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

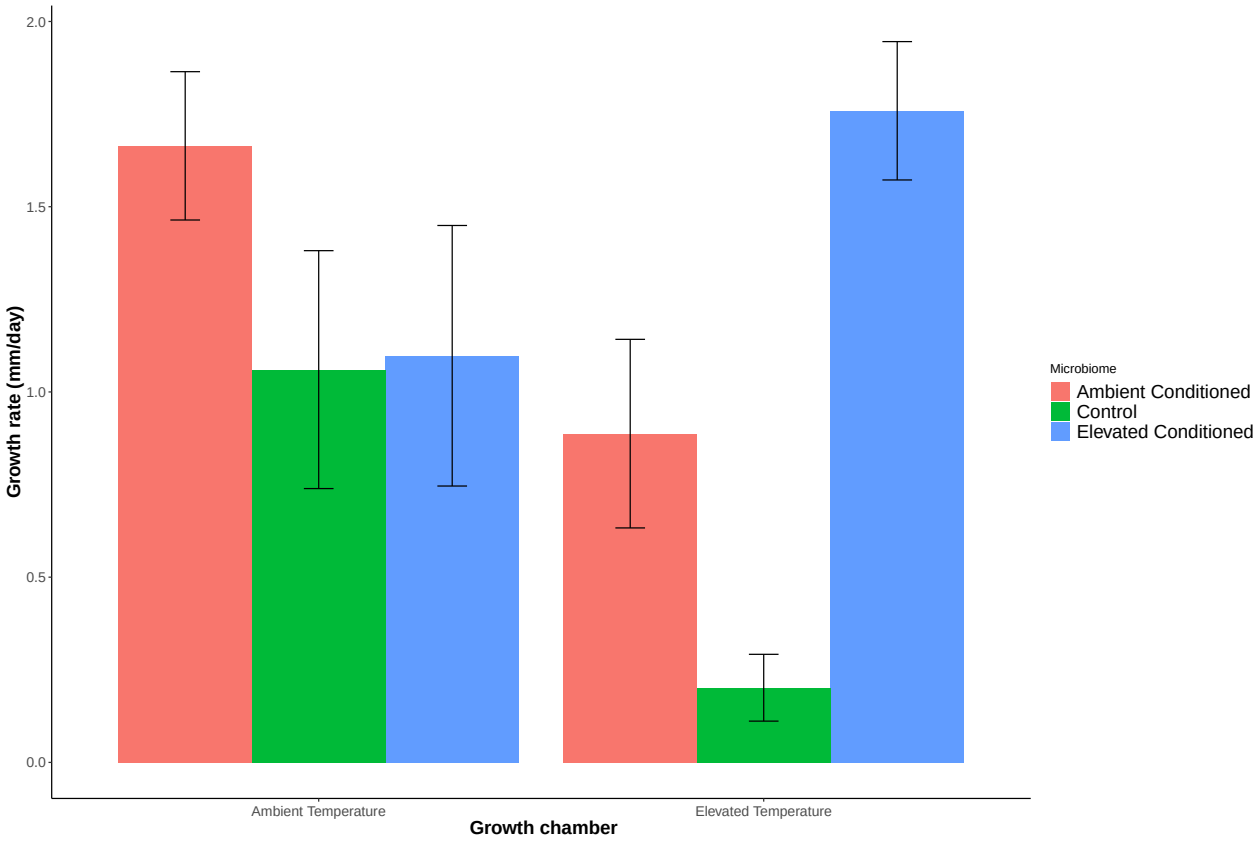


Figure 1: Fig. 1: Growth rate of conditioned microbiome 2016

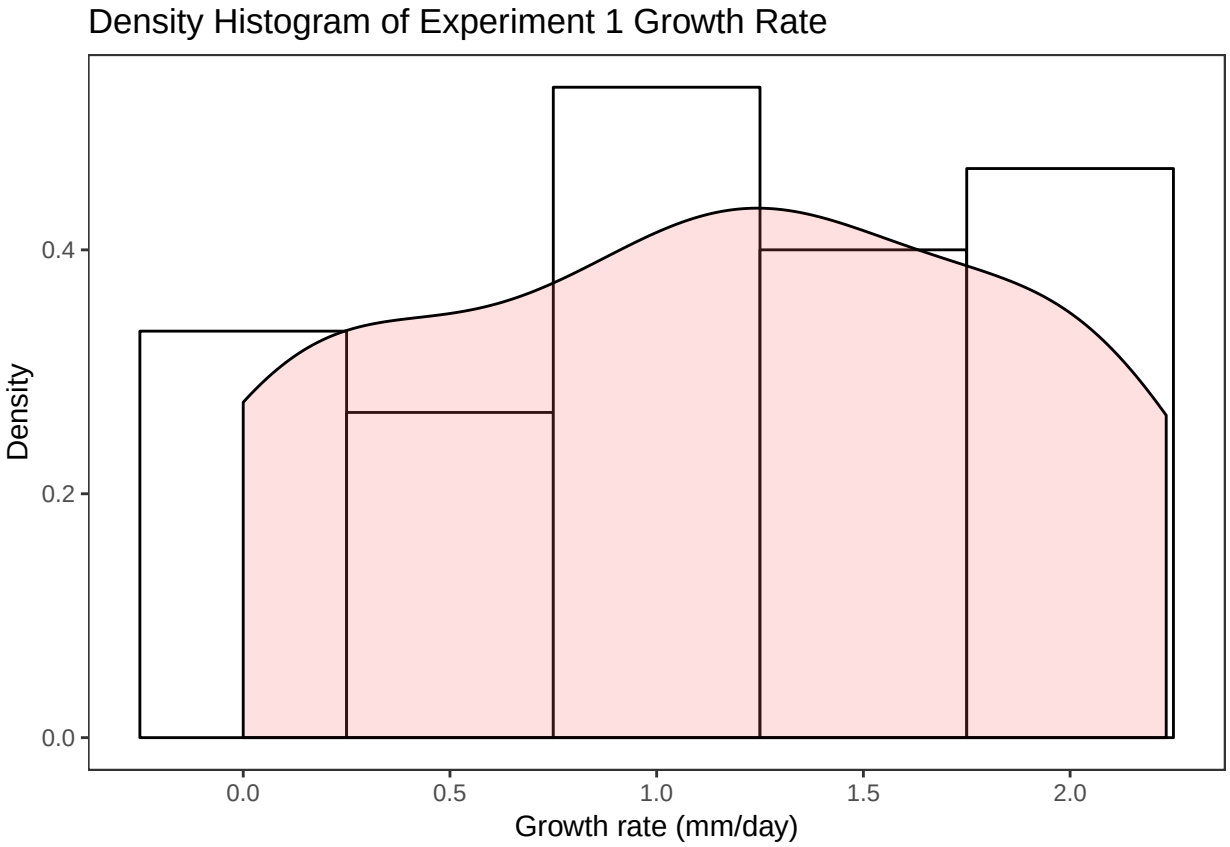
Measurements taken
Experiment 1 - Fluorcam weekly, area growth, 16S profile, RNA not extractable, pH measured
Experiment 2 - Fluorcam weekly, area growth, 16S profile, RNAseq, final pH, 15N and ARA

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
```

Growth data was rank transformed prior to performing a standard two-way ANOVA. Growth rate in each chamber was dependant on microbial community ($p < 0.01$). Within the Ambient Temperature (AT) chamber there were no differences in moss growth rate among the three microbial treatments. Growth rate of moss inoculated with an Ambient Temperature Conditioned microbiome (AC) did not differ from the control or moss inoculated with an Elevated Temperature Conditioned microbiome (EC). However, within the Elevated Temperature (ET) chamber, moss growth rate was significantly different between EC and control ($p < 0.01$). Moss grown with AC and the control had relatively similar growth rates, suggesting that changes in growth rate are not simply due to having a microbiome. Rather, the advantage does appear to be driven by a microbiome pre-adapted to elevated temperatures.

Between the two chambers, there were no differences in growth rate of AC, Control, and EC. For example, moss grown without a microbiome had a similar growth rate regardless of temperature.

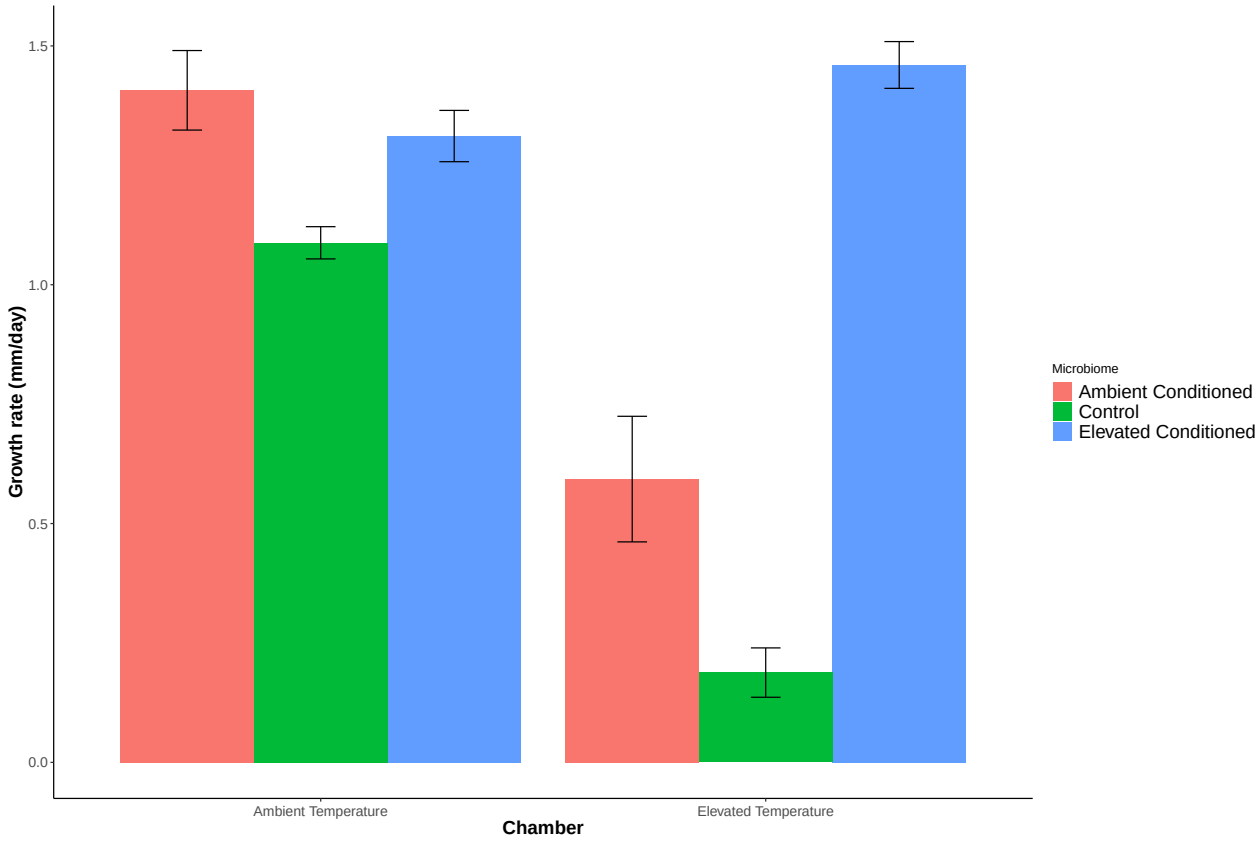
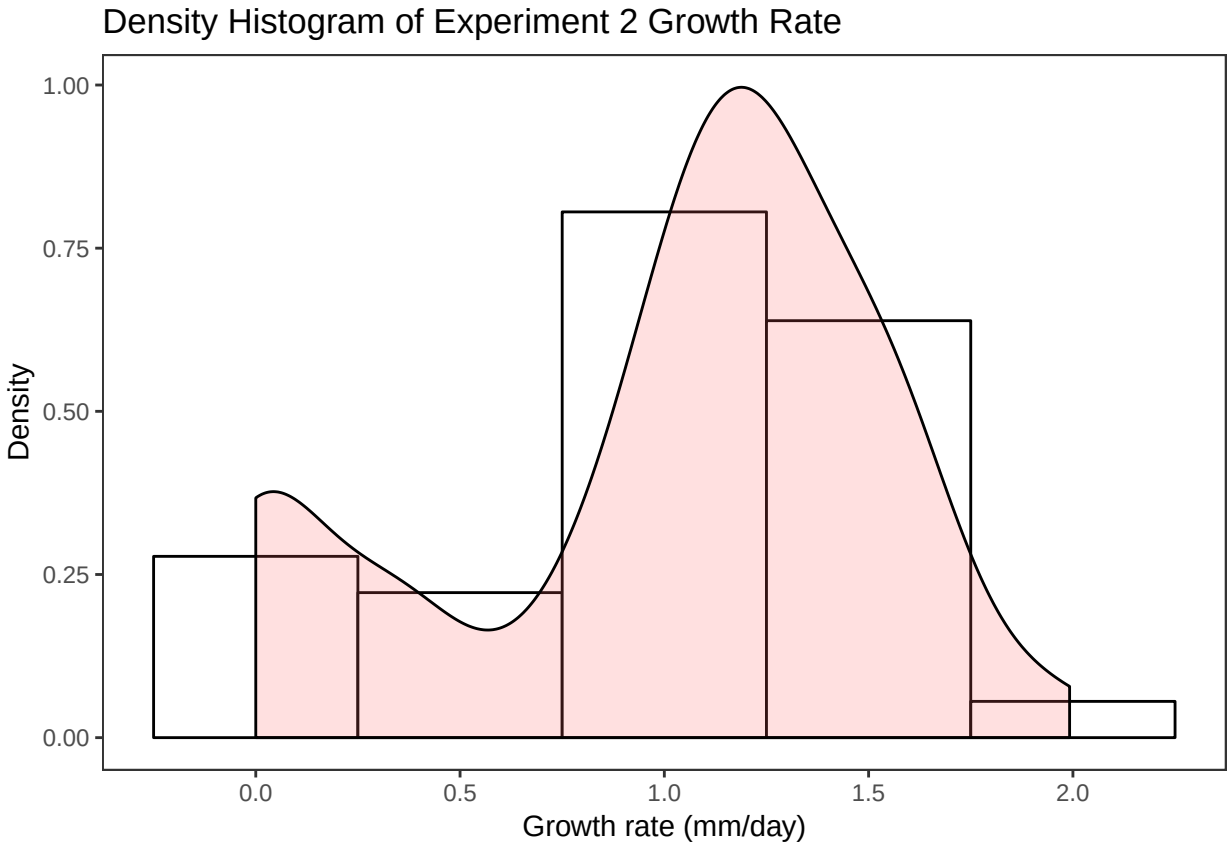


Figure 2: Fig. 2: Growth rate of conditioned microbiome 2017

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
```

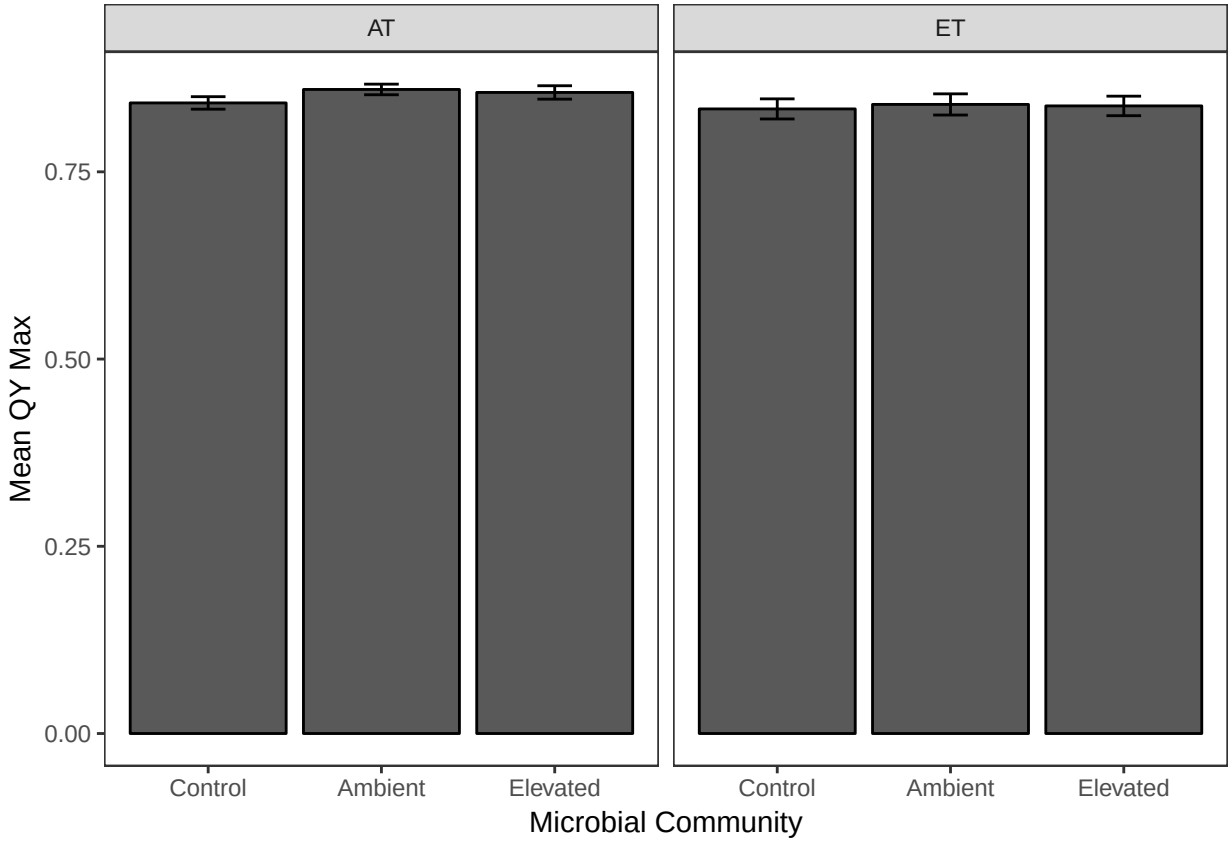
Differences in moss growth rate were based on the influence of microbial treatments in the chambers ($p < 0.001$). Within the ambient temperature chamber (AT), moss grown with AC and moss grown with EC increased growth rate relative to the control ($p < 0.05$). However, there were no differences in growth between AC and EC suggesting that under ambient temperature, microbial inoculation increases growth irregardless of microbial community diversity.

Elevated temperatures differentiated the influence of the microbial community on moss growth rate. Moss inoculated with a microbial community conditioned in elevated temperatures had high rates of growth despite elevated temperatures with respect to moss grown with ambient conditioned microbiomes (AC) and the control ($p < 0.001$). Furthermore, AC moss and control moss did not differ in growth rate suggesting that it is specifically a pre-adapted microbiome that imparts moss with the capacity to tolerate elevated temperatures.

When comparing microbial treatments across chambers, it is apparent that elevated temperatures severely decreased growth of moss with AC and moss control. Only moss inoculated with EC showed no difference in growth regardless of ambient or elevated temperatures. This indicates that the presence of microbial members capable of surviving in elevated temperatures is generally beneficial, but is especially critical in rising temperatures.

Experiment 1 QY

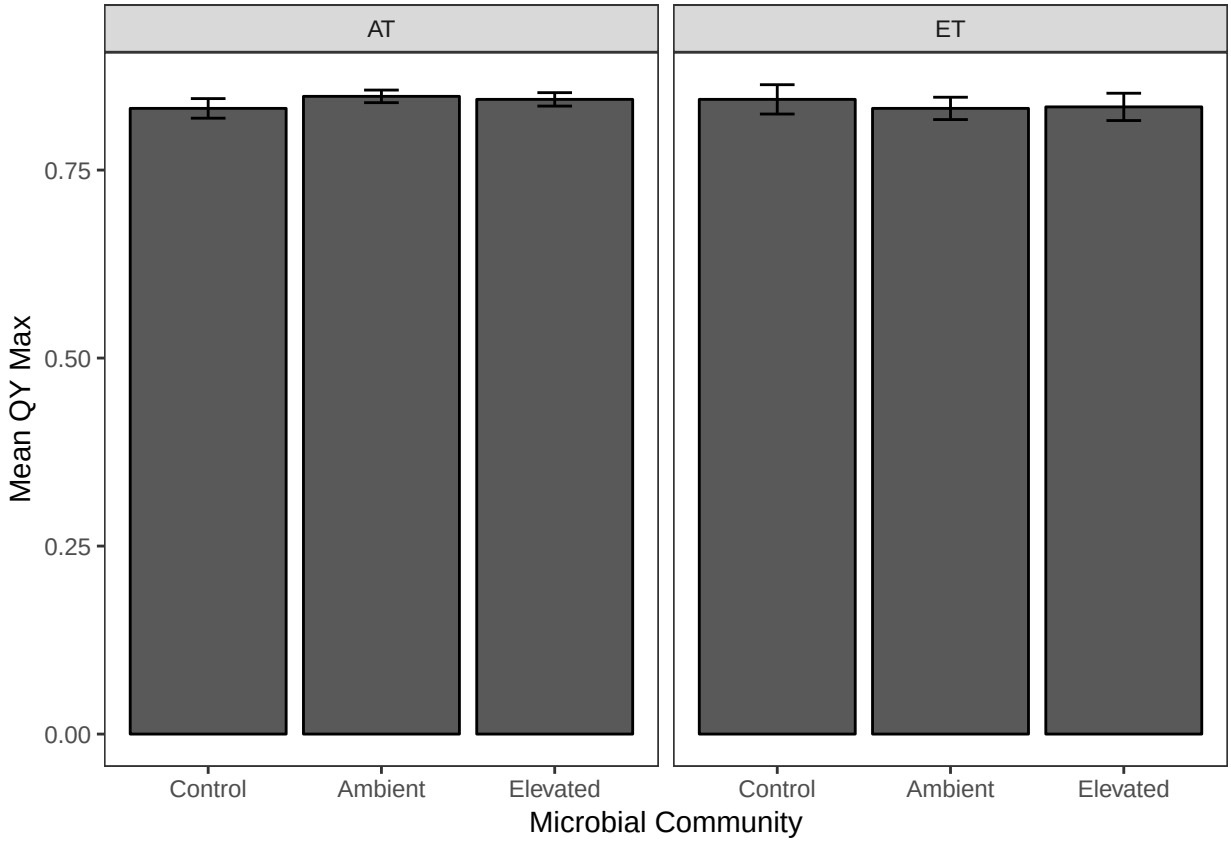
Week 0




```
##
##  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
```

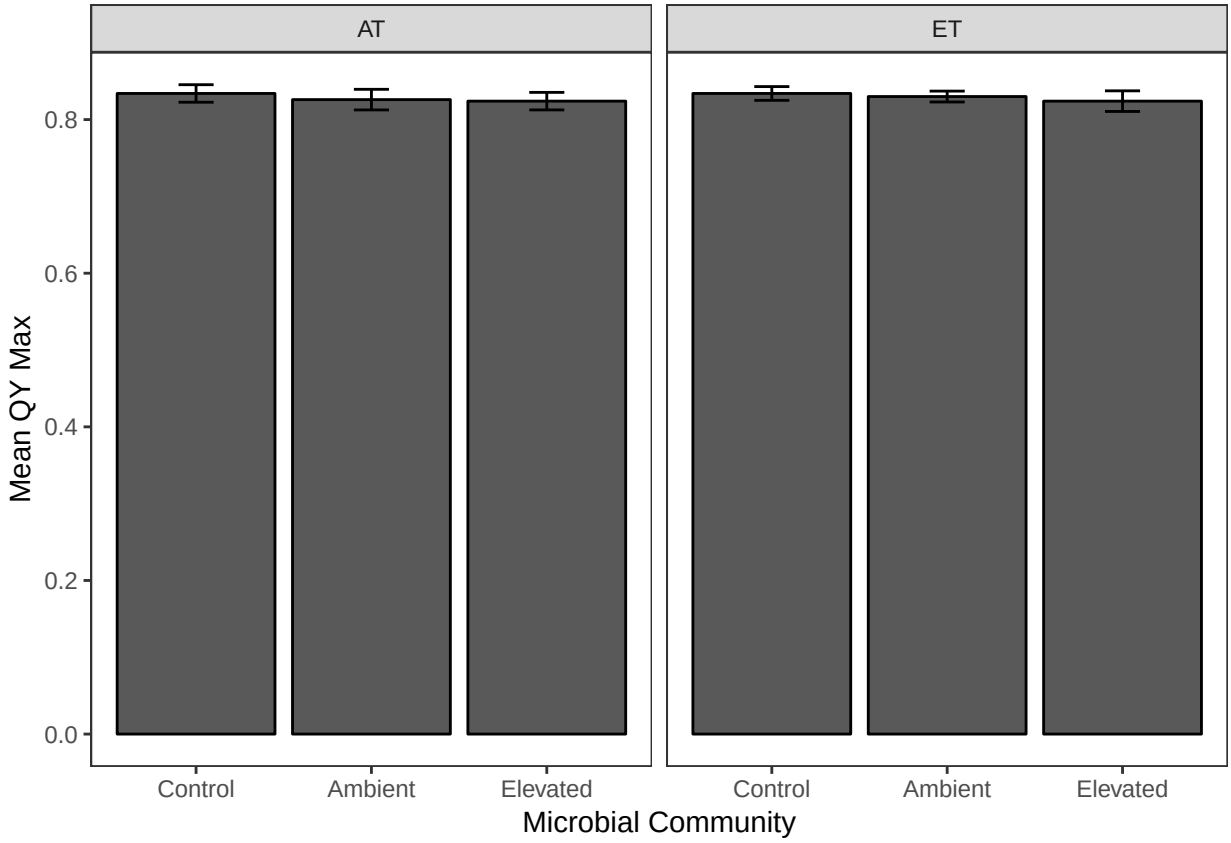
Week 1




```
##
##  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
```

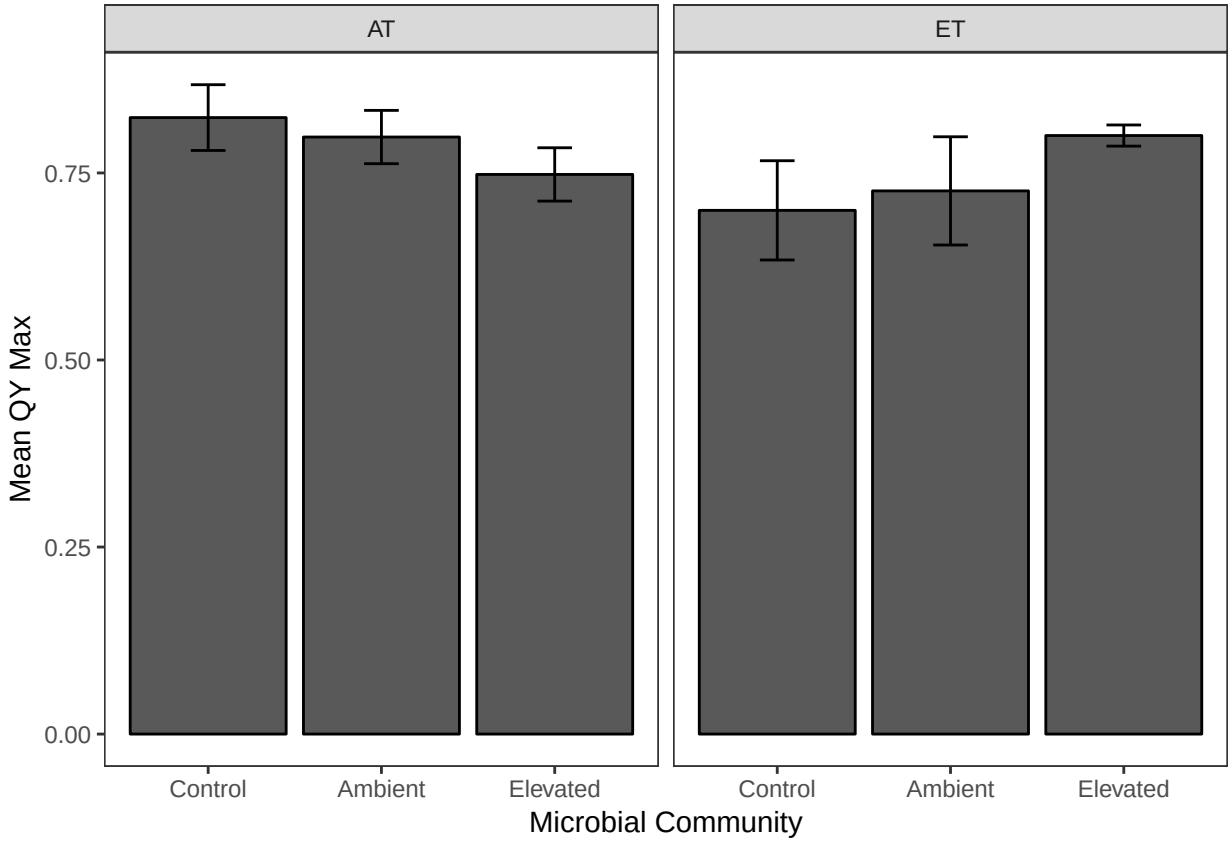
Week 2




```
##
##  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
```

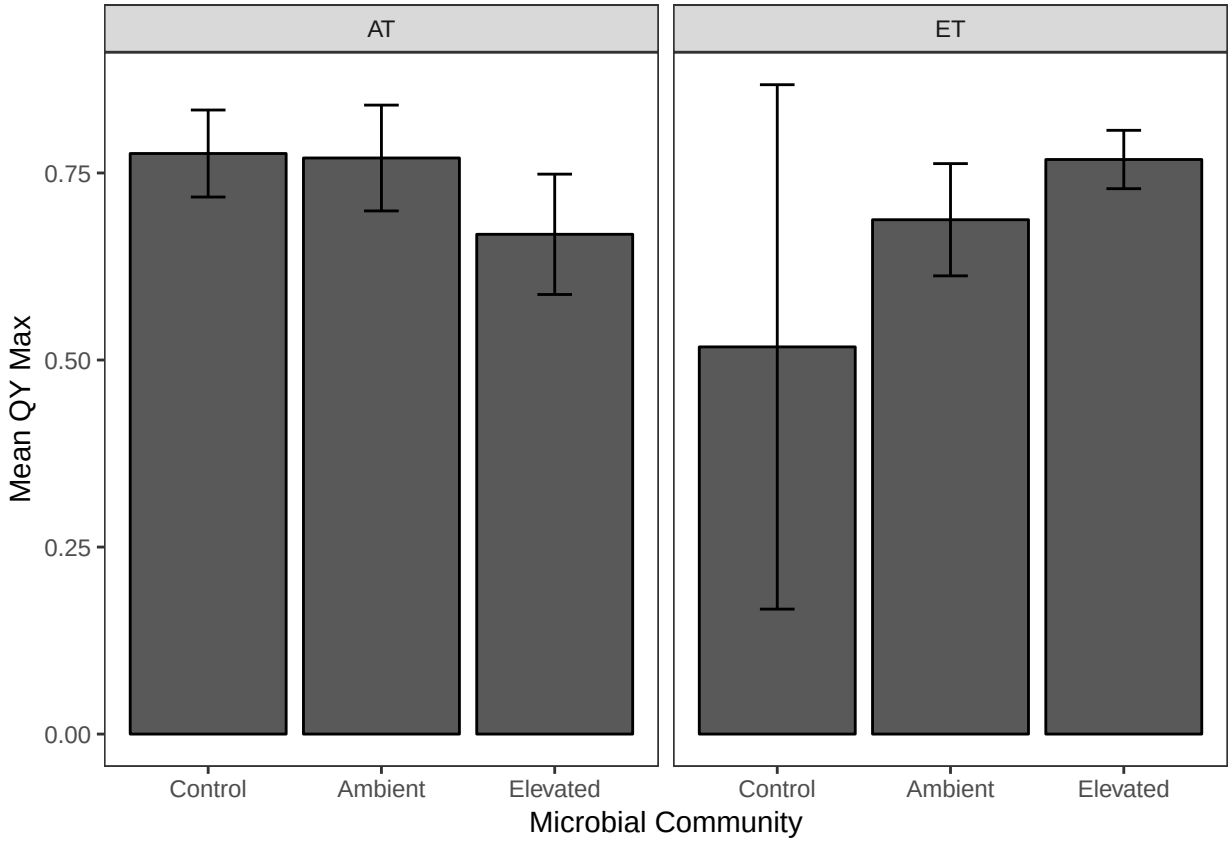
Week 3




```
##
##  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



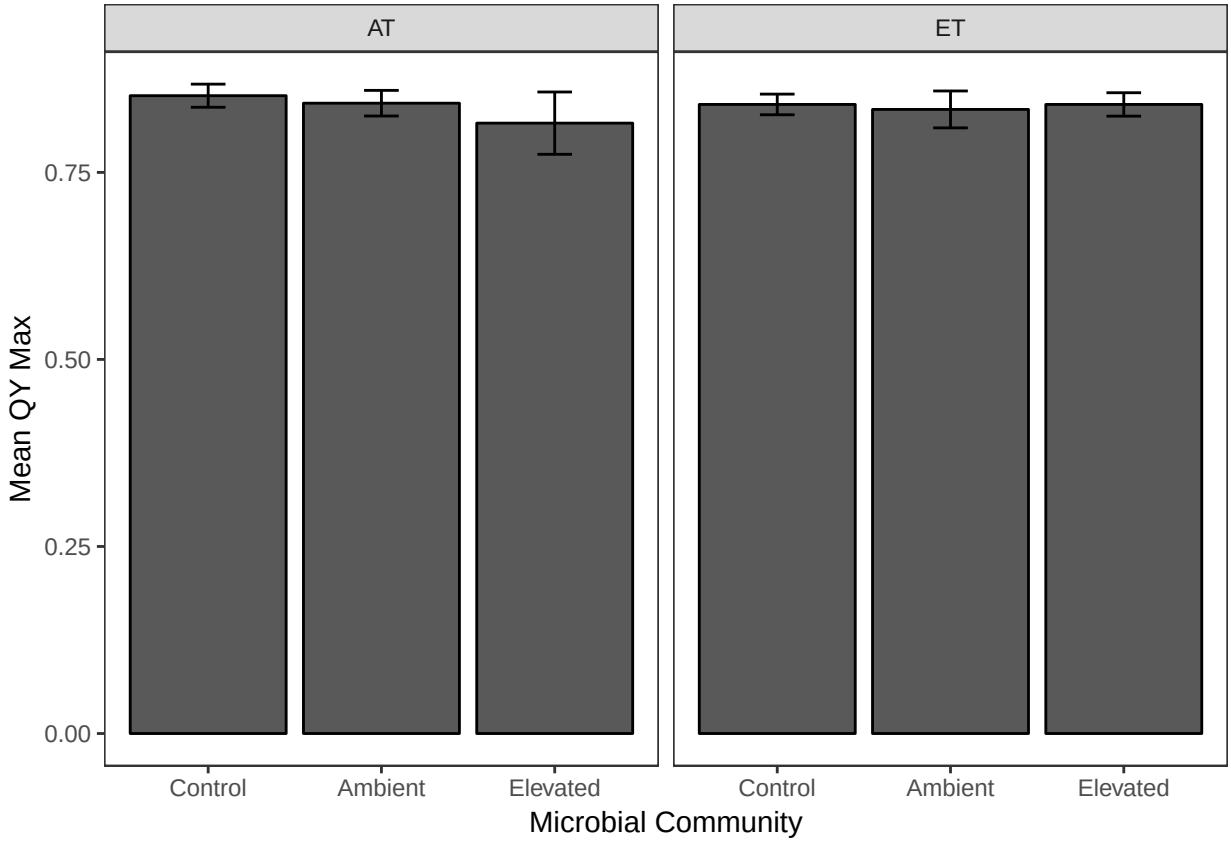

```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.4 by Chamber
## Kruskal-Wallis chi-squared = 0.54578, df = 1, p-value = 0.46
```

```
##
##  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

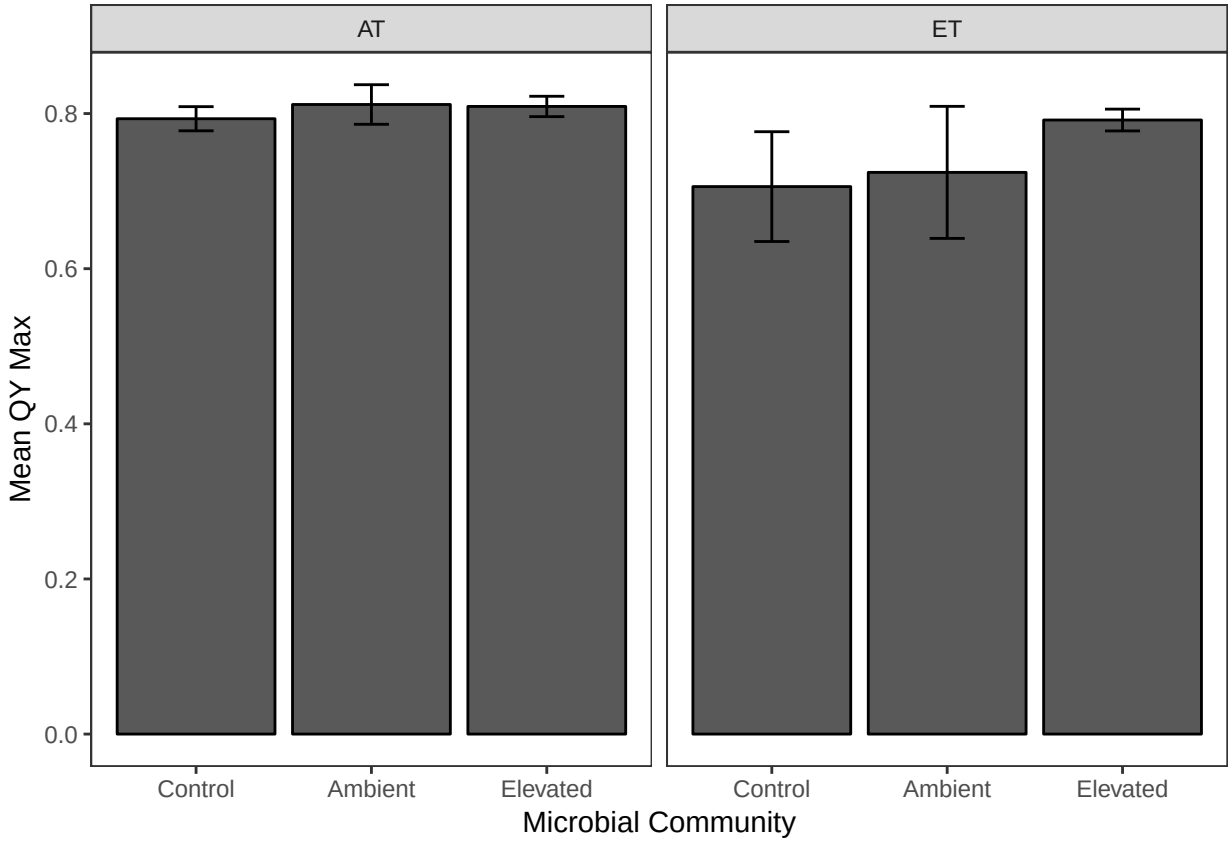
Week 0




```
##
##  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

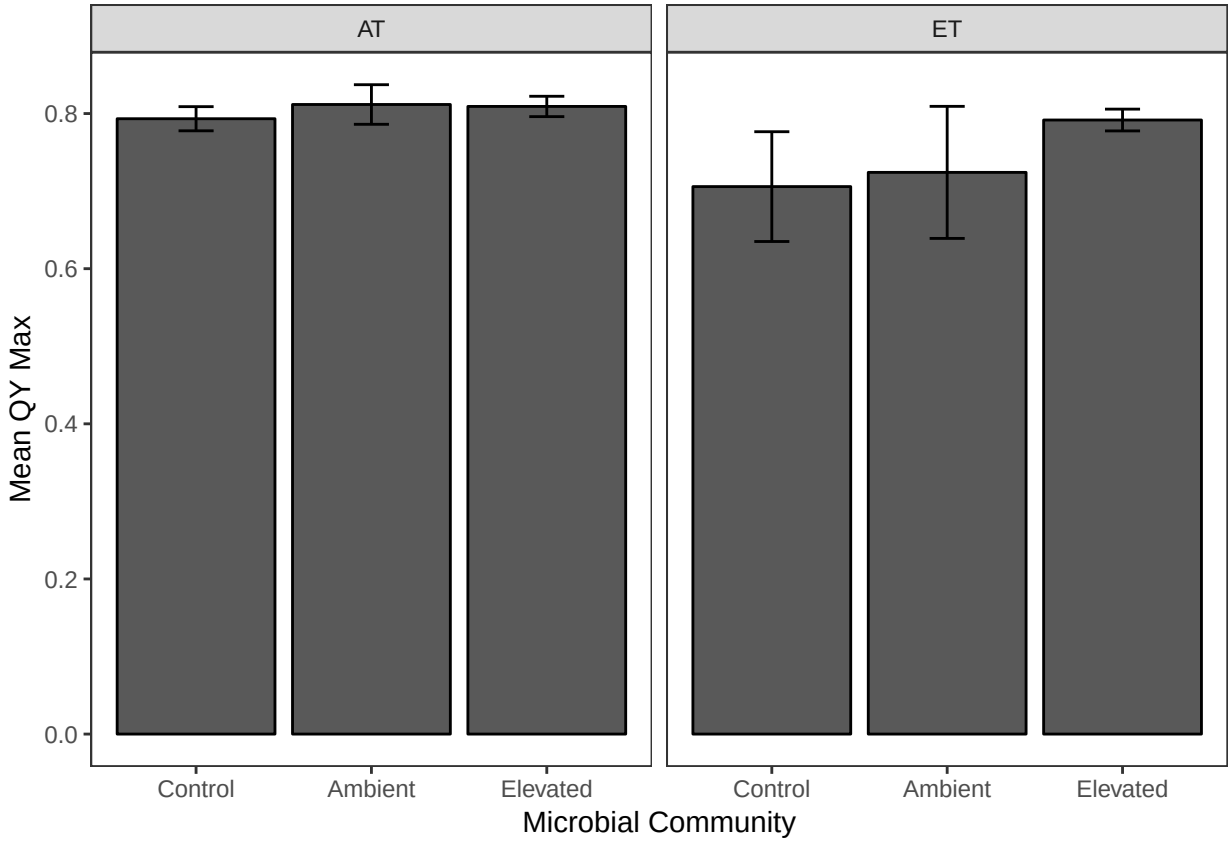



```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.1 by Chamber
## Kruskal-Wallis chi-squared = 0.59473, df = 1, p-value = 0.4406
```

```
##
##  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

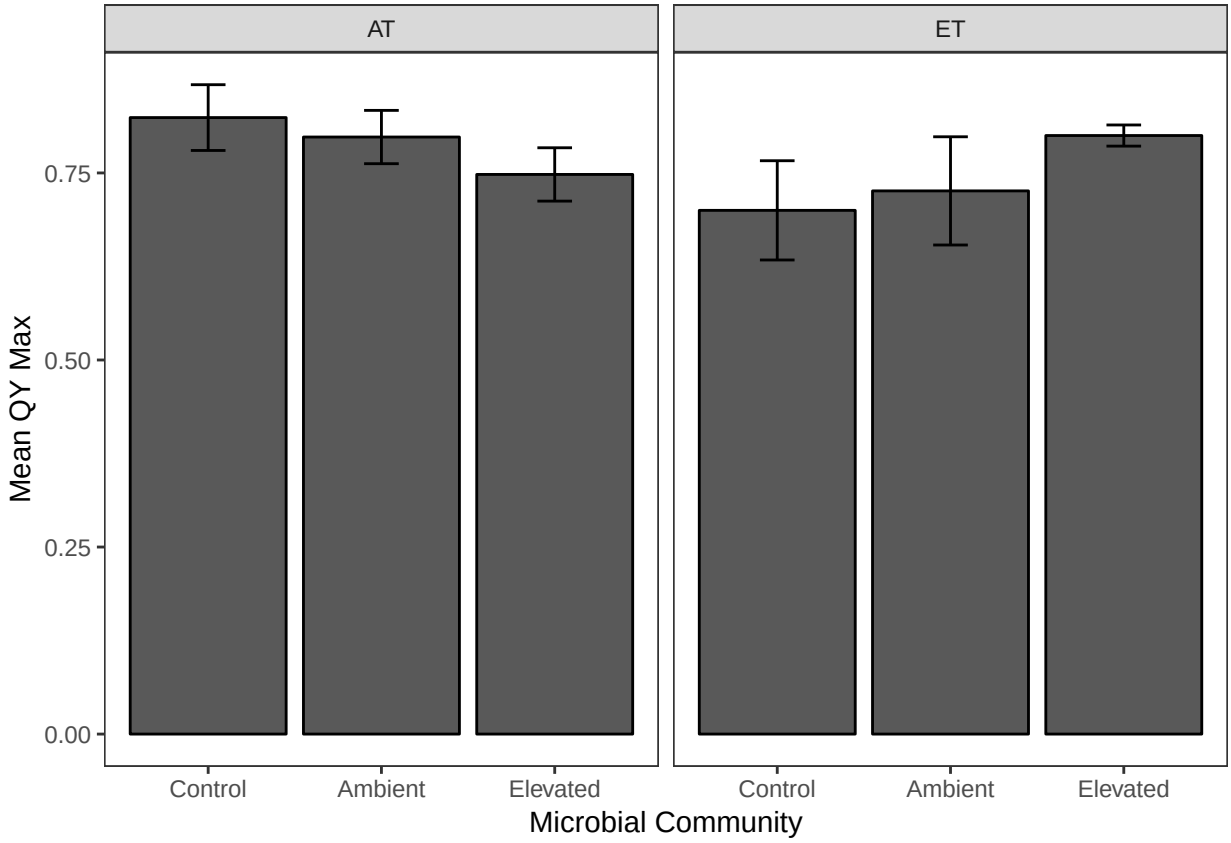



```
##
##  Kruskal-Wallis rank sum test
##
## data:  QY_max.2 by Chamber
## Kruskal-Wallis chi-squared = 23.211, df = 1, p-value = 1.452e-06
```

```
##
##  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.