The Effect of Chili Consumption on the Abundance of Bacteroidetes in the Human Gut Microbiome
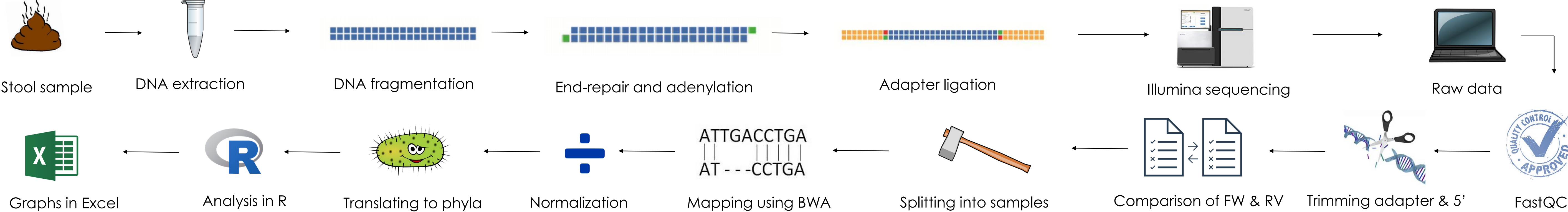
Introduction

Bacteroides is one of the most abundant genera in the human gut microbiome. However, certain *Bacteroides spp.* are opportunistic pathogens and can cause serious infections (1). A study has shown that *Bacteroides spp.* have developed resistance to multiple antibiotics (2), so alternative treatment methods must be found. Capsaicin (8-methyl-N-vanillyl-6-nonenamide), a component of chili, has shown to have antimicrobial effect (3). We thus hypothesize that dietary capsaicin will have an antimicrobial effect on *Bacteroides*. This will be investigated by analyzing the phylum abundance of Bacteroidetes in the human gut microbiome after chili intake.

Sampling

A randomized controlled trial was performed with 21 participants, of which 10 were randomly allocated in the chili intervention and 11 participants in a control group. The chili intervention consisted of a single high-dose chili intake, where the control group would not eat chili. Stool samples were taken before (B) and after (A) chili or no chili consumption. All participants filled in a questionnaire prior to the start of the study in order to obtain metadata. The general workflow of the sample analysis is depicted below. The raw reads were quality trimmed to 30, adapters were cut from both ends and 5 bases were hardtrimmed from the 5' end. Trimmed reads were mapped with BWA to a reference database containing 3.9 million genes and consequently a count matrix was constructed.

Workflow

Stool sample → DNA extraction → DNA fragmentation → End-repair and adenylation → Adapter ligation → Illumina sequencing → Raw data

Graphs in Excel ← Analysis in R ← Translating to phyla ← Normalization ← Mapping using BWA (ATTGACCTGA AT - - -CCTGA) ← Splitting into samples ← Comparison of FW & RV ← Trimming adapter & 5' ← FastQC

Results

Overview of reads

After performing data pre-processing, we wanted to check the reads per sample. As seen in Figure 1, we have few reads in many samples. Therefore, we only chose to normalize and not downsize our data set.

- Average count ~ 396628
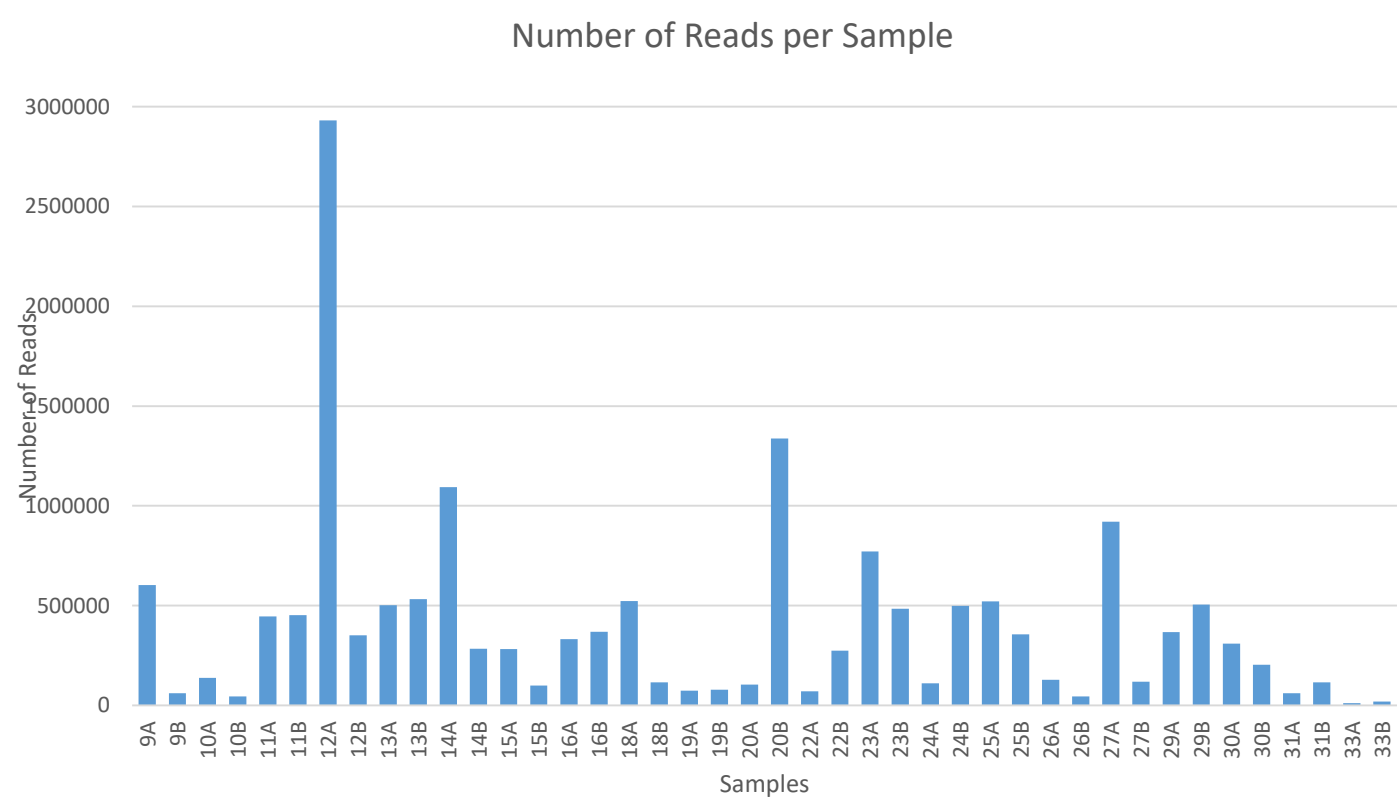- Minimum count ~ 12366
- Maximum count ~ 2930444

Figure 1: The read count of each sample after the preprocessing steps. Before samples are annotated "B" and after samples "A".

Phylum Distribution for Each Participant

The phyla distribution in each of the 42 samples are presented in Figure 2. The samples are sorted with the chili group (10 participants) followed by the control group (11 participants). The colors indicate the phylum present in each sample. There are no significant difference between the before and after samples (t-test, p = 0.99).
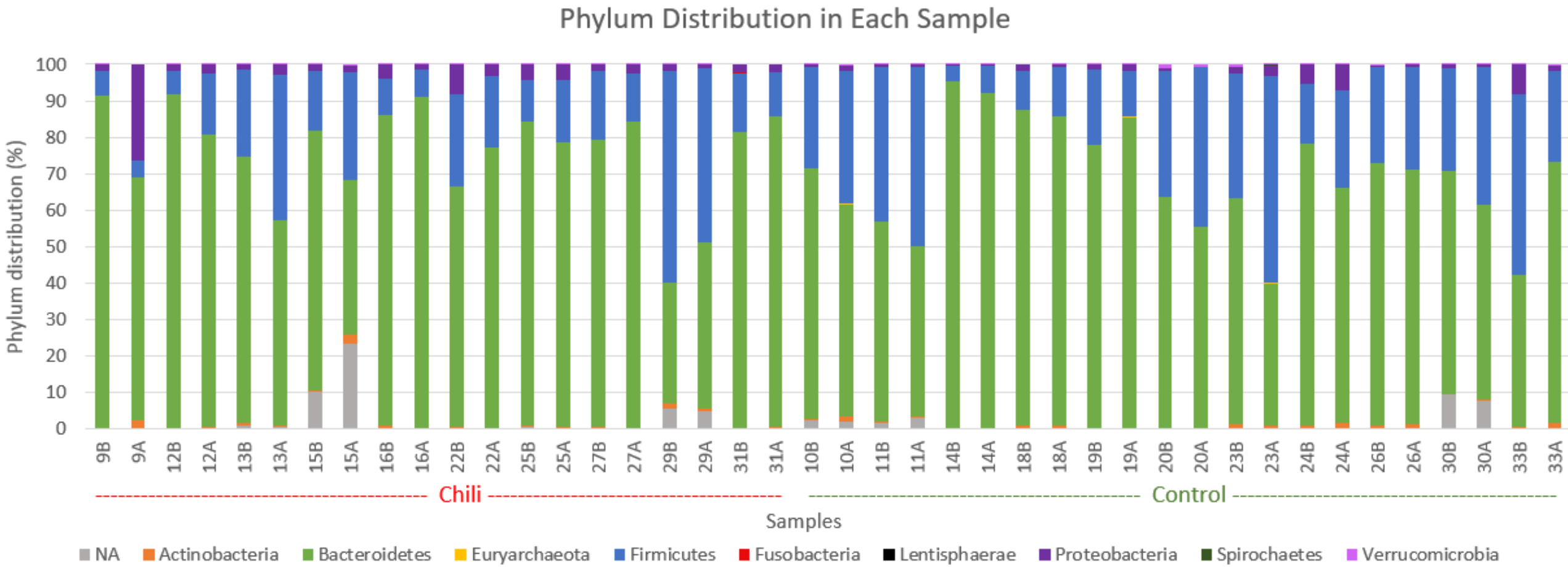

Figure 2: Phylum distribution in for each participant. The figure shows before samples (B) and after samples (A).

Phylum Abundance Distribution

We wanted to investigate the abundance distribution of the three most abundant phyla; Bacteroidetes, Firmicutes and Proteobacteria. The distributions are shown in Figure 3A and 3B.
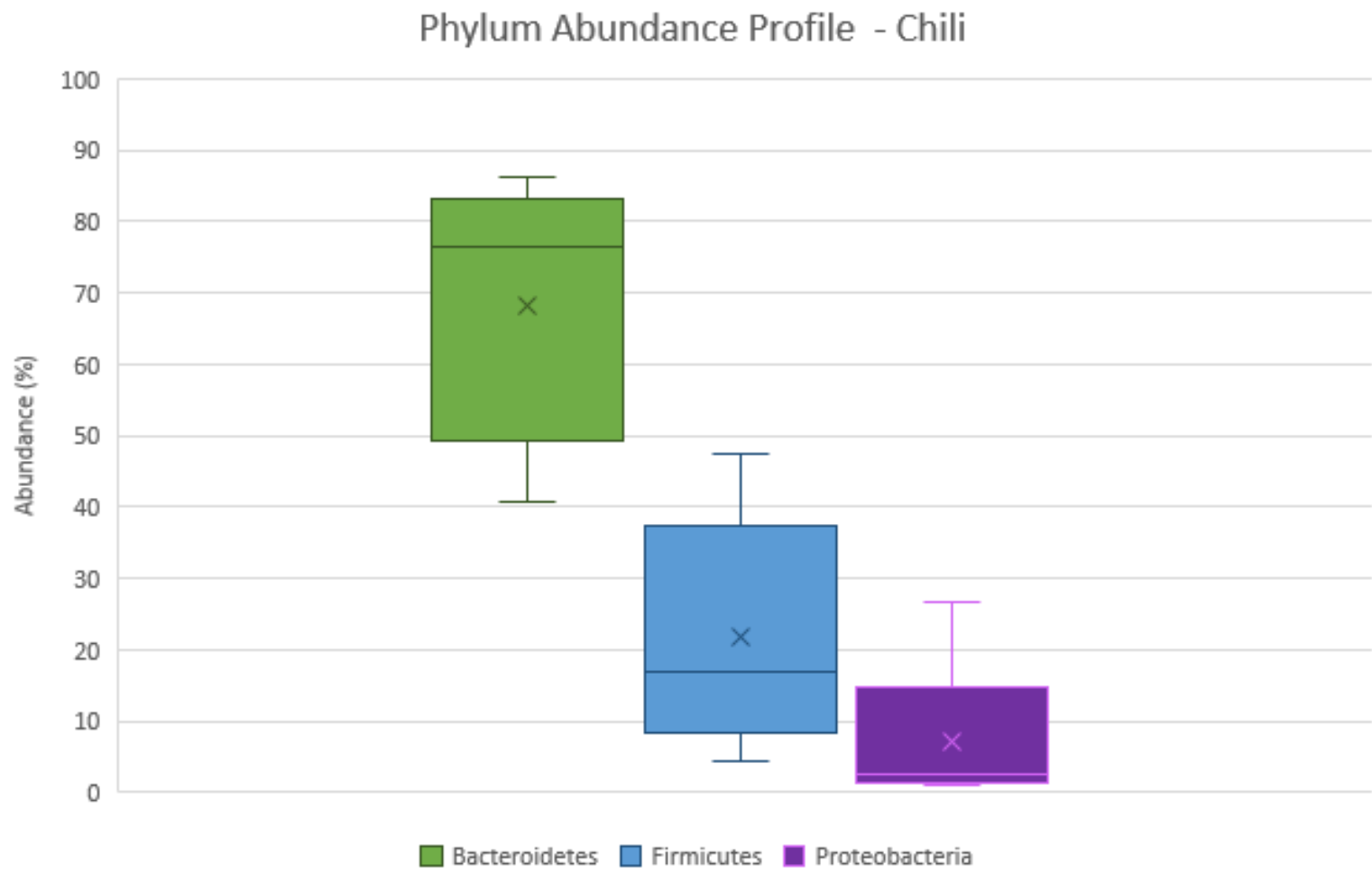

Figure 3A: The distribution among the samples of the three most abundant phyla (Bacteroidetes, Firmicutes and Proteobacteria) in the chili after samples.
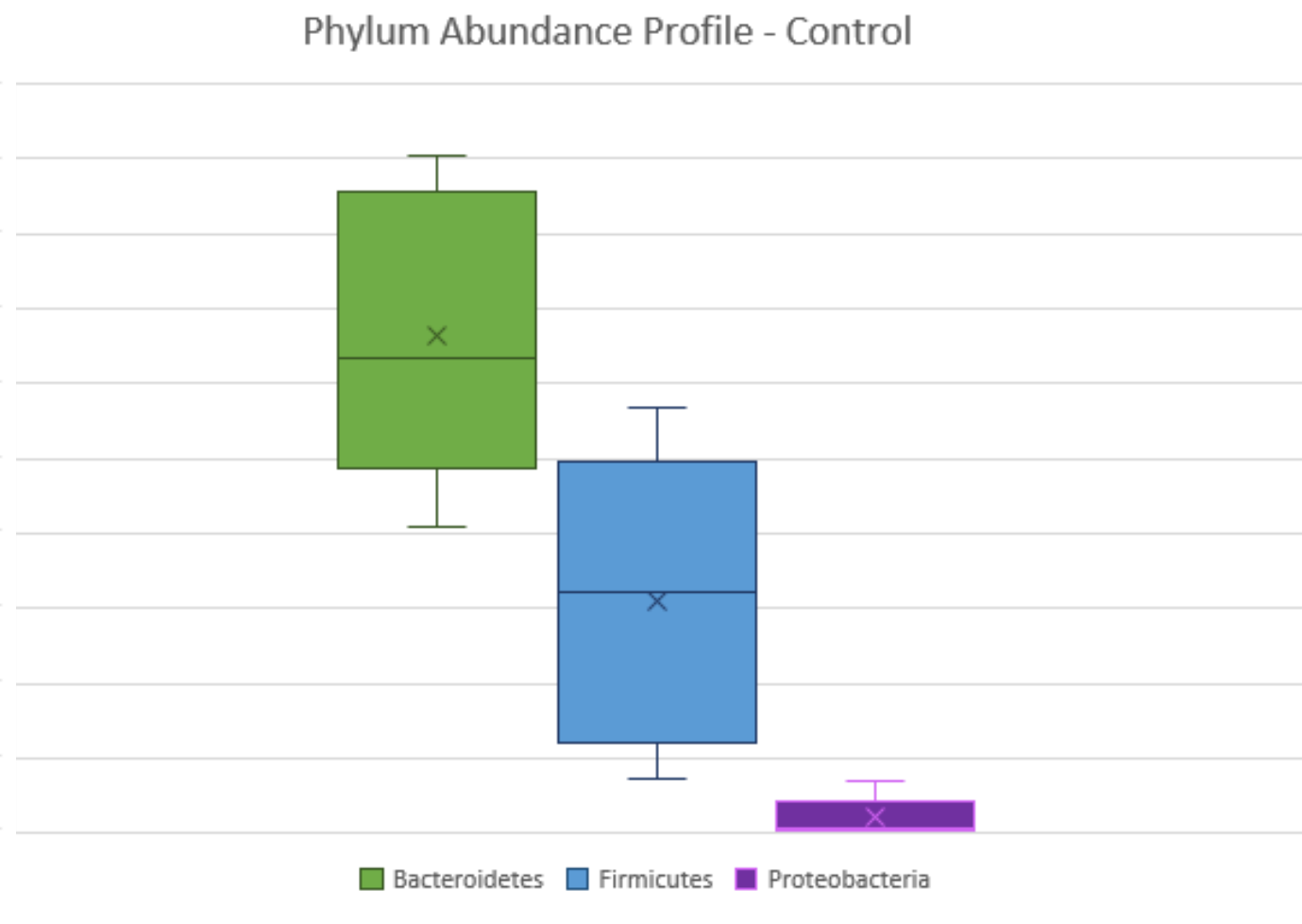
Figure 3B: The distribution among the samples of the three most abundant phyla (Bacteroidetes, Firmicutes and Proteobacteria) in the control after samples.

Phylum Distribution

We wanted to look at the phylum distribution in all the samples for the two intervention groups. We also wanted to check whether there is a difference in the phylum distribution in the before and after samples for each group. The percentages of each phylum were calculated for all the samples. As indicated in the pie charts below, the three most abundant phyla are Bacteroidetes, Firmicutes and Proteobacteria. The phylum distribution is similar for the two intervention groups. Similar phyla distributions are also observed before and after the intervention in each of the groups. The impact significance of these pie charts is described below.
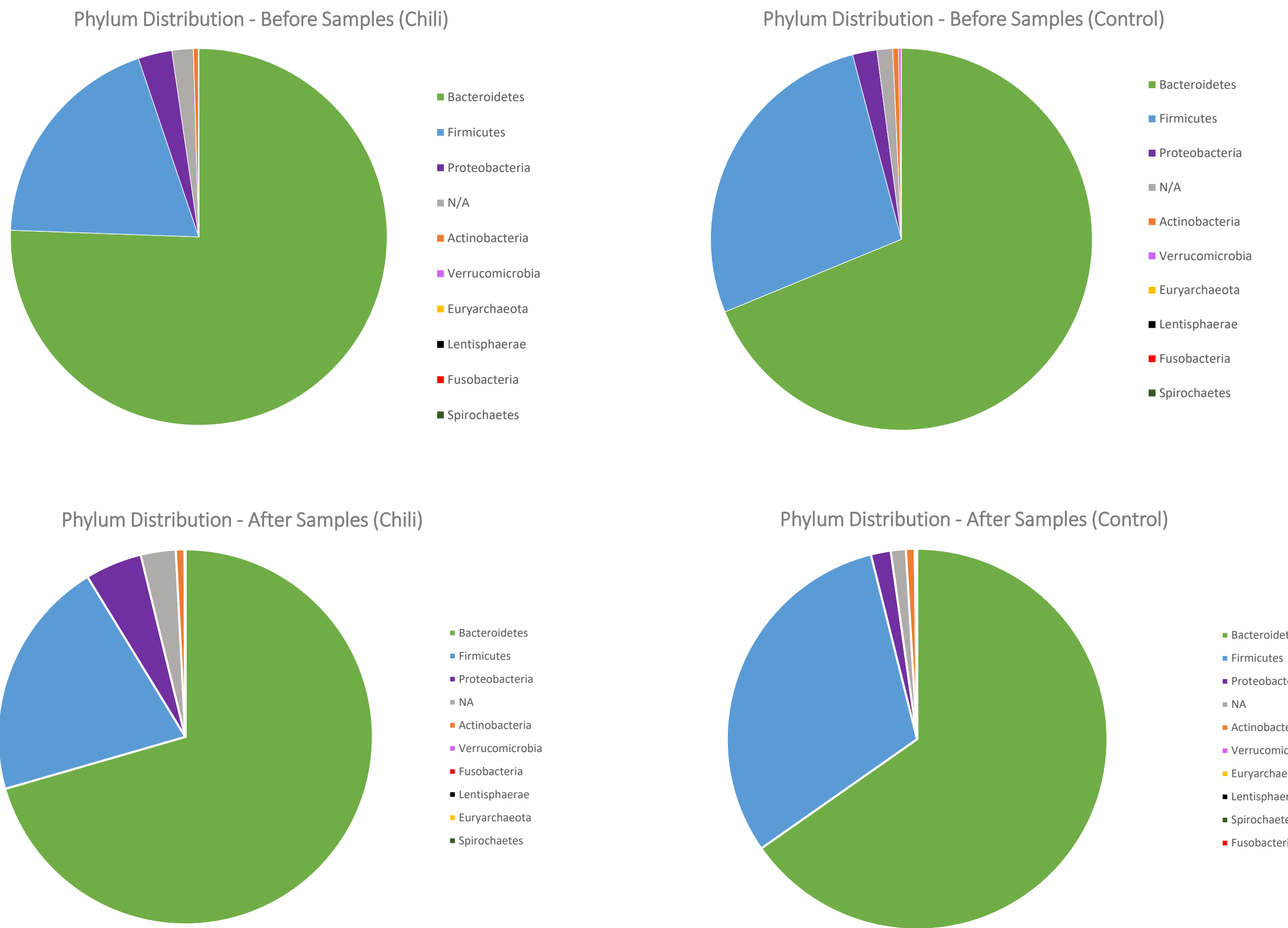

Figure 4: Phylum distribution in percent for before and after samples for each of the two groups (chili/control).

Overview of Impact Significance

After looking at the phylum distribution in percentages, we wanted to calculate if there are any significant differences in the phylum distribution. A paired t-test was performed for the control group and the chili group to verify if any significant differences in the phylum distribution was observed in the before and after samples. Figure 5 gives an overview of the calculated p-values. As it is observed in the chart, none of the p-values are below 5%, which indicates no significant difference in the phylum distribution.
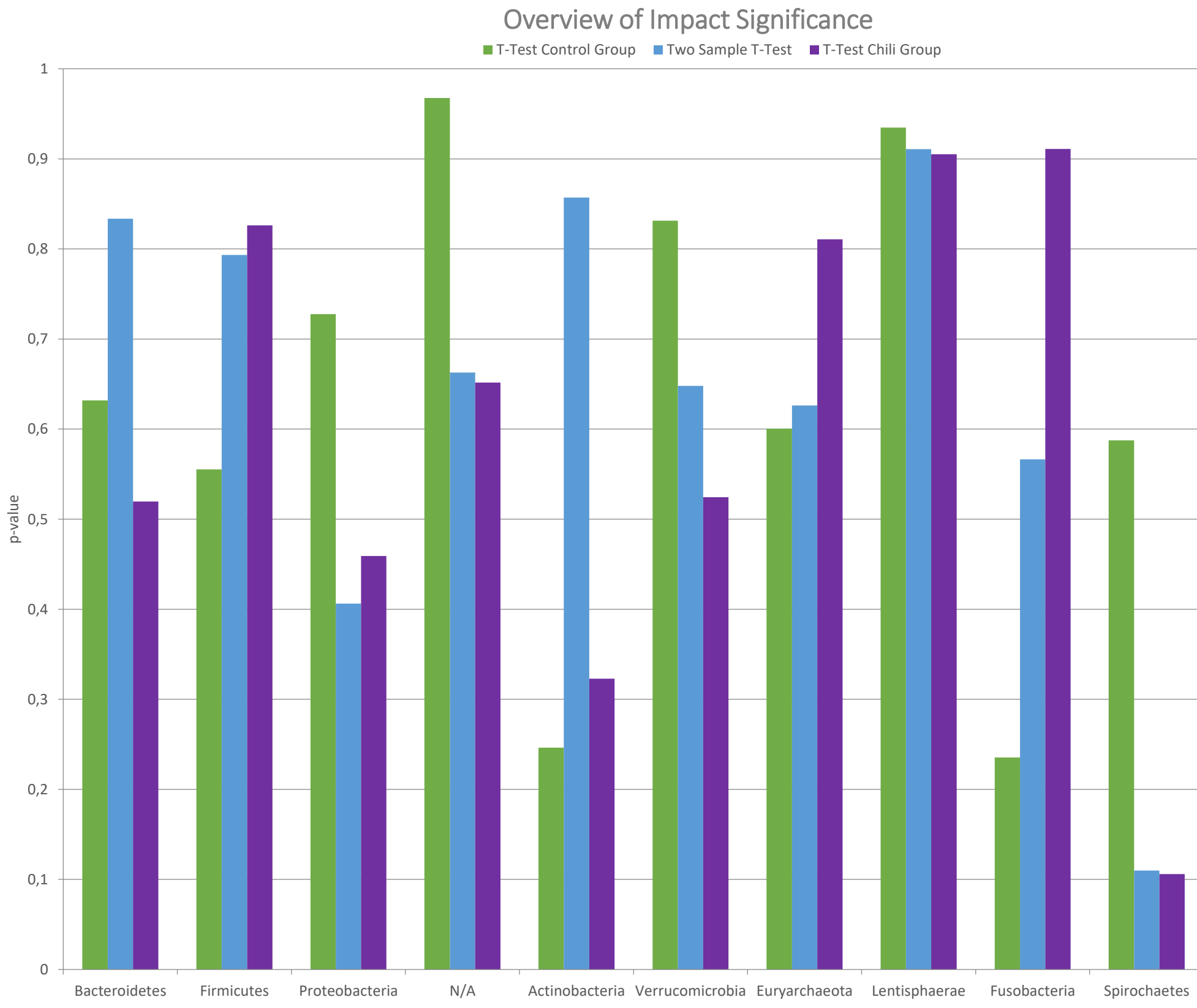

Figure 5: Overview of calculated p-values in the control and chili group, and between the two groups.

Discussion & Conclusion

The aim of this study was to investigate if a single dose of chili would have an inhibitory effect on *Bacteroides* in the human gut. However, we were unable to draw a relevant conclusion to this question, as none of the results we obtained were statistically significant. There are a few factors that have complicated our analysis. During the preprocessing, the computer server that was used ran out of disk space. Consequently, we performed BWA not with the entire data set, resulting in the skewing of our data. We choose not to downsize as this would even further reduce our amount of available data. All in all, even though the obtained reads were of high quality (FastQC), the resolution of our data did not permit us to look at species level. Instead, we looked at phylum level, as we could assign most of our identified MGS to a certain phylum. However, we did not observe any statistically relevant effect of chili consumption on the abundance of the Bacteroidetes phylum. Consequently, we did not draw any conclusion regarding the metadata of the participants. Future work should include redoing the preprocessing steps to obtain more reads and to have a larger study with more participants to achieve a greater diversity.

References

1) Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., ... Meta, H. I. T. C. (2011). **Enterotypes of the human gut microbiome.** *Nature.*
2) Vedantam, G. (2009). **Antimicrobial resistance in Bacteroides spp.: occurrence and dissemination.** *Future Microbiology.*
3) Jones, L.N. et al (1996). **Capsaicin as an inhibitor of the growth of the gastric pathogen Helicobacter pylori.** *FEMS Microbiology letters.*

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