

Comparative Gut Microbiota Profiling of Obese and Normal-Weight Indian Adults Using 16S rRNA Sequencing

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Short Report

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Abstract

Background: Obesity rates are rising globally, placing a significant strain on individuals, society, and economies. The gut microbiota (GM) plays a pivotal role in the development of obesity. Many studies have identified differences in GM composition between obese and normal-weight people worldwide. However, there is limited data on the GM profiles of obese and control Indian individuals.

Methods: Fecal samples from 20 participants (10 obese, 10 control) underwent 16S rRNA gene sequencing. Anthropometric analysis confirmed significant differences in weight and BMI, with no significant variance in age or height. Taxonomic profiling and diversity indices (Chao1, Shannon, Simpson) were evaluated using the NCBI database.

Results: Sequencing identified 1,359 Operational Taxonomic Units. The obese cohort exhibited an elevated *Firmicutes*-to-*Bacteroidetes*(F/B) ratio, with *Firmicutes* increasing to 53.78% and *Bacteroidetes* decreasing to 25.54%. A significant reduction in *Fusobacteria* was observed in the Ob group ($p=0.034$). Seven species were significantly enriched in obese subjects: *Bifidobacterium catenulatum* ($p=0.031$), *Anaerostipes hadrus* ($p=0.014$), *Eggerthella lenta* ($p=0.032$), *Bifidobacterium bifidum* ($p=0.037$), *Clostridium butyricum* ($p=0.012$), *Phascolarctobacterium* sp. ($p=0.046$), and *Alistipes onderdonkii* ($p=0.033$). Rarefaction curves showed higher species richness in the control group, whereas PCA plots indicated greater community similarity (lower beta diversity) in the obese group.

Conclusion: Obesity is associated with distinct microbial dysbiosis, characterized by a significant loss of *Fusobacteria* and an enrichment of SCFA-producing species. These specific taxonomic shifts, rather than broad diversity indices, provide a more sensitive signature for metabolic changes associated with obesity, supporting the “energy harvest” hypothesis.

Introduction

The global landscape of public health is currently facing an unprecedented challenge: the escalating prevalence of obesity and its associated metabolic disorders (1). Once considered a condition primarily affecting high-income nations, obesity has now permeated every corner of the globe, affecting diverse populations regardless of economic status. The World Health Organization (WHO) traditionally defines obesity as a body mass index (BMI) of 30 kg/m² or higher (2). However, recent clinical consensus suggests that this classification must be adapted to account for ethnic and regional variations in body composition. For instance, in China, a BMI is categorized as ≥ 28 kg/m² is considered obese (3), whereas in India, the Association of Physicians defines normal BMI as, with obesity starting at BMI ≥ 25 kg/m² (4–6). These regional adjustments highlight the biological nuances of how excess adiposity manifests across different genetic backgrounds.

Current epidemiological data paints a sobering picture: approximately 1 in 8 people worldwide, roughly 890 million adults are living with obesity (2). Projections suggest that this burden will continue to climb, with nearly 1.13 billion adults expected to meet the criteria for obesity by 2030 (7,8). This trajectory indicates that over half of the global adult population will soon carry excess weight, placing immense pressure on healthcare systems to manage the secondary complications of this “globesity” epidemic.

While the “westernization” of dietary habits and sedentary lifestyles are primary drivers of this trend, the etiology of obesity is far more complex than a simple caloric imbalance (9). Emerging research has identified the human gut microbiota (GM) as a critical mediator of host energy homeostasis and metabolic health (10). Often referred to as a “superorganism” or a “virtual organ,” the GM consists of trillions of microorganisms that influence the host’s physiology, immune responses, and dietary processing (9). With a total genome estimated to have 100 times more genes than the human genome, the GM is seen as an extra organ. On the other hand, alterations in the GM composition, called “dysbiosis,” is linked to a variety of metabolic disorders, including obesity and diabetes (11,12). In the past decade, this microbial community has been repositioned from a passive bystander to a significant factor in the development of obesity and type 2 diabetes (13).

Studies comparing lean and obese phenotypes consistently reveal that obesity is associated with a reduction in microbial diversity (14,15). This “impoverished” microbiota is less efficient at metabolic energy expenditure and is often characterized by

an altered ratio of the two dominant phyla, *Firmicutes* and *Bacteroidetes*, which together comprise approximately 90% of the intestinal bacterial species, followed by other, but less dominant phyla, such as *Actinobacteria*, *Proteobacteria*, *Fusobacteria* and *Verrucomicrobia* (9,12,16). Several studies have also reported that the taxonomic composition and abundance of human GM can vary among individuals or populations according to a range of factors, such as host genetics, ethnic origin, age, sex, dietary and lifestyle habits, geographic location and socioeconomic conditions (11,12). Research in gnotobiotic models has demonstrated that the “obesity microbiota” can extract significantly more energy from food than a “lean microbiota,” leading to increased fat accumulation even when caloric intake is controlled (17). The potential link between obesity and GM has attracted increasing attention in recent years, with studies revealing distinct differences in the composition of GM of obese individuals compared to those with normal weight (18–20). The GM of obese individuals is characterized by a reduced microbial diversity and an increase in the relative abundance of Firmicutes, which have a high potential to harvest energy from diet (11,12,17).

Despite these insights, the complete census of the human gut remains elusive. While 553 species have been successfully cultivated from the human intestinal tract, recent metagenomic efforts by Almeida et al. identified an additional 1,952 potential species that have yet to be cultured (21). This “dark matter” of the microbiome representing 60–80% of anaerobic species cannot be studied through conventional culture techniques (22). However, breakthroughs in next-generation sequencing (NGS) and molecular biology have finally enabled researchers to identify these uncultivable organisms and map their functional pathways (9,23).

Metabolically, the gut microbiota influences the host through the production of short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate (24). These metabolites, synthesized through the hydrolysis of indigestible carbohydrates, act as signaling molecules via receptors such as G-protein-coupled receptor 41 (GPR41). This pathway is linked to the expression of the leptin hormone, enhanced energy expenditure, and reduced food intake (25). Consequently, certain anaerobic microorganisms may serve as a natural defense against obesity.

Thus, understanding the GM composition in obesity is crucial for elucidating the mechanistic underpinnings this multifaceted condition, and has great potential for the development of targeted therapies. Accordingly, further studies are still needed to provide detailed information on variations in GM composition and their impacts on obesity.

In this study, we investigate the diversity and abundance of the microbiota, focusing on specific shifts at the genus and species levels. Understanding these complex interactions is crucial for developing personalized interventions to prevent disease progression and enhance the quality of life for individuals with obesity.

Materials and methods

Ethical approval

The experimental protocol was established according to the ethical guidelines of the Declaration of the Institute of Medical Sciences and approved by the ethics committee of Institute of Medical Sciences, Banaras Hindu University, Varanasi, with approval number Dean/2022/EC/3333.

Sample collection

Ten obese and 10 normal-weight individuals were recruited from Sir Sundar Lal Hospital, Banaras Hindu University, Uttar Pradesh. They were not on any prescribed medications or diet plans to reduce weight, and they abstained from antibiotics for a month before collecting fecal samples. Fecal samples were collected in sterile containers (HIMEDIA PW015) within 2 hours of defecation and preserved at -80°C to investigate gut microbiota variation between obese and control individuals. All participants provided written informed consent. Anthropometric parameters for each participant were measured, including height and weight, following the protocols of the International Society for the Advancement of Kinanthropometry (ISAK). Height was measured to 0.1 cm with subjects barefoot upright on a stadiometer and recorded again. Body mass was

measured to 0.1 kg using a calibrated digital scale (Omron HN 300T), with participants wearing a T-shirt and pants. BMI was calculated using the formula $[\text{weight (Kg)}/\text{height(m}^2\text{)}]$ with the recorded data.

DNA extraction, Quality control, and PCR amplification

Total genomic DNA was extracted from fecal samples using the QIAamp Fast DNA Stool Mini Kit. The quality of the extracted DNA was assessed using NanoDrop and by electrophoresis on 1% agarose gel before PCR amplification. DNA quality was considered acceptable if the NanoDrop 260/280 ratio was between 1.8 and 2. The V3-V4 region of the 16S rRNA gene was amplified using the V34F (5'-AGAGTTTGATGTTGGCTCAG-3') and V34R (5'-TTACCGCGGCMGCSGGCAC-3') primers with High-Fidelity DNA Polymerase. Each reaction contained 40 ng of extracted DNA and 10 pM of each primer. The PCR protocol consisted of 25 cycles with the following steps: initial denaturation at 95°C, followed by cycles of 95°C for 15 seconds (denaturation), 60°C for 15 seconds (annealing), and 72°C for 2 minutes (elongation). A final extension was performed at 72°C for 10 minutes, and the reaction was held at 4°C. The quality of the purified PCR amplicons was confirmed by electrophoresis on a 2% agarose gel and using NanoDrop, which showed a 260/280 ratio of approximately 1.8 to 2, indicating high-quality DNA (Fig S9).

After amplification, the amplicons from each sample were purified with AMPure beads to remove unused primers. An additional 8 PCR cycles were then performed using Illumina barcoded adapters to generate sequencing libraries. Following this, the libraries were again purified with AMPure beads and quantified using the Qubit dsDNA High Sensitivity assay kit. Finally, sequencing was conducted using the Illumina MiSeq i100 Series (Illumina, Inc. San Diego, California).

Bioinformatics analysis

Sequence data were analyzed using NCBI databases. Initially, the bcl data was converted to FASTQ raw data format via demultiplexing. The quality of this data was then checked using FastQC (Version 0.11.9) and MultiQC (Version 1.10.1). Adapters and low-quality reads were subsequently trimmed with TRIMGALORE (for the complete workflow, Fig S1). Samples that met the quality control criteria were then processed using the Biokart Pipeline for 16S metagenomics. This process generated a raw OTU (Operational Taxonomic Unit) table, followed by clustering at a 97% similarity threshold using a reference-based approach with QIIME software. For visualization, the abundance feature table and the top ten genera per sample were compiled in Microsoft Excel (2021). Additional analyses, including Alpha diversity, Beta diversity, and PCoA plots were performed using the online tool Microbiomeanalyst (<https://www.microbiomeanalyst.ca/>) [40,41].

To optimize the results, diversity measures were calculated at the genus level. The data, based on the raw counts in the OTU table from the 16S metagenomic pipeline, were rarefied before analysis. Community diversity profiling was primarily performed using the Vegan R packages within Microbiomeanalyst [42]. A tool that also provided other technical details. Alpha diversity, which quantifies the diversity within individual samples, was assessed using two metrics: the Shannon-diversity index (which accounts for both the number of unique taxa and their richness) and the Shannon-evenness index (which reflects the relative abundance of these exceptional taxa). Outliers on the box plots of the Shannon indices represented samples with lower diversity. A significant issue with other statistical methods for estimating diversity lies in their dependence on the analytical capabilities of the study. For example, the Chao1 statistical method assesses richness by incorporating rare taxa that may have been missed due to under sampling. In contrast, observed genus measures the distinct taxa present in a sample set.

Beta diversity measures the differences in diversity, or the similarity and dissimilarity, between two different samples. This analysis involves two main steps: first, calculating the degree of similarity using a dissimilarity matrix, often with the non-phylogenetic Bray-Curtis index; and second, visualizing this dissimilarity matrix in lower dimensions with Principal Coordinate Analysis (PCoA). PCoA plots, which can be in 2D or 3D, represent the microbial community of each sample as a single point. The axes of these plots show the percentage of variation between samples, with the x-axis representing the most significant dimension of variation and the y-axis representing the second most significant. Points on the plot are typically colored to distinguish between different sample groups. The statistical significance of the clustering seen in these plots was evaluated using Permutational ANOVA (PERMANOVA). Furthermore, PERMDISP was used to test for differences in dispersion (variance)

among groups. The presence, absence, and abundance of taxa, along with their statistical significance, were analyzed using Analysis of Similarities (ANOISM).

Statistical analysis

All statistical analysis was performed using R software (v 2.15.3) and SPSS 25.0. Quantitative variables were presented as mean ± standard deviation (SD) and compared between the obese and control groups using the Mann-Whitney test. Differences in alpha diversity among the groups were analyzed using the Mann-Whitney test. The significance level was set at 5%.

Results

Participant characteristics

In this study, we analyzed 20 fecal samples collected from participants, including 10 from obese participants and 10 from controls. The characteristics of the participants are given in Table 1. Obese participants had an average BMI of 28.42±2.10 kg/m² and a mean age of 27.40±4.006 years, whereas the control subjects had an average BMI of 20.27±2.07 kg/m² and a mean age of 23.70±2.71 years. No significant differences in age and height (p > 0.05) were observed between the two groups. Body weight and BMI were significantly different between the two groups (p < 0.05).

Table 1: Characteristics of the participants.

Variables	control (n=10)	obese (n=10)	p-value
Age	23.70±2.71	27.40±4.006	0.28
Height	172.35±7.02	166.05±6.99	0.60
Weight	60±4.39	78.580±9.65	0.000
BMI	20.27±2.07	28.42±2.10	0.000

Sequencing output, preprocessing and taxonomic assignment

Gut bacteriome taxonomic classification/ microbiota of obese and control individuals

The sequencing output for both the control and obese experimental groups was processed. The total counts of raw and quality-filtered sequences were determined, with a mean length of 300 bp. The Control group yielded a total of 2,350,176 raw sequences, of which 2,291,671 (97.51%) passed quality filtering. Similarly, the Obese group generated 2,350,263 raw sequences, of which 2,291,762 (97.51%) met quality control standards. These comparable sequencing depths and high-quality filtering rates across both groups ensure a robust foundation for downstream taxonomic analysis and facilitate reliable comparisons of microbial community structures between conditions. The overall high quality indicates consistent library preparation and sequencing performance.

The entire sequencing dataset has been deposited in the National Centre for Biotechnology Information (NCBI) repository. After filtering with an approximate default threshold of 0.03%–0.5%, a total of 2,350,176 reads were identified among 1359 OTUs from the 20 samples. The control group contained 1233 OTUs, while the obese group had 1069 OTUs. Overall, 2.43% of the OTUs were classified at the phylum level, 48.42% at the genus level, and 78.22% at the species level (see Supplementary File 2 for more details). Other taxonomic levels, such as class, order, and family, were also included in the analysis (Fig S6, Fig S7, and Fig S8).

Fig S2 shows the gut microbiota composition at the phylum level between the obese and control groups. The obese group exhibited a higher relative abundance of *Firmicutes* (54.1%) and a lower abundance of *Bacteroidetes* (25.8%) than the controls (28%). In addition, Fig S3a and S3b present the top 10 genera identified in each individual from the control and obese groups,

respectively, underscoring the inter-individual variability within each cohort. Collectively, these results demonstrate that the overall gut microbial composition differs markedly between obese and normal individuals.

A total of 33 bacterial phyla were identified, with five dominant phyla representing most of the sequences based on the NCBI dataset. The most prevalent phylum, with a relative abundance exceeding 1%, was *Firmicutes*. This phylum constituted a larger proportion in obese individuals (53.78%) than in the control subjects (46.63%), followed by *Bacteroidetes* (obese 25.54%, control 26.56%), *Proteobacteria* (obese 12.15%, control 19.5%), *Actinobacteria* (obese 7.85%, control 6.24%), and *Cyanobacteria* (obese 0.17%, control 0.74%). Less abundant phyla included *Planctomycetes* (obese 0.18%, control 0.14%), *Chloroflexi* (obese 0.06%, control 0.06%), *Elusimicrobia* (obese 0.004%, control 0.053%), and *Acidobacteria* (obese 0.042%, control 0.045%) (Fig 2 and Fig S4), but no significant differences were found between the groups; *Fusobacteria* (obese 0.012%, control 0.08%, $p=0.034$) showed a significantly higher level in control.

Among the 697 genera identified, *Prevotella* was the most abundant, with percentages of 23.24% and 21.73% in the obese and control groups, respectively. Based on a threshold of >1% abundance, other prominent genera included: *Lactobacillus* (9.23% obese, 7.09% control), *Faecalibacterium* (4.28% obese, 7% control), *Unclassified Clostridiales* (5.9% obese, 6.34% control), *Bifidobacterium* (5.67% obese, 4.85% control), *Escherichia* (2.8% obese, 4.74% control), and *Clostridioides* (8.6% obese, 4.57% control). *Pseudomonas* (0.87% obese, 3.63% control), *Bacteroides* (1.14% obese, 1.44% control), and *Blautia* (3.06% obese, 1.42% control) were non-significant genera found in both groups (Fig 3 and Fig S5 (a), (b)). *Anaerostipes*, *Eggerthella*, and *Phascolarctobacterium* ($p<0.05$) were found significantly more abundant in obese individuals, whereas *Cutibacterium*, *Ezakiella*, and *Rothia* ($p<0.05$) genera were significantly more prevalent in the control group Table S1.

At the species level, a total of 1359 species was recorded including *Prevotella denticola* (obese 18.45%, control 18.18%), *Faecalibacterium prausnitzii* (obese 4.28%, control 7%), *Clostridiales bacterium* CCNA10 (obese 6%, control 6.3%), *Escherichia coli* (obese 2.8%, control 4.7%), *Clostridioides difficile* (obese 8.6%, control 4.5%), *Lactobacillus reuteri* (obese 6.6%, control 4.35%), *Streptococcus mutans* (obese 3.3%, control 2.9%), *Prevotella sp.* (obese 3.4%, control 2.75%) (Fig 4 a) and the following were among the most abundant (relative abundance greater than 1%) were found in non-significant in both group (Fig 4b). *Bifidobacterium catenulatum*, *Anaerostipes hadrus*, *Eggerthella lenta*, *Bifidobacterium bifidum*, *Clostridium butyricum*, *Phascolarctobacterium sp.*, and *Alistipes onderdonkii* ($p<0.05$) were significantly more abundant in the obese group.

Table 2: Bacterial species significantly more abundant in Obese Samples

Species	Ave. Control (%)	Ave. Obese (%)	p-value
<i>Bifidobacterium catenulatum</i>	0.831	2.359	0.031
<i>Anaerostipes hadrus</i>	0.105	0.521	0.014
<i>Eggerthella lenta</i>	0.019	0.095	0.032
<i>Bifidobacterium bifidum</i>	0.018	0.148	0.037
<i>Clostridium butyricum</i>	0.012	0.047	0.012
<i>Phascolarctobacterium sp.</i>	0.007	0.109	0.046
<i>Alistipes onderdonkii</i>	0.002	0.044	0.033

A comparative analysis of the four most abundant phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* showed no significant difference, but *Fusobacteria* increased significantly in the control group, as conducted using NCBI databases. The groups showed a difference in the log-transformed count graph, as evidenced by the median shift; however, the p-value for the inter-group comparison was not statistically significant ($p>0.05$) (Fig 5).

Variation in microbial species diversity between obese and control individuals

Using 16S rRNA gene sequences derived from clone libraries, we evaluated the microbial diversity in control and obese subjects. The assessment, based on genus-level phylotype classification, included measures of both richness and evenness. To evaluate differences in microbial alpha diversity between the control and obese cohorts, we conducted independent two-sample t-tests on four key diversity indices: Chao1, Shannon, Simpson, and Fisher's Alpha. The results, as presented in the table, reveal no statistically significant differences across any of the indices when comparing the two groups.

The t-test for the Chao1 index, a measure of species richness, yielded a p-value of 0.18345 ($t=1.3838$), which is above the standard significance threshold of 0.05. Similarly, the Shannon index, which accounts for both richness and evenness, showed a p-value of 0.96188 ($t=-0.048492$), further indicating no significant difference. The Simpson index also showed no statistical significance, with a p-value of 0.78557 ($t=-0.27647$). Finally, Fisher's Alpha index, another measure of diversity, produced a p-value of 0.37627 ($t=0.91036$) (Fig 6 a, b, c, d).

Collectively, these findings suggest no substantial variation in the overall alpha diversity of microbial communities between the control and obese groups. While there may be other differences not captured by these metrics, the results from this analysis do not support the hypothesis that obesity, as defined in this study, significantly alters the richness or evenness of the gut microbiome.

Comparison of species-level gut bacteria relative abundance between the obese and control groups

The rarefaction curve illustrates species richness (alpha diversity) in the gut microbiomes of control and obese individuals, as a function of sequencing depth. The x-axis indicates the total sampled sequences, and the y-axis displays the count of observed species. Each line corresponds to a single sample, with red representing the control group and blue representing the obese group (Fig 7). The curves for both groups show a sharp initial increase that gradually levels off. This trend indicates that the sequencing depth was sufficient to capture most of the microbial diversity in the samples. The plateauing trends indicate that additional sequencing would contribute little to the discovery of new species, affirming adequate sampling coverage.

A consistent difference emerges between the two groups: at any sequencing depth, the control group's lines are positioned higher than those of the obese group. This means that control individuals harbor more unique microbial species compared to obese individuals, reflecting greater alpha diversity overall. This reduced diversity in the obese group is a hallmark of microbial dysbiosis, often linked to obesity and related metabolic disorders. In contrast, the higher richness observed in the control group suggests a healthier, more stable gut ecosystem. Thus, the rarefaction curves highlight obesity-associated microbial diversity loss and its potential role in disease.

PCA results comparison between obese and control individuals

The provided Principal Component Analysis (PCA) plot and accompanying table reveal significant differences in the gut microbiome composition between control and obese groups. The PCA plot, which accounts for 45.6% of the total variance, shows a clear separation between the two groups. The obese group data points are tightly clustered within a small blue ellipse, indicating a highly similar and less diverse microbial community. In contrast, the control group points are widely dispersed across a larger red ellipse, suggesting a more varied and heterogeneous gut microbiome among these individuals; however, no significant difference was found between the group (T-test for PC1: $t = -0.614$, $p = 0.602$).

The table data provides insights into the specific genera contributing to this separation. While some genera like *Prevotella*, are dominant in both groups, there are notable differences in others. The obese group shows higher relative abundances of genera such as *Clostridioides* (8.60) and *Bifidobacterium* (5.68), along with slightly higher levels of *Streptococcus*. The control group, on the other hand, exhibits greater overall variability, with some individuals showing very high abundances of genera like *Prevotella* (Fig 8).

This analysis suggests that obesity is associated with a more uniform, potentially less diverse, gut microbiome, whereas the control group's microbiome is more varied. This difference in microbial community structure is a key finding that could be further explored in relation to metabolic health.

Distribution of bacterial genera prevalence in microbial communities

The heatmap shows the prevalence of gut bacterial genera (OTUs) across detection thresholds, with the x-axis indicating the minimum relative abundance required for detection and the y-axis listing bacterial genera. The color gradient ranges from dark blue (0.0 prevalence) to dark red (1.0 prevalence), reflecting the prevalence of each bacterium across samples. Certain genera, including *Prevotella*, *Lactobacillus*, and *Bifidobacterium*, remain highly prevalent even at higher thresholds, indicated by persistent dark red coloration. This suggests that these bacteria are not only frequently present but also occur at relatively high abundances in the gut. Their consistent prevalence underscores their established, stable role in the microbiome.

In contrast, bacteria such as *Pseudobutyrvibrio*, *Bacillus*, and *Bacteroides* are less prevalent, particularly under stricter thresholds. Dark blue coloration indicates they are either rare or found only in small amounts, limiting their ecological impact within the studied samples. Other genera, including *Pseudomonas* and *Clostridium*, exhibit a gradient-like pattern, with prevalence decreasing as detection thresholds increase. This suggests that while they may occur across samples, they are often present at lower abundances. Overall, the plot provides valuable insight into core versus rare members of the gut microbiome, distinguishing abundant, common taxa from those present sporadically or minimally, as shown in Fig 9.

Comparative analysis of gut microbiome composition in obese and control individuals using heatmap visualization

The heatmap illustrates the relative abundance of bacterial genera across control (C) and obese (Ob) individuals, highlighting distinct microbial community patterns between the two groups. The color gradient, ranging from red (high abundance) to blue (low abundance), emphasizes differences in microbial composition, while hierarchical clustering on both axes provides insights into sample and genus-level similarities.

Genera that appear enriched in the obese group include *Anaerostipes*, *Blautia*, *Clostridioides*, *Escherichia*, *Staphylococcus*, and *Streptococcus*. These taxa are consistently represented by red-shaded cells across multiple obese samples, suggesting they may be associated with obesity-related alterations in gut ecology that could contribute to metabolic dysregulation or pro-inflammatory states. In contrast, several genera, such as *Ruminococcus*, *Tannerella*, and *Prevotella*, are more abundant in the control group. Their higher presence in lean individuals is indicative of a potentially healthier microbial profile, often linked to fiber fermentation and beneficial SCFAs production.

Clustering on the x-axis (individuals) indicates that most obese samples cluster together, suggesting a relatively homogeneous microbial composition within this cohort. For example, Ob6 and Ob4, as well as Ob1 and Ob10, form tight clusters, underscoring their community resemblance. Interestingly, the control group shows greater diversity, with C2, C4, and C6 clustering separately, while C5 displays a microbial profile more similar to obese individuals, highlighting inter-individual variation within the control population.

Clustering on the y-axis (genera) identifies co-occurrence patterns among bacteria. For instance, *Anaerostipes* clusters with *Blautia*, while *Streptococcus* aligns with *Haemophilus*, reflecting potential ecological or functional similarities. Such patterns suggest that shared niches or host-driven environmental factors may shape the composition of microbial genera. Overall, the heatmap underscores distinct microbial signatures associated with obesity, while also revealing heterogeneity in the control group that could reflect diet, lifestyle, or host-specific factors (Fig 10).

Comparative analysis of microbiomes at the species level

Seven bacterial species were found to be significantly associated with the obese group in a species-level analysis. These included: *Bifidobacterium catenulatum*, *Anaerostipes hadrus*, *Eggerthella lenta*, *Bifidobacterium bifidum*, *Clostridium butyricum*, *Phascolarctobacterium* sp., and *Alistipes onderdonkii* (Table 2 & Table 3). These species, which belong to the Acinetobacter, Bacteroidetes, and Firmicutes phyla, are known to possess genes involved in polysaccharide metabolism. This metabolic function is believed to increase the host's energy harvesting efficiency by facilitating the degradation and fermentation of resistant starches into SCFAs.

Table 3: Taxonomic classification and functional roles of selected gut bacterial strains identified in the human intestinal microbiota.

Phylum	Genus	Bacterial Strain	Habitat	Gut Function	Remarks
Actinobacteria	<i>Bifidobacterium</i> (29)	<i>Bifidobacterium catenulatum</i>	Human Gut/Feces	Carbohydrate fermentation; produces acetate.	Often higher in high-fiber diets; linked to improved glucose metabolism.
Firmicutes	<i>Anaerostipes</i> (30,31)	<i>Anaerostipes hadrus</i>	Human Colon	Butyrate production; degrades dietary fiber.	Key “cross-feeder”; its presence is usually a sign of a functional ecosystem.
Actinobacteria	<i>Eggerthella</i> (32)	<i>Eggerthella lenta</i>	Human Gut	Complex role in metabolic and immune processes, often acting as a “pathobiont”.	Known pathobiont; linked to inflammation if levels are too high.
Actinobacteria	<i>Bifidobacterium</i> (33)	<i>Bifidobacterium bifidum</i>	Human Gut	Mucus degradation; cross-feeding other microbes.	A foundational probiotic species that helps maintain the gut barrier.
Firmicutes	<i>Clostridium</i> (34)	<i>Clostridium butyricum</i>	Human Gut	Strong butyrate producer; inhibits pathogens.	Used as a probiotic; helps regulate insulin sensitivity in some studies.
Firmicutes	<i>Phascolarctobacterium</i> (35)	<i>Phascolarctobacterium</i> sp.	Human/Animal Gut	Converts succinate to propionate.	Propionate is known to help signal “fullness” (satiety) to the brain.
Bacteroidota	<i>Alistipes</i> (36)	<i>Alistipes onderdonkii</i>	Human Gut	Protein fermentation; bile acid metabolism.	Often increased in diets high in animal fats and proteins.

Discussion

The human gut microbiome plays a fundamental role in host metabolic homeostasis, influencing energy balance, nutrient absorption, immune regulation, and inflammatory responses (37,38). Increasing evidence suggests that alterations in gut microbial composition are closely associated with the development of obesity and related metabolic disorders (39). In the present study, we performed 16S rRNA gene sequencing to characterize gut microbial profiles in obese and normal-weight

individuals from an Indian population. Our findings demonstrate distinct microbial signatures associated with obesity, including an increased Firmicutes-to-Bacteroidetes ratio, reduced abundance of Fusobacteria, and enrichment of several species involved in carbohydrate fermentation and short-chain fatty acid (SCFA) metabolism. These findings are consistent with previous reports demonstrating that gut microbiota composition plays an important role in host metabolic regulation and energy balance (12,17,40).

One of the most widely discussed microbiome markers of obesity is the Firmicutes-to-Bacteroidetes (F/B) ratio. In agreement with early landmark studies by Turnbaugh and colleagues, our results show an increased relative abundance of Firmicutes and a modest reduction in Bacteroidetes in obese individuals, resulting in an elevated F/B ratio (17,40). This pattern has been proposed to enhance microbial capacity for dietary polysaccharide degradation and subsequent SCFA production, thereby increasing caloric extraction from otherwise indigestible carbohydrates (41). However, recent meta-analyses have demonstrated that the F/B ratio is not universally consistent across populations and may be influenced by host genetics, diet, and environmental factors (12). Therefore, while the elevated F/B ratio observed in our cohort supports the concept of microbial energy harvest in obesity, it should be interpreted cautiously and in conjunction with species-level microbial alterations.

In addition to phylum-level shifts, our analysis identified a significant reduction in the phylum Fusobacteria among obese individuals. Although Fusobacteria are typically present at relatively low abundance in the gut microbiome, their depletion may reflect broader ecological disturbances within the microbial community. Previous studies have suggested that reduced microbial diversity and altered minor phyla can reflect disrupted microbial equilibrium and may contribute to metabolic dysregulation (26,42,43). Nevertheless, the functional implications of Fusobacteria depletion in obesity remain unclear and warrant further investigation.

A key finding of this study is the species-level enrichment of seven bacterial taxa in obese individuals, including *Bifidobacterium catenulatum*, *Anaerostipes hadrus*, *Eggerthella lenta*, *Bifidobacterium bifidum*, *Clostridium butyricum*, *Phascolarctobacterium* sp., and *Alistipes onderdonkii*. Several of these organisms are known producers of SCFAs such as acetate, propionate, and butyrate, metabolites that play critical roles in host metabolism (12,44). SCFAs serve as energy substrates for colonocytes, regulate gut barrier integrity, and act as signaling molecules through G-protein-coupled receptors such as GPR41 and GPR43. However, under certain physiological conditions, increased microbial fermentation may contribute to excess caloric availability, supporting the “energy harvest hypothesis” of obesity (44,45). According to this model, the microbiota enhances the host’s ability to extract energy from the diet, potentially contributing up to 10–15% of daily caloric intake (45,46).

Interestingly, several of the enriched taxa identified in this study are traditionally regarded as beneficial or probiotic organisms, particularly *Bifidobacterium* species and *Clostridium butyricum*. This apparent paradox highlights the complex and context-dependent nature of host–microbiome interactions. While SCFA-producing bacteria are generally associated with metabolic health, their expansion within a specific dietary environment—particularly one characterized by high carbohydrate or energy intake—may enhance microbial fermentation capacity and thereby increase energy availability to the host. These findings suggest that microbial function, rather than simple taxonomic abundance, may ultimately determine metabolic outcomes (47).

Our analysis of alpha diversity indices (Chao1, Shannon, Simpson, and Fisher’s alpha) did not reveal statistically significant differences between obese and control groups. Although many previous studies have reported reduced microbial diversity in obesity, conflicting results have been observed across populations (17,19,23). The absence of significant diversity differences in our study may be attributed to the relatively small sample size or to population-specific microbiome characteristics (19). Notably, rarefaction curve analysis indicated that control individuals tended to harbor greater species richness compared with obese individuals, suggesting that subtle diversity differences may exist but were not captured by conventional statistical indices.

Beta diversity analysis further revealed differences in microbial community structure between groups. Principal component analysis demonstrated tighter clustering of microbiome profiles among obese individuals, indicating a more homogeneous

microbial composition within this group (48,49). In contrast, control subjects exhibited greater inter-individual variability, reflecting the inherent heterogeneity of healthy gut microbiomes. Similar patterns have been reported in previous microbiome studies, where disease states often correspond to reduced ecological variability and increased dominance of specific microbial taxa (50).

Another notable observation was the detection of unclassified or poorly characterized taxa, including organisms that could not be assigned confidently at the species level. This finding highlights the substantial proportion of the gut microbiome that remains incompletely characterized, particularly in non-Western populations. Emerging metagenomic studies suggest that a large fraction of intestinal microorganisms remain uncultured or genomically unannotated, emphasizing the need for expanded microbiome reference databases and functional characterization (21).

The presence of unclassified OTUs and the detection of genera such as *Thermobaculum* and *Vampirococcus* that could not be assigned at the species level suggest a reservoir of unique, potentially unidentified bacteria within this population. Furthermore, enrichment of *Eggerthella lenta*, a pathobiont associated with pro-inflammatory states (e.g., IBD, autoimmunity), was observed, potentially contributing to the low-grade inflammatory milieu characteristic of obese microbiomes, alongside altered energy-harvest capacity (32). Our study confirms that obesity is associated with a distinct microbial signature characterized by an elevated F/B ratio, reduced species richness, and a specific enrichment of SCFA-producing bacteria.

Despite these insights, several limitations of the present study should be acknowledged. First, the relatively small sample size limits statistical power and may reduce the generalizability of the findings. Second, dietary intake, lifestyle factors, and metabolic parameters were not comprehensively assessed, although these variables are known to strongly influence gut microbiota composition (51). Third, functional metagenomic analyses were not performed; therefore, the metabolic roles of the identified taxa were inferred primarily from previously published literature rather than directly measured pathways.

Future investigations incorporating larger cohorts, longitudinal study designs, and multi-omics approaches including metagenomics, metabolomics, and SCFA quantification will be essential to clarify the mechanistic relationships between gut microbiota and obesity. Such studies could help determine whether the microbial signatures observed here represent causal drivers of obesity or secondary consequences of metabolic alterations (52).

Conclusion

In conclusion, this study demonstrates significant taxonomic divergence in the gut bacteriome of obese individuals compared with that of normal-weight controls. The observed elevation in the Firmicutes-to-Bacteroidetes ratio, alongside a marked reduction in *Fusobacteria*, reinforces the paradigm of phylum-level dysbiosis in metabolic disorders. Furthermore, increasing specific SCFA producers, such as *Anaerostipes hadrus* and *Clostridium butyricum*, supports the “energy harvest” hypothesis, which suggests that microbial fermentation may contribute to the host's caloric surplus and fat expansion.

Although rarefaction analysis shows a decrease in species richness, indicating reduced community stability, the absence of significant differences in common alpha diversity measures suggests microbial changes might occur before a notable loss of diversity. These results highlight the intricate nature of the host-microbe interaction and emphasize the need for additional long-term studies to establish whether these microbial patterns are causes or consequences of obesity.

Declarations

Author contributions

Prof. Gopal Nath: Conceptualization, data curation, writing-review and editing, supervision, visualization. Dr. Bhupendra Singh Yadav: Supervision, visualization, data curation. Ranjeet Kumar Vishwakarma: Writing-original draft preparation, visualization, methodology, investigation, data curation. Priyanka Gautam: Methodology, data curation, review and editing. Minakshi Sahu: Formal analysis and visualization.

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Conflict of interest

The authors declare no competing interests.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Figures

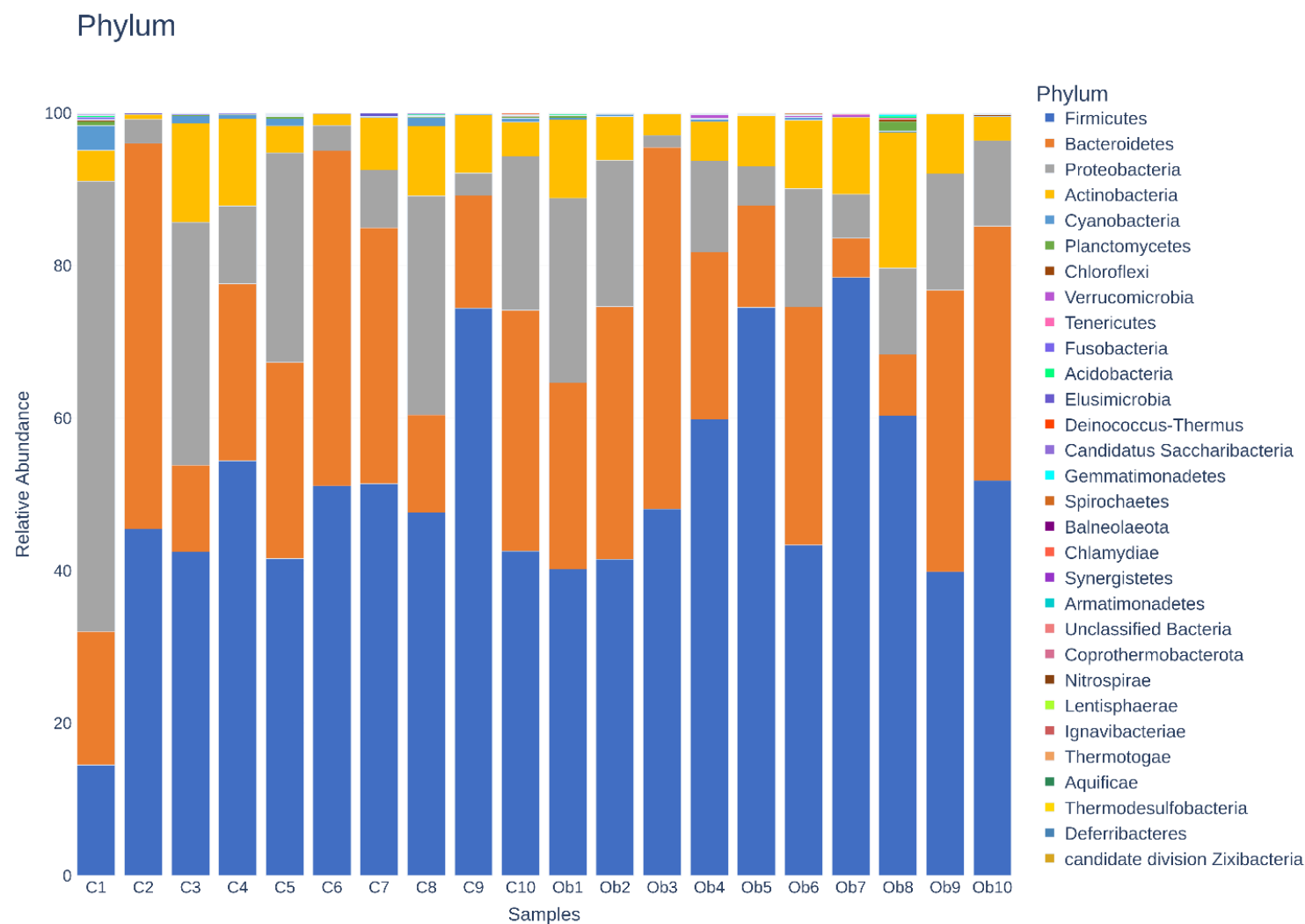


Figure 1

Fig 2: Phylum-level gut microbiome composition of control and obese individuals

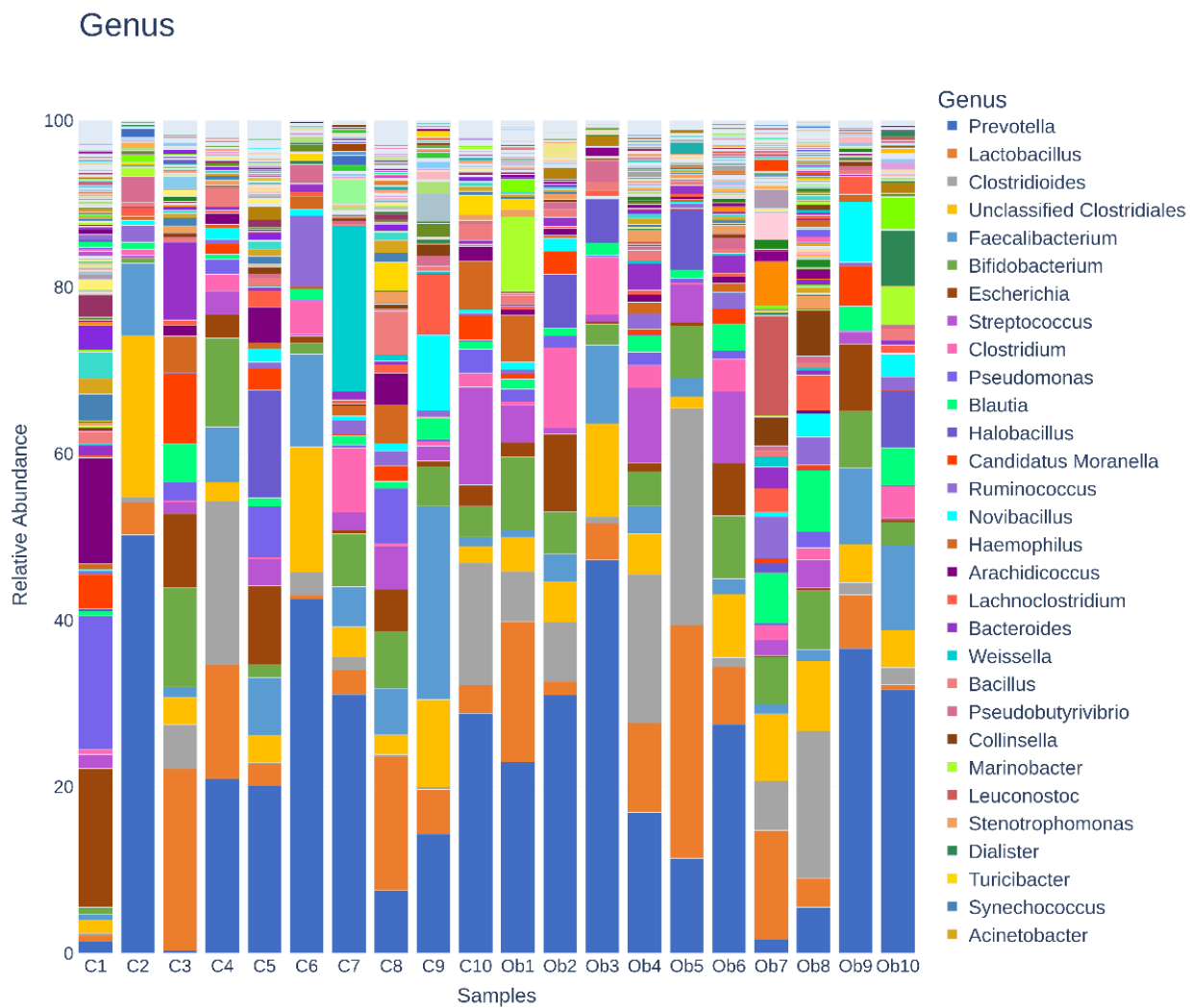


Figure 2

Fig 3: Genus-level gut microbiome composition of control and obese individuals

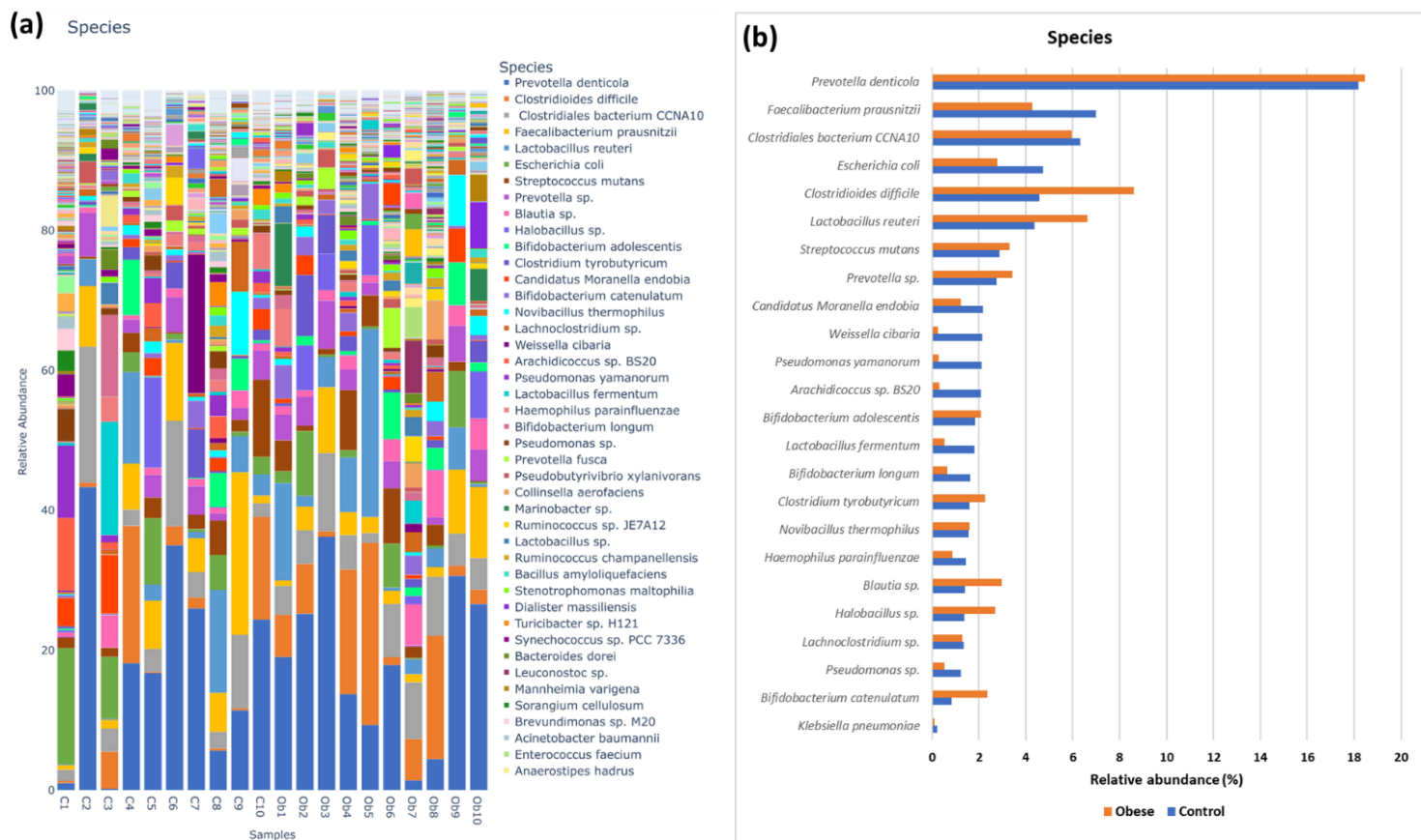


Figure 3

Fig 4: (a) Species-level gut microbiome composition of control and obese individuals, **(b)** Abundant species detected in obese and control individuals (excluding species with less than 1% of relative abundance).

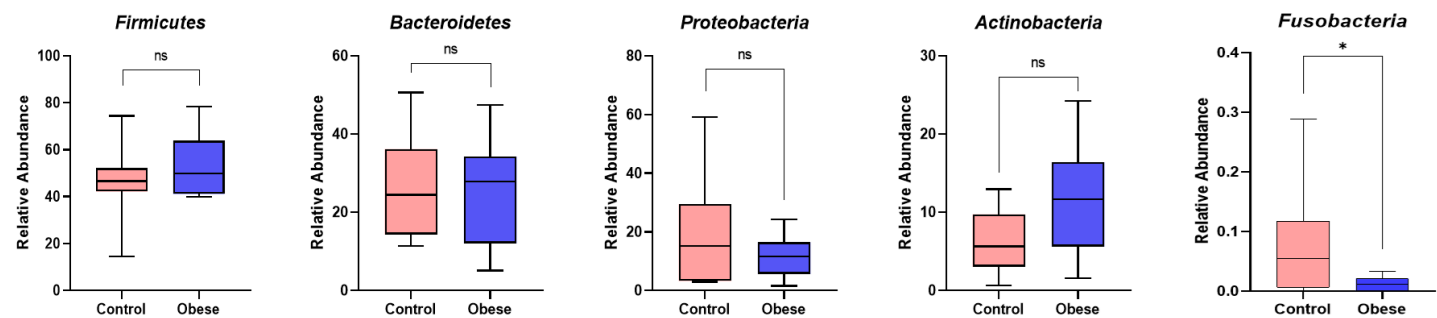


Figure 4

Fig 5: Whisker-box plots based on NCBI taxonomy showed significant differences ($p < 0.05$) in the relative abundance of *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria* and *Fusobacteria*

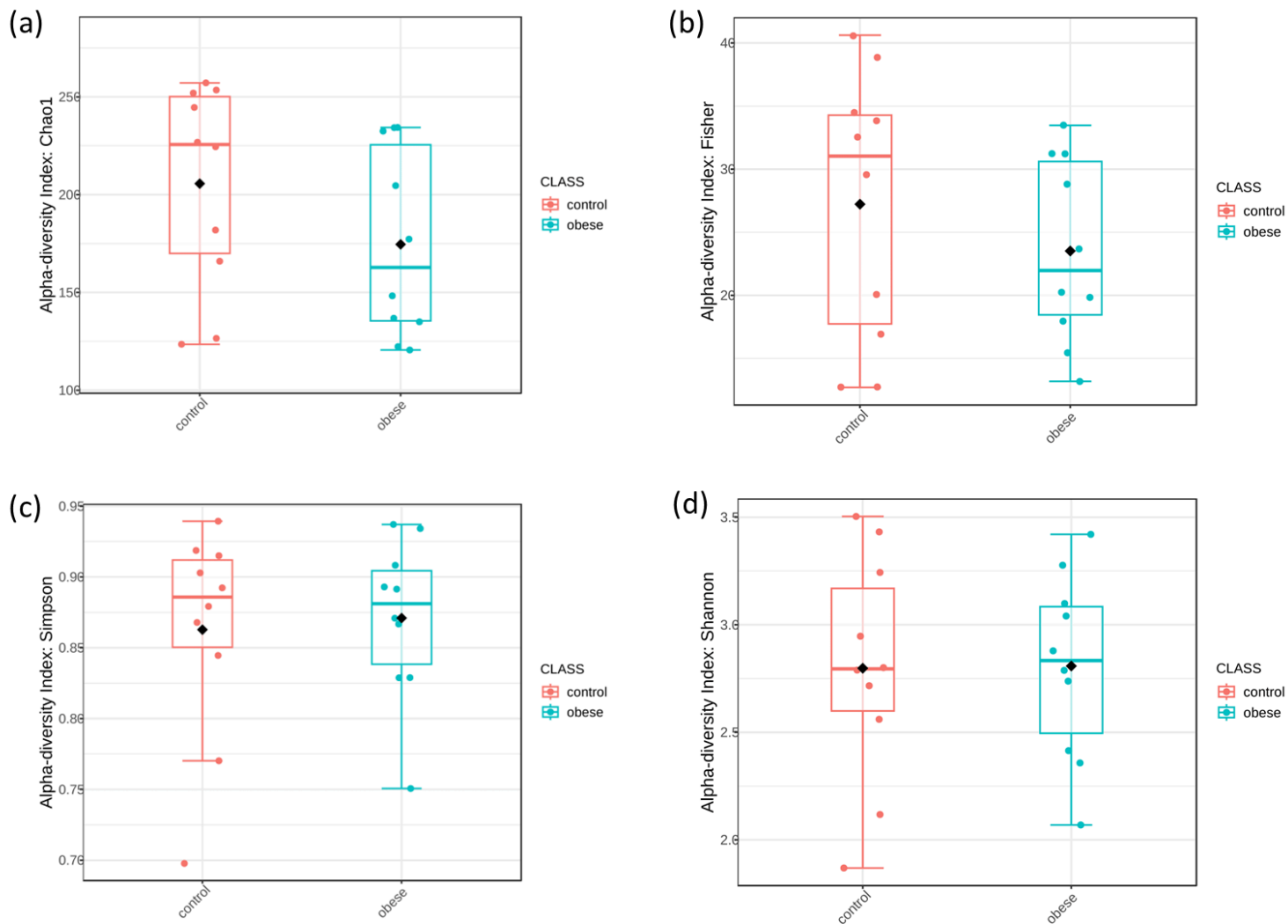


Figure 5

Fig 6: Alpha diversity indices (a. Chao1, b. Fisher, c. Simpson, d. Shannon) reveal microbial diversity differences between control and obese groups

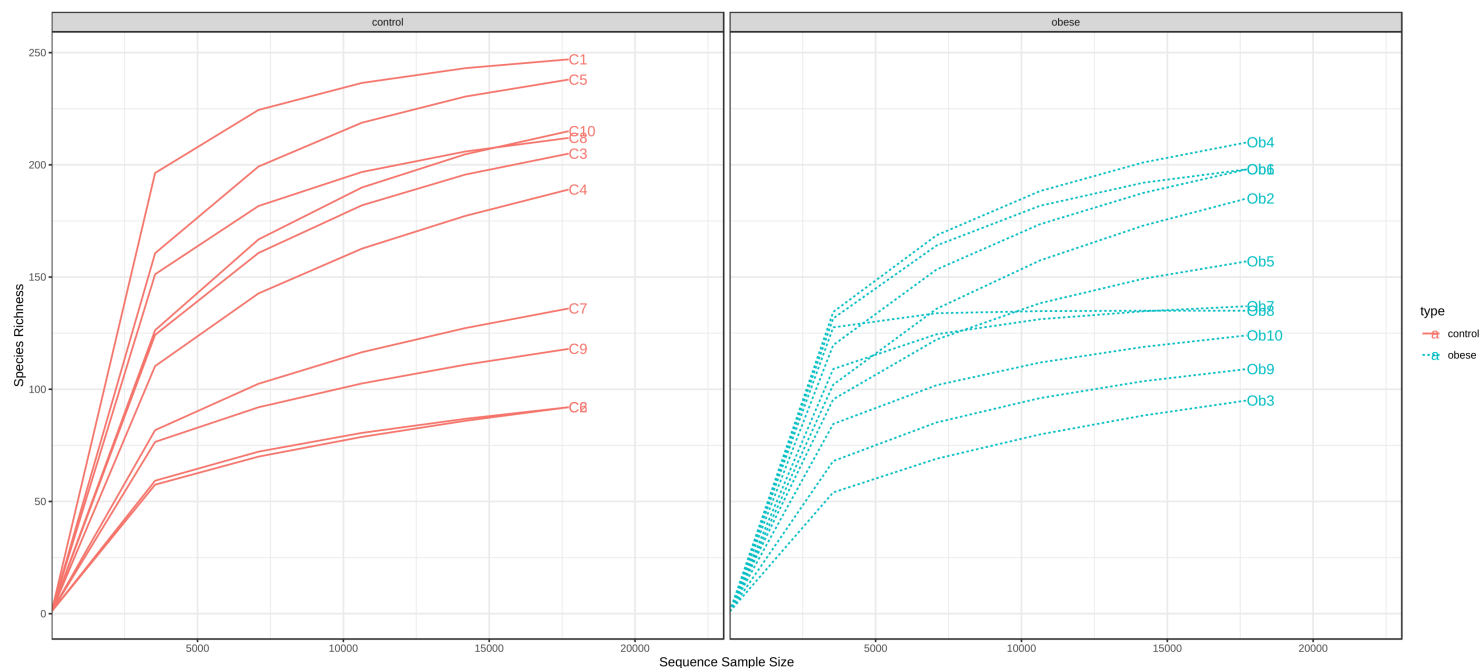


Figure 6

Fig 7: Rarefaction curves using Shannon index illustrate sequencing depth adequacy and microbial richness; similar analyses applied with Chao1, Fisher, and Simpson.

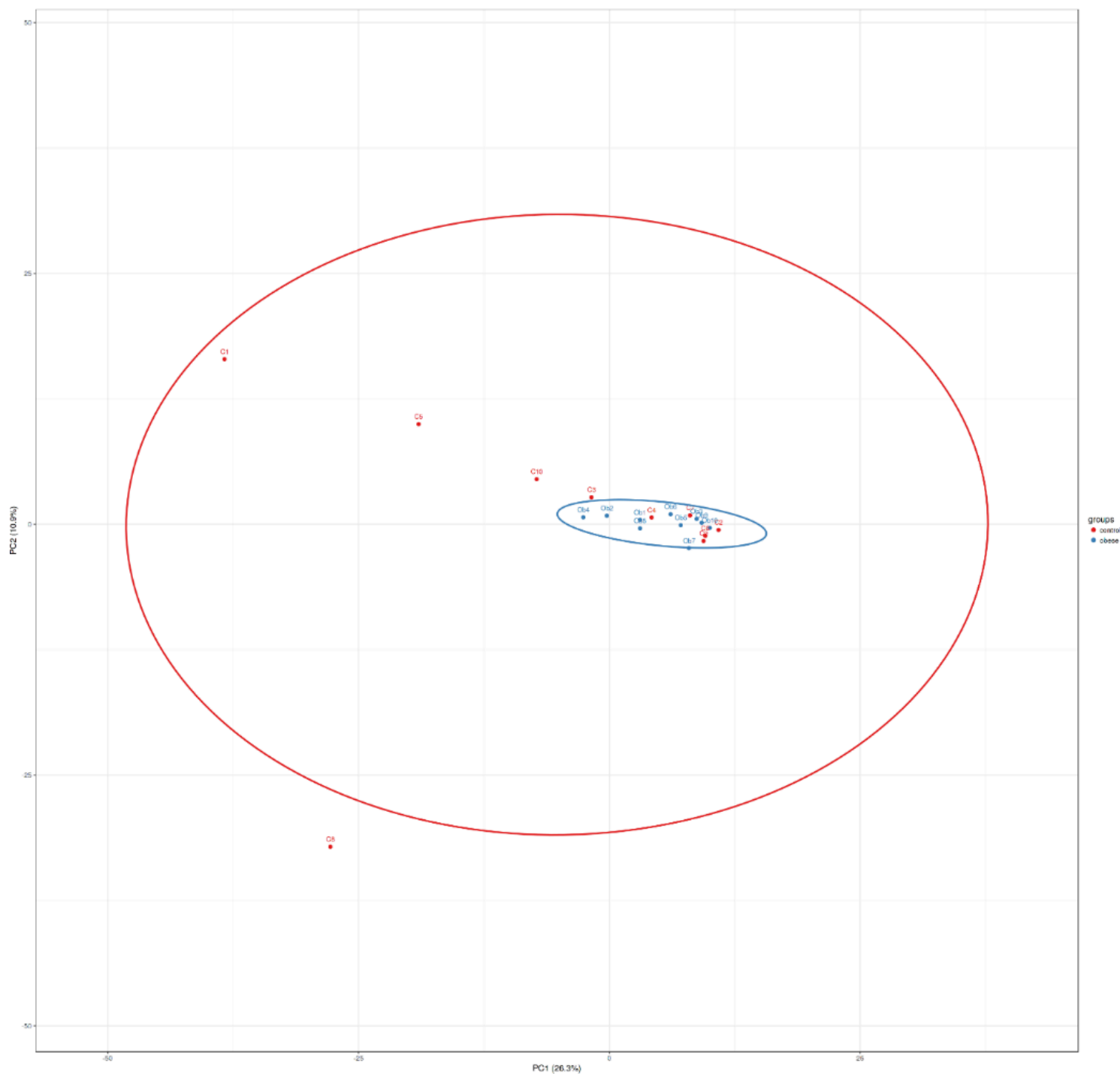


Figure 7

Fig 8: PCA at the genus level shows distinct clustering of obese and control microbiota, driven by dominant genera *Megamonas*, *Bacteroides*, *Blautia*, and *Faecalibacterium*.

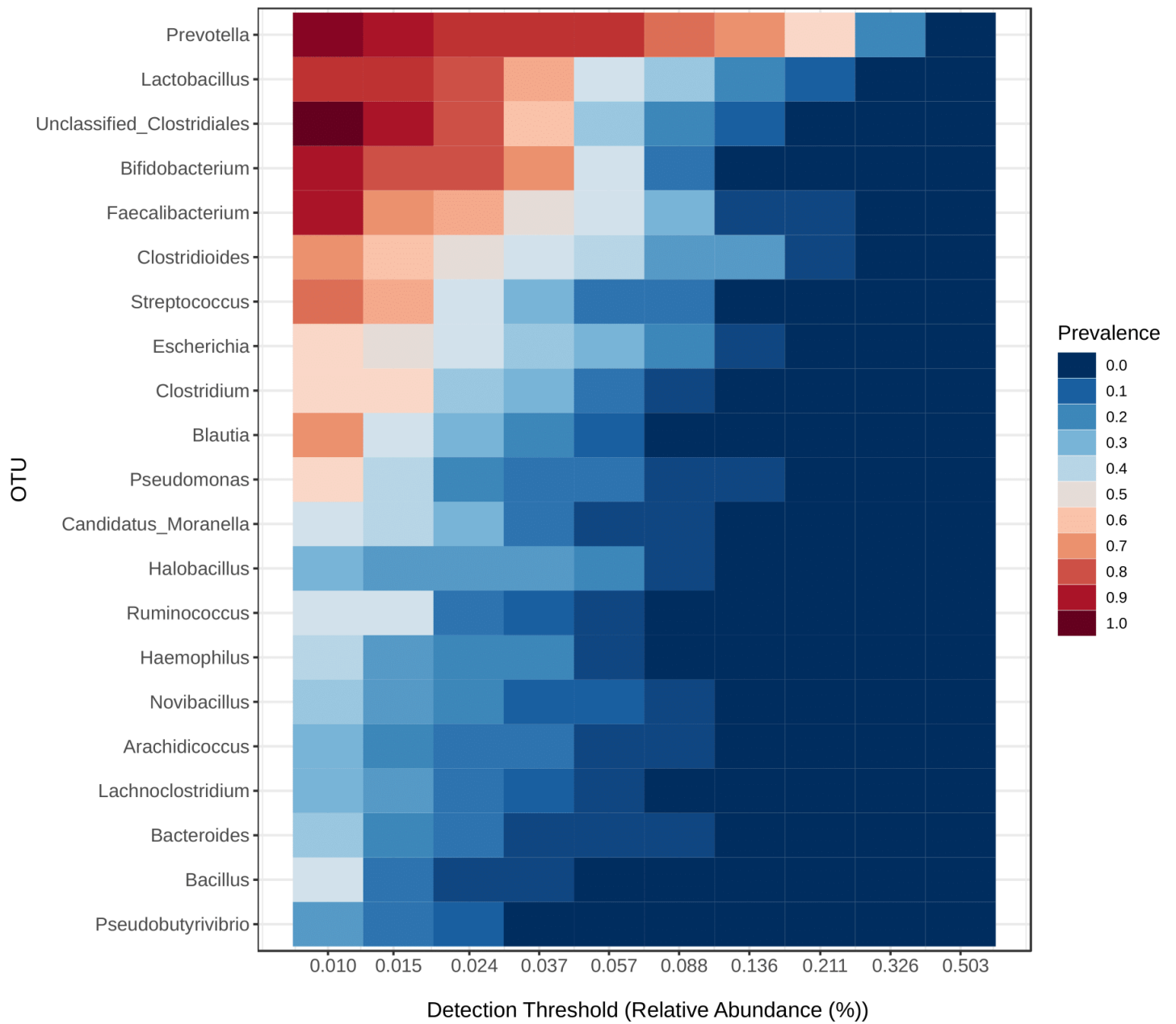


Figure 8

Fig 9: Heatmap of Bacterial Species Prevalence in Microbial Samples

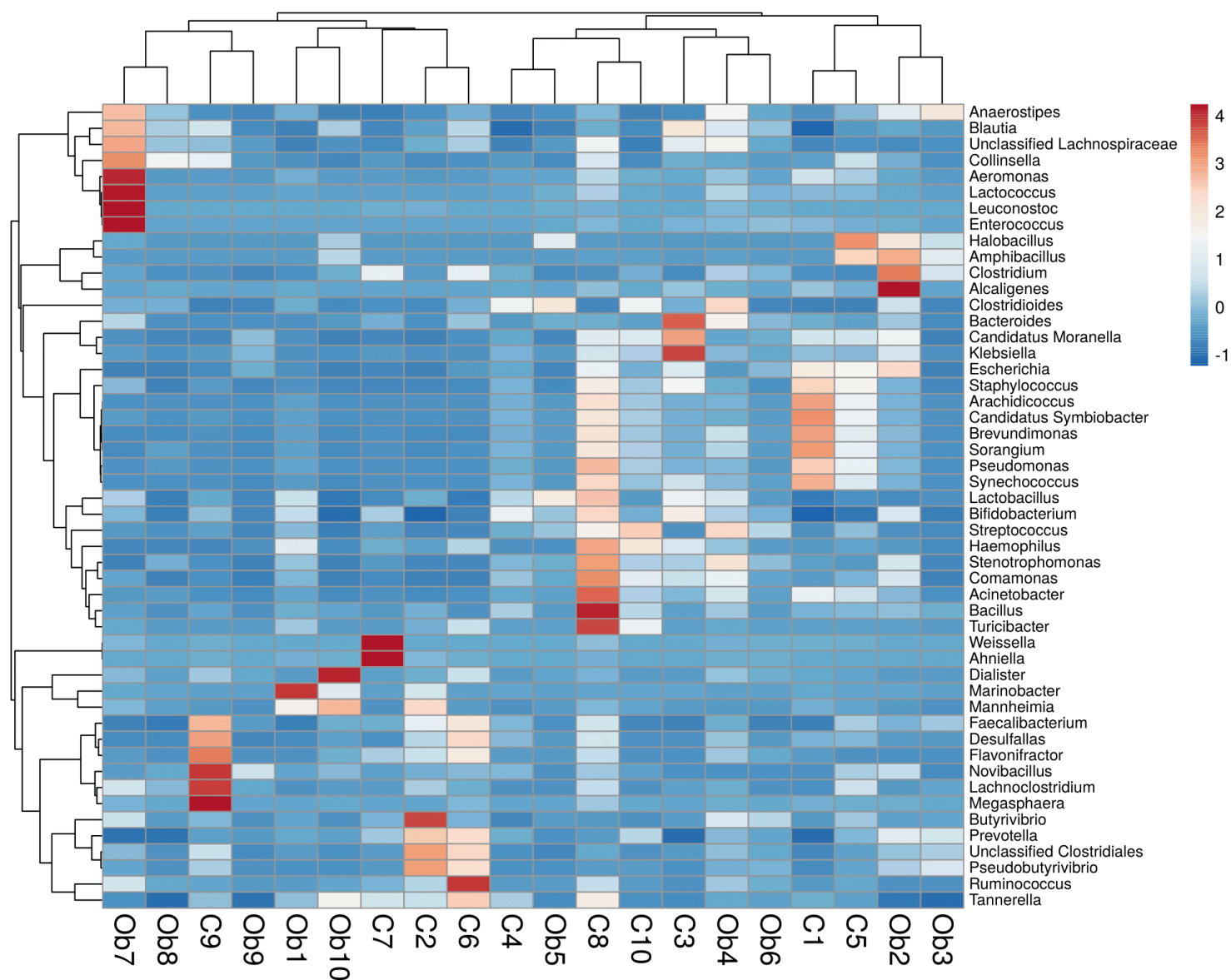


Figure 9

Fig 10: Comparison of microbial profiles between obese and control individuals using heatmap analysis.

Supplementary Files

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