# The Physiological Condition of Host Affects more Significantly Gut Microbiome than Diet

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**Research**

**Keywords:** Gut microbiome, diet shift, physical exercise, gut microbiome diversity, gut microbiome composition

**Posted Date:** August 19th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-56670/v1

# Abstract

**Background:** The gut microbiome, which is composed of trillions of microbes, plays a fundamental role in human health and disease. Each individual has a unique profile of gut microbiome which plays specific functions in its host. Although multiple factors might affect the diversity of gut microbiome, physiological condition and diet are considered as main determining factors for gut microbiome diversity.

**Results:** We performed a comparative study to elucidate which one between physiological condition and diet affects more significantly gut microbiome diversity by comparing the effect of a vegetarian diet and a light physical exercise. Although both Physical exercise and vegetarian diet affected significantly the composition of gut microbiome, the patterns of affection were different. The physical exercise increased the populations of Firmicutes and Actinobacteria, whereas the vegetarian diet increased the populations of Bacteroidetes and Actinobacteria. Flourishing of *Megamonas funiformis* was most notable by the physical exercise while flourishing *Prevotella* and decreasing *Bacteroides* was most notable by the vegetarian diet. Maximum-likelihood phylogenetic tree analysis comprising all of the taxa, the taxonomic α-diversity measurement by ACE richness, Shannon and Simpson, and HCL heatmap analysis at Genus level showed that the physical exercise affected the microbial community more significantly than diet shift, and the diversities of gut microbiome were slightly decreased in both cases. Both NMDS heatmap and Fast UniFrac analysis further confirmed that physical exercise affected the composition of gut microbiome more significantly than vegetarian diet. The NMDS plots based on Bray-Curtis distances validated that the original composition of gut microbiome became diverged into a different direction by either physical exercise or vegetarian diet.

**Conclusion:** The physiological condition of host contributes more significantly to the composition of gut microbiome than diet, which means that the composition of gut microbiome is more significantly affected by a host factor than diet. This work also would provide a new theoretical basis why physical exercises is more health-beneficial than vegetarian diets.


# Background

A typical human intestine contains a massive number of intestinal microbes, 100 trillion microbes, as gut microbiome [1]. Gut microbiome is not also essential to the health and well-being of the host but also even determines the human traits as much as our own genes [2]. Especially, the role of gut microbiome was notable in the case of atherosclerosis, hypertension, obesity, diabetes, metabolic syndrome, inflammatory bowel disease (IBD), gastrointestinal tract malignancies, hepatic encephalopathy, allergies, behavior, intelligence, autism, neurological diseases, and psychological diseases [1-6]. Thus, the composition of gut microbiome and its role on human health and disease became a booming area of research, presenting a new paradigm of opportunities for medical and food applications [1].

Given the intimate and complex interactions between gut microbiome and its host, it is not surprising that the gut microbiome plays many determinant roles in the metabolic activities of human to affect various

traits and diseases [7]. Therefore, the stability and dynamics of the gut microbiome will have not only local but also systemic effects which may determine the health and well-being of the host [8]. Gut microbiome develops rapidly right after birth and fluctuates for ~2 years until it matures [2-6]. It has been generally believed that the composition of gut microbiome remains stably maintained throughout life once a certain type of gut microbiome is established on an individual basis [2-6].

Despite an opinion on immutable nature of gut microbiome during adulthood, recent researches are showing that the composition of gut microbiome could be changed during the lifetime of its host. Jackson and Jewell recently reported that diets containing different amounts of soluble or insoluble fibers affected the composition of gut microbiome [7]. An exercise training while consuming a fat-sugar supplemented diet for 4 weeks influenced the composition of gut microbiome [8]. Another recent study confirmed that exercise had a significant effect on the composition of the gut microbiome [9]. These results not only deny a previous concept of immutable nature of gut microbiome but also indicate that the compositions of gut microbiome could be constantly fluctuating, reflecting physiological condition and/or composition of diet. However, which one between the physiological condition of host and diet composition plays a more significant role in the composition of gut microbiome remains unknown. In this work, we conducted a comparative study to elucidate the effect of the physiological condition of host and diet composition on the diversity of gut microbiome. The controlled comparative study following thorough statistical analyses showed that the physiological condition of host affected more significantly the diversity of gut microbiome than diet composition, which means that the composition of gut microbiome is mainly determined by a host factor.

# Results

**Physical exercise and vegetarian diet affected gut microbiome differently; a physical exercise increased the populations of Firmicutes and Actinobacteria, whereas a vegetarian diet increased the populations of Bacteroidetes and Actinobacteria.**

To investigate the effect of the physiological condition of host and diet composition on the diversity of gut microbiome, we first recruited 30 ~ 50 years old volunteers depending on a meat-containing diet. The 75 volunteers were divided into three groups; one group shifting their diet from meat diet to a vegetarian diet (the VT group), second group adopting a 30 min physical exercise of a guided aerobic exercise in fitness center three times per week without changing their original diets (the EX group), and control continuing their life style (the Ctrl group). The fecal samples from each group were collected for metagenome analyses by NGS (Next-generation sequencing) (the VT group, n=20; the EX group, n=21; the Ctrl group n=22).

The NGS analyses showed that the compositions of gut microbiome changed significantly in both groups after the diet shift or the physical exercise (Fig. 1). The phylogenetic compositions of the microbial communities of the gut microbiome in each group were compared by combining gene abundance data at taxonomic levels from phylum to species. As shown in Fig. 1a, Firmicutes, Bacteroidetes, Actinobacteria

and Proteobacteria were present as main dominant phyla in all of the experimental groups, and the members of 2 major phyla, Bacteroidetes and Firmicutes, dominated the gut microbiome of each groups (>88% of all 16S rRNA sequences), which is consistent with previous human gut microbiome studies [10]. Bacteroidetes were more abundant than Firmicutes at the starting point, and their compositions were not changed in the gut microbiome of the control group. However, in the EX group, the abundance of Bacteroidetes and Proteobacteria decreased while the abundance of Firmicutes and Actinobacteria increased; whereas, in the VT group, the abundance of Bacteroidetes and Actinobacteria increased while correspondingly the abundance of Firmicutes and Proteobacteria decreased. The Bacteroidetes/Firmicutes ratio in the VT group altered 3 times more than that of the EX group during the experimental period (Fig. 1b). Bacteroidetes constituted 66.3% of the VT gut microbiome and 47.9% of the EX gut microbiome at the end of experiment respectively (Fig. 1, Additional file 1: Fig. S1).

**The physical exercise increased the population of *Megamonas funiformis* while decreased *Bacteroides*, whereas a vegetarian diet increased the populations of *Prevotella* while decreased Bacteroides.**

The five most dominant families at starting point of the experiment were Prevotellaceae, Ruminococcaceae, Bacteroidaceae, Lanchonospiraceae, and Bifidobacteriaceae (Fig. 2a). Exercise increased the composition of Bacteroidaceae, Lachnospiraceae, and Enterobacteriaceae while decreased the composition of Prevotellaceae, Ruminococcaceae, and Bifidobacteriaceae. Meanwhile, a vegetarian diet increased the composition of Prevotellaceae, Ruminococcaceae, and Lachnospiraceae while decreased the composition of Bacteroidaceae, Bifidobacteriaceae, and Enterobacteriaceae. The five most abundant genera in the VT group at the end of the experiment were *Prevotella, Bacteroides, Faecalibacterium, Bifidobacterium*, and *Lachnospira,* which accounted for 77.7% of the bacterial population. Although these five genera were also the most abundant in the VT group at the end of the experiment, these genera constituted only 51.6% in the EX group. The relative proportion of *Bacteroides* was decreased while the relative proportion of *Prevotella* and *Faecalibacterium* were increased in the VT group at the end of the experiment (Fig. 2b, Fig. 3, Additional file 2: Fig. S2, Additional file 3: Fig. S3). Interestingly, exercise made *Megamonas* genus flourish to constitute up to 7.0 % in the gut microbiome meanwhile the abundance of *Prevotella* and *Bacteroides* decrease. Although most of the species detected in the VT group were also detected in the EX group, it is worth noting that the relative abundances of *Prevotella spp. (Prevotella copri, FJ678796_s, FJ511060_s and EU462041_s), Bacteroides spp. (Bacteroides vulgatus, Bacteroides coprocola), Faecalibacterium spp. (BABG01000051_s, Faecalibacterium prausnitzii),* and *Bifidobacterium spp. (Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, and Bifidobacterium stercoris)* in the VT group were higher than in the EX group, but the relative abundance of *Prevotella stercorea, Bacteroides plebeius, Bacteroides uniformis, Bacteroides stercoris* and *Faecalibacterium spp.* (*DQ793299_s*) in the VT group were lower than in the EX group (Fig. 4). Especially, exercise increased the population of *Megamonas funiformis* up to 17 times, constituting 6.9% of the gut microbiome, while reducing the population of *Bacteroides_uc spp.* to tenth.

Diet shift decreased *Escherichia coli* 22 times. It has been reported that a vegetarian diet shifts gut microbiome to contain less potentially pathogenic intestinal microbes responsible of diarrhea [11]. This

work showed the same tendency in which Enterobacteriaceae, such as *Shigella* and *Escherichia*, was significantly underrepresented in the VT group (Additional file 4: Fig. S4). The genus *Prevotella*, one of the leading sources of the inter-individual gut microbiome variation [12], has been reported to be dominant in a vegetarian diet [13]. In the same context, another previous study has also shown that an animal-based diet led to the domination of *Bacteroides* genus, while a vegetarian diet led to the domination of *Prevotella* genus in gut microbiome [14]. It is interesting to note that *Prevotella* is the only notable genus, which was significantly increased in its abundance, and that *Bacteroides* is the only notable genus, which was notably decreased in its abundance by diet shift in this work, which confirms the previous report of the positive correlation between a vegetarian diet and domination of *Prevotella.*

**Alpha-diversity analysis showed that physical exercise and vegetarian diet modified the gut microbiomes in a different way.**

Since the gross microbiome analysis showed the transformative effect of physical exercise and diet shift on gut microbiome (Fig. 1 ~ 4), we analyzed further the effect of physical exercise and vegetarian diet on the compositional changes of gut microbiome by using a-diversity analyses. Maximum-likelihood phylogenetic tree comprising all of the taxa in each group showed that the diversity of microbial composition slightly decreased as well as confirming the increase of Firmicutes and Actinobacteria by exercise and increase of Bacteroidetes and Actinobacteria by vegetarian diet (Fig. 5a). The taxonomic α-diversity measurement by ACE richness, Shannon and Simpson showed that the diversities of gut microbiome were slightly decreased in both cases of physical exercise and vegetarian diet although the differences were not statistically significant (Fig. 5b). Interestingly, HCL heatmap at Genus level indicated that exercise affected the microbial community more significantly than diet shift (Fig. 5c). Based on the results of Fig. 4 and Fig. 5, the slight decreases in microbial diversity by physical exercise and vegetarian diet seems to have its roots in inhibition of proliferation of a harmful group of bacteria.

**Beta-diversity analysis confirmed that physical exercise and vegetarian diet modified the gut microbiomes in a different way.**

Different effects of physical exercise and vegetarian diet on gut microbiome became more evident by β analyses which quantitate the ratio between location and local species method (Fig. 6). The NMDS plot based on Bray-Curtis distances showed that the original composition of gut microbiome became diverged into a different direction by either physical exercise or vegetarian diet (Fig. 6a). Also, the NMDS heatmap with Bray-Cirtis distance showed that physical exercise affected the composition of gut microbiome more significantly than vegetarian diet (Fig. 6b).

The information on the relative relatedness of microbial members based on phylogenetic distances between the microbial organisms was evaluated by using Fast UniFrac analysis [15] to analyze the effect of physical exercise and vegetarian diet. As shown in Fig. 6c, both a physical exercise and a vegetarian diet dramatically changed the compositions of the gut microbiome. Interestingly enough, however, the Fast UniFrac analysis showed that the physical exercise affected the composition of gut microbiome much more than the vegetarian diet. Principal coordinate analysis (PCA) also confirmed a same tendency

(Fig. 6d). The first principal component (PC1) was dominant with 75.6% variability, and the microbial compositions of the EX, VT, and Ctrl were different each other. In consistent with the Fast UniFrac analysis, exercise affected the composition of gut microbiome more dramatically than a vegetarian diet.

# Discussion

The human gut microbiome has been shown to differ in almost every different cohort. Also, the human gut microbiome affects our traits as much as our own genome does [1-6,16]. Realization of such significant role of gut microbiome rose to the concept of human as a superorganism that is the conglomerate of human and microbial cells. The composition of human gut microbiome is dependent on the host's diet [17] as well as the physiology of host [18, 19]. This work showed that even light exercise affected the microbial community more significantly than diet shift (Fig. 5 & Fig. 6), confirming that human gut microbiome is mutable by reflecting the physiological condition of its host. Exercise mainly affects the physiological condition of host while diet shift mainly changes the incoming nutritional nature in the gut. The more significant change of gut microbiome by exercise than diet shift in this work would be interpreted as that the main determining factor for the composition of gut microbiome in human could be a physiological condition. Therefore, this finding supports an opinion that a composition of gut microbiome is mainly determined by a communication between microbiome and its host in the gut [1~6].

Both exercise and diet shift made the gut microbiomes less diverse although the differences were not statistically significant. The slight reduction of the diversities was due to relative decrease of *Prevotella_uc, EU531928_s, EU459805_s, DQ796978_s, Paraprevotella clara and EU728713_s, Bacteroides coprocola, Bacteroides coprophilus,Bacteroides_uc, Bacteroides xylanisolvens, Bacteroides eggerthii. Bacteroides massiliensis, Bifidobacterium_uc, Bifidobacterium angulatum and Bifidobacterium kashiwanohense* (Fig. 4). Especially, the abundance of *Bacteroides eggerthii* was decreased up to 20 times by exercise and 17 times by vegetarian diet respectively. However, unlike diet shifts, exercise increased the abundance of *Megamonas funiformis* up to 17 times so that it constituted 6.9% of the gut microbiome. The function of *Megamonas funiformis* in human body is unknown. Therefore, it would be worth to note the role of *Megamonas funiformis* in the gut.

Previous works showed that Bacteroidetes is inversely correlated with obesity while Firmicutes is positively correlated [20~22]. It is interesting to note that exercise decreased the abundance of Bacteroidetes and increased the abundance of Firmicutes (Fig. 1b). Exercise consumes a large quantity of energy so that the body might send a signal to extract thoroughly energy in the GI tract. Since Firmicutes facilitates extraction of energy from food [23, 24], this work suggest that human body achieves the increased energy requirement by stimulating proliferation of Firmicutes. On the other hand, maintaining large quantity of Firmicutes would result a thorough extraction of energy from food, which eventually contribute to development of obesity in the case of no physical exercise.

Finally, this work supports that the composition of gut microbiome is not stable and is heavily affected by physical conditions, dietary lifestyles, and the genome of host. This work also well matched with previous

observations that exercise very effective in weight loss, reducing risk of chronic diseases, relaxation and stress relieve, gain of muscles and bones, *etc*, than simple vegetarian diet [25, 26].

# Conclusions

Gut microbiome plays essential roles in health and well-being of the host, affecting the traits and diseases of humans as much as our genes. There has been a question on which one between the physical condition and diet composition affects more significantly the composition of gut microbiome. This work showed that the physiological condition of host affected more significantly gut microbiome than diet composition, which means that the composition of gut microbiome is mainly determined by a host factor rather than diet. This work also provides a new theoretical basis why physical exercises is more health-beneficial than vegetarian diets.

# Materials And Methods

### Study design

A 12-week, randomized, parallel, controlled clinical trial was carried out with diet interventions at the CTCF2 (Clinical Trial Center for Functional Foods) in the Chonbuk National University Hospital, South Korea. We recruited 30 ~ 50 years old volunteers depending on a meat-containing diet at least twice per day to investigate the compositional change of gut microbiome after diet shift to a vegetarian diet or a physiological shift by exercise. Computer-generated random numbers were used to assign each subject to either the experimental or control groups. The 75 volunteers were divided into three groups; one group shifting their diet from meat diet to a vegetarian diet (the VT group), second group adopting a 30 min physical exercise of a guided aerobic exercise in fitness center three times per week without changing their original diets (the EX group), and control continuing their life style (the Ctrl group). After 3 months, the volunteers were interviewed to ask whether they strictly followed the experimental guideline, and the fecal samples of individuals who followed the guide line were collected for further analysis (the VT group, n=20; the EX group, n=21; the Ctrl group n=22). The study subjects were recruited from the Clinical Trial Center for Functional Foods (CTCF2) in the Chonbuk National University Hospital. Written consents were obtained from all participants. The study was conducted according to the Declaration of Helsinki. This study was carried out in strict accordance with the recommendations in the Guide for the Ethics Committee of Chonbuk National University Hospital.The research protocol was approved by the Institutional Review Board (IRB) of Chonbuk National University Hospital, Republic of Korea (CHU_KOREAN_FOOD_2-2_2010).

### Fecal sample collection and preparation

Fecal samples were freshly collected from each participant at the day beginning (week 0) and the end of the intervention (week 12). Fecal samples were kept in individual sterile feces containers at 4 ℃ and processed within 4 h. Each sample was mixed in an equal volume of sterile phosphate-buffered saline

buffer and homogenized using a Stomacher machine before aliquoting. Aliquots of 1 ml were frozen immediately at -80 ℃ for further processing.

## Microbial genomic sequencing and data analysis

Genomic DNA was extracted from fecal aliquots samples using the Mobio PowerLyzer™ PowerSoil® DNA isolation Kit (MO BIO Laboratories Inc., Qiagen, Hilden, Germany). The DNA extraction procedure followed the standard protocol supplied by the company and final elution of DNA was performed with 100 µl Tris (MoBIO buffer C6). DNA concentrations and purity were determined as stated above, and samples were stored at -20 ℃ until sequencing. Metagenome sequencing analyses of the gut microbiome DNA samples were processed and sequenced by a commercial company, the ChunLab Inc. in South Korea [27]. The metagenome sequencing and basic analysis were described by Chun *et al.* [28]. Libraries for each sample were prepared with a fragment length of approximately 300bp and short reads were excluded from raw data for the metagenome sequencing.

The sequence reads generated from metagenome sequencing were identified using the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net/) on the basis of 16S rRNA sequence data [29, 30]. The number of sequences analyzed, observed diversity richness [Operational Taxonomic Units (OTUs)], estimated OTU richness (ACE and Chao1), and Shannon diversity index indicated in Table S1 were calculated using the Mothur program. A cut-off value of 97% similarity of the 16S rRNA gene sequences was defined as a same species.

The microbial communities of the gut microbiome of each group were analyzed by using the Fast UniFrac Analysis method which is a phylogenetic method to analyze a beta diversity of microbial ecology between groups [15]. Fast Unifrac analysis is a variant of the original Unifrac algorithm designed to handle larger dataset using taxonomic assignment to a phylogenetic tree [31]. The eztaxon-e taxonomic structure as the backbone phylogenetic tree was used to the data.

## Data processing

The metagenomics data, OTU and taxonomic classification tables were imported into phyloseq (1.28.0) package in R version 3.6.1 [32] and the data was processed as described in [33, 34]. In brief, the OTUs that are presents as single unit in each sample were considered as OUTs generated by sequencing errors, thereby removed for further analysis. The OUT data was converted to metagenomeseq object and normalized by cumulative-sum-scaling (CSS), which was specially built for metagenome data in the bioConductor package metagenomeSeq (1.16.0.) [35]. Normalized data was imported as phyloseq class object in R for further analysis and visualization.

## Alpha diversity and abundance evaluation of microbiome

Alpha diversity (ACE richness, Simpson diversity, and Fisher's alpha) metrics were calculated based on CSS normalized values without filtering in phyloseq package. Unclassified phyla were removed from total

samples, and any taxa with a total of less than 0.5% were collected into “other”. Each taxonomic level was glommed for plotting.

### Beta diversity and abundance evaluation of microbiome

Beta diversity metrics were computed and visualized using log transformed, normalized OTU data in phyloseq package including Bray-Curtis dissimilarity [36]. Unweighted PCoA was calculated and visualized by QIIME2 while NMDS, was plotted in the phyloseq package in R.

### Construction of heatmap and phylogenetic tree

A heatmap and cluster analysis were generated using the relative abundances of genera from both all OTU values or core abundant OTU values in Heatplus (2.30.0.) package from bioconductor and vegan package in R. Average linkage hierarchical clustering and Bray-Curtis distance metrix were used for cluster analysis and heatmap generation respectively [37]. U*nsupervised* prevalence filtering was done with threshold 5% in total samples to collect most abundant taxa for heatmap generation.

Phylogenetic trees for each sampling site were constructed from row sequences without any filtering to show direct visualization of sample richness with relation to taxanomy classification. Taxa that could not be classified down to the species level were reclassified based on NCBI accession number using taxonomizr (0.5.3) package in R [38]. 16s rRNA sequences from each sampling site were aligned in ClustalW [39] with default parameter, and resulted alignments were used to construct the Maximum-likelihood phylogenetic trees in MEGAX [40] with 500 bootstraps replicates. All phylogenetic trees were visualized in iTOL [41].

### Statistical analysis

The data are expressed as the means ± standard deviations. Statistical analysis of the data was performed with One-way analysis of variance (ANOVA). Differences with a $p < 0.05$ were considered statistically significant.

## Declarations

### Ethics and consent to participate

The study subjects were recruited from the Clinical Trial Center for Functional Foods (CTCF2) in the Chonbuk National University Hospital. Written consents were obtained from all participants. The study was conducted according to the Declaration of Helsinki. This study was carried out in strict accordance with the recommendations in the Guide for the Ethics Committee of Chonbuk National University Hospital.The research protocol was approved by the Institutional Review Board (IRB) of Chonbuk National University Hospital, Republic of Korea (CHU_KOREAN_FOOD_2-2_2010).

### Consent for publication

Not applicable

### Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

### Competing interests

The authors declare that they have no competing interests.


### Funding

This study was supported by Forest Resources Research Program (2017028A00-1719-BA01) funded by Korea Forest Service, Republic of Korea.


### Authors' contributions

S.T.H. designed the project and supervised the study. H.J.C performed experiments. S.T.H., E.L. and H.J.C. undertook data acquisition, analysis, and interpretation. S.T.H. wrote the manuscript.


### Acknowledgement

The authors wish to acknowledge Ms. Jinny Hong at Case Western Reserve University for her help in through proofreading of the manuscript and for improving the use of English in the manuscript. The authors also appreciate Dr. Hyeon-Jin Kim at JINIS Biopharmaceuticals Co. for her through proofreading of the manuscript and discussion of the experimental data and Professor Cho Back-Hwan at Chonbuk National University Medical School for discussion of the experiments.


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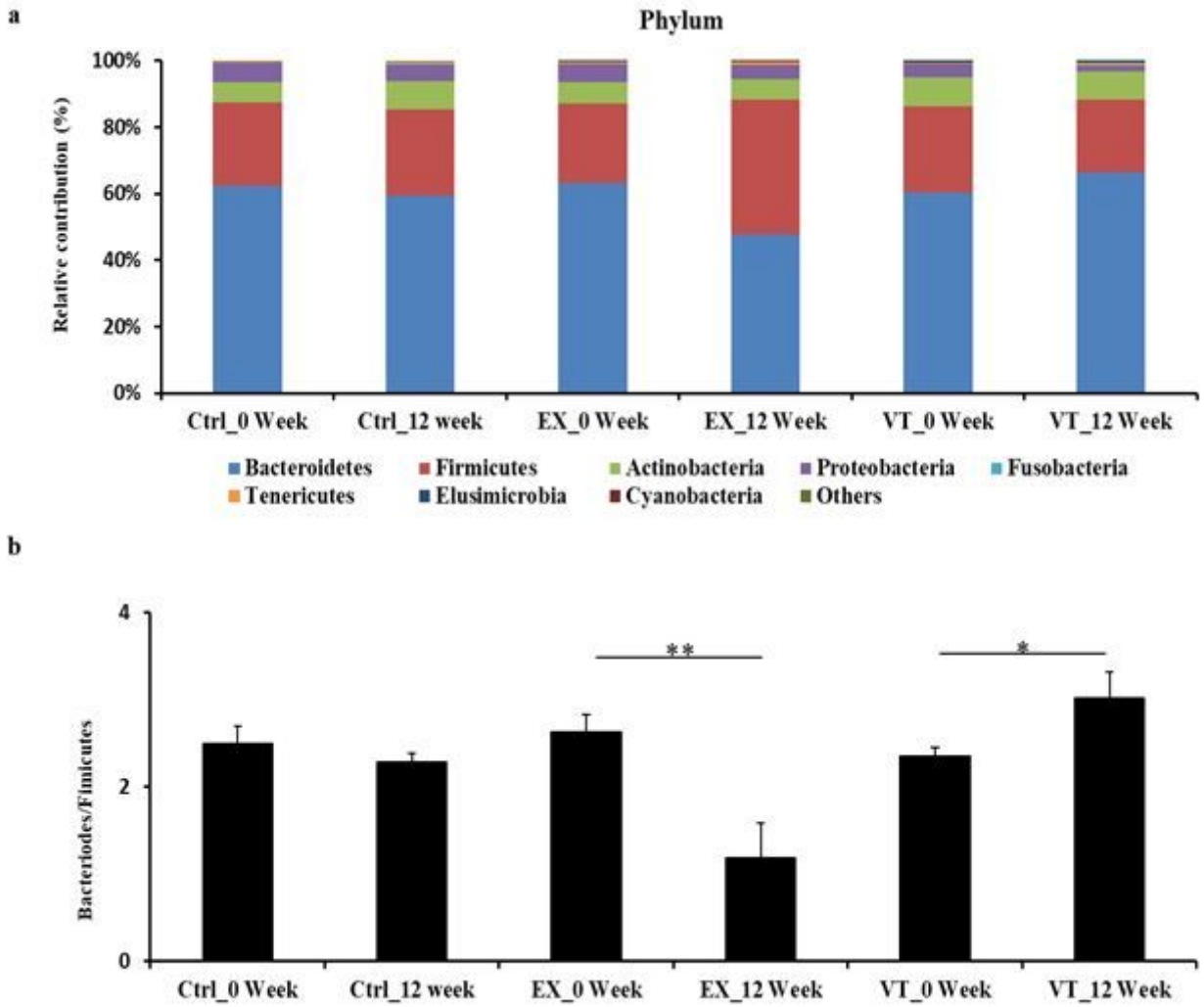
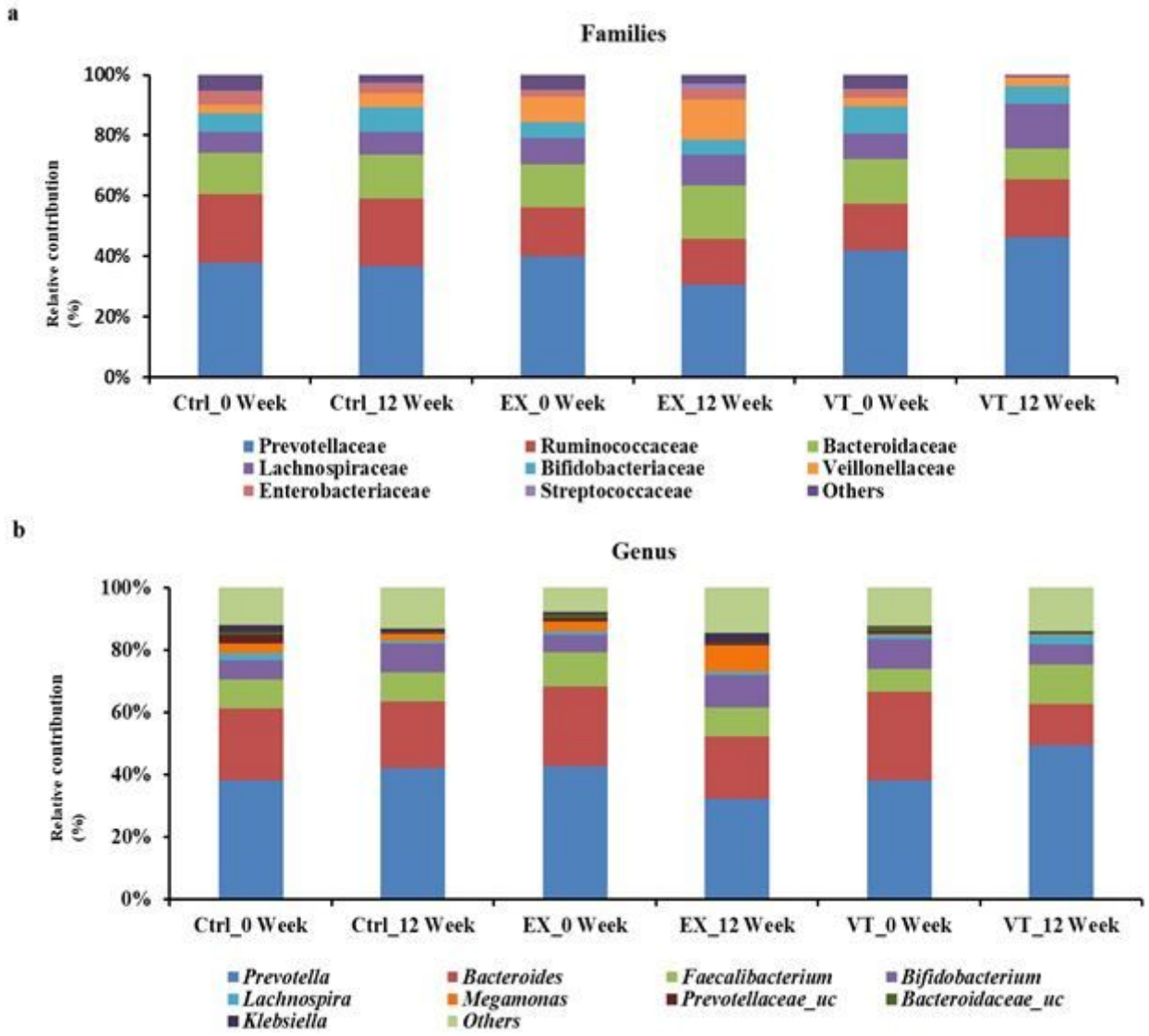
# Figures




## Figure 1

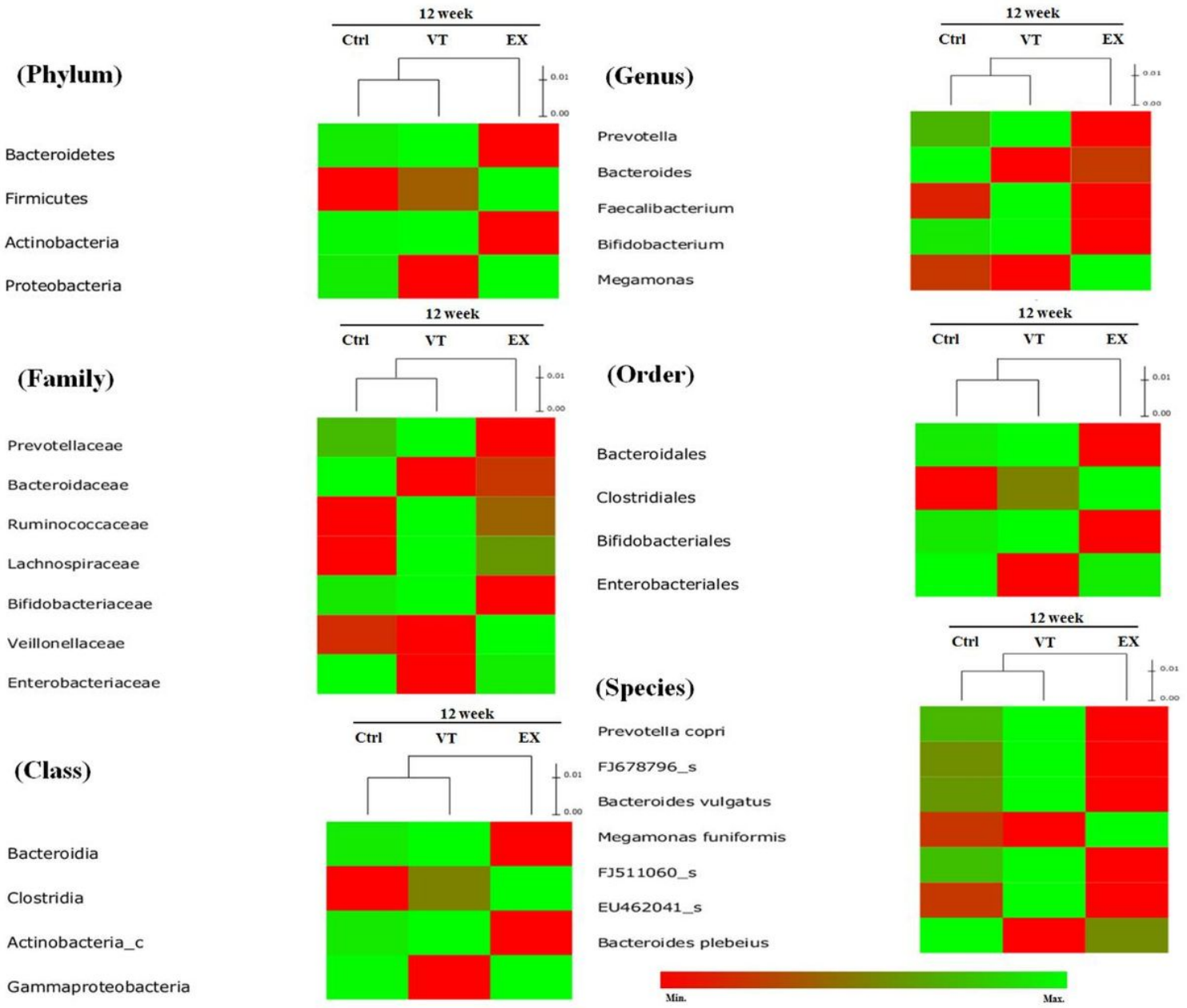
The compositional changes of the gut microbiome at phylum levels after diet shift to the vegetarian diet or the physical exercise during the 12-week experimental period. (a) The relative compositional changes at phylum levels and (b) the relative compositional changes of Bacteriodes to Fimicutes ratio in the gut microbiome are shown. The VT, EX, and Ctrl represent the vegetarian group, the exercise group, and

control respectively. All values are indicated as the mean ± standard deviation. *, p < 0.05, **, p < 0.01 with the division



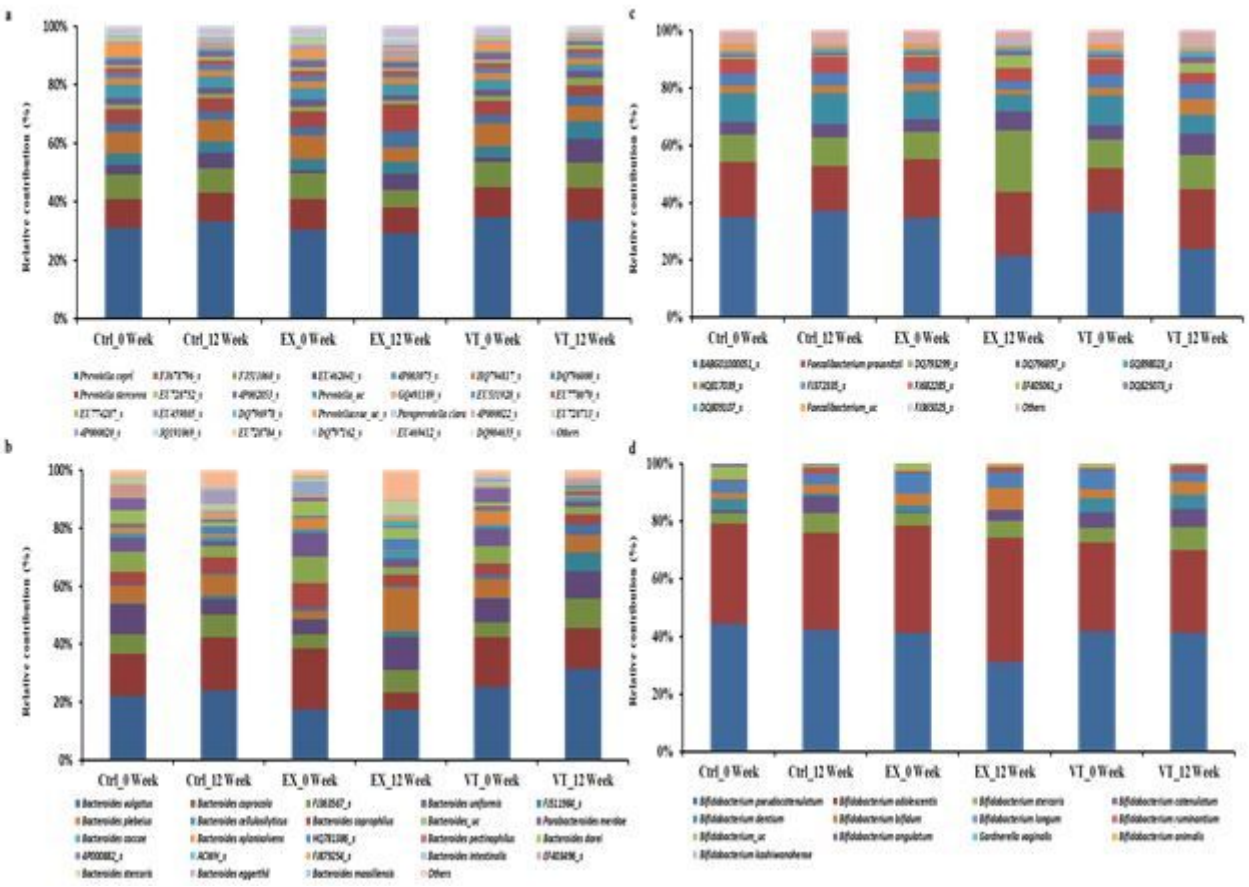

## Figure 2

The compositional changes of gut microbiome at family and genus levels after diet shift to a vegetarian diet or a physical exercise during the 12-week experimental period. (a) The relative compositional changes at family levels and (b) genus levels are shown. The VT, EX, and Ctrl represent the vegetarian group, the exercise group, and control respectively.



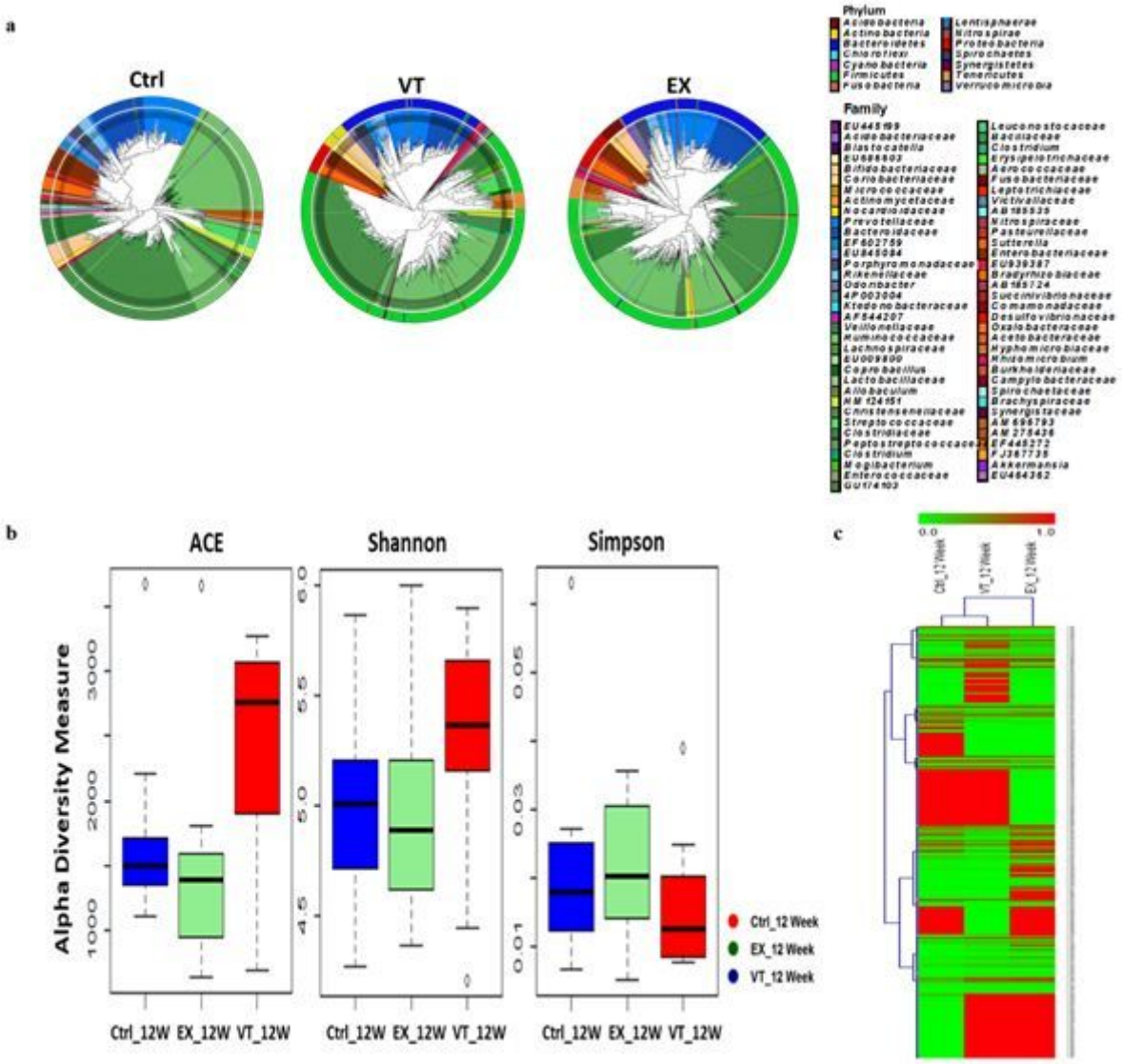

## Figure 3

The relative differences in specific composition of gut microbiome after the 12-week experimental period. Heatmaps show the relative contributions of dominant genus or species. The VT, EX, and Ctrl represent the vegetarian group, the exercise group, and control respectively.




## Figure 4

The compositional change of major species constituting gut microbiome during the 12-week experimental period. The relative compositional changes at species levels in the genus of (a) Prevotella, (b) Bacteroides, (c) Faecalibacterium, and (d) Bifidobacterium are shown. The VT, EX, and Ctrl represent the vegetarian group, the exercise group, and control respectively.




## Figure 5

Comparative analysis on microbiome diversity by α-diversity analyses. (a) Maximum-likelihood phylogenetic tree comprising all of the taxa in tree independent groups respectively. Clades are labelled according to family and the corresponding phylum is depicted in the first outer layer. (b) Species richness and diversity measured by the indices of ACE richness, Shannon, and Simpson alpha. (c) HCL heatmap at Genus level.

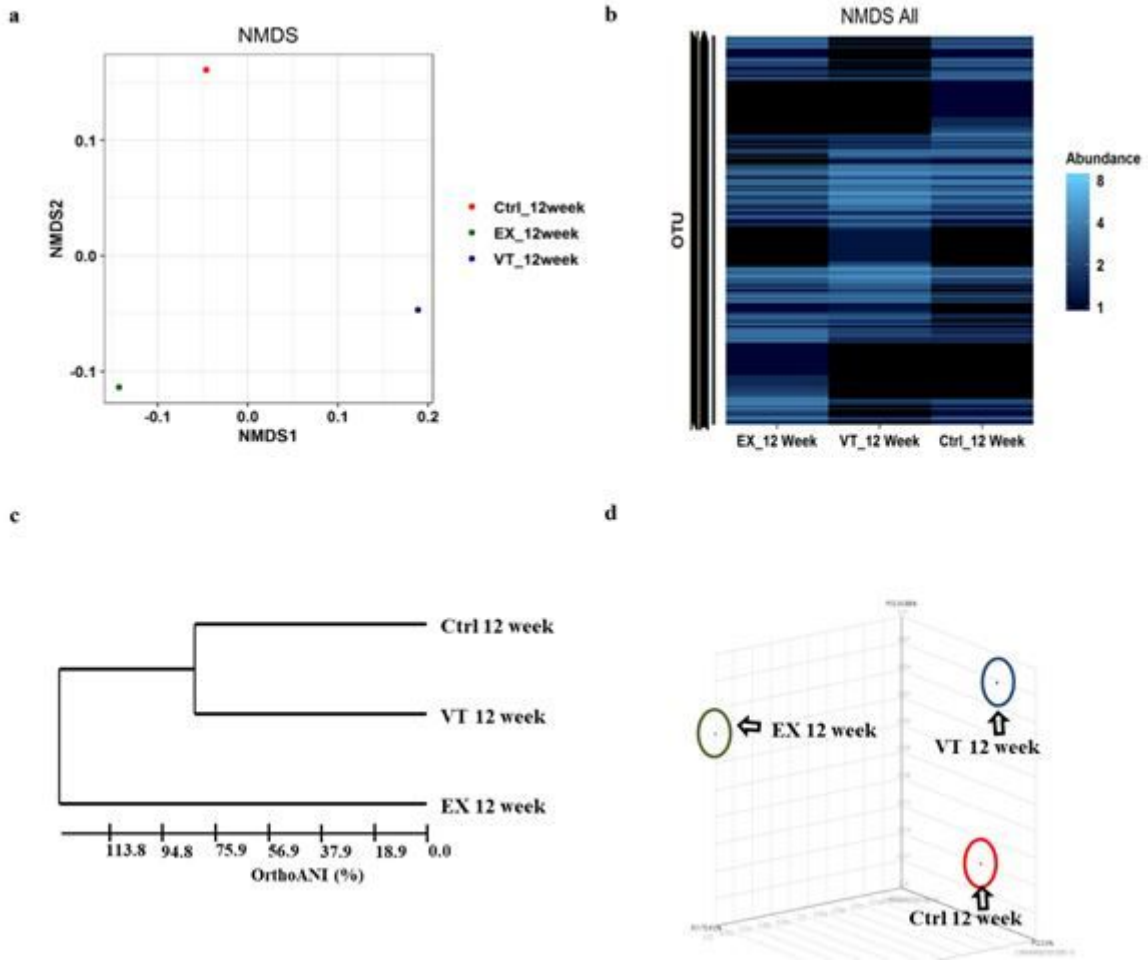



**Figure 6**

The beta diversity analysis results in the gut microbiome of the VT group, the EX group, and the Ctrl group at 12th week. (a) NMDS 2D plot with Bray-Cirtis distance. (b) NMDS heatmap with Bray-Cirtis distance. (c) Fast UniFrac Analysis and (d) The Principal coordinate analysis (PCA). The VT, EX, and Ctrl represent the vegetarian group, the exercise group, and control respectively.

# Supplementary Files

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- Supp.docx