Sphagnum Conditioned Microbiome Data and Figures

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Contents

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 Start Date
Experiment 1 - performed 2016
Experiment 2 - performed 2017

Duration
Experiment 1 - 4 weeks
Experiment 2 - 3 weeks

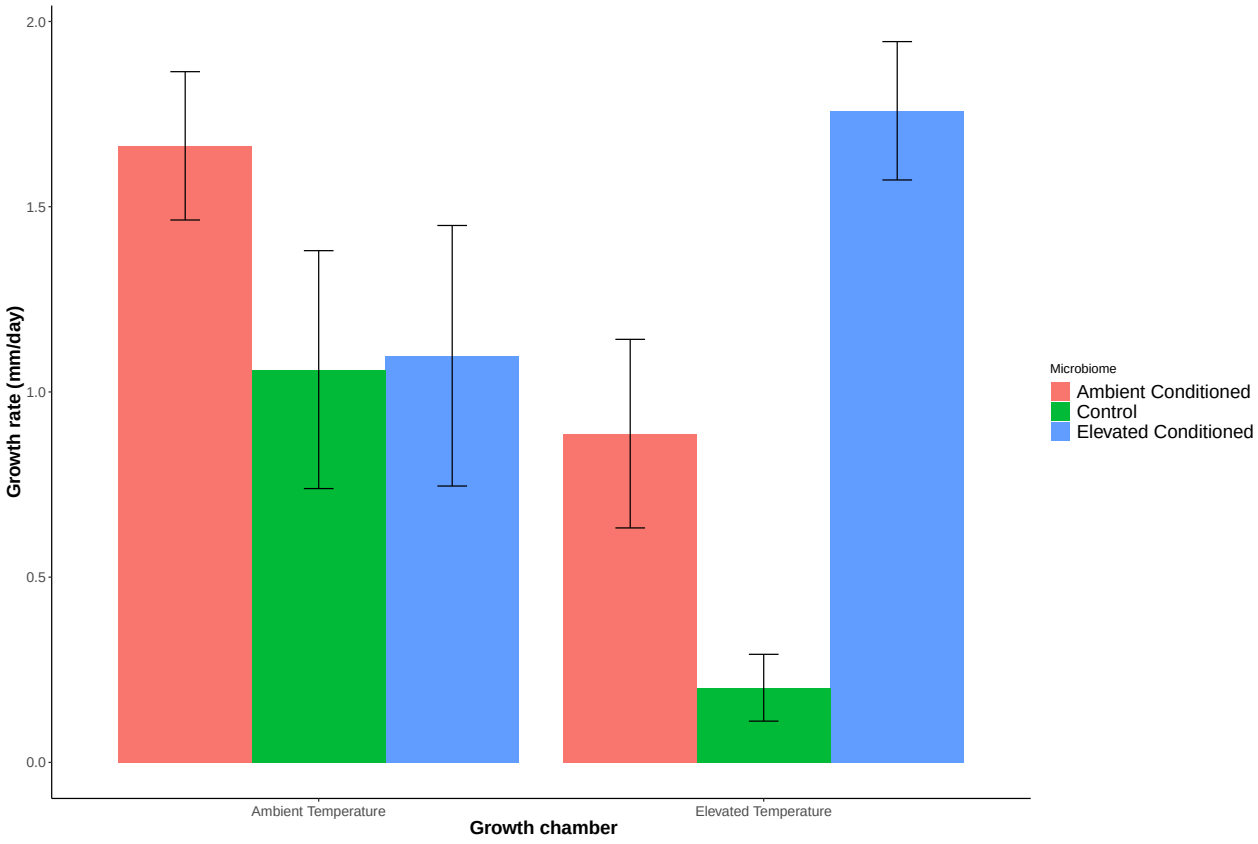


Figure 1: Fig. 1: Growth rate of conditioned microbiome 2016

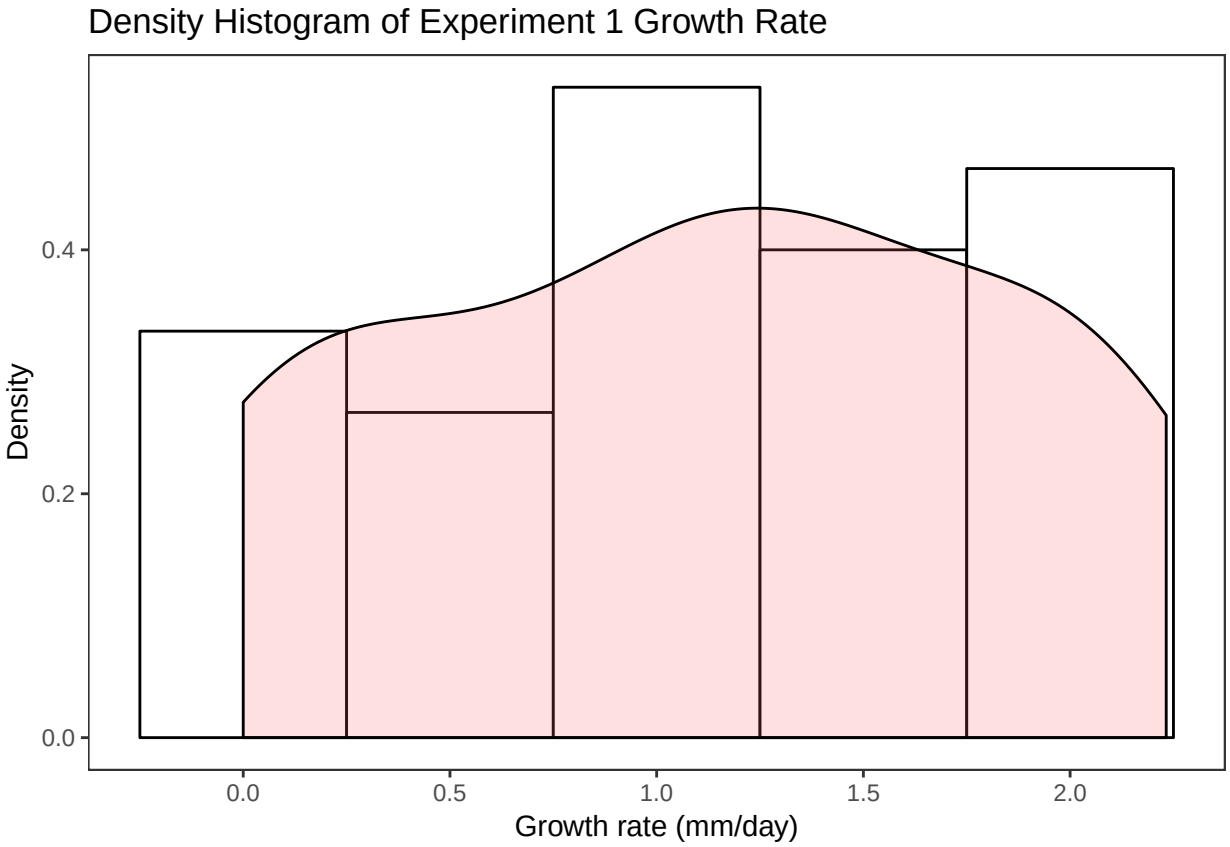
Measurements taken
Experiment 1 - Fluorcam weekly, area growth, 16S profile, RNA not extractable, pH measured
Experiment 2 - Fluorcam weekly, area growth, 16S profile, RNAseq, final pH, 15N and ARA

Methods Adapted microbe extraction and inoculation From each treatment, 100 g of 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 was added to each well of a 12 well plate and inoculated with 2 ml of +0 microbiome, +9 microbiome, or sterile media. Warming conditions were determined from the 2016 summer season temperatures

Packages

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

Experiment 1 Summary Statistics



Exp 1 Rank Transform

```
# rank transform
exp1.rank <- rank(Exp1$Growth.rate.mmperday)

# binding transform
Exp1 <- cbind(Exp1, exp1.rank)

# two-way ANOVA with ranked data
ranked.exp1.aov <- aov(exp1.rank ~ Chamber + Microbe + Chamber:Microbe , data = Exp1)
Anova(ranked.exp1.aov, type = 3)
```

```
## Anova Table (Type III tests)
##
## Response: exp1.rank
##                  Sum Sq Df F value   Pr(>F)
## (Intercept)     2420.00  1 53.2258 1.55e-07 ***
## Chamber          211.60  1  4.6540 0.041211 *
## Microbe          170.53  2  1.8754 0.175082
## Chamber:Microbe  516.47  2  5.6796 0.009561 **
## Residuals       1091.20 24
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(ranked.exp1.aov)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = exp1.rank ~ Chamber + Microbe + Chamber:Microbe, data = Exp1)
##
## $Chamber
##            diff       lwr      upr     p adj
## ET-AT -3.533333 -8.614977 1.548311 0.1641758
##
## $Microbe
##            diff        lwr        upr     p adj
## Control-AC -7.8 -15.330608 -0.2693921 0.0413468
## EC-AC       2.1  -5.430608  9.6306079 0.7678500
## EC-Control  9.9   2.369392 17.4306079 0.0084967
##
## $`Chamber:Microbe`
##                          diff        lwr       upr     p adj
## ET:AC-AT:AC             -9.2 -22.385796  3.985796 0.2933802
## AT:Control-AT:AC        -7.6 -20.785796  5.585796 0.4949848
## ET:Control-AT:AC       -17.2 -30.385796 -4.014204 0.0057197
## AT:EC-AT:AC             -6.6 -19.785796  6.585796 0.6385347
## ET:EC-AT:AC              1.6 -11.585796 14.785796 0.9989007
## AT:Control-ET:AC         1.6 -11.585796 14.785796 0.9989007
## ET:Control-ET:AC        -8.0 -21.185796  5.185796 0.4398671
## AT:EC-ET:AC              2.6 -10.585796 15.785796 0.9892763
## ET:EC-ET:AC             10.8  -2.385796 23.985796 0.1541977
## ET:Control-AT:Control  -9.6 -22.785796  3.585796 0.2523661
## AT:EC-AT:Control         1.0 -12.185796 14.185796 0.9998890
## ET:EC-AT:Control         9.2  -3.985796 22.385796 0.2933802
## AT:EC-ET:Control        10.6  -2.585796 23.785796 0.1680561
## ET:EC-ET:Control        18.8   5.614204 31.985796 0.0022945
## ET:EC-AT:EC              8.2  -4.985796 21.385796 0.4132882
```

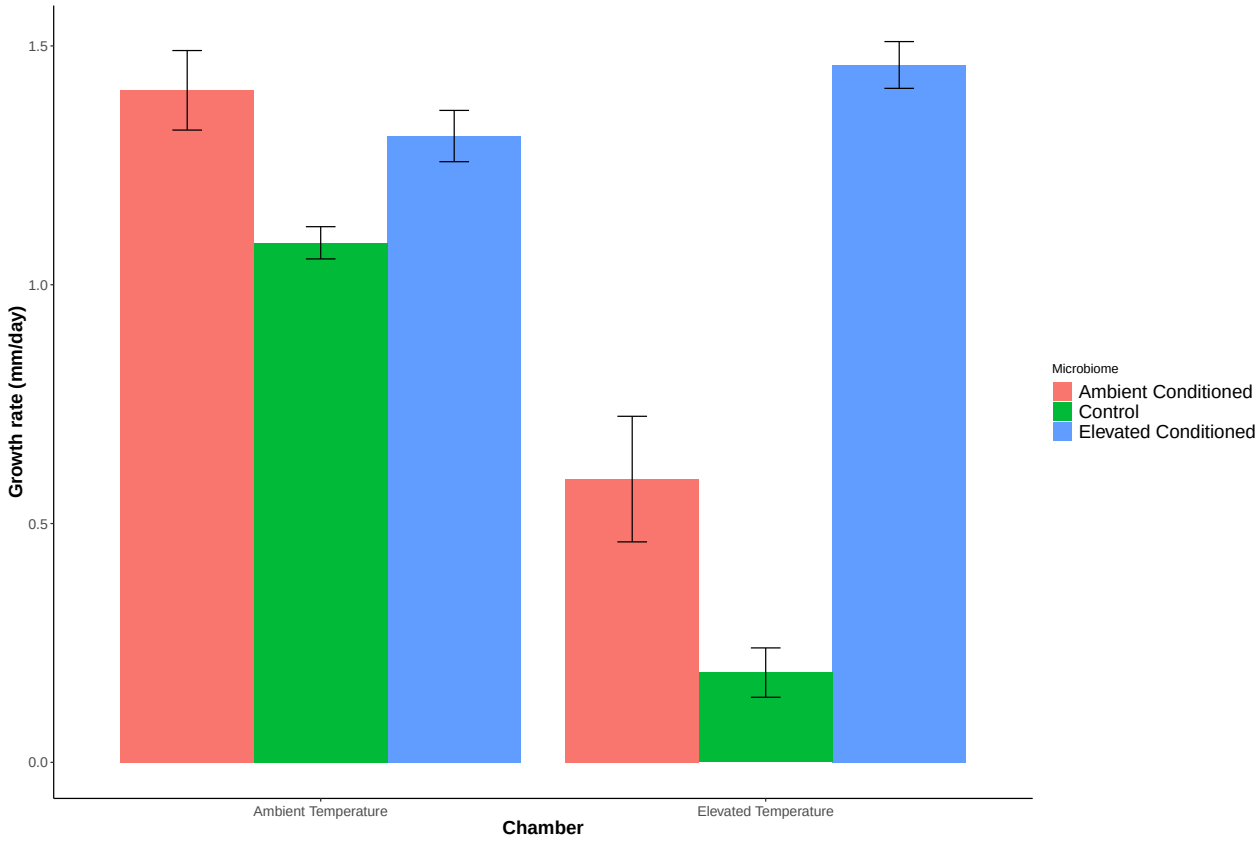
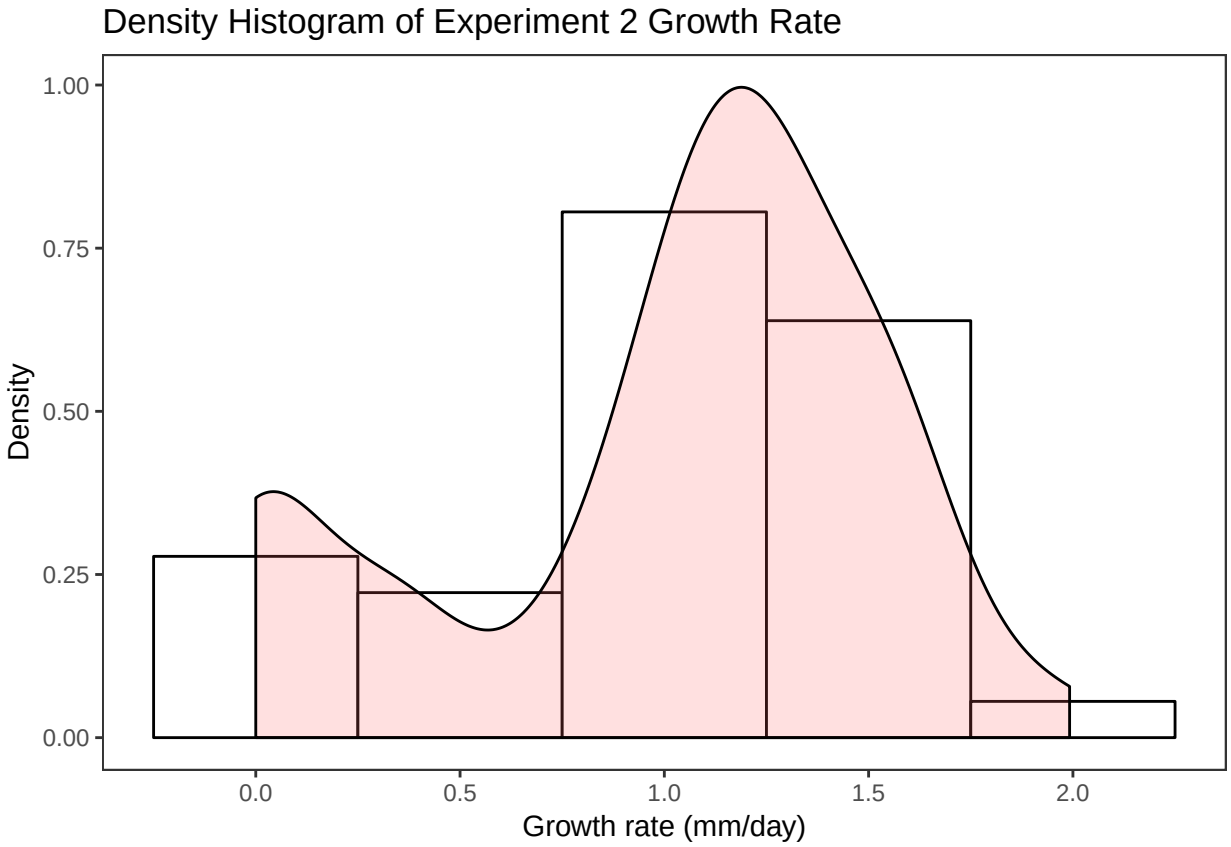


Figure 2: Fig. 2: Growth rate of conditioned microbiome 2017

Experiment 2 Summary Statistics



Exp 2 Rank Transform

```
# rank transform
exp2.rank <- rank(Exp2$Growth.rate.mmperday)

# merge ranked data
Exp2 <- cbind(Exp2, exp2.rank)

# two-way ANOVA with ranked data
ranked.exp2.aov <- aov(exp2.rank ~ Chamber + Microbe + Chamber:Microbe , data = Exp2)
Anova(ranked.exp2.aov, type = 3)
```

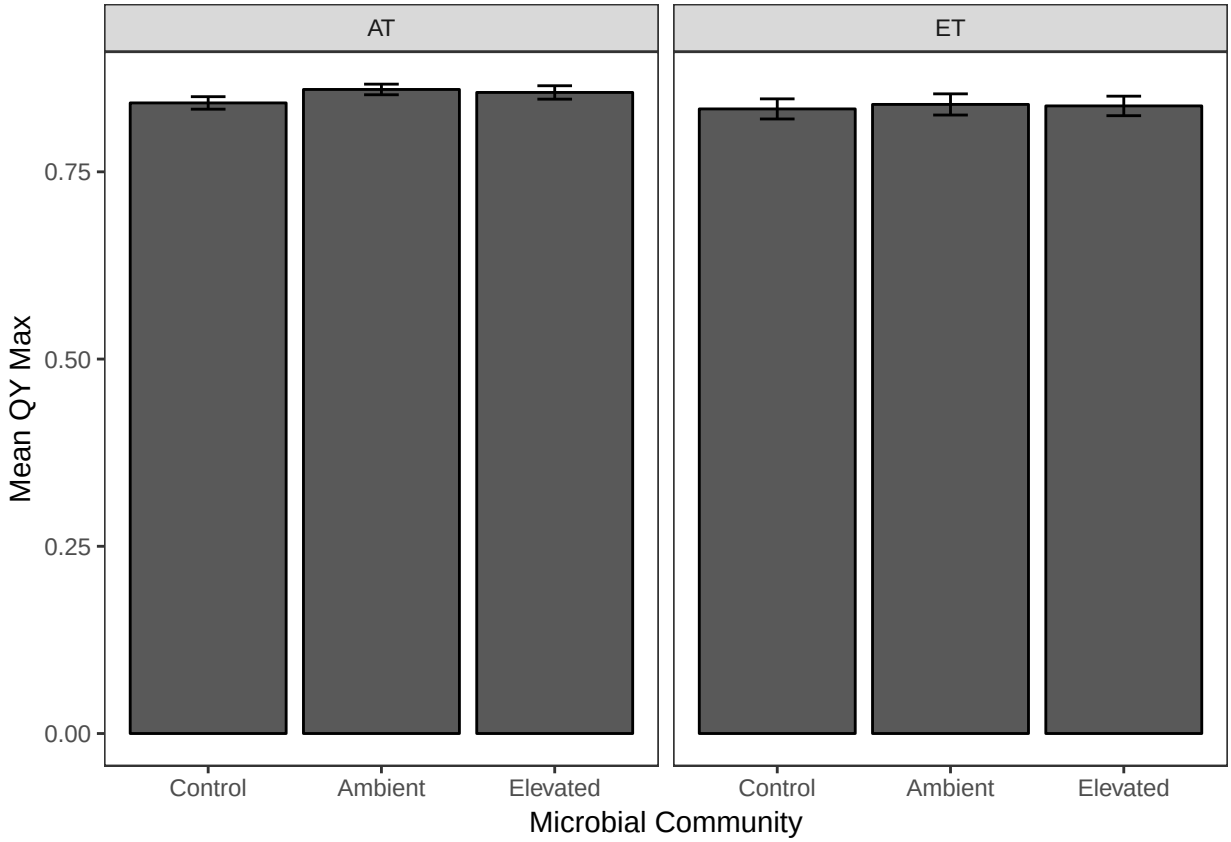
```
## Anova Table (Type III tests)
##
## Response: exp2.rank
##                 Sum Sq Df F value    Pr(>F)
## (Intercept)      32865  1 308.448 < 2.2e-16 ***
## Chamber           7633  1  71.634 3.923e-12 ***
## Microbe           2477  2  11.621 4.743e-05 ***
## Chamber:Microbe   6823  2  32.016 1.911e-10 ***
## Residuals         7032 66
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(ranked.exp2.aov)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = exp2.rank ~ Chamber + Microbe + Chamber:Microbe, data = Exp2)
##
## $Chamber
##            diff      lwr       upr p adj
## ET-AT -16.16667 -21.0243 -11.30903     0
##
## $Microbe
##                 diff       lwr       upr     p adj
## Control-AC -13.04167 -20.18634 -5.896992 0.0001288
## EC-AC       19.04167  11.89699 26.186341 0.0000001
## EC-Control  32.08333  24.93866 39.228008 0.0000000
##
## $`Chamber:Microbe`
##                              diff        lwr        upr     p adj
## ET:AC-AT:AC           -35.666667 -48.035372 -23.297961 0.0000000
## AT:Control-AT:AC      -19.250000 -31.618705  -6.881295 0.0003091
## ET:Control-AT:AC      -42.500000 -54.868705 -30.131295 0.0000000
## AT:EC-AT:AC            -4.000000 -16.368705   8.368705 0.9319339
## ET:EC-AT:AC             6.416667  -5.952039  18.785372 0.6511149
## AT:Control-ET:AC       16.416667   4.047961  28.785372 0.0030445
## ET:Control-ET:AC       -6.833333 -19.202039   5.535372 0.5875372
## AT:EC-ET:AC            31.666667  19.297961  44.035372 0.0000000
## ET:EC-ET:AC            42.083333  29.714628  54.452039 0.0000000
## ET:Control-AT:Control -23.250000 -35.618705 -10.881295 0.0000090
## AT:EC-AT:Control       15.250000   2.881295  27.618705 0.0072775
## ET:EC-AT:Control       25.666667  13.297961  38.035372 0.0000009
## AT:EC-ET:Control       38.500000  26.131295  50.868705 0.0000000
## ET:EC-ET:Control       48.916667  36.547961  61.285372 0.0000000
## ET:EC-AT:EC            10.416667  -1.952039  22.785372 0.1475808
```

Experiment 1 QY

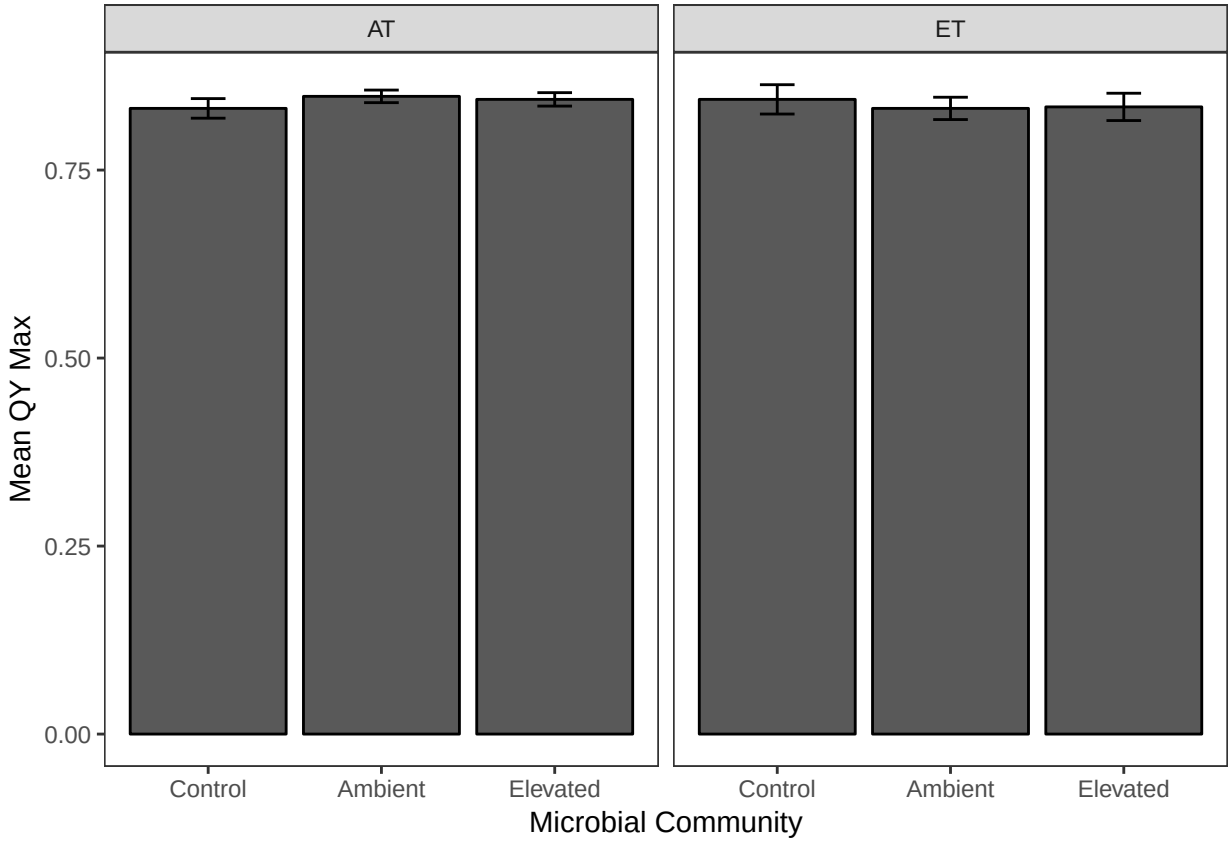
Week 0




```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.0 by Chamber
## Kruskal-Wallis chi-squared = 9.7587, df = 1, p-value = 0.001785
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.0 by Microbe
## Kruskal-Wallis chi-squared = 4.8352, df = 2, p-value = 0.08914
```

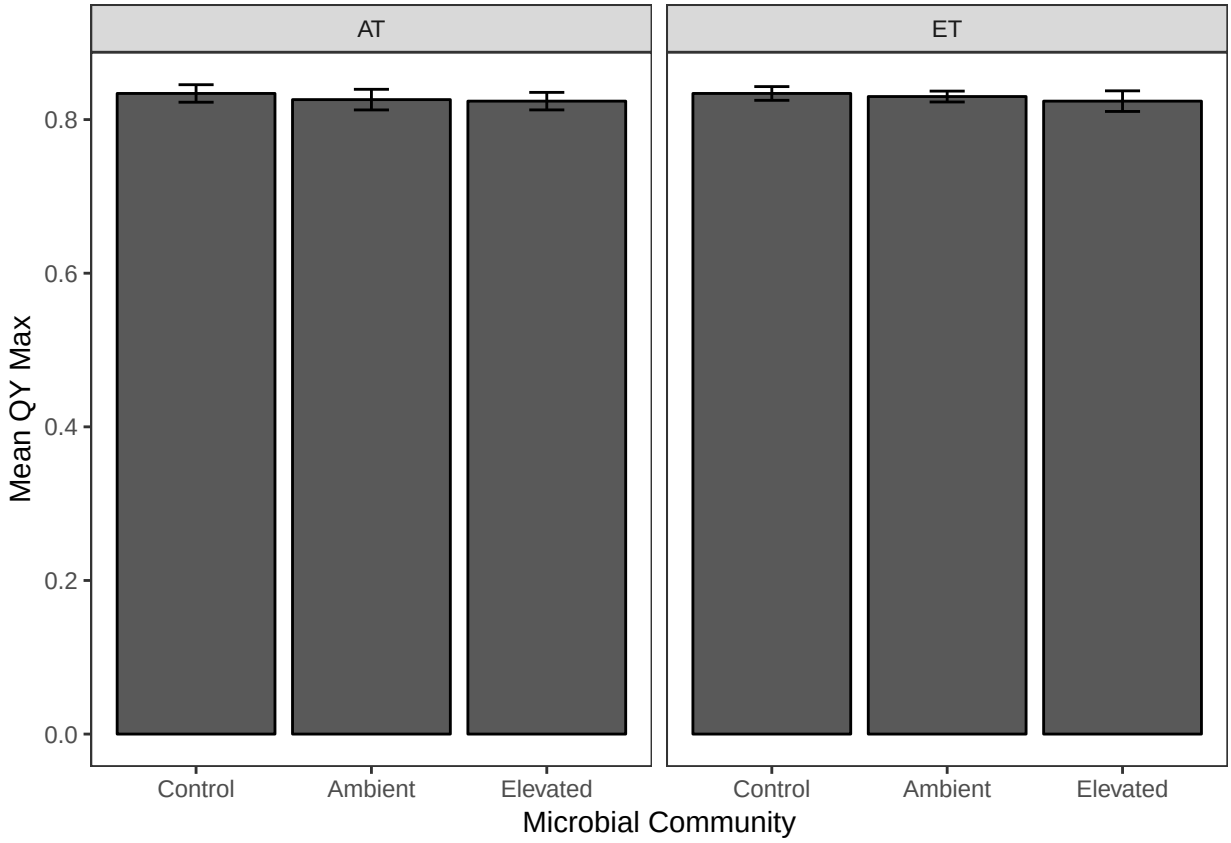
Week 1




```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.1 by Chamber
## Kruskal-Wallis chi-squared = 0.4667, df = 1, p-value = 0.4945
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.1 by Microbe
## Kruskal-Wallis chi-squared = 0.035549, df = 2, p-value = 0.9824
```

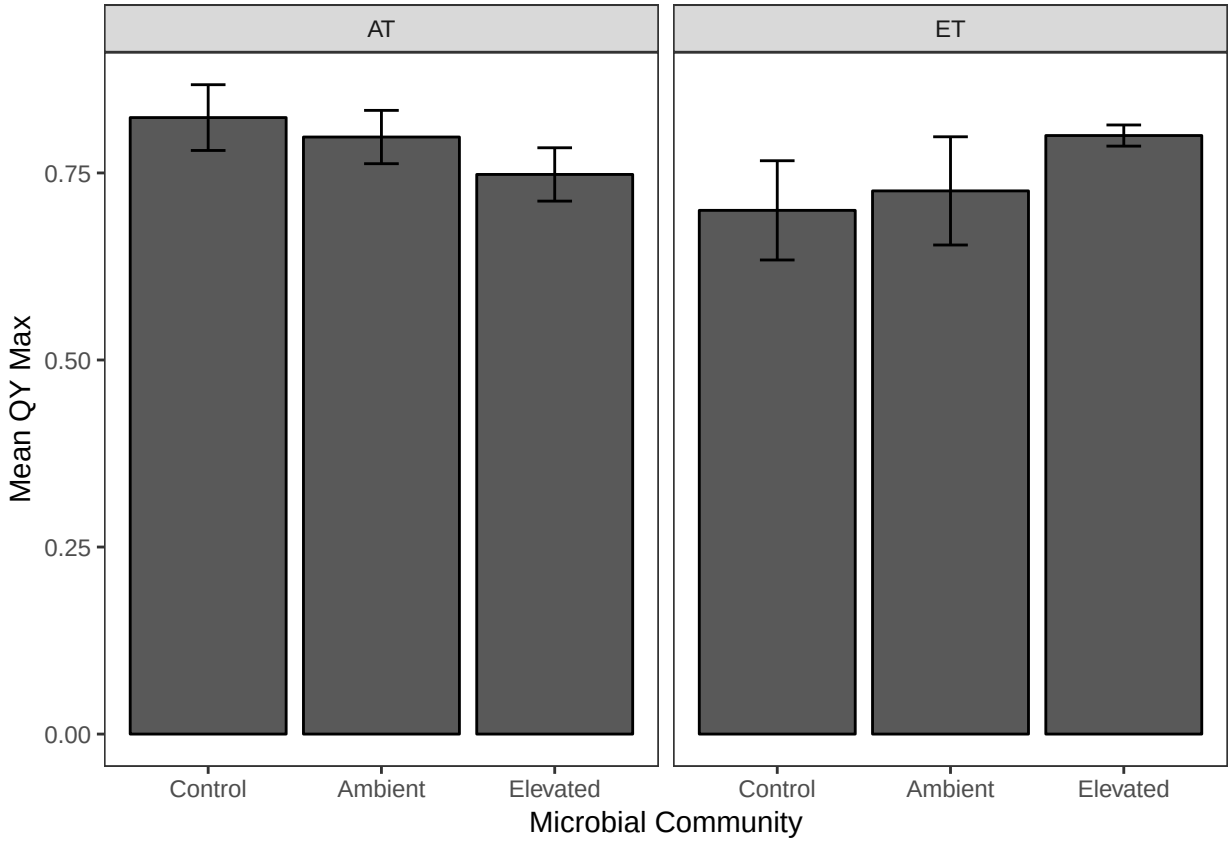
Week 2




```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.2 by Chamber
## Kruskal-Wallis chi-squared = 0.15174, df = 1, p-value = 0.6969
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.2 by Microbe
## Kruskal-Wallis chi-squared = 3.2822, df = 2, p-value = 0.1938
```

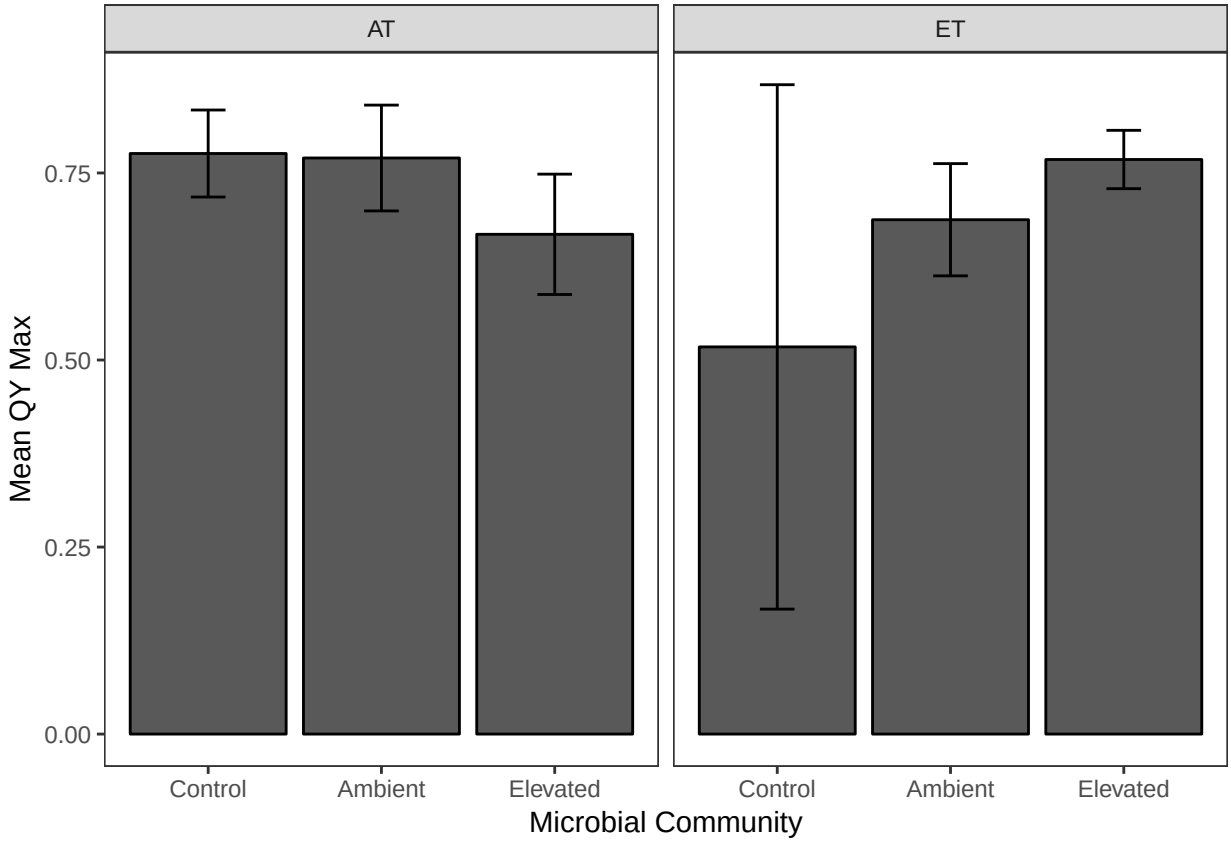
Week 3




```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.3 by Chamber
## Kruskal-Wallis chi-squared = 2.1126, df = 1, p-value = 0.1461
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.3 by Microbe
## Kruskal-Wallis chi-squared = 0.38338, df = 2, p-value = 0.8256
```

Week 4



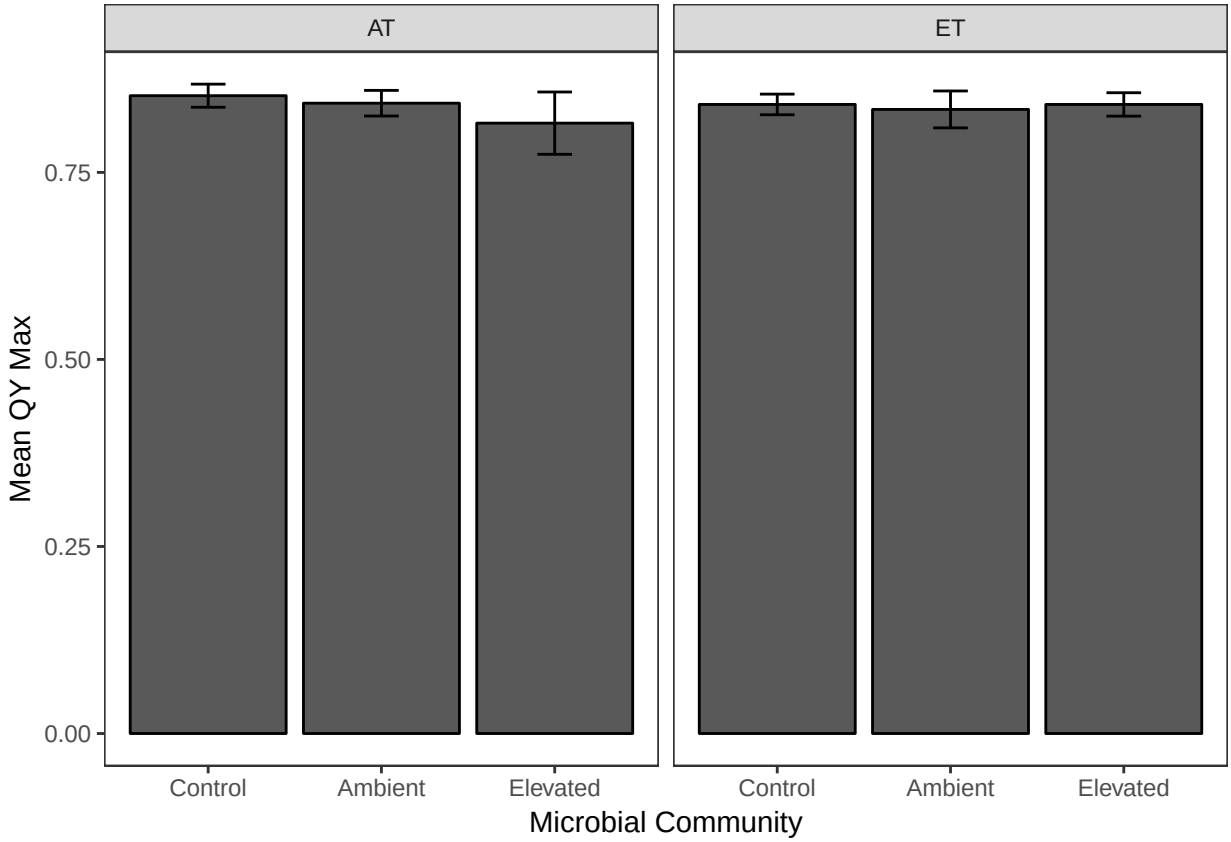

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.4 by Chamber
## Kruskal-Wallis chi-squared = 0.54578, df = 1, p-value = 0.46
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.4 by Microbe
## Kruskal-Wallis chi-squared = 0.046976, df = 2, p-value = 0.9768
```

In general, there was no significant main effects of the microbial community on QY_Max across the four weeks of Experiment 1. The temperature of the chamber, not the microbial community, appeared to regulate QY_Max heavily in Weeks 0 and 1, though only until Week 3. As the experiment progressed, cross-over interactions between chamber temperature and microbial communities led to significant interaction terms ($p < 0.05$), despite no main effect of the microbial community. This suggests that QY_Max likely depends on how microbial communities influence moss within each chamber despite hte lack of a main effect.

Experiment 2 QY

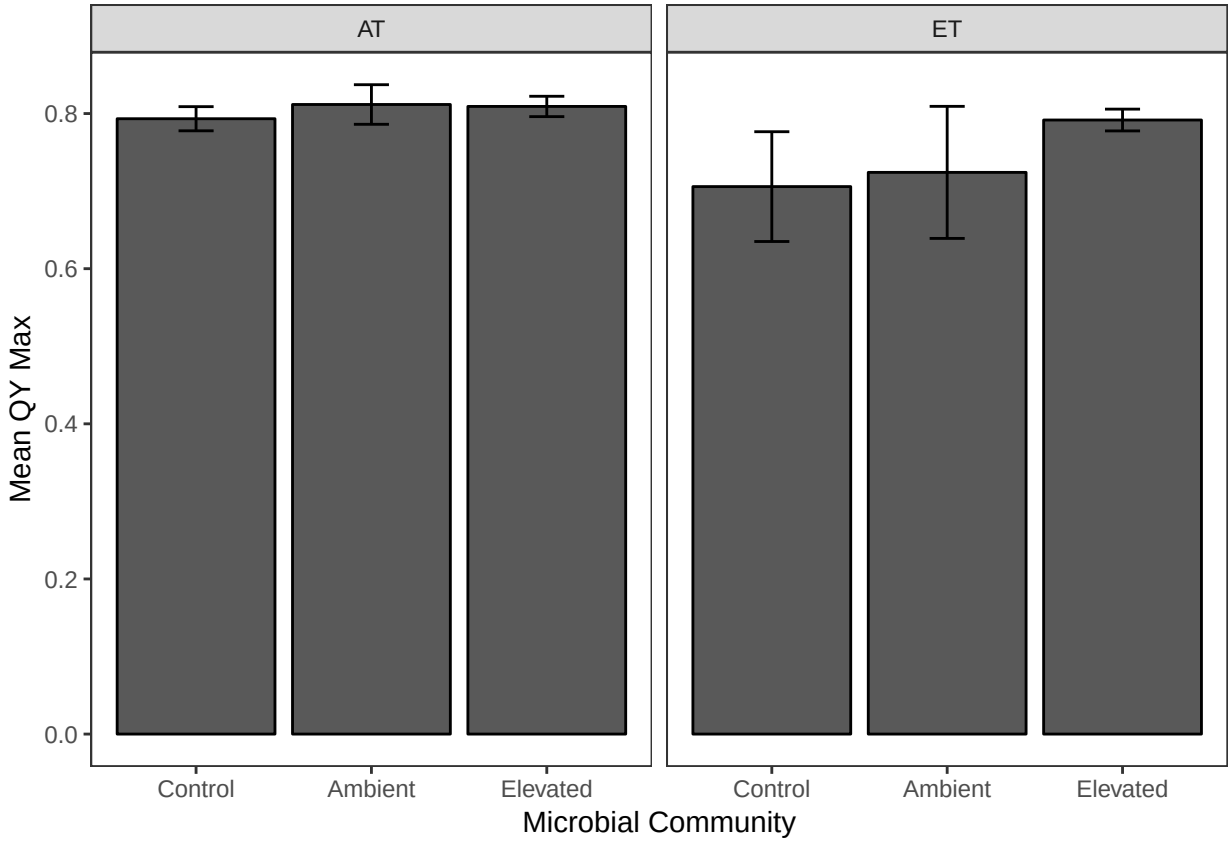
Week 0




```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.0 by Chamber
## Kruskal-Wallis chi-squared = 0.006447, df = 1, p-value = 0.936
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.0 by Microbe
## Kruskal-Wallis chi-squared = 6.9125, df = 2, p-value = 0.03155
```

Week 1

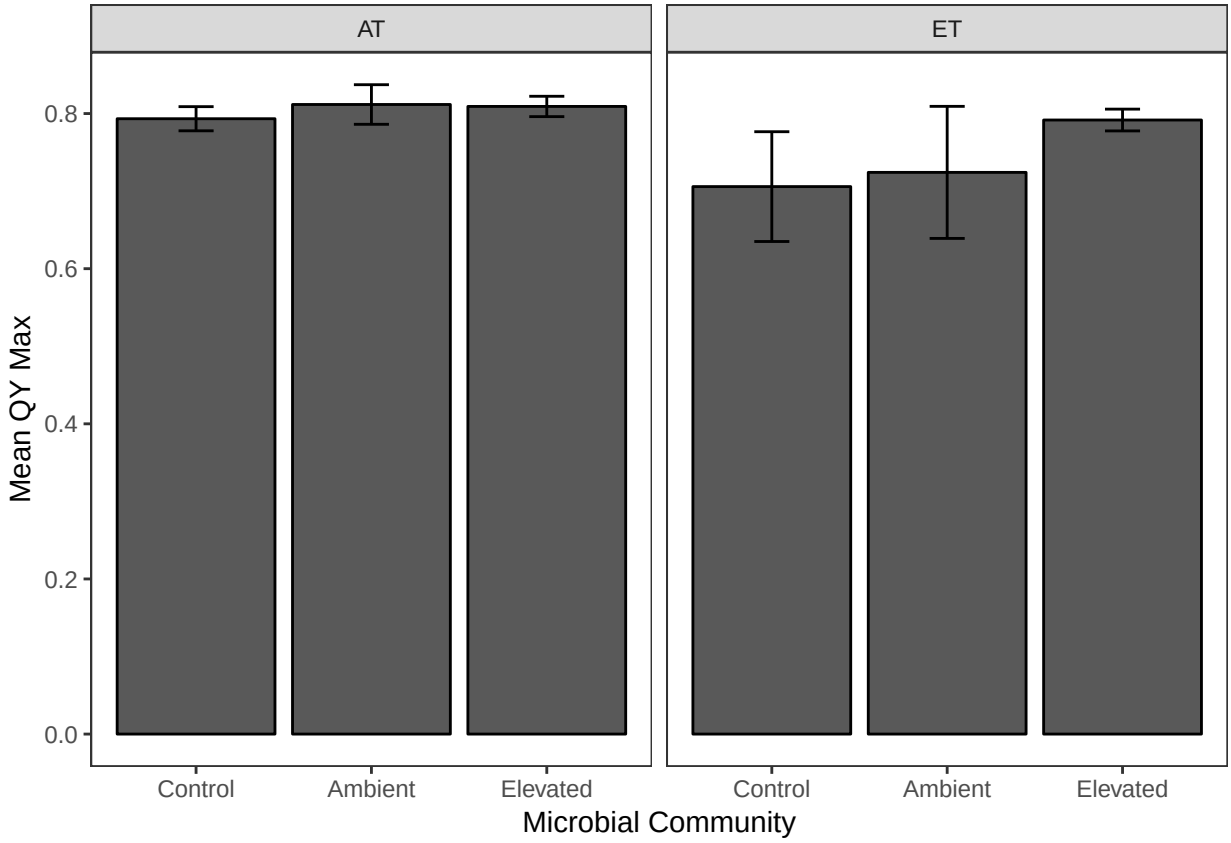



```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.1 by Chamber
## Kruskal-Wallis chi-squared = 0.59473, df = 1, p-value = 0.4406
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.1 by Microbe
## Kruskal-Wallis chi-squared = 8.163, df = 2, p-value = 0.01688
```

```
##     Group Letter MonoLetter
## 1      AC      a          a
## 2 Control      b           b
## 3      EC      b           b
```

Week 2

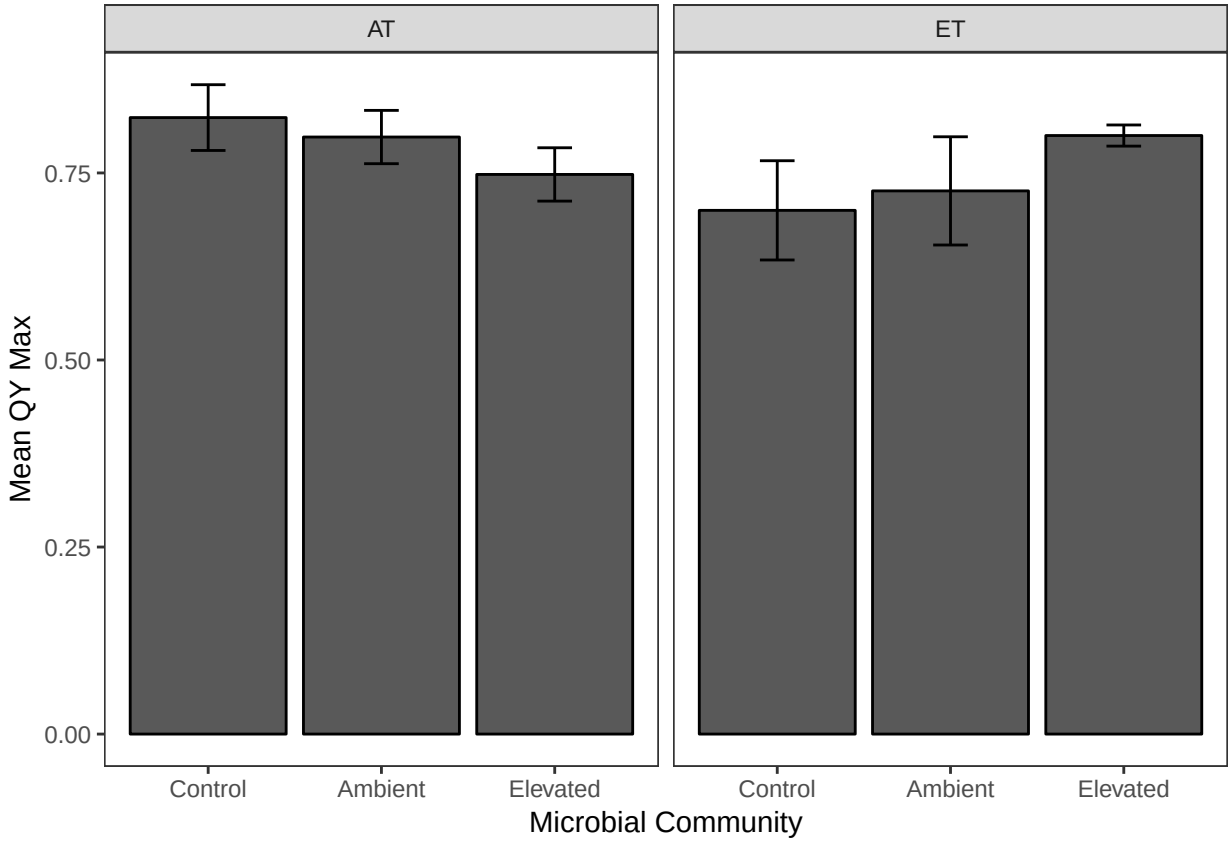



```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.2 by Chamber
## Kruskal-Wallis chi-squared = 23.211, df = 1, p-value = 1.452e-06
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.2 by Microbe
## Kruskal-Wallis chi-squared = 10.392, df = 2, p-value = 0.005538
```

```
##     Group Letter MonoLetter
## 1      AC     ab         ab
## 2 Control      a         a
## 3      EC      b          b
```

Week 3




```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.3 by Chamber
## Kruskal-Wallis chi-squared = 16.886, df = 1, p-value = 3.97e-05
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.3 by Microbe
## Kruskal-Wallis chi-squared = 4.1587, df = 2, p-value = 0.125
```

The conditioned microbiome was a primary determinant of QY_Max in Experiment 2. With the exception of the last week (Week 3), the microbiome was the most significant factor in QY_Max. In contrast to Experiment 1, there was only one instance where there appeared to be a strong interaction between the conditioned microbiome and the chamber (Week 2). Typically, it was the microbiome that drove differences in QY_Max between and within both chambers.