

Freeze-dried vs Frozen samples UniFrac beta diversity

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Introduction

I here calculate weighted and unweighted unifracs 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

```

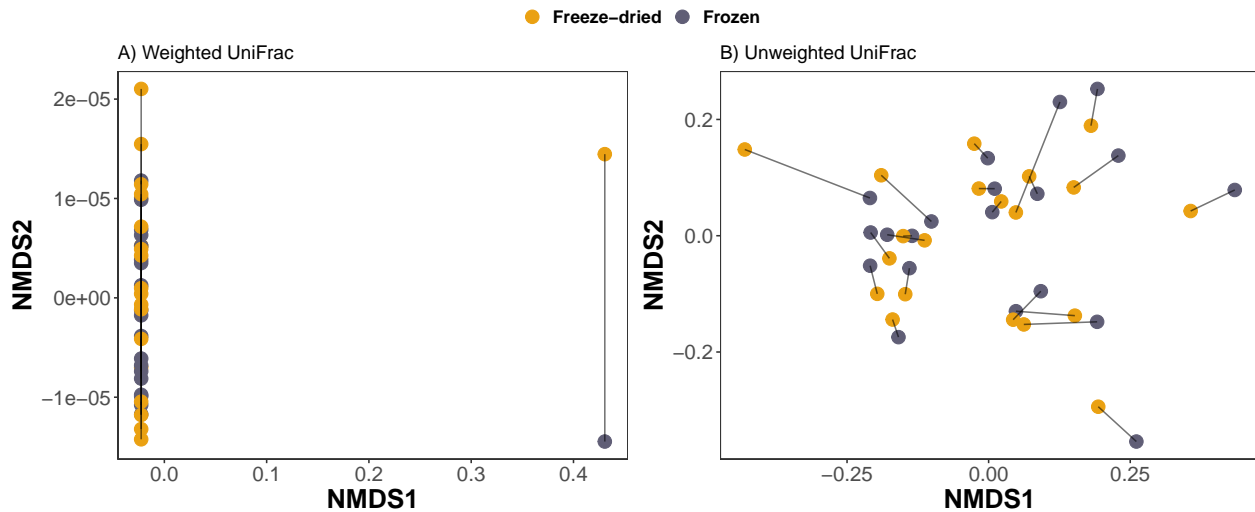


Figure 1: NMDS weighted & unweighted unifrac un-rarefied data. Point colour represent the sample treatment and line between replicates.

```

## 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

```

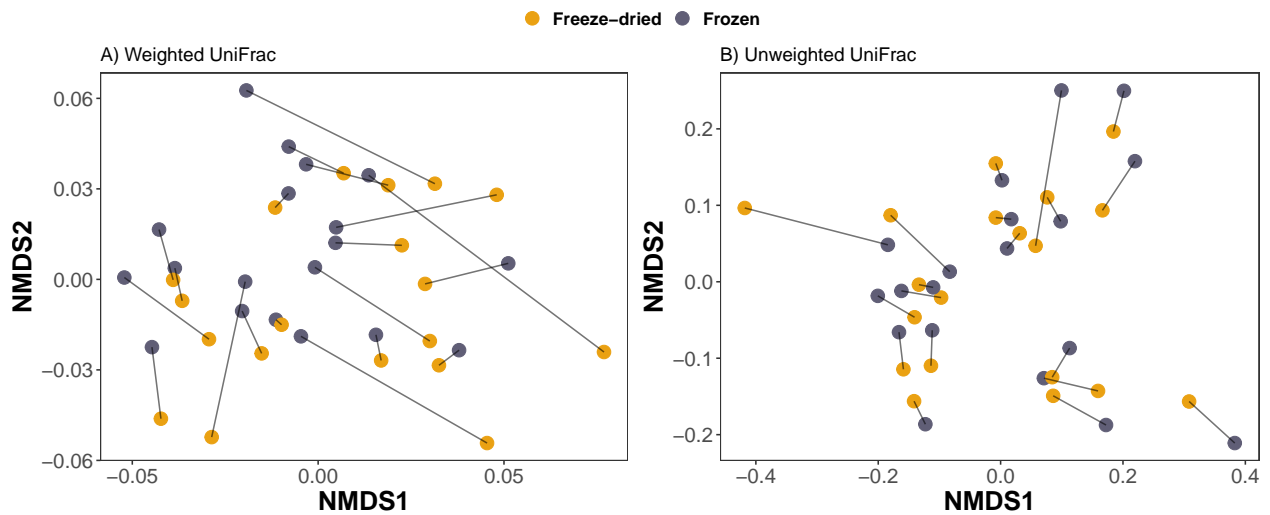


Figure 2: NMDS weighted & unweighted unifracs un-rarefied data and sample 4 excluded. Point colour represent the sample treatment and line between replicates.

PERMANOVAS

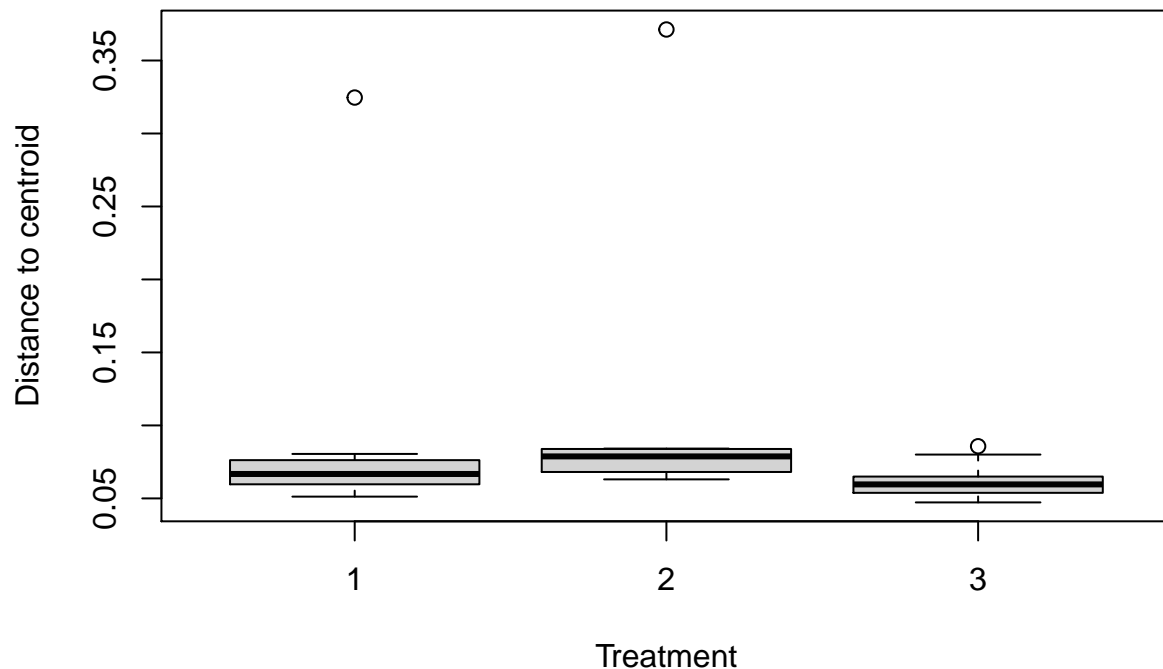
test permanova on un-rarefied and all samples (including sample 4), first only to test Plate Number and second to nest within plate number and test SampleNumber vs treatment:

On weighted unifrac distance

```
# test weighted
perm <- how(nperm = 9999)
permanova <- adonis2(wuni_dist ~ Plate_No, data = as(sample_data(pseq), "data.frame"), permutations = p
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"), p
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

On un-weighted unifrac distance

On un-weighted unifrac distance excluding sample 4