HARVARD
T.H. CHAN
SCHOOL OF PUBLIC HEALTH

Microbiome Analysis Core

James Luo Mice Colitis Colon Cancer Surgery Gut Microbiome 16S

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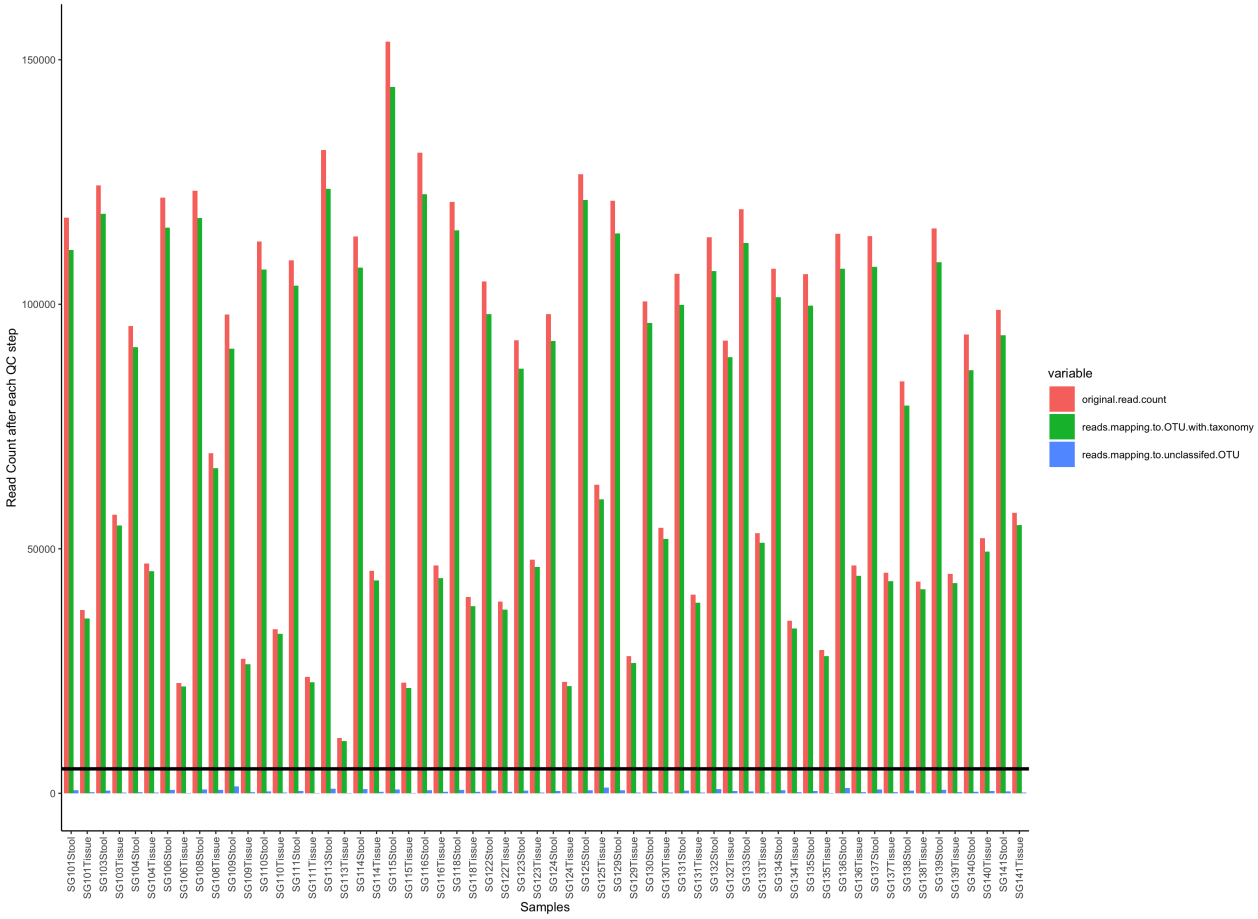
Section 1. Full Dataset

Data QC

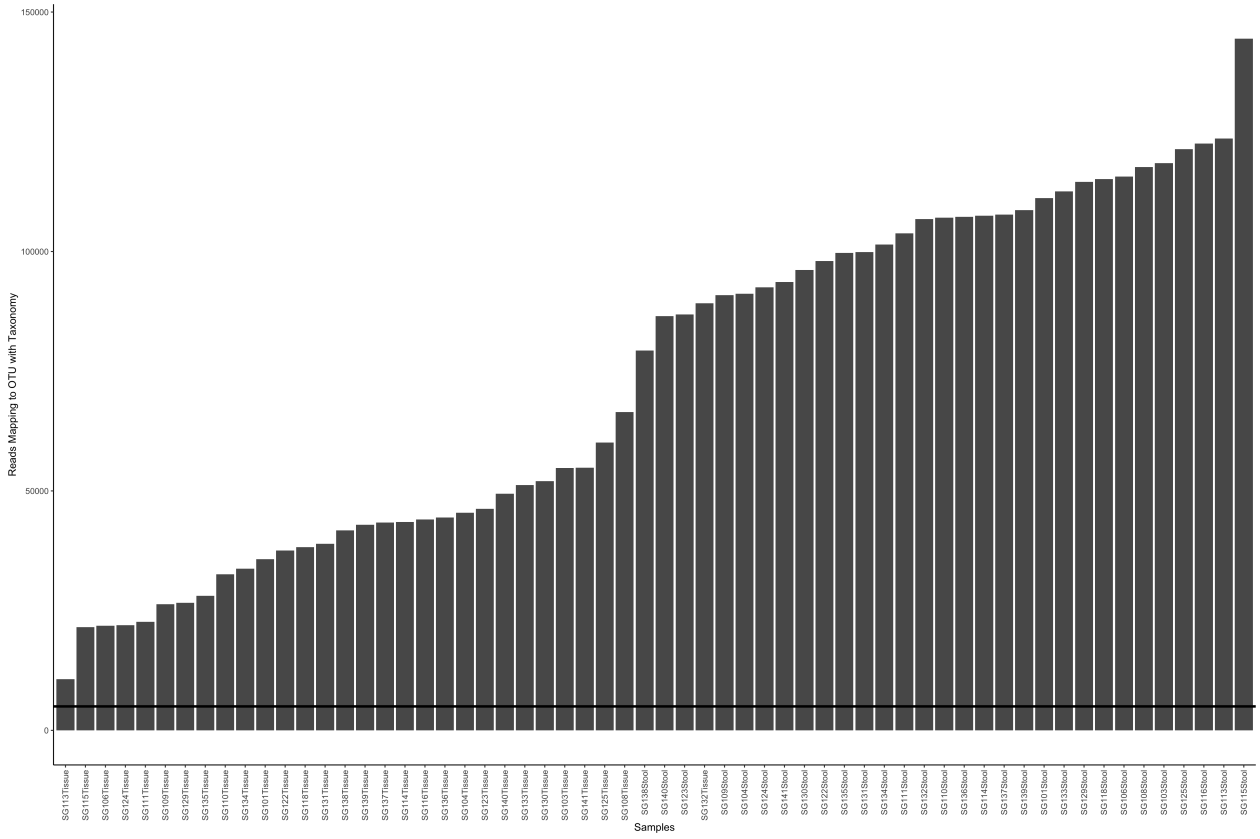
Summary of raw data processing

Data from USEARCH
file = all_samples_read_counts.tsv

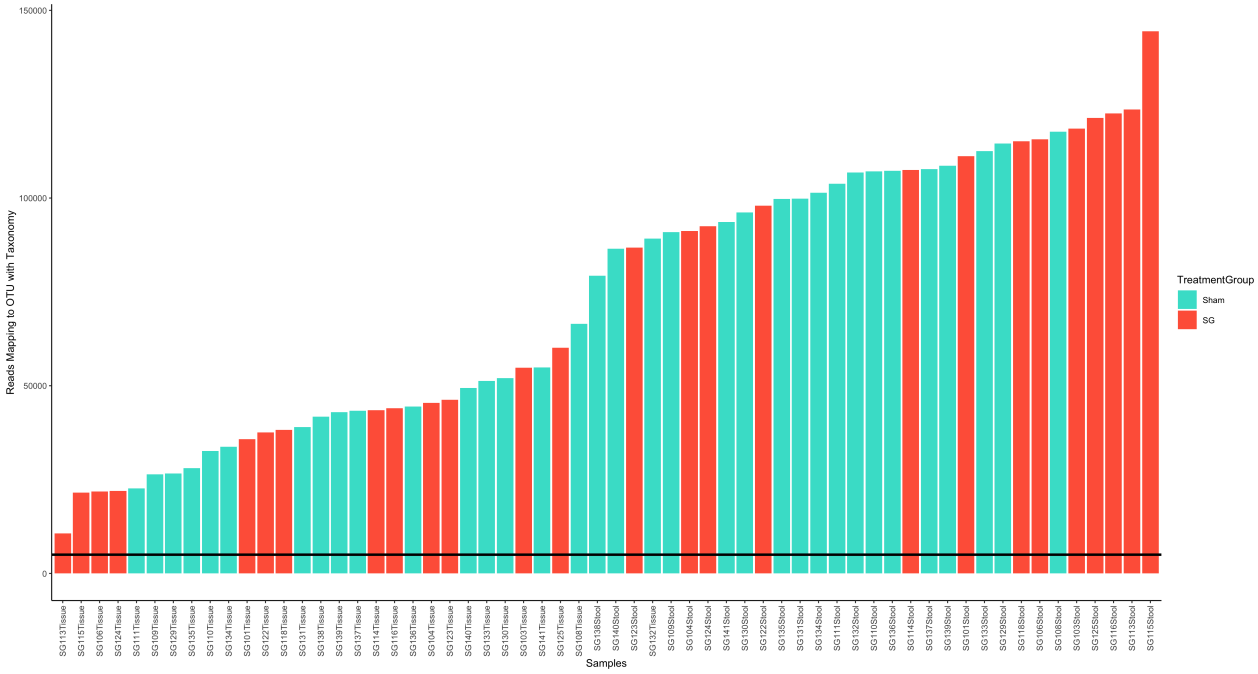
Bar plot of reads per samples at each step of the QC process



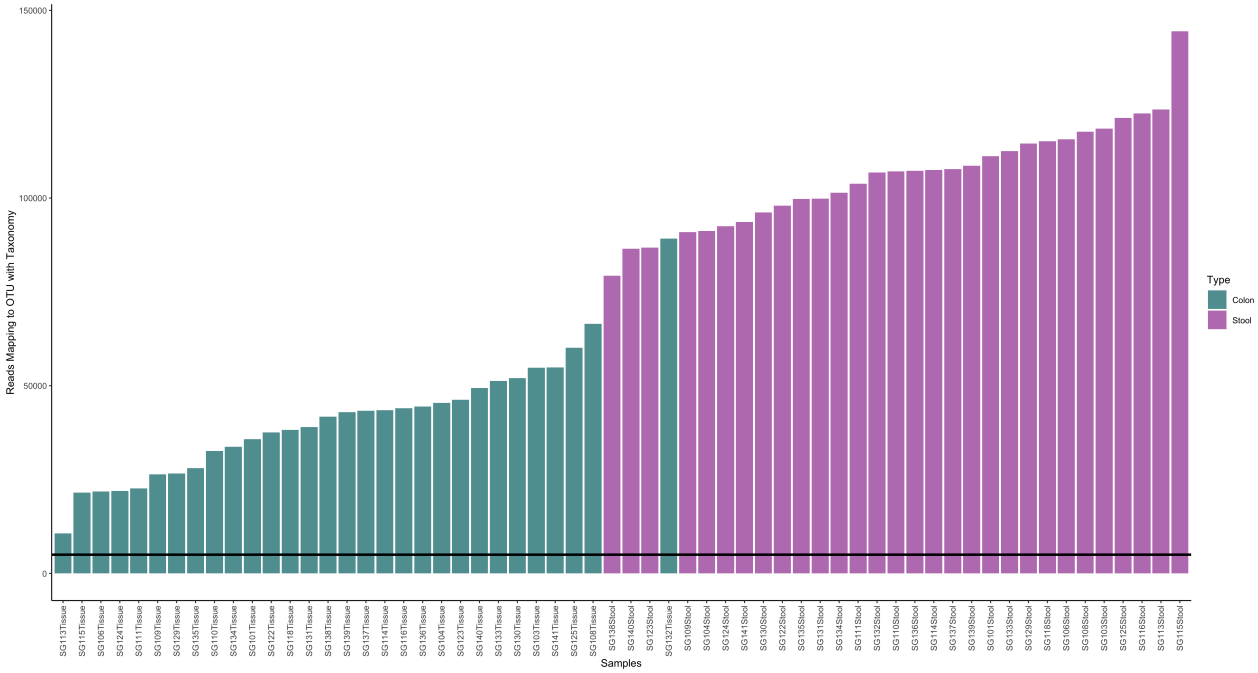
Bar plot of the final reads that were mapped to OTU with taxonomy



This plot shows mapped read count being colored by TreatmentGroup status. Here we are looking for any obvious bias in sequencing depth by TreatmentGroup.



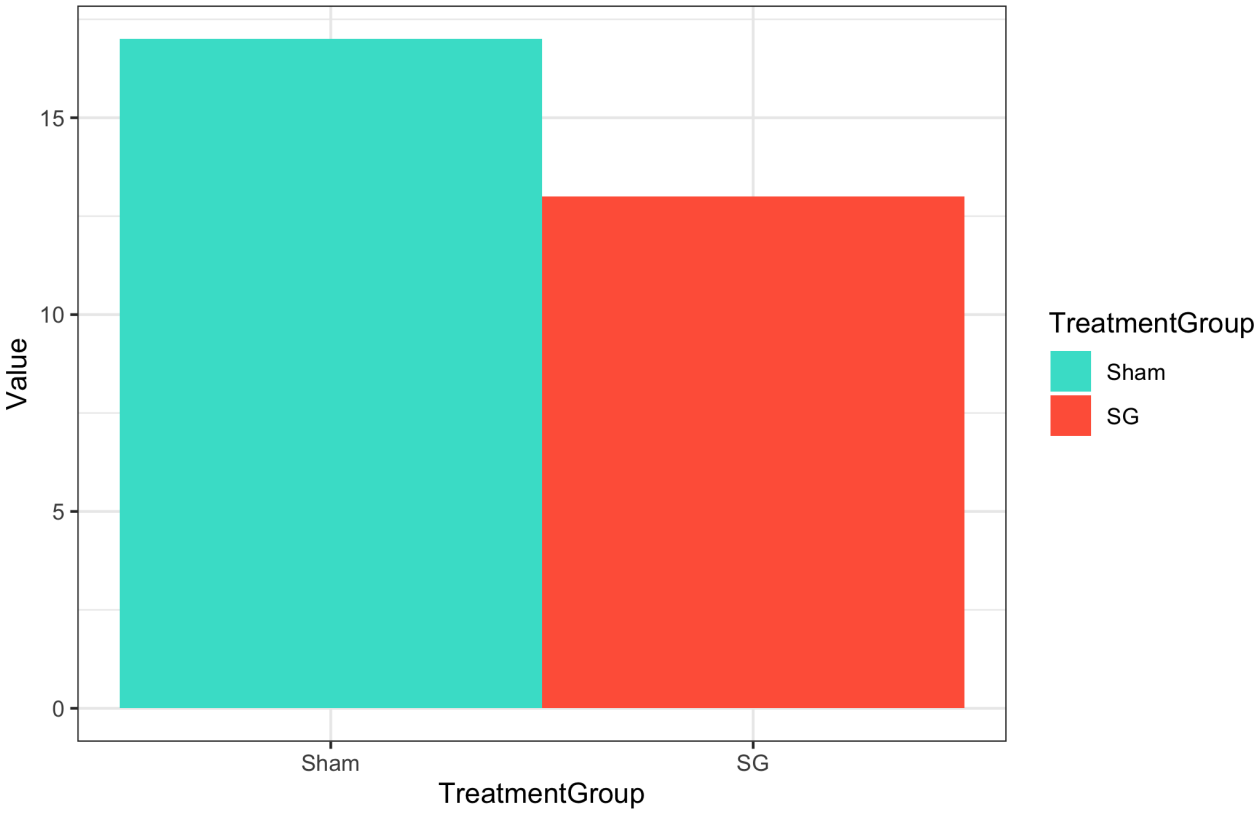
This plot shows mapped read count being colored by Type.



Visualize Covariates

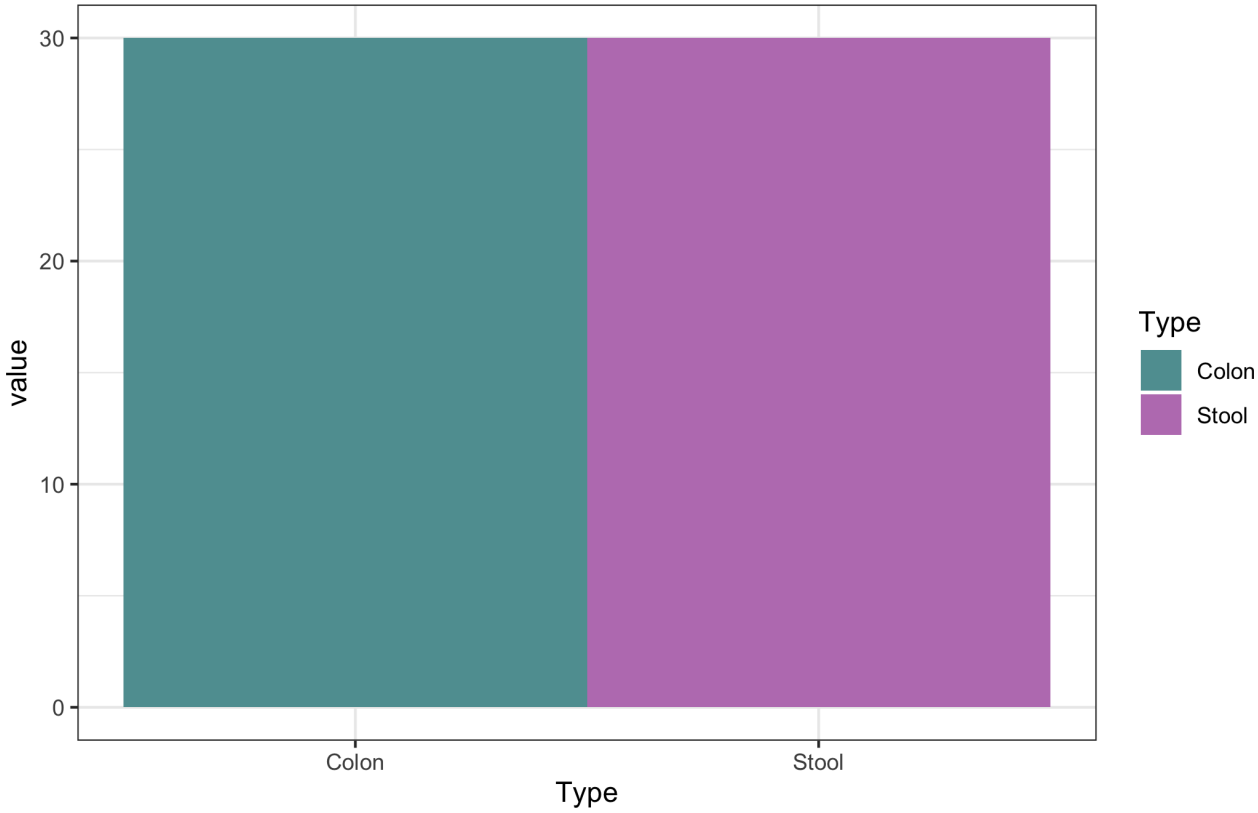
TreatmentGroup

Samples are collected from 17 Sham, 13 SG individuals.



Sample Type

This dataset includes 30 stool and 30 colon samples.



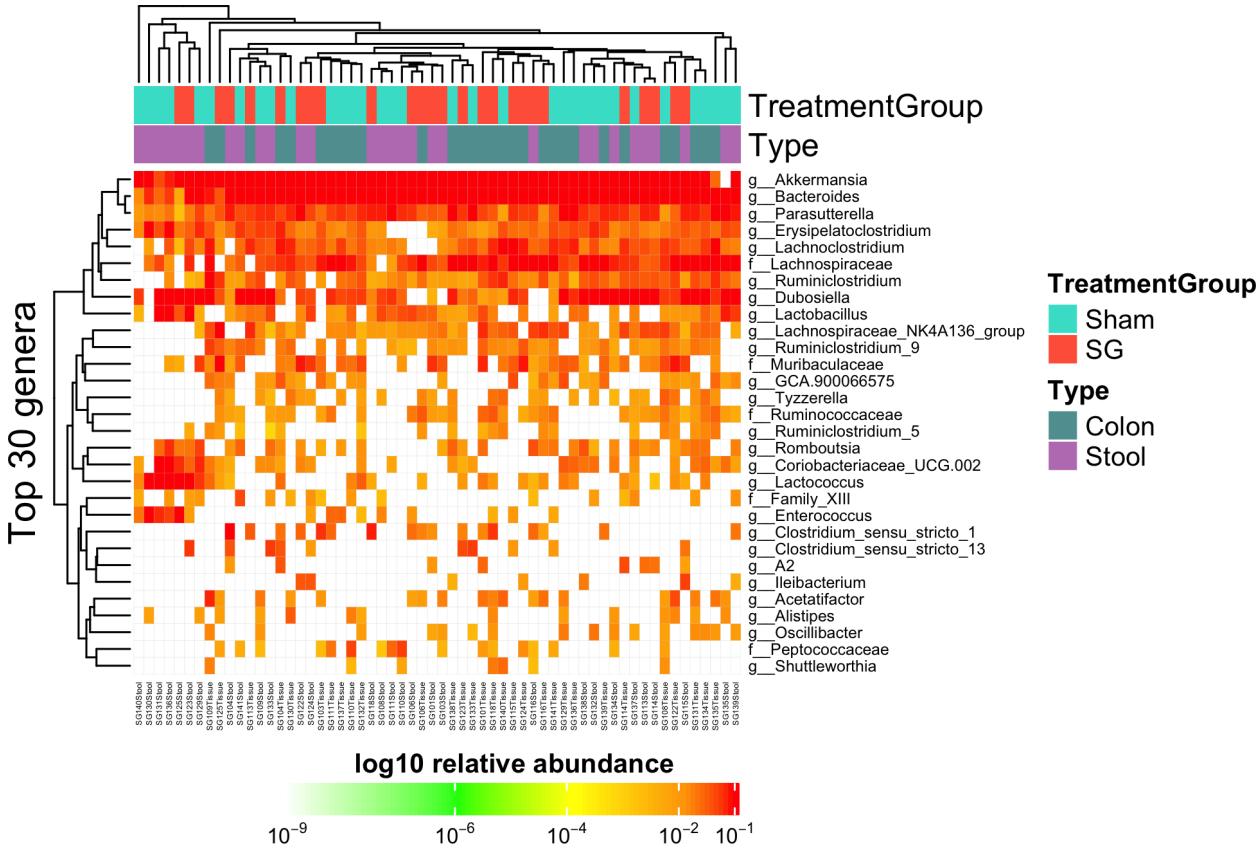
Taxonomy Overview

Data from USEARCH Genera results:
file = all_samples_taxonomy_closed_reference.tsv

Visualized as heatmaps

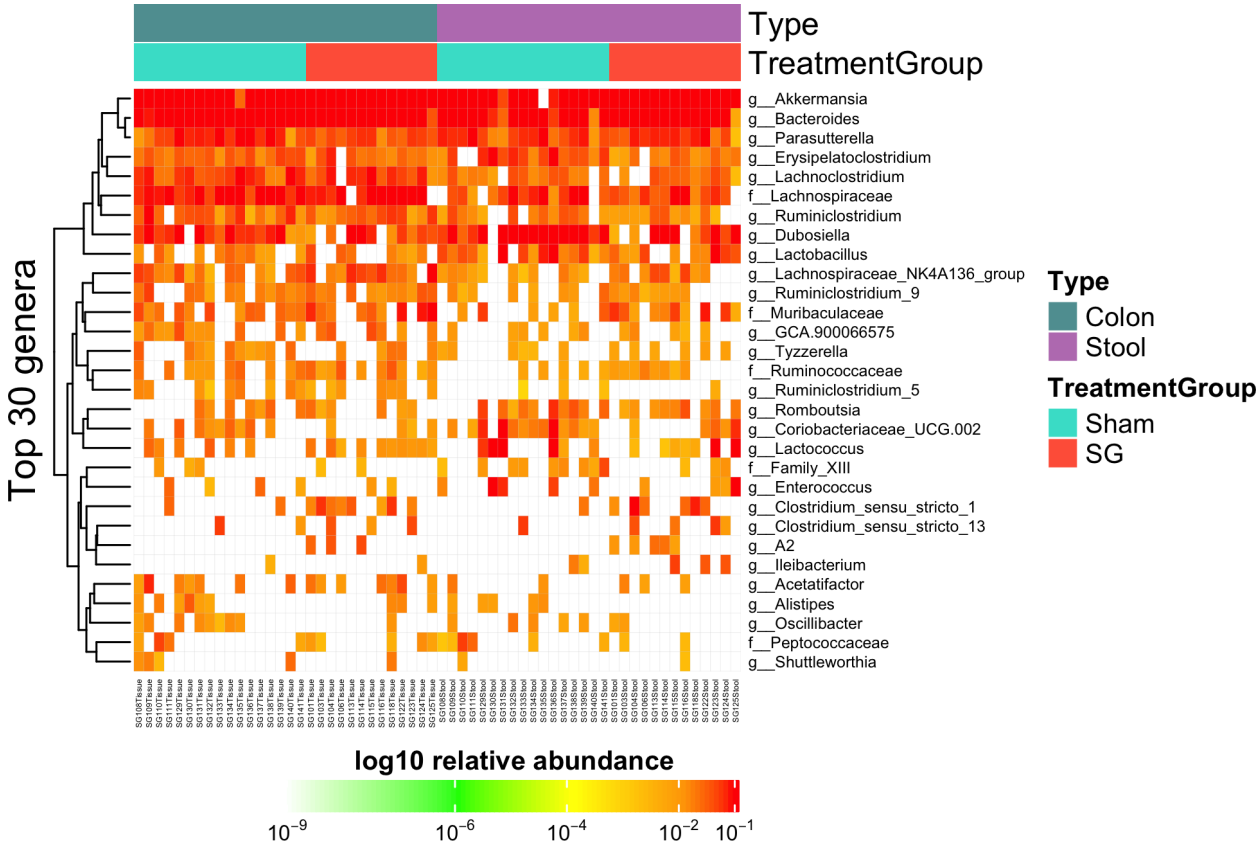
Heatmap clustered based on similar gut compositions

Heatmap of log10 relative abundances of the top 30 genera in samples. Samples (columns) and taxa (rows) are sorted based on Bray-Curtis dissimilarities and Euclidean distances, respectively. These samples display similar to other studies on gut microbiome samples.

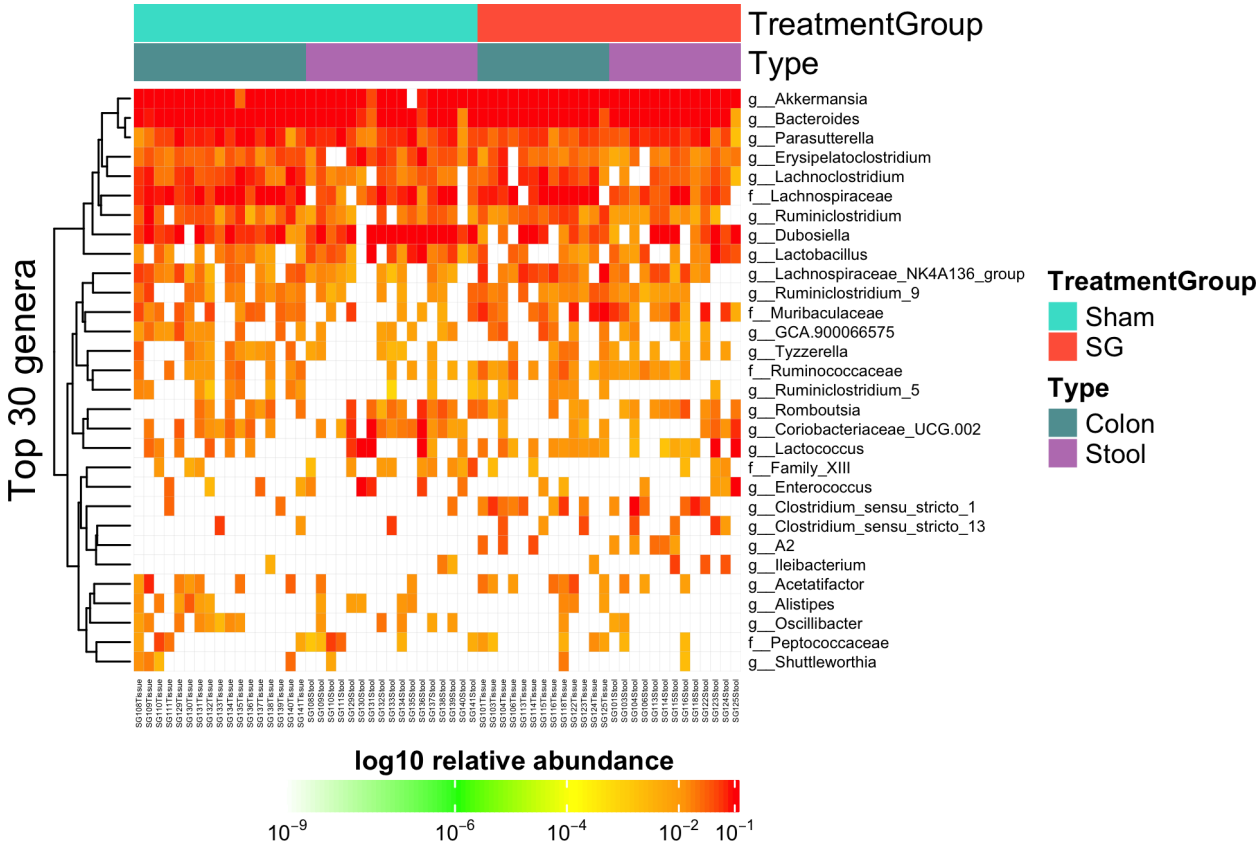


Heatmap clustered based on factors in the metadata

Heatmap of log10 relative abundances of the top 30 genera. Samples (columns) and taxa (rows) are sorted based on Type, TreatmentGroup, and descending overall average relative abundance, respectively.

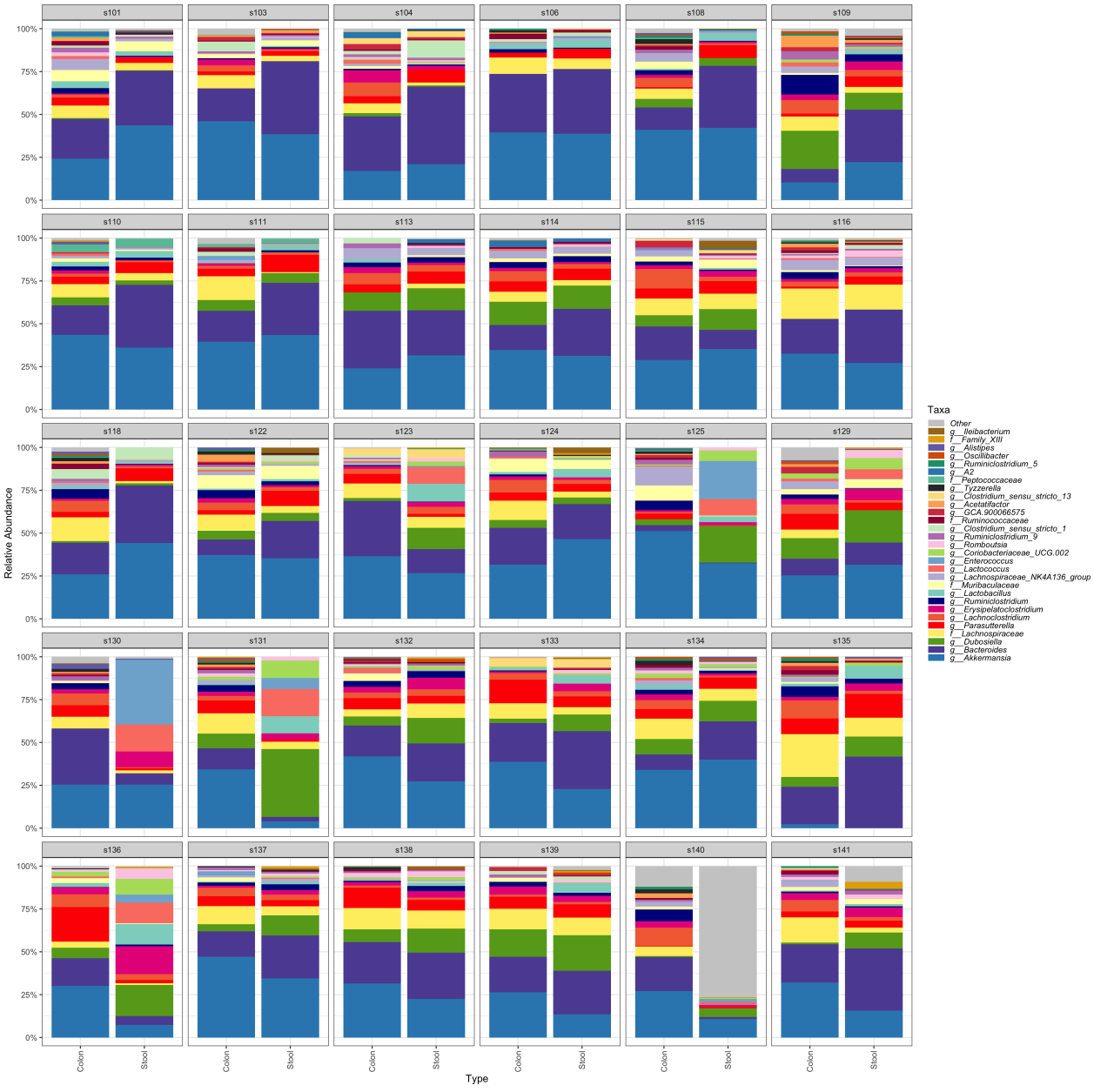


Heatmap of log10 relative abundances of the top 30 genera. Samples (columns) and taxa (rows) are sorted based on TreatmentGroup, Type, and descending overall average relative abundance, respectively.

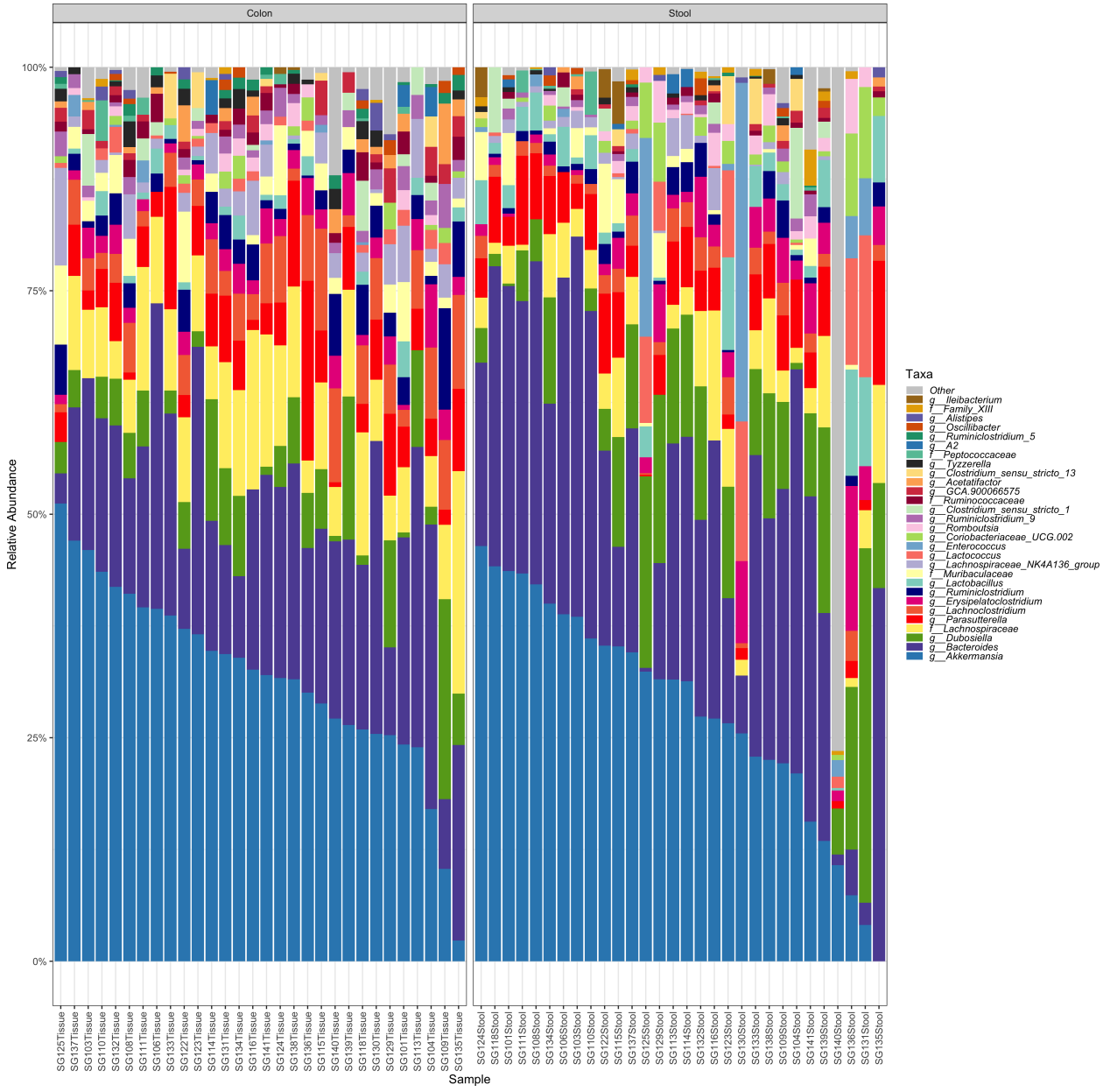


Visualized as stacked barplots

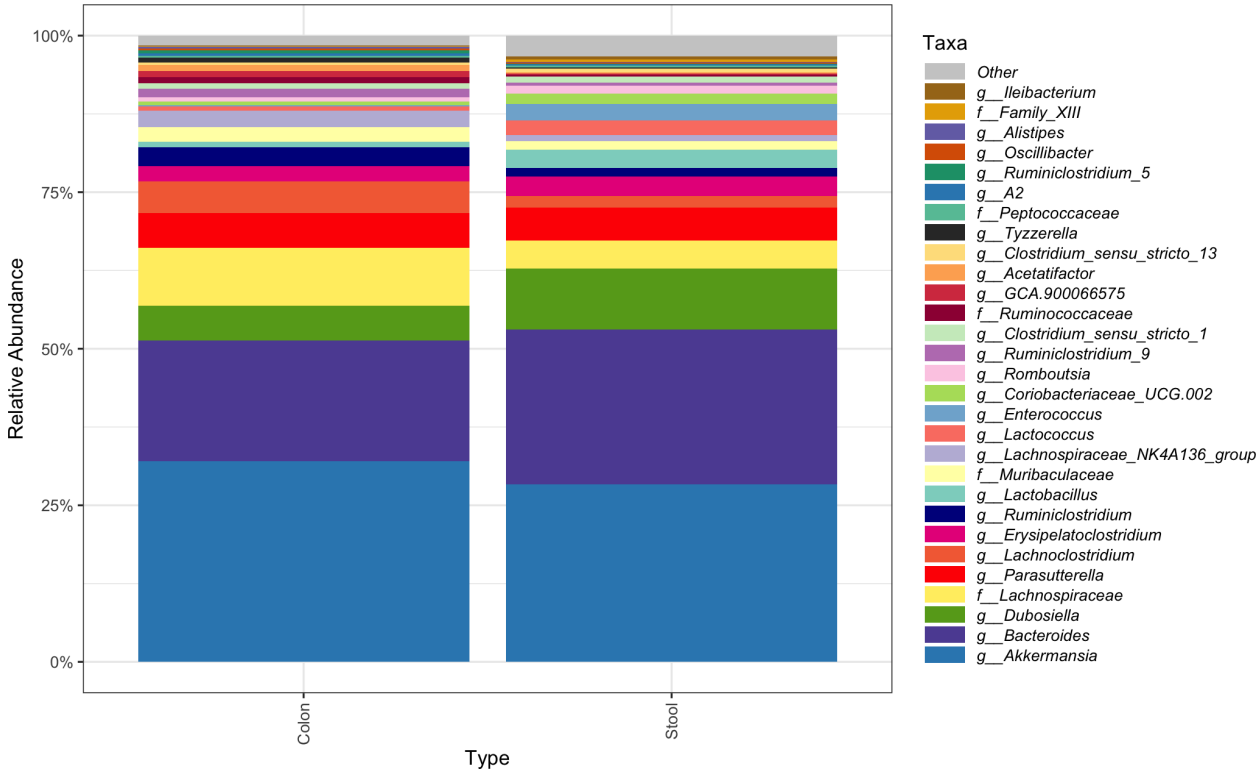
Bar plot of the top 29 abundant taxa in study faceted by StudyID (individual)



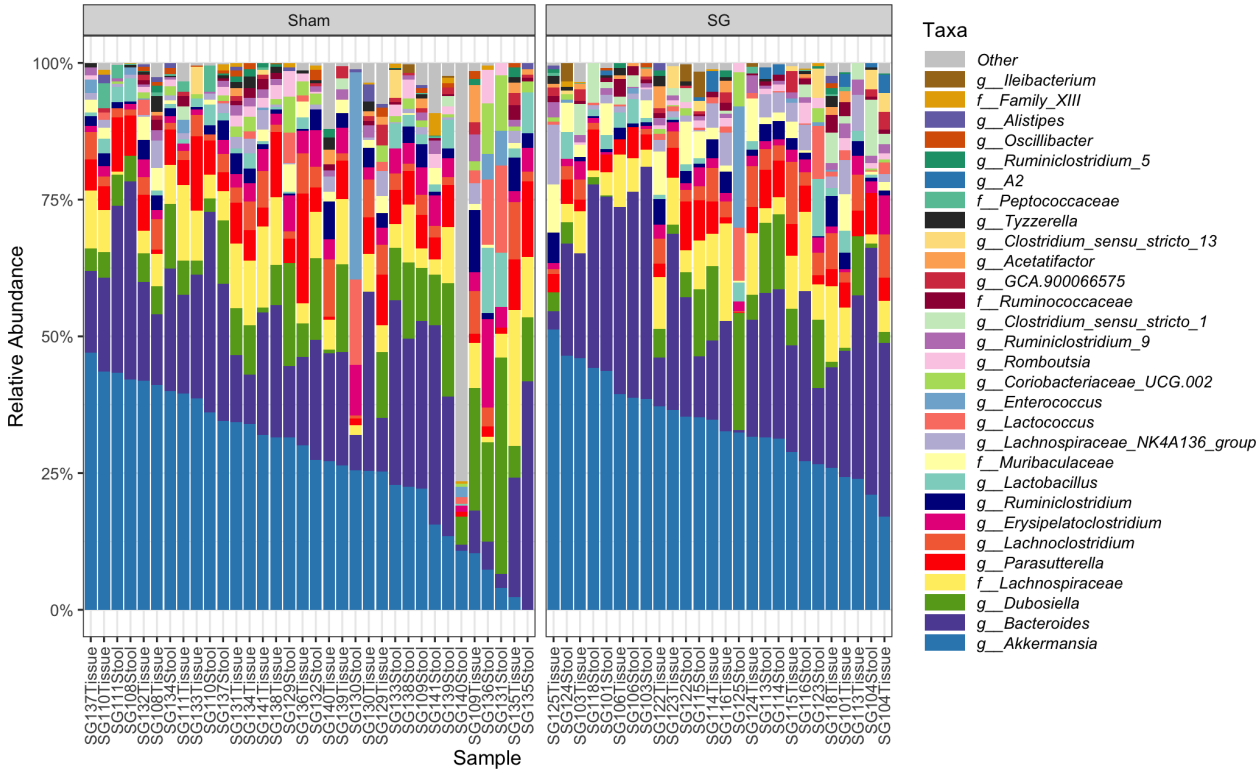
Bar plot of the top 29 abundant taxa in study faceted by Type



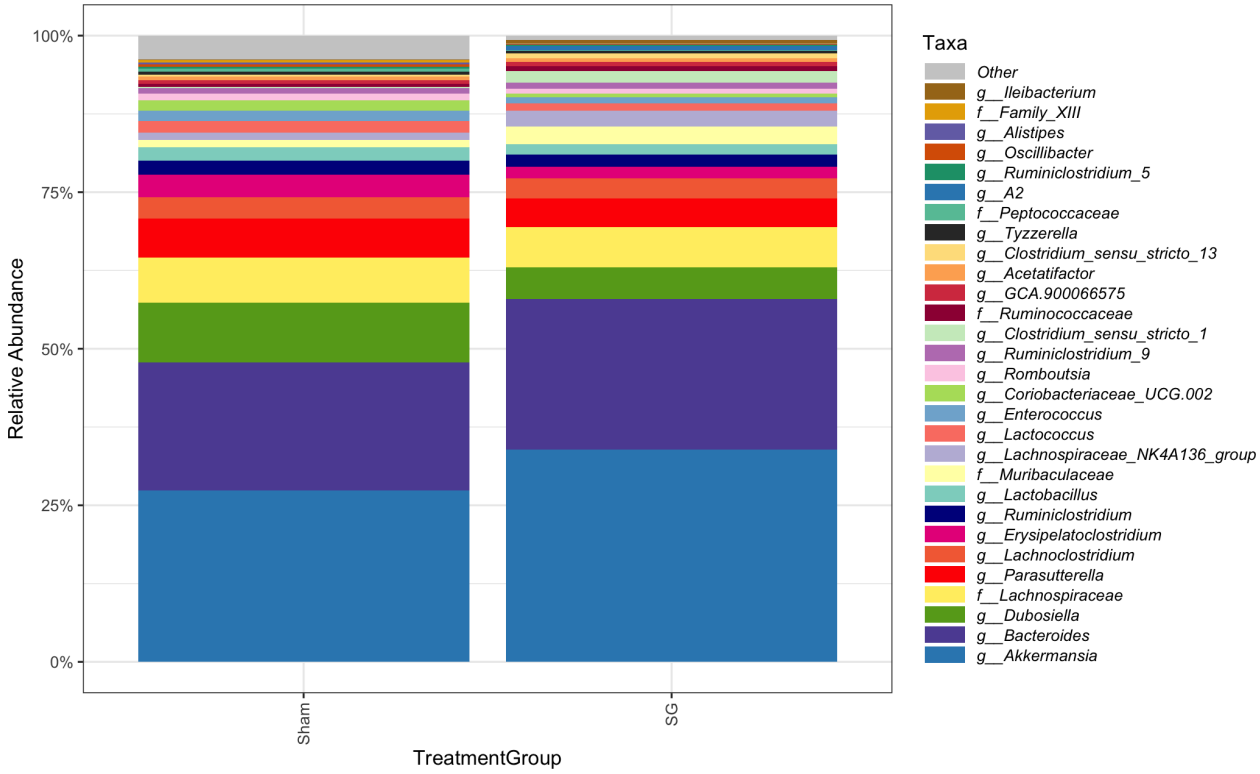
Bar plot of the mean relative abundance of the top 29 taxa in study by Type



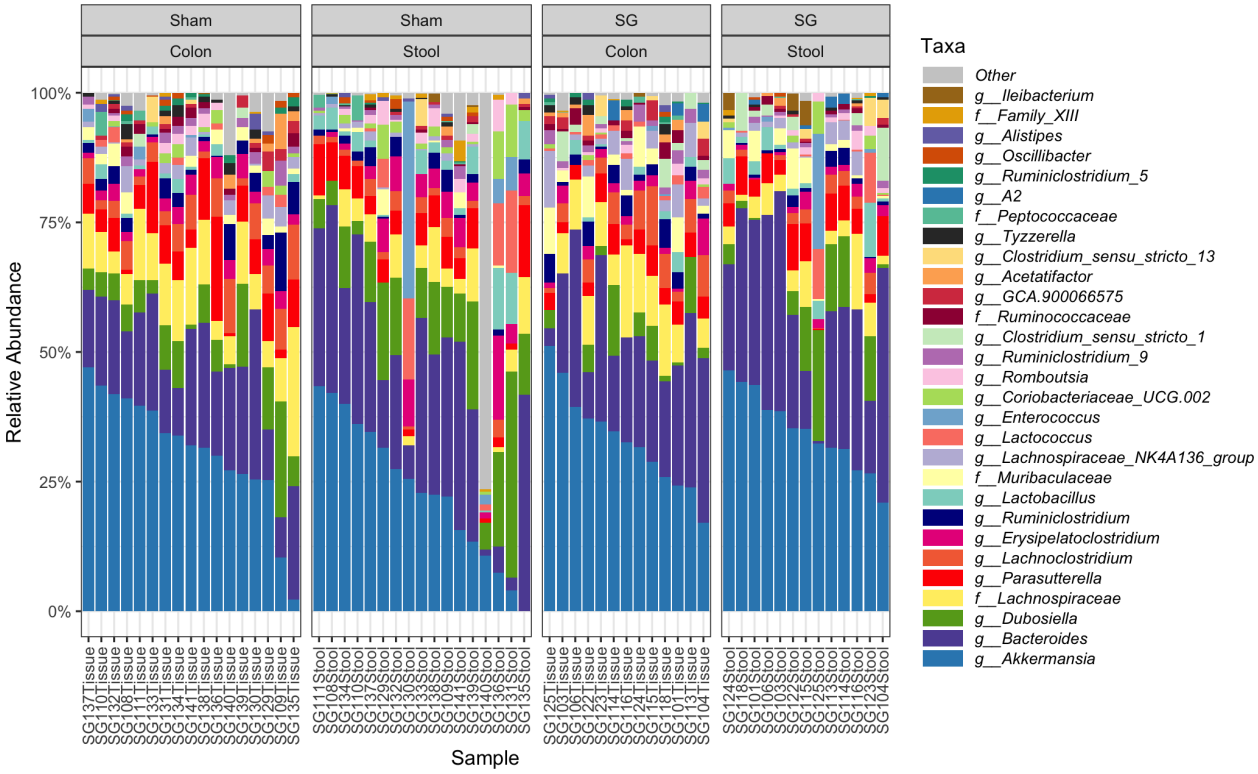
Bar plot of the top 29 abundant taxa in study faceted by TreatmentGroup



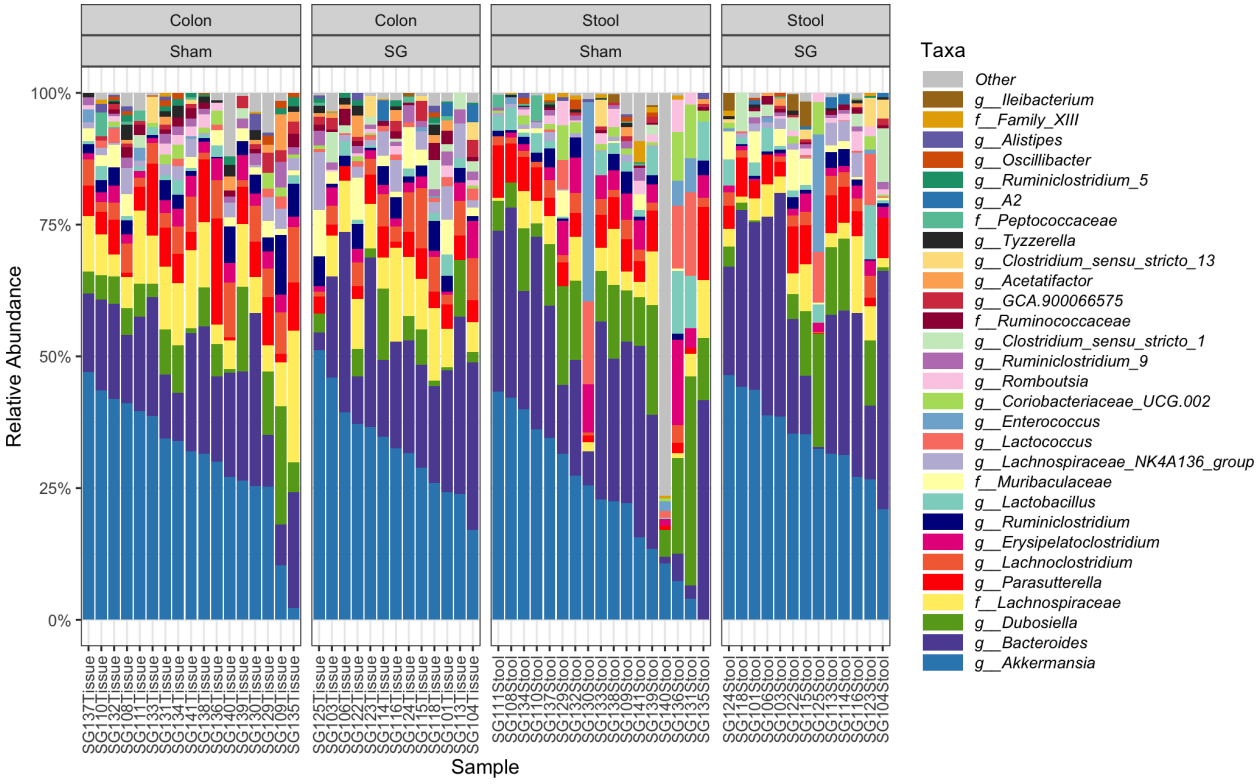
Bar plot of the mean relative abundance of the top 29 taxa in study by TreatmentGroup



Bar plot of the top 29 abundant taxa in study faceted by Type and TreatmentGroup



Bar plot of the top 29 abundant taxa in study faceted by TreatmentGroup and Type

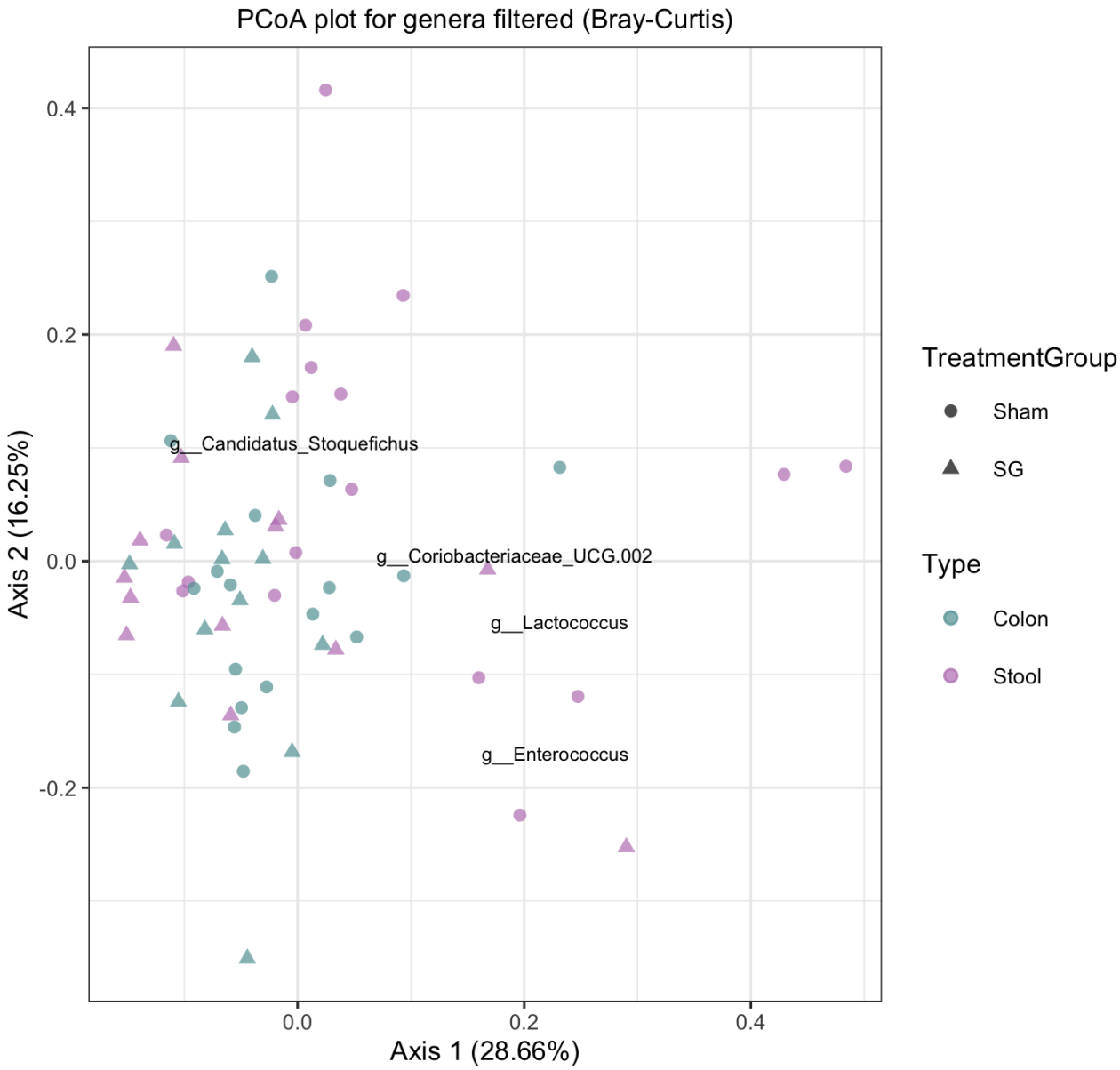


Beta Diversity: Bray-Curtis dissimilarity

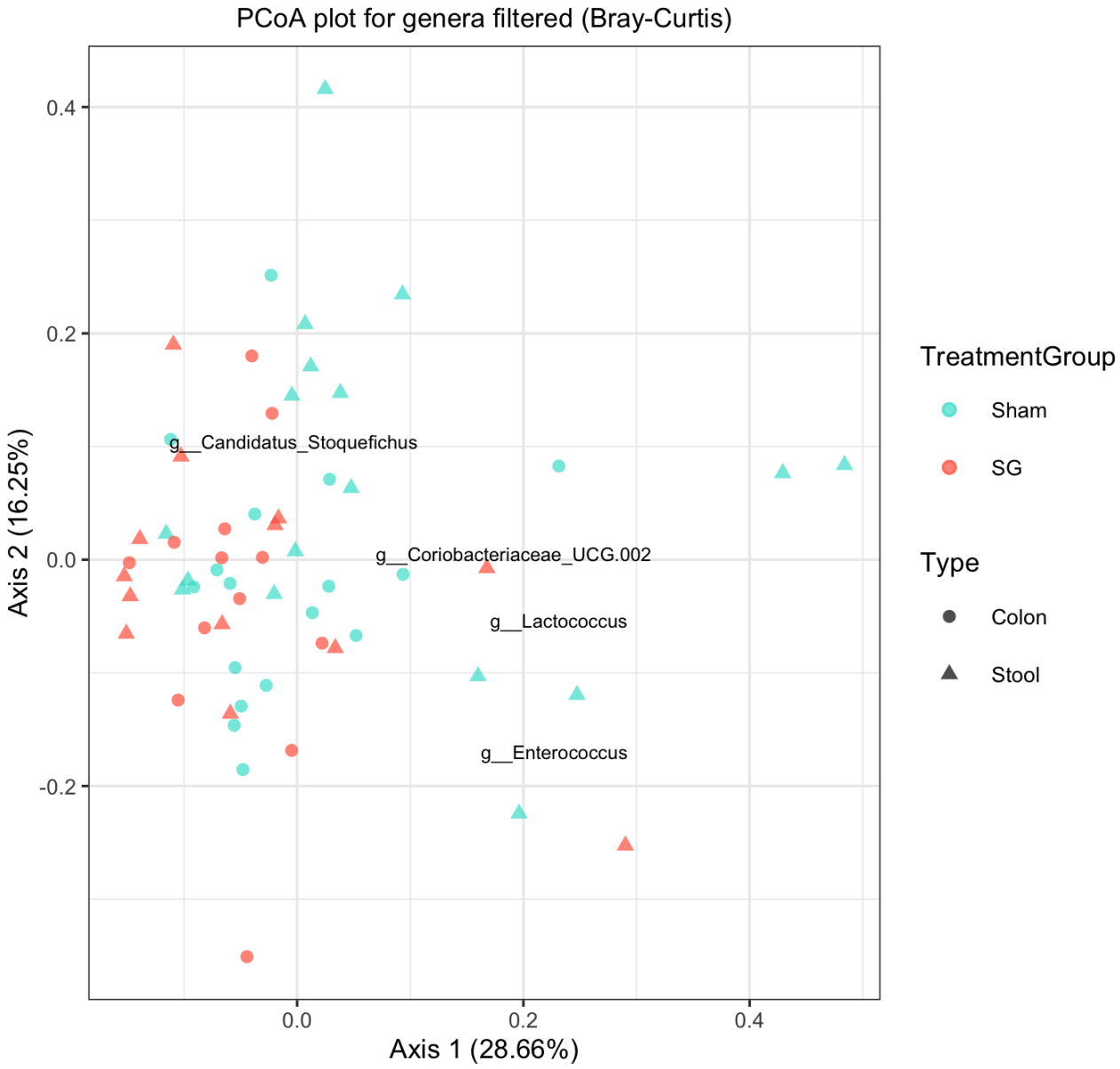
Genus-level abundances are passed through a basic filter requiring each genus to have at least 0.01 % abundance in at least 10 % of all samples. A total of 53 genera were identified. After basic filtering and aggragation of the data 33 genera remained.

Principal coordinates analysis colored by covariates

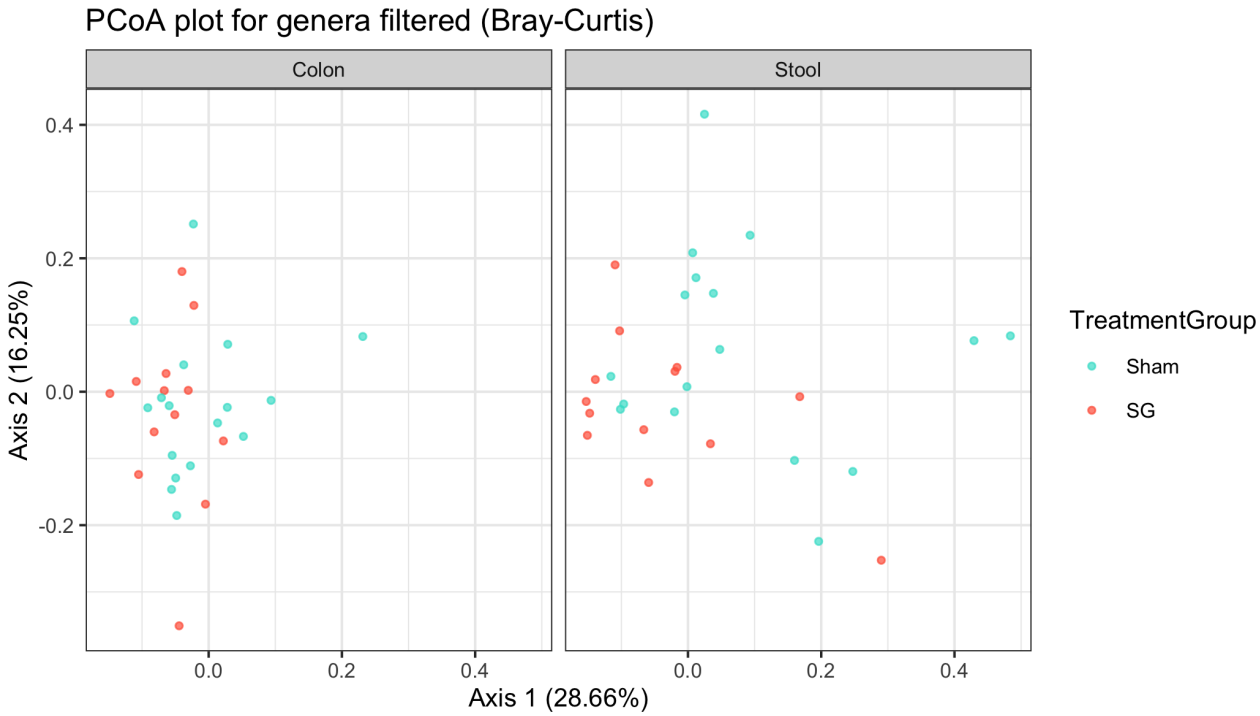
Principal coordinate analysis colored by Type, shaped by TreatmentGroup



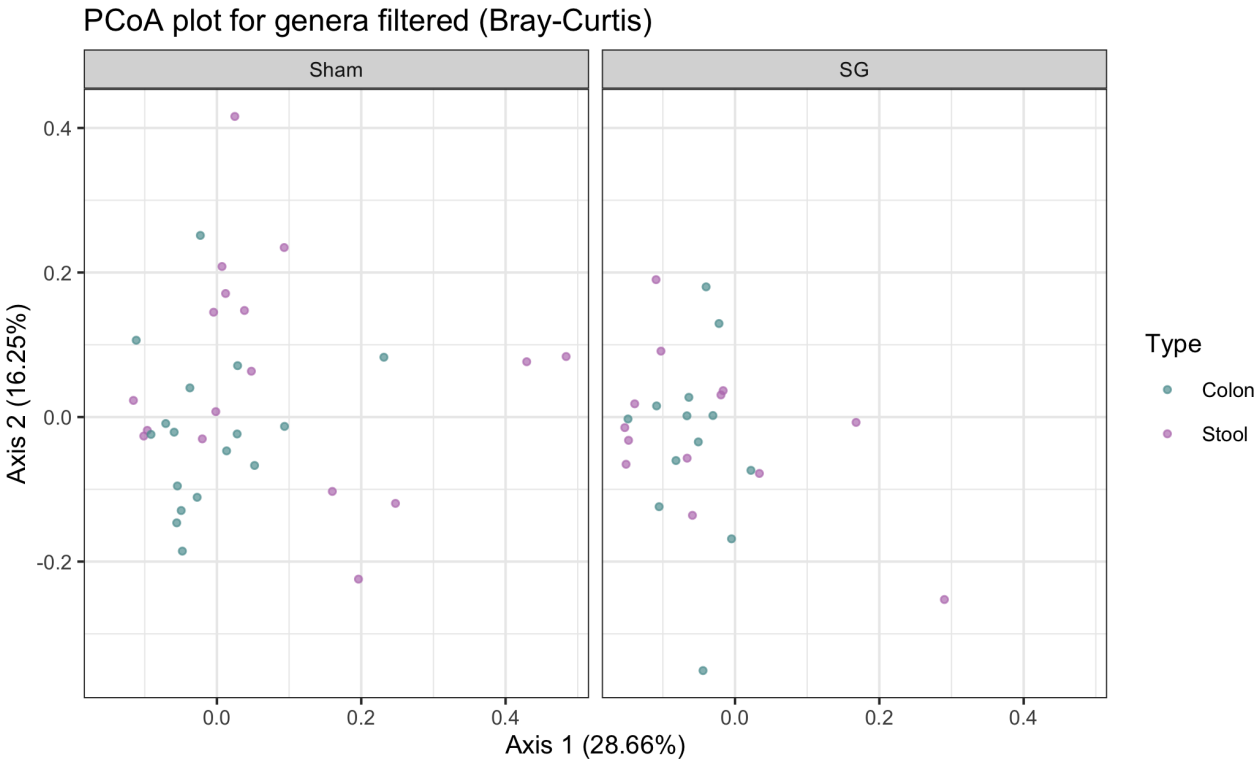
Principal coordinate analysis colored by TreatmentGroup, shaped by Type



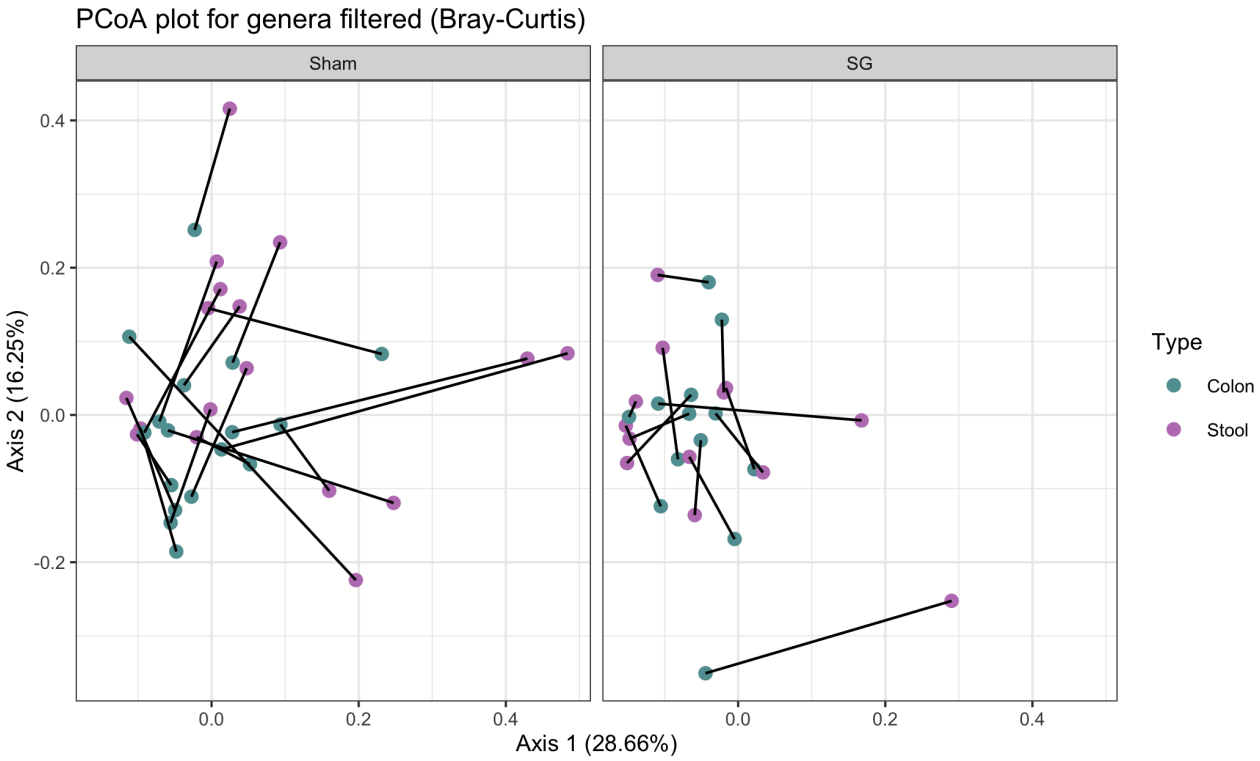
Principal coordinate analysis colored by TreatmentGroup and faceted by Type



Principal coordinate analysis colored by Type and faceted by TreatmentGroup



PCoA colored by Type, faceted by TreatmentGroup, and points are connected if they are from the same individual.

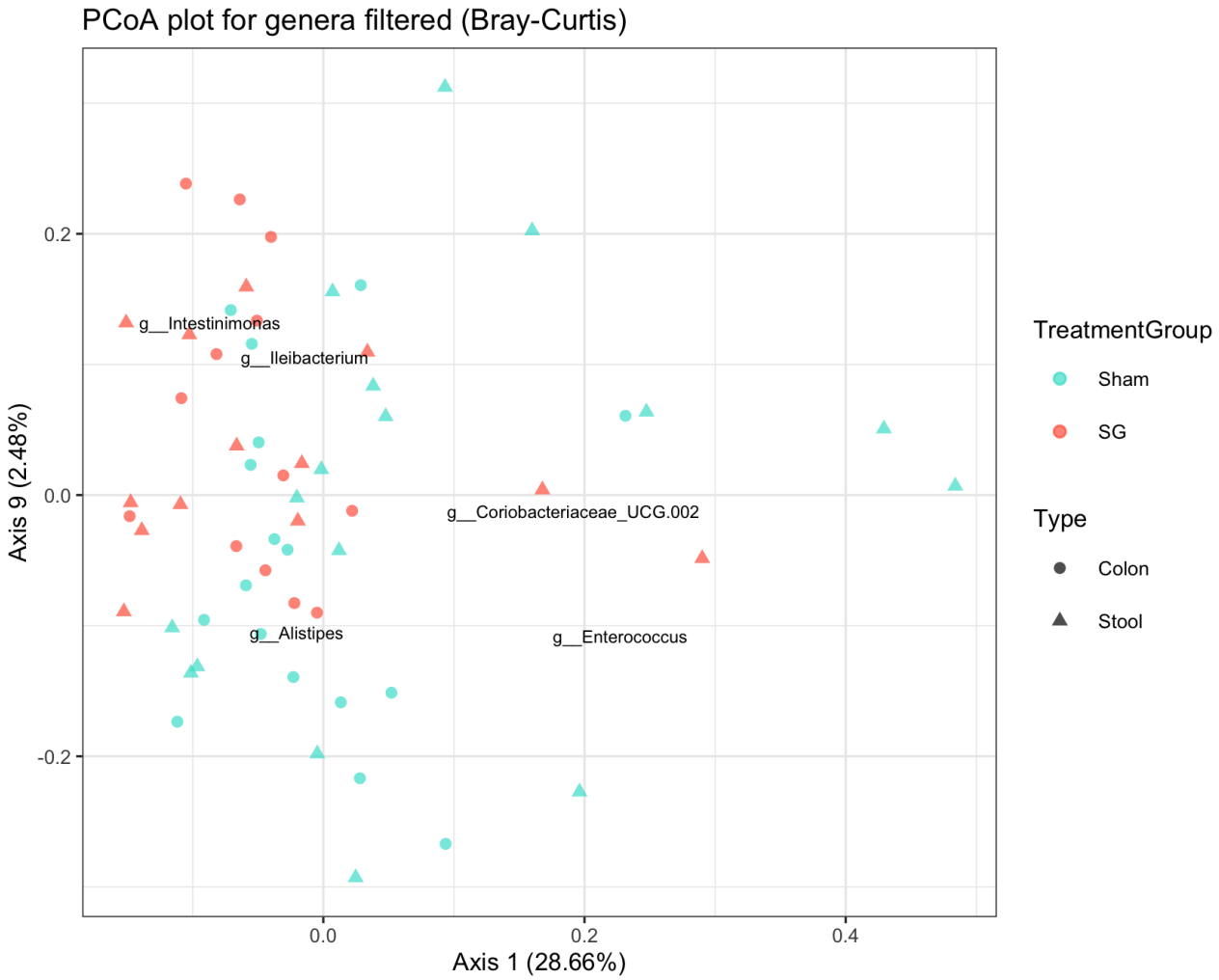


Finding the PCs that are most significantly associated with metadata factors among all samples.

TreatmentGroup

PC	pval
MDS1	0.013526 *
MDS9	0.028257 *
MDS2	0.142933
MDS4	0.17392
MDS6	0.276299
MDS5	0.303177
MDS8	0.35821
MDS7	0.393631
MDS3	0.500367

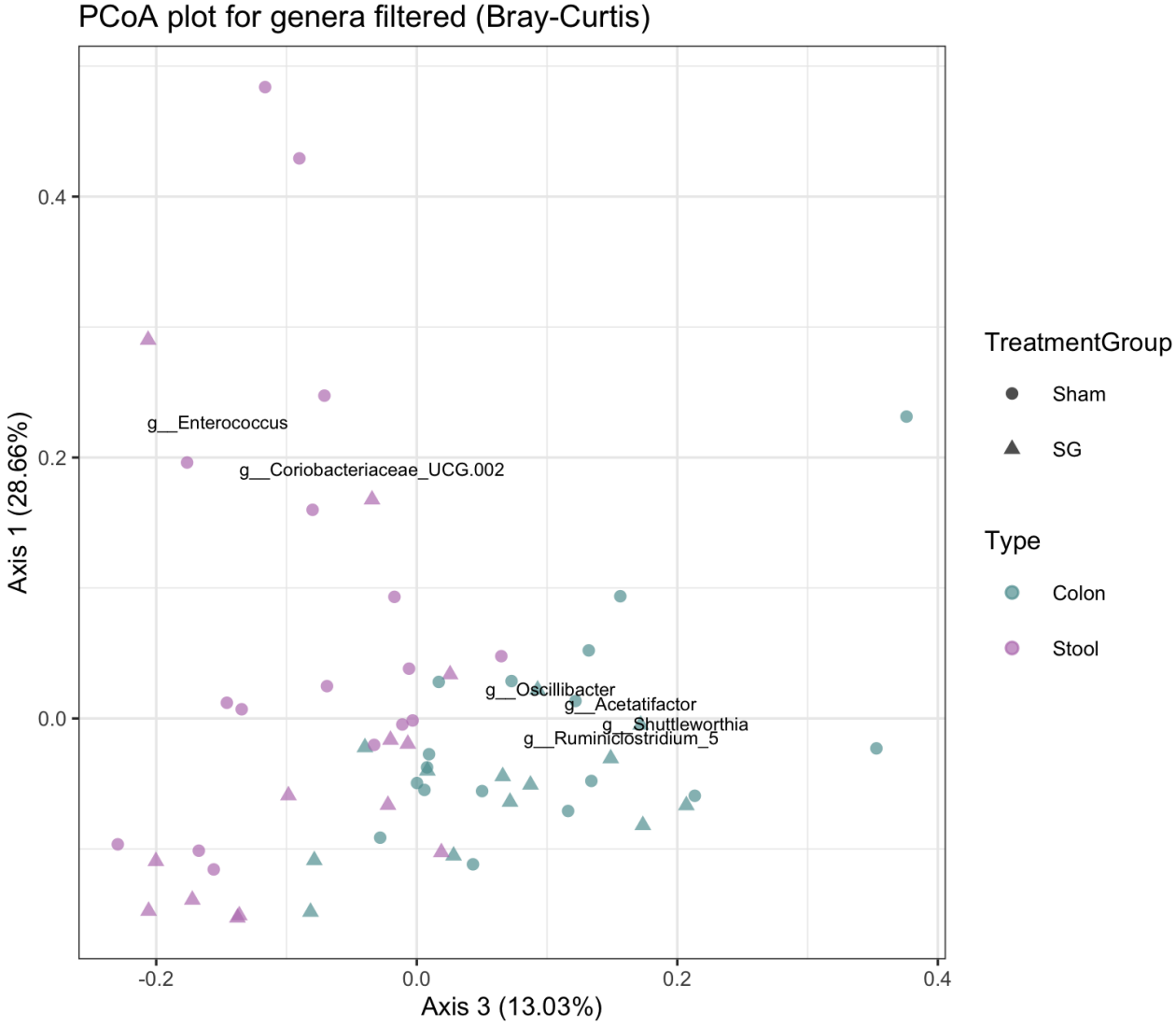
Principal coordinate analysis of MDS1 and MDS9, colored by TreatmentGroup, shaped by Type



Type

PC	pval
MDS3	0 ***
MDS1	0.065364 ‘
MDS2	0.12358
MDS6	0.347818
MDS8	0.478751
MDS4	0.523971
MDS9	0.672311
MDS5	0.83748
MDS7	0.913071

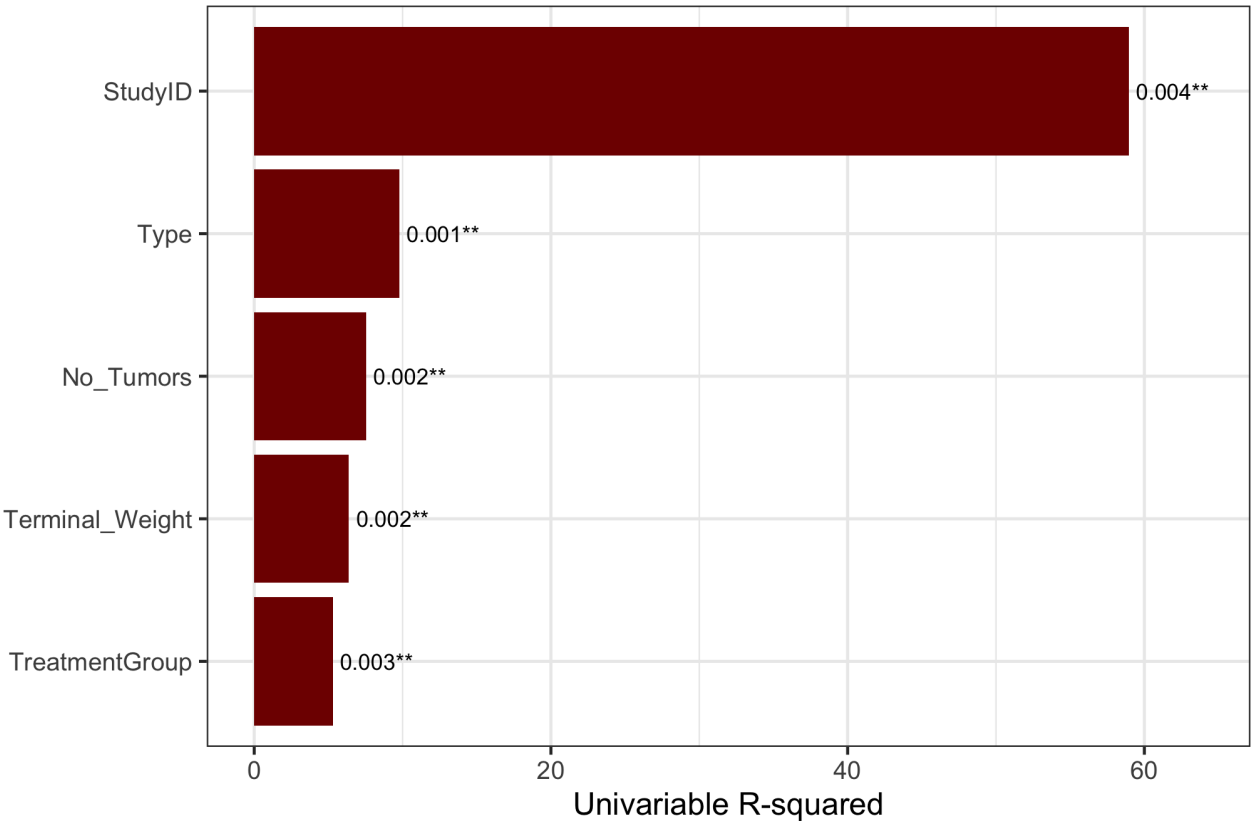
Principal coordinate analysis of MDS3 and MDS1, colored by Type, shaped by TreatmentGroup



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable- Bray-Curtis dissimilarity

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

TreatmentGroup + Type + Standard Clinical Covarites

```
##
## Call:
## adonis(formula = bray ~ TreatmentGroup + Type + No_Tumors + Terminal_Weight,      data = meta_dat)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup   1    0.2813 0.28126  3.6719 0.05291  0.003 **
## Type             1    0.5199 0.51988  6.7871 0.09779  0.001 ***
## No_Tumors        1    0.1967 0.19667  2.5675 0.03699  0.029 *
## Terminal_Weight  1    0.1054 0.10544  1.3766 0.01983  0.194
## Residuals       55    4.2129 0.07660         0.79247
## Total           59    5.3161                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

TreatmentGroup + Type

```
##
## Call:
## adonis(formula = bray ~ TreatmentGroup + Type, data = meta_dat)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup  1    0.2813 0.28126  3.5508 0.05291  0.002 **
## Type            1    0.5199 0.51988  6.5633 0.09779  0.001 ***
## Residuals      57    4.5150 0.07921         0.84930
## Total          59    5.3161                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

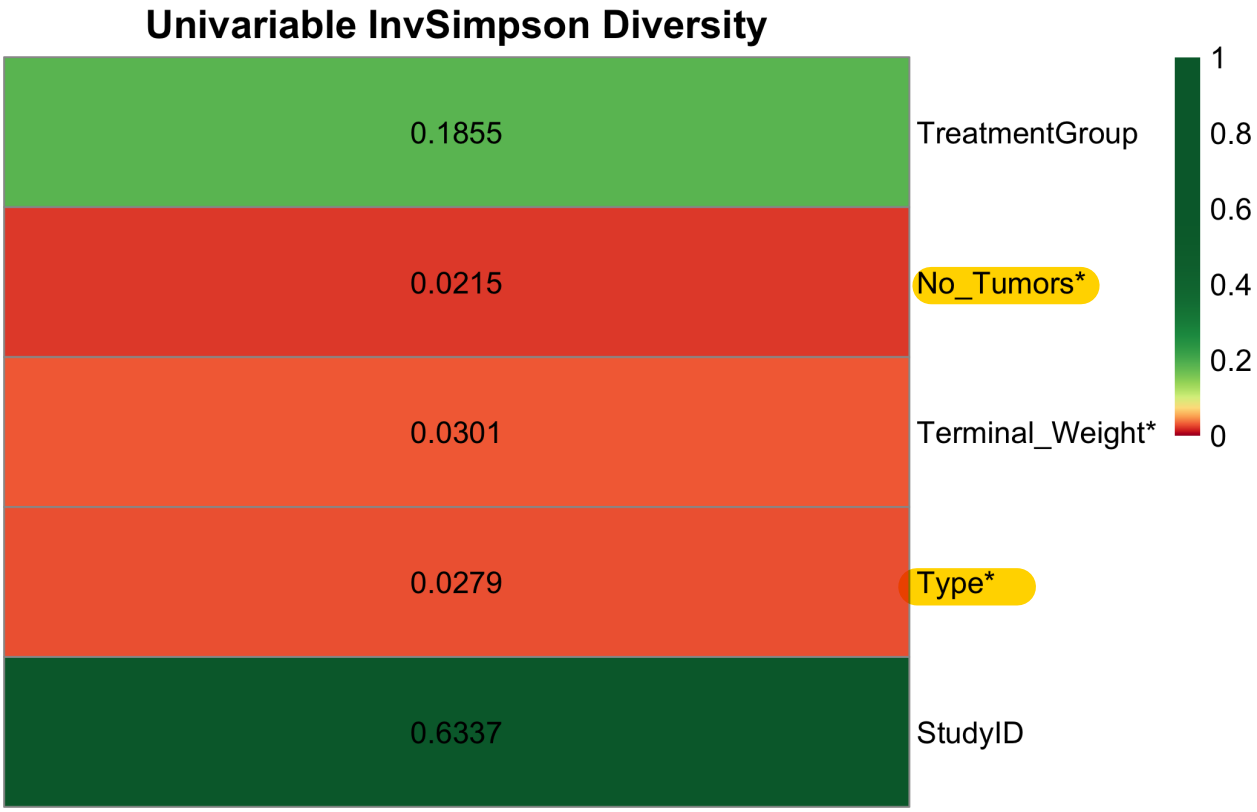
TreatmentGroup + Type + TreatmentGroup*Type

```
##
## Call:
## adonis(formula = bray ~ TreatmentGroup * Type, data = meta_dat)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                     Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup       1    0.2813 0.28126  3.5406 0.05291  0.005 **
## Type                 1    0.5199 0.51988  6.5444 0.09779  0.001 ***
## TreatmentGroup:Type  1    0.0665 0.06646  0.8366 0.01250  0.527
## Residuals           56    4.4485 0.07944         0.83680
## Total               59    5.3161                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Alpha Diversity

Statistics alpha diversity: anova on linear model

Heatmap annotated with the p-value for each covariate



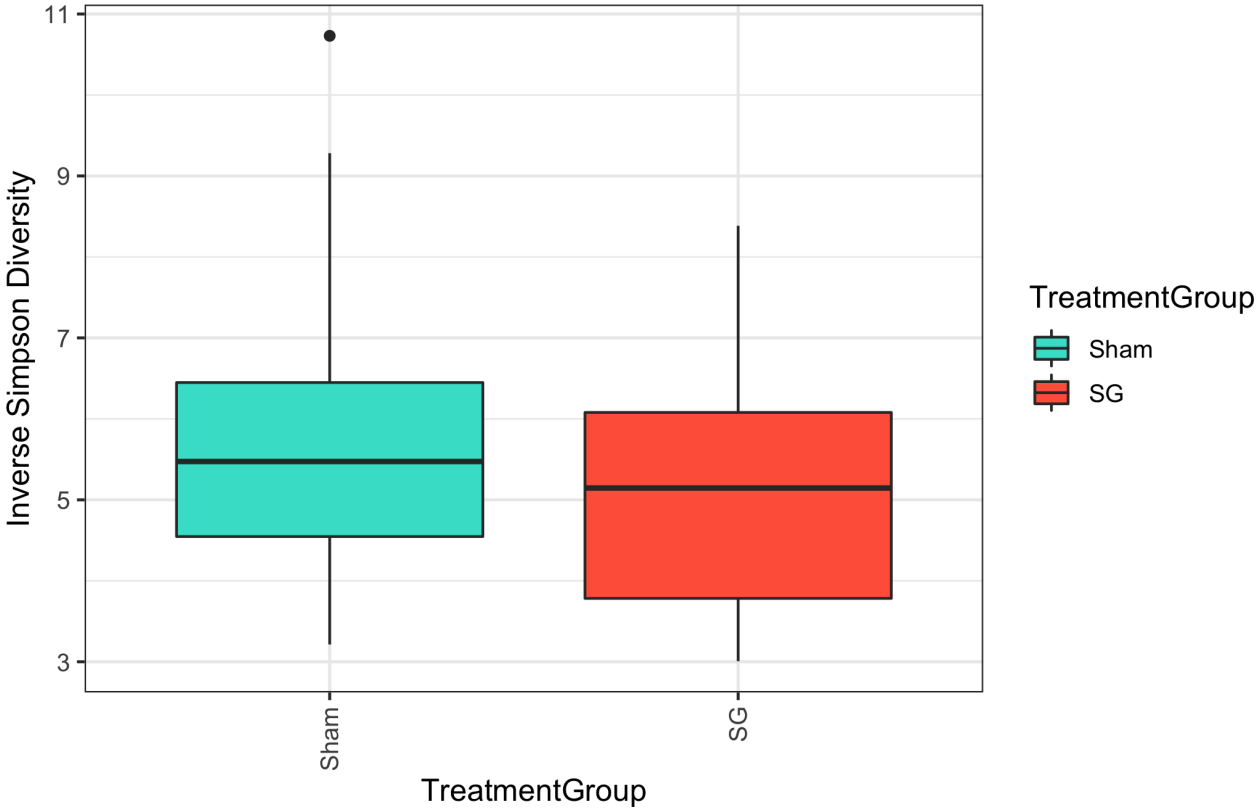
Multivariable model for Alpha diversity

```
## Analysis of Variance Table
##
## Response: InvSimpson
##                  Df  Sum Sq Mean Sq F value  Pr(>F)
## TreatmentGroup    1   5.009  5.0089  2.0228 0.16060
## Type              1  13.452 13.4521  5.4326 0.02346 *
## No_Tumors         1   9.990  9.9900  4.0345 0.04950 *
## Terminal_Weight   1   2.204  2.2037  0.8900 0.34961
## Residuals        55 136.189  2.4762
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

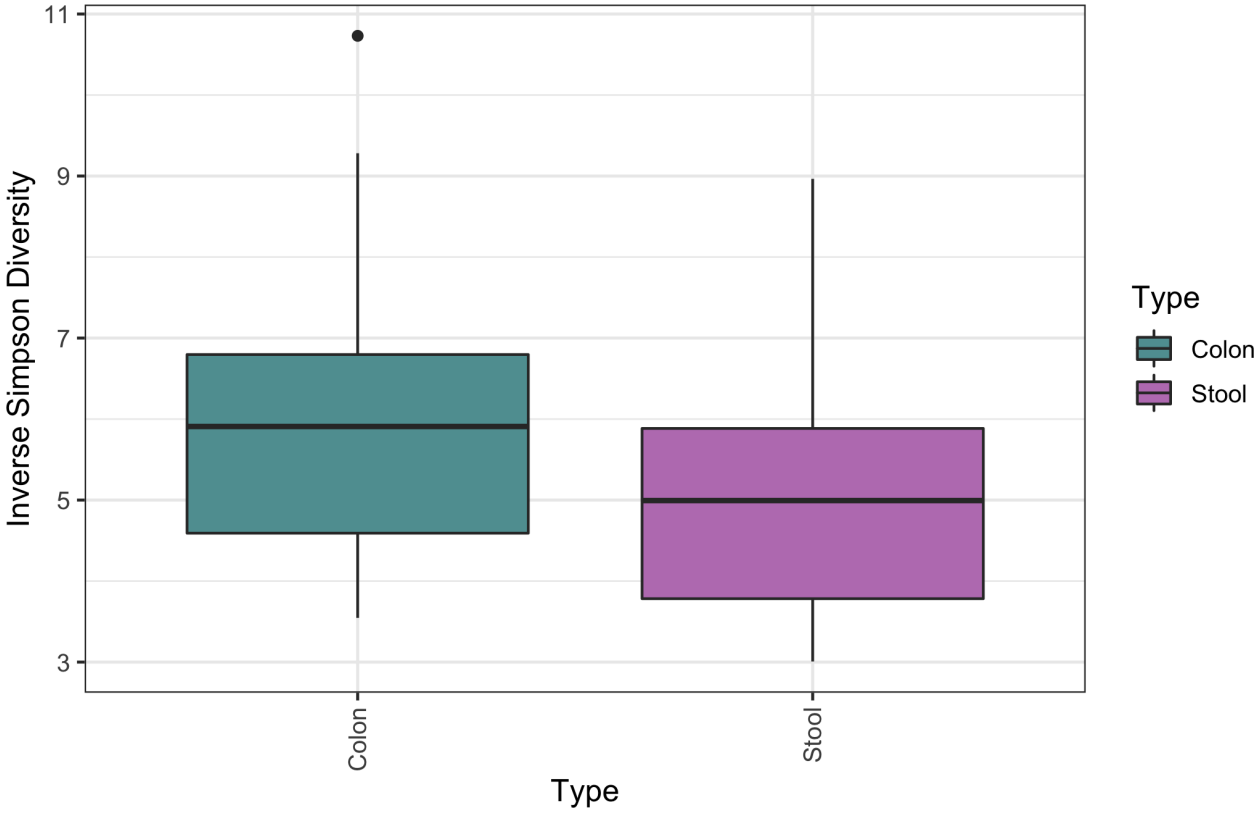
```
## Analysis of Variance Table
##
## Response: InvSimpson
##                 Df  Sum Sq Mean Sq F value Pr(>F)
## TreatmentGroup   1   5.009  5.0089  1.9241 0.1708
## Type             1  13.452 13.4521  5.1675 0.0268 *
## Residuals       57 148.383  2.6032
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Analysis of Variance Table
##
## Response: InvSimpson
##                      Df  Sum Sq Mean Sq F value  Pr(>F)
## TreatmentGroup        1   5.009  5.0089  1.8935 0.17429
## Type                  1  13.452 13.4521  5.0852 0.02806 *
## TreatmentGroup:Type   1   0.244  0.2438  0.0922 0.76258
## Residuals            56 148.139  2.6453
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

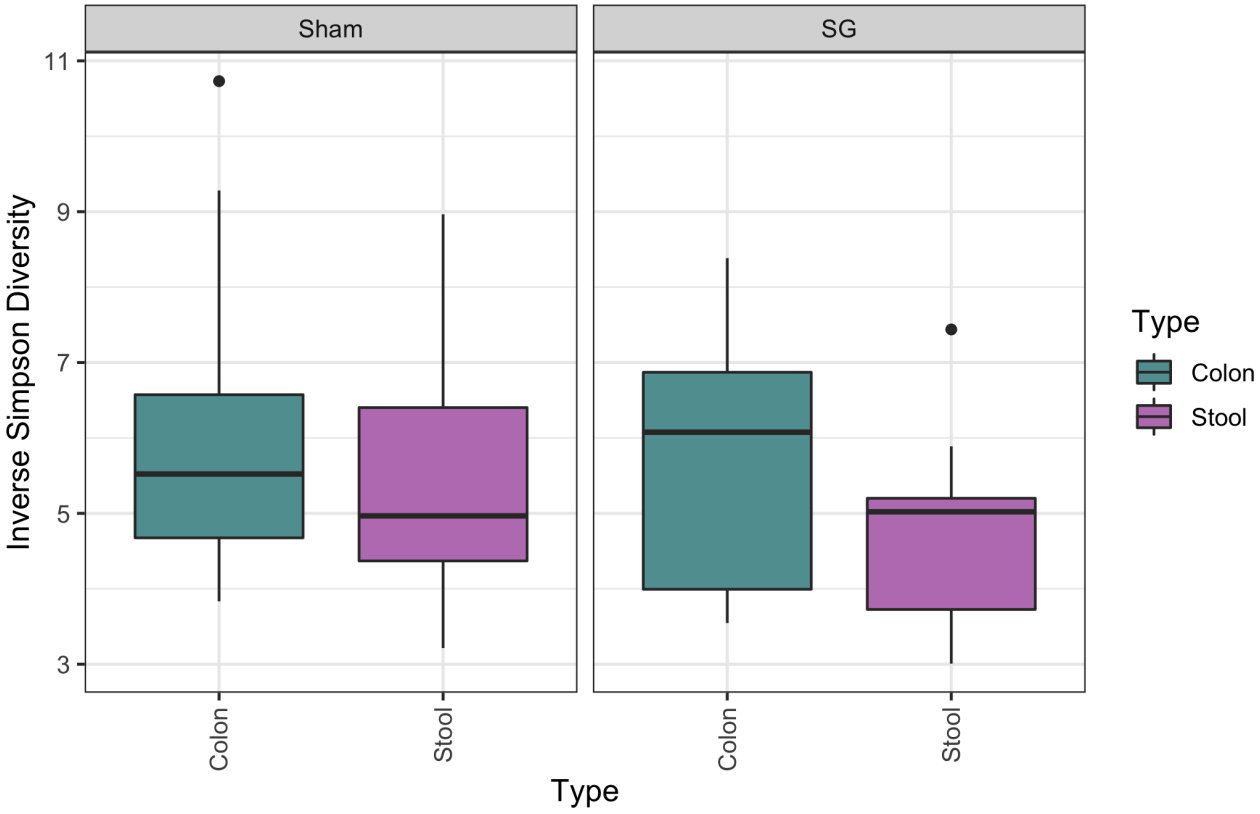
Invsimpson vs. TreatmentGroup



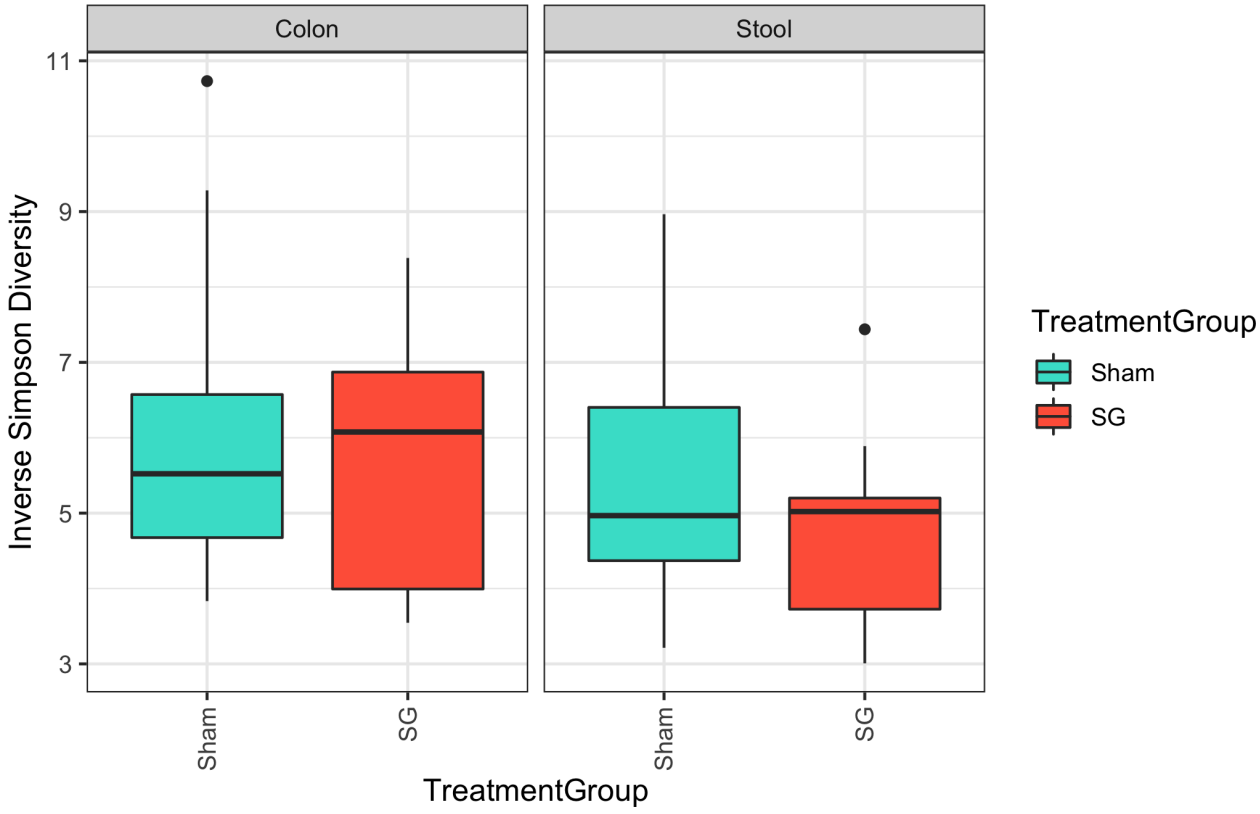
Invsimpson vs. Type



Invsimpson vs. Type by TreatmentGroup



Invsimpson vs. TreatmentGroup by Type

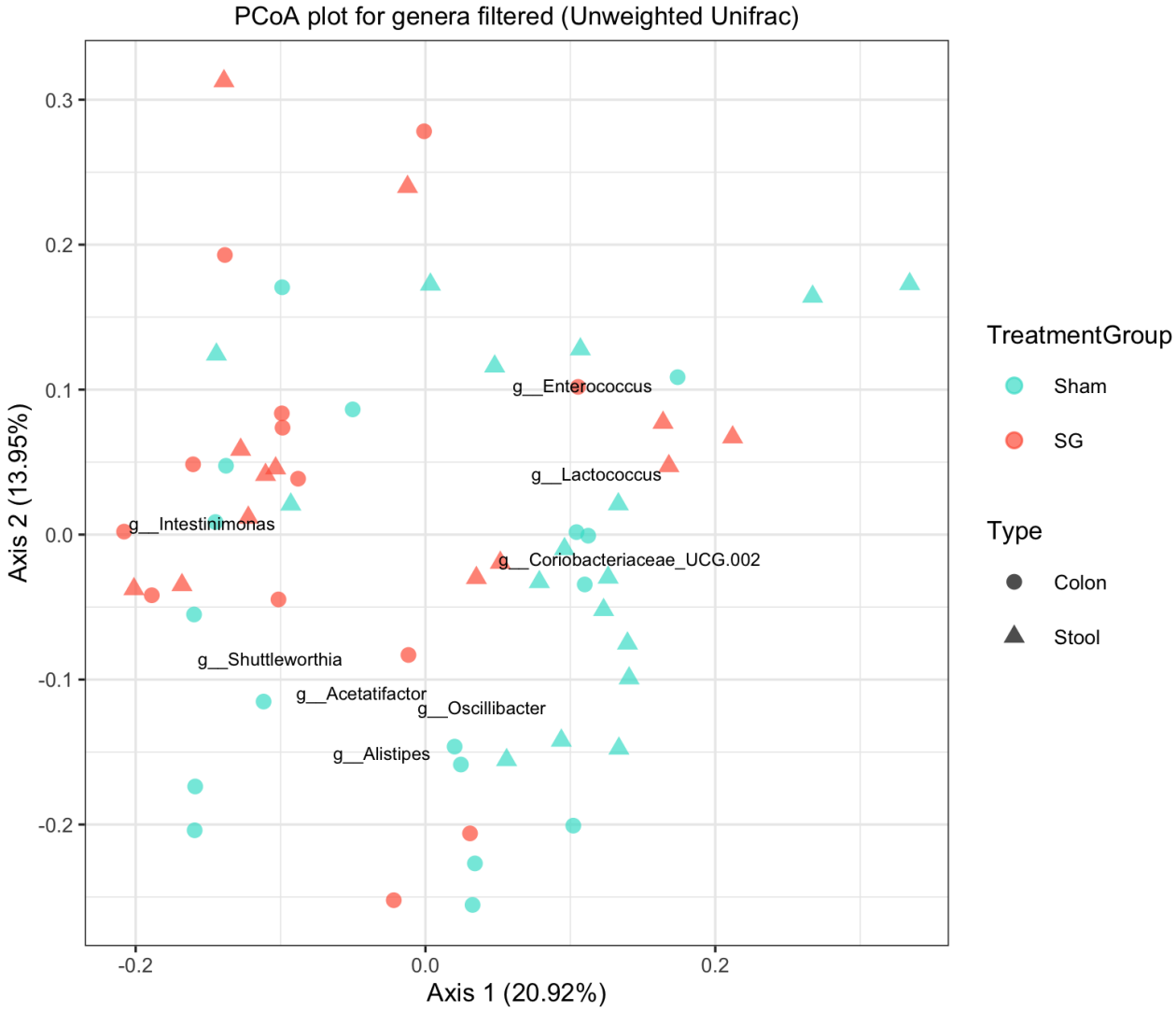


Other Beta Diversity Metrics

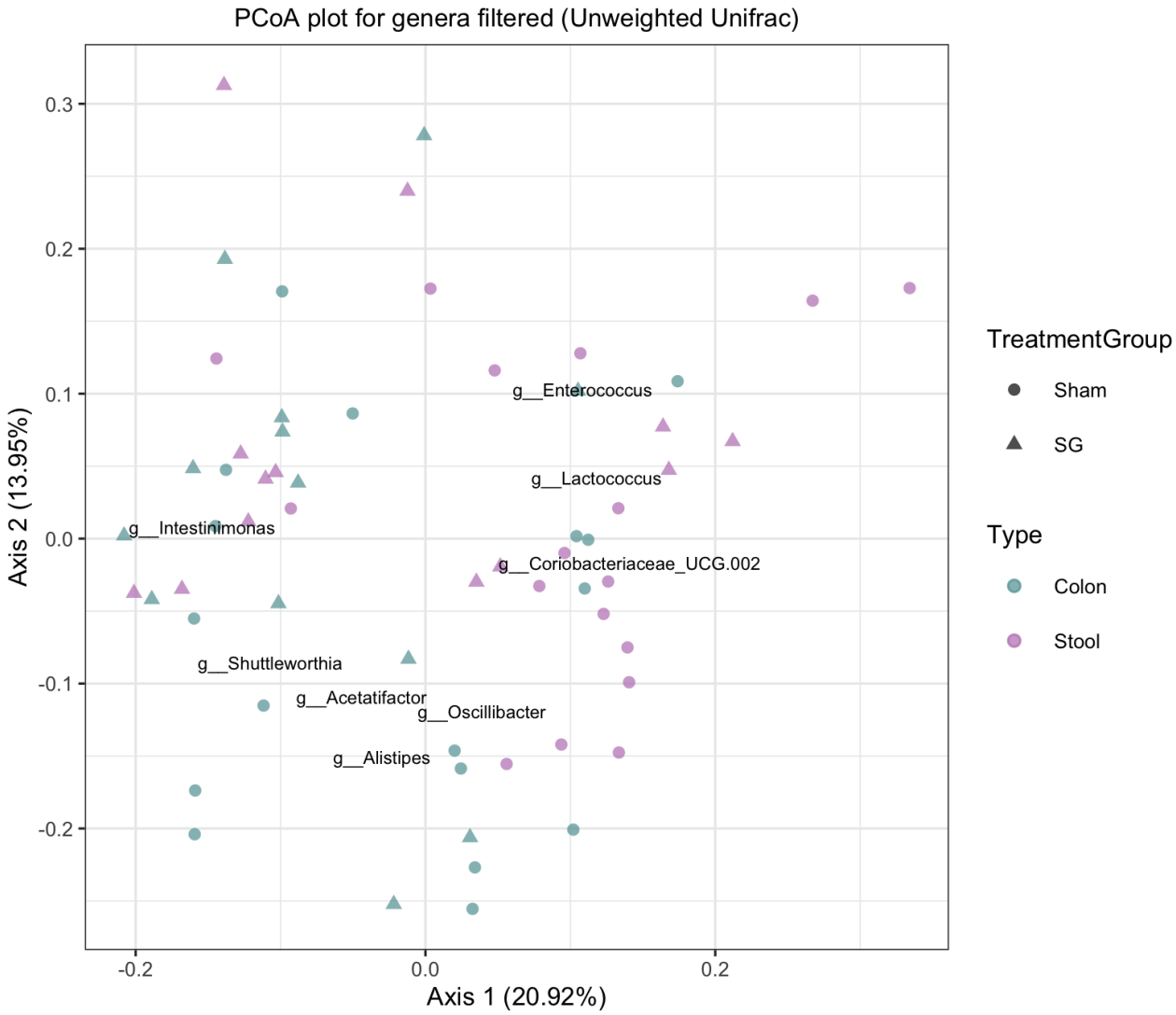
Unweighted UniFrac

Principal coordinates analysis colored by covariates

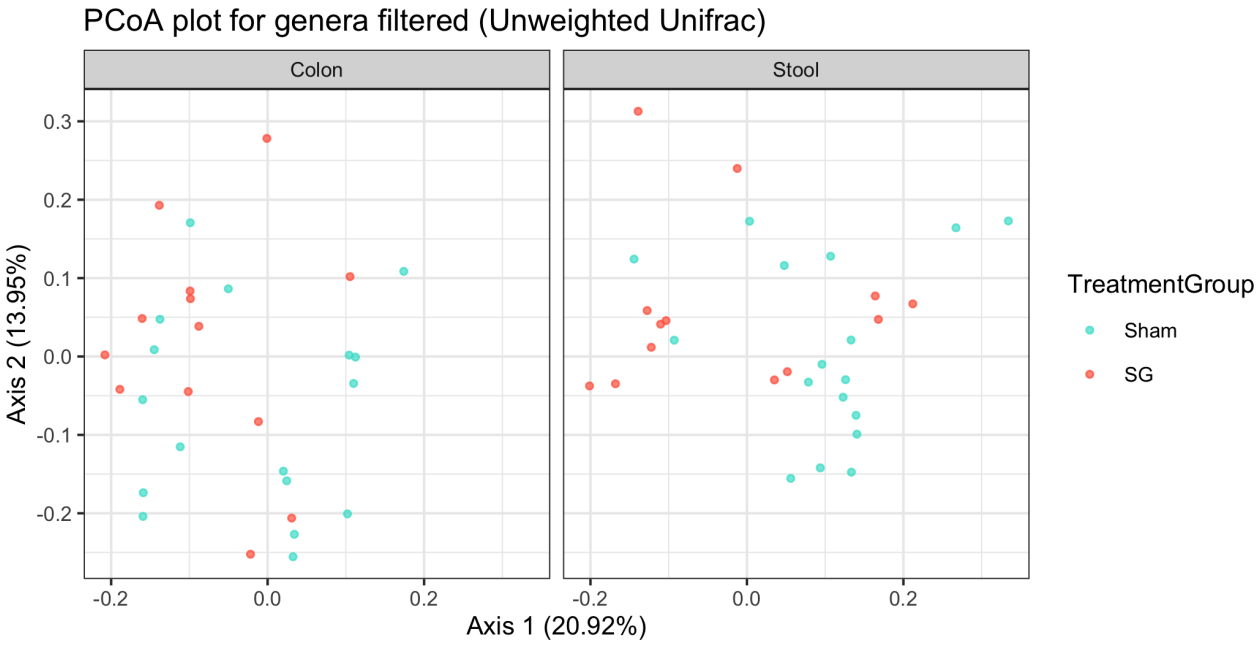
Principal coordinate analysis colored by TreatmentGroup, shaped by Type



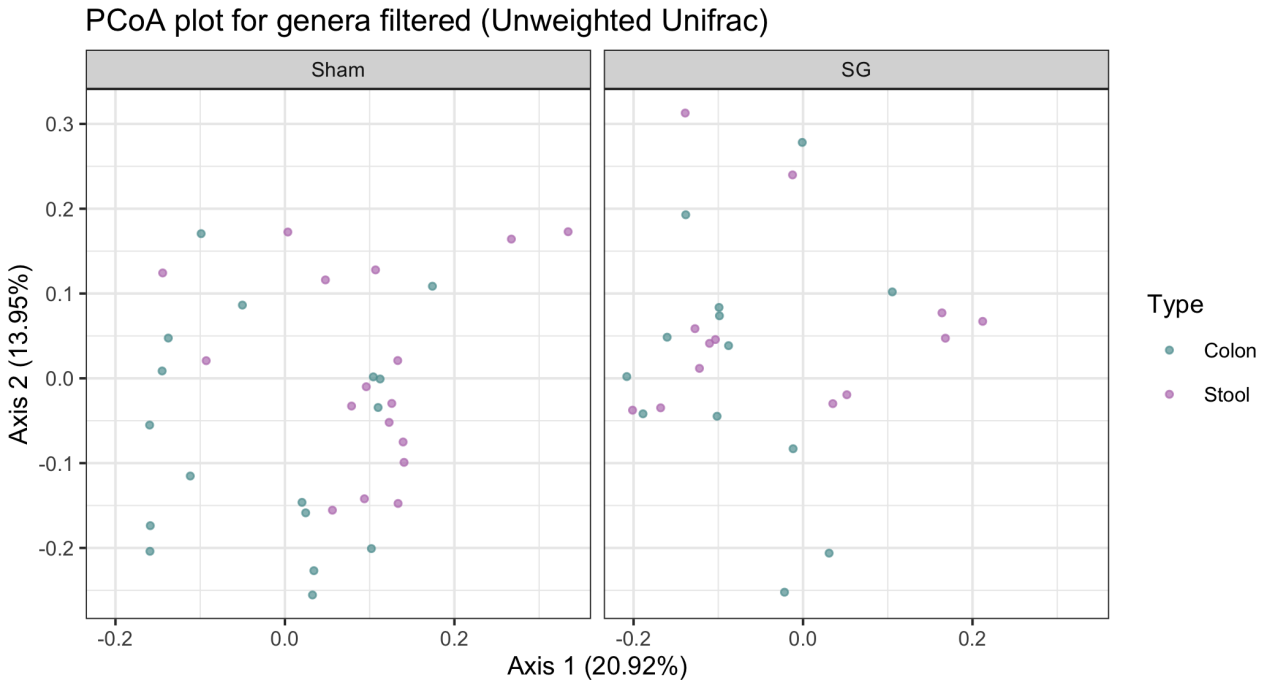
Principal coordinate analysis colored by Type, shaped by TreatmentGroup



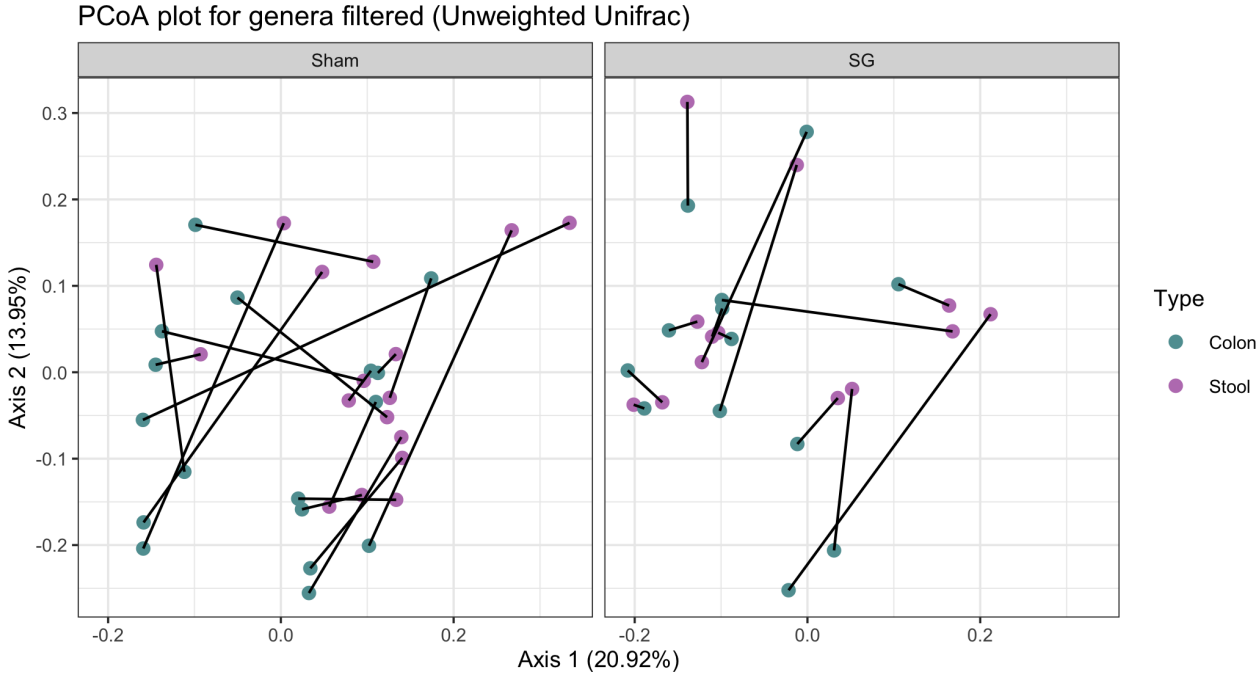
Principal coordinate analysis colored by TreatmentGroup and faceted by Type



Principal coordinate analysis colored by Type and faceted by TreatmentGroup



PCoA colored by Type, faceted by TreatmentGroup, and points are connected if they are from the same individual.

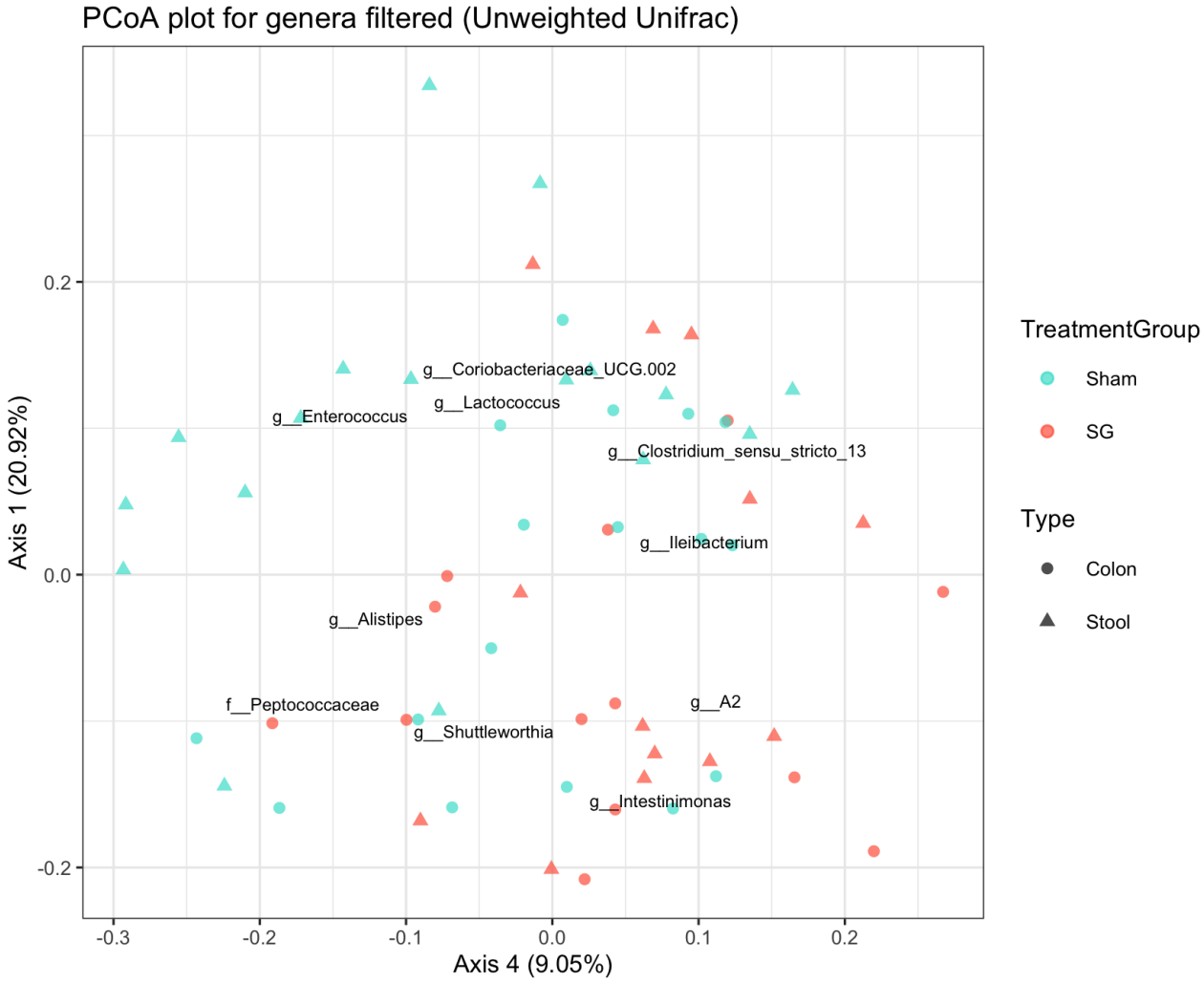


Finding the PCs that are most significantly associated with metadata factors among all samples.

TreatmentGroup

PC	pval
MDS4	0.00648 **
MDS1	0.006481 **
MDS2	0.051022 ‘
MDS8	0.190093
MDS6	0.208457
MDS3	0.459441
MDS7	0.606356
MDS9	0.801038
MDS5	0.893248

Principal coordinate analysis of MDS4 and MDS1, colored by TreatmentGroup, shaped by Type



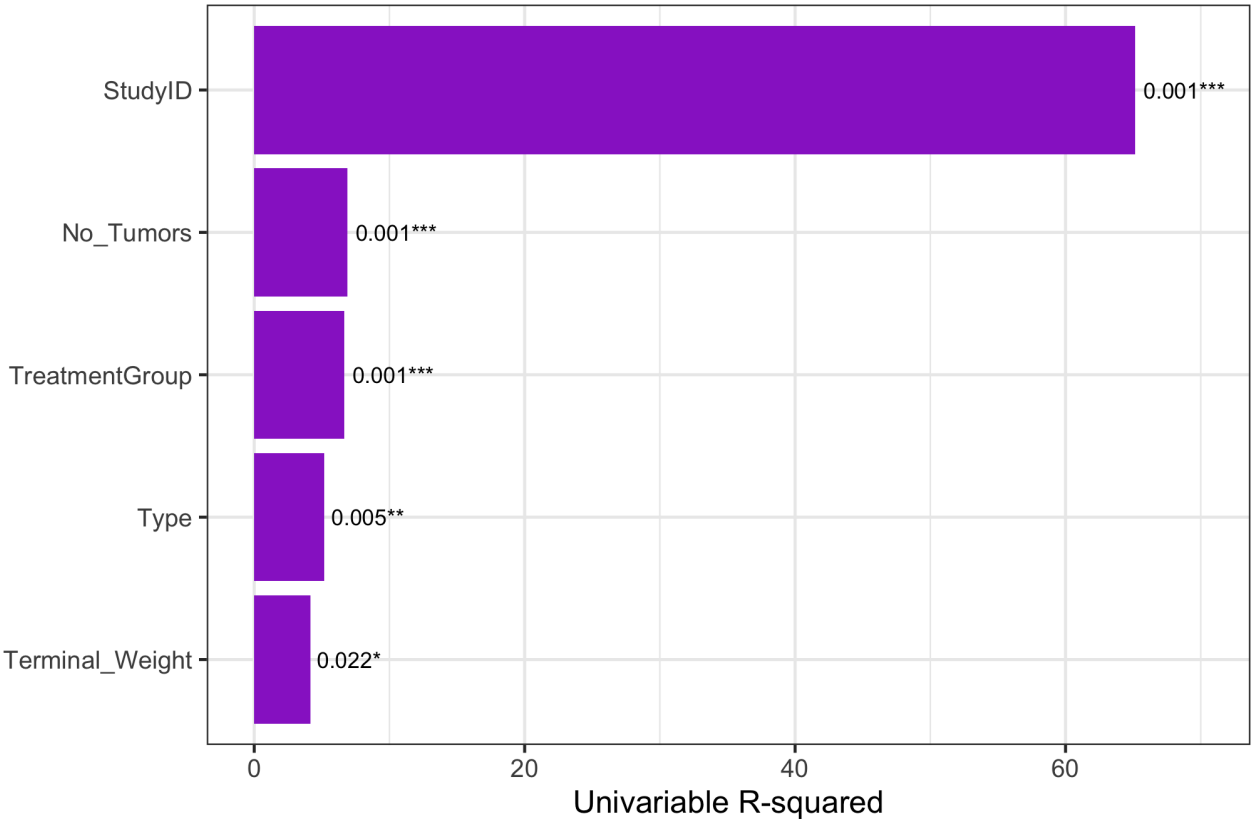
Type

PC	pval
MDS1	0.009378 **
MDS2	0.05718 ‘
MDS6	0.176816
MDS3	0.266827
MDS4	0.285895
MDS5	0.427883
MDS8	0.456857
MDS7	0.64984
MDS9	0.876913

Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Unweighted UniFrac distances

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

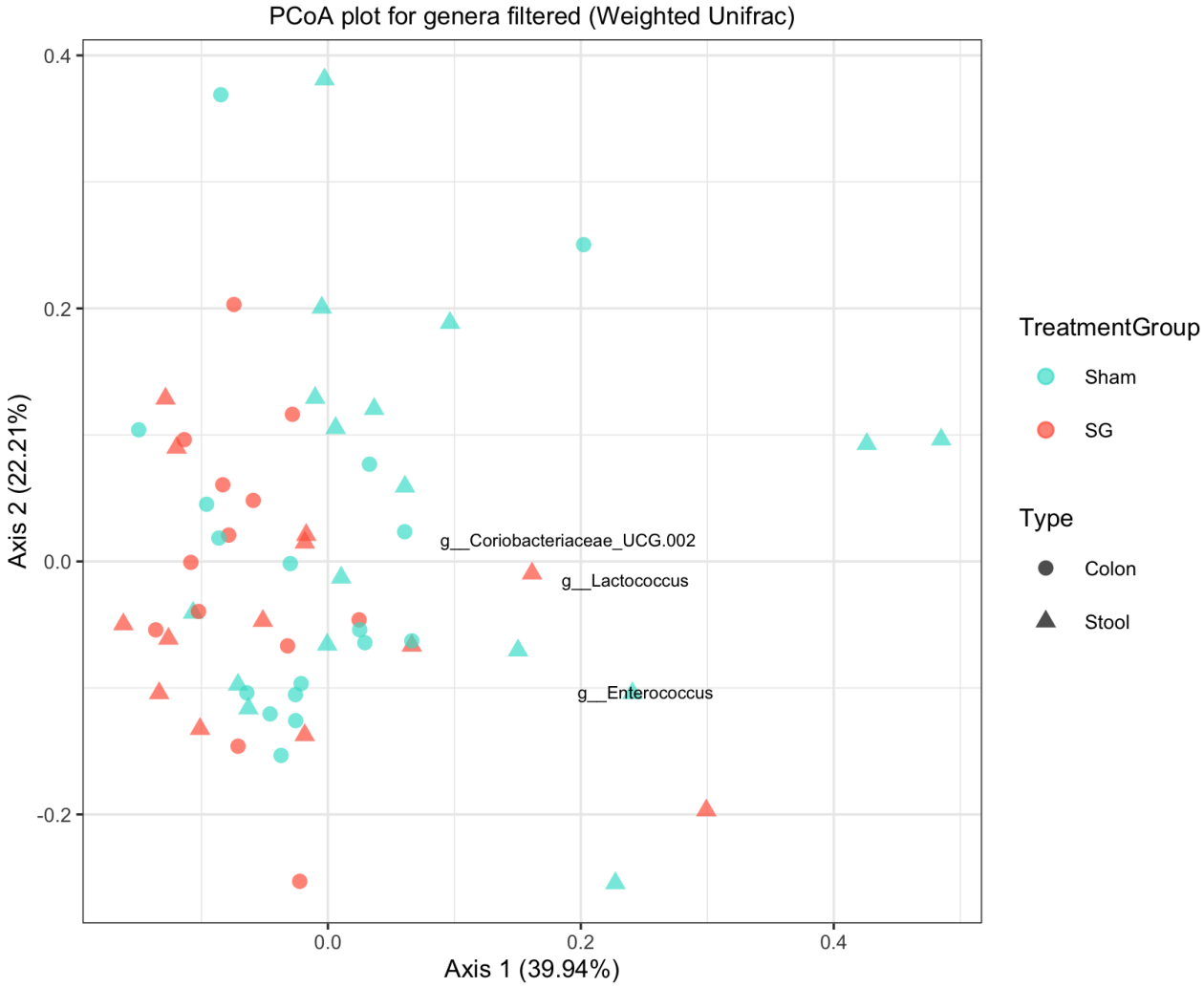
```
##
## Call:
## adonis(formula = duw ~ TreatmentGroup + Type + No_Tumors + Terminal_Weight,      data = meta_dat)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup   1    0.2156 0.215613  4.3921 0.06665  0.001 ***
## Type             1    0.1673 0.167338  3.4087 0.05173  0.006 **
## No_Tumors        1    0.1390 0.138955  2.8306 0.04296  0.007 **
## Terminal_Weight  1    0.0130 0.012953  0.2639 0.00400  0.964
## Residuals       55    2.7000 0.049091         0.83466
## Total           59    3.2349                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Call:
## adonis(formula = duw ~ TreatmentGroup + Type, data = meta_dat)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup  1    0.2156 0.215613  4.3094 0.06665  0.001 ***
## Type            1    0.1673 0.167338  3.3445 0.05173  0.003 **
## Residuals      57    2.8519 0.050034         0.88162
## Total          59    3.2349                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Call:
## adonis(formula = duw ~ TreatmentGroup * Type, data = meta_dat)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                     Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup       1    0.2156 0.215613  4.3042 0.06665  0.001 ***
## Type                 1    0.1673 0.167338  3.3405 0.05173  0.003 **
## TreatmentGroup:Type  1    0.0467 0.046696  0.9322 0.01444  0.510
## Residuals           56    2.8052 0.050093         0.86718
## Total               59    3.2349                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

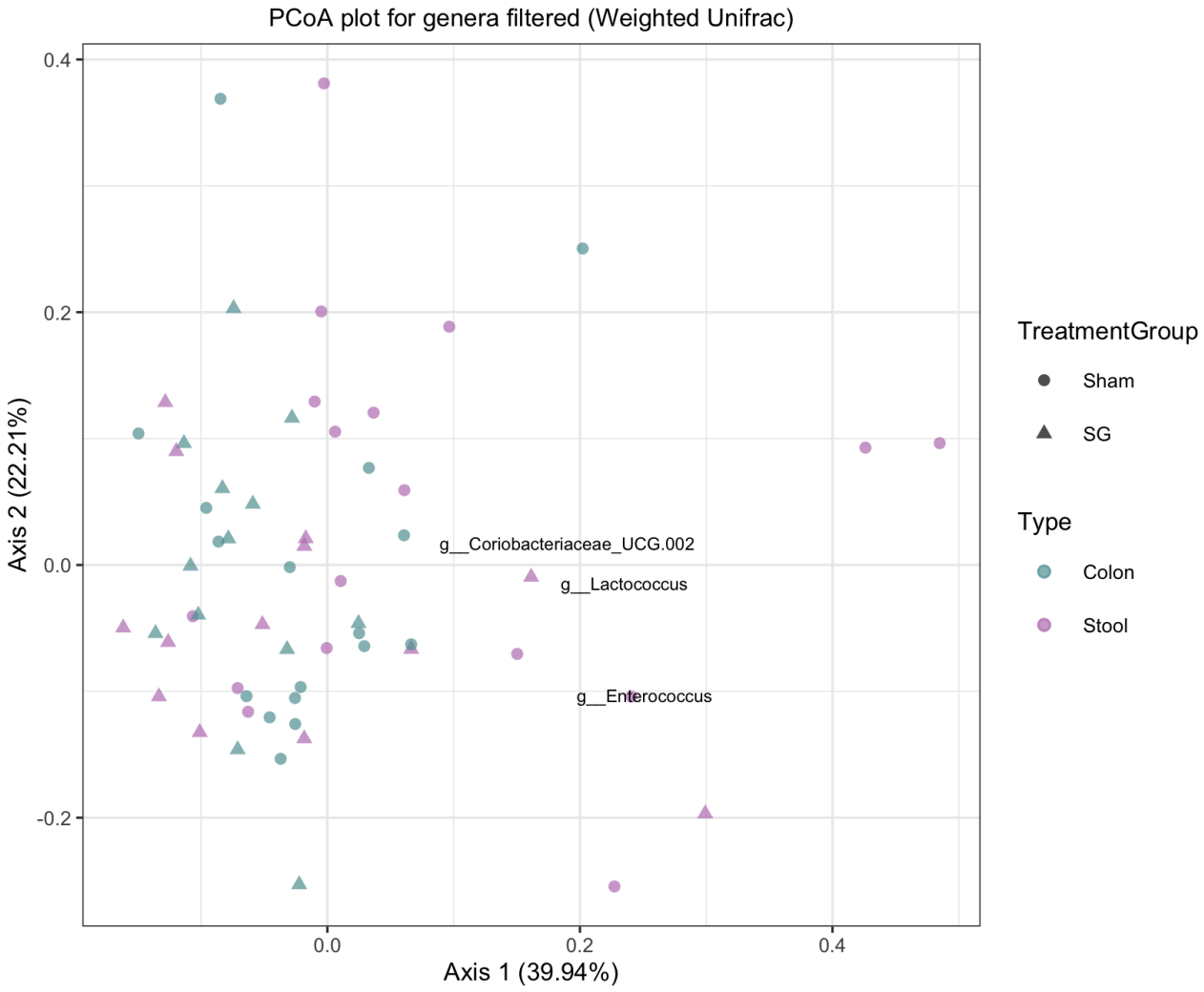
Weighted UniFrac

Principal coordinates analysis colored by covariates

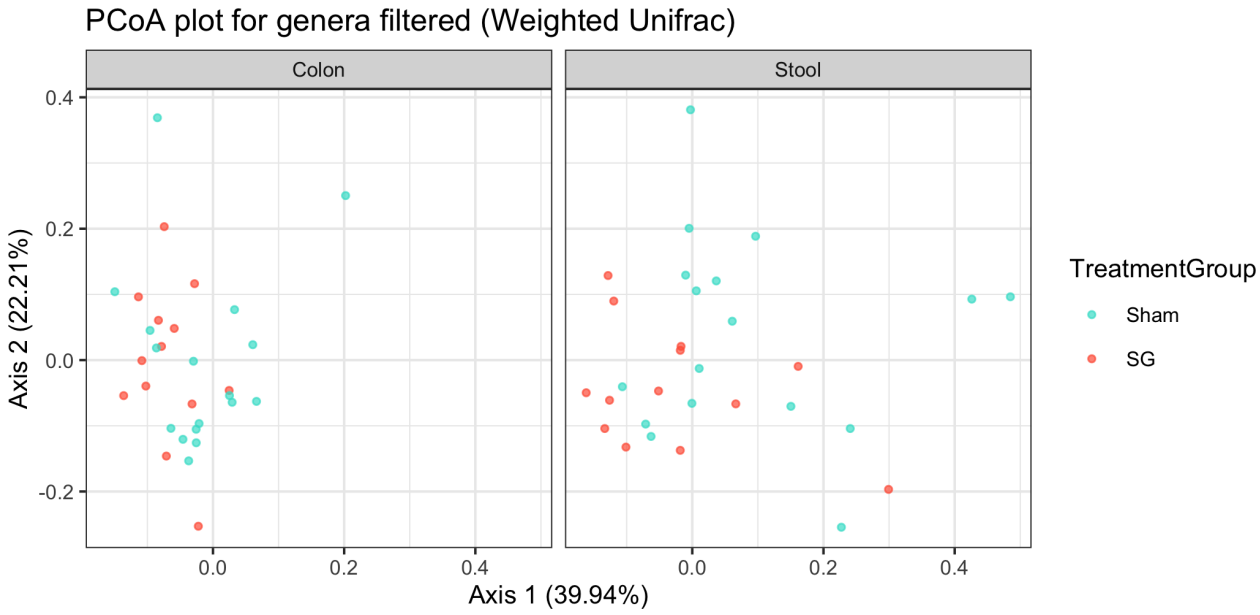
Principal coordinate analysis colored by TreatmentGroup, shaped by Type



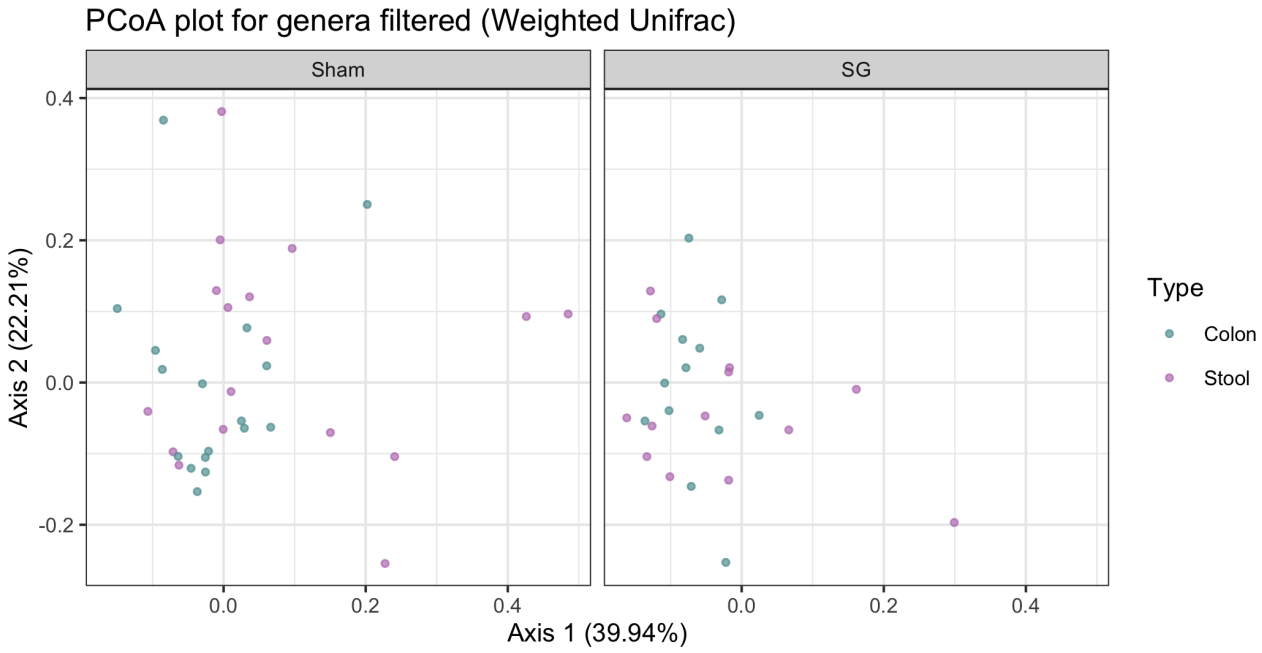
Principal coordinate analysis colored by Type, shaped by TreatmentGroup



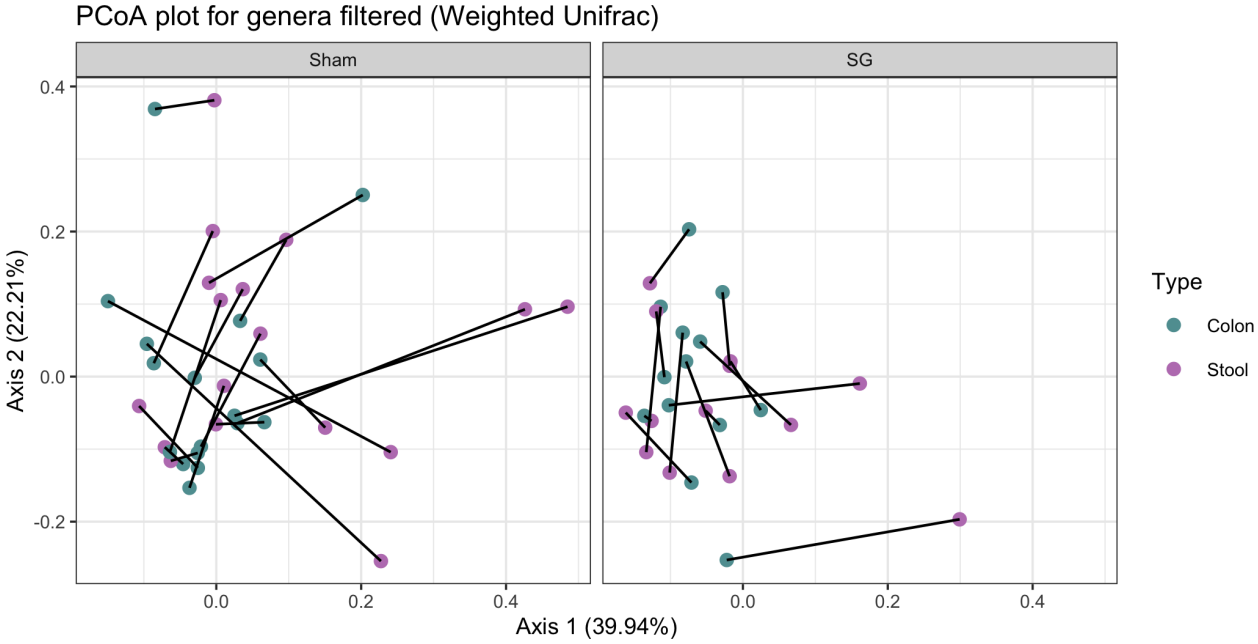
Principal coordinate analysis colored by TreatmentGroup and faceted by Type



Principal coordinate analysis colored by Type and faceted by TreatmentGroup



PCoA colored by Type, faceted by TreatmentGroup, and points are connected if they are from the same individual.

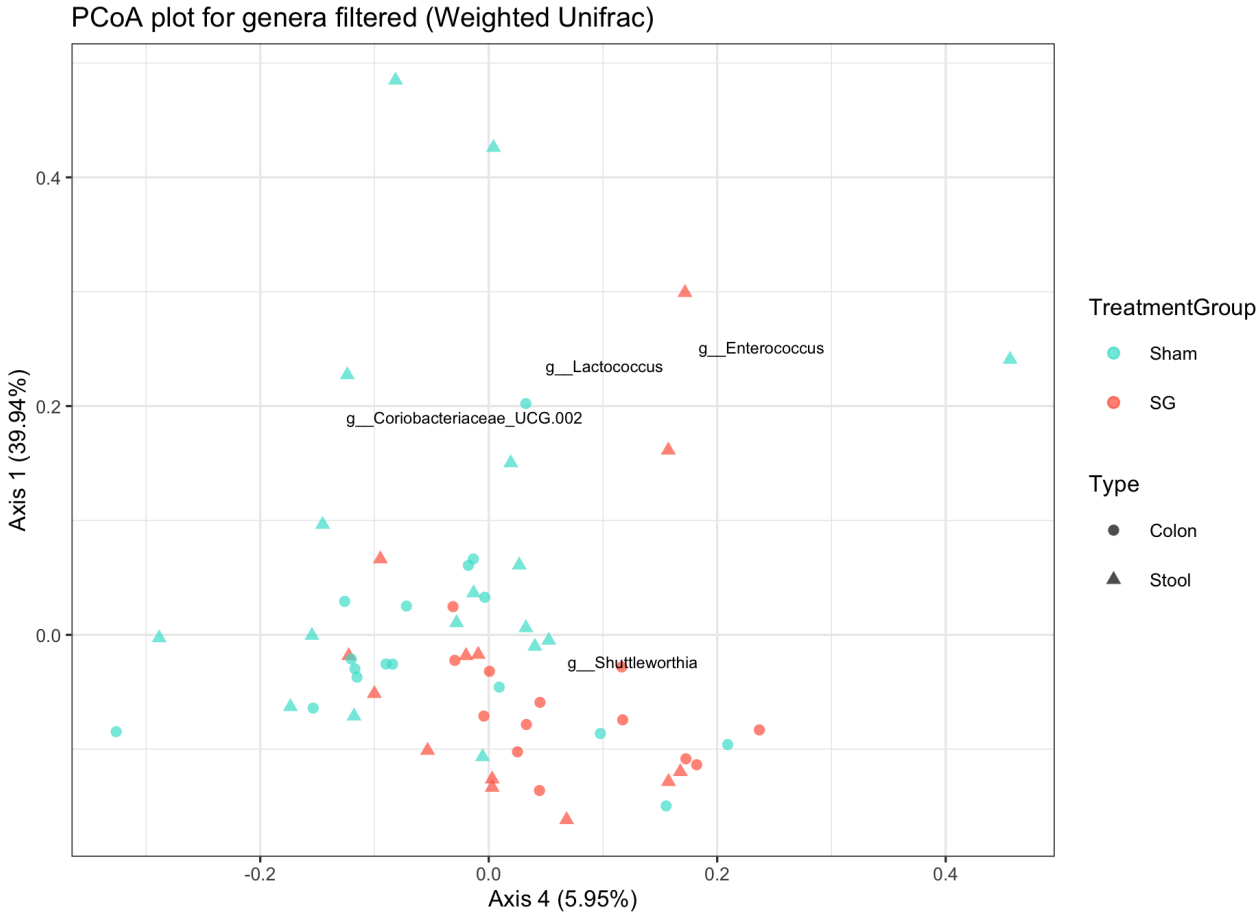


Finding the PCs that are most significantly associated with metadata factors among all samples.

TreatmentGroup

PC	pval
MDS4	0.0121 *
MDS1	0.012362 *
MDS2	0.224199
MDS5	0.339335
MDS8	0.359794
MDS3	0.530253
MDS6	0.677165
MDS7	0.902846
MDS9	0.915688

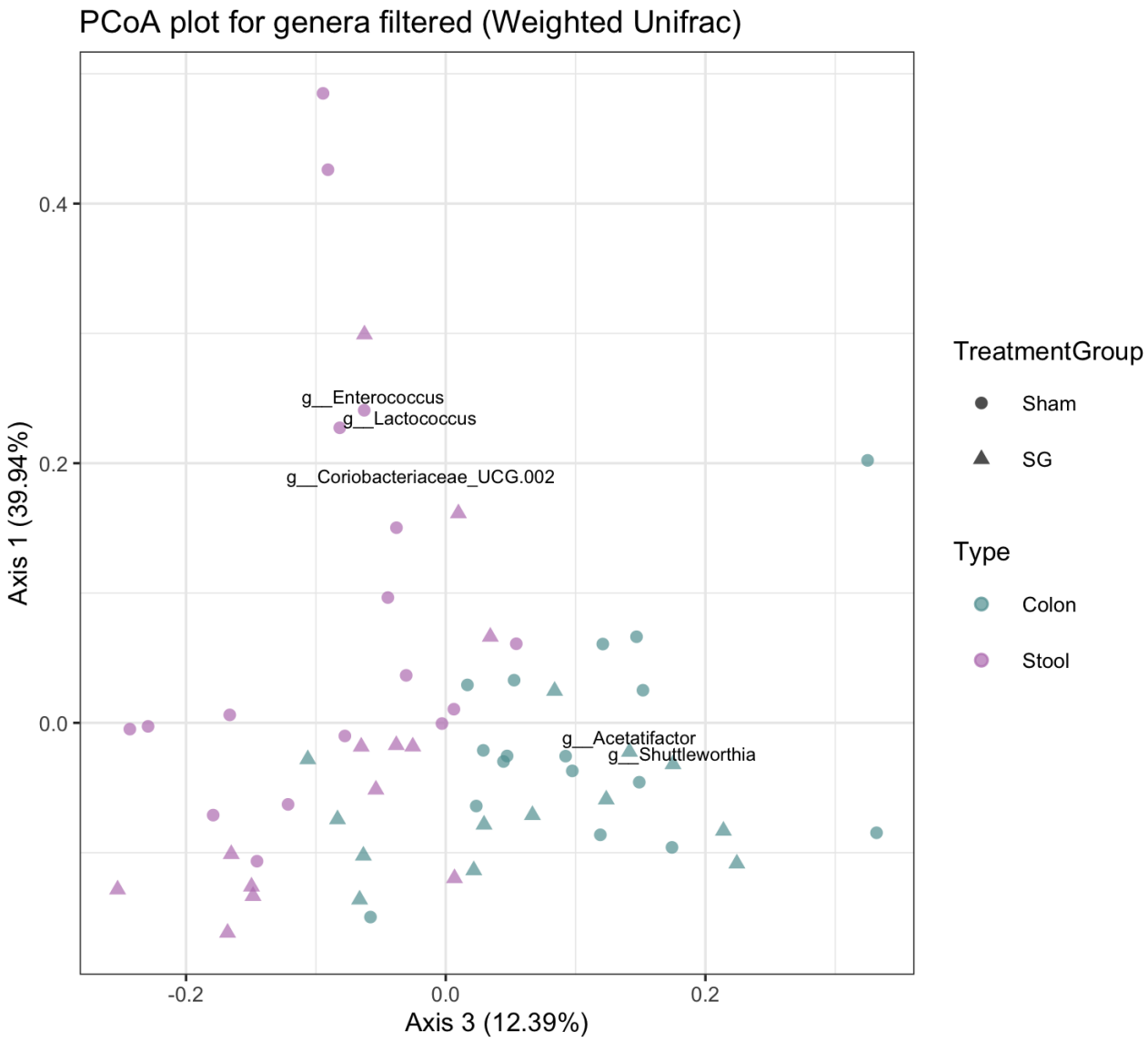
Principal coordinate analysis of MDS4 and MDS1, colored by TreatmentGroup, shaped by Type



Type

PC	pval
MDS3	0 ***
MDS1	0.023337 *
MDS8	0.386979
MDS5	0.54064
MDS6	0.7027
MDS4	0.735958
MDS7	0.757074
MDS2	0.903503
MDS9	0.936604

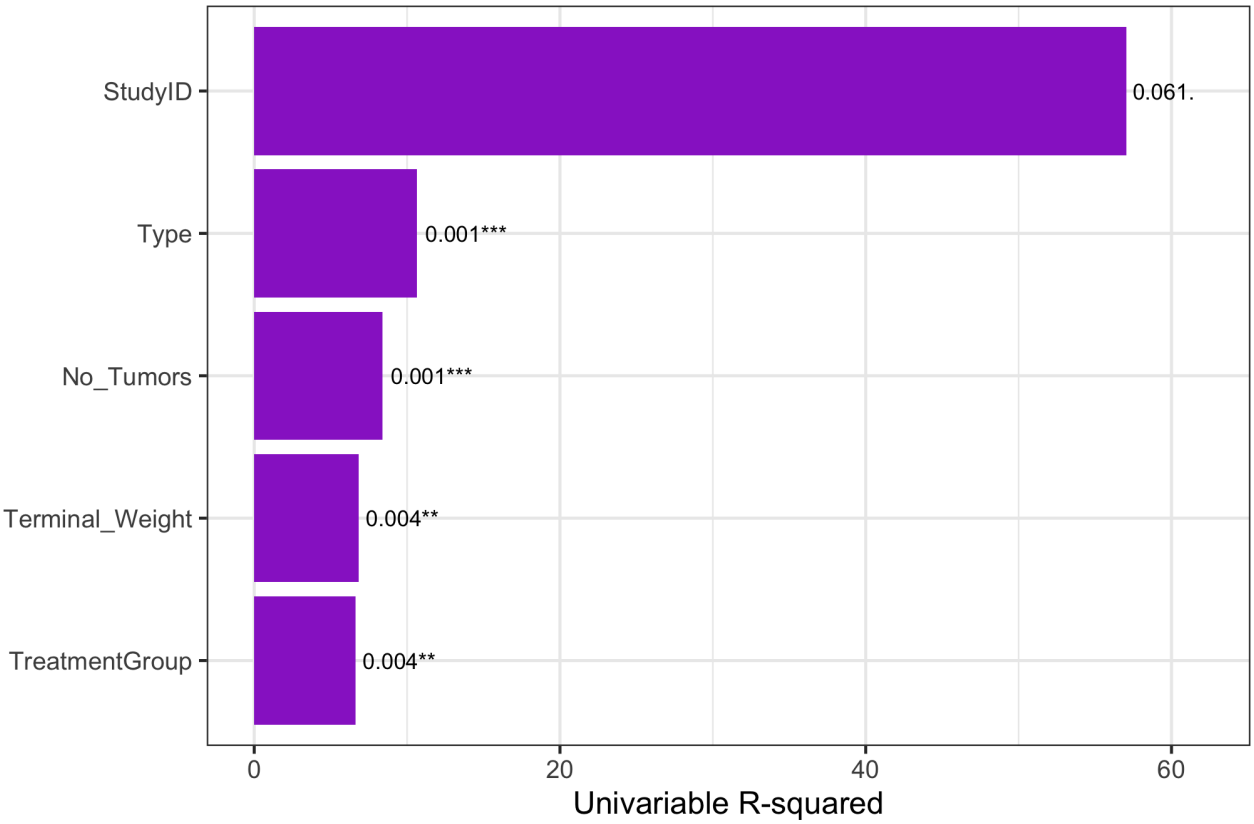
Principal coordinate analysis of MDS3 and MDS1, colored Type, shaped by TreatmentGroup



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Weighted UniFrac distances

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

```
##
## Call:
## adonis(formula = dw ~ TreatmentGroup + Type + No_Tumors + Terminal_Weight,      data = meta_dat)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup   1   0.18179 0.181793  4.6516 0.06632  0.004 **
## Type             1   0.29226 0.292258  7.4780 0.10662  0.001 ***
## No_Tumors        1   0.07975 0.079747  2.0405 0.02909  0.107
## Terminal_Weight  1   0.03783 0.037834  0.9681 0.01380  0.409
## Residuals       55   2.14952 0.039082         0.78417
## Total           59   2.74115                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Call:
## adonis(formula = dw ~ TreatmentGroup + Type, data = meta_dat)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup  1   0.18179 0.181793  4.5707 0.06632  0.003 **
## Type            1   0.29226 0.292258  7.3480 0.10662  0.001 ***
## Residuals      57   2.26710 0.039774         0.82706
## Total          59   2.74115                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Call:
## adonis(formula = dw ~ TreatmentGroup * Type, data = meta_dat)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                     Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup       1   0.18179 0.181793  4.5629 0.06632  0.001 ***
## Type                 1   0.29226 0.292258  7.3355 0.10662  0.003 **
## TreatmentGroup:Type  1   0.03599 0.035987  0.9032 0.01313  0.402
## Residuals           56   2.23111 0.039841         0.81393
## Total               59   2.74115                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

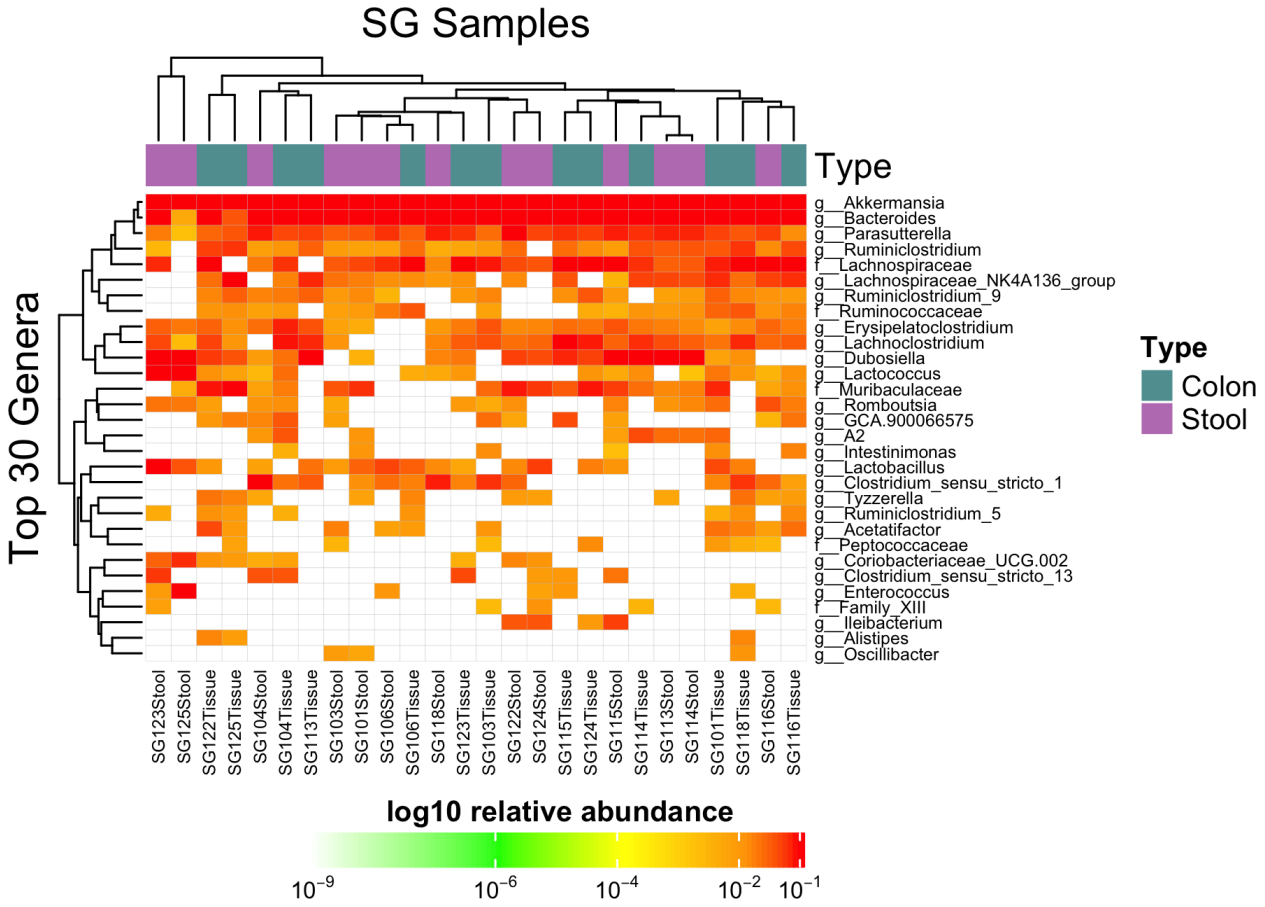
Section 2. SG Samples Only

Taxonomic Overview

Visualized as heatmaps

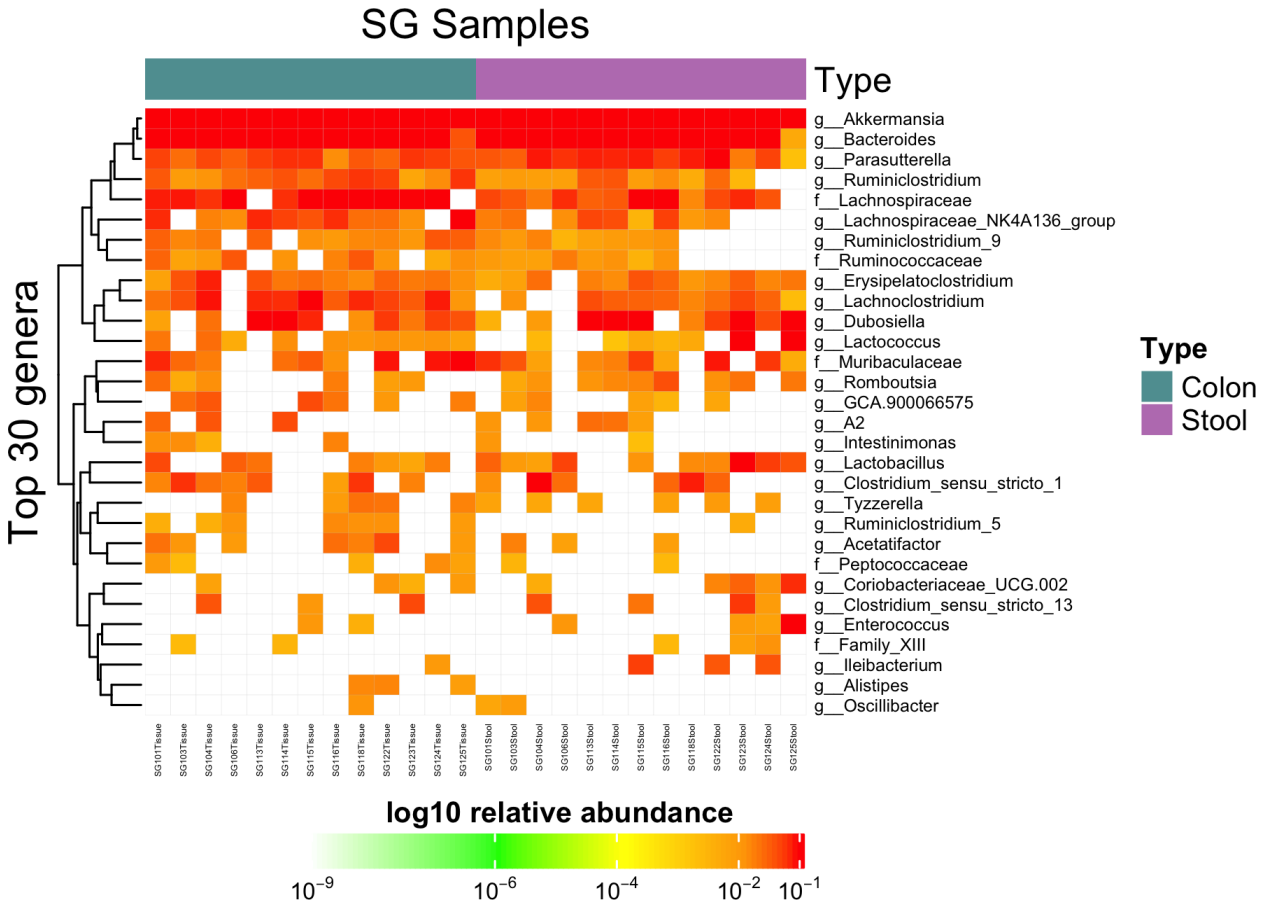
Heatmap clustered based on similar gut compositions

Heatmap of log10 relative abundances of the top 30 genera in SG samples. Samples (columns) and taxa (rows) are sorted based on Bray-Curtis dissimilarities and Euclidean distances, respectively. These samples display similar to other studies on gut microbiome samples.



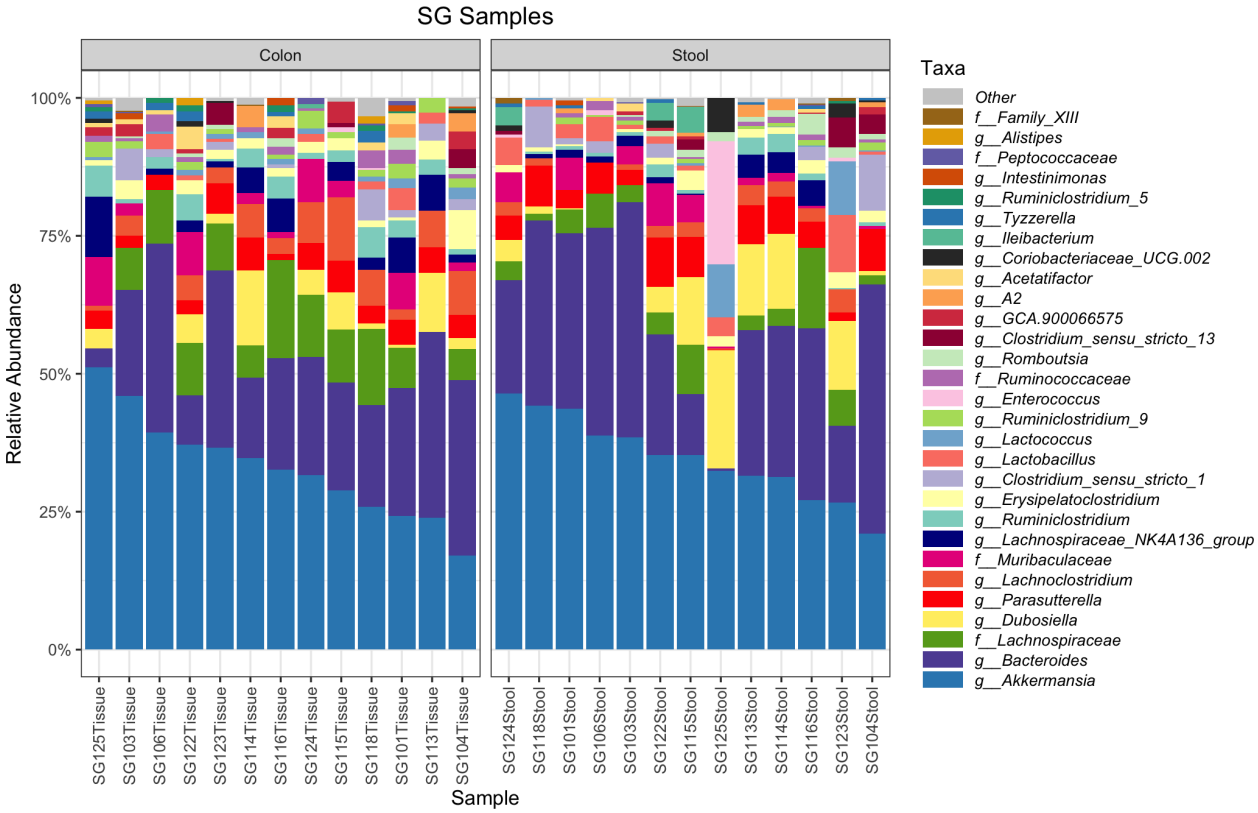
Heatmap clustered based on factors in the metadata

Heatmap of log10 relative abundances of the top 30 genera. Samples (columns) and taxa (rows) are sorted based on Type, and descending overall average relative abundance, respectively.

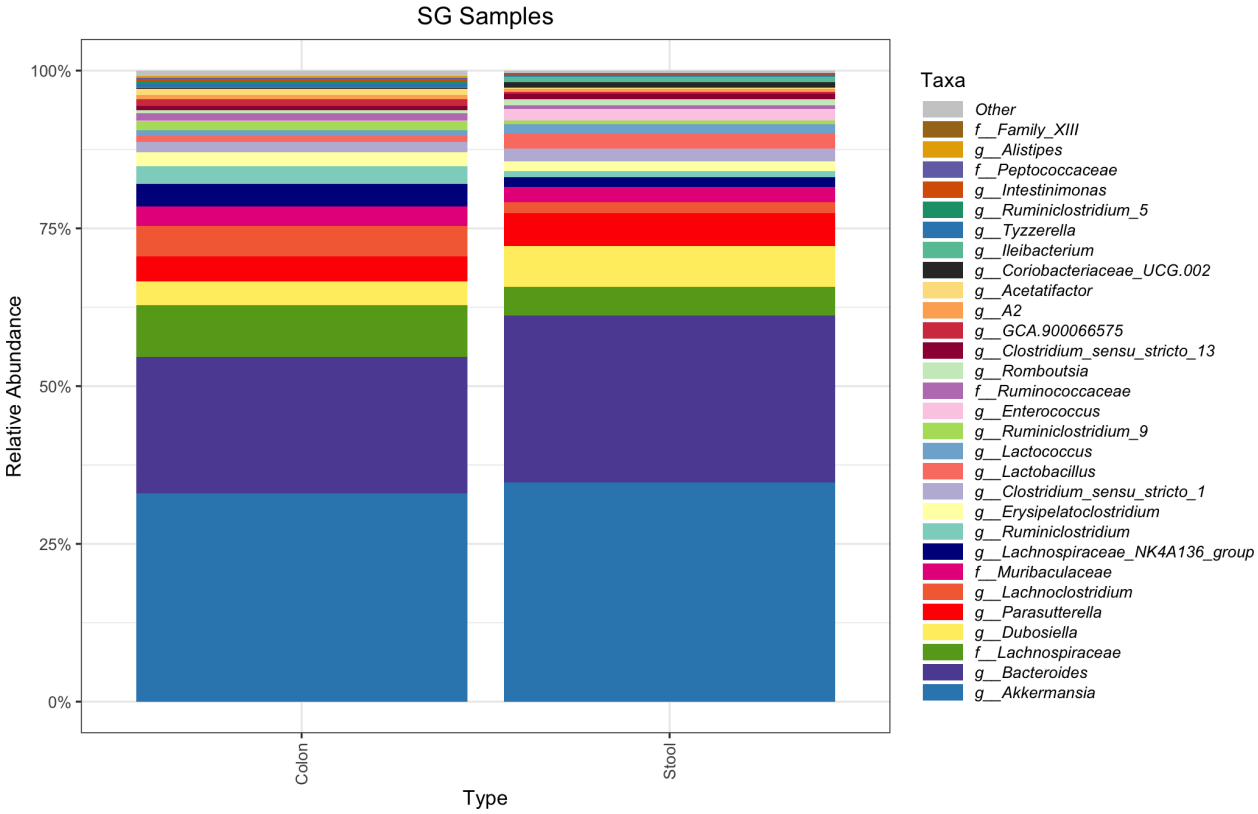


Visualized as stacked barplots

Bar plot of the top 29 abundant taxa among SG samples by Type

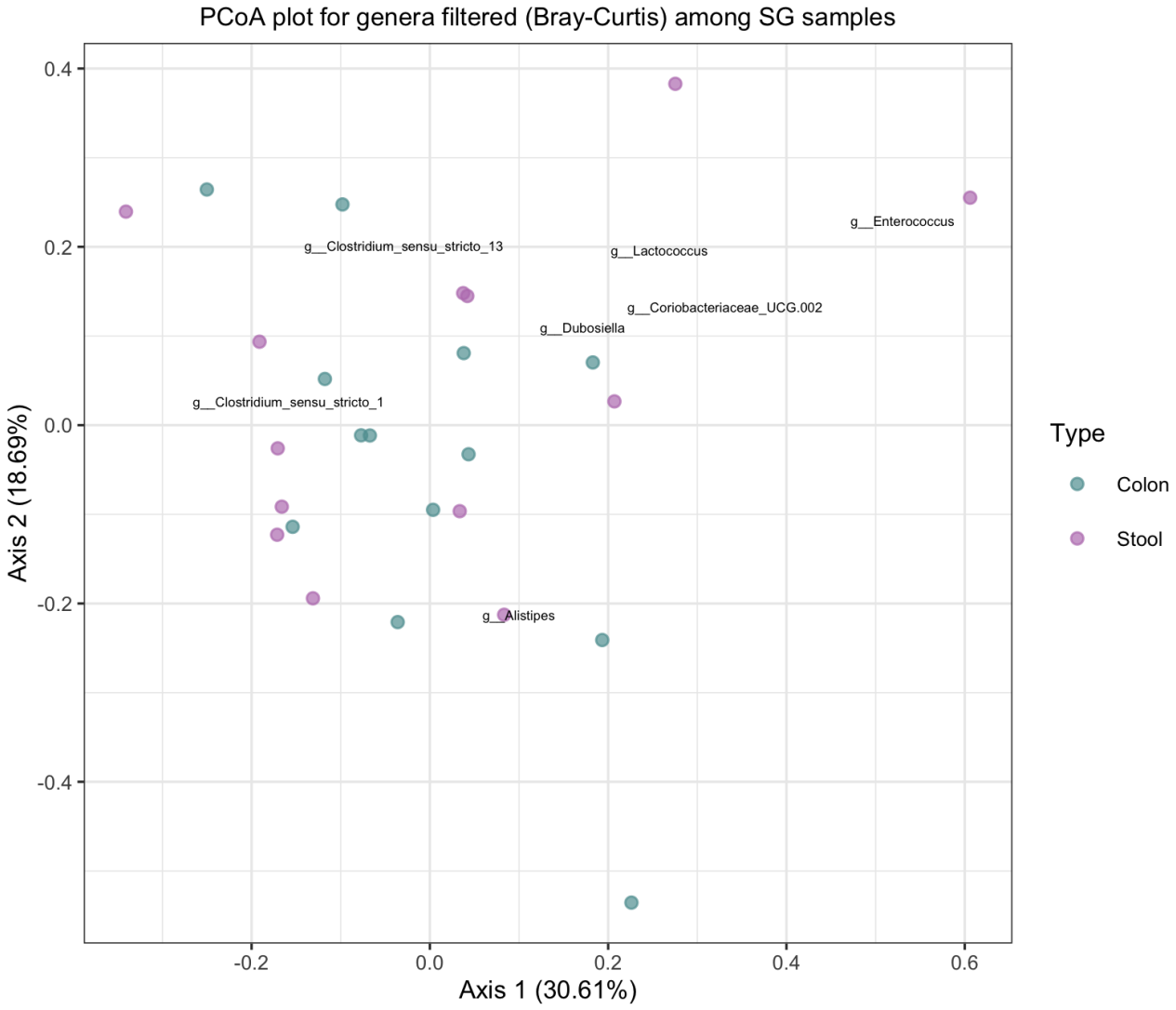


Bar plot of the mean relative abundance of the top 29 taxa among SG samples by Type



Beta Diversity: Bray-Curtis dissimilarity

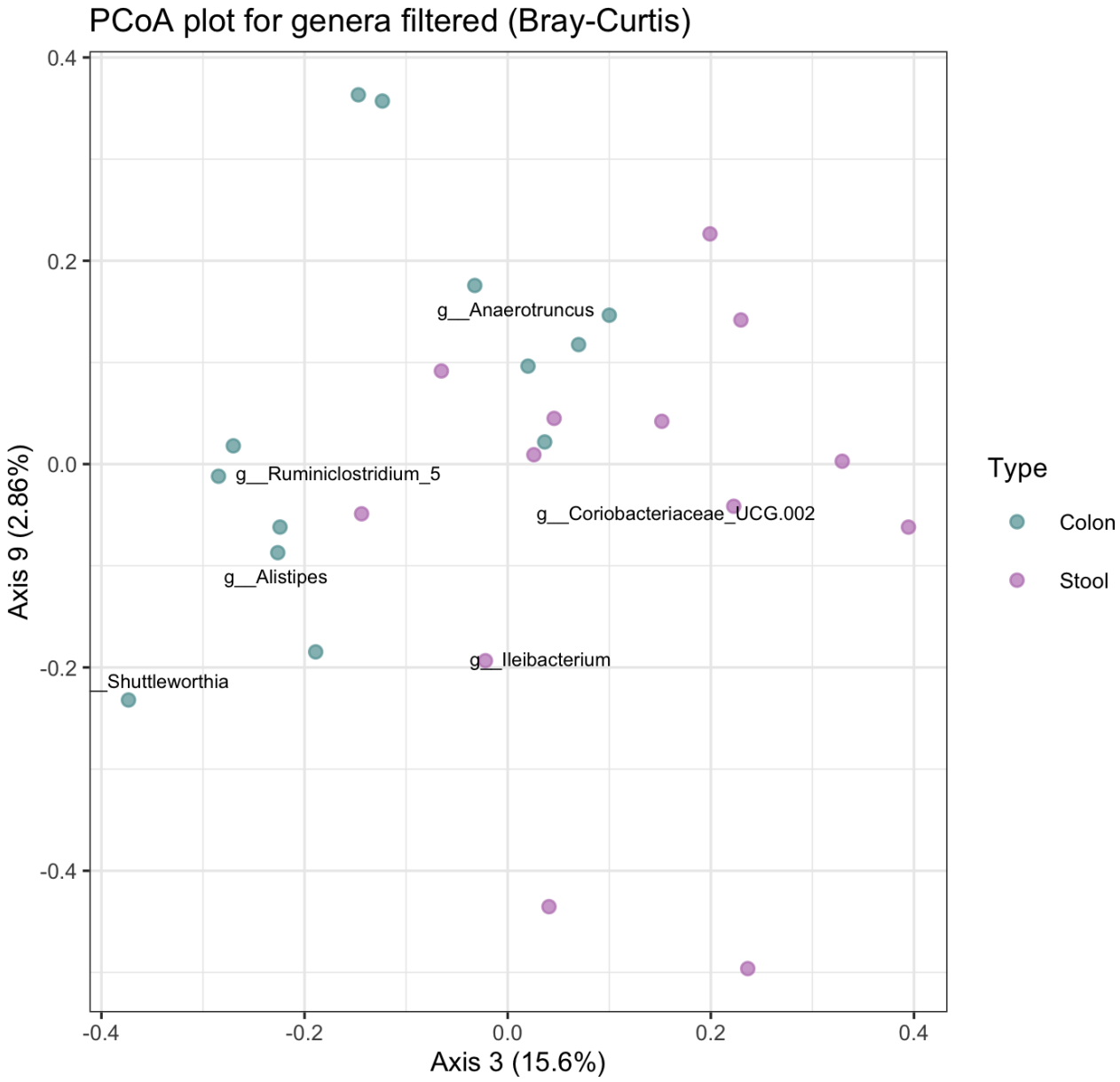
Principal coordinate analysis colored by Type



Finding the PCs that are most significantly associated with Type among SG samples.

PC	pval
MDS3	0.000374 ***
MDS9	0.163141
MDS2	0.292556
MDS6	0.315521
MDS7	0.407907
MDS8	0.447958
MDS5	0.454195
MDS4	0.771888
MDS1	0.829363

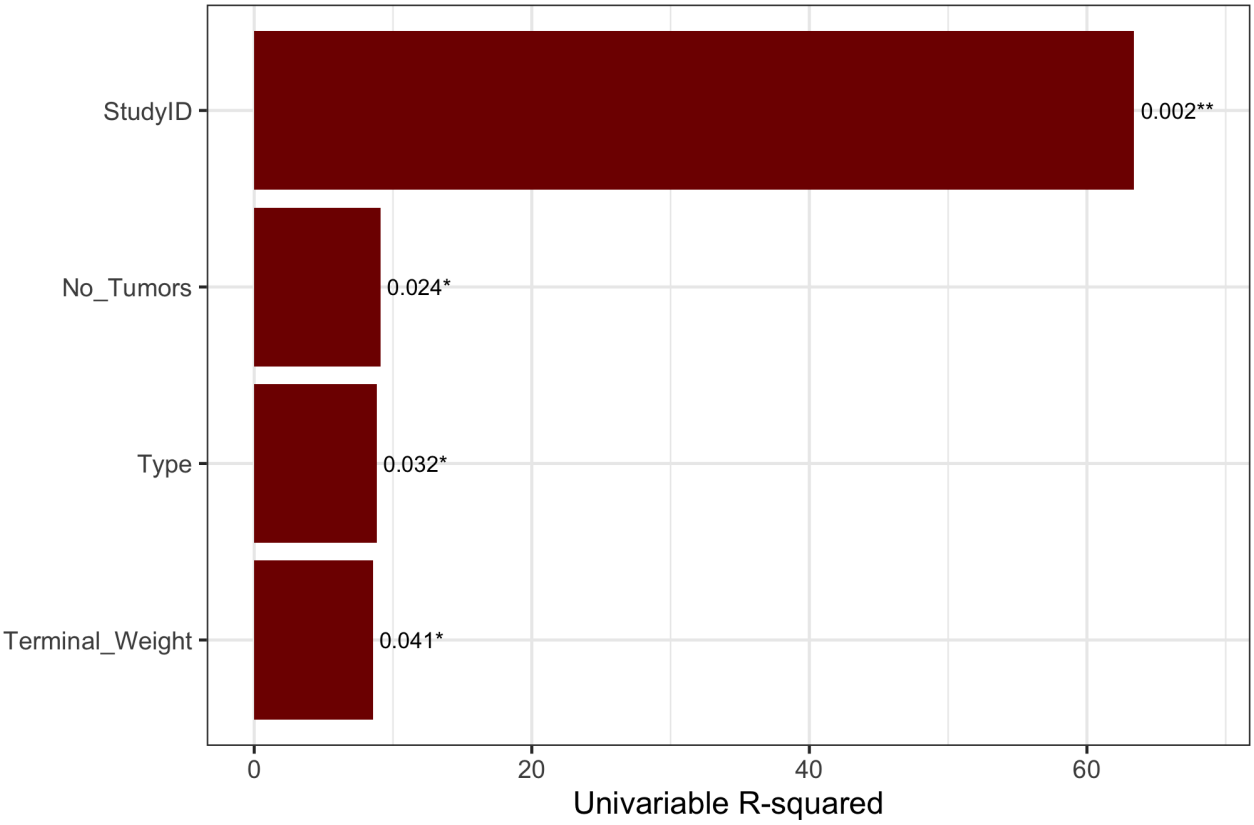
Principal coordinate analysis of MDS3 and MDS9, colored Type



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Bray-Curtis dissimilarity among SG samples

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

Type + Standard Clinical Covarites

```
##
## Call:
## adonis(formula = bray_sub ~ Type + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Type             1   0.15039 0.150392  2.5963 0.08833  0.019 *
## No_Tumors        1   0.15522 0.155216  2.6796 0.09116  0.017 *
## Terminal_Weight  1   0.12269 0.122689  2.1181 0.07206  0.043 *
## Residuals       22   1.27435 0.057925         0.74845
## Total           25   1.70265                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

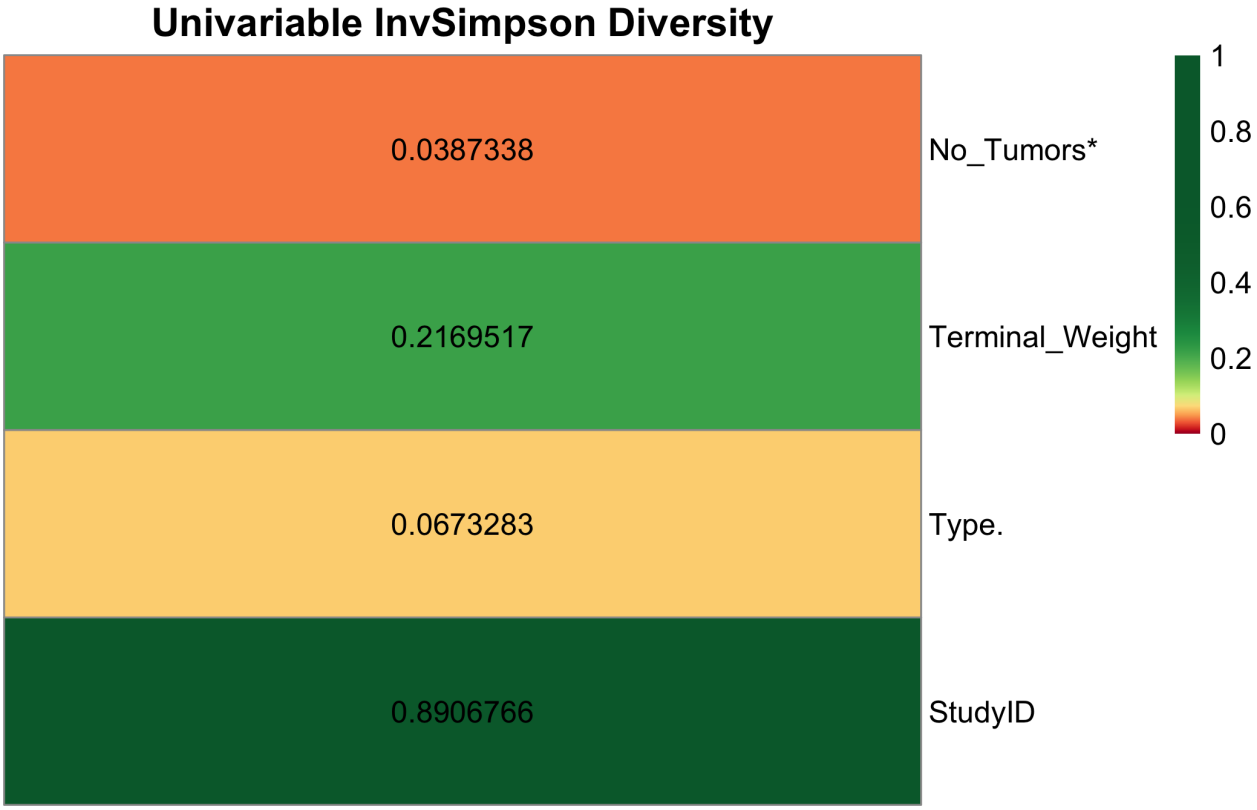
Type

```
##
## Call:
## adonis(formula = bray_sub ~ Type, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Type       1   0.15039 0.150392  2.3253 0.08833  0.027 *
## Residuals 24   1.55226 0.064677         0.91167
## Total     25   1.70265                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Alpha Diversity

Statistics alpha diversity: anova on linear model

Heatmap annotated with the p-value for each covariate

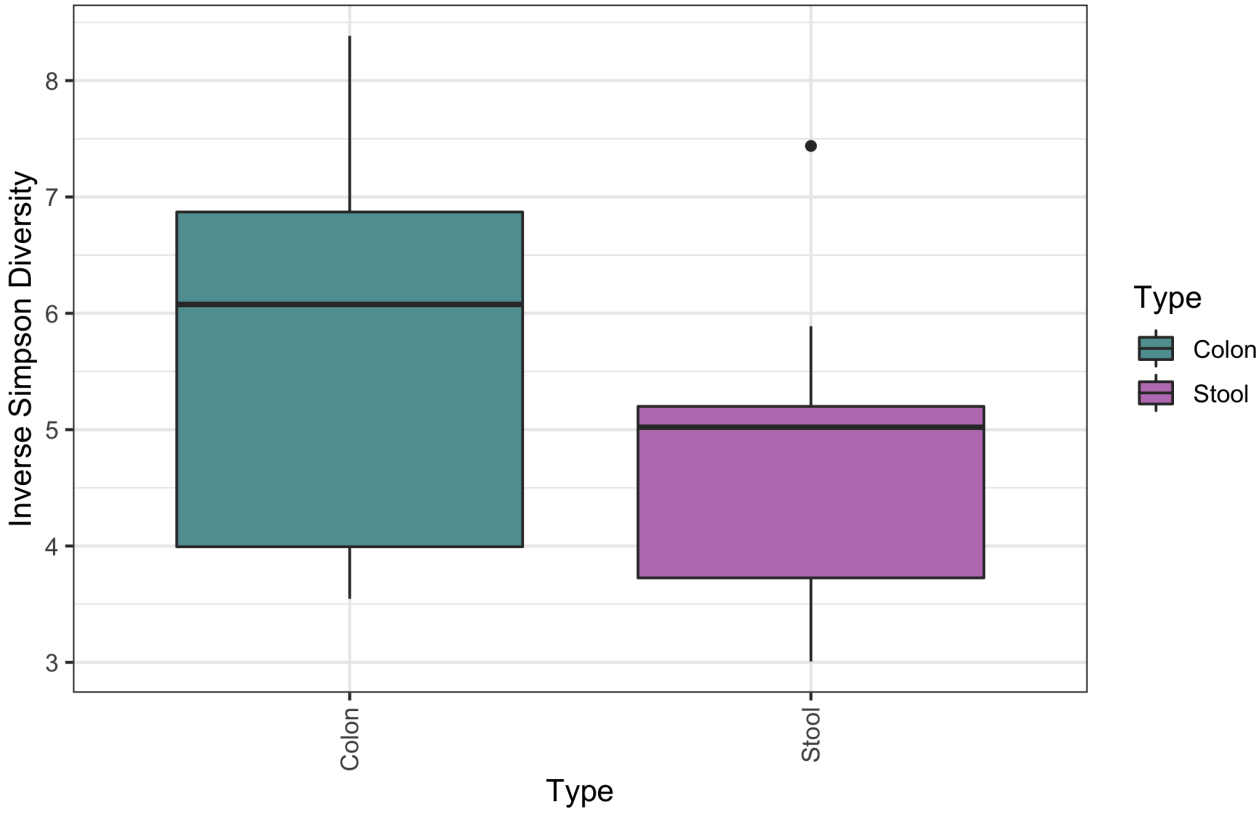


Multivariable model for Alpha diversity

```
## Analysis of Variance Table
##
## Response: InvSimpson
##           Df Sum Sq Mean Sq F value  Pr(>F)
## Type       1  7.762  7.7621  3.6717 0.06733 .
## Residuals 24 50.737  2.1140
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Analysis of Variance Table
##
## Response: InvSimpson
##                 Df Sum Sq Mean Sq F value  Pr(>F)
## Type             1  7.762  7.7621  4.1718 0.05327 .
## No_Tumors        1  9.720  9.7199  5.2241 0.03227 *
## Terminal_Weight  1  0.083  0.0833  0.0448 0.83437
## Residuals       22 40.933  1.8606
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

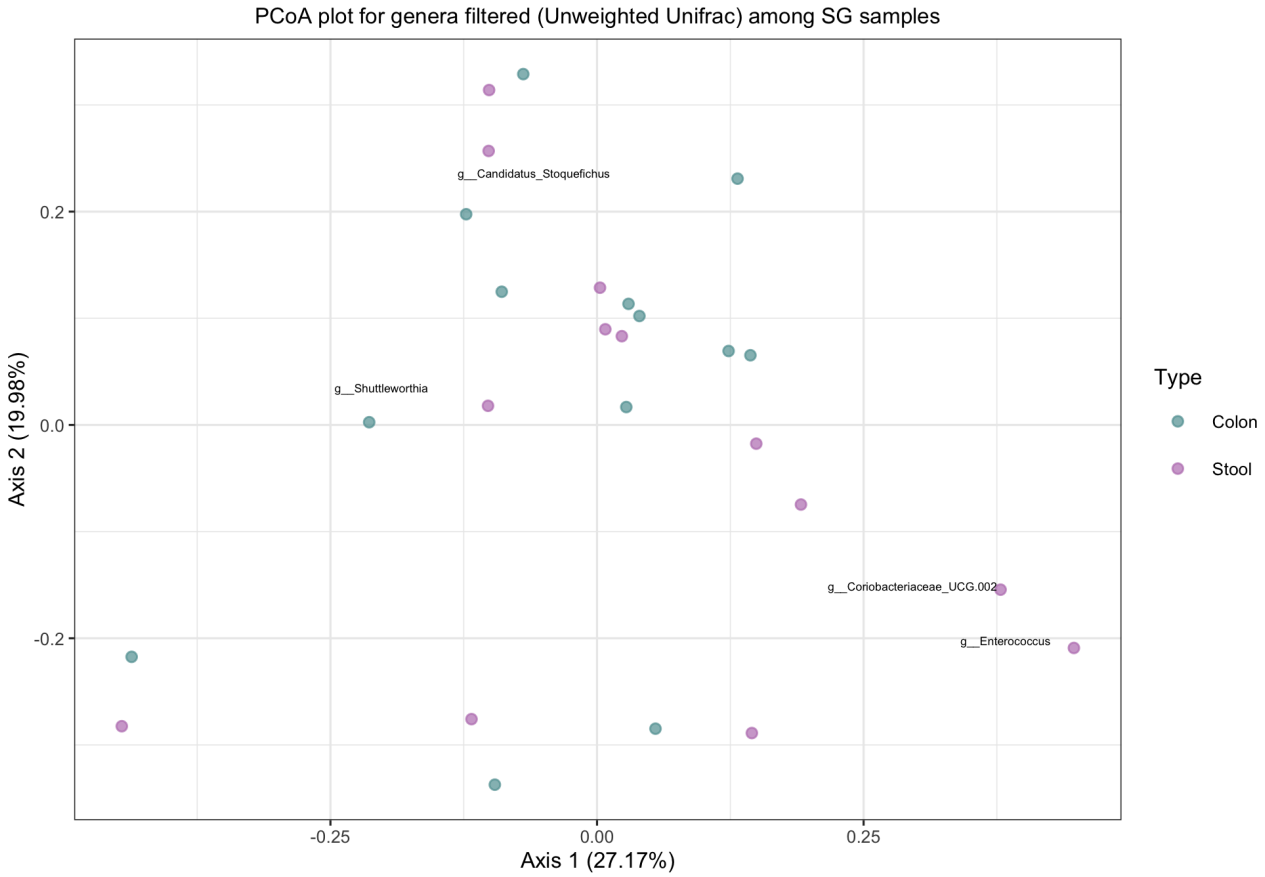
Invsimpson vs. Type



Other Beta Diversity Metrics

Unweighted UniFrac

Principal coordinate analysis colored by Type



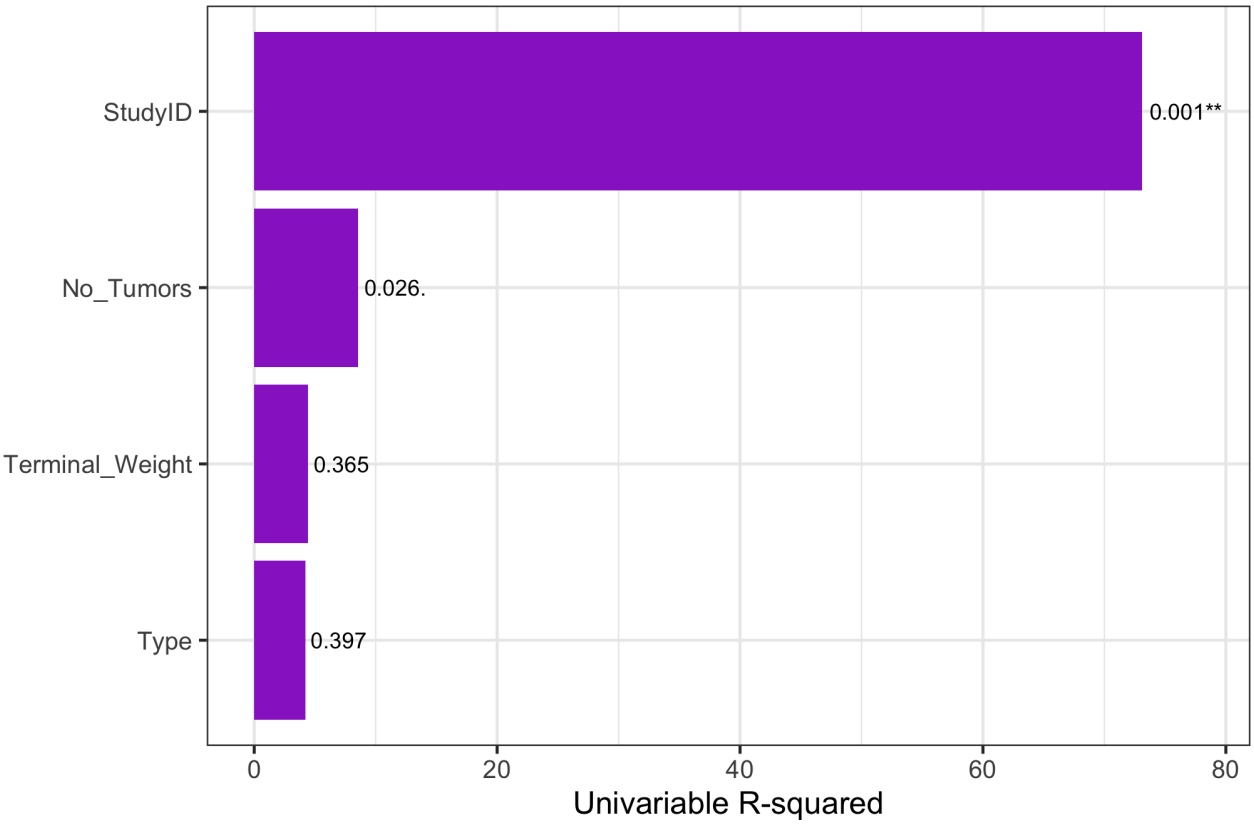
Finding the PCs that are most significantly associated with Type among SG samples.

PC	pval
MDS3	0.101828
MDS7	0.181223
MDS6	0.189006
MDS4	0.349549
MDS1	0.360377
MDS2	0.429784
MDS8	0.912054
MDS9	0.937566
MDS5	0.982227

Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Unweighted UniFrac Distances

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

Type + Standard Clinical Covarites

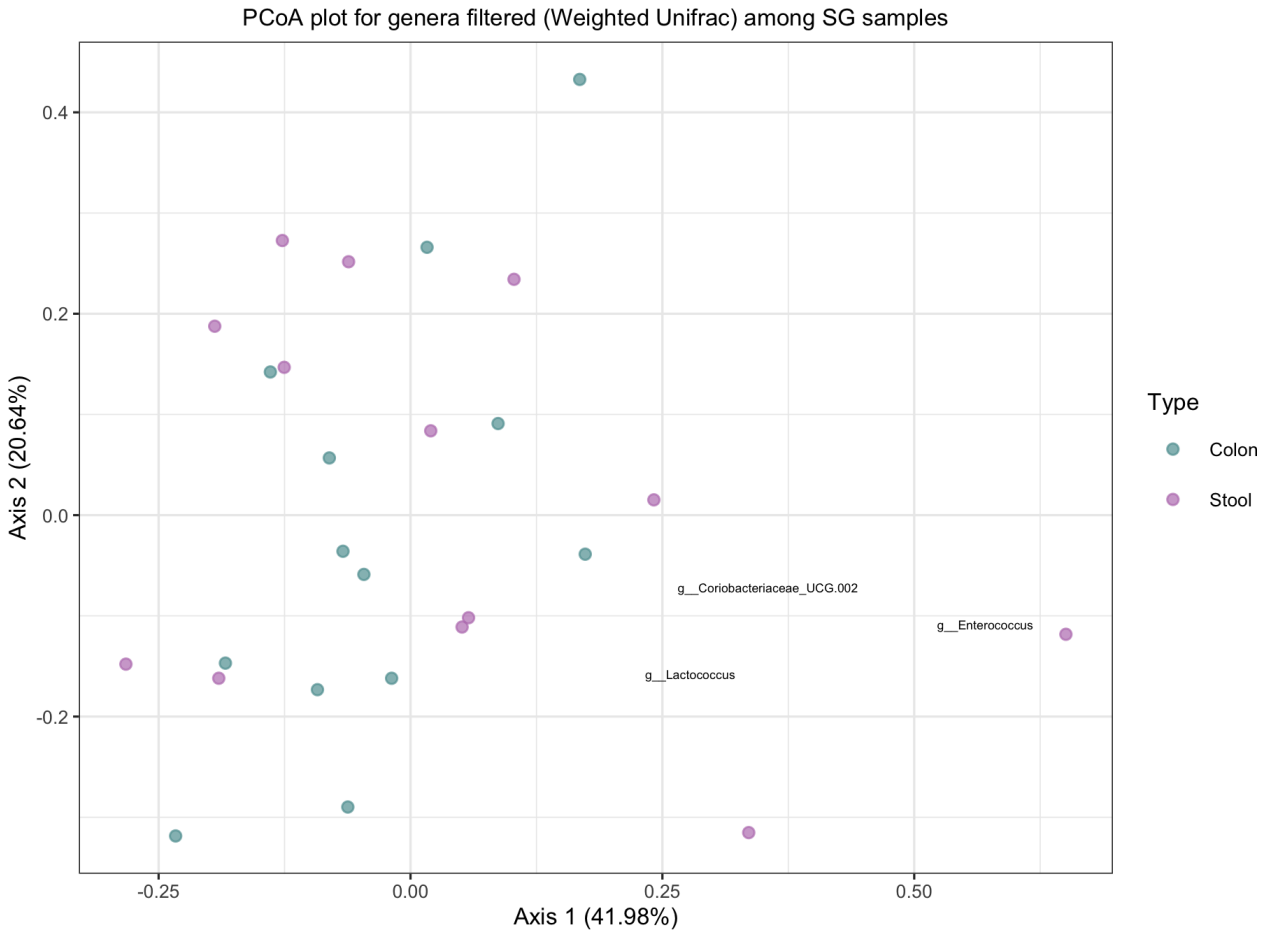
```
##
## Call:
## adonis(formula = duw_sub ~ Type + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Type             1   0.05296 0.052964 1.10410 0.04217  0.386
## No_Tumors        1   0.10758 0.107578 2.24263 0.08566  0.045 *
## Terminal_Weight  1   0.04006 0.040063 0.83518 0.03190  0.560
## Residuals       22   1.05533 0.047970         0.84027
## Total           25   1.25594                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Type

```
##
## Call:
## adonis(formula = duw_sub ~ Type, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Type       1   0.05296 0.052964  1.0567 0.04217  0.399
## Residuals 24   1.20297 0.050124         0.95783
## Total     25   1.25594                  1.00000
```

Weighted UniFrac

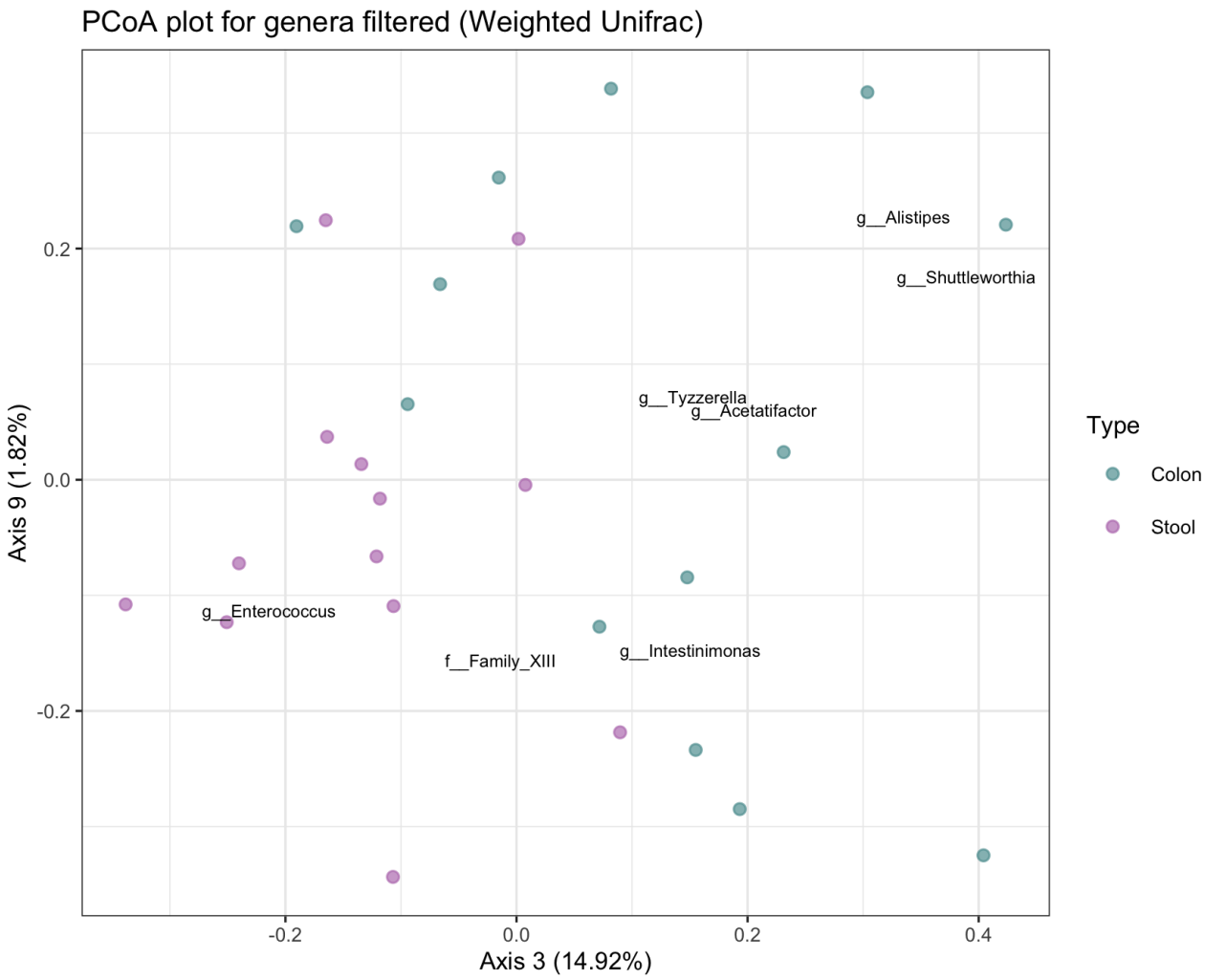
Principal coordinate analysis colored by Type



Finding the PCs that are most significantly associated with Type among SG samples.

PC	pval
MDS3	0.000367 ***
MDS9	0.265129
MDS1	0.358684
MDS8	0.404002
MDS4	0.4956
MDS2	0.653629
MDS7	0.773859
MDS6	0.9021
MDS5	0.924402

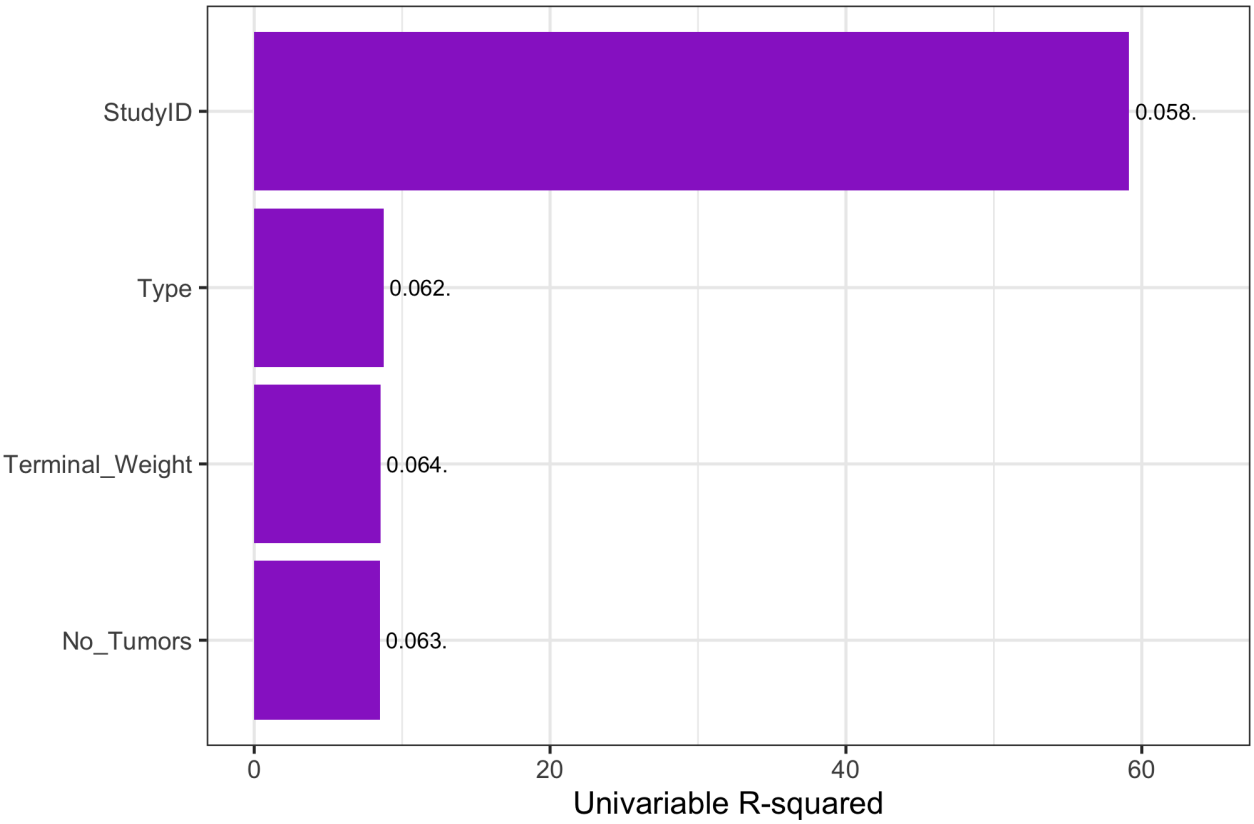
Principal coordinate analysis of MDS3 and MDS9, colored Type



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Weighted UniFrac Distances

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

Type + Standard Clinical Covarites

```
##
## Call:
## adonis(formula = dw_sub ~ Type + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Type             1   0.07060 0.070605  2.5579 0.08738  0.029 *
## No_Tumors        1   0.06866 0.068662  2.4876 0.08497  0.053 .
## Terminal_Weight  1   0.06153 0.061535  2.2294 0.07615  0.055 .
## Residuals       22   0.60725 0.027602         0.75150
## Total           25   0.80805                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Type

```
##
## Call:
## adonis(formula = dw_sub ~ Type, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Type       1   0.07060 0.070605  2.2978 0.08738  0.052 .
## Residuals 24   0.73744 0.030727         0.91262
## Total     25   0.80805                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

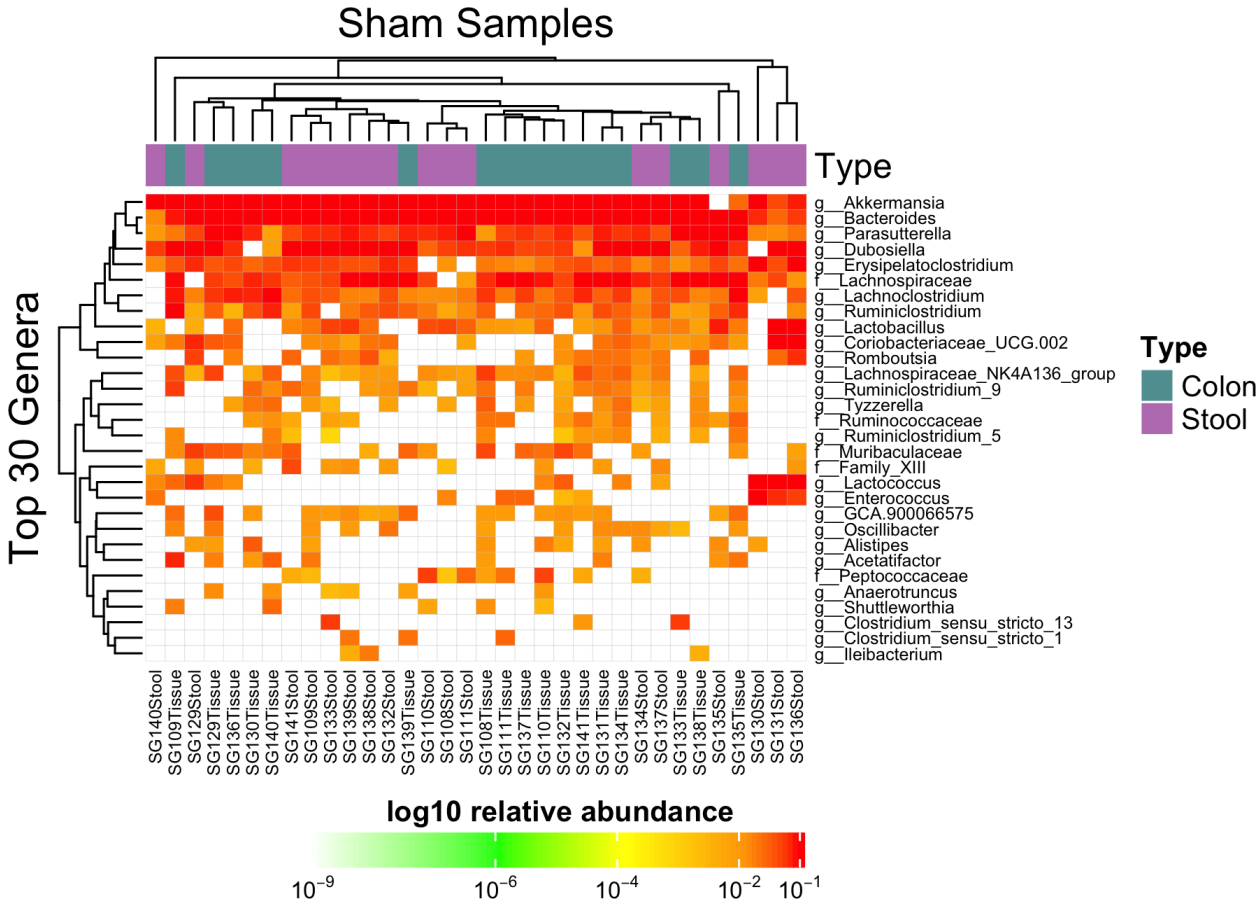
Section 3. Sham Samples Only

Taxonomic Overview

Visualized as heatmaps

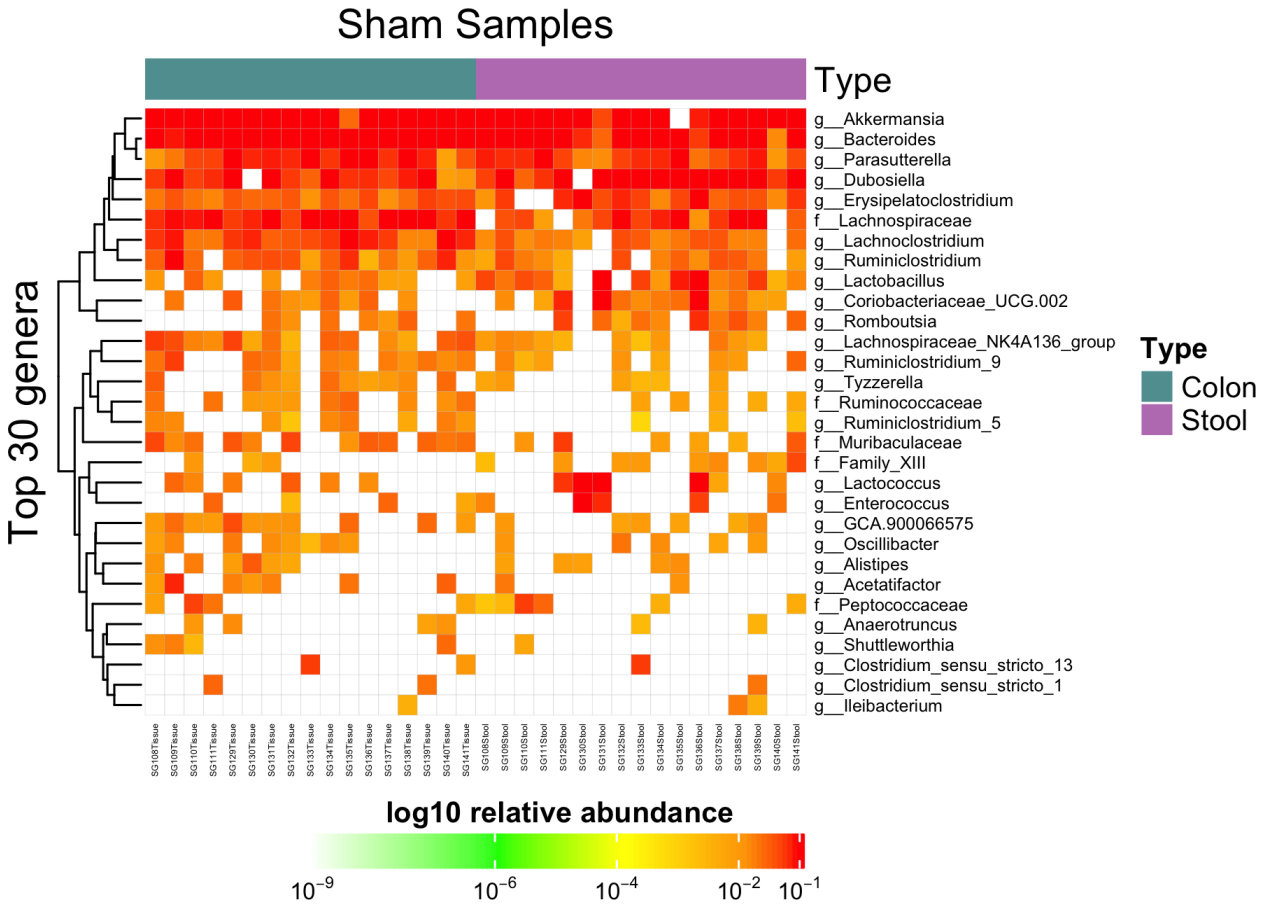
Heatmap clustered based on similar gut compositions

Heatmap of log10 relative abundances of the top 30 genera in Sham samples. Samples (columns) and taxa (rows) are sorted based on Bray-Curtis dissimilarities and Euclidean distances, respectively. These samples display similar to other studies on gut microbiome samples.



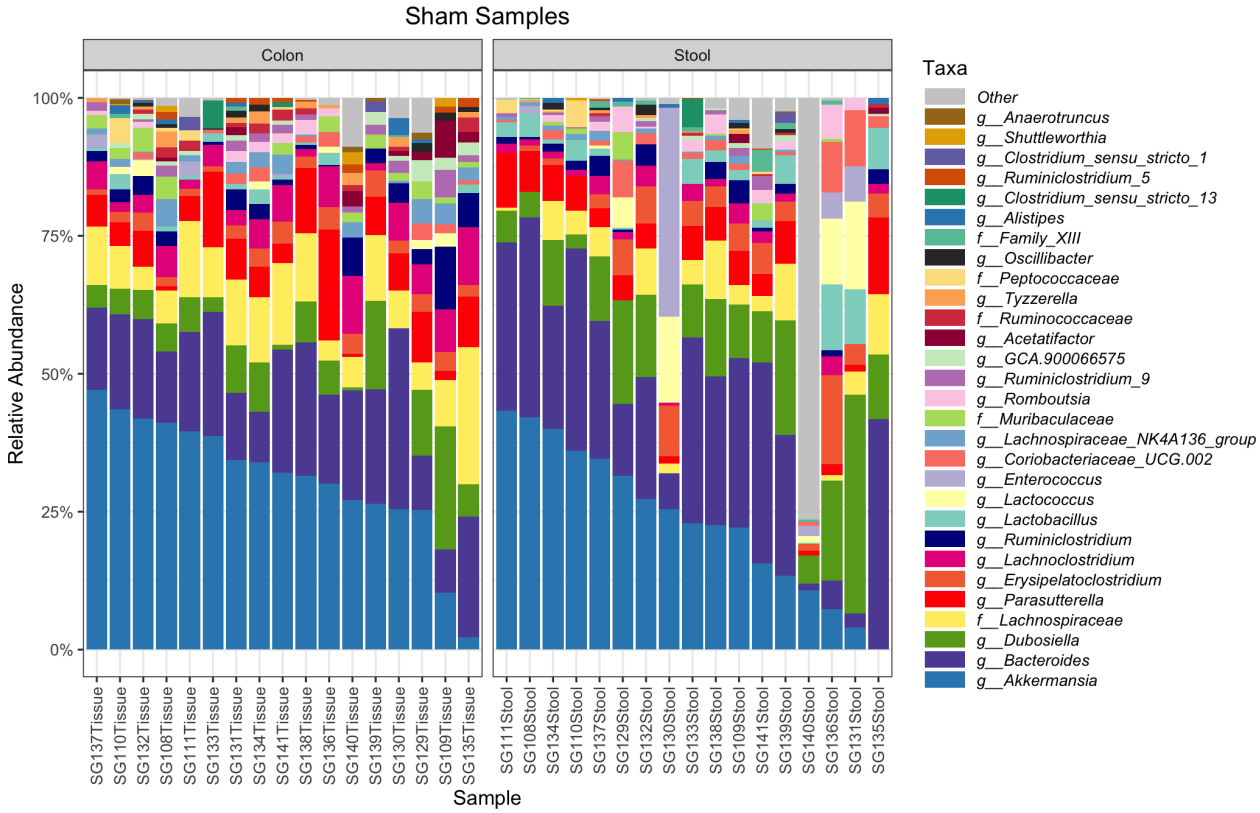
Heatmap clustered based on factors in the metadata

Heatmap of log10 relative abundances of the top 30 genera. Samples (columns) and taxa (rows) are sorted based on Type, and descending overall average relative abundance, respectively.

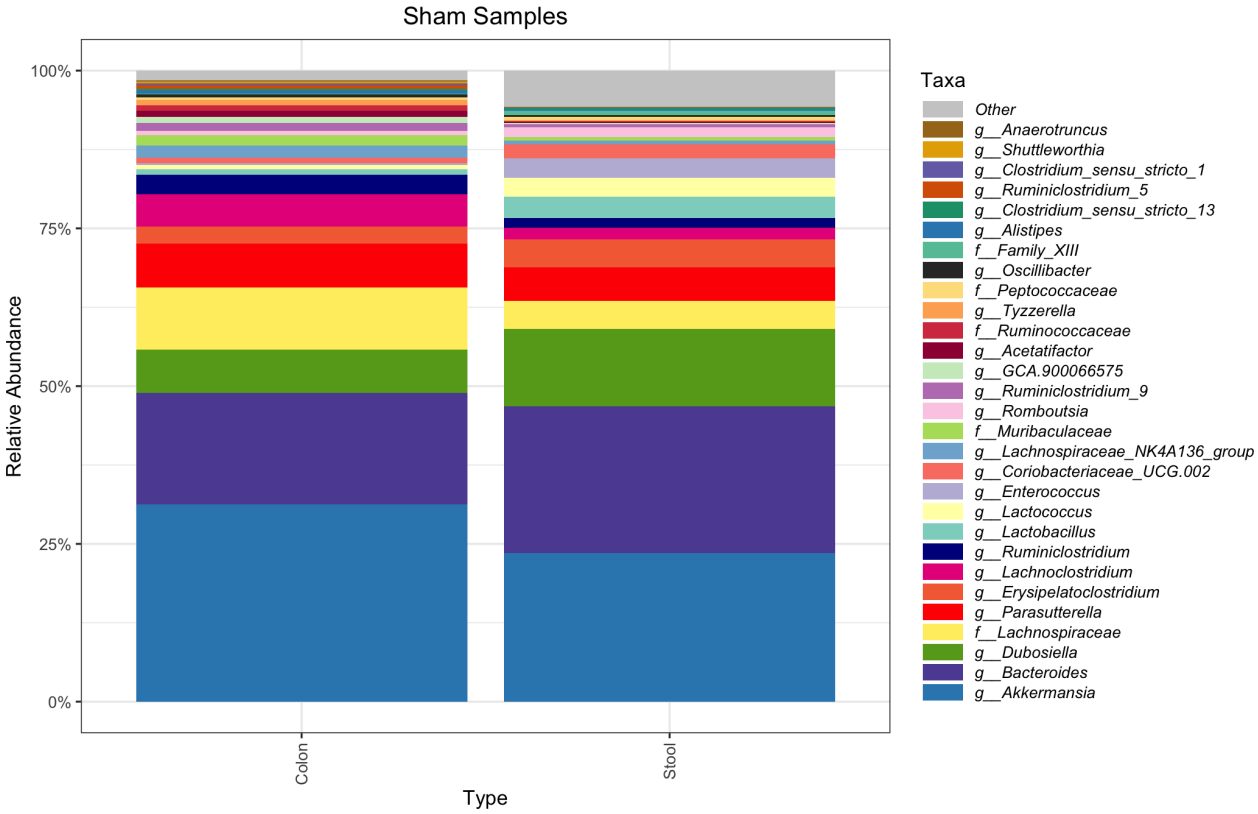


Visualized as stacked barplots

Bar plot of the top 29 abundant taxa among Sham samples by Type

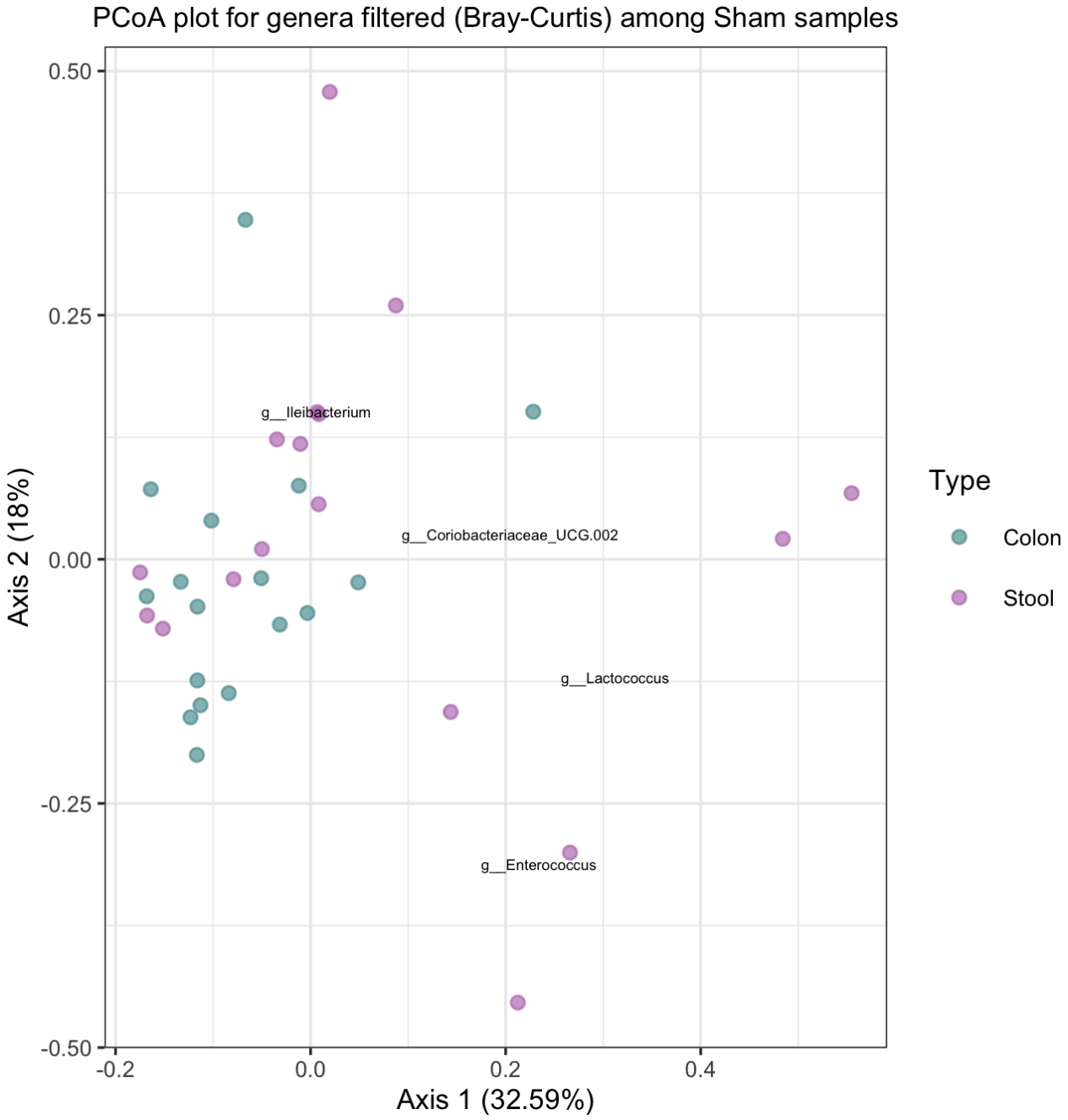


Bar plot of the mean relative abundance of the top 29 taxa among Sham samples by Type



Beta Diversity: Bray-Curtis dissimilarity

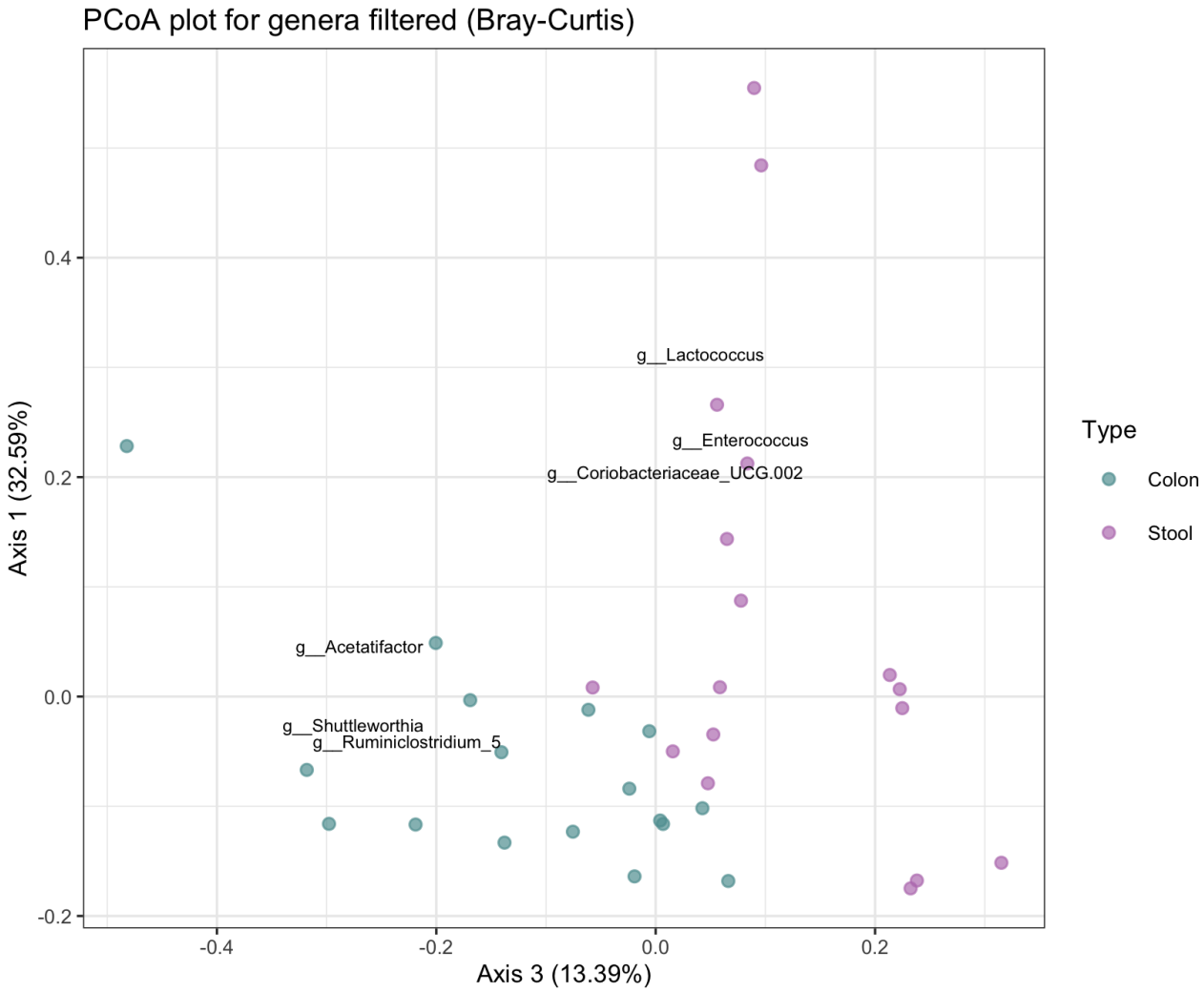
Principal coordinate analysis colored by Type



Finding the PCs that are most significantly associated with Type among Sham samples.

PC	pval
MDS3	5e-06 ***
MDS1	0.024433 *
MDS8	0.265994
MDS6	0.290251
MDS5	0.38276
MDS2	0.484859
MDS7	0.841018
MDS4	0.863285
MDS9	0.935637

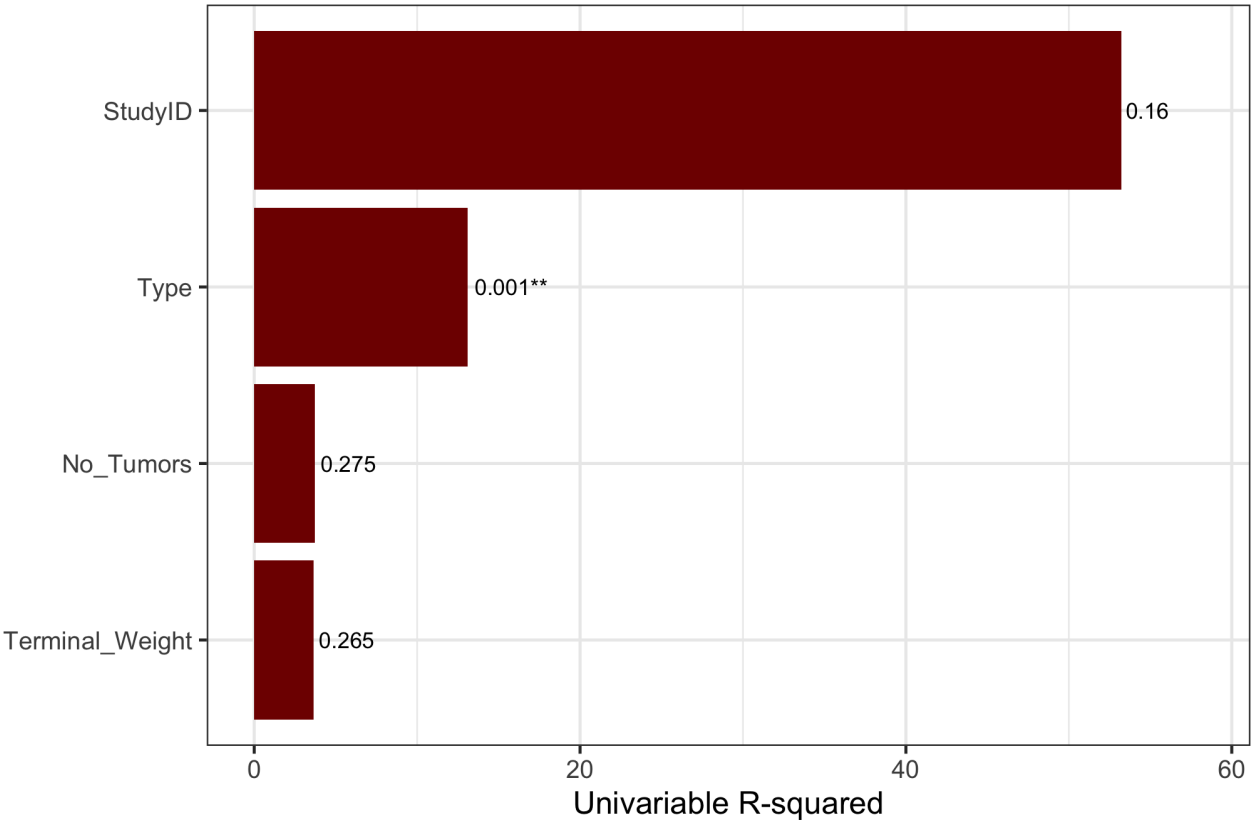
Principal coordinate analysis of MDS3 and MDS1, colored Type



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Bray-Curtis dissimilarity among Sham samples

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

Type + Standard Clinical Covarites

```
##
## Call:
## adonis(formula = bray_sub ~ Type + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## Type             1    0.4359 0.43595  4.8505 0.13083  0.001 ***
## No_Tumors        1    0.1245 0.12451  1.3854 0.03737  0.228
## Terminal_Weight  1    0.0754 0.07544  0.8394 0.02264  0.538
## Residuals       30    2.6963 0.08988         0.80917
## Total           33    3.3322                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

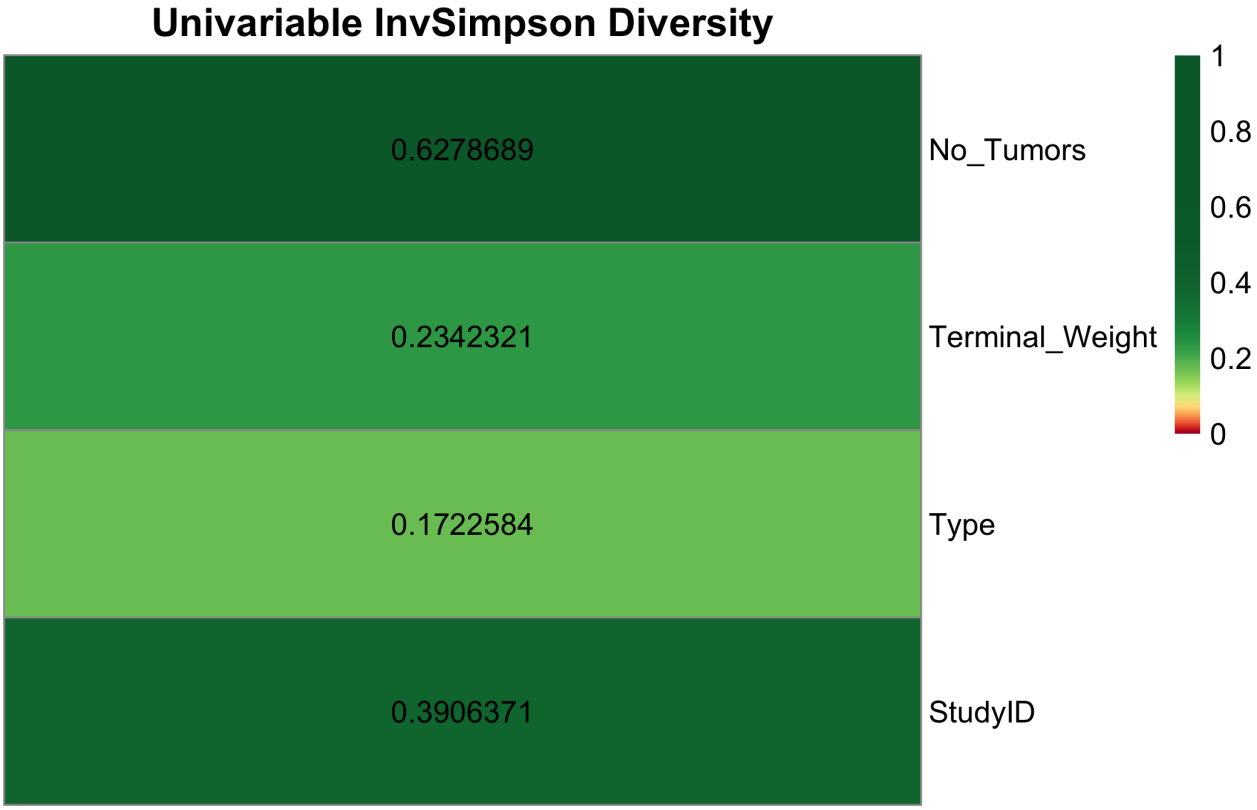
Type

```
##
## Call:
## adonis(formula = bray_sub ~ Type, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## Type       1    0.4359 0.43595  4.8166 0.13083  0.001 ***
## Residuals 32    2.8963 0.09051         0.86917
## Total     33    3.3322                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Alpha Diversity

Statistics alpha diversity: anova on linear model

Heatmap annotated with the p-value for each covariate

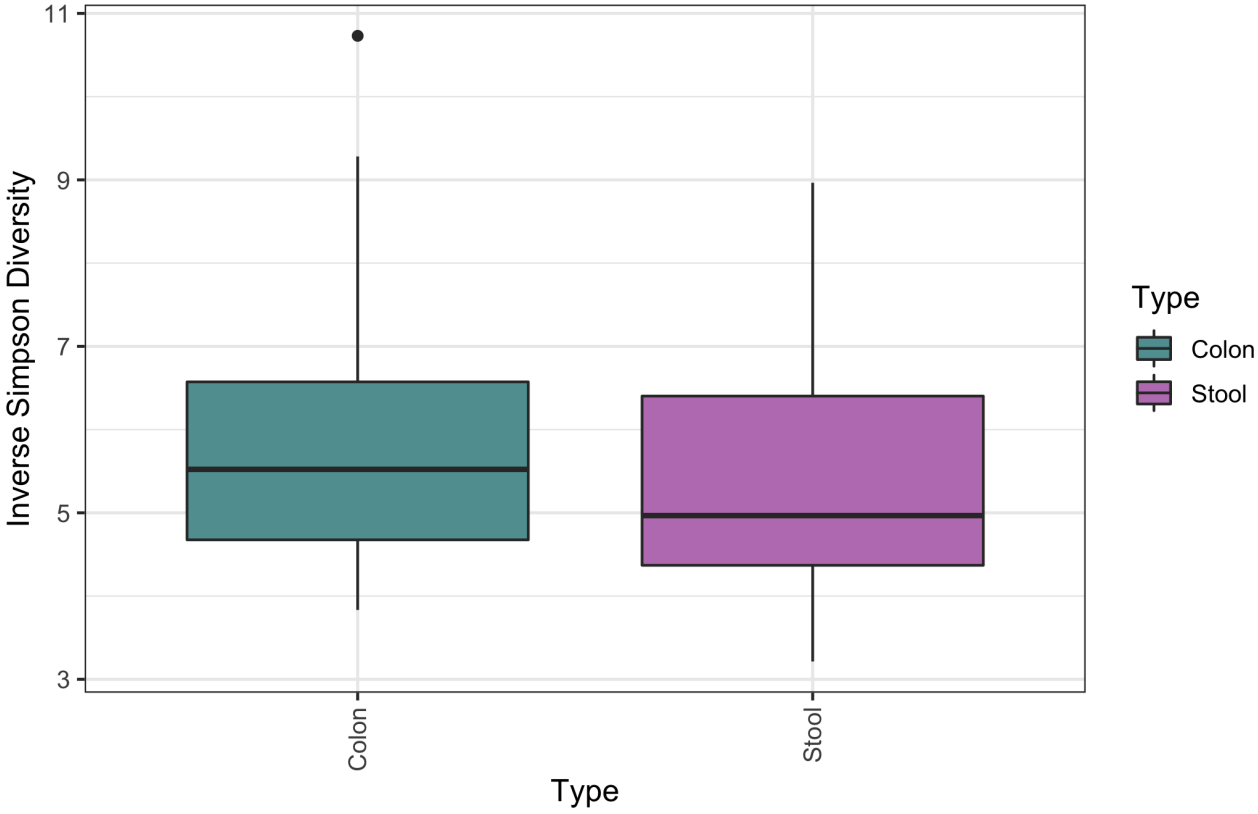


Multivariable model for Alpha diversity

```
## Analysis of Variance Table
##
## Response: InvSimpson
##           Df Sum Sq Mean Sq F value Pr(>F)
## Type       1  5.934  5.9338  1.9494 0.1723
## Residuals 32 97.403  3.0438
```

```
## Analysis of Variance Table
##
## Response: InvSimpson
##                 Df Sum Sq Mean Sq F value Pr(>F)
## Type             1  5.934  5.9338  1.9171 0.1764
## No_Tumors        1  0.768  0.7678  0.2481 0.6221
## Terminal_Weight  1  3.778  3.7782  1.2207 0.2780
## Residuals       30 92.857  3.0952
```

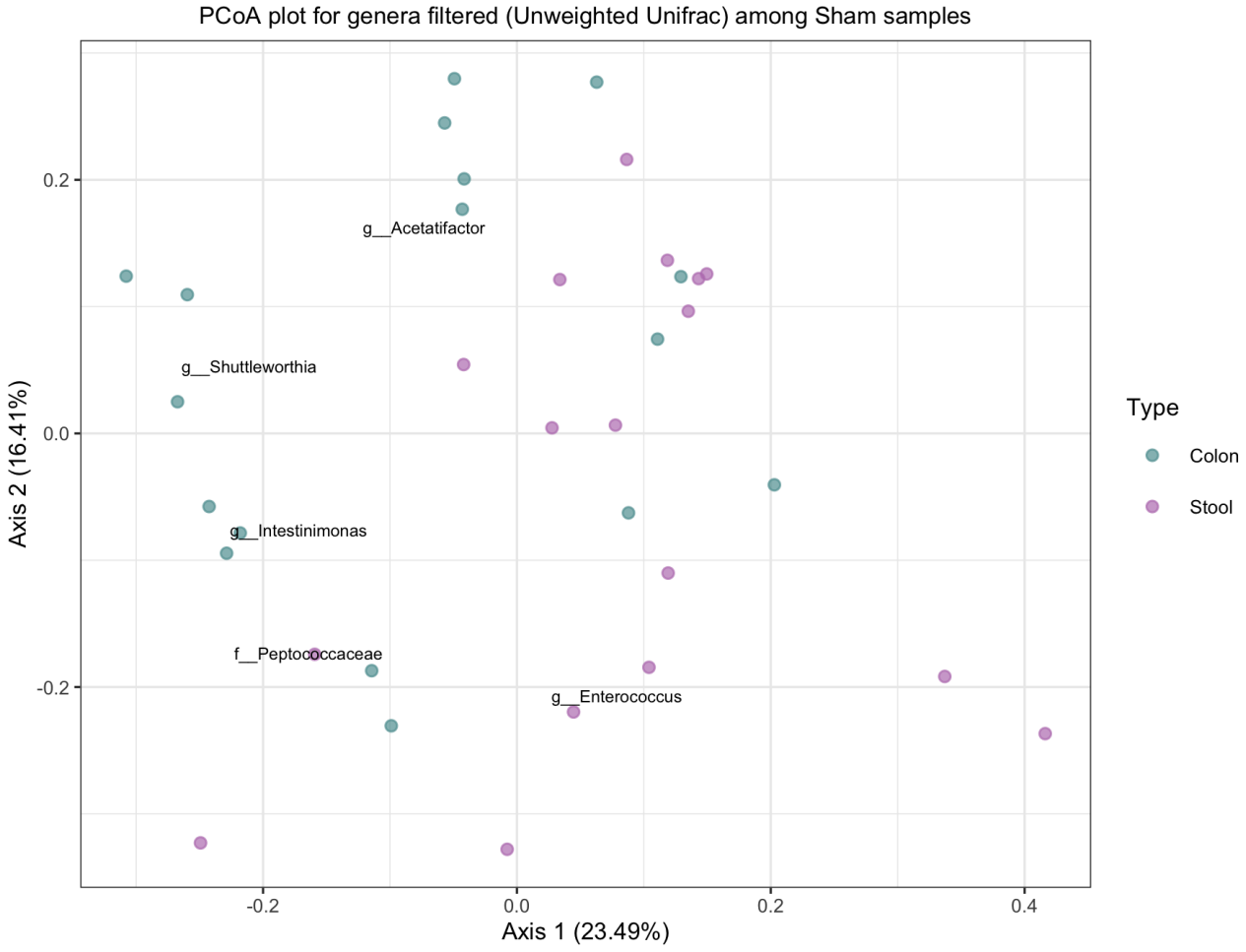
Invsimpson vs. Type



Other Beta Diversity Metrics

Unweighted UniFrac

Principal coordinate analysis colored by Type



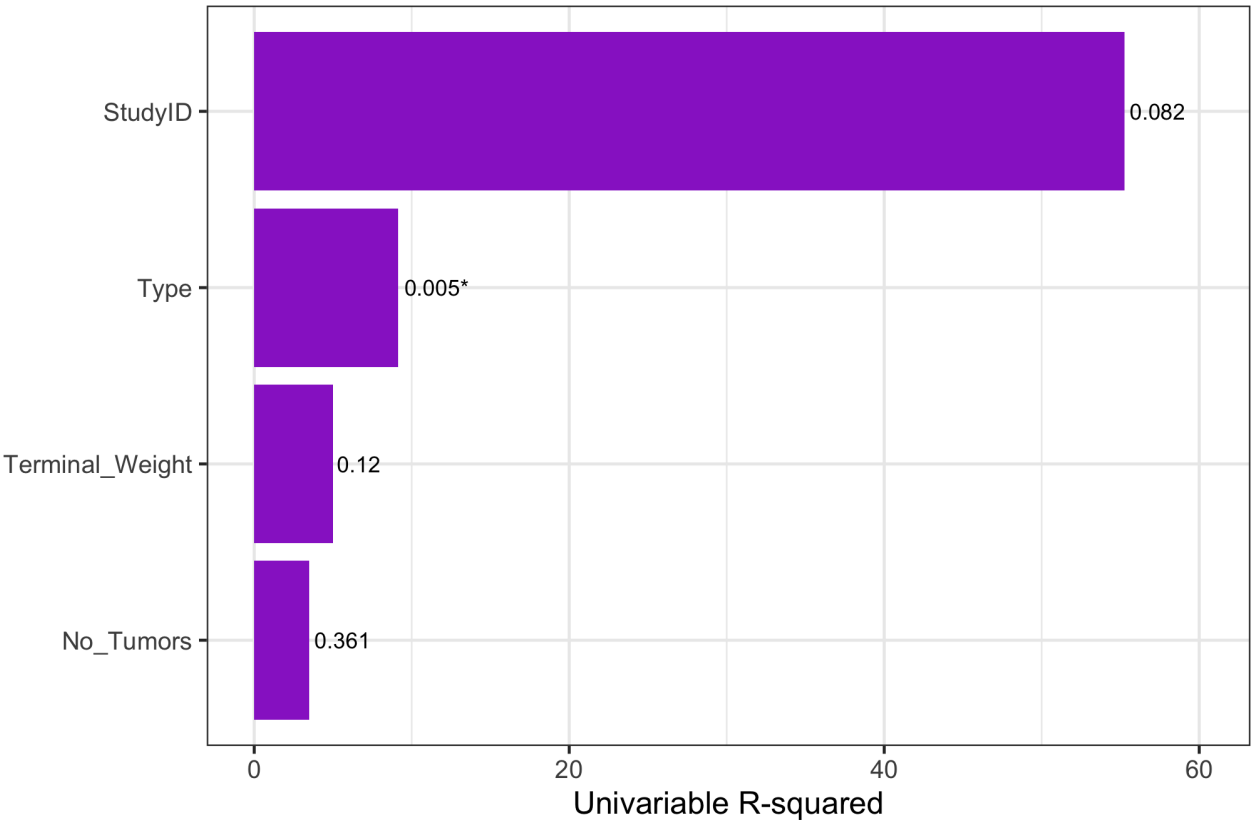
Finding the PCs that are most significantly associated with Type among Sham samples.

PC	pval
MDS1	0.00648 **
MDS2	0.081211 ‘
MDS6	0.115518
MDS7	0.208414
MDS5	0.296494
MDS4	0.554161
MDS3	0.60468
MDS9	0.849537
MDS8	0.871982

Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Unweighted UniFrac Distances

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

Type + Standard Clinical Covarites

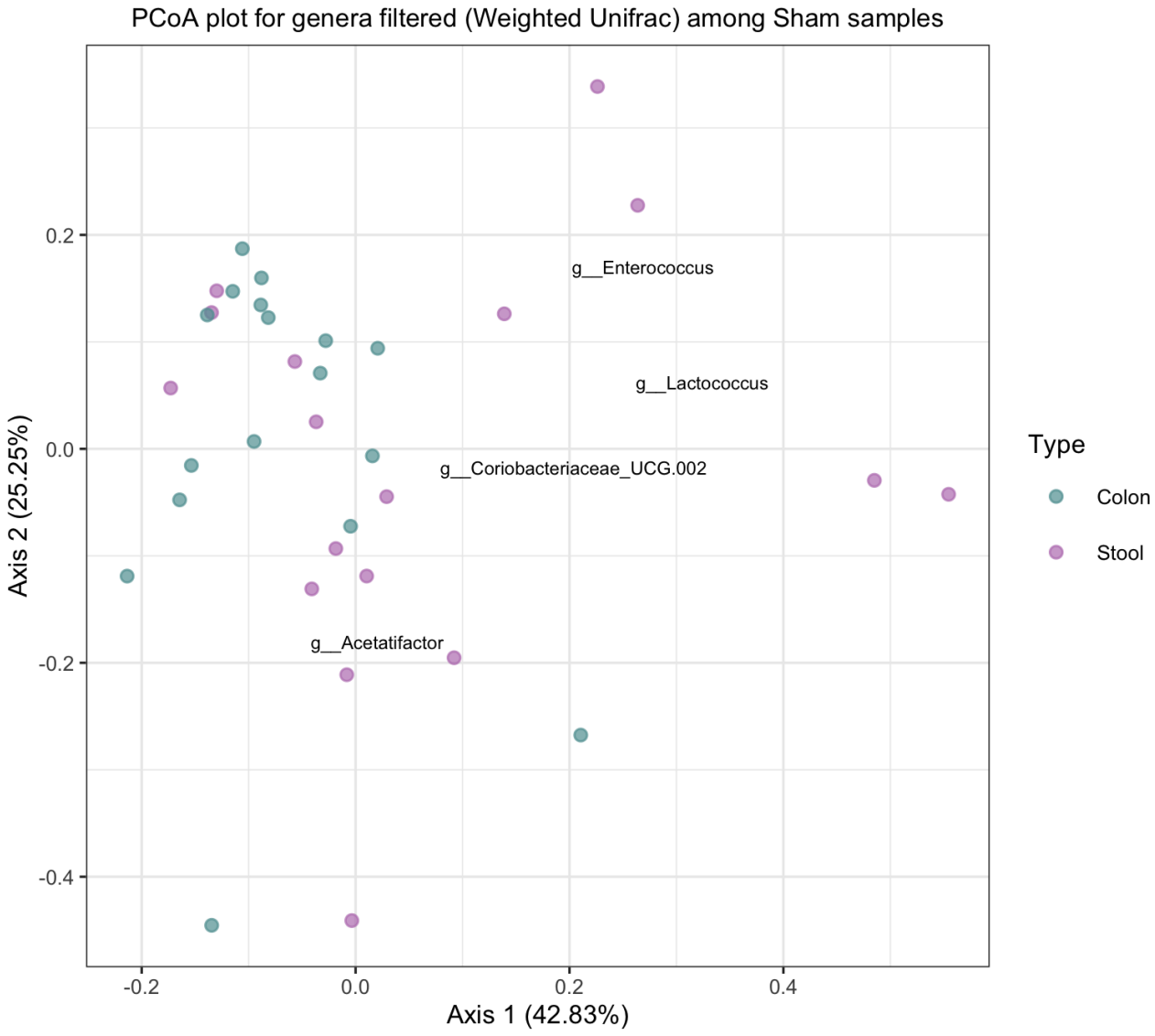
```
##
## Call:
## adonis(formula = duw_sub ~ Type + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Type             1   0.16107 0.161071  3.2491 0.09135  0.005 **
## No_Tumors        1   0.06157 0.061568  1.2420 0.03492  0.315
## Terminal_Weight  1   0.05348 0.053479  1.0788 0.03033  0.395
## Residuals       30   1.48720 0.049573         0.84341
## Total           33   1.76332                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Type

```
##
## Call:
## adonis(formula = duw_sub ~ Type, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## Type       1   0.16107 0.16107  3.2169 0.09135  0.009 **
## Residuals 32   1.60224 0.05007         0.90865
## Total     33   1.76332                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Weighted UniFrac

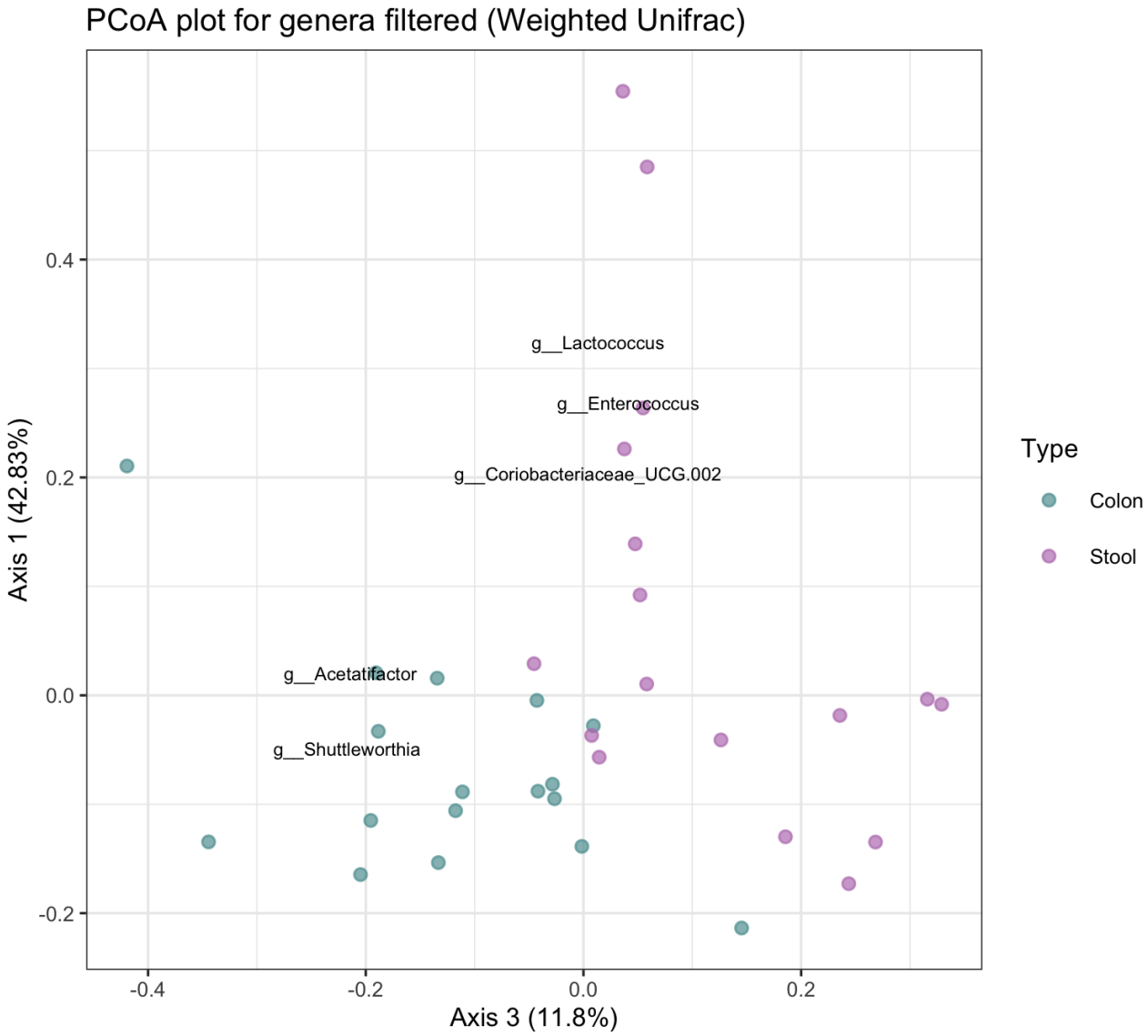
Principal coordinate analysis colored by Type



Finding the PCs that are most significantly associated with Type among Sham samples.

PC	pval
MDS3	5e-06 ***
MDS1	0.015809 *
MDS6	0.357241
MDS5	0.416702
MDS8	0.66671
MDS2	0.735309
MDS9	0.778561
MDS7	0.866899
MDS4	0.99011

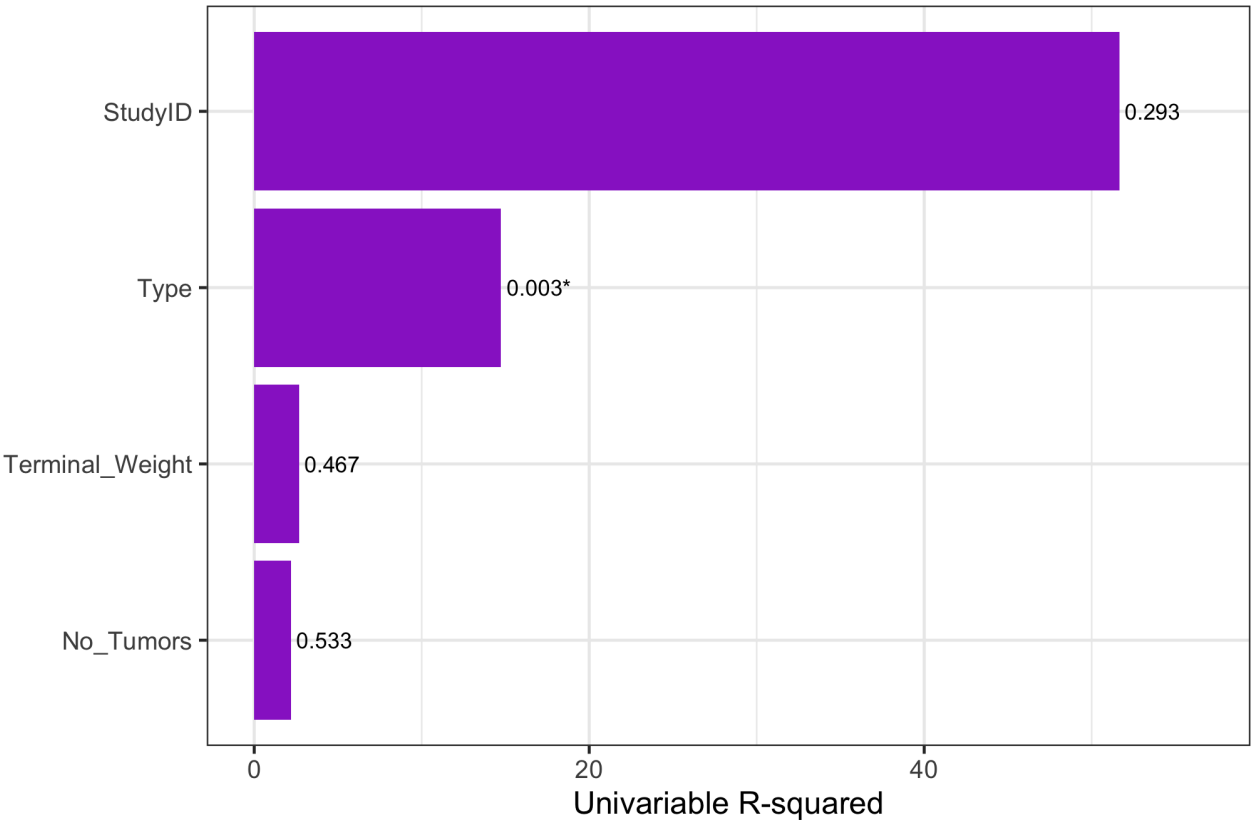
Principal coordinate analysis of MDS3 and MDS1, colored Type



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Weighted UniFrac Distances

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

Type + Standard Clinical Covarites

```
##
## Call:
## adonis(formula = dw_sub ~ Type + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Type             1   0.25764 0.257639  5.4629 0.14711  0.001 ***
## No_Tumors        1   0.03856 0.038563  0.8177 0.02202  0.513
## Terminal_Weight  1   0.04026 0.040264  0.8537 0.02299  0.488
## Residuals       30   1.41484 0.047161         0.80788
## Total           33   1.75131                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Type

```
##
## Call:
## adonis(formula = dw_sub ~ Type, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Type       1   0.25764 0.257639  5.5196 0.14711  0.003 **
## Residuals 32   1.49367 0.046677         0.85289
## Total     33   1.75131                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

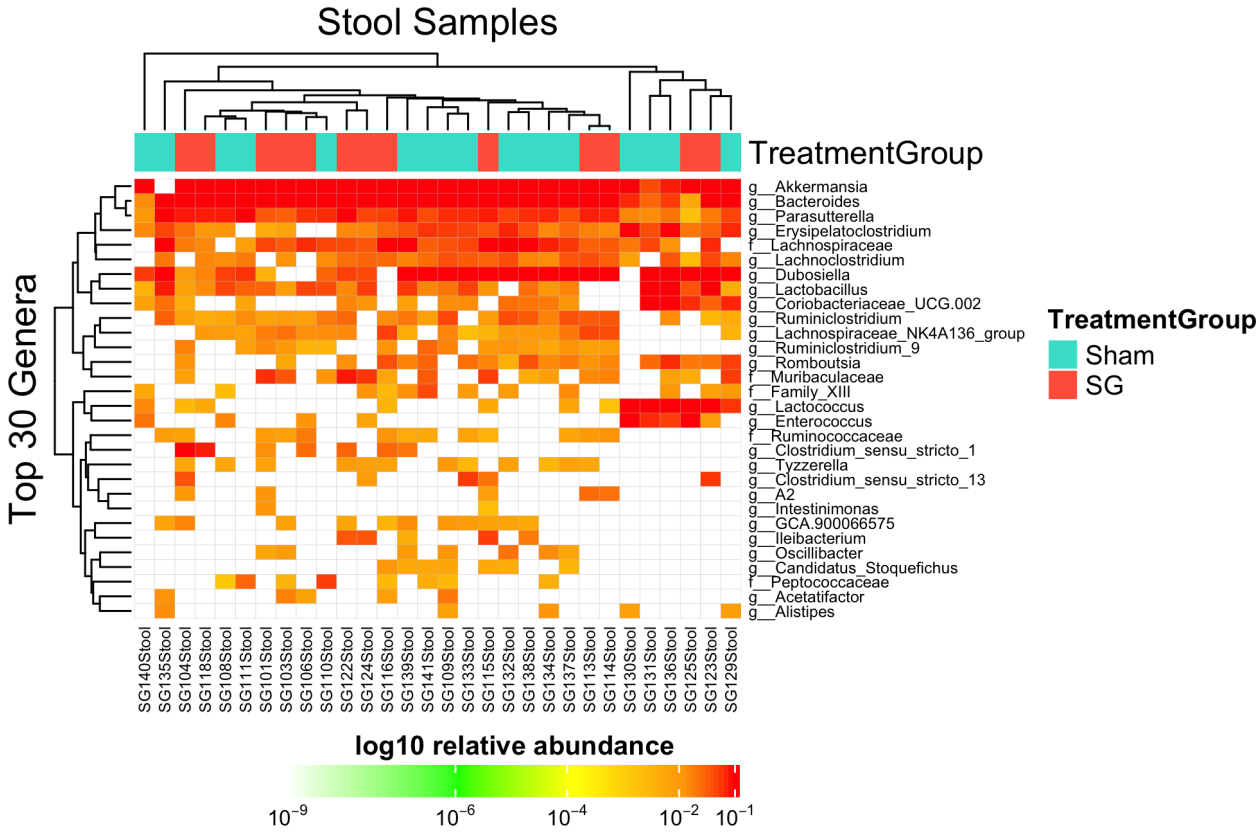
Section 4. Stool Samples Only

Taxonomic Overview

Visualized as heatmaps

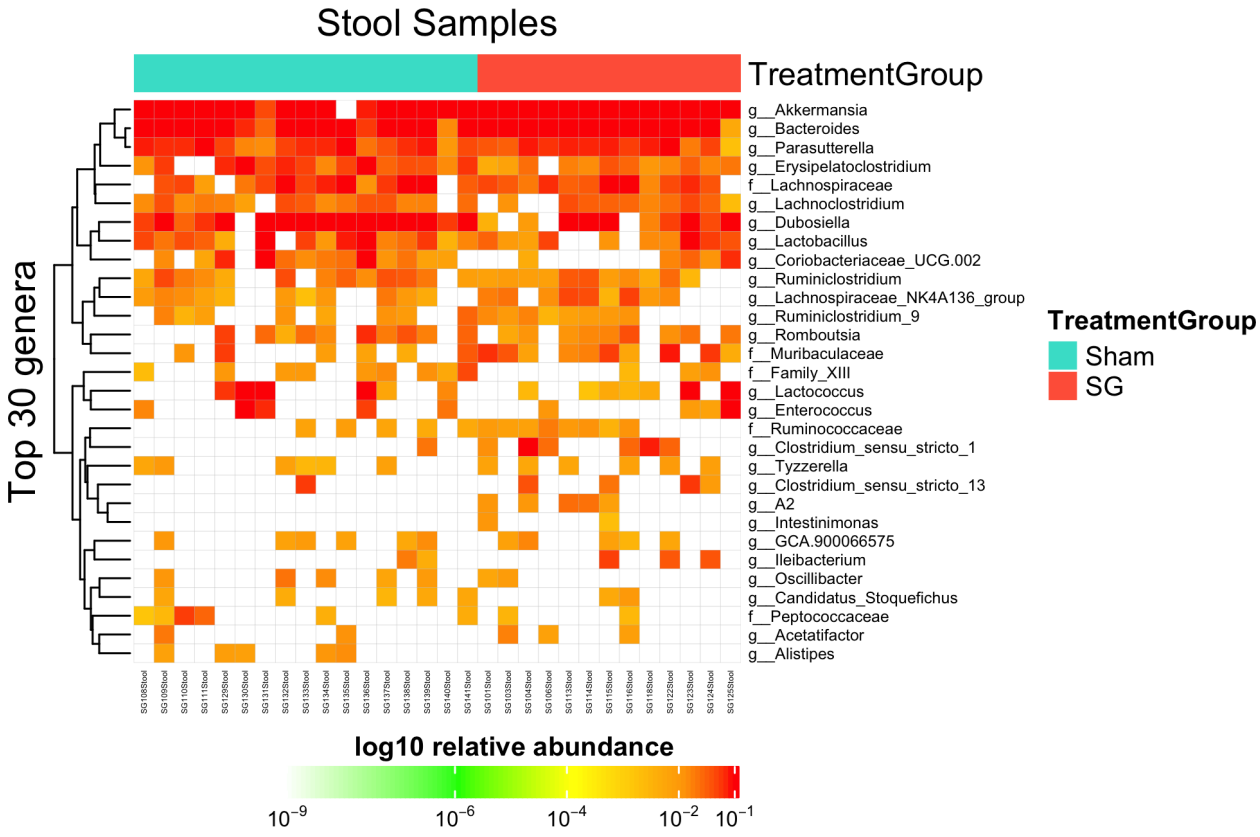
Heatmap clustered based on similar gut compositions

Heatmap of log10 relative abundances of the top 30 genera in Stool Samples. Samples (columns) and taxa (rows) are sorted based on Bray-Curtis dissimilarities and Euclidean distances, respectively. These samples display similar to other studies on gut microbiome samples.



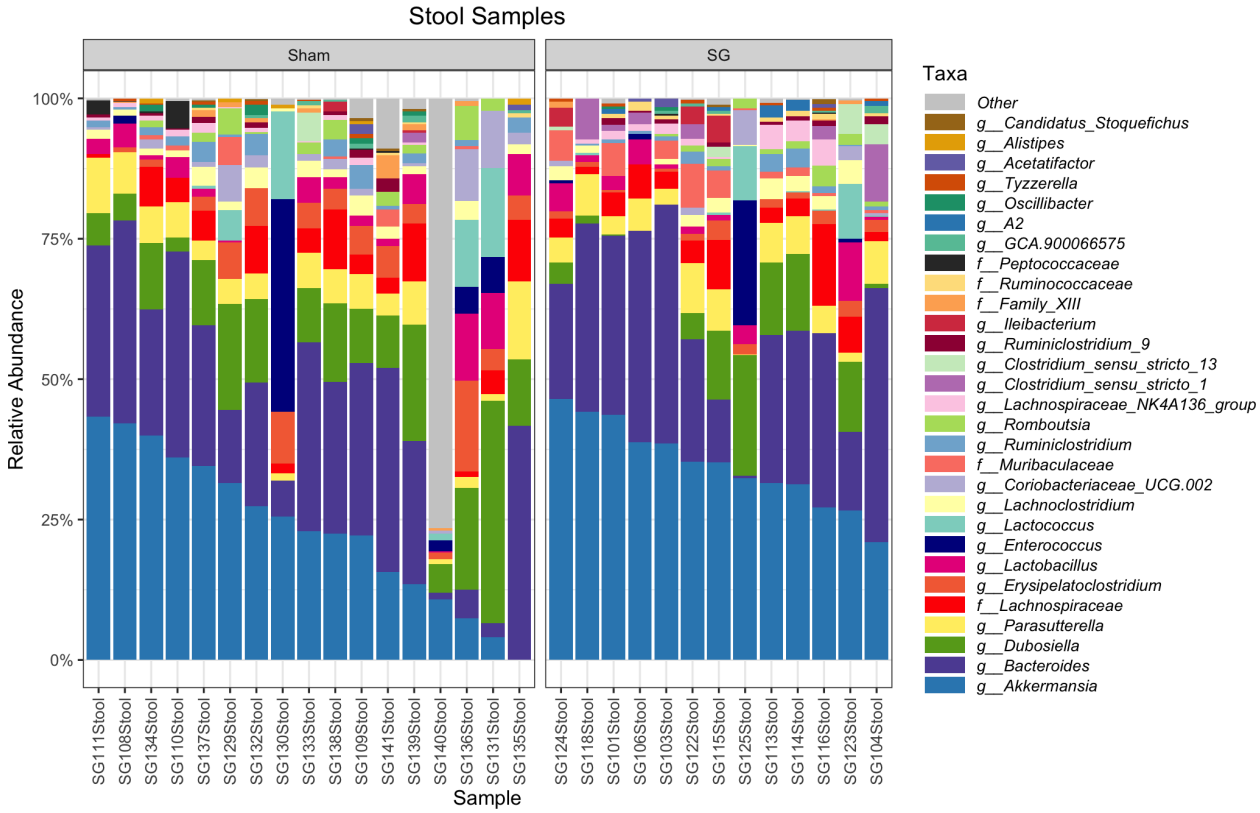
Heatmap clustered based on factors in the metadata

Heatmap of log10 relative abundances of the top 30 genera. Samples (columns) and taxa (rows) are sorted based on TreatmentGroup, and descending overall average relative abundance, respectively.

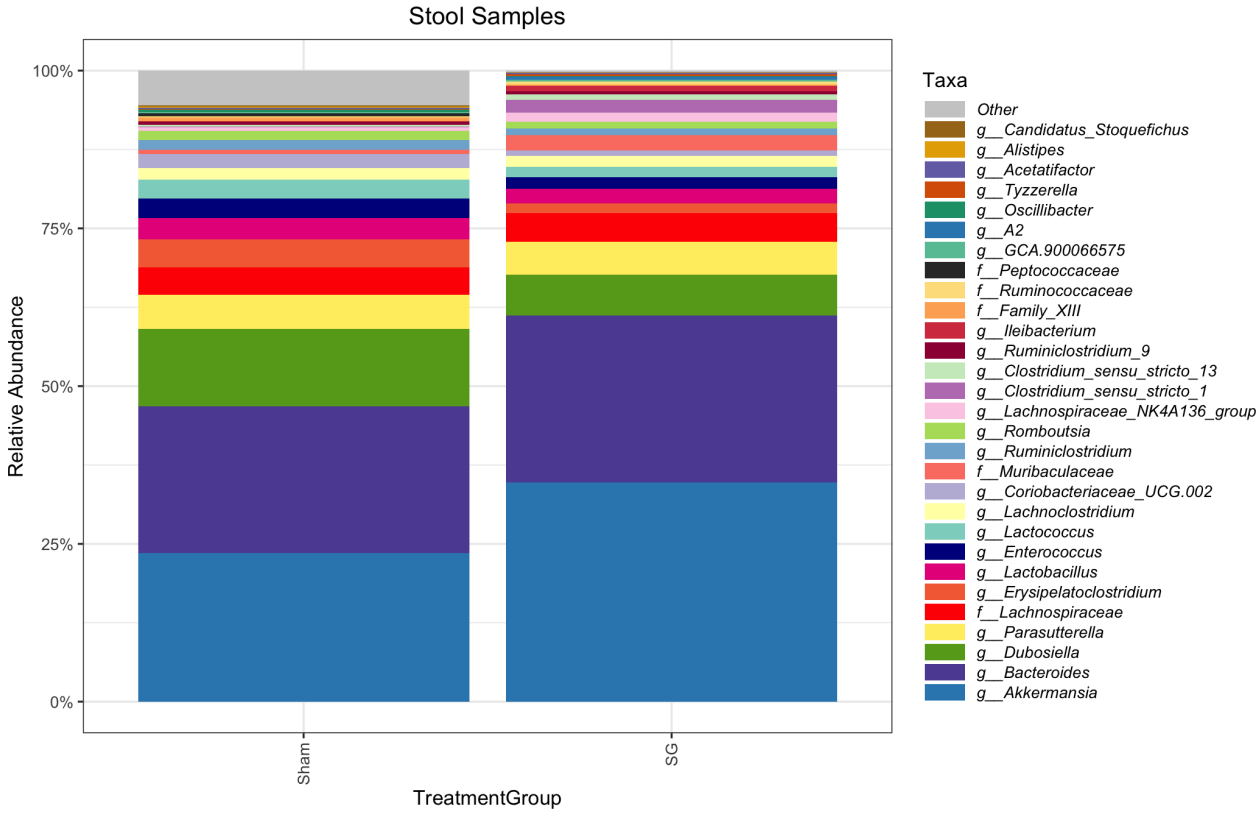


Visualized as stacked barplots

Bar plot of the top 29 abundant taxa among Stool Samples by TreatmentGroup

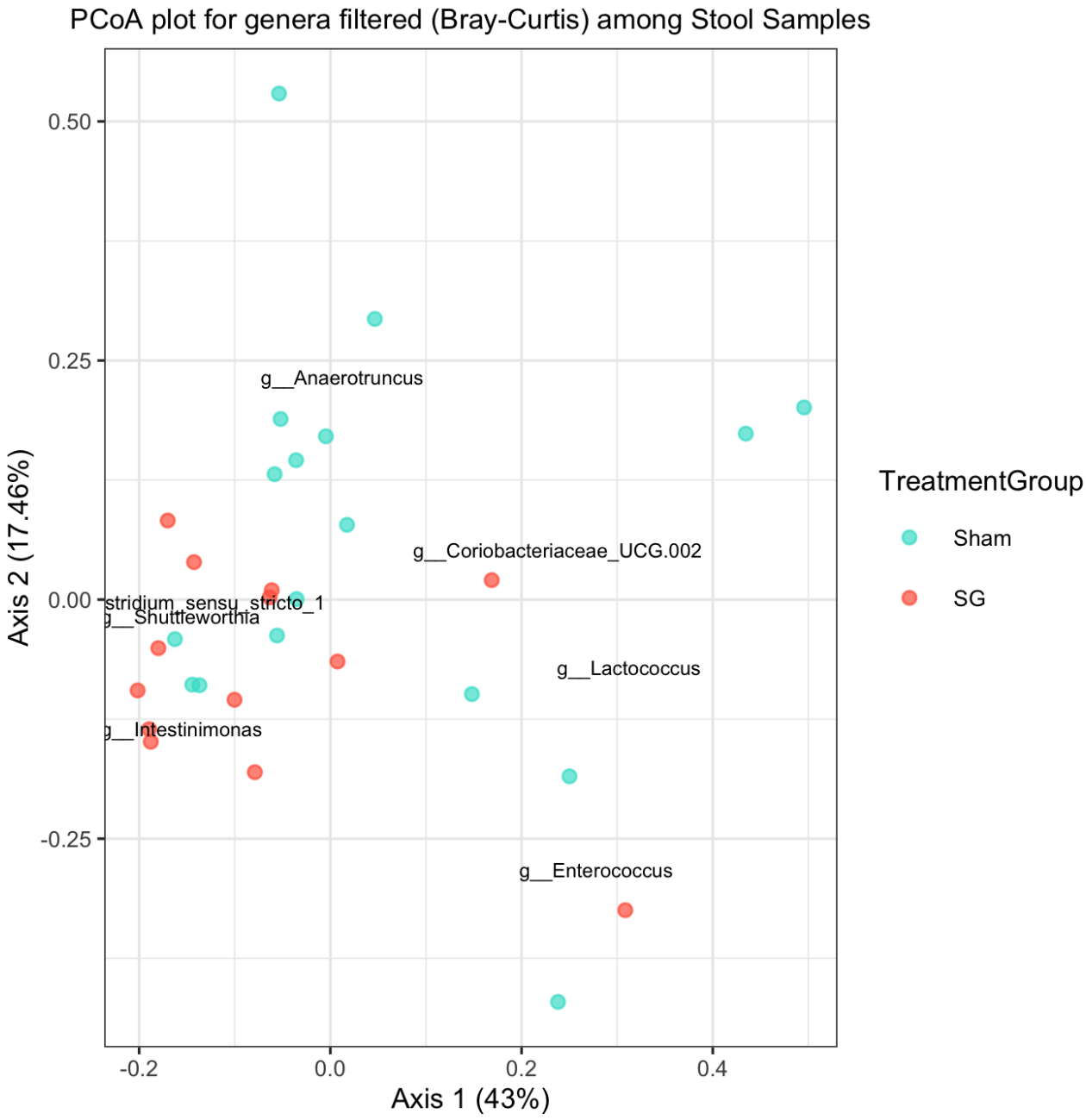


Bar plot of the mean relative abundance of the top 29 taxa among Stool Samples by TreatmentGroup



Beta Diversity: Bray-Curtis dissimilarity

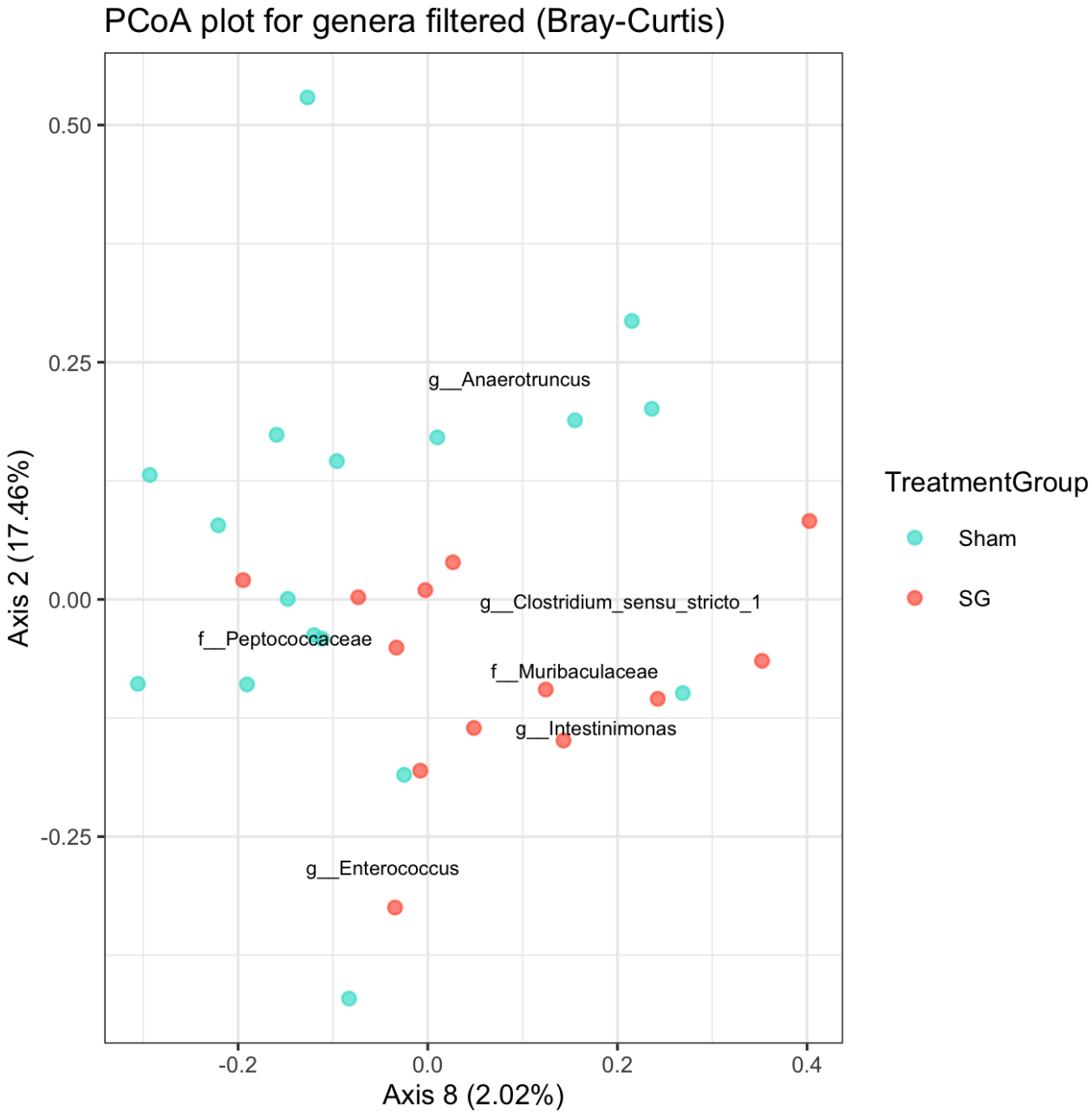
Principal coordinate analysis colored by TreatmentGroup



Finding the PCs that are most significantly associated with TreatmentGroup among Stool Samples.

PC	pval
MDS8	0.046492 *
MDS2	0.057826 ‘
MDS1	0.076558 ‘
MDS9	0.150835
MDS3	0.245101
MDS6	0.350946
MDS7	0.561921
MDS4	0.798893
MDS5	0.962923

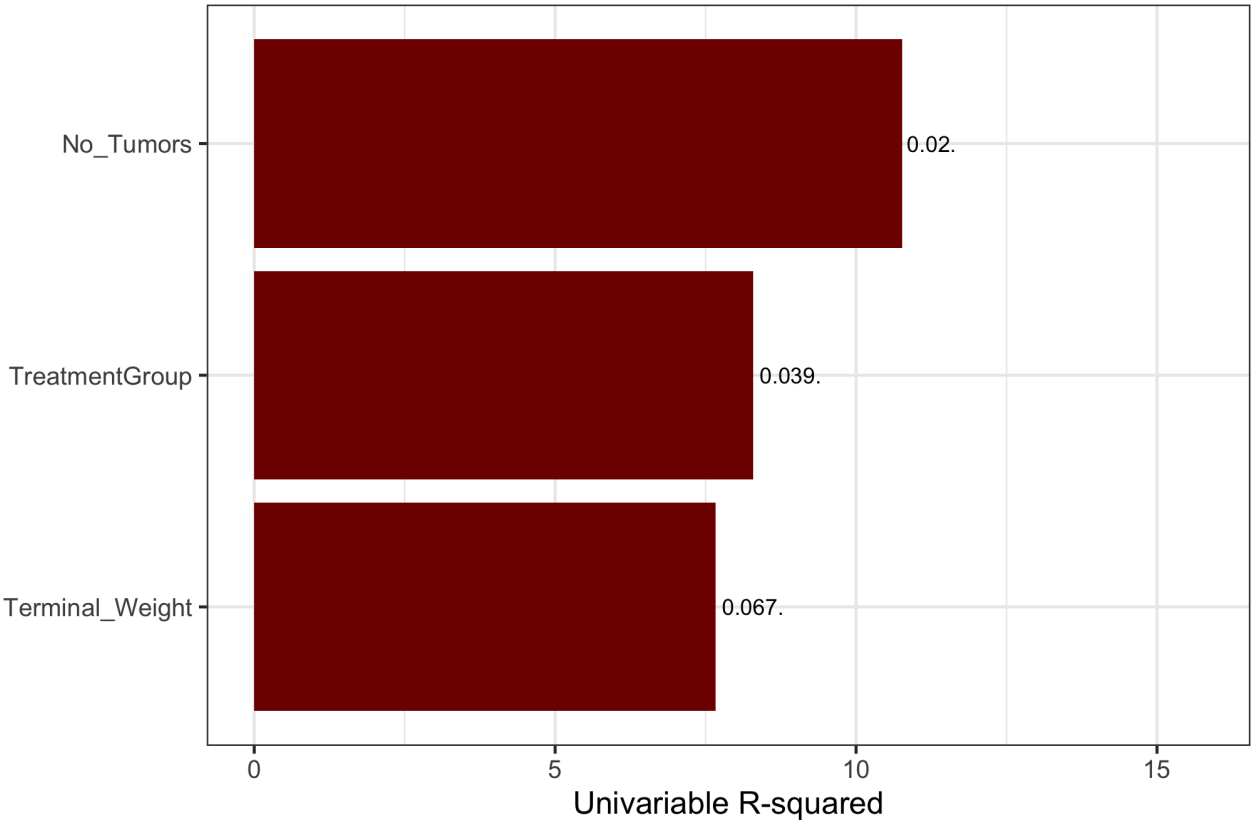
Principal coordinate analysis of MDS8 and MDS2, colored by TreatmentGroup



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Bray-Curtis dissimilarity among Stool Samples

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

TreatmentGroup + Standard Clinical Covarites

```
##
## Call:
## adonis(formula = bray_sub ~ TreatmentGroup + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup   1   0.25391 0.253910 2.57762 0.08295  0.035 *
## No_Tumors        1   0.16489 0.164888 1.67389 0.05387  0.154
## Terminal_Weight  1   0.08102 0.081020 0.82249 0.02647  0.537
## Residuals       26   2.56115 0.098506         0.83671
## Total           29   3.06097                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

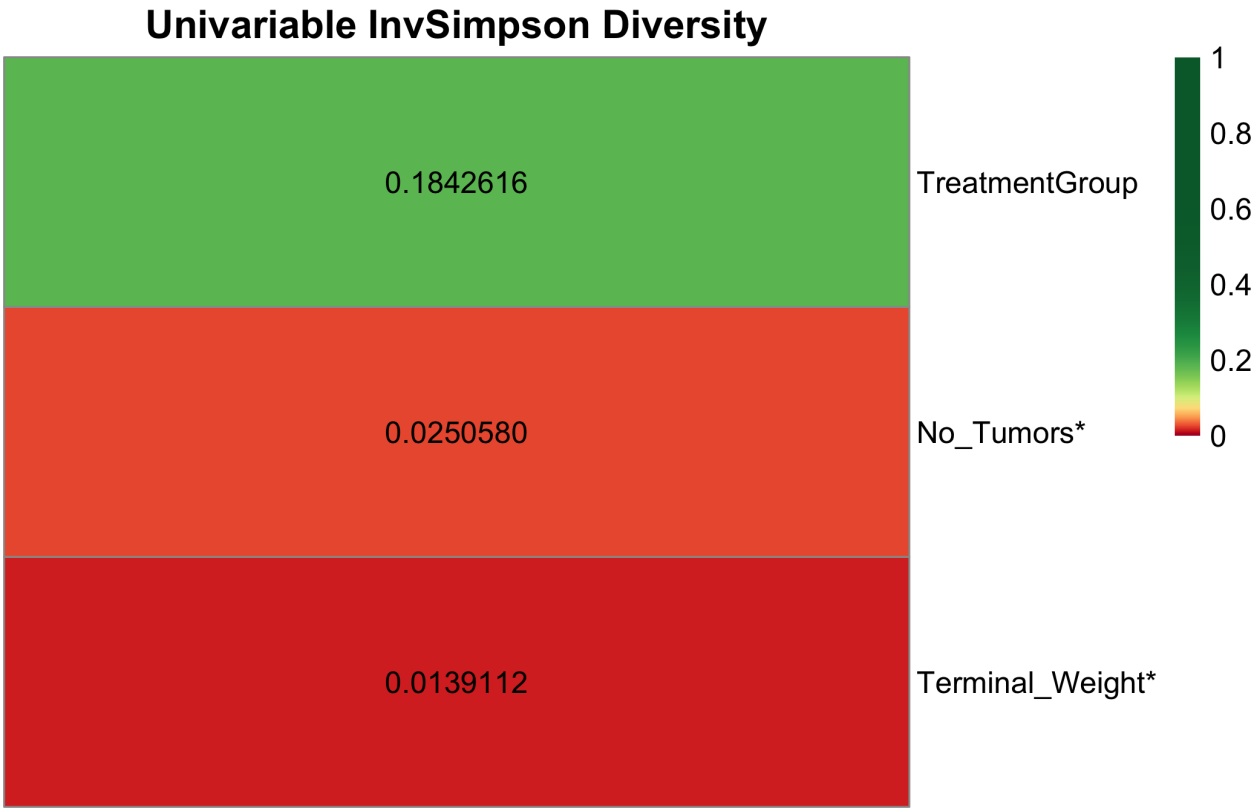
TreatmentGroup

```
##
## Call:
## adonis(formula = bray_sub ~ TreatmentGroup, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup  1   0.25391 0.25391  2.5327 0.08295  0.033 *
## Residuals      28   2.80706 0.10025         0.91705
## Total          29   3.06097                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Alpha Diversity

Statistics alpha diversity: anova on linear model

Heatmap annotated with the p-value for each covariate

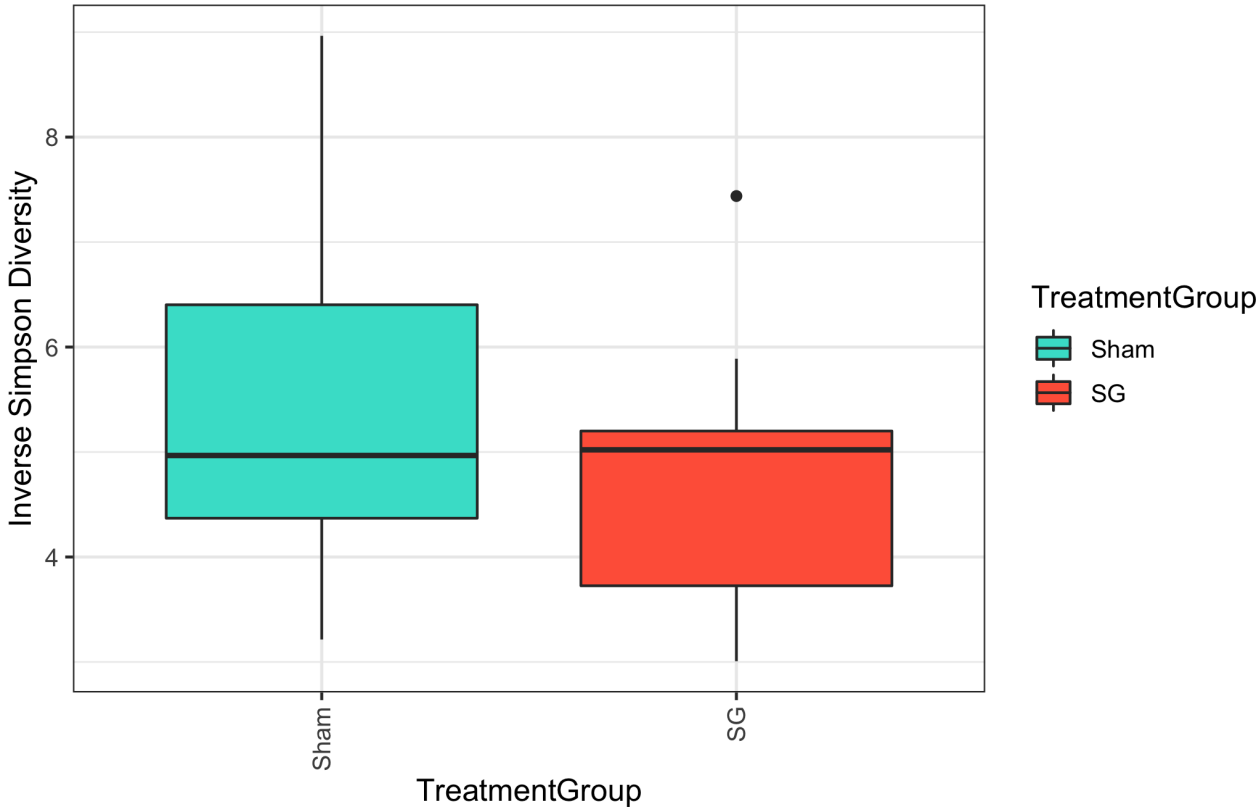


Multivariable model for Alpha diversity

```
## Analysis of Variance Table
##
## Response: InvSimpson
##                Df Sum Sq Mean Sq F value Pr(>F)
## TreatmentGroup  1  3.731  3.7313  1.8532 0.1843
## Residuals      28 56.375  2.0134
```

```
## Analysis of Variance Table
##
## Response: InvSimpson
##                 Df Sum Sq Mean Sq F value  Pr(>F)
## TreatmentGroup   1  3.731  3.7313  2.0842 0.16078
## No_Tumors        1  6.433  6.4331  3.5932 0.06918 .
## Terminal_Weight  1  3.394  3.3935  1.8955 0.18033
## Residuals       26 46.549  1.7903
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

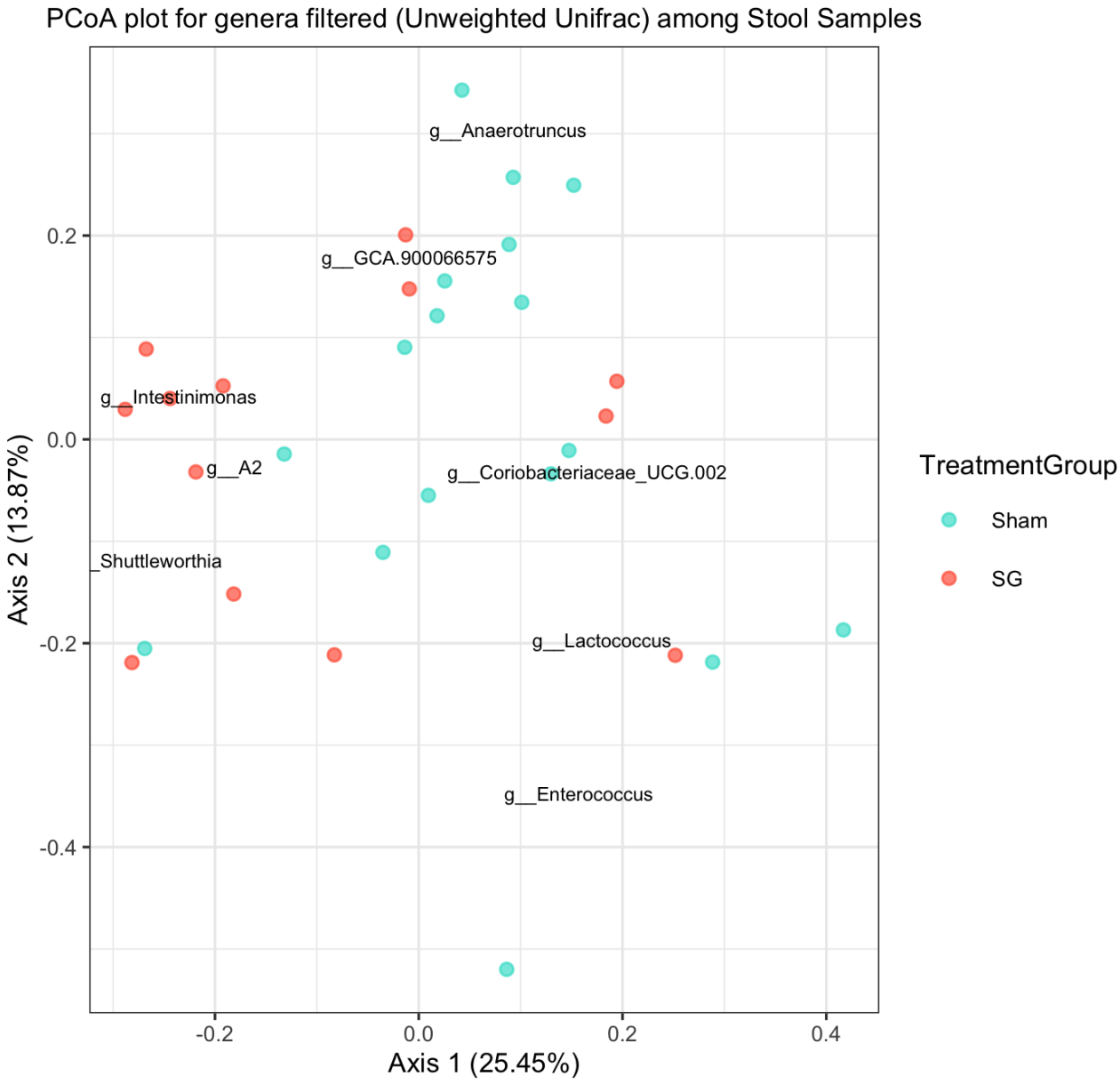
Invsimpson vs. TreatmentGroup



Other Beta Diversity Metrics

Unweighted UniFrac

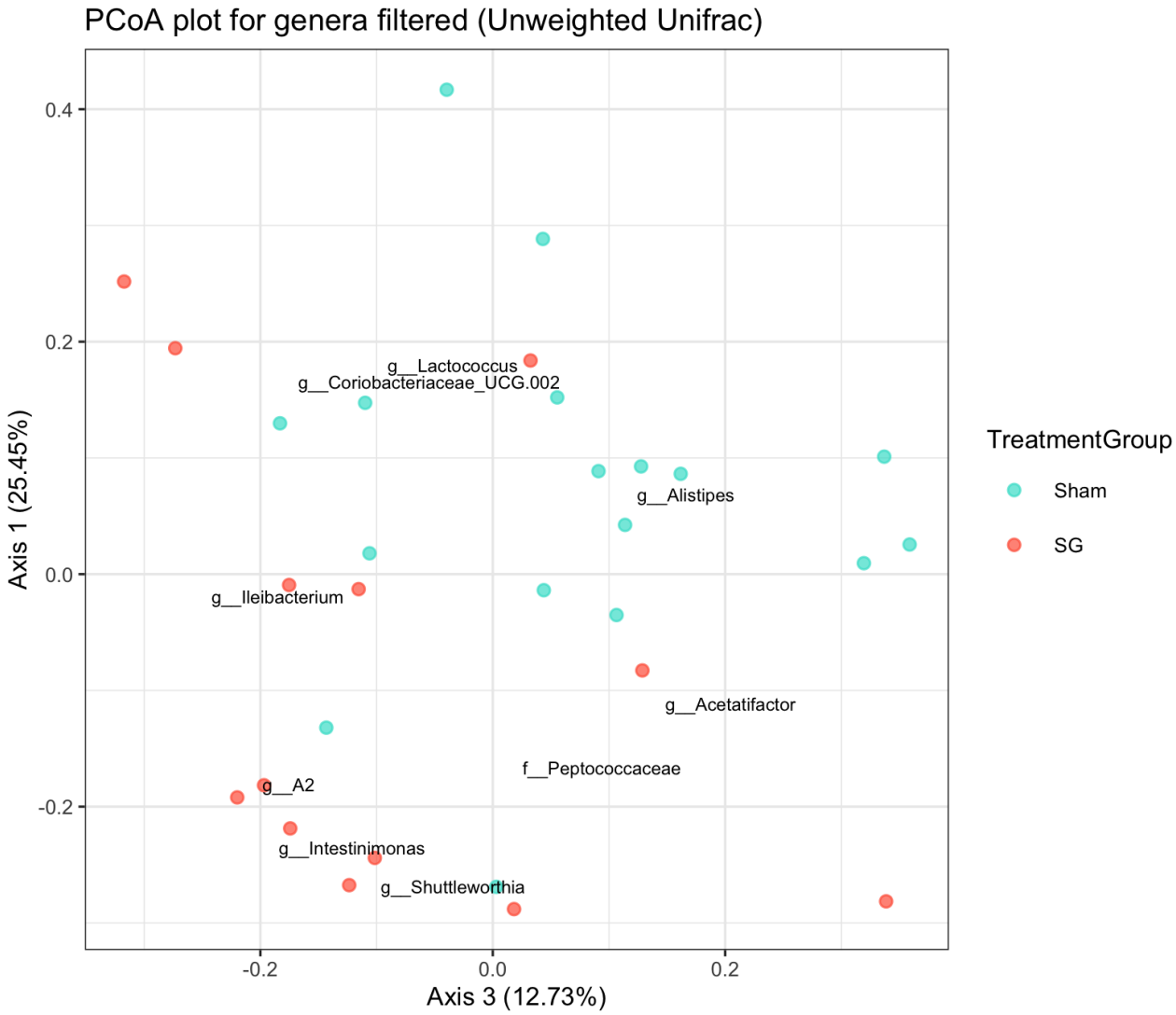
Principal coordinate analysis colored by TreatmentGroup



Finding the PCs that are most significantly associated with TreatmentGroup among Stool Samples.

PC	pval
MDS3	0.016422 *
MDS1	0.019799 *
MDS4	0.033396 *
MDS5	0.191443
MDS7	0.489604
MDS6	0.670308
MDS2	0.71787
MDS9	0.833486
MDS8	0.861267

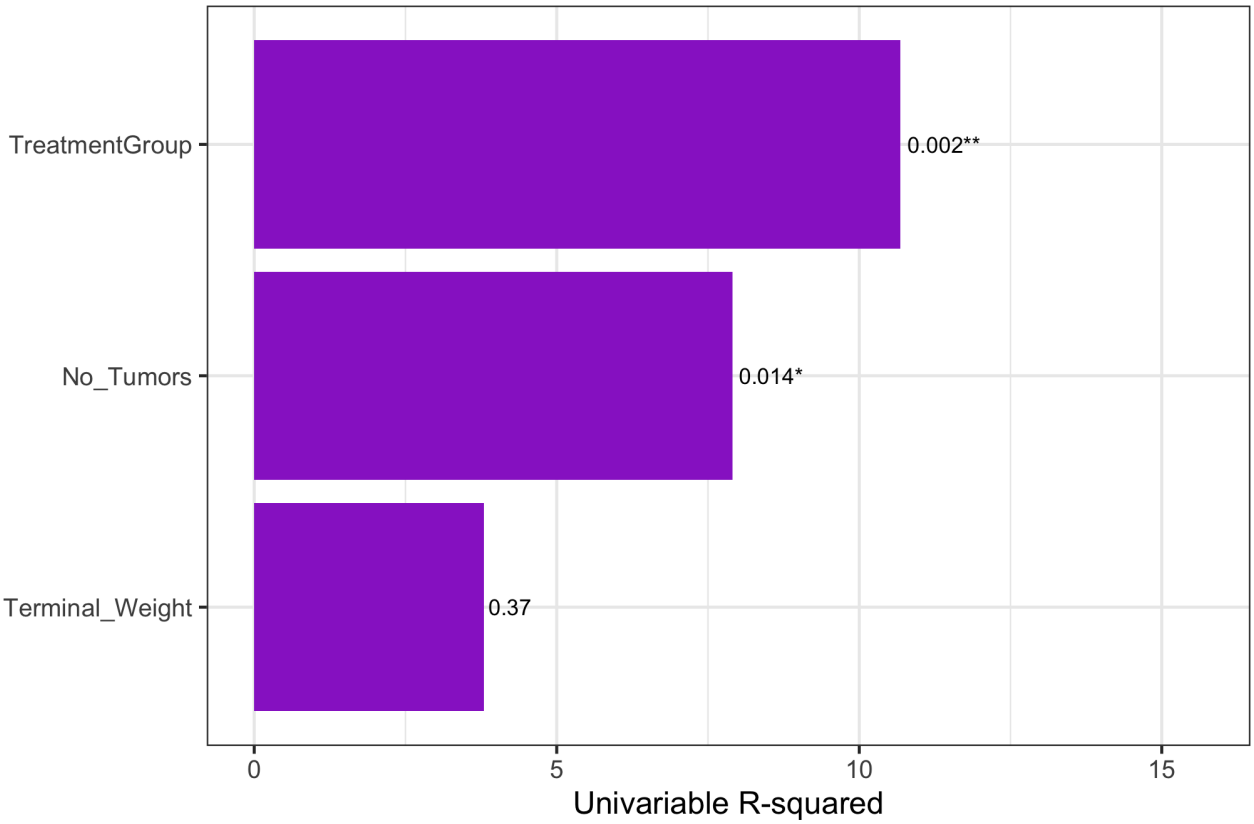
Principal coordinate analysis of MDS3 and MDS1, colored by TreatmentGroup



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Unweighted UniFrac Distances

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

TreatmentGroup + Standard Clinical Covarites

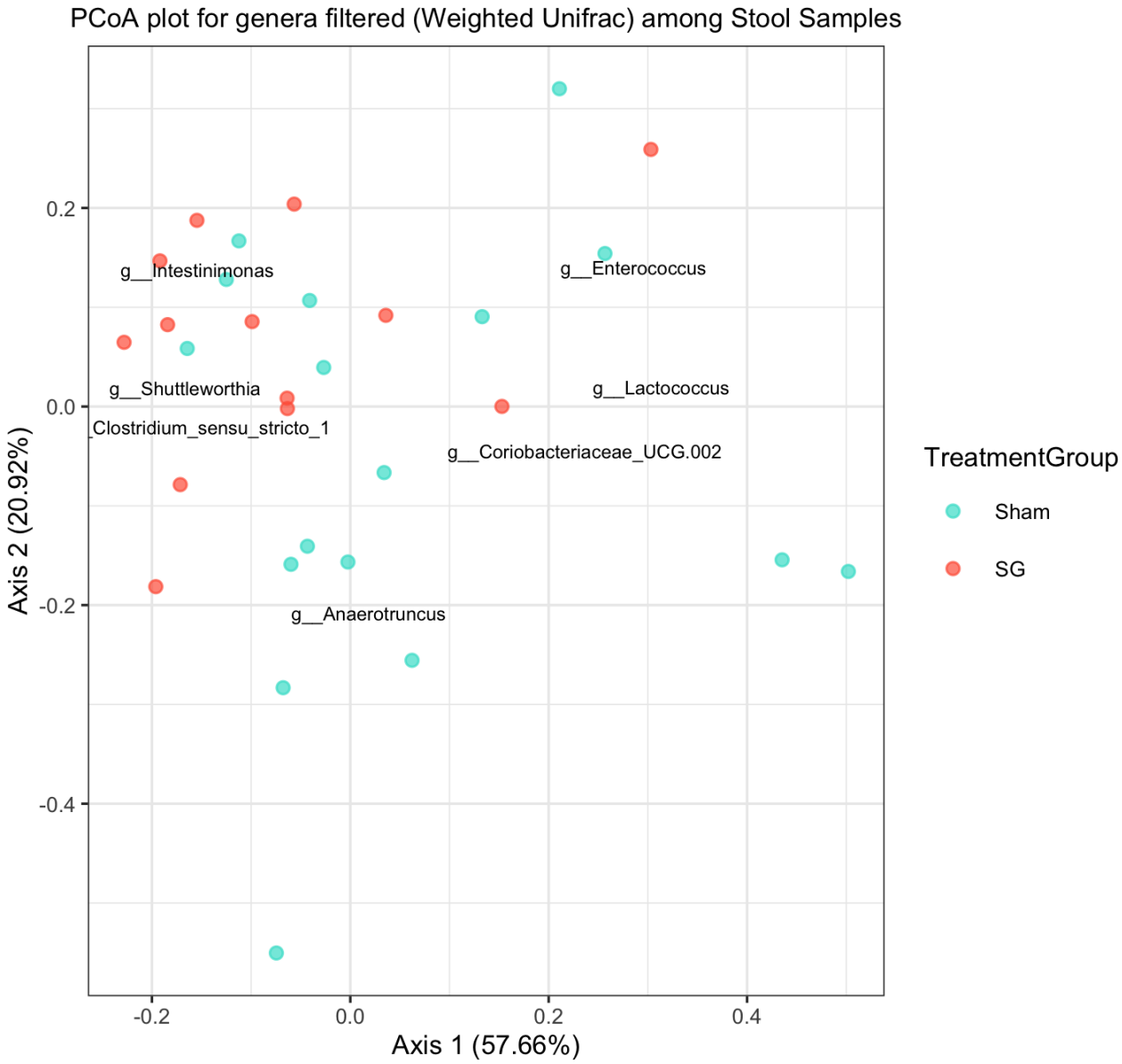
```
##
## Call:
## adonis(formula = duw_sub ~ TreatmentGroup + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup   1   0.18492 0.184921  3.3079 0.10682  0.005 **
## No_Tumors        1   0.07894 0.078943  1.4121 0.04560  0.230
## Terminal_Weight  1   0.01387 0.013873  0.2482 0.00801  0.982
## Residuals       26   1.45349 0.055904         0.83957
## Total           29   1.73123                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

TreatmentGroup

```
##
## Call:
## adonis(formula = duw_sub ~ TreatmentGroup, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup  1   0.18492 0.184921  3.3485 0.10682  0.003 **
## Residuals      28   1.54631 0.055225         0.89318
## Total          29   1.73123                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Weighted UniFrac

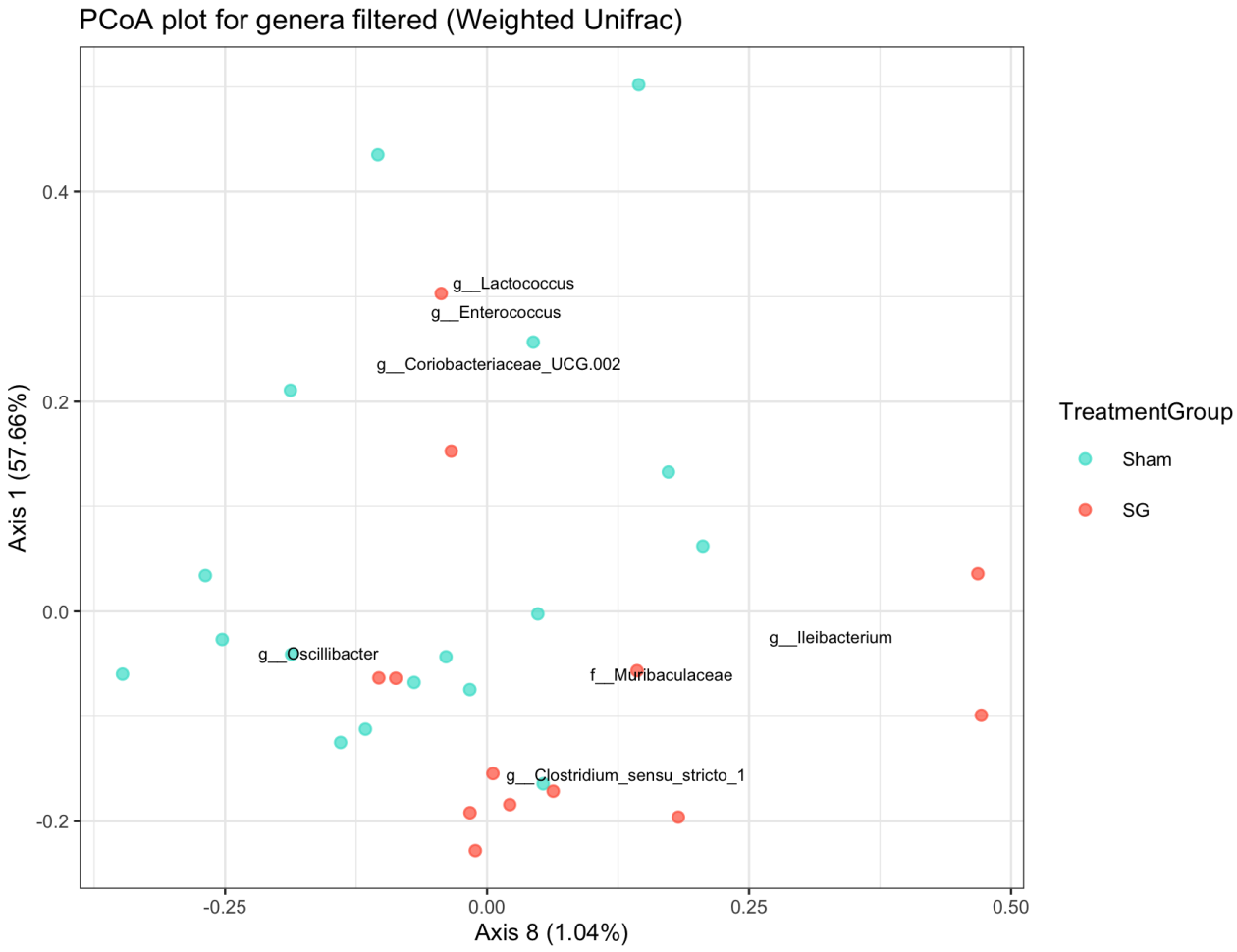
Principal coordinate analysis colored by TreatmentGroup



Finding the PCs that are most significantly associated with TreatmentGroup among Stool Samples.

PC	pval
MDS8	0.032903 *
MDS1	0.067785 ‘
MDS2	0.08488 ‘
MDS4	0.358697
MDS6	0.381037
MDS5	0.432526
MDS3	0.44454
MDS9	0.460329
MDS7	0.869533

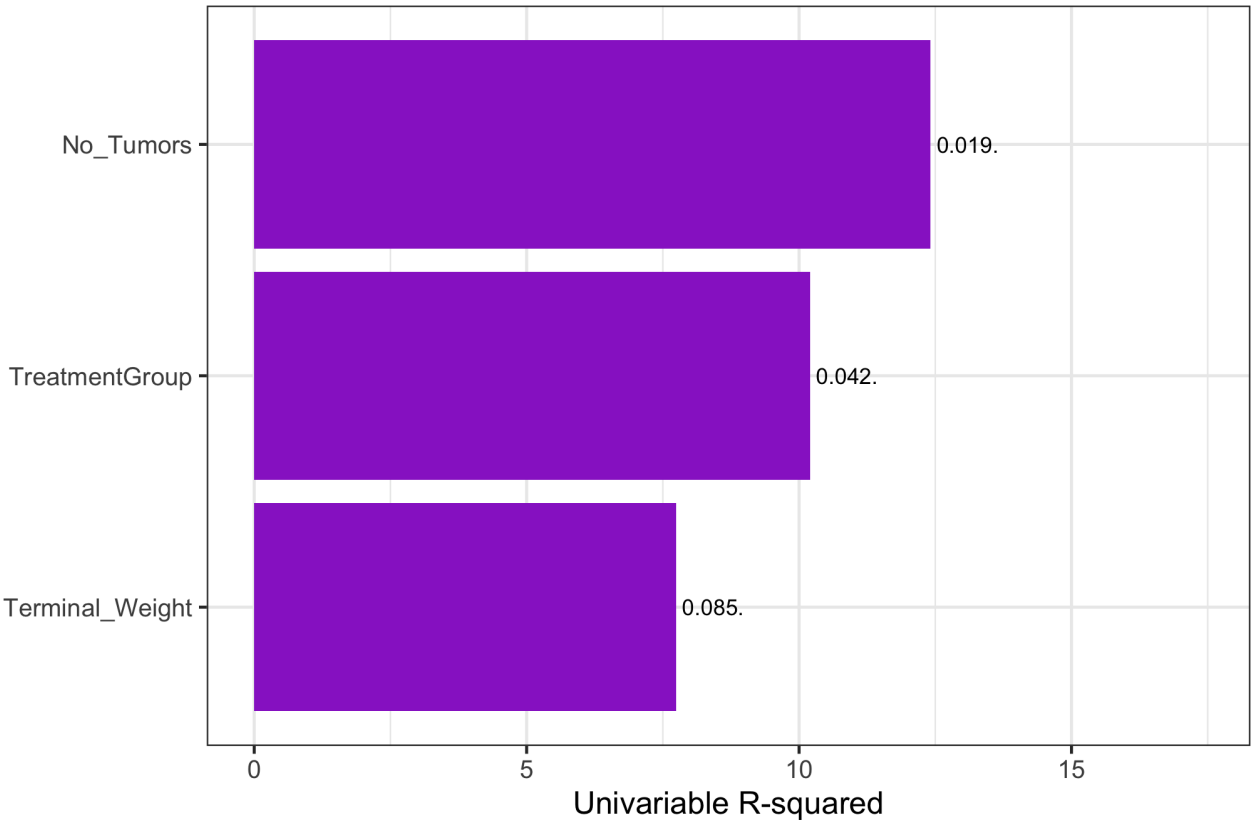
Principal coordinate analysis of MDS8 and MDS1, colored by TreatmentGroup



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Weighted UniFrac Distances

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

TreatmentGroup + Standard Clinical Covarites

```
##
## Call:
## adonis(formula = dw_sub ~ TreatmentGroup + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup   1   0.15854 0.158545  3.1681 0.10201  0.042 *
## No_Tumors        1   0.06350 0.063498  1.2688 0.04086  0.265
## Terminal_Weight  1   0.03097 0.030966  0.6188 0.01992  0.585
## Residuals       26   1.30113 0.050044         0.83720
## Total           29   1.55414                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

TreatmentGroup

```
##
## Call:
## adonis(formula = dw_sub ~ TreatmentGroup, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup  1   0.15854 0.158545  3.1809 0.10201  0.034 *
## Residuals      28   1.39560 0.049843         0.89799
## Total          29   1.55414                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

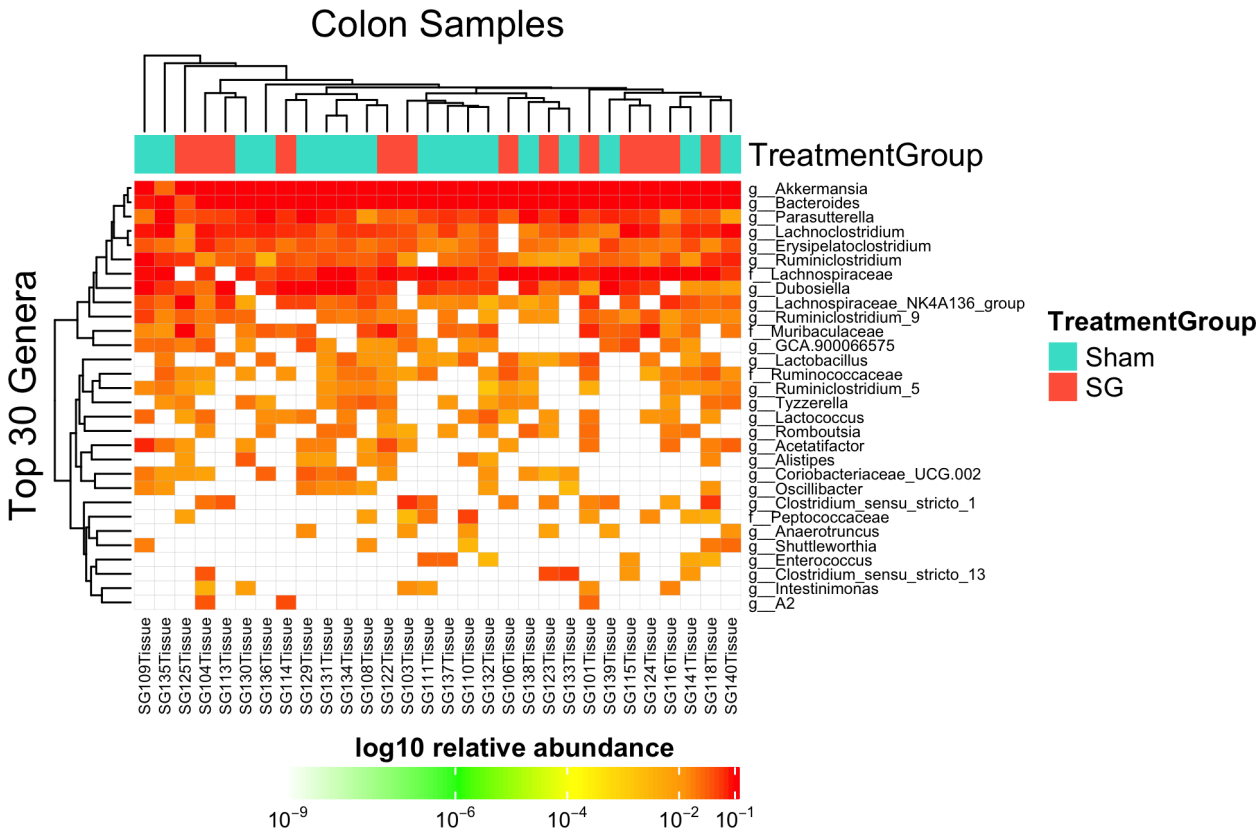
Section 5. Colon Samples Only

Taxonomic Overview

Visualized as heatmaps

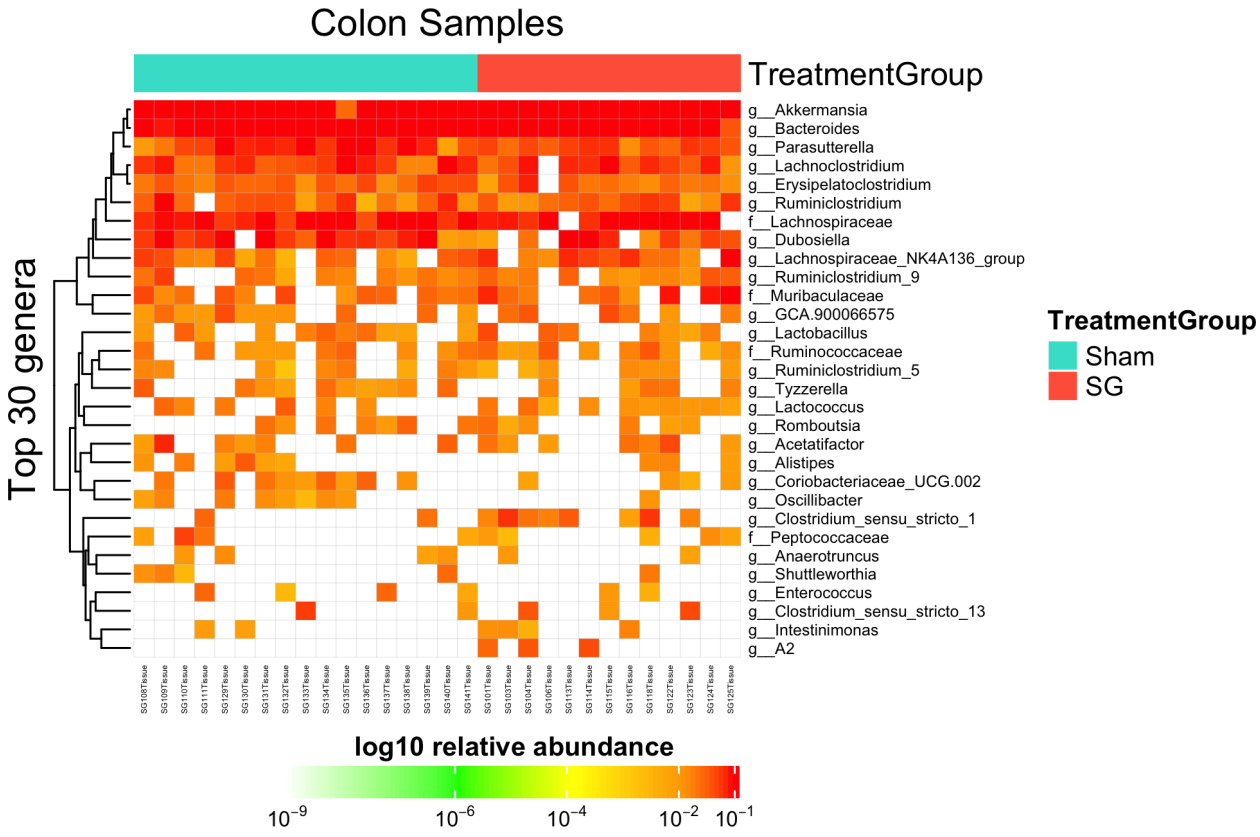
Heatmap clustered based on similar gut compositions

Heatmap of log10 relative abundances of the top 30 genera in Colon Samples. Samples (columns) and taxa (rows) are sorted based on Bray-Curtis dissimilarities and Euclidean distances, respectively. These samples display similar to other studies on gut microbiome samples.



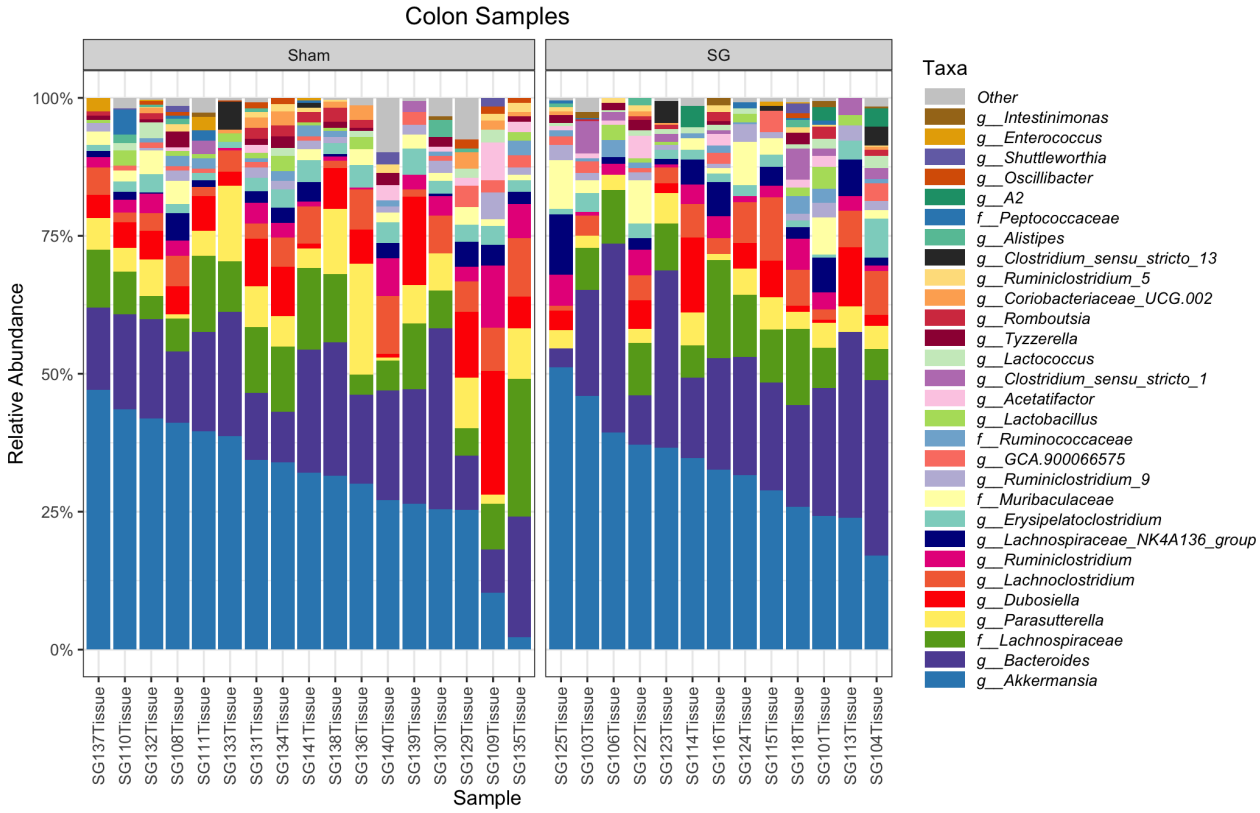
Heatmap clustered based on factors in the metadata

Heatmap of log10 relative abundances of the top 30 genera. Samples (columns) and taxa (rows) are sorted based on TreatmentGroup, and descending overall average relative abundance, respectively.

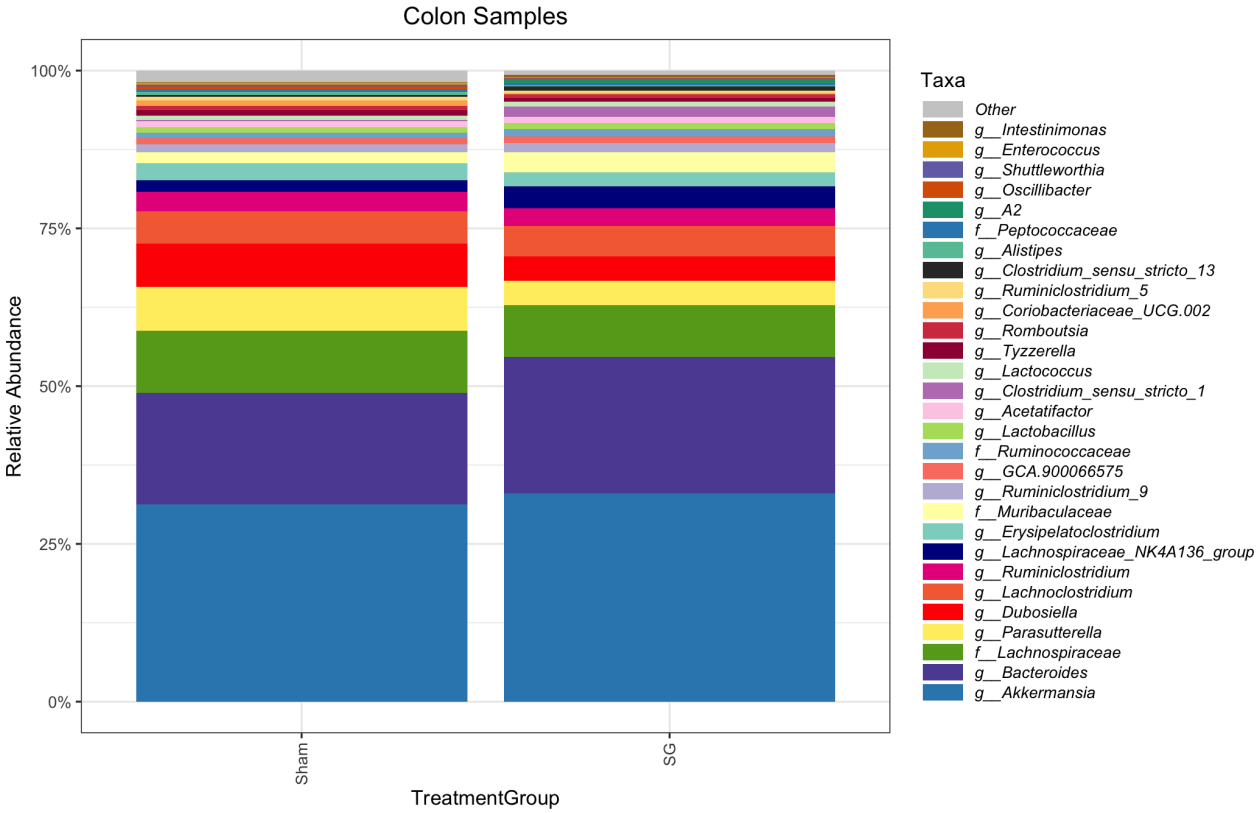


Visualized as stacked barplots

Bar plot of the top 29 abundant taxa among Colon Samples by TreatmentGroup

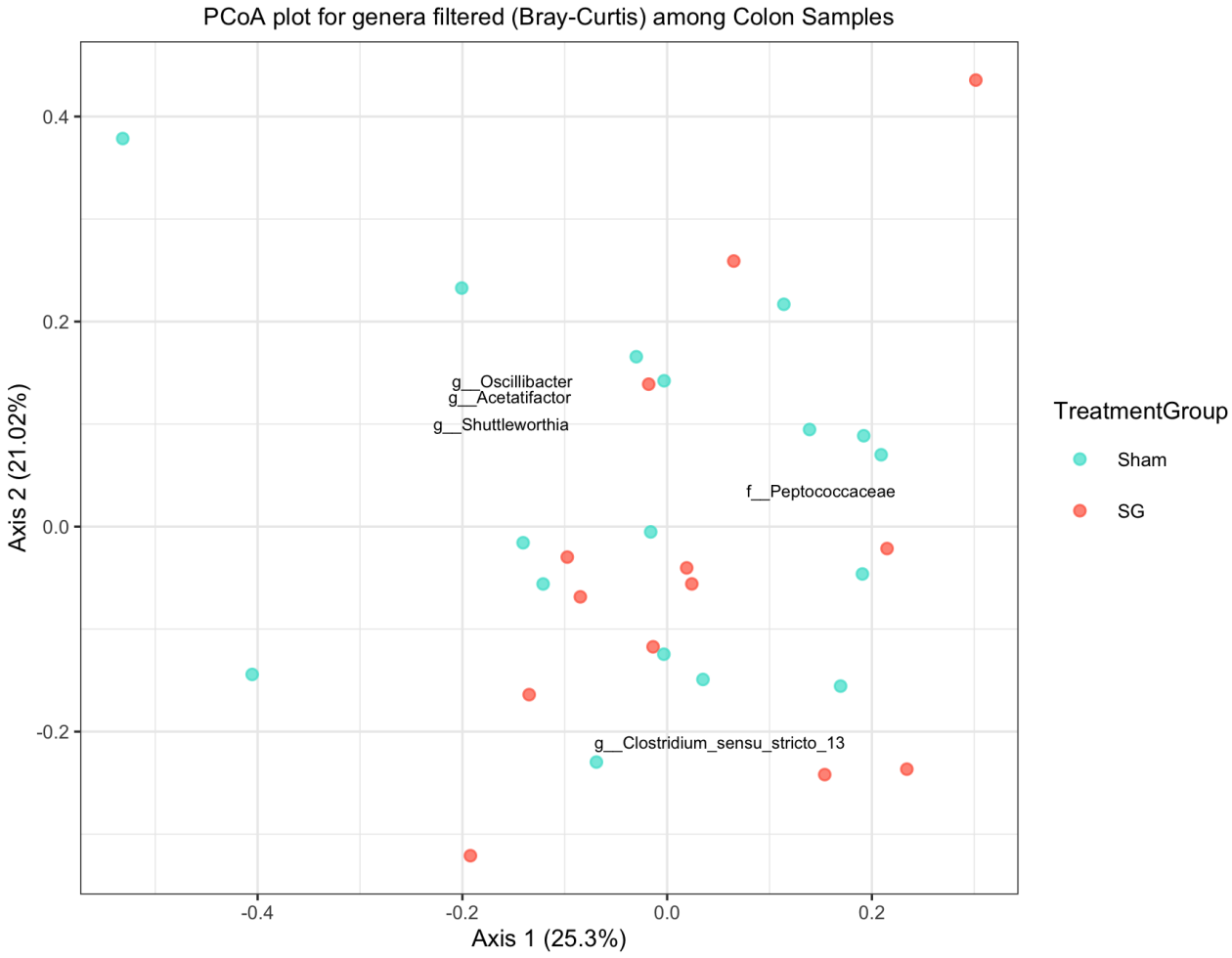


Bar plot of the mean relative abundance of the top 29 taxa among Colon Samples by TreatmentGroup



Beta Diversity: Bray-Curtis dissimilarity

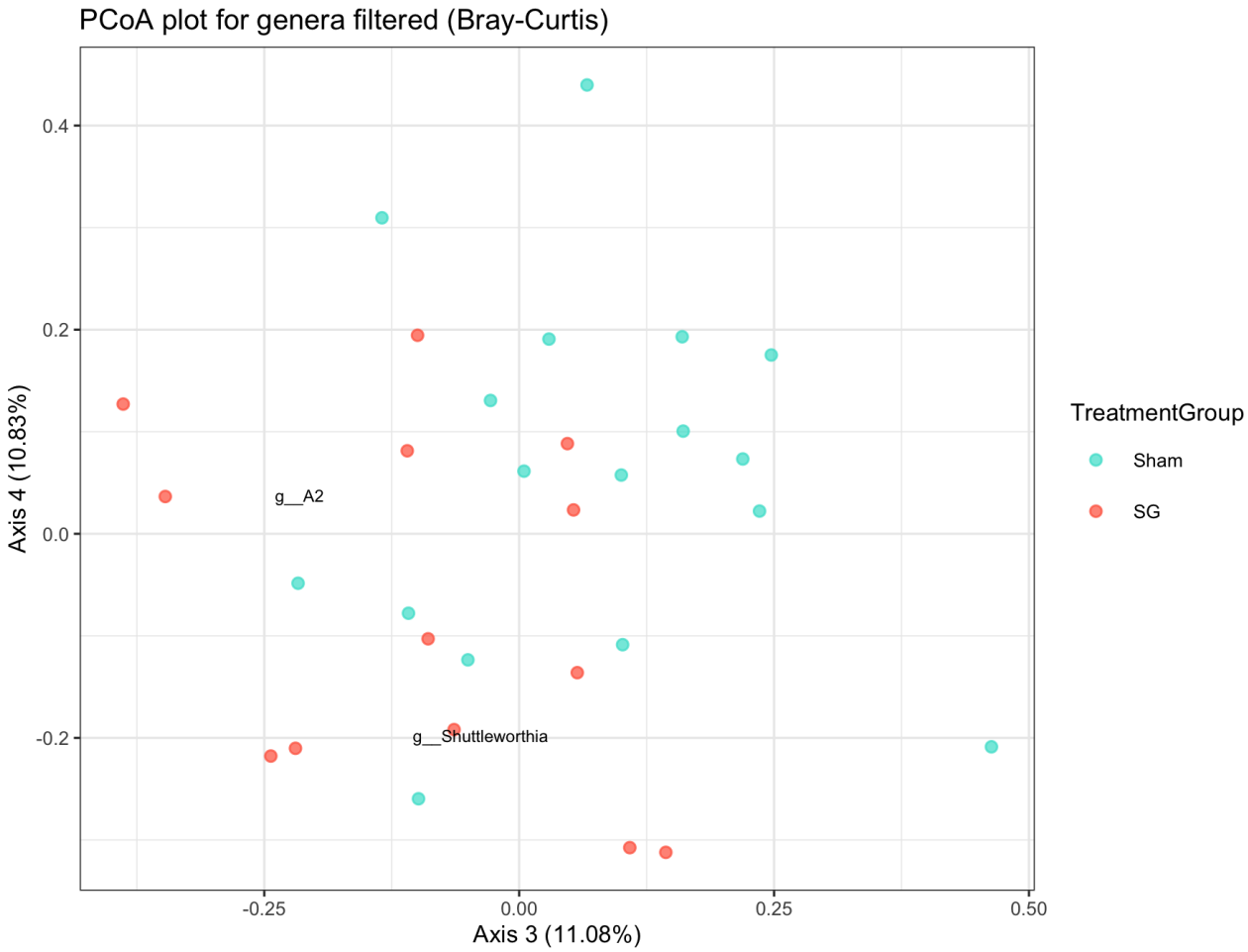
Principal coordinate analysis colored by TreatmentGroup



Finding the PCs that are most significantly associated with TreatmentGroup among Colon Samples.

PC	pval
MDS3	0.019501 *
MDS4	0.064641 ‘
MDS9	0.260267
MDS1	0.358385
MDS2	0.367312
MDS5	0.468651
MDS8	0.482561
MDS7	0.600893
MDS6	0.945259

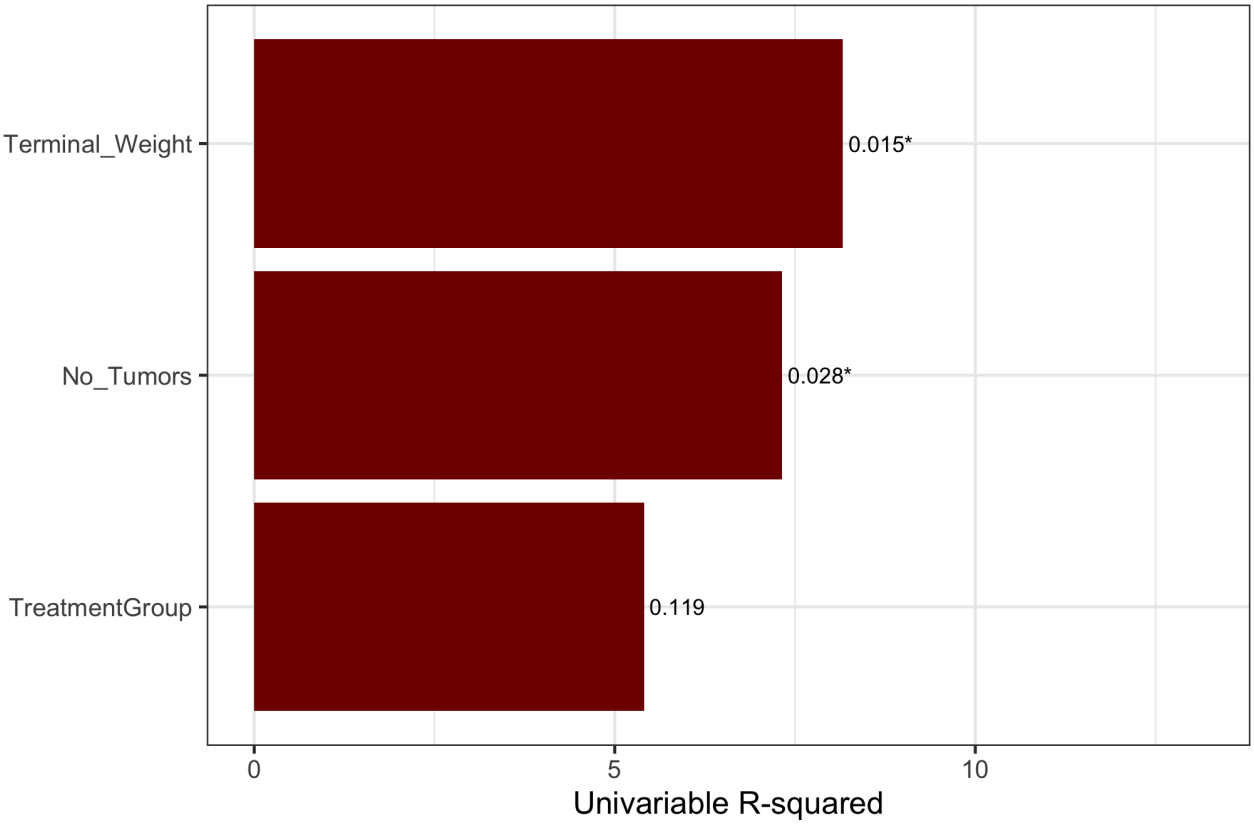
Principal coordinate analysis of MDS3 and MDS4, colored by TreatmentGroup



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Bray-Curtis dissimilarity among Colon Samples

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

TreatmentGroup + Standard Clinical Covarites

```
##
## Call:
## adonis(formula = bray_sub ~ TreatmentGroup + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup   1   0.09381 0.093807  1.6267 0.05406  0.127
## No_Tumors        1   0.08078 0.080784  1.4009 0.04655  0.190
## Terminal_Weight  1   0.06139 0.061389  1.0646 0.03538  0.398
## Residuals       26   1.49930 0.057666         0.86401
## Total           29   1.73528                  1.00000
```

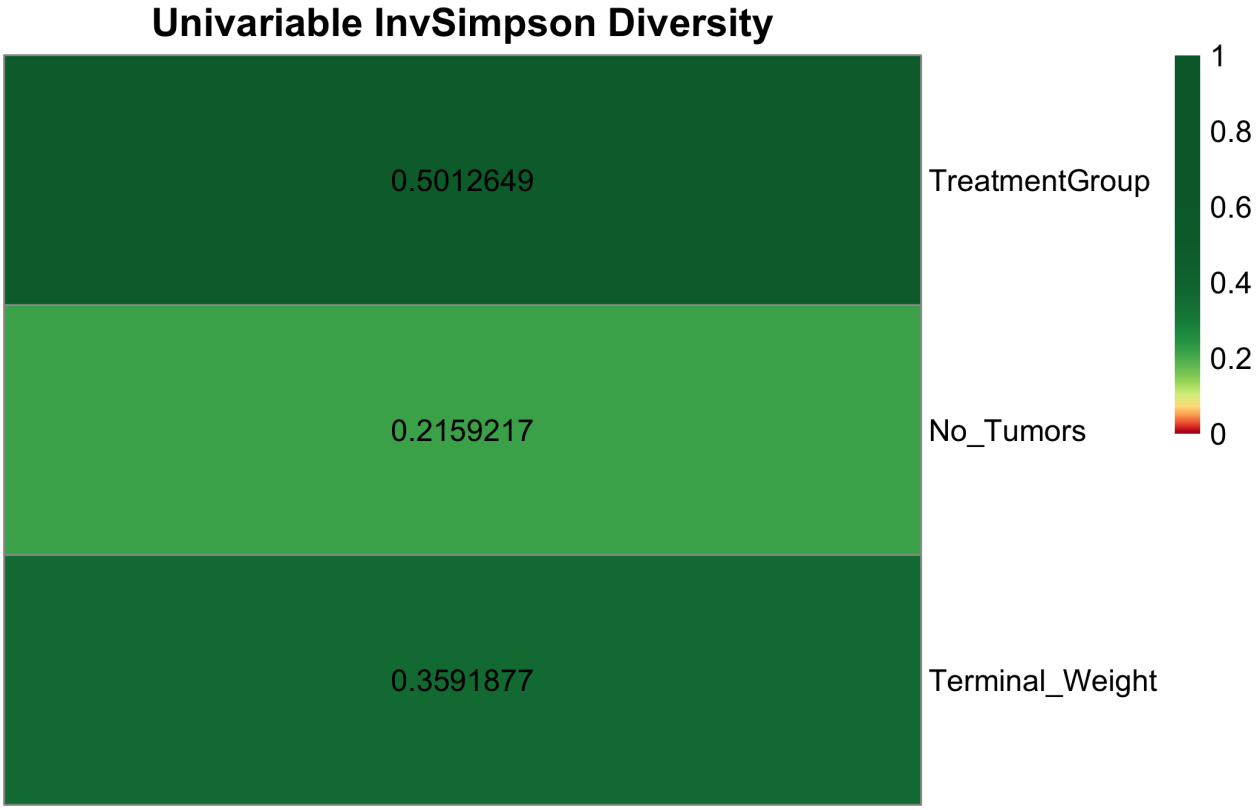
TreatmentGroup

```
##
## Call:
## adonis(formula = bray_sub ~ TreatmentGroup, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup  1   0.09381 0.093807  1.6001 0.05406  0.128
## Residuals      28   1.64148 0.058624         0.94594
## Total          29   1.73528                  1.00000
```

Alpha Diversity

Statistics alpha diversity: anova on linear model

Heatmap annotated with the p-value for each covariate

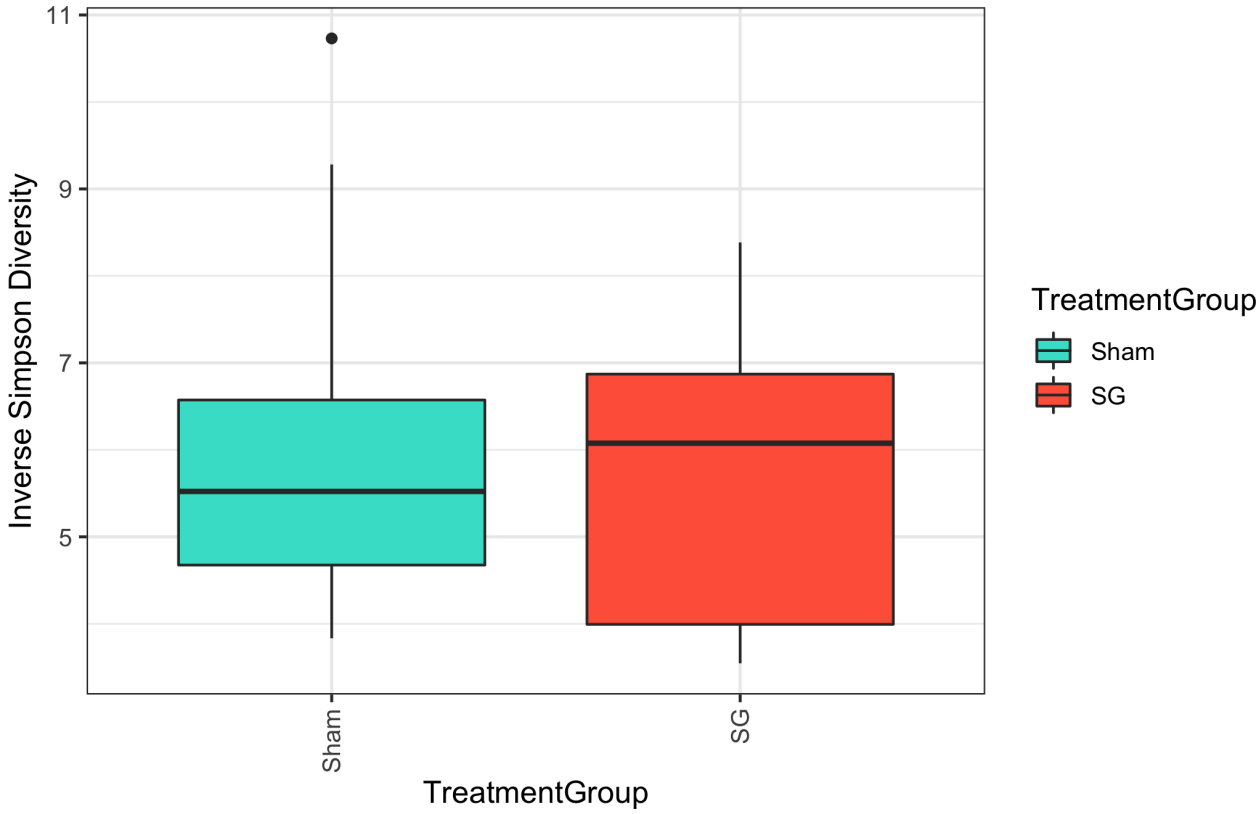


Multivariable model for Alpha diversity

```
## Analysis of Variance Table
##
## Response: InvSimpson
##                Df Sum Sq Mean Sq F value Pr(>F)
## TreatmentGroup  1  1.521  1.5213  0.4642 0.5013
## Residuals      28 91.764  3.2773
```

```
## Analysis of Variance Table
##
## Response: InvSimpson
##                 Df Sum Sq Mean Sq F value Pr(>F)
## TreatmentGroup   1  1.521  1.5213  0.4497 0.5084
## No_Tumors        1  3.739  3.7386  1.1051 0.3028
## Terminal_Weight  1  0.066  0.0662  0.0196 0.8899
## Residuals       26 87.959  3.3831
```

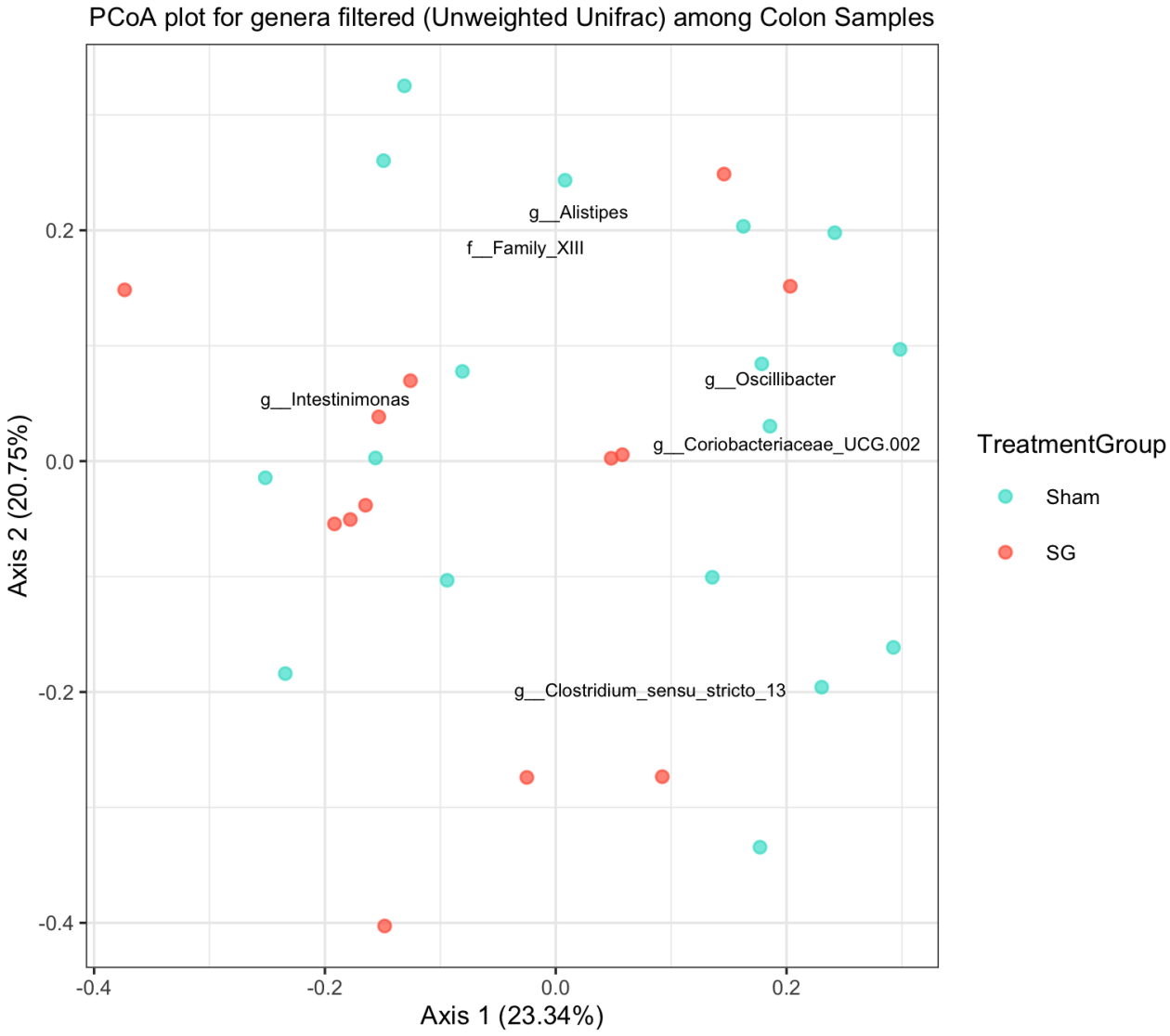
Invsimpson vs. TreatmentGroup



Other Beta Diversity Metrics

Unweighted UniFrac

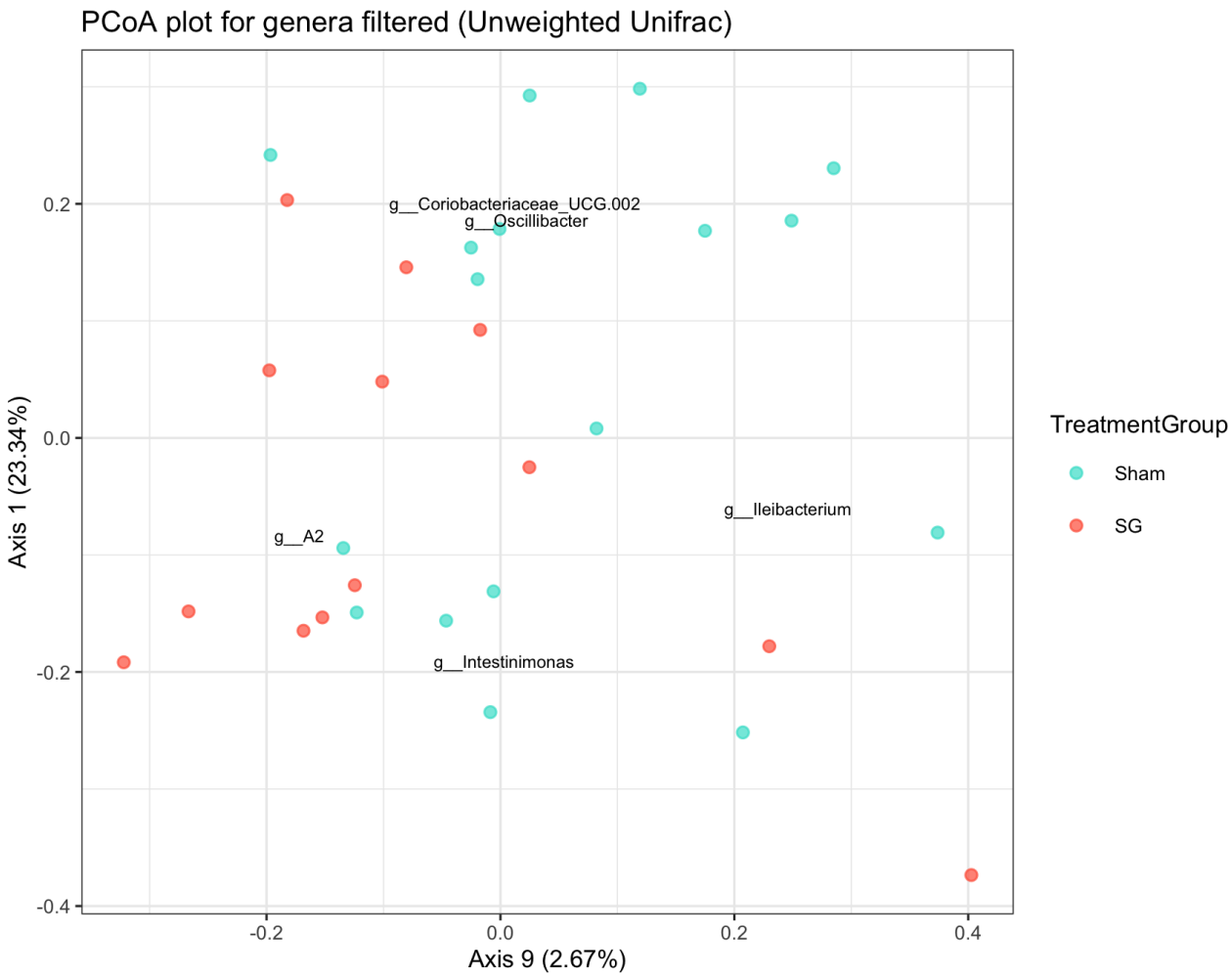
Principal coordinate analysis colored by TreatmentGroup



Finding the PCs that are most significantly associated with TreatmentGroup among Colon Samples.

PC	pval
MDS9	0.056333 ‘
MDS1	0.107685
MDS7	0.129075
MDS6	0.137198
MDS4	0.202361
MDS3	0.286901
MDS2	0.404903
MDS8	0.438138
MDS5	0.713165

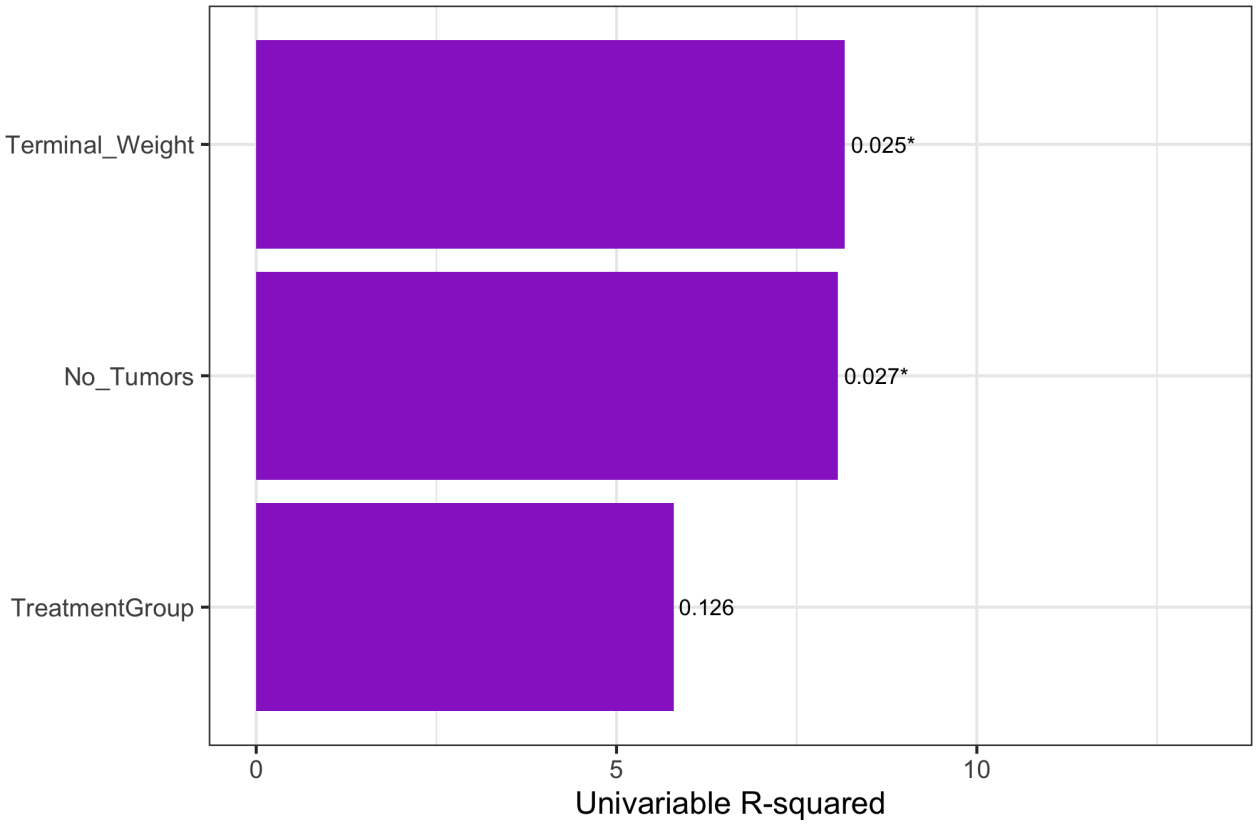
Principal coordinate analysis of MDS9 and MDS1, colored by TreatmentGroup



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Unweighted UniFrac Distances

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

TreatmentGroup + Standard Clinical Covarites

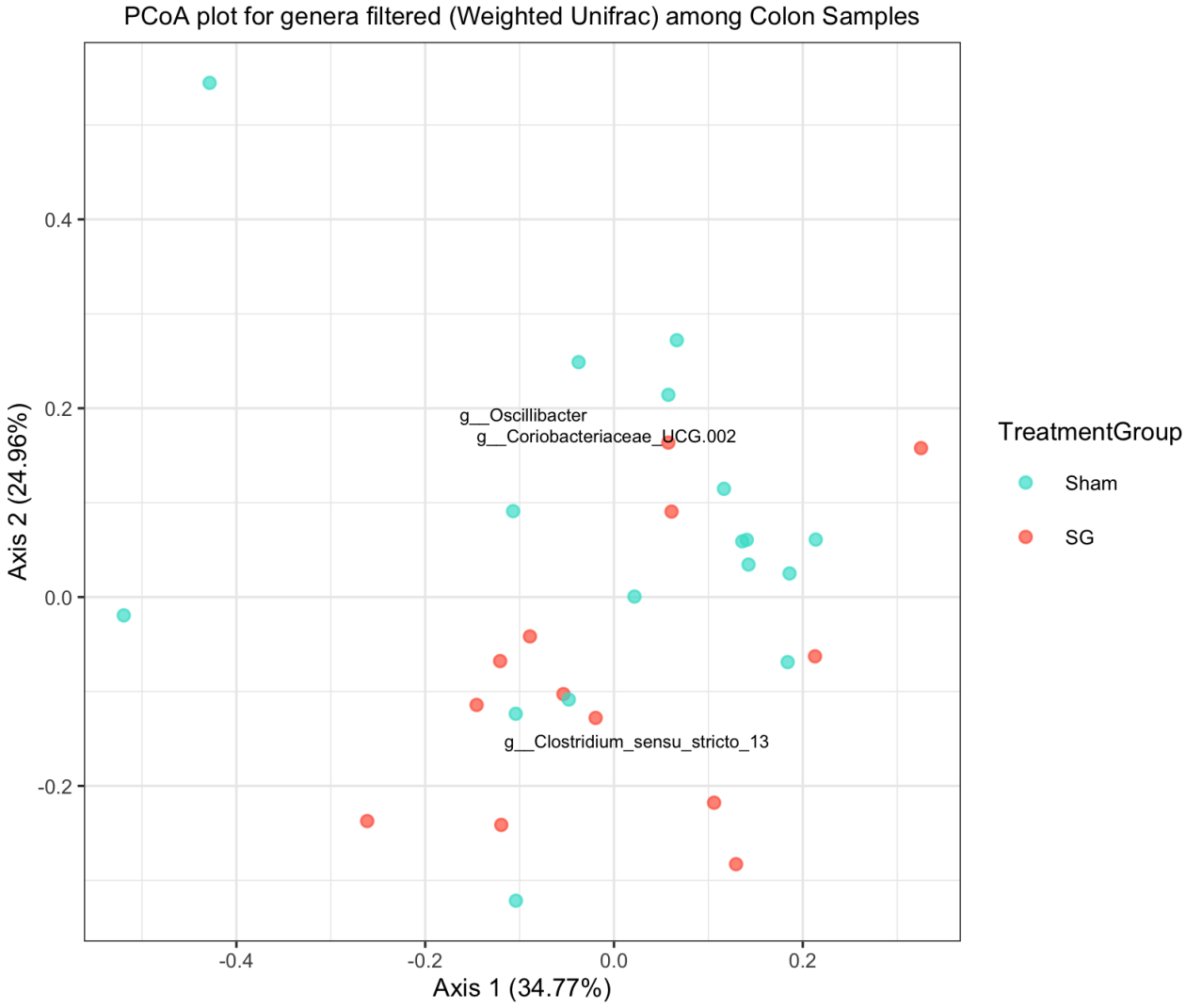
```
##
## Call:
## adonis(formula = duw_sub ~ TreatmentGroup + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup   1   0.07739 0.077388 1.77471 0.05791  0.107
## No_Tumors        1   0.08654 0.086541 1.98462 0.06476  0.078 .
## Terminal_Weight  1   0.03862 0.038618 0.88562 0.02890  0.548
## Residuals       26   1.13375 0.043606         0.84843
## Total           29   1.33630                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

TreatmentGroup

```
##
## Call:
## adonis(formula = duw_sub ~ TreatmentGroup, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup  1   0.07739 0.077388  1.7212 0.05791  0.117
## Residuals      28   1.25891 0.044961         0.94209
## Total          29   1.33630                  1.00000
```

Weighted UniFrac

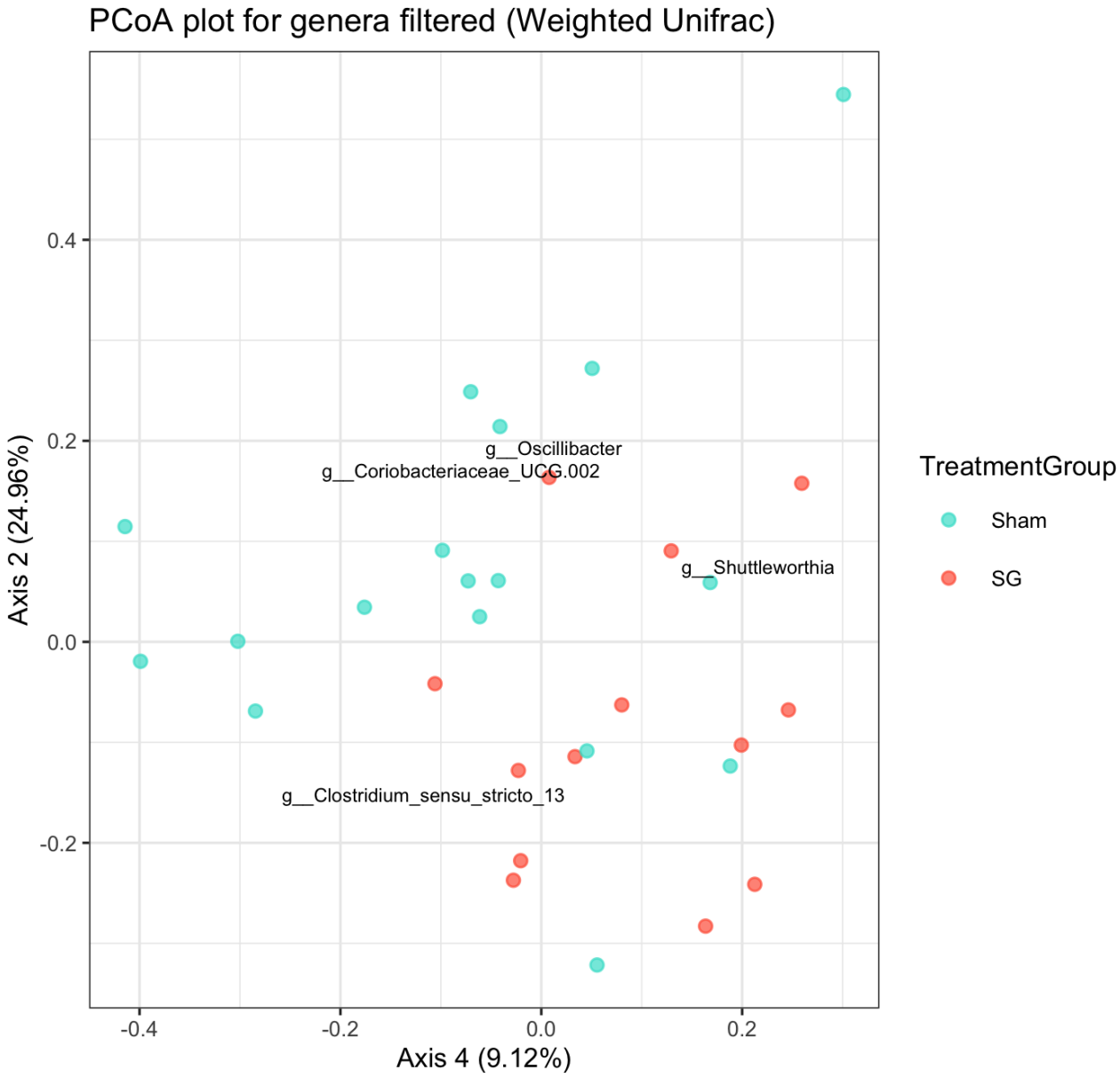
Principal coordinate analysis colored by TreatmentGroup



Finding the PCs that are most significantly associated with TreatmentGroup among Colon Samples.

PC	pval
MDS4	0.019122 *
MDS2	0.028754 *
MDS6	0.360194
MDS7	0.469259
MDS3	0.501126
MDS8	0.616702
MDS9	0.674955
MDS5	0.740985
MDS1	0.872639

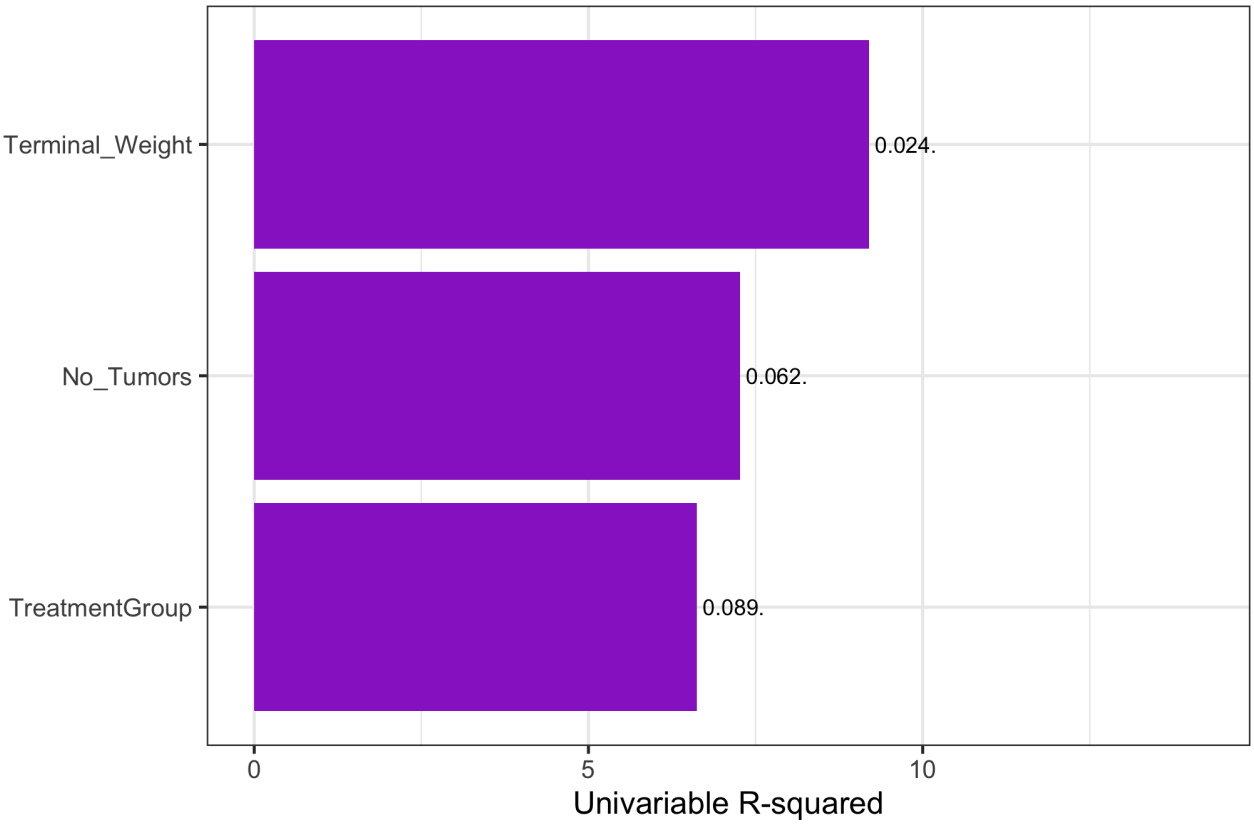
Principal coordinate analysis of MDS4 and MDS2, colored by TreatmentGroup



Beta diversity statistics: Using PERMANOVA on dissimilarity matrix

Univariable-Weighted UniFrac Distances

Bar plot of R-square value, annotated with the p-value for each covariate



Multivariable model for beta diversity

TreatmentGroup + Standard Clinical Covarites

```
##
## Call:
## adonis(formula = dw_sub ~ TreatmentGroup + No_Tumors + Terminal_Weight,      data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## TreatmentGroup   1   0.05923 0.059235  2.0036 0.06620  0.070 .
## No_Tumors        1   0.03610 0.036095  1.2209 0.04034  0.262
## Terminal_Weight  1   0.03075 0.030750  1.0401 0.03437  0.394
## Residuals       26   0.76867 0.029564         0.85909
## Total           29   0.89475                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

TreatmentGroup

```
##
## Call:
## adonis(formula = dw_sub ~ TreatmentGroup, data = meta_dat_sub)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs  MeanSqs F.Model     R2 Pr(>F)
## TreatmentGroup  1   0.05923 0.059235  1.9851 0.0662   0.08 .
## Residuals      28   0.83552 0.029840         0.9338
## Total          29   0.89475                  1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Summary of R session

```
## R version 3.6.2 (2019-12-12)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Mojave 10.14.6
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] foreign_0.8-76       circlize_0.4.8       magick_2.3
##  [4] seriation_1.2-8      ComplexHeatmap_2.2.0 viridis_0.5.1
##  [7] viridisLite_0.3.0    tidyr_1.1.0          qiime2R_0.99.34
## [10] readr_1.3.1          phyloseq_1.30.0      gtools_3.8.2
## [13] gplots_3.0.3         scales_1.1.1         pheatmap_1.0.12
## [16] phytools_0.7-20      maps_3.3.0           GUniFrac_1.1
## [19] matrixStats_0.56.0   ape_5.4              vegan_2.5-6
## [22] lattice_0.20-41      permute_0.9-5        dplyr_1.0.0
## [25] stringr_1.4.0        kableExtra_1.1.0     tibble_3.0.3
## [28] knitr_1.29           RColorBrewer_1.1-2   ggplot2_3.3.2
## [31] reshape2_1.4.4
##
## loaded via a namespace (and not attached):
##   [1] backports_1.1.8         Hmisc_4.4-0             fastmatch_1.1-0
##   [4] plyr_1.8.6              igraph_1.2.5            splines_3.6.2
##   [7] digest_0.6.25           foreach_1.5.0           htmltools_0.5.0
##  [10] gdata_2.18.0            magrittr_1.5            checkmate_2.0.0
##  [13] cluster_2.1.0           gclus_1.3.2             Biostrings_2.54.0
##  [16] jpeg_0.1-8.1            colorspace_1.4-1        rvest_0.3.5
##  [19] xfun_0.16               crayon_1.3.4            jsonlite_1.7.0
##  [22] survival_3.1-11         phangorn_2.5.5          iterators_1.0.12
##  [25] glue_1.4.1              registry_0.5-1          gtable_0.3.0
##  [28] zlibbioc_1.32.0         XVector_0.26.0          webshot_0.5.2
##  [31] GetoptLong_0.1.8        Rhdf5lib_1.8.0          shape_1.4.4
##  [34] BiocGenerics_0.32.0     Rcpp_1.0.5              plotrix_3.7-7
##  [37] htmlTable_2.0.1         clue_0.3-57             Formula_1.2-3
##  [40] stats4_3.6.2            DT_0.14                 truncnorm_1.0-8
##  [43] animation_2.6           htmlwidgets_1.5.1       httr_1.4.1
##  [46] acepack_1.4.1           ellipsis_0.3.1          pkgconfig_2.0.3
##  [49] NADA_1.6-1.1            farver_2.0.3            nnet_7.3-13
##  [52] tidyselect_1.1.0        labeling_0.3            rlang_0.4.7
##  [55] munsell_0.5.0           tools_3.6.2             generics_0.0.2
##  [58] ade4_1.7-15             evaluate_0.14           biomformat_1.14.0
##  [61] yaml_2.2.1              caTools_1.18.0          purrr_0.3.4
##  [64] dendextend_1.13.4       nlme_3.1-145            xml2_1.3.0
##  [67] compiler_3.6.2          rstudioapi_0.11         png_0.1-7
```

```
##  [70] zCompositions_1.3.4     clusterGeneration_1.3.4 stringi_1.4.6
##  [73] Matrix_1.2-18           multtest_2.42.0         vctrs_0.3.2
##  [76] pillar_1.4.6            lifecycle_0.2.0         combinat_0.0-8
##  [79] GlobalOptions_0.1.1     data.table_1.12.8       bitops_1.0-6
##  [82] R6_2.4.1                latticeExtra_0.6-29     TSP_1.1-9
##  [85] KernSmooth_2.23-16      gridExtra_2.3           IRanges_2.20.2
##  [88] codetools_0.2-16        MASS_7.3-51.5           rhdf5_2.30.1
##  [91] rjson_0.2.20            withr_2.2.0             mnormt_1.5-6
##  [94] S4Vectors_0.24.4        mgcv_1.8-31             expm_0.999-4
##  [97] parallel_3.6.2          hms_0.5.3               quadprog_1.5-8
## [100] rpart_4.1-15            coda_0.19-3             rmarkdown_2.1
## [103] numDeriv_2016.8-1.1     scatterplot3d_0.3-41    Biobase_2.46.0
## [106] base64enc_0.1-3
```