Microbial Ecology Analysis Report

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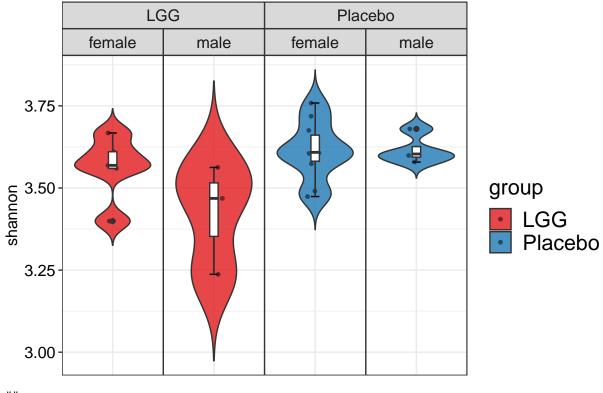
1. Alpha Diversity Analysis

1.1 Alpha Diversity Boxplots

```
alpha_boxplot_results <- generate_alpha_boxplot_single(data.obj = data.obj,</pre>
                                                         alpha.obj = alpha.obj,
                                                         alpha.name = alpha.name,
                                                         subject.var = subject.var,
                                                         time.var = time.var,
                                                         t.level = t.level,
                                                         group.var = group.var,
                                                         strata.var = strata.var,
                                                         base.size = base.size,
                                                         theme.choice = theme.choice,
                                                         custom.theme = custom.theme,
                                                         palette = palette,
                                                         pdf = pdf,
                                                         file.ann = file.ann,
                                                         pdf.wid = pdf.wid,
                                                         pdf.hei = pdf.hei)
alpha_boxplot_results
```

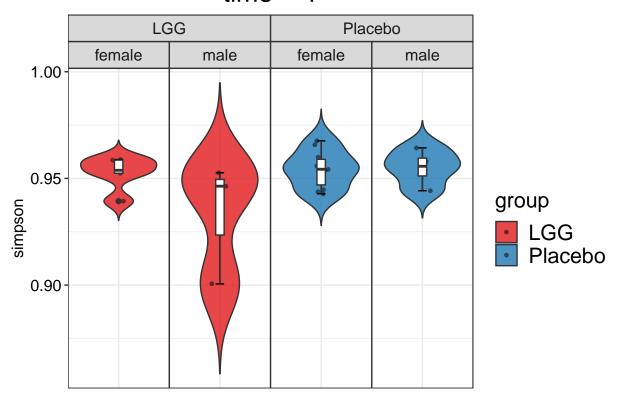
[[1]]

time = 1



[[2]]

time = 1



1.2 Alpha Diversity Test Results

shannon Index

| term | Estimate | Std.Error | Statistic | P.Value |
|----------------------|----------|-----------|-----------|-----------|
| (Intercept) | 3.531 | 0.03964 | 89.09 | 2.234e-26 |
| sexmale | -0.05447 | 0.04633 | -1.176 | 0.2542 |
| ${\tt groupPlacebo}$ | 0.09928 | 0.04486 | 2.213 | 0.03931 |

shannon Index Analysis

The variable (Intercept) has a statistically significant impact on the shannon diversity index with an estimate of 3.53.

The variable sexmale does not appear to have a statistically significant effect on the shannon diversity index. The estimate of its effect is -0.05.

The variable groupPlacebo has a statistically significant impact on the shannon diversity index with an estimate of 0.1.

simpson Index

| term | Estimate | Std.Error | Statistic | P.Value |
|-----------------------|-----------|-----------|-----------|-----------|
| (Intercept) | 0.9481 | 0.005097 | 186 | 1.911e-32 |
| sexmale | -0.007223 | 0.005957 | -1.213 | 0.2401 |
| groupPlacebo | 0.008501 | 0.005768 | 1.474 | 0.1569 |

simpson Index Analysis

The variable (Intercept) has a statistically significant impact on the simpson diversity index with an estimate of 0.95.

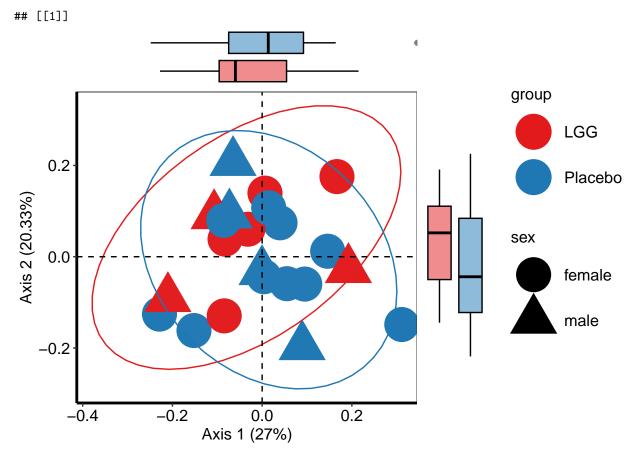
The variable sexmale does not appear to have a statistically significant effect on the simpson diversity index. The estimate of its effect is -0.01.

The variable groupPlacebo does not appear to have a statistically significant effect on the simpson diversity index. The estimate of its effect is 0.01.

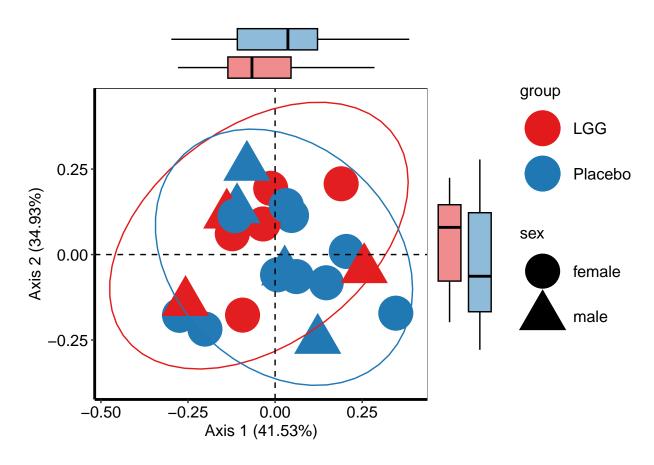
2. Beta Diversity Analysis

2.1 Beta Diversity Ordination

```
beta_ordination_results <- generate_beta_ordination_single(data.obj = data.obj,</pre>
                                                             dist.obj = dist.obj,
                                                             pc.obj = NULL,
                                                             subject.var = subject.var,
                                                             time.var = time.var,
                                                             t.level = t.level,
                                                             group.var = group.var,
                                                             strata.var = strata.var,
                                                             dist.name = dist.name,
                                                             base.size = base.size,
                                                             theme.choice = theme.choice,
                                                             custom.theme = custom.theme,
                                                             palette = palette,
                                                            pdf = pdf,
                                                            file.ann = file.ann,
                                                             pdf.wid = pdf.wid,
                                                            pdf.hei = pdf.hei)
beta_ordination_results
```



[[2]]



2.2 Beta Diversity Test Results

P-Tab Results

| Term | D1.p.value | D2.p.value | omni.p.value |
|-------|------------|------------|--------------|
| sex | 0.785 | 0.832 | 0.809 |
| group | 0.787 | 0.839 | 0.814 |

Beta Diversity PERMANOVA Analysis for Variable: sex

The variable sex does not appear to have a statistically significant effect on the beta diversity according to the PERMANOVA test with the BC distance matrix.

The variable sex does not appear to have a statistically significant effect on the beta diversity according to the PERMANOVA test with the Jaccard distance matrix.

The variable sex does not appear to have a statistically significant effect on the beta diversity according to the omnibus PERMANOVA test.

Beta Diversity PERMANOVA Analysis for Variable: group

The variable group does not appear to have a statistically significant effect on the beta diversity according to the PERMANOVA test with the BC distance matrix.

The variable group does not appear to have a statistically significant effect on the beta diversity according to the PERMANOVA test with the Jaccard distance matrix.

The variable group does not appear to have a statistically significant effect on the beta diversity according to the omnibus PERMANOVA test.

AOV-Tab Results

| Variable | DF | Sum_Sq | $Mean_Sq$ | F_Statistic | $R_Squared$ | P_Value | Distance |
|-----------|----|-----------|------------|-------------|--------------|---------|------------------|
| sex | 1 | 0.043 | 0.043 | 0.644 | 0.032 | 0.785 | BC |
| group | 1 | 0.042 | 0.042 | 0.621 | 0.031 | 0.787 | $_{\mathrm{BC}}$ |
| Residuals | 19 | 1.277 | 0.067 | NA | 0.938 | NA | $_{\mathrm{BC}}$ |
| Total | 21 | 1.362 | NA | NA | 1 | NA | $_{\mathrm{BC}}$ |
| sex | 1 | 0.099 | 0.099 | 0.707 | 0.035 | 0.832 | Jaccard |
| group | 1 | 0.094 | 0.094 | 0.668 | 0.033 | 0.839 | Jaccard |
| Residuals | 19 | 2.669 | 0.14 | NA | 0.933 | NA | Jaccard |
| Total | 21 | 2.862 | NA | NA | 1 | NA | Jaccard |

sex Variable Analysis

The variable sex does not appear to have a statistically significant effect on the beta diversity according to the PERMANOVA test with the BC distance matrix.

The variable sex does not appear to have a statistically significant effect on the beta diversity according to the PERMANOVA test with the Jaccard distance matrix.

group Variable Analysis

The variable group does not appear to have a statistically significant effect on the beta diversity according to the PERMANOVA test with the BC distance matrix.

The variable group does not appear to have a statistically significant effect on the beta diversity according to the PERMANOVA test with the Jaccard distance matrix.

Residuals Variable Analysis

Warning: NAs introduced by coercion

Warning: NAs introduced by coercion

Total Variable Analysis

Warning: NAs introduced by coercion

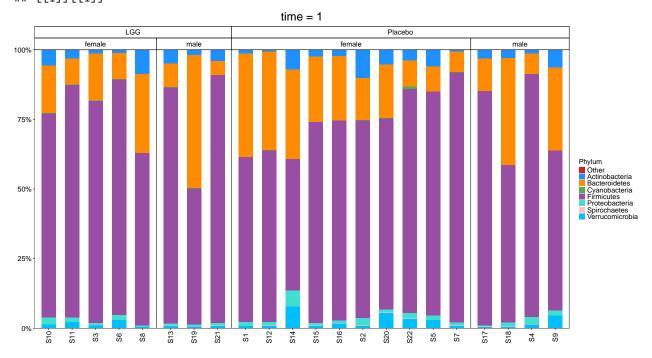
Warning: NAs introduced by coercion

3. Taxonomic Feature Analysis

3.1 Taxa Barplot

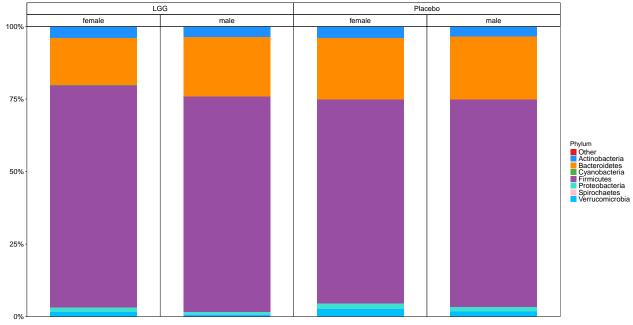
```
t.level = t.level,
                                                      group.var = group.var,
                                                      strata.var = strata.var,
                                                      feature.level = feature.level,
                                                      feature.dat.type = feature.dat.type,
                                                      feature.number = feature.number,
                                                      base.size = base.size,
                                                      theme.choice = theme.choice,
                                                      custom.theme = custom.theme,
                                                      palette = NULL,
                                                      pdf = pdf,
                                                      file.ann = file.ann,
                                                      pdf.wid = pdf.wid,
                                                      pdf.hei = pdf.hei)
taxa_barplot_results
```

[[1]] ## [[1]][[1]]



[[1]][[2]]

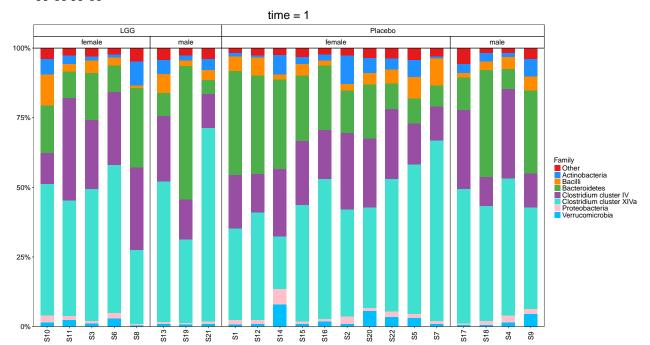




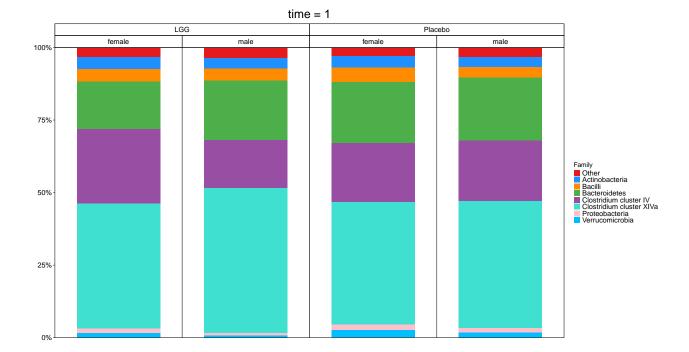
##

[[2]]

[[2]][[1]]



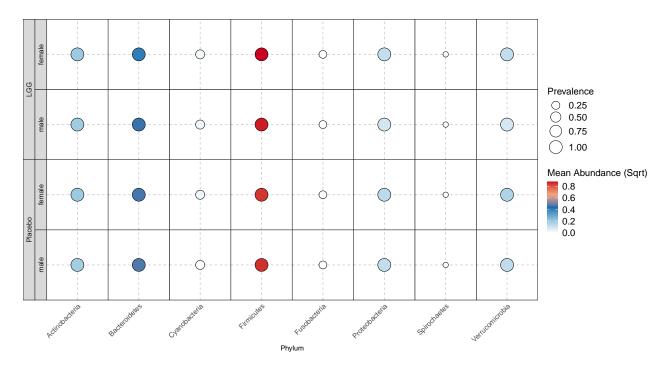
[[2]][[2]]



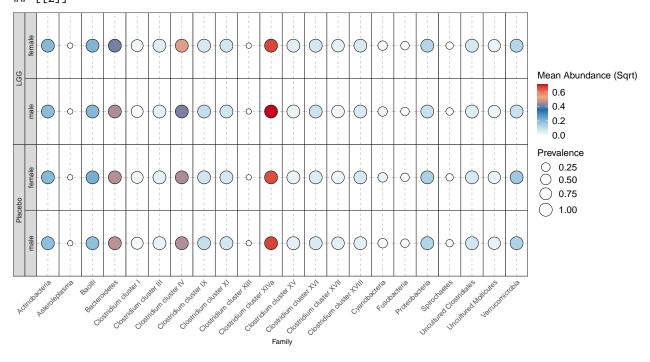
3.2 Taxa Dotplot

```
taxa_dotplot_results <- generate_taxa_dotplot_single(data.obj = data.obj,</pre>
                                                      subject.var = subject.var,
                                                      time.var = time.var,
                                                      t0.level = t.level,
                                                      group.var = group.var,
                                                      strata.var = strata.var,
                                                      feature.level = feature.level,
                                                      feature.dat.type = feature.dat.type,
                                                      features.plot = features.plot,
                                                      top.k.plot = top.k.plot,
                                                      top.k.func = top.k.func,
                                                      prev.filter = prev.filter,
                                                      abund.filter = abund.filter,
                                                      base.size = base.size,
                                                      theme.choice = theme.choice,
                                                      custom.theme = custom.theme,
                                                      palette = palette,
                                                      pdf = pdf,
                                                      file.ann = file.ann,
                                                      pdf.wid = pdf.wid,
                                                      pdf.hei = pdf.hei)
taxa_dotplot_results
```

[[1]]

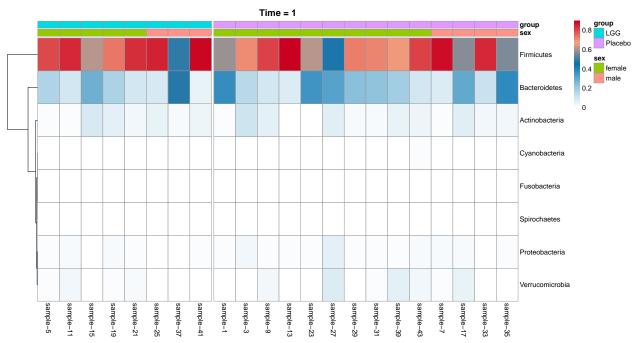


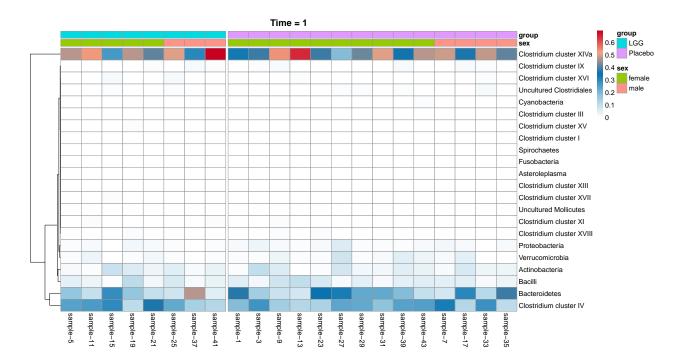
[[2]]



3.3 Taxa Heatmap

```
feature.level = feature.level,
    feature.dat.type = feature.dat.type,
    features.plot = features.plot,
    top.k.plot = top.k.plot,
    top.k.func = top.k.func,
    prev.filter = prev.filter,
    abund.filter = abund.filter,
    base.size = base.size,
    palette = NULL,
    cluster.cols = NULL,
    cluster.rows = NULL,
    pdf = pdf,
    file.ann = file.ann,
    pdf.wid = pdf.wid,
    pdf.hei = pdf.hei)
```





3.4 Taxa Test

```
## For proportion and other data types, posterior sampling will not be performed!
## The data has 22 samples and 8 features will be tested!
## On average, 1 outlier counts will be replaced for each feature!
## Permutation testing ...
## ......
## Completed!
## For proportion and other data types, posterior sampling will not be performed!
## The data has 22 samples and 21 features will be tested!
## On average, 1 outlier counts will be replaced for each feature!
## Permutation testing ...
## ......
## ......
## Completed!
## ## Taxa Test Results
```

• Phylum:

Table 5: Table continues below

| Variable | Group | R.Squared | F.Statistic | Estimate |
|-----------------|-------------|-----------|-------------|----------------------|
| Actinobacteria | Placebo | 0.008281 | 1.622 | 0.0371656495862313 |
| Actinobacteria | LGG | 0.008281 | 1.622 | 0.0371656495862313 |
| Bacteroidetes | Placebo | 3.171e-05 | 0.007819 | -0.00522944015520488 |
| Bacteroidetes | LGG | 3.171e-05 | 0.007819 | -0.00522944015520488 |
| Cyanobacteria | Placebo | 0.007035 | 0.215 | -0.00426210232386587 |
| Cyanobacteria | LGG | 0.007035 | 0.215 | -0.00426210232386587 |
| Firmicutes | Placebo | 1.488e-08 | 3.721e-05 | -0.00021666603778836 |
| Firmicutes | LGG | 1.488e-08 | 3.721e-05 | -0.00021666603778836 |
| Fusobacteria | Placebo | 0.004317 | 0.102 | 0.000446912815084747 |
| Fusobacteria | LGG | 0.004317 | 0.102 | 0.000446912815084747 |
| Proteobacteria | Placebo | 0.009319 | 3.424 | -0.0250120304262438 |
| Proteobacteria | $_{ m LGG}$ | 0.009319 | 3.424 | -0.0250120304262438 |
| Spirochaetes | Placebo | 0.0612 | 1.45 | -0.00213813810466464 |
| Spirochaetes | LGG | 0.0612 | 1.45 | -0.00213813810466464 |
| Verrucomicrobia | Placebo | 0.002485 | 0.3079 | -0.0142760721608877 |
| Verrucomicrobia | LGG | 0.002485 | 0.3079 | -0.0142760721608877 |

Table 6: Table continues below

| P.Value | Adjusted.P.Value | Mean.Proportion | Mean.Prevalence | SD.Abundance |
|---------|------------------|-----------------|-----------------|--------------|
| 0.24 | 0.7104 | 0.03858 | 1 | 0.02768 |
| 0.24 | 0.7104 | 0.03887 | 1 | 0.02551 |
| 0.96 | 0.9874 | 0.2133 | 1 | 0.1164 |
| 0.96 | 0.9874 | 0.1783 | 1 | 0.1418 |
| 0.61 | 0.9815 | 0.0008715 | 0.2857 | 0.002676 |
| 0.61 | 0.9815 | 0.0006973 | 0.5 | 0.0009465 |
| 0.98 | 0.9874 | 0.7056 | 1 | 0.129 |
| 0.98 | 0.9874 | 0.7568 | 1 | 0.1375 |
| 0.76 | 0.9815 | 2.213e-05 | 0.2143 | 5.433e-05 |
| 0.76 | 0.9815 | 0 | 0 | 0 |
| 0.09 | 0.5354 | 0.01829 | 1 | 0.01254 |
| 0.09 | 0.5354 | 0.01256 | 1 | 0.006984 |
| 0.32 | 0.7104 | 2.993e-05 | 0.2143 | 6.179 e - 05 |
| 0.32 | 0.7104 | 0 | 0 | 0 |
| 0.64 | 0.9815 | 0.02332 | 1 | 0.0224 |
| 0.64 | 0.9815 | 0.01274 | 1 | 0.008737 |

| SD.Prevalence |
|---------------|
| 0 |
| 0 |
| 0 |
| 0 |
| 0.4688 |
| 0.5345 |
| 0 |
| 0 |
| 0.4258 |
| |

| SD.Prevalence |
|---------------|
| 0 |
| 0 |
| 0 |
| 0.4258 |
| 0 |
| 0 |
| 0 |

• Family:

Table 8: Table continues below

| Variable | Group | R.Squared | F.Statistic |
|---------------------------|-------------|--------------|-------------|
| Actinobacteria | Placebo | 0.008281 | 1.622 |
| Actinobacteria | LGG | 0.008281 | 1.622 |
| Bacilli | Placebo | 0.007595 | 2.078 |
| Bacilli | $_{ m LGG}$ | 0.007595 | 2.078 |
| Bacteroidetes | Placebo | 3.171e-05 | 0.007819 |
| Bacteroidetes | $_{ m LGG}$ | 3.171e-05 | 0.007819 |
| Clostridium cluster I | Placebo | 0.03478 | 1.937 |
| Clostridium cluster I | $_{ m LGG}$ | 0.03478 | 1.937 |
| Clostridium cluster III | Placebo | 0.0009978 | 0.3254 |
| Clostridium cluster III | LGG | 0.0009978 | 0.3254 |
| Clostridium cluster IV | Placebo | 0.0005453 | 0.3611 |
| Clostridium cluster IV | $_{ m LGG}$ | 0.0005453 | 0.3611 |
| Clostridium cluster IX | Placebo | 0.02899 | 5.118 |
| Clostridium cluster IX | $_{ m LGG}$ | 0.02899 | 5.118 |
| Clostridium cluster XI | Placebo | 0.0002079 | 0.2086 |
| Clostridium cluster XI | $_{ m LGG}$ | 0.0002079 | 0.2086 |
| Clostridium cluster XIII | Placebo | 0.02201 | 0.4589 |
| Clostridium cluster XIII | $_{ m LGG}$ | 0.02201 | 0.4589 |
| Clostridium cluster XIVa | Placebo | 3.245 e - 05 | 0.03652 |
| Clostridium cluster XIVa | $_{ m LGG}$ | 3.245 e - 05 | 0.03652 |
| Clostridium cluster XV | Placebo | 0.00284 | 0.4938 |
| Clostridium cluster XV | $_{ m LGG}$ | 0.00284 | 0.4938 |
| Clostridium cluster XVI | Placebo | 0.001252 | 0.1505 |
| Clostridium cluster XVI | $_{ m LGG}$ | 0.001252 | 0.1505 |
| Clostridium cluster XVII | Placebo | 0.00913 | 0.6283 |
| Clostridium cluster XVII | $_{ m LGG}$ | 0.00913 | 0.6283 |
| Clostridium cluster XVIII | Placebo | 0.0008333 | 0.1064 |
| Clostridium cluster XVIII | $_{ m LGG}$ | 0.0008333 | 0.1064 |
| Cyanobacteria | Placebo | 0.007035 | 0.215 |
| Cyanobacteria | $_{ m LGG}$ | 0.007035 | 0.215 |
| Fusobacteria | Placebo | 0.004317 | 0.102 |
| Fusobacteria | LGG | 0.004317 | 0.102 |
| Proteobacteria | Placebo | 0.009319 | 3.424 |
| Proteobacteria | LGG | 0.009319 | 3.424 |
| Spirochaetes | Placebo | 0.0612 | 1.45 |
| Spirochaetes | LGG | 0.0612 | 1.45 |
| Uncultured Clostridiales | Placebo | 4.582e-05 | 0.004194 |
| Uncultured Clostridiales | $_{ m LGG}$ | 4.582e-05 | 0.004194 |

| Variable | Group | R.Squared | F.Statistic |
|-----------------------|-------------|-----------|-------------|
| Uncultured Mollicutes | Placebo | 0.008615 | 0.4431 |
| Uncultured Mollicutes | $_{ m LGG}$ | 0.008615 | 0.4431 |
| Verrucomicrobia | Placebo | 0.002485 | 0.3079 |
| Verrucomicrobia | LGG | 0.002485 | 0.3079 |

Table 9: Table continues below

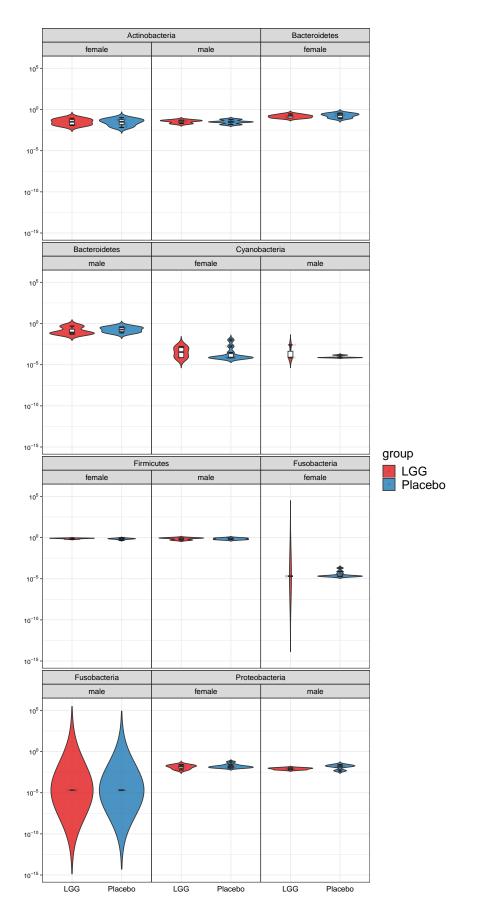
| Estimate | P.Value | Adjusted.P.Value | Mean.Proportion |
|-----------------------|---------|------------------|-----------------|
| 0.0371656495862313 | 0.28 | 0.8956 | 0.03858 |
| 0.0371656495862313 | 0.28 | 0.8956 | 0.03887 |
| -0.0380311099488204 | 0.21 | 0.8956 | 0.04462 |
| -0.0380311099488204 | 0.21 | 0.8956 | 0.04312 |
| -0.00522944015520488 | 0.95 | 0.949 | 0.2133 |
| -0.00522944015520488 | 0.95 | 0.949 | 0.1783 |
| -0.00778505857179085 | 0.18 | 0.8956 | 0.0004309 |
| -0.00778505857179085 | 0.18 | 0.8956 | 0.0003633 |
| 0.00307464254072209 | 0.61 | 0.8984 | 0.001921 |
| 0.00307464254072209 | 0.61 | 0.8984 | 0.002703 |
| 0.022308499587939 | 0.53 | 0.8984 | 0.2038 |
| 0.022308499587939 | 0.53 | 0.8984 | 0.2228 |
| 0.0284837310780048 | 0.07 | 0.6768 | 0.006706 |
| 0.0284837310780048 | 0.07 | 0.6768 | 0.006017 |
| 0.00223243108522325 | 0.64 | 0.8984 | 0.005256 |
| 0.00223243108522325 | 0.64 | 0.8984 | 0.005965 |
| 0.000419187792751176 | 0.71 | 0.8984 | 7.779e-06 |
| 0.000419187792751176 | 0.71 | 0.8984 | 0 |
| -0.00785830208245519 | 0.86 | 0.9484 | 0.4268 |
| -0.00785830208245519 | 0.86 | 0.9484 | 0.4572 |
| -0.00433160436417467 | 0.48 | 0.8984 | 0.001428 |
| -0.00433160436417467 | 0.48 | 0.8984 | 0.001955 |
| 0.00485366929249193 | 0.7 | 0.8984 | 0.003553 |
| 0.00485366929249193 | 0.7 | 0.8984 | 0.005731 |
| 0.00668670952996451 | 0.48 | 0.8984 | 0.001052 |
| 0.00668670952996451 | 0.48 | 0.8984 | 0.00137 |
| -0.00387419196078766 | 0.77 | 0.8984 | 0.004027 |
| -0.00387419196078766 | 0.77 | 0.8984 | 0.004411 |
| -0.00426210232386587 | 0.62 | 0.8984 | 0.0008715 |
| -0.00426210232386587 | 0.62 | 0.8984 | 0.0006973 |
| 0.000446912815084747 | 0.75 | 0.8984 | 2.213e-05 |
| 0.000446912815084747 | 0.75 | 0.8984 | 0 |
| -0.0250120304262438 | 0.11 | 0.7879 | 0.01829 |
| -0.0250120304262438 | 0.11 | 0.7879 | 0.01256 |
| -0.00213813810466464 | 0.44 | 0.8956 | 2.993e-05 |
| -0.00213813810466464 | 0.44 | 0.8956 | 0 |
| -0.000912135473189165 | 0.96 | 0.949 | 0.004694 |
| -0.000912135473189165 | 0.96 | 0.949 | 0.004054 |
| -0.00648233320320809 | 0.51 | 0.8984 | 0.001222 |
| -0.00648233320320809 | 0.51 | 0.8984 | 0.001134 |
| -0.0142760721608877 | 0.6 | 0.8984 | 0.02332 |
| -0.0142760721608877 | 0.6 | 0.8984 | 0.01274 |

| Mean.Prevalence | SD.Abundance | SD.Prevalence |
|-----------------|--------------|---------------|
| 1 | 0.02768 | 0 |
| 1 | 0.02551 | 0 |
| 1 | 0.02371 | 0 |
| 1 | 0.03268 | 0 |
| 1 | 0.1164 | 0 |
| 1 | 0.1418 | 0 |
| 0.8571 | 0.0005898 | 0.3631 |
| 0.625 | 0.0004214 | 0.5175 |
| 1 | 0.0009628 | 0 |
| 1 | 0.001215 | 0 |
| 1 | 0.06958 | 0 |
| 1 | 0.09082 | 0 |
| 1 | 0.005046 | 0 |
| 1 | 0.005356 | 0 |
| 1 | 0.001698 | 0 |
| 1 | 0.001659 | 0 |
| 0.07143 | 2.91e-05 | 0.2673 |
| 0 | 0 | 0 |
| 1 | 0.109 | 0 |
| 1 | 0.1352 | 0 |
| 1 | 0.001012 | 0 |
| 1 | 0.002454 | 0 |
| 1 | 0.00294 | 0 |
| 1 | 0.006604 | 0 |
| 1 | 0.001502 | 0 |
| 1 | 0.002487 | 0 |
| 0.9286 | 0.002957 | 0.2673 |
| 1 | 0.002947 | 0 |
| 0.2857 | 0.002676 | 0.4688 |
| 0.5 | 0.0009465 | 0.5345 |
| 0.2143 | 5.433e-05 | 0.4258 |
| 0 | 0 | 0 |
| 1 | 0.01254 | 0 |
| 1 | 0.006984 | 0 |
| 0.2143 | 6.179e-05 | 0.4258 |
| 0 | 0 | 0 |
| 1 | 0.005238 | 0 |
| 1 | 0.003825 | 0 |
| 0.7857 | 0.003023 | 0.4258 |
| 0.75 | 0.001711 | 0.4629 |
| 1 | 0.0224 | 0.1023 |
| 1 | 0.008737 | 0 |

3.5 Taxa Boxplot for Significant Taxa

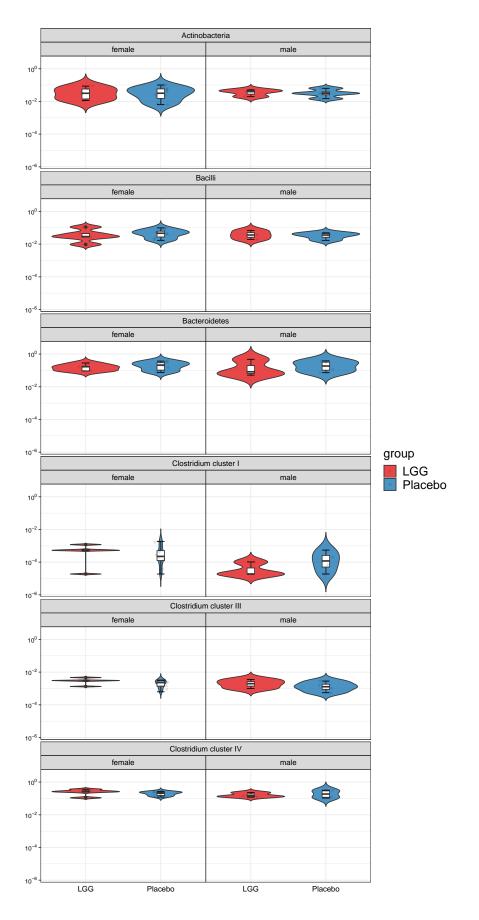
```
group.var = group.var,
                                                               strata.var = strata.var,
                                                               feature.level = feature.level,
                                                               feature.dat.type = feature.dat.type,
                                                               features.plot = significant_taxa,
                                                               top.k.plot = top.k.plot,
                                                               top.k.func = top.k.func,
                                                               Transform = Transform,
                                                               prev.filter = prev.filter,
                                                               abund.filter = abund.filter,
                                                               base.size = 10,
                                                               theme.choice = theme.choice,
                                                               custom.theme = custom.theme,
                                                               palette = palette,
                                                               pdf = pdf,
                                                               file.ann = file.ann,
                                                               pdf.wid = pdf.wid,
                                                               pdf.hei = pdf.hei)
names(taxa_boxplot_results) <- feature.level</pre>
taxa_boxplot_results
```

\$Phylum



##

\$Family



```
taxa_indiv_boxplot_results <- generate_taxa_indiv_boxplot_single(data.obj = data.obj,</pre>
                                    subject.var = subject.var,
                                    time.var = time.var,
                                    t.level = t.level,
                                    group.var = group.var,
                                    strata.var = strata.var,
                                    feature.level = feature.level,
                                    features.plot = significant taxa,
                                    feature.dat.type = feature.dat.type,
                                    top.k.plot = top.k.plot,
                                    top.k.func = top.k.func,
                                    Transform = Transform,
                                    prev.filter = prev.filter,
                                    abund.filter = abund.filter,
                                    base.size = 10,
                                    theme.choice = theme.choice,
                                    custom.theme = custom.theme,
                                    palette = palette,
                                    pdf = TRUE,
                                    file.ann = file.ann,
                                    pdf.wid = pdf.wid,
                                    pdf.hei = pdf.hei)
```

The boxplot results for individual taxa or features can be found in the current working directory. The relevant file is named: taxa_indiv_boxplot_single_subject_subject_time_time_feature_level_Phylum_transform_log_prev_filter_0_abund_filter_0_group_group_strata_sex_Phylum.pdf. Please refer to this file for more detailed visualizations. The boxplot results for individual taxa or features can be found in the current working directory. The relevant file is named: taxa_indiv_boxplot_single_subject_subject_time_time_feature_level_Family_transform_log_prev_filter_0_abund_filter_0_group_group_strata_sex_Family.pdf. Please refer to this file for more detailed visualizations.