Results and Assessment of pH 5 Leave-One-Out data

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Background info

The objective of this experiment was to assess the net contributions of key Bayley species, as identified by strong roles in pairwise interaction assays. To assess their overall contribution in the community, each of the following species was left out of the community when plates in equal ratio on cheese curd agar, pH 5.

Species left out * none ("community" or "comm") * Penicillium JBC ("comm-JBC") * Diutina 135E ("comm-135E") * Staphyloccocus xylosus BC10 ("comm-BC10") * Diutina 135E & S. xylosus BC10 ("community minus early deacidifiers" or "comm-eD")
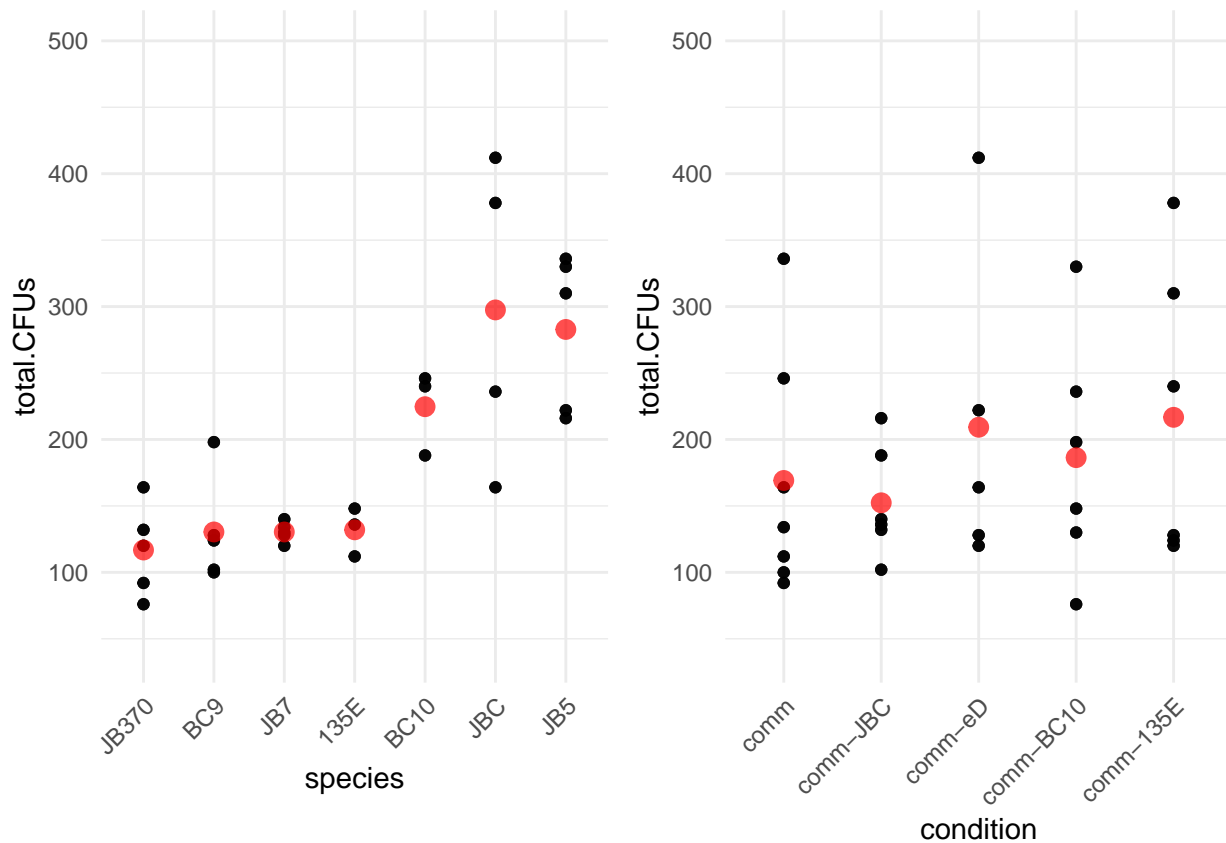
```
d.loo5 <- readRDS(here("wrangled_data/leave-one-out_pH5.rds"))
d.18 <- readRDS(here("wrangled_data/all_pairwise.rds"))
load(here("figures/plotting_objects.RData"))
```

Inoculation quality assessment

Are the inoculations around 200 CFUs each? Any noteable biases?

```
## Warning: `fun.y` is deprecated. Use `fun` instead.

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```

Representative inoculation of each species in each condition in black, and the mean within species (left) or within condition (right) in red.

So there's definitely some species that were inoculated lower than target (mean ~130 CFUs), and some species that were ioculated higher than target, namely Brevibacterium JB5 with average ~300 CFUs. S. xylosus BC10 was right about on target, and then Penicillium JBC was all over the place, from 150 - 400 CFUs inoculated.

However, overall the average inoculation within each community was right about at 200, or at least between 150 - 220 CFUs, which is pretty good.

pH probe variability

Interested in seeing whether pH of "control" (uninoculated but from same 96-well plate as community samples) cheese at time of harvest is changing at different timepoints, given that I've found strong ability of some Bayley species to raise the pH of the media through volatiles alone (namely, Penicillium, Brevibacterium, and Scopulariopsis, and this effect gets stronger between 7 & 10 days of growth).

Note that each "replicate" was the pH of uninoculated cheese at harvest day 3 (rep./harvest # 1), day 10 (rep./harvest # 2), and day 21 (rep./harvest # 3).
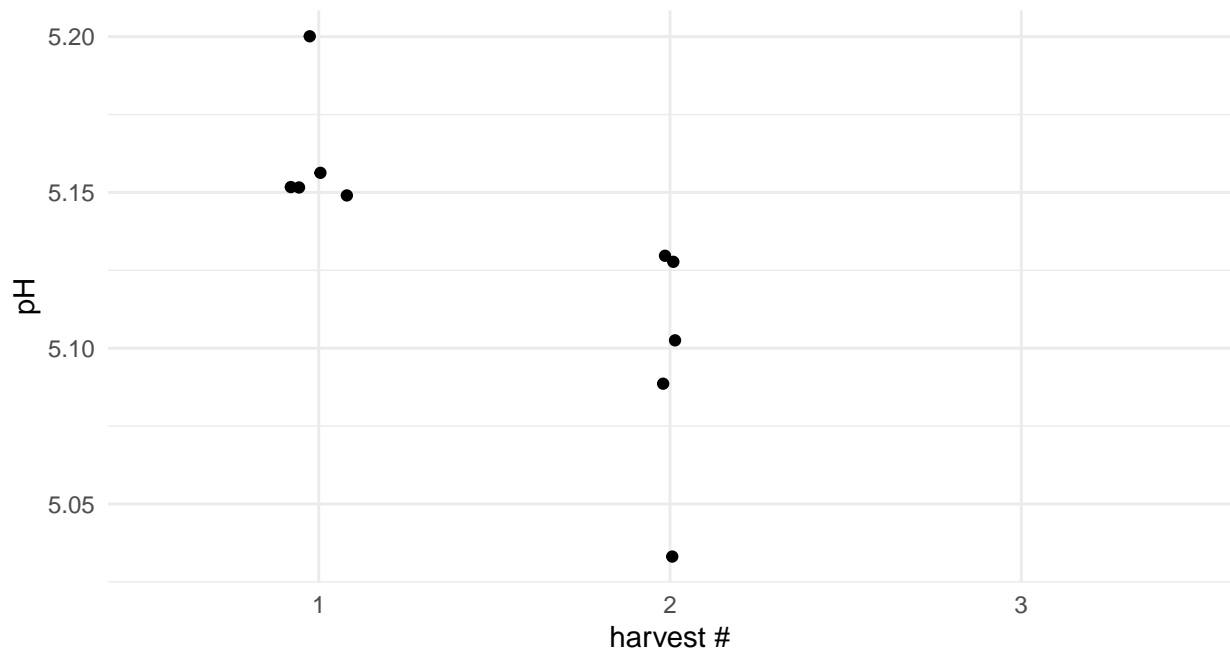
So what I'm really seeing here is just a bias according to harvest date (note, pH probe re-calibrated at day 3 and day 10).

I also wan't to check that there isn't some systematic pH change over time of use.

pH over time measuring: day 10 community samples — day 10 control CC...
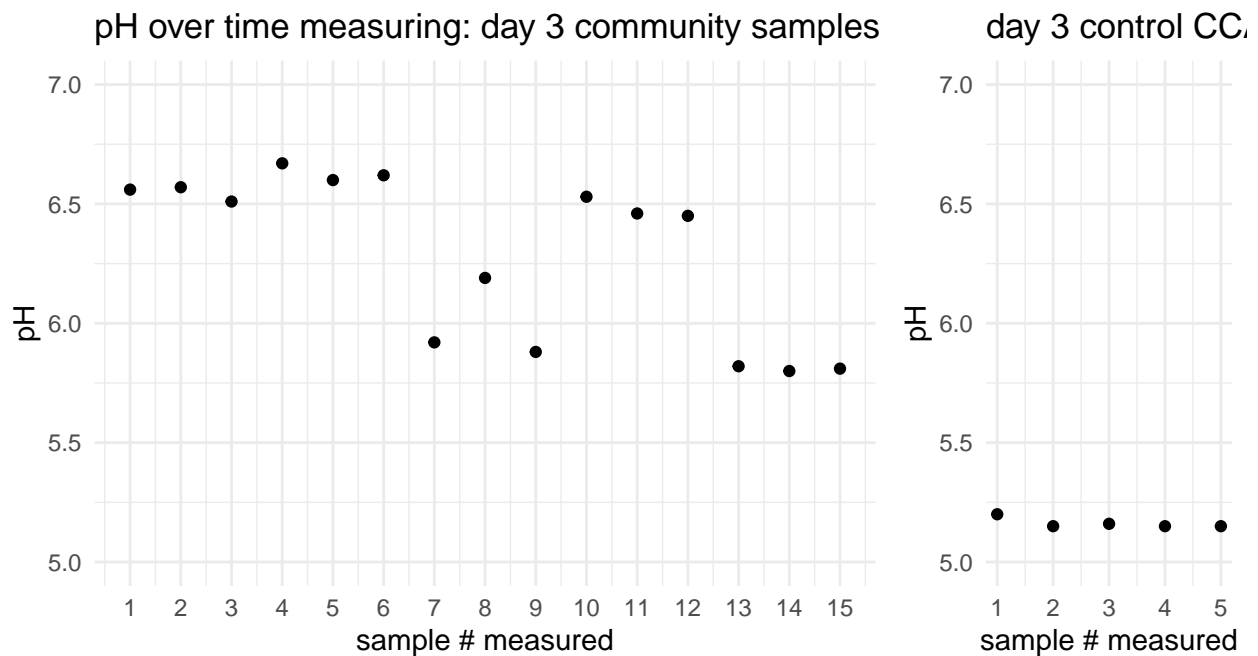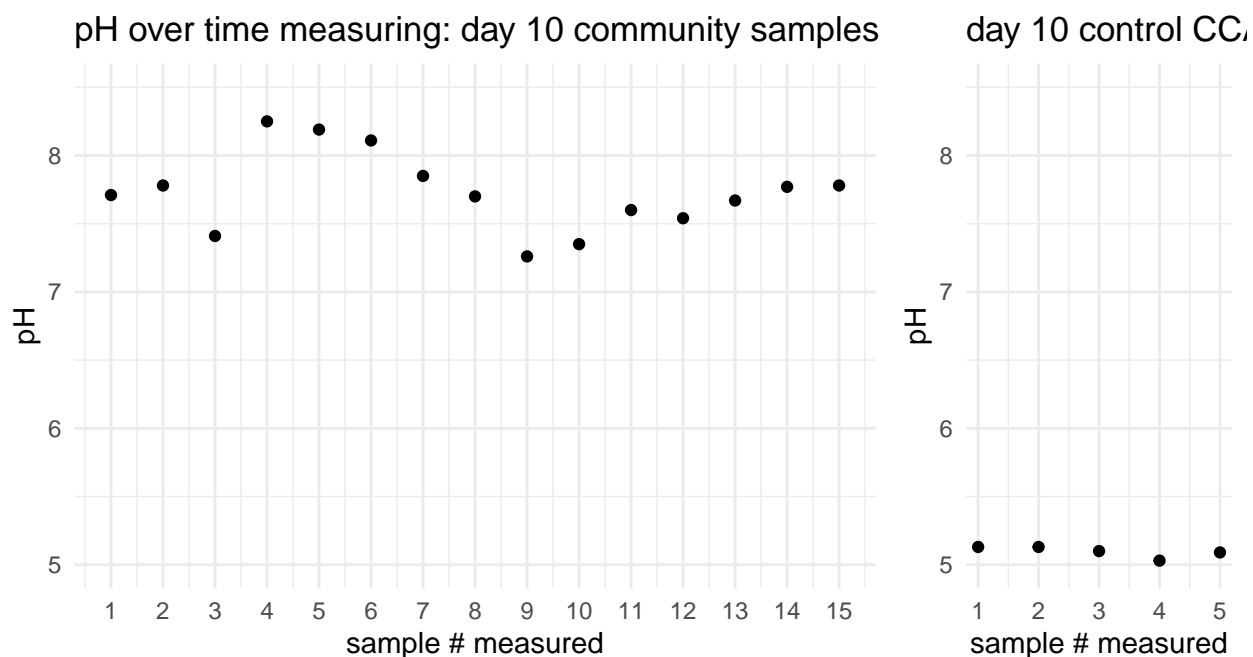
I can't help but feel there is like some smallllll bias towards pH measurement decline over time. But even if so, from looking at any trend in the control (uninocuated) data and comparing it to the scale of differences in the community sample data, I don't think it's going to have an effect in the analysis. (could do a linear model assessment if I really cared to eliminate this).

# Is complete community data replicating what has been seen before (i.e. 08_2018)
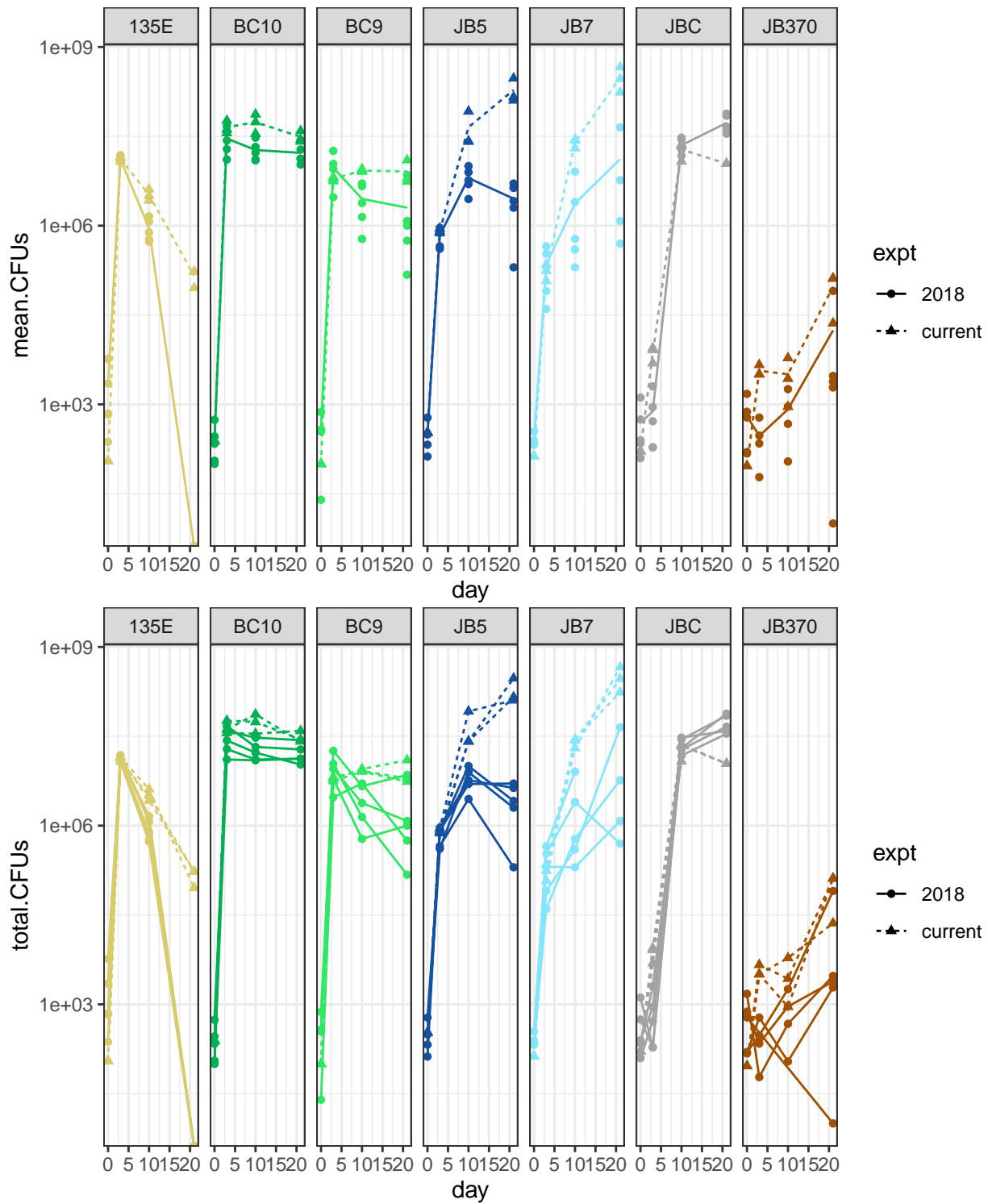
See if there's something predictive about the difference in growth patterns between day3 & day10

```
## # A tibble: 0 x 3
```

```
## # ... with 3 variables: species <fct>, growth_period <fct>, p.value <dbl>
```

```
## # A tibble: 10 x 4
##     species growth_period term          p.value
##     <fct>   <fct>         <chr>           <dbl>
##  1 BC9     0-3           day_0         0.00851
##  2 BC9     3-10          day_0         0.0289
##  3 JB5     3-10          exptcurrent   0.0447
##  4 JB5     0-3           day_0         0.0203
##  5 JB7     3-10          exptcurrent   0.0353
##  6 135E    0-3           exptcurrent   0.000399
##  7 135E    3-10          exptcurrent   0.00237
##  8 135E    10-21         exptcurrent   0.00991
##  9 JBC     0-3           exptcurrent   0.00215
## 10 JB370   0-3           exptcurrent   0.00293
```
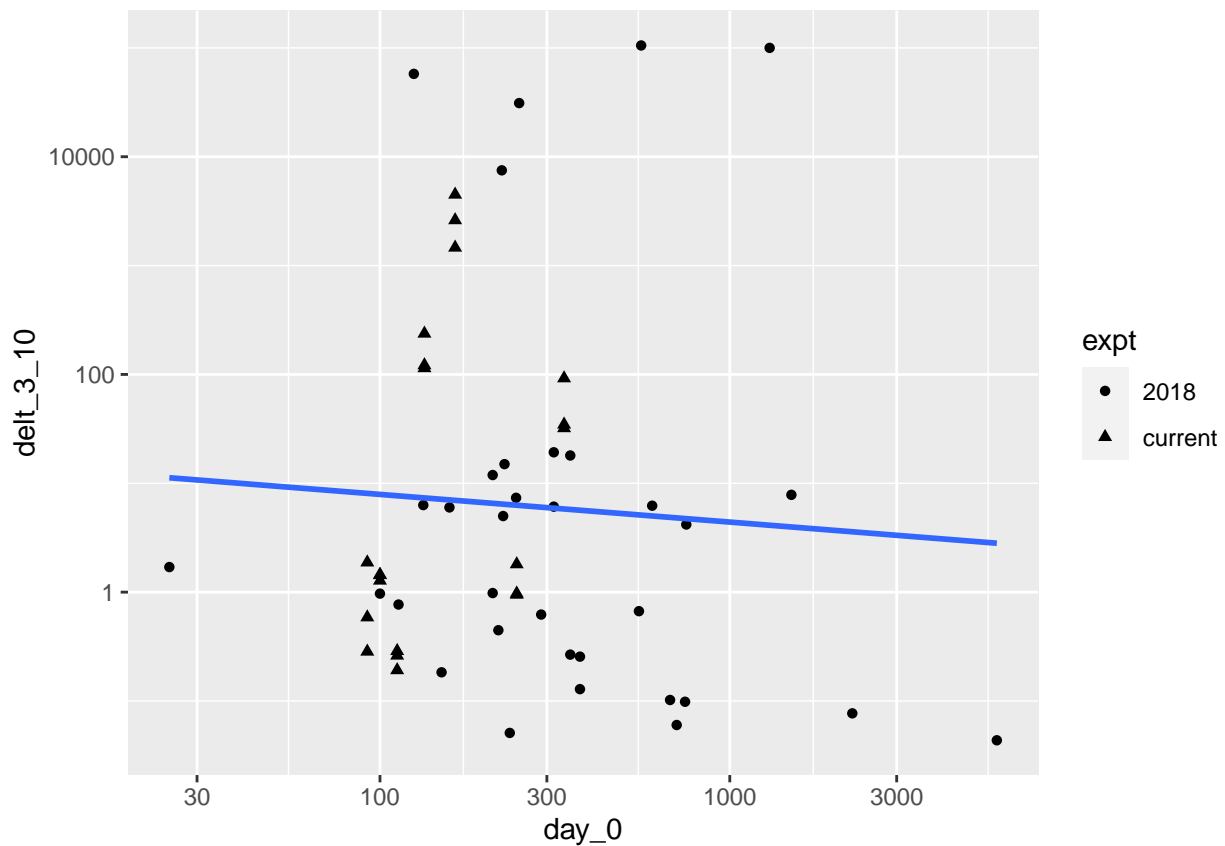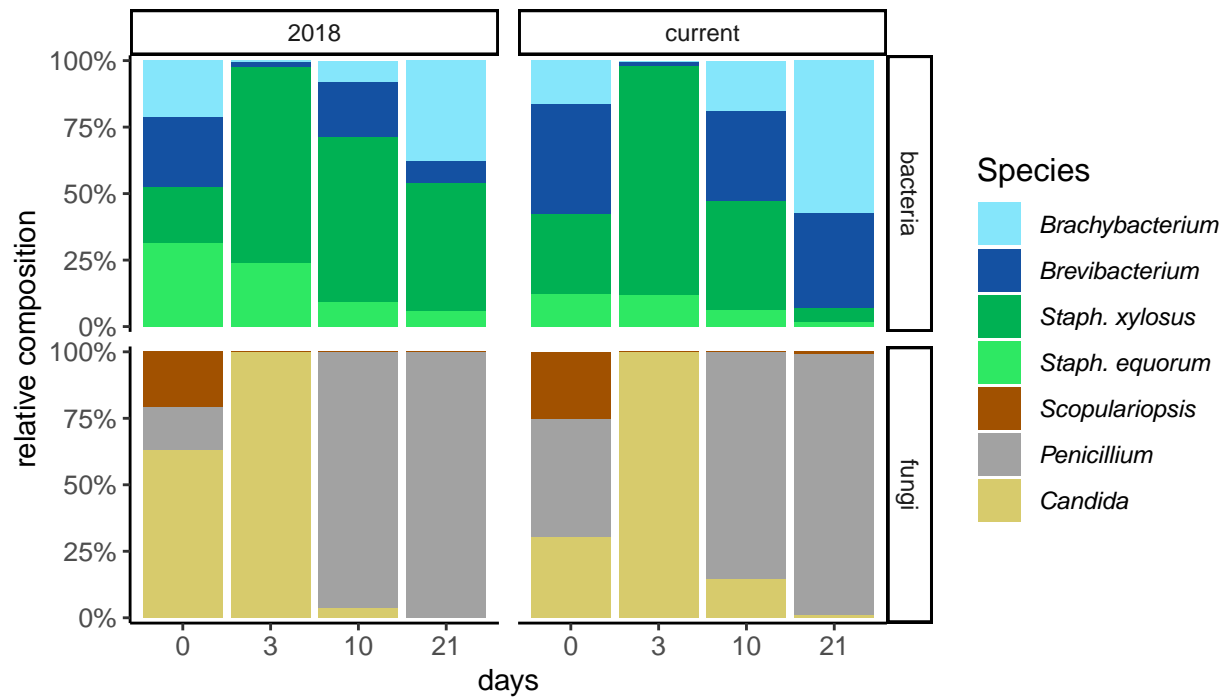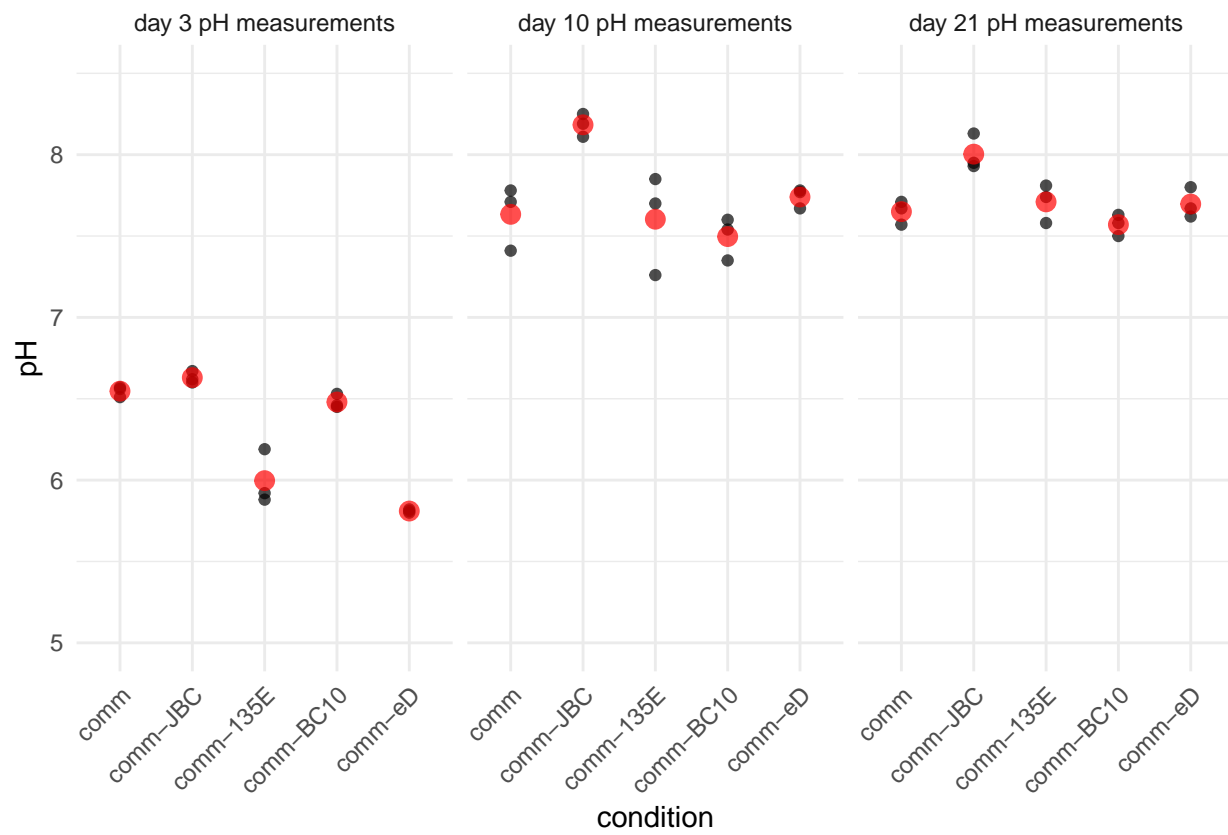

Compare community succession pattern

Relative composition of Bayley community bacteria and fungi
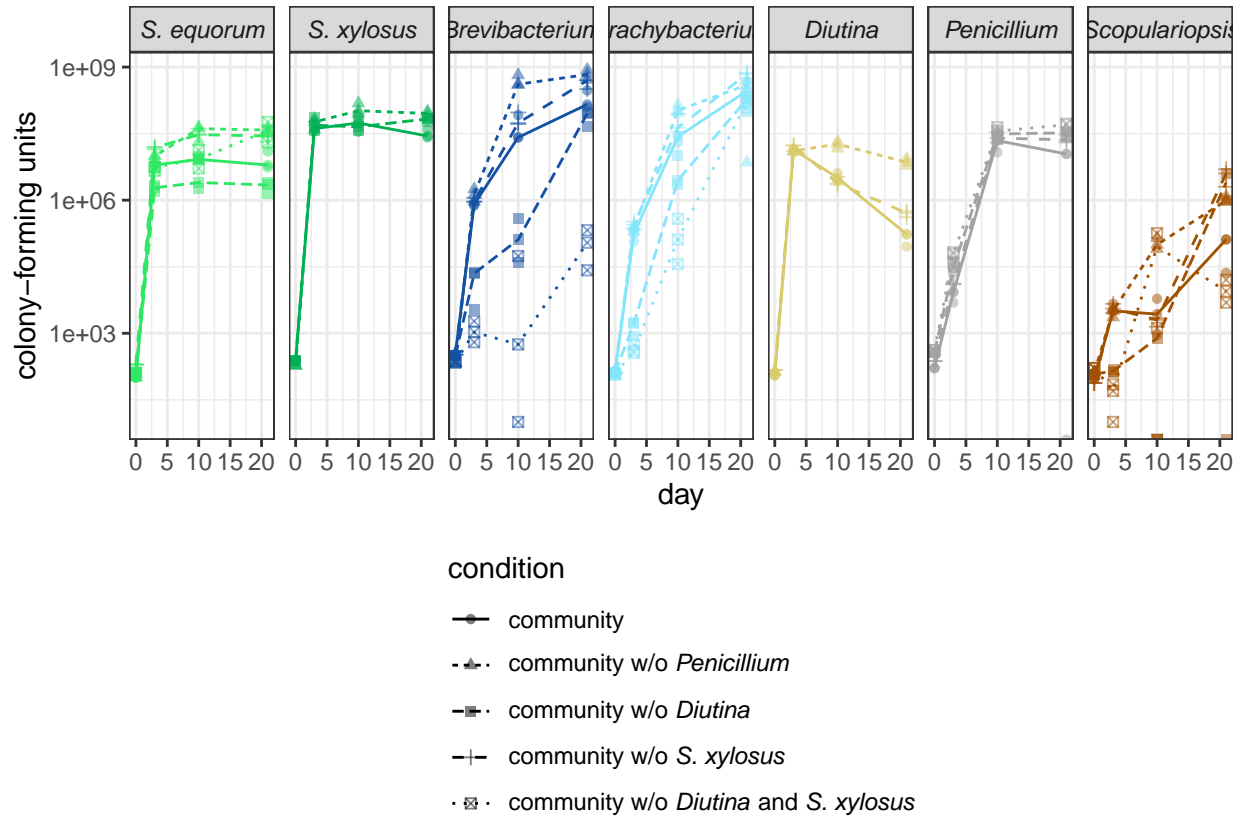

pH data

First let's plot the data to know what we're working with:

growth data & ttests

Comparing growth curves for each species in each condition:

Assess how dropping certain community members from the community affects growth, using growth in the full community as a baseline for comparison (indicated by horizontal gray bar, CFUs of the species in full community at that timepoint). Each large dot is the average of the smaller dots, which show the growth of that species in that community at that time point, and the vertical line connecting the dots to the horizontal gray line highlights the difference in growth between the dropout community and the full community. Points are colored by sample pH.
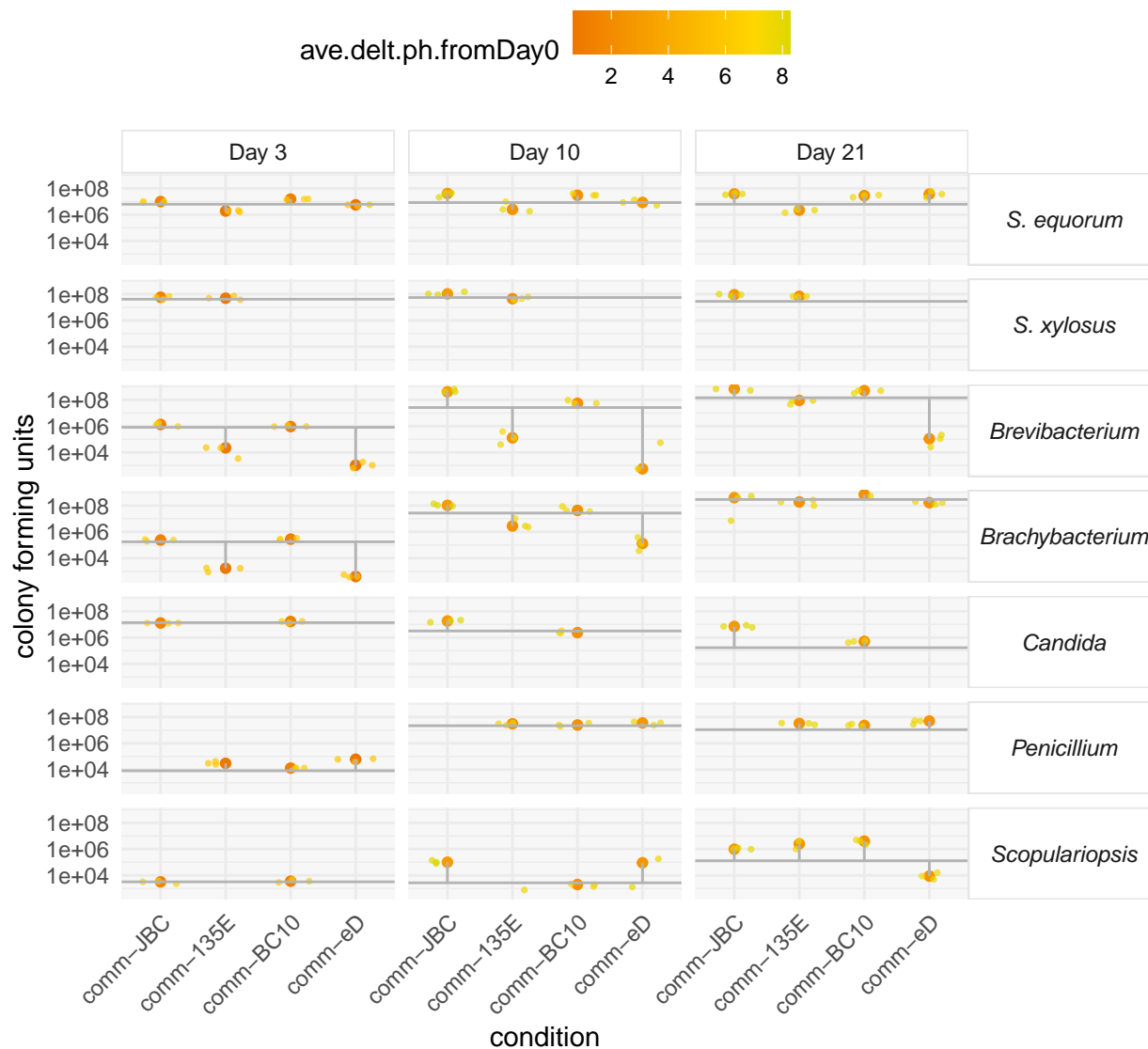
```
## [1] "comparing community pH's at day 3"
## Analysis of Variance Table
##
## Response: pH
##            Df  Sum Sq Mean Sq F value    Pr(>F)
## condition   4 1.60196 0.40049  61.112 5.441e-07 ***
## Residuals  10 0.06553 0.00655
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = pH ~ condition, data = d.loo5 %>% filter(day == d) %>% select(condition, day, rep)
##
## $condition
##                          diff         lwr         upr       p adj
## comm-135E-comm    -0.55000000 -0.76753254 -0.33246746 0.0000635
## comm-BC10-comm    -0.06666667 -0.28419921  0.15086587 0.8458324
## comm-eD-comm      -0.73666667 -0.95419921 -0.51913413 0.0000045
## comm-JBC-comm      0.08333333 -0.13419921  0.30086587 0.7189390
## comm-BC10-comm-135E  0.48333333  0.26580079  0.70086587 0.0001929
```
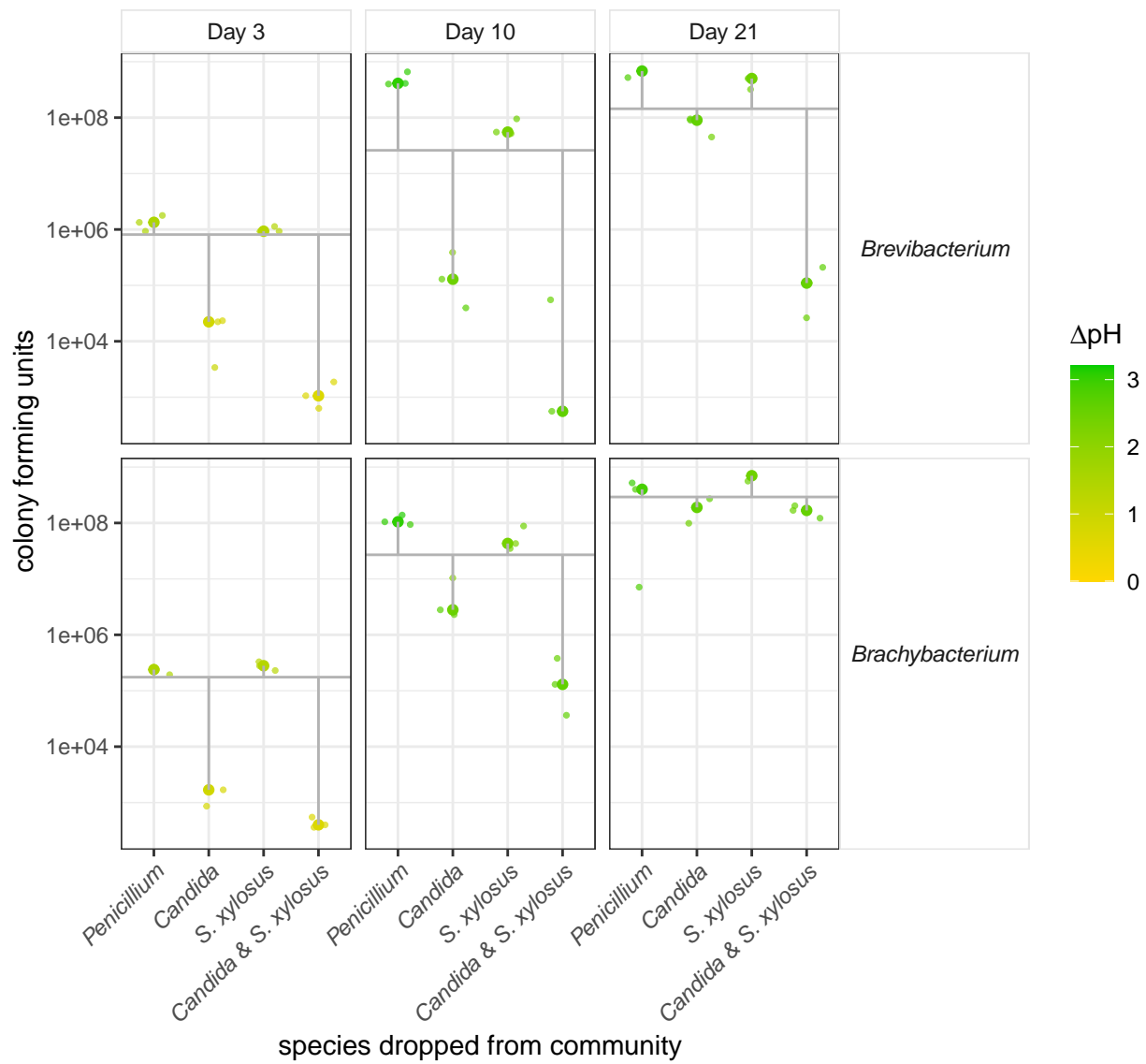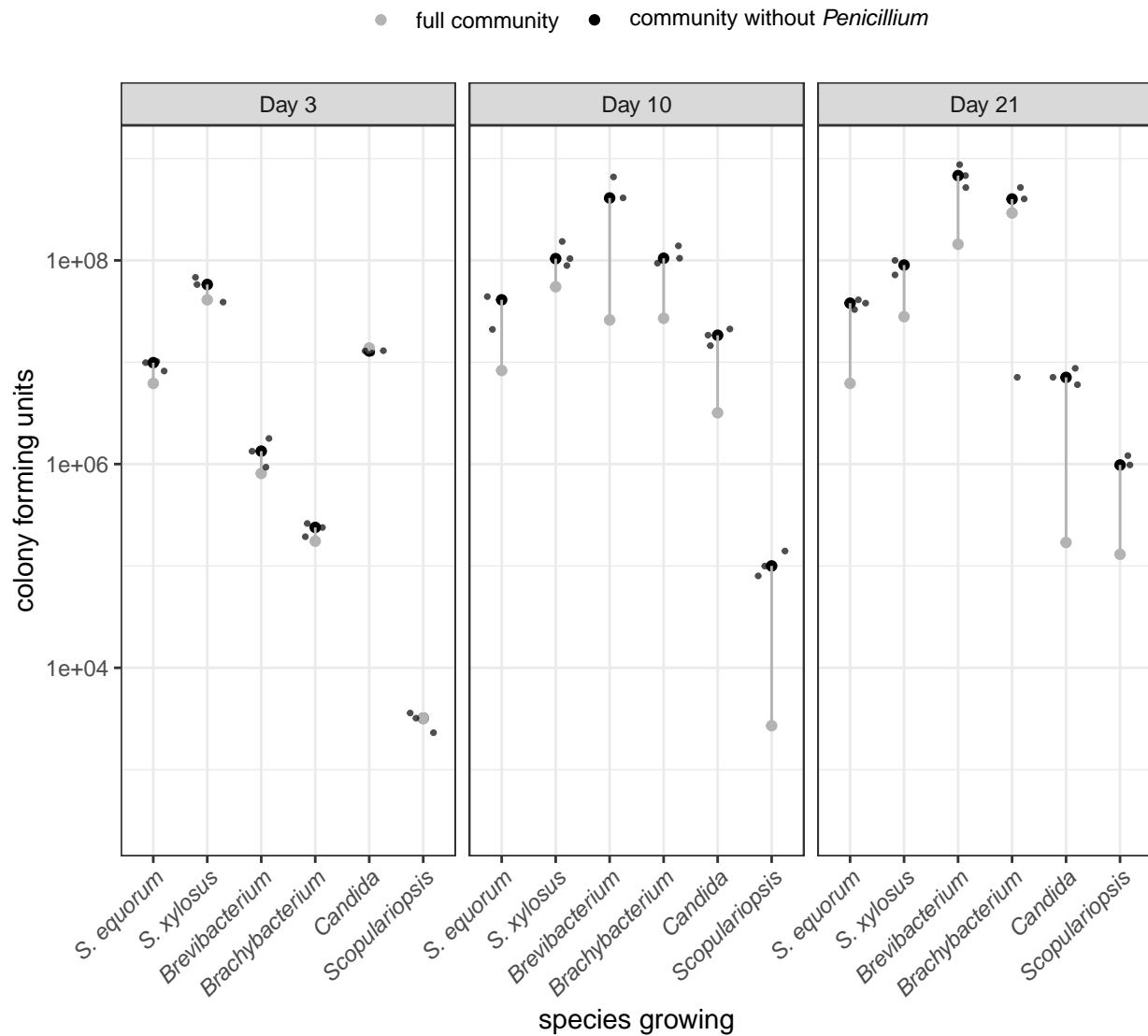
9

```
## comm-eD-comm-135E     -0.18666667 -0.40419921   0.03086587 0.1026261
## comm-JBC-comm-135E      0.63333333  0.41580079   0.85086587 0.0000181
## comm-eD-comm-BC10      -0.67000000 -0.88753254  -0.45246746 0.0000109
## comm-JBC-comm-BC10      0.15000000 -0.06753254   0.36753254 0.2312621
## comm-JBC-comm-eD        0.82000000  0.60246746   1.03753254 0.0000017
##
## [1] "comparing community pH's at day 10"
## Analysis of Variance Table
##
## Response: pH
##            Df  Sum Sq  Mean Sq F value   Pr(>F)
## condition   4 0.85631 0.214077  6.7603 0.006669 **
## Residuals  10 0.31667 0.031667
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = pH ~ condition, data = d.loo5 %>% filter(day == d) %>% select(condition, day, rep
##
## $condition
##                          diff          lwr       upr     p adj
## comm-135E-comm       -0.0300000 -0.50818316 0.4481832 0.9995194
## comm-BC10-comm       -0.1366667 -0.61484982 0.3415165 0.8746923
## comm-eD-comm          0.1066667 -0.37151649 0.5848498 0.9434209
## comm-JBC-comm         0.5500000  0.07181684 1.0281832 0.0232368
## comm-BC10-comm-135E  -0.1066667 -0.58484982 0.3715165 0.9434209
## comm-eD-comm-135E     0.1366667 -0.34151649 0.6148498 0.8746923
## comm-JBC-comm-135E    0.5800000  0.10181684 1.0581832 0.0169203
## comm-eD-comm-BC10     0.2433333 -0.23484982 0.7215165 0.4884159
## comm-JBC-comm-BC10    0.6866667  0.20848351 1.1648498 0.0056383
## comm-JBC-comm-eD      0.4433333 -0.03484982 0.9215165 0.0724789
##
## [1] "comparing community pH's at day 21"
## Analysis of Variance Table
##
## Response: pH
##            Df  Sum Sq  Mean Sq F value   Pr(>F)
## condition   4 0.32443 0.081107  9.1819 0.002213 **
## Residuals  10 0.08833 0.008833
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = pH ~ condition, data = d.loo5 %>% filter(day == d) %>% select(condition, day, rep
##
## $condition
##                           diff          lwr       upr     p adj
## comm-135E-comm        0.06000000 -0.19255467 0.3125547 0.9302083
## comm-BC10-comm       -0.08000000 -0.33255467 0.1725547 0.8304472
## comm-eD-comm          0.04666667 -0.20588801 0.2992213 0.9705356
## comm-JBC-comm         0.35333333  0.10077866 0.6058880 0.0067389
## comm-BC10-comm-135E  -0.14000000 -0.39255467 0.1125547 0.4118736
```

```
## comm-eD-comm-135E    -0.01333333  -0.26588801  0.2392213  0.9997571
## comm-JBC-comm-135E     0.29333333   0.04077866  0.5458880  0.0219446
## comm-eD-comm-BC10      0.12666667  -0.12588801  0.3792213  0.5013370
## comm-JBC-comm-BC10     0.43333333   0.18077866  0.6858880  0.0015458
## comm-JBC-comm-eD       0.30666667   0.05411199  0.5592213  0.0168071
```

colony forming units

species growing

full community   community without *Penicillium*

Statistical tests on differences in growth

```
## # A tibble: 21 x 4
##    species   day `p-value` sig.by.condition
##    <chr>   <dbl>    <dbl> <chr>
##  1 BC9         3  0.00978 "*"
##  2 BC9        10  0.0311  "*"
##  3 BC9        21  0.0186  "*"
##  4 BC10        3  0.646   ""
##  5 BC10       10  0.0665  ""
##  6 BC10       21  0.0265  "*"
##  7 JB5         3  0.0106  "*"
##  8 JB5        10  0.0124  "*"
##  9 JB5        21  0.00907 "*"
## 10 JB7         3  0.0128  "*"
## # ... with 11 more rows

## [1] "Post-hoc analysis for BC9 on day 3"
##        Comparison           Z   P.unadj     P.adj
## 1 comm - comm-135E  1.5574529 0.11936300 0.1989383
```

```
## 2 comm - comm-BC10 -1.7406826 0.08173922 0.2043481
## 3   comm - comm-eD  0.6413041 0.52132511 0.5213251
## 4  comm - comm-JBC -0.9161488 0.35958888 0.5136984
## [1] "Post-hoc analysis for BC9 on day 10"
##        Comparison         Z    P.unadj      P.adj
## 1 comm - comm-135E  0.4572527 0.64748941 0.8093618
## 2 comm - comm-BC10 -1.8747361 0.06082903 0.1216581
## 3   comm - comm-eD -0.1829011 0.85487563 0.8548756
## 4  comm - comm-JBC -2.0576372 0.03962497 0.1320832
## [1] "Post-hoc analysis for BC9 on day 21"
##        Comparison         Z    P.unadj      P.adj
## 1 comm - comm-135E  0.8215838 0.41131379 0.5141422
## 2 comm - comm-BC10 -1.0954451 0.27332168 0.4555361
## 3   comm - comm-eD -1.7344548 0.08283743 0.1656749
## 4  comm - comm-JBC -2.0996031 0.03576377 0.1192126
## [1] "Post-hoc analysis for BC10 on day 21"
##        Comparison         Z    P.unadj      P.adj
## 1 comm - comm-135E -1.347266 0.17789453 0.17789453
## 2  comm - comm-JBC -2.694532 0.00704875 0.02114625
## [1] "Post-hoc analysis for JB5 on day 3"
##        Comparison         Z    P.unadj     P.adj
## 1 comm - comm-135E  0.8223184 0.4108957 0.4565508
## 2 comm - comm-BC10 -0.9593714 0.3373717 0.4819595
## 3   comm - comm-eD  1.6446368 0.1000447 0.2000895
## 4  comm - comm-JBC -1.5075837 0.1316611 0.2194352
## [1] "Post-hoc analysis for JB5 on day 10"
##        Comparison         Z    P.unadj     P.adj
## 1 comm - comm-135E  1.0964245 0.27289305 0.3898472
## 2 comm - comm-BC10 -0.4568435 0.64778351 0.6477835
## 3   comm - comm-eD  1.7360055 0.08256287 0.2064072
## 4  comm - comm-JBC -1.4618993 0.14376880 0.2396147
## [1] "Post-hoc analysis for JB5 on day 21"
##        Comparison         Z   P.unadj     P.adj
## 1 comm - comm-135E  0.8215838 0.4113138 0.4113138
## 2 comm - comm-BC10 -0.8215838 0.4113138 0.4570153
## 3   comm - comm-eD  1.6431677 0.1003482 0.2006965
## 4  comm - comm-JBC -1.6431677 0.1003482 0.2508706
## [1] "Post-hoc analysis for JB7 on day 3"
##        Comparison         Z    P.unadj     P.adj
## 1 comm - comm-135E  0.9128709 0.36131043 0.5161578
## 2 comm - comm-BC10 -1.3693064 0.17090352 0.2848392
## 3   comm - comm-eD  1.7344548 0.08283743 0.1656749
## 4  comm - comm-JBC -0.8215838 0.41131379 0.5141422
## [1] "Post-hoc analysis for JB7 on day 10"
##        Comparison         Z   P.unadj     P.adj
## 1 comm - comm-135E  0.8215838 0.4113138 0.4113138
## 2 comm - comm-BC10 -0.8215838 0.4113138 0.4570153
## 3   comm - comm-eD  1.6431677 0.1003482 0.2006965
## 4  comm - comm-JBC -1.6431677 0.1003482 0.2508706
## [1] "Post-hoc analysis for 135E on day 21"
##        Comparison         Z    P.unadj      P.adj
## 1 comm - comm-BC10 -1.347266 0.17789453 0.17789453
## 2  comm - comm-JBC -2.694532 0.00704875 0.02114625
## [1] "Post-hoc analysis for JBC on day 3"
```

```
##          Comparison           Z     P.unadj      P.adj
## 1 comm - comm-135E -2.151326 0.031450449 0.09435135
## 2 comm - comm-BC10 -1.019049 0.308179547 0.36981546
## 3   comm - comm-eD -2.943920 0.003240835 0.01944501
## [1] "Post-hoc analysis for JBC on day 21"
##          Comparison           Z     P.unadj      P.adj
## 1 comm - comm-135E -2.155097 0.031154220 0.09346266
## 2 comm - comm-BC10 -1.247688 0.212145367 0.31821805
## 3   comm - comm-eD -2.722228 0.006484335 0.03890601
## [1] "Post-hoc analysis for JB370 on day 3"
##          Comparison            Z     P.unadj      P.adj
## 1 comm - comm-135E  1.78971187 0.073500243 0.14700049
## 2 comm - comm-BC10 -0.04589005 0.963397886 0.96339789
## 3   comm - comm-eD  2.61573274 0.008903622 0.04451811
## 4  comm - comm-JBC  0.45890048 0.646305634 0.71811737
```

patterns of succession

Compare patterns of succession when certain species are dropped from the community

Relative composition of Bayley community bacteria and fungi
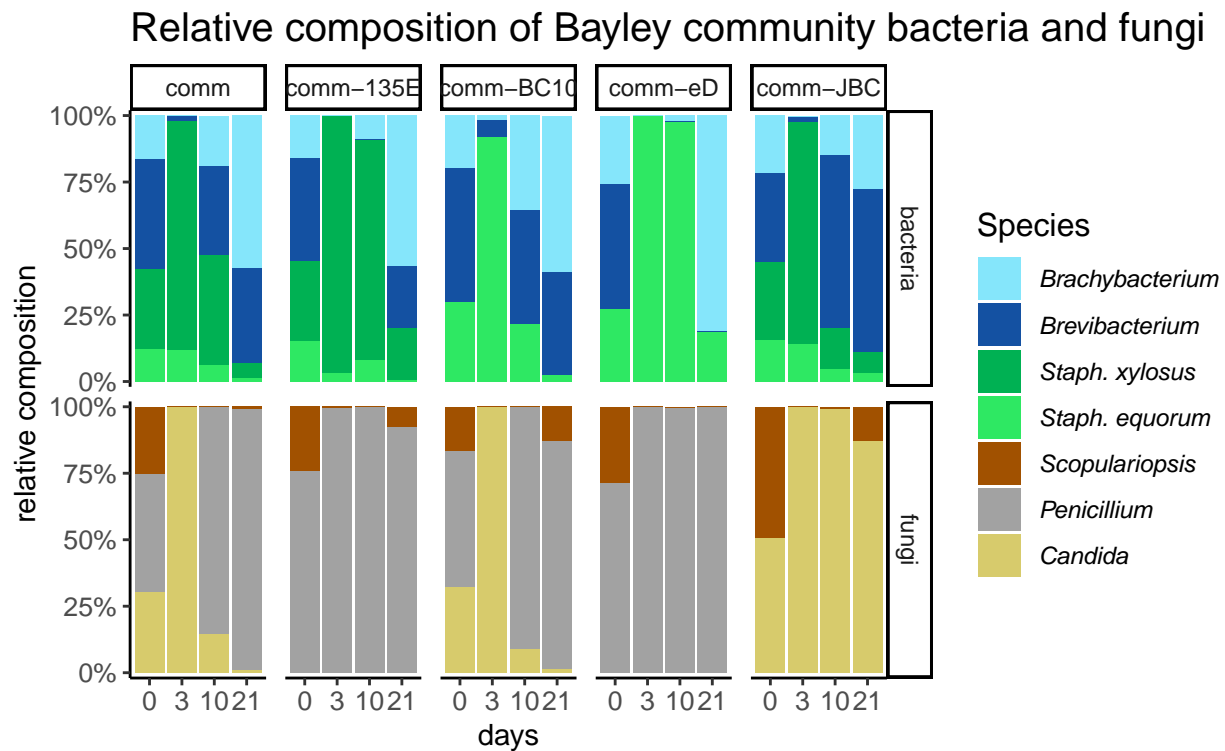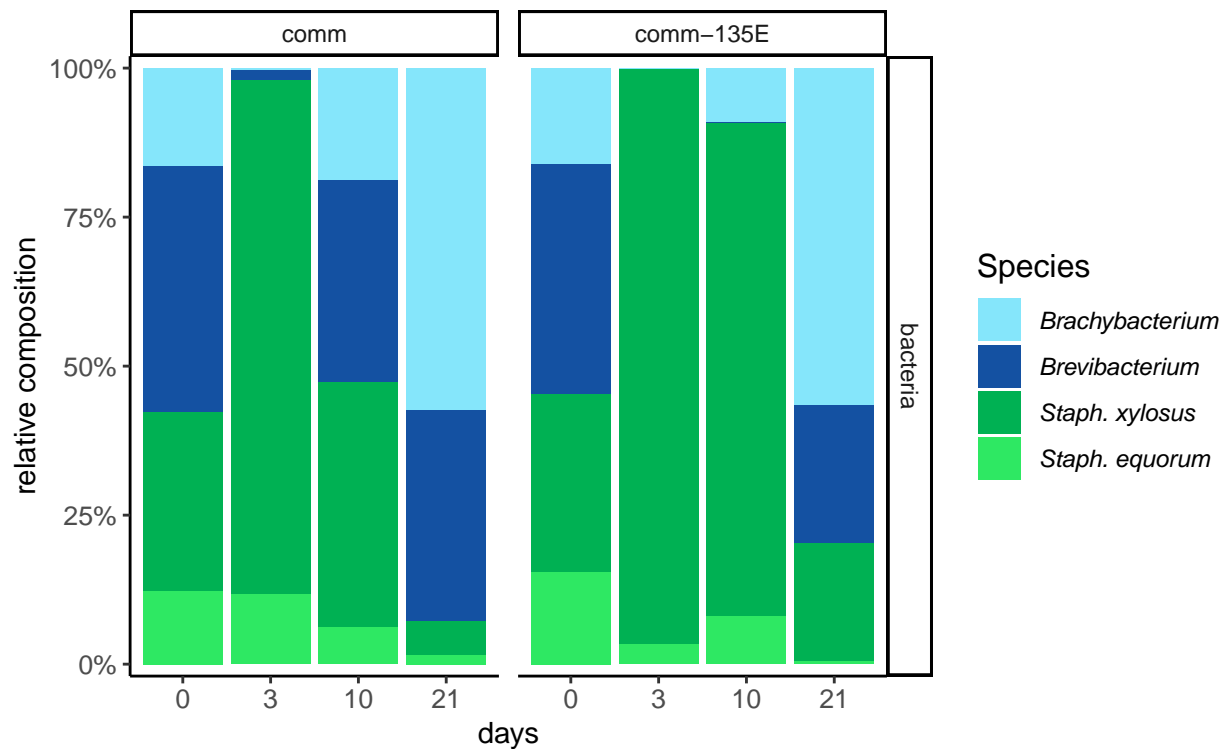
Obviously this is a little hard to interpret because, e.g. in the community minus BC10, there is no bar for BC10 anymore and so the percent abundance of the remaining bacteria can't be directly compared to conditions that also have BC10.

Let's first look at just the bacteria that are in all the community conditions – BC9, JB5, and JB7:

Relative composition of Bayley community bacteria and fungi
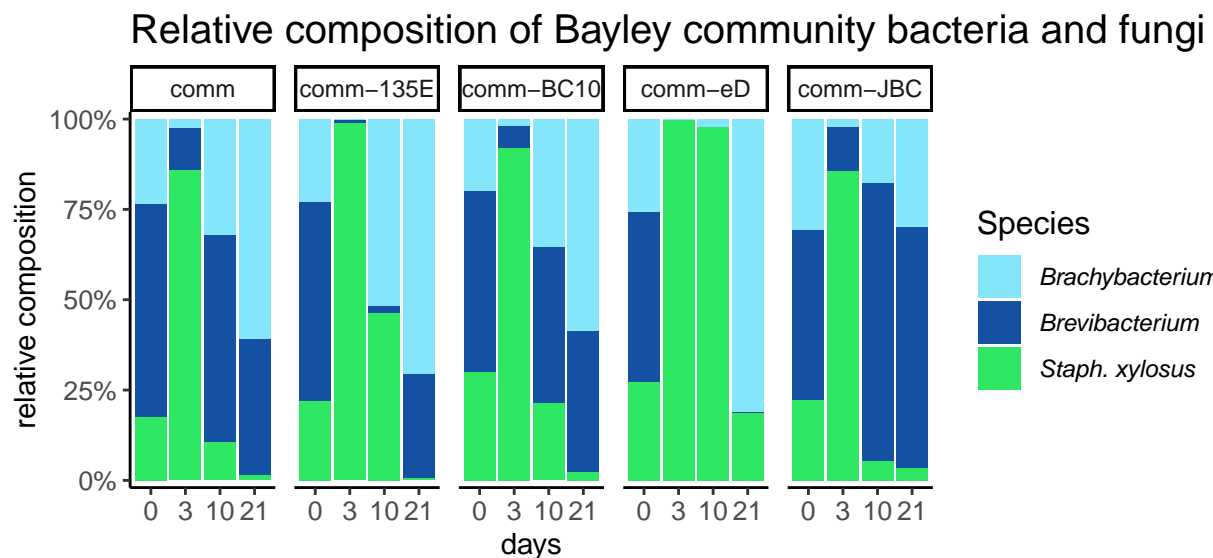
Let's also look at the balance between fungi and bacteria, although I suppose it's a little funny to consider this since the dropout of any one fungus or bacterium necessitates that the amount of fungi vs. bacteria from the start is different as compared to the complete community.

Relative composition of Bayley community bacteria and fungi