D4006- Gut Microbiota Analysis
UC Davis MMPC - Microbiome & Host Response Core

Contents

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Project #: MBP-3995

MMRRC strain ID: MMRRC_050690

Animal Information: The strain was donated to the MMRRC by David Luo at University of California, Irvine. Fecal samples were obtained from animals housed under the care of David Luo at University of California, Irvine consuming Teklad (Envigo) 2020.

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 facilty 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.50690_M3	MMRRC.50690_Tg_M3	M3	Tg	MMRRC.50690	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 2020.8.0. 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 $43079 \pm NA$ (SD) individual sequencing reads per sample (Min= 43079; Max= 43079). After data processing the average number of sequences for each sample passing through to OTU classification was $NA \pm NA$ (SD). The average number of OTUs per sample was 146.

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 14900 sequences per sample.

2.3   Data analysis using taxa abundance data

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 abundace 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.
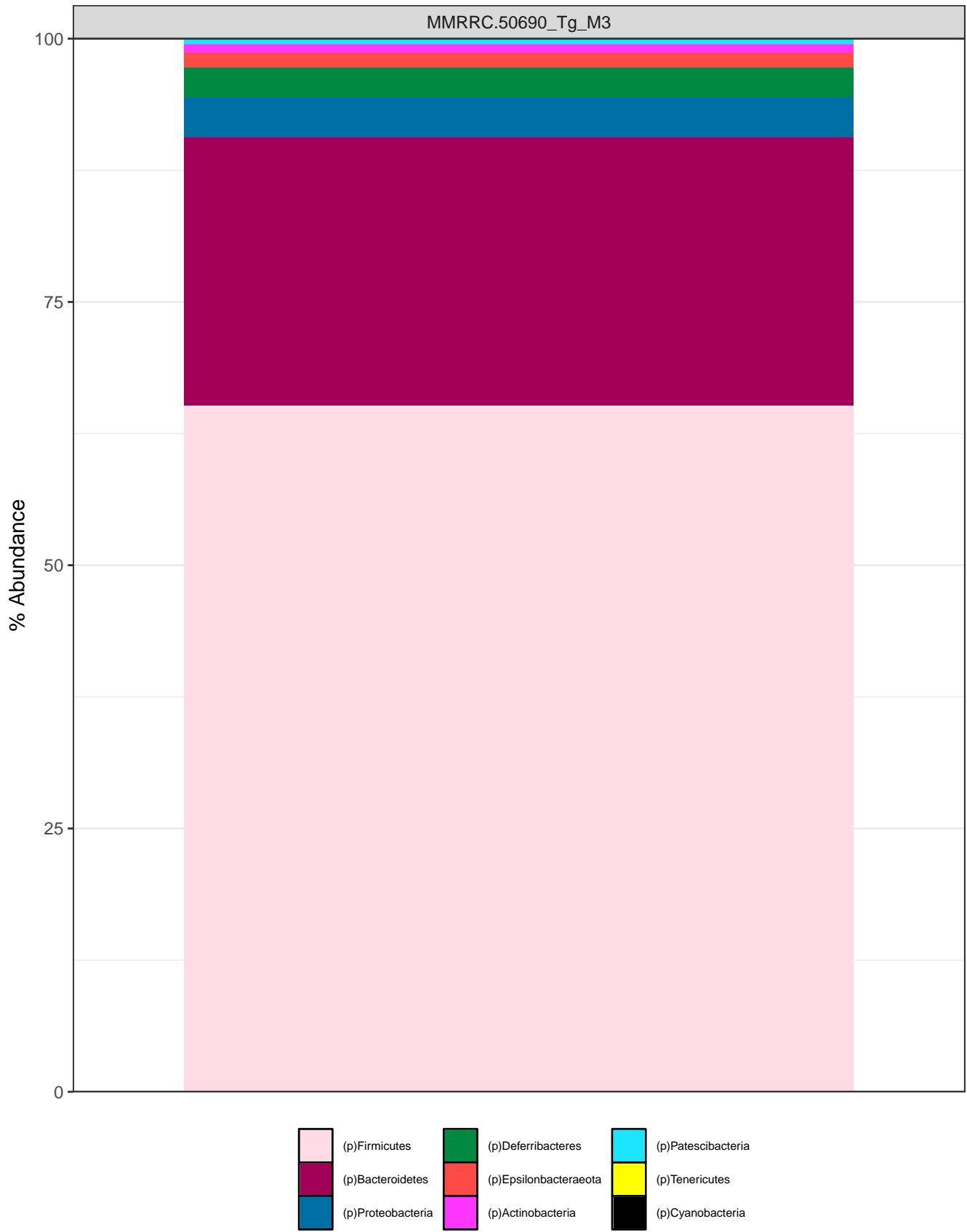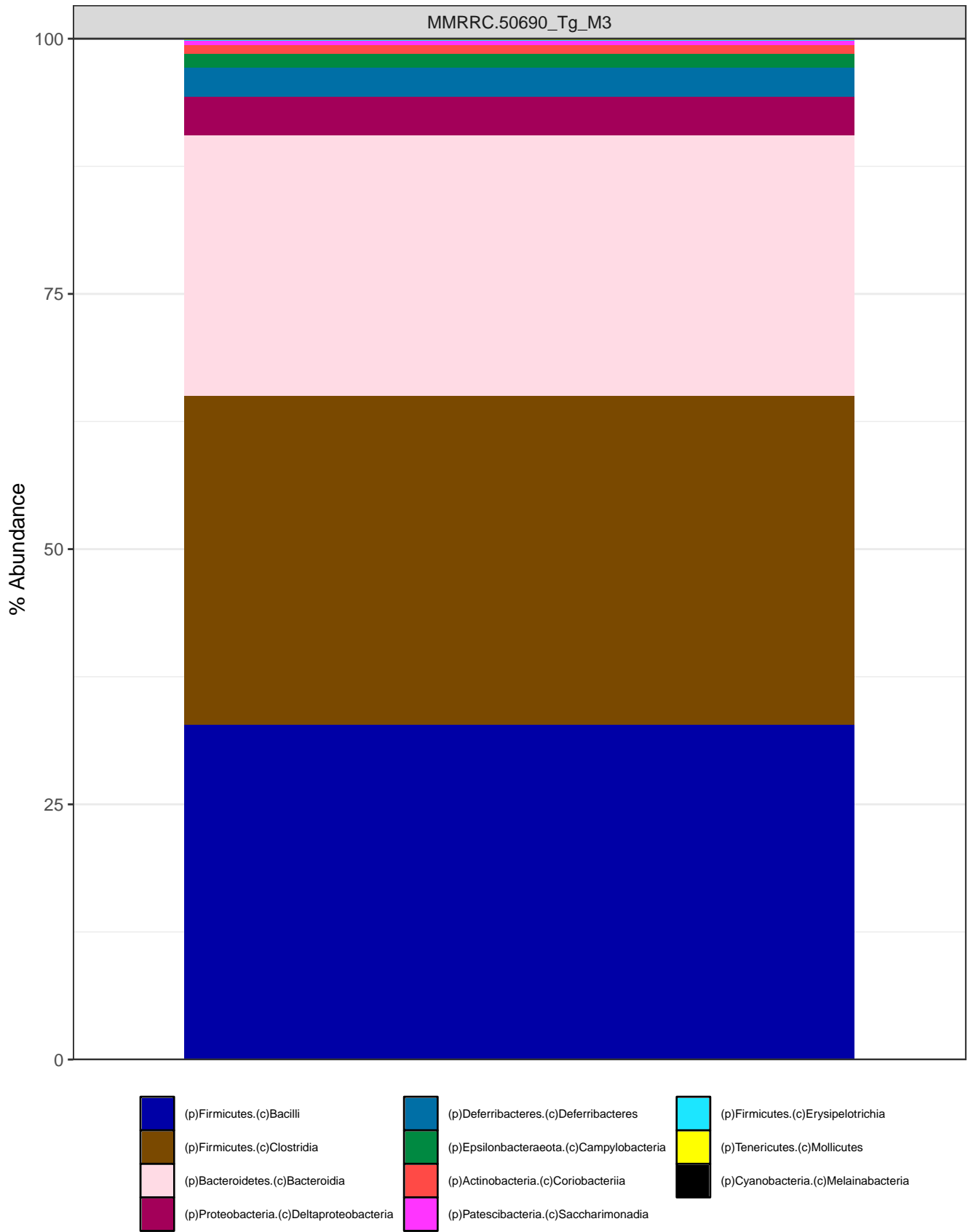
Figure 1: Phylum level abundances-UNFILTERED

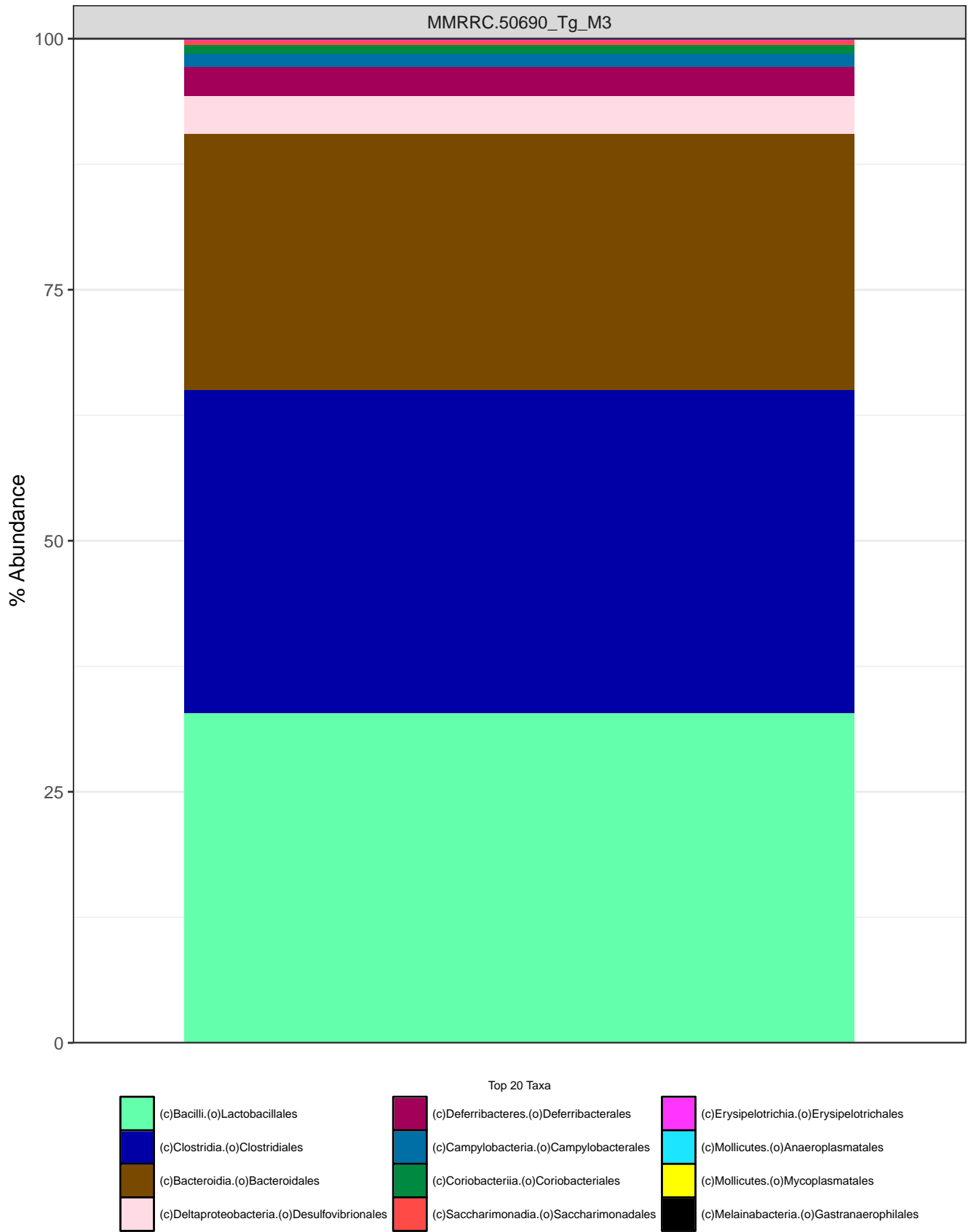Figure 2: Class level abundances-UNFILTERED

Figure 3: Order level abundances-UNFILTERED

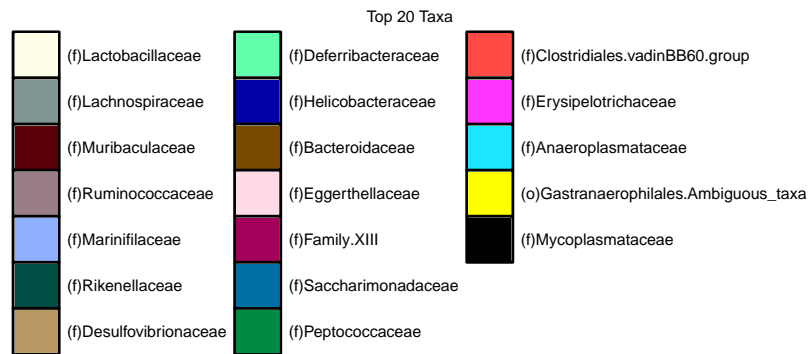Figure 4: Family level abundances-UNFILTERED

MMRRC.50690_Tg_M3

Top 30 Taxa

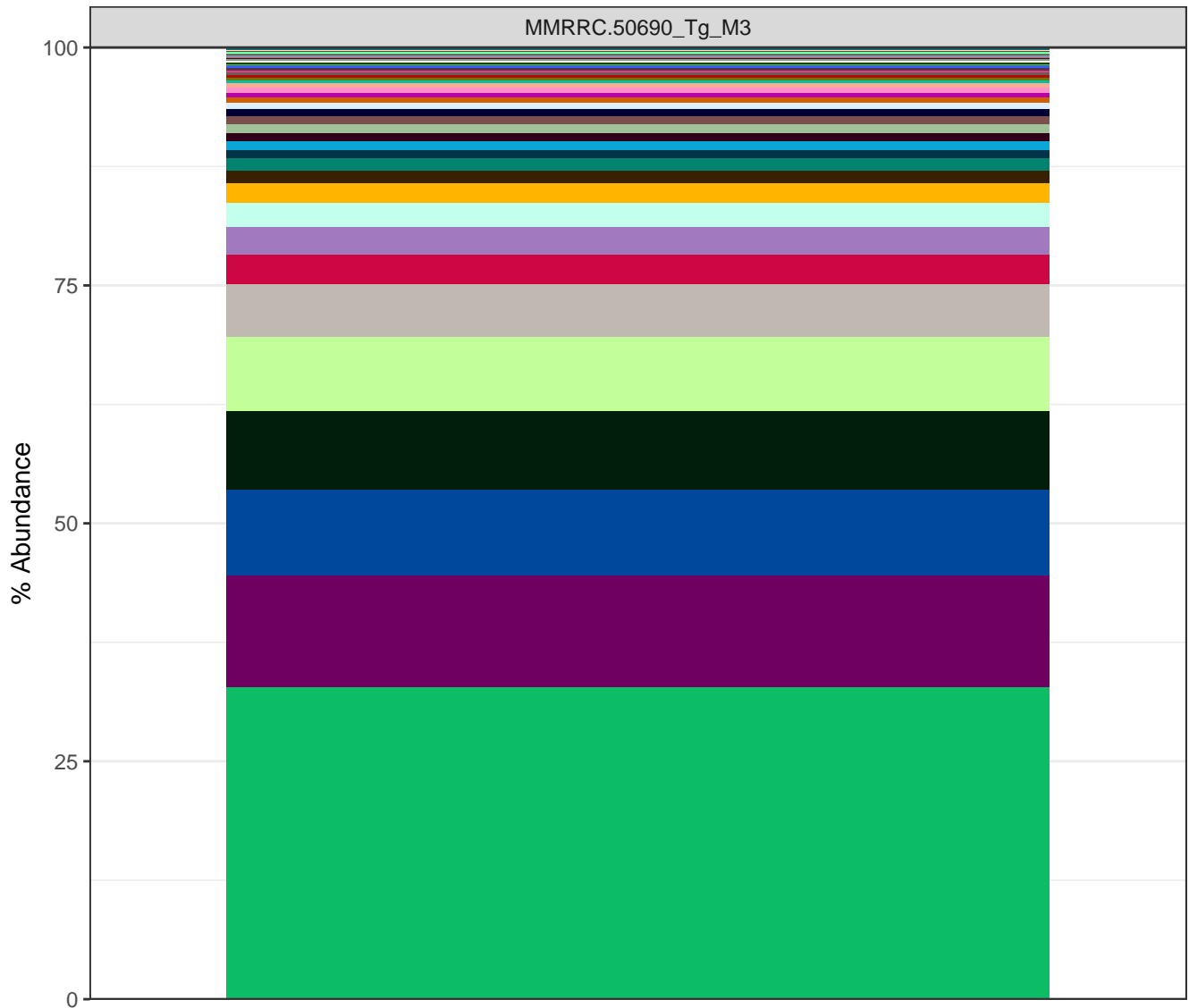|	
(f)Lactobacillaceae.(g)Lactobacillus	(f)Muribaculaceae.(g)uncultured.Bacteroidales.bacterium
(f)Lachnospiraceae.__	(f)Eggerthellaceae.(g)Enterorhabdus
(f)Muribaculaceae.__	(f)Rikenellaceae.(g)Rikenellaceae.RC9.gut.group
(f)Marinifilaceae.(g)Odoribacter	(f)Lachnospiraceae.(g)Lachnoclostridium
(f)Ruminococcaceae.__	(f)Desulfovibrionaceae.(g)uncultured
(f)Lachnospiraceae.(g)Lachnospiraceae.NK4A136.group	(f)Rikenellaceae.(g)Rikenella
(f)Desulfovibrionaceae.(g)Desulfovibrio	(f)Lachnospiraceae.(g)Lachnospiraceae.UCG.006
(f)Deferribacteraceae.(g)Mucispirillum	(f)Saccharimonadaceae.(g)Candidatus.Saccharimonas
(f)Rikenellaceae.(g)Alistipes	(f)Peptococcaceae.(g)uncultured
(f)Muribaculaceae.(g)uncultured.bacterium	(f)Family.XIII.(g)Family.XIII.UCG.001
(f)Helicobacteraceae.(g)Helicobacter	(f)Ruminococcaceae.(g)Ruminococcaceae.UCG.014
(f)Bacteroidaceae.(g)Bacteroides	(f)Ruminococcaceae.(g)Ruminiclostridium.5
(f)Ruminococcaceae.(g)Ruminiclostridium.9	(f)Lachnospiraceae.(g)Roseburia
(f)Ruminococcaceae.(g)Ruminiclostridium	(f)Ruminococcaceae.(g)Intestinimonas
(f)Lachnospiraceae.(g)Tyzzerella	(f)Ruminococcaceae.(g)Angelakisella

Figure 5: Genus level abundances-UNFILTERED

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A   Appendix 1 (Taxa Abundance Tables)

Table 2: Phylum level taxa group mean percent abundances

Taxa	MMRRC.50690_Tg_M3
(p)Cyanobacteria	0.03%
(p)Tenericutes	0.07%
(p)Patescibacteria	0.44%
(p)Actinobacteria	0.86%
(p)Epsilonbacteraeota	1.32%
(p)Deferribacteres	2.83%
(p)Proteobacteria	3.83%
(p)Bacteroidetes	25.47%
(p)Firmicutes	65.13%

Table 3: Class level taxa group mean percent abundances

Taxa	MMRRC.50690__Tg__M3
(p)Cyanobacteria.(c)Melainabacteria	0.03%
(p)Tenericutes.(c)Mollicutes	0.07%
(p)Firmicutes.(c)Erysipelotrichia	0.12%
(p)Patescibacteria.(c)Saccharimonadia	0.44%
(p)Actinobacteria.(c)Coriobacteriia	0.86%
(p)Epsilonbacteraeota.(c)Campylobacteria	1.32%
(p)Deferribacteres.(c)Deferribacteres	2.83%
(p)Proteobacteria.(c)Deltaproteobacteria	3.83%
(p)Bacteroidetes.(c)Bacteroidia	25.47%
(p)Firmicutes.(c)Clostridia	32.25%
(p)Firmicutes.(c)Bacilli	32.77%

Table 4: Order level taxa group mean percent abundances

Taxa	MMRRC.50690_Tg_M3
(c)Melainabacteria.(o)Gastranaerophilales	0.03%
(c)Mollicutes.(o)Mycoplasmatales	0.03%
(c)Mollicutes.(o)Anaeroplasmatales	0.04%
(c)Erysipelotrichia.(o)Erysipelotrichales	0.12%
(c)Saccharimonadia.(o)Saccharimonadales	0.44%
(c)Coriobacteriia.(o)Coriobacteriales	0.86%
(c)Campylobacteria.(o)Campylobacterales	1.32%
(c)Deferribacteres.(o)Deferribacterales	2.83%
(c)Deltaproteobacteria.(o)Desulfovibrionales	3.83%
(c)Bacteroidia.(o)Bacteroidales	25.47%
(c)Clostridia.(o)Clostridiales	32.25%
(c)Bacilli.(o)Lactobacillales	32.77%

Table 5: Family level taxa group mean percent abundances

Taxa	MMRRC.50690__Tg__M3
(f)Mycoplasmataceae	0.03%
(o)Gastranaerophilales.Ambiguous__taxa	0.03%
(f)Anaeroplasmataceae	0.04%
(f)Erysipelotrichaceae	0.12%
(f)Clostridiales.vadinBB60.group	0.19%
(f)Peptococcaceae	0.28%
(f)Saccharimonadaceae	0.44%
(f)Family.XIII	0.51%
(f)Eggerthellaceae	0.86%
(f)Bacteroidaceae	1.28%
(f)Helicobacteraceae	1.32%
(f)Deferribacteraceae	2.83%
(f)Desulfovibrionaceae	3.83%
(f)Rikenellaceae	3.88%
(f)Marinifilaceae	8.33%
(f)Ruminococcaceae	11.25%
(f)Muribaculaceae	11.98%
(f)Lachnospiraceae	20.02%
(f)Lactobacillaceae	32.77%

Table 6: Genus level taxa group mean percent abundances

Taxa	MMRRC.50690__Tg__M3
(f)Lachnospiraceae.(g).Eubacterium..ventriosum.group	0.01%
(f)Ruminococcaceae.(g)Ruminococcaceae.UCG.009	0.02%
(f)Mycoplasmataceae.(g)Mycoplasma	0.03%
(o)Gastranaerophilales.Ambiguous__taxa.Ambiguous__taxa	0.03%
(f)Anaeroplasmataceae.(g)Anaeroplasma	0.04%
(f)Lachnospiraceae.(g)uncultured	0.05%
(f)Erysipelotrichaceae.(g)Turicibacter	0.06%
(f)Erysipelotrichaceae.(g)uncultured.bacterium	0.06%
(f)Clostridiales.vadinBB60.group.___	0.07%
(f)Family.XIII.(g)Anaerovorax	0.07%
(f)Ruminococcaceae.(g)Harryflintia	0.09%
(f)Lachnospiraceae.(g)A2	0.1%
(f)Desulfovibrionaceae.(g)Bilophila	0.11%
(f)Clostridiales.vadinBB60.group.(g)uncultured.bacterium	0.12%
(f)Ruminococcaceae.(g)Ruminococcaceae.NK4A214.group	0.12%
(f)Ruminococcaceae.(g)Ruminococcaceae.UCG.013	0.13%
(f)Ruminococcaceae.(g)Ruminococcaceae.UCG.004	0.15%
(f)Family.XIII.(g).Eubacterium..brachy.group	0.15%
(f)Lachnospiraceae.(g).Eubacterium..xylanophilum.group	0.16%
(f)Ruminococcaceae.(g)Oscillibacter	0.16%
(f)Lachnospiraceae.(g)GCA.900066575	0.19%
(f)Ruminococcaceae.(g)Angelakisella	0.23%
(f)Ruminococcaceae.(g)Intestinimonas	0.23%
(f)Lachnospiraceae.(g)Roseburia	0.26%
(f)Ruminococcaceae.(g)Ruminiclostridium.5	0.26%
(f)Ruminococcaceae.(g)Ruminococcaceae.UCG.014	0.27%
(f)Family.XIII.(g)Family.XIII.UCG.001	0.28%
(f)Peptococcaceae.(g)uncultured	0.28%
(f)Saccharimonadaceae.(g)Candidatus.Saccharimonas	0.44%
(f)Lachnospiraceae.(g)Lachnospiraceae.UCG.006	0.55%
(f)Rikenellaceae.(g)Rikenella	0.55%
(f)Desulfovibrionaceae.(g)uncultured	0.56%
(f)Lachnospiraceae.(g)Lachnoclostridium	0.64%
(f)Rikenellaceae.(g)Rikenellaceae.RC9.gut.group	0.75%
(f)Eggerthellaceae.(g)Enterorhabdus	0.86%
(f)Muribaculaceae.(g)uncultured.Bacteroidales.bacterium	0.88%
(f)Lachnospiraceae.(g)Tyzzerella	0.9%
(f)Ruminococcaceae.(g)Ruminiclostridium	0.9%
(f)Ruminococcaceae.(g)Ruminiclostridium.9	0.92%
(f)Bacteroidaceae.(g)Bacteroides	1.28%
(f)Helicobacteraceae.(g)Helicobacter	1.32%
(f)Muribaculaceae.(g)uncultured.bacterium	2.07%
(f)Rikenellaceae.(g)Alistipes	2.59%
(f)Deferribacteraceae.(g)Mucispirillum	2.83%
(f)Desulfovibrionaceae.(g)Desulfovibrio	3.16%
(f)Lachnospiraceae.(g)Lachnospiraceae.NK4A136.group	5.47%
(f)Ruminococcaceae.___	7.77%
(f)Marinifilaceae.(g)Odoribacter	8.33%
(f)Muribaculaceae.___	9.04%
(f)Lachnospiraceae.___	11.68%

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

Taxa	MMRRC.50690_Tg_M3
(f)Lactobacillaceae.(g)Lactobacillus	32.77%

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