Monolayer Biofilm Experiments

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# Experiment 4: Assessment of AE microorganisms’ effect on monolayer-associated Bd cells

Data wrangling and analysis the Bd monolayer experiment using the SFEB (San Francisco East Bay) water samples for part A and the follow up experiment part B with SBNCOS (Santa Barbara North Campus Open Space) pond water samples. These are the data presented in Figure 7A and 7B in the manuscript, and information is under the section “Experiment 4”.

## 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  
  
# data  
# SFEB  
ns\_ml\_pw\_bd <- read.csv(here("data", "nine-sites-PW-on-MLBd - Sheet1.csv"))  
# SBNCOS  
fig\_4b\_raw <- read.csv(here("data", "final\_NCOS\_2024\_reformatted\_for\_R.xlsx - Fig4B.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 for exploratory data  
myCustomTheme <- function() {  
 theme\_light() +  
 theme(axis.text = element\_text(size = 12, family = "Times", color = "black"),  
 axis.title.x = element\_text(margin = margin(t = 10), size = 14, face = "bold", family = "Times", color = "black"), # Add space between x-axis label and axis  
 axis.title.y = element\_text(margin = margin(r = 10), size = 14, face = "bold", family = "Times", color = "black"), # Add space between y-axis label and axis  
 title = element\_text(size = 12, face = "bold", family = "Times"),  
 plot.caption = element\_text(size = 10, face = "italic", family = "Times"),  
 legend.text = element\_text(size = 10, family = "Times"), # Increase legend text size  
 panel.grid.major.x = element\_blank(), # Remove major vertical grid lines  
 panel.grid.minor.x = element\_blank(), # Remove minor vertical grid lines  
 panel.grid.major.y = element\_blank(), # Remove major horizontal grid lines  
 panel.grid.minor.y = element\_blank(), # Remove minor horizontal grid lines  
 strip.text = element\_text(size = 12, face = "bold", family = "Times", color = "black"), # Set strip text style  
 strip.background = element\_rect(fill = "white", color = "grey"), # Set strip background to white, # color = "black"  
 axis.ticks = element\_blank() # Remove x and y axis ticks  
 )}

# SFEB Monolayer

Scientific Q: Is mono-strain Bd biofilm resistant to microbes in pond water

Statistical question:

Is there a difference in the **gain or loss of ADHERENT Bd over 6 days** between the **filter sizes**?

**There is a significant difference in the change in the quantity of adherent Bd from Day 1 to Day 7 across the filter types (t = -6.45, df = 7, p-value = 0.0003)**

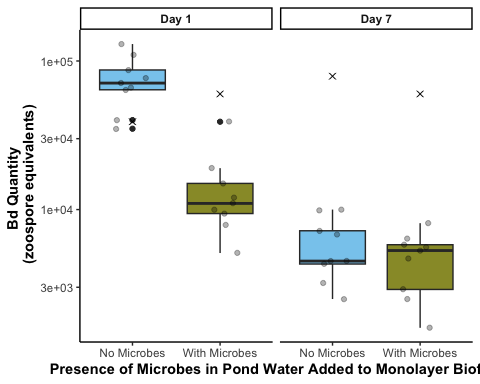
## Data Wrangling SFEB Monolayer

eb\_monolayer <- ns\_ml\_pw\_bd %>%   
 filter(site != "MQ") %>% # remove control  
 filter(bd\_location == "adherent") # only want adherent Bd data (decision by Renwei, most relevant to our scientific question)  
  
# keep control for labeling plot  
eb\_ml\_controls <- ns\_ml\_pw\_bd %>%   
 filter(site =="MQ") %>% # controls only  
 filter(bd\_location == "adherent") # only want adherent Bd data for controls too  
  
# Data type cleaning  
eb\_monolayer$site <- factor(eb\_monolayer$site,  
 levels = c("CABIN", "GRAMPS", "WEST", "GDPND005", "GDPND006", "GDPND009", "PRPND004", "PRPND009", "PRPND010"))  
eb\_monolayer$day <- factor(eb\_monolayer$day, levels = c("Day\_1", "Day\_7"))   
  
# get the total difference across days by combining both locations of Bd then subtracting across days  
eb\_monolayer\_diff <- eb\_monolayer %>%  
 # pivot so each day has its own column  
 pivot\_wider(names\_from = day, values\_from = bd\_qty) %>%  
 # calculate the rate loss by taking the log of each before subtracting  
 mutate(rate\_loss = log(Day\_1) - log(Day\_7))  
  
# Create subsets for each treatment  
eb\_monolayer\_diff\_40um <- eb\_monolayer\_diff %>%   
 filter(filter =="40um\_filter")  
eb\_monolayer\_diff.22um <- eb\_monolayer\_diff %>%   
 filter(filter =="0.22um\_filter")

## EDA SFEB Monolayer

Boxplot

eb\_monolayer %>%  
  
 # plot it  
 ggplot(aes(y= bd\_qty, x = filter, fill = filter)) +   
 geom\_boxplot() +  
 geom\_jitter(width = 0.2, alpha = 0.3) +  
 scale\_y\_log10() +  
 facet\_wrap(~day, labeller = labeller(day = c("Day\_1" = "Day 1",  
 "Day\_7" = "Day 7")))+  
 scale\_fill\_manual(values = c("40um\_filter" = with\_microbes\_40\_color,   
 "0.22um\_filter" = no\_microbes\_.22\_color)) +  
 theme\_classic() +  
 theme(legend.position = "none",  
 strip.text = element\_text(face="bold"),  
 axis.title = element\_text(face = "bold")) +   
 scale\_x\_discrete (labels= c("40um\_filter" = "With Microbes", "0.22um\_filter" = "No Microbes")) +  
 xlab("Presence of Microbes in Pond Water Added to Monolayer Biofilm") +  
 ylab("Bd Quantity \n (zoospore equivalents)") +  
  
 # add controls ad x's  
geom\_point(data = eb\_ml\_controls, aes(x = filter, y = bd\_qty), shape = 4, size = 2)



## SFEB Stats and assumption testing

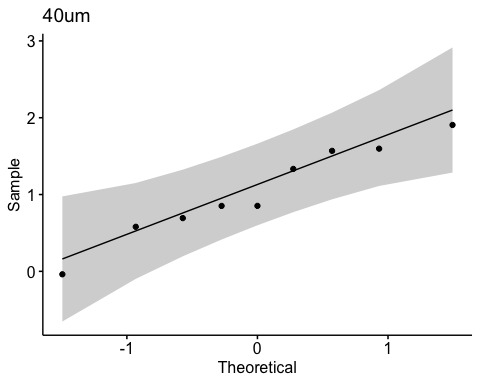
Question:

Question: Does the rate loss in Bd from day 1 to day 7 (aka Bd-inhibitory potency) differ between the two filter types (40 and 0.22 um; aka AE microorganisms + and AE microorganisms -)?

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

Test: Paired t-test on rate loss of Bd between day 1 and 7

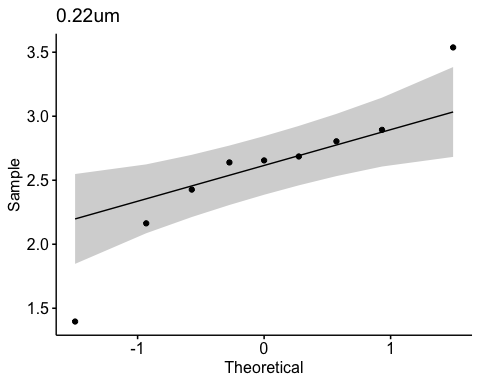
# check normality of the differences across groups  
# With microbes (40 um)  
eb\_monolayer\_diff\_40um %>%   
 ggqqplot("rate\_loss", title = "40um") # normal



shapiro.test(eb\_monolayer\_diff\_40um$rate\_loss) # numeric check using shapiro wilks test, a p > 0.05 says the data is indeed normal

Shapiro-Wilk normality test  
  
data: eb\_monolayer\_diff\_40um$rate\_loss  
W = 0.95903, p-value = 0.7881

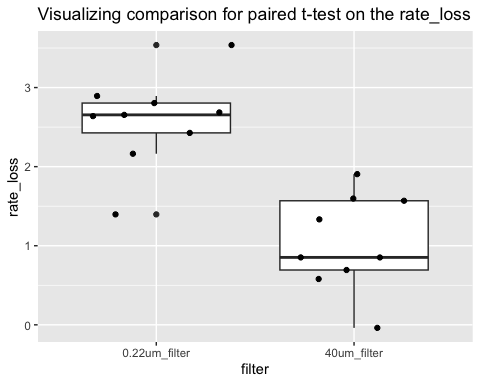
# Without microbes (0.22 um)  
eb\_monolayer\_diff.22um%>%   
 ggqqplot("rate\_loss", title = "0.22um") # looks good



shapiro.test(eb\_monolayer\_diff.22um$rate\_loss) # numeric check using shapiro wilks test, a p > 0.05 says the data is indeed normal

Shapiro-Wilk normality test  
  
data: eb\_monolayer\_diff.22um$rate\_loss  
W = 0.92597, p-value = 0.4439

# visualize the comparison I am making  
eb\_monolayer\_diff %>%  
ggplot(aes(y= rate\_loss, x = filter)) +   
 geom\_boxplot() +  
 geom\_jitter() +  
 ggtitle("Visualizing comparison for paired t-test on the rate\_loss")



# Stats SFEB

# Step 3: run the paired t-test on the difference  
t.test(eb\_monolayer\_diff\_40um$rate\_loss, eb\_monolayer\_diff.22um$rate\_loss, paired = TRUE)

Paired t-test  
  
data: eb\_monolayer\_diff\_40um$rate\_loss and eb\_monolayer\_diff.22um$rate\_loss  
t = -5.3606, df = 8, p-value = 0.0006772  
alternative hypothesis: true mean difference is not equal to 0  
95 percent confidence interval:  
 -2.2020978 -0.8773797  
sample estimates:  
mean difference   
 -1.539739

### Stats results:

When monolayer Bd biofilm is exposed to pond water without microbes has a significantly greater loss of Bd between day 1 to day 7 than pond water with microbes (t = -5.4, df = 8, p = 0.0006772). The t-value is negative, which shows that the first item entered (with microbes) has a smaller loss of Bd than the second item entered (no microbes).

## Planned comparison to look at Day 1

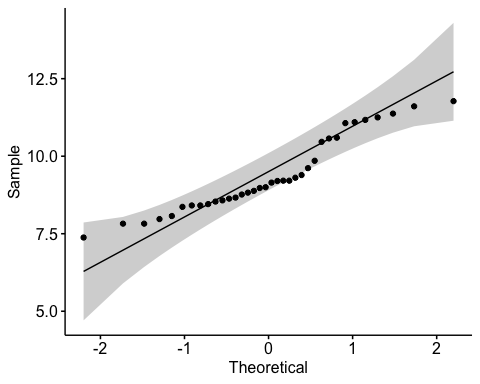
Q: qty ~ factor(day) + microbe\_treatment, random = ~1|factor(site)

Question: does the quantity of Bd differ across days and treatment while controlling for the paired site?

This will be a lme since it is a mixed effects model!

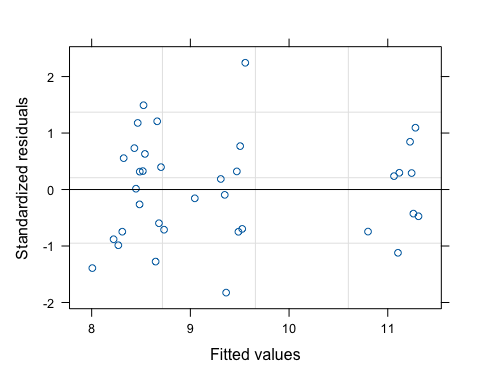
Data wrangling

eb\_monolayer <- eb\_monolayer %>%   
 mutate(log\_bd = log(bd\_qty))  
  
# Exploration: normality of transformed data  
eb\_monolayer %>%   
 ggqqplot("log\_bd") # normal yay



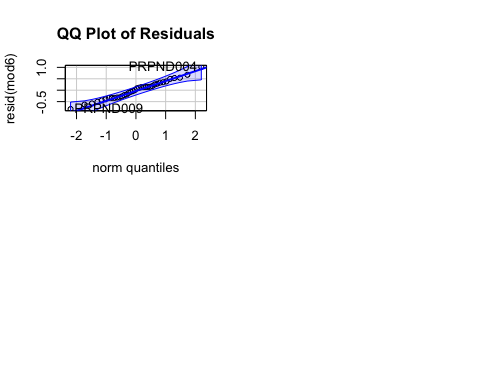
Assumptions testing

par(mfrow = c(2,2))  
# build model  
mod6 <- lme(log\_bd~day\*factor(filter), random = ~1|site, data=eb\_monolayer)  
# model assumptions  
plot(mod6) # passes homogeneity of variances yay



qqPlot(resid(mod6), main="QQ Plot of Residuals") # passes normality of resids yay

PRPND004 PRPND009   
 24 23



Interpret

summary(mod6)

Linear mixed-effects model fit by REML  
 Data: eb\_monolayer   
 AIC BIC logLik  
 66.32921 75.12363 -27.16461  
  
Random effects:  
 Formula: ~1 | site  
 (Intercept) Residual  
StdDev: 0.2252822 0.4522353  
  
Fixed effects: log\_bd ~ day \* factor(filter)   
 Value Std.Error DF t-value p-value  
(Intercept) 11.155886 0.1684138 24 66.24093 0  
dayDay\_7 -2.577871 0.2131858 24 -12.09214 0  
factor(filter)40um\_filter -1.754991 0.2131858 24 -8.23222 0  
dayDay\_7:factor(filter)40um\_filter 1.539739 0.3014902 24 5.10709 0  
 Correlation:   
 (Intr) dyDy\_7 f()40\_  
dayDay\_7 -0.633   
factor(filter)40um\_filter -0.633 0.500   
dayDay\_7:factor(filter)40um\_filter 0.448 -0.707 -0.707  
  
Standardized Within-Group Residuals:  
 Min Q1 Med Q3 Max   
-1.8252309 -0.7197000 0.1006392 0.5741601 2.2442375   
  
Number of Observations: 36  
Number of Groups: 9

anova(mod6)

numDF denDF F-value p-value  
(Intercept) 1 24 7763.082 <.0001  
day 1 24 143.850 <.0001  
factor(filter) 1 24 42.706 <.0001  
day:factor(filter) 1 24 26.082 <.0001

emmeans::emmeans(mod6, pairwise ~ day \* filter) # pairwise comparison

$emmeans  
 day filter emmean SE df lower.CL upper.CL  
 Day\_1 0.22um\_filter 11.16 0.168 8 10.77 11.54  
 Day\_7 0.22um\_filter 8.58 0.168 8 8.19 8.97  
 Day\_1 40um\_filter 9.40 0.168 8 9.01 9.79  
 Day\_7 40um\_filter 8.36 0.168 8 7.97 8.75  
  
Degrees-of-freedom method: containment   
Confidence level used: 0.95   
  
$contrasts  
 contrast estimate SE df t.ratio p.value  
 Day\_1 0.22um\_filter - Day\_7 0.22um\_filter 2.578 0.213 24 12.092 <.0001  
 Day\_1 0.22um\_filter - Day\_1 40um\_filter 1.755 0.213 24 8.232 <.0001  
 Day\_1 0.22um\_filter - Day\_7 40um\_filter 2.793 0.213 24 13.102 <.0001  
 Day\_7 0.22um\_filter - Day\_1 40um\_filter -0.823 0.213 24 -3.860 0.0039  
 Day\_7 0.22um\_filter - Day\_7 40um\_filter 0.215 0.213 24 1.010 0.7454  
 Day\_1 40um\_filter - Day\_7 40um\_filter 1.038 0.213 24 4.870 0.0003  
  
Degrees-of-freedom method: containment   
P value adjustment: tukey method for comparing a family of 4 estimates

Bd abundance was indeed significantly lower on day 1 in the presence of AE microorganisms compared to its abundance in microorganism-depleted pond water (Day\_1 0.22um\_filter - Day\_1 40um\_filter p <.0001)

# SBNCOS

## Data Wrangling SBNCOS

## Monolayer  
# add column for microbes or no  
monolayer <- fig\_4b\_raw %>%   
 rename(sample\_ID = sample.ID) %>%   
 rename(adh\_plus\_sup = ahd\_plus\_sup) %>%   
 # rename sample\_id to only include treatment, not day (we have a column for day already)  
 mutate(sample\_ID = str\_replace(sample\_ID, "-D[0-9]+$", "")) %>%   
  
 # add columns for components y/n  
 mutate(microbes = case\_when(  
 str\_detect(sample\_ID, "\\+microbes") ~ "y",  
 TRUE ~ "n")) %>%   
 # add column for TB or no  
 mutate(TB = case\_when(  
 str\_detect(sample\_ID, "TB") ~ "y",  
 TRUE ~ "n")) %>%   
 mutate(day = case\_when(  
 day == 1 ~ "Day\_1",  
 day == 3 ~ "Day\_3",  
 day == 5 ~ "Day\_5",  
 day == 7 ~ "Day\_7",  
 day == 0 ~ "Day\_0")) %>%   
 mutate(day = factor(day)) %>%   
 mutate(day\_numeric = as.numeric(gsub("Day\_", "", as.character(day)))) # add day numeric for plotting  
  
monolayer\_summary <- monolayer %>%   
 group\_by(day, sample\_ID) %>%   
 reframe(mean\_adh = 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  
 ) %>%   
 # re-create above columns, but in the summary df  
 mutate(microbes = case\_when(  
 str\_detect(sample\_ID, "\\+microbes") ~ "y",TRUE ~ "n")) %>%   
 # add column for TB or no  
 mutate(TB = case\_when(str\_detect(sample\_ID, "TB") ~ "y", TRUE ~ "n")) %>%   
 mutate(day\_numeric = as.numeric(gsub("Day\_", "", as.character(day))))  
  
# no day 0, this is the "initial Bd", not part of the analysis  
ml\_noday0 <- monolayer %>%  
 filter(day != "Day\_0") %>%  
 mutate(log\_adh = log(adh)) %>%   
 mutate(day = as.factor(day))  
  
# set PW-microbes as reference (no milliQ control here)  
ml\_noday0$sample\_ID <- factor(ml\_noday0$sample\_ID) # set as factor  
ml\_noday0$sample\_ID <- relevel(ml\_noday0$sample\_ID, ref = "PW-microbes") # assign reference  
  
# change the names in microbes and tb so there arent 2 levels with y and n (oops that was shortsighted of me!)  
ml\_noday0 <- ml\_noday0 %>%   
 mutate(  
 microbes = case\_when(  
 microbes == "y" ~ "MO present",  
 microbes == "n" ~ "MO absent"),  
 TB = case\_when(  
 TB == "y" ~ "TB present",  
 TB == "n" ~ "TB absent"))

## EDA SBNCOS

visualize y var: bd load

Commented out to save space, but spoiler alert: log transformed is better

# # untransformed  
# ml\_noday0 <- monolayer %>%  
# filter(day != 0) %>%  
# mutate(log\_adh = log(adh)) # note: no zeroes so not log + 1  
# ggqqplot(ml\_noday0, "adh", title = "untransformed")  
# shapiro.test(ml\_noday0$adh) # nope  
# hist(ml\_noday0$adh) # note  
#   
# # transformed  
# ggqqplot(ml\_noday0, "log\_adh", title = "log transformed") # gorgeous  
# hist(ml\_noday0$log\_adh) # better

## Stats SBNCOS

y var: amount of Bd (log transformed)

Model: Bd ~ day\*microbes\*TB

Question: Does the amount of Bd in the sample differ across the treatments of presence of microbes, TB, and day?

Model: 3-way ANOVA

**results**

Results summary

* day (p<0.05)
  + All differ from each other except 5 and 7 (Tukey)
    - Day 1 > Day 3 > Day 5 = Day 7
* microbes (p<0.05)
  + presence microbes has less Bd (Tukey)
* TB (NOT SIG)
* day:microbes (p<0.05)
* day:TB (NOT SIG)
* microbes:TB (p<0.05)
* day:microbes:TB (NOT SIG)

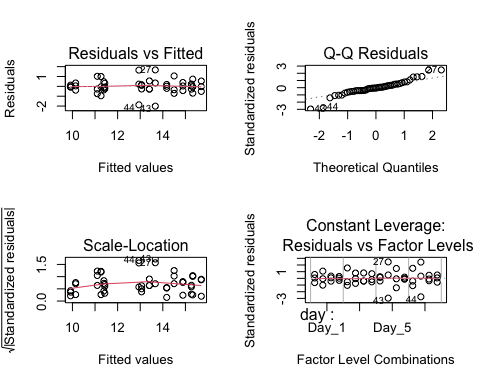
## null

null <- lm(log\_adh ~ 1,  
 data = ml\_noday0)  
AIC(null) #205.4965

[1] 205.4965

## Bd ~ day\*microbes\*TB

# build model  
aov\_4b <- aov(log\_adh ~ day\*microbes\*TB,  
 data = ml\_noday0)  
  
# diagnostic plot  
par(mfrow = c(2,2))  
plot(aov\_4b) # looks good



# look at results  
summary(aov\_4b)

Df Sum Sq Mean Sq F value Pr(>F)   
day 3 93.76 31.25 45.336 1.26e-11 \*\*\*  
microbes 1 37.80 37.80 54.841 2.00e-08 \*\*\*  
TB 1 0.51 0.51 0.746 0.39422   
day:microbes 3 12.21 4.07 5.905 0.00252 \*\*   
day:TB 3 0.94 0.31 0.456 0.71469   
microbes:TB 1 18.12 18.12 26.283 1.37e-05 \*\*\*  
day:microbes:TB 3 1.61 0.54 0.777 0.51570   
Residuals 32 22.06 0.69   
---  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

AIC(aov\_4b) # 132.8989 better than null

[1] 132.8989

## Post hoc

Using a Tukey test on the anova model

TukeyHSD(aov\_4b)

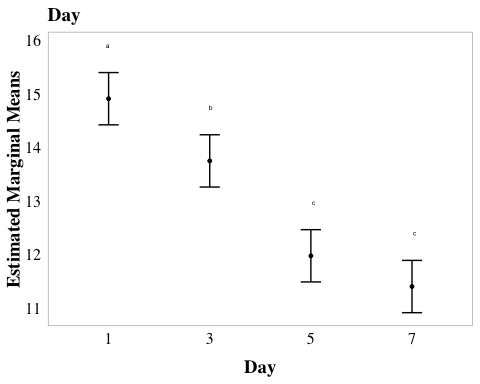
Tukey multiple comparisons of means  
 95% family-wise confidence level  
  
Fit: aov(formula = log\_adh ~ day \* microbes \* TB, data = ml\_noday0)  
  
$day  
 diff lwr upr p adj  
Day\_3-Day\_1 -1.1620166 -2.080368 -0.2436653 0.0087187  
Day\_5-Day\_1 -2.9344665 -3.852818 -2.0161153 0.0000000  
Day\_7-Day\_1 -3.5087930 -4.427144 -2.5904417 0.0000000  
Day\_5-Day\_3 -1.7724499 -2.690801 -0.8540987 0.0000581  
Day\_7-Day\_3 -2.3467763 -3.265128 -1.4284251 0.0000005  
Day\_7-Day\_5 -0.5743264 -1.492678 0.3440249 0.3431674  
  
$microbes  
 diff lwr upr p adj  
MO present-MO absent -1.774918 -2.263125 -1.286711 0  
  
$TB  
 diff lwr upr p adj  
TB present-TB absent -0.2069936 -0.6952002 0.281213 0.3942159  
  
$`day:microbes`  
 diff lwr upr p adj  
Day\_3:MO absent-Day\_1:MO absent -0.004871029 -1.5576434 1.54790136 1.0000000  
Day\_5:MO absent-Day\_1:MO absent -1.742341527 -3.2951139 -0.18956914 0.0191770  
Day\_7:MO absent-Day\_1:MO absent -3.085556125 -4.6383285 -1.53278374 0.0000079  
Day\_1:MO present-Day\_1:MO absent -0.388664202 -1.9414366 1.16410819 0.9912401  
Day\_3:MO present-Day\_1:MO absent -2.707826384 -4.2605988 -1.15505400 0.0000750  
Day\_5:MO present-Day\_1:MO absent -4.515255768 -6.0680282 -2.96248338 0.0000000  
Day\_7:MO present-Day\_1:MO absent -4.320693986 -5.8734664 -2.76792160 0.0000000  
Day\_5:MO absent-Day\_3:MO absent -1.737470497 -3.2902429 -0.18469811 0.0196728  
Day\_7:MO absent-Day\_3:MO absent -3.080685096 -4.6334575 -1.52791271 0.0000082  
Day\_1:MO present-Day\_3:MO absent -0.383793173 -1.9365656 1.16897921 0.9918720  
Day\_3:MO present-Day\_3:MO absent -2.702955355 -4.2557277 -1.15018297 0.0000772  
Day\_5:MO present-Day\_3:MO absent -4.510384738 -6.0631571 -2.95761235 0.0000000  
Day\_7:MO present-Day\_3:MO absent -4.315822957 -5.8685953 -2.76305057 0.0000000  
Day\_7:MO absent-Day\_5:MO absent -1.343214599 -2.8959870 0.20955779 0.1300131  
Day\_1:MO present-Day\_5:MO absent 1.353677324 -0.1990951 2.90644971 0.1243433  
Day\_3:MO present-Day\_5:MO absent -0.965484857 -2.5182572 0.58728753 0.4886753  
Day\_5:MO present-Day\_5:MO absent -2.772914241 -4.3256866 -1.22014185 0.0000509  
Day\_7:MO present-Day\_5:MO absent -2.578352460 -4.1311248 -1.02558007 0.0001622  
Day\_1:MO present-Day\_7:MO absent 2.696891923 1.1441195 4.24966431 0.0000801  
Day\_3:MO present-Day\_7:MO absent 0.377729742 -1.1750426 1.93050213 0.9926085  
Day\_5:MO present-Day\_7:MO absent -1.429699642 -2.9824720 0.12307275 0.0889994  
Day\_7:MO present-Day\_7:MO absent -1.235137861 -2.7879102 0.31763453 0.2015413  
Day\_3:MO present-Day\_1:MO present -2.319162182 -3.8719346 -0.76638979 0.0007513  
Day\_5:MO present-Day\_1:MO present -4.126591565 -5.6793640 -2.57381918 0.0000000  
Day\_7:MO present-Day\_1:MO present -3.932029784 -5.4848022 -2.37925740 0.0000001  
Day\_5:MO present-Day\_3:MO present -1.807429384 -3.3602018 -0.25465700 0.0135812  
Day\_7:MO present-Day\_3:MO present -1.612867602 -3.1656400 -0.06009522 0.0372097  
Day\_7:MO present-Day\_5:MO present 0.194561781 -1.3582106 1.74733417 0.9998954  
  
$`day:TB`  
 diff lwr upr p adj  
Day\_3:TB absent-Day\_1:TB absent -1.1235183 -2.6762907 0.42925405 0.3023905  
Day\_5:TB absent-Day\_1:TB absent -2.9130623 -4.4658347 -1.36028989 0.0000221  
Day\_7:TB absent-Day\_1:TB absent -3.8110976 -5.3638700 -2.25832525 0.0000001  
Day\_1:TB present-Day\_1:TB absent -0.3281947 -1.8809671 1.22457771 0.9968653  
Day\_3:TB present-Day\_1:TB absent -1.5287096 -3.0814819 0.02406283 0.0561428  
Day\_5:TB present-Day\_1:TB absent -3.2840655 -4.8368379 -1.73129311 0.0000025  
Day\_7:TB present-Day\_1:TB absent -3.5346830 -5.0874553 -1.98191056 0.0000006  
Day\_5:TB absent-Day\_3:TB absent -1.7895439 -3.3423163 -0.23677155 0.0149424  
Day\_7:TB absent-Day\_3:TB absent -2.6875793 -4.2403517 -1.13480691 0.0000846  
Day\_1:TB present-Day\_3:TB absent 0.7953237 -0.7574487 2.34809604 0.7119877  
Day\_3:TB present-Day\_3:TB absent -0.4051912 -1.9579636 1.14758117 0.9888085  
Day\_5:TB present-Day\_3:TB absent -2.1605472 -3.7133195 -0.60777477 0.0018907  
Day\_7:TB present-Day\_3:TB absent -2.4111646 -3.9639370 -0.85839223 0.0004372  
Day\_7:TB absent-Day\_5:TB absent -0.8980354 -2.4508077 0.65473703 0.5777381  
Day\_1:TB present-Day\_5:TB absent 2.5848676 1.0320952 4.13763999 0.0001560  
Day\_3:TB present-Day\_5:TB absent 1.3843527 -0.1684197 2.93712511 0.1088829  
Day\_5:TB present-Day\_5:TB absent -0.3710032 -1.9237756 1.18176917 0.9933635  
Day\_7:TB present-Day\_5:TB absent -0.6216207 -2.1743931 0.93115172 0.8933907  
Day\_1:TB present-Day\_7:TB absent 3.4829030 1.9301306 5.03567535 0.0000008  
Day\_3:TB present-Day\_7:TB absent 2.2823881 0.7296157 3.83516047 0.0009319  
Day\_5:TB present-Day\_7:TB absent 0.5270321 -1.0257402 2.07980453 0.9522604  
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Day\_7:TB present-Day\_3:TB present -2.0059734 -3.5587458 -0.45320101 0.0045607  
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$`microbes:TB`  
 diff lwr upr  
MO present:TB absent-MO absent:TB absent -0.5461759 -1.4645271 0.3721754  
MO absent:TB present-MO absent:TB absent 1.0217484 0.1033972 1.9400997  
MO present:TB present-MO absent:TB absent -1.9819115 -2.9002628 -1.0635603  
MO absent:TB present-MO present:TB absent 1.5679243 0.6495730 2.4862756  
MO present:TB present-MO present:TB absent -1.4357356 -2.3540869 -0.5173844  
MO present:TB present-MO absent:TB present -3.0036600 -3.9220112 -2.0853087  
 p adj  
MO present:TB absent-MO absent:TB absent 0.3867876  
MO absent:TB present-MO absent:TB absent 0.0245376  
MO present:TB present-MO absent:TB absent 0.0000098  
MO absent:TB present-MO present:TB absent 0.0003283  
MO present:TB present-MO present:TB absent 0.0009862  
MO present:TB present-MO absent:TB present 0.0000000  
  
$`day:microbes:TB`  
 diff lwr  
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 upr p adj  
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Day\_3:MO absent:TB present-Day\_1:MO absent:TB present 2.8921954 0.9999998  
Day\_5:MO absent:TB present-Day\_1:MO absent:TB present 0.8561742 0.5308207  
Day\_7:MO absent:TB present-Day\_1:MO absent:TB present 0.1302844 0.0777604  
Day\_1:MO present:TB present-Day\_1:MO absent:TB present 1.3602307 0.9314141  
Day\_3:MO present:TB present-Day\_1:MO absent:TB present -1.4192477 0.0001863  
Day\_5:MO present:TB present-Day\_1:MO absent:TB present -2.8939384 0.0000004  
Day\_7:MO present:TB present-Day\_1:MO absent:TB present -2.6692835 0.0000011  
Day\_5:MO absent:TB present-Day\_3:MO absent:TB present 0.4777256 0.2229337  
Day\_7:MO absent:TB present-Day\_3:MO absent:TB present -0.2481642 0.0204568  
Day\_1:MO present:TB present-Day\_3:MO absent:TB present 0.9817820 0.6525195  
Day\_3:MO present:TB present-Day\_3:MO absent:TB present -1.7976963 0.0000387  
Day\_5:MO present:TB present-Day\_3:MO absent:TB present -3.2723870 0.0000001  
Day\_7:MO present:TB present-Day\_3:MO absent:TB present -3.0477321 0.0000002  
Day\_7:MO absent:TB present-Day\_5:MO absent:TB present 1.7878570 0.9990715  
Day\_1:MO present:TB present-Day\_5:MO absent:TB present 3.0178032 0.9999887  
Day\_3:MO present:TB present-Day\_5:MO absent:TB present 0.2383249 0.1102026  
Day\_5:MO present:TB present-Day\_5:MO absent:TB present -1.2363658 0.0003976  
Day\_7:MO present:TB present-Day\_5:MO absent:TB present -1.0117109 0.0010033  
Day\_1:MO present:TB present-Day\_7:MO absent:TB present 3.7436930 0.8934059  
Day\_3:MO present:TB present-Day\_7:MO absent:TB present 0.9642147 0.6356474  
Day\_5:MO present:TB present-Day\_7:MO absent:TB present -0.5104760 0.0075145  
Day\_7:MO present:TB present-Day\_7:MO absent:TB present -0.2858211 0.0177729  
Day\_3:MO present:TB present-Day\_1:MO present:TB present -0.2657316 0.0191605  
Day\_5:MO present:TB present-Day\_1:MO present:TB present -1.7404222 0.0000491  
Day\_7:MO present:TB present-Day\_1:MO present:TB present -1.5157674 0.0001248  
Day\_5:MO present:TB present-Day\_3:MO present:TB present 1.0390561 0.7063594  
Day\_7:MO present:TB present-Day\_3:MO present:TB present 1.2637110 0.8816492  
Day\_7:MO present:TB present-Day\_5:MO present:TB present 2.7384017 1.0000000

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.

# emmeans and cld (compact letter display)  
  
#..............................day...............................  
  
# perform tukey pairwise comparisons across day only & get cld letters  
cld\_day <- emmeans(aov\_4b, pairwise ~ day, adjust = "tukey") %>%  
 cld(Letters = letters, reverse = TRUE)

NOTE: Results may be misleading due to involvement in interactions

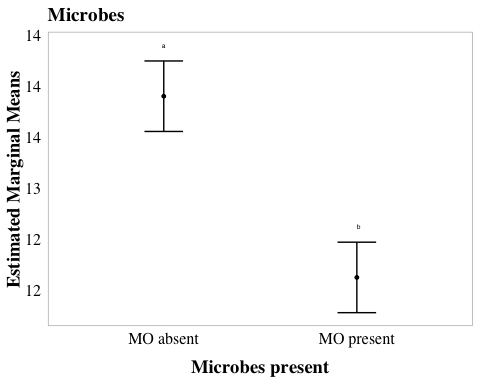
# make a plot of estimated marginal means with CLD letters for each day  
cld\_day\_4b <- ggplot(cld\_day, aes(x = day, y = emmean)) +  
 geom\_point(size = 1) + # Plot the estimated means  
 geom\_errorbar(aes(ymin = lower.CL, ymax = upper.CL), width = 0.2) + # Error bars  
 geom\_text(aes(label = .group), nudge\_y = 1, size = 1.75, color = "black") + # Add CLD letters  
 xlab("Day") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1)) +  
 ggtitle("Day") +  
 scale\_x\_discrete(labels= c("Day\_1" = "1", "Day\_3" = "3", "Day\_5" = "5", "Day\_7" = "7")) +  
 myCustomTheme() + # Use minimal theme for clean look  
 theme(axis.title.y = element\_text(margin = margin(r = 1)))  
cld\_day\_4b



#.........................microbes...............................  
  
cld\_microbes <- emmeans(aov\_4b, pairwise ~ microbes, adjust = "tukey") %>%  
 cld(Letters = letters, reverse = TRUE)

NOTE: Results may be misleading due to involvement in interactions

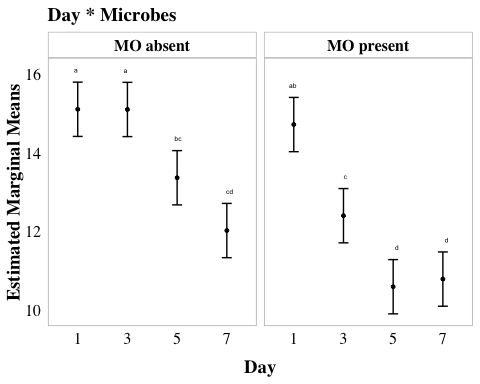
cld\_microbes\_4b <- ggplot(cld\_microbes, aes(x = microbes, y = emmean)) +  
 geom\_point(size = 1) + # Plot the estimated means  
 geom\_errorbar(aes(ymin = lower.CL, ymax = upper.CL), width = 0.2) +   
 geom\_text(aes(label = .group), nudge\_y = 0.5, size = 1.75, color = "black") +   
 xlab("Microbes present") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1)) +  
 ggtitle("Microbes") +  
 scale\_x\_discrete(labels= c("1%TB+AEbiofilm" = "1%TB", "PW+AEBiofilm" = "PW - MO", "MQ+AEbiofilm" = "MQ")) +  
 myCustomTheme() +   
theme(axis.title.y = element\_text(margin = margin(r = 1)))  
cld\_microbes\_4b



#.....................day\*microbes...............................  
  
cld\_day\_microbes <- emmeans(aov\_4b, pairwise ~ day \* microbes, adjust = "tukey") %>%  
 cld(Letters = letters, reverse = TRUE)

NOTE: Results may be misleading due to involvement in interactions

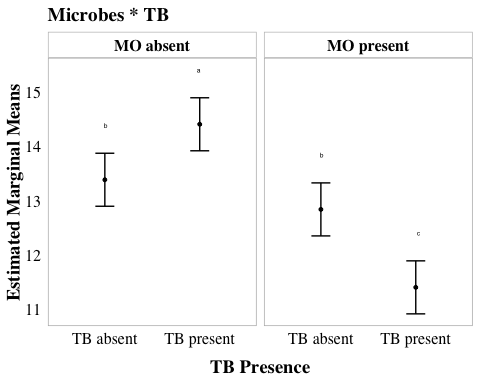
cld\_day\_microbes\_4b <- ggplot(cld\_day\_microbes, aes(x = day, y = emmean)) +  
 geom\_point(size = 1) +   
 geom\_errorbar(aes(ymin = lower.CL, ymax = upper.CL), width = 0.2) +   
 geom\_text(aes(label = .group), nudge\_y = 1, size = 1.75, color = "black") +   
facet\_wrap(~ factor(microbes)) +   
 xlab("Day") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1)) +  
 ggtitle("Day \* Microbes") +  
 scale\_x\_discrete(labels= c("Day\_1" = "1", "Day\_3" = "3", "Day\_5" = "5", "Day\_7" = "7")) +  
 myCustomTheme() +   
theme(axis.title.y = element\_text(margin = margin(r = 1)))  
cld\_day\_microbes\_4b



#.....................microbes\*tb...............................  
  
cld\_TB\_microbes <- emmeans(aov\_4b, pairwise ~ TB \* microbes, adjust = "tukey") %>%  
 cld(Letters = letters, reverse = TRUE)

NOTE: Results may be misleading due to involvement in interactions

cld\_TB\_microbes\_4b <- ggplot(cld\_TB\_microbes, aes(x = TB, 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(microbes)) +   
 xlab("TB Presence") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1)) +  
 ggtitle("Microbes \* TB") +  
 scale\_x\_discrete(labels= c("Day\_1" = "1", "Day\_3" = "3", "Day\_5" = "5", "Day\_7" = "7")) +  
 myCustomTheme() +   
theme(axis.title.y = element\_text(margin = margin(r = 1)))  
cld\_TB\_microbes\_4b



# plot them all together  
# cld\_day\_4b + cld\_microbes\_4b + cld\_day\_microbes\_4b + cld\_TB\_microbes\_4b

# get the stats for each comparison  
f4b\_tukey\_day <- emmeans(aov\_4b, pairwise ~ day, adjust = "tukey")$contrasts

NOTE: Results may be misleading due to involvement in interactions

f4b\_tukey\_microbes <- emmeans(aov\_4b, pairwise ~ microbes, adjust = "tukey")$contrasts

NOTE: Results may be misleading due to involvement in interactions

f4b\_tukey\_microbe\_TB <- emmeans(aov\_4b, pairwise ~ microbes \* TB, adjust = "tukey")$contrasts

NOTE: Results may be misleading due to involvement in interactions

f4b\_tukey\_microbe\_day <- emmeans(aov\_4b, pairwise ~ microbes \* day, adjust = "tukey")$contrasts

NOTE: Results may be misleading due to involvement in interactions

# print the values  
f4b\_tukey\_day

contrast estimate SE df t.ratio p.value  
 Day\_1 - Day\_3 1.162 0.339 32 3.428 0.0087  
 Day\_1 - Day\_5 2.934 0.339 32 8.657 <.0001  
 Day\_1 - Day\_7 3.509 0.339 32 10.352 <.0001  
 Day\_3 - Day\_5 1.772 0.339 32 5.229 0.0001  
 Day\_3 - Day\_7 2.347 0.339 32 6.924 <.0001  
 Day\_5 - Day\_7 0.574 0.339 32 1.694 0.3432  
  
Results are averaged over the levels of: microbes, TB   
P value adjustment: tukey method for comparing a family of 4 estimates

f4b\_tukey\_microbes

contrast estimate SE df t.ratio p.value  
 MO absent - MO present 1.77 0.24 32 7.405 <.0001  
  
Results are averaged over the levels of: day, TB

f4b\_tukey\_microbe\_TB

contrast estimate SE df t.ratio p.value  
 MO absent TB absent - MO present TB absent 0.546 0.339 32 1.611 0.3868  
 MO absent TB absent - MO absent TB present -1.022 0.339 32 -3.014 0.0245  
 MO absent TB absent - MO present TB present 1.982 0.339 32 5.847 <.0001  
 MO present TB absent - MO absent TB present -1.568 0.339 32 -4.626 0.0003  
 MO present TB absent - MO present TB present 1.436 0.339 32 4.236 0.0010  
 MO absent TB present - MO present TB present 3.004 0.339 32 8.862 <.0001  
  
Results are averaged over the levels of: day   
P value adjustment: tukey method for comparing a family of 4 estimates

f4b\_tukey\_microbe\_day

contrast estimate SE df t.ratio p.value  
 MO absent Day\_1 - MO present Day\_1 0.38866 0.479 32 0.811 0.9912  
 MO absent Day\_1 - MO absent Day\_3 0.00487 0.479 32 0.010 1.0000  
 MO absent Day\_1 - MO present Day\_3 2.70783 0.479 32 5.649 0.0001  
 MO absent Day\_1 - MO absent Day\_5 1.74234 0.479 32 3.635 0.0192  
 MO absent Day\_1 - MO present Day\_5 4.51526 0.479 32 9.419 <.0001  
 MO absent Day\_1 - MO absent Day\_7 3.08556 0.479 32 6.437 <.0001  
 MO absent Day\_1 - MO present Day\_7 4.32069 0.479 32 9.014 <.0001  
 MO present Day\_1 - MO absent Day\_3 -0.38379 0.479 32 -0.801 0.9919  
 MO present Day\_1 - MO present Day\_3 2.31916 0.479 32 4.838 0.0008  
 MO present Day\_1 - MO absent Day\_5 1.35368 0.479 32 2.824 0.1243  
 MO present Day\_1 - MO present Day\_5 4.12659 0.479 32 8.609 <.0001  
 MO present Day\_1 - MO absent Day\_7 2.69689 0.479 32 5.626 0.0001  
 MO present Day\_1 - MO present Day\_7 3.93203 0.479 32 8.203 <.0001  
 MO absent Day\_3 - MO present Day\_3 2.70296 0.479 32 5.639 0.0001  
 MO absent Day\_3 - MO absent Day\_5 1.73747 0.479 32 3.625 0.0197  
 MO absent Day\_3 - MO present Day\_5 4.51039 0.479 32 9.409 <.0001  
 MO absent Day\_3 - MO absent Day\_7 3.08068 0.479 32 6.427 <.0001  
 MO absent Day\_3 - MO present Day\_7 4.31582 0.479 32 9.003 <.0001  
 MO present Day\_3 - MO absent Day\_5 -0.96549 0.479 32 -2.014 0.4887  
 MO present Day\_3 - MO present Day\_5 1.80743 0.479 32 3.771 0.0136  
 MO present Day\_3 - MO absent Day\_7 0.37773 0.479 32 0.788 0.9926  
 MO present Day\_3 - MO present Day\_7 1.61287 0.479 32 3.365 0.0372  
 MO absent Day\_5 - MO present Day\_5 2.77291 0.479 32 5.785 0.0001  
 MO absent Day\_5 - MO absent Day\_7 1.34322 0.479 32 2.802 0.1300  
 MO absent Day\_5 - MO present Day\_7 2.57835 0.479 32 5.379 0.0002  
 MO present Day\_5 - MO absent Day\_7 -1.42970 0.479 32 -2.983 0.0890  
 MO present Day\_5 - MO present Day\_7 -0.19456 0.479 32 -0.406 0.9999  
 MO absent Day\_7 - MO present Day\_7 1.23514 0.479 32 2.577 0.2015  
  
Results are averaged over the levels of: TB   
P value adjustment: tukey method for comparing a family of 8 estimates

# \*Publication figures

# set up custom theme  
myCustomTheme <- function() {  
 theme\_light() + # base theme with light background  
 theme(axis.text = element\_text(size = 7, family = "Helvetica", color = "black"), # set font for axis tick labels  
 axis.title.x = element\_text(margin = margin(t = 10), # add spacing between x-axis label and plot  
 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), # add spacing between y-axis label and plot  
 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 gridlines (major and minor, x and y)  
 # axis.line.x = element\_line(color = "grey"), # uncomment if axis lines are needed  
 # axis.line.y = element\_line(color = "grey"), # uncomment if axis lines are needed  
 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"), # formatting for facet labels  
 strip.background = element\_rect(fill = "white", color = "grey", size = 0.5))} # set background of facet strips to white with grey border

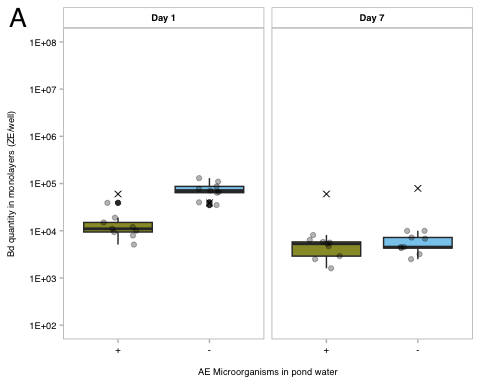
## Fig 7A SFEB Monolayer

fig\_SFEB\_monolayer <- eb\_monolayer %>%   
 mutate(filter = factor(filter, levels = c("40um\_filter", "0.22um\_filter"))) %>% # Switch the order of levels  
 # plot it  
 ggplot(aes(y= bd\_qty, x = filter, fill = filter)) +   
 geom\_boxplot() +  
 geom\_jitter(width = 0.2, alpha = 0.3) +  
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)) +   
 facet\_wrap(~day, labeller = labeller(day = c("Day\_1" = "Day 1",  
 "Day\_7" = "Day 7")))+  
 scale\_fill\_manual(values = c("40um\_filter" = with\_microbes\_40\_color,   
 "0.22um\_filter" = no\_microbes\_.22\_color)) +  
 myCustomTheme()+  
 theme(legend.position = "none",  
 strip.background = element\_rect(fill = "white", color = "grey", size = 0.5)) + # Adjust the facet line thickness  
 scale\_x\_discrete (labels= c("40um\_filter" = "+", "0.22um\_filter" = "-")) +  
 xlab("AE Microorganisms in pond water") +  
 ylab("Bd quantity in monolayers (ZE/well)") +  
  
 # add controls ad x's  
geom\_point(data = eb\_ml\_controls, aes(x = filter, y = bd\_qty), shape = 4, size = 2)

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.

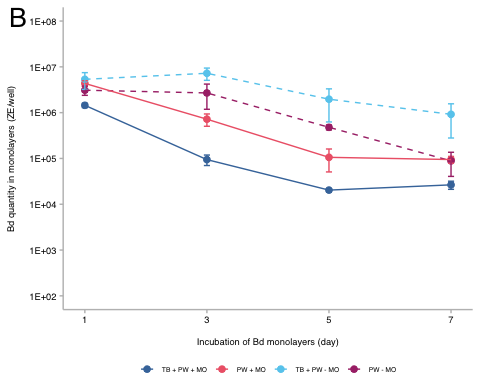
fig\_SFEB\_monolayer <- fig\_SFEB\_monolayer +  
 labs(tag = "A") +  
 theme(  
 plot.tag = element\_text(family = "Helvetica", size = 20, face = "plain", hjust = -0.1, vjust = 1),  
 plot.tag.position = c(0, 1)  
 )  
  
fig\_SFEB\_monolayer



#ggsave("paper-figures/expt4-SFEB\_AE\_monolayer\_fig4a\_updated.pdf", plot = fig\_SFEB\_monolayer, width = 3.46, height = 3.46)

## Fig 7B SBNCOS monolayer

fig\_SBNCOS\_monolayer <- monolayer\_summary %>%   
 # reorder to match Renwei's plot  
 mutate(sample\_ID = factor(sample\_ID,   
 levels = c("TB+PW+microbes","PW+microbes",  
 "TB+PW-microbes", "PW-microbes"))) %>%   
 ggplot(aes(x = day\_numeric,   
 y = mean\_adh,   
 color = sample\_ID)) +  
 geom\_point(size = 2) +  
 geom\_errorbar(aes(ymin = mean\_adh - se, # plot the standard error  
 ymax = mean\_adh + se),  
 width = 0.1) +  
 geom\_line(aes(linetype = microbes), show.legend = FALSE) +   
 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 monolayers (day)",  
 y = "Bd quantity in monolayers (ZE/well)",  
 color = "Medium", # Title for color legend  
 linetype = "Microbes\nPresent") +  
 scale\_color\_manual(values = c("TB+PW+microbes" = "#4477AA",   
 "PW+microbes" = "#EE6677",   
 "TB+PW-microbes" = "#66CCEE",   
 "PW-microbes" = "#AA3377"),   
   
 labels = c("TB+PW+microbes" = "TB + PW + MO",  
 "PW+microbes" = "PW + MO",  
 "TB+PW-microbes" = "TB + PW - MO",  
 "PW-microbes" = "PW - MO")) + # Custom labels  
 myCustomTheme()+  
 theme(legend.position = "bottom",  
 panel.border = element\_blank(),  
 legend.text = element\_text(size = 5), # Set legend text font size to 5pt  
 legend.key.size = unit(0.4, "cm"), # Reduce size of legend keys  
 legend.spacing.y = unit(0.1, "cm"), # Reduce vertical spacing between legend items  
 legend.margin = margin(t = 0, r = 0, b = 0, l = 0),  
 axis.line.x = element\_line(color = "grey", size = 0.5), # Keep the x-axis line  
 axis.line.y = element\_line(color = "grey", size = 0.5)) + # Keep the y-axis line) + # Remove margins around legend  
guides(color = guide\_legend(title = NULL)) +  
  
 scale\_linetype\_manual(values = c("n" = "dashed",   
 "y" = "solid"),  
 labels = c("n" = "N", "y" = "Y")) + # Change labels to uppercase N and Y  
 scale\_x\_continuous(breaks = c(0, 1, 3, 5, 7),  
 labels = c("Initial\nBd", "1", "3", "5", "7"))  
  
fig\_SBNCOS\_monolayer <- fig\_SBNCOS\_monolayer +  
 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\_monolayer

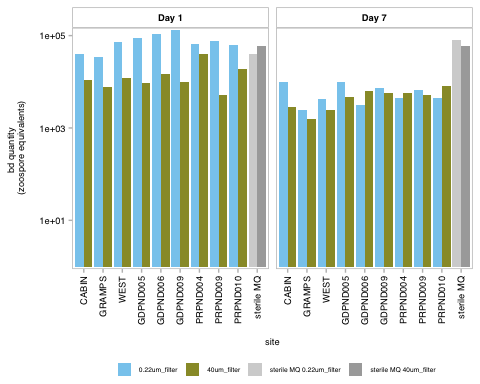


#ggsave("paper-figures/expt4-SBNCOS\_AE\_monolayer\_fig4b\_updated.pdf", plot = fig\_SBNCOS\_monolayer, width = 3.46, height = 3.46)

# \*SI

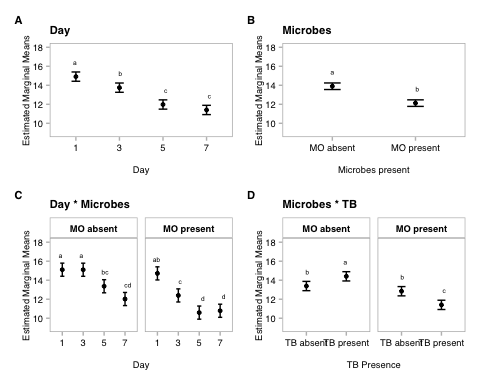
## S8: SFEB

# df for control data  
eb\_ml\_controls <- eb\_ml\_controls %>%  
 mutate(site = case\_when(  
 site == "MQ" ~ "sterile MQ", # rename mq to sterile mq so it's clear  
 TRUE ~ site)) %>%   
 mutate(filter = case\_when(  
 filter == "40um\_filter" ~ "sterile MQ 40um\_filter", # re-label filter as sterile control  
 filter == "0.22um\_filter" ~ "sterile MQ 0.22um\_filter", # same for other filter  
 TRUE ~ filter))   
  
# drop column, don't need it, will allow for combining df's  
eb\_monolayer\_nolog <- eb\_monolayer %>%  
 dplyr::select(-log\_bd)   
  
# cmbine df's of experimental data and controls together  
combined\_data\_4 <- bind\_rows(  
 eb\_monolayer\_nolog, # main data  
 eb\_ml\_controls) # control data already formatted  
  
##### plot it  
  
SI\_4a <- combined\_data\_4 %>%  
 ggplot(aes(y = bd\_qty, x = site, fill = filter)) +   
 geom\_col(position = position\_dodge()) + # side-by-side bars so filters don't stack  
  
 # log y axis  
 scale\_y\_continuous(  
 expand = c(0.01, 0.01), # small padding around bars  
 trans = "log", # log scale   
 breaks = c(1e+01, 1e+03, 1e+05), # specific breaks  
 labels = scales::label\_scientific()) + # use scientific notation (the E's)  
  
 # order sites by property   
 scale\_x\_discrete(limits = c(  
 "CABIN", "GRAMPS", "WEST",  
 "GDPND005", "GDPND006", "GDPND009",  
 "PRPND004", "PRPND009", "PRPND010",  
 "sterile MQ")) + # put sterile MQ at the end  
  
 # facet by day  
 facet\_wrap(~day, labeller = labeller(  
 day = c("Day\_1" = "Day 1", "Day\_7" = "Day 7"))) + # prettier facet labels  
  
 # assign custom colors to each filter type  
 scale\_fill\_manual(values = c(  
 "40um\_filter" = with\_microbes\_40\_color,   
 "0.22um\_filter" = no\_microbes\_.22\_color,   
 "sterile MQ 40um\_filter" = "darkgray",   
 "sterile MQ 0.22um\_filter" = "lightgray")) +   
  
 # apply my custom theme & some extra tweaks  
 myCustomTheme() +   
 theme(  
 axis.text.x = element\_text(angle = 90, vjust = 0.5, hjust = 1), # rotate site names  
 legend.position = "bottom", # move legend under plot  
 panel.border = element\_rect(color = "gray", size = 0.5, fill = NA), # outline each facet  
 legend.text = element\_text(size = 5), # small font in legend  
 legend.key.size = unit(0.4, "cm"), # shrink legend keys  
 legend.spacing.y = unit(0.1, "cm"), # tighter spacing between legend items  
 legend.margin = margin(t = 0, r = 0, b = 0, l = 0)) + # remove extra padding  
 xlab("site") + # x axis label  
 ylab("bd quantity \n (zoospore equivalents)") + # y axis label (with line break)  
 guides(fill = guide\_legend(title = "")) # remove legend title  
  
SI\_4a



## S9: SBNCOS

# set consistent y-axis limits and breaks for all cld plots  
global\_y\_limits <- c(9, 18)  
global\_y\_breaks <- seq(10, 18, by = 2)  
  
# compact letter display plot for day  
cld\_day\_4b <- 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 = 1.5, 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") +  
 scale\_x\_discrete(labels = c("Day\_1" = "1", "Day\_3" = "3", "Day\_5" = "5", "Day\_7" = "7")) +  
 myCustomTheme() +  
 theme(axis.title.y = element\_text(margin = margin(r = 1)))  
  
# compact letter display plot for microbes  
cld\_microbes\_4b <- ggplot(cld\_microbes, aes(x = microbes, 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 = 1.5, size = 1.75, color = "black") +  
 xlab("Microbes present") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1), limits = global\_y\_limits, breaks = global\_y\_breaks) +  
 ggtitle("Microbes") +  
 scale\_x\_discrete(labels = c("1%TB+AEbiofilm" = "1%TB", "PW+AEBiofilm" = "PW - MO", "MQ+AEbiofilm" = "MQ")) +  
 myCustomTheme() +  
 theme(axis.title.y = element\_text(margin = margin(r = 1)))  
  
# compact letter display plot for day \* microbes  
cld\_day\_microbes\_4b <- ggplot(cld\_day\_microbes, 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 = 1.5, size = 1.75, color = "black") +  
 facet\_wrap(~ factor(microbes)) +  
 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 \* Microbes") +  
 scale\_x\_discrete(labels = c("Day\_1" = "1", "Day\_3" = "3", "Day\_5" = "5", "Day\_7" = "7")) +  
 myCustomTheme() +  
 theme(axis.title.y = element\_text(margin = margin(r = 1)))  
  
# compact letter display plot for microbes \* TB  
cld\_TB\_microbes\_4b <- ggplot(cld\_TB\_microbes, aes(x = TB, 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 = 1.5, size = 1.75, color = "black") +  
 facet\_wrap(~ factor(microbes)) +  
 xlab("TB Presence") +  
 ylab("Estimated Marginal Means") +  
 scale\_y\_continuous(labels = scales::label\_number(accuracy = 1), limits = global\_y\_limits, breaks = global\_y\_breaks) +  
 ggtitle("Microbes \* TB") +  
 scale\_x\_discrete(labels = c("Day\_1" = "1", "Day\_3" = "3", "Day\_5" = "5", "Day\_7" = "7")) +  
 myCustomTheme() +  
 theme(axis.title.y = element\_text(margin = margin(r = 1)))  
  
# Combine all plots  
pairwise\_cld\_4b <- cld\_day\_4b + cld\_microbes\_4b + cld\_day\_microbes\_4b + cld\_TB\_microbes\_4b +  
 plot\_annotation(tag\_levels = 'A')  
pairwise\_cld\_4b



#ggsave("SI\_4b\_updated.pdf", plot = pairwise\_cld\_4b, width = 7.09, height = 3.46)

# Appendix

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

## anova table

anova\_output4b <- tidy(aov\_4b) # get model output as a tibble  
  
anova\_output4b <- anova\_output4b %>%  
 mutate(term = gsub(":", " x ", term)) %>% # clean up interaction terms  
 mutate(term = paste0(term, " (\*df = ", df, ", ", anova\_output4b[df == max(df), "df"], "\*)")) %>% # move degrees of freedom into first column with the "term"  
 filter(term != "Residuals (\*df = 32, 32\*)") # drop residual row from table, accounted for in the max (df) above  
  
aov\_4b\_tbl\_b <- anova\_output4b %>%  
 dplyr::select(term, statistic, p.value) %>% # just keep what's needed  
 gt() %>%  
 tab\_header(title = "4b ANOVA Table") %>% # give the table a title  
 fmt\_markdown(columns = term) %>% # render df as italics; tells gt to interpret markdown formatting in the specified column(s), so the \*'s above will be italics  
 fmt\_number(columns = statistic, decimals = 2) %>% # round f value  
 cols\_label( # relabel for clean display  
 term = "",  
 statistic = "F value",  
 p.value = "P-value") %>%  
 fmt\_scientific(columns = p.value, decimals = 1, rows = p.value < 0.001) %>% # sci format for tiny p-value  
 fmt\_number(columns = p.value, decimals = 3, rows = p.value >= 0.001) # normal format for rest of the p values  
  
aov\_4b\_tbl\_b

Table 1: 4b ANOVA Table

|  | F value | P-value |
| --- | --- | --- |
| day (*df = 3, 32*) | 45.34 | 1.3 × 10^-11 |
| microbes (*df = 1, 32*) | 54.84 | 2.0 × 10^-8 |
| TB (*df = 1, 32*) | 0.75 | 0.394 |
| day x microbes (*df = 3, 32*) | 5.90 | 0.003 |
| day x TB (*df = 3, 32*) | 0.46 | 0.715 |
| microbes x TB (*df = 1, 32*) | 26.28 | 1.4 × 10^-5 |
| day x microbes x TB (*df = 3, 32*) | 0.78 | 0.516 |

## post hoc

# # post hoc table  
# # Convert Tukey emmeans results to data frames  
# f4b\_tukey\_day\_df <- as.data.frame(f4b\_tukey\_day)  
# f4b\_tukey\_microbes\_df <- as.data.frame(f4b\_tukey\_microbes)  
# f4b\_tukey\_int\_microbe\_day\_df <- as.data.frame(f4b\_tukey\_int\_microbe\_day)  
# f4b\_tukey\_int\_microbe\_TB\_df <- as.data.frame(f4b\_tukey\_int\_microbe\_TB)  
#   
# # Add labels to indicate which factor the comparison refers to  
# f4b\_tukey\_day\_df <- f4b\_tukey\_day\_df %>% mutate(factor = "Day")  
# f4b\_tukey\_microbes\_df <- f4b\_tukey\_microbes\_df %>% mutate(factor = "Medium")  
# f4b\_tukey\_int\_microbe\_day\_df <- f4b\_tukey\_int\_microbe\_day\_df %>% mutate(factor = "Day x microbes")  
# f4b\_tukey\_int\_microbe\_TB\_df <- f4b\_tukey\_int\_microbe\_TB\_df %>% mutate(factor = "Microbes x TB")  
#   
# f4b\_all\_tukey\_df <- bind\_rows(f4b\_tukey\_day\_df, f4b\_tukey\_microbes\_df, f4b\_tukey\_int\_microbe\_TB\_df, f4b\_tukey\_int\_microbe\_day\_df)  
#   
# f4b\_all\_tukey\_df <- f4b\_all\_tukey\_df %>%   
# mutate(contrast = gsub("MO present TB absent", "microbes only", contrast),  
# contrast = gsub("MO present TB present", "both microbes and TB", contrast),  
# contrast = gsub("MO absent TB absent", "neither microbes nor TB", contrast),  
# contrast = gsub("MO absent TB present", "TB only", contrast))  
#   
#   
# ph4b\_table <- f4b\_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 = "4b 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()))  
#   
# ph4b\_table