$, $p = 0.023$, 95% CI = -.897 - -.070. Supplementary Tables 6 and 7 report descriptive statistics and correlations for key variables in Study 1. The mean relative abundance value of *Clostridium* (prior to CLR transformation) was .488% and the standard deviation was 1.352%. These values are also reported in the legend of Supplemental Table 6 and in Supplemental Table 8.

Aim 11 [Study 2 only]

To Determine Whether RSA at Rest, or in Reactivity to a Laboratory Stressor is Associated with Symbiotic or Pathogenic Bacteria

***Lactobacillus* Predicts RSA Stress Reactivity but not After Adjusting for Covariates.** Pearson correlations were performed to reveal associations between *Lactobacillus, Akkermansia, Bifidobacterium,* and RSA measures at rest and in reactivity to the laboratory stressor. *Lactobacillus* was significantly positively associated with RSA stress reactivity ($r(72) = .269$, $p = .020$). After adjusting for age, BMI, diet, health, and baseline RSA (adjusted $R^2 = .642$, $p < .001$), *Lactobacillus* was no longer significantly associated with RSA stress reactivity, $\Delta R^2 = 0.013$, $b = 1.768$, $SE = .098$, $p = 0.110$, 95% CI = -.037 - .353**.** Supplementary Tables 1 and 2 report descriptive statistics and correlations for key variables in Study 2. The mean relative

abundance value of *Lactobacillus* (prior to CLR transformation) was .070% and the standard deviation was .371%. These values are also reported in the legend of Supplemental Table 1 and in Supplemental Table 8.

No Associations between Select Pathogenic Bacteria and RSA at Rest or in Response to a Laboratory Stressor in Study 2. Pearson correlations were also performed to reveal associations between *Escherichia/Shigella, Streptococcus, Clostridium* and RSA measures surrounding stress. There were no significant associations between select pathogenic bacteria and RSA in Study 2.

Below is a summary of the stress-microbiome findings across both studies. Table 4 provides a global view of the results for ease of comparison. Results will be described in detail in the Discussion section following Table 4.

Table 4

Associations and Group Differences in Microbial Composition by Stressor Type for Study 1 and Study 2

Stress Measures	Study 1	Study 2
Alpha Diversity		
Perceived Stress	Increased in low compared to high	x
Stressful Life Events	x	x
RSA Stress Reactivity	x	x
Beta Diversity		
Perceived Stress	x	x
Stressful Life Events	Group differences	Group differences
RSA Stress Reactivity	Group differences	x
ANCOM-BC		
Perceived Stress	x	x
Stressful Life Events	Differential abundances between groups (present/absent)	Differential abundances between groups (present/absent) *Gemella* ↓ in low stress group *Catabacter* ↑in low stress group
RSA Stress Reactivity	Differential abundances between groups (present/absent) *Eggerthella* ↓ in high RSA group *Lachnoclostridium/Roseburia* ↓ in low RSA	x
	Regressions	
Perceived Stress	x	*Escherichia/Shigella* positively associated with perceived stress
Stressful Life Events	x	x
RSA Stress Reactivity	*Clostridium* negatively associated with RSA	x

Note. This table represents broad findings and displays a comparison of results across Study 1 and Study 2 with 'x' depicting null findings. Details of differential abundances (present/absent) results can be found in Supplemental Tables 3, 4, and 5.

CHAPTER 7: Discussion

Discussion

The overarching aim of this dissertation was to assess mind-microbiome connections within the context of a stress triad consisting of three different domains including environmental, physiological, and psychological stress. This allowed for comparisons across stressor types, across two samples, and added both novel contributions and support to extant literature. While most previous studies have examined the impact of individual stressors or stress-related conditions such as depression and anxiety on the microbiome, this dissertation takes a more comprehensive stress-focused approach by investigating a network of stressors and microbial composition in two-samples of healthy adults. Broadly, results revealed stress-microbiome connections in each domain in at least one study and suggest that these associations are sensitive to stress context. In sum, findings hint at the possibility that beta diversity is most robustly linked to potentially severe, chronic stressors, while connections between beta diversity and biological stress may be more likely to vary by sample type. Additionally, those low in perceived stress had higher alpha diversity compared to those with high perceived stress in Study 1, levels of *Clostridium* were negatively associated with RSA stress reactivity in Study 1, and levels *Escherichia/Shigella* were positively associated with perceived stress in Study 2. Below I review the findings of the current work and describe results across the contexts of each stressor type in each study. By examining differences in microbial composition, I aimed to contribute to the current literature by providing an extensive, yet nuanced understanding of stress-microbiome connections.

Psychological Stress (i.e., Perceived Stress)

As a reminder, perceived stress was indexed using self-reports that assessed feelings of stress and overwhelm over the last month (Cohen et al., 1983). As hypothesized, there were

statistically significant differences in alpha diversity between high and low perceived stress groups for Study 1, such that those lower in perceived stress had greater alpha diversity (i.e., a more diverse microbiome) than those in the high perceived stress group. These results support other studies suggesting that lower levels of perceived stress are linked to increased alpha diversity (Carson et al., 2023; Humble et al., 2020; Sobko et al, 2020). For instance, a study of adults showed inverse relations between perceived stress and alpha diversity (Carson et al., 2023). Similarly, increased alpha diversity was linked to decreased perceived stress in a study of 95 pregnant women (Long et al., 2023). The current work was cross-sectional and could not determine directionality but there are some intriguing bidirectional explanations for these results.

One bottom-up explanation for findings like these is that microbial alpha diversity in the gut may not only commonly support better health outcomes but may also play a role in how we appraise stress possibly through metabolic mechanisms described below. Diversity within the gut microbiome is thought to build resilience into the intestinal ecosystem so that if even certain beneficial microbes expire (e.g., those that digest fiber) when facing certain threats such as antibiotic use or a bloom in a pathogenic microbe, others may survive the perturbation and continue to perform beneficial tasks. Notably, prebiotics (e.g., fiber) have been shown to reduce negative emotions and stimulate the growth of endogenous commensal gut bacteria (Paiva et al., 2020), such as *Bifidobacterium* and *Lactobacillus* which release health promoting metabolites when they consume prebiotics. These metabolites (e.g., neurotransmitters like GABA, serotonin and dopamine) can promote gut health and help to mitigate stress (Wall et al., 2014). Specifically, GABA (Jie et al., 2018), serotonin (Van Den Buuse & Hale, 2019) and dopamine (Stanwood et al., 2019) are associated with the etiology and management of stress. Indeed, research suggests the possibility that bacteria excreted neurotransmitters and neuromodulators

may activate epithelial cells (cells that line the gut) in the gut that then modulate neural signaling in the enteric nervous system (network of neurons that govern the gastrointestinal tract) or that they could influence neurons in the brain by communicating via the vagus nerve (Bravo et al., 2011; Forsythe et al., 2014; Wall et al., 2014). Thus, it is also possible that alpha diversity bidirectionally supports adaptive psychological processes, for example, by creating a biological environment in the host that promotes the growth of more types of bacteria that produce metabolites which could intervene in the stress appraisal process. The current work did not measure metabolic output of bacteria; however, future research should examine whether greater alpha diversity is linked to higher levels of potentially stress mitigating metabolites.

A top-down explanation for the findings between perceived stress and alpha diversity is that psychological perceptions of threat can lead to measurable changes in physiological processes in the gastrointestinal system (Rhee et al., 2009). Specifically, heightened appraisals of threat lead to stress-induced alterations in gut motility, secretions, and permeability (Musial et al., 2008; Rhee et al., 2009). Indeed, a pioneer investigating psychophysiology in the gastrointestinal system found that laboratory induced stress typically increased colonic motility (Almy et al., 1950). Further, evidence suggests that alterations in gut motility, secretions, and permeability may lead to shifts in gut microbial composition and function in both patient and healthy samples (Qin et al., 2014). Thus, prolonged stress can alter the ecological environment in the gut. These stress-induced changes could create a host environment that is hospitable to fewer types of bacteria. Indeed, there is longitudinal work supporting this possibility. For instance, it was found that levels of perceived stress increased in college students and gut bacteria count decreased during exam week when compared to the beginning of the semester (Knowles et al.,

2008) but experimental work is needed to establish causal and directional links between perceived stress and alpha diversity in humans.

Interestingly, there were no statistically significant differences in alpha diversity for perceived stress groups in Study 2 and this pattern persisted even when including rare genera present in less than 10% of the samples. This may be because Study 2 consisted of a more homogenous cohort than Study 1, that is, mothers of children within the ages of 8-12 years old as opposed to a comparatively more diverse sample of healthy adults of any sex across adulthood. Since Study 1 also included men and non-parents, the lack of significant findings (even after adjusting for sex and age) in Study 2 may be partially due to exposure to more similar microbial environments as a result of having school-aged children in Southern California. For example, having children in the public school system, participating in after-school programs or other communal types of activities such as sports (as was common in the Study 2 sample) could mean more exposure to similar types and varieties of microbes. Relatedly, having school age children could mean that the mothers were exposed to more similar foods, lived in close proximity to elementary schools, and exposed them to similar pollutants than someone living on a college campus or in a retirement home which was possible with Study 1 participants. Thus, the influence of the similar exposures in Study 2 might have been more robust in shaping alpha diversity among perceived stress groups than perceived stress itself.

There were no differences in beta diversity between perceived stress groups for either study. The adult microbiome is relatively stable and the communities of bacteria that lay within can generally only be altered by drastic mechanisms such as the use of antibiotics (Bello, 2018) or by a consistent and long-term change in diet (Davani-Davari et al., 2019), and as newly emerging evidence suggests, some types of stress (Karl et al., 2018). Interestingly, perceived

stress was the only stress measurement that was not marked by an acute or episodic stress event and thus appraisals of stress may place differential burdens on both human and microbial systems than stressor types triggered by a distinct incident. Further, since individuals may experience high levels of perceived stress even in the absence of a stressful life event, this method of measurement (PSS) may have also reflected psychological factors not directly included in the measurement of stressful life events such as transient mood, neuroticism, or negative affect. Thus, it might be possible that perceptions of stress represent numerous overlapping psychological constructs tied to alpha but not beta diversity in some samples. It is also possible that the microbiome exerts more influence over stress perceptions than vice versa, especially when dietary habits are considered. For example, one study showed that perceived stress was reduced through a dietary intervention (Berding et al., 2023), thus, some levels of heightened perceived stress may still not be robust enough to consistently alter microbial composition in every sample (top-down) but microbial composition may be powerful enough to more reliably alter perceptions of stress (bottom-up; Berding et al., 2023). One other possibility is that perceived stress is more closely tied to the metabolic output of bacteria instead of the populations of bacteria directly. Examination of metabolic output of bacteria was outside of the scope of this dissertation but this is a promising avenue for future researchers to explore.

Based on past research connecting perceived stress to select bacteria (Carson et al., 2018, Dinan, 2013), I hypothesized that there would be associations between *Lactobacillus, Akkermansia, Bifidobacterium, Clostridium, Streptococcus* and perceived stress, yet none were found in either Study 1 or Study 2. This is inconsistent with numerous intervention studies examining whether the administration of probiotics mitigates perceived stress and stress-adjacent conditions (e.g., depression, anxiety). Indeed, numerous studies suggest that probiotics including

certain strains of *Bifidobacterium* and *Lactobacillus* ameliorate depression, anxiety, and perceived stress (see meta-analysis by McKean et al., 2017; see review by Dinan, 2013). Yet, these findings are not always consistent, for instance, the probiotic *Lactobacillus rhamnosus* failed to mitigate perceived stress in a healthy human sample (Kelly et al., 2017). Only a few studies in humans test associations between perceived stress and the endogenous microbiome (as is assessed in the current work) and they have mixed findings. For example, there were no significant associations between perceived stress, *Clostridium* and *Bifidobacterium* in one sample of healthy women (Kleiman et al., 2017) but perceived stress was positively associated with *Clostridium* and negatively associated with *Bifidobacterium* in another sample of 80 healthy black and white women (Carson et al., 2018). The Kleiman and colleagues (2017) study and the Carson and colleagues (2018) study were the most similar to aims related to perceived stress in the current work with the non-significant findings in this dissertation more closely aligned to those in the Kleiman (2017) study. In contrast, neither Study 1 or Study 2 replicated the findings in the Carson (2018) study. This may be due to racial differences in samples, for instance, the Carson and colleagues (2018) study also examined racial differences in the microbiome between black and whites and thus the sample was 58.8% Black. In the current work, Study 1 participants were 4% Black and there were no Black participants in Study 2. Further, there were no racial or ethnic demographics reported in the Kleiman and colleagues (2017) study, thus, it remains unclear as to whether racial and ethnic differences might be a possible reason for our similar findings. Importantly, research shows that racial and ethnic differences are reflected in microbial composition (Brooks et al., 2018) and this may be one important reason why these patterns were not consistent across these studies. Examining ethnic differences was beyond the scope of the current work but this is an important factor to consider in future research.

Interestingly, *Escherichia/Shigella* was significantly positively associated with perceived stress in Study 2 and these results persisted even after controlling for physical covariates. These findings complement other work linking higher levels of *Escherichia/Shigella* to post-traumatic stress disorder (Bajaj et al., 2019) and anxiety (Chen et al., 2019). Indeed, pathogenic bacteria including certain strains of *Escherichia/Shigella* are linked to detrimental health outcomes such as gut dysbiosis, and irritable bowel syndrome (Ohman & Simren, 2013), and are also linked to worse stress responses across multiple stressor types including psychological and biological stress (see review by Biondi, 1997; Dinan & Cryan, 2016; Galland, 2014; Lyte et al., 1992; Sun et al., 2019). Pathogens such as *Escherichia/Shigella,* when in high levels or in vulnerable hosts, can prompt the immune system to release pro-inflammatory chemicals called cytokines (Konsman et al., 2002). The release of proinflammatory cytokines is known to trigger sickness behavior which is a condition in which an organism seeks to preserve energy by minimizing movement, social contact, and shows signs of fatigue (Konsman et al., 2002). Interestingly, these behaviors resemble stress-adjacent conditions such as depression. Further, perceived stress is linked to biomarkers of inflammation (Jain et al., 2007; Knight et al., 2021; Zou et al., 2020). Thus, one reason for the positive associations between perceived stress and *Escherichia/Shigella* could be that those high in *Escherichia/Shigella* were ill or on the verge of illness at the time of the study and that this illness led them to report more negative affect related constructs, including a higher perception of stress. Interestingly, Study 2 took place from April 2018 to September 2019. This was pre-COVID, thus, there were no quarantine protocols in the Orange and LA County areas. This could mean that participants in Study 2 were exposed to more pathogens when compared to Study 1 which took place from January 2020 to October 2020 since seven of those months were under various degrees of COVID quarantine protocols. Thus, it is possible

that Study 2 participants were harboring a larger pathogenic load when compared to Study 1 participants due to factors such as normal patterns of social interaction and fewer travel restrictions. That said, there are individual differences in inflammation across the general population, especially in comparison to those with autoimmune disorders. Further, it is also important to consider the fact that this sample was all female and research has shown that females tend to experience higher psychological impacts in relation to inflammation (Derry et al., 2015).

To summarize the psychological stress findings, alpha diversity was higher in the low perceived stress group when compared to the high stress group in Study 1 and *Escherichia/Shigella* was positively associated with perceived stress in Study 2. The current work also highlights the possibility that *Escherichia/Shigella* may exacerbate perceptions of stress in some samples, however, this was a cross-sectional study, thus it is also possible that perceived stress influenced levels of *Escherichia/Shigella*. Research has shown that high levels of perceived stress place individuals at greater risk for numerous detrimental health outcomes including metabolic syndrome (Rod et al., 2009) and poorer general health (Flores et al., 2008). Thus, perceived stress may not only contribute to allostatic load in human body systems but may also contribute to the allostatic load placed on the gut microbial ecosystem possibly due to stress-induced alterations in gut motility, secretions, and permeability. In sum, these results showed two nuanced ways in which the gut microbiome may be associated with perceived stress in some samples with perceived stress groups predicting microbial composition in Study 1 and levels of *Escherichia/Shigella* predicting perceived stress in Study 2 in these statistical models.

Environmental Stress (i.e., Stressful Life Events)

Contrary to hypotheses, there were no significant differences in alpha diversity between stressful life events groups in either study. These patterns persisted in both studies even after including rare genus that were only present in less than 10% of samples. While stressful life events groups did not differ in alpha diversity in either sample, there were significant differences in microbial composition (beta diversity) between stressful life events groups for *both* studies. This pattern is interesting due to the lack of differences in beta diversity between perceived stress groups. This could mean that stressful life events impact beta diversity more robustly than one's appraisal of stress. As mentioned previously, stressful life events represent potentially major and long-lasting stressors that are triggered by distinct, objective episodes. In contrast, while perceived stress might capture the response to a major life event, it can also reflect a transient mood in the absence of any event. Thus, these distinctions may reveal nuanced connections between these different types of stress with microbial composition. Indeed, it is possible that alpha diversity was linked to perceived stress in Study 1 but not stressful life events since perceived stress might also reflect numerous psychological constructs such as mood, neuroticism, and trait affect. Thus, alpha diversity may be more sensitive to this constellation of psychological constructs than an objective environmental stressor such as stressful life events in these samples.

As mentioned above, there were meaningful differences in beta diversity between stressful life events groups in both studies. Stressful life events are linked to the onset and progression of disease, sudden cardiac death, and coronary artery disease (Hinkle & Wolff, 1957; Rahe & Lind, 1971; Stantiute, et al., 2013). Indeed, stressful life events play a powerful role in health outcomes and may also play a governing role in shifting microbial composition.

Specifically, this could mean that stressful life events could reshape or perhaps redistribute populations of bacteria that live in the gut, essentially moving those with high levels of stressful life events to more similar places on the tree of life more than they impact the numbers of different types of genera (alpha diversity) within samples. This could mean that stressful life events, via their lasting and robust effects on the stressfulness of everyday life, trigger a repeated influx of stress hormones to which the gut microbiome has not yet become habituated. Indeed, microbial growth and colonization is altered by mammalian stress hormones such as epinephrine and norepinephrine (Boyanova, 2019) and thus may be more likely to influence which microbes are most dominant as opposed to how many different varieties exist.

ANCOM-BC revealed that certain microbes (e.g., *Eubacterium/Roseburia*, *Slackia*, *Odoribacter*) differentiated stressful life events groups in both studies (see Supplemental Tables 3 and 4). Numerous microbes emerged as potential candidates linked to stressful life events, however, exhaustive inferences detailing the role that each microbe might play in the mind-gut connection is not only beyond the scope of this dissertation but beyond the scope of extant literature since many of the microbes listed in Supplemental Tables 3, 4, and 5 have not yet been evaluated within the context of stressful life events. Nevertheless, a few potentially important stress players that are relevant to study aims closely will be discussed.

Interestingly, *Eubacterium/Roseburia* were the only genera that replicated in both studies and distinguished stressful life events groups, specifically, they were absent in both high stressful life events groups. It is worth noting that *Eubacterium* and *Roseburia* are two distinct genus, however, the two were classified together in this instance since they were indistinguishable during taxonomic assignment. Notably, some research suggests that these are beneficial bacteria. Indeed, certain species of *Eubacterium* are thought to contribute to gut health by reducing

inflammation and removing cholesterol formations in the gut (Mukherjee et al., 2020). Similarly, *Roseburia* is also thought to have therapeutic inflammation reducing properties in the gut. Both *Eubacterium* and *Roseburia* include several butyrate producing species. Butyrate is a metabolite that plays a crucial role in the suppression of inflammation (Mukherjee et al., 2020) which supports gut barrier integrity (Geirnaert et al., 2017). Additionally, Butyrate exerts many potentially protective functions against metabolic diseases, insulin resistance and stroke (Canani et al., 2011). Importantly, evidence suggests bidirectional links between *Eubacterium/Roseburia* and stress (Seewoo et al., 2022; Xu et al., 2021). For example, Xu and colleagues (2021) found that, following chronic stress, gut epithelial integrity was restored in rats treated with *Roseburia* (Xu et al., 2021) and another study demonstrated that rats in chronic stress showed decreases in levels of *Roseburia* (Seewoo et al., 2022).

However, some research suggests that connections between *Eubacterium/Roseburia*, health outcomes and stress are not always beneficial. For example, some strains of *Eubacterium* are thought to contribute to inflammation and colorectal cancer (Wang et al., 2021). Further, research examining these connections as they relate to stress in humans is limited and mixed. For instance, studies have shown negative associations between these genera and stress-related conditions, while others have found positive links (Kuo et al., 2019). The findings in the current study support literature revealing an inverse relation between *Eubacterium*/*Roseburia* and stress. Specifically, the current work suggests that abundances of *Eubacterium*/*Roseburia* are sensitive to stressful life events, therefore, could be a primary target for observation or intervention in future work exploring the impact of stressful life events on microbial populations. While this research is promising, studies assessing *Eubacterium/Roseburia* within the context of stressful life events in humans is sparse and more work is needed to establish that patterns are replicable.

Further, the variation in *Eubacterium*/*Roseburia's* outcomes underscores the need for more research exploring these connections on the species level and within the context of genetics, environment, and populations of other microbes present within the gut.

Interestingly, none of the select bacteria were associated with stressful life events as hypothesized in Study 1 or Study 2. It is possible that, in these samples, the ANCOM-BC calculated bacteria that differentiated stressful life events groups played the dominant role in relation to stressful life events over-shadowing the role that the predicted bacteria play in other contexts of stress in extant literature. Further, the few studies assessing relations between stressful life events and select bacteria were conducted in various locations across the world including the Netherlands (Hermes et al., 2020), Australia (Knowles et al., 2008), and Japan (Nishida et al., 2019). Indeed, microbial composition is closely tied to geographic location (Yatsunenko et al., 2012), thus, there may be factors such as climate or population levels related to the geographic location of Orange and LA counties that explain the dominance of the ANCOM-BC determined bacteria as they relate to environmental stress but further research is needed to test that possibility.

To summarize, microbial composition between stressful life events groups were significantly different in both Study 1 and Study 2. Specifically, the current study found that stressful life events explained approximately 5% of microbiota variance in both studies revealing a relatively robust and replicable finding across studies. To provide context, diet explains about 5-20% of gut microbiota variation (see review by Johnson et al., 2020), while host genetics explains from 1.9% to 8.1% of gut microbiome variation (Goodrich et al., 2016; Rothschild et al., 2018) showing that stress may be as important to microbiota composition as the well-established aforementioned factors.

As described previously, the current work operationalized stressful life events by assessing the frequency in which major life changes within the past year such as the death of a loved one, divorce, or loss of employment occurred in participants' lives. Since there was no consideration of the psychological appraisal of these threats, this assessment is typically considered an objective measure of the environmental demand placed on individuals (and the downstream requirement to adapt to the major life change). Interestingly, this objective measure yielded different microbiome related outcomes than the subjective measure of perceived stress. Indeed, there were no differences in beta diversity between perceived stress groups in either study and differences in beta diversity were only evident in stressful life events groups. This is interesting given evidence suggesting that perceived stress is more closely tied to physical symptomatology such as various experiences of pain including headache, backache, stomach ache compared to stressful life events (Cohen et al., 1983). This could mean that microbial composition has a different relation to perceived stress than it does to other physical symptomatology. One explanation for this is that, even if the individual does not perceive life changes as stressful, the gut microbiome *is* sensitive to the demands that major life changes require. This may be due to the burden placed on multiple body systems when confronted by a stressor that requires adaptation to both an episodic event and the long-lasting effects of that event. The incongruent findings between perceived stress groups and stressful life events groups could help shape future treatments directing them to focus gut microbiome interventions at those experiencing high levels of objective environmental stress whether or not they report perceptions of overwhelm.

Biological Stress (i.e., RSA Stress Reactivity)

There were no significant differences in alpha diversity between physiological stress groups in Study 1 or Study 2 at rest or in recovery from the laboratory stressor. However, there were differences in beta diversity across different RSA stress reactivity groups in Study 1 and these differences remained significant after controlling for sex and baseline RSA.

As described in the introduction, high RSA (at rest) is linked to better psychological wellbeing while low RSA is linked to poorer psychological wellbeing (Porges et al., 1994). ANCOM-BC revealed that one of the genera that was only absent in the low RSA (during stress reactivity) group was *Slackia*. *Slackia* is a potentially protective genus that produces dihydroresveratrol (DHR) which was shown to have anti-cancer and anti-inflammatory effects in mice (Li et al., 2022). Further, *Slackia* is negatively associated with cortisol and psychological distress (daily hassles) in humans (Aatsinki et al., 2020). Similarly, *Blautia* was only missing in the low RSA reactivity group. *Blautia* is another butyrate producing bacteria and it was also absent in the low RSA group. *Blautia* is negatively correlated with visceral fat, and is thought to mitigate metabolic syndrome (Lui et al., 2021) but no work, to our knowledge, has connected it with RSA stress reactivity. Thus, this study is among the first to reveal these connections. That said, a recent mindfulness-based intervention was found to significantly increase levels of *Blautia* in pregnant women (Zhang et al., 2022) hinting at the possibility that there may be connections between stress reduction and this bacterium and that this direction may be a promising avenue for future research.

There were no associations between hypothesized symbiotic bacteria and RSA at rest, in reactivity to, or in recovery from either laboratory stressor. The only hypothesized microbe with links to RSA stress reactivity was one of the hypothesized pathogenic bacteria; *Clostridium*.

Higher levels of *Clostridium* were associated with lower levels of RSA reactivity even after controlling for physical covariates. Certain strains of *Clostridium* are linked to increased risk of heart failure (Méndez-Bailón et al., 2020). In relation to stress, one strain of *Clostridium* was significantly increased in students during an examination period (Mullie et al., 2002). However, other strains of *Clostridium* are considered commensal, produce butyrate and are thought to have probiotic properties (Guo et al., 2020). This emphasizes the fact that gut microbes exist within an ecosystem and symbiotic versus pathogenic roles of gut bacteria likely depend on checks and balances such as genetics and populations of other bacteria that exist within the context of each specific ecosystem. Since the current study found that *Clostridium* predicts lower RSA stress reactivity, this could mean that the particular strains of *Clostridium* linking the two variables may play a pathogenic role within the context of cardiovascular (RSA) function during acute stress.

Putting the above findings together, there were differences between RSA reactivity groups and beta diversity and there were also significant negative associations between RSA reactivity and levels of *Clostridium* in Study 1. However, these results were not replicated in Study 2. The TSST was used as the laboratory stressor in Study 1, which is an established task that uses social evaluation focused directly at the participant to effectively induce moderate stress (Kirschbaum & Hellhammer, 1993). In contrast, the laboratory stressor in Study 2 was not focused directly at the participant in this study but rather on the participant's child. Because of the indirect (and likely milder) nature of this stressor, it is thus possible that the task in Study 2 did not have a robust enough effect on RSA. It may also be that it was not a strong enough indicator of RSA stress reactivity as a disposition (given its indirect characteristic) to detect differences in RSA reactivity groups or associations between *Clostridium* and RSA reactivity.

Differences in RSA reactivity groups and the negative association between levels of *Clostridium* and RSA complement established research that reveals connections between higher RSA and better social and emotional function (Geisler et al., 2013) and the ability to respond to environmental demands (Butler et al., 2006; Calkins, 1997). Specifically, the current work showed that those with higher levels of *Clostridium* had lower RSA. As discussed earlier, the vagus nerve can differentiate between pathogenic and non-pathogenic bacteria and can transmit signals that both exacerbate or mitigate anxiety depending on the bacterial stimulus (Forsythe et al., 2014). Thus, the vagus may be a key mediator in these findings and should be considered in future research assessing various types of stress.

Limitations

The current study revealed novel mind-microbiome associations and also supports existing literature linking the gut microbiome to various types of stress, however, there are important limitations to consider. Both studies were cross-sectional, thus, microbial composition was only measured at one-time point limiting causal and directional inferences. Further, the mechanisms that drove these connections were not tested. For example, it is possible that there were health behavioral mechanisms linking stress to the microbiome such as diet. While the current study adjusted for fruit, vegetable, meat, and grain consumption, I did not adjust for fat and sugar consumption which is known to increase in relation to stress (Yau et al., 2013). Additionally, stress measures and fecal samples were not collected on the same day. Specifically, fecal samples were collected within weeks to months of the laboratory visit in Study 1 and a two to three-week time frame in Study 2 following laboratory visits. It is possible that during the period of time between administration of stress surveys, the lab visit, and fecal collection, that other stressful life events occurred, levels of perceived stress varied, or that there was a third-

variable at play that may have altered connections between the microbiome and measures of RSA. Further, it has been shown that the human microbiome expresses some seasonal variation because of changes in diet, weather, and flu-season/medication use (Davenport et al., 2014). Future work should assess stress-microbiome links longitudinally and test whether seasonality alters these connections. Relatedly, Study 1 and Study 2 took place during different periods of time. Specifically, Study 1 occurred from January 2020 to October 2020. COVID quarantine protocols began in March 2020, meaning, seven months of data collection for Study 1 took place during varying degrees of quarantine. Indeed, it has been shown that COVID quarantine protocols are linked to altered microbial composition in humans (Aguilera et al., 2022). Since Study 2 took place pre-COVID from April 2018 to September 2019, this may have impacted the replication of findings between studies. Another important difference between studies is that Study 1 included men and non-parents, while Study 2 was composed of female mothers only. Although the current study adjusted for these differences statistically (e.g., sex and age), this leaves open the possibility for other third-variables that were not adjusted for in this study such as levels of sex hormones. Further, it is possible that mothers are exposed to different communities of microbes particularly from having children that might attend public schools whereas a non-parent may not be exposed to the same microbes. Further, taxa were identified and quantified using 16S sequencing. 16S has been shown to only detect part of the gut microbial community when compared to shotgun sequencing (Durazzi et al., 2021). Further, 16S amplicon sequencing cannot identify or quantify the metabolic output of microbes. This is an important limitation because there is a strong possibility that links between stress and the gut microbiome are more closely tied to bacterial metabolites than the bacteria themselves. For instance, the same bacteria can create different levels and types of metabolites depending on the

host environment (Passalacqua et al., 2016). Future stress and microbiome research should be conducted experimentally and longitudinally (e.g., by measuring microbial alterations pre and post experimental stress exposure or via prospective longitudinal studies with microbiome assessed repeatedly over time in conjunction with stress experiences), in samples that are more similar, occur during the same year, and should include metabolomics data analysis.

Conclusion

The current work is among the first to explore, discover and compare connections between stressful life events, perceived stress, cardiovascular function surrounding acute stress, and microbial composition in two samples of healthy adults. Indeed, this dissertation was designed to assess variations in mind-gut-microbiome links within the context of a stress specific triad that included both objective and subjective measures across environmental, psychological, and physiological stress domains. These domains are multifaceted providing a new context in which to assess these dynamic connections. Specifically, each stress domain reflects distinct but overlapping components of stress with perceived stress reflecting everyday stress appraisals and psychological constructs related to negative affect, RSA stress reactivity reflecting an acute biological response to a moderate social stressor, and finally, stressful life events reflecting a chronic stressor triggered by a potentially major life changing occurrence. Thus, stress-microbiome associations revealed in this dissertation broadly reflect the logic of the aforementioned pattern. That is, the major stressor (i.e., stressful life events; chronic and potentially severe) was linked to beta diversity in both studies. This was the only replication of beta diversity findings between studies and suggests the top-down possibility that intense and prolonged stress may alter microbial composition. Additionally, there were compositional differences between low, mid, and high RSA stress reactivity groups and I also found that

Clostridium predicted lower levels of RSA stress reactivity but both of these findings were only significant in Study 1. The lack of replication from Study 1 to Study 2 may reflect varying levels of intensity between acute stressor types such that the stressor in Study 1 was directed at the participant while the laboratory stressor in Study 2 was directed at the participant's child which may have made it less impactful. Finally, there were significant differences between perceived stress groups for alpha diversity in Study 1 but not Study 2. Study 1 was a more heterogeneous cohort consisting of men, women, and non-parents whereas Study 2 consisted of female mothers only. Thus, the strength of the connections between alpha diversity and perceived stress may vary depending on the characteristics of the sample. In sum, while not all hypotheses were met, it is impressive that across all modalities of stress assessment, some associations were found indicating that regardless of how stress is measured, it may be reflected to some extent in the microbiome, albeit in different ways. Further, the patterns suggest that stress-microbiome links are the most consistent in relation to more objective and sometimes serious stressful life events and that, in general, other stress-microbiome connections are both stressor-type and sample dependent.

Broadly, the patterns revealed in this dissertation replicate past studies that have shown that the gut microbiome is a stress-sensitive system, both *bidirectionally* and *differentially* linked to various types of stress. Bottom-up findings suggest the gut microbiome could hold both therapeutic and nocuous properties that impact both psychological and physiological stress while top-down findings lend support to the concept that the gut microbiome, like other body systems, may also bear an allostatic load. This dissertation lays the groundwork for the discovery of a counterpart to a stress-bearing allostatic load that could be described as a *homeostatic reservoir* housed in the gut, namely, beneficial metabolites. Indeed, work like this opens avenues for the

exciting possibility that the gut contains innumerable microbial internal physicians within. Future research should focus on designing experimental studies and longitudinal interventions that can identify both top-down and bottom-up pathways that promote mutual wellbeing in the mind and the microbiome. Interventions that mobilize these beneficial bidirectional mind-microbiome connections could promote wellbeing in both healthy and patient populations across ethnicities and socioeconomic statuses.

APPENDICES

Appendix A – Supplemental Tables and Figures

Supplemental Table 1. Descriptive Statistics of Key Variables: Study 2

Measures	Total $N = 74$	
	M	*SD*
Age in Years	41.592	6.349
Body Mass Index	25.649	6.551
General Health	73.413	18.283
Meat Consumption	2.299	.533
Vegetable/Fruit Consumption	2.464	.573
Grain Consumption	2.291	.804
RSA Baseline	5.960	1.326
RSA Stress Reactivity	5.649	1.123
Perceived Stress	14.381	6.465
Lactobacillus	-.262	.862
Escherichia Shigella	-.372	.818

Note. Includes descriptive statistics for variables in significant models. Taxa abundance data displayed in this Table were adjusted for compositionally with centered log-ratio transformation. Raw relative abundance values, prior to transformation in decimal form are as follows: *Lactobacillus* ($M = .00070$, $SD = .00368$). *Escherichia /Shigella* ($M = .00147$, $SD = .00371$)

Supplemental Table 2. Correlation Matrix for Key Variables in Study 2 *(n = 74)*

Variables	1	2	3	4	5	6	7	8	9	10
1. Age in Years	--									
2. Body Mass Index	-.129	--								
3. General Health	-.011	-.238*	--							
4. Meat Consumption	.172	-.252*	.151	--						
5. Vegetable/Fruit Consumption	.051	-.089	.013	.077	--					
6. Grain Consumption	.067	.116	-.073	-.066	.313**	--				
7. RSA Baseline	-.305**	-.171	.131	.021	-.013	-.057	--			
8. RSA Stress Reactivity	-.263*	-.084	.078	-.027	-.118	-.003	.807**	--		
9. Perceived Stress	.050	.049	-.365**	.107	-.002	-.004	-.055	-.089	--	
10. Lactobacillus	.004	-.295*	-.196	-.160	-.110	-.151	.193	.269*	.142	--
11. Escherichia Shigella	.155	-.005	-.112	.014	-.053	-.113	.158	.100	.264*	.113

Note. * $p < .05$. ** $p < .01$. *** $p < .001$

Includes correlations between variables in significant models.

Supplemental Table 3. The Detection of Structural Zeros in Stressful Life Events Groups in Study 1

Taxon	Low Stressful Life Events	Mid Stressful Life Events	High Stressful Life Events
Acidaminococcus	false	true	true
Megasphaera	false	true	true
Veillonella	false	true	true
Faecalibacterium/Subdoligranulum	false	false	true
Intestinimonas	false	false	true
Slackia	true	false	false
Anaerofustis	true	false	false
Anaerofilum	true	false	false
Coprobacter	true	false	true
Enterococcus	true	false	true
Eubacterium/Roseburia	true	false	true
Pseudobutyrivibrio	true	false	true
Eubacterium_3	true	false	true
Gelria	true	false	true
**Stoquefichus*	true	false	true
Oxalobacter	true	false	true
Klebsiella	true	false	true
Enterorhabdus	true	true	false
Olsenella	true	true	false
Eubacterium	true	true	false
Howardella	true	true	false
Lactonifactor	true	true	false
Holdemanella	true	true	false
Coprobacillus	true	true	false

Note. Taxa displayed in Table 3 are reported due to ANCOM-BCs identification of structural zeros in either the high or low stressful life events groups for Study 1.

Supplemental Table 4. The Detection of Structural Zeros in Stressful Life Events Groups in Study 2			
Taxon	Low Stressful Life Events	Mid Stressful Life Events	High Stressful Life Events
Blautia	true	true	false
Peptoniphilus	true	true	false
Methanosphaera	true	false	false
Eggerthella	true	false	false
Actinomyces	false	true	true
Weissella	false	true	true
Varibaculum	false	true	true
Eubacterium-Roseburia	false	true	true
Odoribacter	false	false	true
Granulicatella	false	false	true
Methanobrevibacter	false	true	false
Bacteroides	false	true	false
Anaerococcus	false	true	false
Eubacterium_2	false	true	false

Note. Taxa displayed in Table 4 are reported due to ANCOM-BCs identification of structural zeros in either the high or low stressful life events groups for Study 2.

Supplemental Table 5. The Detection of Structural Zeros in RSA Reactivity Groups in Study 1			
Taxon	Low RSA	Mid RSA	High RSA
Slackia	true	false	false
Blautia/Marvinbryantia	true	false	false
Faecalibacterium/Subdoligranulum	true	false	false
Acidaminococcus	true	false	false
Megasphaera	true	false	false
Thalassospira	true	false	false
Pseudobutyrivibrio	true	true	false
Holdemanella	true	true	false
Coprobacillus	false	true	true
Enterorhabdus	true	false	true
Allisonella	true	false	true
Veillonella	true	false	true
Klebsiella	false	true	true
Cloacibacillus	false	true	true
Coprobacter	false	true	true
Enterococcus	false	true	true
Anaerofustis	false	true	true
Eubacterium	false	true	true
Eubacterium_1	false	true	true
Eubacterium/Roseburia	false	true	true
Lactonifactor	false	true	true
Anaerofilum	false	true	true
Gelria	false	true	true
**Stoquefichus*	false	true	false
Escherichia/Shigella	false	true	false

Supplemental Table 5 continued…			
Denitrobacterium	false	false	true
Oxalobacter	false	false	true

Note. Taxa displayed in this table are reported due to ANCOM-BCs identification of structural zeros in either the high or low RSA reactivity group in Study 1.

Supplemental Table 6. Descriptive Statistics of Key Variables by Sex for Study 1 (n = 62)

Measures	Total N = 62 *M(SD)*	Males n = 17	Females n = 45	*Sex differences t*
Age in Years	37.601(11.696)	35.244(10.574)	38.491(12.084)	-1.043
Body Mass Index	25.528(6.420)	27.119(6.097)	24.927(6.502)	1.241
General Health	33.681(13.168)	30.951(12.664)	34.713(13.346)	-1.028
Meat Consumption	2.322(.5132)	2.235(.471)	2.355(.529)	-.863
Vegetable/Fruit Consumption	2.274(.630)	2.117(.740)	2.334(.582)	-1.086
Grain Consumption	2.371(.751)	2.176(.808)	2.444(.724)	-1.196
RSA Baseline	5.725(1.173)	5.920(1.103)	5.651(1.203)	.833
RSA Stress Reactivity	5.778(1.283)	5.930(1.410)	5.721(1.244)	.537
Clostridium	-.478(1.657)	-.404(1.817)	-.506(1.614)	.203

Note. Includes descriptive statistics for variables in significant models. No sex differences were found. Taxa abundance data displayed in this Table were adjusted for compositionally with centered log-ratio transformation. Raw relative abundance values for participants (N = 62) in Study 2, prior to transformation in decimal form are as follows: *Clostridium* (M = .00488, SD = .01352) Abbreviations: Respiratory Sinus Arrhythmia (RSA).

Supplemental Table 7. Correlation Matrix for Key Variables in Study 1

Variables	1	2	3	4	5	6	7	8	9
1. Age in Years	--								
2. Sex	.125	--							
3. Body Mass Index	.030	-.154	--						
4. General Health	-.351**	.129	.032	--					
5. Meat Consumption	-.017	.105	-.368**	-.206	--				
6. Vegetable/Fruit Consumption	.213	.154	-.123	-.124	.175	--			
7. Grain Consumption	.230	.160	-.110	-.069	.088	.315*	--		
8. RSA Baseline	-.187	-.103	-.066	.021	.089	-.023	-.213	--	
9. RSA Stress Reactivity	-.407**	-.073	-.149	.180	.059	-.030	-.140	.593**	--
10. *Clostridium*	-.381**	-.028	-.014	-.045	-.101	-.078	-.072	-.098	-.369**

Note: Sex Coding; 1 = men, 2 = women

* $p < .05$. ** $p < .01$. *** $p < .001$

Includes correlations between variables in significant models.

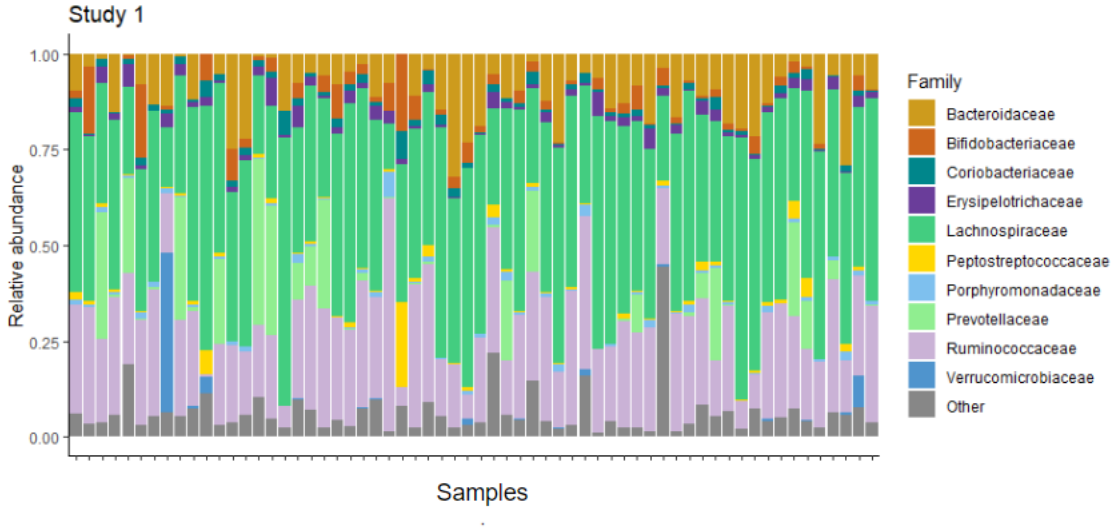
Supplemental Table 8. Relative Abundance and Prevalence of Select Bacteria in Study 1 and Study 2

Select Bacteria	Study 1 (Total $N = 62$) *M(SD)*	Study 2 (Total $N = 74$) *M(SD)*	Study 1 Prevalence	Study 2 Prevalence
Lactobacillus	.140(.689)	.071(.368)	33.87	16.22
Akkermansia	1.003(5.36)	.270(.620)	48.39	43.24
Bifidobacterium	3.010(4.277)	3.126(3.64)	85.48	82.43
Streptococcus	.389(.705)	.962(2.477)	93.55	90.54
Escherichia/Shigella	.601(4.13)	.147(.371)	33.87	35.14
Clostridium	.489(.353)	.273(.619)	98.39	48.65

Note. Values of relative abundances prior to centered log transformation and prevalence of select genus in percentage form are reported in this table.

Supplemental Figure 1

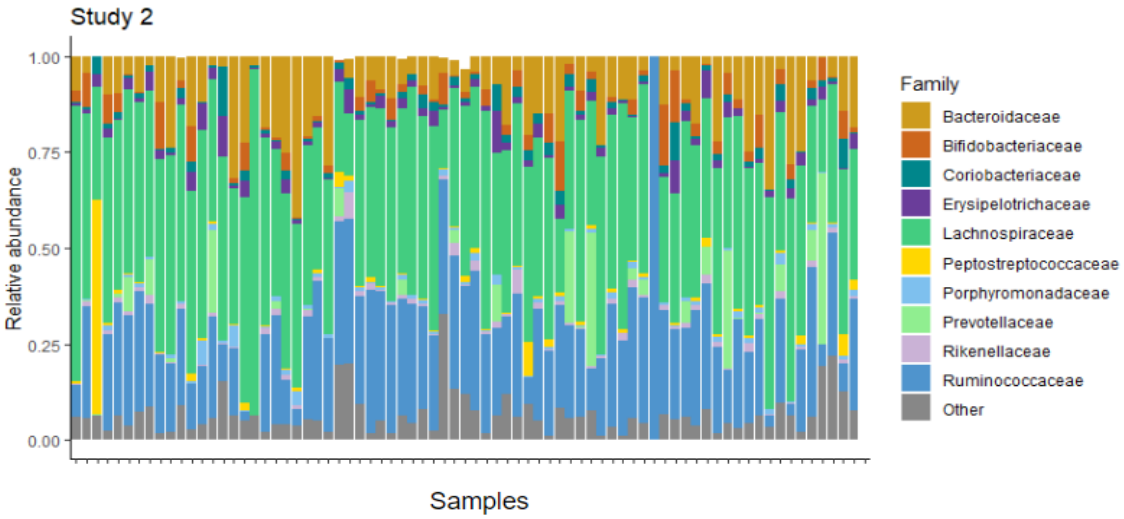
Stacked Bar-Plot of Microbiota Composition: Study 1



Note. The relative abundance of microbial families across all samples. Samples are grouped by each individual with a total of 62 samples depicted. The top 10 most abundant bacterial families present in Study 1 are identical to those in Study 2 with the exception of *Verrucomicrobiaceae*.

Supplemental Figure 2

Stacked Bar-Plot of Microbiota Composition: Study 2



Note. The relative abundance of microbial families across all samples. Samples are grouped by each individual with a total of 74 samples depicted. The top 10 most abundant bacterial families present in Study 2 are identical to those in Study 1 with the exception of *Rikenellaceae*.

References

Aatsinki, A. K., Keskitalo, A., Laitinen, V., Munukka, E., Uusitupa, H. M., Lahti, L., ... & Karlsson, L. (2020). Maternal prenatal psychological distress and hair cortisol levels associate with infant fecal microbiota composition at 2.5 months of age. *Psychoneuroendocrinology*, *119*, 104754.

Aguilera, P., Mascardi, M. F., Belforte, F. S., Rosso, A. D., Quesada, S., Llovet, I., ... & Penas-Steinhardt, A. (2022). A two-time point analysis of gut microbiota in the general population of buenos aires and its variation due to preventive and compulsory social isolation during the COVID-19 pandemic. *Frontiers in Microbiology, 13*.

Aktipis, A., & Guevara Beltran, D. (2021). Can some microbes promote host stress and benefit evolutionarily from this strategy?. *BioEssays*, *43*(1), 2000188.

Allen, A. P., Hutch, W., Borre, Y. E., Kennedy, P. J., Temko, A., Boylan, G., ... & Clarke, G. (2016). Bifidobacterium longum 1714 as a translational psychobiotic: modulation of stress, electrophysiology and neurocognition in healthy volunteers. *Translational psychiatry, 6*(11), e939-e939.

Al Omran, Y., & Aziz, Q. (2014). The brain-gut axis in health and disease. *Microbial endocrinology: the microbiota-gut-brain axis in health and disease*, 135-153.

Almy, T. P., Abbot, F. K., & Hinkle Jr, L. E. (1950). Alterations in Colonic Function in Man Under Stress: IV: Hypomotility of the Sigmoid Colon, and its Relationship to the Mechanism of Functional Diarrhea. *Gastroenterology*, *15*(1), 95-103.

Amato, K. R., Yeoman, C. J., Cerda, G., A. Schmitt, C., Cramer, J. D., Miller, M. E., Gomez, A., R. Turner, T., Wilson, B. A., Stumpf, R. M., Nelson, K. E., White, B. A., Knight, R., & Leigh, S. R. (2015). Variable responses of human and non-human primate gut microbiomes to a western diet. *Microbiome, 3*(1).

American Psychological Association. (2015, February 4). *Stress in America: Paying With Our Health*. Retrieved from https://www.apa.org/news/press/releases/stress/2014/stress-report.pdf.

Arango Duque, G., & Descoteaux, A. (2014). Macrophage cytokines: involvement in immunity and infectious diseases. *Frontiers in immunology, 5*, 491.

Aroniadis, O. C., Drossman, D. A., & Simrén, M. (2017). A Perspective on Brain–Gut Communication: The American Gastroenterology Association and American Psychosomatic Society Joint Symposium on Brain–Gut Interactions and the Intestinal Microenvironment. *Psychosomatic medicine, 79*(8), 847-856.

Aziz, Q., & Thompson, D. G. (1998). Brain-gut axis in health and disease. *Gastroenterology, 114*(3), 559-578.

Bailey, M. T., & Coe, C. L. (1999). Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. Developmental Psychobiology: *The Journal of the International Society for Developmental Psychobiology, 35*(2), 146-155.

Bailey, M. T., Lubach, G. R., & Coe, C. L. (2004). Prenatal stress alters bacterial colonization of the gut in infant monkeys. *Journal of Pediatric Gastroenterology and Nutrition, 38*(4), 414–421.

Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain, behavior, and immunity, 25*(3), 397-407.

Bailey, M. T. (2012). The contributing role of the intestinal microbiota in stressor-induced increases in susceptibility to enteric infection and systemic immunomodulation. *Hormones and behavior, 62*(3), 286-294.

Bajaj, J. S., Sikaroodi, M., Fagan, A., Heuman, D., Gilles, H., Gavis, E. A., ... & Wade, J. B. (2019). Posttraumatic stress disorder is associated with altered gut microbiota that modulates cognitive

performance in veterans with cirrhosis. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 317*(5), G661-G669.

Beaumont, W., & Osler, W. (1996). *Experiments and Observations on the Gastric Juice and the Physiology of Digestion*. Courier Corporation.

Beauchaine, T. P. (2015). Respiratory sinus arrhythmia: A transdiagnostic biomarker of emotion dysregulation and psychopathology. *Current Opinion in Psychology*, *3*, 43–47.

Beaugerie, L., & Petit, J. C. (2004). Antibiotic-associated diarrhoea. *Best practice & research Clinical gastroenterology, 18*(2), 337-352.

Bello, M. G., Knight, R., Gilbert, J. A., & Blaser, M. J. (2018). Preserving microbial diversity. *Science, 362*(6410), 33–34.

Benton, D., Williams, C., & Brown, A. (2007). Impact of consuming a milk drink containing a probiotic on mood and cognition. *European journal of clinical nutrition, 61*(3), 355-361.

Berding, K., Bastiaanssen, T. F., Moloney, G. M., Boscaini, S., Strain, C. R., Anesi, A., ... & Cryan, J. F. (2023). Feed your microbes to deal with stress: A psychobiotic diet impacts microbial stability and perceived stress in a healthy adult population. *Molecular psychiatry, 28*(2), 601-610.

Biondi, M., & Zannino, L. G. (1997). Psychological stress, neuroimmunomodulation, and susceptibility to infectious diseases in animals and man: a review. *Psychotherapy and psychosomatics, 66*(1), 3-26.

Boyanova, L., Markovska, R., & Mitov, I. (2019). Helicobacter pylori growth stimulation by adrenaline detected by two methods. *Diagnostic microbiology and infectious disease*, *93*(1), 30-32.

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., Bienenstock, J., & Cryan, J. F. (2011). Ingestion of lactobacillus strain regulates emotional behavior and central

GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences, 108*(38), 16050–16055. https://doi.org/10.1073/pnas.1102999108

Brooks, A. W., Priya, S., Blekhman, R., & Bordenstein, S. R. (2018). Gut microbiota diversity across ethnicities in the United States. *PLoS biology*, *16*(12), e2006842.

Butler, E. A., Wilhelm, F. H., & Gross, J. J. (2006). Respiratory sinus arrhythmia, emotion, and emotion regulation during social interaction. *Psychophysiology*, *43*(6), 612-622.

Bruno, J. S., Heidrich, V., Knebel, F. H., de Molla, V. C., Parahyba, C. J., Miranda-Silva, W., ... & Fregnani, E. R. (2022). Commensal oral microbiota impacts ulcerative oral mucositis clinical course in allogeneic stem cell transplant recipients. *Scientific Reports, 12*(1), 17527.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., & Johnson, A. (2016). JA, Holmes SP. 2016. *DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods*, *13*(7), 581-583. doi:10.1038/nmeth.3869.

Calkins, S. D. (1997). Cardiac vagal tone indices of temperamental reactivity and behavioral regulation in young children. *Developmental Psychobiology, 31*(2), 125–135.

Campbell, A. A., & Wisco, B. E. (2021). Respiratory sinus arrhythmia reactivity in anxiety and posttraumatic stress disorder: A review of literature. *Clinical Psychology Review, 87*, 102034.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... & Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature methods*, *7*(5), 335-336.doi: 10.1038/nmeth.f.303

Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M., & Owen, L. J. (2015). Dysbiosis of the gut microbiota in disease. *Microbial Ecology in Health & Disease, 26.* https://doi.org/10.3402/mehd.v26.26191

Carson, T. L., Wang, F., Cui, X., Jackson, B. E., Van Der Pol, W. J., Lefkowitz, E. J., ... & Baskin, M. L. (2018). Associations between race, perceived psychological stress, and the gut microbiota in a sample of generally healthy black and white women: a pilot study on the role of race and perceived psychological stress. *Psychosomatic medicine*, *80*(7), 640.

Carson, T. L., Byrd, D. A., Smith, K. S., Carter, D., Abaskaron, M., Little, R. B., ... & Gomez, M. (2023). A case-control study of the association between the gut microbiota and colorectal cancer: exploring the roles of diet, stress, and race.

Casadevall, A., & Pirofski, L. A. (2014). Microbiology: ditch the term pathogen. *Nature*, *516*(7530), 165-166.

Chassard, C., Dapoigny, M., Scott, K. P., Crouzet, L., Del'homme, C., Marquet, P., Martin, J. C., Pickering, G., Ardid, D., Eschalier, A., Dubray, C., Flint, H. J., & Bernalier-Donadille, A. (2012). Functional dysbiosis within the gut microbiota of patients with constipated-irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics*, *35*(7), 828–838. https://doi.org/10.1111/j.1365-2036.2012.05007.x

Chen, Y. H., Bai, J., Wu, D. I., Yu, S. F., Qiang, X. L., Bai, H., ... & Peng, Z. W. (2019). Association between fecal microbiota and generalized anxiety disorder: severity and early treatment response. *Journal of Affective Disorders, 259*, 56-66.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, *24*(4), 385. https://doi.org/10.2307/2136404

Cohen, S., Kessler, R. C., & Gordon, L. U. (1998). *Measuring stress: A guide for health and social scientists*. Oxford University Press.

Cole, C. C., Nikpay, M., Lau, P., Stewart, A. F. R., Davies, R. W., Wells, G. A., ... & McPherson, R. (2015). ADIPOSITY SIGNIFICANTLY MODIFIES GENETIC RISK FOR DYSLIPIDEMIA.

Collins, S. M., Surette, M., & Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. *Nature Reviews Microbiology*, *10*(11), 735–742. https://doi.org/10.1038/nrmicro2876

Cong, X., Henderson, W. A., Graf, J., & McGrath, J. M. (2015). Early life experience and Gut Microbiome. *Advances in Neonatal Care*, *15*(5), 314–323. https://doi.org/10.1097/anc.0000000000000191

Cresci, G. A., & Bawden, E. (2015). Gut microbiome: what we do and don't know. *Nutrition in Clinical Practice*, *30(*6), 734-746.

Crosswell, A. D., & Lockwood, K. G. (2020). Best practices for stress measurement: How to measure psychological stress in health research. *Health psychology open*, *7*(2), 2055102920933072.

Crovesy, L., Masterson, D., & Rosado, E. L. (2020). Profile of the gut microbiota of adults with obesity: A systematic review. *European Journal of Clinical Nutrition*, *74*(9), 1251–1262. https://doi.org/10.1038/s41430-020-0607-6

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature reviews neuroscience*, *13*(10), 701-712.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., ... & Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559-563.

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., ... & Ghasemi, Y. (2019). Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods*, *8*(3), 92.

Davenport, E. R., Mizrahi-Man, O., Michelini, K., Barreiro, L. B., Ober, C., & Gilad, Y. (2014). Seasonal variation in human gut microbiome composition. *PloS one*, *9*(3), e90731.

De Palma, G., Collins, S. M., Bercik, P., & Verdu, E. F. (2014). The microbiota-gut-brain axis in gastrointestinal disorders: Stressed bugs, stressed brain or both? *The Journal of Physiology*, *592*(14), 2989–2997. https://doi.org/10.1113/jphysiol.2014.273995

Derry, H. M., Padin, A. C., Kuo, J. L., Hughes, S., & Kiecolt-Glaser, J. K. (2015). Sex differences in depression: does inflammation play a role?. *Current psychiatry reports*, *17*, 1-10.

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience*, *170*(4), 1179-1188.

Dinan, T. G., & Cryan, J. F. (2016). Gut instincts: Microbiota as a key regulator of brain development, ageing and neurodegeneration. *The Journal of Physiology*, *595*(2), 489–503. https://doi.org/10.1113/jp273106

Dinan, T. G., Stanton, C., & Cryan, J. F. (2013). Psychobiotics: a novel class of psychotropic. *Biological psychiatry*, *74*(10), 720-726.

Dinan, T. G., & Cryan, J. F. (2017). Brain-gut-microbiota axis and mental health. *Psychosomatic medicine*, *79*(8), 920-926.

Duan, H., Cai, X., Luan, Y., Yang, S., Yang, J., Dong, H., ... & Shao, L. (2021). Regulation of the autonomic nervous system on intestine. *Frontiers in Physiology*, *12*.

Durazzi, F., Sala, C., Castellani, G., Manfreda, G., Remondini, D., & De Cesare, A. (2021). Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. *Scientific reports*, *11*(1), 1-10.

Ecologic investigations of the relationship between illness, life experiences and the Social Environment. (1958). *Annals of Internal Medicine*, *49*(6), 1373.

Elliott, D., Kufera, J. A., & Myers, R. A. (2000). The microbiology of necrotizing soft tissue infections. *The American journal of surgery*, *179*(5), 361-366.

Eloe-Fadrosh, E. A., & Rasko, D. A. (2013). The human microbiome: from symbiosis to pathogenesis. *Annual review of medicine*, *64*, 145-163.

Feng, Y., Duan, Y., Xu, Z., Lyu, N., Liu, F., Liang, S., & Zhu, B. (2019). An examination of data from the American Gut Project reveals that the dominance of the genus Bifidobacterium is associated with the diversity and robustness of the gut microbiota. *MicrobiologyOpen*, *8*(12), e939.

Fisher, J. P., Young, C. N., & Fadel, P. J. (2009). Central sympathetic overactivity: maladies and mechanisms. *Autonomic Neuroscience*, *148*(1-2), 5-15.

Forsythe, P., & Kunze, W. A. (2013). Voices from within: gut microbes and the CNS. *Cellular and molecular life sciences*, *70*, 55-69.

Forsythe, P., Bienenstock, J., & Kunze, W. A. (2014). Vagal pathways for microbiome-brain-gut axis communication. *Microbial endocrinology: the microbiota-gut-brain axis in health and disease*, 115-133.

Foster, J. A., & McVey Neufeld, K.-A. (2013). Gut–Brain Axis: How the microbiome influences anxiety and depression. *Trends in Neurosciences*, *36*(5), 305–312.

Foster, J. A., Rinaman, L., & Cryan, J. F. (2017). Stress & the gut-brain axis: regulation by the microbiome. *Neurobiology of stress*, *7*, 124-136.

Flores, E., Tschann, J. M., Dimas, J. M., Bachen, E. A., Pasch, L. A., & de Groat, C. L. (2008). Perceived discrimination, perceived stress, and mental and physical health among Mexican-origin adults. *Hispanic Journal of Behavioral Sciences*, *30*(4), 401-424.

Friedman, B. H., & Thayer, J. F. (1998). Autonomic balance revisited: panic anxiety and heart rate variability. *Journal of psychosomatic research*, *44*(1), 133-151.

Galland, L. (2014). The gut microbiome and the brain. *Journal of Medicinal Food*, *17*(12), 1261–1272.

Ganji, L., Alebouyeh, M., Shirazi, M. H., Eshraghi, S. S., Mirshafiey, A., Ebrahimi Daryani, N., & Zali, M. R. (2016). Dysbiosis of fecal microbiota and high frequency of *Citrobacter, Klebsiella* spp., and Actinomycetes in patients with irritable bowel syndrome and gastroenteritis. *Gastroenterology and hepatology from bed to bench*, *9*(4), 325–330.

Gaudin, C., Safar, M., & Cuche, J. L. (1990). Effects of restraint stress on catecholamine concentrations in the glandular stomach of rats. *Life sciences*, *47*(22), 2003-2008.

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., MacQueen, G., & Sherman, P. M. (2010). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, *60*(3), 307–317.

Gautam, A., Kumar, R., Chakraborty, N., Muhie, S., Hoke, A., Hammamieh, R., & Jett, M. (2018). Altered fecal microbiota composition in all male aggressor-exposed rodent model simulating features of post-traumatic stress disorder. *Journal of neuroscience research*, *96*(7), 1311-1323.

Geirnaert, A., Calatayud, M., Grootaert, C., Laukens, D., Devriese, S., Smagghe, G., ... & Van de Wiele, T. (2017). Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. *Scientific reports*, *7*(1), 1-14.

Geisler, F. C., Kubiak, T., Siewert, K., & Weber, H. (2013). Cardiac vagal tone is associated with social engagement and self-regulation. *Biological psychology*, *93*(2), 279-286.

Gest, H. (2004). The discovery of microorganisms by Robert Hooke and Antoni Van Leeuwenhoek, fellows of the Royal Society. *Notes and records of the Royal Society of London*, *58*(2), 187-201.

Gibson, M. K., Pesesky, M. W., & Dantas, G. (2014). The yin and yang of bacterial resilience in the human gut microbiota. *Journal of molecular biology*, *426*(23), 3866-3876.

Glaser, R. (2005). Stress-associated immune dysregulation and its importance for human health: a personal history of psychoneuroimmunology. *Brain, behavior, and immunity*, *19*(1), 3-11.

GLICK, G. E. R. A. L. D., BRAUNWALD, E. U. G. E. N. E., & Lewis, R. M. (1965). Relative roles of the sympathetic and parasympathetic nervous systems in the reflex control of heart rate. *Circulation Research*, *16*(4), 363–375.

Goodrich, J. K., Davenport, E. R., Beaumont, M., Jackson, M. A., Knight, R., Ober, C., ... & Ley, R. E. (2016). Genetic determinants of the gut microbiome in UK twins. *Cell host & microbe*, *19*(5), 731-743.

Ghosh, T. S., Arnoux, J., & O'Toole, P. W. (2020). Metagenomic analysis reveals distinct patterns of gut lactobacillus prevalence, abundance, and geographical variation in health and disease. *Gut microbes*, *12*(1), 1822729.

Guo, P., Zhang, K., Ma, X., & He, P. (2020). Clostridium species as probiotics: potentials and challenges. *Journal of animal science and biotechnology, 11*(1), 1-10.

Haque, S. Z., & Haque, M. (2017). The ecological community of commensal, symbiotic, and pathogenic gastrointestinal microorganisms–an appraisal. *Clinical and experimental gastroenterology*, *10*, 91.

Hantsoo, L., Jašarević, E., Criniti, S., McGeehan, B., Tanes, C., Sammel, M. D., ... & Epperson, C. N. (2019). Childhood adversity impact on gut microbiota and inflammatory response to stress during pregnancy. *Brain, behavior, and immunity*, *75*, 240-250.

Hantsoo, L., & Zemel, B. S. (2021). Stress gets into the belly: Early life stress and the gut microbiome. *Behavioural Brain Research*, *414*, 113474.

Hawkins, G. M., Burkett, W. C., McCoy, A. N., Nichols, H. B., Olshan, A. F., Broaddus, R., ... & Bae-Jump, V. (2022). Differences in the microbial profiles of early stage endometrial cancers between Black and White women. *Gynecologic Oncology*, *165*(2), 248-256.

Hauschildt, M., Peters, M. J., Moritz, S., & Jelinek, L. (2011). Heart rate variability in response to affective scenes in posttraumatic stress disorder. *Biological Psychology*, *88*(2-3), 215-222.

Henry, J. P. (1993). Biological basis of the stress response. *Physiology*, *8*(2), 69–73.

Hermes, G. D., Eckermann, H. A., de Vos, W. M., & de Weerth, C. (2020). Does entry to center-based childcare affect gut microbial colonization in young infants?. *Scientific reports*, *10*(1), 1-13.

Heym, N., Heasman, B. C., Hunter, K., Blanco, S. R., Wang, G. Y., Siegert, R., ... & Sumich, A. L. (2019). The role of microbiota and inflammation in self-judgement and empathy: implications for understanding the brain-gut-microbiome axis in depression. *Psychopharmacology*, *236*(5), 1459-1470.

Hinkle, L. E., & Wolff, H. G. (1957). The nature of man's adaptation to his total environment and the relation of this to illness. *AMA archives of Internal Medicine*, *99*(3), 442-460.

Holmes, T. H., & Rahe, R. H. (1967). The social readjustment rating scale. *Journal of psychosomatic research.*

Humbel, F., Rieder, J. H., Franc, Y., Juillerat, P., Scharl, M., Misselwitz, B., ... & Swiss IBD Cohort Study Group. (2020). Association of alterations in intestinal microbiota with impaired psychological function in patients with inflammatory bowel diseases in remission. *Clinical gastroenterology and hepatology*, *18*(9), 2019-2029.

Hunter, P. R. (2003). Drinking water and diarrhoeal disease due to Escherichia coli. *Journal of water and health*, *1*(2), 65-72.

Jain, S., Mills, P. J., Von Känel, R., Hong, S., & Dimsdale, J. E. (2007). Effects of perceived stress and uplifts on inflammation and coagulability. *Psychophysiology*, *44*(1), 154-160.

Jašarević, E., Morrison, K. E., & Bale, T. L. (2016). Sex differences in the gut microbiome–brain axis across the lifespan. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1688), 20150122.

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., ... & Ruan, B. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain, behavior, and immunity*, *48*, 186-194.

Jiang, H. Y., Zhang, X., Yu, Z. H., Zhang, Z., Deng, M., Zhao, J. H., & Ruan, B. (2018). Altered gut microbiota profile in patients with generalized anxiety disorder. *Journal of psychiatric research*, *104*, 130-136.

Jie, F., Yin, G., Yang, W., Yang, M., Gao, S., Lv, J., & Li, B. (2018). Stress in regulation of GABA amygdala system and relevance to neuropsychiatric diseases. *Frontiers in neuroscience*, *12*, 562.

Johnson, K. V. A. (2020). Gut microbiome composition and diversity are related to human personality traits. *Human Microbiome Journal*, *15*, 100069.

Johnson, A. J., Zheng, J. J., Kang, J. W., Saboe, A., Knights, D., & Zivkovic, A. M. (2020). A guide to diet-microbiome study design. *Frontiers in nutrition*, *7*, 79.

Juster, R.-P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience & Biobehavioral Reviews*, *35*(1), 2–16.

Karl, J. P., Hatch, A. M., Arcidiacono, S. M., Pearce, S. C., Pantoja-Feliciano, I. G., Doherty, L. A., & Soares, J. W. (2018). Effects of psychological, environmental and physical stressors on the gut microbiota. *Frontiers in Microbiology*, 2013.

Katona, P. G., & Jih, F. (1975). Respiratory sinus arrhythmia: Noninvasive measure of parasympathetic cardiac control. *Journal of Applied Physiology*, *39*(5), 801–805.

Kelly, J. R., Allen, A. P., Temko, A., Hutch, W., Kennedy, P. J., Farid, N., ... & Dinan, T. G. (2017). Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain, behavior, and immunity, 61*, 50-59.

Kelly, J. R., Borre, Y., O'Brien, C., Patterson, E., El Aidy, S., Deane, J., ... & Dinan, T. G. (2016). Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *Journal of psychiatric research, 82*, 109-118.

Kelly, J. R., Kennedy, P. J., Cryan, J. F., Dinan, T. G., Clarke, G., & Hyland, N. P. (2015). Breaking down the barriers: The gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Frontiers in Cellular Neuroscience*, *392*.

Kemeny, M. E. (2003). The psychobiology of stress. *Current Directions in Psychological Science*, *12*(4), 124–129.

Kinross, J. M., Darzi, A. W., & Nicholson, J. K. (2011). Gut microbiome-host interactions in health and disease. *Genome Medicine*, *3*(3), 14.

Kirschbaum, C., Pirke, K.-M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test' – a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1-2), 76–81.

Kleiman, S. C., Bulik-Sullivan, E. C., Glenny, E. M., Zerwas, S. C., Huh, E. Y., Tsilimigras, M. C., ... & Carroll, I. M. (2017). The gut-brain axis in healthy females: lack of significant association between microbial composition and diversity with psychiatric measures. *PLoS One*, *12*(1), e0170208.

Knowles, S. R., Nelson, E. A., & Palombo, E. A. (2008). Investigating the role of perceived stress on bacterial flora activity and salivary cortisol secretion: a possible mechanism underlying susceptibility to illness. *Biological psychology*, *77*(2), 132-137.

Knight, E. L., Jiang, Y., Rodriguez-Stanley, J., Almeida, D. M., Engeland, C. G., & Zilioli, S. (2021). Perceived stress is linked to heightened biomarkers of inflammation via diurnal cortisol in a national sample of adults. *Brain, behavior, and immunity*, *93*, 206-213.

Konsman, J. P., Parnet, P., & Dantzer, R. (2002). Cytokine-induced sickness behaviour: mechanisms and implications. *Trends in neurosciences*, *25*(3), 154-159.

Krajmalnik-Brown, R., Ilhan, Z.-E., Kang, D.-W., & DiBaise, J. K. (2012). Effects of gut microbes on nutrient absorption and energy regulation. *Nutrition in Clinical Practice*, *27*(2), 201–214.

Kristal-Boneh, E., Raifel, M., Froom, P., & Ribak, J. (1995). Heart rate variability in health and disease. *Scandinavian journal of work, environment & health*, 85-95.

Kuo, P. H., & Chung, Y. C. E. (2019). Moody microbiome: Challenges and chances. *Journal of the Formosan Medical Association*, *118*, S42-S54.

Lane, J. D., Adcock, R. A., & Burnett, R. E. (1992). Respiratory sinus arrhythmia and cardiovascular responses to stress. *Psychophysiology*, *29*(4), 461-470.

Lehtoranta, L., Ala-Jaakkola, R., Laitila, A., & Maukonen, J. (2022). Healthy vaginal microbiota and influence of probiotics across the female life span. *Frontiers in Microbiology*, 787.

Lerner, A., Shoenfeld, Y., & Matthias, T. (2019). Probiotics: if it does not help it does not do any harm. Really?. *Microorganisms*, *7*(4), 104.

Levin, I. I., Zonana, D. M., Fosdick, B. K., Song, S. J., Knight, R., & Safran, R. J. (2016). Stress response, gut microbial diversity and sexual signals correlate with social interactions. *Biology letters*, *12*(6), 20160352.

Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., & Gordon, J. I. (2008). Worlds within worlds: evolution of the vertebrate gut microbiota. *Nature Reviews Microbiology*, *6*(10), 776-788.

Li, P., Stuart, E. A., & Allison, D. B. (2015). Multiple imputation: a flexible tool for handling missing data. *Jama*, *314*(18), 1966-1967.

Li, F., Han, Y., Wu, X., Cao, X., Gao, Z., Sun, Y., ... & Xiao, H. (2022). Gut microbiota-derived resveratrol metabolites, dihydroresveratrol and lunularin, significantly contribute to the biological activities of resveratrol. *Frontiers in Nutrition*, *9*, 1002.

Lin, H., Peddada, S. D., & Lin, M. H. (2021). Package 'ANCOM-BC'.

Long, E. S., Penalver Bernabe, B., Xia, K., Azcarate-Peril, M. A., Carroll, I. M., Rackers, H. S., ... & Kimmel, M. C. (2023). The microbiota-gut-brain axis and perceived stress in the perinatal period. *Archives of Women's Mental Health*, *26*(2), 227-234.

Lyte, M., & Ernst, S. (1992). Catecholamine induced growth of gram negative bacteria. *Life sciences*, *50*(3), 203-212.

Mackner, L. M., Hatzakis, E., Allen, J. M., Davies, R. H., Kim, S. C., Maltz, R. M., & Bailey, M. T. (2020). Fecal microbiota and metabolites are distinct in a pilot study of pediatric Crohn's disease patients with higher levels of perceived stress. *Psychoneuroendocrinology*, *111*, 104469.

Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., & Balamurugan, R. (2020). The firmicutes/bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients?. *Nutrients*, *12*(5), 1474.

Magruder, M., Sholi, A. N., Gong, C., Zhang, L., Edusei, E., Huang, J., Albakry, S., Satlin, M. J., Westblade, L. F., Crawford, C., Dadhania, D. M., Lubetzky, M., Taur, Y., Littman, E., Ling, L., Burnham, P., De Vlaminck, I., Pamer, E., Suthanthiran, M., & Lee, J. R. (2019). Gut

uropathogen abundance is a risk factor for development of bacteriuria and urinary tract infection. *Nature Communications, 10*(1).

Malan-Muller, S., Valles-Colomer, M., Raes, J., Lowry, C. A., Seedat, S., & Hemmings, S. M. (2018). The gut microbiome and mental health: implications for anxiety-and trauma-related disorders. *Omics: a journal of integrative biology, 22*(2), 90-107.

Martens, E. C., Chiang, H. C., & Gordon, J. I. (2008). Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. *Cell host & microbe, 4*(5), 447-457.

Matsuki, T., Watanabe, K., Tanaka, R., Fukuda, M., & Oyaizu, H. (1999). Distribution of bifidobacterial species in human intestinal microflora examined with 16S rRNA-gene-targeted species-specific primers. *Applied and environmental microbiology, 65*(10), 4506-4512.

Mayer, E. A. (2018). The Mind-Gut Connection. HarperCollins Publishers

Mayer, E. A., Tillisch, K., & Gupta, A. (2015). Gut/Brain Axis and the microbiota. *Journal of Clinical Investigation, 125*(3), 926–938.

McEwen, B. S. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York academy of sciences, 840*(1), 33-44.

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and Biomedicine. *Hormones and Behavior, 43*(1), 2–15.

McKean, J., Naug, H., Nikbakht, E., Amiet, B., & Colson, N. (2017). Probiotics and subclinical psychological symptoms in healthy participants: a systematic review and meta-analysis. *The Journal of alternative and complementary medicine, 23*(4), 249-258.

Méndez-Bailón, M., Jiménez-García, R., Hernández-Barrera, V., de Miguel-Díez, J., de Miguel-Yanes, J. M., Muñoz-Rivas, N., ... & López-de-Andrés, A. (2020). Heart failure is a risk factor for

suffering and dying of Clostridium difficile infection. Results of a 15-year nationwide study in Spain. *Journal of Clinical Medicine, 9*(3), 614.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., ... & Cazaubiel, J. M. (2011). Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. *British Journal of Nutrition, 105*(5), 755-764.

Michels, N., Van de Wiele, T., Fouhy, F., O'Mahony, S., Clarke, G., & Keane, J. (2019). Gut microbiome patterns depending on children's psychosocial stress: *Reports versus biomarkers. Brain, behavior, and immunity, 80*, 751-762.

Mosca, A., Leclerc, M., & Hugot, J. P. (2016). Gut microbiota diversity and human diseases: should we reintroduce key predators in our ecosystem?. *Frontiers in microbiology*, *7*, 455.

Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., & Cryan, J. F. (2014). The microbiome: Stress, health and disease. *Mammalian Genome*, *25*(1-2), 49–74.

Mukherjee, A., Lordan, C., Ross, R. P., & Cotter, P. D. (2020). Gut microbes from the phylogenetically diverse genus Eubacterium and their various contributions to gut health. *Gut Microbes, 12*(1), 1802866.

Mullie, C., Romond, M. B., Yazourh, A., Libersa, C., Bezirtzoglou, E., & Romond, C. (2002). Influence of stress on faecal carriage of Clostridium perfringens. *Microbial ecology in health and disease, 14*(2), 118-121.

Nagao-Kitamoto, H., Kitamoto, S., Kuffa, P., & Kamada, N. (2016). Pathogenic role of the gut microbiota in gastrointestinal diseases. *Intestinal Research, 14*(2), 127.

Naseribafrouei, A., Hestad, K., Avershina, E., Sekelja, M., Linløkken, A., Wilson, R., & Rudi, K. (2014). Correlation between the human fecal microbiota and depression. *Neurogastroenterology & Motility, 26*(8), 1155-1162.

Nguyen, T. L., Vieira-Silva, S., Liston, A., & Raes, J. (2015). How informative is the mouse for human gut microbiota research? *Disease Models & Mechanisms*, *8*(1), 1–16.

Nishida, K., Sawada, D., Kuwano, Y., Tanaka, H., & Rokutan, K. (2019). Health benefits of Lactobacillus gasseri CP2305 tablets in young adults exposed to chronic stress: a randomized, double-blind, placebo-controlled study. *Nutrients*, *11*(8), 1859.

O'callaghan, A., & Van Sinderen, D. (2016). Bifidobacteria and their role as members of the human gut microbiota. *Frontiers in microbiology, 7*, 925.

Öhman, L., & Simrén, M. (2013). Intestinal microbiota and its role in irritable bowel syndrome (IBS). *Current gastroenterology reports, 15*(5), 323.

Ondicova, K., & Mravec, B. (2010). Multilevel interactions between the sympathetic and parasympathetic nervous systems: A minireview. *Endocrine Regulations*, *44*(2), 69–75.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., ... & Oksanen, M. J. (2013). Package 'vegan'. *Community ecology package, version*, *2*(9), 1-295.

Ottosson, F., Brunkwall, L., Ericson, U., Nilsson, P. M., Almgren, P., Fernandez, C., Melander, O., & Orho-Melander, M. (2018). Connection between BMI-related plasma metabolite profile and gut microbiota. *The Journal of Clinical Endocrinology & Metabolism*, *103*(4), 1491–1501.

Paiva, I. H. R., Duarte-Silva, E., & Peixoto, C. A. (2020). The role of prebiotics in cognition, anxiety, and depression. *European Neuropsychopharmacology, 34*, 1-18.

Pallister, T., Jackson, M. A., Martin, T. C., Zierer, J., Jennings, A., Mohney, R. P., ... & Menni, C. (2017). Hippurate as a metabolomic marker of gut microbiome diversity: Modulation by diet and relationship to metabolic syndrome. *Scientific reports*, *7*(1), 1-9.

Park, C. L., Russell, B. S., Fendrich, M., Finkelstein-Fox, L., Hutchison, M., & Becker, J. (2020). Americans' covid-19 stress, coping, and adherence to CDC guidelines. *Journal of General Internal Medicine*, *35*(8), 2296–2303.

Passalacqua, K. D., Charbonneau, M. E., & O'riordan, M. X. (2016). Bacterial metabolism shapes the host–pathogen interface. *Virulence Mechanisms of Bacterial Pathogens*, 15-41.

Porges, S. W., Doussard-Roosevelt, J. A., & Maiti, A. K. (1994). Vagal tone and the physiological regulation of emotion. *Monographs of the society for research in child development*, 167-186.

Piquer-Esteban, S., Ruiz-Ruiz, S., Arnau, V., Diaz, W., & Moya, A. (2022). Exploring the universal healthy human gut microbiota around the World. *Computational and Structural Biotechnology Journal*, *20*, 421-433.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, *41*(D1), D590-D596.

Qin, H. Y., Cheng, C. W., Tang, X. D., & Bian, Z. X. (2014). Impact of psychological stress on irritable bowel syndrome. *World journal of gastroenterology: WJG*, *20*(39), 14126.

Rahe, R. H., & Lind, E. (1971). Psychosocial factors and Sudden cardiac death: A pilot study. *Journal of Psychosomatic Research*, *15*(1), 19–24.

Rhee, S. H., Pothoulakis, C., & Mayer, E. A. (2009). Principles and clinical implications of the brain–gut–enteric microbiota axis. *Nature reviews Gastroenterology & hepatology*, *6*(5), 306-314.

Rod, N. H., Grønbaek, M., Schnohr, P., Prescott, E., & Kristensen, T. S. (2009). Perceived stress as a risk factor for changes in health behaviour and cardiac risk profile: a longitudinal study. *Journal of internal medicine*, *266*(5), 467-475.

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., ... & Segal, E. (2018). Environment dominates over host genetics in shaping human gut microbiota. *Nature*, *555*(7695), 210-215.

Reyes del Paso, G. A., Godoy, J., & Vila, J. (1993). Respiratory sinus arrhythmia as an index of parasympathetic cardiac control during the cardiac defense response. *Biological Psychology*, *35*(1), 17–35.

Savage, D. C. (1977). Microbial ecology of the gastrointestinal tract. *Annual review of microbiology*, *31*(1), 107-133.

Sharma, V. K., Trakroo, M., Subramaniam, V., Rajajeyakumar, M., Bhavanani, A. B., & Sahai, A. (2013). Effect of fast and slow pranayama on perceived stress and cardiovascular parameters in young health-care students. *International journal of yoga*, *6*(2), 104.

Sharon, G., Sampson, T. R., Geschwind, D. H., & Mazmanian, S. K. (2016). The central nervous system and the gut microbiome. *Cell*, *167*(4), 915-932.

Schmidt, E. K., Torres-Espin, A., Raposo, P. J., Madsen, K. L., Kigerl, K. A., Popovich, P. G., Fenrich, K. K., & Fouad, K. (2020). Fecal transplant prevents gut dysbiosis and anxiety-like behaviour after spinal cord injury in rats. *PLOS ONE*, *15*(1).

Segal-Isaacson, C. J., Wylie-Rosett, J., & Gans, K. M. (2004). Validation of a short dietary assessment questionnaire: THE RAPID eating and activity assessment for participants short version (reap-S). *The Diabetes Educator*, *30*(5), 774–781.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome biology*, *12*(6), 1-18.

Smits, S. A., Leach, J., Sonnenburg, E. D., Gonzalez, C. G., Lichtman, J. S., Reid, G., ... & Sonnenburg, J. L. (2017). Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science*, *357*(6353), 802-806.

Sobko, T., Liang, S., Cheng, W. H., & Tun, H. M. (2020). Impact of outdoor nature-related activities on gut microbiota, fecal serotonin, and perceived stress in preschool children: the Play&Grow randomized controlled trial. *Scientific Reports*, *10*(1), 1-12.

Sonnenburg, J. L., Xu, J., Leip, D. D., Chen, C. H., Westover, B. P., Weatherford, J., ... & Gordon, J. I. (2005). Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science*, *307*(5717), 1955-1959.

Sonnenburg, J. L., & Sonnenburg, E. D. (2019). Vulnerability of the industrialized microbiota. *Science*, *366*(6464), eaaw9255.

Spencer, C. N., McQuade, J. L., Gopalakrishnan, V., McCulloch, J. A., Vetizou, M., Cogdill, A. P., ... & Wargo, J. A. (2021). Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. *Science*, *374*(6575), 1632-1640.

Staniute, M., Brozaitiene, J., & Bunevicius, R. (2013). Effects of social support and stressful life events on health-related quality of life in coronary artery disease patients. *Journal of Cardiovascular Nursing*, *28*(1), 83-89.

Stanwood, G. D. (2019). Dopamine and stress. In *Stress: Physiology, biochemistry, and pathology* (pp. 105-114). Academic Press.

Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain research, 1693*, 128-133.

Steptoe, A., & Kivimäki, M. (2012). Stress and cardiovascular disease. *Nature Reviews Cardiology*, *9*(6), 360-370.

Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain research, 1693*, 128-133.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., ... & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *The Journal of physiology, 558*(1), 263-275.

Suez, J., Zmora, N., Zilberman-Schapira, G., Mor, U., Dori-Bachash, M., Bashiardes, S., ... & Elinav, E. (2018). Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. *Cell, 174*(6), 1406-1423.

Suez, J., Zmora, N., Segal, E., & Elinav, E. (2019). The pros, cons, and many unknowns of probiotics. *Nature medicine*, *25*(5), 716-729.

Sun, L., Zhang, H., Cao, Y., Wang, C., Zhao, C., Wang, H., ... & Nie, Y. (2019). Fluoxetine ameliorates dysbiosis in a depression model induced by chronic unpredicted mild stress in mice. *International journal of medical sciences, 16*(9), 1260.

Sun, J., Wang, F., Hong, G., Pang, M., Xu, H., Li, H., ... & Liu, J. (2016). Antidepressant-like effects of sodium butyrate and its possible mechanisms of action in mice exposed to chronic unpredictable mild stress. *Neuroscience letters*, *618*, 159-166.

Sun, L., Zhang, H., Cao, Y., Wang, C., Zhao, C., Wang, H., ... & Nie, Y. (2019). Fluoxetine ameliorates dysbiosis in a depression model induced by chronic unpredicted mild stress in mice. *International journal of medical sciences*, *16*(9), 1260.

Svensson, E., Horváth-Puhó, E., Thomsen, R. W., Djurhuus, J. C., Pedersen, L., Borghammer, P., & Sørensen, H. T. (2015). Vagotomy and subsequent risk of Parkinson's disease. *Annals of neurology, 78*(4), 522-529.

Vincent, C., Boerlin, P., Daignault, D., Dozois, C. M., Dutil, L., Galanakis, C., ... & Manges, A. R. (2010). Food reservoir for Escherichia coli causing urinary tract infections. *Emerging infectious diseases, 16*(1), 88.

Takeshita, T., Kageyama, S., Furuta, M., Tsuboi, H., Takeuchi, K., Shibata, Y., ... & Yamashita, Y. (2016). Bacterial diversity in saliva and oral health-related conditions: the Hisayama Study. *Scientific reports, 6*(1), 1-11.

Tillisch, K., Mayer, E., Gupta, A., Gill, Z., Brazeilles, R., Le Nevé, B., ... & Labus, J. (2017). Brain structure and response to emotional stimuli as related to gut microbial profiles in healthy women. *Psychosomatic medicine, 79*(8), 905.

Travagli, R. A., & Anselmi, L. (2016). Vagal neurocircuitry and its influence on gastric motility. *Nature Reviews Gastroenterology & Hepatology, 13*(7), 389-401.

Van den Buuse, M., & Hale, M. W. (2019). Serotonin in stress. In *Stress: Physiology, Biochemistry, and Pathology* (pp. 115-123). Academic Press.

Vonck, K. E. J., & Larsen, L. E. (2018). Vagus nerve stimulation. *Neuromodulation*, 211–220.

Wall, R., Cryan, J. F., Ross, R. P., Fitzgerald, G. F., Dinan, T. G., & Stanton, C. (2014). Bacterial neuroactive compounds produced by psychobiotics. *Microbial endocrinology: The microbiota-gut-brain axis in health and disease*, 221-239.

Wang, Y., Wan, X., Wu, X., Zhang, C., Liu, J., & Hou, S. (2021). Eubacterium rectale contributes to colorectal cancer initiation via promoting colitis. *Gut pathogens, 13*(1), 1-11.

Ware, J. E., & Sherbourne, C. D. (1992). The MOS 36-ltem short-form health survey (SF-36). *Medical Care*, *30*(6), 473–483.

Wechsler, D. (1991). Wechsler intelligence scale for children—Third edition (WISC-III). San Antonio, TX: *Psychological Corporation*.

Wexler, H. M. (2007). Bacteroides: the good, the bad, and the nitty-gritty. *Clinical microbiology reviews*, *20*(4), 593-621.

Yang, J., & Yu, J. (2018). The Association of Diet, gut microbiota and colorectal cancer: What we eat may imply what we get. *Protein & Cell*, *9*(5), 474–487.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., ... & Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, *486*(7402), 222-227.

Yau, Y. H., & Potenza, M. N. (2013). Stress and eating behaviors. *Minerva endocrinologica*, *38*(3), 255.

Yoshioka, M., Ito, T., Miyazaki, S., & Nakajima, Y. (1998). The release of tumor necrosis factor-α, interleukin-1, interleukin-6 and prostaglandin E2 in bovine Kupffer cells stimulated with bacterial lipopolysaccharide. *Veterinary immunology and immunopathology*, *66*(3-4), 301-307.

Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., ... & Xie, P. (2016). Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Molecular psychiatry*, *21*(6), 786-796.

Zhou, Yingting, and Fachao Zhi. "Lower Level of *Bacteroides* in the Gut Microbiota Is Associated with Inflammatory Bowel Disease: A Meta-Analysis." *BioMed Research International* 2016 (2016): 5828959–9. Web.

Zhou, K. (2017). Strategies to promote abundance of *Akkermansia* muciniphila, an emerging probiotic in the gut, evidence from dietary intervention studies. *Journal of functional foods*, *33*, 194-201.

Zou, B., Miao, C., & Chen, J. (2020). Depression and perceived stress, but not anxiety, are associated with elevated inflammation in an obese adult population. *Risk Management and Healthcare Policy*, 1489-1497.