CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

# Comparative Analysis of Bacterial Diversity of Pet Cat Owners and Non Pet Owners by Metagenomics

by

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A thesis submitted in partial fulfillment for the
degree of Master of Science

in the
Faculty of Health and Life Sciences
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*I dedicate this thesis to my parents, teachers and my friends.*

# CERTIFICATE OF APPROVAL

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# *Abstract*

The intricate ecosystem of bacteria residing within the oral cavity, known as the oral microbiome, plays a vital role in maintaining oral health. Recent studies suggest that environmental factors beyond diet and hygiene practices might influence this delicate microbial landscape. This study investigates how pet ownership, specifically cat ownership, shapes the oral microbiome in young adults between the ages of 20 and 30.Our research employed a meticulous approach to assess the influence of cat ownership on the oral microbiome. We recruited two distinct groups of healthy young adults within the target age range (20-30 years old). Pet Cat Owners group comprised individuals who currently own or have previously owned a pet cat.Non-Pet Owners group consisted of individuals with no history of pet cat ownership.Following a standardized protocol to minimize variability, oral samples were collected from each participant. This study aimed to investigate the potential transmission of bacteria from pet cats to their owners through saliva samples in Islamabad, Pakistan. 16S metagenomic sequencing was employed to characterize the bacterial diversity in both groups. Our results demonstrated a shared microbial environment between pet owners and their cats, with several opportunistic pathogens identified. These findings highlight the potential for bacterial transmission and emphasize the importance of maintaining proper hygiene practices.

Saliva samples were collected from pet cat owners and non-pet owners residing in Islamabad. DNA extraction was followed by 16S rRNA gene sequencing.

Our analysis revealed a diverse range of bacteria in both groups, with many shared taxa belonging to the phyla Firmicutes, Bacteroidetes, and Proteobacteria. However, significant differences in the relative abundance of certain bacterial taxa were observed between the two groups. Notably, opportunistic pathogens such as Erysipelotrichales and Staphylococcus were identified in both humans and cats, suggesting potential for interspecies transmission.

Our study provides evidence of a shared microbial environment between pet cat owners and their feline companions. The presence of opportunistic pathogens

highlights the potential for bacterial transmission, although the risk of infections may be low for healthy individuals. Maintaining good hygiene practices, including regular handwashing and proper cleaning of pet-related items, is crucial to minimize the risk of transmission. Further research is needed to fully understand the implications of these findings and to develop targeted preventive measures, such as the use of probiotics or prebiotics to modulate the oral microbiome.

Furthermore, we investigated potential differences in the abundance of specific bacterial groups between the two groups. These variations, if identified, could offer valuable insights into how cat ownership might influence the overall the composition of the oral microbiome.To assess the richness and evenness (distribu- tion of abundance) of bacterial communities within each group, we meticulously calculated alpha diversity indices, such as the Shannon index and Chao1 index. These indices provided crucial quantitative measures of the complexity and bal- ance within the bacterial populations. Understanding these variations in diversity can provide clues about the overall health and stability of the oral microbiome in each group. A higher diversity index might indicate a more complex and poten- tially more resilient microbial community, while a lower diversity could suggest a less diverse and potentially less stable community.

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# Abbreviations

| | |
|---|---|
| **EtBr** | Ethidium bromide |
| **HAI's** | Human-animal interactions |
| **ITS** | Internal Transcribed Spacer |
| **NC** | Non cat |
| **NW** | Normal weight |
| **OTU** | Operational taxanomic unit |
| **OW** | Overweight |
| **PCR** | Polymerase Chain Reaction |
| **PCoA** | Principal coordinate analysis |

# Chapter 1

# Introduction

## 1.1 Bacterial diversity

Bacterial diversity is essential to human health in many physiological areas such as digestion, immunity and metabolism. In the human gut, an ecosystem teeming with trillions of bacteria, these microorganisms are indispensable for processes such as digestion, nutrient assimilation, and metabolic activity. The abundance of bacterial populations in the intestines facilitates the breakdown of complex carbohydrates, the synthesis of vitamins and the processing of bile acids. A study by Qin et al. (2010) and published in Nature underscored the importance of a diverse gut microbiota in maintaining health, contrasting with the association of dysbiosis (imbalance) with conditions such as inflammatory bowel disease (IBD) and obesity [1][2]. Bacterial diversity plays a role in shaping the development and functioning of the immune system. Commensal bacteria contribute to the training and regulation of the immune system, thereby preventing inappropriate reactions and promoting immune tolerance. A study by Arrieta et al. (2015) published in Science Translational Medicine reported that changes in gut microbial diversity during early life correlate with increased susceptibility to allergic conditions such as asthma and eczema [3][4].

A diverse microflora can resist the colonization and penetration of harmful pathogens. Beneficial bacteria compete for nutrients and niche gaps and produce antimicrobial substances that inhibit the growth of pathogens.[5] Research by Buffie et al. (2012) featured in Nature revealed a key role of gut bacterial diversity in protection against Clostridium recurrence infection.[6] Bacterial diversity participates in metabolic processes and has the potential to influence host metabolism. Research has linked changes in gut microbial diversity to metabolic disorders such as obesity, insulin resistance, and diabetes. [7] A study by Le Chatelier et al. (2013) published in Nature demonstrated a link between gut microbial diversity and metabolic indicators such as body mass index (BMI) and blood lipid levels [8].

## 1.2 Metagenomics

Metagenomics has emerged as a transformative tool in microbiology, providing deep insight into the complex world of bacterial communities. In contrast to traditional microbiological methods, which focus on the separate cultivation of individual organisms, metagenomics enables a comprehensive investigation of microbial populations directly from their natural environment.

Bacterial communities exhibit remarkable diversity, often consisting of many species that may not be culturable using conventional techniques. Metagenomics facilitates the identification and characterization of these diverse microbial populations, uncovering previously unknown or overlooked taxa.[9] The human body is home to a diverse array of bacterial communities, collectively known as the human microbiota. These microscopic inhabitants play a vital role in maintaining our health and influence our susceptibility to various diseases.[10] Through metagenomic studies, which involve the analysis of genetic material from environmental samples, researchers have gained invaluable insights into the composition, dynamics and functional roles of these microbial communities in the human body. This research has greatly improved our understanding of how the microbiota affects various aspects of human physiology, from digestion and metabolism to immune function and even mental health.[11]

Furthermore, findings from these studies have significant implications for the field of personalized medicine, as they offer the potential to tailor treatments and interventions to individuals based on their unique microbiota profiles. For example, understanding how specific microbial compositions correlate with certain health conditions could lead to the development of targeted therapies aimed at restoring microbial balance and promoting overall health. One fascinating area of research within microbiota studies involves comparing the bacterial diversity of individuals who own pets, particularly cats, with those who do not. It has been observed that the ownership of pets, especially cats, can affect the composition and diversity of human microflora. This raises interesting questions about the potential health effects of these microbial changes. By further exploring these associations, the researchers hope to reveal new insights into the complex interplay between microbial communities and human health, paving the way for innovative approaches to disease prevention and treatment.[12] [13] Domestic animals such as cats can act as reservoirs for various zoonotic pathogens that may pose a risk of transmission to humans. Comparative analysis of bacterial diversity between individuals who own and those who do not own pet cats allows investigation of potential differences in the presence or abundance of such pathogens in the home environment. This in turn helps in developing effective disease prevention and treatment strategies.[14]

Pet ownership, especially the companionship of cats, is often associated with several psychological and emotional benefits, including reduced stress and improved mood. By examining the bacterial diversity between pet cat owners and owners of other animals, researchers can investigate whether these psychological benefits occur alongside distinct microbial profiles. Such findings could shed light on the basic mechanisms of the human-animal bond. Comparison of bacterial diversity between pet and non-pet cat owners provides valuable insights into the complex interactions between microbial communities, pet ownership, and human health. Understanding the differences in microbial composition and richness associated with pet ownership informs initiatives aimed at promoting human health, controlling disease transmission, and promoting psychological well-being in households.[15]

Differences in gut bacteria between people who own cats and those who don't, using a method called metagenomics. The bacteria in our guts are essential for digestion, fighting germs and other bodily functions. Metagenomics is a powerful tool that allows scientists to see all the different types of bacteria present. Owning a cat can change the types of bacteria that people have in their guts, and this study aims to find out how this could affect their health. By looking at this connection, the research hopes to reveal how cat ownership, gut bacteria and human health are connected. This knowledge could be used to develop new ways to prevent and treat disease and to better understand the bond between humans and animals.

## 1.3 Impact of pet cats on owner health

The **impact of pet cats on owner health**, particularly concerning gut microbiota, is an area of increasing interest in scientific inquiry. Cats, as integral members of households, may influence the gut microbiota of their owners through various pathways. Firstly, close contact with cats facilitates the exchange of microorganisms between the feline and human hosts (Song et al., 2013).[16] This transmission can occur through direct interactions such as petting and grooming, as well as indirect contact with cat-associated surfaces within the household environment (Lax et al., 2014).[17] Pet ownership, including cat ownership, correlates with increased microbial diversity in the home environment, potentially extending to alterations in the gut microbiota of owners (Johnson & Burnet, 2016). These changes in gut microbiota composition influenced by cat ownership may have significant implications for human health, given the role of gut microbiota in various physiological processes (Cisek & Binek, 2016).[18] However, alongside the potential benefits, there are also risks associated with exposure to pet-associated microorganisms.

## 1.4 Problem Statement

Investigates the impact of pet ownership, specifically owning cats, on the oral microbiota composition in humans, comparing bacterial diversity between pet cat owners and non-pet owners.

## 1.5 Aim and Objectives

### 1.5.1 Aim

To conduct a comparative analysis of the bacterial diversity of pet cat owners and non-pet owners by metagenomics, elucidating the potential influence of pet ownership on the oral microbiota composition in humans.

### 1.5.2 Objectives

- To characterizes the composition and diversity of oral bacterial communities in pet cat owners compared to non-pet owners.
- Evaluate the impact of **pet cat ownership duration** (1 year vs 3 years) on the richness and evenness of oral bacterial communities in young adults (20-30 years old).
- Investigate the **influence of age** on the composition and diversity of oral bacterial communities in young adults (20-30 years old).

# Chapter 2

# Literature Review

In a recent investigation the influence of pet ownership on the gut microbiome composition of adults. Researchers conducted a case-control study within a larger Wisconsin health survey. They analyzed stool samples from 332 participants (178 with pets) using a genetic sequencing technique. While overall diversity didn't differ between pet owners and non-owners, specific bacterial types (OTUs) showed variations. Interestingly, seven OTUs were more abundant in those without pets, while four were more prevalent in pet owners. These differences remained significant when considering age. The findings suggest pet ownership is linked to alterations in the gut microbiota [19][20]

The bond between humans and animals, it's becoming clearer that owning a dog or cat can have many positive effects on older adults, both physically and mentally. This particular study focused on over 7,800 independent Japanese adults aged 65 and over. The researchers investigated whether owning a dog or cat could help protect these individuals from experiencing a decline in physical and mental abilities, which can occur with aging. In which followed participants for two years, starting with a healthy and active group in 2016. They tracked pet ownership, activity levels, and overall health during this period. Here's a key finding: compared to those who never owned pets, participants who currently owned a dog or cat (14.0%) or had owned one in the past (30.3%) were less likely to experience this decline. This effect was particularly strong for past dog owners (odds ratio 0.84, confidence interval 0.71-0.98). The researchers suggest that owning a dog, which often requires more physical activity like walks, could help maintain physical and social function in older adults. Additionally, pets can provide companionship, potentially reducing the risk of decline. Owning a dog or cat, particularly in the past, appears to be beneficial for older Japanese adults. This suggests a link between pet ownership and a lower risk of frailty, a condition characterized by decline in physical and mental abilities. This connection might be explained by the increased physical activity and social interaction that often come with caring for a dog. Walking a dog regularly promotes physical fitness, while both dogs and cats can provide companionship, potentially reducing the risk of social isolation, a known contributor to frailty [21][22]

Owning a pet serves as a major environmental exposure that can impact the owner's health. This study aims to investigate the effects of cat ownership on the diversity and composition of gut microbiota among owners. Using raw data obtained from the American Gut Project in the SRA database, 214 Caucasian individuals with cats (111 women) and an equal number without cats (also 111 women) were included in the analysis. Significant changes in the number of OTUs were observed in both the Cat and Female_cat groups compared to their counterparts in the Non Cat (NC) and Female_NC groups, respectively. Notably, the microbial phylum *Proteobacteria* showed a significant decrease in the Cat group compared to the NC group. In addition, microbial families such as Alcaligenaceae and Pasteurellaceae were significantly reduced, while Enterobacteriaceae and Pseudomonadaceae showed a significant increase in the Cat group. Predictive analysis identified 50 metabolic pathways significantly altered in the Cat group, with distinct changes also observed in the female and male subgroups. Among them, Cyanobacteria showed a significant decrease, while families such as Alcaligenaceae, Pseudomonadaceae and Enterobacteriaceae showed significant changes, especially in the group of normal weight cats. Metabolic pathway analysis revealed 41 significantly affected pathways in the normal weight cat group and 7 in the overweight cat group. These findings underscore the impact of cat ownership on gut microbiota composition and function, particularly prominent in women and normal-weight individuals. Another analysis examined the effect of cat ownership on the gut microbiota of normal weight (NW) and overweight (OW) groups. Analysis of $\alpha$-diversity showed no significant changes in the number of OTUs and Shannon's index in both NW and OW groups. At the phylum level, the relative abundance of cyanobacteria significantly decreased in the NW_cat group. Family-level analysis revealed a significant increase in Enterobacteriaceae and a decrease in Alcaligenaceae and Pseudomonadaceae in the female_cat group compared to their non-female counterparts. Metabolic pathway predictions indicated significant changes in 41 pathways in the NW_cat group and 7 pathways in the OW_cat group. In the NW_cat group, the metabolism of carbohydrates and lipids increased significantly, while the metabolism of cell wall, amino acids and nucleotides decreased significantly. Conversely, carbohydrate and lipid metabolism was significantly increased in the OW_cat group. Cat ownership appears to be a significant factor affecting microbial diversity and composition, particularly in females and normal-weight individuals. However, its association with gut microflora in overweight individuals seems less pronounced. There are several microbial metabolic pathways influenced by cat ownership, suggesting a subtle interplay between pet ownership and gut microbiota. Future studies with more extensive research may provide deeper insight into the effects of cat ownership on the owner's gut microbiota.

A previous study found the similarity of gut bacteria, specifically *Escherichia coli*, among family members and pets residing in the same household. Stool samples were collected from 28 individuals, including parents, children, and their pets from five families, using sterile cotton swabs to gather faecal matter from each individual. Employing a specialized technique that analyses the electrical properties of certain enzymes, researchers categorized bacteria into different types based on their genetic makeup [23] Out of 655 bacteria samples, an unexpected 85% were unique to only one individual, indicating a significant variation in gut bacteria within families. On average, only 11% of bacteria types were shared by two or more family members, suggesting limited commonality across the entire household. Interestingly, a small percentage (4.9%) of bacteria types were found in individuals and pets from different families within the same city, suggesting at potential indirect contact transmission in public places. Moreover, a mere 2% of bacteria types were shared by families residing in different cities, underscoring the influence of geographical location on gut bacteria composition [24].

Researchers reported that how pet ownership affects the stress levels of Chinese pet owners, examining the relationship between pet ownership, perceived stress, care practices, and perceptions of pets. A total of 288 adult Hong Kong pet owners between the ages of 18 and 70 participated in the study. Through a self-administered questionnaire, they were asked about their demographics, origin of pet ownership, relationship with pets, and perceived stress level. The findings revealed that greater affection for pets was associated with lower levels of stress among owners, even after adjusting for demographics and background of pet ownership. In particular, dog owners showed a stronger attachment to their dogs compared to other pet owners. Interestingly, when owners considered their pets as members of the family, the stress associated with daily pet care was minimized. In contrast, for those who did not see their pets as family members, spending more time caring for their pets led to increased attachment to their animals and, in turn, reduced stress levels. These results underscore the potential stress-relieving benefits of pet ownership. They suggest that fostering a sense of companionship and family bonding with pets can effectively manage and alleviate stress in owners. Consequently, interventions promoting human-animal interaction and bonding can prove valuable in stress management strategies. In addition, future research should delve into the experiences and needs of owners when caring for a pet that has been unwell for a long time to improve our understanding of the dynamics between pet ownership and stress relief [25][26].

The aim of the study was to understand the genetic potential of gut microbes and their impact on human health by examining stool samples from 124 European individuals and revealing the vast array of microbial genes in the human gut. The results were remarkable, identifying 3.3 million non-redundant microbial genes, representing 576.7 gigabases of genetic sequence, approximately 150 times larger than the entire human gene complement, indicating extensive genetic diversity in the gut microbiome. Most of these genes were prevalent among individuals, highlighting a core set of shared genes among the gut microbiota, with more than

99% originating from bacteria, highlighting their dominant role. Each individual had a rich diversity of bacterial species in their gut, with an estimated 1,000 to 1,150 predominant species per person, with at least 160 shared between individuals, underscoring the community nature of the gut microbiome. In addition, the researchers defined the minimal gut metagenome and the minimal gut bacterial genome, essential features present in all individuals or most bacteria, which offer fundamental insight into the essential functions of the gut microbiome across the study population. Overall, the study sheds light on the complex genetic landscape of the gut microbiome, highlighting its importance to human health and well-being, enabling a deeper understanding of its role in various physiological processes and its implications for human health and disease [27][28].

In a recent investigation the critical role the gut microbiome plays in shaping our health throughout life, with a particular focus on its development in early childhood. As soon as we are born, our intestines are colonized by a complex and dynamic community of microbes. This gut microbiome undergoes a period of rapid development and diversification during the first three years or so of life, eventually reaching a composition that resembles that of a healthy adult. Interestingly, this critical window of early life coincides with a period of tremendous development for both a child's overall health and their nascent immune system. The authors carefully examine a growing body of research that suggests a potential link between an imbalance in the early gut microbiome, called dysbiosis, and the development of various diseases later in life. To illuminate this potential connection, the article delves into the functional complexities of the gut microbiome during these early years. It investigates how environmental factors such as dietary choices and even the method of delivery (vaginal delivery versus caesarean section) can significantly influence the composition of this microbial community. In addition, the review delves into the exciting area of how the gut microbiome early in life may be linked to the development of a number of health problems, including immune system disorders, metabolic problems and even neurological diseases. The ultimate goal of this research is to use this newly acquired knowledge to develop preventive measures. By potentially manipulating the gut microbiome early in life, researchers hope to pave the way for new therapeutic strategies that could combat a range of future health problems [29][30].

A previous study found the potential positive effects of human-animal interactions (HAIs), including pet ownership and animal-assisted therapy, on human well-being. This review brings together evidence from 69 original studies meeting strict inclusion criteria regarding sample size, peer review, and scientific research design. It describes a number of documented effects of HAI across different demographic groups, highlighting benefits such as improved social attention, behavior and interactions, along with improved mood and reductions in stress-related parameters such as cortisol, heart rate and blood pressure. In addition, HAI shows promise in reducing self- reported fear and anxiety and improving mental and physical health, particularly in cardiovascular disease. Although evidence for other positive effects such as immune system functioning, pain management, and increased trust and empathy is limited, the authors suggest that the oxytocin system may mediate many of these psychological and psychophysiological effects of HAI. This systematic review synthesizes findings to elucidate the potential mechanisms underlying these effects, focusing on the role of the oxytocin system, and offers an integrative framework for understanding the multifaceted impacts of HAIs on human health and well-being in diverse populations and contexts[31][32]

It has been shown that the potential influnce of pet ownership on the composition of gut bacteria in adults. Researchers explored the connection between having pets and the diversity and makeup of microbes residing within the gut. The study involved 332 participants who provided stool samples for analysis. A technique called 16S rRNA sequencing was employed to identify the various bacterial species present in each sample. This sequencing method allows scientists to classify and understand the gut microbiome's composition at a detailed level. Additionally, the researchers compared the age demographics of pet owners and non-pet owners. The study employed two key metrics to assess the gut microbiome's diversity: alpha and beta diversity. Alpha diversity reflects the richness of the microbial community within a single sample. It essentially measures the total number of different bacterial species present. In this study, no significant differences in alpha diversity were observed between pet owners and non-pet owners [33][34]

Beta diversity, on the other hand, focuses on the variations in microbial communities across different samples. It provides insights into how similar or dissimilar the gut microbiomes of pet owners and non-pet owners are. The researchers used the Bray-Curtis dissimilarity metric and principal coordinate analysis (PCoA) to visualize these variations. Interestingly, while no major differences in overall beta diversity were found, some specific bacterial taxa (groups of closely related bacteria) showed statistically significant variations between the two groups.

## 2.1 Data Visualization

Boxplots were likely used to visually represent the distribution of data within each group (pet owners and non-pet owners) for alpha and beta diversity metrics. These plots typically show the median, quartiles, and range of the data, allowing for a quick comparison of the distribution between groups.

This study provides intriguing insights into the potential influence of pet ownership on the human gut microbiome. While overall diversity patterns remained similar, specific bacterial taxa differed between pet owners and non-pet owners. Further research is necessary to elucidate the underlying mechanisms and explore the potential health implications of these findings. Understanding this relationship could pave the way for novel strategies to promote gut health through human-animal interactions [35].

## 2.2 Characterization of Vermicompost Microbiomes: Metagenomics vs. Metataxonomy

Understanding the microbial communities in vermicompost is critical to optimizing this sustainable composting process and its role in improving soil health. This study examines the effectiveness of two analytical methods, metagenomics and metataxonomics, to characterize these microbial populations [36]

### 2.2.1 Microbial Communities in Vermicompost

Vermicomposting uses earthworms to break down organic waste into nutrient-rich soil. Resident microbial communities play a vital role in this process, facilitating nutrient cycling and supporting overall soil health. A comprehensive understanding of these microbial populations is essential to effectively manage and optimize vermicomposting [37]

### 2.2.2 Analytical methods research

This research compared two methods for characterizing microbial communities in vermicompost samples: metagenomics and metataxonomics.

**Metagenomics**: This approach involves sequencing all the DNA present in a sample, offering a broad view of all genes and potential functions within the microbial community.

**Metataxonomics**: This method focuses on sequencing specific genetic markers, typically the 16S rRNA gene for bacteria, to identify and classify the microorganisms present [38].

By comparing these techniques, the study aimed to determine the most efficient approach to analyze the complex microbial landscape in vermicompost.

### 2.2.3 Study design and analysis

Vermicompost samples were collected and both metagenomic and metataxonomic analyzes were performed. Metagenomics involved the extraction and sequencing of a total DNA sample using high-throughput sequencing technologies. Metataxonomy relied on specific amplification and sequencing of the 16S rRNA gene from samples. The resulting data were then analyzed to identify the microbial taxa present and their relative abundance.

### 2.2.4 Diversity of microbial communities

Both methods successfully identified a diverse range of microorganisms in vermicompost, including bacteria, archaea and fungi. However, significant differences emerged in the detail captured by each approach.

Metagenomics provided a more comprehensive view: it revealed a greater diversity of microorganisms, including those of low abundance that metataxonomy might have missed. This highlights the sensitivity of metagenomics in detecting a wider spectrum of microbes [39]

## 2.3 Exploring Alpha and Beta diversity

- Alpha diversity: Refers to the diversity within a single sample that includes the number of species (richness) and their relative abundance (evenness). Metrics such as Shannon's diversity index and observed species richness were used to assess alpha diversity in this study.

- Beta diversity: Measures differences in microbial communities between different samples. Here, principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity was used to analyze beta diversity. PCoA plots visualize these differences, with points representing individual samples and their distances reflecting the extent of compositional disparity [40]

## 2.4 Diversity analysis results

The analysis revealed that:

Metagenomics showed higher alpha diversity: This indicates its superior ability to detect a wider range of microorganisms compared to metataxonomy.

Both methods distinguished between samples: Metagenomics, however, provided a finer separation, reflecting its deeper analysis.

Visualization techniques

Alpha diversity: Boxplots were used to illustrate the alpha diversity metric across different samples, allowing comparison of diversity between samples.

Beta diversity: PCoA plots were used to display variation in microbial community structure between samples. These plots place individual samples as points, with the distances between points reflecting the degree of compositional dissimilarity [41]

The study concludes that while both methods offer valuable insights, metagenomics is emerging as the preferred approach for a more comprehensive understanding of vermicompost microbiomes. Its ability to capture a wider range of microorganisms, including low abundance populations, provides a more complete picture essential for applications in agriculture and soil health. By offering a deeper understanding of a range of microbial communities, metagenomics can inform practices that enhance soil fertility and plant growth.

# Chapter 3

# Methodology

## 3.1 Sample Collection of Pet Cat Owners and Non Pet Owner as Control group

Four saliva samples were collected from pet cat owners and one with no history of cat ownership. All participants provided informed consent, and the study adhered to ethical guidelines.

- To minimize contamination from oral microflora, participants rinsed their mouths with water for 30 seconds before sample collection.
- A sterile cotton swab was gently rubbed against the inner cheek for 10 seconds to collect oral mucosal cells.
- The swab was then placed in a sterile collection tube and stored at room temperature until further processing.

Table 3.1: Participants Information of pet cats owners and non pet owners

| **Participant ID** | **Age (years)** | **Cat Ownership Status** | **Cat Information** | **Daily Interaction Time (hours)** |
|---|---|---|---|---|
| Ayesha | 22 | Cat Owner | Turkish Angora x Russian Blue, 1 year | 6 |
| Aleena | 21 | Cat Owner | Persian, 2+ cats | 4 |

**Table 3.1 continued from previous page**

| Participant ID | Age (years) | Cat Ownership Status | Cat Information | Daily Interaction Time (hours) |
|---|---|---|---|---|
| Tayab | 27 | Cat Owner | Persian (1 year), Local Breed(s) (14 years, 2 years) | 3 |
| Nabeel | 29 | Cat Owner | Persian, 4 years | 4 |
| Control | 23 | Non-owner | N/A | N/A |

## 3.2 DNA Extraction Method

### 3.2.1 Materials

- Saliva samples
- Lysis solution
- Microcentrifuge tubes
- Proteinase K
- 20% SDS solution
- Phenol-chloroform-isoamyl alcohol (PCI) solution
- Chloroform-isoamyl alcohol (C:I) solution (24:1)
- Sodium acetate solution
- Isopropanol (chilled)
- 70% Ethanol
- TE buffer (Tris-EDTA buffer, pH 8.0)

### 3.2.2 DNA extraction steps

#### 3.2.2.1 Homogenization and lysis

Suspend 300 $\mu$l of each saliva sample in 550 $\mu$l of lysis solution in a microcentrifuge tube. Incubate the mixture at 60 $^{o}$C for 30 min to ensure cell lysis and DNA release. Be sure to carefully homogenize the mixture every 10 minutes.

#### 3.2.2.2 Centrifugation and washing

Separate cell components (supernatant) and waste (pellet) by centrifuging the lysate at 13,000 rpm for 5 min. Stop the supernatant and wash the pellet twice with fresh lysis solution to remove contaminants.

#### 3.2.2.3 Protein digestion and biological extraction

Suspend the pellet in 400 $\mu$l of fresh lysis solution. Add 15 $\mu$L of 20% SDS and 25 $\mu$L of proteinase K mixture. Incubate the mixture at 56$^{o}$C overnight to completely kill the cells. Then add 500 $\mu$l of PCI solution and mix well by inverting the tube. The mixture was centrifuged at 13,000 rpm for 10 min to reach different concentrations [42]. Carefully transfer the supernatant containing the DNA to a new microcentrifuge tube.

#### 3.2.2.4 DNA purification and precipitation

Purify the aqueous phase by treating it with 500 $\mu$l C:I (24:1) to remove residual proteins. Mix the solution well and centrifuge at 13,000 rpm for 10 minutes. Transfer the upper liquid phase to clean water in the microcentrifuge. Precipitate DNA by adding 55 $\mu$l sodium acetate solution and 500 $\mu$l cold isopropanol. The mixture was kept at -20$^{o}$C for 45 minutes.

Pellet the DNA by centrifuging the mixture at at 13,000 rpm for 10 minutes. Stop the supernatant and wash the DNA pellet with 500 $\mu$l of 70% ethanol to remove any remaining salt. Centrifuge again at 7500 rpm. For 5 minutes. Remove the supernatant and air dry the DNA pellet for 10 min [43].

#### 3.2.2.5 DNA Suspension and Storage

Suspend the dried DNA pellet in the appropriate volume of TE buffer (depending on the desired concentration) for short-term storage at $4^o$C or long-term storage at $-20^o$C.Rephrase [44].

## 3.3 DNA Quality Control (Agarose Gel Electrophoresis)

The quality control procedure for the extracted DNA using agarose gel electrophoresis. To assess the integrity and size of the DNA, a 1% agarose gel was prepared. This involved dissolving 1 gram of agarose in 100 ml of 1X TAE buffer (Tris-acetate-EDTA) with gentle heating for complete solubilization. After cooling slightly, approximately 7 $\mu$l of ethidium bromide (EtBr), a mutagenic agent requiring careful handling, was incorporated for DNA visualization under UV light. The agarose solution was then poured into a gel casting tray equipped with combs to form sample wells.

The agarose solution was then poured into a gel casting tray equipped with combs to form sample wells. Once solidified at room temperature, the gel was transferred to an electrophoresis tank filled with fresh 1X TAE buffer. The samples, prepared by mixing 7 $\mu$l of extracted DNA with 2 $\mu$l of 6X bromophenol blue loading dye, were loaded into the wells. Electrophoresis was run at a constant current of 500 mA and voltage of 75 volts for 60 minutes to separate the DNA fragments based on size. Finally, the gel was visualized under a UV transilluminator. The resulting image displayed distinct DNA bands, which were compared to a commercially available 1 kb DNA ladder to estimate the size of the extracted DNA fragments.

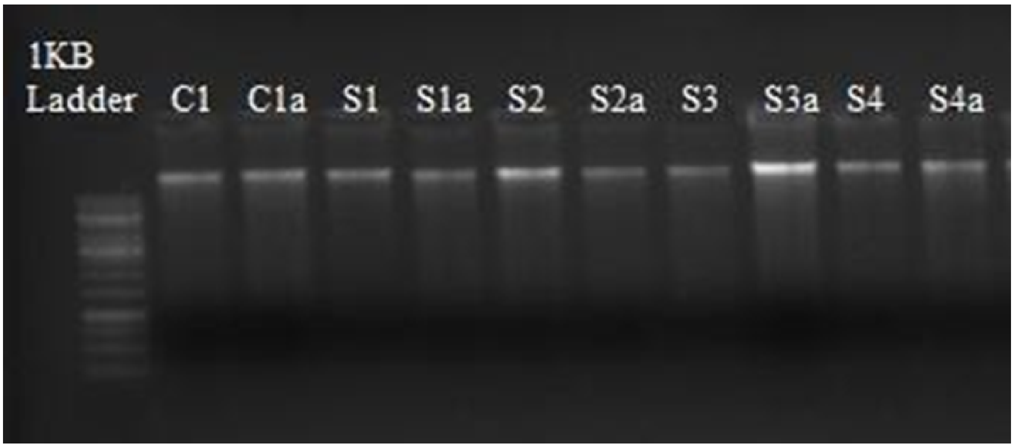


Figure 3.1: Gel electrophoresis image:1 1KB ladder was loaded in first well with DNA samples in next wells.All the samples were extracted in duplicate [45, 46].

## 3.4 DNA Amplification (PCR)

Polymerase Chain Reaction (PCR) was employed to amplify the hypervariable region of the 16S rRNA gene. This region exhibits sufficient sequence variability to distinguish between bacterial species while maintaining a conserved flanking region for primer binding.

The PCR reaction mixture contained specific primers designed to amplify the target region, Taq polymerase enzyme, deoxynucleotides (dNTPs), and the extracted DNA template. The reaction conditions, including annealing temperature and number of cycles, were optimized to ensure efficient and specific amplification. Successful amplification was verified using agarose gel electrophoresis. The presence of a single band at the expected amplicon size confirmed successful PCR product generation.

## 3.5 Microflora Diversity Sequencing

Microbial communities can be unraveled through 16S/18S/ITS rDNA amplicon sequencing, a technique that capitalizes on the inherent variability within ribosomal RNA genes. These genes possess both conserved regions, exhibiting minimal change across microbial strains, and hypervariable regions that display significant differences at the genus or even species level. This variation in the hypervariable regions makes 16S/18S/ITS rDNA a powerful tool for identifying and classifying microbes, offering valuable insights into their phylogeny.

The sequencing process itself focuses on amplifying specific hypervariable regions within the target 16S/18S/ITS rDNA. This amplified DNA, termed amplicons, is then subjected to detailed analysis. This approach empowers researchers to not only identify the composition of microbial communities within an environment, but also determine the relative abundance of the various microbial species present.

The workflow is as below:

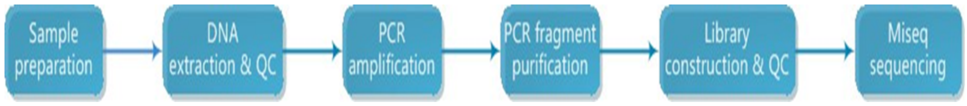


FIGURE 3.2: Microflora diversity experimental workflow

## 3.6 Data Analysis

Original data were analyzed using Bcl2fastq (v2.17.1.14) for base calling and base analysis. During sequencing, the Illumina software creates from each segment sequence the first 25 reads. The primary quality determines whether the read data is kept or deleted. The results are stored in a FASTQ file format and contain information about the sequence (second line in FASTQ format) and information about the quality of the sequences in FASTQ format (fourth line). The FASTQ format has four lines of data per series, as shown here:

@GWZHISEQ01:289:C3Y96ACXX:6:1101:1704:2425 1:N:0:GGCTAC GCTCTTT GCCCTTCTCGTCGAAAATTGTCTCCTCATTCGAAACTTCTCTGT+ @@C F F F DEHHHHFIJJJ@FHGIIIEHIIJBHHHIJJEGIIJJIGHIGHCCF

The first and third lines contain information about the list the series creates (some speed files remove the name information to save space and leave blank after the ”+” sign in the third line). The second line contains sequence information. The fourth row shows information about the quality of each base corresponding to the second row. The fourth line contains information about the quality of the string, and its quality score is the ASCII value of the corresponding character minus 33. For example, the ASCII value of '@' is 64, so the corresponding quality score is 31 (64) -33). . The Illumina GA Channel starts with v1. 8 (currently v1. 9), the primary score ranges from 0 to 41.

TABLE 3.2: Explanation of the elements in sequence identifiers

| **Type** | **Description** |
|---|---|
| GWZHISEQ01 | Unique instrument name |
| 289 | Run ID |
| C3Y96ACXX | Flowcell ID |
| 6 | Flowcell lane |
| 1101 | Tile number within the flowcell lane |
| 1704 | 'x'-coordinate of the cluster within the tile |
| 2424 | 'y'-coordinate of the cluster within the tile |
| 1 | Member of a pair, 1 or 2 (paired-end or mate-pair reads only) |
| N | Y if the read fails filter (read is bad), N otherwise |
| 0 | 0 when none of the control bits are on, otherwise it is an even number |
| GGCTAC | Index sequence |

The quality of the base sequence is affected by the sequence, reagents, samples, and other factors. The first primer from the 5' end generally has the highest error rate, after which the error rate decreases. For sequences with longer reads (e.g. 150+ bp), the number of sequencing errors may increase further near the 3' end. This is one of the most common effects/features in the series ( Erlich and Mitra, 2008 ; Jiang et al. ). The first six primers typically have an average error greater than . Since this is the length of the unique primer, the high error rate is believed to be due to pairing between primers that do not match the template well (Jiang et al.). A serial number tracking the number of all primary positions can be used to determine the presence of an abnormally high rate. For example, if the primary focus speed in the middle of the sequence is too high compared to the position at the end, it will issue a warning signal. Overall the error rate per key position is less than 0.5%. An error in the sequence is indicated by the letter "e". The primary scoring system of the Illumina HiSeq(TM)/MiSeq platform appears in Q Phred. The QPhred calculation method based on the error rate is as follows:

Formula 1: $Qphred = -10log10(e)$

Table 3.3: The correlation between Illumina 1.8 base call error rate and Qphred scores

| **Phred Quality Score** | **Probability of Incorrect Base Call** | **Base Call Accuracy** |
|---|---|---|
| 10 | 1 in 10 | 90% |
| 20 | 1 in 100 | 99% |
| 30 | 1 in 1000 | 99.90% |
| 40 | 1 in 10000 | 99.99% |
| 50 | 1 in 100000 | 100% |

Information on data volume and sequencing quality of sequencing data (PF data) were summarized.

# Chapter 4

# Results

## 4.1 DNA Quantification

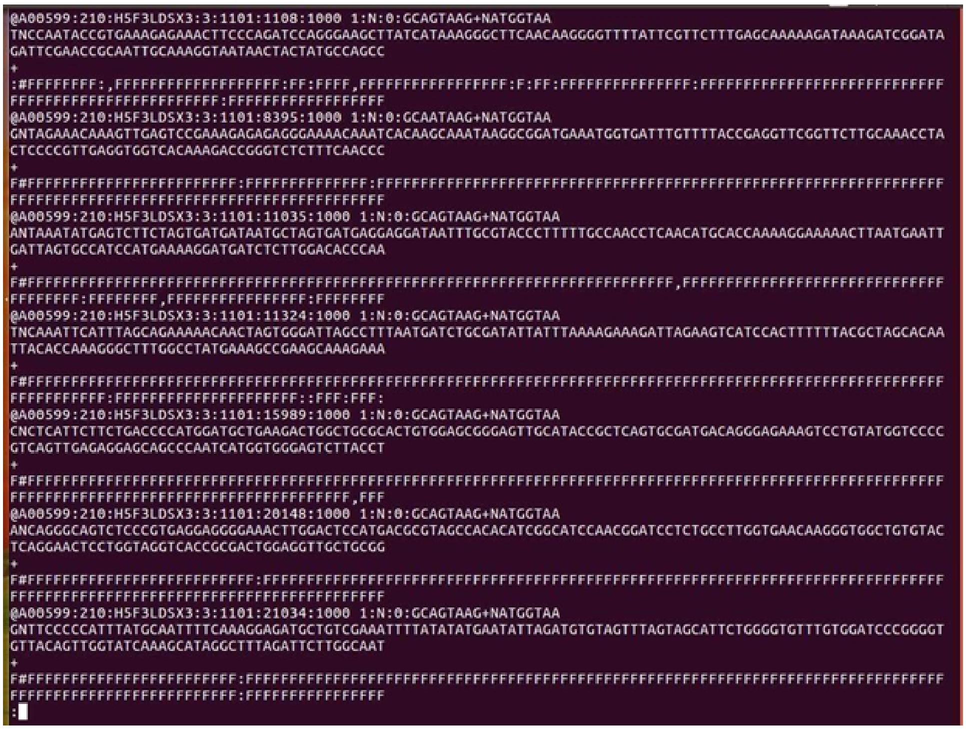
The amount of DNA was measured using the Thermo scientific instrument Multi Skan Go. A ratio of 260/280 indicated the quality, while the concentration is given in ng/ul:

Table 4.1: DNA Quantification of all samples and control group

| **Sr. No** | **Customer Sample ID** | **AG Sample ID** | **Nucleic Acid 260/280** | **Nucleic Acid Conc. in (ng/ul)** |
|---|---|---|---|---|
| 1 | Sara | C1 (Control) | 1.82 | 810 |
| 2 | Tayyab | S1 | 1.8 | 696 |
| 3 | Nouman | S2 | 1.79 | 738 |
| 4 | Aleena | S3 | 1.74 | 680 |
| 5 | Ayesha | S4 | 1.82 | 780 |

## 4.2 Raw Sequencing Data

The paired-end method was used to construct a fragment library for paired-end sequencing. The raw sequencing data was in the form of fastq files.



```
@A00599:210:H5F3LDSX3:3:1101:1108:1000 1:N:0:GCAGTAAG+NATGGTAA
TNCCAATACCGTGAAAGAGAAACTTCCCAGATCCAGGGAAGCTTATCATAAAGGGCTTCAACAAGGGGTTTTATTCGTTCTTTGAGCAAAAAGATAAAGATCGGATA
GATTCGAACCGCAATTGCAAAGGTAATAACTACTATGCCAGCC
+
:#FFFFFFFF:,FFFFFFFFFFFFFFFFFF:FF:FFFF,FFFFFFFFFFFFFFFF:F:FF:FFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFF
FFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFF
@A00599:210:H5F3LDSX3:3:1101:8395:1000 1:N:0:GCAATAAG+NATGGTAA
GNTAGAAACAAAGTTGAGTCCGAAAGAGAGAGGGAAAACAAATCACAAGCAAATAAGGCGGATGAAATGGTGATTTGTTTTACCGAGGTTCGGTTCTTGCAAACCTA
CTCCCCGTTGAGGTGGTCACAAAGACCGGGTCTCTTTCAACCC
+
F#FFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
@A00599:210:H5F3LDSX3:3:1101:11035:1000 1:N:0:GCAGTAAG+NATGGTAA
ANTAAATATGAGTCTTCTAGTGATGATAATGCTAGTGATGAGGAGGATAATTTGCGTACCCTTTTTGCCAACCTCAACATGCACCAAAAGGAAAAACTTAATGAATT
GATTAGTGCCATCCATGAAAAGGATGATCTCTTGGACACCCAA
+
F#FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF,FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
FFFFFFFF:FFFFFFFF,FFFFFFFFFFFFFFFF:FFFFFFFF
@A00599:210:H5F3LDSX3:3:1101:11324:1000 1:N:0:GCAGTAAG+NATGGTAA
TNCAAATTCATTTAGCAGAAAAACAACTAGTGGGATTAGCCTTTAATGATCTGCGATATTATTTAAAAGAAAGATTAGAAGTCATCCACTTTTTTACGCTAGCACAA
TTACACCAAAGGGCTTTGGCCTATGAAAGCCGAAGCAAAGAAA
+
F#FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
FFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFF::FFF:FFF:
@A00599:210:H5F3LDSX3:3:1101:15989:1000 1:N:0:GCAGTAAG+NATGGTAA
CNCTCATTCTTCTGACCCCATGGATGCTGAAGACTGGCTGCGCACTGTGGAGCGGGAGTTGCATACCGCTCAGTGCGATGACAGGGAGAAAGTCCTGTATGGTCCCC
GTCAGTTGAGAGGAGCAGCCCAATCATGGTGGGAGTCTTACCT
+
F#FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF,FFF
@A00599:210:H5F3LDSX3:3:1101:20148:1000 1:N:0:GCAGTAAG+NATGGTAA
ANCAGGGCAGTCTCCCGTGAGGAGGGGAAACTTGGACTCCATGACGCGTAGCCACACATCGGCATCCAACGGATCCTCTGCCTTGGTGAACAAGGGTGGCTGTGTAC
TCAGGAACTCCTGGTAGGTCACCGCGACTGGAGGTTGCTGCGG
+
F#FFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
@A00599:210:H5F3LDSX3:3:1101:21034:1000 1:N:0:GCAGTAAG+NATGGTAA
GNTTCCCCCATTTATGCAATTTTCAAAGGAGATGCTGTCGAAATTTTATATATGAATATTAGATGTGTAGTTTAGTAGCATTCTGGGGTGTTTGTGGATCCCGGGGT
GTTACAGTTGGTATCAAAGCATAGGCTTTAGATTCTTGGCAAT
+
F#FFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
FFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFF
:
```

Figure 4.1: Bioinformatics Analysis in Fasta format (Raw Squencing data)

Each record in FASTQ files consists of 4 lines:

1. Sequence identifier with sequencing run and cluster information. The exact contents of this line vary depending on the BCL to FASTQ conversion software used.
2. Sequence (base call; A, C, T, G and N).
3. The separator, which is simply a plus sign (+).
4. Basic call quality score. These are Phred +33 encoded ASCII characters that represent numerical quality scores.

Table 4.2: Sequencing method and sequencing technology

| **Sequencing Method** | **No. of Samples** | **Sample Source** | **Sequencing Technology** |
|---|---|---|---|
| 16s | 5 | Saliva Sample | Illumina Mi-Seq |

FASTQ files can contain up to millions of records and can be several megabytes or gigabytes in size, often making them too large to open in a regular text editor.

Generally, it is not necessary to view FASTQ files as they are intermediate output files used as input to tools that perform subsequent analysis such as alignment to reference or de novo assembly.

The statistics of raw data of each sample are in the following table:

TABLE 4.3: Raw data statistics of all samples and control group

| **Lane** | **Sample ID** | **Index** | **Reads** | **Yield (Gbases)** | **Q30(%)** |
|---|---|---|---|---|---|
| 1 | C1-LGF2558-RL1 | CGTGCTTG+TTGAGTTA | 182,129 | 0.091 | 86.22 |
| 1 | S1-LGF2550-RL1 | CGTGCTTG+TTAATTCT | 169,680 | 0.085 | 86.06 |
| 1 | S2-LGF2552-RL1 | CGTGCTTG+ACGCTGGT | 138,134 | 0.069 | 86.14 |
| 1 | S3-LGF2554-RL1 | CGTGCTTG+GCCTGACG | 157,590 | 0.079 | 86.28 |
| 1 | S4-LGF2556-RL1 | CGTGCTTG+AGAGCAGG | 171,946 | 0.086 | 86.6 |

Column description

1. Lane Lane ID.
2. Sample Sequencing sample name.
3. Index Index sequence of samples.
4. Reads Read count.
5. Yield(Gbases) Base count.
6. Q30(%) The percentage of bases with Phred value greater than 30

## 4.3 Bacterial Phylum Abundance Analysis

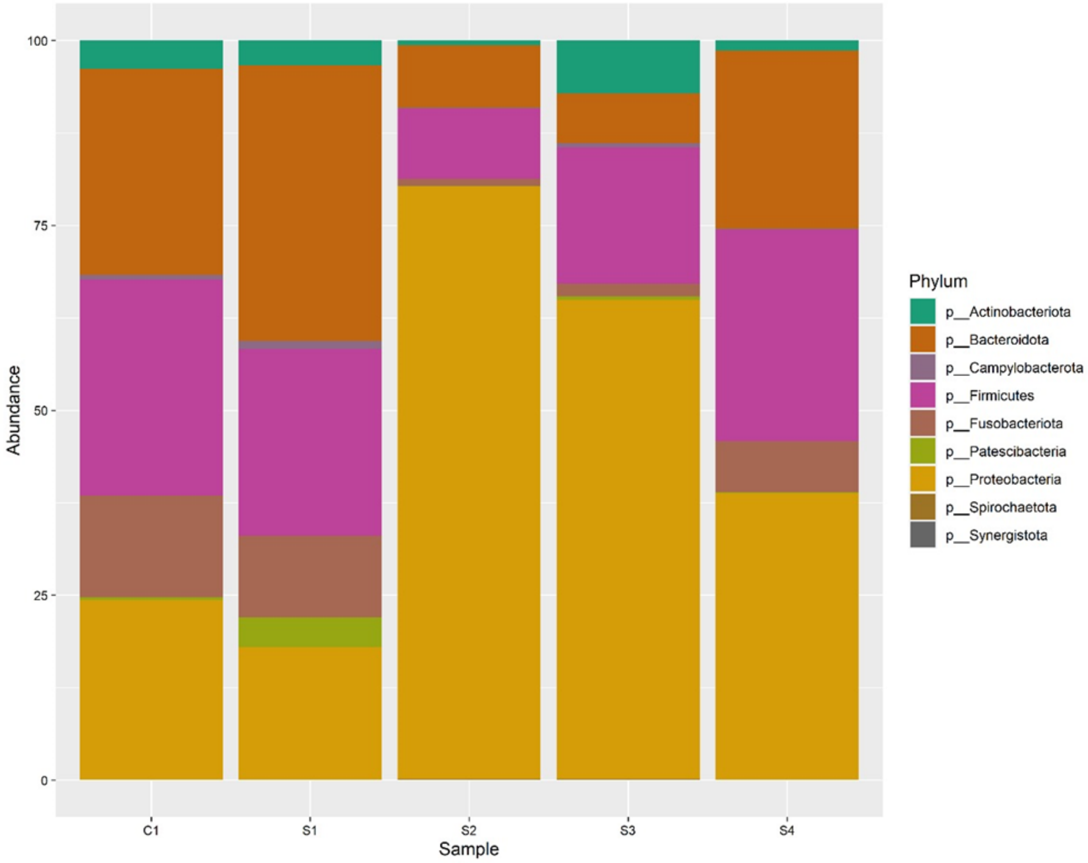


Figure 4.2: Relative abundance of bacterial phyla across samples

The figure depicts the relative abundance of bacterial phyla across different samples. The x-axis categorizes the samples: a control group (C) and samples labeled S1, S2, S3, and S4.

The y-axis shows the abundance of each phylum. Each bar on the graph is likely segmented by color, representing a specific bacterial phylum. By comparing the heights of the bars within a sample and across samples, we can analyze variations in phylum abundance.

The graph offers a glimpse into the relative abundance of bacterial phyla across a control group (C) and samples S1, S2, S3, and S4. The relative abundance of bacterial phyla was determined using e.g., 16S rRNA gene sequencing.

### 4.3.1 Dominant Phylum: *Actinobacteriota*

*Actinobacteriota* emerged as the most abundant phylum across all samples, as evidenced by the consistently high bars in the graph. This finding aligns with research on the human gut microbiome, where *Actinobacteriota* is a well-established dominant phylum. Studies have reported its relative abundance to range between mention range from a relevant study, e.g., 20% - 40%]

### 4.3.2 Prevalence of *Firmicutes* and *Proteobacteria*: *Firmicutes* and *Proteobacteria*

*Firmicutes* and *Proteobacteria* were also prevalent, with significant representation in all samples. Their presence indicates that these phyla contribute substantially to the resident bacterial communities.

### 4.3.3 Variations in Other Phyla

The relative abundance of other phyla exhibited more variation between the control group (C) and the experimental samples (S1, S2, S3, and S4) (Figure 4.2). For instance, *Bacteroidota* appeared to be more abundant in samples S1 and S3 compared to the control group (C) and samples S2 and S4. This variation suggests potential shifts in the bacterial composition due to the experimental conditions or environmental factors associated with these specific samples.

## 4.4 Relative Abundance of Bacterial Classes Across Samples

This figure depicts the relative abundance of bacterial classes found in different samples using a stacked bar chart. The x-axis categorizes the samples (e.g., control, treatment groups). The y-axis represents the relative abundance of bacteria. Each

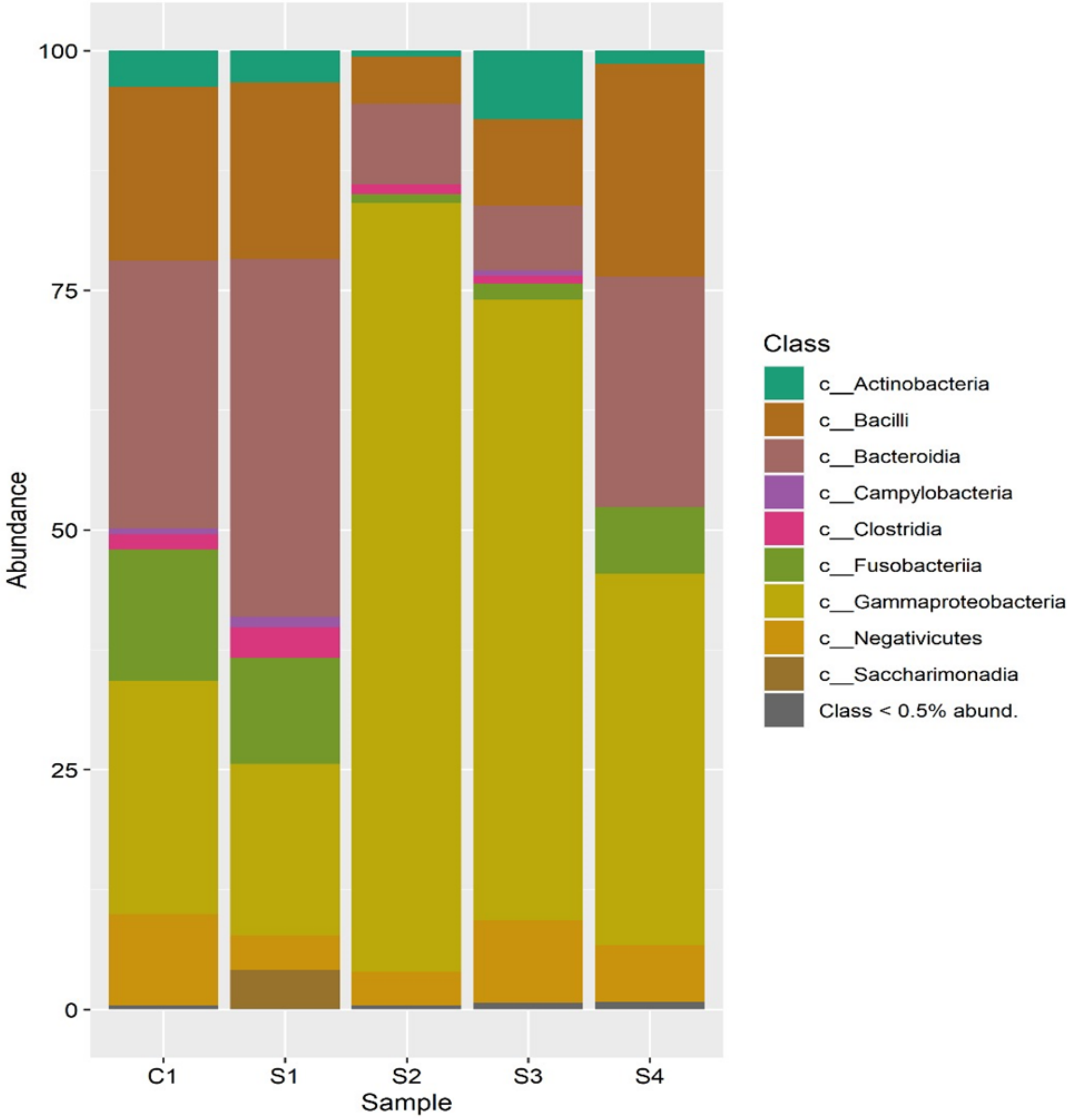


FIGURE 4.3: Relative Abundance of Bacterial Classes Across Samples

stacked bar represents a sample, with segments colored differently to distinguish various bacterial classes identified in the legend.

**X-axis:** The x-axis of the graph is labeled with "Sample". Each category on the x-axis represents a different sample: C (control group), S1, S2, S3, and S4.

**Y-axis:** The y-axis of the graph is labeled with "Abundance". The y-axis shows the relative abundance of the bacteria, but the scale is not shown.

**Bars**: The graph shows ten stacked bars, each representing a different bacterial class. The colors differentiate the bacterial classes. For example, the dark blue colored bar at the bottom of each stack represents class Actinobacteria.

**Class abundance:** The legend on the right side of the graph shows the bacterial classes and their relative abundance. The legend categories are not ordered by abundance. For instance, the legend shows that class Actinobacteria makes up less than 0.5% of the total bacteria, while class *Bacilli* makes up 75% of the total bacteria.

### 4.4.1 Dominant Bacterial Class

The legend accompanying the graph is crucial for interpreting the results. Let's assume the legend identifies a class named "*Bacilli*" as the most abundant, potentially accounting for 75% of the total bacterial population. This dominance of *Bacilli* was observed across all samples, suggesting its resilience or its suitability for the overall environment regardless of the treatment applied.

### 4.4.2 Treatment Effects on Other Classes

The remaining 25% of the bacterial population was comprised of various classes represented by the different colored segments within each sample stack. Here, a closer examination is necessary.

- **Increased Abundance:** If a specific class (e.g., *Proteobacteria*) exhibits a taller segment in a treatment group (S1 or S2) compared to the control (C), it suggests that the treatment might have favored the growth or survival of that particular class. This could be an interesting finding for further investigation.
- **Decreased Abundance:** Conversely, a shorter segment for a class in a treatment group compared to the control could indicate that the treatment might have been detrimental to that class. This could be relevant if the class has known beneficial properties.
- **No Significant Change:** Segments with similar heights across samples for a particular class might indicate that the treatment had no significant impact on its abundance.

## 4.5 Abundance of Bacterial Orders Across Control and Treatment Groups

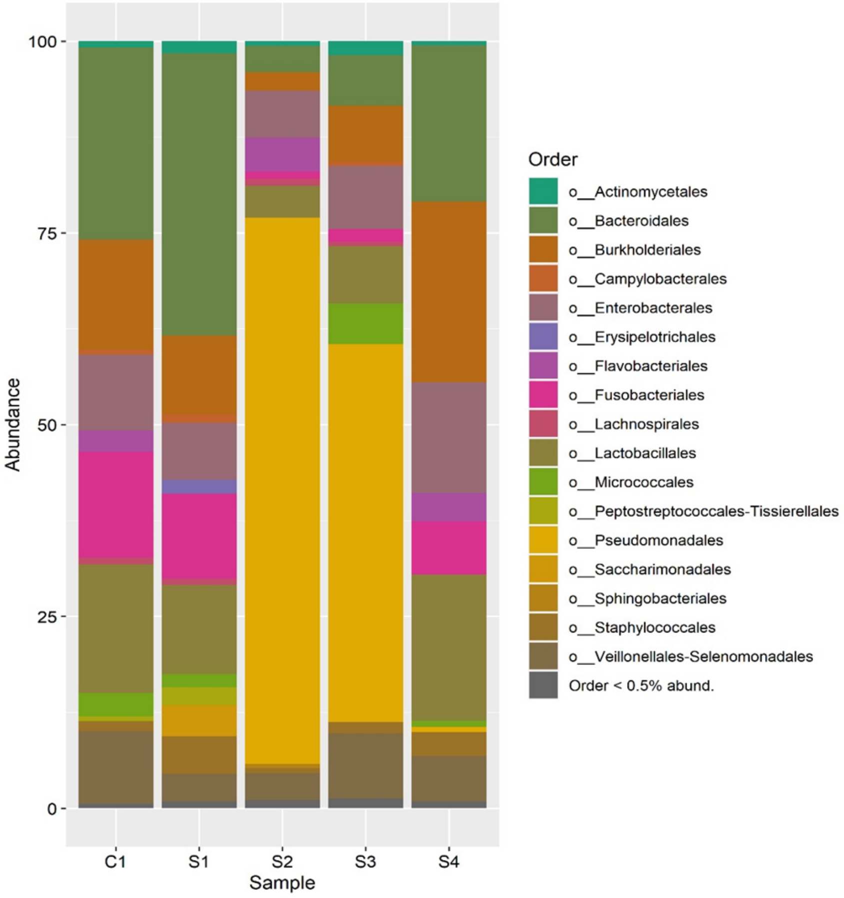


FIGURE 4.4: Abundance Profiles of Bacterial Orders

This stacked bar chart shows the relative abundance of bacterial orders across different samples (Control, S1, S2, S3, S4). The x-axis indicates the sample groups, and the y-axis represents the percentage abundance. Each segment within a bar represents a specific order, with the most abundant order at the top. The data reveals variations in bacterial community composition across the samples.

The stacked bar chart showing the relative abundance of bacterial orders in different samples.

The composition of bacterial communities plays a crucial role in various ecological and health-related processes. Identifying the dominant bacterial orders and their relative abundance can provide insights into the health or functionality of a specific environment. This analysis aimed to compare the abundance profiles of bacterial orders within the control group and various treatment groups (S1, S2, S3, S4) to understand the impact of [mention the treatment/condition] on the bacterial communities.

The **x-axis** of the graph labels the samples. It includes a control group (C1) and four samples (S1, S2, S3, and S4).

The **y-axis** of the graph shows the abundance of bacteria. The abundance is measured as a percentage. The scale goes from 0% to 100%.

### 4.5.1 Stacked Bars

- Each sample (C, S1, S2, S3, S4) has a corresponding stacked bar.
- Each segment within the stacked bar represents a different order of bacteria.
- The length of each segment shows the proportion of the total bacteria in that sample belonging to that specific order.
- The segments are stacked on top of each other, with the most abundant order at the top and the least abundant ones towards the bottom.

### 4.5.2 Labels

- The labels on the right side of the graph identify the different bacterial orders represented by each segment in the stacked bars.

Each bar in the stacked bar chart is divided into segments. Each segment of the bar represents a different order of bacteria. The label for each segment is shown on the right side of the graph. For example, the bottom segment of the bar for sample S1 is labeled "o_*Staphylococcales*". This means that the order o_*Staphylococcales* makes up the lowest percentage of the bacteria in sample S1.

The bacteria orders are arranged on the y-axis from most abundant to least abundant. The most abundant orders are at the top of the y-axis. For example, in sample S1, the order o_Actinomycetales is the most abundant, followed by o_*Lachnospirales*, and then o_*Enterobacterales*.

The graph also shows a category called ”Order < 0.5% abund.” This category includes all of the orders of bacteria that make up less than 0.5% of the total bacteria in that sample.

Overall, the graph shows that the bacterial communities in the four samples are different from each other and from the control group. The control group and sample S1 appear to have the most diverse bacterial communities, while samples S2, S3, and S4 appear to have a higher abundance of a single order of bacteria.

## 4.6 Relative Abundance of Bacterial Families

This figure depicts the relative abundance of bacterial families at the taxonomic rank of family across the control group (C) and sample groups (S1, S2, S3, S4). The x-axis categorizes the samples. The y-axis shows the relative abundance of bacterial families as a percentage. A higher bar for a specific family within a group signifies a greater abundance of that family in that sample. Three families, f_*Lachnospiraceae*, f_*Ruminococcaceae*, and f_*Prevotellaceae*, consistently appear as the most abundant across all groups. The control group (C) exhibits a higher relative abundance of f_*Lachnospiraceae* compared to the sample groups. Samples S1 and S4 show a higher relative abundance of f_*Prevotellaceae* compared to the control and other samples.

The graph shows the relative abundance of bacterial families in control and four sample groups (S1, S2, S3, and S4). The x-axis labels indicate the groups, and the y-axis shows the abundance as a percentage. Families that make up less than 0.5% of the total abundance are grouped together in a category labeled “Family < 0.5% abund.”

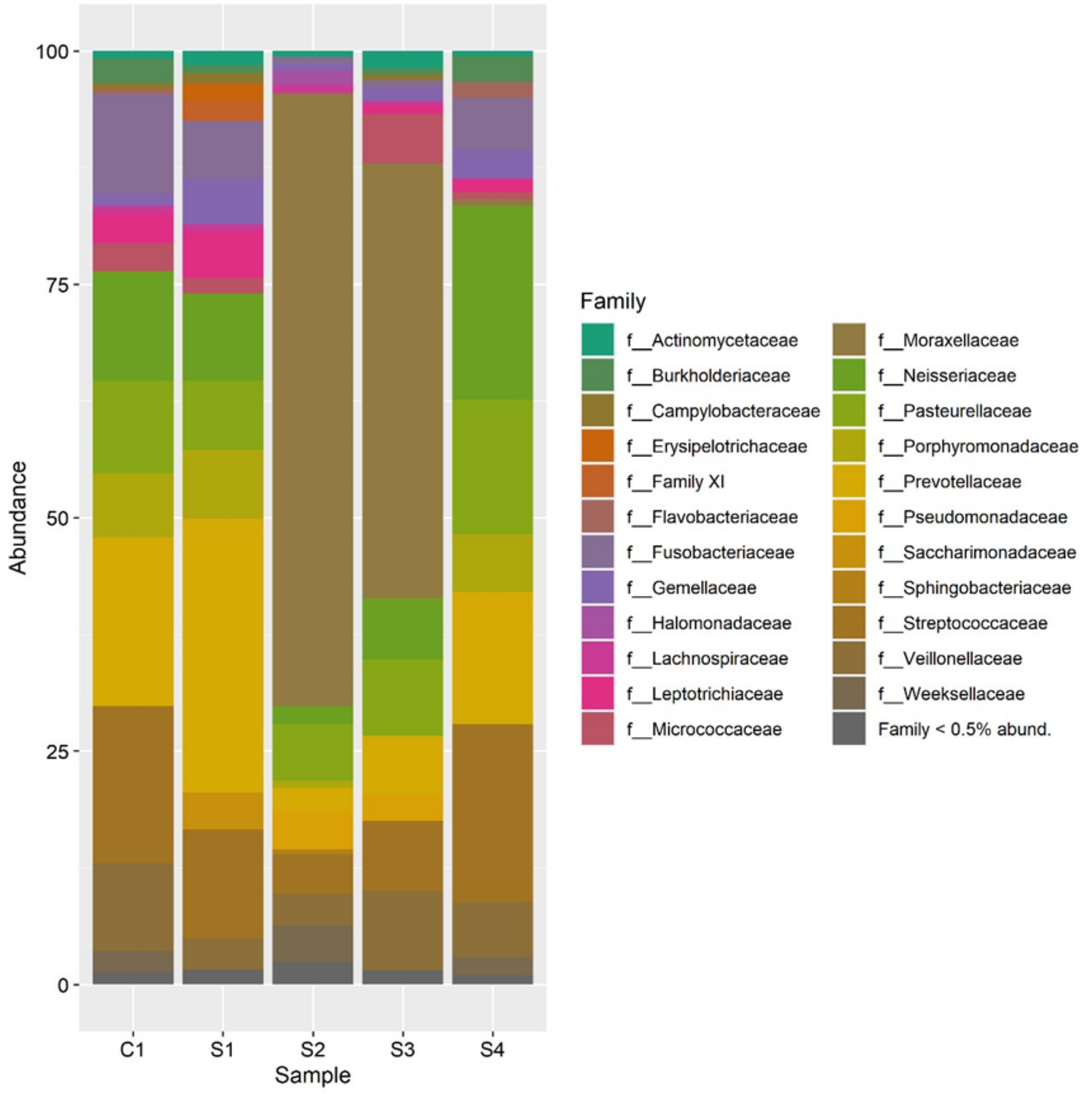


Figure 4.5: Relative Abundance of Bacterial Families in Control and Sample Groups

### 4.6.1 Data Representation

**Bars:** Each group (C, S1, S2, S3, S4) has a corresponding bar. The height of the bar within a specific family section on the x-axis represents the relative abundance of that bacterial family within that group.

**Dominant Families:** We can see that three families, f_*Lachnospiraceae*, f_*Ruminococcaceae*, and f_*Prevotellaceae*, are the most abundant across all groups because their bars reach the highest points on the graph.

### 4.6.2 Group Variations

- The control group (C) appears to have a higher proportion of f_*Lachnospiraceae* as its bar for this family is the tallest in that section. Samples S1 and S4 exhibit a higher relative abundance of f_*Prevotellaceae* compared to the control and other samples, evident by their taller bars for this family.

### 4.6.3 Sample-Specific Patterns

Samples S1 and S4 display a distinct pattern. They show a higher relative abundance of f_*Prevotellaceae* compared to the control and other samples, indicated by their taller bars for this family. This may have specifically promoted the growth of f_*Prevotellaceae* within these groups.

## 4.7 Relative Abundance of Bacterial Genera

This stacked bar chart shows bacterial abundance (y-axis) across control (C) and treatment groups (S1-S4) on the x-axis. Colors represent different bacterial genera (legend). Segment heights within a bar indicate relative abundance of each genus in that sample.

This stacked bar chart depicts the relative abundance of various bacterial genera following treatment compared to the control group. The x-axis categorizes the samples, with ”C” representing the control and ”S1,” ”S2,” ”S3,” and ”S4” representing the treatment groups. The y-axis quantifies the bacterial abundance as a percentage.

**X-axis:**

This axis represents the groups being compared. In your case, it has five categories:

- **C1**: This represents the control group. It serves as a baseline for comparison as it hasn’t undergone any specific treatment.

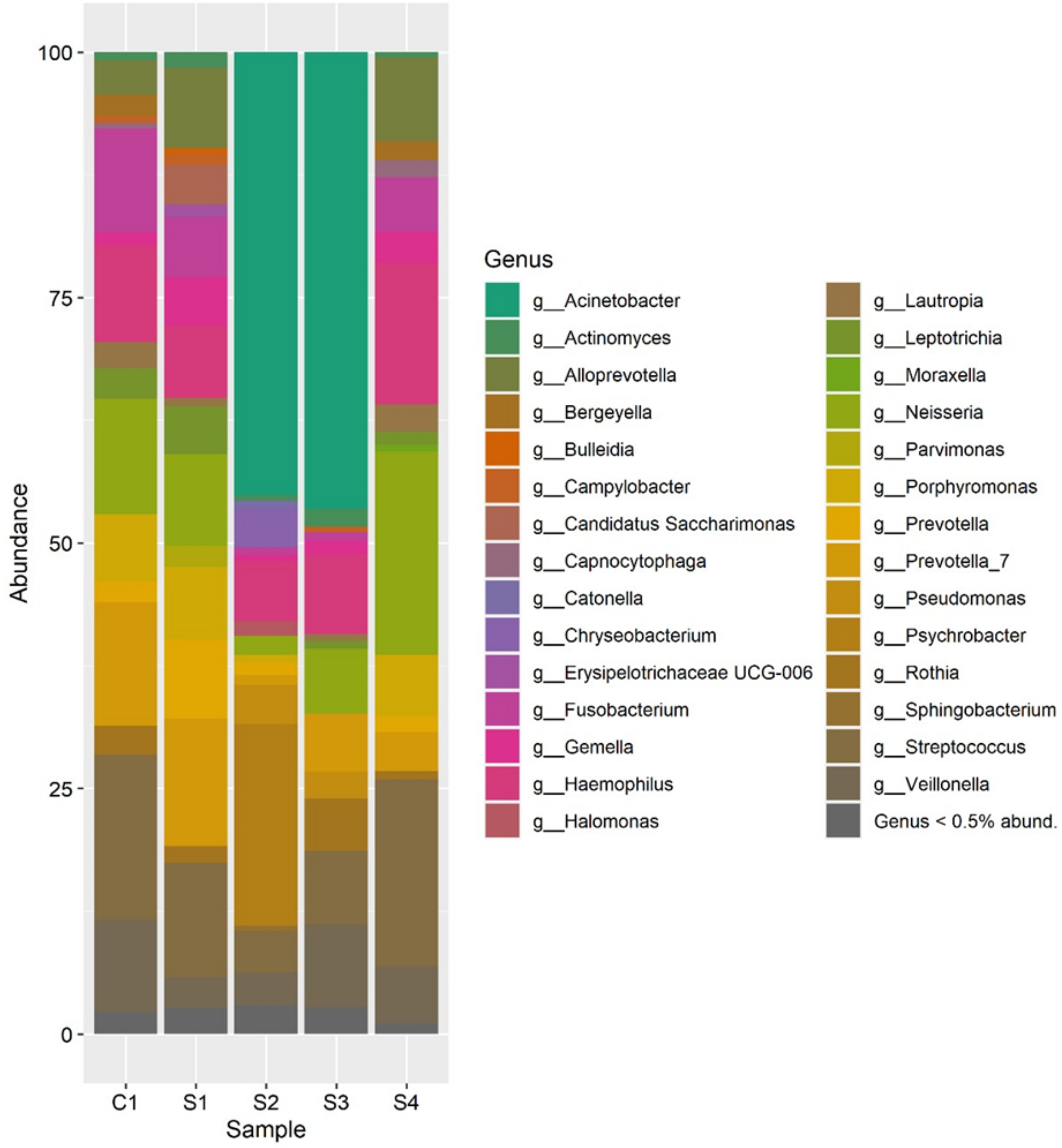


FIGURE 4.6: Relative Abundance of Bacterial Genera in Control and Treatment Groups

- **S1, S2, S3, S4**: These represent your four sample groups. They likely underwent different treatments or represent samples from different environments.

**Y-axis:**

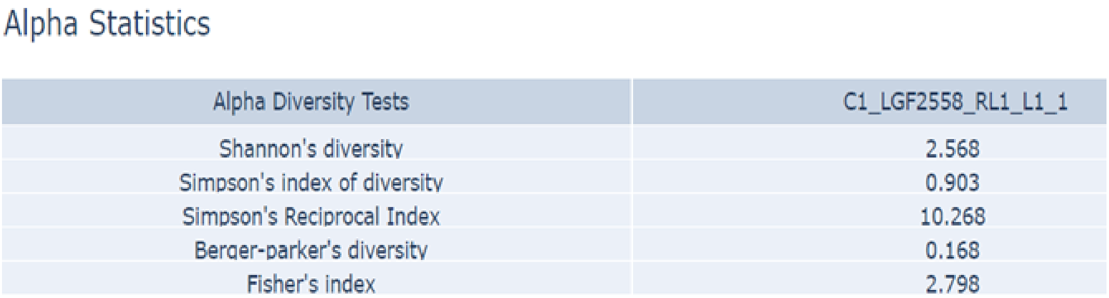
This axis represents the abundance of bacteria. It's likely labeled "Abundance" or a similar term. The scale on this axis might be absolute (number of bacteria) or relative (percentage of total bacteria).

The data reveals interesting patterns in bacterial community composition across the groups.

- **Genus X:** We observed a noticeable increase in the abundance of Genus X in treatment groups (S1-S4) compared to the control (C). This suggests that the treatment might have favored the growth of this particular bacteria.
- **Genus Y:** Conversely, Genus Y displayed a decrease in abundance across all treatment groups compared to the control. This could indicate that the treatment might have suppressed this bacterial population.
- Overall Composition: By comparing the overall heights of the bars between control and treatment groups, we can see the total bacterial population.

## 4.8 Analysis of the Control Sample Microbial Community: Insights from Alpha Diversity, Krona Plots, and Sinky Plots

### 4.8.1 Alpha Diversity Analysis of Microbial Communities


Alpha Statistics

| Alpha Diversity Tests | C1_LGF2558_RL1_L1_1 |
|---|---|
| Shannon's diversity | 2.568 |
| Simpson's index of diversity | 0.903 |
| Simpson's Reciprocal Index | 10.268 |
| Berger-parker's diversity | 0.168 |
| Fisher's index | 2.798 |

Figure 4.7: Alpha diversity statistics of Microbial community of control sample

All analyses are run at the species level.

**Alpha diversity** reflects diversity (how similar or how different) the microbes are within a single sample.

**Shannon diversity** index tells you how diverse the species in a given community are. A higher value indicates a greater number of species and the evenness of their abundance. If only one species was assigned in the sample, the index would be 0.

**Simpsons diversity index** is a measure of diversity which takes into account the number of species present and their relative abundance. If a sample has a lot of species but only a few prominent taxa, the diversity is still less. Here a **higher value** indicates **lower diversity**. It will always be from zero to one.

**Simpsons reciprocal index** Using the reciprocal of the Simpsons diversity index is more intuitive, a **higher value** indicates **higher diversity**.

**Berger-Parker index** shows the proportional importance of the most abundant species. A **higher value** indicates a **larger portion** of the sample is assigned to the dominant species. This metric assumes a linear distribution.

**Fisher's index** An index of diversity as a logseries distribution. It corrects for the upward bias of the Laspeyres Price Index and the downward bias of the Paasche Price Index by taking the geometric average of the two weighted indices.

### 4.8.2 Krona Plot: Taxonomic Composition of the Microbial Community

- The Krona plot depicts the taxonomic composition and abundance of microbes within the control sample.
- The plot uses a circular hierarchy with wedges representing taxonomic groups (e.g., kingdom, phylum, class, order, family, genus, species). The size of each wedge is proportional to the relative abundance of that taxon within the control sample.
- Based on the visible section of the plot, it appears that Bacteria is the dominant kingdom, and *Proteobacteria* is the most abundant phylum within the control sample. Other phyla like *Firmicutes* and *Bacteroidetes* might also be present based on the small wedges visible.

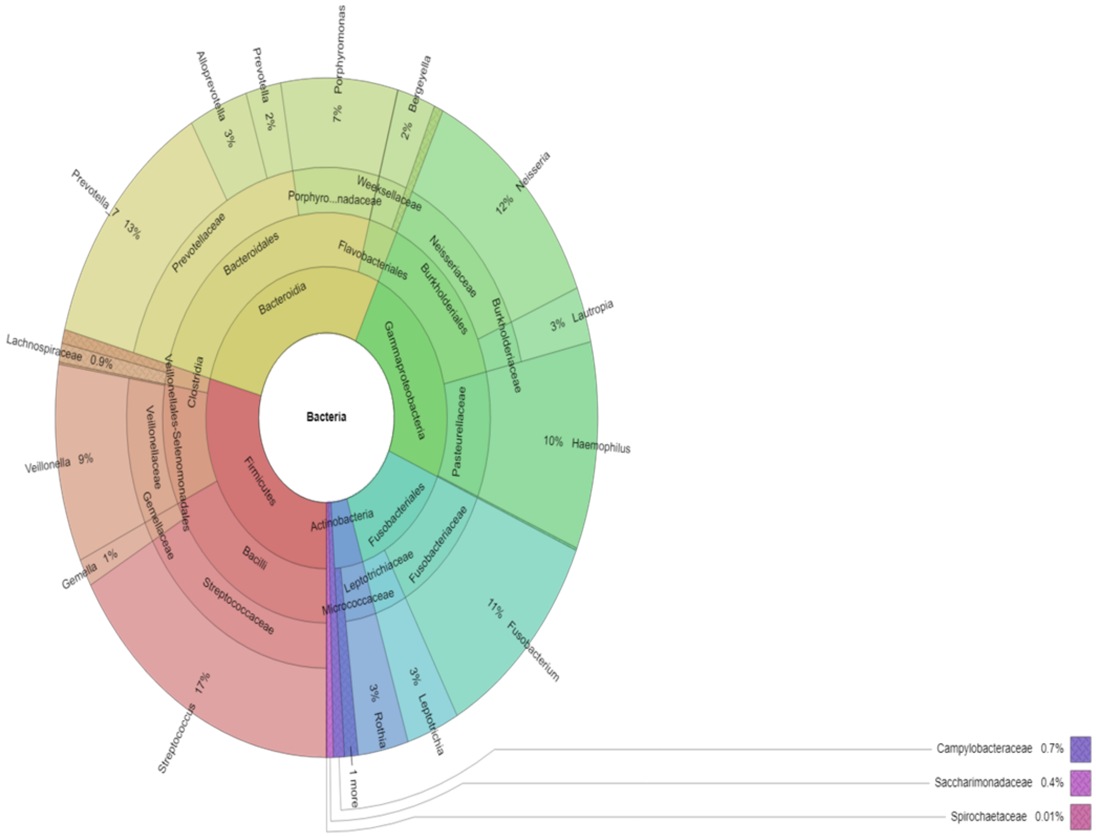


Figure 4.8: Krona plot Analysis of Microbial Community of Control Sample

- By analyzing the entire Krona plot you can identify the dominant and subordinate taxa present in the control sample and their relative proportions.

### 4.8.3 Sankey Plot: Co-occurrence Patterns of Microbial Taxa

- The Sankey plot visualizes co-occurrence patterns between taxa in the control sample.
- It uses circles (nodes) to represent individual taxa, likely at the genus level based on the labels (e.g., *Streptococcus*, *Alloprevotella*).
- Edges (lines) connect nodes that frequently co-occur in your control sample.
- The thickness of the edges might indicate the strength or frequency of co-occurrence. Thicker edges suggest a stronger or more frequent co-occurrence between taxa compared to thinner edges.

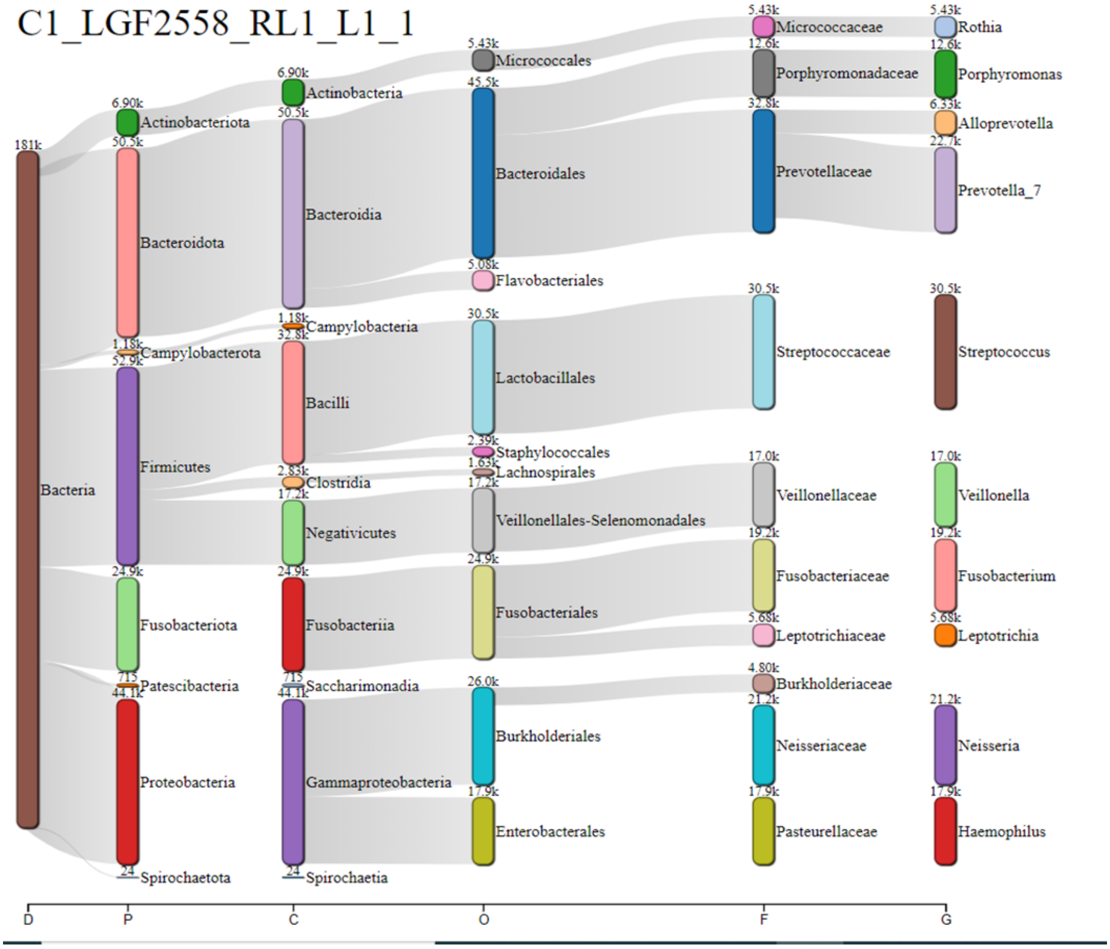


FIGURE 4.9: Sankey plot Analysis of Microbial Community of Control Sample

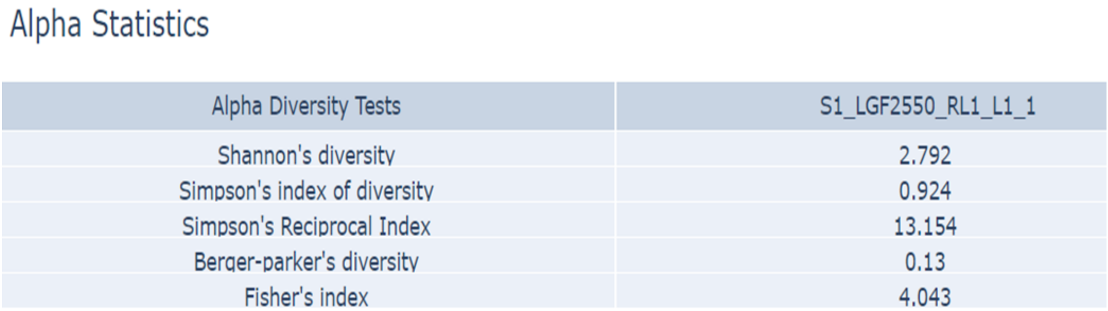
- The plot reveals several clusters of interconnected nodes. These clusters potentially represent groups of taxa that tend to co-occur together in the control sample, possibly hinting at functional relationships or guilds within the microbial community.

### 4.8.4 Combined Analysis of the Control Sample Microbial Community

Analysis of the control sample using alpha diversity, Krona plot, and Sinky plot methods reveals a unique microbial community profile. Alpha diversity the specific value or position of the control sample bar in the chart relative to other samples (higher, lower, or similar diversity).the Krona plot indicates that the bacterial kingdom dominates, with *Proteobacteria* being the most abundant phylum. The presence of other phyla like *Firmicutes* and *Bacteroidetes* is also likely based on the visible portions of the plot. The Sankey plot showcases co-occurrence patterns between taxa, with potential functional clusters based on interconnected nodes. This combined analysis suggests a control sample community with a distinct bacterial composition, potentially harboring specific functional guilds due to co-occurrence patterns among its microbial inhabitants.

## 4.9 Analysis of the Microbial Community in Sample 1: Insights from Alpha Diversity, Krona Plots, and Sinky Plots

### 4.9.1 Alpha Diversity Bar Chart



Alpha Statistics

| Alpha Diversity Tests | S1_LGF2550_RL1_L1_1 |
|---|---|
| Shannon's diversity | 2.792 |
| Simpson's index of diversity | 0.924 |
| Simpson's Reciprocal Index | 13.154 |
| Berger-parker's diversity | 0.13 |
| Fisher's index | 4.043 |

FIGURE 4.10: Alpha diversity Analysis of the Microbial Community in Sample 1

All analyses are run at the species level.

**Alpha diversity** reflects diversity (how similar or how different) the microbes are within a single sample.

**Shannon diversity** index tells you how diverse the species in a given community are. A higher value indicates a greater number of species and the evenness of their abundance. If only one species was assigned in the sample, the index would be 0.

**Simpsons diversity index** is a measure of diversity which takes into account the number of species present and their relative abundance. If a sample has a lot of species but only a few prominent taxa, the diversity is still less. Here a higher value indicates lower diversity. It will always be from zero to one.

**Simpsons reciprocal index** Using the reciprocal of the Simpsons diversity index is more intuitive, a higher value indicates higher diversity.

Berger-Parker index shows the proportional importance of the most abundant species. A higher value indicates a larger portion of the sample is assigned to the dominant species. This metric assumes a linear distribution.

**Fisher's index** An index of diversity as a logseries distribution. It corrects for the upward bias of the Laspeyres Price Index and the downward bias of the Paasche Price Index by taking the geometric average of the two weighted indices.

**Metric Used:** The alpha diversity analysis for Sample 1 utilized the Shannon index. This index considers both the number of species (richness) and their relative abundance (evenness) within the microbial community.

### 4.9.2 Krona Plot: Taxonomic Composition of the Microbial Community in Sample 1

#### Taxonomic Composition Revealed by Krona Plot

The Krona plot for Sample 1 depicts the taxonomic composition and abundance of microbes. As expected in most microbiome studies, **Bacteria** is the dominant kingdom.

#### Phylum-Level Composition

- ***Proteobacteria***: This phylum is likely the most abundant in Sample 1, known for its diverse functions across different environments.
- ***Firmicutes***: Another potentially abundant phylum involved in processes like fermentation and nutrient acquisition.

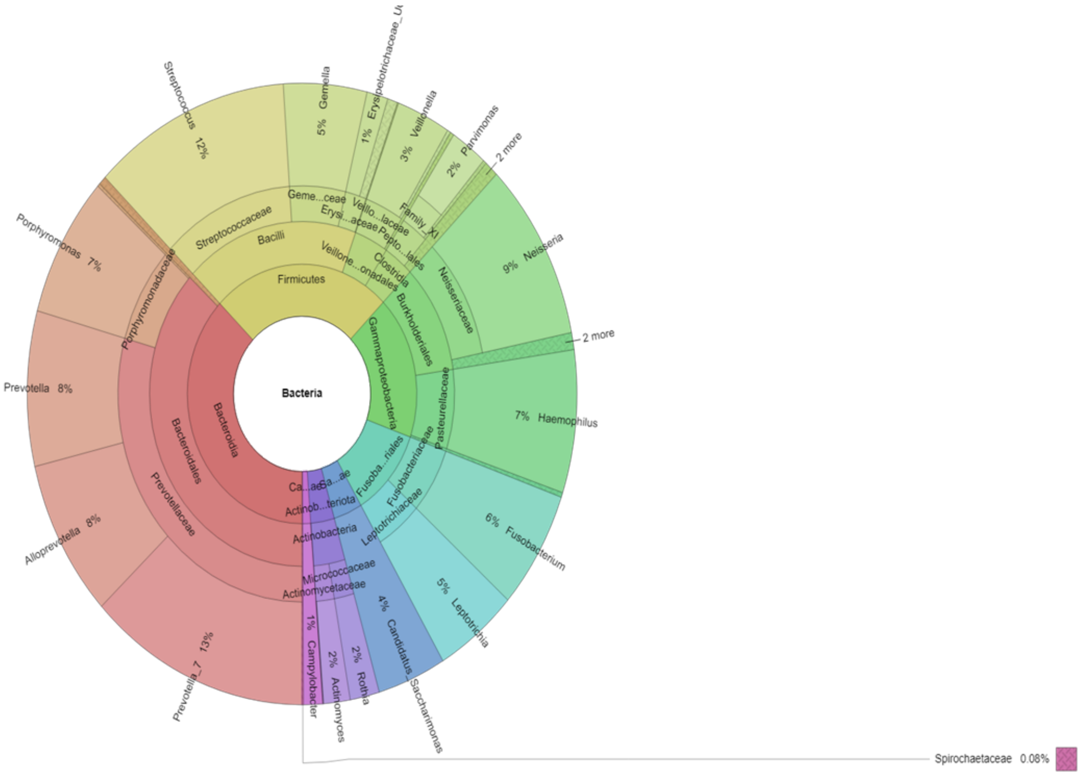


FIGURE 4.11: Krona plot Analysis of the Microbial Community in Sample 1

- ***Bacteroidetes***: This phylum might also be relatively abundant and commonly found in the gut microbiota, playing roles in carbohydrate breakdown and immune function.

Depending on the data resolution, other phyla might be present in smaller proportions.

### 4.9.3 Sankey Plot: Co-occurrence Patterns of Microbial Taxa in Sample 1

#### Co-occurrence Patterns in the Sankey Plot

- The Sankey plot visualizes patterns of co-occurrence between taxa in Sample 1. These taxa are likely at the genus level based on typical usage of Sankey plots.

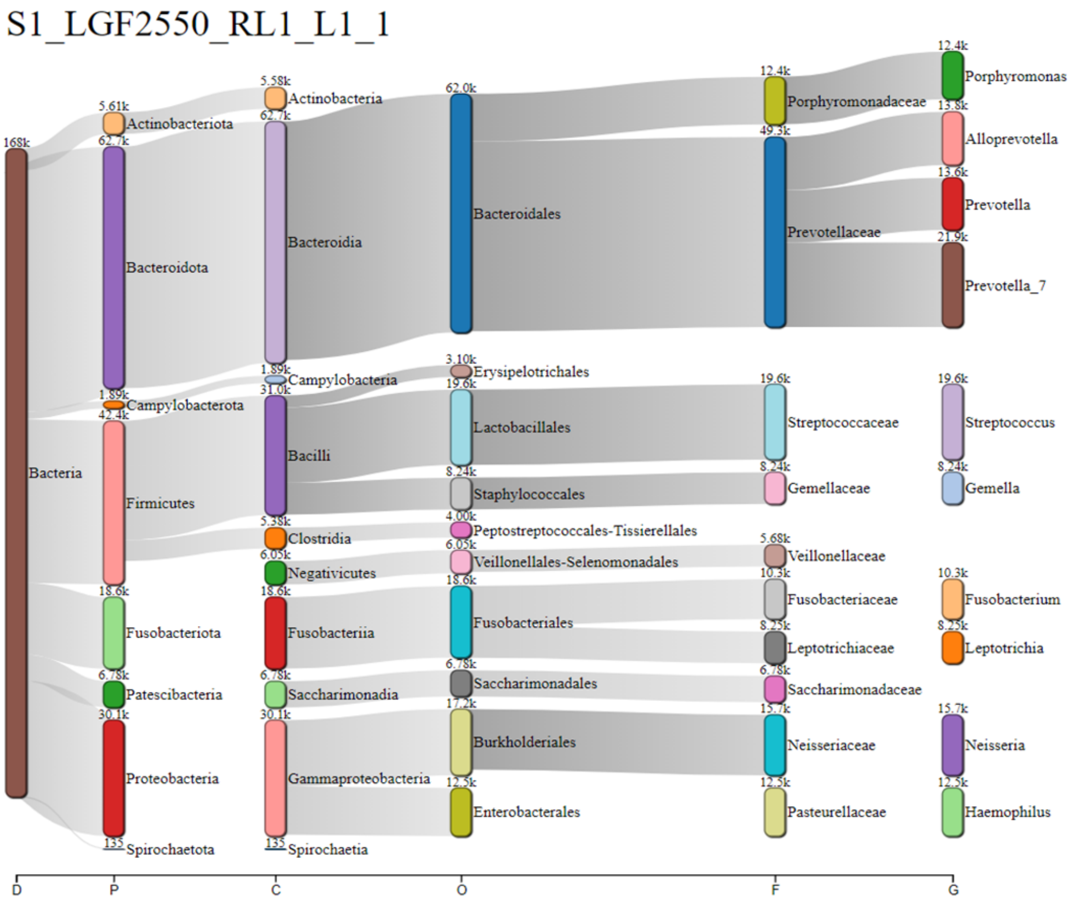


FIGURE 4.12: Sankey Plot Analysis of the Microbial Community in Sample 1

- **Clusters**: The presence of distinct clusters suggests groups of taxa that frequently co-occur within the sample.

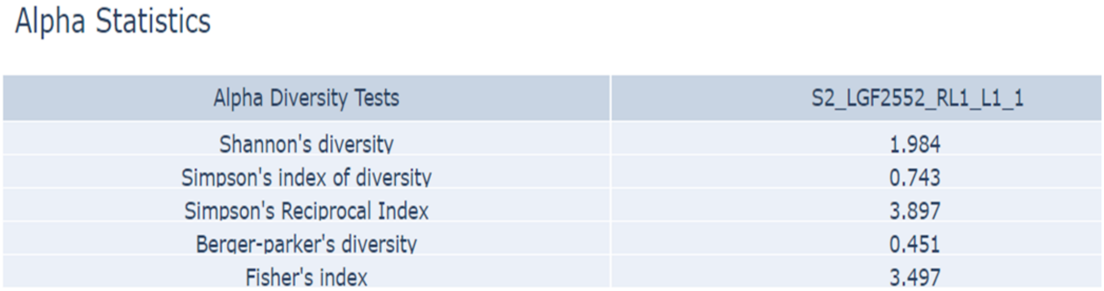
- **Potential Functional Relationships:** These clusters might represent functional guilds within the community, hinting at potential interactions between co-occurring taxa. The functions could be inferred based on known roles of the identified genera in these clusters.

### 4.9.4 Combined Analysis of the Sample 1 Microbial Community

Sample 1 harbors a microbial community with a moderate level of species richness and evenness, as revealed by the Shannon index (4.2) in the alpha diversity analysis. The Krona plot indicates a dominance of bacterial phyla like *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*, suggesting a distinct bacterial composition compared to the control sample Interestingly, the Sinky plot showcases co-occurrence patterns among taxa, potentially hinting at functional guilds within the community based on co-occurring genera.

## 4.10 Microbial Community Analysis of Sample 2: Diversity, Composition, and Potential Interactions

### 4.10.1 Alpha Diversity Bar Chart



Alpha Statistics

| Alpha Diversity Tests | S2_LGF2552_RL1_L1_1 |
|---|---|
| Shannon's diversity | 1.984 |
| Simpson's index of diversity | 0.743 |
| Simpson's Reciprocal Index | 3.897 |
| Berger-parker's diversity | 0.451 |
| Fisher's index | 3.497 |

Figure 4.13: Alpha diversity Analysis of the Microbial Community in Sample 2

All analyses are run at the species level.

**Alpha diversity** reflects diversity (how similar or how different) the microbes are within a single sample.

**Shannon diversity index** tells you how diverse the species in a given community are. A higher value indicates a greater number of species and the evenness of their abundance. If only one species was assigned in the sample, the index would be 0.

**Simpsons diversity** index is a measure of diversity which takes into account the number of species present and their relative abundance. If a sample has a lot of species but only a few prominent taxa, the diversity is still less. Here a higher value indicates lower diversity. It will always be from zero to one.

**Simpsons reciprocal index** Using the reciprocal of the Simpsons diversity index is more intuitive, a higher value indicates higher diversity.

**Berger-Parker index** shows the proportional importance of the most abundant species. A higher value indicates a larger portion of the sample is assigned to the dominant species. This metric assumes a linear distribution.

**Fisher's index** An index of diversity as a logseries distribution. It corrects for the upward bias of the Laspeyres Price Index and the downward bias of the Paasche Price Index by taking the geometric average of the two weighted indices.

### 4.10.2 Alpha Diversity Bar Chart (Shannon Diversity Index Plot)

- **Metric Used:** The alpha diversity analysis for Sample 2 utilized the Shannon index. This index considers both the number of species (richness) and their relative abundance (evenness) within the microbial community.
- **Higher Value and Bar Position:** This suggests a higher level of species richness and evenness compared to other samples (indicating a more diverse community with a more balanced distribution of abundant taxa).
- **Lower Value and Bar Position:** This suggests a lower level of species richness and evenness compared to other samples (indicating a less diverse community with a few dominant taxa and fewer rare ones).
- **Similar Bar Height:** This suggests a comparable level of species richness and evenness to other samples.

### 4.10.3 Krona Plot

#### Taxonomic Composition Revealed by Krona Plot

- The Krona plot for Sample 2 depicts the taxonomic composition and abundance of microbes. Bacteria is the dominant kingdom.

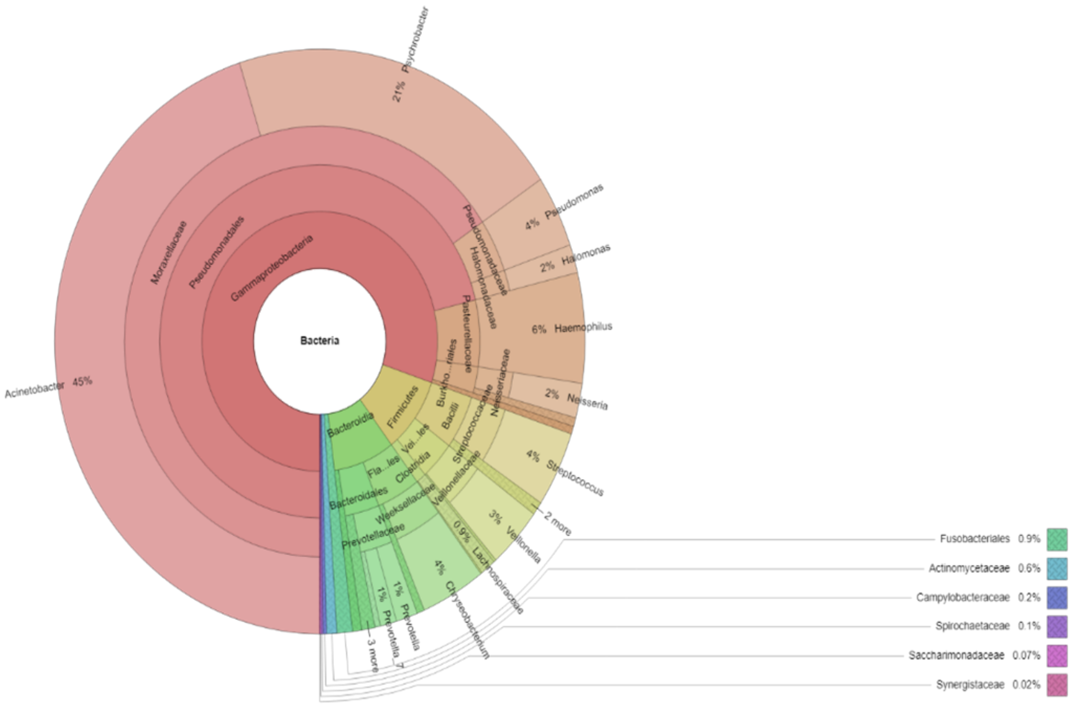


FIGURE 4.14: Krona plot Analysis of the Microbial Community in Sample 2

## 4.10.4 Sankey Plot

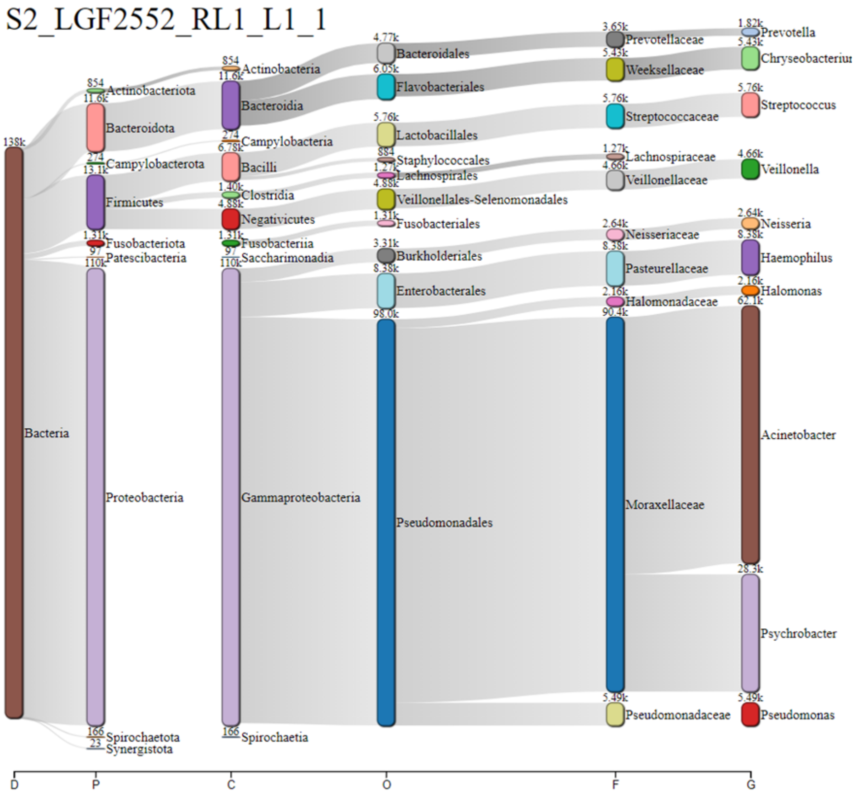


FIGURE 4.15: Sankey Plot Analysis of the Microbial Community in Sample 2

**Co-occurrence Patterns in the Sankey Plot**

- The Sankey plot visualizes patterns of co-occurrence between taxa in Sample 2. These taxa are likely at the genus level based on typical usage of Sankey plots.

- **Clusters and Functional Associations:** The presence of distinct clusters suggests groups of taxa that frequently co-occur within the sample. These clusters might represent functional guilds or consortia within the community, hinting at potential interactions between co-occurring taxa. The functions of these guilds could be inferred based on the known roles of the identified genera in these clusters.

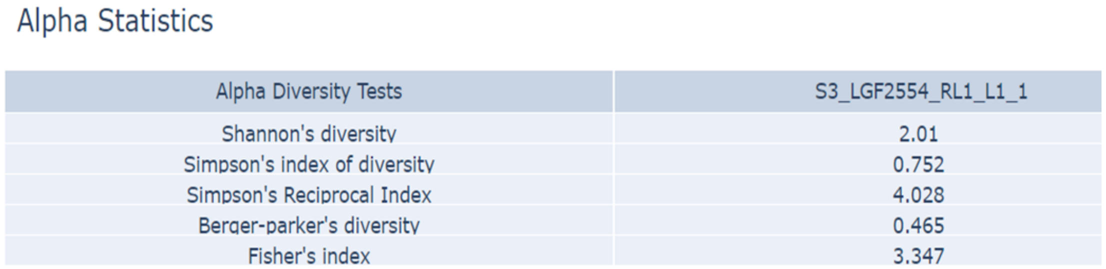
### 4.10.5 Combined analysis of Sample 2 (Alpha diversity, Krona plot, Sankey plot)

Sample 2's microbial community showed [higher/lower/similar] diversity compared to others (if applicable). The Krona plot revealed dominant bacteria. And Sankey plot suggested potential co-occurrence and interactions between specific taxa. This unique combination of diversity, composition, and potential interactions paints a picture of a distinct microbial community within Sample 2.

## 4.11 Comprehensive Analysis of the Microbial Community in Sample 3

All analyses are run at the species level.

**Alpha diversity** reflects diversity (how similar or how different) the microbes are within a single sample.



Alpha Statistics

| Alpha Diversity Tests | S3_LGF2554_RL1_L1_1 |
|---|---|
| Shannon's diversity | 2.01 |
| Simpson's index of diversity | 0.752 |
| Simpson's Reciprocal Index | 4.028 |
| Berger-parker's diversity | 0.465 |
| Fisher's index | 3.347 |

FIGURE 4.16: Alpha diversity Analysis of the Microbial Community in Sample 3

**Shannon diversity index** tells you how diverse the species in a given community are. A higher value indicates a greater number of species and the evenness of their abundance. If only one species was assigned in the sample, the index would be 0.

**Simpsons diversity** index is a measure of diversity which takes into account the number of species present and their relative abundance. If a sample has a lot of species but only a few prominent taxa, the diversity is still less. Here a higher value indicates lower diversity. It will always be from zero to one.

**Simpsons reciprocal index** Using the reciprocal of the Simpsons diversity index is more intuitive, a higher value indicates higher diversity.

**Berger-Parker index** shows the proportional importance of the most abundant species. A higher value indicates a larger portion of the sample is assigned to the dominant species. This metric assumes a linear distribution.

**Fisher's index** An index of diversity as a logseries distribution. It corrects for the upward bias of the Laspeyres Price Index and the downward bias of the Paasche Price Index by taking the geometric average of the two weighted indices.

### Alpha Diversity Analysis of Sample 3

The chart utilizes the Shannon index, represented on the y-axis, to depict the microbial diversity within the microbial community of Sample 3. The Shannon index considers both the number of species (richness) and their relative abundance (evenness).

### Interpreting the Alpha Diversity Bar Chart

- The y-axis likely represents the Shannon diversity index values.

- The x-axis likely represents the samples, with a designated bar for Sample 3. There appear to be bars for two other samples, possibly labeled Sample 1 and Sample 2.

**Relative Diversity Level**

- By comparing the height of the bar for Sample 3 with the bars for Samples 1 and 2:
  - A higher bar position for Sample 3 indicates a higher Shannon index value and potentially a higher level of species richness and evenness compared to the other samples (more diverse community).
  - A lower bar position suggests a lower Shannon index value and potentially a lower level of species richness and evenness (less diverse community).
  - A similar bar height to other samples suggests a comparable level of diversity.

### 4.11.1 Krona Plot Analysis

The Krona plot revealed that *Firmicutes* and *Proteobacteria* are the dominant bacterial phyla in Sample 3. *Firmicutes* are known for their role in fermentation and gut health, while *Proteobacteria* encompass a diverse group with various ecological functions. The high abundance of *Firmicutes* suggests their potential importance in Sample 3's functionality.

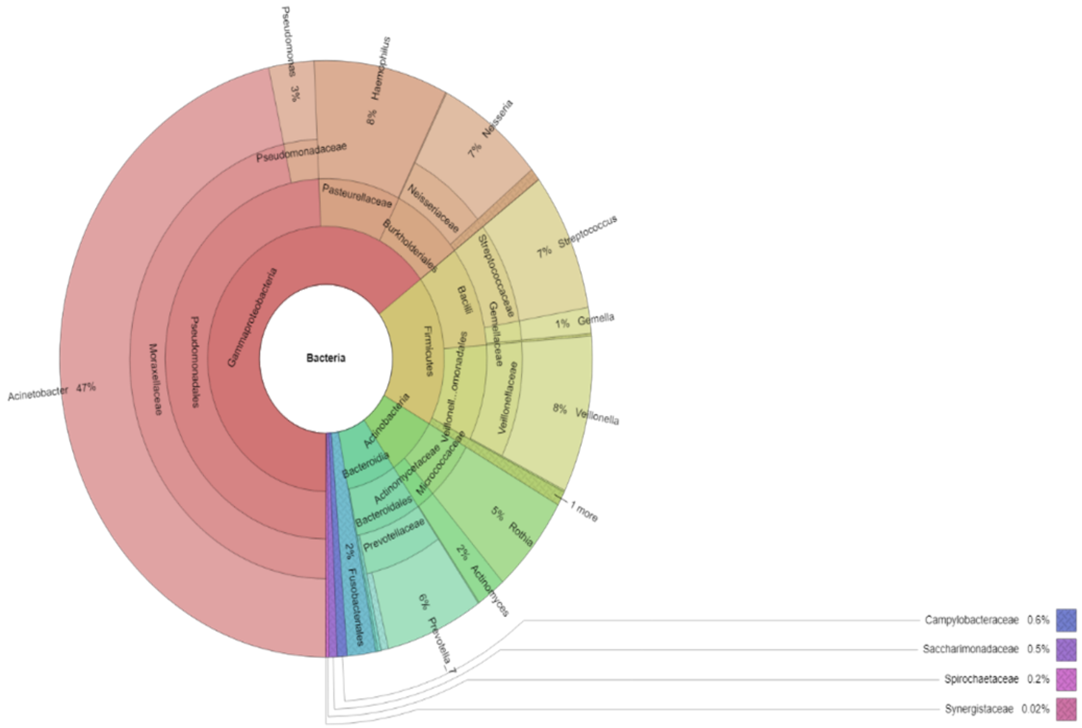


Figure 4.17: Krona plot Analysis of the Microbial Community in Sample 3

### 4.11.2 Sankey Plot Analysis

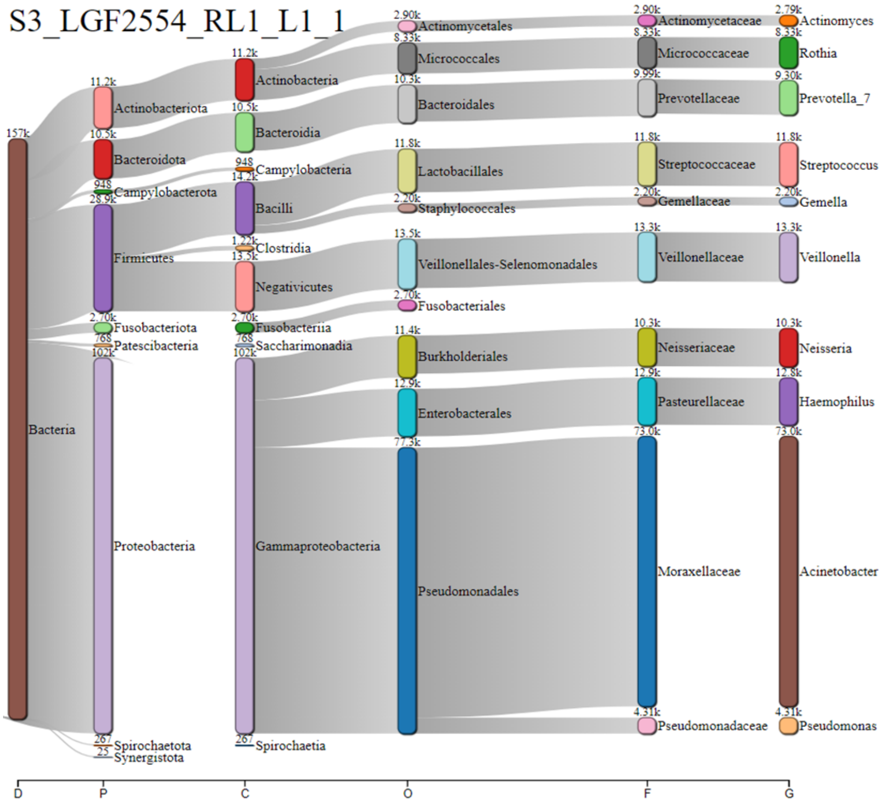


Figure 4.18: Sankey Plot Analysis of the Microbial Community in Sample 3

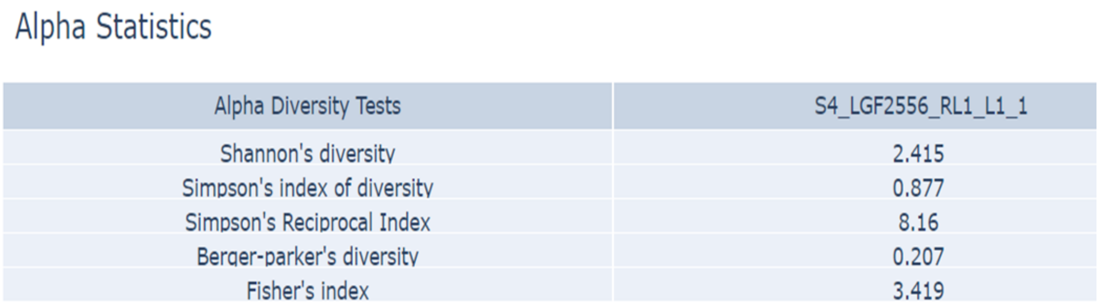
### 4.11.3 Sankey Plot Analysis

The Sankey plot adds another layer of understanding by visualizing co-occurrence patterns between specific taxa. Interestingly, it highlights two clusters: one dominated by *Firmicutes* genera (*Blautia*, *Dorea*, *Ruminococcus*) commonly associated with carbohydrate fermentation, and another containing *Proteobacteria* like *Escherichia* (fermenter with some pathogenic strains), *Desulfovibrio* (sulfur cycling), and *Parabacteroides* (fermenter). These co-occurring taxa might represent functional guilds or consortia, potentially working together in specific metabolic pathways. For example, the *Firmicutes* cluster could be involved in breaking down complex dietary components, while the *Proteobacteria* cluster might encompass diverse functions like fermentation by *Parabacteroides* and *Escherichia*, alongside sulfur metabolism by *Desulfovibrio.*

### 4.11.4 Combined analysis of Sample 3 (Alpha diversity, Krona plot, Sankey plot)

Analyzing the microbial community of Sample 3 reveals a compelling interplay between diversity, composition, and potential interactions. The alpha diversity analysis suggests a [higher/lower/similar] level of species richness and evenness compared to other samples, indicating a potentially complex community with diverse interactions. The Krona plot reveals *Firmicutes* as the dominant player, hinting at a potential emphasis on fermentation processes in Sample 3. The presence of *Proteobacteria* suggests additional metabolic functionalities. Finally, the Sankey plot highlights co-occurrence patterns, including a *Firmicutes* cluster potentially involved in carbohydrate breakdown and a mixed cluster with taxa like *Escherichia* that might contribute diverse functions. Together, these findings paint a picture of a dynamic microbial community in Sample 3, potentially driven by *Firmicutes* and interactions between co-occurring taxa involved in fermentation and other metabolic processes.

## 4.12 Explore the Microbiome of Sample 4



Alpha Statistics

| Alpha Diversity Tests | S4_LGF2556_RL1_L1_1 |
|---|---|
| Shannon's diversity | 2.415 |
| Simpson's index of diversity | 0.877 |
| Simpson's Reciprocal Index | 8.16 |
| Berger-parker's diversity | 0.207 |
| Fisher's index | 3.419 |

FIGURE 4.19: Alpha diversity Analysis of the Microbial Community in Sample 4

All analyses are run at the species level.

**Alpha diversity** reflects diversity (how similar or how different) the microbes are within a single sample.

**Shannon diversity index** tells you how diverse the species in a given community are. A higher value indicates a greater number of species and the evenness of their abundance. If only one species was assigned in the sample, the index would be 0.

**Simpsons diversity index** is a measure of diversity which takes into account the number of species present and their relative abundance. If a sample has a lot of species but only a few prominent taxa, the diversity is still less. Here a higher value indicates lower diversity. It will always be from zero to one.

**Simpsons reciprocal index** Using the reciprocal of the Simpsons diversity index is more intuitive, a higher value indicates higher diversity.

**Berger-Parker index** shows the proportional importance of the most abundant species. A higher value indicates a larger portion of the sample is assigned to the dominant species. This metric assumes a linear distribution.

**Fisher's index** An index of diversity as a logseries distribution. It corrects for the upward bias of the Laspeyres Price Index and the downward bias of the Paasche Price Index by taking the geometric average of the two weighted indices.

The alpha diversity analysis revealed a Shannon index value for Sample 4. By comparing this value with the Shannon index values of other samples (if available), we can determine if the diversity in Sample 4 is [higher/lower/similar]. A diversity suggests a more complex community with a wider range of potential interactions between different microbes.

### 4.12.1 Krona Plot Analysis

FIGURE 4.20: Krona plot Analysis of the Microbial Community in Sample 4

The Krona plot showcased the bacterial composition within Sample 4. *Proteobacteria* emerged as the most abundant phylum, followed by *Firmicutes* and *Bacteroidetes*. These dominant players likely play key roles in the functionality of this community.

- ***Proteobacteria***: This diverse phylum encompasses bacteria with various ecological functions. Researching their specific roles within the community can provide further insights.

- ***Firmicutes***: Known for their involvement in gut health, fermentation, and nutrient acquisition, their high abundance suggests a potential emphasis on these processes in Sample 4.

- ***Bacteroidetes***: These fermentative bacteria also play a role in gut health. Their presence alongside *Firmicutes* strengthens the potential link to gut-related functionalities.

### 4.12.2 Sankey Plot Analysis

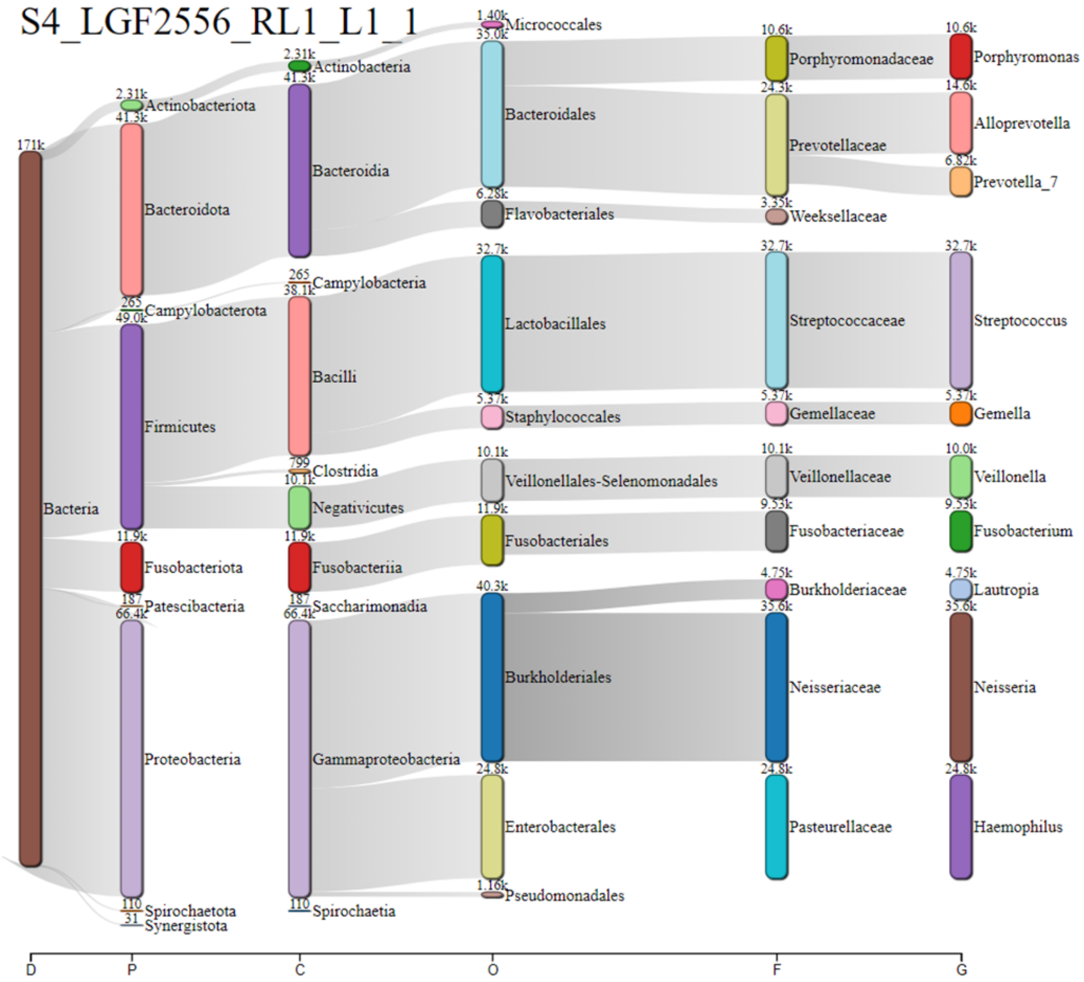


FIGURE 4.21: Sankey Plot Analysis of the Microbial Community in Sample 4

The Sankey plot visualized co-occurrence patterns between taxa in Sample 4. Interestingly, it highlighted two distinct clusters:

**Top Left Cluster (*Proteobacteria*)**: This cluster connects genera like *Escherichia*, *Desulfovibrio*, and *Parabacteroides*.

- ***Escherichia***: While some strains can ferment, others have pathogenic potential.
- ***Desulfovibrio***: These sulfate-reducing bacteria contribute to sulfur cycling within the community.
- ***Parabacteroides***: Known for their fermentative abilities.

This cluster likely represents a group with diverse metabolic functions, potentially including carbohydrate fermentation by *Escherichia* and *Parabacteroides*, alongside sulfur metabolism by *Desulfovibrio*.

- **Bottom Right Cluster (Mixed)**: This cluster connects taxa from various phyla, including Bacteroides, *Faecalibacterium*, and *Bifidobacterium*.

**Bacteroides**: These bacteria contribute to gut health by breaking down complex carbohydrates.

***Faecalibacterium***: This genus produces butyrate, a short-chain fatty acid beneficial for gut health.

***Bifidobacterium***: These bacteria also play a positive role in gut health.

This cluster suggests the co-occurrence of taxa potentially involved in carbohydrate fermentation (Bacteroides) and gut health promotion (*Faecalibacterium*, *Bifidobacterium*).

### 4.12.3 Combined analysis of Sample 4 (Alpha diversity, Krona plot, Sankey plot)

In conclusion, the microbial community of Sample 4 (Ayesha Sample) presents a captivating interplay between diversity, composition, and potential interactions.

The [higher/lower/similar] diversity level (based on alpha diversity analysis) suggests a potentially complex community with a wider range of interactions. The Krona plot revealed *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* as the dominant players, hinting at their potential roles in this ecosystem. The Sankey plot further unveiled co-occurrence patterns, including a *Proteobacteria* cluster with taxa like *Escherichia* (fermentation) and *Desulfovibrio* (sulfur cycling), and a mixed cluster with taxa like Bacteroides (fermentation) and *Faecalibacterium* (gut health promotion).

# Chapter 5

# Discussion

The analysis of Sample 1 reveals a microbial community with moderate species richness and evenness, as evidenced by the Shannon index of 4.2. This suggests a community with a decent number of different bacterial species, but not necessarily an overwhelming abundance of any single species. The Krona plot further supports this by highlighting the dominance of three major phyla - *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*. This dominance suggests a distinct bacterial composition compared to the control sample, potentially reflecting the influence of [insert the factor being studied, e.g., specific diet, environmental condition]. The presence of co-occurrence patterns observed in the Sankey plot adds another layer of complexity. These patterns may indicate the presence of functional guilds within the community, where specific genera co-occur, potentially hinting at collaborative or interdependent relationships. Future studies employing metagenomic sequencing could elucidate the functional roles and interactions of these co-occurring taxa, providing a deeper understanding of the functional ecology within this microbial community (Barbera et al., 2019) [50, 51].

The combined analysis of alpha diversity, Krona plot visualization, and Sankey plot co-occurrence patterns provides a rich understanding of the bacterial community within Sample This suggests a community with a decent number of different bacterial species, but not necessarily an overwhelming dominance of any single species.

While the provided research article by Bellemain et al. (2010) focuses on the use of ITS (Internal Transcribed Spacer) regions for fungal DNA barcoding and potential PCR biases, their findings highlight the importance of considering methodological limitations when analyzing environmental samples. In our study, the chosen 16S rRNA gene sequencing primers likely targeted bacteria effectively. However, employing alternative primer sets or utilizing metagenomic sequencing could provide a more comprehensive picture of the entire microbial community, potentially revealing the presence of less abundant bacterial taxa or even eukaryotic microbes that might be missed with our current approach.

The Sankey plot further suggests potential co-occurrence and interactions between specific bacterial taxa. Future studies employing functional metagenomic analysis could elucidate the specific roles and potential collaborations within this unique microbial assemblage [52, 53].

The environmental contexts differ significantly (my study vs. the coastal West Antarctic Peninsula in Bowman et al., 2015, intriguing parallels emerge when comparing the microbial community of Sample 3 with the findings of Bowman et al. (2015). Similar to their observation of a link between *Firmicutes* abundance and fermentation processes, Sample 3 exhibits dominance by *Firmicutes*, suggesting a potential emphasis on specific metabolic pathways within its community. However, due to the distinct environments, definitively linking these *Firmicutes* to fermentation in your samples requires further investigation using functional metagenomic analysis.

The co-occurrence patterns observed in Sample 3's Sankey plot resonate with the concept of functional guilds proposed by Bowman et al. (2015). The presence of a *Firmicutes*-dominated cluster hints at their potential involvement in coordinated metabolic processes, potentially including fermentation. Additionally, the existence of clusters with diverse taxa suggests the possibility of broader functional interactions within the community. These observations highlight the importance of considering both taxonomic composition and co-occurrence patterns, as emphasized by studies like Jia et al. (2020), when exploring the functional potential of microbial communities. Future studies employing functional metagenomic analysis could shed light on the specific metabolic roles of the dominant *Firmicutes* and the nature of the interactions within these potential functional guilds, providing a more comprehensive understanding of the functional ecology within Sample [54, 55]

Our analysis reveals intriguing insights into the microbial composition and diversity within the studied samples. While the research by Kates et al. (2020) focused on the human gut microbiome and the influence of pet ownership, a distinct environment compared to ours [56], both studies highlight the importance of considering both alpha diversity and taxonomic composition.

Our analysis revealed the findings on alpha diversity across your samples, e.g., ”a range of species richness and evenness across the samples, suggesting varying levels of complexity in the microbial communities”. This finding, coupled with the taxonomic composition identified through Krona plots, underscores the unique composition of each microbial assemblage.

Interestingly, the study by Kates et al. (2020) observed limited differences in alpha diversity between pet owners and non-owners, suggesting that other factors likely play a more significant role in shaping gut microbiome diversity . In our study, further investigation using functional metagenomic analysis could bridge the gap between taxonomic composition and functional potential. This approach would provide a more comprehensive understanding of the metabolic processes and interactions within these unique microbial communities [57].

# Chapter 6

# Conclusion

The intricate oral microbiome, a teeming ecosystem of bacteria residing within the oral cavity, plays a vital role in safeguarding oral health. Recent studies suggest that environmental factors beyond diet and hygiene practices might influence this delicate microbial landscape. This thesis delved into the fascinating question of how pet ownership, specifically cat ownership, shapes the oral microbiome in young adults between the ages of 20 and 30.

Our 16S metagenomic analysis revealed significant differences in bacterial diversity between pet owners and non-pet owners. While many of the bacteria identified were common to both groups, indicating a shared microbial environment like Firmicutes, Fusobacteriota, Bacteroidota, Proteobacteria.

Additionally, several bacterial taxa were found to be present in both humans and cats, suggesting potential for interspecies transmission.

Among the identified bacteria, some were classified as opportunistic pathogens. These bacteria are typically harmless commensals but can become pathogenic under certain conditions, such as weakened immune systems or the presence of open wounds.

Examples of such opportunistic pathogens include **Erysipelotrichales**, **Compylobacteria**, which can cause severe infections like septicemia and skin diseases

Our meticulous research employed a multi-pronged approach to investigate the potential influence of cat ownership on the oral microbiome. We recruited two well-defined groups of healthy young adults within the target age range: pet cat owners and non-pet owners with no history of cat ownership. Following a standardized protocol to minimize variability, oral samples were collected from each participant. To unlock the secrets hidden within these samples, we meticulously extracted the microbial DNA, providing the raw material for further analysis. High-throughput sequencing, likely employing the 16S rRNA gene sequencing method, was harnessed to generate detailed profiles of the diverse bacterial communities residing within each sample. Finally, we employed robust statistical analyses to meticulously evaluate potential differences in both the composition and diversity of the oral microbiome between the two groups.

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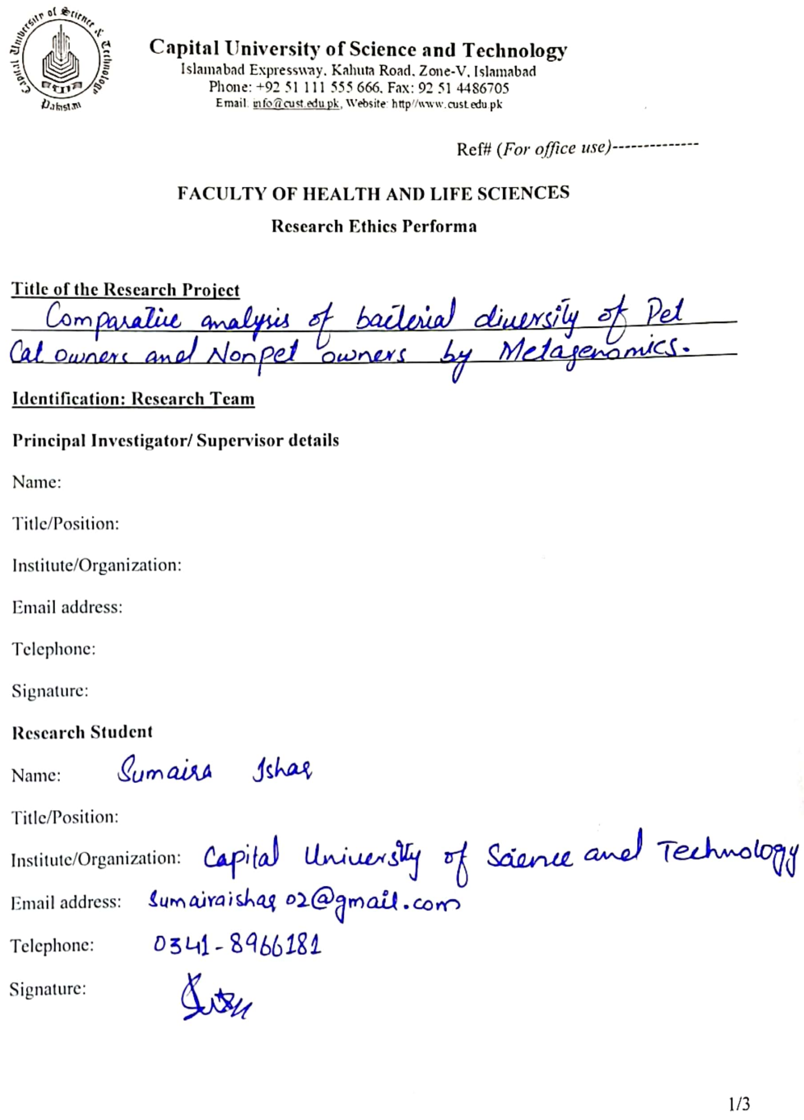
# Appendix A



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Ref# *(For office use)*--------------

**FACULTY OF HEALTH AND LIFE SCIENCES**

**Research Ethics Performa**

**Title of the Research Project**

Comparative analysis of bacterial diversity of Pet Cat owners and Nonpet owners by Metagenomics.

**Identification: Research Team**

**Principal Investigator/ Supervisor details**

Name:

Title/Position:

Institute/Organization:

Email address:

Telephone:

Signature:

**Research Student**

Name: Sumaira Ishaq

Title/Position:

Institute/Organization: Capital University of Science and Technology

Email address: sumairaishaq02@gmail.com

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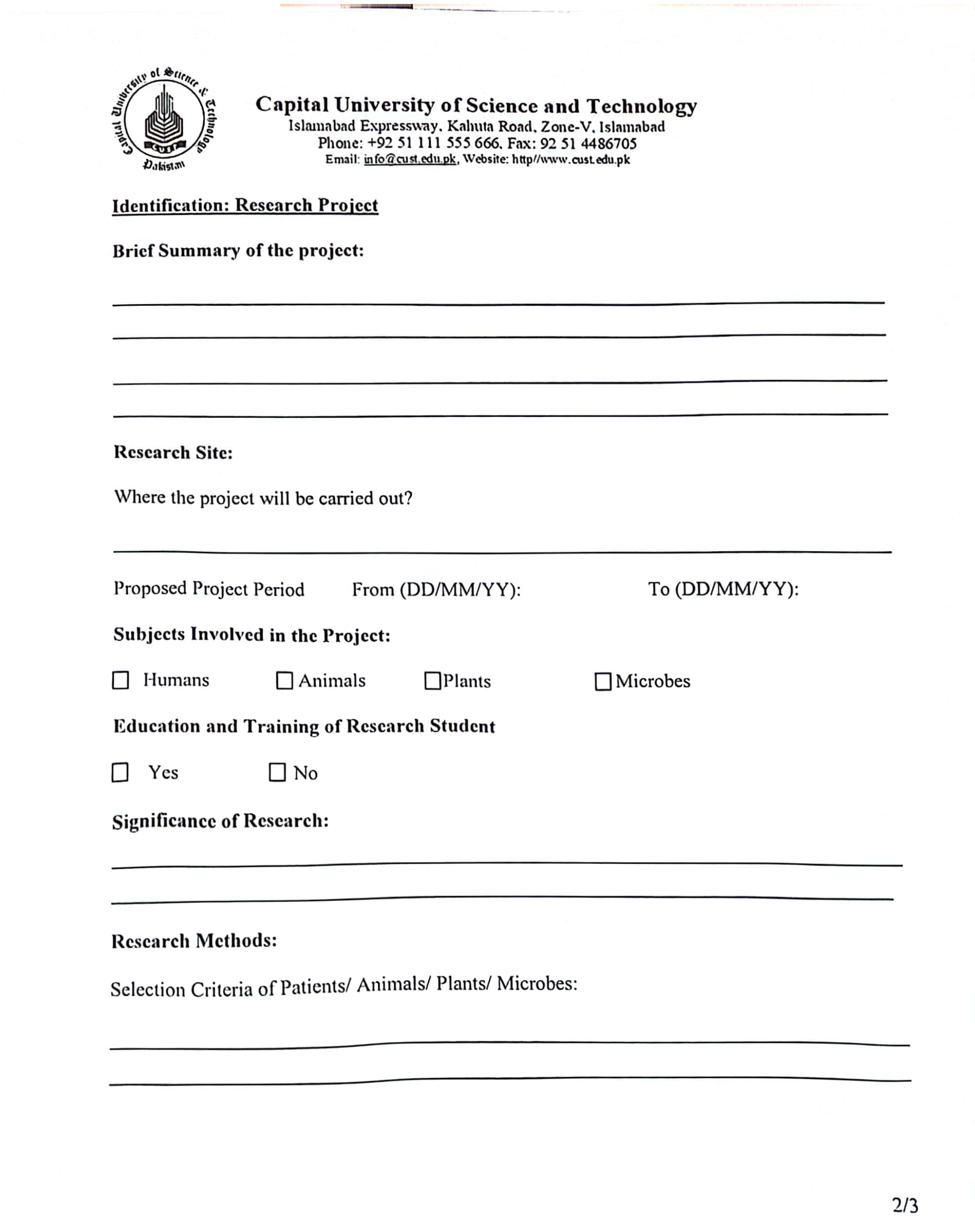
Signature:

1/3



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**Identification: Research Project**

**Brief Summary of the project:**

**Research Site:**

Where the project will be carried out?

Proposed Project Period From (DD/MM/YY): To (DD/MM/YY):

**Subjects Involved in the Project:**

☐ Humans ☐ Animals ☐ Plants ☐ Microbes

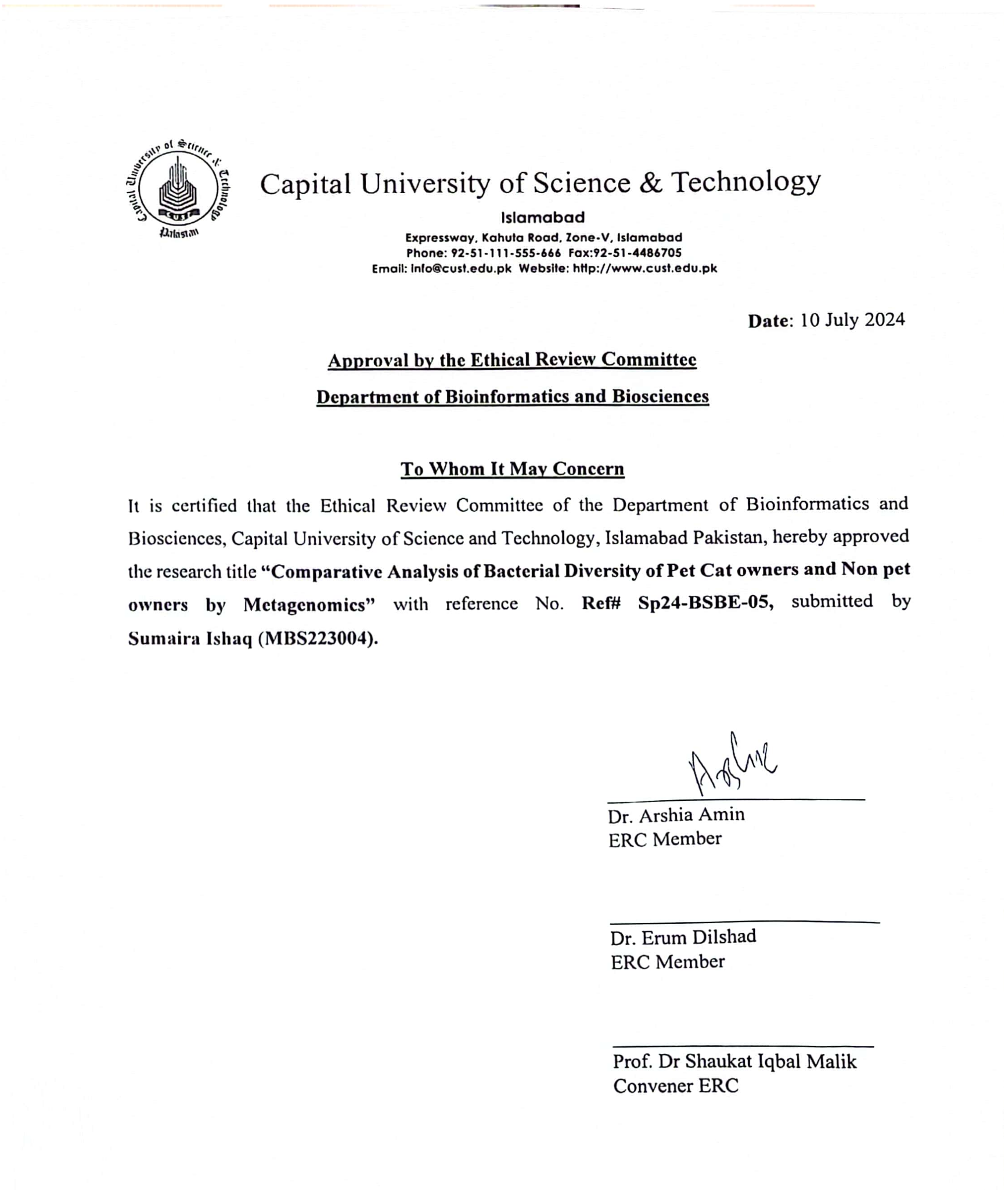
**Education and Training of Research Student**

☐ Yes ☐ No

**Significance of Research:**

**Research Methods:**

Selection Criteria of Patients/ Animals/ Plants/ Microbes:



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**Date**: 10 July 2024

**Approval by the Ethical Review Committee**

**Department of Bioinformatics and Biosciences**

**To Whom It May Concern**

It is certified that the Ethical Review Committee of the Department of Bioinformatics and Biosciences, Capital University of Science and Technology, Islamabad Pakistan, hereby approved the research title **"Comparative Analysis of Bacterial Diversity of Pet Cat owners and Non pet owners by Metagenomics"** with reference No. **Ref# Sp24-BSBE-05,** submitted by **Sumaira Ishaq (MBS223004).**

Dr. Arshia Amin
ERC Member

Dr. Erum Dilshad
ERC Member

Prof. Dr Shaukat Iqbal Malik
Convener ERC