

The Impact of Aging on Gut Microbiota Diversity and Function: A Comparative Study Across Age Groups

Introduction

The human gut microbiota, a complex ecosystem of microorganisms residing in the gastrointestinal tract, plays a vital role in host health and disease. It contributes to various physiological processes, including digestion, immune function, and metabolism. Recent research underscores that the composition and functionality of the gut microbiome are influenced by several factors, with age being a significant determinant (1, 2).

Aging is associated with profound changes in the gut microbiota, which can affect microbial diversity and functional capabilities. Early life, middle age, and advanced age represent distinct physiological stages with varying impacts on the microbiome. In the young, gut microbiota is highly dynamic and susceptible to environmental influences and dietary changes (3). Middle age often sees stabilization of the microbiome, although lifestyle factors and health conditions may still exert considerable influence (4). In contrast, older age is characterized by decreased microbial diversity and shifts towards a less balanced microbiota composition, which is thought to contribute to age-related health issues such as frailty, cognitive decline, and increased susceptibility to infections (5, 6).

Understanding these age-related microbiome alterations is critical for elucidating their implications for health and disease. For instance, a decline in microbial diversity in older adults has been linked to reduced metabolic function and increased incidence of inflammatory conditions (7). Moreover, age-related shifts in microbiota composition can impact the efficacy of therapeutic interventions and contribute to the pathogenesis of age-associated diseases (8).

In this study, we conducted a comparative analysis of gut microbiota across three distinct age groups: young adults, middle-aged individuals, and older adults. Utilizing comprehensive methodologies—including taxonomic profiling, functional analysis, and diversity assessments—we aim to elucidate how aging affects microbial diversity and functionality. Our approach integrates differential abundance analysis, alpha and beta diversity metrics, and phylogenetic tree construction to provide an in-depth understanding of age-related microbiome dynamics.

This research contributes to the growing body of literature on gut microbiota and aging, offering insights into how age influences microbial ecosystems and their functional outcomes. By bridging microbiome science with gerontology, our findings have the potential to inform strategies for maintaining gut health throughout the lifespan and addressing age-related health challenges (9, 10).

Materials and Methods

Data Acquisition

Gut microbiota sequence data were obtained from the National Center for Biotechnology Information (NCBI) database. Specifically, we accessed publicly available datasets from NCBI's Sequence Read Archive (SRA) corresponding to three distinct age groups: young adults, middle-aged individuals, and older adults. The datasets were selected based on their relevance and quality, as indicated by accompanying metadata and initial quality assessments.

Data Processing and Quality Control

The raw sequence data were retrieved from the NCBI SRA using the **sratoolkit** ([version 2.11.0](#)). Following retrieval, data quality was assessed using **FastQC** (version 0.11.9). Sequences were then trimmed and filtered to remove low-quality reads and adapter sequences using **Trimmomatic** (version 0.39).

Data Integration and Analysis

Taxonomic Profiling:

Kraken2 (version 2.1.2) was utilized for taxonomic classification of the microbial sequences.

The taxonomic results were visualized with **Krona** ([version 2.8](#)), generating interactive pie charts to represent the microbial composition.

Functional Profiling:

PICRUSt2 ([version 2.4.0-b](#)) was used to predict the functional content of the microbiota based on 16S rRNA gene sequences.

HUMAnN3 ([version 3.0.0](#)) was employed for profiling the functional capabilities of the microbiota, including pathway and gene family abundances.

Metagenomic Assembly:

MetaSPAdes ([version 3.15.3](#)) was used for metagenomic assembly to reconstruct microbial genomes from the sequencing data.

Denoising and Feature Table Construction:

DADA2 ([version 1.24.0](#)) was used for denoising the raw sequencing data and constructing amplicon sequence variant (ASV) tables.

Diversity and Differential Abundance Analysis:

QIIME 2 ([version 2023.2](#)) was used to analyze microbial diversity, including alpha and beta diversity metrics.

ANCOM ([version 2.1](#)) was employed to assess differential abundance across the different age groups.

Statistical and Graphical Analysis

Statistical analyses and data visualization were performed using **R** ([version 4.3.1](#)). Key packages included:

- **ggplot2** ([version 3.4.2](#)) for creating detailed and customizable plots.
- **phyloseq** ([version 1.42.0](#)) for handling and analyzing microbiome data.
- **vegan** ([version 2.6-4](#)) for diversity analysis and ecological statistics.

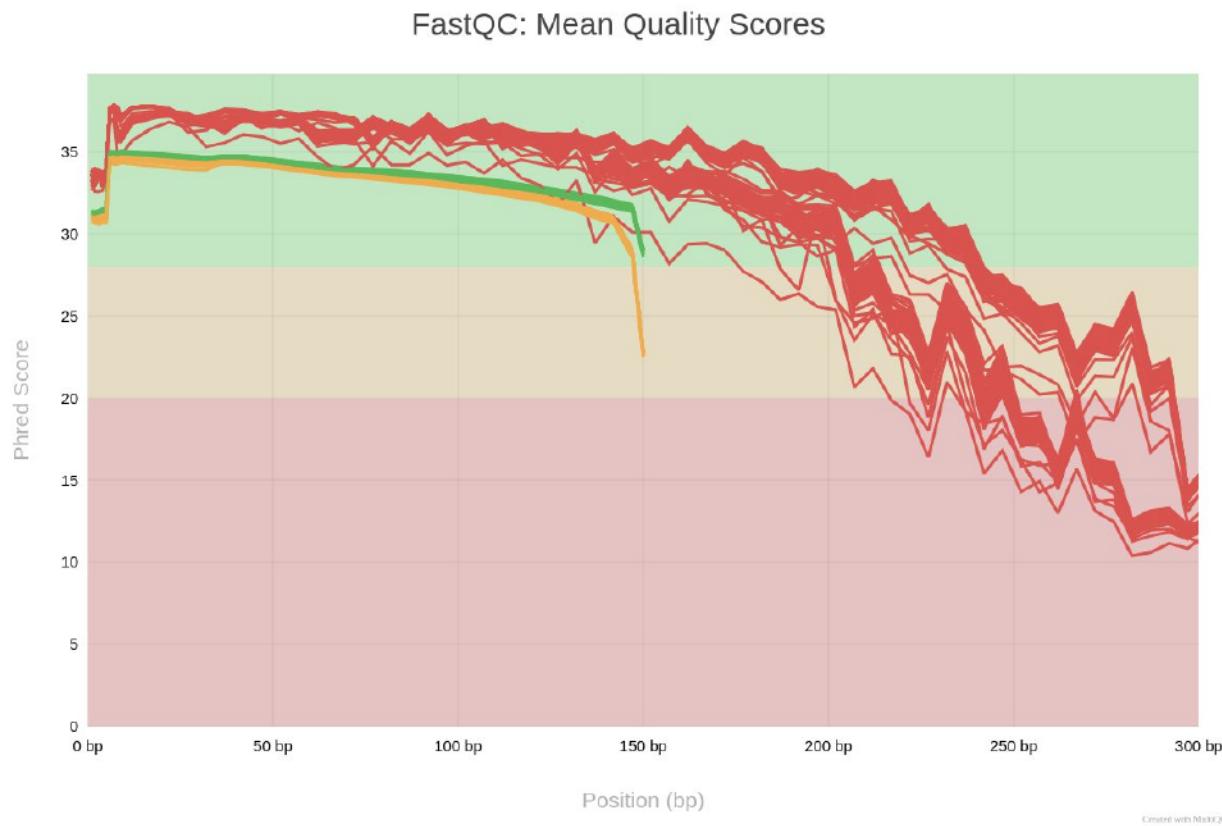
Python ([version 3.11.4](#)) was used with the **Biopython** module ([version 1.82](#)) for sequence data handling and additional analyses, including sequence manipulation and parsing.

Integration and Reporting

MultiQC ([version 1.14](#)) was used to aggregate and visualize quality control metrics from multiple analysis steps. The final dataset was carefully compiled to ensure consistency and accuracy across all analytical stages.

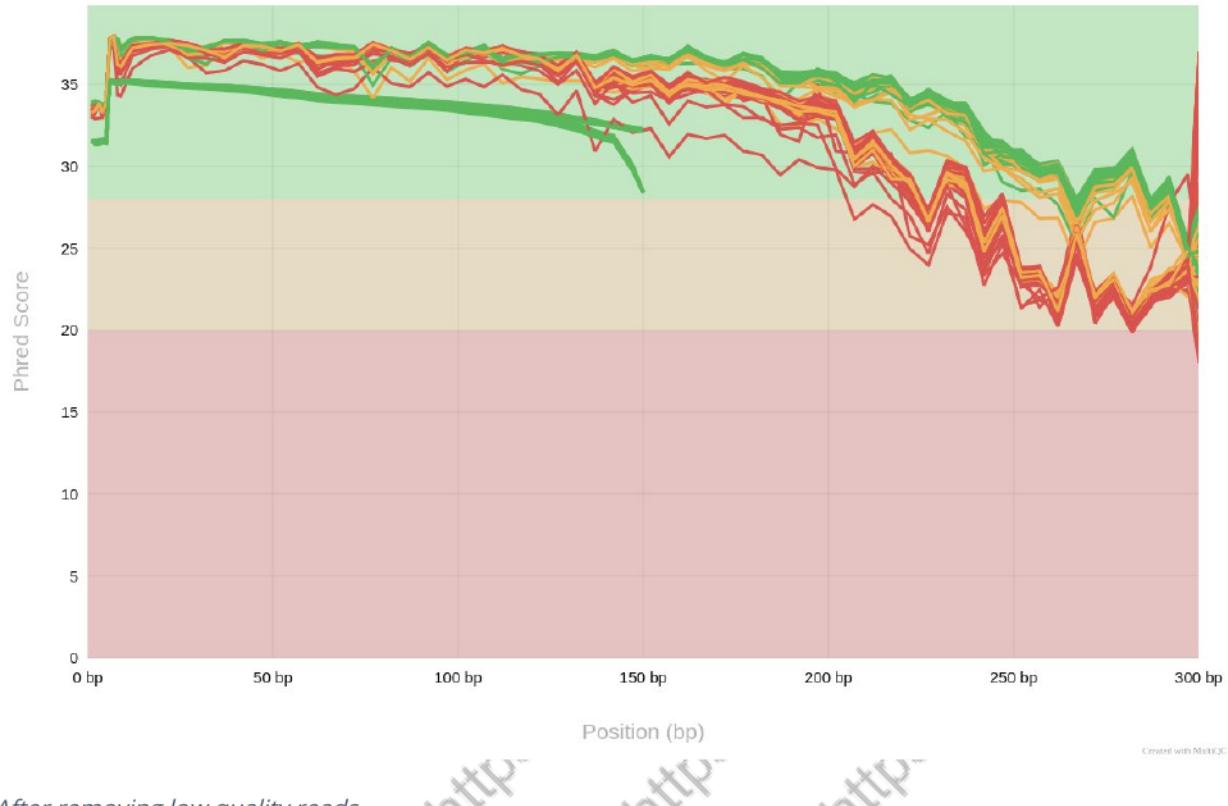
Results and Discussion

MultiQC Report: Quality Control Overview



Created with MultiQC

FastQC: Mean Quality Scores



After removing low quality reads

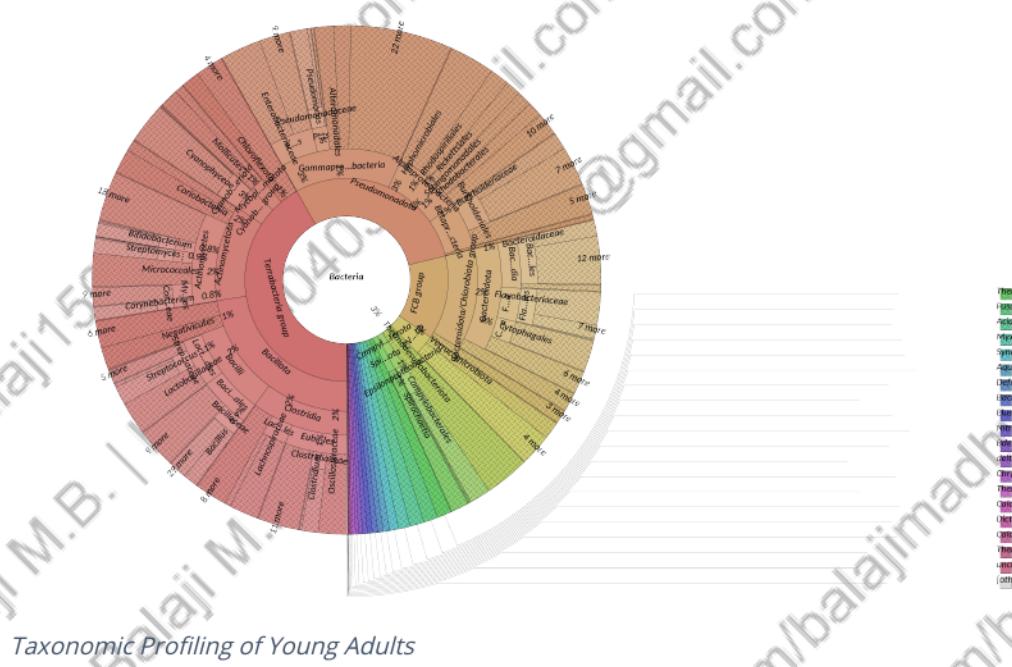


Figure X: Taxonomic Composition of Human Gut Microbiota in Young Individuals

Taxonomic Profiling of Young Adults

This figure presents the taxonomic composition of the gut microbiota derived from a group of 15 young individuals. The radial diagram highlights the diversity and hierarchical

relationships among microbial taxa within this age group. Each segment represents a distinct taxonomic group, with color variations indicating the relative abundance and diversity of microbial species in the gut. This visualization provides a foundational reference for subsequent comparisons with middle-aged and older populations.

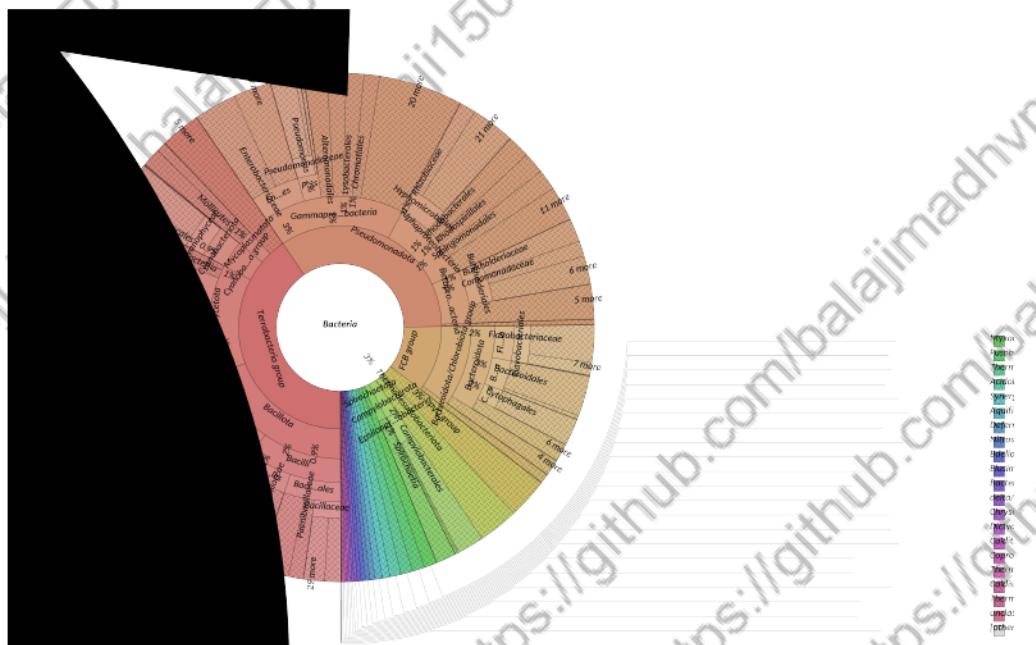


Figure X: Taxonomic Composition of Human Gut Microbiota in Middle-Aged Individuals

This figure illustrates the taxonomic composition of the gut microbiota from a group of 13 middle-aged individuals. The radial diagram displays the diversity and hierarchical relationships among microbial taxa within this age group. Each segment corresponds to a distinct taxonomic category, with color coding representing the relative abundance and diversity of microbial species. This visualization serves as a comparative reference for examining changes in gut microbiota composition across different age groups, including future comparisons with young and older populations.

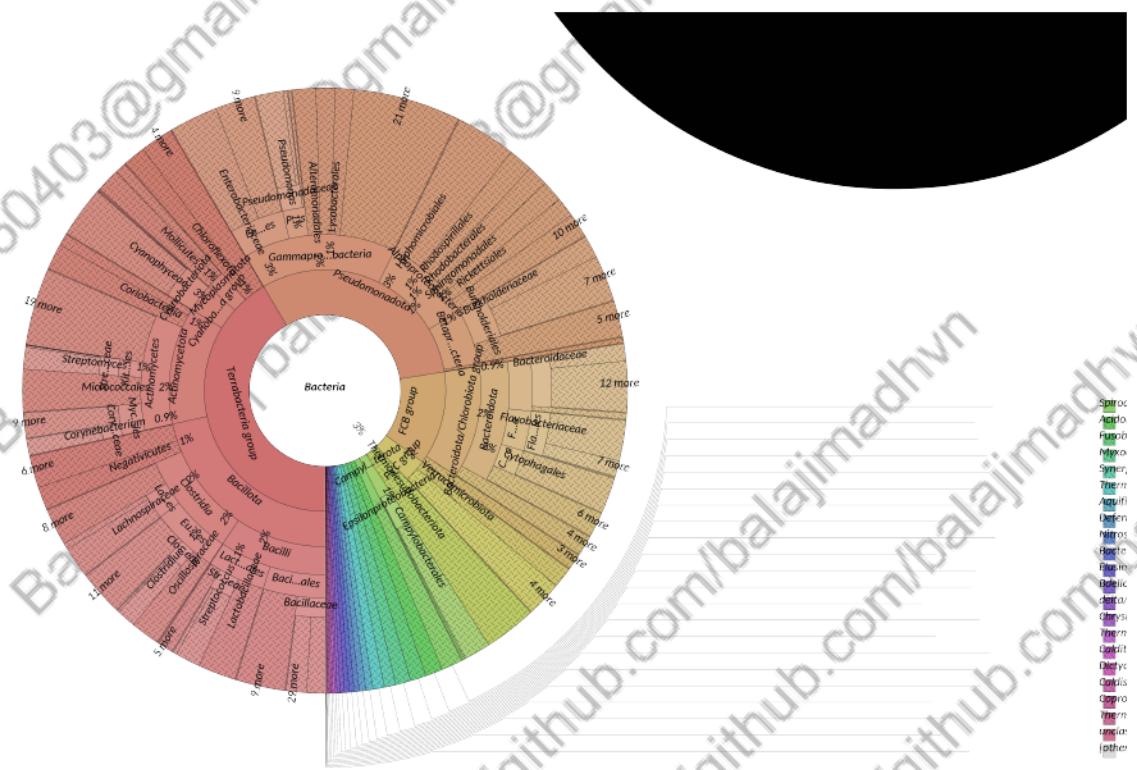


Figure X: Taxonomic Composition of Human Gut Microbiota in Older-Aged Individuals

This figure illustrates the taxonomic composition of the gut microbiota from a group of 48 older individuals. The radial diagram displays the diversity and hierarchical relationships among microbial taxa within this age group. Each segment corresponds to a distinct taxonomic category, with color coding representing the relative abundance and diversity of microbial species. This visualization serves as a critical reference for examining changes in gut microbiota composition across different age groups, including comparisons with young and middle-aged populations.

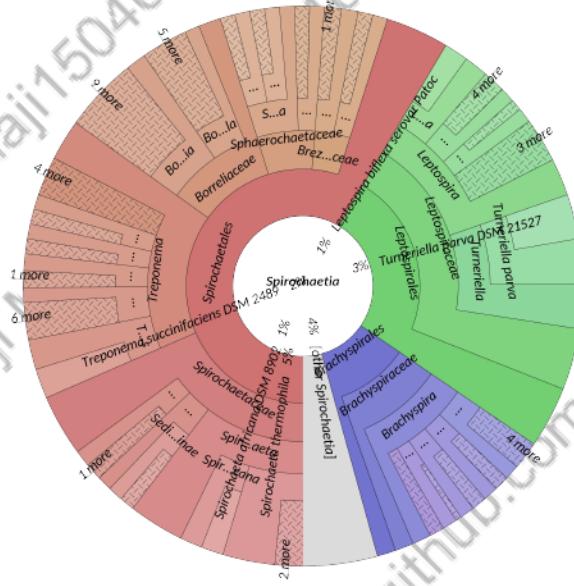
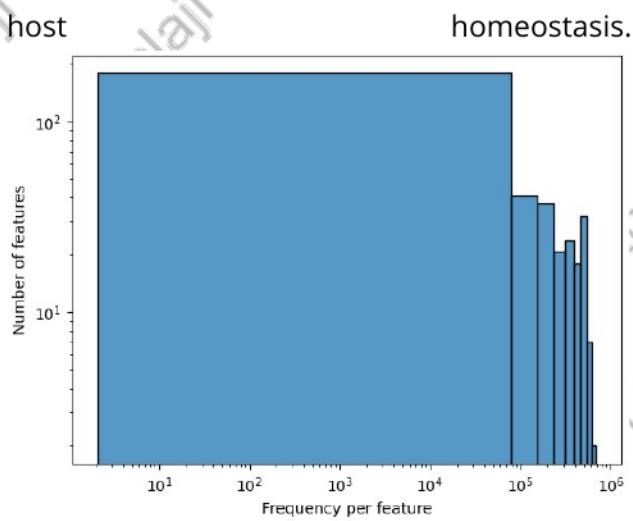


Figure X: Taxonomic Composition in Older-Aged Individuals - Spirochaetota

In the given analysis, the image indicates that Spirochaetota comprises 1% of the microbial composition in the older age group, a notably higher percentage compared to the younger and middle-aged cohorts. This increased prevalence of Spirochaetota in older individuals may be attributed to age-related changes in the gut environment, such as alterations in immune function, diet, and gut motility, which can create a more favorable niche for these microorganisms. Additionally, the aging process often involves a shift in the gut microbiome, potentially allowing Spirochaetota to thrive more readily in older adults.

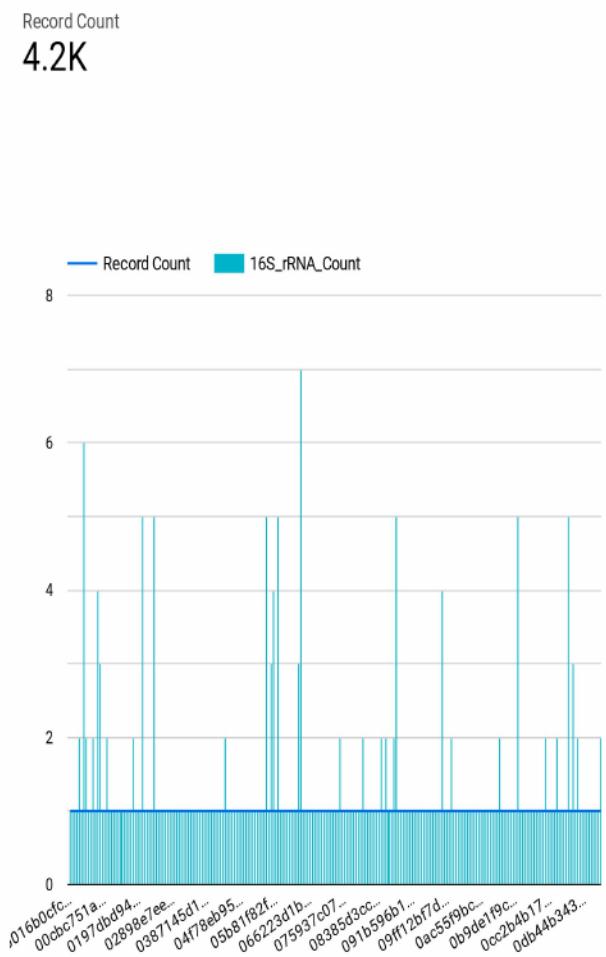
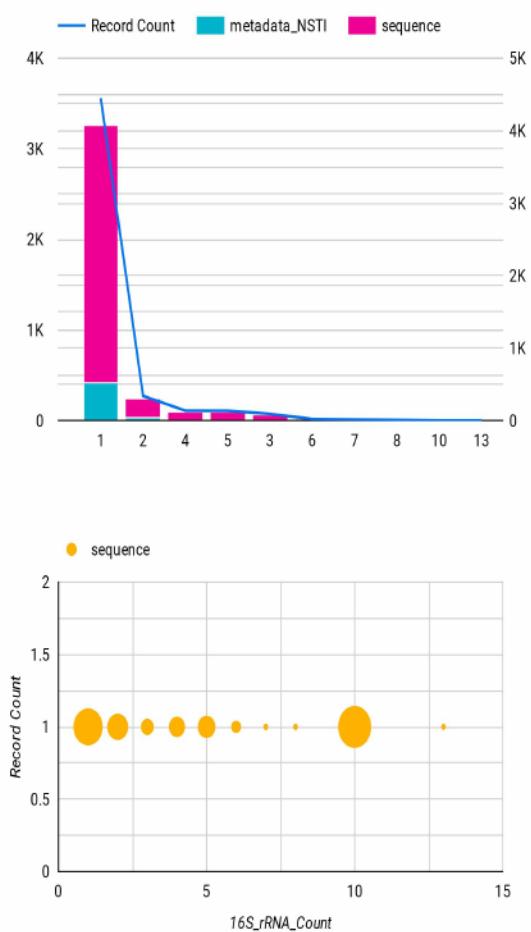


The KEGG pathway analysis of the human gut microbiome reveals the functional capabilities encoded by microbial genes, allowing for the identification of key metabolic pathways involved in host-microbe interactions. By quantifying pathway abundance, researchers can assess the contributions of the gut microbiota to processes such as carbohydrate metabolism, amino acid synthesis, and lipid metabolism, which are crucial for maintaining



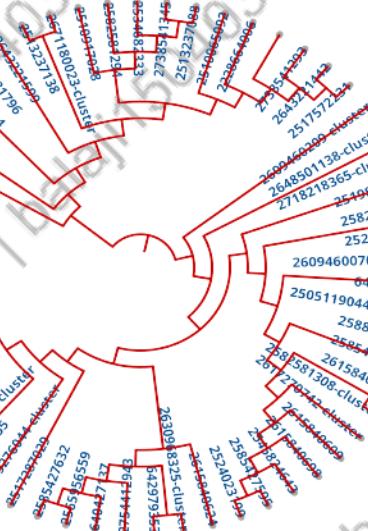
In particular, this analysis enables the detection of functional shifts in the gut microbiome associated with various factors such as age, diet, or disease. For instance, an increased abundance of pathways related to short-chain fatty acid production may be linked to beneficial metabolic effects, while changes in pathways related to bile acid metabolism could indicate alterations in gut microbial composition associated with metabolic disorders.

NSTI Abundance Analysis for Human Gut Microbiome



In human gut microbiome studies, the Nearest Sequence Taxon Index (NSTI) is a critical metric for evaluating the reliability of functional predictions derived from 16S rRNA gene sequences using tools like PICRUSt. NSTI values measure the evolutionary distance between query sequences and the closest reference sequences, with lower scores indicating higher confidence in the predicted functional profiles. This is particularly relevant for understanding microbial contributions to health and disease, as accurate functional predictions can reveal insights into metabolic functions, nutrient processing, and disease associations. Analyzing NSTI scores alongside functional abundances helps differentiate between robust predictions and those with less reliability, thus enhancing the accuracy of microbiome studies and their implications for human health.

Phylogenetic Tree Representation of Gut Microbiome Diversity

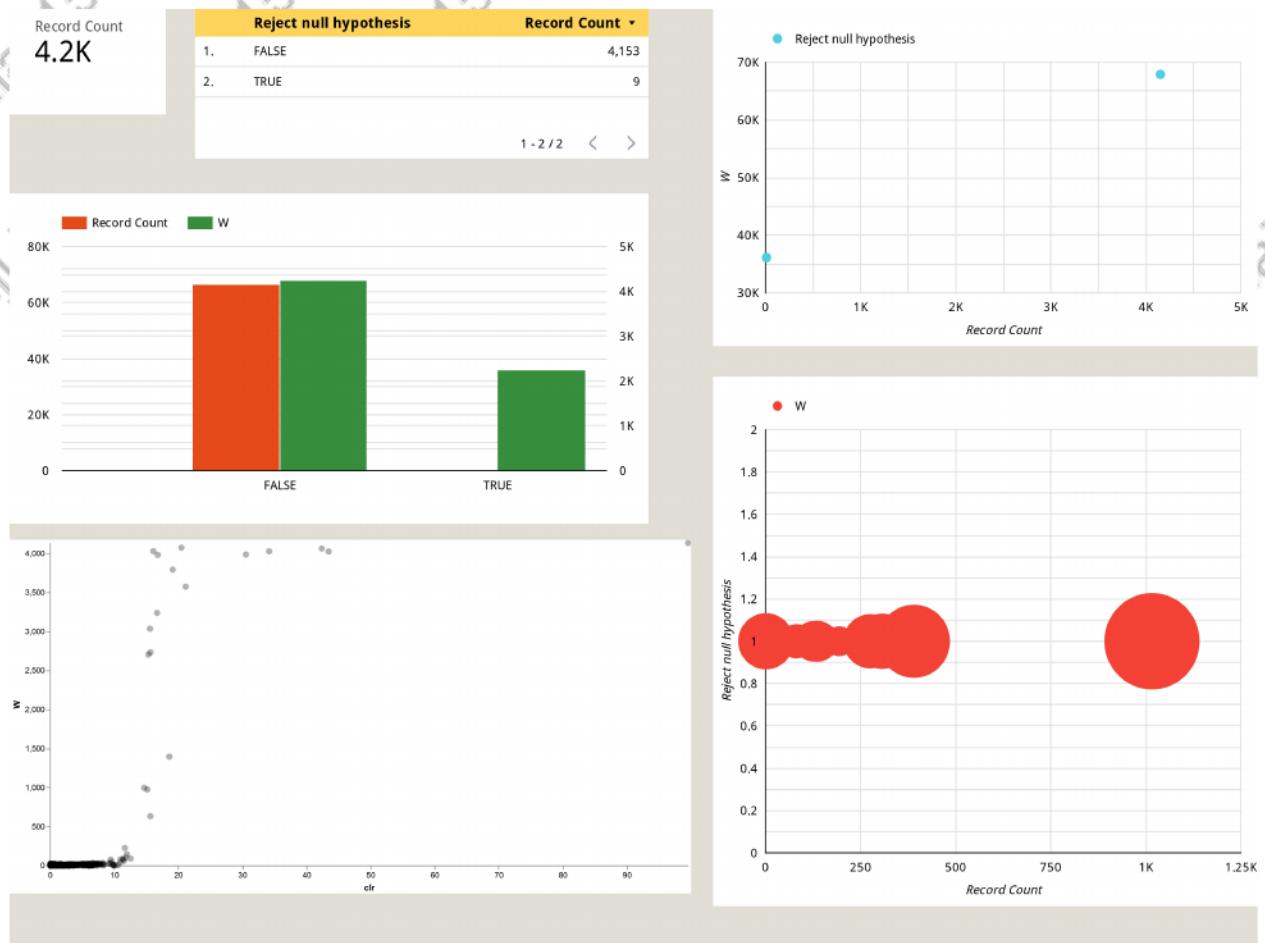


The phylogenetic tree depicted in the circular image represents a condensed visualization of the complex relationships among microbial taxa identified across the 76 samples studied. This tree illustrates the hierarchical clustering and evolutionary distances among different microbial species, with branches indicating taxonomic similarities and differences. The circular format offers a compact yet comprehensive view, emphasizing key nodes that denote significant clusters or lineage divergences within the gut microbiome. The full phylogenetic tree, though not displayed in its entirety due to its complexity, underpins the broader diversity and structure of the microbiome. Analyzing these phylogenetic relationships helps elucidate patterns of microbial diversity, identify core microbiome components, and understand the functional implications of microbial interactions in the gut ecosystem. This approach is crucial for revealing insights into how microbial community structures correlate with health and disease states, highlighting the dynamic nature of gut microbiome composition and its impact on human physiology.

Differential Abundance Analysis

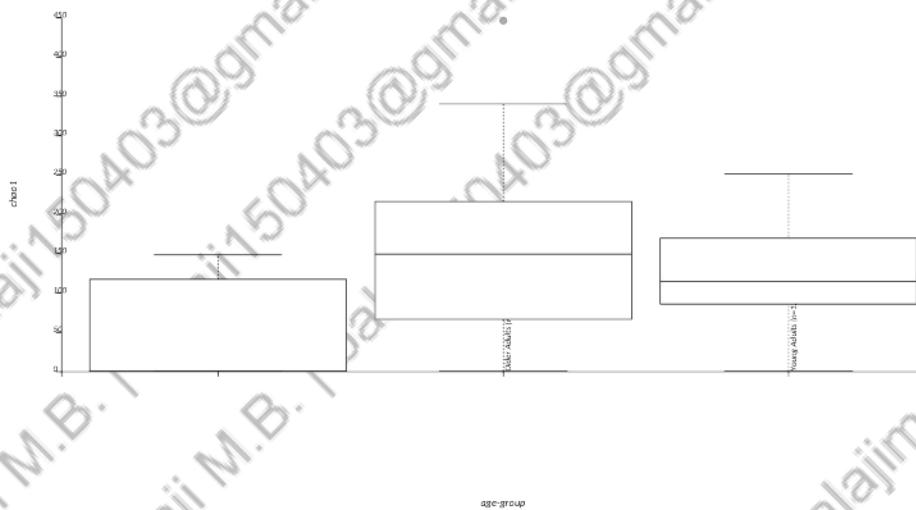
ANCOM is a robust statistical framework designed to address the challenges of compositional data, which is particularly relevant for microbiome studies where the relative abundances of taxa are measured. This approach enables us to determine which microbial taxa are differentially abundant between groups, providing insights into how specific bacteria may be associated with various health conditions or environmental factors. The results of this analysis reveal taxa that exhibit statistically significant changes in abundance, highlighting potential biomarkers or microbial shifts linked to the gut microbiome's

functional dynamics. This differential abundance analysis is instrumental in understanding the relationships between microbial community composition and host health, offering a detailed view of how microbial populations interact and vary in different conditions.



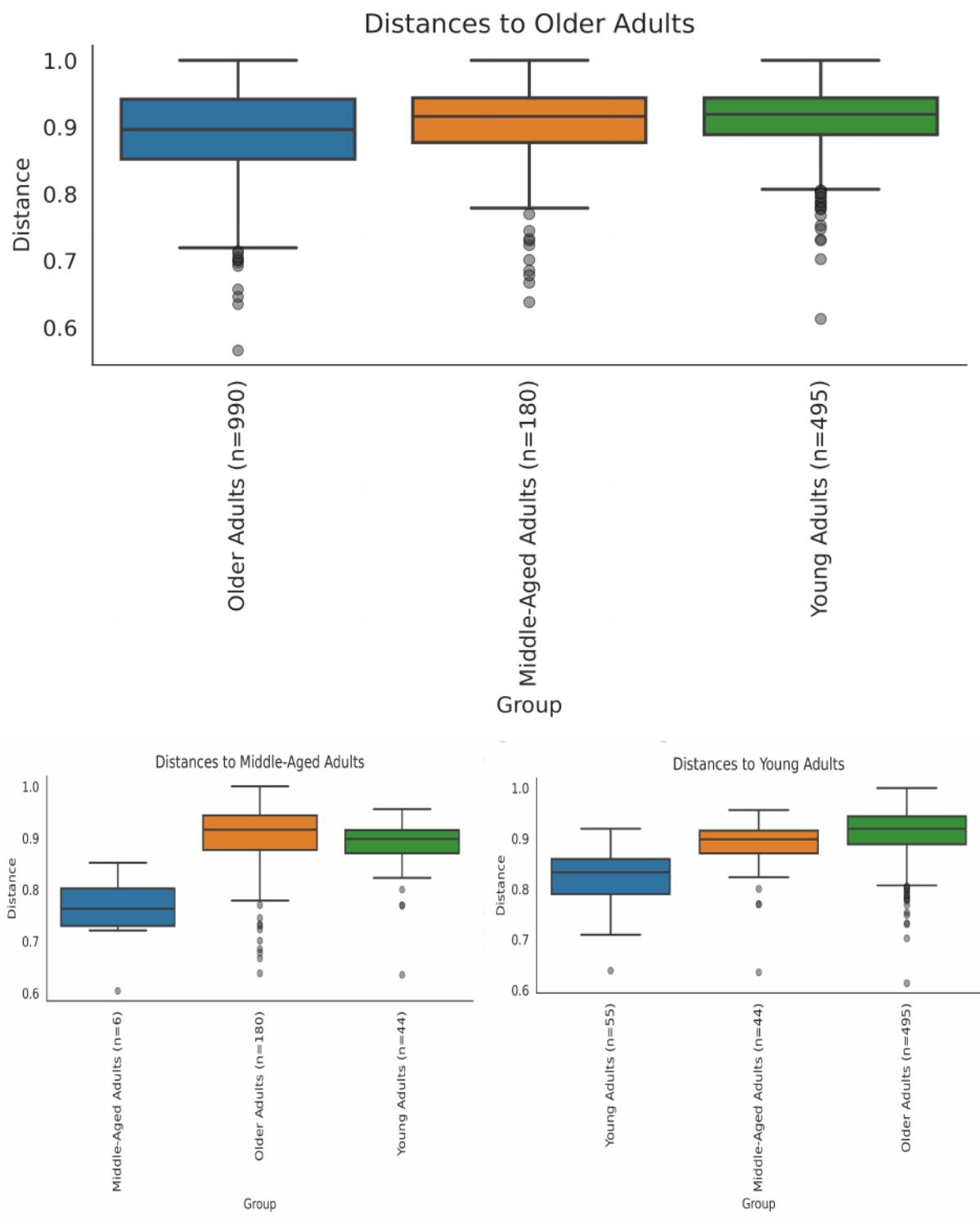
Diversity Analysis of Human Gut Microbiota Across Age Groups

Alpha Diversity Analysis of Human Gut Microbiota Using Shannon metrics



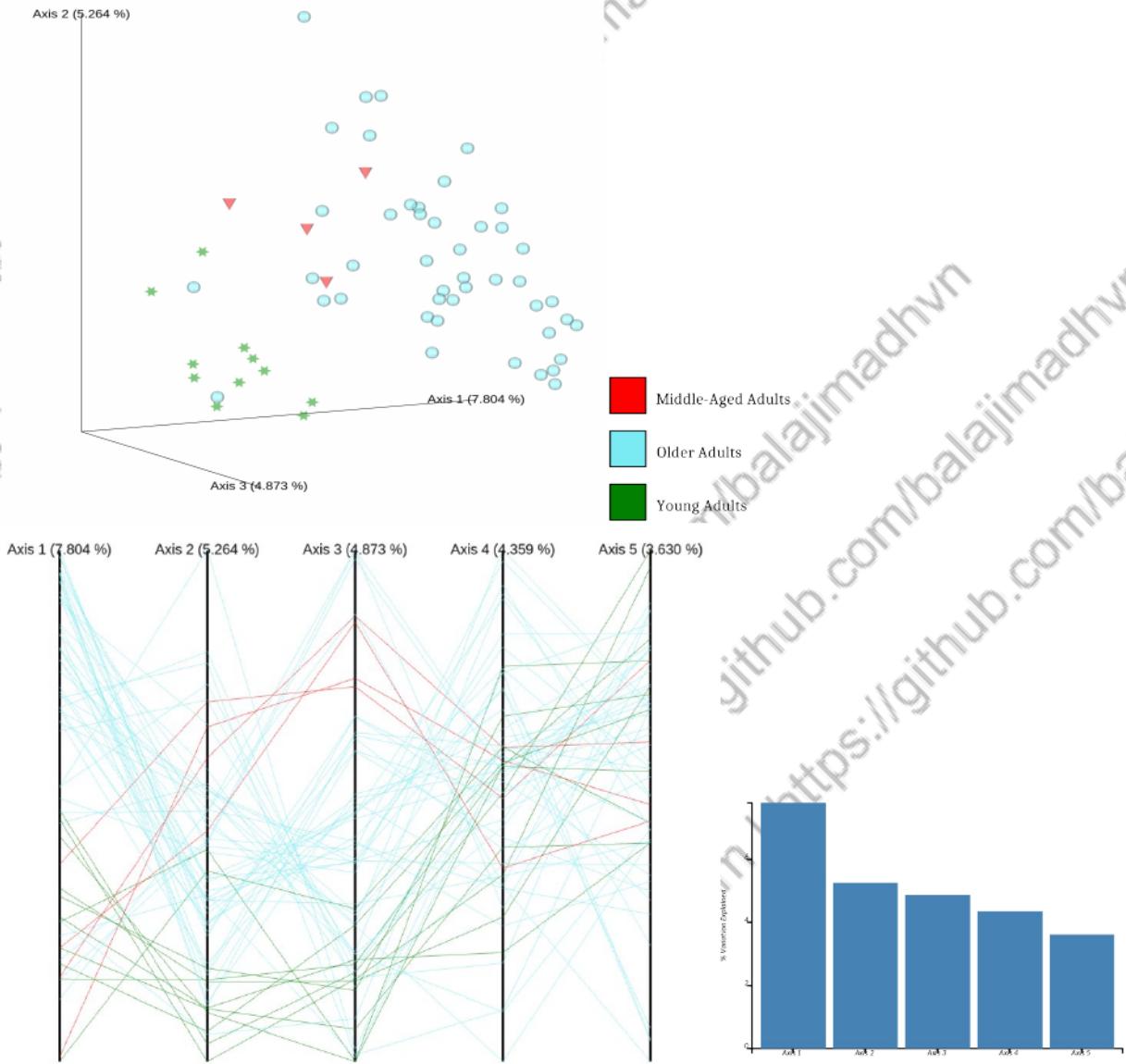
Alpha diversity, which measures the richness and evenness of microbial species within a single sample, is crucial for understanding the gut ecosystem's stability and health. Variations in alpha diversity across age groups can reflect age-related shifts in gut microbiota composition and function, potentially influencing overall health and disease susceptibility. By examining these diversity metrics, researchers can delve into how microbial diversity contributes to the ultimate maintenance of gut homeostasis and its implications for age-associated health outcomes.

Beta Diversity Analysis of Human Gut Microbiota Using Bray-Curtis Distances



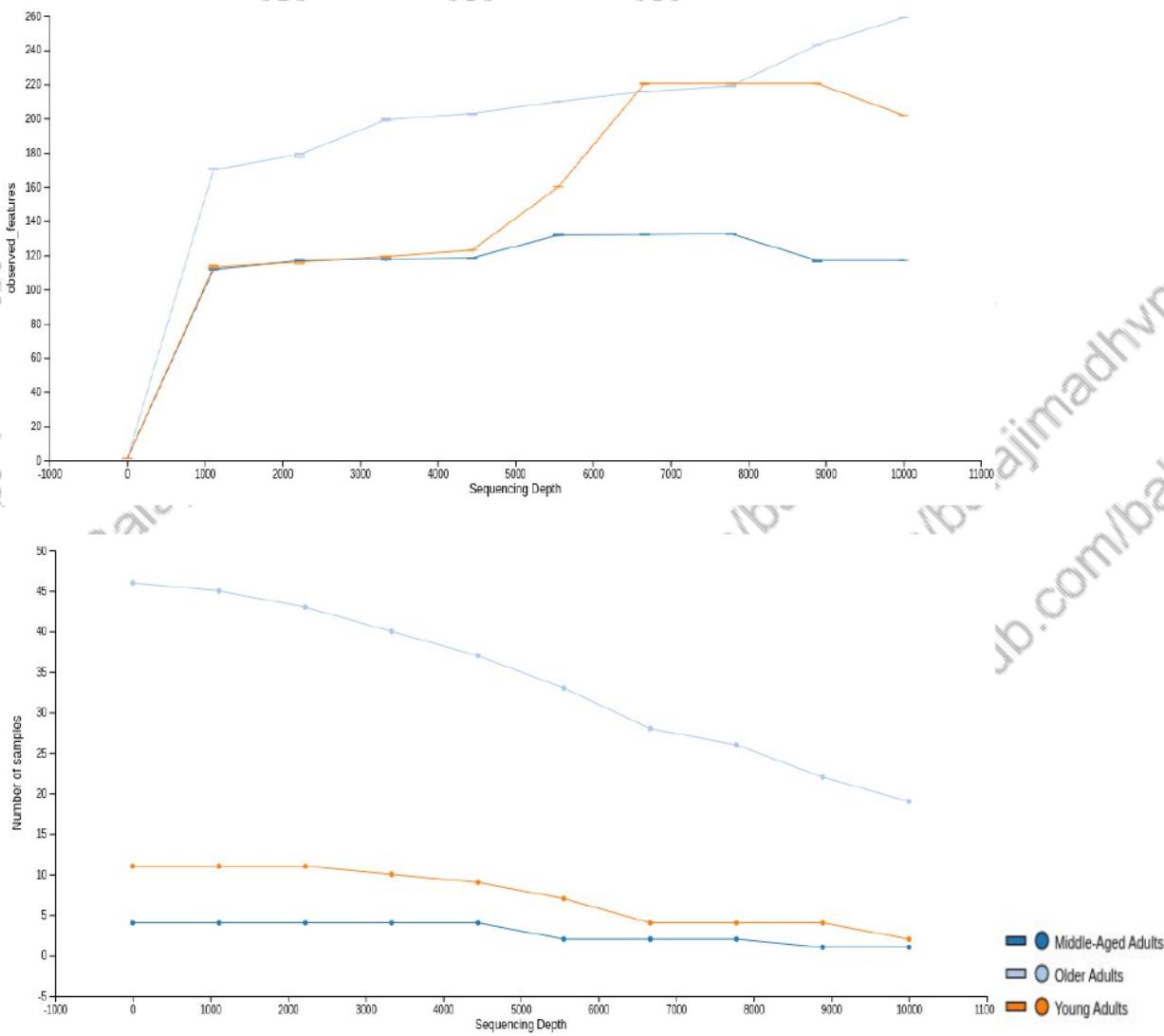
Bray-Curtis distances quantify the dissimilarity between pairs of samples based on species presence and abundance, offering insights into how microbiota composition varies with age. This analysis helps to elucidate the extent of microbial turnover and community structure changes, contributing to a deeper understanding of how aging influences gut microbiota dynamics and potentially affects overall health. By examining these differences, researchers can delve into the ultimate implications for gut microbiota stability and function throughout the human lifespan.

Principal Coordinate Analysis (PCA) of Gut Microbiota Diversity Across Age Groups



The Principal Coordinate Analysis (PCA) of human gut microbiota, applied to Bray-Curtis distance metrics for samples across young, middle-aged, and older age groups, visualizes the multidimensional compositional differences among microbial communities. PCA reduces the complexity of microbial diversity data into principal components, enabling a clearer interpretation of how microbial communities cluster or diverge based on age. By mapping these components, PCA reveals underlying patterns and variations in gut microbiota composition, offering insights into how age-related changes influence microbial community structure and potentially impact gut health. This analysis enhances our understanding of the relationships between microbial diversity and age, providing a visual representation of the ecological shifts in the human gut microbiome throughout the lifespan.

Rarefaction Curve Analysis of Gut Microbiota Diversity Across Age Groups



The rarefaction curve analysis of human gut microbiota, conducted across 76 samples from young, middle-aged, and older age groups, assesses the adequacy of sampling depth in capturing microbial diversity. Rarefaction curves plot the number of observed species against the number of sequences sampled, providing insights into the completeness of species detection and the sufficiency of the sample size. By comparing these curves across different age groups, researchers can evaluate whether the sampling efforts adequately represent the diversity of gut microbiota and identify potential differences in microbial richness and evenness associated with age. This analysis is crucial for understanding the robustness of the observed microbial profiles and ensuring that the diversity metrics accurately reflect the underlying microbial community structure throughout the human lifespan.

Conclusion

In this study, we conducted a comprehensive analysis of the human gut microbiome across young, middle-aged, and older age groups using functional profiling, taxonomic profiling, differential abundance analysis, and rarefaction curve analysis.

Our findings reveal significant age-related variations in both microbial diversity and composition. Functional profiling highlighted differences in key metabolic pathways, while taxonomic profiling identified shifts in microbial taxa associated with aging. Differential abundance analysis pinpointed specific taxa and functions that varied significantly with age, suggesting potential implications for gut health and disease susceptibility. Rarefaction curve analysis confirmed that our sampling depth was adequate for capturing the diversity of gut microbiota across age groups. These results provide valuable insights into how aging impacts gut microbiota dynamics and underscore the need for further investigation into the role of the microbiome in age-related health outcomes.

Future Directions

Future research should aim to elucidate the mechanisms underlying the observed age-related changes in gut microbiota. Longitudinal studies could provide insights into how microbial diversity and function evolve over time and their implications for health. Additionally, investigating the functional consequences of specific microbial shifts could enhance our understanding of how these changes impact metabolic and immune functions. Exploring interventions, such as dietary modifications or probiotics, to mitigate adverse effects associated with aging could also be valuable. Finally, expanding the study to include a broader range of ages and diverse populations will help validate findings and improve the generalizability of the results.