Summary

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```
## converting counts to integer mode

## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing

## 2 rows did not converge in beta, labelled in mcols(object)$betaConv. Use larger maxit argument with n

## -- replacing outliers and refitting for 3285 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)

## estimating dispersions

## fitting model and testing

## 2 rows did not converge in beta, labelled in mcols(object)$betaConv. Use larger maxit argument with n

##          baseMean log2FoldChange      lfcSE     stat       pvalue
## ASV939  5.5121024     0.07364395 0.01824878 4.035555 5.447348e-05
## ASV996  4.2464048     0.06004654 0.01825309 3.289664 1.003070e-03
## ASV1000 3.9296182     0.05919934 0.01825280 3.243302 1.181531e-03
## ASV1090 4.4169160     0.15014245 0.01825358 8.225369 1.945916e-16
## ASV1233 3.3082249     0.06349022 0.01825193 3.478548 5.041383e-04
## ASV1356 2.7268086     0.14530799 0.01825370 7.960469 1.713885e-15
## ASV1391 2.4497731     0.06052116 0.01825308 3.315669 9.142405e-04
## ASV1424 1.5939402     0.10427375 0.01825367 5.712483 1.113396e-08
## ASV1527 2.6538568     0.14489347 0.01825370 7.937759 2.058675e-15
## ASV1585 1.9696934     0.13042651 0.01825370 7.145210 8.985858e-13
## ASV1730 1.0897346     0.09191796 0.01825378 5.035559 4.764570e-07
## ASV1794 1.0246758     0.08980433 0.01825380 4.919762 8.664949e-07
## ASV1826 1.5527660     0.12936125 0.01825374 7.086833 1.372156e-12
## ASV1843 1.5275178     0.12887834 0.01825375 7.060377 1.660511e-12
## ASV1844 0.9840141     0.08854072 0.01825381 4.850533 1.231298e-06
## ASV1857 1.8408255     0.13457572 0.01825373 7.372506 1.674505e-13
## ASV1885 1.4103978     0.12044251 0.01825374 6.598236 4.160777e-11
```

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## ASV1888 0.9352200     0.08687899 0.01825383 4.759493 1.940796e-06
## ASV1920 1.5277556     0.12886798 0.01825375 7.059810 1.667307e-12
## ASV2034 0.8213671     0.08269904 0.01825389 4.530488 5.884766e-06
## ASV2068 1.3249562     0.12480135 0.01825377 6.837019 8.085777e-12
## ASV2102 1.1992908     0.12196370 0.01825378 6.681559 2.364143e-11
## ASV2128 1.1740426     0.12135379 0.01825378 6.648144 2.968110e-11
## ASV2186 1.1109220     0.11976062 0.01825379 6.560862 5.349771e-11
## ASV2211 1.0991378     0.08415185 0.01825378 4.610106 4.024637e-06
## ASV2245 1.0478014     0.11807512 0.01825380 6.468521 9.896679e-11
## ASV2333 1.0410370     0.11787371 0.01825381 6.457487 1.064560e-10
## ASV2350 0.9594326     0.11554324 0.01825382 6.329811 2.454622e-10
## ASV2388 1.1351757     0.12081634 0.01825379 6.618698 3.623759e-11
## ASV2393 0.9457697     0.07905400 0.01825385 4.330812 1.485603e-05
## ASV2405 0.9329891     0.07861488 0.01825386 4.306754 1.656676e-05
## ASV2436 0.8632607     0.10557014 0.01825384 5.783450 7.318405e-09
## ASV2448 0.9463973     0.11514756 0.01825383 6.308133 2.824217e-10
## ASV2477 0.8389435     0.10468839 0.01825384 5.735143 9.743031e-09
## ASV2533 0.8307437     0.07480601 0.01825393 4.098077 4.165964e-05
## ASV2555 0.7659919     0.10201886 0.01825387 5.588889 2.285264e-08
## ASV2579 0.7416747     0.10103846 0.01825388 5.535177 3.109145e-08
## ASV2611 0.7173575     0.10002482 0.01825389 5.479643 4.261844e-08
## ASV2614 0.7540597     0.07152640 0.01825400 3.918396 8.914029e-05
## ASV2701 0.6901563     0.06000211 0.01825435 3.287003 1.012597e-03
## ASV2702 0.6901563     0.06000211 0.01825435 3.287003 1.012597e-03
## ASV2719 0.6773756     0.06792155 0.01825410 3.720893 1.985198e-04
## ASV2793 0.6262529     0.06530744 0.01825419 3.577669 3.466716e-04
## ASV2826 0.5714543     0.09308918 0.01825399 5.099661 3.402623e-07
## ASV2882 0.5623496     0.06159704 0.01825434 3.374378 7.398263e-04
## ASV2957 0.5407984     0.09888600 0.01825402 5.417217 6.053393e-08
## ASV3291 0.3134288     0.09750605 0.01825474 5.341409 9.222699e-08
##                 padj  Kingdom        Phylum            Class
## ASV939  5.777134e-03 Bacteria Bacteroidetes      Bacteroidia
## ASV996  8.637894e-02 Bacteria    Firmicutes       Clostridia
## ASV1000 9.864524e-02 Bacteria   Tenericutes       Mollicutes
## ASV1090 7.635774e-13 Bacteria    Firmicutes       Clostridia
## ASV1233 4.824973e-02 Bacteria    Firmicutes       Clostridia
## ASV1356 2.692747e-12 Bacteria Bacteroidetes      Bacteroidia
## ASV1391 8.342976e-02 Bacteria Bacteroidetes      Bacteroidia
## ASV1424 2.080459e-06 Bacteria    Firmicutes       Clostridia
## ASV1527 2.692747e-12 Bacteria Bacteroidetes      Bacteroidia
## ASV1585 7.052101e-10 Bacteria    Firmicutes       Clostridia
## ASV1730 6.677205e-05 Bacteria    Firmicutes       Clostridia
## ASV1794 1.172457e-04 Bacteria    Firmicutes       Clostridia
## ASV1826 8.178141e-10 Bacteria Bacteroidetes      Bacteroidia
## ASV1843 8.178141e-10 Bacteria Bacteroidetes      Bacteroidia
## ASV1844 1.610538e-04 Bacteria    Firmicutes       Clostridia
## ASV1857 1.642689e-10 Bacteria Bacteroidetes      Bacteroidia
## ASV1885 1.255914e-08 Bacteria    Firmicutes          Bacilli
## ASV1888 2.456672e-04 Bacteria    Firmicutes       Clostridia
## ASV1920 8.178141e-10 Bacteria Bacteroidetes      Bacteroidia
## ASV2034 6.997522e-04 Bacteria    Firmicutes       Clostridia
## ASV2068 3.525399e-09 Bacteria    Firmicutes       Clostridia
## ASV2102 9.276899e-09 Bacteria    Firmicutes       Clostridia
## ASV2128 1.058806e-08 Bacteria Bacteroidetes      Bacteroidia
```

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## ASV2186 1.499465e-08 Bacteria    Firmicutes       Clostridia
## ASV2211 4.935211e-04 Bacteria    Firmicutes       Clostridia
## ASV2245 2.588971e-08 Bacteria    Firmicutes       Clostridia
## ASV2333 2.610833e-08 Bacteria    Firmicutes       Clostridia
## ASV2350 5.665846e-08 Bacteria Bacteroidetes      Bacteroidia
## ASV2388 1.184969e-08 Bacteria Bacteroidetes      Bacteroidia
## ASV2393 1.714560e-03 Bacteria    Firmicutes       Clostridia
## ASV2405 1.857370e-03 Bacteria Bacteroidetes      Bacteroidia
## ASV2436 1.511443e-06 Bacteria    Firmicutes Erysipelotrichia
## ASV2448 6.156793e-08 Bacteria    Firmicutes       Clostridia
## ASV2477 1.911583e-06 Bacteria    Firmicutes       Clostridia
## ASV2533 4.540901e-03 Bacteria Bacteroidetes      Bacteroidia
## ASV2555 4.076079e-06 Bacteria Bacteroidetes      Bacteroidia
## ASV2579 5.304471e-06 Bacteria    Firmicutes       Clostridia
## ASV2611 6.968116e-06 Bacteria Bacteroidetes      Bacteroidia
## ASV2614 9.204908e-03 Bacteria    Firmicutes       Clostridia
## ASV2701 8.637894e-02 Bacteria    Firmicutes       Clostridia
## ASV2702 8.637894e-02 Bacteria    Firmicutes Erysipelotrichia
## ASV2719 1.997415e-02 Bacteria    Firmicutes       Clostridia
## ASV2793 3.400848e-02 Bacteria    Firmicutes       Clostridia
## ASV2826 4.945145e-05 Bacteria    Firmicutes       Clostridia
## ASV2882 6.912092e-02 Bacteria    Firmicutes       Clostridia
## ASV2957 9.501405e-06 Bacteria    Firmicutes       Clostridia
## ASV3291 1.391918e-05 Bacteria    Firmicutes       Clostridia
##                      Order              Family
## ASV939       Bacteroidales      Muribaculaceae
## ASV996       Clostridiales     Lachnospiraceae
## ASV1000    Mycoplasmatales    Mycoplasmataceae
## ASV1090      Clostridiales     Lachnospiraceae
## ASV1233      Clostridiales     Lachnospiraceae
## ASV1356      Bacteroidales      Prevotellaceae
## ASV1391      Bacteroidales      Muribaculaceae
## ASV1424      Clostridiales     Lachnospiraceae
## ASV1527      Bacteroidales      Bacteroidaceae
## ASV1585      Clostridiales     Ruminococcaceae
## ASV1730      Clostridiales     Lachnospiraceae
## ASV1794      Clostridiales     Lachnospiraceae
## ASV1826      Bacteroidales      Prevotellaceae
## ASV1843      Bacteroidales      Prevotellaceae
## ASV1844      Clostridiales     Lachnospiraceae
## ASV1857      Bacteroidales      Bacteroidaceae
## ASV1885    Lactobacillales    Lactobacillaceae
## ASV1888      Clostridiales     Lachnospiraceae
## ASV1920      Bacteroidales      Muribaculaceae
## ASV2034      Clostridiales     Lachnospiraceae
## ASV2068      Clostridiales     Lachnospiraceae
## ASV2102      Clostridiales     Lachnospiraceae
## ASV2128      Bacteroidales      Prevotellaceae
## ASV2186      Clostridiales     Ruminococcaceae
## ASV2211      Clostridiales     Lachnospiraceae
## ASV2245      Clostridiales     Ruminococcaceae
## ASV2333      Clostridiales     Ruminococcaceae
## ASV2350      Bacteroidales      Muribaculaceae
## ASV2388      Bacteroidales      Bacteroidaceae
```
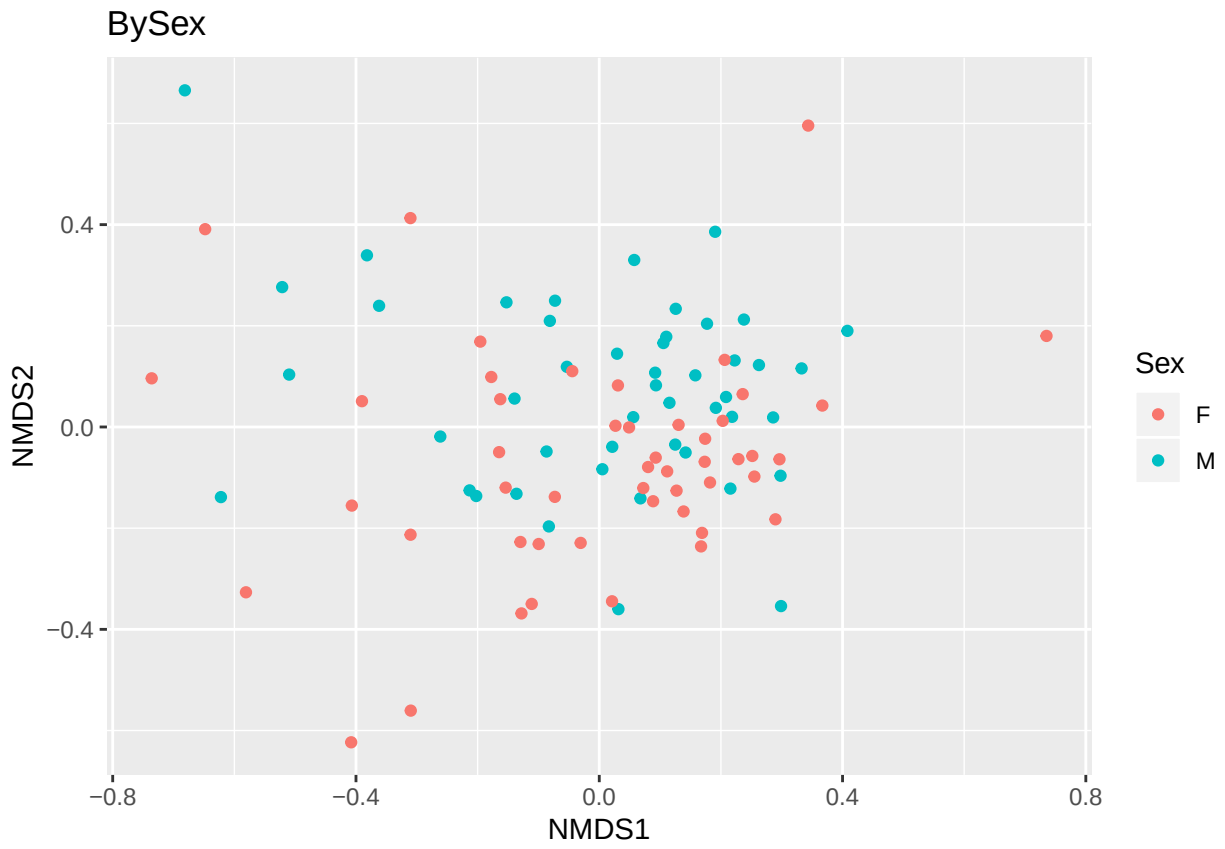
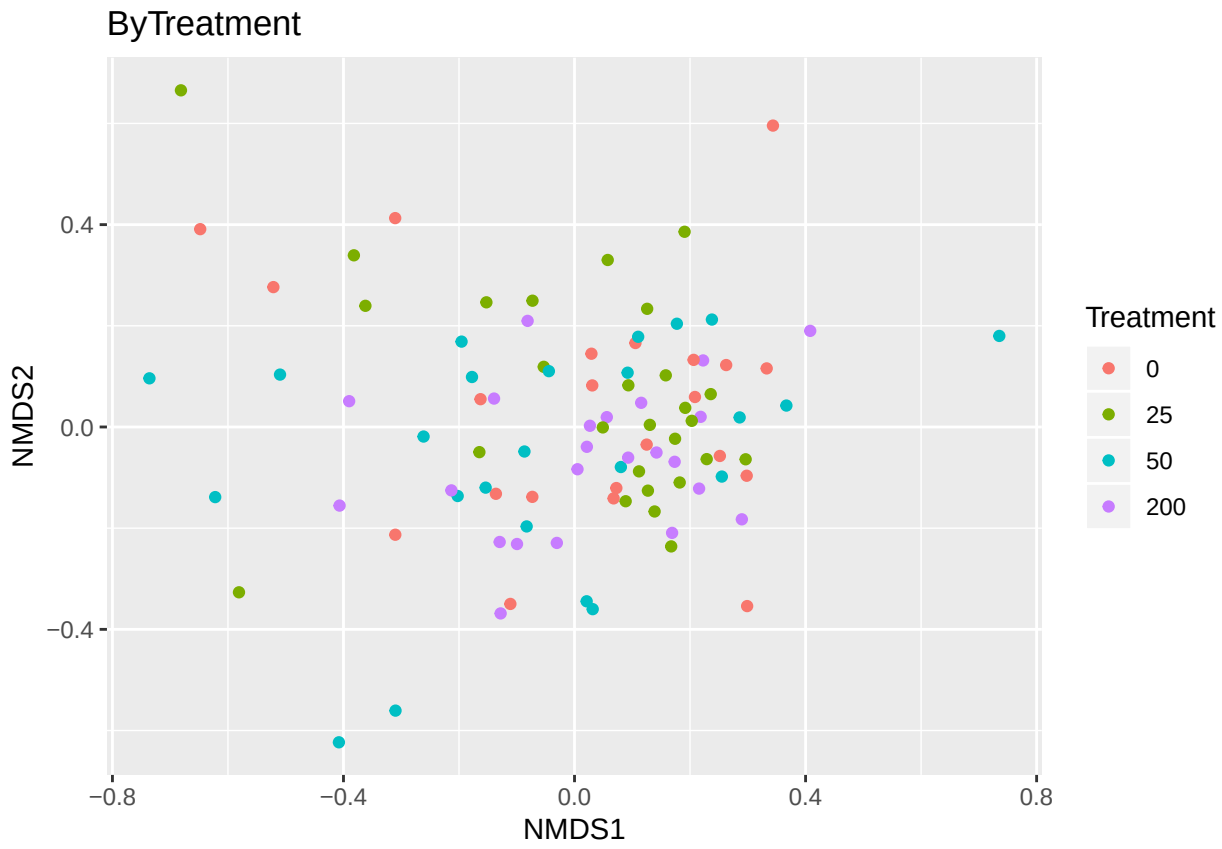

```
## ASV2393      Clostridiales     Ruminococcaceae
## ASV2405      Bacteroidales      Tannerellaceae
## ASV2436 Erysipelotrichales Erysipelotrichaceae
## ASV2448      Clostridiales     Lachnospiraceae
## ASV2477      Clostridiales     Lachnospiraceae
## ASV2533      Bacteroidales      Muribaculaceae
## ASV2555      Bacteroidales       Rikenellaceae
## ASV2579      Clostridiales     Lachnospiraceae
## ASV2611      Bacteroidales      Muribaculaceae
## ASV2614      Clostridiales     Lachnospiraceae
## ASV2701      Clostridiales     Ruminococcaceae
## ASV2702 Erysipelotrichales Erysipelotrichaceae
## ASV2719      Clostridiales     Lachnospiraceae
## ASV2793      Clostridiales     Ruminococcaceae
## ASV2826      Clostridiales     Lachnospiraceae
## ASV2882      Clostridiales     Lachnospiraceae
## ASV2957      Clostridiales     Lachnospiraceae
## ASV3291      Clostridiales     Lachnospiraceae
##                                Genus Species
## ASV939                           <NA>    <NA>
## ASV996                           <NA>    <NA>
## ASV1000                     Mycoplasma    <NA>
## ASV1090 Lachnospiraceae_NK4A136_group    <NA>
## ASV1233                          <NA>    <NA>
## ASV1356   Prevotellaceae_NK3B31_group    <NA>
## ASV1391                          <NA>    <NA>
## ASV1424                          <NA>    <NA>
## ASV1527                    Bacteroides    <NA>
## ASV1585       Ruminococcaceae_UCG-014    <NA>
## ASV1730 Lachnospiraceae_NK4A136_group    <NA>
## ASV1794                          <NA>    <NA>
## ASV1826   Prevotellaceae_NK3B31_group    <NA>
## ASV1843                Alloprevotella    <NA>
## ASV1844                          <NA>    <NA>
## ASV1857                    Bacteroides    <NA>
## ASV1885                  Lactobacillus    <NA>
## ASV1888                          <NA>    <NA>
## ASV1920                          <NA>    <NA>
## ASV2034                          <NA>    <NA>
## ASV2068                          <NA>    <NA>
## ASV2102       Lachnospiraceae_UCG-001    <NA>
## ASV2128   Prevotellaceae_NK3B31_group    <NA>
## ASV2186           Ruminiclostridium_9    <NA>
## ASV2211                            A2    <NA>
## ASV2245           Ruminiclostridium_9    <NA>
## ASV2333           Ruminiclostridium_5    <NA>
## ASV2350                          <NA>    <NA>
## ASV2388                    Bacteroides    <NA>
## ASV2393           Ruminiclostridium_9    <NA>
## ASV2405               Parabacteroides    <NA>
## ASV2436                          <NA>    <NA>
## ASV2448                          <NA>    <NA>
## ASV2477                          <NA>    <NA>
## ASV2533                          <NA>    <NA>
```

```
## ASV2555                      Alistipes    <NA>
## ASV2579                           <NA>    <NA>
## ASV2611                           <NA>    <NA>
## ASV2614                           <NA>    <NA>
## ASV2701           Ruminiclostridium_9    <NA>
## ASV2702                Faecalibaculum    <NA>
## ASV2719 Lachnospiraceae_NK4A136_group    <NA>
## ASV2793                           <NA>    <NA>
## ASV2826 Lachnospiraceae_NK4A136_group    <NA>
## ASV2882       Lachnospiraceae_UCG-001    <NA>
## ASV2957                           <NA>    <NA>
## ASV3291                  Tyzzerella_3    <NA>

## converting counts to integer mode

## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing

## -- replacing outliers and refitting for 27 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)

## estimating dispersions

## fitting model and testing

## [1] "No significant taxa were identified using the specified formula"
```

```
##Alpha diversity is a metric that describes the diversity of taxa in a specific sample or set. Alpha d

##Beta diversity describes the diversity between different samples or sets of samples. Beta diversity w

##The first figure shows an ordination of between sample diversity according to Sex. The second figure
plot(physeq2.ord.plot)
```

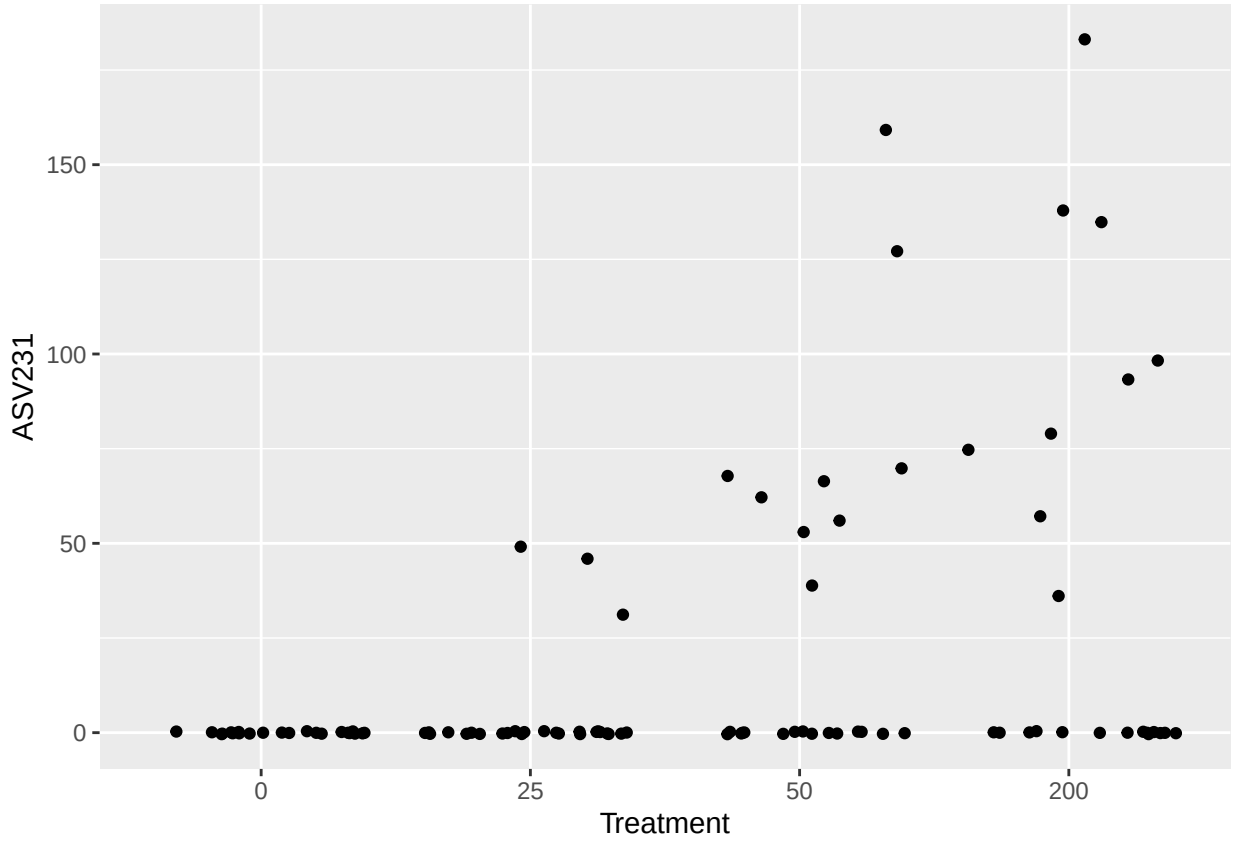



```
plot(physeq2.ord.treatment)
```



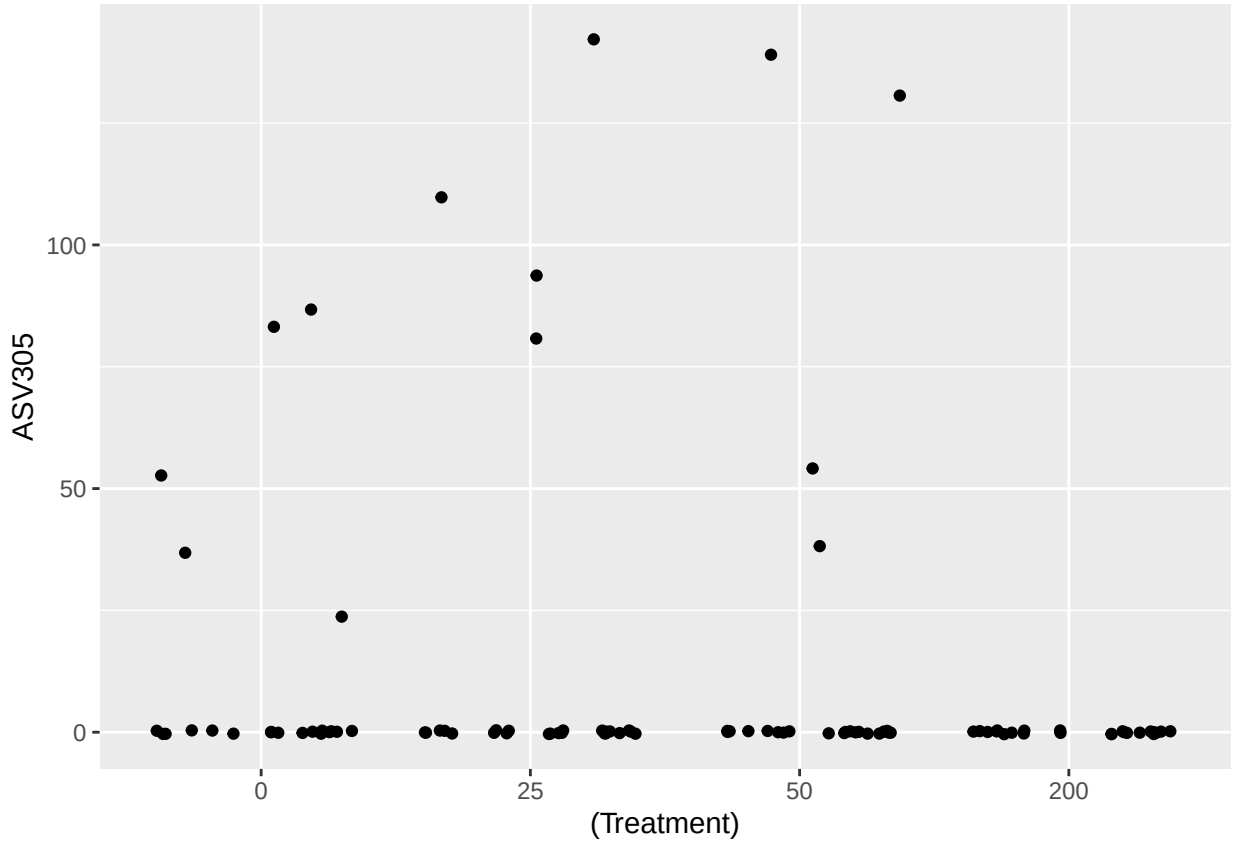

```
## There were associations between covariates of interest and taxa. DESeq2 was used to analyze the asso
##print(Treatment_Sex_ASVs)
##print(Treatment_Sex_genus)
plotting_ASV231 <- ggplot(taxa_table_plus_metadata_asv_rare, aes(x=Treatment, y=ASV231)) + geom_jitter()
plotting_ASV231
```

```
## `geom_smooth()` using method = 'loess' and formula 'y ~ x'
```



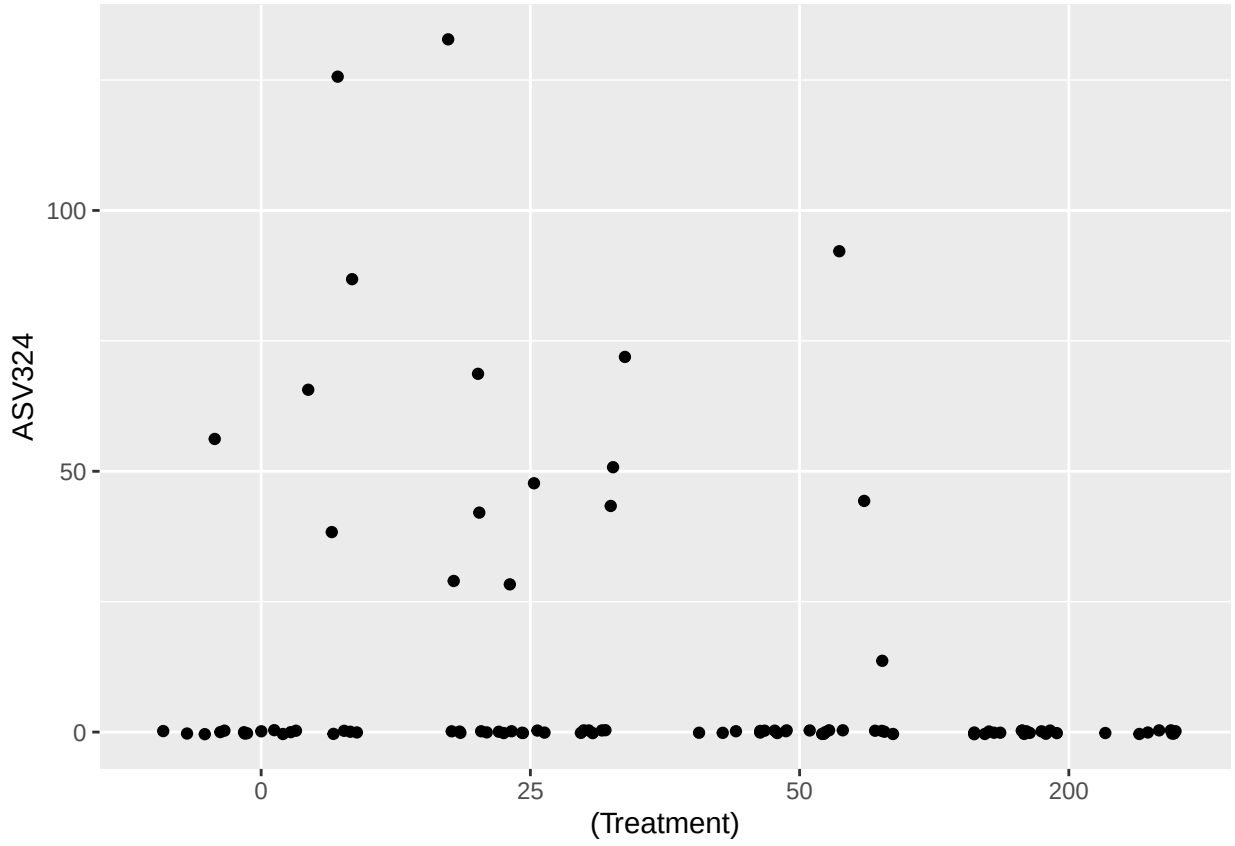

```
plotting_ASV305 <- ggplot(taxa_table_plus_metadata_asv_rare, aes(x=(Treatment), y=ASV305)) + geom_jitte
plotting_ASV305
```

```
## `geom_smooth()` using method = 'loess' and formula 'y ~ x'
```



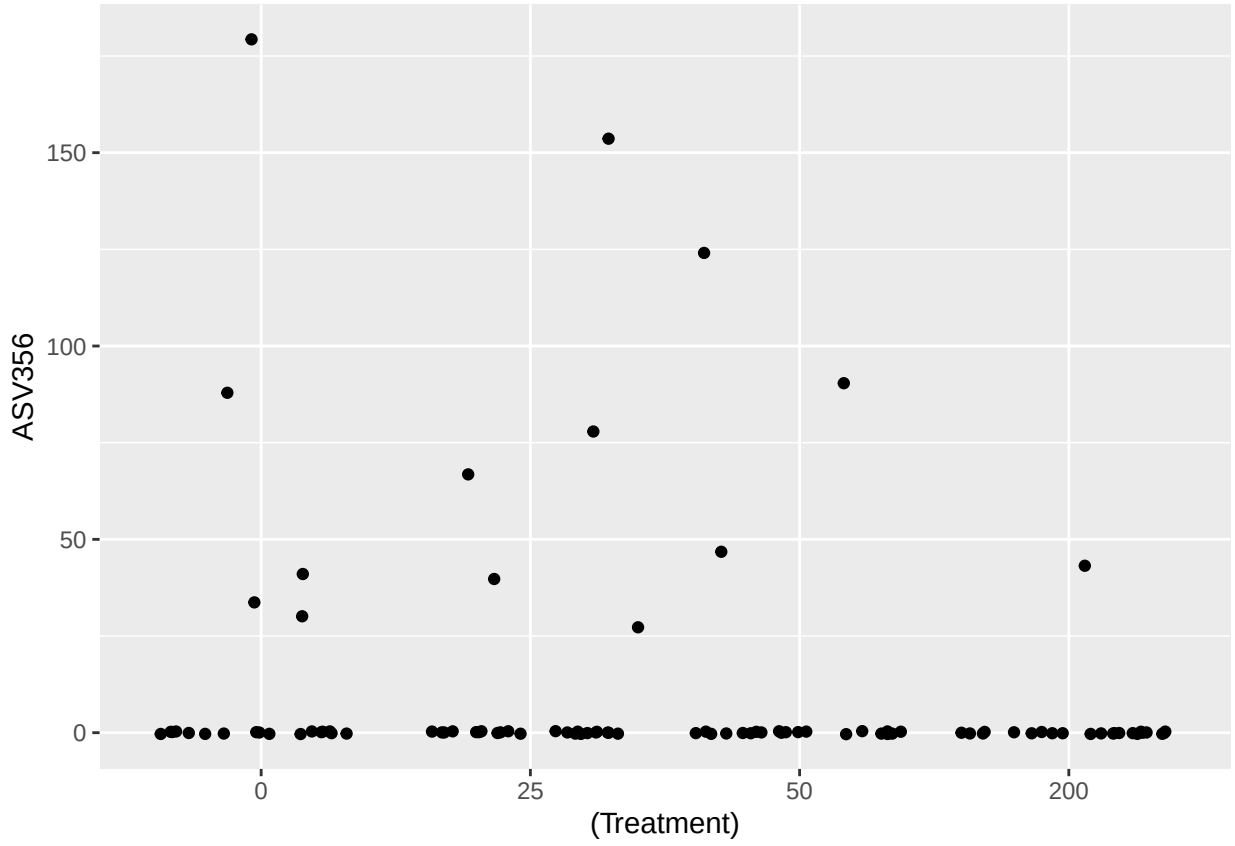

```
plotting_ASV324 <- ggplot(taxa_table_plus_metadata_asv_rare, aes(x=(Treatment), y=ASV324)) + geom_jitte
plotting_ASV324
```

```
## `geom_smooth()` using method = 'loess' and formula 'y ~ x'
```



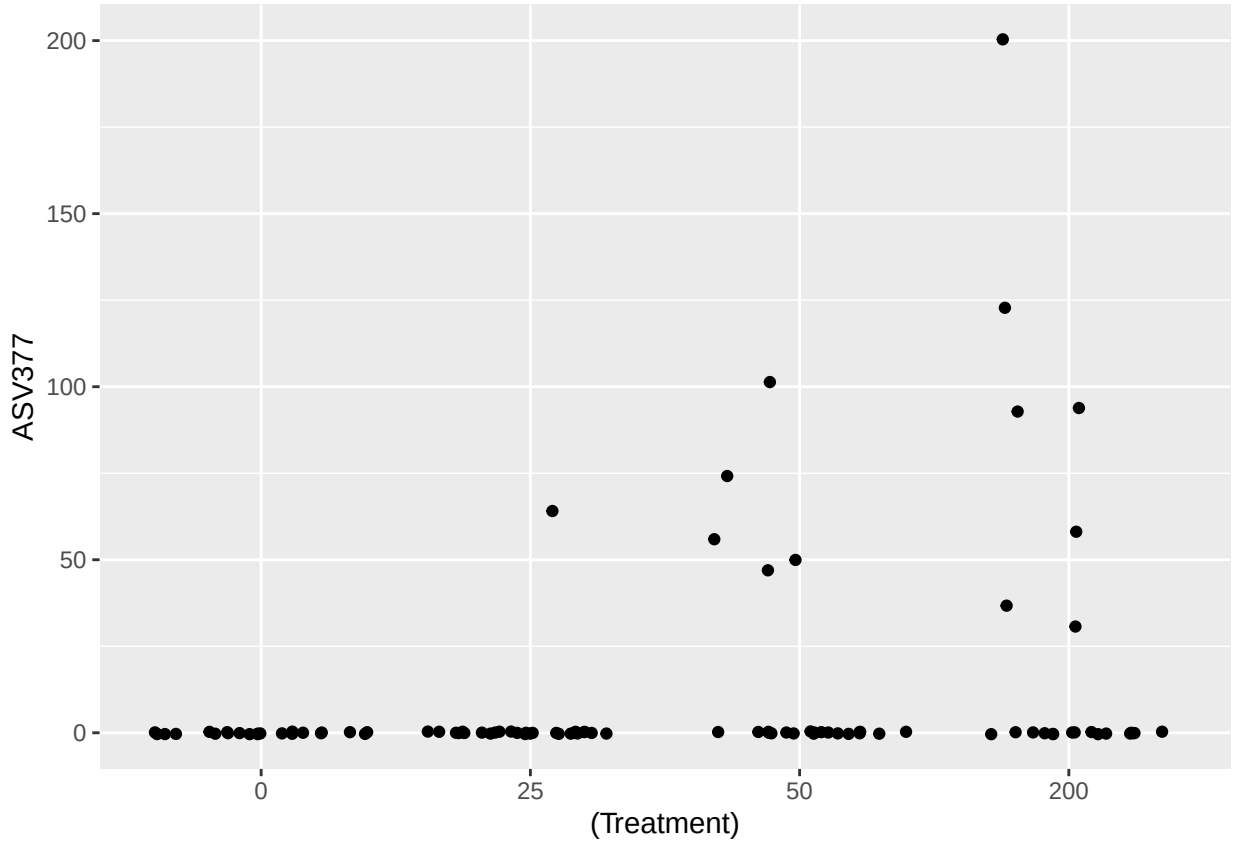

```
plotting_ASV356 <- ggplot(taxa_table_plus_metadata_asv_rare, aes(x=(Treatment), y=ASV356)) + geom_jitte
plotting_ASV356
```

```
## `geom_smooth()` using method = 'loess' and formula 'y ~ x'
```



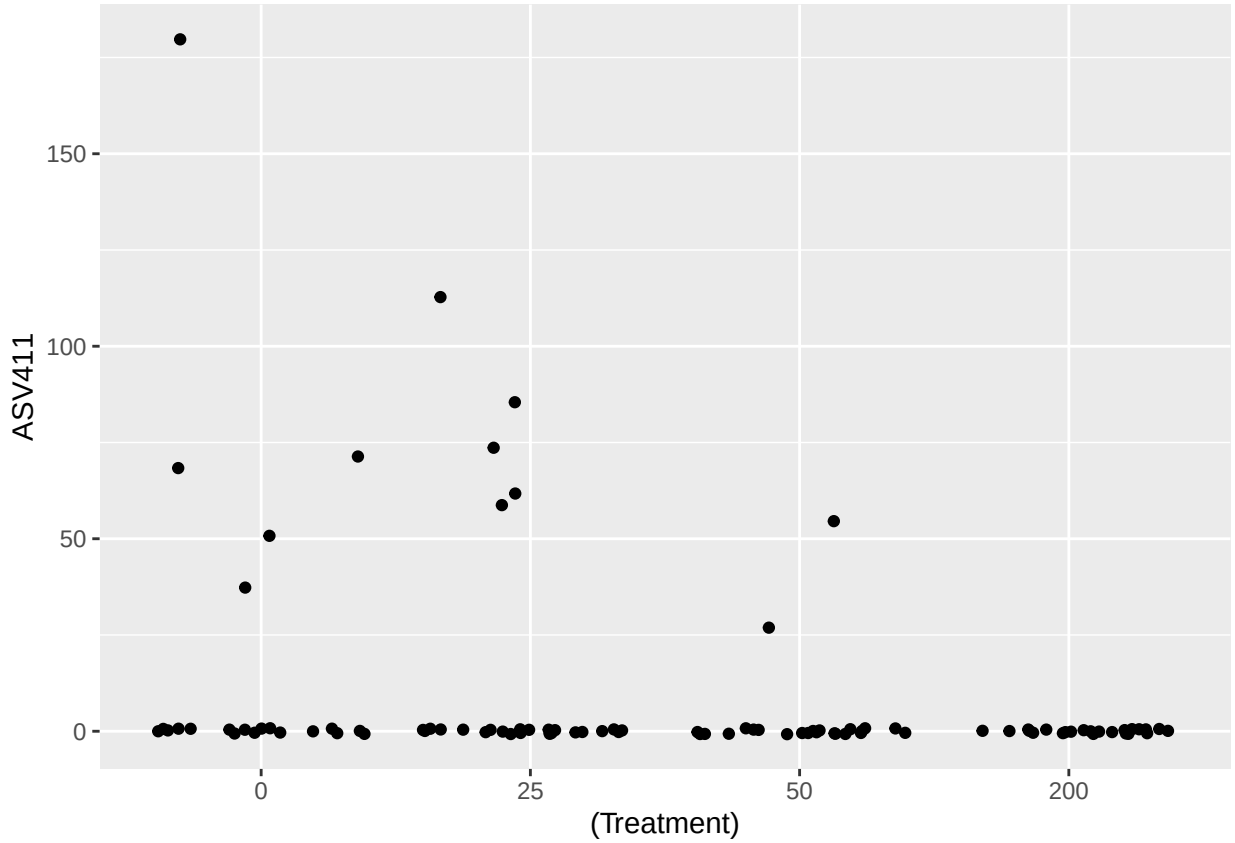

```
plotting_ASV377 <- ggplot(taxa_table_plus_metadata_asv_rare, aes(x=(Treatment), y=ASV377)) + geom_jitte
plotting_ASV377
```

```
## `geom_smooth()` using method = 'loess' and formula 'y ~ x'
```




```
plotting_ASV411 <- ggplot(taxa_table_plus_metadata_asv_rare, aes(x=(Treatment), y=ASV411)) + geom_jitte
plotting_ASV411
```

```
## `geom_smooth()` using method = 'loess' and formula 'y ~ x'
```




```
##Note to Tom, PALatCross shows a somewhat large number of taxa where the fold change is associated wit
```