

Sphagnum Conditioned Microbiome Across Moss Species Data and Figures

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Introduction

The use of microbiomes to select for specific plant host phenotypic response to environment have been demonstrated in recent studies. However, the underlying mechanisms for microbial mediated plant phenotypic response is unknown. Here we explore the mechanisms for microbial mediated host tolerance of elevated temperature. Microbiomes conditioned to ambient or elevated temperature were applied to moss and subjected to ambient or elevated temperatures. Fluorcam measurements were performed each week and the experiment was harvested at 4 weeks to assess plant growth and microbiome composition.

Experimental details

Experiment duration was 4 weeks with material analyzed or collected for: Fluorcam, area growth, 16S profile, and RNAseq

Methods Conditioned microbe extraction and inoculation

From each treatment, 100 g of *S. fallax* tissue was diced with sterile razor blade and pulverized in BG11 -N, pH 5.5 with a mortar and pestle. The resulting suspension was filtered through Mira Cloth and stored at 4 C until application. The microbes were then pelleted and resuspended in BG11 -N medium (pH5.5). A single capitula of axenic *Sphagnum fallax* or *magellanicum* was added to each well of a 12 well plate and inoculated with 2 ml of +0 microbiome, 4.5 microbiome +9 microbiome, or sterile media. Additionally, *Sphagnum fuscum* were inoculated with +0 or +9 microbiome. Warming conditions were determined from June 2019 temperatures

```
library(tidyverse)
library(car)
library(rcompanion)
library(FSA)
library(agricolae)
library(reshape2)
```

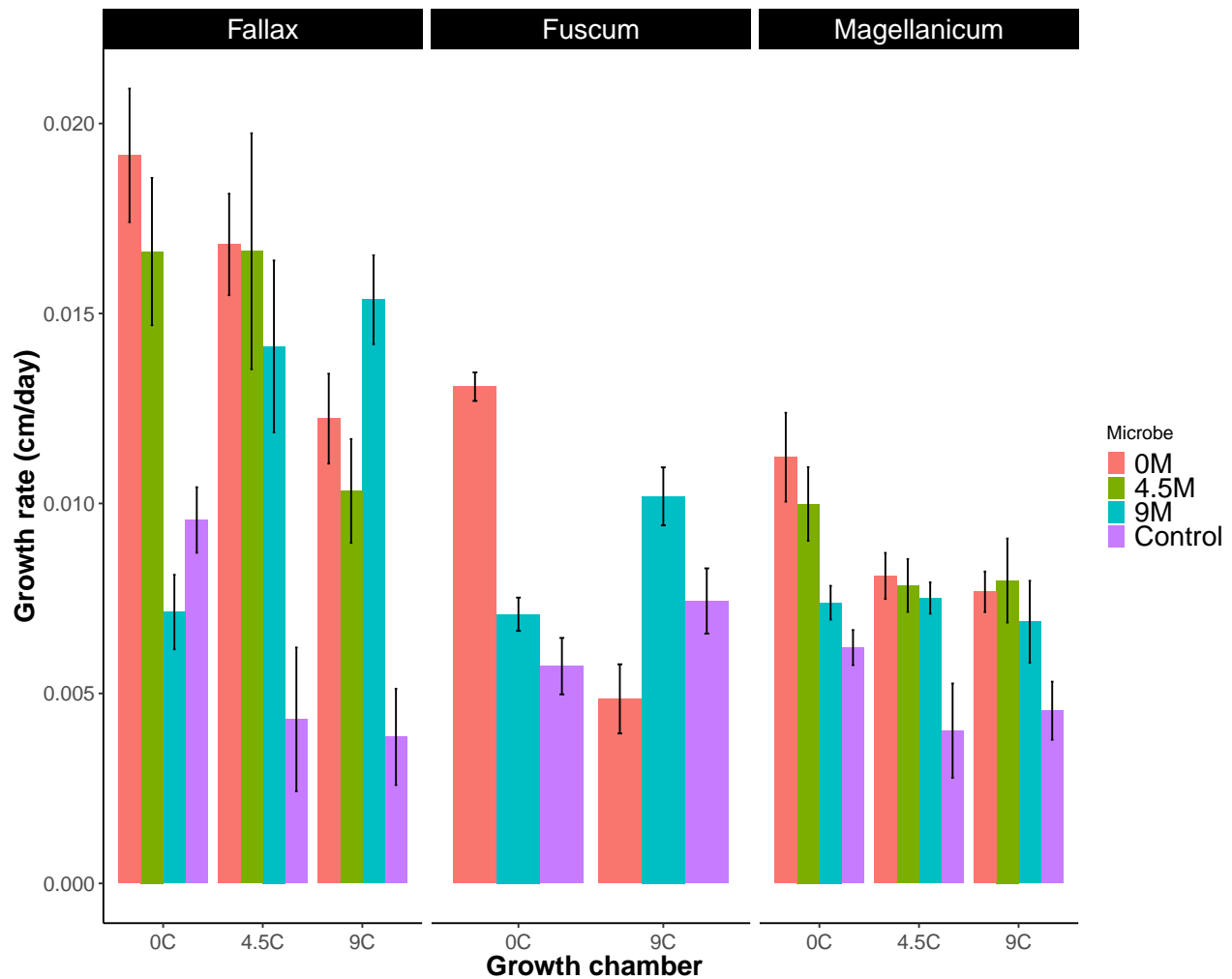


Figure 1: Fig. 1: Growth rate of conditioned microbiome applied to *Sphagnum fallax*, *Sphagnum Magellanicum* or *Sphagnum fuscum*

Growth Phenotypes for each moss across growth chamber treatments

Fallax statistics

```
CM.Fallax<- CM %>%
  filter(Moss=="Fallax")

CM.Fall.rank <- rank(CM.Fallax$Growth.rate.cmperday)
CM.Fallax <- cbind ( CM.Fallax, CM.Fall.rank)
ranked.CM.Fall.aov <- aov(CM.Fall.rank ~ Chamber + Microbe + Chamber:Microbe, data = CM.Fallax)
Anova(ranked.CM.Fall.aov, type = 3)

## Anova Table (Type III tests)
##
## Response: CM.Fall.rank
```

```
##              Sum Sq Df F value    Pr(>F)
## (Intercept)   149410  1 143.3110 < 2.2e-16 ***
## Chamber        8275   2   3.9686   0.0212 *
## Microbe       40959   3  13.0956 1.540e-07 ***
## Chamber:Microbe 39693   6   6.3454 6.893e-06 ***
## Residuals    137618 132
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(ranked.CM.Fall.aov)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = CM.Fall.rank ~ Chamber + Microbe + Chamber:Microbe, data = CM.Fallax)
##
## $Chamber
##              diff          lwr          upr          p adj
## 4.5C-0C    -0.5833333 -16.20670 15.040030 0.9956908
## 9C-0C     -12.2291667 -27.85253  3.394196 0.1558857
## 9C-4.5C   -11.6458333 -27.26920  3.977530 0.1847518
##
## $Microbe
##              diff          lwr          upr          p adj
## 4.5M-0M    -13.86111 -33.66412  5.941902 0.2679666
## 9M-0M      -24.22222 -44.02523 -4.419209 0.0097272
## Control-0M -58.36111 -78.16412 -38.558098 0.0000000
## 9M-4.5M    -10.36111 -30.16412  9.441902 0.5257556
## Control-4.5M -44.50000 -64.30301 -24.696987 0.0000002
## Control-9M  -34.13889 -53.94190 -14.335876 0.0000915
##
## $`Chamber:Microbe`
##              diff          lwr          upr          p adj
## 4.5C:0M-0C:0M    -9.166667 -53.0301537 34.69682034 0.9999185
## 9C:0M-0C:0M     -35.750000 -79.6134870  8.11348701 0.2322231
## 0C:4.5M-0C:0M   -13.666667 -57.5301537 30.19682034 0.9965280
## 4.5C:4.5M-0C:0M -24.500000 -68.3634870 19.36348701 0.7820080
## 9C:4.5M-0C:0M   -48.333333 -92.1968203 -4.46984633 0.0177090
## 0C:9M-0C:0M     -72.500000 -116.3634870 -28.63651299 0.0000121
## 4.5C:9M-0C:0M   -28.166667 -72.0301537 15.69682034 0.5976712
## 9C:9M-0C:0M     -16.916667 -60.7801537 26.94682034 0.9798911
## 0C:Control-0C:0M -53.083333 -96.9468203 -9.21984633 0.0051845
## 4.5C:Control-0C:0M -79.750000 -123.6134870 -35.88651299 0.0000009
## 9C:Control-0C:0M  -87.166667 -131.0301537 -43.30317966 0.0000001
## 9C:0M-4.5C:0M   -26.583333 -70.4468203 17.28015367 0.6813520
## 0C:4.5M-4.5C:0M  -4.500000 -48.3634870 39.36348701 1.0000000
## 4.5C:4.5M-4.5C:0M -15.333333 -59.1968203 28.53015367 0.9907935
## 9C:4.5M-4.5C:0M -39.166667 -83.0301537  4.69682034 0.1291446
## 0C:9M-4.5C:0M   -63.333333 -107.1968203 -19.46984633 0.0002536
## 4.5C:9M-4.5C:0M -19.000000 -62.8634870 24.86348701 0.9528855
## 9C:9M-4.5C:0M    -7.750000 -51.6134870 36.11348701 0.9999850
## 0C:Control-4.5C:0M -43.916667 -87.7801537 -0.05317966 0.0494227
## 4.5C:Control-4.5C:0M -70.583333 -114.4468203 -26.71984633 0.0000234
## 9C:Control-4.5C:0M -78.000000 -121.8634870 -34.13651299 0.0000017
## 0C:4.5M-9C:0M    22.083333 -21.7801537 65.94682034 0.8761212
```

```
## 4.5C:4.5M-9C:0M      11.250000 -32.6134870 55.11348701 0.9994079
## 9C:4.5M-9C:0M        -12.583333 -56.4468203 31.28015367 0.9983339
## 0C:9M-9C:0M          -36.750000 -80.6134870 7.11348701 0.1974765
## 4.5C:9M-9C:0M        7.583333 -36.2801537 51.44682034 0.9999880
## 9C:9M-9C:0M          18.833333 -25.0301537 62.69682034 0.9557000
## 0C:Control-9C:0M     -17.333333 -61.1968203 26.53015367 0.9757999
## 4.5C:Control-9C:0M   -44.000000 -87.8634870 -0.13651299 0.0485297
## 9C:Control-9C:0M     -51.416667 -95.2801537 -7.55317966 0.0080876
## 4.5C:4.5M-0C:4.5M    -10.833333 -54.6968203 33.03015367 0.9995858
## 9C:4.5M-0C:4.5M     -34.666667 -78.5301537 9.19682034 0.2742097
## 0C:9M-0C:4.5M       -58.833333 -102.6968203 -14.96984633 0.0010089
## 4.5C:9M-0C:4.5M     -14.500000 -58.3634870 29.36348701 0.9942232
## 9C:9M-0C:4.5M       -3.250000 -47.1134870 40.61348701 1.0000000
## 0C:Control-0C:4.5M   -39.416667 -83.2801537 4.44682034 0.1232760
## 4.5C:Control-0C:4.5M -66.083333 -109.9468203 -22.21984633 0.0001050
## 9C:Control-0C:4.5M   -73.500000 -117.3634870 -29.63651299 0.0000085
## 9C:4.5M-4.5C:4.5M    -23.833333 -67.6968203 20.03015367 0.8107507
## 0C:9M-4.5C:4.5M     -48.000000 -91.8634870 -4.13651299 0.0192137
## 4.5C:9M-4.5C:4.5M    -3.666667 -47.5301537 40.19682034 1.0000000
## 9C:9M-4.5C:4.5M      7.583333 -36.2801537 51.44682034 0.9999880
## 0C:Control-4.5C:4.5M -28.583333 -72.4468203 15.28015367 0.5751916
## 4.5C:Control-4.5C:4.5M -55.250000 -99.1134870 -11.38651299 0.0028489
## 9C:Control-4.5C:4.5M -62.666667 -106.5301537 -18.80317966 0.0003128
## 0C:9M-9C:4.5M       -24.166667 -68.0301537 19.69682034 0.7966237
## 4.5C:9M-9C:4.5M     20.166667 -23.6968203 64.03015367 0.9295227
## 9C:9M-9C:4.5M       31.416667 -12.4468203 75.28015367 0.4246933
## 0C:Control-9C:4.5M   -4.750000 -48.6134870 39.11348701 0.9999999
## 4.5C:Control-9C:4.5M -31.416667 -75.2801537 12.44682034 0.4246933
## 9C:Control-9C:4.5M   -38.833333 -82.6968203 5.03015367 0.1373051
## 4.5C:9M-0C:9M       44.333333 0.4698463 88.19682034 0.0450956
## 9C:9M-0C:9M         55.583333 11.7198463 99.44682034 0.0025930
## 0C:Control-0C:9M     19.416667 -24.4468203 63.28015367 0.9452917
## 4.5C:Control-0C:9M   -7.250000 -51.1134870 36.61348701 0.9999924
## 9C:Control-0C:9M     -14.666667 -58.5301537 29.19682034 0.9936379
## 9C:9M-4.5C:9M       11.250000 -32.6134870 55.11348701 0.9994079
## 0C:Control-4.5C:9M   -24.916667 -68.7801537 18.94682034 0.7630902
## 4.5C:Control-4.5C:9M -51.583333 -95.4468203 -7.71984633 0.0077408
## 9C:Control-4.5C:9M   -59.000000 -102.8634870 -15.13651299 0.0009600
## 0C:Control-9C:9M     -36.166667 -80.0301537 7.69682034 0.2172750
## 4.5C:Control-9C:9M   -62.833333 -106.6968203 -18.96984633 0.0002969
## 9C:Control-9C:9M     -70.250000 -114.1134870 -26.38651299 0.0000262
## 4.5C:Control-0C:Control -26.666667 -70.5301537 17.19682034 0.6770579
## 9C:Control-0C:Control -34.083333 -77.9468203 9.78015367 0.2986425
## 9C:Control-4.5C:Control -7.416667 -51.2801537 36.44682034 0.9999904
```

Fuscum statistics

```
CM.Fuscum<- CM %>%
  filter(Moss=="Fuscum")

CM.Fuscum.rank <- rank(CM.Fuscum$Growth.rate.cmperday)
CM.Fuscum <- cbind ( CM.Fuscum, CM.Fuscum.rank)
```

```
ranked.CM.Fussum.aov <- aov(CM.Fussum.rank ~ Chamber + Microbe + Chamber:Microbe, data = CM.Fussum)
Anova(ranked.CM.Fussum.aov, type = 3)
```

```
## Anova Table (Type III tests)
##
## Response: CM.Fussum.rank
##           Sum Sq Df F value    Pr(>F)
## (Intercept)    48769  1 245.147 < 2.2e-16 ***
## Chamber        12060  1  60.623 6.394e-11 ***
## Microbe         11823  2  29.715 6.277e-10 ***
## Chamber:Microbe 14855  2  37.336 1.426e-11 ***
## Residuals      13130 66
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(ranked.CM.Fussum.aov)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = CM.Fussum.rank ~ Chamber + Microbe + Chamber:Microbe, data = CM.Fussum)
##
## $Chamber
##           diff          lwr          upr          p adj
## 9C-0C -4.388889 -11.02639  2.248614  0.1913352
##
## $Microbe
##           diff          lwr          upr          p adj
## 9M-0M      -0.916667 -10.67920  8.845862  0.9724566
## Control-0M -13.583333 -23.34586 -3.820804  0.0039458
## Control-9M -12.666667 -22.42920 -2.904138  0.0076593
##
## $`Chamber:Microbe`
##           diff          lwr          upr          p adj
## 9C:0M-0C:0M    -44.833333 -61.734011 -27.9326552  0.0000000
## 0C:9M-0C:0M    -32.916667 -49.817345 -16.0159885  0.0000042
## 9C:9M-0C:0M    -13.750000 -30.650678  3.1506782  0.1755284
## 0C:Control-0C:0M -42.250000 -59.150678 -25.3493218  0.0000000
## 9C:Control-0C:0M -29.750000 -46.650678 -12.8493218  0.0000344
## 0C:9M-9C:0M     11.916667  -4.984011  28.8173448  0.3157888
## 9C:9M-9C:0M     31.083333  14.182655  47.9840115  0.0000143
## 0C:Control-9C:0M  2.583333 -14.317345  19.4840115  0.9976115
## 9C:Control-9C:0M  15.083333  -1.817345  31.9840115  0.1068955
## 9C:9M-0C:9M     19.166667  2.265989  36.0673448  0.0172051
## 0C:Control-0C:9M  -9.333333 -26.234011  7.5673448  0.5879622
## 9C:Control-0C:9M  3.166667 -13.734011  20.0673448  0.9937685
## 0C:Control-9C:9M -28.500000 -45.400678 -11.5993218  0.0000774
## 9C:Control-9C:9M -16.000000 -32.900678  0.9006782  0.0737051
## 9C:Control-0C:Control 12.500000  -4.400678  29.4006782  0.2652630
```

Magellanicum statistics

```
CM.Mag<- CM %>%
  filter(Moss=="Magellanicum")

CM.Mag.rank <- rank(CM.Mag$Growth.rate.cmperday)
CM.Mag <- cbind ( CM.Mag, CM.Mag.rank)
ranked.CM.Mag.aov <- aov(CM.Mag.rank ~ Chamber + Microbe + Chamber:Microbe, data = CM.Mag)
Anova(ranked.CM.Mag.aov, type = 3)

## Anova Table (Type III tests)
##
## Response: CM.Mag.rank
##
```

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	150080	1	112.1695	< 2.2e-16 ***
Chamber	7371	2	2.7544	0.0673078 .
Microbe	25796	3	6.4267	0.0004269 ***
Chamber:Microbe	3741	6	0.4660	0.8324611
Residuals	176613	132		

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

TukeyHSD(ranked.CM.Mag.aov)

## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = CM.Mag.rank ~ Chamber + Microbe + Chamber:Microbe, data = CM.Mag)
##
## $Chamber
##
```

	diff	lwr	upr	p adj
4.5C-0C	-16.166667	-33.86568	1.532343	0.0810463
9C-0C	-22.708333	-40.40734	-5.009324	0.0079479
9C-4.5C	-6.541667	-24.24068	11.157343	0.6562775

```
##
## $Microbe
##
```

	diff	lwr	upr	p adj
4.5M-0M	-4.75000	-27.18395	17.6839481	0.9461480
9M-0M	-22.58333	-45.01728	-0.1493852	0.0478323
Control-0M	-50.00000	-72.43395	-27.5660519	0.0000003
9M-4.5M	-17.83333	-40.26728	4.6006148	0.1689021
Control-4.5M	-45.25000	-67.68395	-22.8160519	0.0000035
Control-9M	-27.41667	-49.85061	-4.9827186	0.0098095

```
##
## $`Chamber:Microbe`
##
```

	diff	lwr	upr	p adj
4.5C:0M-0C:0M	-27.33333333	-77.02432	22.3576507	0.7982955
9C:0M-0C:0M	-32.66666667	-82.35765	17.0243174	0.5615495
0C:4.5M-0C:0M	-9.08333333	-58.77432	40.6076507	0.9999788
4.5C:4.5M-0C:0M	-32.41666667	-82.10765	17.2743174	0.5734798
9C:4.5M-0C:0M	-32.75000000	-82.44098	16.9409841	0.5575721
0C:9M-0C:0M	-38.16666667	-87.85765	11.5243174	0.3157793
4.5C:9M-0C:0M	-35.16666667	-84.85765	14.5243174	0.4439272
9C:9M-0C:0M	-54.41666667	-104.10765	-4.7256826	0.0190506

## 0C:Control-0C:0M	-58.25000000	-107.94098	-8.5590159	0.0080832
## 4.5C:Control-0C:0M	-75.25000000	-124.94098	-25.5590159	0.0000939
## 9C:Control-0C:0M	-76.50000000	-126.19098	-26.8090159	0.0000653
## 9C:0M-4.5C:0M	-5.33333333	-55.02432	44.3576507	0.9999999
## 0C:4.5M-4.5C:0M	18.25000000	-31.44098	67.9409841	0.9862775
## 4.5C:4.5M-4.5C:0M	-5.08333333	-54.77432	44.6076507	1.0000000
## 9C:4.5M-4.5C:0M	-5.41666667	-55.10765	44.2743174	0.9999999
## 0C:9M-4.5C:0M	-10.83333333	-60.52432	38.8576507	0.9998762
## 4.5C:9M-4.5C:0M	-7.83333333	-57.52432	41.8576507	0.9999954
## 9C:9M-4.5C:0M	-27.08333333	-76.77432	22.6076507	0.8076659
## 0C:Control-4.5C:0M	-30.91666667	-80.60765	18.7743174	0.6444348
## 4.5C:Control-4.5C:0M	-47.91666667	-97.60765	1.7743174	0.0697973
## 9C:Control-4.5C:0M	-49.16666667	-98.85765	0.5243174	0.0552741
## 0C:4.5M-9C:0M	23.58333333	-26.10765	73.2743174	0.9135409
## 4.5C:4.5M-9C:0M	0.25000000	-49.44098	49.9409841	1.0000000
## 9C:4.5M-9C:0M	-0.08333333	-49.77432	49.6076507	1.0000000
## 0C:9M-9C:0M	-5.50000000	-55.19098	44.1909841	0.9999999
## 4.5C:9M-9C:0M	-2.50000000	-52.19098	47.1909841	1.0000000
## 9C:9M-9C:0M	-21.75000000	-71.44098	27.9409841	0.9493545
## 0C:Control-9C:0M	-25.58333333	-75.27432	24.1076507	0.8590306
## 4.5C:Control-9C:0M	-42.58333333	-92.27432	7.1076507	0.1712823
## 9C:Control-9C:0M	-43.83333333	-93.52432	5.8576507	0.1408628
## 4.5C:4.5M-0C:4.5M	-23.33333333	-73.02432	26.3576507	0.9192038
## 9C:4.5M-0C:4.5M	-23.66666667	-73.35765	26.0243174	0.9115969
## 0C:9M-0C:4.5M	-29.08333333	-78.77432	20.6076507	0.7271029
## 4.5C:9M-0C:4.5M	-26.08333333	-75.77432	23.6076507	0.8428686
## 9C:9M-0C:4.5M	-45.33333333	-95.02432	4.3576507	0.1100420
## 0C:Control-0C:4.5M	-49.16666667	-98.85765	0.5243174	0.0552741
## 4.5C:Control-0C:4.5M	-66.16666667	-115.85765	-16.4756826	0.0011450
## 9C:Control-0C:4.5M	-67.41666667	-117.10765	-17.7256826	0.0008240
## 9C:4.5M-4.5C:4.5M	-0.33333333	-50.02432	49.3576507	1.0000000
## 0C:9M-4.5C:4.5M	-5.75000000	-55.44098	43.9409841	0.9999998
## 4.5C:9M-4.5C:4.5M	-2.75000000	-52.44098	46.9409841	1.0000000
## 9C:9M-4.5C:4.5M	-22.00000000	-71.69098	27.6909841	0.9452286
## 0C:Control-4.5C:4.5M	-25.83333333	-75.52432	23.8576507	0.8510731
## 4.5C:Control-4.5C:4.5M	-42.83333333	-92.52432	6.8576507	0.1648390
## 9C:Control-4.5C:4.5M	-44.08333333	-93.77432	5.6076507	0.1353077
## 0C:9M-9C:4.5M	-5.41666667	-55.10765	44.2743174	0.9999999
## 4.5C:9M-9C:4.5M	-2.41666667	-52.10765	47.2743174	1.0000000
## 9C:9M-9C:4.5M	-21.66666667	-71.35765	28.0243174	0.9506792
## 0C:Control-9C:4.5M	-25.50000000	-75.19098	24.1909841	0.8616277
## 4.5C:Control-9C:4.5M	-42.50000000	-92.19098	7.1909841	0.1734708
## 9C:Control-9C:4.5M	-43.75000000	-93.44098	5.9409841	0.1427529
## 4.5C:9M-0C:9M	3.00000000	-46.69098	52.6909841	1.0000000
## 9C:9M-0C:9M	-16.25000000	-65.94098	33.4409841	0.9947295
## 0C:Control-0C:9M	-20.08333333	-69.77432	29.6076507	0.9713651
## 4.5C:Control-0C:9M	-37.08333333	-86.77432	12.6076507	0.3597025
## 9C:Control-0C:9M	-38.33333333	-88.02432	11.3576507	0.3092947
## 9C:9M-4.5C:9M	-19.25000000	-68.94098	30.4409841	0.9791898
## 0C:Control-4.5C:9M	-23.08333333	-72.77432	26.6076507	0.9246148
## 4.5C:Control-4.5C:9M	-40.08333333	-89.77432	9.6076507	0.2459610
## 9C:Control-4.5C:9M	-41.33333333	-91.02432	8.3576507	0.2062745
## 0C:Control-9C:9M	-3.83333333	-53.52432	45.8576507	1.0000000
## 4.5C:Control-9C:9M	-20.83333333	-70.52432	28.8576507	0.9625899

## 9C:Control-9C:9M	-22.08333333	-71.77432	27.6076507	0.9438020
## 4.5C:Control-0C:Control	-17.00000000	-66.69098	32.6909841	0.9922949
## 9C:Control-0C:Control	-18.25000000	-67.94098	31.4409841	0.9862775
## 9C:Control-4.5C:Control	-1.25000000	-50.94098	48.4409841	1.0000000