3A & B: AE Biofilm

Caitlin Nordheim-Maestas

# Experiment 3: Assessment of AE biofilms’ inhibitory impact on Bd

Data wrangling and analysis the AE biofilms using the SFEB (San Francisco East Bay) biofilms for part A (nicknamed “nine sites” in our lab) and the follow up experiment part B with SBNCOS (Santa Barbara North Campus Open Space) biofilm samples. These are the data presented in Figure 4A and 4B in the manuscript, and information is under the section “Experiment 3”.

# Load in data and libraries

## read in and clean data  
library(tidyverse) # for cleaning and viewing data  
library(gt) # pretty stats tables  
library(broom) # cleaning for gt  
library(here) # for importing data  
library(car) # stats tests  
library(multcompView) # view cld  
library(multcomp) # stats  
library(nlme) # mixed effects models  
library(emmeans) # for pairwise comparisons, especially on mixed effects models and glms  
library(ggpubr) # for making ggqq plot  
library(patchwork) # for combining figures  
  
# import data for SFEB  
ns\_biofilm\_bd <- read.csv(here("data", "nine-sites-biofilm-on-Bd - Sheet1.csv"))  
# import data for SBNCOS  
fig\_3b\_raw <- read.csv(here("data", "final\_NCOS\_2024\_reformatted\_for\_R.xlsx - Fig3B.csv"))  
  
# Colors: these are from Paul Tol's colorblind friendly palette  
with\_microbes\_40\_color <- "#999933"  
no\_microbes\_.22\_color <- "#88ccee"  
  
# set up custom theme  
myCustomTheme <- function() {  
 theme\_light() +  
 theme(axis.text = element\_text(size = 7, family = "Helvetica", color = "black"),  
 axis.title.x = element\_text(margin = margin(t = 10), size = 7, face = "bold", family = "Helvetica", color = "black"), # Add space between x-axis label and axis  
 axis.title.y = element\_text(margin = margin(r = 10), size = 7, face = "bold", family = "Helvetica", color = "black"), # Add space between y-axis label and axis  
 title = element\_text(size = 7, face = "bold", family = "Helvetica"),  
 plot.caption = element\_text(size = 7, face = "italic", family = "Helvetica"),  
 legend.text = element\_text(size = 7, family = "Helvetica"), # Increase legend text size  
 panel.grid = element\_blank(), # Remove all grid lines (both major and minor)  
 # axis.line.x = element\_line(color = "grey"), # Keep the x-axis line  
 # axis.line.y = element\_line(color = "grey"), # Keep the y-axis line  
 axis.ticks = element\_line(color = "grey", size = 0.5), # Keep tick markers  
 strip.text = element\_text(size = 7, face = "bold", family = "Helvetica", color = "black"), # Set strip text style  
 strip.background = element\_rect(fill = "white", color = "grey", size = 0.5) # Set strip background to white, outline grey  
 )  
}

# 3A

Effect of the East Bay aquatic environmental biofilm on Bd growth - “9 sites”

Does the difference in rate loss of Bd in the **adherent** AE biofilm from day 1 to day 7 differ between the two filter types?

## 3A Data Wrangling

eb\_ae <- ns\_biofilm\_bd # rename  
  
# set as factors with levels so they appear in order in plots later  
eb\_ae$site <- factor(eb\_ae$site,  
 levels = c("CABIN", "GRAMPS", "WEST", "GDPND005", "GDPND006", "GDPND009", "PRPND004", "PRPND009", "PRPND010"))  
eb\_ae$bd\_location <- factor(eb\_ae$bd\_location, levels = c("supernatant", "biofilm")) # set bd location as factor  
eb\_ae$day <- factor(eb\_ae$day, levels = c("Day\_0", "Day\_1", "Day\_7")) # set day as factor  
  
# Biofilm only, no supernatant, and days 1 and 7 only, no day 0  
eb\_ae\_bf\_only <- eb\_ae %>%   
 filter(bd\_location == "biofilm") %>% # only the adherent fraction  
 filter(day != "Day\_0") %>% # no day 0 data, decided by Renwei  
 mutate(log\_qty = log(bd\_qty)) # log transform (no plus one because no zeroes)  
  
# export clean data  
write.csv(eb\_ae\_bf\_only, "data/eb\_ae\_bf\_only.csv", row.names = FALSE)

## EDA

## Stats and assumption testing

Question:

Does the amount of Bd in the biofilm differ between day 1 and day 7?

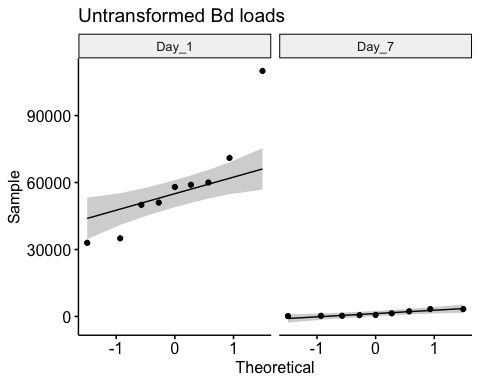
The samples are essentially paired by site, so a paired t-test is most appropriate

Assumptions:

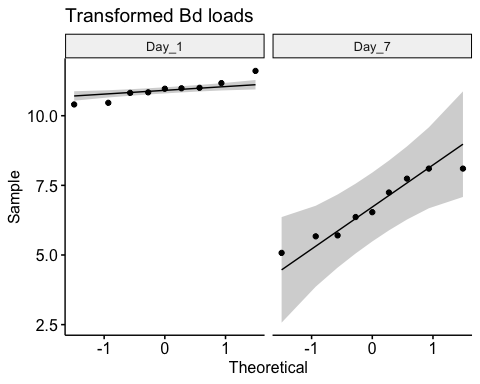
Assumes that the observations from each group represent a random sample from the population. Assumes that the difference of the two observations follow a normal distribution.

Check assumptions

# check normality of the differences across groups let's try untransformed  
eb\_ae\_bf\_only %>%   
 ggqqplot("bd\_qty", title = "Untransformed Bd loads") +  
 facet\_wrap(~day) # gotta transform the data, day 1 is not normal



eb\_ae\_bf\_only %>%   
 ggqqplot("log\_qty", title = "Transformed Bd loads") +  
 facet\_wrap(~day) # not perfect but closer



# Shapiro tests  
day\_one <- eb\_ae\_bf\_only %>%   
 filter(day == "Day\_1") %>% # filter to only include day 1  
 pull(log\_qty)  
  
shapiro.test(day\_one) # p >> 0.05, it's normal!

Shapiro-Wilk normality test  
  
data: day\_one  
W = 0.93796, p-value = 0.5606

day\_seven <- eb\_ae\_bf\_only %>%   
 filter(day == "Day\_7") %>% # filter to only include day 7  
 pull(log\_qty)  
  
shapiro.test(day\_seven) # p >> 0.05, it's normal!

Shapiro-Wilk normality test  
  
data: day\_seven  
W = 0.92235, p-value = 0.412

Stats

# Step 3: run the paired t-test  
t.test(day\_one, day\_seven, paired = TRUE)

Paired t-test  
  
data: day\_one and day\_seven  
t = 10.094, df = 8, p-value = 7.915e-06  
alternative hypothesis: true mean difference is not equal to 0  
95 percent confidence interval:  
 3.233993 5.149159  
sample estimates:  
mean difference   
 4.191576

**There is significantly more Bd in the biofilm on Day 1 than on Day 7 (t = 10.094, df = 8, p-value < 0.0001)**

# 3b NCOS AE biofilm

All microbe-depleted, NO pw microbe+ treatment. There is a treatment of tryptone broth.

## 3b Data wrangling

ae <- fig\_3b\_raw %>%   
 rename(sample\_ID = Adherent.sample.ID) %>% # cleaner name  
 mutate(TB = case\_when( # add column for TB or no  
 str\_detect(sample\_ID, "TB") ~ "y",  
 TRUE ~ "n")) %>%   
 mutate(PW = case\_when( # add column for PW (pond water) or no  
 str\_detect(sample\_ID, "PW") ~ "y",  
 TRUE ~ "n")) %>%   
 mutate(day = case\_when( # cleaner day name  
 day == 1 ~ "Day\_1",  
 day == 3 ~ "Day\_3",  
 day == 5 ~ "Day\_5",  
 day == 7 ~ "Day\_7",  
 day == 0 ~ "Day\_0")) %>%   
 mutate(day = factor(day)) %>% # day as a factor  
 mutate(day\_numeric = as.numeric(gsub("Day\_", "", as.character(day)))) # day as numeric for plotting  
  
# control data for ae  
ae\_control\_data <- ae %>%   
 filter(day == "Day\_0") %>% # no initial dose amount   
 mutate(day\_numeric = as.numeric(gsub("Day\_", "", as.character(day)))) %>% # day as numeric for plotting  
 dplyr::select(day, adh, day\_numeric) # select only needed columns  
  
ae\_summary <- ae %>%  
 group\_by(day, sample\_ID) %>% # this groups all the replicates of the same day and treatment together  
 reframe(mean = mean(adh), # calculate the mean  
 n = length(adh), # count the number of observations within the day and sample id  
 df = n - 1, # calculate the degrees of freedom  
 sd = sd(adh), # calculate the standard deviation  
 se = sd/sqrt(n)) %>% # calculate the standard error  
 ## Now that it is in a new summarized format, recreate the columns made above  
 mutate(TB = case\_when(str\_detect(sample\_ID, "TB") ~ "y", TRUE ~ "n")) %>% # add column for TB or no  
 mutate(PW = case\_when(str\_detect(sample\_ID, "PW") ~ "y", TRUE ~ "n")) %>% # add column for PW or no  
 mutate(day\_numeric = as.numeric(gsub("Day\_", "", as.character(day)))) # day as numeric for plotting  
  
ae\_noday0 <- ae %>%   
 filter(day != "Day\_0") %>% # no initial dose amount   
 mutate(log\_adh = log(adh)) %>% # note: no zeroes so not log + 1'# column for medium  
 mutate(medium = sample\_ID) # medium  
  
write.csv(ae\_noday0, "data/ae\_noday0.csv", row.names = FALSE)

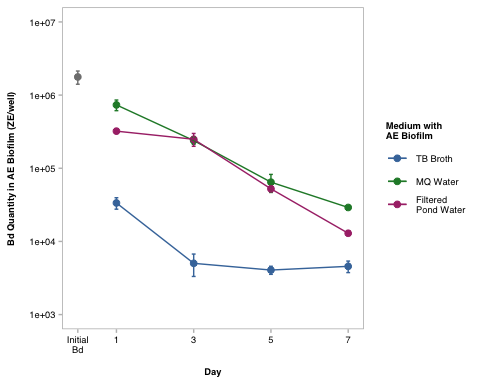
## 3b EDA

Caitlin’s version AE

ae\_summary %>%  
 # reorder to match Renwei's plot  
 mutate(sample\_ID = factor(sample\_ID,  
 levels = c("1%TB+AEbiofilm", "MQ+AEbiofilm",  
 "PW+AEBiofilm", "Added Bd" ))) %>%  
 ggplot(aes(x = day\_numeric,  
 y = mean,  
 color = sample\_ID)) +  
 geom\_point(size = 2) +  
 geom\_errorbar(aes(ymin = mean - se, # plot the standard error  
 ymax = mean + se),  
 width = 0.1) +  
 geom\_line() +  
 scale\_y\_log10(limits = c(1e3, 1e7),  
 breaks = c(1e3, 1e4, 1e5, 1e6, 1e7)) +  
 # vibes  
 labs(x = "Day",  
 y = "Bd Quantity in AE Biofilm (ZE/well)",  
 color = "Medium with\nAE Biofilm")+ # Title for color legend  
  
 scale\_color\_manual(values = c("1%TB+AEbiofilm"= "#4477AA",  
 "MQ+AEbiofilm" = "#228833",  
 #"Added Bd" = "darkgrey",  
 "PW+AEBiofilm" = "#AA3377"),  
 labels = c("1%TB+AEbiofilm" = "TB Broth",  
 "MQ+AEbiofilm" = "MQ Water",  
 "PW+AEBiofilm" = "Filtered\nPond Water",  
 "Added Bd" = "Initial Bd")) + # Custom labels  
  
 myCustomTheme()+  
 scale\_x\_continuous(breaks = c(0, 1, 3, 5, 7),  
 labels = c("Initial\nBd", "1", "3", "5", "7"))

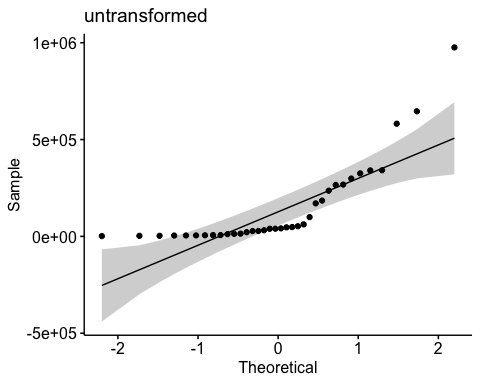
Warning: The `size` argument of `element\_line()` is deprecated as of ggplot2 3.4.0.  
ℹ Please use the `linewidth` argument instead.

Warning: The `size` argument of `element\_rect()` is deprecated as of ggplot2 3.4.0.  
ℹ Please use the `linewidth` argument instead.



visualize y var: bd load

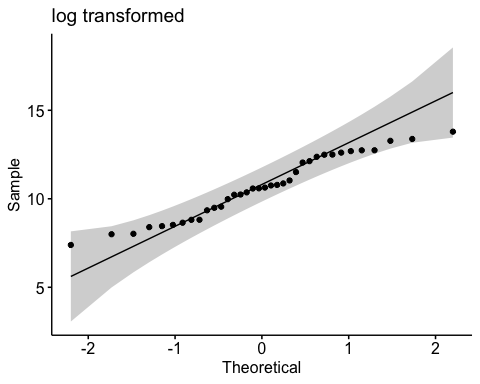
# untransformed  
ggqqplot(ae\_noday0, "adh", title = "untransformed")



shapiro.test(ae\_noday0$adh) # nope

Shapiro-Wilk normality test  
  
data: ae\_noday0$adh  
W = 0.69154, p-value = 2.089e-07

# transformed  
ggqqplot(ae\_noday0, "log\_adh", title = "log transformed") # gorgeous



shapiro.test(ae\_noday0$log\_adh) # p-value = 0.1699 def normal

Shapiro-Wilk normality test  
  
data: ae\_noday0$log\_adh  
W = 0.95671, p-value = 0.1699

## 3b Stats

y var: amount of Bd in adherent

x vars: day & medium with the AE biofilm and Bd (MQ, TB, PW)

Bd ~ day\*medium

Question: Does the amount of Bd in the aquatic environmental biofilm differ across the media tested and across the days, and do they interact with each other?

Model: 2-way ANOVA

**Summary of results**

Bd qty Day 1 > Day 3 > Day 5 > Day 7 (all p <0.005) in other words, Bd significantly reduced each day

MQ+AEbiofilm > PW+AEBiofilm > 1%TB+AEbiofilm (all p <0.005) In other words, TB plus biofilm has most Bd inhibition power, followed by pond water, then by milliQ with the least inhibition power

null

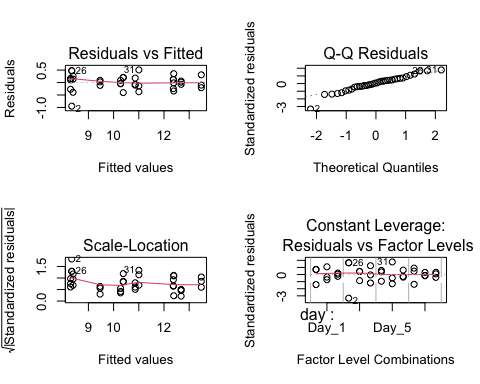
null <- lm(log\_adh ~ 1,  
 data = ae\_noday0)  
AIC(null) #146.5865

[1] 146.5865

## Bd ~ day\*medium

Note: Not perfectly homoskedstic but “good enough”

# set day 1 as reference  
ae\_noday0$day <- factor(ae\_noday0$day, levels = c("Day\_1", "Day\_3", "Day\_5", "Day\_7")) # set as factor  
ae\_noday0$day <- relevel(ae\_noday0$day, ref = "Day\_1") # set as reference  
  
# set MQ as reference  
ae\_noday0$sample\_ID <- factor(ae\_noday0$sample\_ID) # set as factor  
ae\_noday0$sample\_ID <- relevel(ae\_noday0$sample\_ID, ref = "MQ+AEbiofilm") # set as reference  
  
# build model  
aov\_3b <- aov(log\_adh ~ day\*medium, data = ae\_noday0)  
  
# diagnostic plot  
par(mfrow = c(2,2))  
plot(aov\_3b) # diagnostic plot



# look at results  
summary(aov\_3b)

Df Sum Sq Mean Sq F value Pr(>F)   
day 3 40.24 13.413 110.10 3.64e-14 \*\*\*  
medium 2 60.07 30.036 246.55 < 2e-16 \*\*\*  
day:medium 6 7.42 1.236 10.14 1.30e-05 \*\*\*  
Residuals 24 2.92 0.122   
---  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

anova(aov\_3b) # all significant and interaction sig

Analysis of Variance Table  
  
Response: log\_adh  
 Df Sum Sq Mean Sq F value Pr(>F)   
day 3 40.239 13.4129 110.100 3.642e-14 \*\*\*  
medium 2 60.073 30.0364 246.553 < 2.2e-16 \*\*\*  
day:medium 6 7.415 1.2359 10.145 1.301e-05 \*\*\*  
Residuals 24 2.924 0.1218   
---  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

AIC(aov\_3b) # 37.78083 better than null

[1] 37.78083

### Posthoc

Using a Tukey test on the anova model

# post hoc  
TukeyHSD(aov\_3b)

Tukey multiple comparisons of means  
 95% family-wise confidence level  
  
Fit: aov(formula = log\_adh ~ day \* medium, data = ae\_noday0)  
  
$day  
 diff lwr upr p adj  
Day\_3-Day\_1 -1.1493419 -1.603234 -0.6954497 0.0000018  
Day\_5-Day\_1 -2.1306208 -2.584513 -1.6767286 0.0000000  
Day\_7-Day\_1 -2.8046545 -3.258547 -2.3507623 0.0000000  
Day\_5-Day\_3 -0.9812789 -1.435171 -0.5273867 0.0000210  
Day\_7-Day\_3 -1.6553126 -2.109205 -1.2014204 0.0000000  
Day\_7-Day\_5 -0.6740337 -1.127926 -0.2201415 0.0021838  
  
$medium  
 diff lwr upr p adj  
MQ+AEbiofilm-1%TB+AEbiofilm 2.9323391 2.5764935 3.28818480 0.0000000  
PW+AEBiofilm-1%TB+AEbiofilm 2.4957994 2.1399538 2.85164510 0.0000000  
PW+AEBiofilm-MQ+AEbiofilm -0.4365397 -0.7923854 -0.08069402 0.0142222  
  
$`day:medium`  
 diff lwr upr  
Day\_3:1%TB+AEbiofilm-Day\_1:1%TB+AEbiofilm -2.04493896 -3.07249206 -1.01738586  
Day\_5:1%TB+AEbiofilm-Day\_1:1%TB+AEbiofilm -2.09173123 -3.11928433 -1.06417813  
Day\_7:1%TB+AEbiofilm-Day\_1:1%TB+AEbiofilm -1.99414642 -3.02169952 -0.96659332  
Day\_1:MQ+AEbiofilm-Day\_1:1%TB+AEbiofilm 3.09761537 2.07006227 4.12516848  
Day\_3:MQ+AEbiofilm-Day\_1:1%TB+AEbiofilm 1.98759548 0.96004238 3.01514858  
Day\_5:MQ+AEbiofilm-Day\_1:1%TB+AEbiofilm 0.61984147 -0.40771163 1.64739458  
Day\_7:MQ+AEbiofilm-Day\_1:1%TB+AEbiofilm -0.10651243 -1.13406553 0.92104068  
Day\_1:PW+AEBiofilm-Day\_1:1%TB+AEbiofilm 2.29587354 1.26832044 3.32342665  
Day\_3:PW+AEBiofilm-Day\_1:1%TB+AEbiofilm 2.00280682 0.97525371 3.03035992  
Day\_5:PW+AEBiofilm-Day\_1:1%TB+AEbiofilm 0.47351640 -0.55403671 1.50106950  
Day\_7:PW+AEBiofilm-Day\_1:1%TB+AEbiofilm -0.91981565 -1.94736875 0.10773746  
Day\_5:1%TB+AEbiofilm-Day\_3:1%TB+AEbiofilm -0.04679227 -1.07434537 0.98076083  
Day\_7:1%TB+AEbiofilm-Day\_3:1%TB+AEbiofilm 0.05079254 -0.97676056 1.07834564  
Day\_1:MQ+AEbiofilm-Day\_3:1%TB+AEbiofilm 5.14255433 4.11500123 6.17010744  
Day\_3:MQ+AEbiofilm-Day\_3:1%TB+AEbiofilm 4.03253444 3.00498134 5.06008754  
Day\_5:MQ+AEbiofilm-Day\_3:1%TB+AEbiofilm 2.66478043 1.63722733 3.69233354  
Day\_7:MQ+AEbiofilm-Day\_3:1%TB+AEbiofilm 1.93842653 0.91087343 2.96597964  
Day\_1:PW+AEBiofilm-Day\_3:1%TB+AEbiofilm 4.34081250 3.31325940 5.36836561  
Day\_3:PW+AEBiofilm-Day\_3:1%TB+AEbiofilm 4.04774578 3.02019267 5.07529888  
Day\_5:PW+AEBiofilm-Day\_3:1%TB+AEbiofilm 2.51845536 1.49090225 3.54600846  
Day\_7:PW+AEBiofilm-Day\_3:1%TB+AEbiofilm 1.12512331 0.09757021 2.15267642  
Day\_7:1%TB+AEbiofilm-Day\_5:1%TB+AEbiofilm 0.09758481 -0.92996829 1.12513791  
Day\_1:MQ+AEbiofilm-Day\_5:1%TB+AEbiofilm 5.18934660 4.16179350 6.21689970  
Day\_3:MQ+AEbiofilm-Day\_5:1%TB+AEbiofilm 4.07932671 3.05177361 5.10687981  
Day\_5:MQ+AEbiofilm-Day\_5:1%TB+AEbiofilm 2.71157270 1.68401960 3.73912581  
Day\_7:MQ+AEbiofilm-Day\_5:1%TB+AEbiofilm 1.98521880 0.95766570 3.01277191  
Day\_1:PW+AEBiofilm-Day\_5:1%TB+AEbiofilm 4.38760477 3.36005167 5.41515788  
Day\_3:PW+AEBiofilm-Day\_5:1%TB+AEbiofilm 4.09453805 3.06698494 5.12209115  
Day\_5:PW+AEBiofilm-Day\_5:1%TB+AEbiofilm 2.56524763 1.53769452 3.59280073  
Day\_7:PW+AEBiofilm-Day\_5:1%TB+AEbiofilm 1.17191558 0.14436248 2.19946869  
Day\_1:MQ+AEbiofilm-Day\_7:1%TB+AEbiofilm 5.09176179 4.06420869 6.11931489  
Day\_3:MQ+AEbiofilm-Day\_7:1%TB+AEbiofilm 3.98174190 2.95418880 5.00929500  
Day\_5:MQ+AEbiofilm-Day\_7:1%TB+AEbiofilm 2.61398789 1.58643479 3.64154100  
Day\_7:MQ+AEbiofilm-Day\_7:1%TB+AEbiofilm 1.88763399 0.86008089 2.91518710  
Day\_1:PW+AEBiofilm-Day\_7:1%TB+AEbiofilm 4.29001996 3.26246686 5.31757307  
Day\_3:PW+AEBiofilm-Day\_7:1%TB+AEbiofilm 3.99695324 2.96940013 5.02450634  
Day\_5:PW+AEBiofilm-Day\_7:1%TB+AEbiofilm 2.46766282 1.44010971 3.49521592  
Day\_7:PW+AEBiofilm-Day\_7:1%TB+AEbiofilm 1.07433077 0.04677767 2.10188388  
Day\_3:MQ+AEbiofilm-Day\_1:MQ+AEbiofilm -1.11001989 -2.13757300 -0.08246679  
Day\_5:MQ+AEbiofilm-Day\_1:MQ+AEbiofilm -2.47777390 -3.50532700 -1.45022079  
Day\_7:MQ+AEbiofilm-Day\_1:MQ+AEbiofilm -3.20412780 -4.23168090 -2.17657470  
Day\_1:PW+AEBiofilm-Day\_1:MQ+AEbiofilm -0.80174183 -1.82929493 0.22581127  
Day\_3:PW+AEBiofilm-Day\_1:MQ+AEbiofilm -1.09480855 -2.12236166 -0.06725545  
Day\_5:PW+AEBiofilm-Day\_1:MQ+AEbiofilm -2.62409897 -3.65165208 -1.59654587  
Day\_7:PW+AEBiofilm-Day\_1:MQ+AEbiofilm -4.01743102 -5.04498412 -2.98987792  
Day\_5:MQ+AEbiofilm-Day\_3:MQ+AEbiofilm -1.36775401 -2.39530711 -0.34020090  
Day\_7:MQ+AEbiofilm-Day\_3:MQ+AEbiofilm -2.09410791 -3.12166101 -1.06655480  
Day\_1:PW+AEBiofilm-Day\_3:MQ+AEbiofilm 0.30827806 -0.71927504 1.33583117  
Day\_3:PW+AEBiofilm-Day\_3:MQ+AEbiofilm 0.01521134 -1.01234177 1.04276444  
Day\_5:PW+AEBiofilm-Day\_3:MQ+AEbiofilm -1.51407908 -2.54163219 -0.48652598  
Day\_7:PW+AEBiofilm-Day\_3:MQ+AEbiofilm -2.90741113 -3.93496423 -1.87985802  
Day\_7:MQ+AEbiofilm-Day\_5:MQ+AEbiofilm -0.72635390 -1.75390700 0.30119920  
Day\_1:PW+AEBiofilm-Day\_5:MQ+AEbiofilm 1.67603207 0.64847897 2.70358517  
Day\_3:PW+AEBiofilm-Day\_5:MQ+AEbiofilm 1.38296534 0.35541224 2.41051845  
Day\_5:PW+AEBiofilm-Day\_5:MQ+AEbiofilm -0.14632508 -1.17387818 0.88122803  
Day\_7:PW+AEBiofilm-Day\_5:MQ+AEbiofilm -1.53965712 -2.56721022 -0.51210402  
Day\_1:PW+AEBiofilm-Day\_7:MQ+AEbiofilm 2.40238597 1.37483287 3.42993907  
Day\_3:PW+AEBiofilm-Day\_7:MQ+AEbiofilm 2.10931925 1.08176614 3.13687235  
Day\_5:PW+AEBiofilm-Day\_7:MQ+AEbiofilm 0.58002883 -0.44752428 1.60758193  
Day\_7:PW+AEBiofilm-Day\_7:MQ+AEbiofilm -0.81330322 -1.84085632 0.21424988  
Day\_3:PW+AEBiofilm-Day\_1:PW+AEBiofilm -0.29306673 -1.32061983 0.73448638  
Day\_5:PW+AEBiofilm-Day\_1:PW+AEBiofilm -1.82235715 -2.84991025 -0.79480404  
Day\_7:PW+AEBiofilm-Day\_1:PW+AEBiofilm -3.21568919 -4.24324229 -2.18813609  
Day\_5:PW+AEBiofilm-Day\_3:PW+AEBiofilm -1.52929042 -2.55684352 -0.50173732  
Day\_7:PW+AEBiofilm-Day\_3:PW+AEBiofilm -2.92262246 -3.95017557 -1.89506936  
Day\_7:PW+AEBiofilm-Day\_5:PW+AEBiofilm -1.39333205 -2.42088515 -0.36577894  
 p adj  
Day\_3:1%TB+AEbiofilm-Day\_1:1%TB+AEbiofilm 0.0000113  
Day\_5:1%TB+AEbiofilm-Day\_1:1%TB+AEbiofilm 0.0000078  
Day\_7:1%TB+AEbiofilm-Day\_1:1%TB+AEbiofilm 0.0000169  
Day\_1:MQ+AEbiofilm-Day\_1:1%TB+AEbiofilm 0.0000000  
Day\_3:MQ+AEbiofilm-Day\_1:1%TB+AEbiofilm 0.0000179  
Day\_5:MQ+AEbiofilm-Day\_1:1%TB+AEbiofilm 0.5802121  
Day\_7:MQ+AEbiofilm-Day\_1:1%TB+AEbiofilm 0.9999997  
Day\_1:PW+AEBiofilm-Day\_1:1%TB+AEbiofilm 0.0000016  
Day\_3:PW+AEBiofilm-Day\_1:1%TB+AEbiofilm 0.0000158  
Day\_5:PW+AEBiofilm-Day\_1:1%TB+AEbiofilm 0.8683022  
Day\_7:PW+AEBiofilm-Day\_1:1%TB+AEbiofilm 0.1093965  
Day\_5:1%TB+AEbiofilm-Day\_3:1%TB+AEbiofilm 1.0000000  
Day\_7:1%TB+AEbiofilm-Day\_3:1%TB+AEbiofilm 1.0000000  
Day\_1:MQ+AEbiofilm-Day\_3:1%TB+AEbiofilm 0.0000000  
Day\_3:MQ+AEbiofilm-Day\_3:1%TB+AEbiofilm 0.0000000  
Day\_5:MQ+AEbiofilm-Day\_3:1%TB+AEbiofilm 0.0000001  
Day\_7:MQ+AEbiofilm-Day\_3:1%TB+AEbiofilm 0.0000266  
Day\_1:PW+AEBiofilm-Day\_3:1%TB+AEbiofilm 0.0000000  
Day\_3:PW+AEBiofilm-Day\_3:1%TB+AEbiofilm 0.0000000  
Day\_5:PW+AEBiofilm-Day\_3:1%TB+AEbiofilm 0.0000003  
Day\_7:PW+AEBiofilm-Day\_3:1%TB+AEbiofilm 0.0234402  
Day\_7:1%TB+AEbiofilm-Day\_5:1%TB+AEbiofilm 0.9999999  
Day\_1:MQ+AEbiofilm-Day\_5:1%TB+AEbiofilm 0.0000000  
Day\_3:MQ+AEbiofilm-Day\_5:1%TB+AEbiofilm 0.0000000  
Day\_5:MQ+AEbiofilm-Day\_5:1%TB+AEbiofilm 0.0000001  
Day\_7:MQ+AEbiofilm-Day\_5:1%TB+AEbiofilm 0.0000182  
Day\_1:PW+AEBiofilm-Day\_5:1%TB+AEbiofilm 0.0000000  
Day\_3:PW+AEBiofilm-Day\_5:1%TB+AEbiofilm 0.0000000  
Day\_5:PW+AEBiofilm-Day\_5:1%TB+AEbiofilm 0.0000002  
Day\_7:PW+AEBiofilm-Day\_5:1%TB+AEbiofilm 0.0161067  
Day\_1:MQ+AEbiofilm-Day\_7:1%TB+AEbiofilm 0.0000000  
Day\_3:MQ+AEbiofilm-Day\_7:1%TB+AEbiofilm 0.0000000  
Day\_5:MQ+AEbiofilm-Day\_7:1%TB+AEbiofilm 0.0000001  
Day\_7:MQ+AEbiofilm-Day\_7:1%TB+AEbiofilm 0.0000404  
Day\_1:PW+AEBiofilm-Day\_7:1%TB+AEbiofilm 0.0000000  
Day\_3:PW+AEBiofilm-Day\_7:1%TB+AEbiofilm 0.0000000  
Day\_5:PW+AEBiofilm-Day\_7:1%TB+AEbiofilm 0.0000004  
Day\_7:PW+AEBiofilm-Day\_7:1%TB+AEbiofilm 0.0349376  
Day\_3:MQ+AEbiofilm-Day\_1:MQ+AEbiofilm 0.0264194  
Day\_5:MQ+AEbiofilm-Day\_1:MQ+AEbiofilm 0.0000004  
Day\_7:MQ+AEbiofilm-Day\_1:MQ+AEbiofilm 0.0000000  
Day\_1:PW+AEBiofilm-Day\_1:MQ+AEbiofilm 0.2352787  
Day\_3:PW+AEBiofilm-Day\_1:MQ+AEbiofilm 0.0297786  
Day\_5:PW+AEBiofilm-Day\_1:MQ+AEbiofilm 0.0000001  
Day\_7:PW+AEBiofilm-Day\_1:MQ+AEbiofilm 0.0000000  
Day\_5:MQ+AEbiofilm-Day\_3:MQ+AEbiofilm 0.0031814  
Day\_7:MQ+AEbiofilm-Day\_3:MQ+AEbiofilm 0.0000076  
Day\_1:PW+AEBiofilm-Day\_3:MQ+AEbiofilm 0.9927584  
Day\_3:PW+AEBiofilm-Day\_3:MQ+AEbiofilm 1.0000000  
Day\_5:PW+AEBiofilm-Day\_3:MQ+AEbiofilm 0.0009231  
Day\_7:PW+AEBiofilm-Day\_3:MQ+AEbiofilm 0.0000000  
Day\_7:MQ+AEbiofilm-Day\_5:MQ+AEbiofilm 0.3586125  
Day\_1:PW+AEBiofilm-Day\_5:MQ+AEbiofilm 0.0002348  
Day\_3:PW+AEBiofilm-Day\_5:MQ+AEbiofilm 0.0027988  
Day\_5:PW+AEBiofilm-Day\_5:MQ+AEbiofilm 0.9999930  
Day\_7:PW+AEBiofilm-Day\_5:MQ+AEbiofilm 0.0007432  
Day\_1:PW+AEBiofilm-Day\_7:MQ+AEbiofilm 0.0000007  
Day\_3:PW+AEBiofilm-Day\_7:MQ+AEbiofilm 0.0000067  
Day\_5:PW+AEBiofilm-Day\_7:MQ+AEbiofilm 0.6677092  
Day\_7:PW+AEBiofilm-Day\_7:MQ+AEbiofilm 0.2194307  
Day\_3:PW+AEBiofilm-Day\_1:PW+AEBiofilm 0.9952000  
Day\_5:PW+AEBiofilm-Day\_1:PW+AEBiofilm 0.0000692  
Day\_7:PW+AEBiofilm-Day\_1:PW+AEBiofilm 0.0000000  
Day\_5:PW+AEBiofilm-Day\_3:PW+AEBiofilm 0.0008115  
Day\_7:PW+AEBiofilm-Day\_3:PW+AEBiofilm 0.0000000  
Day\_7:PW+AEBiofilm-Day\_5:PW+AEBiofilm 0.0025645

Now let’s get this in a format to visualize this data. I will use the emmeans function to extract the estimated marginal means using a tukey adjustment, and from that, will use R to differentiate between the ones with significant differences, and show that with a compact letter display (cld) plot for each comparison type.

#..............................day...............................  
# perform tukey pairwise comparisons across day only & et cld letters  
em\_day <- emmeans(aov\_3b, ~ day)

NOTE: Results may be misleading due to involvement in interactions

tukey\_day <- pairs(em\_day, adjust = "tukey")  
  
#...........................medium...............................  
em\_medium <- emmeans(aov\_3b, ~ medium)

NOTE: Results may be misleading due to involvement in interactions

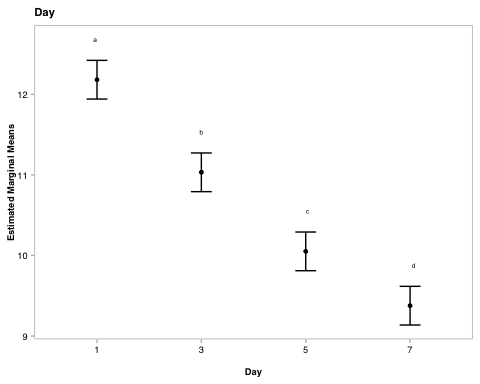
tukey\_medium <- pairs(em\_medium, adjust = "tukey")  
  
#..........................interaction...............................  
em\_interaction <- emmeans(aov\_3b, ~ day \* medium)  
tukey\_interaction <- pairs(em\_interaction, adjust = "tukey")

#### cld

#..............................day...............................  
# perform tukey pairwise comparisons across day only & et cld letters  
cld\_day <- emmeans(aov\_3b, pairwise ~ day, adjust = "tukey") %>%  
 # add compact letter display (cld) to group days that are not significantly different  
 cld(Letters = letters, reverse = TRUE) # reverse = TRUE ensures "a" goes to the highest mean

NOTE: Results may be misleading due to involvement in interactions

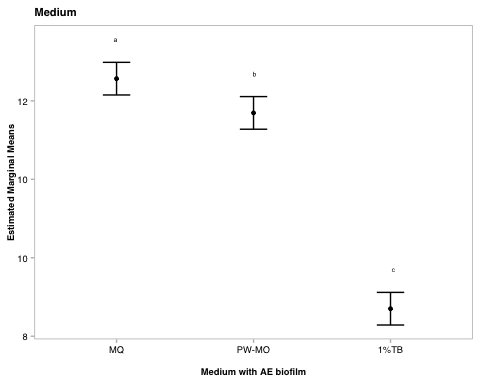
# make a plot of estimated marginal means with CLD letters for each day  
cld\_day\_3b <- ggplot(cld\_day, aes(x = day, y = emmean)) +  
 geom\_point(size = 1) + # Plot the estimated means  
 geom\_errorbar(aes(ymin = lower.CL, ymax = upper.CL), width = 0.2) + # add 95% confidence intervals  
 geom\_text(aes(label = .group), nudge\_y = 0.5, size = 1.75, color = "black") + # add cld group letters above points  
 xlab("Day") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1)) + # format y-axis: whole numbers  
 ggtitle("Day") +  
 myCustomTheme() +  
 scale\_x\_discrete(labels= c("Day\_1" = "1", "Day\_3" = "3", "Day\_5" = "5", "Day\_7" = "7")) +  
 theme(axis.title.y = element\_text(margin = margin(r = 1))) # give y-axis label a little breathing room  
cld\_day\_3b



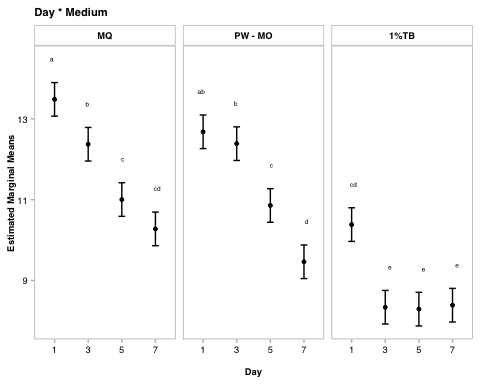
#..........................medium...............................  
## same as above but for another factor, see "day" example for code comments  
cld\_medium <- emmeans(aov\_3b, pairwise ~ medium, adjust = "tukey") %>%  
 cld(Letters = letters, reverse = TRUE)

NOTE: Results may be misleading due to involvement in interactions

cld\_medium\_3b <- ggplot(cld\_medium, aes(x = factor(medium, levels = c("MQ+AEbiofilm", "PW+AEBiofilm", "1%TB+AEbiofilm")), y = emmean)) +  
 geom\_point(size = 1) +   
 geom\_errorbar(aes(ymin = lower.CL, ymax = upper.CL), width = 0.2) +   
 geom\_text(aes(label = .group), nudge\_y = 0.5, size = 1.75, color = "black") +   
 xlab("Medium with AE biofilm") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1)) +  
 ggtitle("Medium") +  
 myCustomTheme() +  
 scale\_x\_discrete(labels= c("1%TB+AEbiofilm" = "1%TB", "PW+AEBiofilm" = "PW-MO", "MQ+AEbiofilm" = "MQ")) +  
 theme(axis.title.y = element\_text(margin = margin(r = 1)))  
cld\_medium\_3b



#.......................medium \* day............................  
cld\_day\_medium <- emmeans(aov\_3b, pairwise ~ day \* medium, adjust = "tukey") %>%  
 cld(Letters = letters, reverse = TRUE)  
  
## make a plot of estimated marginal means across day, faceted by medium  
cld\_day\_medium\_3b <- ggplot(cld\_day\_medium, aes(x = factor(day), y = emmean)) +  
 geom\_point(size = 1) + # Plot the estimated means  
 geom\_errorbar(aes(ymin = lower.CL, ymax = upper.CL), width = 0.2) +   
 geom\_text(aes(label = .group), nudge\_y = 1, size = 1.75, color = "black") +   
 facet\_wrap(~ factor(medium, # facet by medium  
 levels = c("MQ+AEbiofilm", "PW+AEBiofilm", "1%TB+AEbiofilm")), # order  
 labeller = as\_labeller(c("1%TB+AEbiofilm" = "1%TB", # custom labels  
 "PW+AEBiofilm" = "PW - MO",   
 "MQ+AEbiofilm" = "MQ"))) +   
 xlab("Day") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1)) +  
 ggtitle("Day \* Medium") +  
 myCustomTheme() +  
 scale\_x\_discrete(labels= c("Day\_1" = "1", "Day\_3" = "3", "Day\_5" = "5", "Day\_7" = "7")) +  
 theme(axis.title.y = element\_text(margin = margin(r = 1)))  
cld\_day\_medium\_3b



Results write up

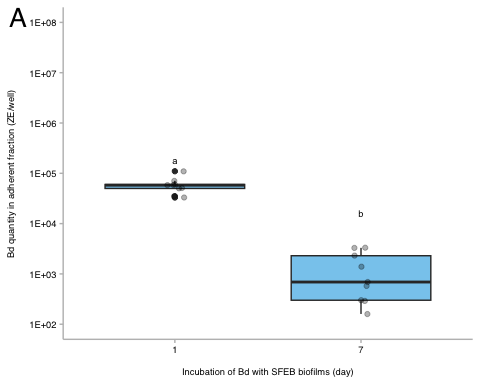
A two-way ANOVA revealed that there was a statistically significant difference in Bd load across days (F(3, 24) = 110.100, p = p <0.0001), across the media (F(2, 24) = 246.55, p <0.0001), and the interaction between the effects of day and medium were also significant (F(6, 24) = 10.145, p = p <0.0001). Bd was significantly lower with each day (Tukey test, p <0.005 for all) and TB plus biofilm has most Bd inhibition power, followed by pond water with no microbes, then by milliQ with the least inhibition power (Tukey test, p <0.05 for all).

# \*PUBLICATION FIGURES

# set up custom theme  
myCustomTheme <- function() {  
 theme\_light() +  
 theme(axis.text = element\_text(size = 7, family = "Helvetica", color = "black"),  
 axis.title.x = element\_text(margin = margin(t = 10), size = 7, face = "plain", family = "Helvetica", color = "black"), # Add space between x-axis label and axis  
 axis.title.y = element\_text(margin = margin(r = 10), size = 7, face = "plain", family = "Helvetica", color = "black"), # Add space between y-axis label and axis  
 title = element\_text(size = 7, face = "bold", family = "Helvetica"),  
 plot.caption = element\_text(size = 7, face = "italic", family = "Helvetica"),  
 legend.text = element\_text(size = 7, family = "Helvetica"), # Increase legend text size  
 panel.grid = element\_blank(), # Remove all grid lines (both major and minor)  
 # axis.line.x = element\_line(color = "grey"), # Keep the x-axis line  
 # axis.line.y = element\_line(color = "grey"), # Keep the y-axis line  
 axis.ticks = element\_line(color = "grey", size = 0.5), # Keep tick markers  
 axis.ticks.x = element\_line(color = "grey", size = 0.5), # ensure bottom axis ticks  
 axis.ticks.y = element\_line(color = "grey", size = 0.5), # ensure side axis ticks  
 strip.text = element\_text(size = 7, face = "bold", family = "Helvetica", color = "black"), # Set strip text style  
 strip.background = element\_rect(fill = "white", color = "grey", size = 0.5) # Set strip background to white, outline grey  
 )  
}

## SFEB AE Biofilm

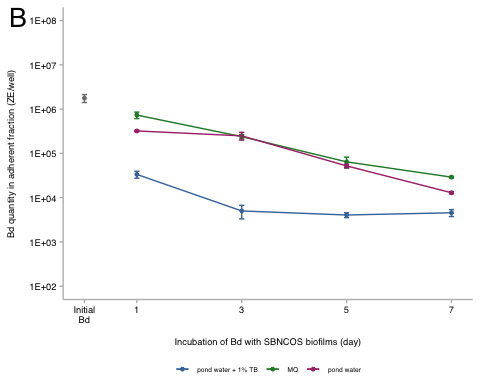
fig\_SFEB\_biofilm\_boxplot <- eb\_ae\_bf\_only %>%  
ggplot(aes(y= bd\_qty, x = day)) +   
 geom\_boxplot(fill = no\_microbes\_.22\_color) +  
 geom\_jitter(alpha = 0.3, width = 0.05) +  
 myCustomTheme() +  
 scale\_y\_log10(labels = function(x) {gsub("e", "E", scales::scientific\_format()(x))},   
 # update to consistent scale per reviewer request  
 limits = c(1e+02, 1e+08),  
 breaks = c(1e2, 1e3, 1e4, 1e5, 1e6, 1e7, 1e8)) +   
 xlab("Incubation of Bd with SFEB biofilms (day)") +  
 ylab("Bd quantity in adherent fraction (ZE/well)") +  
 scale\_x\_discrete (labels= c("Day\_1" = "1", "Day\_7" = "7")) +  
   
 theme(legend.position = "none",  
 panel.border = element\_blank(),  
 axis.line.x = element\_line(color = "grey", size = 0.5),   
 axis.line.y = element\_line(color = "grey", size = 0.5))  
  
# Let's add significance letters  
significance\_data <- tibble(  
 day = factor(c("Day\_1", "Day\_7"), levels = c("Day\_1", "Day\_7")),  
 y\_position = c(1.6e+05, 1.4e+04),   
 label = c("a", "b"))  
  
fig\_SFEB\_biofilm <- fig\_SFEB\_biofilm\_boxplot +   
geom\_text(data = significance\_data, aes(x = day, y = y\_position, label = label),  
 position = position\_dodge(width = 0.75), vjust = 0, size = 7 / 2.85) # /2.85 to convert to the same "pt" text sizing the axes use  
  
#fig\_SFEB\_biofilm  
  
fig\_SFEB\_biofilm <- fig\_SFEB\_biofilm +  
 labs(tag = "A") +  
 theme(  
 plot.tag = element\_text(family = "Helvetica", size = 20, face = "plain", hjust = -0.1, vjust = 1),  
 plot.tag.position = c(0, 1))  
  
fig\_SFEB\_biofilm



# 3a alone  
#ggsave("paper-figures/expt3-SFEB-biofilms\_fig4a\_updated.pdf", plot = fig\_SFEB\_biofilm, width = 3.46, height = 3.46)

## 3B: SBNCOS Biofilm

# add column for microbes or no  
fig\_SBNCOS\_biofilm <- ae\_summary %>%  
 # reorder to match Renwei's plot  
 mutate(sample\_ID = factor(sample\_ID,  
 levels = c("1%TB+AEbiofilm", "MQ+AEbiofilm",  
 "PW+AEBiofilm", "Added Bd" ))) %>%  
  
 ggplot(aes(x = day\_numeric,  
 y = mean,  
 color = sample\_ID)) +  
 geom\_point(size = 1) +  
 geom\_errorbar(aes(ymin = mean - se, # standard error  
 ymax = mean + se), # standard error  
 width = 0.1) +  
 geom\_line() +   
 ## update scale for reviewer  
 scale\_y\_log10(labels = function(x) {gsub("e", "E", scales::scientific\_format()(x))},   
 # update to consistent scale per reviewer request  
 limits = c(1e+02, 1e+08),  
 breaks = c(1e2, 1e3, 1e4, 1e5, 1e6, 1e7, 1e8)) +   
 # vibes  
 labs(x = "Incubation of Bd with SBNCOS biofilms (day)",  
 y = "Bd quantity in adherent fraction (ZE/well)",  
 color = "Medium with\nAE Biofilm")+   
   
 scale\_color\_manual(values = c("1%TB+AEbiofilm"= "#4477AA",  
 "MQ+AEbiofilm" = "#228833",  
 #"Added Bd" = "darkgrey",  
 "PW+AEBiofilm" = "#AA3377"),  
 labels = c("1%TB+AEbiofilm" = "pond water + 1% TB",  
 "MQ+AEbiofilm" = "MQ",  
 "PW+AEBiofilm" = "pond water",  
 "Added Bd" = "Initial Bd")) +   
 myCustomTheme()+  
 scale\_x\_continuous(breaks = c(0, 1, 3, 5, 7),  
 labels = c("Initial\nBd", "1", "3", "5", "7")) +  
 theme(legend.position = "bottom",  
 panel.border = element\_blank(),  
 legend.text = element\_text(size = 5),   
 legend.key.size = unit(0.4, "cm"),   
 legend.spacing.y = unit(0.1, "cm"),   
 legend.margin = margin(t = 0, r = 0, b = 0, l = 0),  
 axis.line.x = element\_line(color = "grey", size = 0.5),   
 axis.line.y = element\_line(color = "grey", size = 0.5)) +   
guides(color = guide\_legend(title = NULL))  
  
#fig\_SBNCOS\_biofilm  
  
fig\_SBNCOS\_biofilm <- fig\_SBNCOS\_biofilm +  
 labs(tag = "B") +  
 theme(  
 plot.tag = element\_text(family = "Helvetica", size = 20, face = "plain", hjust = -0.1, vjust = 1),  
 plot.tag.position = c(0, 1)  
 )  
  
fig\_SBNCOS\_biofilm

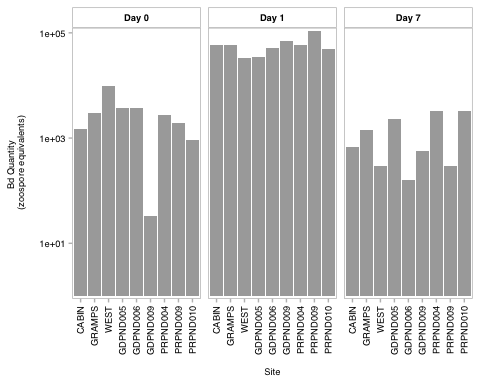


#ggsave("paper-figures/expt3-SBNCOS-biofilms\_fig4b\_updated.pdf", plot = fig\_SBNCOS\_biofilm, width = 3.46, height = 3.46)

# \*SI figures and tables

## Fig S6

SI\_6 <- eb\_ae %>%  
 filter(bd\_location == "biofilm") %>% # no supernatant  
ggplot(aes(y= bd\_qty, x = site)) +   
 geom\_col(fill = "darkgrey") +  
 facet\_wrap(~day, labeller = labeller(day = c("Day\_0" = "Day 0", "Day\_1" = "Day 1", "Day\_7" = "Day 7"))) +  
 scale\_y\_continuous(expand = c(0.01, 0.01), # get rid of weird gap  
 trans = "log", # Natural logarithmic scale  
 breaks = c(1e+01, 1e+03, 1e+05), # Set exact breaks  
 labels = scales::label\_scientific()) + # Use scientific notation 1e+01, 1e+03, 1e+05  
 #scale\_y\_log10() + produces same as above but has weird spacing between facet and bottom of bar  
 xlab("Site") +  
 ylab("Bd Quantity \n (zoospore equivalents)") +  
 myCustomTheme() +   
 theme(axis.text.x = element\_text(angle = 90, vjust = 0.5, hjust = 1),  
 panel.border = element\_rect(color = "gray", size = 0.5, fill = NA))  
SI\_6

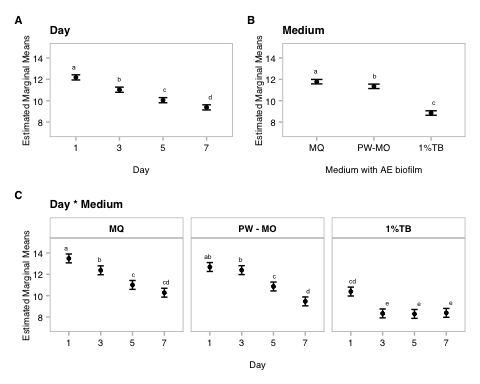


#ggsave("SI\_6.pdf", plot = SI\_6, width = 7.09, height = 3.46)

## Fig. S7

Here I updated the y axis limits, this is the difference from the prior cld plots in the exploratory and analysus phase

global\_y\_limits <- c(7, 15)  
global\_y\_breaks <- seq(8, 14, by = 2)  
  
# Compute CLD letters for 'day'  
cld\_day\_3b <- ggplot(cld\_day, aes(x = day, y = emmean)) +  
 geom\_point(size = 1) +  
 geom\_errorbar(aes(ymin = lower.CL, ymax = upper.CL), width = 0.2) +  
 geom\_text(aes(label = .group), nudge\_y = 1, size = 1.75, color = "black") +  
 xlab("Day") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1), limits = global\_y\_limits, breaks = global\_y\_breaks) +  
 ggtitle("Day") +  
 myCustomTheme() +  
 scale\_x\_discrete(labels = c("Day\_1" = "1", "Day\_3" = "3", "Day\_5" = "5", "Day\_7" = "7")) +  
 theme(axis.title.y = element\_text(margin = margin(r = 1)))  
  
# Compute CLD letters for 'medium'  
cld\_medium\_3b <- ggplot(cld\_medium, aes(x = factor(medium, levels = c("MQ+AEbiofilm", "PW+AEBiofilm", "1%TB+AEbiofilm")), y = emmean)) +  
 geom\_point(size = 1) +  
 geom\_errorbar(aes(ymin = lower.CL, ymax = upper.CL), width = 0.2) +  
 geom\_text(aes(label = .group), nudge\_y = 1, size = 1.75, color = "black") +  
 xlab("Medium with AE biofilm") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1), limits = global\_y\_limits, breaks = global\_y\_breaks) +  
 ggtitle("Medium") +  
 myCustomTheme() +  
 scale\_x\_discrete(labels = c("1%TB+AEbiofilm" = "1%TB", "PW+AEBiofilm" = "PW-MO", "MQ+AEbiofilm" = "MQ")) +  
 theme(axis.title.y = element\_text(margin = margin(r = 1)))  
  
# Compute CLD letters for 'day \* medium'  
cld\_day\_medium\_3b <- ggplot(cld\_day\_medium, aes(x = factor(day), y = emmean)) +  
 geom\_point(size = 1) +  
 geom\_errorbar(aes(ymin = lower.CL, ymax = upper.CL), width = 0.2) +  
 geom\_text(aes(label = .group), nudge\_y = 1, size = 1.75, color = "black") +  
 facet\_wrap(~ factor(medium, levels = c("MQ+AEbiofilm", "PW+AEBiofilm", "1%TB+AEbiofilm")),   
 labeller = as\_labeller(c("1%TB+AEbiofilm" = "1%TB",   
 "PW+AEBiofilm" = "PW - MO",   
 "MQ+AEbiofilm" = "MQ"))) +  
 xlab("Day") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1), limits = global\_y\_limits, breaks = global\_y\_breaks) +  
 ggtitle("Day \* Medium") +  
 myCustomTheme() +  
 scale\_x\_discrete(labels = c("Day\_1" = "1", "Day\_3" = "3", "Day\_5" = "5", "Day\_7" = "7")) +  
 theme(axis.title.y = element\_text(margin = margin(r = 1)))  
  
# Combine plots  
pairwise\_cld\_3b <- (cld\_day\_3b + cld\_medium\_3b) / cld\_day\_medium\_3b +  
 plot\_annotation(tag\_levels = 'A')  
  
pairwise\_cld\_3b



#ggsave("SI\_3b\_updated.pdf", plot = pairwise\_cld\_3b, width = 7.09, height = 3.46)

# Appendix

Not used in publication but I like these so I’ll keep them here

## anova table

# anova table  
anova\_output <- tidy(aov\_3b)  
  
aov\_3b\_tbl <- anova\_output %>%  
 dplyr::select(term, df, sumsq, meansq, statistic, p.value) %>%  
 gt() %>%  
 tab\_header(  
 title = "ANOVA Table"  
 ) %>%  
 fmt\_number(  
 columns = c(sumsq, meansq, statistic),  
 decimals = 2  
 ) %>%  
 cols\_label(  
 term = "Term",  
 df = "Df",  
 sumsq = "Sum Sq",  
 meansq = "Mean Sq",  
 statistic = "F value",  
 p.value = "P-value"  
 ) %>% # scientific number format for values <0.001 in p values  
 fmt\_scientific(  
 columns = c(p.value),  
 decimals = 1,  
 rows = p.value < 0.001  
 ) %>%  
 # 3 decimals for p values >=0.001  
 fmt\_number(  
 columns = c(p.value),  
 decimals = 3,  
 rows = p.value >= 0.001  
 )  
aov\_3b\_tbl

Table 1: ANOVA Table

| Term | Df | Sum Sq | Mean Sq | F value | P-value |
| --- | --- | --- | --- | --- | --- |
| day | 3 | 40.24 | 13.41 | 110.10 | 3.6 × 10^-14 |
| medium | 2 | 60.07 | 30.04 | 246.55 | 1.0 × 10^-16 |
| day:medium | 6 | 7.42 | 1.24 | 10.14 | 1.3 × 10^-5 |
| Residuals | 24 | 2.92 | 0.12 | NA | NA |

# prettier, simplified  
anova\_output <- tidy(aov\_3b)  
  
# Modify term to include degrees of freedom in \*italics\*  
anova\_output <- anova\_output %>%  
 mutate(term = ifelse(grepl("day:medium", term), "day x medium", term)) %>%   
 mutate (term = paste0(term, " (\*df = ", df, ", ", anova\_output[df == max(df), "df"], "\*)")) %>%   
 filter(term != "Residuals (\*df = 24, 24\*)")  
  
# Create the gt table with selected columns  
aov\_3b\_tbl\_b <- anova\_output %>%  
 dplyr::select(term, statistic, p.value) %>%  
 gt() %>%  
 tab\_header(  
 title = "ANOVA Table"  
 ) %>%  
 fmt\_markdown(  
 columns = c(term)  
 ) %>%  
 fmt\_number(  
 columns = c(statistic),  
 decimals = 2  
 ) %>%  
 cols\_label(  
 term = "",  
 statistic = "F value",  
 p.value = "P-value"  
 ) %>%  
 fmt\_scientific(  
 columns = c(p.value),  
 decimals = 1,  
 rows = p.value < 0.001  
 ) %>%  
 fmt\_number(  
 columns = c(p.value),  
 decimals = 3,  
 rows = p.value >= 0.001  
 )  
  
aov\_3b\_tbl\_b

Table 1: ANOVA Table

|  | F value | P-value |
| --- | --- | --- |
| day (*df = 3, 24*) | 110.10 | 3.6 × 10^-14 |
| medium (*df = 2, 24*) | 246.55 | 1.0 × 10^-16 |
| day x medium (*df = 6, 24*) | 10.14 | 1.3 × 10^-5 |

## posthoc table

# post hoc table  
# Convert Tukey emmeans results to data frames  
tukey\_day\_df <- as.data.frame(tukey\_day)  
tukey\_medium\_df <- as.data.frame(tukey\_medium)  
tukey\_interaction\_df <- as.data.frame(tukey\_interaction)  
  
# Add labels to indicate which factor the comparison refers to  
tukey\_day\_df <- tukey\_day\_df %>% mutate(factor = "Day")  
tukey\_medium\_df <- tukey\_medium\_df %>% mutate(factor = "Medium")  
tukey\_interaction\_df <- tukey\_interaction\_df %>% mutate(factor = "Interaction")  
  
all\_tukey\_df <- bind\_rows(tukey\_day\_df, tukey\_medium\_df, tukey\_interaction\_df)  
ph3b\_table <- all\_tukey\_df %>%  
 dplyr::select(factor, contrast, estimate, SE, df, t.ratio, p.value) %>%  
 gt() %>%  
 # change column names  
 cols\_label(  
 factor = "Comparison",  
 contrast = "Group Comparison",  
 estimate = "Estimate",  
 SE = "Standard Error",  
 df = "Degrees of Freedom",  
 t.ratio = "t-Ratio",  
 p.value = "p-value"  
 ) %>%  
 # update header for table  
 tab\_header(  
 title = "Emmeans Post-hoc Test Results"  
 ) %>%  
 # 3 decimal places  
 fmt\_number(  
 columns = c(estimate, SE, t.ratio),  
 decimals = 3  
 ) %>%  
 # scientific number format for values <0.001 in p values  
 fmt\_scientific(  
 columns = c(p.value),  
 decimals = 1,  
 rows = p.value < 0.001  
 ) %>%  
 # 3 decimals for p values >=0.001  
 fmt\_number(  
 columns = c(p.value),  
 decimals = 3,  
 rows = p.value >= 0.001  
 ) %>%  
 #make the headers bold  
 tab\_style(  
 style = list(  
 cell\_text(weight = "bold")  
 ),  
 locations = cells\_column\_labels(everything()))  
ph3b\_table

Table 1: Emmeans Post-hoc Test Results

| Comparison | Group Comparison | Estimate | Standard Error | Degrees of Freedom | t-Ratio | p-value |
| --- | --- | --- | --- | --- | --- | --- |
| Day | Day\_1 - Day\_3 | 1.149 | 0.165 | 24 | 6.985 | 1.8 × 10^-6 |
| Day | Day\_1 - Day\_5 | 2.131 | 0.165 | 24 | 12.949 | 1.5 × 10^-11 |
| Day | Day\_1 - Day\_7 | 2.805 | 0.165 | 24 | 17.046 | 6.2 × 10^-14 |
| Day | Day\_3 - Day\_5 | 0.981 | 0.165 | 24 | 5.964 | 2.1 × 10^-5 |
| Day | Day\_3 - Day\_7 | 1.655 | 0.165 | 24 | 10.060 | 2.5 × 10^-9 |
| Day | Day\_5 - Day\_7 | 0.674 | 0.165 | 24 | 4.097 | 0.002 |
| Medium | (1%TB+AEbiofilm) - (MQ+AEbiofilm) | -2.932 | 0.142 | 24 | -20.579 | 2.1 × 10^-14 |
| Medium | (1%TB+AEbiofilm) - (PW+AEBiofilm) | -2.496 | 0.142 | 24 | -17.515 | 3.2 × 10^-14 |
| Medium | (MQ+AEbiofilm) - (PW+AEBiofilm) | 0.437 | 0.142 | 24 | 3.064 | 0.014 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_3 1%TB+AEbiofilm) | 2.045 | 0.285 | 24 | 7.176 | 1.1 × 10^-5 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_5 1%TB+AEbiofilm) | 2.092 | 0.285 | 24 | 7.340 | 7.8 × 10^-6 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_7 1%TB+AEbiofilm) | 1.994 | 0.285 | 24 | 6.997 | 1.7 × 10^-5 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_1 MQ+AEbiofilm) | -3.098 | 0.285 | 24 | -10.869 | 5.6 × 10^-9 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_3 MQ+AEbiofilm) | -1.988 | 0.285 | 24 | -6.974 | 1.8 × 10^-5 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_5 MQ+AEbiofilm) | -0.620 | 0.285 | 24 | -2.175 | 0.580 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_7 MQ+AEbiofilm) | 0.107 | 0.285 | 24 | 0.374 | 1.000 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_1 PW+AEBiofilm) | -2.296 | 0.285 | 24 | -8.056 | 1.6 × 10^-6 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_3 PW+AEBiofilm) | -2.003 | 0.285 | 24 | -7.028 | 1.6 × 10^-5 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_5 PW+AEBiofilm) | -0.474 | 0.285 | 24 | -1.662 | 0.868 |
| Interaction | (Day\_1 1%TB+AEbiofilm) - (Day\_7 PW+AEBiofilm) | 0.920 | 0.285 | 24 | 3.228 | 0.109 |
| Interaction | (Day\_3 1%TB+AEbiofilm) - (Day\_5 1%TB+AEbiofilm) | 0.047 | 0.285 | 24 | 0.164 | 1.000 |
| Interaction | (Day\_3 1%TB+AEbiofilm) - (Day\_7 1%TB+AEbiofilm) | -0.051 | 0.285 | 24 | -0.178 | 1.000 |
| Interaction | (Day\_3 1%TB+AEbiofilm) - (Day\_1 MQ+AEbiofilm) | -5.143 | 0.285 | 24 | -18.045 | 1.4 × 10^-13 |
| Interaction | (Day\_3 1%TB+AEbiofilm) - (Day\_3 MQ+AEbiofilm) | -4.033 | 0.285 | 24 | -14.150 | 2.3 × 10^-11 |
| Interaction | (Day\_3 1%TB+AEbiofilm) - (Day\_5 MQ+AEbiofilm) | -2.665 | 0.285 | 24 | -9.351 | 1.0 × 10^-7 |
| Interaction | (Day\_3 1%TB+AEbiofilm) - (Day\_7 MQ+AEbiofilm) | -1.938 | 0.285 | 24 | -6.802 | 2.7 × 10^-5 |
| Interaction | (Day\_3 1%TB+AEbiofilm) - (Day\_1 PW+AEBiofilm) | -4.341 | 0.285 | 24 | -15.232 | 4.7 × 10^-12 |
| Interaction | (Day\_3 1%TB+AEbiofilm) - (Day\_3 PW+AEBiofilm) | -4.048 | 0.285 | 24 | -14.203 | 2.1 × 10^-11 |
| Interaction | (Day\_3 1%TB+AEbiofilm) - (Day\_5 PW+AEBiofilm) | -2.518 | 0.285 | 24 | -8.837 | 3.0 × 10^-7 |
| Interaction | (Day\_3 1%TB+AEbiofilm) - (Day\_7 PW+AEBiofilm) | -1.125 | 0.285 | 24 | -3.948 | 0.023 |
| Interaction | (Day\_5 1%TB+AEbiofilm) - (Day\_7 1%TB+AEbiofilm) | -0.098 | 0.285 | 24 | -0.342 | 1.000 |
| Interaction | (Day\_5 1%TB+AEbiofilm) - (Day\_1 MQ+AEbiofilm) | -5.189 | 0.285 | 24 | -18.209 | 1.2 × 10^-13 |
| Interaction | (Day\_5 1%TB+AEbiofilm) - (Day\_3 MQ+AEbiofilm) | -4.079 | 0.285 | 24 | -14.314 | 1.8 × 10^-11 |
| Interaction | (Day\_5 1%TB+AEbiofilm) - (Day\_5 MQ+AEbiofilm) | -2.712 | 0.285 | 24 | -9.515 | 7.5 × 10^-8 |
| Interaction | (Day\_5 1%TB+AEbiofilm) - (Day\_7 MQ+AEbiofilm) | -1.985 | 0.285 | 24 | -6.966 | 1.8 × 10^-5 |
| Interaction | (Day\_5 1%TB+AEbiofilm) - (Day\_1 PW+AEBiofilm) | -4.388 | 0.285 | 24 | -15.396 | 3.7 × 10^-12 |
| Interaction | (Day\_5 1%TB+AEbiofilm) - (Day\_3 PW+AEBiofilm) | -4.095 | 0.285 | 24 | -14.368 | 1.6 × 10^-11 |
| Interaction | (Day\_5 1%TB+AEbiofilm) - (Day\_5 PW+AEBiofilm) | -2.565 | 0.285 | 24 | -9.001 | 2.1 × 10^-7 |
| Interaction | (Day\_5 1%TB+AEbiofilm) - (Day\_7 PW+AEBiofilm) | -1.172 | 0.285 | 24 | -4.112 | 0.016 |
| Interaction | (Day\_7 1%TB+AEbiofilm) - (Day\_1 MQ+AEbiofilm) | -5.092 | 0.285 | 24 | -17.867 | 1.7 × 10^-13 |
| Interaction | (Day\_7 1%TB+AEbiofilm) - (Day\_3 MQ+AEbiofilm) | -3.982 | 0.285 | 24 | -13.972 | 3.0 × 10^-11 |
| Interaction | (Day\_7 1%TB+AEbiofilm) - (Day\_5 MQ+AEbiofilm) | -2.614 | 0.285 | 24 | -9.172 | 1.5 × 10^-7 |
| Interaction | (Day\_7 1%TB+AEbiofilm) - (Day\_7 MQ+AEbiofilm) | -1.888 | 0.285 | 24 | -6.624 | 4.0 × 10^-5 |
| Interaction | (Day\_7 1%TB+AEbiofilm) - (Day\_1 PW+AEBiofilm) | -4.290 | 0.285 | 24 | -15.053 | 6.0 × 10^-12 |
| Interaction | (Day\_7 1%TB+AEbiofilm) - (Day\_3 PW+AEBiofilm) | -3.997 | 0.285 | 24 | -14.025 | 2.8 × 10^-11 |
| Interaction | (Day\_7 1%TB+AEbiofilm) - (Day\_5 PW+AEBiofilm) | -2.468 | 0.285 | 24 | -8.659 | 4.3 × 10^-7 |
| Interaction | (Day\_7 1%TB+AEbiofilm) - (Day\_7 PW+AEBiofilm) | -1.074 | 0.285 | 24 | -3.770 | 0.035 |
| Interaction | (Day\_1 MQ+AEbiofilm) - (Day\_3 MQ+AEbiofilm) | 1.110 | 0.285 | 24 | 3.895 | 0.026 |
| Interaction | (Day\_1 MQ+AEbiofilm) - (Day\_5 MQ+AEbiofilm) | 2.478 | 0.285 | 24 | 8.694 | 4.0 × 10^-7 |
| Interaction | (Day\_1 MQ+AEbiofilm) - (Day\_7 MQ+AEbiofilm) | 3.204 | 0.285 | 24 | 11.243 | 2.8 × 10^-9 |
| Interaction | (Day\_1 MQ+AEbiofilm) - (Day\_1 PW+AEBiofilm) | 0.802 | 0.285 | 24 | 2.813 | 0.235 |
| Interaction | (Day\_1 MQ+AEbiofilm) - (Day\_3 PW+AEBiofilm) | 1.095 | 0.285 | 24 | 3.842 | 0.030 |
| Interaction | (Day\_1 MQ+AEbiofilm) - (Day\_5 PW+AEBiofilm) | 2.624 | 0.285 | 24 | 9.208 | 1.4 × 10^-7 |
| Interaction | (Day\_1 MQ+AEbiofilm) - (Day\_7 PW+AEBiofilm) | 4.017 | 0.285 | 24 | 14.097 | 2.5 × 10^-11 |
| Interaction | (Day\_3 MQ+AEbiofilm) - (Day\_5 MQ+AEbiofilm) | 1.368 | 0.285 | 24 | 4.799 | 0.003 |
| Interaction | (Day\_3 MQ+AEbiofilm) - (Day\_7 MQ+AEbiofilm) | 2.094 | 0.285 | 24 | 7.348 | 7.6 × 10^-6 |
| Interaction | (Day\_3 MQ+AEbiofilm) - (Day\_1 PW+AEBiofilm) | -0.308 | 0.285 | 24 | -1.082 | 0.993 |
| Interaction | (Day\_3 MQ+AEbiofilm) - (Day\_3 PW+AEBiofilm) | -0.015 | 0.285 | 24 | -0.053 | 1.000 |
| Interaction | (Day\_3 MQ+AEbiofilm) - (Day\_5 PW+AEBiofilm) | 1.514 | 0.285 | 24 | 5.313 | 9.2 × 10^-4 |
| Interaction | (Day\_3 MQ+AEbiofilm) - (Day\_7 PW+AEBiofilm) | 2.907 | 0.285 | 24 | 10.202 | 1.9 × 10^-8 |
| Interaction | (Day\_5 MQ+AEbiofilm) - (Day\_7 MQ+AEbiofilm) | 0.726 | 0.285 | 24 | 2.549 | 0.359 |
| Interaction | (Day\_5 MQ+AEbiofilm) - (Day\_1 PW+AEBiofilm) | -1.676 | 0.285 | 24 | -5.881 | 2.3 × 10^-4 |
| Interaction | (Day\_5 MQ+AEbiofilm) - (Day\_3 PW+AEBiofilm) | -1.383 | 0.285 | 24 | -4.853 | 0.003 |
| Interaction | (Day\_5 MQ+AEbiofilm) - (Day\_5 PW+AEBiofilm) | 0.146 | 0.285 | 24 | 0.513 | 1.000 |
| Interaction | (Day\_5 MQ+AEbiofilm) - (Day\_7 PW+AEBiofilm) | 1.540 | 0.285 | 24 | 5.403 | 7.4 × 10^-4 |
| Interaction | (Day\_7 MQ+AEbiofilm) - (Day\_1 PW+AEBiofilm) | -2.402 | 0.285 | 24 | -8.430 | 7.0 × 10^-7 |
| Interaction | (Day\_7 MQ+AEbiofilm) - (Day\_3 PW+AEBiofilm) | -2.109 | 0.285 | 24 | -7.401 | 6.7 × 10^-6 |
| Interaction | (Day\_7 MQ+AEbiofilm) - (Day\_5 PW+AEBiofilm) | -0.580 | 0.285 | 24 | -2.035 | 0.668 |
| Interaction | (Day\_7 MQ+AEbiofilm) - (Day\_7 PW+AEBiofilm) | 0.813 | 0.285 | 24 | 2.854 | 0.219 |
| Interaction | (Day\_1 PW+AEBiofilm) - (Day\_3 PW+AEBiofilm) | 0.293 | 0.285 | 24 | 1.028 | 0.995 |
| Interaction | (Day\_1 PW+AEBiofilm) - (Day\_5 PW+AEBiofilm) | 1.822 | 0.285 | 24 | 6.395 | 6.9 × 10^-5 |
| Interaction | (Day\_1 PW+AEBiofilm) - (Day\_7 PW+AEBiofilm) | 3.216 | 0.285 | 24 | 11.284 | 2.6 × 10^-9 |
| Interaction | (Day\_3 PW+AEBiofilm) - (Day\_5 PW+AEBiofilm) | 1.529 | 0.285 | 24 | 5.366 | 8.1 × 10^-4 |
| Interaction | (Day\_3 PW+AEBiofilm) - (Day\_7 PW+AEBiofilm) | 2.923 | 0.285 | 24 | 10.255 | 1.8 × 10^-8 |
| Interaction | (Day\_5 PW+AEBiofilm) - (Day\_7 PW+AEBiofilm) | 1.393 | 0.285 | 24 | 4.889 | 0.003 |