Results from Pairwise Growth Assays

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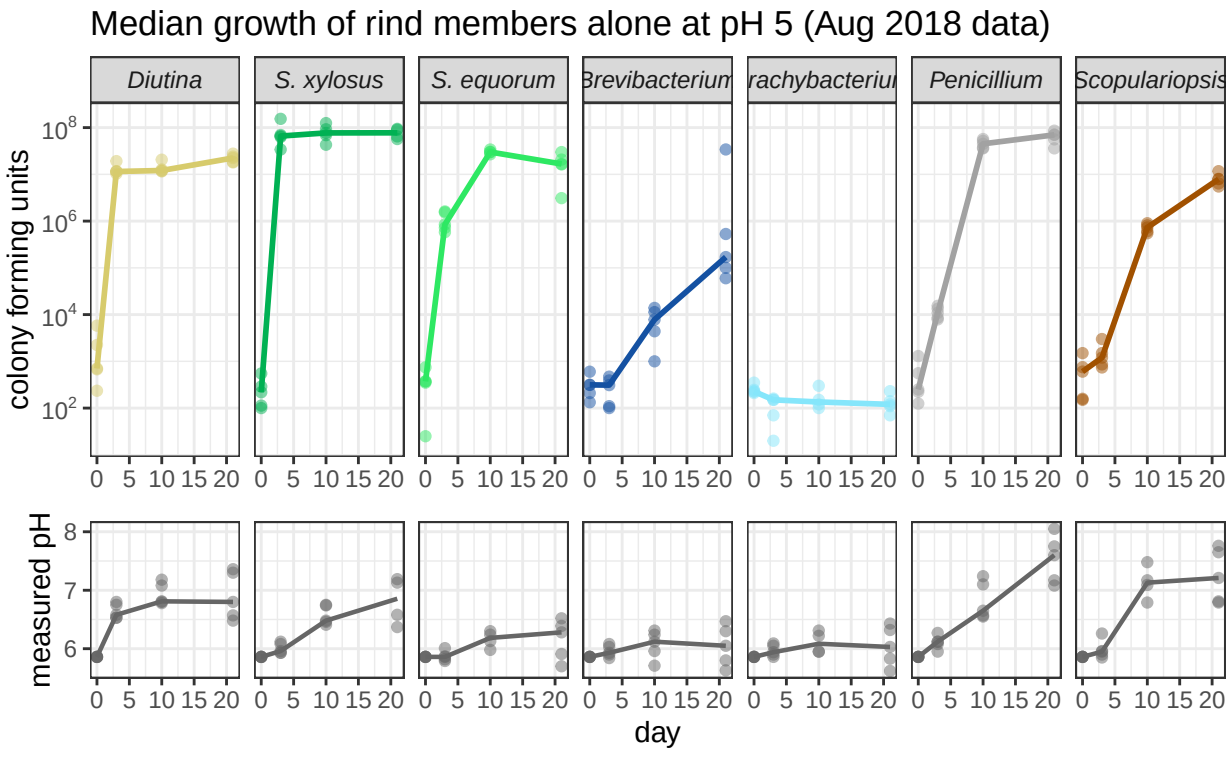
7/14/2021

```
d.18 <- readRDS(here("wrangled_data/all_pairwise.rds"))
d.18.summ <- readRDS(here("wrangled_data/pairwise_summary_stats.rds"))
load(here("wrangled_data/pairwise_test_summaries.RData"))
```

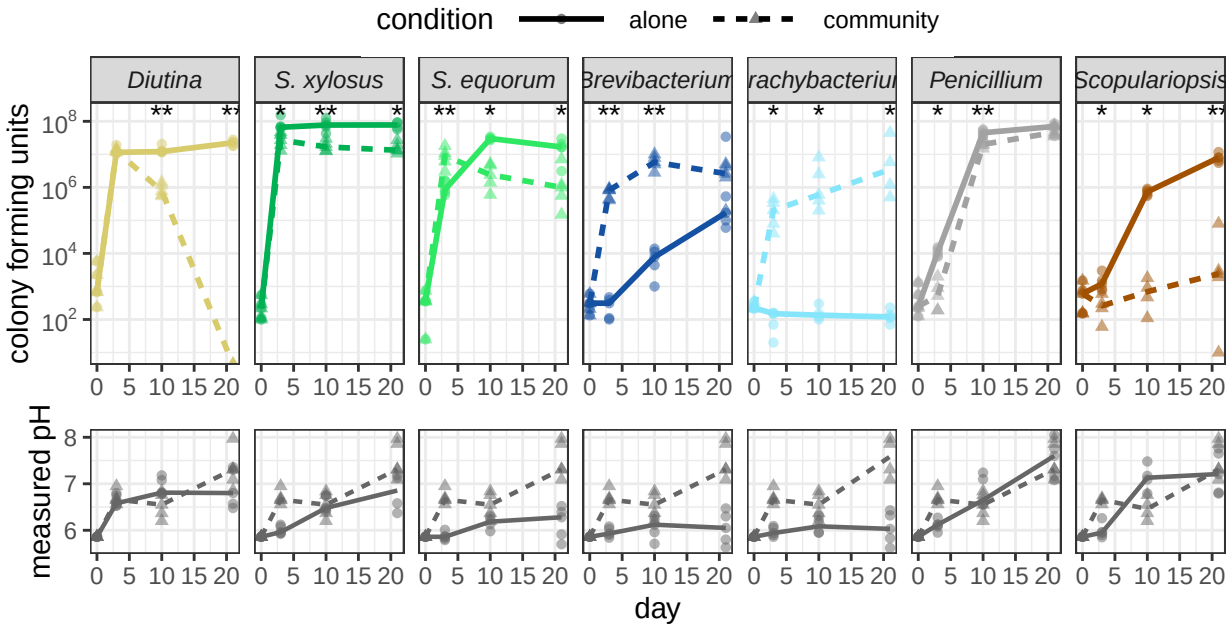
```
load(here("figures/plotting_objects.RData"))
```

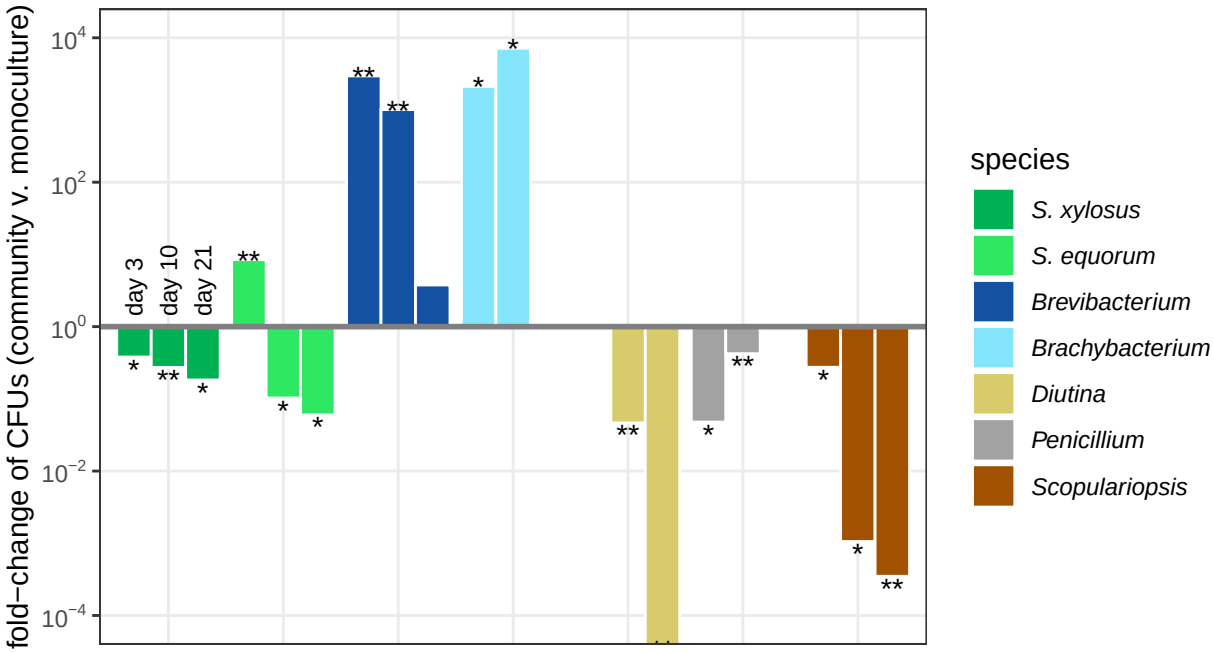
The rind community modulates growth phenotypes of each individual species

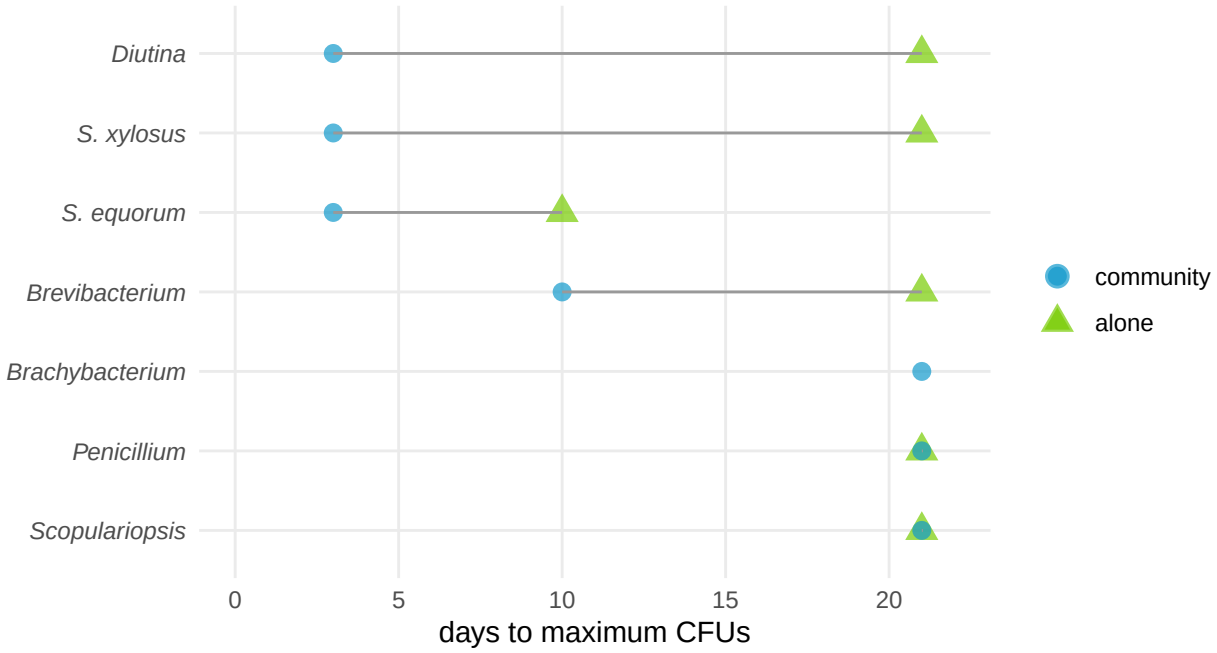
##Each species can be isolated and all but one grow alone on cheese



Community impacts individual member's growth



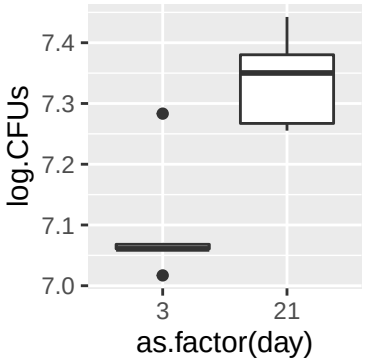




There are both net inhibitory and net facilitory interactions occuring in the community.

Side inquiry....

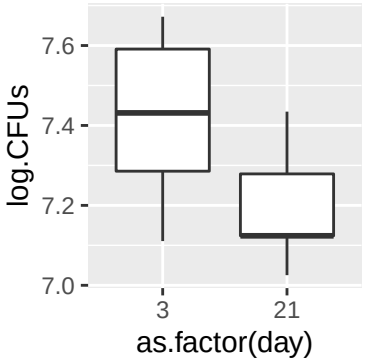
Is the increase in Candida cell counts alone significant?




```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  log.day3 and log.day21
## W = 2, p-value = 0.03615
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  -0.3817732 -0.0669523
## sample estimates:
## difference in location
##              -0.2501462
```

There is a significant (p-value `round(ttest$p.value, 3)`) increase in Candida growth alone. Backcalulated, Candida declines by a factor of `10^(ttest$conf.int[1])` to `10^(ttest$conf.int[2])`

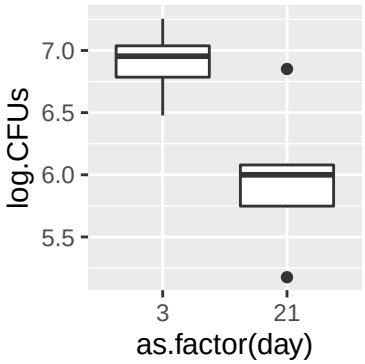
Is the decrease in S. xylosus cell counts in the community significant?




```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  log.day3 and log.day21
## W = 19, p-value = 0.2087
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  -0.1489551  0.5482531
## sample estimates:
## difference in location
##              0.2375453
```

There is not a significant decrease in S. xylosus over community growth

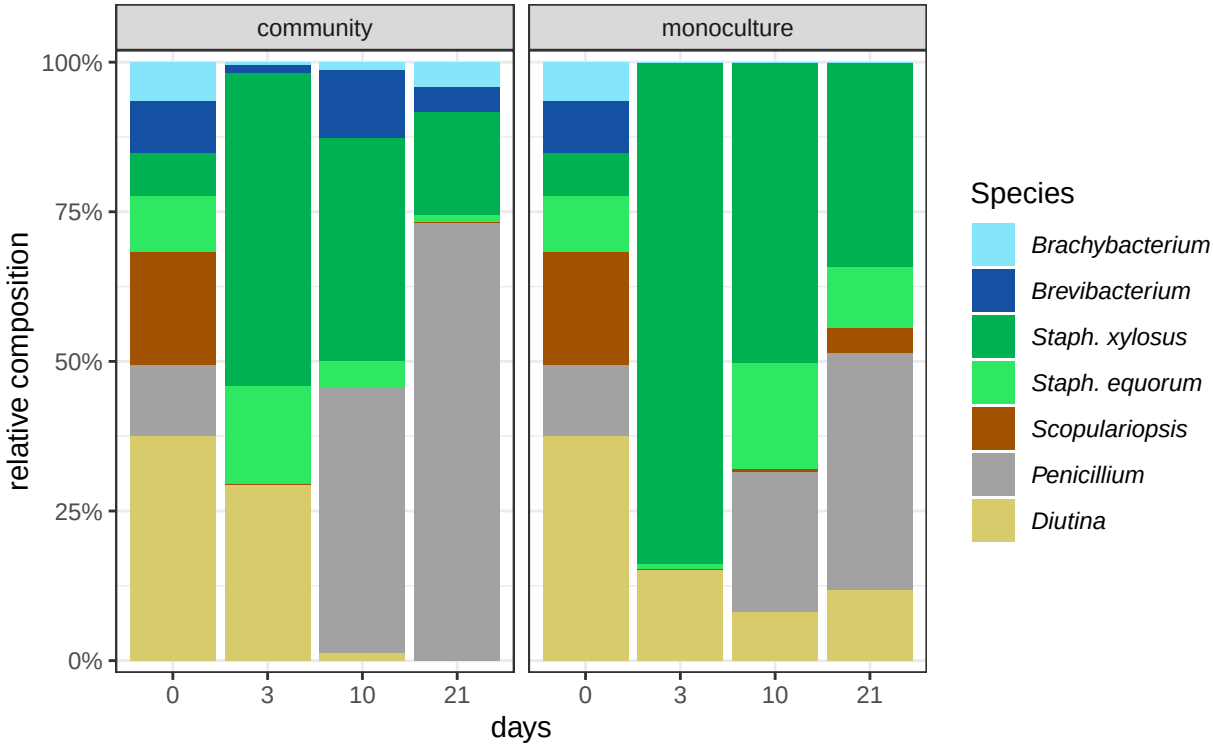
Is the decrease in S. equorum cell counts in the community significant?



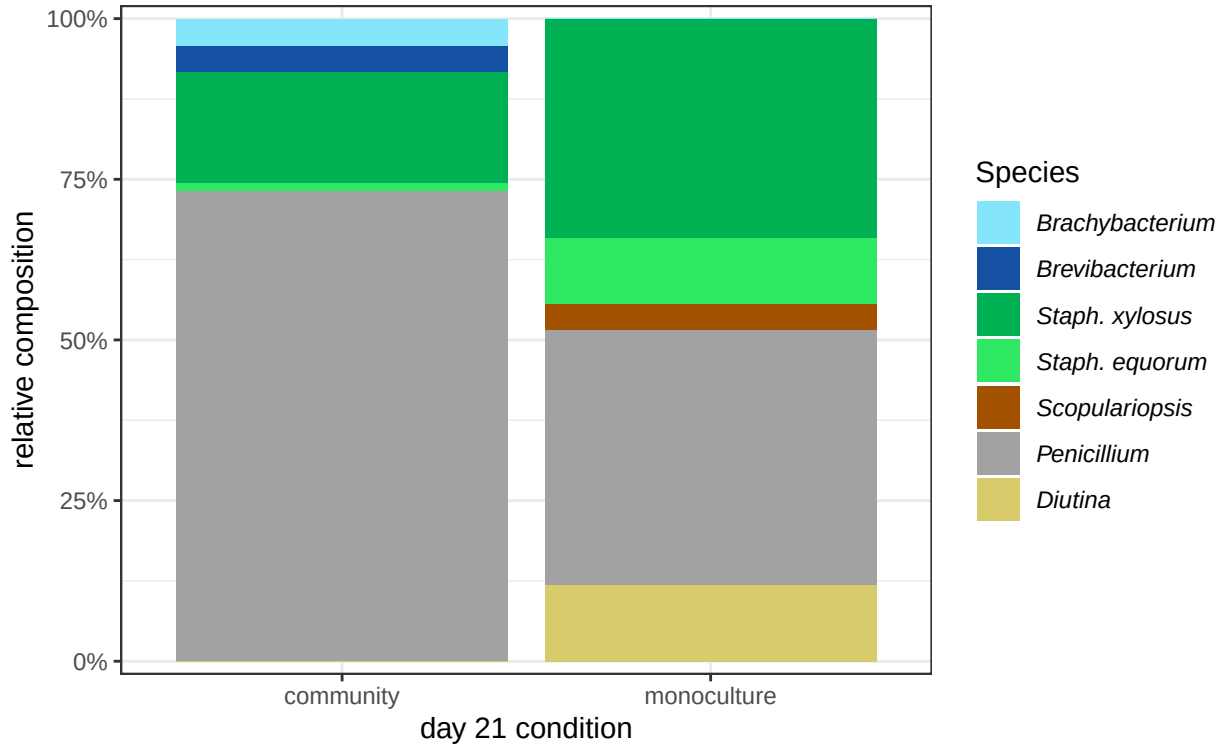

```
##
##  Wilcoxon rank sum test
##
## data:  log.day3 and log.day21
## W = 23, p-value = 0.03175
## alternative hypothesis: true location shift is not equal to 0
## 95 percent confidence interval:
##  0.1029842 1.7781513
## sample estimates:
## difference in location
##              0.9582453
```

So yup, there's a significant (p-value `round(ttest$p.value, 3)`) decline in S. equorum population over community growth

Interactions contribute to the pattern of succession.

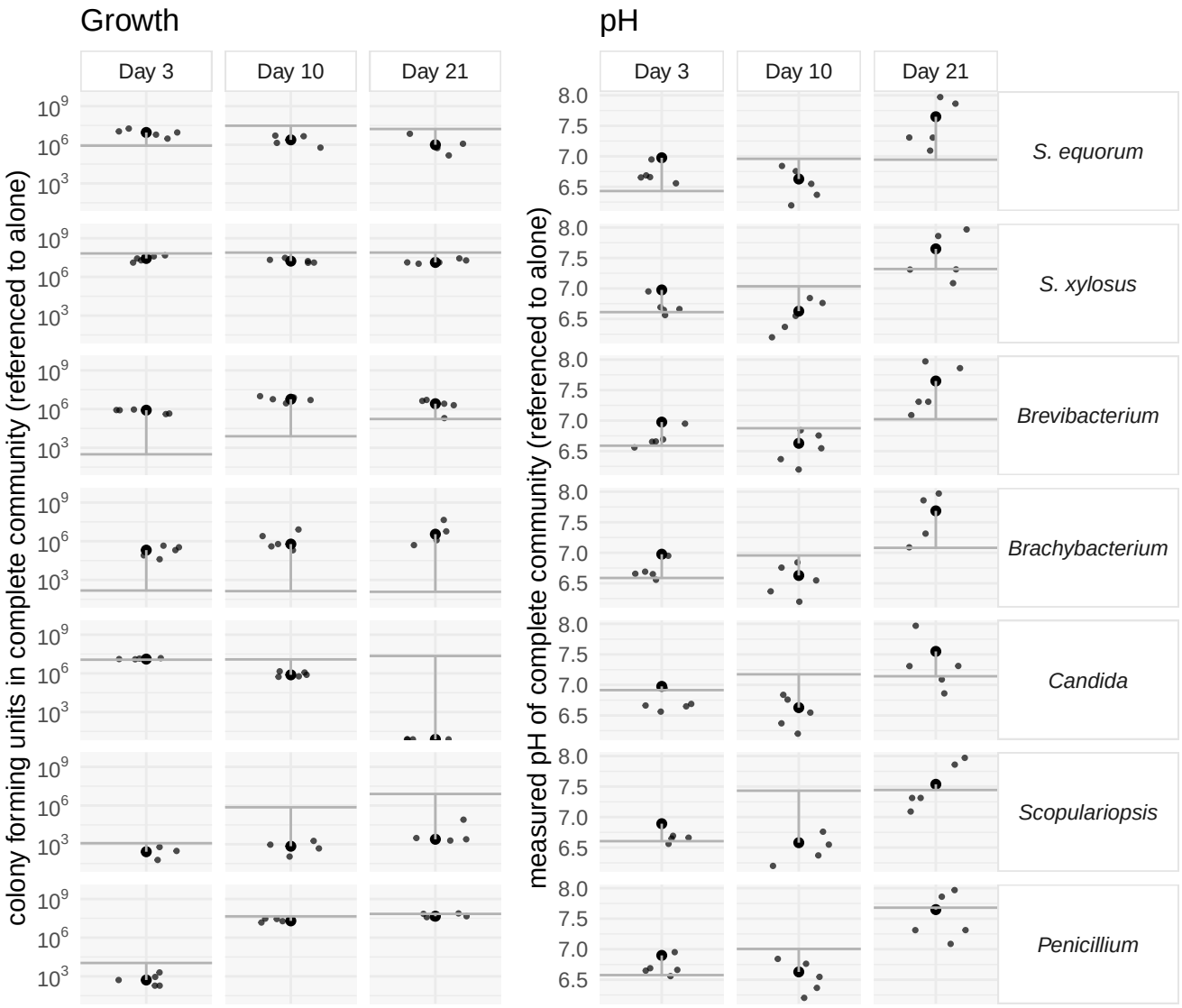



```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = rel.abund.matrix[4:10] ~ condition + day, data = rel.abund.matrix[1:2])
##           Df SumOfSqs      R2       F Pr(>F)
## condition  1   0.3518 0.12783  6.8848  0.007 **
## day        1   1.3271 0.48225 25.9727  0.001 ***
## Residual  21   1.0730 0.38992
## Total     23   2.7520 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



species	day	p.val	sig	condition	pH	med.rel.abund	cum.abund
135E	3	0.0758002	NA	alone	5	0.1528640	0.0464320
135E	10	0.0090234	**	alone	5	0.0833107	0.0116553
135E	21	0.0053457	**	alone	5	0.1175721	0.0287860
BC10	3	0.0090234	**	alone	5	0.8374289	0.5512737
BC10	10	0.0758002	NA	alone	5	0.5099368	0.7298167
BC10	21	0.0864107	NA	alone	5	0.3379923	0.7938618
BC9	3	0.0090234	**	alone	5	0.0095706	0.1277740
BC9	10	0.0143059	*	alone	5	0.1797391	0.3849788
BC9	21	0.0282801	*	alone	5	0.1014059	0.5741627
JB5	3	0.0090234	**	alone	5	0.0000024	0.9699893
JB5	10	0.0090234	**	alone	5	0.0000513	0.9848108
JB5	21	0.1745253	NA	alone	5	0.0010331	0.9633745
JB7	3	0.0090234	**	alone	5	0.0000009	0.9699910
JB7	10	0.0143059	*	alone	5	0.0000007	0.9848368
JB7	21	0.0143059	*	alone	5	0.0000008	0.9638914
JBC	3	0.0090234	**	alone	5	0.0001143	0.1229212
JBC	10	0.0758002	NA	alone	5	0.2373089	0.1719651
JBC	21	0.0758002	NA	alone	5	0.3942102	0.2846772
JB370	3	0.0864107	NA	alone	5	0.0000104	0.1229835
JB370	10	0.0143059	*	alone	5	0.0044897	0.2928644
JB370	21	0.0090234	**	alone	5	0.0416775	0.5026210

fold-change lollipop graph style: difference between community growth and alone growth at pH 5. Community growth (CFUs) is marked by points, colored by pH. Larger points mark the median CFUs in the community and are colored by average pH. Horizontal gray lines mark growth alone.



Notable from these graphs is that the community seems to slow deacidification compared to every alone growth. Implies that this delay in deacidification can't be attributed to one single species.

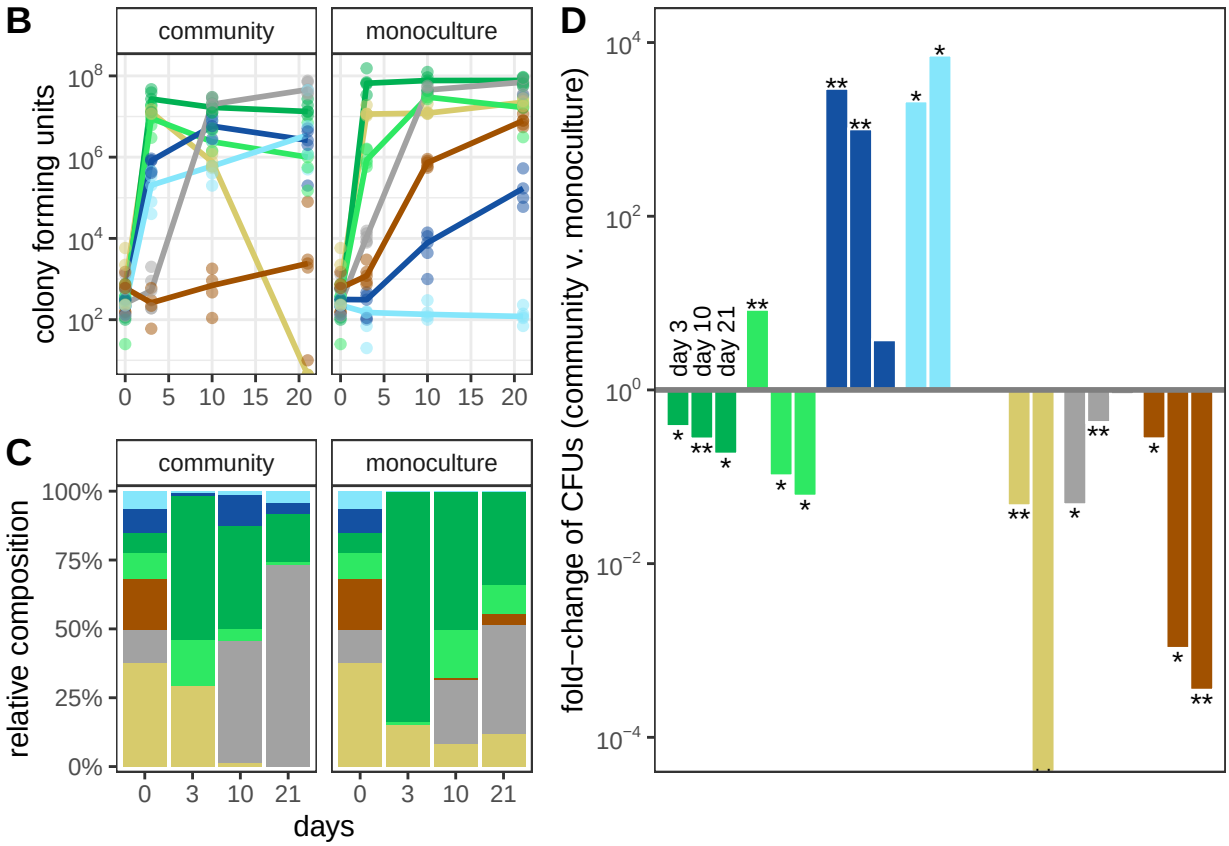
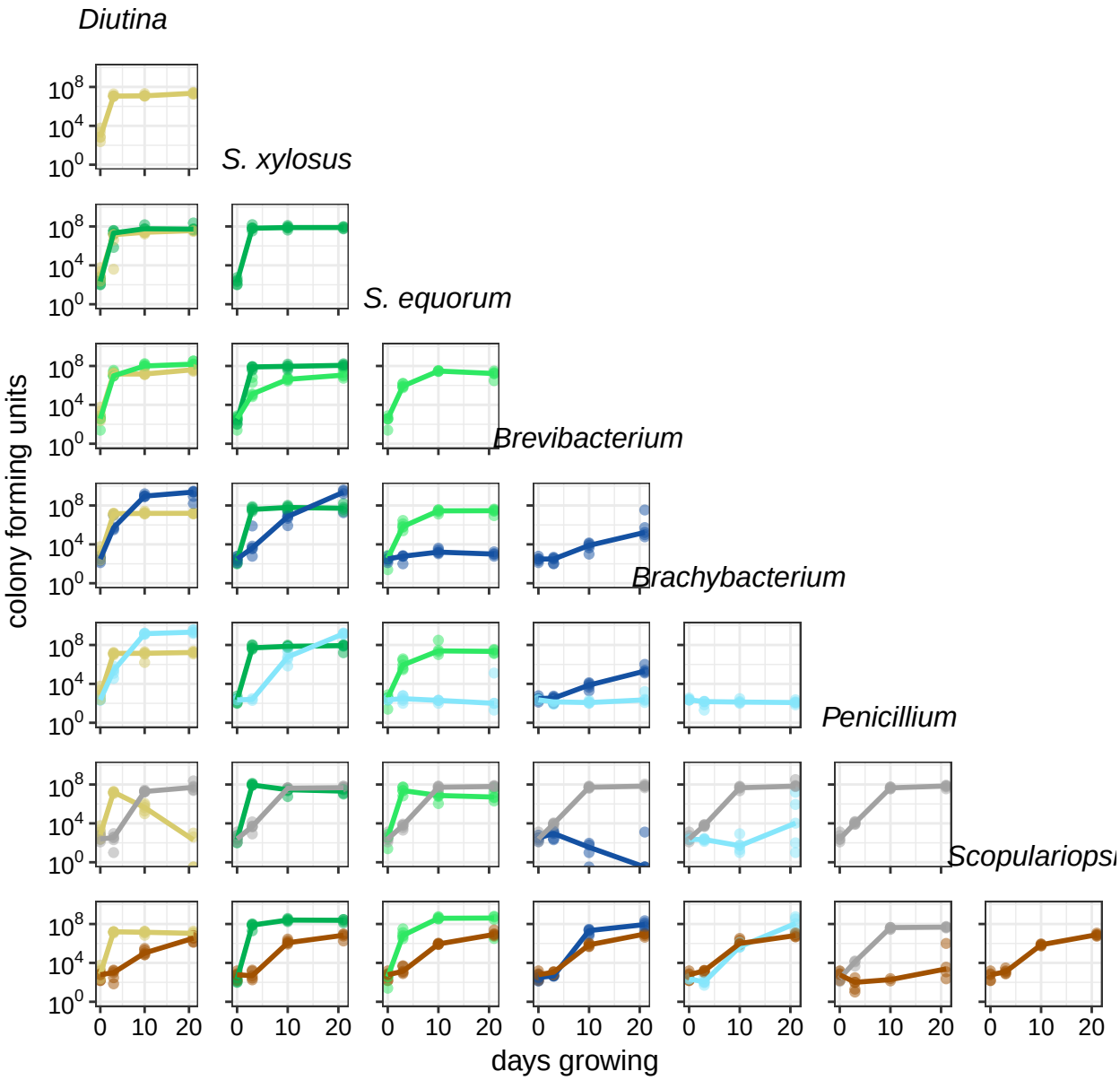
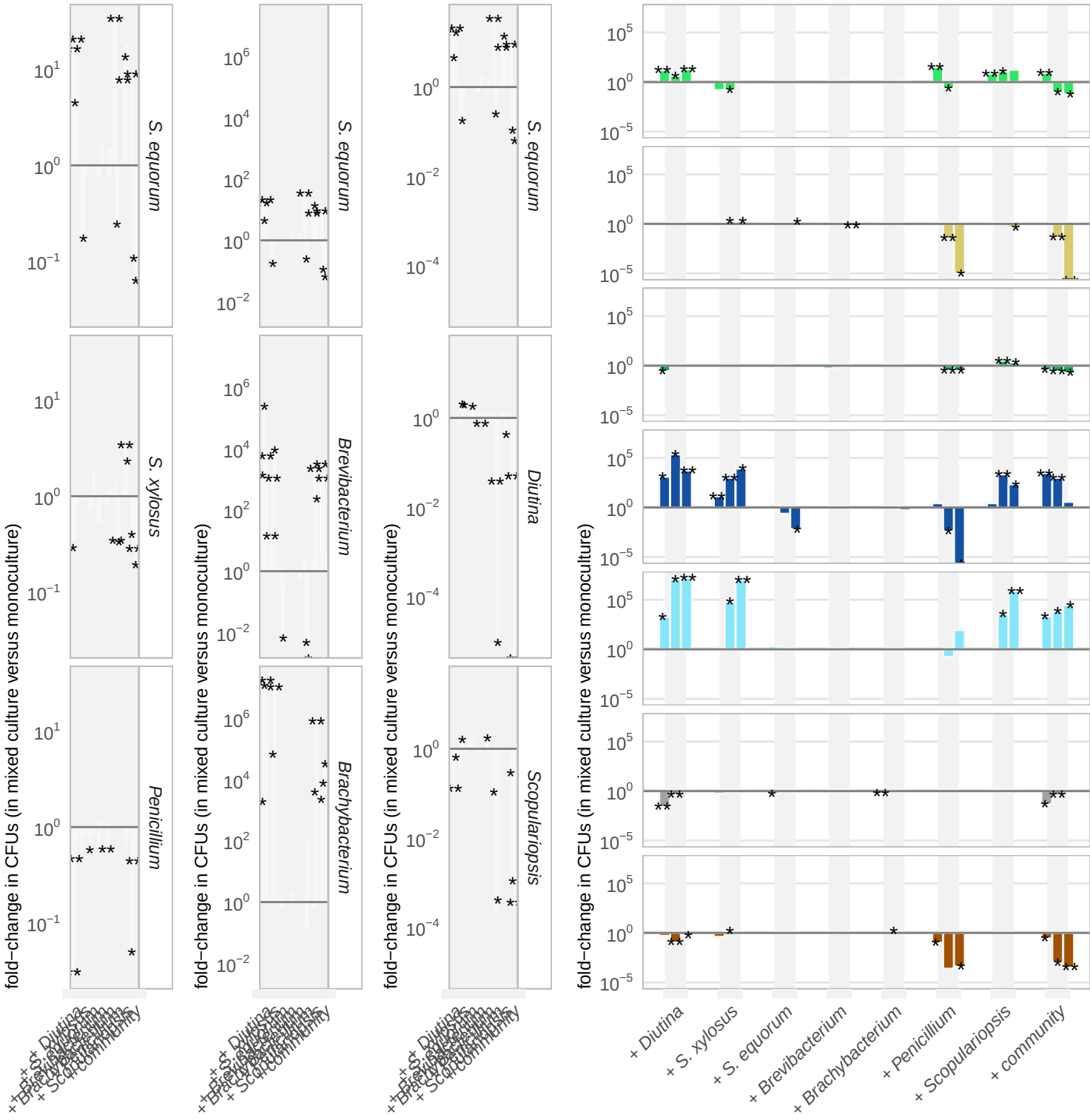

B
community
monoculture
colony forming units
10^8
10^6
10^4
10^2
0 5 10 15 20
C
community
monoculture
relative composition
100%
75%
50%
25%
0%
0 3 10 21
days
D
fold−change of CFUs (community v. monoculture)
10^4
10^2
10^0
10^{-2}
10^{-4}
day 3
day 10
day 21

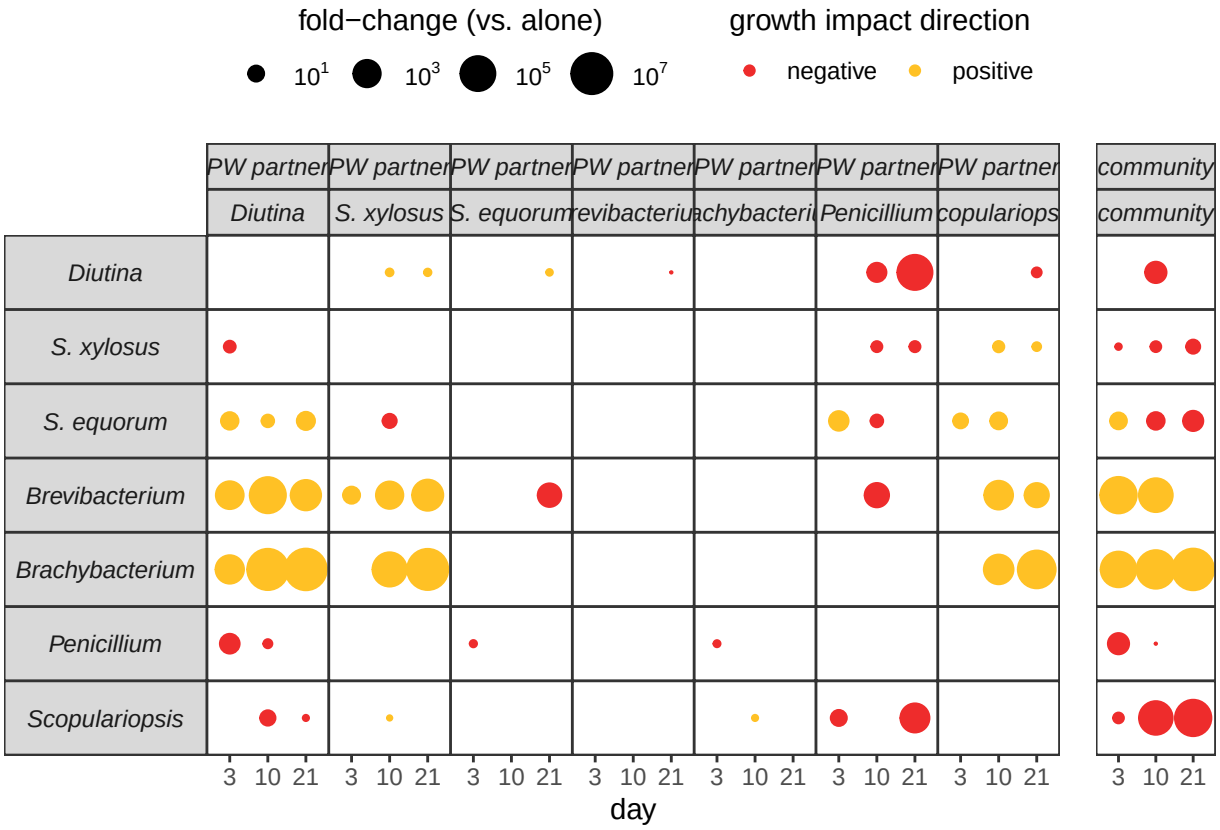
Figure 1:

Pairwise interactions:

both inhibitory and stimulatory interactions between players




fold−change in CFUs (in mixed culture versus monoculture)
S. equorum
S. xylosus
Penicillium
Brevibacterium
Brachybacterium
Diutina
Scopulariopsis
+ Diutina
+ S. xylosus
+ S. equorum
+ Brevibacterium
+ Brachybacterium
+ Penicillium
+ Scopulariopsis
+ community



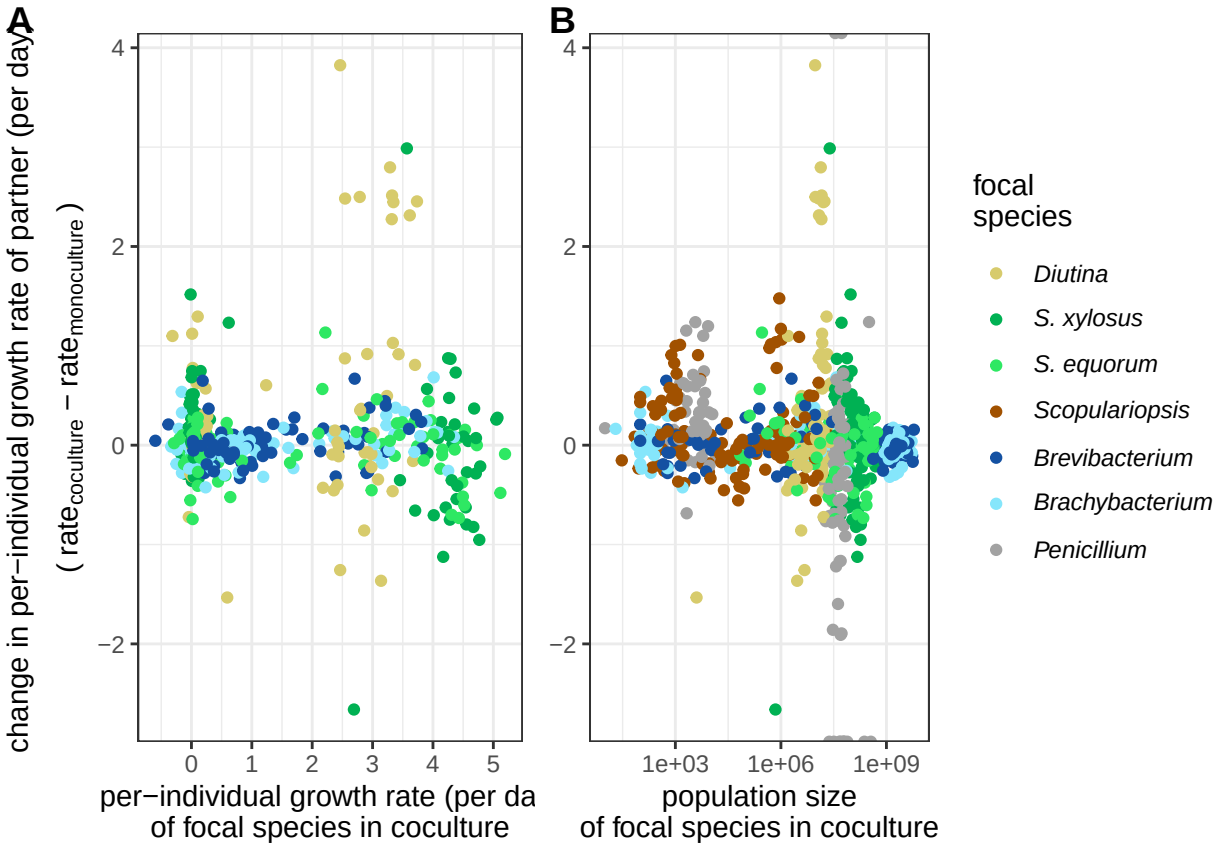
Observations:

- Early colonizers mostly stimulated late colonizers
- Fungi inhibit each other
- Penicillium inhibits everyone
- Scopulariopsis has surprisingly widespread effects on bacterial species, stimulating each, for being such a late-colonizer.
- Late colonizing bacteria don't have much effect on coculture parnters.

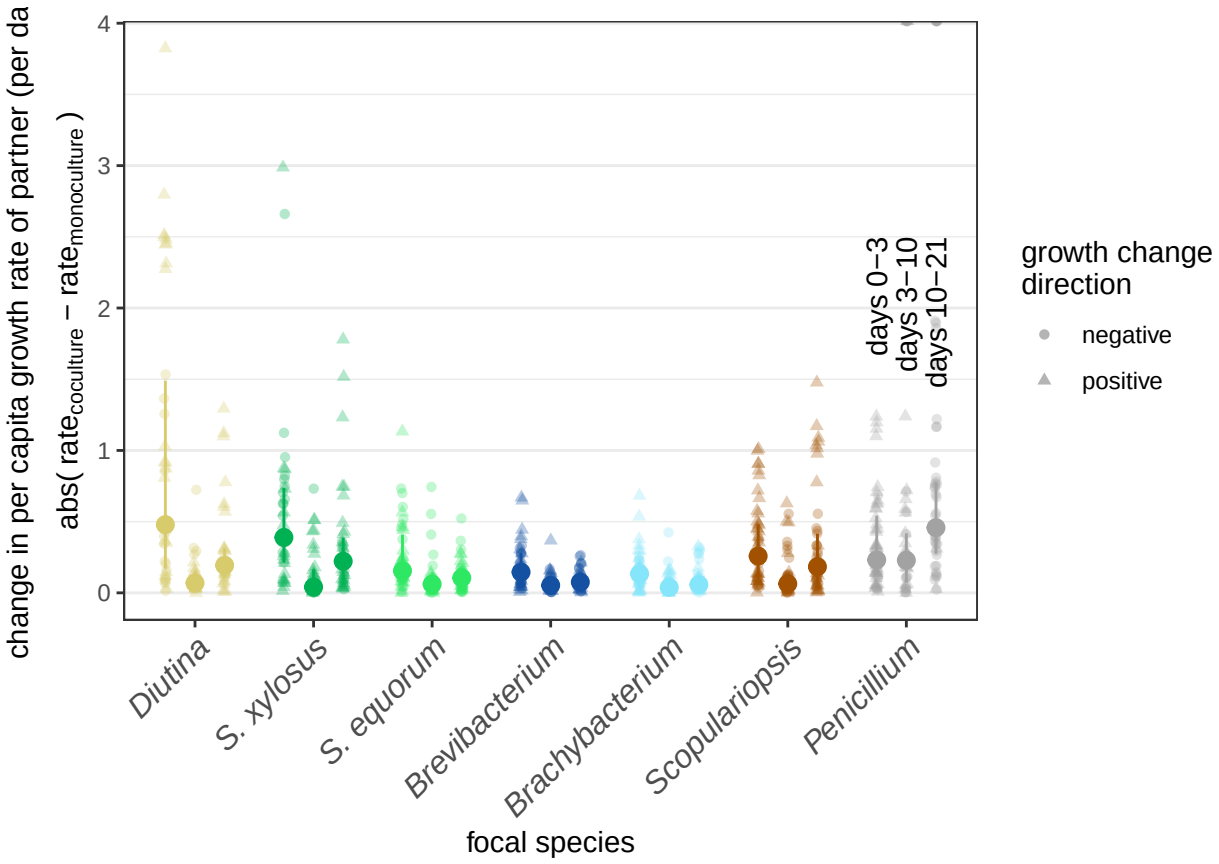
What interactions are same at pH 5 & pH 7 versus different?

```
## # A tibble: 63 x 9
##    species condition   day sig.pH5 sig.pH7 intx.dir.pH5 med.fc.pH5 intx.dir.pH7
##    <fct>   <fct>     <dbl> <chr>   <chr>   <chr>             <dbl> <chr>
##  1 JB5     JBC          21 *       *       neg           0          neg
##  2 135E    JBC          21 *       **      neg           0.00000893 neg
##  3 JB370   JBC          21 *       *       neg           0.000390   neg
##  4 JB5     JBC          10 *       **      neg           0.00434    neg
##  5 JB5     BC9          21 *       <NA>    neg           0.00626    <NA>
##  6 JBC     135E          3 **      <NA>    neg           0.0293     <NA>
##  7 135E    JBC          10 **      **      neg           0.0364     neg
##  8 JB370   JBC           3 *       *       neg           0.1        neg
##  9 JB370   135E         10 **      **      neg           0.122      neg
## 10 BC9     BC10         10 *       **      neg           0.165      neg
## # ... with 53 more rows, and 1 more variable: med.fc.pH7 <dbl>
```

Ben specifically asked whether the magnitude of partner effects was correlated with the *growth rate* – and so let's consider change in population size over time vs. change in affected species growth rate, or change in focal species growth rate vs. change in partner species growth rate.



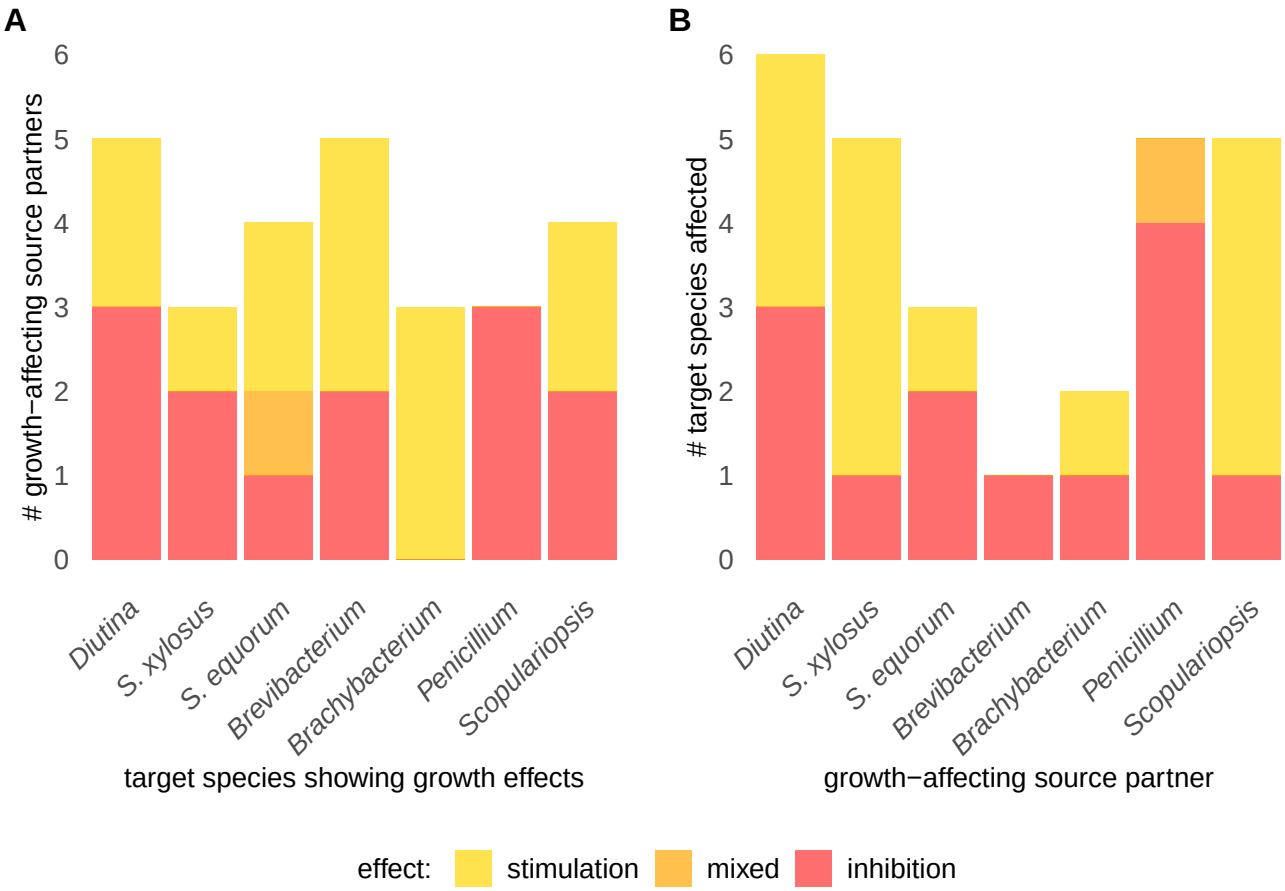
Does interaction effect on growth increase with time, presumably because population size of focal species increases?



```
## # A tibble: 251 x 7
## # Groups:   species, partner, pH [84]
##    species partner pH    timeframe median.delt.r  p.val sig
##    <fct>   <chr>   <fct> <chr>             <dbl>  <dbl> <chr>
##  1 135E    BC10    5     0-3            -0.0801  0.690  <NA>
##  2 135E    BC10    5     10-21           0.00446 0.886  <NA>
##  3 135E    BC10    5     3-10            0.0881  0.0556 <NA>
##  4 135E    BC10    7     0-3             0.233   0.421  <NA>
##  5 135E    BC10    7     10-21          -0.00472 0.841  <NA>
##  6 135E    BC10    7     3-10            0.0302  1      <NA>
##  7 135E    BC9     5     0-3             0.0828  0.690  <NA>
##  8 135E    BC9     5     10-21           0.0231  0.151  <NA>
##  9 135E    BC9     5     3-10            0.0185  0.841  <NA>
## 10 135E    BC9     7     0-3             0       0.917  <NA>
## # ... with 241 more rows
```

How frequently is a species' growth affected by a coculture partner, and is each species more frequently inhibited or stimulated by partners? What about the vive-versa, what species are frequently or infrequently affecting the growth of their coculture partner, and is that species usually inhibiting or stimulating partner species?

Will only consider significant interactions in the following (p-value from Wilcoxon ranked sums test < 0.05).



So we can see that all species are frequently affected by partners, with average ~4 partner affecting growth. *Penicillium* is only ever ever significantly inhibited, and *Brachybacterium* is only ever stimulated by coculture partners. The rest are mostly evenly split between being stimulated or inhibited by partners.

Penicillium stands out as a widespread and devoted inhibitor, whereas *Scopulariopsis* & *S. xylosus* both stimulate 4/5 of the coculture partners they interact with. *Candida*, suprisingly, is an even split between stimulating and inhibiting its coculture partners. There's a wide range of total number of species affected, from just 1 partner

species affected by *Brevibacterium* to all 6 partners affected in cocultures with *Candida*. Noteably, all three fungi significantly affect 5-6 of their coculture partners, thought through diverse ranges of effects. The later colonizers, including *S. equorum*, *Brevibacterium*, and *Brachybacterium*, all exert the fewest number of growth effects.

Are Staphs more inhibited in community than could be predicted from coculture pairs alone?

```
## [1] "The total positive interactions, multiplied up for S. xylosus, are:"
## [1] "day 3 = 1.59259259259259, day 10 = 3.79455914805631, and day 21 = 4.53376187763174"
## [1] "The total negative interactions, multiplied up for S. xylosus, are:"
## [1] "day 3 = 0.0876815290956705, day 10 = 0.11982889204675, and day 21 = 0.267209467455621"
## [1] "The total product of interactions, multiplied up for S. xylosus, are:"
## [1] "day 3 = 0.139640953744957, day 10 = 0.454697818517447, and day 21 = 1.21146409689258"
## [1] "The in vitro result of all interactions in the community for S. xylosus, are:"
## [1] "day 3 = 0.379411764705882, day 10 = 0.272727272727273, and day 21 = 0.182602291325696"
## [1] "--------------------"
```

```
## [1] "The total positive interactions, multiplied up for S. equorum, are:"
## [1] "day 3 = 3776.95425996486, day 10 = 53.6204047021346, and day 21 = 904.270385375711"
## [1] "The total negative interactions, multiplied up for S. equorum, are:"
## [1] "day 3 = 0.122352941176471, day 10 = 0.0290944675844046, and day 21 = 0.620718562874252"
## [1] "The total product of interactions, multiplied up for S. equorum, are:"
## [1] "day 3 = 462.1214623957, day 10 = 1.56005712646891, and day 21 = 561.297414060157"
## [1] "The in vitro result of all interactions in the community for S. equorum, are:"
## [1] "day 3 = 8.47222222222222, day 10 = 0.10268897149938, and day 21 = 0.0598802395209581"
## [1] "--------------------"
```

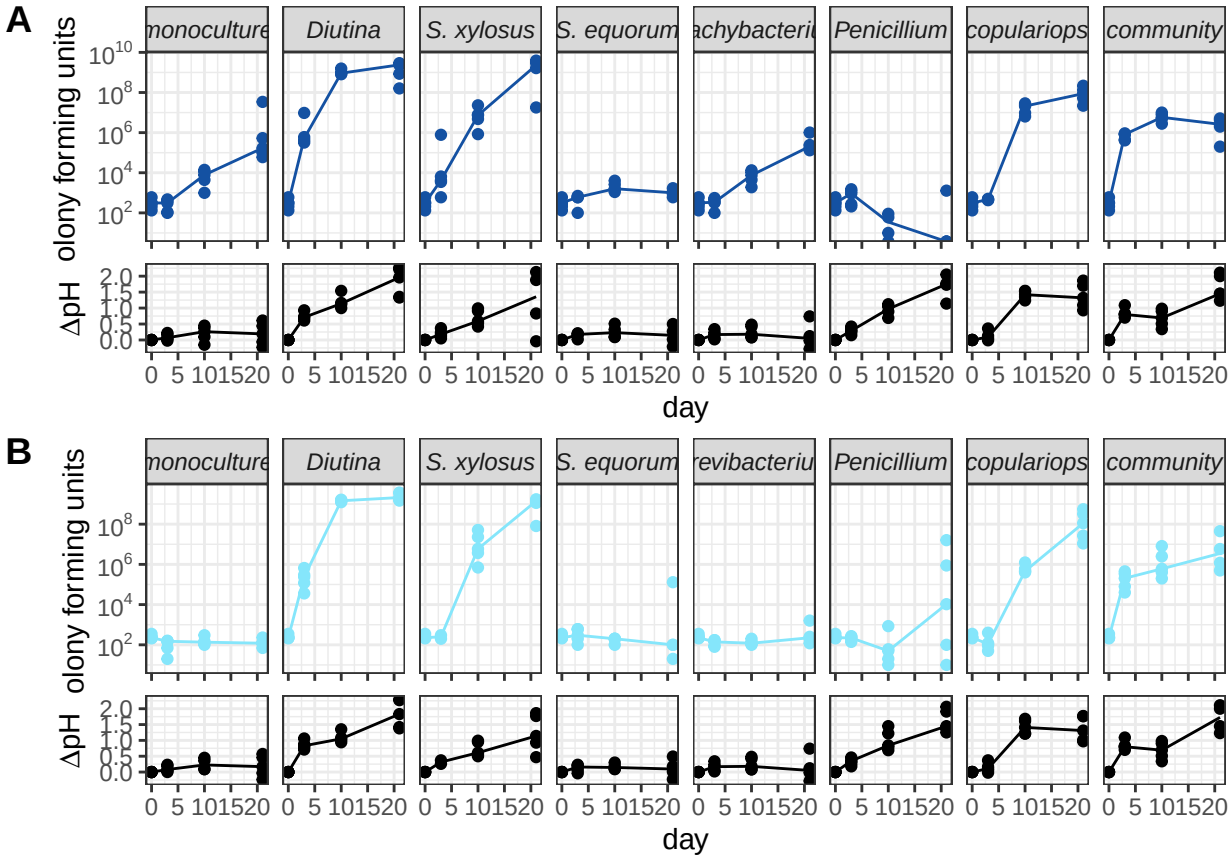
S. xylosus is more stimulated than inhibited. Nonetheless, it's inhibited in the community at scales similar to the product of all negative interactions, suggesting that negative interactions are dominant.

S. equorum is very much stimulated, but to at some timepoints it is inhibited at equal scales (order of 100-fold). Overall in the community, it is, like *S. xylosus*, predominantly inhibited, at scales similar to the product of inhibitory pairwise interactions. So again, negative interactions are dominant.

Late-growing species are stimulated by deacidifiers & neutral starting pH compensates

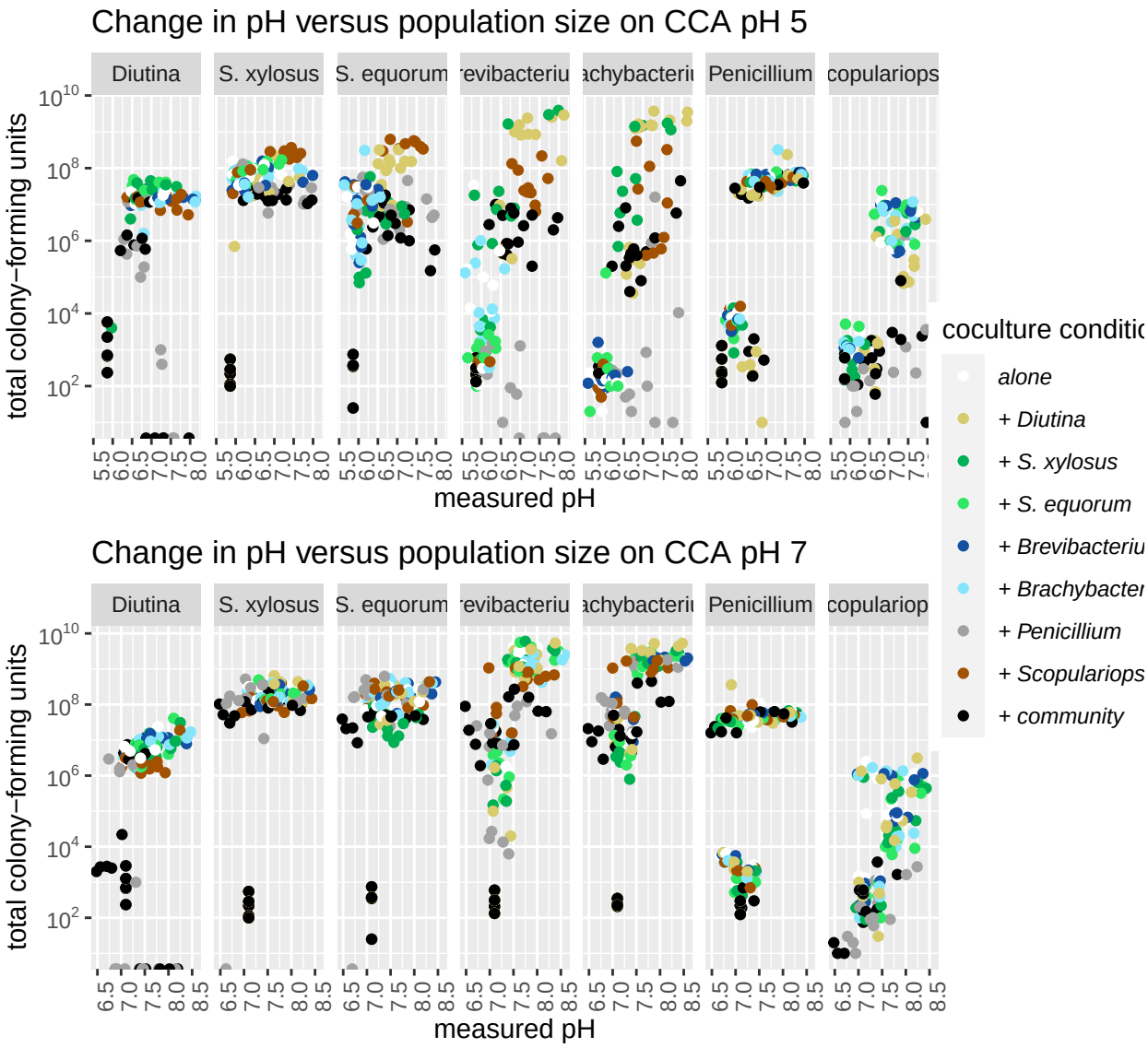
Since, again, the pH of these samples was measured after homogenizing in buffer and therefore is not an accurate measurement of the pH of the sample, I'll just consider the magnitude of the buffered pH change. So measure difference in pH (delta.pH) from day 0.

Deacidifying pairwise interactions correlate with stimulation of Brevi/Brachy



Can we quantitatively observe this correlation between pH & population size for acid-sensitive species in pairwise cocultures?

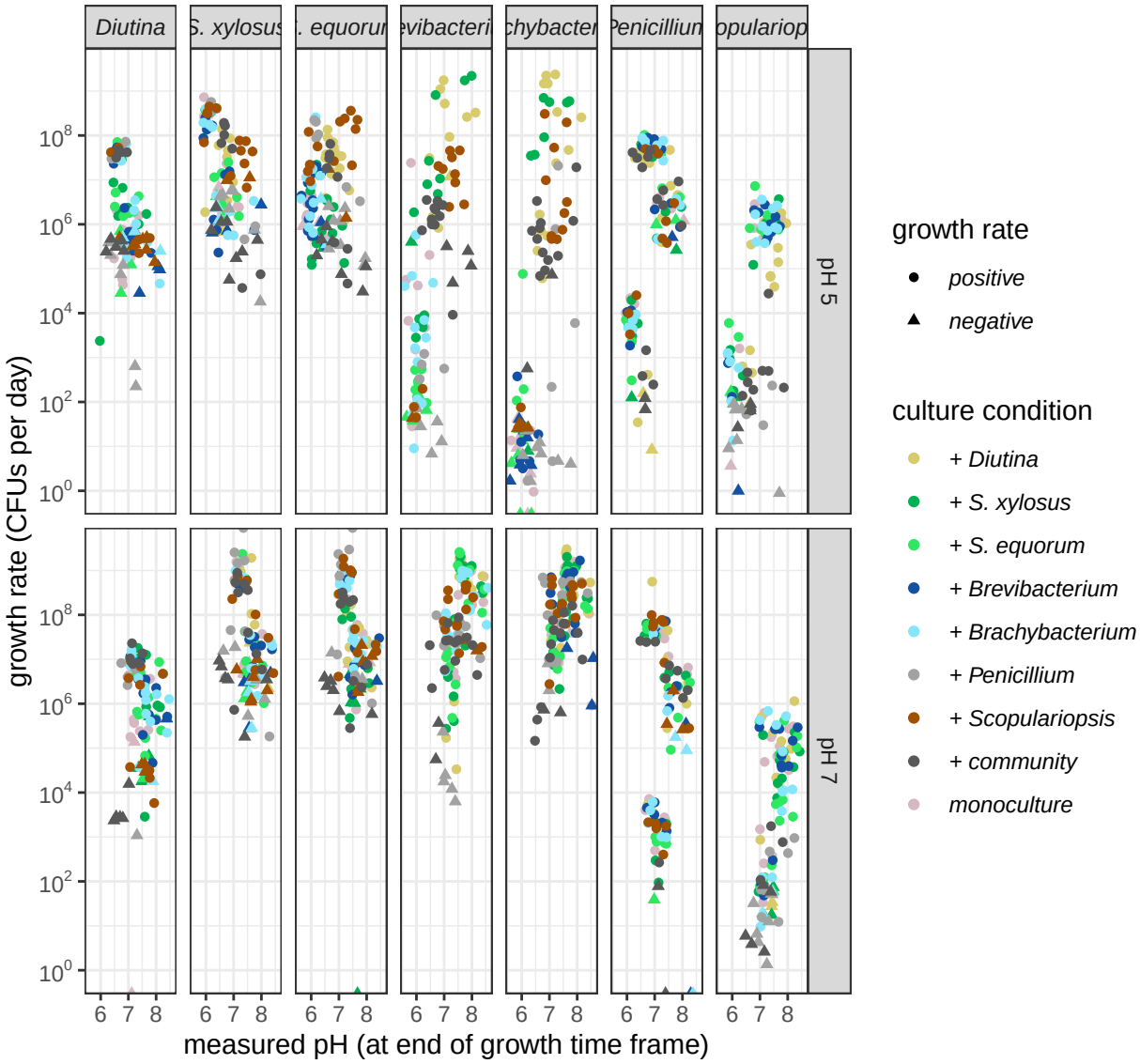
look at correlation between population size & pH: hypothesis is that we would see a positive correlation for pH 5, and no correlation at pH 7



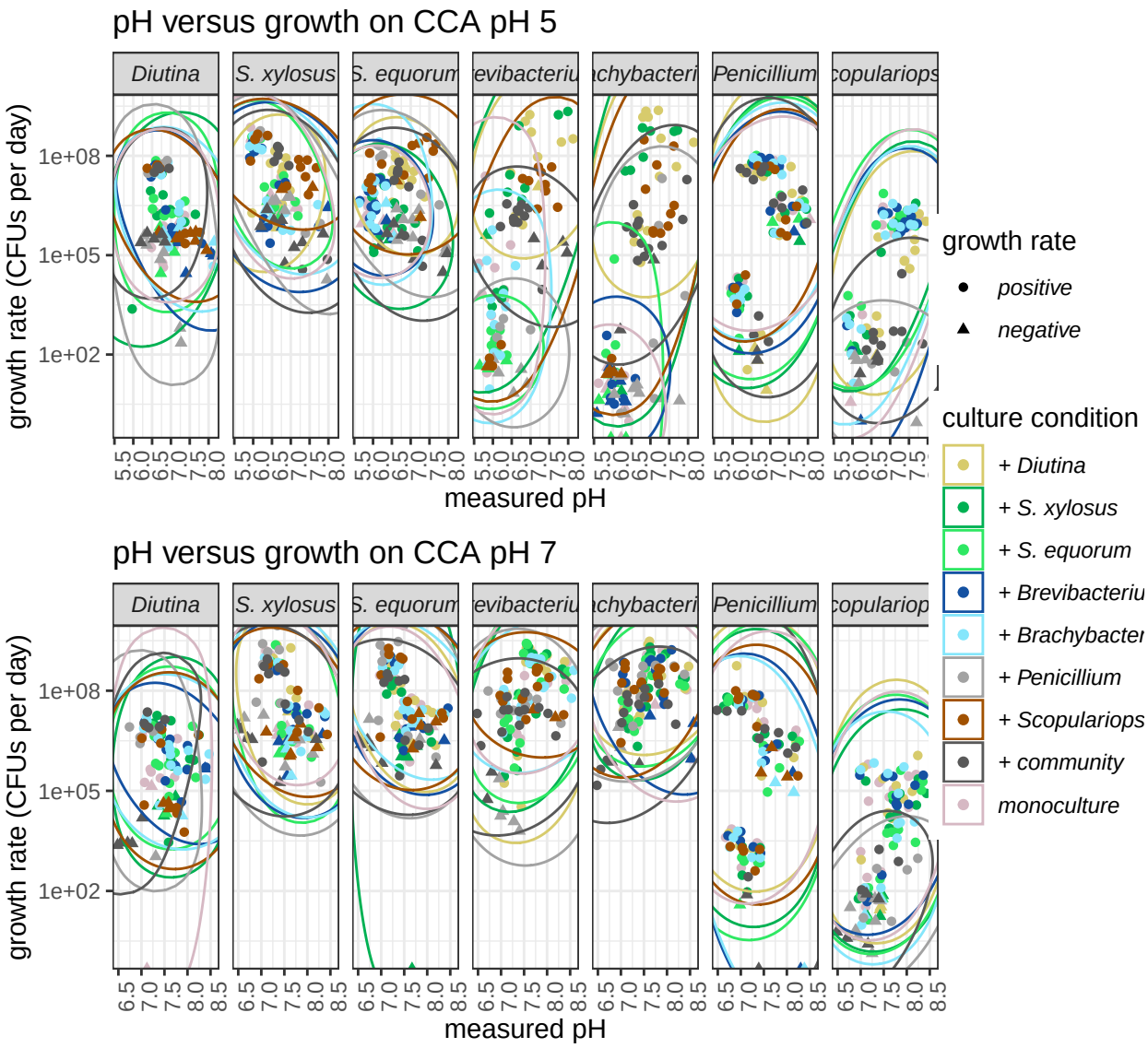
So it definitely feels like I'm seeing a positive correlation for pH 5 *Brevi* and *Brachy*. However, the trend isn't completely eliminated for pH7 cocultures, though much of this seems to be coming from the *Penicillium* coculture, which is probably inhibitory for reasons unrelated to pH.

Also worth noting that at pH 7, *Scopulariopsis* has a rather steady positive correlation trend between pH and population size, though there might also be correlation with time.

But should I be considering more a relationship between *growth* and pH? Let's take a look at the correlations between pH the difference in growth rates between coculture and monoculture (growth rate generated by per-capita growth rate from the exponential growth equation * population size (i.e. capitol) at the later time point of the two measured.)

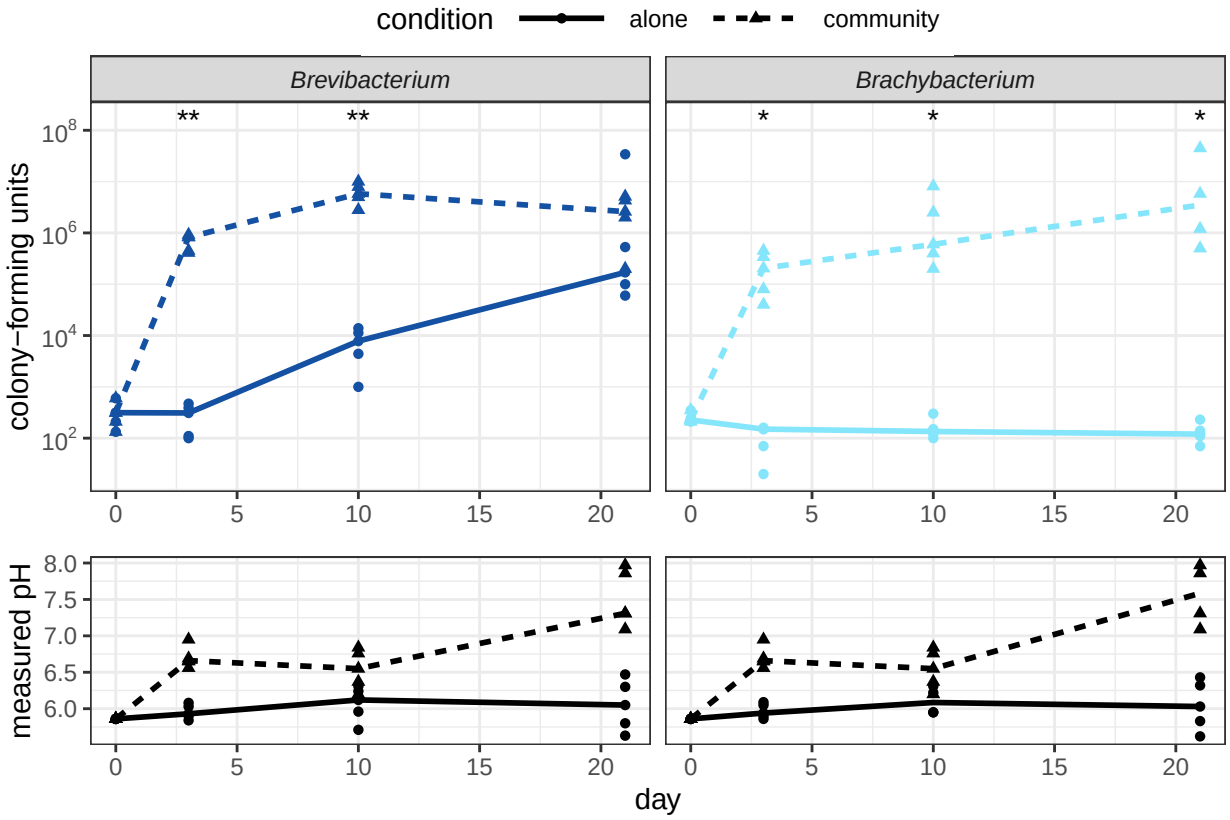


The same graph, but points clustered by partner species to show relationships.



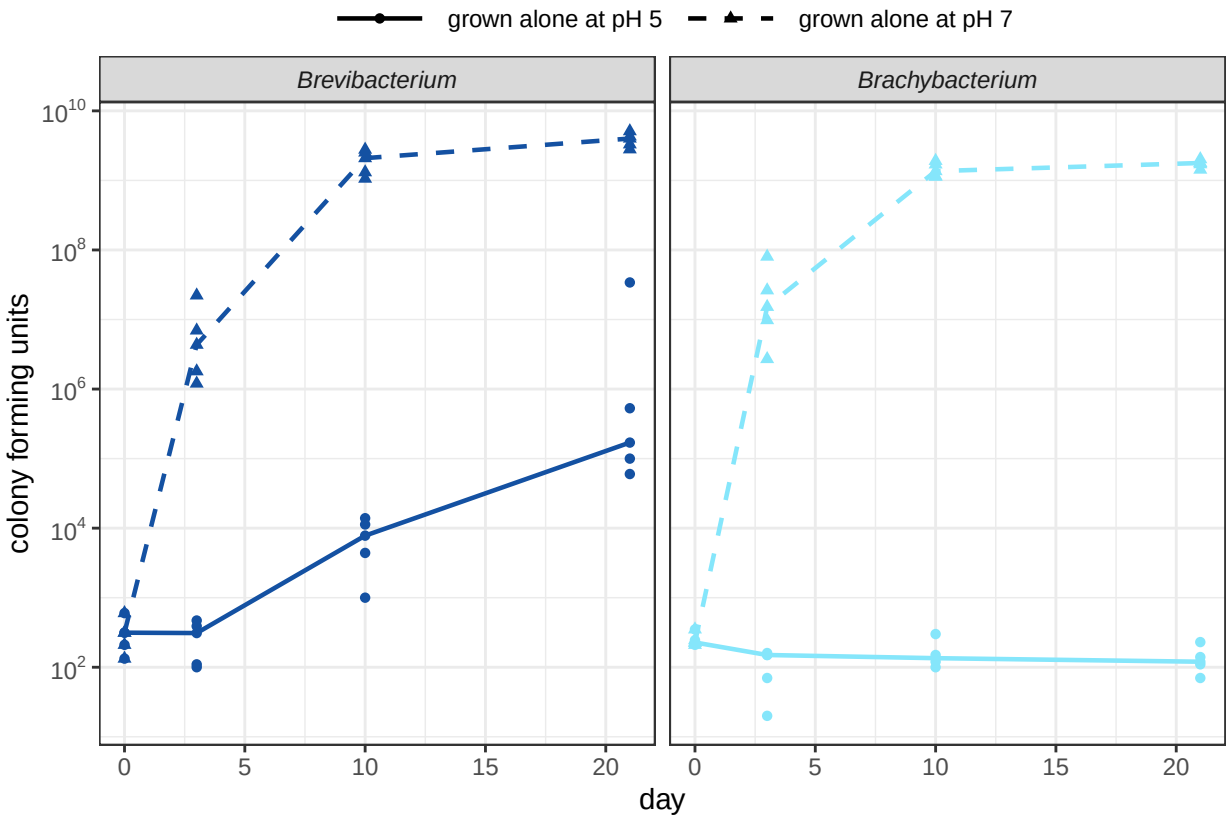
Overall, pH isn't a complete descriptor/predictor of *Brevibacterium* and *Brachybacterium* growth, though it seems to be involved. Let's get back to what we *can* learn about the acid-sensitivity of these two Actinobacteria

Brevi/Brachy are sensitive to acidic pH, but grow in community with increasing pH

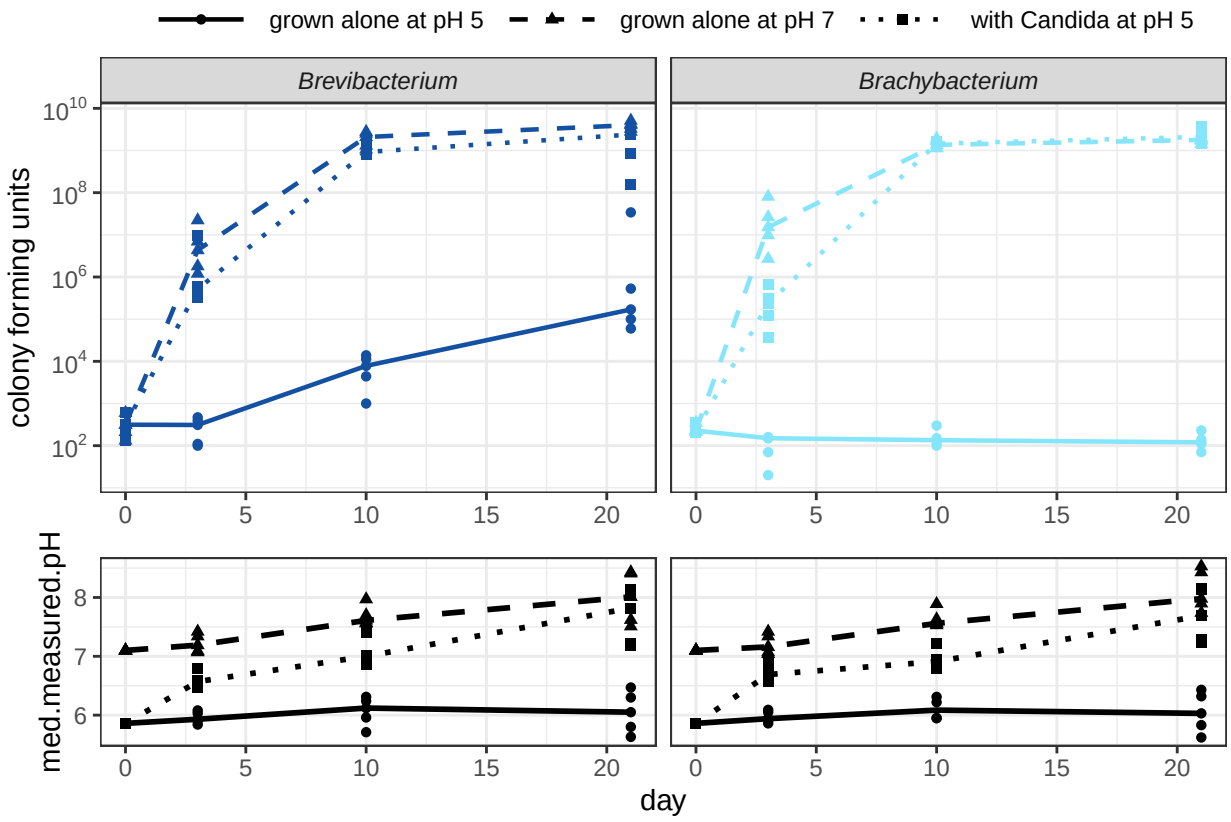


Model Actinobacteria are acid-sensitive

Inoculation on CCA adjusted to pH 7 allows for rapid growth of both Actinobacteria

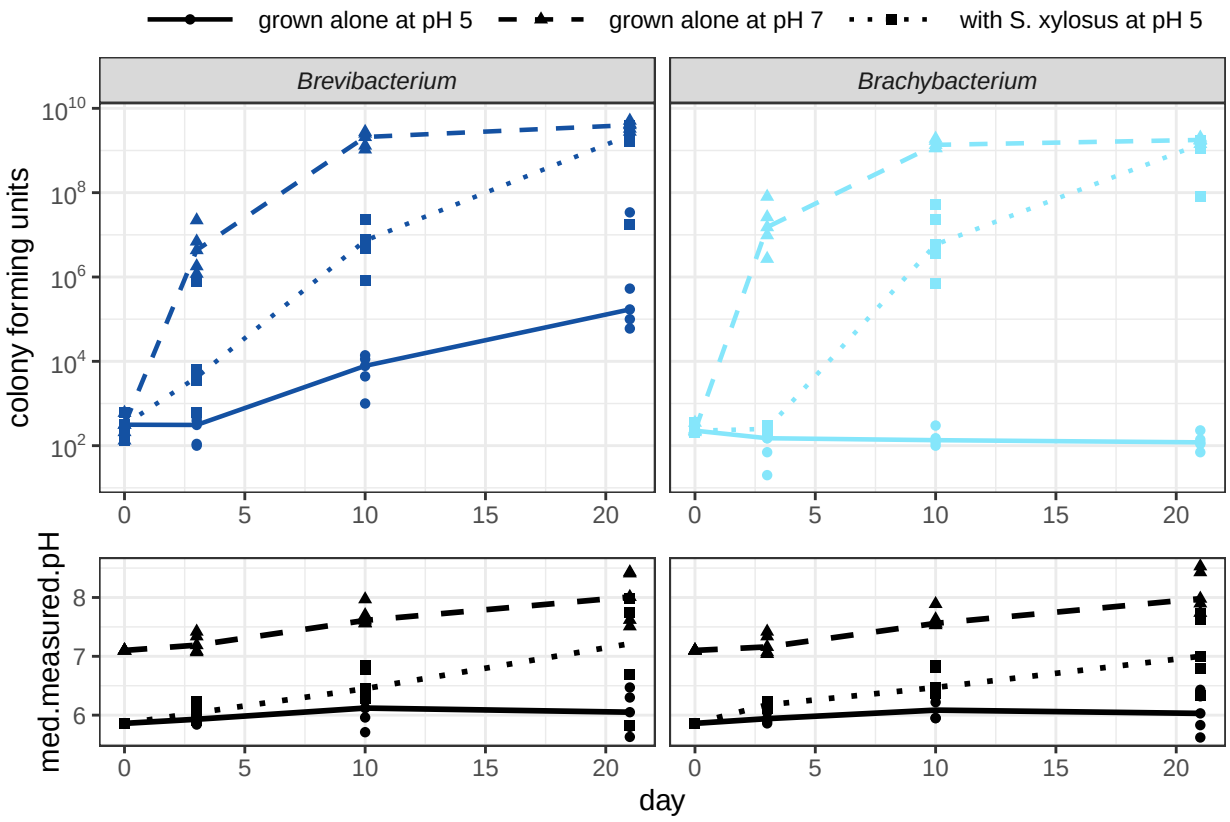

grown alone at pH 5
grown alone at pH 7
Brevibacterium
Brachybacterium
colony forming units
10^{10}
10^{8}
10^{6}
10^{4}
10^{2}
0
5
10
15
20
day

increased pH is sufficient to simulate Candida's stimulation of Brevi/Brachy



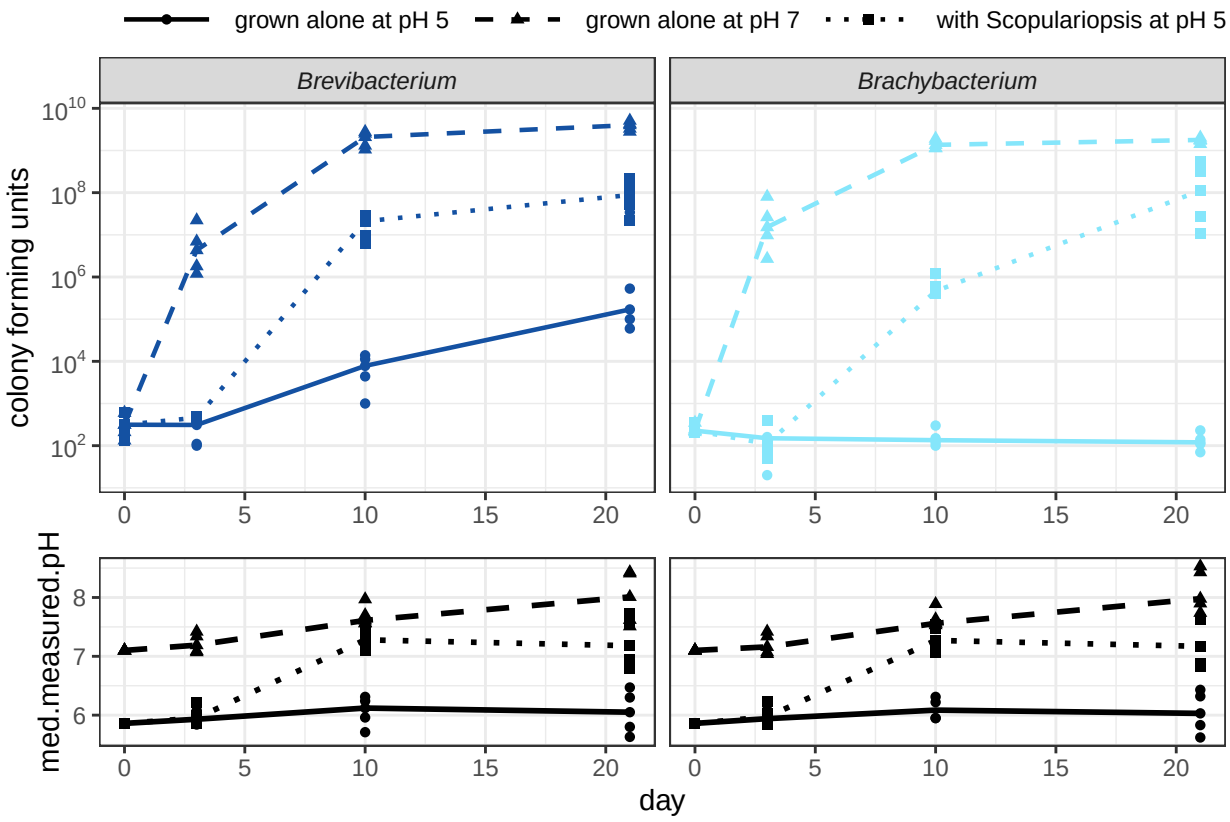
Growth on CCA pH 5 in coculture with *Candida* is very similar to growth alone on CCA pH 7. Suggests that deacidification might be the main stimulating mechanism by *Candida*

Brevi/Brachy + S. xylosus



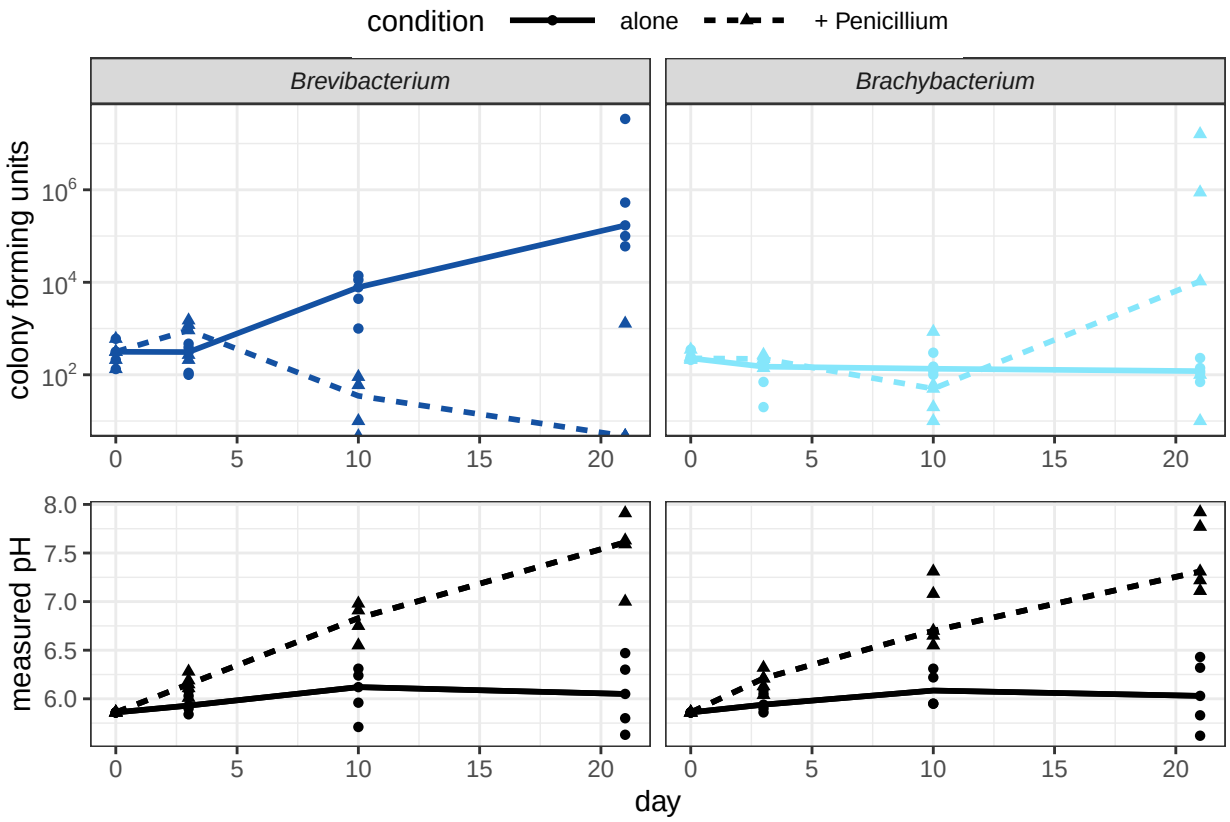
Coculture with *S. xylosus* is not quite as powerful in stimulating Actinobacterial growth, but neither is the deacidification as strong/quick as in coculture with *Candida*

Brevi/Brachy + Scopulariopsis



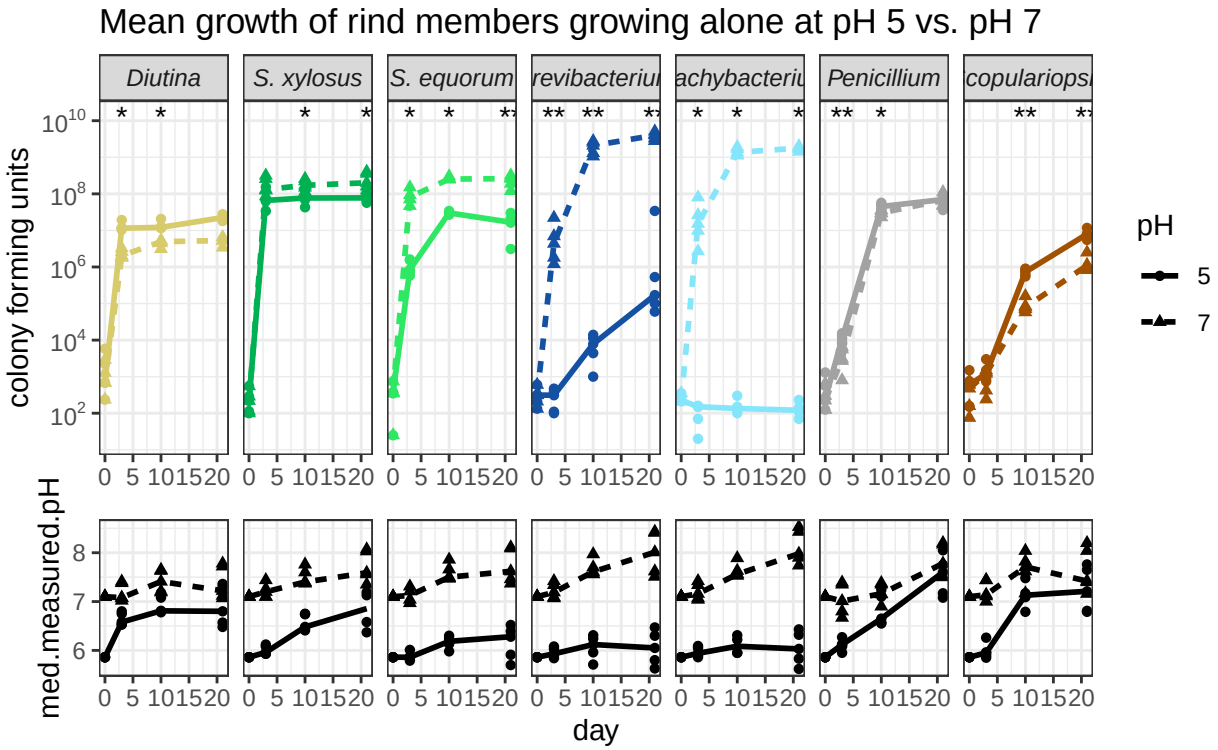
Similar story for cocultures with *Scopulariopsis* as for *S. xylosus*: slower deacidifcation correlates with slower stimulation of Actinobacterial growth.

Brevi + Penicillium

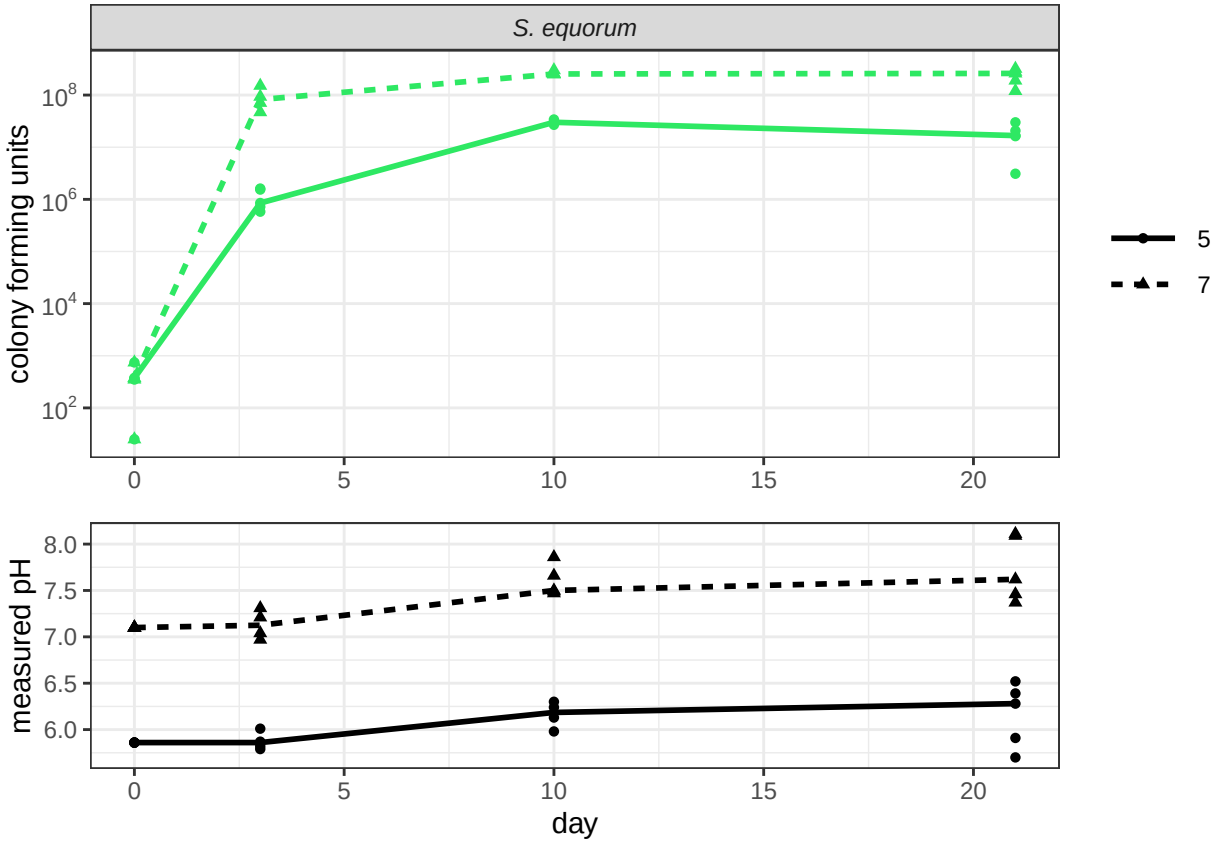


pH affects rind members' growth

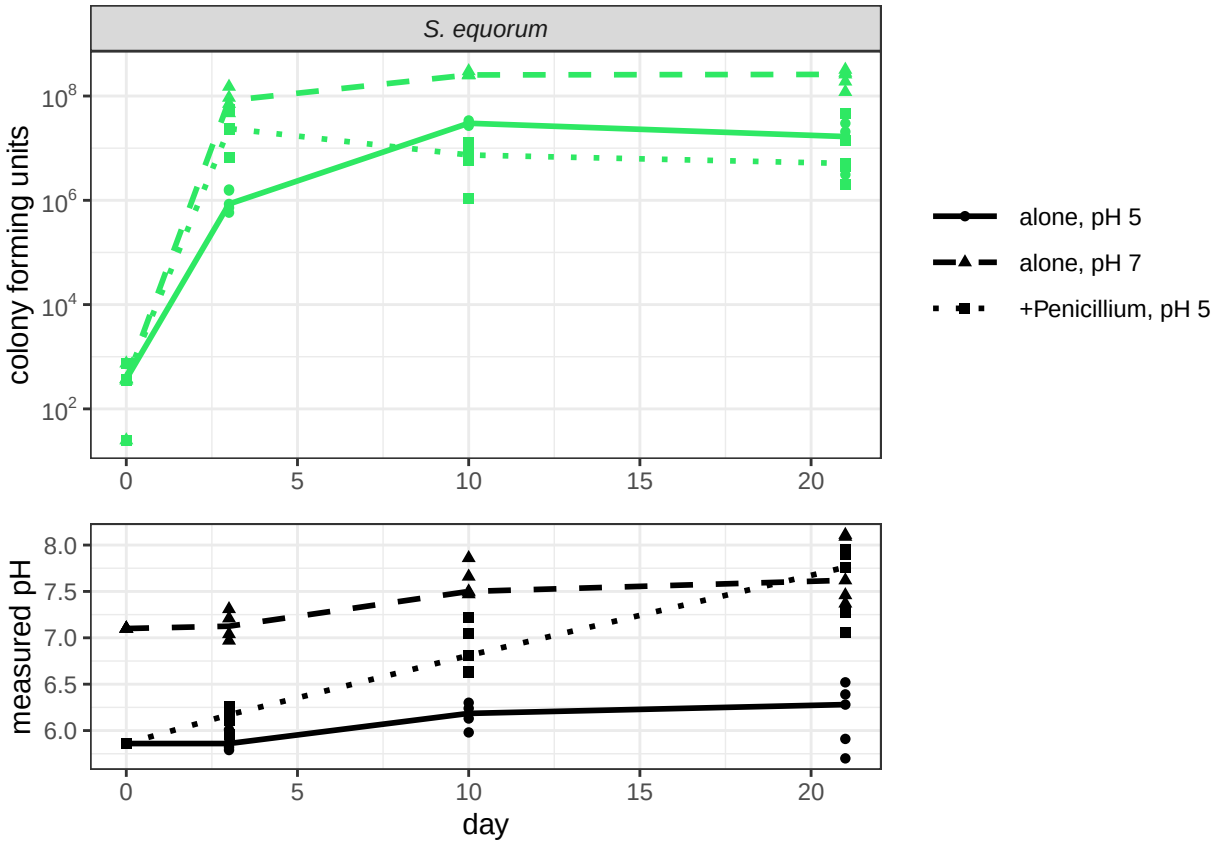
Fungi are generally inhibited and bacteria stimulated by a neutral vs. acidic pH



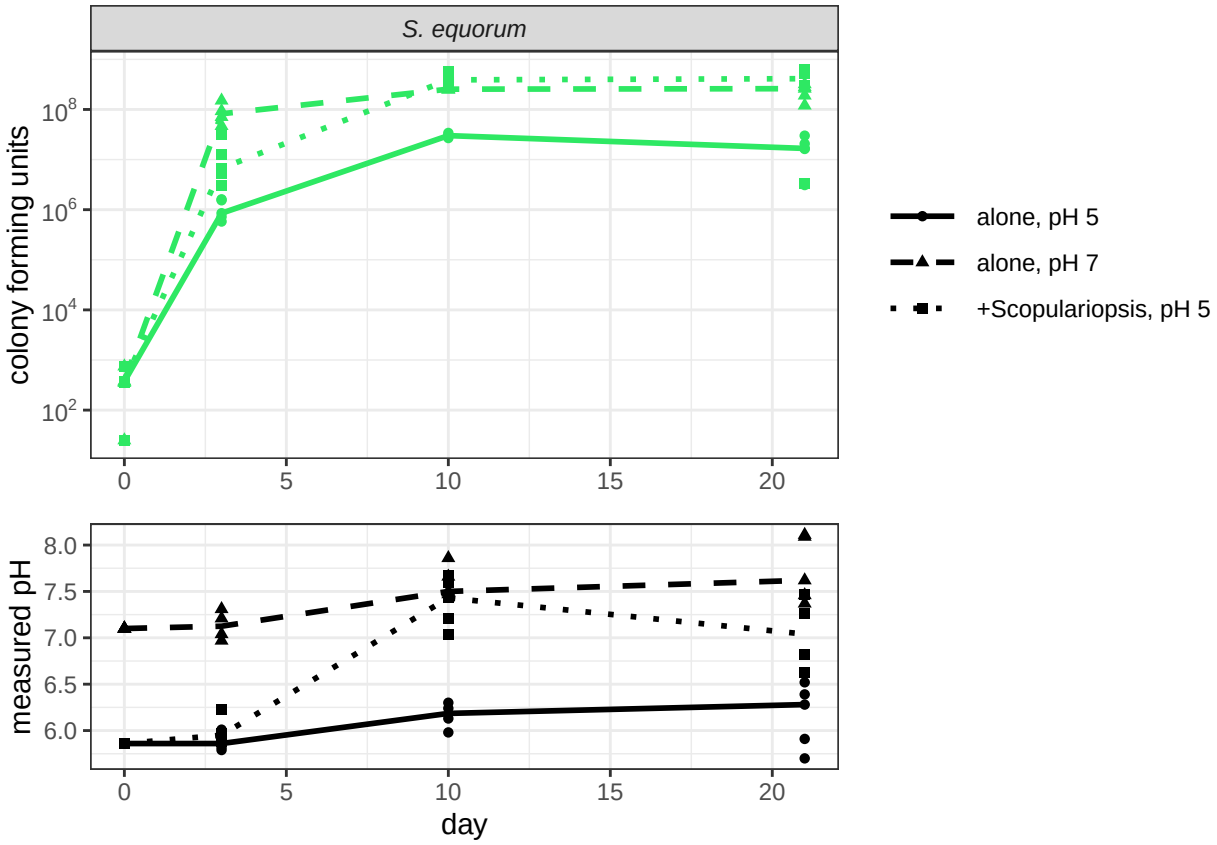
***S. equorum* also demonstrates improved growth at neutral pH compared to acidic.**



S. equorum* in coculture with *Penicillium

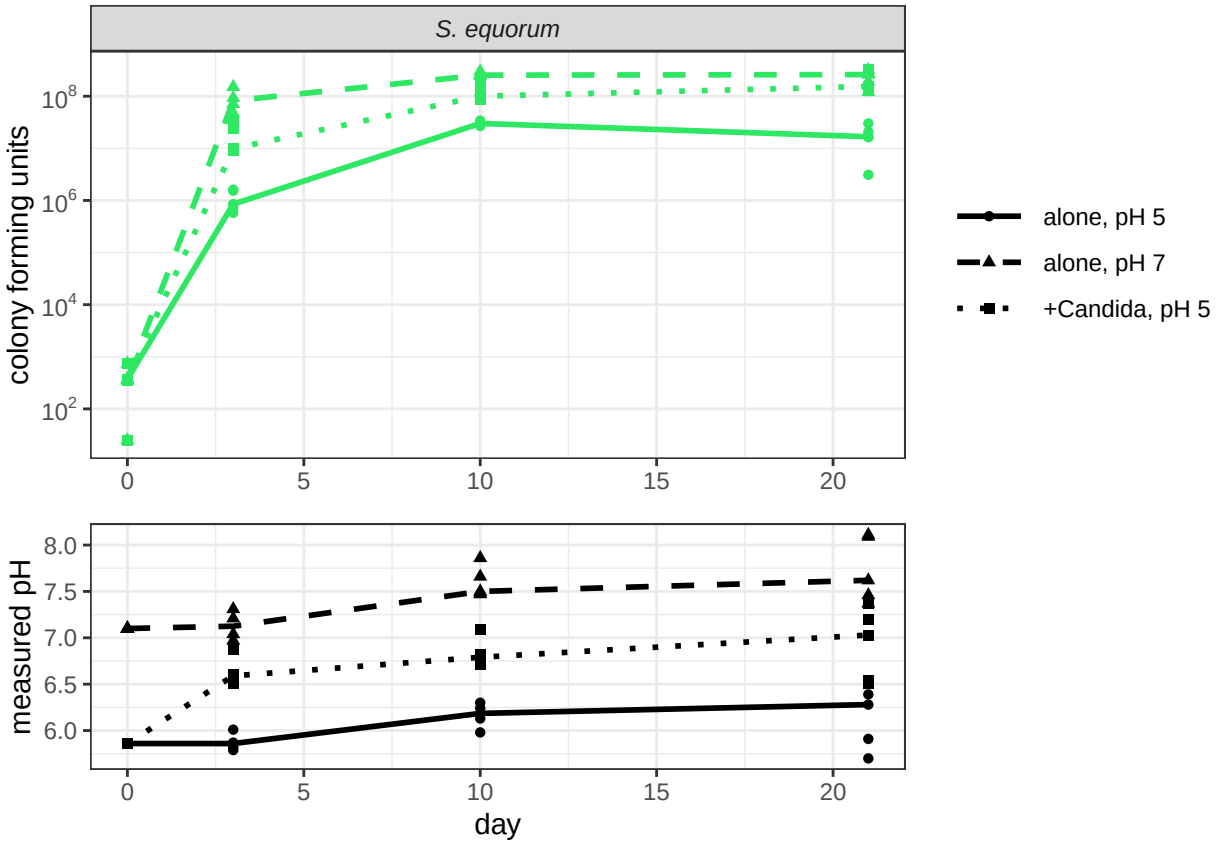


S. equorum* in coculture with *Scopulariopsis



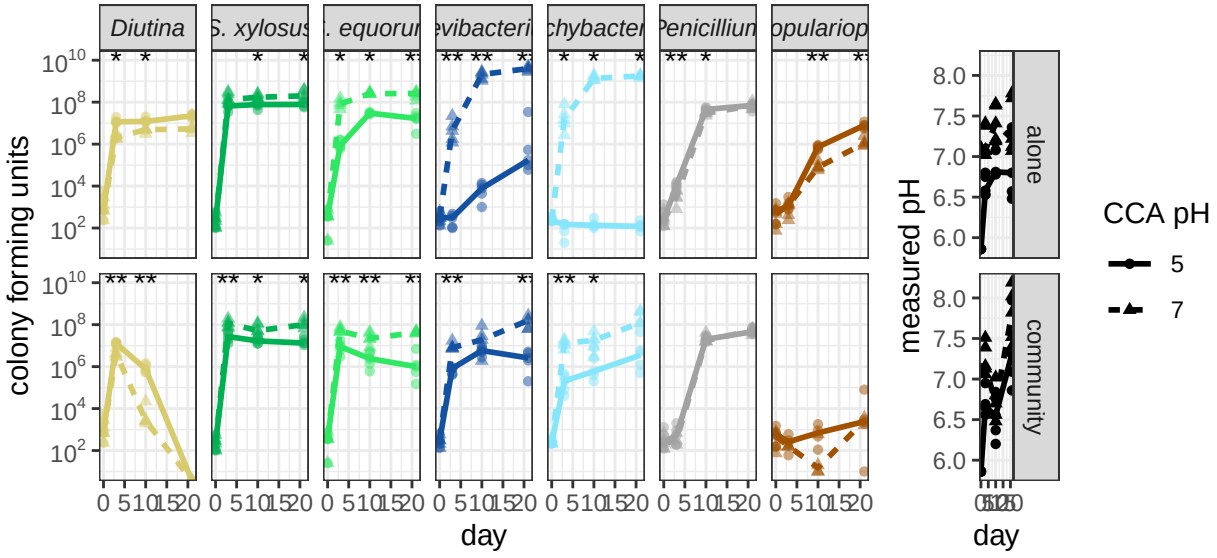
Should be noted that these results are in line with the Wolfe lab paper showing that *Scopulariopsis* can stimulated *S. equorum* growth, potentially through cross-feeding iron scavenging molecules.

S. equorum* in coculture with *Candida



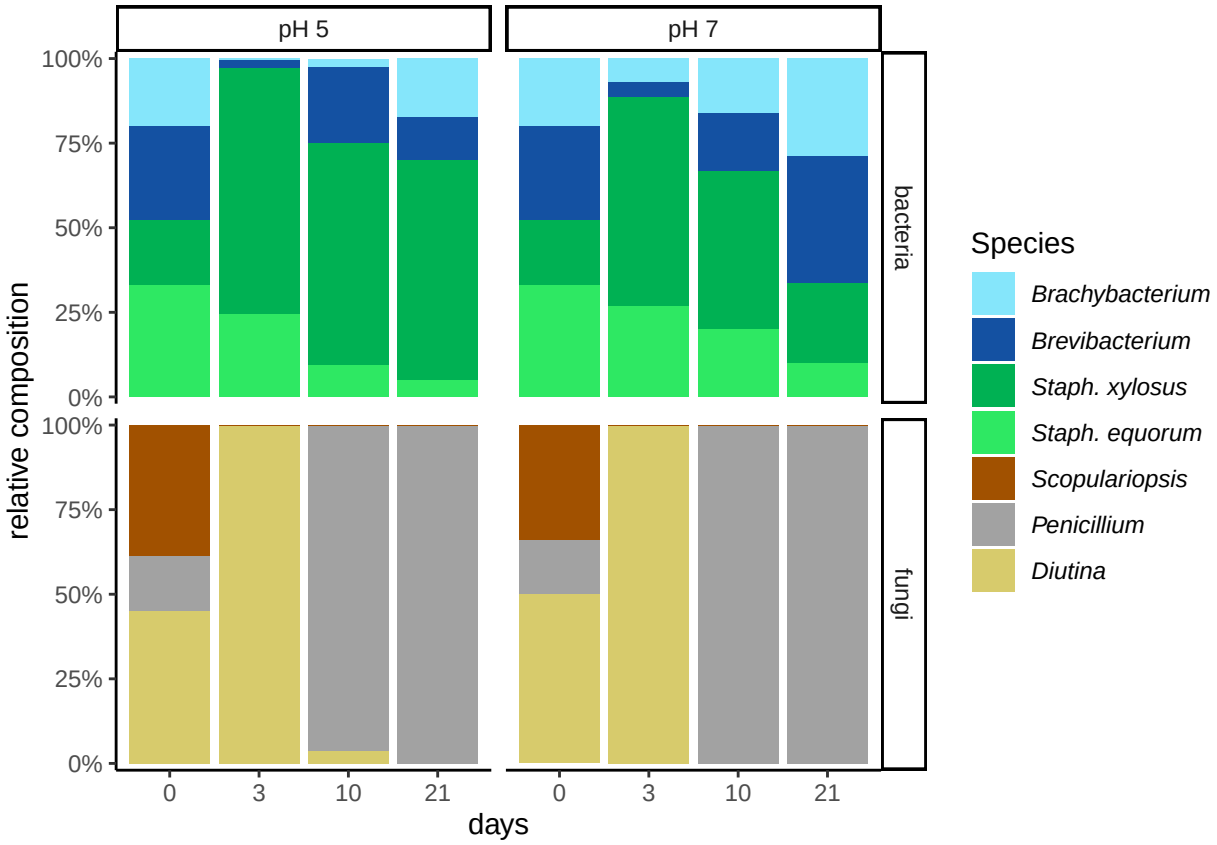
Adjusting starting pH changes absolute growth of most species in community

Overall, bacteria grow better at pH 7 than pH 5 (prefer nettural starting pH), and fungi tend to grow worse or the same at pH 7 compared to pH 5 (slight preference acidic start)



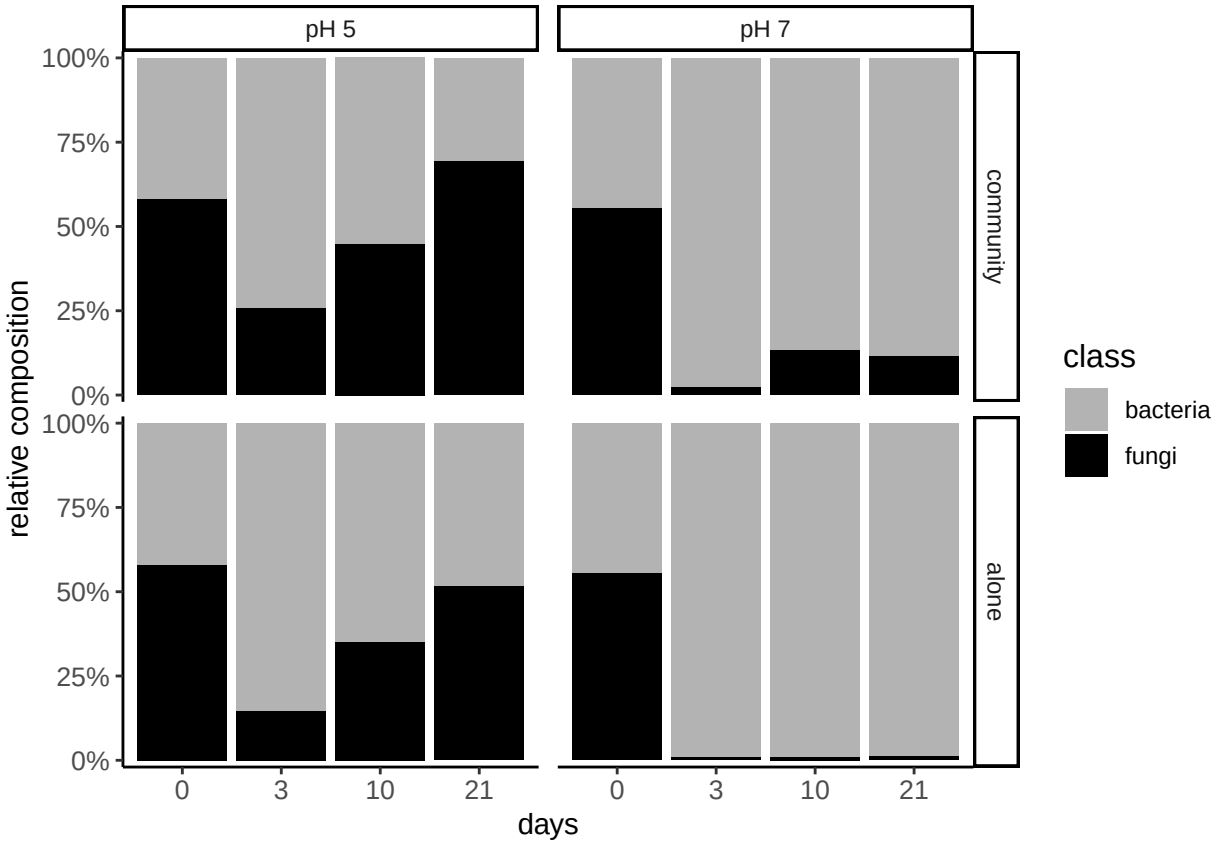
Successional patterns

despite improvement in Actinobacterial growth at pH 7, pattern of succession doesn't qualitatively change between pH 5 & pH 7. While Actinobacteria *do* pop up at higher abundance a bit earlier in the pH 7 condition, there still exists two phases of bacterial growth, first dominated by *Staphylococcus* species and then later arrival of Actinobacteria as a significant proportion of the community.



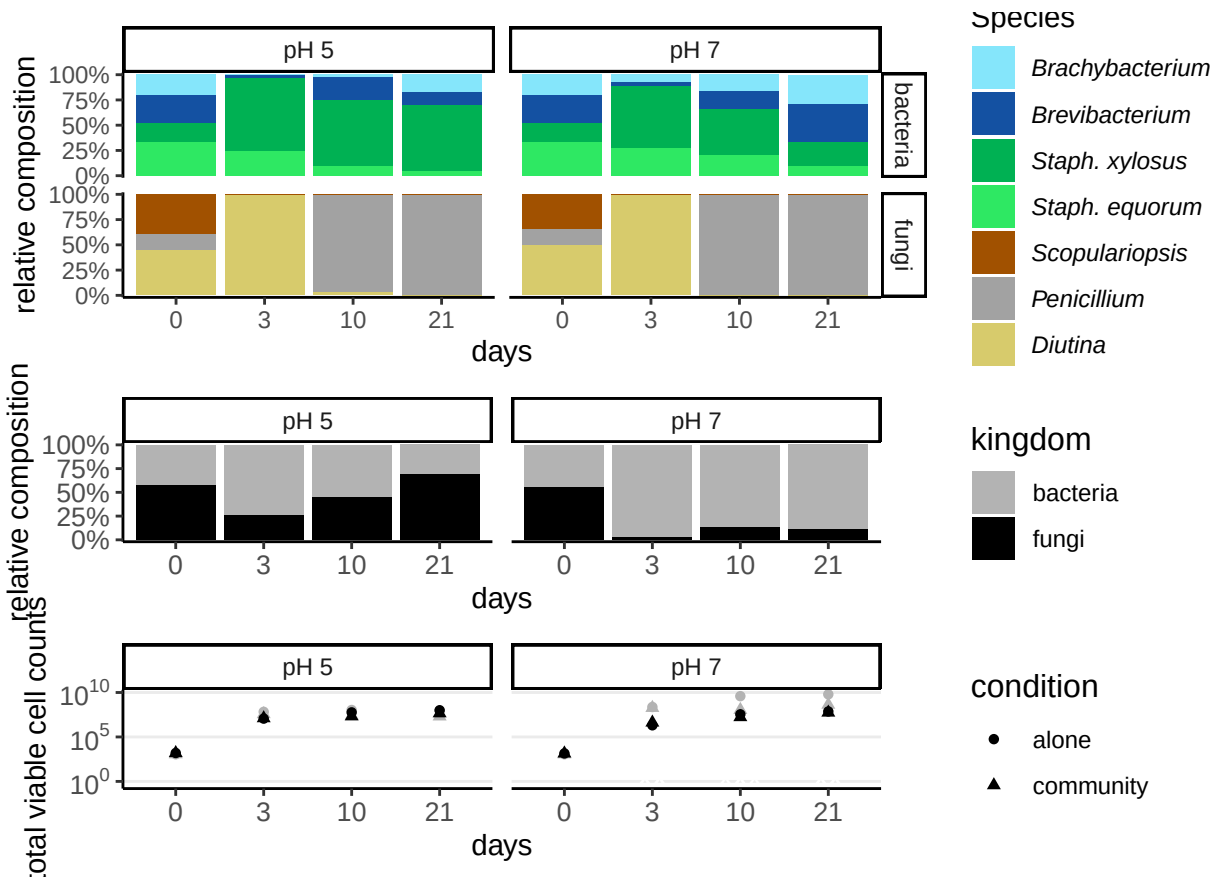
inter-kingdom successional pattern: fungi-vs-bacteria

Pattern of succession at kingdom level on CCA pH 5 vs. pH 7



Fungi are far less abundant in communities started on pH 7 medium. Not entirely surprising given the increased growth of all bacteria when grown on pH 7. However, at both pH 5 and pH 7, the proportion of fungi in the community is higher than what would be predicted from alone growth, suggesting that the community has something of a balancing effect on kingdom-level abundance.

Let's plot colony forming units as well – are there more fungi in communities than when grown alone? Here's a nice plot summarizing all the pH 5 vs. pH 7 dynamics:



So here we see that fungi aren't increased in population in communities, but rather just that bacteria are reduced in communities, leading to the effective increases in fungal relative abundance.

So let's check out why bacteria might be so inhibited in communities!

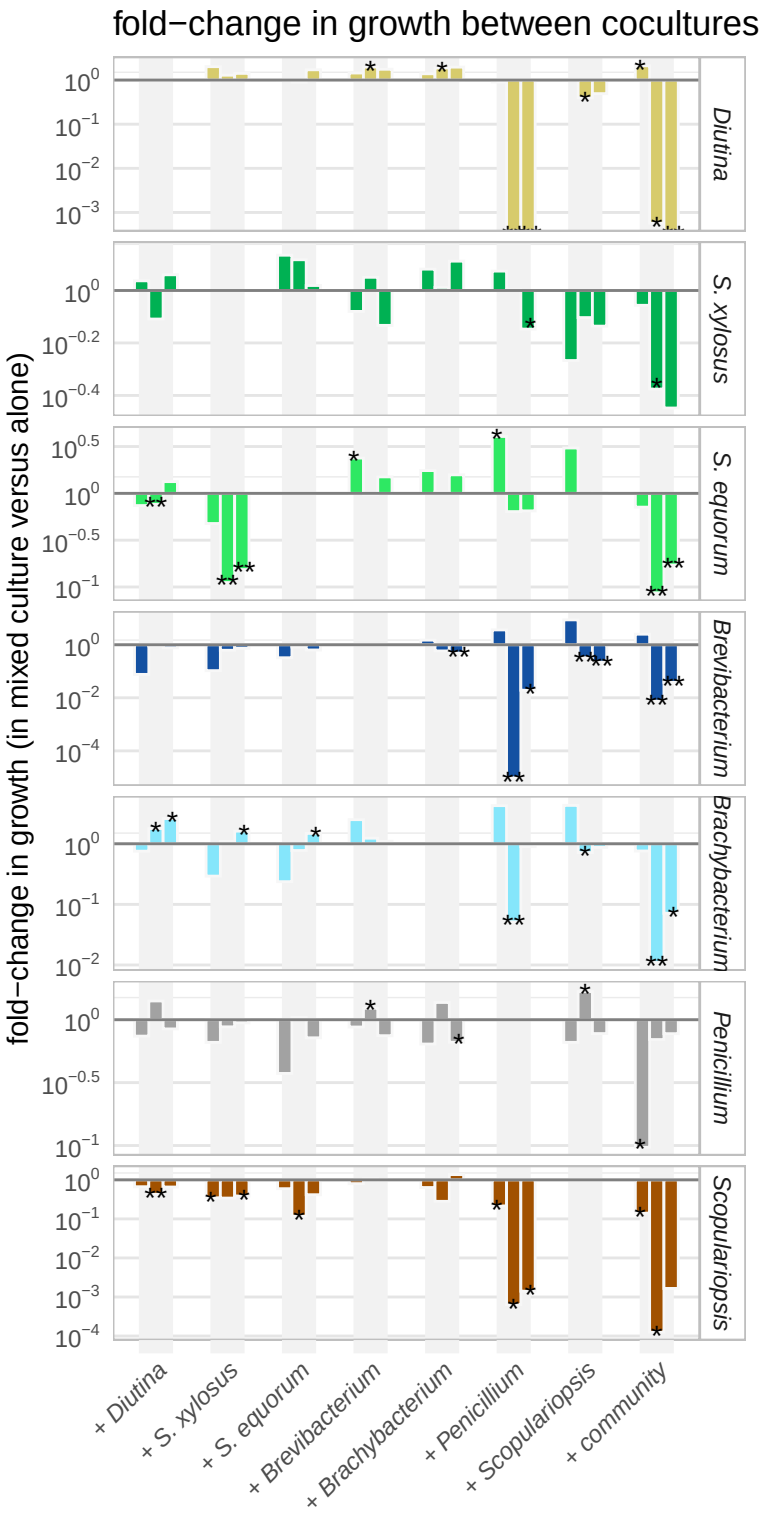
Inhibitory forces in community

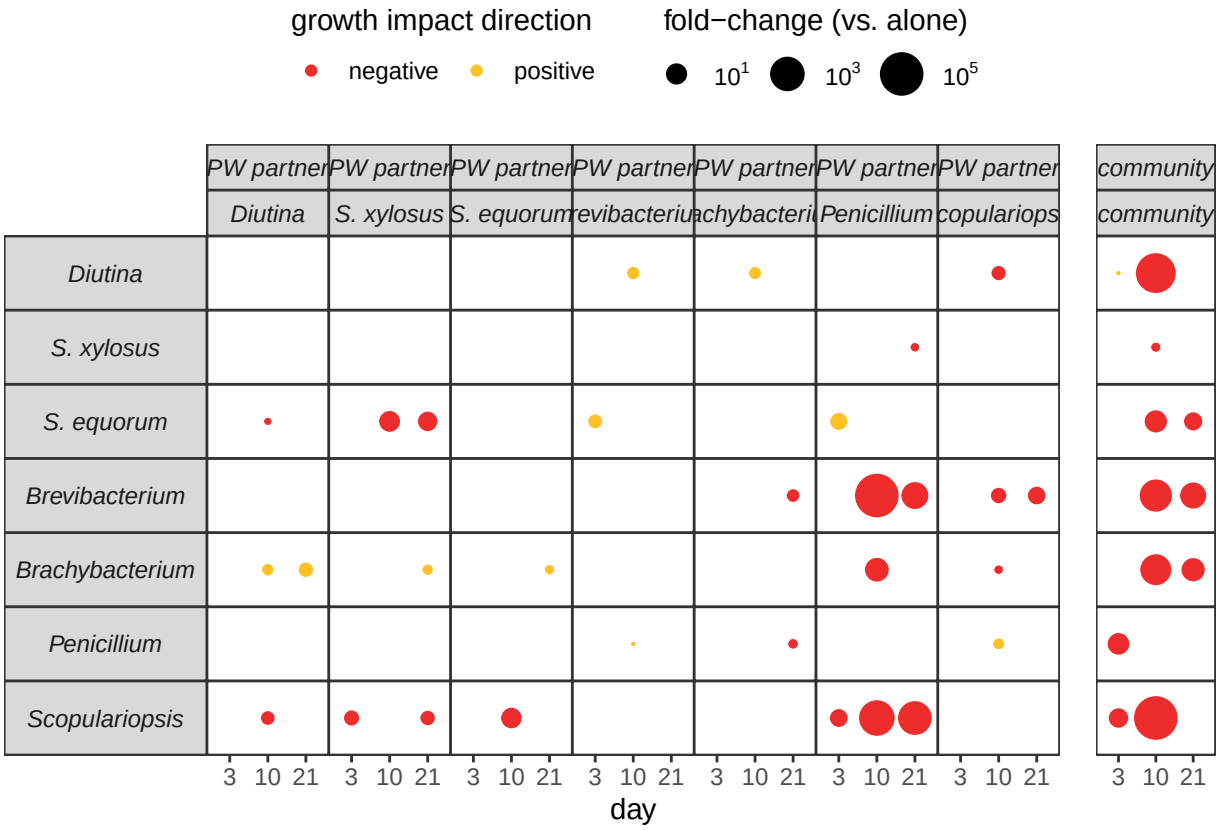
Earlier we showed that there are a lot of pairwise interactions that are inhibitory, mostly coming from *Penicillium* as a partner.

While running experiments at pH 5 showed both stimulatory and inhibitory forces in the community, we were curious what we would see when we removed deacidification dynamics from the community.

Unfortunately, we had little luck with buffering media – increased amounts of MES buffer ended up somehow liquifying the cheese curd agar, and still it wasn't an effective enough buffer to counter microbial deacidification.

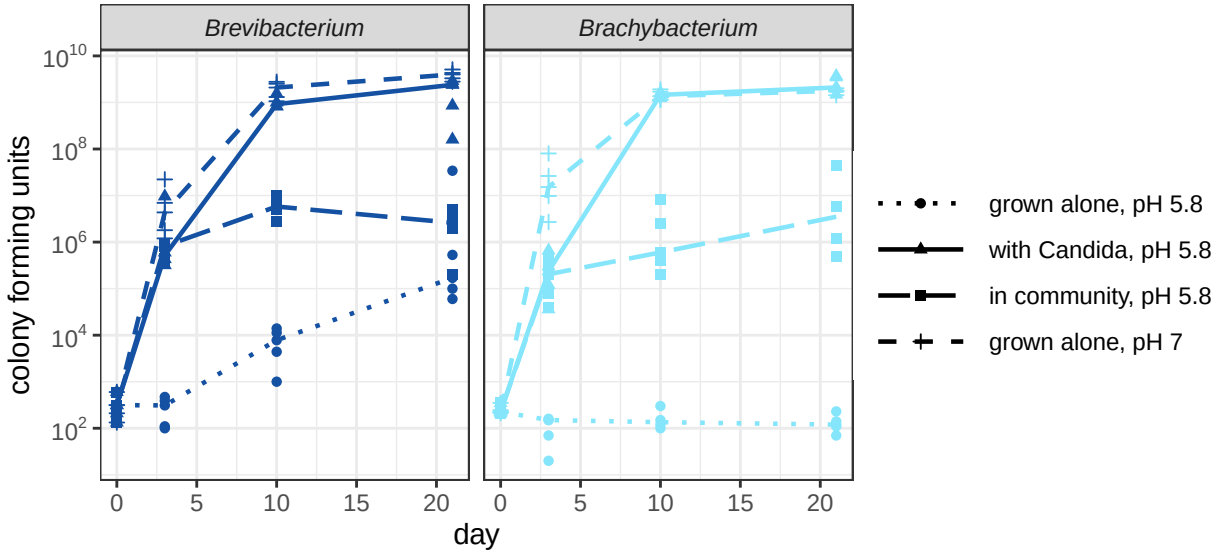
So instead we ran experiments at pH 7, to understand what's happening in the community when deacidification isn't a factor.


fold−change in growth between cocultures
fold−change in growth (in mixed culture versus alone)
Diutina
S. xylosus
S. equorum
Brevibacterium
Brachybacterium
Penicillium
Scopulariopsis
+ Diutina
+ S. xylosus
+ S. equorum
+ Brevibacterium
+ Brachybacterium
+ Penicillium
+ Scopulariopsis
+ community

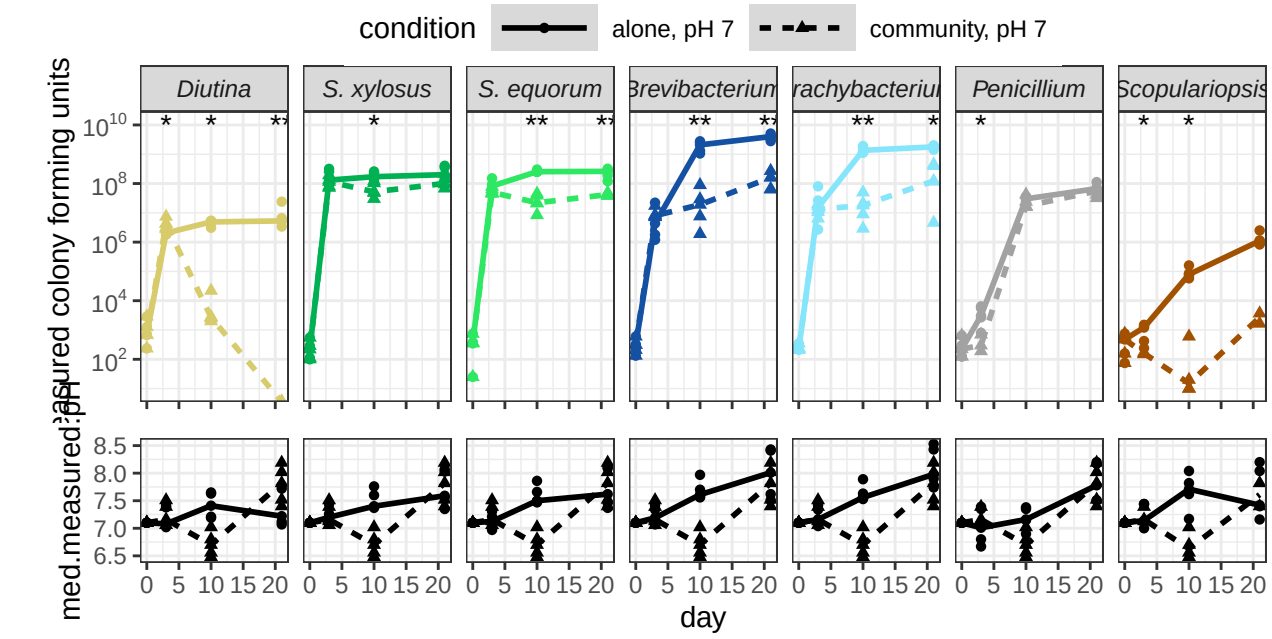


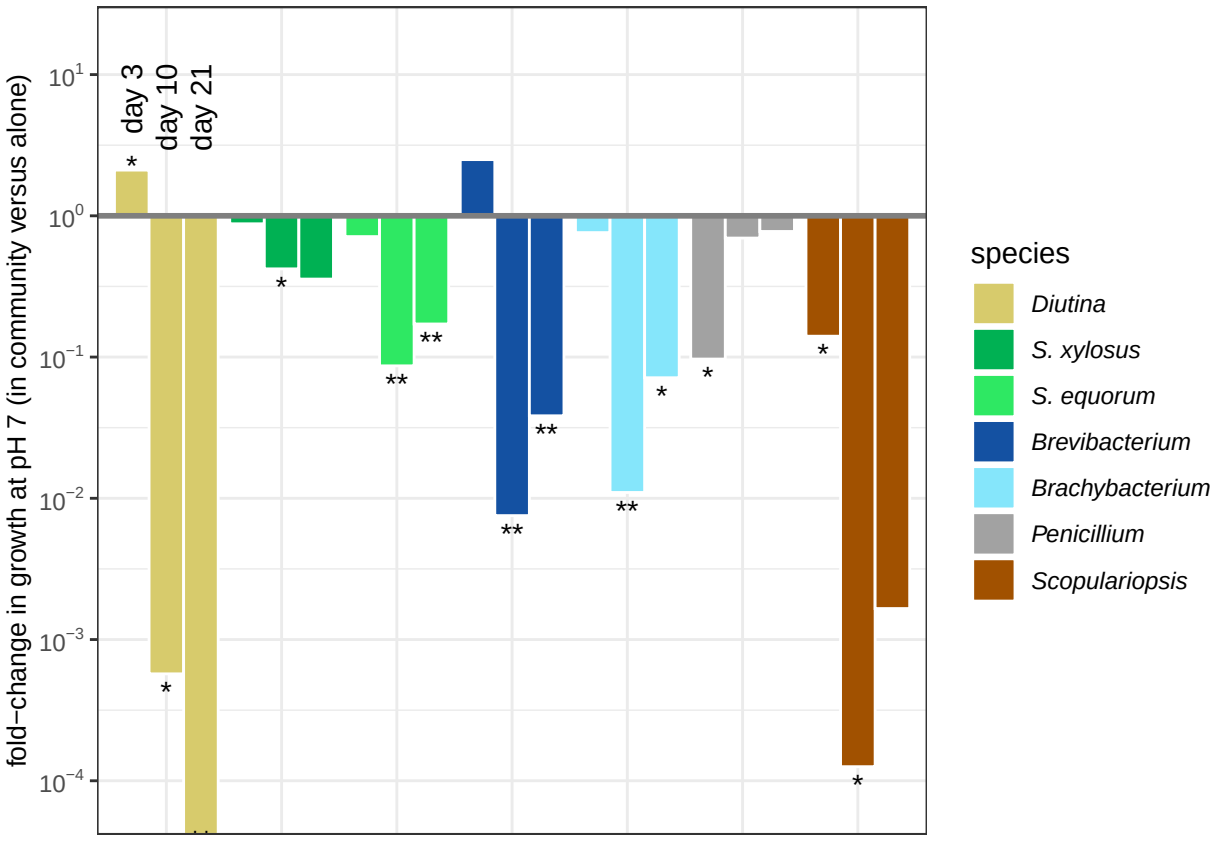
So from both of these, we're really just seeing a lot of strong inhibitory interactions now.

adjusting the pH overaccounts for growth in the community, despite deacidification past pH 7.

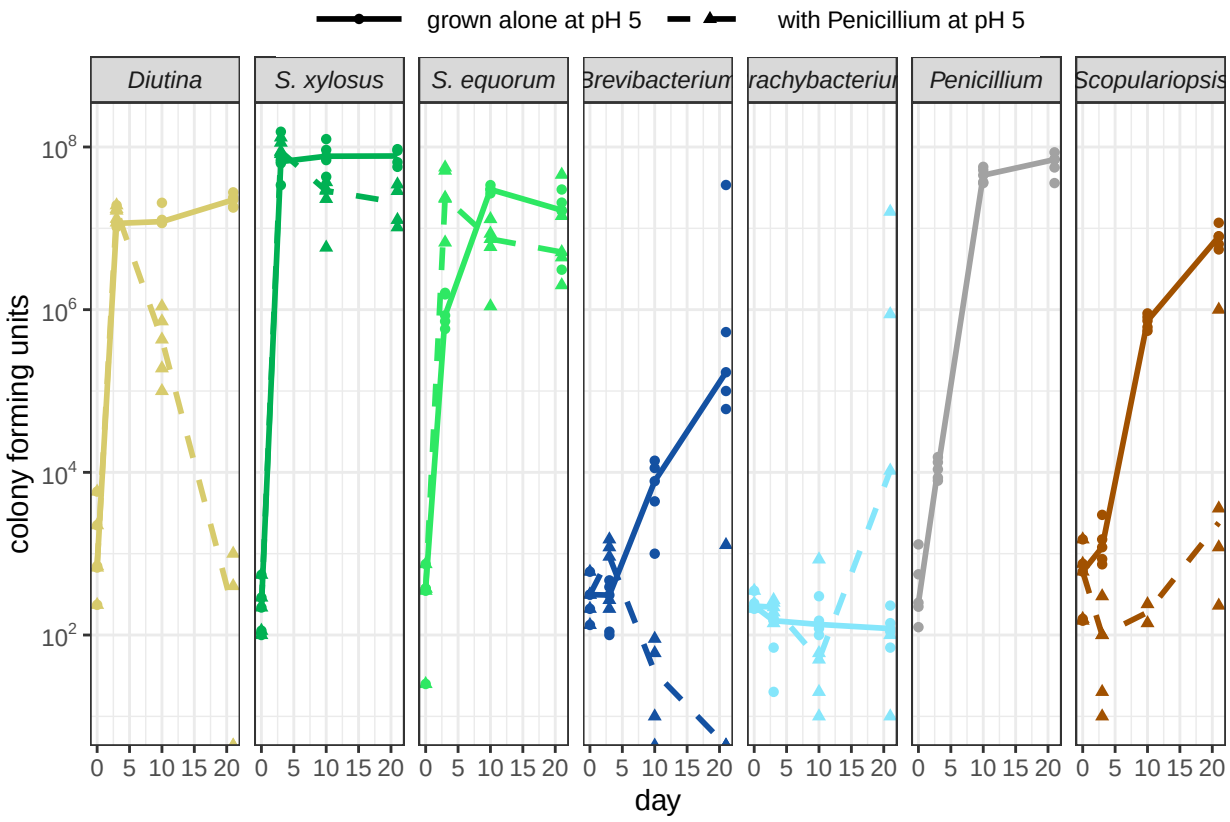


There are suppressive forces across the board in the community

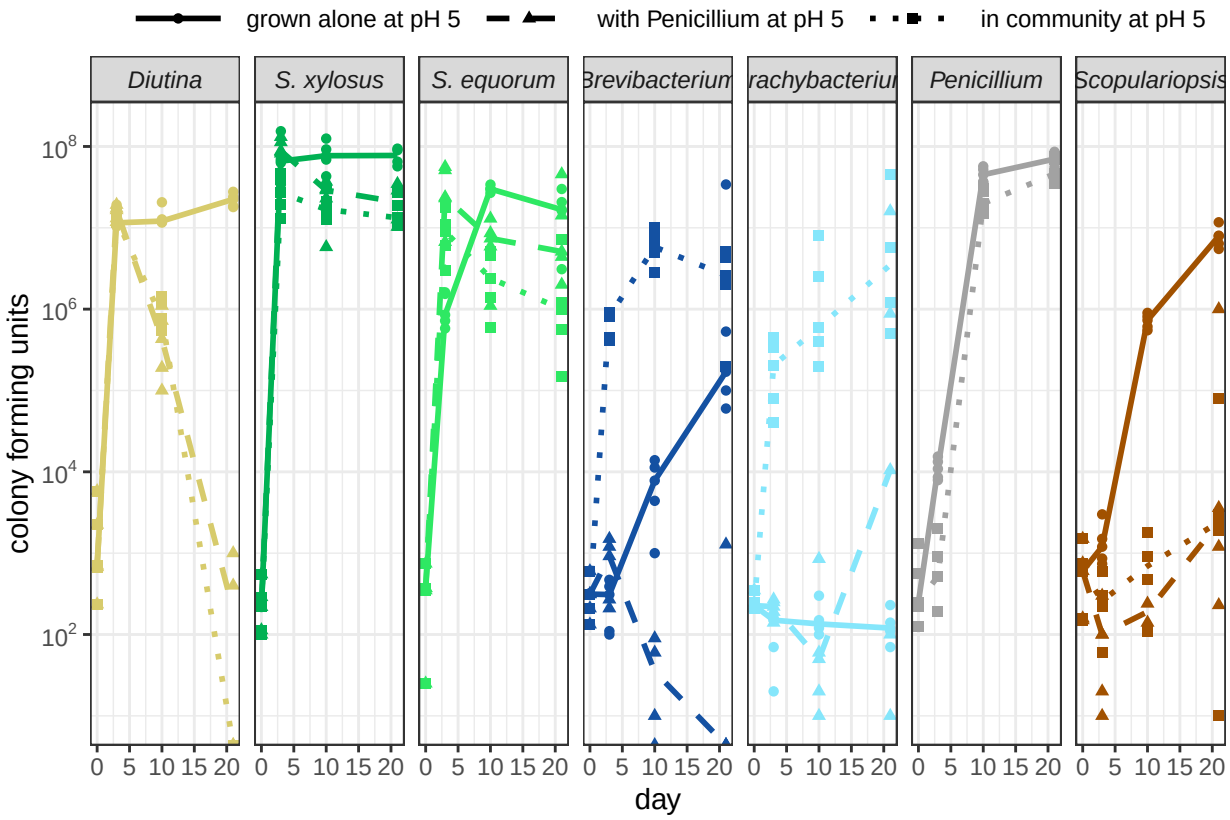




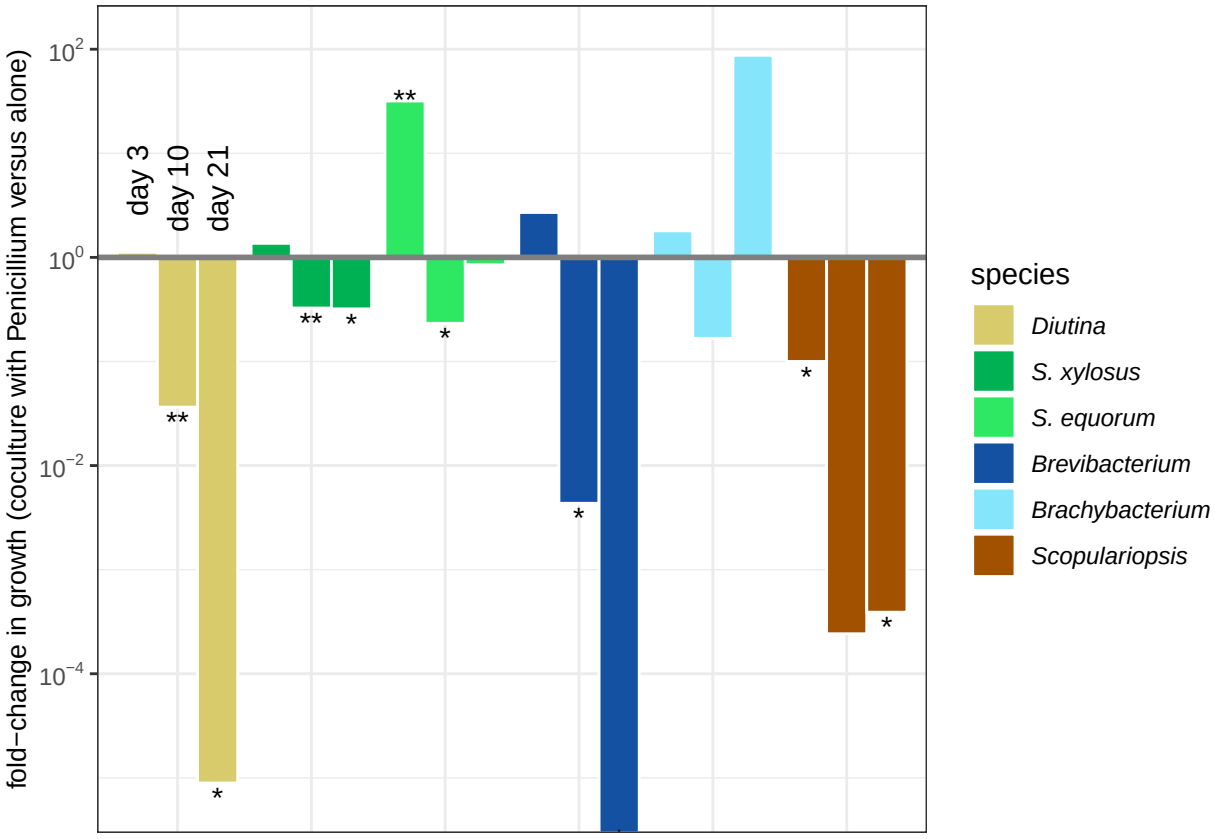
JBC is a major suppressor



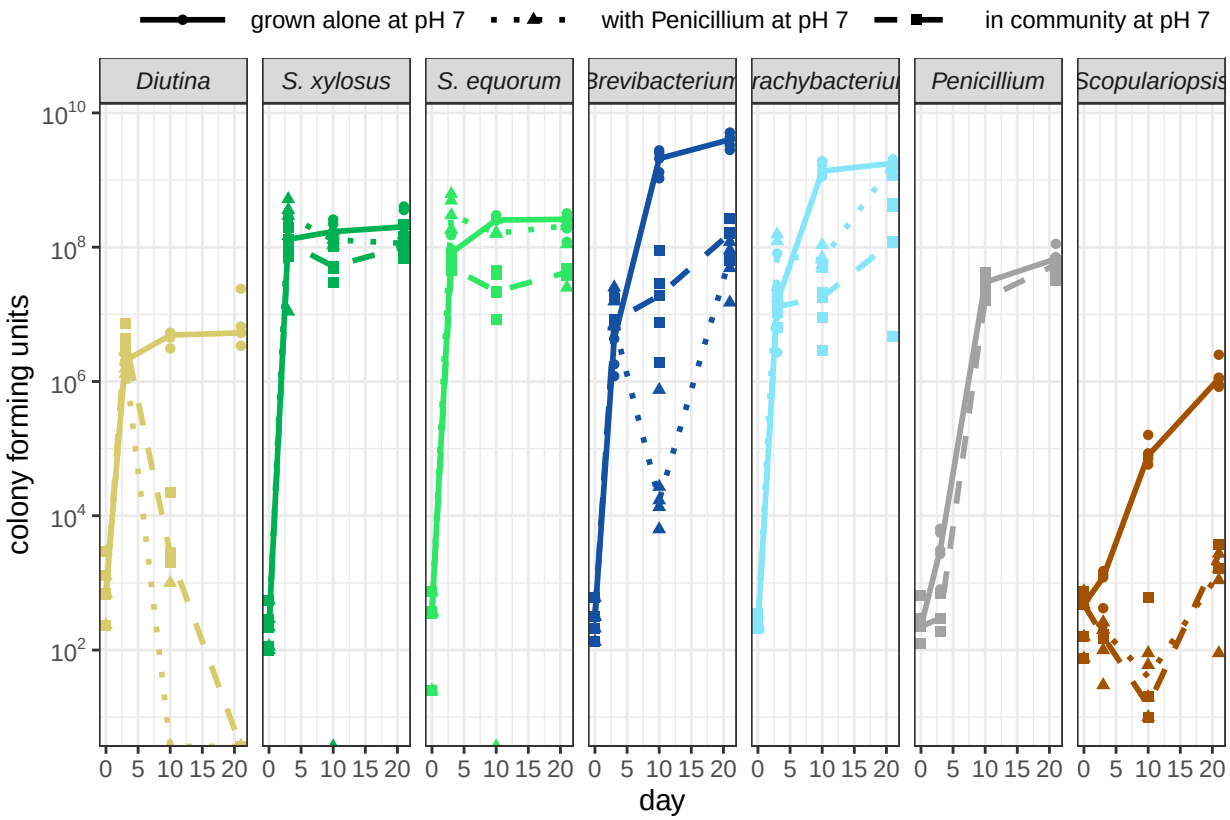
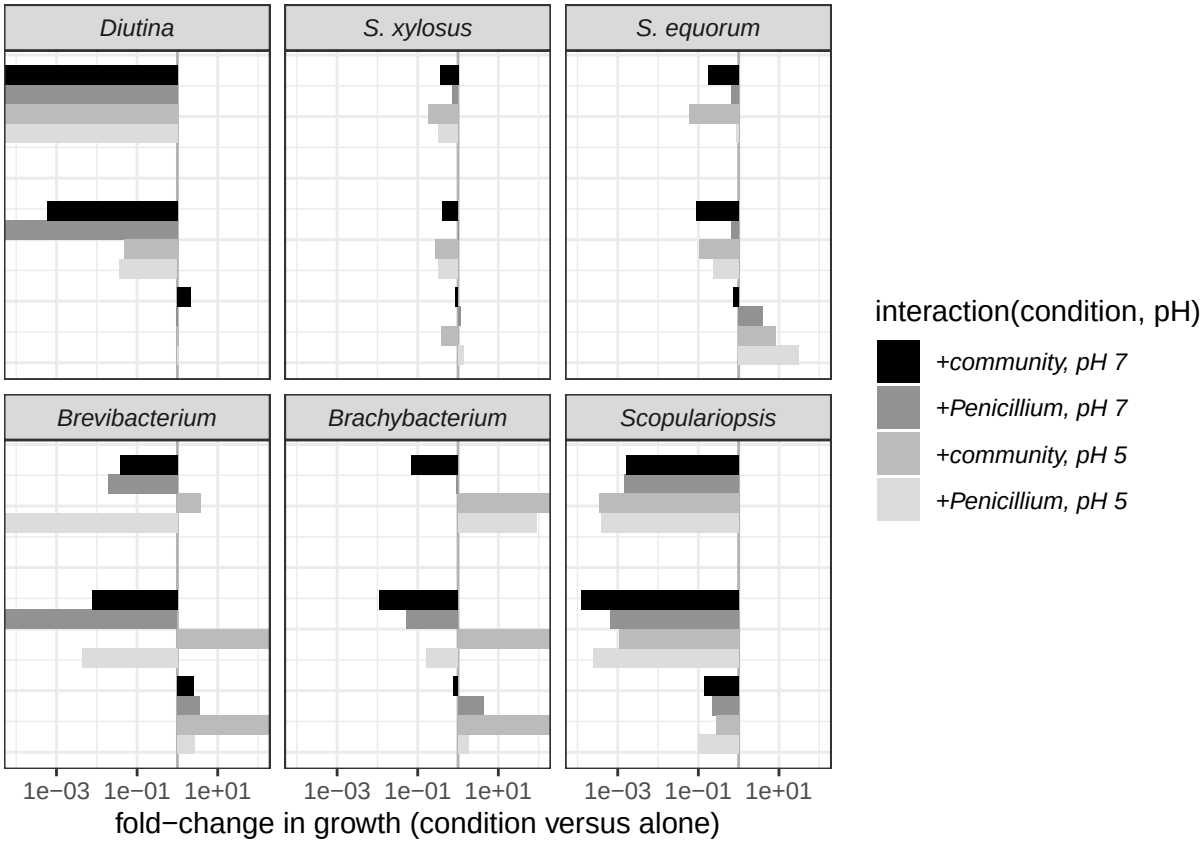
Pairwise inhibition is comparable to what is seen in the community.



If we look at the effect of *Penicillium* in pairwise coculture on CCA pH 5, we also see lots of significant inhibition



Pattern of inhibition by *Penicillium* holds on CCA pH 7



Penicillium suppression: different levels in different conditions