

Beta diversity: unconstrained ordination (NMDS)

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Introduction

Non-metric multidimensional scaling (NMDS) of weighted UniFrac distances based on relative abundance data at genus level. We used NMDS because semimetric distance matrices give negative eigenvalues and imaginary axes in MDS/PCoA, NMDS is more robust since it is rank-based. We used weighted UniFrac because this gave the best fit (Shepard stressplot, compared to Bray-Curtis and unweighted UniFrac).

Load packages

```
library(phyloseq)
library(microbiome)
library(microbiomeutilities)
library(vegan)
library(plyr)
library(ggplot2)
library(ggpubr)
library(viridis)
```

Input files

```
pstot <- readRDS("./phyobjects/ps1.work.rds")
print(pstot)
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 2222 taxa and 93 samples ]
## sample_data() Sample Data:      [ 93 samples by 16 sample variables ]
## tax_table()   Taxonomy Table:    [ 2222 taxa by 6 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 2222 tips and 2221 internal nodes ]
```

1. Prepare data

1.1. Input total

```
# genus level, relative abundance
pstot.g <- microbiome::aggregate_taxa(pstot, "Genus")
pstot.g.r <- microbiome::transform(pstot.g, "compositional")

# tax table with OTU column and best_hit
tot.tax <- as.data.frame(tax_table(pstot.g.r))
tot.tax$OTU <- rownames(tot.tax)
tax_table(pstot.g.r) <- tax_table(as.matrix(tot.tax))
pstot.g.bh <- format_to_besthit(pstot.g.r)
tot.tax.bh <- as.data.frame(tax_table(pstot.g.bh))
colnames(tot.tax.bh)[7] <- "OTU"
```

1.2. Subsets per diet

```
# chicken feed, day 0
CF0 <- subset_samples(pstot.g.r, Diet == "CF" & Timepoint == 0)
CF0 <- prune_taxa(taxa_sums(otu_table(CF0)) > 0, CF0)
wuf.cf0 <- distance(CF0, "wunifrac")
```

```

# chicken feed, day 15
CF <- subset_samples(pstot.g.r, Diet == "CF" & Timepoint == 15)
CF <- prune_taxa(taxa_sums(otu_table(CF)) > 0, CF)
wuf.cf <- distance(CF, "wunifrac")

# chicken manure, day 0
CM0 <- subset_samples(pstot.g.r, Diet == "CM" & Timepoint == 0)
CM0 <- prune_taxa(taxa_sums(otu_table(CM0)) > 0, CM0)
wuf.cm0 <- distance(CM0, "wunifrac")

# chicken manure, day 15
CM <- subset_samples(pstot.g.r, Diet == "CM" & Timepoint == 15)
CM <- prune_taxa(taxa_sums(otu_table(CM)) > 0, CM)
wuf.cm <- distance(CM, "wunifrac")

```

1.3. Plot presets

```

theme_nmDS <- theme_classic() +
  theme(panel.grid = element_blank(),
        panel.spacing = unit(.5, "lines"),
        panel.border = element_rect(color = "black", fill = NA, size = .5),
        strip.background = element_blank(),
        text = element_text(size = 25))

```

2. NMDS

2.1. Chicken feed, day 0

```

# ordination
set.seed(200)
nmDS.cf0 <- metaMDS(comm = wuf.cf0, autotransform = F, k = 2, try = 20, trymax = 200)

```

```

## Run 0 stress 0
## Run 1 stress 0
## ... Procrustes: rmse 0.228562 max resid 0.3604641
## Run 2 stress 8.74163e-05
## ... Procrustes: rmse 0.2395805 max resid 0.3438549
## Run 3 stress 0
## ... Procrustes: rmse 0.1964422 max resid 0.343503
## Run 4 stress 8.467509e-05
## ... Procrustes: rmse 0.2613045 max resid 0.3657644
## Run 5 stress 0
## ... Procrustes: rmse 0.2289098 max resid 0.3512099
## Run 6 stress 9.91371e-05
## ... Procrustes: rmse 0.1097549 max resid 0.1842366
## Run 7 stress 6.725892e-05
## ... Procrustes: rmse 0.2662093 max resid 0.3571294

```

```

## Run 8 stress 0
## ... Procrustes: rmse 0.153927 max resid 0.2913723
## Run 9 stress 6.593682e-05
## ... Procrustes: rmse 0.2221177 max resid 0.3485877
## Run 10 stress 0
## ... Procrustes: rmse 0.04405324 max resid 0.05478391
## Run 11 stress 8.856886e-05
## ... Procrustes: rmse 0.233199 max resid 0.3275207
## Run 12 stress 0
## ... Procrustes: rmse 0.05557775 max resid 0.1040111
## Run 13 stress 0.2597189
## Run 14 stress 0
## ... Procrustes: rmse 0.1899572 max resid 0.3106847
## Run 15 stress 8.927172e-05
## ... Procrustes: rmse 0.1734481 max resid 0.2715742
## Run 16 stress 0
## ... Procrustes: rmse 0.2196399 max resid 0.3534134
## Run 17 stress 0
## ... Procrustes: rmse 0.2259144 max resid 0.3550062
## Run 18 stress 9.668465e-05
## ... Procrustes: rmse 0.01724377 max resid 0.02523754
## Run 19 stress 9.836048e-05
## ... Procrustes: rmse 0.2129762 max resid 0.3365775
## Run 20 stress 0
## ... Procrustes: rmse 0.1830041 max resid 0.3421766
## Run 21 stress 7.196122e-05
## ... Procrustes: rmse 0.1841279 max resid 0.3039579
## Run 22 stress 0.0002731136
## ... Procrustes: rmse 0.1773497 max resid 0.291633
## Run 23 stress 0
## ... Procrustes: rmse 0.1424323 max resid 0.2674175
## Run 24 stress 0
## ... Procrustes: rmse 0.2270665 max resid 0.3460085
## Run 25 stress 0
## ... Procrustes: rmse 0.2470076 max resid 0.3776333
## Run 26 stress 0
## ... Procrustes: rmse 0.01099215 max resid 0.02028587
## Run 27 stress 6.163346e-06
## ... Procrustes: rmse 0.1946193 max resid 0.3087366
## Run 28 stress 9.762747e-05
## ... Procrustes: rmse 0.2223207 max resid 0.3808739
## Run 29 stress 0
## ... Procrustes: rmse 0.2303563 max resid 0.3790022
## Run 30 stress 9.694898e-05
## ... Procrustes: rmse 0.2399623 max resid 0.4222304
## Run 31 stress 0
## ... Procrustes: rmse 0.2522573 max resid 0.3654963
## Run 32 stress 9.902424e-05
## ... Procrustes: rmse 0.1837417 max resid 0.3580471
## Run 33 stress 0
## ... Procrustes: rmse 0.2388171 max resid 0.3616596
## Run 34 stress 8.639461e-05
## ... Procrustes: rmse 0.03983119 max resid 0.07060907
## Run 35 stress 0

```

```

## ... Procrustes: rmse 0.2161221 max resid 0.346367
## Run 36 stress 0
## ... Procrustes: rmse 0.2202842 max resid 0.3543041
## Run 37 stress 0
## ... Procrustes: rmse 0.04803283 max resid 0.08605552
## Run 38 stress 2.418386e-05
## ... Procrustes: rmse 0.2067428 max resid 0.3523926
## Run 39 stress 0
## ... Procrustes: rmse 0.2397843 max resid 0.375024
## Run 40 stress 0
## ... Procrustes: rmse 0.1219408 max resid 0.2322868
## Run 41 stress 5.616633e-05
## ... Procrustes: rmse 0.1695097 max resid 0.2746496
## Run 42 stress 0
## ... Procrustes: rmse 0.08642681 max resid 0.1649992
## Run 43 stress 0
## ... Procrustes: rmse 0.1259268 max resid 0.20667
## Run 44 stress 3.555702e-05
## ... Procrustes: rmse 0.2460747 max resid 0.3721048
## Run 45 stress 2.759495e-05
## ... Procrustes: rmse 0.06718156 max resid 0.1245618
## Run 46 stress 9.670363e-05
## ... Procrustes: rmse 0.229789 max resid 0.3384224
## Run 47 stress 0
## ... Procrustes: rmse 0.1250092 max resid 0.2307544
## Run 48 stress 0
## ... Procrustes: rmse 0.06982334 max resid 0.1238624
## Run 49 stress 4.632543e-05
## ... Procrustes: rmse 0.2597406 max resid 0.3613647
## Run 50 stress 0.000199807
## ... Procrustes: rmse 0.2342798 max resid 0.3661237
## Run 51 stress 9.819462e-05
## ... Procrustes: rmse 0.2456678 max resid 0.4007097
## Run 52 stress 0
## ... Procrustes: rmse 0.2219696 max resid 0.3457354
## Run 53 stress 0
## ... Procrustes: rmse 0.1190007 max resid 0.2124133
## Run 54 stress 9.114963e-05
## ... Procrustes: rmse 0.2109131 max resid 0.3509642
## Run 55 stress 9.021569e-05
## ... Procrustes: rmse 0.04146339 max resid 0.04895566
## Run 56 stress 9.63785e-05
## ... Procrustes: rmse 0.1900758 max resid 0.3286408
## Run 57 stress 5.647813e-05
## ... Procrustes: rmse 0.2434664 max resid 0.3384288
## Run 58 stress 0
## ... Procrustes: rmse 0.162051 max resid 0.2904102
## Run 59 stress 0
## ... Procrustes: rmse 0.1118455 max resid 0.1846526
## Run 60 stress 0
## ... Procrustes: rmse 0.1959049 max resid 0.327194
## Run 61 stress 0
## ... Procrustes: rmse 0.06271467 max resid 0.10392
## Run 62 stress 0

```

```

## ... Procrustes: rmse 0.1556135  max resid 0.2736861
## Run 63 stress 8.73898e-05
## ... Procrustes: rmse 0.2245865  max resid 0.3352093
## Run 64 stress 9.66444e-05
## ... Procrustes: rmse 0.1958781  max resid 0.3638581
## Run 65 stress 9.975116e-05
## ... Procrustes: rmse 0.1891181  max resid 0.2835842
## Run 66 stress 0
## ... Procrustes: rmse 0.04887354  max resid 0.09194969
## Run 67 stress 9.845822e-05
## ... Procrustes: rmse 0.2021636  max resid 0.313725
## Run 68 stress 4.444121e-05
## ... Procrustes: rmse 0.0989943  max resid 0.1832611
## Run 69 stress 8.181588e-05
## ... Procrustes: rmse 0.01191428  max resid 0.01715698
## Run 70 stress 4.313395e-05
## ... Procrustes: rmse 0.1769997  max resid 0.3115096
## Run 71 stress 0
## ... Procrustes: rmse 0.2129501  max resid 0.3295142
## Run 72 stress 0
## ... Procrustes: rmse 0.1895534  max resid 0.3258
## Run 73 stress 0
## ... Procrustes: rmse 0.1669863  max resid 0.2557453
## Run 74 stress 0
## ... Procrustes: rmse 0.1998433  max resid 0.3445825
## Run 75 stress 3.798273e-05
## ... Procrustes: rmse 0.1872212  max resid 0.2941365
## Run 76 stress 0
## ... Procrustes: rmse 0.05713263  max resid 0.1011321
## Run 77 stress 0
## ... Procrustes: rmse 0.2339642  max resid 0.3624277
## Run 78 stress 9.308565e-05
## ... Procrustes: rmse 0.200305  max resid 0.3332306
## Run 79 stress 9.696363e-05
## ... Procrustes: rmse 0.08124465  max resid 0.1341824
## Run 80 stress 0
## ... Procrustes: rmse 0.2662001  max resid 0.4271577
## Run 81 stress 0
## ... Procrustes: rmse 0.1610487  max resid 0.2949789
## Run 82 stress 8.649814e-05
## ... Procrustes: rmse 0.1973695  max resid 0.3432388
## Run 83 stress 9.920175e-05
## ... Procrustes: rmse 0.2395287  max resid 0.384674
## Run 84 stress 0
## ... Procrustes: rmse 0.172373  max resid 0.3246368
## Run 85 stress 0
## ... Procrustes: rmse 0.2135064  max resid 0.3577576
## Run 86 stress 0
## ... Procrustes: rmse 0.1978902  max resid 0.3493126
## Run 87 stress 3.127053e-05
## ... Procrustes: rmse 0.03220457  max resid 0.0518989
## Run 88 stress 9.730964e-05
## ... Procrustes: rmse 0.2146633  max resid 0.3561649
## Run 89 stress 0

```

```

## ... Procrustes: rmse 0.2527524 max resid 0.361534
## Run 90 stress 9.426645e-05
## ... Procrustes: rmse 0.2090876 max resid 0.3808565
## Run 91 stress 9.559176e-05
## ... Procrustes: rmse 0.1382658 max resid 0.2278675
## Run 92 stress 0
## ... Procrustes: rmse 0.1541029 max resid 0.2695283
## Run 93 stress 0
## ... Procrustes: rmse 0.1863178 max resid 0.3078146
## Run 94 stress 0
## ... Procrustes: rmse 0.1764898 max resid 0.2783756
## Run 95 stress 0
## ... Procrustes: rmse 0.1667213 max resid 0.2600174
## Run 96 stress 0
## ... Procrustes: rmse 0.101403 max resid 0.1729013
## Run 97 stress 0
## ... Procrustes: rmse 0.1935244 max resid 0.3307905
## Run 98 stress 0
## ... Procrustes: rmse 0.2453192 max resid 0.3720356
## Run 99 stress 0
## ... Procrustes: rmse 0.2360854 max resid 0.3463608
## Run 100 stress 7.800361e-05
## ... Procrustes: rmse 0.2310609 max resid 0.4063056
## Run 101 stress 0
## ... Procrustes: rmse 0.2301529 max resid 0.3723066
## Run 102 stress 0
## ... Procrustes: rmse 0.225801 max resid 0.3632086
## Run 103 stress 0
## ... Procrustes: rmse 0.2186088 max resid 0.3756255
## Run 104 stress 0
## ... Procrustes: rmse 0.1530707 max resid 0.2699976
## Run 105 stress 0
## ... Procrustes: rmse 0.1655924 max resid 0.2930361
## Run 106 stress 0
## ... Procrustes: rmse 0.04500268 max resid 0.06392797
## Run 107 stress 9.780233e-05
## ... Procrustes: rmse 0.1278221 max resid 0.2141657
## Run 108 stress 9.551874e-05
## ... Procrustes: rmse 0.2557371 max resid 0.3498257
## Run 109 stress 0
## ... Procrustes: rmse 0.1126856 max resid 0.2071466
## Run 110 stress 0
## ... Procrustes: rmse 0.1780938 max resid 0.28404
## Run 111 stress 0
## ... Procrustes: rmse 0.23688 max resid 0.3820532
## Run 112 stress 0
## ... Procrustes: rmse 0.2069934 max resid 0.3564612
## Run 113 stress 0
## ... Procrustes: rmse 0.2399883 max resid 0.3728016
## Run 114 stress 7.753438e-05
## ... Procrustes: rmse 0.2033319 max resid 0.2991604
## Run 115 stress 0
## ... Procrustes: rmse 0.2005717 max resid 0.3749141
## Run 116 stress 2.946635e-05

```

```

## ... Procrustes: rmse 0.02917329  max resid 0.05265491
## Run 117 stress 1.206656e-10
## ... Procrustes: rmse 0.00883221  max resid 0.01322424
## Run 118 stress 0
## ... Procrustes: rmse 0.2228591  max resid 0.3702225
## Run 119 stress 0
## ... Procrustes: rmse 0.2010341  max resid 0.339454
## Run 120 stress 0
## ... Procrustes: rmse 0.248392  max resid 0.3934128
## Run 121 stress 0.0002570731
## ... Procrustes: rmse 0.2498979  max resid 0.3830421
## Run 122 stress 0
## ... Procrustes: rmse 0.128946  max resid 0.2446446
## Run 123 stress 0
## ... Procrustes: rmse 0.2392694  max resid 0.3821472
## Run 124 stress 9.792191e-05
## ... Procrustes: rmse 0.1877481  max resid 0.2898109
## Run 125 stress 0
## ... Procrustes: rmse 0.2039278  max resid 0.3349234
## Run 126 stress 0
## ... Procrustes: rmse 0.02546071  max resid 0.04419093
## Run 127 stress 0
## ... Procrustes: rmse 0.1713478  max resid 0.2812879
## Run 128 stress 0
## ... Procrustes: rmse 0.2285814  max resid 0.3606162
## Run 129 stress 8.677745e-05
## ... Procrustes: rmse 0.2476092  max resid 0.3895701
## Run 130 stress 0
## ... Procrustes: rmse 0.2386508  max resid 0.369306
## Run 131 stress 9.339251e-05
## ... Procrustes: rmse 0.2167698  max resid 0.3941571
## Run 132 stress 0
## ... Procrustes: rmse 0.02496573  max resid 0.04649564
## Run 133 stress 0
## ... Procrustes: rmse 0.1453381  max resid 0.2493844
## Run 134 stress 8.99929e-05
## ... Procrustes: rmse 0.1907984  max resid 0.3739615
## Run 135 stress 9.89213e-05
## ... Procrustes: rmse 0.1233667  max resid 0.2106497
## Run 136 stress 0
## ... Procrustes: rmse 0.1575748  max resid 0.2585705
## Run 137 stress 9.369014e-05
## ... Procrustes: rmse 0.2805556  max resid 0.3698445
## Run 138 stress 0
## ... Procrustes: rmse 0.01175307  max resid 0.01637863
## Run 139 stress 0
## ... Procrustes: rmse 0.2456515  max resid 0.3577918
## Run 140 stress 0.0003406598
## ... Procrustes: rmse 0.2355424  max resid 0.3717813
## Run 141 stress 0
## ... Procrustes: rmse 0.1695195  max resid 0.3169812
## Run 142 stress 6.935432e-05
## ... Procrustes: rmse 0.1964762  max resid 0.3241416
## Run 143 stress 0

```



```

## ... Procrustes: rmse 0.08322238 max resid 0.1391509
## Run 144 stress 9.833596e-05
## ... Procrustes: rmse 0.2464106 max resid 0.332386
## Run 145 stress 8.303863e-05
## ... Procrustes: rmse 0.2451619 max resid 0.3521217
## Run 146 stress 0
## ... Procrustes: rmse 0.05592709 max resid 0.1048324
## Run 147 stress 9.433263e-05
## ... Procrustes: rmse 0.2423714 max resid 0.3445274
## Run 148 stress 0
## ... Procrustes: rmse 0.181644 max resid 0.297759
## Run 149 stress 9.992721e-05
## ... Procrustes: rmse 0.2436419 max resid 0.3703442
## Run 150 stress 9.969618e-05
## ... Procrustes: rmse 0.1856587 max resid 0.2968689
## Run 151 stress 0.0001682157
## ... Procrustes: rmse 0.1844863 max resid 0.3106872
## Run 152 stress 0
## ... Procrustes: rmse 0.1831929 max resid 0.326561
## Run 153 stress 9.936275e-05
## ... Procrustes: rmse 0.1833994 max resid 0.3282392
## Run 154 stress 0
## ... Procrustes: rmse 0.2332371 max resid 0.3829285
## Run 155 stress 0
## ... Procrustes: rmse 0.2554911 max resid 0.4035206
## Run 156 stress 2.533488e-06
## ... Procrustes: rmse 0.03076423 max resid 0.05668731
## Run 157 stress 0
## ... Procrustes: rmse 0.03920969 max resid 0.06036805
## Run 158 stress 0
## ... Procrustes: rmse 0.1067841 max resid 0.2033349
## Run 159 stress 9.795989e-05
## ... Procrustes: rmse 0.2337358 max resid 0.3168466
## Run 160 stress 0
## ... Procrustes: rmse 0.1263252 max resid 0.2147422
## Run 161 stress 0
## ... Procrustes: rmse 0.259484 max resid 0.3808204
## Run 162 stress 0
## ... Procrustes: rmse 0.2401067 max resid 0.3579949
## Run 163 stress 0
## ... Procrustes: rmse 0.2119571 max resid 0.3337576
## Run 164 stress 0
## ... Procrustes: rmse 0.1626224 max resid 0.2663022
## Run 165 stress 0
## ... Procrustes: rmse 0.1757776 max resid 0.2960212
## Run 166 stress 9.94459e-05
## ... Procrustes: rmse 0.1455819 max resid 0.2487673
## Run 167 stress 9.159902e-05
## ... Procrustes: rmse 0.2848241 max resid 0.3839814
## Run 168 stress 4.20967e-05
## ... Procrustes: rmse 0.2259784 max resid 0.3692864
## Run 169 stress 2.102984e-05
## ... Procrustes: rmse 0.219622 max resid 0.3903331
## Run 170 stress 0

```

```

## ... Procrustes: rmse 0.09834145  max resid 0.1715306
## Run 171 stress 6.43133e-05
## ... Procrustes: rmse 0.2621705  max resid 0.4178327
## Run 172 stress 0.2597188
## Run 173 stress 0
## ... Procrustes: rmse 0.2059358  max resid 0.3343037
## Run 174 stress 0
## ... Procrustes: rmse 0.258455  max resid 0.4007597
## Run 175 stress 0
## ... Procrustes: rmse 0.1777428  max resid 0.3125838
## Run 176 stress 8.771136e-05
## ... Procrustes: rmse 0.2871603  max resid 0.3891855
## Run 177 stress 0
## ... Procrustes: rmse 0.219739  max resid 0.3474274
## Run 178 stress 9.470911e-05
## ... Procrustes: rmse 0.2418164  max resid 0.3670042
## Run 179 stress 0
## ... Procrustes: rmse 0.1624096  max resid 0.2701915
## Run 180 stress 0
## ... Procrustes: rmse 0.1719223  max resid 0.2978086
## Run 181 stress 8.682707e-05
## ... Procrustes: rmse 0.2603989  max resid 0.4154787
## Run 182 stress 9.98135e-05
## ... Procrustes: rmse 0.2105121  max resid 0.3668693
## Run 183 stress 5.755859e-05
## ... Procrustes: rmse 0.2463816  max resid 0.3289625
## Run 184 stress 0
## ... Procrustes: rmse 0.1425236  max resid 0.2454216
## Run 185 stress 3.12141e-06
## ... Procrustes: rmse 0.0156084  max resid 0.0282102
## Run 186 stress 3.339974e-05
## ... Procrustes: rmse 0.2163464  max resid 0.3848432
## Run 187 stress 1.795015e-06
## ... Procrustes: rmse 0.05941985  max resid 0.1001215
## Run 188 stress 0
## ... Procrustes: rmse 0.2432568  max resid 0.3916338
## Run 189 stress 0
## ... Procrustes: rmse 0.2342729  max resid 0.3473706
## Run 190 stress 8.612658e-05
## ... Procrustes: rmse 0.1981463  max resid 0.3290125
## Run 191 stress 7.306131e-05
## ... Procrustes: rmse 0.2457737  max resid 0.3725648
## Run 192 stress 0
## ... Procrustes: rmse 0.2607352  max resid 0.3672686
## Run 193 stress 9.808423e-05
## ... Procrustes: rmse 0.2382727  max resid 0.381677
## Run 194 stress 5.277554e-05
## ... Procrustes: rmse 0.2372273  max resid 0.3596257
## Run 195 stress 0
## ... Procrustes: rmse 0.237818  max resid 0.3608334
## Run 196 stress 9.846104e-05
## ... Procrustes: rmse 0.170399  max resid 0.248768
## Run 197 stress 5.363205e-06
## ... Procrustes: rmse 0.1671834  max resid 0.3017068

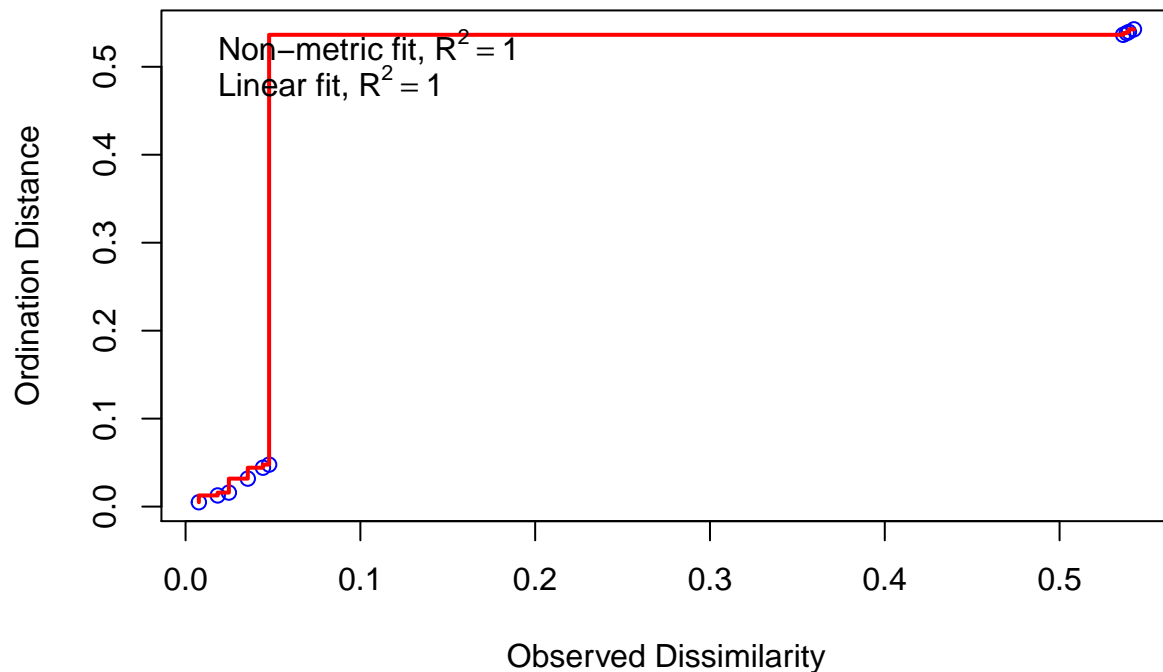
```

```
## Run 198 stress 0
## ... Procrustes: rmse 0.05839899  max resid 0.1005377
## Run 199 stress 0
## ... Procrustes: rmse 0.06729649  max resid 0.1275423
## Run 200 stress 0
## ... Procrustes: rmse 0.1924267  max resid 0.324689
## *** No convergence -- monoMDS stopping criteria:
##      5: no. of iterations >= maxit
##     193: stress < smin
##      2: stress ratio > sratmax
```

```
nm.ds.cf0 # no convergent solution, stress = 0. Likely because only 5 samples.
```

```
##
## Call:
## metaMDS(comm = wuf.cf0, k = 2, try = 20, trymax = 200, autotransform = F)
##
## global Multidimensional Scaling using monoMDS
##
## Data:      wuf.cf0
## Distance: user supplied
##
## Dimensions: 2
## Stress:      0
## Stress type 1, weak ties
## No convergent solutions - best solution after 200 tries
## Scaling: centring, PC rotation
## Species: scores missing
```

```
# stress plot
stressplot(nm.ds.cf0) # fit OK, limited points.
```



2.2. Chicken feed, day 15

```
set.seed(200)
nmms.cf <- metaMDS(comm = wuf.cf, autotransform = F, k = 2, try = 100, trymax = 200)
```

```
## Run 0 stress 0.06326327
## Run 1 stress 0.06369218
## ... Procrustes: rmse 0.06580854 max resid 0.2106057
## Run 2 stress 0.3841094
## Run 3 stress 0.07209186
## Run 4 stress 0.06944783
## Run 5 stress 0.06326321
## ... New best solution
## ... Procrustes: rmse 0.0006901645 max resid 0.001533823
## ... Similar to previous best
## Run 6 stress 0.06347078
## ... Procrustes: rmse 0.009523649 max resid 0.03873413
## Run 7 stress 0.08404561
## Run 8 stress 0.06717137
## Run 9 stress 0.06368889
## ... Procrustes: rmse 0.06586403 max resid 0.2103063
## Run 10 stress 0.07487769
## Run 11 stress 0.06416525
```

```

## Run 12 stress 0.0849259
## Run 13 stress 0.06728866
## Run 14 stress 0.08209283
## Run 15 stress 0.06928529
## Run 16 stress 0.06382571
## Run 17 stress 0.08488529
## Run 18 stress 0.06368992
## ... Procrustes: rmse 0.06586306  max resid 0.2103749
## Run 19 stress 0.06416337
## Run 20 stress 0.06347006
## ... Procrustes: rmse 0.009519948  max resid 0.03926308
## Run 21 stress 0.0747662
## Run 22 stress 0.0780191
## Run 23 stress 0.06368968
## ... Procrustes: rmse 0.06586386  max resid 0.2103613
## Run 24 stress 0.06416662
## Run 25 stress 0.06728908
## Run 26 stress 0.08455807
## Run 27 stress 0.06347138
## ... Procrustes: rmse 0.009536283  max resid 0.0386153
## Run 28 stress 0.06416279
## Run 29 stress 0.07431522
## Run 30 stress 0.06368699
## ... Procrustes: rmse 0.0658754  max resid 0.2093625
## Run 31 stress 0.07488354
## Run 32 stress 0.06326257
## ... New best solution
## ... Procrustes: rmse 0.0001282029  max resid 0.000267755
## ... Similar to previous best
## Run 33 stress 0.07801933
## Run 34 stress 0.06422889
## Run 35 stress 0.07488969
## Run 36 stress 0.06416305
## Run 37 stress 0.07655724
## Run 38 stress 0.06368991
## ... Procrustes: rmse 0.06586608  max resid 0.2103669
## Run 39 stress 0.0737894
## Run 40 stress 0.0692844
## Run 41 stress 0.06416691
## Run 42 stress 0.07348417
## Run 43 stress 0.07323953
## Run 44 stress 0.063689
## ... Procrustes: rmse 0.06585722  max resid 0.2103354
## Run 45 stress 0.06368771
## ... Procrustes: rmse 0.06585764  max resid 0.2102304
## Run 46 stress 0.07488603
## Run 47 stress 0.06416542
## Run 48 stress 0.06326368
## ... Procrustes: rmse 0.000662465  max resid 0.001508104
## ... Similar to previous best
## Run 49 stress 0.06416275
## Run 50 stress 0.07477298
## Run 51 stress 0.07348456
## Run 52 stress 0.06368845

```

```

## ... Procrustes: rmse 0.06585726  max resid 0.210293
## Run 53 stress 0.06928391
## Run 54 stress 0.06326281
## ... Procrustes: rmse 9.796441e-05  max resid 0.0002364107
## ... Similar to previous best
## Run 55 stress 0.08591553
## Run 56 stress 0.07559069
## Run 57 stress 0.06326283
## ... Procrustes: rmse 0.0004617133  max resid 0.001058894
## ... Similar to previous best
## Run 58 stress 0.0743136
## Run 59 stress 0.06717303
## Run 60 stress 0.07173862
## Run 61 stress 0.08591375
## Run 62 stress 0.07801408
## Run 63 stress 0.07654142
## Run 64 stress 0.07801489
## Run 65 stress 0.06326332
## ... Procrustes: rmse 0.0002194084  max resid 0.0005056514
## ... Similar to previous best
## Run 66 stress 0.07179312
## Run 67 stress 0.08208947
## Run 68 stress 0.3842588
## Run 69 stress 0.07654398
## Run 70 stress 0.06717223
## Run 71 stress 0.0720937
## Run 72 stress 0.06326289
## ... Procrustes: rmse 6.195343e-05  max resid 0.0001803232
## ... Similar to previous best
## Run 73 stress 0.07487485
## Run 74 stress 0.07801795
## Run 75 stress 0.06717228
## Run 76 stress 0.06368843
## ... Procrustes: rmse 0.06586513  max resid 0.209252
## Run 77 stress 0.06416689
## Run 78 stress 0.07550603
## Run 79 stress 0.06369078
## ... Procrustes: rmse 0.06585826  max resid 0.2104197
## Run 80 stress 0.07547977
## Run 81 stress 0.06369007
## ... Procrustes: rmse 0.06585784  max resid 0.2104094
## Run 82 stress 0.07173946
## Run 83 stress 0.06717129
## Run 84 stress 0.07476051
## Run 85 stress 0.06717375
## Run 86 stress 0.06326263
## ... Procrustes: rmse 3.38206e-05  max resid 7.57382e-05
## ... Similar to previous best
## Run 87 stress 0.08488214
## Run 88 stress 0.06421319
## Run 89 stress 0.07378906
## Run 90 stress 0.06416566
## Run 91 stress 0.07547701
## Run 92 stress 0.0765507

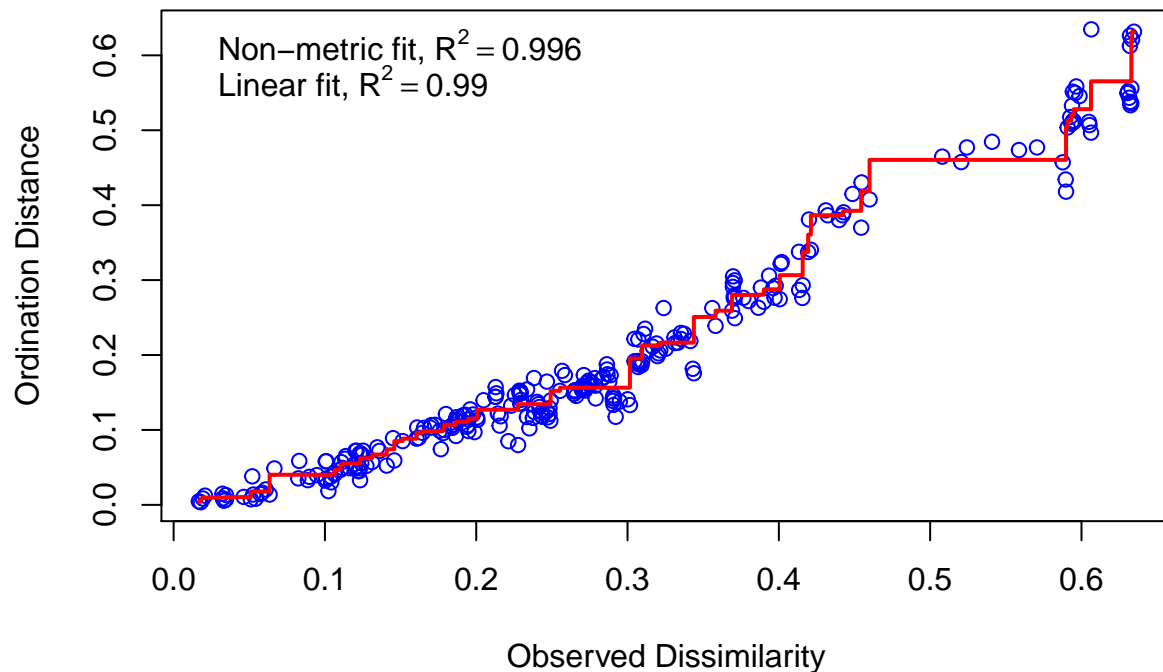
```

```
## Run 93 stress 0.06416817
## Run 94 stress 0.06928981
## Run 95 stress 0.06326259
## ... Procrustes: rmse 1.650661e-05  max resid 5.321113e-05
## ... Similar to previous best
## Run 96 stress 0.06929248
## Run 97 stress 0.06717089
## Run 98 stress 0.0692926
## Run 99 stress 0.06326298
## ... Procrustes: rmse 0.000148737  max resid 0.0003698982
## ... Similar to previous best
## Run 100 stress 0.0692893
## *** Solution reached
```

```
nmds.cf # convergent solution, stress = 0.063
```

```
##
## Call:
## metaMDS(comm = wuf.cf, k = 2, try = 100, trymax = 200, autotransform = F)
##
## global Multidimensional Scaling using monoMDS
##
## Data:      wuf.cf
## Distance: user supplied
##
## Dimensions: 2
## Stress:    0.06326257
## Stress type 1, weak ties
## Two convergent solutions found after 100 tries
## Scaling: centring, PC rotation
## Species: scores missing
```

```
# stress plot
stressplot(nmds.cf) # good fit.
```



2.3. Chicken manure, day 0

```
# in 2 dimensions
set.seed(200)
nmfs.cm0 <- metaMDS(comm = wuf.cm0, autotransform = F, k = 2, try = 100, trymax = 200)

## Run 0 stress 0.06590622
## Run 1 stress 0.06590563
## ... New best solution
## ... Procrustes: rmse 0.0006473271  max resid 0.001552661
## ... Similar to previous best
## Run 2 stress 0.06590599
## ... Procrustes: rmse 0.0006025085  max resid 0.001438833
## ... Similar to previous best
## Run 3 stress 0.1117637
## Run 4 stress 0.07841613
## Run 5 stress 0.07841607
## Run 6 stress 0.07841669
## Run 7 stress 0.0920718
## Run 8 stress 0.09206904
## Run 9 stress 0.1028092
## Run 10 stress 0.102502
## Run 11 stress 0.09434566
## Run 12 stress 0.09207459
```



```

## Run 13 stress 0.06590538
## ... New best solution
## ... Procrustes: rmse 0.0001639888 max resid 0.0003709482
## ... Similar to previous best
## Run 14 stress 0.06590577
## ... Procrustes: rmse 0.0003141768 max resid 0.0007542271
## ... Similar to previous best
## Run 15 stress 0.07841606
## Run 16 stress 0.07841626
## Run 17 stress 0.06590574
## ... Procrustes: rmse 0.0003500028 max resid 0.0008444831
## ... Similar to previous best
## Run 18 stress 0.06590534
## ... New best solution
## ... Procrustes: rmse 9.502679e-05 max resid 0.0002304173
## ... Similar to previous best
## Run 19 stress 0.09207313
## Run 20 stress 0.09207587
## Run 21 stress 0.06590534
## ... Procrustes: rmse 2.382065e-05 max resid 5.599338e-05
## ... Similar to previous best
## Run 22 stress 0.09207565
## Run 23 stress 0.07841606
## Run 24 stress 0.107002
## Run 25 stress 0.09434673
## Run 26 stress 0.0992663
## Run 27 stress 0.07841606
## Run 28 stress 0.09434685
## Run 29 stress 0.09434699
## Run 30 stress 0.0659054
## ... Procrustes: rmse 9.923141e-05 max resid 0.0002300977
## ... Similar to previous best
## Run 31 stress 0.1070018
## Run 32 stress 0.07841611
## Run 33 stress 0.06590534
## ... Procrustes: rmse 4.981417e-05 max resid 0.0001200093
## ... Similar to previous best
## Run 34 stress 0.06590541
## ... Procrustes: rmse 0.0001002292 max resid 0.0002374948
## ... Similar to previous best
## Run 35 stress 0.1028093
## Run 36 stress 0.09207228
## Run 37 stress 0.06590534
## ... Procrustes: rmse 9.041565e-06 max resid 1.737647e-05
## ... Similar to previous best
## Run 38 stress 0.09207145
## Run 39 stress 0.06590537
## ... Procrustes: rmse 3.822516e-05 max resid 8.748092e-05
## ... Similar to previous best
## Run 40 stress 0.07841606
## Run 41 stress 0.09207218
## Run 42 stress 0.07654648
## Run 43 stress 0.363261
## Run 44 stress 0.07654654

```

```

## Run 45 stress 0.06590539
## ... Procrustes: rmse 7.95197e-05  max resid 0.0001891233
## ... Similar to previous best
## Run 46 stress 0.1024128
## Run 47 stress 0.1024131
## Run 48 stress 0.06590534
## ... New best solution
## ... Procrustes: rmse 7.586069e-06  max resid 1.71556e-05
## ... Similar to previous best
## Run 49 stress 0.06590552
## ... Procrustes: rmse 0.0001845636  max resid 0.0004413046
## ... Similar to previous best
## Run 50 stress 0.09434781
## Run 51 stress 0.1028693
## Run 52 stress 0.09434569
## Run 53 stress 0.09434547
## Run 54 stress 0.07654648
## Run 55 stress 0.07841605
## Run 56 stress 0.09207196
## Run 57 stress 0.1050411
## Run 58 stress 0.06590542
## ... Procrustes: rmse 0.0001132609  max resid 0.0002674095
## ... Similar to previous best
## Run 59 stress 0.06590539
## ... Procrustes: rmse 9.248554e-05  max resid 0.0002181469
## ... Similar to previous best
## Run 60 stress 0.06590538
## ... Procrustes: rmse 8.37497e-05  max resid 0.0001975751
## ... Similar to previous best
## Run 61 stress 0.1028649
## Run 62 stress 0.06590539
## ... Procrustes: rmse 0.0001090542  max resid 0.0002550597
## ... Similar to previous best
## Run 63 stress 0.0784161
## Run 64 stress 0.09207349
## Run 65 stress 0.06590578
## ... Procrustes: rmse 0.0002907754  max resid 0.0006766269
## ... Similar to previous best
## Run 66 stress 0.06590546
## ... Procrustes: rmse 0.0001681182  max resid 0.0003908231
## ... Similar to previous best
## Run 67 stress 0.1024063
## Run 68 stress 0.06590593
## ... Procrustes: rmse 0.0002763168  max resid 0.0006635147
## ... Similar to previous best
## Run 69 stress 0.06590536
## ... Procrustes: rmse 6.449714e-05  max resid 0.0001531688
## ... Similar to previous best
## Run 70 stress 0.06590555
## ... Procrustes: rmse 0.0001187025  max resid 0.0002719258
## ... Similar to previous best
## Run 71 stress 0.09434584
## Run 72 stress 0.09207054
## Run 73 stress 0.07841627

```

```

## Run 74 stress 0.1025029
## Run 75 stress 0.09434599
## Run 76 stress 0.1117734
## Run 77 stress 0.07654647
## Run 78 stress 0.07841605
## Run 79 stress 0.09434714
## Run 80 stress 0.0943476
## Run 81 stress 0.06590661
## ... Procrustes: rmse 0.0005232419 max resid 0.00121716
## ... Similar to previous best
## Run 82 stress 0.1028652
## Run 83 stress 0.0784162
## Run 84 stress 0.09207161
## Run 85 stress 0.06590563
## ... Procrustes: rmse 0.0002344657 max resid 0.0005632292
## ... Similar to previous best
## Run 86 stress 0.07841629
## Run 87 stress 0.06590597
## ... Procrustes: rmse 0.0003721581 max resid 0.0008653802
## ... Similar to previous best
## Run 88 stress 0.1024115
## Run 89 stress 0.07654675
## Run 90 stress 0.1024042
## Run 91 stress 0.09207485
## Run 92 stress 0.1025018
## Run 93 stress 0.06590541
## ... Procrustes: rmse 0.0001138233 max resid 0.0002691852
## ... Similar to previous best
## Run 94 stress 0.1183697
## Run 95 stress 0.06590556
## ... Procrustes: rmse 0.000176323 max resid 0.0004108561
## ... Similar to previous best
## Run 96 stress 0.1028086
## Run 97 stress 0.06590535
## ... Procrustes: rmse 4.022094e-05 max resid 8.931572e-05
## ... Similar to previous best
## Run 98 stress 0.09207325
## Run 99 stress 0.1024048
## Run 100 stress 0.1183685
## *** Solution reached

```

```

nmds.cm0 # convergent solution, stress = 0.066

```

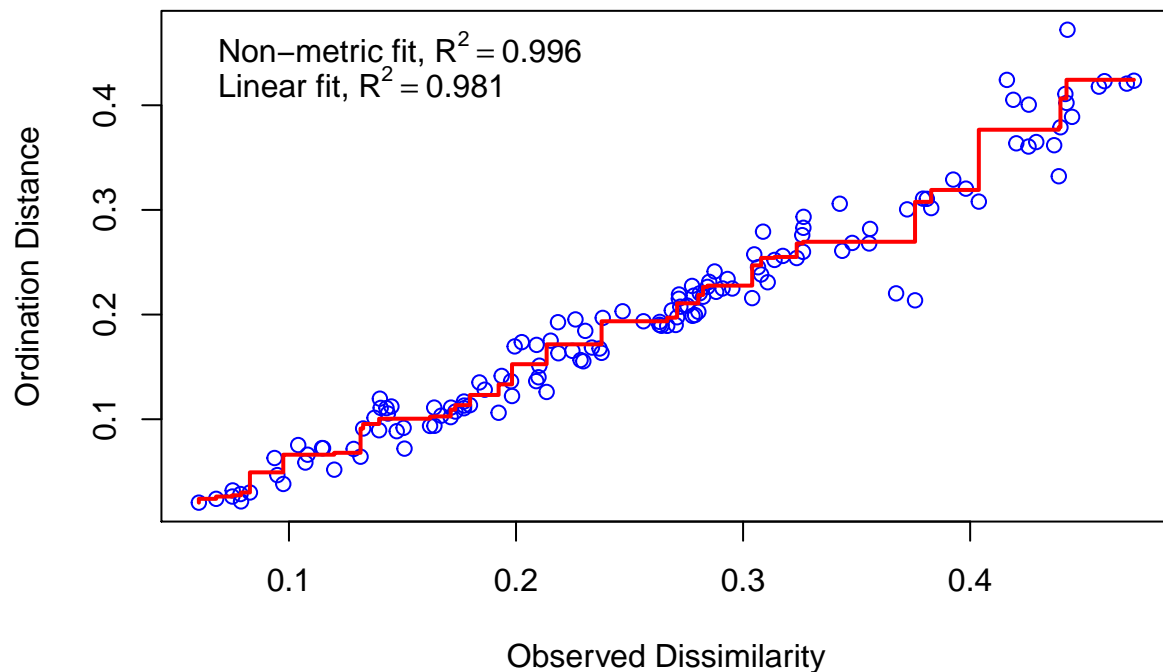
```

##
## Call:
## metaMDS(comm = wuf.cm0, k = 2, try = 100, trymax = 200, autotransform = F)
##
## global Multidimensional Scaling using monoMDS
##
## Data:      wuf.cm0
## Distance:  user supplied
##
## Dimensions: 2
## Stress:      0.06590534

```

```
## Stress type 1, weak ties
## Two convergent solutions found after 100 tries
## Scaling: centring, PC rotation
## Species: scores missing
```

```
# stress plot
stressplot(nmds.cm0) # good fit.
```



2.4. Chicken manure, day 15

```
# in 2 dimensions
set.seed(200)
nmds.cm <- metaMDS(comm = wuf.cm, autotransform = F, k = 2, try = 100, trymax = 200)
```

```
## Run 0 stress 0.05986316
## Run 1 stress 0.0805724
## Run 2 stress 0.08382086
## Run 3 stress 0.0639782
## Run 4 stress 0.06197006
## Run 5 stress 0.06193159
## Run 6 stress 0.06197012
## Run 7 stress 0.06193218
## Run 8 stress 0.06193179
```

```

## Run 9 stress 0.06697932
## Run 10 stress 0.06197012
## Run 11 stress 0.06193198
## Run 12 stress 0.06193195
## Run 13 stress 0.06397827
## Run 14 stress 0.06197013
## Run 15 stress 0.06759582
## Run 16 stress 0.08091707
## Run 17 stress 0.06397792
## Run 18 stress 0.06193303
## Run 19 stress 0.05986149
## ... New best solution
## ... Procrustes: rmse 0.0003316239 max resid 0.001837423
## ... Similar to previous best
## Run 20 stress 0.0598616
## ... Procrustes: rmse 0.0001026646 max resid 0.0005284039
## ... Similar to previous best
## Run 21 stress 0.0800834
## Run 22 stress 0.06197064
## Run 23 stress 0.06397794
## Run 24 stress 0.06397931
## Run 25 stress 0.08081416
## Run 26 stress 0.05986176
## ... Procrustes: rmse 0.0001746782 max resid 0.0009912582
## ... Similar to previous best
## Run 27 stress 0.06193224
## Run 28 stress 0.06197047
## Run 29 stress 0.07983006
## Run 30 stress 0.06588584
## Run 31 stress 0.06193189
## Run 32 stress 0.06197058
## Run 33 stress 0.0619323
## Run 34 stress 0.059862
## ... Procrustes: rmse 0.0001882975 max resid 0.001047776
## ... Similar to previous best
## Run 35 stress 0.06197153
## Run 36 stress 0.05986221
## ... Procrustes: rmse 0.0002577665 max resid 0.001374166
## ... Similar to previous best
## Run 37 stress 0.06690515
## Run 38 stress 0.0821169
## Run 39 stress 0.05986227
## ... Procrustes: rmse 0.0002858865 max resid 0.001529021
## ... Similar to previous best
## Run 40 stress 0.06197017
## Run 41 stress 0.06197005
## Run 42 stress 0.08024629
## Run 43 stress 0.05986183
## ... Procrustes: rmse 0.000185235 max resid 0.001117957
## ... Similar to previous best
## Run 44 stress 0.06197039
## Run 45 stress 0.06197011
## Run 46 stress 0.06584926
## Run 47 stress 0.1586908

```

```

## Run 48 stress 0.05986168
## ... Procrustes: rmse 6.847418e-05 max resid 0.0003641802
## ... Similar to previous best
## Run 49 stress 0.05986188
## ... Procrustes: rmse 0.0001892824 max resid 0.001041791
## ... Similar to previous best
## Run 50 stress 0.06193154
## Run 51 stress 0.06397841
## Run 52 stress 0.08320028
## Run 53 stress 0.06193156
## Run 54 stress 0.06193187
## Run 55 stress 0.0639798
## Run 56 stress 0.06397922
## Run 57 stress 0.05986166
## ... Procrustes: rmse 0.0001218898 max resid 0.0004812525
## ... Similar to previous best
## Run 58 stress 0.08332806
## Run 59 stress 0.08135468
## Run 60 stress 0.06193203
## Run 61 stress 0.06398002
## Run 62 stress 0.06517708
## Run 63 stress 0.05986161
## ... Procrustes: rmse 8.741944e-05 max resid 0.000476245
## ... Similar to previous best
## Run 64 stress 0.05986194
## ... Procrustes: rmse 0.000216158 max resid 0.001164939
## ... Similar to previous best
## Run 65 stress 0.06397845
## Run 66 stress 0.06397811
## Run 67 stress 0.06197097
## Run 68 stress 0.06690635
## Run 69 stress 0.0619316
## Run 70 stress 0.0639797
## Run 71 stress 0.06197016
## Run 72 stress 0.05986162
## ... Procrustes: rmse 0.0001282847 max resid 0.0007057577
## ... Similar to previous best
## Run 73 stress 0.06525726
## Run 74 stress 0.06193216
## Run 75 stress 0.05986215
## ... Procrustes: rmse 0.0002152846 max resid 0.001212603
## ... Similar to previous best
## Run 76 stress 0.06398052
## Run 77 stress 0.06197031
## Run 78 stress 0.06397833
## Run 79 stress 0.06397923
## Run 80 stress 0.08246316
## Run 81 stress 0.08155779
## Run 82 stress 0.05986168
## ... Procrustes: rmse 0.0001438661 max resid 0.000807251
## ... Similar to previous best
## Run 83 stress 0.08128504
## Run 84 stress 0.05986169
## ... Procrustes: rmse 0.0001115971 max resid 0.0005978414

```

```

## ... Similar to previous best
## Run 85 stress 0.06397793
## Run 86 stress 0.05986213
## ... Procrustes: rmse 0.0002549806  max resid 0.001304809
## ... Similar to previous best
## Run 87 stress 0.06193179
## Run 88 stress 0.05986171
## ... Procrustes: rmse 0.0001146246  max resid 0.0006398224
## ... Similar to previous best
## Run 89 stress 0.06193201
## Run 90 stress 0.06397924
## Run 91 stress 0.06517718
## Run 92 stress 0.06690495
## Run 93 stress 0.06197017
## Run 94 stress 0.06193226
## Run 95 stress 0.08322354
## Run 96 stress 0.06197241
## Run 97 stress 0.06197109
## Run 98 stress 0.0639793
## Run 99 stress 0.08003001
## Run 100 stress 0.06397827
## *** Solution reached

```

```
nmfs.cm # convergent solution, stress = 0.060
```

```

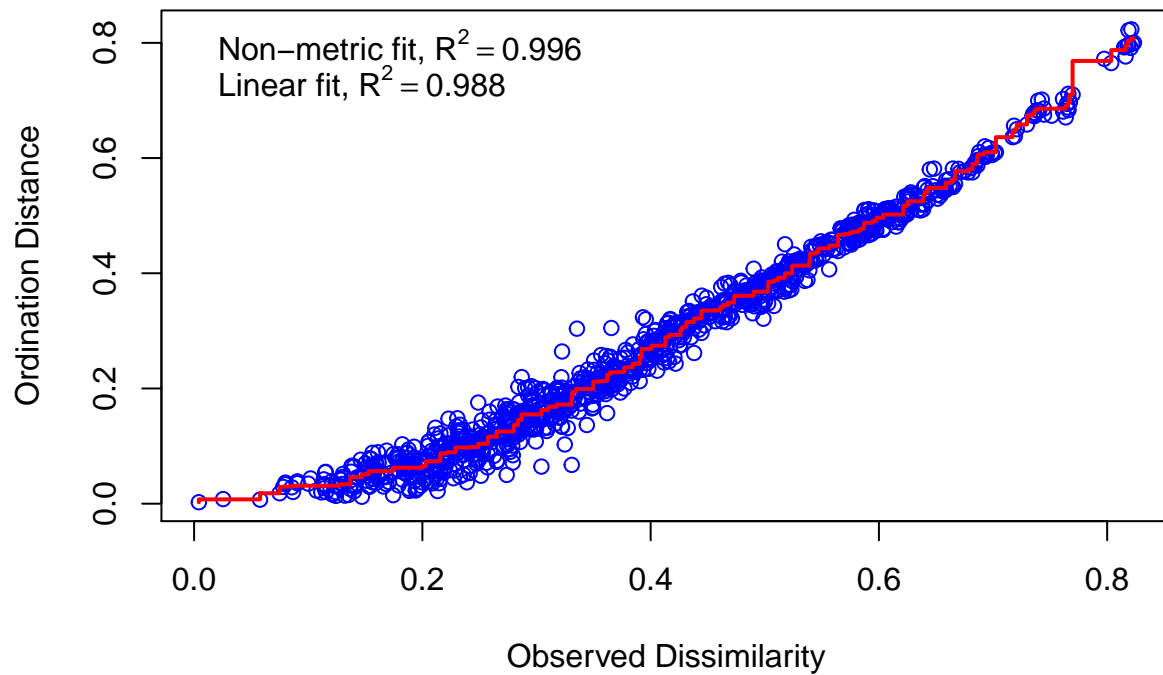
##
## Call:
## metaMDS(comm = wuf.cm, k = 2, try = 100, trymax = 200, autotransform = F)
##
## global Multidimensional Scaling using monoMDS
##
## Data:      wuf.cm
## Distance: user supplied
##
## Dimensions: 2
## Stress:    0.05986149
## Stress type 1, weak ties
## Two convergent solutions found after 100 tries
## Scaling: centring, PC rotation
## Species: scores missing

```

```

# stress plot
stressplot(nmfs.cm) # good fit.

```

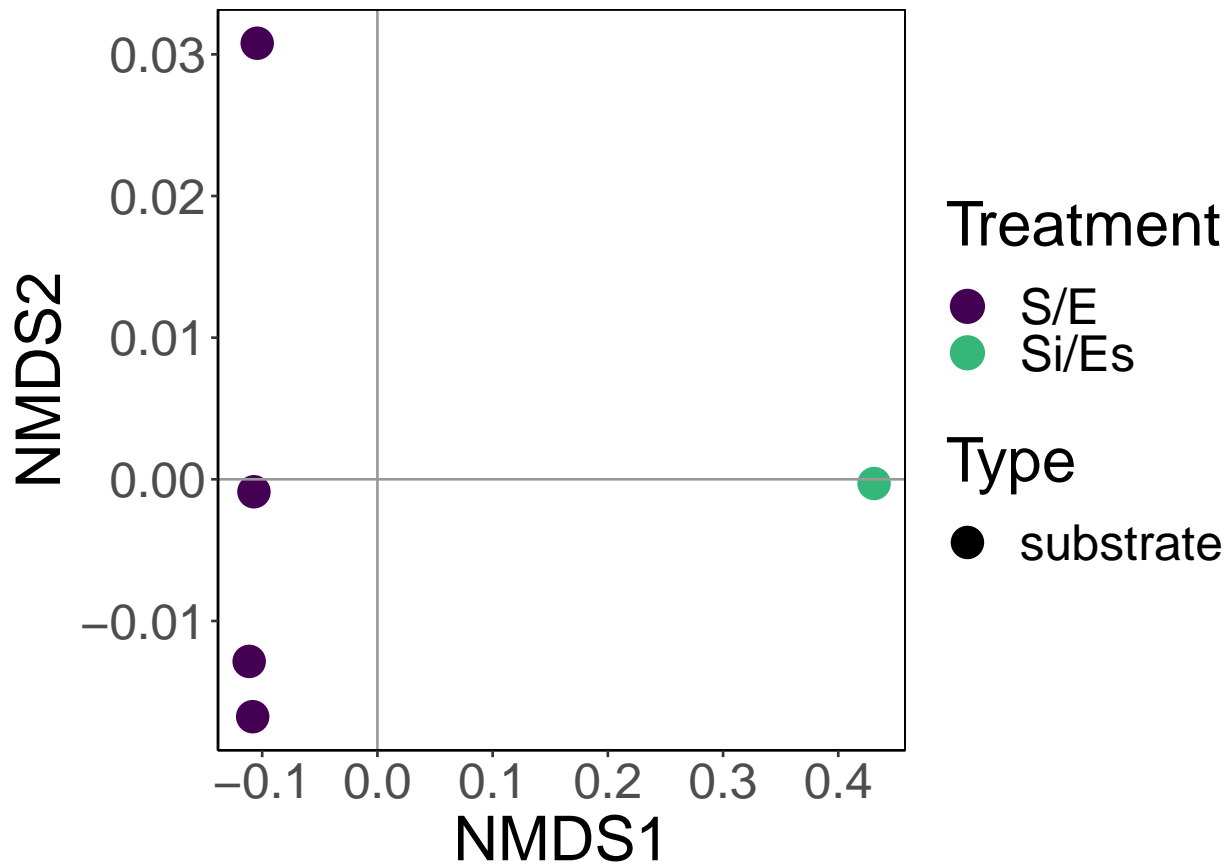


3. Custom NMDS plots

Chicken feed day 0 (see below):

```
# custom plot
nm.ds.cf0.df <- plot_ordination(CF0, nm.ds.cf0, "samples", axes = c(1,2), justDF = T)

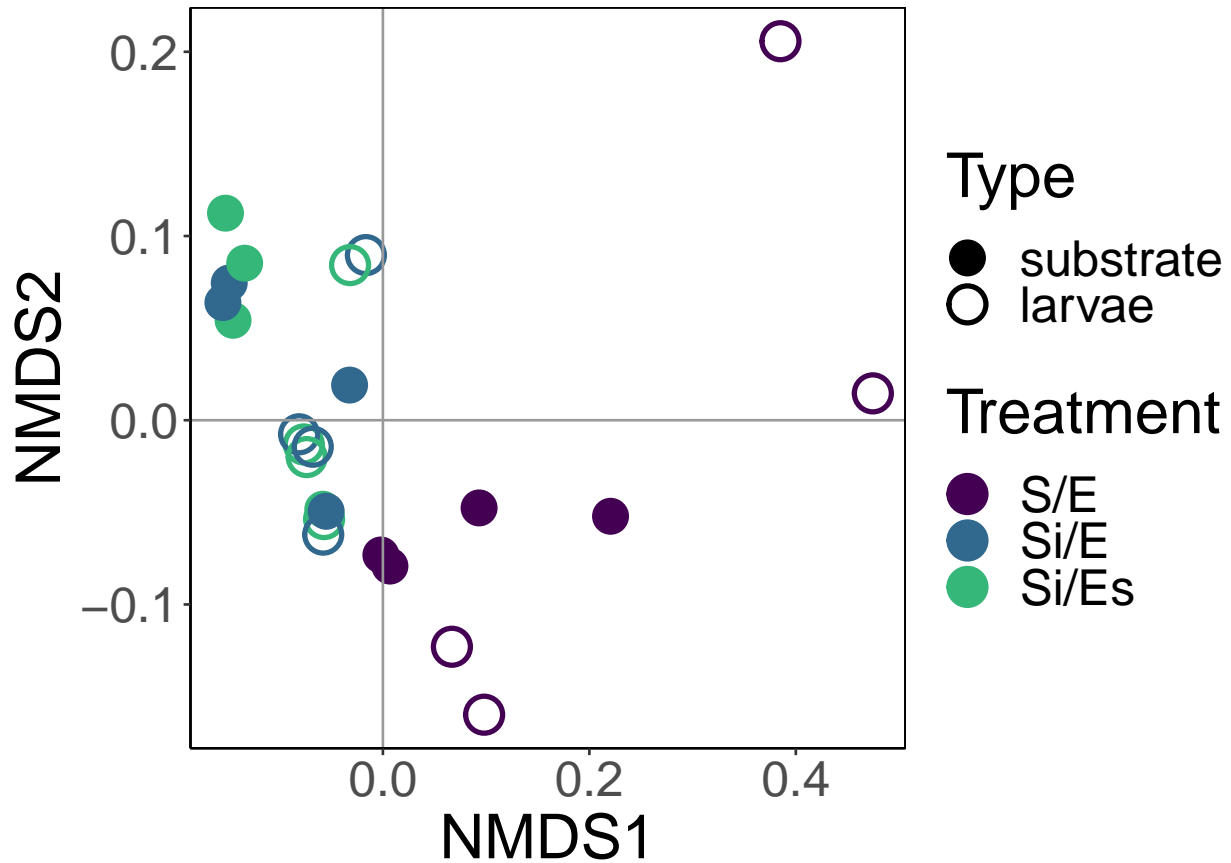
# plot
pNMDS.cf0 <- ggplot(nm.ds.cf0.df, aes(x = NMDS1, y = NMDS2, colour = Treatment)) +
  geom_point(aes(shape = Type), size = 6) +
  scale_color_manual(values = c("#440154FF", "#35B779FF")) +
  xlab("NMDS1") + ylab("NMDS2") +
  geom_hline(yintercept = 0, linetype = "solid", color = "grey60") +
  geom_vline(xintercept = 0, linetype = "solid", color = "grey60") +
  theme_nm.ds
pNMDS.cf0
```

Chicken feed day 15 (see below):

```
# custom plot
nm.ds.cf.df <- plot_ordination(CF, nm.ds.cf, "samples", axes = c(1,2), justDF = T)

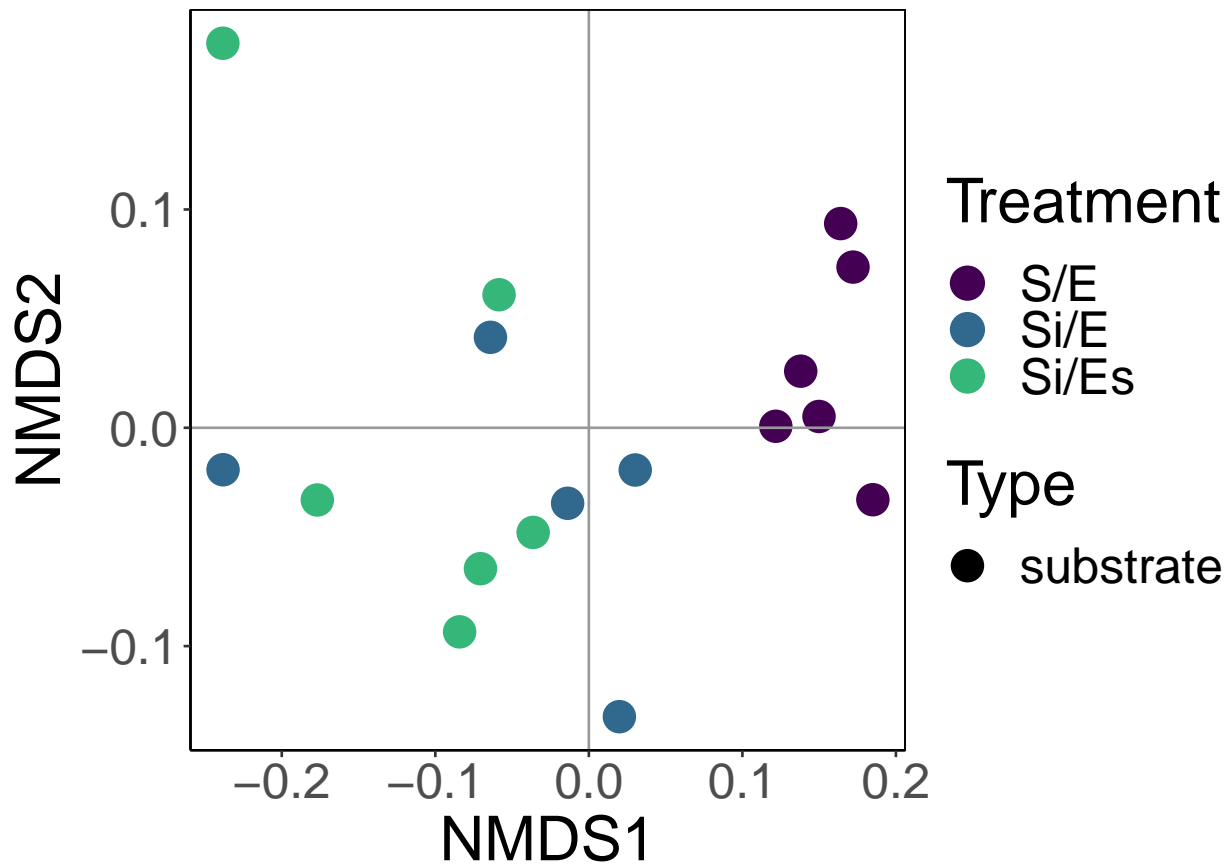
# plot
pNMDS.cf <- ggplot(nm.ds.cf.df, aes(x = NMDS1, y = NMDS2, colour = Treatment)) +
  geom_point(aes(shape = Type), size = 6, stroke = 1.5) +
  scale_shape_manual(values = c(16, 1)) +
  scale_color_manual(values = c("#440154FF", "#31688EFF", "#35B779FF")) +
  xlab("NMDS1") + ylab("NMDS2") +
  geom_hline(yintercept = 0, linetype = "solid", color = "grey60") +
  geom_vline(xintercept = 0, linetype = "solid", color = "grey60") +
  theme_nm.ds
pNMDS.cf
```



Chicken manure day 0 (see below):

```
# custom plot
nm.ds.cm0.df <- plot_ordination(CM0, nm.ds.cm0, "samples", axes = c(1,2), justDF = T)

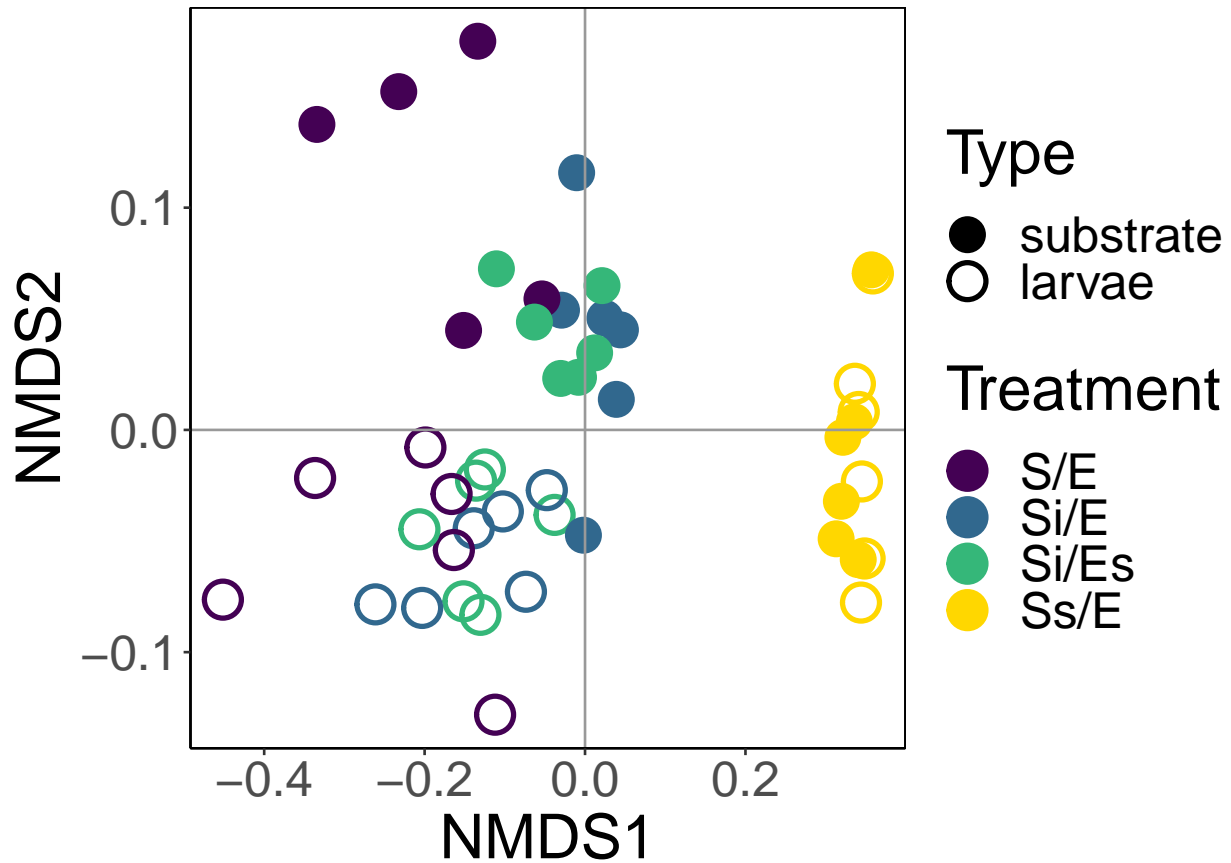
# plot
pNMDS.cm0 <- ggplot(nm.ds.cm0.df, aes(x = NMDS1, y = NMDS2, colour = Treatment)) +
  geom_point(aes(shape = Type), size = 6) +
  scale_color_manual(values = c("#440154FF", "#31688EFF", "#35B779FF")) +
  xlab("NMDS1") + ylab("NMDS2") +
  geom_hline(yintercept = 0, linetype = "solid", color = "grey60") +
  geom_vline(xintercept = 0, linetype = "solid", color = "grey60") +
  theme_nm.ds
pNMDS.cm0
```



Chicken manure day 15 (see below):

```
# custom plot
nm.ds.cm.df <- plot_ordination(CM, nm.ds.cm, "samples", axes = c(1,2), justDF = T)

# plot
pNMDS.cm <- ggplot(nm.ds.cm.df, aes(x = NMDS1, y = NMDS2, colour = Treatment))+
  geom_point(aes(shape = Type), size = 6, stroke = 1.5) +
  scale_shape_manual(values = c(16, 1)) +
  scale_color_manual(values = c("#440154FF", "#31688EFF", "#35B779FF", "gold")) +
  xlab("NMDS1") + ylab("NMDS2") +
  geom_hline(yintercept = 0, linetype = "solid", color = "grey60") +
  geom_vline(xintercept = 0, linetype = "solid", color = "grey60") +
  theme_nm.ds
pNMDS.cm
```



Legend (see below):

```
# extract colour codes
g <- ggplot_build(pNMDS.cm)
unique(g$data[[1]]["colour"])
```

```
##      colour
## 1 #31688EFF
## 2 #35B779FF
## 4      gold
## 6 #440154FF
```

```
# viridis colour codes Treatments
# DnEn, S/E   = #440154FF (purple)
# DiEn, Si/E  = #31688EFF (cyan)
# DiEs, Si/Es = #35B779FF (green)
# DsEn, Ss/E  = #FDE725FF (yellow), changed to "gold".
```

```
# plot legend
pLeg <- ggplot(nmds.cm.df, aes(x = NMDS1, y = NMDS2, colour = Treatment))
pLeg <- pLeg +
  geom_point(aes(shape = Type), size = 6) +
  scale_shape_manual(name = "Sample type", values = c(16, 1)) +
  scale_color_manual(values = c("#440154FF", "#31688EFF", "#35B779FF", "gold")) +
  guides(colour = guide_legend(order = 1), shape = guide_legend(order = 2)) +
```

```

theme_nmds
# plot only legend
nmds.legend <- get_legend(pLeg)
pNMDS.leg <- as_ggplot(nmds.legend)
pNMDS.leg

```

Treatment

- S/E
- Si/E
- Si/Es
- Ss/E

Sample type

- substrate
- larvae

4. Export plots

NMDS plots: Figure 5 in manuscript.

```

# NMDS plots
ggsave(plot = pNMDS.cf0, "./figures/Fig_5A_NMDS_CF_day0.png", h = 5, w = 7)
ggsave(plot = pNMDS.cf, "./figures/Fig_5B_NMDS_CF_day15.png", h = 5, w = 7)
ggsave(plot = pNMDS.cm0, "./figures/Fig_5C_NMDS_CM_day0.png", h = 5, w = 7)

```

```

ggsave(plot = pNMDS.cm, "./figures/Fig_5D_NMDS_CM_day15.png", h = 5, w = 7)
ggsave(plot = pNMDS.leg, "./figures/Fig_5_NMDS_legend.png", h = 6, w = 4)

# high-res format
ggsave(plot = pNMDS.cf0, "./figures/Fig_5A_NMDS_CF_day0.pdf", h = 100, w = 160, u = "mm")
ggsave(plot = pNMDS.cf, "./figures/Fig_5B_NMDS_CF_day15.pdf", h = 100, w = 160, u = "mm")
ggsave(plot = pNMDS.cm0, "./figures/Fig_5C_NMDS_CM_day0.pdf", h = 100, w = 160, u = "mm")
ggsave(plot = pNMDS.cm, "./figures/Fig_5D_NMDS_CM_day15.pdf", h = 100, w = 160, u = "mm")
ggsave(plot = pNMDS.leg, "./figures/Fig_5_NMDS_legend.pdf", h = 80, w = 60, u = "mm")

```