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Meditation Induced Changes in the Human Gut Microbiota

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Abstract

This study investigates the effects of a seven-day meditation retreat on the gut microbiome, with a particular focus on individuals with Post-Traumatic-Stress-Disease (PTSD). While there were no significant changes in overall microbial diversity as measured by alpha diversity indices, notable shifts in beta diversity were observed. The PERMANOVA analysis revealed a significant difference in microbiome composition between pre- and post-retreat samples from participants with PTSD and Anxiety, with p-values of < 0.001 and 0.043, respectively. This suggests that meditation may induce specific alterations in the gut microbiome of PTSD and Anxiety patients, even though the changes were subtle. Of particular interest was the increase in *Roseburia*, a genus linked to anti-inflammatory effects and improved gut barrier function, alongside a decrease in *Bacteroides*, which is sometimes associated with inflammation. These shifts suggest a potential benefit for gut health in these cohorts. Furthermore, a global increase in the *Bacillota:Bacteroidota* (former *F:B*) ratio was observed, a shift often associated with a more youthful microbiome profile. This change could be indicative of a rejuvenating effect of meditation on the gut microbiome. Species-level analysis revealed that PTSD patients experienced shifts toward a microbiome composition similar to that of healthy controls. Several species, including *Gemmiger formicilis* and *Subdoligranulum sp.*, showing alterations that are known to be associated with metabolic health and gut integrity. These findings suggest that meditation may not only affect microbial diversity but also potentially "normalize" the microbiome in PTSD patients, contributing to both physical and mental health improvements. Future studies should explore the long-term effects and mechanisms behind these changes, as well as the broader implications for using meditation as a potential therapeutic intervention for individuals with PTSD or other health conditions.

Zusammenfassung

Diese Studie untersucht die Auswirkungen eines sieben-tägigen Meditations-Retreats auf das Mikrobiom des Darms, mit besonderem Fokus auf Personen mit Posttraumatischer Belastungsstörung (PTBS). Während es keine signifikanten Veränderungen in der Gesamt-Mikrobiomdiversität, gemessen durch Alpha-Diversitätsindizes, gab, wurden bemerkenswerte Veränderungen in der Beta-Diversität beobachtet. Die PERMANOVA-Analyse ergab einen signifikanten Unterschied in der Mikrobiomzusammensetzung zwischen den vor- und nach-Retreat Proben von PTBS-Teilnehmer:innen mit einem p-Wert kleiner 0,001, sowie von Probanden mit Angststörung ($p = 0.043$). Dies deutet darauf hin, dass Meditation spezifische Veränderungen im Mikrobiom von PTBS-Patienten hervorrufen kann, auch wenn diese Veränderungen subtil waren. Besonders interessant war die Zunahme von *Roseburia*, einer Gattung, die mit entzündungshemmenden Effekten und einer verbesserten Darmbarrierefunktion in Verbindung steht. Gleichzeitig wurde eine Abnahme von *Bacteroides*, das typischerweise mit Entzündungen assoziiert wird, gemessen. Diese Verschiebungen deuten auf einen potenziellen Nutzen für die Darmgesundheit der PTBS-Gruppe hin. Darüber hinaus wurde ein globaler Anstieg des *Bacillota:Bacteroidota*-Verhältnisses beobachtet, eine Veränderung, die häufig mit einem 'jugendlicheren' Mikrobiomprofil verbunden ist. Diese Veränderung könnte einen verjüngenden Effekt der Meditation auf das Mikrobiom des Darms anzeigen. Eine Analyse auf Spezies-Ebene ergab, dass PTBS-Patient:innen Verschiebungen in Richtung einer Mikrobiomzusammensetzung erlebten, die der von gesunden Kontrollpersonen ähnelt. Hierbei haben mehrere Arten, darunter *Gemmiger formicilis* und *Subdoligranulum sp.*, Veränderungen gezeigt, die mit metabolischer Gesundheit und der Integrität des Darms in Verbindung stehen. Diese Ergebnisse deuten darauf hin, dass Meditation nicht nur die mikrobielle Diversität beeinflussen, sondern auch das Mikrobiom von PTBS-Patienten „normalisieren" könnte, was sowohl körperliche als auch geistige Gesundheitsverbesserungen zur Folge haben könnte. Zukünftige Studien sollten die langfristigen Auswirkungen und Mechanismen hinter diesen Veränderungen sowie die breiteren Implikationen der Meditation als potenzielle therapeutische Intervention für Personen mit PTBS und anderen Krankheiten weiter untersuchen.

1 Introduction

1.1 The Human Gut Microbiome

The historical development of microbiome research is tracing back to the late 1800s in Europe. The discovery of microorganisms in the human gut however, can be followed back to Antonie van Leeuwenhoek's observations in the late 1600s (Ford, 1982a), but it was in the mid-19th century that significant progress was made. Studies throughout the 19th century supported the presence of bacteria in human faeces, gradually building the case for the human gut flora (Hentges, 2012). The terms "microbiota" and "microbiome" can be traced to 1927 and 1949, respectively (Appanna, 2018).

Four European scientists — Theodor Escherich, Henry Tissier, Ilya Metchnikov, and Alfred Nissle — were pioneering gut microbiome research with their efforts to improve health and extend lifespan.

Theodor Escherich was a German pediatrician, who is perhaps best known for his discovery of *Escherichia coli* (*E. coli*) in the human gut back in 1885. His work helped solidify the understanding of gut flora. He identified the bacterium as part of the normal intestinal flora in infants, linking it to healthy digestion. This discovery contributed to the recognition of the critical role gut microorganisms play in maintaining health. Escherich's research was instrumental in advancing the idea that beneficial microorganisms could be important for human well-being, laying the groundwork for future probiotic research (Friedmann, 2006).

Henry Tissier was a French pediatrician, who is credited with advancing the concept of probiotics. He conducted studies in the late 19th and early 20th centuries on the microbiota of infants and found that children with diarrhea often had an imbalance in their gut microbiota. Tissier introduced the idea of using beneficial bacteria to treat gastrointestinal conditions, famously administering *Lactobacillus* strains as a probiotic treatment for both children and adults to restore healthy gut flora and improve digestive health. His pioneering work demonstrated the therapeutic potential of using microorganisms to restore health, particularly in managing gastrointestinal disorders (Farré-Maduell & Casals-Pascual, 2019).

Ilya Metchnikov was a Russian immunologist and winner of the Nobel price in 1908, who is best known for his work on immunity and for popularizing the consumption

of fermented milk as a way to improve health and extend lifespan. He hypothesized that certain microorganisms, particularly those found in fermented foods like yogurt, could have beneficial effects on the gut. Metchnikov believed that these "friendly" bacteria could help delay the aging process by promoting a healthy gut microbiota and improving overall immune function. His theories on the benefits of probiotics laid the foundation for later research on gut health and the use of probiotics in modern medicine (Caramia, 2008).

Alfred Nissle was a German microbiologist, who made a major contribution to the therapeutic use of probiotics by discovering and isolating a beneficial *E. coli* strain in 1917. During World War I, Nissle observed that soldiers suffering from bloody diarrhea showed improvement when treated with gelatine capsules containing this strain (Schultz, 2008).

These four scientists' work laid the foundations for modern microbiome research, particularly the understanding that gut bacteria are crucial for health and the potential of manipulating the microbiome to treat diseases. Although these early studies had significant clinical application potential, microbiome research faded in Western medicine for a time.

Interest in the microbiome resurged with the introduction of advanced sequencing technologies, the launch of the Human Microbiome Project in 2007, and the successful use of fecal microbiota transplants to treat conditions like *Clostridium difficile* infection. Since the Human Microbiome Project's first results were published in 2012, the realization that bacteria outnumber human cells has renewed both scientific and popular interest in the microbiome. It has been estimated that the human microbiome contributes 2 to 20 million genes, vastly outnumbering the around 20,000 genes present in the human genome. This project provided a comprehensive look at the diversity of the human microbiome and began to reveal its crucial roles in human health and disease (Turnbaugh et al., 2007).

In the 2010s and beyond, high-throughput sequencing techniques, such as 16S rRNA sequencing and metagenomics, revolutionized the study of the gut microbiome. This has led to an explosion of research into its links to various health conditions, including digestive disorders, metabolic diseases, mental health, and immune function (Chuang, Walsh, Yen, & Cho, 2014).

Clinical relevance became evident in 2013, when a randomized controlled trial showed superior results for fecal transplants over standard antibiotic treatments for *Clostridium difficile* infections (Karadsheh & Sule, 2013).

Today, the human gut microbiome is a dynamic and rapidly growing area of

research, with increasing focus on its potential in personalized medicine and disease prevention. However, challenges persist in converting this knowledge into practical clinical applications. Additional research is essential to comprehensively explore the complexities of microbial interactions and their implications for precision medicine (Ford, 1982b).

1.2 Studies on Meditation

The term "meditation" originates from the Latin word "meditari", which translates to "to actively participate in contemplation or thought." The word "meditation" has its origins in the shared Greek and Latin roots associated with the word "medicine." Meditation is a distinct and clearly defined experience characterized by a state of "thoughtless awareness" or mental stillness (Sharma & Sharma, 2024).

In recent years, meditation has shifted from mainly a spiritual practice to becoming an important tool in various health-related fields. Initial studies primarily focused on the psychological effects of meditation, but as interest expanded, the absence of a clear definition became evident in research (Cardoso, Souza, Camano, & Leite, 2004a). Wallace, Benson, & Wilson (1971) introduced the idea of a "wakeful hypometabolic physiologic state," highlighting meditation's physical aspects without explaining the methods used. Other early views included Goleman's description of meditation as "a consistent attempt to reach a specific attention position" and Craven's view, which focused on key elements like relaxation, concentration, altered consciousness, and self-observation (Craven, 1989; Goleman, 1976).

In 1998, the Department of Psychobiology at the Universidade Federal de São Paulo began systematically researching meditation, proposing a structured definition to standardize the practice. They argued that meditation requires a well-defined technique combining both physical and mental relaxation, free from analysis, judgment, or expectations. It should also be self-sustained, using concentration to minimize reliance on an instructor. This framework highlights the core elements of meditation, such as relaxation and focused attention, which foster a calm, present awareness. Techniques fitting this definition include mindfulness, mantra-based practices, breath work, and movement practices like yoga and qigong (Cardoso, Souza, Camano, & Leite, 2004b).

Regular meditation has been consistently associated with benefits for personal well-being across both psychological and physiological dimensions. Psychologically, it may help reduce stress, strengthen emotional resilience, boost cognitive function,

improve sleep quality, and reduce symptoms of anxiety and depression (Goyal et al., 2014; Toneatto & Nguyen, 2007; Waechter & Wekerle, 2015).

Physiologically, meditation can lower blood pressure, aid in pain management, enhance immune function, reduce inflammation, balance stress hormones, and even slow certain aging processes (Black & Slavich, 2016; Buric, Farias, Jong, Mee, & Brazil, 2017; Klimecki et al., 2019; Nascimento, Oliveira, & DeSantana, 2018). A recent study by Zuniga-Hertz et al. (2023) suggest meditation as an adjuvant treatment to viral infections, such as the Covid-19 disease.

Reducing stress through meditation has been shown to positively impact heart health, potentially helping to prevent heart disease by improving risk factors like high blood pressure, smoking, alcohol use, blood sugar, and cholesterol levels (Barnes & Orme-Johnson, 2012). Movement-based practices, like yoga, are linked to lower cholesterol and triglycerides, with similar benefits found in qigong, a traditional Chinese practice that combines meditation with movement (Antonelli et al., 2024).

In addition to meditation, breath work practices, particularly those involving deep, rhythmic, or intense breathing patterns, can play a role in inducing altered states of consciousness by increasing cerebrospinal fluid (CSF) flow. Through focused breathing, the natural rise and fall of intracranial pressure are amplified, promoting the movement of CSF through the brain and spinal cord (Kollmeier et al., 2022). This enhanced circulation may impact the brain's chemical environment, potentially influencing neurotransmitter activity and stimulating the parasympathetic nervous system. The result can be a heightened sense of awareness, relaxation, and clarity, allowing practitioners to access altered states that promote introspection and emotional release (Drigas & Mitsea, 2022).

1.3 Next Generation Sequencing (NGS)

Whole genome sequencing (WGS) has evolved significantly, with techniques such as Illumina and nanopore sequencing leading the way. Illumina's short-read sequencing is widely used for its high throughput and accuracy, while nanopore sequencing offers unique advantages, including long-read capabilities and real-time analysis (Hasman et al., 2020a).

Illumina, Inc. was established in 1998 and was co-founded by Jay Flatley, who played a significant role in its development and growth (Chuang et al., 2014). Illumina's sequencing technology primarily utilizes a method known as short-read sequencing. This process involves fragmenting DNA into smaller pieces, which are then attached to

a flow cell. During sequencing, fluorescently labeled nucleotides are added, and as they incorporate into the growing DNA strands, they emit signals that are captured by a camera. This enables the accurate identification of the DNA sequence. Illumina's high-throughput capabilities enable the simultaneous processing of millions of fragments, establishing it as a powerful tool for diverse genomic applications, including research and clinical diagnostics (Croucher et al., 2009).

Nanopore sequencing is a revolutionary technique in the field of genomics that allows for the direct sequencing of DNA or RNA strands. It was developed by researchers at Oxford Nanopore Technologies, which was founded in 2005. The technology utilizes a nanopore, a tiny hole in a membrane, through which single strands of DNA or RNA are passed. As the nucleotides move through the nanopore, they cause characteristic disruptions in an ionic current, which are measured in real-time. These disruptions allow for the identification of the sequence of nucleotides, offering significant advantages such as long-read capabilities and the ability to analyze samples on-the-go, making it a valuable tool for various applications in research and clinical settings (Rhee & Burns, 2006).

Moreover, WGS has had a profound impact on gut microbiome studies, revolutionizing our understanding of the complex communities of microorganisms living in the human gastrointestinal tract. WGS allows researchers to identify and characterize the diverse microbial populations present, uncovering their roles in health and disease while providing insights into interactions between different species (Maccaferri, Biagi, & Brigidi, 2011).

Additionally, WGS allows researchers to obtain detailed genetic information from all organisms present in a gut sample. Unlike conventional approaches, which frequently depend on culturing or targeted sequencing, WGS provides a complete picture of the microbial diversity, including bacteria, archaea, viruses, and fungi. This thorough characterization is essential for gaining an understanding of the complex interactions within the gut microbiome and how they relate to health and disease (Hasman et al., 2020b).

Furthermore, WGS enables the discovery of previously uncharacterized microbial species. Many gut microbes are difficult to culture in laboratory settings, leading to gaps in knowledge about their functions and roles. With WGS, researchers can sequence DNA directly from fecal samples, uncovering novel taxa and expanding the known diversity of the gut microbiome. This is particularly important for identifying microorganisms that may play a role in various health conditions (Jansen van Rensburg, Swift, Cody, Jenkins, & Maiden, 2016).

In addition, WGS facilitates functional genomics studies by allowing researchers to analyze the metabolic capabilities of gut microbiota. By examining the genes present in a microbiome sample, scientists can infer the metabolic pathways and functions of microbial communities. This metagenomic approach helps in understanding how gut microbes contribute to processes such as digestion, nutrient absorption, and the production of metabolites that influence host health (W.-L. Wang et al., 2015).

What is more, WGS has improved our understanding of the interactions between gut microbes and their human hosts. By analyzing the genetic material of both the microbiome and the host, researchers can investigate how microbial communities influence host physiology, immune responses, and even mental health. This research is advancing personalized medicine, using microbiome profiles to guide treatment strategies for a range of diseases (Fung, Olson, & Hsiao, 2017).

Another important aspect of WGS is, that it allows the tracking of shifts of specific microbes in the gut over time, providing insights into how diet, lifestyle, and environmental factors influence microbial composition. Longitudinal studies using WGS can reveal patterns of stability and variability in the microbiome, helping to identify factors that contribute to dysbiosis (imbalanced microbiome) and its association with health conditions such as inflammatory bowel disease (Frank et al., 2007), obesity (Cînpeanu, Rus, & Tarcea, 2019), cancer (García-Castillo, Sanhueza, McNerney, Onate, & García, 2016) and other metabolic diseases.

The integration of WGS in gut microbiome research has significant implications for personalized medicine. By analyzing individual microbiome profiles, healthcare providers are able to tailor dietary recommendations, probiotics, and other interventions to optimize gut health. For example, specific microbial signatures associated with certain diseases can guide clinicians in predicting disease risk and developing preventive strategies (L. Wang et al., 2023).

To summarize, WGS has been instrumental in establishing connections between gut microbiota and various diseases. Studies have shown that dysbiosis - in addition to the diseases mentioned above - is linked to conditions such as diabetes, cardiovascular diseases, and autoimmune disorders. By leveraging WGS data, researchers can identify microbial biomarkers that may serve as diagnostic tools or therapeutic targets (Gupta, Singh, & Mani, 2022).

2 Material and Methods

2.1 Study Design and Demographics

The GLOBAL study was designed to investigate the impact of meditation on the gut microbiome, using a randomized selection of participants from Dr. Joe Dispenza's advanced meditation retreats. A previous study by Zuniga-Hertz et al. (2023) has already been published, examining participants from these same meditation retreats and using an identical meditation intervention to assess related health outcomes. Participants were randomly chosen from attendees across five retreat locations—Vienna, Austria; Maroochydore, Australia; Cancun, Mexico; Nashville, Tennessee, USA; and Denver, Colorado, USA—ensuring a diverse sample pool. This randomization aimed to reduce selection bias, enhancing the generalizability of the findings. An overview of the study design is presented in Figure 2.1.

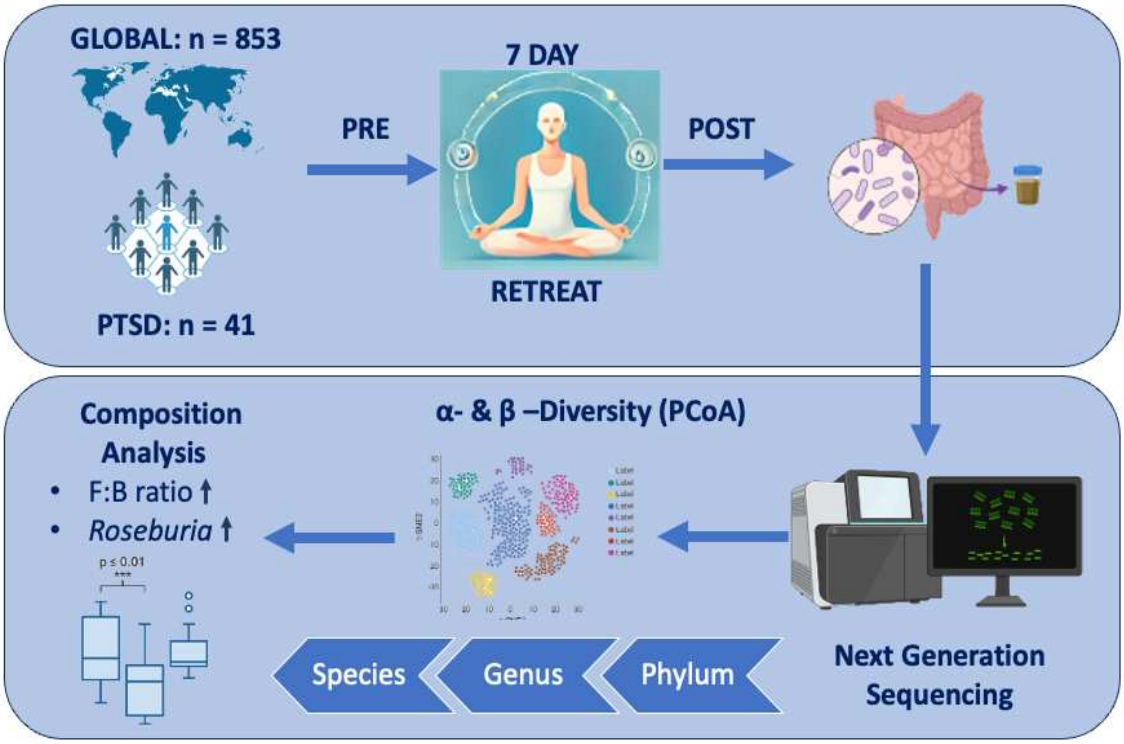

Figure 2.1: Infographics of a global microbiome study on meditation.

As shown in Figure 2.2, the distribution of subjects across the five locations reveals that Vienna has the highest number of participants, while Denver has the fewest.

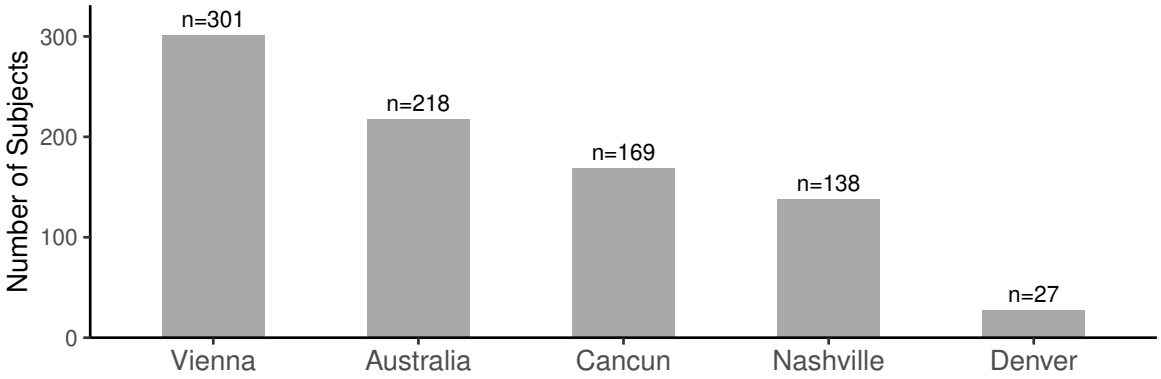

Figure 2.2: Location-based Subject Distribution: The bar chart shows the distribution of study subjects across five locations: Vienna, Australia, Cancun, Nashville, and Denver.

The gender distribution shows a strong female majority across all age categories, with the highest concentration in the 40-50 and 50-60 age ranges. These demographics suggest a somehow balanced representation of age groups but a higher prevalence of female participants, which may influence the analysis of gender-based outcomes in this study shown in Figure 2.3.

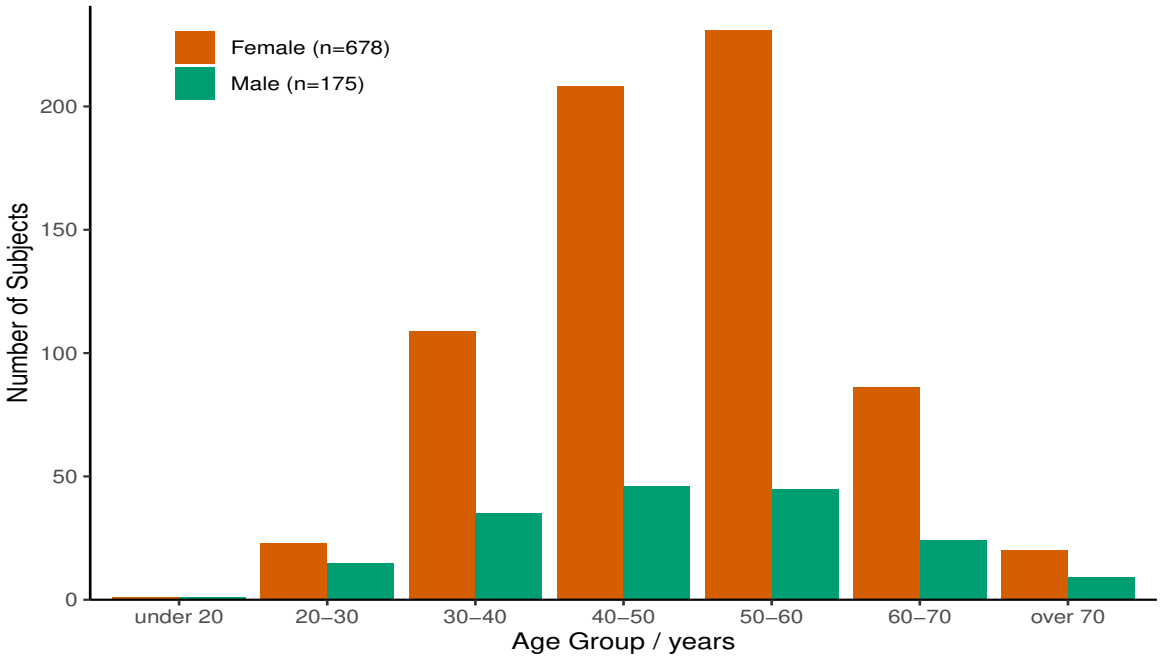

Figure 2.3: Illustration of the distribution of subjects by age group and gender. Female subjects dominate across all age groups, particularly in the 40-50 and 50-60 age ranges.

Figure 2.4 presents the participant counts for various health conditions as well as a healthy control group. The left-hand side of the graph depicts the total number of participants categorized as having a "Disease" condition, which amounts to 534 individuals.

In contrast, the right-hand side showcases the breakdown of participants across specific health diagnoses. We investigated the patterns of microbiome changes across five distinct conditions: Cancer, Multiple Sclerosis (MS), Depression, PTSD, and Anxiety. Anxiety emerges as the most prevalent condition, with 146 participants. Depression follows with 74 participants, while Cancer is represented by 60 individuals. PTSD and MS have the lowest participant counts, at 41 and 5, respectively. Notably, the Healthy control group comprises 319 participants.

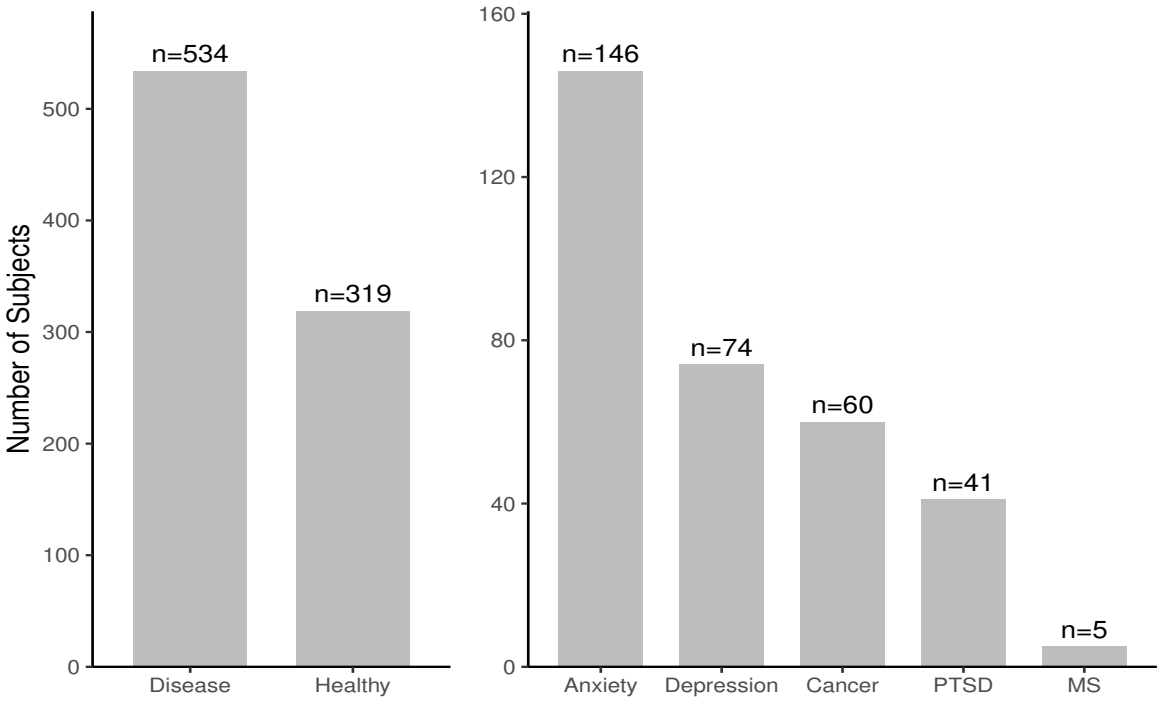

Figure 2.4: Illustration of the distribution of healthy and sick subjects as well as participant counts with the specific diseases Anxiety, Depression, Cancer, PTSD and MS.

2.2 Meditation Intervention and Sample Collection

In this study, participants were recruited from Dr. Joe Dispenza's advanced meditation retreats, where stool samples were collected to investigate gut microbiome changes. The GLOBAL study cohort initially included samples from 1,161 participants. However, after filtering and rarefaction to improve data quality, the final dataset

comprised 853 participants. This produced 1,706 stool samples in total, with two collections for each participant: one at the start of the retreat ("pre") and another seven days later ("post"). Each retreat involved seven days of structured practice, combining approximately 25 hours of instructional sessions with 35 hours of meditation focused on Kundalini techniques. These sessions included components of focused attention, non-dual awareness, and loving-kindness or compassion meditation, alongside breathwork, having a huge focus during the course of the seven days. Participants practiced meditation in various postures - seated, standing, lying down, and walking - while maintaining a consistent schedule and consuming standardized breakfasts and lunches. Both male and female participants were included to ensure scientific rigor and reproducibility.

Stool samples where collected and shipped to the University of California, San Diego (UCSD) for microbiome studies. Specialized collection kits were utilized, for long term storage and shipping. During transport, 70% ethanol was used as a stabilizing agent, and the samples were securely packaged with dry ice to prevent leakage and maintain microbial diversity. The samples were initially stored at -80°C for long-term preservation. However, they were collected in the incorrect vials. As a result, they were transferred into new vials provided by the sequencing company OneCodex® before being shipped for sequencing. The new vials also contained 70% ethanol for stabilization.

2.3 Sequencing and Bioinformatic Analysis

Fecal samples were collected in individual tubes containing DNA stabilization buffer to ensure reproducibility, stability, and traceability. The samples were then shipped for DNA extraction, library preparation, and sequencing. DNA extraction was optimized and fully automated to ensure reproducible isolation of inhibitor-free, high molecular weight genomic DNA, accurately reflecting the microbial diversity in stool samples. This process was carried out using the Qiagen DNeasy 96 PowerSoil Pro QIAcube HT extraction kit and protocol. Following DNA extraction and quality control (QC), the genomic DNA was converted into sequencing libraries using a method designed to minimize bias. Unique dual-indexed (UDI) adapters were employed to prevent misassignment of reads or organisms. Library preparation was performed using the KAPA HyperPlus library preparation protocol.

Shotgun metagenomic sequencing was conducted on the Illumina NextSeq 2000 platform following the manufacturer's protocol. Libraries underwent QC before being

sequenced to a depth of 3–6 million 2x150 bp read pairs, enabling species- and strain-level taxonomic resolution. Nucleotide reads were mapped against an extensive database of bacterial, viral, fungal, and archaeal genomes, as well as metagenome-assembled genomes (MAGs). The database (November 2024) includes ~134,000 microbial assemblies across ~118,000 organisms and incorporates human, mouse, chicken, and rat host genomes. Reads are classified by exact k-mer matches, with each k-mer assigned to the lowest common ancestor (LCA) within a taxonomic tree, ensuring precise identification.

Comparing a microbial sample against the OneCodex database involves three sequential steps. First, sequences are classified using k-mer-based alignment, where each sequence is matched exactly against the database using k-mers of length 31. Next, artifact filtering is applied to remove sequencing artifacts based on the relative frequency of unique k-mers in the sample. This automated process, designed for most WGS data, specifically targets probable sequencing or reference genome artifacts while preserving low-abundance or low-confidence hits. Finally, species-level abundance is estimated by calculating the relative abundance of microbial species based on the depth and coverage of sequencing across all available reference genomes.

Subsequent analyses were restricted to sequences classified within the bacterial domain, with all non-bacterial assignments, including host-derived DNA, phages, and fungi, excluded to ensure focus on bacterial community composition. The resulting sequencing data was analyzed using the vegan package (version 2.6-4) in R for ecological analyses. Alpha diversity indices were calculated, and beta diversity analyses were performed using Bray-Curtis dissimilarity matrices. Community composition differences between samples were statistically assessed using permutational multivariate analysis of variance (PERMANOVA) implemented through the adonis2 function.

2.4 Statistical Analysis

Statistical analyses were performed using R (version 4.2.0). Data normality was assessed using the Kolmogorov-Smirnov test. Non-parametric tests were used for subsequent analyses because the data did not follow a normal distribution. Differences between groups were evaluated using the Kruskal-Wallis test, followed by post-hoc pairwise comparisons when significant differences were detected. The Wilcoxon signed-rank test was applied for paired comparisons. Multiple testing corrections were performed using the Benjamini-Hochberg method to control for false discovery rate (FDR). Multivariate analysis of community composition was conducted using

permutational multivariate analysis of variance (PERMANOVA) executed through the adonis2 function in the vegan package, with 1000 permutations and Bray-Curtis dissimilarity matrices.Effect sizes were calculated using Cohen's d to quantify the magnitude of differences between groups.

3 Results and Discussion

3.1 Rarefaction

The rarefaction analysis was conducted using the `rrarefy()` function from the vegan package in R. This function was applied at the phylum, genus, and species levels to standardize the sequencing depth across all samples. Figure 3.1 shows rarefaction curves at the species level plotted against the number of sequences.

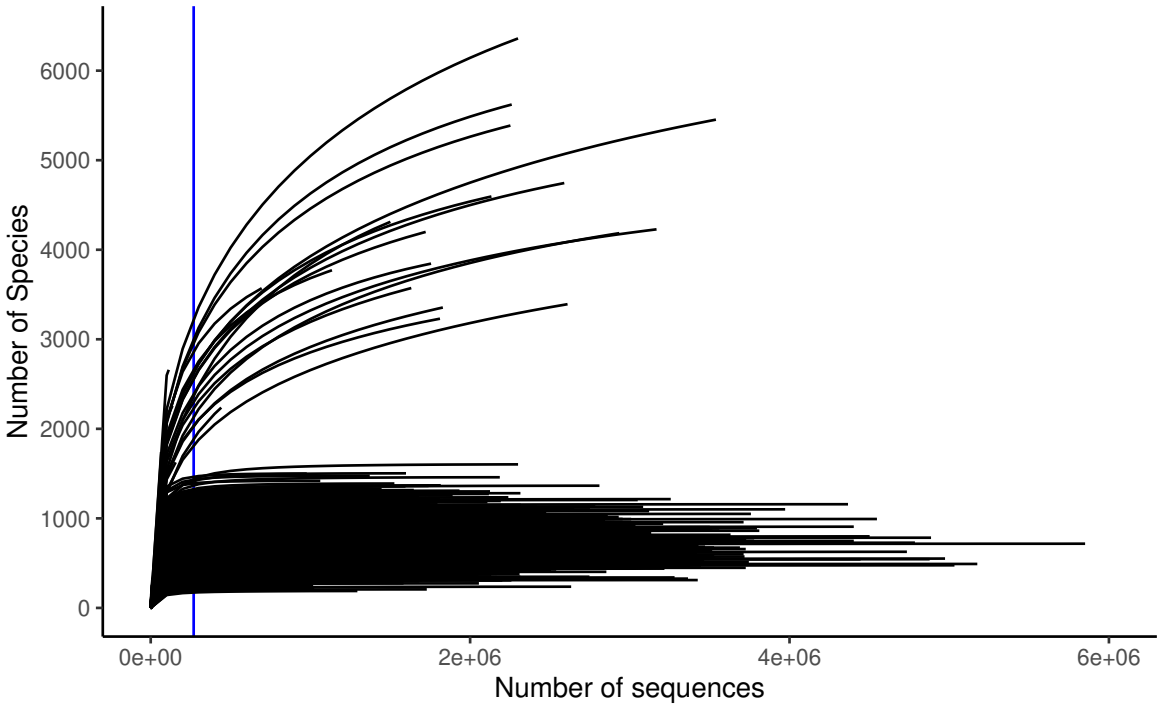

Figure 3.1: Rarefaction curves at the species level. The vertical blue line depicts the cutoff value at the 10th percentile.

Each black line represents an individual sample's accumulation of unique species with increasing sequencing depth. The vertical blue line indicates the sequence count cutoff at the 10th percentile (269,002 sequences), which was used to standardize sampling depth across samples. The curves display varying levels of species richness, with some samples reaching over 2,000 species while others plateau at lower richness levels. The asymptotic shape of most curves suggests that the sequencing depth was

sufficient to capture the majority of species diversity in most samples, though some curves haven't fully plateaued, indicating potential for additional species detection with deeper sequencing.

The histogram in Figure 3.2 depicts the distribution of sequencing effort (number of sequences) across all samples, with a fitted density curve shown in red. The vertical blue line marks the 10th percentile of sequencing depth, which was used as a cutoff threshold for downstream analyses. The distribution shows a right-skewed pattern, with most samples having between 500,000 and 2,000,000 sequences, and a peak frequency around 1.2 million sequences.

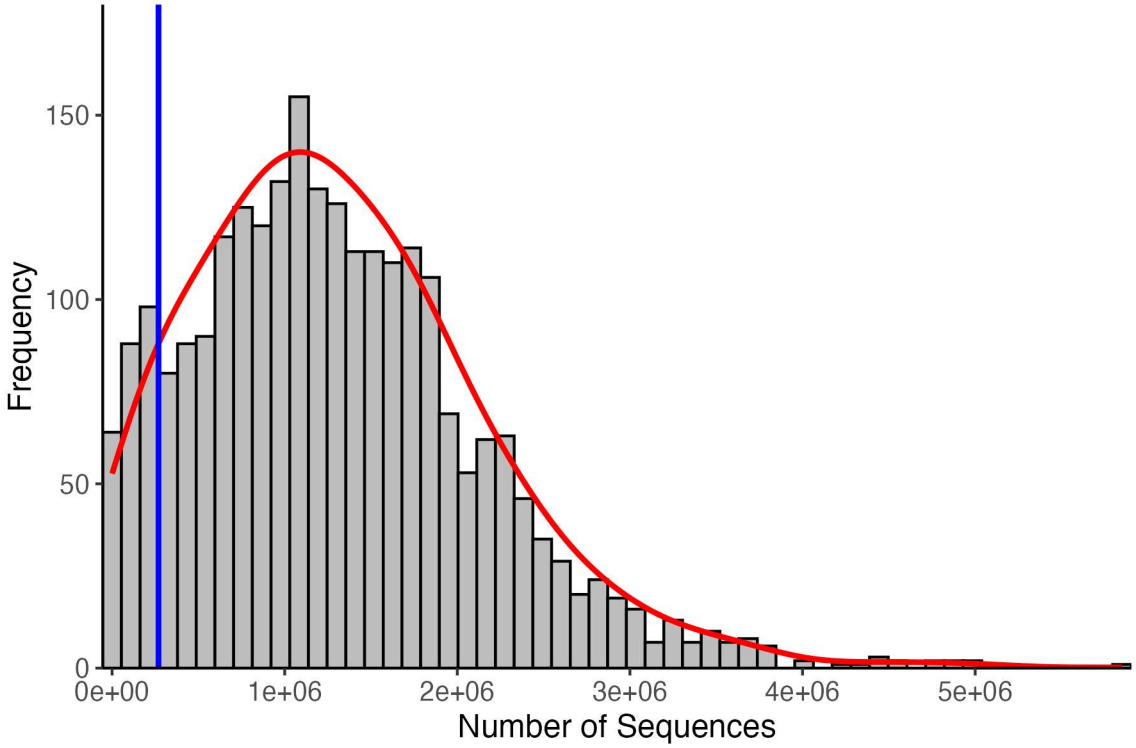

Figure 3.2: Histogram of sequencing depth at the species leve. The vertical blue line depicts the cutoff value at the 10th percentile.

The rarefaction curves and sequencing depth histogram both illustrate the sequencing variation across the dataset, helping to choose a suitable standardization threshold. The chosen 10th percentile cutoff represents a careful balance between two competing factors: retaining a sufficient number of samples in the analysis while maximizing the sequence information captured from each sample. This standardization decision inherently involves a trade-off between excluding samples below the threshold and discarding additional sequences from deeply sequenced samples.

After applying the 10th percentile cutoff, 248 samples were filtered out for failing to meet this threshold. Additionally, during the filtering process, any unpaired samples were also removed. This resulted in a final dataset of 853 paired samples at the species level that passed the quality control criteria and were carried forward for downstream community composition analyses.

The standardization process was applied consistently across different taxonomic levels. At the phylum level, a sequence depth cutoff of 927,990 was used, which resulted in 855 samples being retained for subsequent analyses.

Similarly, at the genus level, the sequence depth threshold was set to 508,883, after which 853 samples met the criteria and were included in the downstream analyses.

3.2 Alpha Diversity

Alpha diversity analysis in microbiome research is a key method for assessing the diversity within a single microbial community. It measures the variety and abundance of species in a sample, typically using indices like Shannon, Simpson, or Richness, which capture different aspects of microbial diversity. These metrics help researchers understand how diverse and complex the microbial communities are in relation to environmental factors, treatments, or diseases. Changes in alpha diversity can indicate shifts in the microbial ecosystem, which may influence health outcomes or reflect responses to interventions. By comparing alpha diversity across different conditions or time points, researchers can gain insights into the stability and resilience of microbial communities (Willis, 2019).

First, the relationship between sequencing depth and various alpha diversity metrics, including the Shannon index, inverse Simpson index, and number of observed taxa (Richness) was examined (Figure 3.3). This type of analysis is commonly used in microbial ecology studies to assess whether the sequencing effort is sufficient to capture the full taxonomic diversity present in the samples.

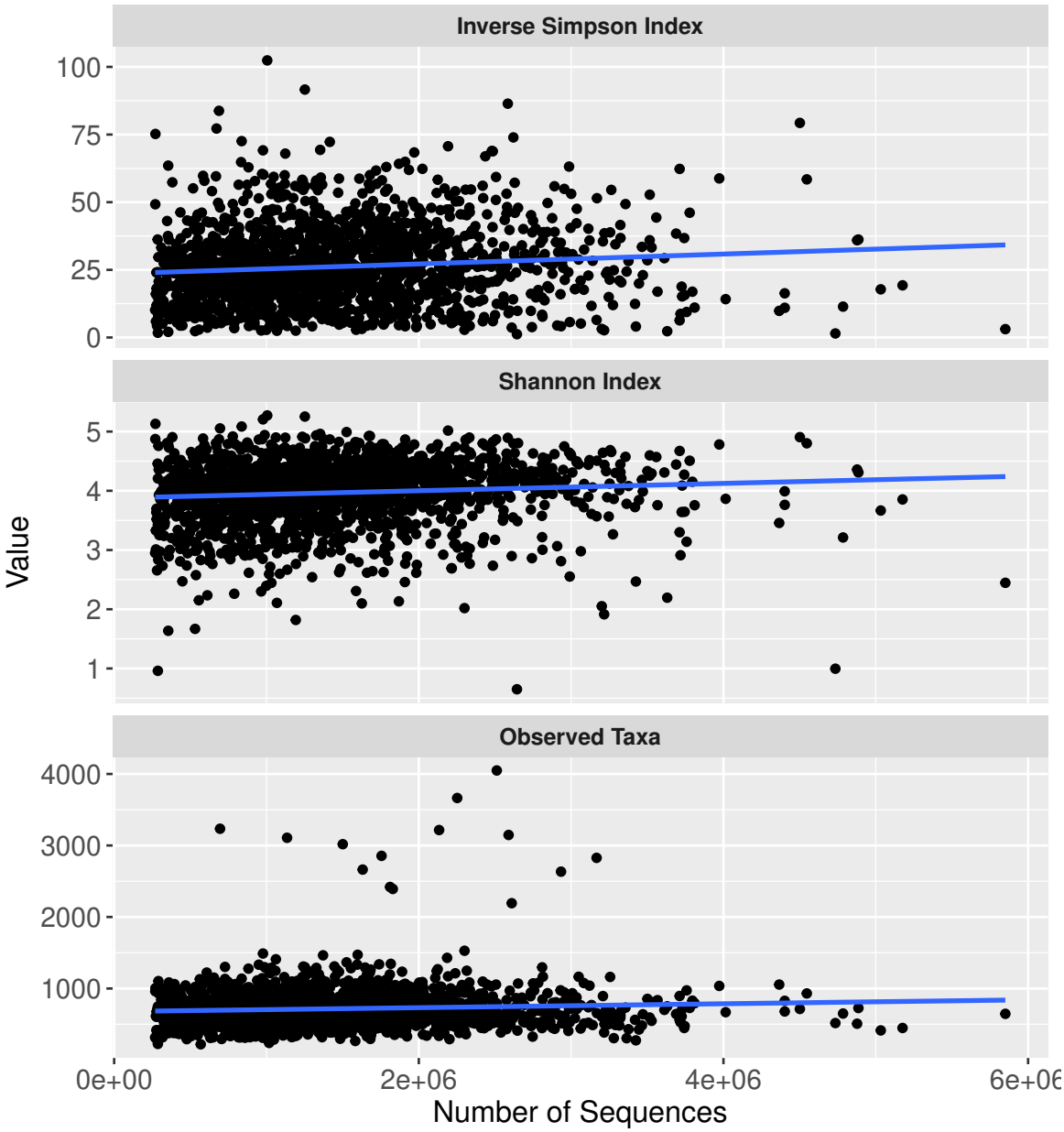

Figure 3.3: Relationship between sequencing depth and alpha diversity metrics.

The results show that as sequencing depth increases, there is no substantial change in the alpha diversity measures. All measured indices remain relatively stable across the range of sequencing depths.

These findings indicate that the sequencing depth was adequate, and further increasing the sequencing effort would not significantly alter the alpha diversity measures for these samples. This suggests the community composition was well-characterized without the need for deeper sequencing.

3.2.1 Global Analysis

The violin plot in Figure 3.4 indicates that there is no statistically significant difference in the inverse Simpson index between the pre- and post-retreat samples analyzing the global community. This finding aligns with the results of other alpha diversity indices, as shown in the Appendix. Specifically, the violin plots (Figure 5.1) of the Shannon Index (a) and Richness values (b) from pre- and post-retreat samples on a global scale further support this conclusion, suggesting that meditation did not significantly alter the overall diversity of the global community being studied.

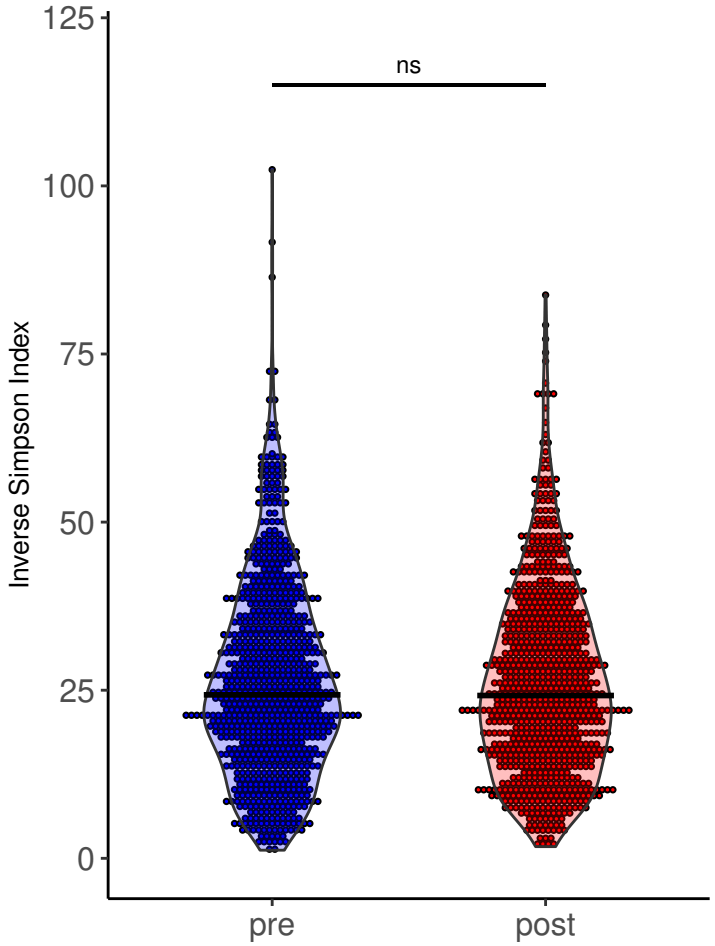

Figure 3.4: Violin plot of the Inverse Simpson Index from pre and post retreat samples on a global scale.

3.2.2 Location-Specific Microbiome Dynamics

Next, a detailed analysis was conducted to examine the effects of location on participants' alpha diversity. Within samples from Australia, Denver and Nashville, the

median inverse Simpson index appears to increase slightly from the pre to post condition, suggesting a potential increase in community diversity following the meditation intervention (Figure 3.5).

Conversely, for Cancun and Vienna, the median inverse Simpson index values show a slight decrease from pre to post, hinting at a potential reduction in overall diversity after the treatment.

In addition to the inverse Simpson index, the Richness values and Shannon Index were also calculated and are presented in Figures 5.2 and 5.3 in the Appendix. The results show that, apart from a significant reduction in Richness within the Denver group, no significant changes were detected in the other indices.

Examining the paired samples more closely, as shown by the connecting lines, reveals huge shifts in both directions within individuals following the seven-day intervention. This and the varied directional changes in the median inverse Simpson index values, even when not statistically significant, suggest that meditation may have had differential impacts on the microbial community structure across the diverse geographic regions sampled. Nevertheless, further investigation is needed to elucidate the underlying drivers of these trends.

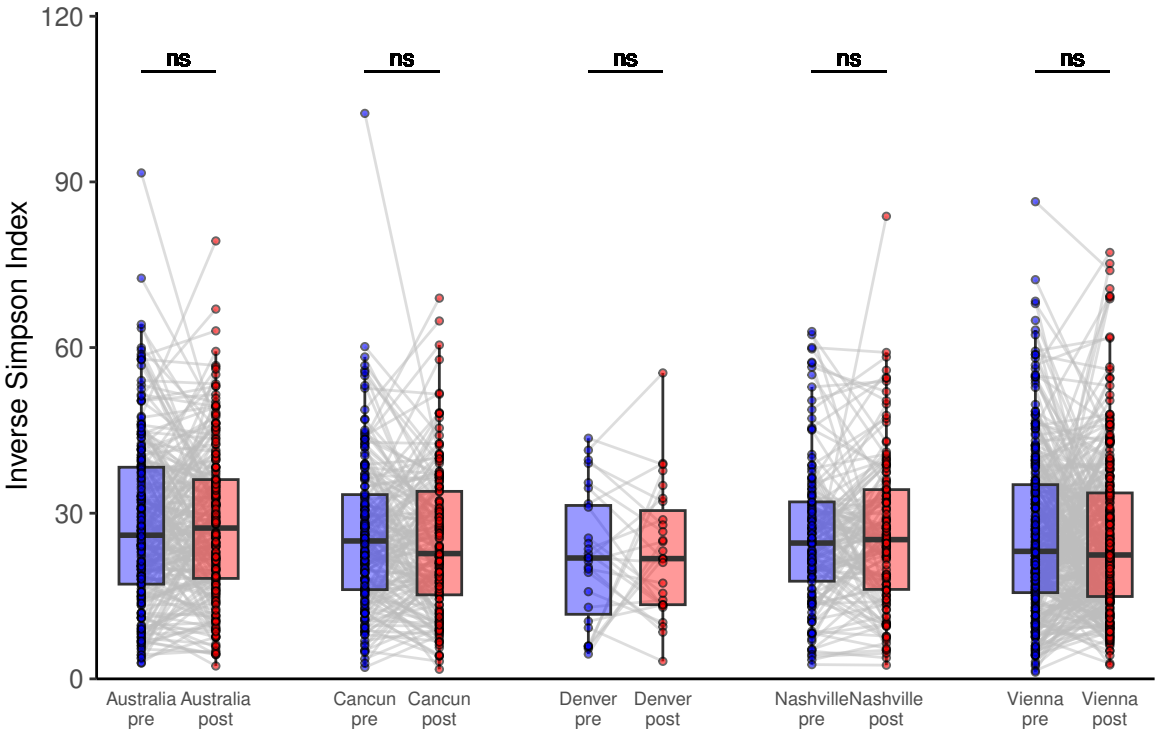

Figure 3.5: Inverse Simpson Index of pre- and post-retreat samples across different locations.

3.2.3 Health-Dependent Microbiome Responses

To see whether the health status has an influence on the alpha diversity, the inverse Simpson index was calculated for both healthy and disease groups (Figure 3.6). The gray connecting lines show that while some individuals experienced increases and others decreases, overall there was no consistent directional change in alpha diversity after the meditation intervention in either the healthy or disease groups. The healthy cohort shows a slight decrease in the median inverse Simpson index from pre to post, whereas the disease group displays the opposite trend.

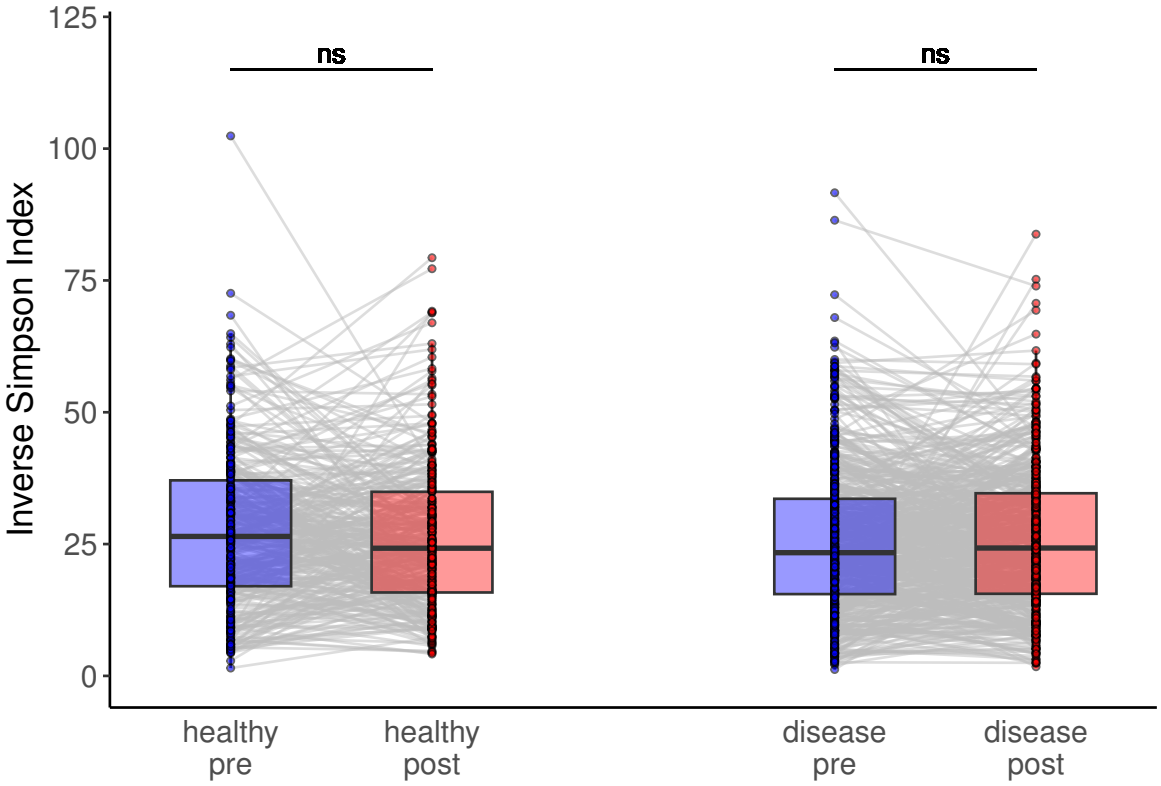

Figure 3.6: Inverse Simpson Index of pre- and post-retreat samples from healthy and disease subjects.

However, as already seen in the previous chapter, there is considerable individual variation, shown by the vertical spread of individual data points and some outliers reaching values over 50.

In addition to the Inverse Simpson index, two other alpha diversity metrics—species Richness and Shannon diversity index—were analyzed to comprehensively assess microbial community diversity. These additional measures exhibited patterns consistent with the Inverse Simpson index findings showing no significant trends or changes following the 7-day meditation retreat in either healthy individuals or those

with disease (see Appendix for corresponding figures 5.4 and 5.5).

While these alpha diversity indices collectively demonstrate no significant shifts at the community level, it's important to note that this lack of overall change does not exclude the occurrence of meaningful alterations within individual participants' microbiomes during the retreat. Indeed, the substantial individual variation observed, suggests that participant-specific changes may be occurring that are not captured by these broad diversity metrics alone. To better understand these individual-level dynamics and potential compositional shifts, the next analytical step will focus on beta diversity measurements. This will allow us to examine differences in microbial community composition both between participants and across time points.

3.3 Beta Diversity

The Bray-Curtis distance metric, calculated using the `vegdist()` function from the vegan R package, was used for analyzing microbial community dissimilarities and visualized in PCoA plots. Bray-Curtis considers both the presence/absence and relative abundances of taxa, providing a robust measure of compositional differences between samples (Kers & Saccenti, 2022).

The dimensionality reduction and ordination of the Bray-Curtis distance matrix was then performed using the `cmdscale()` function, which generates the principal coordinate (PCo) axes displayed in the following visualizations.

3.3.1 Global Analysis

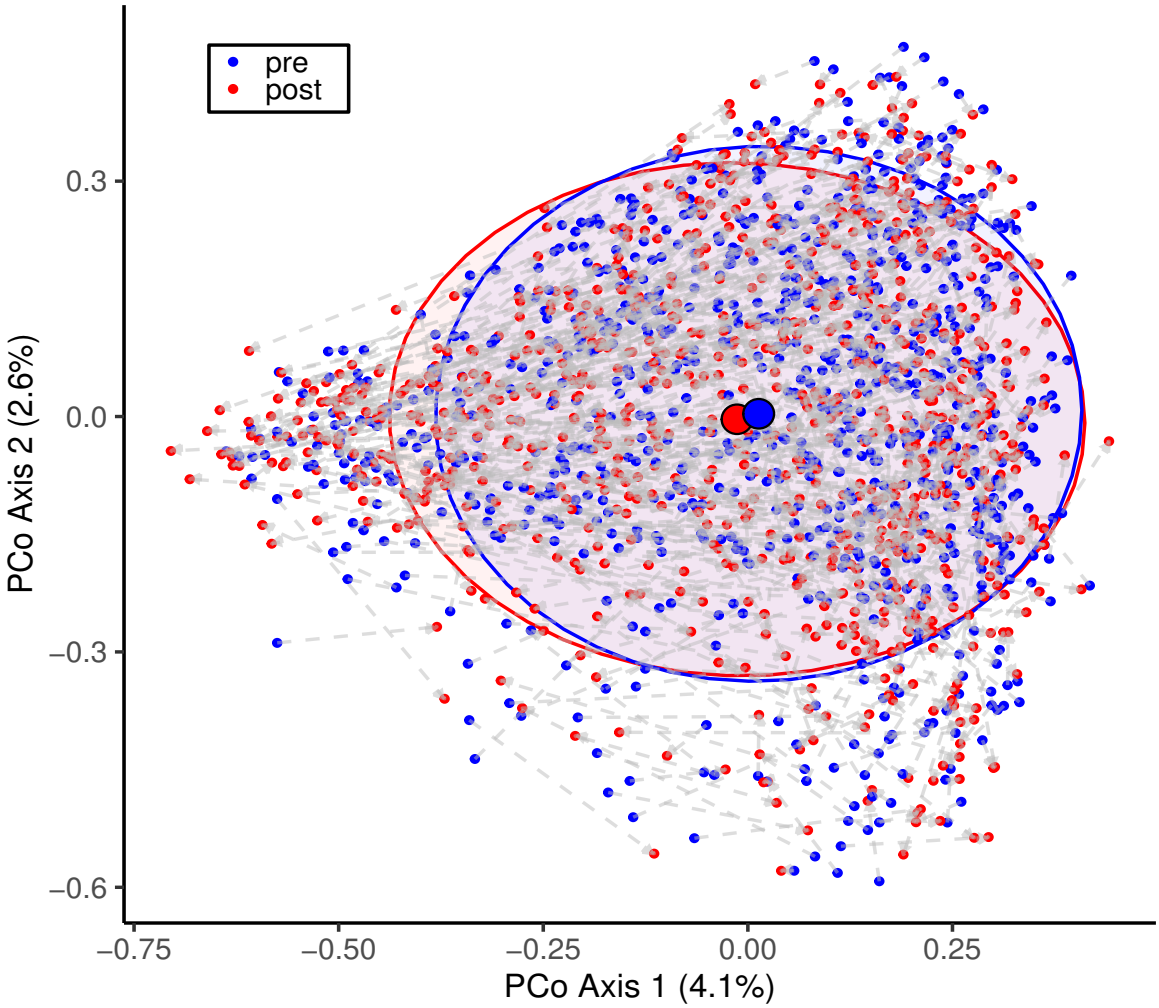

Figure 3.7: Global PCoA plot using Bray-Curtis distance metric.

The PCoA plot of all 1,706 measured samples is displayed in Figure 3.7. The dashed lines connect paired samples from the same individual pre- and post-retreat. The presence of some long dashed connection lines between individual data points indicates that despite the lack of clear clustering in the overall trend, there are some individual samples that exhibit large distances or differences in their microbial community composition. This suggests that while the centroids and ellipsoids do not depict drastic shifts, the intervention did cause significant changes for certain individuals. This implies that the impact of the intervention, while not uniform across all samples, was quite dramatic for some participants. This emphasizes the importance of looking at both the general and individual-level responses in microbiome studies.

The use of PERMANOVA, specifically the `adonis2()` function from the `vegan` R package, using the Bray-Curtis distance metric provides further insights into the microbiome changes observed in this study. PERMANOVA analysis revealed a statistically significant difference in microbial community composition between the pre- and post-intervention groups (p-value < 0.001), indicating that the 7-day meditation retreat had a measurable impact on the overall microbiome structure. The use of the `strata` argument in the `adonis2()` function allows to account for the paired nature of the samples, which can increase the power of the statistical test.

Importantly, the authors mention the need to consider potential confounding dispersion effects, as significant changes observed in the PERMANOVA analysis may be driven by differences in within-group variation rather than true compositional shifts (Anderson, 2001). To address this, the `betadisper()` function was used to test for homogeneity of multivariate dispersions, which revealed no significant differences (p-value = 0.635).

Despite the significant PERMANOVA result, the Cohen's d effect size (cohan d estimate = -0.026) and the R-squared value ($R^2 = 0.0008$) from the `adonis2()` function showed negligible results, suggesting that the observed changes may be relatively small in magnitude. Therefore, the explanatory variables included in the model are not the primary drivers of the community differences. This raises the question of where the significance is originating from. One can say that the unexplained variance can always be attributable to 'dark matter', meaning that there may be underlying factors or complexities in the data that are not fully understood.

Therefore, other explanatory variables were included in the test. The code below specifies the explanatory variables (Timepoint, Gender, Age Group and Location) and their interactions. The '*' operator indicates that all main effects and interactions between the variables should be included in the model.

```
# Code for running the PERMANOVA test using the adonis2 function
Global_time_test <- adonis2(bray_dist_species ~
                                timepoint*Gender*`Age Group`*Location,
                            data = meta_bray_all_ordered,
                            permutations = 1000,
                            strata = meta_bray_all_ordered$Partp_ID)
```

Corresponding R^2 and p-values are depicted in Table 3.1. In addidtion to `Timepoint`, the factors `Gender` ($R^2 = 0.00480$, $p < 0.001$) and `Age Group` ($R^2 = 0.00767$, $p = 0.027$) also display a statistically significant effect on microbiome composition, indicating some variations based on these influences. Though statistically meaningful, the small effect size suggests that any gender- or age-group-based differences in the microbiome are subtle.

The effect of `Location`, representing retreat sites across continents, showed a relatively higher R^2 value (0.01767). However, this effect was not statistically significant ($p = 0.254$). While the effect size for location is larger than that of the other main effects, the lack of statistical significance suggests that any observed location-based differences might be due to chance rather than a consistent or meaningful pattern in microbiome composition. However, Figure 3.8 showing the PCoA Plot of the five locations depicting some shifts in the centroids between the different sites. The clustering and distribution of the points suggests there are distinct microbial community profiles associated with some of the locations. Indeed, without using `strata` and accounting for the paired nature of the samples, the PERMANOVA test resulted in significant differences between some locations (p-value < 0.001).

Among interaction effects, the relationship between `Timepoint` and `Location` was statistically significant ($R^2 = 0.0014$, $p < 0.001$). This suggests that changes in microbiome composition pre and post may vary slightly depending on location. However, the minimal effect size again implies that any practical or biological significance is likely limited.

The `Gender` and `Age group` interaction also showed statistical significance ($R^2 = 0.00731$, $p = 0.003$), suggesting that the relationship between gender and microbiome composition might differ across age groups. However, given the small effect size, the influence of age on gender-related differences in microbiome composition is subtle. Additionally, the interaction between `Age group` and `Location` was significant ($R^2 = 0.0183$, $p = 0.014$), suggesting that the distribution of age groups within each retreat site may contribute to slight differences in microbiome composition across locations.

Higher-order interactions, such as `timepoint:Gender:Location` and `timepoint:Gender:Age Group`, were not statistically significant. This implies that the combined effect of multiple factors on microbiome composition is limited, and there are no strong or consistent patterns emerging when these factors are considered in combination.

Notably, the residuals account for the vast majority of variation in microbiome composition, with 91.43% of the total R^2 attributable to unmeasured factors or inherent individual variation. This large residual component suggests that the factors included in the model explain only a small portion of the total variation in microbiome composition. It may be that additional unmeasured factors ('dark matter') are influencing the microbiome, or that individual differences are a major source of variation.

Table 3.1: PERMANOVA Results: Factors, R^2 and p-values

Factor	**R^2**	**p-value**
Timepoint	0.00088	0.000999 *
Gender	0.00480	0.000999 *
Age Group	0.00767	0.026973 *
Location	0.01767	0.253746
Timepoint:Gender	0.00049	0.263736
Timepoint:Age Group	0.00123	0.457542
Gender:Age Group	0.00731	0.002997 *
Timepoint:Location	0.00140	0.000999 *
Gender:Location	0.00408	0.525475
Age Group:Location	0.01834	0.013986 *
Timepoint:Gender:Age Group	0.00157	0.300699
Timepoint:Gender:Location	0.00080	0.611389
Timepoint:Age Group:Location	0.00328	0.907093
Gender:Age Group:Location	0.01410	0.865135
Timepoint:Gender:Age Group:Location	0.00334	0.260739
Residual	0.91304	NA

So in summary, it is important to explain the statistical significance in the context of the effect size, as a significant result does not necessarily imply a large or meaningful difference in community composition. This is highlighting the need to carefully interpret the practical significance—and therefore also the biological relevance—of the findings.

Further exploration of the data, including the consideration of additional explanatory variables or alternative analytical approaches, may be necessary to better understand the factors shaping the observed community patterns.

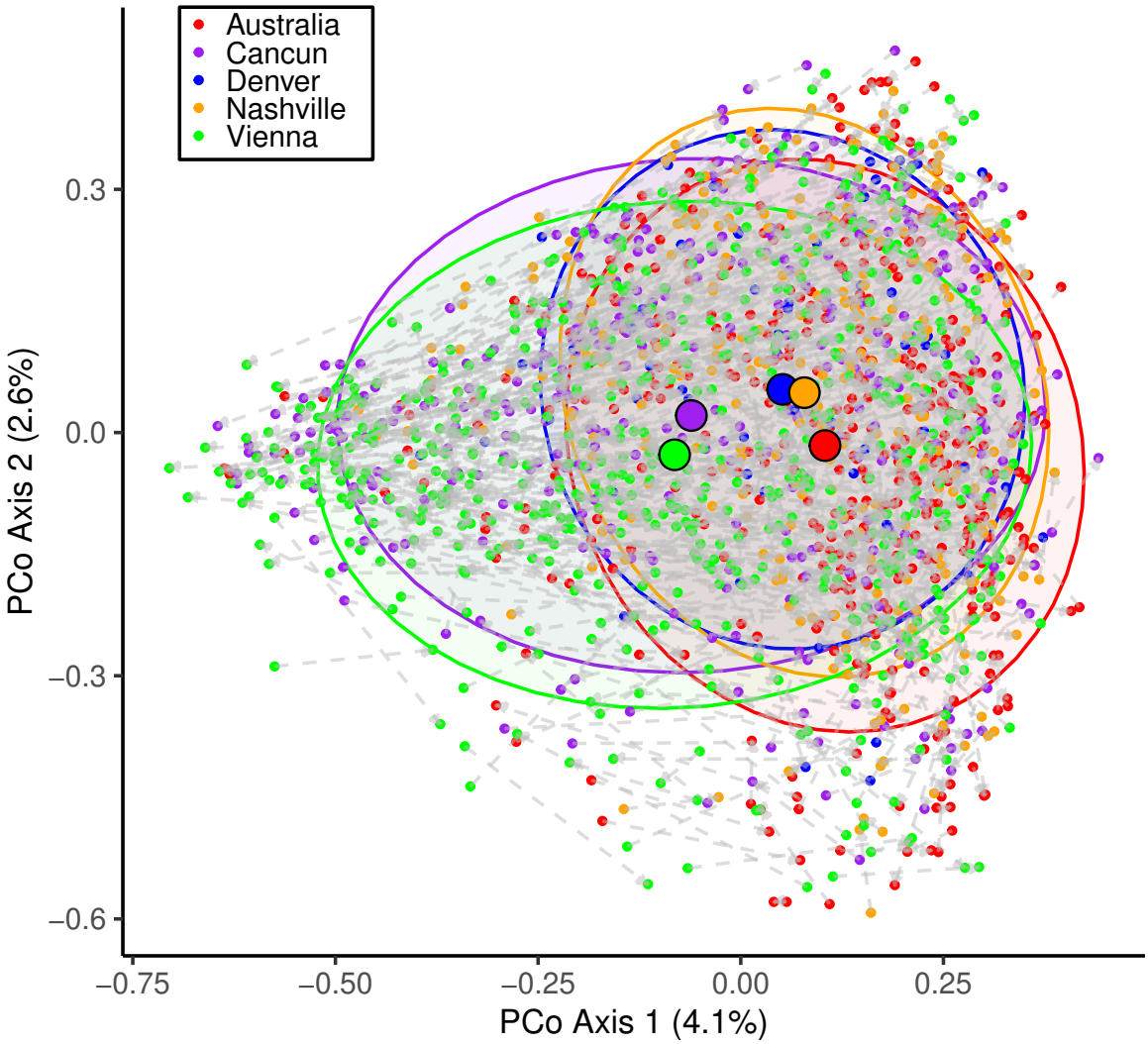

Figure 3.8: PCoA plot of microbiome changes following a 7-day meditation retreat across five locations, using Bray-Curtis distance metric.

3.3.2 Intra- and Inter-Human Changes

Rather than using a PCoA plot, Turnbaugh et al. (2009) applied another way of depicting the distances between individuals.

Figure 3.9 effectively illustrates three distinct perspectives on microbial community relationships in the context of a meditation retreat intervention. All subsequent plots in this section display light gray dots representing individual data points, along with black or colored medians and error bars indicating the 50% interquartile range.

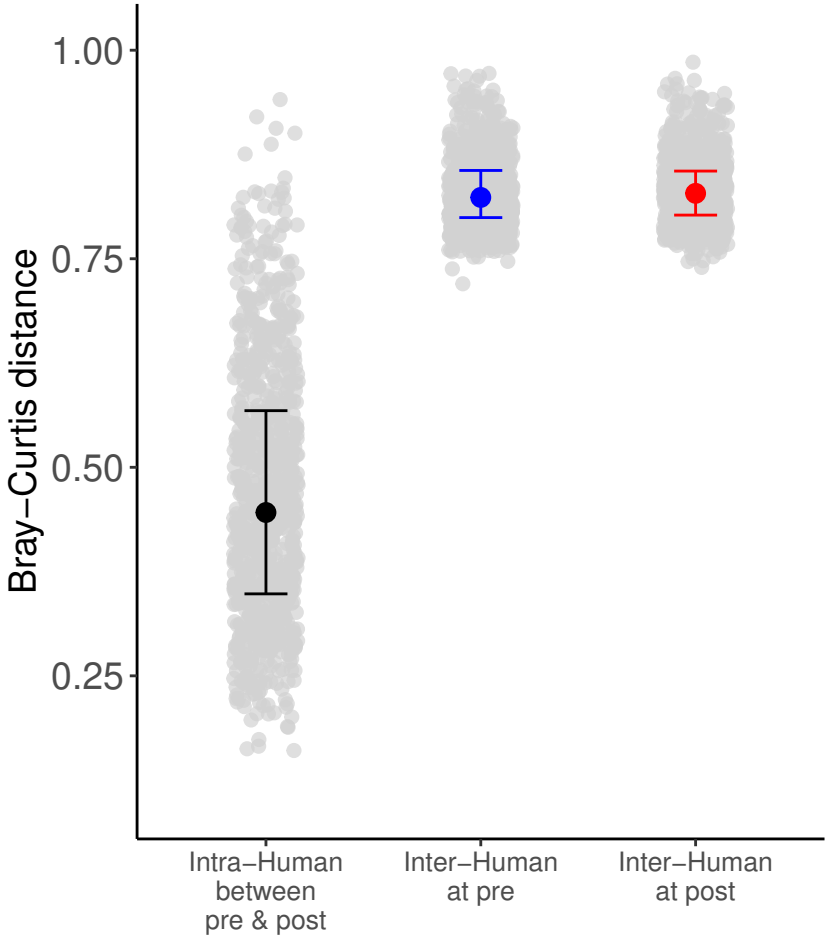

Figure 3.9: Comparison of intra-individual and inter-individual microbiome differences before and after a meditation retreat using Bray-Curtis dissimilarity measures.

The first comparison shows the "Intra-Human" distances between samples from the same individual before and after the meditation retreat. These measurements, represented by a black median with error bars, demonstrate a lower average dissimilarity value of 0.446, but with notably high dispersion. This pattern validates the observed trends in the PCoA plot (Figure 3.7) that while individuals generally maintain some consistency in their microbiome composition pre and post, there is considerable variation in how much change occurs at the individual level.

The "Inter-Human" comparisons at both pre and post-intervention timepoints, shown with blue and red dots respectively, reveal higher dissimilarity values around 0.82. The values were calculated using the mean distance from one person to all other participants at one timepoint. These elevated values indicate that different individuals harbor distinctly different microbiome compositions, a pattern that remains stable after the retreat. The similarity between pre and post measurements suggests

that while individuals may have experienced personal changes during the retreat, the fundamental differences between individuals' microbiomes remained largely unchanged.

Looking at the health status comparisons in Figure 3.10, we observe similar patterns when separating the data between healthy individuals and those with disease. In the left "Intra-Human distance" plot, both healthy and disease groups show comparable median dissimilarity values around 0.45, with similar distributions of individual data points as shown by the grey dots. This suggests that the degree of microbiome variation within individuals remains consistent regardless of health status.

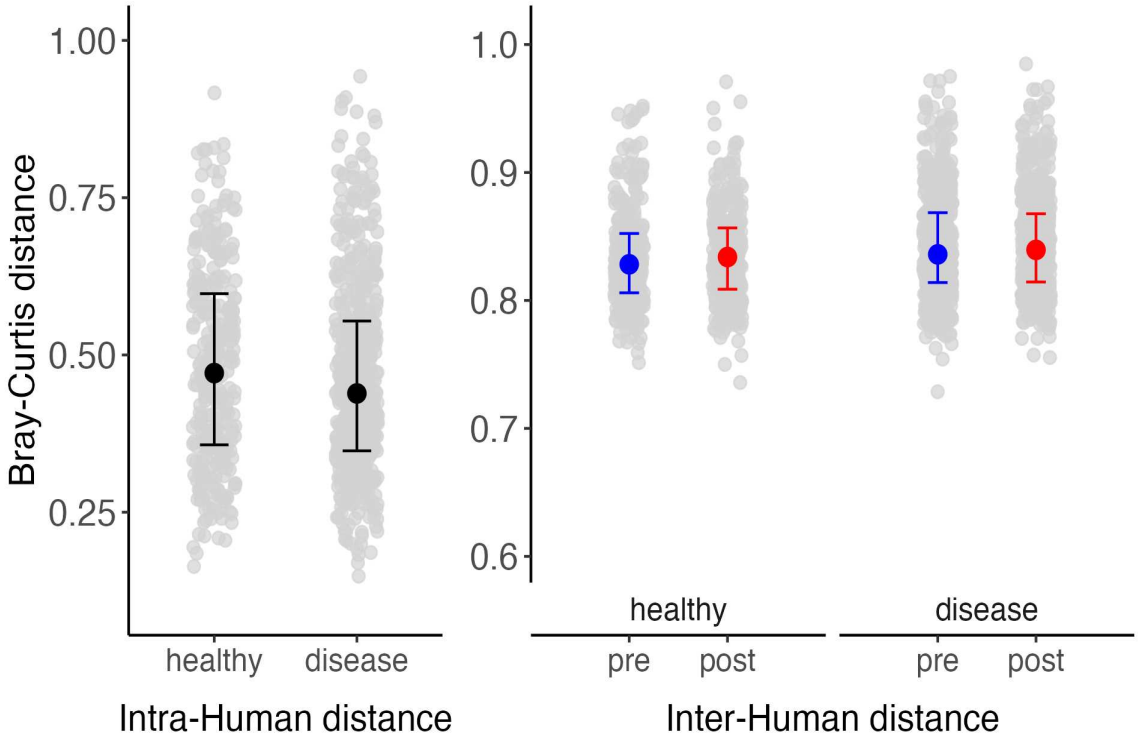

Figure 3.10: Comparison of intra-individual and inter-individual microbiome differences before and after a meditation retreat using Bray-Curtis dissimilarity measures.

The right "Inter-Human distance" plot further reinforces this pattern when examining the healthy and disease groups separately. For healthy as well as sick individuals, the inter-human distances show high dissimilarity values of approximately 0.83 both before (blue) and after (red) the intervention. This parallel pattern between health status groups indicates that regardless of whether individuals are healthy or have disease, they maintain distinct personal microbiome signatures that differ substantially from other people. The consistency of these inter-individual differences before and after the intervention demonstrates that the fundamental uniqueness of each person's

microbiome remains stable, independent of their health status.

3.3.3 Inter-Human Location Effects

Figure 3.11 shows the mean inter-human distance at the pre- and post-intervention timepoints for each location. This metric captures the average dissimilarity between an individual's microbiome and the microbiomes of all other participants within the same location pre and post. By calculating the mean distance from one individual to all others, the visualization provides insights into the overall degree of variation in microbial communities within each geographic setting.

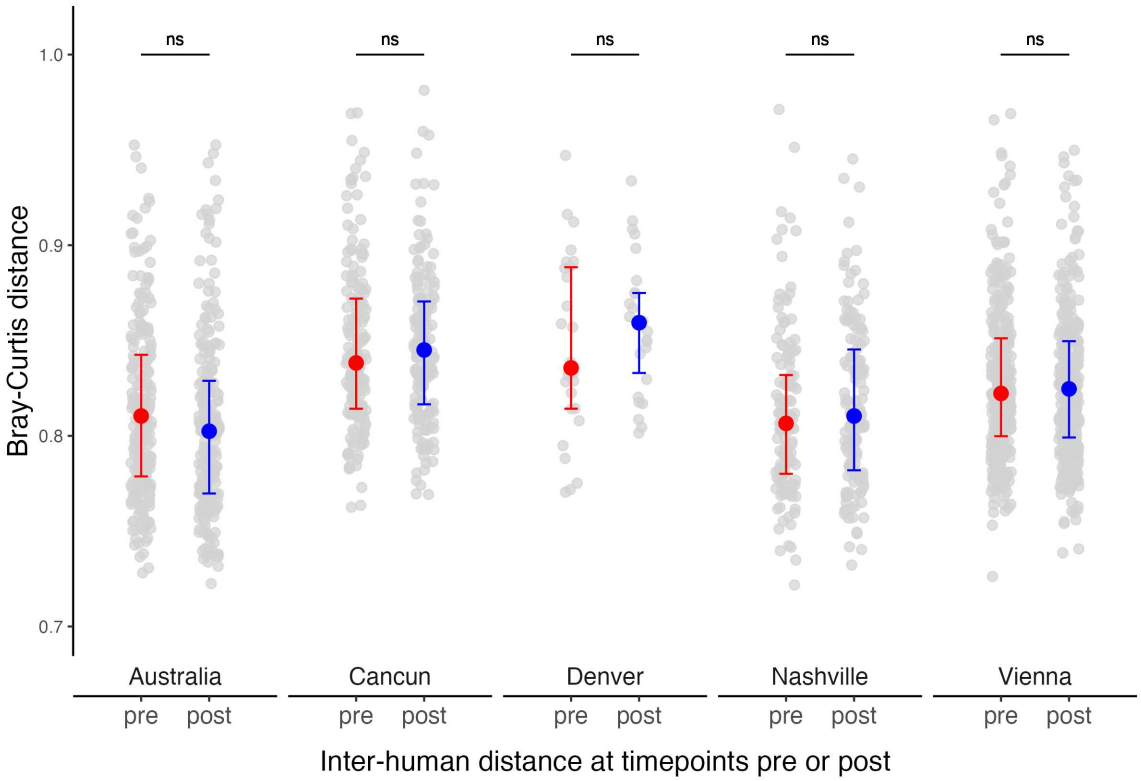

Figure 3.11: Comparison of inter-individual microbiome differences before and after a meditation retreat using Bray-Curtis dissimilarity measures across different locations.

These between-person variations provides valuable context for interpreting any potential location specific effects like eating the same food for seven days that may have occurred due to staying at the same venue.

If the shared diet during the 7-day meditation retreat had a substantial impact on the participants' microbiomes, then we would expect to see a reduction in the

inter-human distances at the post-timepoint compared to pre. The fact that the inter-human distances remain relatively stable suggests that the shared dietary exposure over the course of the retreat did not drive a significant convergence of the microbial communities between individuals across the different locations.

The persistence of the high inter-human distances does imply that location effects, at least in terms of dietary changes, were likely negligible in this case.

3.3.4 Specific Disease Group Changes

In order to better understand how different disease states might influence microbiome stability, a more detailed analysis was conducted, examining specific disease groups separately. Following the observations in the general comparison between healthy individuals and those with disease, we investigated the patterns of microbiome changes across five distinct conditions: Cancer, Multiple Sclerosis (MS), Depression, PTSD, and Anxiety. This disease-specific analysis allowed us to determine whether particular health conditions might be associated with unique patterns of microbiome variation over time, while also enabling direct comparisons both between different disease groups and against the healthy reference cohort.

Figure 3.12 provides a detailed breakdown of the intra-human distances across these different disease groups. When comparing these disease-specific patterns to the healthy cohort from the previous plot (Figure 3.10), we observe some interesting similarities and variations.

All disease groups show median Bray-Curtis dissimilarity values hovering around 0.45-0.50, which is remarkably similar to the healthy cohort's median value of approximately 0.5. Cancer patients show a particularly wide distribution of individual responses, with whiskers—indicating the 50% interquartile—ranging from 0.35-0.65, revealing substantial variability in how individual cancer patients' microbiomes changed before and after the retreat.

The mental health conditions—Depression, PTSD, and Anxiety—display similar median values to each other and to the healthy cohort.

What's particularly striking is that despite these being distinct disease states, the overall pattern of intra-human distances remains consistent with what we observed in the healthy cohort. This reinforces the earlier observation that the fundamental dynamics of microbiome change appear to be relatively stable across health conditions, with individual variation being a more prominent factor than disease status in determining the degree of microbiome change between pre and post.

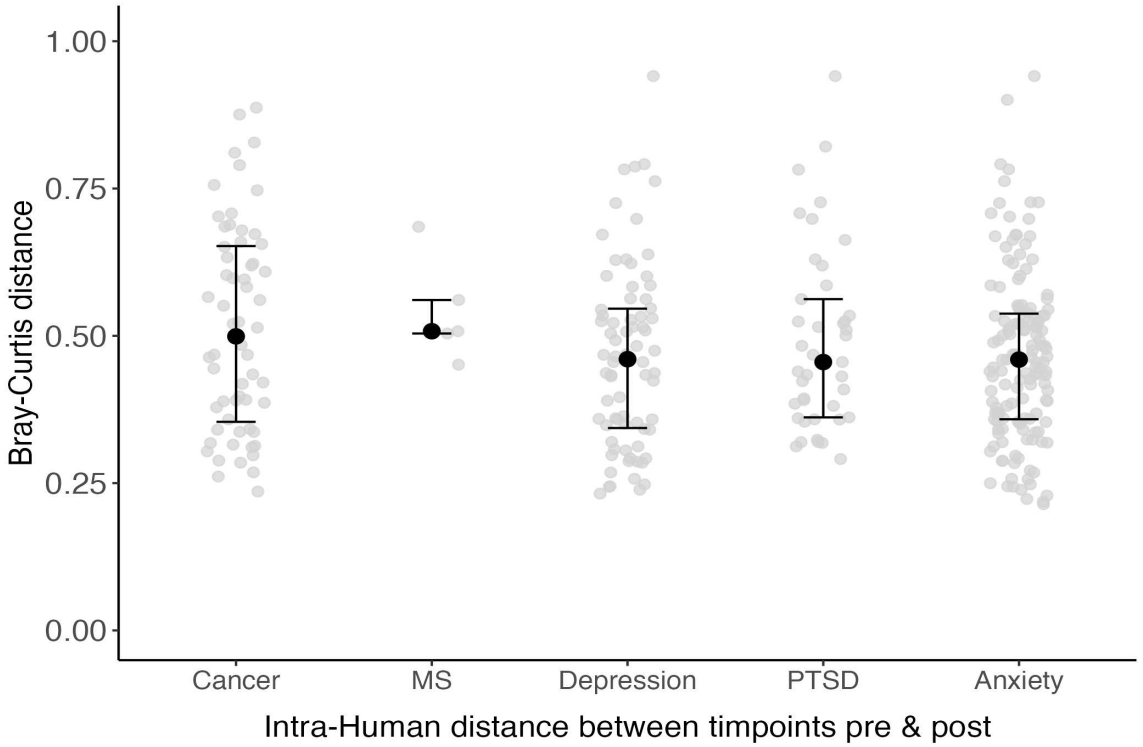

Figure 3.12: Comparison of intra-individual microbiome variation between pre- and post-intervention timepoints across five disease groups (Cancer, MS, Depression, PTSD, and Anxiety).

Figure 3.13 expands our understanding of inter-individual microbiome differences by examining how individuals with specific diseases compare to a healthy cohort, both before and after the intervention.

To ensure a robust and controlled comparison, the healthy cohort was carefully matched to each disease group using specific criteria. For each disease group, control participants were selected based on three key matching factors: (1) the number of participants was matched one-to-one with the disease group size, (2) controls were chosen from the same geographical location as the disease group participants to account for potential environmental influences, and (3) the gender distribution was matched between the disease and control groups. This matched-control design is particularly important as it helps minimize potential confounding factors that could arise from demographic or environmental differences, allowing for more direct comparisons of how different diseases affects microbiome composition. By implementing these matching criteria, any observed differences between disease groups and their respective healthy controls are more likely to reflect true disease-associated variations rather than differences due to external factors or sampling bias.

Looking at the detailed patterns, all disease groups show remarkably similar mean Bray-Curtis distances of approximately 0.83-0.87 when compared to the healthy cohort. This is highly consistent with the inter-human distances we observed in the previous plot for the general healthy versus disease comparison (Figure 3.10).

Furthermore, the lack of significant changes between pre and post measurements suggests that the intervention did not substantially alter how different these disease groups' microbiomes were from the healthy cohort.

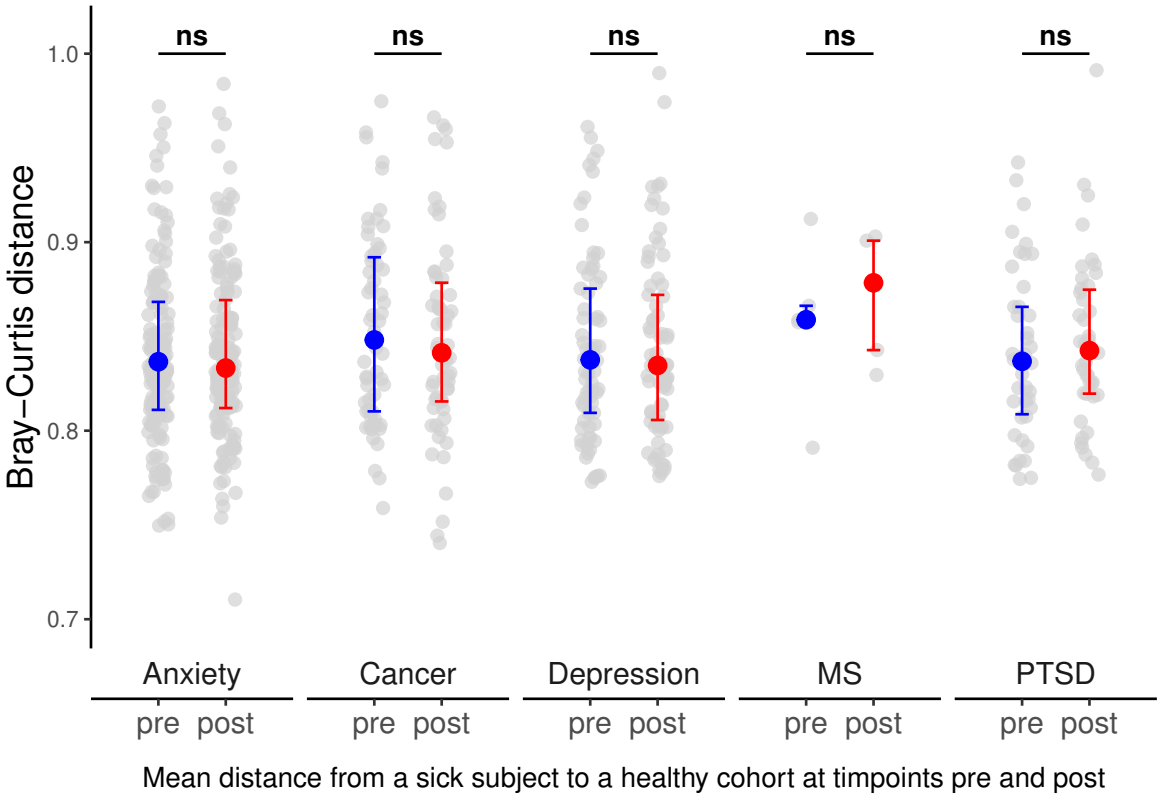

Figure 3.13: Comparison of inter-individual microbiome variation between pre- and post-intervention timepoints across five disease groups (Cancer, MS, Depression, PTSD, and Anxiety) compare to a healthy cohort.

The disease-specific analysis shwon in Figure 3.13 strengthens our previous conclusion that individual microbiome signatures remain distinct regardless of health status or intervention, while adding the detail that this holds true even when examining specific disease conditions separately.

PERMANOVA was again used to examine differences in gut microbiome composition between pre- and post-meditation samples from these different disease groups (Table 3.2).

Table 3.2: PERMANOVA Results: R^2 and p-values across disease states.

Disease State	R^2	p-value
PTSD	0.0087	0.0010 *
Cancer	0.0030	0.6603
Anxiety	0.0016	0.0430 *
Depression	0.0025	0.2967
MS	-	-

Overall, these results suggest that the intervention had the strongest impact on microbiome changes for the PTSD group, followed by people with anxiety, as indicated by the significant findings from statistical testing. Corresponding PCoA analysis for the PTSD cohort is shown in Figure 3.14.

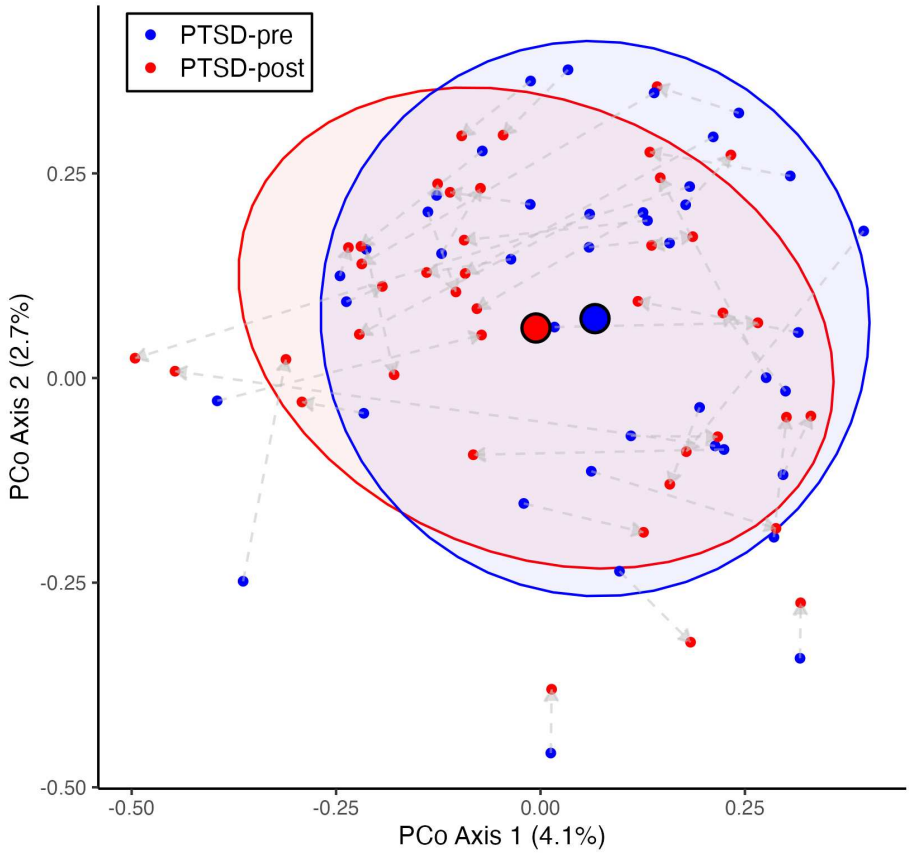

Figure 3.14: PCoA ordination of gut microbiome samples from PTSD patients before and after meditation intervention.

The other disease conditions did not show statistically significant alterations in microbiome composition between the pre and post timepoints. The MS group could not be measured due to the small sample size. The low R^2 values across all groups indicate that the factor timepoint is not a major driver of microbiome variation within each disease state.

This finding is particularly interesting as it suggests that meditation practice can induce measurable changes in the gut microbiome of individuals with PTSD and anxiety, even though the shifts are modest. The statistical significance of the PERMANOVA test—$p = 0.001$ for PTSD and $p = 0.043$ for anxiety—indicates these changes are unlikely to be due to chance, despite the considerable overlap we see in the visualization. This adds to our understanding of the potential biological mechanisms through which meditation might influence these stress-related conditions.

Additional analysis is required to gain a deeper understanding of the factors influencing the observed patterns. Therefore, differential abundance analysis complemented alpha and beta diversity by identifying specific taxa driving these community changes. This helps in identifying key taxa that contributed to the observed shifts in the microbial community.

3.4 Differential Abundance Analysis

Differential abundance analysis complements alpha and beta diversity metrics by providing insight into specific taxa driving changes in microbial communities. While alpha diversity assesses overall richness and evenness, and beta diversity evaluates differences in community composition between samples and groups, these metrics do not identify which microbes are contributing to observed patterns. Differential abundance analysis pinpoints individual taxa that significantly vary between conditions or groups, offering a deeper understanding of microbial shifts and their potential functional implications. This targeted approach is essential for linking community changes to biological factors and for generating hypotheses about microbial roles in health and disease.

3.4.1 Phylum Level Analysis

The analysis began at the Phylum level. Figure 3.15 shows the *Bacillota:Bacteroidota* ratio across pre- and post-samples from a 7-day meditation retreat. The y-axis, on a log10 scale, highlights that the ratio spans several orders of magnitude. A significant increase was observed ($P < 0.001$), suggesting meditation influenced microbiome composition, with a higher abundance of *Bacillota* relative to *Bacteroidota*.

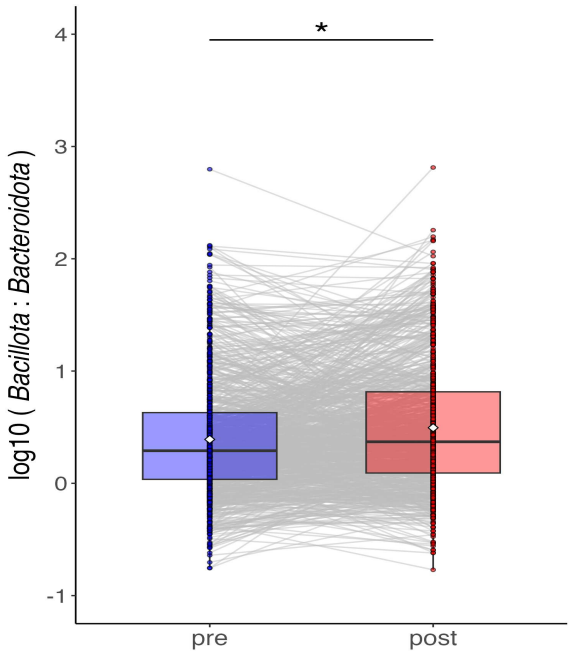

Figure 3.15: Changes in the *Bacillota* to *Bacteroidota* ratio before and after a meditation intervention.

This ratio, identical to the commonly studied *Firmicutes:Bacteroidota* (*F:B*) ratio, is a key indicator of gut microbial balance and health. The ratio was proposed by Ley, Turnbaugh, Klein, & Gordon (2006), who observed an increased proportion of *Firmicutes* and a decreased proportion of *Bacteroidota* in the gut microbiota of obese compared to lean humans. It suggested that these differences in microbiota composition might influence the host's ability to extract energy from the diet, contributing to obesity.

Since then, the *F:B* ratio has become a widely used metric in gut microbiome research, often employed as a general marker for gut microbial composition, metabolic health, and disease states. However, subsequent studies have shown that the significance of the *F:B* ratio can vary depending on the context (e.g., diet, geography, health status), leading to discussions about its limitations as a universal biomarker (Kusnadi et al., 2023).

The *Bacillota:Bacteroidota* ratio essentially serves as an updated representation of the *F:B* ratio, reflecting the taxonomic reclassification of *Firmicutes* into *Bacillota* (An, Kwon, & Kim, 2023). In this study, it is noteworthy that the highest density of *Bacillota* to *Bacteroidota* is observed at a ratio of approximately 60% *Bacillota* to 40% *Bacteroidota* (Figure 3.16). The observed global rise in the *Bacillota:Bacteroidota* ratio is driven by both a significant increase in *Bacillota* and a significant decrease in *Bacteroidota*, with corresponding p-values of 4.01×10^{-6} and 4.44×10^{-8}, respectively.

A loss of *Bacillota* and an increase in *Bacteroidota* is often also associated with older individuals, reflecting changes in gut microbiota composition linked to aging (Mariat et al., 2009). This shift, frequently accompanied by a decline in butyrate-producing bacteria like *Faecalibacterium* and *Roseburia*, can impair gut barrier function and contribute to systemic inflammation. However, these changes are also influenced by diet, lifestyle, and health status, with some studies suggesting they are less age-specific and more reflective of dietary patterns, such as fiber intake. The relationship between microbiome composition and aging remains complex, requiring further research to clarify its implications for health and disease prevention (Badal et al., 2020).

Figure 3.16 shows another depiction of the *Bacillota:Bacteroidota* ratio as a 2D contour plot, which confirms the findings from the previous graph. In addition, the histograms in the plot confirm both, a general increase in *Bacillota* and a decrease in *Bacteroidota*. The post-retreat panel shows a shift of data points towards the top left—high *Bacillota* and low *Bacteroidota* values—confirming the higher *Bacillota:Bacteroidota* ratio after the meditation retreat.

Remarkably, these changes were consistent across all subgroups analyzed, regardless

of location, health status, or disease group shown in Figures 5.6, 5.7 and 5.8 in the Appendix. While the magnitude of change varied slightly between categories, the overall trend remained uniform.

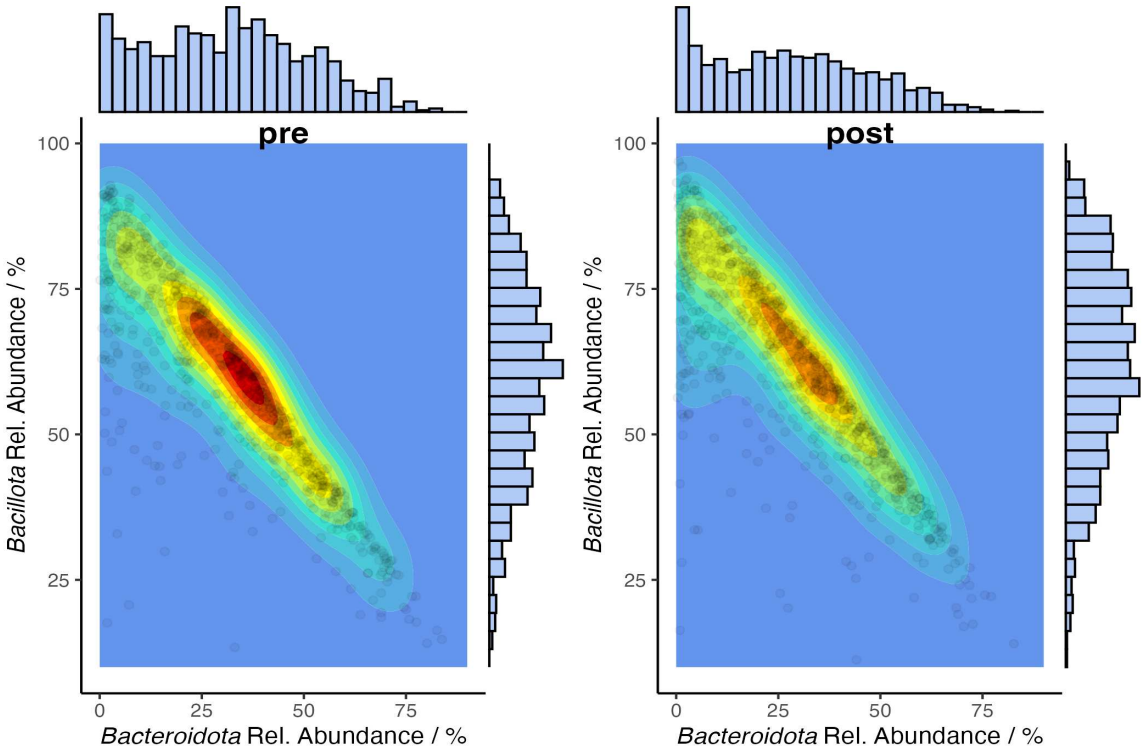

Figure 3.16: 2D contour plot showing the distribution of the *Bacillota* to *Bacteroidota* ratio in pre- and post-retreat samples.

In summary, while the *F:B* ratio has been proposed as a potential marker for obesity, significant methodological and interpretative challenges remain, making it difficult to establish a clear link between this ratio and health outcomes. Therefore, a more detailed analysis at genus and species levels was conducted and presented in following chapters.

3.4.2 Genus Level Analysis

Next, analysis on the genus level were performed.The stacked bar plot (Figure 3.17) illustrates the relative abundances of bacterial genera (> 2% abundance) before and after the meditation retreat, with additional stratification by health status.

The plot presents six conditions: overall pre/post, healthy-pre/post, and disease-pre/post. Notably, Bacteroides (green) shows relatively high abundance across all conditions but appears to decrease post-retreat regardless if people were healthy or

not. This pattern was consistently observed across all five retreat locations, further highlighting a potential common effect of the intervention on this microbial group (see Appendix Figure 5.9). This findings align with the previous phylum-level analysis showing a decrease in *Bacteroidota.* Several *Firmicutes* (now *Bacillota*) genera like *Faecalibacterium*, *Roseburia*, and *Ruminococcus* maintain at least consistently present across conditions, supporting the earlier observed increase in the *Bacillota:Bacteroidota* ratio.

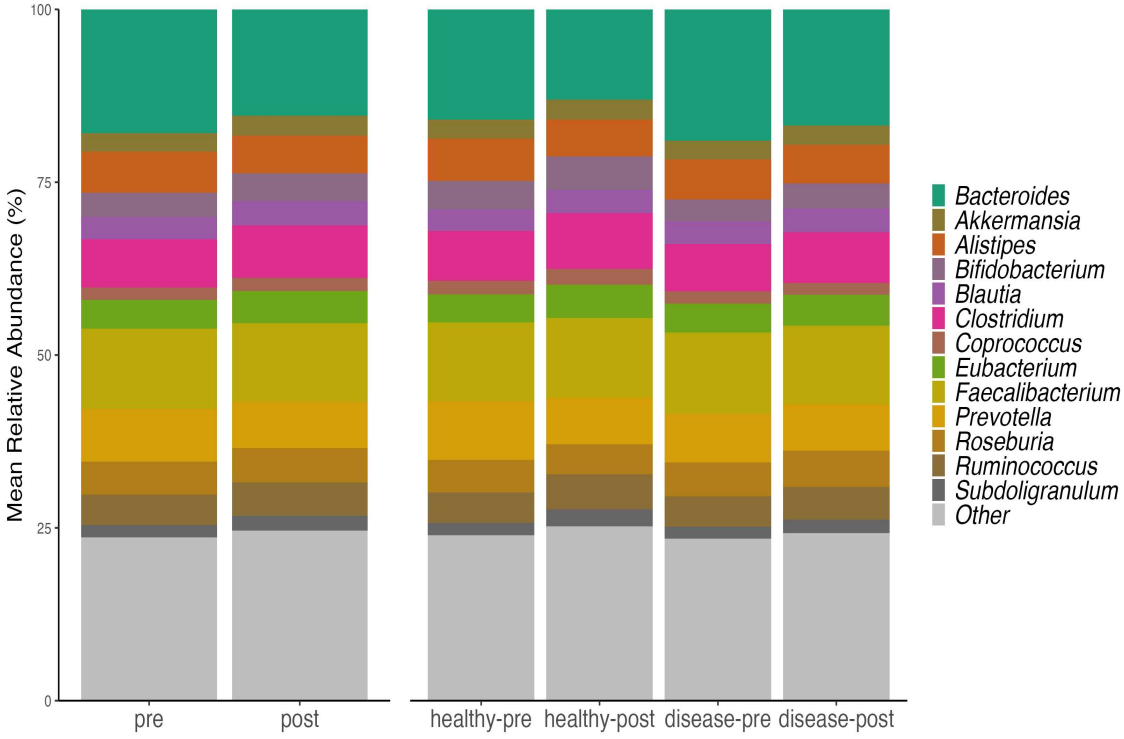

Figure 3.17: Mean relative abundances of dominant bacterial genera (>2%) in fecal samples before and after the meditation retreat, classified by health status.

Interestingly, the healthy and disease subgroups show different levels of *Bacteroides* before going into the retreat, with higher levels in people having a disease. These findings align with recent results from various studies. The genus *Bacteroides* exhibits characteristics that contribute to its increased prevalence in individuals with various diseases. Alterations in *Bacteroides* species composition, its ability to evade the immune system, and the production of pro-inflammatory cytokines are key factors that enable *Bacteroides* to thrive in dysbiotic gut environments and potentially worsen certain health conditions. Studies have shown that *Bacteroides* is significantly over-represented in conditions like Autism Spectrum Disorder (ASD) and coeliac disease,

indicating a potential link to disease pathology (Carmel et al., 2023; Sánchez, Donat, Ribes-Koninckx, Calabuig, & Sanz, 2010).

Conversely, while *Bacteroides* is often associated with disease, it also plays a crucial role in maintaining gut homeostasis under normal conditions. This duality highlights the complexity of *Bacteroides* in health and disease contexts.

Figure 3.18 presents a more detailed genus-level analysis of the relative abundance changes between pre- and post-retreat samples.

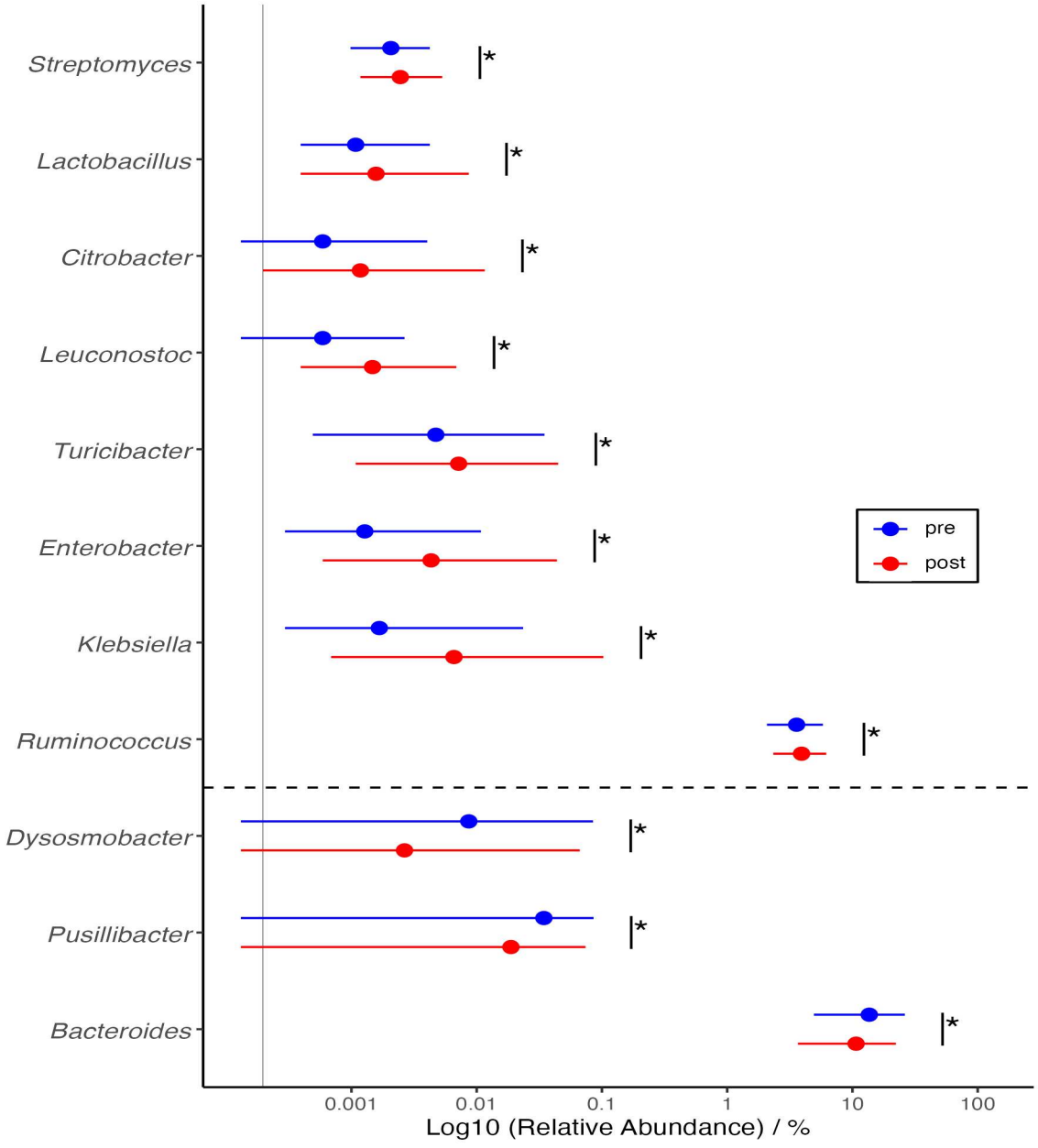

Figure 3.18: Log10 Rel. Abundances (%) of bacterial genera significantly changing before and after a meditation retreat on the global scale.

Eleven genera showed statistically significant changes: *Streptomyces, Lactobacillus, Citrobacter, Leuconostoc, Turicibacter, Enterobacter, Klebsiella and Ruminococcus* all increased post-retreat, while *Dysosmobacter, Pusillibacter and Bacteroides* decreased.

The Limit of Detection (LOD) was calculated based on a cutoff value of 508,833 reads using the formula LOD $= \frac{100}{\text{cutoff value}}$ and is represented by the gray vertical line in the plot. Pointranges in blue and red represent the median value along with the range covering the middle 50% of the data. Above the dashed line median values are increasing, below a decrease was observed.

The effects of these genera on gut health are context-dependent, shaped by host factors, diet, and microbial interactions. While some genera, such as *Streptomyces* and *Lactobacillus*, generally play beneficial roles through short-chain fatty acid (SCFA) production, others like *Klebsiella* and *Turicibacter* exhibit more ambiguous or dual effects, contributing to both health and disease. The variability in this findings underscores the importance of personalized approaches in microbiome research and interventions (Al Bander, Nitert, Mousa, & Naderpoor, 2020).

However, I want to highlight the shifts of the two enterotypes *Ruminococcus* and *Bacteroides* between pre and post measurements.

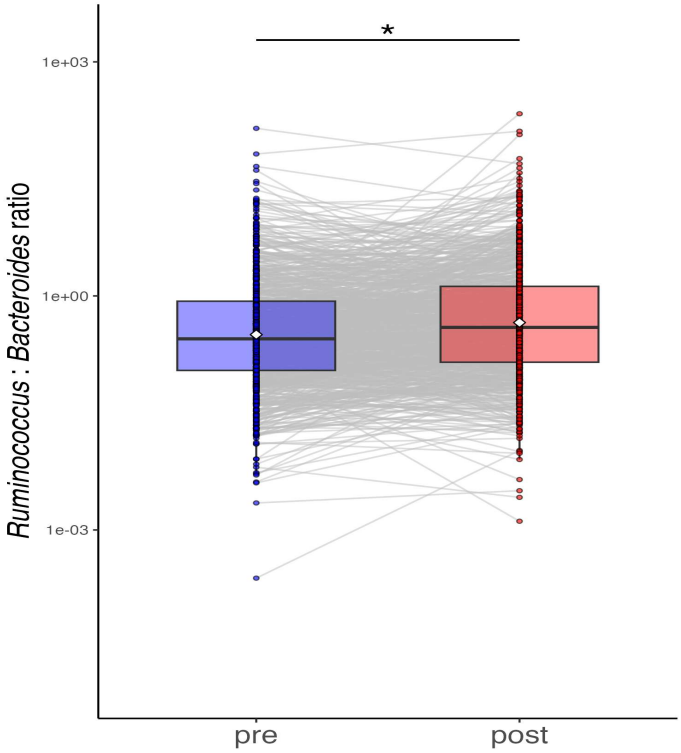

Figure 3.19: Global changes in the *Ruminococcus* to *Bacteroides* ratio before and after a meditation intervention.

These divergent changes in two dominant genera align with the previously observed increase in the *Bacillota:Bacteroidota* ratio at the phylum level. To further examine this, the *Ruminococcus:Bacteroides* ratio was calculated (Figure 3.19) and accounted for potential confounding factors such as location, health status, and different disease groups (see Appendix).

Figure 3.19 shows a significant global increase in the *Ruminococcus:Bacteroides* ratio. To better understand the community composition, the contour plot (Figure 3.20) illustrates a slight increase in *Ruminococcus* density and a corresponding decrease in *Bacteroides* density. Overall, it is noteworthy that *Ruminococcus* exhibits a relative abundance of less than 5% in most individuals, with only a few exceeding 10%. In contrast, *Bacteroides* demonstrates a broader range of relative abundance, reaching up to 70%, although the majority of individuals fall within the 1–25% range. A more detailed representation of the changes in these two genera within specific disease groups is provided in Figure 5.12 in the Appendix. Similar shifts can be observed across all disease groups.

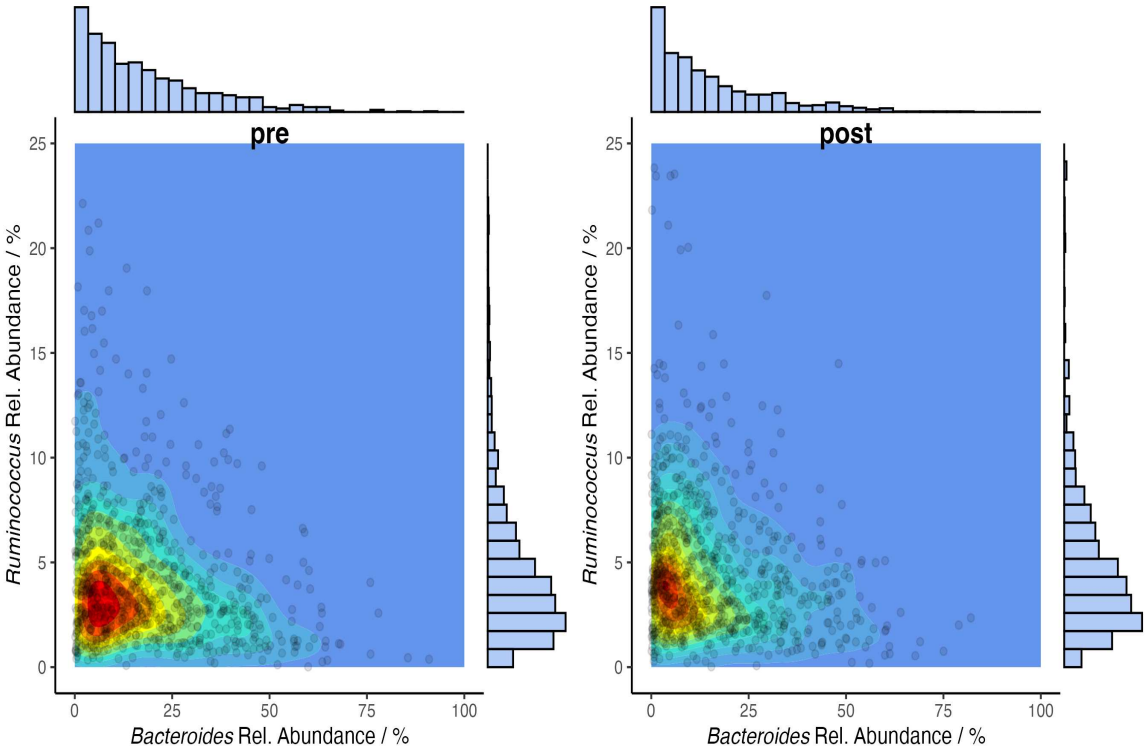

Figure 3.20: 2D contour plot showing the distribution of the *Ruminococcus* to *Bacteroides* ratio in pre- and post-retreat samples.

The observed shifts in the *Ruminococcus:Bacteroides* ratio appear to be largely independent of location, health status, and disease state. Notably, the increase in

Ruminococcus and decrease in *Bacteroides* was consistent across the different subgroups analyzed. Figures 5.10 and 5.11 in the Appendix illustrate the influence of location and health status on this ratio, respectively.

Interestingly, the changes in this ratio seem to be particularly noticeable in individuals with anxiety (Figure 3.21), a group that showed alreday significant changes in PERMANOVA testing. This suggests the gut microbiome shifts may have significant implications for mental health conditions, at least regarding this two genera.

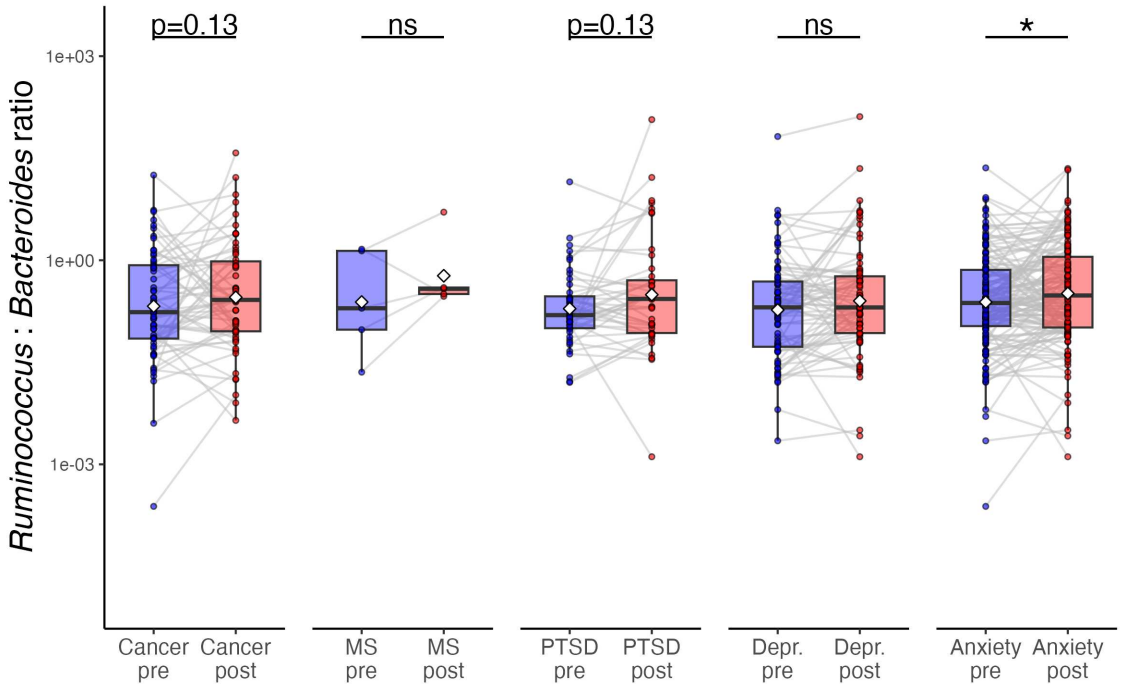

Figure 3.21: Changes in the *Ruminococcus* to *Bacteroides* ratio associated with different medical conditions before and after a meditation intervention.

Indeed, the *Ruminococcus* and *Bacteroides* genera are known to impact various physiological and neurological processes, so their altered balance could play an important role in the gut-brain axis (GBA) for these disorders. They are associated with metabolic pathways that influence immune function, gut barrier integrity, and the release of neuroactive compounds like short-chain fatty acids (SCFAs). These compounds play a crucial role in mediating gut-brain communication and have been connected to neurological and psychiatric conditions. Studies suggest that an imbalance in this ratio could lead to dysbiosis, contributing to neurological disorders like autism spectrum disorder (ASD), depression, and Parkinson's disease. For example, *Ruminococcus* species are often related to the production of butyrate, a key SCFA

that strengthens the mucus layer and reduces inflammation. On the other hand, an overrepresentation of *Bacteroides* has been linked to a pro-inflammatory state and potential disruption of the gut-brain communication (Nakhal et al., 2024).

Furthermore, the gut microbiota significantly impacts T-cell differentiation, a key element of the adaptive immune system. SCFA producing bacteria indirectly support the development of regulatory T-cells while reducing systemic inflammation. SCFAs also influence cellular metabolism, encouraging regulatory B cell formation and inhibiting pro-inflammatory Th17 cells. This interplay is vital for maintaining immune balance and managing autoimmune diseases (Mann, Lam, & Uhlig, 2024).

In summary, the Ruminococcus to Bacteroides ratio is crucial for understanding microbial interactions in the gut. However, the complexity of these interactions suggests that a simplistic view of this ratio may overlook the complex roles these bacteria play in the gut ecosystem.

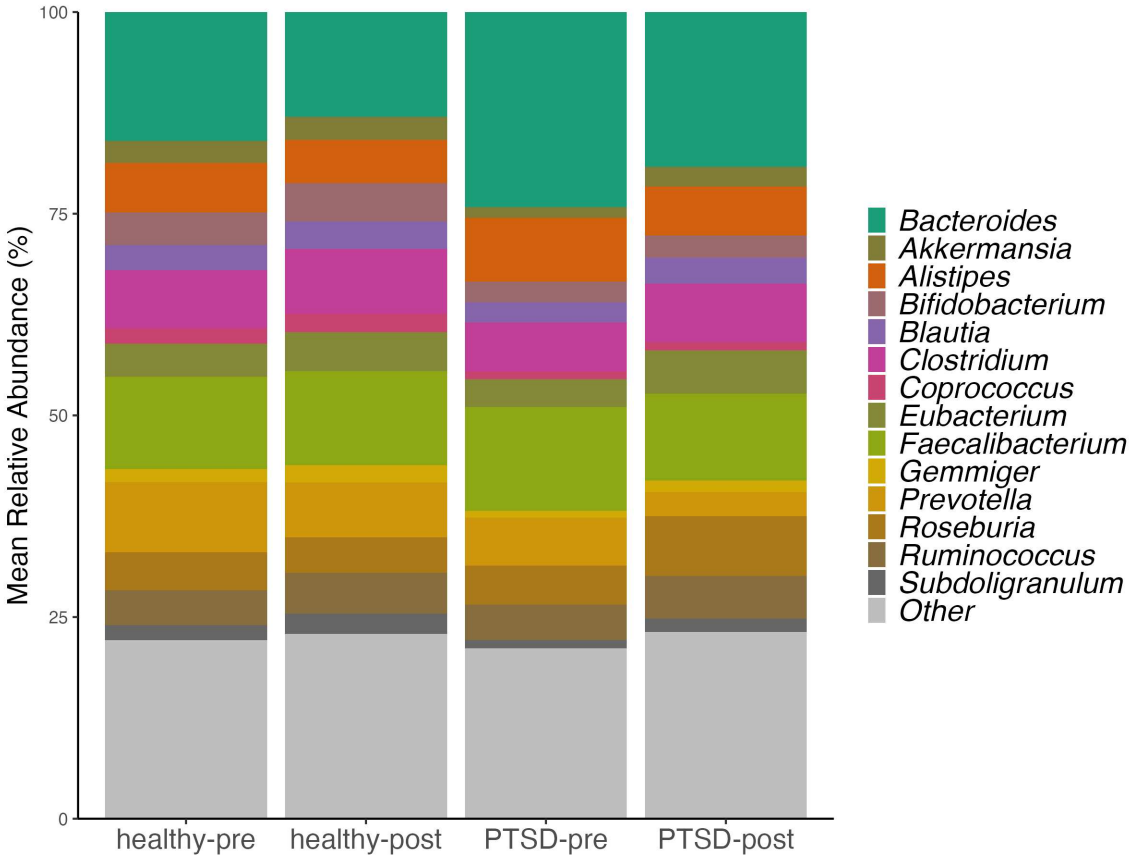

Figure 3.22: Relative abundance of bacterial genera (>2%) in fecal samples from PTSD patients and healthy controls before and after a meditation retreat.

Next, particular attention was focused on the microbial changes in the PTSD group due to significant findings from PERMANOVA testing within this cohort as discussed in Subsection 3.3.4. The stacked bar plot (Figure 3.22) presents a comprehensive analysis of bacterial genera abundances ($> 2\%$) comparing both PTSD patients and healthy controls.

Following the meditation retreat, the PTSD group exhibited notable shifts in their gut microbial composition. The most striking change was a substantial decrease in the relative abundance of *Bacteroides*, accompanied by an apparent increase in *Roseburia.* Interestingly, these alterations appeared to shift the overall microbial community structure of PTSD patients toward a profile more closely resembling that of healthy controls. The healthy control group maintained a relatively stable bacterial compositions between pre- and post-retreat measurements, but showing a major decrease in *Bacteroides* as well.

Figure 3.23 depicts a detailed analysis of the genus-level comparisons between healthy controls and PTSD patients before and after the meditation retreat: The plot presents log10-transformed relative abundances of bacterial genera, with median values and error bars indicating the 50% interquartile range. A dashed horizontal line divides the genera into two groups—those that increased in abundance after the retreat (above) and those that decreased (below). The comparison with the healthy cohort highlighted patterns in the pre- and post-PTSD groups that were not evident when analyzed alone, as no significant changes were detected within these groups after applying Benjamini-Hochberg corrections for multiple comparisons. In this analysis, the healthy controls were taken from the pre retreat timepoint.

A particularly noteworthy pattern emerged for several genera: *Collinsella, Subdoligranulum, Gemmiger, Intestinimonas*, and *Parabacteroides.* Initially, these genera showed significant differences when comparing healthy controls to pre-retreat PTSD patients. However, these significant differences disappeared when comparing healthy controls to post-retreat PTSD samples. This shift toward a more "healthy-like" microbiome composition suggests that the meditation retreat may have had a normalizing effect on the gut microbiota of PTSD patients.

Let us discuss some of the genera mentioned and their potential impect on health. The *Collinsella* genus of bacteria plays a significant role in modulating the human immune system, with implications for disease prevention and treatment. *Collinsella's* production of ursodeoxycholate may mitigate COVID-19 severity by inhibiting viral binding and reducing pro-inflammatory cytokines, thus potentially preventing severe outcomes (Hirayama et al., 2021).

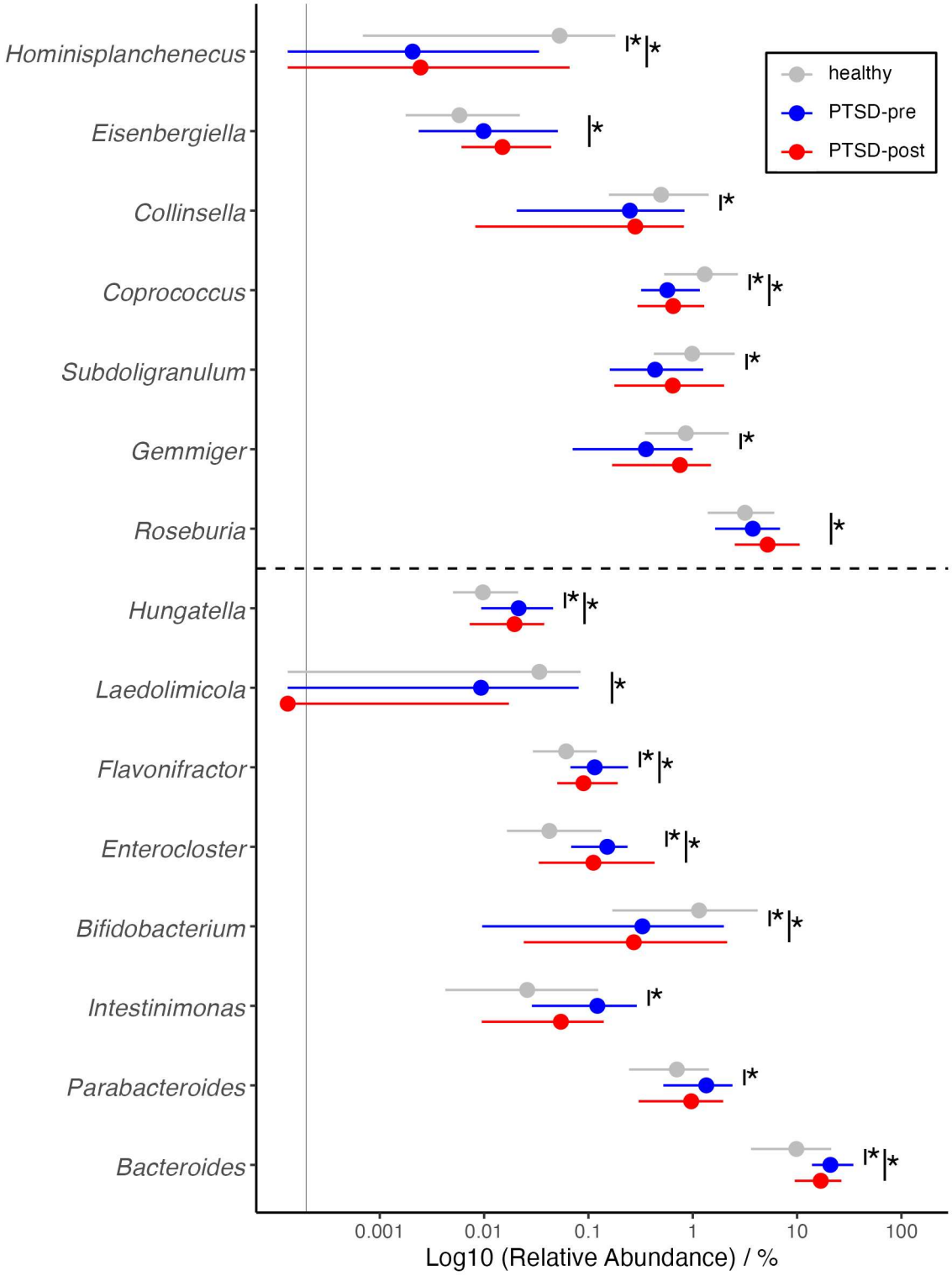

Figure 3.23: Log10-transformed relative abundances of bacterial genera in healthy controls and PTSD patients before and after a meditation retreat. Points represent the median and error bars represent the 50% interquartile range. Genera above the dashed line increased in abundance post-retreat, while those below decreased.

Research indicates that the genus *Subdoligranulum* can influence immune responses and metabolic pathways, suggesting a protective role in maintaining gut health. It has been shown to promote TSG-6 secretion, which is associated with reduced inflammation in rheumatoid arthritis (RA) (Li et al., 2024).

The genus *Gemmiger* helps maintain a healthy gut microbiota by promoting the growth of beneficial bacteria and inhibiting pathogenic strains, thus preventing dysbiosis. It is associated with the alleviation of conditions such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) by restoring microbial balance (Sundin, Öhman, & Simrén, 2017).

The genus *Intestinimonas* interacts with the human immune system in complex ways, influencing both immune responses and disease outcomes. It has been shown to be elevated in people with Huntington's disease (HD) and was found to correlate with IL-4 concentrations, suggesting a connection between gut microbiota alterations and systemic chronic inflammation (Du et al., 2021).

The current understanding of *Parabacteroides*, particularly *Parabacteroides distasonis*, highlights its significant role in maintaining gut health and preventing various diseases. This bacterium is recognized for its anti-inflammatory properties, metabolic benefits, and potential as a probiotic. Despite its benefits, *P. distasonis* may also pose risks, such as antimicrobial resistance and potential pathogenic effects in certain contexts, including autoimmune disease triggers (Ezeji et al., 2021). This duality necessitates careful consideration in therapeutic applications.

The observed increase in *Roseburia* in the bar chart (Figure 3.22) following the meditation retreat, particularly in the PTSD group, deserved a closer examination of this genus due to its recognized role in gut and overall health. *Roseburia*, a butyrate-producing genus within the *Lachnospiraceae* family, is associated with anti-inflammatory effects and the maintenance of gut barrier integrity. Additionally, higher abundances of *Roseburia* have been linked to healthier gut microbial ecosystems and have been inversely associated with conditions such as inflammatory bowel disease (IBD) and metabolic syndrome (Tamanai-Shacoori et al., 2017).

Figure 3.24 illustrates the relative abundance of *Roseburia* across different groups. Panel (a) shows the global community, while panel (b) focuses on the PTSD group pre- and post-retreat. A statistically significant increase in *Roseburia* abundance post-treatment is observed in the PTSD-group, while the global community showed no changes in median or mean values. Corresponding p-values can be seen in Table 3.3. The paired lines highlight individual trajectories, showing that while some individuals experience an increase and others a decrease in *Roseburia* abundance, the magnitude

of change varies widely, ranging from minimal to substantial shifts.

Wilcoxon signed-rank test results for *Roseburia* relative abundance across various disease states pre and post are shown in Table 3.3. Significant shifts in microbial composition were observed in PTSD, Cancer and Anxiety groups, with post mean values significantly higher than pre, as evidenced by p-values of 0.0117, 0.0131 and 0.0012, respectively.

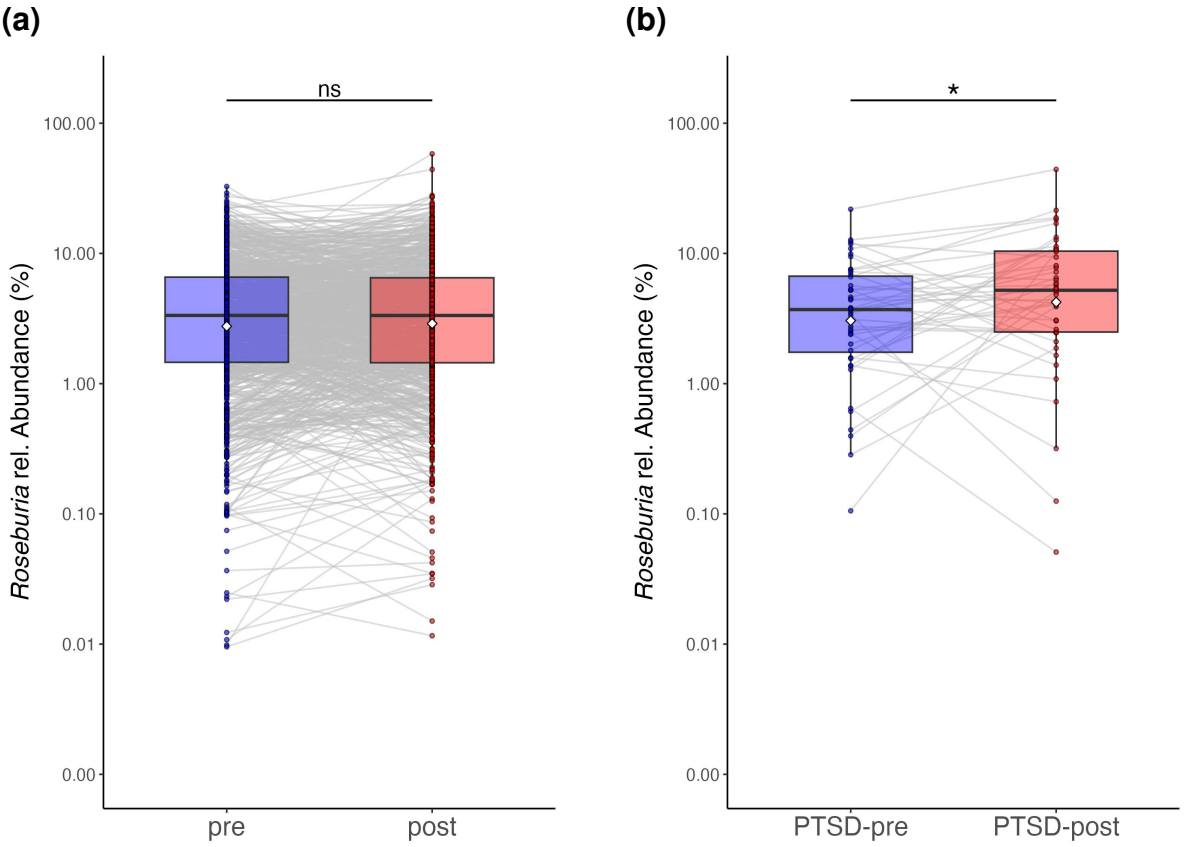

Figure 3.24: Changes in *Roseburia* rel. abundance in the global community (a) and the PTSD subgroup (b) from pre- and post-retreat samples.

In contrast, the Depression and MS groups did not show significant changes. However, MS followed the trend of an increase in *Roseburia* abundance, while Depression was the only disease group with a slight decrease in mean values. The global results also indicate an overall modest shift across all conditions, highlighting a general change in *Roseburia* relative abundance. Notebly, the standard deviation values are relatively large in comparison to the means for all groups, suggesting considerable variability within each disease state.

Given these beneficial roles, the rise in *Roseburia* in PTSD, Cancer and Anxiety

Table 3.3: Results from Wilcoxon signed-rank test: Changes in *Roseburia* relative abundance (%) for various disease states, showing the mean, standard deviation (SD), and p-values for pre- and post-retreat measurements, as well as the sample size (n).

Disease State	**n**	**mean-pre (%)**	**mean-post (%)**	**p-value**
PTSD	41	4.79 ± 4.31	7.34 ± 7.77	0.0117*
Cancer	60	4.08 ± 4.71	6.00 ± 6.21	0.0131*
Anxiety	146	4.30 ± 4.07	5.42 ± 5.38	0.0012*
Depression	74	5.08 ± 5.21	4.94 ± 3.96	0.3044
MS	5	2.06 ± 2.14	3.84 ± 3.57	0.6250
Global	853	4.81 ± 4.80	4.95 ± 5.22	0.3110

patients could suggest a potential shift toward a more resilient and metabolically supportive gut environment post-retreat. This finding, alongside the concurrent decrease in *Bacteroides*, highlights the potential of behavioral interventions like meditation to influence gut microbial dynamics. This leads us to further detailed analyses at the species level to explore the mechanisms of these changes more deeply.

3.4.3 Species Level Analysis

Analyzing taxonomic differences at the species level is essential for gaining a deeper understanding of microbiome dynamics, as it reveals specific microbial contributors to health and disease that may be obscured at higher taxonomic ranks. While phylum and genus-level analyses provide valuable insights into broader trends, species-level resolution allows for the identification of functional roles, strain-specific behaviors, and precise associations with environmental or clinical factors. This depth is particularly important for linking microbial shifts to physiological effects and potential therapeutic targets.

Figure 3.25 shows changes in the relative abundance of four bacterial species in the gut microbiome before and after meditation intervention. All species displayed very low relative abundances (below 0.1% in median values) as indicated by the log10 scale. After the meditation intervention, all four species—*Flavonifractor plautii*, *Clostridium phocensis*, *Lachnospiraceae bacterium 7_1_58FAA*, and *Pusillibacter faecalis*—showed significant decreases in their relative abundance. The data are presented as median with their corresponding 50% interquartile range. Notably, the median values for *Flavonifractor plautii* and *Lachnospiraceae bacterium 7_1_58FAA* in the post group

fell below the LOD, indicating that over half of the community effectively had zero counts for these species after the retreat.

Flavonifractor plautii has been shown to suppress interleukin (IL)-17 signaling, which is crucial in inflammatory responses, thereby promoting recovery from acute colitis in mice (Mikami et al., 2021). Moreover, the bacterium's ability to metabolize flavonoids, such as catechins, further supports gut health by potentially improving nutrient absorption and reducing oxidative stress (Rodriguez-Castaño, Rey, Caro-Quintero, & Acosta-González, 2020). Conversely, its pathogenic potential in certain contexts, such as bloodstream infections in immunocompromised individuals, raises concerns about its safety in vulnerable populations (Karpat et al., 2021).

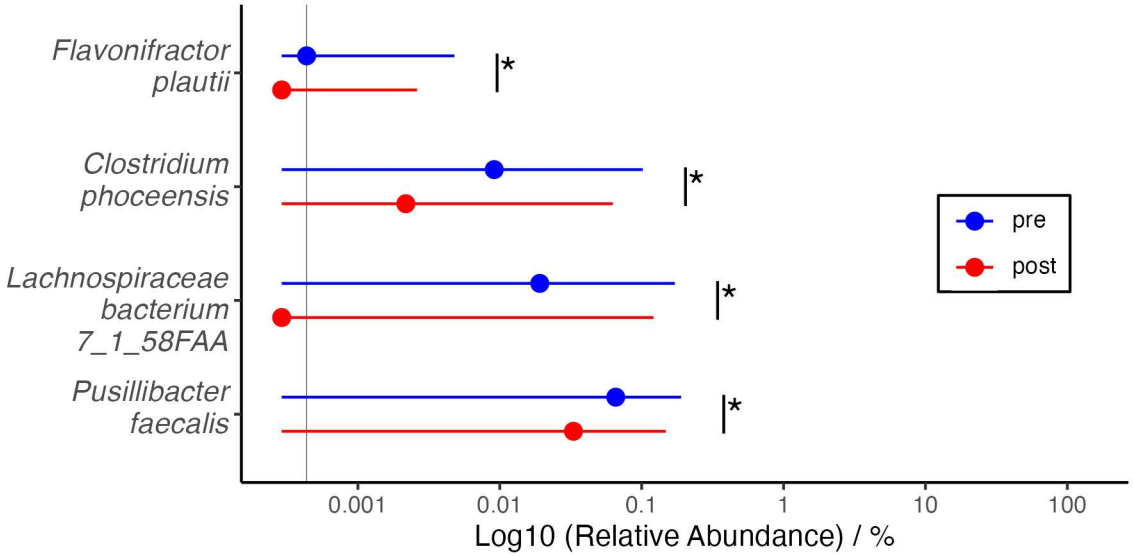

Figure 3.25: Log10 Rel. Abundances (%) of bacterial species before and after a meditation retreat in the global community.

Clostridium spp. play a dual role in the gut, supporting immune regulation and gut health through SCFA production, regulatory T cell (Treg) activation, and intestinal barrier maintenance. However, their therapeutic use as probiotics is tempered by safety concerns, as excessive colonization could lead to dysbiosis (P. Guo, Zhang, Ma, & He, 2020). While Clostridium spp. are widely studied, there are no specific studies on *Clostridium phocensis*, leaving its exact role in the gut microbiota unclear.

Lachnospiraceae bacterium 7_1_58FAA has been shown to be significantly increased in children with IBS and was linked to abdominal pain severity and frequency. Its functions in carbohydrate metabolism and potential immune activation suggest a role in IBS symptomatology. Sharing high genetic similarity with *Flavonifractor*

plautii—a pairwise average nucleotide identity exceeding 98%—its precise classification and mechanisms require further research (Hollister et al., 2019). It is interesting that exactly these two species, *Flavonifractor plautii* and *Lachnospiraceae bacterium 7_1_58FAA*, showed significant alterations after the meditation intervention on a global scale. The findings suggest that these species might play a crucial role in the observed microbiome changes following the retreat.

A recent study by Felicianna et al. (2024) showed that *Pusillibacter faecalis* was significantly enriched in mice following a high-fat diet, contributing to the development of metabolic dysfunction-associated steatotic liver disease (MASLD). This finding indicates that the presence of this bacterium may contribute to the development of metabolic disorders associated with diet-related liver disease.

The meditation intervention resulted in significant decreases in the relative abundance of several bacterial species within the gut microbiome of the global community. These changes, observed at very low abundance levels, highlight the potential for behavioral interventions to influence microbial dynamics. Notably, some species showed such substantial decreases that their abundance fell below detection limits in many individuals. These findings emphasize the importance of examining specific bacteria to understand their role in gut health and metabolic processes.

Building on this, the next analysis focuses on species-level differences between the PTSD group and a healthy control group (Figure 3.26), providing deeper insight into condition-specific microbiome shifts. In this case, the healthy control group corresponds to samples taken at the pre-treatment timepoint. The plot compares the relative abundance (on a log10 scale) of various bacterial species across three conditions: healthy controls (grey), PTSD patients before (blue), and PTSD patients after the retreat (red). The data includes only bacterial species that exhibit significant differences in abundance across at least two groups. The species are organized vertically, with horizontal lines representing the 50% interquartile range around median values. The figure is divided into two sections by a dashed line: species above the line show an increase in relative abundance between the pre- and post-PTSD groups, while species below the line demonstrate a decrease in their median relative abundances.

Looking at the species-level data in the plot, only *Laedolimicola ammoniilytica* shows a significant difference in abundance between pre and post in PTSD patients, with a notable decrease following the meditation retreat. While other species show various patterns between healthy controls and PTSD groups, they did not demonstrate significant changes between pre- and post conditions.

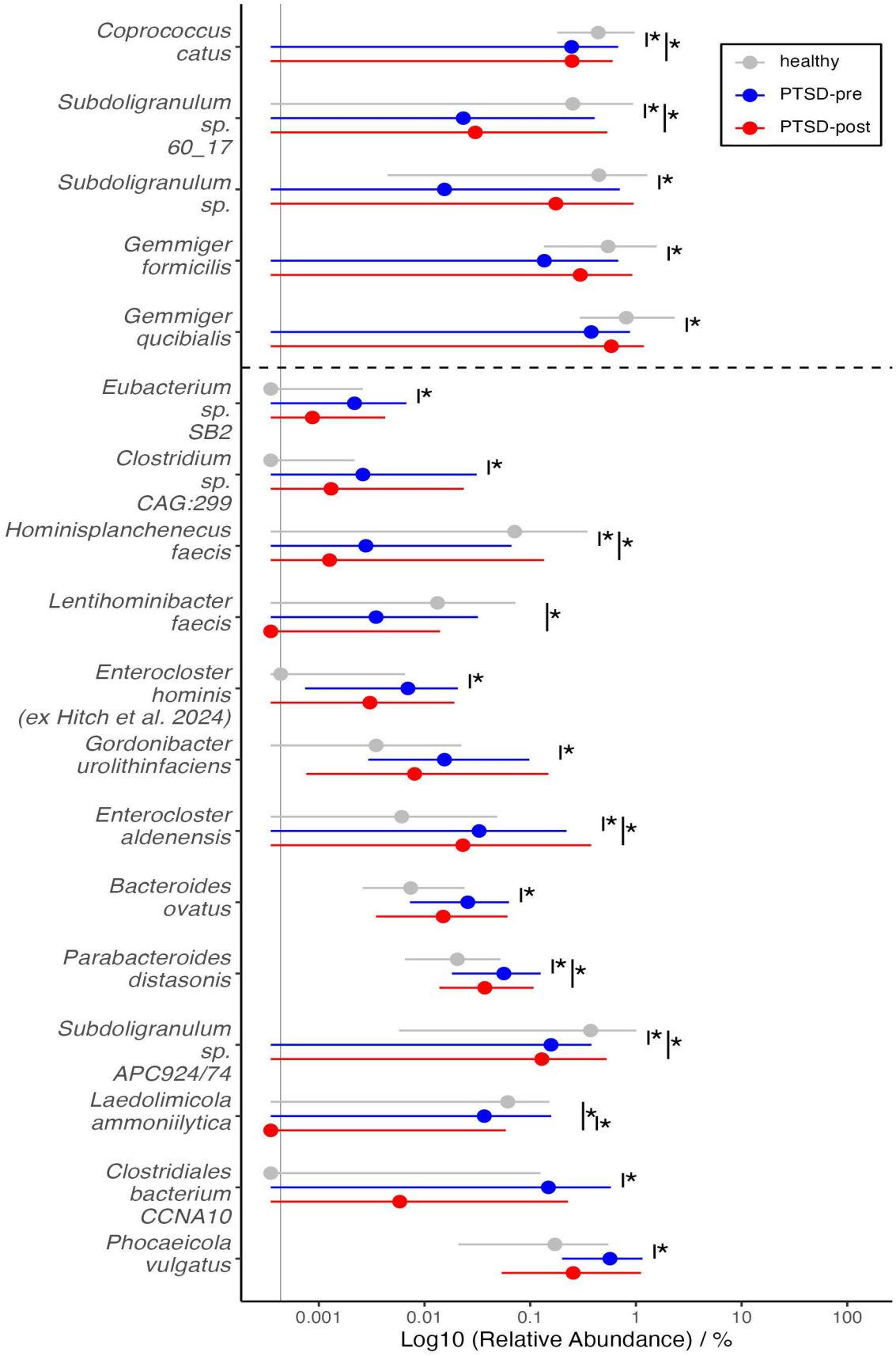

Figure 3.26: Log10 Rel. Abundances (%) of bacterial species in healthy controls and PTSD patients before and after a meditation retreat.

Interestingly, several bacterial species show a pattern where PTSD post values shift closer to healthy control values compared to PTSD pre: *Subdoligranulum sp., Gemmiger formicilis,* and *Gemmiger qucibialis* increased, whereas *Eubacterium sp. SB2, Clostridium sp. CAG:299, Enterocloster hominis (ex Hitch et al. 2024), Gordonibacter urolithinfaciens, Bacteroides ovatus, Clostridiales bacterium CCNA10,* and *Phocaeicola vulgatus* decreased, moving toward patterns characteristic of the healthy group. This suggests a normalization of the microbiome composition after the meditation retreat, similar to what was observed at the genus level (Figure 3.23).

The *Gemmiger* genus is part of the *Firmicutes* phylum and is commonly associated with a healthy gut microbiome. Members of this genus are known for producing SCFAs, particularly butyrate, through the fermentation of dietary fibers. A recent study from W. Guo et al. (2024) found that in Obstructive Sleep Apnea (OSA) patients, the fecal microbiome showed a decrease in the abundance of the *Gemmiger* genus and its species *Gemmiger formicilis*.

Research suggest that *Subdoligranulum sp.* are positively associated with metabolic health, correlating with improved markers such as higher HDL cholesterol levels and lower glycated hemoglobin (HbA1c). It increases in abundance with prebiotic treatment and its lower abundance is linked to diseases like type-2 diabetes and inflammatory bowel diseases. However, it is also underrepresented in conditions like non-alcoholic fatty liver disease (NAFLD) and cirrhosis, where its reduced levels are associated with metabolic dysfunction and poor gut integrity (Van Hul et al., 2020).

Over the past two decades, *Eubacterium* has been recognized as a key genus in the human gut microbiome, contributing to health through its phylogenetically diverse species. Many Eubacterium species produce butyrate, and they also participate in bile acid and cholesterol metabolism. However, specific functional details about Eubacterium sp. SB2 are not readily available in the literature.

Liu et al. (2023) showed that individuals exposed to environmental risk factors, such as passive smoking or house decoration, showed an increased abundance of *Clostridium sp. CAG:299*. This change suggests that these environmental exposures may influence the composition and function of the gut microbiota, potentially affecting gut health in individuals with atopic dermatitis (AD).

The bacterium *Enterocloster hominis* was recently classified and named by Hitch et al. (2023) as part of a broader reorganization of bacterial taxonomy, moving certain species from the *Clostridium* and *Lachnoclostridium* genera to the new *Enterocloster* genus. This change reflects advances in phylogenetic analysis and the need to better categorize these organisms based on their genetic and functional characteristics. While

its specific role in host health is not yet well understood, further studies are necessary to explore its potential impacts, particularly within the human gut microbiome, where related species have shown significant roles in metabolism and immune interactions.

Gordonibacter urolithinfaciens plays a key role in the gut microbiome's metabolism of dietary phenolic compounds, particularly ellagic acid and related polyphenols. This bacterium is capable of transforming ellagic acid into bioactive metabolites called urolithins by removing hydroxyl groups from catechol moieties and reducing double bonds in the phenolic structures. However, the presence and abundance of *G. urolithinfaciens* in the human gut vary significantly among individuals, contributing to differences in how people metabolize and respond to polyphenol-rich diets (García-Villalba et al., 2020).

Bacteroides ovatus has shown significant therapeutic potential in various applications. The strain *B. ovatus* D-6 expresses the tumor-specific Thomsen-Friedenreich antigen (TFα), which activates anti-TFα antibodies, offering promise as a cancer vaccine candidate. Additionally, *B. ovatus* V975 has been developed to treat bowel diseases such as colitis and inflammatory bowel disease (IBD) by delivering therapeutic agents like human growth factors and TGF-β in connection with prebiotics. These attributes make *B. ovatus* a key player among next-generation probiotics for promoting gut health and systemic benefits (Tan, Zhao, Zhang, Zhai, & Chen, 2019).

In a study with post-weaning piglets, Zinc oxide (ZnO) treatment was given to effectively reduced the abundance of *Escherichia coli* across samples, particularly in diarrheal cases. *Clostridiales bacterium CCNA10* was uniquely detected in zinc-treated diarrheal samples at 7 days post-weaning (dpw) and persisted at 14dpw in these groups. While its role remains uncertain, the bacterium's association with zinc-treated environments suggests it may adapt to gut conditions influenced by zinc supplementation, suggests it might coexist in a protective microbial context. However, more research is needed to confirm its precise functional role in gut health (Ortiz Sanjuán et al., 2024).

Phocaeicola vulgatus ferments carbohydrates, yielding organic acids such as succinate and acetate, which are crucial for maintaining gut homeostasis. It produces metabolites like 3-Hydroxyphenylacetic acid (3-HPAA), which can regulate lipid metabolism and reduce conditions like metabolic dysfunction-associated steatotic liver disease (MASLD) (Jin et al., 2024).

Summarizing the analysis of species-level microbiome differences between PTSD patients and healthy controls, the data reveal significant shifts in microbiome composition following a meditation retreat. It shows that, while only one species exhibit

significant changes in abundance between pre- and post-PTSD conditions, many species demonstrate a trend where PTSD post values shift closer to those of healthy controls. This suggests a partial normalization of the microbiome composition in PTSD patients after the retreat, aligning their gut microbiomes more closely with the patterns seen in healthy individuals. This finding points to a potential recovery or balancing effect on the microbiome following the intervention.

4 Conclusion

The analysis of sequencing variation helped set a 10th percentile cutoff, with 269,002 sequences at the species level, retaining 853 paired samples for further analysis. Consistent thresholds were applied at other taxonomic levels, with 927,990 sequences at the phylum level and 508,883 at the genus level, retaining 855 and 853 samples, respectively. This approach ensured that enough high-quality samples were included for studying community composition.

The analysis of alpha diversity indices, including the inverse Simpson index, Shannon index, and species richness, revealed no significant changes in overall microbial community diversity following the seven-day meditation retreat, either globally or within specific locations. While minor location-specific trends were observed, such as slight increases in diversity in Australia, Denver, and Nashville and decreases in Cancun and Vienna, these patterns were not consistently significant. Similarly, comparisons between healthy and disease groups showed no significant directional changes in diversity, although individual participants displayed substantial variation. This highlights the potential for meaningful participant-specific microbiome changes that may not be reflected in community-wide alpha diversity metricies. These findings suggest that while meditation did not broadly alter microbial diversity, individual-level dynamics require further investigation.

The PERMANOVA results together with PCoA from beta diversity analysis using Bray-Curtis distances indicate that certain factors (e.g., timepoint, gender, and age group) and their interactions (e.g., Timepoint:Location and Gender:Age Group) have statistically significant effects on microbiome composition on a global scale. Moreover, a significant change in the PTSD-group (n=41) pre- vs. post-retreat ($p = 0.001$) and in the Anxiety cohort ($p = 043$) was measured. This suggests meditation practice induced subtle but consistent variations in the gut microbial communities of individuals with PTSD and Anxiety. Nontheless, their effect sizes—reflected in the R^2 values—are generally small as the residuals account for the vast majority of variation in microbiome composition, with 91.43% of the total R^2. This is consistent with most microbiome studies, where only up to 25% of the variation in species abundance can be explained (Johnson et al., 2020). However, even low R^2 values can highlight measurable impacts on beta diversity, emphasizing the complex and multifactorial nature of microbial

communities. Statistically significant results, while informative, should be interpreted cautiously, as they may not necessarily indicate meaningful or impactful biological differences in the context of microbiome changes. This underlines the importance of considering both statistical significance and effect size when drawing conclusions about microbiome dynamics.

One of the most striking aspects of the beta diversity measurements is the contrast between within-person and between-person variations. The notably lower dissimilarity values (around 0.5) observed in intra-human comparisons suggest that individual microbiomes may experience changes, but these changes are generally less clear than the natural variations between different individuals, which show dissimilarity values around 0.85. The greater dispersion in intra-human comparisons further emphasizes the complexity and variability of microbiomes within individuals compared to across individuals. This finding aligns with our understanding of the human microbiome as being relatively personalized and stable over time.

Furthermore, our analysis reveals that intra-human Bray-Curtis dissimilarity patterns are remarkably consistent across different disease groups—Cancer, MS, Depression, PTSD, and Anxiety—when compared to a healthy cohort. All groups, including the healthy cohort, exhibit similar median dissimilarity values around 0.45-0.50, suggesting that the degree of microbiome change over time is not strongly influenced by disease status. Cancer patients display the widest variability, indicating highly individual microbiome responses and mental health conditions like Depression, PTSD, and Anxiety show distributions almost equal to each other. Overall, these findings highlight that individual variation plays a more significant role than disease state in microbiome dynamics, emphasizing the stability of intra-human microbiome changes across diverse health conditions. These findings suggest that health status does not significantly impact the degree of individual microbiome changes as both healthy and disease groups show remarkably similar patterns in both intra-human and inter-human comparisons.

Analyzing the inter-human distances at timepoints before and after offers valuable insight into understanding the overall impact of the meditation retreat. If the food or other factors at the retreat center significantly influence the microbiome, we would expect to see the microbiomes of participants become more similar after the retreat than before. Seeing that this is not the case indicates that location effects, particularly related to diet, were likely minimal in this study. This is an important insight that helps framing the broader findings. The unchanged between-person variations suggest that the intervention, not location-driven factors, primarily influenced the observed

microbiome changes.

The *Bacillota:Bacteroidota* ratio (former *F:B* ratio) observed to increase significantly after the 7-day meditation retreat, suggests a shift in the gut microbiome that favors *Bacillota* over *Bacteroidota*. The observed changes were consistent across different subgroups, regardless of location or health status, indicating a possible impact of meditation on microbiome composition. Interestingly, aging is generally associated with a decrease in this ratio. Older individuals tend to exhibit lower levels of *Bacillota* and higher levels of *Bacteroidota*. This can reflect changes in gut microbiome composition linked to aging, such as a reduction in beneficial, butyrate-producing bacteria like *Faecalibacterium* and *Roseburia*. Could the observed increase in the *Bacillota:Bacteroidota* ratio following the meditation retreat be interpreted as a shift toward a more youthful microbiome profile? Can this change suggest that the meditation retreat had an effect on the microbiome similar to that of younger individuals, potentially indicating rejuvenating effects on gut health? Further analysis at the genus and species levels helped to understand these microbiome changes and their health implications.

Globally, eleven genera displayed statistically significant changes: *Streptomyces, Lactobacillus, Citrobacter, Leuconostoc, Turicibacter, Enterobacter, Klebsiella* and *Ruminococcus* all increased post-retreat, while *Dysosmobacter, Pusillibacter* and *Bacteroides* decreased.

The analysis of the *Ruminococcus:Bacteroides* ratio revealed significant changes in gut microbiome composition following the meditation retreat. The ratio increased globally, with *Ruminococcus* rising and *Bacteroides* decreasing, consistent across all locations, health status, and disease groups. Especially, participants with mental health conditions such as PTSD and anxiety had a notable shift post retreat, suggesting potential benefits for gut-brain interactions. These findings align with earlier results showing changes in the *Bacillota:Bacteroidota* ratio, indicating that meditation could lead to a more youthful, balanced microbiome. However, the relationship between these genera is complex, influenced by various factors like diet and health status. Further research is needed to explore the broader implications of these microbiome shifts for health and disease prevention.

Looking more closely at PTSD patients, significant changes in bacterial genera such as *Bacteroides* and *Roseburia* could be observed. These shifts brought the PTSD group's microbial composition closer to that of healthy controls, suggesting a potential "normalizing" effect. The decrease in *Bacteroides* and increase in *Roseburia*, particularly in the PTSD cohort, aligns with observed health benefits, as *Roseburia* is

associated with anti-inflammatory effects and improved gut barrier integrity. Further species-level analyses are needed to explore these changes and their health implications in greater detail.

The meditation intervention significantly reduced the relative abundance of four bacterial species in the global community's gut microbiome: *Flavonifractor plautii*, *Clostridium phocensis*, *Lachnospiraceae bacterium 7_1_58FAA*, and *Pusillibacter faecalis*. While some of these species, such as *Flavonifractor plautii* and *Clostridium phocensis*, are known for their potential health benefits through immune regulation and gut barrier support, their roles can also be context-dependent, posing risks under certain conditions. Conversely, *Lachnospiraceae bacterium 7_1_58FAA* and *Pusillibacter faecalis* have been linked to disease states, including IBS and metabolic dysfunction. These findings suggest that the intervention may reduce disease-associated species while simultaneously lowering potentially beneficial ones, indicating complex dynamics that require further investigation to better understand their overall effect on health.

Species-level analysis of the gut microbiome in PTSD patients reveals significant changes after a meditation retreat. While only *Laedolimicola ammoniilytica* exhibited a significant decrease in abundance between pre- and post-retreat, other species, such as *Subdoligranulum sp.*, *Gemmiger formicilis*, and *Eubacterium sp. SB2*, showed shifts toward patterns seen in healthy controls—as already witnessed on the genus level. This suggests a normalization of the microbiome composition in PTSD patients following the retreat, potentially contributing to improved gut health. These findings align with previous studies linking specific bacterial species, such as *Gemmiger sp.* and *Subdoligranulum sp.*, with metabolic health and gut integrity. However, as microbiome data are compositional, careful interpretation is needed.

In summary, these findings show how the gut microbiome affects both physical and mental health. They suggest that interventions, like meditation, which influence the microbiome, could be a new way to improve outcomes for people with PTSD. By promoting healthier microbial communities, such interventions may help restore balance in the gut, reduce inflammation, and improve overall resilience in both the mind and body.

4.1 Limitations

The application of False Discovery Rate (FDR) corrections is a critical aspect of microbiome studies to manage multiple comparisons. However, it can lead to overlooking

significant trends in specific genera. For example, despite a clear increase in *Roseburia* for the PTSD-group post-retreat visible in box-plots in Figure 3.24(b), strict FDR adjustments removed its significance in group-level analyses. To address this limitation, some studies use a relaxed threshold ($q < 0.25$) to identify potential trends without entirely dismissing relevant biological changes (Knights et al., 2014). In this study, however, the approach of using FDR-corrected q-values < 0.25 wasn't applied. Instead, our analysis followed a stricter approach, making the detected significant changes even more compelling. Nonetheless, this approach likely caused us to miss additional biologically relevant patterns. This underscores the trade-off between statistical rigor and the ability to detect subtle, yet potentially meaningful, microbiome shifts.

Additionally, tt is crucial to recognize that interpreting relative abundance in microbiome data is complex due to its compositional nature. Relative abundance represents the proportion of a specific microbe within the total microbial community. A decrease in relative abundance does not necessarily indicate a reduction in absolute microbial numbers—it may reflect shifts in the overall community composition. A taxon's relative abundance can decline while its absolute abundance remains stable or even increases, depending on the dynamics of other taxa in the community. While relative abundance provides insights into community structure, it may mask the true dynamics of microbial populations, requiring a careful approach for accurate data interpretation (Gloor, Macklaim, Pawlowsky-Glahn, & Egozcue, 2017).

Another limitation of this microbiome study is the absence of a proper control group, which would have included people who visited the retreat venues for vacation purposes but did not participate in the retreat activities. Without such a control group, it is challenging to isolate the specific effects of the meditation retreat from other potential confounding factors, such as vacation effects or changes in diet due to the food provided at the hotels. Without accounting for these variables, it is difficult to clearly state whether the observed microbiome shifts were solely due to the retreat experience. It might have been influenced by other factors such as travel, stress reduction, or changes in food intake, which may also have beneficial effects on the microbiome. Therefore, future studies should include a more comprehensive control group to better isolate the effects of the intervention on microbiome changes. However, as discussed previously, the lack of decreased between-person microbiome variation post-retreat suggests that the intervention itself, rather than location-driven factors like diet, was likely the primary factor influencing microbiome changes.

4.2 Outlook

The growing body of research linking gut microbiota to human health highlights the need for understanding causal relationships between specific microbial species and health outcomes. Although correlations between certain bacteria and metabolic parameters are well-established, these associations do not always imply causality. This fact is illustrated for example by the lack of impact from *Subdoligranulum* supplementation in animal models (Van Hul et al., 2020). These findings underscore the critical need for rigorous in vivo studies to confirm the causative roles of microbial species in human health. In addition to therapeutic interventions, the potential benefits of meditation on the microbiome are increasingly recognized, making it a valuable area for further study to explore its therapeutic possibilities and broader health impacts.

Future studies should consider conducting longitudinal research to fully explore the long-term effects of meditation interventions on health. A well-designed longitudinal study could track participants over a period of a year or more, assessing how sustained meditation practices impact the gut microbiome, physical health, and mental well-being. By collecting periodic data, researchers could better understand whether the observed changes in microbial composition, such as the increase in anti-inflammatory species like *Roseburia* and a healthier *F:B* ratio, persist over time or require continued practice to maintain. Additionally, a longitudinal approach would allow for the evaluation of secondary health benefits, such as improvements in metabolic markers, immune function, and psychological resilience. For individuals with PTSD or other chronic conditions, these studies could clarify whether meditation not only normalizes gut microbiota composition but also contributes to reducing symptoms and enhancing quality of life over the long term. Such insights would provide valuable evidence for integrating meditation into therapeutic protocols and could help identify the duration and frequency of interventions needed for optimal health outcomes.

5 Appendix

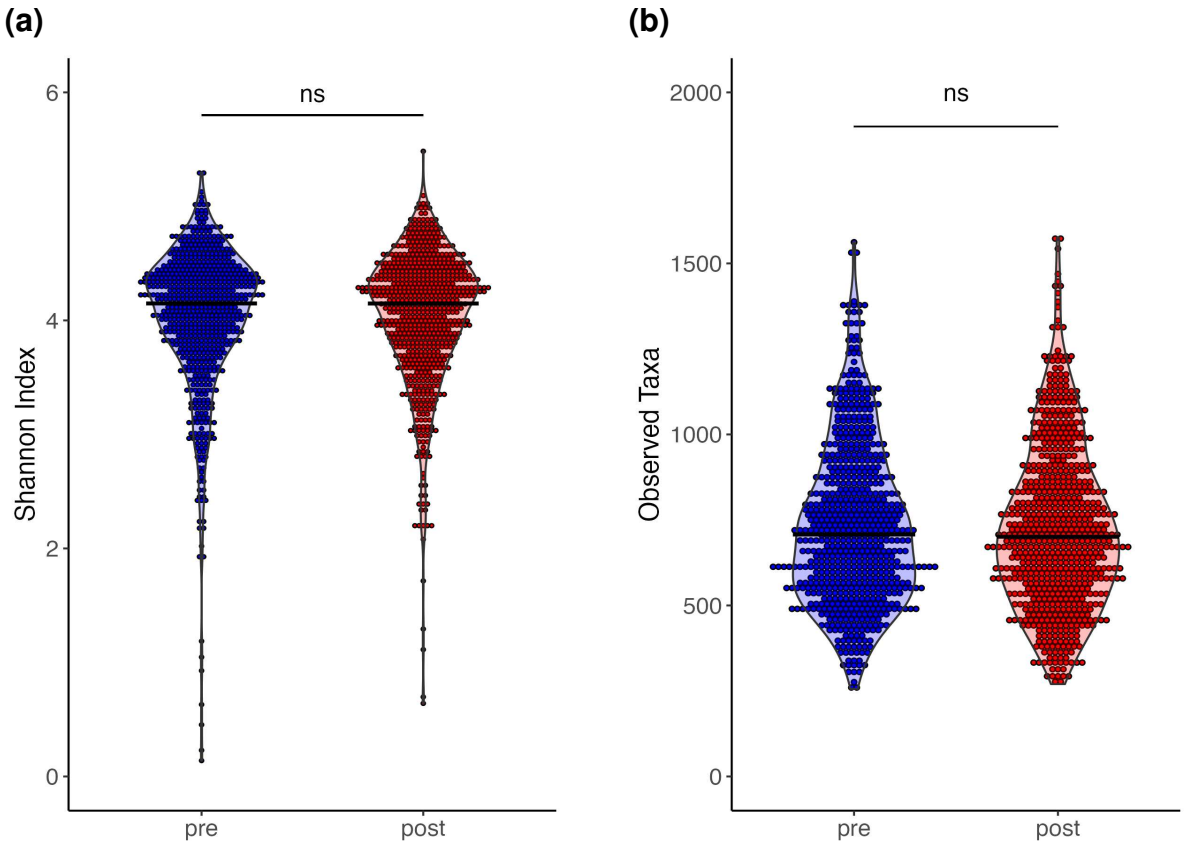

Figure 5.1: Violin plots of the Shannon Index (a) and Richness value (b) from pre- and post-retreat samples on a global scale.

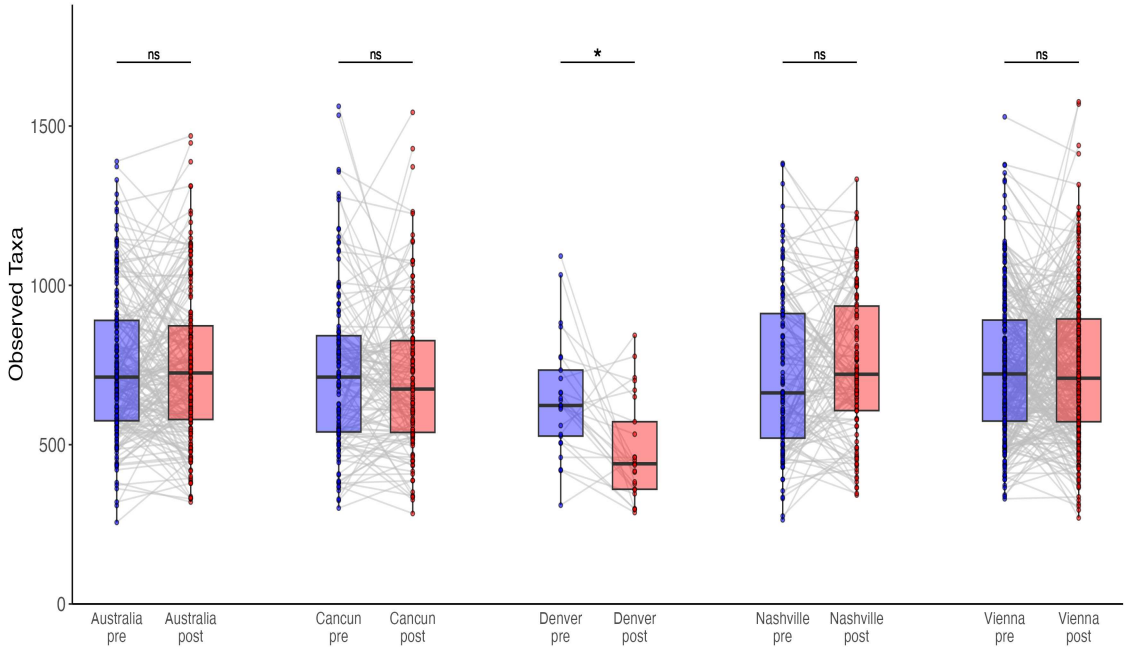

Figure 5.2: Richness values of pre- and post-retreat samples across various locations.

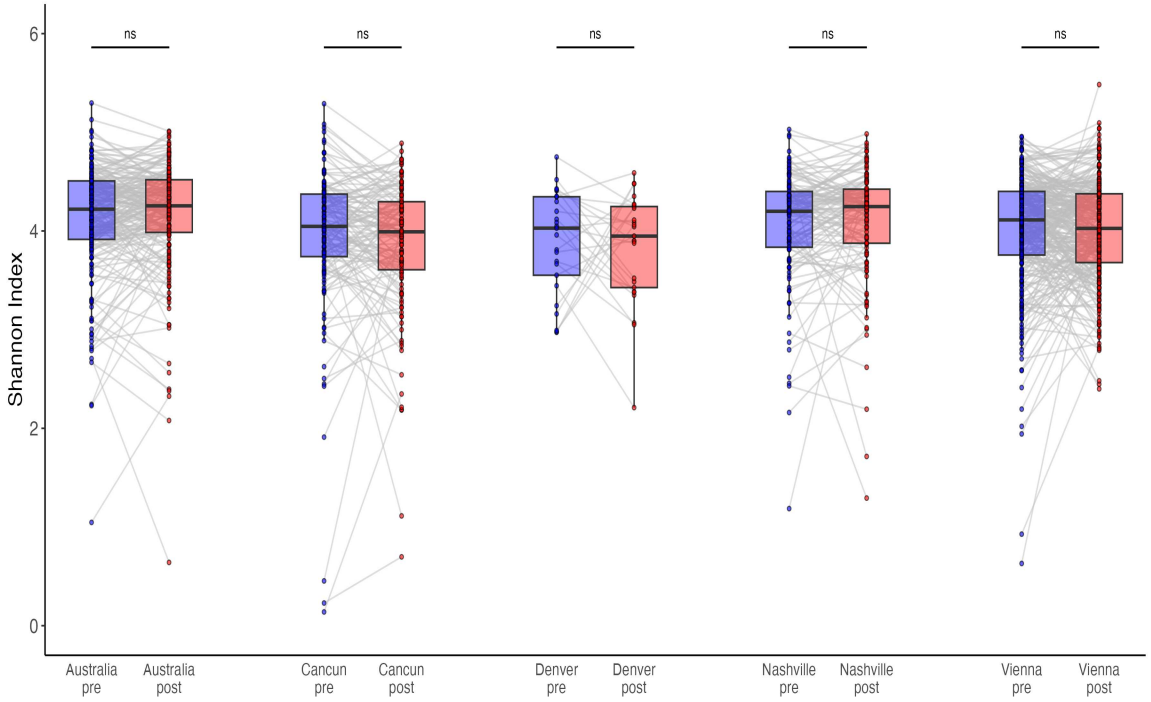

Figure 5.3: Shannon Index of pre- and post-retreat samples across various locations.

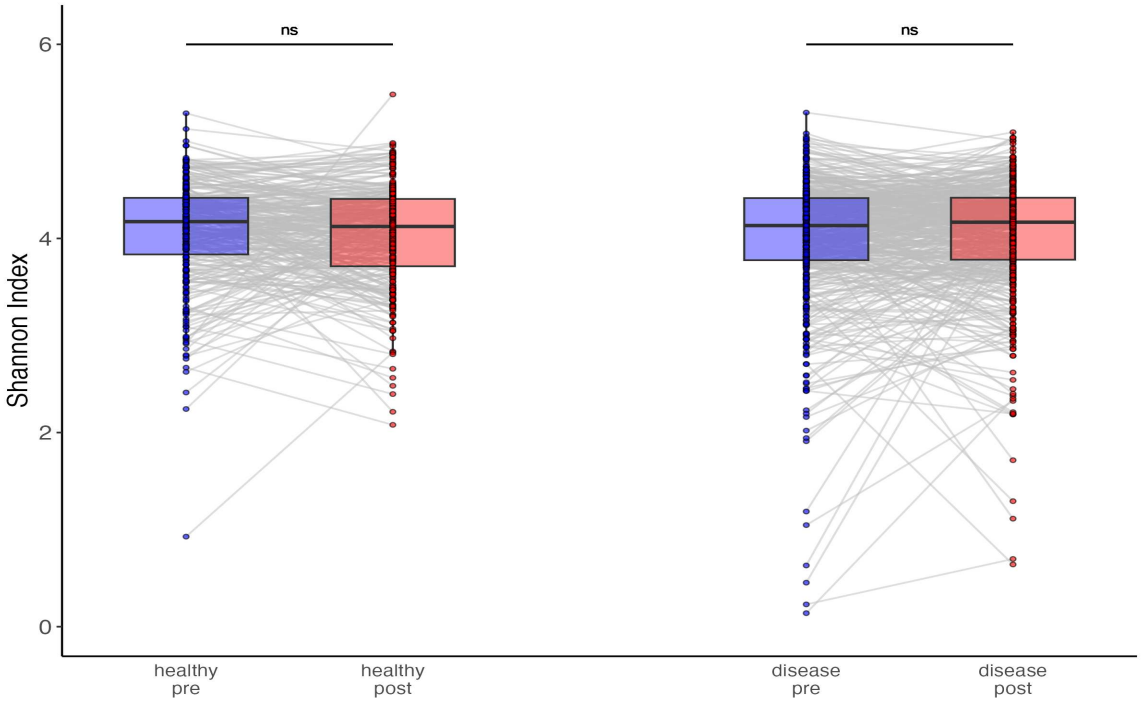

Figure 5.4: Inverse Simpson Index of pre- and post-retreat samples from healthy and sick subjects.

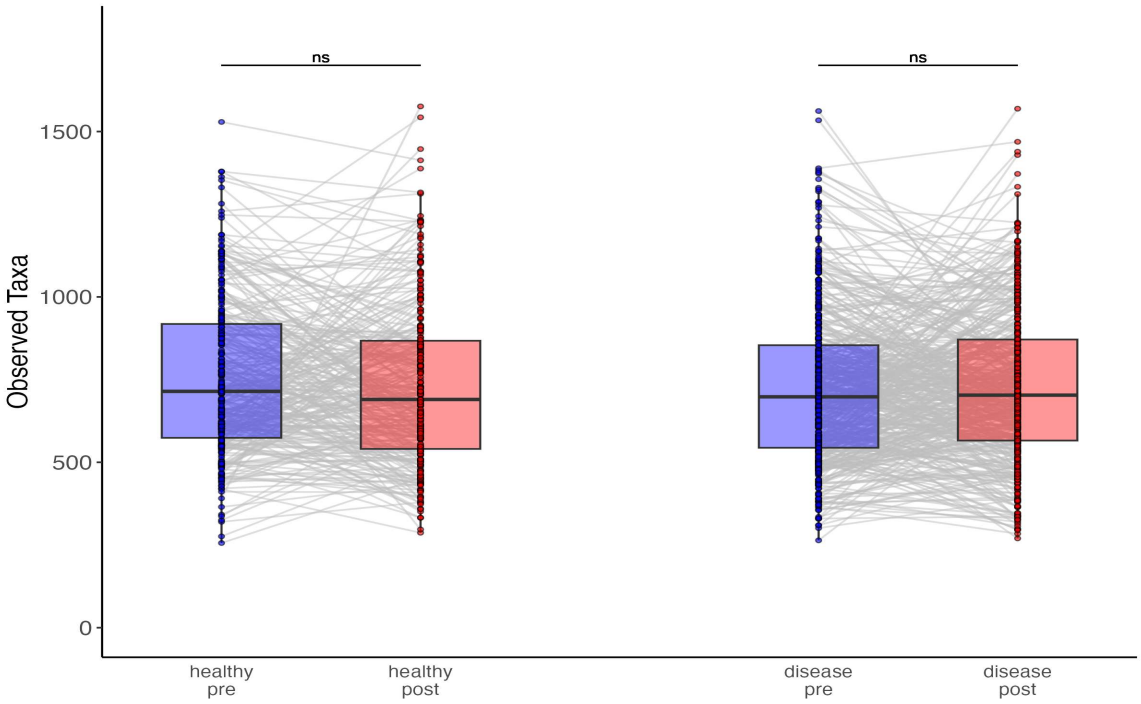

Figure 5.5: Inverse Simpson Index of pre- and post-retreat samples from healthy and sick subjects.

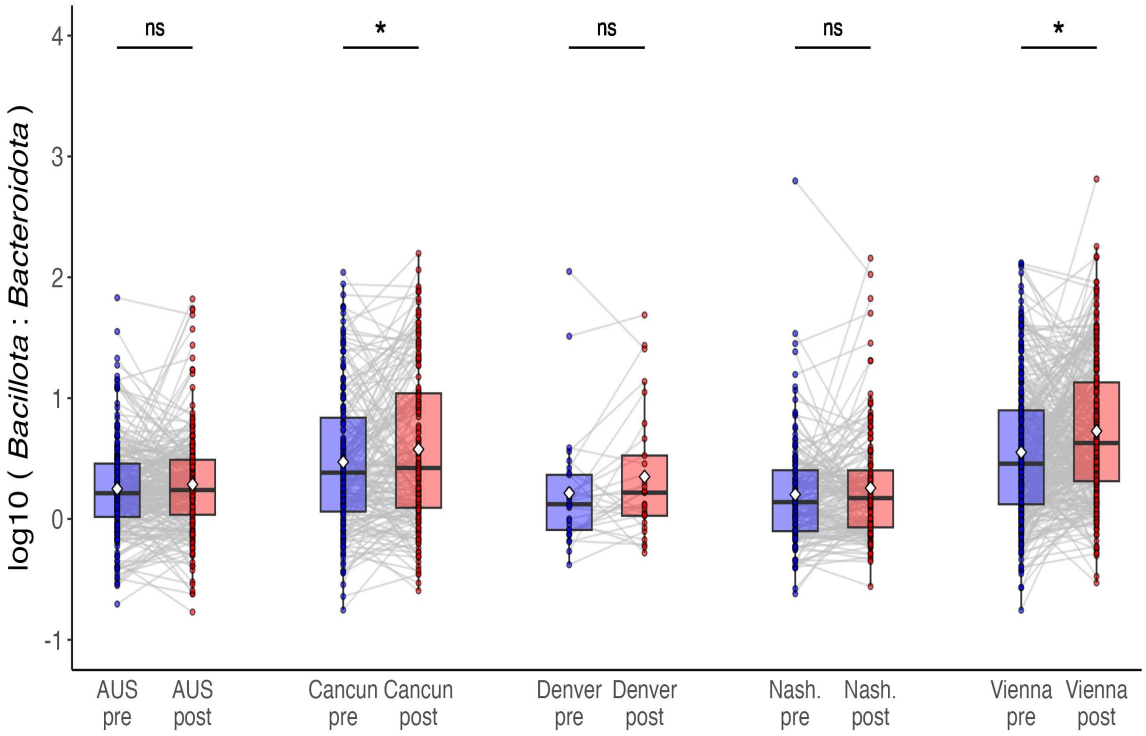

Figure 5.6: Changes in the *Bacillota* to *Bacteroidota* ratio across different locations before and after a meditation intervention.

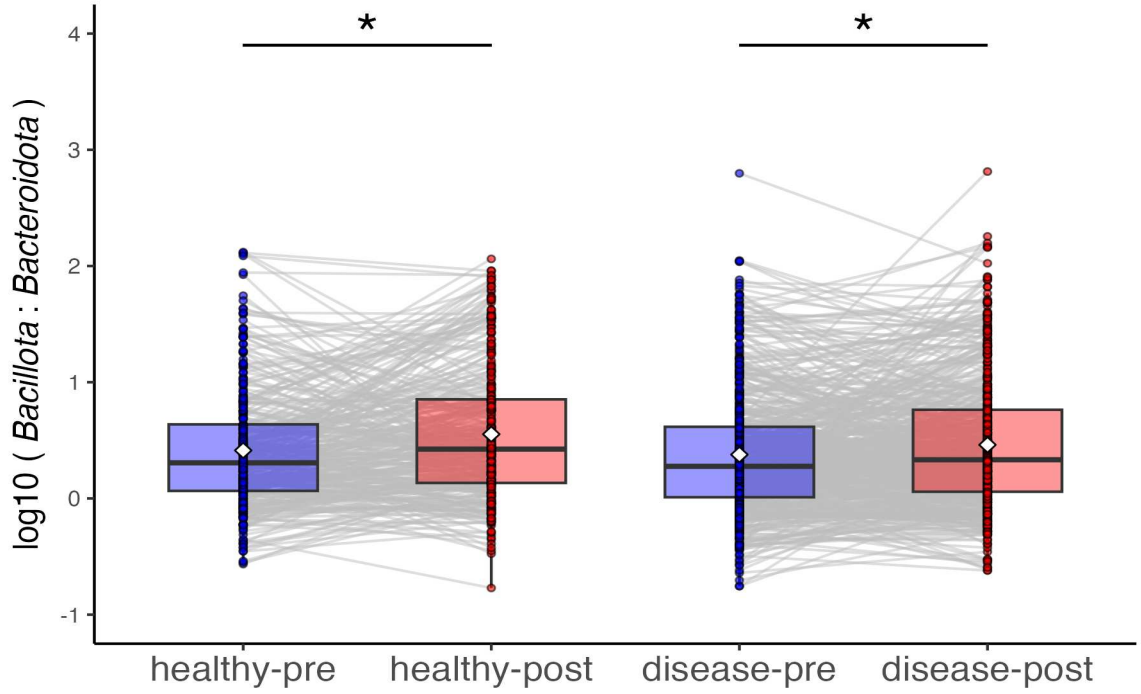

Figure 5.7: Changes in the *Bacillota* to *Bacteroidota* ratio before and after a meditation intervention for healthy and disease subjects.

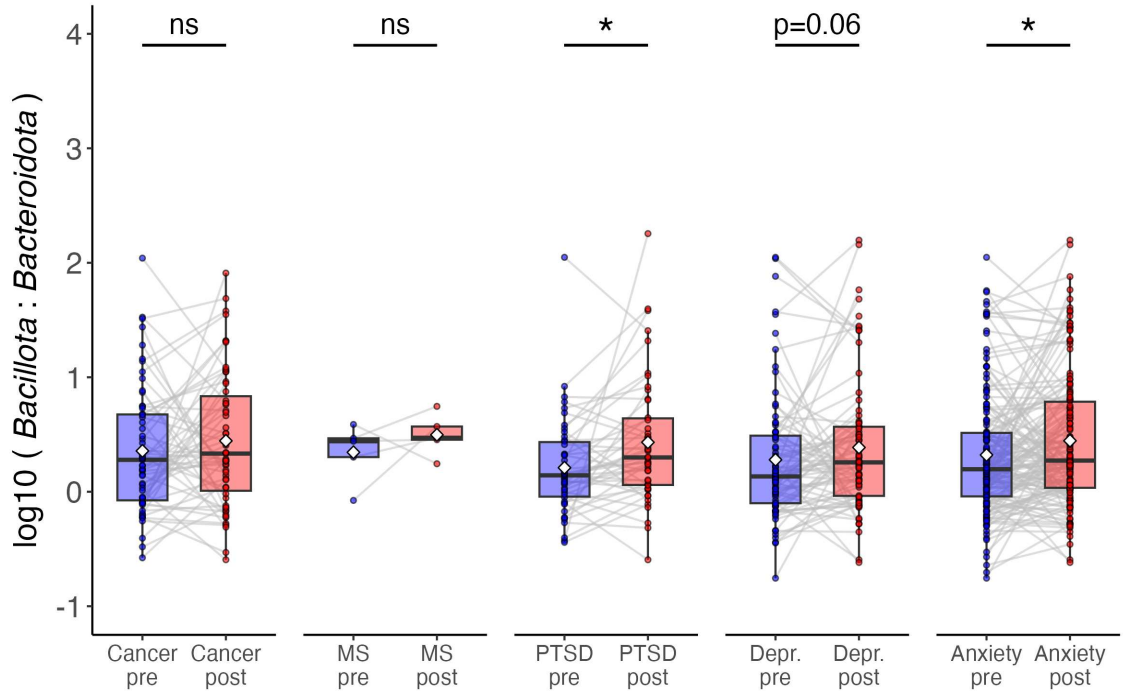

Figure 5.8: Changes in the *Bacillota* to *Bacteroidota* ratio before and after a meditation intervention for different disease groups.

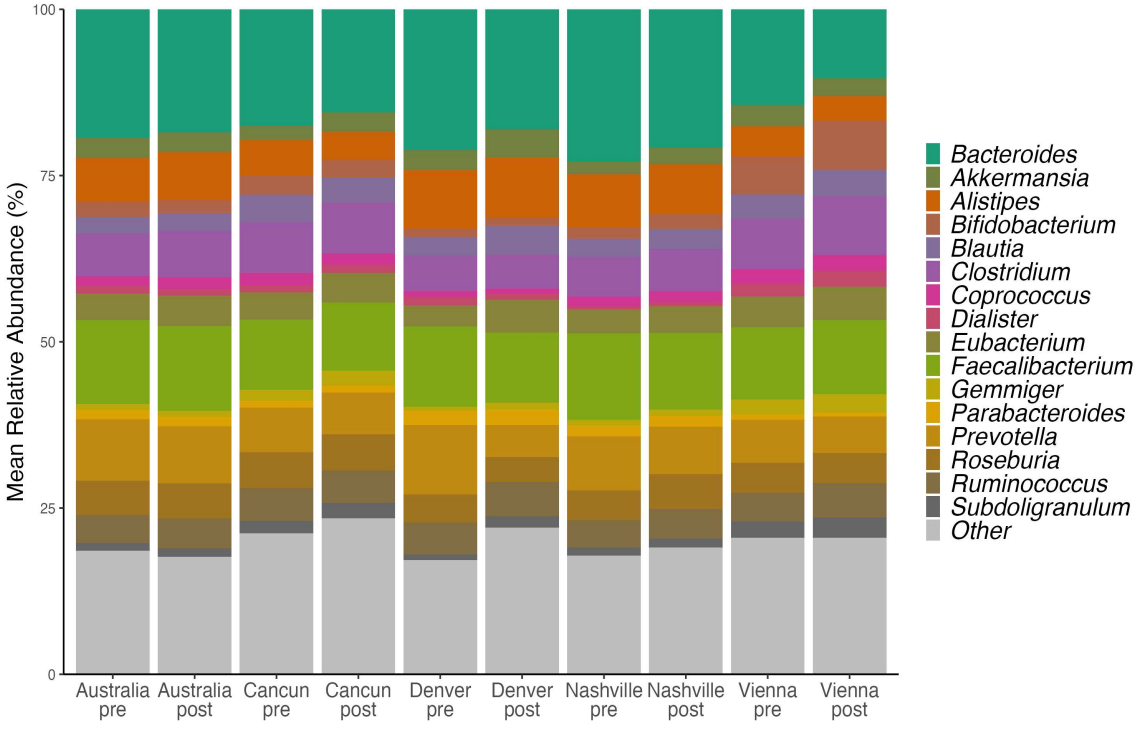

Figure 5.9: Mean relative abundances of dominant bacterial genera (>2%) before and after a meditation retreat, classified by location.

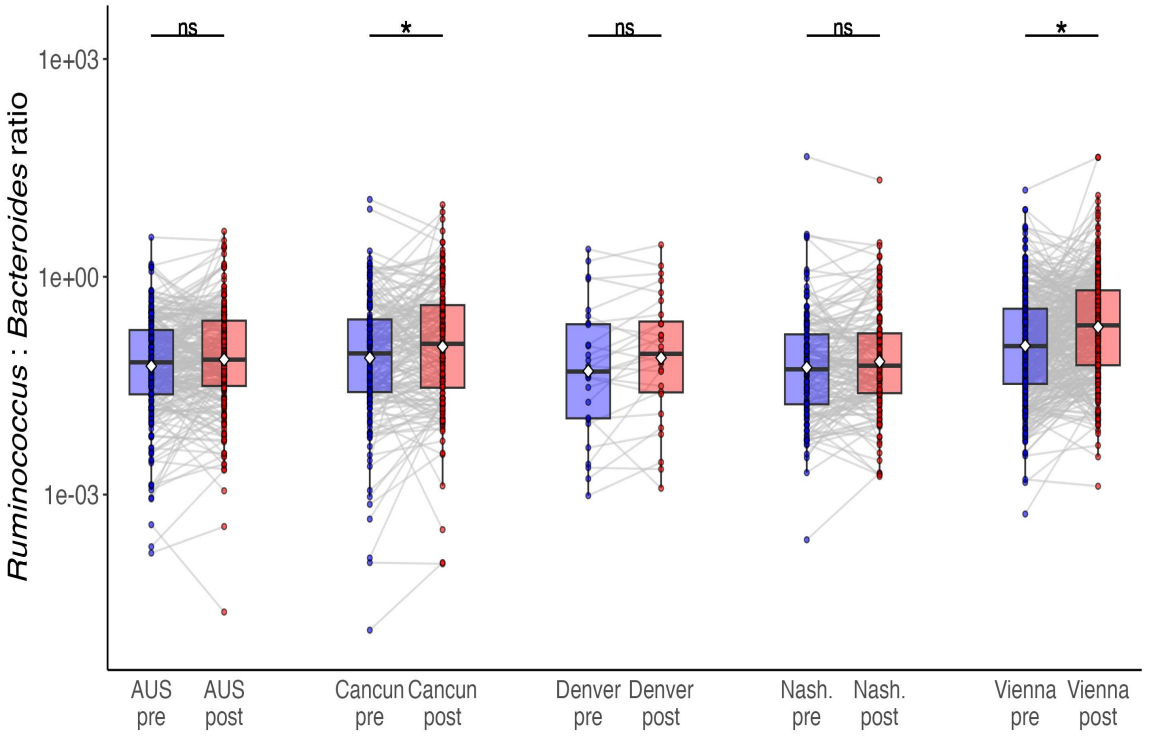

Figure 5.10: Changes in the *Ruminococcus* to *Bacteroides* ratio before and after a meditation intervention across different location.

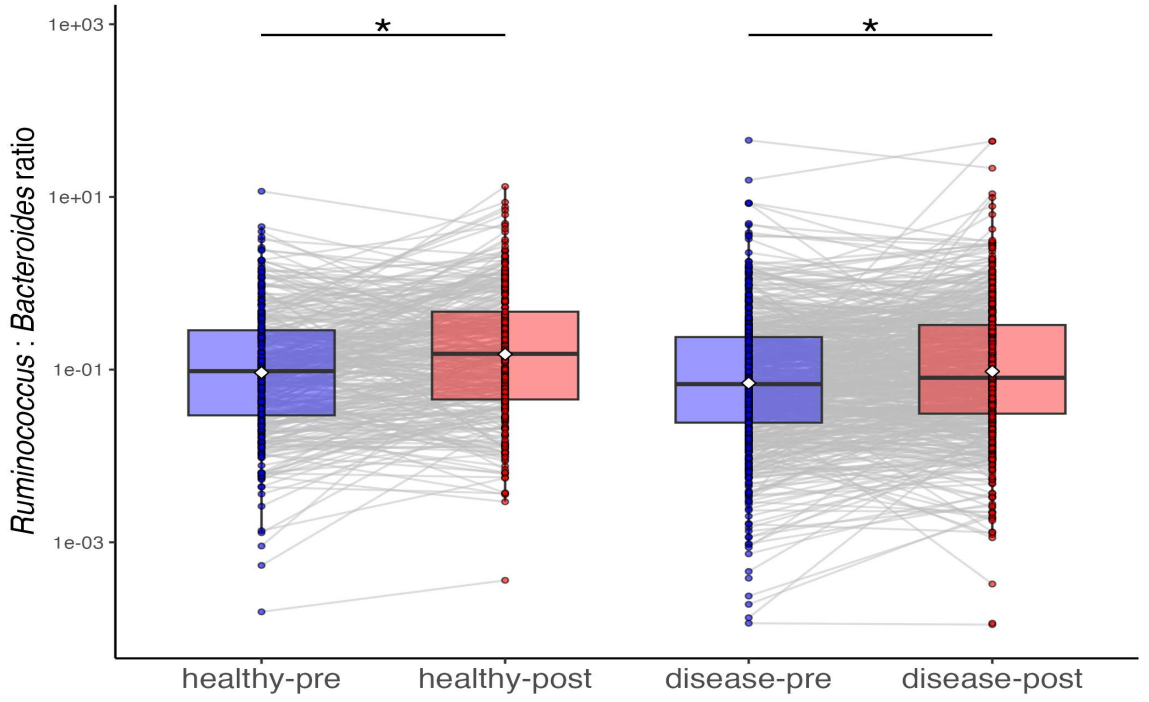

Figure 5.11: Changes in the *Ruminococcus* to *Bacteroides* ratio before and after a meditation intervention for healthy and disease subjects.

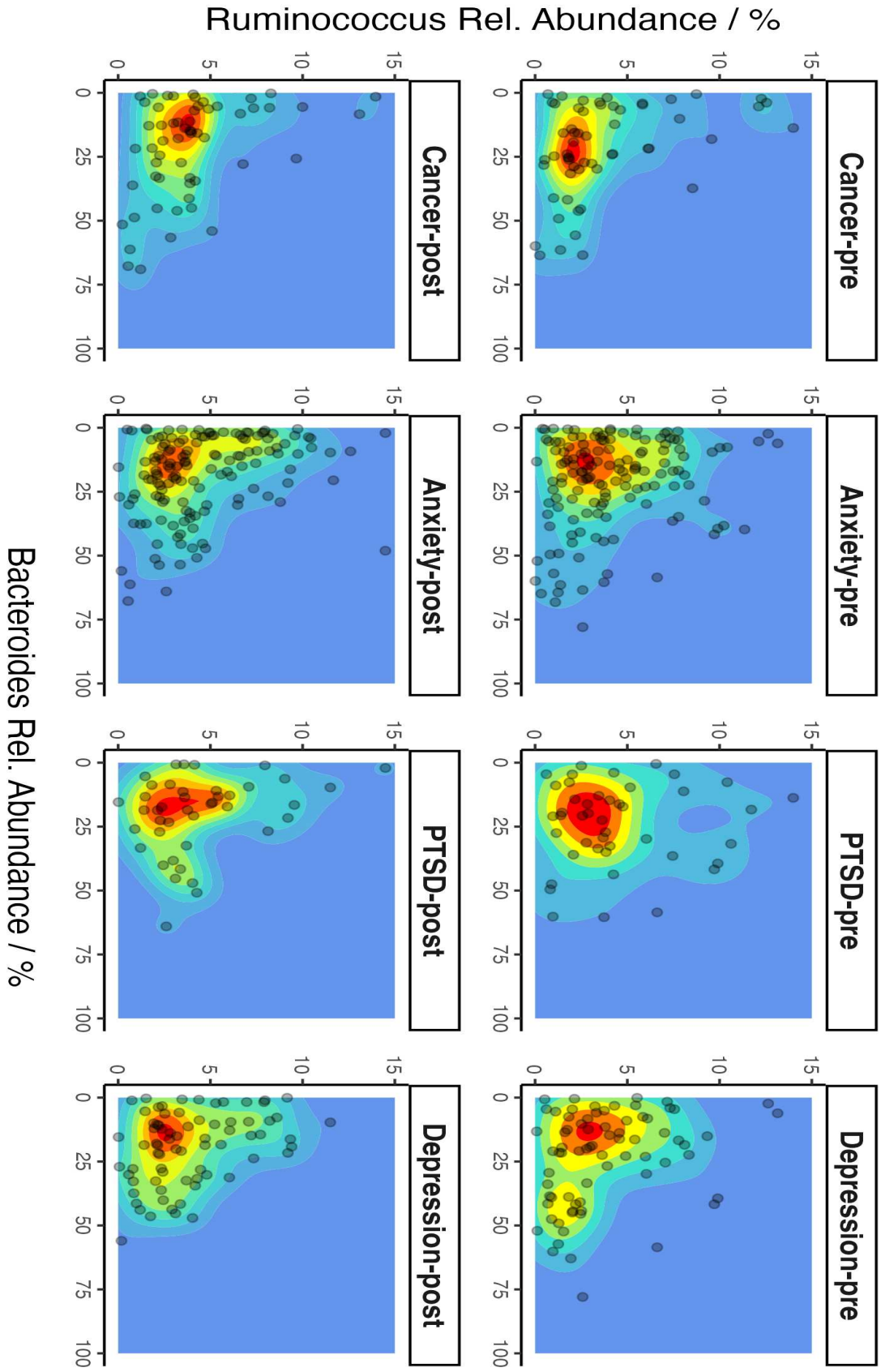

Figure 5.12: 2D contour plot showing the distribution of the *Ruminococcus* to *Bacteroides* ratio in pre- and post-retreat samples across various disease groups.

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