Spatial distribution - Section 3

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Effect of community complexity on intraspecific spatial relations

```
fractions %>% head
```

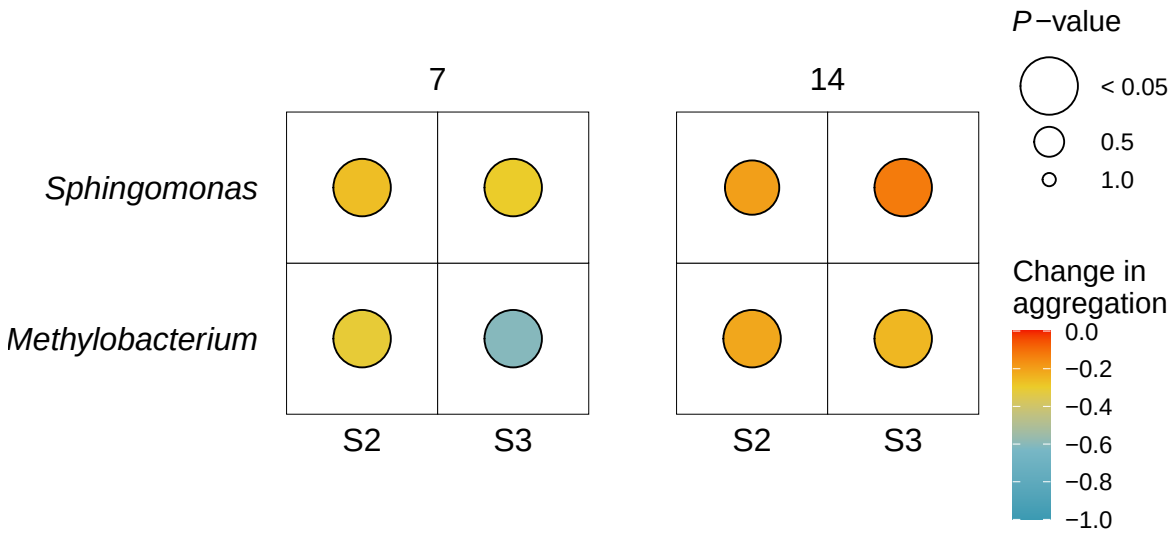
```
## # A tibble: 6 x 8
##   syncom synID strain dpi       r type               fraction taxa
##   <chr>  <chr> <chr>  <chr> <dbl> <chr>                 <dbl> <chr>
## 1 C.01   C     meL85  07dpi   0   aggregate_fraction   0      Methylobacterium
## 2 C.01   C     meL85  07dpi   0   regular_fraction     0      Methylobacterium
## 3 C.01   C     meL85  07dpi   0   random_fraction      0      Methylobacterium
## 4 C.01   C     meL85  07dpi   0.2 aggregate_fraction   0.0179 Methylobacterium
## 5 C.01   C     meL85  07dpi   0.2 regular_fraction     0.357  Methylobacterium
## 6 C.01   C     meL85  07dpi   0.2 random_fraction      0.625  Methylobacterium
```

Community context was expected to influence the spatial distribution patterns (aggregation, randomness, regularity) within bacterial populations in the phyllosphere. To evaluate this, we first determined relative frequencies of a spatial pattern based on K(r) for every strain in each community context. We then determined the area under the curve of each spatial pattern and calculated the fractional change compared to the near-isogenic control condition, C (Fig 6a).

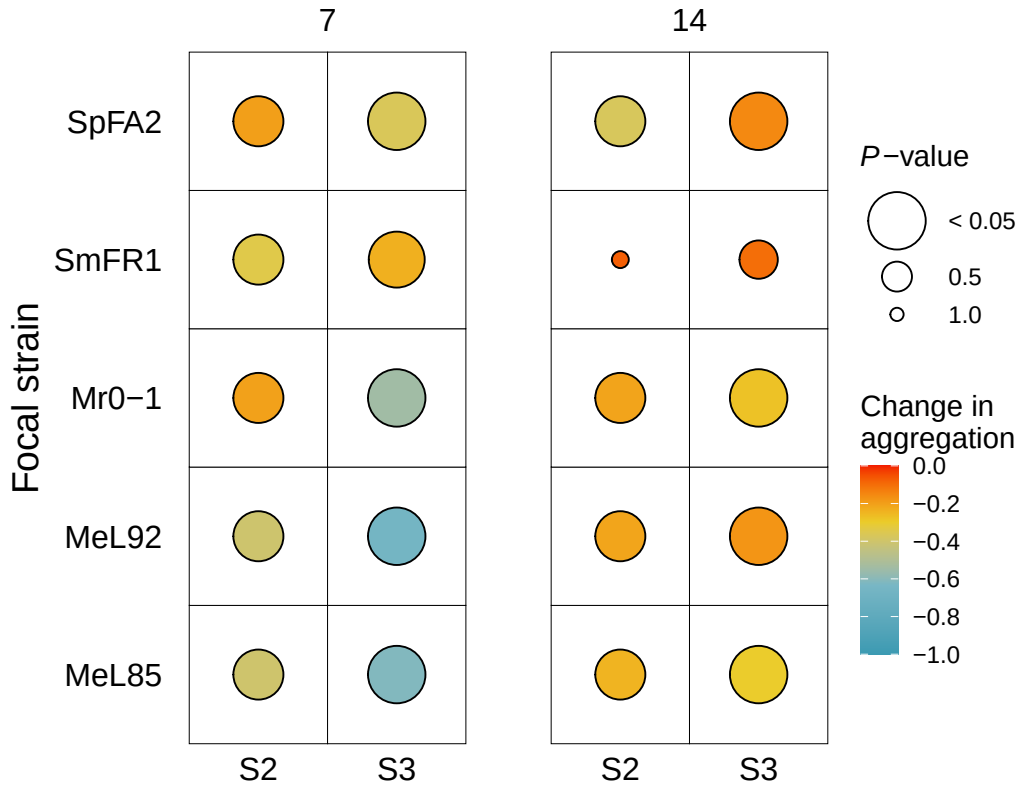
```
## Taxa
wilcox_one_taxa = auc_fold_change %>%
    group_by(synID, dpi, type, taxa) %>%
    wilcox_test(fractional_change ~ 1, mu = 0, detailed = TRUE) %>%
    select(synID, dpi, type, taxa, estimate, statistic, p) %>%
    mutate(
        p_size = case_when(p < 0.05 ~ 0.05, TRUE ~ p),
        p_label = case_when(p < 0.05 ~ "< 0.05", TRUE ~ as.character(p)))

## Strain
wilcox_one_strain = auc_fold_change %>%
    group_by(synID, dpi, type, strain) %>%
    wilcox_test(fractional_change ~ 1, mu = 0, detailed = TRUE) %>%
    select(synID, dpi, type, strain, estimate, statistic, p) %>%
    mutate(
        p_size = case_when(p < 0.05 ~ 0.05, TRUE ~ p),
        p_label = case_when(p < 0.05 ~ "< 0.05", TRUE ~ as.character(p)))
```

```
wilcox_one_taxa %>%
    filter(type == "aggregate_fraction") %>%
    ggplot(aes(synID, taxa))+
    facet_wrap(~dpi, ncol = 2, labeller = labeller(dpi=dpi.lab))+
    geom_tile(color = "black", fill = "white", linewidth = 0.1)+
    geom_point(aes(fill = estimate, size = p_size), shape = 21)+
    coord_fixed()+
```

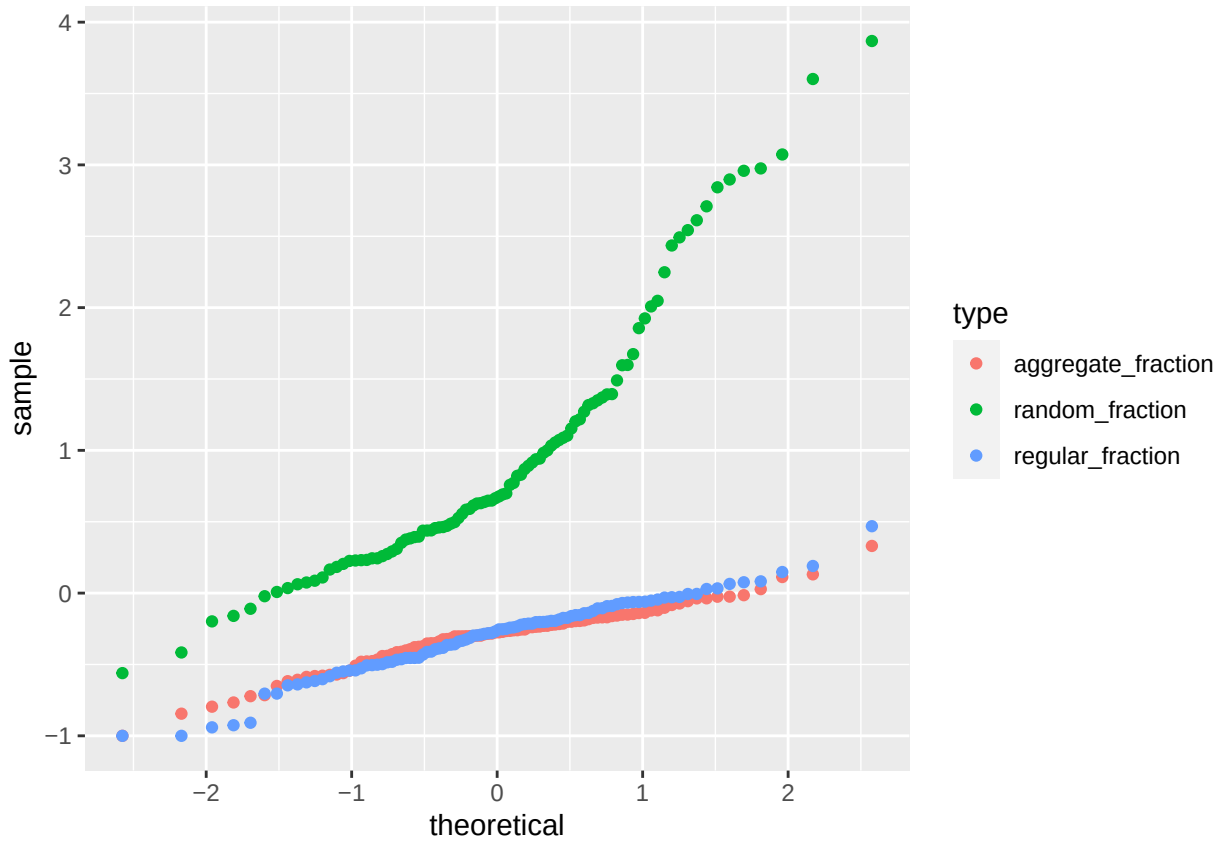
```
    scale_fill_gradientn(name = "Change in\naggregation",
        colours = wes_palette("Zissou1")[c(1,2,3,5)], values=c(0,0.55,1),
        limits = c(-1,0), breaks = seq(-1,0, 0.2))+
    scale_size_continuous(name = expression(paste(italic("P"), "-value")),
                          range = c(12,2), breaks = c(0.05, 0.5, 1), limits = c(0,1),
                          labels = c("< 0.05", "0.5", "1.0"))+
    labs(x="", y="")+
    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"))
```




```
wilcox_one_strain %>%
    filter(type == "aggregate_fraction") %>%
    ggplot(aes(synID, strain))+
    facet_wrap(~dpi, ncol = 2, labeller = labeller(dpi=dpi.lab))+
    geom_tile(color = "black", fill = "white", linewidth = 0.1)+
    geom_point(aes(fill = estimate, size = p_size), shape = 21)+
    coord_fixed()+
    scale_fill_gradientn(name = "Change in\naggregation",
        colours = wes_palette("Zissou1")[c(1,2,3,5)], values=c(0,0.55,1),
        limits = c(-1,0), breaks = seq(-1,0, 0.2))+
    scale_size_continuous(name = expression(paste(italic("P"), "-value")),
                          range = c(12,2), breaks = c(0.05, 0.5, 1), limits = c(0,1),
                          labels = c("< 0.05", "0.5", "1.0"))+
    scale_y_discrete(name = "Focal strain", labels = sp.lab)+
    labs(x="")+
    theme_rs()+
    theme(panel.border = element_blank(),
          axis.text.x = element_text(hjust=0.5, vjust=3),
          strip.text = element_text(face="plain"))
```




```
## Summary
auc_fold_change %>%
    ggplot(aes(sample = fractional_change, color = type))+
    geom_qq()
```




```
summary_type <- auc_fold_change %>%
    group_by(type) %>%
    summarise(median = median(fractional_change),
              q1 = format(round(quantile(fractional_change, 0.25), 2), nsmall = 2),
              q3 = format(round(quantile(fractional_change, 0.75), 2), nsmall = 2),
              range = paste0(q1,"-(",q3,")", sep=''))

## Kruskal-Wallis
kw_type <- auc_fold_change %>%
    kruskal_test(fractional_change ~ type) %>%
    mutate(
        p_size = case_when(p < 0.05 ~ 0.05, TRUE ~ p),
        p_label = case_when(p < 0.05 ~ "< 0.05", TRUE ~ as.character(p)))

kw_eff_type <- auc_fold_change %>%
    kruskal_effsize(fractional_change ~ type, ci=TRUE, nboot=100)

## One-sample Wilcoxon test
w1_type <-  auc_fold_change %>%
    group_by(type) %>%
    wilcox_test(fractional_change ~ 1, mu = 0, detailed = TRUE) %>%
    select(type, estimate, statistic, p) %>%
    mutate(
        p_size = case_when(p < 0.05 ~ 0.05, TRUE ~ p),
        p_label = case_when(p < 0.05 ~ "< 0.05", TRUE ~ as.character(p)))
```

Our initial analysis showed that spatial distribution patterns within populations differed from their respective

controls (Kruskal-Wallis, $H(2) = 177.1$, $p < 0.05$). Generally, aggregation and regularity were lower (-0.28 (-0.41-(-0.17)) and -0.26 (-0.46-(-0.12)), respectively, median (IQR)), while randomness was higher.

```
## One sample Wilcoxon test
wilcox_one_dpi = auc_fold_change %>%
    group_by(dpi, type) %>%
    wilcox_test(fractional_change ~ 1, mu = 0, detailed = TRUE) %>%
    select(dpi, type, estimate, statistic, p) %>%
    mutate(
        p_size = case_when(p < 0.05 ~ 0.05, TRUE ~ p),
        p_label = case_when(p < 0.05 ~ "< 0.05", TRUE ~ as.character(p)))

## Two samples Wilcoxon test
w_dpi <- auc_fold_change %>%
    wilcox_test(fractional_change ~ dpi, p.adjust.method = "holm", detailed = TRUE)

auc_fold_change %>%
    group_by(type) %>%
    wilcox_test(fractional_change ~ dpi, p.adjust.method = "holm", detailed = TRUE)
```

```
## # A tibble: 3 x 13
##   type       estimate .y.   group1 group2    n1    n2 statistic       p conf.low
## * <chr>         <dbl> <chr> <chr>  <chr>  <int> <int>     <dbl>   <dbl>    <dbl>
## 1 aggregate~   -0.181 frac~ 07dpi  14dpi     50    50       578 3.67e-6  -0.264
## 2 random_fr~    0.274 frac~ 07dpi  14dpi     50    50      1510 7.36e-2  -0.0229
## 3 regular_f~   -0.179 frac~ 07dpi  14dpi     50    50       747 5.32e-4  -0.281
## # i 3 more variables: conf.high <dbl>, method <chr>, alternative <chr>
```

```
## taxa
auc_fold_change %>%
    #filter(type == "aggregate_fraction") %>%
    group_by(dpi, type) %>%
    wilcox_test(fractional_change ~ taxa, p.adjust.method = "holm")
```

```
## # A tibble: 6 x 9
##   dpi   type              .y.       group1 group2    n1    n2 statistic       p
## * <chr> <chr>             <chr>     <chr>  <chr>  <int> <int>     <dbl>   <dbl>
## 1 07dpi aggregate_fraction fraction~ Methy~ Sphin~    30    20       133 6.91e-4
## 2 07dpi random_fraction    fraction~ Methy~ Sphin~    30    20       553 3.03e-8
## 3 07dpi regular_fraction   fraction~ Methy~ Sphin~    30    20       105 1.17e-4
## 4 14dpi aggregate_fraction fraction~ Methy~ Sphin~    30    20       207 6.67e-2
## 5 14dpi random_fraction    fraction~ Methy~ Sphin~    30    20       479 2.5 e-4
## 6 14dpi regular_fraction   fraction~ Methy~ Sphin~    30    20       255 3.82e-1
```

```
## synID
auc_fold_change %>%
    filter(type == "aggregate_fraction") %>%
    group_by(dpi) %>%
    wilcox_test(fractional_change ~ synID, p.adjust.method = "holm")
```

```
## # A tibble: 2 x 8
##   dpi   .y.               group1 group2    n1    n2 statistic       p
## * <chr> <chr>             <chr>  <chr>  <int> <int>     <dbl>   <dbl>
## 1 07dpi fractional_change S2     S3        20    30       452 0.00218
## 2 14dpi fractional_change S2     S3        20    30       269 0.549
```

```
## strain
auc_fold_change %>%
    filter(type == "aggregate_fraction") %>%
    group_by(dpi) %>%
    kruskal_test(fractional_change ~ strain)
```

```
## # A tibble: 2 x 7
##   dpi   .y.                   n statistic    df       p method
## * <chr> <chr>             <int>     <dbl> <int>   <dbl> <chr>
## 1 07dpi fractional_change    50     13.9      4 0.00776 Kruskal-Wallis
## 2 14dpi fractional_change    50      8.36     4 0.0794  Kruskal-Wallis
```

```
auc_fold_change %>%
    filter(type == "aggregate_fraction" & dpi == "07dpi") %>%
    group_by(synID) %>%
    dunn_test(fractional_change ~ strain, p.adjust.method = "holm")
```

```
## # A tibble: 20 x 10
##    synID .y.    group1 group2    n1    n2 statistic       p   p.adj p.adj.signif
##  * <chr> <chr>  <chr>  <chr>  <int> <int>     <dbl>   <dbl>   <dbl> <chr>
##  1 S2    fract~ meL85  meL92      4     4    -0.359 7.20e-1 1       ns
##  2 S2    fract~ meL85  mr01       4     4     1.55  1.20e-1 0.842   ns
##  3 S2    fract~ meL85  smfr1      4     4     0.299 7.65e-1 1       ns
##  4 S2    fract~ meL85  spfa2      4     4     1.79  7.30e-2 0.584   ns
##  5 S2    fract~ meL92  mr01       4     4     1.91  5.58e-2 0.502   ns
##  6 S2    fract~ meL92  smfr1      4     4     0.657 5.11e-1 1       ns
##  7 S2    fract~ meL92  spfa2      4     4     2.15  3.14e-2 0.314   ns
##  8 S2    fract~ mr01   smfr1      4     4    -1.25  2.09e-1 1       ns
##  9 S2    fract~ mr01   spfa2      4     4     0.239 8.11e-1 1       ns
## 10 S2    fract~ smfr1  spfa2      4     4     1.49  1.35e-1 0.842   ns
## 11 S3    fract~ meL85  meL92      6     6    -0.557 5.77e-1 1       ns
## 12 S3    fract~ meL85  mr01       6     6     0.525 6.00e-1 1       ns
## 13 S3    fract~ meL85  smfr1      6     6     2.89  3.91e-3 0.0352  *
## 14 S3    fract~ meL85  spfa2      6     6     2.07  3.88e-2 0.233   ns
## 15 S3    fract~ meL92  mr01       6     6     1.08  2.79e-1 1       ns
## 16 S3    fract~ meL92  smfr1      6     6     3.44  5.75e-4 0.00575 **
## 17 S3    fract~ meL92  spfa2      6     6     2.62  8.71e-3 0.0697  ns
## 18 S3    fract~ mr01   smfr1      6     6     2.36  1.82e-2 0.128   ns
## 19 S3    fract~ mr01   spfa2      6     6     1.54  1.23e-1 0.616   ns
## 20 S3    fract~ smfr1  spfa2      6     6    -0.820 4.12e-1 1       ns
```

These observations were consistent between time points (Wilcoxon test, $W =$ `w_dpi$statistic`, $p = 0.01$). Aggregation and regularity decreased, while randomness increased from 7 to 14 dpi.

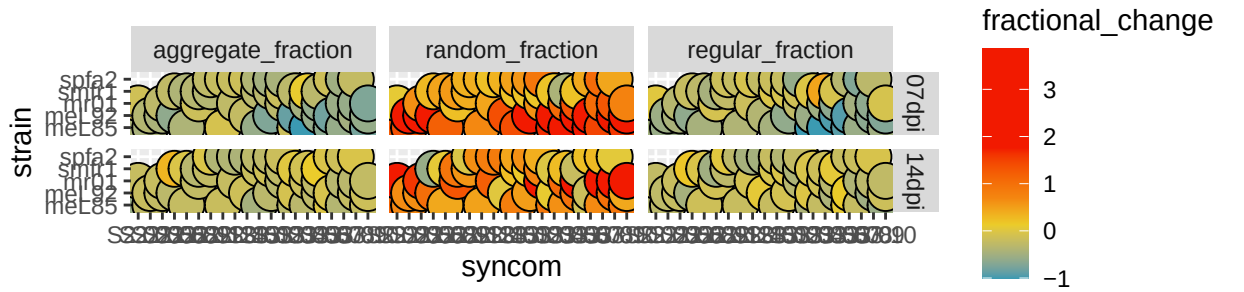
We used a one-sample Wilcox test to evaluate if the spatial pattern of a population in a community was different from its near-isogenic control (C). The null hypothesis was that there is no change of a spatial pattern within a population compared to C.

In general, both taxa decreased their self-aggregation pattern. *Methylobacterium* showed the largest decrease in aggregation, which was observed in every strain (Mr0-1, MeL92, and MeL85)

On average, we observed a 23.9% increase in aggregation of Methylobacterium strains (t17 = 6.58, p < 0.05) at 14 dpi in S3 (Table SX). This increase was present in every Methylobacterium strain (Fig. 6b, MeL85 = +28.6%; MeL92 = +16.6%; Mr0-1 = +26.6%). Within the sphingomonads, SmFR1 decreased its aggregation pattern by 11.6% at 7 dpi in S3 communities (t5 = -5.03, p < 0.05), while SpFA2 remained unchanged (Fig 6b).

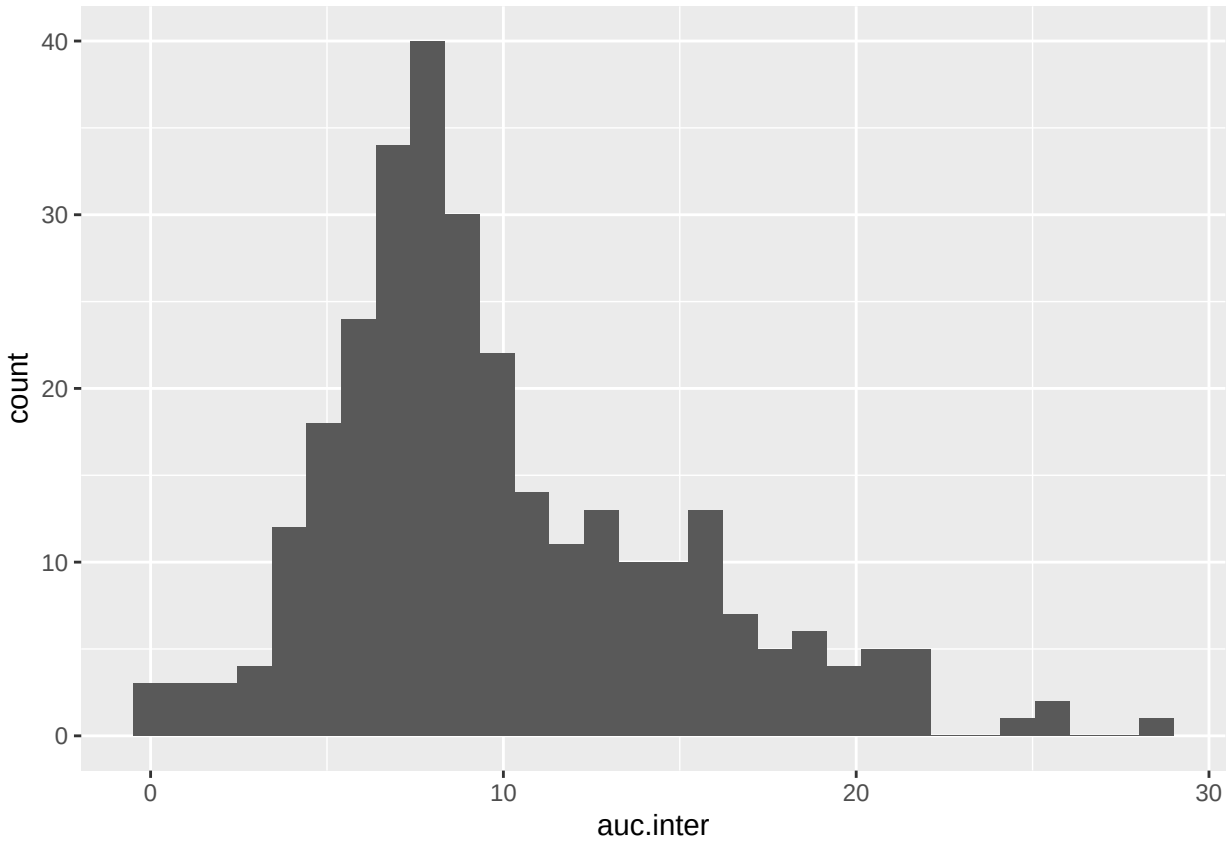
We defined the maximal intraspecific aggregation distances of a population as the maximal distance in which K(r) is higher than the upper limit of the estimator Kinhom(r) envelope, which indicates spatial aggregation (Fig 2b, Fig 6a). We determined the intraspecific aggregation distances for each interaction until they reached the maximal aggregation distance using the empirical cumulative distribution function (Fig. 6a), in which 95% of the aggregation was observed up to a given distance. We observed differences in intraspecific aggregation distances between strains: MeL85, MeL92, and SpFA2 showed the largest aggregation ranges of, in a few cases, up to 35 um, while Mr0-1 and SmFR1 showed the shorter aggregation ranges of 0–15.5 um and 0–18 um, respectively (Fig. 6c). These ranges were not explained by the treatments or sampling points, but only by strain differences ($F_{4,81} = 6.53$, $p < 0.05$).

```
auc_fold_change %>%
    ggplot(aes(syncom, strain))+
    facet_grid(dpi~type)+
    geom_tile(colour='black', fill='white')+
    geom_point(aes(fill=fractional_change), shape = 21, size = 6)+
    scale_fill_gradientn(colours = wes_palette("Zissou1")[c(1,3,5,5)], values = c(0,0.35,1))+
    coord_fixed()
```




```
auc_fold_change %>%
    ggplot(aes(auc.inter))+
    geom_histogram()
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



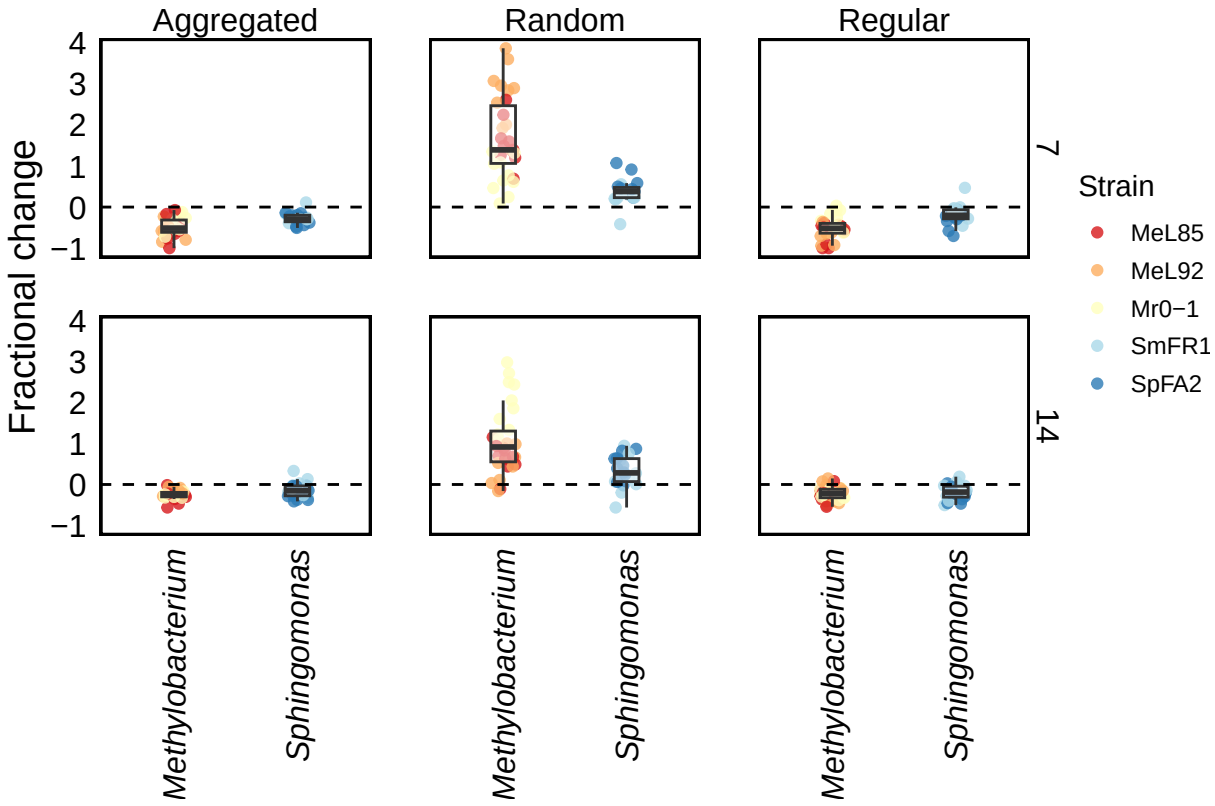

```
lmauc = lm(fractional_change ~ synID + dpi + type + strain, data = auc_fold_change)
shapiro.test(rstandard(lmauc))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  rstandard(lmauc)
## W = 0.9, p-value = 2e-13
```

```
ncvTest(lmauc)
```

```
## Non-constant Variance Score Test
## Variance formula: ~ fitted.values
## Chisquare = 175, Df = 1, p = <2e-16
```

```
auc_fold_change %>%
    ggplot(aes(taxa, fractional_change))+
    facet_grid(dpi ~ type, labeller = labeller(dpi = dpi.lab, type = pattern.lab))+
    geom_jitter(aes(color = strain), width = 0.1, alpha = 0.8)+
    geom_boxplot(alpha = 0.5, fill = "white", width = 0.2, outlier.alpha = 0)+
    geom_hline(yintercept = 0, linetype = "dashed")+
    theme_rs()+
    theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1, face="italic"),
          strip.text = element_text(face = "plain"))+
    labs(x = "", y = "Fractional change")+
    scale_color_manual(name = "Strain", values=sp.pal, labels=sp.lab)
```


Aggregated
Random
Regular
Fractional change
4
3
2
1
0
−1
7
14
Strain
MeL85
MeL92
Mr0−1
SmFR1
SpFA2
Methylobacterium
Sphingomonas