Freeze-dried vs Frozen samples UniFraq beta diversity

hanna.bensch@lnu.se

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Introducion

I here calculate weighted an unweighted unifrac beta diversity based on the .tre file from the SPP analysis using the GG taxonomy.

I calculated these with ordinate() on a phyloseq object because I failed to figure out how to do it with vegan and tidyverse, following examples such as https://www.nicholas-ollberding.com/post/introduction-to-the-statistical-analysis-of-microbiome-data-in-r/ & https://mibwurrepo.github.io/Microbial-bioinformatics-introductory-course-Material-2018/beta-diversity-metrics.html#phylogenetic-beta-diversity-metrics

```
## Run 0 stress 9.95722e-05
## Run 1 stress 6.112179e-05
## ... New best solution
## ... Procrustes: rmse 8.439116e-05  max resid 0.0001630675
## ... Similar to previous best
## Run 2 stress 9.134744e-05
## ... Procrustes: rmse 4.344606e-05  max resid 0.0001413326
## ... Similar to previous best
## Run 3 stress 7.15492e-05
## ... Procrustes: rmse 2.545011e-05  max resid 5.178076e-05
## ... Similar to previous best
## Run 4 stress 8.478219e-05
## ... Procrustes: rmse 2.743932e-05  max resid 6.215119e-05
## ... Similar to previous best
## Run 5 stress 8.879704e-05
## ... Procrustes: rmse 2.885047e-05  max resid 0.0001153759
## ... Similar to previous best
## Run 6 stress 9.685545e-05
## ... Procrustes: rmse 3.346691e-05  max resid 0.0001337882
## ... Similar to previous best
## Run 7 stress 8.95672e-05
## ... Procrustes: rmse 2.943453e-05  max resid 0.0001133146
## ... Similar to previous best
## Run 8 stress 8.633531e-05
## ... Procrustes: rmse 3.11118e-05  max resid 6.475785e-05
## ... Similar to previous best
## Run 9 stress 9.506785e-05
## ... Procrustes: rmse 3.334854e-05  max resid 0.0001389695
## ... Similar to previous best
```

```
## Run 10 stress 8.416207e-05
## ... Procrustes: rmse 2.389432e-05  max resid 7.593601e-05
## ... Similar to previous best
## Run 11 stress 7.613958e-05
## ... Procrustes: rmse 2.171232e-05  max resid 6.540668e-05
## ... Similar to previous best
## Run 12 stress 9.662229e-05
## ... Procrustes: rmse 3.453503e-05  max resid 0.0001451088
## ... Similar to previous best
## Run 13 stress 6.300052e-05
## ... Procrustes: rmse 4.097601e-05  max resid 0.000145911
## ... Similar to previous best
## Run 14 stress 8.885492e-05
## ... Procrustes: rmse 2.578297e-05  max resid 5.36761e-05
## ... Similar to previous best
## Run 15 stress 9.746946e-05
## ... Procrustes: rmse 3.207172e-05  max resid 9.480206e-05
## ... Similar to previous best
## Run 16 stress 8.678075e-05
## ... Procrustes: rmse 2.83002e-05  max resid 0.0001109818
## ... Similar to previous best
## Run 17 stress 5.011812e-05
## ... New best solution
## ... Procrustes: rmse 3.674689e-05  max resid 0.0001385445
## ... Similar to previous best
## Run 18 stress 8.472657e-05
## ... Procrustes: rmse 5.389594e-05  max resid 0.0002102433
## ... Similar to previous best
## Run 19 stress 9.289489e-05
## ... Procrustes: rmse 6.327435e-05  max resid 0.0002704463
## ... Similar to previous best
## Run 20 stress 7.177815e-05
## ... Procrustes: rmse 4.776438e-05  max resid 0.0001972116
## ... Similar to previous best
## *** Solution reached
```

```
## Run 0 stress 0.1765873
## Run 1 stress 0.173262
## ... New best solution
## ... Procrustes: rmse 0.05063993  max resid 0.2349191
## Run 2 stress 0.1758716
## Run 3 stress 0.197007
## Run 4 stress 0.1762391
## Run 5 stress 0.173262
## ... New best solution
## ... Procrustes: rmse 0.0002865445  max resid 0.001642931
## ... Similar to previous best
## Run 6 stress 0.1795293
## Run 7 stress 0.2135731
## Run 8 stress 0.2237027
## Run 9 stress 0.1771
## Run 10 stress 0.1732619
## ... New best solution
## ... Procrustes: rmse 9.341373e-05  max resid 0.0005236204
```

```
## ... Similar to previous best
## Run 11 stress 0.1765087
## Run 12 stress 0.1734655
## ... Procrustes: rmse 0.009895176  max resid 0.05063226
## Run 13 stress 0.1734655
## ... Procrustes: rmse 0.009907548  max resid 0.05069099
## Run 14 stress 0.175927
## Run 15 stress 0.173262
## ... Procrustes: rmse 0.0001205665  max resid 0.0006867077
## ... Similar to previous best
## Run 16 stress 0.1734655
## ... Procrustes: rmse 0.009896733  max resid 0.0506405
## Run 17 stress 0.1734655
## ... Procrustes: rmse 0.009894149  max resid 0.05062306
## Run 18 stress 0.2138672
## Run 19 stress 0.1734656
## ... Procrustes: rmse 0.009890629  max resid 0.0506061
## Run 20 stress 0.1764339
## *** Solution reached
```

```
## [1] 5.011812e-05
```

```
## [1] 0.1732619
```

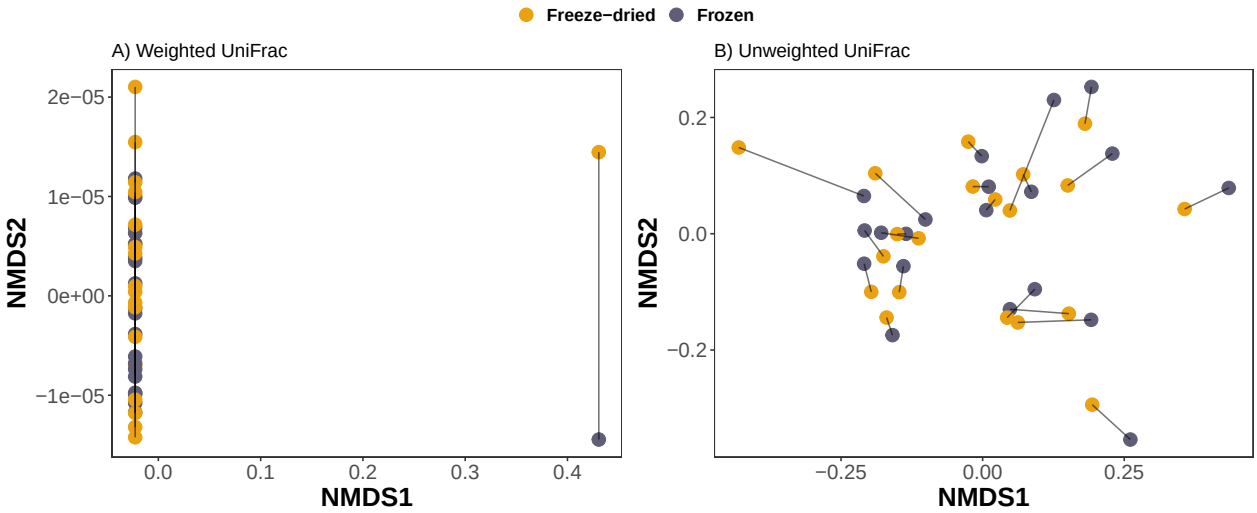


Figure 1: **NMDS weighted & unweigted unifrac un-rarefied data. Point colour represent the sample treatment and line beteween replicates.**

```
## Run 0 stress 0.1721682
## Run 1 stress 0.1721682
## ... Procrustes: rmse 6.670404e-06  max resid 2.761557e-05
## ... Similar to previous best
## Run 2 stress 0.1721682
## ... New best solution
## ... Procrustes: rmse 5.681972e-06  max resid 2.237393e-05
## ... Similar to previous best
## Run 3 stress 0.1721682
## ... Procrustes: rmse 2.746619e-05  max resid 0.0001364699
## ... Similar to previous best
## Run 4 stress 0.1721682
```

```
## ... Procrustes: rmse 1.116075e-06  max resid 4.234217e-06
## ... Similar to previous best
## Run 5 stress 0.1721682
## ... Procrustes: rmse 5.49911e-06  max resid 2.378332e-05
## ... Similar to previous best
## Run 6 stress 0.1721682
## ... New best solution
## ... Procrustes: rmse 3.981129e-06  max resid 1.901418e-05
## ... Similar to previous best
## Run 7 stress 0.1721682
## ... Procrustes: rmse 2.412487e-05  max resid 0.0001201939
## ... Similar to previous best
## Run 8 stress 0.1721682
## ... Procrustes: rmse 4.804048e-06  max resid 2.429335e-05
## ... Similar to previous best
## Run 9 stress 0.1721682
## ... Procrustes: rmse 4.935774e-06  max resid 2.020623e-05
## ... Similar to previous best
## Run 10 stress 0.1803746
## Run 11 stress 0.1721682
## ... Procrustes: rmse 2.358127e-06  max resid 1.022763e-05
## ... Similar to previous best
## Run 12 stress 0.2334473
## Run 13 stress 0.1721682
## ... Procrustes: rmse 3.070771e-06  max resid 1.417787e-05
## ... Similar to previous best
## Run 14 stress 0.1721682
## ... Procrustes: rmse 4.031323e-06  max resid 1.633056e-05
## ... Similar to previous best
## Run 15 stress 0.1721682
## ... Procrustes: rmse 3.674729e-06  max resid 1.566649e-05
## ... Similar to previous best
## Run 16 stress 0.1721682
## ... Procrustes: rmse 1.067492e-06  max resid 4.775583e-06
## ... Similar to previous best
## Run 17 stress 0.1721682
## ... Procrustes: rmse 5.123504e-06  max resid 1.511232e-05
## ... Similar to previous best
## Run 18 stress 0.1721682
## ... Procrustes: rmse 1.160031e-05  max resid 5.702718e-05
## ... Similar to previous best
## Run 19 stress 0.1721682
## ... Procrustes: rmse 3.791834e-06  max resid 1.803504e-05
## ... Similar to previous best
## Run 20 stress 0.1721682
## ... Procrustes: rmse 3.127655e-06  max resid 1.579066e-05
## ... Similar to previous best
## *** Solution reached
```

```
## Run 0 stress 0.1835217
## Run 1 stress 0.1791546
## ... New best solution
## ... Procrustes: rmse 0.06222282  max resid 0.2651071
## Run 2 stress 0.1832647
```

```
## Run 3 stress 0.2123475
## Run 4 stress 0.1811417
## Run 5 stress 0.1791544
## ... New best solution
## ... Procrustes: rmse 0.0006309196  max resid 0.003437614
## ... Similar to previous best
## Run 6 stress 0.208347
## Run 7 stress 0.2387347
## Run 8 stress 0.1815884
## Run 9 stress 0.2435301
## Run 10 stress 0.1864088
## Run 11 stress 0.1791543
## ... New best solution
## ... Procrustes: rmse 0.0003691556  max resid 0.001804482
## ... Similar to previous best
## Run 12 stress 0.1874767
## Run 13 stress 0.1836908
## Run 14 stress 0.1816496
## Run 15 stress 0.2099464
## Run 16 stress 0.1833727
## Run 17 stress 0.1791542
## ... New best solution
## ... Procrustes: rmse 0.0002517528  max resid 0.001134877
## ... Similar to previous best
## Run 18 stress 0.181147
## Run 19 stress 0.1791544
## ... Procrustes: rmse 0.0002853774  max resid 0.001544248
## ... Similar to previous best
## Run 20 stress 0.1899012
## *** Solution reached
```

```
## [1] 0.1721682
```

```
## [1] 0.1791542
```

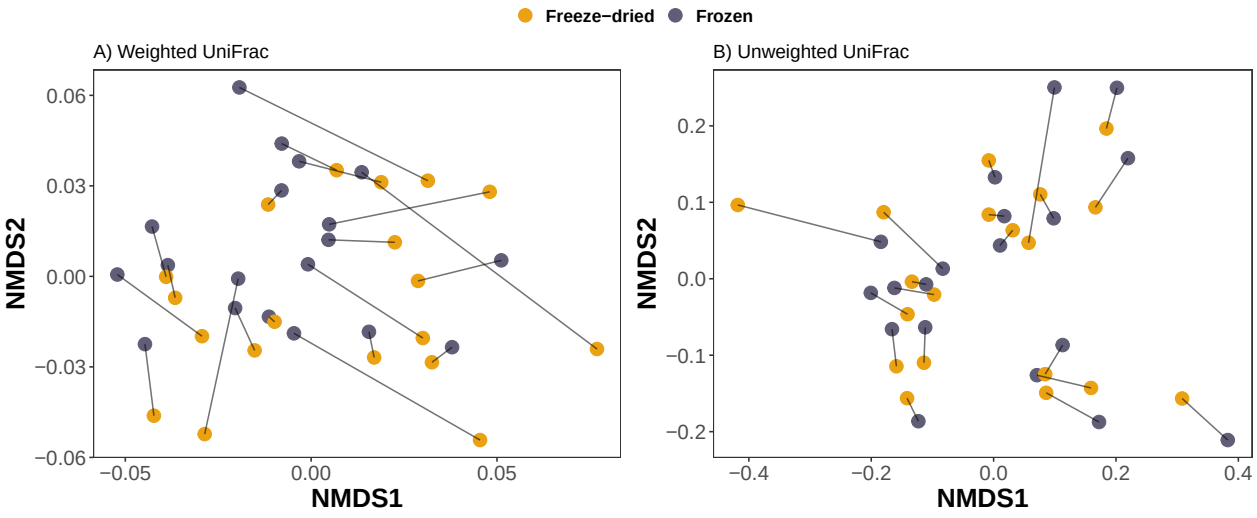


Figure 2: **NMDS weighted & unweigted unifrac un-rarefied data and sample 4 excluded. Point colour represent the sample treatment and line beteween replicates.**

PERMANOVAS

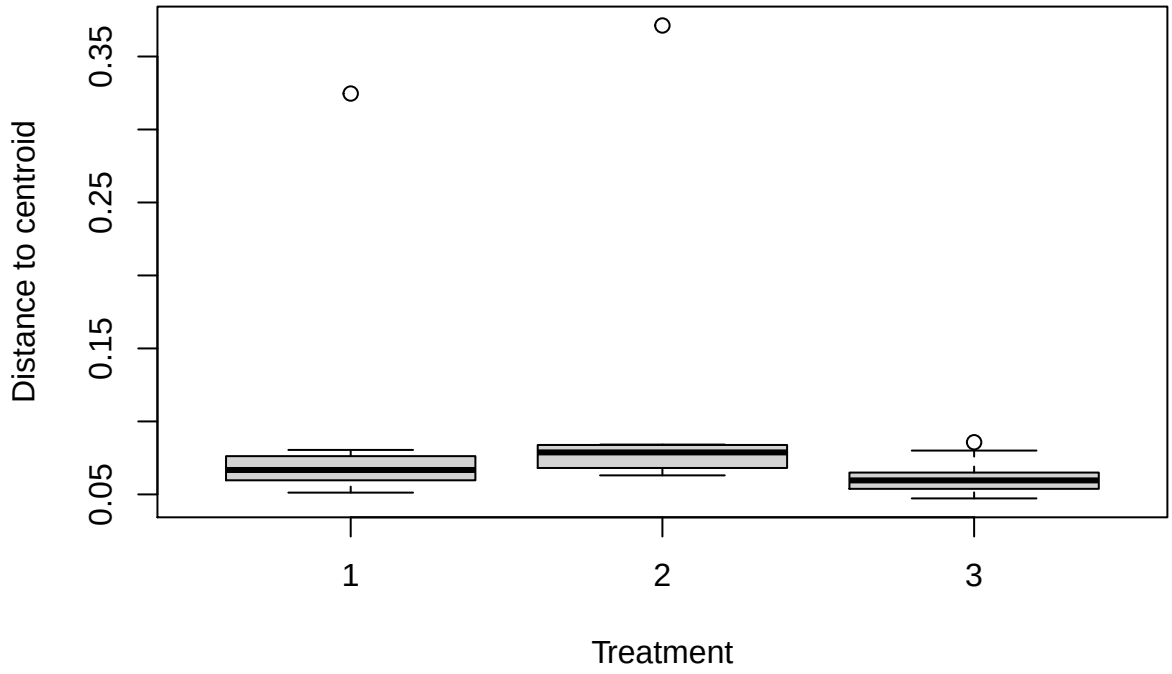
test permanova on un-rarefied and all samples (including sample 4), first only to test Plate Number and second to nest within plate number and test SampleNumber vs treatment:

On weighted unifrac distance

```
# test weighted
perm <- how(nperm = 9999)
permanova <- adonis2(wuni_dist ~ Plate_No, data = as(sample_data(pseq), "data.frame"), permutations = pe
print(as.data.frame(permanova)) # Plate No NS
```

```
##          Df   SumOfSqs         R2        F Pr(>F)
## Plate_No  2 0.03153913 0.07127529 1.419789 0.1157
## Residual 37 0.41095825 0.92872471       NA     NA
## Total    39 0.44249738 1.00000000       NA     NA
```

```
betadisp <- betadisper(wuni_dist, as(sample_data(pseq), "data.frame")$Plate_No, type = "centroid", bias
"boxplot"(betadisp, ylab = "Distance to centroid", xlab = "Treatment") # Doesn't look OK...
```




```
anova(betadisp)
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value Pr(>F)
## Groups     2 0.014896 0.0074482  1.9428 0.1576
## Residuals 37 0.141851 0.0038338
```

```
setBlocks(perm) <- with(pseq@sam_data, Plate_No)
permanova <- adonis2(wuni_dist ~ Treatment + SampleNumber,data = as(sample_data(pseq), "data.frame"), pe
print(as.data.frame(permanova)) # Treatment significant but just small proportion
```

```
##              Df    SumOfSqs         R2         F Pr(>F)
## Treatment     1 0.005529424 0.01249595  4.768283  1e-04
## SampleNumber 19 0.414935067 0.93771192 18.832531  1e-04
```

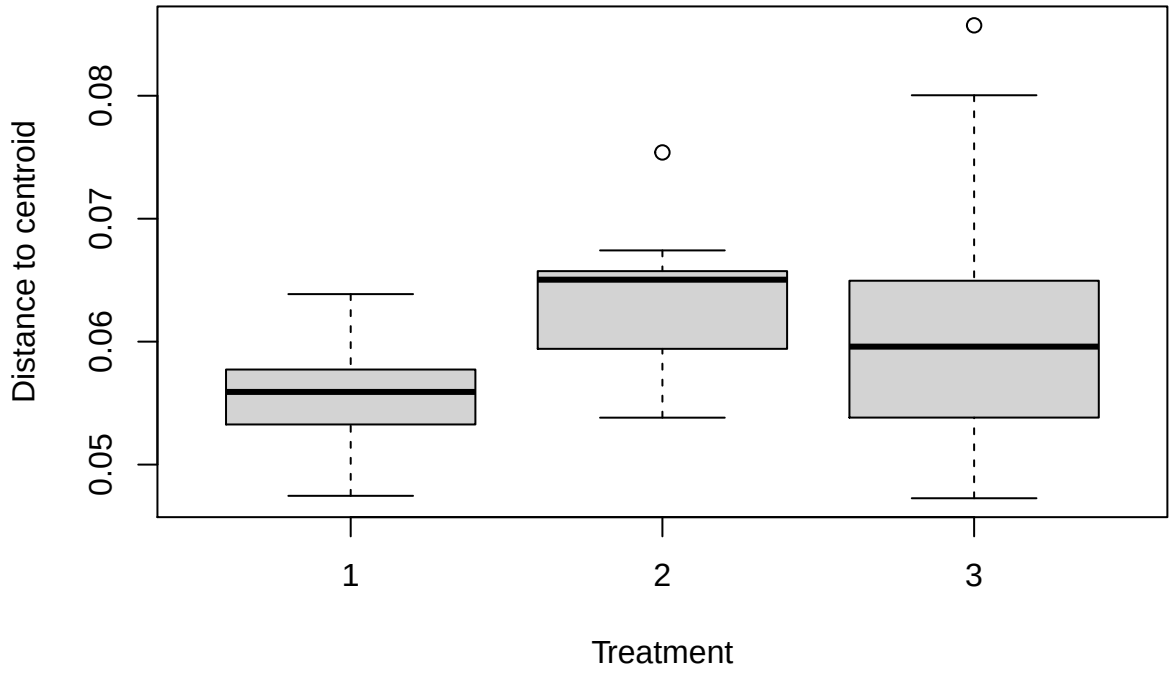
```
## Residual     19 0.022032889 0.04979213        NA     NA
## Total        39 0.442497381 1.00000000        NA     NA
```

On weighted unifrac distance excluding sample 4

```
# test weighted
perm <- how(nperm = 9999)
permanova <- adonis2(wuni_dist2 ~ Plate_No, data = as(sample_data(subset_samples(pseq, SampleNumber !=
print(as.data.frame(permanova)) # Plate No NS
```

```
##          Df   SumOfSqs         R2        F Pr(>F)
## Plate_No  2 0.01247426 0.08141663 1.551074 0.0413
## Residual 35 0.14074090 0.91858337       NA     NA
## Total    37 0.15321517 1.00000000       NA     NA
```

```
betadisp <- betadisper(wuni_dist2, as(sample_data(subset_samples(pseq, SampleNumber != "4")), "data.fra
                                      type = "centroid", bias.adjust = FALSE, sqrt.dist = FALSE, add = F
"boxplot"(betadisp, ylab = "Distance to centroid", xlab = "Treatment") # Doesn't look OK...
```




```
anova(betadisp)
```

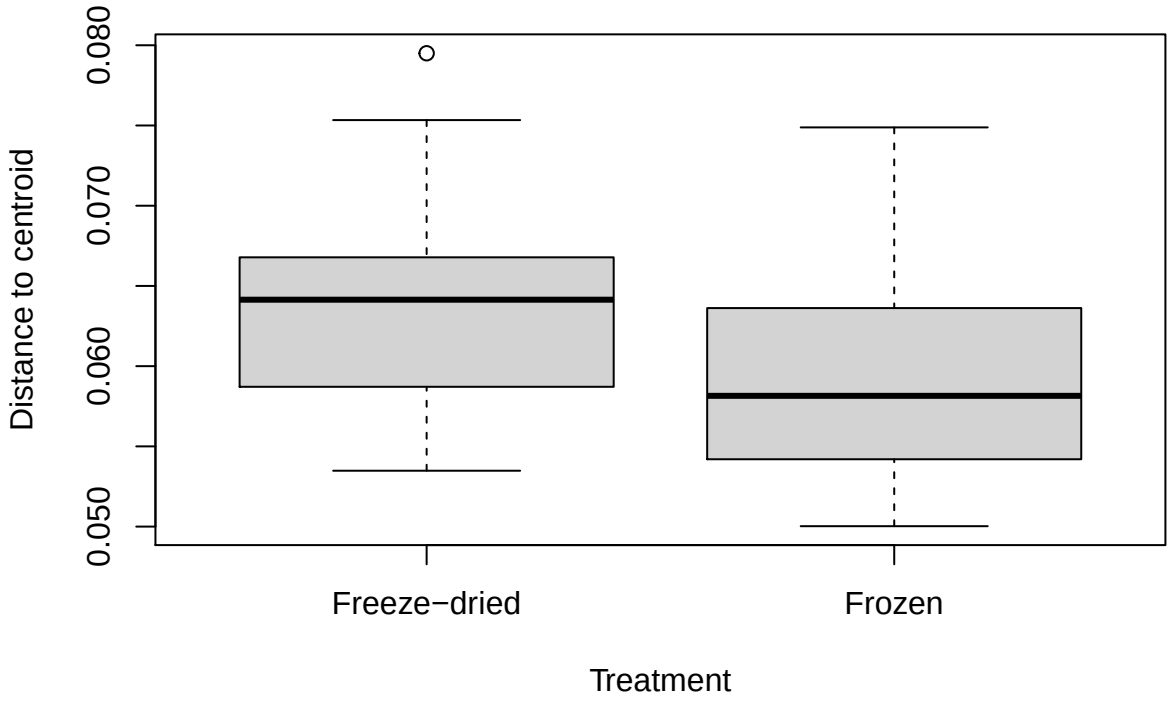
```
## Analysis of Variance Table
##
## Response: Distances
##           Df    Sum Sq    Mean Sq F value  Pr(>F)
## Groups     2 0.0003075 1.5375e-04  2.5422 0.09314 .
## Residuals 35 0.0021168 6.0478e-05
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#no strata argument
permanova <- adonis2(wuni_dist2 ~ Treatment + SampleNumber,data = as(sample_data(subset_samples(pseq, Sa
print(as.data.frame(permanova)) # Treatment significant but just small proportion
```

```
##              Df    SumOfSqs         R2        F Pr(>F)
## Treatment     1 0.005369491 0.03504543 4.750954  1e-04
```

```
## SampleNumber 18 0.127502214 0.83217750 6.267479  1e-04
## Residual     18 0.020343460 0.13277707       NA     NA
## Total        37 0.153215165 1.00000000       NA     NA
```

```
betadisp <- betadisper(wuni_dist2, as(sample_data(subset_samples(pseq, SampleNumber != "4")), "data.fra
                                      type = "centroid", bias.adjust = FALSE, sqrt.dist = FALSE, add = F
"boxplot"(betadisp, ylab = "Distance to centroid", xlab = "Treatment") # Doesn't look OK...
```




```
anova(betadisp)
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df     Sum Sq    Mean Sq F value  Pr(>F)
## Groups     1 0.00018997 1.8997e-04  3.3891 0.07388 .
## Residuals 36 0.00201790 5.6053e-05
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#plate number as strata
setBlocks(perm) <- with(as.tibble(pseq@sam_data) %>% filter( SampleNumber != "4"), Plate_No)
permanova <- adonis2(wuni_dist2 ~ Treatment + SampleNumber,data = as(sample_data(subset_samples(pseq, Sa
print(as.data.frame(permanova)) # Treatment significant but just small proportion
```

```
##              Df    SumOfSqs         R2        F Pr(>F)
## Treatment     1 0.005369491 0.03504543 4.750954  1e-04
## SampleNumber 18 0.127502214 0.83217750 6.267479  1e-04
## Residual     18 0.020343460 0.13277707       NA     NA
## Total        37 0.153215165 1.00000000       NA     NA
```

On un-weighted unifrac distance

```
# test unweighted
perm <- how(nperm = 9999)
```

```
permanova <- adonis2(uni_dist ~ Plate_No, data = as(sample_data(pseq), "data.frame"), permutations = pe
print(as.data.frame(permanova)) # Plate No almost significant
```

```
##           Df  SumOfSqs         R2        F Pr(>F)
## Plate_No   2  0.708301 0.06810129 1.351943 0.0211
## Residual  37  9.692398 0.93189871       NA     NA
## Total     39 10.400699 1.00000000       NA     NA
```

```
setBlocks(perm) <- with(pseq@sam_data, Plate_No)
permanova <- adonis2(uni_dist ~ Treatment + SampleNumber,data = as(sample_data(pseq), "data.frame"), pe
print(as.data.frame(permanova)) # Treatment significant but just small proportion
```

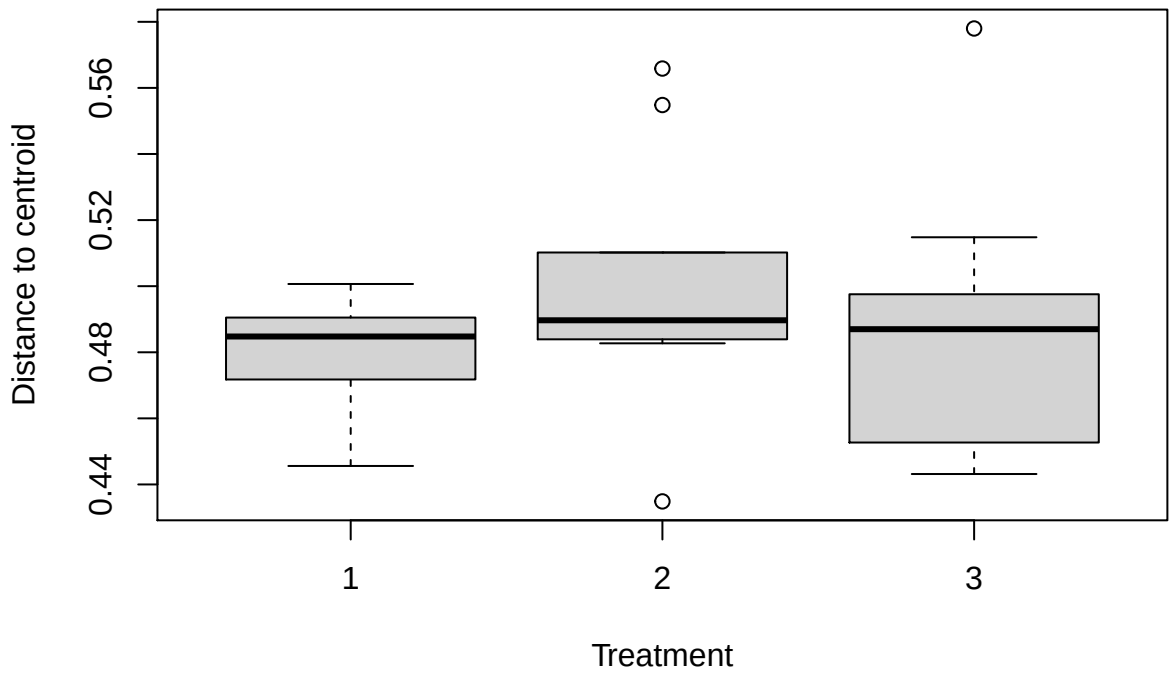
```
##               Df   SumOfSqs         R2        F Pr(>F)
## Treatment      1  0.1928182 0.01853897 1.398961 0.0414
## SampleNumber  19  7.5891188 0.72967393 2.897980 0.0001
## Residual      19  2.6187619 0.25178710       NA     NA
## Total         39 10.4006989 1.00000000       NA     NA
```

On un-weighted unifrac distance excluding sample 4

```
# test weighted
perm <- how(nperm = 9999)
permanova <- adonis2(uni_dist2 ~ Plate_No, data = as(sample_data(subset_samples(pseq, SampleNumber != "
print(as.data.frame(permanova)) # Plate significant
```

```
##           Df  SumOfSqs         R2        F Pr(>F)
## Plate_No   2 0.6702307 0.06922256 1.301487 0.0334
## Residual  35 9.0120278 0.93077744       NA     NA
## Total     37 9.6822585 1.00000000       NA     NA
```

```
betadisp <- betadisper(uni_dist2, as(sample_data(subset_samples(pseq, SampleNumber != "4")), "data.frame
                                     type = "centroid", bias.adjust = FALSE, sqrt.dist = FALSE, add = FA
"boxplot"(betadisp, ylab = "Distance to centroid", xlab = "Treatment") # Doesn't look OK...
```



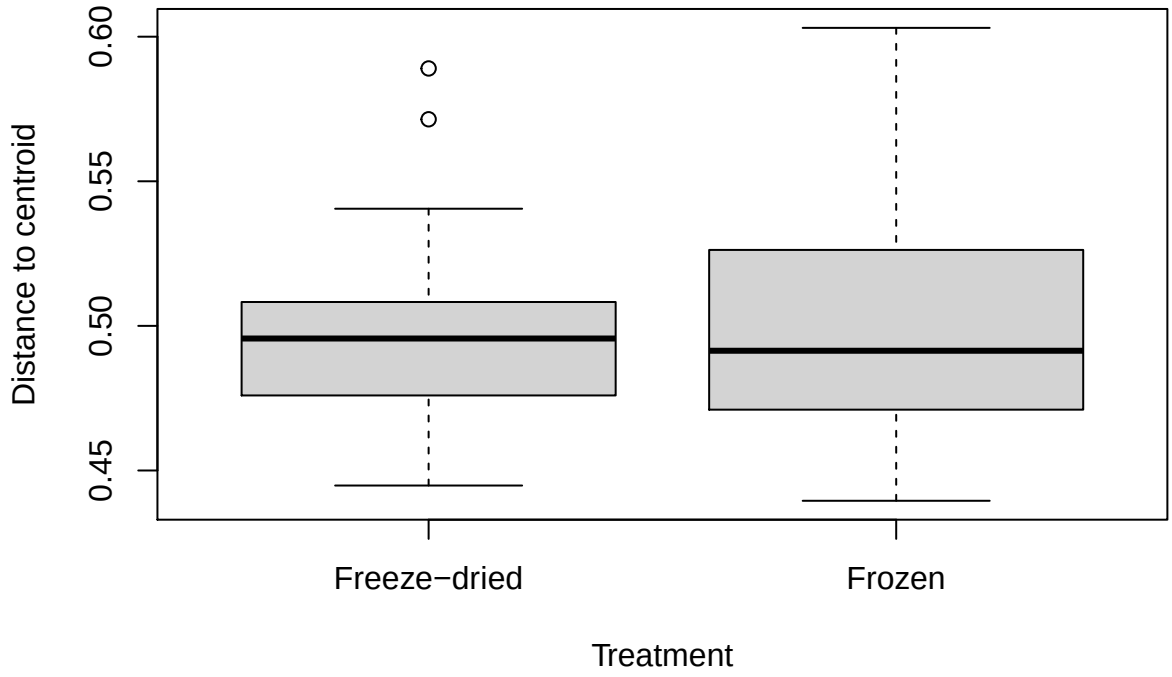
```
anova(betadisp)
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value Pr(>F)
## Groups     2 0.002266 0.0011332  1.1313 0.3341
## Residuals 35 0.035059 0.0010017
```

```
permanova <- adonis2(uni_dist2 ~ Treatment + SampleNumber,data = as(sample_data(subset_samples(pseq, Sa
print(as.data.frame(permanova)) # Treatment significant but just small proportion
```

```
##              Df SumOfSqs         R2        F Pr(>F)
## Treatment     1 0.185176 0.01912529 1.353340 0.0632
## SampleNumber 18 7.034162 0.72650017 2.856026 0.0001
## Residual     18 2.462920 0.25437454       NA     NA
## Total        37 9.682258 1.00000000       NA     NA
```

```
betadisp <- betadisper(uni_dist2, as(sample_data(subset_samples(pseq, SampleNumber != "4")), "data.frame
                                     type = "centroid", bias.adjust = FALSE, sqrt.dist = FALSE, add = FA
"boxplot"(betadisp, ylab = "Distance to centroid", xlab = "Treatment") # Doesn't look OK...
```




```
anova(betadisp)
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq    Mean Sq F value Pr(>F)
## Groups     1 0.000054 0.00005394  0.0357 0.8512
## Residuals 36 0.054387 0.00151075
```

```
# with plate number as strata argument
setBlocks(perm) <- with(as.tibble(pseq@sam_data) %>% filter( SampleNumber != "4"), Plate_No)
permanova <- adonis2(uni_dist2 ~ Treatment + SampleNumber,data = as(sample_data(subset_samples(pseq, Sa
```

```
print(as.data.frame(permanova)) # Treatment significant but just small proportion
```

```
##              Df SumOfSqs         R2        F Pr(>F)
## Treatment     1 0.185176 0.01912529 1.353340 0.0553
## SampleNumber 18 7.034162 0.72650017 2.856026 0.0001
## Residual     18 2.462920 0.25437454       NA     NA
## Total        37 9.682258 1.00000000       NA     NA
```