



# D4006- Gut Microbiota Analysis

*UC Davis MMPC - Microbiome & Host Response Core*

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### Core Contacts:

Helen E. Raybould, Ph.D., Core Leader (heraybould@ucdavis.edu)  
 Trina A. Knotts, Ph.D., Core Co-Leader (taknotts@ucdavis.edu)  
 Michael L. Goodson, Ph.D., Core Scientist (mlgoodson@ucdavis.edu)

**Client(s):** Kent Lloyd, DVM PhD ;MMRRC; UC Davis

**Project #:** MBP-2079

**MMRRC strain ID:** MMRRC\_043515

**Animal Information:** The strain was donated to the MMRRC by Russell Ray at Baylor College of Medicine. Fecal samples were obtained from animals housed under the care of Russell Ray at Baylor College of Medicine.

## 1 Methods:

Brief Project Description: MMRRC strains are often contributed to the MMRRC to fulfill the resource sharing aspects of NIH grants. Since transporting mice to another facility often causes a microbiota shift, having a record of the original fecal microbiota from the donor institution where the original phenotyping or testing was performed may prove helpful if a phenotype is lost after transfer. Several MMRRC mouse lines were selected for fecal microbiota profiling of the microbiota.

Table 1: Animal-Strain Information

X.SampleID	TreatmentGroup	Animal_ID	Genotype	Line	Sex
MMRRC.043515.M918	MMRRC.043515_Het_M	M918	Het	MMRRC.043515	M
MMRRC.043515.M921	MMRRC.043515_Het_M	M921	Het	MMRRC.043515	M

## 1.1 Sequencing

Frozen fecal or regional gut samples were shipped on dry ice to UC Davis MMPC and Host Microbe Systems Biology Core. Total DNA was extracted using Mo-Bio (now Qiagen) PowerFecal kit. Sample libraries were prepared and analyzed by barcoded amplicon sequencing. In brief, the purified DNA was amplified on the V4 region of the 16S rRNA genes via PCR using the following primers: F319 (5'-ACTCCTACGGGAGGCAGCAGT-3') and R806 (5'-GGACTACNVGGGTWTCTAAT-3'). High-throughput sequencing was performed with Illumina MiSeq paired end 250-bp run.

## 1.2 Data processing

The data derived from sequencing was processed using QIIME2 for 16S based microbiota analyses (QIIME 2 Development Team (2017)). Demultiplexed paired end sequences that already had barcodes and adapters removed were analyzed using QIIME 2 version 2019.1.0. For quality filtering and feature (OTU) prediction, we used DADA2 (Callahan et al. (2016)). Upon reviewing the sequence quality data, we trimmed 0 nucleotides (nts) from the 5' end of the forward and 0 nts from the reverse reads. Forward reads were truncated to 270 nts and reverse reads to 200 nts. Representative sequences were aligned using MAFFT (Katoh and Standley (2013)). A phylogenetic tree of the aligned sequences was made using FastTree 2 (Price, Dehal, and Arkin (2010)). OTUs/features were taxonomically classified using a pre-trained Naive Bayes taxonomy classifier. The classifier was trained using the Silva 128 97% OTUs (Quast et al. (2013)) for the 319F-806R region. Tables of taxonomic counts and percentage (relative frequency) were generated. Diversity analyses were run on the resulting OTU/feature .biom tables to provide both phylogenetic and non-phylogenetic metrics of alpha and beta diversity (Lozupone et al. (2011)). Additional data analysis (PLS-DA) and statistics were performed with R. Visualization files (.qzv) can be viewed through <http://view.qiime2.org>. The most relevant files for browsing in this manner are the feature tables (table.qzv (seq count data by taxa) and relative\_freq\_table.qzv (% abundance by taxa)), and taxa bar plots (taxa-bar-plots.qzv).

# 2 Summary of Findings:

## 2.1 Sequencing analysis

We obtained a mean of  $65703 \pm 884$  (SD) individual sequencing reads per sample (Min= 65078; Max= 66328). After data processing the average number of sequences for each sample passing through to OTU classification was  $40196 \pm 861$  (SD). The average number of OTUs per sample was 282.

## 2.2 Microbial diversity

Alpha and beta diversity measures were calculated using the QIIME pipeline (QIIME 2). Rarefaction was used to sample the same number of random reads from each sample for the diversity analyses. The sampling depth was set at 39500 sequences per sample.

### 2.2.1 Alpha Diversity

Alpha diversity is a measure of a specific sample's richness or diversity. Six different measures of alpha diversity were analyzed (Chao1, Good's coverage, Observed species, Faith's phylogenetic diversity (PD)), Shannon index, and Simpson's index. To account for differences in the number of reads in a sample, we performed rarefaction to collect a random set of sequences on a set number of sequences to rule out sample depth bias.

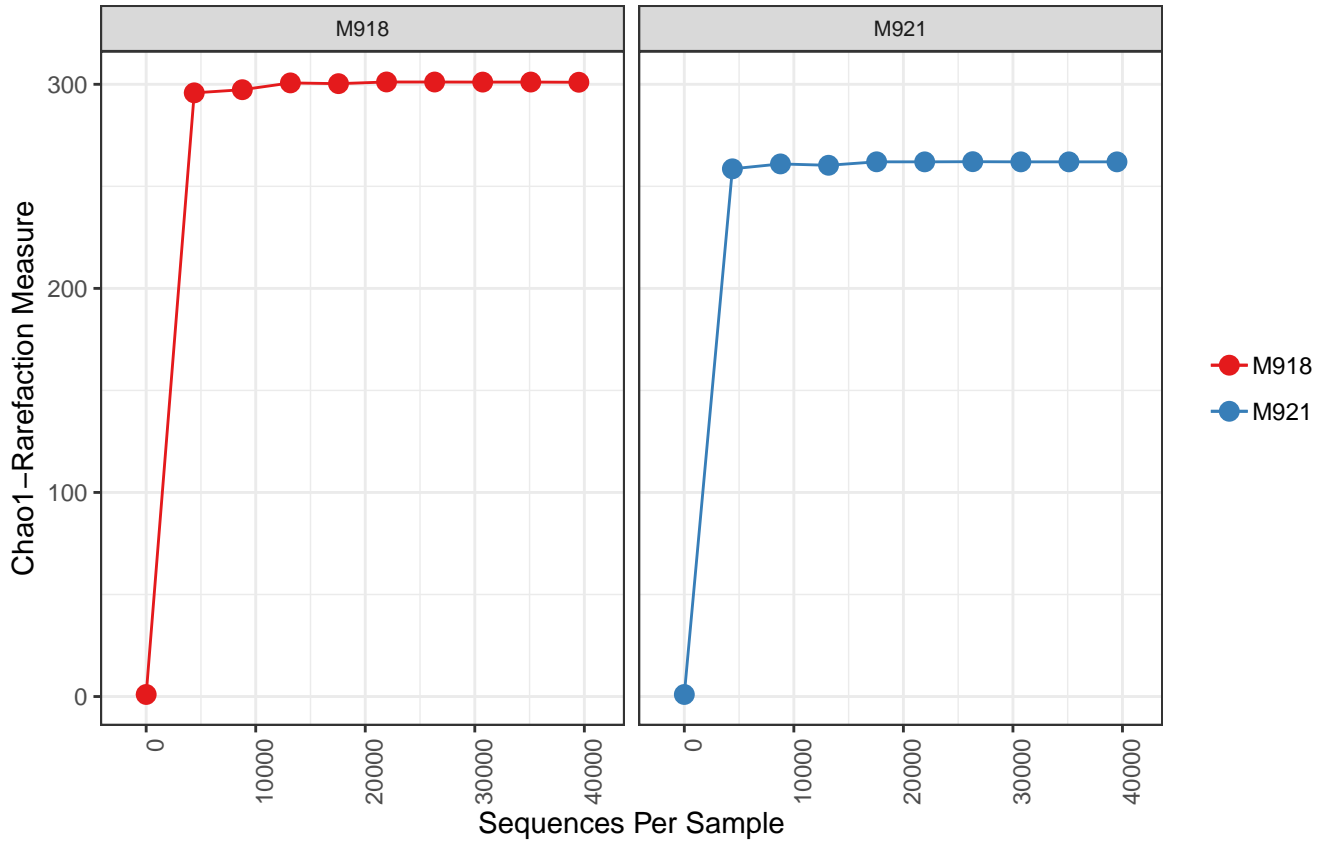


Figure 1: Chao1 Measure of Alpha Diversity- Rarefaction curve-Animal\_ID.

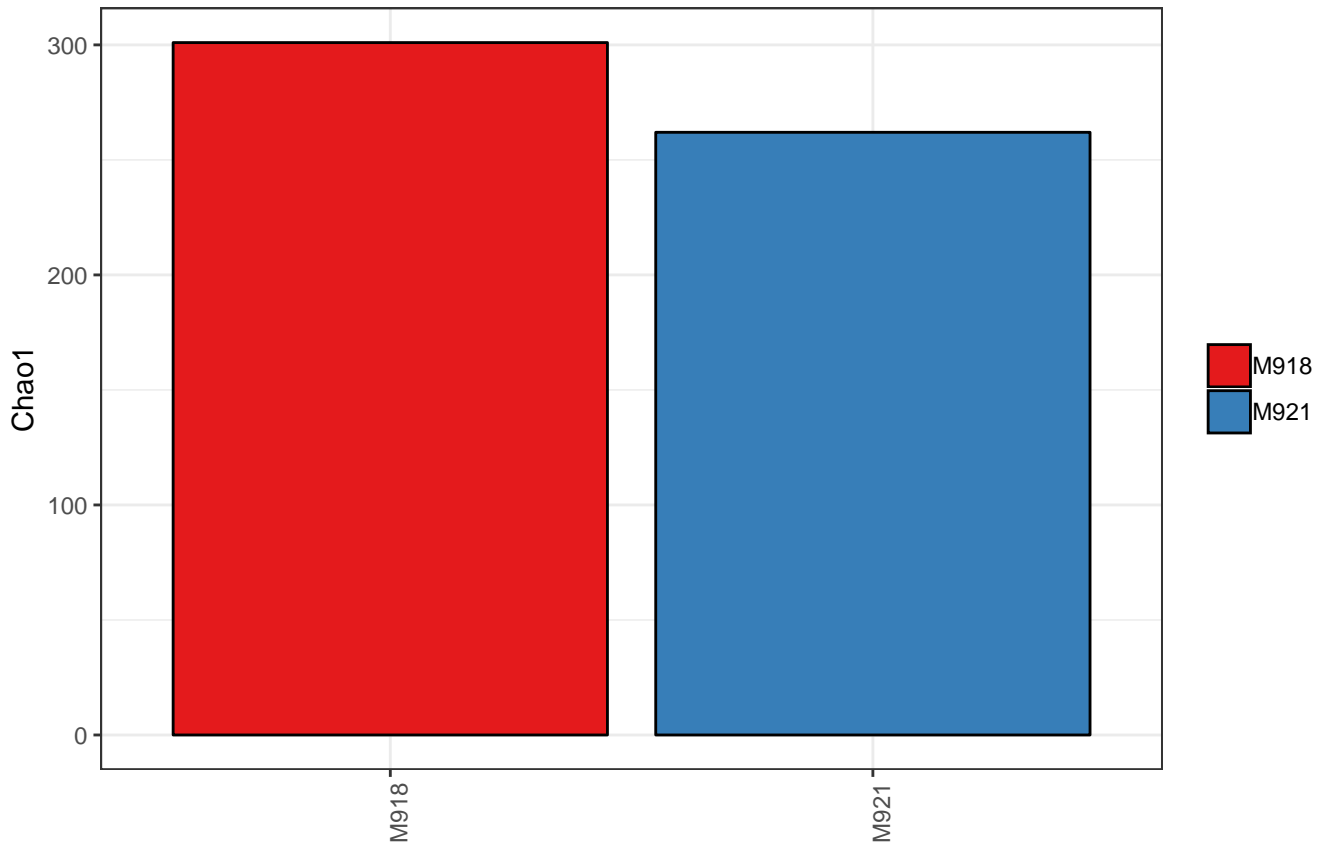


Figure 2: Chao1 Measure of Alpha Diversity- Boxplots-Animal\_ID.

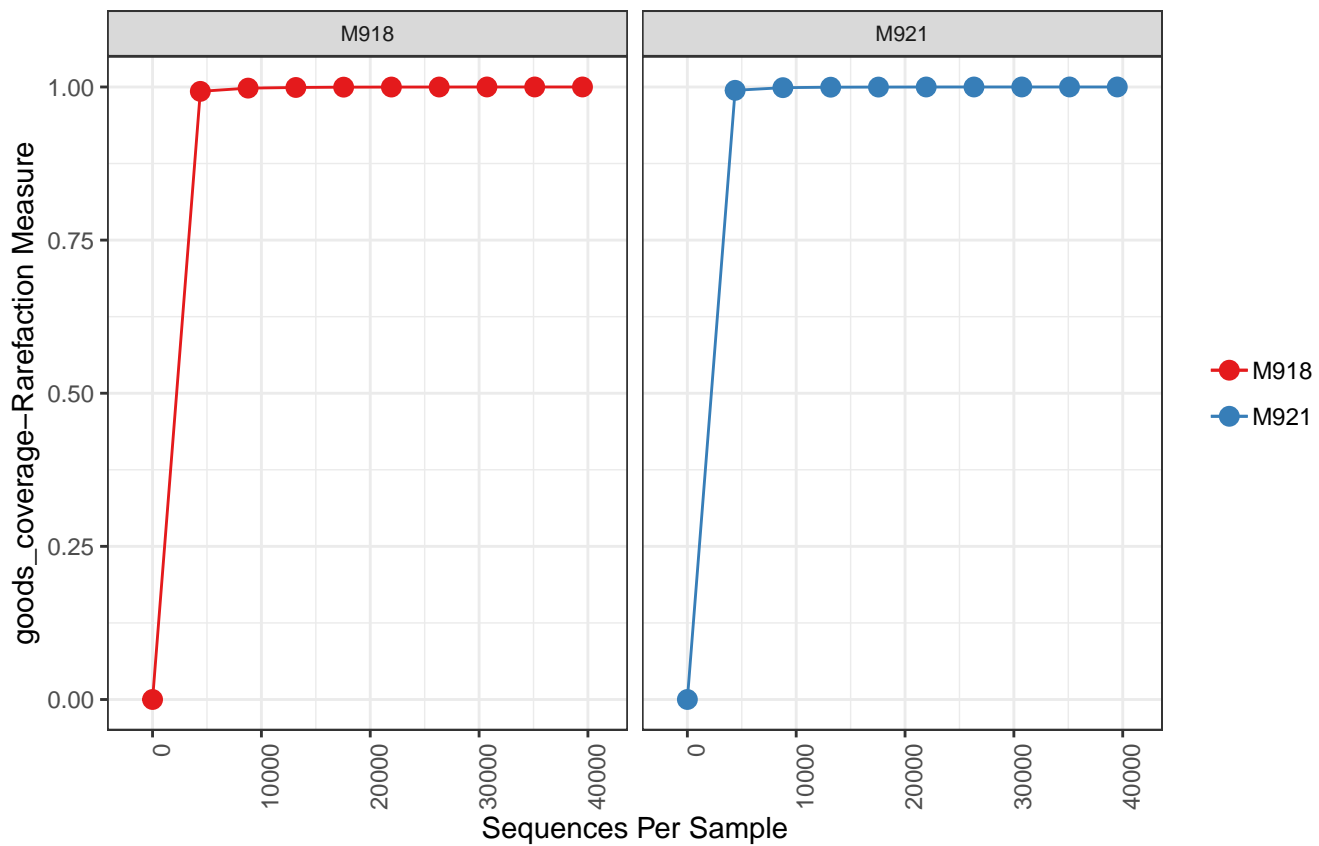


Figure 3: Goods Coverage Measure of Alpha Diversity- Rarefaction curve-Animal\_ID.

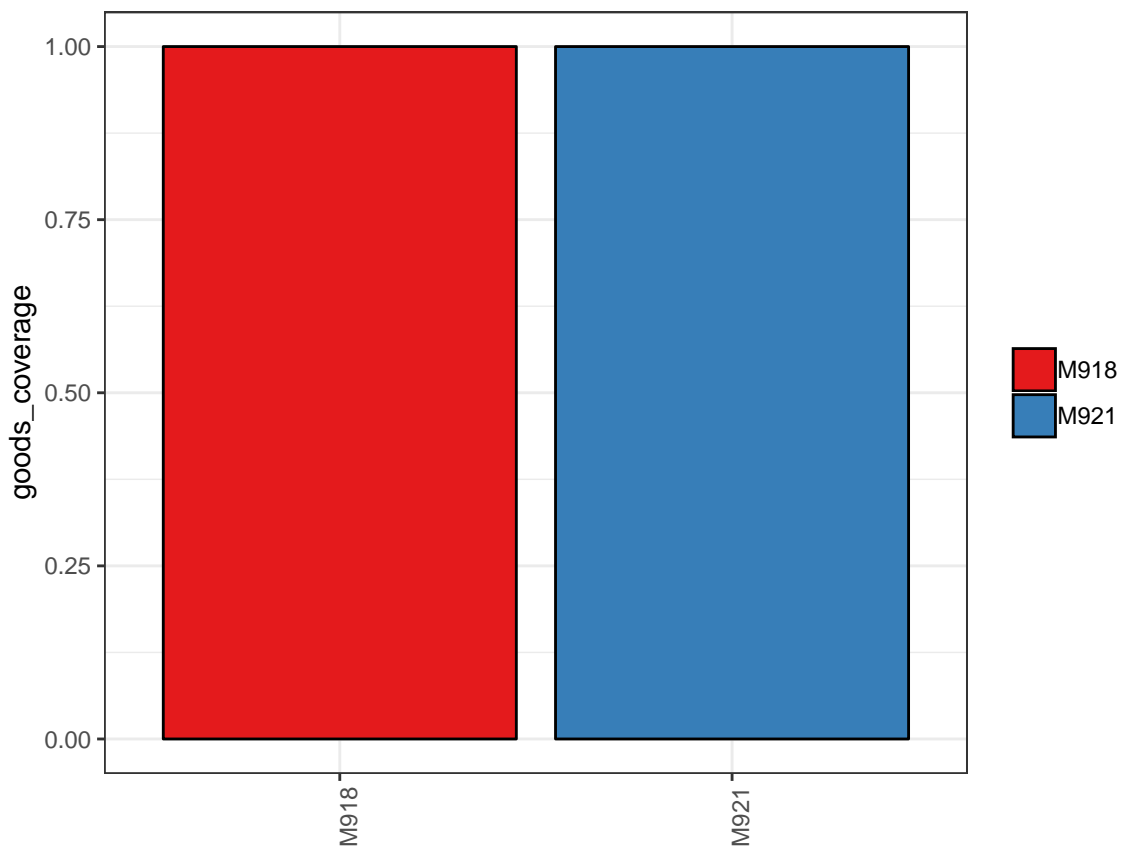


Figure 4: Goods Coverage Measure of Alpha Diversity- Boxplots-Animal\_ID.

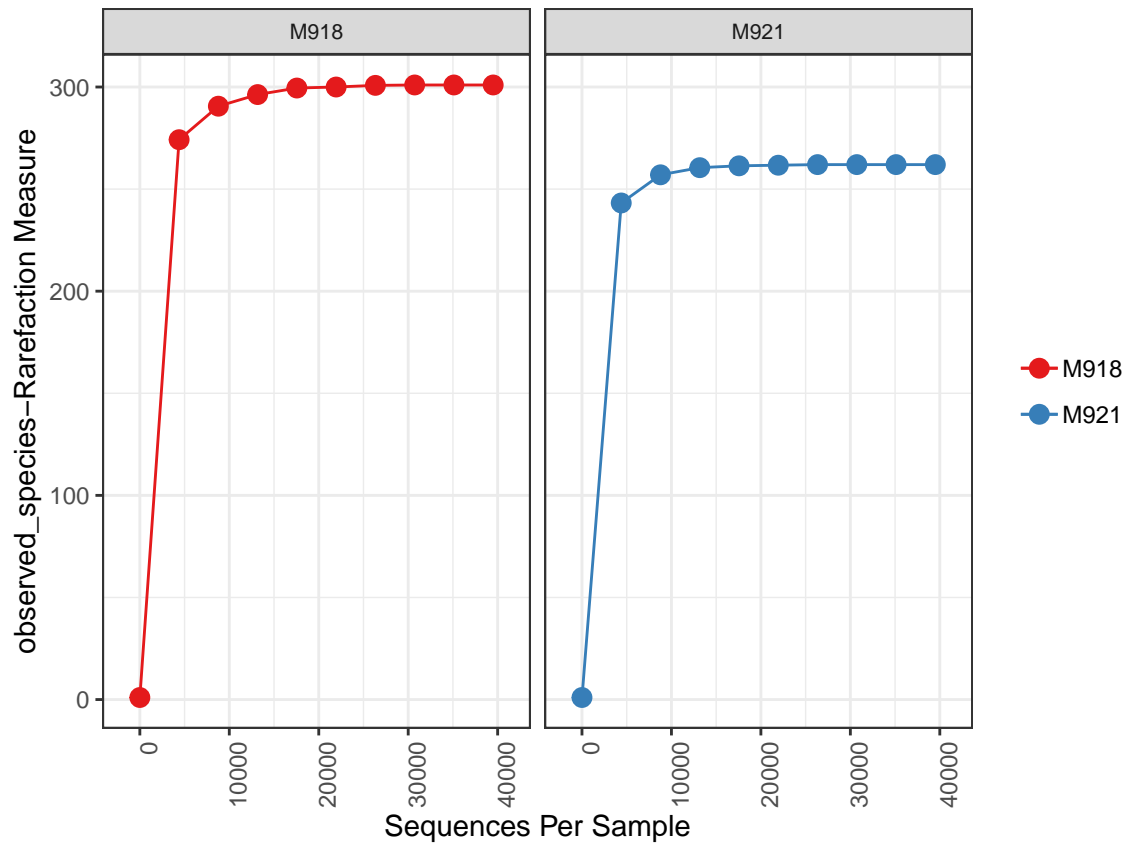


Figure 5: Observed Species Measure of Alpha Diversity- Rarefaction curve-Animal\_ID.

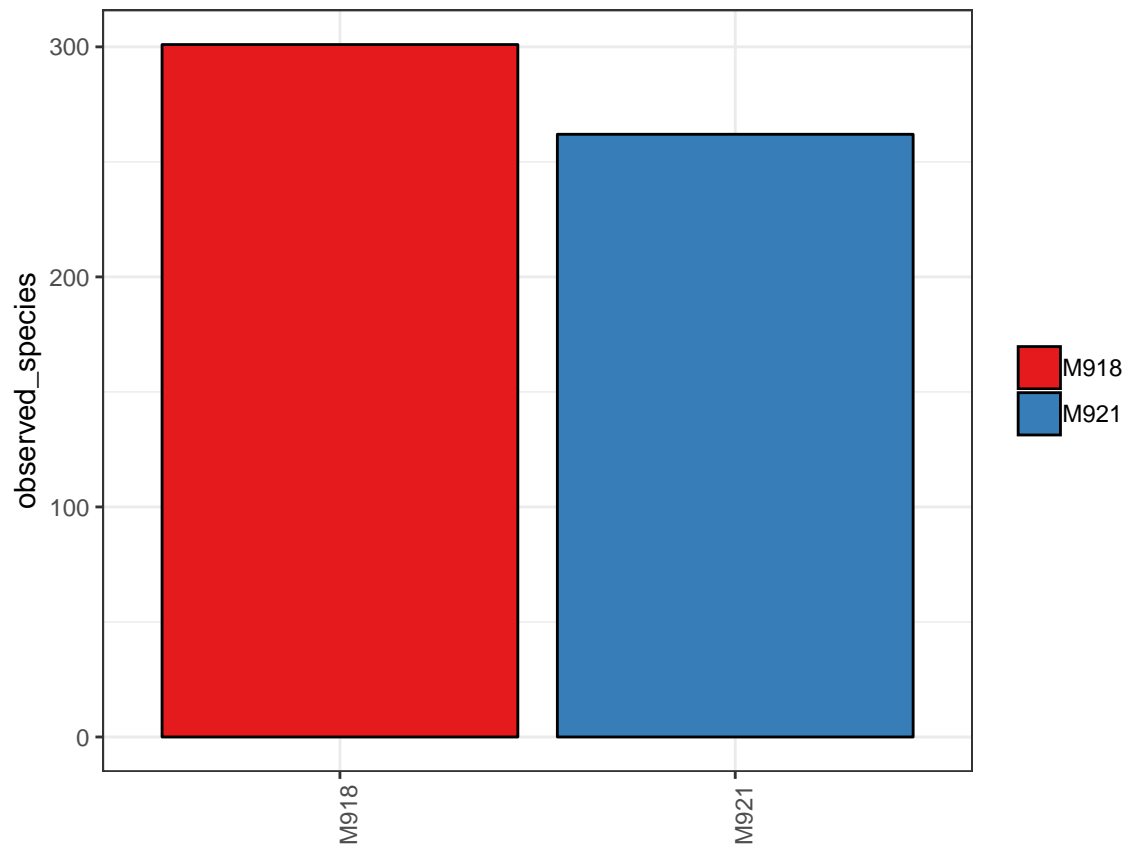


Figure 6: Observed Species Measure of Alpha Diversity- Boxplot-Animal\_ID.

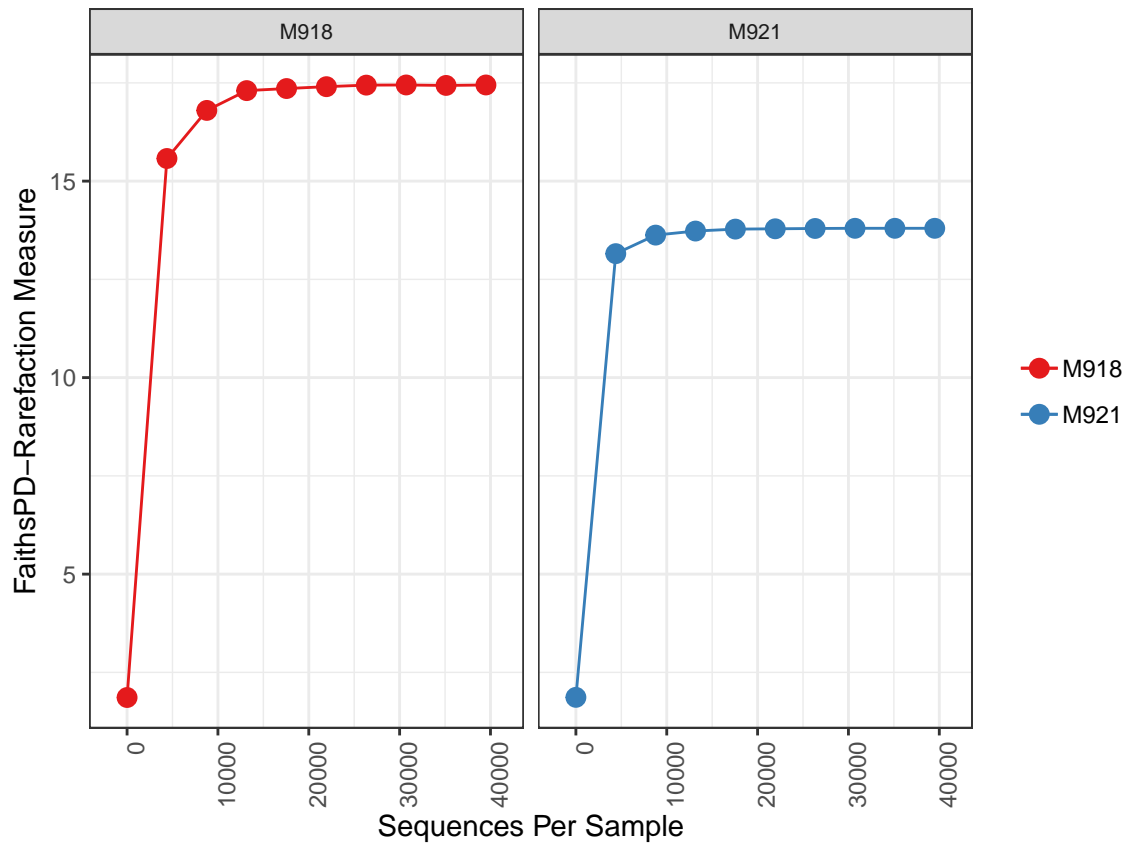


Figure 7: Faith's Phylogenetic Diversity Measure of Alpha Diversity- Rarefaction curve-Animal\_ID.

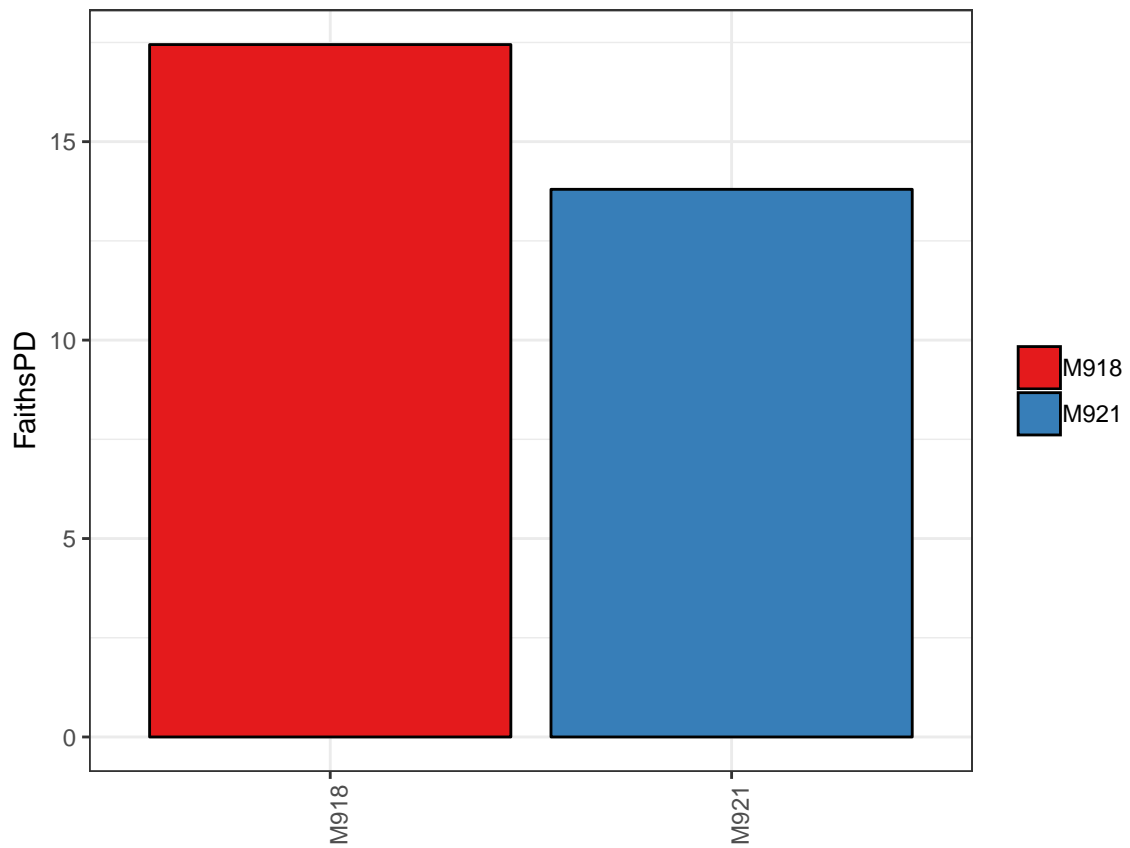


Figure 8: Faith's Phylogenetic Diversity Measure of Alpha Diversity- Boxplots-Animal\_ID.

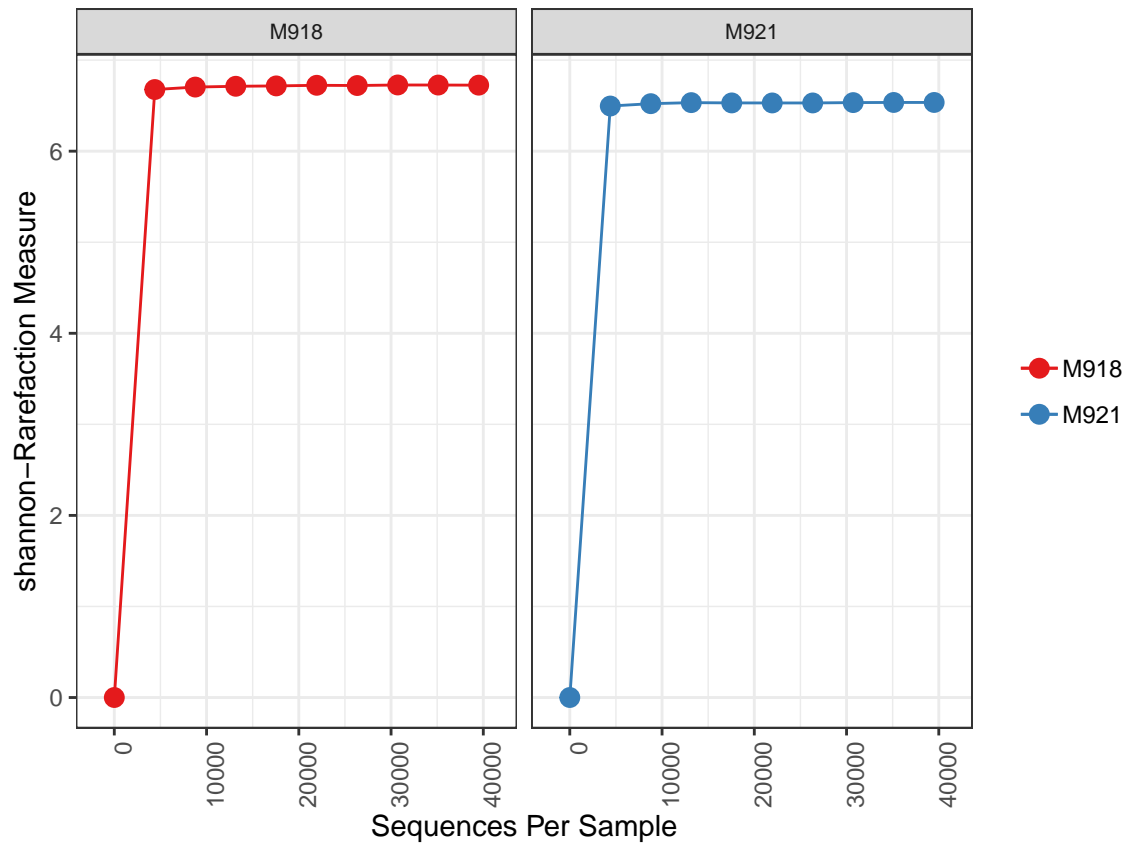


Figure 9: Shannon Measure of Alpha Diversity- Rarefaction curve-Animal\_ID.

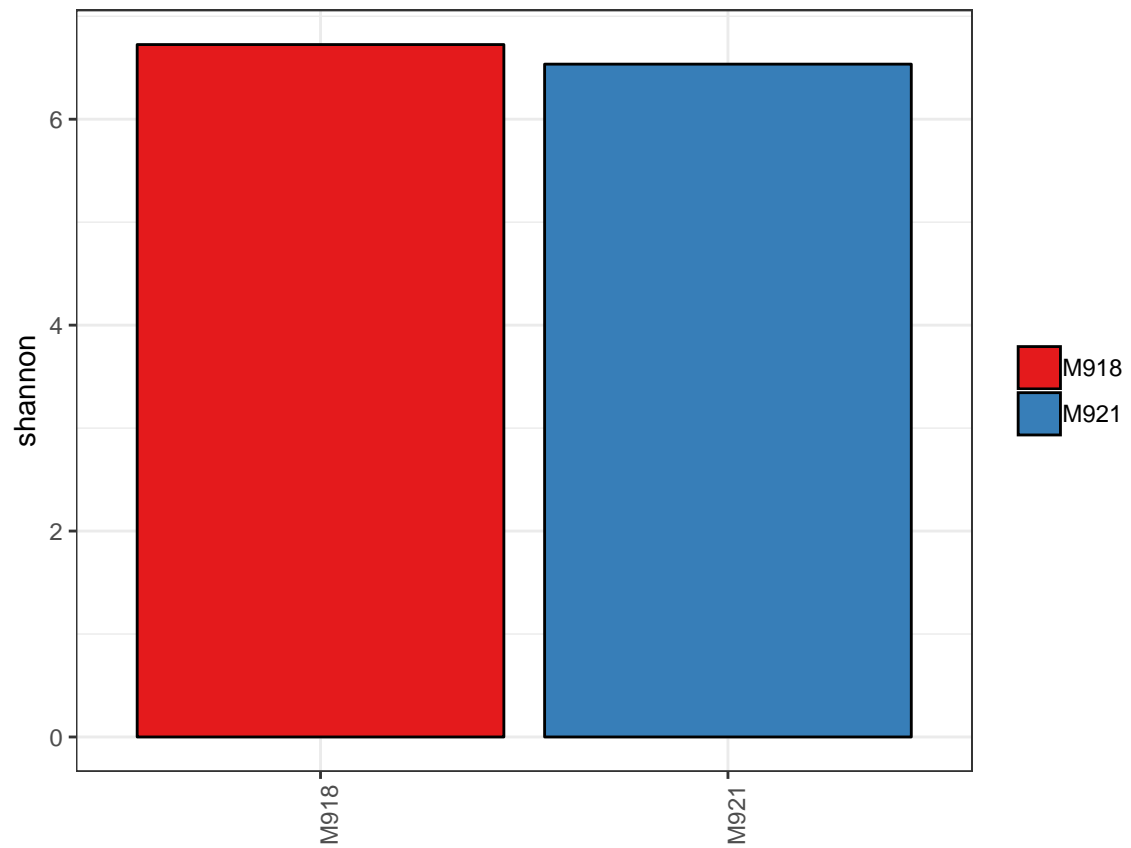


Figure 10: Shannon Measure of Alpha Diversity- Boxplots-Animal\_ID.

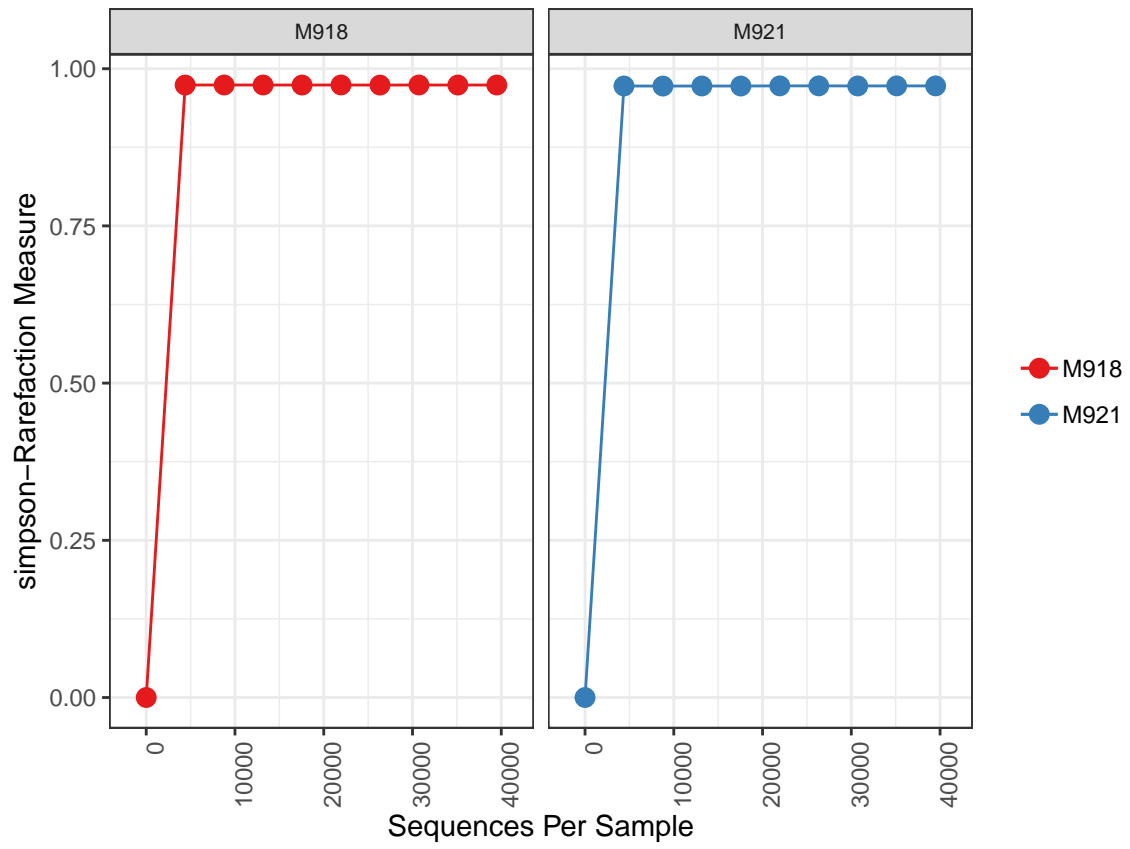


Figure 11: Simpson Measure of Alpha Diversity- Rarefaction curve-Animal\_ID.

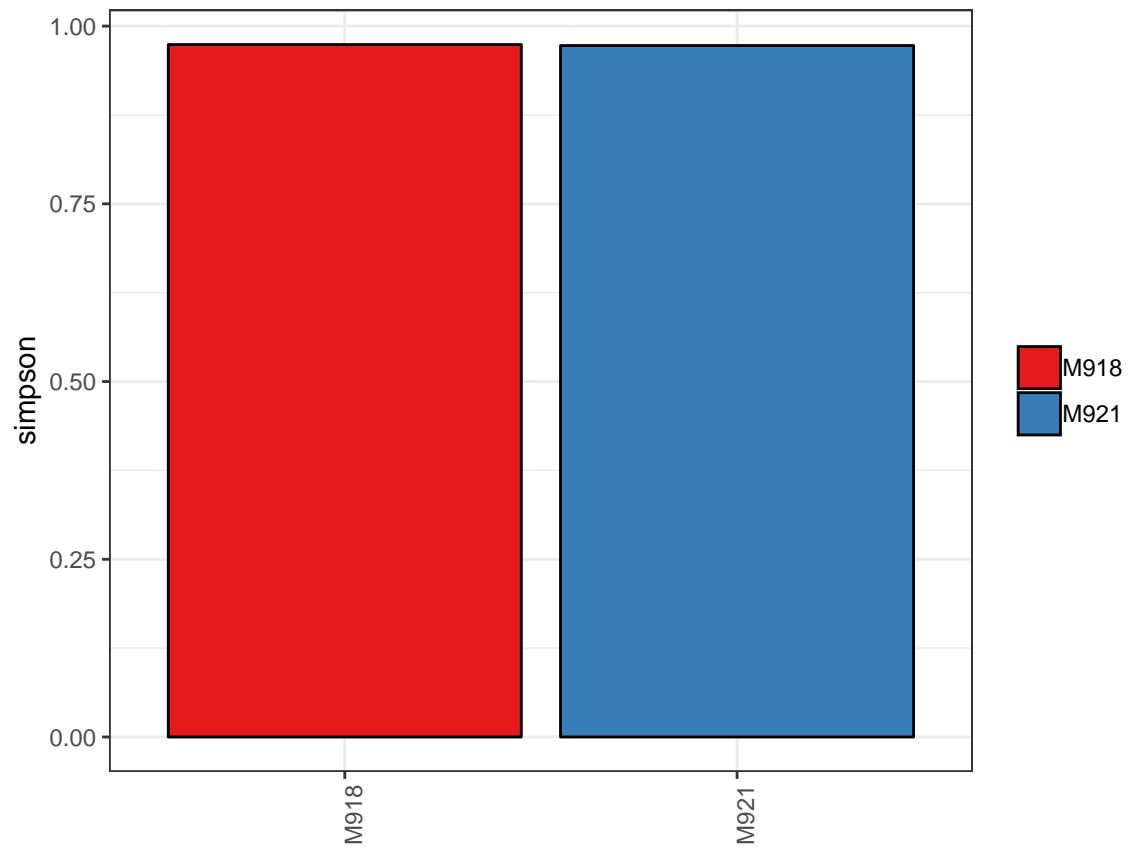


Figure 12: Simpson Measure of Alpha Diversity- Boxplots-Animal\_ID.



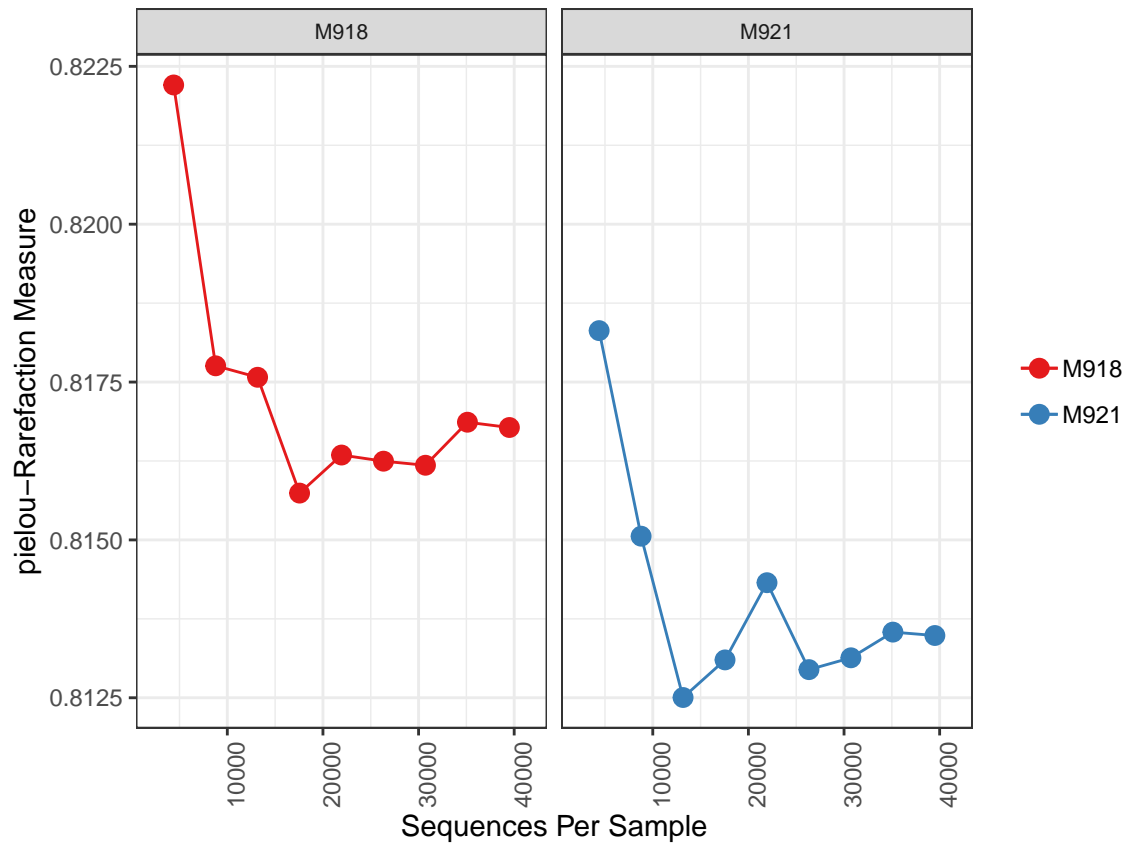


Figure 13: Pielou's Evenness Measure of Alpha Diversity- Rarefaction curve-Animal\_ID.

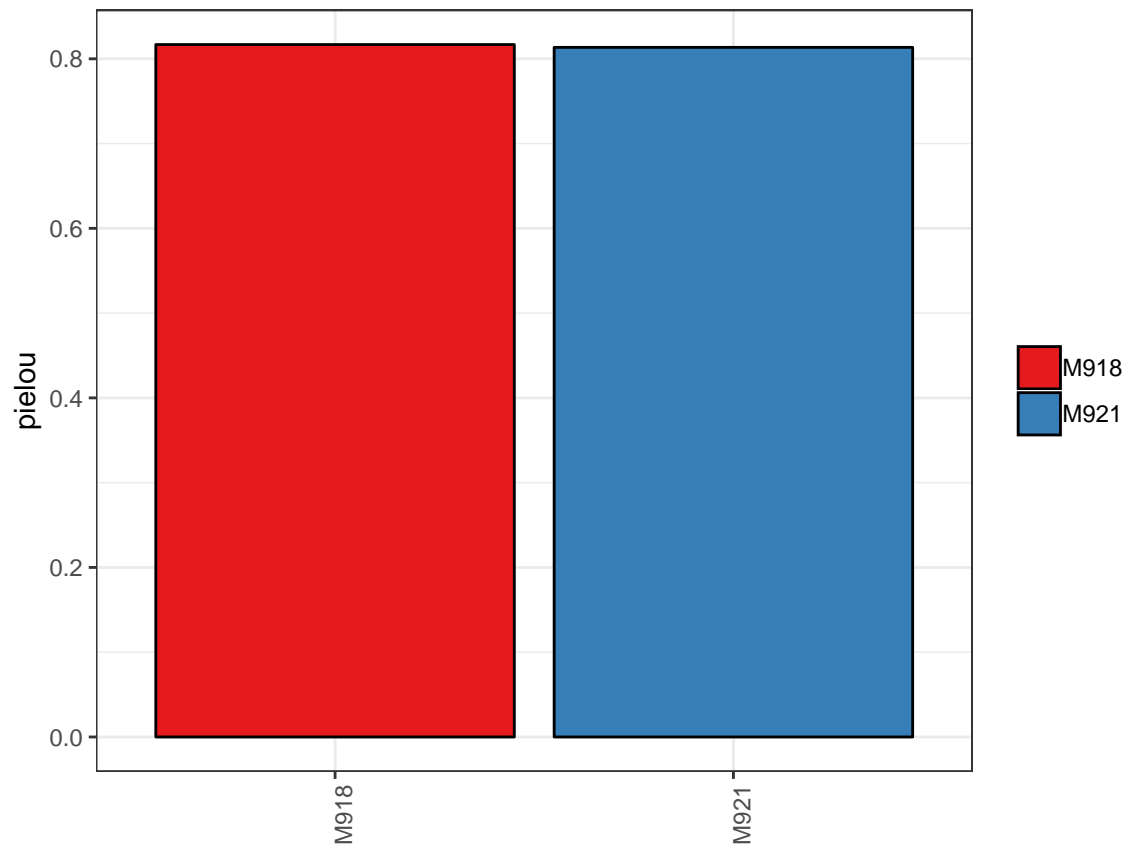


Figure 14: Pielou's Evenness Measure of Alpha Diversity- Boxplots-Animal\_ID.

### 2.2.2 Beta Diversity

Beta diversity is the diversity between different treatment groups. Beta diversity analyses include the phylogenetic measure of unweighted and weighted UniFrac distances and the non-phylogenetic Bray-Curtis and Jaccard methods. Principal coordinates analysis (PCoA) of the first 2 coordinates of the distance matrices are plotted. The shaded ellipse depicts the 95% confidence interval. For more information on the UniFrac distance metric for community comparisons, please see (Lozupone et al., 2011). Comparing the sample beta diversity was performed with PERMANOVA (anderson).

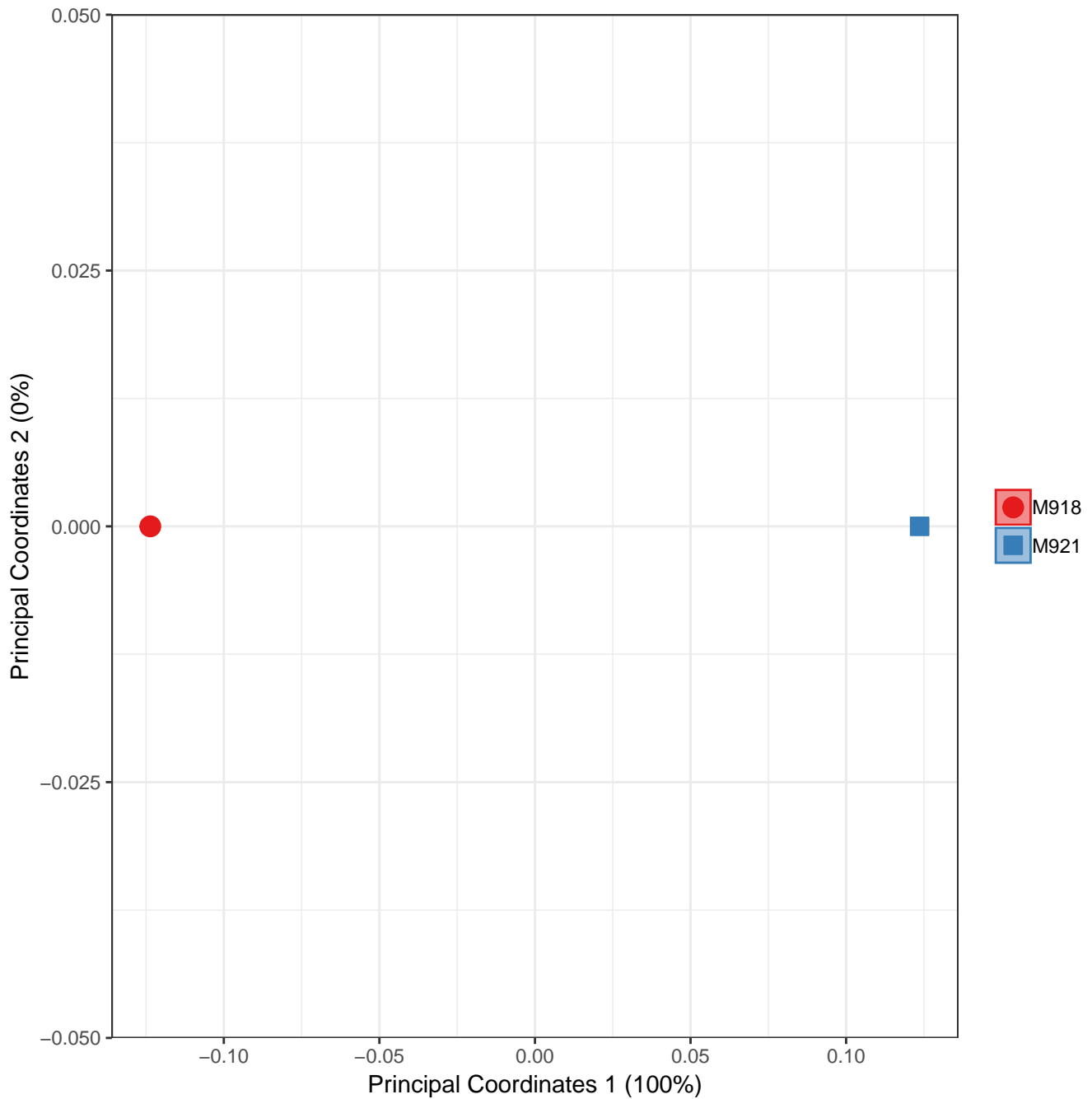


Figure 15: Unweighted Unifrac Measure of Beta Diversity-Animal\_ID.

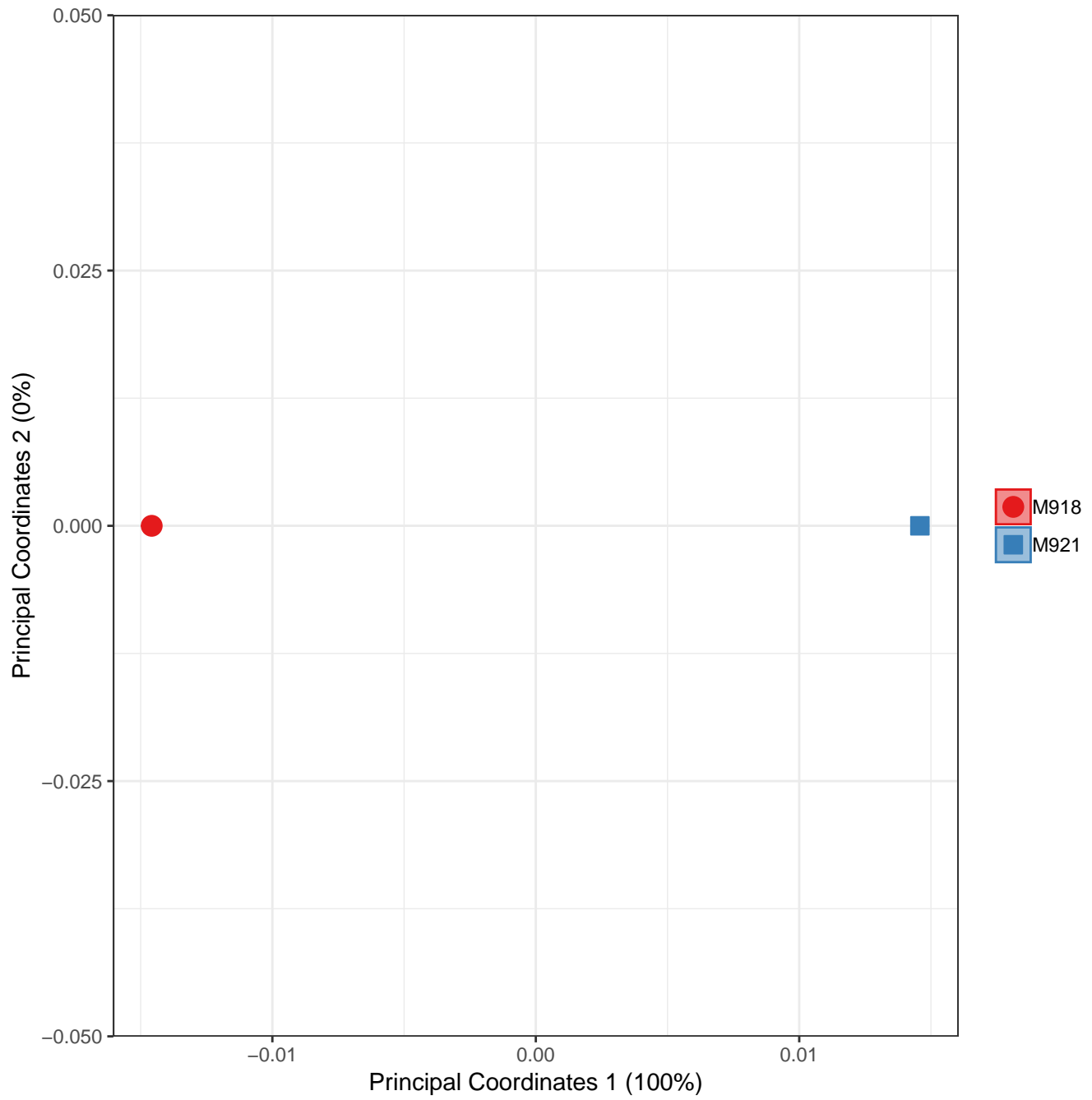


Figure 16: Weighted Unifrac Measure of Beta Diversity-Animal\_ID.

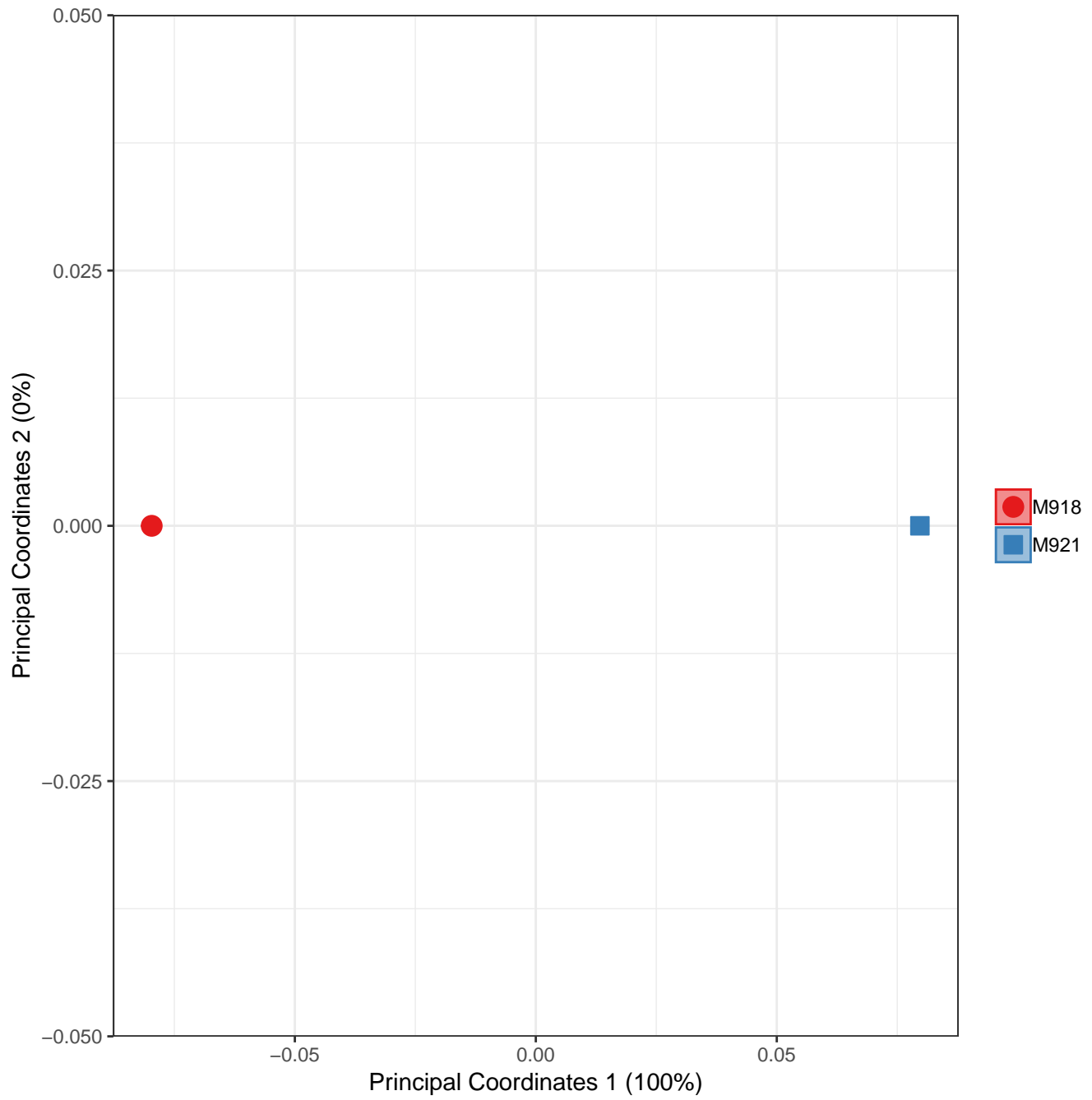


Figure 17: Bray-Curtis Measure of Beta Diversity-Animal\_ID.

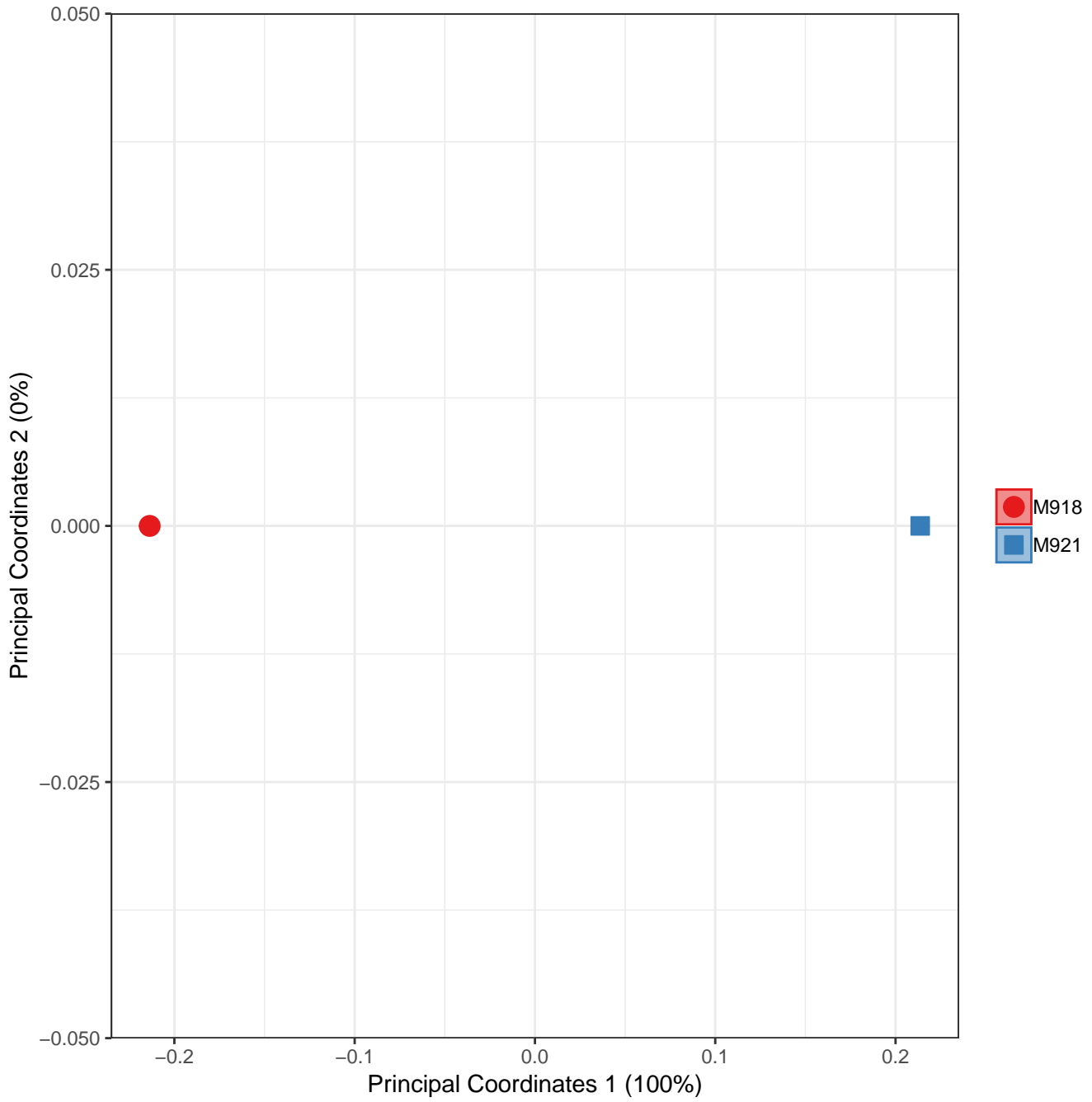


Figure 18: Jaccard Measure of Beta Diversity-Animal\_ID.

### 2.3 Data analysis using taxa abundance data

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### 2.3.1 Stacked bar graphs of Taxa abundances at each level

Sequence counts are converted to relative abundance (% abundance). Taxa abundance data is presented in 3 ways: unfiltered, filtered, and filtered/rescaled. "Unfiltered" is the unmanipulated taxa abundance data. "Filtered" data removes low abundance taxa (mean of any group < 0.05%) and genera not present in at least 50% of samples within an experimental group. Filtering out taxa will then reduce the overall abundance to less than 100%. "Filtered /Rescaled" renormalizes the filtered data back to 100% after the low abundance taxa are removed.

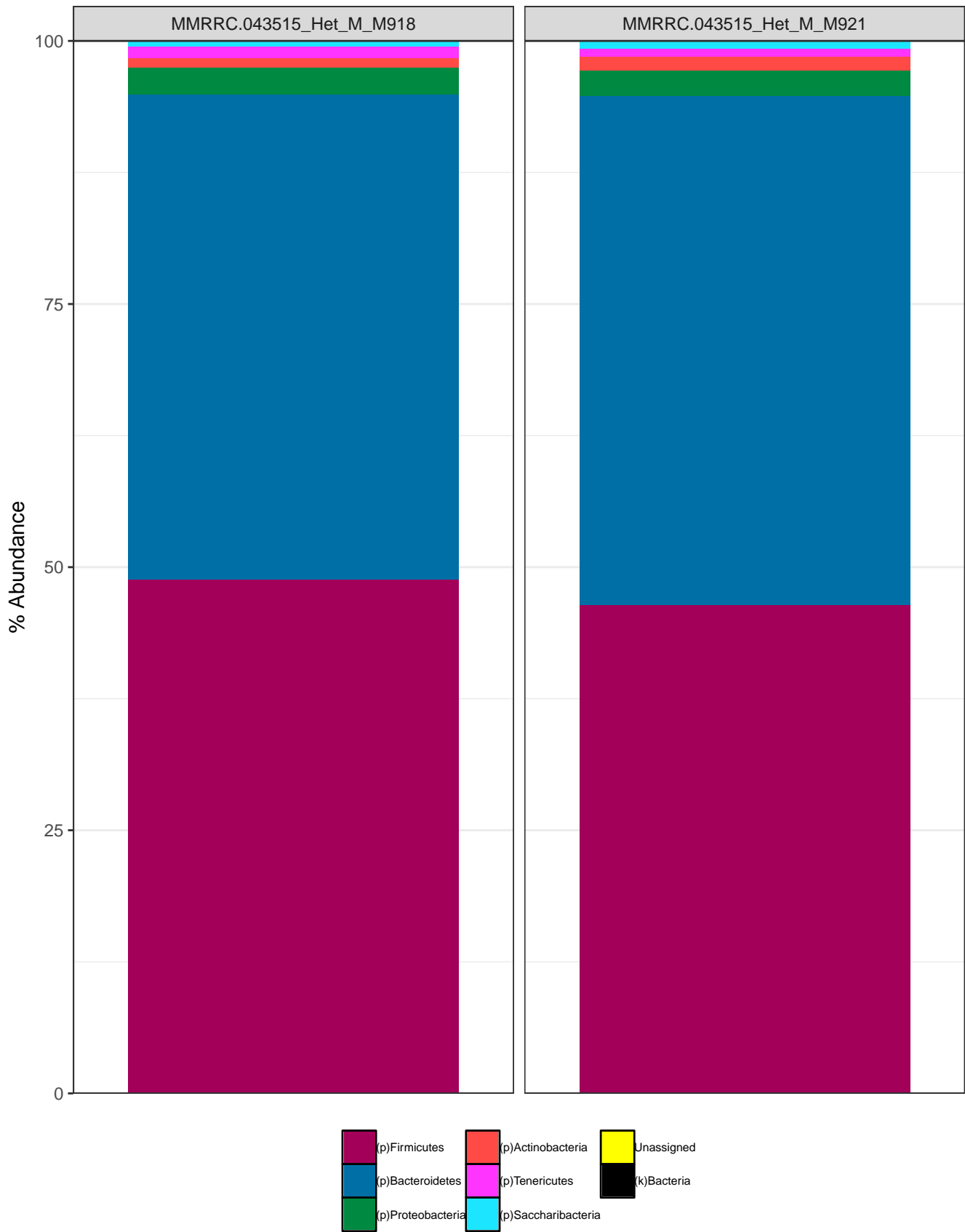


Figure 19: Phylum level abundances-UNFILTERED

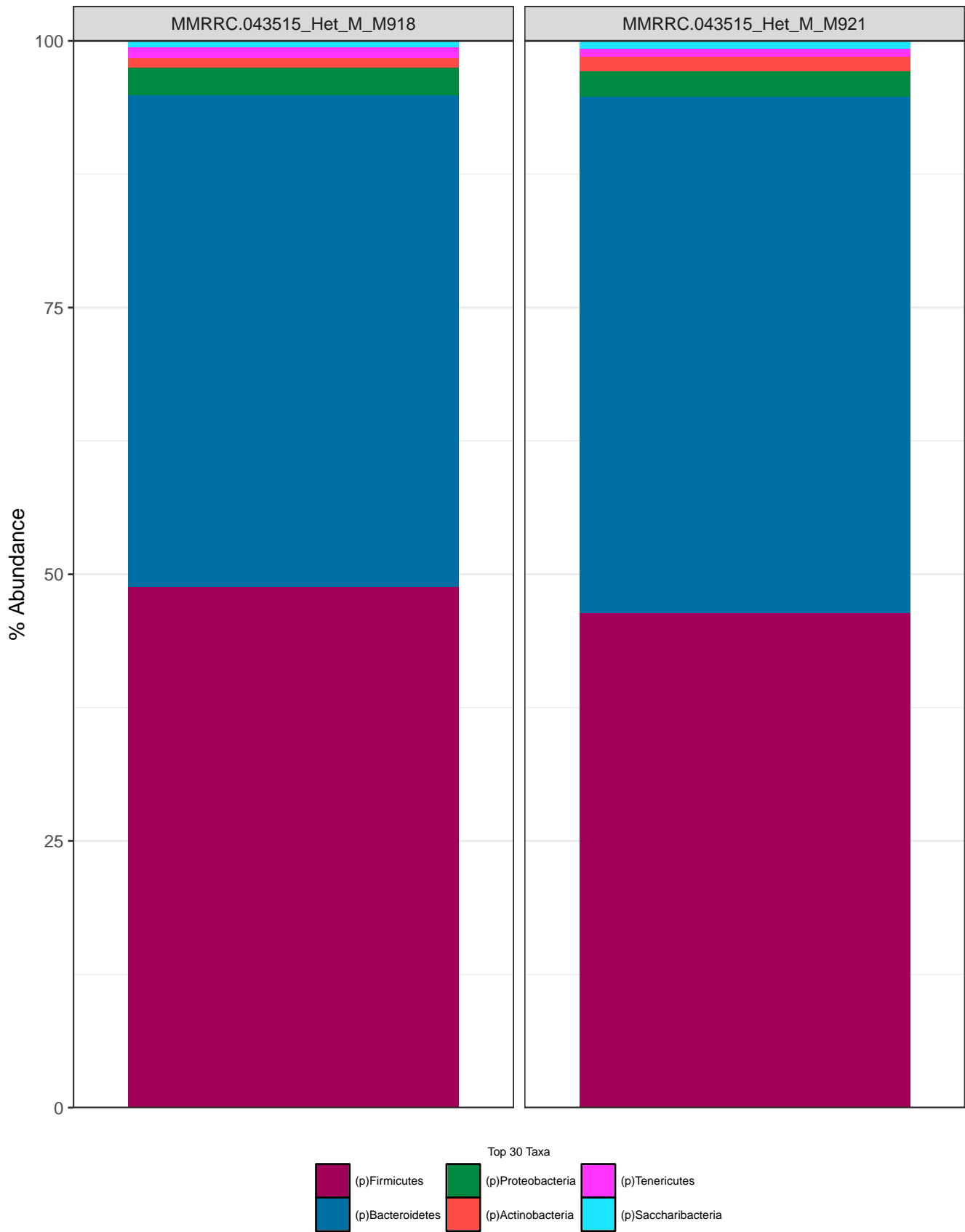


Figure 20: Phylum level abundances-FILTERED: 2 phylum level taxa removed



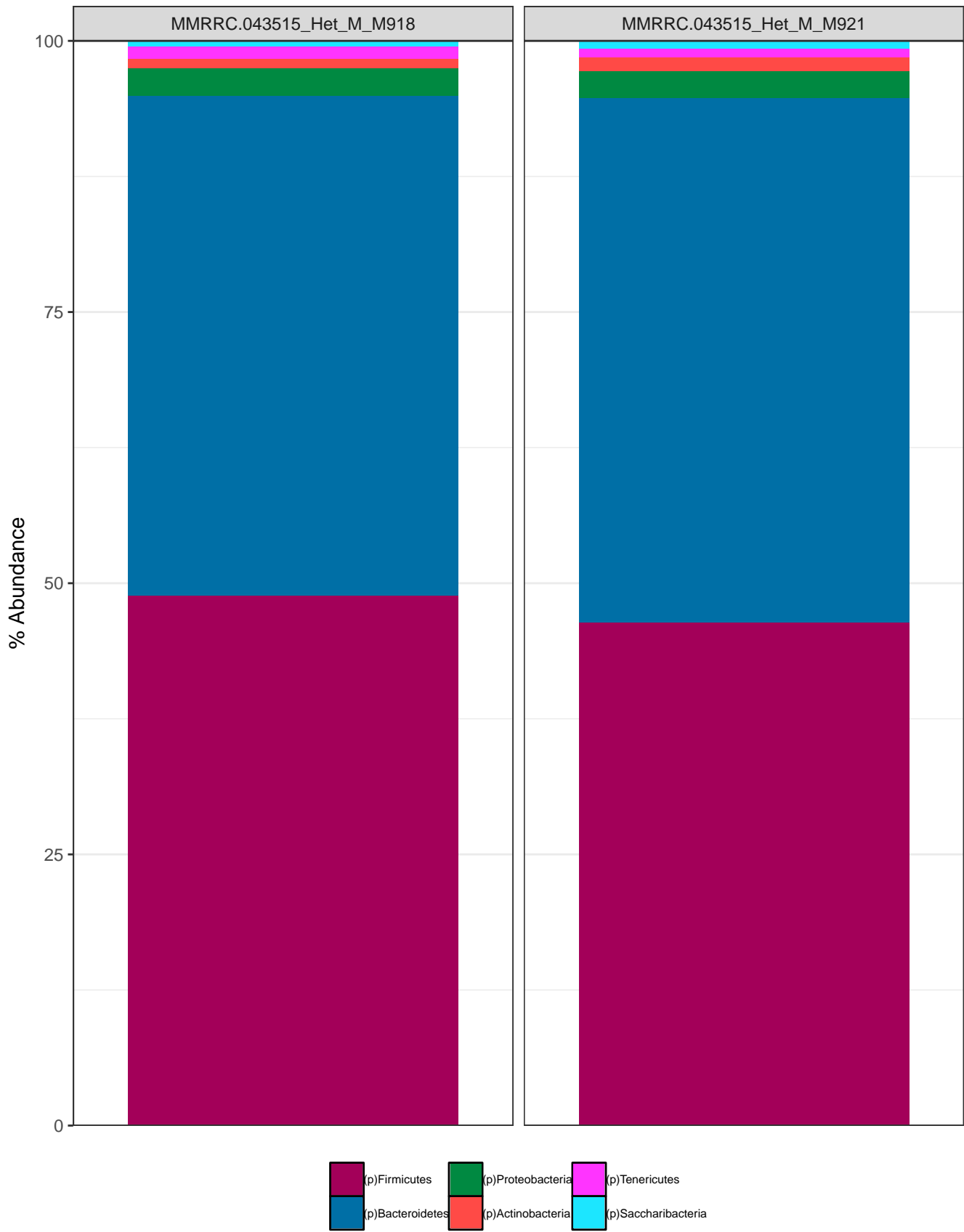


Figure 21: Phylum level abundances-FILTERED & RESCALED

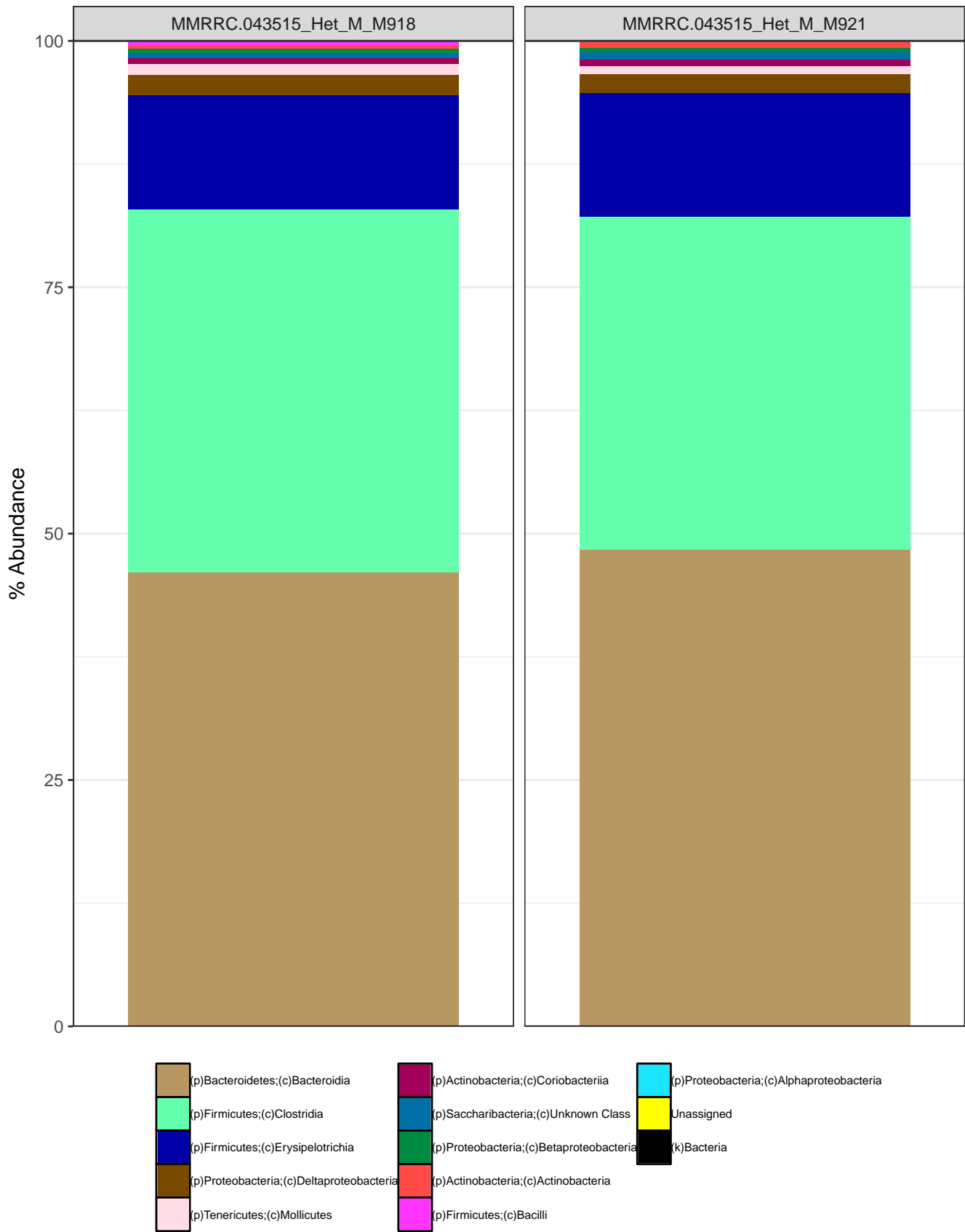


Figure 22: Class level abundances-UNFILTERED

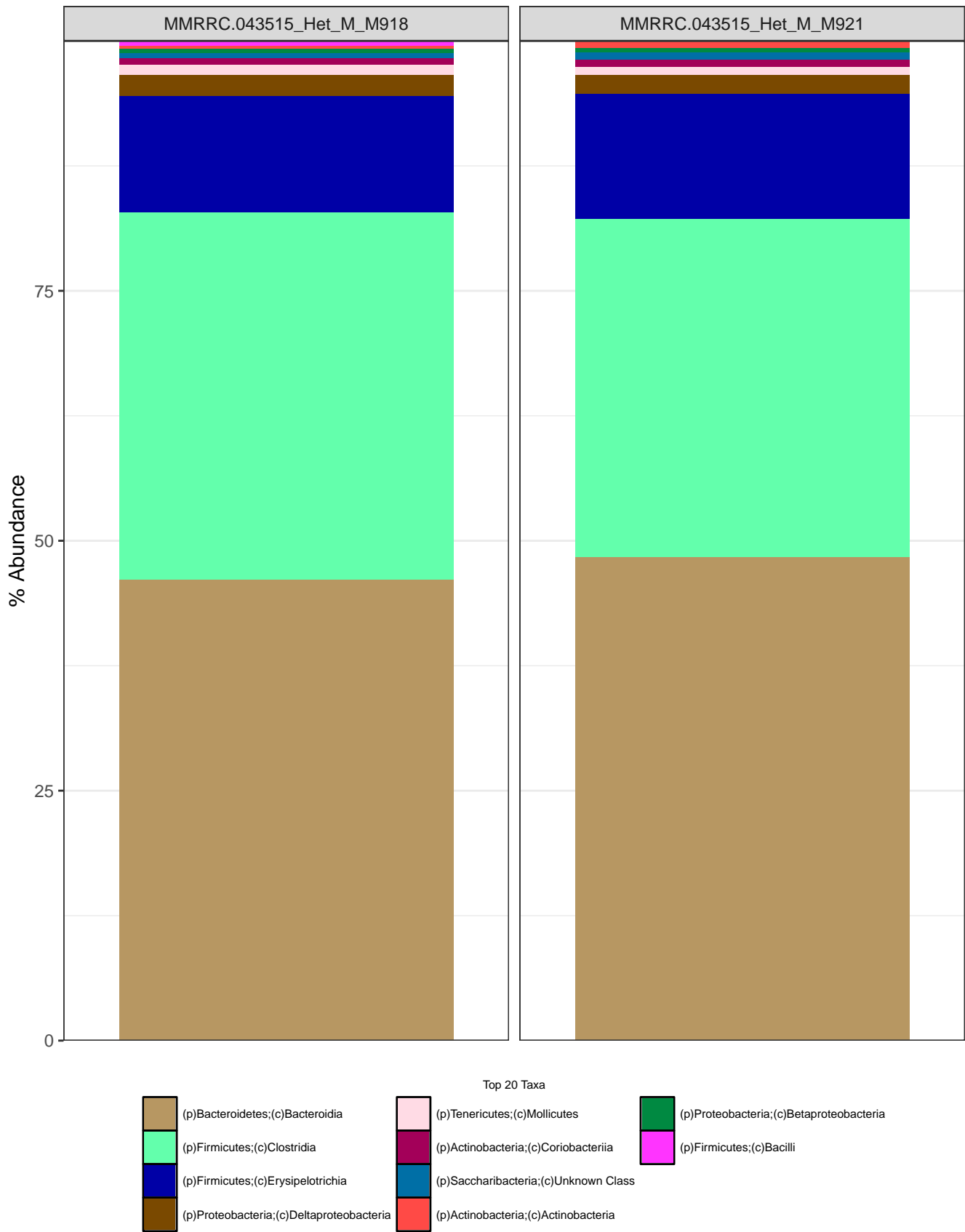


Figure 23: Class level abundances-FILTERED: 3 class level taxa removed

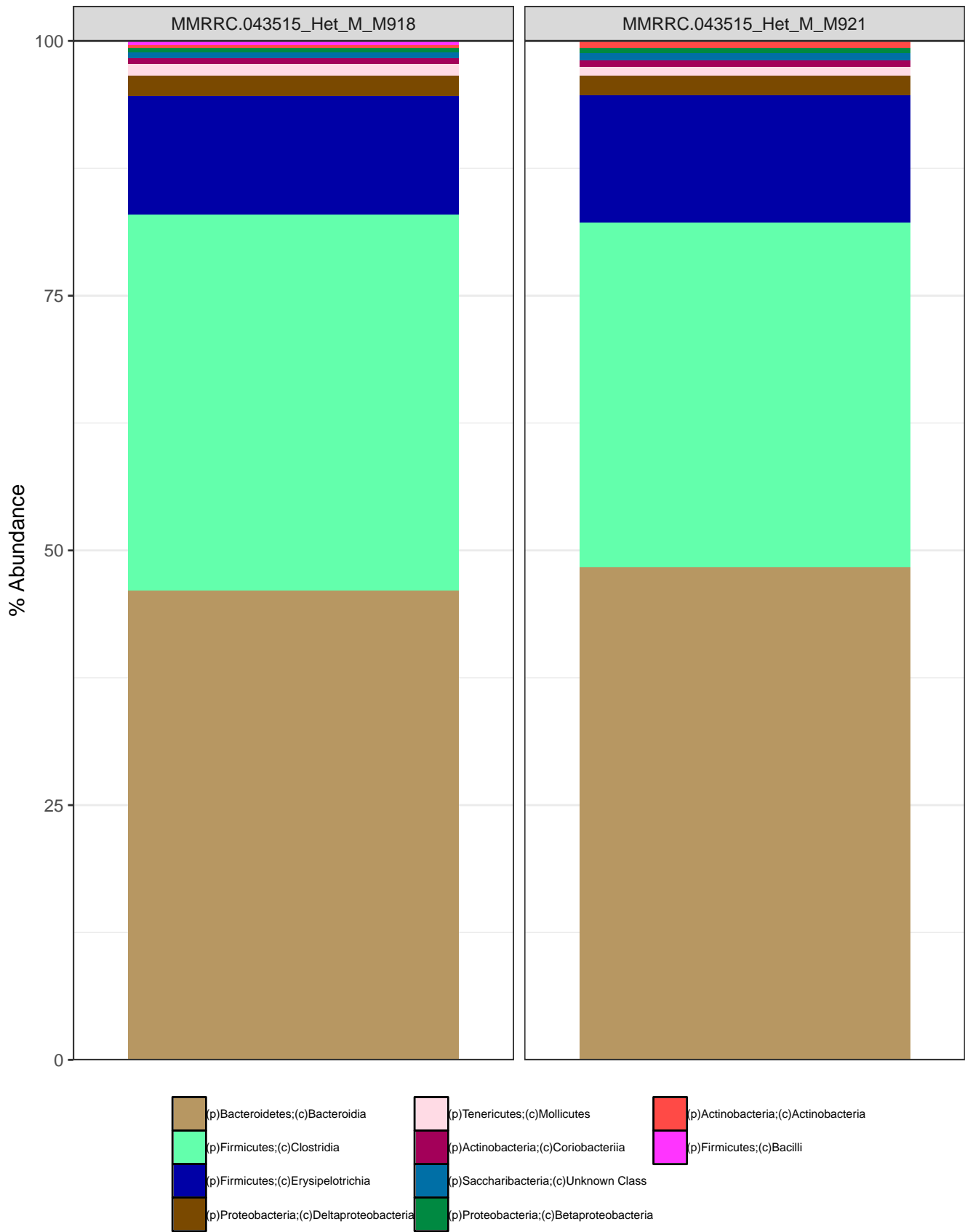


Figure 24: Class level abundances-FILTERED & RESCALED

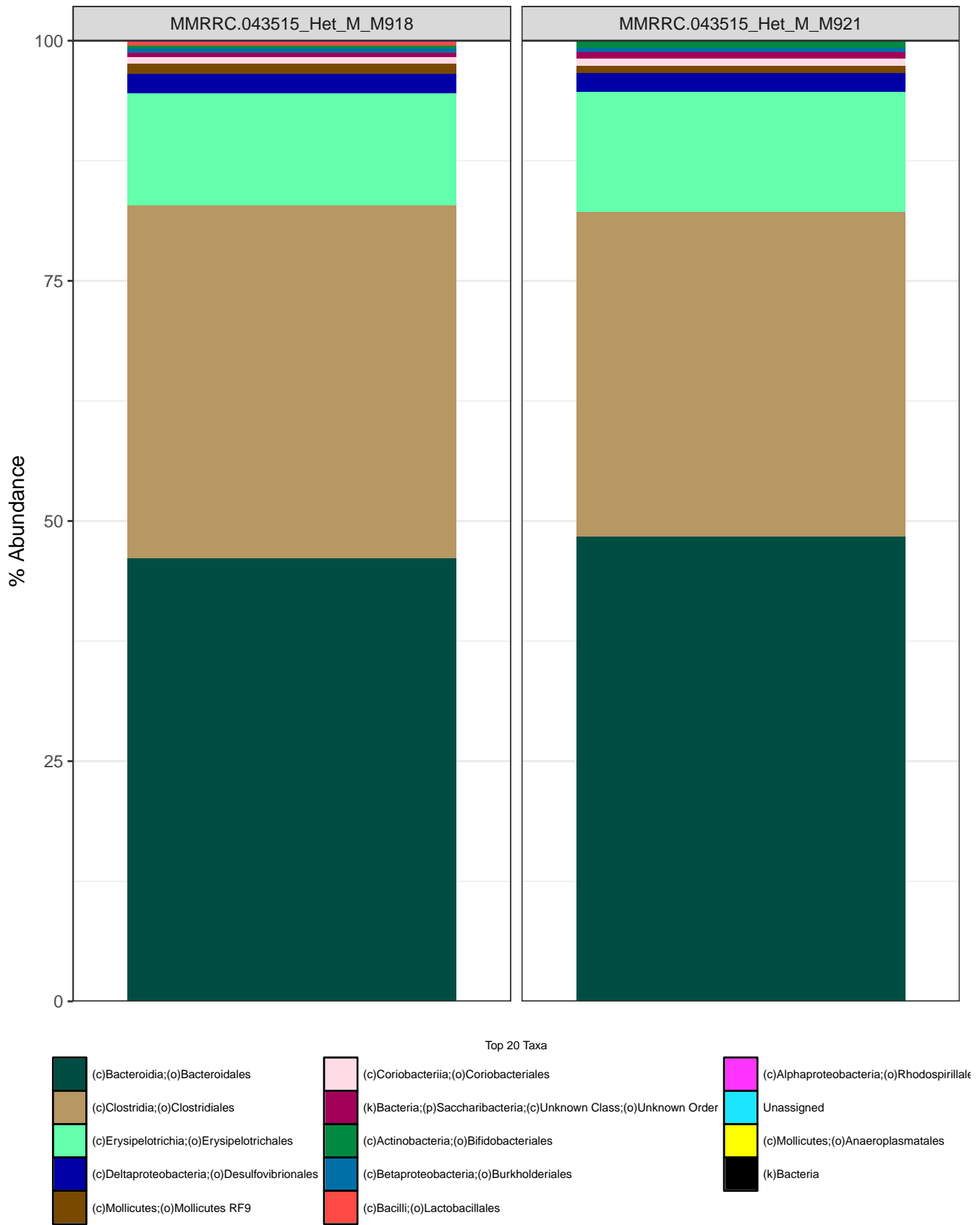


Figure 25: Order level abundances-UNFILTERED

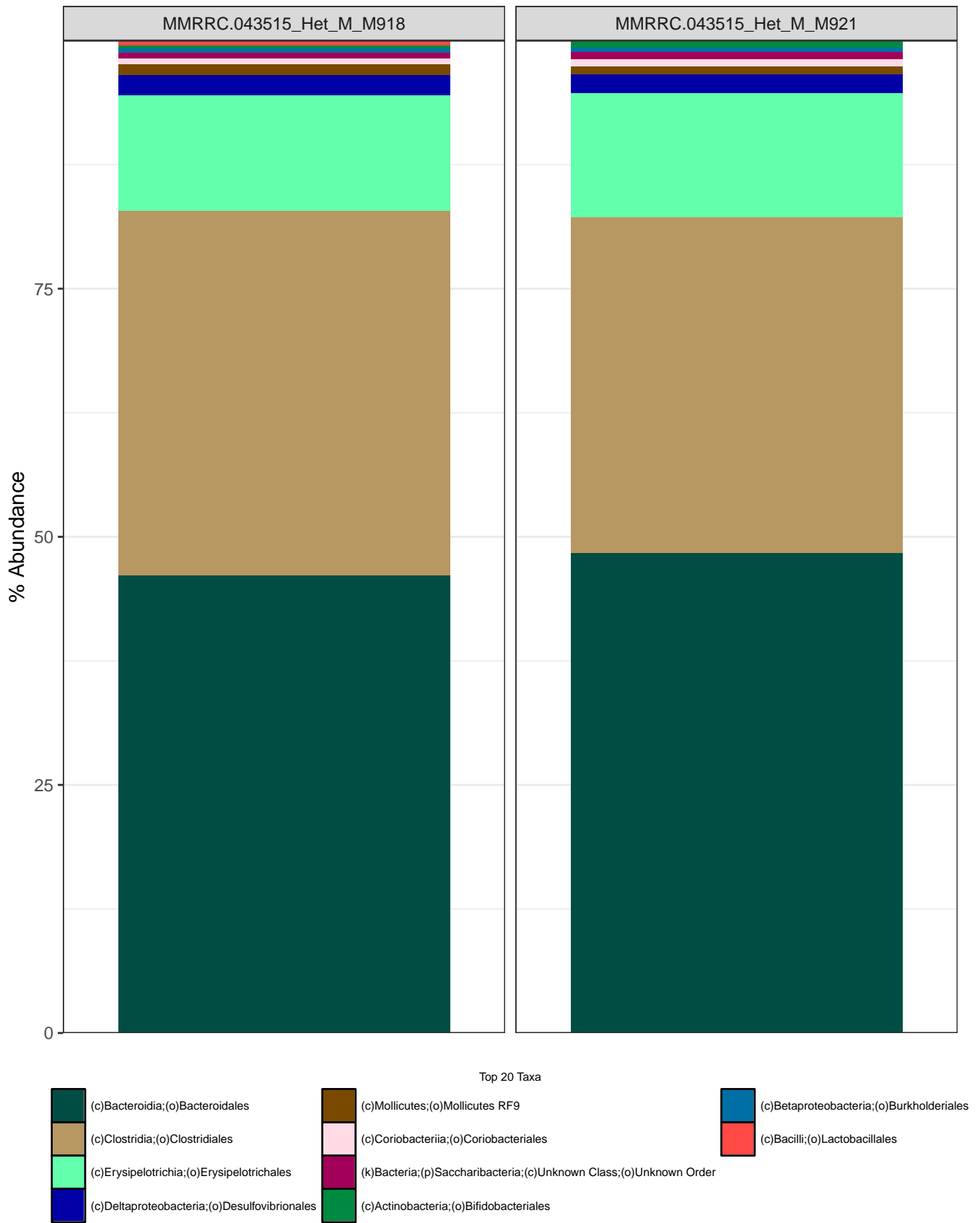


Figure 26: Order level abundances-FILTERED: 4 order level taxa removed

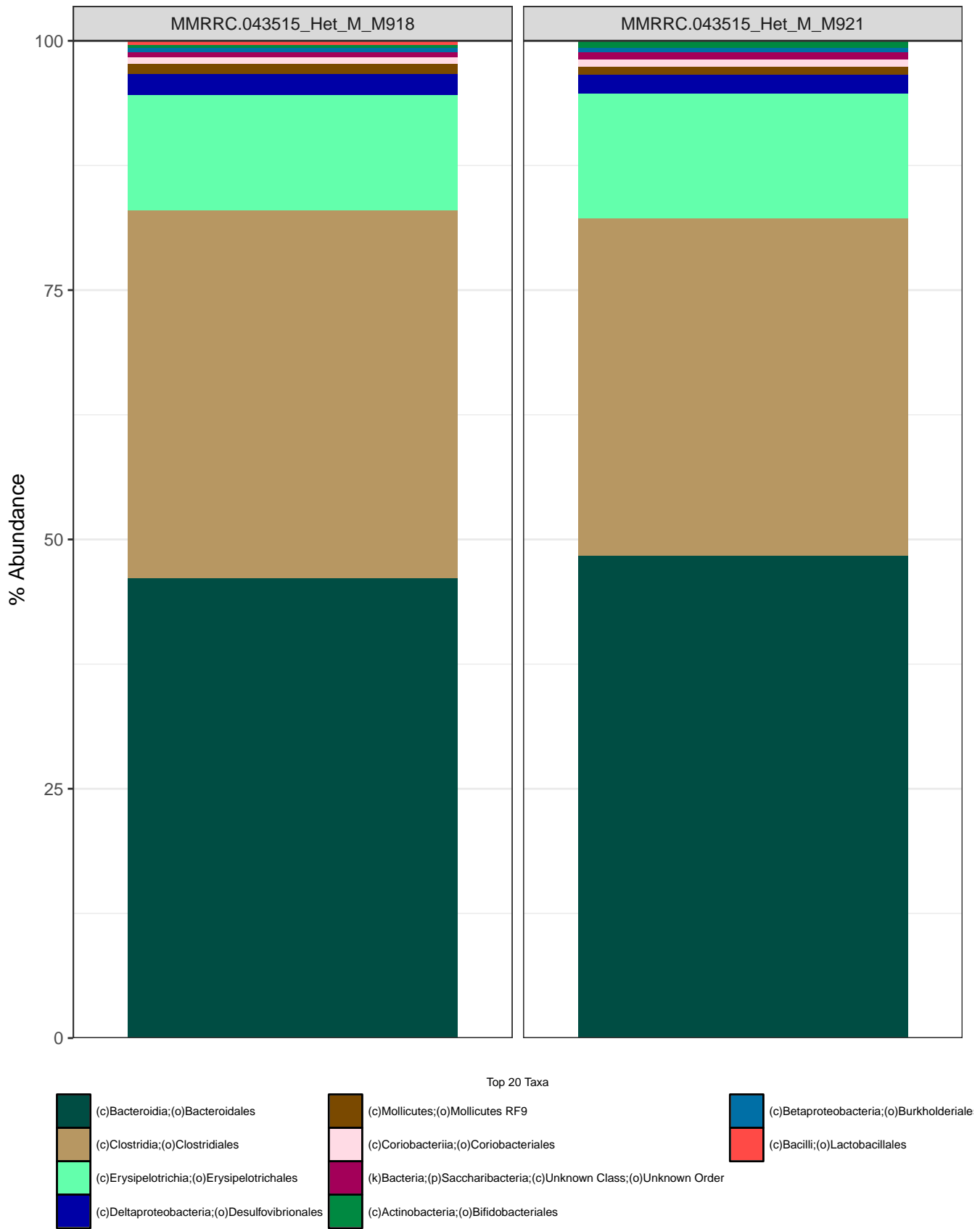


Figure 27: Order level abundances-FILTERED & RESCALED

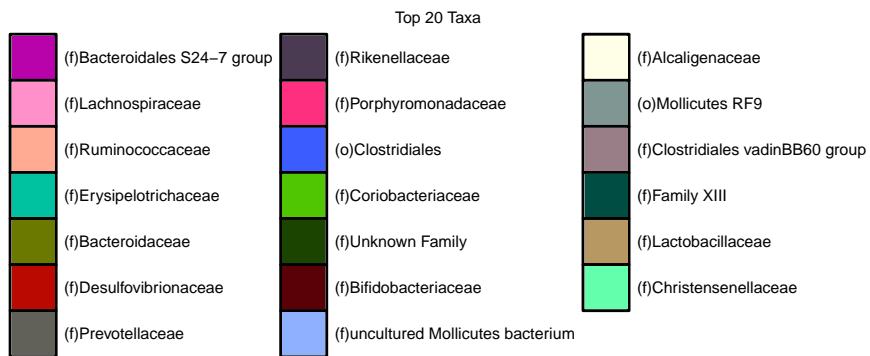
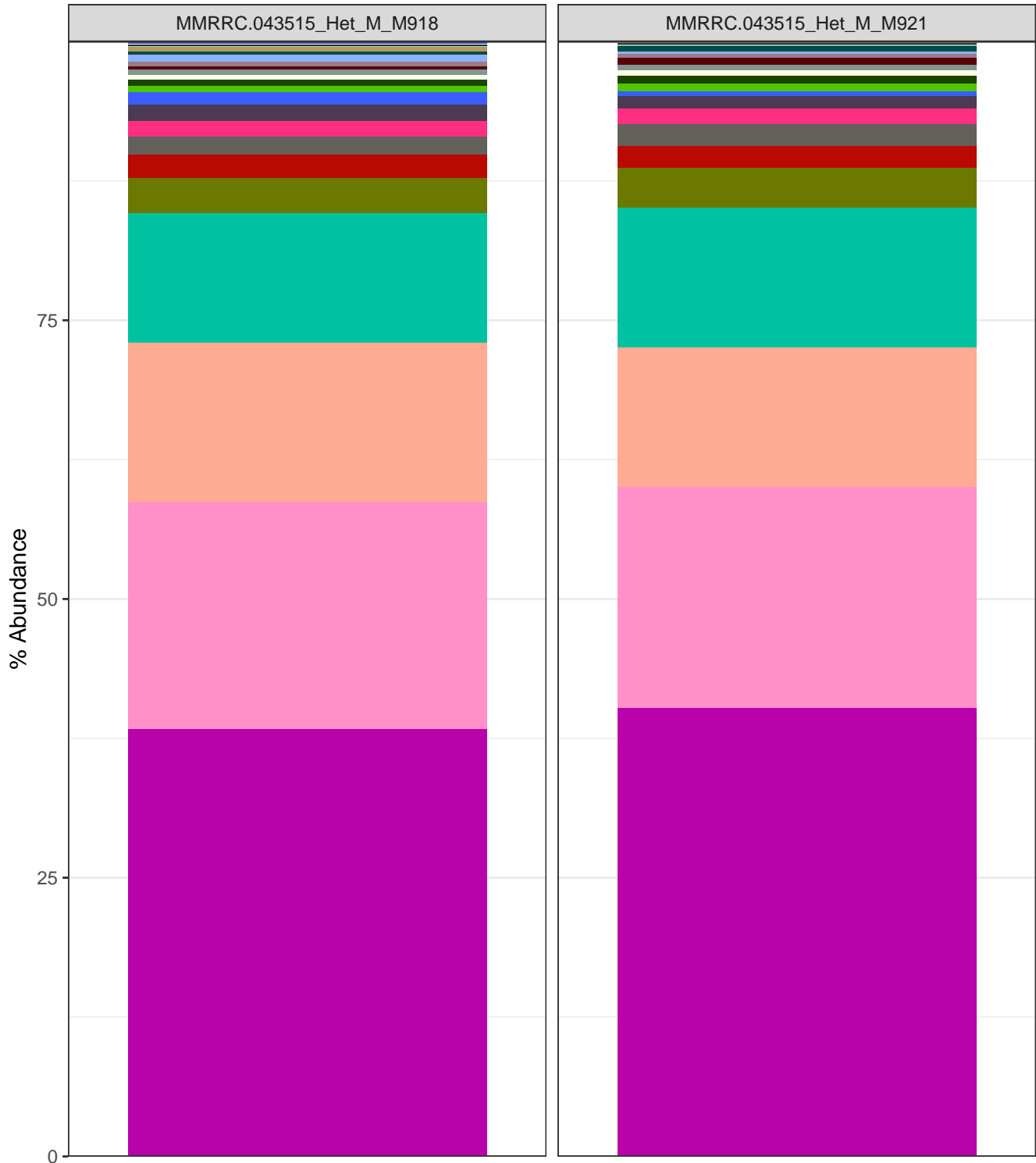


Figure 28: Family level abundances-UNFILTERED



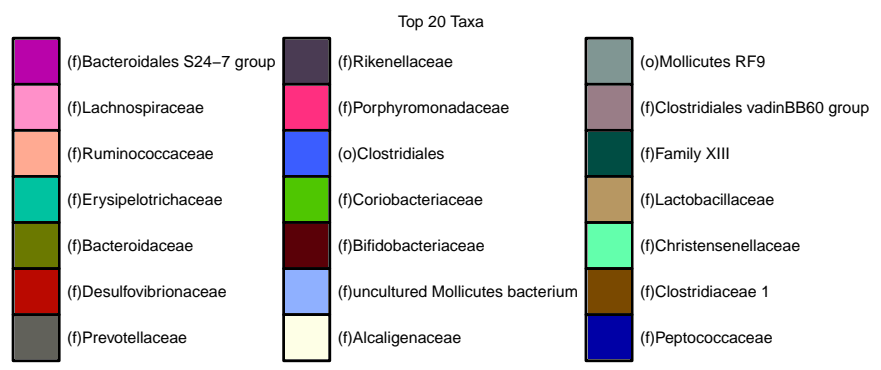
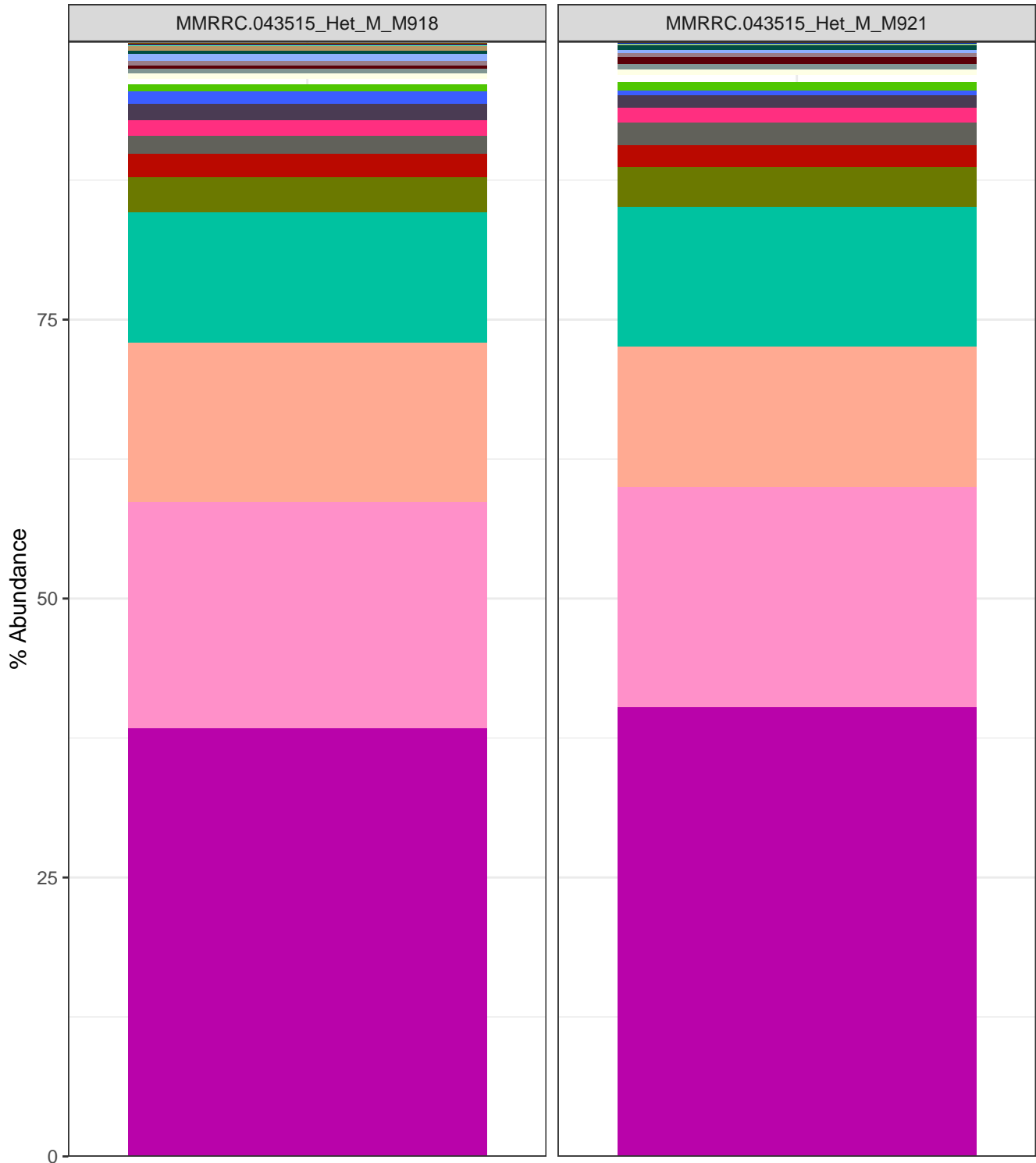


Figure 29: Family level abundances-FILTERED: 10 family level taxa removed

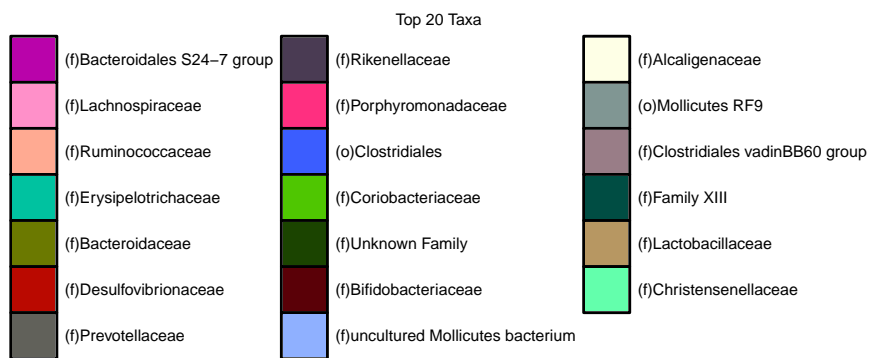
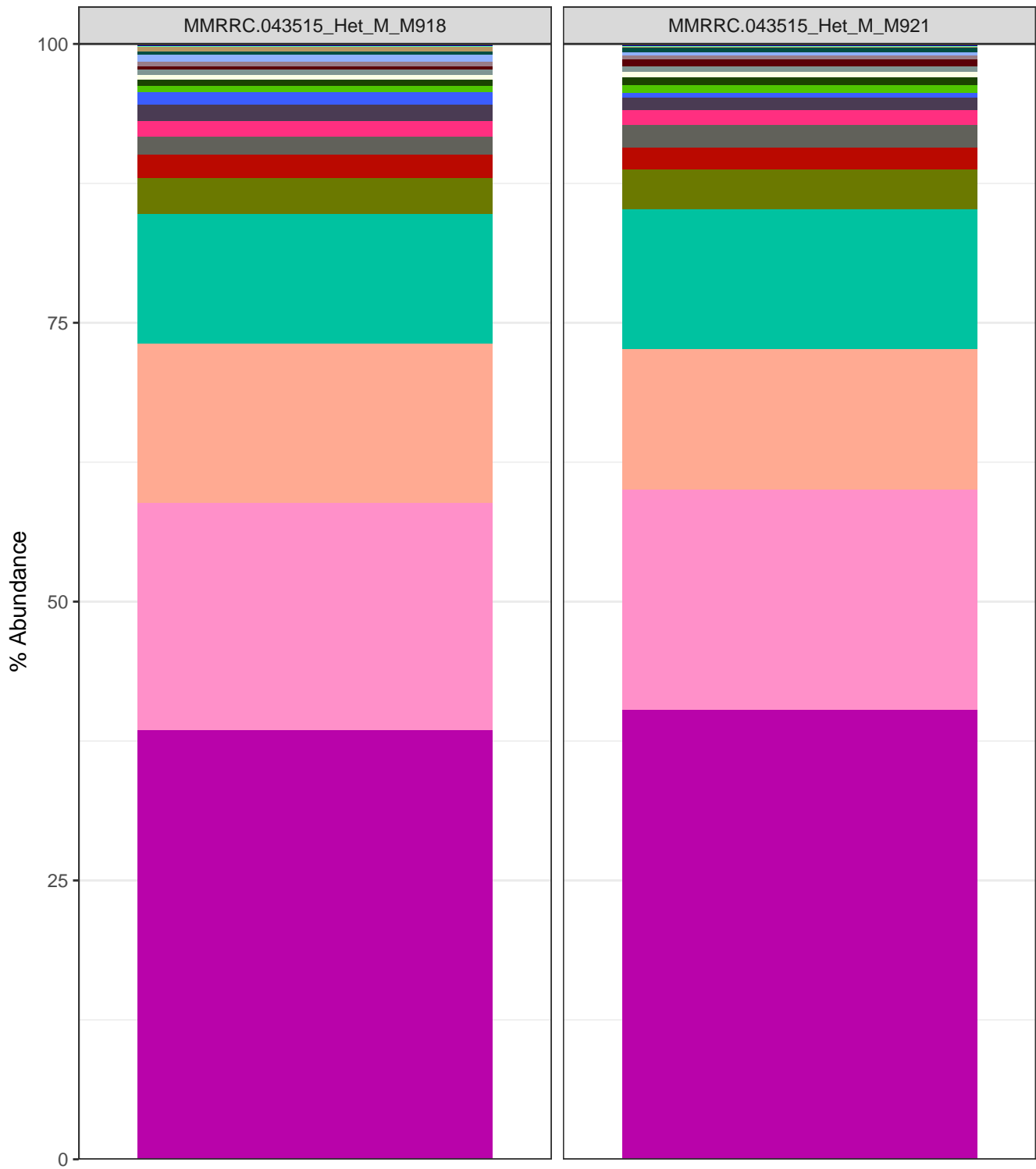


Figure 30: Family level abundances-FILTERED & RESCALED

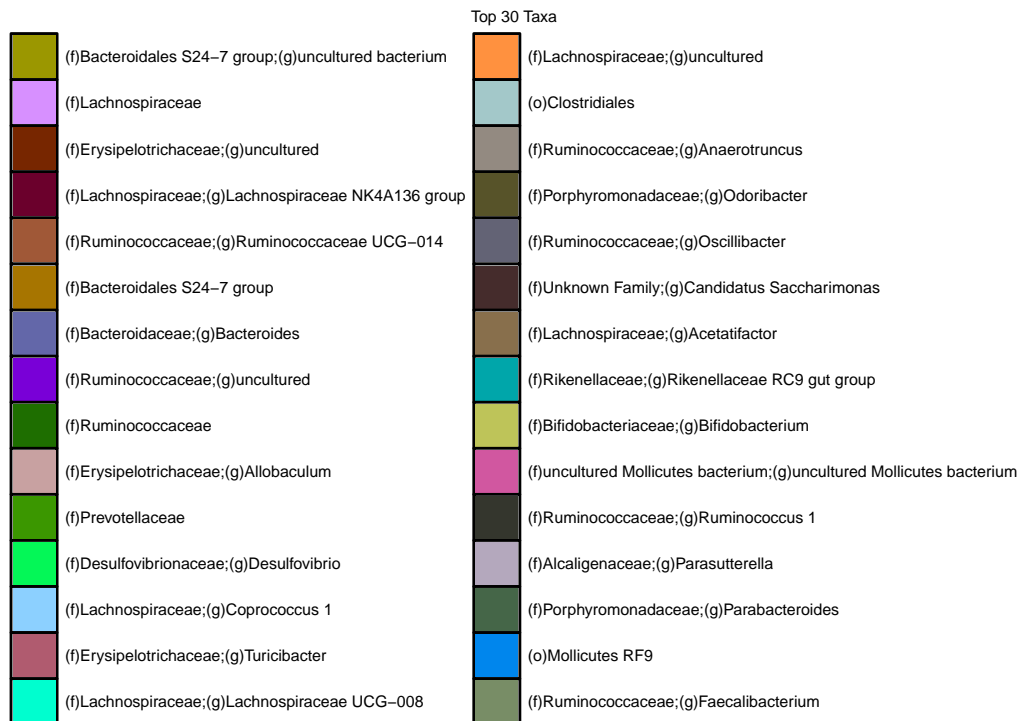
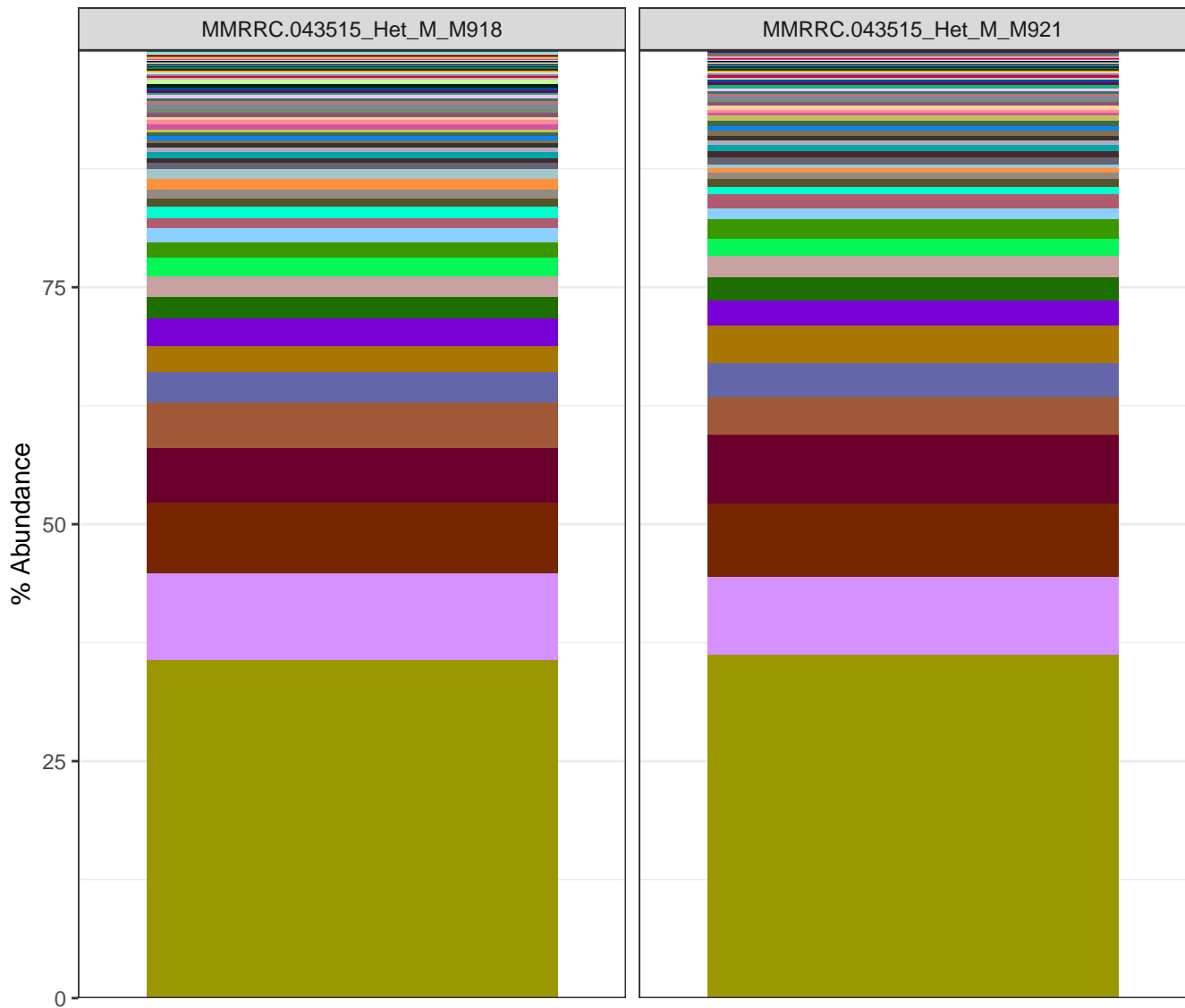


Figure 31: Genus level abundances-UNFILTERED

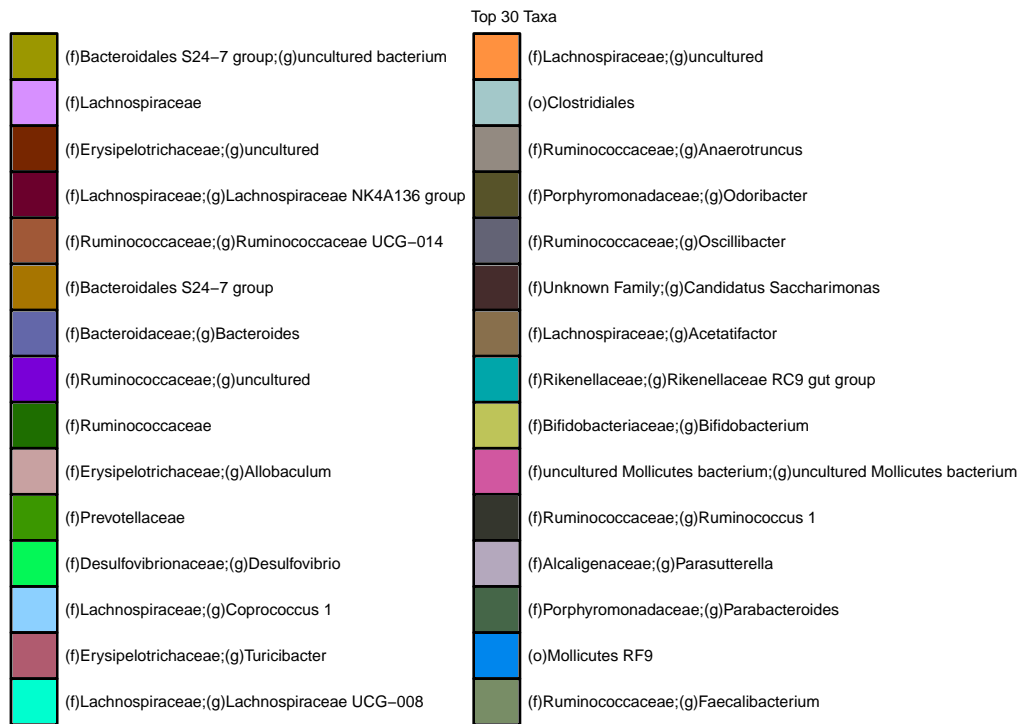
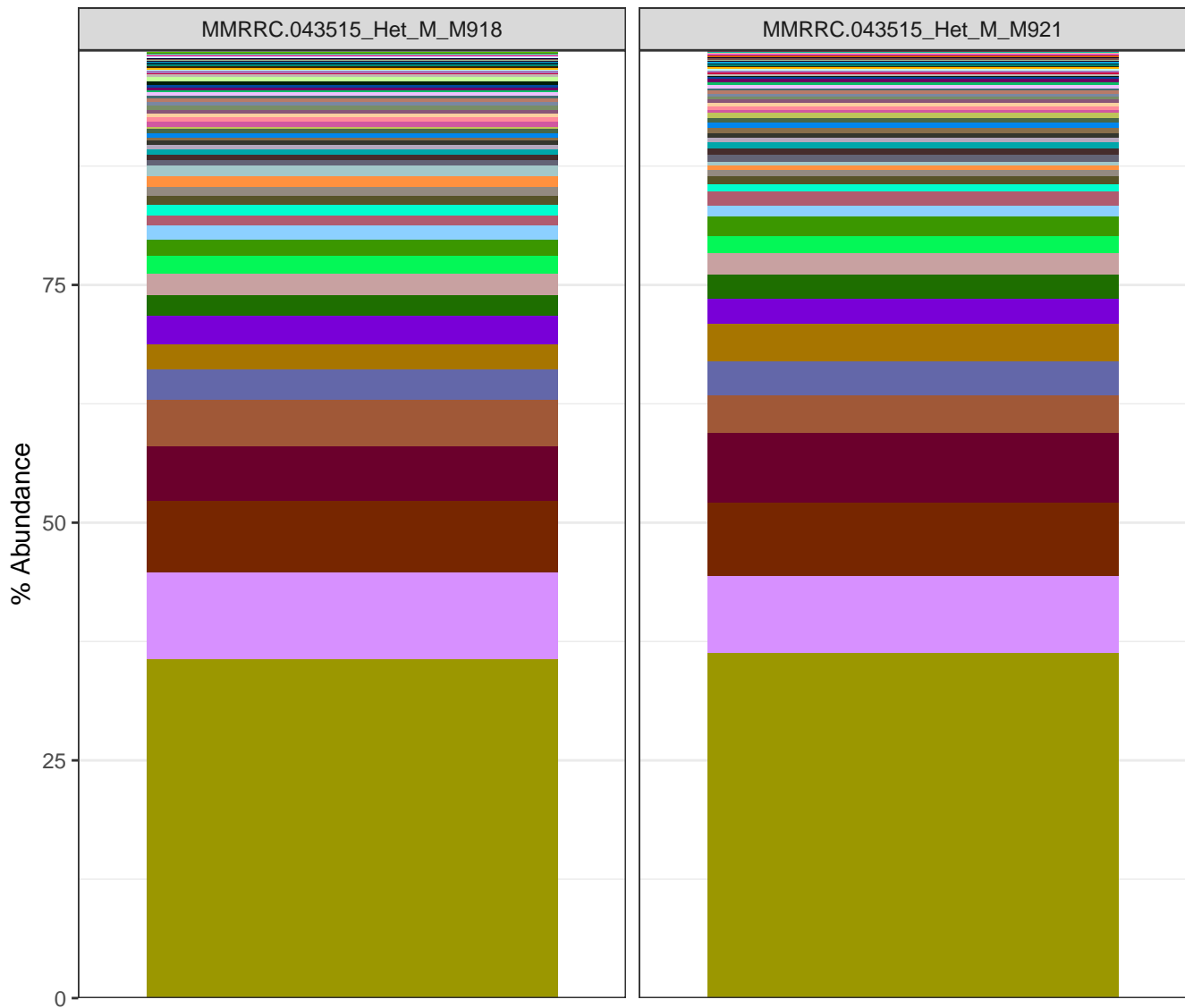


Figure 32: Genus level abundances-FILTERED: 23 genus level taxa removed

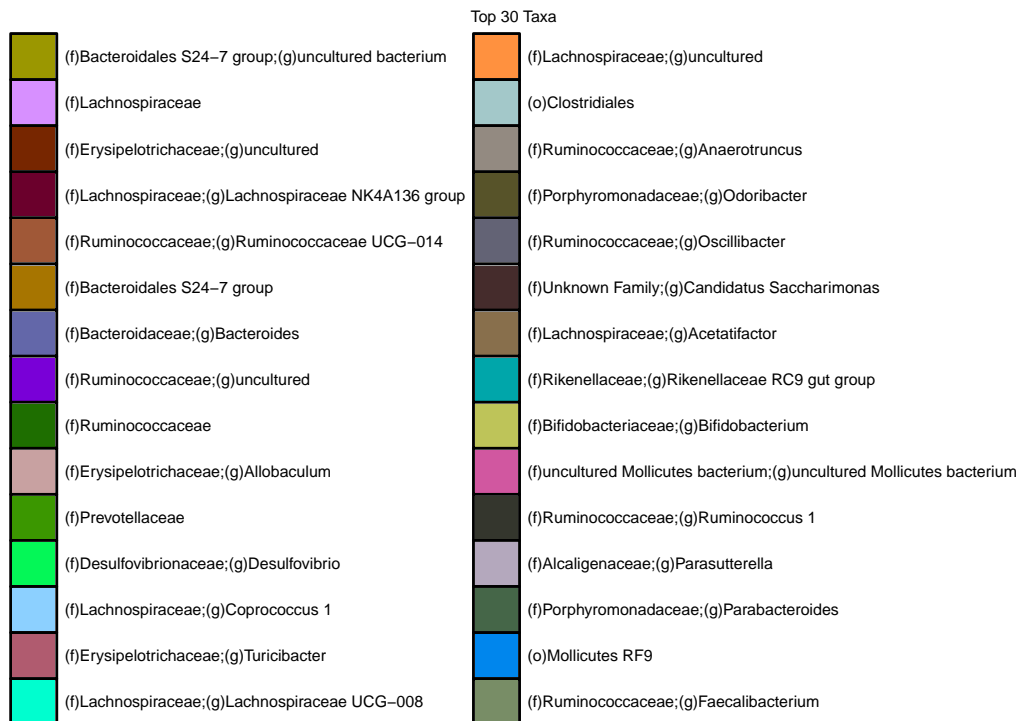
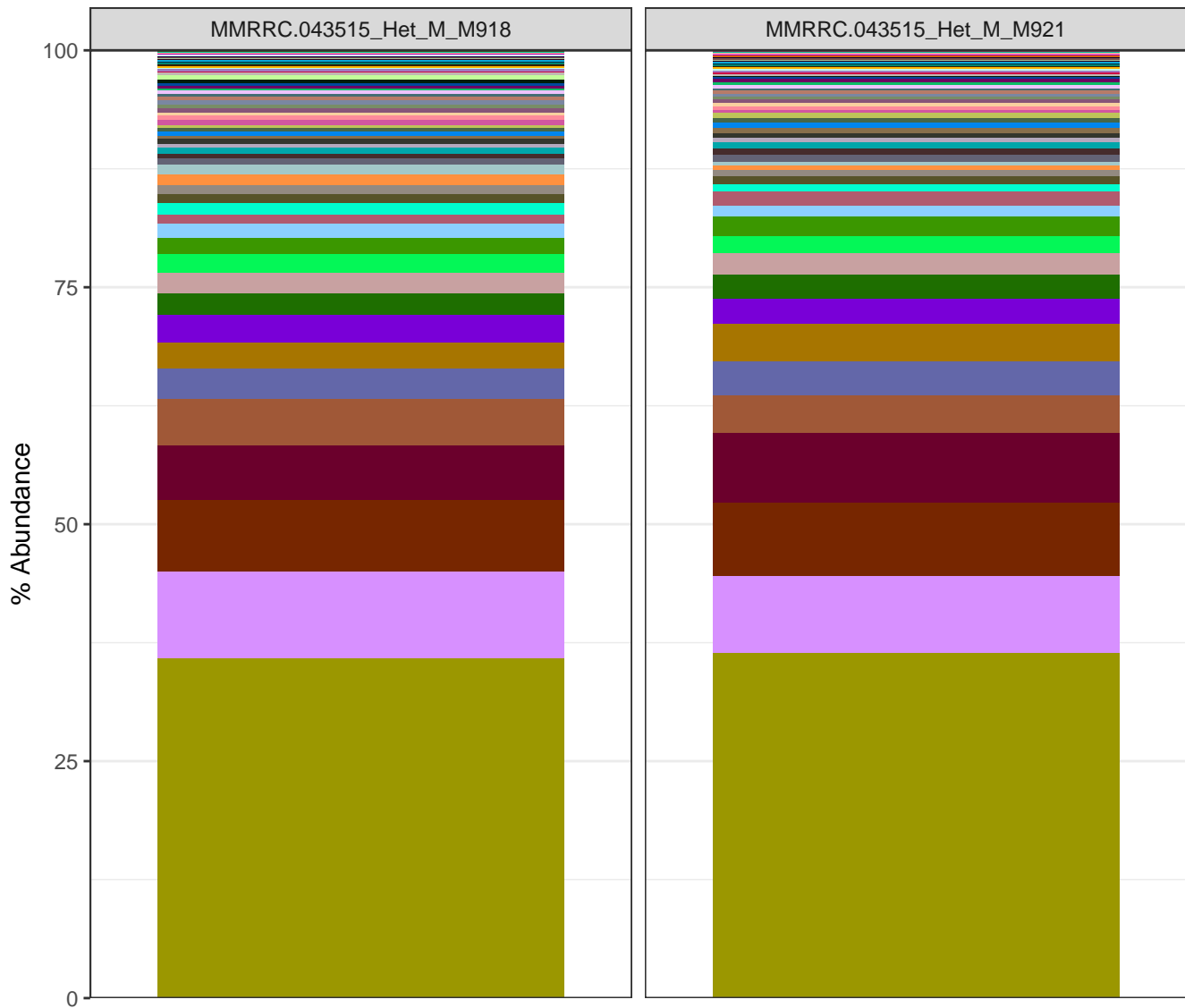


Figure 33: Genus level abundances-FILTERED & RESCALED

## A Appendix 1 (Taxa Abundance Tables)

Table 2: Phylum (Filtered) level taxa group mean percent abundances

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(p)Actinobacteria	0.88%	1.31%
(p)Bacteroidetes	46.09%	48.37%
(p)Firmicutes	48.82%	46.41%
(p)Proteobacteria	2.56%	2.43%
(p)Saccharibacteria	0.52%	0.7%
(p)Tenericutes	1.09%	0.79%
NA	99.968	100

Table 3: Class (Filtered) level taxa group mean percent abundances

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(p)Actinobacteria;(c)Actinobacteria	0.26%	0.6%
(p)Actinobacteria;(c)Coriobacteriia	0.62%	0.71%
(p)Bacteroidetes;(c)Bacteroidia	46.09%	48.37%
(p)Firmicutes;(c)Bacilli	0.4%	0.07%
(p)Firmicutes;(c)Clostridia	36.8%	33.83%
(p)Firmicutes;(c)Erysipelotrichia	11.62%	12.51%
(p)Proteobacteria;(c)Betaproteobacteria	0.46%	0.5%
(p)Proteobacteria;(c)Delta proteobacteria	2.06%	1.93%
(p)Saccharibacteria;(c)Unknown Class	0.52%	0.7%
(p)Tenericutes;(c)Mollicutes	1.09%	0.79%
<b>NA</b>	<b>99.926</b>	<b>100</b>



Table 4: Order (Filtered) level taxa group mean percent abundances

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(c)Actinobacteria;(o)Bifidobacteriales	0.26%	0.6%
(c)Bacilli;(o)Lactobacillales	0.4%	0.07%
(c)Bacteroidia;(o)Bacteroidales	46.09%	48.37%
(c)Betaproteobacteria;(o)Burkholderiales	0.46%	0.5%
(c)Clostridia;(o)Clostridiales	36.8%	33.83%
(c)Coriobacteriia;(o)Coriobacteriales	0.62%	0.71%
(c)Deltaproteobacteria;(o)Desulfovibrionales	2.06%	1.93%
(c)Erysipelotrichia;(o)Erysipelotrichales	11.62%	12.51%
(c)Mollicutes;(o)Mollicutes RF9	1.07%	0.79%
(k)Bacteria;(p)Saccharibacteria;(c)Unknown Class;(o)Unknown Order	0.52%	0.7%
<b>NA</b>	<b>99.912</b>	<b>100</b>

Table 5: Family (Filtered) level taxa group mean percent abundances

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(f)Alcaligenaceae	0.46%	0.5%
(f)Bacteroidaceae	3.2%	3.6%
(f)Bacteroidales S24-7 group	38.38%	40.25%
(f)Bifidobacteriaceae	0.26%	0.6%
(f)Christensenellaceae	0.11%	0.1%
(f)Clostridiaceae 1	0.08%	0.07%
(f)Clostridiales vadinBB60 group	0.48%	0.38%
(f)Coriobacteriaceae	0.62%	0.71%
(f)Desulfovibrionaceae	2.06%	1.93%
(f)Erysipelotrichaceae	11.62%	12.51%
(f)Family XIII	0.3%	0.45%
(f)Lachnospiraceae	20.33%	19.79%
(f)Lactobacillaceae	0.39%	0.07%
(f)Peptococcaceae	0.08%	0.08%
(f)Porphyromonadaceae	1.33%	1.36%
(f)Prevotellaceae	1.67%	2.01%
(f)Rikenellaceae	1.52%	1.14%
(f)Ruminococcaceae	14.29%	12.55%
(f)uncultured Mollicutes bacterium	0.58%	0.24%
(k)Bacteria;(p)Saccharibacteria;(c)Unknown Class;(o)Unknown Order;(f)Unknown Family	0.52%	0.7%
(o)Clostridiales	1.07%	0.39%
(o)Mollicutes RF9	0.44%	0.48%
NA	99.787	99.919

Table 6: Genus (Filtered) level taxa group mean percent abundances

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(f)Alcaligenaceae;(g)Parasutterella	0.46%	0.5%
(f)Bacteroidaceae;(g)Bacteroides	3.2%	3.6%
(f)Bacteroidales S24-7 group	2.72%	3.94%
(f)Bacteroidales S24-7 group;(g)uncultured bacterium	35.65%	36.3%
(f)Bifidobacteriaceae;(g)Bifidobacterium	0.26%	0.6%
(f)Christensenellaceae;(g)uncultured	0.07%	0.1%
(f)Clostridiaceae 1;(g)Sarcina	0.07%	0.07%
(f)Clostridiales vadinBB60 group	0.01%	0.06%
(f)Clostridiales vadinBB60 group;(g)uncultured bacterium	0.4%	0.31%
(f)Clostridiales vadinBB60 group;Ambiguous_taxa	0.07%	0.01%
(f)Coriobacteriaceae	0.11%	0.09%
(f)Coriobacteriaceae;(g)Enterorhabdus	0.1%	0.14%
(f)Coriobacteriaceae;(g)Parvibacter	0.1%	0.14%
(f)Coriobacteriaceae;(g)Senegalimassilia	0.15%	0.18%
(f)Coriobacteriaceae;(g)uncultured bacterium	0.16%	0.15%
(f)Desulfovibrionaceae;(g)Bilophila	0.11%	0.08%
(f)Desulfovibrionaceae;(g)Desulfovibrio	1.95%	1.85%
(f)Erysipelotrichaceae	0.26%	0.14%
(f)Erysipelotrichaceae;(g)Allobaculum	2.19%	2.25%
(f)Erysipelotrichaceae;(g)Faecalibaculum	0.22%	0.31%
(f)Erysipelotrichaceae;(g)Holdemanella	0.16%	0.17%
(f)Erysipelotrichaceae;(g)Holdemania	0.34%	0.43%
(f)Erysipelotrichaceae;(g)Turicibacter	0.98%	1.45%
(f)Erysipelotrichaceae;(g)uncultured	7.44%	7.71%
(f)Family XIII;(g)[Eubacterium] nodatum group	0.04%	0.13%
(f)Family XIII;(g)Family XIII UCG-001	0.18%	0.22%
(f)Lachnospiraceae	9.18%	8.14%
(f)Lachnospiraceae;(g)[Eubacterium] fissicatena group	0.02%	0.05%
(f)Lachnospiraceae;(g)[Eubacterium] oxidoreducens group	0.08%	0.07%
(f)Lachnospiraceae;(g)[Eubacterium] ventriosum group	0.27%	0.32%
(f)Lachnospiraceae;(g)Acetatifactor	0.29%	0.64%
(f)Lachnospiraceae;(g)Blautia	0.19%	0.14%
(f)Lachnospiraceae;(g)Coprococcus 1	1.52%	1.17%
(f)Lachnospiraceae;(g)Lachnospiraceae FCS020 group	0.2%	0.17%
(f)Lachnospiraceae;(g)Lachnospiraceae NK4A136 group	5.8%	7.34%
(f)Lachnospiraceae;(g)Lachnospiraceae UCG-008	1.19%	0.8%
(f)Lachnospiraceae;(g)Lactonifactor	0.04%	0.12%
(f)Lachnospiraceae;(g)Roseburia	0.13%	0.1%
(f)Lachnospiraceae;(g)Tyzzerella	0.31%	0.31%
(f)Lachnospiraceae;(g)uncultured	1.08%	0.43%
(f)Lactobacillaceae;(g)Lactobacillus	0.39%	0.07%
(f)Peptococcaceae;(g)uncultured	0.05%	0.06%
(f)Porphyromonadaceae;(g)Odoribacter	0.91%	0.87%
(f)Porphyromonadaceae;(g)Parabacteroides	0.42%	0.5%
(f)Prevotellaceae	1.67%	2.01%
(f)Rikenellaceae	0.1%	0.12%
(f)Rikenellaceae;(g)Alistipes	0.39%	0.35%
(f)Rikenellaceae;(g)Rikenella	0.45%	0.06%
(f)Rikenellaceae;(g)Rikenellaceae RC9 gut group	0.58%	0.61%
(f)Ruminococcaceae	2.24%	2.48%

Table 6: Genus (Filtered) level taxa group mean percent abundances (*continued*)

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(f)Ruminococcaceae;(g)Anaerotruncus	0.98%	0.67%
(f)Ruminococcaceae;(g)Faecalibacterium	0.45%	0.26%
(f)Ruminococcaceae;(g)Intestinimonas	0.37%	0.33%
(f)Ruminococcaceae;(g)Oscillibacter	0.64%	0.74%
(f)Ruminococcaceae;(g)Ruminiclostridium 5	0.34%	0.28%
(f)Ruminococcaceae;(g)Ruminiclostridium 6	0.18%	0.12%
(f)Ruminococcaceae;(g)Ruminiclostridium 9	0.44%	0.36%
(f)Ruminococcaceae;(g)Ruminococcaceae UCG-010	0.28%	0.24%
(f)Ruminococcaceae;(g)Ruminococcaceae UCG-014	4.83%	3.91%
(f)Ruminococcaceae;(g)Ruminococcus 1	0.51%	0.44%
(f)Ruminococcaceae;(g)uncultured	2.93%	2.66%
(f)uncultured Mollicutes bacterium;(g)uncultured Mollicutes bacterium	0.58%	0.24%
(f)Unknown Family;(g)Candidatus Saccharimonas	0.52%	0.7%
(o)Clostridiales	1.07%	0.39%
(o)Mollicutes RF9	0.44%	0.48%
NA	99.488	99.672

## B Appendix 2 (Taxa removed from filtered datasets at each level)

Table 7: Phylum level taxa removed via filtering- group mean abundances

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(k)Bacteria	0.0098	0
Unassigned	0.0221	0
<b>Total % Removed</b>	<b>0.0319</b>	<b>0</b>

Table 8: Class level taxa removed via filtering- group mean abundances

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(k)Bacteria	0.0098	0
(p)Proteobacteria;(c)Alphaproteobacteria	0.0417	0
Unassigned	0.0221	0
<b>Total % Removed</b>	<b>0.0735</b>	<b>0</b>

Table 9: Order level taxa removed via filtering- group mean abundances

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(c)Alphaproteobacteria;(o)Rhodospirillales	0.0417	0
(c)Mollicutes;(o)Anaeroplasmatales	0.0147	0
(k)Bacteria	0.0098	0
Unassigned	0.0221	0
<b>Total % Removed</b>	<b>0.0882</b>	<b>0</b>



Table 10: Family level taxa removed via filtering- group mean abundances

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(f)Acholeplasmatales bacterium canine oral taxon 316	0.0270	0.0429
(f)Anaeroplasmataceae	0.0147	0.0000
(f)Carnobacteriaceae	0.0172	0.0000
(f)Defluviitaleaceae	0.0221	0.0126
(f)Peptostreptococcaceae	0.0368	0.0000
(f)Rhodospirillaceae	0.0417	0.0000
(f)uncultured Paenibacillaceae bacterium	0.0221	0.0253
(k)Bacteria	0.0098	0.0000
Unassigned	0.0221	0.0000
<b>Total % Removed</b>	<b>0.2132</b>	<b>0.0808</b>

Table 11: Genus level taxa removed via filtering- group mean abundances

Taxa	MMRRC.043515_Het_M_M918	MMRRC.043515_Het_M_M921
(f)Acholeplasmatales bacterium canine oral taxon 316;(g)Acholeplasmatales bacterium canine oral taxon 316	0.0270	0.0429
(f)Anaeroplasmataceae;(g)Anaeroplasma	0.0147	0.0000
(f)Bacteroidales S24-7 group;(g)mouse gut metagenome	0.0098	0.0076
(f)Carnobacteriaceae;(g)Atopostipes	0.0172	0.0000
(f)Christensenellaceae	0.0098	0.0000
(f)Christensenellaceae;Ambiguous_taxa	0.0319	0.0000
(f)Clostridiaceae 1;(g)Candidatus Arthromitus	0.0098	0.0000
(f)Defluviitaleaceae;(g)Defluviitaleaceae UCG-011	0.0221	0.0126
(f)Erysipelotrichaceae;(g)Candidatus Stoquefichus	0.0245	0.0404
(f)Family XIII	0.0147	0.0354
(f)Family XIII;(g)[Eubacterium] brachy group	0.0417	0.0455
(f)Family XIII;(g)Anaerovorax	0.0196	0.0177
(f)Lachnospiraceae;(g)mouse gut metagenome	0.0221	0.0000
(f)Peptococcaceae;(g)Peptococcus	0.0245	0.0202
(f)Peptostreptococcaceae;(g)Romboutsia	0.0368	0.0000
(f)Rhodospirillaceae;(g)uncultured	0.0417	0.0000
(f)Ruminococcaceae;(g)Butyricoccus	0.0319	0.0354
(f)Ruminococcaceae;(g)Candidatus Soleaferrea	0.0000	0.0202
(f)Ruminococcaceae;(g)Hydrogenoanaerobacterium	0.0147	0.0000
(f)Ruminococcaceae;(g)Ruminococcaceae UCG-013	0.0441	0.0253
(f)uncultured Paenibacillaceae bacterium;(g)uncultured Paenibacillaceae bacterium	0.0221	0.0253
(k)Bacteria	0.0098	0.0000
Unassigned	0.0221	0.0000
<b>Total % Removed</b>	<b>0.5122</b>	<b>0.3284</b>

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