

Spatial distribution paper - Section 1

Rudolf Schlechter

Taxon-specific population density changes correlate with community complexity

```
data_cfu %>% head
```

```
##   exp sample   dpi synID comID syncom strain          taxa channel    cfu
## 1   e1      1 07dpi    C Com01   C.01  meL85 Methylobacterium    C0 8800000
## 2   e1      2 07dpi    C Com01   C.01  meL85 Methylobacterium    C0 15400000
## 3   e1      3 07dpi    C Com01   C.01  meL85 Methylobacterium    C0 19900000
## 4   e1      4 07dpi    C Com01   C.01  meL85 Methylobacterium    C0 9040000
## 5   e2      1 07dpi    C Com01   C.01  meL85 Methylobacterium    C0 769000
## 6   e2      2 07dpi    C Com01   C.01  meL85 Methylobacterium    C0 2660000

##   cfu_log
## 1      6.9
## 2      7.2
## 3      7.3
## 4      7.0
## 5      5.9
## 6      6.4
```

```
linear_cfu = lm(cfu_log ~ synID + dpi + taxa, data_cfu)
```

```
# Shapiro-Wilk test for normality
```

```
cfu_normality = shapiro.test(rstandard(linear_cfu))
```

```
# Breusch-Pagan test for homogeneity of variances
```

```
cfu_homoskedasticity = ncvTest(linear_cfu)
```

We first investigated the effect of community complexity on bacterial population densities from two bacterial taxa (*Methylobacterium* and *Sphingomonas*) *in planta* using a full factorial design (Fig. 1). Bacterial densities were determined for each strain at different community complexities (C = near isogenic control; S2 = two-species SynCom; S3 = three-species SynCom) after 7 and 14 days post-inoculation (dpi).

We used non-parametric methods to analyse the CFU data, considering violation of normality (Shapiro-Wilk test, $W = 0.94$, $p = 3.67 \times 10^{-19}$) and homogeneity of variance (Breusch-Pagan test, $X^2 = 31.1$, $p = 2.45 \times 10^{-8}$)

```
# Kruskal-Wallis test and effect size for community complexity (synID)
kw_synID = kruskal.test(cfu_log ~ synID, data_cfu) %>% tidy %>%
  mutate(p_label = case_when(p.value < 0.05 ~ "< 0.05", TRUE ~ as.character(p.value)))
keff_synID = kruskal_effsize(formula = cfu_log ~ synID, data = data_cfu, ci=TRUE, nboot=100)

# Dunn's Test
dunn_synID = dunn_test(cfu_log ~ synID, p.adjust.method = "holm", data=data_cfu) %>% tibble %>%
  mutate(p_label = case_when(p.adj < 0.05 ~ "< 0.05", TRUE ~ as.character(p.adj)))

# Fold change of population density by SynCom complexity (synID)
fc_cfu_synID = data_cfu %>%
  group_by(synID) %>%
  summarise(median_cfu = median(cfu)) %>%
  mutate(FC = median_cfu/median_cfu[1],
         logFC = log2(FC))
```

We tested how the community complexity influenced changes in individual bacterial populations. Community complexity had a large effect on bacterial populations (Kruskal-Wallis, $H(2) = 307.02$, $p = < 0.05$, $\eta^2(2) = 0.34$ [0.29-0.4]). This was reflected as a significant 2.29-fold increase in population densities composed of two-species communities ($Z = 4.48$, $p < 0.05$), and a pronounced 4.71-fold decrease in S3 ($Z = 8.62$, $p < 0.05$), both compared to C.

(Fig. 3a): CFU vs SynCom

```
# Wilcoxon test and effect size for sampling time (dpi)
w_dpi = wilcox.test(formula = cfu_log ~ dpi, data = data_cfu) %>% tidy %>%
  mutate(p_label = case_when(p.value < 0.05 ~ "< 0.05", TRUE ~ as.character(p.value)))
```

```
weff_dpi = wilcox_effsize(formula = cfu_log ~ dpi, data = data_cfu, ci=TRUE, nboot=100)

# Fold change of population density by time of sampling (dpi)
fc_cfu_dpi = data_cfu %>%
  group_by(dpi) %>%
  summarise(median_cfu = median(cfu)) %>%
  mutate(FC = median_cfu/median_cfu[1],
         logFC = log2(FC))
```

Changes in population density could be related to temporal changes. Thus, we evaluated how population density changed over the two times of sampling, 7 dpi and 14 dpi. Here we observed a small yet significant effect of time of sampling (Wilcoxon, $Z = 84973$, $p = 3.21 \times 10^{-6}$, $r = 0.15$ [0.07-0.21]). This represented an increase in 1.69-fold between 7 and 14 dpi.

```
# Wilcoxon test and effect size for bacterial group (taxa)
w_taxa = wilcox.test(formula = cfu_log ~ taxa, data = data_cfu) %>% tidy %>%
  mutate(p_label = case_when(p.value < 0.05 ~ "< 0.05", TRUE ~ as.character(p.value)))
weff_taxa = wilcox_effsize(formula = cfu_log ~ taxa, data = data_cfu, ci=TRUE, nboot=100)

# Fold change of population density by bacterial group (taxa)
fc_cfu_taxa = data_cfu %>%
  group_by(taxa) %>%
  summarise(median_cfu = median(cfu)) %>%
  mutate(FC = median_cfu/median_cfu[1],
         logFC = log2(FC))
```

We wanted to test whether the observed change in population density was associated to specific bacterial taxa. Consequently, we observed a difference between populations that belonged to *Methylobacterium* and *Sphingomonas* (Wilcoxon, $Z = 49825.5$, $p = < 0.05$, $r = 0.42$ [0.36-0.48]). The population densities of *Sphingomonas* were 5.11 times larger than that of *Methylobacterium*.

Within *Sphingomonas*, SmFR1 consistently increased population sizes in S2, regardless of the presence of a second species, which was observed to a lesser extent in S3 (Fig. Sup). For SpFA2, while population sizes increased in S2, they generally decreased lightly in S3 (Log2FC = -1.31331028731873 – -2.17640903501251))

and S3 communities compared to C, while SpFA2 increased in S2 but decreased lightly in S3.

By contrast, all *Methylobacterium* species (MeL85, MeL92, and Mr0-1) consistently decreased in population sizes in S3. Within *Methylobacterium*, MeL92 and Mr0-1 benefited in S2 communities while MeL85 was the most susceptible species to decrease in population size.

Fig.3b: Fold change taxa

```
plt1 <- inner_join(dunntest_taxa_dpi, fold_taxa_dpi, by = c("taxa", "dpi", "synID")) %>%
  filter(group1 == "C") %>%
  ggplot(aes(x=synID, y=taxa))+
  facet_wrap(~dpi, ncol=2, labeller = labeller(dpi=dpi.lab2))+
  geom_tile(colour= "black", fill= "white", linewidth = 0.1)+
  geom_point(aes(fill = log2FC, size = p_size), shape = 21)+
  coord_fixed()+
  scale_fill_gradientn(name = bquote(Log[2]~"FC"), colours = wes_palette("Zissou1")[c(1,2,3,5)],
    values = c(0,0.55,1), limits=c(-8,4), breaks=seq(-8,4,4), na.value = 'grey90'),
  scale_size_continuous(range = c(15,3), breaks = c(0.05, 0.5, 1), limits = c(0,1),
    label = c("< 0.05", "0.5", "1.0"), name = expression(paste(italic("P"), "-adj"))),
  scale_y_discrete(name="Taxa", labels = taxa.lab)+
  labs(x = "")+
  theme_rs()+
  theme(panel.border = element_blank(),
    axis.text.x = element_text(hjust=0.5, vjust=3),
    axis.text.y = element_text(face="italic"),
    strip.text = element_text(face="plain"))
```

Warning: The `size` argument of `element_rect()` is deprecated as of ggplot2 3.4.0.

i Please use the `linewidth` argument instead.

This warning is displayed once every 8 hours.

Call `lifecycle::last_lifecycle_warnings()` to see where this warning was

generated.

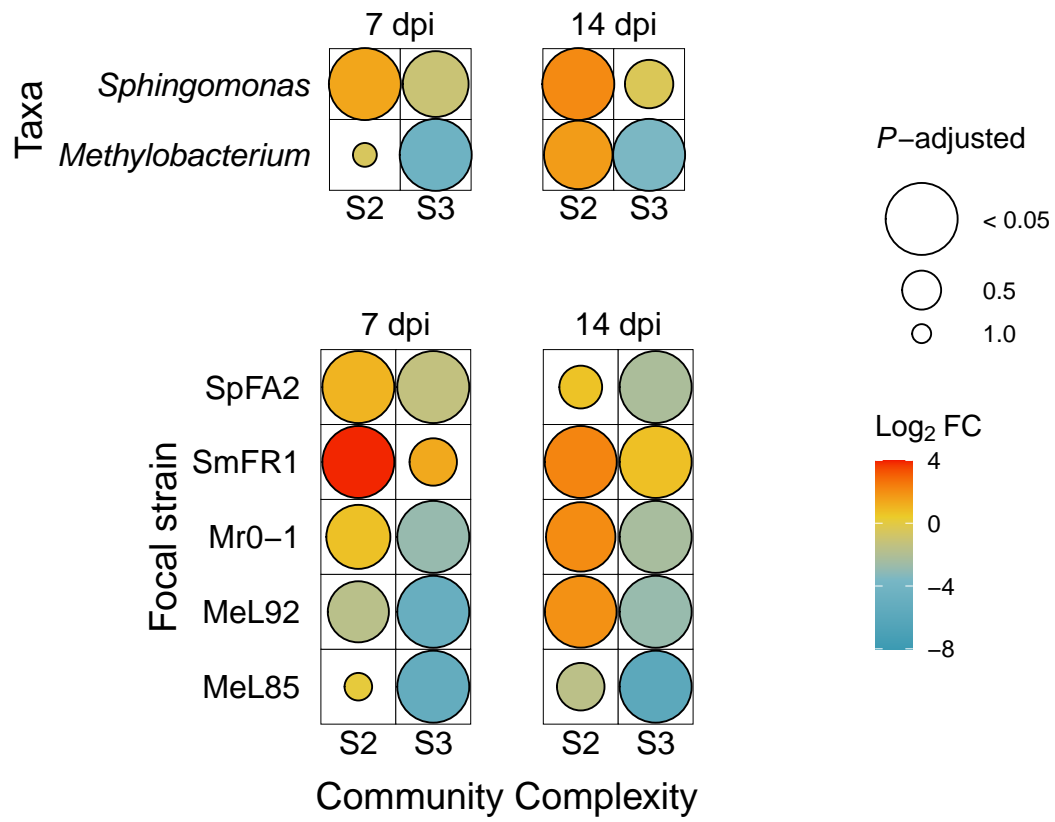
```
plt2 <- inner_join(dunntest_strain_dpi, fold_strain_dpi, by = c("strain", "dpi", "synID")) %>%
  filter(group1 == "C") %>%
  ggplot(aes(x=synID, y=strain))+
```

```

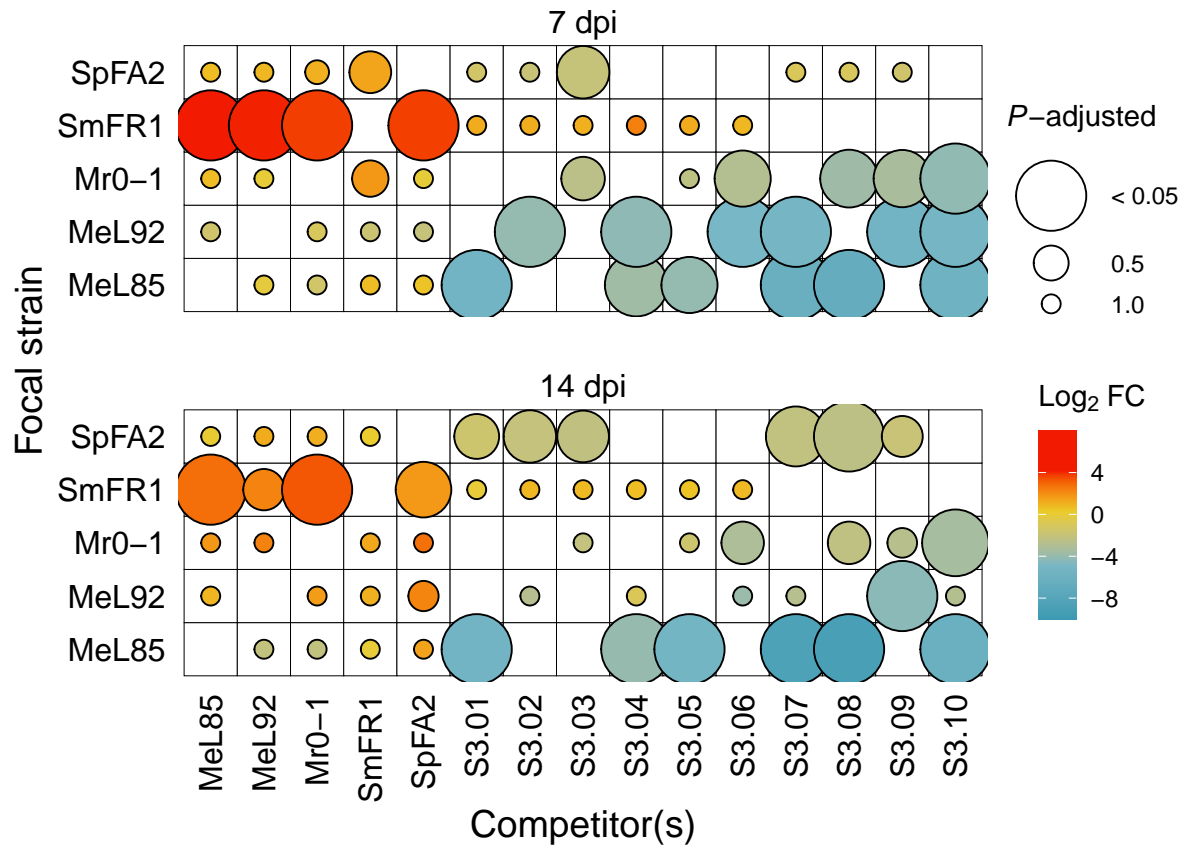
facet_wrap(~dpi, ncol=2, labeller = labeller(dpi=dpi.lab2))+
geom_tile(colour= "black", fill= "white", linewidth = 0.1)+
geom_point(aes(fill = log2FC, size = p_size), shape = 21)+
coord_fixed()+
scale_fill_gradientn(name = bquote(Log[2]~"FC"), colours = wes_palette("Zissou1")[c(1,2,3,5)],
                      values = c(0,0.55,1), limits=c(-8,4), breaks=seq(-8,4,4), na.value = 'grey90')+
scale_size_continuous(range = c(15,3), breaks = c(0.05, 0.5, 1), limits = c(0,1),
                      label = c("< 0.05", "0.5", "1.0"), name = expression(paste(italic("P"), "-adj")))+
scale_y_discrete(name="Focal strain", labels = sp.lab)+
scale_x_discrete(name="Community Complexity", labels = sp.lab)+
theme_rs()+
theme(panel.border = element_blank(),
      axis.text.x = element_text(hjust=0.5, vjust=3),
      strip.text = element_text(face="plain"))+
guides(size = "none")

plt1/plt2+plot_layout(heights = c(2,5), guides="collect")

```



plt.sup1



The increase in population density in S2 was observed for populations of both *Methylobacterium* and *Sphingomonas* (Fig. 3c). However, within *Methylobacterium*, MeL92 and Mr0-1 benefited the most in S2 (Fig. S1a). In particular, Mr0-1 consistently benefited from the presence of individual *Sphingomonas* strains. However, this effect on methylobacteria was lost when a third competitor was present (S3), regardless of the competitor's identity (Fig. S1b).

The increase in *Sphingomonas* population density in S2 was mainly associated with an increase in SmFR1 populations (Fig. S1a), which was higher in every S2 compared to C (Fig. S1b). For SpFA2, only a transient effect was observed when this strain was paired with SmFR1 or MeL92; however, both populations in each pair increased in density compared to C, suggesting facilitative interactions. The decrease in population density in S3 communities was observed only for *Methylobacterium* (Fig. 3c). The decrease in *Methylobacterium* population density was accompanied by an increase in their coefficient of variation (Fig. 3d). The increased coefficient of variation can be attributed to differences in densities between the different *Methylobacterium* strains: MeL85 was most impacted and had the lowest mean densities, while Mr0-1 and MeL92 were less impacted (Fig. S1a). Within methylobacteria, the largest mean difference was between MeL85 (1.58×10^5 CFU gFW⁻¹) and Mr0-1 (2.51×10^6 CFU gFW⁻¹) in S3 ($t_{546} = 11.94$, $p < 0.001$). However, the largest mean differences were observed between MeL85 and SmFR1 ($75.75 \times$, $t_{894} = 25.79$, $p < 0.001$).

and with SpFA2 (73.13×, $t_{894} = 25.58$, $p < 0.001$) in S3. In general, the decrease in each *Methylobacterium* population density was observed for every S3 community (Fig. S1b). These results indicate that bacterial taxa differentially respond to community complexity in the phyllosphere and that methylobacteria are more affected compared to sphingomonads.