GUT MICROBIOME CHANGES IN RESPONSE TO SHIFTS FROM RURAL TO URBAN ENVIRONMENTS OF THE TURKANA COMMUNITY IN KENYA

ORINA JEREMIAH

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Declaration

This thesis is my original work and has not been presented for a degree in any other University for examination.

Orina Jeremiah

I56/6978/2017

University of Nairobi

Signature:  Date: 29th July 2021

This thesis has been submitted for examination with our approval as University supervisors.

Dr. Joseph Kamau

University of Nairobi

Signature:  Date: 30th July 2021

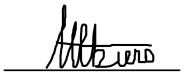
Dr. Julien F Ayroles

Princeton University

Signature:  Date: 29th July 2021

Dr. George Obiero

Center for Biotechnology and Bioinformatics, University of Nairobi

Signature:  Date: __30th July 2021_____

Dedication

This work is dedicated to my God, for it is His breath in our mind that makes men of understanding, and also to my family for their encouragement and support, I honor you guys.

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List of Abbreviations

DNA – Deoxyribonucleic acid

PCoA – Principal coordinates analysis

PERMANOVA – Permutational multivariate analysis of variance

FAO – Food and agricultural organization

WHO – World health organization

RNA – Ribonucleic acid

16s rRNA – 16 subunit ribosomal RNA

PCR – Polymerase chain reaction

QIIME – Quantitative insights into microbial ecology

OTU – Operational taxonomic units

BMI – Basal metabolic index

HDL – High-density lipoprotein

ASV – Absolute sequence variants

ANCOVA – Analysis of covariance

DF – Degree of freedom

BP – Blood pressure

ABSTRACT

The human gut microbiome has emerged as an important factor in many traits, including those associated with human health. Change in diversity and community composition of the gut microbiome is linked continuously to various health implications; recently, we are witnessing the rise of a variety of complex diseases related to dramatic changes in daily environments that are maybe related to mismatches between human and microbial evolution as population transit from rural to urban areas.

The human gut microbiome composition in industrialized populations is hypothesized to be different from those living in traditional lifestyles. To test this, I studied the Turkana community with an aim of finding out the gut microbiome variations of the traditional and urban group, and the various phenotypic factors that influence the changes. The Turkana gave me an opportunity to understand this, in that it is a unique Nilotic group in Kenya whose population has a common ancestry, but different levels of modernization. Some individuals live a traditional pastoralist lifestyle, while others are sedentary within the Turkana region or urban centers. The transitions from a pastoralist diet centered on meat, blood and milk to an urban diet rich in carbohydrates and fats can give a better understanding of the rising epidemic of chronic metabolic-related diseases around the world.

I characterized fecal microbiota of 133 individuals from the Turkana community, 90 - traditional and 43 – urban by paired-end v4 region of 16s rRNA region in the Illumina MiSeq platform. With this data, I asked two main questions: 1) How does the community composition and diversity of the Turkana microbiome compare between traditional and urban groups? 2) How are changes in the microbiome associated with phenotypic variations and the environment?

Community structure comparison of the traditional and urban population using UniFrac and Bray Curtis distances showed in the composition of gut microbiome between the two groups. Bacteroidetes, Firmicutes, and Proteobacteria were more abundant in the two populations; the Bacteroidetes were dominant in the urban population while the Firmicutes and Spirochetes dominated the traditional community. For alpha diversity, the traditional and urban people had a different

microbial richness and Fisher diversity, but no observed differences with the Shannon and Simpson diversity measures. The ANCOVA correlation results showed that BMI, Age, blood pressure, and residence time in current location correlate microbial richness and Fisher diversity, but Cholesterol and HDL do not. But none of the phenotypes associated with Inverse Simpson, Shannon, and Simpson, could have resulted from not having the sample size necessary to perform the comparisons.

In conclusion, the rural to urban areas transition has an impact on the gut microbiome both within and between the populations. I observed a gradual reduction in the microbial diversity in the Turkana population who've moved to urban areas. These, coupled with the loss of beneficial microbiome as has been observed in populations that transited to urban areas several years ago, indicate a possible increased risk to metabolic diseases. Therefore, Future studies should perform a complete sampling of both groups; and determine the metabolic profiles of every sample in order to provide a better understanding of the microbial community structure of the Turkana population.

CHAPTER ONE: INTRODUCTION

1.1 Background

The human microbiota describes a community of microorganisms comprised of bacteria, archaea, fungi, viruses and protists coinhabiting the host's body surfaces(Nibali & Henderson, 2016). The collection of the microbiota and their genes are termed as the human microbiome(Al Khodor et al., 2017). The gut microbiome of humans has beneficial role to the host in digestion, training the host immunity, protection against the colonization of pathogens, and lastly the regulation of the central nervous system (Gorvitovskaia et al., 2016; Tyakht et al., 2013). The microbial cells colonizing a healthy human body, inclusive of mucosal and skin environments, are equally abundant to our somatic cells(Gilbert et al., 2018). The composition of the microbiome is influenced by lifestyle, environment, genetics, antimicrobials treatment, mode of new born delivery, diet, and other factors(Lundgren et al., 2018; Oduaran et al., 2020). Though, the contribution of factors associated with rural to urban migration, such as diet, climate, host genetics, hygiene practices, medication and phenotypic traits is not apparent (Morton et al., 2015)

Transplantation of the microbiome can transfer beneficial phenotypes to the recipient from the donor such as nutritional status(Foo et al., 2017; Kachrimanidou & Tsintarakis, 2020). Indicating that altered microbiome can cause or be a result of changed physiological states (Clemente et al., 2015). Changes in human microbiome composition and structure of a given site termed as dysbiosis (Pessemier et al., 2021; Vangay et al., 2018a), might provide an understanding of why some individuals are more likely to be predisposed to some infections or a severe form of an illness(Carding et al., 2015). Variations in the microbiome are progressively linked to several non-communicable diseases development, and this includes; obesity,

diabetes, cardiovascular diseases, cancer, inflammatory bowel disease, asthma, and kidney disease (Al Khodor et al., 2017).

The host genetics and the gut microbiome can both impact metabolic phenotypes. The gut microbial communities of humans has been associated with metabolic disease(Carding et al., 2015), while dissimilarities in host genetics could also underlie susceptibility to metabolic disorders(Brüssow, 2020). Despite these shared effects, the relationship between diversity of gut microbiome and host genetic variation is largely unknown(Lamichhane et al., 2018; Scepanovic et al., 2019). A new-born comes into contact with the microbial communities of the uterus and the mother's birth canal during delivery, and subsequently with atmospheric microbial communities at birth(Deo & Deshmukh, 2019a). The gut microbiota is acquired environmentally from the time of birth. It may play a role as an environmental factor associating with the genetics of a host to shape phenotypes, and also a genetically determined attribute that is determined by and interacts with its host (Goodrich et al., 2014; Xia & Sun, 2017). Since the microbiome can be manipulated for therapeutic applications (Foo et al., 2017), it is composed of an appealing target for manipulation. Once the relations of host genetics and the gut microbiome are clearly understood, its manipulation could be optimized for a particular genome to minimize disease risk (Goodrich et al., 2014).

In this study, we will focus on the Turkana community, an eastern Nilotic group that practices nomadic pastoralism for subsistence. In their traditional settings, the Turkana community depend on almost entirely on their livestock for food (Barkey et al., 2016). Though the pastoralists occasionally trade with agriculturalists to obtain grains. Majority of the agriculturalists population were once pastoralists, until during the severe droughts of the 1960s and 1970s combined with livestock raiding, that forced them to settle and become farmers (Corbett et al., 2003). The recent development of roads, with the introduction of county governments and the discovery of oil, has further facilitated the transition of the Turkana to urban areas(Johannes et al., 2015). However, gut microbiome composition in urban

inhabitants is different from those practicing traditional lifestyles. Moreover, it hasn't been easy to disconnect human genetic, phenotypic expressions, geographic aspects contributions from lifestyle. Whether transitions from traditional lifestyle that is characteristic of humanity history influence the human gut microbiome, and to what extent remains unclear (Jha et al., 2018).

The Turkana community offers us an opportunity to understand the gut microbiome transition over a single generation of a population with similar ancestral descent. This study aims to analyse the gut microbiome variations across various lifestyles of the Turkana people, and to understand the relationship of various environmental and phenotypic traits with the microbiome. To do so, I collected stool samples, phenotypic data, and extensive interview information. I then extracted Deoxyribonucleic acid (DNA) that I later sequenced to obtain microbiome data. These involved the Turkana inhabiting their historical range (n=90) and second-generation immigrants of the same ancestry that now reside in major cities (n=43). I used the data obtained to characterize the microbial community composition of the two populations. These allowed my investigation on how the gut microbiome changes in response to transitions from rural to urban environments. I further analysed the data to give an understanding of the association between the gut microbiome, genetic variations, phenotypic traits, and the environment of the Turkana population. Then I will also determine unique genes in the gut microbiome of the Turkana community.

1.2 Research questions

What differences exist in the microbiome composition of rural and urban Turkana communities? How are they associated with genetic traits, phenotypic variations, and the environment?

What unique microbial communities exist across the Turkana community, and how significant are they?

1.3 Research Objectives

1.3.1 General Objective

To evaluate the gut microbiome variation of the traditional and urban Turkana people and the various factors that influence the changes.

1.3.2 Specific Objectives

i. To determine the diversity and community composition of the microbiome within and between traditional and urban populations of the Turkana.
ii. Evaluate the influence of various phenotypic factors on the gut microbiome of both populations.

1.4 Null Hypothesis

There are no variations in gut microbiome composition of the traditional and urban Turkana people.

1.5 Problem statement and Justification

1.5.1 Problem statement

Changes in gut microbiome are known to cause undesirable health implications. Recently we are witnessing the rise of a variety of complex diseases related to dramatic changes in daily environments as people transit from rural to urban environments. These transitions cause populations to experience a radically different set of conditions, including exposure to new diets and pathogens that plays a role in altering the gut microbiome. Transitions in the human microbial communities are increasingly associated to the development of some non-communicable diseases, including inflammatory bowel disease, diabetes, obesity, cardiovascular diseases, cancer, and asthma and kidney disease.

1.5.2 Justification

The Turkana have a common origin and ancestry, yet there are very different levels of modernization across the populations. Whereby some live a traditional pastoralist lifestyle, while others are sedentary within the Turkana region or urban centers. These allow us to understand gut microbiome transitions within a population of common genetic background.

The transition from a pastoralist diet centered on meat, blood, and milk to an urban diet rich in carbohydrates and fats should be particularly insightful in our quest to understand the rising epidemic of chronic metabolic-related diseases around the world.

CHAPTER TWO: LITERATURE REVIEW

2.1 The Human microbiome

The human microbiota describes a mixed community of microorganisms coinhabiting the human body surfaces(Kachrimanidou & Tsintarakis, 2020). A collection of the microbiota and their genes are known as the microbiome (Al Khodor et al., 2017; Matsuki & Tanaka, 2014; Ursell et al., 2012; Wang et al., 2017). The microbial cells colonizing the human body, inclusive of mucosal and skin environments, are as abundant as our somatic cells(Gilbert et al., 2018; Sender et al., 2016).

The human microbial communities' development follows trajectories that are site-specific to everybody site(Henry et al., 2019; Knights et al., 2017; Olivares et al., 2018). The skin microbiota of healthy adults are primarily dependent on the skin site physiology, with bacterial taxa relative abundance changes that are associated with dry, sebaceous, and moist environments. Lipophilic *Propionibacterium* species dominate sebaceous sites(Grice & Segre, 2011; Oh et al., 2012; Rosenthal et al., 2011). Whereas in damp areas, like the feet and bends of the elbows are preferentially dominated by microorganisms that thrive in wet or humid environments, such as *Corynebacterium* and *Staphylococcus* species(Byrd et al., 2018; Costello et al., 2009). However, in longitudinal sampling, the skin microbiome is relatively stable over a study period of 2 years despite changes in the environment(Oh et al., 2016; Sharma et al., 2019). Based on single nucleotide and strain level analyses, the stability is on strain maintenance over time instead of reacquiring common species in the environment(Byrd et al., 2018).

2.2 Human Gut, Oral, and Skin microbiota

The gut microbiota is the largest and most diverse microbiome in the human body, comprising of all microorganisms colonizing the gastrointestinal tract. It is capable of modulating multiple neural, immune and endocrine pathways of the host thus

influencing essential functions of the human body including energy metabolism, digestion and inflammation. Two of the twenty-nine known bacterial phyla are dominant in the gut of healthy humans: Bacteroidetes (mainly Gram-negative bacteria) and Firmicutes (majorly Gram-Positive clostridia) whereas Actinobacteria, Proteobacteria, Verrucomicrobia and Fusobacteria phyla are found less frequently.

The oral microbiota is the second largest in diversity and composition after the gut with over 700 bacteria species(Dewhirst et al., 2010; Kilian et al., 2016). This is due to the various niches in the mouth, leading to a rich habitat with microbes colonizing the teeth, cheeks, tongue, tonsils, gingival sulcus, soft palate, and hard palate(Deo & Deshmukh, 2019a; Gao et al., 2018). The oral cavity and nasopharyngeal regions provide ideal conditions for microorganisms growth; the average normal temperature of 37^0C, salivary pH of 6.5-7, saliva also keeps microorganisms hydrated in addition to being a medium for transportation(Deo & Deshmukh, 2019a). Microorganisms colonization of the mouth begins from first feeding after birth onwards, commencing the resident oral microbiome acquisition process(Deo & Deshmukh, 2019b; Mason et al., 2018; Sampaio-Maia & Monteiro-Silva, 2014).

Gut microbiome longitudinal studies show that adult microbiome is fairly stable and distinct to every person(Derrien et al., 2019; Lozupone et al., 2012; Martinson et al., 2019). In comparison to the extreme variability that takes place during the first three years of life(Caporaso et al., 2011).

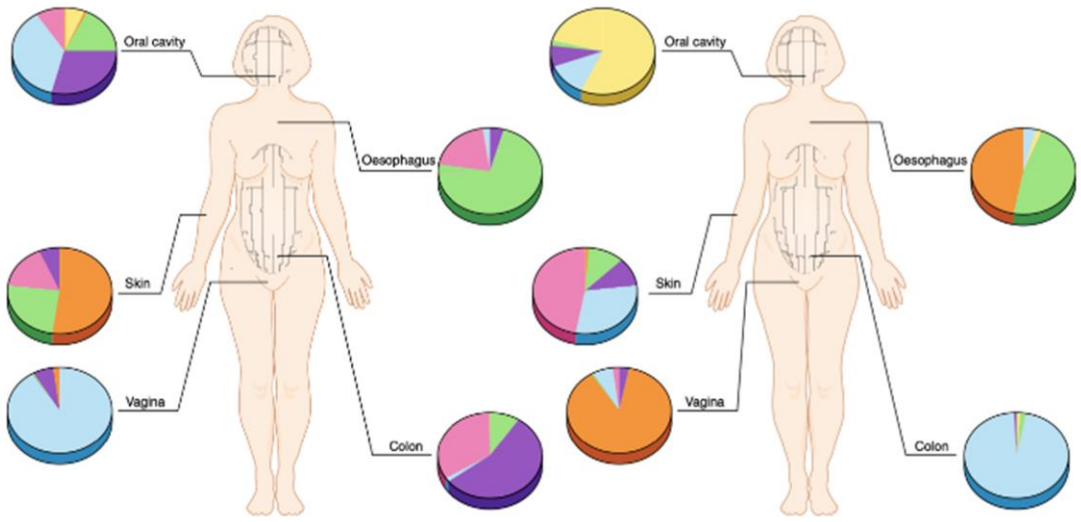

Figure 1: Shows the uniqueness of an individual's microbiome. The different colors in the pie chart represent different kinds of bacteria(Gilbert et al., 2018)

The microbiome being a living ecosystem, it does undergo fluctuations in each of its constituents in survival and growth rate(Gilbert et al., 2018; Prakash et al., 2020). For example, vigorous cleaning can, for a limited period, change the skin microbial community(Bouslimani et al., 2019; Longo & Zamudio, 2017; Yu et al., 2018). However, the original structure of the microbiota will re-emerge after the restoration of the original conditions. This is similar to the effects of diet changes to the gut microbiome(Clemente et al., 2012; Gagliardi et al., 2018; Hannigan et al., 2015; Milani et al., 2017). However, the most noticeable characteristic of the interaction between the human body and microbes is their inextricable link with each other(Inda et al., 2019). Thus they influence metabolism, immunity, resistance to pathogens at all ages, development, and other aspects of human health(Belkaid & Hand, 2014; Dominguez-Bello et al., 2019; Gill et al., 2006; Lazar et al., 2018; Ley et al., 2006; Wang et al., 2017; Zhang et al., 2019).

Humans have been subjected to a great diversity of environments since about 12,000 years ago in the Neolithic revolution(Henke et al., 2007). Thus the modes of subsistence known today are a result of various human populations trying to

diversify their dietary regimes(Morton et al., 2015). These significant cultural transitions have generated metabolic constraints in addition to novel pathogens as a result of the increasing population density and the proximity of livestock(Gordo, 2019; Jha et al., 2018). Such environmental and cultural differences amid populations have lead to physiological adaptations that can be detected in individuals' genome(Morton et al., 2015), which have also influenced the community dynamics of an individual's gut microbial ecosystem(Gordo, 2019).

The gut microbiome of humans is a highly diverse ecosystem with extreme inter-individual diversity (Huttenhower et al., 2012; Lloyd-Price et al., 2017; Mosca et al., 2016; Thursby & Juge, 2017). Following the second wave of data by the National Institutes of Health Human Microbiome Project, comprising of 1,631 new metagenomes in 265 individuals within multiple time points. That targeted various body sites. The study applied updated assembly and profiling methods to provide one of the broadest characterizations in baseline microbiome and functional diversity of the human microbial communities. The identification of strains revealed subspecies clades that are body part specific. Besides, it quantified species with underrepresented phylogenetic diversity in isolated genomes. Not only are the microbial communities different across different body sites, but also functionally distinct. Lastly, temporal microbiome analysis decomposed microbiome variation into stable, moderately variable, and rapidly variable subsets. These together, increase complexity in the human microbiome. Nonetheless, gaps still exist in the understanding of the processes that shape the gut microbiota throughout evolutionary timescales and also the consequence to human adaptation(Amato et al., 2019; Gordo, 2019; Henry et al., 2019; Quercia et al., 2014; Scanlan, 2019). The diversity and composition of gut microbial communities are attributed to various extrinsic and intrinsic factors that include host genes, environmental exposures (including diet and medication), gene expression patterns, and lifestyle factors(Cuesta-Zuluaga et al., 2019; Hasan & Yang, 2019; Leeming et al., 2019; Wen & Duffy, 2017; Zhu et al., 2020). Though the relative influence of factors associated with rural to urban migration, such as host genetics, diet, climate, medication, hygiene practices, and

phenotypic traits, is not clear(Morton et al., 2015). Factors that influence the microbiome composition must be well understood, in order to manipulate the microbiome for therapeutic, preventative, or for understanding a particular medical condition(Gilbert et al., 2018; Sarin et al., 2019).

2.3 Factors associated with gut microbiome

2.3.1 Host genetics and gut microbiome

The human microbiome composition is unique in every individual, and the differences are pronounced than the usual biochemical differences that come up within an individual over time(Lax et al., 2014). The genetics of the host and gut microbiome has been shown to influence metabolic phenotypes(Goodrich et al., 2014). Further, the microbial structure and composition of the identical and nonidentical twins in the same study, showed that the genetic effects of the host are variable, and certain taxa such as *Christensenella* are highly heritable(Goodrich et al., 2014).

Understanding how environmental factors and host genetics interact in shaping the gut microbiota composition is still a challenge(Org et al., 2015; Ussar et al., 2016). Others have said that the environment is more important than the genetics of the host(Phillips, 2009; Wen & Duffy, 2017). In a study examining microbiome data and genotype from 1,046 healthy participants, with a few distinct ancestral origins sharing a fairly common environment. Revealed that genetic ancestry does not have a significant association with the gut microbiome. Moreover, the genetics of the host plays a minimal role in microbiome composition determination(Rothschild et al., 2018). In contrast, the study demonstrated significant similarities of microbiome compositions in individuals who are genetically unrelated but sharing a household. More than 20% of microbiome variation between individuals is as a result of association with factors linked to anthropometric measurements, drugs, and diet(Rothschild et al., 2018). These results are in agreement with a primate microbiome study of 14 baboon populations across native hybrid zones. Which

demonstrated that the baboon's environment best-explained gut microbiota. More so, the exchangeable sodium and soil's geologic history, but little evidence in host genetic effects as a significant predictor of the baboon's gut microbiota(Grieneisen et al., 2019).

This knowledge is paramount in designing strategies aiming to modify the composition of gut microbial communities to improve health outcomes(Kemis et al., 2019). However, whether the genetic variation of the host plays a role in shaping the gut microbiome and also interacts with it to influence the host phenotype is unclear(Wang et al., 2018).

2.3.2 Effects of Diet on human microbiome

The effects of diet on the microbiome are extensively studied (Conlon & Bird, 2015; Rajoka et al., 2017). Diet modulation offers an ideal chance for psychologically and culturally tolerable low-risk intervention to alter the microbiome (Chey & Menees, 2018; Singh et al., 2017). Current evidence suggests huge effects of long term diet in the composition of the gut microbiome (David et al., 2014). However, a concise term change in diet can cause a resemblance in the gut microbiome of different people within days. This study showed that consumption of a diet composed of exclusive plant or animal-based products in the short term changes the microbiome structure, and overwhelms microbial gene expression differences between individuals. The diet based on animal products leads to an increase in microorganisms that are bile tolerant (*Bacteroides, Bilophila, Alistipes*) and a decrease in *Firmicutes* that are involved in the metabolism of plant polysaccharides (*Eubacterium rectale, Ruminococcus bromii, Roseburia)*(David et al., 2014).

The gut microbiome influences the leptin concentration in humans, thus also influencing appetite (Cani & Knauf, 2016; Rajala et al., 2014; van de Wouw et al., 2017). Interestingly, the effects of similar dietary ingredients on the measurements of blood glucose level can be variable in different people, a microbiome mediated effect (Zeevi et al., 2015). Whether the gut microbiome can influence dietary preferences is

still an open question, which probably can provide positive feedback when such diet changes make alterations to the microbiome (Gilbert et al., 2018).

2.3.3 Lifestyle as a factor in human microbiome

Lifestyle is known to posses a significant influence on the diversity and composition of the microbiome(Rodríguez et al., 2015; Wen & Duffy, 2017). Cohabitation with a spouse or pets such as dogs also correlates with the microbiome composition(Finnicum et al., 2019; Song et al., 2013). The ownership of a pet, exposure to livestock are linked with a decreased risk of asthma(Kemis et al., 2019; Stein et al., 2016).

Several lifestyle traits relate to the microbiota composition. Lack of exercise and smoking have a significant effect on the large bowel, and this potentially extends to the microbial community(Biedermann et al., 2013; Li et al., 2020). Smoking has a significant influence on the composition of the microbiota, leading to an increase in Bacteroides-Prevotella in healthy people and those with Crohn's disease(Conlon & Bird, 2015). Exercise influence the microbiome structure through a reduction in inflammation; this produces a subtle alteration in the composition of community microbiota that correlates with cytokine profile changes(Cook et al., 2016; Kemis et al., 2019). Stress has been shown to cause an increase in intestinal permeability, and this correlates with alteration in Actinobacteria and Bacteroidetes with a similar change in inflammatory markers and metabolite concentrations(Karl et al., 2017). Sleep loss and sleep deprivation correlate with changes in the gut microbiome. These lead to an elevated abundance of Erysipelotrichacea and Coriobacteriaceae and an increased ratio of Firmicutes to Bacteroidetes(Benedict et al., 2016).

Travel and occupation expose an individual to different places of residence and environments, thus influencing the microbiome(Leung & Lee, 2016). Circadian disorganization that occurs because of shift work, travel, or other reasons affect gut health and causes an alteration in the gut microbial populations(Voigt et al., 2014).

The state of personal hygiene and sanitary conditions can also facilitate the spread of infectious agents(Conlon & Bird, 2015).

2.4 Role of the microbiome in human health

An alteration in the gut microbiome could lead to or result from altered physiological states(Clemente et al., 2015). The alteration in the microbiome composition and structure of a particular site termed as dysbiosis (Vangay et al., 2018b), might provide an understanding of why some people are more likely to be predisposed to certain illnesses or a more severe form of illness(Carding et al., 2015).

The gut microbial community impact diverse physiological processes that range from obesity, adiposity, energy metabolism, glucose homeostasis, blood pressure control, clotting risks, or even behaviour(Dominguez-Bello et al., 2019). In each of these cases, mechanistic ties exist between gut microbes, host receptors, metabolites they generate, and phenotypic responses(Dominguez-Bello et al., 2019). Transplantation of the fecal microbiota can transfer phenotypes such as nutritional status from donor to recipient(Foo et al., 2017), this treatment is effective in individuals having dysbiotic microbiome with recurrent *Clostridium difficile* overgrowth (Jin Song et al., 2019).

A distal gut microbiota study comparing obese vs. lean mice and human subjects, demonstrated that obesity is associated with relative abundance changes of two bacterial divisions that are also most dominant, the Firmicutes and the Bacteroidetes(Tseng & Wu, 2019; Turnbaugh et al., 2006). Results from this study showed that the microbiome that is associated with obesity has a higher capacity of energy harvesting from the diet(Davis, 2016). Besides, the trait is also transmissible; germ-free mice colonization with a microbiota that is obese resulted in a notably increase in gross body fat in comparison to lean microbiota colonization(Turnbaugh et al., 2006). Which raises the possibility that gut microbiota manipulation could prevent obesity or facilitate weight loss in humans.(Davis, 2016; Tseng & Wu, 2019)

Studies have also shown that antibiotics cause relatively large effects on all microbiome in comparison to the other factors(Modi et al., 2014; Zarrinpar et al., 2018). Antibiotics are currently not only considered useful, but also as potentially harmful agents. Since their abuse appears to be significant in the pathogenesis of several disorders (metabolic disorders or *Clostridium difficile* infection) associated with microbial community impairment(Ianiro et al., 2016). The adult gut microbiome appears to be non-resilient to repeated administration of antibiotics; this may be attributed to metabolic states, different growth phases, or the network in the context of the microorganism's existence(Langdon et al., 2016; Lozupone et al., 2012; Singh et al., 2017). Increasing evidence suggests that antibiotics taken in the early life of an individual have an extreme impact on the microbiota that can lead to later development of asthma, inflammatory bowel disease, obesity, and other disorders(Ianiro et al., 2016; Lange et al., 2016).

2.5 Gut microbiome variation across the transition from subsistence to urban lifestyles

In studies comparing communities from unindustrialized rural areas; in Africa, Asia, western industrialized societies from North America, and Europe have disclosed specific gut microbiome adaptations to the respective lifestyles(Afolayan et al., 2019; Morton et al., 2015). These adaptations are inclusive of higher biodiversity and enrichment of Actinobacteria, Bacteroidetes in rural areas communities. On the other hand, western populations have an overall reduction in stability and microbial diversity(Mcdonald et al., 2018; Schnorr et al., 2014). A gut microbiome study of the Hadza community, whose lifestyle represents over 90% of the evolutionary history of humans(Rampelli et al., 2015). Found out that gut microbial communities of the Hadza have adapted to broad-spectrum metabolism of carbohydrates that reflect the complex polysaccharides of their diet(Rampelli et al., 2015). The enrichment in *Treponema*, *Prevotella*, peculiar *Clostridiales* taxa arrangement and unclassified

Bacteroidetes, might facilitate the ability of the Hadza to digest as well as extract valuable nutrition of fibrous plant foods(Schnorr et al., 2014). The Hadza microbiome is equipped for the biosynthesis of aromatic and the degradation of branched-chain amino acids. However, with limited exposure to antibiotics, the resistome functionality of the Hadza demonstrated the existence of antibiotic resistance genes. Which indicates the universal presence of resistances derived from the environment(Rampelli et al., 2015). In a study comparing the Hadza hunter-gatherer, an Italian, and African agricultural society subjects investigating gut microbiota variation, metabolite production and phylogenetic diversity. The study demonstrated higher levels of biodiversity and microbial richness than the Italian urban subjects. And that, further comparisons to two rural African farming groups showed more features that are unique to Hadza and also associated to foraging lifestyle. These were inclusive of the absence of *Bifidobacterium*, and microbial composition differences between the two genders reflect division of labour on the basis of sex(Schnorr et al., 2014).

Unindustrialized societies in rural areas are the primary targets. In understanding the interactions of humans and their gut microbiome. Because these populations have less reliance on sterile cleaners and antibiotics, they consume a lot of unrefined foods(Schnorr et al., 2014). A study that was examining lifeways and gut microbiome relationship through functional potential and taxonomic characterization of faecal microbial communities, from urban-industrialized US community and traditional agriculturalist and hunter-gatherer communities in Peru. The study showed metabolic and taxonomic differences between traditional and urban lifestyles. Additionally, the hunter-gatherers formed a clear-cut sub-group amid traditional peoples. In agreement with observations from previous studies, the study found out that *Treponema* is typical of traditional gut microbiota. Moreover, functional potential characterization and genome reconstruction discovered this *Treponema* to be of non-pathogenic clades. They are diverse with similarity to *Treponema succinifaciens*, known to metabolize carbohydrates in swine. *Treponema* is present in the gut of all traditional populations and non-human primates studied to

date. Thus they are symbionts non-existent in industrialized urban communities(Obregon-Tito et al., 2015).

A study of the Yanomami mountain people microbiome and resistome, who have retained their lifestyle as a seminomadic hunter-gatherer in the Amazon forest, with no contact to a modern lifestyle(Clemente et al., 2015). Yanomami microbiome has the highest bacterial diversity and genetic functions in comparison with what has ever been reported in human groups. However, their microbiome carries genes with functional antibiotic resistance, despite living in isolation from time past. These results demonstrated that human microbial community diversities are significantly affected by westernization. The functional antibiotic resistance genes are a characteristic of the microbiome regardless of commercial antibiotics exposure(Clemente et al., 2015).

Despite the recent focus on rural societies, a gap remains in our understanding of the microbiome host relationship among nomadic pastoralists inhabiting extreme conditions. The Turkana community, having retained their traditional lifestyle, provide us an opportunity to understand how ecological pressures can shape the human gut microbiome.

The traditional Turkana population are known to be healthier but that changes as they move to urban areas. Understanding the gut microbiome of this population could play a role in the search for microbiome-based therapy, in addition to understanding the role of the microbiome in the health of an individual. This study will also add to the microbiome research findings that have been conducted in Africa. Africa is currently considered as an understudied continent in microbiome research.

2.6 Methods in microbiome studies

2.6.1 Alpha Diversity

Alpha diversity measures give a summary of the structure of ecological communities for evenness (abundance distribution of the groups) and richness (measures the number of taxonomic groups)(Willis, 2019). Because any changes to a community influence the community's alpha diversity, comparing and summarizing community structures using alpha diversity measures is a ubiquitous approach of community surveys analysis. In microbial ecology, alpha diversity analysis of sequencing data is the most common primary approach to compute differences between environments meaningfully.

Rarefaction adjusts for the library size differences across samples to facilitate alpha diversity comparisons(Brewer & Williamson, 1994). Rarefaction method entails selecting a designated number of samples that is less than or equal to the number of samples contained by the smallest sample, then discarding reads randomly in the larger samples till the remaining number of samples equals to the set threshold(Hurlbert, 1971). Therefore with these equal size subsamples, diversity measures can be computed that can give a fair distinction of ecosystems unrestrained by sample size differences(McMurdie & Holmes, 2014; Weiss et al., 2017).

2.6.1.1 Diversity Indices

Diversity index normally refers to the mathematical measure of the species diversity in a microbial community. The indices provide additional information on the microbial community composition than only species richness (number of species present in a microbial community). Diversity indices also take into account different species' relative abundance. They play an important role in providing rarity and commonness information of species present in a community. The ability to determine diversity is a useful tool in understanding community structure(Jost, 2006).

Shannon and Simpson diversity methods are the most common indices in characterizing the diversity of species in a community. These methods account for both the evenness and richness of the present species, but the Simpson index offers more weight to evenness. Additionally, units are not similar across the various measures. The observed species gives a count, Shannon diversity measure has a logarithmic value while Simpson measure is an addition of the squared proportions.(Jost, 2006) These weighting and unit differences offer an explanation for the observed differences in results by each of the measure(Wagner et al., 2018).

2.6.2 Beta diversity

The diversity in association with an individual sample is the local or alpha component. The diversity for sample collections is called regional, or gamma component, while the relationship between the two components is beta diversity. Beta diversity is computed for every pair of samples and is a representation of either a distance or similarity between two samples(Tuomisto & Ruokolainen, 2006; Wagner et al., 2018).

2.6.2.1 Bray Curtis

Bray Curtis dissimilarity is a statistic that quantifies the species differences in populations between two distinct sites. It is normally a number in the range of 0 - 1. If 0, the two sites share all the species. If 1, they share none of the species. For easy interpretation, it is frequently multiplied by 100 and expressed as a percentage. In computing the Bray Curtis dissimilarity distances between two sites, the assumption is that both sites are similar in volume(Michie, 1982; Ricotta & Podani, 2017).

2.6.2.2 UniFrac distance measures

UniFrac is a β-diversity method to computes dissimilarities between microbial communities based on phylogenetic information(C. Lozupone & Knight, 2005). UniFrac determines the phylogenetic distances linking taxa sets in a tree of

phylogeny, the method coupled with multivariate standard statistical techniques including principal coordinates analysis (PCoA) can help determine whether microbial communities are significantly different(Lozupone et al., 2011). It compares several communities at the same time using ordination and clustering techniques, in addition to the relative contribution of various factors, such as geography and chemistry, to the similarities existing between samples(C. Lozupone & Knight, 2005). In 2007 a proportional weighting was added to the original UniFrac, and the two are weighted UniFrac and unweighted UniFrac(Lozupone et al., 2007). Weighted UniFrac distance measure uses the abundance information of species. It weighs the branch length with differences in abundance, and unweighted UniFrac distance measure puts into consideration only species absence and presence information and counts the branch length fraction unique to either of the communities.

Although both unweighted and weighted UniFrac methods have been used widely in measuring phylogenetic distances, their limitations as well have been noticed. The weighted and unweighted UniFrac measures assign too much weight to most abundant lineages (weighted) or rare lineages (unweighted). Hence, their power of detecting changes in abundance within lineages that are moderately abundant is limited. The generalized UniFrac distance is on the basis variance adjusted weighted UniFrac extending the unweighted and weighted UniFrac(Chang et al., 2011). Hence, generalized UniFrac distance measure through incorporating multivariate analysis of variance with permutation (PERMANOVA) is potent in detecting such changes, in addition to retaining almost all its power for the detection of highly and rarely abundant lineages, and it also possesses an overall better power in comparison to the joint usage of weighted/ unweighted UniFrac distance measure(Xia & Sun, 2017)

2.7 Study Areas and Population

The Turkana people are an eastern Nilotic group inhabiting the northwest region of Kenya in Turkana County that covers an estimated area of approximately 77,000km. Turkana County is one of the aridest ecosystems in East Africa; it experiences high temperatures year-round, with an average daytime high of 36^0C and daily averages of 30.5^0C. Turkana County generally is hot and dry most of the year, with a rainfall average of approximately 220mm per year. The rainfall distribution and patterns are unreliable and erratic with both time and space. Although the annual precipitation is meager, the events normally occur with high intensities in the short duration that lead to flash floods(Galvin et al., 2001). These conditions make subsistence in this region to be difficult to this poorly studied nomadic pastoralist population in addition to shaping

Subsistence in the Turkana region is difficult. The Turkana people are pastoral nomads and represent the second largest pastoral community in Kenya after the Maasai. Their diet is unusual protein-rich, with 62% derived from milk or milk products, and ~70-80% of the diet is from animal products. Daily protein intake exceeds the FAO/WHO requirements by >300%. However, the total caloric intake is limited, with dietary estimates of 1,300-1,600 kcal/day for adults(Kaye-Zwiebel & King, 2014; Popkin et al., 2019). The Turkana are thus lean and have limited energy reserves, yet they undertake the difficult task of collecting water daily. The process involves walking several miles to wells dug in dry riverbeds, and hauling water up from the bottom of a well, which can exceed 30 feet in dry seasons. Water must then be carried home and shared among family and livestock. As a result, the Turkana drink relatively little daily, while tolerating extreme heat and exerting considerable energy, they do so despite limited caloric reserves and a protein-rich diet, which takes considerably more energy to digest than carbohydrates or fats (Popkin et al., 2019). This extreme lifestyle has likely selected for a unique microbiome adaptation that we aim to uncover.

2.7.1 Lifestyle gradient in the Turkana community

The Turkana are majorly subsistence level nomadic pastoralists. They are transiting to a sedentary lifestyle, in their traditional lifestyle, the Turkana pastoralists herd goats, camels, cattle, and sheep(Galvin, 1992). However, many get forced to settle since they have lost their livestock to raiding, drought, poor management, and bad luck(Barkey et al., 2016). In their traditional settings, the Turkana relies almost entirely on their livestock for food, which is equivalent to 70-80% of their diet coming from some animal products; milk, meat, and blood. Though the pastoralists sometimes trade with agriculturalists to obtain grains. Many of the agriculturalists are Turkana, who were once themselves pastoralists, until during the severe droughts of the 1960s and 1970s combined with livestock raiding that forced them to settle and become farmers(Corbett et al., 2003). Farming has good potential in Turkana in areas where farmers have access to water from rivers, underground water, and impounded runoffs. Drought-tolerant crops like green grams and sorghum, as well as *Aloe Vera* and mangoes, are the crops mostly farmed. However, the choice of crops is dependent on water requirements and soil type.

Coupled with drought effects, from independence, a majority of African nations emphasized a nation-state inclusive of Kenya. Which lead to a highly centralizing effect negatively impacting efficiency in service delivery of local governance and public services, and Turkana county was one of the most affected areas in the nation(Hope, 2014). As the nation of Kenya embraced devolved government and introduced counties to attempt an improvement; in service delivery of local governance, public services, as well as increasing the administrative capacity and public sector productivity in a cost-efficient manner(Hope, 2014). Thus leading to improved infrastructure, an expansion of small scale markets into northwest Kenya, and the recent discovery of oil has facilitated the transition of the Turkana from their traditional to urban life. The urban population harvest and sell sand or make and sell woven baskets or charcoal or keep animals in fixed locations in various markets for trade instead of subsistence level nomadism. Additionally, some individuals have

completely left their homelands and are now inhabiting highly urbanized parts of the rift valley and central Kenya(Lea et al., 2019). Studies have shown that the gut microbiome composition in urban populations is not similar to those living traditional lifestyles. Although few studies have focused on pastoralist communities, it is difficult to separate human genetic, geographic factors, and phenotypic expressions contributions from lifestyle. Whether transitions from traditional lifestyle that is characteristic of humanity's past influence the gut microbiota and to what extent remains unclear(Jha et al., 2018).

The Turkana community provides several unique situations in understanding gut microbiome transition. They have a common origin and ancestry, with different levels of modernization across the population, this gradient provides us the opportunity to understand the effects of lifestyle changes to the microbiome of a population within a common genetic background. Secondly, the demographic history of the Turkana appears to include a recent bottleneck, leading to a relatively homogeneous genetic background among the Turkana, which makes the study of their microbiome even more appealing. Thirdly the Turkana have subsisted in a very harsh environment with extreme water scarcity and constant high temperatures for many years. Gut microbiome study will thus allow us to uncover signatures of selection. Thus understanding the uniqueness of their microbiome, which have evolved to confer a survival advantage in the harsh environment. The transition from a pastoralist diet centered on meat, blood and milk to a diet rich in carbohydrates and fats as is common in urban settings, is particularly insightful in our desire to understand the relationship of the microbiome with the rising epidemic of chronic metabolism-related diseases around the world and the development of fecal microbiota transfer therapy

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area, participating individuals and sample collection

Figure 2: A map of Kenya, showing sampling locations in Turkana County and Laikipia indicated by a star

I collected a total of 165 fecal samples from healthy individuals of the Turkana tribe with informed consent. One group came from the Northwestern part of the Turkana region (n=106), where most of the Turkana have maintained a traditional lifestyle. Another group came from the city of Lodwar and Nanyuki (n=59). Participants were men and women aged above 18 years. I collected the samples over a period of three months, from December 2018, then February and March 2019. I refrigerated the samples in cool boxes and took them to Turkana Basin Institute (TBI) and air lifted

them to Mpala research centre; at Mpala I stored the samples at -20^{0}C until DNA extraction.

I conducted in-depth interviews with each participant. This included requests for information such as: self-reported ancestry, age, and place of birth, occupation, summary medical history, diet, education, and number of children. I also collected basic anthropometric data that included; height, weight, waist circumference, skinfolds, and blood pressure. I used this data to prepare the metadata for the analysis section.

3.2 Fecal DNA extraction, Library preparations and 16S rRNA gene sequencing

I extracted total DNA from the 165 samples using the Quick-DNA^{TM} Fecal/Soil Microbe Microprep kit according to the manufacturer's protocol (Jha et al., 2018). I suspended pea size faeces in 750µl of lysis buffer, placed in a bashing bead lysis tube. I stored the extracted DNA at -20^{0}C until sequencing. I shipped the extracted DNA to Princeton University on dry ice and stored it at -20^{0}C until when I sequenced.

I did Qubit quantification to quantify the DNA in each sample. I repeated the process two times to be sure of the figures I was getting, I repeated three times for the samples that had a quantity of greater than 80ng/µl and those with a quantity less than 1 ng/µl. I quantified samples with a quantity of less than 1ng/µl without dilution, and those with a quantity greater than 80ng/µl I further diluted to 1/100 then quantified

I amplified the V4 region of the 16s rRNA of the normalized DNA in triplicates using the primers 515F/806R, targeting bacteria Achaea, following Earth Microbiome project modification (Caporaso et al., 2012). I prepared the master mix by adding 5 µl 2x buffer, 0.4 µl 515F forward primer (10µM), 0.4 µl 806R reverse primer (10µM) and 3.2 µl of PCR grade water.

I indexed the amplified DNA fragments. I prepared the master mix for indexing by 5 µl 2x buffer, 1 µl i5 (5µM by plate), 2 µl PCR grade water and 1 µl i7 (5 µM by individual sample), then I added 8 µl of the master mix and 1 µl of i7 to 1 µl of PCR amplicon. I then PCR amplified the products under cycling conditions 95^0C for 3 minutes, then 95^0C 30 seconds, 55^0C-30seconds, 68^0C 30s, 68^0C - 5 minutes I repeated 25 times then held at 12^0C for ∞. I ran a gel to confirm the success of the step.

After indexing, the samples, I cleaned them by Ampure XP beads. First, I let the beads stand for 30 minutes after taking them out of the 4^0C storage, then I created a pool from every indexed plate of the 6 plates, with each pool having 1.1 µl of every PCR index, that gave me a total of 7 pools in addition to 1 pool of unindexed plate with 96 µl volume. I added 1x beads to every pool and mixed thoroughly, and then I incubated at room temperature for 10 minutes. I placed the solution in a magnet for 5 minutes; I took off the supernatant and washed with 80% ethanol for 30 s and then air-dried for 5 minutes. I took off the magnet, and resuspended the product in 96 µl PCR grade water and incubated for another 5 minutes. I put the magnet back until it was clear after 5 minutes, then I took off the supernatant. I eluted the product in 25µl PCR grade water, the beads resuspended off the magnet for 5 minutes, then I put back the magnet, and kept the supernatant. I qubit quantified the cleaned libraries in 1:10 dilutions. Then I did further quality check by tape station. I paired-end sequenced the cleaned libraries using Illumina MiSeq at the Princeton University Genomics Core.

3.3 Bioinformatics analysis of 16s rRNA and statistical methods

I analyzed the paired-end reads using the QIIME2 version; 2019.7 pipeline, as described previously by (Bolyen et al., 2019). I followed the following criteria in filtering the sequences; I first demultiplexed the sequences and in this step I used the metadata file; to obtain high quality sequences, I trimmed read length not shorter than 50 base pairs (bp) and not longer than 250bp, I then denoised the reads using the

denoising package DADA2 an implement of q2-dada2 plugin(Callahan et al., 2016). I trimmed samples with less than 20000 reads, and I retained only OTUs with more than ten occurrences. I filtered negative controls and rep - seqs for the phylogenetic tree, and I was left with a total of 133 samples n=90 for the traditional population and n=43 samples for the urban population for further analysis. I did subsequent statistical analysis to determine alpha, beta diversity and alpha diversity correlation with the various phenotypes using R-studio version 1.2.5001 and several other packages but majorly phyloseq(McMurdie & Holmes, 2013).

3.4 Ethical Approval.

Maseno University Ethical Review Committee approval number: MSU/DRPI/MUERC/00519/18, Princeton University Institutional Review board approval number 10237 (IRB # 10237) and NACOSTI/P/18/46195/24671 for evolutionary and functional genomics of the Turkana: signatures of past selection and responses to modern urbanization.

CHAPTER FOUR: RESULTS

4.1 Lifestyle aspects of the Turkana community

To explore the lifestyle of the Turkana community, we collected biomarker data. We conducted an extensive interview of the sampled Turkana population. From this data, we tried to determine how the two populations relate to one another phenotypically.

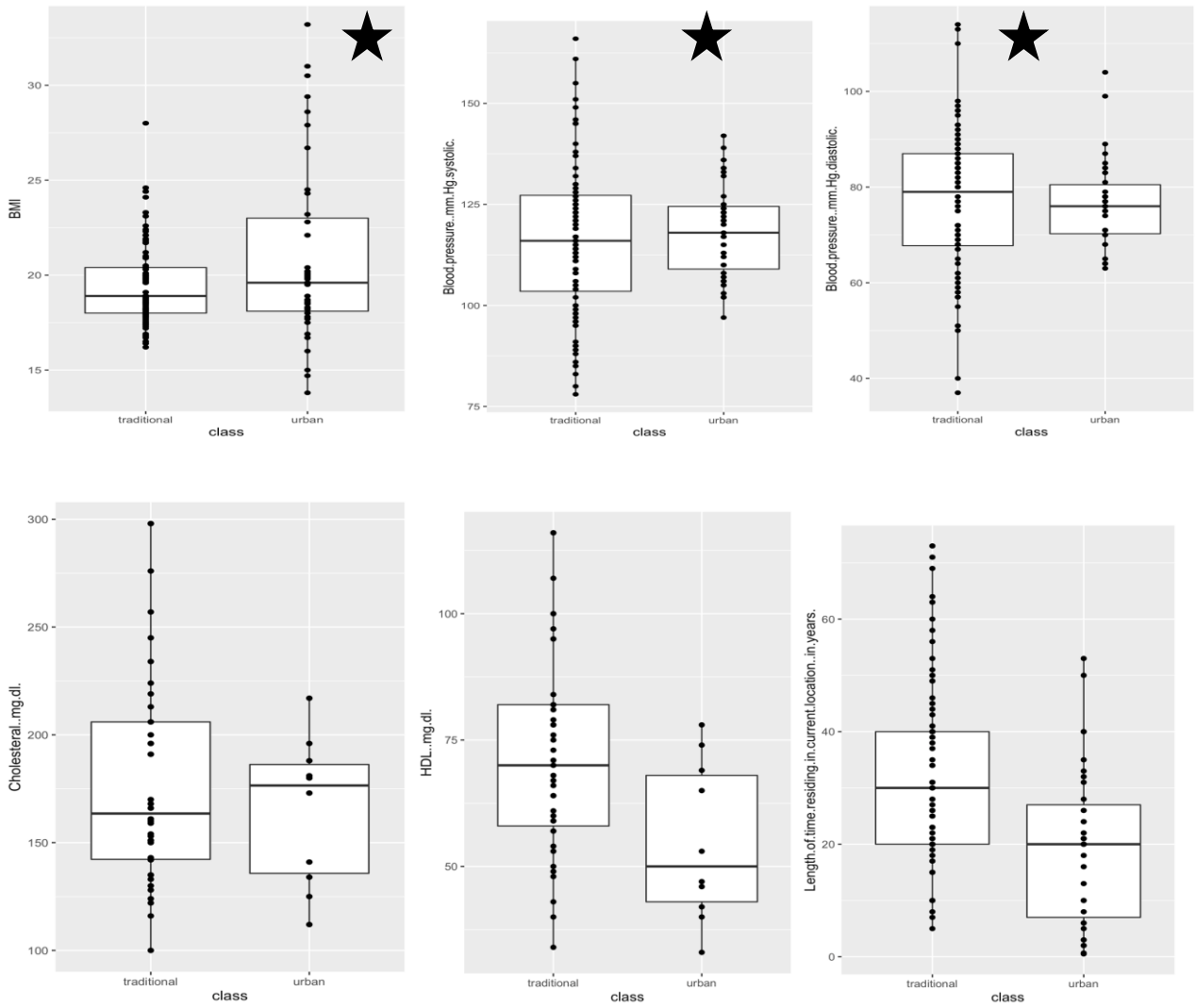

Figure 3: Box plot visualization of the phenotypic differences between the traditional and urban population from top left in clockwise direction a) BMI, b) systolic blood pressure, c) diastolic blood pressure, d) cholesterol, e) high-density lipoprotein and g) length of time residing in the current location. Every dot represents an individual in the population, the star represent those with significant differences, and not all individuals were sampled for all measures

The results showed that only one individual had a BMI above 25 for the traditional community. However, the urban population had several individuals that had a BMI value above 30. However, the BMI differences between the traditional and urban populations were not significant (Figure 3A, t-test, p=0.0641). The mean BMI value for the urban population was 21.01 and 19.49 for the traditional community.

We also checked the blood pressure variation differences between the two populations for both diastolic and systolic blood pressure. There was a more considerable variation in the blood pressure of the traditional population as compared to the urban population. Systolic blood pressure was not significantly different between urban and traditional communities (Figure 3C, t-test, p=0.4958). The diastolic blood pressure also didn't show a significant difference between the urban and traditional population (Figure 3D, t-test, p=0.8243)

The cholesterol of the traditional population is distributed more evenly as compared to that of the urban population. The cholesterol differences between the traditional and urban people were not significant (Figure 3F, t-test, p=0.307). However, only 36.09% of participants from both urban and traditional populations were sampled for cholesterol. On the other hand, the high-density lipoprotein (HDL) distribution in the two communities was different, with the traditional society having a higher HDL in comparison to the urban population (Figure 3G, t-test, p=0.0094). The HDL results represent 36.84% of the participants from both the traditional and urban populations.

The length of time residing in the current location in years is significantly different between the traditional and urban communities (Figure 3H, t-test, p = < 0.0001) with a mean value of 32.43 years for the traditional population and 19.65 years for the urban population. These show that the Turkana population started transiting to urban areas recently.

4.2 Sample sequencing depth distribution

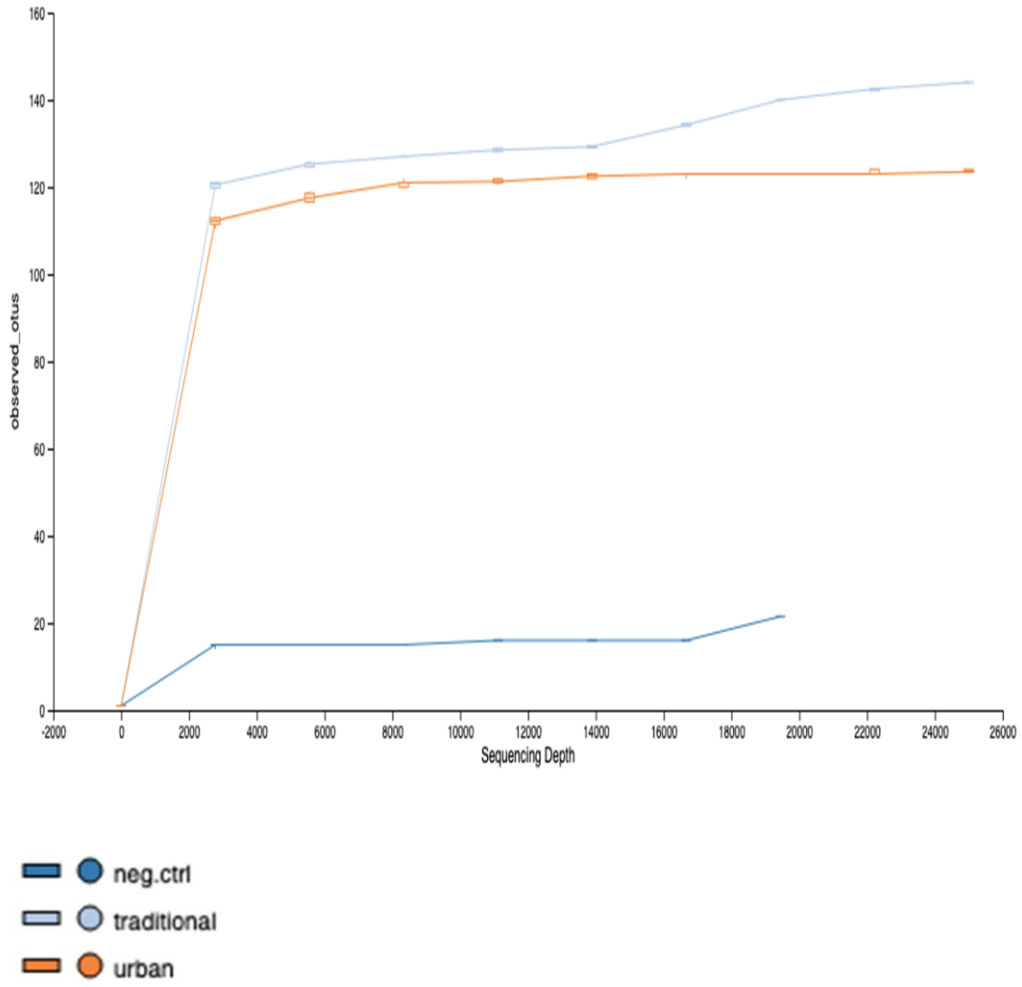

Figure 4: Alpha rarefaction curve showing the observed taxonomic units (OTUs) against sample sequencing depth for every individual in the population

In this study, rarefaction leveled off at a sequencing depth of 6000, suggesting we fully sampled the microbial community.

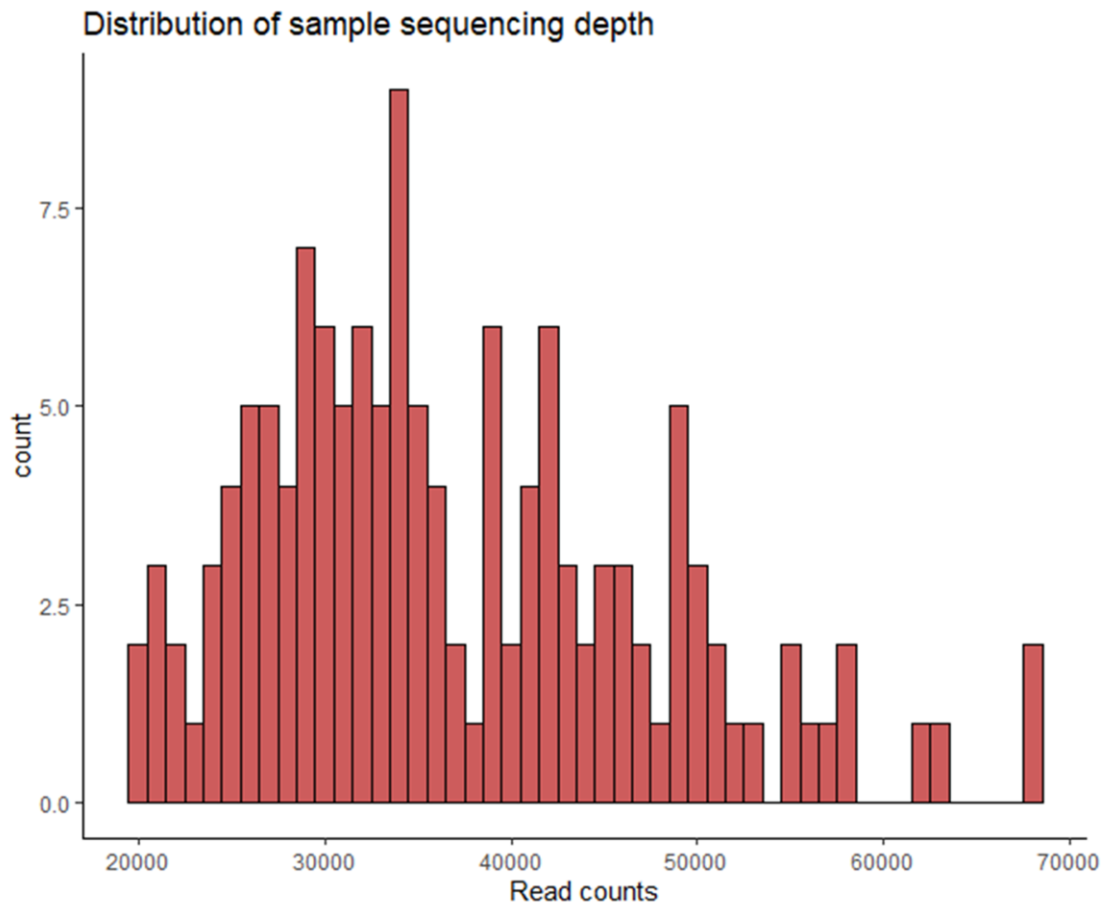

Figure 5: Histogram showing the read counts distribution of samples sequenced for the microbiome. The results represent merged data of each sample for the triplicate plates.

In this study, we sampled deeply and merged the data from the three plates. The average read count of all the samples was 36,961.37 counts, the highest read count was 67,715, and the minimum was 19,785 counts. The total reads for all the samples were 4,915,862 reads, 1250 absolute sequence variants (ASVs) were present across 15 phyla.

4.3 The relative abundance of the Turkana microbiome

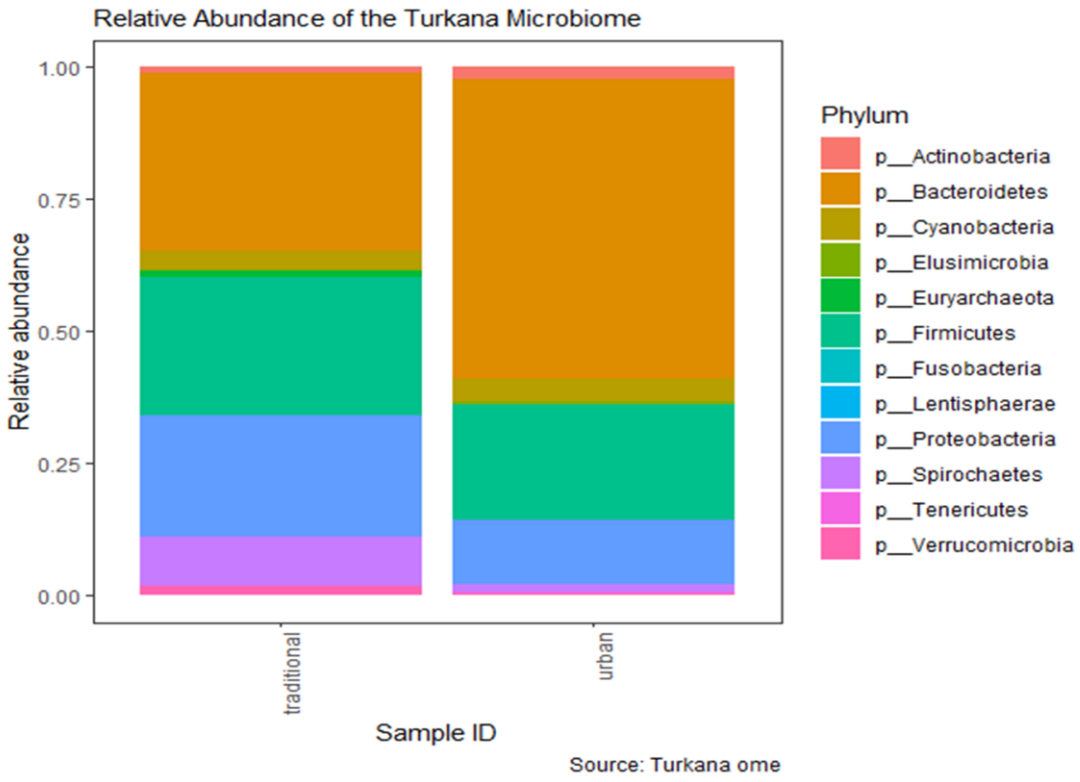

Figure 6: Relative abundance of the traditional and urban population at the phyla level, the different colors indicates the different phyla present.

Several phyla were present in the data of the urban population that included; Actinobacteria, Firmicutes, Spirochaetes, Bacteroidetes, Lentisphaerae, Cyanobacteria, Euryarchaeota, Proteobacteria, Fusobacteria, Elusimicrobia, and Tenericutes that were equally present in the traditional population, with the addition of Verrucomicrobia with differences in the abundances between the two groups.

The most prevalent phyla in the communities were Bacteroidetes, with a more pronounced abundance in the urban population; the Firmicutes that were more abundant in the rural population followed this. The Spirochaetes and Verrucomicrobia were also more abundant in the rural community. There was an equal abundance of cyanobacteria between the communities.

We also performed a t-test to check whether differences between the most abundant phyla were significant. The urban group had significantly more Bacteroidetes than the traditional group (Figure 6, t-test, p = < 0.0001). Bacteroidetes in the urban group is made up of 38 individuals representing 88.37% of the participants, while only 64 individuals in the traditional group representing 71.11% of the participants.

There was no significant difference between Firmicutes in the traditional and urban populations (Figure 6, t-test, p=0.4046). However, there was a considerable difference for Firmicutes, between the urban and traditional communities. When checking the number of individuals between the two populations that had the presence of Firmicutes, 75 individuals that represent 82.42 % of the sampled individuals in the traditional community had Firmicutes. And in the urban population, only 22 individuals that represent 50 % of the urban population participants had the presence of Firmicutes as described in figure 6 below.

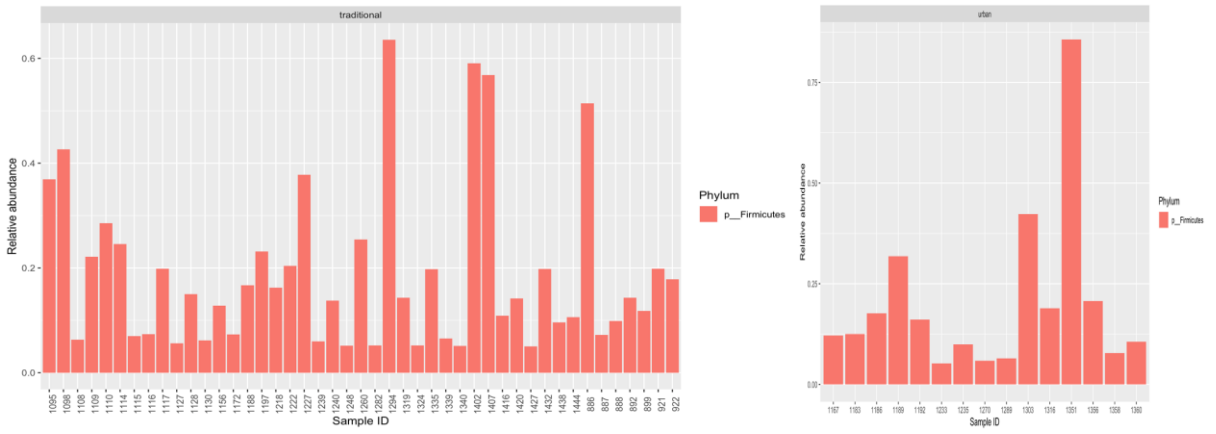

Figure 7: Relative abundance of Firmicutes across individuals from (A) traditional and (B) urban groups. Firmicutes were in 82.42% of the traditional population, but only 50% of the urban population.

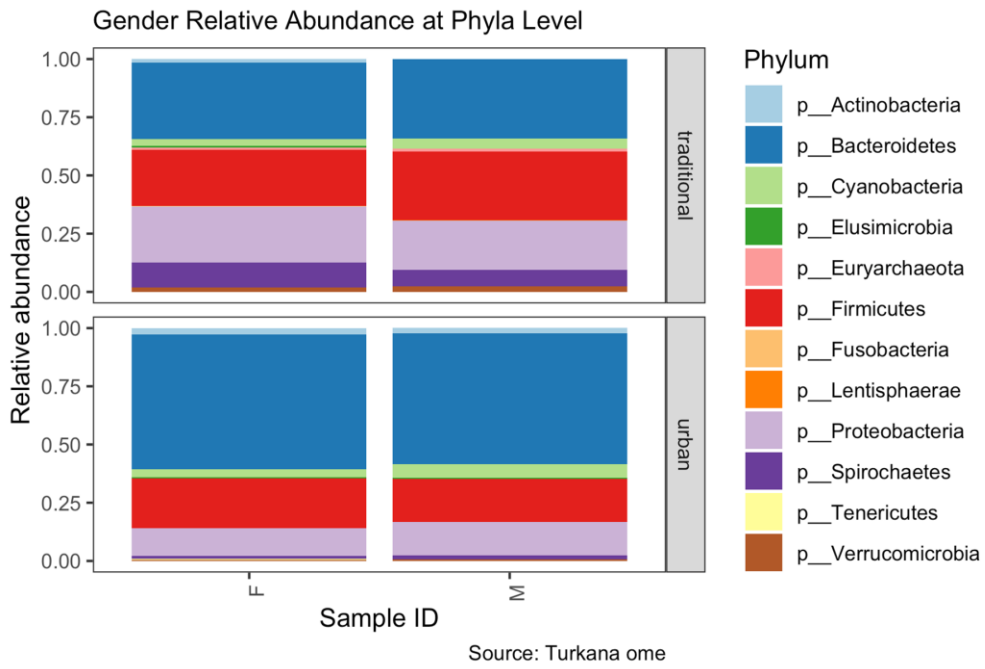

Figure 8: Relative abundance differences between male and female gut microbiota in traditional and urban populations. The Top chart, A represents a variation within the traditional community, and the bottom chat, B represents the variation within the urban population.

The relative abundance of the urban population seemed uniform regardless of the gender of the individual. However, the rural community showed a difference in microbiome abundance between the males and the females. The Firmicutes were more abundant in males than in females; the Proteobacteria were more abundant in females than in males. Then the Spirochaetes were more abundant in females than males; nevertheless, the Spirochaetes were only present in the rural population. The Verrucomicrobia were also present only in the traditional community.

Additionally, the traditional population microbial communities showed a variation of Spirochaetes, Proteobacteria, Firmicutes, Cyanobacteria, Bacteroidetes, and Actinobacteria between males and females.

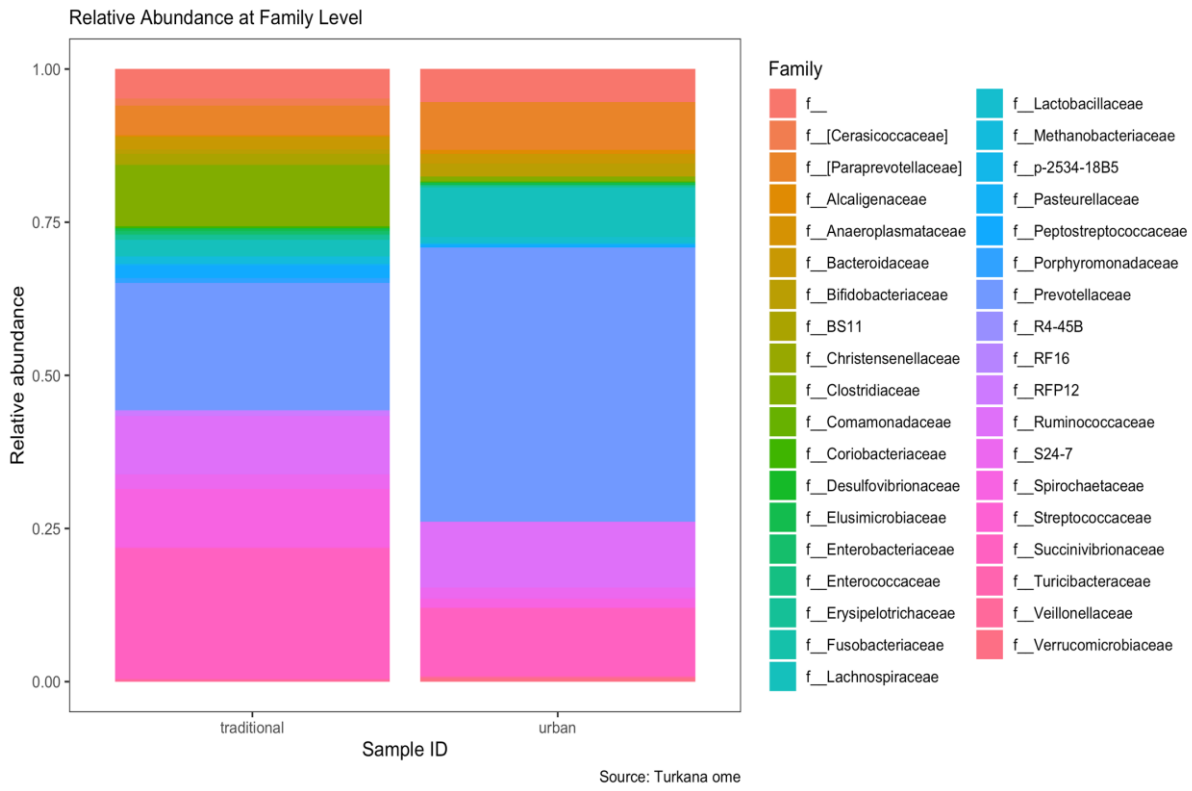

Figure 9: The Microbiome relative abundance at the family level of the traditional and urban Turkana population.

4.3.1 Relative abundance at the family level

The relative abundance bar plot (Figure 9) showed differences between the urban and traditional populations. The most abundant family in the two groups was the family Prevotellaceae, other families that were available include, Bacteroidaceae, Porphyromonadaceae and [Paraprevotellaceae] (that is a recommended annotation) with three other unknown groups.

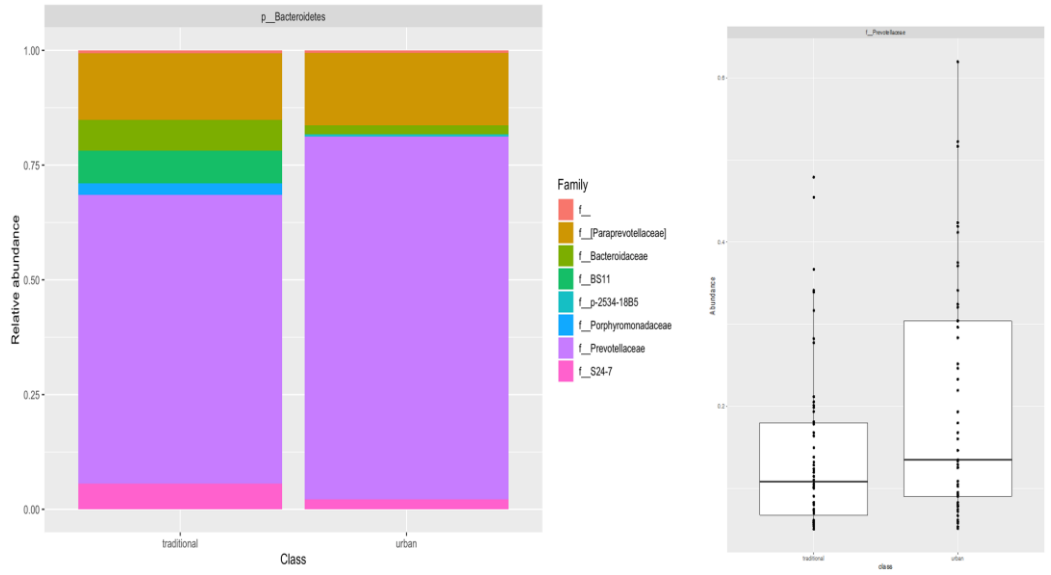

Figure 10: A: First figure shows relative abundance of the Bacteroidetes at the family level for the urban and traditional population. B: Boxplot of Prevotellaceae family variation between the traditional and urban populations; each point represents an individual.

The urban group had significantly more Prevotellaceae than the traditional group (Figure 10A, t-test, p=0.0216), further visualizing the data using a boxplot (Figure 10B) also demonstrated the observed distribution differences in the individuals of the two populations. Another group that we visualized was the family Spirochaetaceae. The traditional community had significantly more Spirochaetaceae than the urban group (Figure 9, t-test, p=0.0412). A boxplot distribution visualization of the Spirochaetaceae (Figure 11) showed the same difference, and only three individuals in the urban group had the family.

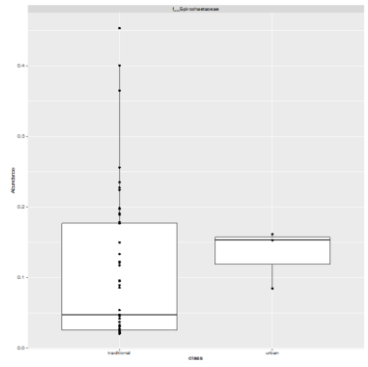

Figure 11: The Spirochaetaceae family variation between the traditional and urban populations; each point represents an individual.

4.4 Gut microbiome variation across the Turkana population

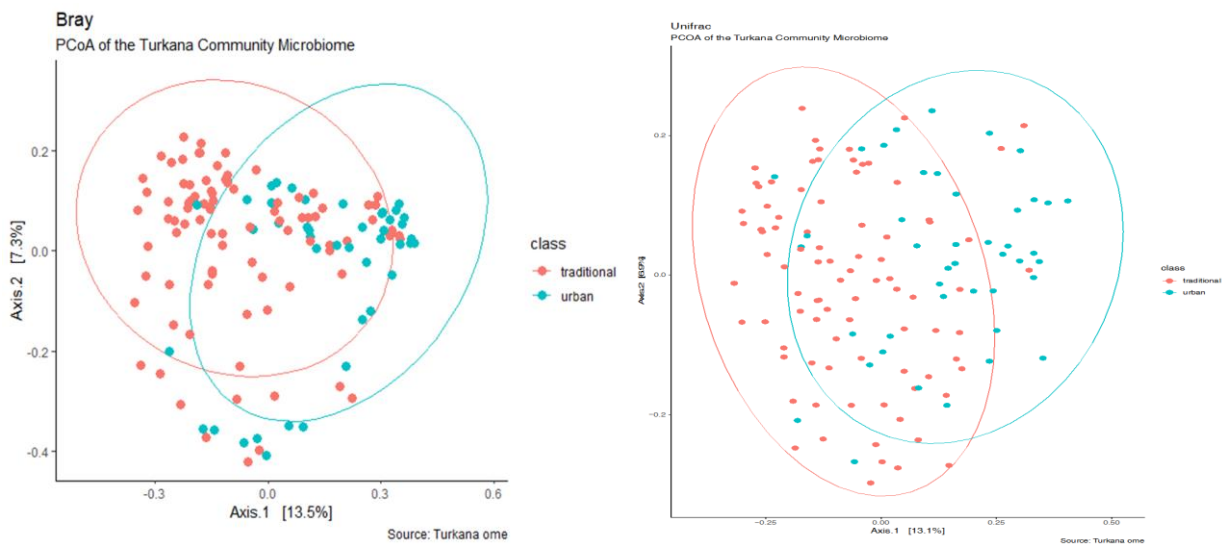

Figure 12: Beta diversity between the traditional and urban populations. A: Bray curtis diversity differences between the traditional and urban populations, each blue dot represent an individual in the urban community and a red dot an individual in the traditional community, B; UniFrac diversity between the urban and traditional populations, each blue dot represent an individual in the urban population and a red dot represents an individual in the traditional population

Community structure comparison in the two study populations using Bray Curtis and UniFrac distances showed a variation in gut microbiome composition between the populations. UniFrac is a method that computes microbial communities' differences by incorporating phylogenetic information. UniFrac measures phylogenetic distances between taxa sets in a tree of phylogeny as a fraction of branch length of a tree leading to descendants from one environment or the other(C. Lozupone & Knight, 2005). The method revealed a difference between urban and traditional populations using clustering and ordination techniques. The results were similar to the bray Curtis dissimilarity test that also demonstrated a difference in diversity between the urban and traditional populations of the Turkana community. Unlike UniFrac, Bray Curtis quantifies the differences using sequence abundance(Goodrich, Di Rienzi, et al., 2014). We tested the significance of the differences using Permanova, Adonis

test with 999 permutations that confirmed that the differences between the two populations were significant.

Table 1: Adonis test for Bray Curtis distance, showing the significance of the differences between the traditional and urban populations.

	Df	Sums Of Sqs	Mean Sqs	F. Model	R2	Pr (>F)
Class	1	1.897	1.89651	5.862	0.04347	0.001***
Residuals	129	41.735	0.32353		0.95653	
Total	130	43.632			1.00000	
Signif Codes	0 '***'	0.001 '**'	0.01'*'	0.05 '.'	0.1 ' '	1

Table 2: Bray Curtis betadisper homogeneity test among the urban and traditional group

	Df	Sum sq.	Mean sq.	F	N. Perm	Pr (>F)
Groups	1	0.08458	0.084582	15.536	999	0.001***
Residuals	129	0.70230	0.005444			
Signif. Codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ''	1

The beta dispersion results for Bray Curtis distance were significant, showing that there is a significantly unequal variance between the traditional and urban groups, stating that the two populations have the same dispersions. The results make us confident that the traditional and urban communities have differences in-group dispersions.

Table 3: Adonis test for UniFrac distances, showing the significance of the differences between the traditional and urban populations.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr (>F)
Class	1	1.596	1.5963	6.6266	0.04886	0.001***
Residuals	129	31.076	0.2409		0.95114	
Total	130	32.672			1.00000	
Signif. Codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' '	1

Table 4: UniFrac betadisper test for the traditional and urban groups

	Df	Sum Sq	Mean Sq	F	N. Perm	Pr (>F)
Groups	1	0.08458	0.084582	15.536	999	0.001***
Residuals	129	0.70230	0.005444			

The beta dispersion results for UniFrac distances were significant, showing a significantly unequal variance between the traditional and urban Turkana groups.

4.5 Gut bacterial diversity (alpha) across the Turkana population

Alpha diversity measures were employed to determine the ecological community structure about its richness (the number of available taxonomic groups), evenness (abundance distribution of the groups), or both(Willis, 2019).

Inverse Simpson, Fisher, Shannon, and Simpson's measures were also applied to determine richness and evenness within every group. Richness measures helped us to characterize communities by the number of species that were available in the group. While Species diversity or evenness helped us determine how equally abundant the species were within an individual population in every group.

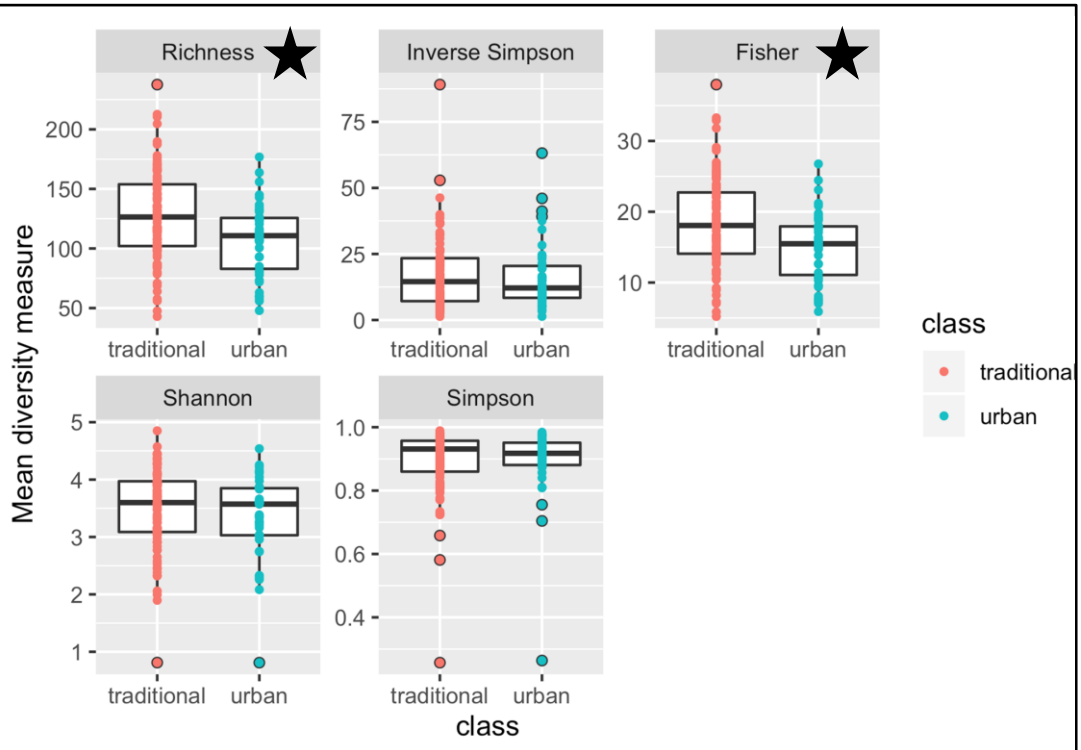

Figure 13: The alpha diversity of the Turkana population microbiome, the first figure shows richness, then evenness by Inverse Simpson, Fisher, Shannon, and Simpson measures respectively. Each point represents an individual in the urban and traditional group and the black star indicate measures that had significant p value.

The number of species in every group (alpha diversity) was measured using Inverse Simpson, Fisher, Shannon, and Simpson measures. The general trend was that the samples in the traditional population are distributed more evenly in comparison to the urban population. We also measured richness in different ways, and the general trend was more richness in the traditional community than the urban population. A T-test for the differences reflected the same results, whereby the traditional group had a more significantly richness than the urban group (Figure 13A, t-test, p=0.0015).

We also measured the significance of the diversity differences between the two groups using the different measures of diversity. The results for diversity

measurement using the Fisher measure showed that the microbial community distribution in the traditional population was more even than the urban population (Figure 13C, t-test, p=0.0011).

The evenness results by Inverse Simpson, Shannon, and Simpson showed that the traditional community is highly diverse compared to the urban population. However, the differences between the traditional and urban populations were not significant for Inverse Simpson (Figure 13B, t-test, p=0.7862), Shannon (Figure 13D, t-test, p=0.3183) and Simpson measure (Figure 13E, t-test, p=0.9599).

4.6 Alpha Diversity Correlation with Phenotypes

We also checked the correlation between phenotypes and the various diversity measures to find out the differences between the traditional and urban populations.

4.6.1 Alpha diversity Correlation with BMI

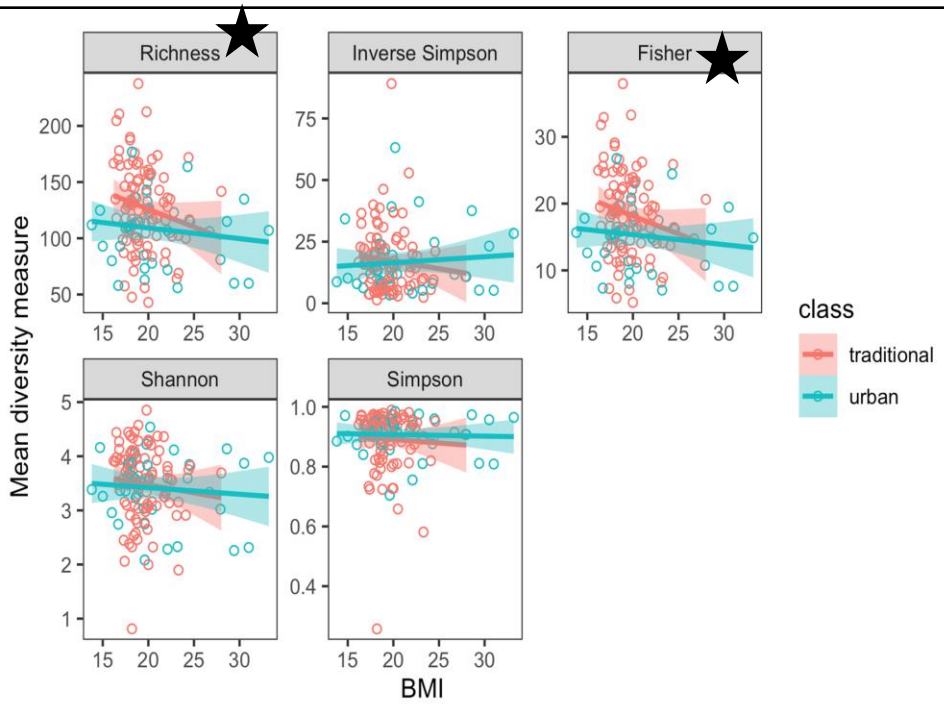

Figure 14: The correlation between microbial diversity with BMI. The green line represents the correlation between BMI and average diversity measures in the urban population. The red line represents BMI and diversity measure correlation in the traditional group. The red and green points represent the participants in the study. The black star shows correlations with significant p values.

The ANCOVA correlation results between alpha diversity and BMI showed that mean microbial richness (Supp. Table 6, F = 4.873, p = 0.0291) and Fisher diversity (Supp. Table 6, F = 4.886, p = 0.0289) are in correlation with BMI. There was no correlation between Inverse Simpson, Shannon, and Simpson's measures with BMI. The urban and traditional population had different Richness (Supp. Table 6, F =

5.538, p = 0.0202) and Fisher (Supp. Table 6, F = 5.668, p = 0.0188) diversity values but similar for Inverse Simpson, Shannon and Simpson diversity measures. No interaction existed between BMI and the two populations for all alpha diversity measures.

4.6.2 Alpha diversity in correlation with age

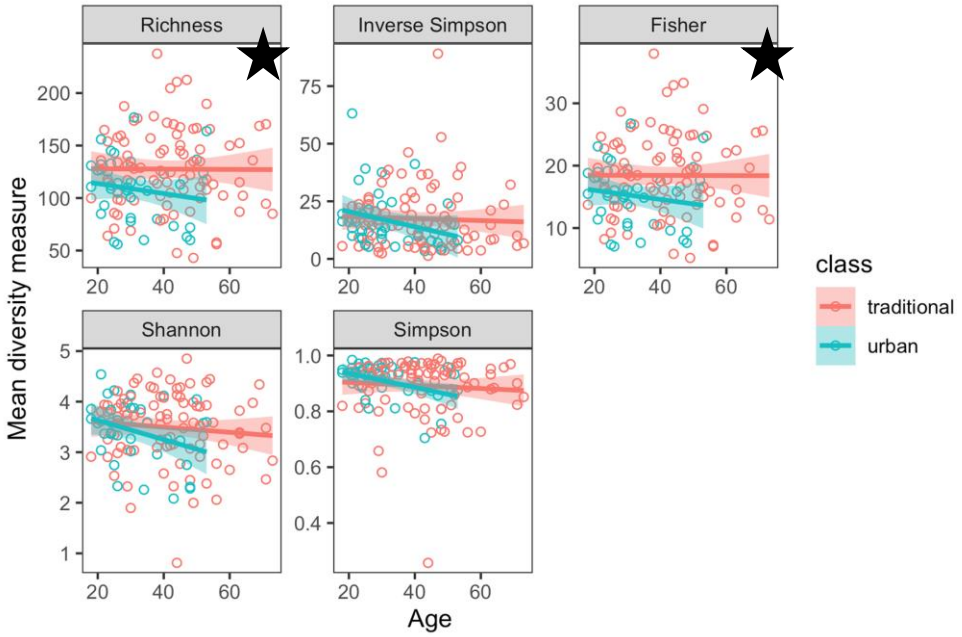

Figure 15: The correlation between microbial diversity with age in years. The green line represents; the relationship between age in years and mean alpha diversity measure in the traditional population, and the red line represents age and average alpha diversity measure correlation in the urban population. The red and green dots represent the participants in the study. The black star shows correlations with significant p values.

The ANCOVA alpha diversity correlation with age indicated that there is no correlation between alpha diversity and age. The urban and traditional population had different Richness (Supp. Table 7, F = 7.345, p = 0.0077) and Fisher (Supp. Table 7, F = 7.384, p = 0.0075) values but similar values for Inverse Simpson,

Simpson and Shannon. No interaction exists between age and the two population groups for all the diversity measures.

4.6.3 Alpha diversity in correlation with cholesterol

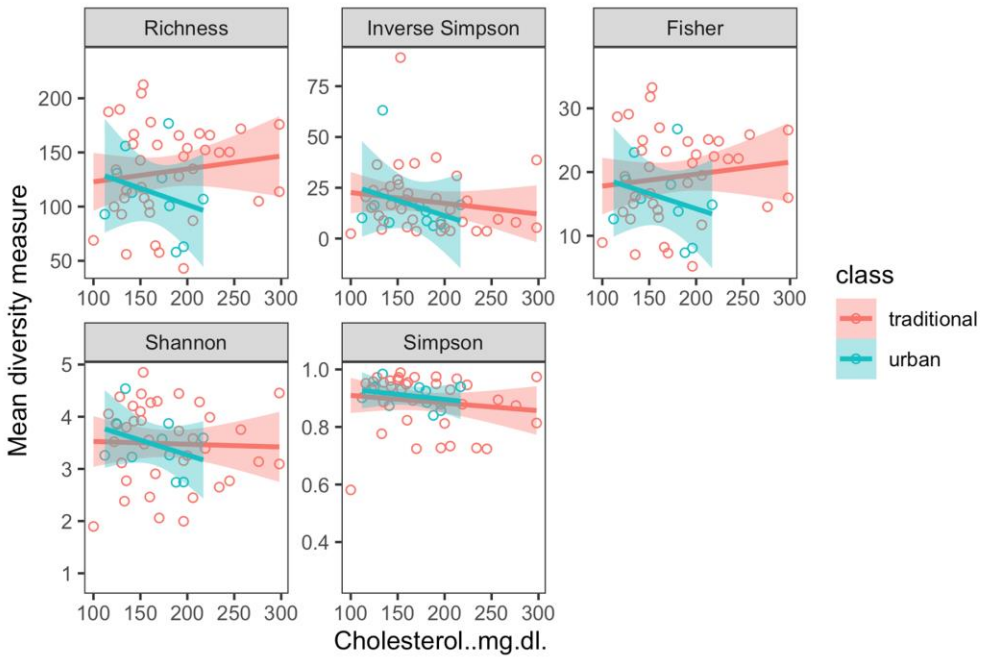

Figure 16: The correlation between microbial diversity with cholesterol. The green line represents the correlation between cholesterol and average diversity measure in the urban population, and the red line represents cholesterol and average diversity measure correlation in the traditional community. The red and green dots represent the participants in the study.

The ANCOVA alpha diversity correlation with cholesterol showed that cholesterol does not correlate with alpha diversity measures. Additionally, no interaction existed between cholesterol and the two population groups. However, the results represent 48 participants sampled for cholesterol out of the total 133.

4.6.4 Alpha diversity in correlation with HDL

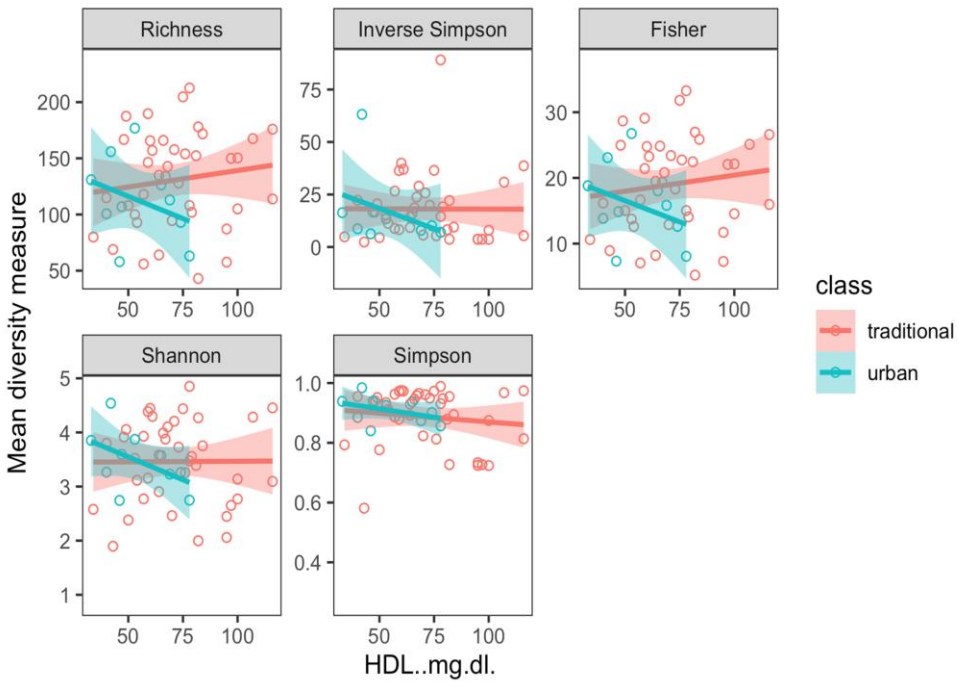

Figure 2: The correlation between microbial diversity with high-density lipoprotein. The green line represents HDL and average diversity measure correlation in the urban population, and the red line represents HDL and average diversity measure correlation in the traditional society. The red and green dots represent the participants in the study.

The ANCOVA alpha diversity correlation with HDL showed that HDL does not correlate with alpha diversity measures. Additionally, no interaction existed between HDL and the two population groups. The results represent 49 individuals sampled for HDL out of the total 133 in the study.

4.6.5 Alpha diversity in correlation with diastolic blood pressure

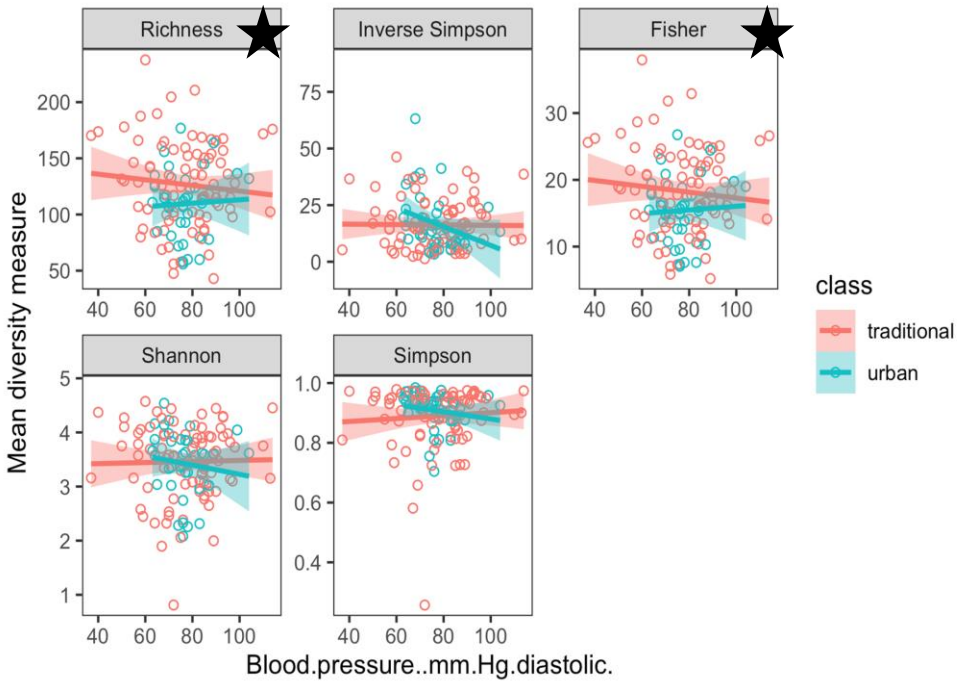

Figure 18: Mean microbial diversity correlation with diastolic blood pressure. The green line represents the correlation between diastolic blood pressure and alpha diversity measures in the urban population. The red line represents diastolic blood pressure correlation with mean alpha diversity measure in the urban population. The red and green dots represent the participants in the study, and the black star shows correlations with significant p values.

The ANCOVA correlation results between alpha diversity and diastolic blood pressure showed that diastolic blood pressure does not correlate with alpha diversity. No interaction existed between diastolic blood pressure and the two populations for all alpha diversity measures.

4.6.6 Alpha diversity in correlation with systolic blood pressure

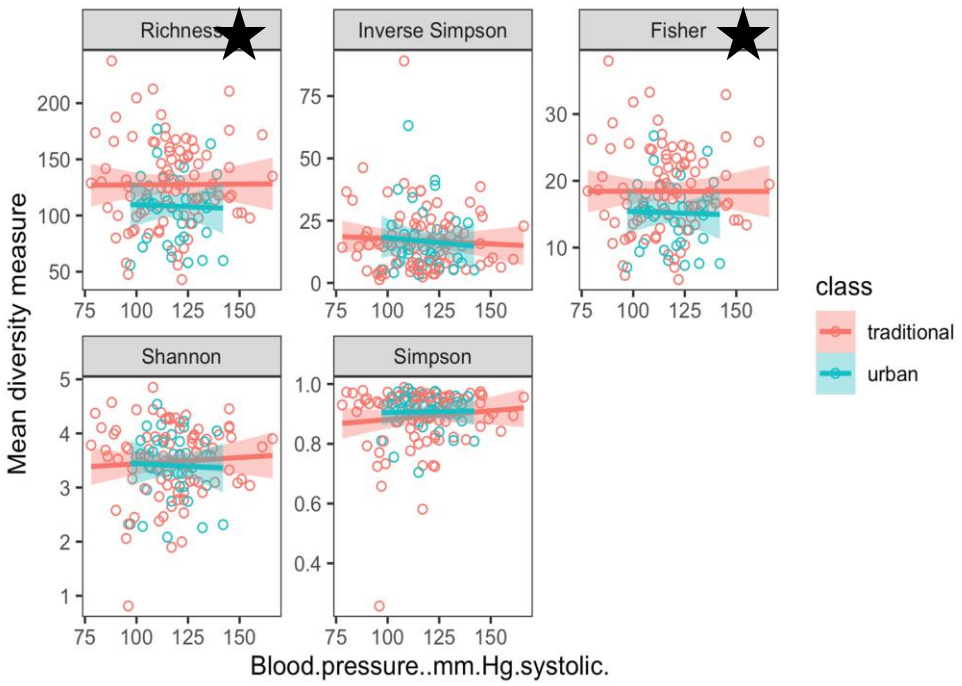

Figure 19: Mean microbial diversity correlation with systolic blood pressure. The green line represents the correlation between systolic blood pressure and alpha diversity in the urban population, and the red line represents systolic blood pressure correlation with mean alpha diversity in the traditional community. The red and green dots represent the participants in the study, and the black star shows correlations with significant p values.

The ANCOVA correlation results between alpha diversity and systolic blood pressure showed that systolic blood pressure does not correlate with alpha diversity. However, no interaction existed between systolic blood pressure and the two populations for all alpha diversity measures.

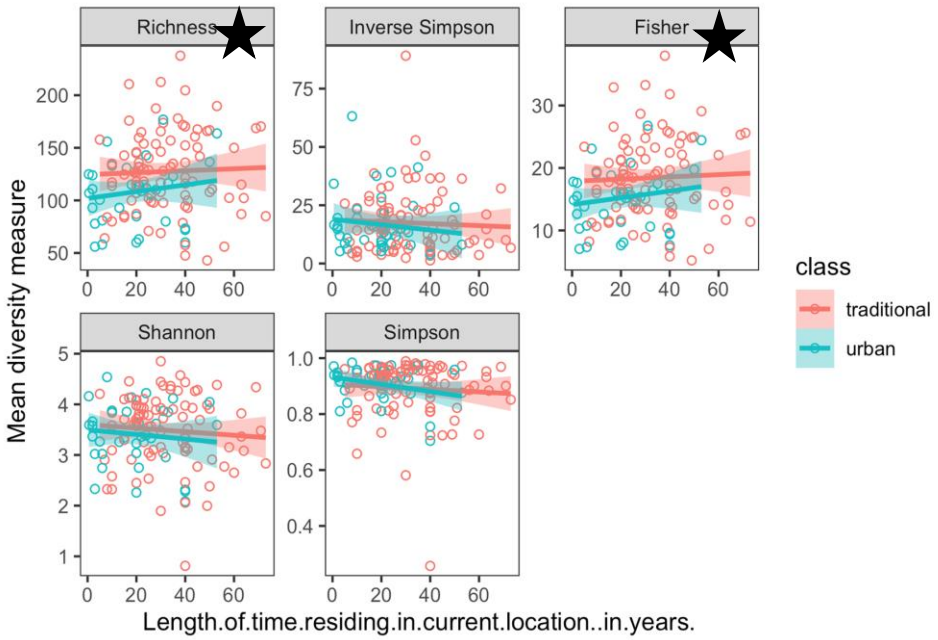

Figure 20: The correlation between microbial diversity with the length of time residing in the current location. The red line represents the correlation between period living in current location with average diversity in the traditional population, and the green line represents the length of time residing in the current site with mean diversity correlation in the urban population. The red and green dots represent the participants in the study, and the black star shows correlations with significant p values.

4.6.7 Alpha diversity in correlation with length of time residing in the current location

The ANCOVA alpha diversity correlation with length of time residing in the current location showed that alpha diversity measures did not correlate with the period an individual has stayed in the current location. No interaction existed between the time of residing in the current location with the two population groups.

4.7 Summary of the ANCOVA Correlation between alpha diversity with phenotypes

Table 5: Summary of the ANCOVA test of the correlation between alpha diversity and various phenotypes: ✓ shows significant correlation and x shows that the association is not significant (details for the correlation between phenotype and alpha diversity are in the supplementary section)

	Richness	Inverse Simpson	Fisher	Shannon	Simpson
BMI	✓	x	✓	x	x
Age	x	x	x	x	x
Cholesterol	x	x	x	x	x
HDL	x	x	x	x	x
Diastolic bp	x	x	x	x	x
Systolic bp	x	x	x	x	x
Time in Cur.loc.	x	x	x	x	x

CHAPTER FIVE: DISCUSSION

The results demonstrated a distinction in the various lifestyle aspects of the urban and traditional Turkana population (Figure 3). In the traditional population, similar to other subsistence-level populations, only one individual had a BMI above 25, and none met obesity criteria (BMI>30)(Grundy et al., 2005). However, the urban population had several individuals with an obese BMI. The differences were however insignificant for BMI (p=0.064), body fat percentage (p = 0.08521), Cholesterol (p = 0.4958) and blood pressure diastolic (p = 0.8243). Significant differences were observed in blood pressure systolic (P = 0.0495), average waist circumference (p = 0.0004), HDL (p= 0.0004) and the number of years an individual has resided in current location in years (p = < 0.0001). The two populations in the past shared similar lifestyles and diet until recently when they started moving to urban areas. Even though previously the population shared the same geographical environment, currently, their diet and lifestyles are different. The results from the microbiome analysis we obtained indicate that gut microbial communities reflect the lifestyles and diet of the population. These show that the human gut microbiome can vary within a few years of departure from nomadic pastoralism.

The variation in the gut microbiome of the Turkana population coincides with the general patterns observed in a majority of studies comparing the gut microbial communities of industrialized and traditional populations (Clemente et al., 2015; Conteville et al., 2019; Fragiadakis et al., 2019; Gomez et al., 2016; Gupta et al.,' 2017; Hansen et al., 2019; Jandhyala et al., 2015; Martínez et al., 2015; Moeller et al., 2014; Obregon-Tito et al., 2015; Oduaran et al., 2020; Schnorr et al., 2014; Sonnenburg & Sonnenburg, 2019; Yatsunenko et al., 2012). These studies have revealed that gut microbial composition varies between these populations; higher alpha diversity in traditional populations, and the variations can be attributed to diet. In this study, the Bacteroidetes and Firmicutes were more abundant in the two populations. Other studies have shown the Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria(Jandhyala et al., 2015) to be the most dominant phyla

representing 90% of the gut microbial communities. Fusobacteria, Verrucomicrobia, and Cyanobacteria combine in low numbers for the rest of the remaining bacteria (Arumugam et al., 2013).

The Bacteroidetes were the most abundant phyla in both the urban and traditional populations, with pronounced abundance in the urban population (Figure 6, $p = < 0.0001$) in comparison to the traditional. Additionally, Prevotellaceae, a member of the Bacteroidetes phylum, was the most highly enriched family in both the traditional and urban populations ($p = 0.0216$). The Prevotellaceae family has also been reported in other African populations, including the Hadza hunter and gatherers, *Prevotella* genera are also associated with a vegetarian diet in populations that are industrialized(David et al., 2014). The taxa *Bacteroides* and *Blautia* that are highly associated with diets rich in animal protein were also present in the Turkana population, and animal protein is a major dietary component of the Turkana population.

There was a pronounced abundance of Proteobacteria in this study, similar to previous studies done in African populations. Individuals with a high abundance of Proteobacteria were associated with infection by the gastrointestinal parasite *Entamoeba* in the hunters and gatherers of the central African rainforest(Morton et al., 2015). Enterobacteriaceae family also tends to increase in number during dysbiosis, as was observed in a study that was suggesting that a dysbiotic expansion of Proteobacteria is a potential microbial signature for the diagnosis of epithelial dysfunction. Anaerobsis, antibiotic treatment, and intestinal inflammation increase the oxygenation of the epithelial lining in the colon, creating a more aerobic environment. Promoting the proliferation of the facultative anaerobes (Proteobacteria members) over the obligate(Litvak et al., 2017).

The Spirochaetaceae family was more abundant in the traditional population in comparison to the urban population (Figure 9, t-test, $p = 0.04122$). The same results have been reported previously from the gut microbial communities of non-human

primates and human populations in Africa whose lifestyles are non urban. These studies reported the presence of Spirochaetes in high abundance in populations with lifestyles that are non urban. These include the traditional communities in Burkina Faso(Hansen et al., 2019), the Hadza hunter and gatherers, and other populations with traditional lifestyle. However, the family is almost undetected in industrialized populations(Smits et al., 2017; Yatsunenko et al., 2012). Additionally, the Matses hunter-gatherer from Peruvian Amazon and the Tunapuco, a traditional community of agriculturalists from Andean highlands, show similar results(Obregon-Tito et al., 2015). The results are in agreement with what we observed in the Turkana population from rural areas. The Spirochaetes may represent a part of the ancestry of human gut microbiome symbionts that was lost as populations started adopting industrial agriculture, or other lifestyle changes as individuals transition to urban and western-like lifestyles(Gomez et al., 2016; Obregon-Tito et al., 2015; Schnorr et al., 2014). *Treponema*, an important genus in Spirochaetes, is implicated in diverse functions, but may include the metabolism of carbohydrates(Afolayan et al., 2019). The traditional Turkana population is enriched with the Spirochaetes that has genera associated with the metabolism of uncultivated plant products. This enrichment can be as a result of individuals in the Turkana population practicing farming, as well as the habit of feeding on wild plants and fruits, common with pastoralists in Turkana.

The relative abundance of the microbiome of males and females of the traditional group had slight differences; however, for the urban population, the relative abundance was uniform for both males and females. The abundance differences may result from gender differences in activities within the traditional Turkana community. The females are involved in every activity at home; they travel long distances to fetch water, milk cows, cook meals, and even construct their homestead. Conversely, men's activities involve feeding animals, whereby they can travel for three days before returning. In this period, they feed on blood and milk, or they can spend long periods fasting, in addition to raiding neighbouring communities and playing games. Thus the diet, level of activities and the environments of the males and female of the traditional Turkana community could be periodically different, that

may explain the gender differences in the relative abundance of the traditional population microbiome.

5.1 Alpha diversity

Despite the observed differences in the composition of the gut microbiome, only the Fisher ($p = 0.00214$), and Richness ($p = 0.00214$), diversity metric showed significant differences in alpha diversity. No significant differences in the gut microbial alpha diversity measured by Simpson ($p = 0.8287$), Inverse Simpson ($p = 0.8287$), and Shannon ($p = 0.2834$) existed across the Turkana populations. Previous studies comparing populations living in similar geographical areas with different subsistence strategies showed similar results; urban Nigerians and Bassa farmers(Ayeni et al., 2018), Tunapuco farmers, and Matses hunter-gatherers(Obregon-Tito et al., 2015), as well as Bantu farmers and BaAka hunter and gatherers(Gomez et al., 2016). These populations and other traditional communities like Hadza(Schnorr et al., 2014), had a higher richness and diversity in comparison with urban populations. The higher microbial richness in traditional Turkana population could be facilitated with the frequent use of antibiotics in the urban population unlike in the traditional community. The traditional community uses a wide variety of natural remedies for treatment and in some cases an individual will be cut to shed blood, believing it removes sicknesses. Such practices could facilitate a higher richness that was observed in this study. However, no diversity differences were observed with Shannon and Simpson measures. These could be due to the limitation in the sample size that didn't allow us to perform the necessary comparisons.

5.1.1 Cardio metabolic risk factors contribution to alpha diversity

The slope for the mean alpha diversity and BMI (Figure 14), age (Figure 15), cholesterol (Figure 16), HDL (Figure 17), diastolic blood pressure (Figure 18) and systolic blood pressure (Figure 19) for the two study populations had a lot of overlap for Simpson, Inverse Simpson, and Shannon. The ANCOVA values for the slope of

the regression lines were also insignificant, indicating that the slopes may not be different. However, Richness and Fisher's diversity was different, and the ANCOVA values for the slope of the regression lines were significant.

The Turkana population with an underweight BMI had a higher alpha diversity, and the diversity decreased with an increase in BMI. The results are similar to a gut microbiome pilot study of 170 female individuals in Bushbuckridge and Soweto representing a rural, and urban South African cohort, the mean alpha diversity of the Bushbuckridge and Soweto were also not significant(Oduaran et al., 2020). However, a large Chinese cohort that aimed to find the differences in the microbiome as a function of BMI found out that underweight individuals had a significantly high gut microbial diversity compared to the normal, overweight, or obese(Gao et al., 2018). Moreover, other comparison studies of Non-Hispanic whites and blacks showed that black obese individuals still have a higher mean alpha diversity(Peters et al., 2018; Stanislawski et al., 2019). The high alpha diversity in this study was mostly from the traditional group, whose daily routines involve a lot of activities. The activities range from walking long distances in search of pasture or water, irregular feeding patterns of a wide variety of meals, and less number of meals in comparison to the urban group.

This study showed a gradual decrease in the Turkana community average alpha diversity with an increase in the level of cholesterol, HDL, diastolic blood pressure, age, and a lot of overlap in the length of time residing in current location and systolic blood pressure with alpha diversity change. Previous studies have shown significantly lower gut microbial alpha diversity with an increase in cardio metabolic risk factors. However, the associations were mostly dependent on the type of cardio metabolic risk factor and geographical locations; for example, a higher blood pressure association among Ghanaians and South Africans(Fei et al., 2019; Le Chatelier et al., 2013; Turnbaugh et al., 2009). In this study Age, cholesterol, HDL, systolic and diastolic blood pressure did not correlate with alpha diversity. These may have resulted from not having the sample size necessary to perform the

comparisons. However, age, diastolic and systolic blood pressure had different values for the two populations. The differences may give an understanding of the current increase in metabolic diseases within the urban Turkana community in comparison to the traditional population that remains to be healthier.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

In conclusion, this project is one of the first microbiome work conducted among the Turkana population in Kenya, aiming to find out the effects of transiting from a nomadic pastoralist lifestyle to urban areas on the gut microbiome.

To achieve the objectives, I explored the microbiome changes as the traditional population transit from their traditional lifestyle to urban lifestyle in urban areas. The results showed that these transitions have an impact on both the alpha and beta microbial diversity. I observed a gradual reduction in the microbial alpha diversity in the Turkana population who've moved to urban areas. In evaluating the various phenotypic factors that affect the microbial alpha diversity, the Turkana population with an underweight BMI had a higher alpha diversity, and the diversity decreased with an increase in BMI. The study also showed a gradual decrease in the Turkana community average alpha diversity with an increase in the level of cholesterol, HDL, diastolic blood pressure, age, and a lot of overlap in the length of time residing in current location and systolic blood pressure with alpha diversity change.

These, coupled with the loss of beneficial microbiome as has been observed in urban populations, may indicate a possible increased risk to metabolic diseases. Moreover, the microbiome composition of the traditional population, represent a unique microbial community that is also present in traditional populations. These microbial communities could be helpful in the understanding of human health relationships and even in the development of microbiome-based therapies. Additionally, the observed differences helped to reject the null hypothesis by indicating an existence of differences in gut microbiome composition of the traditional and Urban Turkana people.

Recommendations

- A complete sampling in the future for both urban and traditional settings can provide a better understanding of the microbial community structure of the Turkana population, and this can extend to other comparative communities within Kenya.
- Determine the metabolic profiles of every sample in order to better understand the correlations with alpha diversity.

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APPENDIX

1.1 Supplementary Material

1.2 Alpha Diversity Correlation with Phenotypes

1.2.1 Alpha diversity ANCOVA with BMI

Table 6: Alpha diversity Richness, Inverse Simpson, Fisher, Shannon and Simpson in correlation with BMI

Richness correlation with BMI					
	Df	Sum sq.	Mean Sq	F value	Pr (>F)
BMI	1	6382	6382	4.873	0.0291*
Class	1	7252	7252	5.538	0.0202*
BMI: class	1	1371	1371	1.047	0.3083
Residuals	124	162383	1310		
Signif. Codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' ' 1
Inverse Simpson diversity correlation with BMI					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
BMI	1	4	4.48	0.025	0.875
Class	1	12	11.79	0.066	0.798
BMI: class	1	207	206.68	1.149	0.286
Residuals	124	22298	179.82		
Fisher diversity correlation with BMI					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
BMI	1	176	176.32	4.886	0.0289*
Class	1	205	204.55	5.668	0.0188*
BMI: class	1	43	42.90	1.189	0.2777
Residuals	124	4475	36.09		
Signif.	0 '***'	0.001	0.01 '*'	0.05 '.'	0.1 ' ' 1

Codes		‘**’			
Shannon diversity correlation with BMI					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
BMI	1	0.51	0.5111	1.098	0.297
Class	1	0.07	0.0695	0.149	0.700
BMI: class	1	0.09	0.0855	0.184	0.669
Residuals	124	57.72	0.4655		
Simpson diversity correlation with BMI					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
BMI	1	0.0005	0.0005	0.055	0.816
Class	1	0.0072	0.0072	0.782	0.378
BMI: class	1	0.0009	0.0009	0.099	0.753
Residuals	124	1.1471	0.0092		

The ANCOVA results in table 6 for the correlation between mean Richness and BMI indicated that. Mean microbial richness and BMI are correlated (Table 6, F = 4.873, p = 0.0291), the traditional and urban groups have different Richness values (Table 6, F = 5.538, p = 0.0202) the result also match the boxplot in figure 12. The results also showed no interaction between BMI and class (Table 6, F = 1.047, p = 0.3083), indicating that the correlation between urban and traditional people is the same.

The ANCOVA correlation results between Inverse Simpson and BMI in table 6 showed that; Inverse Simpson does not correlate with BMI (Table 6, F = 0.025, p = 0.875). The urban and traditional group have similar Inverse Simpson diversity (Table 6, F = 0.066, p = 0.798) matching the boxplots results (Figure 12B, t-test, p=0.7862), and no interaction existed between BMI and the two population groups (Table 6, F = 1.149, p = 0.286).

The ANCOVA correlation results for the Fisher diversity measure with BMI for the urban and traditional population showed that; Fisher diversity correlates with BMI (Table 6, F = 4.886, p = 0.0289). The urban and traditional population have different

Fisher diversity values (Table 6, F = 5.668, p = 0.0188) similar to the boxplot results (Figure 13C, t-test, p=0.0011), and no interaction existed between BMI and the two populations (Table 6, F = 1.189, p = 0.2777).

The ANCOVA Shannon diversity correlation with BMI results for the two populations showed that; Shannon diversity does not correlate with BMI (Table 6, F = 1.098, p = 0.297). The urban and traditional populations have similar Shannon diversity (Table 6, F = 0.149, p = 0.700) the results are similar to the boxplot (Figure 12D, t-test, p=0.3183). No interactions existed between BMI and the two populations (Table 6, F = 0.184, p = 0.669).

The ANCOVA correlation results between the Simpson diversity measure and BMI showed that; Simpson diversity does not correlate with BMI (Table 6, F = 0.055, p = 0.816). The traditional and urban population have similar Simpson diversity (Table 6, F = 0.782, p = 0.378). No interaction existed between BMI and the two population groups (Table 6, F = 0.099, p = 0.753) as neither BMI nor class influence Simpson diversity.

1.2.2 Alpha diversity ANCOVA with age

Table 7: Alpha diversity in correlation with age for the urban and traditional population

Richness in correlation with age					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Age	1	441	441	0.329	0.5675
Class	1	9857	9857	7.345	0.0077**
Age: class	1	668	668	0.498	0.4818
Residuals	124	166421	1342		
Signif. Codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' ' 1
Inverse Simpson diversity measure in correlation with age					
	Df	Sum Sq	Mean Sq	F value	Pr (>)

Age	1	141	141.46	0.796	0.374
Class	1	72	72.14	0.406	0.525
Age: class	1	259	258.72	1.455	0.230
Residuals	124	22048	177.81		
Fisher diversity measure in correlation with age					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Age	1	14	14.45	0.390	0.5334
Class	1	274	273.59	7.384	0.0075**
Age: class	1	16	16.39	0.442	0.5072
Residuals	124	4594	37.05		
Signif. Codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' '1
Shannon diversity measure in correlation with age					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Age	1	0.81	0.8086	1.779	0.185
Class	1	0.60	0.5991	1.318	0.253
Age: class	1	0.61	0.6056	1.332	0.251
Residuals	124	56.37	0.4546		
Simpson diversity measure in correlation with age					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Age	1	0.0229	0.0229	2.534	0.114
Class	1	0.0007	0.0007	0.082	0.776
Age: class	1	0.0122	0.0122	1.351	0.247
Residuals	124	1.1199	0.0090		

The ANCOVA tests for the regression lines of the traditional and urban populations were as follows. Richness measure is not correlated with age (Table 7, $F = 0.329$, $p = 0.5675$), the urban and traditional population have different Richness values (Table 7, $F = 7.345$, $p = 0.0077$) and no interaction existed between age and the two population groups (Table 7, $F = 0.498$, $p = 0.4818$).

The ANCOVA results for the correlation between Inverse Simpson diversity and age showed that; Inverse Simpson diversity doesn't correlate with age (Table 7, F = 0.796, p = 0.374). The traditional and urban population do not have different Fisher diversity values (Table 7, F = 0.406, p = 0.525). No interaction existed between age and the two groups of the population (Table 7, F = 1.455, p = 0.230).

The ANCOVA correlation results for Fisher and age showed that; Fisher mean diversity is not correlated with age (Table 7, F = 0.390, p = 0.5334), the traditional and urban population have different Fisher mean diversity values (Table 7, F = 7.384, p = 0.0075) however no interaction existed between age and the two groups of population (Table 7, F = 0.442, p = 0.5072).

The ANCOVA correlation results for Shannon diversity and age were; Shannon diversity is not correlated with age (Table 7, F = 1.779, p = 0.185), the urban and traditional population do not have different Shannon diversity values (Table 7, F = 1.318, p = 0.253) and no interaction existed between age and the two population groups (Table 7, F = 1.332, p = 0.251).

The Simpson diversity and age ANCOVA correlation for the regression lines between the two populations were as follows; Simpson diversity does not correlate with age (Table 7, F = 2.534, p = 0.114). The urban and traditional population do not have different Simpson diversity values (Table 7, F = 0.082, p = 0.776). No interaction existed between age and the two population groups (Table 7, F = 1.351, p = 0.247).

1.2.3 Alpha diversity ANCOVA with cholesterol

Table 8: Alpha diversity in correlation with cholesterol for the urban and traditional population (Note: only 48 were samples for cholesterol out of the total 133).

Richness in correlation with cholesterol					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Cholesterol	1	976	975.6	0.541	0.466

Class	1	2740	2740.1	1.519	0.224
Cholesterol: class	1	1719	1719.1	0.953	0.334
Residuals	44	79387	1804.2		
Inverse Simpson diversity measure in correlation with cholesterol					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Cholesterol	1	380	380.2	1.384	0.246
Class	1	62	61.6	0.224	0.638
Cholesterol: class	1	90	89.9	0.327	0.570
Residuals	44	12083	274.6		
Fisher in correlation with cholesterol					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Cholesterol	1	25.1	25.12	0.507	0.480
Class	1	78.6	78.57	1.585	0.215
Cholesterol: class	1	42.6	42.56	0.858	0.359
Residuals	44	2181.4	49.58		
Shannon diversity measure in correlation with cholesterol					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Cholesterol	1	0.111	0.1109	0.198	0.658
Class	1	0.008	0.0077	0.014	0.907
Cholesterol: class	1	0.253	0.2530	0.452	0.505
Residuals	44	24.63	0.5597		
Simpson diversity measure in correlation with cholesterol					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Cholesterol	1	0.009	0.009	1.074	0.306
Class	1	0.0019	0.0019	0.227	0.636
Cholesterol	1	0.0001	0.0001	0.009	0.923
Residuals	44	0.3690	0.0084		

The ANCOVA results for alpha diversity correlation with cholesterol for both the urban and traditional group were as follows [Richness (Table 8, F = 0.541, p = 0.466), Inverse Simpson (Table 8, F = 1.384, p = 0.246), Fisher (Table 8, F = 0.507, p = 0.480), Shannon (Table 8, F = 0.198, p = 0.658) and Simpson (Table 8, F = 1.074, p = 0.306)]. The alpha diversity of the traditional and urban population is not different [Richness (Table 8, F = 0.541, p = 0.466), Inverse Simpson (Table 8, F = 0.224, p = 0.638), Fisher (Table 8, F = 1.585, p = 0.215), Shannon (Table 8, F = 0.014, p = 0.907) and Simpson (Table 8, F = 0.227, p = 0.636)]. Finally, no interaction exists between cholesterol and the two population groups [Richness (Table 8, F = 0.541, p = 0.466), Inverse Simpson (Table 8, F = 0.327, p = 0.570), Fisher (Table 8, F = 0.858, p = 0.359), Shannon (Table 8, F = 0.452, p = 0.505) and Simpson (Table 8, F = 0.009, p = 0.923)].

1.2.4 Alpha diversity ANCOVA with HDL

Table 9: Alpha diversity in correlation with high-density lipoprotein (HDL) (Note: not all samples were sampled for HDL so 84 observations out of 133 are missing in this data)

Richness in correlation with high density lipoprotein (HDL) mg/dl					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
HDL	1	1445	1445	0.797	0.377
Class	1	1736	1736	0.957	0.333
HDL: class	1	2285	2285	1.260	0.268
Residuals	45	81628	1814		
Inverse Simpson in correlation with HDL mg/dl					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
HDL	1	22	22.50	0.081	0.777
Class	1	41	41.31	0.149	0.701
HDL: class	1	285	284.59	1.030	0.316

Residuals	45	12437	276.39		
Fisher diversity measure in correlation with HDL					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
HDL	1	41.3	41.27	0.830	0.367
Class	1	49.6	49.57	0.997	0.323
HDL: class	1	61.2	61.17	1.230	0.273
Residuals	45	2237.7	49.73		
Shannon measure in correlation with HDL					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
HDL	1	0.059	0.0594	0.106	0.746
Class	1	0.005	0.0047	0.008	0.928
HDL: class	1	0.570	0.5703	1.020	0.318
Residuals	45	25.16	0.5592		
Simpson diversity measure in correlation with HDL					
	Df	Sum Sq	Mean sq.	F value	Pr (>F)
HDL	1	0.0106	0.0106	1.268	0.266
Class	1	0.0008	0.0008	0.097	0.757
HDL: class	1	0.0006	0.0006	0.068	0.796
Residuals	45	0.3778	0.0084		

The ANCOVA test for the regression lines between the two groups were as follows; Alpha diversity is not correlated with HDL [Richness (Table 9, F = 0.797, p = 0.377), Inverse Simpson (Table 9, F = 0.081, p = 0.777), Fisher (Table 9, F = 0.830, p = 0.367), Shannon (Table 9, F = 0.106, p = 0.746) and Simpson (Table 9, F = 1.268, p = 0.266)]. The traditional and urban populations do not have different alpha diversity [Richness (Table 9, F = 0.957, p = 0.333), Inverse Simpson (Table 9, F = 0.149, p = 0.701), Fisher (Table 9, F = 0.997, p = 0.323), Shannon (Table 9, F =

0.008, p = 0.928) and Simpson (Table 9, F = 0.097, p = 0.757)]. No interaction exists between HDL and the two population groups [Richness (Table 9, F = 1.260, p = 0.268), Inverse Simpson (Table 9, F = 1.030, p = 0.316), Fisher (Table 9, F = 1.230, p = 0.273), Shannon (Table 9, F = 1.020, p = 0.318) and Simpson (Table 9, F = 0.068, p = 0.796)].

1.2.5 Alpha diversity ANCOVA with diastolic blood pressure

Table 10: Alpha diversity correlation with average waist circumference in both the urban and traditional groups

Richness in correlation with diastolic blood pressure					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Diastolic bp	1	754	754	0.569	0.4522
Class	1	7809	7809	5.894	0.0167*
Diastolic: class	1	425	425	0.321	0.5722
Residuals	118	156341	1325		
Signif. Codes	0 '***'	0.001'**'	0.01 '*'	0.05 '.'	0.1 ' ' 1
Inverse Simpson diversity in correlation with diastolic blood pressure					
	Df	Sum Sq	Mean Sq	F value	Pr (>)
Diastolic bp	1	90	90.0	0.699	0.4047
Class	1	11	10.8	0.084	0.7727
Diastolic: class	1	423	422.9	3.287	0.0724
Residuals	118	15180	128.6		
Fisher diversity in correlation with diastolic blood pressure					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Diastolic bp	1	23	23.22	0.638	0.426
Class	1	222	221.65	6.092	0.015*
Diastolic: class	1	13	13.12	0.361	0.549
Residuals	118	4293	36.39		
Signif. Codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 '' 1

Shannon diversity in correlation with diastolic blood pressure					
Diastolic bp	1	0.00	0.0017	0.004	0.952
Class	1	0.03	0.0308	0.067	0.797
Diastolic: class	1	0.23	0.2329	0.504	0.479
Residuals	118	54.52	0.4620		
Simpson Diversity in correlation with diastolic blood pressure					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Diastolic bp	1	0.0014	0.0014	0.145	0.704
Class	1	0.0093	0.0093	0.987	0.322
Diastolic: class	1	0.0075	0.0075	0.796	0.374
Residuals	118	1.1080	0.0094		

The ANCOVA tests for the regression lines of the traditional and urban populations were as follows. Richness measure is not correlated with diastolic blood pressure (Table 10, F = 0.569, p = 0.4522), the urban and traditional population have different Richness values (Table 10, F = 5.894, p = 0.0167) and no interaction existed between diastolic blood pressure and the two population groups (Table 10, F = 0.321, p = 0.572).

The ANCOVA results for the correlation between Inverse Simpson diversity and diastolic blood pressure showed that; Inverse Simpson diversity does not correlate with diastolic blood pressure (Table 10, F = 0.699, p = 0.4047). The traditional and urban population do not have different Inverse Simpson diversity values (Table 10, F = 0.084, p = 0.7727). No interaction existed between diastolic blood pressure and the two-class of population (Table 10, F = 3.287, p = 0.0724).

The ANCOVA correlation results for Fisher and diastolic blood pressure showed that; Fisher diversity does not correlate with diastolic blood pressure (Table 10, F = 0.638, p = 0.426). The traditional and urban population have different Fisher mean diversity values (Table 10, F = 6.092, p = 0.015) however no interaction existed

between diastolic blood pressure and the two-class of population (Table 10, F = 0.361, p = 0.549).

The ANCOVA correlation results for Shannon diversity and diastolic blood pressure were; Shannon diversity does not correlate with diastolic blood pressure (Table 10, F = 0.004, p = 0.952). The urban and traditional population do not have different Shannon diversity values (Table 10, F = 0.067, p = 0.797). No interaction existed between diastolic blood pressure and the two population groups (Table 10, F = 0.504, p = 0.479).

The Simpson diversity and diastolic blood pressure ANCOVA correlation for the regression lines between the two populations were as follows; Simpson diversity does not correlate with diastolic blood pressure (Table 10, F = 0.145, p = 0.704). The urban and traditional population do not have different Simpson diversity values (Table 10, F = 0.987, p = 0.322). No interaction existed between diastolic blood pressure and the two population groups (Table 10, F = 0.796, p = 0.374).

1.2.6 Alpha diversity ANCOVA with systolic blood pressure

Table 11: Alpha diversity correlation systolic blood pressure in both the urban and traditional groups

Richness in correlation with systolic blood pressure					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Systolic bp	1	26	26	0.019	0.8911
Class	1	9962	9962	7.326	0.0078**
Systolic: class	1	34	34	0.025	0.8755
Residuals	123	167259	1360		
Signif. Codes	0 ‘***’	0.001‘**’	0.01 ‘*’	0.05 ‘.’	0.1 ‘ ’ 1
Inverse Simpson diversity in correlation with systolic blood pressure					
	Df	Sum Sq	Mean Sq	F value	Pr (>)
Systolic bp	1	79	78.79	0.458	0.500

Class	1	2	1.58	0.009	0.924
Systolic: class	1	7	7.45	0.043	0.836
Residuals	123	21151	171.96		
Fisher diversity in correlation with systolic blood pressure					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Systolic bp	1	1	1.49	0.040	0.8423
Class	1	280	279.72	7.456	0.0073**
Systolic: class	1	0	0.50	0.013	0.9084
Residuals	123	4615	37.52		
Signif. Codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 '' 1
Shannon diversity in correlation with systolic blood pressure					
Systolic bp	1	0.09	0.0926	0.199	0.656
Class	1	0.14	0.1408	0.303	0.583
Systolic: class	1	0.08	0.0789	0.170	0.681
Residuals	123	57.26	0.4655		
Simpson Diversity in correlation with systolic blood pressure					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Systolic bp	1	0.0105	0.0105	1.139	0.288
Class	1	0.0061	0.0061	0.665	0.416
Systolic: class	1	0.001	0.001	0.108	0.743
Residuals	123	1.1309	0.0092		

The ANCOVA results for alpha diversity correlation with systolic blood pressure for both the urban and traditional group were as follows; no correlation existed between alpha diversity and systolic blood pressure [Richness (Table 11, F = 0.019, p = 0.8911), Inverse Simpson (Table 11, F = 0.458, p = 0.500), Fisher (Table 11, F = 0.040, p = 0.8423), Shannon (Table 11, F = 0.199, p = 0.656) and Simpson (Table 11, F = 1.139, p = 0.288)]. The alpha diversity of the traditional and urban population is not different, except for Richness and Fisher diversity [Richness (Table 11, F = 7.326, p = 0.0078), Inverse Simpson (Table 11, F = 0.009, p = 0.924), Fisher

(Table 11, F = 7.456, p = 0.0073), Shannon (Table 11, F = 0.303, p = 0.583) and Simpson (Table 11, F = 0.665, p = 0.416)]. Finally, no interaction exists between systolic blood pressure and the two population groups [Richness (Table 11, F = 0.025, p = 0.8755), Inverse Simpson (Table 11, F = 0.043, p = 0.836), Fisher (Table 11, F = 0.013, p = 0.9084), Shannon (Table 11, F = 0.170, p = 0.681) and Simpson (Table 11, F = 0.108, p = 0.743)].

1.2.7 Alpha diversity ANCOVA with length of time residing in current location

Table 12: Alpha diversity in correlation with length of time residing in current location for the traditional and urban group

Richness in correlation with length of time residing in current location in years					
	Df	Sum Sq	Mean Sq	F value	Pr (>)
Time in current loc.	1	3633	108.35	2.929	0.1024
Class	1	7098	196.18	5.303	0.0231 *
Time in cur loc.: class	1	284	7.26	0.196	0.6461
Residuals	124	166372	36.99		
Signif. Codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' ' 1
Inverse Simpson measure in correlation with length of time residing in the current location in years					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Time in current loc.	1	70	69.76	0.387	0.535
Class	1	54	53.82	0.298	0.586
Time in cur loc.: class	1	29	29.31	0.163	0.688
Residuals	124	22368	180.39		
Fisher diversity measure with the length of time residing in the current location in years					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Time in current loc.	1	108	108.35	2.929	0.0895
Class	1	196	196.18	5.303	0.0230*

Time in cur loc.: class	1	7	7.26	0.196	0.6586
Residuals	124	4587	36.99		
Signif. Codes	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' ' 1
Shannon diversity correlation with length of time residing in the current location in years					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Time in current loc.	1	0.21	0.2079	0.446	0.505
Class	1	0.38	0.3831	0.822	0.366
Time in cur loc.: class	1	0.01	0.0061	0.013	0.909
Residuals	124	57.79	0.4661		
Simpson diversity correlation with length of time residing in the current location in years					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Time in current loc.	1	0.0182	0.0182	1.989	0.161
Class	1	0.001	0.001	0.109	0.742
Time in cur loc.: class	1	0.0038	0.0038	0.420	0.518
Residuals	124	1.1328	0.0091		

The ANCOVA tests for the regression lines of the traditional and urban populations were as follows. Richness measure is not correlated with length of time residing in current location (Table 12, F = 2.929, p = 0.1024), the urban and traditional population have different Richness values (Table 12, F = 5.303, p = 0.0231) and no interaction existed between length of time residing in current location and the two population groups (Table 12, F = 0.196, p = 0.6461).

The ANCOVA results for the correlation between Inverse Simpson diversity and length of time residing in current location showed that; Inverse Simpson diversity does not correlate with the length of time residing in current location (Table 12, F = 0.387, p = 0.535). The traditional and urban population do not have different Inverse Simpson diversity values (Table 12, F = 0.298, p = 0.586). No interaction existed

between the length of time residing in the current location and the two classes of population (Table 12, F = 0.163, p = 0.68).

The ANCOVA correlation results for Fisher and length of time residing in current location showed that; Fisher diversity does not correlate with the length of time residing in current location (Table 12, F = 2.929, p = 0.0895). The traditional and urban population have different Fisher mean diversity values (Table 12, F = 5.303, p = 0.0230) however no interaction existed between the length of time residing in current location and the two-class of population (Table 12, F = 0.196, p = 0.6586).

The ANCOVA correlation results for Shannon diversity and diastolic blood pressure were; Shannon diversity is not correlated with diastolic blood pressure (Table 12, F = 0.446, p = 0.505), the urban and traditional population do not have different Shannon diversity values (Table 12, F = 0.822, p = 0.366) and no interaction existed between length of time residing in current location and the two population groups (Table 12, F = 0.013, p = 0.909).

The Simpson diversity and length of time residing in current location ANCOVA correlation for the regression lines between the two populations were as follows; Simpson diversity does not correlate with the length of time residing in current location (Table 12, F = 1.989, p = 0.161). The urban and traditional population do not have different Simpson diversity values (Table 12, F = 0.109, p = 0.742). No interaction existed between the length of time residing in the current location and the two population groups (Table 12, F = 0.420, p = 0.518).

1.3 Project's consent form and datasheet

Link: https://drive.google.com/file/d/17HjTbWDhG8rQ7Rk_x9XP-8vKFd8Bp7A8/view?usp=sharing

1.4 Metadata file

Link:https://drive.google.com/file/d/0B0N47HvqhSaabW9BcWJZQzdFQ0pZLVpDUTRvVGY1XzY5MlJj/view?usp=sharing&resourcekey=0-AIVy7LEBa2emjf-d5wipvQ